Saudi Journal of Biological Sciences 29 (2022) 101670

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Antiproliferative activity and interaction with proteins of N-cyclohexylacrylamide

Nevin Çankaya [a,*], Serap Yalçin [b]

[a] Department of Chemistry, Usak University, Uşak, Turkey
[b] Department of Molecular Biology and Genetic, Kırsehir Ahi Evran University, Kırşehir, Turkey

ARTICLE INFO

Article history:
Received 11 November 2019
Revised 15 April 2020
Accepted 16 April 2020
Available online 23 April 2020

Keywords:
N-cyclohexylacrylamide
HeLa cell line
Antiproliferative activity
Molecular docking
Anti-apoptotic proteins

ABSTRACT

N-cyclohexylacrylamıde (NCA), the synthesized compound, was evaluated for their cytotoxic activities against HeLa cancer cell line. Also, the current study has been analyzed by the use of molecular docking as protein-ligand interactions play a vital role in drug design. The docking study of NCA was performed with BCL-2, BCL-W, MCl-1, AKT, BRAF, CDK2, VEGFR, EGFR PARP1, CDK6 proteins. The 3D structures of proteins were obtained from the protein data bank and 3D structure of NCA compounds using GAUSSIAN. The in silico molecular docking results indicated that NCA compound can inhibit cancer-related proteins and can play a role as potential lead compounds for developing new drugs for cancer therapy with chemical modification.

1. Introduction

According to laboratory studies on animals, acrylamide monomer has been reported as a neurotoxin agent, moreover, it is thought to be carcinogenic to both animals and humans (Tanış et al., 2019; http://who.int/foodsafety/fs_management/No_02_Acrylamide_Mar05_en_rev1.pdf; Baskaya et al., 2009; Dybing and Sanner, 2003). The health effects of acrylamide can be classified as toxic and carcinogenic due to their carcinogenic properties (Ruden, 2004; http://www.who.int/foodsafety/areas_work/chemicalrisks/acrylamide/; Rydberg et al., 2003). The acrylamide monomer consists of acryl and amide groups containing vinyl groups. The acrylamide-based N-cyclohexylacrylamide (NCA) monomer, synthesized by our team, carries the cyclohexyl group in its structure. Therefore, it is expected that it will exhibit different properties than acrylamide containing only the vinyl group. Our team has done a lot of work on amide monomers, in which NCA is one of the important monomers (Tanış et al., 2019; Çankaya and Temüz, 2014; Daşbaşı et al., 2016; Çankaya and Temüz, 2012).

The tumor formation and progression is a multi-step process including the proliferation, invasion, angiogenesis, and metastasis (Hanahan and Weinberg, 2000; Gupta et al., 2010). The genetic alterations that regulate proliferation, invasion, angiogenesis, and metastasis have been associated with the progression of cancer in cellular signaling pathways (Gupta et al., 2010). For instance, cyclin-dependent kinases (CDK/Cyclins; such as CDK2, CDK6) have important roles in regulation of cell cycle progression, transcription, and other major biological processes but mutations in these genes contributes to the proliferation of cancer cells (Peyressatre et al., 2015). These genes involved in the progression of cancer may constitute pharmacological targets for the development of anticancer therapeutics. The aim of the study has been on NCA which has actions on BCL-2, BCL-W, MCl-1, AKT, BRAF, CDK2, VEGF, EGFR PARP 1, CDK6 inhibition. For the first time, we performed molecular docking of NCA on human protein structures from the Protein Data Bank resulting in target proteins of NCA. Furthermore, antiproliferative effects of NCA monomer has been investigated on HeLa cancer cell line, and their interaction with proteins using molecular insertion analysis software has been investigated.

2. Materials and methods

2.1. The synthesis of N-cyclohexylacrylamide (NCA)

Acryloyl chloride was added drop by drop to a solution of cyclohexylamine and triethylamine, over a one-hour period at 0–5 °C, and these were stirred, and the reaction was continued to stir at

* Corresponding author.
E-mail address: nevin.cankaya@usak.edu.tr (N. Çankaya).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2020.04.030
1319-562X/

room temperature. The precipitate was filtered off, the excess solution was removed and the reaction mixture was precipitated in ice-water, so that the NCA molecule was synthesized high yield (Çankaya and Temüz, 2014; Daşbaşı et al., 2016). Synthesis reaction of NCA was given Fig. 1.

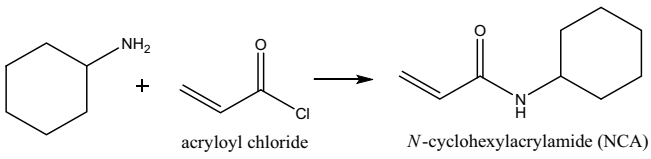
2.2. Antiproliferative effect of NCA on HeLa cell line

The cytotoxic activity of synthesized NCA molecule was assessed against HeLa cancer cells. HeLa cancer cells were obtained from the Kırsehir-Ahi Evran University, Cancer and Stem cell laboratory and grown in RPMI-1640 medium with L-glutamine (Sigma-Aldrich, USA). Exponentially growing cells (5×10^4 cells/well) were seeded in 96-well plates in RPMI1640 medium at 37 °C under 5% CO_2 supplemented with 10% FBS, and gentamicin and incubated overnight. The cells were treated by different concentrations of NCA molecule, were dissolved in DMSO, and allowed to incubate for 48 h. To determine cell viability, cell proliferation assay, XTT (Biological Industries) was used according to the manufacturer's protocol that was described for HeLa cells. The absorbance values were measured using a multi-well plate reader (BioTek ELX808, USA) at 480 nm wavelengths. The IC50 values were compared with the control and expressed in mean ± SD from the dose–response logarithmic curves of at least three independent experiments.

2.3. Molecular docking of NCA molecule

For ligand preparation, all the molecular structure of NCA were sketched using GAUSSIAN09 (Frisch, 2009). The intended crystal protein structures were extracted from the protein data bank (www.rcsb.org) (BCL-2 PDB ID: 4MAN, BCL-W PDB ID: 2Y6W, MCl-1 PDB ID: 5FDO; AKT PDB ID: 4GV1, BRAF PDB ID: 5VAM, CDK2 PDB ID: 2UZO; VEGFR PDB ID: 3CP9; EGFR PDB ID: 4HJO; PARP1 PDB ID: 5DS3; CDK6 PDB ID: 5I2İ). Molecular docking calculations were performed via Lamarckian Generic Algorithm (Morris et al., 1998) in Autodock Vina (Trott and Olson, 2010). All bound water molecules and ligands were removed from the proteins, non-polar hydrogen atoms were merged and the polar hydrogen atoms were added. The VMD and Molegro Molecular Viewer 2.5 (Molegro Molecular viewer free software) programs were used in the visualization of protein a ligand (Humphrey et al., 1996; Thomsen and Christensen, 2006).

In this study, the grid size was set to 68X126X72 points with 1.000 Å spacing centered on AKT-1, 84X80X90 points with 1.000 Å spacing centered on BRAF, 72X68X68 points with 1.000 Å spacing centered on BCL-W, 72X66X72 points with 1.000 Å spacing centered on BCL-2, 70X96X90 points with 1.000 Å spacing centered on CDK2, 70X74X80 points with 1.000 Å spacing centered on CDK6, 74X94X 106 points with 1.000 Å spacing centered on PARP1, 112X116X 104 points with 1.000 Å spacing centered on MCL-1, 92X70X78 points with 1.000 Å spacing centered on EGFR and 112X116X120 points with 1.000 Å spacing centered on VEGFR. Lamarckian Genetic Algorithm (LGA) was implemented to analyze protein-ligand interactions.



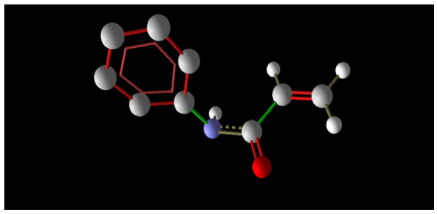


Fig. 1. The synthesis scheme of NCA and 3D structure.

3. Results and discussion

3.1. The cytotoxic effect of NCA molecule

The cell viability for HeLa cell line after 48 h exposure to NCA molecule is illustrated in Fig. 2. The IC_{50}, dose of HeLa cell lines after exposure to NCA, was extrapolated from the dose-response graph. In this study, NCA were found significantly cytotoxic up to 0.3 M. The determined IC_{50} values for HeLa cells were 0.07 M.

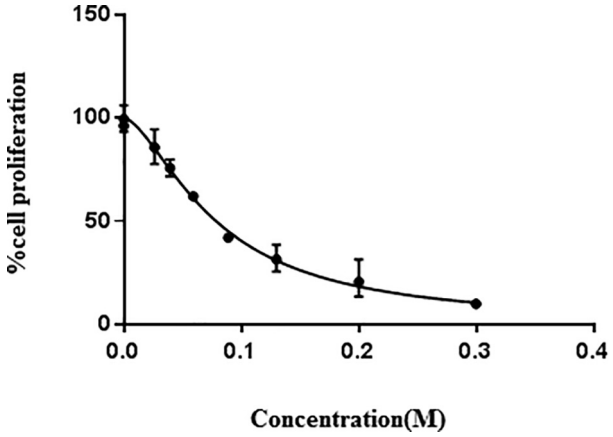


Fig. 2. HeLa cells treated with different concentrations of NCA molecule for 48 h.

Table 1
Docking binding energy results of novel NCA molecule with proteins.

Molecule	Protein	Binding Energy (K.Cal/mol)	PDB ID
NCA	**AKT-1**	−6.4	**4GVI**
NCA	**Bcl-2**	−5.8	**4MAN**
NCA	**Bcl-w**	−6.2	**2Y6W**
NCA	**BRAF**	−6.2	**5VAM**
NCA	**CDK2**	−6.4	**2UZO**
NCA	**CDK6**	−6.0	**5I2İ**
NCA	**EGFR**	−6.9	**4HJO**
NCA	**MCL-1**	−6.1	**5FDO**
NCA	**PARP-1**	−6.2	**5DS3**
NCA	**VEGFR**	−6.8	**3CP9**

Table 2
Docking binding energy results of Doxorubicin (Control) as inhibitor with proteins.

Molecule	Protein	Binding Energy (K.Cal/mol)	PDB ID
NCA	**AKT-1**	−11.2	**4GVI**
NCA	**Bcl-2**	−9.0	**4MAN**
NCA	**Bcl-w**	−9.0	**2Y6W**
NCA	**BRAF**	−9.7	**5VAM**
NCA	**CDK2**	−8.8	**2UZO**
NCA	**CDK6**	−9.7	**5I2İ**
NCA	**EGFR**	−12.4	**4HJO**
NCA	**MCL-1**	−9.1	**5FDO**
NCA	**PARP-1**	−9.1	**5DS3**
NCA	**VEGFR**	−10.3	**3CP9**

Several traditional chemotherapeutic drugs have been applied for treatment of cancer (Doxorubicin, Palbociclib, Apatinib, Paclitaxel etc.) (Serra et al., 2019; Zhang et al., 2019). The chemotherapeutic drugs have an IC_{50} value against various cancer cells and these - drugs effectively kill cancer cells. Nevertheless, in this study, we analyzed both synthesized NCA molecule and Doxorubicin, anti-cancer drugs, and results showed that NCA was not as active as anticancer drugs.

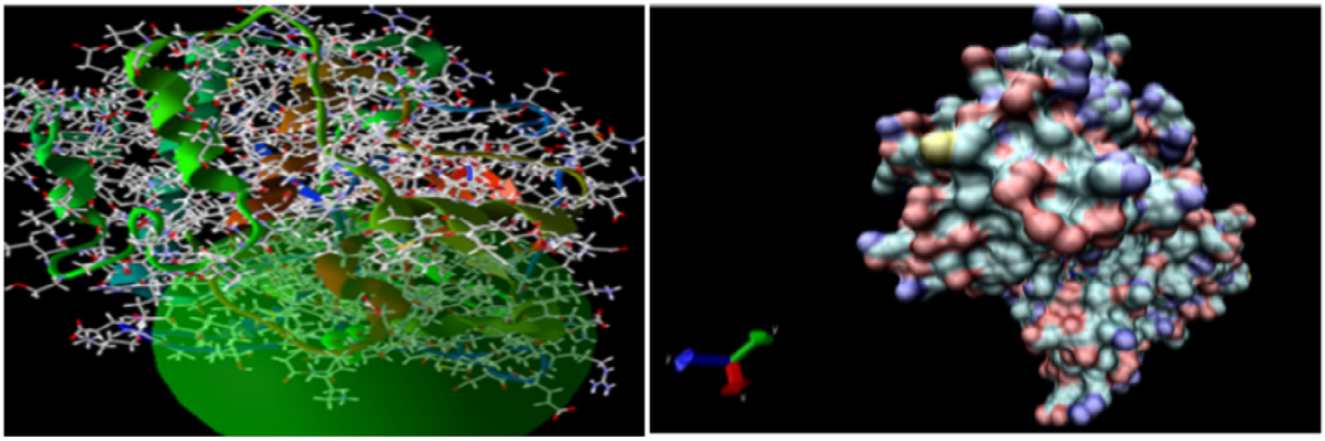


Fig. 3. The interaction of AKT-1 and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

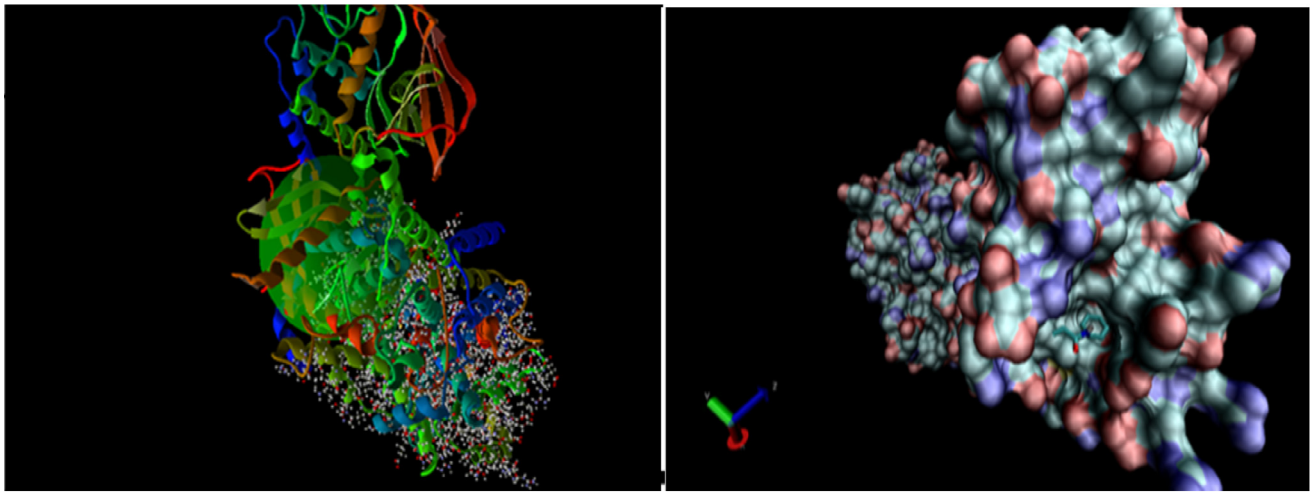


Fig. 4. The interaction of Bcl-2 and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

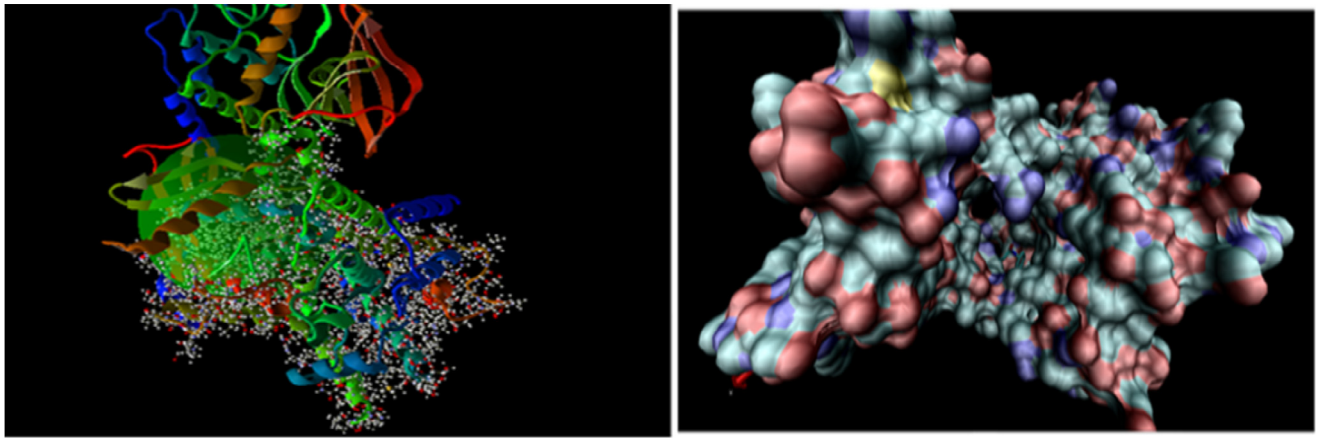


Fig. 5. The interaction of Bcl-w and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

3.2. Molecular docking of NCA molecule

The docking simulation results are shown in Tables 1 and 2. Figures show the hydrogen bond interactions associated with the NCA and the protein residues in the Autodock result (Figs. 3–10). In the result, the residues (Tyr 326, Lys 297 and Arg 273) of AKT-1 made H-bond interaction to NCA. The interaction of NCA and BRAF, Bcl-w and Bcl-2 represent with residue (Thr529), the interaction of NCA and CDK1 represent with residue (Lys33), the residue (Phe832) of EGFR made H-bond interaction to NCA, the interaction of NCA and PARP-1 represent with residue (Gly863, Ser804 and Tyr 898), the interaction of NCA and VEGFR represent with residue (Lys868). There was no hydrogen bond between NCA and CDK6 and Mcl-1 protein. These hydrogen bond networks are thought to play an important role in strengthening the binding effect between the protein and ligand. The protein-ligand binding predic-

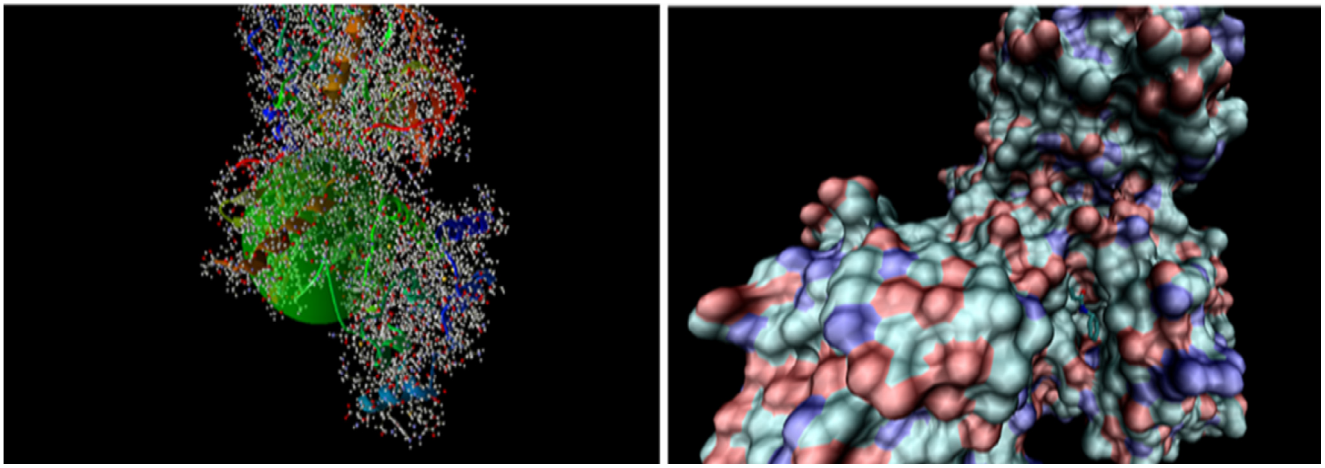


Fig. 6. The interaction of BRAF and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

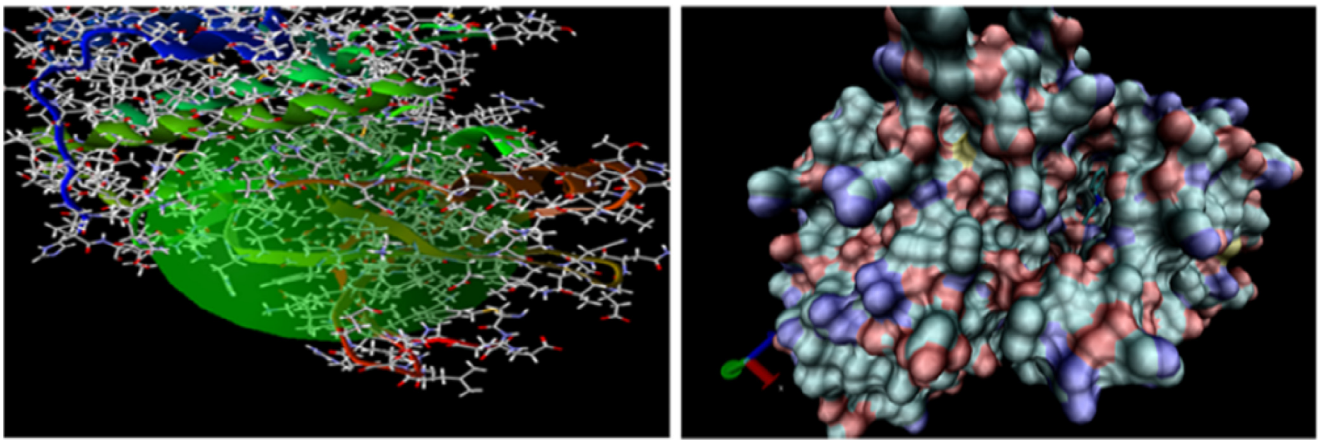


Fig. 7. The interaction of CDK-2 and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

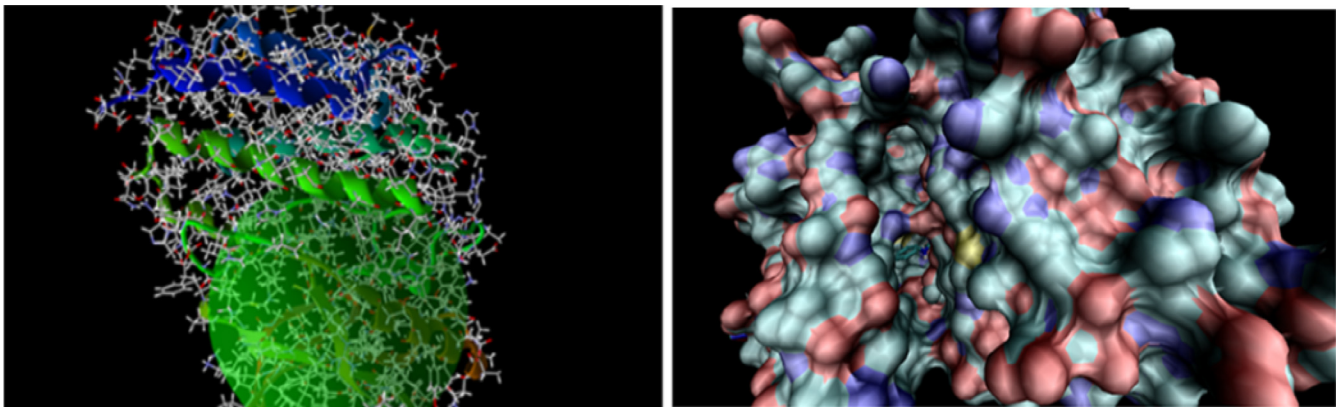


Fig. 8. The interaction of EGFR and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

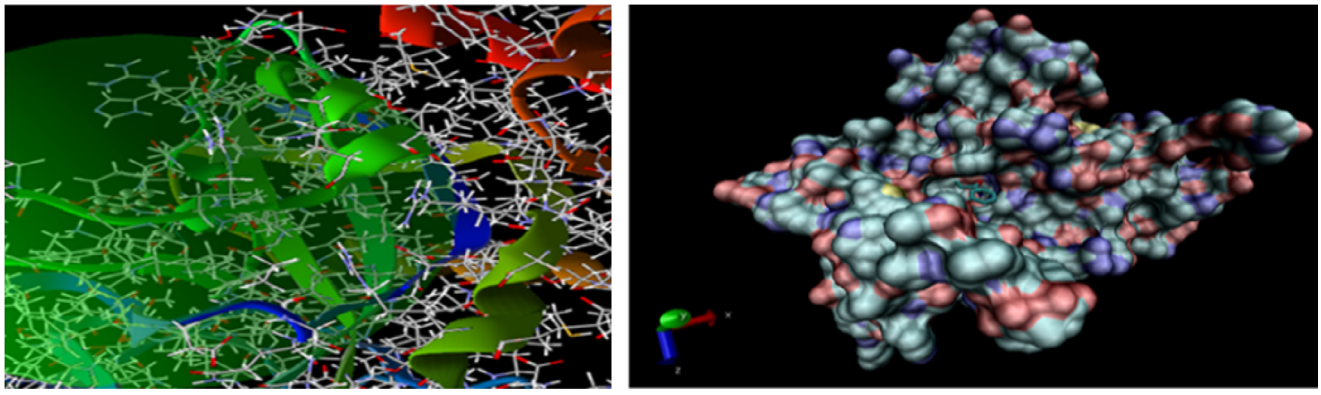


Fig. 9. The interaction of PARP-1 and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

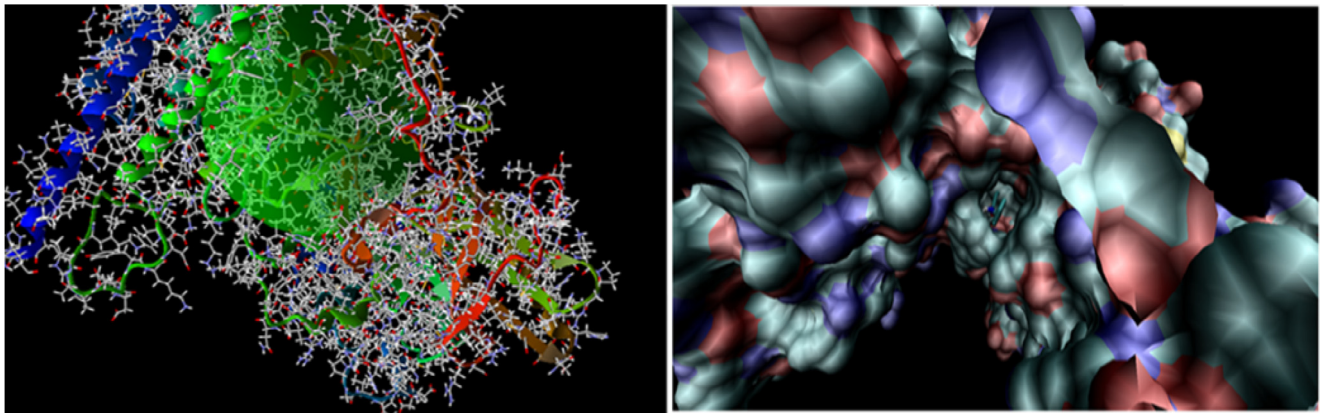


Fig. 10. The interaction of VEGFR and NCA molecule were visualized by Molegro Molecular Viewer and VMD.

tions are very important for the development of the drug discovery process (Trott and Olson, 2010).

4. Conclusion

The N-cyclohexylacrylamide (NCA) molecule was previously synthesized and characterization by our team and this study the anti-proliferative activity of this molecule and its interaction with human proteins were investigated. According to the results, it is concluded that the NCA molecule was not as effective as a cancer drug. However, NCA may have a high potential to become a drug active substance with various modifications of the molecule in future studies and in vitro and in vivo studies can be carried out to produce supporting information.

References

Baskaya, R., Keskin, Y., Karagoz, A., Koc, H.İ., 2009. Prevent. Medic. Bullet. 8, 2.

Çankaya, N., Temüz, M.M., 2012. Characterization and monomer reactivity ratios of grafted cellulose with N-(4-nitrophenyl)acrylamide and Methyl Methacrylate by Atom Transfer Radical Polymerization. Cellul. Chem. Technol. 46 (9–10), 551–558.

Çankaya, N., Temüz, M.M., 2014. Monomer reactivity ratios of cellulose grafted with N-cyclohexylacrylamide and methyl methacrylate by atom transfer radical polymerization. Cellul. Chem. Technol. 48 (3–4), 209–215.

Daşbaşı, T., Saçmacı, Ş., Çankaya, N., Soykan, C., 2016. Synthesis, characterization and application of a new chelating resin for solid phase extraction, preconcentration and determination of trace metal ions in some dairy samples by flame atomic absorption spectrometry. Food Chem. 211, 68–73.

Dybing, E., Sanner, T., 2003. Toxicol. Sci. 75, 1.

Frisch, M.J. et al., 2009. Gaussian 09, Revision A.1. Gaussian Inc., Wallingford, CT.

Gupta, S.C., Kim, J.H., Prasad, S., Aggarwal, B.B., 2010. Regulation of survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastasis Rev. 29 (3), 405–434.

Hanahan, D., Weinberg, R.A., 2000. The hallmarks of cancer. Cell 100, 57–70.

Humphrey, W., Dalke, A., Schulten, K., 1996. VMD: visual molecular dynamics. J. Mol. Graph. 14 (33e38). 27e38.

Morris, G.M., Goodsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A. J., 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J. Comput. Chem. 19 (14), 1639e1662.

Marion Peyressatre, Camille Prével, Morgan Pellerano, May C. Morris. Targeting Cyclin-Dependent Kinases in Human Cancers: From Small Molecules to Peptide Inhibitors Cancers (Basel). 2015; 7(1): 179–237.

Ruden, C., 2004. Food Chemist. Toxicol. 42, 3.

Rydberg, P., Ericson, S., Tareke, E., Karlsson, P., Ehrenberg, L., Tornquvist, M., 2003. J. Agric. Food Chem. 5, 7012.

Serra, F., Lapidari, P., Quaquarini, E., Tagliaferri, B., Sottotetti, F., Palumbo, R., 2019. Palbociclib in metastatic breast cancer: current evidence and real-life data. Drugs Context. 16, (8) 212579.

Tanış, E., Çankaya, N., Yalçın, S., 2019. Synthesis, characterization, computation of global reactivity descriptors and Antiproliferative Activity of N-(4-nitrophenyl) acrylamide. Russian J. Phys. Chem. B 13 (1), 49–61.

Thomsen, R., Christensen, M.H., 2006. MolDock: a new technique for high-accuracy molecular docking. J. Med. Chem. 49 (11), 3315–3321.

Trott, O., Olson, A.J., 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 31 (2), 455–461. 30.

Zhang, Q., Song, Y., Cheng, X., Xu, Z., Matthew, O.A., Wang, J., Sun, Z., Zhang, X., 2019. Apatinib Reverses Paclitaxel-resistant Lung Cancer Cells (A549) Through Blocking the Function of ABCB1 Transporter. Anticancer Res. 39 (10), 5461–5471. https://doi.org/10.21873/anticanres.13739.