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Araştırma Makalesi

Antiproliferative Activity of α-Tomatine and Molecular Target Identification

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Abstract

α-tomatine is a glycoalkaloid derived from tomato varieties that has been reported to possess various anticancer properties. However, its inhibitory effects on epidermal growth factor receptor is still poorly understood. The aim of this study is to investigate the anticancer effect of α-tomatine and its related mechanisms in lung cancer cells. Cytotoxicity and apoptosis induction of α-tomatine were determined by MTT assay and annexin V-FITC staining methods, respectively. For tyrosine kinase activity, TK-1 kinase selectivity profiling assay and molecular modelling study were performed. The DNA cleavage activity of α-tomatine was investigated using agarose gel electrophoretic method. α-tomatine proved to possess an outstanding antiproliferative activity against A549 and Jurkat cells without noticeable toxicity on PBMC. The results indicated that α-tomatine has a significant inhibition effect on both EGFR and HER2. α-tomatine formed some key interaction into ATP binding sites of EGFR and HER2. Furthermore, α-tomatine strongly disintegrated DNA at low concentrations in the presence of iron(II) complexes. The current findings suggest that α-tomatine has a distinguished receptor tyrosine kinase inhibition profile from erlotinib and might be a potential drug candidate for treatment of NSCLC.

Keywords: α-Tomatine, Lung cancer, NSCLC, EGFR, HER2, DNA cleavage

α-Tomatin`nin Antiproliferatif Aktivitesi ve Moleküler Hedef Tanımlaması

Özet

Domates çeşitlerinden elde edilen bir glikoalkaloid olan α-tomatin`nin çeşitli antikanser özelliklere sahip olduğu bildirilmiştir. Fakat, onun epidermal büyüme faktörü reseptörü üzerindeki inhibitör etkileri hala tam olarak anlaşılamamıştır. Bu çalışma, α-tomatin`nin akciğer kanser hücreleri üzerindeki antikanser etkisi ve ilgili mekanizmalarını araştırmayı amaçlamaktadır. α-tomatin`nin sitotoksisitesi ve apoptoz tetiklenmesi sırasıyla MTT ve anneksin V-FITC boyama yöntemleriyle belirlendi. Tirozin kinaz aktivitesi için, sırasıyla TK-1 kinaz seçicilik profil tahlili ve moleküler modelleme çalışmaları yapıldı. α-tomatin`nin DNA parçalanma aktivitesi, agaroz jel elektroforezi yöntemi kullanılarak araştırıldı. α-tomatin`in A549 ve Jurkat hücrelerine karşı üstün bir antiproliferatif aktiviteye sahip olduğu ve PBMC'de gözle görülür toksisitesinin olmadığı kanıtlandı. Sonuçlar, α-tomatin`nin hem EGFR hem de HER2 kinazların üzerinde önemli bir inhibisyon etkisine sahip olduğunu gösterdi. α-tomatin, EGFR ve HER2'nin ATP bağlayıcı bölgelerine bazı önemli etkileşimler meydana getirdi. Ayrıca, α-tomatin, düşük konsantrasyonlarda, demir(II) kompleksleri varlığında, DNA`yı güçlü bir şekilde parçalamıştır. Mevcut bulgular α-tomatin`nin erlotinib'den ayırt edici bir reseptör tirozin kinaz inhibisyon profiline sahip olduğunu ve KHDAK tedavisi için potansiyel bir ilaç adayı olabileceğini göstermektedir.

Anahtar kelimeler: α-Tomatin, Akciğer kanseri, KHDAK, EGFR, HER2, DNA parçalanması

Introduction

Lung cancer is the most commonly diagnosed cancer type and the second deadliest disease in the world, causing more than 18% of all cancer deaths (Goebel et al., 2019). In 2018, the annual estimated new cases of lung cancer were 2.09 million worldwide, with 1.76 million deaths (Goebel et al., 2019). Lung cancer is classified into two main pathological classes for treatment and prognostic factors; SCLC (small-cell lung carcinoma) and NSCLC (non-small-cell lung carcinoma). The main type of lung cancer is NSCLC, which accounts for around 85% of all lung cancer cases (Inamura, 2017; Travis, 2015) and frequently develops resistance to chemotherapy and radiation. The majority of NSCLC patients (around %70) are diagnosed in advanced stage when surgery is not suitable. Despite extensive research and many efforts on lung carcinoma therapeutics in the last decades, the treatment of NSCLC has evolved remarkable from the traditional approach to molecular targeted drugs, due to technological advances on cancer research (Sun et al., 2007; Koinis et al., 2016; Denisenko et al., 2018). Thus, effective treatment in NSCLC would shrink lung cancer death rates.

EGFR/HER1 (the epidermal growth factor receptor) is a receptor tyrosine kinase that plays important roles in migration, apoptosis, cell proliferation, metastasis and angiogenesis. The family of EGFR/HER is classified into four members; EGFR (HER1), HER2, HER3, and HER4. Among them, EGFR is the most common genetic driver and has been overexpressed in NSCLC (about 40–80%), which made EGFR as a potential therapeutic target (Chan and Hughes, 2015; Prabhakar, 2015). The introduction of first EGFR inhibitor erlotinib has been important advancements for the treatment NSCLC patients in targeted chemotherapy. However, some patients with NSCLC start to develop resistance to erlotinib after 10-14 months of its treatment, which results in recurrence of NSCLC (Tang et al., 2015; Riely et al., 2015; Dong et al., 2019). Several new generation drugs have been developed to overcome the problems of drug resistance and reduce side effects, but there are still no alternative long-term chemotherapeutic inhibitors that efficiently treat NSCLC patients (Wang et al., 2016; Takeda and Nakagawa, 2019; Tan et al., 2018).

HER2 is amplified, mutated and overexpressed in several types of cancers. In NSCLC, HER2 aberrations have been identified as another oncogenic driver and present in 2% to 6% of NSCLC patients (Liu et al., 2018; Zimmermann, 2014). Furthermore, HER2 amplification plays an important role in NSCLC by which patients develop disease progression because of secondary drug resistance to EGFR TKIs (Liu et al., 2018). Although there is currently no identified direct ligand for HER2, it has confirmed that dimerization is essential for activation of HER2 with its preferred partner for other HER family members, primarily EGFR. The heterodimers formed by EGFR and HER2, are considered to have higher signal transduction and stability than EGFR homodimer and showed good responses in patients with NSCLC (Arkhipov et al., 2013; Hou et al., 2015; Citri and Yarden, 2006). Thus, targeting EGFR and HER2 receptors may have important implications and may overcome EGFR-TKI drug resistance problem. It was reported that combination of some drugs with erlotinib inhibit formation of heterodimers by disrupting of interaction between EGFR-HER2 and/or HER2-HER3 and showed synergistic anticancer effects on NSCLC cells (Banappagari et al., 2012; Kanthala et al., 2015). Therefore, the discovery of potent and selected anticancer agents for blocking EGFR/HER family pathways are necessary.

α-tomatine is a naturally occurring glycoalkaloid, found in roots, stems and leaves, pointing out that it may have an important role in decreasing drug resistance to potential pathogens (Huang et al., 2015; Shieh et al., 2011; Chao et al., 2012). α-tomatine consist of one tomatidine (aglycon moiety), and one b-lycotetraose (tetrasaccharide moiety) that contains two glucose molecules, a xylose and a galactose. Previous studies showed that α-tomatine has antiproliferative effects on cancer cell lines (HT-29 colon, MCF-7 breast, HL-60 myeloid leukemia, HepG2 liver, and PC-3 prostate) (Lee et al., 2004; Lee et al., 2011; Sucha et al., 2013). It also showed inhibition of proliferation on lymphoma and lung cancer cell lines (Shieh et al., 2011; Chao et al., 2012; Yang et al., 2004; Shih et al., 2009). Herein, we study antiproliferative activity of α-tomatine against A549 human NSCLC cells with an exploration of its dual mechanism as multiple kinase inhibition and DNA cleavage.

Materials and Methods

Materials

α-tomatine (Figure 1) and erlotinib were purchased from Tokyo Chemical and Santa Cruz Biotechnology respectively, dissolved in DMSO (dimethyl sulfoxide) and kept at -20°C.

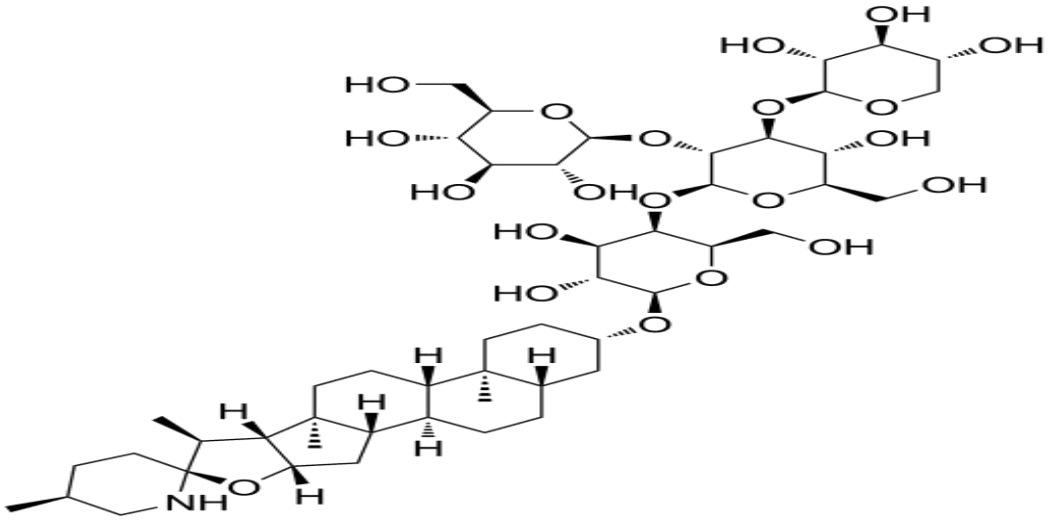


Figure 1. Structure of α-tomatine

Cell culture and drug treatment

A549 cell line was incubated in DMEM (Wako Pure Chemical Industries, Osaka, Japan) medium and supplemented with 10% fetal bovine serum (FBS) (Sigma Aldrich, MO, USA). Human leukemic Jurkat and normal blood (Precision Bioservices, Frederic, MD) cells were cultured with medium RPMI 1640 (Wako Pure Chemical Industries) with 10% FBS. All cells were maintained in a 5% CO_2 incubator at 37 °C under a humid atmosphere. The stock solution of α-tomatine and erlotinib dissolved in DMSO (Wako Pure Chemical Industries) between 0.1-10 mM concentrations and further diluted 100 fold with fresh media. DMSO concentration was 1% in the final culture medium which was reported to has no toxic effect on the cell viability (Koga et al., 2017).

Cell viability assay

The effect of α-tomatine and Erlotinib on cell viability was assessed by using MTT assay as previously described (Radwan et al., 2019; Ali et al., 2019).

Detection of cell death

Microscopic observations for detection of cell death were performed using Biorevo Fluorescence BZ-9000 (Keyence, Osaka, Japan), as previously described (Ciftci et al., 2019; Tateishi et al., 2017; Shida et al., 2019).

Kinase inhibition assay

The kinase profiling assay was performed as described previously (Ciftci et al., 2018).

Molecular modeling

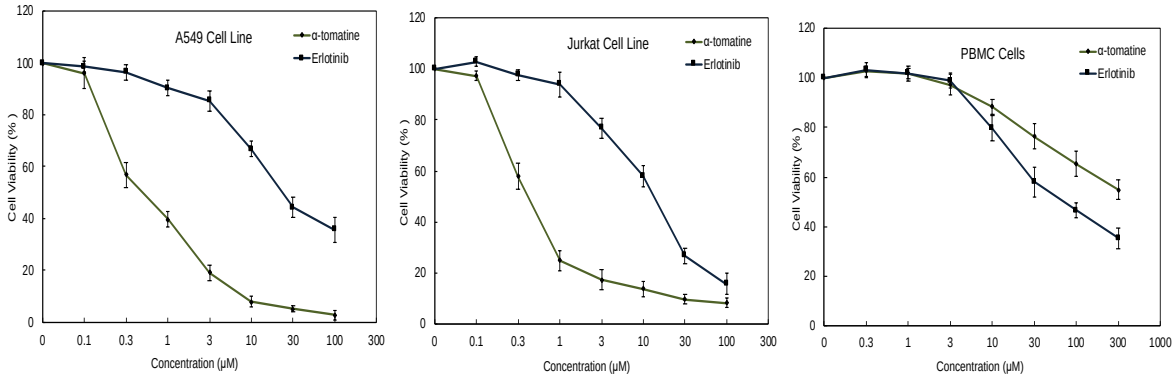
The X-ray structures of EGFR (using the PDB entry 4HJO co-crystallized with Erlotinib) (Park et al., 2012), HER2 (using the PDB entry 3RCD co-crystallized with TAK-285) (Ishikawa et al., 2011) and HER4 (using the PDB entry 2R4B co-crystallized with GW7) (Wood et al., 2008) were retrieved from the protein data bank. The protein structures were prepared by using the Structure Preparation module of MOE 2019.01 (Molecular Operating Environment, Chemical Computing Group Inc., Montreal, Canada) (Ciftci et al., 2019; Bayrak et al., 2019). Before docking, all water molecules were removed from the structure. Then the co-crystallized ligands were defined as the center of the binding site. In the current docking simulation, the Triangle Matcher method was used to place ligand conformations in the site then ranked using the London ΔG scoring function. To validate our docking method, the co-crystallized ligands were also docked with α-tomatine. Thirty docking poses were calculated for α-tomatine; then the generated docking poses were visualized using MOE 2019.01. The binding free energy (ΔG) in kcal/mol of α-tomatine and the co-crystallized ligands were calculated using the top-scored docking poses (Radwan et al., 2019; Ibrahim et al., 2017).

DNA Cleavage Assay

The DNA cleavage assay was performed as described previously (Ciftci et al., 2019; Bayrak et al., 2019).

Results and Discussion

In the current study, the antiproliferative activity of α-tomatine against A549 human lung adenocarcinoma cell line was investigated using erlotinib as a control. Erlotinib was chosen as a model drug because of its wide use in the management of EGFR and lung cancer. First, A549 cells were incubated with α-tomatine and erlotinib at various concentration (0.1 μM – 100 μM) for 72 h and then the cytotoxicity of compounds were examined by MTT assay. α-tomatine decreased viability of cells in a dose dependent manner in A549 cells. As shown Figure 2a, α-tomatine has a very potent cytotoxic activity of EGFR inhibitor-sensitive A549 cell line with IC_{50} value of 0.57±0.05 μM, ~40 times stronger activity than positive control erlotinib (IC_{50}=23.65±2.18 μM). Next, the cytotoxic effects of α-tomatine was examined on normal blood (PBMC) and cancer (Jurkat) cells for selectivity of tumor (Figure 2b and 2c). The SI of tested compounds were calculated between the PBMC and Jurkat cells as the ratio of the IC_{50} values. The IC_{50} and selectivity index (SI) values of α-tomatine in comparison with erlotinib were shown in Table 1. These results exhibited that α-tomatine has a high safety profile against PBMC and showed ~6 times lower cytotoxicity than erlotinib (Figure 2c).



(a) **(b)** **(c)**

Figure 2. Dose-dependent cytotoxic activity of α-tomatine and Erlotinib on A549 (a), Jurkat (b) and PBMC (c) cells after 72 h of drug treatment.

Table 1. IC_{50} values of α-tomatine and Erlotinib against A549, Jurkat and PBMC cells and selectivity index (SI). The data represents the mean ±SD (standard deviation) for each compound performed in triplicate.

IC_{50} Values (μM)				
	A549	**Jurkat**	**PBMC**	**SI**
α-tomatine	0.57±0.05	0.39±0.04	>300	>769.23
Erlotinib	23.65±2.18	11.91±1.72	52.05±8.94	4.37

On the basis of MTT assay results, α-tomatine concentration was chosen for further investigation whether its cytotoxic effect is attributed to the induction of apoptosis or not. Therefore, A549 cell-treated α-tomatine or erlotinib at 3 μM and 1 μM concentrations were incubated for 24 h. After treatment, the cells were washed, stained with the Hoechst 33342, annexin V and EtD-III and then observed under a fluorescence microscope. Figure 3a shows the representative microscopic field. Some cells were colored with green (annexin V) and not with red (EtD-III), meaning necrosis. On the contrary, the totally opposite results showed apoptosis. In addition, some cells were colored with both red and green, pointing late apoptosis/necrosis and many cells were colored with only blue (Hoechst 33342), meaning healthy. The response of A549 cells upon 24 h of α-tomatine treatment at 3 μM concentration was 7.21% apoptosis, 6.01% necrosis and 2.70% late apoptosis/necrosis as shown in Figure 3b. On the contrary, erlotinib (3 μM) had 2.06% apoptosis, 0.55% necrosis and 1.24% late apoptosis/ necrosis. α-tomatine and Erlotinib both at 1 μM concentration were not shown significant cell death against A549 cells after 24 h incubation (data not shown). The results showed that α-tomatine induced significant cell apoptosis than erlotinib on A549 cells at 3 μM concentration after 24 h of drug treatment (Figure 3c).

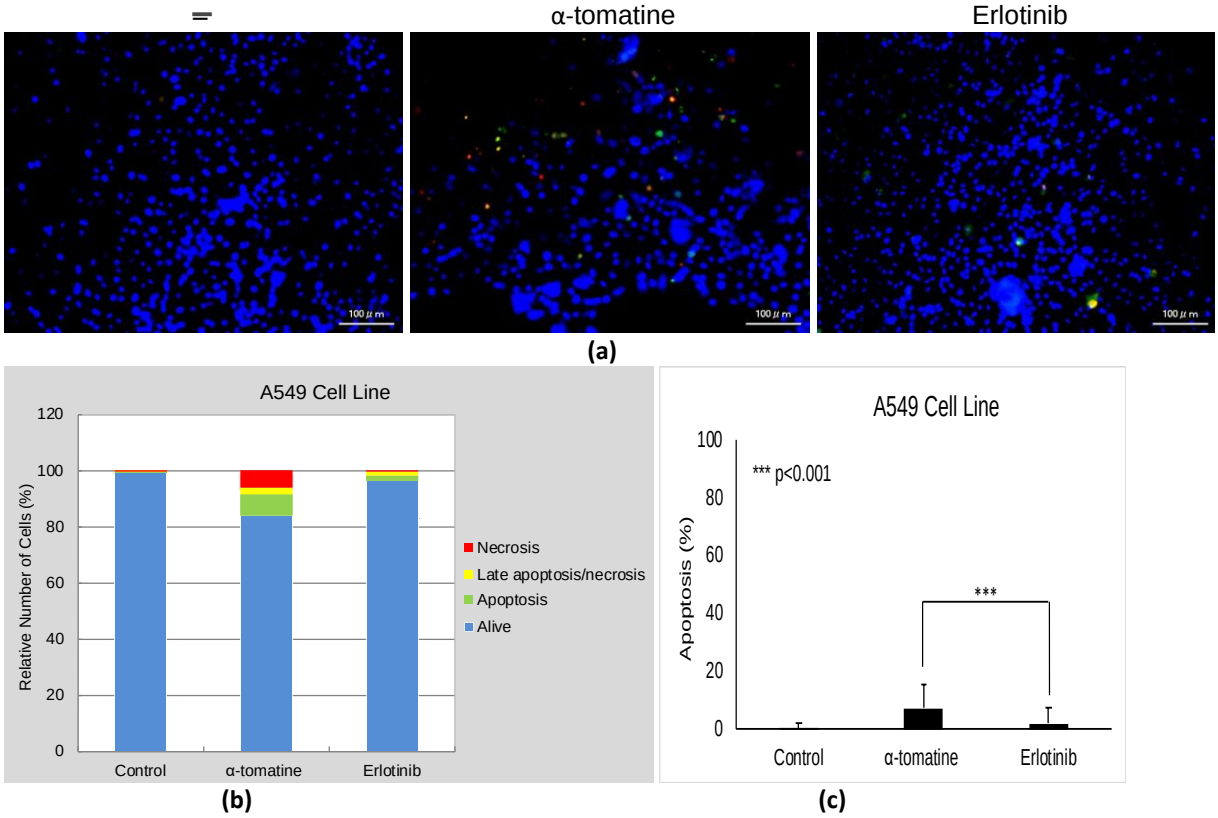


Figure 3. The apoptosis-inducing effect of **α-tomatine** and **Erlotinib** at 3 μM concentration after 24 h treatment (a). More than 100 stained cells were randomly chosen from each experiment of (a) and divided into four categories: apoptosis, necrosis, late apoptosis/necrosis and alive (b). Quantify of α-tomatine and **Erlotinib**-induced cell death (apoptosis) in A549 cells (c). The reported values are representative of three independent experiments and are shown as means ± SD (error bars). p values were explored by statistical calculation using t-test.

Based on those finding, we speculate that the outstanding anticancer effect of α-tomatine on EGFR-sensitive A549 might be through the inhibition of receptor tyrosine kinases. Therefore, a panel of kinases including EGFR, HER2, HER4, IGF1R, InsR, KDR and PDGFR-alpha and PDGFR-beta were selected. In this kinase selectivity profiling system, the inhibition of α-tomatine at two concentration (3 μM and 1 μM) were examined on eights tyrosine kinase enzymes compared to erlotinib (Figure 4).

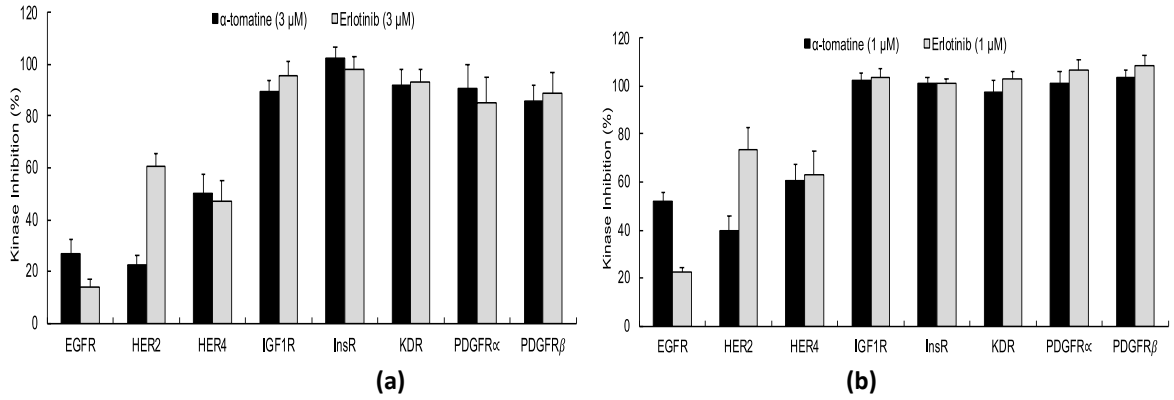


Figure 4. Inhibition of a panel of kinases by α-tomatine and Erlotinib at 3 μM (a) and 1 μM (b) concentrations.

Inhibitory effect of α-tomatine (3 μM) on eight kinases exposed the following potency order: HER2 > EGFR > HER4 > PDGFR-beta > IGF1R > PDGFR-alpha > KDR > InsR (Figure 4a). Among these kinases, α-tomatine displayed the most potent inhibitory activity on the HER2, and showed stronger inhibition than erlotinib at both 3 μM and 1 μM concentrations (Figure 4a and 4b). Erlotinib was used for comparison and exhibited stronger inhibitory effect than α-tomatine on the EGFR. Although the EGFR inhibitory activity of α-tomatine is still incomparable to erlotinib, they showed similar and moderate HER4 inhibition. Furthermore, α-tomatine and erlotinib were found to be inactive on IGF1R, InsR, KDR, PDGFR-alpha and PDGFR-beta at 1 μM concentration (Figure 4b). It can be concluded that α-tomatine might be as an effective multi-targeted tyrosine kinase inhibitor with a distinct inhibition profile from erlotinib.

Because *in vitro* study revealed that α-tomatine possesses potent and direct inhibition of EGFR, and HER2; *in silico* study was performed to get deep insights on how α-tomatine interact with the active sites of the two kinases at molecular level. The co-crystallized ligands (Erlotinib, and TAK-285) were initially re-docked in their corresponding co-crystal structures (PDB code 4HJO, and 3RCD, respectively) to examine whether MOE is capable of reproducing the default binding mode of the co-crystallized inhibitors. Figures 5a, and 6a demonstrated the superimposition of the co-crystallized ligands (Erlotinib, and TAK-285) and their superposed docking conformation, where all the co-crystallized ligands were perfectly docked into their crystal structures with acceptable RMSD values (0.3, and 0.4 Å, respectively) and they formed a hydrogen bond with the crucial key amino acid residues (Met769, Met801 and Met799, respectively) in accordance with the co-crystallized ligands.

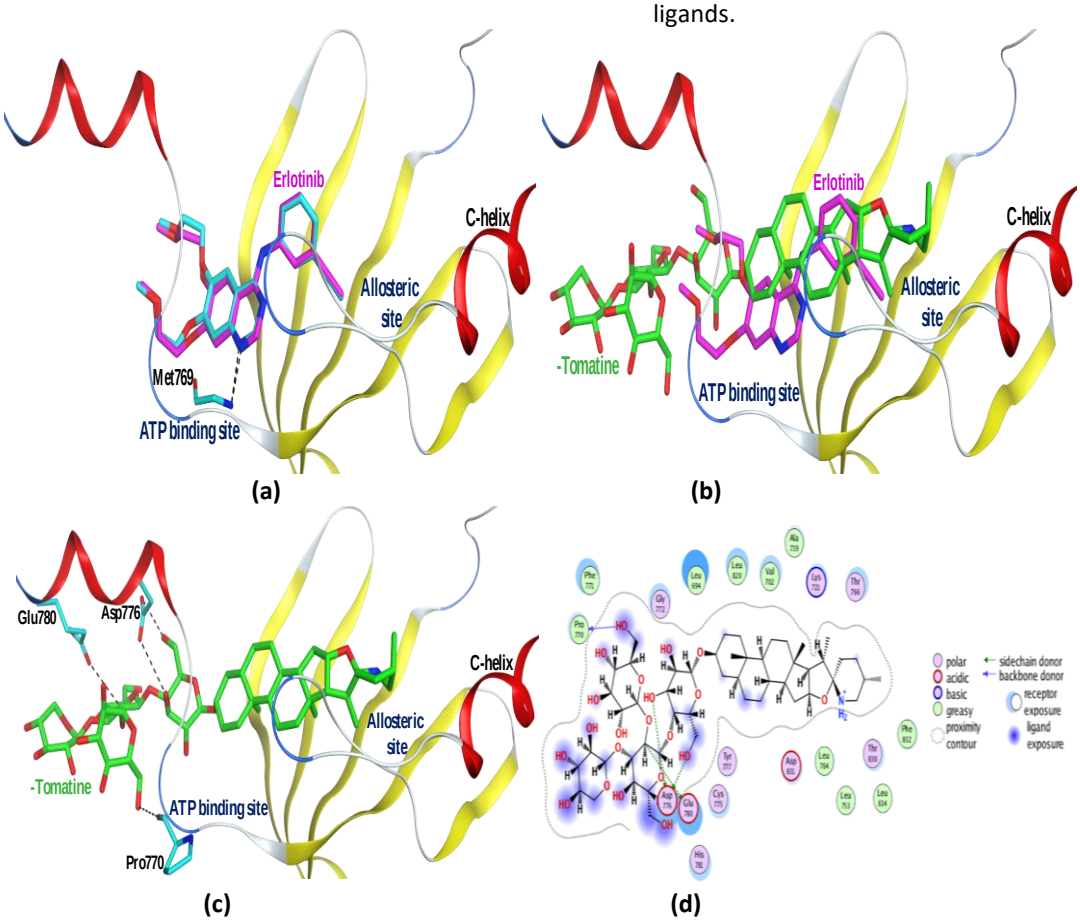


Figure 5. The top-scoring docked pose of **α-tomatine** to the EGFR active site (PDB code 4HJO) as predicted by MOE 2019.01. (a) Comparison of modeled binding mode of the co-crystallized ligand **Erlotinib** (magenta sticks) and its superposed docking conformation (cyan sticks). (b) Comparison of modeled binding mode of **α-tomatine** (green sticks) and **Erlotinib** (magenta sticks). (c) Detailed binding mode of **α-tomatine** (green sticks) displaying hydrogen bonds (black dashed line) with the key amino acid residues (cyan sticks). (d) 2D depiction of **α-tomatine** binding interactions with the key amino acid residues.

Table 2: The binding free energy (ΔG) in kcal/mol for **α-tomatine** with the active site of EGFR (PDB 4HJO; co-crystallized with **Erlotinib**), and HER2 (PDB 3RCD; co-crystallized with **TAK-285**) compared to the co-crystalized ligands.

Ligand	ΔG (kcal/mol) **EGFR**	ΔG (kcal/mol) **HER2**
α-tomatine	-8.3	-9.2
Co-crystalized ligand	-9.0 (**Erlotinib**)	-10.2 (**TAK-285**)

Molecular docking of α-tomatine into EGFR, and HER2 successfully predicted its binding mode and orientation within the active site of these kinases. The binding free energy (ΔG) in kcal/mol of α-tomatine to EGFR, and HER2 compared to the co-crystalized ligands were summarized in Table 2. α-Tomatine has higher binding affinities for both EGFR and HER2.

As illustrated in Figure 5, the glycoside moiety of α-tomatine forms four hydrogen bonds with the key amino acid residues (Pro770, Asp776 and Glu780) to the EGFR active site. Also, the aglycone part of α-tomatine goes deeply in the ATP-binding pocket and allosteric binding site. Figure 6 demonstrates the binding mode and orientation of α-tomatine within the active site of Her2. The high binding affinity of α-tomatine to Her2 can be explained by its ability to make six strong hydrogen bonds with the key amino acids (Leu726, Asp808, Arg811 and Asp863) in addition to a CH-π interaction with the key residue Phe1004. Those theoretical data are in a complete agreement with the above mentioned *in vitro* assay and helped us comprehend and rationalize the high affinity of EGFR and HER2.

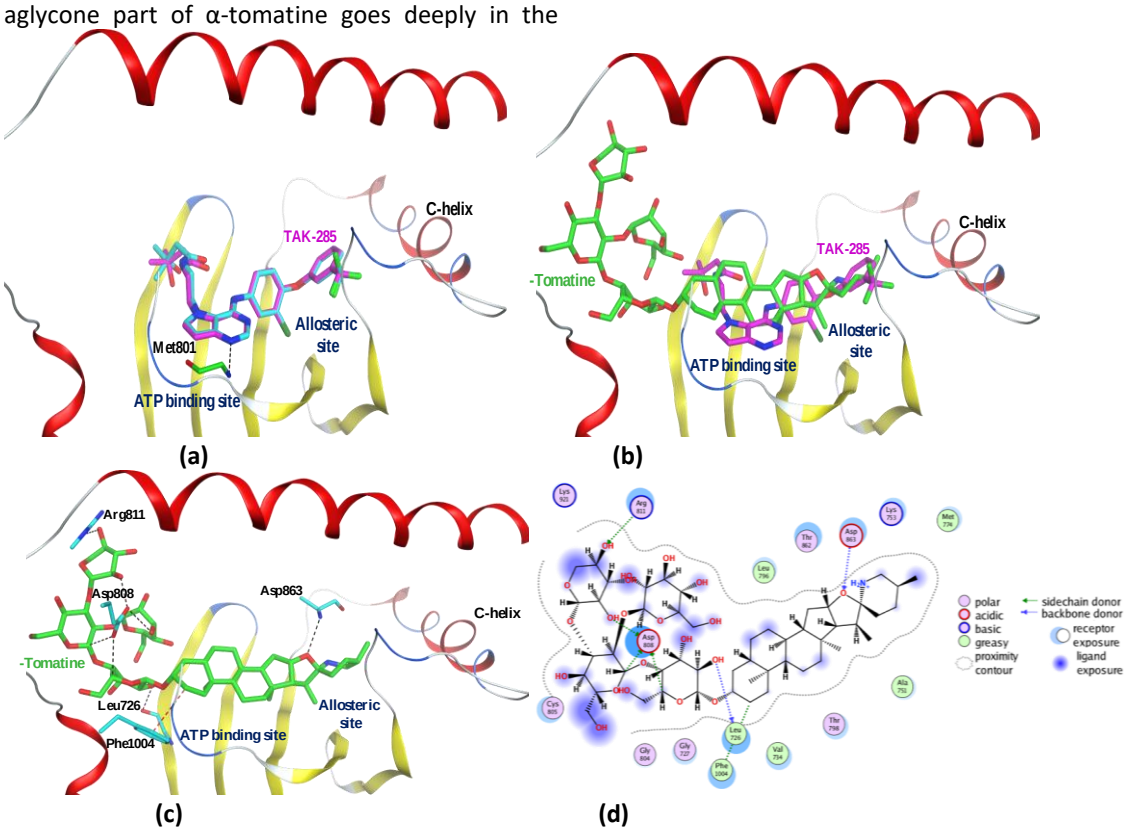


Figure 6. The top-scoring docked pose of **α-tomatine** to the HER2 active site (PDB code 3RCD) as predicted by MOE 2019.01. (a) Comparison of modeled binding mode of the co-crystallized ligand **TAK-285** (magenta sticks) and its superposed docking conformation (cyan sticks). (b) Comparison of modeled binding mode of **α-tomatine** (green sticks) and **TAK-285** (magenta sticks). (c) Detailed binding mode of **α-tomatine** (green sticks) displaying hydrogen bonds (black dashed line) and CH-π interactions (red dashed

line) with the key amino acid residues (cyan sticks). (d) 2D depiction of **α-tomatine** binding interactions with the key amino acid residues.

Overall, the molecular docking output were matched with biological data and proved that inhibition of kinases (EGFR and HER2) by α-tomatine is one of its mechanism for its prominent cytotoxicity on A549. To explain the high cytotoxic activity of α-tomatine against A549, we then investigated another mechanism of action. To this end, the inhibitory effect of α-tomatine on DNA cleavage at 3 µM and 1 µM were performed using plasmid DNA (pUC19 DNA) with and without the iron (II) ($FeSO_4$), hydrogen peroxide (H_2O_2), and ascorbic acid complex as shown in Figure 7.

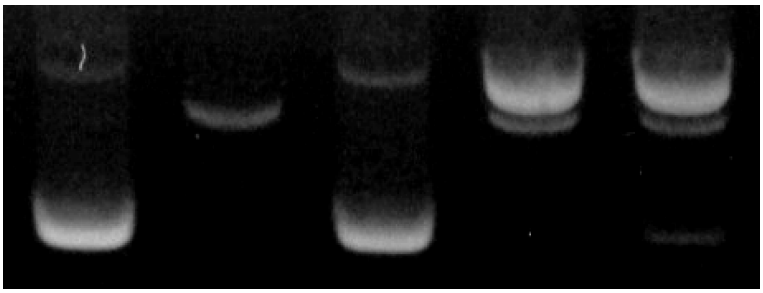


Figure 7. The DNA cleavage activity of **α-tomatine** at 3 µM and 1 µM concentrations with and without of $FeSO_4$, H_2O_2, and ascorbic acid system. Supercoil pUC19 DNA (form I), single strand break DNA (form II), and double strand break DNA (form III) bands after an agarose gel electrophoresis. Form III (the second lane from the left) was generated by the pUC19 DNA cut using restriction enzyme *Eco*R I.

The reaction was accomplished at 37°C for 2 h, and then the samples were loaded to 1 % agarose gel for electrophoresis (100 V for 30 min). After that, the gel was stained and the bands of the supercoiled DNA (form I), single strand break DNA (form II), and double strand break DNA (form III) were visualized under ultraviolet radiation (UV) illuminator. α-tomatine strongly cleaved the DNA in a dose dependent manner through the activation of the iron II complex. Thus, it is considered that α-tomatine may generated activated oxygen and cleaved the DNA. These results suggest that high cytotoxic activity of α-tomatine on A549 may correlate with its dual effects as multiple kinase inhibition and DNA cleavage.

Conclusion

The present study showed for the first time, to the best of our knowledge, that α-tomatine inhibited efficiently EGFR and HER2 tyrosine kinases. It was also found that α-tomatine strongly disintegrated DNA at low concentration with iron (II) complex system as an activator. Taken together, α-tomatine has multi-faceted mechanism of action against NSCLC and two of them are anti-EGFR/HER2 and DNA-cleaving activity. These results suggest that α-tomatine highly surpasses the gold standard erlotinib indicating the presence of other potential molecular targets that we will explore, in the near future.

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References

Ali, T. F. S., Ciftci, H. I., Radwan, M. O., Koga, R., Ohsugi, T., Okiyama, Y., Honma, T., Nakata, A., Ito, A., Yoshida, M., Fujita, M., Otsuka, M. 2019. New SIRT2 inhibitors: Histidine-based bleomycin spin-off. Bioorgan Med Chem, 27, (9), 1767-1775.

Arkhipov, A., Shan, Y. B., Kim, E. T., Dror, R. O., Shaw, D. E. 2013. Her2 activation

mechanism reflects evolutionary preservation of asymmetric ectodomain dimers in the human EGFR family. Elife, 2.

Banappagari, S., Corti, M., Pincus, S., Satyanarayanajois, S. 2012. Inhibition of protein-protein interaction of HER2-EGFR and HER2-HER3 by a rationally designed peptidomimetic. J Biomol Struct Dyn, 30, (5), 594-606.

Bayrak, N., Yildirim, H., Yildiz, M., Radwan, M. O., Otsuka, M., Fujita, M., Tuyun, A. F., Ciftci, H. I., 2019. Design, synthesis, and biological activity of Plastoquinone analogs as a new class of anticancer agents. Bioorg Chem, 92.

Chan, B. A., Hughes, B. G. M. 2015. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. Transl Lung Cancer R, 4, (1), 36-54.

Chao, M. W., Chen, C. H., Chang, Y. L., Teng, C. M., Pan, S. L. 2012. Alpha-Tomatine-Mediated Anti-Cancer Activity In Vitro and In Vivo through Cell Cycle- and Caspase-Independent Pathways. Plos One, 7, (9), 1-13.

Ciftci, H. I., Bayrak, N., Yildirim, H., Yildiz, M., Radwan, M. O., Otsuka, M., Fujita, M., Tuyun, A. F. 2019. Discovery and structure-activity relationship of plastoquinone analogs as anticancer agents against chronic myelogenous leukemia cells. Arch Pharm.

Ciftci, H. I., Ozturk, S. E., Ali, T. F. S., Radwan, M. O., Tateishi, H., Koga, R., Ocak, Z., Can, M., Otsuka, M., Fujita, M. 2018. The First Pentacyclic Triterpenoid Gypsogenin Derivative Exhibiting Anti-ABL1 Kinase and Anti-chronic Myelogenous Leukemia Activities. Biol Pharm Bull, 41, (4), 570-574.

Ciftci, H.I., Radwan, M. O., Ozturk, S.E., Ulusoy, N. G., Sozer, E., Ellakwa, D. E., Ocak, Z., Can, M., Ali, T. F. S., Abd-Alla, H. I., Yayli, N., Tateishi, H., Otsuka, M., Fujita, M. 2019. Design, Synthesis and Biological Evaluation of Pentacyclic Triterpene Derivatives: Optimization of Anti-ABL Kinase Activity. Molecules, 24, (19), 1-15.

Citri, A., Yarden, Y. 2006. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Bio, 7, (7), 505-516.

Denisenko, T. V., Budkevich, I. N., Zhivotovsky, B. 2018. Cell death-based treatment of lung adenocarcinoma. Cell Death Dis, 9.

Dong, Q. J., Yu, P. F., Ye, L., Zhang, J. Z., Wang, H. B., Zou, F. X., Tian, J. W., Kurihara, H. 2019. PCC0208027, a novel tyrosine kinase inhibitor, inhibits tumor growth of NSCLC by targeting EGFR and HER2 aberrations. Sci Rep-Uk, 9.

Goebel, C., Louden, C. L., Mckenna, R., Onugha, O., Wachtel, A., Long, T. 2019. Diagnosis of Non-small Cell Lung Cancer for Early Stage Asymptomatic Patients. Cancer Genom Proteom, 16, (4), 229-244.

Hou, X. J., Zhang, L., Han, L. S., Ge, J., Ma, R. J., Zhang, X. S., Moley, K., Schedl, T., Wang, Q. 2015. Differing roles of pyruvate dehydrogenase kinases during mouse oocyte maturation. J Cell Sci, 128, (13), 2319-2329.

Huang, H. R., Chen, S. H., Van Doren, J., Li, D. L., Farichon, C., He, Y., Zhang, Q. Y., Zhang, K., Conney, A. H., Goodin, S., Du, Z. Y., Zheng, X. 2015. Alpha-tomatine inhibits growth and induces apoptosis in HL-60 human myeloid leukemia cells. Mol Med Rep, 11, (6), 4573-4578.

Ibrahim, M. A., El-Alfy, A. T., Ezel, K., Radwan, M. O., Shilabin, A. G., Kochanowska-Karamyan, A. J., Abd-Alla, H. I., Otsuka, M., Hamann, M. T. 2017. Marine Inspired 2-(5-Halo-1H-indol-3-yl)-N, N-dimethylethanamines as Modulators of Serotonin Receptors: An Example Illustrating the Power of Bromine as Part of the Uniquely Marine Chemical Space. Mar Drugs, 15, (8).

Inamura, K. 2017. Lung Cancer: Understanding its Molecular Pathology and the 2015 WHO Classification. Front Oncol, 7.

Ishikawa, T., Seto, M., Banno, H., Kawakita, Y., Oorui, M., Taniguchi, T., Ohta, Y., Tamura, T., Nakayama, A., Miki, H., Kamiguchi, H., Tanaka, T., Habuka, N., Sogabe, S., Yano, J., Aertgeerts, K., Kamiyama, K. 2011. Design and Synthesis of Novel Human Epidermal Growth Factor Receptor 2 (HER2)/Epidermal Growth Factor Receptor (EGFR) Dual Inhibitors Bearing a Pyrrolo[3,2-d]pyrimidine Scaffold. J Med Chem, 54, (23), 8030-8050.

Kanthala, S., Banappagari, S., Gokhale, A., Liu, Y. Y., Xin, G., Zhao, Y. F., Jois, S. 2015. Novel Peptidomimetics for Inhibition of HER2:HER3 Heterodimerization in HER2-Positive Breast Cancer. Chem Biol Drug Des, 85, (6), 702-714.

Koga, R., Radwan, M. O., Ejima, T., Kanemaru, Y., Tateishi, H., Ali, T. F. S., Ciftci, H. I., Shibata, Y., Taguchi, Y., Inoue, J., Otsuka, M., Fujita, M. 2017. A Dithiol Compound Binds to the Zinc Finger Protein TRAF6 and Suppresses

Its Ubiquitination. Chemmedchem, 12, (23), 1935-1941.

Koinis, F., Kotsakis, A., Georgoulias, V. 2016. Small cell lung cancer (SCLC): no treatment advances in recent years. Transl Lung Cancer R, 5, (1), 39-50.

Lee, K. R., Kozukue, N., Han, J. S., Park, J. H., Chang, E. Y., Baek, E. J., Chang, J. S., Friedman, M. 2004. Glycoalkaloids and metabolites inhibit the growth of human colon (HT29) and liver (HepG2) cancer cells. J Agr Food Chem, 52, (10), 2832-2839.

Lee, S. T., Wong, P. F., Cheah, S. C., Mustafa, M. R. 2011. Alpha-Tomatine Induces Apoptosis and Inhibits Nuclear Factor-Kappa B Activation on Human Prostatic Adenocarcinoma PC-3 Cells. Plos One, 6, (4).

Liu, S. W., Li, S., Hai, J., Wang, X., Chen, T., Quinn, M. M., Gao, P., Zhang, Y. X., Ji, H. B., Cross, D. A. E., Wong, K. K. 2018. Targeting HER2 Aberrations in Non-Small Cell Lung Cancer with Osimertinib. Clin Cancer Res, 24, (11), 2594-2604.

Liu, Q., Yu, S. N., Zhao, W. H., Qin, S., Chu, Q., Wu, K. M. 2018. EGFR-TKIs resistance via EGFR-independent signaling pathways. Mol Cancer, 17.

Park, J. H., Liu, Y. T., Lemmon, M. A., Radhakrishnan, R. 2012. Erlotinib binds both inactive and active conformations of the EGFR tyrosine kinase domain. Biochem J, 448, 417-423.

Prabhakar, C. N. 2015. Epidermal growth factor receptor in non-small cell lung cancer. Transl Lung Cancer R, 4, (2), 110-118.

Radwan, M. O., Ciftci, H. I., Ali, T. F. S., Ellakwa, D. E., Koga, R., Tateishi, H., Nakata, A., Ito, A., Yoshida, M., Okamoto, Y., Fujita, M., Otsuka, M. 2019. Antiproliferative S-Trityl-l-Cysteine -Derived Compounds as SIRT2 Inhibitors: Repurposing and Solubility Enhancement. Molecules, 24, (18).

Radwan, M. O., Koga, R., Hida, T., Ejima, T., Kanemaru, Y., Tateishi, H., Okamoto, Y., Inoue, J., Fujita, M., Otsuka, M. 2019. Minimum structural requirements for inhibitors of the zinc finger protein TRAF6. Bioorg Med Chem Lett, 29, (16), 2162-2167.

Riely, G. J.; Yu, H. A., EGFR: The Paradigm of an Oncogene-Driven Lung Cancer. Clin Cancer Res 2015, 21, (10), 2221-2226.

Shida, W., Tateishi, H., Tahara, Y., Fujita, M., Husham Majeed Alsaadi, D., Watanabe, M., Koga, R., Radwan, M. O., Ciftci, H. I., Gezici, S., Kurauchi, Y., Katsuki, H., Otsuka, M., Sugimura, K., Wada, M., Sekeroglu, N., Watanabe, T. 2019. Antileukemic Activity of Twig Components of Caucasian Beech in Turkey. Molecules, 24, (21).

Shieh, J. M., Cheng, T. H., Shi, M. D., Wu, P. F., Chen, Y., Ko, S. C., Shih, Y. W. 2011. Alpha-Tomatine Suppresses Invasion and Migration of Human Non-Small Cell Lung Cancer NCI-H460 Cells Through Inactivating FAK/PI3K/Akt Signaling Pathway and Reducing Binding Activity of NF-kappa B. Cell Biochem Biophys, 60, (3), 297-310.

Shih, Y. W., Shieh, J. M., Wu, P. F., Lee, Y. C., Chen, Y. Z., Chiang, T. A. 2009. Alpha-Tomatine inactivates PI3K/Akt and ERK signaling pathways in human lung adenocarcinoma A549 cells: Effect on metastasis. Food Chem Toxicol, 47, (8), 1985-1995.

Sucha, L., Hroch, M., Rezacova, M., Rudolf, E., Havelek, R., Sispera, L., Cmielova, J., Kohlerova, R., Bezrouk, A., Tomsik, P. 2013. The cytotoxic effect of alpha-tomatine in MCF-7 human adenocarcinoma breast cancer cells depends on its interaction with cholesterol in incubation media and does not involve apoptosis induction. Oncol Rep, 30, (6), 2593-2602.

Sun, S., Schiller, J. H., Spinola, M., Minna, J. D. 2007. New molecularly targeted therapies for lung cancer. J Clin Invest, 117, (10), 2740-2750.

Takeda, M., Nakagawa, K. 2019. First- and Second-Generation EGFR-TKIs Are All Replaced to Osimertinib in Chemo-Naive EGFR Mutation-Positive Non-Small Cell Lung Cancer? Int J Mol Sci, 20, (1).

Tan, C. S., Kumarakulasinghe, N. B., Huang, Y. Q., Ang, Y. L. E., Choo, J. R. E., Goh, B. C., Soo, R. A. 2018. Third generation EGFR TKIs: current data and future directions. Mol Cancer, 17.

Tang, J., Salama, R., Gadgeel, S. M., Sarkar, F. H., Ahmad, A. 2013. Erlotinib resistance in lung cancer: current progress and future perspectives. Front Pharmacol, 4.

Tateishi, H., Monde, K., Anraku, K., Koga, R., Hayashi, Y., Ciftci, H. I., DeMirci, H., Higashi, T., Motoyama, K., Arima, H., Otsuka, M., Fujita, M. 2017. A clue to unprecedented strategy to HIV eradication: "Lock-in and apoptosis". Sci Rep-Uk, 7.

Travis, W. D. 2015. WHO Classification of the Pathology and Genetics of Tumors of the Lung. J Thorac Oncol, 10, (9), S68-S68.

Wang, X., Teng, F. F., Kong, L., Yu, J. M. 2016. PD-L1 expression in human cancers and its association with clinical outcomes. Oncotargets Ther, 9, 5023-5039.

Wood, E. R., Shewchuk, L. M., Ellis, B., Brignola, P., Brashear, R. L., Caferro, T. R., Dickerson, S. H., Dickson, H. D., Donaldson, K. H., Gaul, M., Griffin, R. J., Hassell, A. M., Keith, B., Mullin, R., Petrov, K. G., Reno, M. J., Rusnak, D. W., Tadepalli, S. M., Ulrich, J. C., Wagner, C. D., Vanderwall, D. E., Waterson, A. G., Williams, J. D., White, W. L., Uehling, D. E. 2008. 6-Ethynylthieno[3,2-d]- and 6-ethynylthieno[2,3-d]pyrimidin-4-anilines as tunable covalent modifiers of ErbB kinases. P Natl Acad Sci USA, 105, (8), 2773-2778.

Yang, Y. W., Wu, C. A., Morrow, W. J. W. 2004. The apoptotic and necrotic effects of tomatine adjuvant. Vaccine, 22, (17-18), 2316-2327.

Zimmermann, S. P. a. S. 2014. Targeted therapy in SCHLOCK driven by HER 2 insertions. Transl Lung Cancer R, 3, (2), 84–88.