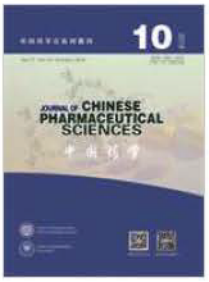

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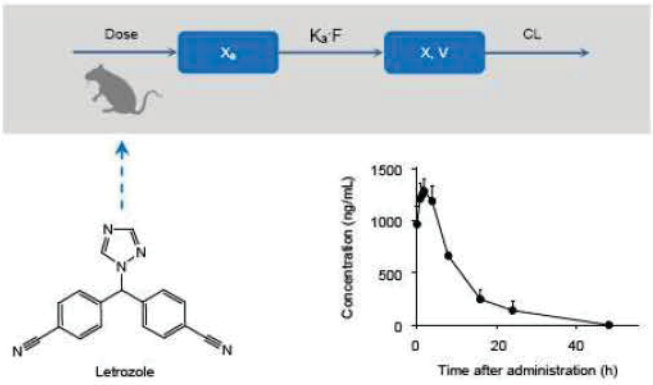
665 **Development and validation of a sensitive LC/MS-MS method for the determination of letrozole in nude mice plasma and its application to a pharmacokinetic study** Hot!

Junsheng Xue, Qingyu Yao, Jian Li, Wenjun Chen, Hong Su, Xiuyun Tian, Chunyi Hao, Tianyan Zhou

DOI: 10.5246/jcps.2018.10.068

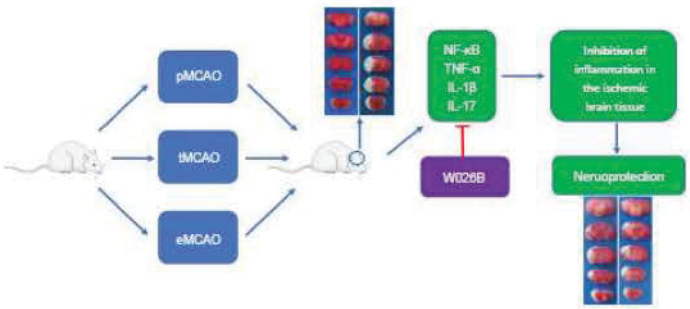


2018 Vol. 27 (10): 665-674 [Abstract] (3) HTML (113 KB) PDF (1283 KB) (7)

675 **A new compound W026B alleviates ischemic brain injury through inhibiting the production of inflammatory cytokines in pMCAO and tMCAO, and enhances the beneficial effect of tPA** Hot!

Ye Zhang, Danping Zheng, Mengyang Shui, Ye Liu, Xiaoyan Liu, Yinye Wang

DOI: 10.5246/jcps.2018.10.069

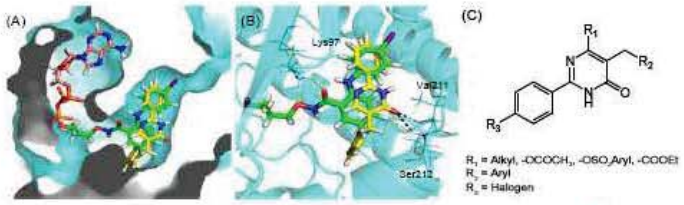


2018 Vol. 27 (10): 675-685 [Abstract] (3) HTML (109 KB) PDF (1608 KB) (2)

666 **Structure-based design and synthesis of 5-benzyl-2-phenylpyrimidin-4(3H)-one derivatives as novel MEK1 inhibitors** Hot!

Can Li, Hongyue Li, Jing Sun, Dandan Xi, Chao Wang, Lei Liang, Fengrong Xu, Yan Niu, Ping Xu

DOI: 10.5246/jcps.2018.10.070

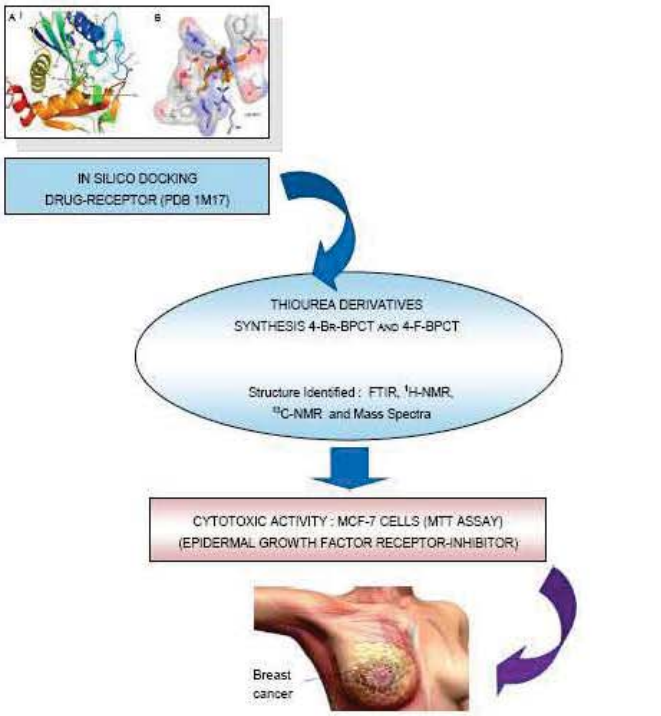


2018 Vol. 27 (10): 686-695 [Abstract] (8) HTML (137 KB) PDF (1544 KB) (3)

696 **Synthesis of N-(phenylcarbamothioyl)-benzamide derivatives and their cytotoxic activity against MCF-7 cells** Hot!

Dini Kesuma, Siswandono, Bambang Tri Purwanto, Marcellino Rudyanto

DOI: 10.5246/jcps.2018.10.071

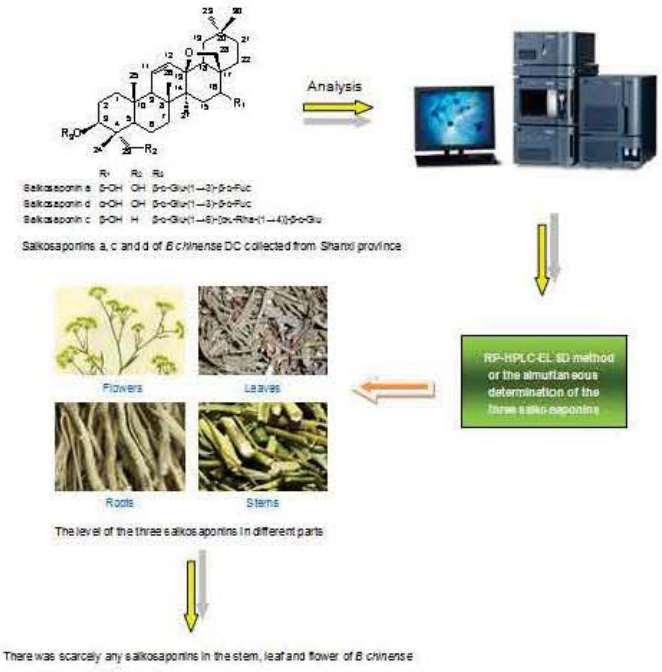


2018 Vol. 27 (10): 696-702 [Abstract] (2) HTML (119 KB) PDF (1224 KB) (7)

703 **Simultaneous determination of Saikosaponin a, c and d in different parts of wild bupleurum from Shanxi Province** Hot!

Rui Wang, Ximei Yuan, Boya Ju, Rui Liu, Hongda Yuan

DOI: 10.5246/jcps.2018.10.072

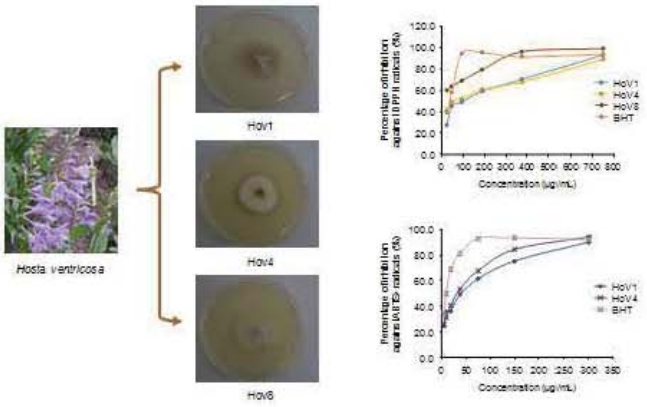


2018 Vol. 27 (10): 703-710 [Abstract] (3) HTML (85 KB) PDF (1339 KB) (0)

711 **Isolation, characterization and antioxidant activities of the endophytic fungi from Hosta. Ventricosa** Hot!

Nan Chen, Xiaoxia Liang

DOI: 10.5246/jcps.2018.10.073



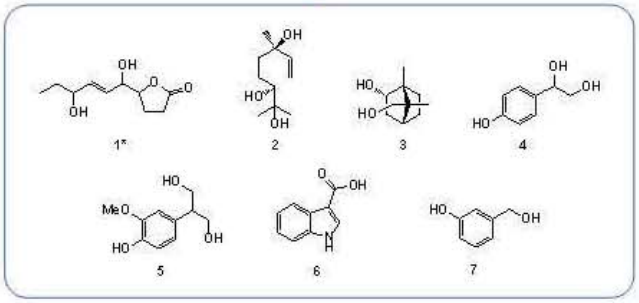
2018 Vol. 27 (10): 711-718 [Abstract] (3) HTML (91 KB) PDF (1593 KB) (0)

Short communication

719 **Chemical constituents from Teucrium viscidum** Hot!

Li Chang, Jing Chen, Yan Tan, Fuqian Wang, Xuanbin Wang, Yongbo Xue, Xincai Hao, Yonghui Zhang

DOI: 10.5246/jcps.2018.10.074



2018 Vol. 27 (10): 719-723 [Abstract] (5) HTML (98 KB) PDF (1215 KB) (0)

Others

724 **Information from US FDA** Hot!

http://www.fda.gov

2018 Vol. 27 (10): 724-731 [Abstract] (4) HTML (73 KB) PDF (977 KB) (0)

732 **The research group of Prof. Tao Liu published the new technique of protein phosphorylation in Journal of the American Chemical Society** Hot!

School of Pharmaceutical Sciences, Peking University Health Science Center

2018 Vol. 27 (10): 732-733 [Abstract] (7) HTML (20 KB) PDF (1282 KB) (0)

734 **The group of Prof. Zhongjun Li made progress in the research of novel anticoagulant** Hot!

School of Pharmaceutical Sciences, Peking University Health Science Center

2018 Vol. 27 (10): 734-734 [Abstract] (4) HTML (11 KB) PDF (1350 KB) (0)

Synthesis of *N*-(phenylcarbamothioyl)-benzamide derivatives and their cytotoxic activity against MCF-7 cells

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Abstract: Cancer is one of the leading causes of death both in developing countries and across the globe. In Indonesia, cancer ranks as the fifth primary cause of death following heart disease, stroke, respiratory tract and diarrhea. Therefore, studies on thiourea derivative compounds as anticancer agents have been profoundly conducted but still require further continuous development. In the present study, we aimed to synthesize new anticancer compounds of *N*-(phenylcarbamothioyl)-benzamide derivatives, namely *N*-(phenylcarbamothioyl)-4-bromobenzamide and *N*-(phenylcarbamothioyl)-4-fluorobenzamide compounds and assess their activities against MCF-7 breast cancer cells. The initial step was to predict the drug-receptor activity through docking between the tested compounds using epidermal growth factor receptor (EGFR) (PDB code: 1M17). The compounds were futher synthesized from the reactions between benzoyl chloride derivatives and *N*-phenylthiourea. The structures of the new compounds were identified using FTIR, ^{1}H NMR, ^{13}C NMR and mass spectra. The cytotoxic activities (IC_{50}) to breast cancer cells of MCF-7 *N*-(phenylcarbamothioyl)-4-bromobenzamide compound and *N*-(phenylcarbamothioyl)-4-fluorobenzamide were 0.27 mM and 0.31 mM, respectively. These two new compounds had better cytotoxic activities than those of the current hydroxyurea-based anticancer drugs (the reference compound) with an IC_{50} value of 9.76 mM. Furthermore, these two new compounds were not toxic to Vero normal cells. Therefore, they possessed tremendous potentials as the candidates for new drugs against breast cancer.

Keywords: *N*-(phenylcarbamothioyl)-benzamide derivatives; Cytotoxic; MCF-7 cells; EGFR

CLC number: R916 **Document code:** A **Article ID:** 1003–1057(2018)10–696–07

1. Introduction

The International Agency for Research on Cancer (IARC) has found that there were 14 067 894 new cases of cancer and 8 201 575 cancer-related deaths worldwide in 2012. Both lung and breast cancer are the leading causes of death compared with other types of cancer, with breast cancer ranks the top, especially in women. Ironically, it has been proposed that the prevalence of breast cancer is increased, and it becomes one of serious problems in healthcare systems globally[1].

Thiourea is an organic compound consisting of carbon, nitrogen, sulfur and hydrogen atoms. The compound shares similarities to urea except for the oxygen atoms, which are then replaced by sulfur. Hydroxyurea, nitrosourea and 5-fluorouracil are urea compounds still used today as anticancer drugs[2–4]. However, it has been widely reported that patients, particularly those with essential thrombocythaemia, have some adverse drug reactions when treated using hydroxyurea[5,6]. This results in the diminishing numbers of the clinical use of hydroxyurea, although, as a matter of fact, it is still used as a DNA replication inhibitor in biochemical research and development of

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the anticancer drugs[7]. Findings of research data have suggested that the hydrophilic properties of hydroxyurea are associated with the less optimal activity of this compound due to its poor membrane penetration ability. Therefore, it can be suggested that development of new anticancer drugs of urea and thiourea derivatives which have hydrophobic properties will result in better membrane penetration ability[8–11]. Li[9] has synthesized urea and thiourea derivatives and proved that phenylthiourea derivatives, *N*-(5-chloro-2-hydroxybenzyl)-*N*-(4-hydroxybenzyl)-*N*′-phenylthiourea, have cytotoxic activity on MCF-7 cells by inhibiting EGFR and HER-2. Nakisah[10] has also shown that the compounds of 2-[3-(2-methyl benzoyl)-thioureido]-acetic acid and 2-[3-(4-methyl benzoyl)-thioureido]-acetic acid have cytotoxic activity against MCF-7 cells as well.

In this present study, we synthesized *N*-(phenyl-carbamothioyl)-4-bromobenzamide/4-Br-BPCT and *N*-(phenylcarbamothioyl)4-fluorobenzamide/4-F-BPCT. The presence of the substituents of bromo and fluoro at the benzoyl ring could enhance the lipophilic and electronic properties of these two compounds compared with their lead compound (BPCT). As a result, the drug and receptor bonds were improved, thus leading to the increased activities among these two compounds[12–14]. This study was different from the study of Li[9] in regards to the different modification substrates. Study by Li has modified the benzyl groups, while our present study modified the benzoyl groups. This study was initiated with activity predictions using molecular modeling in silico and docking test compound with EGF (epidermal growth factor) Receptor PDB code: 1M17 of Protein Data Bank (PDB). Molecular modeling was analyzed using Molegro Virtual Docker (MVD) program 5.5[15]. The activity prediction was carried out using EGFR receptor (1M17) because its ligand is Erlotinib[9], an anticancer drug which inhibits the EGFR pathway[4,9,16]. The test compounds were synthesized from *N*-phenylthiourea with R-benzoyl chloride (R = 4-Br and 4-F) using an acyl nucleophilic substitution reaction[17,18]. The structures of the synthesized compounds were then identified with IR spectrophotometers, ^{1}H NMR Spectrometers, ^{13}C NMR and mass spectrometers[19].

The cytotoxic activities of the two test compounds were observed through cytotoxic assay using MTT method (3-(4,5-dimetylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) *in vitro* on breast cancer cells MCF-7 and Vero normal cells[20]. After the identification of cytotoxic activity test on MCF-7 cells, the IC_{50} was then compared with hydroxyurea. Ourstudy might provide candidates of anticancer drugs from new thiourea derivatives, which have potent cytotoxic activity in breast cancer MCF-7 cells.

2. Materials and methods

2.1. Materials and instruments

Materials for synthesis included phenylthiourea, 4-bromobenzoyl chloride, 4-fluorobenzoyl chloride (Sigma Aldrich), tetrahydrofuran (THF), triethylamine (TEA), acetone, ethyl acetate, *n*-hexane, chloroform and ethanol. Materials for activity test included test compounds and HU, cell cultures of MCF-7 and Vero, culture medium DMEM and M199, buffer saline phosphate (PBS), FBS (fetal bovine serum), trypsin, penicillin-streptomycin, fungizon, DMSO, 0.5 mg/mL MTT (3-(4,5-dimetylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and SDS 10% in HCl 0.01 N. Glass tools for synthesis were Corning hot plate P351, Fisher-John Electrothermal Mel-Temp, Jasco FT-IR 5300 Spectrophotometer, ^{1}H NMR Spectrometer and ^{13}C NMR Agilent 500 MHz with DD2 console system at 500 MHz (^{1}H) and 125 MHz (^{13}C) and mass Spectrometer (Waters). Tools for cytotoxic test included 5% CO_2 incubator, LAF, micropipet with blue and yellow tip, test tube,

vortex, 96-well microplate, conical tube, inverted microscope, hemocytometer and ELISA-reader. Molecular modeling: ChemBioDraw Ultra 15.0, Molegro Virtual Docker (MVD) 5.5.

2.2. Methods

2.2.1. Molecular modeling

Activities of new compounds were predicted using molecular modeling in silico, and docking of two test compounds with EGFR (epidermal growth factor receptor) PDB code: 1M17 of Protein Data Bank (PDB) was carried out using computer program Molegro Virtual Docker (MVD) 5.5. The EGFR receptor (1M17) was chosen because its ligand is Erlotinib[9], an anticancer drug, which inhibits EGFR pathway[4]. Hydroxyurea was used as a reference compound.

2.2.2. Synthesis of 4-Br-BPCT and 4-F-BPCT compounds

N-Phenylthiourea was mixed with THF and TEA in a round flask, and a solution of *R*-benzoyl chloride (R = 4-Br; 4-F) in THF was added into the mixture over the ice bath through a dropping funnel using a magnetic stirrer. The mixture was refluxed and stirred on top of a water bath. The reaction was terminated when the stain in the TLC formed a single stain. After the termination, THF was evaporated in the rotary evaporator. Then recrystallization was carried out[21].

The structures of new compounds were identified using spectroscopy: infrared, ^{1}H NMR, ^{13}C NMR and HRMS[19].

2.2.3. Cytotoxicity test of MTT assay method

MCF-7 and M199 cells were seeded into 96-well plates and then incubated for 24 h in 5% CO_2 incubators. Furthermore, test solutions, positive and negative controls of various concentrations were added. Each concentration was replicated for three times. Wells containing no cells and only filled with medium were used as medium controls. At the end of incubation, each well was added with 100 μL of 0.5 mg/mL MTT, followed by incubation for 3 h, and then the MTT reaction was discontinued by adding 100 μL of 10% SDS in 0.01 N HCI into each well. The microplate was wrapped in paper and incubated at 37 ºC for 24 h. The live cells converted MTT into a dark blue formazan. Elisa reader was utilized to identify the absorption at λ = 595 nm. The IC_{50} values of the two test compounds and the reference compound were obtained by using probit analysis[20,21].

3. Results and discussion

Drug activity was predicted in silico, and the RS value was used as the indicator. According to the result of in silico test (Table 1), the values of RS BFTU, 4-Br-BPCT, 4-F-BPCT, RS HU were –76.9757, –85.4741, –83.5488 and –38.4495, respectively. The smaller RS value indicated the more stable bonds between drug-receptors, leading to better activities[22]. The RS values of the two test compounds were smaller than those of the lead compound and the reference compound. The smaller values suggested their better activities.

The numbers and types of amino acids involved are listed in Table 2 and Figure 2. Based on the bonding of drugs and amino acids, it was predicted that the greater number of hydrogen bonds and steric bonds (Van der Waals and Hydrophobic) resulted in the more stable bonding between drugs and receptors, which might further affect the greater biological activity. The 4-Br-BPCT compound produced the largest number of bonds with amino acids at the EGFR receptor. Therefore, it was predicted that its activity as cytotoxic agents would be better than 4-F-BPCT, BFTU and HU. The better activity of the 4-Br-BPCT could be attributed to the higher lipophilic value of 4-Br (0.86) compared with 4-F (0.14). Therefore, the membrane penetration of 4-Br-BPCT was better than 4-F- BPCT, and the activities of 4-Br-BPCT were also improved[13,14].

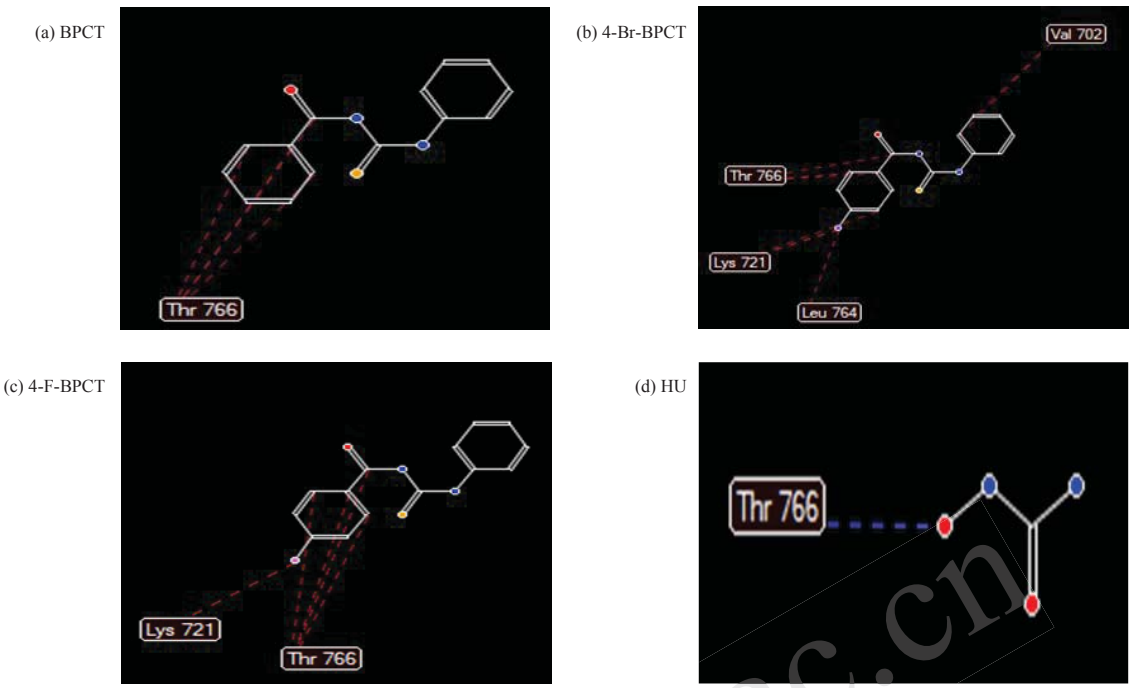

Figure 1. Ligand interaction with amino acids at the EGFR binding sites where hydrogen bonds are indicated by blue dashed-lines, while steric interruptions are indicated by red dashed-lines: (a) BPCT compound, (b) 4-Br-BPCT compound, (c) 4-F-BPCT compound and (d) HU reference compound.

Table 1. Rerank score (RS) value.

Compounds	BPCT	4-Br-BPCT	4-F-BPCT	HU
RS (kkal/mol)	–76.9757	–85.4741	–83.5488	–38.4495

Table 2. Chemical and amino acid bonds involved in the interaction of 4-Br-BPCT and 4-F-BPCT compounds with EGFR (1M17).

Compounds	Amino acids			
	Thr 766	Val 702	Lys 721	Leu 764
BPCT	3S	–	–	–
4-Br-BPCT	3S	1S	3S	1S
4-F-BPCT	4S	–	1S	–
HU	1H	–	–	–

Description: H: Hydrogen bond and S: Steric bond (Van der Waals and Hydrophobic).

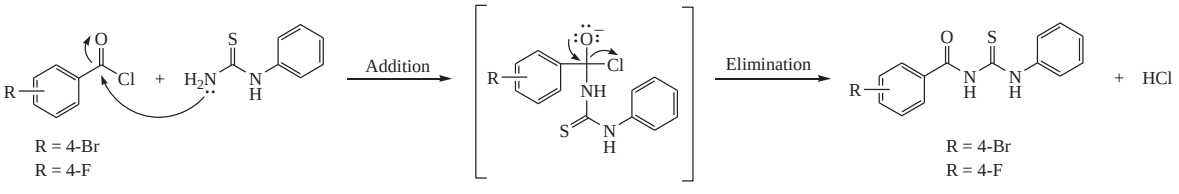

Figure 2. Reaction mechanism underlying the synthesis of 4-Br-BPCT and 4-F-BPCT.

The 4-Br-BPCT and 4-F-BPCT compounds were synthesized from R-benzoyl chloride (R = 4-Br and 4-F) with *N*-phenylthiourea in one stage. The two compounds were yellow light crystals luster and insoluble in water. The structure of the synthesized compounds was identified by IR, ^{1}H NMR, ^{13}C NMR, and HRMS spectroscopy as follows:

***N*-(Phenylcarbamothioyl)-4-bromobenzamide:** It was obtained as a yellow crystal, yield 67%, m.p. 132–133 ºC. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.29 (dd, J_1 7.5 Hz, J_2 2.0 Hz, 1H, Ar-H), 7.43 (t, J 7.5 Hz, 2H, Ar-H), 7.67 (dd, J_1 7.5 Hz, J_2 2.0 Hz 2H, Ar-H), 7.69 (d, J 8.5 Hz, 2H, Ar-H), 7.77 (d, J 8.5 Hz, 2H, Ar-H), 9.10 (s, 1H, O=C-NH-C=S), 12.50 (s, 1H, S=C-NH-Ar). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 124.27 (1C, Ar), 127.16 (2C, Ar), 129.08 (1C, Ar), 129.11 (2C, Ar), 129.14 (2C, Ar), 130.55 (2C, Ar), 132.68 (1C, Ar), 137.59 (1C, Ar), 166.16 (1C, C=O), 178.26 (1C, C=S). IR (KBr), ν_{max} (cm^{-1}): 1667 (C=O amide), 1596 and 1479 (C=C Aromatic); 3328 and 1596 (NH strech sec. amides); 1077 and 830 (C=S). HRMS (*m/z*): $C_{14}H_{10}N_2OSBr$ $(M-H)^-$ = 332.9687 and Calc. Mass = 332.9697.

***N*-(Phenylcarbamothioyl)-4-fluorobenzamide:** It was obtained as a yellow crystal, yield 45%, m.p. 123–124 ºC. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 7.21 (dd, J_1 8.5 Hz, J_2 2.0 Hz, 1H, Ar-H), 7.29 (d, J 7.6 Hz, 2H, Ar-H), 7.42 (t, J 8.5 Hz, 2H, Ar-H), 7.70 (dd, J_1 8.5 Hz, J_2 2.0 Hz, 2H, Ar-H), 7.93 (d, J 7.6 Hz, 2H, Ar-H), 9.12 (s, 1H, O=C-NH-C=S), 12.54 (s, 1H, S=C-NH-Ar). ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 116.62 (2C, Ar), 124.26 (2C, Ar), 127.50 (1C, Ar), 129.04 (1C, Ar), 130.29 (2C, Ar), 130.37 (2C, Ar), 137.62 (1C, Ar), 165.52 (1C, C=O), 167.12 (1C, Ar), 178.37 (1C, C=S). IR (KBr), ν_{max} (cm^{-1}): 1663 (C=O amide), 1663 and 1498 (C=C Aromatic), 3269 and 1598 (NH strech sec. amides), 1108 and 805 (C=S). HRMS (*m/z*): $C_{14}H_{10}N_2OSF$ $(M-H)^-$ = 273.0507 and Calc. Mass = 273.0498.

Table 3 presents the cytotoxic test results (IC_{50}) of the two test compounds in MCF-7 cancer cells and Vero normal cells. IC_{50} values of two test compounds were better than reference compound of hydroxyurea. The IC_{50} value of the 4-Br-BPCT compound was better than that of the 4-F-BPCT compound. This finding was similar to the activity predictions performed using in silico (Table 3). The smaller value of RS (stable bonding of drugs and receptors) could result in the better cytotoxic activities. In addition, the 4-Br-BPCT compound also had a better lipophilic value (0.86) compared with the 4-F-BPCT (0.14)[13,14].

Table 3. RS, IC_{50} MCF-7 and Vero cells values of two test compounds and reference compound.

Compounds	RS (kcal/mol)	IC_{50} MCF-7 cells (mM)	IC_{50} Vero cells (mM)
4-Br-BPCT	–85.4741	0.27	108.06
4-F-BPCT	–83.5488	0.31	25.28
HU	–38.4495	9.76	22.59

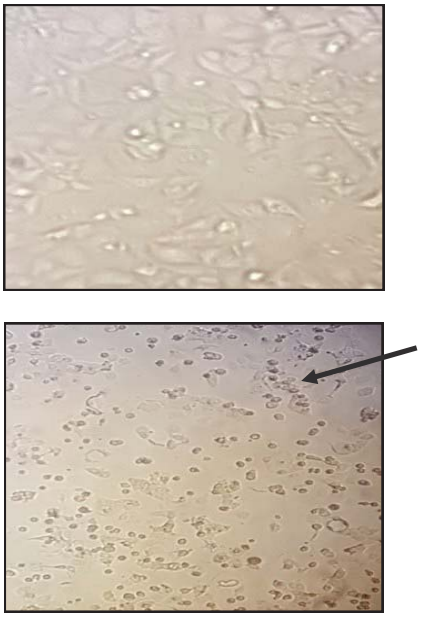

Figure 3. MCF-7 cells before administration of a test compound (4-Br-BPCT): living cells condition (a) and black arrow shows: MCF-7 cells after administration of a test compound (4-Br-BPCT) with a dose of 1000 μg/mL: the presence of dead cells after administration of a test compound (4-Br-BPCT) (b).

4. Conclusions

In this study, we synthesized two new compounds, namely *N*-(phenylcarbamothioyl)-4-bromobenzamide and *N*-(phenylcarbamothioyl)-4-fluorobenzamide. They had *in vitro* cytotoxic activities against human breast cancer cells (MCF-7), which were higher than those of hydroxyurea-based anticancer drugs. The in silico prediction results proved that the RS values of the two new compounds were lower than those of the lead compound and HU. The RS of 4-Br-BPCT compound was lower than of 4-F-BPCT compound. The IC_{50} values of *N*-(phenylcarbamothioyl)-3-bromobenzamide and *N*-(phenylcarbamothioyl)-4-fluorobenzamide were 0.27 mM and 0.31 mM, respectively, and both were more active than hydroxyurea (IC_{50} = 9.76 mM). The cytotoxic effect of 4-Br-BPCT was higher than that of 4-F-BPCT, and it may be related to 4-Br lipophilic values, which were higher than 4-F. These two new compounds were more suitable for binding enzymes compared with hydroxyurea, as they had better inhibitory activities. Collectively, these two new compounds could be used as new targets because they had toxic effects on cancer cells but not Vero normal cells. Further studies are required to examine the molecular mechanisms on EGFR receptor of these two new compounds.

Acknowledgements

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References

[1] World Health Organization. Cancer. **2014**. Retrieved from www.who.int on March 13, 2017.

[2] Bell, F.W.; Cantrell, A.S.; Hogberg, M.; Jaskunas, S.R.; Johansson, N.G.; Jordan, C.L; Kinnick M.D.; Lind P.; Morin Jr. J.M.; Noréen R.; Öberg B.; Palkowitz J.A.; Parrish C.A.; Pranc P.; Sahlberg C.; Ternansky R.J.; Vasileff R.T.; Vrang L.; West S.J.; Zhang H.; Zhou, X.X. Phenethylthiazolethiourea (PETT) compounds, a new class of HIV-1 reverse transcriptase inhibitors 1. Synthesis and basic structure-activity relationship studies of PETT analog. *J. Med. Chem*. **1995**, *38*, 4929–4936.

[3] Mutschler, E. Dynamics of Pharmacology and Toxicology Drugs. Bandung. ITB. **1999**, 56–62.

[4] Avendaño, C.; Menéndez, J.C. *Medicinal Chemistry of Anticancer Drugs*. 2nd ed. Amsterdam: Elsevier. **2015**, *15–19*, 396–406.

[5] Barosi, G.; Besses, C.; Birgegard, G.; Briere, J.; Cervantes, F.; Finazzi, G.; Gisslinger H.; Griesshammer M.; Gugliotta L.; Harrison C.; Hasselbalch H.; Lengfelder E.; Reilly J.T.; Michiels J.J.; Barbui T. A unified definition of clinical resistance/intolerance to hydroxyurea in essential thrombocythemia: results of a consensus process by an international working group. *Bioorg. Med. Chem. Lett*. **2009**, *19*, 755–758.

[6] Tibes R.; Mesa R.A. Blood consult: resistant and progressive essential thrombocythemia. *Blood*. **2001**, *118*, 240–242.

[7] Koç, A.; Wheeler, L.J.; Mathews, C.K.; Merrill, G.F. Hydroxyurea arrests DNA replication by a mechanism that preserves basal dNTP pools. *J. Biol. Chem.* **2004**, *279*, 223–230.

[8] Bielenica, A.; Stefańska, J.; Stępień, K.; Napiórkowska, A.; Augustynowicz-Kopeć, E.; Sanna, G.; Madeddu S.; Boi S.; Giliberti G.; Wrzosek M.; Struga M. Synthesis, cytotoxicity and antimicrobial activity of thiourea derivatives incorporating 3-(trifluoromethyl)phenyl moiety. *Eur. J. Med. Chem*. **2015**, *101*, 111–125.

[9] Li, H.Q.; Yan, Y.; Shi, L.; Zhou, C.F.; Zhu, H. Synthesis and structure-activity relationships of *N*-benzyl-(X-2-hydroxybenzyl)-*N′*-phenylureas and thioureas as antitumor agents. *Bioorg. Med. Chem*. **2010**, *18*, 305–313.

[10] Nakisah, Tan, J.W.; dan Shukri, Y.M. Anti-Cancer Activities of Several Synthetic Carbonylthiourea Compounds on MCF-7 Cells, UMTAS. *Malaysia*. **2011**.

[11] Song, D.Q.; Du, N.N.; Wang, Y.M.; He, W.Y.; Jiang, E.Z.; Cheng, S.X.; Wang, Y.X.; Li, Y.H.; Wang, Y.P.; Li, X.; Jiang, J.D. Synthesis and activity evaluation of phenylurea derivatives as potent antitumor agents. *Bioorg. Med. Chem*. **2009**, *17*, 3873–3878.

[12] Kar, A. *Medicinal Chemistry*, 4th ed. New Delhi: New Age International Ltd Publishers. **2007**, 794–810.

[13] Siswandono, Development of New Drugs. Surabaya: Airlangga University Press. **2014**.

[14] Topliss, J.G. Utilization of Operational Schemes for Analog Synthesis in Drug Design. *J. Med. Chem*. **1972**, *15*, 1006–1009.

[15] *Manual Software Molegro Virtual Docker*. http://www.molegro/mvd-technology.php. **2011**.

[16] Tartarone A.; Lazzari, C.; Lerose, R.; Conteduca, V.; Improta, G.; Zupa, A.; Bulotta, A; Aieta, M; Gregorc, V. Mechanisms of resistance to EGFR tyrosine kinase inhibitors gefitinib/erlotinib and to ALK inhibitor crizotinib. *Lung Canc*. **2013**, *81*, 328–336.

[17] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*, 2nd ed. New York: Oxford University Press. **2012**, 279–289.

[18] McMurry, J.M. *Fundamental of Organic Chemistry*, 7th ed., Belmont: Brooks/Cole. **2011**, 349.

[19] Pavia, D.L.; Lampman, G.M.; Kriz, G.S.; James R.; Vyvyan, J.R. *Spectroscopy*. 4th ed. Belmont: Brooks/Cole. **2009**, 851–886.

[20] Cancer Chemoprevention Research Center Faculty of Pharmacy UGM (CCRC-UGM) Fixed procedure Cytotoxic Test Method MTT. **2012**.

[21] Widiandani T.; Arifianti L.; Siswandono. Docking, Synthesis, and Cytotoxicity Test Human Breast Cancer Cell Line T47D of *N*-(Allylcarbamothioyl)benzamide. *Int. J. Pharm. Clin*. **2016**, *8*, 372–376.

[22] Hinchliffe, A. *Molecular Modelling for Beginners*, 2nd ed. Chichester: John Wiley and Sons Ltd. **2008**.