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Research Article

Synthesis, Characterization, And Bio-Evaluation Of Arylvinylypyrimidine

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ABSTRACT

Water pollution due to increasing anthropological activities, made compromised health conditions of aquatic animals. Synthesis and characterization of a hybrid arylvinylypyrimidine compound, 4,6-bis((E)-2,5-dimethoxystyryl)pyrimidin-2-ol (AVP), by integrating curcumin and pyrimidine pharmacophores may contribute positively in aquaculture. In silico docking data supported that AVP's efficacy arises from synergistic interactions between the arylvinyl and pyrimidine moieties, improving cell permeability and bioavailability. In vitro, AVP showed antibacterial (*Escherichia coli*) and cytotoxic effects against carcinoma cells, with nil toxicity to healthy cells. In vivo, AVP significantly enhanced tested health parameters (hematological, immunological) compared to untreated freshwater catfish (*Heteropneustes fossilis*) with nil lethality or behavioral abnormalities in concentration, duration and post-exposure recovery study. Further extended research required to explore possibility in aquaculture health management and biomedical research.

1. INTRODUCTION

Pyrimidines are integral building blocks of DNA and RNA and hence play a vital role in various biological processes and have considerable chemical and pharmacological importance (Amr et

al., 2006, Boncel et al., 2008). Scaffolds containing the pyrimidine structural unit exhibit various biological effects including anti-tumour, anti-inflammatory, anti-viral, antiproliferative, analgesic, antipyretic, antiarrhythmic, antimicrobial, anticancer activities (Otmir et al., 2004; Bryzgalov et al., 2006; Amin et al., 2009;

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Kim et al., 2009; Keri et al., 2010; Al-Abdullah et al., 2011, Kumar et al., 2011). In particular, aminopyrimidines constitute a common structural design in a large number of naturally occurring and biologically active compounds and have been widely used as pharmacophore for drug discovery. More importantly, aminopyrimidines with substituents either at N or at 4th-position are present in many drug-like scaffolds with great chemotherapeutic potential. Several 2-aminopyrimidines are known as broad spectrum antibacterial agents (Nurfinal et al., 1997). Pyrimidine improves neuro-configuration (Ali et al., 2025a), and even resist hypoxia induced damage in fresh water fish (Ali et al., 2026).

Curcumin [diferuloyl methane; 1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], a natural phenolic diarylheptanoid, is an active phytochemical constituent of turmeric (*Curcuma longa*). Indian medical scripture 'Ayurveda' has well documented the therapeutic properties of turmeric. The numerous biological properties including antioxidant (Menon et al., 1999), anticancer (Aggarwal et al., 2003; Anand et al., 2008; Wilken et al., 2011), anti-inflammatory (Maheshwari et al., 2006), anti-metastatic (Yang et al., 2005), anti-HIV protease (Mazumder et al., 1997), anti-alzheimer's activities (Baum, & Ng, 2004), anti-arthritis, anti-amyloid (Yang et al., 2005), anti-ischemic (Maheshwari et al., 2006), and others, make curcumin a promising lead compound for structural modification to develop novel biological agents in the medicinal world (Jiang et al., 2012). In addition, curcumin also has interesting optical properties as it absorbs and emits strongly in the visible spectral region and can be employed for one and two-photon imaging applications (Kumar et al., 2012). Curcumin, a hydrophobic molecule, has poor water solubility due to its rigid and planar structure, which affects its absorption from the

digestive system into the blood circulation (Anand et al., 2007). Unsuitability with water results in low potency, poor bioavailability and absorption and prevented it from being used as a potent therapeutic agent (Dhillon et al., 2008). Curcumin's bio-stimulant properties were screened in fish for overall health, immunity, oxidative stress, nutritional quality impact (Manju et al., 2013; Yonar et al., 2019; Ashry et al., 2021; Pandey & Mishra, 2022; Pandey et al., 2022,2024).

The biological properties and therapeutic applications of pyrimidines depend upon the pattern of substitution and have attracted intense interest in recent years because of their diverse pharmaceutical properties (Kenner et al., 1943; Joffe et al., 1989; Nadal & Olavarria, 2004). Therefore, it is essential requisite to synthesize and develop novel analogs of pyrimidines with multiple targets. The major active component of turmeric and N-C-N and C-N fragment containing compounds will be utilized for the synthesis of 2-aminopyrimidines. Several elegant approaches for the synthesis of pyrimidines involving different types of reactant and catalysts have been reported (Kenner et al., 1943). The great relevance to both synthetic and medicinal chemists, and diverse pharmacological properties of curcumin and pyrimidines prompted the design of curcumin-substituted arylvinylpyrimidines and exploration of biological activities.

Fresh water catfish, *Heteropneustes fossilis* (Bloch, 1794) is highly recommended edible fish because of its rich nutritional value (less in fat, more in protein, minerals with essential omega 3-fatty acids) (Paul et al., 2016; Pegu et al., 2023). But in its culture and developmental process, deteriorating environmental conditions are presenting a huge hurdle. Improving health of this species through novel therapeutic strategies is



crucial to enhance resilience under deteriorating aquatic conditions.

A step toward bioremediation, the synthesis of a bioactive hybrid molecule that contains the therapeutic properties from well-known pharmacophores like pyrimidine and curcumin was synthesized. Further, the hybrid molecule was screened for preliminary anti-cancerous, qualitative anti-bacterial activity and impact on health of fresh water catfish *H. fossilis*.

2. Materials and methods

2.1. Chemicals

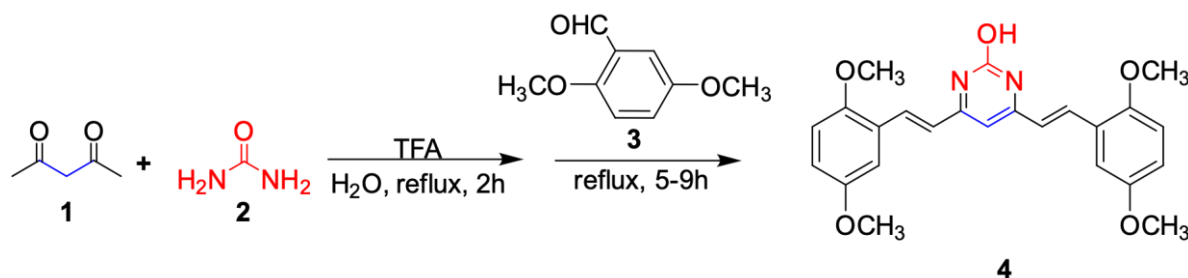
All required chemicals were of analytical grade and purchased from a scientific supplier.

All experimental protocols involving animals were reviewed and approved by the Institutional Animal

Ethics Committee (IAEC) (2352/PO/Re/S/2025/CCSEA). The study was performed in compliance with the applicable institutional guidelines and relevant regulatory standards.

2.2. Synthesis of arylvinylpyrimidine

Arylvinylpyrimidine prepared with one-pot protocol (Singh et al., 2022), briefly, compounds **1** (acetylacetone, 2.5 mmol) and **2** (urea, 2.5 mmol) were added in water (5 ml), and refluxed in the presence of trifluoroacetic acid (TFA, **3**) (0.5 ml) after 2 h, 2,5-dimethoxy benzaldehyde (**3**, 5.5 mmol) was added and the reaction mixture was further refluxed for 5 h results in compound **4** (4,6-bis((E)-2,5-dimethoxystyryl) pyrimidin-2-ol; arylvinylpyrimidine) in 87% yield, pale yellow solid.



Scheme 1. Synthesis of arylvinylpyrimidine (4,6-bis((E)-2,5-dimethoxystyryl) pyrimidin-2-ol: **4**); where as, **1**: acetylacetone, **2**: urea, **3**: trifluoroacetic

2.3. Properties of synthetic arylvinylpyrimidine

Melting point is determined using the Buchi-500 MMP apparatus (194-197 °C). NMR spectra were recorded on the Bruker 500 MHz NMR spectrometer while using tetramethylsilane (TMS) as an internal standard. The chemical shift (δ) and the coupling constant (J) are given in ppm and hertz, respectively. The reaction was monitored on pre-coated TLC silica gel 60 F254 plates (Merck) and visualized with iodine vapor. Column chromatography was performed on silica gel (100–

200 mesh, Merck) using methanol/chloroform as the eluent. ^1H NMR (500 MHz, DMSO- d_6), δ 11.71 (s, ^1H), 7.99 (d, $J = 15.7$ Hz, ^2H), 7.24 (s, ^2H), 7.12 (d, $J = 16.2$ Hz, ^2H), 7.04 (d, $J = 8.8$ Hz, 2H), 6.99 (d, $J = 8.9$ Hz, ^2H), 6.83 (s, ^1H), 3.84 (s, ^6H), 3.77 (s, ^6H); ^{13}C NMR (126 MHz, DMSO- d_6): δ 153.78, 152.66, 124.81, 117.32, 113.62, 56.63, 56.05; HRMS (ESI) m/z calc. for $\text{C}_{24}\text{H}_{24}\text{N}_2\text{O}_5$ $[\text{M} + \text{H}]^+$, 421.1770.

2.4. In silico Study

2.4.1. Protein structure retrieval



The sequence of CXCR4 chemokine receptor protein was retrieved from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The protein model with PDB ID: 3OE0 (PDB DOI: <https://doi.org/10.2210/pdb3OE0/pdb>) was chosen for active site predictions and further docking studies. For molecular docking the receptor structure was prepared by removing the co-crystallized ligand and other non-receptor heteroatoms (e.g., water, ions), using Argus Lab software (Hubbard, & Haider, 2010).

2.4.2. Protein active site predictions

Potential ligand-binding regions of CXCR4 were examined using Computed Atlas of Surface Topography of proteins (CASTp) database (<http://sts-fw.bioengr.uic.edu/castp/calculation.php>). The active site was analyzed and site having the binding amino acid Asp171 and Glu288 was chosen as for the docking study based on the predicted binding cavity and previously reported information on CXCR4 ligand recognition (Patwardhan et al., 2019). Analysis of the catalytic amino acids was done through Uniprot (<http://www.uniprot.org/uniprot/> P61073) (Primary Accession Number: P61073).

2.4.3. Ligand preparation

The three-dimensional (3D) structures of arylvinylpyrimidine and Doxorubicin were obtained from the PubChem database (<http://pubchem.ncbi.nlm.nih.gov/>). The positional coordinates of each molecule were retrieved in SDF format and converted into the required PDB format using the PRODRG server (<http://davapc1.bioch.dundee.ac.uk/prodrg/>). The molecular structures were subsequently optimized using Argus Lab software. Polar hydrogen bonds

were added during ligand preparation prior to docking (Kitchen et al., 2004).

2.4.4. Molecular docking

Molecular docking was performed using AutoDock 4.2 with the Lamarckian Genetic Algorithm (LGA). A population size of 150 and an energy evaluation limit of 2.5×10^7 were used (Zhang et al., 2012; Zhang et al., 2019). Ten independent docking runs were performed for each ligand, and the resulting poses were clustered using a 1.0 Å RMSD criterion. Grid maps were generated using AutoGrid with a $60 \times 60 \times 60$ point grid and a spacing of 0.375 Å. Polar hydrogens were added and Kollman charges were assigned during the docking preparation. The docked complexes were visualized using UCSF Chimera, and receptor residues located within approximately 5 Å of the docked ligand were examined for predicted ligand–receptor contacts (Zhang et al., 2019).

2.4.5. Interaction study

The 3D and 2D structure of the protein receptor–ligand complexes, which include, CXCR4–arylvinylpyrimidine and CXCR4–doxorubicin complexes, were examined using UCSF Chimera and LigPlot+, to identify predicted ligand–receptor interactions (Zhang et al., 2012). Hydrogen-bonding and hydrophobic contacts observed in the docked poses were analyzed (Hubbard & Haider, 2010).

2.5. In vitro bio-efficiency of arylvinylpyrimidine

2.5.1. Viability analysis

In this assay, the optimum number of cells were seeded in each well of 96 well plates after cell counting using viability dye such as trypan blue. Cells were counted by using a hemocytometer, under bright field microscopy. These experiments



were performed in triplicates and repeated six times.

For checking cellular toxicity, compound (AVP) were tested against normal cell lines (HEK-293 and VERO) with a fixed ($10 \mu\text{g mL}^{-1}$) or variable concentration (80, 40, 20, 10, 5 and $2.5 \mu\text{g mL}^{-1}$). In this experiment, we used DMSO as a growth control. The compounds were dissolved in DMSO and compared for growth in the presence of the anti-cancer cytotoxic compound, doxorubicin at $10 \mu\text{g mL}^{-1}$, which served as a positive cytotoxic control.

2.5.1.1. Seeding of cells in 96-well plates

After counting cells, a cell suspension was prepared (ranging from 5×10^3 to 10×10^3 cells mL^{-1}) in 10% FBS-containing cell growth medium (RPMI1640, EMEM and McCoy's medium). The desired number of cells (from 5×10^3 to 10×10^3 cells mL^{-1}) were added to each of 96 well plates in a $100 \mu\text{L}$ volume, and the cells were allowed to attach to the 96 well plates. Time for adherence of cells varies from 12 to 24 h, which depends on the doubling time of each cell.

2.5.1.2. Treatment in the cells

After appropriate hours of incubation till adherence, the cells were treated with compounds by preparing $10 \mu\text{g mL}^{-1}$ (from 10 mg mL^{-1} stock in DMSO) concentration in 5% FBS containing desired medium, a DMSO-containing suspension as a growth control and $10 \mu\text{g mL}^{-1}$ concentration of doxorubicin (from 10 mg mL^{-1} stock in DMSO) which used as a positive cytotoxicity control. $100 \mu\text{L}$ of prepared drug was transferred to the desired wells of 96 well plates. After treatment, plates were incubated at 37°C temperature in CO_2 incubator (5% CO_2 level), for 24-72 h, depending on the growth rate of the cells. After treatment during incubation, every day, the cells were

monitored for increased proliferation. The assay was stopped just before the cells reached confluency by removing the treatment media and rinse the cells with 1X Dulbecco's PBS, then completely aspirate off the PBS from each well of 96 well plates.

2.5.1.3. Effects on cancerous cell lines

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay performed for checking the anticancer activity of each compound at the desired concentration. After incubation, the absorption was measured at 590 nm by using Epoch™ Microplate spectrophotometer.

Because of the good anti-cancer activity in the primary screening, the compound further tested in different cancer cell lines, breast cancer cell lines (MCF-7, estrogen receptor positive and MDA-MB-468, triple negative breast cancer-TNBC), colon cancer cell lines (DLD-1 and HCT116) as well as lung cancer cell line (A549) at different concentrations ($2.5, 5, 10, 20, 40$ and $80 \mu\text{g mL}^{-1}$). These experiments were performed in triplicates and repeated six times.

2.5.2. Effects on bacteria (*Escherichia coli*) activity

Antibacterial activity was recorded for *E. coli* (DH5-Alpha) upon treatment with 4,6-bis((E)-2,5-dimethoxystyryl) pyrimidin-2-ol ($10 \mu\text{g mL}^{-1}$) on *E. coli* culture on agar plate. 100 mL of LB liquid was prepared in a 500 mL Erlenmeyer flask then added 1.5% of agar powder and mixed by swirling. Autoclaved at 121°C for 15 minutes. Poured 25 mL LB agar into the petri dish to completely cover the bottom of the dish and replaced the lid. Growth of *E. coli* upon treatment with 4,6-bis((E)-2,5-dimethoxystyryl) pyrimidin-2-ol ($10 \mu\text{g mL}^{-1}$) on *E. coli* culture on LB agar plate and control non-treated LB plate. These



experiments were performed in triplicates and repeated six times.

2.6. In vivo bio-efficiency of arylvinylpyrimidine

Adult *H. fossilis* (25-30g) irrespective of sex, were collected live from the local fisherman. They were acclimatized for one week, under natural photoperiod (12h:12h; light: dark) and temperature (23 ± 2 °C) in laboratory conditions. They were fed ad libitum with goat liver. After 24 h interval water was changed to avoid waste accumulation.

All experimental protocols involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) (2352/PO/Re/S/2025/CCSEA). The study was performed in compliance with the applicable institutional guidelines and relevant regulatory standards.

2.6.1. Concentration Study

To get effective concentration, acclimatized adult catfish *H. fossilis* were incubated for 24h in different concentrations of synthesised arylvinylpyrimidine molecule (1.0 pg mL^{-1} - 1.0 mg mL^{-1} , stock solution in water, dissolved in luke warm water) (five fish in each concentration and in control) to get effective concentration based on hematology. Experimental sets maintained in triplicates.

2.6.2. Duration Study

Effective concentration of synthesized arylvinylpyrimidine ($0.1 \text{ } \mu\text{g mL}^{-1}$) was tested further for effective duration study (1, 5, and 21 day) of drug over fish (five fish in each duration set, maintained in triplicates) along with control set for comparison.

2.6.3. Post-exposure Recovery Study

In this experimental setup, five fish was treated for 21 days with arylvinylpyrimidine ($0.1 \text{ } \mu\text{g mL}^{-1}$) then fish was maintained in fresh water for seven day (one week) without drug to assess its post-exposure recovery from fish system (to assess drug's short term or long-term effect in fish health). Experimental sets maintained in triplicates. The media was changed with fresh dose of chemicals every 24 h to maintain the same chemical constituents and to avoid any accumulation of metabolic wastes. After the completion of the experiment, fish were anaesthetized with clove oil, sacrificed and sampling was done as per analysis requirements.

2.6.4. Sample collection

After the completion of the experiments, fish were anaesthetized with clove oil, and fish blood was taken in heparinized tube for direct hematological parameter study. The RBC/WBC was counted with hemocytometer slide (Neuber chamber). Hemoglobin estimation (Hb) was done by Sahli pipette. Mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), hematocrit (Hct) estimated indirectly (formula-based quantification).

Anaesthetized fish were sacrificed and vital tissues (liver, kidney, gill) responsible for detoxification and muscle as main edible portion of fish were dissected out for myeloperoxidase (MPO) and nitroblue tetrazolium (NBT) activities. The NBT assay (Kumari, & Sahoo, 2006) performed to measure produced reactive oxygen species. The MPO is a peroxidase enzyme and produces hypochlorous acid (HOCl) from hydrogen peroxide (H_2O_2) and chloride anion (Cl^-) during the neutrophil's respiratory burst (Palic et al., 2005).

2.7. Statistical analysis



Data were analyzed with Microsoft Excel version 2407 and SPSS software. Data were represented as Means $\pm$ SEM. The significance of values obtained from different groups tested by using one-way analysis of variance (ANOVA; $P < 0.001$). Intergroup comparisons were done by Newman-Keuls' test ($P < 0.05$). Groups sharing the same letters or numbers or roman numerals indicate no significant differences, while different letters or numbers or roman numerals show significant differences.

3. Results

3.1. Chemistry of arylvinylpyrimidine

The synthesis of target compound was outlined in scheme 1. Firstly, acetylacetone and urea react in the presence of Lewis acid TFA, and form an intermediate which undergoes reaction with aldehyde in the same pot to give arylvinylpyrimidine in good yield. In the ^1H NMR spectrum of compound 4, the signal at δ 11.71 ppm is attributed to the phenolic OH protons. The signals in the aromatic/vinylic region were observed at δ 7.99, 7.24, 7.12, 7.04, 6.99, and 6.83 ppm, while the methoxy groups appeared at δ 3.84 and 3.77 ppm. The large coupling constants of the vinylic protons (approximately $J = 16.2$ Hz) support the trans configuration of C=C bonds. Similarly, in the ^{13}C NMR spectrum, seven clearly resolved resonances were observed at approximately δ 153.78, 152.66, 124.81, 117.32, 113.62, 56.63, and 56.05 ppm. The resonances around δ 56–57 ppm are attributed to methoxy carbons. The signals in the δ 113–154 ppm region correspond to the pyrimidine, aromatic, and vinylic carbon environments. Because the molecule contains two identical aryl-vinyl units, several carbon atoms are chemically equivalent or have similar chemical environments (Figure 1-4).

The hybrid molecule arylvinylpyrimidine, with two methoxy group at 2nd and 5th position of benzene ring increase the electron density of the molecule which in turn increase the liberation of hydrogen radical from the hydroxyl group present at the 2nd position of the pyrimidine ring and hence help improved solubility (Scheme 1, Table 1).

3.2. Molecular docking study

The CXCR4 protein (3OE0) was selected for computational docking study. Three dimensional structures of the test ligand, arylvinylpyrimidine and the reference standard, doxorubicin were selected from PubChem databases and were screened against the functional site of 3OE0 through AutoDock 4.2 software.

The active site predictions carried out using CASTp clearly showed the possible binding sites. The ADMET analysis of the test ligand showed that it not only follows the Lipinski Rule, rather also shows clogP and molecular weight to be approximately 4.675 and 421.17 respectively. On the other hand, ADMET analysis of the reference standard, doxorubicin showed value of 1.208 and 543.17, respectively. In this study, it was found that the arylvinylpyrimidine showed better binding affinity as compared to the reference ligand (Figure 5). After molecular docking analysis, arylvinylpyrimidine and doxorubicin, showed the binding energy of -7.73 and -5.30 KCal/mol, respectively (Table 2).

The amino acids involved in the interactions of arylvinylpyrimidine and CXCR4 are Leu253, Leu208, Phe249, Leu246, Ile245, Tyr219, Val242, Ile215, Gly212, Ile213, Ile209, with no hydrogen bonds. On the other hand, doxorubicin showed one hydrogen bond interaction with Ser260 and together with hydrophobic interactions involving Leu216, Leu253, Leu246, Phe249, Ile215, Leu208, Gly212, Ile209 and Ile213 (Table 2).



3.3. In vitro bio-efficiency of arylvinylpyrimidine

3.3.1. Viability and anti-cancer activity

Arylvinylpyrimidine showed 57.81 ± 6.04 % cell growth inhibition in estrogen receptor-positive MCF-7 breast cancer cell line, 73.36 ± 2.83 % inhibition in triple-negative cell line (TNBC) MDAMB468, 67.83 ± 0.47 % inhibition in DLD-1 colon cancer cell line and 36.10 ± 0.77 % inhibition in lung cancer cell line at $10 \mu\text{g mL}^{-1}$ concentration. Arylvinylpyrimidine showed 56.29 ± 1.11 % cytotoxicity in HEK293 cell line whereas in VERO (African green monkey kidney) cell line the compound showed 2.59 ± 0.48 % cytotoxicity at $10 \mu\text{g mL}^{-1}$ concentration. In this experiment, we used doxorubicin as a positive control which showed 60.38 ± 9.5 % inhibition in MCF-7 cell line, 84.79 ± 2.26 % inhibition in MDAMB468 cell line, 62.73 ± 13.58 % inhibition in DLD-1 cell line, 69.78 ± 10.78 % inhibition in HCT116 cell line, 52.07 ± 1.19 % inhibition in A549 cancer cell line whereas in normal cell line doxorubicin showed 58.71 ± 0.00 % cytotoxicity in HEK293 cell ($F=11.32$; $P < 0.05$; Figure 6 and 7).

3.3.2. Antibacterial activity

To analyses the antibacterial potential of the synthesized compound AVP, *E. coli* (DH5-Alpha) was cultured on LB agar plates supplemented with the compound at a concentration of $10 \mu\text{g mL}^{-1}$. Control plates were prepared using LB agar without the test compound. Following incubation at 37°C for 18–24 h, a significant reduction in bacterial colony formation was observed on the treated plates compared to controls ($F=19.9$; $P < 0.05$; Figure 8). The compound-treated plates showed clear growth inhibition zones and decreased colony density, indicating effective suppression of *E. coli* growth. In contrast, control plates without the compound displayed uniform and dense bacterial growth across the agar surface.

This inhibitory effect was reproducible across all replicates, with consistent outcomes observed in triplicate experiments repeated six times independently. These findings suggest that 4,6-bis((E)-2,5-dimethoxystyryl) pyrimidin-2-ol exhibits reliable antibacterial activity against *E. coli* (DH5-Alpha) at the tested concentration.

3.4. In-vivo bio-efficiency of arylvinylpyrimidine in catfish *H. fossilis*

3.4.1. Effect on blood parameters (RBC, Hb, MCV, MCHC, hematocrit, MCH)

The hybrid molecule, arylvinylpyrimidine, demonstrated a concentration dependent ($1 \mu\text{g mL}^{-1}$, 1 ng mL^{-1} , $0.1 \mu\text{g mL}^{-1}$, 0.01 mg mL^{-1} , 1 mg mL^{-1}) effect on the hematological parameters of catfish. RBC increased in all tested concentrations with a significant increase ($P < 0.05$) in $0.1 \mu\text{g mL}^{-1}$ concentration of drug ($F = 15.54$; Table 3). Hb, Hct, and MCHC levels were high across all concentrations compared to the control, with significant increases ($P < 0.05$) at 1 ng mL^{-1} and $0.1 \mu\text{g mL}^{-1}$ (Hb, $F = 15.67$; Hct, $F = 14.40$; MCHC, $F = 16.42$). The rest of the concentrations showed no significant changes compared to the control. Indirect haematology parameters, MCV ($F = 17.27$) and MCH ($F = 16.42$) remained significantly low ($P < 0.05$) in 0.01 mg mL^{-1} , 1 mg mL^{-1} concentrations as compared to control and insignificant with $0.01 \mu\text{g mL}^{-1}$, 1 pg mL^{-1} , 1 ng mL^{-1} . Concentration of $0.1 \mu\text{g mL}^{-1}$ considered as the effective concentration to assess the duration and post-exposure recovery effect of AVP. We did not find fish mortality in any tested concentrations of hybrid compound arylvinylpyrimidine. All fish were in normal behavior and swimming pattern in all tested concentrations of arylvinylpyrimidine.

In a duration study, an effective concentration of $0.1 \mu\text{g mL}^{-1}$, arylvinylpyrimidine significantly



increased ($P < 0.05$) the total RBC count from day 1 of treatment, and this increase was maintained throughout the recovery period ($F = 168.83$; Figure 9). Similarly, Hb, Hct, and MCHC levels showed a significant ($P < 0.05$), time-dependent increase, reaching high levels at 21 days of treatment. Even in the recovery group, these parameters remained elevated compared to the control and remained same as day 21 (Hb, $F = 24.63$; Hct, $F = 24.623$; MCHC, $F = 23.16$; Figure 9). However, MCV and MCH show a significant decline ($P < 0.05$) starting from day 5 of treatment, and this reduction persisted in the recovery group compared to both day 1 and control (MCV, $F = 25.284$; MCH, $F = 24.223$; Figure 9). All these parameter's changes indicate a progressive hematological response to arylvinylpyrimidine exposure, with prolonged effects that persist even after discontinuation of treatment (Figure 9).

3.4.2. Effect on non-specific immunity parameters (WBC, NBT, MPO)

The hybrid molecule, arylvinylpyrimidine, significantly reduced ($P < 0.05$) total WBC count in a duration dependent manner from day 5 onwards and low count maintained in recovery group ($F = 11.64$; Figure 10 A). Both MPO and NBT decreased significantly ($P < 0.05$) in the tested tissues and muscles, showing supportive to health after AVP treatment to catfish. In muscles, MPO and NBT decreased within one day of hybrid compound treatment in a duration dependent manner and maintained a significant low level ($P < 0.05$) in recovery period as compared to control group (MPO, $F = 10.46$; NBT, $F = 12.13$; Figure 10 B, C). In the kidney, MPO decreased significantly ($P < 0.05$) after day 5 onwards but NBT registered a significant fall ($P < 0.05$) from day 1 onwards (MPO, $F = 11.81$; NBT, $F = 12.34$; Figure 10 B, C). In the liver, MPO decreased significantly ($P < 0.05$) after day 5 onwards but

NBT registered significant fall ($P < 0.05$) from day 1 onwards (MPO, $F = 11.27$; NBT, $F = 13.78$; Figure 10 B, C). In gill, MPO decreased significantly ($P < 0.05$) after day 5 onwards but NBT registered significant fall ($P < 0.05$) on day 1 and so on (MPO, $F = 18.44$; NBT, $F = 14.11$; Figure 10 B, C). In the post-exposure recovery group the level of NBT and MPO were lower as compared to control in tested tissues (Figure 10).

4. Discussion

The development of multifunctional therapeutic or nutraceutical agents is crucial for aquaculture health challenges, particularly for economically demanding species like *H. fossilis*. A arylvinylpyrimidine compound that combines the structural frameworks of curcumin and pyrimidine to overcome monomolecules bio-restrictions with enhance bioavailability, to increase synthetic drug potential as a synergic impact. The results of the study suggested that the AVP has shown anticancer and antibacterial properties *in vitro* and improved bio-potential in aquatic animal health contexts. The compound was found to be soluble in multiple solvents compared to curcumin, which likely contributes to its improved bioavailability. This is consistent with earlier observations that methoxy substitutions increase the electron density and biological interactions of curcumin analogs (Sandur, 2011).

The current synthesis of arylvinylpyrimidine involved comparatively economic and single pot system compared to earlier reported work for the synthesis of pyrimidine derivatives (Liu et al., 2007; Gao et al., 2012). Molecular docking studies with CXCR4 (3OE0), a chemokine receptor implicated in tumor growth and immune cell migration, suggested that the hybrid molecule effectively binds (7.73 kcal/mol), interacting with key residues. The interactions are comparable to those seen with known anticancer drugs like



doxorubicin (Kitchen, et al. 2004). A computation technique is used to predict how a protein (target) interacts with molecules (legend). The molecular docking study showed the binding affinity of the legend. Calculations through CASTp showed the binding sites, containing the catalytic aspartic acid and glutamic acid residues that were found to be highly conserved in CXCR4 and were further taken into consideration for docking. The test ligand had high lipophilicity, which is good membrane permeability as compared to the standard. On the other hand, molecular weight of the test ligand is less as compared to the standard, making it less bulky. These results make the test ligand, a better candidate for the further analysis. The computational analysis predicts the affinity of the test ligand towards the target receptor, CXCR4, in terms of the binding energy of the ligand, for that protein.

More the negative binding energy, higher is the affinity of the test ligand towards the receptor. In our study, we found out that the arylvinylpyrimidine showed better binding strength as compared to doxorubicin. After molecular docking analysis, arylvinylpyrimidine and doxorubicin, showed the binding energy of -7.73 and -5.30 KCal/mol, respectively. While binding energy is a key factor, it's not the sole determinant of a legend's inhibitory potential. The stability of protein-legend interactions, particularly hydrogen bonding, also plays a critical role. Analysis of the protein-ligand complex using Chimera revealed key hydrogen bond interactions that contribute to the binding strength. Adding to this, hydrogen bonds often are specific and stronger but, binding as well as structure stability is provided by the hydrophobic interactions (Hubbard & Haider, 2010; Patwardhan et al., 2019). Interestingly, both the test ligand and the standard interacted with the receptor near the same binding pocket. The amino acids involved in the

interactions of arylvinylpyrimidine and CXCR4 are Leu253, Leu208, Phe249, Leu246, Ile245, Tyr219, Val242, Ile215, Gly212, Ile213, Ile209, with no hydrogen bonds. On the other hand, doxorubicin showed one hydrogen bond with Ser260 and lesser hydrophobic interactions with Leu216, Leu253, Leu246, Phe249, Ile215, Leu208, Gly212, Ile209 and Ile213. In the present investigation, through an *in silico* approach, arylvinylpyrimidine could provide a base for the development of novel leads with better affinity and specificity against CXCR4 as compared to the well-known standard drug, doxorubicin.

In vitro viability assays validated will cytotoxicity among normal cell lines. Whereas, the hybrid compound showed inhibition in tested cancer cell lines, including MDA-MB-468 and DLD-1, which was nearly equal to doxorubicin. Meanwhile, in colorectal carcinoma cell line, AVP showed higher cytotoxicity as compared to doxorubicin, while in VERO cells, a normal cell line, no significant cytotoxicity was found. Its selective cytotoxicity opens new exploration sites (Riss et al., 2004).

As germs become more resistant to antibiotics, researchers are synthesizing new compounds with high property of antibiotics. Therefore, synthesis of heterocyclic compounds with antimicrobial properties gained much attention in recent years (Gupta et al., 2013; Adamczak et al., 2020; Khanam et al., 2025). Arylvinylpyrimidine compound qualitatively inhibits *E. coli* growth, showing a reduction in bacterial proliferation compared to control. Inhibition of bacterial colony growth, in presence of the compound, making it a good candidate for further investigation of antimicrobial properties.

Hybrid compound with curcumin and pyrimidine moieties (AVP) showed a concentration dependent effect on haematological parameters of adult catfish *H. fossilis*. It was effective in moderate



concentration. Though there were no visible changes in fish behavior in higher or lower concentrations. The effective concentration of arylvinylpyrimidine positively influences various hematological parameters, including RBC count, Hb levels, Hct and indices such as MCV, MCH, and MCHC as curcumin (Ashry et al., 2021; Ali et al., 2025b). The RBC increase suggested compound effect in recruitment of RBC to support increased oxygen demand of improved tissue action or metabolism by increasing Hb, Hct and MCHC (Manju et al., 2013). Curcumin controls haematological parameters to support overall health in fish (Yonar et al., 2019), mammal (Yousef et al., 2010). The MCV, and MCH low value after arylvinylpyrimidine treatment supported further improved health status. These findings suggest that arylvinylpyrimidine supports the blood quality of catfish, which is crucial for maintaining nutritional and productivity of aquaculture.

The AVP effect on non-specific immunity, as shown by its impact on WBC count, NBT activity, and MPO levels, suggest involvement in fish health (Cheng et al., 2011; Tahmasebi-Kohyani et al., 2012; Hossain et al., 2016, Mokhtar et al., 2023). Arylvinylpyrimidine treatment led to a gradual decrease in NBT and MPO activity over time in the muscles and kidneys, compared to the untreated control fish, with a mild increase during the recovery period. However, in liver and gills, remained low as long as under compound exposure, and maintained during the recovery phase, which was still lower than in the control group. The supplementation of a nucleotide-based diet led to a decrease in non-specific immune parameters, indicating a time-dependent reduction in oxidative stress and immune activity (Sakai et al., 2001; Rohmah et al., 2022). It is reported that pyrimidine and its derivative registered repair in

hypoxia induced neuro-damage in freshwater catfish (Ali and Mishra, 2026). In the present study different parameters improvement suggest that AVP may reduce inflammatory activity or immune suppression that may further help fish to strengthen defense against pathogens, which may support aquaculture practices.

The lower concentrations of the AVP had more pronounced biological effects than higher doses. This inverse dose-response has been noted in studies involving other phytochemical-based therapeutics (Maheshwari et al., 2006). The post-exposure recovery study suggested that effects gradually returned to baseline levels of treatment, a desirable trait for sustainable aquaculture treatments.

CONCLUSION

The synthetic arylvinylpyrimidine molecule has both moieties of curcumin and pyrimidine, and shown their synergistic effect by improved bioavailability and *invitro* and *invivo* studied parameters. Based on bio-screening, hybrid AVP, shown preliminary anti-cancerous, anti-bacterial properties and a health supporter for edible freshwater catfish *H. fossilis*. Further detailed study is required to explore possible nutraceutical or therapeutic role in aquaculture.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, or analysis, and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work.



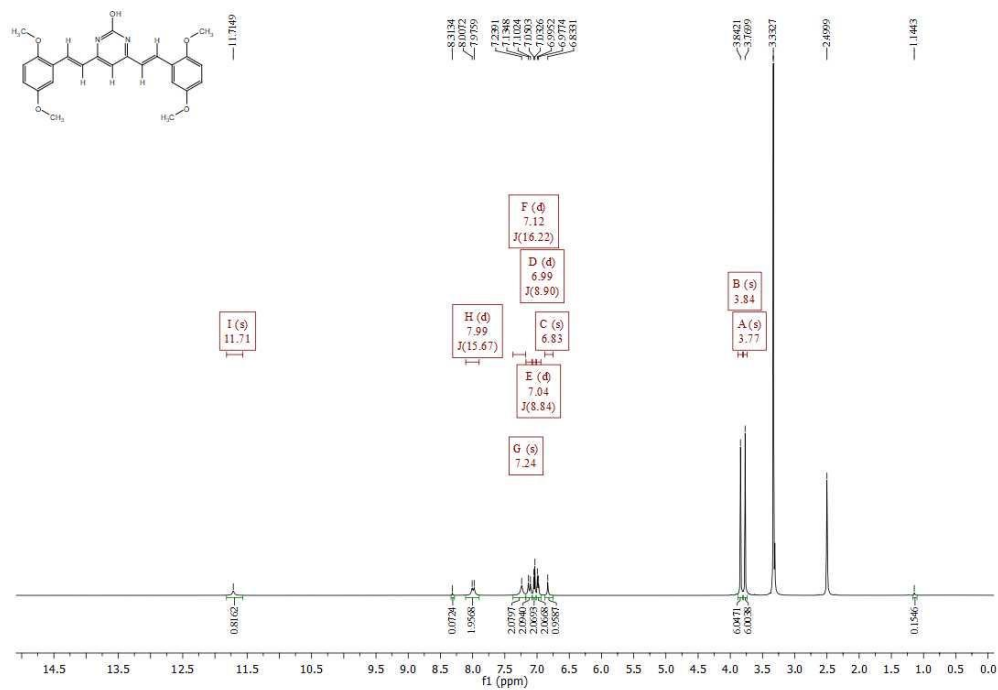


Figure 1. ^1H NMR spectrum of arylvinylpyrimidine recorded in DMSO-d_6 .

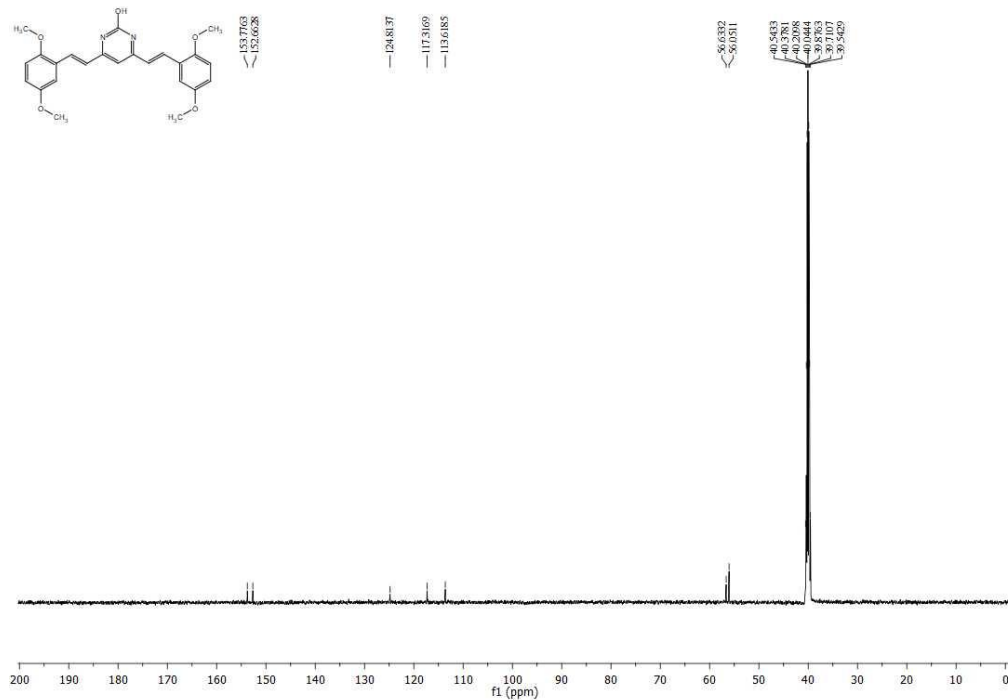


Figure 2. ^{13}C NMR spectrum of arylvinylpyrimidine recorded in DMSO-d_6 .

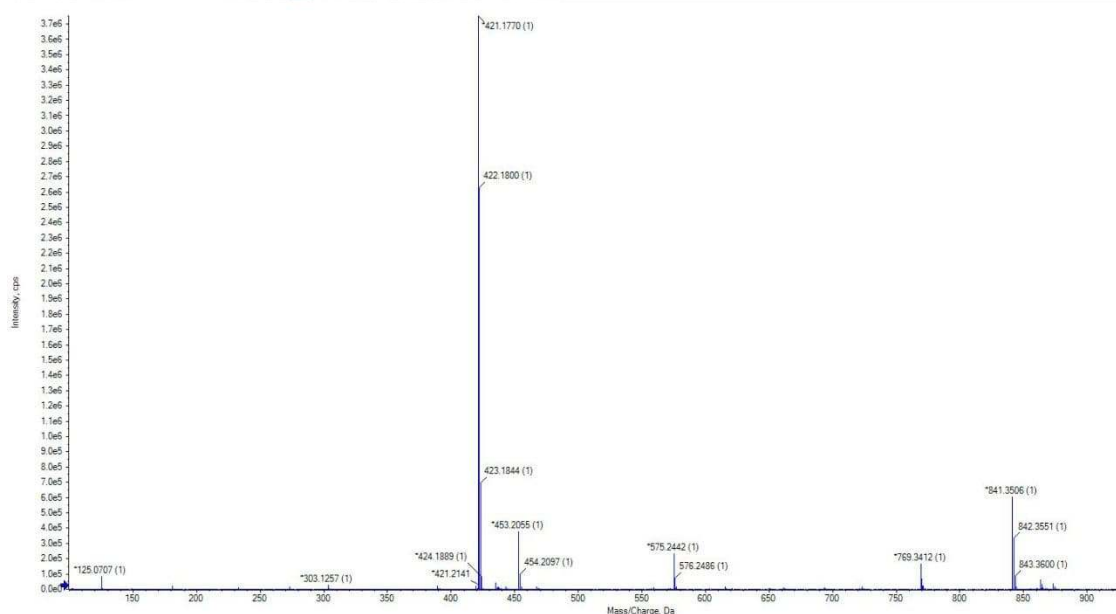


Figure 3. HRMS (ESI) spectrum of arylvinylpyrimidine.

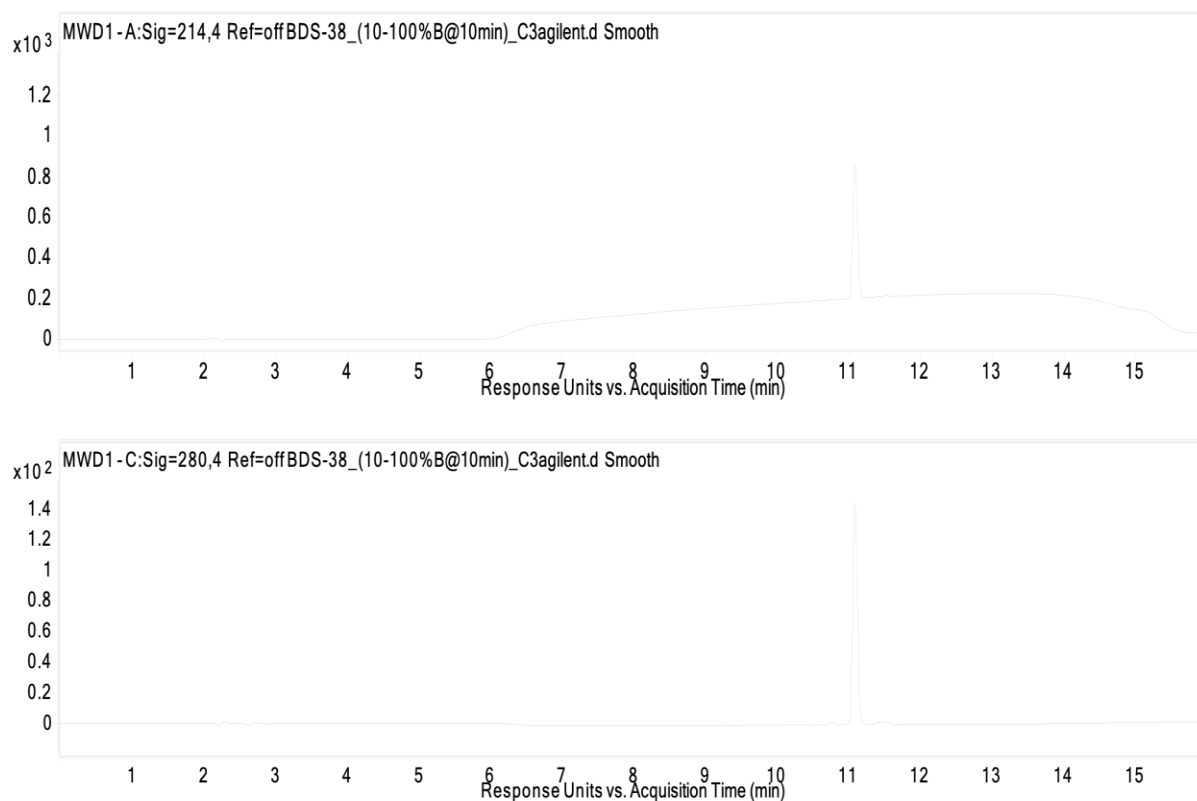
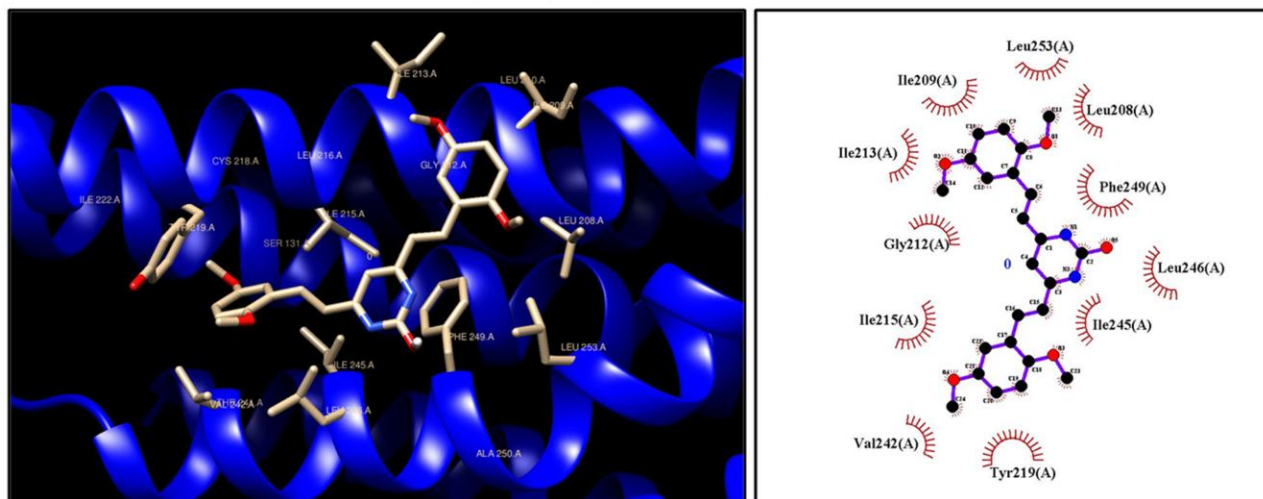


Figure 4. HPLC chromatograms of arylvinylpyrimidine recorded at (a) 214.4 nm and (b) 280.4 nm.

ArylVinylPyrimidine – CXCR4



Doxorubicin – CXCR4

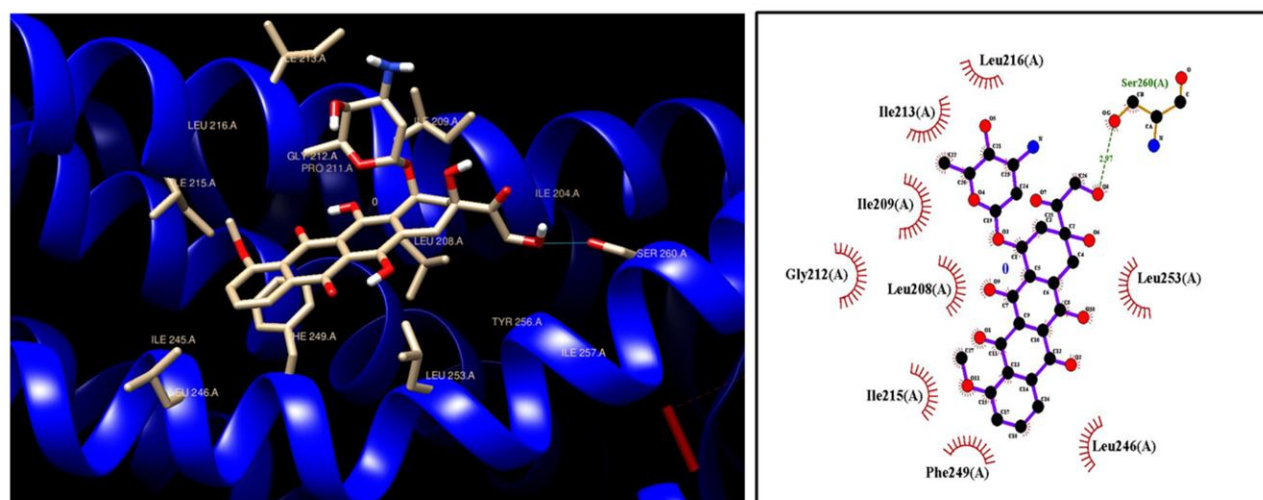


Figure 5. Arylvinylpyrimidine with an *in silico* approach to find affinity and specificity against CXCR4 as compared to standard drug doxorubicin.

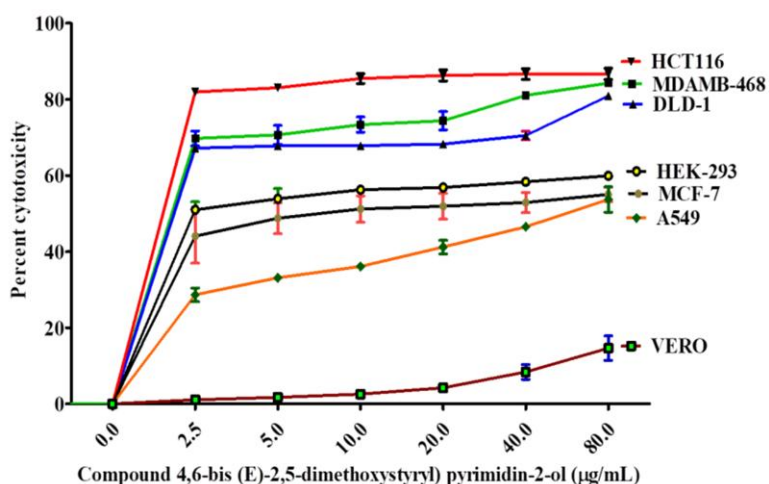


Figure 6. Concentration-dependent activity of 4,6-bis(E)-2,5-dimethoxystyryl pyrimidin-2-ol (arylvinylypyrimidine) against different cancer cell lines {Breast cancer cell line, MCF-7 (estrogen receptor-positive) and MDA-MB-468 (triple-negative breast cancer, TNBC); colon cancer cell lines, DLD-1 and HCT116; lung cancer cell line, A549} as well as in the normal cell lines (HEK-293 and VERO). These experiments were performed in triplicate and repeated six times. The data are expressed as Mean $\pm$ SEM, indicating the percentage of cytotoxicity.

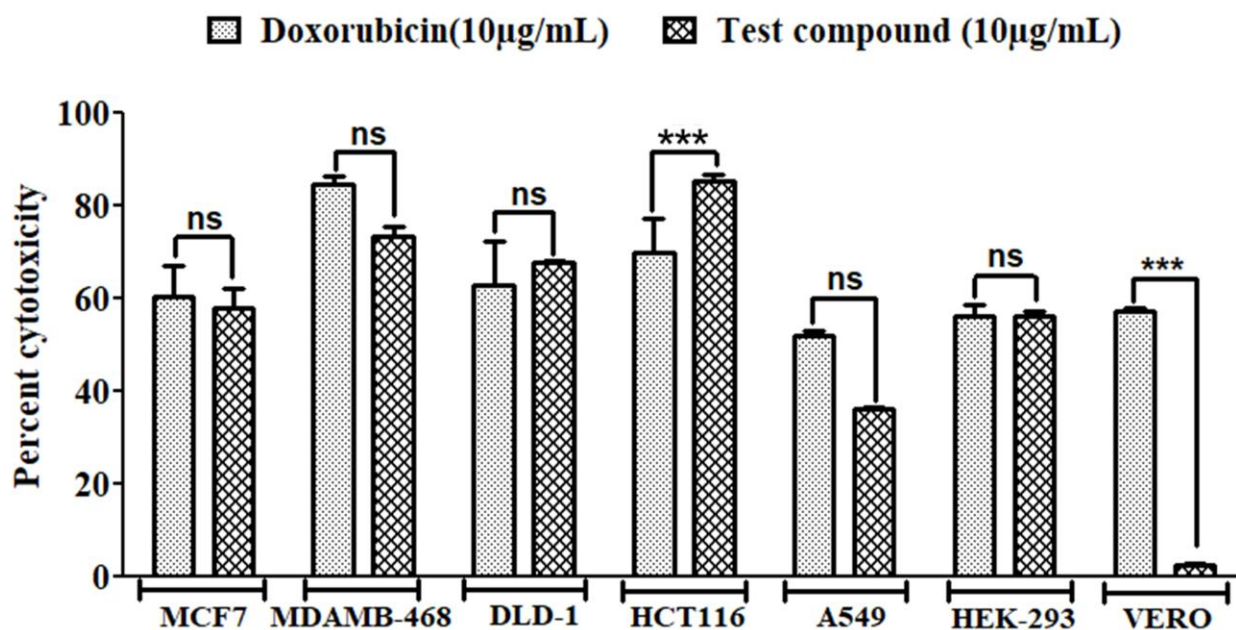


Figure 7. Cytotoxicity of 4,6-bis(E)-2,5-dimethoxystyryl pyrimidin-2-ol (arylvinylypyrimidine, 10 $\mu\text{g mL}^{-1}$) and doxorubicin (10 $\mu\text{g mL}^{-1}$) against different cancer cell lines {breast cancer cell line, MCF-7 (estrogen receptor-positive) and MDA-MB-468 (triple-negative breast cancer, TNBC); colon cancer cell lines, DLD-1 and HCT116; lung cancer cell line, A549} as well as in the normal cell lines (HEK-293 and VERO). These experiments were performed in triplicate and repeated six times. The data are presented as Mean $\pm$ SEM. Statistical significance between groups was evaluated using one-way ANOVA ($P < 0.001$). Astrisks shows the significant and 'ns' shows not significant in between the doxorubicin and arylvinylypyrimidine compound.

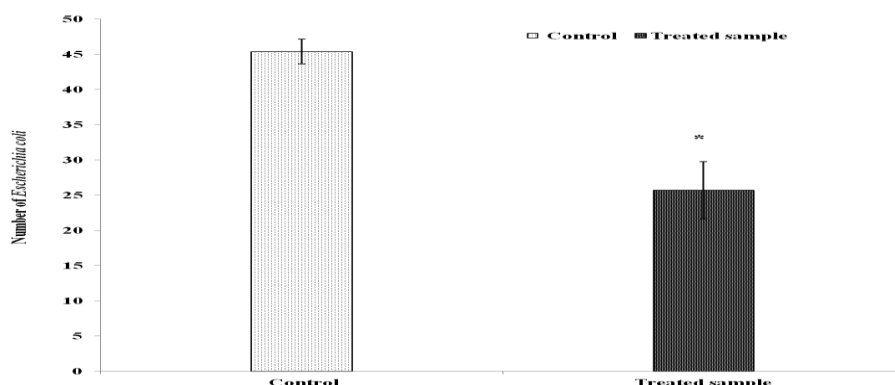


Figure 8. Effect of 4,6-bis(E)-2,5-dimethoxystyryl pyrimidin-2-ol (arylvinylypyrimidine, 10 $\mu\text{g mL}^{-1}$) on growth of Escherichia coli (DH5-Alpha) (A) control, (B) treated samples, arylvinylypyrimidine (10 $\mu\text{g mL}^{-1}$). These experiments were performed in triplicate and repeated six times. The data are presented as Mean $\pm$ SEM. Statistical significance between groups was evaluated using one-way ANOVA ($P < 0.001$). Astrisks shows the significant between without and with arylvinylypyrimidine compound treated Escherichia coli (DH5-Alpha) growth.

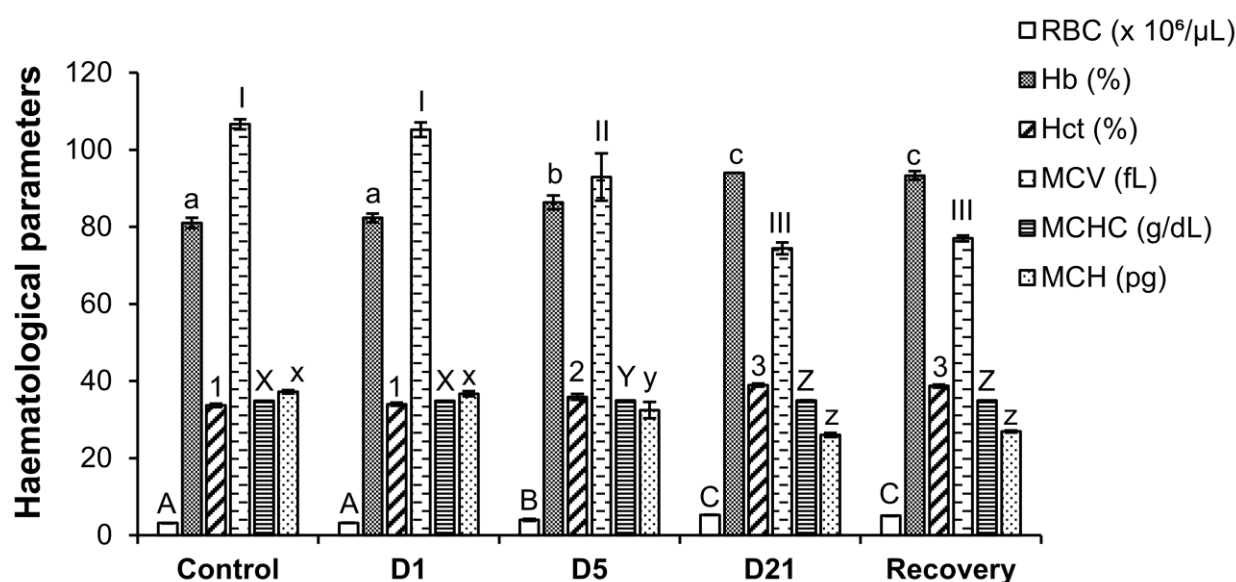


Figure 9. Effect of exposure time of arylvinylypyrimidine ($0.1 \mu\text{g mL}^{-1}$) on haematology of freshwater catfish, *Heteropneustes fossilis*. Recovery group, had 21 days pre-treated fish [with arylvinylypyrimidine ($0.1 \mu\text{g mL}^{-1}$)], followed by maintenance for one week in water only. Each set had five fish ($n=5$). Experiments repeated in triplicates. Values expressed as Mean $\pm$ SEM.

Data were analyzed by one way ANOVA ($P < 0.001$). Capital letters A, B and C shows intergroup comparison between RBC. Small letters a, b, and c show an intergroup comparison between Hb. Numbers 1, 2, and 3 show an intergroup comparison between Hct. Roman I, II and III show an intergroup comparison between MCV. Capital letters X, Y and Z shows intergroup

comparison between MCHC. Small letters x, y, and z show an intergroup comparison between MCH. Groups superscripted with the same letters, Roman, numbers are non-significant, and those

with different letters, Roman, numbers are significantly different in intergroup comparison (Newman-Kuels test, $P < 0.05$).

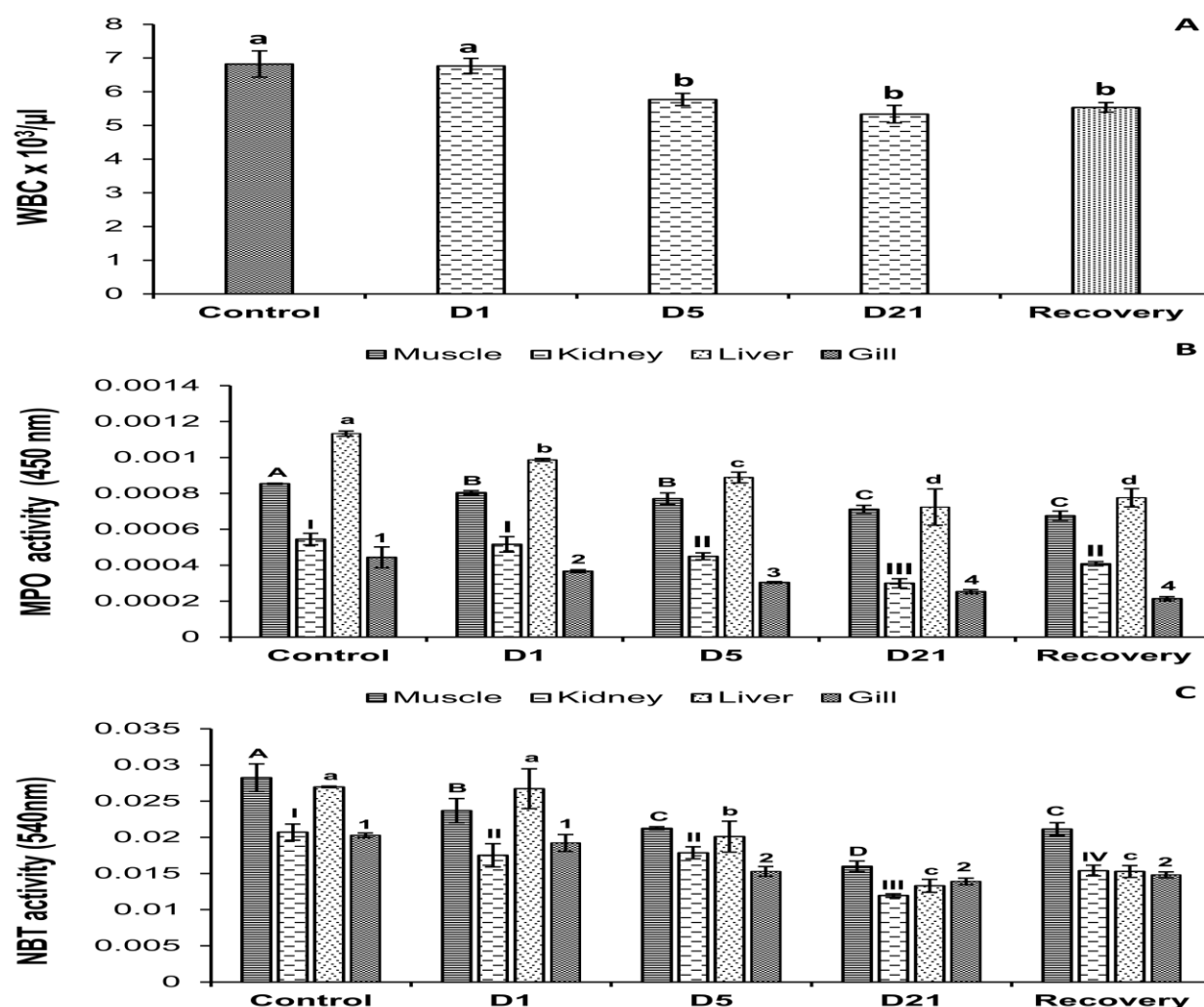


Figure 10. Effect of exposure time of arylvinylpyrimidine ($0.1 \mu\text{g mL}^{-1}$) on indirect immunity parameters of freshwater catfish, *Heteropneustes fossilis*. Recovery group, had 21 day pre-treated fish [with arylvinylpyrimidine ($0.1 \mu\text{g mL}^{-1}$)], for one week in water only. (A) white blood corpuscles (WBC); (B) myeloperoxidase (MPO); and (C) nitroblue tetrazolium (NBT) assay in muscle, liver, kidney, and gill. Each duration set had five fish along with control ($n=5$). Experiments repeated in triplicates. Values expressed as Mean $\pm$ SEM. Data were analyzed by

one way ANOVA ($P < 0.001$). Capital letters A, B and C shows intergroup comparison between muscle. Roman I, II and III show an intergroup comparison between kidney tissue. Small letters a, b, and c show an intergroup comparison between liver tissue. Numbers 1, 2, and 3 show an intergroup comparison between gill tissue. Groups superscripted with the same letters, Roman, numbers are non-significant, and those with different letters, Roman, numbers are significantly different in intergroup comparison (Newman-Kuels test, $P < 0.05$).

Table 1. Qualitative solubility screening of curcumin and arylvinylpyrimidine at a test concentration of 1 mg/mL

S.No.	Solvent	Curcumin	Arylvinylpyrimidine
	Chloroform	Insoluble	Soluble
	Ethyl acetate	Insoluble	Soluble
	DMSO	Insoluble	Soluble
	Ethanol	Soluble	Soluble
	Water	Insoluble	Partially soluble

Solubility was assessed by adding 1 mg of sample to 1 mL of each solvent (1 mg/mL) under the experimental conditions used for the screening.

Table 2. Molecular docking analysis showing binding energies and interacting amino acid residues of CXCR4 with arylvinylpyrimidine and doxorubicin used for comparison.

S.No.	Ligand	Binding Energy (KCal/mol)	Interacting amino acids of Receptor
1	Arylvinylpyrimidine	-7.73	Leu253, Leu208, Phe249, Leu246, Ile245, Tyr219, Val242, Ile215, Gly212, Ile213, Ile209
2	Doxorubicin	-5.30	Leu216, Leu253, Leu246, Phe249, Ile215, Leu208, Gly212, Ile209 and Ile213

Table 3. Concentration effects of arylvinylpyrimidine, 4,6-bis((E)-2,5-dimethoxystyryl)pyrimidin-2-ol, (1 pg/mL, 1 ng/mL, 0.1 µg/mL, 0.01 mg/mL, 1 mg/mL) on direct and indirect haematological parameters of catfish *Heteropneustes fossilis* after 24 h.

Blood parameters	Control	Concentrations of arylvinylpyrimidine				
		1 pg/ml	1 ng/ml	0.1 µg/ml	0.01mg/ml	1 mg/ml
RBC ($\times 10^6/\mu\text{L}$)	4.4 $\pm$ 0.1	4.7 $\pm$ 0.1 ^{NS}	4.6 $\pm$ 0.9 ^{NS}	4.9 $\pm$ 0.9*	4.5 $\pm$ 0.9 ^{NS}	4.7 $\pm$ 0.6 ^{NS}



Hb (%)	71.2±1.0	76.2±1.0 ^N _S	78.6±1.0 [*]	79.0±1.1 [*]	72.2±1.0 ^N _S	74.8±1.1 _{NS}
Hct (%)	29.6±0.9	31.7±0.3 ^N _S	32.6±0.9 [*]	32.8±0.6 [*]	30.1±0.1 ^N _S	31.1±0.6 _{NS}
MCHC (g/dL)	34.7±0.6	34.8±0.1 _{NS}	34.8±0.4 [*]	34.8±0.5 [*]	34.7±0.7 _{NS}	34.7±0.9 _{NS}
MCV (fL)	67.2±1.3	67.1±1.4 ^N _S	70.2±0.9 _{NS}	66.1±0.1 ^N _S	65.6±0.9 [*]	65.8±0.1 [*]
MCH (pg)	23.3±0.7	23.5±0.3 ^N _S	24.4±0.9 _{NS}	23.0±0.4 ^N _S	22.8±0.4 [*]	22.9±0.1 [*]
WBC (×10 ³ /μL)	7.4±0.2	6.0±0.2 ^{NS}	5.2±0.8 ^N _S	4.4±0.2 [*]	6.6±0.2 _{NS}	6.3±0.1 _{NS}

Each concentration had minimum five fish (n=5). Experiment repeated in triplicates. Values expressed as mean ± SEM. Where, asterisk (*) shows level of significance as compared to control group (p<0.05). NS: Not significant as compared to control group. Where, RBC: Red blood cells, Hb: Haemoglobin, Hct: Hematocrit, MCHC: Mean corpuscular Haemoglobin concentration, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, WBC: White blood cell.

REFERENCES

- Adamczak, A., Ożarowski, M., Karpiński, T.M., 2020. Curcumin, a natural antimicrobial agent with strain-specific activity. *Pharmaceuticals*, 13, 153. <https://doi.org/10.3390/ph13070153>
- Aggarwal, B.B., Kumar, A., Bharti, A.C., 2003. Anticancer potential of curcumin: Preclinical and clinical studies. *Anticancer Research*, 23, 363–398. PMID: 12680238
- Al-Abdullah, E.S., Al-Obaid, A.R., Al-Deeb, O. A., Habib, E.E., El-Emam, A.A., 2011. Design, synthesis and biological evaluation of novel 4-aryl-1,2,3-triazole derivatives as potential anticancer agents. *European Journal of Medicinal Chemistry*, 11, 4642–4648. <https://doi.org/10.1016/j.ejmech.2011.08.003>
- Ali, B., Mishra, A., 2026. Mitigation of hypoxia-induced brain damage by pyrimidine and its derivative in *Heteropneustes fossilis*. *Journal of Basic and Applied Zoology*, 87, 42 10.1186/s41936-026-00581-7.
- Ali, B., Pandey, P., Pandey, J., Mishra, A., 2025b. Pyrimidine analogue effect on health and nutritional value of fresh water catfish, *Heteropneustes fossilis*. *Journal of aquatic Animal Health*, 37(1), 11–20. <https://doi.org/10.1093/jahafs/vsae003>
- Ali, B., Sharma, N., Gautam, R.K., Mishra, A., 2025a. Effects of pyrimidine on cellular and neuronal arrangement, oxidative stress and energy content in the brain of the freshwater catfish, *Heteropneustes fossilis*. *Fish*



- Physiology and Biochemistry, 51(2), 65. doi: 10.1007/s10695-025-01481-3.
7. Ali, B., Sharma, N., Gautam, R.K., Arya, S., Mishra, A., 2026. Remediation of hypoxia induced brain alteration with pyrimidine treatment in the freshwater catfish, *Heteropneustes fossilis*. *Fish Physiology and Biochemistry*, 52, 5. DOI:10.1007/s10695-025-01627-3
8. Amin, K.M., Hanna, M.M., Abo-Youssef, H. E., George, R.F., 2009. Synthesis and biological evaluation of some novel pyrazoline derivatives as potential anticancer agents. *European Journal of Medicinal Chemistry*, 12, 4572–4579. <https://doi.org/10.1016/j.ejmech.2009.06.028>
9. Amr, A.G.E., Mohamed, A.M., Mohamed, S.F., Abdel-Hafez, N.A., Hammam, A.E.F.G., 2006. Anticancer activities of some newly synthesized pyridine, pyrane, and pyrimidine derivatives. *Bioorganic & Medicinal Chemistry*, 14, 5481–5488. <https://doi.org/10.1016/j.bmc.2006.04.045>
10. Anand, P., Kunnumakkara, A.B., Newman, R.A., Aggarwal, B.B., 2007. Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics*, 4, 807–818. <https://doi.org/10.1021/mp700113r>
11. Anand, P., Sundaram, C., Jhurani, S., Kunnumakkara, A.B., Aggarwal, B.B., 2008. Curcumin and cancer: An ‘old-age’ disease with an ‘age-old’ solution. *Cancer Letters*, 267, 133–164. <https://doi.org/10.1016/j.canlet.2008.03.025>
12. Ashry, A.M., Hassan, A.M., Habiba, M.M., El-Zayat, A., El-Sharnouby, M.E., Sewilam, H., Dawood, M.A., 2021. The impact of dietary curcumin on the growth performance, intestinal antibacterial capacity, and haemato-biochemical parameters of gilthead seabream (*Sparus aurata*). *Animals*, 11, 1779. <https://doi.org/10.3390/ani11061779>
13. Baum, L., Ng, A., 2004. Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. *Journal of Alzheimer's Disease*, 6, 367–377. <https://doi.org/10.3233/JAD-2004-6403>
14. Boncel, S., Gondela, A., Walczak, K., 2008. Uracil as a target for nucleophilic and electrophilic reagents. *Current Organic Synthesis*, 4, 365–380. <https://doi.org/10.2174/157017908786241581>
15. Bryzgalov, A.O., Dolgikh, M.P., Sorokina, I.V., Tolstikova, T.G., Sedova, V.F., Shkurko, O.P., 2006. Antiarrhythmic activity of 4,6-di(het)aryl-5-nitro-3,4-dihydropyrimidin-(1H)-2-ones and its effects on arterial pressure in rats. *Bioorganic & Medicinal Chemistry Letters*, 6, 1418–1422. <https://doi.org/10.1016/j.bmcl.2005.11.043>
16. Cheng, Z., Buentello, A., Gatlin, III D.M., 2011. Dietary nucleotides influence immune responses and intestinal morphology of red drum *Sciaenops ocellatus*. *Fish and Shellfish Immunology*, 30, 143–147. <https://doi.org/10.1016/j.fsi.2010.09.019>
17. Dhillon, N., Aggarwal, B.B., Newman, R.A., Wolff, R.A., Kunnumakkara, A.B., Abbruzzese, J.L., Ng, C.S., Badmaev, V., Kurzrock, R., 2008. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clinical Cancer Research*, 14, 4491–4499. <https://doi.org/10.1158/1078-0432.CCR-08-0024>
18. Gao, L., Liu, Q., Ren, S., Wan, S., Jiang, T., Wong, I.L.K., Chow, L.M.C., 2012. Synthesis of a novel series of (E,E)-4,6-bis(styryl)-2-o-glucopyranosyl-pyrimidines and their potent multidrug resistance (MDR) reversal activity against cancer cells. *Journal of Carbohydrate Chemistry*, 31, 620–631. <https://doi.org/10.1080/07328303.2012.689041>



19. Gupta, Y.K., Gupta, V., Singh, S., 2013. Synthesis, characterization and antimicrobial activity of pyrimidine-based derivatives. *Journal of Pharmaceutical Research*, 7, 491–495. <https://doi.org/10.1016/j.jopr.2013.05.020>
20. Hossain, M.S., Koshio, S., Ishikawa, M., Yokoyama, S., Sony, N.M., 2016. Dietary nucleotide administration influences growth, immune responses and oxidative stress resistance of juvenile red sea bream (*Pagrus major*). *Aquaculture*, 455, 41–49. <https://doi.org/10.1016/j.aquaculture.2016.01.008>
21. Hubbard, R., Haider, M., 2010. Hydrogen bonds in proteins: role and strength. *Encyclopedia of Life Sciences*, 1, 1-6. <https://doi.org/10.1002/9780470015902.a0003011.pub2>
22. Jiang, T., Zhou, G.R., Zhang, Y.H., Sun, P.C., Du, Q.M., Zhou, P., 2012. Influence of curcumin on the Al(iii)-induced conformation transition of silk fibroin and resulting potential therapy for neurodegenerative diseases. *RSC Advances*, 2, 9106–9114. <https://doi.org/10.1039/c2ra21453a>
23. Joffe, A.M., Farley, J.D., Linden, D., Goldsand, G., 1989. Chemotherapeutic agents in cancer therapy. *The American Journal of Medicine*, 87, 332–338.
24. Kenner, G.W., Lythgoe, B., Todd, A.R., Topham, A.A., 1943. Some reactions of amidines with derivatives of malonic acid. *Journal of the Chemical Society*, 102, 388–395. <https://doi.org/10.1039/jr9430000388>
25. Keri, R.S., Hosamani, K.M., Shingalapur, R.V., Hugar, M.H., 2010. Analgesic, anti-pyretic and DNA cleavage studies of novel pyrimidine derivatives of coumarin moiety. *European Journal of Medicinal Chemistry*, 6, 2597–2602. <https://doi.org/10.1016/j.ejmech.2010.02.048>
26. Khanam, H., Pandey, J., Singh, B.D., Fatma, S., Pathak, A., Sharma, A., et al., 2025. synthesis and photophysical properties of D- π -A- π -D-2-hydroxy-4,6-bis-(arylvinyl)-pyrimidines based push-pull fluorophores: DFT studies and antibacterial activity. *Journal of Molecular Structure*, 1338, 142334. <https://doi.org/10.1016/j.molstruc.2025.142334>
27. Kim, S., Park, D.H., Kim, T.H., Hwang, M., Shim, J., 2009. Functional analysis of pyrimidine biosynthesis enzymes using the anticancer drug 5-fluorouracil in *Caenorhabditiselegans*. *The Federation of European Biochemical Societies Journal*, 276, 4715–4723. <https://doi.org/10.1111/j.1742-4658.2009.07168.x>
28. Kitchen, D.B., et al., 2004. Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature Reviews Drug Discovery*, 3, 935–949. <https://doi.org/10.1038/nrd1549>
29. Kumar, A., Li, L., Chaturvedi, A., Brzostowski, J., Chittigori, J., Pierce, S., et al., 2012. Two-photon fluorescence properties of curcumin as a biocompatible marker for confocal imaging. *Applied Physics Letters*, 100, 203701. <https://doi.org/10.1063/1.4717753>
30. Kumar, N., Chauhan, A., Drabu, S., 2011. Synthesis of cyanopyridine and pyrimidine analogues as new anti-inflammatory and antimicrobial agent. *Biomedicine & pharmacotherapy*, 5, 375–380. <http://dx.doi.org/10.1016/j.biopha.2011.04.023>
31. Kumari, J., Sahoo, P.K., 2006. Non-specific immune response of healthy and immunocompromised Asian catfish (*Clarias batrachus*) to several immunostimulants. *Aquaculture*, 255, 133–141.



- <https://doi.org/10.1016/j.aquaculture.2005.12.012>
32. Liu, Z., Chen, T., Liu, B., Huang, Z.L., Huang, T., Li, S., et al., 2007. Two-photon absorption of a series of V-shape molecules: the influence of acceptor's strength on two-photon absorption in a non-centrosymmetric D- π -A- π -D system. *Journal of Materials Chemistry*, 17, 4685–4690. <https://doi.org/10.1039/b707909e>
33. Maheshwari, R.K., Singh, A.K., Gaddipati, J., Srimal, R.C., 2006. Multiple biological activities of curcumin: A short review. *Life sciences*, 78, 2081–2087. <https://doi.org/10.1016/j.lfs.2005.12.007>
34. Manju, M., Vijayasree, A.S., Akbarsha, M.A., Oommen, O.V., 2013. Protective effect of dietary curcumin in *Anabas testudineus* (Bloch) with a special note on DNA fragmentation assay on hepatocytes and micronucleus assay on erythrocytes in vivo. *Fish Physiology and Biochemistry*, 39, 1323–1330. <https://doi.org/10.1007/s10695-013-9786-6>
35. Mazumder, A., Neamati, N., Sunder, S., Schulz, J., Pertz, H., Eich, E., Pommier, Y., 1997. Curcumin analogs with altered potencies against HIV-1 integrase as probes for biochemical mechanisms of drug action. *Journal of Medicinal Chemistry*, 40, 3057–3065. <https://doi.org/10.1021/jm970190x>
36. Menon, L.G., Kuttan, R., Kuttan, G., 1999. Anti-tumor and antioxidant activity of curcumin. *Cancer Letters*, 141, 159–165. [https://doi.org/10.1016/0304-3835\(95\)03827-j](https://doi.org/10.1016/0304-3835(95)03827-j)
37. Mokhtar, D.M., Zacccone, G., Alesci, A., Kuciel, M., Hussein, M.T., Sayed, R.K.A., 2023. Main components of fish immunity: an overview of the fish immune system. *Fishes*, 8, 93. <https://doi.org/10.3390/fishes8020093>
38. Nadal, E., Olavarria, E., 2004. Imatinib mesylate (Gleevec/Glivec) a molecular-targeted therapy for chronic myeloid leukaemia and other malignancies. *International Journal of Clinical Practice*, 58, 511–515. <https://doi.org/10.1111/j.1368-5031.2004.00173.x>
39. Nurfinal, A.N., Reksohadiprodjo, M.S., Timmerman, H., Jenie, U.A., Sugiyant, D., van der Goot, H., 1997. Synthesis and evaluation of new heterocyclic compounds. *European Journal of Medicinal Chemistry*, 32, 321–328. [https://doi.org/10.1016/s0223-5234\(97\)89084-8](https://doi.org/10.1016/s0223-5234(97)89084-8)
40. Otmar, M., Masojidkova, M., Votruba, I., Holy, A., 2004. Synthesis and antiproliferative activity of 2,6-diamino-9-benzyl-9-deazapurine and related compounds. *Bioorganic & Medicinal Chemistry*, 13, 3187–3193. <https://doi.org/10.1016/j.bmc.2004.04.003>
41. Palic, D., Andreasen, C.B, Menzel, B.W., Roth, J.A., 2005. A rapid, direct assay to measure degranulation of primary granules in neutrophils from kidney of Fathead Minnow (*Pimephales promelas* Rafinesque, 1820). *Fish and Shellfish Immunology*, 19, 217–227. <https://doi.org/10.1016/j.fsi.2004.12.003>
42. Pandey, P., Mishra, A., 2022. Curcumin influence on antioxidative stress and energy macromolecules of stinging catfish, *Heteropneustes fossilis* (Bloch, 1794). *Journal of Environment and Bio-sciences*, 36 (2), 105–113. <https://connectjournals.com/03843.2022.36.105>
43. Pandey, P., Mishra, A., 2022. Curcumin influence on antioxidative stress and energy macromolecules of stinging catfish, *Heteropneustes fossilis* (Bloch, 1794). *Journal of Environment and Bio-Sciences*, 36, 105–113. <https://connectjournals.com/pages/article/details/toc036932>



44. Pandey, P., Ali, B., Mishra, A., 2024. Bioaccumulation and clearance of curcumin from gill, liver, kidney, and muscle of freshwater catfish, *Heteropneustes fossilis*. *Journal of Zoology*, 13, 110-115. (ISSN: 2319-3883).
45. Pandey, P., Mishra, A., Pandey, J., 2022. Immunity and overall tissue health of stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) in curcumin medium. *Indian Journal of Nutrition and Dietetics*, 59 (2), 169-186. <https://doi.org/10.21048/IJND.2022.59.2.29282>.
46. Patwardhan, R.S., Checker, R., Sharma, D., Kohli, V., Priyadarsini, K.I., Bulusu, G., Desiraju, G., 2019. Strong and weak hydrogen bonds in protein-ligand recognition. *Journal of the Indian Institute of Science*, 100, 31-41. <https://doi.org/10.1007/s41745-019-00141-9>
47. Paul, B.N., Chanda, S., Sridhar, N., Saha, G.S., Giri, S.S., 2016. Fatty acid, amino acid and vitamin composition of Indian catfish, magur (*Clarias batrachus*) and singhi (*Heteropneustes fossilis*). *South Asian Association for Regional Cooperation Journal of Agriculture*, 14(2), 189-199. <https://doi.org/10.3329/sja.v14i2.31258>
48. Pegu, A., Kalita, R., Das, P., Baruah, C., 2023. Nutritional composition of small indigenous species of fishes of Northeast India: A systematic review. *Journal of Applied and Natural Science*, 15(2), 649. <https://doi.org/10.31018/jans.v15i2.4490>
49. Riss, T.L., Moravec, R.A., Niles, A.L., Duellman, S., Benink, H.A., Worzella, T.J., Minor, L., 2004. Assay guidance manual: cell viability assays. National Library of Medicine, Bethesda, MD, USA.
50. Rohmah, M.K., Salahdin, O.D., Gupta, R., Muzammil, K., Qasim, M.T., Al-Qaim, Z.H., et al., 2022. Modulatory role of dietary curcumin and resveratrol on growth performance, serum immunity responses, mucus enzymes activity, antioxidant capacity and serum and mucus biochemicals in the common carp, *Cyprinus carpio* exposed to abamectin. *Fish and Shellfish Immunology*, 129, 221-230. <https://doi.org/10.59429/ace.v8i1.5613>
51. Sakai, M., Taniguchi, K., Mamoto, K., Ogawa, H., Tabata, M., 2001. Immunostimulant effects of nucleotide isolated from yeast RNA on carp, *Cyprinus carpio* L. *Journal of Fish Diseases*, 24, 433-438. <https://doi.org/10.1046/j.1365-2761.2001.00314.x>
52. Sandur, S.K., 2011. Demethoxycurcumin, a metabolically stable analogue of curcumin, exhibits anti-inflammatory activities in murine and human lymphocytes. *Biochemical Pharmacology*, 82, 642-657. <https://doi.org/10.1016/j.bcp.2011.06.024>
53. Singh, B.D., Pandey, J., Khanam, H., Singh, S., 2022. Lewis acid catalyzed [3+ 3] annulation/aldol condensation in water: one pot synthesis of (E, E)-4, 6-bis (styryl)-pyrimidines. *Asian Journal of Organic Chemistry*, 11, p.e202200479. <https://doi.org/10.1002/ajoc.202200479>
54. Tahmasebi-Kohyani, A., Keyvanshokoo, S., Nematollahi, A., Mahmoudi, N., Pasha-Zanoosi, H., 2012. Effects of dietary nucleotides supplementation on rainbow trout (*Oncorhynchus mykiss*) performance and acute stress response. *Fish Physiology and Biochemistry*, 38, 431-440. <https://doi.org/10.1007/s10695-011-9524-x>
55. Wilken, R., Veena, M.S., Wang, M.B., Srivatsan, E.S., 2011. Curcumin: a review of anti-cancer properties and therapeutic activity in head and neck squamous cell Carcinoma. *Molecular Cancer*, 10, 12. <https://doi.org/10.1186/1476-4598-10-12>
56. Yang, F., Lim, G.P., Begum, A.N., Ubeda, O.J., Simmons, M.R., Ambegaokar, S.S.,



- Chen, P. P., Kayed, R., Glabe, C.G., Frautschy, S.A., Cole, G.M., 2005. Curcumin inhibits formation of amyloid β oligomers and fibrils, binds plaques, and reduces amyloid in vivo. *Journal of Biological Chemistry*, 280, 5892–5901.
<https://doi.org/10.1074/jbc.M404751200>
57. Yonar, M.E., Yonar, S.M., İspir, U., Ural, M.S., 2019. Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (*Oncorhynchus mykiss*) against *Aeromonas salmonicida* subsp. *Achromogenes*. *Fish and Shellfish Immunology*, 89, 83–90.
<https://doi.org/10.1016/j.fsi.2019.03.038>
58. Yousef, M.I., Omar, S.A., El-Guendi, M.I., Abdelmegid, L.A., 2010. Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haemato toxicity in rat. *Food and Chemical Toxicology*, 48, 3246–3261.
<https://doi.org/10.1016/j.fct.2010.08.034>
59. Zhang, S., et al., 2012. CD133+ CXCR4+ colon cancer cells exhibit metastatic potential and predict poor prognosis of patients. *BMC Medicine*, 10, 85.
<https://doi.org/10.1186/1741-7015-10-85>
60. Zhang, Y., Forli, S., Omelchenko, A., Sanner, M.F., 2019. Auto grid FR: Improvements on auto dock affinity maps and associated software tools. *Journal of Computational Chemistry*, 40, 2882–2886.
<https://doi.org/10.1002/jcc.26054>

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