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Schiff base metal complexes as potential anticancer agents: Biological evaluation, and in silico studies

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Abstract

The development of novel anticancer agents with improved efficacy and selectivity remains a significant challenge in medicinal chemistry. In this study, a series of Schiff base ligands (IAP, TAP, and FAP) and their corresponding cobalt(II) and nickel(II) complexes were synthesized and evaluated for their anticancer potential. The cytotoxic activity of the synthesized compounds was investigated against human cancer cell lines (MCF-7, MDA-MB-231, HeLa, and PC-3), along with a normal cell line (HEK-293) at 10 μ M concentration. The results demonstrated that metal complexation significantly enhances cytotoxic activity compared to the free ligands. Among the tested compounds, Co-TAP and Co-FAP complexes exhibited the most promising anticancer activity, showing higher inhibition of cancer cell proliferation while maintaining relatively lower toxicity toward normal cells. In addition, in silico ADME analysis revealed that the ligands possess favorable physicochemical properties, good gastrointestinal absorption, and compliance with drug-likeness criteria. Toxicity prediction suggested moderate acute toxicity with low risks of carcinogenicity and immunotoxicity, although potential hepatotoxicity and mutagenicity were indicated for some compounds. Overall, the combined experimental and computational findings suggest that these Schiff base metal complexes represent promising candidates for further development as anticancer agents.

Keywords: Schiff base, Metal complexes, Anticancer activity, ADME analysis, Toxicity

1. Introduction

Cancer continues to be one of the leading causes of mortality worldwide, posing a major challenge to global healthcare systems. Despite substantial progress in early diagnosis and therapeutic strategies, the effectiveness of current anticancer treatments remains limited by several factors, including drug resistance, poor selectivity, and severe adverse effects on normal tissues. Conventional chemotherapeutic agents often lack the ability to discriminate between cancerous and healthy cells, resulting in systemic toxicity and reduced patient compliance. In addition, tumor heterogeneity and the emergence of multidrug resistance further compromise therapeutic outcomes, highlighting the urgent need for the development of novel anticancer agents with improved efficacy, selectivity, and safety profiles [1, 2]. Heterocyclic compounds play a pivotal role in medicinal chemistry and constitute the core structure of many clinically approved drugs [3-5]. Among them, Schiff base derivatives have gained significant attention due to their structural versatility, ease of synthesis, and diverse biological activities, including anticancer, antimicrobial, antiviral, and anti-inflammatory properties. The presence of the azomethine ($-C=N-$) functional group enables Schiff bases to interact with various biological targets through hydrogen bonding and coordination interactions, thereby enhancing their pharmacological potential. Furthermore, the incorporation of different substituents within the ligand framework allows fine-tuning of their electronic and steric properties, which is crucial for optimizing biological activity [6-10]. In recent years, metal-based drug design has emerged as a promising approach in anticancer research. The coordination of organic ligands with transition metal ions can significantly alter their physicochemical and biological properties. Metal complexes often exhibit enhanced lipophilicity, improved cellular uptake, and the ability to interact with biomolecules such as DNA and proteins, leading to improved cytotoxic activity [11-14]. In particular, cobalt(II) and nickel(II) complexes have attracted considerable interest due to

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their redox properties and their ability to induce oxidative stress, DNA damage, and apoptosis in cancer cells. Several studies have demonstrated that metal complexes of Schiff base ligands exhibit superior anticancer activity compared to their corresponding free ligands, emphasizing the importance of metal coordination in drug development [15-18]. Alongside experimental approaches, computational methods have become indispensable tools in modern drug discovery. The evaluation of absorption, distribution, metabolism, and excretion (ADME) properties at an early stage helps in identifying compounds with favorable pharmacokinetic profiles and reduces the likelihood of late-stage failure [19-22].

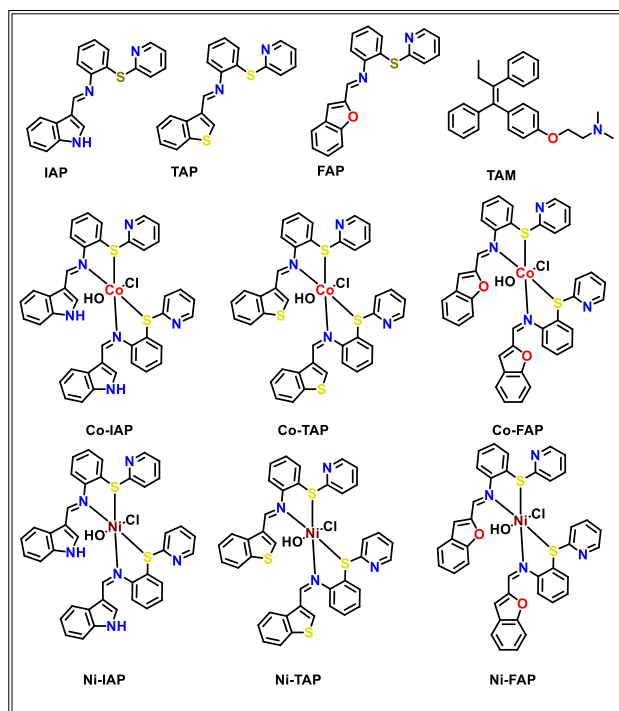


Fig 1: Structure of various Schiff bases, control drug tamoxifen (TAM), cobalt(II) and nickel(II) metal complexes of corresponding Schiff bases.

SwissADME is widely used for predicting key physicochemical and pharmacokinetic parameters, such as lipophilicity, gastrointestinal absorption, and drug-likeness [23, 24]. Similarly, toxicity prediction tools such as ProTox (III) provide valuable insights into the safety profile of compounds, including acute toxicity, organ toxicity, and potential mutagenicity [25, 26]. These *in silico* approaches offer a cost-effective and efficient strategy to complement experimental studies and guide the rational design of drug candidates.

In this context, the present study focuses on the design, synthesis, and biological evaluation of novel Schiff base ligands and their corresponding cobalt(II) and nickel(II) complexes as potential anticancer agents (Figure 1). The synthesized compounds were evaluated for their cytotoxic activity against multiple human cancer cell lines, along with a normal cell line to assess selectivity. Furthermore, *in silico* ADME and toxicity analyses were performed to investigate

their pharmacokinetic behavior and safety profiles. The integration of synthetic chemistry, biological evaluation, and computational modeling in this study is expected to provide valuable insights into the development of new metal-based anticancer agents with improved therapeutic potential.

2. Results and Discussion

The cytotoxic activity of the synthesized ligands and their corresponding metal complexes was evaluated against human cancer cell lines including MCF-7, MDA-MB-231, HeLa, and PC-3, along with the normal cell line HEK-293 at a concentration of 10 μ M after 48 h incubation. The obtained results are summarized in Table 1 and Figure 2. Among the tested ligands, IAP exhibited moderate cytotoxic activity with percentage inhibition values of 32.25%, 29.62%, 26.12%, and 25.56% against MCF-7, MDA-MB-231, HeLa, and PC-3 cell lines, respectively, while showing relatively low toxicity toward the normal HEK-293 cells (12.42%). Coordination with metal ions significantly enhanced the cytotoxic activity. In particular, Co-IAP showed improved inhibition against MCF-7 (44.12%) compared with the parent ligand, indicating that metal complexation may enhance biological activity. Similarly, Ni-IAP demonstrated higher activity against MDA-MB-231 (36.56%) and PC-3 (32.86%) cell lines relative to the free ligand. The TAP ligand displayed moderate inhibition toward the tested cancer cell lines, with the highest activity observed against MCF-7 (40.72%) and HeLa (38.53%). Notably, the Co-TAP complex exhibited enhanced cytotoxic activity, especially against MCF-7 (48.34%) and HeLa (40.55%), suggesting that cobalt coordination improves the anticancer potential of the ligand framework. The Ni-TAP complex also showed good activity against PC-3 cells (36.26%) while maintaining relatively low toxicity toward HEK-293 cells (8.18%), indicating a favorable selectivity profile. Similarly, the FAP ligand demonstrated moderate cytotoxic effects across the tested cancer cell lines. However, its metal complexes showed improved activity. The Co-FAP complex exhibited significant inhibition against MCF-7 (44.96%), MDA-MB-231 cells (42.84%), while Ni-FAP showed comparatively higher activity against HeLa cells (42.14%). Importantly, most of the synthesized compounds showed lower inhibition toward the normal HEK-293 cell line compared with cancer cell lines, suggesting selective cytotoxic effects. For comparison, the reference drug Tamoxifen exhibited the highest inhibition against MCF-7 (56.62%) and MDA-MB-231 (44.46%) cell lines, which is consistent with its well-known anticancer activity against breast cancer cells (Table 1 and Figure 2). Overall, the results indicate that coordination of the Schiff base ligands with transition metals, particularly cobalt and nickel, generally enhances cytotoxic activity against the tested cancer cell lines. Among the investigated compounds, Co-TAP and Co-FAP complexes exhibited comparatively stronger anticancer activity, while maintaining relatively lower toxicity toward normal HEK-293 cells, highlighting their potential as promising candidates for further anticancer studies.

Table 1: Cytotoxic activity of the synthesized ligands against human cancer and normal cell lines at 10 μ M concentration after 48 h incubation.

Compound	% Inhibition at 10 μ M concentration				
	MCF-7	MDA-MB-231	HeLa	PC-3	HEK-293
IAP	32.25 $\pm$ 2.32	29.62 $\pm$ 2.42	26.12 $\pm$ 2.42	25.56 $\pm$ 1.68	12.42 $\pm$ 1.82
Co-IAP	44.12 $\pm$ 2.28	32.35 $\pm$ 1.58	30.64 $\pm$ 1.88	28.12 $\pm$ 1.78	10.42 $\pm$ 1.92
Ni-IAP	38.65 $\pm$ 1.86	36.56 $\pm$ 2.10	34.15 $\pm$ 2.35	32.86 $\pm$ 2.64	18.42 $\pm$ 1.58
TAP	40.72 $\pm$ 2.45	30.25 $\pm$ 2.15	38.53 $\pm$ 2.86	28.32 $\pm$ 1.58	16.12 $\pm$ 1.08
Co-TAP	48.34 $\pm$ 1.92	36.42 $\pm$ 1.85	40.55 $\pm$ 2.14	ND	ND
Ni-TAP	42.86 $\pm$ 2.86	32.42 $\pm$ 1.54	ND	36.26 $\pm$ 1.24	8.18 $\pm$ 1.96
FAP	28.56 $\pm$ 1.85	34.84 $\pm$ 1.62	32.64 $\pm$ 1.98	25.28 $\pm$ 1.66	16.36 $\pm$ 2.62
Co-FAP	44.96 $\pm$ 1.74	42.84 $\pm$ 1.94	ND	30.26 $\pm$ 1.86	10.36 $\pm$ 2.43
Ni-FAP	36.42 $\pm$ 2.64	38.84 $\pm$ 2.86	42.14 $\pm$ 2.35	32.24 $\pm$ 1.96	12.86 $\pm$ 2.56
Tamoxifen	56.62 $\pm$ 3.45	44.46 $\pm$ 2.95	ND	ND	ND

Notes: Values are expressed as mean $\pm$ SD (n = 3). ND = Not determined.

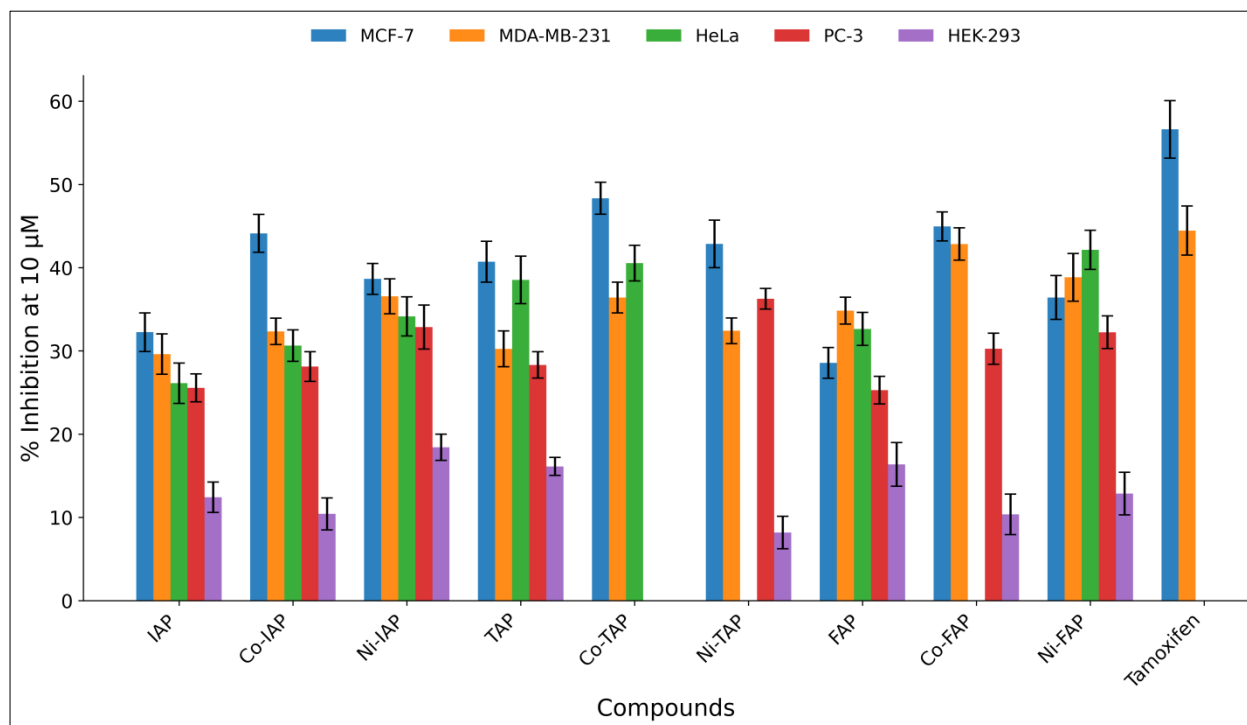


Fig 2: Cytotoxic activity of the synthesized ligands and their metal complexes against human cancer cell lines (MCF-7, MDA-MB-231, HeLa, PC-3) and the normal cell line HEK-293 at 10 μ M concentration after 48 h incubation. Values represent mean $\pm$ SD (n = 3).

3. In-silico ADME Analysis

Absorption, distribution, metabolism, and excretion (ADME) properties play a crucial role in determining the pharmacokinetic behavior, bioavailability, and therapeutic effectiveness of drug candidates. Evaluation of these parameters at an early stage of drug discovery helps in identifying compounds with favorable drug-like characteristics and reduces the risk of late-stage failure. Several physicochemical properties, including molecular weight, lipophilicity, polarity, hydrogen bonding capacity, and molecular flexibility, significantly influence membrane permeability, solubility, and oral absorption of drug molecules. In recent years, computational approaches have become valuable tools for predicting ADME properties prior to experimental studies. Among these, SwissADME is widely used to estimate physicochemical descriptors, pharmacokinetic parameters, and drug-likeness of small molecules [23, 24]. Therefore, in the present study, the ADME properties of the investigated ligands were predicted using SwissADME to evaluate their pharmacokinetic behavior and potential as drug-like molecules.

4. Discussion

The in-silico ADME analysis was carried out to evaluate the physicochemical and pharmacokinetic properties of the ligands IAP, TAP and FAP in comparison with the reference drug Tamoxifen (TAM). The predicted parameters summarized in Table 2 provide insight into the drug-likeness, absorption behavior and pharmacokinetic characteristics of the investigated compounds. For ligand IAP, the molecular weight is 329.42 g/mol, which lies within the acceptable range for drug-like molecules. The compound possesses four rotatable bonds, indicating moderate molecular flexibility. The hydrogen bonding parameters also satisfy Lipinski criteria, with two hydrogen bond acceptors and one hydrogen bond donor. The TPSA value of 66.34 $\text{\AA}^2$ falls within the optimal range for good membrane permeability and oral absorption. The predicted iLOGP value of 2.75 indicates balanced lipophilicity. Water solubility prediction suggests that IAP is moderately soluble. Pharmacokinetic prediction indicates high gastrointestinal absorption and no penetration across the blood-brain barrier. The BOILED-Egg model further supports these findings, where IAP is positioned in the white

region of the plot indicating high probability of passive gastrointestinal absorption without BBB permeation (Fig. 3,

Table 2).

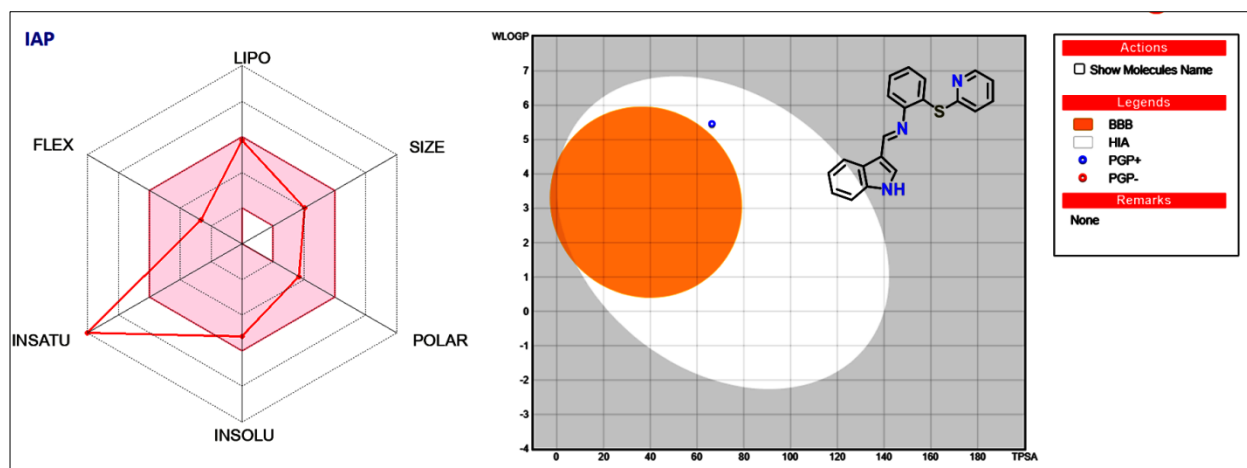


Fig 3: SwissADME bioavailability radar and BOILED-Egg plot for ligand IAP showing the predicted drug-likeness profile, gastrointestinal absorption, and blood-brain barrier permeability.

For ligand TAP, the molecular weight is 346.47 g/mol, which also lies within the recommended range for drug candidates. TAP contains four rotatable bonds and two hydrogen bond acceptors with no hydrogen bond donors. The TPSA value of 78.79 Å² indicates suitable polarity for efficient intestinal absorption. The predicted iLOGP value of 3.41 suggests moderate lipophilicity. TAP is predicted to

be moderately soluble in water and exhibits high gastrointestinal absorption. Similar to IAP, TAP is not predicted to cross the blood-brain barrier. In the BOILED-Egg plot, TAP is also located in the white region, indicating good gastrointestinal absorption with low probability of brain penetration (Fig. 4, Table 2).

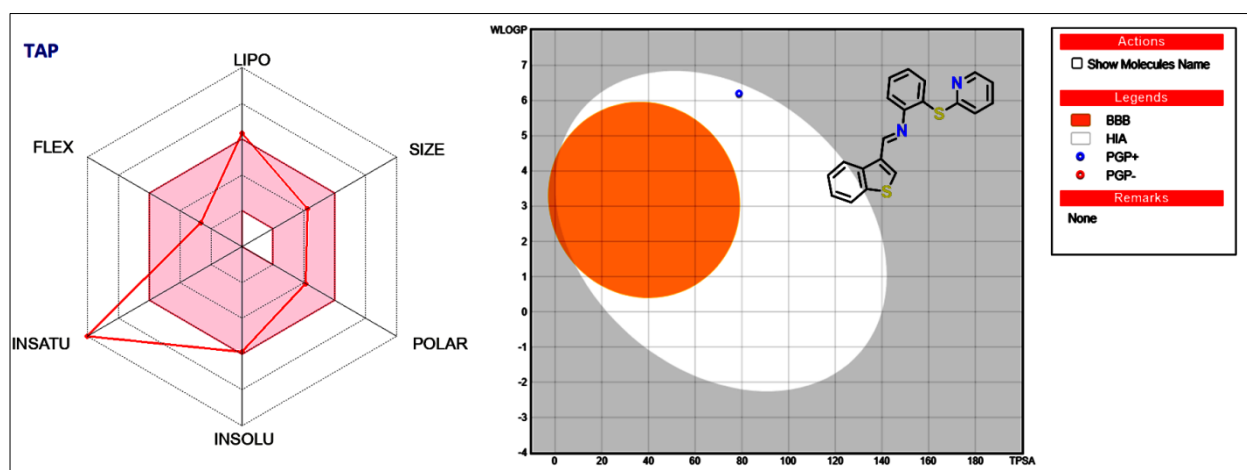


Fig 4: SwissADME bioavailability radar and BOILED-Egg model for ligand TAP illustrating the predicted physicochemical properties and pharmacokinetic behavior.

For ligand FAP, the molecular weight is 330.40 g/mol with four rotatable bonds. The compound contains three hydrogen bond acceptors and no hydrogen bond donors, which remain within acceptable limits. The TPSA value of 63.69 Å² suggests favorable permeability across biological membranes. The iLOGP value of 2.96 indicates balanced lipophilicity and the compound is predicted to be

moderately soluble. Pharmacokinetic prediction shows high gastrointestinal absorption and absence of blood-brain barrier penetration. The BOILED-Egg model also places FAP in the white region of the diagram, confirming a high probability of gastrointestinal absorption without central nervous system exposure (Fig. 5, Table 2).

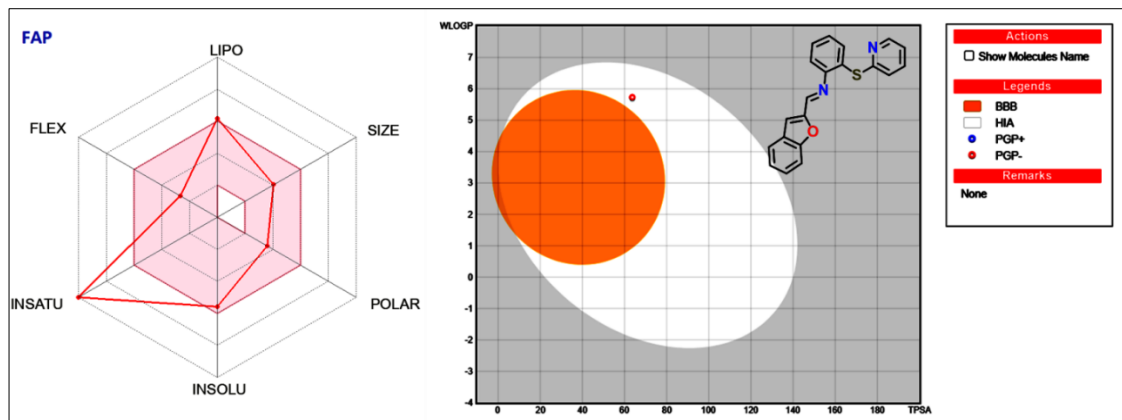


Fig 5: SwissADME bioavailability radar and BOILED-Egg plot for ligand FAP representing the predicted drug-likeness and gastrointestinal absorption profile.

In comparison, the reference drug tamoxifen (TAM) shows a molecular weight of 371.51 g/mol and eight rotatable bonds, indicating higher molecular flexibility. The TPSA value of 12.47 Å² reflects its highly lipophilic nature, which is consistent with its higher lipophilicity values. Tamoxifen

is predicted to be poorly soluble and shows low gastrointestinal absorption in the model. It also exhibits one Lipinski rule violation, mainly due to its high lipophilicity, although the predicted bioavailability score remains comparable to the ligands (Fig 6, Table 2).

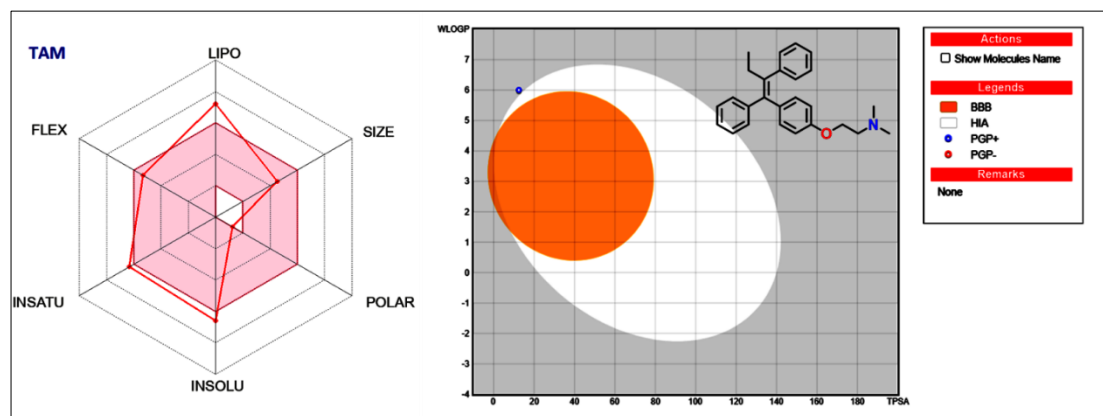


Fig 6: SwissADME bioavailability radar and BOILED-Egg plot of Tamoxifen showing the predicted physicochemical properties and pharmacokinetic profile.

Table 2: In-silico assessment of physicochemical properties, pharmacokinetic parameters, and drug-likeness of the ligands in comparison with the reference drug Tamoxifen (TAM).

Parameters (Optimal range)		IAP	TAP	FAP	TAM
Physicochemical properties					
Molecular Weight (150-500 g/mol)		329.42	346.47	330.40	371.51
Fraction Csp ³ (not less than 0.25)		0.00	0.00	0.00	0.23
Num. rotatable bonds ≤ 9		4	4	4	8
H- Bond Acceptors (HBAs) ≤ 10		2	2	3	2
H- Bond Donors (HBDs) ≤ 5		1	0	0	0
Topological polar surface area (TPSA) 20 - 130 Å ²		66.34	78.79	63.69	12.47
Lipophilicity					
LogP _{o/w} -0.7 to +5.0	iLOGP	2.75	3.41	2.96	4.64
	XLOGP3	4.68	5.57	5.29	7.14
Water solubility					
LogS (ESOL) ≤ 6		-5.21	-5.88	-5.60	-6.59
Solubility		Moderately soluble	Moderately soluble	Moderately soluble	Poorly Soluble
Pharmacokinetics					
GI absorption		High	High	High	Low
Blood brain barrier (BBB)		No	No	No	No
P-gp substrate		Yes	Yes	No	Yes
CYP1A2 inhibitor		Yes	Yes	Yes	No
CYP2C19 inhibitor		Yes	Yes	Yes	Yes
CYP2C9 inhibitor		Yes	Yes	Yes	No
CYP2D6 inhibitor		Yes	No	No	Yes
CYP3A4 inhibitor		Yes	Yes	Yes	No

Log K_p (skin permeation) cm/s	-4.99	-4.46	-4.56	-3.50
Drug-likeness				
Lipinski violation	No	No	No	Yes
Bioavailability Score 0.55	0.55	0.55	0.55	0.55

Overall, the ADME predictions and BOILED-Egg model analysis indicate that the synthesized ligands IAP, TAP and FAP possess favorable physicochemical properties, good gastrointestinal absorption and acceptable pharmacokinetic profiles, supporting their potential as promising candidates for further pharmacological investigation.

5. In-silico Toxicity Assessment

Evaluation of the toxicity profile of newly designed compounds is a crucial step in drug discovery, as potential toxic effects can significantly influence their therapeutic applicability and safety. Early prediction of toxicological properties helps to identify possible adverse effects and reduce the risk of failure during later stages of drug development. In recent years, computational toxicity prediction has emerged as an efficient and cost-effective approach for evaluating the safety of chemical compounds prior to experimental studies. Various in-silico tools are now widely used to estimate different toxicity parameters such as acute toxicity, organ toxicity, carcinogenicity, mutagenicity, and immunotoxicity based on the molecular structure of compounds. Among these tools, ProTox-3.0 provides reliable predictions of oral LD_{50} values, toxicity classes, organ toxicity, and stress response pathways using advanced machine-learning models [25, 26]. These predictions offer valuable insights into the potential toxicological behavior of

compounds and assist in evaluating their safety profiles. Therefore, in the present study, the toxicity properties of the investigated ligands were predicted using ProTox-3.0 to assess their potential toxicological risks. The predicted parameters including LD_{50} values, organ toxicity, toxicity end points, and stress response pathways were analyzed to evaluate the safety profile of the studied compounds.

The toxicity profile of the investigated ligands was predicted using the ProTox-3.0 platform. The predicted toxicity parameters, including LD_{50} values, organ toxicity, and toxicity end points, are summarized in Table X and illustrated in Figures 5-7. For compound IAP, the predicted oral LD_{50} value was 680 mg/kg, indicating moderate acute toxicity. The compound showed predicted hepatotoxicity and neurotoxicity with moderate probability values, suggesting possible effects on liver and nervous system functions. Respiratory toxicity was also predicted to be active. However, nephrotoxicity and cardiotoxicity were predicted to be inactive, indicating a lower risk of kidney and heart toxicity. In terms of toxicity end points, IAP was predicted to be inactive for carcinogenicity and immunotoxicity, while mutagenicity was predicted to be active. Additionally, the compound showed potential interaction with the mitochondrial membrane potential stress response pathway, while the p53 signaling pathway remained inactive (Fig 7, Table 3).

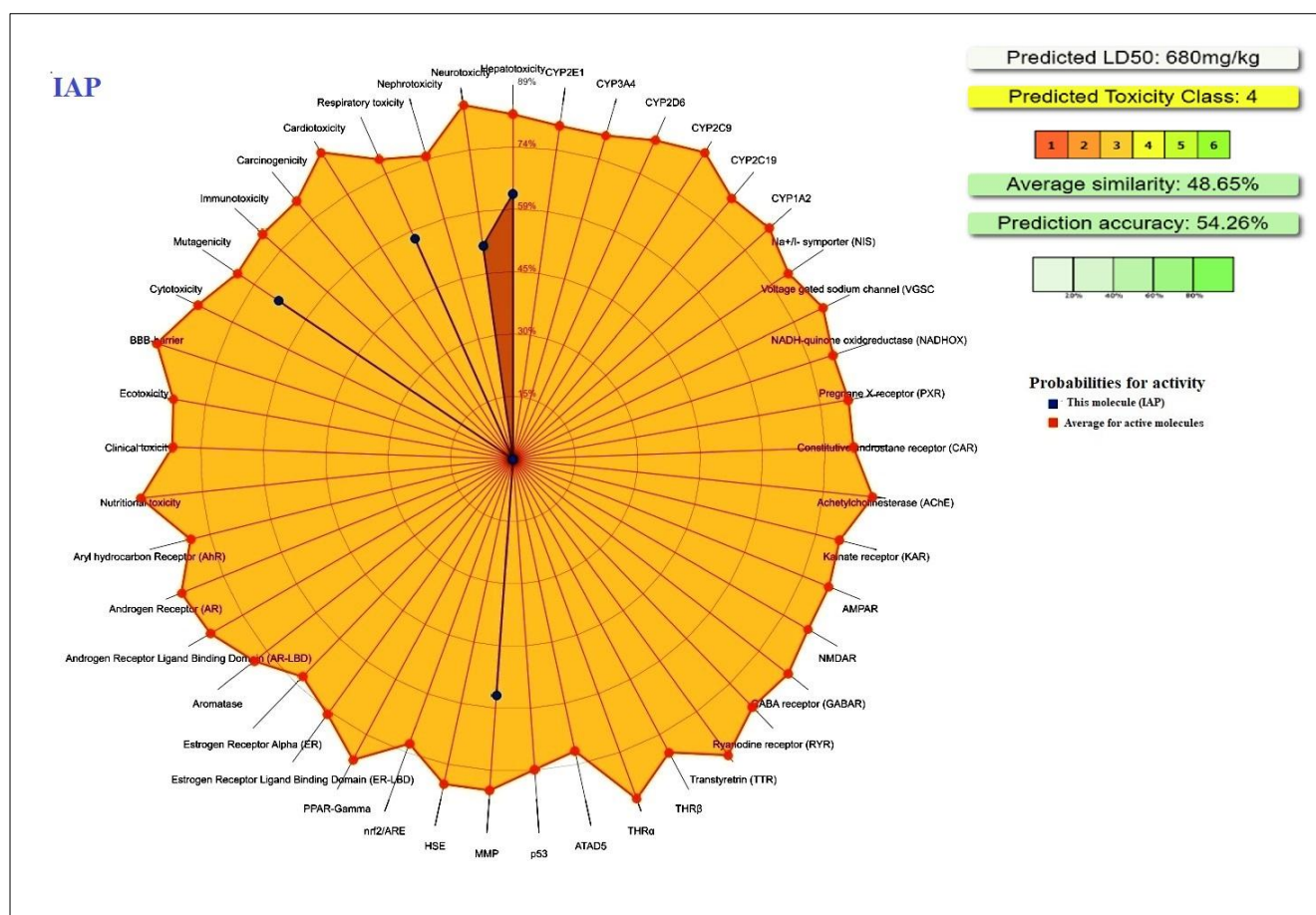


Fig 7: Predicted toxicity profile of ligand IAP obtained from ProTox-3.0.

For compound TAP, the predicted LD₅₀ value was 600 mg/kg, suggesting slightly higher toxicity compared with IAP but still within a moderate toxicity range. Similar to IAP, TAP exhibited predicted hepatotoxicity, neurotoxicity, and respiratory toxicity. However, nephrotoxicity and cardiotoxicity were predicted to be inactive. The compound was also predicted to be inactive for carcinogenicity and

immunotoxicity, indicating a favorable toxicity profile in these aspects. Mutagenicity was predicted to be active with moderate probability, while mitochondrial membrane potential stress response was also predicted to be active. The p53 pathway remained inactive for this compound as well (Fig 8, Table 3).

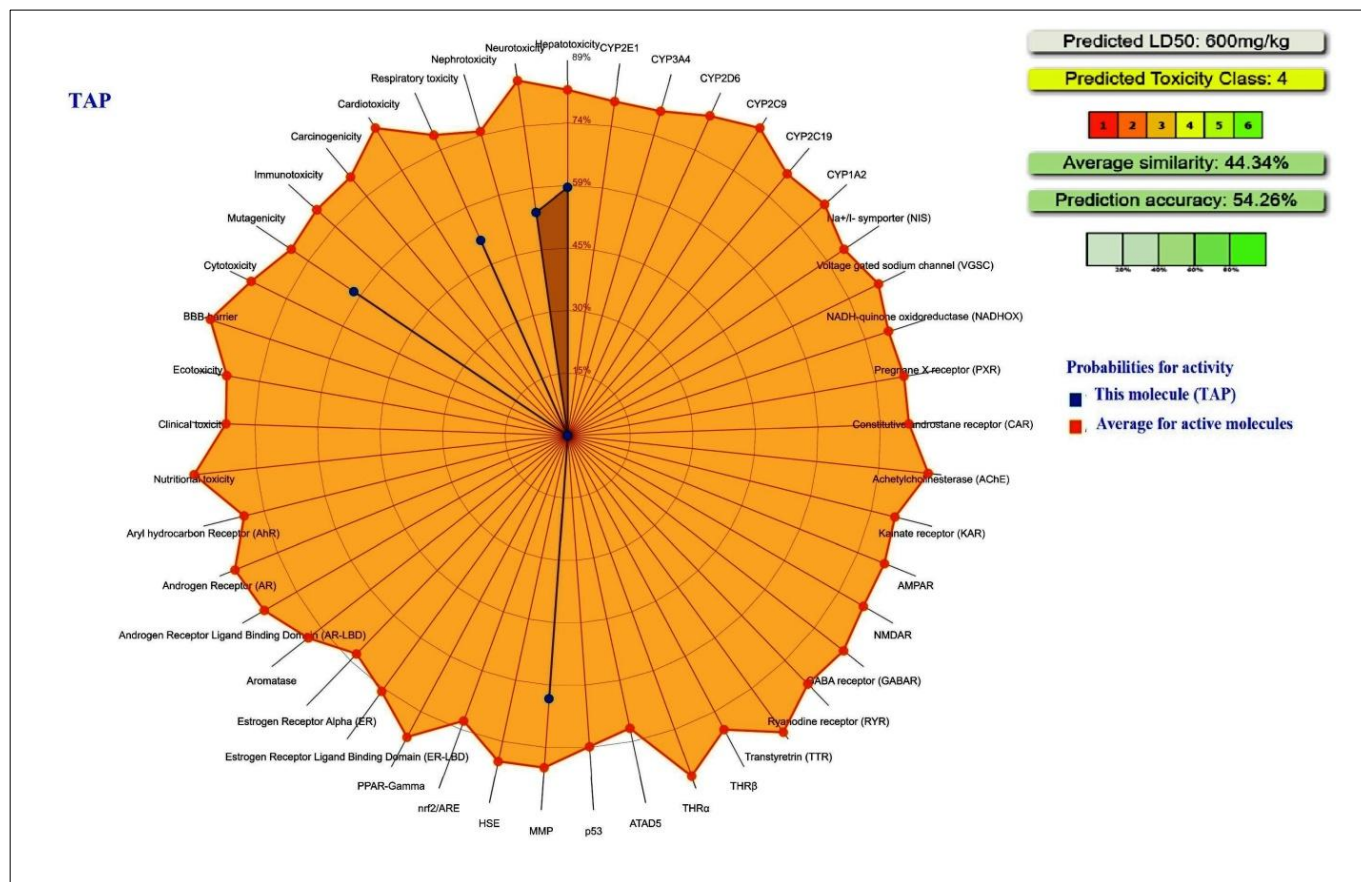


Fig 8: Predicted toxicity profile of ligand TAP obtained from ProTox-3.0.

For compound FAP, the predicted LD₅₀ value was 600 mg/kg, similar to TAP, indicating moderate toxicity. The compound showed predicted hepatotoxicity, neurotoxicity, and respiratory toxicity, while nephrotoxicity and cardiotoxicity were predicted to be inactive. The toxicity end point analysis indicated that FAP is likely non-carcinogenic and non-immunotoxic. However, mutagenicity was predicted to be active. Similar to the other ligands, FAP was predicted to influence the mitochondrial membrane potential stress response pathway, while no significant

interaction with the p53 pathway was predicted (Fig 9, Table 3).

Overall, the toxicity prediction results indicate that the investigated ligands exhibit moderate acute toxicity with relatively low risks of carcinogenicity, immunotoxicity, nephrotoxicity, and cardiotoxicity. However, the predicted hepatotoxicity, neurotoxicity, and mutagenicity suggest that further experimental toxicological evaluation is required to confirm the safety profile of these compounds.

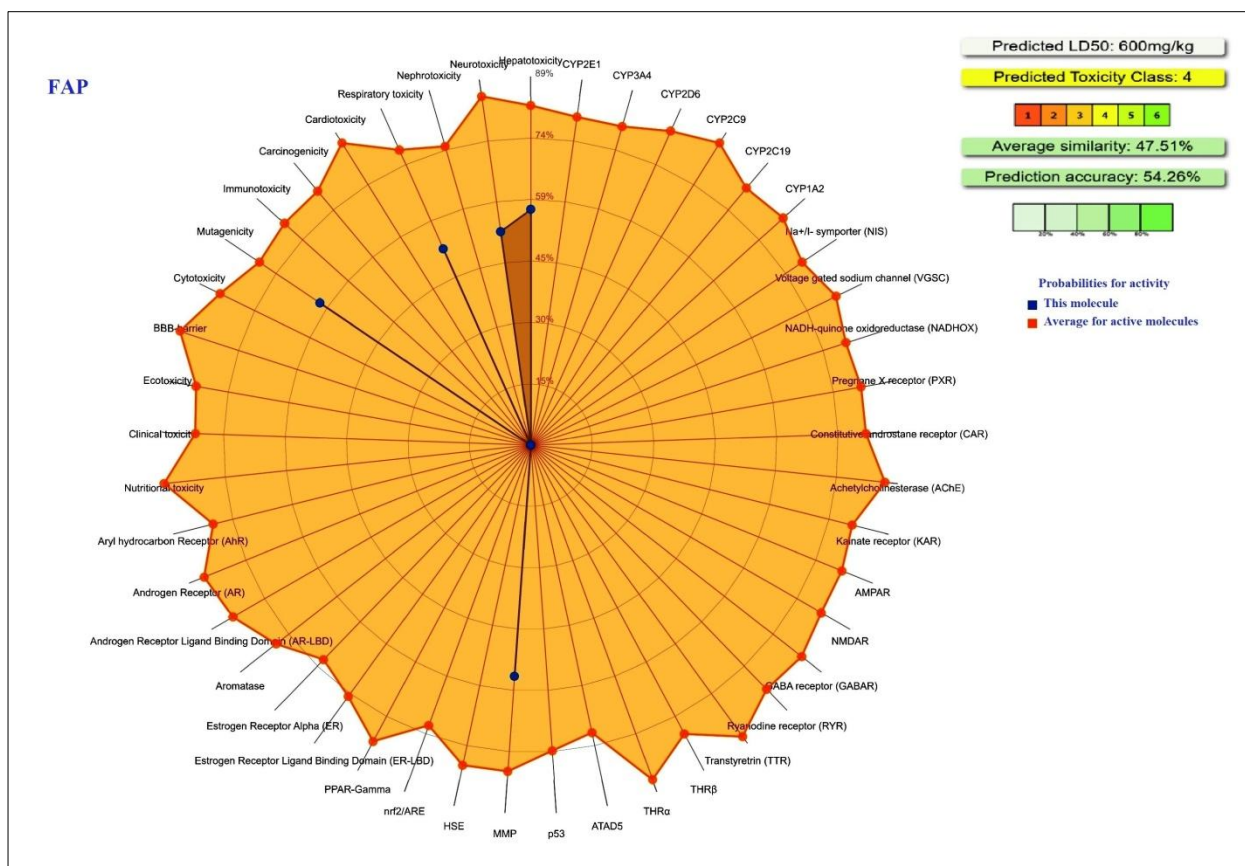


Fig 9: Predicted toxicity profile of ligand FAP obtained from ProTox-3.0.

Table 3: ProTox-3.0 - Prediction of Toxicity of chemicals

Classification	Target	Shorthand	Prediction	Probability
IAP; LD₅₀ = 680 mg/kg				
Organ toxicity	Hepatotoxicity	dili	Active	0.63
Organ toxicity	Neurotoxicity	neuro	Active	0.51
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.81
Organ toxicity	Respiratory toxicity	respi	Active	0.57
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.92
Toxicity end points	Carcinogenicity	carcino	Inactive	0.56
Toxicity end points	Immunotoxicity	immuno	Inactive	0.91
Toxicity end points	Mutagenicity	mutagen	Active	0.67
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr mmp	Active	0.56
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr p53	Inactive	0.60
TAP; LD₅₀ = 600 mg/kg				
Organ toxicity	Hepatotoxicity	dili	Active	0.58
Organ toxicity	Neurotoxicity	neuro	Active	0.53
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.8
Organ toxicity	Respiratory toxicity	respi	Active	0.50
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.91
Toxicity end points	Carcinogenicity	carcino	Inactive	0.60
Toxicity end points	Immunotoxicity	immuno	Inactive	0.86
Toxicity end points	Mutagenicity	mutagen	Active	0.61
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr mmp	Active	0.62
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr p53	Inactive	0.71
FAP; LD₅₀ = 600 mg/kg				
Organ toxicity	Hepatotoxicity	dili	Active	0.56
Organ toxicity	Neurotoxicity	neuro	Active	0.52
Organ toxicity	Nephrotoxicity	nephro	Inactive	0.75
Organ toxicity	Respiratory toxicity	respi	Active	0.51
Organ toxicity	Cardiotoxicity	cardio	Inactive	0.81
Toxicity end points	Carcinogenicity	carcino	Inactive	0.51
Toxicity end points	Immunotoxicity	immuno	Inactive	0.91
Toxicity end points	Mutagenicity	mutagen	Active	0.61
Tox21-Stress response pathways	Mitochondrial Membrane Potential (MMP)	sr mmp	Active	0.55
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	sr p53	Inactive	0.73

6. Conclusion

In summary, a series of Schiff base ligands and their corresponding cobalt(II) and nickel(II) complexes were successfully designed, synthesized, and evaluated for their anticancer potential. The biological studies revealed that metal complexation significantly enhances cytotoxic activity compared to the free ligands, highlighting the importance of coordination chemistry in drug design. Among the investigated compounds, Co-TAP and Co-FAP complexes demonstrated the most promising anticancer activity against tested cancer cell lines, along with relatively lower toxicity toward normal cells, indicating a favorable selectivity profile. Furthermore, *in silico* ADME analysis suggested that the ligands possess desirable physicochemical properties and good gastrointestinal absorption, supporting their drug-likeness. Toxicity prediction indicated moderate acute toxicity with low risks of carcinogenicity and immunotoxicity, although potential hepatotoxicity and mutagenicity warrant further experimental validation. Overall, the combined experimental and computational findings suggest that these Schiff base metal complexes represent promising scaffolds for the development of new anticancer agents. Further studies, including detailed mechanistic investigations and *in vivo* evaluation, are required to fully explore their therapeutic potential.

7. Materials and methods

7.1 in-silico study

The *in-silico* pharmacokinetic properties of the studied ligands were evaluated using the SwissADME web server. Initially, the chemical structures of the ligands were drawn and converted into SMILES (Simplified Molecular Input Line Entry System) notation using chemical drawing software (Table 4). The generated SMILES strings for each ligand were then used as input in the SwissADME online platform [23, 24]. The SwissADME tool was employed to predict various physicochemical descriptors and pharmacokinetic parameters including molecular weight, number of rotatable bonds, hydrogen bond donors and acceptors, topological polar surface area (TPSA), lipophilicity (iLOGP and XLOGP3), water solubility (LogS), gastrointestinal absorption, blood-brain barrier permeability, P-glycoprotein interaction, cytochrome P450 enzyme inhibition, and skin permeation coefficient (log Kp). In addition, drug-likeness was evaluated using Lipinski's rule of five and bioavailability score. Furthermore, SwissADME generated graphical representations such as the bioavailability radar and BOILED-Egg model to visualize the drug-likeness profile and the probability of gastrointestinal absorption and blood-brain barrier penetration of the investigated compounds. The predicted results were compiled and summarized in Tables 2, while the radar plots and BOILED-Egg diagrams are presented in Fig 3-6.

Table 4: Structures and SMILES representations of the studied ligands used for *in-silico* ADME analysis.

S. No	Ligands	SMILES
1	IAP	<chem>C1(N=C/C2=CNC3=C2C=CC=C3)=CC=CC=C1SC4=CC=CC=N4</chem>
2	TAP	<chem>C1(N=C/C2=CSC3=C2C=CC=C3)=CC=CC=C1SC4=CC=CC=N4</chem>
3	FAP	<chem>C1(N=C/C2=CC(C=CC=C3)=C3O2)=CC=CC=C1SC4=CC=CC=N4</chem>
4	TAM	<chem>CN(CCOC1=CC=C/C(C2=CC=CC=C2)=C(CC)C3=CC=CC=C3)C=C1)C</chem>

7.2 Cell culture

MCF-7 and MDA-MB231 human breast cancer cell lines, along with the normal human kidney cell line HEK-293 were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% (v/v) Penicillin-Streptomycin solution and grown in a humidified environment in 5% CO₂ incubator at 37 °C. Prior to experiments, cells were cultured in phenol red-free DMEM supplemented with 10% charcoal-stripped fetal bovine serum.

7.3 MTT assay

The antiproliferative activity of the compounds was evaluated using the MTT reduction assay [27, 28]. Cells (2.5 × 10³ per well) were seeded in 96-well plates containing 100 µL of DMEM supplemented with 10% FBS and incubated for 24 h at 37 °C in a CO₂ incubator. The compounds were then added at the desired concentrations, and cells were further incubated for 48 h. Subsequently, the medium was removed and 100 µL of MTT solution (0.5 mg mL⁻¹) was added to each well, followed by incubation for 3 h. The supernatant was carefully discarded, and the resulting formazan crystals were dissolved in 100 µL of DMSO. Absorbance was measured at 540 nm using a Labman LMSPUV1900 spectrophotometer.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

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