Drug and Chemical Toxicology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/idct20

The molecular interaction of human anti-apoptotic proteins and *in silico* ADMET, drug-likeness and toxicity computation of N-cyclohexylmethacrylamide

Nevin Çankaya, Serap Yalçın Azarkan & Emine Tanış

To cite this article: Nevin Çankaya, Serap Yalçın Azarkan & Emine Tanış (2021): The molecular interaction of human anti-apoptotic proteins and *in silico* ADMET, drug-likeness and toxicity computation of N-cyclohexylmethacrylamide, Drug and Chemical Toxicology, DOI: 10.1080/01480545.2021.1894711

To link to this article: https://doi.org/10.1080/01480545.2021.1894711

DRUG AND CHEMICAL TOXICOLOGY
https://doi.org/10.1080/01480545.2021.1894711

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

The molecular interaction of human anti-apoptotic proteins and *in silico* ADMET, drug-likeness and toxicity computation of N-cyclohexylmethacrylamide

Nevin Çankaya[a], Serap Yalçın Azarkan[b] and Emine Tanış[c]

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

ABSTRACT

Cancer is an uncontrolled growth of normal cells and apoptosis has an important role in cancer progression and cancer treatment. Antiapoptotic proteins are overexpressed in several tumors including breast, brain, lung cancer cells. The protein-ligand interaction has a critical role in drug designing. The present study aims to evaluate the interaction of synthesized N-cyclohexylmethacrylamide (NCMA) with proteins using *in silico* molecular docking and toxicity analyses. The NCMA monomer was synthesized and characterized by our team, previously. Kinetics stability, binding affinities and toxic potential of protein-NCMA complex were examined with the aid of molecular simulation. The toxicity results of this study indicate that NCMA is a sample with low toxic potential. According to the docking results, NCMA may be a drug active substance with chemical modifications and toxicity results support this situation. The drug-likeness and ADMET parameters were screened properties of NCMA.

ARTICLE HISTORY
Received 16 September 2020
Revised 18 December 2020
Accepted 1 March 2021

KEYWORDS
Molecular docking; anti-apoptotic proteins; ADMET; drug-likeness; toxicology

Introduction

The reason meth/acrylamide attracts the attention of the scientific community is that it is a neurotoxic compound and is present in high concentrations in thermally processed foods (Tareke *et al.* 2002). Neurotoxicity has been well identified by epidemiologic studies not only on laboratory animals, but also on human population (Hanaa *et al.* 2010). Therefore, the monomer and polymer structures of the amide derivatives having functional groups are noteworthy. Our team has many studies on meth/acrylamide monomers and polymers (Akman and Cankaya 2016, Erdogan *et al.* 2018, Cankaya and Temüz 2014, Cankaya and Tanıs 2018).

Due to both cost and intensive labor, experimental toxicity studies cannot be conducted at a sufficiently high level. Therefore, in recent years, computational toxicology studies have been carried out using various silico techniques, which are quite compatible with experimental studies (Bhardwaj *et al.* 2020, Bhardwaj and Purohit 2020, Losson *et al.* 2020, Singh *et al.* 2021). In these studies, by using computational techniques such as molecular docking and MD simulation, important results were obtained in terms of molecule-protein free binding energies, toxicity of molecules from RMSD analysis results, biological applicability and development of new therapeutic agents. Silico toxicology is a field of study that allows researchers to visualize, analyze, simulate, and predict the potential toxicity of chemicals before any cell or animal studies are conducted to determine the safety and toxicity of chemicals. In this study, the toxic effects of new chemicals were investigated with the help of computational approaches with a software from the silico Toxicology class, which is an alternative and guide to experimental research. This calculation tool is *VirtualToxLab*, a silico concept for estimating the toxic potential (TP) of natural samples and drugs (Vedani and Smieško 2009, Vedani *et al.* 2012, 2015). Toxic effect or TP can be defined as the destruction caused by any chemical on humans, animals, plants, or the environment. *VirtualToxLab* software includes a set of 16 proteins that are thought to be precursors of adverse effects. These include: Mineralocorticoid (MR), androgen (AR), estrogen beta (ERβ), estrogen alpha (ERα), peroxisome proliferator-activated receptor (PPAR), liver X (LXR), thyroid alpha (TRα), thyroid beta (PR), glucocorticoid (GR), 10 nuclear receptors which are 2D6, 1A1, 3A4, 2C9, P450 enzyme family, aryl hydrocarbon receptor (AhR) and a potassium ion channel (hERG). Also, this program is one of the software that provides the most accurate results in toxicity estimation. It calculates the binding affinity (IC_{50}) between the target protein and the sample using of intermolecular interactions such as H bond, hydrophobic, covalent and non-covalent bonds, Van der Waals interactions and electrostatic interactions (Vedani *et al.* 2012).

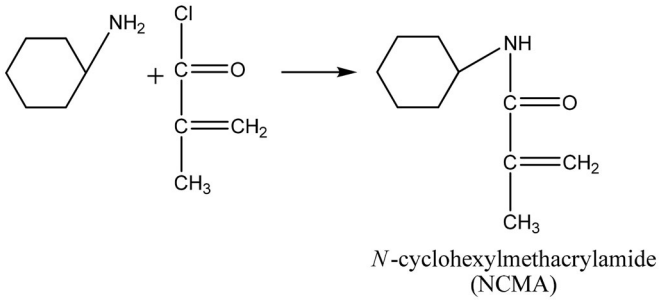
In the present study, we also investigated whether NCMA could be a potential anticancer drug target with *in silico* molecular docking, ADMET and toxic effect. In addition, we performed molecular docking analyses of NCMA molecule with human protein structures including Bcl-2, Bcl-w, Mcl-1, AKT, and BRAF.

Materials and methods

The synthesis of NCMA

The NCMA molecule has been previously synthesized and characterized by our team. (Akman and Cankaya 2016,

CONTACT Nevin Çankaya nevin.cankaya@usak.edu.tr Department of Chemistry, Usak University, Uşak, Turkey



N-cyclohexylmethacrylamide (NCMA)

Figure 1. Synthesis of the NCMA.

Erdogan *et al.* 2018) The synthesis reaction and formula of the molecule are given in Figure 1.

In silico *prediction of the IC_{50} and toxic potential values of NCMA*

The NCMA was optimized with the Gaussian09 program (Frisch *et al.* 2009). IC_{50} values were obtained from the interaction of the optimized molecule with 16 target proteins in aqueous solution mimicking the cytoplasm on the Open Virtual platform using the 'ab initio' type approach. As a result of the 3D dimensional molecule-protein interaction in the user interface, H bonds and molecule-protein interactions were visualized.

Molecular docking procedure of NCMA

The molecular structure of NCMA was drawn using GAUSSIAN09. The designed crystal protein structures were attracted from the protein data bank (www.rcsb.org) (Bcl-2 PDB ID: 4MAN, Bcl-w PDB ID: 2Y6W, Mcl-1 PDB ID: 5FDO; AKT-1 PDB ID: 4gv1, BRAF PDB ID: 5vam). Molecular docking analyses were calculated via the Lamarckian Generic Algorithm (Morris *et al.* 1998) in Autodock Vina (Trott and Olson 2010, Thomsen and Christensen, 2006). All bond water molecules were removed from the proteins, non-polar hydrogen atoms were fused, and the polar hydrogen atoms were attached.

Molegro Molecular Viewer 2.5 (Molegro Molecular viewer free software, http://www.molegro.com) (Thomsen and Christensen 2006) was used to visualize protein and ligand interaction. In addition, Paclitaxel was chosen as a control drug. (https://pubchem.ncbi.nlm.nih.gov/compound/taxol). In this study, setting the grid and center of proteins was given in Table 1.

ADMET and drug-likeness analysis

Currently, computer-based analyses are an important part of pharmacology (Ntie-Kang *et al.* 2013). ADMET analysis is used to determine the pharmacological properties in drug discovery (http://biosig.unimelb.edu.au/pkcsm/prediction). The drug-likeness prediction of NCMA was performed by an online tool SwissADME (http://www.sib.swiss) (http://www.swissadme.ch/index.php), pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) and admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) (Zoete *et al.* 2016, Daina *et al.* 2017, Cheng *et al.* 2012). Furthermore, toxicological predictions of NCMA were applied to Lipinski, Ghose, and Veber rules and bioavailability scores (Lipinski *et al.* 2001, Veber *et al.* 2002, Ghose *et al.* 1999).

Table 1. Position of the Grid box center in proteins.

Proteins	Grid	Center
AKT-1	72X90X70	1.000 Å
BRAF	94X92X88	1.000 Å
Bcl-w	60X56X76	1.000 Å
Bcl-2	84X80X64	1.000 Å
Mcl-1	124X112X118	1.000 Å

Table 2. IC_{50} nanomolar values of the NCMA in the *VirtualToxLab*.

Proteins	Ligand
AR	Not binding
AhR	0.0682
CYP1A2	Not binding
CYP2C9	Not binding
CYP2D6	Not bonding
CYP3A4	Not binding
ERα	Not binding
ERβ	Not binding
GR	Not binding
hERG	Not binding
LXR	Not binding
MR	Not binding
PPARγ	Not binding
PR	0.0139
TRα	Not binding
TRβ	Not binding

Results and discussion

In silico *prediction of the IC_{50} and toxic potential values of NCMA*

Here, we evaluated the interaction result of optimized NCMA using the DFT/B3LYP/6–311 ++ G (d, p) basis set with 16 proteins identified in *VirtualToxLab*. From the interaction results, the IC_{50} and TP values of each protein of the ligand were obtained. The value of TP varies from 0.0 to 1.0. TP $\leq$ 0.3 (low), 0.3 $<$ TP $\leq$ 0.6 (moderate), 0.6 $<$ TP $\leq$ 0.8 (high) and TP $>$ 0.8 (extreme) (Vedani *et al.* 2012). The total toxic TP value was calculated as 0.278 (low). Table 2 lists the IC_{50} values for 16 target proteins for NCMA. The table also defines the 'not binding' $IC_{50}>$ 100 μM. The highest affinity PR (0.0139 nM) and AhR (0.0682 nM) were also observed. Thus, ligand binds with the strongest PR and AhR. The kinetic stability of ligand was generated according to the results and is shown in Figure 2(A) (AhR) and Figure 2(B) (PR). In both ligand-protein complexes, it appears that they are stabilized by weak H bond interaction from the O terminal, which is an electron donor atom of NCMA.

Molecular docking

Docking studies were performed on NCMA and MCl-1, Bcl-2, Bcl-w, AKT-1, and BRAF proteins. AutoDock results were investigated based on the interactions between anti-apoptotic proteins and NCMA and the binding energies of the complexes. The definiteness of the results was approved as

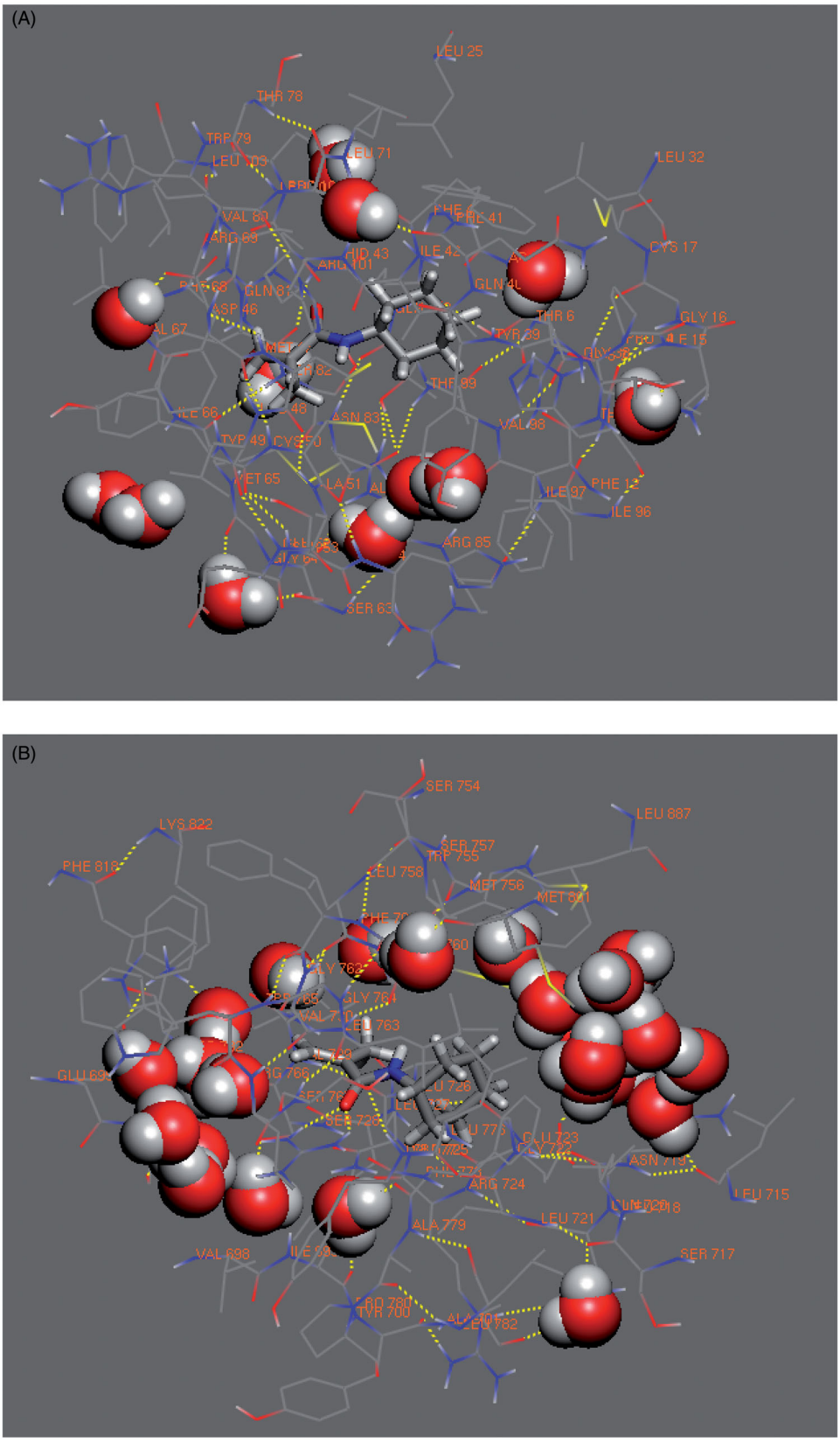


Figure 2. Binding of ligand to the (A) arylhydrocarbon receptor (AhR) (B) thyroid beta (PR). The protein is shown with a rod-like structure around the ligand. The water molecules are represented by red-gray beads, and the hydrogen bond interaction are represented by yellow dotted lines.

binding free energy and the hydrogen bonds between the proteins and NCMA (Table 3).

During the docking Glu191 was involved in the formation of hydrogen bonds with AKT-1. Thr529 was involved in BRAF and Thr266 was involved MCl-1. NCMA exhibited no hydrogen bond formation with Bcl-2 and Bcl-w (Figure 3). The protein-ligand binding results are significant for the development of the drug discovery process (Trott and Olson

Table 3. Docking binding energy results of novel NCMA with anti-apoptotic proteins and Paclitaxel (control drug).

Molecule	Protein	Binding energy (K.Cal/mol)	PDB ID
NCMA	**AKT-1**	−6.3	**4GVI**
NCMA	**Bcl-2**	−6.6	**4MAN**
NCMA	**Bcl-w**	−6.5	**2Y6W**
NCMA	**BRAF**	−6.9	**5VAM**
NCMA	**Mcl-1**	−6.5	**5FDO**
Paclitaxel	**AKT-1**	−12.2	**4GVI**
Paclitaxel	**Bcl-2**	−11.0	**4MAN**
Paclitaxel	**Bcl-w**	−13.6	**2Y6W**
Paclitaxel	**BRAF**	−12.3	**5VAM**
Paclitaxel	**Mcl-1**	−11.4	**5FDO**

PDB ID (Protein Data bank ID): A unique accession or identification code (https://www.rcsb.org/).

Binding energy (kcal/mol): The molecular docking simulation results have similar poses and the calculated binding energy for each docking pose within each cluster. If the binding energy has low energy, protein-ligand interaction more stable (Pradeep *et al*. 2015).

2010). Figure 4 demonstrates the prediction of the bonds of ligands for a targeted protein.

ADMET and drug-likeness analysis

In this study, admetSAR, pkCSM and SwissADME servers were used to determine pharmacokinetic profile. NCMA passes the drug-like rules namely Lipinski's rule, Veber rule, Ghose rule and has a creditable drug-likeness attribute (Table 4). In addition, ADMET results show that all calculated values were positive (Table 5).

The physiochemical properties of NCMA have number of 12 heavy atoms, 1 hydrogen bond acceptors, 1 hydrogen bond donors, molar refractivity of 51.07 and topological polar surface area (TPSA) of the molecule is found to be 29.10 Å2.

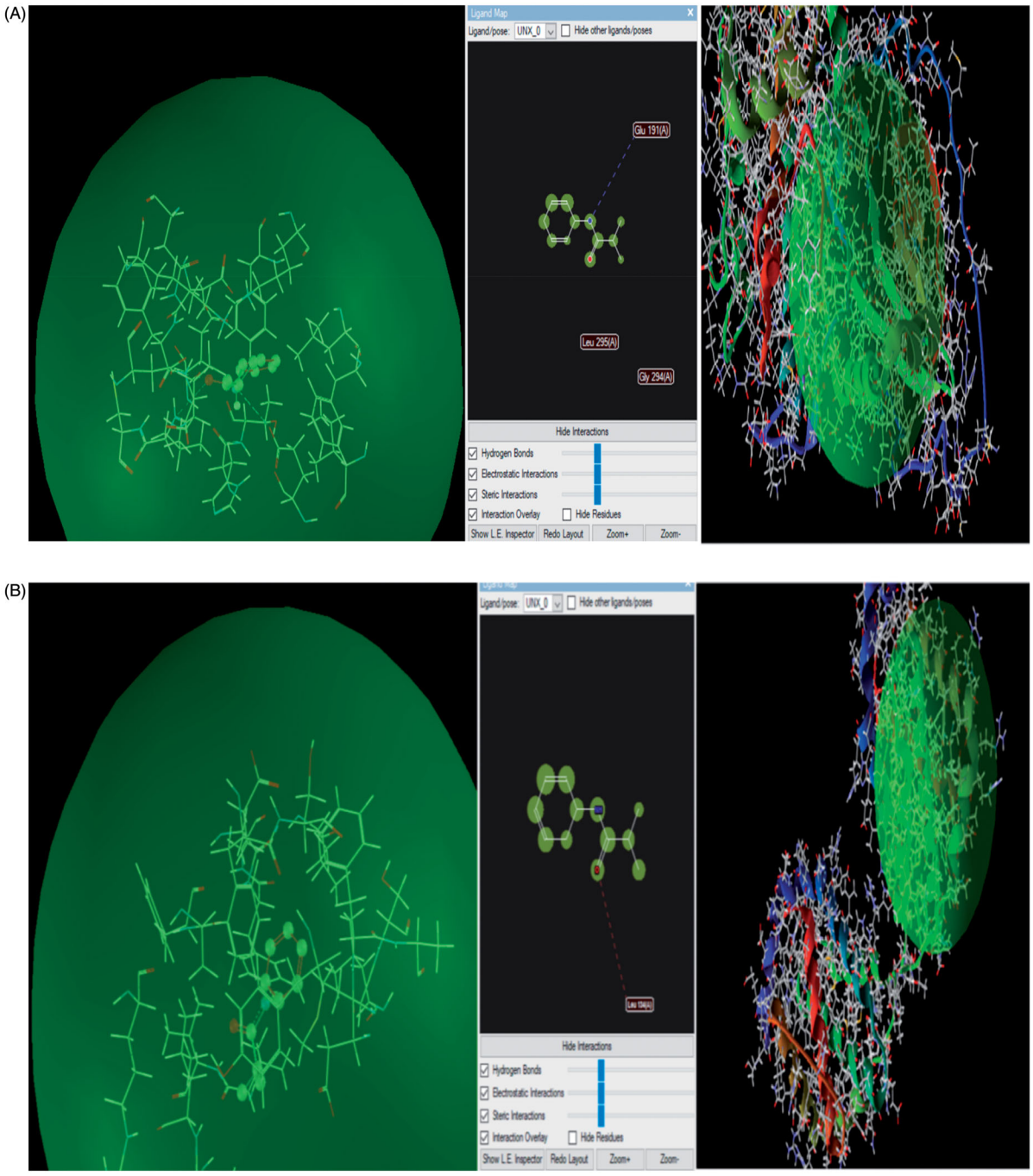


Figure 3. The 2D and 3D interaction of (A) AKT-1 (B) Bcl-2 (C) Bcl-w (D) BRAF (E) Mcl-1 proteins and NCMA were visualized by Molegro Molecular Viewer.

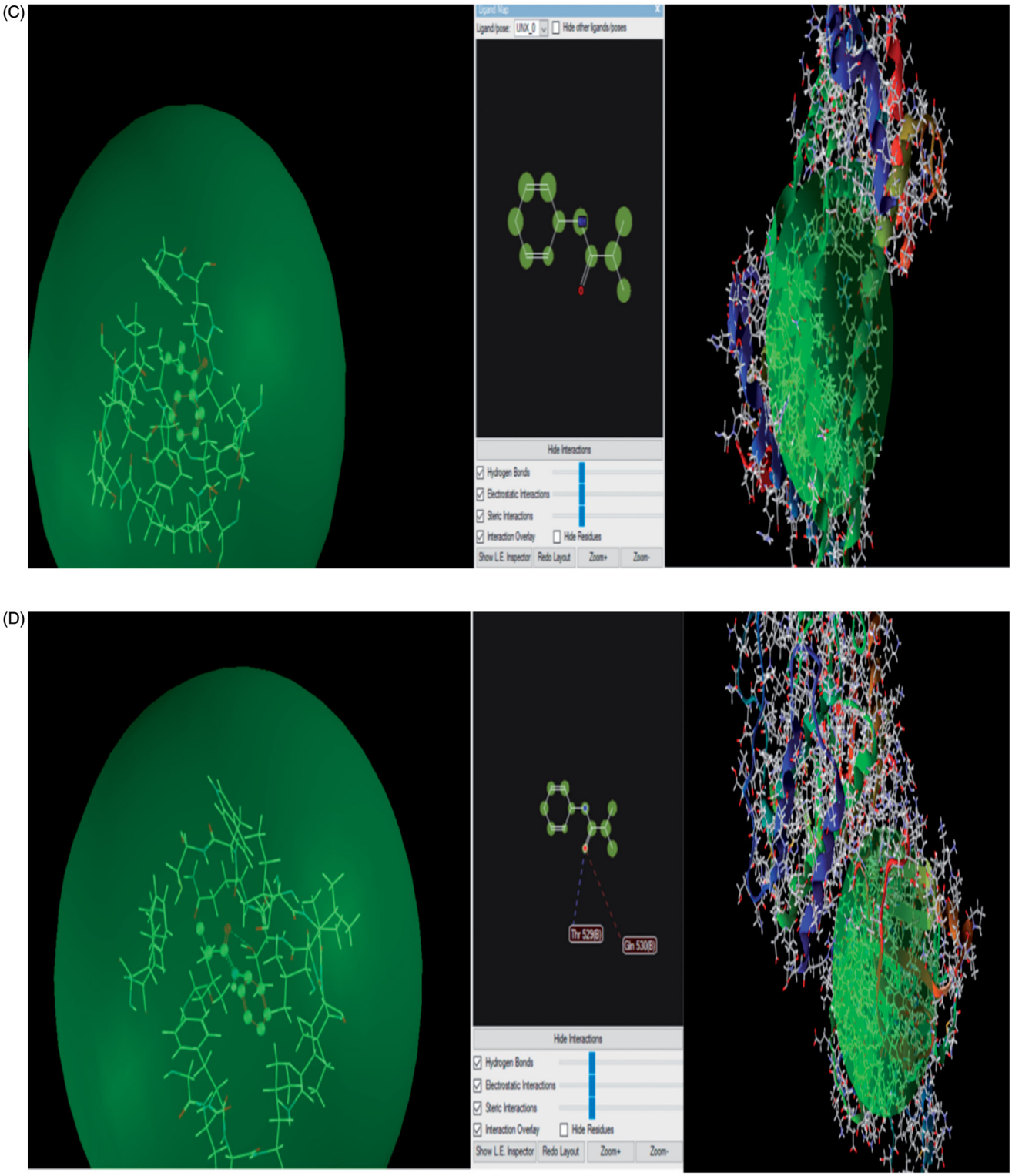


Figure 3. (Continued).

Water Solubility properties calculated are ESOL −2.2, solubility of 1.08e + mg/ml and of soluble class; Ali −2.64, solubility of 3.86e-01 mg/ml and of soluble class, SILICOS-IT −2.13, solubility of 1.24e + 00 mg/ml and of soluble class.

Pharmacokinetic data of NCMA was predicted to be of high Gastrointestinal absorption (GI), do not act as a P-gp substrate, do not inhibit CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 cytochromes. Skin permeation kinetics (Log Kp) was predicted to be −5.64 cm/s (http://www.swissadme.ch/index.php)

Drug-likeness factors was found to be of drug like compound, which obeys Lipinski's Rules, with no violation, similarly, Ghose, Veber's rules are the same (Bioavailability score: 0.55).

The bioavailability radar (Figure 5) demonstrated that the pink zone is the suitable physicochemical area for

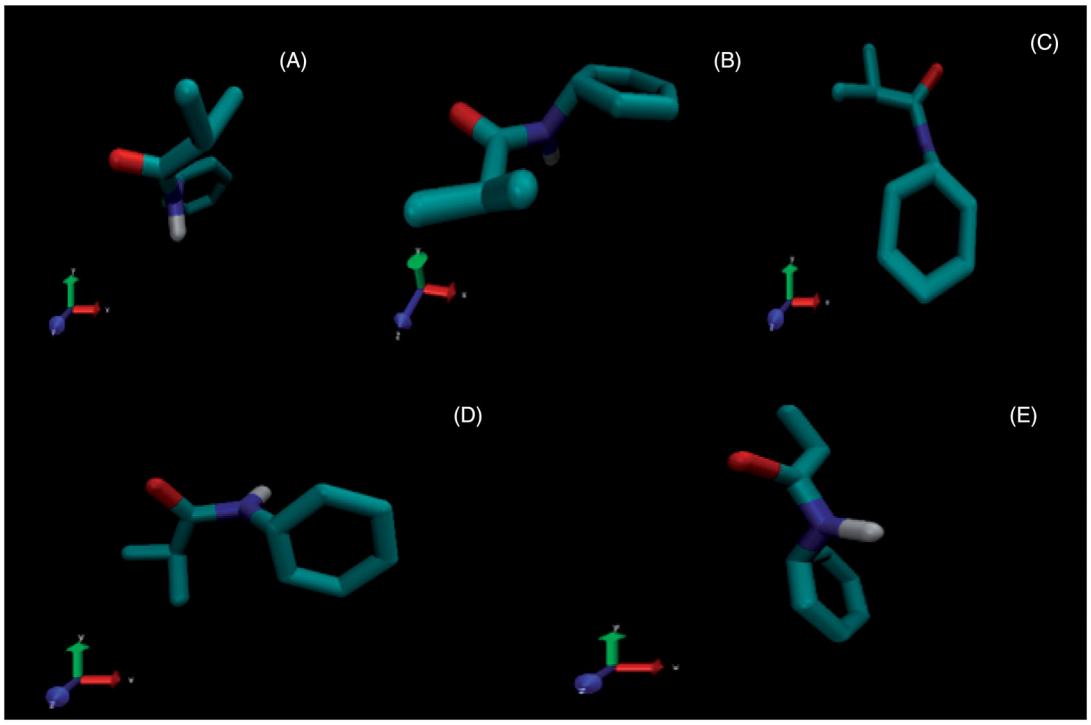


Figure 4. The prediction of ligand binding poses (A) AKT-1 (B) Bcl-2 (C) Bcl-w (D) BRAF (E) Mcl-1.

Table 4. Drug-likeness results of compounds.

	Drug-likeness			
Ligand	Lipinski	Ghose	Veber	Bioavalibility Score
NCMA	Yes	Yes	Yes	0.55

Lipinski's filter includes molecular weight $\leq$ 500, MLOGP (lipophilicity) $\leq$ 4.15, hydrogen bond acceptors, $\leq$ 10, and hydrogen bond donors $\leq$ 5 (Lipinski *et al.* 2001); Ghose's filter includes 160 $\leq$ molecular weight $\leq$ 480, $-0.4 \leq$ WLOGP (lipophilicity) $\leq$ 5.6, 40 $\leq$ the molar refractivity $\leq$ 130, and 20 $\leq$ number of atoms $\leq$ 70 (Ghose *et al.* 1999); Veber's filter includes the number of rotatable bonds $\leq$ 10 and the total polar surface area $\leq$ 140 (Veber *et al.* 2002). The bioavailability score defined the permeability and bioavailability properties of a potential drug molecule (Martin 2005).

Table 5. ADMET prediction for the components, predicted by pkCSM and SwissADME.

Model name	Predicted value	Unit
Water solubility		
Log S (ESOL)	−2.20 Soluble	log mol/L
Log S (Ali)	−2.64 Soluble	log mol/L
Log S (SILICOS-IT)	−2.13 Soluble	log mol/L
Intestinal absorption (human)	94.278 High	Numeric (Absorbed%)
P-glycoprotein substrate	No	Categorical (Yes/No)
P-glycoprotein I inhibitor	No	Categorical (Yes/No)
P-glycoprotein II inhibitor	No	Categorical (Yes/No)
VDss (human)	0.079	Numeric (log L/kg)
Fraction unbound (human)	0.563	Numeric (Fu)
BBB permeability	0.431	Numeric (log BB)
Renal OCT2 substrate	No	Categorical (Yes/No)
AMES toxicity	No	Categorical (Yes/No)
Max. tolerated dose (human)	0.731	Numeric (log mg/kg/day)
Oral Rat Acute Toxicity (LD50)	2.502	Numeric (mol/kg)
Oral Rat Chronic Toxicity (LOAEL)	1.563	Numeric (log mg/kg bw/day)
Hepatotoxicity	No	Categorical (Yes/No)
Carcinogenity	No	Categorical (Yes/No)

ADMET: Absorption, distribution, metabolism, excretion, and toxicity; AMES: Assay of the ability of a chemical compound to induce mutations in DNA; BBB: Blood–brain barrier (log BB $>$ 0.3 (cross BBB), log BB$<$-1 (poorly distributed brain); LD: Lethal dose; LOAEL: Lowest-observed-adverse-effect level; VDss: The steady state volume of distribution (log VDss$<$-0.15 (low), log VDss $>$ 0.45 (high). (http://biosig.unimelb.edu.au/pkcsm/prediction).

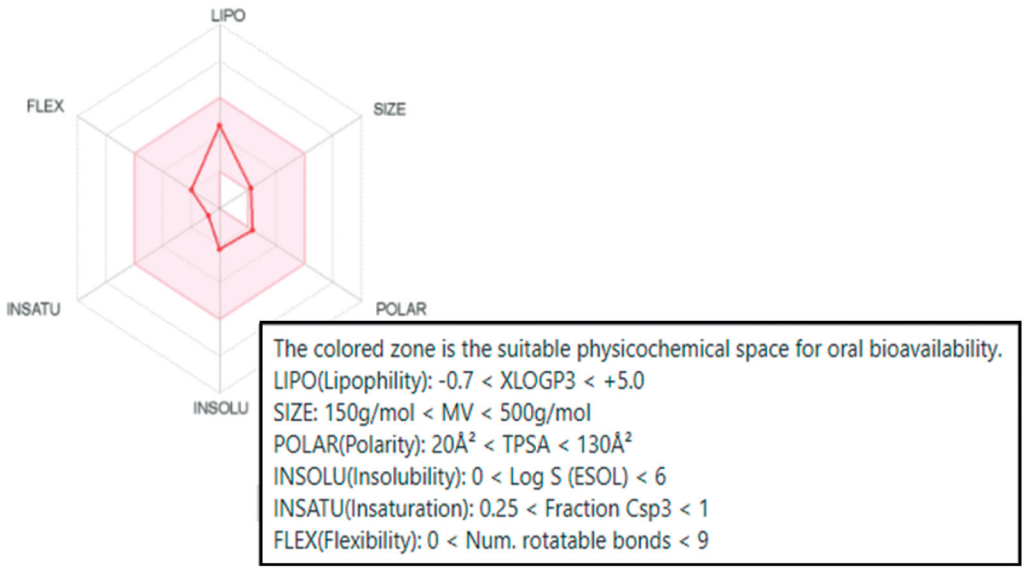


Figure 5. Bioavailability Radar graph of NCMA (http://www.swissadme.ch/index.php).

lipophilicity, flexibility, saturation, size, polarity, and solubility. In addition, the lipophilicity of the NCMA can range from −0.7 to +5.0. The molecular size can range from 150 g/mol to 500 g/mol. TPSA (topological polar surface area ranges) can range from 20 to 130 A° 2. The log S (ESOL) ranges between 0 and 6. The number of flexibility must be between 0–9 and the unsaturation ranges from 0.25 to 1.0 (Figure 5).

Conclusion

In the present work, we explore the human MCl-1, Bcl-2, Bcl-w, AKT-1, and BRAF protein-ligand interactions with synthesized N-cyclohexylmethacrylamide (NCMA) using theoretical toxic properties computational molecular docking. According to the *in silico* results, it is concluded that the NCMA molecule was not effective on anti-apoptotic proteins, and also it had no toxic properties. From the results obtained with *VirtualToxLab* software, a silico tool, it was found that the total toxicity potential of NCMA was very low with a value of 0.278. In addition, it was determined that NCMA ligand was only binding to aryl hydrocarbon and thyroid beta proteins out of 16 controlled proteins, albeit at low affinity values. In the future, NCMA may have a high potential to become a drug active substance with various modifications of the molecule. In addition, the results need both *in vitro* and *in vivo* study to prove the effective development of molecules.

Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Nevin Çankaya http://orcid.org/0000-0002-6079-4987
Serap Yalçın Azarkan http://orcid.org/0000-0002-9584-266X
Emine Tanış http://orcid.org/0000-0001-6815-9286

References

Akman, F., and Cankaya, N., 2016. A study of experimental and theoretical analysis of N–cyclohexylmethacrylamide monomer based on DFT and HF computation. *Pigment & Resin Technology*, 45 (5), 301–307.

Bhardwaj, V.K., and Purohit, R., 2020. Structural changes induced by substitution of amino acid 129 in the coat protein of Cucumber mosaic virus. *Genomics*, 112 (5), 3729–3738.

Bhardwaj, V.K., *et al.*, 2020. Structural based study to identify new potential inhibitors for Dual Specificity tyrosine-phosphorylation- regulated kinase . *Computer Methods and Programs in Biomedicine*, 194, 105494.

Cankaya, N., and Tanış, E., 2018. Synthesis, characterization and in-silico estimation of the toxic potential of N-(4-nitrophenyl)methacrylamide. *Materials Research Express*, 6 (2), 025310.

Cankaya, N., and Temüz, M.M., 2014. Monomer reactivity ratios of cellulose grafted with N-cyclohexylacrylamide and methyl methacrylate by atom transfer radical polymerization. *Cellulose Chemistry and Technology*, 48 (3–4), 209–215.

Daina, A., Michielin, O., and Zoete, V., 2017. Swissadme: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific Reports*, 7, 42517–42717.

Erdogan, M., *et al.*, 2018. Optical and chemical sensor properties of Langmuir-Blodgett thin films coated with N-cyclohexylmethacrylamide monomer. *Journal of Optoelectronics and Advanced Materials*, 20, 520–525.

Cheng, F., *et al.*, 2012. AdmetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. *Journal of Chemical Information and Modeling*, 52 (11), 3099–3105.

Frisch, M. J., *et al.*, 2009. *Gaussian 09, Revision D. 01*. Wallingford, CT: Gaussian. Inc.

Ghose, A.K., Viswanadhan, V.N., and Wendoloski, J.J., 1999. A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *Journal of Combinatorial Chemistry*, 1 (1), 55–68.

Hanaa, H.A., *et al.*, 2010. Potent neuroprotective role of novel melatonin derivatives for management of central neuropathy induced by acrylamide in rats. *European Journal of Medicinal Chemistry*, 45, 5452–5459.

Lipinski, C.A., *et al.*, 2001. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews*, 46 (1–3), 3–26.

Losson, H., *et al.*, 2020. HDAC6—an emerging target against chronic myeloid leukemia? *Journal of Biomolecular Structure and Dynamics*, 38 (17), 5126–5135.

Martin, Y.C., 2005. A bioavailability score. *Journal of Medicinal Chemistry*, 48 (9), 3164–3170.

Morris, G.M., *et al.*, 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry*, 19 (14), 1639–1662.

Ntie-Kang, F., *et al.*, 2013. In silico drug metabolism and pharmacokinetic profiles of natural products from medicinal plants in the Congo basin. *In Silico Pharmacology*, 1, 12.

Pradeep, P., *et al.*, 2015. A novel scoring based distributed protein docking application to improve enrichment. *IEEE/ACM Transactions on Computational Biology and Bioinformatics*, 12 (6), 1464–1469.

Singh, R., *et al.*, 2021. Discovery and in silico evaluation of aminoarylbenzosuberene molecules as novel checkpoint kinase 1 inhibitor determinants. *Genomics*, 113 (1), 707–715.

Tareke, E., *et al.*, 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. *Journal of Agricultural and Food Chemistry*, 50 (17), 4998–5006.

Thomsen, R., and Christensen, M.H., 2006. MolDock: a new technique for high-accuracy molecular docking. *Journal of Medicinal Chemistry*, 49 (11), 3315–3321.

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

Veber, D.F., *et al.*, 2002. Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry*, 45 (12), 2615–2623.

Vedani, A., *et al.*, 2015. OpenVirtualToxLab-A platform for generating and exchanging in silico toxicity data. *Toxicology Letters*, 232 (2), 519–532.

Vedani, A., Dobler, M., and Smieško, M., 2012. VirtualToxLab - a platform for estimating the toxic potential of drugs, chemicals and natural products . *Toxicology and Applied Pharmacology*, 261 (2), 142–153.

Vedani, A., and Smieško, M., 2009. In silico toxicology in drug discovery - concepts based on three-dimensional models . *Alternatives to Laboratory Animals : ATLA*, 37 (5), 477–496.

Zoete, V., *et al.*, 2016. SwissSimilarity: a web tool for low to ultra high throughput ligand-based virtual screening. *Journal of Chemical Information and Modeling*, 56 (8), 1399–1404.