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Potential compound of *Curcuma xanthorrhiza* and *Curcuma zedoaria* as Mortalin inhibitor to control cancer cell growth through computational study

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Abstract. *C. xanthorrhiza* and *C. zedoaria*, anticancer agent widely used in Asia. Previous studies have identified that active compounds from both Curcuma are capable of inhibiting cancer cell growth and inducing apoptosis. However, the roles from its active compound for Mortalin inhibition in carcinogenesis process was unknown. The recent study shows that Mortalin also known as HSPA9/GRP75/mtHSP70 is a highly conserved heat-shock protein 70 family has various roles in carcinogenesis process in multiple ways and very complicated. This study analyzed the potential of active compound from both curcuma as Mortalin inhibitor to control cancer cell growth with the computational study. the results of binding affinity analysis through molecular docking show that some active compounds from both Curcuma have the potential as Mortalin inhibitor with smallest energy and same binding site with commercial Mortalin inhibitors. protein interaction analysis illustrates that Mortalin can interact with various protein including hspd1, timm17a, and hspa4. upregulation is it proteins expression have been identified as a diagnostic marker and poor prognosis factor for cancer. these data indicate that active compounds from both Curcuma have a promising opportunity as anticancer through Mortalin inhibitor mechanism. additional research is needed to validate the in vitro activity of the compounds.
Keyword: anticancer, *C. xanthorrhiza*, *C. zedoaria*, Mortalin inhibitor

1. Introduction

Cancer is one of the leading causes of death in the world [1]. Since cancer began to be detected in Indonesia in 1970 there was an increase in the number of patients each year [2]. In 2019 there were 348,809 cases of cancer with cases of death reaching 207,210. The prevalence for the past 5 years is 775,120 cases. The most common cancer cases are breast, cervical, throat, colorectal and liver.

There are about 35,000 species of plants that are used as treatment materials including Curcuma xanthorrhiza and Curcuma zedoaria. Both plants come from the Zingiberaceae family. Curcuma zedoaria is traditionally used by people in various countries as food and treatment for diseases such as abdominal pain, fever, antiseptic, expectorant. People in the Asian region also use curcuma as a cancer treatment such as cervical cancer, breast cancer [3], colorectal cancer [4]. This is related to the ability of active compounds in curcuma that can kill cancer cells. Curcuma xanthorrhiza is a native plant of Indonesia called by the local name of ginger. It is also able to grow in the Philippines, Thailand, Malaysia and Sri Lanka. Curcuma xanthorrhiza is known to have pharmacological effects as anti-

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inflammatory, antioxidant, antiplatelet, anticancer, antibacterial, antihypertensive, hepatoprotective, estrogenic, antiestrogenic and neproprotective [5].

Mortalin / mtHsp70 is a group of Hsp70 proteins that play a role in inducing carcinogenesis by deactivating tumor suppressor protein p53, activation of EMT signaling and deregulation of apoptosis [6]. Curcuma zedoaria has a role in providing antimetastasis in colorectal cancer by inducing downregulation of signaling EMT.4 Curcuma xanthorrhiza containing Xantiz known to have Xantiz which has known activity to have antimetastasis in colorectal cancer by inducing EMT signaling downregulation. antimetastasis in vivo [7]. At present many researchers are developing anticancer drugs with both curcuma, but the mechanism as an anti-mortalin is unknown. This study aims to determine the potential of active compounds from both curcuma as mortalin inhibitors to control the growth of cancer cells.

2. Material and methods

2.1. Preparation of molecular structures and codes

The ligands used in this study were compounds from both Curcuma, including α-curcumene, Ar-turmerone, β-atlantone, β-curcumene, Bisdemethoxycurcumin, Curcumin, D-camphor, Demethoxycurcumin, Germacrol, Germacrone, Xanthorrizol, β-sitosterol, Campesterol, Curcumenol, Curcumenone, Curdione, Curzerenone, Dehydrocurdione, Isocurcumenol, Neocurdione, Stigmasterol, and Zederone, CAFE and MKT-077 as Mortalin inhibitor. Sybil Data Files (SDF) format of the compounds retrieved from PubChem (http://pubchem.ncbi.nlm.nih.gov) were converted to 3D structures in Protein Data Bank (PDB) format using BIOVIA Discovery Studio 16.1. The detailed information for all ligand are shown in table 1. The receptor used in this study was Mortalin protein retrieved from PDB with PDB ID 4KBO. The protein then prepared using PyMOL v1.74.

2.2. Ligand docking and interaction prediction

Interactions between ligands and receptor were analyzed through molecular docking by AutoDock Vina integrated in PyRx 0.8 [8]. Docking results and bonds formed were analyzed using BIOVIA Discovery Studio version 16.1 [9]. Ligand's atoms and interacted residues were determined by LigPlot+ 2.1 [10].

2.3. Protein interaction network

Protein interaction networks used to identify protein were interacted with Mortalin in cancer cell developed. Protein interaction network was determined by STRING (https://string-db.org) [11].

3. Result and discussion

3.1. Ligand docking analysis

Molecullar docking results between Mortalin, active compounds of C. xanthorrhiza, C. zedoaria and 2 known Mortalin inhibitor (Café and MKT-077) show different activities. Total of 11 active compounds from the two Curcuma species were analyzed with Mortalin protein. There are 5 active compounds of C. xanthorrhiza that have the same binding position with Mortalin inhibitors (Café; data not shown). Two compounds that the most potential to anticancer in C. xanthorrhiza are Curcumin and Xanthorrhizol. Curcumin has a higher binding value (-7.9 kcal/mol) compared to Café (-7.3 kcal/mol) and Xanthorrhizol has a binding value close to café (-6.7 kcal/mol). In contrast to the active compound C. xanthorrhiza, the active compound C. zedoaria has the potential as an anticancer because it has a different affinity for the same protein. Some compounds have the same binding position as Café and MKT-077 (respectively). Five of the 11 compounds used have an affinity value more than -6.0 kcal/mol (data not shown) and have the same binding position with Café. Curzerenone has a higher binding value (-7.4 kcal/mol) compared to Café and Campesterol has a binding value close to Café (-7.1 kcal/mol). Only 2 compounds from C. zedoaria, β-sitosterol and Dehydrocurdione have the same

binding position with MKT-077 (-6.6 kcal/mol and -6.2 kcal/mol, respectively) (Table 1). Curcumin is a compound with the strongest inhibitory activity compared to other compounds from the binding affinity value.

Table 1. Binding affinity between Mortalin and all molecules

Ligand name	PubChem CID	Molecular Weight (g/mol)	Binding Affinity (mol/kcal)
Curcuma xanthorrhiza			
Curcumin	969516	368.4	-7.9
Xanthorrhizol	93135	218.33	-6.7
Curcuma zedoaria			
Curzerenone	10376566	230.3	-7.4
Campesterol	173183	400.7	-7.1
β-sitosterol	222284	414.7	-6.6
Dehydrocurdione	14191392	234.33	-6.2
Mortalin Inhibitor			
CAFÉ	5281787	284.31	-7.3
MKT-077	6912334	396.6	-7.2

Further analysis focused on determining the orientation of each compound that has the potential of being an inhibitor when interacting with the active side of Mortalin. The results illustrate that the four compounds of C. xanthorrhiza and C. zedoaria (Curcumin, Xanthorrhizol, Curzerenone and Campesterol) bind to the active side of Mortalin in the same position with Café (Fig. 1). Other illustration results show that β-sitosterol and Dehydrocurdione also bind to the active side of Mortalin in the same position with MKT-077 (Fig. 2). The six compounds show the same position as a known inhibitor and have a relatively high hydrophobic value. These data indicate that the six compounds most potential as Mortalin inhibitors.

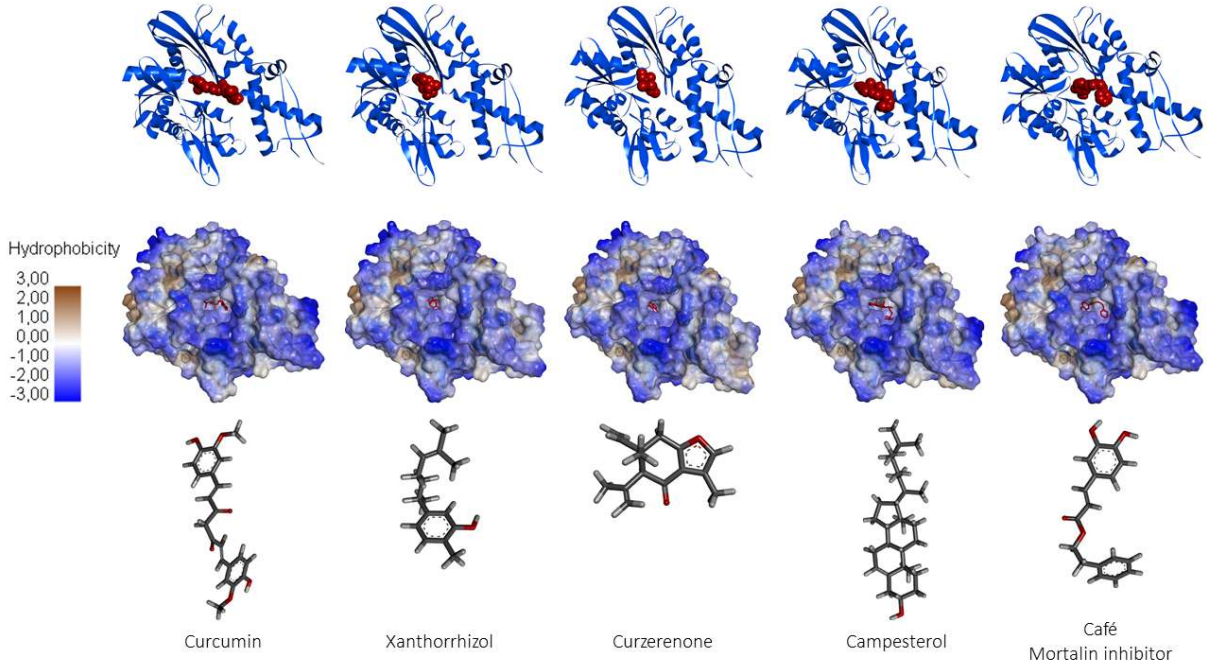

Figure 1. Interaction of Mortalin with active compounds from *C. xanthorrhiza*, *C. zedoaria* and Mortalin inhibitor. (A-B) Curcumin and Xanthorrhizol from *C. xanthorrhiza*, (C-D) Curzerenone and Campesterol from *C. zedoaria* and (E) Café. At the top, Mortalin presented in blue ribbon structure with ligands presents as red spheres. In the middle, Hidrophobicity surface map of the Mortalin active site with the ligands presented as red cylinders. At the bottom, the ligands presented with cylinder.

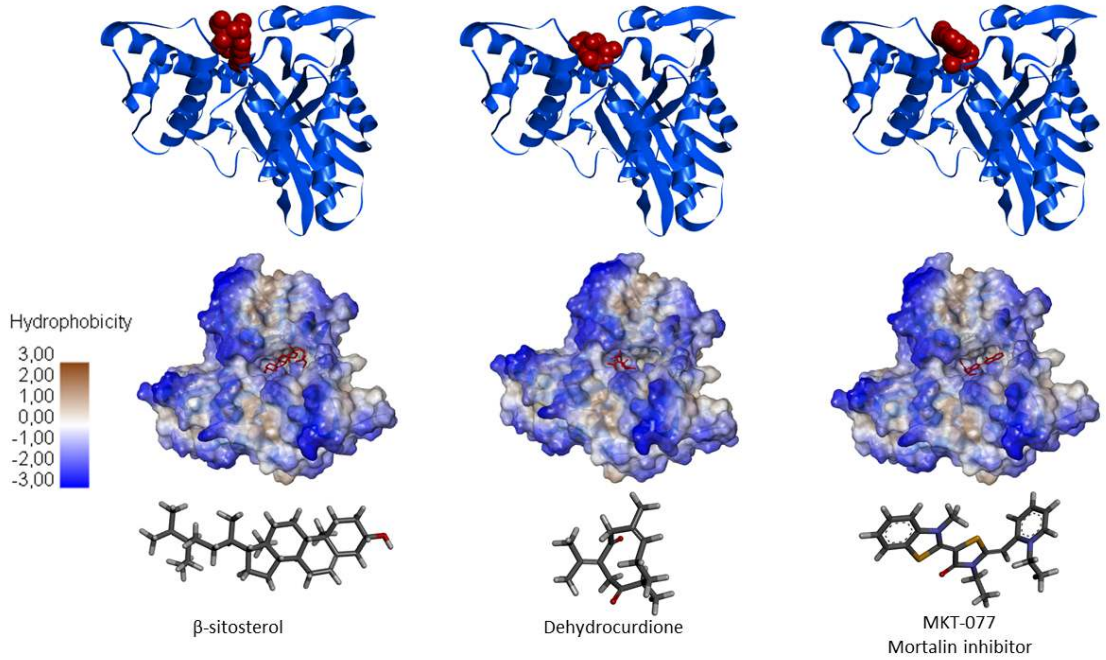

Figure 2. Interaction of Mortalin with active compounds from *C. zedoaria* and Mortalin inhibitor. (A) β-sitosterol, (B) Dehydrocurdione and (C) MKT-077. At the top, Mortalin presented in blue ribbon structure with ligands presents as red spheres. In the middle, Hydrophobicity surface map of the Mortalin active site with the ligands presented as red cylinders. At the bottom, the ligands presented with cylinder.

Amino acid residues formed in interactions between Mortalin and all molecules are used to determine the ability of a ligand binding to its receptors and the binding position to the same as a known inhibitor. All active compounds have the same binding position with known mortalin inhibitors. This is indicated by the presence of similar amino acid residues that bind through hydrogen and hydrophobic bonds. There are 4 amino acid residues that interact in the Mortalin-Curcumin complex. Five similar amino acid residues interact in the Mortalin-Xanthorrhizol complex. The active compounds of C. zedoaria such as Curzerenone and Campesterol have a similar binding position. This is demonstrated by the similarity of hydrophobic-bound amino acid residues, but the amino acid residues that are hydrogen-bound different (Asan64 and Asp59, respectively).

β-sitosterol is predicted most potential as an anticancer by inhibiting Mortalin. This is indicated by the number of amino acid residues formed. In addition to the Mortalin-β-sitosterol complex, there are amino acid residues that bind to hydrogen bonds that are not present in the inhibitors that have been used (MKT-077). There are 8 amino acid residues that interact in the Mortalin-Dehydrocurdine complex and bind hydrophobically. The results of the analysis showed that the hydrogen bonds formed in the Mortalin-active compound of the two Curcuma active compounds had a smaller average distance than the control compounds (Table 2). The same amino acid residues between the two active compounds of Curcuma compared to controls in Mortalin interactions indicated that the compounds of Curcuma most

potential as an anticancer. Hydrogen bonds formed in ligands-protein complexes can increase the affinity of ligand bonds in proteins [12, 13]. The formed hydrophobic interactions play an important role to stabilize ligand-protein bonds and help increase the affinity of ligand binding to proteins [14].

Table 2. Amino acid residues formed from ligands-protein interactions

Ligand Name	Interaction
Curcuma xanthorrhiza	
Curcumin	Hydrogen bond (length) : Glu313 (2.76 Å), Met389 (2.97 Å), **Gly388 (3.05 Å)**, Glu222 (3.07 Å), Hydrophobic bond : **Lys316**, **Gly247**, Asn64, Gly246, Thr62, **Gly61**, Lys121, Asn244, Val417, Val386, Asp59, Cys317
Xanthorrhizol	Hydrogen bond (length) : **Thr63 (2.81 Å)**, **Asn64 (2.80 Å, 3.13 Å)** Hydrophobic bond : Thr249, Gly248, Thr62, Gly246, **Asp414**, Gly388, Val386, **Gly61**, Asp59, Gly387, Val417, Asp244, **Gly247**
Curcuma zedoaria	
Curzerenone	Hydrogen bond (length) : **Asn64 (3.11 Å)** Hydrophobic bond : **Gly247**, Gly248, Thr63, Thr62, **Asp414**, Asp59, Pro413, Val386, Gly387, Val417, Asp244, Thr249
Campesterol	Hydrogen bond (length) : **Asp59 (2.89 Å)** Hydrophobic bond : Gly246, **Gly247**, Asn64, **Asp414**, Lys106, Asp279, Glu313, Lys316, Gly388, Thr86, Gly387
β-sitosterol	Hydrogen bond (length) : Asp277 (2.33 Å) Hydrophobic bond : **Gln280**, **Asn139**, Pro141, Leu305, Thr299, **Asn302**, **Val110**, **Pro113**, **Arg309**, **Glu276**, **Tyr118**
Dehydrocurdione	Hydrogen bond (length) : - Hydrophobic bond : Val140, **Asn139**, **Glu276**, **Gln280**, **Arg309**, Tyr118, **Asp277**, **Arg122**, **Arg284**
Mortalin Inhibitor	
CAFÉ	Hydrogen bond (length) : **Gly388 (3.33 Å)**, **Thr63 (3.06 Å)**, **Asn64 (2.96 Å)**, **Asp59 (3.13 Å)** Hydrophobic bond : Glu276, Gly275, **Lys316**, Lys106, **Gly247**, **Gly61**, **Asp414**, Met389
MKT-077	Hydrogen bond (length) : - Hydrophobic bond : **Tyr118**, **Asn139**, **Glu276**, Asp277, **Arg309**, **Val110**, **Asn302**, **Pro113**, **Gln280**, **Arg284**, **Arg122**

Our results show that the active compounds in both Curcuma highly potential as an anticancer by inhibiting Mortalin, but there are no studies. Mortalin is one of the markers that plays a role in many cellular processes and has an important role in promoting carcinogenesis, is also one of the targets of therapy for cancer sufferers. The results of previous studies showed that overexpression of Mortalin was one of the causes of the poor development of cancer in sufferers. Mortalin overexpression wasknown increase cell proliferation and resistance in human medullary thyroid cancer (MTC). Mortalin inhibition in these studies is able to induce various effects in the cell arrest and increase level TP 53 [15, 16]. Other studies showed that Mortalin overexpression in breast cancer is a very important

marker to determine the level of malignancy and poor prognosis in patients. Inhibition of binding of the Mortalin-p53 complex in breast cancer cells indicates the translocation and activation of p53 transcription which suppresses the growth of cancer cells, these results also indicate inactivation of the metastatic signal [17, 18]. Mortalin upregulation is also known contribute to cancer cell stemness, demonstrated by increased expression of cancer cell markers such as ABCG2, OCT-4, CD133, ALDH1, CD9 and MRP1. Thus inhibition of Mortalin became a very effective candidate for cancer chemotherapy [19].

3.2. Protein interaction networks

Protein network analysis by STTRING shows that there are 10 protein interacts with Mortalin, but only 2 proteins that directly interact with Mortalin. Two of the 10 proteins including HSPD1 and VDAC1, that interact have direct involvement in the carcinogenesis process (Fig. 3). It is known that Mortalin depletion induces transient MEK / ERK activation and altered bioenergetics mitochondria in cancer cells. This is demonstrated by mitochondrial membrane depolarization, decreased oxygen consumption and extracellular acidification [16]. These results are supported by evidence that ERK phosphorylation in cells underlying hypoxia will activate gene transcription that encodes various anti-apoptotic factors or proteins involved in metabolic reprogramming to protect cells from apoptosis when mediated by mitochondria. In cells underlying apoptosis, Mortalin formed the Mortalin / HKII (Hexokinase II) / HIF-1α (Hypoxia inducible factor-1) complex that binds to the mitochondrial membrane protein (VDAC1; Voltage-dependent anion channel) to protect cells from hypoxia and prevent apoptosis [20].

Other studies showed that HKII can bind ATP and glucose into cells to produce glucose-6-phosphate. These products are precursors of biosynthesis to support cell proliferation. Glucose-6-phosphate is also a precursor for the formation of lactic acid which will exit cancer cells and cause an unfavorable environment for normal cells [21]. Besides VDAC1, HSPD1 is also a protein that interacts directly with Mortalin. It is known that HSPD1 (Heat shock protein family D (Hsp60) member 1) is one of the proteins responsible for maintaining mitochondrial function. The results of previous studies showed that knockdown of HSP60 and OXPHOS (Oxidative phosphorylation) by metformin decreased Erk1 / 2 phosphorylation and induced apoptosis and cell cycle arrest [22]. It is known that inhibition of Mortalin can suppress the MEK / ERK signaling pathway is one of the potential therapeutic targets for cancer treatment.

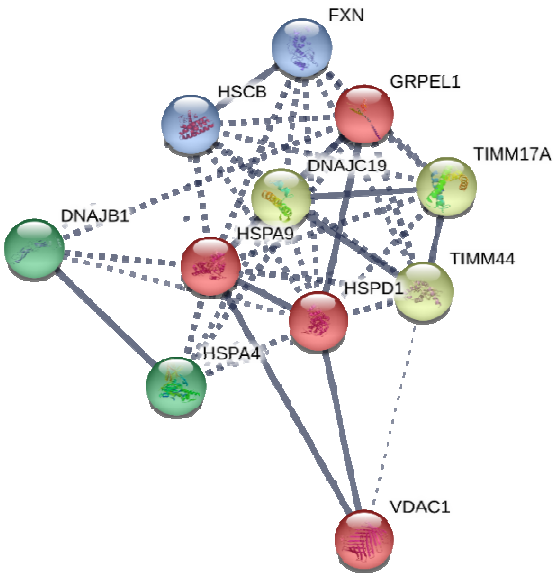

Figure 3. Mortalin protein interaction network. HSPD1 and VDAC1 direct interaction with Mortalin.

4. Conclusion

The active compounds in C. xanthorrhiza and C. zedoaria highly potential as anticancer agents by inhibiting Mortalin. Mortalin inhibition affects the level of cancer malignancy of patients through the MEK / ERK signaling pathway. However, further research in vitro or in vivo must be carried out to validate the potential of the active compounds.

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References

[1] A. Bunevicius, "The Association of Digit Ratio (2D : 4D) with Cancer: A Systematic Review and Meta-Analysis," *Dis. Markers*, vol. 2018, pp. 1–9, 2018.

[2] M. Wahidin, R. Noviani, S. Hermawan, V. Andriani, A. Ardian, and H. Djarir, "Population-based cancer registration in Indonesia," *Asian Pac. J. Cancer Prev. APJCP*, vol. 13, no. 4, pp. 1709–1710, 2012.

[3] O. A. Ahmed Hamdi *et al.*, "Cytotoxic constituents from the rhizomes of Curcuma zedoaria," *ScientificWorldJournal*, vol. 2014, p. 321943, 2014.

[4] X. Tan, M. Xu, F. Liu, M. Xu, Y. Yao, and D. Tang, "Antimetastasis Effect of Astragalus membranaceus-Curcuma zedoaria via β-Catenin Mediated CXCR4 and EMT Signaling Pathway in HCT116," *Evid.-Based Complement. Altern. Med. ECAM*, vol. 2019, p. 9692350, 2019.

[5] S. F. Oon *et al.*, "Xanthorrhizol: a review of its pharmacological activities and anticancer properties," *Cancer Cell Int.*, vol. 15, Oct. 2015.

[6] C.-O. Yun *et al.*, "Relevance of mortalin to cancer cell stemness and cancer therapy," *Sci. Rep.*, vol. 7, p. 42016, 06 2017.

[7] M.-A. Choi, S. H. Kim, W.-Y. Chung, J.-K. Hwang, and K.-K. Park, "Xanthorrhizol, a natural sesquiterpenoid from Curcuma xanthorrhiza, has an anti-metastatic potential in experimental mouse lung metastasis model," *Biochem. Biophys. Res. Commun.*, vol. 326, no. 1, pp. 210–217, Jan. 2005.

[8] O. Trott and A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading," *J. Comput. Chem.*, vol. 31, no. 2, pp. 455–461, Jan. 2010.

[9] "Discovery Studio Predictive Science Application | Dassault Systèmes BIOVIA." [Online]. Available: https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/. [Accessed: 30-Aug-2019].

[10] R. A. Laskowski and M. B. Swindells, "LigPlot+: multiple ligand-protein interaction diagrams for drug discovery," *J. Chem. Inf. Model.*, vol. 51, no. 10, pp. 2778–2786, Oct. 2011.

[11] D. Szklarczyk *et al.*, "STRING v10: protein-protein interaction networks, integrated over the tree of life," *Nucleic Acids Res.*, vol. 43, no. Database issue, pp. D447-452, Jan. 2015.

[12] Y. Itoh *et al.*, "N+-C-H···O Hydrogen bonds in protein-ligand complexes," *Sci. Rep.*, vol. 9, Jan. 2019.

[13] D. Chen, N. Oezguen, P. Urvil, C. Ferguson, S. M. Dann, and T. C. Savidge, "Regulation of protein-ligand binding affinity by hydrogen bond pairing," *Sci. Adv.*, vol. 2, no. 3, Mar. 2016.

[14] R. Patil, S. Das, A. Stanley, L. Yadav, A. Sudhakar, and A. K. Varma, "Optimized Hydrophobic Interactions and Hydrogen Bonding at the Target-Ligand Interface Leads the Pathways of Drug-Designing," *PLoS ONE*, vol. 5, no. 8, Aug. 2010.

[15] D. Starenki, N. Sosonkina, S.-K. Hong, R. V. Lloyd, and J.-I. Park, "Mortalin (GRP75/HSPA9) Promotes Survival and Proliferation of Thyroid Carcinoma Cells," *Int. J. Mol. Sci.*, vol. 20, no. 9, Apr. 2019.

[16] D. Starenki, S.-K. Hong, R. V. Lloyd, and J.-I. Park, "Mortalin (GRP75/HSPA9) upregulation promotes survival and proliferation of medullary thyroid carcinoma cells," *Oncogene*, vol. 34, no. 35, pp. 4624–4634, Aug. 2015.
[17] N. Nigam *et al.*, "Targeting Mortalin by Embelin Causes Activation of Tumor Suppressor p53 and Deactivation of Metastatic Signaling in Human Breast Cancer Cells," *PloS One*, vol. 10, no. 9, p. e0138192, 2015.
[18] H. Jin *et al.*, "The clinicopathological significance of Mortalin overexpression in invasive ductal carcinoma of breast," *J. Exp. Clin. Cancer Res. CR*, vol. 35, Mar. 2016.
[19] C.-O. Yun *et al.*, "Relevance of mortalin to cancer cell stemness and cancer therapy," *Sci. Rep.*, vol. 7, p. 42016, 06 2017.
[20] I. Mylonis, M. Kourti, M. Samiotaki, G. Panayotou, and G. Simos, "Mortalin-mediated and ERK-controlled targeting of HIF-1α to mitochondria confers resistance to apoptosis under hypoxia," *J. Cell Sci.*, vol. 130, no. 2, pp. 466–479, 15 2017.
[21] S. P. Mathupala, Y. H. Ko, and P. L. Pedersen, "Hexokinase II: Cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria," *Oncogene*, vol. 25, no. 34, pp. 4777–4786, Aug. 2006.
[22] C. Zhou *et al.*, "Oncogenic HSP60 regulates mitochondrial oxidative phosphorylation to support Erk1/2 activation during pancreatic cancer cell growth," *Cell Death Dis.*, vol. 9, no. 2, pp. 1–14, Feb. 2018.