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Research Article

Investigating the anti-carcinogenic potential action of 1,2,3 triazole core compounds: impact of introducing an aldehyde or Nitro group, integrating cell line studies, and in silico ADME and protein target prediction

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Abstract

The development of novel chemotherapeutic drugs begins with the suppression of cancer and angiogenesis. Ringed compounds with one or more heteroatoms are known as heterocyclic compounds. In organic chemistry and the pharmaceutical sector, heterocyclic compounds containing nitrogen atoms are valuable. In pharmaceutical chemistry, molecules containing a 1,2,3-triazole skeleton are particularly favored. They have great stability, making it simple to bind them to biomolecular targets. In this work, two 1,2,3-triazole scaffolds containing new chemical entities were assessed using the MTT assay against two malignant (MCF-7 and HCT116) and one normal (HUVECs) cell lines with the goal of creating a new leading prodrug for cancer treatment. The ligands were well characterized by FTIR and 1HNMR. In silico ADMET studies show acceptable pharmacokinetic properties. With the aid of the ligands' SWISS target protein prediction, the in silico binding to target proteins was examined. The two compounds exhibited a dose-dependent cytotoxic effect, with the H4 compound demonstrating a favorable selectivity index against MCF-7 breast cancer, indicating its potential as a leading compound for anticancer prodrugs.

Keywords

cancer, 1,2,3 triazole scaffolds, ADME (absorption, distribution, metabolism, and excretion), selectivity index

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PENSOFT

Introduction

Globally, infectious illnesses and cancer represent a significant health burden on humanity (Kamal et al. 2010). The WHO predicts that in the upcoming years, the death rate from cancer will double (Parkin 2001). The cornerstone of cancer treatment, chemotherapy, has drawbacks such as poor selectivity, unfavorable side effects, and multidrug resistance (Hassan et al. 2015), as also confirmed by Hanahan and Weinberg (2011). However, infectious disorders also present a significant challenge to the medical community because of microbial resistance to the antimicrobial medications that are already on the market (Aslam et al. 2018), as established by Sekyere and Asante (2018). Due to weakened immunity, cancer patients who have had chemotherapy are more susceptible to microbial infections (Rostom et al. 2011). As a result, monotherapy with dual actions as an antibacterial and an anticancer agent may be more affordable and lessen the frequency of medication administration, adverse effects, and antimicrobial resistance. Since most anticarcinogenic medications are extremely toxic, efforts to create new, safer, and less toxic substances that can prevent or reduce the growth of cancer are accelerated (Sak 2012).

Herbs have long been used to treat cancer; in fact, the use of herbs is the primary source of many traditional medicines used to treat a wide range of ailments. Even though plant-derived molecules, such as those from Phoenix Dactylifera seeds (Husseina et al. 2018), Cuminum cyminum seeds extract confirmed by Mahmood A and Sahib H (2017), Zizyphus spinachristi leaf extracts (Abu-Raghif et al. 2015), Chalcones (Raghif), and Prunus Dulcis seed oil as mentioned by Ali ZK and Sahib HB (2023), are not commonly used as pharmaceuticals, researchers still consider plants to be essential sources for the discovery of novel medications as confirmed by Ahmad et al. 2018; Phukan and Sarma 2021.

The development of drugs has greatly benefited from the use of heterocycles to diminish side effects and overcome drug resistance. Many previous studies have proved that the flexible scaffold 1,2,3-triazole demonstrates potential pharmacological activity and has received growing attention as a source of both antimicrobial and anticancer drugs. It is present in many bioactive compounds, including antiviral (Gonzaga et al. 2018), antitubercular (Shaikh et al. 2015), anticancer (Ashour et al. 2020), antibacterial (Lal et al. 2018), and anti-HIV properties (Brik et al. 2005). Numerous marketed medications, including furamizole (an antibiotic), zibotentan (an anticancer medication), and raltegravir (an antiviral), have the 1,2,3-triazole core in their structures (Siddiqui et al. 2009). The process of creating novel therapeutic medications is usually costly and time-consuming. In silico technology may shorten the time and cost involved in drug discovery, but it has become indispensable in the modern pharmaceutical industry. Today, computational prediction tools are included in each step of the drug discovery process, thanks to developments in computational algorithms and knowledge databases. Effective application of computational drug discovery techniques has been demonstrated (Shaker et al. 2021). In the current study, we compared the anticancer activity of two N-benzyl phenyl isoserine derivatives that are related to the 1,2,3 triazoles, and they showed anti-inflammatory properties (Sabbar Omran et al. 2022; Sabbar Omran et al. 2023) against a variety of cancer cell lines. The structures of the two tested compounds are given in Figs 1, 2. In light of the compounds' potential effect as anti-tumor medicines, we evaluated their efficacy using preclinical cell-line cultures generated from human colon cancer lines (HCT116), breast cancer cells (MCF-7), and human umbilical ventricular endothelial cell lines (HUVECs).

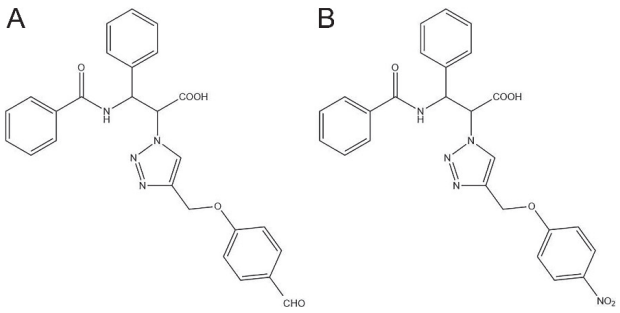

Figure 1. Chemical structure of H1(**A**) and H4(**B**) compounds.

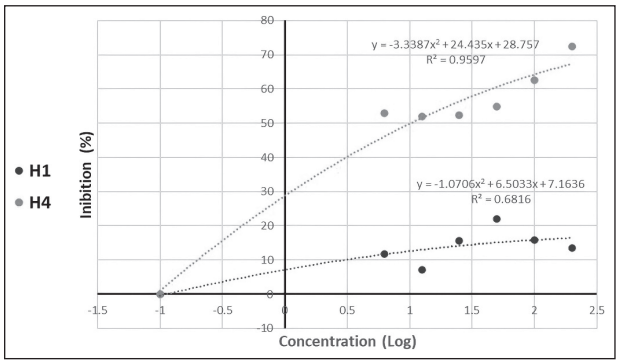

Figure 2. Comparison between H1 and H4 compounds on the inhibition of normal cells (HUVECs).

In the field of in silico prediction, various methods are employed to predict off-target hits in drug discovery, like the similarity ensemble approach (SEA), which has the advantages of utilizing ligand-based similarity to predict off-target interactions and can identify unreported targets of FDA-approved and investigational drugs. While the disadvantages are illustrated by the fact that the SEA approach relies on known ligand-target interactions, limiting its applicability to novel compounds, it also may not capture structural features critical for target binding (Keiser et al. 2009).

Machine learning models are other methods for in silico prediction. It comes with the advantages of the capability to analyze large datasets, identify complex patterns, and predict off-target interactions based on diverse data sources. Disadvantages include: performance may be limited by incomplete or irrelevant training data; and

challenges in predicting outside the training set due to chemical space and target diversity (Rao et al. 2019). The integration of multiple computational methods has been used for in silico prediction, with the benefits of combining ligand- and structure-based approaches for comprehensive predictions and the ability to improve predictive performance by considering drug-protein interactions in three dimensions. Its limitation is that it requires expertise in multiple computational techniques, and the integration complexity may impact the interpretability of results (Takai et al. 2023). Tanimoto similarity analysis, mentioned by Takai et al. (2023) in their retrospective analysis, has the advantages of evaluating chemical space coverage by the training set for prediction performance and providing insights into the relationship between compound similarity and prediction accuracy. While limitations to assessing chemical similarity may not capture target-specific interactions, the requirement for a reference training set for comparison, which may not always be comprehensive, is considered a disadvantage.

Within the FDA-approved target coverage, the Swiss Target Prediction has a bigger pharmacological space and has demonstrated a strong capacity to forecast the "old" targets for novel medications. Moreover, broad chemical space coverage with high computational efficiency is limited by the limitations of predictive accuracy and incomplete target coverage.

Each method has its strengths and limitations, and the choice of method depends on the specific research question and available data. Integrating multiple approaches can enhance the robustness of predictions and provide a more comprehensive understanding of off-target interactions in drug discovery (Ji et al. 2023).

This study aims to determine the effects of 1,2,3 triazole-related compounds as anti-cancer and antiangiogenic using three different cell lines, along with the ADME (absorption, distribution, metabolism, and excretion) prediction for the pharmacokinetic profile and targeted protein prediction for a possible explanation of the results.

Methods

This study was carried out at Al-Nahrain University, College of Medicine, Department of Pharmacology, during the period from the 1st of October 2022 to the 1st of April 2023. The study was given approval by the institutional Scientific and Ethical Committees. The new chemical entities will be referred to in the article as H1 and H4. Both compounds were generously provided by the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Al-Kafeel, Najaf, Iraq. The chemical structures of H1 are (3-benzamido-2-(4-((4-formylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylpropanoic acid) (Fig. 1A), and those of H4 are (3-benzamido-2-(4-((4-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylpropanoic acid) (Fig. 1B). The structure of the compounds was characterized by FTIR and ^{1}HNMR.

Analytical and spectral data for each compound, as presented by the supplier, are:

For H1 compound: FT-IR (KBr, cm-1): 1215-1237 ((N-N=N) stretching vibration), 1732 ((C=O) stretching vibration of carboxylic acid), 1656 ((C=O) stretching vibration of amide), 3024 ((C-H) aromatic stretching vibration), 3410 (Broad (O-H) stretching of carboxylic acid), 3352 (N-H stretching vibration of secondary amide), 1030 ((C-O) stretching vibration). 1H-NMR (300 MHz, DMSO-d6) δ 9.87 (s, 1H, -CHO), 8.75 (s, 1H, triazole ring), 7.96–7.09 (aromatic protons), 5.94 (d, J = 4.0 Hz, 1H, CH-triazole ring), 5.45 (d, J = 8.1, 3.9 Hz, 1H, Ph- CH-), 3.52 (s, 3H, -OCH3).

For H4 compound: FT-IR (KBr, cm-1): 1246 ((N-N=N) stretching vibration), 1739 ((C=O) stretching vibration of carboxylic acid), 1643 ((C=O) stretching vibration of amide), 3032 ((C-H) aromatic stretching vibration), 3363 (Broad (O-H) stretching of carboxylic acid), 3255 (N-H stretching vibration of secondary amide),1180 ((C-O-C) stretching vibration). ^{1}H-NMR (300 MHz, DMSO-d6) δ 9.27 (s, 1H, -CHO), 7.53 (d, 1H, triazole ring), 7.88–7.26 (aromatic protons), 4.99 (m, 1H, CH-N), 8.22 (m, 2H, C-NO2), 5.26 (s, 2H, -O-CH2), 9.27 (s, 1H, CHO).

Cell validity assay

MTT assay and % of cell growth inhibition

The cytotoxic effect of both compounds (H1 and H4) on cell viability and proliferation was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, according to the Mosmann method (Mosmann 1983). The American Type Culture Collection provided the human umbilical ventricular endothelial cell line (HUVEC), breast cancer cell line (MCF-7), and colon cancer cell line (HCT116). Every cell line was kept alive in its own medium using RPMI-1640. Additionally, 10% heat-inactivated fetal calf serum (HIFCS) was bought and used. To create the full growth medium specified in the growth medium sheet included with the cell line, 1% penicillin or streptomycin was added to those mediums. Before the trials, each prepared medium had a full growth medium. at a concentration of 1 × 104 /mL were planted into 96-well culture plates with 200 µl of complete growth medium and incubated with various concentrations (6.25, 12.5, 25, 50, 100, and 200 µg/mL) of the tested compounds (H1 and H4) each alone for 24 h. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess the test chemicals' effects on human cancer cell viability, which was expressed as the percentage of cell survival.

Cell growth viability and inhibition were calculated using the following formula:

$$\text{Cell viability (\%)} = (A_0/A) \times 100$$

$$\text{Cell inhibition (\%)} = 1\text{- cell viability (\%)}$$

Where: A_0 = absorbance of the samples A = absorbance of the negative control.

Using a microplate reader, the absorbance (A) was measured at 570 nm and the reference at 650 nm. Inhibitory concentration (IC_{50}) values were calculated by using linear and logarithmic equations.

2.1b. Calculation of selectivity index (SI)

The SI value can be calculated using the following equation (López-Lázaro 2015; Peña-Morán et al. 2016):

$$SI = IC_{50} \text{ (non-target cell line)}/IC_{50} \text{ (target cell line)}$$

Selectivity Index = IC_{50} for normal cells and IC50 for cancer cells.

ADME and target protein prediction

The absorption, distribution, metabolism, and excretion (ADME) pharmacokinetic profiles of the potential compounds were computed using Swiss ADME (Daina et al. 2017). The chemical structure of both tested compounds (H1 and H4) was drawn and sent to Swiss ADME (http://www.swissadme.ch) for SMILES file and group parameter calculations to assess drug-likeness, pharmacokinetics, lipophilicity, and physicochemical characteristics. Molecular weight (MW), specific atom counts (H-bond donors and acceptors), and topological polar surface area (TPSA) were used to represent the physicochemical features of the candidate molecule. The expected partition coefficient (LOGPo/w) of n-octanol and water was used to represent lipophilicity. The decimal logarithm of a molar solubility in water, or LOGS, was used to represent water solubility. Moreover, the Swiss ADME offered qualitative solubility classes, such as soluble, moderately soluble, poorly soluble, and insoluble, to describe a compound's overall water solubility. The skin permeability coefficient (Kp), passive human gastrointestinal absorption, and blood–brain barrier (BBB) penetration were used to illustrate the pharmacokinetic features. Additional pharmacokinetic characteristics involved cytochromes P450, five primary isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4) as well as substrates or non-substrates of the permeability glycoprotein (P-gp). The oral bioavailability of the compounds was predicted using bioavailability. The Lipinski filter is often used to evaluate drug-likeness, which is the chance (from least to most probable) that a molecule will become an oral drug (Park et al. 2006).

Statistical analysis

A two-sample, two-tailed t-test was used for testing the significant differences in the inhibitory effects of H1 and H4. P-values of < 0.05 are the lowest limit of significance. The Excel 2010 program was used for computing the data (Microsoft Cooperation, Redmond, Washington, U.S.). Figs 3–5 were generated by a polynomial curve of order 2 because they did not follow linear regression and there was no 100% inhibition. Swiss ADME, a free and easily accessible web software, was used for predicting the physicochemical properties and pharmacokinetics (Riyadi et al. 2021). Swiss ADME is an online resource and service platform for bioinformatics for scientists, managed by the Swiss Institute of Bioinformatics (SIB) and available at http://www.swissadme.ch/.

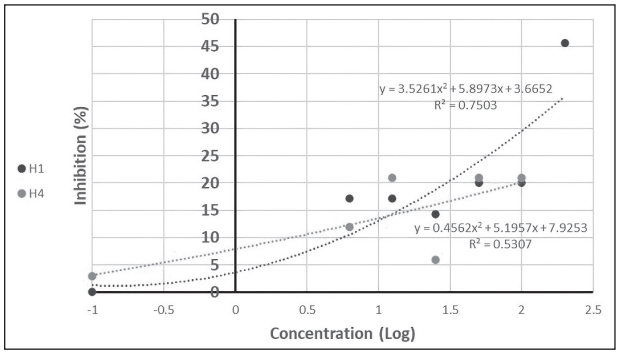

Figure 3. Comparison between H1 and H4 compounds on the inhibition of MCF-7 cancer cells.

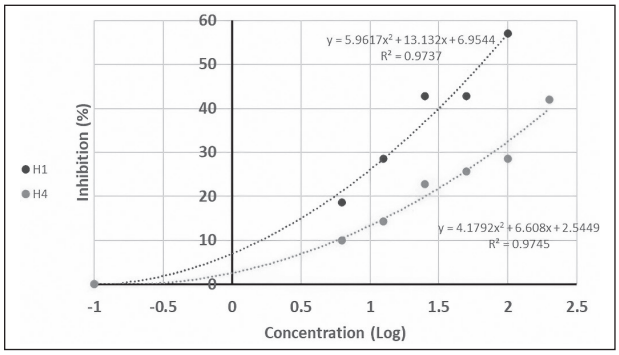

Figure 4. Comparison between H1 and H4 compounds on the inhibition of human colon cancer cells.

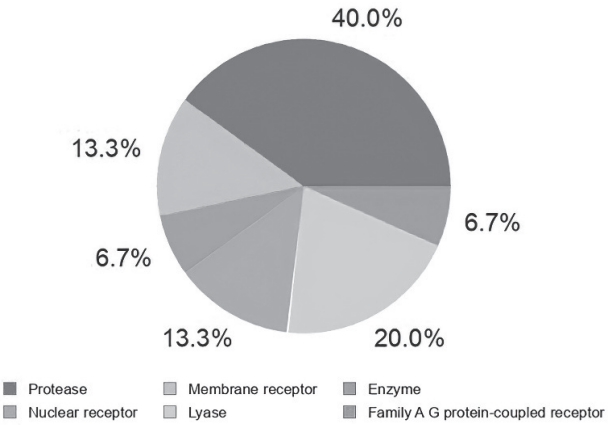

Figure 5. SWISS target prediction for the H1 compound.

Results

Concentration response curve and $IC_{50\%}$ determination by MTT assay

The concentration of a plant extract, drug, or chemical entity (µg/mL) at which 50% of tested animals or cells

in cell lines die is known as $IC_{50\%}$. The IC_{50} value was calculated for three cell lines by MTT assay: two cancerous ones (MCF-7 and HCT-116) and one normal cell line (HUVECs), as illustrated in Table 1. The results of the cytotoxicity screening of both triazole-related compounds showed a dose-dependent inhibition, as shown in Tables 2, 3, and 4 with different potencies. On HUVECs, H4 was more potent by 4.1 folds than H1, as shown in Fig. 3. While H1 results were superior to H4 on McF-7 cells with 1.3 folds and also on human colon cancer, the results were 1.7 fold more potent than H4, as shown in Fig. 4.

Table 1. IC_{50} values for both compounds on different cell lines.

		IC_{50} (µg\mL)	
Cell type	**Cell line**	**H1**	**H4**
Breast cancer	MCF-7	24.62585028	15.45714282
Human colon cancer	HCT116	47.14285701	26.84353733
Human umbilical vein endothelial cells	HUVEC	14.89455781	59.75272103

Table 2. Inhibition activity of H1 and H4 compounds on human umbilical vein endothelial cells (HUVECs).

Dose (µg\mL)	Percentage of inhibition of H1	Percentage of inhibition of H4
200	13	73
100	16	63
50	22	55
25	16	52
12.5	7	52
6.25	12	53

Each concentration was tested in triplicate, and the test was repeated twice. The data is represented as the mean percent of inhibition.

Table 3. Inhibition activity of the H1 compound on breast cancer (MCF-7).

Dose (µg\ml)	Percentage of inhibition of H1	Percentage of inhibition of H4
200	46	21
100	20	21
50	20	6
25	14	21
12.5	17	12
6.25	17	3

Each concentration was tested in triplicate, and the test was repeated twice. The data is represented as the mean percent of inhibition.

Table 4. Inhibition activity of the H1 compound on human colon cancer (HCT116).

Dose (µg\ml)	Percentage of inhibition of H1	Percentage of inhibition of H4
200	57	42
100	43	29
50	43	26
25	29	23
12.5	19	14
6.25	10	10

Each concentration was tested in triplicate, and the test was repeated twice. The data is represented as the mean percent of inhibition.

Selectivity index calculation results

The cytotoxic extracts' level of selectivity was quantified as SI = IC_{50} in normal cells/IC50 in tumor cells. The results shown in Table 5 reveal that on the MCF-7 cell line, H4 compounds can be classified as "prospective anti-cancer samples," according to Weerapreeyakul et al. (2012), who proposed that at SI values (≥ 3), the leading compound will be considered a prospective anti-cancer sample.

Table 5. Selectivity index values for the cancerous cells.

Compound	Cell lines			
	MCF-7		HCT116	
	IC_{50}	SI	IC_{50}	SI
H1	24.62	0.604	47.14	0.315
H4	15.45	3.865	26.84	2.226

Selectivity index = IC_{50} for normal cells/IC_{50} for cancer cells.

Prediction and assessment of pharmacokinetics and the target proteins

Both compounds showed low skin permeability, moderate water solubility, no BBB permeation, and similar effects on the five major isoforms of cytochromes P450 as inhibitors for CYP2C19, CYP2C9, and CYP3A4. H1 compounds show higher GI absorption and better bioavailability than H4, and they serve as substrates for the P-gp efflux pump. All pharmacokinetic results are illustrated in Table 6. Regarding the prediction of possible target proteins by both entities, we have chosen targets with the highest number of known actives (3D and 2D) as d for dimensions. Having a higher number of known actives (3D/2D) suggests that there is more experimental data available for that target protein, which can be helpful for assessing the reliability of the predicted target and facilitating further investigation. All targets mentioned in Table 7 have a ratio of >200/0 of known actives (3D/2D). Figs 5, 6 demonstrate SWISS target predictions for H1 and H4 compounds, respectively.

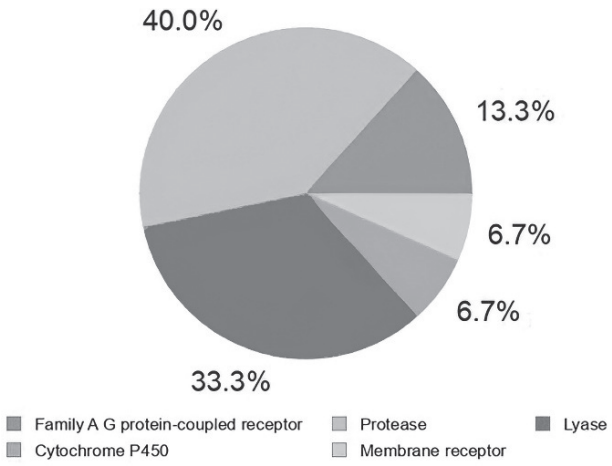

Figure 6. SWISS target prediction for the H4 compound.

Table 6. The pharmacokinetic characteristics of the potential candidate compounds predicted by Swiss ADME.

Name	MW g/mol	Rotatable bonds	#H-bond ACC	#H-bond Don	TPSA (A2)	LOGP. (o/w)	LOGS (ESOL)	Water sol.	GI abs.
H1	470.48	11	7	2	123.41	2.63	-4.33	Moderate	High
H4	487.46	11	8	2	152.16	2.11	-4.66	Moderate	low

Table 6. Continued.

BBB perm.	P-gp substrate	CYP1A2 inh.	CYP2C19 inh.	CYP2C9 inh.	CYP2D6 inh.	CYP3A4 inh.	Log Kp cm/s	Lipinski	Bioavailability
No	Yes	No	Yes	Yes	No	Yes	-7.13	0	0.56
No	No	No	Yes	Yes	No	Yes	-6.97	1	0.11

[1]Molecular weight (MW).
[2]#H-bond ACC (H-bond acceptors) and #H-bond DON (H-bond donors).
[3]Topological polar surface area (TPSA).
[4]Lipophilicity (LOGPo/w), partition coefficient between n-octanol and water.
[5]Water solubility (LOGS), the decimal logarithm of the molar solubility in water.
[6]The pharmacokinetic characteristics: Gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeation and skin permeability coefficient (Kp), permeability glycoprotein (P-gp) substrates or non-substrates, and five major isoforms of cytochromes P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4).
[7]Bioavailability predicts the oral bioavailability of a compound (0, least probable to 1, most probable).
[8]H1 is (3-benzamido-2-(4-((4-formylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylpropanoic acid) C26H22N4O5.
[9]H4 is (3-benzamido-2-(4-((4-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylpropanoic acid) C25H21N5O6.

Table 7. Pharmacodynamics of the identified candidate compounds by Swiss Target Prediction (the common targets between the two compounds were colored the same).

	Protein Target	Target Class	CHEMBL ID
H1	Integrin alpha-4/ beta-1	Membrane receptor	CHEMBL1907599
	Integrin alpha-4/ beta-7	Membrane receptor	CHEMBL2095184
	Integrin alpha-V/ beta-3	Membrane receptor	CHEMBL1907598
	Peroxisome proliferator-activated receptor gamma	Nuclear receptor	CHEMBL235
	Peroxisome proliferator-activated receptor alpha	Nuclear receptor	CHEMBL239
	Matrix metalloproteinase 12	Protease	CHEMBL4393
	5-lipoxygenase activating protein	cytosolic protein	CHEMBL4550
	G protein-coupled receptor 44	Family A G protein coupled receptor	CHEMBL5071
	Caspase-1	Protease	CHEMBL4801
H4	Endothelin receptor ET-A	Family A G protein coupled receptor	CHEMBL252
	Matrix metalloproteinase 3	Protease	CHEMBL283
	Integrin alpha-4/ beta-	Membrane receptor	CHEMBL2095184
	Peroxisome proliferator-activated receptor gamma	Nuclear receptor	CHEMBL235
	Peroxisome proliferator-activated receptor alpha	Nuclear receptor	CHEMBL239
	Angiotensin converting enzyme	Protease	CHEMBL1808
	Caspase-1	Protease	CHEMBL4801
	G protein-coupled receptor 44	Family A G protein coupled receptor	CHEMBL5071

ChEMBL ID: identifier from a chemical database of bioactive molecules with drug-like properties.
H1 is (3-benzamido-2-(4-((4-formylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylpropanoic acid).
H4 is (3-benzamido-2-(4-((4-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)-3-phenylpropanoic acid).

Discussion

In our study, we offer a workflow for in silico target prediction that can predict and suggest new molecular targets for new chemical entities. The results revealed the polypharmacology of both H1 and H4 compounds as follows:

MTT cell line assay results

Regarding the anti-cancer effects, both compounds could serve as a lead to developing multitarget inhibitors directed against cancer. Molecules containing a 1,2,3-triazole skeleton are exceedingly favored in medicinal chemistry. They bind to biomolecular targets with ease and have a stable nature (Rani et al. 2021). Combining 1, 2, and 3-triazole rings with chemical structures in medicinal chemistry has a variety of biological effects, such as the suppression of HIV-1 protease, anticancer efficacy, and radiolabeling ability for tumor identification (Hein et al. 2008). The most popular and useful indicator of a drug's effectiveness is its half-maximal inhibitory concentration, or IC50. It shows how much medication is required to cut down a biological process in half. The majority of methods for calculating a pharmacological compound's IC50 rely on tests that make use of entire cell systems. Although results can vary depending on the experimental cell line employed and may not distinguish between a compound's ability to inhibit specific interactions, they generally provide excellent potency information, as confirmed by Aykul and Martinez-Hackert (2016). Table 2 shows the percentage of cell validity inhibition of H1 and H4 compounds, respectively, on human umbilical vein endothelial cells (HUVECs) using different concentrations. Fig. 2 shows the difference between H1 and H4 compounds on the inhibition of normal cells (HUVECs), with H4 being

more potent by 64%/15.7% = 4.1-fold than H1. The results demonstrate that both tested chemical entities are potent vascular disrupting agents. Motility and migration of vascular endothelial cells are important in the angiogenic process.

Swiss Target Prediction is a web server that uses strategies for assessing chemical similarity through the use of molecular fingerprints, often referred to as 2D similarity. Compounds that are highly similar according to these metrics typically exhibit an increased propensity to interact with similar molecular targets (Willett 2011). However, considering the complex nature of molecular recognition, which also involves ligand shape and electrostatics, 3D structural similarity methods have been devised to evaluate how molecules compare spatially (Armstrong et al. 2011). Recent findings indicate that the integration of both 2D and 3D similarity evaluations considerably improves the precision of target prediction, particularly when the compound in question is novel and does not fall within a chemical class that has been extensively researched (Sucularl et al. 2022). We kept in mind that the presence of known active ingredients can provide some confidence in the prediction, but experimental validation is still necessary to confirm the actual activity of your compound against the target protein.

With the help of the SWISS target prediction shown in Table 7, both H1 and H4 targeted Integrin alpha-4 and beta-1. Integrins are regarded as one of the most vital families of cell adhesion receptors mediating cell-to-cell as well as cell-extracellular matrix interactions (Humphries et al. 2006). This can explain the effect of both H1 and H4 compounds in preventing HUVECs from organizing into vessel-like tubes, disturbs the establishment of the endothelial cell tubes in a concentration-dependent manner, and may have an inhibitory effect on endothelial cell migration. Another possible target shown by the SWISS target prediction shown in Table 7 is the endothelin receptor ET-A as the target for the H4 compound only. Endothelin-1 (ET-1) is a vital signaling messenger in many pathological conditions, including human cancers (Rosanò et al. 2013; Harrison et al. 2024), resulting in a range of pleiotropic responses, including inhibiting apoptosis or programmed cell death, leading to boosting the survival and growth of tumor cells as well as promoting the proliferation of cancer cells. ET-A signaling aids the release of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), and encourages the development of new blood vessels to supply the expanding tumor with oxygen and nutrients (Yanagisawa et al. 2018). This could be a possible explanation for the higher effect seen by the H4 compound on both HUVEC cell lines. Targeting ET-A receptors has been explored as a potential therapeutic strategy in cancer treatment to inhibit tumor growth and metastasis (Harrison et al. 2024). SWISS target predictions for H1 and H4 compounds are shown in Figs 5, 6, respectively.

Table 3 shows the percentage inhibition of H1 and H4 compounds, respectively, on breast cancer (MCF-7) using different concentrations. Fig. 3 shows the difference between H1 and H4 compounds on the inhibition of Mc7 cancer cells, with H1 being more potent than H4 by 1.3-fold. Table 4 shows the percentage inhibition of H1 and H4 compounds, respectively, on human colon cancer (HCT116) using different concentrations. Fig. 4 shows the difference between H1 and H4 compounds in the inhibition of colonic cancer cells. H1 shows higher potency than H4 by 1.7 fold.

Selectivity index

The ratio of the toxic concentration of a plant or drug sample against its effective bioactive concentration is known as the selectivity index (Pritchett et al. 2014). In order to decide if more research on herbal drugs or isolated compounds can be conducted, the selectivity index value evaluation is essential. It is commonly known that a medication must have both biological efficacy and good pharmacokinetic properties, or the capacity of a substance to reach therapeutic concentrations in the body. Many therapeutic plants, herbal medications, and isolated compounds have been shown to be bioactive in countless scientific publications; sadly, the majority of these reports ignore the SI data. In 2006, Cos et al. noted that the presented paper had barely any significance without the SI data of the reported bioactive sample(s). According to Nogueira and Rosário (2010), SI should not be lower than 2, while other authors like Adamu et al. in 2013 and 2014 stated that the extracts might be expected to be bioactive, safe, and non-toxic if SI was higher than 1. If the SI value was lower than 1, it indicated the presence of a toxic component and that the sample might be toxic and not be able to be used as a therapeutic herb or drug. Chemical methods can be applied to remove the toxic compound and render the compound more active, safer, and with a higher selectivity index (Indrayanto et al. 2021). As an alternate strategy to support drug discovery and development, we employed computer models in our study to forecast and assess the possible characteristics of a candidate molecule in absorption, distribution, metabolism, and excretion, also abbreviated as ADME. SwissADME software was utilized to estimate the identified candidate's pharmacokinetic and physiochemical characteristics. Our tested chemical entities both have the 1,2,3 triazole core, but the H1 compound has an aldehyde (CHO) side group while the H4 compound has a nitic oxide (NO_2) side group. Adding a nitro group (NO2) to the 1,2,3-triazole core of a chemical compound has potentially increased its activity in both normal and cancerous cell lines. This enhanced cytotoxicity effect can be explained by the fact that nitrogen-containing compounds can undergo metabolic reduction within cells, leading to the formation of reactive intermediates. These reactive species can cause cellular damage, induce DNA strand breaks, disrupt redox balance, or interfere with essential cellular processes, altering target engagement and resulting in heightened cytotoxicity (Penning et al. 2022). Adding a CHO (aldehyde) group to a chemical compound appears to result in a more pronounced inhibition of cancer cells compared to incorporating a NO_2 (nitro) group in a colon cancer cell line, as shown in Fig. 4. This observation may be explained by factors such as structural impact, since introducing a CHO or aldehyde group could alter the compound's shape or electronic properties, improving its binding to cancer cell targets. Moreover, dis-

tinct molecular affinity impacts suggest that the aldehyde group could potentially form preferential interactions with certain proteins, enzymes, or receptors that are critical in cancer pathways, thereby more effectively suppressing the growth of cancer cells and may even enhance metabolic resilience, making it less vulnerable to breakdown by cellular enzymes, as confirmed by Gampe and Verma (2020). This improved stability could translate to a prolonged presence in cancerous cells, giving the compound an extended duration to act on its targets, thereby amplifying its anticancer actions. The potential of aldehydes in medication development was demonstrated in numerous cases. Aldehydes offer a multitude of options for interacting with biological targets to produce extremely targeted effects. Aldehydes have been used by both naturally occurring substances—such as vitamins A (McDowell 2000) and B6 (Parra et al. 2018) —and creative drug development projects. Thanks to their unique chemical characteristics, aldehydes can be quite useful in these kinds of projects.

Conclusion

The use of chemical side groups in prodrug synthesis in medicine is expanding on a daily basis, and the variety of their analogues offers a significant and feasible avenue for the discovery of pharmaceuticals with a range of biological uses. When it comes to creating structurally varied heterocyclic compounds with uses in combinatorial chemistry, diversity-oriented synthesis, bioconjugation chemistry, and drug discovery, the method of creating triazole derivatives may have important implications and may be considered a new hope for curing cancer.

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