RESEARCH ARTICLE
Bull Biotechnol (2021) 2(1):6-9
https://doi.org/10.51539/biotech.734817 Journal homepage: www.dergipark.org.tr/biotech

Bulletin of Biotechnology

Computational study on pazopanib and pemetrexed anticancer drug molecules interacting with a small peptide link

Merve GÜREŞCİ*, Güliz AK[2], Habibe YILMAZ[2], Şenay HAMARAT ŞANLIER[2], Armağan KINAL[1]

*[1]*Ege University, Science Faculty Department of Chemistry, IZMIR, TURKEY*
[2] Ege University Science Faculty Department of Biochemistry, IZMIR, TURKEY

*Corresponding author : merveguresci@windowslive.com
Orcid No: https://orcid.org/0000-0002-9268-6518

Received : 09/05/2020
Accepted : 11/05/2021

Abstract: Cancer is a group of diseases that are defined as uncontrolled cell proliferation, impaired function of vital tissues, and cell death. Chemotherapy is a treatment using anti-cancer drugs to destroy cancer cells or control the growth of these cells. In chemotherapy applications pharmacologically active anticancer drugs reach with low specificity to tumor tissue, and their toxicity is dose dependent. Classical drug administration routes are either oral or intravenous. Orally taken pills result in irregular pharmacokinetics due to the passages of different metabolic pathways and their low specificity. This leads to frequent damage to healthy tissues. Nanoparticle-containing drug delivery systems may overcome these harmful side effects partially (or sometimes totally). Binding peptide-drug conjugates inside of some appropriate nanoparticles is one of the prominent methods among targeted drug delivery systems. Such a system containing Pazopanib (Pz) and Pemetrexed (Pm) drug complexes attached to magnetite nanoparticles with a short polypeptide chain (Ala-Lys-Ala-Leu-Arg-Cys) were designed in our laboratory. In the present study, we have computationally investigated the conjugation mechanisms of Pz and Pm drug molecules to the above-mentioned polypeptide chain. The stable structures on the complex formation pathways and their free energy values were obtained at the B3LYP/6-31G(d) level in the water. The mechanism of Pept-Pz complex formation has two steps whose free energy barriers are found to be 21.37 and 27.72 kcal/mol. On the other hand, the free energy barrier of the Pept-Pm complex having a single-step mechanism was calculated as 28.16 kcal/mol.

Keywords: Anticancer drugs; Pemetrexed; Pazopanib; DFT-B3LYP.

1 Introduction

Cancer is a critical and complex disease that occurs when the genes that control cell growth and division are damaged. The most important descriptive feature of cancer is abnormal cell divisions that occur in various parts of the body and can spread to other organs (Nayak and Pal 2010).

Today cancer is one of the diseases that pose a grave problem for all countries of the world. The International Cancer Research Organization (IARC) affiliated with the World Health Organization (WHO) for 2030 predicts that cancer will be in the first place among the causes of death (Tuncer 2008).

Cancer is a disease that takes a long time to develop. It provides convenience in the treatment of diagnosis in the early stages. If damaged cells can be intervened in the early stages of the mutation, cancer development can be stopped (Auyang 2006). Radiotherapy, chemotherapy, and surgery are the main methods used in cancer treatment. Surgical methods consist of resection (removal) of cancerous tissue. The disadvantage of this method is the loss of an organ, risk of recurrence of cancer and inability to apply to all types of cancer (Thorn et al. 2017). In radiotherapy, the radiation distribution is not equal in density to all cancer cells, while in chemotherapy, cancer cells are killed by drugs that have toxic effects, causing healthy cells as well as cancer cells (Nehru and Singh 2008). The side effects that occur are caused by the fact that the methods in the treatments do not have a tissue-specific effect. Therefore, they became the most important medical problem in recent years.

Although there are many anti-cancer drugs on the market, it is realized that high toxicity and poor bioavailability pose the most important problem. At the same time, these drugs are not selective and affect both cancer cells and normal cells. Therefore, new, and selective and tumor-targeted drug delivery systems (Ak et al. 2019) need to be developed to combat such a lethal disease.

Advances in nanotechnology have brought important innovations in targeted drug delivery (Goel et al. 2009). In this way, while the intracellular concentrations of drugs in

cancer cells can be increased, their toxic effects on healthy cells can be minimized.

Today, it enables the diagnosis of diseases, determination of drug interactions, and delivery of coded packages with smart carrier systems to the relevant part of the body, especially due to its nanoparticular properties (Liu et al. 2008). As drug carriers, the reasons for using nanoparticles are as follows: their loading capacity is high, their surfaces can be modified by means of various molecules in order to reach the target more effectively due to not being recognized by the immune system. Since they cannot be recognized by the immune system, they can be designed to allow passage through physiological barriers such as blood-brain barrier and tight connections between cells.

Polymer-coated superparamagnetic materials can be used in diagnostic applications by allowing magnetic resonance visualization, and they have features such as enabling highly specific tissue targeting via conjugation with a ligand. (Soloviev 2007). The drug is directed to the target cell by ensuring that it is targeted to a specific area within the body with external stimuli (Vasir and Labhasetwar 2005). The magnetic targeting (Hamarat Şanlıer et al 2019) approach involves the injection of a therapeutic agent attached to or encapsulated into the carrier of the magnetic drug and orientation to the tumor tissue by externally applied localized magnetic field (Zhang et al. 2008). Magnetic targeting is a targeting method applied by using magnetic particles and magnetic field in order to concentrate the drugs in the targeted area and control the release of the drug (Widder and Senyei 1984). These drugs are generally toxic, non-durable, expensive and intended to be directed to a specific area for treatment. The magnetic field sensitive drug carriers contain materials such as magnetite, iron, nickel, and cobalt. Drug carriers can be magnetic liposomes, microspheres, nanospheres and colloidal iron oxide solutions.

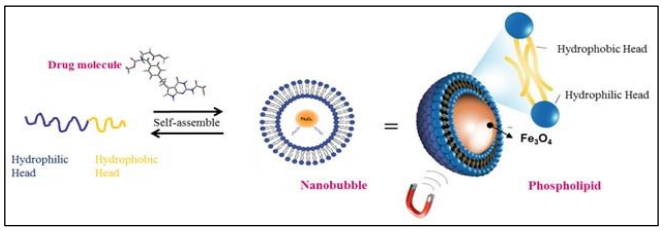

Fig. 1 Material formats of nanoparticle

Magnetic nanoparticle containing nano-conjugate is a hydrophilic material. The system called nano-bubble (Lin et al. 2014) consists of a short polypeptide chain and an anticancer drug complex bound to magnetite nanoparticles (Miklán et al. 2011). These nanoparticles are targeted by an external magnetic field. In addition, the binding of pharmaceutical carrier systems of different forms directly onto the biomolecule takes place via conjugation. Here, we present a computational study on conjugation mechanisms of anti-cancer Pemetrexed (Pm) and Pazopanib (Pz) drug molecules that are used in tumor targeted drug delivery systems to a short polypeptide chain (Ala-Lys-Ala-Leu-Arg-Cys).

2 Materials and Method

The most stable conformers of the peptide link, Pm, Pz, and their complexes were determined by the conformational analysis available in the Spartan'08 suite (Henre et al. 1986). The B3LYP method together with the 6-31G(d) basis set was employed for the determination of ground-state energies of the conformers. All the drug molecules, peptide and complexes as well as the transition state structures involved in the formation reaction mechanisms of the complexes have been optimized and characterized at B3LYP/6-31G(d) level of theory in water. The transition state structures connecting the reactants (intermediates) to the corresponding products were validated by the IRC (Internal Reaction Coordinate) calculations. The solvent effect was introduced with the Integral Equation Formalism Polarizable Continuum Model (IEFPCM, for water ε=80.6) (Tomasi et al. 1999). All calculations were performed with Gaussian 09 computational chemistry suite (Frisch et al. 2009).

3 Results and Discussion

The geometries of the polypeptide chain, Pm, and Pz molecules optimized at B3LYP/6-31G(d) level are shown in Figure 2. The polypeptide chain employed here is composed of Ala-Lys-Ala-Leu-Arg-Cys units. The chloro-acetylated forms of the drug molecules were utilized to be able to fuse with the polypeptide chain for the formation of the Pept-Pz and Pept-Pm complexes, respectively.

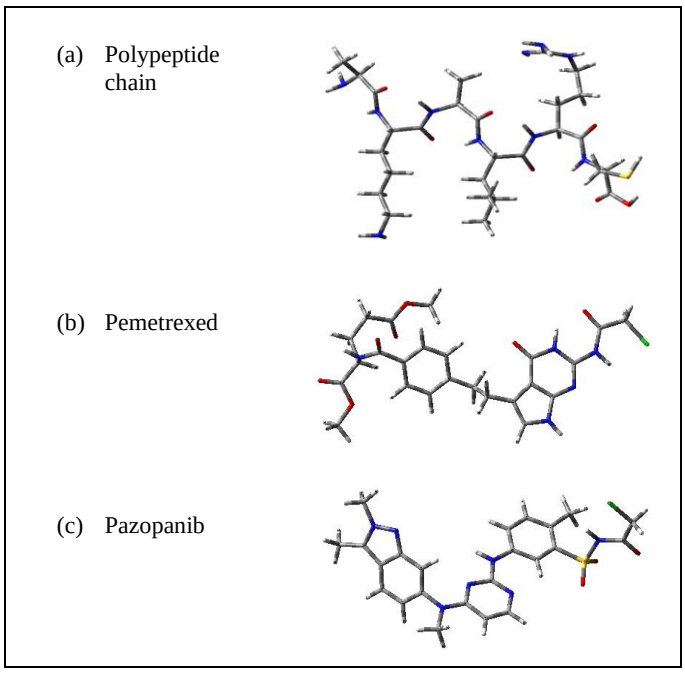

Fig. 2 Optimized geometries of (a) polypeptide chain, (b) Pm, and (c) Pz molecules calculated at the B3LYP/6-31G(d) level.

Figure 3 depicts the optimized geometries and frontier orbitals of the Pm, and Pz molecules calculated at the B3LYP/6-31G(d) level. In the PM molecule, both HOMO and LUMO are on the pyrrolo pyrimidine ring and chloroacetate. Similarly, the HOMO and LUMO of the Pz molecule are located on the dimethyl indazole and the adjacent pyrimidine ring. Consequently, the electron

densities of both drugs are mainly found to be concentrated on the fused five- and six-member ring moieties.

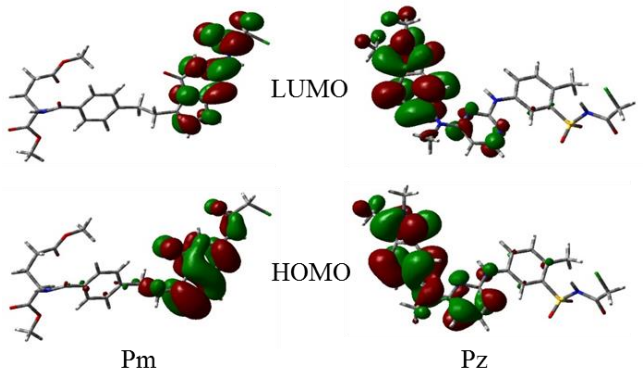

Fig. 3 Optimized geometries and frontier orbitals of the Pm and Pz molecules obtained at the B3LYP/6-31G(d) level.

Figure 4 shows the B3LYP optimized geometries of the Pept-Pz and Pept-Pz complexes. There are no significant conformational changes between the geometries of the free Pm and free Pz molecules in water media and their geometries combined with the peptide. Conversely, the peptide molecule has subjected to a significant conformational change after combining with the drug molecules. This change stands out as elongation. Namely, the peptide molecule elongates when it is fused with the drug while it is compact when it is alone.

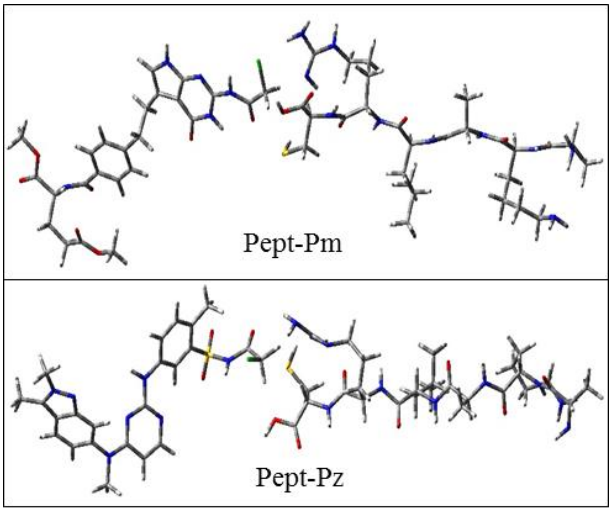

Fig. 4 Optimized geometries of Pept-Pm and Pept-Pz complex obtained at the B3LYP/6-31G(d) level.

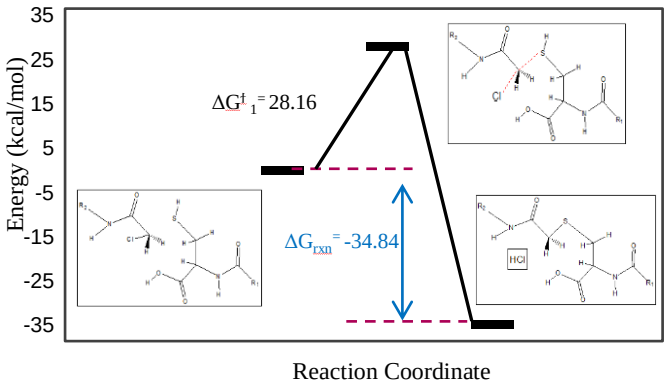

Fig. 5 The mechanism of Pept-Pm complex obtained at the B3LYP/6-31G(d) level in water.

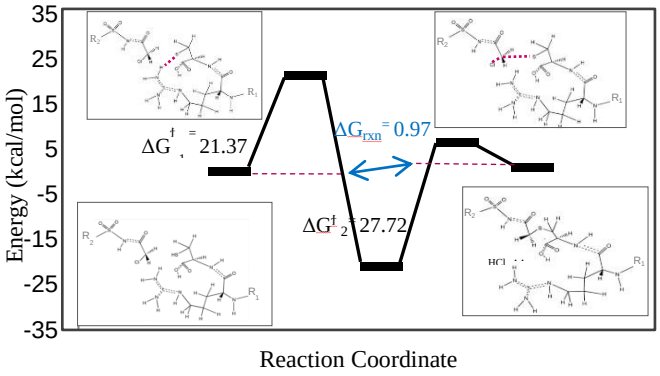

Fig. 6 The mechanism of Pept-Pz complex obtained at the B3LYP/6-31G(d) level in water.

The formation of the Pept-Pm complex has an exothermic single-step mechanism. The activation free energy barrier and the reaction free energy were calculated as 28.16 kcal/mol and -34.84 kcal/mol, respectively (Fig. 5). On the other hand, the formation of Pept-Pz complex occurs via a two-step exothermic mechanism as shown in Figure 6. As seen from the figure, the second step is the rate-determining step of the reaction. The activation free energy barrier and the reaction free energy were calculated as 27.72 kcal/mol and 0.97 kcal/mol, respectively. Table 1 gives activation and reaction enthalpies of the Pept-Pm complex.

Table 1 The activation and reaction enthalpies of the Pept-Pm and Pept-Pz complex obtained at B3LYP/6-31G(d) level

Complex	$\Delta H^{\ddagger}_{298}$ (kcal/mol)	ΔH^{rxn}_{298} (kcal/mol)
Pept-Pm	25.57	-38.11
Pept-Pz	23.58	-1.87

Pept-Pm and Pept-Pz complexes are formed by conjugation reaction of the chloro-acetylated Pm and Pz molecules with the peptide link. The possible reaction mechanism for the formation of both complexes is the sulfur-carbon bond formation mechanism (R'-S-CH2-R'') where the thiol group (–SH) of the cysteine amino acid in the peptide attacks the carbon of the chloroacetyl group of the drug.

4 Conclusion

In this study, the conjugation mechanisms of Pm and Pz drug molecules to the specified polypeptide chain (Ala-Lys-Ala-Leu-Arg-Cys) in an aqueous medium have been studied using the DFT-B3LYP method.

- Pept-Pz and Pept-Pm complexes are formed via the reactions between chloro-acetylated forms of the Pz, and Pm drug molecules and a specifically designed peptide link.
- The stable structures on the complex formation pathways and their free energies values were obtained at the B3LYP/6-31G(d) level.
- The free energy barrier of the Pept-Pm complex having a single-step mechanism was calculated as 28.16 kcal/mol.
- The mechanism of Pept-Pz complex formation has two steps whose free energy barriers were calculated as 21.37 and 27.72 kcal/mol. The second step is found to be the rate-determining step of the reaction.

Acknowledgements

We would like to thank for financial support received from The Scientific and Technological Research Council of Turkey (Project ID: 213M672). The numerical calculations reported in this paper were fully performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

Authors' contributions: The experimental part of the study was performed by Şenay HŞ, GA, HY, while the computational part was completed by AK and MG.

References

Ak G, Aksu D, Çapkın E, Sarı Ö, Kımız Gebelioğlu I, Hamarat Şanlıer Ş (2019) Delivery of pemetrexed by magnetic nanoparticles: design, characterization, in vitro and in vivo assessment. Preparative Biochemistry & Biotechnology 50:3, 215-225

Auyang YS (2006) Cancer Causes and Cancer Research on Many Levels of Complexity. Available at: http://www.creatingtechnology.org/biomed/cancer Accessed 20 Apr 2015

Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR et al. (2009) Gaussian 09, Revision D.01.Wallingford CT: Gaussian, Inc.

Goel HC, Kumar B, Yadav PR, Moshahid M, Rizvi A (2009) Recent developments in cancer therapy by use of nanotechnology. Digest J Nanomater Biostr 4(1):1-12

Hamarat Şanlıer Ş, Ak G, Yılmaz H, Ünal A, Bozkaya ÜF, Tanıyan G, Yıldırım Y, Türkyılmaz GY (2019) Development of Ultrasound-Triggered and Magnetic-Targeted Nanobubble System for Dual-Drug Delivery. Journal of Pharmaceutical Sciences 108-3 1272-1283

Hehre WJ, Radom L, Schleyer PVR, Pople JA (1986) Ab Initio Molecular Orbital Theory. John Wiley & Sons, Inc. USA 11-88

Lin CY, Javadi M, Belnap DM, Barrow JR, Pitt WG (2014) Ultrasound sensitive Liposomes containing doxorubicin for drug targeting therapy. Nanomedicine: Nanotechnology, Biology, and Medicine 10(1):67-76

Liu HL, Wu JH, Min JH, Kim YK (2008) Synthesis of monosized magnetic-optical AuFe alloy nanoparticles. Journal of Applied Physics Part 103(7):1-3

Miklán Z, Orbán E, Bánóczi Z, Hudecz F (2011) New pemetrexed-peptide conjugates: synthesis, characterization and in vitro cytostatic effect on non-small cell lung carcinoma (NCI-H358) and human leukemia (HL-60) cells. J Pept Sci 17(12):805-811

Nayak KA, Pal D (2010) Nanotechnology for targeted delivery in cancer therapeutics. Seeman-ta Institute of Pharmaceutical Sciences 1:1

Nehru MR, Singh PO (2008) Nanotechnology and cancer treatment. Asian J Exp Sci 22-2:45-50

Soloviev M (2007) Nanobiotechnology today: focus on nanoparticles. Journal of Nanobiotechnology 5(11):1-3

Thorn CF, Sharma MR, Altman RB, KleinTE (2017) PharmGKB summary: pazopanib pathway, pharmacokinetics. Pharmacogenet Genomics. 27(8): 307–312

Tomasi, J, Mennucci B, Cances E (1999) The IEF version of the PCM solvation method: an overview of a new method addressed to study molecular solutes at the QM Ab initio level. J. Mol. Struct, Theochem 464:211–226

Tuncer M (2008) Globalleşen Kanser ve Türkiye. Available at: www.nukte.org Accessed: 15 Mar 2015

Vasir JK, Labhasetwar V (2005) Targeted drug delivery in cancer therapy. Technology in Cancer Research and Treatment 4(4):363-374

Zhang L, Gu FX, Chan JM, Wang AZ, Langer RS, Farokhzad OC (2008) Nanoparticles in medicine: Therapeutic applications and developments. Clinical Pharmacology and Therapeutics 83:761-769

Widder KJ, Senyei AE (1984)'Magnetic albumin microspheres in drug delivery' Microsheres and Drug Therapy. Eda.: S.S. Davis, L.Illum, J.G.McVie, E. Tomlinson, Elsevier, Amsterdam 393-411