

Potential Inhibitors of The OTUB1 Catalytic Site to Develop an Anti-Cancer Drug Using *In-Silico* Approaches

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Abstract

Background: Cancer continues worldwide. It has been reported that OTUB1, a cysteine protease, plays a critical role in a variety of tumors and is strongly related to tumor proliferation, migration, and clinical prognosis by its functions on deubiquitination. Drug advances continue against new therapeutic targets. In this study we used OTUB1 to develop a specific pharmacological treatment to regulate deubiquitination by OTUB1. The aim of this research is to regulate OTUB1 functions.

Methods: By molecular docking in a specific potential OTUB1 interaction site between Asp88, Cys91, and His26 amino acids, using a chemical library of over 500,000 compounds, we selected potential inhibitors of the OTUB1 catalytic site.

Results: Ten compounds (OT1 - OT10) were selected by molecular docking to develop a new anti-cancer drug to decrease OTUB1 functions in cancer processes.

Conclusions: OT1 – OT10 compounds could be interacting in the potential site between Asp88, Cys91, and His265 amino acids in OTUB1. This site is necessary for the deubiquitinating function of OTUB1. Therefore, this study shows another way to attack cancer.

Keywords: Anti-cancer, Dub inhibitor, Molecular docking, OTUB1.

Introduction

Otubain 1 (OTUB1) is a deubiquitinating enzyme that belongs to the OTU family of cysteine proteases (1, 2). OTUB1 has multiples functions; it can decrease the ubiquitination state of a protein by removing ubiquitin (canonical activity) or inhibiting ubiquitin conjugation (non-canonical activity) (3, 4). For example, OTUB1 regulates the UBC13 and the E2 enzymes (2, 5, 6), regulating diverse processes including immune responses and factors that regulate interferon and NF- κ B (7, 8). OTUB1 regulates many cancer-associated signaling pathways including MAPK, ERa, EMT, RHOa, mTORC1, FOXM1, and P53 to promote tumor cell survival, proliferation, invasiveness, and therapeutic resistance (9-13). In addition,

clinical studies have associated elevated OTUB1 expression with high-grade nodal invasiveness and metastasis in some tumor types, including lung, breast, prostate, ovary, glioma, colon, and gastric (3, 14, 15). Therefore, OTUB1 has been studied to understand its role in cancer development. In vitro and in vivo invasion and migration assays revealed that OTUB1 overexpression promoted tumor development while decreasing the functions or silencing of OTUB1 inhibited tumor development and invasion (15, 16).

Protein ubiquitination is a dynamic and reversible process that can be reversed by deubiquitinating enzymes (DUBs) (1, 3), DUBs are considered therapeutic targets due to the

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cysteines in their active sites; this favors work that can determine drugs/compounds that inhibit the activity of these DUBs such as OTUB1. Asp88, Cys91, and His265 have been identified in the OTUB1 catalytic site (1, 3, 4), and mutation studies have shown that Ser16 and Asn22 are important for its functions (3, 17).

Immunotherapies and drugs have been designed to treat cancer, but their mechanisms of action may cause some cancer cells to generate resistance or mutations that allow them to escape the pharmacological effect. Selective protein degradation has emerged as an alternative therapy that requires only a small molecule to interact with a protein, thus causing the desired loss of the protein's function (18). Therefore, inhibition of deubiquitinating proteins such as OTUB1 has a high potential for developing an anti-cancer drug, in which small molecules and drugs have been tested to regulate their functions (3, 11, 12, 14, 19, 20).

In this study, OTUB1 was used as a therapeutic target to develop an anti-cancer drug, in which molecular docking was performed using a library of 500,679 compounds. In this way, OTUB1 was evaluated as a therapeutic target. In addition, compounds with inhibitory potential are proposed.

Materials and Methods

Preparation of receptor protein and selection of the binding site

Atomic coordinates were obtained from the Protein Data Bank (PDB) (21). OTUB1 (PDB code 3VON) was used for molecular docking using Molecular Operating Environment (MOE) software following procedures previously reported (22-24). The binding site was selected in the catalytic site between amino acids D88, C91, and H265 (3).

Compound library used for molecular docking

The EXPRESS-pick Collection Stock screening library (Chembridge Corp.) (25) was used for molecular docking. This collection of molecules contains 502,350 compounds to evaluate the interaction with OTUB1.

Molecular docking

For molecular docking, up to 100 conformers were generated from each compound to interact in the potential OTUB1 site following procedures previously reported (22, 24). The high-throughput molecular docking was performed using MOE software, and ligand interaction analysis per amino acid with MOE and Protein-Ligand Interaction Profiler (25-28).

Selection of the ten best compounds

To select the ten best compounds, the results of up to 28 conformers of each compound were used. Using MOE (29, 30), the binding free energy (ΔG binding) of each ligand-protein complex was determined as previously reported (22, 24). With these results, the best ΔG binding averages and standard deviations were determined between OTUB1 and each compound using Excel software (Microsoft-365). In addition, chemical properties (31), BOILED-Egg (32), and theoretical toxicity (carcinogenicity and mutagenicity) were described (32-34).

Results

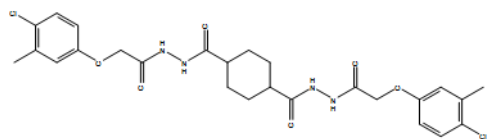
Selection of compounds by Molecular Docking

By molecular docking (22, 24), the selection criteria for the ten best compounds were based on the calculation of the ΔG binding average for each compound using the values of their conformers (16 to 28 conformers) and determining an average range from -5.49 to -5.31 kcal/mol for the ten best compounds (Fig. 1). This study used the EXPRESS-pick Collection Stock library from Chembridge Corp. (25) with 502,350 compounds and up to 100 conformers generated from each compound to interact in the region between amino acids Lys84, Asp88, Cys91, and His264 (Fig. 2). Ten compounds were selected, depicted here as OT1 - OT10, and the analysis of the interaction of each with OTUB1 was determined from the interaction report. All calculated ΔG binding averages were related with the number of interactions generated by the conformers analyzed from the molecular docking results, mainly via hydrogen bonding, in which the ten compounds mainly interacted

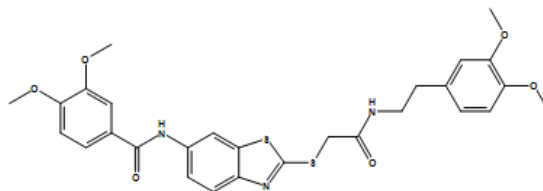
with Asn45, Arg86, Pro87, Asp88, Gln165, Cys212, Arg262, Pro263, Gly264, and His265 (Table 1). In addition, the chemical properties,

BOILED-Egg, theoretical toxicity, and ADME characteristics of each compound are presented.

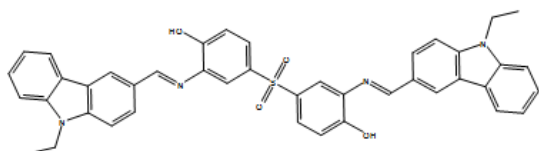
OT1.- 2983702



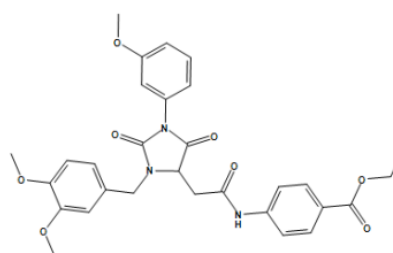
OT2.- 2941180



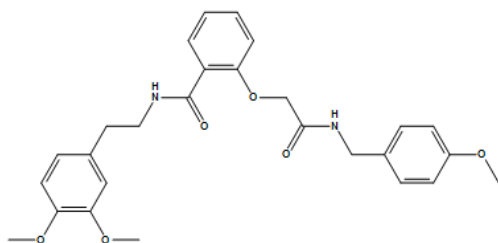
OT3.- 2833016



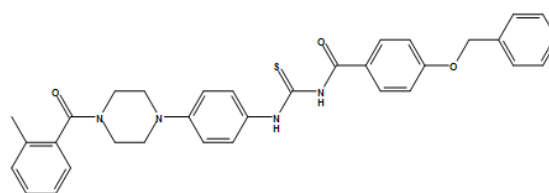
OT4.- 2983215



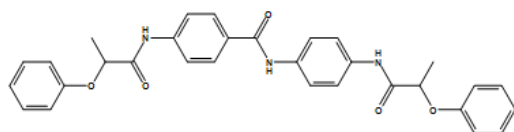
OT5.- 2923590



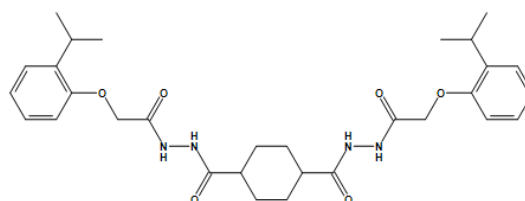
OT6.- 2948528



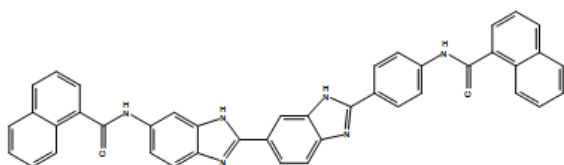
OT7.- 2857071



OT8.- 2983549



OT9.- 2832141



OT10.- 2946215

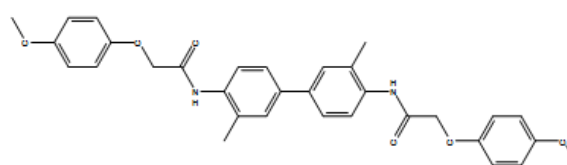


Fig. 1. PubChem CID and Structure of compounds OT1 – OT10.

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Table 1. PubChem CID - ID Chembridge, Canonical SMILES, Interaction with amino acids in OTUB1, Number of conformers analyzed, Average of $\Delta G_{\text{binding}}$ (kcal/mol) with standard deviation (SD) and Ames test and strain used (positive or negative) (33) and LD₅₀ (34).

PubChem CID, ID Chembridge	Canonical SMILES	Interaction with amino acids in OTUB1	Number of conformers	Average of $\Delta G_{\text{binding}} \pm \text{SD}$	PreADMETAmes's test and LD ₅₀
					TA100_10RL
					TA100_NA
					TA1535_10R
					TA1535_NA
					Predicted LD50 (mg/kg)
OT1.- 2983702, 7988434	CC1=C(C=CC(=C1)OCC(=O)NNC(=O)C2CCC(CC2)C(=O)NNC(=O)COC3=CC(=C(C=C3)Cl)C)Cl	His265 Arg86 Pro87 Cys212 Pro263	16	5.49 ± 0.43	Mutagen Negative Negative Negative Negative 2500
OT2.- 2941180, 7501037	COC1=C(C=C(C=C1)CCNC(=O)CSC2=NC3=C(S2)C=C(C=C3)NC(=O)C4=CC(=C(C=C4)OC)OC)OC	Lys84 Pro87 Asp88 Pro263 His265	28	5.47 ± 0.37	Mutagen Negative Negative Negative Negative 2500
OT3.- 2833016, 5194547	CCN1C2=C(C=C(C=C2)C=NC3=C(C=CC(=C3)S(=O)(=O)C4=CC(=C(C=C4)O)N=C5=CC6=C(C=C5)N(C7=CC=CC=C76)CC)O)C8=CC=C(C=C81	Asn45, Glu60, Lys84, Arg86 Glu214 Pro263 His265	22	5.42 ± 0.36	Non-mutagen Negative Negative Negative 2000
OT4.- 2983215, 7986805	CCOC(=O)C1=CC=C(C=C1)NC(=O)CC2C(=O)N(C(=O)N2CC3=CC(=C(C=C3)OC)OC)C4=CC(=CC=C4)OC	Asn45 Lys84 Pro87 Gly264 His265	27	5.39 ± 0.49	Mutagen Negative Negative Negative Negative 1000

OT5.- 2923590, 7013154	<chem>COC1=CC=C(C=C1)CNC(=O)COC2=CC=CC=C2C(=O)NCCCC3=CC(=C(C=C3)OC)OC</chem>	Asn45, Lys84	21	5.39 ± 0.40	Mutagen
		Arg262			Negative
		Pro263			Negative
		Gly264			Negative
		His265			Negative
1420					
OT6.- 2948528, 7699311	<chem>CC1=CC=CC=C1C(=O)N2C CN(CC2)C3=CC=C(C=C3)N C(=S)NC(=O)C4=CC=C(C=C4)OCC5=CC=CC=C5</chem>	Asn45	21	5.34 ± 0.43	Mutagen
		Arg86			Negative
		Pro87			Positive
		Asp88			Negative
168					
OT7.- 2857071, 5634266	<chem>CC(C(=O)NC1=CC=C(C=C1)C(=O)NC2=CC=C(C=C2)NC(=O)C(C)OC3=CC=CC=C3)OC4=CC=CC=C4</chem>	Asn45	19	34 ± 0.32	Mutagen
		Gln165			Negative
		Arg262			Negative
		Pro263			Negative
1600					
OT8.- 2983549, 7987933	<chem>CC(C)C1=CC=CC=C1OCC(=O)NNC(=O)C2CCC(CC2)C(=O)NNC(=O)COC3=CC=CC=C3C(C)C</chem>	Glu60, Pro87	21	5.33 ± 0.29	Non-mutagen
		Cys212			Negative
		His265			Negative
2500					
OT9.- 2832141, 5181121	<chem>C1=CC=C2C(=C1)C=CC=C2C(=O)NC3=CC=C(C=C3)C4=NC5=C(N4)C=C(C=C5)C6=NC7=C(N6)C=C(C=C7)NC(=O)C8=CC=CC9=CC=CC=C9</chem>	Glu60, Arg86	19	5.32 ± 0.38	Non-mutagen
		Pro87, Asp88			Negative
		Gln165			Negative
		Cys212			Negative
		His265			Negative
200					
OT10.- 2946215, 7661200	<chem>CC1=C(C=CC(=C1)C2=CC(=C(C=C2)NC(=O)COC3=C(C=C(C=C3)OC)C)NC(=O)COC4=CC=C(C=C4)OC</chem>	Asn45, Arg86	24	5.31 ± 0.34	Non-mutagen
		Pro87			Negative
		Cys212			Negative
		Gly264			Negative
		His265			Negative
1600					

Interaction of compounds OT1 – OT10 with OTUB1

To describe the probable interaction of compounds OT1 – OT10 with OTUB1, up to 28 conformers of each compound interacting in the potential site were analyzed. This was the region between Lys84, Asp88, Cys91, and His265 (Fig. 2). From molecular docking results (not shown here), it was determined the main amino acids in OTUB1 interacting with

OT1 – OT10 were Asn45, Lys84, Arg86, Pro87, Asp88, Gln165, Cys212, Arg262, Pro263, Gly264, and His265 (Table 1). These ten compounds had the strongest interactions in the potential site (Fig. 3), particularly with Arg87, Pro263 and His265, mainly via hydrogen bonding. With these results, the catalytic site could be blocked, which is essential for the deubiquitination of OTUB1.

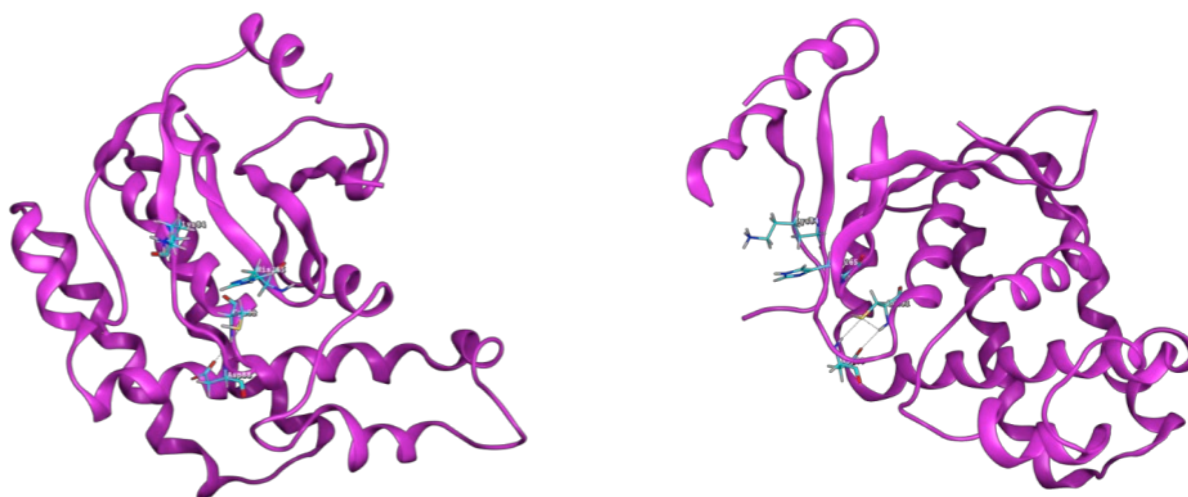


Fig. 2. OTUB1 ribbon diagrams. The main amino acids in the potential binding site are Lys84, Asp88, Cys91, and His265 (Cyan).

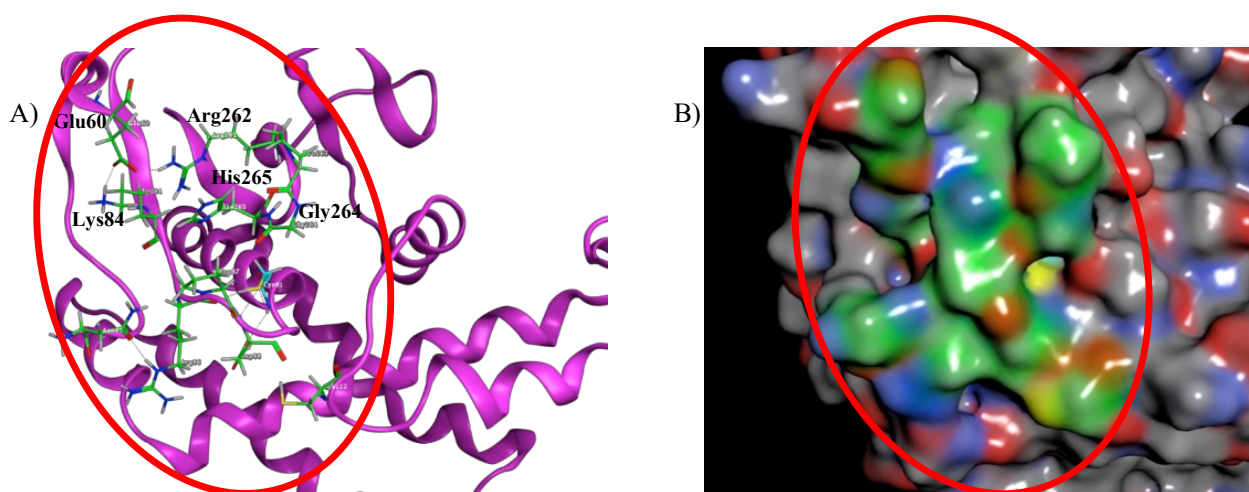


Fig. 3. A) OTUB1 ribbon diagram. The main amino acids in the binding site that could interact with OT1 - OT10 compounds are Asn45, Glu60, Lys84, Pro87, Asp88, Cys91, Cys212, Arg262, Gly264, and His265 (Green), B) The pocket is displayed in the binding site.

Discussion

Cancer continues worldwide and a growing number of studies have identified OTUB1 as a regulator of important pathways in cancer development and progression. It has been reported that OTUB1 plays a critical role in a variety of tumors and is strongly related to tumor proliferation, migration (3, 4), and clinical prognosis via its deubiquitination function, and drug development advances continue against new therapeutic targets. In this study we used OTUB1 to develop a specific pharmacological treatment to regulate deubiquitination by this enzyme.

OTUB1 has characteristics that might help to develop a specific OTU/DUB inhibitor. Studies propose that amino acids in the catalytic site generate interactions and selectivity between potential compounds and OTUB1 (3, 12). These compounds can inhibit or alter OTUB1 functions in the cancer cell. Herbal acevaltrate (35) and nanchangmycin (36) are two inhibitors that bind the OTUB1 catalytic site (12). Studies with these inhibitors have shown promising results demonstrating that OTUB1 might be a good therapeutic target.

Therefore, we propose ten compounds selected to bind OTUB1 via molecular docking at the catalytic site (Fig. 3). The docking results indicate the main OTUB1 amino acids

interacting with the ten compounds were Asn45, Lys84, Arg86, Pro87, Asp88, Gln165, Cys212, Arg262, Pro263, Gly264, and His265, which are in the catalytic site region (Table 1 and Fig. 3). Lys71, with Asp88 and Cys91, is necessary in the OTUB1/c-Maf axis (12). Blocking the catalytic site with the compounds could generate a dysfunctional OTUB1, inhibiting its deubiquitinating functions. Crystal structures (PDB: 4DDI and 4I6L) (37, 38) demonstrate the interaction of the OTUB1-ubiquitin complex (Fig. 4), as well as the amino acids involved in the interaction. We propose that the compounds have the greatest interaction with Arg87, Pro263, and His265, mainly via hydrogen bonding in the conformers analyzed. We propose these ten compounds inhibit accessibility to the OTUB1 catalytic site and affect its functions, including deubiquitination. Our proposal could most likely reduce this canonical function, and we will evaluate the impact it could have on the non-canonical function (3, 4), due to the compounds' interactions with Asn, Lys, Arg, Pro, Asp, Gln, Cys, Arg, Pro, Gly, and His in OTUB1. It is important to consider other consequences that some compounds could facilitate by interacting with certain amino acids, particularly Arg, Asp, and Lys, since these amino acids could influence protein conformational stability (39).

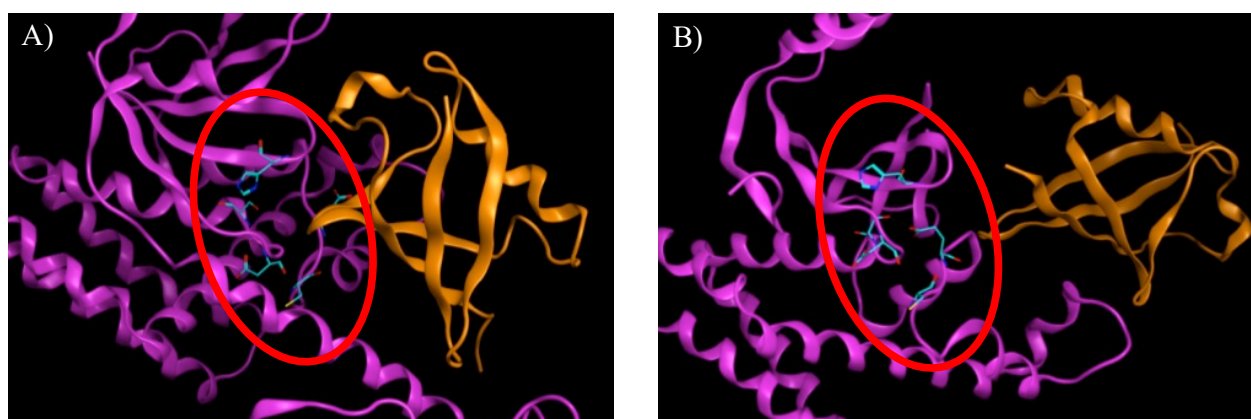


Fig. 4. OTUB1-ubiquitin complex ribbon diagrams. The red circle shows the OTUB1 catalytic site pocket. The main amino acids in the potential binding site are Asp88, Cys212, Glu214 and His265 (Cyan), interacting with ubiquitin (Orange). A) PDB:4DDI, B) PDB:4I6L.

The ADME theoretical results show the capability of the ten compounds to be delivered as an oral drug in accordance with

bioavailability. In addition, the BOILED-Egg profile, which enables consideration of passive gastrointestinal absorption (HIA) and brain

penetration were screened for the ten compounds selected. All ten were estimated to be well-absorbed but not did not access the brain, possibly due to the blood-brain barrier. Also, toxicity was predicted; the theoretical LD50 was 168 – 2500 mg/kg (toxicity class: 3-5) (34).

Some current anti-cancer drugs use OTUB1 as a therapeutic target (12, 35, 36), but our study identified ten compounds that may be better than those currently used from a library of over 500,000 compounds. As mentioned, OTUB1 has been demonstrated as a therapeutic target in various in vitro and in vivo cancer cell assays (14, 15). Therefore, OTUB1 inhibition may affect cancer cell viability, and this study may contribute to the development of more effective anti-cancer drugs than are presently available.

This study used OTUB1 as therapeutic cancer target because this protein has functions involved in cancer processes, which could be altered with new specific drugs against OTUB1.

We identified ten compounds with high

probability to be selective against OTUB1, to block the OTUB1 catalytic site (Asp88, Cys91, and His265 amino acids), this site is necessary for the deubiquitinating function of OTUB1. Therefore, this study proposes another way to attack cancer. Future research, such as in vitro and in vivo assays, will be necessary to evaluate these compounds' effectiveness against this therapeutic target to develop new anti-cancer drugs.

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Conflict of Interest

The authors declare no conflict of interest.

References

- Balakirev MY, Tcherniuk SO, Jaquinod M, Chroboczek J. Otubains: a new family of cysteine proteases in the ubiquitin pathway. *EMBO Rep.* 2003;4(5):517–22.
- Wiener R, DiBello AT, Lombardi PM, Guzzo CM, Zhang X, Matunis MJ, et al. E2 ubiquitin-conjugating enzymes regulate the deubiquitinating activity of OTUB1. *Nat Struct Mol Biol.* 2013;20(9):1033–9.
- Saldana M, VanderVorst K, Berg AL, Lee H, Carraway KL. Otubain 1: a non-canonical deubiquitinase with an emerging role in cancer. *Endocr Relat Cancer.* 2019;26(1):R1–14.
- Wu Q, Huang Y, Gu L, Chang Z, Li G-M. OTUB1 stabilizes mismatch repair protein MSH2 by blocking ubiquitination. *J Biol Chem.* 2021;296:100466.
- Wiener R, Zhang X, Wang T, Wolberger C. The mechanism of OTUB1-mediated inhibition of ubiquitination. *Nature.* 2012;22;483(7391):618–22.
- Pasupala N, Morrow ME, Que LT, Malynn BA, Ma A, Wolberger C. OTUB1 non-catalytically stabilizes the E2 ubiquitin-conjugating enzyme UBE2E1 by preventing its autoubiquitination. *J Biol Chem.* 2018;293(47):18285–95.
- Jahan AS, Biquand E, Muñoz-Moreno R, Le Quang A, Mok CK-P, Wong HH, et al. OTUB1 Is a Key Regulator of RIG-I-Dependent Immune Signaling and Is Targeted for Proteasomal Degradation by Influenza A NS1. *Cell Rep.* 2020;30(5):1570-1584.e6.
- Mulas F, Wang X, Song S, Nishanth G, Yi W, Brunn A, et al. The deubiquitinase OTUB1 augments NF-κB-dependent immune responses in dendritic cells in infection and inflammation by stabilizing UBC13. *Cell Mol Immunol.* 2021;18(6):1512–27.
- Sun X-X, Dai M-S. Deubiquitinating enzyme regulation of the p53 pathway: A lesson from Otub1. *World J Biol Chem.* 2014;5(2):75–84.
- Baghal-Sadriforoush S, Bagheri M, Abdi Rad I, Sotoodeh Nejadnematalahi F. PI3K Inhibition Sensitize the Cisplatin-resistant Human Ovarian Cancer Cell OVCAR3 by Induction of Oxidative Stress. *Rep Biochem Mol Biol.* 2022;10(4):675–85.
- Sun X-X, Challagundla KB, Dai M-S. Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1. *EMBO J.* 2012;31(3):576–92.

12. Xu Y, Xu M, Tong J, Tang X, Chen J, Chen X, et al. Targeting the Otub1/c-Maf axis for the treatment of multiple myeloma. *Blood*. 2021;137(11):1478–90.
13. Rezai M, Saravani R, Sargazi S, Moudi M, Jafari Shahroudi M, Saravani R. Achillea Wilhelmsii C. Koch Hydroalcoholic Extract Induces Apoptosis and Alters LIN28B and p53 Gene Expression in Hela Cervical Cancer Cells. *Reports Biochem Mol Biol*. 2019;8(3):318–25.
14. Lee B-S, Kang SU, Huang M, Kim YS, Lee Y-S, Park J-Y, et al. OTUB1 knockdown promotes apoptosis in melanoma cells by upregulating TRAIL expression. *BMB Rep*. 2021;54(12):608–13.
15. Liao Y, Wu N, Wang K, Wang M, Wang Y, Gao J, et al. OTUB1 Promotes Progression and Proliferation of Prostate Cancer via Deubiquitinating and Stabilizing Cyclin E1. *Front Cell Dev Biol*. 2021;8.
16. Liu T, Jiang L, Tavana O, Gu W. The Deubiquitylase OTUB1 Mediates Ferroptosis via Stabilization of SLC7A11. *Cancer Res*. 2019;79(8):1913–24.
17. Scholz CC, Rodriguez J, Pickel C, Burr S, Fabrizio J-A, Nolan KA, et al. FIH Regulates Cellular Metabolism through Hydroxylation of the Deubiquitinase OTUB1. *PLoS Biol*. 2016(1):e1002347.
18. Salami J, Crews CM. Waste disposal-An attractive strategy for cancer therapy. *Science*. 2017;355(6330):1163–7.
19. Doherty LM, Mills CE, Boswell SA, Liu X, Hoyt CT, Gyori BM, et al. Integrating multi-omics data reveals function and therapeutic potential of deubiquitinating enzymes. *eLife*. 2022;11:e72879.
20. Sivakumar D, Stein M. Binding of SARS-CoV Covalent Non-Covalent Inhibitors to the SARS-CoV-2 Papain-Like Protease and Ovarian Tumor Domain Deubiquitinases. *Biomolecules*. 2021;11(6):802.
21. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. *Nucleic Acids Res*. 2000;28:235–242.
22. Benítez-Cardoza CG, Vique-Sánchez JL. Potential inhibitors of the interaction between ACE2 and SARS-CoV-2 (RBD), to develop a drug. *Life Sci*. 2020;256:117970.
23. Halgren TA. Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J Comput Chem*. 1996;17(5–6):490–519.
24. Vique-Sánchez JL. Potential inhibitors interacting in Neuropilin-1 to develop an adjuvant drug against COVID-19, by molecular docking. *Bioorg Med Chem*. 202;33:116040.
25. ChemBridge Corp.. EXPRESS-Pick Stock. 2021.
26. Soga S, Shirai H, Kobori M, Hirayama N. Use of Amino Acid Composition to Predict Ligand-Binding Sites. *J Chem Inf Model*. 2007;47(2):400–6.
27. Protein-Ligand Interaction Profiler. <https://plip-tool.biotec.tu-dresden.de/plip-web/plip/index>.
28. Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M. PLIP: fully automated protein–ligand interaction profiler. *Nucleic Acids Res*. 2015;43(W1):W443–7.
29. Labute P. The generalized Born/volume integral implicit solvent model: estimation of the free energy of hydration using London dispersion instead of atomic surface area. *J Comput Chem*. 2008;29(10):1693–8. 9
30. Wadood A, Ghufuran M, Hassan SF, Khan H, Azam SS, Rashid U. In silico identification of promiscuous scaffolds as potential inhibitors of 1-deoxy- d -xylulose 5-phosphate reductoisomerase for treatment of Falciparum malaria. *Pharm Biol*. 2017;55(1):19–32.
31. www.acdlabs.com/. Available from: <https://www.acdlabs.com/products/percepta/index.php>.
32. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep*. 2017;7(1):42717.
33. PreADMET. <https://preadmet.webservice.bmdrc.org/>.
34. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*. 2018;46(W1):W257–63.

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35. Sun T, Xu Y, Xu Z, Cao B, Zhang Z, Wang Q, et al. Inhibition of the Otub1/c-Maf axis by the herbal acevaltrate induces myeloma cell apoptosis. *Cell Commun Signal*. 2021;19(1):24.
36. Xu Y, Sun T, Zeng K, Xu M, Chen J, Xu X, et al. Anti-bacterial and anti-viral nanchangmycin displays anti-myeloma activity by targeting Otub1 and c-Maf. *Cell Death Dis*. 2020;11(9):818.
37. Juang Y-C, Landry M-C, Sanches M, Vittal V, Leung CCY, Ceccarelli DF, et al. OTUB1 Co-opts Lys48-Linked Ubiquitin Recognition to Suppress E2 Enzyme Function. *Mol Cell*. 2012;45(3):384–97.
38. Ernst A, Avvakumov G, Tong J, Fan Y, Zhao Y, Alberts P, et al. A Strategy for Modulation of Enzymes in the Ubiquitin System. *Science*. 2013;339(6119):590–5.
39. Meuzelaar H, Vreede J, Woutersen S. Influence of Glu/Arg, Asp/Arg, and Glu/Lys Salt Bridges on α -Helical Stability and Folding Kinetics. *Biophys J*. 2016;110(11):2328–41.