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Novel Anthraquinone-Based Heterocyclic Compounds as Potential Inhibitors of PKM2 and Topoisomerase: Synthesis, Cytotoxicity, Docking, and ADME Profiling

Mazin N. Mousa [1,2*], Mohammed Hasan Mohammed [2]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq

[2]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad, Baghdad, Iraq

***Corresponding Author Email: mazin.mousa@uobasrah.edu.iq**

ORCID: 0000-0001-8092-7428

Abstract

This study aimed to develop novel anticancer agents by synthesizing and evaluating anthraquinone-based heterocyclic compounds as potential inhibitors of Pyruvate Kinase M2 (PKM2) and topoisomerase. Three target compounds incorporating 1,3,4-oxadiazole (Compound 3), 1,2,4-triazole (Compound 5), and 1,3,4-thiadiazole (Compound 6) moieties with an anthraquinone core were synthesized and characterized using spectroscopic methods (FT-IR, 1H-NMR, 13C-NMR). In silico molecular docking revealed that Compound 6 exhibited the highest binding affinity for both topoisomerase II (ΔG: -8.313 kcal/mol) and PKM2 (ΔG: -9.342 kcal/mol). ADME predictions indicated all compounds possess high gastrointestinal absorption, are not BBB permeant, and adhere to Lipinski's rule of five. Cytotoxicity studies (MTT assay) against A549 lung and HepG2 liver cancer cell lines, along with normal HDF cells, demonstrated Compound 6 as the most potent, with IC_{50} values of 104.37 µg/ml (A549) and 126.59 µg/ml (HepG2). Compound 6 also showed the highest selectivity for A549 cells (SI: 2.61981). These integrated findings identify Compound 6 as a promising lead candidate for further investigation in anticancer drug development.

Keywords: Anthraquinone, Cytotoxicity, Docking, Pyruvate Kinase, Topoisomerase

Introduction

The search for novel and effective therapeutic agents remains a cornerstone of medicinal chemistry, particularly in the context of complex diseases such as cancer[1]. Heterocyclic compounds have consistently demonstrated a broad spectrum of pharmacological activities, making them privileged scaffolds in drug discovery[2]. Among these, anthraquinone derivatives have garnered significant attention due to their diverse biological properties, including anticancer[3], antimicrobial[4], antiviral, and anti-inflammatory activities[5]. The planar anthraquinone ring system is known to intercalate with DNA and inhibit enzymes crucial for cell proliferation, such as topoisomerases.[6] Further functionalization of the anthraquinone core with various heterocyclic moieties can modulate its physicochemical properties, biological activity, and target specificity. [7]Specifically, five-membered nitrogen, oxygen, and sulfur-containing heterocycles like 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, and 1,2,4-triazoles are well-established pharmacophores[8][9][10]. These rings are frequently incorporated into drug candidates owing to their ability to participate in hydrogen bonding, enhance metabolic stability, and improve pharmacokinetic profiles.[11][12] The unique electronic and structural features of these heterocycles have been associated with a wide array of biological activities, including potent anticancer effects[13]. The strategic combination of the anthraquinone scaffold with these bioactive heterocyclic rings presents a promising avenue for the development of novel therapeutic agents with potentially enhanced efficacy and selectivity. Such hybrid molecules may exhibit synergistic effects or novel mechanisms of action arising from the interplay between the different pharmacophoric units. In the context of cancer, targeting key enzymes involved in tumor progression and survival is a validated therapeutic strategy[14]. Pyruvate Kinase M2 (PKM2) is a critical enzyme in cancer cell metabolism, promoting aerobic glycolysis (the Warburg effect) and providing building blocks for anabolic processes, thereby supporting rapid cell proliferation[15]. Topoisomerase enzymes (e.g., Topoisomerase I and II) are essential for DNA replication, transcription, and repair, and their inhibition leads to DNA damage and apoptotic cell death, making them well-established targets for anticancer drugs.[16] Therefore, the present study focuses on the rational design and synthesis of novel hybrid compounds incorporating an anthraquinone moiety linked to 1,3,4-oxadiazole, 1,3,4-thiadiazole, or 1,2,4-triazole rings. The synthesized compounds were evaluated for their cytotoxic potential using the MTT assay. To gain insights into their potential mechanisms of action and drug-likeness, in silico molecular docking studies were performed against Pyruvate Kinase M2 (PKM2) and topoisomerase enzymes. Furthermore, ADME (Absorption, Distribution, Metabolism, and Excretion) predictions were carried out to assess the pharmacokinetic profiles of these novel entities. This multifaceted approach aims to identify promising lead candidates for further development as potential anticancer agents.

Experimental and Methods

Chemistry

The starting materials were sourced from a commercial supplier, and their purity was assessed by measuring melting points. Reaction progress was monitored using TLC, with spots visualized by iodine vapor. The chemical structures of the intermediate and final compounds were identified through FT-IR, H-NMR, C-NMR, and mass spectroscopy. Figure 1 illustrates the chemical synthesis of the desired compounds.

Synthesis of (ethyl9,10-dioxo-9,10-dihydroenthracene -2-carboxylate.) (comp. 1)

In a 500 ml round-bottom flask, 20 mmole of Anthraquinone (ATQ)-2 carboxylic acid was suspended in 150 ml of ethanol (99%) with five drops of sulfuric acid. The suspension was refluxed for 18 hours until a clear solution had been obtained. TLC was used to track the progress of the reactions. After cooling the solution, the resulting product was taken, washed, dried, and recrystallized from hot ethanol. Yield 87% white powder, m.p. 144-146 °C , Rf value 0.68 using 9:1 v/v of chloroform and ethanol.

FT-IR: 3075 (=C-H), 2991-2825 (-C-H), 1772 (C=O ester), 1678 (C=O), 1484, 1455 (-C=C-).

1H.NMR: 1.41 (t, 3H, -CH3), 4.3 (q, 2H, -CH2-), 7.7-8.41 (m,7H, =C-H).

Synthesis of (9,10-dioxo-9 ,10-dihydroanthracene -2-carbohydrazide.) (comp. 2)

Compound 1 (15 mmole) was added to 125 ml of absolute ethanol. An excess amount of Hydrazine hydrate (2.5 equivalents, 1.2 ml) was added gradually with continuous stirring. The color immediately converted into purple, and the content was refluxed for 48 hours. The suspension gradually changed into a pale and thick yellow. The mixture, latterly, was cooled and the product collected, washed, dried, and recrystallized from pyridine. Yield 75%, m.p. 239-241°C , Rf value 0.37 from using methanol as a mobile phase.

FT-IR: 3334,3313 (=NH2), 3253 (-NH-), 1678.07 (=C=O amide),1612.74 (C=O), 1591, 1560 (-C=N-), 1479,1451 (-C=C-). 1H-NMR (400MHz, δ,ppm, DMSO-d6): 4.71 (s,2H,-NH2), 8.1- 8.8 (m, 7H, =C-H), 10.3 (s, 1H, -NH-)

Synthesis of (2-(5-mercapto-1, 3 ,4 -oxadiazole- 2-yl)anthracene-9,10-dione)) (comp. 3)

Compound 2 (15 mmole) was suspended in a flask with 100 ml of ethanol (95%). An ethanolic solution (10 ml) of KOH (10% w/v) had been added to the content and agitated for 15 minutes. The color turned immediately into deep dark. An excess amount (1.4 ml) of Carbon Disulfide (CS2) had been added gradually to the suspension and refluxed overnight. Later, the content converted into a very thick yellow-orange suspension. Finally, the content was cooled and neutralized with 5 N HCL. The yellow suspension is filtered, and the formed product is taken apart, washed twice with distilled water (D.W.), and dried. Absolute ethanol was used for recrystallization. Yield: 80%, m.p.196-

200°C, Rf value 0.56 from using dichloromethane: methanol (1:1).[17]

FT-IR: 3072 (=C-H), 2537 (-SH), 1675 (carbonyl -C=O), 1589 (-C=N-), 1492,1475 (=C-H). 1H.NMR: (8.9-9.7) (m, 7H, =C-H), 14(s, 1H, -SH). 13C-NMR : 125-135 (=C-H), 146 (=C-SH), 165 (Ar-C=N), 182 (-C=O). M/Z+ 308.21

Synthesis of 2-(9,10-dioxo-9,10-dihydroanthracene-2-carbonyl)-N-phenylhydrazine-1-carbothioamide-formyl-N-phenylhydrazine-1-carbothioamide (comp. 4)

Two grams of compound 2 (8 mmole) and 1.5 equivalents of phenyl isothiocyanate were combined in 50 mL of absolute ethanol, and the mixture was refluxed for 12 hours before being cooled and poured over crushed ice, resulting in a solid precipitate. The precipitate was isolated, dried, and used immediately without purification in the next step. The melting point was 181-184°C, yielding a 79% return.

Synthesis of 2-(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)anthracene-9,10-dione (comp. 5)

Compound 4, 2 grams (5 mmole), was transferred into a round-bottom flask. A 15 % solution of potassium hydroxide (20 ml) was added to the dried precipitate. The resulting mixture was then refluxed for 6 hours, and after cooling, it was poured over crushed ice. The suspension was neutralized with concentrated hydrochloric acid (HCl), leading to the formation of a precipitate, which was isolated through vacuum filtration, dried in an oven, and purified by recrystallization from ethanol. The melting point was found to be 200°C , yielding 67%. FT-IR: 3070 (-C=H), 2549 (-SH), 1647.2 (-C=O), 1589, 1546, 1589,1546,1498 (-C=C-). 1H-NMR: 7.4- 8.3 (m,12H,-C=H), 14.4 (s,1H,-SH). 13C-NMR : 125-136 (=C-H), 148 (=C-SH), 167 (Ar-C=N), 182 (-C=O)[18]. M/Z+ 383.18

Synthesis of 2-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)anthracene-9,10-dione (comp. 6)

Compound 4, weighing 2 grams (5 mmole), was transferred into a round-bottom flask. A concentrated sulfuric acid (20 ml) was added to the dried precipitate. The resulting mixture was refluxed for 6 hours. After cooling, the solution was poured over crushed ice, resulting in the formation of a precipitate. This precipitate was isolated through vacuum filtration, dried in an oven, and purified by recrystallization from ethanol. The melting point was found to be 184-186°C, yielding 76%. FT-IR: 3248 (-NH-), 3062 -C=H), 1672 (- C=O), 1591, 1571,1546,1498 1H-NMR: 5.8-8.3 (m,12H,-C=H), 10.6 (s,1H,-NH-). 13C-NMR : 117-130 (=C-H), 158 (-N=C-NH), 165 (Ar-C=N), 182 (-C=O). M/Z+ 383.10

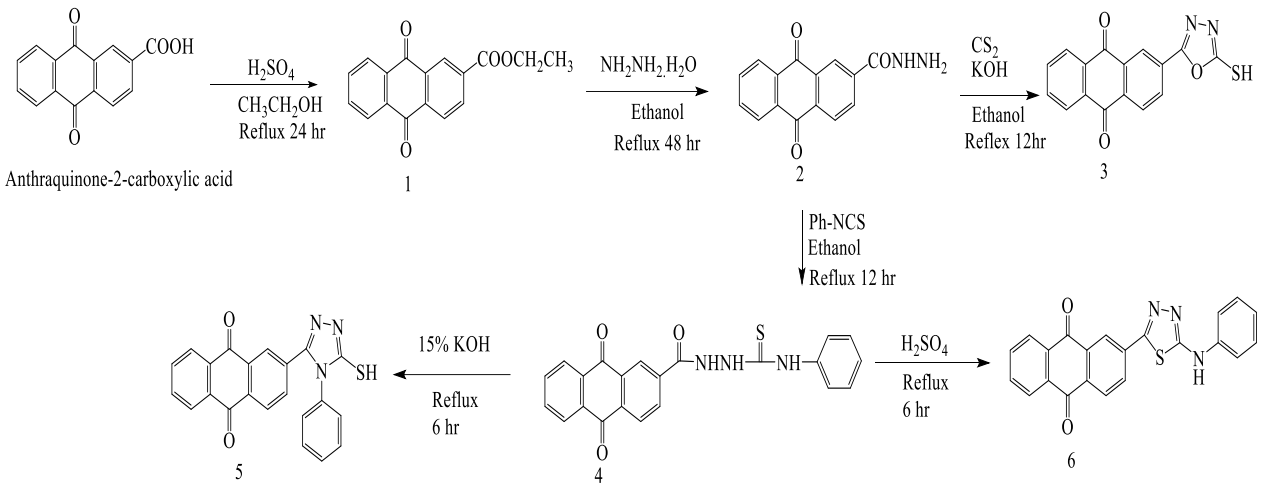

Figure 1: Chemical Synthesis of the Desired Compounds

Docking study:

A docking study was performed using SwissDock Online. The crystallized target proteins were downloaded from the Protein Data Bank (https://www.rcsb.org) (PDB ID: 3QX3 and 4G1N). The target protein was prepared using the Protein Preparation Wizard in Biovia Discovery Studio. The ligand water molecules were removed, and the structure was saved in PDBQT format. The active site used for docking was defined through grid generation for the co-crystallized ligand obtained from the PDB using Pymol software, with the coordinates of 30, 120, 75 for 3QX3 and 15,-35,45 for 4G1N with dimensions of (30 Å x 30 Å x 30 Å). The ligands were sketched in ChemOffice, converted to SMILES notation, and docked against topoisomerase and PKM2. The docking results were visualized to assess the ligand's affinity for the protein's active site and the docking score. [19][20]

ADME Study:

Pharmacokinetic properties, including potential sites of metabolism, CNS penetration, intestinal absorption, and adherence to the rule of five, can be virtually determined using ligand-based ADME prediction through online SwissADME software. [21][22]

Cytotoxicity

A549 (lung cancer), HepG2 (hepatocarcinoma), and HDF (normal fibroblast) cell lines, all of human origin, were acquired from the Iranian National Cell Bank (Pasteur Institute). These cell lines were cultured and propagated according to Eneama et al. Cell viability was assessed using the MTT assay, in which cells were seeded in 96-well plates, treated with varying concentrations of compounds (600, 200, 66.66, 22.22, and 7.4 µg/ml), and then incubated with MTT solution. A UV spectrophotometer measured absorbance to

determine cell viability at 570 nm, and IC50 values were calculated to represent the concentration causing 50% cell death (Table 3)[23]. The Index of Selectivity was calculated using the formula:

Selectivity Index (SI)=IC50 (HDF)/IC50 (Cancer Cell Line)[24]

Results:

The study successfully synthesized three target anthraquinone-based heterocyclic compounds: 2-(5-mercapto-1,3,4-oxadiazol-2-yl)anthracene-9,10-dione (Compound 3), 2-(5-mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)anthracene-9,10-dione (Compound 5), and 2-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)anthracene-9,10-dione (Compound 6). Their structures were confirmed using FT-IR, 1H-NMR, and 13C-NMR spectroscopy. Molecular docking studies predicted that Compound 6 exhibited the highest binding affinity for both topoisomerase II (PDB ID: 3qx3, ΔG: -8.313 kcal/mol) and PKM2 (PDB ID: 4G1N, ΔG: -9.342 kcal/mol). Compound 5 also demonstrated strong affinities, particularly for PKM2 (ΔG: -9.024 kcal/mol). The docking scores are shown in Table 1.

Table 1: The Docking scores (ΔG) kcal/mol of the prepared compounds, topoisomerase II (PDB ID: 3qx3), and Pyruvate Kinase M2 (PKM2. (PDB ID: 4G1N)

	Docking scores (ΔG)	
	3qx3	4G1N
Compound 3	-7.639	-7.826
Compound 5	-7.809	-9.024
Compound 6	-8.313	-9.342

Predicted ADME properties (Table 2) indicated that all three compounds have high gastrointestinal absorption, are not BBB permeant, and do not act as P-gp substrates. They complied with Lipinski's rule of five, with no violations, and had a bioavailability score of 0.55. Compound 3 (Molecule 1) showed no CYP2C19 inhibition and zero lead-likeness violations. Compounds 5 (Molecule 2) and 6 (Molecule 3) were predicted to inhibit CYP2C19, and each had two lead-likeness violations (MW>350, XLOGP3>3.5). All compounds showed a PAINS alert for "quinone_A," while compounds 3 and 5 also had a "thiol_2" alert, which compound 6 lacked.

Table 2: Predicted Physicochemical and ADME Properties of Compounds 3,5, and 6

Molecule	GI absorption	BBB permeant	Pgp substrate	CYP2C19 inhibitor	Lipinski #violations	Bioavailability Score	Leadlikeness #violations
Molecule 1	High	No	No	No	0	0.55	0
Molecule 2	High	No	No	Yes	0	0.55	2
Molecule 3	High	No	No	Yes	0	0.55	2

In vitro cytotoxicity assays (MTT) revealed that Compound 6 was the most potent against A549 lung cancer cells (IC_{50}: 104.37 µg/ml) and HepG2 liver cancer cells (IC_{50}: 126.59 µg/ml). Compound 6 also displayed the highest selectivity index for A549 cells (SI: 2.61981). Compound 5, while slightly less potent, demonstrated the highest selectivity for HepG2 cells (SI: 2.20771). These cytotoxic activities were generally consistent with the docking results, highlighting Compound 6 as a promising lead candidate.

Table (3): IC50 Values (µg/ml) and Selectivity Indices (SI) of the prepared Compounds Against HDF, A549, and HepG2 Cell Lines.

	HDF (µg/ml)	A549 (µg/ml)	SI (A549)	HepG2 (µg/ml)	SI (HepG2)
Comp 3	246.56	178.89	1.37828	129.44	1.90482
Comp 5	296.23	219.23	1.35123	134.18	2.20771
Comp 6	273.43	104.37	2.61981	126.59	2.15997

Discussion

This study aimed to synthesize, biologically evaluate, and in silico profile of novel anthraquinone derivatives bearing 1,3,4-oxadiazole (compound 3), 1,2,4-triazole (compound 5), and 1,3,4-thiadiazole (compound 6) heterocyclic moieties as potential anticancer agents targeting PKM2 and topoisomerase enzymes. The successful synthesis of these target compounds was confirmed by comprehensive spectroscopic analysis. The molecular docking studies provided valuable insights into the potential interactions of these compounds with the active sites of topoisomerase II and PKM2. Compound 6 emerged as the most promising candidate in silico, exhibiting the lowest binding energies for both enzymes. This suggests a potentially stronger inhibitory action compared to compounds 3 and 5. The phenylamino substituent on the thiadiazole ring of compound 6, and the phenyl substituent on the triazole ring of compound 5, appear to contribute favorably to the binding affinity. The interaction between the ligand and target protein is shown in Figure 2.

The predicted ADME properties indicated that all synthesized compounds possess favorable characteristics for drug development, including high GI absorption and negligible BBB permeability. All compounds conform to Lipinski's rule of five, further supporting their drug-likeness with radar and boiled egg graphs (Figures 3 and 4). While compounds 5 and 6 showed some lead-likeness violations and potential for CYP enzyme inhibition, these aspects can be addressed in future lead optimization studies

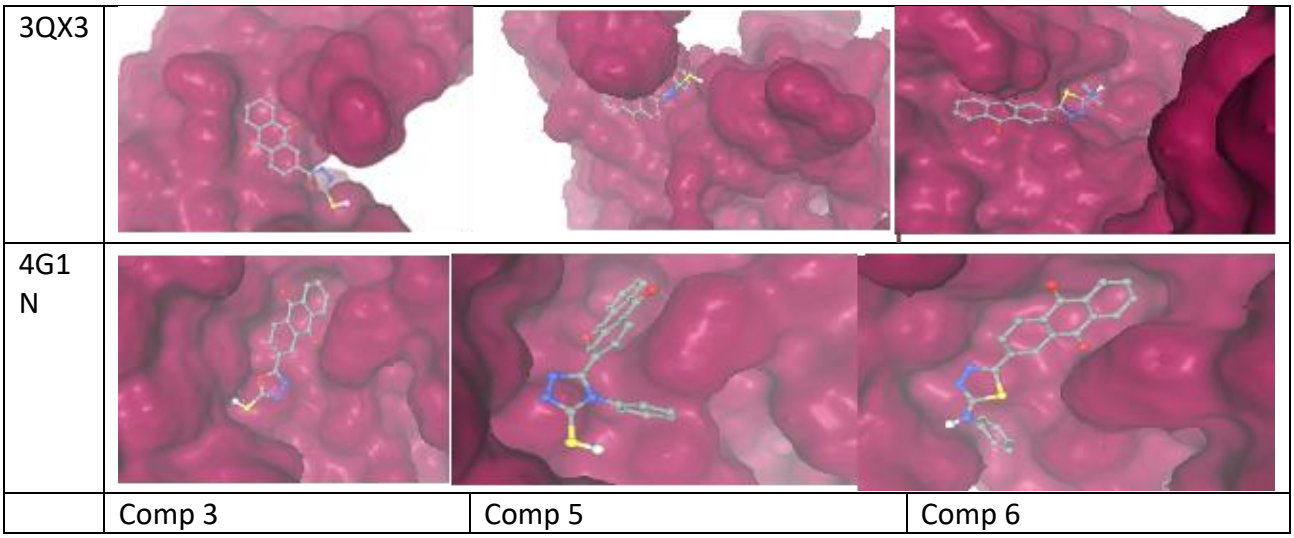

Figure 2: 3D interaction of the synthesized compounds with the target proteins 3QX3 and 4G1N.

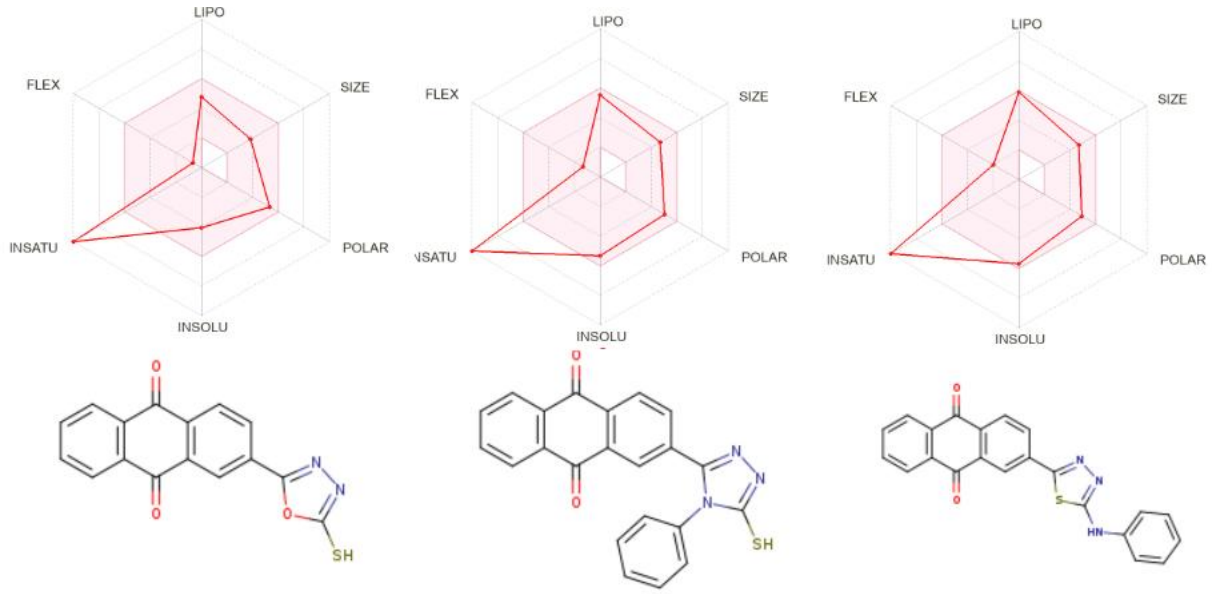

Figure 3: Radar graph of compounds 3,5, and 6. All parameters of the physicochemical properties of the tested compounds are within the normal margins, except the degree of unsaturation.

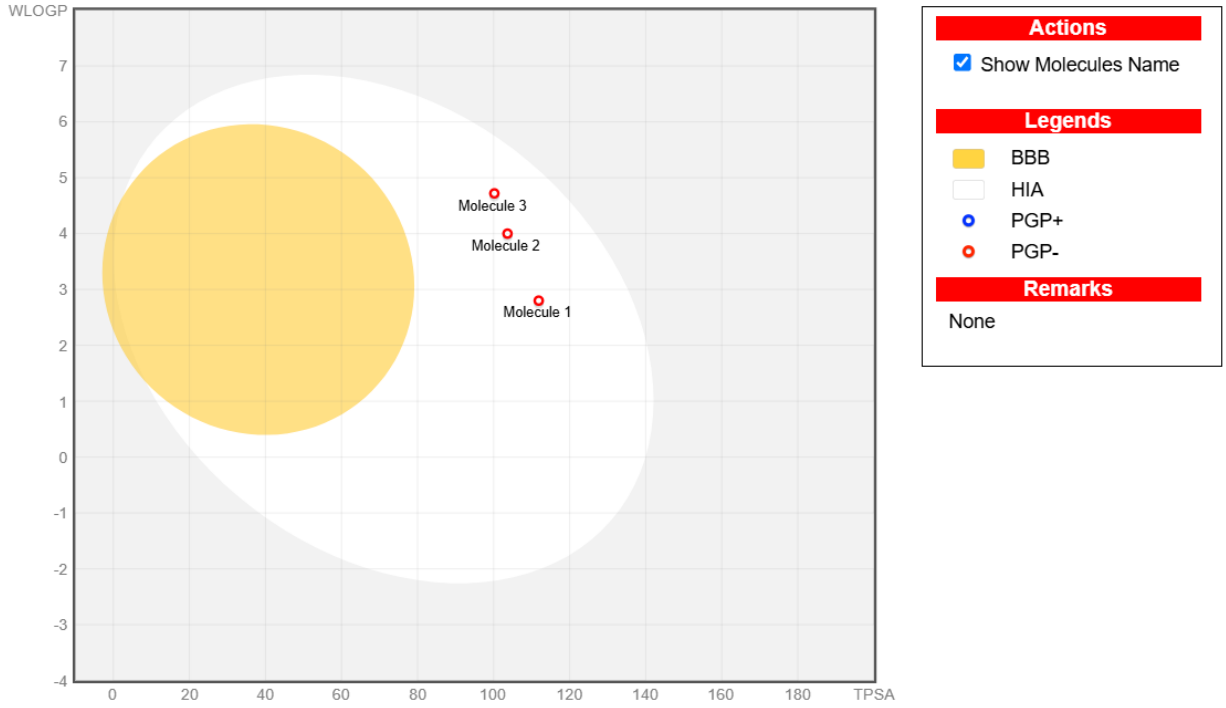

Figure 4: Boiled egg graph. All compounds are out of the yellow area (BBB), and all of them are substrates for PGP (red color)

The in vitro cytotoxicity results largely corroborated the docking studies. Compound 6 demonstrated the most potent cytotoxic effects against both A549 and HepG2 cancer cell lines and also exhibited the highest selectivity index against A549 lung cancer cells. Compound 5, while slightly less potent, showed good cytotoxicity and the best selectivity for HepG2 liver cancer cells. These findings suggest that the incorporation of the 1,3,4-thiadiazole and 1,2,4-triazole moieties, particularly with phenyl substituents, onto the anthraquinone scaffold can lead to compounds with significant anticancer activity and some degree of selectivity. The observed cytotoxicity against cancer cells at concentrations less toxic to normal HDF cells is a positive indicator for potential therapeutic application. Comparing the heterocyclic systems, the 1,3,4-thiadiazole derivative (compound 6) generally outperformed the 1,3,4-oxadiazole (compound 3) and 1,2,4-triazole (compound 5) derivatives in terms of docking scores and overall cytotoxicity against A549 cells. The phenylamino group in compound 6 may play a crucial role in its enhanced activity. In conclusion, the strategic combination of the anthraquinone scaffold with bioactive heterocyclic rings, particularly the 1,3,4-thiadiazole moiety in compound 6, has yielded a promising lead compound with significant cytotoxic activity against lung and liver cancer cell lines and favorable docking scores against PKM2 and topoisomerase II. The multifaceted approach combining

synthesis, in silico studies, and in vitro biological evaluation has successfully identified compound 6 as a candidate for further investigation and optimization in the development of novel anticancer agents. Future studies should focus on elucidating the precise mechanism of action, exploring further structural modifications to improve potency and selectivity, and conducting in vivo efficacy studies.

Conclusions:

This study successfully synthesized novel anthraquinone-based heterocyclic compounds (Compounds 3, 5, and 6) as potential anticancer agents. Compound 6 demonstrated the highest binding affinity for both topoisomerase II and PKM2 in molecular docking studies. All compounds exhibited favorable ADME properties, including high gastrointestinal absorption and adherence to Lipinski's rule of five. In cytotoxicity assays, Compound 6 was the most potent against A549 lung and HepG2 liver cancer cells, also showing the highest selectivity for A549 cells. These findings identify Compound 6 as a promising lead candidate for further anticancer drug development.

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