DRUG DISCOVERY

In silico screening and molecular docking of spiroindimicin A-H targeting Insulin Growth Factor 1 Receptor (IGF-1R) for cancer treatment

Thet Htwe Aung[1*], **Chandan Shivamallu**[2], **Shiva Prasad Kollur**[3]

To Cite:
Aung TH, Shivamallu C, Kollur SP. In silico screening and molecular docking of spiroindimicin A-H targeting Insulin Growth Factor 1 Receptor (IGF-1R) for cancer treatment. *Drug Discovery* 2023; 17: e20dd1923
doi: https://doi.org/10.54905/disssi.v17i39.e20dd1923

Author Affiliation:
[1]Marine Science Department, Mawlamyine University, Mawlamyine, Myanmar
[2]Department of Biotechnology and Bioinformatics, School of Life Science, Mysore, India
[3]Department of Chemistry, School of Physical Sciences, Mysuru, India

[*]**Corresponding author**
Marine Science Department, Mawlamyine University, Mawlamyine, Myanmar
Email: thethtweaung101@gmail.com
ORCID: 0000-0002-4612-2789

Peer-Review History
Received: 18 February 2023
Reviewed & Revised: 21/February/2023 to 30/April/2023
Accepted: 03 May 2023
Published: 07 May 2023

Peer-Review Model
External peer-review was done through double-blind method.

Drug Discovery
pISSN 2278–540X; eISSN 2278–5396

ABSTRACT

Spirocyclic compounds have the potential to become anticancer drugs, hence it is anticipated that spiroindimicin A-H, which has moderate cytotoxicity against numerous cancer cell lines, can be a promising anticancer treatment for drug discovery. Insulin- growth factor 1 receptor (IGF-1R) is currently one of the most desired targets for cancer treatments. The aim of this work is to identify spiroindimicin A-H as potential lead compounds for the development of anticancer medicines using in silico research. Spiroindimicin A-H were docked against IGF-1R using the mcule (one-click docking server) and ligPlot+ software. SwissADME, Molinspiration and ProTox II computational tools were used to predict their physicochemical, pharmacokinetic, bioactive and toxicity properties. In this work, spiroindimicin C had the highest affinity score out of all the compounds, which all displayed high affinity, with a binding energy of 9.1 kcal/mol via three hydrogen bonds (GLN24C, LYS50C and ASP165C). Although all spiroindimicin A-H adhered to the (Rule of five) Ro5 filter, they might have low bioavailability and undesirable pharmacokinetic consequences.

Keywords: Spiroindimicin, docking, IGF1R, ADME, cancer treatment.

1. INTRODUCTION

Cancer is the second leading cause of death in the world, with 11.5 million deaths expected by 2030. Of the 36 different types of cancer, men are more likely to develop colorectal, lung, liver, prostate and stomach cancers than women are to develop breast, colorectal, cervical, lung and thyroid cancers (Bray et al., 2018). Chemotherapy, radiation therapy and surgery are only a few of the traditional and contemporary methods that have been utilized to treat cancer (Karpuz et al., 2018). These methods have a number of drawbacks, such as toxicity and side effects related to the use of traditional chemicals for the treatment of cancer (Nobili et al., 2009). The ineffectiveness of traditional chemotherapeutic strategies

DISCOVERY SCIENTIFIC SOCIETY

needs the development of new, effective medications for the prevention and treatment of this disease with few adverse effects (Gurung et al., 2021).

In general, the word "in silico" is related to the more well-known biological terms "in vivo" and "in vitro" and describes computational models that assess pharmacological concepts. In silico is a synonym for "in vivo," which refers to living things and "in vitro," which refers to test tubes. The phrase "in silico" refers to the silicon chemical element, which is frequently utilized to create computer chips. In silico models are simple to use, rapid and economical compared to "in vivo" and "in vitro" experiments. One of the most significant benefits is that one may anticipate ADME qualities using only the chemical structure, even before the molecule is synthesized (Waterbeemd and Gifford, 2003).

Spirocyclic compounds are common in medicinal chemistry and are currently getting increasing attention as novel drugs are being developed. Today, both approved drugs and therapeutic candidates contain a range of spirocyclic scaffolds (Hiesinger et al., 2021). Moreover, the spiro compounds have been widely employed for many years in the treatment of cancer due to their persistent ubiquity as pharmacophores with strong biological activity. Hence, the development of anticancer drugs based on spiro molecules is a potential strategy in the area of medicinal chemistry and drug discovery (Bora et al., 2021).

Spiroindimicins are a special class of natural compounds with spirocyclic scaffolds produced by the oxidative dimerization of tryptophan. The earliest members of this family, spiroindimicins A-D, were first reported by Zhang et al., (2012). Spiroindimicins E and F, two monochlorinated members of this family, were described by Paulus et al., (2017). The Zhang et al., (2012) also recovered two deschloro congeners, spiroindimicin G and H, from a bacterial mutant with an inactivated halogenase gene (Liu et al., 2019). The spiroindimicins showed moderate cytotoxicity against a number of cancer cell lines in biological experiments performed thus far (IC50 = 9–44 M) (Zhang et al., 2012; Liu et al., 2019).

The development of numerous cancers is thought to be influenced by the insulin-like growth factor-1 receptor (IGF-1R), a tyrosine kinase receptor (Liao et al., 2005). Many human solid tumors, including sarcomas, hepatocellular carcinoma, pancreatic, ovarian and gastrointestinal malignancies, as well as breast, non-small cell lung and prostate cancer, have been found to significantly overexpress IGF-IR (Rikhof et al., 2009; Jia et al., 2013; Tsuta et al., 2013; Ucar et al., 2013). IGF-1R can start the PI3K/AKT/mTOR signaling and Ras/Raf/MEK/MAPK pathways after ligand binding, which activates a number of transcription factors as ELK-1, CREB and AP-1 to influence cell proliferation, survival, differentiation, motility, invasion and angiogenesis (Wilson and Chia, 2013; Sharmila et al., 2014). Additionally, increasing research shows that IGF-1R participates in the crucial stages of the metastatic cascade and is a requirement for tumor growth (Zhao et al., 2013; Werner and Bruchim, 2009). IGF-1R is therefore among the most desirable targets for cancer treatment approaches (Wei et al., 2012).

Due to the fascinating spiro-fused scaffold and promising bioactivity, spiroindimicin A-H were tested against the insulin-like growth factor-1 receptor (IGF-1R) in this experiment to find potential lead compounds for the development of anticancer drugs. In order to compare the binding affinities to the Insulin-like growth factor-1 receptor, this study used Linsitinib (OSI-906) for the treatment of various cancer, as positive control. It is also a potent, selective and orally bioavailable dual inhibitor of the Insulin-like growth factor-1 receptor (IGF-1R) and insulin receptor (IR) (Mulvihill et al., 2009).

2. MATERIAL AND METHODS

Molecular Docking Analysis

When designing drugs, docking involves producing a non-covalent protein ligand. Predicting the structure of the binder complex is the task, which has the stated structures of a protein and a ligand. The forces involved in protein-ligand recognition, including as hydrogen-based van der wall bonds, electrostatic forces and the proper placement of the molecule in the active site, are assessed using a docking approach (Weber et al., 1998).

IGF1R's crystal structure was discovered using the mcule one-click docking server (https://mcule.com/apps/1-click-docking/). The structures of spiroindimicin A-H were created by drawing the structures in Chem Sketch and then converting them to SMILES notation based on words. With the help of the quick and easy mcule 1-click docking server, docking simulations were carried out. The program displayed four different docking scores for compounds by assessing the affinities of the compounds. However, for this study, the docking poses with the highest binding affinities and the most negative docking scores (kcal/mol) were chosen. The IGF1R target at the binding site of X= 6.5577, Y = 43.8166 and Z= -7.3536 showed rather excellent affinity in a docking analysis. Using LigPlot+, the selected docking results were made visible for 2D hydrogen bond interaction.

Pharmacokinetics, physicochemical and drug-like properties

The canonical SMILES of spiroindimicin A-H were later added to the SwissADME tool (http://www.swissadme.ch/). Then, it was possible to forecast the compounds' physicochemical properties, including their molecular weight (MV), number of hydrogen bond acceptors (nHBA), number of hydrogen bond donors (nHBD) and number of rotational bonds (nRB). The Moriguchi octanol-water partition coefficient lipophilicity (MLogP), solubility, gastrointestinal absorption (GIA), blood brain barrier (BBB), p-glycoprotein substrate, inhibition of isoforms of cytochrome P450 (CYP) and skin permeability (LogKp) were among the ADME parameters that were estimated by SwissADME (Daina et al., 2017).

Bioactivity scores

The bioactivity scores of the spiroindimicin A-H for G protein coupled receptor (GPCR) ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand and protease inhibitor were evaluated using Molinspiration Online tool (http://www.molinspiration.com). The canonical SMILES strings of the compounds were put into the Molinspiration tool before the prediction of bioactivity scores (Maliehe et al., 2020).

Toxicity analysis

The canonical SMILES of spiroindimicin A-H were then incorporated into the ProTox-II tool. The ProTox-II is a free online server (http://tox.charite.de/protox_II/) that forecasts many toxicological endpoints for a variety of chemical substances (Drwal et al., 2014; Banerjee et al., 2018). This tool, which represents a novel approach in toxicity prediction, combines molecular similarity, pharmacophores, fragment propensities and machine-learning models for the prediction of some toxicity endpoints, including acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity and immunotoxicity. The ProTox-II platform is divided down into five classification steps created by various computational models: (1) acute toxicity (oral toxicity model with five different toxicity classes); (2) organ toxicity model; (3) toxicological and genotoxicological end points, primarily immunotoxicity, cytotoxicity, mutagenicity and carcinogenicity; (4) toxicological pathways; and (5) toxicity targets. Toxic doses are provided as LD50 values in mg/Kg for oral acute toxicity.

3. RESULTS AND DISCUSSION

Docking Analysis

Insulin-like growth factor-1 receptor (IGF-1R) play in the development and progression of cancer. Affinity describes how much a drug interacts with a receptor (Aung, 2022). Binding affinity between ligands and receptors is determined by the binding energy, the lower the energy, the higher the binding affinity (Sarkar et al., 2021). In order to estimate the binding sites and affinities, spiroindimicin A-H were molecularly docked with Insulin-like growth factor-1 receptor (IGF-1R).

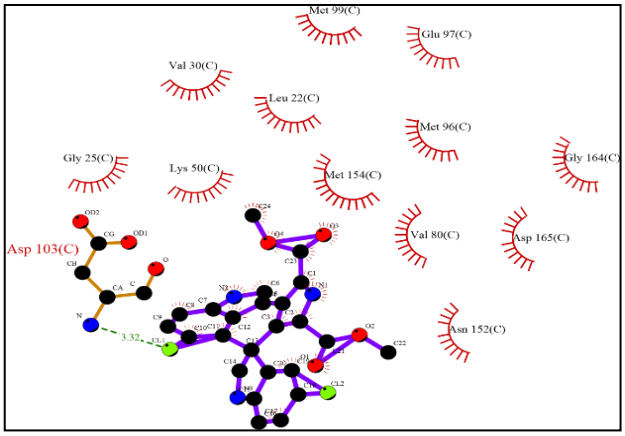
As a result, spiroindimicin C had the highest affinity score and was the most stable complex for IGF1R, with a binding energy of 9.1 kcal/mol. Spiroindimicin D also exhibited a significant high affinity score with a binding energy of 8.5 kcal/mol, followed by Spiroindimicin F with -8.0 kcal/mol, Spiroindimicin A and B with -7.8 kcal/mol, Spiroindimicin E with -7.6 kcal/mol, Spiroindimicin H with -7.5 kcal/mol and Spiroindimicin G with -7.2 kcal/mol. Linsitinib, the positive control, showed the binding affinity score with -8.9 kcal/mol. Therefore, spiroindimicin C has the higher binding affinity than the positive control.

Four of the eight ligands that bind to the IGF1R interact through an only one hydrogen bond. The four are spiroindimicin A through ASP103, spiroindimicin B through SER 26C, spiroindimicin F through GLN24C and spiroindimicin G through LYS50C. Three of them formed three hydrogen connections with the IGF1R. They are spiroindimicin C through GLN24C, LYS50C, ASP165C; spiroindimicin D through SER26C, SER26C, LYS50C; and spiroindimicin H through SER26C, ASP147C, LYS176C (Figure 1). Among them, spiroindimicin E, however, did not exhibit any hydrogen bond interactions. The majority of ligands with high affinity need strong hydrogen bonds (Pantsar and Poso, 2018). As a result, ligands with strong affinity for IGF1R include spiroindimicin C-D and H. However, neither linsitinib (standard drug) nor spiroindimicin E demonstrated any hydrogen bond interaction with IGF1R.

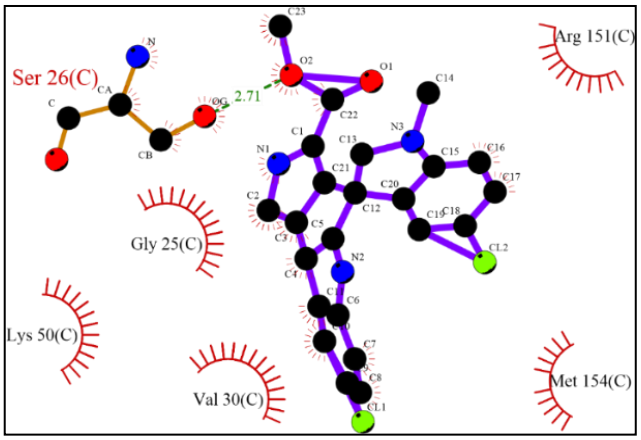
The selection of drug candidates begins with ligands that bind firmly to the target protein because stronger bonds will have a greater impact on the target proteins' physiological function. Typically, a high affinity results in a lower dose requirement (Aung, 2022). Spiroindimicin C-D and H are therefore concluded to be the best drug candidates for the treatment of cancer that target IGF1R based on the outcomes of molecular docking and protein-ligand interaction.

Table 1 Binding interactions of the spiroindimicin A-H and linsitinib (standard drug) with IGF1R by molecular docking

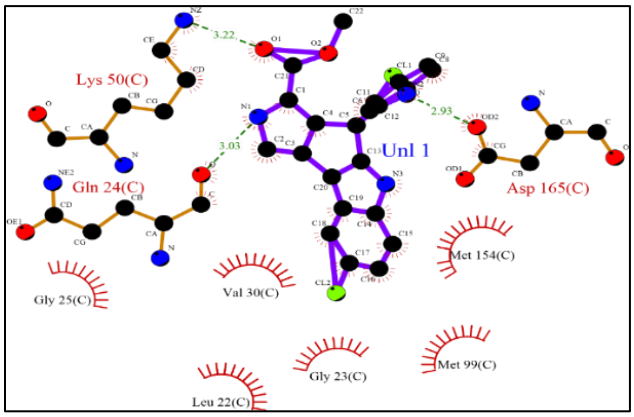
Spiroindimicin	Compound atoms	Receptor atoms	Receptor Residues	Chain	Distance (A^0)	Energy (Kcal/mol)
A	CL1	N	ASP103	C	3.32	-7.8
B	O2	OG	SER26	C	2.71	-7.8
C	N1	O	GLN24	C	3.03	-9.1
	O1	NZ	LYS50	C	3.22	
	N2	OD2	ASP165	C	2.93	
D	O1	N	SER26	C	2.88	-8.5
	O1	OG	SER26	C	3.02	
	O2	NZ	LYS50	C	3.18	
E	No hydrogen bond contact					-7.6
F	N1	O	GLN24	C	3.06	-8.0
G	O1	NZ	LYS50	C	3.19	-7.2
H	N2	OG	SER26	C	2.90	-7.5
	N3	OD2	ASP147	C	2.98	
	O1	NZ	LYS176	C	2.95	
Linsitinib (standard drug)	No hydrogen bond contact					-8.9



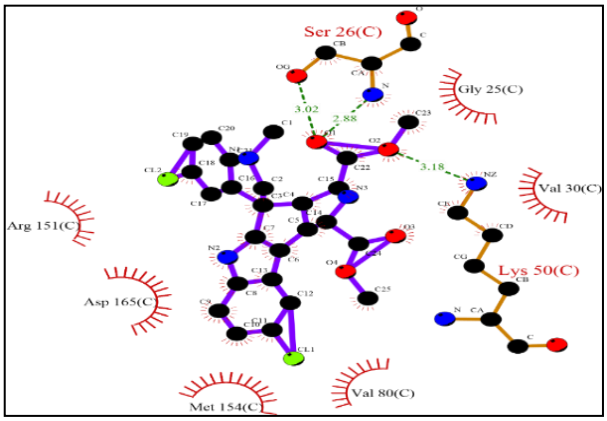
Spiroindimicin A



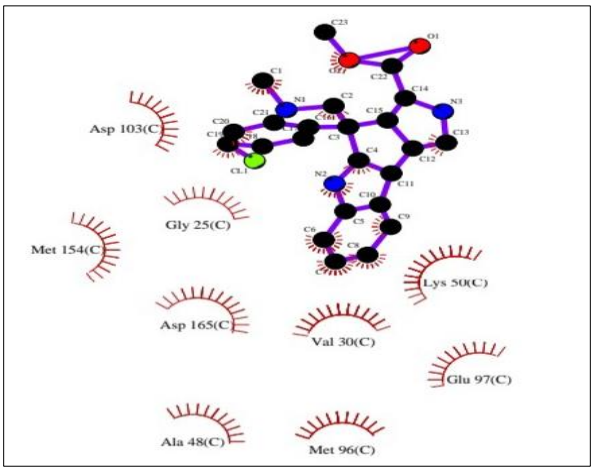
Spiroindimicin B



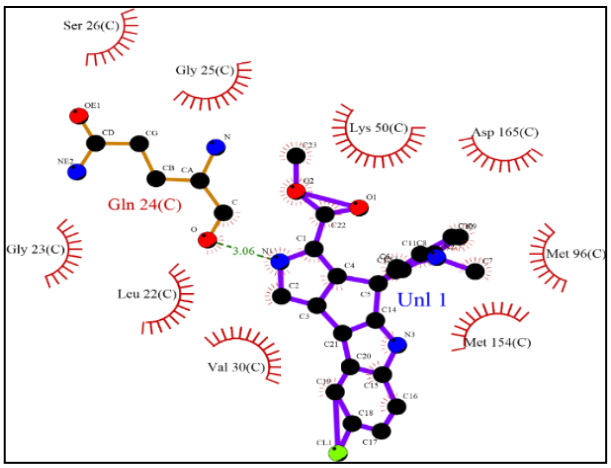
Spiroindimicin C



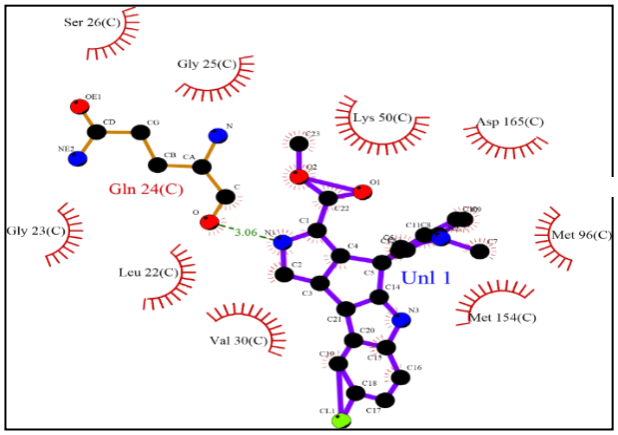
Spiroindimicin D



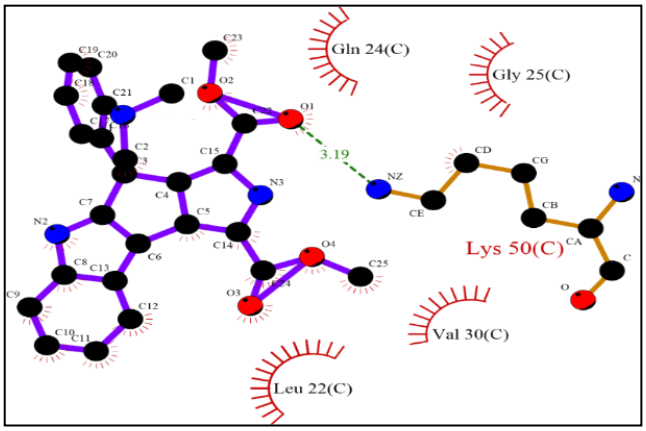
Spiroindimicin E



Spiroindimicin F



Spiroindimicin G



Spiroindimicin H

Figure 1 2D hydrogen bond interactions of spiroindimicin A-H with IGF1R

Physicochemical properties

All of the spiroindimicin A-H had molecular weights that are within the permitted limit (MW ≤ 500 g/mol) (Table 2). As a result, it was inferred that all the compounds with MW lower than 500 have the ability to be rapidly absorbed, dispersed and transported. (Srimai et al., 2013). The drug-like compounds should have number of hydrogen bond acceptor (nHBA) ≤ 10 and number of hydrogen bond donar (nHBD) ≤ 5, according to the rule of five (Ro5) (Lipinski et al., 1997). All spiroindimicins' nHBA and nHBD values were discovered to be within the Lipinski's limit range (Table 2). This suggests that spiroindimicins can be easily absorbed or pass through the digestive tract when administering them (Lipinski et al., 1997). As a measure of molecular flexibility, the number of rotatable bonds (nRB) is one of the often employed filters in the drug discovery process (Veber et al., 2002). Good bioavailability compounds have ≤ 15 rotatable bonds (Muegge et al., 2001). The fact that all spiroindimicin A-H were within the permissible range (nRB ≤ 15) suggests that they may be permeable and orally bioavailable.

Table 2 The physicochemical properties and lipophilicity of spiroindimicin A-H

Spiroindimicin	Properties				
	MW (g/mol)	nHBD	Nhba	nRB	MlogP
A	480.30	2	5	4	2.98
B	438.31	2	2	2	3.72
C	424.28	3	2	2	3.51
D	496.34	2	4	4	3.25
E	408.86	2	2	2	3.24
F	403.86	2	2	2	3.24
G	427.45	2	4	4	2.58

H	427.45	2	4	4	2.58

Lipophilicity and solubility

The solubility, selectivity, potency, permeability and promiscuity of lead compounds are all influenced by lipophilicity (Arnott and Planey, 2012). The MLogP of the spiroindimicin A-H are demonstrated (Table 3). High lipophilicity (MlogP > 4.15) is a common source of substances with fast metabolic turnover, poor solubility and poor absorption. Moreover, chemicals are more likely to bind to hydrophobic protein targets other than the intended ones when their lipophilicity increases (MlogP > 4.15), which can have harmful consequences on biological systems. However, in Table 3, all compounds adhered to the rule of five (Ro5) (MlogP ≤ 4.15).

One of the elements influencing drug distribution and absorption is solubility (Kerns and Di, 2003). According to the LogS prediction model, the spiroindimicin A-H are either moderately soluble or poorly soluble in water, according to the estimation of the aqueous solubility (Table 3). To cross cell membranes and be absorbed, compounds must be water soluble (Stegemann et al., 2007). As a result, none of the spiroindimicins showed any indication that they might be absorbed and spread.

Table 3 Solubility predictions of the spiroindimicin A-H

	Properties		
Spiroindimicin	LogS (ESOL) Class	LogS (Ali) Class	LogS SILICOS-IT Class
A	-5.81 (Moderately soluble)	-6.21 (poorly soluble)	-9.10 (poorly soluble)
B	-6.26 (poorly soluble)	-6.33 (poorly soluble)	-8.98 (poorly soluble)
C	-6.10 (poorly soluble)	-6.37 (poorly soluble)	-9.32 (poorly soluble)
D	-6.55 (poorly soluble)	-7.08 (poorly soluble)	-8.99 (poorly soluble)
E	-5.66 (Moderately soluble)	-5.68 (Moderately soluble)	-8.39 (poorly soluble)
F	-5.66 (Moderately soluble)	-5.68 (Moderately soluble)	-8.39 (poorly soluble)
G	-5.35 (Moderately soluble)	-5.78 (Moderately soluble)	-7.83 (poorly soluble)
H	-5.33 (Moderately soluble)	-5.75(Moderately soluble)	-8.39 (poorly soluble)

Pharmacokinetic properties

The findings of the prediction of the spiroindimicins' gastrointestinal absorption (GIA) are shown (Table 4). All of the compounds showed substantial probabilities of being absorbed in the digestive system. This suggests that when administered orally, these compounds may have the ability to be absorbed in the gastrointestinal tract (Daisy et al., 2011). The BBB, which divides the brain from the blood, is a layer of microvascular endothelial cells in the brain (Wang et al., 2015). The capacity of substances to cross the BBB was assessed and the outcomes are displayed (Table 4). The results show that spiroindimicin B, C, E and F have the ability to cross the BBB. Only substances that target the central nervous system (CNS) are required to penetrate BBB (Borra and Kuna, 2013). Spiroindimicin A, D, G and H did not have BBB-crossing capability; hence, this can be advantageous as they are less likely to cause negative effects in the CNS (Daina et al., 2017). Membrane transporters of substances in either an intracellular or extracellular route are known as P-glycoproteina (P-gp) (Levin, 2012). Consequently, only the efficacy of all compounds may be blocked at various target sites (Khan et al., 2017). One of the top priorities during the drug discovery process is the prediction of the metabolism of lead compounds (Wang et al., 2015). CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 are the five isoforms of the cytochrome P450 (CYP) monooxygenase family that were used to predict how the compounds would be metabolized. The results are shown (Table 4). All spiroindimicins inhibited CYP1A2, CYP2C19, CYP2C9 and CYP3A4, while only spiroindimicin C and E-H inhibited CYP2D6. In biological systems, the enzyme Cytochrome P450 monooxygenase is essential for drug metabolization and excretion. The fact that the compounds don't inhibit these enzymes suggests that they are highly likely to undergo transformation and, as a result, be accessible when taken orally (Lynch and Price, 2007).

On the other hand, the chemicals' inhibition of the CYP isomers might result in side effects that are hazardous due to their accumulation and low bioavailability as a result of their failure to be metabolized (Chow et al., 2010). As a result, the majority of spiroindimicins show that they have the potential to cause adverse effects and have a low bioavailability with regard to metabolism. Depending on their physicochemical characteristics, various substances can pass through the skin's selective barrier at varying rates (Ng and Lau, 2015). Skin permeability (LogKp) is a crucial measure for evaluating drugs that may need to be administered transdermally. Table 4 displays the compounds' LogKp values. Given that all of the compounds have negative LogKp values, they are all predicted to be impermeable. This suggests that spiroindimicins could not be delivered through the skin in an effective way.

Table 4 The pharmacokinetic parameters of the Spiroindimicin A-H

	Properties								
Spiroindimicin	GIA	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 Inhibitor	CYP2C9 inhibitor	CYP2C6 inhibitor	CYP3A4 inhibitor	LogKp (cm/s)
A	High	No	Yes	Yes	Yes	Yes	No	Yes	-6.06
B	High	Yes	Yes	Yes	Yes	Yes	No	Yes	-5.21
C	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-5.22
D	High	No	Yes	Yes	Yes	Yes	No	Yes	-5.43
E	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-5.45
F	High	Yes	Yes	Yes	Yes	Yes	Yes	Yes	-5.45
G	High	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.90
H	Low	No	Yes	Yes	Yes	Yes	Yes	Yes	-5.93

Drug-likeness properties and bioavailability

If the compounds violate more than one Ro5, there is a reduced ability of medication molecules to be absorbed orally (Paramashivam et al., 2015). Table 5 displays the outcomes of using Ro5 as a filter to find compounds that are very likely to be drug candidates. All spiroindimicins can be categorized as drug-like substances because they all adhere to the five Lipinski rules. When a substance is taken orally, bioavailability describes the amount and rate at which the compound enters the bloodstream and ultimately reaches the desired areas (El-Kattan and Varm, 2012). Table 5 displays the oral bioavailability data of spiroindimicins. The majority of spiroindimicins have a bioavailability between 0.55 and 0.56. The numbers of 0.55 and 0.56 indicate that the compounds follow the Lipinski rule of five and have probabilities of being bioavailable of 55 and 56%, respectively (Lipinski et al., 1997). It was determined that all spiroindimicins had good probability of achieving the bioavailability endpoints (0.5).

Table 5 Drug-likeness and bioavailability properties of the spiroindimicin A-H

Spiroindimicin	Lipinski's rule		
	Satisfactory	Number of violations	Bioavailability
A	Yes	0	0.55
B	Yes	0	0.55
C	Yes	0	0.55
D	Yes	0	0.55
E	Yes	0	0.55
F	Yes	0	0.55
G	Yes	0	0.55
H	Yes	0	0.55

The bioavailability radar is a feature exclusive to swissADME and offers a graphical picture of the drug-likeness properties of an orally accessible bioactive drugs. Each vertex on the drug-likeness graph, which is shown as a hexagon in Figure 2, represents a characteristic that describes a bioavailable drug. The pink area of the hexagon represents the ideal range for each property, including flexibility (no more than 9 rotatable bonds), size (MW between 150 and 500 g/mol), polarity (TPSA between 20 and 130° A^2), solubility (log S not exceeding 6) and lipophilicity (XLOGP3 between -0.7 and +5.0) (Daina et al., 2017). The five characteristics of each compound in this study are within the optimum range, yet they all exhibit unsaturation, as seen by an off shoot from a vertex that indicates saturation (INSATU). The ratio of sp3 hybridized C atoms to the total number of C atoms is what is meant by the INSATU violation in the radar graphic. These results suggest that increasing the saturation would enhance this compound's bioavailability.

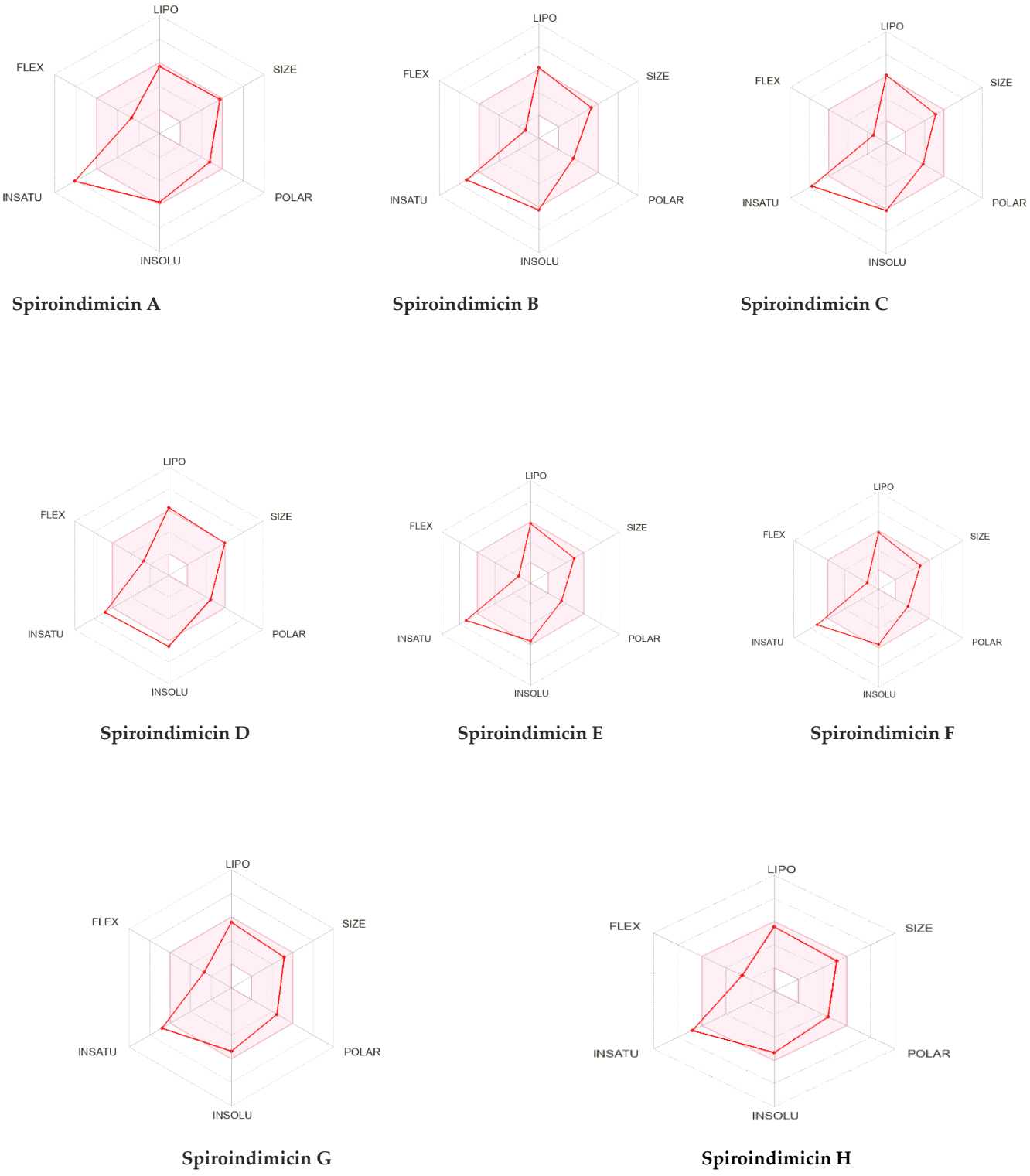


Figure 2 Radar plot of spiroindimicin A-H

Bioactivity scores

The computed bioactivity scores for the substances are shown (Table 6). According to Singh et al., (2013), bioactivity scores are divided into three categories: Actively active (scores > 0), moderately active (scores: -5.0-0.0) and inactive (scores -5.0). Spiroindimicin B, C, E and F were discovered to be active G protein coupled receptor ligands (> 0) among the examined substances, whereas spiroindimicin A, D, G and H are moderate ligands. While the other six showed only moderate potential to influence ion channels, spiroindimicin C and H showed active activity as ion channel modulators. Spiroindimicin B, C, E and F is the compound

that is active against the kinase inhibitor, with a bioactivity score ranging from 0.10 to 0.06; other compounds, on the other hand, exhibited moderate activity, with bioactivity values ranging from -0.00 to -0.04. Only spiroindimicin A were expected to exhibit active nuclear receptor ligand while the other spiroindimicin B-H had moderate activity. Our findings indicated that all spiroindimicin A-H had a modest ability to block protease inhibitors and enzyme inhibitors. The potential of these bioactives as effective therapeutic agents were highlighted by high bioactivity scores; the higher the scores, the better the activity. This suggests that spiroindimicin A-H are also effective therapeutic candidates for managing various metabolic diseases.

Table 6 Bioactivity score of the spiroindimicin A-H

Spiroindimicin	Bioactivity					
	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
A	-0.05	-0.04	-0.00	0.25	-0.34	-0.08
B	0.02	0.00	0.05	-0.02	-0.35	-0.05
C	0.04	0.07	0.10	`-0.15	-0.30	-0.01
D	-0.01	-0.05	-0.04	-0.21	-0.33	-0.13
E	0.03	0.01	0.05	-0.21	-0.38	-0.06
F	0.02	-0.01	0.06	-0.20	-0.36	-0.06
G	-0.01	-0.05	-0.02	-0.20	-0.33	-0.12
H	-0.05	-0.02	- 0.01	-0.27	-0.39	-0.09

Toxicological predictions

Toxicology prediction was done to determine the toxicity and dangers associated with the compound that might affect humans. The ability to predict toxicity was crucial since compounds with low toxicity were also required because a compound's activity alone was insufficient to qualify it as a drug candidate (Spinu et al., 2020). Table 7 displays the results of oral acute toxicity expressed as LD50 (mg/Kg) and the associated toxicity class for each compound. All spiroindimicin A-H were classified as classes V in the toxicity prediction results table. The Globally Harmonized System (GHS), which was separated into 6 groups, formed the basis for the classification of toxicity classes. Classes I to III are quite hazardous due to their high levels of toxicity. Classes IV to VI have a modest level of toxicity, making it somewhat hazardous (GHS, 2019). Research demonstrated that these compounds had a modest level of toxicity, making them moderately hazardous. The lower the toxicity, the higher the LD50 number (Silva et al., 2021). However, the toxicity class of the standard drug, Linsitinib was also IV.

Table 8 reports the findings on organ toxicity as well as the estimated forecasts for several toxicological endpoints made using the ProTox-II web server. Spiroindimicin C, D and F were anticipated to be inactive compounds for all five toxicological endpoints, according to these data. Spiroindimicin A was an active compound for both hepatotoxicity and immunotoxicity, while spiroindimicin B and E were expected to be active compounds for an endpoint of immunotoxicity. It was hypothesized that the active spiroindimicin G and H were carcinogenic. Based on these presumptions, spiroindimicin C, D and F are safe for use although they show lowest level of oral acute toxicity.

Table 7 Acute oral toxicity prediction obtained by using ProTox-II web server

Spiroindimicin	Oral Toxicity Prediction Results			
	Predicted LD50 (mg/Kg)	Predicted Toxicity Class	Average Similarity (%)	Prediction Accuracy (%)
A	2000	IV	40.45	54.26
B	1800	IV	45.94	54.26
C	1800	IV	45.94	54.26
D	1800	IV	43.66	54.26
E	1800	IV	46.78	54.26
F	1700	IV	47.4	54.26
G	2000	IV	46.24	54.26
H	2000	IV	50.27	67.38

	1000	IV	59.25	67.38

Table 8 Organ toxicity and toxicological endpoint prediction calculated using ProTox-II web server

Spiroindimicin	Classification				
	Organ Toxicity (% Probability)		Toxicity Endpoint (% Probability)		
	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
A	Active (55%)	Inactive (50%)	Active (92%)	Inactive (66%)	Inactive (71%)
B	Inactive (56%)	Inactive (53%)	Active (59%)	Inactive (69%)	Inactive (68%)
C	Inactive (58%)	Inactive (54%)	Inactive (67%)	Inactive (71%)	Inactive (61%)
D	Inactive (56%)	Inactive (51%)	Inactive (64%)	Inactive (68%)	Inactive (70%)
E	Inactive (56%)	Inactive (53%)	Active (82%)	Inactive (69%)	Inactive (68%)
F	Inactive (56%)	Inactive (53%)	Inactive (54%)	Inactive (69%)	Inactive (68%)
G	Inactive (69%)	Active (50%)	Inactive (77%)	Inactive (62%)	Inactive (70%)
H	Inactive (71%)	Active (52%)	Inactive (82%)	Inactive (63%)	Inactive (69%)
	Active (50%)	Active (51%)	Active (83%)	Active (52%)	Inactive (73%)

4. CONCLUSIONS

Spiroindimicin C-D and H have been suggested to be among the best cancer treatment drugs candidates in the current study. All spiroindimicin A-H are safe and have a promising oral bioavailability, according to physicochemical, solubility and drug-like properties. Pharmacokinetic properties, however, indicate that compounds may have adverse effects and have a low bioavailability. Increasing the saturation would, however, improve the compounds' bioavailability, according to bioavailability radar. Compounds will have the lowest amount of toxicity, according to predictions about their toxicity. Spiroindimicin A-H are the first report to identify them as prospective lead compounds for the development of anticancer drugs. In vivo and in vitro research are also necessary to validate in silico results.

Acknowledgements

We gratefully acknowledge the research computer services provided by mcule one click dock, SwissADME, Molinspiration and Protox-II.

Informed consent

Not applicable.

Ethical approval

Not applicable.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Funding

The study has not received any external funding.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Arnott JA, Planey SL. The influence of lipophilicity in drug discovery and design. Expert Opin Drug Discov 2012; 7(10):863-75. doi: 10.1517/17460441.2012.714363
2. Aung TH. Proximicin A-C as prospective HER2-positive and negative breast cancer drugs: Molecular docking and in silico ADME modeling. IPS J Mol Dock Sim 2022; 1(1):1–11. doi: 10.54117/ijmds.v1i1.9

3. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: A webserver for the prediction of toxicity of chemicals. Nucleic Acids Res 2018; 46(W1):W257-W263. doi: 10.1093/nar/gky318
4. Borra NK, Kuna Y. Evolution of toxic properties of antialzheimer's drugs through Lipinski's rule of five. Int J Pure App Biosci 2013; 1:28-36.
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68(6):394-424. doi: 10.3322/caac.21492
6. Chow HH, Garland LL, Hsu CH, Vining DR, Chew WM, Miller JA, Perloff M, Crowell JA, Alberts DS. Resveratrol modulates drug- and carcinogen-metabolizing enzymes in a healthy volunteer study. Cancer Prev Res (Phila) 2010; 3(9):1168-75. doi: 10.1158/1940-6207.CAPR-09-0155
7. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep 2017; 7:42717. doi: 10.1038/srep42717
8. Daisy P, Singh SK, Vijayalakshmi P, Selvaraj C, Rajalakshmi M, Suveena S. A database for the predicted pharmacophoric features of medicinal compounds. Bioinformation 2011; 6(4):167-8. doi: 10.6026/97320630006167
9. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R. ProTox: A web server for the in-silico prediction of rodent oral toxicity. Nucleic Acids Res 2014; 42(Web Server issue):W53-8. doi: 10.1093/nar/gku401
10. El-Kattan A, Varm M. Oral absorption, intestinal metabolism and human oral bioavailability. Topics on Drug Metabolism 2012. doi: 10.5772/31087
11. GHS (Rev.8). In: UNECE 2019. https://unece.org/ghs-rev8-2019.
12. Gurung AB, Ali MA, Lee J, Farah MA, Al-Anazi KM. Molecular docking and dynamics simulation study of bioactive compounds from *Ficus carica L.* with important anticancer drug targets. PLoS One 2021; 16(7):e0254035. doi: 10.1371/journal.pone.0254035
13. Hiesinger K, Darin D, Proschak E, Krasavin M. Spirocyclic Scaffolds in Medicinal Chemistry. J Med Chem 2021; 64(1):150-183. doi: 10.1021/acs.jmedchem.0c01473
14. Jia Y, Zhang Y, Qiao C, Liu G, Zhao Q, Zhou T, Chen G, Li Y, Feng J, Li Y, Zhang Q, Peng H. IGF-1R and ErbB3/HER3 contribute to enhanced proliferation and carcinogenesis in trastuzumab-resistant ovarian cancer model. Biochem Biophys Res Commun 2013; 436(4):740-5. doi: 10.1016/j.bbrc.2013.06.030
15. Karpuz M, Silindir-Gunay M, Ozer AY. Current and future approaches for effective cancer imaging and treatment. Cancer Biother Radiopharm 2018; 33:39-51. doi: 10.1089/cbr.2017.2378
16. Kerns EH, Di L. Pharmaceutical profiling in drug discovery. Drug Discov Today 2003; 8(7):316-23. doi: 10.1016/s1359-6446(03)02649-7
17. Khan MF, Bari MA, Islam MK, Kayser MS, Nahar N, Faruk MA, Rashid MA. The natural anti-tubercular agents: In silico study of physicochemical, pharmacokinetic and toxicological properties. J Appl Pharm Sci 2017. doi: 10.7324/japs.2017.70506
18. Liao Y, Abel U, Grobholz R, Hermani A, Trojan L, Angel P, Mayer D. Up-regulation of insulin-like growth factor axis components in human primary prostate cancer correlates with tumor grade. Hum Pathol 2005; 36(11):1186-96. doi: 10.1016/j.humpath.2005.07.023
19. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001; 46(1-3):3-26. doi: 10.1016/s0169-409x(00)00129-0
20. Liu Z, Ma L, Zhang L, Zhang W, Zhu Y, Chen Y, Zhang W, Zhang C. Functional characterization of the halogenase SpmH and discovery of new deschloro-tryptophan dimers. Org Biomol Chem 2019; 17(5):1053-1057. doi: 10.1039/c8ob02775g
21. Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions and adverse effects. Am Fam Physician 2007; 76(3):391-6.
22. Maliehe TS, Tsilo PH, Shandu JS. Computational Evaluation of ADMET Properties and Bioactive Score of Compounds from Encephalartos ferox. Pharmacogn J 2020; 12(6):1357–1362. doi: 10.5530/pj.2020.12.187
23. Muegge I, Heald SL, Brittelli D. Simple selection criteria for drug-like chemical matter. J Med Chem 2001; 44(12):1841-6. doi: 10.1021/jm015507e
24. Mulvihill MJ, Cooke A, Rosenfeld-Franklin M, Buck E, Foreman K, Landfair D, O'Connor M, Pirritt C, Sun Y, Yao Y, Arnold LD, Gibson NW, Ji QS. Discovery of OSI-906: A selective and orally efficacious dual inhibitor of the IGF-1 receptor and insulin receptor. Future Med Chem 2009; 1(6):1153–71. doi: 10.4155/fmc.09.89
25. Ng KW, Lau WM. Skin deep: The basics of human skin structure and drug penetration. Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement 2015; 3–11. doi: 10.1007/978-3-662-45013-0_1
26. Pantsar T, Poso A. Binding Affinity via Docking: Fact and Fiction. Molecules 2018; 23(8):1899. doi: 10.3390/molecules23081899
27. Paramashivam SK, Elayaperumal K, Natarajan BB, Ramamoorthy MD, Balasubramanian S, Dhiraviam KN. In silico pharmacokinetic and molecular docking studies of small

molecules derived from Indigofera aspalathoides Vahl targeting receptor tyrosine kinases. Bioinformation 2015; 11 (2):73-84. doi: 10.6026/97320630011073

28. Paulus C, Rebets Y, Tokovenko B, Nadmid S, Terekhova LP, Myronovskyi M, Zotchev SB, Rückert C, Braig S, Zahler S, Kalinowski J, Luzhetskyy A. New natural products identified by combined genomics-metabolomics profiling of marine Streptomyces sp. MP131-18. Sci Rep 2017; 7:42382. doi: 10.1038/srep42382
29. Rikhof B, De-Jong S, Suurmeijer AJ, Meijer C, Graaf WTV. The insulin-like growth factor system and sarcomas. J Pathol 2009; 217(4):469-82. doi: 10.1002/path.2499
30. Sarkar B, Ullah MA, Islam SS, Rahman MH, Araf Y. Analysis of plant-derived phytochemicals as anti-cancer agents targeting cyclin dependent kinase-2, human topoisomerase IIa and vascular endothelial growth factor receptor-2. J Recept Signal Transduct Res 2021; 41(3):217-233. doi: 10.1080/10799893.2020.1805628
31. Sharmila G, Bhat FA, Arunkumar R, Elumalai P, Raja-Singh P, Senthilkumar K, Arunakaran J. Chemopreventive effect of quercetin, a natural dietary flavonoid on prostate cancer in in vivo model. Clin Nutr 2014; 33(4):718-26. doi: 10.1016/j.clnu.2013.08.011
32. Silva GM, Federico LB, Alves VM, Silva CHDP. In silico methods to predict relevant toxicological endpoints of bioactive substances. Functional Properties of Advanced Engineering Materials and Biomolecules 2021; 649–676. doi: 10.1007/978-3-030-62226-8_22
33. Spinu N, Cronin MTD, Enoch SJ, Madden JC, Worth AP. Quantitative adverse outcome pathway (qAOP) models for toxicity prediction. Arch Toxicol 2020; 94(5):1497-1510. doi: 10.1007/s00204-020-02774-7
34. Stegemann S, Leveiller F, Franchi D, Jong H, Lindén H. When poor solubility becomes an issue: From early stage to proof of concept. Eur J Pharm Sci 2007; 31(5):249-61. doi: 10.1016/j.ejps.2007.05.110
35. Tsuta K, Mimae T, Nitta H, Yoshida A, Maeshima AM, Asamura H, Grogan TM, Furuta K, Tsuda H. Insulin-like growth factor-1 receptor protein expression and gene copy number alterations in non-small cell lung carcinomas. Hum Pathol 2013; 44(6):975-82. doi: 10.1016/j.humpath.2012.09.002
36. Ucar DA, Magis A, He DH, Ostrov D, Hochwald SN. Abstract 3582: Targeting the FAK amino terminus F2 subdomain to disrupt growth factor receptor protein interactions, signaling and function. Cancer Res 2011; 71(8 Supplement):3582–3582. doi: 10.1158/1538-7445.am2011-3582
37. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem 2002; 45(12):2615-23. doi: 10.1021/jm020017n
38. Wang Y, Xing J, Xu Y, Zhou N, Peng J, Xiong Z, Liu X, Luo X, Luo C, Chen K, Zheng M, Jiang H. In silico ADME/T modelling for rational drug design. Q Rev Biophys 2015; 48(4):488-515. doi: 10.1017/S0033583515000190
39. Waterbeemd HV, Gifford E. ADMET in silico modelling: Towards prediction paradise? Nat Rev Drug Discov 2003; 2(3):192-204. doi: 10.1038/nrd1032
40. Weber IT, Harrison RW, Kubinyi H, Folkers G, Martin YC. Molecular Mechanics Calculations on Protein-Ligand Complexes. London Kluwer Academic Publishers 1998; 2:115-27.
41. Wei Z, Hurtt R, Bodzin AS, Gu T, Doria C. Abstract 1234: GRK2 negatively regulates IGF-1R signaling pathway and cyclins in HepG2 cells. Cancer Res 2012; 72(8 Supplement):1234–1234. doi: 10.1158/1538-7445.am2012-1234
42. Werner H, Bruchim I. The insulin-like growth factor-I receptor as an oncogene. Arch Physiol Biochem 2009; 115(2):58-71. doi: 10.1080/13813450902783106
43. Wilson S, Chia SK. IGF-1R inhibition: Right direction, wrong pathway? Lancet Oncol 2013; 14(3):182-3. doi: 10.1016/S1470-2045(13)70019-6
44. Zhang W, Liu Z, Li S, Yang T, Zhang Q, Ma L, Tian X, Zhang H, Huang C, Zhang S, Ju J, Shen Y, Zhang C. Spiroindimicins AD: New bisindole alkaloids from a deep-sea-derived actinomycete. Org Lett 2012; 14(13):3364-7. doi: 10.1021/ol301343n
45. Zhao X, Dou W, He L, Liang S, Tie J, Liu C, Li T, Lu Y, Mo P, Shi Y, Wu K, Nie Y, Fan D. MicroRNA-7 functions as an anti-metastatic microRNA in gastric cancer by targeting insulin-like growth factor-1 receptor. Oncogene 2013; 32(11):1363-72. doi: 10.1038/onc.2012.156