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Bioassessment of Acetylide-Imine in *Acanthamoeba* sp. Through In Vitro and In Silico Investigations for Its Environmental Contamination Implications from Electronic Waste

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FYD4A, an organically synthesised molecule featuring an acetylide-imine motif in its molecular backbone, has potential as an electronic component. The compound's safety was evaluated on the eukaryotic microorganism, *Acanthamoeba* sp., which is highly sensitive to environmental changes. Dose-response test using the MTT assay, and morphological observations via light and fluorescence microscopy, assess the cytoplasmic activity. Apoptosis confirmation was through DNA fragmentation analysis. Finally, in silico analysis examines the interaction between **FYD4A** and *Acanthamoeba* profilin II protein. The IC_{50} value indicates that **FYD4A** is moderately toxic to *Acanthamoeba* (9.15 µg/mL). **FYD4A** caused cell shrinkage and led to the encystment stage. Fluorescent staining revealed that the majority of the cells experienced membrane leakage, indicating signs of necrosis and random fragmentation in the DNA structure and smearing, which is another indicator of necrotic effects by **FYD4A**. Observation of **FYD4A**'s interaction with *Acanthamoeba* profilin protein (II) showed strong binding to the following amino acids: Ile70, Arg71, Arg75, Tyr78, Pro106, Ala110, Glu114, Asp118, and Ile121. Understanding the detailed effects of synthetic compounds, particularly for electronic applications, is crucial, as improper disposal could lead to adverse environmental impacts, starting with their effects on microorganisms in the environment, such as *Acanthamoeba*.

Keywords: Acetylide-Imine; necrosis; protein; microscopy techniques; molecular docking

I. INTRODUCTION

The growing reliance on solar energy has led to the widespread deployment of photovoltaic (PV) panels. While this shift towards renewable energy is environmentally beneficial, increasing electronic waste (e-waste) from end-of-life (EoL) PV panels presents a significant ecological challenge. Conventional PV panels contain hazardous materials, including heavy metals and toxic compounds, which, when improperly discarded, contribute to environmental contamination and potential health hazards. The absence of efficient recycling systems further exacerbates

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this issue, necessitating the development of alternative, eco-friendly photovoltaic technologies. The utilisation of organic photovoltaic (OPV) as active material in its panels have attracted high interests among researchers and stakeholders in recent years. The use of organic semiconducting materials, which exhibit biodegradable properties and being less hazardous than conventional PV panels are much favoured by many. These organic compounds consist of functional moieties that impact the efficiency, stability, and environmental impact of solar cells. However, despite their organic composition, the potential toxicity of these hybrid moieties when released into the environment remains largely unexplored. Understanding their impact is crucial, as degradation or leaching of these compounds into ecosystems could disrupt microbial communities and ecological balances.

Bioindicators, particularly microorganisms, have been increasingly recognised as effective tools for assessing environmental toxicity without being time-consuming (Kharkova *et al.*, 2022) and low-cost (Devi *et al.*, 2024). *Acanthamoeba* sp., a free-living protozoan, is particularly suitable as a bioindicator due to its sensitivity to pollutants and as a typical amoeba, the eukaryotic microorganism, allowing for a comparative assessment of cytotoxic effects relevant to higher organisms (Kosakyan & Lara, 2019). The ability of *Acanthamoeba* sp. to respond to environmental stressors by exhibiting physiological and morphological changes (Wanner *et al.*, 2020) makes it an ideal model for evaluating the toxicity of hybrid moieties derived from OPV panels. This study investigates the cytotoxicity of a hybrid moiety synthesised for OPV applications by assessing its effects on *Acanthamoeba* sp. through in vitro and in silico analyses. On a previous occasion, we have reported that the derivatives of acetylide-imine have shown great potential and performance as electronic transporters in conductive films, an essential investigation in any molecular electronics development (Khairul *et al.*, 2018). However, there are no known such studies involving this motif in its bioassessment towards Acanthamoeba, which evaluates their biosafety measures, and may have implications for electronic waste.

Thus, this research aims to determine the IC_{50} values of the compound, evaluate morphological alteration in treated cells, and elucidate the mode of cell disruption via apoptosis, necrosis, or autophagy. Furthermore, in silico work simulations were employed to examine the interaction of **FYD4A** with *Acanthamoeba* sp. profilin protein, providing insights into their biochemical impact at the molecular level. By integrating cytotoxicity assessments with bioindicator-based evaluations, this study aims to contribute valuable knowledge to ecological toxicology and bioindicator research, ultimately guiding the development of safer materials for next-generation photovoltaic technologies.

II. MATERIALS AND METHODS

A. Media Preparation

Peptone yeast extract glucose (PYG) culture media (1 L) was prepared by mixing 8 mg of anhydrous magnesium sulphate, 8mg of anhydrous calcium chloride, 40 mg of dipotassium phosphate, 40mg of monopotassium phosphate, 80 mg of sodium chloride, 400 mg of sodium bicarbonate, 10 g of 0.1 M glucose, 10 g/L yeast extract, and 20 g/L proteose peptone in one litre of distilled water. The solution mixture was autoclaved at 121 °C for 15 minutes and kept at 4 °C in the chiller.

B. Treatment for Dose-Response Analysis

Healthy trophozoites of *Acanthamoeba* cells were first harvested and seeded into a 96-well plate (2.5 x 10^4 cells/ well) and incubated for approximately 24 hours. One milligram of **FYD4A** was dissolved in 0.03 mL, and then 0.970 µL dimethyl sulfoxide (DMSO). Dose-response was based on the serial dilution to prepare a serial dilution of **FYD4A** (ranging from 100 µg/mL to 1.563 µg/mL), and from 100% to 1.85% for H_2O_2 as it is in solution form. Each well containing attached *Acanthamoeba* cells was incubated with 100 µL of the **FYD4A** and positive control at the respective concentration, with five replicates each, and incubated for another 24 hours in a 30 °C incubator.

C. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) Assay

Following 24 hours of **FYD4A** exposure time, MTT (20 µL) was added to each well. The plate was left to incubate for 4 hours, then 100 µL of DMSO was added. After the addition of DMSO, the plate was incubated for an additional 10 minutes

and read with an ELISA microplate reader to obtain the absorbance reading of the compound. The IC_{50} values of **FYD4A** and the positive control, hydrogen peroxide (H2O2) was obtained after completing One-way ANOVA statistical analysis, using GraphPad Prism software.

D. Light Microscopy

The *Acanthamoeba* sp. cells' morphology after treatment with **FYD4A** was observed after 24 hours of incubation with **FYD4A** at its IC_{50} value in a 6-well plate. An inverted light microscope with a magnification of 400x was used to capture and record the alteration to the *Acanthamoeba* cell morphology.

E. DNA Laddering Assay

Acanthamoeba sp. cell suspension after treatment with **FYD4A** at the IC_{50} value for 24 hours was prepared by centrifugation at 3000 rpm at room temperature for 15 min. TES lysis buffer (0.5 mL) was added, and the mixture was vortexed vigorously. Then, 20 µL of RNase cocktail was added. The cell suspension was then incubated at 37 °C for 30-120 minutes. After that, proteinase K (20 µL) was incubated again at 50 °C for 90 minutes to complete the DNA extraction process. One microlitre of loading buffer was mixed with 10-20 µL of the DNA samples and carefully inserted into each well of a 1% agarose gel containing. The gel is then run at a low voltage, 20 to 35 V, for 3 to 4 hours or until the loading dye has run two-thirds of the way down the gel, to ensure that the formation of DNA fragments is located at the correct size. The gel was then placed in a UV light Gel-Doc (Biorad, USA), and the image was captured for further analysis.

F. Molecular Docking

The crystal structures of *Acanthamoeba* profilin IB (PDB ID: 1ACF) and profilin II (PDB ID: 2ACG) were obtained from the RCSB Protein Data Bank. Sequence alignment between both proteins was performed using the Clustal Omega online tool to identify conserved residues within the actin-binding region. This conserved region, previously validated as the functional binding site in profilin IB (Zamli *et al*., 2023), was selected as the docking site for profilin II. Ligand structures (hybrid moieties) were built and energy-minimised using Avogadro software. Protein preparation was carried out in UCSF Chimera v.1.17, where nonprotein molecules were removed and polar hydrogens and charges were added. Both protein and ligand structures were then converted into PDBQT format for docking analysis. Molecular docking of **FYD4A** and other hybrid moieties into the actin-binding site of profilin II (2ACG) was performed using AutoDock Vina with the default scoring function. Binding affinities were reported in kcal·mol^{-1}. Visualisation of ligand–protein interactions was carried out in UCSF Chimera (3D conformation) and LigPlot+ (2D interaction profiles).

III. RESULT AND DISCUSSION

*A. IC_{50} Value Determination of the **FYD4A** against Acanthamoeba sp.*

The IC_{50} value of **FYD4A** was determined based on the MTT assay, which is the concentration needed to inhibit at least 50% of the *Acanthamoeba* sp. population. According to the graph presented (Figure 1), the IC_{50} value of **FYD4A** after 24 hours of treatment was 9.155 µg/mL. In comparison, hydrogen peroxide (H_2O_2), used as the positive control, showed a significantly higher IC_{50} value of 19.22% (Figure 1a). These results suggest that **FYD4A** exhibits moderate toxicity towards *Acanthamoeba* sp.

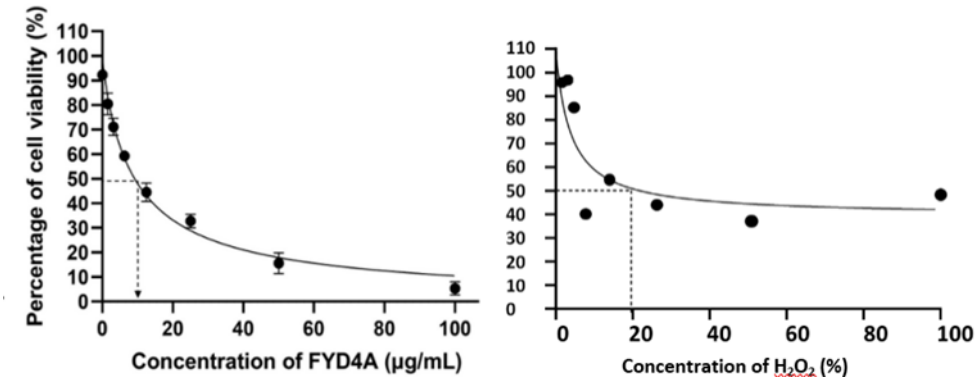


Figure 1. *Acanthamoeba* sp. cell viability percentage after 24 hours of treatment with **FYD4A** was analysed using the MTT assay. The IC_{50} values for (a) **FYD4A** (9.155 µg/ml) and (b) H_2O_2 (19.22%).

The IC_{50} value represents the concentration required to inhibit 50% of cell viability and is a key parameter in evaluating cytotoxic potential. The half-maximal inhibitory concentration (IC_{50}) values represent the concentration required to reduce cell viability by 50%. These values fall within the moderately toxic range in in vitro cytotoxicity assessments. The results indicated the IC_{50} value for **FYD4A** was 9.155 µg/mL. Hydrogen peroxide (H_2O_2) was used as a positive control to compare cytotoxicity levels. Results indicated vital insights into the efficacy of hybrid moieties in inhibiting *Acanthamoeba* sp. The IC_{50} values obtained from the MTT assay for H_2O_2 were 19.22%. Acetylide-imine caused cell death in cancer cells at a concentration range of 3.5–3.8 µg/mL; however, a concentration of more than 100 µg/mL is required to induce cell death in normal cells, resulting in a selectivity index greater than 25 (Iliev *et al.*, 2023). Acetylide-imine derivatives have significant environmental implications due to their cytotoxic effects and potential to leach into ecosystems, particularly when used in industrial applications such as electronic components. This group of compounds exhibits cytotoxicity by interfering with cellular functions, often through the induction of oxidative stress, DNA damage, or the disruption of enzymatic activities (Pero *et al.*, 2009). The presence of the acetylide group enables strong interactions with biomolecules, resulting in cell death via apoptosis or necrosis (Bofonco *et al.*, 1995).

Studies have shown that transition metal-acetylide complexes, including those with imine ligands, induce cytotoxic effects by binding to DNA and proteins, altering their function (Lee *et al.*, 2021). For example, platinum (II) acetylide-imines have demonstrated DNA cross-linking mechanisms, similar to cisplatin but with enhanced specificity and reduced toxicity to normal cells (Wang *et al.*, 2022). Additionally, acetylide imines may affect mitochondrial function, leading to the activation of caspase-dependent apoptosis pathways by releasing cytochrome C (Manisekaran *et al.*, 2024).

In aquatic environments, acetylide imines may interact with organic matter, forming reactive intermediates that can be toxic to aquatic organisms (Kim *et al.*, 2024). Studies suggest that certain metal-acetylide complexes can induce oxidative stress in fish and algae, thereby disrupting cellular metabolism and enzyme activity (Jones & Carter, 2020). Additionally, their ability to chelate metals may alter nutrient availability in soil and water, thereby impacting microbial communities that are essential for maintaining ecosystem balance (Lee *et al.*, 2022).

In terrestrial environments, acetylide-imines could affect soil microbial diversity and plant growth. If these compounds persist, they may interfere with nitrogen-fixing bacteria, leading to soil fertility issues (Wang *et al.*, 2023). Moreover, their potential to degrade into toxic byproducts, such as nitriles and amines, could further contribute to environmental pollution and toxicity risks (Garcia *et al.*, 2024). In summary, acetylide-imines occupy a middle range in cytotoxic efficacy but offer distinct advantages in ecological safety studies.

B. Internal Cell Morphology and Mode of Cell Death Assessment by Fluorescence Microscopy using Double Staining Acridine Orange and Propidium Iodide (AO/PI).

Cytoplasmic changes in *Acanthamoeba* sp. cells following treatment with **FYD4A** were assessed using a fluorescence light microscope after staining with AOPI dye (Figure 3).

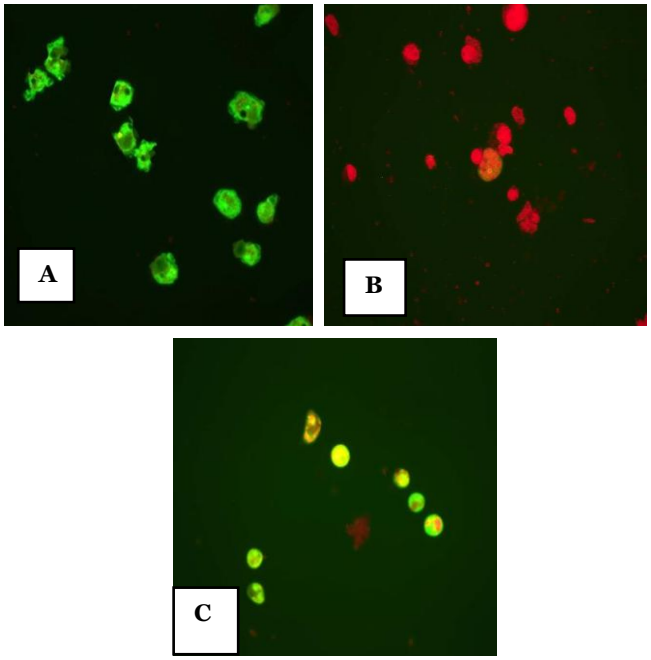


Figure 3. Internal Assessment of *Acanthamoeba* cell morphology and mode of cell death visualisation by fluorescence microscopy using AO/PI (a) Untreated *Acanthamoeba;* (b) **FYD4A**-treated and (c) H_2O_2-treated *Acanthamoeba.*

The dye's diffusion into the cytoplasm enabled the identification of cell death modes such as necrosis and autophagy after 24 hours of treatment. Untreated cells served as the negative control for comparison with the **FYD4A**. Observation under fluorescence microscopy revealed internal morphological changes in Acanthamoeba after 24 hours of treatment with **FYD4A**. AO/PI staining using a blue filter on untreated cells appears with green-fluorescent cells, which were stained with AO dye. AO is a membrane-permeable dye that will stain green in an *Acanthamoeba* cell-neutral environment, including organelles such as the nucleus and lysosome (Figure 3a).

The **FYD4A**-treated cells, as shown in Figure 3b, displayed red fluorescence in all cells, resulting from necrotic cell death. The lysosomal activity in cells increases the pH of the cytoplasm, making it more acidic due to heightened secretion of hydrolytic enzymes. This process stained the lysosomes red as the cytoplasmic cavity of the treated amoebic cells underwent rupture and may burst due to cytoplasmic membrane breakage, thereby losing their integrity, as indicated by the penetration of PI into the cell. The event of necrosis was explained in mammalian cell culture by Proskuryakov (2003). In the necrosis event, the cell first swells, followed by the breakdown of the plasma membrane, and eventually the cell will lyse. Additionally, mammalian cell apoptosis is characterised by cell shrinkage and nuclear condensation. The formation of apoptosis, characterised by well-wrapped damaged organelles, is the hallmark of apoptosis morphologically.

Treatment with H_2O_2, however, displayed a green to orange *Acanthamoeba* cell. The mixture colour is due to the AO dye that stains the neutral cytoplasmic area, but the organelles in the cell, such as the lysosome, become an acidic compartment due to hydrolysis enzyme activity when reacting with the AO dye. For an orange-coloured nucleus, the mechanism of AO is when the DNA (deoxyribonucleic acid) breaks between the H-bonds of A-T (two hydrogen bonds) and C-G (three hydrogen bonds). The AO molecules intercalate into the breakage of the double helix strands. In the case of **FYD4A**, the *Acanthamoeba* cells experienced similar damage with the formation of H+ ions resulting from the breakage of the H bonds. This was also mentioned by Harding *et al.* (2018) in the situation of a highly acidic environment when the DNA linkage or H-bond breaks.

*C. Observation on Acanthamoeba DNA Fragmentation Treated with **FYD4A***

The DNA fragmentation analysis is a hallmark technique used to distinguish between apoptosis and necrosis in cell death by analysing the patterns of DNA fragmentation. Apoptotic cells exhibit characteristic ladder-like DNA fragmentation due to the activation of endogenous endonucleases, whereas necrotic cells typically show random DNA degradation (Rahbar *et al.*, 2015). In this study, the confirmation of the DNA pattern was used to distinguish the type of cell death in *Acanthamoeba* sp. following treatment with **FYD4A**. The results provide insights into whether the compounds induce programmed cell death or trigger necrosis.

Understanding the mode of cell death is crucial in evaluating the efficacy and mechanism of action of potential chemical substances. Additionally, hydrogen peroxide (H_2O_2) was used as a positive control to compare DNA degradation patterns. The findings contribute to a deeper understanding of how these hybrid moieties of **FYD4A** interact with *Acanthamoeb*a sp.

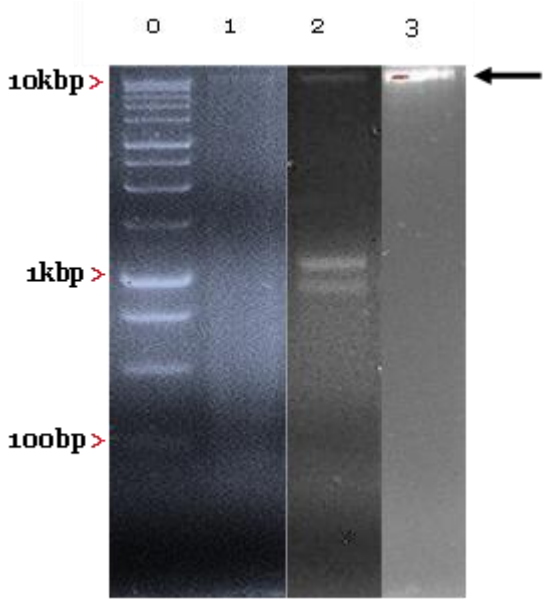


Figure 4. Electrophoresis of *Acanthamoeba* DNA using 2% agarose gel at 35 V for 4 hours. Lane 0 represents a 10 kbp DNA laddering marker. Lane 1 is H_2O_2, with smeary effects of the DNA. Lane 2 is **FYD4A,** showing fragmentation of DNA at a 1-1.2kbp band. Lane 3 is an untreated *Acanthamoeba* that appears with a single band of DNA on top of the lane indicated by the arrow.

The genomic DNA laddering assay is a method that can confirm the mode of cell death in *Acanthamoeba* sp. following treatment with **FYD4A**. This technique provides insight into whether the observed cytotoxic effects were associated with apoptotic DNA fragmentation or random degradation of the DNA (Yakovlev *et al.*, 1999). The DNA samples were analysed using agarose gel electrophoresis, with Lane M representing a molecular weight marker ranging from 10 kbp to 100bp. A single, intact DNA band was observed at the top of the lane in the negative control sample (Lane 3), representing DNA extracted from untreated, healthy *Acanthamoeba* cells. The absence of smearing or additional fragmented bands indicates that the DNA remained undamaged, confirming the healthy state of the control cells (Gomez *et al.*, 2021). This suggests that untreated *Acanthamoeba* cells did not undergo apoptosis or necrosis and retained their genomic integrity.

Conversely, DNA extracted from *Acanthamoeba* cells treated with **FYD4A** (Lane 2) exhibited distinct patterns indicative of DNA fragmentation. Two prominent DNA bands and a characteristic smeary effect were observed near the 1 kbp marker in these lanes. The presence of these bands suggests that DNA fragmentation occurred at approximately 1100 bp, 900 bp, and smaller fragments, including those around 100 bp. The smeary appearance is a hallmark of extensive DNA degradation, commonly associated with DNA cleavage (Zrimec *et al.*, 2013) and typically observed in late-stage apoptosis. The double bands near 1 kbp, seen consistently across all treated samples, indicate a controlled pattern of DNA fragmentation, which is typically associated with apoptotic cell death. Apoptosis is characterized by the activation of endogenous nucleases that cleave DNA into oligonucleosomal fragments, resulting in a characteristic DNA laddering pattern. The presence of these bands suggests that treatment with the **FYD4A** triggered apoptosis and necrosis in *Acanthamoeba* sp. cells.

Additionally, the smeary effects observed in the gel indicate that some degree of necrotic DNA degradation has occurred. Necrosis is typically characterised by random and extensive DNA breakdown, resulting in a diffuse smear rather than distinct laddering (Costigan *et al.*, 2023). Both laddered DNA bands and smearing suggest that a mixed mode of cell death, apoptosis accompanied by secondary necrosis, might have occurred following treatment. These findings align with previous studies demonstrating that certain cytotoxic compounds can induce apoptosis in *Acanthamoeba* sp. rather than purely necrotic cell death. The fragmentation at 1100 bp and 900 bp may represent characteristic cleavage sites specific to the degradation of *Acanthamoeba* DNA upon exposure to these compounds. The smaller 100 bp fragments could correspond to further degradation into oligonucleotides, which is a key feature of late-stage apoptosis (Lyubov *et al.*, 2024).

The molecular weight marker (Lane M) provided a reference for estimating the sizes of the fragments. Since the observed bands were located close to the 1 kb region, this suggests that the DNA degradation pattern is consistent with

apoptotic fragmentation rather than necrotic smearing alone. This further strengthens the conclusion that apoptosis was the dominant induction of death by the **FYD4A**. The results of the DNA laddering assay complement previous findings from fluorescence microscopy, which revealed hallmarks of necrosis and apoptosis, including nuclear condensation and membrane disruption. The combination of these observations provides strong evidence that the hybrid moieties effectively induce programmed cell death in *Acanthamoeba* sp. The summary of events that occurred in this study is depicted in Figure 4 below.

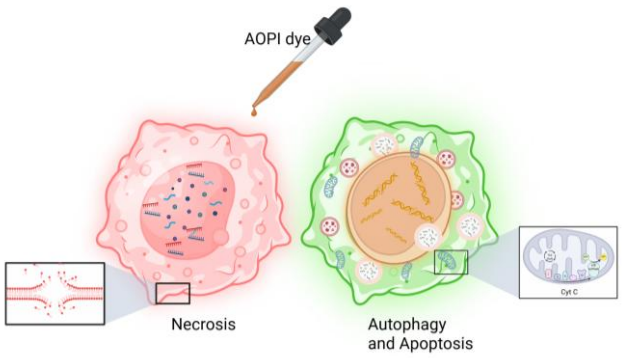


Figure 4. The actual observations occurring in amoeba cells upon treatment with **FYD4A** indicate necrosis, whereas treatment with H_2O_2 triggers autophagy and apoptosis. The red colouration is caused by PI penetration, resulting from damage to the membrane layer, which stains the cells red. Random DNA fragmentation also occurs, as evidenced by the DNA laddering results, which display a smear-like DNA pattern. In the case of H_2O_2, organelles such as lysosomes appear orange and red due to the acidic environment activated by hydrolytic enzymes and their reaction with AO dye. DNA fragmentation is also observed.

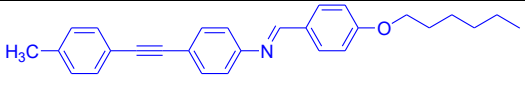
In summary, the DNA laddering assay confirmed that treatment with **FYD4A** resulted in significant DNA fragmentation in *Acanthamoeba* sp., producing a characteristic apoptotic laddering pattern alongside some smeared degradation. Distinct DNA bands at approximately 1100 bp and 900 bp suggest a controlled apoptotic process, while the smear indicates possible secondary necrosis.

*D. In Silico Interaction of **FYD4A** on Profilin II of Acanthamoeba*

Docking simulation between profilin II of *Acanthamoeba* sp. and **FYD4A** demonstrated a binding affinity of −4.9 kcal·mol^{-1} (Table 1, Figure 4a). In the molecular docking analysis, **FYD4A** exhibited multiple hydrophobic interactions (shown as red arcs in LigPlot+) with surrounding residues, including Ile70, Arg71, Arg75, Tyr78, Pro106, Ala110, Glu114, Asp118, and Ile121 (Figure 5b). These interactions indicate that **FYD4A** was accommodated within the hydrophobic pocket of profilin II and stabilised by a combination of nonpolar and polar contacts. Hydrophobic interactions play a major role in stabilising **FYD4A** within the actin-binding site of profilin II, supported by contacts with both nonpolar residues (e.g., Ile70, Tyr78, Pro106) and polar residues (e.g., Arg71, Glu114, Asp118). Such stabilisation suggests that **FYD4A** may interfere with profilin's ability to regulate actin polymerisation, potentially impairing cytoskeletal function and contributing to the necrotic cell death observed in vitro.

Importantly, the docking site was defined based on previously validated findings. Zamli *et al.* (2023) demonstrated that the actin-binding site of *Acanthamoeba* profilin (PDB ID: 1ACF) is conserved and functionally relevant for ligand recognition. By aligning interacting residues between profilin IB (1ACF) and profilin II (2ACG) (Figure 6), **FYD4A** was docked into this conserved actin-binding region, ensuring biological relevance and strengthening the interpretation of our docking results.

Table 1. Molecular docking simulation between *Acanthamoeba* profilin II (PDB ID:2ACG) and hybrid moieties.

Code	Molecular structure of compound	Binding affinity (Kcal mol^{-1})	Interacted residues
FYD4A	 Acetylide-imine	-4.9	Ile70, Arg71, Arg75, Tyr78, Pro106, Ala110, Glu114, Asp118, Ile121

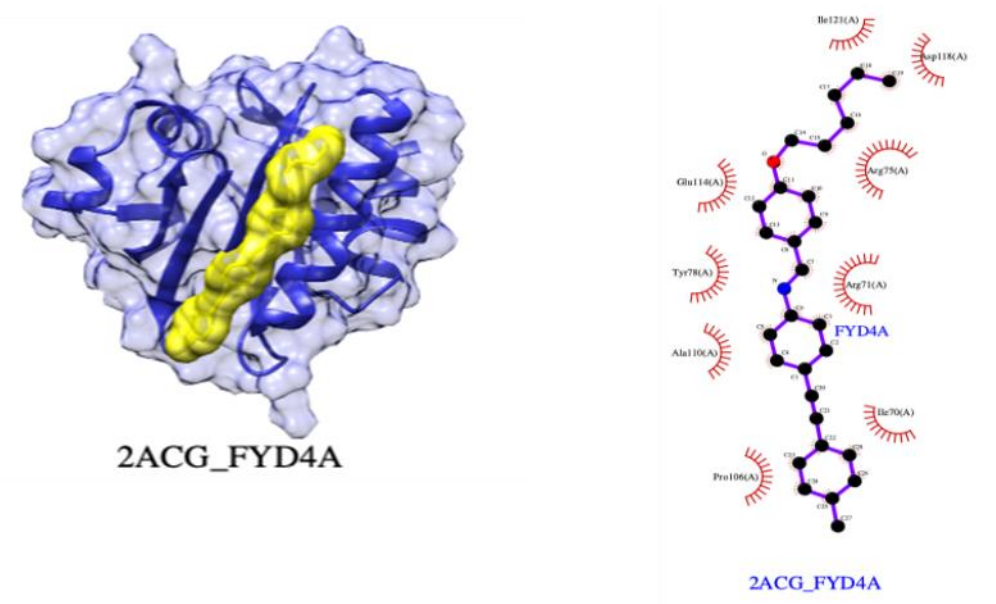


Figure 5. (a) Docked complex of **FYD4A** (yellow) with *Acanthamoeba* profilin II (PDB ID: 2ACG, blue surface and ribbon). (b) 2D LigPlot+ diagram illustrating hydrogen bonds (green dotted lines) and hydrophobic interactions (red arcs) between **FYD4A** and surrounding amino acid residues.

Our findings suggest that even at low concentrations, acetylide imine induces significant cellular stress, leading to both apoptosis and necrosis. MTT assay results indicate a biphasic response, where an initial increase in metabolic activity is observed, followed by a decline at prolonged exposure, suggesting mitochondrial dysfunction and oxidative stress-induced cytotoxicity.

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                    **********:* ***********:**:*::**.**:
1ACF_1|Chain SWQTYVDTNLVGTGAVTQAAILGLDGNTWATSAGFAVTPAQGTTLAGAFN    50
2ACG_1|Chain SWQTYVDTNLVGTGAVTQAAIIGHDGNTWATSAGFAVSPANGAALANAFK    50
             1.......10........20........30........40........50

                    :* ***:.**:***.:***:******:******:***********:***
1ACF_1|Chain NADAIRAGGFDLAGVHYVTLRADDRSIYGKKGSSGVITVKTSKAILVGVY   100
2ACG_1|Chain DATAIRSNGFELAGTRYVTIRADDRSVYGKKGSAGVITVKTSKAILIGVY   100
             ........60........70........80........90.......100

                    ************************
1ACF_1|Chain NEKIQPGTAANVVEKLADYLIGQGF   125
2ACG_1|Chain NEKIQPGTAANVVEKLADYLIGQGF   125
             .......110.......120.....
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Figure 6. Sequence alignment between *Acanthamoeba* profilin IB (PDB ID: 1ACF) and profilin II (PDB ID: 2ACG). Residues involved in ligand interaction in profilin II (this study) are highlighted in blue, while corresponding residues previously identified in profilin IB (Zamli *et al.*, 2023) are shown in red. The alignment demonstrates conservation of the actin-binding region across profilin isoforms, supporting the selection of this binding site for docking **FYD4A**. This comparative approach ensures that the docking analysis was carried out in a biologically validated and functionally relevant region of profilin.

These findings align with previous studies on other toxic metal-organic compounds that exhibit similar metabolic perturbations (Smith *et al.*, 2022). Among the other compounds that cause cytotoxicity to *Acanthamoeba* are azole compounds (Qubais Saeed *et al.*, 2024), dihydropyridine (DHP) compounds (Anwar *et al.*, 2020a), and plant-based compounds with silver nanoparticles (Anwar *et al.*, 2020b). Light microscopy analysis revealed a marked increase in encystment upon exposure to acetylide imine, suggesting that the compound triggers stress-induced differentiation as a survival mechanism. The ability of *Acanthamoeba* to form cysts under chemical stress poses environmental concerns, as resistant cysts may facilitate the persistence and dissemination of the pathogen in

contaminated sites (Jones & Patel, 2021). Additionally, AO/PI staining demonstrated an increase in necrotic cell populations, further confirming the cytotoxic effects of acetylide imine. The predominance of necrosis over apoptosis at higher concentrations suggests that excessive oxidative damage may overwhelm cellular repair mechanisms, leading to irreversible cell death (Wang *et al.*, 2023).

DNA fragmentation analysis via laddering assays confirmed, both apoptotic and necrotic modes of cell death, indicative of genotoxic stress. Acetylide imine exposure led to chromatin condensation and DNA fragmentation patterns consistent with caspase-dependent apoptosis, further corroborating its cytotoxic potential. The observed dual mechanism of cell death suggests that acetylide imine disrupts key regulatory pathways involved in cell survival and stress responses (Lee *et al*., 2024).

Molecular docking studies provided additional insights into these biochemical effects. **FYD4A** strongly interacted with key residues (Ile70, Arg71, Arg75, Tyr78, Pro106, Ala110, Glu114, Asp118, and Ile121), reinforcing the hypothesis that disruption of profilin function may compromise cytoskeletal regulation. Such interference could contribute to downstream effects on metabolism, stress response, and programmed cell death (Garcia *et al.*, 2023).

Given the widespread use of acetylide imines in the electronic and solar cell industries, their environmental persistence and potential toxicity warrant further investigation. The ability of these compounds to induce encystment in *Acanthamoeba* raises concerns regarding their long-term ecological impact, as cysts serve as reservoirs for pathogenic amoebae in water systems. Future studies should focus on biodegradation pathways and potential mitigation strategies to reduce environmental risks associated with these compounds.

IV. CONCLUSION

This study demonstrates the cytotoxic effects of acetylide-imine (**FYD4A**) on *Acanthamoeba* cells, particularly in the context of environmental exposure to electronic waste and solar cell components. Our findings reveal that even at low concentrations, acetylide-imine induces apoptosis and necrosis, as evidenced by MTT assays, AO/PI staining, and DNA fragmentation analysis. The compound also triggers encystment, highlighting its potential to promote the environmental persistence of *Acanthamoeba*.

Molecular docking analysis further suggests that acetylide imine interacts with critical amino acid residues, potentially disrupting essential cellular functions and stress response pathways. These findings raise concerns regarding the environmental impact of acetylide-imine, as its persistence in aquatic and terrestrial ecosystems may contribute to disruptions to the ecosystem. Given the widespread use of acetylide imine in industrial applications, further studies are needed to evaluate its biodegradability, long-term ecological effects, and potential mitigation strategies. Understanding the mechanisms underlying its cytotoxicity can aid in developing safer alternatives or remediation approaches to minimise environmental risks, particularly in the development of OPVs towards safer and minimal health risks for use by all walks of life.

V. ACKNOWLEDGEMENT

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VI. REFERENCES

Alam, MM, Varala, R & Seema, V 2024, 'Zinc acetate in organic synthesis and catalysis: A 'review', Mini-Reviews in Organic Chemistry, vol. 21, no. 5, pp. 555-587. doi: 10.2174/1570193X20666230507213511

Anwar, A, Siddiqui, R, Hameed, A, Shah, MR & Khan, NA 2020, Synthetic dihydropyridines as novel antiacanthamoebic agents, vol. 16, no. 7, pp. 841 – 847. doi: 0.2174/1573406415666190722113412

Anwar, A, Ting, ELS, Anwar, A, Ain, N, Faizi, S, Shah, MR, Khan, NA, Siddiqui, R 2020, Antiamoebic activity of plant-based natural products and their conjugated silver nanoparticles against *Acanthamoeba castellanii* (ATCC 50492), vol. 10, no. 1, art. no. 24. doi: 10.1186/s13568-020-0960-9

Bonfoco, E, Krainc, D, Ankarcrona, M, Nicotera, P & Lipton, SA 1995, 'Apoptosis and necrosis: two distinct events induced, respectively, by mild and intense insults with N-methyl-D-aspartate or nitric oxide/superoxide in cortical cell cultures', Proc. Natl. Acad. Sci. USA., vol. 92, no. 16, pp. 7162-6. doi: 10.1073/pnas.92.16.7162. PMID: 7638161;

Costigan, A, Hollville, E & Martin, SJ 2023, 'Discriminating between apoptosis, necrosis, necroptosis, and ferroptosis by microscopy and flow cytometry', Current protocols, vol. 3, no. 12. doi: 10.1002/cpz1.951

Devi, P, Kumar, P & Singh, J 2024, 'Application of potential microbes in bioremediation', Development in Waste Water Treatment Research and Processes: Role of Environmental Microbiology in Industrial Wastewater Research, pp. 201 – 225. doi: 10.1016/B978-0-443-13609-2.00021-5

Flefel, E, Abbas, HA, Abdel, MR & Zaghary, W 2015, 'Synthesis and Cytotoxic Effect of Some Novel 1,2-Dihydropyridin-3-carbonitrile and Nicotinonitrile 'Derivatives', Molecules, vol. 21, no. 1. doi: 10.3390/molecules21010030

García-Peña, LM, Abel, ED & Pereira, RO 2024, 'Mitochondrial Dynamics, Diabetes, and Cardiovascular 'Disease', Diabetes, vol. 73, no. 2, pp. 151–161. Doi: 10.3390/molecules21010030

Gomez Martinez, AE & Herr, AE 2021, 'Programmed Cell-Death Mechanism Analysis Using Same-Cell, Multimode DNA and Proteoform 'Electrophoresis', ACS Meas Sci Au, vol. 1, no. 3, pp. 139-146. Doi: 10.1021/acsmeasuresciau.1c00014

Kharkova, A, Arlyapov, V, Medvedeva, A, Lepikash, R, Melnikov, P & Reshetilov, A 2022, 'Mediator Microbial Biosensor Analyzers for Rapid Determination of Surface Water Toxicity', Sensors, vol. 22, no. 21, art. no. 8522. doi: 10.3390/s22218522

Iliev, I, Mavrova, A, Yancheva, D, Dimov, S, Staneva, G, Nesheva, A, Tsoneva, I & Nikolova, B 2023, '2-Alkyl-Substituted-4-Amino-Thieno[2,3-d]Pyrimidines: Anti-Proliferative Properties to In Vitro Breast Cancer 'Models', Molecules, vol. 28, no. 17, p. 6347. Doi: 10.3390/molecules28176347

Jones, ND, Wolf, MO & Giaquinta, DM 1997, 'Synthesis of a ferrocenyl-capped ruthenium (II) bis (acetylide) complex: A model for organometallic molecular wires', Organometallics, vol. 16, no. 7, pp. 1352-1354. Doi: 10.1021/om960849h

Khairul, WM, Foong, YD, Sarih, NM, Lee, OJ, Lim, SKJ, Daud, AI & Rahamathullah, R 2018, 'Elucidating Mesomorphic and Electrical Properties of Conjugated Acetylide-Imine as Electrochemically Deposited Electron Transporting Materials in Organic Conductive 'Film', ASM Sci. J, pp. 124-135.

Kim, Y-S, Nagahawatta, DP, Kurera, MJMS, Jayawardhana HHACK, Liyanage, NM, Dissanayake, D, Amarasiri, RPGSK, Kim, C-Y, Je, J-G, Lee, SG, Jo, HY, Kim, HJ, Oh, MJ, Seo, N, An, HJ & Jeon, Y-J 2024, 'Detection of Cyclic Imines (CIs) Toxins in Whole Body of Shellfishes: First Monitoring Report of CIs in South Korea Shellfishes', Ocean Science Journal, vol. 59, no. 3. Doi: 10.1007/s12601-024-00151-4

Kosakyan, A & Lara, E 2019, 'Using testate amoebae communities to evaluate environmental stress: A molecular biology perspective', Encyclopedia of Environmental Health, pp. 308 – 313. Doi: 10.1016/B978-0-12-409548-9.11589-1

Wanner, M, Birkhofer, K, Puppe, D, Shimano, SD & Shimizu, M 2020, 'Tolerance of testate amoeba species to rising sea levels under laboratory conditions and in the South Pacific', Pedobiologia, vol. 79, art. no. 150610. Doi: 10.1016/j.pedobi.2019.150610

Lyubov, EA, Dmitry, DZ, Yulia, AG, Anastasia, NS & Victoria, VS 2024, 'Electrochemical approach for the analysis of DNA degradation in native DNA and apoptotic 'cells', Heliyon, vol. 10, no. 3. Doi: 10.1016/j.heliyon.2024.e25602

Manisekaran, T, Khairul, WM, Foong, YD, Johari, SA, Hashim, F, Rahamathullah, R & Mohammed, M 2024, 'Assessment on functionalized conjugated acetylide and its designated acetylide-imine moieties towards *Acanthamoeba* sp.: An in vitro bioindicator study', Chemosphere, vol. 1, no. 364, p. 143220. Doi: 10.1016/j.chemosphere.2024.143220

Pero, RW, Anderson, MW, Doyle, GA, Anna, CH, Romagna, F, Markowitz, M & Bryngelsson, C 1990, 'Oxidative stress induces DNA damage and inhibits the repair of DNA lesions induced by N-acetoxy-2-acetylaminofluorene in human peripheral mononuclear leukocytes', Cancer Res., vol. 50, no. 15, pp. 4619-25. PMID: 2114943.

Qubais Saeed, B, Hamdy, R, Akbar, N, Sajeevan, SE, Khan, NA & Soliman, SSM 2024, 'Azole-based compounds as potential anti-Acanthamoeba agents', RSC Medicinal Chemistry, vol. 15, no. 5, pp. 1578 – 1588. Doi: 10.1039/d4md00029c

Rahbar, SY, Saeidi, N, Zununi, VS, Barzegari, A & Barar, J 2015, 'An update to DNA ladder assay for apoptosis 'detection', Bioimpacts, vol. 5, no. 1, pp. 25-8. Doi: 10.15171/bi.2015.01

Sayed, M, Krishnamurthy & B, Pal, H 2016, 'Unravelling multiple binding modes of acridine orange to DNA using a multispectroscopic 'approach', Phys Chem Chem Phys, vol. 18, no. 35. Doi: 10.1039/c6cp03716j

Wang, H et al. 2022, 'Platinum(II) acetylide imines and their interaction with DNA', Journal of Inorganic Biochemistry, vol. 235, p. 110325. Doi: 10.1021/ic0263084

Yakovlev, AG, Wang, G, Stoica, BA & Smulson, ME 1999, 'Role of DNAS1L3 in Ca2+- and Mg2+-dependent cleavage of DNA into oligonucleosomal and high molecular mass fragments', Nucleic Acids 1999. Doi: 10.1093/nar/27.9.1999

Zamli, KM, Hashim, F, Razali, SA, Yusoff, HM, Mohamad, H, Abdullah F & Asari, A 2023, 'Synthesis, anti-amoebic activity and molecular docking simulation of eugenol derivatives against *Acanthamoeba* sp.', Saudi Pharmaceutical Journal, vol. 31, no. 9. Doi: 10.1016/j.jsps.2023.101703

Zrimec, J, Kopinč, R, Rijavec, T, Zrimec, T & Lapanje, A, 2013 'Band smearing of PCR amplified bacterial 16S rRNA genes: Dependence on initial PCR target diversity', Journal of Microbiological Methods, vol. 95, no. 2, pp. 186 – 194. Doi: 10.1016/j.mimet.2013.08.002