Novel Cinnamic Acid Derivatives as Potential Anticancer Agents: Synthesis, In Vitro Cytotoxicity and Molecular Docking Studies

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Abstract: Inhibition of matrix metalloproteinase-9 (MMP-9) can be an emerging strategy for a cancer cure as overexpression of MMP-9 is associated with several types of malignancies, including cancers related to lungs, breasts, and prostate. The present work was proposed to design and synthesize some novel cinnamic acid derivatives as potential anticancer agents. Novel cinnamic acid derivatives were designed using a structure-based drug design approach, and a series of 16 newer cinnamic acid analogs was prepared and evaluated for in vitro cytotoxicity (lung cancer cell line, A-549), followed by docking studies to explore binding interactions of designed compounds in the binding site of MMP-9. In the in vitro cytotoxicity assay, compound 5 was found to be most potent, with an IC50 value of 10.36 µM amongst the synthesized analogs. The designed molecules showed appreciable docking interactions and binding patterns with MMP-9 protein supporting the in vitro cytotoxicity outcomes. Compound 5 can be further explored as a possible lead molecule to develop potent and selective MMP-9 inhibitors as potential antineoplastic agents.

Keywords: anticancer; A-549; cinnamic acid; lung cancer; matrix metalloproteinase-9; MMP-9 inhibitors.

1. Introduction

Matrix metalloproteinases (MMPs) are the members of the zinc-containing neutral endo-peptidases family capable of degrading several elements of the extra-cellular matrix (ECM) and the basement membrane. MMPs play a significant part in numerous biological and pathogenic operations and are over-expressed in nearly every kind of human malignancies, and it associated with dissimilar phases of tumor [1,2]. Among various types of MMPs, MMP-9 (gelatinase B) is a 92 kDa protein belonging to the gelatinase group, capable of degrading ECM. MMP-9 portrays crucial functions in several biological processes, too, including ECM breakdown; controlling stem cells and epithelial cells; secretion of strong inducers of angiogenesis stimulators such as vascular EGFA (“epithelial growth factor-A”) and “fibroblast growth factor” [3]. Over-expressed MMP-9 guides tissue’s regular condition to malignant condition. Large cells and vascular incursion are characteristic of malignancy, and it is an interesting point that over-expression of MMP-9’s had previously been evidenced [4].
the tumor grows, through the aid of MMP-9, proangiogenic actions conquer the efficacy of angiogenic blockers [5]. MMP-9 over-expression was reported in several types of malignancies, including cancers of the lungs, prostate, and mammary glands [3]. A divergent range of nature-based (including marine products [6], flavonoids [7], artemisinins [8,9], catechins [10-12], caffeic acid [13] and iridoid glycosides [14-16]) as well as synthetic compounds (including barbiturates [17], caffeïoyl/cinnamic acid derivatives [18], phosphoramidates [19], phosphonate derivatives [20], sulphones [21] and heteroaryl compounds containing N and S-atoms [22-29]) were reported as inhibitors of MMP-9 valuable in management of numerous cancer situations [30]. Numerous cinnamic/caffeic acid derivatives were documented as strong and specific inhibitors of MMP-9 (evaluated in vitro or in silico) for cancer therapy recently [31-41]. Owing to the potential of cinnamic acid derivatives as MMP-9 inhibitors and pharmacophoric necessities for MMP-9 inhibition, the cinnamic acid scaffold was preferred for designing newer derivatives, and hydroxamate group was introduced on the aromatic ring of cinnamic acid (to interact with zinc through metal interaction and H-bond interaction with Leu188, Ala189 and Glu227 residues of MMP-9 protein). Based on the pharmacophoric features, functions of MMP-9 in the development of tumors, and potential of cinnamic acid analogs as potent inhibitors of MMP-9; a few newer cinnamic acid derivatives were designed and synthesized as potential antineoplastic agents and further accessed for in vitro anticancer activity (A-549, lung cancer cell line) as well as MMP-9 inhibition by the docking studies (Figure 1).

![Figure 1](https://biointerfaceresearch.com/Markush_structure.png)

**Figure 1.** Markush structure of the cinnamic acid analogs designed as probable MMP-9 inhibitors.

2. Materials and Methods

2.1. General.

All the reagents employed were purchased from HiMedia, Spectrochem, Merck, SRL, etc., and employed for experiments. The melting point was determined using Veego V MP-d apparatus (uncorrected). 1H-NMR spectra were recorded on Bruker Avance II 300 MHz NMR spectrophotometer and documented as chemical shift (δ) in parts per million (ppm) downfield from internal standard, and infrared (IR) spectra were taken on Shimadzu FTIR spectrophotometer using KBr pellet approach.

2.2. Synthesis.

A mixture of benzaldehydes (7.8 mmol), malonic acid (19.2 mmol), pyridine (49.4 mmol), and piperidine (0.2 ml) was heated on a water bath (80-90°C) for 3 h and then poured
to 2N HCl solution. The acid was precipitated instantly and kept aside for proper separation, filtered, and dried. The substituted cinnamic acids (0.1 mmol) obtained above were then refluxed with SOCl₂ (0.1 mmol) for 4 h to acquire respective acid chlorides. After that, respective amines (0.1 mmol) were added to the acid chlorides attained above, refluxed for 3 h, and the product (cinnamic acid amides) was dried. A mixture of methanol (12 ml) and hydroxylamine HCl in a flask was placed on a heated magnetic plate with stirring for 5 minutes. While stirring, a previously prepared methanolic solution of KOH (0.05 M, 2.81 g) was added dropwise. After the complete addition of KOH solution, the product was filtered, and the filtrate was collected. Chloroacetyl chloride (0.07 M) was added dropwise to methanol (25 ml) while stirring on a magnetic stirrer. The NH₂OH stock solution was added dropwise to chloroacetyl chloride solution and stirred for 2 h. The product was filtered and dried. The 2-chloro-N-hydroxyacetamide (0.1 mmol) was added to cinnamic acid amides (0.1 mmol) obtained above and refluxed for 8 h. The final products obtained were dried, collected, and recrystallized using methanol [42-44].

(2E)-N-Methyl-N-((hydroxycarbamoyl)methyl)-3-phenylprop-2-enamide (1): IR (KBr pellet): Yield 57%; m.p.: 113-116 ºC; 1629.58 (C=O), 1793.8 (C=O), 3462.22 (N=H), 1278.81 (N=C), 1535.34 (C=C), 3757.33 (O-H), 1278.81 (C-C), 1458.18 (CH₂), 1319.31 (CH₃); ¹H-NMR (δ ppm, DMSOδ₆): 7.69 (d, 1H, NH), 2.47 (d, 1H, OH), 2.51 (s, 3H, CH₃), 7.42 (dd, 2H, CH), 7.41 (tt, 3H, CH), 7.14 (d, 1H, CH), 6.55 (d, 1H, α-CH), 7.57 (d, 1H, β-CH).

(2E)-N-Ethyl-N-((hydroxycarbamoyl)methyl)-3-phenylprop-2-enamide (2): IR (KBr pellet): Yield 47%; m.p.: 108-111 ºC; 1627.92 (C=O), 1793.8 (C=O), 3219.19 (N=H), 1024.20 (N=C), 1680 (C=O), 3753.48 (O-H), 1276.88 (C-C), 1319.31 (CH₂), 1458.18 (CH₃); ¹H-NMR (δ ppm, DMSOδ₆): 7.7 (d, 1H, NH), 2.49 (d, 1H, OH), 2.47 (q, 2H, CH₂), 2.50 (t, 3H, CH₃), 7.40 (dd, 2H, CH), 7.21 (tt, 2H, CH), 7.38 (t, 1H, CH), 6.55 (d, 1H, α-CH), 7.57 (d, 1H, β-CH).

(2E)-N-Butyl-N-((hydroxycarbamoyl)methyl)-3-phenylprop-2-enamide (3): Yield 35%; m.p.: 107-110 ºC; IR (KBr pellet): 1627.92 (C=O), 1703.14 (C=O), 2993.52 (N=H), 1026.13 (N=C), 1703.14 (C=C), 3755.4 (O-H), 1276.88 (C-C), 1375.25 (CH₂), 1465.9 (CH₃).

(2E)-N-Benzyl-N-((hydroxycarbamoyl)methyl)-3-phenylprop-2-enamide (4): Yield 45%; m.p.: 112-115 ºC; IR (KBr pellet): 1535.34 (C=O), 1720.5 (C=O), 3520.09 (N=H), 1315.45 (N=C), 1645.28 (C=O), 3649.32 (O-H), 1282.66 (C-C), 1496.76 (CH₂).

(2E)-N-Methyl-N-((hydroxycarbamoyl)methyl)-3-(3-hydroxyphenyl)prop-2-enamide (5): Yield 64%; m.p.: 117-120 ºC; IR (KBr pellet): 1517.98 (C=O), 1703.14 (C=O), 3105.39 (N=H), 1373.52 (N=C), 1660.71 (C=O), 3583.74 (O-H), 1280.73 (C-C), 1333.02 (C=O), 1454.33 (CH₂), 1483.26 (CH); ¹H-NMR (δ ppm, DMSOδ₆): 7.62 (d, 1H, NH), 2.55 (d, 1H, OH), 2.51 (s, 3H, CH₃), 7.54 (dd, 2H, CH), 7.46 (s, 1H, CH), 7.14 (d, 1H, CH), 6.59 (d, 1H, α-CH), 7.53 (d, 1H, β-CH), 4.44 (s, 1H, OH).

(2E)-N-Ethyl-N-((hydroxycarbamoyl)methyl)-3-(3-hydroxyphenyl)prop-2-enamide (6): Yield 53%; m.p.: 123-126 ºC; IR (KBr pellet): 1598.99 (C=O), 1865.17 (C=O), 3132.4 (N-H), 1365.6 (N-C), 1672.28 (C=O), 3753.48 (O-H), 1215.15 (C-C), 1325.1 (C=O), 1448.54 (CH₂), 1365.6 (CH₃); ¹H-NMR (δ ppm, DMSOδ₆): 7.50 (d, 1H, NH), 2.50 (d, 1H, OH), 2.55 (q, 2H, CH₂), 2.50 (t, 2H, CH₃), 6.85 (s, 1H, CH), 6.42 (d, 1H, CH), 6.84 (t, 1H, CH), 6.82 (d, 1H, CH), 6.96 (d, 1H, α-CH), 7.50 (d, 1H, β-CH), 4.38 (s, 1H, OH).

(2E)-N-Butyl-N-((hydroxycarbamoyl)methyl)-3-(3-hydroxyphenyl)prop-2-enamide (7): Yield 44%; m.p.: 107-110 ºC; IR (KBr pellet): 1593.2 (C=O), 1865.17 (C=O), 3506.59 (N-H), 1325.1 (N-C), 1676.14 (C=O), 3585.67 (O-H), 1226.73 (C-C), 1305.81 (C-O), 1452.4 (CH₂), 1325.1 (CH₃); ¹H-NMR (δ ppm, DMSOδ₆): 8.87 (d, 1H, NH), 2.50 (d, 1H, OH), 2.96
(s, 1H, CH2), 2.50 (t, 2H, CH2), 1.55 (m, 2H, CH2), 1.33 (m, 2H, CH2), 0.96 (q, 3H, CH3), 6.83 (s, 1H, CH), 6.38 (d, 1H, CH), 7.03 (t, 1H, CH), 6.85 (d, 1H, CH), 6.86 (d, 1H, α-CH), 7.50 (d, 1H, β-CH), 6.31 (s, 1H, OH).

(2E)-N-Benzyl-N-((hydroxycarbamoyl)methyl)-3-(3-hydroxyphenyl)prop-2-enamide (8): Yield 76%; m.p.: 118-121 °C; IR (KBr pellet): 1593.2 (C=C), 1710.86 (C=O), 3516.23 (N-H), 1338.6 (N-C), 1676.14 (C=C), 3558.67 (O-H), 1280.73 (C-C), 290.38 (C-C), 1313.52 (C-O), 1442.75 (CH2).

(2E)-N-Methyl-N-((hydroxycarbamoyl)methyl)-3-(4-hydroxyphenyl)prop-2-enamide (9): Yield 66%; m.p.: 117-120 °C; IR (KBr pellet): 1676.14 (C=C), 1577.77 (C=O), 3132.75 (N-H), 1328.95 (N-C), 1479.4 (C=C), 3606.89 (O-H), 1213.23 (C-C), 1406.11 (C-O), 1479.4 (CH2), 1375.5 (CH3).

(2E)-N-Ethyl-N-((hydroxycarbamoyl)methyl)-3-(4-hydroxyphenyl)prop-2-enamide (10): Yield 65%; m.p.: 116-119 °C; IR (KBr pellet): 1691.57 (C=C), 1793.8 (C=O), 3273.2 (N-H), 1371.39 (N-C), 1598.99 (C=C), 3709.11 (O-H), 1222.87 (C-C), 1371.39 (C-O), 1450.47 (CH2), 1371.39 (CH3).

(2E)-N-Butyl-N-((hydroxycarbamoyl)methyl)-3-(4-hydroxyphenyl)prop-2-enamide (11): Yield 68%; m.p.: 118-121 °C; IR (KBr pellet): 1463.97 (C=C), 1909 (C=O), 3448.72 (N-H), 1409.96 (N-C), 1695.43 (C=C), 3448.72 (O-H), 1224.8 (C-C), 1409.96 (C-O), 1463.97(CH2), 1261.45 (CH3).

(2E)-N-Benzyl-N-((hydroxycarbamoyl)methyl)-3-(4-hydroxyphenyl)prop-2-enamide (12): Yield 78%; m.p.: 123-126°C; IR (KBr pellet): 1512.19 (C=C), 1602.85 (C=O), 3462.22 (N-H), 1409.16 (N-C), 1602.85 (C=C), 3763.12 (O-H), 1259.52 (C-C), 1409.96 (C-O), 1462.04 (CH3).

(2E)-3-(3,4-Dihydroxyphenyl)-N-((hydroxycarbamoyl)methyl)-N-methylprop-2-enamide (13): Yield 41%; m.p.: 122-125 °C; IR (KBr pellet): 1463.97 (C=C), 1705.07 (C=O), 3441.01 (N-H), 1284.59 (N-C), 1606.7 (C=C), 3433.29 (O-H), 1244.09 (C-C), 1371.39 (C-O), 1463.97 (CH2), 1371.39 (CH3).

(2E)-3-(3,4-Dihydroxyphenyl)-N-ethyl-N-((hydroxycarbamoyl)methyl)prop-2-enamide (14): Yield 55%; m.p.: 120-123 °C; IR (KBr pellet): 1517.98 (C=C), 1851.66 (C=O), 3203.76 (N-H), 1371.39 (N-C), 1606.7 (C=C), 3687.9 (O-H), 1195.87 (C-C), 1278.81 (C-O), 1448.54 (CH2), 1371.39 (CH3); 1H-NMR (δ ppm, DMSOδ6): 7.51 (d, 1H, NH), 3.01 (d, 1H, OH), 3.03 (q, 2H, CH2), 1.25 (t, 3H, CH3), 6.72 (s, 1H, CH), 6.71 (d, 1H, CH), 6.84 (d, 1H, CH), 6.95 (d, 1H, α-CH), 7.48 (d, 1H, β-CH), 4.21 (s, 1H, OH), 3.96 (s, 1H, OH), 3.75 (s, 2H, CH3).

(2E)-N-Butyl-3-(3,4-dihydroxyphenyl)-N-((hydroxycarbamoyl)methyl)prop-2-enamide (15): Yield 35%; m.p.: 148-151 °C; IR (KBr pellet): 1579.7 (C=C), 1845.88 (C=O), 3423.65 (N-H), 1282.66 (N-C), 1602.85 (C=C), 3685.97 (O-H), 1199.72 (C-C), 1394.53 (C-O), 1462.04 (CH2), 1375.25 (CH3).

(2E)-N-Benzyl-3-(3,4-dihydroxyphenyl)-N-[(hydroxycarbamoyl)methyl]prop-2-enamide (16): Yield 62%; m.p.: 140-143 °C; IR (KBr pellet): 1604.77 (C=C), 1843.95 (C=O), 1282.66 (N-H), 3180.62 (N-C), 1660.71 (C=C), 3685.97 (O-H), 1199.72 (C-C), 1379.1 (C-O), 1458.18 (CH2), 1379.1 (CH3).

2.3. In vitro cell viability assay.

In vitro cell viability was investigated by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The lung cancer cell line (A-549) was obtained
from National Centre for Cell Science, Pune (India), having passage no. of 32, and was developed using DMEM media escorted by 10% FBS (fetal bovine serum) and antibiotic mix (1X Penstrip, Invitrogen). The cells were incubated for 24-hours at 37 °C with 5% CO₂ and 95% humidity settings. For tests, cells were planted in identical quantities post trypan blue cell numeration (8000-10000 cells/well of the 96-well plate). Next, cells were cleaned one time using 1X PBS (sterilized) and cultivated in serum-free media for two days to synchronize. MTT assay was performed employing a 96-well-plate; in each well, 100 μL media was added, in which cells were treated with the test compounds for 48 h (compounds (1, 5, and 25 μM) were dissolved in a minimum amount of biological grade DMSO). The analysis was performed in triplicates, with colchicine serving as a positive control. Following 2-days, media was scrapped and then cleaned using 1X PBS and were then treated with MTT dye (5 mg per 10 mL of 1X PBS) at 100 μL/well concentration and incubated (4 h) at room temperature to allow formazan crystal formation. Crystals were then liquified in DMSO (100 μL), and readings were taken employing a microplate reader at 570 nm. The outcomes were then denoted as half-maximal inhibitory concentration (IC₅₀) [34].

2.4. In silico docking studies.

The docking investigations were performed using AutoDock Vina [45] and Auto-dock tools [46]. The 2-D structures of ligands were drawn in MarvinSketch (Ver 18.5.0) and transformed to 3-D with Frog2 server [47]. The co-crystallized info of MMP-9 protein was gained from the protein data bank, and after assessing numerous entries, the ligand-bound complex with the top resolution was selected (PDB entry: 4H3X). An analogous method for docking (using AutoDock Vina) of designed molecules was used as previously described. In the end, the poses of ligand-protein complexes with the utmost favorable binding energy (ΔG, kcal per mol) were elected. The bindings of protein-ligand were explored further for the docked poses employing PyMOL [48-51].

3. Results and Discussion

3.1. Chemistry.

Cinnamic acid amides were prepared by reaction of benzaldehydes and malonic acid, followed by refluxing with SOCl₂ and then with amines.

![Scheme 1](https://biointerfaceresearch.com/)

Scheme 1. Synthetic procedure for designed derivatives. Reagents and reaction conditions: (i) Malonic acid, pyridine, piperidine, 80-90 °C; (ii) SOCl₂, reflux; (iii) R³-NH₂, reflux; (iv) NH₂OH, methyl alcohol, KOH, stirring; (v) Dimethylformamide.

Lastly, 2-chloro-N-hydroxy acetamide was reacted with cinnamic acid amides to obtain the designed derivatives (Scheme 1). The purity of the prepared compounds was detected using
silica gel-G TLC and was further verified by FTIR and $^1$H-NMR spectra. The yield and physicochemical characteristics of the prepared derivatives are presented in Table 1.

### Table 1. Physicochemical properties, % yield and in vitro cytotoxicity (IC$_{50}$ values) of the synthesized derivatives.

| Compound | R$^1$ | R$^2$ | R$^3$ | Mol. Formula | M. Pt. (°C) | R$^4$ | % Yield | IC$_{50}$ (µM)$^*$ |
|----------|-------|-------|-------|-------------|------------|-------|--------|------------------|
| 1        | H     | H     | CH$_1$| C$_8$H$_{12}$N$_2$O$_3$ | 113-116    | 0.65  | 57     | 11.38 ± 0.16     |
| 2        | H     | H     | C$_8$H$_{12}$N$_2$O$_3$ | 108-111    | 0.55  | 47     | 11.89 ± 0.21     |
| 3        | H     | H     | C$_8$H$_{12}$N$_2$O$_3$ | 107-110    | 0.42  | 35     | 18.73 ± 0.16     |
| 4        | H     | H     | C$_8$H$_{12}$N$_2$O$_3$ | 112-115    | 0.43  | 45     | 12.06 ± 0.08     |
| 5        | OH    | H     | CH$_1$ | C$_8$H$_{12}$N$_2$O$_4$ | 117-120    | 0.56  | 67     | 10.36 ± 0.27     |
| 6        | OH    | H     | C$_8$H$_{12}$N$_2$O$_4$ | 123-126    | 0.78  | 53     | 18.62 ± 0.33     |
| 7        | OH    | H     | C$_8$H$_{12}$N$_2$O$_4$ | 107-110    | 0.44  | 44     | 16.98 ± 0.23     |
| 8        | OH    | H     | C$_8$H$_{12}$N$_2$O$_4$ | 118-121    | 0.50  | 76     | 13.90 ± 0.12     |
| 9        | H     | OH    | CH$_1$ | C$_8$H$_{12}$N$_2$O$_4$ | 117-120    | 0.35  | 66     | 11.06 ± 0.23     |
| 10       | H     | OH    | C$_8$H$_{12}$N$_2$O$_4$ | 116-119    | 0.65  | 65     | 12.98 ± 0.21     |
| 11       | H     | OH    | C$_8$H$_{12}$N$_2$O$_4$ | 118-121    | 0.48  | 68     | 18.75 ± 0.28     |
| 12       | H     | OH    | C$_8$H$_{12}$N$_2$O$_4$ | 123-126    | 0.75  | 78     | > 25.00         |
| 13       | OH    | OH    | CH$_1$ | C$_8$H$_{12}$N$_2$O$_5$ | 122-125    | 0.28  | 41     | 18.80 ± 0.31     |
| 14       | OH    | OH    | C$_8$H$_{12}$N$_2$O$_5$ | 120-123    | 0.30  | 55     | 16.33 ± 0.13     |
| 15       | OH    | OH    | C$_8$H$_{12}$N$_2$O$_5$ | 148-151    | 0.47  | 35     | 14.52 ± 0.12     |
| 16       | OH    | OH    | C$_8$H$_{12}$N$_2$O$_5$ | 140-143    | 0.49  | 62     | 13.95 ± 0.10     |

| Colchicine | | | | | | | 0.632 ± 0.09 |

$^*$TLC mobile phase: Dichloromethane: methanol (19:1); $^*$Values are presented as the mean ± standard deviation (n = 3) of in vitro cytotoxicity assay.

The $^1$H-NMR spectra of prepared molecules displayed -NH signal near δ 8 ppm validated the existence of hydroxamate (-NHOH) moiety. The $^1$H-NMR spectra contained a doublet signal near δ 2 ppm validating the occurrence of OH group in -NHOH moiety. The $^1$H-NMR spectra displayed a doublet signal near δ 6.50 ppm, confirming the presence of H on α-C to amide carbonyl and doublet around δ 7.50 ppm H on β-C to amide carbonyl. The protons belonging to the aromatic ring were observed with the expected chemical shift and integral values. The coupling constant (J) value was above 12 Hz for ethylenic carbons (CH=CH), which is related to trans-configuration, thus, endorsing the trans-configuration of the prepared molecules (i.e., E). The FTIR spectra of the synthesized molecules revealed absorption bands which give conformation about the presence of various functional groups in the synthesized compounds. The FTIR spectra exhibited the absorption band in the region 1600-1475 cm$^{-1}$ corresponding to the C=C stretching band, suggesting aromatic moiety in the prepared derivatives. The C-C stretching in region 1680-1600 cm$^{-1}$ shows the existence of an aliphatic chain. The absorption band in regions 3500-3100 and 1350-1000 cm$^{-1}$ indicated the existence of N-H and N-C stretch, respectively. The absorption band in the region 3650-3600 cm$^{-1}$ specified the incidence of OH of -NHOH moiety. The absorption bands in the region 1740-1705 and 1350-800 cm$^{-1}$ revealed the incidence of C=O and C-C stretch, respectively. The presence of the phenolic -OH group was learned by the band in the region 1410-1300 cm$^{-1}$ for -OH groups at C$_3$ and C$_4$ of the phenyl ring.

3.2. In vitro cytotoxicity.

The cytotoxicity of the synthesized cinnamic acid derivatives was evaluated using MTT assay. The repressive growth impact was evaluated for the synthesized molecules on the human lung cancer cell line (A-549). The outcomes of this study showed that almost all of the tested derivatives significantly diminished the cell viability of A-549 cells with an IC$_{50}$ value in the range 10 µM to 18 µM except compound 12 (IC$_{50}$ value > 25 µM), while IC$_{50}$ value of
colchicine (positive control) was 6.32 µM (Table 1). Compound 5 showed the most potent cytotoxicity in A-549 cells. Amongst the compounds tested in vitro, compounds bearing methyl-substituted amide group (1, 5, and 9) showed the most potent in vitro cytotoxicity with IC₅₀ values of 11.38, 10.36, and 11.06 µM, respectively. Amongst the synthesized molecules, compounds bearing ethyl substituted amide scaffold (2 and 10) showed potent cytotoxicity but were less active when compared to the compounds with a methyl-substituted amide group. The in vitro cytotoxicity assay outcomes depicted that substituting phenyl ring with a hydroxyl group at both C-3 and C-4 resulted in decreased cytotoxicity, as can be seen for the compounds 13-16. Replacement of methyl or ethyl group at amide ‘N’ with benzyl (C₆H₅CH₂) ring or butyl group resulted in decreased activity, as can be seen from in vitro cytotoxicity assay results for the compounds 8 and 15.

3.3. In silico studies.

Hit optimization of the prepared derivatives was carried out using calculating drug-like parameters (molecular weight (Mol. Wt.), partition coefficient (log P), hydrogen bond donors (HBD), and hydrogen bond acceptors (HBA)). Most derivatives showed drug-likeness as derived by “Lipinski’s rule of 5” (Table 2). The docking simulations were performed using AutoDock Vina in the active site of MMP9 protein, and the docking method was authenticated by docking of 4H3X ligand with MMP9 (ΔG value of -7.8). Almost all the prepared ligands displayed appreciable bonding in the active site as determined by analyzing the H-bonding, hydrophobic bonds, and binding energy (ΔG values) of the finest docked ligand-protein poses (Table 2).

Table 2. Molecular properties, ΔG values, and H-bond interactions of the synthesized molecules with the target (MMP-9 protein).

| Ligand | Mol. Wt. | Log P | HBA | HBD | ΔG  |
|--------|----------|-------|-----|-----|-----|
| 1      | 234      | 0.44  | 2   | 3   | -8.1|
| 2      | 248      | 0.80  | 2   | 3   | -8.0|
| 3      | 276      | 1.77  | 2   | 3   | -7.8|
| 4      | 310      | 2.17  | 2   | 3   | -8.5|
| 5      | 250      | 0.14  | 4   | 3   | -8.6|
| 6      | 264      | 0.50  | 4   | 3   | -8.1|
| 7      | 292      | 1.46  | 4   | 3   | -8.2|
| 8      | 326      | 1.87  | 4   | 3   | -8.2|
| 9      | 250      | 0.14  | 4   | 3   | -8.3|
| 10     | 266      | 0.50  | 4   | 3   | -8.2|
| 11     | 292      | 1.46  | 4   | 3   | -7.6|
| 12     | 283      | 1.87  | 4   | 3   | -7.1|
| 13     | 266      | 0.16  | 5   | 4   | -8.3|
| 14     | 280      | 0.19  | 5   | 4   | -8.6|
| 15     | 308      | 1.16  | 5   | 4   | -8.4|
| 16     | 342      | 1.56  | 5   | 4   | -7.8|

Colchicine | -       | -     | -   | -   | -   |

*Mol. Wt., log P, HBA, and HBD were computed using MarvinSketch.

The best-docked poses for compounds 1, 5, and 9 were further studied in minitue employing PyMOL for exploring the positioning, style, and binding contacts of the prepared ligands in the active site of MMP-9. A superpose of docked poses of compounds 1, 5, and 9 on the docked pose of 4H3X ligand (‘N-2-(biphenyl-4-ylsulfonyl)-N sol-pyloxyacetoxyhydroxamic acid’) showed a comparable binding style in the active site of MMP-9 as that of the x-ray crystallized inhibitor (Figure 2A).
Figure 2. (A) Superimpose of docked poses for compounds 1, 5, and 9 (black sticks) on that of Ligand 4WZV (grey sticks); (B) Docked pose for compounds 1; (C) 5, and (D) 9 showing H-bonds with active site residues of MMP-9.

The docked pose of compound 1 disclosed the H-bonding amongst the ‘NH’ and ‘OH’ of hydroxamate and backbone amide ‘carbonyl’ and ‘COOH’ of Ala189 and Glu227 residues in the active site of MMP-9 with H-bond distance of 2.8 and 2.9 Å, correspondingly. The ‘carbonyl’ group of compound-1 also showed the H-bond interaction with amide ‘NH’ of Leu188 residue of MMP-9 with an H-bond distance of 3.9 Å. The ‘NH’ of hydroxamate also showed a good metal interaction with Zn$^{2+}$ of the MMP-9. The phenyl moiety obtruded in the hydrophobic region, revealing hydrophobic bonds with Leu188, Val223, His226, Met247, and Tyr248 residues of MMP-9, whereas the methyl group showed hydrophobic interaction with Pro246 residue of MMP-9 (Figure 2B).

Compounds 5 and 9 also exhibited strong H-bond interactions (with Leu188, Ala189, and Glu227 residues), hydrophobic bonds (with Leu188, Val223, His226, Pro246, Met247, and Tyr248 residues), and metal interaction with Zn in the active site of MMP-9 supporting the in vitro cytotoxic effects of these compounds (Figure 2C and 2D). Thus, in silico docking studies supported the in vitro cytotoxicity of the synthesized compounds via inhibition of the MMP-9 protein.

4. Conclusions

A series of newer cinnamic acid analogs were designed, prepared, and evaluated for the in vitro cytotoxicity on human lung cancer cell lines (A-549). Amongst the synthesized compounds, compound 5 exhibited the most potent cytotoxicity on lung cancer cell lines with an IC$_{50}$ value of 10.36 µM. The synthesized derivatives were evaluated in silico to evaluate their binding interactions with the MMP-9 protein. The designed molecules showed H-bond interactions...
interactions with Leu188, Ala189, and Glu227 residues of the MMP-9 protein. The NH of the hydroxamate group showed good metal-interaction with Zn$^{2+}$ ion of MMP-9 protein. All the synthesized compounds showed appreciable drug-like characteristics. The in vitro cytotoxicity and in silico docking results showed the potential of these molecules to act as strong MMP-9 inhibitors, and these derivatives could serve as preliminary molecules for the development of effective antineoplastic agents.

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**Conflicts of Interest**

The authors declare no conflict of interest.

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