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# CORRELATION BETWEEN *IN SILICO* AND *IN VITRO* RESULTS OF 1-(BENZOYLOXY)UREA AND ITS DERIVATIVES AS POTENTIAL ANTI-CANCER DRUGS

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**Abstract.** 1-(Benzoyloxy)urea and its derivatives were synthesized by modified Scotten-Bauman reaction with adding benzoyl chloride or homologs to hydroxyurea in tetrahydrofuran. Structure characterization was conducted based on ultra-violet (UV-VIS) spectrum, infrared (FT-IR), H nucleus magnetic resonance ($^1$H NMR), C nuclear magnetic resonance ($^{13}$C NMR) and mass spectrometry (MS). *In silico* test to predict anti-cancer activity of 1-(benzoyloxy)urea and its derivatives in ribonucleotide reductase enzyme (PDB: 2EUD) was done using Molegro Program. The anti-cancer activity test was performed *in vitro* by using MTT method to HeLa cell lines. *In silico* test result (Rerank Score) was correlated relative to anti-cancer activity ($\log 1/IC_{50}$). There was a significant linear relationship between *in vitro* and *in silico* anti-cancer activity.

**Keywords:** 1-(benzoyloxy)urea, derivatives, *in silico* test, *in vitro* test, rerank score.

## 1. Introduction

In Indonesia cancer is the fifth leading cause of death after heart disease, stroke, respiratory disease and diarrhea. Nearly six percent or 13.2 million of Indonesian people suffer from cancer and need early treatment. According to WHO, one person is dying every 11 minutes in the world because of cancer, and there is one new cancer patient every 3 minutes [1]. Based on the Report of National Basic Health Research 2013, the prevalence of tumor/cancer in Indonesia is 1.4 % per 1,000 people [2].

Cancer treatment can be carried out in several forms: surgery, radiation photo, immunotherapy, stem cell and chemotherapy [3]. Chemotherapy still remains one of the alternatives in cancer treatment, whether it can be done alone or collectively with other forms of treatment. There are some anti-cancer drugs used, including hydroxyurea.

Hydroxyurea was first synthesized in 1869, and its effect in slowing down the growth of leukocyte cells was first observed in 1928. Its clinical use as an anti-cancer compound began in the 1960s [4]. Hydroxyurea is antineoplastic that performs an activity to slow down the work of ribonucleotide reductase enzyme. The enzyme function is to convert ribonucleotide into deoxyribonucleotide. If the enzyme's work slows down, DNA biosynthesis will also slow down. The activities of hydroxyurea are called cytotoxic and antineoplastic that exhibits a special effect on S phase and disturbs cell cycle in phases of G2 and S [5]. Hydroxyurea is a derivative of urea that is used in myeloproliferative syndrome, chronic myelogenous leukemia (CML), polycythemia vera, and essential thrombocytosis [6].

To predict the activity of the compound, *in silico* test is carried out *via* computer simulation. *In silico* test is the method used to initiate discovery of new medicines and improve efficiency in the optimization of the main compounds activity [7]. The activity of synthesized medicinal compound can be predicted from the energy of molecular interaction between a receptor and ligand. The interaction energy can be illustrated with rerank score. *In silico* test is administered by docking molecule of the potential medicinal compound with the selected receptor. Docking is an attempt to streamline ligand that is a small molecule into the receptor that is a big protein by

considering the characteristics of both and the relation between each other [8].

The substitution of the lead compound will result in changes in physicochemical properties, namely lyophilic, electronic, and steric characteristics [9]. To design and develop new drugs, the physicochemical properties of drug molecules can be predicted before a new compound is synthesized and purified. In 1972, Topliss proposed operational scheme or diagram to synthesize the analog in drug design. The diagram was built on the basic assumption of Hänsch method, a certain substituent of which can alter relative activity towards the main compound based on the change of hydrophobic, electronic effect, and steric effect [10]. In this study hydroxyurea derivative was synthesized with the addition of benzoyl and homolog that boost lipophilic, electronic, and steric characteristics to improve cytotoxic activities. The synthesized homolog was 1-(benzoyloxy)urea with its derivatives: 1-(2-chlorobenzoyloxy)urea, 1-(4-chlorobenzoyloxy)urea, 1-(2,4-dichlorobenzoyloxy)urea, 1-(4-bromobenzoyloxy)urea, 1-(4-trifluoromethyl-benzoyloxy)urea, 1-(4-methylbenzoyloxy)urea, 1-(4-t-butylbenzoyloxy)urea, 1-(4-methoxybenzoyloxy)urea and 1-(4-fluorobenzoyloxy)urea.

Before synthesis the impact of *in silico* test of the compound 1-(benzoyloxy)urea and its nine derivatives and ribonucleotide reductase enzyme (PDB:2EUD) was assayed. Ribonucleotide reductase enzyme was used as the main target or anticancer compound receptor-like HU, 1-(benzoyloxy)urea, and its derivatives. The compound formed a complex structure with the crystal structure of ribonucleotide reductase enzyme I, which was 2EUD. 2EUD was selected because it is the receptor of gemcitabine [11]. The acquired result was the number of hydrogen bonds between the investigated compound and 2EUD, and the interaction energy value between them, which was reranked score. The aim of *in silico* test in the present study is to scrutinize the interaction between 1-(benzoyloxy)urea and its derivatives with 2EUD, from the standpoint of predicting anti-cancer activities.

## 2. Experimental

### 2.1. Materials

The used materials were: benzoyl chloride and its derivatives (Sigma-Aldrich, p.s.), tetrahydrofuran (Merck, p.a.), triethylamine (Merck, p.s.), ethanol (Merck, p.a.), DMEM (Dulbecco's Modified Eagle Medium*)* culture media, Methylthiazolyldiphenyl-tetrazolium bromide (MTT) reagent (Sigma-Aldrich), Sodium dodecyl sulfate (SDS) reagent (Sigma-Aldrich).

### 2.2. Methods

*In silico* test was performed by complexing the test compound with GCQ that is ligand found in 2EUD. GCQ is the depiction of gemcitabine, cancer that resembles the action like hydroxyurea. The computer programs that were used were ChemBio Draw and Molegro Virtual Docker. *In silico* test, the test compound resided in the cavity like GCQ. The yielded result was hydrogen bond number between the test compound and 2EUD, and the number of interaction energy between the test compound and 2EUD in the form of Rerank Score. In the present study, *in silico* test aims to understand the interaction between 1-(benzoyloxy)urea and its derivatives and 2EUD in order to forecast anti-cancer activities.

The synthesis was done by adding benzoyl chloride or homolog to tetrahydrofuran, to the mixture of hydroxyurea in tetrahydrofuran. Triethylamine was used as the catalyst. The mixter was stirring constantly with a magnetic stirrer at 268 K. [12, 13]. The purity of the results was determined with the melting point and thin-layer chromatography. Recrystallization was carried out with a hot ethanol. To ensure that the synthesized compound was in accordance with the expected one, structure characterization was conducted based on UV-VIS, FT-IR, $^{1}$H NMR, $^{13}$C NMR and MS analyses [14]. Synthesis reaction mechanism of 1-(benzoyloxy)urea and its derivatives can be seen in Fig. 1.

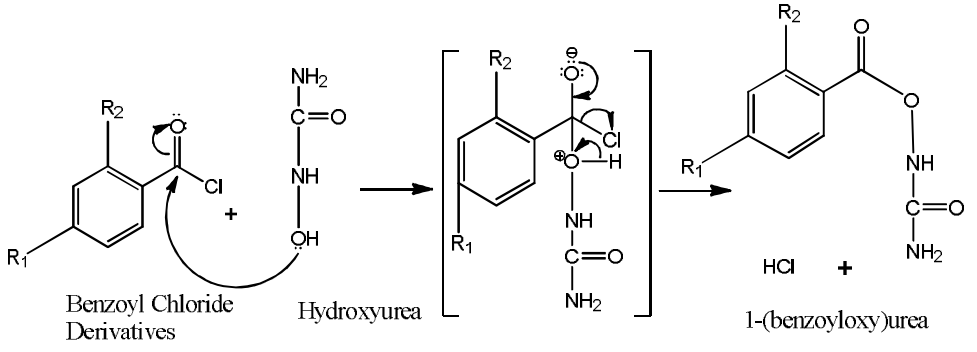


**Fig. 1.** Synthesis reaction mechanism of 1-(benzyloxy)urea and its derivatives

*Table 1*

**The results of the determination of percent yield, melting point and Rf of 1-(benzoyloxy)urea derivatives**

| Compound | Symbol | Yield, % | Melting point, K | Rf | | |
|---|---|---|---|---|---|---|
| | | | | Eluent 1 | Eluent 2 | Eluent 3 |
| 1-(Benzoyloxy)urea | BOU | 53.3 | 401–402 | 0.80 | 0.89 | 0.90 |
| 1-(4-Chloro-benzoyloxy)urea | 4-ClBOU | 59.5 | 462–463 | 0.73 | 0.70 | 0.89 |
| 1-(4-Methyl-benzoyloxy)urea | 4-$CH_3$BOU | 22.8 | 434–435 | 0.84 | 0.80 | 0.88 |
| 1-(4-Methoxybenzoyloxy)urea | 4-$OCH_3$BOU | 21.4 | 449–450 | 0.82 | 0.78 | 0.84 |
| 1-(4-*Tert*-butylbenzoyloxy)urea | 4-t-$C_4H_9$BOU | 46.8 | 395–396 | 0.86 | 0.84 | 0.84 |
| 1-(4-Trifluoromethylbenzoyloxy)urea | 4-$CF_3$BOU | 14.7 | 453–454 | 0.92 | 0.72 | 0.81 |
| 1-(4-Bromobenzoyloxy)urea | 4-BrBOU | 13.4 | 471–472 | 0.64 | 0.70 | 0.81 |
| 1-(4-Fluorobenzoyloxy)urea | 4-FBOU | 37.1 | 437–438 | 0.80 | 0.78 | 0.87 |
| 1-(2,4-Dichlorobenzoyloxy)urea | 2,4-DiClBOU | 25.3 | 405–406 | 0.89 | 0.87 | 0.76 |
| 1-(2-Chlorobenzoyloxy)urea | 2-ClBOU | 10.4 | 402–403 | 0.75 | 0.69 | 0.80 |

Cytotoxic activity was determined *via in vitro* test by using HeLa cells. The steps were conducted based on the protocol of Cancer Chemoprevention Research Center [15]. The main solution was dissolved gradually with DMEM culture media, so a series of standard solution was obtained. HeLa cell culture was prepared on the microplate with 96 wells, in the form of cell suspension with the density of 8000 cells/well. With probit analysis, the value of $IC_{50}$ that illustrates activities of the test compound was earned. To calculate quantitative structure-cytotoxic activity relationships, $IC_{50}$ was modified to log ($1/IC_{50}$).

The correlation between the *in silico* test result of rerank score (RS) of 1-(benzoyloxy)urea compound and its derivatives with log ($1/IC_{50}$) which depicted the cytotoxic activity of *in vitro* test result, was calculated with SPSS program.

## 3. Results and Discussion

The results of the structure characterization can be seen below [16].

*1-(Benzoyloxy)urea*: white crystal; UV spectrum, λ max (nm) in ethanol 204 and 232; IR spectrum, ν ($cm^{-1}$) in KBr pellet : 3131 and 3205 (-$NH_2$), 3273 (-NH), 1747 (C=O ester) 1686 (C=O amide), 1581 (-C=C- aromatic) and 1103 (-C-O-), 703 (-C-H aromatic); $^1$H NMR spectrum, δ (ppm) in DMSO-D6 : 9.77, s, H (–NH); 7.99, d, J = 6.3 Hz, 2H (Ar-H); 7.63, m, J = 2.9 Hz, 3H (Ar-H), 6.56, s, 2H (-$NH_2$); $^{13}$C-NMR spectrum (δ ppm) in DMSO-D6: C atom in 164.9, 159.2, 133.7, 129.3, 128.7, and 127.6; Mass Spectrometer spectrum (m/e): 135 ($C_7H_5NO_2^+$); 105 ($C_7H_5O^+$); 77 ($C_6H_5^+$).

*1-(4-Chlorobenzoyloxy)urea*: fine white crystal; UV spectrum, λ max (nm) in ethanol: 204 and 244; IR spectrum, ν ($cm^{-1}$) in KBr pellet : 3407 and 755 (-C-H aromatic); 3092 (-NH); 3221 and 3184 (-$NH_2$), 1750 (-C=O ester), 1723 (-C=O amide);1596 (-C=C- aromatic) and 1011 (-C-O-); $^1$H NMR spectrum, δ (ppm) in DMSO-D6: 9.81, s, 1H (-NH); 8.00, d, J = 9 Hz, 2H (Ar-H); 7.66, d, J = 8.6 Hz, 2H (Ar-H); and 6.60, s, 2H (-$NH_2$); $^{13}$C-NMR spectrum (δ ppm) in DMSO-D6: C atom in 164.1, 159.1, 138,6, 131.2, 128.8, and 126.4; Mass Spectrometer spectrum (m/e): HRMS (m/z): calculated mass for $C_8H_8N_2O_3Cl$ ($M^+$+H) 215.0223 and measured mass 215.0193.

*1-(4-Methylbenzoyloxy)urea*: white crystal; UV spectrum, λ max (nm) in ethanol: 206 and 242; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3449 (-$NH_2$), 3333 (-NH), 1749 (-C=O ester), 1683 (-C=O amide), 1589(-C=C-aromatic) and 1013(-C-O-), 747 (-C-H aromatic); $^1$H NMR spectrum, δ (ppm) in DMSO-D6: 9.70, s, 1H (NH), 7.96, d, J = 7.9 Hz, 2H (Ar-H); 7.41, d, J = 7.8 Hz, 2H (Ar-H), 6.52, s, 2H ($NH_2$); 2.40, s, 3H ($CH_3$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 164.9, 159.2, 144.1,129.4 ,129.2, 125.0 and 21.2; Mass Spectrometer spectrum (m/e): HRMS (m/z): calculated mass for $C_9H_{11}N_2O_3$ ($M^+$+H) 195.0769 and measured mass 195.0798.

*1-(Methoxybenzoyloxy)urea*: white crystal; UV spectrum, λ max (nm) in ethanol: 210 and 260; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3446 (-NH), 3171 (-$NH_2$), 3013 (-C-H aromatic), 1757 (-C=O ester), 1687 (-C=O amide), 1607 (-C-H aromatic); 1509 (-C=C- aromatic) and 1112 (-C-O-); $^1$H NMR spectrum, δ (ppm) in DMSO-D6: 9.67, s, 1H (NH); 7.94, d, J = 9 Hz, 2H (Ar-H); 7.02, d, J = 9 Hz, 2H (Ar-H); 6.52, s, 2H ($NH_2$) and 3.85, s, 3H ($CH_3$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 164.6 ,163.4, 159.3, 131.5, 119.5, 113.9, and 55.5. Mass Spectrometer spectrum (m/e): 135 m/z ($C_8H_7O_2^+$), 120 m/z ($C_7H_4O_2^{\cdot+}$) and 107 ($C7H7O^+$).

*1-(4-Tert-butylbenzoyloxy)urea*: fine yellowish white crystal; UV spectrum, λ max (nm) in ethanol: 204 and 242; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3505 (-NH); 3285 and 3215 (-$NH_2$); 1750 (-C=O ester); 1699 (-C=O amide); 1582 and 1459 (-C=C- aromatic); 1012 (-C-O-);

and 702 (-CH aromatic); $^{1}$H NMR spectrum, δ (ppm) in DMSO-D6: 9.73, s (NH);7.92, d, J = 8.55 Hz, 2H (Ar-H); 7. 53, d, J = 8.64 Hz, 2H (Ar-H); 6.53, s, 2H ($NH_2$), and 1.32, s, 9H ($3CH_3$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 164.8, 159.2, 156.8, 129.2, 125.4, 124.8, 34.9, and 30.3. Mass Spectrometer spectrum (m/e): HRMS (m/z): calculated mass for $C_{12}H_{17}N_2O_3$ ($M^+$+H) 237.1239 and measured mass 237.1252.

*1-(4-Trifluoromethylbenzoyloxy)urea*: white crystal; UV spectrum, λ max (nm) in ethanol: 226 and 274; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3438, (-NH); 3231and 3186 (-$NH_2$); 1751 (-C=O ester); 1717 (-C=O amide); 1515 and 1432 (-C=C- aromatic); 1014 (-C-O-) and 771 (-C-H aromatic); $^{1}$H NMR spectrum, δ (ppm) in DMSO-D6: 9.89, s, 1H, (NH); 8.18, d, J = 8.1 Hz, 2H (Ar-H); 7.88, d J = 9 Hz, 2H (Ar-H); 6.65, s, 2H ($NH_2$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 163.9, 159.1, 132.4, 131.5, 131.4, 130.2, and 125.7; Mass Spectrometer spectrum (m/e): HRMS (m/z): calculated mass for $C_9H_8N_2O_3F_3$ 249.0487 and measured mass 249.0458.

*1-(4-Bromobenzoyloxy)urea*: white crystal; UV spectrum, λ max (nm) in ethanol: 206 and 244; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3469 (-NH); 3225 (-$NH_2$); 1746 (-C=O ester); 1720 (-C=O amide); 1589.67 (-C=C- aromatic); 1008.68 (-C-O-); 750 (-C-H aromatic); $^{1}$H NMR spectrum, δ (ppm) in DMSO-D6: 9.79, s, 1H (-NH), 7.90, d, J = 3.42 Hz, 2H (Ar-H), 7.71, d, J = 8.82 Hz, 2H (Ar-H), 6.57, s, 2H (-$NH_2$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 165.7, 159.1, 131.8, 131.2, 129.4, 129.2, 127.8 and 126.8; Mass Spectrometer spectrum (m/e): HRMS (m/z) calculated mass for $C_8H_8N_2O_3Br$ ($M^+$+H) 258.9718 and measured mass 258.9738.

*1-(4-Fluorobenzoyloxy)urea*: fine white crystal; UV spectrum, λ max (nm) in ethanol: 204 and 234; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3418 (-NH); 3175 and 3083 (-$NH_2$); 1746 (-C=O ester); 1715 (-C=O amide); 1606 and 1507 (-C=C- aromatic); 1160.47 (-C-O-) and 760 (-C-H aromatic); $^{1}$H NMR spectrum, δ (ppm) in DMSO-D6: 9.78, s, 1H (NH); 8.06, m, J = 17.1 Hz, 2H (Ar-H); 7.38, m, J = 20.7 Hz, 2H (Ar-H); 6.68, s, 2H ($NH_2$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 170.7, 164.0, 159.4, 132.1, 133.0, 124.2,115.3 and 116.3; Mass Spectrometer spectrum (m/e): HRMS (m/z): calculated mass for $C_8H_8N_2O_3F$ ($M^+$+H) 199.0519 and measured mass 199.0511.

*1-(2,4-Dichlorobenzoyloxy)urea*: fine yellowish white crystal; UV spectrum, λ max (nm) in ethanol: 223 and 242; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3411 (-NH); 3314 and 3170 (-$NH_2$); 1755 (-C=O ester); 1682 (-C=O amide); 1556 and 1470 (-C=C- aromatic); 1111 (-C-O-) and 758 (-C-H aromatic); $^{1}$H NMR spectrum, δ (ppm) in DMSO-D6: 9.93, s, H (NH); 7.93, d, J = 21.5 Hz, 1H (Ar-H) 7.79, s, 1H (Ar-H), 7.56, d, J = 10.53 Hz, 1H (Ar-H), 6.59, s, 2H (-$NH_2$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 163.1, 158.9, 137.8, 133.7, 132.8, 130.3, 127.5, 126.3; Mass Spectrometer Spectrum (m/e): HRMS (m/z): calculated mass for $C_8H_7N_2O_3Cl_2$ ($M^+$) 248.9823 and measured mass 248.9841.

*1-(2-Chlorobenzoyloxy)urea*: white crystal; UV spectrum, λ max (nm) in ethanol: 230 and 280; IR spectrum, ν ($cm^{-1}$) in KBr pellet: 3481 (-NH); 3276 and 3207 (-$NH_2$); 1739 (-C=O ester); 1685 (-C=O amide); 1590 and 1437 (-C=C- aromatic); 1116.67 (-C-O-) and 744 (-C-H aromatic); $^{1}$H NMR spectrum, δ (ppm) in DMSO-D6: 9.91, s, 1H (NH); 7.99, d, J = 7.47 Hz,1H (Ar-H ); 7.54, m, J = 18.45 Hz, 3H (Ar-H); 6.56, s, 2H ($NH_2$); $^{13}$C NMR spectrum (δ ppm) in DMSO-D6: C atom in 163.9, 159.0, 133.7, 132.8, 131.4, 130.7, 127.6, and 127.2; Mass Spectrometer spectrum (m/e): HRMS (m/z): calculated mass for $C_8H_8N_2O_3Cl$ ($M^+$+H) 215.0233 and measured mass 215.0192.

The results of *in silico* and *in vitro*tests on 1-(benzyloxy)urea compound and its derivatives are presented in Table 2.

*Table 2*

**Log (1/$IC_{50}$) value and rerank score (RS) value from 1-(benzyloxy)urea compound and its derivatives**

| Compound | Symbol | RS | $IC_{50}$, µM/ml | Log (1/$IC_{50}$) |
|---|---|---|---|---|
| 1-(Benzoyloxy)urea | BOU | -79.9432 | 0.42 | 0.3768 |
| 1-(4-Chlorobenzoyloxy)urea | 4-ClBOU | -82.7887 | 0.47 | 0.3279 |
| 1-(4-Methylbenzoyloxy)urea | 4-$CH_3$BOU | -85.2089 | 0.43 | 0.3665 |
| 1-(4-Methoxybenzoyloxy)urea | 4-$OCH_3$BOU | -86.5856 | 0.40 | 0.3979 |
| 1-(4-*Tert*-butylbenzoyloxy)urea | 4-t-$C_4H_9$BOU | -91.4471 | 0.25 | 0.6021 |
| 1-(4-Trifluoromethylbenzoyloxy)urea | 4-$CF_3$BOU | -86.0949 | 0.33 | 0.4815 |
| 1-(4-Bromobenzoyloxy)urea | 4-BrBOU | -85.1651 | 0.37 | 0.4318 |
| 1-(4-Fluorobenzoyloxy)urea | 4-FBOU | -82.9755 | 0.42 | 0.3768 |
| 1-(2,4-Dichlorobenzoyloxy)urea | 2,4-DiClBOU | -81.0833 | 0.49 | 0.3098 |
| 1-(2-Chlorobenzoyloxy)urea | 2-ClBOU | -81.1349 | 0.44 | 0.3565 |

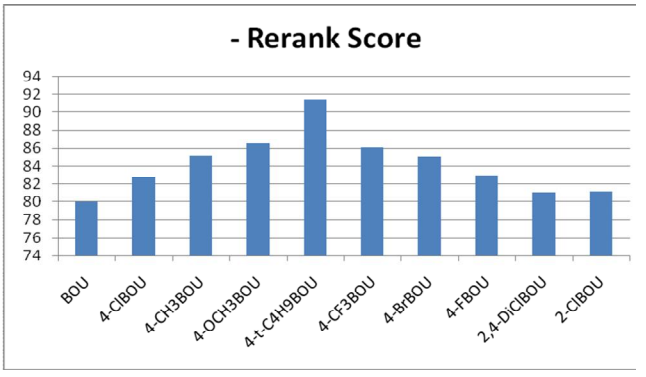


**Fig. 2.** RS values of 1-(benzoyloxy)urea and its derivatives

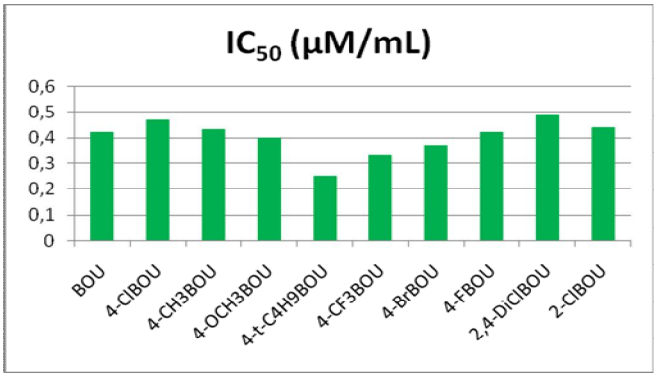


**Fig. 3.** $IC_{50}$ values of 1-(benzoyloxy)urea and its derivatives

Modification of the parent structure of 1-(benzoyloxy)urea was done by using a Topliss model approach. It is expected to increase the activity of the structure modifications, namely: Cl; $CH_3$; $OCH_3$; *t*-$C_4H_9$; $CF_3$; Br and F at the *para*-position; two Cl-groups at the *ortho*- and *para*-position; and Cl-group at the *ortho*-position. Each group was added to the benzene of 1-(benzoyloxy)urea, by nucleophilic substitution reactions between hydroxyurea and nine benzoyl chloride derivatives containing this cluster.

1-(2-Chlorobenzoyloxy)urea and 1-(4-chlorobenzoyloxy)urea were more lipophilic than 1-(benzoyloxy)urea, therefore it was expected they penetrate more easily the cell walls. The Cl-group was added into 1-(benzoyloxy)urea to strengthen the bonds between compounds and the receptor. 1-(2,4-Dichlorobenzoyloxy)urea had lipophilic properties to penetrate more easily the cell walls. 1-(4-Bromobenzoyloxy)urea with Br-group had also lipophilic properties, therefore it was able to penetrate easily the cell walls. 1-(4-Trifluoromethylbenzoyloxy)urea had a high lipophilicity in order to penetrate easily the cell walls. 1-(4-Fluorobenzoyloxy)urea contained fluoro-groups which had the nature of electron-withdrawing groups larger than chlorine groups. 1-(4-Methoxybenzoyloxy)urea had methoxy groups of poor lipophilicity resulted in poor penetration to the cell walls. However, this compound had a high electron booster to facilitate drug binding receptor. 1-(4-*Tert*-butilbenzoyloxy)urea with *tert*-butyl groups had high lipophilic properties to be able to penetrate cell walls as well as electron booster to facilitate drug binding receptor.

The illustration of activity prediction *via in silico*, which is rerank score and activities of test compound in the form of $IC_{50}$ value of 1-(benzoyloxy)urea and its derivatives can be seen in Figs. 2 and 3.

It is noticeable from Fig. 2, that 1-(4-*tert*-butylbenzoyloxy)urea has the smallest RS. This demonstrates that the energy bond between the compound and 2EUD is the smallest or the bond between both is the most stable one. It illustrates that 1-(4-*tert*-butylbenzoyloxy)urea is predicted to exhibit the highest activity.

It is obvious from Fig. 3 that 1-(4-*tert*-butylbenzoyloxy)urea has the smallest value of $IC_{50}$ or the smallest value compared to other derivative is required to kill 50 % of HeLa cells. The result indicates that 1-(4-*tert*-butylbenzoyloxy)urea exhibits the highest activity.

The linear correlation between log ($1/IC_{50}$) and RS value of 1-(benzoyloxy)urea and its derivatives was analyzed with SPSS, and the result can be illustrated below:

$$\log 1/IC_{50} = -0.022 \cdot (\pm 0.005) RS - 1.414 \cdot (\pm 0.383) \quad (1)$$

where RS is Rerank Score

This equation was obtained by calculating from 10 compounds ($n$ =10) resulting in regression coefficient $R$ = 0.859 at significance of 0.001.

Linear regression curve is depicted in Fig. 4.

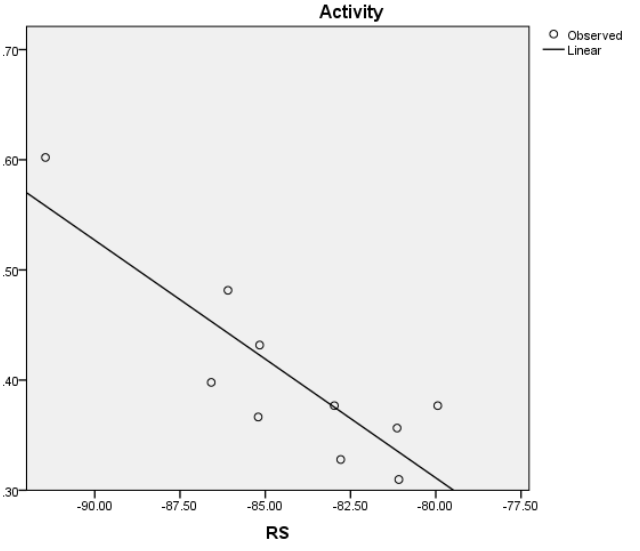


**Fig. 4.** Linear regression between log ($1/IC_{50}$) and RS value of 1-(benzoyloxy)urea and its derivatives

According to the linear regression curve between $\log(1/IC_{50})$ and the RS value of 1-(benzoyloxy)urea and its derivatives, it can be inferred that the smaller the RS value, the bigger the value of $\log(1/IC_{50})$ and *vice versa*. The smaller the value of $IC_{50}$, the greater the activity.

On the measurement value Rerank Score, binding energy indicates the amount of energy required to form a bond between the ligand to the receptor. The smaller the binding energy means that more stable bonds. The more stable binding of ligands to the receptor, it can be predicted that its activity is also getting bigger. The smaller the RS value of a compound, the greater the *in silico* predictive activity. It shows a negative correlation between *in silico* and *in vitro* tests of 1-(benzoyloxy)urea and its derivatives. This means that the lower RS value shows the higher anti-cancer activity.

## 4. Conclusions

There is a linear correlation between activity prediction *via in silico* that is RS and cytotoxic activities *via in vitro* on HeLa cells from a series of 1-(benzoiloksi)urea and its derivatives. The correlation can be illustrated by the equation

$$\log 1/IC_{50} = -0.022\ (\pm\ 0.005)RS - 1.414(\pm\ 0.383).$$

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**КОРЕЛЯЦІЯ *IN SILICO* ТА *IN VITRO* РЕЗУЛЬТАТІВ ДОСЛІДЖЕННЯ 1-(БЕНЗОЇЛОКСИ)СЕЧОВИНИ ТА ЇЇ ПОХІДНИХ ЯК ПОТЕНЦІЙНИХ ПРОТИРАКОВИХ ПРЕПАРАТІВ**

***Анотація.*** *За модифікованою реакцією Шоттена-Баумана з додаванням хлористого бензоїлу або його гомологів до гідроксисечовини в тетрагідрофурані синтезовано 1-(бензоїлокси)сечовину та її похідні. Структуру синтезованих речовин підтверджено UV-Vis та інфрачервоною спектроскопією, методами $^{1}H$ ЯМР, $^{13}C$ ЯМР і мас-спектроскопією. Дослідження in silico стосовно протипухлинної активності 1-(бензоїлокси)сечовини та її похідних у ферменті рібонуклеотідредуктази (PDB:2EUD) проведено за допомогою програми Molegro. Протиракову активність за методом in vitro визначали за допомогою методу ММТ до колоній клітин HeLa. Показано, що результати in silico (Rerank Score) корелюють з результатами in vitro (log1/IC50). Визначено лінійну залежність між результатами in silico та in vitro.*

***Ключові слова:*** *1-(бензоїлокси)сечовина, похідні, in vitro, in silico.*