Synthesis of 6-Ethoxy-4-(Ureido) Indole-3-Sulfonic Acid and its Computational Evaluation against Prostate Cancer

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ABSTRACT

This research focused on developing a new prostate cancer drug to overcome the side effects and resistance associated with current therapies like Olaparib. The study leveraged the indole moiety, a scaffold renowned for its favourable pharmacokinetic properties in anticancer drug development, to synthesise a new compound: 6-ethoxy-4-(ureido)indole-3-sulfonic acid. A three-step synthetic route was employed, starting with the sulfonation of indole, followed by regioselective C-4 ureidation with urea and culminating in ethoxylation using sodium ethoxide. The final product was purified via vacuum liquid chromatography, and its chemical structure was confirmed through characterisation using FT-IR and NMR spectroscopy.

The FT-IR spectrum showed critical absorptions for N-H stretching (3431.83, 3337.16, 3260 cm^{-1}) and the C=O of the ureido amide (1676.5 cm^{-1}). Furthermore, the ^{1}H NMR spectrum displayed resonances for the ethoxy protons at 3.81 ppm, while the ^{13}C NMR spectrum confirmed eleven carbons, including the key ureido-substituted carbon at C-4 (148–157 ppm). The compound's potential was then evaluated through computational methods. Molecular docking studies against the PARP enzyme (PDB ID: 3L3M) demonstrated that the novel indole derivative possessed an enhanced binding affinity and formed more interactions with the target protein compared to Olaparib. Also, ADME-Toxicity (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis predicted favourable pharmacokinetics. Based on these computational results, which demonstrate promising drug-like properties, the study strongly recommends further investigation and development of this synthesised indole derivative as a leading candidate for the treatment of prostate cancer.

Keywords: Prostate cancer, poly(ADP-ribose) polymerase (PARP) inhibitor, olaparib, ADME-T analysis.

INTRODUCTION

Prostate cancer is the most prevalent cancer disease and the eighth leading cause of death among men globally (Ferlay *et al.*, 2024). One possible treatment for prostate cancer that has shown significant promise is a poly(ADP) ribose polymerase (PARP) inhibitor. PARP inhibitors are drugs that block the activity of these enzymes, and some are being studied as potential cancer treatments. Olaparib (Lynparza) is an example of a PARP inhibitor. It is an effective oral PARP inhibitor, approved for the treatment of prostate cancer and other cancer-related diseases by the National Agency for Food and Drug Administration and Control (NAFDAC) in Nigeria and the U.S. Food and Drug Administration (FDA).

Notwithstanding recent advancements in the treatment of prostate cancer, most patients constantly develop mild or acute resistance to current therapies. Furthermore, some patients experience unyielding side effects and recurrence of the ailment in advanced stages. This research aims to investigate a novel drug compound with effective anti-prostate cancer properties, using indole as the starting material for laboratory synthesis and the molecular docking of the ligand via the Maestro Schrodinger Suite 2023-1.

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MATERIALS AND METHODS

Experimental

Synthesis of Indole-3-Sulfonic Acid

Indole (0.64 g) was weighed into a 100 ml Quick-fit two-neck round-bottom flask. Glacial acetic acid (10 ml) and concentrated sulfuric acid (3 drops) were added. The mixture was thereafter heated under reflux for 3 hours at 50 ^{o}C. The reaction was monitored by Thin Layer Chromatography [silica gel; hexane: ethyl acetate 70:30, v/v].

Synthesis of 4-(Ureido) Indole-3-Sulfonic Acid

Sulfonic acid of indole (20 g), as obtained from the above synthesis, was weighed into a 100 ml Quick fit two-neck round-bottom flask and dissolved in 20 ml of distilled water. Urea (2.1 g) was added and stirred to dissolve. While being stirred, 4 drops of concentrated H_2SO_4 were added to catalyse the chemical reaction. The reaction was allowed to proceed for 15 hours, under reflux at 45 °C, and was monitored by Thin Layer Chromatography [silica gel; hexane: ethyl acetate 70:30, v/v] until it had gone to completion as observed by the presence of some products.

To the residue, diethyl ether (20 ml) was added and stirred to wash away organic impurities. The mixture was then transferred to a separatory funnel and allowed to separate into two distinct layers. The aqueous layer was confirmed by TLC to contain the product.

Introduction of Ether Moiety to 4-(Ureido) Indole-3-Sulfonic Acid

Sodium metal (0.23 g) was weighed into a 25 ml beaker. Dry glassware was used because the metal is highly reactive with water. Ethanol (0.5 ml) was carefully added, and there was a release of hydrogen gas in the course of the reaction, resulting in the formation of sodium ethoxide.

10 g of the products from the second synthesis [4-(Ureido) Indole-3-Sulfonic Acid and byproduct] was weighed into a 100 ml Quick fit two-neck round-bottom flask. 10 ml of Dimethyl Formamide (DMF) was added to dissolve the products. The mixture was stirred for 5 minutes and sodium ethoxide (0.5 g) was added under a $CaCl_2$ atmosphere, using a reflux setup. The reaction was heated for 29 hours at 30 °C and was monitored by TLC [silica gel; hexane: ethyl acetate 70:30, v/v].

After the completion of the reaction, as observed by the formation of products, diethyl ether (15 ml) was added and stirred to wash away organic impurities. The mixture was transferred to a separatory funnel and allowed to separate into two distinct layers. The aqueous layer was found to contain the products as monitored by TLC. The crude material was further purified by Vacuum Liquid Chromatography. 30 g of Silica gel (TLC) was added to a gram of the crude material until a homogeneous mixture in powdery form was obtained. The prepared sample was poured into the column, carefully positioned between the silica gel (TLC). The solvent system, hexane: ethyl acetate 70:30, v/v, was continuously added to the top of the column and the solvent was sucked through the column with the aid of a vacuum pump. Sample bottles were sequentially used to collect the mobile phase as it drained from the column. Additional solvent was added to the top of the column until all the desired compounds had eluted from the column. Solvent was removed from the pooled product fractions, and each batch was monitored with TLC to ascertain the batch with the expected product. The resultant product was analysed by NMR and FTIR.

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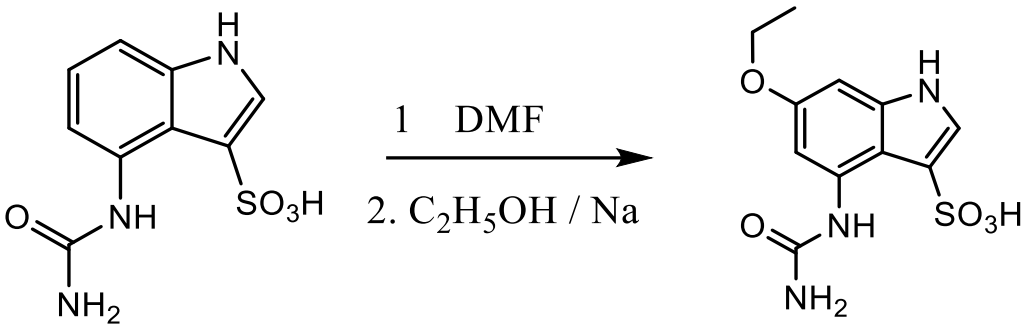

Figure 1: Synthesis of 6-Ethoxy-4-(Ureido)Indole-3-Sulfonic Acid

Ligand Preparation

The ligand was created using the 2D builder in Maestro Schrodinger Suite 2023-1. Olaparib, the reference drug, was downloaded in the structure-data file (SDF) format from the NCBI PubChem database. Ligand preparation of the reference compound was performed on the downloaded SDF file to assign proper bond orders, generate a minimised energy and create a three-dimensional geometry.

Protein Preparation

The 3-dimensional (3D) structure of PARP, poly (ADP-ribose) polymerase complexed with A927929 (PDB ID: 3L3M), was retrieved from the Protein Data Bank (PDB). After considering residual factors such as R-value free, R-value work, R-value observed and overall resolution of the PARP enzyme structures on PDB 4DQY, 4OQA, 3L3M and 6VKO, structure 3L3M was found to have the lowest values in all the parameters [R-value free (0.275), R-value work (0.202), R-value observed (0.205) and overall resolution (2.50 Å)] which indicated the possibility of being a good target. The PARP enzyme (PDB ID 3L3M) was refined, and hydrogen atoms were added. The root-mean-square deviation (RMSD) was fixed at 0.30Å for liquid simulations (OPLS4) force field using the Protein Preparation Wizard of Schrödinger Maestro Release 2023–1.

The PDB ID 3L3M was split into ligand, water molecules and protein structure. Site mapping was performed to precisely map protein-interacting binding sites and reveal the fine details of these interactions. This generates the site volume, site score, size, D-score, philic and phobic values of the sitemap.

A receptor glide grid was generated for each of the sitemaps; subsequently, molecular docking of the enzyme with the prepared ligands was performed separately.

Extra precision (XP) ligand docking

A molecular docking experiment was performed by using the Glide executed in the Schrödinger Suite. The enzyme was treated as a rigid structure, while the ligand was treated as being flexible. The enzyme grid was given a dimension suitable to accommodate the ligand structure. The Van der Waals scaling factor was set to 0.80, and the partial charges limit value was set at 0.15.

Calculation of Physicochemical Properties

For the analysis of the pharmaceutical, physiological, biochemical and molecular effects of the compound, the adsorption, distribution, metabolism, excretion and toxicity (ADME-Tox) properties were calculated with the QikProp program of Maestro Schrodinger Suites. The QikProp predicted the physicochemical and pharmacokinetic properties of the compounds.

It also assessed the tolerability of the analogues based on Lipinski's rule of five (that is, it does not violate more than one of the following criteria: no more than five hydrogen bond donors; no more than ten hydrogen bond

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acceptors; a molecular mass of less than 500 Daltons and a calculated octanol-water partition coefficient, ClogP, that does not exceed 5).

RESULTS AND DISCUSSION

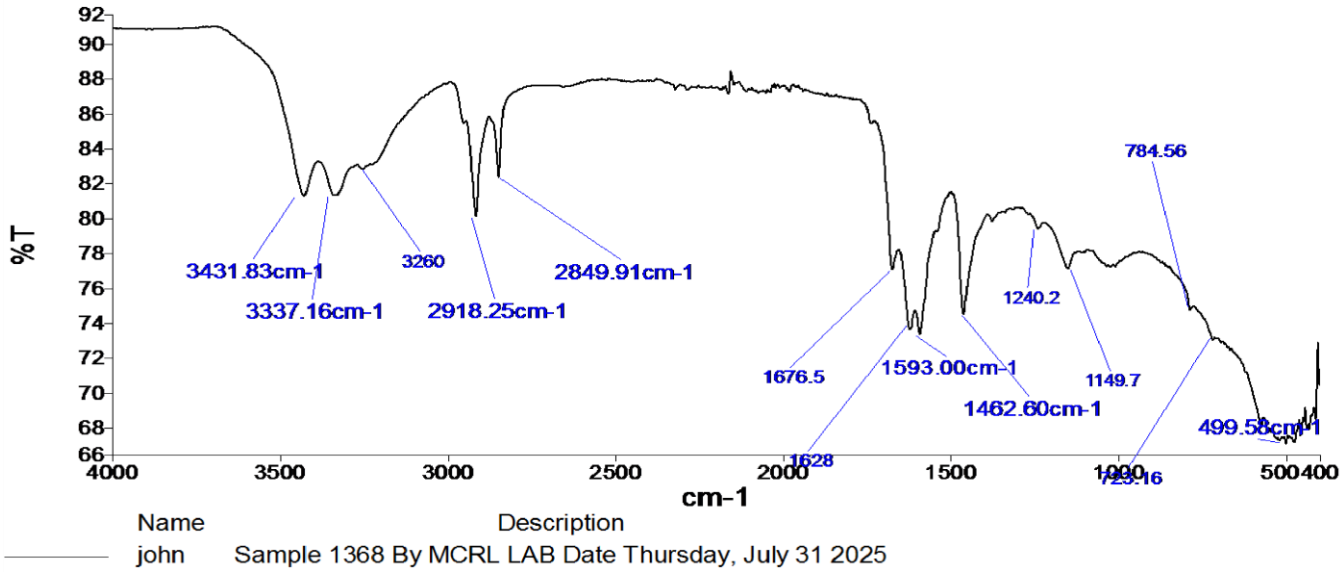

Figure 2: FTIR Analysis of 6-Ethoxy-4-(Ureido)Indole-3-Sulfonic Acid

Multiple peaks at 3431.83, 3337.16 and 3260 cm^{-1} depict the presence of N-H stretching. The absorptions observed at 2918.25 cm^{-1} and 2849.91 cm^{-1} indicate the presence of Aliphatic C-H stretching, while absorption at 1676.5 cm^{-1} suggests the presence of C=O (ureido amide 1). Intense peaks at 1628 cm^{-1} and 1593 cm^{-1} confirm aromatic C=C stretching and N-H bending (amide II band). Furthermore, absorptions at 1240.2 cm^{-1} and 1149.7 cm^{-1} reveal the presence of C-O stretch (ethoxy), while those of 784.56 cm^{-1} and 725.16 cm^{-1} signify the presence of substituted benzene.

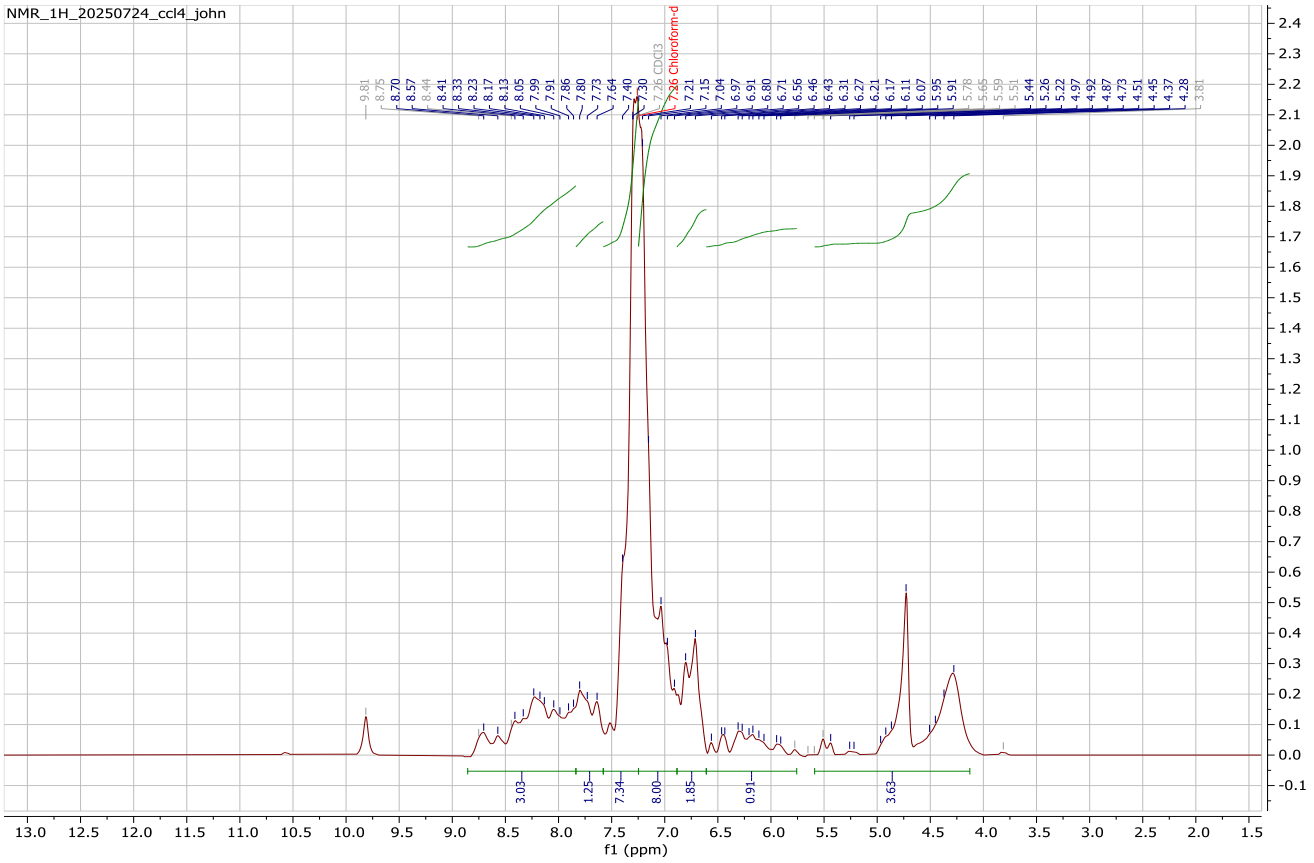

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Figure 3: Proton NMR analysis of 6-Ethoxy-4-(Ureido)Indole-3-Sulfonic Acid

Table 1 Observed Peaks of ^{1}H NMR Spectrum of 6-Ethoxy-4-(Ureido)Indole-3-Sulfonic Acid

Observed Peak (ppm)	Matches Expected
9.81	Indole NH (Strongly deshielded)
8.75 - 8.0	Ureido NH (Broad, 1H)
7.0 – 6.5	Ureido NH_2 (Broad, 2H)
7.40 – 6.71	H5, H7 (2H, dd/m)
3.81	-OR (Ethoxy functional group)

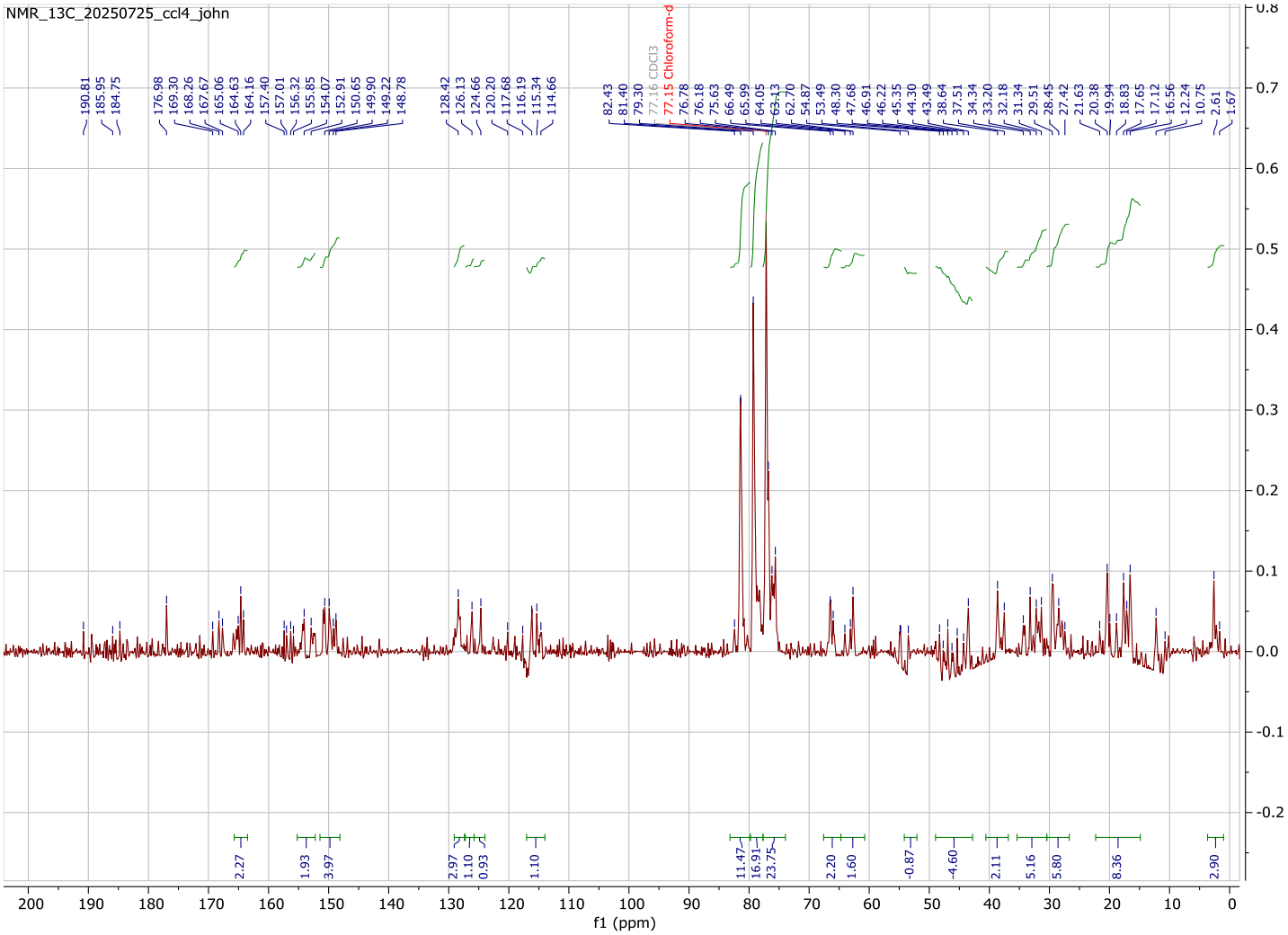


Figure 4:^{13}C NMR analysis of 6-Ethoxy-4-(Ureido)Indole-3-Sulfonic Acid

Table 2: Observed Peaks of ^{13}C NMR Spectrum of 6-Ethoxy-4-(Ureido)Indole-3-Sulfonic Acid

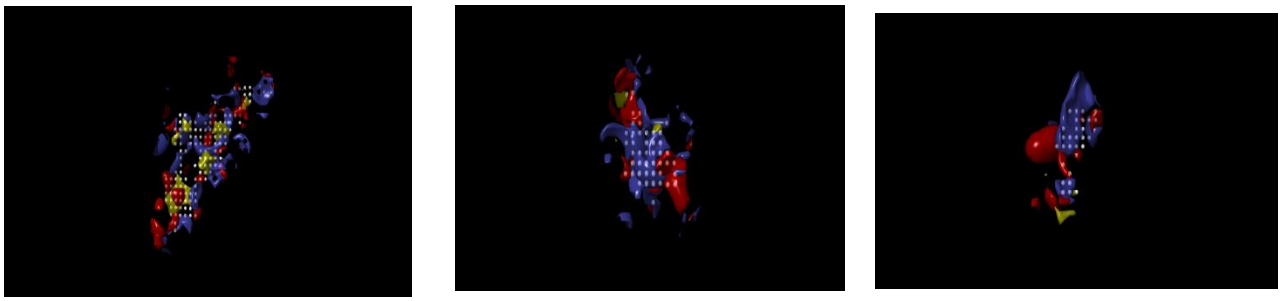
Observed Peak (ppm)	Matches Expected
10 – 22	-CH_3 possibly attached to heteroatom
62 – 66	-OCH_3 (Ethoxy)
114 – 128	Indole aromatic carbon
148 – 157	C4 (Ureido–substituted)

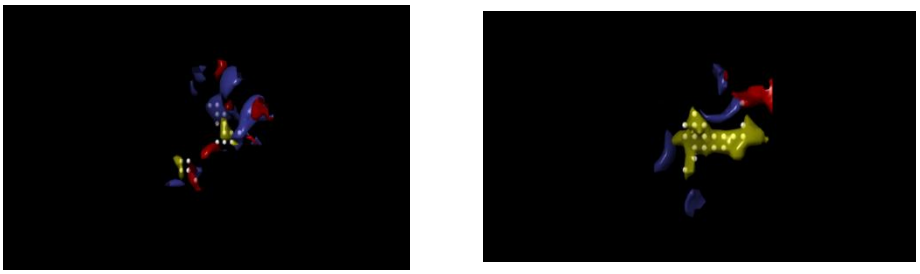
164 – 169	Ureido C=O
184 – 191	Degraded product (e.g., oxidised indole)

Table 3: Binding site scores of PARP enzyme (PDB ID: 3L3M)

Entry	Site score	Size	Dscore	Volume	Phobic	Philic
Site 1	1.070	260	1.088	721.329	0.853	1.003
Site 2	1.005	99	0.904	212.317	0.144	1.405
Site 3	0.705	45	0.585	110.103	0.159	1.277
Site 4	0.617	28	0.602	91.581	0.443	0.655
Site 5	0.838	32	0.842	78.890	2.921	0.449

Sitemap_1 Site_1 Sitemap_1 Site_2 Sitemap_1 Site_3





Sitemap_1 Site_4 Sitemap_1 Site_5

Figure 5. Sitemaps of PARP Inhibitor (PDB ID: 3L3M)

Table 4: Docking results of the ligands with the five sitemaps

Sitemap	Ligand	Docking Score	Ligand Efficiency	E Model
1	Proposed Compound	-5.438	-0.272	-57.731
	Reference	-10.498	-0.328	-93.885
2	Proposed Compound	-4.198	-0.210	-46.965
	Reference	-2.049	-0.064	-52.395
3	Proposed Compound	-4.327	-0.216	-41.103
	Reference	-3.682	-0.115	-42.260
4	Proposed Compound	-4.907	-0.245	-44.118
	Reference	-1.392	-0.044	-43.288

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5	Proposed Compound	-3.858	-0.193	-39.875
	Reference	-1.840	-0.058	-50.688

Table 5: Ligand interactions with amino acids at the five sitemaps

Sitemap	Ligand	Hydrogen Bond Distance	Pi-Pi Stacking Distance	Pie-Cation Distance
1	Proposed Compound	GLY_{227} 1.96 Å LYS_{242} 1.92 Å GLU_{327} 1.89 Å	TYR_{246} 4.02 Å TYR_{246} 3.96 Å	
	Reference	GLY_{202} 2.07 Å GLY_{202} 2.01 Å SER_{243} 1.83 Å	HIS_{201} 5.15 Å TYR_{246} 4.22 Å TYR_{246} 3.87 Å	
2	Proposed Compound	THR_{205} 2.57 Å ILE_{255} 2.09 Å ASP_{253} 2.02 Å		LYS_{23} 6.26 Å LYS_{23} 5.69 Å
	Reference	ILE_{255} 2.12 Å		
3	Proposed Compound	GLU_{148} 2.11 Å GLU_{148} 1.80 Å GLU_{148} 1.79 Å		
	Reference	GLU_{151} 2.25 Å		
4	Proposed Compound	ARG_{145} 2.14 Å VAL_{172} 1.91 Å VAL_{143} 1.78 Å ILE_{173} 1.73 Å		
	Reference	VAL_{172} 2.77 Å ILE_{175} 2.35 Å ARG_{145} 1.87 Å		
Sitemap	Ligand	Hydrogen Bond Distance	Pi-Pi Stacking Distance	Pi-Cation Distance
5	Proposed Compound	ASP_{122} 2.61 Å ASN_{132} 2.28 Å ASP_{123} 2.01 Å ASP_{122} 1.99 Å		
	Reference	ASN_{132} 2.57 Å ASN_{132} 2.45 Å ASN_{132} 2.36 Å		

Table 6: ADME-Tox Properties of the Proposed and Reference Compounds

Ligand	Mol MW	Dipole	DonorHB	AcceptorHB	QPlogP_0/w	%Human Oral Absorption	PSA	Rule of five
Reference Structure	434.469	9.891	1.000	8.000	2.865	83.736	112.349	0
Proposed Compound	299.301	6.736	5.000	8.350	-0.918	48.422	134.371	0

CONCLUSION

The results obtained from FT-IR and NMR analyses of the synthesised compound confirmed the presence of the anticipated key functional groups. The docking scores and the ligand efficiency were used to evaluate the binding affinity and the overall stability of the ligand-amino acid complex. The analysis revealed that the reference compound has a lower HBdonor compared to the hit compound. In addition, the interactions of the designed compound with the PARP inhibitor (PDB ID: 3L3M) showed that it has several π-π cation distance values in binding site 2, thus stabilising the "interaction" that significantly influences a ligand's binding affinity and stability within a protein's active site. Also, the designed ligand had better docking scores and better interactions on all the sitemaps except sitemap 1. The ADMET analysis further supports the potential of the proposed compound, underscoring its lower energy and better hydrophilicity, which align with the requirements for cancer drug delivery.

RECOMMENDATIONS

Following the findings of this research, the recommendations highlighted below are hereby put forward:

The proposed ligand should be further optimised for more computational studies to enrich its drug-likeness.

In-vitro and *in-vivo* studies should be carried out to validate its binding affinity, toxicity profile, pharmacokinetics and efficacy as a prostate cancer therapeutic agent. This is necessary to translate these findings into clinical applications.

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