Jurnal Ilmiah Mahasiswa Kedokteran Universitas Airlangga

JUXTA: Jurnal Ilmiah Mahasiswa Kedokteran Universitas Airlangga
2022 August, XIII (02)

ORIGINAL ARTICLE

In-Silico Study: Potential Inhibitor of Cyclin-Dependent Kinase 6 (CDK6) from Natural Plant Compounds for Melanoma Treatment

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ABSTRACT

Introduction: Melanoma is the most aggressive and dangerous type of skin cancer. It usually occurs in the skin because melanocytes originate from the neural crest cells that migrate. A previous study stated misregulation of cyclin-dependent kinase 6 (CDK6) had a role in melanoma progression. This study aimed to identify the potential natural compound targeting and modulating the CDK6.

Methods: This was an investigative study using in-silico docking analysis to search for compatible ligands and potential inhibitors to CDK6 protein. This study screened 46 natural compounds based on the drug-likeliness based on Lipinski's rules of five and used PyRx (AutoDock Vina) software for the initial screening. 10 compounds with the highest binding energy underwent docking simulation using Molecular Operating Environment (MOE) software.

Results: Chlorogenic acid, guattegaumerine, luteolin, and acronycine were potential natural compounds in plants as CDK6 inhibitors.

Conclusion: This study found that chlorogenic acid was the most potential to be an inhibitor of CDK6 compared to other compounds screened.

ARTICLE INFO

Article history:

Received 2 June 2022

Received in revised form
28 June 2022

Accepted 28 July 2022

Available online 10 August 2022

Keywords:
CDK6,
In-silico,
Life expectancy,
Melanoma,
Natural plant compounds.

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JUXTA: Jurnal Ilmiah Mahasiswa Kedokteran Universitas Airlangga
p-ISSN: 1907-3623; e-ISSN: 2684-9453
DOI: 10.20473/juxta.V13I12022.93-99

Introduction

Melanoma is a malignant tumor originating from melanocytes and is also a rare malignancy.[1] It usually occurs in the skin because melanocytes originate from the neural crest cells migrate. The incidence of melanoma has steadily increased worldwide over the last few decades. Although the prevalence is only 4% of all cases of skin cancer, this disease is very deadly and accounts for 75% of deaths.[1]

The increasing number of cases, the highest contributor to skin cancer deaths, poor prognosis, and limited treatment make new therapy necessary for this disease.[2] Various treatments are used to treat melanoma. Current therapeutic approaches include surgical resection, chemotherapy, photodynamic therapy, immunotherapy, biochemotherapy, and targeted therapy. The choice of treatment depends on the patient's health status, stage, and tumor location. Therapeutic efficacy may decrease as resistance develops.[3] Despite advances in adjuvant therapy, the 5-year relative survival is only 15.3% compared to other malignancies, melanomas refractory to chemotherapy, and emerging immunotherapy.[4] This underlies the need for the development of melanoma treatment.

Cyclin-dependent kinase 6 (CDK6) cooperates with cyclin D in controlling cell cycle progression through the phosphorylation of Retinoblastoma protein (Rb).[5] The role of CDK6 is to promote development through cell cycle overload, increase cell proliferation, cell migration, and angiogenesis of cancer.[6] Dysregulation of CDK6 pathways is found in up to 90% of melanoma.[7] UV light promotes CDK6 amplification, then induces UV-lesion repairment and melanomagenesis.[7] Knock-down of CDK6 has shown to reduce the angiogenic potential of the tumor cells, decrease cell proliferation, reduce cell proliferation migration, and also decrease tumor growth in melanoma.[5] The previous explanation shows that CDK6 can be a therapeutic target in treating melanoma. Flavonoid and phenolic compounds on plants are known to have anticancer effects.[5,8] Therefore, in this study, the in-silico analysis aimed to find the potential plant-based compound targeting CDK6 for melanoma treatment. Hopefully, this study could be used as the basis for future in-vivo and in-vitro studies about melanoma treatment targeting CDK6.

Methods

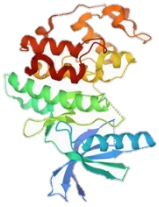
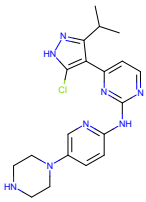
This was an investigative study using in-silico docking analysis to search for compatible ligands and potential inhibitors to CDK6 protein. This study searched the herbal compound with potential anti-melanoma and anticancer in the skin which acts as the ligand for the study from Dr. Duke's Phytochemical and Ethnobotanical Databases.[9] A total of 46 compounds were listed.

The structure of each available natural compound was downloaded from PubChem in .sdf format.[10] This study also retrieved the canonical Simplified Molecular Input Line Entry System (SMILES) from PubChem. It was used to examine the drug-likeness criteria by Lipinski's rule of five using the SwissADME web server.[11] The compound with two or more violations of Lipinski's Rule was excluded.

The molecular structure of CDK6 (PDB 3NUX) was obtained from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank in .pdb format.[12] The active site was predicted using the Computed Atlas of Surface Topography of Proteins (CASTp).[13] The initial screening was performed using the PyRx software (AutoDock Vina). The ligand's position on the binding site was determined by arranging the grid box sized 18.9641x15.5527x19.2737 with the center of coordinates in 14.7076x27.9003x8.6742. Then, 10 compounds with the highest binding affinity will be docked using Molecular Operating Environment (MOE) software. Ligand and protein preparation were prepared beforehand.

Docking calculation was performed by the Lamarckian algorithm in MOE software. Each ligand was docked into protein CDK6 at the pocket site of the protein. Molecular docking was performed using Triangle Matcher for Placement and Forcefield for Refinement. The parameter used for pose generation was the London dG score. For each ligand, the top poses were selected from 100 poses

Table 1. Protein target, native ligands, active site

Protein Target	PDB ID	Native Ligand	Active Site
CDK6 **CDK6 (monomeric) in complex with inhibitor** 	3NUX	**3NV** 4-[5-chloro-3-(1-methylethyl)-1H-pyrazol-4-yl]-N-(5-piperazin-1-ylpyridin-2-yl)pyrimidin-2-amine  C_{19} H_{23} Cl N_8	ILE19, GLY20, GLU21, GLY22, ALA23, GLY25, LYS26, VAL27, ALA41, LYS43, VAL45, ARG46, PRO55, THR58, GLU61, LEU65, LEU68, GLU69, VAL76, VAL77, LEU79, LYS93, LEU94, PHE98, GLU99, HIS100, VAL101, ASP102, ASP104, PHE135, LEU136, VAL141, VAL142, HIS 143, ARG144, ASP145, LYS147, GLN149, ASN150, LEU152, ALA162, ASP163, PHE164, GLY165, LEU166, ALA167, VAL181, THR182, ARG186

Results

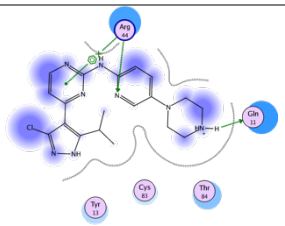
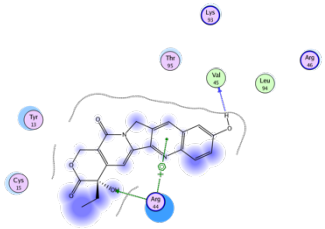
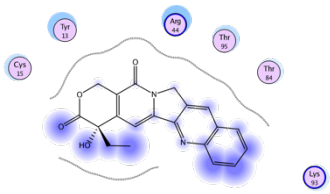
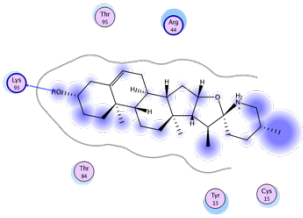
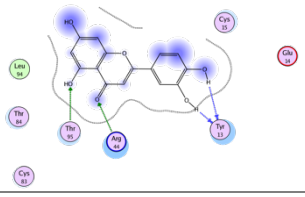
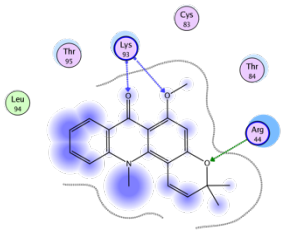
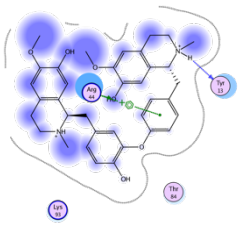
Table 1 shows the structures and amino acids found in the active site pockets of 3NUX. This protein was found in humans, structured and repositioned in PDB, and could be accessed by the public since December 2010.

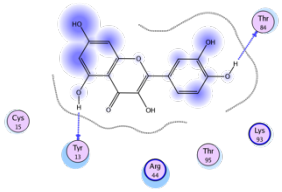
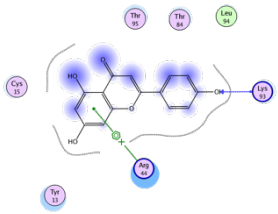
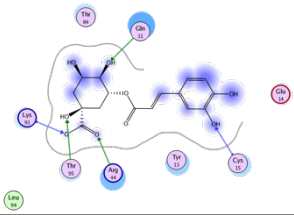
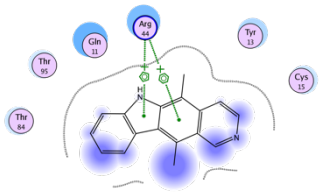
Table 2 shows some ligands and several drug candidates with the lowest binding affinity through PyRx screening and met the requirements of Lipinski's rule of five. Table 3 shows molecular docking analysis results for several compounds against 3NUX, including Gibbs Energy, affinity, H donor/H acceptor interaction, and the amino acid involved. Figure 1 shows the 3D visualization of the 3 best potential ligands on CDK6 pocket site.

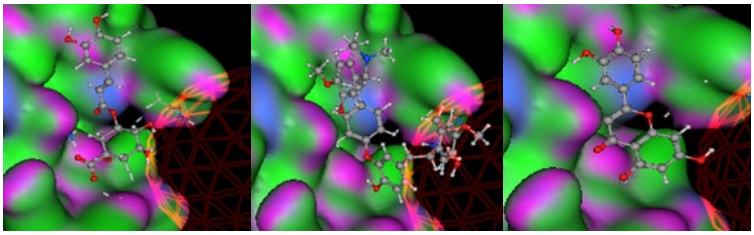
Table 2. Properties of CDK6 potential inhibitor candidates

No.	Compound	Lipinski's Rule of Five	
		Properties	Value
1	10-Hydroxycamptothecin (CID 97226)	Molecular weight (<500 Da)	364.35
		LogP (<5)	1.11
		H-bond donor (5)	2
	$C_{20}H_{16}N_2O_5$	H-bond acceptor (<10)	6
		Violation	0
2	Camptothecin (CID 24360)	Molecular weight (<500 Da)	348.35
		LogP (<5)	1.64
	$C_{20}H_{16}N_2O_4$	H-bond donor (5)	1
		H-bond acceptor (<10)	5
		Violation	0
3	Solasodine (CID 5280445)	Molecular weight (<500 Da)	413.64
		LogP (<5)	4.94
	$C_{27}H_{43}NO_2$	H-bond donor (5)	2
		H-bond acceptor (<10)	3
		Violation	1
4	Luteolin (CID 5281807)	Molecular weight (<500 Da)	286.24
		LogP (<5)	-0.03
	$C_{15}H_{10}O_6$	H-bond donor (5)	4
		H-bond acceptor (<10)	6
		Violation	0
5	Acronycine (CID 345512)	Molecular weight (<500 Da)	321.37
		LogP (<5)	2.50
	$C_{20}H_{19}NO_3$	H-bond donor (5)	0
		H-bond acceptor (<10)	3
		Violation	0
6	Guattegaumerine (CID 159911)	Molecular weight (<500 Da)	596.71
		LogP (<5)	3.11
	$C_{36}H_{40}N_2O_6$	H-bond donor (5)	3
		H-bond acceptor (<10)	8
		Violations	1
7	Quercetin (CID 5280343)	Molecular weight (<500 Da)	302.24
		LogP (<5)	-0.56
	$C_{15}H_{10}O_7$	H-bond donor (5)	5
		H-bond acceptor (<10)	7
		Violation	0
8	Apigenin (CID 5280443)	Molecular weight (<500 Da)	270.24
		LogP (<5)	0.52
	$C_{15}H_{10}O_5$	H-bond donor (5)	3
		H-bond acceptor (<10)	5
		Violation	0
9	Cholorogenic acid (CID 1794427)	Molecular weight (<500 Da)	354.31
		LogP (<5)	-1.05
	$C_{16}H_{18}O_9$	H-bond donor (5)	6
		H-bond acceptor (<10)	9
		Violation	1
10	Ellipticine (CID 3213)	Molecular weight (<500 Da)	246.31
		LogP (<5)	3.00
	$C_{17}H_{14}N_2$	H-bond donor (5)	1
		H-bond acceptor (<10)	1
		Violation	0

Table 3. Molecular docking results and visualization of 5 best phytochemicals

Ligand	1DEH-Ligand		H-Bond
Native Ligand: 3NV (CID 49800100)		ΔG: -18.875 kcal/mol Affinity: 6.123pKi H donor/ H acceptor interaction: 2	GLN11 (1.64), ARG44 (2.85)
10-Hydroxy-camptothecin (CID 97226)		ΔG: -18.059 kcal/mol Affinity: 6.812pKi H donor/ H acceptor interaction: 2	VAL46 (1.66), ARG44 (3.00)
Camptothecin (CID 24360)		ΔG: -10.320 kcal/mol Affinity: 4.901pKi H donor/ H acceptor interaction: 0	-
Solasodine (CID 5280445)		ΔG: -16.438 kcal/mol Affinity: 5.086pKi H donor/ H acceptor interaction: 1	LYS93 (2.52)
Luteolin (CID 5281807)		ΔG: -11.356 kcal/mol Affinity: 7.552pKi H donor/ H acceptor interaction: 5	TYR13 (3.59,1.67), THR95 (2.99), ARG44 (2,77), THR95 (2.99)
Acronycine (CID 345512)		ΔG: -10.154 kcal/mol Affinity: 5.141pKi H donor/ H acceptor interaction: 3	ARG44 (2.96, 2.97), YS93 (2.60)
Guattegaumerine (CID 159911)		ΔG: -20.245 kcal/mol Affinity: 6.894pKi H donor/ H acceptor interaction: 2	TYR13 (1.67), ARG44 (2.68)
Quercetin (CID 5280343)		ΔG: -13.330 kcal/mol Affinity: 5.587pKi	TYR13 (1.78), THR84 (2.16)

		H donor/ H acceptor interaction: 2	
Apigenin (CID 5280443)		ΔG: -9.713 kcal/mol Affinity: 5.330pKi H donor/ H acceptor interaction: 1	LYS93 (2.59)
Cholorogenic acid (CID 1794427)		ΔG: -28.273 kcal/mol Affinity: 9.776pKi H donor/ H acceptor interaction: 7	GLN11 (2.93), CYS15 (3.10), ARG44 (2.58, 2.53), LYS93 (2.45), THR95 (2.79, 2.79)
Ellipticine (CID 3213)		ΔG: -14.085 kcal/mol Affinity: 4.813pKi H donor/ H acceptor interaction: 0	-



a b c

Figure 1. Conformation of chlorogenic acid (a), guattegaumerine (b), and luteolin (c) on pocket site of CDK6

Discussion

CDK6 are cell cycle kinases that facilitate the progression of cells forming complexes with D-type cyclin. Disordered cell regulation leads to uncontrolled cell proliferation and cancer development, including melanoma.[6] CDK6 inhibitor has a good therapeutic effect in human melanoma. This monotherapy offers a great simple benefit.[7] Knock-down of CDK6 has shown to reduce the angiogenic potential of the tumor cells, decrease cell proliferation, reduce cell migration, and also decrease tumor growth.[5]

This study investigated 10-hydroxycamptothecin, camptothecin, solasodine, luteolin, acronycine, guattegaumerine, quercetin, apigenin, chlorogenic acid, and ellipticine as potential inhibitors of the CDK6 protein. Those compounds can be found in various plants (Table 4). The binding energies obtained from docking 3NUX with the native ligand, 10-hydroxycamptothecin, camptothecin, solasodine, luteolin, acronycine, guattegaumerine, quercetin, apigenin, chlorogenic-acid, and ellipticine were -18.875, -18.059,-10.320, -16.438, -11.356, -10.154, -20.245, -13.330, -9.713, -28.273, and -14.085 kcal/mol, respectively (Table 3). The docking analysis showed the inhibition potential of several compounds, ranked by binding energies (ΔG); chlorogenic acid > guattegaumerine > 10-hydroxycamptothecin > solasodine > ellipticine > quercetin > luteolin > camptothecin > acronycine > apigenin.

Table 4. The natural compound sources

Compound	Plants
10-Hydroxycamptothecin (CID 97226)	*Camptotheca acuminata;* families of Apocynaceae, Olacaceae, and Rubiaceae[14]
Camptothecin (CID 24360)	*Camptotheca acuminata;* families of Apocynaceae, Olacaceae, and Rubiaceae[14]
Solasodine (CID 5280445)	*Solanum torvum Sw.*[15]
Luteolin (CID 5281807)	*Apium graveolens, Petroselinum crispum, Brassica oleracea, Allium fistulosum, Daucus carota subsp. Sativus, Capsicum annuum group, Brassica oleracea,* and *Chrysanthemum*[16]
Acronycine (CID 345512)	*Acronychia baueri*[17]
Guattegaumerine (CID 159911)	*Berberis stolonifera*[18]
Quercetin (CID 5280343)	*Citrus, Malus domestica, Rubus idaeus, Allium cepa,* and *Vitis vinifera*[19,20]
Apigenin (CID 5280443)	*Matricaria recutita, Citrus reticulata, Allium cepa, Apium graveolens,* and *Petroselinum crispum*[20,21]
Cholorogenic acid (CID 1794427)	*Salvia rosmarinus, Eugenia uniflora, Coffea,* and *Citrus limon*[22]
Ellipticine (CID 3213)	*Ochrosia vieillardii, Ochrosia acuminata, Ochrosia moorei, Strychnos dinkagei,* and families of Apocynaceae [23,24]

The results of docking analysis (Table 3) showed that the native ligand formed H-bonds with the 3NUX amino acid GLN11 and ARG44. The 10-Hydroxycamptothecin formed H-bonds with the 3NUX amino acids VAL46 and ARG44. Camptothecin did not have H-bonds interaction with the 3NUX amino acid. Solasodine formed H-bonds with the 3NUX amino acid LYS93. Luteolin created H-bonds with the 3NUX amino acids TYR13, ARG44, and THR95. Acronycine formed H-bonds with the 3NUX amino acids ARG44 and LYS93. Guattegaumerine formed H-bonds with the 3NUX amino acids TYR13 and ARG44. Quercetin formed H-bonds with the 3NUX amino acids TYR13 and THR84. Apigenin created H-bonds with the 3NUX amino acid LYS93. Chlorogenic acid formed H-bonds with the 3NUX amino acid GLN11, CYS15, ARG44, LYS93, and THR95. Ellipticine did not have an H-bonds interaction with the 3NUX amino acid. The high affinity of drug compounds depended on the type and amount of bonding with the active site of the protein. The 3NV native ligand had less hydrogen interaction than chlorogenic acid, luteolin, and acronycine. Chlorogenic acid on 3NUX had the most hydrogen interactions.

Based on the binding energy, chlorogenic acid and guattegaumerine had lower binding affinity than native ligands with protein 3NUX. Based on the binding energy, affinity, and hydrogen bond interaction, chlorogenic acid had promising potential to be an inhibitor of CDK6. Chlorogenic acid is a natural product that is spread in the kingdom Plantae.[22] It can be found in various plants, like rosemary, cherry, coffee, and lemon (Table 4). Those plants are easily found in everyday life and in various countries.

The results of this study are in line with other studies regarding the potential of phenolic and flavonoid compounds as anticancer. Plant-derived phenolic is shown to inhibit the initiation and progression of cancers by inducing apoptosis, promoting cell survival, and inhibiting the cell cycle, invasion, metastasis, and angiogenesis.[8] Phenolic acid has some toxicity effects that are easy to manage, making it potential as a therapeutic option for human by paying attention to certain things such as dose.[8] Flavonoids modulate Reactive Oxygen Species (ROS) scavenging enzyme activities, arrest the cell cycle, induce apoptosis, autophagy, and suppress cancer cell proliferation and invasiveness.[14] These compounds are found in a wide variety of fruits, vegetables, beverages, and human foods, hence they do not have dangerous side effects.[21] Flavonoids are also able to bind on the CDK6/cyclin D complex via hydrogen and van der Walls bonds on amino acids, allowing it to be an inhibitor for this protein.[25] Therefore, chlorogenic acid as a phenolic compound and guattegaumerine, luteolin, and acronycine as flavonoids were the most recommended natural compounds found in plants as potential inhibitors of plant CDK6 which should be explored in future studies.

Conclusion

In conclusion, this study docked the best 10 compounds with CDK6. Chlorogenic acid was the most suitable ligand for this protein. It had the lowest ΔG binding energy, better affinity, and most hydrogen bonding interaction. Therefore, it could be the most suitable inhibitor on CDK6. Overall, chlorogenic acid, guattegaumerine, luteolin, and acronycine were the most recommended natural compounds found in plants as potential inhibitors of CDK6 for melanoma treatment. Hopefully, this study could be used as the basis for both in-vivo and in-vitro studies, hence new herbal plant-based therapies can be found for treating melanoma.

Acknowledgments

The authors would like to acknowledge the support from Faculty of Medicine Universitas Airlangga and Universitas Indonesia that have supported and facilitated this study.

Conflict of Interest

The author declared there is no conflict of interest.

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