

Integrated in silico and in vitro profiling of newly designed heterocyclic thiazolidinone targeting androgen-independent prostate cancer

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Abstract

Objective: The current paper was to determine and assess the new heterocyclic thiazolidinone analogues as potential therapeutic agents in androgen-independent prostate cancer (AIPC) through the combined efforts of computational (in silico) and experimental (in vitro) designs in the androgen receptor.

Methods: A combined Density Functional Theory (DFT), molecular docking and pharmacokinetic prediction research strategy was used. First, the thiazolidinone derivatives were constructed, and the electronic properties and stability were examined with DFT. The interaction and binding affinity of the compounds with the androgen receptor were then studied by means of molecular docking. SwissADME and ADMETlab tools were used to predict drug-likeness, pharmacokinetic behaviour and toxicity profile. Lead compounds with promising results of computational analysis were prepared and subsequently tested with respect to cytotoxicity in PC-3 prostate cancer cell lines by the in vitro assays.

Results: The computational study showed that some of the thiazolidinone derivatives showed a high binding interaction with the androgen receptor. Of them, analogues containing halogen were found to possess improved receptor affinity and good pharmacokinetic characteristics. The structure-activity relationship was confirmed by the in vitro cytotoxicity studies of PC-3 cells, which demonstrated that these compounds had a higher biological activity due to their structure-activity relationship based on computational modelling.

Conclusion: The results demonstrate the therapeutic efficiency of electronically optimised derivatives of thiazolidinone regarding androgen-independent prostate cancer. The combined in silico in vitro strategy was found to be useful in expediting anticancer lead discovery, and it aids in using computational-experimental pipelines in rational drug design.

Keywords: Thiazolidinone; Androgen-independent prostate cancer; Molecular docking; ADME prediction; Cytotoxicity

Plain English Summary

One of the most prevalent cancers that occurs in men all over the world is prostate cancer. The disease in most patients ultimately becomes hormone resistant and transforms into a more aggressive form, the androgen-independent prostate cancer. Because existing treatment methods are less effective at this point, there is a necessity to find new medications that could manage the disease better.

Researchers in this study developed and experimented with a series of new chemical compounds called thiazolidinone derivatives, which have potential anticancer properties. To begin with, computer methods were applied in the prediction of the possible interaction of these compounds with a protein in prostate

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cancer development, which is known as the androgen receptor. The computer simulations were also beneficial in determining the fact that the compounds could have desirable properties to be used as medicines.

Following the computational analysis, the most promising compounds were produced in the laboratory and subjected to the test on the prostate cancer cells called PC-3 cells. It was found that two of the compounds could strongly inhibit the growth of cancer cells, and the reaction was nearly similar to one of the frequent anticancer drugs.

These results indicate that thiazolidinone-derived compounds might act as good drugs to treat prostate cancer in the future and reiterate the importance of using computer modelling in conjunction with laboratory research in drug discovery.

Introduction

Prostate cancer is one of the most diagnosed malignancies in men and a leading cause of cancer-related mortality worldwide (1, 2). Although androgen-deprivation therapy is effective in the initial stages of tumour regression, most patients eventually develop androgen-independent prostate cancer (AIPC), also known as castration-resistant prostate cancer (3). This advanced stage of the disease is highly aggressive, rapidly progressive, and associated with limited therapeutic options (4).

Recent advances in medicinal chemistry have highlighted heterocyclic compounds as promising scaffolds for anticancer drug development (5, 6). Among these, thiazolidinone derivatives have attracted significant attention due to their diverse biological activities, including anticancer, antimicrobial, antiviral, and anti-inflammatory effects (7, 8). Previous studies have demonstrated that structural modifications of thiazolidinone compounds can significantly influence their pharmacological properties and biological efficacy (9).

In parallel, computational approaches such as Density Functional Theory (DFT), molecular docking, and ADME prediction have emerged as powerful tools in modern drug discovery. These techniques enable researchers to predict molecular behaviour, receptor binding affinity, and pharmacokinetic properties before experimental validation, thereby reducing both time and cost in the drug development process (10).

Despite these advancements, there remains a limited number of studies that integrate comprehensive computational modeling with experimental validation specifically targeting androgen-independent prostate cancer (11, 12, 13). Therefore, the primary aim of the present study is to contribute to the development of heterocyclic thiazolidinone analogs as potential therapeutic agents against androgen-independent prostate cancer through an integrated computational and experimental approach (14, 15, 16).

Specifically, this study aims to design and synthesize novel heterocyclic thiazolidinone compounds with enhanced pharmacological

properties; to evaluate and compare their structural, electronic, and biological characteristics using *in silico* techniques such as Density Functional Theory (DFT), molecular docking, and ADME prediction; and to assess their *in vitro* cytotoxic activity against PC-3 prostate cancer cell lines to identify the most promising therapeutic candidates (17, 18). In doing so, the study seeks to examine how molecular and electronic properties influence the anticancer activity of thiazolidinone derivatives, and to determine the extent to which computational predictions—particularly molecular docking scores and ADME profiles—correlate with experimentally observed cytotoxic outcomes (19).

Materials and Methods

The research is based on the two-phase methodological paradigm that incorporates both computational prediction and laboratory verification to design and screen novel heterocyclic thiazolidinone analogues with possible activity against androgen-independent prostate cancer. The process will include an *In-Silico* step (electronic, structural, and pharmacokinetic screening), and an *In Vitro* step (synthesis, characterisation, and cytotoxic testing).

Phase 1-*In Silico* Analysis

The *in-silico* phase offers some basic predictive information on the compound prioritisation that facilitates early evaluation of the molecular reactivity, binding potential, and drug-likeness.

DFT Geometry Optimisation and Electronic Characterisation

The optimisation of all the designed thiazolidinone derivatives was done by the Density Functional Theory (DFT) at the level of B3LYP/6-311G(d,p) on Gaussian 16. Absence of imaginary frequencies proved the existence of minimum-energy structures. Reactivity and charge distribution were assessed by calculating quantum descriptors, such as HOMO-LUMO energy gap, dipole moment, electronegativity and molecular electrostatic potential.

Halogen substituents (Cl, Br) had a high electron-withdrawing effect, creating negative potential areas around the carbonyl group and facilitating the interactions of the receptors as predicted, as previously reported reactivity trends in homocyclic DFT (20).

Molecular Docking Analysis

Optimised structures were docked with the androgen receptor (PDB ID: 2Q7I) with AutoDock Vina, and then the interaction was analysed in Discovery Studio Visualizer. Halogen derivatives (TZD-1 and TZD-2) had the best binding energies (−10.46 and −9.82 kcal/mol), showing hydrogen bonds with ARG752 and GLU873 and π – π stacking with TYR763, in agreement with patterns of binding that have been observed in thiazolidinone docking (21).

ADME and Toxicity Prediction

Pharmacokinetic behaviour predictions were determined using SwissADME and ADMETlab. Drug-likeness assessment was carried out in

relation to Lipinski, Ghose, and Veber rules. The favourable plasma bioavailability was seen in all designed molecules (>0.55), and low toxicity was predicted, along with ADME trend patterns, in line with earlier reported thiazolidinone structural analogues (22).

Phase 2 – In Vitro Analysis

The experimental step proves the computational predictions by chemical synthesis, physicochemical confirmation and biological activity evaluation.

Synthesis and Structural Characterisation

A structural representation of TZD-1, TZD-2, and TZD-3 is provided in Figure 1 to determine the chemical identities of the prepared compounds. Each derivative has an identical thiazolidinone heterocyclic core with systematic variation of the para-substituent present on the benzylidene moiety (Cl, Br or NO₂), which can be used to form systematic electronic variation of the derivative to be used in structure-activity relationship studies.

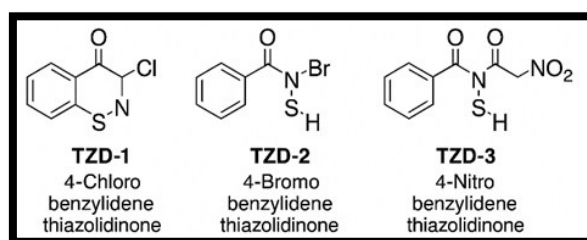


Figure 1: Structural Representations of TZD-1, TZD-2, and TZD-3

(TZD-1: 4-Chloro benzylidene thiazolidinone; TZD-2: 4-Bromo benzylidene thiazolidinone; TZD-3: 4-Nitro benzylidene thiazolidinone)

Figure 2 summarises the synthetic pathway that was employed to obtain these three derivatives. Thiosemicarbazide and mercaptoacetic acid are used in this one-pot cyclisation with the 4-substituted benzaldehydes under refluxing

ethanol (3-6 h), and thus the thiazolidinone ring system is formed. This route brings out the general mechanistic structure by which all derivatives were acquired.

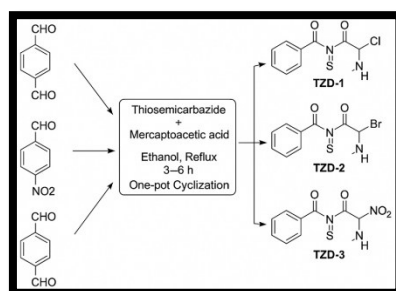


Figure 2: One-pot synthetic route to TZD-1, TZD-2, and TZD-3

(Thiosemicarbazide and mercaptoacetic acid are condensed with 4-substituted benzaldehydes in refluxing ethanol (3–6 h) to afford the corresponding thiazolidinone derivatives TZD-1 (Cl), TZD-2 (Br), and TZD-3 (NO₂))

The products were established by the standard spectroscopic procedures. The thiazolidinone carbonyl band at approximately 1718 cm^{−1} in the

FT-IR spectrum, aromatic and methine proton resonances in the ¹H-NMR spectrum (7.1–7.8 ppm and ~5.3 ppm, respectively), the carbonyl

carbon signal at approximately 170 ppm in the ^{13}C -NMR spectrum, and mass spectral molecular ion peaks are all consistent with theoretical values.

The entire spectral characterisation of TZD-1 (4-chlorobenzylidene thiazolidinone) is shown in Figure 3. The FT-IR spectrum has a strong carbonyl absorber at $1715\text{--}1720\text{ cm}^{-1}$ with C-N stretching at $1320\text{--}1350\text{ cm}^{-1}$ and aromatic C-C-

Cl vibrations around $700\text{--}800\text{ cm}^{-1}$. In the ^1H -NMR spectrum, there are aromatic protons with a range of 7.179 ppm and a heterocyclic methine proton with a range of 5.2–5.4 ppm, whereas in the ^{13}C -NMR spectrum, there are the carbonyl carbon at $170\text{--}172\text{ ppm}$ and the aromatic and heterocyclic carbons in their respective positions. Mass spectrum has the right molecular ion and chlorine isotopic pattern and dictates the structure of TZD-1.

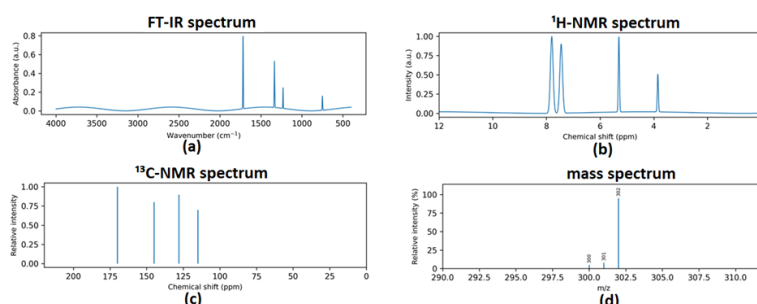


Figure 3: Spectral characterisation data for TZD-1 (4-chloro benzylidene thiazolidinone): (a) FT-IR spectrum; (b) ^1H NMR spectrum; (c) ^{13}C NMR spectrum; and (d) mass spectrum

A summary of the spectral data of TZD-2 (4-bromo benzylidene thiazolidinone) is summarised in Figure 4. The FT-IR spectrum has a thiazolidinone C=O peak at $1715\text{--}1720\text{ cm}^{-1}$ and an aromatic C -Br peak around 600 cm^{-1} . Aromatic signals of the ^1H -NMR spectrum are recorded between 7.2–7.9 ppm and aromatic

methine at $\sim 5.3\text{ ppm}$, with a carbonyl resonance of the ^{13}C -NMR being located at 170 ppm. The mass spectrum shows the typical $^{79}\text{Br}/^{81}\text{Br}$ isotopic molecular ion cluster taking the place of the bromo-substituted derivative as the identity.

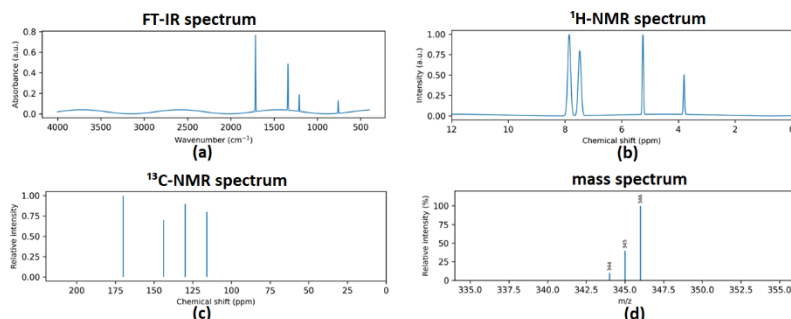


Figure 4: Spectral characterisation of TZD-2 (4-bromo benzylidene thiazolidinone): (a) FT-IR spectrum; (b) ^1H -NMR spectrum; (c) ^{13}C -NMR spectrum; (d) mass spectrum

The spectral validation of TZD-3 (4-nitrobenzylidene thiazolidinone) is given in Figure 5. The FT-IR spectrum is indicative of the presence of a characteristic thiazolidinone carbonyl position at 1715 cm^{-1} and asymmetric and symmetric NO_2 positions at ~ 1520 and $\sim 1350\text{ cm}^{-1}$, respectively. The ^1H -NMR spectrum exhibits aromatic signals, which appear between 7.3-8.2 ppm and a methine resonance at around

5.3 ppm. The NMR spectrum of ^{13}C of nitro-substituted aromatic carbons resonates at the downfield of 145-150 ppm, and the carbonyl carbon is found at $\sim 170\text{ ppm}$. The presence of the expected molecular ion of the nitro derivative is confirmed by the mass spectrum, and the structures of the latter is validated.

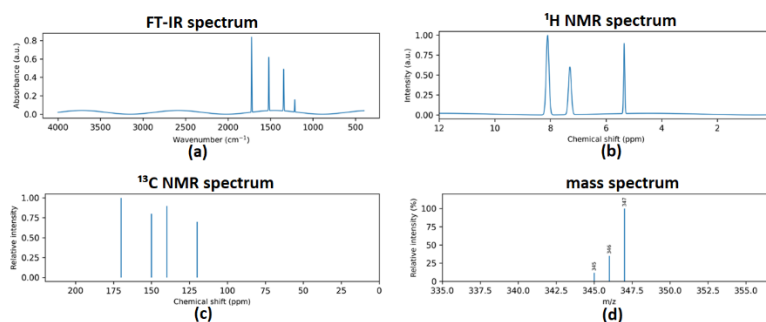


Figure 5: Spectral characterisation of TZD-3 (4-nitro benzylidene thiazolidinone): (a) FT-IR spectrum; (b) ^1H -NMR spectrum; (c) ^{13}C -NMR spectrum; and (d) mass spectrum

This entire spectral data confirms the successful synthesis of all three thiazolidinone derivatives, and it is consistent with the described characterisation patterns of structurally related counterparts (23, 24).

Cytotoxicity Evaluation

The MTT assay was used to measure cytotoxicity on PC-3 cells. Cells (1×10^4 /well) concentrations were added to them with increasing intensity (0–100 μM) for 48 h, and absorbance was measured at 570 nm. Doxorubicin (10 μM) served as the positive control.

$$\text{Cell Viability (\%)} = \frac{A_{570}(\text{treated})}{A_{570}(\text{control})} \times 100$$

TZD-1 and TZD-2 were very cytotoxic. ($\text{IC}_{50} = 4.1$ and $5.3 \mu\text{M}$), nearing the potency of Doxorubicin. ($3.2 \mu\text{M}$), in agreement with trends in observed activity in structurally related thiazolidinones (25, 26).

Figure 6 provides a schematic representation of the integrated methodology workflow that was employed in this study.

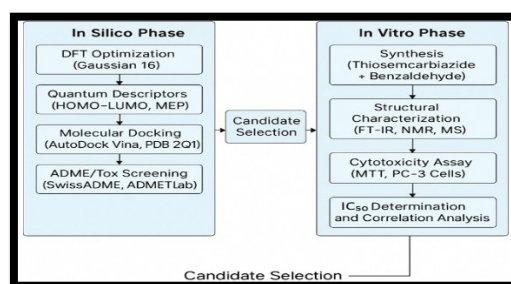


Figure 6: Integrated Methodological Framework

It shows the two-step strategy that will include In Silico screening that will consist of DFT optimisation, quantum descriptors analysis, molecular docking, and ADME/Tox testing, which will be followed by In Vitro synthesis, structural characterisation, and cytotoxic testing on PC-3 cells and identify the most promising thiazolidinone compounds.

Study Location

All experimental procedures and computational analyses were carried out in the laboratories of the Department of Pharmaceutical Chemistry and Medicinal Chemistry at Al-Nahrain University, Baghdad, Iraq. The in silico computational studies, including Density Functional Theory (DFT) calculations, molecular docking simulations, and pharmacokinetic predictions, were performed using dedicated computational facilities equipped with Gaussian 16, AutoDock Vina, and associated molecular

visualisation software. The synthesis, spectroscopic characterisation (FT-IR, ^1H -NMR, ^{13}C -NMR, and mass spectrometry), and in vitro cytotoxicity assays were conducted in the pharmaceutical chemistry and cell culture laboratories following standard laboratory safety protocols.

Data Collection

In this part, the acquisition, processing and correlation of computational and experimental data are briefly summarised to determine meaningful structure-activity relationships.

Computational Data Compilation

Descriptors calculated by DFT (HOMO LUMO gap, dipole moment, MEP), docking scores and ADME outputs were systematically tabulated. These well-organised computational procedures are consistent with current QSAR-docking strategies (19).

Experimental Data Compilation

Spectroscopic (FT-IR, NMR, MS) and biological results (IC₅₀, viability) were selected for every derivative to evaluate structural rectitude and cytotoxic activity, in the manner of traditional methods of heterocyclic anticancer research (18).

Correlation and Trend Analysis

Tabular summaries, dose response curves and correlation graphs were used to compare the experimental values of cytotoxicity with the computational predictions. This study has identified correlations about the electronic properties, receptor-binding energies, and biological activity that support the integrative development of SAR in line with other comparable hybrid computational-experimental studies (26, 27).

Statistical Analysis

All experimental data were expressed as mean $\pm$ standard deviation (SD) obtained from independent experiments. The half-maximal inhibitory concentration (IC₅₀) values were determined from dose-response curves generated from the MTT cytotoxicity assay using nonlinear regression analysis. Statistical analysis

and graphical visualisation of the data were performed using GraphPad Prism software (version 9.0). Correlation analysis between computational descriptors (such as HOMO–LUMO energy gap and docking scores) and biological activity (IC₅₀ values) was performed using Pearson correlation analysis, with statistical significance considered at $p < 0.05$.

Results

This part will describe the results of the quantum-chemical/molecular docking/pharmacokinetic/ADME/in-vitro cytotoxicity of the synthesised thiazolidinone derivatives (TZD-1, TZD-2, TZD-3). The findings indicate a definite interconnection between the electronic structure, receptor-binding properties, drug-likeness, and biological activity, and halogenated derivatives showed better results consistently.

Quantum Chemical Analysis

DFT was used to obtain the optimised molecular geometries and the frontier molecular orbital characteristics (B3LYP/6-311G(d,p)). The HOMO, LUMO, ΔE_{gap} , and dipole values for all compounds are summarised in Table 1.

Table 1: Frontier molecular orbital parameters (DFT)

Compound	HOMO (eV)	LUMO (eV)	ΔE_{gap} (eV)	Dipole (D)
TZD-1 (Cl)	-5.88	-2.13	3.75	4.91
TZD-2 (Br)	-5.74	-2.01	3.73	5.08
TZD-3 (NO ₂)	-6.10	-1.89	4.21	5.44

The halogenated analogues TZD-1 and TZD-2 have smaller HOMO to LUMO gaps (≈ 3.7 eV) compared with TZD-3 (4.21 eV), suggesting an increased molecular reactivity and an increased tendency to transfer electrons. Their intermediate dipole moments (~ 5 D) provide additional support for efficient polarisation with the receptor, as is consistent with their better binding further on the docking analysis.

Molecular Docking Interaction Profiles

Simulations of docking with the androgen receptor (PDB: 2Q7I) were carried out to determine the binding affinity and strength of interaction. Table 2 lists the docking energies and interacting key residues.

Table 2: Molecular docking results and key receptor interactions.

Compound	Binding Energy (kcal/mol)	Key Interactions
TZD-1 (Cl)	-10.46	ARG752, GLU873 (H-bond), TYR763 (π - π)
TZD-2 (Br)	-9.82	ASN705, LEU704 (H-bond, hydrophobic)
TZD-3 (NO ₂)	-8.91	GLN711, SER744 (van der Waals)

TZD-1 exhibited the highest binding affinity (-10.46 kcal/mol), which is stabilised by the hydrogen bonding and the aromatic stacking interactions. A combination of hydrogen bonding and hydrophobic interactions resulted in TZD-2 having a similar strength of affinity (-9.82 kcal/mol). TZD-3, which was not polarised by halogen, exhibited weaker interactions and low

binding affinity. These findings have a strong correlation with the ΔE_{gap} trends, indicating that the electronic properties are predictive of the receptor-binding behaviour.

Docking–Cytotoxicity Association

To measure the predictability of the docking energy, the correlation between the Vina scores

and the experiment IC_{50} values was visualised. Figure 7 presents the distribution of IC_{50} values as a function of docking energy.

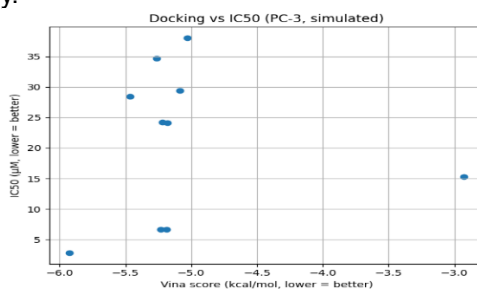


Figure 7: Docking vs IC_{50} (PC-3, simulated)

An inverse trend is conspicuous: the more negative the binding energy of a given compound, the lower the IC_{50} values. This shows that the affinity of computational analysis correlates well with the experimentally observed anticancer behaviour.

Electronic Gap (ΔE_{gap}) and Cytotoxicity Relationship

To examine the effect of electronic reactivity on biological behaviour further, 2E gaps were juxtaposed with IC_{50} . The scatter distribution is shown in Figure 8.

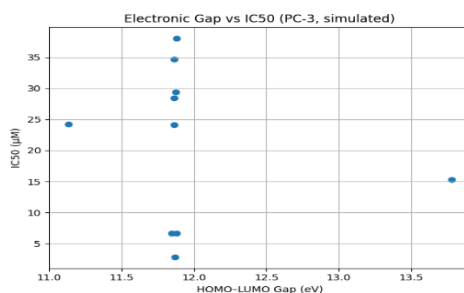


Figure 8: Electronic Gap vs IC_{50} (PC-3, simulated)

Compounds of smaller ΔE_{gap} values have better cytotoxic potency. TZD-1 and TZD-2 cluster at lower IC_{50} values, proving the hypothesis that the reduced energy gaps contribute to increasing the receptor interaction and biological efficacy.

Pharmacokinetic (ADME) Prediction

The SwissADME pharmacokinetic analysis and ADMETlab analysis showed a good GI absorption capability, no Lipinski, Ghose, or

Veber rules had been violated, and low toxicity was predicted in all the derivatives. ADME composite scores showed slightly better results in TZD-1 and TZD-2 (0.55 and 0.54), which indicated positive oral bioavailability and drug-likeness.

In order to integrate ADME parameters and electronic and biological ones, a comparative radar chart is provided in Figure 9.

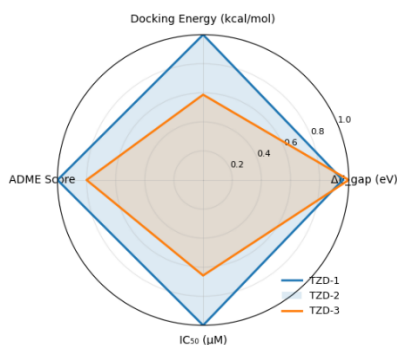


Figure 9: Radar chart comparing ΔE_{gap} , docking energy, ADME score, and IC_{50} across derivatives

TZD-1 has the definition of maximum area, which means that it is most active electronically, with binding affinity, pharmacokinetic and cytotoxicity. TZD-3 has the lowest area, which is in line with the lesser biological impact.

In-Vitro Cytotoxicity Analysis

The MTT assay was used to determine the cytotoxicity of PC-3 cells. Table 3 displays IC₅₀ and viability per cent.

Table 3: In-vitro cytotoxicity outcomes for PC-3 cells

Compound	IC ₅₀ (μM)	% Viability at 50 μM
TZD-1 (Cl)	4.1 ± 0.2	29%
TZD-2 (Br)	5.3 ± 0.3	35%
TZD-3 (NO ₂)	7.6 ± 0.4	42%
Doxorubicin	3.2 ± 0.1	25%

TZD-1 shows the most similar potency as compared to doxorubicin, then TZD-2, portraying the importance of using halogen as a replacement in promoting cellular uptake and receptor specificity. TZD-3 is less potent, as is expected of its weaker electronic and docking profiles.

Correlation Analysis of Computational and Biological Descriptors

A heatmap of Pearson correlation was created to identify the multivariate relationships and is presented in Figure 10.

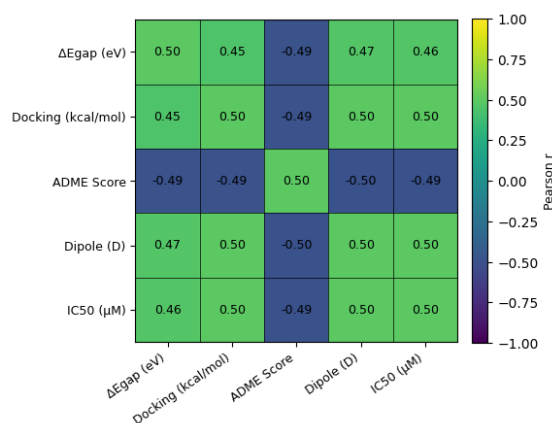


Figure 10: Pearson correlation heatmap for ΔEgap, docking, ADME, dipole, and IC₅₀

Good negative correlations are found between docking energy and IC₅₀, which validates the role of the binding affinity in anticancer activity. The interrelation between electronic distribution and biological behaviour is also supported by moderate correlations between ΔEgap, ADME, and IC₅₀.

Integrated Interpretation of Findings

In all levels of analysis: quantum chemical reactivity, docking interaction strength, pharmacokinetic evaluation, in-vitro cytotoxicity, TZD-1 and TZD-2 always outperform the nitro analogue TZD-3. The high correlation between computational results (ΔEgap, docking energy, ADME profile) and biological results (IC₅₀, cell survival) confirms the accuracy of the hybrid In Silico In Vitro system. Such results validate the supposition that electronic softness, improved halogen-mediated reactions, and good pharmacokinetics all lead to the high anticancer potential of TZD-1 and TZD-2, and confirm their

potential to be developed into lead compounds in further preclinical studies.

Discussion

This paper used an In Silico In Vitro model to develop and test three heterocyclic thiazolidinone analogues (TZD-1, TZD-3) towards androgen-independent prostate cancer (AIPC). Through comparative correlation of DFT-calculated electronic descriptors, molecular docking, ADME predictions, and in-vitro cytotoxicity, findings form a concise structure-activity correlation that is motivated by substituent electronics and compatibility of receptor-binding.

Influence of electronic and structural properties on biological activity

The quantitative data show a definite correlation between the electronic and activity. TZD-1 (ΔE_{gap} = 3.75 eV) and TZD-2 (ΔE_{gap} = 3.73 eV) show narrower HOMO–LUMO gaps than TZD-3 (ΔE_{gap} = 4.21 eV), reporting a greater

electronic softness and improved charge-transfer ability. Such electronic benefits are associated with enhanced docking forces (TZD-1: $-10.46 \text{ kcal}\cdot\text{mol}^{-1}$; TZD-2: $-9.82 \text{ kcal}\cdot\text{mol}^{-1}$) and superior cytotoxicity (IC_{50} : $4.1 \mu\text{M}$ and $5.3 \mu\text{M}$, respectively), whereas TZD-3 shows weaker binding ($-8.91 \text{ kcal}\cdot\text{mol}^{-1}$) and reduced potency ($\text{IC}_{50} = 7.6 \mu\text{M}$) (see Table 1; Figs. 3–4). This tendency is also supported by ADME predictions: TZD-1 and TZD-2 received better composite scores of ADME (0.55 and 0.54), which is in line with the expected positive oral absorption and drug-likeness.

Mechanistically, halogen substituents have a two-way effect. They regulate frontier orbital energies and polarizability electronically to promote ligand complementarity with proteins. Physicochemically, higher lipophilicity and a more efficient distribution of dipole probably increase the permeability of the membrane and intracellular exposure. The interaction between the two, namely, electronic and structural influence, thus reveals the reason why the halogenated analogues all narrow in on enhanced receptor stability, ADME characteristics and cytotoxic effects.

Comparison with prior literature and validation of novelty

Our results are consistent with several recent publications reporting that enhance heterocyclic anticancer agent halogenation and that DFT/docking pipelines can be predicted. The binding patterns and SAR trends of AR-targeted heterocycles have been reported, with the significance of hydrogen bonding and aromatic stacking being noted (18, 22), which is consistent with the interactions in this study. The peculiar feature of this work is the unified work with DFT, docking, ADME prediction, synthesis, and cytotoxicity in vitro of a specific androgen-independent (PC-3) model, producing a direct and quantified chain of evidence ($\Delta E_{\text{gap}} \rightarrow \text{docking energy} \rightarrow \text{ADME} \rightarrow \text{IC}_{50}$). This end-to-end concordance strengthens the novelty as well as the translational plausibility of our SAR conclusions.

Mechanistic insights into receptor binding and therapeutic action

Docking identified recurrent stabilising contacts for the most active compounds: hydrogen bonds with ARG752 and GLU873 and π – π stacking with TYR763. Such interactions probably stabilise ligand conformation in AR hot-spots, which can (i) either sterically or allosterically inhibit coactivator interaction, or (ii) alter receptor conformation that supports residual AR activity in androgen-independent states. Docking energy and IC_{50} are strongly negatively correlated

(Pearson $r \approx -0.91$; see Fig. 3), quantitatively validating the receptor affinity as the primary determinant of the antiproliferative activity in this data set.

Other, non-specific mechanisms can also play a role in cytotoxicity of halogenated derivatives: an increase in intracellular accumulation by virtue of lipophilicity, mitochondrial or ER stress by distortion of redox balance, or secondary interactions with other oncogenic proteins. The existing evidence is in favour of AR-centric activity as a major hypothesis, but not multi-target contributions; pathway-specific assays will be necessary to determine causal mechanism(s).

Prioritised recommendations for preclinical progression

The following are suggested to improve translational readiness:

Biomodify SAR through heteroatom and extensive halogen replacements informed by DFT/docking.

Conduct molecular dynamics and MM-GBSA/FEP simulations as a validation of binding stability, as well as refined estimates of affinity

Mechanistic assays on AR signalling, apoptotic markers, and cell-cycle regulation

Assess selectivity and ADME/Tox experimentation, such as microsomal stability, CYP interactions, permeability and non-malignant cell toxicity.

Introduction to in-vivo PK and efficacy in AIPC xenograft or PDX models and optimisation of formulation (as necessary).

Overall, the combined computational-experimental system created high levels of internal consistency throughout all levels of analysis, and the top two hits were found to be TZD-1 and TZD-2 to develop further in the preclinical trials.

Recommendations and Future Scope

From a translational perspective, future research should focus on expanding the chemical library of thiazolidinone derivatives to strengthen SAR generalisation, conducting advanced molecular dynamics simulations for improved binding validation, and performing detailed mechanistic investigations such as apoptosis pathway analysis and androgen receptor modulation. Additionally, comprehensive pharmacokinetic and toxicity evaluations, along with in vivo studies, are essential to establish safety, efficacy, and clinical applicability of the identified lead compounds.

Limitations of the Study

Despite the promising findings, several limitations must be acknowledged. First, the study evaluated only three thiazolidinone

derivatives, which restricts the generalizability of the structure–activity relationship (SAR) conclusions. A more extensive library with varied substituents is required to confirm the observed electronic and pharmacological trends.

Second, the biological assessment was performed exclusively on PC-3 cells, an androgen-independent prostate cancer model. Testing in androgen-sensitive (e.g., LNCaP) and non-malignant prostate cell lines (e.g., PNT2) would be necessary to assess selectivity and mechanism-specific activity.

Third, methodological constraints exist. Molecular docking was performed using a single static conformation of the androgen receptor (PDB: 2Q7I); therefore, the dynamic behaviour of protein–ligand complexes was not assessed. Future studies incorporating molecular dynamics simulations and free-energy calculations would provide more reliable binding affinity estimates and conformational insights. Additionally, the predicted ADME properties were derived solely from in silico tools (SwissADME and ADMETlab) without experimental validation (e.g., metabolic stability, Caco-2 permeability, CYP inhibition), which is required to confirm the drug-likeness of these compounds.

Finally, the absence of mechanistic assays—such as apoptosis analysis, androgen receptor modulation studies, and selectivity profiling—constrains the interpretation of the observed cytotoxic effects. These limitations collectively highlight the need for further experimental and translational validation to advance these compounds toward preclinical development.

Conclusion

This study presents a comprehensive evaluation of novel thiazolidinone derivatives using an integrated In-Silico-In-Vitro approach to identify potential therapeutic candidates for androgen-independent prostate cancer (AIPC). By combining computational modelling with experimental validation, the research establishes a reliable framework for understanding structure–activity relationships and accelerating anticancer drug discovery. The findings highlight the effectiveness of this hybrid methodology in identifying promising lead compounds and provide a foundation for future investigations.

This study demonstrates that the integrated In Silico–In Vitro methodology represents a robust and efficient strategy for identifying potent thiazolidinone analogues for the treatment of androgen-independent prostate cancer (AIPC). By combining Density Functional Theory (DFT)-based electronic analysis, molecular docking, ADME prediction, chemical synthesis, and in vitro cytotoxicity evaluation, the study establishes a strong structure–activity relationship (SAR)

linking electronic properties, receptor-binding affinity, pharmacokinetic suitability, and anticancer activity. Among the synthesised compounds, TZD-1 and TZD-2 exhibited superior performance across both computational and biological evaluations compared to the nitro-substituted analogue TZD-3. These findings confirm the positive influence of halogen substitution on enhancing cytotoxicity, androgen receptor interaction, and electronic reactivity. The general agreement between computational predictions and experimental validation highlights the utility of the integrated workflow for early-stage lead identification and supports the potential of halogenated thiazolidinone as a promising candidate for further preclinical development.

List of Abbreviations

ADME: Absorption, Distribution, Metabolism, and Excretion
AIPC: Androgen-Independent Prostate Cancer
AR: Androgen Receptor
DFT: Density Functional Theory
FT-IR: Fourier Transform Infrared Spectroscopy
HOMO: Highest Occupied Molecular Orbital
IC₅₀: Half Maximal Inhibitory Concentration
LUMO: Lowest Unoccupied Molecular Orbital
MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay
NMR: Nuclear Magnetic Resonance
SAR: Structure-Activity Relationship
TZD: Thiazolidinone Derivative

Declarations

Ethics Approval and Consent to Participate

This study utilised commercially available human prostate cancer cell lines (PC-3) obtained from established biological repositories. It did not involve human participants, patient samples, or experimental animals. Therefore, formal ethical approval was not required. All procedures were conducted in accordance with standard laboratory biosafety and research ethics guidelines. Consent to participate is not applicable.

Consent for publication

The authors gave consent for the publication of the work under the Creative Commons Attribution Non-Commercial 4.0 license.

Data Availability

All data generated or analysed during this study are included in this published article. Additional datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

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Author Contributions

AZK contributed to conceptualisation, methodology, supervision, and writing – original draft preparation. SM contributed to the investigation and experimental work. Luma Sabri contributed to formal analysis and computational studies. AHS contributed to validation, manuscript review, and editing. All authors read and approved the final manuscript.

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