第 48 卷 第 2 期
2021 年 2 月

湖南大学学报（自然科学版）
Journal of Hunan University（Natural Sciences）

Vol. 48. No. 2.
Feb. 2021

Open Access Article

In Silico Study and ADMET prediction of N-(4-fluorophenylcarbamothioyl) Benzamide Derivatives as Cytotoxic Agents

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Abstract: Structure modification is one of the ways to obtain new derivative compounds. This current study presents pharmacophore-based structure modification of active compounds. Pharmacophore groups are found in urea and phenyl carbamothioyl benzamide (PCTB) compounds, with their derivatives sharing similar pharmacophore groups to that of urea. Any structure modification of a compound will alter its physicochemical properties. In turn, this will also modify the pharmacokinetics of a compound as well as its toxicity. Both the pharmacological activity and pharmacokinetic properties of a compound can be predicted using in-silico molecular modeling studies. This research aims to examine the anticancer activities exhibited by PCTB compounds against HeLA cells by inhibiting CheckPointKinase-1 (CHK1) PDB A42_1 enzymes. The physicochemical properties such as LogP, MR and Etotal of PCTB derivative compounds are also determined. Pharmacokinetic properties are predicted using the pKCSM program. From the results of the in-silico study using Molegro Virtual Docker (MVD) version 5.5, Rerank Scores of PCTB derivative compounds were obtained, for which smaller scores predict higher pharmacological activity. PCTB derivative compounds exhibit higher anticancer activity and pharmacokinetic properties compared to Hydroxyurea. All PCTB compounds and derivatives show lower toxicity except for 4-CF3-PCTB, which shows hepatotoxicity. Compared to the original ligand (A42_1), all PCTB compounds and derivatives indicate higher anticancer activity. Among all of them, 4-Cl-PCTB is the best potential candidate anticancer agent due to its strong activity; it displays no toxicity in its pharmacokinetic properties.

Keywords: In silico; PCTB and its derivatives; anticancer prediction, ADMET prediction, physicochemical properties.

在计算机模拟研究和 ADMET 中预测 N-（4-氟苯基氨基甲硫酰基）苯甲酰胺衍生物作为细胞毒剂

摘要：结构修饰是获得新的衍生物化合物的方法之一。这项当前的研究提出了基于药效团的活性化合物的结构修饰。在尿素和苯基氨基甲硫基苯甲酰胺（PCTB）化合物中发现了药效基团，其衍生物具有与尿素相似的药效基团。化合物的任何结构修饰都会改变其理化性质。反过来，这也将改变化合物的药代动力学及其毒性。可以使用计算机模拟分子模型研究来预测化合物的药理活性和药代动力学性质。这项研究旨在通过抑制检查点激酶-1（CHK1）PDB 一种 42_1 酶来检查 PCTB 化合物对海拉细胞的抗癌活性。还确定了 PCTB 衍生物化合物的理化性质，例如对数，先生和总计。使用 pKCSM 程序可预测药代动力学特性。从使用莫莱格罗虚拟码头工人

Received: 2 January 2021 / Revised: 13 January 2021 / Accepted: 13 February 2021 / Published: 28 February 2021

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（MVD）5.5 版进行的计算机模拟研究的结果中，可以获得 PCTB 衍生物化合物的重新评分，分数较小则表明其药理活性较高。与羟基脲相比，PCTB 衍生物具有更高的抗癌活性和药代动力学特性。除 4-碳纤维 3-PCTB 表现出肝毒性外，所有 PCTB 化合物和衍生物均显示出较低的毒性。与原始配体（一种 42_1）相比，所有 PCTB 化合物和衍生物均具有更高的抗癌活性。其中，4-Cl-PCTB 活性强，是潜在的最佳候选抗癌剂。它的药代动力学特性没有毒性。

关键词：计算机技术； PCTB 及其衍生物；抗癌预测，ADMET 预测，理化性质。

1. Introduction

Research on cancer cures remains ongoing. Cancer is triggered by damage on DNA cells that results in genetic mutation in cell replications, initiating uncontrolled cell proliferation [1]. Of the different types of cancer, breast cancer dominates, representing 43.3% of all cancers in 2012, as reported by the Globocan International Agency for Research on Cancer (IARC) [2].

The development of anticancer agents remains a priority as the number of people diagnosed with this disease continues to grow. Urea and its derivatives have long been recognized for their anticancer activities. Hydroxyurea, nitrosourea, and 5-fluorouracil are among the most frequently used in drug design today [3, 4].

Drug development aims to derive new drugs with the most anticancer activity and the minimum side effects. One way to conduct a drug design in by molecular structure modification followed by a synthetic process of derivative compounds. After structure identification, an assay of pharmacological activity proceeds to examine the efficacy. New compounds resulting from structure modification will change their physicochemical properties, including lipophilic, electronic, and steric properties, which in turn influence pharmacological activity [5, 6]. The initial step to predict pharmacological activity is an in-silico study, an important stage in medicinal chemistry mainly in developing a drug compound structure, predicting pharmacological activity, and discovering active compounds. This process includes incorporating active compounds with selected cell targets or receptors to obtain an energy bonding value known as a Rerank Score (RS) [7, 8]. It is required for PCTB and their derivatives, as new compounds, to have their physicochemical and pharmacokinetic properties determined to ensure their pharmacological effectiveness. A method applicable to this purpose is the online program pkCSM that can be used to predict pharmacokinetic properties, such as absorption, distribution, metabolism, excretion, and toxicity, and physicochemical properties, such as molecular weight (MW), octanol-water partition coefficient (LogP), the number of rotating atomic bonds (torsion), Hydrogen Bond Acceptor (HBA), Hydrogen Bond Donor (HBD), and Polar Surface Activity (PSA).

This study develops thiourea-based compounds, a urea derivative with anticancer activity, by substituting oxygen atoms on urea with sulfur atoms. This changes the lipophilic properties of the urea derivatives, which enables better penetration into biological membranes, thus resulting in better cytotoxic activity. Thiourea derivatives as anticancer agents continue to be developed in efforts to obtain more potent anticancer compounds with minimum side effects. Urea and thiourea have been successfully synthesized by numerous studies. Lie *et al.* revealed the cytotoxic activity of the phenylthiourea derivative, *N*-(5-chloro-2-hydroxybenzyl)-*N*-(4-hydroxybenzyl)-*N*'phenylthiourea, against MCF7 cell lines by inhibiting EGFR and HER2 [10]. Also, a study by Nakisah [11] indicates that both 2-(3-(2-methylbenzylthioureido)-acetic acid and 2-(3-(4-methylbenzylthioureido)-acetic acid compounds demonstrate cytotoxic activity on MCF7 cancer cell lines. A cytotoxicity assay conducted by Kesuma *et al.* found that synthesized *N*-(3-chlorobenzoyl)-*N*'-phenylthiourea and *N*-(3,4-dichlorobenzoyl)-*N*'-phenylthiourea compounds result in higher cytotoxic activity against T47D breast cancer cells than hydroxyurea [12]. Moreover, several synthesized *N*-allylthiourea derivatives, namely 3,4-dichlorobenzoyl-3-allylthiourea, 2,4-dichlorobenzoyl-3-allylthiourea, 4-fluorobenzoyl-3-allylthiourea, and 4-methylbenzoyl-3-allylthiourea showed better anticancer activity against MCF7 cancer lines than hydroxyurea compounds [13]. Other thiourea-derived compounds, *N*-(phenylcarbamothioyl) benzamide, also yield a higher activity against T47D cell lines in comparison to hydroxyurea compounds after synthesized [14]. This current study aims to develop other thiourea derivatives—*N*-(4-fluorophenylcarbamothioyl) benzamide (F-PCTB), 4-trifluoromethyl-*N*-(4-fluorophenylcarbamothioyl) benzamide (4-CF_3-PCTB), 4-bromo-*N*-(4-fluorophenylcarbamothioyl) benzamide (4-Br-PCTB), 4-chloro-*N*-(4-fluorophenylcarbamothioyl) benzamide (4-Cl-PCTB), and 2,4-dichloro-*N*-(4-

fluorophenylcarbamothioyl) benzamide (2,4-diCl-PCTB)—via an in-silico ADMET prediction assay to examine their anticancer activity. Cytotoxic activity against breast cancer cells is predicted by a molecular docking method using the Molecular Virtual Docker program (version 5.5). This molecular docking process results in Rerank Score value of the synthesized compounds, which are then compared to hydroxyurea. This is a standard methodology for predicting the cytotoxic activity of a compound. The enzyme used in this study is Checkpoint kinase 1(CHK1), a human nuclear serine/threonine-protein kinase. On damaged DNA, CHK1 will be activated and phosphorylation can be regulated, which is required for S and G2 cell-cycle arrest. CHK1 inhibition inflicts damage on DNA cells and initiates mitosis and apoptosis. For this reason, S and G2 checkpoint abrogation will trigger the elevation and selective sensitivity of cancer cells in response to DNA damage on p53-deficient cells. Thus, selectivity for inhibition of CHK1 is a major breakthrough in cancer treatment [15, 16].

2. Research Methodology

2.1. Downloading the Target Protein Checkpoint Kinase1 (CHK1 Enzyme)

The molecular structure of the CHK1 enzyme is downloadable from the Protein Data Bank (PDB) (http://www.rcsb.org/pdb/home/home.do). PDB code of CHK1 enzyme (A42_1) is 2YWP.

2.2. Molecular Docking

Lenovo computer, operating system Windows 10, 64 bit, Intel Core i-5-7200 U, CPU@250 GHz, 8.00 GB RAM. ChemBioDraw Ultra version 12 (Cambridge Soft); ChemBio3D Ultra version 12 (Cambridge Soft); Molegro Virtual Docker version 5.5 (CLC bio). Molecular modelling techniques used in this study involve in silico assay with molecular docking method. To understand the 2D structures of the compounds, at first ChemBio Ultra program version 12.0 was run and saved, then continued with drawing those 2D models into 3D structure with ChemBio3D ultra programme to stabilize the energy. The structures of checkpoint kinase1 (CHK1) ID PDB: 2YWP ligand 1-(5-chloro-2,4-dimethoxy phenyl)-3-(5-cyanopyrazin-2-yl) urea (A42_1) were also identified. Subsequently, the alignment between CHK1 and the compounds was performed. In their 3D structures, the compounds display synchronized bonding with the receptors in particular and suitable cavities. Then, the binding of the compounds with a number of amino acids – taking the form of hydrogen bonds – and the steric interactions were verified. Finally, to obtain the Rerank Score, docking protocol of the compounds was performed using MVD program version 5.5 [17, 18, 19].

2.3. Molecular Dynamics Simulation

The calculated binding energy between the ligand-receptor using the molecular docking procedure must then be validated using Molecular Dynamics Simulation [20], which in this study was carried out by Amber Molecular Dynamics package for 10 ns simulation [21]. The trajectory results in this simulation were processed to count the free energy binding of receptors and the ligands. Model Python, completely included in Amber Molecular Dynamics Package Program, was applied to calculate the simulations [22]. Before the calculation took place, the receptor-native ligand complex model (PDB ID 2YWP) was downloaded from PDB server. This procedure uses UCSF Chimera [23]. With Molecular Mechanics (MM) of AMBER ff14SB force field, the atomic partial charge was supplemented to each standardized residual of the receptor. As for non-standardized residuals of the ligand, semi-empirical AM1-BCC was performed [24]. In this study, other ligands, PCTB, 4-CF3-PCTB, 4-Br-PCTB, 4-Cl-PCTB, 2,4-diCl-PCTB, A42_1, HU had their structures modified from 2D to 3D using the semi-empirical PM6 method.

2.4. ADMET Prediction

Lenovo computer, operating system Windows 10, 64 bit, Intel Core i5-7200U, CPU @ 250 GHz, 8.00 GB RAM. ChemBioDraw Ultra version 12 (Cambridge Soft); ChemBio3D Ultra version 12 (Cambridge Soft); Online SMILES Translator, and pkCSM online tool. With pKCSM online tool software, pharmacokinetic properties, i.e ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity), of the active compounds were predicted. The 2D structures of the active compounds were determined using ChemBioDraw ultra program version 12.0 before being transferred to ChemBio3D ultra software version 12.0 to obtain the 3D structures, and then saved as *.sdf files. Then, Online SMILES translator (http://cactus.nci.nih.gov/translate/) was used to interpret the active compounds in SMILE format, with which ADMET prediction was carried out with pkCSM online tool (htpp://biosig.unimelb.edu.au/pkCSM/ prediction) [25, 26].

2.5. Prediction of Physicochemical Properties

Physicochemical properties of the compounds predicted include molecular mass (MM), octanol-water partition coefficient (LogP). Molar Refraction (MR) and total energy. After determining the two and three-dimensional structures of the compounds using

Chem.Bio.Draw Ultra 2D and 3D, the prediction of physicochemical properties was obtained [27].

3. Results and Discussion

The in silico and ADMET study results were to discover PCBT compounds and their derivatives that were predicted as potent anticancer activity compared with Hydroxyurea.

3.1. Molecular Docking and Molecular Dynamics Simulation

The largest number of bond pairings between 4-CF_3-PCTB and amino acid receptor grounds the prediction of higher cytotoxic activity of the compounds. Table 1 also indicates all PCTB derivative compounds are in smaller affinity/Rerank Score compared to HU compounds. Rerank Score demonstrates harmonious bonding between the active compounds, which are micro molecules with molecular protein cell targets. A stabilized bonding is shown by a small affinity in the binding of molecular protein and the compounds. This leads to higher cytotoxic activity predictions on all PCTB derivative compounds in comparison to HU.

Table 1 Cytotoxic activity (RS) of Phenylthiourea derivatives using Molegro Virtual Docker program

No	Code	Rerank Score (kcal/mol)	Energy Hydrogen Bond (kcal/mol)
1	F-PCTB	-90.6735	-2.5
2	4-CF_3-PCTB	-96.7176	-3.71269
3	4-Br-PCTB	-93.2095	-2.00923
4	4-Cl-PCTB	-87.2554	-2.59481
5	2,4-diCl-PCTB	-86.1768	-3.84184
6	A42_1 [A]	-103.639	-6.81075
7	HU	-50	-1

The lowest affinity/Rerank Score was shown on 4-CF_3-PCTB compounds, suggesting a prediction that they are the most active compounds as anticancer agents. Moreover, higher log P values of PCTB compounds compared to HU enable better penetrations of active compounds into biological membranes, resulting in more interactions of active compounds and the receptors. The MR value, which is also higher in PCTB compounds compared to HU, indicates stronger and more stabilized compound-receptor interactions and leads to a better cytotoxic activity.

Table 2 The types of interaction and the atom that interact with amino acids

Code	The Atoms that interact	Types of Interaction	Amino acids
F-PCTB	Oxygen atom in the carbonyl group	Acceptor hydrogen bond	Lys38A
	Fluoro atom and benzene ring	Hydrophobic Interaction	Leu137A; Leu15A; Ala36A Val23A
	Benzene ring	Hydrophobic Interaction	Phe149A; Val68A; Leu84A
4-CF_3-PCTB	Oxygen atom in the carbonyl group	Acceptor hydrogen bond	Asn135A
	2 Fluoro atoms in CF_3 group	Acceptor hydrogen bond	Gly21A; Gly18A
	Fluoro atom in the aromatic benzene group	Acceptor hydrogen bond	Cys87A
	The nitrogen atom on the amino group	Donor hydrogen bond	Ser147A
	Fluoro atom on the CF3 group and aromatic benzene	Hydrophobic Interaction	Val23A; Tyr20A
	Fluoro atom and benzene group	Hydrophobic Interaction	Val23A; Ala36A; Tyr86A; Leu137A; Leu15A
4-Br-PCTB	The oxygen atom in the carbonyl group that turns into a hydroxy group when interacting	Acceptor hydrogen bond	Lys38A
	Fluoro atom and aromatic benzene ring	Hydrophobic Interaction	Leu137; Leu15A; Ala36A; Tyr 86A
	Bromo atom and aromatic benzene ring	Hydrophobic Interaction	Phe149A; Val86A; Leu84A
4-Cl-PCTB	The oxygen atom in the carbonyl group that turns into a hydroxy group when interacting	Acceptor hydrogen bond	Asp148A
	Fluoro atom and aromatic benzene ring	Hydrophobic Interaction	Leu137A; Leu15A; Ala36A; Tyr86A
	Chloro atom and aromatic benzene ring	Hydrophobic Interaction	Val23A: Tyr20A
2,4-diCl-PCTB	Sulfur atom on thiourea group	Acceptor hydrogen bond	Asp148A
	Fluoro atom on benzene group	Acceptor hydrogen bond	Cys87A
	The oxygen atom in the carbonyl group	Acceptor hydrogen bond	Asn135A
	The Nitrogen atom in the amino group	Donor hydrogen bond	Ser147A
	Chloro atom and aromatic benzene ring	Hydrophobic Interaction	Val23A; Tyr20A
	Fluoro atom and aromatic benzene ring	Hydrophobic Interaction	Leu15A; Val36A; Cys87A; Tyr86A; Leu137A
A42_1	Oxygen atom	Acceptor hydrogen bond	Lys38A
	Chloride atom	Hydrophobic Interaction	Leu137A; Val23A
	Nitrogen atom	Acceptor hydrogen bond	Cys87A

Table 2 demonstrates three types of interactions that occur among PCTB derivative compounds with receptors, i.e., the hydrogen acceptor bond, hydrogen-donor interaction (only in 4-CF_3-PCTB compounds) and

hydrophobic interaction. The occurring reactions on all PCTB derivatives are more frequent compared to those of HU compounds. This indicates an appropriate energy used to interact with the receptors, enabling stabilized bonding on all PCTB derivative compounds and the receptors. Moreover, compared to HU derivatives, PCTB derivative compounds-amino acid receptors indicate more reactions, thus suggesting higher cytotoxic activities.

3.2. ADMET Prediction

The World Drug Index is known as Lipinski's Rule of 5 due to the analysis of 2,245 drug compounds whose results depict multiples of five. A molecular mass larger than 500 for a drug compound implies that the value of the octanol-water partition coefficient (logP) is higher than +5, the value of the Hydrogen bond donor (HBOND=HBD), which is expressed with the number of bindings of the OH and NH groups, is higher than 5, and the Hydrogen Receptor (HBA), expressed with the number of O and N atoms, is larger than 10. Thus, the drug compound will be difficult to absorb [28].

As shown in Table 3, *N*-(4-fluorophenylcarbamothioyl) benzamide derivative compounds have a molecular mass smaller than 500, with the octanol-water partition coefficient (logP) value being smaller than +5. It is observed that the number of OH and NH groups is not more than 5, while the number of O and N atoms is fewer than 10. Resultantly, the compound is predicted to have better absorption along with high permeability.

Table 3 Prediction of pharmacokinetic properties (ADMET) of *N*- (4-fluorophenyl carbamothioyl) benzamide derivatives and standard compound using the pkCSM online tool

	Absorption		**Distribution**		**Metabolism**		**Excretion**		**Toxicity**		
No	**Intestinal absorption (%)**	**Skin Permeability (log Kp, cm/h)**	**VDss (Log L/kg)**	**BBB Permeability (log BB)**	**CYP2D6 substrate**	**CYP2 D6 inhibitor**	**Total Clearance (log ml/min/kg)**	**Renal OCT substrate**	**Ames toxicity**	**LD_{50} (mol/kgBW)**	**Hepatotoxic**
1	89.798	-3.054	-0.176	0.359	No	No	-0.431	No	No	2.092	No
2	87.496	-3.064	-0.228	0.307	No	No	-0.513	No	No	2.228	Yes
3	88.988	-3.076	-0.085	0.337	No	No	-0.584	No	No	2.225	No
4	89.055	-3.078	-0.1	0.338	No	No	-0.563	No	No	2.221	No
5	87.853	-3.046	-0.102	0.243	No	No	-0.37	No	No	2.264	No
6	90.052	-2.979	-0.802	0.027	No	No	0.674	Yes	Yes	3.024	No
7	73.127	-4.319	-0.495	-0.545	No	No	0.659	No	Yes	2.116	No

The small intestine is the primary location where drug compounds are absorbed following oral administration. An absorption below 30% is categorized as poor [28].

Drug compounds ideally have 90% bioavailability with no existing individual variation [29]. Table 3 displays *N*-(4-fluorophenylcarbamothioyl)benzamide derivative compounds with the human intestinal absorption above 30% and, thus, is predicted to have good oral bioavailability.

Skin permeability is a highly relevant topic to transdermal drug development. The skin permeation coefficient is denoted with log Kp (cm/h) values capable of predicting the skin permeability of a drug compound. A compound is predicted to have a low skin permeability with the value of log Kp > -2.5. As shown in Table 3, *N*-(4-fluorophenylcarbamothioyl)benzamide derivative compounds carry a lower value of log Kp < -3.0 (cm/h), indicating that the compounds have low skin permeability.

Volume distribution (VDss) is the theoretical volume necessary to contain the total amount of administered drug to distribute, providing the same blood concentration. A compound with a high VDss value means the compound is distributed more in the body rather than in blood plasma. If the value of log VDss is < -0.15, it means the drug has a low distribution volume. In contrast, with a log value of VDss > 0.45, it has a greater distribution volume [30]. Table 3 shows that the *N*-(4-fluorophenylcarbamothioyl)benzamide derivative compound has log VDss values between 0.100 and 0.200, which means that the compound has a low distribution volume.

Drug compounds with log BB > 0.3 are able to penetrate the blood-brain barrier well, yet they cannot be distributed well with log BB < -1. Table 3, showing the *N*-(4-fluorophenylcarbamothioyl) benzamide derivative compounds, indicates that the log BB value is between 0.300 and 0.359. With this value, these compounds, as shown on number 1 to 4, can penetrate BBB. As for compound number 5, the value of log BB is 0.243, which means that this compound is unable to penetrate the blood-brain barrier [30].

As for the detoxification process, the crucial and primary enzyme in the liver cells is cytochrome P450. The enzyme plays major roles in the oxidation process and facilitates the excretion of foreign organic compounds, including drugs. It is essential to know that drug compounds may inhibit the function of the cytochrome P450 enzyme. In this study, it is represented by cytochrome P3D6 isoform (CYP2D6). Table 3 indicates that *N*-(4-fluorophenylcarbamothioyl) benzamide derivative compounds show no inhibition on cytochrome P450 enzyme function.

The excretion process of drug compounds can be predicted by measuring the total clearance (Cltot) and the renal organic cation transporter 2 (OCT2) substrate. C1 total is the combination of hepatic clearance (liver and bile) and renal excretion. In Table 3, *N*-(4-fluorophenylcarbamothioyl) benzamide derivative compounds have values ranging from -0.370 to -0.584, indicating the compound speed of excretion. OCT2 is a transporter in the kidney with essential roles in the disposition and clearance of drug compounds and endogen compounds in the body. *N*-(4-fluorophenylcarbamothioyl)benzamide derivative compounds, as shown in Table 3, have no effect on OCT2, enabling the compound to be easily secreted [30].

The Ames toxicity test is an assay to determine the existing toxicity of a compound and has been extensively used to assess any mutagenic potentials of a compound, using bacteria. A positive result indicates the mutagenic properties of the compound which may act as carcinogens. Table 3 shows that the *N*-(4-fluorophenylcarbamothioyl) benzamide derivative compounds are non-toxic, except for compound number 2. This means that the compounds are safe, except for compound number 2. They were tested both with Ames and hepatoxicity tests. When the compounds were tested with LD_{50} in mice, the values were between 2.0 and 2.264 mol/kgBW. This shows a low toxicity value, since to eliminate 50% of the mice, 457 mol/kgBW dosage is required [30].

4. Conclusions

On the basis of the analyses above, all of the *N*-(4-fluorophenylcarbamothioyl) benzamide derivative compounds indicate a good pharmacokinetic prediction and 4-chloro-*N*-(4-fluorophenylcarbamothioyl)benzamide compound demonstrates a high cytotoxic activity, causing no mutagenicity nor hepatoxicity.

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