Current Drug Discovery Technologies, 2016, *13*, 101-108

RESEARCH ARTICLE

Quantitative Structure-Cytotoxic Activity Relationship 1-(Benzoyloxy)urea and Its Derivative

Suko Hardjono[1,*], Siswandono Siswodihardjo[1], Purwanto Pramono[1] and Win Darmanto[2]

[1]*Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia;*
[2]*Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia*

Suko Hardjono

ARTICLE HISTORY

Received: February 1, 2016
Revised: May 16, 2016
Accepted: May 20, 2016

DOI:
10.2174/1570163813666160525112327

Abstract: Drug development is originally carried out on a trial and error basis and it is cost-prohibitive. To minimize the trial and error risks, drug design is needed. One of the compound development processes to get a new drug is by designing a structure modification of the mother compound whose activities are recognized. A substitution of the mother compounds alters the physicochemical properties: lipophilic, electronic and steric properties. In Indonesia, one of medical treatments to cure cancer is through chemotherapy and hydroxyurea. Some derivatives, phenylthiourea, phenylurea, benzoylurea, thiourea and benzoylphenylurea, have been found to be anticancer drug candidates. To predict the activity of the drug compound before it is synthesized, the in-silico test is required. From the test, Rerank Score which is the energy of interaction between the receptor and the ligand molecule is then obtained. Hydroxyurea derivatives were synthesized by modifying Schotten-Baumann's method by the addition of benzoyl group and its homologs resulted in the increase of lipophilic, electronic and steric properties, and cytotoxic activity. Synthesized compounds were 1-(benzoyloxy)urea and its derivatives. Structure characterization was obtained by the spectrum of UV, IR, H NMR, C NMR and Mass Spectrometer. Anticancer activity was carried out using MTT method on HeLa cells. The Quantitative Structure-Cytotoxic Activity Relationships of 1-(benzoyloxy)urea compound and its derivatives was calculated using SPSS. The chemical structure was described, namely: ClogP, π, σ, RS, CMR and Es; while, the cytotoxic activity was indicated by log (1 / IC50). The results show that the best equation of Quantitative Structure-Cytotoxic Activity Relationships (QSAR) of 1- (benzoyloxy)urea compound and its derivatives is Log 1/IC50 = - 0.205 (+ 0.068) σ – 0.051 (+ 0.022) Es – 1.911 (+ 0.020).

Keywords: Physicochemical parameters, cytotoxic activity, 1- (benzoyloxy)urea, QSAR.

INTRODUCTION

Drug development is originally conducted based on trial and error. To minimize the negative effects of trial and error, such as prohibitive cost, drug design is made. A drug design is an attempt to develop drugs which have existed and whose molecule structure and biological activity have been identified on the basis of systematic and rational reasoning [1]. There are some steps required to get a new drug. The first step is to modify the structure of the mother compound with identified activities. The next step is to synthesize the derivatives of the mother compound, to confirm the structure, and to do biological activity test. The last step is to formulate the quantitative relationship between structure, physicochemical properties and biological activity, QSAR, by using statistical regression analysis [2]. It is a statistical model that relates a set of structural descriptors of a chemical compound to its biological activity. To provide some guide, it is widely accepted that between five and ten compounds are required for every descriptor in a QSAR [3]. Some researchers use QSAR as research models. Bharate S.P, *et al* studied the anti-leishmanial activity of several structurally similar naturally occurring global's from Myrtaceae family, which have also been predicted using developed QSAR model [4]. Rolli E, *et al* studied N-phenyl-N'-benzothiazol-6-ylurea (PBU) derivatives and observed thea, adventitious rooting

*Address correspondence to this author at the Pharmaceutical Chemistry Department, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia; E-mail: suko.hardjono@yahoo.com

1875-6220/16 $58.00+.00

adjuvant activity was strictly dependent on their chemical structure [5]. Yamuna E, *et al* studied cyclohepta [b] indole derivatives by QSAR [6]. Shalini Singh studied computational design and chemometric QSAR modeling of Plasmodium falciparum carbonic anhydrase inhibitors [7].

Topliss (1972) proposed a scheme or an operational diagram to synthesize analogs in drug design. This diagram is based on the basic assumption of Hänsch's method that a particular substituent may alter the relative activity to the mother compound based on changes in the hydrophobic, electronic effects, and steric [8]. A substitution on the guiding compounds alters the physicochemical properties which include lipophilic, electronic, and steric properties [9]. Until now, researchers are still using the Topliss' diagram for designing a drug because the theory is found to be relatively understandable and easy to follow. Kakwani, studied N-(3-aryl-1,2,4-triazol-5-yl) Cinnamamide Derivatives as Potential Antimycobacterial Agents [10]. Lazzarotto M, studied Optimized modified Topliss method [11].

In Indonesia, cancer is the fifth leading cause of death [12]. Based on the data from a health research conducted in 2013, the prevalence of tumor or cancer in Indonesia was 1.4 per 1000 individuals in 2013 [13]. One of the medical attempts to cure cancer is through chemotherapy and hydroxyurea is one of the drugs used for cancer chemotherapy. Hydroxyurea is an antineoplastic inhibiting the action of the ribonucleotide reductase enzyme. The enzyme function is to convert the ribonucleotide to deoxyribonucleotides. Thus, if the action of the enzyme is inhibited, DNA biosynthesis inhibition will occur. Hydroxyurea activities are called cytotoxic, and antineoplastic have special effects at S phase and will disrupt the cell cycle at the G2 phase and S [14]. Hydroxyurea is a urea derivative used in myeloproliferative syndrome, Chronic Myelogenous Leukemia (CML), polycythemia vera, and essential thrombocytosis [15]. Some urea derivatives have been identified effective for anticancer drug candidates. They are phenylthiourea [16], benzoylphenylurea [17] phenylurea [18], benzoylurea [19], thiourea [20], N,N-diarylureas [21], acyl thiourea [22] and thiourea α-aminophosphonates [23].

This study used Topliss approach model in choosing the group that was used in the structure modification. Hydroxyurea derivatives were synthesized by the addition of benzoyl group and its homologs. This synthesis results in the increase of lipophilic, electronic and steric properties and as a result the cytotoxic activity is also expected to increase. Synthesized compounds were 1-(benzoyloxy)urea and its derivatives, namely: 1-(2-chlorobenzoyloxy)urea, 1-(4-chloro-benzoyloxy) urea, 1-(2,4-dichlorobenzoyloxy)urea, 1-(4-bromo-benzoyloxy)urea, 1-(4-tri-fluoromethylbenzoyloxy) urea, 1-(4-methylbenzoyloxy)urea, 1-(4-t-buthyl-benzoyloxy)urea, 1-(4-methoxybenzoyloxy)urea and 1-(4-fluorobenzoyloxy) urea. The synthesis was done by modifying Schotten-Baumann method [24-26].

To predict the activity of a compound before the synthesis, in silico test was conducted. In silico test is a test which is done through computer simulation. In silico test has become the method used to initiate the discovery of new drug compounds and to improve efficiency in the optimization of mother compound activity [27]. The activity of the drug compound synthesized can be predicted from the energy of interaction between site of action and ligand molecules. The energy of interaction is indicated by Rerank Score. In silico test is done by molecular docking of drug compound candidate with the site of action selected. Docking is an attempt to harmonize between ligands, a small molecule to the receptor and a large protein molecule, with regard to the nature of both to each other [28]. In this study, the in silico test of 1-(benzoyloxy)urea compound and its derivatives was done against ribonucleotide reductase enzyme site of action 2EUD. 2EUD is the code contained in Protein Data Bank. Ribonucleotide reductase enzyme was used as the main target or anticancer compounds site of action, such as HU and 1-(benzoyloxy)urea and its derivatives. These compounds formed complexes with the crystal structure of ribonucleotide reductase enzyme I which was 2EUD. 2EUD was chosen because it was the site of action of Gemcitabine Diphosphate (GDP) [29]. Hydroxyurea has a mechanism similar to gemcitabine.

The cytotoxic activity test was conducted with cancer cell killing methods, and which was carried out by determining IC_{50} based on the amount of 50% of dead cancer cells due to the compound solution. Anticancer activity test was performed

with in vitro using MTT method. Cancer cells used were HeLa cells. HeLa cells are cancer cells selected for a standard used to determine the activity of Hydroxyurea [30]. HeLa cells are safe for human cells commonly used for the benefit of cell culture therefor often used in research. To identify the surviving cells, the result of reaction absorbance of 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromide (MTT) reagent with alive HeLa cells was detected by using Eliza Reader which was read at a wavelength of 595 nm, as mentioned in the Cancer Chemoprevention Research Center (CCRC) protocols [31]. IC_{50} value of test compounds was obtained through probit analysis. QSAR of 1- (benzoyloxy)urea and its derivatives were determined bu using physicochemical parameters with cytotoxic activity. Physicochemical parameters consist of lipophilic parameters, namely ClogP and π, electronic parameters: σ and RS, and steric parameters: CMR and Es. Cytotoxic activity is indicated by IC_{50} or log ($1/IC_{50}$). In addition, to determine the Quantitative Structure-Cytotoxic Activity Relationships, SPSS program was used.

MATERIALS AND METHODS

The activity prediction of 1-(benzoyloxy)urea compound and its derivatives was examined through in silico tests conducted by complexing test compounds with GCQ which was a ligand found on 2EUD. GCQ is a picture of gemcitabine, a cancer drug which has a mechanism of action similar to hydroxyurea. Computer programs used were ChemBio Draw Ultra and Molegro Virtual Docker. On in silico test, test compounds were in the same cavity with GCQ. The result of the test was the amount of interaction energy between test compounds with 2EUD in the form of Rerank Score.

Furthermore, synthesis was done by adding benzoyl chloride or its homologs in tetrahydrofuran into a mixture of hydroxyurea in tetrahydrofuran and triethylamine as a catalyst while stirring [24-26]. The purity of the synthesized product was determined by a melting point and a thin layer chromatography. Recrystallization was done with hot ethanol. To ascertain whether the synthesized compounds were in accordance with the desired compounds, structure characterization was conducted by using spectral of Ultra Violet (UV-VIS), Infrared (FT-IR), H/C Nuclear Magnetic Resonance (H/C-NMR) and Mass Spectrometer (MS) [32].

Cytotoxic activity was determined by in vitro using HeLa cells. The steps were performed based on the CCRC protocols [31].The mother compound was diluted in stages with Dulbecco's Modified Eagle Medium (DMEM) culture media, so a series of working standard solution was gained. HeLa cell cultures were prepared in a microplate with 96 wells in the form of a cell suspension with a density of 8000 cells /well, **t**hen incubated for 24 hours in 5% CO_2 incubator. Surviving cancer cells were identified by using a reduced MTT reagent in the mitochondria of cancer cells that were still alive so as to form violet crystals. The crystals would become purple solution with the addition of Sodium Dedocyl Sulfonate (SDS) reagent that was detergent. The absorbance of purple color was read with Elisa Reader or Microplate Reader, at a wavelength of 595 nm. The activity of the test compound was described by probit analysis obtained IC_{50} value. To calculate the Quantitative Structure-Cytotoxic Activity Relationship, IC_{50} was converted into Log ($1 / IC_{50}$).

Quantitative Structure-Cytotoxic Activity Relationship of 1-(benzoyloxy)urea and its derivatives was calculated by using SPSS. The chemical structure was described based on physicochemical parameters, namely: ClogP, π, σ, RS, CMR, and Es. Physicochemical parameters theoretically obtained from textbooks or ChemBio Draw Ultra, except RS obtained from the in silico test. Cytotoxic activity was indicated by Log ($1/IC_{50}$).

RESULTS AND DISCUSSIONS

In Silico Test

From the results of in silico tests for all substances on 2EUD, the number of hydrogen bonds and the value Rerank Score have been observed as shown in Table **1**. An example of docking the compound 1- (4-t-butyl-benzoyloxy)urea with 2EUD can be seen in Figs. **1** and **2**.

Synthesis

10 (ten) compounds including 1-(benzoyloxy)urea and its derivatives have been synthesized by nucleophilic substitution of

Table 1. Amino acids are involved in hydrogen bonding and Rerank Score on interaction 1- (benzoiloksi)urea and its derivatives with 2EUD.

No	R_2	R_4	CYS428	CYS218	SER217	ASN426	LEU427	Rerank Score
1	H	H	++	+	+	+	+	-79.9432
2	H	Cl	++	+	+	+	+	-82.7887
3	H	CH_3	++	+	+	+	+	-85.2089
4	H	OCH_3	++	+	+	-	-	-86.5856
5	H	t-C_4H_9	++	+	+	+	-	-91.4471
6	H	CF_3	++	+	+	+	+	-86.0949
7	H	Br	++	+	+	+	+	-85.1651
8	H	F	++	+	+	+	+	-82.9755
9	Cl	Cl	++	+	+	+	-	-81.0833
10	Cl	H	++	+	+	+	-	-81.1349

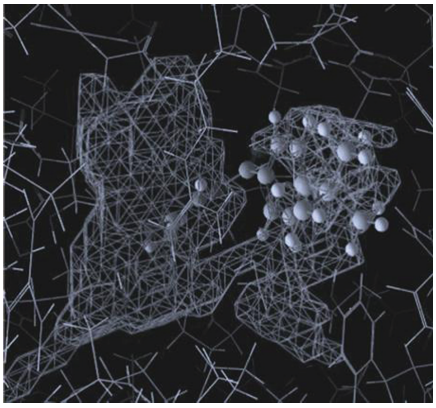


Fig. (1). The position of the 1-(4-t-buthylbenzoyloxy)urea in 2EUD.

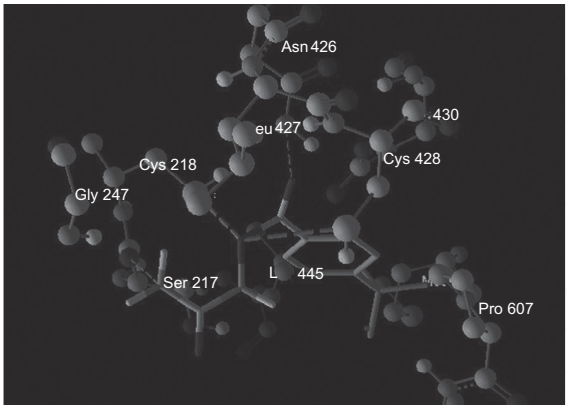


Fig. (2). Hydrogen bond between 1-(4-t-buthylbenzoyloxy)urea and 2EUD.

hydroxyurea to benzoylchloride and its derivatives. Having characterized the structure could be concluded that the 10 compounds were produced according to the target. The results of the structure characterization can be seen in the explanation below:

1-(benzoyloxy)urea

White crystal, yield 53.3%, melting point 128-129 °C; 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 esther) 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=6.3Hz, 2H(Ar-H =d); 7.63, m, J= 2.9 Hz, 3H (Ar-H =e,f), 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 (f); 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, yield 59.5%, melting point 189-190 °C; 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 esther), 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= e); 7.66, d, J=8.6 Hz, 2H (Ar-H=d); and 6.60, s,2H (-NH2); ^{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 for 215.0193.

1-(4-methylbenzoyloxy)urea

White crystal, yield 22.8%, melting point 161-162 °C; UV Spectrum, λ max (nm) in ethanol: 206 nm and 242 nm; IR Spectrum, ν (cm^{-1}) in KBr pellet: 3449 (-NH_2), 3333 (-NH), 1749(-C=O esther), 1683 (-C=O amide), 1589(-C=C-aromatic) and1013(-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= e); 7.41, d, J=7.8 HZ, 2H (Ar-H= d), 6.52,s, 2H (NH2); 2.40,s, 3H CH_3 =g); ^{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 for 195.0798.

1-(methoxybenzoyloxy)urea

White crystal, yield 21.4%, melting point 176-177 °C; 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 esther), 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= e); 7.02,d, J= 9 Hz, 2H (Ar-H=d); 6.52,s,2H (NH2) 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, 113.9,55.5. Mass Spectrometer Spectrum (m/e): 135 m/z ($C_8H_7O_2^+$), 120 m/z ($C_7H_4O_2^{\bullet+}$) and 107 (C7H7O$^+$).

1-(4-tertbutylbenzoyloxy)urea

Fine yellowish white crystal, yield 46.8%, melting point 122-123 °C; 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 esther); 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=d); 7. 53, d,J= 8.64 Hz, 2H (Ar-H=e); 6.53,s, 2H (NH2), and 1.32,s, 9H ($3CH_3$=h); ^{13}C-NMR Spectrum, δ ppm) in DMSO-D6: C atom in164.8 ppm, 159.2 ppm, 156.8 ppm, 129.2,125.4 ppm. 124.8 ppm, 34.9 ppm and 30.3 ppm. 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 for 237.1252.

1-(4-trifluoromethylbenzoyloxy)urea

White crystal, yield 14.7%, melting point 180-181 °C; 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 esther); 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=8.1Hz, 2H (Ar-H=d); 7.88, d J= 9 Hz, 2H (Ar-H=e); 6.65, s, 2H (NH2); ^{13}C-NMR Spectrum, δ ppm) in DMSO-D6: C atom in 163.9 ppm,159.1 ppm,132.4 ppm,131.5 ppm,131.4 ppm, 130.2 ppm, 125.7 ppm; Mass Spectrometer Spectrum (m/e): HRMS (m/z): calculated mass for $C_9H_8N_2O_3F_3$ 249.0487 and measured mass for 249.0458.

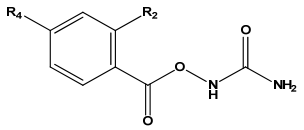
1-(4-bromobenzoyloxy)urea

White crystal, yield 13.4%, melting point 198-199 °C; 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 esther); 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=d), 7.71, d,J=8.82 Hz, 2H (Ar-H= e), 6.57,s, 2H (-NH2); ^{13}C-NMR Spectrum, δ ppm) in DMSO-D6: C atom in 165.7, 159.1, 131.8 and 131.2, 129.4 and 129.2,127.8,126.8; Mass Spectrometer Spectrum (m/e): HRMS (m/z) calculated mass for $C_8H_8N_2O_3Br$ (M^++H): 258.9718 and measured mass for 258.9738.

1-(4-fluorobenzoyloxy)urea

Fine white crystal, yield 37.1%, melting point 164-165 °C; 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 esther); 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= 17.1Hz,2H (Ar-H=d); 7.38, m, J=20.7 Hz,2H (Ar-H=e); 6.68,s,2H (NH2); ^{13}C-NMR Spectrum, δ ppm) in DMSO-D6: C atom in 170.7 ppm, 164.0 ppm, 159.4 ppm, 132.1 ppm, 133.0 ppm, 124.2 ppm,115.3 ppm and 116.3 ppm; Mass Spectrometer Spectrum (m/e): HRMS (m/z): calculated mass for $C_8H_8N_2O_3F$ (M^++H): 199.0519 and measured mass for 199.0511.

Table 2. The physicochemical parameters and the cytotoxic activity of 1-(benzoyloxy)urea and its derivatives.



No	R_2	R_4	ClogP	$(ClogP)^2$	π	$π^2$	σ	RS	CMR	Es	Log ($1/IC_{50}$)
1	H	H	0.566	0.3204	0.01	0.0001	0.01	-79.9432	4.5781	1.24	-1.8827
2	H	Cl	1.279	1.6358	0.71	0.5041	0.23	-82.7887	5.0695	0.27	-2.0044
3	H	CH_3	1.065	1.1342	0.56	0.3136	-0.17	-85.2089	5.0419	0.00	-1.9245
4	H	OCH_3	0.485	0.2352	-0.02	0.0004	-0.27	-86.5856	5.1950	0.69	-1.9245
5	H	t-C_4H_9	2.392	5.7217	1.98	3.9204	-0.20	-91.4471	6.4333	-1.54	-1.7721
6	H	CF_3	1.449	2.0996	0.88	0.7744	0.54	-86.0949	5.0884	-1.16	-1.9172
7	H	Br	1.429	2.0420	0.86	0.7396	0.23	-85.1651	5.3551	0.08	-1.9914
8	H	F	0.709	0.5027	0.14	0.0196	0.06	-82.9755	4.5396	0.78	-1.9172
9	Cl	Cl	1.992	3.968064	1.42	2.0164	0.46	-81.0833	5.5609	0.54	-2.0915
10	Cl	H	1.279	1.635841	0.71	0.5041	0.23	-81.1349	5.0695	0.27	-1.9706

1-(2,4-dichlorobenzoyloxy)urea

Fine yellowish white crystal, yield 25.3%, melting point 132-133 °C; 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 esther); 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=e) 7.79,s 1H (Ar-H=f), 7.56, d,J=10.53 Hz, 1H (Ar-H=g), 6.59,s,2H (-NH2); ^{13}C-NMR Spectrum, δ ppm) in DMSO-D6: C atom in 163.1, 158.9 (a), 137.8 (d) 133.7 (e), 132.8 (h), 130.3 (c), 127.5 (f), 126.3 (g); Mass Spectrometer Spectrum (m/e): HRMS (m/z): calculated mass for $C_8H_7N_2O_3Cl_2$ (M^+): 248,9823 and measured mass for 248,9841.

1-(2-chlorobenzoyloxy)urea

White crystal, yield 10.4%, melting point 129-130 °C; 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 esther); 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 ppm,d, J=7.47 Hz,1H (Ar-H = e); 7.54, m,J= 18.45 Hz, 3H (Ar-H=f,g); 6.56, s, 2H (NH2); ^{13}C-NMR Spectrum, δ ppm) in DMSO-D6: C atom in 163.9 ppm, 159.0 ppm, 133.7 ppm, 132.8, 131.4, 130.7 ppm, 127.6 ppm and 127.2 ppm; Mass Spectrometer Spectrum (m/e): HRMS (m/z): calculated mass for $C_8H_8N_2O_3Cl$ (M^++H): 215,0233 and measured mass for 215,0192.

Cytotoxic Activity Test

The physicochemical parameters and the cytotoxic activity of 1-(benzoyloxy)urea and its derivatives can be seen in Table **2**.

From the table above, it can be seen that the greatest cytotoxic activity is shown by the 1-(4-*t*-buthylbenzoyloxy)urea compound because it has the greatest Log (1 / IC_{50}) value. The table also shows that the compound has the greatest lipophilic parameter value indicated by ClogP, the smallest electronic parameter value: RS, and the greatest steric parameter: CMR.

Quantitative Structure-Cytotoxic Activity Relationship

Determined regression equations are the regression equations which have good and

meaningful regression coefficients. The equations are shown in Table **3**.

Table 3. The regression equations of 1-(benzoyloxy)urea and its derivatives.

One Physicochemical Parameter

1	$Log\ 1/IC_{50} = 0.002\ (\pm\ 0.049)\ CLog\ P - 0.1942\ (\pm\ 0.068)$ (n =10, R = 0.012, F = 0.001, sig = 0.973, s = 0.0892)
2	$Log\ 1/IC_{50} = 0.007\ (\pm\ 0.017)\ (CLog\ P)^2 - 0.1953\ (\pm\ 0.043)$ (n =10, R = 0.146, F = - 45.315, sig =0,688, s = 0.0882)
3	$Log\ 1/IC_{50} = 0.010\ (\pm\ 0.047)\ \pi - 0.1947\ (\pm\ 0.044)$ (n =10, R = 0.074, F = - 43.884, sig = 0.839, s = 0.0889)
4	$Log\ 1/IC_{50} = 0.021\ (\pm\ 0.022)\ \pi^2 - 0.1958\ (\pm\ 0.034)$ (n =10, R = 0.309, F = - 58.176, sig = 0.384, s = 0.0848)
5	$Log\ 1/IC_{50} = 0.191\ (\pm\ 0.084)\ \sigma - 0.1918\ (\pm\ 0.024)$ (n =10, R = 0.624, F = - 79.940, sig = 0.054, s = 0.0697)
6	$Log\ 1/IC_{50} = -\ 0.016\ (\pm\ 0.007)\ RS - 3.269\ (\pm\ 0.566)$ (n =10, R = 0.639, F = 5.520, sig = 0.047, s = 0.0686)
7	$Log\ 1/IC_{50} = 0.039\ (\pm\ 0.054)\ CMR - 0.2144\ (\pm\ 0.281)$ (n =10, R = 0.250, F = - 7.624, sig = 0.486, s = 0.0863)
8	$Log\ 1/IC_{50} = (0.045\pm\ 0.031)\ Es - (0.1934\ \pm\ 0.025)$ (n =10, R = 0.457, F = 2.114, sig = 0.184, s = 0.0793)

Two Physicochemical Parameters

1	$Log\ 1/IC_{50} = 0.135\ (\pm\ 0.066)\ (CLog\ P)^2 - 0.373\ (\pm 0.189)\ CLog\ P - 1.728\ (\pm\ 0.120)$ (n=10, R= 0.609, F=3.190, sig = 0.198, s= 0.0757)
2	$Log\ 1/IC_{50} = 0.135\ (\pm\ 0.054)\ \pi^2 - 0.237\ (\pm\ 0.105)\ \pi - 1.887\ (\pm\ 0.042)$ (n=10, R= 0.691, F=2.060, sig = 0.104, s= 0.0689)
3	$Log\ 1/IC_{50} = 0.031\ (\pm\ 0.041)\ (CLog\ P)^2 - 0.211\ (\pm\ 0.091)\ \sigma - 1.956\ (\pm\ 0.055)$ (n=10, R= 0.659, F= 2.683, sig = 0.136, s= 0.0717)
4	$Log\ 1/IC_{50} = -\ 0.044\ (\pm\ 0.041)\ ClogP - 0.069\ (\pm\ 0.007)\ RS - 3.496\ (\pm\ 0.600)$ (n=10, R= 0.701, F= 3.383, sig = 0.094, s= 0.0680)
5	$Log\ 1/IC_{50} = -\ 0.104\ (\pm\ 0.090)\ ClogP - 0.140\ (\pm\ 0.102)\ CMR - 2.535\ (\pm\ 0.436)$ (n=10, R= 0.461, F= 0.947, sig = 0.433, s= 0.0846)
6	$Log\ 1/IC_{50} = -\ 0.091\ (\pm\ 0.058)\ ClogP - 0.091\ (\pm\ 0.041)\ Es - 1.813\ (\pm\ 0.080)$ (n=10, R= 0.674, F= 2.514, sig = 0.150, s = 0.0727)
7	$Log\ 1/IC_{50} = -\ 0.031\ (\pm\ 0.039)\ \pi - 0.207\ (\pm\ 0.089)\ \sigma - 1.939\ (\pm\ 0.036)$ (n=10, R= 0.663, F= 2.750, sig = 0.131, s= 0.0713)
8	$Log\ 1/IC_{50} = -\ 0.041\ (\pm\ 0.042)\ \pi - 0.019\ (\pm\ 0.008)\ RS - 3.542\ (\pm\ 0.688)$ (n=10, R=0.692, F= 3.218, sig = 0.102, s= 0.0688)
9	$Log\ 1/IC_{50} = -\ 0.091\ (\pm\ 0.100)\ \pi - 0.134\ (\pm\ 0.117)\ CMR - 2.569\ (\pm\ 0.545)$ (n=10, R=0.403, F= 0.678, sig = 0.538, s= 0.0872)
10	$Log\ 1/IC_{50} = -\ 0.079\ (\pm\ 0.060)\ \pi - 0.087\ (\pm\ 0.044)\ Es - 1.872\ (\pm\ 0.053)$ (n=10, R=0.604, F= 2.012, sig = 0.204, s= 0.0760)
11	$Log\ 1/IC_{50} = -\ 0.164\ (\pm\ 0.089)\ \sigma - 0.027\ (\pm\ 0.046)\ CMR - 2.058\ (\pm\ 0.241)$ (n=10, R= 0.646, F= 2.508, sig = 0.151, s= 0.0728)
12	$Log\ 1/IC_{50} = -\ 0.205\ (\pm\ 0.068)\ \sigma - 0.051\ (\pm\ 0.022)\ Es - 1.911\ (\pm\ 0.020)$ (n=10, R= 0.808, F= 6.601, sig = 0.024, s= 0.0561)
13	$Log\ 1/IC_{50} = -\ 0.018\ (\pm\ 0.012)\ RS + 0.012\ (\pm\ 0.046)\ Es - 3.477\ (\pm\ 0.985)$ (n=10, R= 0.644, F= 2.475, sig = 0.154, s= 0.0730)
14	$Log\ 1/IC_{50} = -\ 0.023\ (\pm\ 0.074)\ CMR - 0.055\ (\pm\ 0.046)\ Es - 1.185\ (\pm\ 0.390)$ (n=10, R= 0.469, F= 0.785, sig = 0.420, s= 0.0843)

Of the 22 regression equations, an equation was selected based on the best correlation coefficient value and its significance. The best equation was:

$$Log\ 1/IC_{50} = -\ 0.205\ (\pm\ 0.068)\ \sigma - 0.051\ (\pm\ 0.022)\ Es - 1.911\ (\pm\ 0.020)$$
(n=10, R= 0.808, F= 6.601, sig = 0.024, s= 0.0561)

The above equation was chosen because it had the greatest correlation coefficient (r = 0.808), and little significance, which is 0.0561 (≈ 0.05). From the selected equation, it could be explained that the physicochemical properties affect cytotoxic activity of 1-(benzoyloxy)urea derivatives were electronic properties (σ) and the steric properties (Es).

CONCLUSION

Last analysis, the best equation of Quantitative Structure-Cytotoxic Activity Relationship of 1-(benzoyloxy)urea and its derivatives is:

$Log\ 1/IC_{50} = -\ 0.205\ (\pm\ 0.068)\ \sigma - 0.051\ (\pm\ 0.022)\ Es - 1.911\ (\pm\ 0.020)$ (n=10, R= 0.808, F= 6.601, sig = 0.024, s= 0.0561)

CONFLICT OF INTEREST

Authors report no conflict of interest.

ACKNOWLEDGEMENTS

I thank Higher Degree Directorate (DIKTI) for funding this research. I am grateful to Prof. Dr.

Siswandono, Apt., MS. from the Faculty of Pharmacy, Universitas Airlangga as a promoter and the one who has the Molegro program license, Prof. Dr. Puwanto, Apt. from the Faculty of Pharmacy, Universitas Airlangga as co-promoter, Prof. Drs. Win Darmanto, MSi., Ph.D. from the Faculty of Science and Technology, Universitas Airlangga as co-promoter, Prof. Honda from Hoshi University, Japan, who has helped in interpreting the MS spectra data, Prof Supargiyono, DTM & H, SU., Sp.Par (K), of the Department of Parasitology, Faculty of Medicine, Universitas Gadjah Mada, which has allowed me to study and determine the cytotoxic activity.

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