SECTION 1. DRUG DEVELOPMENT AND EFFECTS

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IN SILICO IDENTIFICATION AND BIOCHEMICAL VALIDATION OF PLAUSIBLE MOLECULAR TARGETS OF 4-THIAZOLIDINONE DERIVATIVE LES-3833 AS A POTENTIAL ANTICANCER AGENT

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Synthetic 4-thiazolidinone derivatives have a broad range of pharmacologic activities. Thus, 4-thiazolidinones are being investigated to create new molecules and develop active pharmaceutical substances for anticancer treatment. In our previous study, we investigated the pyrazoline-thiazolidinone-isatin conjugates, and determined that Les-3833 was the most active compound and might act through inhibition of PARP-, MAPK-, JNK-, Bcl-2-, CDK1/cyclin B, and/or the caspase family. The aim of this research was to perform molecular docking studies to enable the construction of a pharmacophore model for the Les-3833 compound and investigate probable biological targets. Pharmacophore modeling software packages performed molecular docking studies of probable biological targets and enabled the construction of a pharmacophore model. Docking models of Les-3833 with 11 enzymes involved in apoptotic mechanisms were studied. Based on the pharmacophore modeling results for all 11 enzymes, Les-3833 is predicted to be most active in Chk-1, caspase-6, and caspase-8. Immunoblot analysis proved that the application of Les-3833 led to inhibition of Ser345 phosphorylation, which is induced by etoposide, the most important modification responsible for Chk-1 activity. Taken together with the results of the docking studies, several mechanisms for the expression of antitumor activity by 4-thiazolidinones are suggested, and such multi-affinity is a characteristic feature of all these derivatives. The docking analysis confirmed the affinity of test compound Les-3833 for a topoisomerase II inhibitor and a high possibility of inhibitory interaction with Chk-1, caspase-6, and caspase-8.

K e y w o r d s: thiazolidinones, molecular docking, pharmacophore modeling, apoptosis.

Introduction

The development of innovative antitumor agents is an actual challenge for modern medicinal chemistry. Synthetic heterocyclic compounds are widely used in medical practice. Their advantages are the options for chemical modification of their molecules by organic synthesis for the enhancement of their biological activity. The combination of various chemical substituents by the pharmacophore-hybrid approach is useful for the generation of novel substances that can increase the efficacy of the active substance [1].

The 4-thiazolidinone derivatives are characterized by high pharmacological activity, low toxicity,

and the possibility for extensive chemical modification [2,3]. Synthetic 4-thiazolidinone derivatives have a broad range of pharmacologic activities [2,4]. Therefore, 4-thiazolidinones and related heterocycles are being intensively investigated to create new molecules and develop active pharmaceutical substances [5].

Among the non-fused 4-thiazolidinone derivatives, considerable attention is drawn to the pyrazoline-thiazolidinone-isatin conjugates due to their high antitumor activity. 4-Thiazolidinone-induced apoptosis has been demonstrated in various tumor cell lines [6]. The induction of apoptosis by 4-thiazolidinones might occur through inhibition of PARP-, MAPK-, JNK-, Bcl-2-, CDK1-cyclin B or dependence on the caspase family [5]. The mechanism by which apoptosis is triggered is primarily associated with inhibition of the Bcl-2/Bcl-XL function, although other effects of 5-ene-4-thiazolidinones on mitochondrial-mediated apoptotic signaling pathways have been described [2, 3]. Homodimerized Bax acts on the anion channel localized in the outer membrane of the mitochondria, leading to the release of cytochrome *c*, which activates the caspase cascade [7].

4-Thiazolidinones have also been found to reduce the potential of the mitochondrial membrane in leukemic cells, which is one of the most important mechanisms of apoptotic cell death mediated by mitochondria [8]. Similar data were obtained by the action of 2-heterylamino-4-thiazolidinones, which caused the scattering of the mitochondrial membrane potential, as well as redox changes in the treated HT29 cells that had accumulated in the G2/M and sub-G0/G1 cell cycle phases [9].

The G1 checkpoint determines whether all conditions are favorable for cell division to proceed. External influences, such as growth factors, play a large role in carrying the cell past the G1 checkpoint. If a cell meets the requirements for the G1 checkpoint, the cell will enter the S phase and begin DNA replication. This transition, as with all of the major checkpoint transitions in the cell cycle, is signaled by cyclins and cyclin-dependent kinases (CDKs). Cyclins are cell-signaling molecules that regulate the cell cycle.

In our previous studies, we reported the design and synthesis of noncondensed heterocyclic compounds containing 4-thiazolidinone, 2,3-dihydro-1*H*-indol-2-one, and pharmacologically attractive pyrazoline moieties [10]. Among this series of pyrazoline-thiazolidinone-isatin conjugates, we determined that Les-3833 was the most active compound [11]. In biological studies, we compared Les-3833 with a less active compound from the same group – Les-3288 (Fig. 1).

The molecular mechanisms of action of 4-thiazolidinone derivatives remain unclear. While the most extensive investigations in the world of the cytotoxic actions of potential drugs take place at the National Cancer Institute (Bethesda, Maryland, USA), this testing does not involve studying the molecular mechanisms of action, without which it is impossible to determine effects on molecular targets or to justify chemotherapy regimens.

Molecular docking is an *in silico* receptor-directed virtual screening method designed to evaluate the level and energy of binding of a protein-ligand complex. This method is aimed at molecular recognition between the ligand and the target protein to

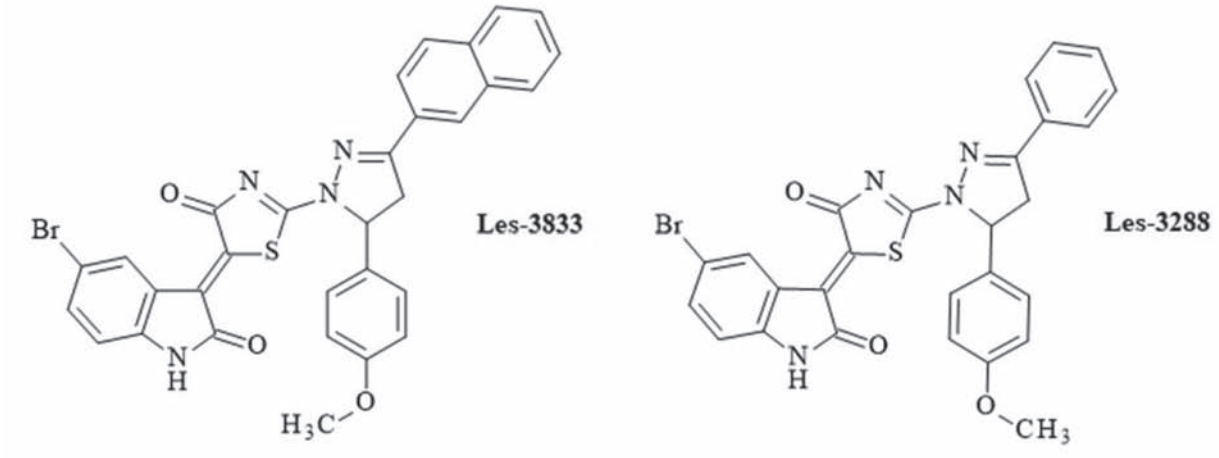


Fig. 1. Structures of the Les-3833 and Les-3288 compounds

select chemical compounds that bind most energetically to the active sites of biologically important targets. A combinatorial library of test substances can be created from which leading compounds can be identified for further studies of biological activity.

Pharmacophore modeling software packages (such as LigandScout) perform molecular docking studies of probable biological targets and enable the construction of a pharmacophore model. When constructing the model, the conformation of the known ligand in combination with the enzyme is used as the basis. The model includes data on the relative location of different functional groups. Importantly, the functional groups are separated into proton donors, proton acceptors and aromatic substituents. The construction of the pharmacophore model is based on several ligands with a similar structure, stacking them one on one. Moreover, the similar relative positions of the donors and proton acceptors, and aromatic fragments are taken into account, rather than the similarity in the chemical structure. In the next step, spatial models of the pharmacophore and test substance are superimposed. The program visualizes a qualitative result as an image in which the match of key snippets of molecular structures can be seen. The quantitative results are provided as the percentage of coincidence with the model and the number of overlapping pharmacophores. Based on these results, conclusions about the activity of the compound can be made.

The docking studies could provide qualitative and quantitative results for Les-3833 to explore the possible mechanisms of the core biological activities. Therefore, the aim of this research was to perform molecular docking studies for probable biological targets and enable the construction of a pharmacophore model *in silico.* The results should be confirmed with further investigations in cell cultures.

Materials and Methods

In silico studies

Enzyme structures used for modeling. 11 enzymes involved in apoptotic mechanisms were obtained from the Protein Data Bank (PDB, https://www.rcsb.org) and used in modeling (Table 1): CheckPoint kinase-1 (Chk-1, PDB ID: 2HXQ) [12], CheckPoint kinase-2 (Chk-2, PDB ID: 2XBJ), topoisomerase II (PDB ID: 5GWK), tyrosine kinase, (PDB ID: 1XBB), serine/threonine protein kinase (PDB ID: 4RF4), mitogen-activated protein kinase (PDB ID: 4ZSG), murine double minute 2 (MDM2) tumor protein p53 (MDM2-tp53, PDB ID: 5LAW), caspase-3, (PDB ID: 2XYG), caspase-6 (PDB ID: 4HVA) [13], caspase-8 (PDB ID: 3KJQ), and caspase-9 (PDB ID: 1TFQ) [14].

3D-structure optimization, molecular docking studies and virtual pharmacophore modeling. Spatial optimization of the structure of the molecule under study was performed using the HyperChem 7.5 software package (Hypercube, Inc., Gainesville, FL, USA; www.hyper.com). The method of molecular mechanics (MM) was used, with a root mean square (RMS) gradient of less than 0.1 kcal/(mole Å). The final minimization of the energies was carried out by the semi-empirical quantum chemical parametric method 3 (PM3) to achieve an RMS gradient of less than 0.01 kcal/(mole Å).

Docking studies were conducted using the AutoDock Vina® program (designed and implemented by Dr. Oleg Trott in the Molecular Graphics Laboratory at The Scripps Research Institute, La Jolla, CA, USA; vina.scripps.edu).

For visualization of the AutoDock results, we used BIOVIA Discovery Studio v20.1.0.19295 (https://www.3dsbiovia.com/products/collaborative-science/biovia-discovery-studio/visualization.html, Dassault Systèmes, San Diego, CA, USA)

For virtual pharmacophore modelling, we used the LigandScout 4.4.3 software package (Software-Entwicklungs und Consulting GmbH, Maria Enzersdorf, Austria). Pharmacophore modeling studies of Les-3833 binding with Chk-1, caspase-6 and caspase-8 involved inhibitors of these enzymes. The Chk-1 inhibitor contains a quinolone nucleus and an indole nucleus (full chemical name 3-(5-{[4-(aminomethyl)piperidin-1-yl]methyl}-1*H*-indol-2yl)quinolin-2(1*H*)-one) [12]. The caspase-6 inhibitor is furan derivative (N-[(1R)-1-[(3-cyanophenyl)methyl]-2-hydroxyethyl]-5-(3,4-dimethoxyphenyl)furan-3-carboxamide) [13]. The crystal structure of caspase-8 from the PDB contains only part of the inhibitor synthesized by the authors. This is because the whole molecule is relatively large and the allosteric site of enzyme can bind only its fragment – 2-(4-chlorophenyl)-N-(2-hexahydropyridazin-3-ylethyl)acetamide [14].

Cell culture

The glioma U251 cell line was derived from a malignant glioblastoma tumor by explant technique. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 2 mM glutamine, 1% non-

essential amino acids (NEAA), 1 mM sodium pyruvate (NaP), and 10% fetal bovine serum (FBS). All media were supplemented with 100 μg/ml penicillin/streptomycin (Gibco, Schwalbach, Germany). Cell lines were incubated at 37°C with 5% CO_2 in 95% humidity. Dimethyl sulfoxide (DMSO) solutions of Chk-1 inhibitor, etoposide (Chk-1 activator), Les-3833, and Les-3288 were stored at 4°C until use.

Experimental procedure. Cells were seeded 24 h prior to treatment, and were treated for 4 h at about 50-75% confluence. Chk-1 inhibitor (AZD7762, Sigma; Hamburg, Germany), Les-3833, and Les-3288 at concentrations of 1 μM were applied simultaneously with etoposide at a concentration of 10 μM.

Immunoblot and Western-blot analysis

Cells were treated with substances of interest or control substances and harvested. The pellet was washed with PBS at 4°C, resuspended in 200 μl of lysis buffer (PBS with protease and phosphatase inhibitor; Roche, Boulogne-Billancourt, France), lysed twice using ultrasonication for 10 s each, and cleared by centrifugation (10,000 g, 20 min, 4°C). For immunoblot analysis, 15 μg of protein were denatured in sodium dodecyl sulfate (SDS) sample buffer for 10 min at 98°C before loading onto a 12% NuPAGE Bis-Tris-protein gel (Thermo Fisher Scientific, Waltham, MA, USA). Proteins were transferred to polyvinylidene difluoride (PVDF) membranes via electroblotting for 2 h at 220 mA, and membranes were blocked overnight in TBS-T buffer (20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 1 ml of Tween 20) with 5% bovine serum albumin (BSA). All antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA) and used according to the manufacturer's recommendations. We used phospho-Chk1 (Ser345) (133D3) rabbit monoclonal antibody (mAb), horseradish peroxidase (HRP)-linked anti-rabbit IgG secondary antibody, and HRP-linked anti-mouse IgG secondary antibody. Equal loading was confirmed with β-actin (13E5) rabbit mAb. Clarity Western ECL Substrate (Bio-Rad, Munich, Germany) was used for detection.

Results

Docking models of Les-3833 (Fig. 1) with 11 enzymes involved in apoptotic mechanisms were studied. The results are shown in Table 1. Based on published reports in the literature and a preliminary COMPARE analysis [10], these enzymes could potentially be affected by Les-3833.

The docking studies showed a probable high-level affinity of Les-3833 for topoisomerase II with a binding energy of -12.9 kcal/mole and for mitogen-activated protein kinase at -11.5 kcal/mole (Table 1). However, when assessing the potential affinity for the other enzymes, there was also a high likelihood of inhibitory interaction, in particular for Chk-1 and Chk-2 (binding energy = -10.0 and -10.2 kcal/mole, respectively) and for serine/threonine protein kinase (binding energy = -10.2 kcal/mole) (Table 1).

Based on the pharmacophore modelling results from the LigandScout program for all 11 enzymes, Les-3833 is predicted to be most active in Chk-1, caspase-6 and caspase-8. For each enzyme, a pharmacophore model was constructed, upon which an optimized 3D structure of Les-3833 was overlaid.

The modeling of Les-3833 binding with Chk-1 is presented in Table 2. A virtual pharmacophore was based on the structure of the Chk-1 inhibitor obtained by Huang et al. [12]. This model showed five points of possible interaction with Chk-1: three of them are hydrophobic interactions from the benzene cycle of isatin and the 4-methoxyphenol ring with the amino acids leucine 15, 84 and 137, valine 23 and 68, and alanine 36 in the Chk-1 A-chain, and two possible hydrogen bonds from glutamic acid 85 and cysteine 87 of the Chk-1 A-chain with a secondary nitrogen and carbonyl group of the quinolone core of the inhibitor. The Pharmacophore-Fit Score of 47.45 calculated by the LigandScout program coincided with four of the five active points. Thus, Les-3833 was suggested to be an active compound.

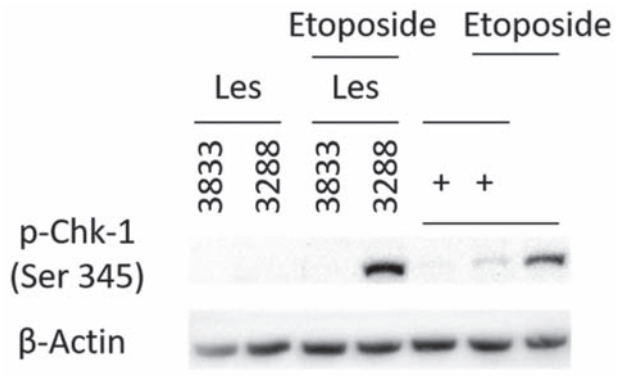


Fig. 2. Western-blot analysis of proteins of glioma U251 cells after treatment with Les-3833, Les-3288, etoposide and CheckPoint kinase-1 (Chk-1) inhibitor (indicated by "+"). Phospho-Chk-1 (p-Chk-1) (Ser345) was probed with rabbit monoclonal antibody (mAb)

T a b l e 1. Spatial structure optimization and binding energy for Les-3833[a] with 11 enzymes involved in apoptotic mechanisms

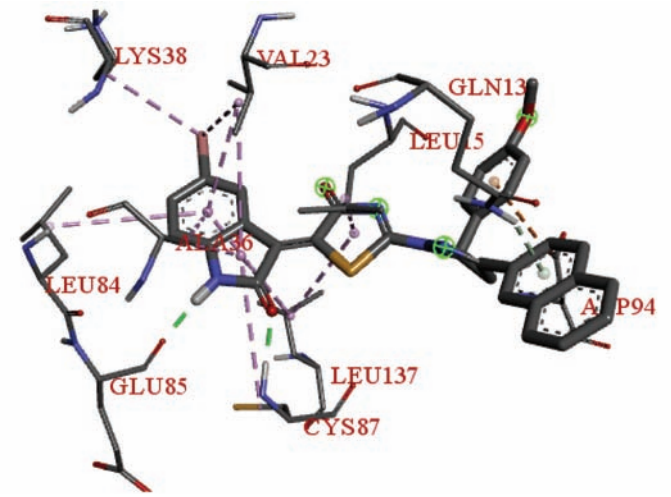
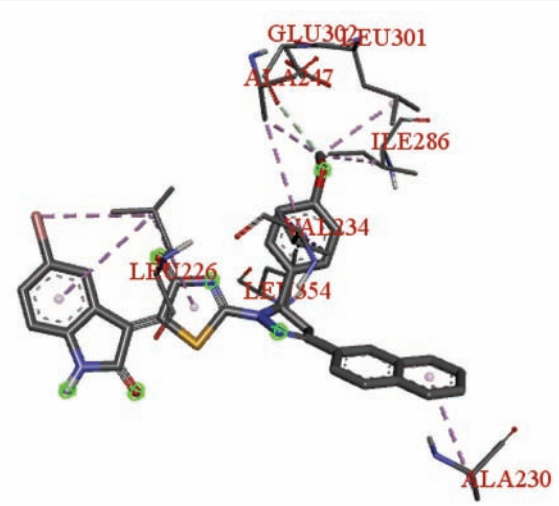
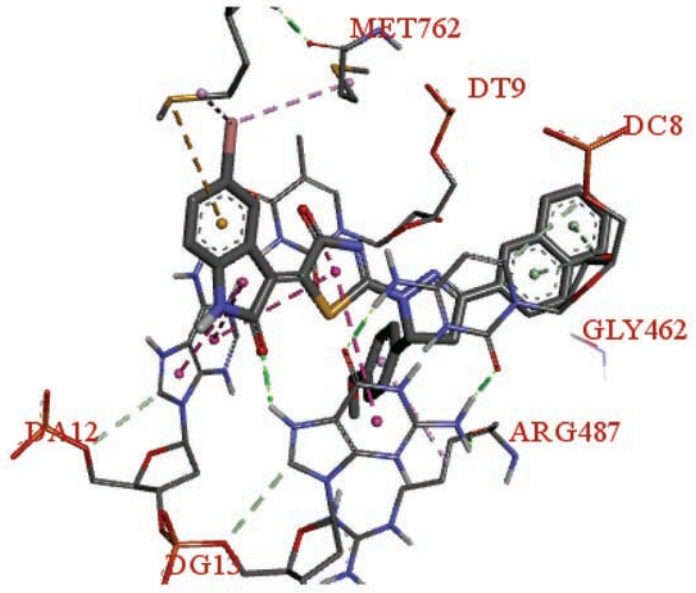
Enzyme	Visualization
CheckPoint kinase-1 (PDB ID:[b] 2HXQ) Binding energy = -10.0 kcal/mole NH (isatin) – CO (GLU85) Bond length = 1.863 Å CO (isatin) – NH (CYS87) Bond length = 1.792 Å	
CheckPoint kinase-2 (PDB ID: 2XBJ) Binding energy = -10.2 kcal/mole	
Topoisomerase II (PDB ID: 5GWK) kcal/mole Binding energy = -12.9 kcal/mole CO (isatin) – NH (Guanine 13) Bond length = 2.202 Å	

Table 1. Continuation

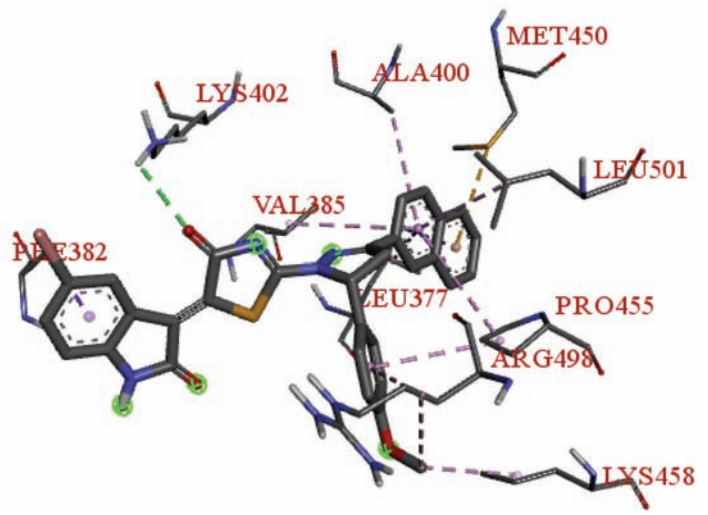
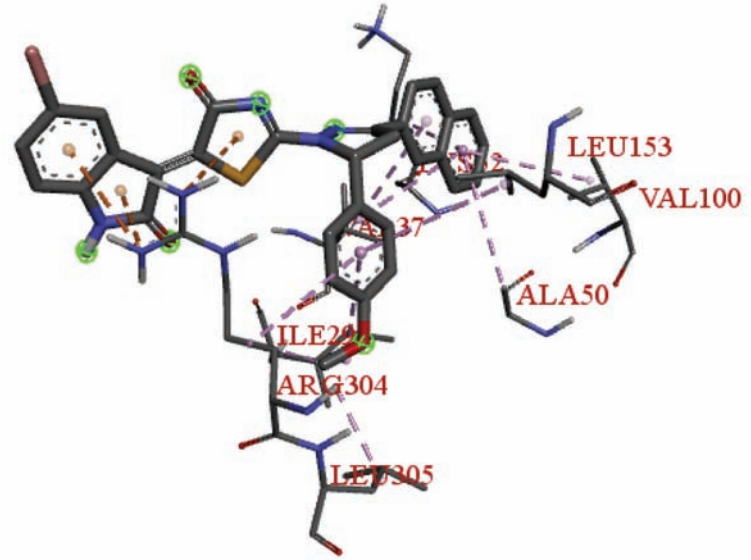
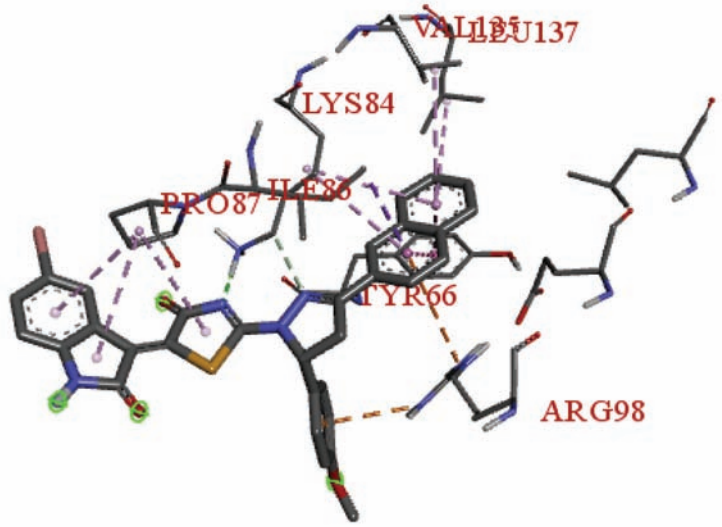
Tyrosine kinase (PDB ID: 1XBB) Binding energy = -9.8 kcal/mole CO (thiazoline) – NH (LYS402) Bond length = 2.704 Å	
Serine/threonine protein kinase (PDB ID: 4FR4) Binding energy = -10.2 kcal/mole	
Mitogen-activated protein kinase (PDB ID: 4ZSG) Binding energy = -11.5 kcal/mole N (thiazoine) – NH (LYS84) Bond length = 2.079 Å	

Table 1. Continuation

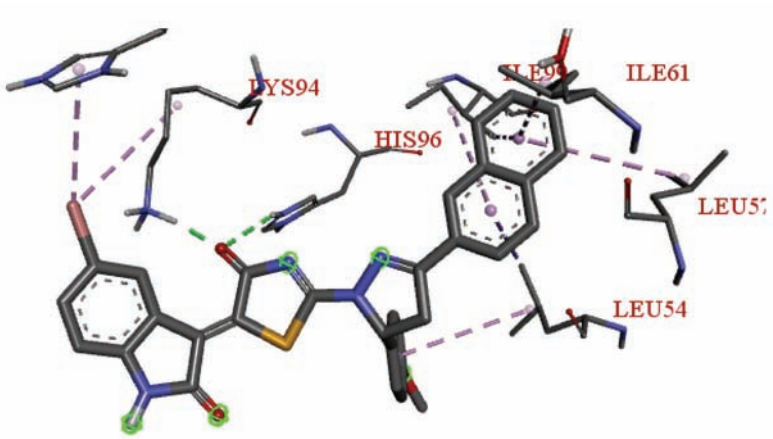
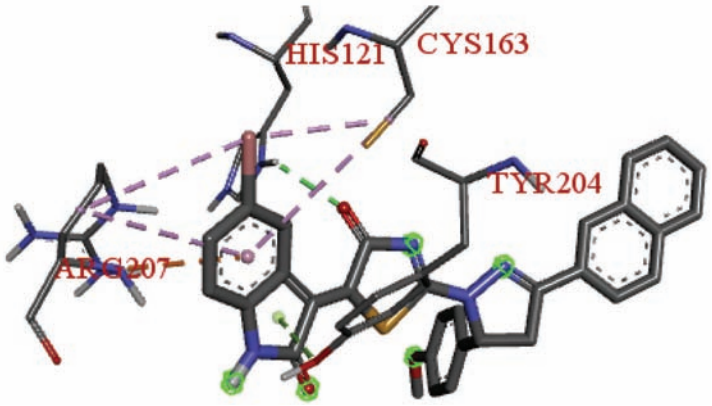
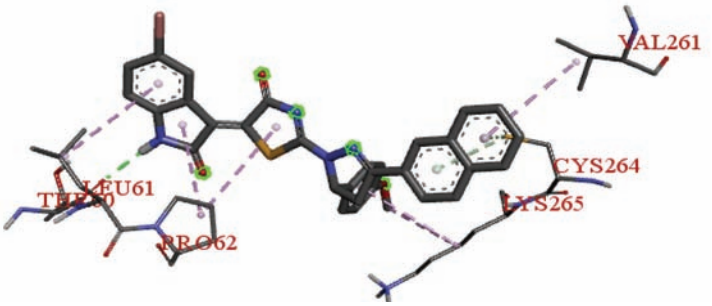
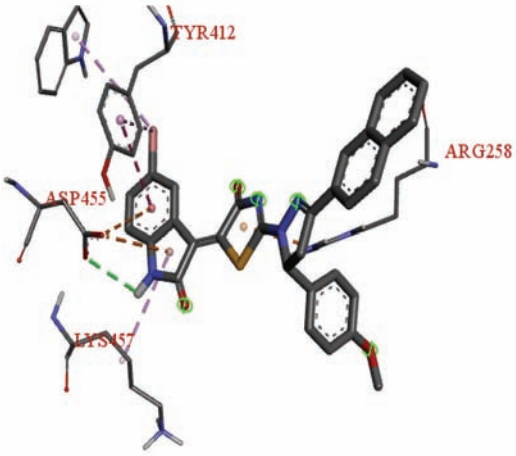
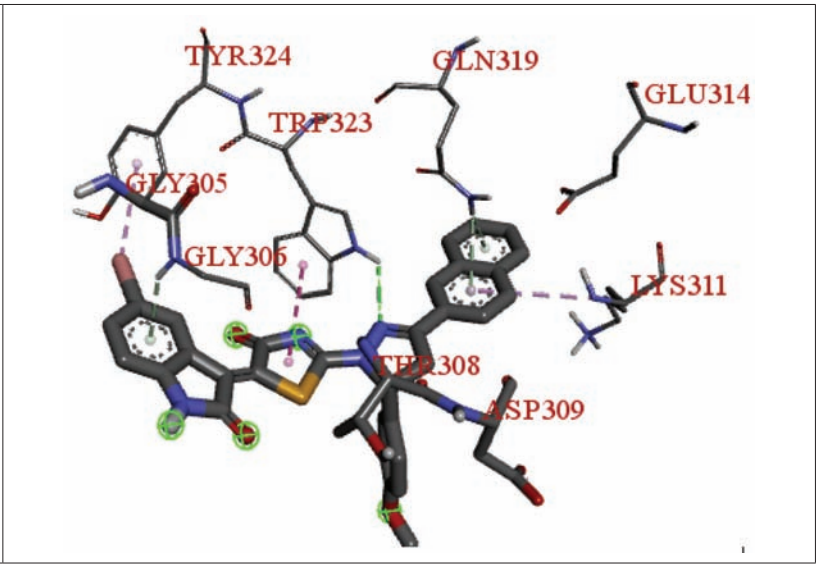
MDM2-tp53[c] (PDB ID: 5LAW) Binding energy = -7.8 kcal/mole O= (thiazoline) - LYS94 Bond length = 1.879 Å O= (thiazoline) – HIS96 Bond length = 2.749 Å	
Caspase-3 (PDB ID: 2XYG) Binding energy = -4.7 kcal/mole O= (thiazoline) – NH (HIS121) Bond length = 2.395 Å	
Caspase-6 (PDB ID: 4HVA) Binding energy = -7.8 kcal/mole NH (isatin) – O(THR60) Bond length = 2.15 Å	
Caspase-8 (PDB ID: 3KJQ) Binding energy = -7.8 kcal/mole NH (isatin) – O(ASP455) Bond length = 3.00 Å	

Table 1. Continuation

Caspase-9 (PDB ID: 1TFQ) Binding energy = -7.0 kcal/mole N1= (pyrazoline) – NH (TRP323) Bond length = 2.490 Å	

[a]Optimized 3D structure of Les-3833 is shown (skeletal structure with thicker lines), along with important segments of each enzyme structure. Lime-green circles indicate heteroatoms that make it possible for the Les-3833 molecule to form hydrogen bonds. Green dashes indicate potential hydrogen bonds between Les-3833 and the active centers of the enzymes. [b]Protein Data Bank identifier (https://www.rcsb.org/). [c]Murine double minute 2 (MDM2) tumor protein p53 (MDM2-tp53). *Abbreviations for amino acids:* ALA, alanine; ARG, arginine; ASN, asparagine; ASP, aspartic acid; CYS, cysteine; GLN, glutamine; GLU, glutamic acid; GLY, glycine; HIS, histidine; ILE, isoleucine; LEU, leucine; LYS, lysine; MET, methionine; PHE, phenylalanine; PRO, proline; SER, serine; THR, threonine; TRP, tryptophan; TYR, tyrosine; VAL, valine. *For topoisomerase II*: DA - adenine, DC - cytosine, DG - guanine, DT - thymine

Table 2. Model of the binding of Les-3833 with CheckPoint kinase-1 (Chk-1)

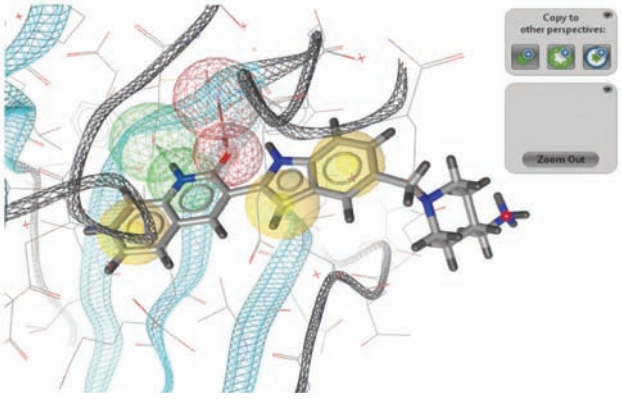
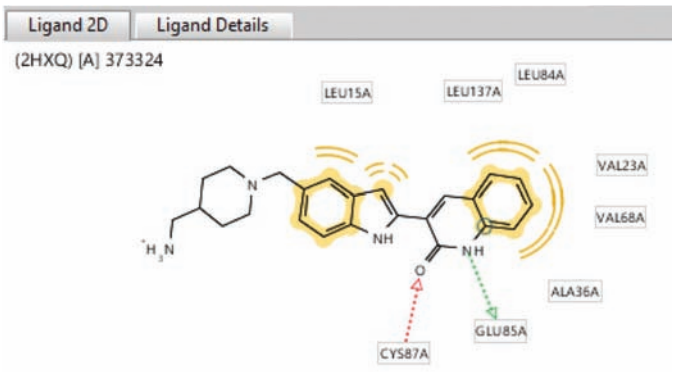
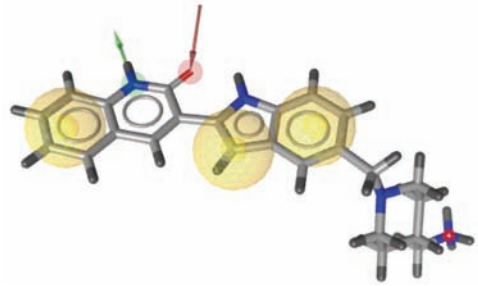
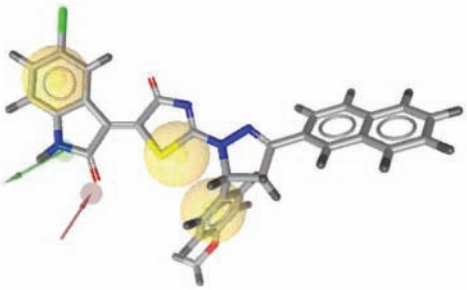
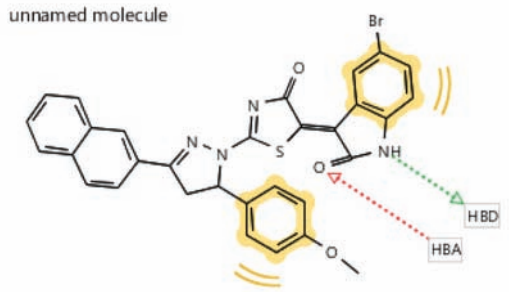
View of the Chk-1 active site with the Chk-1 inhibitor from Protein Data Bank (PDB ID: 2HXQ)	
2D model of the Chk-1 inhibitor with interaction points in the active site of Chk-1	

Table 2. Continuation

Spatial structure of Chk-1 inhibitor overlaided with a virtual pharmacophore	
Les-3833 is combined with a virtual pharmacophore	
2D model of Les-3833 with points of interaction in the Chk-1 active site	

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920	☐	unnamed molec...	920		44.06	3852

The Pharmacophore-Fit Score[a] of **47.45** suggests that the probable activity of Les-3833 coincides with four of the five active points

[a]Calculated by the LigandScout 4.4.3 software package. Yellow spheres, shading or curved lines indicate possible hydrophobic interactions. Green and red spheres or arrows indicate possible hydrogen bonds.

Abbreviations for amino acids: ALA, alanine; CYS, cysteine; GLU, glutamic acid; LEU, leucine; VAL, valine. HBA and HBD denote hydrogen bond

T a b l e 3. Model of the binding of Les-3833 with caspase-6

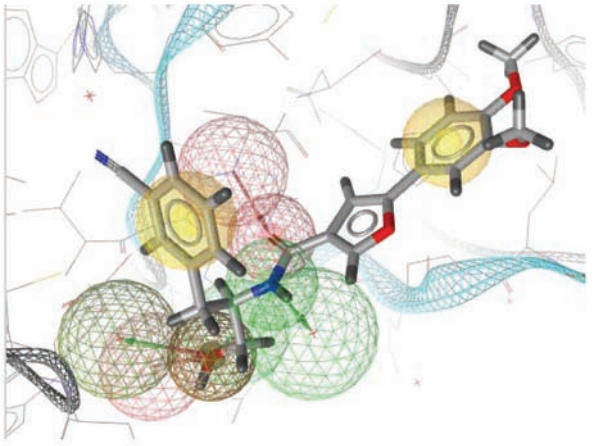
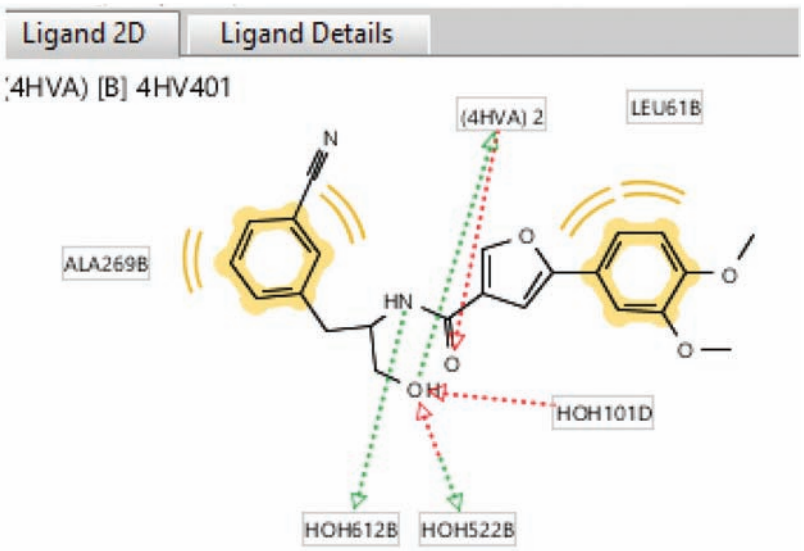
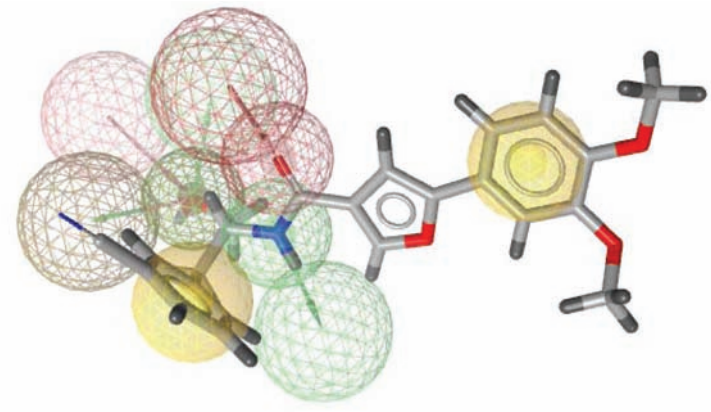
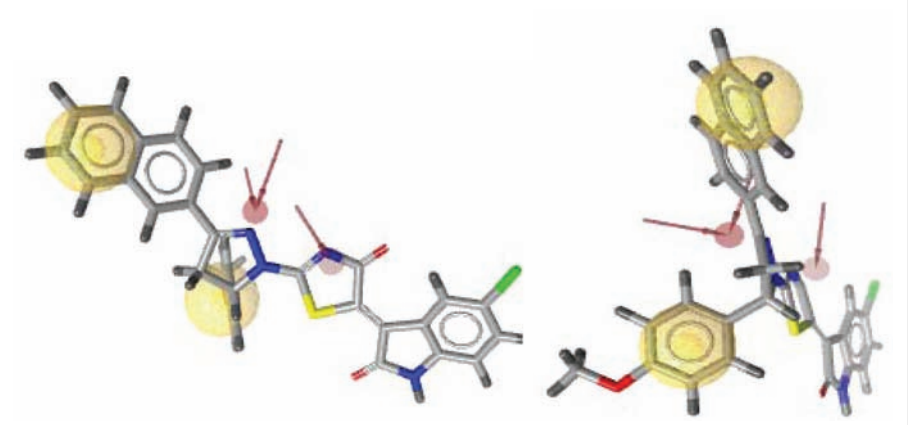
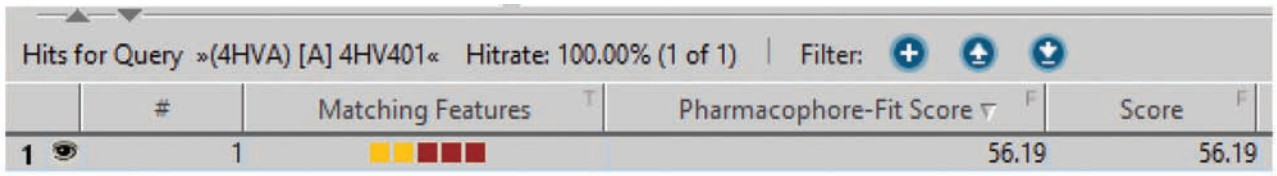
View of the caspase-6 active site with the caspase-6 inhibitor	
2D model of the caspase-6 inhibitor with points of interaction in the active site of caspase-6	
3D model of caspase-6 inhibitor is overlaid with a virtual pharmacophore	

Table 3. Continuation

Different views of Les-3833 combined with a virtual pharmacophore	
	
The Pharmacophore-Fit Score[a] of **56.19** and the coincidence with five of the six active points suggest the probable activity of Les-3833	

[a]Calculated by the LigandScout 4.4.3 software package. Yellow spheres, shading or curved lines indicate possible hydrophobic interactions. Green and red spheres or arrows indicate possible hydrogen bonds. *Abbreviations for amino acids*: ALA, alanine; LEU, leucine. 4HVA is the PDB ID for caspase-6. HOH and HBA denote hydrogen bonds

Immunoblot analysis showed that application of Les-3833 led to inhibition of Ser345 phosphorylation, which is induced by etoposide, the most important modification responsible for Chk-1 activity (Fig. 2). In contrast, no inhibition of this phosphorylation was observed by treatment with the similar 4-thiazolidinone derivative Les-3288.

The modeling of Les-3833 binding with caspase-6 is presented in Table 3. A virtual pharmacophore was based on the structure of the caspase-6 inhibitor synthesized by Heise et al. [13]. The modeling showed six points of possible inhibitor interaction with caspase-6: two of them are hydrophobic interactions from the naphthyl and phenol substituents with leucine 61 and alanine 269 of the caspase-6 B-chain, and four possible hydrogen bonds with the alcohol hydroxyl and carbonyl group as intermediate, and the nitrogen in the amide group. The Pharmacophore-Fit Score of 56.19 and the coincidence with five of the six active points suggest the probable activity of Les-3833.

The model of Les-3833 binding with caspase-8 is presented in Table 4. A virtual pharmacophore was based on the urazolopyridazine moiety of the caspase-8 inhibitor in the allosteric site of this protein as determined by Wang et al. [14]. The modeling showed five likely sites of its interaction with caspase-8: three of them are hydrophobic interactions of the 4-bromo-phenyl substituent with leucine 401, tryptophan 476 and 496, and phenylalanine 499 of the caspase-8 B-chain, and two possible hydrogen bonds of tryptophan 337 of the caspase-8 A-chain with the amide nitrogen and glutamine 396 of the caspase-8 B-chain with the carbonyl group. The Pharmacophore-Fit Score for Les-3833 of 44.23 and the coincidence with four of the five active points suggest the probable activity of Les-3833.

Discussion

Apoptosis is defined by morphological and biochemical changes mediated by the cysteine caspase family, which are expressed as inactive zymogens and are proteolytically converted to the active state after the action of the apoptotic stimulus [15]. There are two alternative pathways that lead to caspase activation. The extrinsic pathway is initiated by the binding of extracellular “death ligands” (most commonly Fas, DR3, TRAIL-R1/2(DR4/5), Apo-3L, DR6) to the transmembrane receptors of tumor necrosis factor TNF-R1, which leads to the activa-

Table 4. Model of the binding of Les-3833 with caspase-8

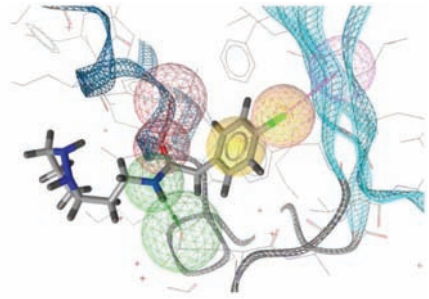
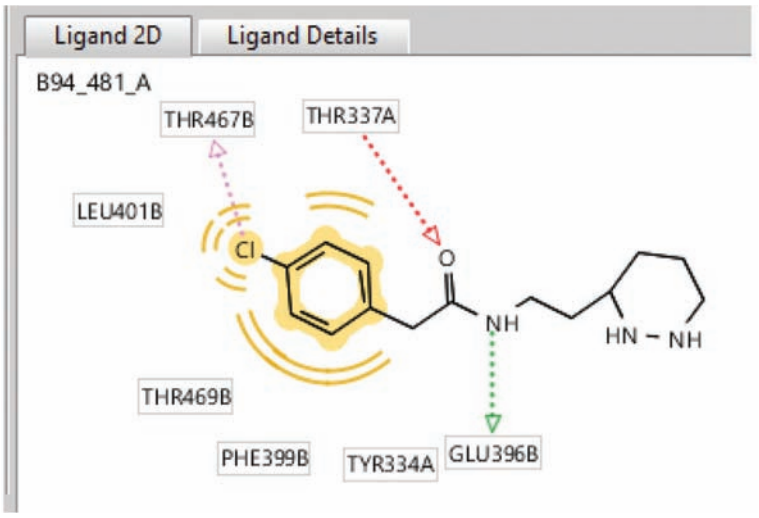
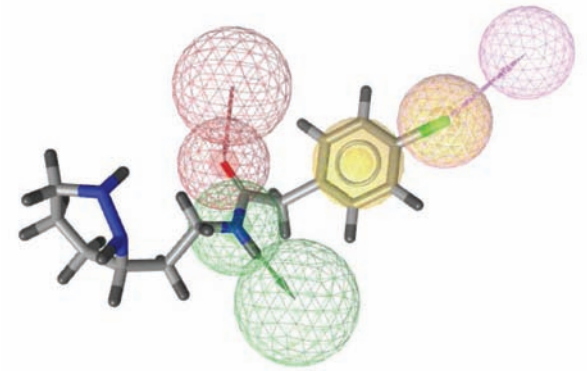
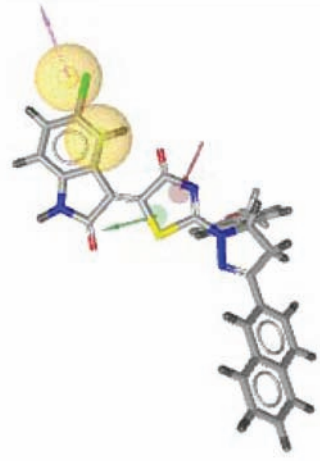
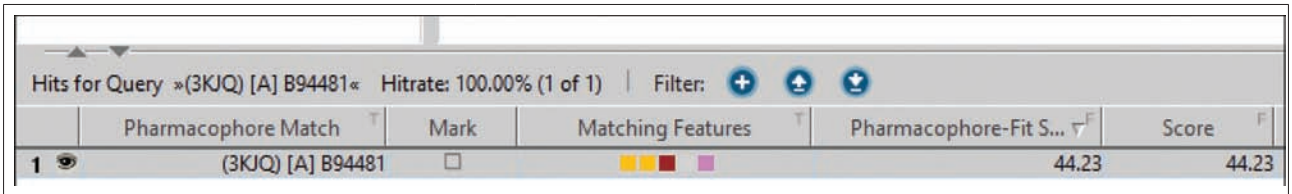
View of the caspase-8 active site with the caspase-8 inhibitor	
2D model of the caspase-8 inhibitor with points of interaction in the active site of caspase-8	
3D model of caspase-8 inhibitor overlapped with a virtual pharmacophore	
Les-3833 is combined with a virtual pharmacophore	

Table 4. Continuation



The Pharmacophore-Fit Score[a] of **44.23** and the coincidence with four of the five active points suggest the probable activity of Les-3833

[a]Calculated by the LigandScout 4.4.3 software package. Yellow spheres, shading or curved lines indicate possible hydrophobic interactions. Green and red spheres or arrows indicate possible hydrogen bonds. Lavender spheres or arrows indicate interaction with the halogen (Cl, Br). *Abbreviations for amino acids*: GLU, glutamic acid; LEU, leucine; PHE, phenylalanine; THR, threonine; TYR, tyrosine

tion of membrane-proximal initiating caspases (caspase-6 and caspase-8) [15,16]. The inner pathway requires damage to the mitochondrial membrane and release of mitochondrial proteins, including Smac/DIABLO, HtRA2, and cytochrome *c*. The function of cytochrome *c* from Apaf-1 is to induce the activation of caspase-9, which initiates the apoptotic cascade [8, 15]. There is switching between the external and internal paths. For example, caspase-8 can proteolytically activate the pro-apoptotic protein Bid, which facilitates the release of cytochrome *c* [15, 17].

To study the molecular mechanisms of induction of apoptosis by the action of Les-3833 in T-leukemia cells of the human Jurkat line, a Western blot analysis was performed on proteins involved in the regulation of apoptosis [18]. It was found that at a concentration of 1.5 μM Les-3833 leads to the activation of initiator caspase-2 and caspase-9 only for 24 h of incubation, whereas the activation of these caspases at a level of inhibition concentration 75 (IC75, 4.5 μM) was observed much earlier – for 12 h [18]. We speculated that the antitumor activity of Les-3833 might be due to a mechanism of caspase-2-mediated enhanced permeability and retention (EPR)-dependent apoptosis [18, 19].

To establish that apoptosis is actually induced by a specific agent, apoptosis expression needs to be confirmed by using at least three alternative approaches. In our previous study, we conducted a Western-blot analysis of apoptosis-related proteins in Les-3833-treated melanoma cells [20]. The levels of activated (cleaved) caspase-3 and inactivated (cleaved) poly-[ADP-ribose]-polymerase-1 (PARP-1) were increased in melanoma cells treated for 72 h with Les-3833 [20]. In addition, this compound stimulated phosphorylation of the extracellular-regulated kinase ½ (ERK½) that belongs to the mitogen-activated protein kinase (MAPK) family, induced the Endo G proteins, and decreased the level of STAT3 protein.

In order to obtain more direct evidence of apoptosis induced by Les-3833 in human melanoma WM793 cells, the activation of caspase-3 and cleavage of the repair enzyme PARP-1 were assessed by Western blot analysis. In addition, ERK½ /MAPKs, and Endo G levels were increased under the action of Les-3833. Both caspase-3 (a key member of pro-apoptotic caspase cascade) and PARP-1 are principal biochemical markers of apoptosis, while the protein kinases ERK½/MAPKs were shown to respond to the action of various stressing agents, including the anticancer drugs [20].

These results allow us to substantiate the incomplete coincidence of correlation coefficients of topoisomerase II inhibitor doxorubicin and Les-3833, since Les-3833 probably affects an additional 4 units of carcinogenesis, which causes a difference in the coefficient of correlation. According to the National Cancer Institute, Les-3833 is a bifunctional compound with a membrane-binding domain and inhibitory activity of the cytosolic ATPase p97/VCP, which exhibits its mechanism of action through the endoplasmic reticulum, GP (growth percent) = -36.64% [8].

In response to DNA damage, cells activate a defense mechanism that requires phosphorylation of Chk-1 by ATP which activates Chk-1 [21]. It was shown that using Chk-1 inhibitors such as AZD7762 can block phosphorylation of Ser296, but not Ser345. Phosphorylation of Ser345 is crucial for Chk-1 activity, as this event increases the basal activity of Chk-1 by order of magnitude [22]. Etoposide can lead to phosphorylation of Chk-1 and Chk-2 and can be used as a model compound to induce the DNA damage

response [23]. Immunoblot analysis showed that Les-3833, but not Les-3288, led to inhibition of Ser345 phosphorylation, which was induced by etoposide. Conformational change or an additional site of binding may explain these results. Interpretation of these preliminary data requires further investigation.

Taken together, the results of the docking studies suggested several mechanisms for expression of antitumor activity by 4-thiazolidone derivatives, and that such multi-affinity is a characteristic feature of all these derivatives. However, it should be noted that according to the results of the preliminary COMPARE analysis [10], the highest Pearson correlation coefficients were observed with antitumor agents that interfere with transcription or translation processes such as actinomycin D (a DNA transcription inhibitor), echinomycin (an RNA synthesis inhibitor), bruceantin (a protein synthesis inhibitor), and deochromomycin (chromomyubin, a topoisomerase II inhibitor).

Caspase-8 has two pockets for binding: an active center and an allosteric site [8]. The allosteric site is located at the junction of two protein subunits. The active site is placed completely [8]. The allosteric site is rather small and contains only the urazolopyridazine fragment of the compound synthesized by the authors. In fact, this site in caspase-8 is a possible center of action for small molecules such as the 4-thiazolidinone derivatives we synthesized.

Conclusions. The molecular docking studies of the compound Les-3833 showed the affinity to topoisomerase II inhibitor, and a high opportunity for inhibitory interaction with Chk-1, caspase-6, and caspase-8.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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ПОШУК *IN SILICO* І БІОХІМІЧНЕ ОБГРУНТУВАННЯ ЙМОВІРНИХ МОЛЕКУЛЯРНИХ МІШЕНЕЙ ПОХІДНОГО 4-ТІАЗОЛІДИНОНУ LES-3833 ЯК ПОТЕНЦІЙНОЇ ПРОТИПУХЛИННОЇ СПОЛУКИ

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Синтетичні похідні 4-тіазолідинону мають широкий спектр фармакологічної дії, тому їх активно досліджують для створення нових молекул і розробки активних фармацевтичних інгредієнтів для хіміотерапії. У нашому попередньому дослідженні піразолін-тіазолідинон-ізатинових кон'югатів було встановлено, що Les-3833 є найактивнішою сполукою, яка може діяти шляхом інгібування біологічних мішеней PARP-, MAPK-, JNK-, Bcl-2-, CDK1/циклін B, та/або сімейства каспаз. Метою цього дослідження було проведення молекулярного докінгу, що дозволило побудувати модель фармакофору для сполуки Les-3833 і дослідити ймовірні біологічні мішені. Використовували програмний пакет AutoDock Vina ®. Просторову оптимізацію структури досліджуваної молекули виконували за допомогою програмного пакету HyperChem 7.5. Проведено дослідження молекулярного докінгу ймовірних біологічних мішеней, що дало змогу побудувати модель фармакофору. Вивчено молекулярні моделі Les-3833 із 11 ензимами, які беруть участь у механізмах апоптозу. За результатами фармакофорного моделювання цих 11 ензимів встановлено, що Les-3833 буде найактивнішим для Chk-1, каспази-6 та каспази-8. Імуноблот аналіз засвідчив, що Les-3833 призводить до пригнічення фосфорилування Ser345, яке індукується етопозидом, найважливішою модифікацією, яка відповідає за активність Chk-1. Пропонується кілька механізмів вираження протипухлинної

активності похідними 4-тіазолідинону, така мультиафінність є характерною особливістю для цих похідних. Докінг-аналіз підтвердив спорідненість досліджуваної сполуки Les-3833 до інгібітора топоізомерази II та високу можливість інгібувальної взаємодії з ензимами Chk-1, каспазою-6 і каспазою-8.

Ключові слова: тіазолідинони, молекулярний докінг, фармакофорне моделювання, апоптоз.

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