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ORIGINAL ARTICLE

In silico Activity and Target Prediction Analyses of Three Triazolothiadiazine Derivatives

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This study has been presented previously in 5th International BAU Drug Design Congress.

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Received: 7 February 2022, Accepted: 18 March 2022, Published online: 16 June 2022

ABSTRACT

Objective: Polypharmacology, interaction of one drug with multiple targets, emerged as an effective approach in drug discovery and development. Bioinformatics and cheminformatics methods are essential tools for determination of polypharmacological profiles of newly synthesized or known compounds and drugs. Previously, three novel triazolothiadiazine derivatives; **1h**, **3c** and **3h**, have been shown to induce apoptosis and cause cell cycle arrest on liver cancer cells. The aim of this study is to find possible action mechanisms and potential targets for these three triazolothiadiazine derivatives, and to investigate their potential as new therapeutic agents by using computational methods.

Materials and Methods: PASS software was used to identify biological activities and Swiss Target Prediction and BindingDB databases to predict potential targets for **1h**, **3c** and **3h**. PDE4A, ALR and DUSP1 proteins were selected for molecular docking analysis following the protein modeling of the three proteins.

Results: Activity prediction results show that **1h**, **3c** and **3h** might have phosphatase and signal transduction pathway inhibitor, hepatocyte growth factor antagonist, anti-inflammatory and antifungal activities. These derivatives are predicted as inhibitors of several phosphodiesterases by activity and target prediction tools.

Conclusion: Based on prediction and molecular docking results, it is proposed that these compounds may have therapeutic properties through new predicted targets.

Keywords: Molecular docking, pharmacology, similarity searching, triazolothiadiazines

INTRODUCTION

Polypharmacology is considered as an emerging approach in discovery and development of new drugs. The idea behind polypharmacology comes from the notion; a drug can act on different targets of a disease pathway or several disease pathways [1]. Bioinformatics approaches provide tools for drug development processes, such as target discovery and prediction of drug-target interactions [2,3]. Using computational methods for such studies ease the drug discovery and development process, and reduce the expanses on drug discovery [4,5]. Therefore, *in silico* activity and target prediction methods are valuable tools to estimate probable activities and targets for newly synthesized compounds.

For newly synthesized compounds, *in silico* target fishing is used to predict potential targets by mining chemical databases. Similarity searching is one of the approaches for searching potential targets for

a compound. The main idea for similarity searching is chemically similar compounds may interact with similar protein targets [6]. Therefore, by comparing the structure of newly synthesized compound with the structure of the known compounds with known targets, the potential targets for novel compound can be predicted.

Molecular docking is performed to assess the potential interaction or binding geometries according to the structure of the novel compound and target protein. Potential targets are virtually docked to novel compounds to identify potential interactions [7,8].

Previously, some novel triazolothiadiazine derivatives (**1a–3j**) have been synthesized, characterized and searched for their anti-proliferative effects on liver cancer cells [9]. Among 30 compounds, 5 of them, **1e**, **1g**, **1h**, **3c** and **3h** have been shown to cause apoptosis and cell cycle arrest at SubG1 phase of cell cycle. Two of the derivatives (**1g** and **1h**) have been studied in detail and proposed as promising anti-proliferative compounds acting by activating ASK-1 and inactivating Akt [9]. In this study, three of these derivatives **1h**, **3c** and **3h**, which may be potential therapeutic agents for cancers were selected. Detailed action mechanisms of these derivatives have not been shown in previous studies, since experimentally testing all possible interactions is not feasible. Biological activities and potential targets of **1h**, **3c** and **3h** were searched by using prediction tools. In order to show the interaction of the derivatives to predicted targets, molecular docking between three of the compounds were performed.

MATERIALS AND METHODS

Simplified Molecular Input Line Entry Specification (SMILES) generation for the compounds 1h, 3c and 3h

A SMILE is an ASCII string, which is used to represent the chemical structure of the compound. SMILES strings of three compounds were generated by using Swiss Target Prediction according to the structure of each molecule ([10], http://www.swisstargetprediction.ch/).

Druglikeness of the compounds 1h, 3c and 3h

Druglikeness of **1h**, **3c** and **3h** according to Lipinski's rule of five for evaluated with SwissADME ([11], http://www.swissadme.ch/index.php).

Computational determination of biological activities of the compounds 1h, 3c and 3h

Biological activity prediction for triazolothiadiazine derivatives was performed by using PASS online version 2.0 ([12], http://www.way2drug.com/passonline/). In order to estimate the probable activities of **1h**, **3c** and **3h**, SMILES strings for each compound loaded to PASS online. The prediction results were provided as predicted activities and corresponding probabilities as Pa (to be active) and Pi (to be inactive), ranging from 0 to 1. In the PASS prediction approach, biological activity is predicted based on the structural formula of a compound for more than 4000 kinds of biological activity with an average accuracy above 95%. Structure-activity relationships are considered for prediction in the training set involving more than 300.000 organic compounds [12]. When the Pa>Pi, the activity is probable for the compound. Pa>0.7 means in an experiment the chance of finding the predicted activity is high. When Pa<0.5, the chance of finding the predicted activity in an experiment is low, however, it also means that there is a chance of finding a structurally new compound. Between those values, which are 0.5<Pa<0.7, the probability of finding the activity in an experiment is less, since the compound is not similar to known pharmaceuticals [13, 14]. Therefore, the higher Pa values increase the chance to find the activity experimentally; also indicate the compound is similar to known pharmaceutical agents.

Target prediction for the compounds 1h, 3c and 3h

Swiss Target Prediction [10] (based on ChEMBL16) and BindingDB ([15], https://www.bindingdb.org/) were used to predict potential targets for each of the three compounds. The SMILES strings of the compounds were inputted to the databases separately and prediction results were reported.

Swiss Target Prediction computes 2D and 3D similarity values for the query compounds against the known ligands. In 2D similarity, FP2

fingerprints are used to define the molecules and Tanimoto coefficient (the number of shared fingerprint patterns/total number of fingerprint patterns) is used to quantify the similarity between molecules. In 3D similarity, different conformations of molecules are produced and Manhattan distances are calculated for all the conformations of each molecule. The target scores are calculated according to the logistic regressions with the 2D and 3D similarity scores of most similar ligands. Target scores rank the possible targets (between 0 to 1, larger when the query compound is the known ligand of the target) and are used to obtain the probability, which assesses the likelihood of the correct prediction [10].

Find My Compound's Targets (FMCT) tool of the BindingDB database was used to find targets for the compounds **1h**, **3c** and **3h**, according to the notion that similar compounds might bind the same proteins. FMCT provided the proposed protein targets with the similarity scores (max similarity) of query compounds to compounds in the database which bind to proposed protein targets, based on Similarity Ensemble Approach (SEA) [15].

Molecular Docking

Phyre2, Protein Homology/analogY Recognition Engine V 2.0, ([16], http://www.sbg.bio.ic.ac.uk/phyre2)) was used to prepare the models of human PDE4A, ALR and DUSP1, since the crystal structures of these proteins in PDB database were partial and/or in complex with other molecules or carried mutated residues. Docking calculations of the predicted target proteins to **1h**, **3c** and **3h** were performed using SwissDock ([8,17], http://www.swissdock.ch/). Following parameters were used for dockings; docking type "Accurate" and flexibility for side chains within 0Å. SwissDock reported FullFitness and Gibbs free energy (ΔG) to evaluate favorable bindings for each molecular docking. For each docking, the most energetically favorable binding, which has a greater negative FullFitness score, of the triazolothiadiazine derivative to the target protein was selected and visualized. UCSF Chimera [18], developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco was used to visualize the results generated by SwissDock and to prepare MOL2 files of the compounds.

RESULTS

Druglikeness of 1h, 3c and 3h

Lipinski's rule of five, which has been used evaluate the druglikeness of a chemical compound, predicts that if a compound has more than 5 H-bond donors, 10 H-bond acceptors, a molecular weight higher than 500 and calculated Log P greater than 5, it may show poorer absorption or permeation [19]. According to predictions, all three compounds met the criteria by having molecular weight between 406-449 (<500), H-bond donors 0 (<5) and H-bond acceptors between 4-5 (<10), although they did not have Log P less than 5, except 1h (Table 1).

Table 1. Physicochemical Properties of 1h, 3c and 3h

Descriptor	**Value 1h**	**Value 3c**	**Value 3h**
Molecular Weight (g/mol)	406.54	448.94	444.52
Consensus Log P	4.85	5.87	5.25
Rotatable Bonds	6	4	5
H-bond Acceptors	4	4	5
H-bond Donors	0	0	0

Biological activity prediction for the compounds 1h, 3c and 3h

Previously, the apoptotic and anti-proliferative activities of the compounds **1h**, **3c** and **3h** were shown experimentally [9]. In order to estimate new possible activities for these derivatives, PASS prediction tool was used. According to PASS predictions, compounds **1h**, **3c** and **3h** might have several biological activities, such as phosphatase and signal transduction pathway inhibitors, had anti-inflammatory effects, and were hepatocyte growth factor and Neuropeptide Y2 antagonists (Pa>0.5, Table 2). All three derivatives predicted as inhibitors of several phosphodiesterases (PDEs), Cyclin-dependent kinase 5 (CDK5), Protein-tyrosine phosphatase 2C (PTP2C, also known as SHP2 and PTPN11) and Dual specificity phosphatase 1 (DUSP1), which regulate removal of phosphate groups from tyrosine residues (Table 2).

Table 2. The predicted activities for 1h, 3c and 3h

	1h		3c		3h	
Activity	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
Phosphatase inhibitor	0.721	0.009	0.735	0.007	0.705	0.012
Hepatocyte growth factor antagonist	0.635	0.002	0.697	0.002	0.665	0.002
Neuropeptide Y2 antagonist	0.54	0.01	0.57	0.008	0.52	0.013
Signal transduction pathways inhibitor	0.526	0.026	0.779	0.008	0.718	0.011
Antiinflammatory	0.51	0.054	0.562	0.04	0.553	0.043
Calpain inhibitor	0.415	0.012	0.304	0.042	0.296	0.045
Anesthetic general	0.422	0.034	0.189	0.145	0.186	0.149
Growth factor antagonist	0.349	0.013	0.457	0.008	0.459	0.007
Respiratory distress syndrome treatment	0.345	0.016	0.301	0.028	0.303	0.028
Antifungal	0.369	0.057	0.29	0.085	0.263	0.099
Amyloid beta precursor protein antagonist	0.31	0.02	0.511	0.005	0.496	0.005
Alkaline phosphatase inhibitor	0.265	0.021	0.387	0.006	0.36	0.008
Anesthetic	0.256	0.05	-	-	-	-
5 Hydroxytryptamine release inhibitor	0.325	0.12	-	-	-	-
Phosphodiesterase 4A inhibitor	0.207	0.006	0.162	0.009	0.192	0.007
Phosphodiesterase 10A inhibitor	0.194	0.004	0.223	0.004	0.247	0.003
Phosphodiesterase X inhibitor	0.194	0.004	0.223	0.004	0.247	0.003
Cyclin-dependent kinase 5 inhibitor	0.182	0.005	0.214	0.004	0.19	0.004
Protein-tyrosine phosphatase 2C inhibitor	0.175	0.004	0.192	0.004	0.185	0.004
Sphingosine 1-phosphate receptor antagonist	0.206	0.04	0.189	0.054	0.138	0.108
Protein phosphatase inhibitor	0.176	0.039	0.142	0.053	0.16	0.045
Macrophage elastase inhibitor	0.138	0.01	0.099	0.018	0.099	0.018
Neuropeptide Y1 antagonist	0.149	0.03	0.148	0.031	0.148	0.032
Cyclin-dependent kinase inhibitor	0.14	0.023	0.192	0.015	0.159	0.019
Phosphodiesterase IV inhibitor	0.132	0.016	-	-	0.12	0.018
ATPase stimulant	0.22	0.106	-	-	-	-
Protein-tyrosine phosphatase inhibitor	0.152	0.039	0.134	0.047	0.149	0.04
Mucolytic	0.19	0.078	-	-	-	-
Phosphodiesterase 3A inhibitor	0.124	0.016	0.119	0.018	0.124	0.016
Sphingosine 1-phosphate receptor 1 antagonist	0.174	0.072	0.159	0.093	-	-
Mcl-1 antagonist	0.165	0.063	0.155	0.069	0.159	0.066
Dual specificity phosphatase 1 inhibitor	0.179	0.08	0.197	0.056	0.192	0.062
CC chemokine receptor 2B antagonist	0.167	0.069	-	-	-	-
Phosphodiesterase 4D inhibitor	0.108	0.017	0.065	0.037	0.103	0.019
Expectorant	0.181	0.093	-	-	-	-
Phosphodiesterase 4B inhibitor	0.101	0.018	0.066	0.038	0.104	0.017
HIF1A expression inhibitor	0.277	0.204	-	-	-	-
Phosphodiesterase inhibitor	0.114	0.043	-	-	0.098	0.053
Sphingosine 1-phosphate receptor 4 antagonist	0.142	0.075	0.12	0.11	-	-
Cyclooxygenase 3 inhibitor	0.087	0.021	-	-	-	-
Serum-glucocorticoid regulated kinase 1 inhibitor	0.23	0.165	-	-	-	-
Fibrosis treatment	0.116	0.068	0.11	0.077	0.126	0.054
Linoleate diol synthase inhibitor	0.235	0.187	-	-	-	-
Cardiotonic	0.208	0.16	-	-	-	-
Phosphodiesterase III inhibitor	0.08	0.032	-	-	0.067	0.042
Preneoplastic conditions treatment	0.259	0.212	-	-	-	-
Angiotensin II receptor agonist	0.138	0.1	-	-	-	-
Phosphodiesterase 4C inhibitor	0.066	0.029	-	-	0.061	0.033
Complement C5a chemotactic receptor antagonist	0.059	0.025	0.04	0.038	-	-
Antiviral (Rhinovirus)	0.295	0.263	-	-	-	-

Pa, probability to be active; Pi, probability to be inactive

Table 2. Continued

	1h		3c		3h	
Activity	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
Calcium channel N-type blocker	0.123	0.092	0.144	0.068	0.153	0.061
Biotinidase inhibitor	0.236	0.207	-	-	-	-
Platelet aggregation inhibitor	0.181	0.154	-	-	-	-
PRL phosphatase inhibitor	0.081	0.058	-	-	0.084	0.048
Benzoin aldolase inhibitor	0.054	0.033	-	-	-	-
Sphingosine 1-phosphate receptor 5 antagonist	0.041	0.026	-	-	-	-
Matrix metalloproteinase 1 (membrane-type) inhibitor	0.035	0.024	-	-	-	-
Vesicle monoamine transporter inhibitor	0.032	0.022	-	-	-	-
T-cell protein-tyrosine phosphatase inhibitor	0.032	0.022	-	-	0.03	0.027
Sphingosine 1-phosphate receptor 3 antagonist	0.061	0.056	-	-	-	-
Calcium channel (voltage-sensitive) activator	0.302	0.301	-	-	-	-
Plastoquinol-plastocyanin reductase inhibitor	0.180	0.180	-	-	-	-
Glycogen synthase stimulant	-	-	0.295	0.081	0.27	0.111
Diabetic neuropathy treatment	-	-	0.338	0.134	-	-
Janus tyrosine kinase 2 inhibitor	-	-	0.229	0.052	0.21	0.066
GABA receptor agonist	-	-	0.225	0.056	0.148	0.138
Histamine H1 receptor agonist	-	-	0.218	0.05	-	-
5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor	-	-	0.328	0.202	-	-
Fibromyalgia syndrome treatment	-	-	0.192	0.066	-	-
Chloride channel activator	-	-	0.218	0.1	-	-
Muscle relaxant	-	-	0.217	0.101	-	-
Dihydroorotase inhibitor	-	-	0.168	0.084	-	-
Rheumatoid arthritis treatment	-	-	0.202	0.127	0.169	0.163
Anticonvulsant	-	-	0.237	0.161	-	-
Sporulation kinase A inhibitor	-	-	0.101	0.034	-	-
Autophagy inducer	-	-	0.099	0.047	-	-
Lysyl oxidase inhibitor	-	-	0.227	0.203	-	-
Antianemic	-	-	0.123	0.099	-	-
Cognition disorders treatment	-	-	0.183	0.166	-	-
Premenstrual syndrome treatment	-	-	0.092	0.076	0.091	0.08
Skeletal muscle relaxant	-	-	0.179	0.163	-	-
Lanosterol 14 alpha demethylase inhibitor	-	-	0.111	0.097	-	-
Neuropeptide Y antagonist	-	-	0.085	0.078	-	-
Transglutaminase 2 inhibitor	-	-	0.09	0.085	0.096	0.068
Alpha 2d adrenoreceptor agonist	-	-	0.023	0.019	-	-
Scytalone dehydratase inhibitor	-	-	0.069	0.066	-	-
Potassium channel small-conductance Ca-activated activator	-	-	0.055	0.053	-	-
Glutamate release inhibitor	-	-	0.102	0.1	-	-
Antinociceptive	-	-	-	-	0.334	0.157
CYP2D15 substrate	-	-	-	-	0.264	0.255
Interleukin 1 antagonist	-	-	-	-	0.109	0.097
Antineoplastic	-	-	-	-	0.266	0.175
Calcium channel activator	-	-	-	-	0.203	0.138
Aspulvinone dimethylallyltransferase inhibitor	-	-	-	-	0.304	0.267

Pa, probability to be active; Pi, probability to be inactive

Target prediction for the compounds 1h, 3c and 3h

In order to predict the potential molecular targets of the compounds, Swiss Target Prediction [10] and BindingDB [15] were used. Swiss Target Prediction predicted muscleblind-like proteins (encoded by *MBNLs*), FAD-linked sulfhydryl oxidase ALR (ALR, which is encoded by *GFER*), several phosphodiesterases (encoded by *PDEs*) and microtubule-associated protein tau (encoded by *MAPT*) as potential targets for compounds **1h**, **3c** and **3h** (Table 3).

BindingDB couldn't predict any target for compound **3c**, while Cholinesterases were predicted as a target for both compounds **1h** and **3h**. cAMP-specific 3',5'-cyclic phosphodiesterase 4A (PDE4A), Carbonic anhydrases and Steroidogenic factor-1 (SF-1, encoded by *NR5A1*) were predicted as targets for only **1h** by BindingDB (Table 4).

Molecular Docking for Selected Targets with the compounds 1h, 3c and 3h

Molecular dockings were performed with the selected targets, PDE4A, ALR and DUSP1, which were predicted as targets for all three triazolothiadiazine derivatives by activity and/or target predictions. The most favorable scores between target proteins and compounds were selected and visualized. According to docking results compounds **3c** and **3h** might interact to the same region on DUSP1 protein (Figure 1). All derivatives predicted to interact at the same interaction site on ALR (Figure 2), while they predicted to interact at the different interaction region on PDE4A protein (Figure 3).

Table 4. Target prediction of 1h and 3h, by BindingDB (similarity 0.7)

Predicted targets	Max Similarity	
	1h	3h
Cholinesterases	0.70	**0.70**
cAMP-specific 3',5'-cyclic phosphodiesterase 4A	0.70	-
Carbonic anhydrase	0.70	-
Carbonic anhydrase 2	0.70	-
Carbonic anhydrases; II & IX	0.70	-
Steroidogenic Factor 1	0.70	-

Max Similarity, maximum similarity of the query compounds to BindingDB compounds tested against Targets

Table 3. The predicted targets of 1h, 3c and 3h by Swiss Target Prediction (based on ChEMBL16)

Gene	Target	Prediction probability		
		1h	3c	3h
MBNL1	Muscleblind-like protein 1	0.77	0.55	0.75
MBNL2	Muscleblind-like protein 2	0.77	0.55	0.75
MBNL3	Muscleblind-like protein 3	0.77	0.55	0.75
GFER	FAD-linked sulfhydryl oxidase ALR	0.64	0.53	0.63
PDE4A	cAMP-specific 3', 5'-cyclic phosphodiesterase 4A	0.51	0.34	0.63
PDE4B	cAMP-specific 3', 5'-cyclic phosphodiesterase 4B	0.51	0.34	0.63
PDE4C	cAMP-specific 3', 5'-cyclic phosphodiesterase 4C	0.51	0.34	0.63
PDE4D	cAMP-specific 3', 5'-cyclic phosphodiesterase 4D	0.51	0.34	0.63
PDE10A	cAMP and cAMP-inhibited cGMP 3', 5'-cyclic phosphodiesterase 10A	0.49	0.28	0.46
MAPT	Microtubule-associated protein tau	0.49	0.53	0.53
ALPL	Alkaline phosphatase tissue-nonspecific isozyme	0.4	0.1	0.37
ALPP	Alkaline phosphatase placental type	0.4	0.1	0.37
ALPI	Intestinal-type alkaline phosphatase	0.4	-	-
ALPPL2	Alkaline phosphatase placental-like	0.4	-	-
DYRK1A	Dual specificity tyrosine-phosphorylation-regulated kinase 1A	0.37	0.32	0.41
MCL1	Induced myeloid leukemia cell differentiation protein Mcl-1	-	0.16	-
PGR	Progesterone receptor	-	0.11	-
PDE3B	cGMP-inhibited 3', 5'-cyclic phosphodiesterase B	-	-	0.41
PDE3A	cGMP-inhibited 3', 5'-cyclic phosphodiesterase A	-	-	0.39

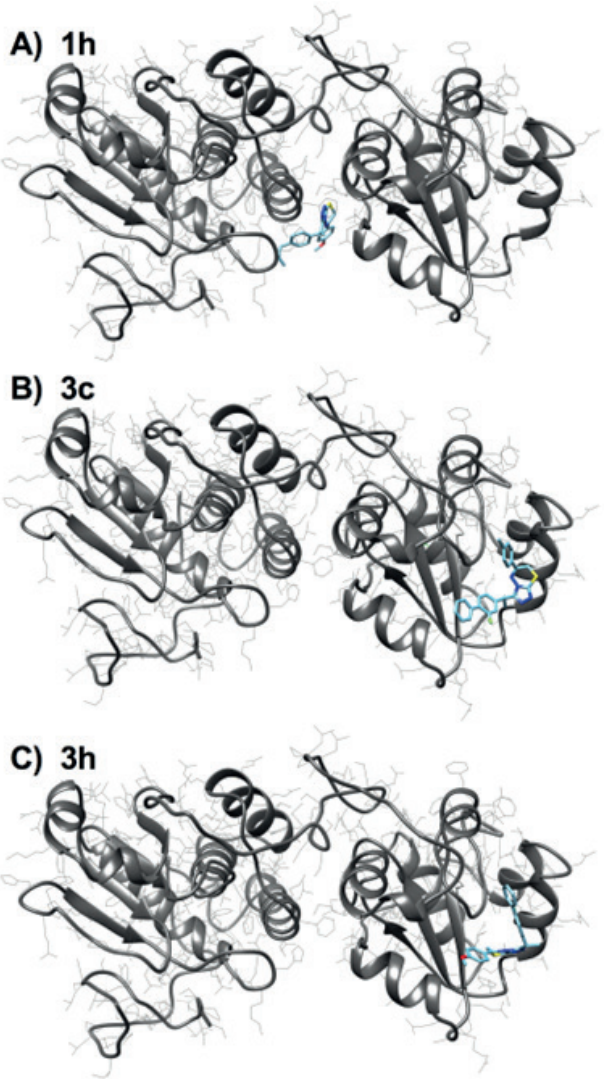

Figure 1. The most favorable molecular docking of DUSP1 and **1h** (A), **3c** (B) and **3h** (C). The modeling of DUSP1 was generated with Phyre2 [16]. SwissDock [8, 17] was used for dockings and the molecular docking results were visualized by UCSF Chimera package [18]. The FullFitness and deltaG Kcal/mol for 1h, -1705.54 and -8.20, for 3c -1680.96 and -8.16 and for 3h -1677.17 and -7.98.

DISCUSSION

Although, conventionally drugs or compounds have been designed to object a single biological target with high selectivity to avoid interaction of a drug with other biological molecules, the complexity of many diseases highlighted the importance of multi target drugs as effective therapeutic candidates [1,20,21]. Computational studies to predict novel activity and targets for known compounds are valuable instruments in using these compounds as therapeutics for diseases.

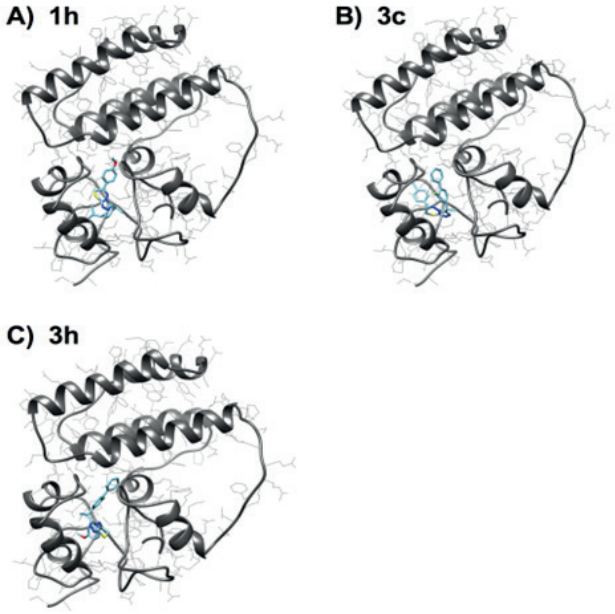

Figure 2. The most favorable molecular docking of ALR and **1h** (A), **3c** (B) and **3h** (C). The modeling of ALR was generated with Phyre2 [16]. SwissDock [8,17] was used for dockings and the molecular docking results were visualized by UCSF Chimera package [18]. The FullFitness and deltaG Kcal/mol for 1h, -1786.17 and -8.39, for 3c -1756.90 and -9.10 and for 3h -1759.01 and -9.17.

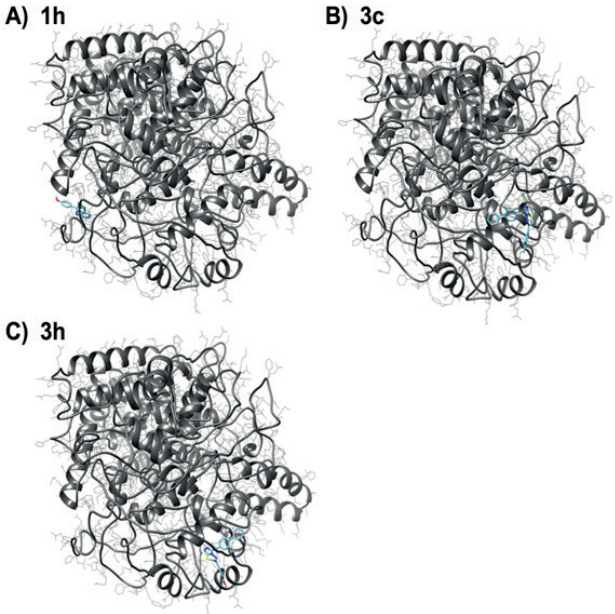

Figure 3. The most favorable molecular docking of PDE4A and **1h** (A), **3c** (B) and **3h** (C). The modeling of PDE4A was generated with Phyre2 [16]. SwissDock [8,17] was used for dockings and the molecular docking results were visualized by UCSF Chimera package [18]. The FullFitness and deltaG Kcal/mol for 1h, -5792.27 and -8.39, for 3c -5764.08 and -8.36 and for 3h -5762.75 and -8.62.

In this study, three triazolothiadiazine derivatives, **1h**, **3c** and **3h**, which have been demonstrated to cause cell cycle arrest at SubG1, indication of apoptosis, in liver cancer cells, was selected [9]. Possible action mechanisms and protein targets of these derivatives, were investigated by using computational methods. According to activity and target prediction results, potential targets and activities of **1h**, **3c** and **3h** were reported. PDE proteins, especially PDE4A, emerged from both biologic activity and target prediction results. Molecular docking analysis showed the potential interaction regions for three derivatives with PDE4A. In addition to PDE4A, possible interactions of three derivatives with ALR and DUSP1, which function in liver regeneration and cell signaling, respectively, were shown by molecular docking analysis. Based on the results presented here, these compounds may have therapeutic properties through new predicted targets.

Results for pharmacokinetic property predictions of 1h, 3c and 3h showed that these compounds might be considered as drug candidates, since they mostly met the Lipinski's rule of five (Table 1).

According to the activity prediction results, all three triazolothiadiazine derivatives had phosphatase inhibitor, alkaline phosphatase inhibitor, hepatocyte growth factor antagonist, Neuropeptide Y2 antagonist, signal transduction pathways inhibitor and anti-inflammatory activities (Table 2). In addition to activity prediction, target prediction results also proposed alkaline phosphatases as targets of **1h**, **3c** and **3h** (Table 3). Phosphatase, especially alkaline phosphatase inhibitor activity of three compounds might be relevant for therapeutic potential of these derivatives in cancers, since the coumarin–triazolothiadiazine hybrid compounds, which have alkaline phosphatase inhibitor activity, were reported as potential anticancer agents [22].

These three triazolothiadiazine derivatives, **1h**, **3c** and **3h**, have been shown to cause cell cycle arrest at SubG1 stage in liver carcinoma cells experimentally [9]. According to results presented in this study, these three triazolothiadiazine derivatives may have potential involvement in liver cell proliferation or regeneration with both activity and target prediction analyses (Table 2 and Table 3). ALR is a critical protein for hepatocyte survival and depletion of ALR caused apoptosis and necrosis in rat hepatocytes [23]. ALR depletion not only affects liver cells, in human derived glioma cells decreased ALR expression caused an increase in apoptosis [24]. Overexpression of ALR induced cell proliferation and inhibited cell death induced by H2O2 in normal human hepatic cell line [25]. In addition to intracellular functions, ALR has extracellular effects; ALR is released from damaged hepatocytes and has been proposed as a hepatic stress/injury marker [26]. In this study, ALR was predicted as target for all three compounds (Table 3). Also in molecular docking analyses, all three derivatives predicted to interact at the same region on ALR protein (Figure 2). These results suggest that in addition to reported factors [9], compounds 1h, 3c and 3h might effect the cell cycle progression in liver cancer cells via their interaction with ALR.

Previously, the action mechanism of one of the compounds, **1h**, was shown to be through the activation ASK-1 and inactivation of Akt proteins [9]. Activity prediction results estimated that all three compounds were protein-tyrosine phosphatase 2C (SHP2) and DUSP1 inhibitors (Table 2). SHP2, a mediator of Erk and PI3K/Akt signaling activation, has been related to cancer by its role in increasing cell proliferation and preventing apoptosis [27]. Therefore, several inhibitors have been developed to target SHP2 [28-30]. Since SHP2 has been shown to activate Akt signaling and prevent apoptosis [27], PASS prediction results on the inhibition of SHP2 by **1h**, **3c** and **3h** might be related with the previous report indicating **1h** inactivated Akt and increased apoptosis [9]. Compound **1h** has also been shown to decrease phosphorylation of ASK-1 and leads to ASK-1 activation. Activated ASK-1 activates JNK protein and causes apoptosis in liver cancer cells [9]. It has been reported that activated PI3-K/Akt signaling pathway cause a decrease in ASK-1 induced apoptosis through ASK-1 phosphorylation by Akt [31]. Therefore, by inhibiting SHP2 activity, compounds **1h**, **3c** and **3h** may inactivate Akt, which increase apoptosis in cells. However, it should be noted that, in addition to Akt, SHP2 is one of the regulators of ASK-1. SHP2 activates ASK1-JNK signaling pathway, by dephosphorylating ASK-1 [32]. PASS prediction tool also proposed compounds **1h**, **3c** and **3h** as DUSP-1 inhibitors (Table 2). In addition, according to molecular docking results, compounds **3h** and **3c** predicted to interact with the same region on DUSP1 protein, while **1h** interacted with a different region (Figure 1). DUSP1 also regulates

JNK mediated apoptosis; DUSP1 inactivates JNK by dephosphorylation and protects cancer cells from apoptosis [33]. Therefore, prediction results propose another action mechanism for these novel compounds on Akt and JNK signaling pathways.

The PDE4 family members that appeared in both activity and target prediction results (Table 2 and Table 3) coded by four genes; *PDE4A*, *PDE4B*, *PDE4C* and *PDE4D* [34]. PDE4A, which has been predicted to be a common target of all three derivatives by activity and target predictions, belongs to a protein family functioning in the cell signaling by hydrolyzing cyclic AMP (cAMP) and cyclic GMP (cGMP) [35]. PDE4A has been proposed as a potential therapeutic target for the anxiety and central nervous system disorders with its role in regulation of anxiety and emotional memory [36]. In addition, the therapeutic effects of inhibition of PDE family members have been shown in several health problems [37]. Phosphodiesterase inhibitors have been used as therapeutics for autoimmune diseases [38] and cancers [39]. Previously, triazolothiadiazines were shown to bind and inhibit PDE4 [40]. According to target and activity prediction and molecular docking results (Figure 3, Table 2 and Table 3), compounds 1h, 3c and 3h might interact with PDE4A. Therefore, **1h**, **3c** and **3h** might be PDE4A inhibitors and their potential therapeutic effect on PDE4A related diseases is worth to evaluate with further experimental studies.

In conclusion, activity and target prediction results proposed new possible activities and targets for the compounds 1h, 3c and 3h. Due to the relevance of predicted activities and targets with cellular mechanisms, all three derivatives might have different therapeutic activities, which need to be tested with experimental studies.

Author contribution

Study conception and design: CS and BT; data collection: CS and SPA; analysis and interpretation of results: CS; draft manuscript preparation: CS. All authors reviewed the results and approved the final version of the manuscript.

Funding

The authors declare that the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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