

# Next-Generation Small-Molecule ALK Inhibitors for Non-Small Cell Lung Cancer Treatment

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## Article

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# Abstract

A novel series of ferulic acid–based hydrazide derivatives (FBH1–FBH5) was rationally designed and synthesized with the aim of targeting anaplastic lymphoma kinase (ALK) in non-small cell lung cancer (NSCLC). NSCLC accounts for nearly 85% of all lung cancer cases, highlighting the urgent need for more effective and selective therapeutic options. Building on the pharmacological potential of the ferulic acid scaffold, these derivatives were developed to enhance anticancer activity and improve target specificity. Structural confirmation was achieved using FT-IR, NMR, and mass spectrometry. Molecular docking, ADMET analysis, MM-GBSA calculations, and 100 ns molecular dynamics simulations showed favourable ALK binding for all compounds, with FBH4 exhibiting the strongest and most stable interactions. ADME profiling confirmed good drug-likeness and compliance with Lipinski's rule. In vitro studies on A549 cells revealed anticancer activity for all derivatives, and FBH4 demonstrated the highest potency ( $IC_{50} = 71.33 \mu\text{M/ml}$ ), approaching the activity of crizotinib. All things considered, FBH4 shows promise as a lead candidate for more preclinical research as a new ALK inhibitor.

## Introduction

A tumor is a complex illness marked by unchecked cell proliferation, tissue invasion, & metastasis. Its progression is influenced by genetic mutations, environmental exposures, and lifestyle factors, involves important routes including Wnt, Notch, PI3K-Akt-mTOR, Ras-MAPK, and Hedgehog contributing towards tumor development. [1] Major risk factors include smoking, alcohol use, obesity, infections, and hereditary predisposition, while prevention through healthy diet, physical activity, screening, and vaccination can help reduce overall cancer risk. [2]

With more than two million new cases each year, lung cancer continues to be the biggest driver of fatalities due to malignancy worldwide. NSCLC, which stands for non-small cell lung cancer which is frequently discovered at a late stage, accounts for roughly eighty-five percent of these instances. [3] Smoking is the predominant cause, though radon, asbestos, air pollution, chronic lung diseases, and genetic susceptibility also contribute. [4–6] According to SEER estimates, there will be 124,730 instances of deaths due to lung cancer and 226 thousand additional cases in the United States in 2025, while India is expected to report over 81,000 cases in males, largely linked to tobacco use and pollution. [7]

Molecular profiling has identified several actionable driver mutations in NSCLC; ALK rearrangements occur in approximately 3–7% of patients and activate proliferative mechanisms such as JAK-STAT, PI3K-AKT, & RAS-MAPK. [8] Although targeted ALK inhibitors such as crizotinib and alectinib have improved patient outcomes, resistance remains a major clinical challenge. Ferulic acid, a plant-derived phenolic compound, possesses antioxidant, anti-inflammatory, and anticancer properties. [9] Its hydrazide derivatives (FAHs), containing a reactive  $-\text{CONHNH}_2$  group, have demonstrated the ability to bind DNA, inhibit replication, and induce apoptosis. [10–11] Owing to these biological activities, FAH derivatives are

being explored as potential ALK-targeted agents and have shown antimicrobial, anticancer, antiviral, and anti-inflammatory effects. [12–13]

## Materials and methods

### In Silico Drug Design

Ferulic acid hydrazide derivatives exhibit notable anticancer and other biological activities, making ferulic acid a suitable scaffold for developing ALK-targeted ligands. Accordingly, new ligands were designed (Figs. 1 & 2) and evaluated through computational and biological studies for their potential activity against NSCLC. [14–20]

### Figure 2: List of Designed Compounds (FBH1-FBH5)

## Molecular Docking

Molecular docking was carried out to evaluate the ligand–receptor affinity of the newly designed compounds toward ALK. The 2D structures were sketched in ChemDraw Pro 8.0 and converted to 3D using LigPrep (Schrödinger 2021-4), generating low-energy tautomers and conformers at pH 7.0  $\pm$  2.0 with OPLS4 minimization. The ALK crystal structure (PDB ID: 4CCB) was obtained from the RCSB database and prepared using the Protein Preparation Wizard and Prime, involving removal of water molecules, addition of hydrogens, correction of bond orders, and refinement at pH 7.0. Energy minimization was performed using OPLS4 with a 0.30 Å RMSD threshold. Van der Waals scaling of 0.8 and a partial charge cutoff of 0.15 were used to create a receptor grid around the active site. The best-scoring ligand postures were further examined using binding free-energy estimates after docking in Glide (XP mode). [21–23]

## ADME Screening

Key pharmacokinetic parameters, including hydrogen-bond donors/acceptors, Lipinski parameters, and Caco-2 permeability, were predicted using the QikProp module (Schrödinger 2021-4).

## MM-GBSA Binding Free Energy Calculation

Utilizing the OPLS4 force field, Prime rotamer sampling and the VSGB 2.0 solvation model, PRIME MM-GBSA was utilized to evaluate the respective binding independent energies of ligands exhibiting profound interactions alongside ALK.

## Molecular Dynamics

To assess complex stability and interaction behavior within the ALK active site (PDB: 4CCB), 100 ns MD simulations were performed using Desmond using the force field from OPLS4. The BH1–4CCB complex has been neutralized with Na<sup>+</sup> ions and allowed to equilibrate in a TIP4P water box containing a 10 Å buffer. With a 2-fs timestep and NPT conditions, the system was heated to 300 K. PME with a 9 Å cutoff was used to handle long-range electrostatics. The Nose–Hoover thermostat and Martyna–Tobias–Klein barostat were used to conduct production runs for 100 ns at 300 K and 1 atm. Trajectories were analyzed in Maestro, focusing on RMSD, RMSF, and protein–ligand interaction profiles.

### **In vitro Cytotoxic Studies**

A549 is a type II pulmonary epithelial human lung cancer cell line procured from Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, Ooty, The Nilgiris, Tamil Nadu, India. These cell lines commonly grown in F-12K media that contains 1% L-glutamine, 10% FBS, and penicillin–streptomycin. The potential of cells with metabolic activity to transform yellow MTT dye into purple formazan crystals is the basis for the MTT assay, which evaluates cell viability. The viable cell count is calculated by measuring absorbance at 570 nm following solubilization with DMSO. Standard CO<sub>2</sub> incubation conditions were used to cultivate A549 cells in F-12K medium. The cells had been trypsinized and seeded into wells of 96 at an average density of 1×10<sup>2</sup> cells per well after reaching the proper confluency.

The cultures were given treatment with various amounts of the produced compounds after a 24-hour incubation period to facilitate cell adhesion. They were then incubated for an additional 48 hours while being periodically seen under a microscope to track morphological changes. After administration, 20 µL of the MTT solution (2 mg per milliliter) were added every well to encourage formazan formation. The combination was then incubated for an additional between and four hours. The formazan crystals were dissolved by adding one hundred micro-liters of DMSO afterwards the media was carefully removed. Finally, the index of absorbance at a wavelength of 570 nm was calculated to determine the vitality of the cells. The viability of cells (%) had been evaluated. [24–25]

## **Results and Discussion**

Novel ferulic acid hydrazide derivatives were synthesized through a stepwise procedure, with reaction progress monitored by TLC. The crude products were purified by recrystallization to obtain well-defined crystals. Structural confirmation was carried out using IR spectroscopy to verify key functional groups, NMR to determine proton and carbon environments, and mass spectrometry to confirm molecular weight and fragmentation. Together, these analyses confirmed the successful synthesis and purity of the new hydrazide derivatives.

## **Molecular Docking**

The docking results (Table 1 & Fig. 3) showed that the FBH series demonstrated favorable interactions compared to the reference drug crizotinib. While crizotinib exhibited a Glide XP score of – 6.980

kcal/mol, the FBH ligands showed scores ranging from – 7.124 to – 4.663 kcal/mol (Table 1). The binding affinity of the derivatives was largely attributed to the hydrazide functional group, heterocyclic moieties, and the formation of stable hydrogen-bond interactions within the active pocket. Overall, the docking analysis suggests that FBH1–FBH5 possess promising ALK-binding characteristics, supporting their potential as new inhibitors for NSCLC therapy.

## ADME Screening

The proposed compounds' ADME features, such as molecular mass (< 500), dipole (1–10), the hydrogen bond donor (< 5), the hydrogen bond acceptor (< 10), QP log P o/w (-2-6.5), and Lipinski rule of five (Max 4), have been determined to be below the recommended values (Table 2).

## MM-GBSA Binding Free Energy Calculation

*In silico* ADME profiling indicated that the synthesized ligands exhibited favorable drug-likeness comparable to crizotinib. MM-GBSA analysis supported the docking results, showing binding free energies ( $\Delta G_{\text{lipo}}$ ,  $\Delta G_{\text{coulomb}}$ ,  $\Delta G_{\text{vdW}}$ ,  $\Delta G_{\text{bind}}$ ) between – 49.77 and – 41.11 kcal/mol for ALK (4CCB), suggesting strong ligand–protein interactions. The lowest-energy poses also showed good consistency between Glide XP scores and MM-GBSA values, confirming a stable and well-aligned docking complex (Table 3).

## Molecular Dynamics

The 100 ns MD simulation of the 4CCB–FBH4 complex showed that the protein C $\alpha$  RMSD remained within 4–7.4 Å, indicating overall structural stability (Figs. 4 & 5). RMSD gradually increased during the first 55 ns (1.5–2.4 Å) and stabilized between 55–100 ns with only minor fluctuations (~ 0.4 Å). The ligand also showed initial variation (1.4–2.4 Å) up to 55 ns before achieving stable motion from 60–100 ns with an RMSD shift of about 1.0 Å. RMSF analysis revealed that most C $\alpha$  residues fluctuated between 0.4–2.4 Å, while active-site residues showed slightly higher flexibility (1.5–2.4 Å), with notable variations beyond residue 250.

Table 1  
Docking studies of compounds FBH (1–5) against ALK (4CCB.pdb)

| Compound                    | GlideXP<br>g-score<br>(kcal/mol) | Glide<br>evdw | Glide XP<br>Hbond | Glide<br>ecoul | Glide-<br>energy |
|-----------------------------|----------------------------------|---------------|-------------------|----------------|------------------|
| FBH1                        | -5.59                            | -37.101       | -1.10312          | -6.31629       | -43.4173         |
| FBH2                        | -6.74                            | -39.101       | -1.10412          | -6.41110       | -43.4453         |
| FBH3                        | <b>-7.26</b>                     | -36.6608      | -0.91096          | -8.17129       | -44.8321         |
| FBH4                        | -7.14                            | -30.9000      | -1.49125          | -9.59010       | -40.4905         |
| FBH5                        | -4.66                            | -36.6382      | -1.62132          | -9.59010       | -40.3811         |
| <b>Crizotinib<br/>(Std)</b> | <b>-9.67</b>                     | -41.6179      | -1.30496          | -8.87550       | -50.4934         |

<sup>a</sup>glide score; <sup>b</sup>glide van der Waals energy, <sup>c</sup>glide Hydrogen bonding; <sup>d</sup>glide Coulomb energy; <sup>e</sup>glide energy.

Table 2  
*In silico* ADME Screening

| Compounds                     | Mol.<br>Wt.     | Donor<br>HB   | Accept<br>HB   | Qp log<br>o/w | Rule of<br>five | %Human oral<br>Absorption                 |
|-------------------------------|-----------------|---------------|----------------|---------------|-----------------|-------------------------------------------|
| FBH1                          | 326.35          | 3             | 4              | 2.183         | 4               | 70.8                                      |
| FBH2                          | 342.35          | 3             | 5              | 1.883         | 4               | 82.4                                      |
| FBH3                          | 374.30          | 3             | 8              | 2.373         | 4               | 76.5                                      |
| FBH4                          | 329.31          | 3             | 6              | 2.465         | 4               | 94.7                                      |
| FBH5                          | 362.40          | 3             | 5              | 1.947         | 4               | 86.1                                      |
| <b>Crizotinib (std)</b>       | 450.34          | 2             | 6              | 5.037         | 3               | 100                                       |
| <b>Recommended<br/>values</b> | <b>&lt; 500</b> | <b>&lt; 5</b> | <b>&lt; 10</b> | <b>-2-6.5</b> | <b>Max 4</b>    | <b>&gt; 85% high<br/>&amp;&gt;25%poor</b> |

<sup>a</sup>Mol. Wt. - molecular weight of the molecules; <sup>b</sup>donorHB: Estimated number of hydrogen bonds that would be donated by the molecule; <sup>c</sup>accptHB: Estimated number of hydrogen bonds that would be accepted by the molecule; <sup>d</sup>QLogPo/w: Predicted octanol/water partition coefficient; <sup>e</sup>Rule of five: Number of violations of Lipinski's rule of five; <sup>f</sup>% human oral abs.: Percent human oral absorption.

Table 3  
Binding free energy calculation approach for ALK (4CCB)

| Compound                | dG_Bind kcal/mol | dG_Bind Coulomb | dG_Bind Covalent | dG_Bind H bond | dG_Bind lipophilic |
|-------------------------|------------------|-----------------|------------------|----------------|--------------------|
| FBH1                    | -44.6431         | -20.2399        | 6.721416         | -1.85435       | -13.1625           |
| FBH2                    | -43.332          | -20.2022        | 6.721312         | -1.80090       | -13.1145           |
| FBH3                    | -41.1166         | -14.406         | 10.87197         | -1.96204       | -11.399            |
| FBH4                    | -49.773          | -17.7284        | 5.573458         | -2.15174       | -12.3307           |
| FBH5                    | -44.799          | -12.0219        | 1.573597         | -1.46145       | -15.9612           |
| <b>Crizotinib (Std)</b> | -52.3207         | -13.6075        | 2.63615          | -1.83669       | -18.5273           |

<sup>a</sup>free energy of binding; <sup>b</sup>Coulomb energy; <sup>c</sup>Covalent bonding energy; <sup>d</sup>hydrogen bonding energy;

<sup>e</sup>hydrophobic energy (non-polar contribution estimated by solvent accessible surface area).

## Spectral Studies

Spectral characterization was carried out for all five synthesized compounds to verify their structure and purity. <sup>1</sup>H and <sup>13</sup>C NMR spectra confirmed the proton and carbon arrangements, while IR analysis identified key functional groups. Mass spectrometry further validated each compound by confirming its molecular weight and fragmentation pattern. Collectively, these techniques supported the successful synthesis and structural confirmation of the target molecules.

### 1. FBH 1

Yellow-green solid; Yield- 80%, Melting point- 182-185<sup>0</sup>C; FT-IR (KBr, cm<sup>-1</sup>) -3388.01(Ar- OH),1628.13 (-NH-), 1506.00 (Ar- C-C), 1689.03 (C = O), 1194.94 (Ar- O-CH<sub>3</sub>). LC-MS (ESI): Calculated for C<sub>18</sub>H<sub>18</sub>N<sub>2</sub>O<sub>4</sub> m/z: 326.35; Found m/z: 324.10; <sup>1</sup>H NMR (400MHz, DMSO) δ ppm- 9.73 (NH), 10.38 (OH), 3.84 (OCH<sub>3</sub> (s, 3H)), 2.36 CH<sub>3</sub> (aryl methyl, s, 3H), 7.17 (Ar H, multiplet, ~8H total), 6.85 (Vinyllic H); <sup>13</sup>C NMR (400MHz, DMSO) δ- 142.91 (Ar C),129.49 (Vinyllic C), 56.21 (OCH<sub>3</sub>), 21.50 (Aryl CH<sub>3</sub>), 165.11(Carbonyls).

### 2. FBH 2

Yellow-green solid; Yield- 85%, Melting point- 193-195<sup>0</sup>C; FT-IR (KBr, cm<sup>-1</sup>) -3158.6(Ar- OH),1629.09 (-NH-), 1499.15 (Ar- C-C), 1691.05 (C = O), 1193.37 (Ar- O-CH<sub>3</sub>). LC-MS (ESI): Calculated for C<sub>18</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub> m/z: 342.35; <sup>1</sup>H NMR (400MHz, DMSO) δ ppm- 7.19 (Ar H. multiplet), 9.73 (NH), 10.46 (OH), 3.81 (OCH<sub>3</sub> (s, 6H)); 6.64 (Vinyllic H) <sup>13</sup>C NMR (400MHz, DMSO) δ- 168.09 (Carbonyls), 147.73 (Ar C), 129.71 (Vinyllic C), 56.21 (OCH<sub>3</sub>).

### 3. FBH 3

Yellow solid; Yield- 65%, Melting point- 210-216<sup>0</sup>C; FT-IR (KBr, cm<sup>-1</sup>) -3158.6(Ar- OH),1619.46 (-NH-), 1498.35 (Ar- C-C), 1691.05 (C = O), 1194.51 (Ar- O-CH<sub>3</sub>), 1368.48 (Ar-NO<sub>2</sub>); LC-MS (ESI): Calculated for C<sub>16</sub>H<sub>14</sub>N<sub>4</sub>O<sub>7</sub>, m/z: 374.30, m/z found: 373.05; <sup>1</sup>H NMR (400MHz, DMSO) δ ppm- 8.152 (NO<sub>2</sub> substituted Ar H), 7.195 (Ar H), 8.9(NH), 3.816 (OCH<sub>3</sub>), 6.753 (Vinylic H),8.73 (NH); <sup>13</sup>C NMR (400MHz, DMSO) δ- 144.97 (Nitro Ar C), 168.45 (Carbonyls), 125.84 (Vinylic C), 56.45 (OCH<sub>3</sub>).

### 4. FBH 4

Yellow solid; Yield- 70%, Melting point- 197-203<sup>0</sup>C; FT-IR (KBr, cm<sup>-1</sup>) -3158.6(Ar- OH),1613.25 (-NH-), 1506.16 (Ar- C-C), 1723.35 (C = O), 1193.22 (Ar- O-CH<sub>3</sub>), 1388.43 (Ar-NO<sub>2</sub>); LC-MS (ESI): Calculated for C<sub>16</sub>H<sub>15</sub>N<sub>3</sub>O<sub>5</sub>, m/z: 329.31, m/z:327.00; <sup>1</sup>H NMR (400MHz, DMSO) δ ppm- 7.885 (NO<sub>2</sub> substituted Ar H), 7.29 (Ar H), 8.9(NH), 3.84 (OCH<sub>3</sub>), 6.83 (Vinylic H), 9.73 (OH); <sup>13</sup>C NMR (400MHz, DMSO) δ- 125.87 (Nitro Ar C), 168.08 (Carbonyls), 125.87 (Vinylic C), 56.58 (OCH<sub>3</sub>).

### 5. FBH 5

Yellow solid; Yield- 75%, Melting point- 198-201<sup>0</sup>C; FT-IR (KBr, cm<sup>-1</sup>) - 3158.6(Ar- OH),1594.02 (-NH-), 1507.29 (Ar- C-C), 1723.03 (C = O), 1197.05 (Ar- O-CH<sub>3</sub>), 111.37 (Ar-SO<sub>2</sub>); LC-MS (ESI): Calculated for C<sub>18</sub>H<sub>18</sub>N<sub>2</sub>O<sub>5</sub>S, m/z: 362.40; <sup>1</sup>H NMR (400MHz, DMSO) δ ppm-7.28 ( Ar H), 7.195 (Ar H), 8.56(OH), 3.83 (OCH<sub>3</sub>), 6.824 (Vinylic H), 2.39 (CH<sub>3</sub>); <sup>13</sup>C NMR (400MHz, DMSO) δ- 130.02 (Sulfonyl attached Ar C), 165.11 (Carbonyls), 133.62 (Vinylic C), 56.45 (OCH<sub>3</sub>), 21.48 (Aryl CH<sub>3</sub>).

### In vitro Cytotoxicity Studies

Figure 6 shows that, in comparison to the traditional IC<sub>50</sub> value of Crizotinib, which was 71.33 μM/ml in A549 cells after treatment for 24 hours, the chemical FBH 3 is showing mid-toxicity to cancer cells ALK. When comparing the IC<sub>50</sub> values of the compounds in A549 with the standard, all of the compound's function well. Moreover, FBH4 was a more effective and safer chemical for A549 cells. After that, the materials were converted to micromolar (μM) concentrations.

## Conclusion

Lung cancer remains a major health burden, and current therapies often offer limited long-term benefits due to toxicity and drug resistance. This has increased interest in safer, naturally inspired molecules. In this study, new ALK-targeted ligands were designed and synthesized using ferulic acid hydrazide as the core scaffold. Five derivatives (FBH1–FBH5) were prepared and evaluated against the EML4-ALK fusion in A549 cells.

Docking studies (PDB: 4CCB) showed good binding energies (−49.77 to − 41.11 kcal/mol), with FBH4 and FBH5 displaying the strongest interactions. ADME profiling confirmed acceptable drug-likeness. FBH4, which had favorable Glide XP and binding-energy scores, also showed stable interactions in molecular dynamics simulations.

All compounds were structurally characterized (melting point, R<sub>f</sub>, ATR-IR, NMR, MS) and screened biologically. Among them, FBH4 showed the highest anticancer activity and better safety, performing comparably to crizotinib (IC<sub>50</sub> = 71.33 μM/ml). Overall, FBH4 emerges as a promising lead ALK inhibitor for further anticancer development.

## Declarations

### Conflict of Interest

The authors declared that they had no conflicts of interest.

### Funding

No Funding

### Data Availability Statement

All data generated or analysed during this study are included in this published article.

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## Figures

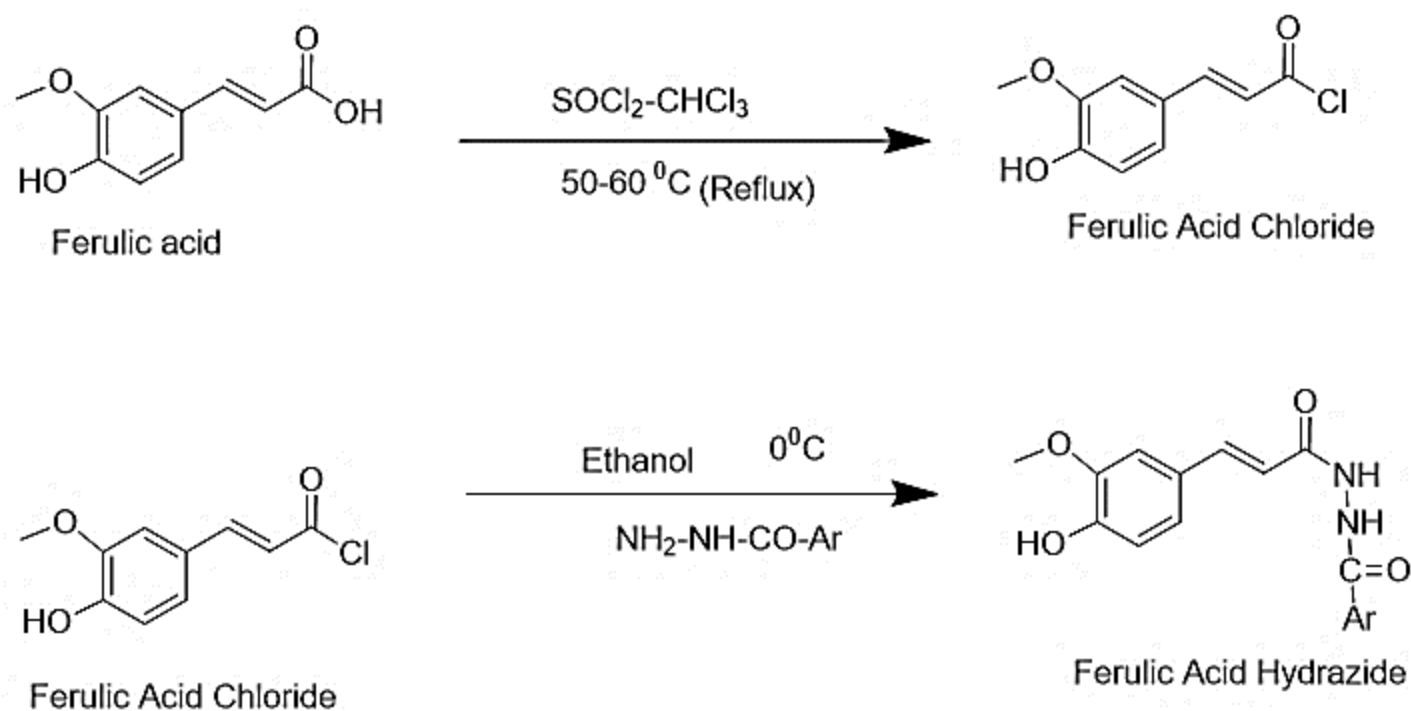
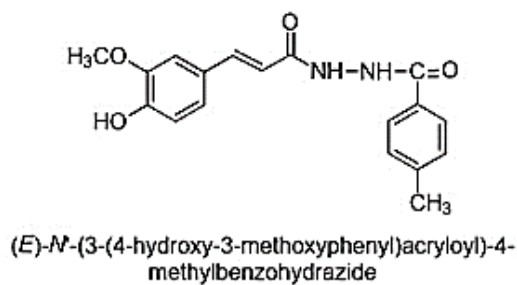
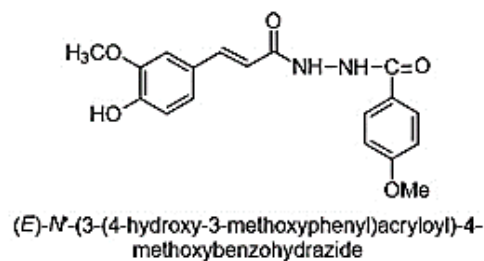


Figure 1

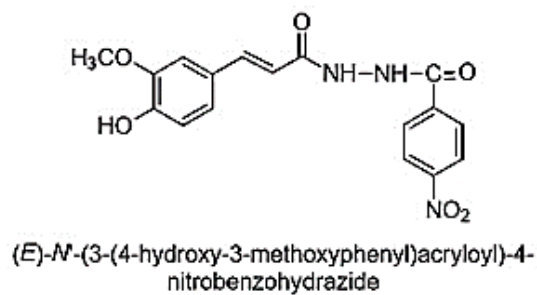
### Scheme of Synthesis



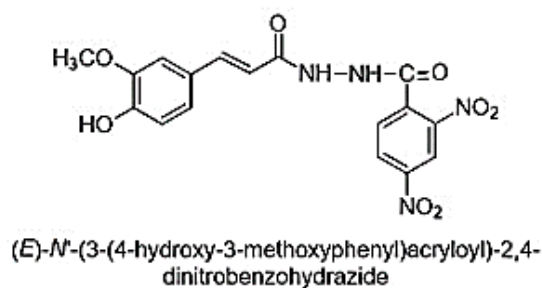
**FBH 1**



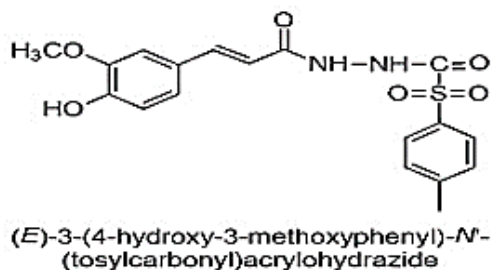
**FBH 2**



**FBH 3**



**FBH 4**



**FBH 5**

Figure 2

### List of Designed Compounds (FBH1-FBH5)

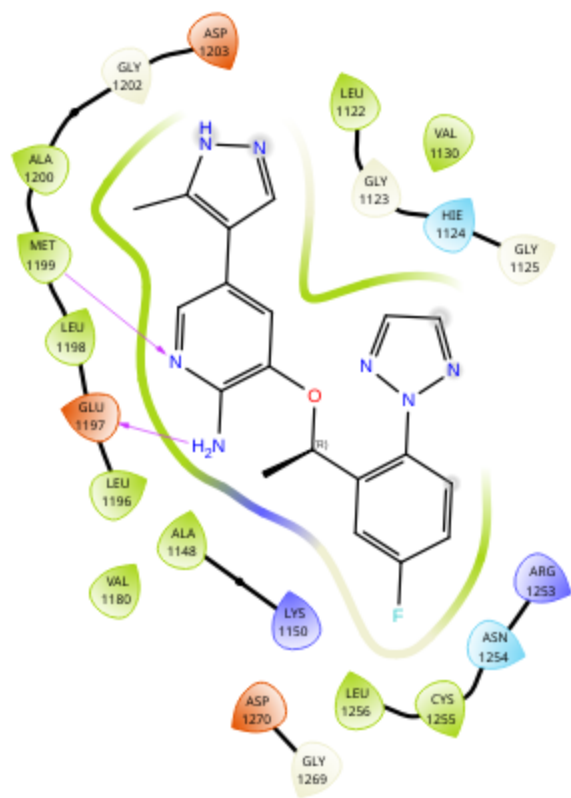


Figure 3

2D interaction of FBH3 compounds against ALK (4CCB)

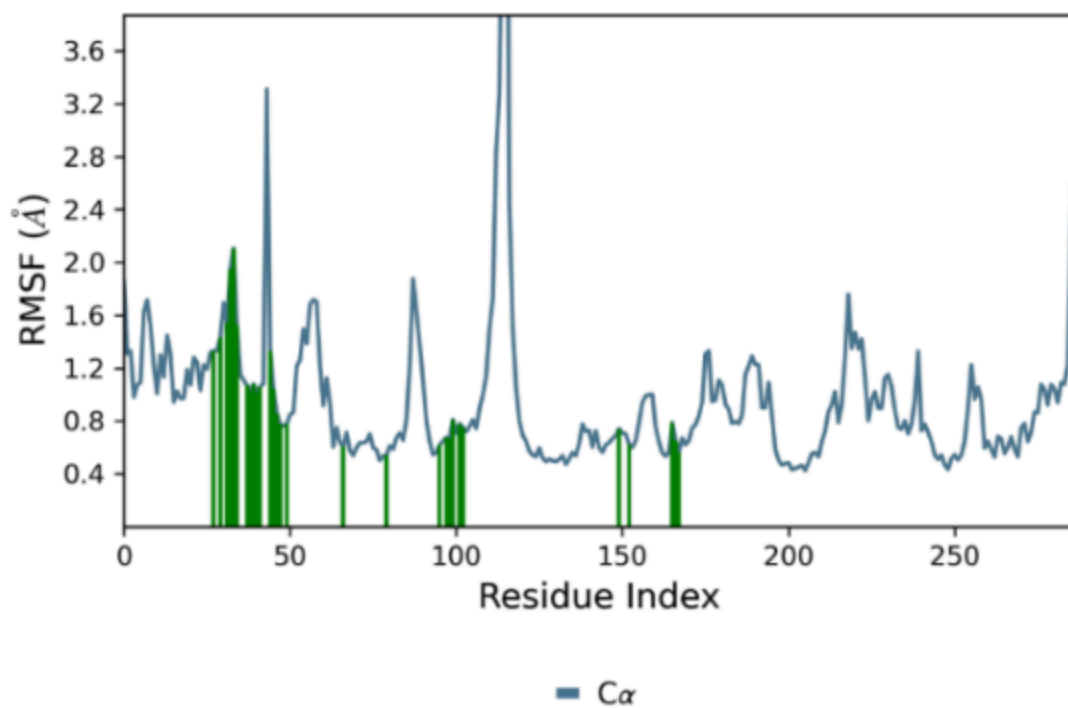


**Figure 4**

**RMSD of FBH4 4CCB.pdb complex during100ns MD simulation**

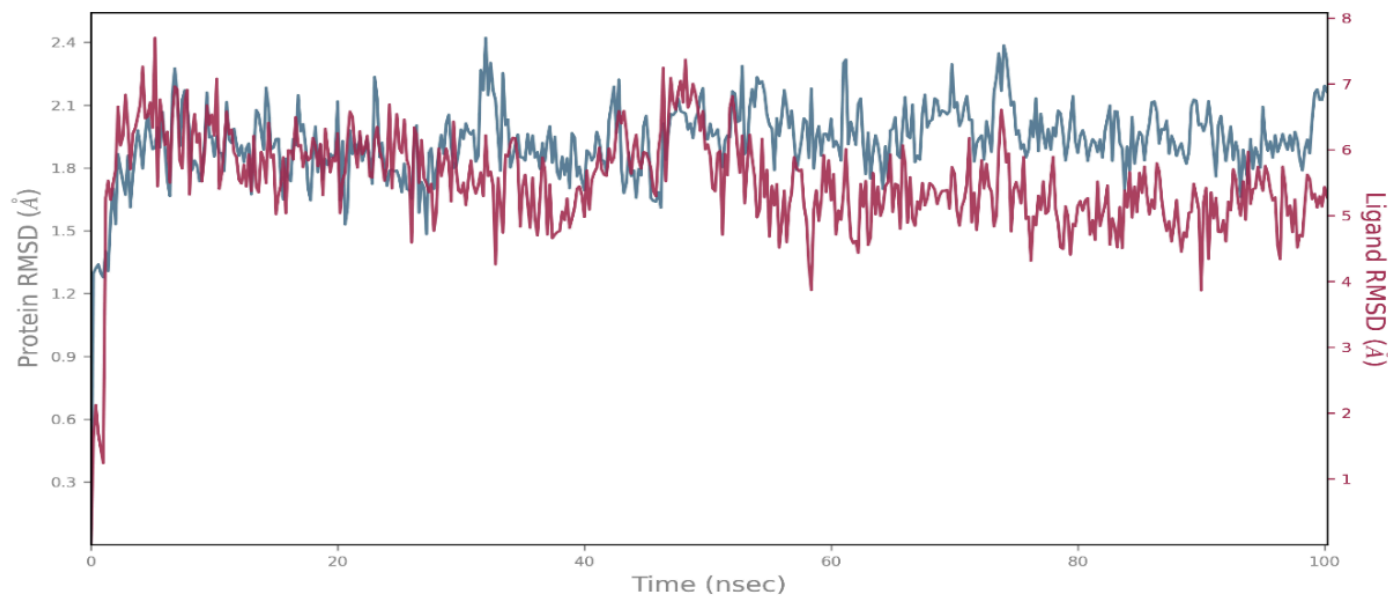


**Figure 5**

**RMSF of FBH4 4CCB.pdb complex during100ns MD simulation**

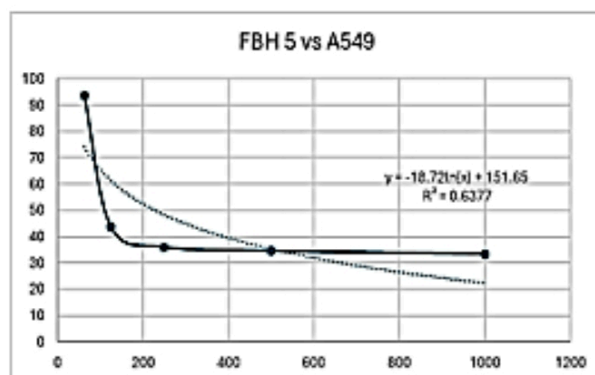
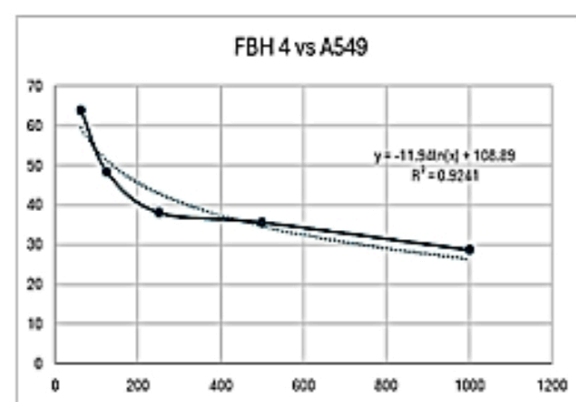
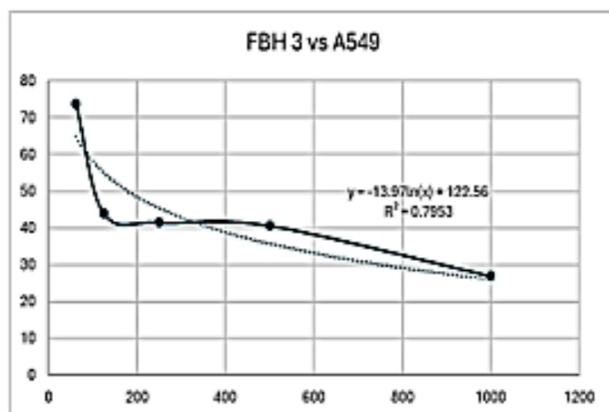
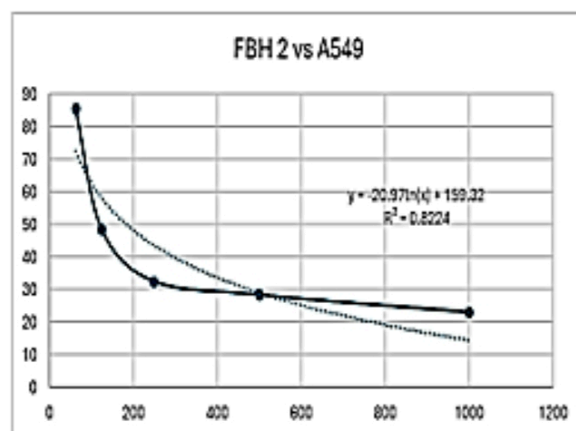
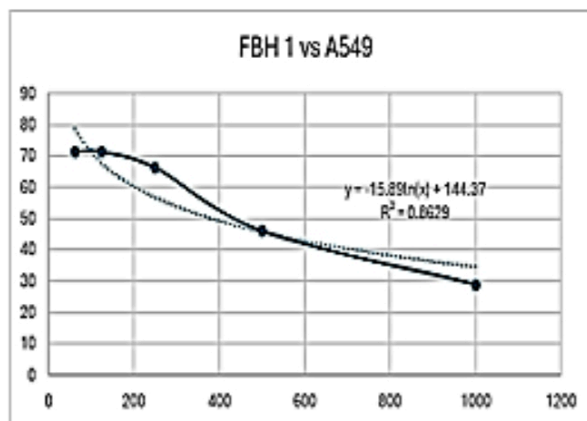


Figure 6

6: % viability of compound **FBH 1 – FBH 5** vs conc. (µg/ml) for **A549** cells