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Full Length Research Article

IN SILICO PROFILING OF THE ADMET PROPERTIES AND INHIBITORY POTENTIALS OF ALKALOIDS AGAINST IFIT5 IN RENAL CELL CARCINOMA

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ABSTRACT:
Alkaloids from diverse classes have demonstrated efficacy in anticancer treatments. Interferon-induced protein with tetratricopeptide repeats 5 (IFIT5) is implicated in the pathogenesis of renal cell carcinoma (RCC). Less is known about the inhibitory effects of these pharmacologically relevant classes of alkaloids on IFIT5. Thus, we determined the ADMET properties of these alkaloids, as well as their inhibitory potentials on IFIT5. Fifty alkaloids were retrieved from PubChem. The structure of IFIT5 was obtained from Protein Data Bank and processed using Biovia Discovery Studio to eliminate nonstandard molecules. Molecular docking was performed using PyRx to assess the binding affinities of protein-ligand complexes, while ADMET analysis was conducted using AdmetSAR and swissADME. Docking results revealed that all 50 selected alkaloids demonstrated high IFIT5 binding effects, ranging from -7.1 to -11.0 kcal/mol, surpassing the di(hydroxyethyl)ether cocrystallized ligand (-3.9 kcal/mol), with nortopsentin A emerging as the most promising hit. Additionally, four of the top five IFIT5 binding alkaloids (nortopsentin A, stylopine, oxymatrine, and deoxytubulosine) displayed favourable drug-like properties. The propitious drug-like properties and strong IFIT5 binding impacts displayed by these alkaloids, particularly nortopsentin A underscore their potential for advancement into pre-clinical/clinical trials for developing selective IFIT5 inhibitors for targeted RCC treatment.

Keywords: Renal cell carcinoma, IFIT5, alkaloids, binding affinity, pharmacokinetics, toxicity

INTRODUCTION

Renal cell carcinoma (RCC) is an insidious neoplasm, accounting for approximately 2% of global cancer diagnoses and deaths (Padala et al., 2020). A unique characteristic of RCC is that many patients are often asymptomatic; it is estimated that more than 50% of patients are diagnosed with RCC incidentally during imaging for an unrelated issue (Rosner et al., 2021). Several major risk factors associated with RCC include smoking, excess body weight, alcohol consumption, hypertension, diabetes, chronic kidney disease, as well as genetic factors, such as mutations in the von Hippel–Lindau (VHL) tumour suppressor gene (Al-Bayati et al., 2019). Most RCCs occur in the cortex of the kidney, which is composed of the glomerulus, tubular apparatus and collecting duct (El-Zaatari et al., 2020). RCC cases in developed countries are often diagnosed through imaging, with only 10% presenting with the classic triad symptoms, including hematuria, flank pain, palpable masses, fever, weight loss, and leukocytosis (Satwikananda et al., 2023).

About 90% of RCCs are of the clear cell, papillary and chromophobe histological subtypes, with the clear cell being the most common and aggressive (Low et al., 2016). These subtypes have significant prognostic and treatment-predictive value. RCC etiology is a complex process that is regulated by several molecular variables (Keefe et al., 2013). The interferon-induced protein with tetratricopeptide repeats (IFIT) is one such factor that has attracted a lot of interest. (Rethnam, 2022). The IFIT family consists of a group of proteins that are upregulated in response to interferon signaling, primarily in the presence of viral infections (Tan et al., 2021). Tetratricopeptide repeat (TPR) motifs are commonly found in members of the IFIT family, including IFIT1, IFIT2, IFIT3, and IFIT5. These motifs are implicated in protein-protein interactions. (Pidugu et al., 2019). IFIT proteins now play a unique role in non-viral diseases including cancer, according to recent investigations (Kwon et al., 2022; Moulin et al., 2023)

IFIT5 has emerged as a pivotal player in RCC progression (Kang et al., 2022). IFIT5 acts solely as a monomer that can not only bind directly to viral RNA molecules via its convoluted RNA-binding cleft but also endogenous cellular RNAs with a 5'-end phosphate cap, including transfer RNAs (tRNA) (Lo, Pong, et al., 2019). IFIT5 has been implicated exclusively in an innate immune response. However, a newly identified mechanism of IFIT5 is regulation of the turnover of tumour suppressor microRNAs (miRNAs), including miR-363 and miR-128, resulting in increased expression of transcription factors of epithelial-to-mesenchymal transition (EMT) such as slug and ZEB1, thereby enhancing invasion in RCC (Lo et al., 2018). EMT plays an essential role in RCC pathogenesis, invasion and response to therapies (Guarino et al., 2007). It has also been shown that EMT correlated with an increased recurrence risk and worst overall survival (OS) in patients with RCC (Rasti et al., 2018).

Conventional chemotherapeutic drug therapy has shown low success against RCC, most of these treatment modalities are associated with complications such as diarrhoea, nausea, and mouth sores, that subsequently affect the quality of life and overall mental status of cancer patients (Regassa et al., 2022). Also, compounds with great specificity are the focus of contemporary drug discovery techniques including high-throughput screening and structure-based drug design (Vázquez et al., 2020). However, anticipating and minimizing off-target effects is still a challenging task, particularly in light of the intricate network of cellular pathways (Newman et al., 2020). IFIT5 is recognized for its intricate involvement in various cellular mechanisms, including antiviral defense and innate immune response (Bela-Ong et al., 2020; Chico et al., 2019; Wu et al., 2022; Zhang et al., 2021). However,

its role in cancer pathogenesis has only recently come to light (Huang et al., 2019; Lo, Bao, et al., 2019; Lo, Pong, et al., 2019; Pidugu et al., 2019; Tan et al., 2021). This could be responsible for the paucity of research attempts pursuing the development of selective inhibitors targeting the cancer-promoting effects of IFIT5. Renal carcinoma cells are among the cancer cells that can become resistant to targeted therapy through a variety of mechanisms, such as the activation of alternative signaling pathways or mutations in the target protein (Posadas et al., 2017). In addition, delivering drug compounds specifically to renal carcinoma cells while minimizing systemic toxicity and off-target effects presents a significant challenge (Yang et al., 2022). Thus, searching for novel therapeutic strategies with minimum side effects has become an urgent requirement in the treatment of renal cancer and there is growing interest in the use of natural product compounds to search for useful candidates as a result of minimal side effects (Garrido-Laguna & Hidalgo, 2015; Gomez et al., 2016).

Natural products have attracted significant interest among researchers for the prevention and treatment of various human cancers. Natural products have been utilized in the discovery and development of drugs, especially for cancer and infectious diseases (Bernardini et al., 2018). Approximately 60% of current cancer chemotherapeutic drugs were derived directly or indirectly from natural sources (Cragg & Pezzuto, 2016). Examples of the associations between natural products and anticancer agents include the vinca alkaloids (vincristine and vinblastine) isolated from the Madagascar periwinkle plant, taxanes (paclitaxel) derived from the bark of a Pacific yew tree, and camptothecins (irinotecan and topotecan) isolated from a Chinese ornamental tree (Nerkar et al., 2021). natural products like alkaloids have been found to provide therapeutic benefits by modulating a wide variety of cellular processes, such as apoptotic pathways, glucose metabolism, cell cycle progression, DNA synthesis and damage repair pathways, and redox regulation pathways (Braicu et al., 2022). Alkaloids encompass an enormous class of approximately 12,000 natural products (Bribi, 2018). The principal requirement for classification as an alkaloid is the presence of a basic nitrogen atom at any position in the molecule, which does not include nitrogen in an amide or peptide bond. Alkaloids sourced from various classes have exhibited notable anticancer and anti-proliferative properties across different cancer types. For instance, compounds such as berbamine, lycorine, and berberine, derived from natural sources, have demonstrated efficacy against glioblastoma and gastrointestinal cancers, including colon cancer. They achieve this by targeting crucial signaling pathways such as Wnt/β-catenin, inducing cell cycle arrest, and promoting apoptosis. Moreover, alkaloids like colchicine, vinblastine, and vincristine exert their anticancer effects by modulating key signaling pathways. The diverse chemical structures of these alkaloids hold promise for future anticancer therapies, particularly in the context of gastrointestinal cancers (Byun et al., 2022; Dhyani et al., 2022; Kadir & Murugappan, 2022; Khan et al., 2022; Varela et al., 2023). Despite the role of the IFIT5 in the progression of RCC, there is a dearth of research that has attempted to develop inhibitors from natural products to selectively counteract its cancer-promoting effects (Gorgulla et al., 2021; Lane, 2018). Given the fascinating and pharmacologically relevant anticancer properties of alkaloids, this study will assess the pharmacokinetic, and toxicity properties, as well as the inhibitory potentials of different classes of alkaloids against IFIT5 to identify potential compounds, which could be developed further as potent, selective IFIT5 inhibitors in (pre)clinical trials for targeted RCC treatment.

MATEIALS AND METHODS

Identification and Preparation of IFIT 5

IFIT5 was retrieved from the Research Collaboratory for Structural Bioinformatics (RSCB) Protein Data Bank (PDB). This online free-access depository stores thousands of protein structures (Rose et al., 2016). The protein was prepared following the method described by Kanmodi, Bankole, et al. (2023). Briefly, the 3D structure of the protein, IFIT 5 (PDB ID: 3ZGQ, resolution: 2.20 Å) was downloaded in PDB format and prepared using Biovia Discovery Studio 4.5 software (https://discover.3ds.com/discovery-studio-visualizer-download) to remove water molecules and hetatoms, and also to add polar hydrogen atoms. Prior to molecular docking, the PDB format of the protein was converted to the Protein Data Bank, Partial Charge (Q), and Atom Type (T) format (PDBQT) using the integrated Autodock wizard tool in PyRx.

Identification and Preparation of Ligands for Docking

The 3D structures of fifty (50) alkaloids of various classes with reported anticancer properties (Mondal et al., 2019) were downloaded in structured data format (SDF) from PubChem (Kim et al., 2016) (https://pubchem.ncbi.nlm.nih.gov); a free, user-friendly database storing millions of chemical compounds. These alkaloids include: indoles (aplicyanins B (CID: 16718384), arctigenin (CID: 64981), camptothecin (CID: 24360), coronaridine (CID: 73489), isoplysin A (CID: 135465870), 9-methoxy-camptothecin (CID: 123617), moschamine (CID: 5969616), nortopsentins A (CID: 179268), nortopsentins B (CID: 456387), pericalline (CID: 6436240), sewarine (CID: 5458504), tetrahydrosecamine (CID: 169527), vallesiachotamine (CID: 5384527)); isoquinoline (berberine (CID: 2353), chelerythrine (CID: 2703), chelidonine (CID: 197810), cribostatins 1 (CID: 10330480), cycleanine (CID: 121313), jorumycin (CID: 9849761), liriodenine (CID: 10144), mimosamycin (CID: 4198), sanguinarine (CID: 5154), stylopine (CID: 6770)); pyrrole and pyrrolizidine (homoharringtonine (CID: 285033), prodigiosin (CID: 135455579), clivorine (CID: 5363807), clathrodin (CID: 5388709), dibromophakellstatin (CID: 10500579), Discorhabdin L (CID: 135451013), discorhabdin W (CID: 136117846), tambjamine I (CID: 381030), tambjamine K (CID: 135934866), indicine N-oxide (CID: 280564)); phenanthroindolizidine alkaloids (antofine (CID: 639288), tylophorine (CID: 92114), tylophorinidine (CID: 161749), hypoestestatin 2 (CID: 126663)); indoloquinoline alkaloids (5,11-dimethylindolo[2,3-b]quinoline (CID: 133982), 3,11-dichloro-6H-indolo[2,3-b]quinoline (CID: 42637308), cryptolepine (CID: 82143), 6H-indolo[2,3-b]quinoline (CID: 67484), neocryptolepine (CID: 390526) and β-Carboline – benzoquinolizidine (harmine (CID: 5280953), harmane (CID: 5281404), harmalol (CID: 3565), harmaline (CID: 35634), tryptoline (CID: 107838), deoxytubulosine (CID: 165003), 1',2',3',4'-tetrahydrotubulosine (CID: 21668793), and oxymatrine (CID: 114850)). Additionally, we downloaded the 3D-SDF format of the co-crystallized ligand di(hydroxyethyl)ether (CID: 8117) of IFIT 5 from PubChem. Before molecular docking (blind docking), energy minimization was carried out, thereby converting the ligands from SDF to PDB format; lastly, we converted them to PDBQT format.

Molecular Docking

Autodock Vina, an integral component of the PyRx software

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package (https://pyrx.sourceforge.io/), was employed to carry out the multiple docking of ligands against the IFIT5 protein. PyRx, a computer-based drug discovery software, is capable of screening compound libraries against potential therapeutic targets and stands out as one of the few docking software packages suitable for multiple docking. In terms of optimization and multithreading, the integrated Autodock Vina in PyRx demonstrates significantly enhanced speed and efficiency. It internally determines grid charges and establishes the docking space (Lobo, 2020). Following the preparation of the ligands and protein, the docking process was executed using Vina Wizard. The residues in the active site of the target protein were chosen to create a grid box, and the Vina Wizard was employed to complete the docking procedure. Subsequently, the binding energy scores and root mean square deviation (RMSD) of the docked complex were generated and downloaded in CSV format.

Absorption, distribution, metabolism elimination, and toxicity (ADMET) investigation.

The ADMET properties are crucial to the discovery and development of industrial chemicals, insecticides, food additives, consumer goods, and pharmaceuticals (Trott & Olson, 2009). The top five (5) ligands with the lowest binding energies were chosen, and canonical SMILES were then generated and uploaded to admetSAR and SwissADME servers (http://lmmd.ecust.edu.cn/admetsar2) and (http://www.swissadme.ch) respectively, for analysis of their druglike characteristics and other pharmacokinetic parameters. admetSAR is a completely free web service tool for estimating chemical characteristics such as absorption, distribution, metabolism, excretion, and toxicity (Cheng et al., 2012). SwissADME is a free online tool to assess the pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of small molecules (Daina et al., 2017).

Analysis of Ligand–Protein Interaction

After docking with the target protein, ligands that showed the lowest binding energy and hence highest binding affinities were found and chosen to examine the associated protein-ligand complex structures and bond interactions. The hydrogen bonding and hydrophobic interactions between the ligand and amino acid residues of the protein-ligand complex were visualized using the Biovia Discovery Studio software. The Biovia Discovery Studio software was also used to obtain the 2D structures illustrating the interactions of the protein-ligand complex.

RESULTS AND DISCUSSION

Binding Affinities

The pharmacological pipeline benefits from in-silico modelling in many ways, such as reduced failure rates, shorter clinical trial durations, and lower costs for research and development (Pushpakom et al., 2019; Roessler et al., 2021). As a result of earlier studies demonstrating the anticancer actions of the various alkaloid ligands (Mondal et al., 2019), we assess the binding energies of the alkaloids with IFIT5 in Table I.

The best five IFIT5 binding ligands were selected, and their binding affinities were compared to the co-crystallized ligand Table II. The results showed that all the ligands exhibited low binding energies (ranging from -11 to -9.8 Kcal/mol), hence, high binding affinities for IFIT5 compared to the co-crystallized ligand (-3.9 kcal/mol).

Table 1: Binding energies (ΔG) in Kcal/mol of the interactions of the fifty alkaloids with IFIT5

Ligands	Binding energies (Kcal/mol)
Indole Alkaloids	
Arctigenin	-8
Aplicyanin B	-7.5
Camptothecin	-9.7
Coronaridine	-8.2
Isoplysin A	-7.4
9-Methoxycamptothecin	-9
Moschamine	-8.8
Nortopsentin A	-11
Nortopsentin B	-9.4
Pericalline	-8.3
Sewarine	-8.3
Tetrahydrosecamine	-8.6
Vallesiachotamine	-8.2
Isoquinoline Alkaloids	
Berberine	-8.9
Chelerythrine	-7.4
Chelidonine	-9
Cribostatin	-7.4
Cycleanine	-9.2
Jorumycin	-8.1
Liriodenine	-9.5
Mimosamycin	-8
Sanguinarine	-8.5
Stylopine	-10.6
Homoharringtonine	-8.5
Prodigiosin	-9.5
Pyrrole and Pyrrolizidine	
Clivorine	-8.2
Clathrodine	-7.5
Dibromophakellstatin	-8
Tamjamine I	-7.4
Discorhabdin W	-10.7
Discorhabdin L	-8.7
Tamjamine k	-7.1
Indicine N-oxide	-7.2
Phenanthroindolizidine	
Antofine	-9.3
Tylophorine	-8.1
Tylophorinidine	-8.4
Hypoestestatin 2	-8.6
Indoquinoline	
3,11-dichloro-6H-indolo[2,3-b] quinoline	-9.6
Cryptolepine	-9.5
Neocryptolepine	-9.8
6-H-indolo [2,3-b] quinoline	-9.5
5,11-dimethylindolo[2,3-b]quinoline	-9.4
β-Carboline – benzoquinolizidine	
Harmine	-8.3
Harmane	-8.2
Harmalol	-8.2
Harmaline	-8.4
Tryptoline	-7.5

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Deoxytubulosine	-9.8
1',2',3',4' - tetrahydrotubulosine	-9.7
Oxymatrine	-10

The implications of the low binding energies and hence high binding affinities of the best five ligands are a tendency for increased potency (requiring low concentrations to achieve a significant effect on the target protein), specificity (the likelihood of the ligand binding with other proteins in the body is lower and it is more likely to bind precisely with the target protein.), and longer duration of action (due to the formation of exceptionally stable complex with the target which takes longer to dissociate). Compounds demonstrating potent activity often display selectivity towards their target protein. For instance, imatinib demonstrates notable selectivity for ABL and its activated derivatives like v-ABL, BCR-ABL, and TEL-ABL, as evidenced by in vitro kinase assays using purified proteins, with IC_{50} values ranging from 0.25 to 0.5 µM. Conversely, the IC_{50} values for numerous other tyrosine and serine/threonine kinases were generally at least 100-fold higher. Furthermore, Cellular studies showed that imatinib specifically inhibited the proliferation of myeloid cell lines that express BCR-ABL with complete inhibition of proliferation occurring between 0.5 and 1 µM (Druker, 2004).

Table 2: Binding energies (ΔG) in Kcal/mol of the interaction of the best five alkaloids with IFIT5 compared to that of the co-crystallized ligand.

S/N	Ligands	PyRx
1.	Nortopsentin A	-11
2.	Discorhabdin W	-10.7
3.	stylopine	-10.6
4.	Oxymatrine	-10
5.	deoxytubulosine	-9.8
Cocrystallized Ligand		
1.	di(hydroxyethyl)ether	-3.9

Analysis of ADME/T Properties

ADME/T evaluation revealed that all the ligands except discorhabdin W complied with Lipinski rule of five Table III.

Table III: Drug-likeness of the best five (5) ligands

S/N	Ligands	Lipinski's Rule of Five
1.	Nortopsentin A	Yes; 1 violation: AlogP>5
2.	Discorhabdin W	No; 2 violations: MW>500, HBA>10
3.	stylopine	Yes; 0 violation
4.	Oxymatrine	Yes; 0 violation
5.	deoxytubulosine	Yes; 1 violation: AlogP>5

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Table IV: The adsorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of the best 10 ligands.

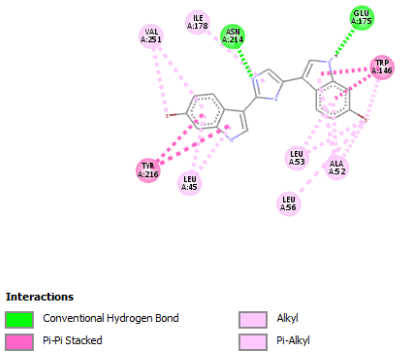
Ligands	Deoxytubolusine	Discorhabdin W	Nortopsentin A	Stylopine	Oxymatrine
MW	459.63	940.57	456.14	323.35	264.37
AlogP	5.41	5.44	6.23	2.80	1.88
H-bond Acceptor	4	13	1	5	2
H-bond Donor	2	5	3	0	0
Rotatable bonds	5	3	2	0	0
CaCo-2	+0.5537	-0.8733	-0.6138	+0.8156	+0.6933
Acute Oral Toxicity	III0.5316	III0.5777	III0.4428	III0.6766	III0.6521
Tetrahymena pyriformis [pigc50(ug/L)	1.615	1.231	1.979	1.452	1.296
BBB	+0.8250	-0.6000	+0.8250	+8500	+0.9250
HIA	+0.9853	+0.9304	+0.9955	+0.9782	-0.4669
p-gp substrate	Substrate0.9137	Substrate0.5765	Non0.8115	Non0.7798	Non0.7675
P-gp Inhibitor	Inhibitor0.8487	Inhibitor0.7645	Non0.6532	Non0.5684	Non0.9404
Subcellular Localization	Mitochondria (0.4020)	Mitochondria (0.5991)	Lysosomes (0.3813)	Mitochondria (0.5937)	Mitochondria (0.5182)
Aqueous Solubility	-2.826	-3.83	-3.166	-2.961	-2.532
CYP2C9 substrate	Non0.8185	Non 1.0000	Non 1.0000	Non0.5820	Non0.5898
CYP2D6 substrate	Substrate0.7349	Non0.8438	Non0.8270	Substrate0.6456	Non0.8403
CYP3A4 Substrate	Substrate0.7231	Substrate0.6757	Non0.5088	Non0.5000	Substrate0.5095
CYP1A2 Inhibitor	Non0.7956	Non0.5000	Inhibitor0.9442	Inhibitor0.9345	Non0.7902
CYP2C9 Inhibitor	Non0.8975	Non0.5051	Inhibitor0.8805	Non0.8860	Non0.8528
CYP2D6 Inhibitor	Inhibitor0.8991	Non0.7973	Non0.9278	Inhibitor0.8397	Non0.8884
CYP2C19 Inhibitor	Non0.8873	Non0.5103	Inhibitor0.7877	Non0.5469	Non0.7644
CYP3A4 Inhibitor	Non0.7319	Non0.7314	Inhibitor 0.7739	Inhibitor0.5325	Non0.8031
CYP inhibitory promiscuity	Low0.8080	High0.6687	High 0.8889	High0.6570	Low0.8276
Ames toxicity	Non0.6100	Non0.5600	Non0.5300	Non0.5300	Non0.7700
Carcinogenicity (three class)	Non-required 0.6763	Non-required 0.5285	Non-required 0.6378	Non-required 0.5621	Non-required 0.4668

Legend: MW: Molecular weight; BBB: Blood brain barrier; P-gp: Permeability glycoprotein; AlogP: Octanol-water partition coefficient; Caco-2: Colon carcinoma cell permeability; CYP: Cytochrome P450; $pIGC_{50}$ negative logarithm of 50% growth inhibitory concentration

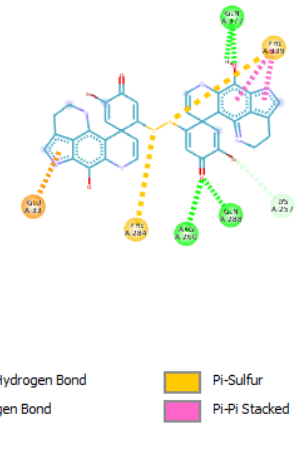
Protein-Ligand Interactions

The protein-ligand binding interactions are shown in Fig 1**(a – e)**. The ligands interacted with the IFIT5 via conventional hydrogen bonds, carbon-hydrogen bond, pi – pi stacked interactions, pi – alkyl interactions, pi – cation and pi – sigma interactions, and pi – sulphur interactions etc.

a



b



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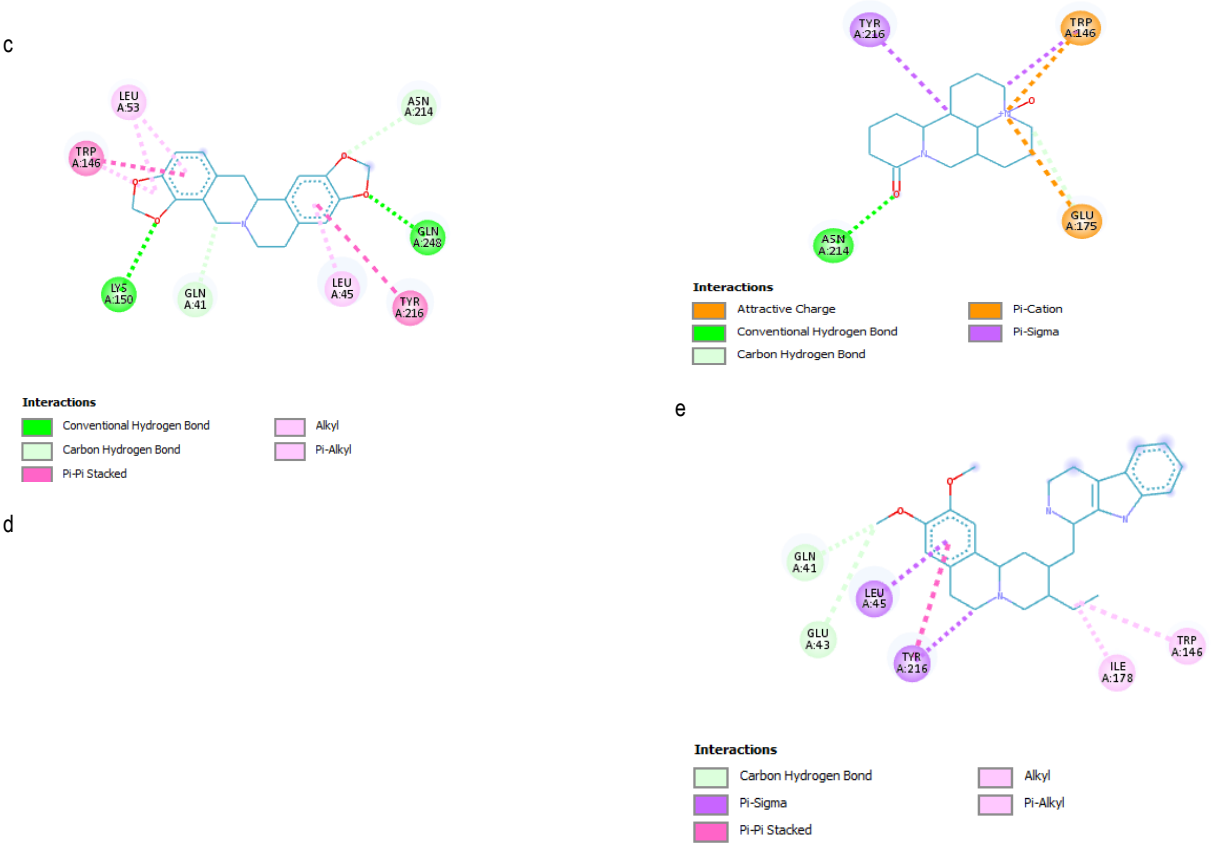
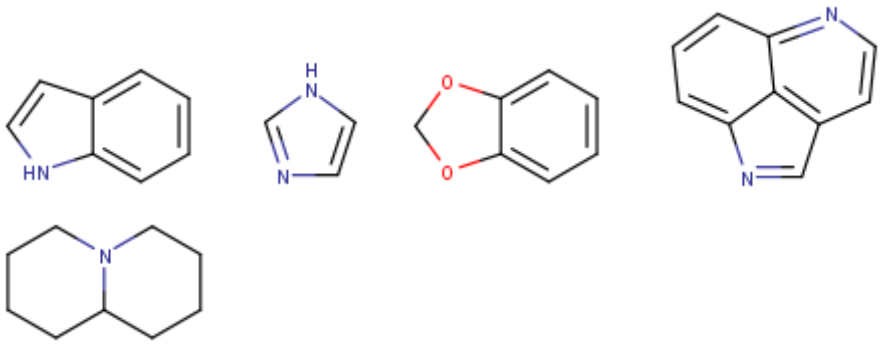


Figure 1: 2D protein-ligand interactions of nortopsentin A and IFIT5 (a); Discorhabdin W and IFIT5 (b); stylopine and IFIT5 (c); oxymatrine and IFIT5 (d); and deoxytubulosine and IFIT5 (e).



a. Indole **b.** Imidazole **c.** Benzodioxole **d.** pyrrolo [4,3,2-de]- quinoline **e.** quinolizidine

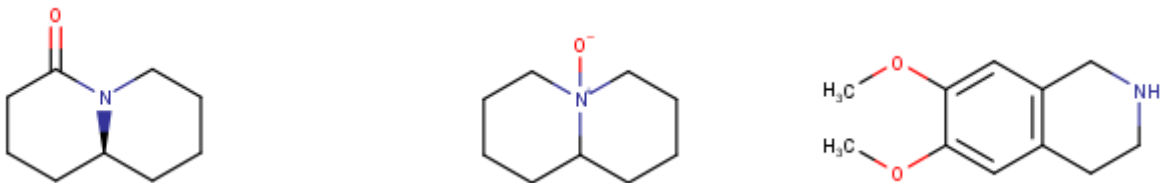


Figure 2: The various pharmacological scaffolds observed to be responsible for the binding interactions of the best five ligands with IFIT5.

DISCUSSION

Targeted therapy has revolutionized the way we approach developing advanced and potent anti-cancer drugs(Kanmodi, Oddiri, et al., 2023). The pharmacological pipeline benefits from in-silico modelling in many ways, such as reduced failure rates, shorter clinical trial durations, and lower costs for research and development (Pushpakom et al., 2019; Roessler et al., 2021). As a result of earlier studies demonstrating the anticancer actions of the various alkaloidal ligands (Mondal et al., 2019), we assess the binding energies of the alkaloids with IFIT1 in this study. Based on our findings, Nortopsentin A had the most desirable binding effect on IFIT5 (-11 Kcal/mol) of the best five ligands and co-crystallized ligands as shown in Table II. Compounds exhibiting low binding energy tend to effectively inhibit their targets at lower concentrations, resulting in stronger inhibitory effects (Umamaheswari et al., 2012).

The interactions leading to the superior binding effects observed in the best ten ligands on IFIT5 are illustrated in the 2D and diagrams of the protein-ligand complexes shown in Fig.1. The presence of alkyl, pi-alkyl bonds, and pi – pi stacked bonds characterized the interaction between aliphatic (valine, isoleucine, and leucine) and aromatic (tyrosine and tryptophan) amino acid residues of IFIT5 and the indole ring (Fig 2a) of nortopsentin A. Further, conventional hydrogen bonds were observed between IFIT5 polar (asparagine), negatively charged (glutamate) amino acid and the imidazole nitrogen and bromine atom attached to the indole ring of nortopsentin A respectively. Imidazole and indole rings have been reported to be crucial rings in the development of anticancer drugs (Dhingra et al., 2022; Godge et al., 2023; Mehra et al., 2022; Salerno et al., 2023). Stylopine benzodioxole ring interacted with IFIT5 amino acid residues via alkyl, pi – alkyl, and pi – pi stacked interactions. A conventional hydrogen bond is observed between the benzodioxole oxygen and lysine. Compounds containing the benzodioxole moiety exhibit a spectrum of biological activities, including anticancer, anti-tuberculosis, antimicrobial, analgesic, and anti-epileptic effects, reflecting their multifaceted pharmacological potential within scientific research and drug discovery endeavours (Hawash et al., 2020).

The pyrrolo [4,3,2-de]- quinoline ring of discorhabdin W (Fig 2) interacted with IFIT5's glutamate residue via the pi-anion bond while interacting with the aromatic phenylalanine residue via pi-pi stacked bonds. Additionally, the disulfide bond of discorhabdin W (a discorhabdin dimer), is observed to interact with IFIT5's phenylalanine residue via a pi-sulfur bond. Numerous derivatives of pyrroloquinoline have exhibited promising pharmacological properties, particularly demonstrating strong antitumor effects across a broad spectrum of cancer cell lines. This underscores their potential as candidates for therapeutic applications in oncology (Francesco et al., 2023). The presence of pi-sigma and carbon-hydrogen bonds characterised the interaction between TYR and GLU residues of IFIT5 and the quinolizidine ring of oxymatrine (Fig 2). The oxo group of (9aS)-octahydro-1H-quinolizin-4-one (a modification of the quinolizidine ring) (Fig 2)interacted with the polar uncharged side chain of an asparagine residue through a conventional hydrogen bond. The octahydro-1H-quinolizin-5-ium-5-olate of oxymatrine (Fig 2) interacted with the negatively charged side chain of GLU residue, and the indole ring of TRP residue via attractive charge interaction and pi-cation bond respectively. Compounds containing quinolizidine demonstrate diverse pharmacological activities, encompassing a wide array of bioactive properties including anticancer, antiviral, anti-inflammatory, and antibacterial effects as discussed by Tang et al and Zhang et al, etc. (Huang et al., 2022; Tang et al., 2023; Zhang et al., 2023). The heliamine moiety of deoxytubulosine interacted with the aromatic amino acid residue TYR via pi-pi stacked and pi-sigma bonds; the aliphatic side chain of LEU via pi-sigma bonds; and the residues GLN and GLU via carbon-hydrogen bonds. Also, the ethyl group attached to the quinolizidine moiety of deoxytubulosine interacted with ILE and TYR residues using alkyl and pi-alky bonds, respectively.

Assessing the ADMET properties is pivotal in drug discovery and development. It serves to ascertain the safety profile and drug-likeness of chemical compounds, thereby guiding decisions throughout the drug development pipeline (Akinlalu et al., 2021; Guan et al., 2019). According to our results, only discorhabdin W did not comply with the Lipinski rule of five as shown in Table III. Thus, the other four alkaloids in the best five category demonstrated propitious drug-likeness properties (Chen et al., 2020). The Lipinski Rule of Five provides a robust framework for predicting that compounds with two or more violations are likely to exhibit low oral bioavailability (Ibrahim et al., 2020). Furthermore, all other ligands ranked within the best five category except discorhabdin W displayed notable permeability across the blood-brain barrier (BBB), as indicated in Table IV. This finding underscores their potential to elicit effects within central nervous system (CNS) regions (Wu et al., 2023). Based on our findings, deoxytubulosine, stylopine, and oxymatrine have high Caco-2 permeability indicating their ability to cross the human intestinal epithelial cell barrier, while only oxymatrine had a low human intestinal absorption of the best five alkaloids.

The status of a compound as either a P-glycoprotein (P-gp) inhibitor or substrate holds paramount importance in drug development, particularly in combatting multidrug resistance (MDR) within cancer therapy. P-gp, an ATP-binding cassette transporter, plays a pivotal role in restricting the absorption and bioavailability of drugs, thereby contributing to MDR. However, inhibitors of P-gp can counteract this resistance mechanism by impeding the efflux of drugs from cells, consequently augmenting their bioavailability and therapeutic efficacy (Rathod et al., 2022; Xing et al., 2020). Conversely, drug molecules that serve as substrates for P-gp undergo active transport out of cells, leading to a decrease in their intracellular concentration and subsequent efficacy (Lee et al., 2019; Rathod et al., 2022). Only deoxytubulosine, and Discorhabdin W, are inhibitors of the P-gp, while the others including nortopsentin A, stylopine and oxymatrine are non-substrates and non-inhibitors of P-gp, as shown in Table 5. This implies that nortopsentin A, stylopine and oxymatrine may be retained in the cells for a longer time increasing their efficiency. Understanding how different compounds interact with CYP (cytochromes P450) is crucial. The metabolic biotransformation (including hydroxylation of aliphatic and aromatic carbon, epoxidation of the aromatic or olefinic double bond, heteroatom oxidation and dealkylation, and dehydrogenation) carried out by this superfamily of isoenzymes is crucial for the removal of drugs (Testa & Kraemer, 2008). According to several reports, it is estimated that between 50 and 90 percent of pharmaceutical compounds are substrates of one of five main isoforms (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) (Di, 2014; Wolf et al., 2000). Different CYP isoforms have varying susceptibilities to inhibition, with 1A2 being the most susceptible and 2D6 being the least susceptible (Hayes et al., 2014). Keeping drug-metabolizing CYPs uninhibited is a common concern in the development of new

drugs (Lamb et al., 2007). The decreased clearance and buildup of the drug or its metabolites due to the inhibition of these isoenzymes is undoubtedly one of the main contributors to pharmacokinetics-related drug-drug interactions that can have toxic or other undesirable side consequences (Kirchmair et al., 2015). In our study, deoxytubulosine and oxymatrine exhibited low CYP inhibitory promiscuity, while nortopsentin A, stylopine and discorhabdin W showed high promiscuity. The broader the spectrum of Cytochrome P450 (CYP) isoforms inhibited by a specific small molecule, the greater the potential for it to engage in drug-drug interactions with a wide range of other medications (Cheng, Yu, et al., 2011). Oxymatrine and discorhabdin W are non-inhibitors of all the major CYP isoforms and are not likely to be involved in any adverse drug reaction related to drug-drug interactions. Deoxytubulosine inhibits only CYP2D6, a particularly important isoform due to its function in the metabolism of a variety of drugs, including antiarrhythmics, and anticancer treatments (Kumar & Surapaneni, 2001). Stylopine demonstrates inhibition of the CYP isoforms 1A2, 2D6, and 3A4, while nortopsentin A inhibits 1A2, 2C9, 2C19, and 3A4. Consequently, the likelihood of causing side effects or adverse drug reactions is highest with nortopsentin A, which inhibits four isoforms. Conversely, oxymatrine and discorhabdin W are less likely to induce such effects.

According to Cheng, Shen, et al. (2011), compounds with positive $pIGC_{50}$ values are generally considered toxic or weakly toxic to aquatic life. Thus, the best five IFIT5 binding ligands with $pIGC_{50}$ values ranging from 1.231 to 1.979 may exert toxic effects on aquatic life. However, our toxicity prediction showed that these five aforementioned compounds are neither carcinogenic nor Ames mutagenic. The Ames test provides valuable information about a potential drug candidate's ability to induce genetic mutations, which lead to cancer or other health problems. It suffices for the substance to be deemed an *in vitro* mutagen if this core test yields a positive result (Hakura et al., 2021; Savale, 2018). Additional investigations are required to evaluate the pharmacokinetic profiles and safety profiles of the best five ligands identified, to advance their candidacy for anti-IFIT5 drug development and targeted RCC therapy.

Conclusion

Our findings demonstrate that all ligands docked against IFIT5 exhibit high IFIT5 binding affinities (ranging from -11 to -7.1 kcal/mol) compared to the di(hydroxyethyl)ether cocrystallized ligand (-3.9 kcal/mol), with nortopsentin A emerging as the most promising hit. Additionally, four of the top five IFIT5 binding ligands—nortopsentin A, stylopine, oxymatrine, and deoxytubulosine — obeyed Lipinski's rule and displayed favourable drug-like properties. Furthermore, toxicity tests revealed that these hit compounds are neither Ames mutagenic nor carcinogenic. The propitious drug-like properties and strong IFIT5 binding impacts displayed by these alkaloids, particularly, nortopsentin A, underscores their potential for advancement into pre-clinical/clinical trials for developing selective IFIT5 inhibitors for targeted RCC treatment.

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REFERENCES

Akinlalu, A. O., Chamundi, A., Yakumbur, D. T., Afolayan, F. I. D., Duru, I. A., Arowosegbe, M. A., & Enejoh, O. A. (2021). Repurposing FDA-approved drugs against multiple proteins of SARS-CoV-2: an in silico study. *Scientific African*, *13*, e00845.

Al-Bayati, O., Hasan, A., Pruthi, D., Kaushik, D., & Liss, M. A. (2019). Systematic review of modifiable risk factors for kidney cancer. Urologic Oncology: Seminars and Original Investigations,

Bela-Ong, D. B., Greiner-Tollersrud, L., van der Wal, Y. A., Jensen, I., Seternes, O. M., & Jørgensen, J. B. (2020). Infection and microbial molecular motifs modulate transcription of the interferon-inducible gene ifit5 in a teleost fish. *Developmental & Comparative Immunology*, *111*, 103746.

Bernardini, S., Tiezzi, A., Laghezza Masci, V., & Ovidi, E. (2018). Natural products for human health: an historical overview of the drug discovery approaches. *Natural product research*, *32*(16), 1926-1950.

Braicu, C., Zanoaga, O., Zimta, A.-A., Tigu, A. B., Kilpatrick, K. L., Bishayee, A., Nabavi, S. M., & Berindan-Neagoe, I. (2022). Natural compounds modulate the crosstalk between apoptosis-and autophagy-regulated signaling pathways: Controlling the uncontrolled expansion of tumor cells. Seminars in Cancer Biology,

Bribi, N. (2018). Pharmacological activity of alkaloids: a review. *Asian journal of botany*, *1*(1), 1-6.

Byun, W. S., Bae, E. S., Kim, W. K., & Lee, S. K. (2022). Antitumor activity of rutaecarpine in human colorectal cancer cells by suppression of Wnt/β-catenin signaling. *Journal of Natural Products*, *85*(5), 1407-1418.

Chen, X., Li, H., Tian, L., Li, Q., Luo, J., & Zhang, Y. (2020). Analysis of the physicochemical properties of acaricides based on Lipinski's rule of five. *Journal of computational biology*, *27*(9), 1397-1406.

Cheng, F., Li, W., Zhou, Y., Shen, J., Wu, Z., Liu, G., Lee, P. W., & Tang, Y. (2012). admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. In: ACS Publications.

Cheng, F., Shen, J., Yu, Y., Li, W., Liu, G., Lee, P. W., & Tang, Y. (2011). In silico prediction of Tetrahymena pyriformis toxicity for diverse industrial chemicals with substructure pattern recognition and machine learning methods. *Chemosphere*, *82*(11), 1636-1643.

Cheng, F., Yu, Y., Zhou, Y., Shen, Z., Xiao, W., Liu, G., Li, W., Lee, P. W., & Tang, Y. (2011). Insights into molecular basis of cytochrome p450 inhibitory promiscuity of compounds. *Journal of chemical information and modeling*, *51*(10), 2482-2495.

Chico, V., Salvador-Mira, M. E., Nombela, I., Puente-Marin, S., Ciordia, S., Mena, M. C., Perez, L., Coll, J., Guzman, F., & Encinar, J. A. (2019). IFIT5 participates in the antiviral mechanisms of rainbow trout red blood cells. *Frontiers in immunology*, *10*, 613.

Cragg, G. M., & Pezzuto, J. M. (2016). Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. *Medical Principles and Practice*, *25*(Suppl. 2), 41-59.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness

and medicinal chemistry friendliness of small molecules. *Scientific reports*, *7*(1), 42717.

Dhingra, A. K., Chopra, B., Sidhu, A., Guarve, K., & Gupta, A. (2022). Imidazole Showing its Therapeutic Voyage as Anticancer Heterocyclic Ring. *Key Heterocyclic Cores for Smart Anticancer Drug–Design Part II*, 105.

Dhyani, P., Quispe, C., Sharma, E., Bahukhandi, A., Sati, P., Attri, D. C., Szopa, A., Sharifi-Rad, J., Docea, A. O., & Mardare, I. (2022). Anticancer potential of alkaloids: a key emphasis to colchicine, vinblastine, vincristine, vindesine, vinorelbine and vincamine. *Cancer cell international*, *22*(1), 206.

Di, L. (2014). The role of drug metabolizing enzymes in clearance. *Expert opinion on drug metabolism & toxicology*, *10*(3), 379-393.

Druker, B. J. (2004). Imatinib as a paradigm of targeted therapies. *Advances in cancer research*, *91*(1), 1-30.

El-Zaatari, Z. M., Arora, K., Divatia, M. K., & Ro, J. Y. (2020). Normal Anatomy and Histology of the Kidney: Importance for Kidney Tumors. *Kidney Cancer: Recent Advances in Surgical and Molecular Pathology*, 33-45.

Francesco, M., Caterina, D., Sano, Claudia, D. A., Marco, F., Luigi, M., Alessia, B., Gabriele, L., Monica, Annamaria, M., Anna, M., Almerico, & Antonino, L. (2023). Synthesis of new antiproliferative 1,3,4-substituted-pyrrolo[3,2-c]quinoline derivatives, biological and in silico insights. *European journal of medicinal chemistry*. https://doi.org/doi: 10.1016/j.ejmech.2023.115537

Garrido-Laguna, I., & Hidalgo, M. (2015). Pancreatic cancer: from state-of-the-art treatments to promising novel therapies. *Nature reviews Clinical oncology*, *12*(6), 319-334.

Godge, R., Dighe, A., & Kolhe, P. (2023). An overview of imidazole, derivatives ofimidazole and its pharmacological applications.

Gomez, I. G., Nakagawa, N., & Duffield, J. S. (2016). MicroRNAs as novel therapeutic targets to treat kidney injury and fibrosis. *American Journal of Physiology-Renal Physiology*, *310*(10), F931-F944.

Gorgulla, C., Das, K. M. P., Leigh, K. E., Cespugli, M., Fischer, P. D., Wang, Z.-F., Tesseyre, G., Pandita, S., Shnapir, A., & Calderaio, A. (2021). A multi-pronged approach targeting SARS-CoV-2 proteins using ultra-large virtual screening. *Iscience*, *24*(2).

Guan, L., Yang, H., Cai, Y., Sun, L., Di, P., Li, W., Liu, G., & Tang, Y. (2019). ADMET-score–a comprehensive scoring function for evaluation of chemical drug-likeness. *Medchemcomm*, *10*(1), 148-157.

Guarino, M., Rubino, B., & Ballabio, G. (2007). The role of epithelial-mesenchymal transition in cancer pathology. *Pathology*, *39*(3), 305-318.

Hakura, A., Awogi, T., Shiragiku, T., Ohigashi, A., Yamamoto, M., Kanasaki, K., Oka, H., Dewa, Y., Ozawa, S., & Sakamoto, K. (2021). Bacterial mutagenicity test data: collection by the task force of the Japan pharmaceutical manufacturers association. *Genes and Environment*, *43*, 1-16.

Hawash, M., Eid, A. M., Jaradat, N., Abualhasan, M., Amer, J., Naser Zaid, A., Draghmeh, S., Daraghmeh, D., Daraghmeh, H., & Shtayeh, T. (2020). Synthesis and biological evaluation of benzodioxole derivatives as potential anticancer and antioxidant agents. *Heterocyclic Communications*, *26*(1), 157-167.

Hayes, C., Ansbro, D., & Kontoyianni, M. (2014). Elucidating substrate promiscuity in the human cytochrome 3A4. *Journal of chemical information and modeling*, *54*(3), 857-869.

Huang, J., Lo, U.-G., Wu, S., Wang, B., Pong, R.-C., Lai, C.-H., Lin, H., He, D., Hsieh, J.-T., & Wu, K. (2019). The roles and mechanism of IFIT5 in bladder cancer epithelial–mesenchymal transition and progression. *Cell death & disease*, *10*(6), 437.

Huang, L., Zhu, L., Xie, H., Goodwin, J. S., Rana, T., Xie, L., & Chen, C.-H. (2022). Quinolizidines as novel SARS-CoV-2 entry inhibitors. *International Journal of Molecular Sciences*, *23*(17), 9659.

Ibrahim, Z. Y. u., Uzairu, A., Shallangwa, G., & Abechi, S. (2020). Molecular docking studies, drug-likeness and in-silico ADMET prediction of some novel β-Amino alcohol grafted 1, 4, 5-trisubstituted 1, 2, 3-triazoles derivatives as elevators of p53 protein levels. *Scientific African*, *10*, e00570.

Kadir, A. S., & Murugappan, S. (2022). Alkaloid Containing Natural Products for Therapeutic Use in Cancer. In *Handbook of Research on Natural Products and Their Bioactive Compounds as Cancer Therapeutics* (pp. 352-383). IGI Global.

Kang, J. A., Kim, Y. J., & Jeon, Y. J. (2022). The diverse repertoire of ISG15: More intricate than initially thought. *Experimental & Molecular Medicine*, *54*(11), 1779-1792.

Kanmodi, R., Bankole, H., Oddiri, R., Arowosegbe, M., Alabi, R., Rahmon, S., Ahmodu, O., AbdulRasheed, B., & Muritala, R. (2023). Repurposing Antipsychotic Agents Against Targets of Angiogenesis Pathways for Cancer Therapy: An in-silico Approach. *Current Drug Discovery Technologies*, *20*(6), 38-46.

Kanmodi, R., Oddiri, R., Arowosegbe, M., & Rahmon, S. (2023). Exploring the pharmacokinetic properties and inhibitory potentials of plant-derived alkaloids against nuclear protein targets in triple-negative breast cancer: An In Silico approach. *Scientific African*, *22*, e01940.

Keefe, S. M., Nathanson, K. L., & Rathmell, W. K. (2013). The molecular biology of renal cell carcinoma. Seminars in oncology,

Khan, H., Alam, W., Alsharif, K. F., Aschner, M., Pervez, S., & Saso, L. (2022). Alkaloids and colon cancer: molecular mechanisms and therapeutic implications for cell cycle arrest. *Molecules*, *27*(3), 920.

Kim, S., Thiessen, P. A., Bolton, E. E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., & Shoemaker, B. A. (2016). PubChem substance and compound databases. *Nucleic Acids Research*, *44*(D1), D1202-D1213.

Kirchmair, J., Göller, A. H., Lang, D., Kunze, J., Testa, B., Wilson, I. D., Glen, R. C., & Schneider, G. (2015). Predicting drug metabolism: experiment and/or computation? *Nature reviews Drug discovery*, *14*(6), 387-404.

Kumar, G. N., & Surapaneni, S. (2001). Role of drug metabolism in drug discovery and development. *Medicinal research reviews*, *21*(5), 397-411.

Kwon, S., Kwon, M., Im, S., Lee, K., & Lee, H. (2022). mRNA vaccines: the most recent clinical applications of

synthetic mRNA. *Archives of pharmacal research*, *45*(4), 245-262.

Lamb, D. C., Waterman, M. R., Kelly, S. L., & Guengerich, F. P. (2007). Cytochromes P450 and drug discovery. *Current opinion in biotechnology*, *18*(6), 504-512.

Lane, W. C. (2018). *Temperature-Dependent Efficacy of the IFN-α/β Response Against Chikungunya and Other Arboviruses* University of Pittsburgh].

Lee, T. D., Lee, O. W., Brimacombe, K. R., Chen, L., Guha, R., Lusvarghi, S., Tebase, B. G., Klumpp-Thomas, C., Robey, R. W., & Ambudkar, S. V. (2019). A high-throughput screen of a library of therapeutics identifies cytotoxic substrates of P-glycoprotein. *Molecular Pharmacology*, *96*(5), 629-640.

Lo, U.-G., Bao, J., Cen, J., Yeh, H.-C., Luo, J., Tan, W., & Hsieh, J.-T. (2019). Interferon-induced IFIT5 promotes epithelial-to-mesenchymal transition leading to renal cancer invasion. *American journal of clinical and experimental urology*, *7*(1), 31.

Lo, U.-G., Pong, R.-C., Yang, D., Gandee, L., Hernandez, E., Dang, A., Lin, C.-J., Santoyo, J., Ma, S.-H., & Sonavane, R. (2018). IFN-γ induces epithelial-to-mesenchymal transition of cancer cells via an unique microRNA processing. *bioRxiv*, 355263.

Lo, U.-G., Pong, R.-C., Yang, D., Gandee, L., Hernandez, E., Dang, A., Lin, C.-J., Santoyo, J., Ma, S., & Sonavane, R. (2019). IFNγ-induced IFIT5 promotes epithelial-to-mesenchymal transition in prostate cancer via miRNA processing. *Cancer research*, *79*(6), 1098-1112.

Lobo, S. (2020). Is there enough focus on lipophilicity in drug discovery? *Expert opinion on drug discovery*, *15*(3), 261-263.

Low, G., Huang, G., Fu, W., Moloo, Z., & Girgis, S. (2016). Review of renal cell carcinoma and its common subtypes in radiology. *World journal of radiology*, *8*(5), 484.

Mehra, A., Sharma, V., Verma, A., Venugopal, S., Mittal, A., Singh, G., & Kaur, B. (2022). Indole derived anticancer agents. *ChemistrySelect*, *7*(34), e202202361.

Mondal, A., Gandhi, A., Fimognari, C., Atanasov, A. G., & Bishayee, A. (2019). Alkaloids for cancer prevention and therapy: Current progress and future perspectives. *European journal of pharmacology*, *858*, 172472.

Moulin, C., Crupi, M. J., Ilkow, C. S., Bell, J. C., & Boulton, S. (2023). Extracellular vesicles and viruses: two intertwined entities. *International Journal of Molecular Sciences*, *24*(2), 1036.

Nerkar, A., Chakraborthy, G., & Ukirde, R. (2021). Anti-cancer agents from natural sources: a review. *Current Trends in Pharmacy and Pharmaceutical. Chemistry*, *3*(4), 38-46.

Newman, A., Starrs, L., & Burgio, G. (2020). Cas9 Cuts and Consequences; Detecting, Predicting, and Mitigating CRISPR/Cas9 On-and Off-Target Damage: Techniques for Detecting, Predicting, and Mitigating the On-and off-target Effects of Cas9 Editing. *Bioessays*, *42*(9), 2000047.

Padala, S. A., Barsouk, A., Thandra, K. C., Saginala, K., Mohammed, A., Vakiti, A., Rawla, P., & Barsouk, A. (2020). Epidemiology of renal cell carcinoma. *World journal of oncology*, *11*(3), 79.

Pidugu, V. K., Pidugu, H. B., Wu, M.-M., Liu, C.-J., & Lee, T.-C. (2019). Emerging functions of human IFIT proteins in cancer. *Frontiers in molecular biosciences*, *6*, 148.

Posadas, E. M., Limvorasak, S., & Figlin, R. A. (2017). Targeted therapies for renal cell carcinoma. *Nature Reviews Nephrology*, *13*(8), 496-511.

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., & McNamee, C. (2019). Drug repurposing: progress, challenges and recommendations. *Nature reviews Drug discovery*, *18*(1), 41-58.

Rasti, A., Madjd, Z., Abolhasani, M., Mehrazma, M., Janani, L., Saeednejad Zanjani, L., & Asgari, M. (2018). Cytoplasmic expression of Twist1, an EMT-related transcription factor, is associated with higher grades renal cell carcinomas and worse progression-free survival in clear cell renal cell carcinoma. *Clinical and Experimental Medicine*, *18*, 177-190.

Rathod, S., Desai, H., Patil, R., & Sarolia, J. (2022). Non-ionic surfactants as a P-glycoprotein (P-gp) efflux inhibitor for optimal drug delivery—a concise outlook. *Aaps Pharmscitech*, *23*(1), 55.

Regassa, H., Sourirajan, A., Kumar, V., Pandey, S., Kumar, D., & Dev, K. (2022). A review of medicinal plants of the himalayas with anti-proliferative activity for the treatment of various cancers. *Cancers*, *14*(16), 3898.

Rethnam, M. (2022). *Prmt5 Is a Potential Therapeutic Vulnerability in Blastic Plasmacytoid Dendritic Cell Neoplasm* National University of Singapore (Singapore)].

Roessler, H. I., Knoers, N. V., van Haelst, M. M., & van Haaften, G. (2021). Drug repurposing for rare diseases. *Trends in pharmacological sciences*, *42*(4), 255-267.

Rose, P. W., Prlić, A., Altunkaya, A., Bi, C., Bradley, A. R., Christie, C. H., Costanzo, L. D., Duarte, J. M., Dutta, S., Feng, Z., Green, R. K., Goodsell, D. S., Hudson, B., Kalro, T., Lowe, R., Peisach, E., Randle, C., Rose, A. S., Shao, C., . . . Burley, S. K. (2016). The RCSB Protein Data Bank: Integrative view of Protein, gene and 3D structural information. *Nucleic Acids Research*, *45*(D1), D271-281. https://doi.org/https://doi.org/10.1093/nar/gkw1000

Rosner, M. H., Jhaveri, K. D., McMahon, B. A., & Perazella, M. A. (2021). Onconephrology: the intersections between the kidney and cancer. *CA: a cancer journal for clinicians*, *71*(1), 47-77.

Salerno, S., Barresi, E., Baglini, E., Poggetti, V., Da Settimo, F., & Taliani, S. (2023). Target-based anticancer indole derivatives for the development of anti-glioblastoma agents. *Molecules*, *28*(6), 2587.

Satwikananda, H., Wiratama, M. A., Putri, K. T. C., & Soebadi, D. M. (2023). Renal cell carcinoma in a patient with staghorn stones: A case report. *International Journal of Surgery Case Reports*, *110*, 108678.

Savale, S. K. (2018). Genotoxicity of drugs: Introduction, prediction and evaluation. *Asian Journal of Biomaterial Research*, *4*(6), 1-29.

Tan, X. F., Chen, Q., Hua, S. H., & Yip, G. W. (2021). Roles of interferon induced protein with tetratricopeptide repeats (IFIT) family in cancer. *Current Medicinal Chemistry*, *28*(25), 5034-5047.

Tang, Q., Liu, Y., Peng, X., Wang, B., Luan, F., & Zeng, N. (2023). Research progress in the pharmacological activities, toxicities, and pharmacokinetics of sophoridine and its derivatives. *Drug Design, Development and Therapy*, 191-212.

Testa, B., & Kraemer, S. D. (2008). The biochemistry of drug metabolism–an introduction: part 4. reactions of conjugation and their enzymes. *Chemistry & biodiversity*, *5*(11), 2171-2336.

Trott, O., & Olson, A. J. (2009). Software news and update AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.*, *31*, 455–461.

Umamaheswari, M., Aji, C., Asokkumar, K., Sivashanmugam, T., Subhadradevi, V., Jagannath, P., & Madeswaran, A. (2012). Docking studies: In silico aldose reductase inhibitory activity of commercially available flavonoids. ||| *Bangladesh Journal of Pharmacology*, *7*(2), 108-113.

Varela, C., Silva, F., Costa, G., & Cabral, C. (2023). Alkaloids: Their relevance in cancer treatment. In *New Insights Into Glioblastoma* (pp. 361-401). Elsevier.

Vázquez, J., López, M., Gibert, E., Herrero, E., & Luque, F. J. (2020). Merging ligand-based and structure-based methods in drug discovery: An overview of combined virtual screening approaches. *Molecules*, *25*(20), 4723.

Wolf, C. R., Smith, G., & Smith, R. L. (2000). Science, medicine, and the future-Pharmacogenetics. *British Medical Journal*, *320*(7240), 987-990.

Wu, D., Chen, Q., Chen, X., Han, F., Chen, Z., & Wang, Y. (2023). The blood–brain barrier: structure, regulation, and drug delivery. *Signal transduction and targeted therapy*, *8*(1), 217.

Wu, Y., Song, X., Cui, D., & Zhang, T. (2022). IFIT3 and IFIT5 play potential roles in innate immune response of porcine pulmonary microvascular endothelial cells to highly pathogenic porcine reproductive and respiratory syndrome virus. *Viruses*, *14*(9), 1919.

Xing, J., Huang, S., Heng, Y., Mei, H., & Pan, X. (2020). Computational Insights into allosteric conformational modulation of P-glycoprotein by substrate and inhibitor binding. *Molecules*, *25*(24), 6006.

Yang, L., Gong, L., Wang, P., Zhao, X., Zhao, F., Zhang, Z., Li, Y., & Huang, W. (2022). Recent advances in lipid nanoparticles for delivery of mRNA. *Pharmaceutics*, *14*(12), 2682.

Zhang, J., Liu, Y.-Q., & Fang, J. (2023). The biological activities of quinolizidine alkaloids. In *The Alkaloids: Chemistry and Biology* (Vol. 89, pp. 1-37). Elsevier.

Zhang, N., Shi, H., Yan, M., & Liu, G. (2021). Ifit5 negatively regulates the type I Ifn pathway by disrupting Tbk1–Ikkε–Irf3 signalosome and degrading Irf3 and Ikkε. *The Journal of Immunology*, *206*(9), 2184-2197.