ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Molecular Docking of 1-Benzoyl-3-Methylthiourea as Anti Cancer Candidate and Its Absorption, Distribution, and Toxicity Prediction

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Abstract

Thiourea is one of the organic compounds are widely used in drug research. From some of the results of existing research produced that thiourea derivatives have activity as an analgesic, antibiotics, antimalarial and anticancer. This study has been designed thiourea to be fourteen 1-benzoyl-3-methylthiourea compounds. These compounds were studied its interaction with ribonucleotide reductase receptor by docking methods, tested its absorption-distribution and toxicity. From the docking results with ribonucleotide reductase receptor produced that all of the 1-benzoyl-3-methylthiourea compound has ΔG (binding energy) is lower than the comparison (hydroxyurea). And from the adsorption and distribution prediction indicate that all of compounds have good adsorption and distribution. And from the toxicity prediction indicate that there are six compounds (**a, f, i, k, l** and **n)** have low toxicity. So we concluded that some 1-benzoyl-3-methylthiourea derivatives could be novel inhibitors for ribonucleotide reductase as anticancer candidate.

Keywords: Thiourea, anticancer, ribonucleotide reductase, toxicity

INTRODUCTION

Ribonucleotide Reductase (RNR) is an enzyme responsible for the reduction of ribonucleotides to their corresponding Deoxyribonucleotides (DNA), which is a building block for DNA replication and repair mechanisms. The key role of RNR in DNA synthesis and control in cell growth has made this an important target for anticancer therapy **[1-4]**.

Recently, three RNR inhibitor used clinically (HU, 3-Aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP), and GTI2040) each has significant drawbacks. Therefore, there is a need in the art for compositions and methods to target and treat cancer more effectively based RR [5]. Hydroxyurea (HU) is the hydroxylated analogue of urea and is the first RR inhibitor to be studied extensively. It has directed tyrosine radical activity in the parts of the R2 and the first found to be active against cancer cells in 1963 [6]. Subsequent experiments in vitro showed that in addition to the direct inhibition of DNA synthesis, it also sensitizer cell killing by X-ray, especially if given before or after IR [7].

In this paper, we have designed some molecules similar with hydroxyurea to be 1-benzoyl-3-methylthiourea derivatives, were identified using structure-based virtual screening approach is based on the crystal structure of Ribonucleotide Reductase. We hope that this study will provide a useful approach for anticancer drug discovery.

MATERIALS AND METHODS

Preparation of Protein Structure

The crystal structure of Ribonucleotide Reductase in complex with Gemcitabine (PDB entry 2EUD) **[8]** recovered from the Brookhaven Protein Data Bank was used as a target for virtual screening using ArgusLab 4.0.1.

Preparation of Compounds

Structure of ligands were drawn using Marvin Sketch software. The structure was cleaned in 3D format and energy was minimized using Marvin Sketch software. The resulting structure was then saved in ".mol" file formats for molecular docking studies, and compounds can be shown in figure 1.

Molecular Docking

ArgusLab 4.0.1. has fast become a favourite introductory molecular modelling package with academics mainly because of its user-friendly interface and intuitive calculation menus **[9]**. Docking was done using the 'ArgusDock engine' exhaustive search docking function of ArgusLab with a grid resolution of 0.4 Å. The default settings of the scoring function and adjusted functions were used for the study. Docking precision was set to 'Regular precision' and the 'Flexible' ligand-docking mode was employed for each docking run. Furthermore, the size of the binding site bounding box was determined automatically using ArgusLab (16.5 × 17.25 × 16.75 Å). The root-mean-square deviation (RMSD) between the experimental and computational ligand structures were computed, and evaluated based on a hypothesis that an RMSD of less than or equal to 2.0 Å was defined as reasonable. The validated method of the docking calculation was then used to perform docking of 1-benzoyl-3-methylthiourea derivatives to the RR binding site. Binding affinity was characterised by binding energy values (ΔG) and hydrogen bonds between ligands and the enzyme **[10]**. The free binding energy results are presented in Table 1.

Predicting the absorption, distribution and toxicity properties

The PreADMET program was accessed at http://preadmet.bmdrc.org/. The 1-benzoyl-3-methylthioureas were used in this study. The structure of all compounds were converted into mol file (*.mol). The program automatically calculated the predictive absorption for Caco-2 cell, HIA (human intestinal absorption), and plasma protein binding **[11]**. Predicting the toxicity properties was done using ECOSAR **[12]**.

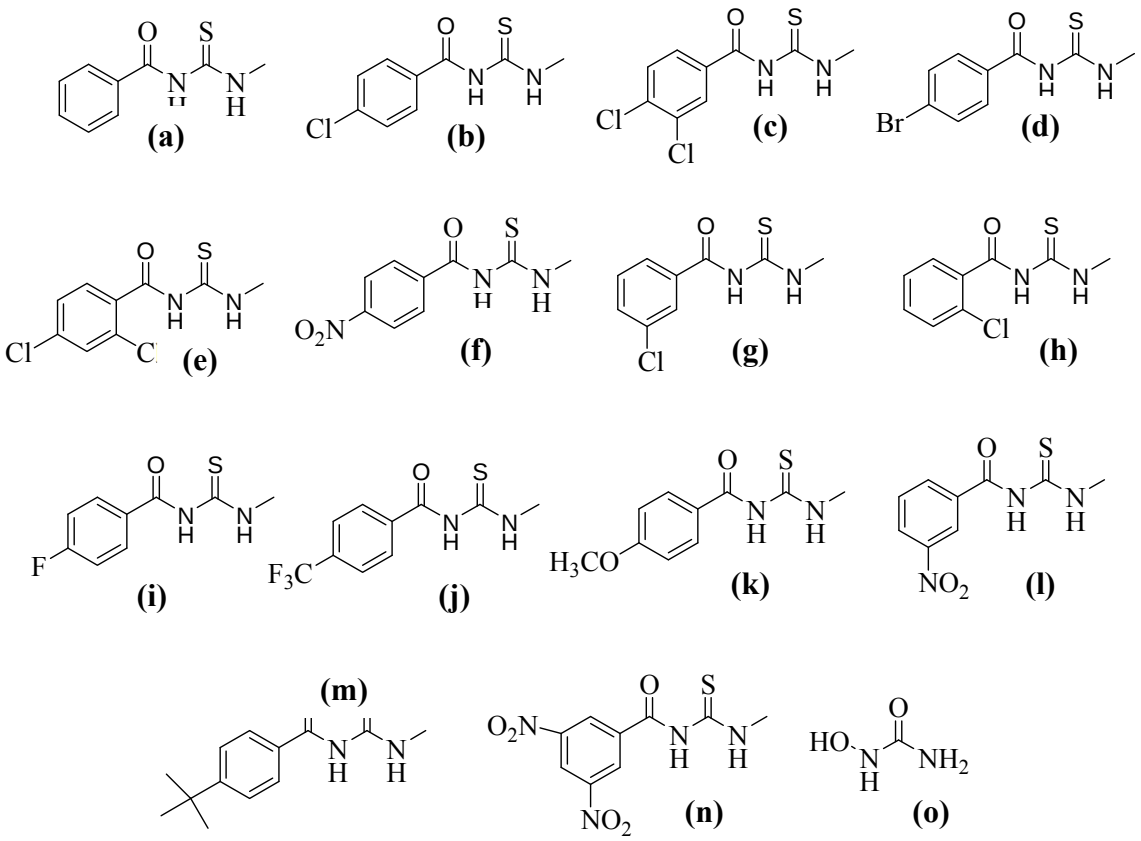

Figure 1: Structure of 1-benzoyl-3-methylthiourea derivatives (a-n) and Hydroxyurea (o)

RESULTS AND DISCUSSION

Validation of The Docking Method

ArgusLab has two docking engine types, i.e., ArgusDock and GaDock, and we used the ArgusDock engine type and then measuring the Root-mean-square Deviation (RMSD) of the Cartesian coordinates of the atoms of the reference ligand (Gemcitabine) in the docked and crystallographic conformations. In the present study, ArgusDock engine gave a good result. Figure 2 shows the conformation superposition of Gemcitabine from the X-ray crystal structure of Gemcitabine -RR complex and that from the docking calculation by ArgusDock engine. An RMSD value between the conformations is only 1.7045 Å, indicating that the parameter set for docking is capable to be used for performing the docking calculation of the 1-benzoyl-3-methylthiourea derivatives.

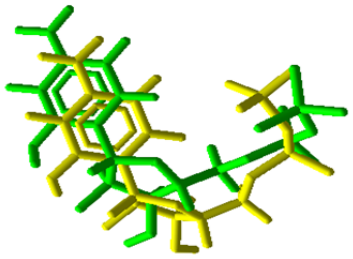

Figure 2. Overlay of the original structure poses obtained from the crystal structure (yellow) and the predicted docking pose obtained from the docking simulations (green)

Docking Results

The docking results of 1-benzoyl-3-methylthyourea and derivatives on ribonucleotide reductase receptor compared with Hydroxyurea compound which is a class of anticancer drugs. Based on the docking results obtained 1-benzoyl-3-methylthyourea and derivatives have free binding affinity (ΔG) which is lower than Hydroxyurea. Free binding affinity (ΔG) is a strength of binding affinity parameter from the compound to the receptor. The lower ΔG then the strength of the higher binding affinity ligand-receptor so more stable. ΔG values of the docking results can be shown in Table 1.

Tabel 1. The Binding affinity (ΔG) of 1-benzoyl-3-methylthyoureas and controls on ribonucleotide reductase

code of compound	ΔG (kcal/mole)
a	-7,69631
b	-7,74926
c	-8,20484
d	-8,2132
e	-8,02125
f	-7,56982
g	-7,62751
h	-7,73248
i	-7,518
j	-8,03418
k	-7,43964
l	-8,24917
m	-8,8014
n	-6,18551
Control	
o	-5,30536

Based on table 1, can be explained that the binding affinity of 1-(4-t-butylbenzoyl)-3-methylthiourea is the best if compared to the others and hydroxyurea. But when compared with hydroxyurea then all of the 1-benzoyl-3-metiltiourea derivatives had a lower binding affinity (ΔG) so it can be explained that all compounds having interaction with ribonucleotide reductase that is more stable than hydroxyurea.

In addition, the docking results can be analyzed interactions between compound to receptor by means of visualization using molegro molecular viewer program. By visualization can be observed amino acid residues contact and the hydrogen bonds formed between the compound to the receptor. The number and length of hydrogen bonds and the amino acid residues that interact with receptors of the compounds can be shown in Table 2.

Table 2. The number and length of hydrogen bonding and the amino acid residues that interact with ribonucleotide reductase

Code of compound	Length of hydrogen bonding (Å)	Amino acid residues contact		Number of Amino acid residues contact
		Hydrogen bonding	Steric interaction	
a	2.6331 3.15187	Ser 217 (S) Cys 428 (N)	Pro 203, Ser 202, Asn 291, Phe 206, Lys 292, Ala 245, Ser 216, Ile 248, Ser 217, Gly 246, Gly 247, Cys 428, Leu 427, Asn 426, Cys 218, Arg 293	16
b	2.94179 2.35988	Cys 218 (O) Ser 217 (S)	Thr 611, Thr 608, Met 606, Ser 217, Cys 218, Leu 445	6
c	2.71389 2.17339	Cys 428 (O) Arg 293 (N)	Pro 607, Ser 610, Thr 608, Ala 446, Thr 611, Ser 442, His 200, Ser 217, Leu 445, Ala 201, Pro 203, Met 606, Ser 202, Glu 430, Cys 218, Leu 427, Cys 428, Ala 609, Ala 296, Arg 293	20
d	1.95998 3.09997	Arg 293 (O) Gly 247 (N)	Phe 329, Tyr 742, Phe 403, Pro 607, Tyr 741, Ala 296, Arg 293, Ser 217, Cys 428, Gly 246, Cys 218, Pro 203, Leu 427, Ala 245, Ser 216, Gly 247	16
e	2.99952 2.86169	Arg 293 (O) Cys 428 (N)	Ala 296, Cys 428, Pro 607, Thr 608, Ala 446, leu 445, Glu 430, Met 606, Ser 217, Thr 611, Pro 203, Ser 202, Ser 610, Arg 293, Ala 201, Ala 609, leu 427, Tyr 741	18
f	2.41995 2.92561 2.01648	Thr 611 (O) Cys 428 (O) Arg 293 (N)	Ala 446, His 200, Pro 203, Ser 202, Thr 608, Ser 447, Thr 611, Ala 201, Met 606, Ser 610, Ala 609, Leu 445, Glu 430, Pro 607, Cys 218, Ser 217, Cys 428, Leu 427, Ala 296, Arg 293	20
g	3.22176	Cys 218 (N)	Thr 611, Ser 217, Thr 608, Ser 447, Ala 201, His 200, Met 606, leu 445, Ala 609, Ala 446, Ser 610, Ser 202, Arg 293, Pro 607, Leu 427, Cys 428, Cys 218, Gly 247	18
h	2.64827	Ala 296 (O)	Leu 427, Ala 296, Arg 293, Cys 428, Tyr 742, Phe 329, Gly 295, Pro 294	8
i	3.35278 2.91686	Cys 428 (O) Arg 293 (N)	Ser 202, Ser 447, Thr 611, Thr 199, Met 606, Thr 608, Ala 446, His 200, Glu 430, Ala 201, Ser 217, Ala 609, Cys 218, Pro 607, Arg 293, Cys 428, Leu 427, Leu 445, Pro 203	19
j	3.2062 2.50443	Cys 218 (O) Ser 217 (S)	Phe 403, Tyr 741, Ala 296, Tyr 742, Phe 329, Leu 427, Gly 247, Cys 428, Cys 218, Gly 246, Arg 293, Pro 203, Ser 217	13
k	2.99977 3.0827 3.10333	Thr 608 (O) Cys 218 (O) Arg 293 (N)	Ala 609, Ala 201, Thr 608, Thr 611, Ser 447, Met 606, Ala 446, Glu 430, Leu 445, Ser 217, Cys 218, Gly 247, Arg 293, Cys 478, Leu 427, Ala 296, Pro 607	17
l	2.86866 3.00019 3.11631 2.14424	Arg 293 (O) Ser 202 (O) Thr 611 (N) Thr 611 (O)	Gly 246, Gly 247, Cys 428, Ala 296, Cys 218, Arg 293, Pro 607, Ala 609, Leu 427, Ser 217, Glu 430, Met 606, Ser 202, Leu 445, Ser 610, Pro 203, Thr 611, Thr 608, His 200, Ala 201	20
m	2.3029 3.08109 2.87392	Cys 428 (O) Cys 218 (N) Gly 247 (N)	Gly 247, Asn 426, Phe 248, Ala 296, Cys 218, Cys 428, Glu 430, Arg 293, Gly 247, Leu 427, Pro 607, Ser 217, Ser 447, Thr 611, Ser 202, Met 606, Ala 609, Leu 445, Thr 608, His 200, Ala 446, Ala 201	22
n	2.42259 3.10687 3.19843 2.35558 3.21826	Arg 293 (O) Cys 428 (N) Cys 218 (O) Tyr 742 (O) Tyr 741 (N)	Ala 296, Gly 295, Arg 293, Pro 294, Cys 218, Cys 428, Ser 217, Leu 427, Tyr 742, Phe 329, Phe 403, Tyr 741	12
o	2.94696 3.13786 2.97558 2.51548 3.17127	Ala 609 (N) Ser 610 (N) Thr 611 (O) Ser 202 (O) Ser 202 (N)	Ser 206, Pro 607, Ala 609, Thr 608, Ala 201, Ser 610, Arg 393, Thr 611	9

From the table 1 can be explained that **1-(4-t-butylbenzoyl)-3-methylthiourea** compound interaction with the lowest free binding energy occurs formation of three hydrogen bonding with Cys 428 attached to the O atom, Cys 218 to the N atom and Gly 247 to the N atom and each having a length of hydrogen bonding 2.3029Å, 3.08109Å and 2.87392Å.

The Adsorption and Distribution Prediction

To predict the absorption and distribution parameters through http://preadmet.bmdrc.kr/. Caco2 cell parameter used to determine the permeability of the compound, the parameters of human intestinal absorption (HIA) can predict the percent of absorption the human intestine (% HIA), and plasma protein binding (%PPB) is used to determine the value of a drug is bound available for diffusion or transport across the membrane cells and also the interaction with the target pharmacology. The predicted value of the absorption and distribution of 1-benzoyl-3-methylthiourea derivatives can be shown in Table 3.

Tabel 3. The **Absorption and Distribution** data prediction of the 1-benzoyl-3-methylthiourea derivatives

Code of compound	Absorption		Distribution
	Caco-2	%HIA	%PPB
a	22.8174	93.212747	59.504748
b	29.1161	94.006304	76.918270
c	39.5905	94.798781	83.466229
d	32.5708	94.561760	80.810759
e	39.1821	94.798739	85.376094
f	17.0508	86.077493	45.504375
g	6.47461	94.006304	74.806518
h	29.6163	94.006246	77.903676
i	23.7916	93.223012	70.464457
j	25.4574	93.406867	82.446175
k	24.0992	93.594419	72.235978
l	20.8938	86.077493	72.574710
m	25.6117	93.864927	92.642787
n	20.9855	80.750572	81.053162

From the table 3 can be explained that all compounds with caco-2 cell parameters were ranged 4-70 nm/sec including middle permeability classification **[13-15]**, % HIA value more than 70% including well absorbed compound classification **[16, 17**], and % PPB value less than 90% including chemical weakly bound classification.

The Toxicity Prediction

Toxicity prediction of 1-benzoyl-3-methylthiourea derivatives used ECOSAR program. ECOSAR predict the potential toxicity of the compounds against living organisms in water and predict toxicity estimate acute toxicity (short-term) and chronic toxicity (long-term). Table 4 shows the results of toxicity prediction of 1-benzoyl-3-methylthiourea derivatives.

Based on the neutral organic SAR values (baseline toxicity), there are six compounds (**a, f, i, k, l** and **n**), which is predicted to have low toxicity because it has LC_{50} values greater than 100 mg/L, while the eight others (**b, c, d, e, g, h, j** and **m**) including moderate toxicity category **[18]**.

CONCLUSION

From this study, fourteen 1-benzoyl-3-methylthiourea compounds have been docked into ribonucleotide reductase. All of them had better free binding energy (ΔG) more than hydroxyurea. The results of adsorption and distribution prediction indicate that all of compounds have good adsorption and distribution. And from the toxicity prediction indicate that there are six compounds (**a, f, i, k, l** and **n)** have low toxicity. So we concluded that some 1-benzoyl-3-methylthiourea derivatives could be novel inhibitors for ribonucleotide reductase as anticancer candidate.

Table 4. Toxicity prediction data by ECOSAR program

Code of compound	MW (g/ mol)	*Water Solubility* (mg/ L)	Log Kow	*Neutral organic SAR* (*baseline toxicity*) (mg/ L) *Fish* LC_{50}, 96 h
a	194.25	1743	1.673	313.600
b	228.70	324.3	2.318	97.375
c	263.14	58.98	2.962	29.549
d	273.15	113.5	2.563	70.000
e	263.14	58.98	2.962	29.549
f	239.25	588.6	1.491	563.100
g	228.70	324.3	2.318	97.375
h	228.70	324.3	2.318	97.375
i	212.24	949.7	1.874	226.393
j	262.25	113.3	2.636	57.790
k	224.28	1037	1.754	306.296
l	239.25	588.6	1.491	563.100
m	250.36	20.52	3.583	7.792
n	284.25	470.7	1.309	975.334
o	76.06	$9.911x10^5$	-1.679	$1.26x10^5$

ACKNOWLEDGEMENT

We would like to specially thank DRPM Kemenristek Dikti for doctoral grant, and the School of Pharmacy ITB, Airlangga University Surabaya and STIKes BTH Tasikmalaya for all facilities in the completion of this project.

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