

# Synthesis, Characterization Anti-Prostate Cancer Activity and Molecular Docking of New Succinimide Derivatives

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## Abstract

Prostate cancer is a leading cause of mortality among men worldwide and is a disease characterized by genetic and epidemiological heterogeneity. Androgen receptors play a pivotal role in the pathogenesis and progression of prostate cancer to its aggressive form. Studies indicate that combining chemotherapy with androgen receptor-targeted therapy improves patient survival, prompting organic chemists to develop novel, effective agents against prostate cancer. In this study, a series of succinimide derivatives were synthesized and characterized using mass spectrometry, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and FT-IR. Their biological activity against castration-resistant prostate cancer (PC-3 cells) was evaluated using the MTT assay. Based on IC<sub>50</sub> values, several synthesized compounds (C<sub>2</sub>, C<sub>5</sub>, F<sub>4</sub>, R<sub>2</sub>) demonstrated anti-prostate cancer activity; notably, compounds C<sub>5</sub>, C<sub>2</sub>, and F<sub>4</sub> exhibited the highest cytotoxicity (IC<sub>50</sub> values of 39, 46, and 49 µg/mL, respectively). Additionally, molecular docking simulations were performed using the MOE software (2024 version) to investigate the binding affinity and mode of interaction within the active site of the AKT1 protein. However, this class of compounds requires further study to optimize various properties and ultimately develop effective treatments for androgen-independent prostate cancer.

**Keywords:** Castration-resistant prostate cancer, succinimide derivatives, AKT1 protein, molecular docking.

## Introduction

Prostate cancer (PCa) is one of the most common malignancies among men worldwide, particularly those over the age of 65, and is a leading cause of cancer-related mortality (1). Globally, the number of cases stands at 1,467,854 representing 29.4% of all cancers with 397,430 deaths occurring annually (2). A distinctive feature of prostate cancer is that not all patients require active treatment; some cases can be managed through active surveillance. Nevertheless, the field has witnessed significant advancements in both diagnosis and treatment, including Positron Emission Tomography (PET), multiparametric MRI, hypofractionated image-guided radiotherapy, stereotactic imaging, and robotic surgery.(3) Regarding patients currently requiring treatment, therapies exist that can cure localized prostate cancer such as radiotherapy and prostatectomy whereas castration-resistant prostate cancer (CRPC) is considered incurable and fatal. CRPC can develop even after androgen deprivation therapy (via chemical or surgical castration), signaling disease progression into a form resistant to hormonal therapy (4). It becomes increasingly aggressive, exhibiting a strong tendency toward metastasis and patient relapse. Historically, the castration-resistant type has been characterized by limited targeted treatment options; the emergence of treatment resistance poses a major challenge, necessitating the deciphering of the complex underlying mechanisms and the identification of the associated genomic landscape to develop novel therapeutic strategies capable of overcoming this resistance (5). The protein kinase Akt1 is a key regulator of cell survival, metabolism, growth, and proliferation, functioning through phosphorylation at Serine 473 and Threonine 308. It also regulates the cell cycle by interacting with cyclins and CDK inhibitors thereby facilitating the G1 to S phase transition which leads to uncontrolled division in cancer cells; furthermore, Akt1 promotes cancer metastasis by inducing the epithelial-mesenchymal transition. However, the AKT1 protein prevents DNA damage-induced apoptosis, thereby conferring resistance to radiotherapy and chemotherapy; consequently, various biological processes such as proliferation, survival, metastasis, and treatment resistance driven by the constitutive activation of AKT1 make it an effective target for prostate cancer therapies (6). Heterocyclic compounds particularly those containing nitrogen serve as the core of these treatments and are integral to the synthesis of numerous pharmaceutical products. Consequently, organic chemists have dedicated significant effort to discovering novel, efficient synthetic methods for these heterocyclic compounds (7,

8). In this context, rational drug design and strategies targeting specific molecular entities have recently garnered considerable attention in the development of potential new cancer therapies. Furthermore, new compounds whose interactions were pre-screened using advanced algorithms are opening up fresh avenues for successful drug discovery, building upon currently available and safety-proven medications (9). Succinimides five-membered heterocyclic compounds containing nitrogen and two carbonyl groups have diverse pharmacological applications, making them important structural motifs in medicinal chemistry. They are characterized by structural flexibility and possess favorable physicochemical properties. In recent decades, compounds featuring the succinimide core have garnered significant interest due to their broad therapeutic potential (10). As derivatives of monocyclic imides, they represent a compelling class of compounds with extensive biological activity (11) and are being explored for various medical applications, including as lipid-lowering agents and for their protective effects on the heart and liver against drug-induced toxicity (12). ...antidepressants (13), antiepileptics, antipsychotics, treatments for smallpox and cancer (14), inhibitors of cholinesterase, alpha-glucosidase, and alpha-amylase, antioxidants (15, 16, 17), antibacterials (18, 19), and antitumor agents (20, 21). However, the F876L mutation has recently emerged, conferring resistance in prostate cancer patients to prescribed drugs such as enzalutamide and apalutamide a development that poses a challenge to modern medicine (22, 23). These challenges prompt researchers to synthesize and design a series of novel succinimide derivatives. These compounds are subsequently characterized using spectroscopic techniques specifically FT-IR,  $^1\text{H}$ -NMR, and  $^{13}\text{C}$ -NMR and their physicochemical properties are investigated. Through this research paper, we aim to evaluate the biological activity of these compounds against the CP-3 cancer cell line to determine their potential to inhibit the growth of malignant cells and to compare their efficacy with currently used standard drugs.

## Materials and Methods

Thin-layer chromatography (TLC) was used to monitor the reaction progress, utilizing UV light and iodine for visualization. Melting points of the resulting compounds were determined using a Gallenkamp apparatus. The compounds were characterized via Fourier-Transform Infrared (FT-IR) spectroscopy using a Bruker instrument (400–4000  $\text{cm}^{-1}$  range) with KBr pellets at room temperature. Nuclear Magnetic Resonance (NMR) analysis was performed using a Bruker instrument operating at 500 MHz ( $^1\text{H}$ -NMR) and 125 MHz ( $^{13}\text{C}$ -NMR) with deuterated DMSO- $d_6$  as the solvent; chemical shifts were measured in ppm. Mass spectral analysis was conducted using an Agilent Technologies 5975C spectrometer via the electron ionization (EI) method at 70 eV.

### General procedure for the synthesis of compounds (C<sub>2</sub>–Z<sub>4</sub>):

The synthesis method described in the literature was employed. A mixture of 0.001 mol of the hydrazide derivative and 0.001 mol of the substituted maleimide in 10 mL of dry ethanol was stirred continuously using a magnetic stirrer at a temperature of 70–80°C. The reaction proceeded for 16 hours, with progress monitored by thin-layer chromatography (TLC). A white precipitate insoluble in ethanol was obtained and washed several times with ethanol to yield pure crystals (24, 25).

#### N'-(1-(3-chloro-4-methylphenyl)-2,5-dioxopyrrolidin-3-yl)-2-(2,4,6-trichlorophenoxy)acetohydrazide(C<sub>2</sub>)

Mixing (0.001 mol) of 2-(2,4,6-trichlorophenoxy)acetohydrazide with (0.001 mol) of 1-(3-chloro-4-methylphenyl)-1H-pyrrole-2,5-dione in 10 mL of absolute ethanol yielded a white solid (70%) with a melting point of 179–181°C. FT-IR (KBr,  $\text{cm}^{-1}$ ): 3412 (NH amide), 3342 (NH), 3252 (C-H Ar), 1715 (C=O), 1497, 1454 (C=C Ar), 1380 (C-N), 1169 (C-O).  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  9.84 (d,  $J$  = 5.3 Hz, 1H,  $\text{NH}_a$ ), 7.73 (s, 2H,  $\text{H}_{Ar}$ ), 7.48 (d,  $J$  = 5.1 Hz, 1H,  $\text{H}_{Ar}$ ), 7.37 (s, 1H,  $\text{H}_{Ar}$ ), 7.18 (d,  $J$  = 9.3 Hz, 1H,  $\text{H}_{Ar}$ ), 5.93 (s, 1H, NH), 4.49 (s, 2H,  $\text{H}_c$ ), 4.19 (d,  $J$  = 4.6 Hz, 1H,  $\text{H}_d$ ), 3.00 (d,  $J$  = 18.1 Hz, 1H,  $\text{H}_e$ ), 2.78 (d,  $J$  = 18.0 Hz, 1H,  $\text{H}_f$ ), 2.38 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (101 MHz, DMSO)  $\delta$  175.70 ( $\text{C}_1$ ), 174.99 ( $\text{C}_2$ ), 166.22 ( $\text{C}_3$ ), [149.86, 136.24, 133.42, 131.76, 131.66, 129.84, 129.66, 129.43, 127.55, 126.14] (C-Ar), 71.22 (O- $\text{CH}_2$ ), 58.12 ( $\text{C}_4$ ), 34.65 ( $\text{C}_5$ ), 19.76 ( $\text{CH}_3$ ) MS(EI)  $m/z$ : 491 [ $\text{M}$ ] $^+$ .

#### acetohydrazide(C<sub>5</sub>) N'-(2,5-dioxo-1-(p-tolyl)pyrrolidin-3-yl)-2-(2,4,6-trichlorophenoxy)

Mixing (0.001 mol) of 2-(2,4,6-trichlorophenoxy)acetohydrazide with (0.001 mol) of 1-(p-tolyl)-1H-pyrrole-2,5-dione in 10 mL of absolute ethanol yielded a white solid (71%) with a melting point of 192–193°C. FT-IR (KBr,  $\text{cm}^{-1}$ ): 3411 (NH amide), 3292 (NH), 3065 (C-H Ar), 1711 (C=O), 1507, 1449 (C=C Ar), 1377 (C-N), 1176 (C-O).  $^1\text{H}$  NMR (400 MHz, DMSO)  $\delta$  9.84 (d,  $J$  = 6.1 Hz, 1H,  $\text{NH}_a$ ), 7.73 (s, 2H,  $\text{H}_{Ar}$ ), 7.29 (d,  $J$  = 8.4 Hz, 2H,  $\text{H}_{Ar}$ ), 7.15 (d,  $J$  = 8.3 Hz, 2H,  $\text{H}_{Ar}$ ), 5.89 (t,  $J$  = 5.4 Hz, 1H,  $\text{NH}_b$ ), 4.50 (s, 2H,  $\text{H}_c$ ), 4.20 (p,  $J$  = 4.3 Hz, 1H,  $\text{H}_d$ ), 3.01 (dd,  $J$  = 18.0, 8.6 Hz, 1H,  $\text{H}_e$ ), 2.78 (dd,  $J$  = 17.9, 4.2 Hz, 1H,  $\text{H}_f$ ), 2.35 (s, 3H,  $\text{CH}_3$ ).  $^{13}\text{C}$  NMR (101 MHz, DMSO)  $\delta$  176.01 ( $\text{C}_1$ ), 175.27 ( $\text{C}_2$ ), 166.24 ( $\text{C}_3$ ), [149.87, 138.26,

130.16, 129.84, 129.76, 129.66, 129.42, 127.18](C-Ar), 71.24(O-CH<sub>2</sub>), 58.06(C<sub>4</sub>), 34.66(C<sub>5</sub>), 21.209(CH<sub>3</sub>) MS(EI) m/z: 457 [M]<sup>+</sup>.

#### **2-(2,5-dimethylphenoxy)-N'-(2,5-dioxo-1-(p-tolyl)pyrrolidin-3-yl)acetohydrazide(F<sub>4</sub>)**

Mixing (0.001 mol) of 2-(2,5-dimethylphenoxy)acetohydrazide with (0.001 mol) of 1-(p-tolyl)-1H-pyrrole-2,5-dione in 10 mL of absolute ethanol yielded a white solid (71%) with a melting point of 129–131°C. FT-IR (KBr, cm<sup>-1</sup>): 3288 (NH amide), 3215 (NH), 2916 (C-H Ar), 1708 (C=O), 1517, 1451 (C=C Ar), 1382 (C-N), 1170 (C-O). <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.64 (d, J = 5.9 Hz, 1H, NH<sub>a</sub>), 7.29 (d, J = 8.3 Hz, 2H, H<sub>Ar</sub>), 7.12 (d, J = 8.4 Hz, 2H, H<sub>Ar</sub>), 7.02 (d, J = 7.3 Hz, 1H, H<sub>Ar</sub>), 6.68 (d, J = 7.5 Hz, 2H, H<sub>Ar</sub>), 5.85 (t, J = 5.3 Hz, 1H, NH<sub>b</sub>), 4.53 (s, 2H, H<sub>c</sub>), 4.17 (p, J = 4.3 Hz, 1H, H<sub>d</sub>), 2.96 (dd, J = 17.9, 8.5 Hz, 1H, H<sub>e</sub>), 2.70 (dd, J = 17.9, 4.2 Hz, 1H, H<sub>f</sub>), 2.35 (s, 3H, CH<sub>3</sub>), 2.22 (s, 3H, CH<sub>3</sub>), 2.15 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, DMSO) δ 176.06(C<sub>2</sub>), 175.23(C<sub>3</sub>), [167.89, 156.24, 138.25, 136.59, 130.76, 130.15, 129.76, 127.18, 123.39, 121.89, 112.78](C-Ar), 66.83(O-CH<sub>2</sub>), 58.01(C<sub>4</sub>), 34.60(C<sub>5</sub>), 21.44(CH<sub>3</sub>), 21.20(CH<sub>3</sub>), 16.18(CH<sub>3</sub>) MS(EI) m/z: 381 [M]<sup>+</sup>.

#### **2-(3,4-dimethylphenoxy)-N'-(2,5-dioxo-1-(p-tolyl)pyrrolidin-3-yl)acetohydrazide(R<sub>2</sub>)**

Mixing (0.001 mol) of 2-(3,4-dimethylphenoxy)acetohydrazide with (0.001 mol) of 1-(p-tolyl)-1H-pyrrole-2,5-dione in 10 mL of absolute ethanol yielded a white solid (68%) with a melting point of 198–199°C. FT-IR (KBr, cm<sup>-1</sup>): 3352 (NH amide), 3211 (NH), 2910 (C-H Ar), 1692(C=O), 1512, 1440 (C=C Ar), 1393 (C-N), 1172 (C-O). <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.74 (d, J = 6.0 Hz, 1H, NH<sub>a</sub>), 7.28 (d, J = 8.4 Hz, 2H, H<sub>Ar</sub>), 7.12 (d, J = 8.4 Hz, 2H, H<sub>Ar</sub>), 7.02 (d, J = 8.4 Hz, 1H, H<sub>Ar</sub>), 6.76 (s, 1H, H<sub>Ar</sub>), 6.66 (d, J = 8.3 Hz, 1H, H<sub>Ar</sub>), 5.86 – 5.79 (m, 1H, NH<sub>b</sub>), 4.48 (s, 2H, H<sub>c</sub>), 4.20 – 4.14 (m, 1H, H<sub>d</sub>), 2.96 (dd, J = 18.0, 8.5 Hz, 1H, H<sub>e</sub>), 2.70 (dd, J = 17.9, 4.1 Hz, 1H, H<sub>f</sub>), 2.35 (s, 3H, CH<sub>3</sub>), 2.17 (s, 3H, CH<sub>3</sub>), 2.13 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, DMSO) δ 175.95(C<sub>1</sub>), 175.01(C<sub>2</sub>), 167.84(C<sub>3</sub>), [156.33, 137.76, 130.31, 129.75, 129.17, 127.18, 116.47, 112.07](C-Ar), 66.71(O-CH<sub>2</sub>), 58.03(C<sub>4</sub>), 21.20(C<sub>5</sub>), 20.08(CH<sub>3</sub>), 18.89(CH<sub>3</sub>) MS(EI) m/z: 381 [M]<sup>+</sup>.

#### **N'-(1-(2-methyl-3-nitrophenyl)-2,5-dioxopyrrolidin-3-yl)-2-phenoxyacetohydrazide(Z<sub>4</sub>)**

Mixing (0.001 mol) of 2-phenoxyacetohydrazide with (0.001 mol) of 1-(2-methyl-3-nitrophenyl)-1H-pyrrole-2,5-dione in 10 mL of absolute ethanol yielded a white solid (76%) with a melting point of 180–182°C. FT-IR (KBr, cm<sup>-1</sup>): 3372 (NH amide), 3228(NH), 3086 (C-H Ar), 1700(C=O), 1519, 1476 (C=C Ar), 1359 (C-N), 1163 (C-O). <sup>1</sup>H NMR (400 MHz, DMSO) δ 9.86 (t, J = 5.7 Hz, 1H, NH<sub>a</sub>), 8.03 (d, J = 7.9 Hz, 1H, H<sub>Ar</sub>), 7.64 – 7.52 (m, 2H, H<sub>Ar</sub>), 7.29 (t, J = 8.0 Hz, 2H, H<sub>Ar</sub>), 6.99 – 6.94 (m, 3H, H<sub>Ar</sub>), 5.98 (q, J = 5.0, 4.4 Hz, 1H, NH<sub>b</sub>), 4.54 (s, 2H, H<sub>c</sub>), 4.27 (p, J = 4.1 Hz, 1H, H<sub>d</sub>), 3.04 (dd, J = 18.0, 8.5 Hz, 1H, H<sub>e</sub>), 2.87 (dd, J = 18.1, 4.0 Hz, 1H, H<sub>f</sub>), 2.17 (s, 3H, CH<sub>3</sub>). <sup>13</sup>C NMR (101 MHz, DMSO) δ 175.70(C<sub>1</sub>), 174.97(C<sub>2</sub>), 167.79(C<sub>3</sub>), [158.21, 151.11, 134.55, 133.87, 133.73, 130.96, 129.95, 129.93, 128.02, 127.89, 127.76, 125.30, 124.69, 121.67, 115.15](C-Ar), 66.56(O-CH<sub>2</sub>), 58.44(C<sub>4</sub>), 34.81(C<sub>5</sub>), 13.95(CH<sub>3</sub>) MS(EI) m/z: 398 [M]<sup>+</sup>.

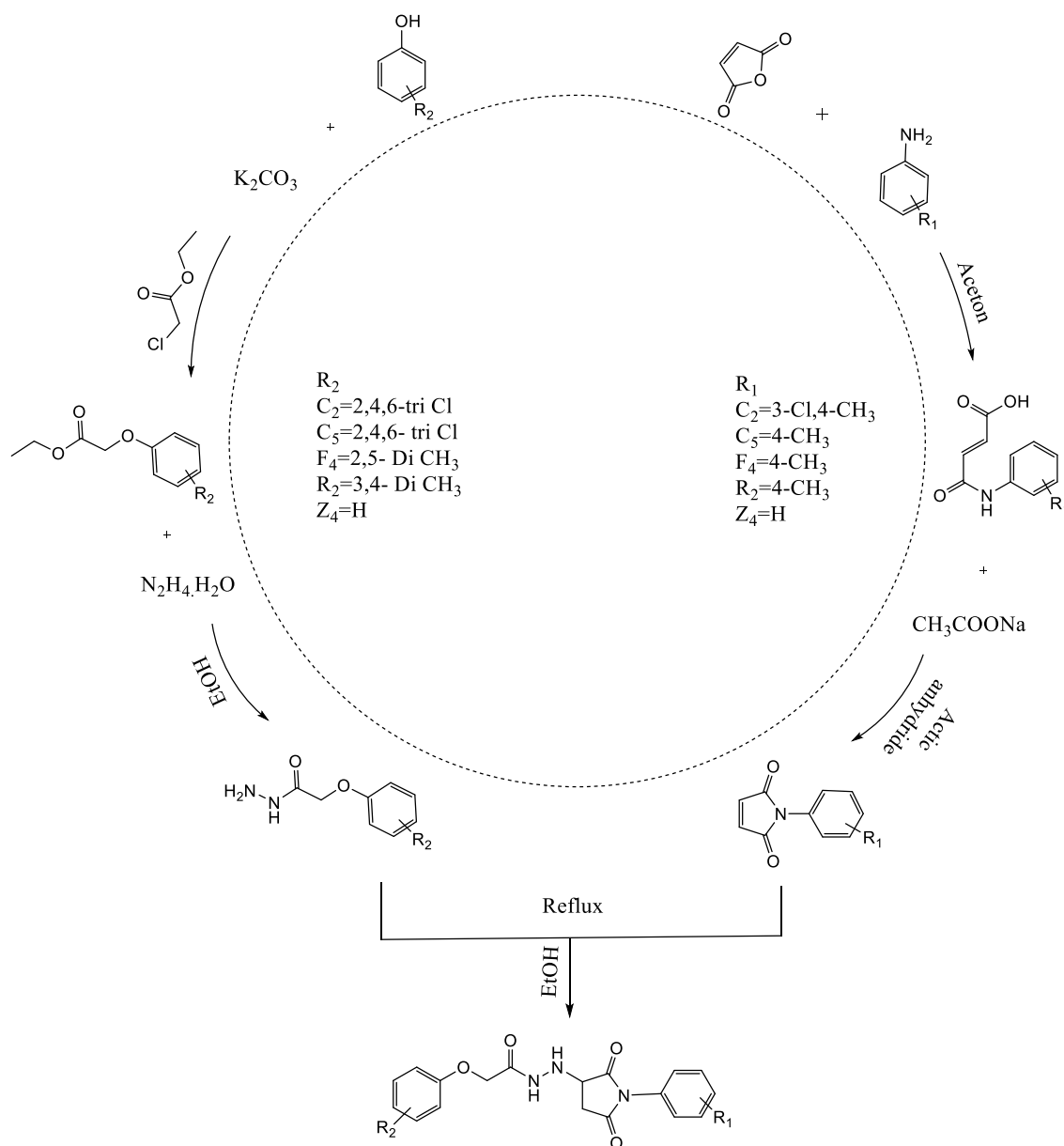
#### **Molecular Docking Study**

Molecular docking was performed on the series of synthesized compounds (C<sub>2</sub>–Z<sub>4</sub>) using the MOE2024 software to elucidate the binding mechanism and stability of the compounds within the binding pocket of the AKT1 protein selected as a drug target (PDB ID: 3O96) which was retrieved from the Protein Data Bank in PDB format. Active amino acid residues within the protein pocket were identified based on the reference co-crystallized ligand found in the AKT1 structure. Subsequently, the crystal structure of the target protein (AKT1) was processed by removing water molecules, adding hydrogen atoms and assigning charges, and repairing broken amino acid chains (28). The co-crystallized ligand was then re-docked into the protein to serve as a reference for evaluating the molecular docking of the synthesized compounds; this evaluation involved comparing binding energy and RMSD values against the crystal structure, while the synthesized compounds were observed to fit into the protein's binding pocket through the formation of hydrogen bonds and hydrophobic interactions (29).

#### **Results and Discussion**

The synthesis of succinimide derivatives was carried out in three steps. In the first step, the maleimide derivative is obtained by reacting maleic anhydride with a substituted amine using acetone as a solvent to yield the corresponding maleamic acid. This intermediate is then reacted with acetic anhydride (acting as both solvent and reactant) in the presence of anhydrous sodium acetate as a catalyst to produce the desired N-substituted maleimide via ring closure. In the second step, hydrazide derivatives are prepared by reacting phenol or one of its derivatives with ethyl chloroacetate in the presence of potassium carbonate to obtain the ester derivative; the resulting ester is then reacted with hydrazine hydrate to yield the hydrazide derivative. In the third step, an N-substituted maleimide is reacted with the hydrazide derivatives in the presence of absolute ethanol to obtain succinimide derivatives (C<sub>2</sub>–Z<sub>4</sub>) via a Michael addition reaction (Figure 1). The resulting compounds were characterized using FT-IR, <sup>1</sup>H-NMR, and <sup>13</sup>C-NMR spectroscopy. Their chemical structures were confirmed by identifying functional groups via IR spectra:

imide N-H and N-H groups appeared in the 3412–3211  $\text{cm}^{-1}$  range; C=O functional groups (stretching and bending modes) were observed between 1717 and 1550  $\text{cm}^{-1}$ ; C=C absorption bands appeared in the 1519–1440  $\text{cm}^{-1}$  range; C-N groups showed absorption bands at 1393–1359  $\text{cm}^{-1}$ ; and absorption bands corresponding to C-O groups were observed at 1188–1163  $\text{cm}^{-1}$ . The synthesized succinimide derivatives were characterized using  $^1\text{H}$ -NMR. Signals corresponding to protons  $\text{H}_a$  and  $\text{H}_b$  appeared as a triplet and a doublet at 9.84–9.47 ppm and 5.98–5.82 ppm, respectively. Doublet signals corresponding to protons  $\text{H}_d$  and  $\text{H}_e$  were observed at 4.29–4.16 ppm and 3.09–3.01 ppm, respectively, while proton  $\text{H}_f$  exhibited a doublet signal in the 2.83–2.70 ppm range. Proton  $\text{H}_c$  showed singlet and doublet signals between 4.55 and 4.46 ppm, and aromatic ring protons displayed singlet, doublet, and multiplet signals in the 8.04–6.66 ppm range. The  $^{13}\text{C}$ -NMR spectra of compounds ( $\text{C}_2$ - $\text{Z}_4$ ) showed a signal ranging from 156 to 176  $\text{cm}^{-1}$ , which is attributed to the carbonyl groups. The aromatic ring carbons showed signals ranging from 112 to 158  $\text{cm}^{-1}$ , while the aflatoxycarboxylates showed signals ranging from 34.60 to 71.22  $\text{cm}^{-1}$ . The mass spectra of compounds ( $\text{C}_2$ - $\text{Z}_4$ ) showed the following molecular ions ( $\text{M}/\text{Z}$ ): 491 $[\text{M}]^+$ , 457 $[\text{M}]^+$ , 381 $[\text{M}]^+$ , 381 $[\text{M}]^+$ , and 398 $[\text{M}]^+$ .

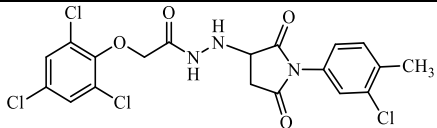
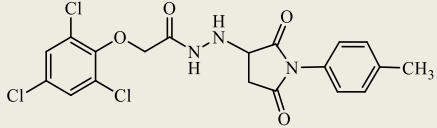
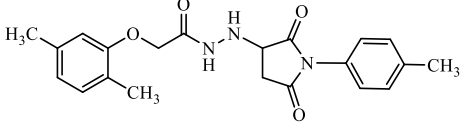
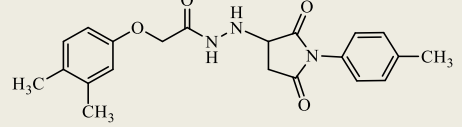
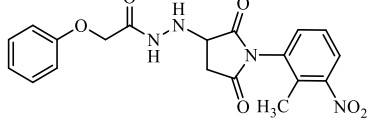


**Scheme 1.** shows the steps for preparing the compounds

### Anti-Prostate Cancer Activity

The cytotoxicity of the synthesized series of compounds (C<sub>2</sub>–Z<sub>4</sub>) was evaluated against the highly metastatic, androgen-independent prostate cancer cell line PC-3. This assessment utilized the MTT assay an effective tool for measuring cell viability in vitro across various culture media—which relies on the ability of living cells to produce formazan; consequently, it serves as a reliable method for determining IC<sub>50</sub> values, as well as assessing cell growth and survival (26). In this study, we tested a new group of succinimide derivatives against PC-3 prostate cancer cells, yielding varying IC<sub>50</sub> values as detailed in Table 5 and Figure 1. Data indicate that compounds C<sub>5</sub>, C<sub>2</sub>, and F<sub>4</sub> exhibited the highest anti-prostate cancer activity among the synthesized compounds. The notable efficacy of compounds C<sub>2</sub> and C<sub>5</sub> is attributed to the presence of electron-withdrawing chlorine groups and electron-donating methyl groups substituted on the aryl ring. Overall, testing of the synthesized compounds on the PC-3 prostate cancer cell line demonstrated greater efficacy than that observed for the same class of compounds tested on the MCF-7 breast cancer cell line in a previous study (Ahmed, F. A., & Mutlaq, D. 2024) (27).

**Table 1.** shows the values of IC<sub>50</sub> for the compounds under study against prostate cancer cells

| Compound symbol | Compound structure                                                                  | PC3 cells IC <sub>50</sub> in µg/mL | Activity |
|-----------------|-------------------------------------------------------------------------------------|-------------------------------------|----------|
| C <sub>2</sub>  |    | 46                                  | Activet  |
| C <sub>5</sub>  |   | 39                                  | Activet  |
| F <sub>4</sub>  |  | 49                                  | Activet  |
| R <sub>2</sub>  |  | 75                                  | Activet  |
| Z <sub>4</sub>  |  | 95                                  | Activet  |

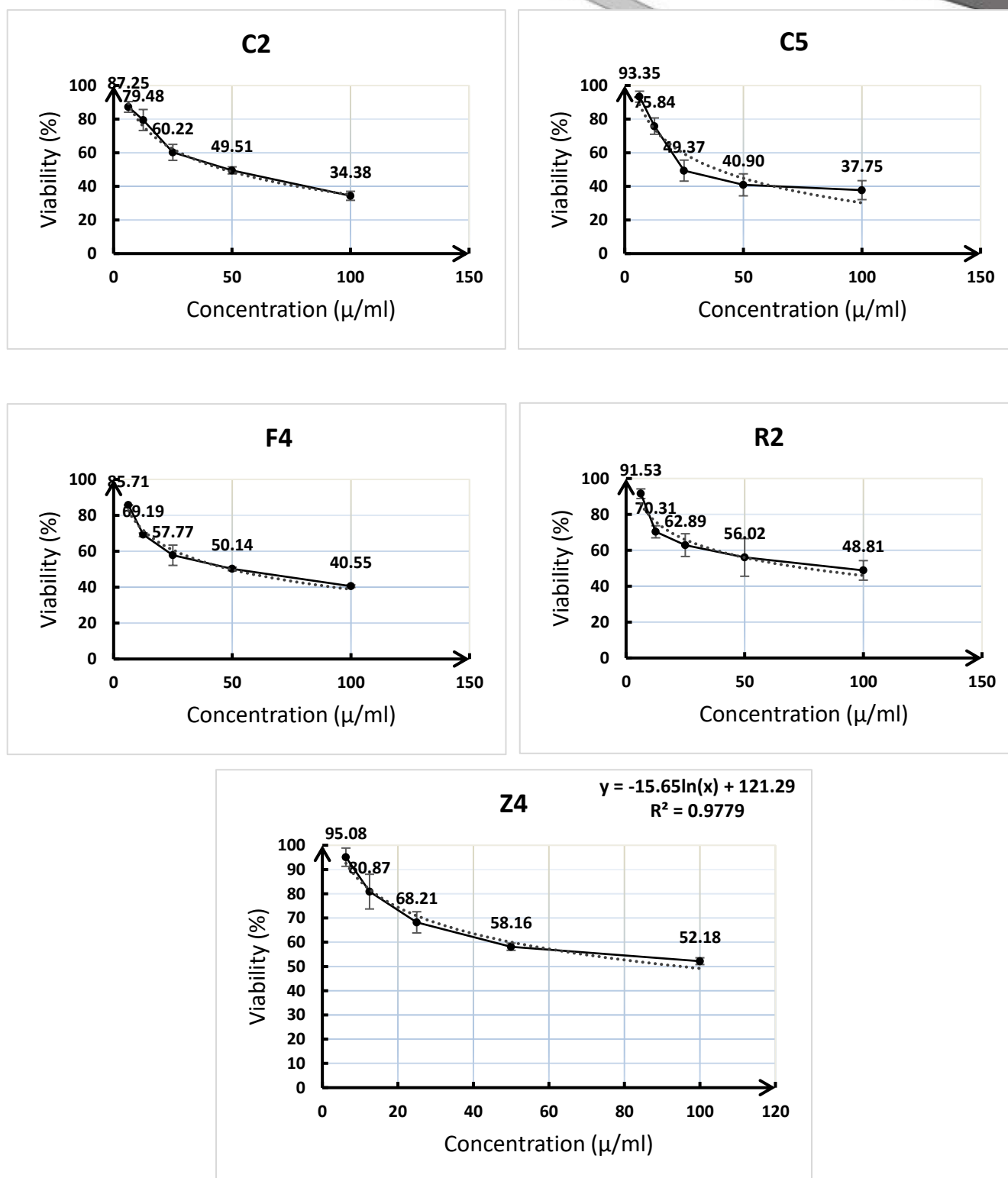


Figure 1. Graph of the response of the prepared compounds against prostate cancer cell lines

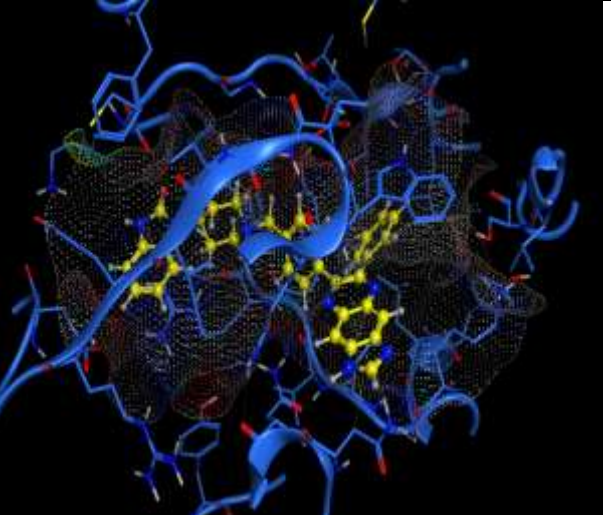
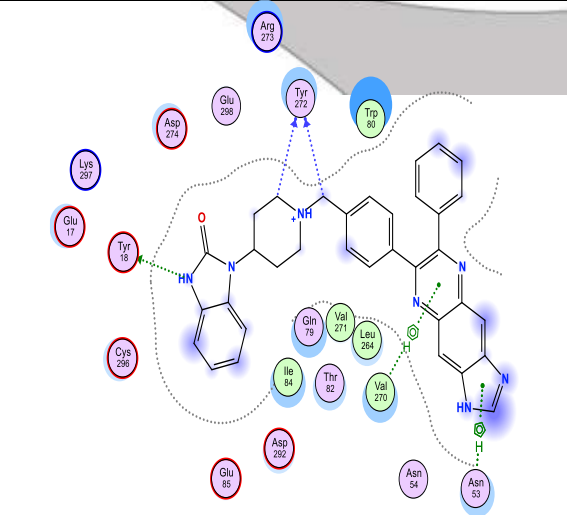
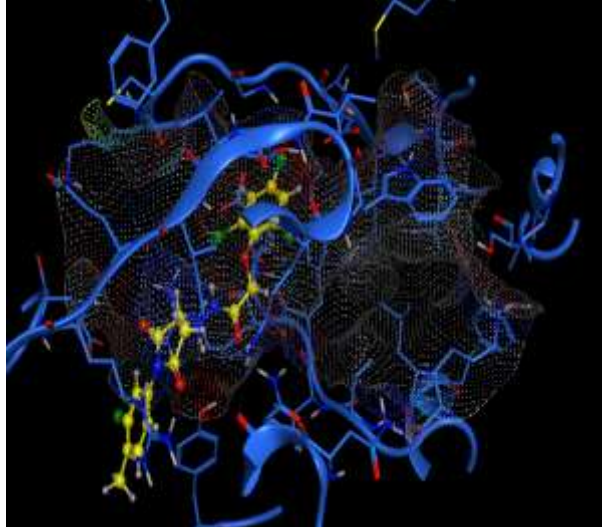
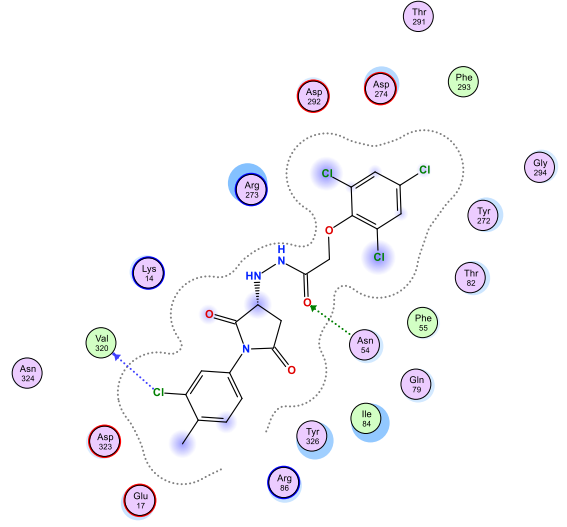
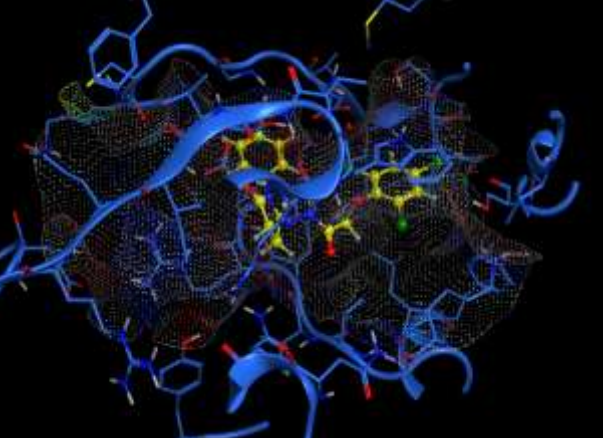
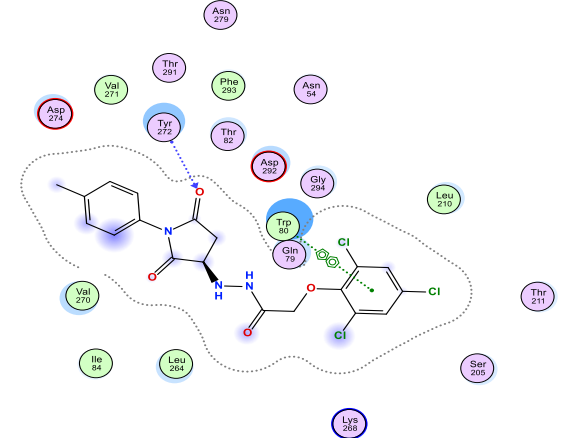
#### Molecular Docking Study

Molecular docking simulations were performed for the synthesized compounds (C<sub>2</sub>–Z<sub>4</sub>) targeting the AKT1 protein (PDB ID: 3O96) using MOE software (version 2024). As a preliminary docking step, the co-crystallized ligand was re-docked into the AKT1 protein.

The active binding pocket was defined based on the co-crystallized ligand within the AKT1 kinase structure. Subsequently, water molecules were removed and hydrogen atoms were added to the AKT1 protein to prepare it for the docking of the synthesized compounds (C2–Z4) into the active site. Binding energies ranged from -7.723 kcal/mol for compound C2 to -7.5236 kcal/mol for compound R2. RMSD values fell within the acceptable limit (2 Å) for some compounds, while being slightly higher for others. These results indicate that the docking of the synthesized compounds into the target AKT1 protein yielded satisfactory performance, as detailed in Table (1) and Figure (1). Compound C2 exhibited the highest binding energy (-7.723 kcal/mol) and an RMSD of 1.812 Å, forming two hydrogen bonds with amino acid residues VAL320 and ASN54. It was followed by compound C5, which showed the second-strongest binding energy (-7.623 kcal/mol); this compound anchored itself within the protein's active pocket via two interactions—a hydrogen bond with TYR272 and a pi-pi stacking interaction with TRP80—while maintaining an acceptable RMSD of 1.723 Å. However, a comparison of binding energy with the cytotoxicity of compounds C2 and C5 (IC50 = 39.46 mg/mL, respectively) in the MTT assay reveals a discrepancy in results, which may be attributed to unfavorable physicochemical properties such as permeability and solubility. Compound Z4 stabilized within the protein pocket by forming four hydrogen bonds with the amino acid residues TYR272, GLY294, TRP80, and LEU210, with an RMSD value of 2.052 Å. Similarly, compounds F4 and R2 exhibited weak binding energy among the synthesized series; specifically, compound R2 showed a binding energy of -7.543 kcal/mol, which aligns with the weak cytotoxicity observed in the MTT assay for this compound. Compound R2 formed a hydrogen bond with the amino acid residue ASN54, and the RMSD value was 2.123 Å. While compound F4 bound to the TRP80 amino acid residue via hydrophobic interactions with a weak binding affinity of -7.543 kcal/mol (and an RMSD value of 1.932 Å), it demonstrated good cytotoxicity (49 mg/mL); this deviation from expectations may be attributed to unfavorable physicochemical properties. Molecular docking results indicate that the compounds in this series require further study and modification to yield promising candidates as effective agents against castration-resistant prostate cancer.

**Table.2: of compound analysis upon binding to the AKT1 protein**

| Compound | Ligand Atom | Protein Atom | Residue | Interaction Type | Distance Å | RMSD Å | Binding Energy |
|----------|-------------|--------------|---------|------------------|------------|--------|----------------|
| Control  | N5          | OH           | TYR 18  | H-donor          | 3.14       | 1.732  | -9.108         |
|          | C18         | O            | TYR 272 | H-donor          | 3.25       |        |                |
|          | C32         | O            | TYR 272 | H-donor          | 3.28       |        |                |
|          | 5-ring      | CB           | ASN53   | $\pi$ - H        | 4.09       |        |                |
|          | 5-ring      | ND2          | ASN53   | $\pi$ - H        | 3.36       |        |                |
|          | 6-ring      | CG1          | VAL270  | $\pi$ - H        | 3.61       |        |                |
| C2       | CL45        | O            | VAL320  | H-donor          | 3.32       | 1.812  | -7.723         |
|          | O19         | ND2          | ASN54   | H-acceptor       | 2.94       |        |                |
| C5       | O29         | CA           | TYR272  | H-acceptor       | 3.27       | 1.723  | -7.623         |
|          | 6-ring      | 5-ring       | TRP80   | $\pi$ - $\pi$    | 3.73       |        |                |
| F4       | 6-ring      | 5-ring       | TRP80   | $\pi$ - $\pi$    | 3.92       | 1.932  | -7.543         |
|          | 6-ring      | 6-ring       | TRP80   | $\pi$ - $\pi$    | 4.11       |        |                |
| R2       | O33         | ND2          | ASN54   | H-acceptor       | 2.87       | 2.123  | -7.5236        |
| Z4       | O31         | CA           | TYR272  | H-acceptor       | 3.29       | 2.052  | -7.637         |
|          | O46         | N            | GLY294  | H-acceptor       | 2.90       |        |                |
|          | C13         | 5-ring       | TRP80   | H- $\pi$         | 3.79       |        |                |
|          | 6-ring      | CD2          | LEU210  | $\pi$ - H        | 4.57       |        |                |

| com                  | Protein Active Site                                                                 | Ligand-Protein Interaction                                                           |
|----------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| Control (co crystal) |    |    |
| C <sub>2</sub>       |   |   |
| C <sub>5</sub>       |  |  |

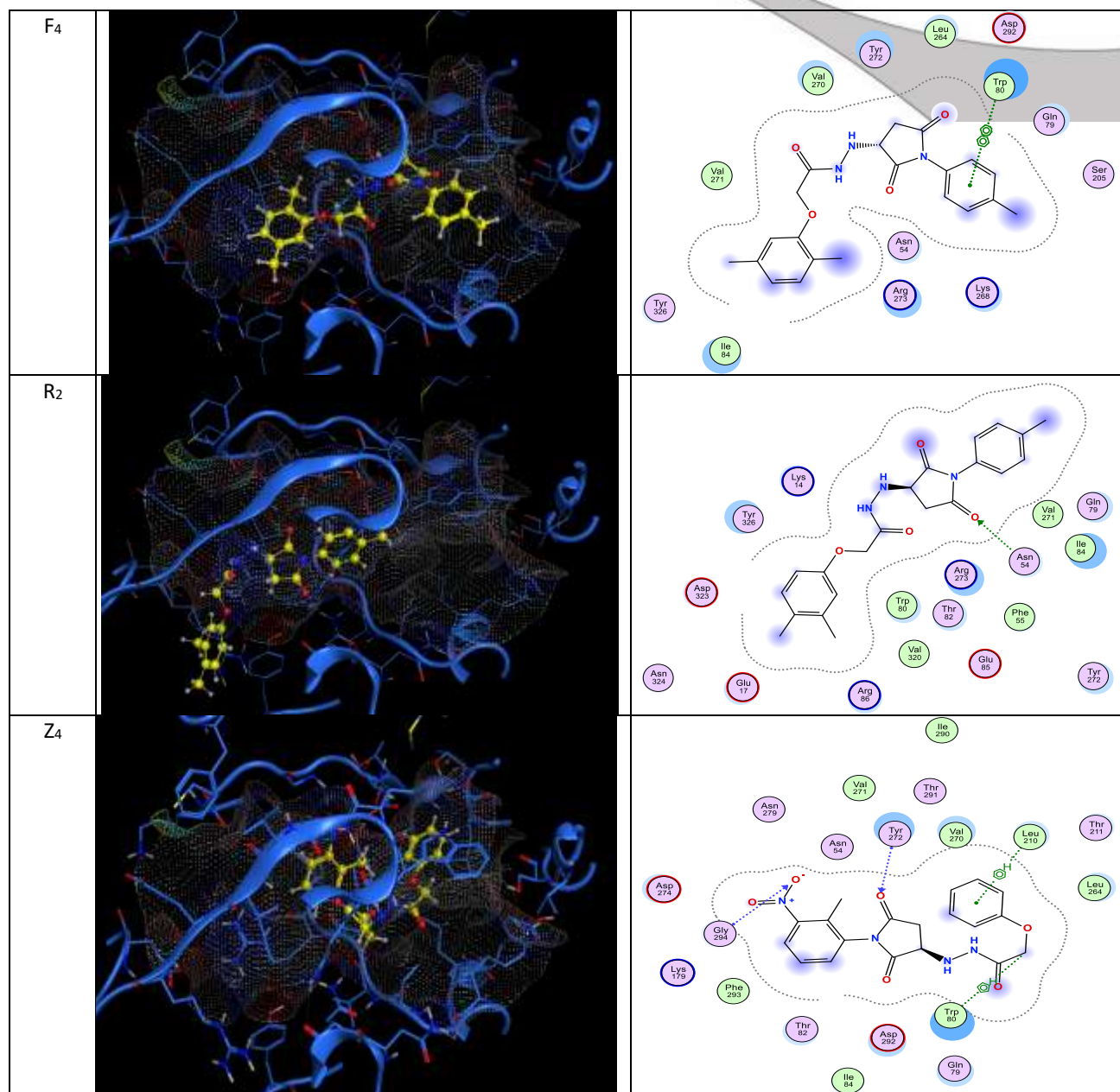


**Fig.2.2D and 3D visualization of the interaction between the compounds and the AKT1 protein**

### Conclusion

Heterocyclic compounds have long served as a source of inspiration in the fields of chemistry and medicine. Here, we report the synthesis of a new series of succinimide derivatives prepared from N-substituted maleimides and various hydrazide derivatives due to the therapeutic potential inherent in their structural scaffold. Consequently, this class of compounds can be evaluated as inhibitors against castration-resistant prostate cancer (PC-3) cells. These compounds were characterized using FT-IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and mass spectrometry. Their biological activity against PC-3 prostate cancer cells was evaluated; based on IC<sub>50</sub> values, varying levels of activity were observed. Compounds C<sub>2</sub>, C<sub>5</sub>, and F<sub>4</sub> demonstrated good efficacy against the disease compared to the other synthesized compounds in the series, whereas compounds R<sub>2</sub> and Z<sub>4</sub> showed weak activity. To understand the mechanism of action, molecular docking simulations were performed for compounds (C<sub>2</sub>–Z<sub>4</sub>) against the AKT1 protein. Although the binding results were modest potentially due to unfavorable physicochemical factors the analysis indicates that these compounds are capable of forming hydrophobic interactions and hydrogen bonds within the active site of the

AKT1 protein. Consequently, the experimental results from the MTT toxicity assay and molecular docking provide insights that support the potential for further study and development of this class of compounds to enhance their efficacy against castration-resistant prostate cancer.

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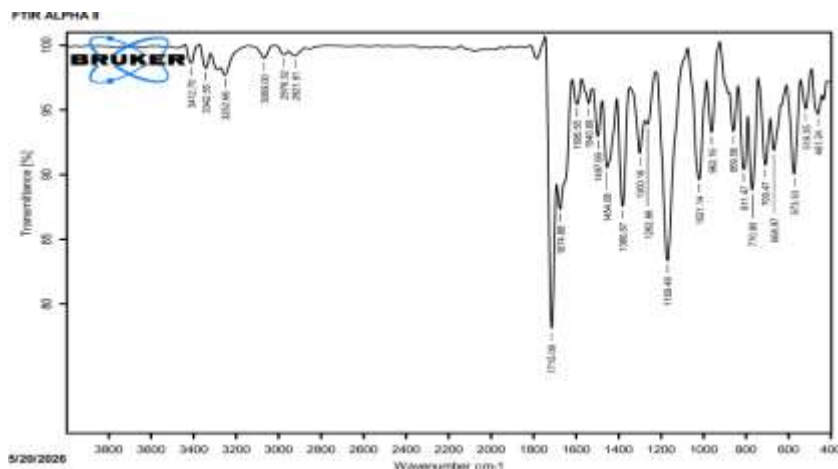


Fig. 5: FT-IR of C<sub>2</sub>

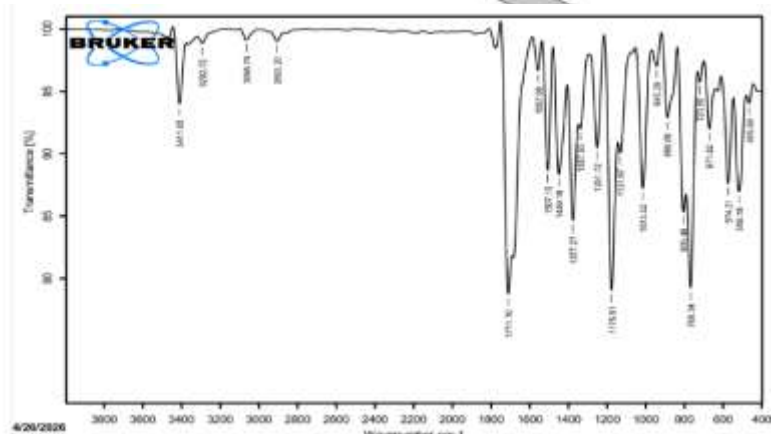


Fig. 5: FT-IR of Cs

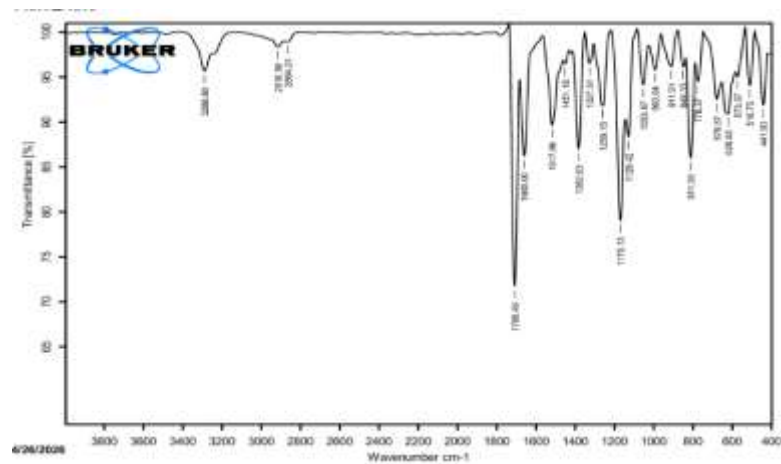
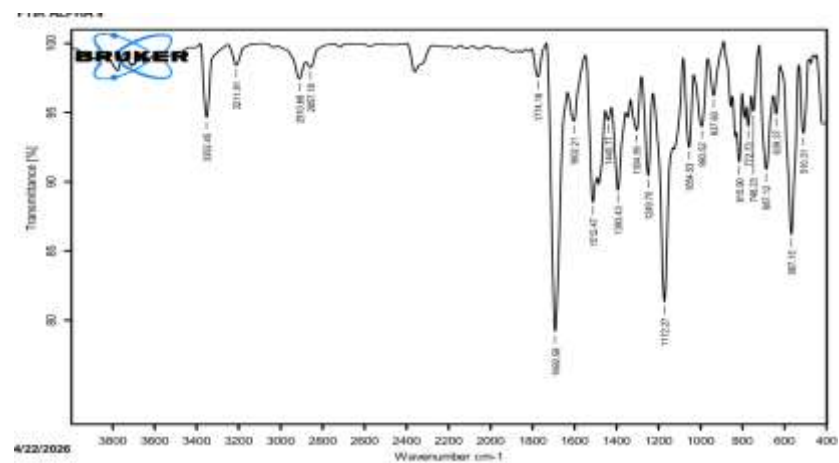


Fig. 5: FT-IR of F4



R2  
Fig. 5: FT-IR of R2

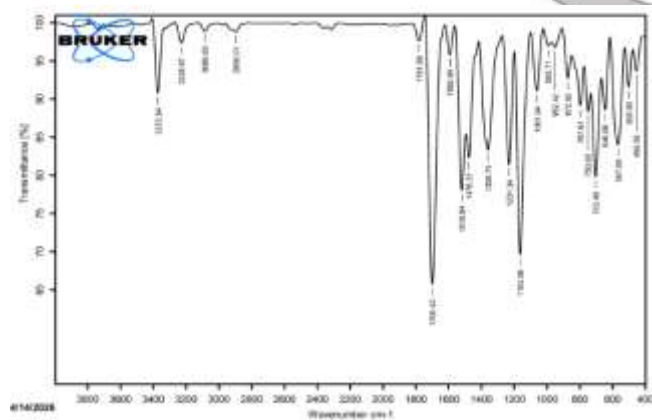


Fig. 5: FT-IR of Z4

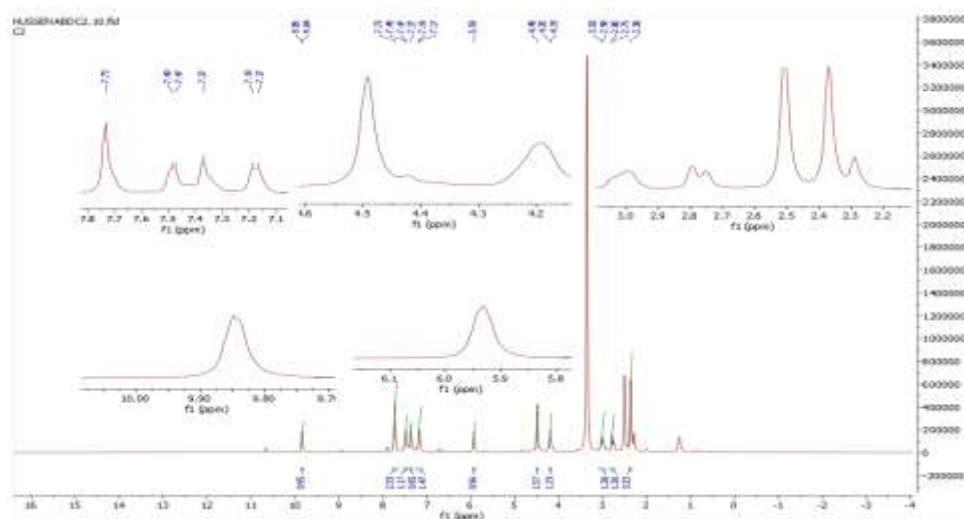


Fig. 9: <sup>1</sup>H NMR of C<sub>2</sub>

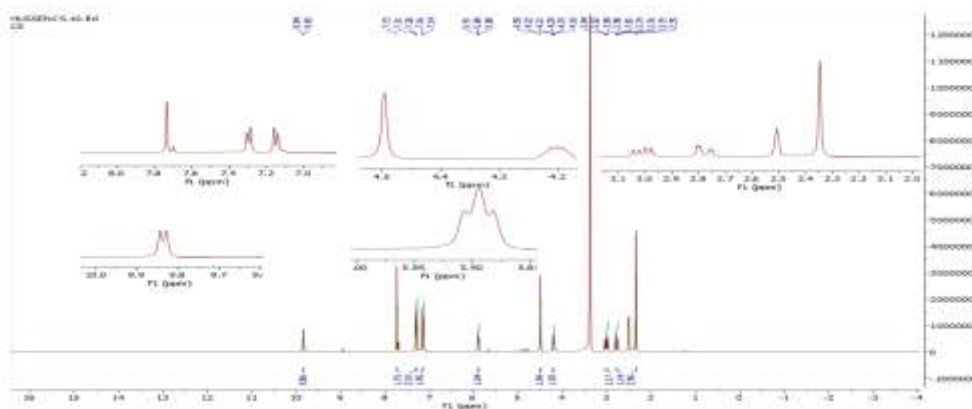


Fig. 9: <sup>1</sup>H NMR of C<sub>5</sub>

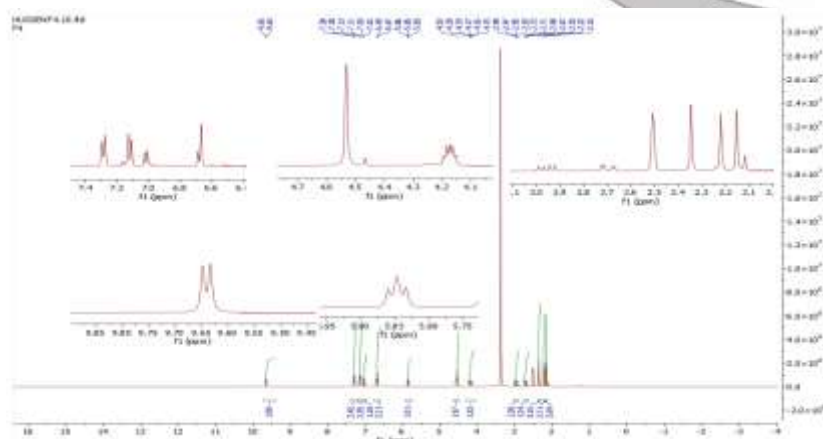


Fig. 9:  $^1\text{H}$ NMR of  $\text{F}_4$

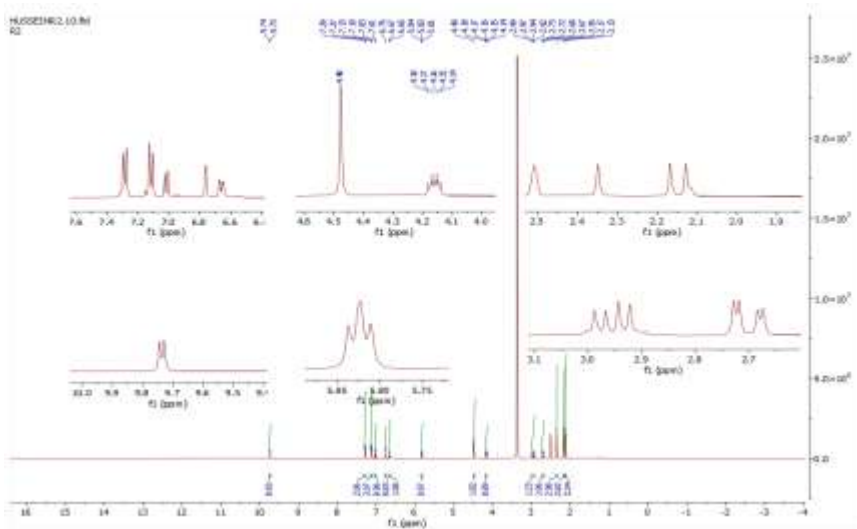


Fig. 9:  $^1\text{H}$ NMR of  $\text{R}_2$

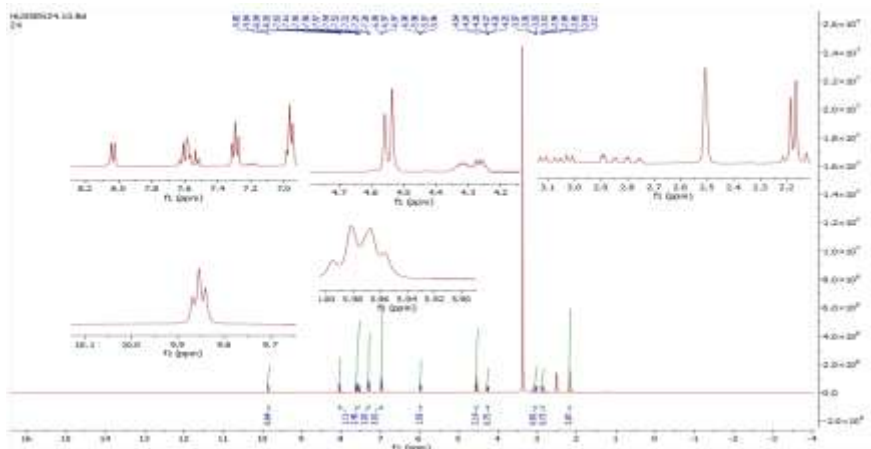


Fig. 9:  $^1\text{H}$ NMR of  $\text{Z}_4$

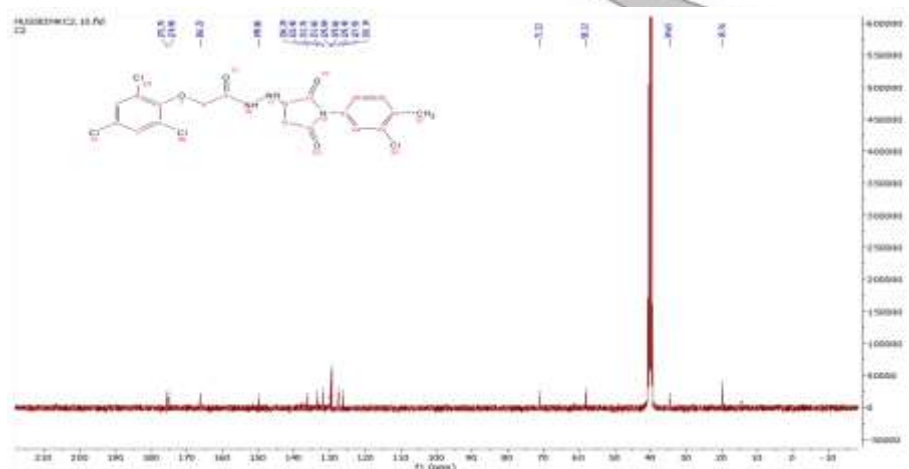


Fig16:  $^{13}\text{C}$ -NMR of  $\text{C}_2$

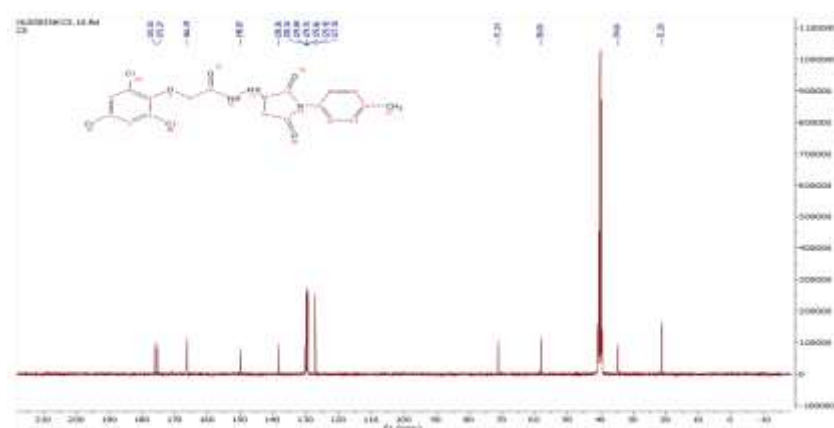


Fig16:  $^{13}\text{C}$ -NMR of  $\text{C}_5$

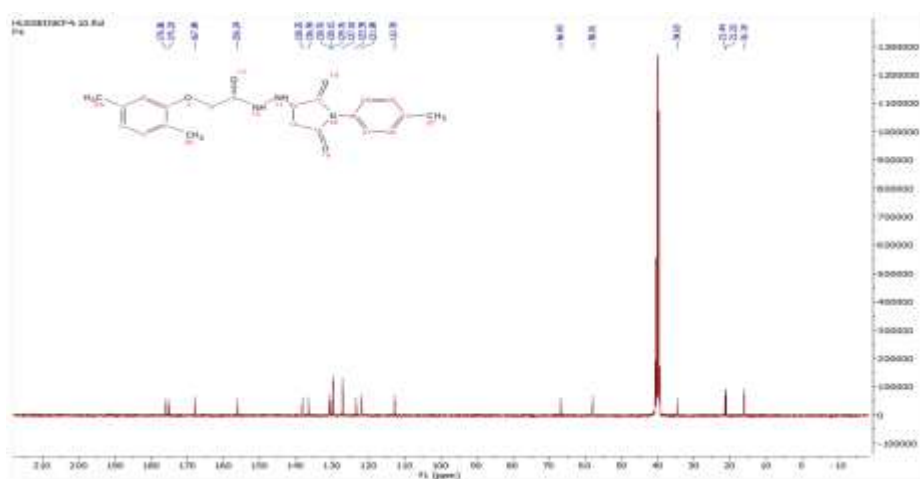


Fig16:  $^{13}\text{C}$ -NMR of  $\text{F}_4$

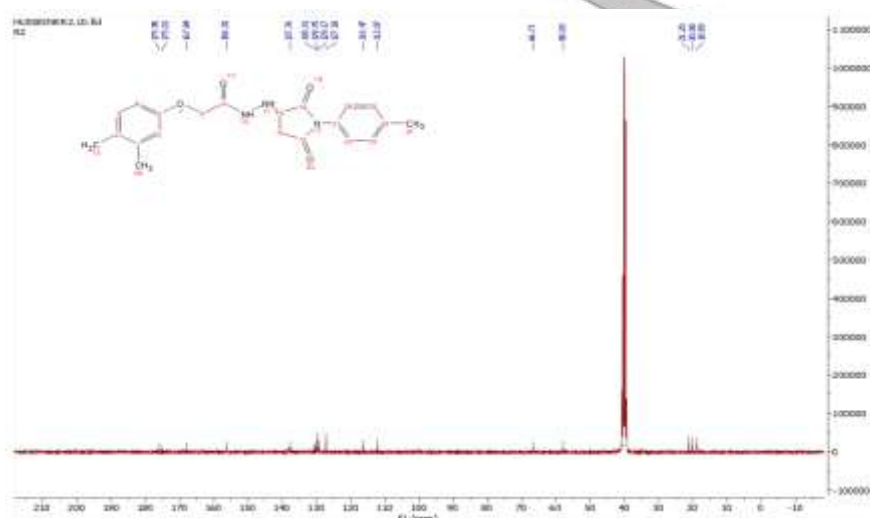


**Fig16: 13C-NMR of R<sub>2</sub>**

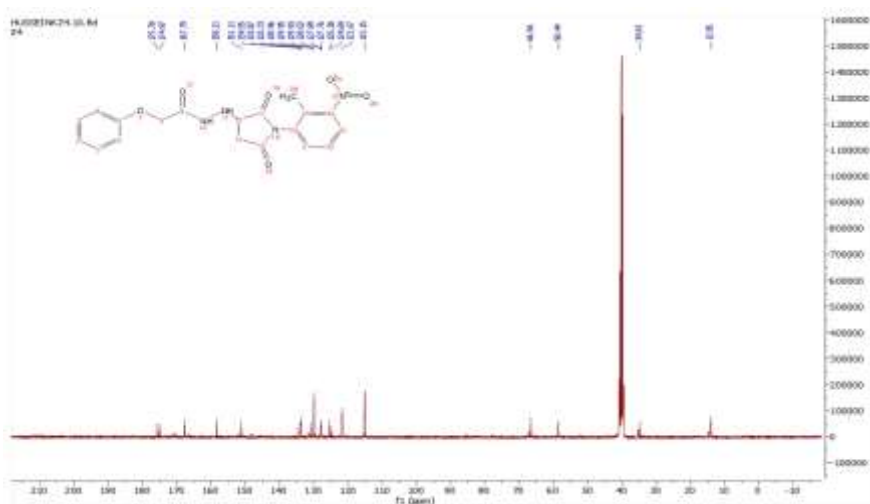
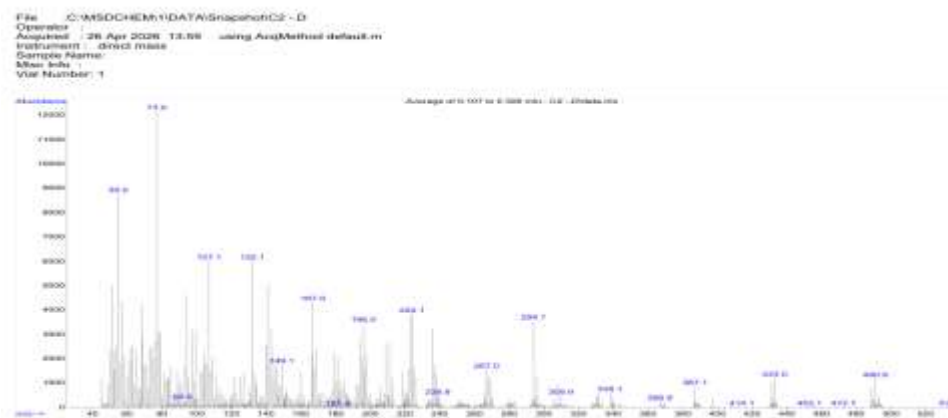


**Fig16: 13C-NMR of Z<sub>4</sub>**



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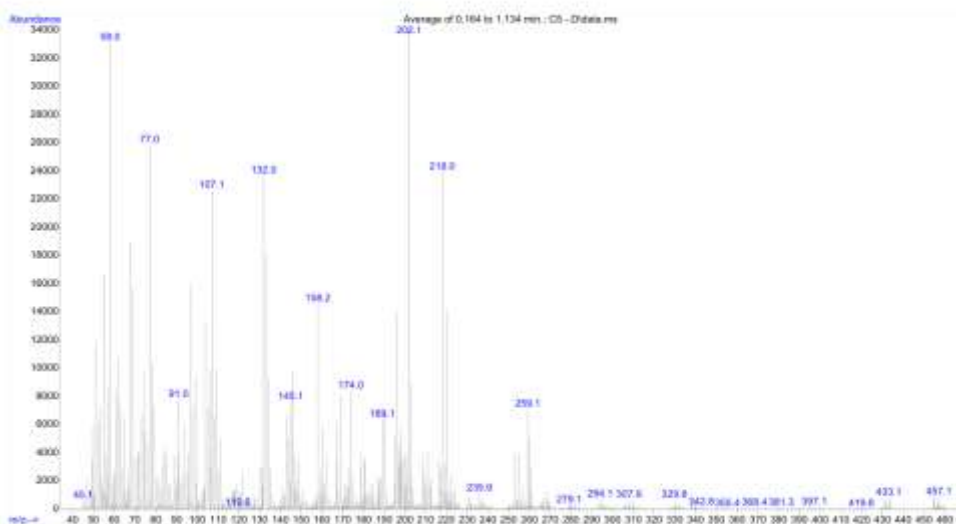


Fig. 19: Mass spectrum of Cs

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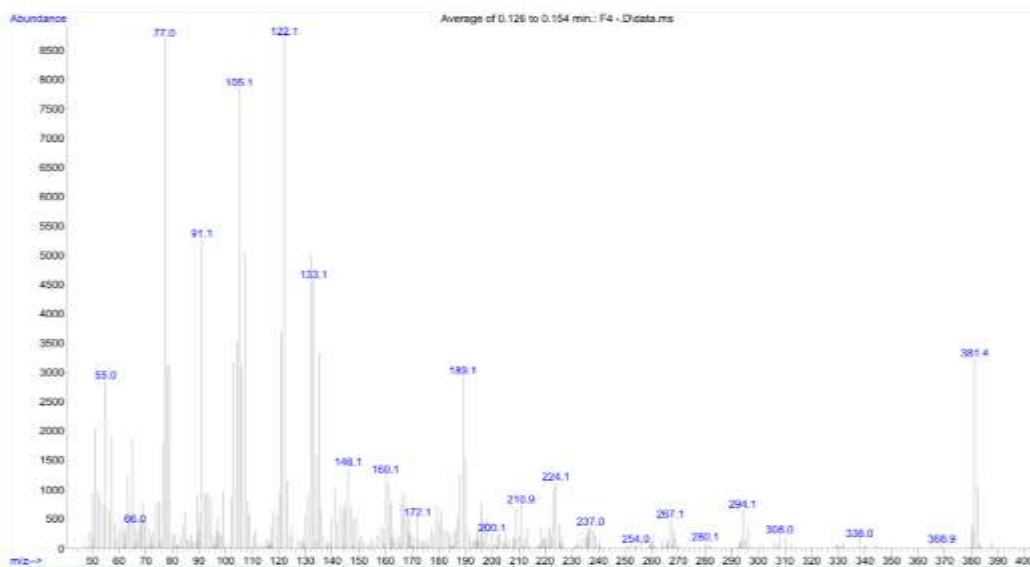


Fig. 19: Mass spectrum of F4

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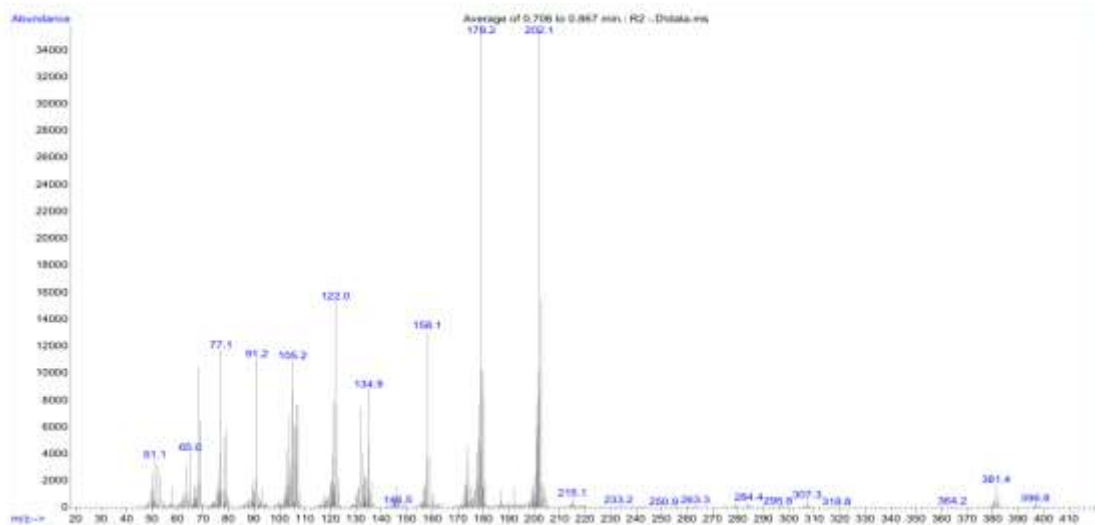


Fig. 19: Mass spectrum of R<sub>2</sub>

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Fig. 19: Mass spectrum of Z<sub>4</sub>