

Assessing the Chemosensitizing effect of Neferine on Cisplatin Resistant Colorectal Cancer Cells through Molecular Docking Studies

ABSTRACT

Colorectal cancer (CRC) is the second deadliest diseases next to lung cancer. Cisplatin is the first generation platinum based alkylating agent using for treatment of advance CRC patients. But the development of cisplatin resistance due to the continuous usage limits its therapeutic efficacy. Recent research is focused on studying the chemotherapeutic efficacy of the phytochemicals as they are less toxic compared to the conventional chemotherapeutic drugs. Neferine is a bisbenzylisoquinoline alkaloid extracted from the embryo of *Nelumbo nucifera*. The anticancer and chemosensitizing effect of neferine has been well reported in several cancer cells. However, there are no reports on the chemosensitizing effect of neferine on cisplatin resistant colorectal cancer cells (CRCs). Hence, the present study aims at identification of target proteins responsible for cisplatin resistance in colorectal cancer cells. The present investigation elucidates the specific interaction of neferine with various cell surface receptor proteins related to cisplatin resistance, multi-drug resistance (MDR) proteins, signal transduction protein and transcription factors *via* molecular docking approach. The interaction between neferine and the target proteins of cisplatin resistant colorectal cancer was analyzed through Schrodinger Maestro 11.9 module. MTT assay was performed for assessing the effects of neferine on cisplatin resistant HCT-15 cells. The results show that 24 hours of post neferine treatment could inhibit cell proliferation in a dose-dependent manner in both cisplatin sensitive and resistant CRCs. From our docking studies we could suggest that neferine is most active for IGF1R receptor, FGFR2 receptor, SNAIL1 protein, STAT3 protein and TGF β R1 receptor when sorted according to their docking score.

Keywords: Colorectal cancer; Neferine; Molecular docking; Cisplatin; IGF1R; Multi-drug resistance

1. INTRODUCTION

Globally, cancer is the leading cause of death and severely affects the quality of life. As per the estimate of World Health Organization (WHO) in the year 2025 there will be 21.9 million new cancer incidences and 11.4 million cancer deaths in the world [1]. Colorectal cancer (CRC) is the third most common and second lethal disease around the world. The current treatment for CRC mainly

includes radiotherapy, surgical resection and chemotherapy [2]. Even though better treatment options have improved overall survival rates in the early stages, 40-50% of all CRC patients have metastasis at the time of diagnosis or as a recurrent disease after receiving chemotherapy [3].

Chemotherapy is the one of the main components in the treatment of cancer. However, the curative responses of chemotherapeutic agents are limited due to drug resistance and therapeutic side effects like non-targeted organ toxicities [4,5]. Most of the cancer cells which initially respond to the traditional chemotherapeutic drugs, eventually develop resistance over a period of treatment. Cancer cells can develop resistance to the treatment through various molecular mechanisms such as decreased drug uptake, elevated drug efflux, alteration of drug target, detoxification, increased DNA repair, apoptosis inhibition and epithelial to mesenchymal transition [6]. Cisplatin remains the most traditional chemotherapeutic drug for solid tumour treatments, including CRC [7]. It is a platinum-based drug which binds with DNA, forming a DNA-Platinum adduct, leading to inhibition of transcription and translation, inducing mitochondrial-mediated cell death [8]. Unfortunately, continuous treatment of cisplatin leads to cell resistance which is a frequent occurrence in CRC clinical chemotherapy.

Recent studies show that the combination of phytochemicals with traditional chemotherapy improves the curative response in cancer patients [9]. Neferine, an alkaloid derivative from lotus (*Nelumbo nucifera*) seed embryo (Figure - 1), exhibits various pharmacological effects including, cardioprotection [10], anti-cancer [11], reversal of multi-drug resistance (MRD) in various cancer [12,13]. Our preliminary study found that neferine reverses the cisplatin-resistant CRCs by inducing apoptosis, but the molecular mechanism of reversal of cisplatin resistance is still unclear. In this present *in-silico* study, we aim to investigate the effect of neferine against cell surface receptor proteins, MDR proteins, signal transduction protein and transcription factors which are related to cisplatin resistance *via* molecular docking approach based on the cytotoxic cell assay results.

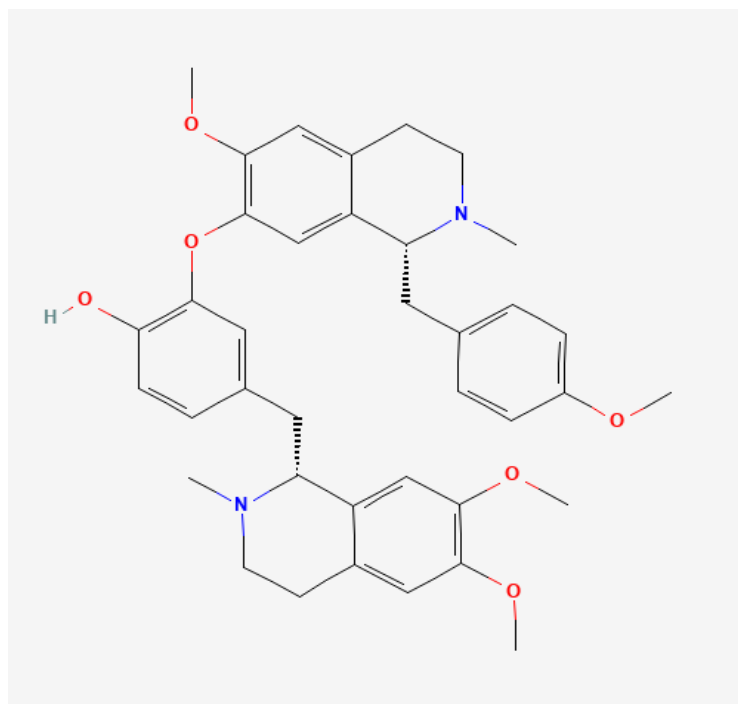


Fig.1. The structure of Neferine

2. METHODOLOGY

2.1 Chemicals and Reagents

Neferine and Cisplatin were purchased from Sigma Aldrich, USA. RPMI 1640 media was purchased from Gibco, USA. Fetal Bovine Serum (FBS), antibiotics and other fine chemicals were purchased from HiMedia Laboratories (Mumbai, India).

2.2 Animal cell culture maintenance

Human colorectal cancer cells (HCT-15) cell line was purchased from National Centre for Cell Science (NCCS), Pune, India. HCT-15 cells and cisplatin resistant HCT-15 were cultured in RPMI-1640 media supplemented with 10% FBS and Penicillin (100Units/ml), Streptomycin (30 μ g/ml) and Gentamycin (20 μ g/ml).

2.3 Cytotoxicity assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was performed for assessing the effect of neferine on cisplatin resistant HCT-15 cells. The 1×10^5 cells/well were seeded in 96 well plates and allowed to attach by incubating overnight at 37°C in CO₂ incubator. After 24 hours of treatment time, media (neferine) was replaced with 20 μ l of (MTT 5mg/ml) dissolved in of phosphate buffer saline (PBS) and incubated for 4 hours in CO₂ incubator. Then, 200 μ l of DMSO was added to dissolve purple formazan crystals formation, and the optical density (OD) was observed at 570nm wavelength in a microplate reader (BioTek, USA). And the results were expressed in terms of percentage viability.

2.4 Preparation of Protein and Receptor Grid

The proteins considered for the study are IGF-1R (PDB id: 2OJ9), FGFR2 (PDB id: 3B2T), SNAIL1 (PDB id:3W5K), STAT3 (PDB id:6QHD), TGFBR1 (PDB id:3KCF), $\alpha 5\beta 6$ (PDB id:4UM9), TGFBR1 (PDB id:3KFD), SMAD (PDB id:1U7F), $\alpha 5\beta 3$ (PDB id:1L5G), TGFBR2 (PDB id:5E8V), GSK3 β (PDB id:1I09), FOXO3 (PDB id: 2K86), Gamma-Secretase (PDB id:4R12), MDR1/ABCB1 (PDB id: 4Q9L) and ABCG2 (PDB id: 5NJG). The protein coordinates were collected from PDB (Protein Data Bank, [www. \(http://www.rcsb.org/pdb/-home/home.do\)](http://www.rcsb.org/pdb/-home/home.do)). Preparation of the proteins prior to docking was done using the protein preparation tab of Maestro 11.9 module of the Schrodinger suite. Corrections such as addition of hydrogen, assignment of bond orders, searching overlaps and water molecules within a range of 5 Å were deleted. RMSD minimization up to 0.03Å and OLPS-2005 (optimized potential for liquid simulation) was used to perform the minimization [14].

The receptor grid was prepared using the grid generation tool of Maestro 11.9 of the Schrödinger suite. The ligand is able to bind forming the achievable conformation using this receptor grid which also highlights the active site of the protein as with the co-crystallized ligand molecule. This co-crystallized ligand will then be expelled from the active site to be occupied by our ligand of interest [14]. Scaling factor of 1.0 Å and van der waals radius of 0.25 Å were used to prepare the grid while other parameters were set to default.

2.5 Ligand Preparation

The structural definition file (sdf) of Neferine was retrieved from PubChem followed by the ligand preparation by the LigPrep wizard Maestro 11.9 (Glide). Corrections such as 2D to 3D conversion, addition of hydrogen, stereochemistry, low energy state, corrections of bond lengths and bond angles, ring conformations along with minimization and optimizations were done using OPLS3 force field [15].

2.6 Molecular Docking

Maestro-GLIDE module of the Schrödinger suite was used to carry out the molecular docking using the optimized ligand, which was docked flexibly within the grid box of each protein considered. The best scoring pose of the ligand were ranked using the G-Score (Glide Score) and H-bond formation along with their corresponding binding affinities. Visualizations were done using the XP (extra precision) module of GLIDE for analyzing each protein-ligand interaction.

3. RESULTS AND DISCUSSION

Colorectal cancer (CRC) is the third most common malignancy in worldwide. Cisplatin is the first generation platinum based chemotherapeutic drug used for CRC treatment. However the development of cisplatin resistance is the common cause of cancer recurrence for CRC patients [16]. Several studies from the past decade revealed that treatment with combinatorial therapy of

chemotherapeutics and phytochemical or individual treatment with phytochemical improve the CRC patient's life expectancy [2].

Nelumbo nucifera is commonly used in traditional Indian and Chinese medicine to treat cardiovascular disease, neuronal disorder and insomnia [11]. Neferine is one of the active biomolecule (bisbenzylisoquinoline alkaloid) from the lotus seed embryo, which has several pharmacological effects such as- antioxidant, anti-inflammatory, anticancer and chemosensitizing ability in cancer cells [17]. A previous study from our lab showed that neferine sensitizes the doxorubicin resistant A549 lung cancer cells by increasing accumulation of inter/intracellular doxorubicin through downregulation of lung resistance protein (LRP) mediated by NRF2 inhibition [13]. Another study demonstrated that neferine downregulates the multidrug resistance (P-glycoprotein) gene expression in the hyperthermal state that synergistically reverses the MDR in human gastric cancer cells [12]. Combinatorial treatment of neferine with oxaliplatin has been shown to increase the chemotherapeutic sensitivity and suppress the epithelial to mesenchymal transition by inhibiting Snail protein expression in hepatocellular carcinoma cells [18].

3.1 Establishment of cisplatin – resistant CRCs

To establish the cisplatin resistant CRCs, 50% cell proliferation inhibition concentration (IC₅₀) of cisplatin was determined by cytotoxic (MTT) assay. HCT – 15 cells were treated with cisplatin (0 – 60µM concentration) for 24 hours and the results showed that it induces cytotoxicity in a dose dependent manner. The IC₅₀ value of cisplatin for HCT-15 was found to be 32 ± 3.4µM conc. (Figure – 2A).

The HCT-15 cells were treated with IC₅₀ dose (32µM) of cisplatin continuously for six months to attain the cisplatin resistant CRC cells and were termed as HCT – 15/R cells. Later, to confirm the resistance of cisplatin and resistance index, the HCT – 15/R cells were subjected to cytotoxic (MTT) assay. The HCT – 15/R cells were treated with different concentration of cisplatin from 0 – 200µM for 24 hours. The IC₅₀ value of cisplatin resistant HCT-15/R cells was found to be 120 ± 5.1µM. The resistance index was 3.75 fold increased compared to cisplatin sensitive HCT-15 cells (Figure – 2B).

Figure – 2:

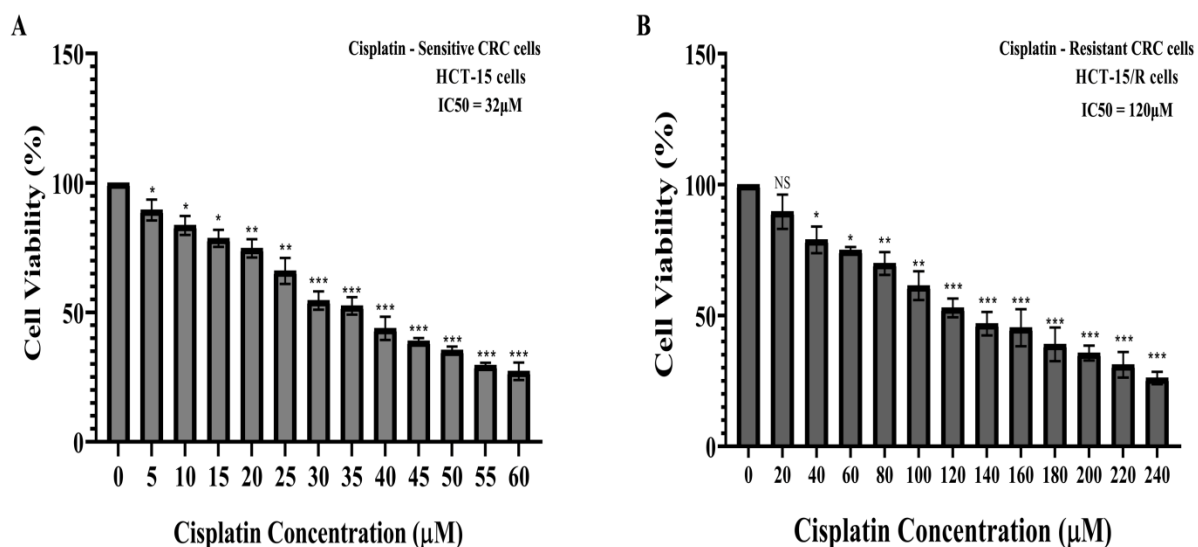


Fig.2. Establishment of cisplatin resistant colorectal cancer cells: (A & B) the cell viability was determined by MTT assay and the results reveal the IC50 value of cisplatin sensitive HCT-15 cells and cisplatin resistant HCT-15/R cells to be 32μM and 120μM respectively. Results shown are mean ± SEM, which are three separate experiments performed in triplicate. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ verse control (One-way ANOVA followed by Tukey's multiple comparison test).

3.2 The cytotoxic effect of neferine on cisplatin-sensitive and resistant CRCs

In this present study, we aimed to investigate the interaction of neferine with transcription factors, signal transductions proteins and surface receptor proteins, which positively correlated with multidrug resistance via molecular docking in cisplatin-resistant CRCs. Initially, the cytotoxic effect of neferine on cisplatin sensitive and resistant CRCs was determined by MTT assay. The results showed that 24 hours of neferine treatment could inhibit cell proliferation in a dose-dependent manner in both cisplatin sensitive and resistant CRCs. Surprisingly, the IC50 dose of neferine in cisplatin-resistant CRCs was relatively low (6μM concentration) compared to IC50 of neferine (8.95μM) on cisplatin sensitive CRCs cells (Figure – 3). The distinct morphological changes like rounding and floating cells, loss of cell membrane integrity were observed after neferine treatment on cisplatin resistant CRCs (Figure – 4).

Figure – 3:

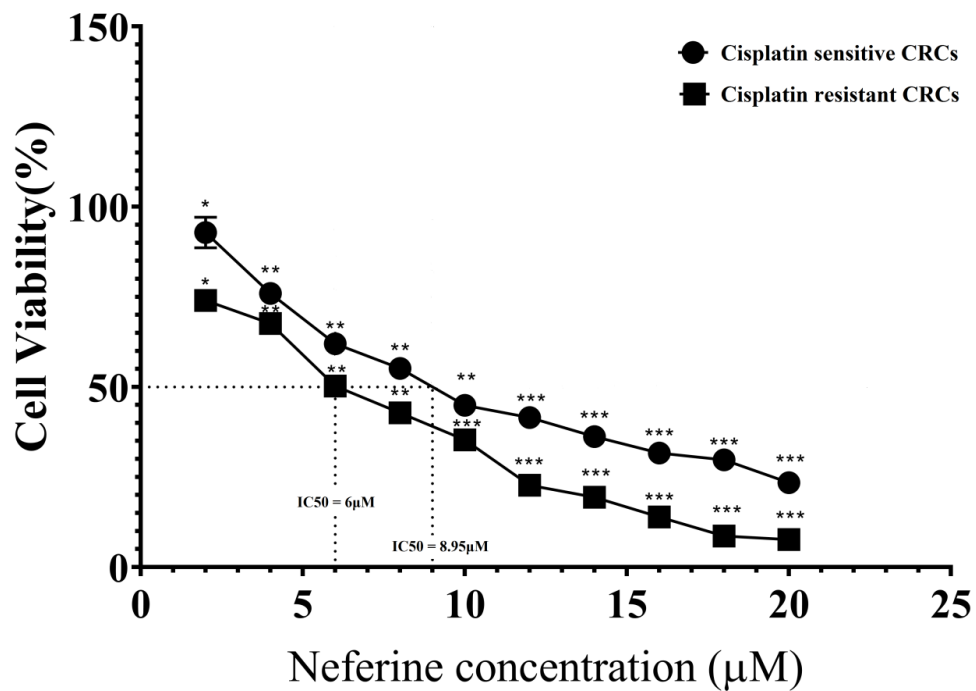


Fig.3. Cytotoxic effect of neferine on cisplatin-sensitive and resistant CRC cells: Cisplatin-sensitive (HCT-15) and resistant (HCT-15/R) CRCs were treated with various concentrations of neferine for 24 hours. IC₅₀ values for neferine in sensitive and resistant cells were determined by MTT assay. Results shown are mean ± SEM, which are three separate experiments performed in triplicate. **p*<0.05, ***p*< 0.01 and ****p*< 0.001 versus control (One-way ANOVA followed by Tukey's multiple comparison test).

Figure – 4:

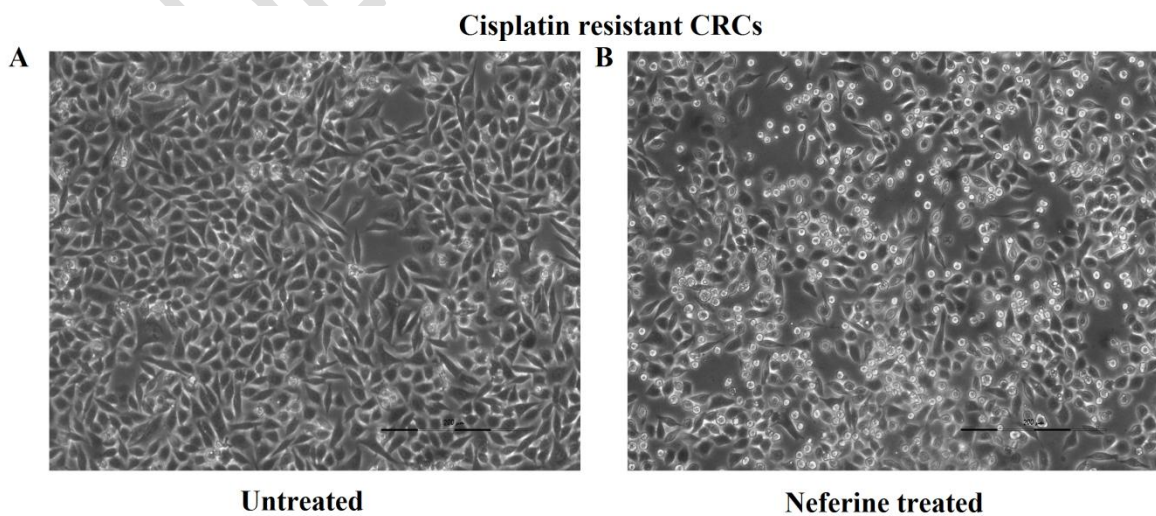


Fig.4. Effect of neferine on cell morphology of cisplatin resistant (HCT-15/R) CRCs. (A) Untreated cells **(B)** Neferine treated cells.

Molecular docking is an in-depth investigation using bioinformatics tools and is based on the theoretical simulation approach [19]. Molecular docking studies reveal the specific interaction of molecules such as proteins with ligands and proteins with proteins, as well as the details of affinity, binding orientation, and biological activity of a drug and its target proteins [20].

Insulin-like Growth Factor type-I Receptor (IGF1R) is a transmembrane receptor, belong to the receptor tyrosine kinase class, which is a crucial factor in the IGF signalling pathway. Overexpression of IGF1R was frequently observed in various cancer cells, including CRCs [21]. The key roles of IGF1R are cell proliferation, differentiation, survival, apoptosis, anchorage-independent growth, angiogenesis and metastasis. The cell survival pathways like PI3K/AKT and MAPK pathways were mediated by activating IGF1R by ligands like IGF1, IGF2 and insulin [22]. A previous study reported that overexpression and nuclear translocation of IGF1R was associated with resistance to conventional chemotherapy in metastatic CRC patients [23]. Targeting IGF1R by small molecules has been considered as a novel therapeutic option to overcome chemoresistance in cancer by inhibiting tyrosine kinase activity [24].

Fibroblast Growth Factor Receptor 2, a class of tyrosine kinase receptor family (FGFR2), is one of the vital receptors in the fibroblast growth factor (FGF) signalling pathway [25]. It controls multiple physiological processes, including cell proliferation, endocrine homeostasis, cell survival, and wound healing via activation of MAPK/ERK1/2, PI3K/AKT pathways, and signal transduction protein STAT3 [26]. Mutation in FGFR2, abnormality in FGFR binding ligands, overexpression, and nuclear translocation leads to cancer progression [27]. In CRC patients, a high level of expression of FGFR2 is positively correlated with the advanced stage of cancer progression and cancer cell metastasis [28]. Recent studies reported that activation of pro-survival transcription factor STAT3 was associated with the abnormal expression of FGFR2 and FGFR4 in 5-fluorouracil and oxaliplatin resistant CRCs [29,27].

Transforming growth factor (TGF- β) is a multifactorial cytokine with three isoforms TGF- β 1, 2 and 3 encoded by TGFB1, TGFB2 and TGFB3 genes, which play essential roles in cell proliferation, differentiation, migration, stem cell maintenance, epithelial to mesenchymal transition and apoptosis [30]. These mechanisms are the result of the sequential activation of numerous components of the TGF- β pathway, which leads to gene expression regulation [31]. The TGF- β signaling is activated by TGF- β cytokines binding to type 1 and type 2 TGF- β receptors (TGF β R1 and TGF β R2), respectively [32]. The normal growth of colonic crypt and villi was regulated by TGF- β signalling, frequent dysregulation of TGF- β leads to the loss of SMAD proteins and TGF- β receptor-2 mediated cell cycle dysregulation in CRC [33].

The overexpression of TGF- β 1, TGF β R1 and TGF β R3 induce the stem cell phenotype through the increased expression of stem cell markers such as Snai1, CD44, CD133, Sox-2, N-

cadherin and Twist1 [34]. The overexpression of proteins TGF β R1 and TGF β R2 correlates with the upregulation of various stem cells markers such as Snai1, CD44, Sox-2, N-cadherin, CD133 and Twist1, causing stem cell phenotype [32].

In order to understand the possible inhibition pattern of neferine, molecular docking studies were used to predict a ligand-receptor interaction. In this present study, docking was performed for various cell surface receptor proteins and downstream proteins responsible for cancer progression, drug resistance, migration and invasion. Abnormal expression of IGF1R [35], FGFR2 [36], TGF β R1, TGF β R2, TGF β 1 [37,38], α 5 β 6, α 5 β 3 [39] and MDR1/ABCB1 [40] were the pivotal surface proteins found to be responsible for cisplatin-resistance in various cancers. Likewise, SNAIL1 [41], STAT3 [40], SMAD [37], GSK3 β [42] and FOXO3 [43] are the crucial proteins involved in signal transduction which were found to be expressed abnormally in various cisplatin-resistant cancer cells.

3.3 Molecular docking results

The compound neferine was docked individually to the active site of each protein using Maestro-GLIDE module of the Schrödinger suite (Table 1). Post docking analyses were based on docking score, Glide evdw (Van Der Waals energy), Glide energy, ecoul (Coulomb energy) and the interacting residues forming hydrogen bond. The highest docking score was observed for IGF1R (PDB ID = 2OJ9) showing hydrogen bonds with Leu975, Asp1056 (Figure - 5). It has been observed that overexpression of many components of the IGF family seem to be involved in tumourigenic mechanisms. IGF1R, IGF1 and IGF2 are frequently overexpressed in a large number of tumor types including CRCs [44]. IGF1R is also associated with resistance to both radiation and chemical based therapies [45]. Thus, compound Neferine could be a potential candidate for targeting IGF1R in CRCs which however requires further studies.

Table 1. Molecular Docking results of Neferine along with the hydrogen bonding residues

Ligand	Protein name	PDB ID	Docking score	No of hydrogen bonds	Interacting residues
	Insulin-like growth factor 1-receptor (IGF1R)	2OJ9	-7.376	2	LEU975, ASP1056
	Fibroblast growth receptor 2 (FGFR2)	3B2T	-6.813	3	LEU 481, LYS517, ASP 644
	Zinc finger protein (SNAIL1)	3W5K	-6.221	2	GLY623, SER621
	Signal Transducer And Activator of Transcription 3 (STAT3)	6QHD	-6.216	4	GLN247, ILE258, GLU324, ARG325
	Transforming growth factor beta receptor 1 (TGF β R1)	3KCF	-6.068	1	PHE216
	Integrin (α 5 β 6)	4UM9	-5.955	1	VAL 226
	Transforming growth factor beta 1 (TGF β 1)	3KFD	-4.581	1	MET104
Neferine	SMAD Family Member (SMAD3/4)	1U7F	-4.319	2	THR 371, GLU397

Ser62. Ser621 (Figure - 7) is one of the prominent residue of the three zinc fingers namely zinc finger 2, 3 and 4 which are interwoven to form a compact ball structure with a tight and compact base for importin- β interaction [47]. Thus, our compound has been shown to interact specifically with the very core interaction site in the Snail1 ZF–importin complex.

Figure – 6:

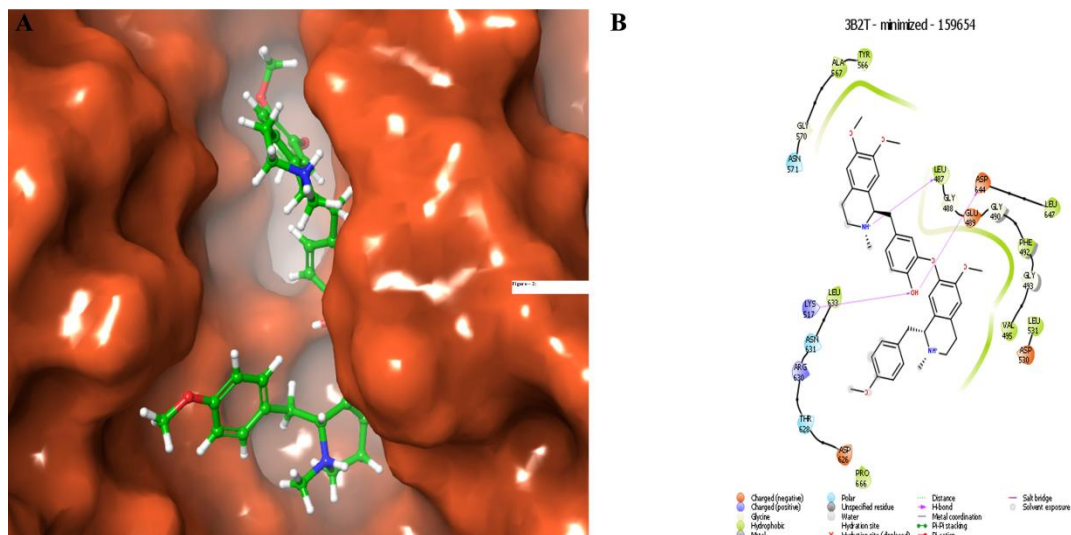


Fig.6: Docking studies of Neferine with fibroblast growth factor receptor (PDB id: 3B2T). (A) Neferine within the active site (B) Hydrogen bonding residues Leu481, Lys517, Asp644 with the ligand.

Figure – 7:

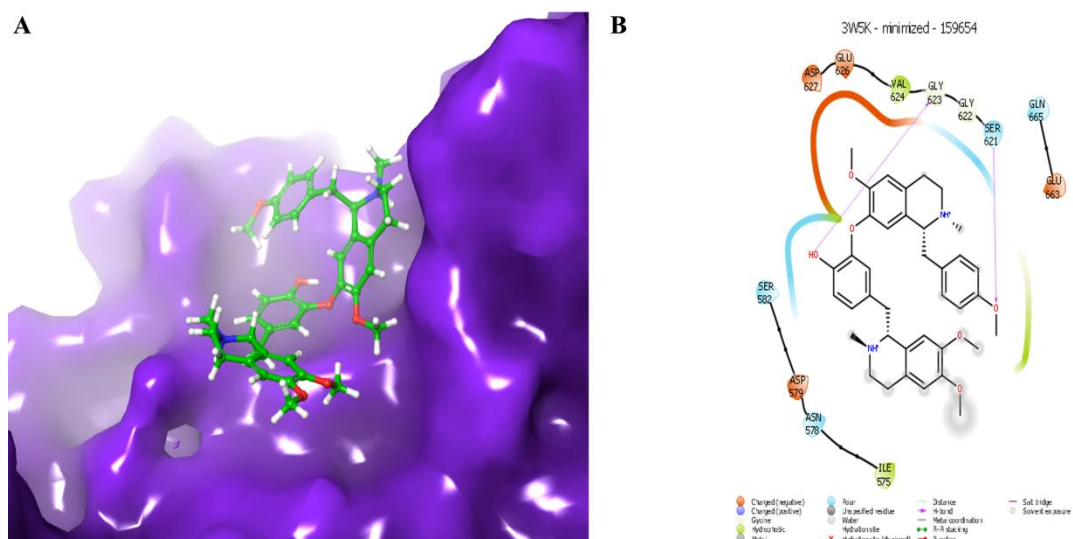


Fig.7: Docking studies of Neferine with SNAIL1 (PDB id: 3W5K). (A) Neferine within the active site **(B)** Hydrogen bonding residues Gly623, Ser621 with the ligand.

Protein STAT3 (PDB ID = 6QHD) and TGF β R1 (PDB ID = 3KCF) are the next two proteins targeted by the compound when sorted according to the docking score, scoring -6.216 and -6.068 respectively (Figure - 8). The former protein formed four hydrogen bonds with residues Gln247, Ile258, Glu324, Arg325 while the later formed a single bond with Phe216. Thus, from our docking studies we could suggest that neferine is most active for IGF1R receptor, FGFR2 receptor, SNAIL1 protein, STAT3 protein and TGF β R1 receptor when sorted according to their docking score.

Figure – 8:

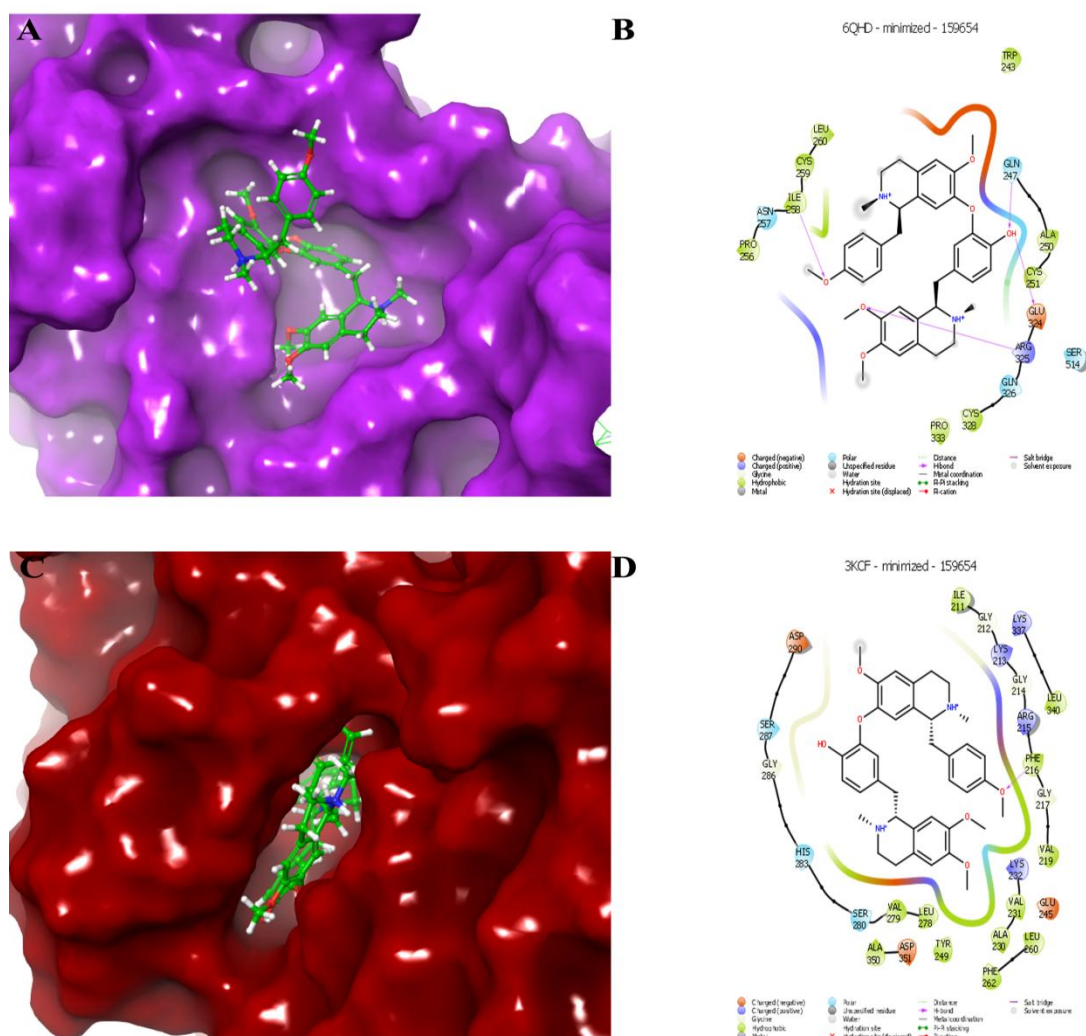


Fig.8: Docking studies of Neferine with STAT3 (PDB id: 6QHD) and TGFβR1 (PDB id: 3KCF). (A & C) Neferine within the active site (B) Hydrogen bonding residues Gln247, Ile258, Glu324, Arg325with the ligand (D) Hydrogen bonding residues Phe216with the ligand.

4. CONCLUSION

The present study evaluated the cytotoxic effect of neferine on cisplatin-resistant CRCs. Further, molecular docking study was carried out to assess the ability of neferine to interact specifically with various target proteins such as IGF-1R, FGFR2, SNAIL1, STAT3, TGFβR1, α5β6, TGFβ1, SMAD, α5β3, TGFβR2, GSK3B, FOXO3, Gamma-Secretase, MDR1/ABCB1 and ABCG2 based on the protein – ligand (neferine) interaction. Neferine interacted with proteins related to cisplatin – resistance with low energy. The binding interaction with target proteins is in the following order, IGF1R > FGFR2 > SNAIL > STAT3 > TGFβR1 > α5β6 > TGFβ1 > SMAD3/4 > α5β3 > TGFβR2 > GSK3β > FOXO3 > gamma-secretase > MDR/ABCB1 > ABCG2. Further, from the docking studies it is evident that Neferine interaction with insulin-like growth factor 1-receptor is strongest compared to

all the proteins considered for the study. This shows that neferine may have the ability to target these proteins which have abnormal expression in CRCs which requires validation through and in vitro and in vivo studies.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, 71(3), 209-249.
2. Wang, Y., Lina, L., Xu, L., Yang, Z., Qian, Z., Zhou, J., & Suoni, L. (2019). Arctigenin enhances the sensitivity of cisplatin resistant colorectal cancer cell by activating autophagy. *Biochemical and biophysical research communications*, 520(1), 20-26.
3. Calon, A., Espinet, E., Palomo-Ponce, S., Tauriello, D. V., Iglesias, M., Céspedes, M. V., & Batlle, E. (2012). Dependency of colorectal cancer on a TGF- β -driven program in stromal cells for metastasis initiation. *Cancer cell*, 22(5), 571-584.
4. Eghtedar, A., Kantarjian, H., Jabbour, E., O'Brien, S., Burton, E., Garcia-Manero, G., & Cortes, J. (2013). Outcome after failure of second generation tyrosine kinase inhibitors treatment as first-line therapy for patients with chronic myeloid leukemia. *Clinical Lymphoma Myeloma and Leukemia*, 13(4), 477-484.

5. Vasan, N., Baselga, J., & Hyman, D. M. (2019). A view on drug resistance in cancer. *Nature*, 575(7782), 299-309.
6. Housman, G., Byler, S., Heerboth, S., Lapinska, K., Longacre, M., Snyder, N., & Sarkar, S. (2014). Drug resistance in cancer: an overview. *Cancers*, 6(3), 1769-1792.
7. Yao, H., Sun, Q., & Zhu, J. (2019). miR-1271 enhances the sensitivity of colorectal cancer cells to cisplatin. *Experimental and therapeutic medicine*, 17(6), 4363-4370.
8. Deng, Z., Wang, H., Guo, G., Li, X., Cai, Y., Tang, Y., & Li, L. (2019). Next-generation sequencing analysis of mRNA profile in cisplatin-resistant gastric cancer cell line SGC7901. *Medical science monitor: international medical journal of experimental and clinical research*, 25, 2386.
9. Chaudhary, T., Chahar, A., Sharma, J. K., Kaur, K., & Dang, A. (2015). Phytomedicine in the treatment of cancer: a health technology assessment. *Journal of clinical and diagnostic research: JCDR*, 9(12), XC04.
10. Priya, L. B., Baskaran, R., Huang, C. Y., & Padma, V. V. (2017). Neferine ameliorates cardiomyoblast apoptosis induced by doxorubicin: possible role in modulating NADPH oxidase/ROS-mediated NFκB redox signaling cascade. *Scientific reports*, 7(1), 1-13.
11. Asokan, S. M., Mariappan, R., Muthusamy, S., & Velmurugan, B. K. (2018). Pharmacological benefits of neferine-A comprehensive review. *Life sciences*, 199, 60-70.
12. Huang, C., Li, Y., Cao, P., Xie, Z., & Qin, Z. (2011). Synergistic effect of hyperthermia and neferine on reverse multidrug resistance in adriamycin-resistant SGC7901/ADM gastric cancer cells. *Journal of Huazhong University of Science and Technology [Medical Sciences]*, 31(4), 488-496.
13. Paramasivan, P., Kumar, J. D., Baskaran, R., Weng, C. F., & Padma, V. V. (2020). Reversal of doxorubicin resistance in lung cancer cells by neferine is explained by nuclear factor erythroid-derived 2-like 2 mediated resistance protein down regulation. *Cancer Drug Resistance*, 3(3), 647-665.
14. Anonymous. Schrödinger release 2018-4: Protein Preparation Wizard; Epik, Schrödinger, LLC, New York, NY. Impact, Schrödinger, LLC, New York, NY, 2018; Prime, Schrödinger, LLC, New York, NY, 2018.
15. Anonymous. Schrödinger release. LigPrep, Schrödinger, LLC. New York, NY. 2018-4
16. Zheng, Z. H., You, H. Y., Feng, Y. J., & Zhang, Z. T. (2021). LncRNA KCNQ1OT1 is a key factor in the reversal effect of curcumin on cisplatin resistance in the colorectal cancer cells. *Molecular and cellular biochemistry*, 476(7), 2575-2585.
17. Manogaran, P., Beeraka, N. M., & Padma, V. V. (2019). The cytoprotective and anti-cancer potential of bisbenzylisoquinoline alkaloids from *Nelumbonucifera*. *Current topics in medicinal chemistry*, 19(32), 2940-2957.
18. Deng, G., Zeng, S., Ma, J., Zhang, Y., Qu, Y., Han, Y., & Shen, H. (2017). The anti-tumor activities of Neferine on cell invasion and oxaliplatin sensitivity regulated by EMT via Snail signaling in hepatocellular carcinoma. *Scientific reports*, 7(1), 1-14.

19. Pagadala, N. S., Syed, K., & Tuszynski, J. (2017). Software for molecular docking: a review. *Biophysical reviews*, 9(2), 91-102.
20. Tao, X., Huang, Y., Wang, C., Chen, F., Yang, L., Ling, L., & Chen, X. (2020). Recent developments in molecular docking technology applied in food science: a review. *International Journal of Food Science & Technology*, 55(1), 33-45.
21. Singh, R. K., Gaikwad, S. M., Jinager, A., Chaudhury, S., Maheshwari, A., & Ray, P. (2014). IGF-1R inhibition potentiates cytotoxic effects of chemotherapeutic agents in early stages of chemoresistant ovarian cancer cells. *Cancer letters*, 354(2), 254-262.
22. Baserga, R. (2009). Customizing the targeting of IGF-1 receptor. *Future Oncology*, 5:43–50.
23. Codony-Servat, J., Cuatrecasas, M., Asensio, E., Montironi, C., Martínez-Cardús, A., Marín-Aguilera, M., & Maurel, J. (2017). Nuclear IGF-1R predicts chemotherapy and targeted therapy resistance in metastatic colorectal cancer. *British journal of cancer*, 117(12), 1777-1786.
24. Packham, S., Warsito, D., Lin, Y., Sadi, S., Karlsson, R., Sehat, B., & Larsson, O. (2015). Nuclear translocation of IGF-1R via p150 Glued and an importin- β /RanBP2-dependent pathway in cancer cells. *Oncogene*, 34(17), 2227-2238.
25. Babina, I. S., & Turner, N. C. (2017). Advances and challenges in targeting FGFR signalling in cancer. *Nature Reviews Cancer*, 17(5), 318-332.
26. Turner, N., & Grose, R. (2010). Fibroblast growth factor signalling: from development to cancer. *Nature Reviews Cancer*, 10(2), 116-129.
27. Zhou, Yangyang, Chengyu Wu, Guangrong Lu, Zijing Hu, Qiuxiang Chen, and Xiaojing Du. "FGF/FGFR signaling pathway involved resistance in various cancer types." *Journal of Cancer* 11, no. 8 (2020): 2000.
28. Li, C. F., He, H. L., Wang, J. Y., Huang, H. Y., Wu, T. F., Hsing, C. H., & Chen, S. H. (2014). Fibroblast growth factor receptor 2 overexpression is predictive of poor prognosis in rectal cancer patients receiving neoadjuvant chemo-radiotherapy. *Journal of clinical pathology*, 67(12), 1056-1061.
29. Turkington, R. C., Longley, D. B., Allen, W. L., Stevenson, L., McLaughlin, K., Dunne, P. D., & Johnston, P. G. (2014). Fibroblast growth factor receptor 4 (FGFR4): a targetable regulator of drug resistance in colorectal cancer. *Cell death & disease*, 5(2), e1046-e1046.
30. Zhao, D., Zhai, B., He, C., Tan, G., Jiang, X., Pan, S., & Sun, X. (2014). Upregulation of HIF-2 α induced by sorafenib contributes to the resistance by activating the TGF- α /EGFR pathway in hepatocellular carcinoma cells. *Cellular signalling*, 26(5), 1030-1039.
31. Katz, L. H., Likhter, M., Jogunoori, W., Belkin, M., Ohshiro, K., & Mishra, L. (2016). TGF- β signaling in liver and gastrointestinal cancers. *Cancer letters*, 379(2), 166-172.
32. Chruścik, A., Gopalan, V., & Lam, A. K. Y. (2018). The clinical and biological roles of transforming growth factor beta in colon cancer stem cells: A systematic review. *European journal of cell biology*, 97(1), 15-22.
33. Buhmann, C., Kraehe, P., Lueders, C., Shayan, P., Goel, A., & Shakibaei, M. (2014). Curcumin suppresses crosstalk between colon cancer stem cells and stromal fibroblasts in the tumor microenvironment: potential role of EMT. *PloS one*, 9(9), e107514.

34. Kim, Y. H., Kim, G., Kwon, C. I., Kim, J. W., Park, P. W., & Hahm, K. B. (2014). TWIST1 and SNAI1 as markers of poor prognosis in human colorectal cancer are associated with the expression of ALDH1 and TGF- β 1. *Oncology reports*, 31(3), 1380-1388.
35. Selfe, J., Goddard, N. C., McIntyre, A., Taylor, K. R., Renshaw, J., Popov, S. D., & Shipley, J. M. (2018). IGF1R signalling in testicular germ cell tumour cells impacts on cell survival and acquired cisplatin resistance. *The Journal of pathology*, 244(2), 242-253.
36. Pu, L., Su, L., & Kang, X. (2019). The efficacy of cisplatin on nasopharyngeal carcinoma cells may be increased via the downregulation of fibroblast growth factor receptor 2. *International journal of molecular medicine*, 44(1), 57-66.
37. Xie, Yu, Shuai Zhu, Jinglei Zang, Guanlin Wu, Yuheng Wen, Yu Liang, Ying Long et al. "ADNP prompts the cisplatin-resistance of bladder cancer via TGF- β -mediated epithelial-mesenchymal transition (EMT) pathway." *Journal of Cancer* 12, no. 17 (2021): 5114.
38. Wang, J., Chen, Y., Xiang, F., Li, M., Li, H., Chi, J., & Ren, K. (2018). Suppression of TGF- β 1 enhances chemosensitivity of cisplatin-resistant lung cancer cells through the inhibition of drug-resistant proteins. *Artificial cells, nanomedicine, and biotechnology*, 46(7), 1505-1512.
39. Ngaokrajang, U., Janvilisri, T., Sae-Ueng, U., Prungsak, A., & Kiatwuthinon, P. (2021). Integrin α 5 mediates intrinsic cisplatin resistance in three-dimensional nasopharyngeal carcinoma spheroids via the inhibition of phosphorylated ERK/caspase-3 induced apoptosis. *Experimental Cell Research*, 406(2), 112765.
40. Fang, Z., Chen, W., Yuan, Z., Liu, X., & Jiang, H. (2018). LncRNA-MALAT1 contributes to the cisplatin-resistance of lung cancer by upregulating MRP1 and MDR1 via STAT3 activation. *Biomedicine & Pharmacotherapy*, 101, 536-542.
41. Kielbik, M., Szulc-Kielbik, I., & Klink, M. (2021). Impact of Selected Signaling Proteins on SNAIL 1 and SNAIL 2 Expression in Ovarian Cancer Cell Lines in Relation to Cells' Cisplatin Resistance and EMT Markers Level. *International Journal of Molecular Sciences*, 22(2), 980.
42. Pan, C. H., Chen, S. Y., Wang, J. Y., Tsao, S. P., Huang, H. Y., Chiu, P. W. C., & Wu, C. H. (2020). Sclareol ameliorated ERCC1-mediated cisplatin resistance in A549 human lung adenocarcinoma cells and a murine xenograft tumor model by suppressing AKT-GSK3 β -AP1/Snail and JNK-AP1 pathways. *Chemico-Biological Interactions*, 332, 109304.
43. An, Y., Wang, B., Wang, X., Dong, G., Jia, J., & Yang, Q. (2020). SIRT1 inhibits chemoresistance and cancer stemness of gastric cancer by initiating an AMPK/FOXO3 positive feedback loop. *Cell death & disease*, 11(2), 1-14.
44. Weber, MM., Fottner, C., Bin.LS., Jung, MC., Engelhardt, D., & Baretton, GB. (2002) Overexpression of the insulin-like growth factor I receptor in human colon carcinomas. *Cancer*, 95, 2086–2095.
45. Jones HE, Gee JMW, Barrow D, Tonge D, Holloway B & Nicholson RI. (2006) Inhibition of insulin receptor isoform-A signalling restores sensitivity to gefitinib in previously de novo resistant colon cancer cells. *Br J Cancer* 95, 172–180.

46. Lew, E.D., Bae, J.H., Rohmann, E., Wollnik, B., Schlessinger, J. Structural basis for reduced FGFR2 activity in LADD syndrome: Implications for FGFR autoinhibition and activation. (2007) Proc Natl Acad Sci U S A 104: 19802-19807.
47. Choi, Saehae; Yamashita, Eiki; Yasuhara, Noriko; Song, Jinsue; Son, Se-Young; Won, Young Han; Hong, Hye Rim; Shin, Yoon Sik; Sekimoto, Toshihiro; Park, Il Yeong; Yoneda, Yoshihiro; Lee, Soo Jae (2014). Structural basis for the selective nuclear import of the C2H2 zinc-finger protein Snail by importin β . Acta Crystallographica Section D Biological Crystallography, 70(4), 1050–1060.

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