

## Synthesis, characterization and biological evaluation of novel ferulic acid-based alkyl-1,2,3-triazole analogs

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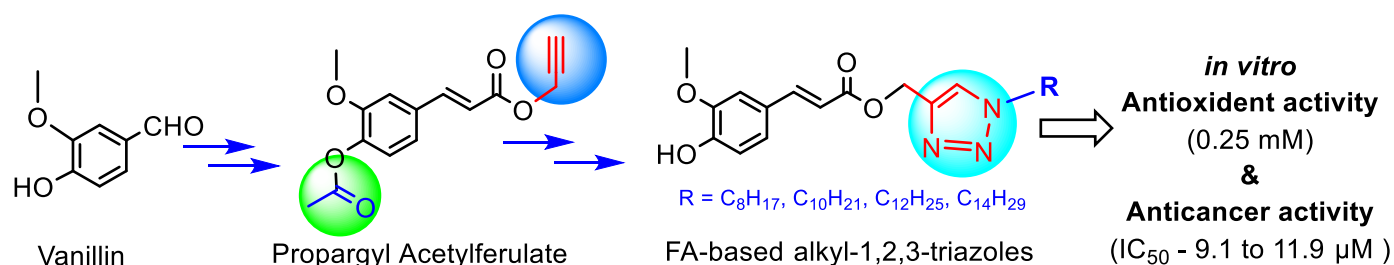
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### Abstract

The work reports the synthesis of novel series of biologically active ferulic acid-based alkyl-1,2,3-triazole analogs from vanillin, for the first time. The synthetic protocol comprises of four steps involving *in situ* acylation and carboxylation of vanillin to 4-acetyl ferulic acid, etherification with propargyl bromide to 4-acetyl propargyl ferulate, followed by 'click' reactions with some alkyl azides to give 4-acetyl alkyl-1,2,3-triazole ferulates and then deacetylation to produce alkyl-1,2,3-triazole ferulates in very good yields (>80%). Antioxidant activity of these ferulate analogs was determined by DPPH radical scavenging assay in comparison with BHT. All these compounds exhibited moderate activity at higher concentrations and the C<sub>8</sub>- and C<sub>10</sub>-1,2,3-triazole ferulates exhibited very good antioxidant activity at lower concentrations (0.25 mM). In addition, these alkyl-triazole ferulates also exhibited promising anticancer activity against DU-145, Hela with MIC values ranging from 9.1 to 11.9 μM and moderate activity against MDAMB-231.



**Keywords:** Vanillin, ferulic acid, alkyl-1,2,3-triazole, antioxidant, cytotoxicity

## Introduction

The human body is constantly subjected to significant oxidative stress (OS) because of imbalance between oxidation and antioxidative protective systems. The antioxidation mechanism of the body itself can resist its oxidation thereby preventing the accumulation of reactive oxygen species (ROS) in the body and repair the damage caused by it. Prolonged higher levels of ROS leads to various pathological conditions such as, aging, Parkinson's disease, cardiovascular, cancer, diabetes, autoimmune diseases, neurological disorders etc.<sup>1-4</sup> Among these, cancer is a life-threatening disease and about 10 million deaths were reported worldwide in 2022, in spite of great advancements in cancer research and treatment. 1,2,3-Triazole compounds have gained lot of attention for the development of new anticancer agents due to their diverse therapeutic potential and easy preparation *via* Cu(I)-catalyzed Azide–Alkyne Cycloaddition (CuAAC) reactions.<sup>5,6</sup> Such 1,2,3-triazole scaffolds are found as major structural components in various class of drugs with a broad spectrum of pharmacological properties such as, anticancer, antimicrobial, antibacterial, anti-tubercular, antioxidant and antiinflammatory.<sup>7-10</sup>

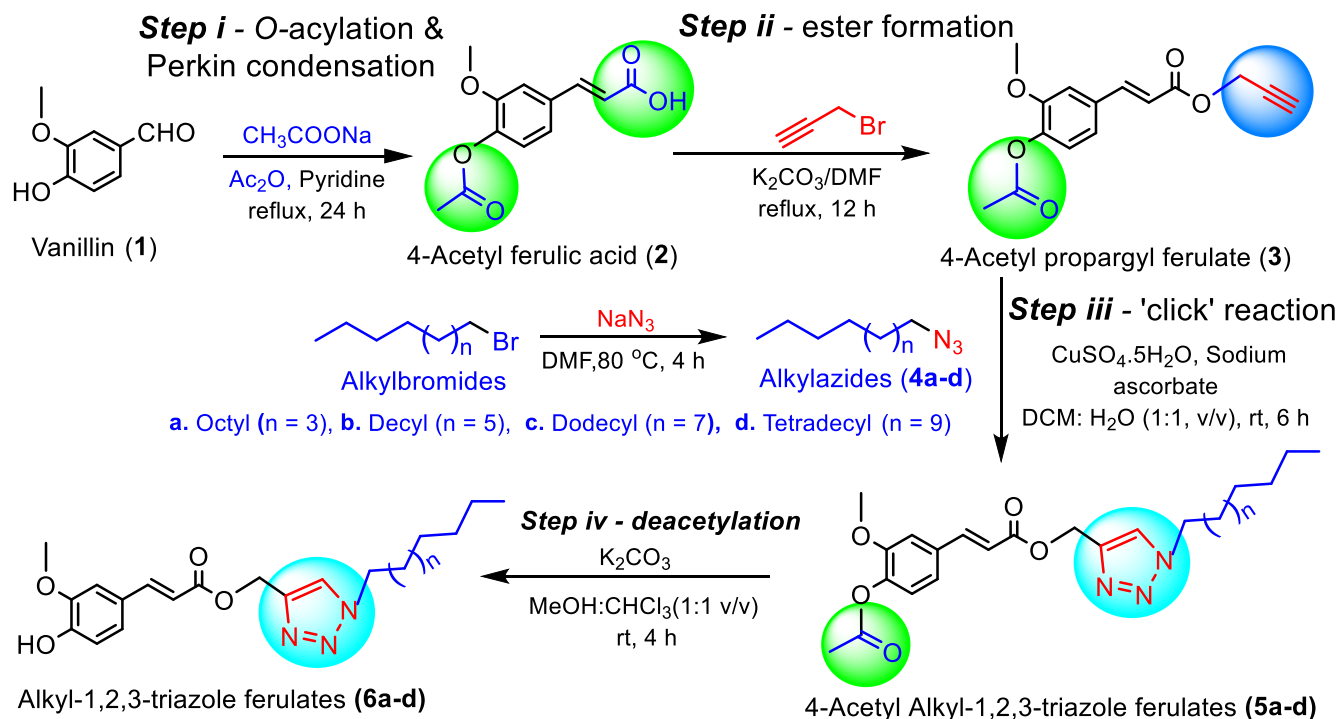
Phenolipids, such as *p*-coumaric, ferulic, caffeic and sinapic acids, are natural hydrophilic antioxidants which occur in fruits, vegetables, spices and herbs. Synthetic phenolipids obtained by lipophilization of natural phenolics find widespread applications in food and cosmetics areas due to their enhanced antioxidant property with improved bioavailability and bioefficacy.<sup>11-13</sup> Lipophilization enhances the solubility of the phenolics in apolar media without compromising the basic core of the molecule responsible for antioxidant activity.<sup>14-16</sup> Among these, ferulic acid has extensive promising therapeutic effects in managing diabetes, cancer, pulmonary and CVS diseases primarily due to its antioxidant and anti-inflammatory action, in addition to food and pharmaceutical applications.<sup>17,18</sup> There are very few reports in the field of phenolipid-based triazole analogs having good anticancer and anti-inflammatory activities.<sup>19-21</sup> In continuation to our ongoing research in the area of bioactive lipid-based triazole derivatives,<sup>22,23</sup> the present study reports the synthesis of novel alkyl-1,2,3-triazole ferulate analogs **6a-d** using a cheaper and commercially available vanillin (**1**, Scheme 1). The synthesized alkyl-1,2,3-triazole ferulate analogs **6a-d** were screened for DPPH radical scavenging activity and *in vitro* cytotoxicity towards DU145, HeLa, MDAMB231 and MCF7 human cancer cell lines.

## Results and Discussion

### Synthesis and characterization

We envisioned the retrosynthesis of alkyl-1,2,3-triazole ferulates **6a-d** from the 4-acetyl propargyl ferulate (**3**), which can be easily prepared from a commercially available vanillin (**1**) by adopting *in situ* O-acetylation and Perkin condensation followed by propargyl ester formation and a 'click' reaction, as depicted in Scheme 1. Accordingly, the synthesis of alkyl-1,2,3-triazole ferulate analogs **6a-d** commenced from vanillin (**1**) and took four steps the last being deacetylation. The 4-acetyl ferulic acid (**2**) was prepared by Perkin condensation, with *concurrent* O-acetylation, of vanillin (**1**), in presence of sodium acetate, acetic anhydride and pyridine at reflux temperature for 24 h in ~65% yield.<sup>24</sup> The structure was confirmed by the presence of a broad peak at 3451 cm<sup>-1</sup> and two sharp peaks at 1762 and 1689 cm<sup>-1</sup> in FT-IR corresponding to OH and carbonyl stretching of acetyl and carboxylic acid functional groups of compound **2**, respectively. <sup>1</sup>H NMR spectrum of **2** showed singlets at  $\delta$  2.31 and 3.86 corresponding to -COCH<sub>3</sub> and -OCH<sub>3</sub> protons. The signals for the *trans* double bond protons (-CH=CH-COOH) were found at  $\delta$  6.39 and 7.61, as doublets with coupling constant of 15.8 Hz. <sup>13</sup>C-NMR spectrum also

showed peaks corresponding to the acetyl carbonyl carbon ( $-\text{COCH}_3$ ) and methoxy carbon ( $-\text{OCH}_3$ ) at  $\delta$  168.7 and 55.3 respectively.



**Scheme 1.** Synthesis of alkyl-1,2,3-triazole ferulates **6a-d**.

Conversion of the carboxyl functionality into an ester **2** with propargyl bromide using  $\text{K}_2\text{CO}_3$  in anhydrous DMF at reflux temperature for 12 h, resulted the 4-*O*-acetyl propargyl ferulate (**3**) in 82% yield. The  $^1\text{H}$  NMR spectrum of the compound **3** showed singlet peak at  $\delta$  3.84 and 4.80 for alkyne- $\text{CH}$  and  $-\text{OCH}_2$ -alkyne protons confirming the propargyl functionality along with the acetyl and double bond protons as discussed for compound **2**. The  $^{13}\text{C}$  NMR spectrum showed the carbonyl carbon peaks of acetyl and ester groups at  $\delta$  168.7 and 165.8, alkyne carbons at  $\delta$  77.6 and 74.9 along with  $-\text{OCH}_2$  carbon at  $\delta$  55.3.

Alkyne-azide 'click' reaction of the acetylenic compound **3** with alkyl azides of octyl ( $\text{C}_8$ , **4a**), decyl ( $\text{C}_{10}$ , **4b**), dodecyl ( $\text{C}_{12}$ , **4c**) and tetradecyl ( $\text{C}_{14}$ , **4d**) under 'click' dipolar cycloaddition conditions using sodium ascorbate and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ <sup>25</sup> resulted the corresponding 4-acetyl alkyl-1,2,3-triazole ferulates **5a-d** in 92-98% isolated yields. During optimization of reaction conditions, several reactions were conducted by varying the concentration of reagents, reaction time, and temperature. This study revealed that the 'click' reaction of compound **3** with alkyl azides **4a-d** in 1:1 mole equivalents using 0.1:0.01 mole ratios of sodium ascorbate and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in DCM/ $\text{H}_2\text{O}$  mixture (1:1 v/v) at ambient temperature was advantageous in resulting the corresponding 1,2,3-triazoles **5a-d** within six hours. The  $^1\text{H}$  NMR spectra of octyl-1,2,3-triazoles of acetyl ferulic acid (**5a**) showed a singlet at  $\delta$  7.63 for  $-\text{C}=\text{CH}-\text{N}-$  of 1,2,3-triazole ring and a triplet at  $\delta$  4.33-4.34 for 1,2,3-triazole attached  $-\text{CH}_2$  in addition to the alkyl chain proton, at higher field. The presence of additional peaks in the  $^{13}\text{C}$  NMR spectra of **5a** at lower field, at  $\delta$  142 and 123, give evidence for the two hetero-aromatic carbons of the 1,2,3-triazole ring along with the alkyl chain carbons at higher field. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the decyl, dodecyl and tetradecyl-1,2,3-triazoles of acetyl ferulic acid **5b-d** also showed a similar pattern to that of **5a**.

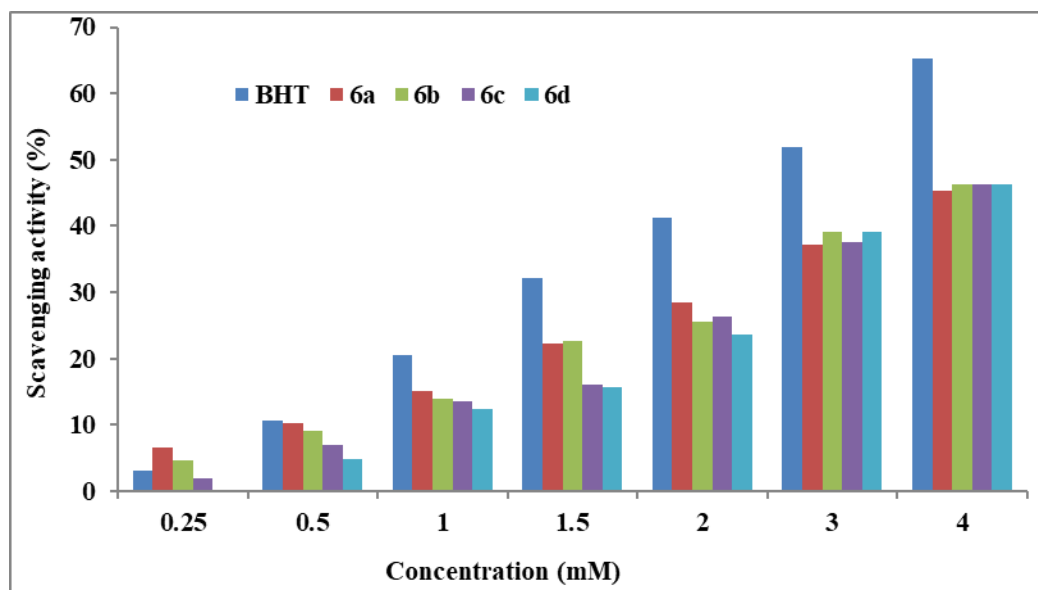
Finally, the targeted alkyl-1,2,3-triazole ferulates **6a-d** were obtained in 81-85% isolated yields by deacetylation of the phenolic ester group of **5a-d** using  $\text{K}_2\text{CO}_3$  in  $\text{MeOH}:\text{CHCl}_3$  (1:1, v/v) at ambient temperature

for 4 hours. The structure of the octyl-1,2,3-triazole ferulate **6a** was confirmed by the spectral data as evidenced from the absence of  $-\text{COCH}_3$  proton at  $\delta$  2.31 (singlet) and carbon peak at  $\delta$  168.7 in  $^1\text{H}$  &  $^{13}\text{C}$  NMR spectra respectively. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the decyl-, dodecyl- and tetradecyl-1,2,3-triazole ferulates **6b-d** also showed the absence of acetyl protons and carbon peaks confirming the complete deacetylation. All the compounds were further confirmed by ESI-MS and HRMS data and are found to be in good agreement with the proposed structures.

## Biological studies

### Antioxidant activity

Antioxidant activity of the alkyl 1,2,3-triazole ferulates **6a-d** were determined by their free radical scavenging ability (FRSA %) using the stable DPPH radical assay.<sup>26</sup> The commercially available synthetic antioxidant BHT was used as positive control for comparison purpose to that of test compounds **6a-d**. The antioxidant activity data, as means of three independent assays are depicted in Figure 1. The assay was conducted at different concentrations of antioxidants (0.25, 0.5, 1, 1.5, 2, 3 and 4 mM) in methanol. The compounds **6a** and **6b** having 1,2,3-triazoles with  $\text{C}_8$  and  $\text{C}_{10}$  alkyl chains were found to exhibit superior FRSA % of 6.6 and 4.7 respectively at lower concentration of 0.25 mM compared to that of BHT (3.1%). Whereas, the FRSA % of all the compounds at 2 mM concentration was found to be in the range of 24-29, which are nearer to that of BHT at 1 mM concentration. Similarly, the FRSA % of all the compounds at 4 mM concentration was found to be in the range of 45 to 46, which is also nearer to that of BHT at 2 mM concentration.



**Figure 1.** Antioxidant activity of the FA-based alkyl 1,2,3-triazoles (**6a-d**).

These results indicate that, the antioxidant activity of all the alkyl-1,2,3-triazoles ferulates **6a-d** was approximately 15-20% less to that of the BHT. In addition, all the compounds exhibited moderate antioxidant activity at higher concentrations (>3 mM), and at lower concentrations only **6a** and **6b** were comparatively active with respect to BHT. The study also revealed that, with increase of chain length from  $\text{C}_8$  to  $\text{C}_{14}$  in the triazole functionality, there is a considerable decrease in the antioxidant activity at lower concentrations (0.25-1.5 mM) and the similar analogy was not observed at higher concentrations ( $\geq 2$  mM).

### Anticancer activity

The alkyl-1,2,3-triazole ferulates **6a-d** were evaluated for anticancer activity against four human cancer cell lines including DU145 (human prostate cancer cell line), Hela (human cervical cancer cell line), MCF-7 (human breast cancer cell line) and MDAMB-231 (human breast cancer cell line) using the MTT assay.<sup>27</sup> and the results are summarized in Table 1. All the compounds exhibited significant anticancer activity against DU145 and Hela cell lines, with IC<sub>50</sub> values in the range from 10.17 to 10.95 μM and 9.11 to 11.64 μM respectively. Moderate activity was observed against MDAMB-231 cells with IC<sub>50</sub> values between 23.71 and 39.01 μM. No significant activity was observed against MCF-7 cells with IC<sub>50</sub> values >170 μM. This study also indicated that variations in the length of alkyl chain in the 1,2,3,-triazole functionality do not significantly impact the proliferation of the cancer cell lines tested.

**Table 1.** Anticancer activity of alkyl-1,2,3-triazole ferulates **6a-d**

Entry	Compound	IC <sub>50</sub> (μM) <sup>a</sup>			
		DU145	Hela	MDAMB231	MCF7
1	<b>6a</b>	10.2±1.4	9.1±0.5	31.9±2.2	170.6±16.5
2	<b>6b</b>	10.2±0.9	9.7±0.8	23.8±5.8	181.9±21.4
3	<b>6c</b>	11.9±0.8	10.2±0.5	23.7±3.6	226.9±16.4
4	<b>6d</b>	11.0±1.0	11.6±1	39.0±2.9	172.5±15.5
5	Doxorubicin	7.8±0.7	5.8±0.1	6.7±0.1	7.4±0.2

<sup>a</sup>Inhibitory activity was expressed as concentration required to inhibit tumor cell proliferation by 50% (IC<sub>50</sub>)

Data are presented as the mean ± SDs of three independent experiments

### Drug-likeness properties

Drug-likeness can predict a gentle balance among the molecular properties of a compound that directly influence its pharmaco-dynamics and pharmaco-kinetics and ultimately affect their absorption, distribution, metabolism, and excretion (ADME) in the human body, like a drug.<sup>28</sup> In general, these parameters allow one to ascertain a poor oral absorption, or membrane permeability, that occurs when the evaluated molecules present values higher than 5H-bond donors (HBD), 10H-bond acceptors (HBA), molecular weight (MW) >500 Da and Log P (c Log P) >5 (Lipinski's 'rule-of-five').<sup>29</sup> There are many violations of this 'rule of five' among existing drugs and vice versa, i.e., this rule does not certify that a molecule is ideal "drug-like". Topological polar surface area (TPSA) are now recognized as a good indicator of drug absorbance in the intestines, Caco-2 monolayers penetration, and blood brain barrier crossing. These parameters were calculated using mol inspiration for the compounds **6a-d**, and the results are depicted in Table 2. As ferulic acid obeys the Lipinski's 'rule-of-five', it can be considered as a good drug candidate and was also well established.<sup>30</sup> The presence of sufficient number of proton acceptor and proton donor groups in the triazoles ensures, efficient interaction with the hydrogen bonding groups of the receptors. Hydrogen-bonding capacity has been also identified as an important parameter for describing drug permeability.<sup>28,29</sup> In spite of the solubility in protic solvents, all the triazole compounds **6a-d** were found to violate one of the Lipinski's parameter miLog P value >5. According to their predictive TPSA data, it seems that this type of compounds could have a good capacity for penetrating cell membranes.

**Table 2.** Structural properties of ferulic acid (FA) and alkyl 1,2,3-triazole ferulates **6a-d**<sup>a</sup>

Entry	Compound	n-Viol Acceptable range	n- atoms —————>	miLog <i>P</i> <5	MW <500	n-HBA <10	n-HBD <5	n-ROTB	TPSA
1	<b>FA</b>	0	14.0	1.24	194.186	4	2	3	66.761
2	<b>6a</b>	1	27.0	5.06	373.453	7	1	12	86.485
3	<b>6b</b>	1	29.0	6.07	401.507	7	1	14	86.485
4	<b>6c</b>	1	31.0	7.08	429.561	7	1	16	86.485
5	<b>6d</b>	1	33.0	8.08	457.615	7	1	18	86.485

<sup>a</sup> n-Viol: no. of violations; n-atoms: no. of atoms; miLog *P*: mol inspiration predicted Log *P*  
 MW: molecular weight; n-HBA: no. of hydrogen acceptor; n-HBD: no. of hydrogen bond donors  
 n-ROTB: no. of rotational bonds; TPSA: topological polar surface

## Conclusions

In this study, we described the synthesis of alkyl-1,2,3-triazole ferulate derivatives **6a-d** with varying alkyl chain length from C<sub>8</sub> to C<sub>14</sub> of the triazole alkyl substituent, in good yields by using vanillin as the starting material. All the synthesized compounds **6a-d** were evaluated for their antioxidant activity (FRSA) using DPPH radical scavenging assay by taking BHT as positive control. The FRSA results showed that the octyl (C<sub>8</sub>) and decyl (C<sub>10</sub>)-1,2,3-triazole ferulate compounds (**6a**) & (**6b**) are comparatively active with BHT at lower concentrations and moderately active at higher concentrations. There is a considerable decrease in the activity with increase of alkyl chain length from C<sub>8</sub> to C<sub>14</sub> at lower concentrations (0.25 & 1.5 mM) and similar analogy was not observed at higher concentrations (>2 mM). These alkyl-1,2,3-triazole ferulates **6a-d** were also evaluated for anticancer activity against four human cancer cell lines and were found to be potentially active against DU145 and Hela with IC<sub>50</sub> values ranging from 9.11 to 11.87 μM, moderately active against MDAMB-231 and inactive against MCF7 cancer cell lines. These compounds **6a-d** may become potentially active drug candidates, based on their solubility in protic solvents and predictive TPSA data for penetrating into cell membranes, in spite of being at some distance from the Lipinski's parameter miLog *P* value >5.

## Experimental Section

**General.** The 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH), butylated hydroxy-toluene (BHT), propargyl bromide, sodium ascorbate were purchased from Sigma-Aldrich, St.Louis, USA. Vanillin, sodium acetate (CH<sub>3</sub>COONa), pyridine, potassium carbonate (K<sub>2</sub>CO<sub>3</sub>), cupric sulphate pentahydrate (CuSO<sub>4</sub> · 5H<sub>2</sub>O) were purchased from SD Fine chemical laboratories, Mumbai, India. Alkyl bromides namely, octyl (C<sub>8</sub>), decyl (C<sub>10</sub>), dodecyl (C<sub>12</sub>) and tetradecyl (C<sub>14</sub>) bromides were purchased from Sigma–Aldrich, St. Louis, USA. All other reagents and solvents were procured from M/s SDFCL (Mumbai, India) and were of highest grade of purity. Progress of the reactions was monitored by using TLC plates (coated with TLC grade silica gel, obtained from Merck, India). The spots were located by exposure to iodine vapours or under UV- light. Column chromatography was performed over silica gel (100-200 mesh) procured from Qualigens (India) using freshly distilled solvents. Melting points were determined in open capillaries on Barnstead Electrothermal's melting

point apparatus (India) and are uncorrected. IR spectra were recorded on a Perkin Elmer (model: spectrum BX) FT-IR Spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded at 300 K Bruker UXNMR (operating at 300 MHz for  $^1\text{H}$  and 75 MHz for  $^{13}\text{C}$  NMR) in  $\text{CDCl}_3$  as solvents with TMS as an internal standard. Chemical shifts ( $\delta$ ) are given in ppm and coupling constants ( $J$ ) in Hz. The splitting pattern abbreviations are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Mass spectra were recorded using Waters, Micromass-Quatromicro electron spray ionization (ESI-MS). HRMS data were recorded on a Thermo Scientific Exactive Orbitrap Mass spectrometer (Germany) and are given in mass units ( $m/z$ ). UV absorbance was measured on a Lambda-35 UV-Vis spectrophotometer from Perkin Elmer (Connecticut, USA). Structures of all the compounds were drawn using the program CS Chem Draw Ultra version 21.0.0.

**Synthesis of *E*-3-(4-acetoxy-3-methoxyphenyl)acrylic acid (2).** The synthesis of 4-acetyl ferulic acid (**2**) was carried out from vanillin (**1**) as per previously reported procedure.<sup>24</sup> To a mixture of vanillin (**1**, 10 g, 65.7 mmol) and sodium acetate (8.63 g, 10.5 mmol) dissolved in acetic anhydride ( $\text{Ac}_2\text{O}$ , 75 mL), pyridine (0.5 mL) was added and the reaction mixture was heated to reflux. After 24 h, the brown solution was poured over crushed ice and the solution was stirred until the appearance of a yellow-brown solid. The flask was left overnight in the freezer and the dark yellow solid obtained was separated by filtration. The crude product was re-crystallized in acetic acid/water to obtain pure compound **2**. Light yellow powder. Yield: 65%, MP 73.5–74 °C. IR (KBr,  $\text{cm}^{-1}$ ): 3451, 3011, 2946, 2849, 1762, 1689, 1633;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  H ppm 2.31 (s, 3H), 3.86 (s, 3H), 6.39 (d,  $J$  15.8 Hz, 1H), 7.02–7.17 (m, 3H), 7.61 (d,  $J$  15.8 Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.0, 167.8, 150.7, 143.1, 140.6, 132.9, 122.5, 120.5, 118.6, 110.6, 55.3, 20.0; ESI-MS:  $m/z$  259 [ $\text{M}+\text{Na}$ ] $^+$ ; HRMS (ESI): calculated for  $\text{C}_{12}\text{H}_{12}\text{O}_5\text{Na}^+$   $m/z$  259.0576, found 259.0575.

**Synthesis of Prop-2-yn-1-yl (*E*)-3-(4-acetoxy-3-methoxyphenyl) acrylate (3).** Propargyl bromide (3.37 mL, 42.3 mmol) was added drop wise to the mixture of **2** (10 g, 42.3 mmol) and  $\text{K}_2\text{CO}_3$  (8.77 g, 63.5 mmol) in dimethyl formamide (DMF) at ambient temperature and the reaction mixture was magnetically stirred at reflux temperature for 12 h. Progress of the reaction was monitored by TLC (on pre-coated glass plate with silica) using hexane/EtOAc (9:1). After completion of the reaction, DMF was removed under vacuum and the residue was extracted with ethyl acetate followed by washing with brine solution. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography to obtain pure 4-acetyl propargyl ferulate (**3**) in 82% yield. Solid; MP; 100–101 °C; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3267, 3072, 2950, 2854, 2125, 1755, 1714;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  H ppm 2.30 (s, 3H), 2.50 (s, 1H), 3.84 (s, 3H), 4.80 (s, 2H), 6.40 (d,  $J$  16.0 Hz, 1H), 7.04 (d, 1H), 7.08–7.14 (m, 2H), 7.68 (d,  $J$  16.0 Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.7, 165.8, 151.3, 145.1, 141.5, 133.0, 123.2, 121.3, 117.1, 111.2, 77.6, 74.9, 55.8, 52.0, 20.6; ESI-MS:  $m/z$  297 [ $\text{M}+\text{Na}$ ] $^+$ ; HRMS (ESI): cald for  $\text{C}_{15}\text{H}_{14}\text{O}_5\text{Na}^+$   $m/z$  297.0733, found 297.0733.

**General procedure for the synthesis of alkyl azides (4a-d).** Alkyl azides namely octyl ( $\text{C}_8$ ), decyl ( $\text{C}_{10}$ ), dodecyl ( $\text{C}_{12}$ ), and tetradecyl ( $\text{C}_{14}$ ) azides were prepared by using the classic procedure for nucleophilic substitution of alkyl bromides.<sup>25</sup> Alkyl bromides were reacted with sodium azide ( $\text{NaN}_3$ ) in DMF at 80 °C for 4 h to get alkyl azides **4(a-d)** as colorless oily liquids in excellent yields.

**General procedure for the synthesis of 4-acetyl alkyl-1,2,3-triazole ferulates (5a-d).** To a stirred suspension of **3** (0.2 g, 72.9 mmol) and alkyl azide (**4**, 1 mol eq.) in DCM:  $\text{H}_2\text{O}$  (1:1, v/v),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (0.01 mol eq.) and sodium ascorbate (0.1 mol eq.) were added and magnetically stirred at ambient temperature for 6 h. Progress of the reaction was monitored by TLC (on pre-coated glass plate with silica) using hexane/EtOAc (9:1) as the eluting system.. After completion of the reaction, the product was extracted into DCM followed by washing with brine solution. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The sticky residue obtained was further purified by silica gel column chromatography to get pure desired products.

Finally the products were verified using the ESI-MS and HRMS methods. The analytical and spectroscopic data for the synthesized 4-acetyl alkyl-1,2,3-triazole ferulates (**5a-d**) are given below.

**1-(1-Octyl)-(1H-1,2,3-triazol-4-yl)methyl-(E)-3-(4-acetoxy-3-methoxyphenyl) acrylate (5a).** Compound **5a** was prepared in 95% yield from octyl azide (**4a**), alkyne **3**; viscous liquid; IR (neat,  $\nu$ ,  $\text{cm}^{-1}$ ): 2930, 2852, 1765, 1716, 1634;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.17-1.42 (m, 10H), 1.84-2.00 (m, 2H), 2.32 (s, 3H), 3.85 (s, 3H), 4.35 (t,  $J$  7.35 Hz, 2H), 5.36 (s, 2H), 6.40 (d,  $J$  16.0 Hz, 1H), 7.01-7.14 (m, 3H), 7.63 (s, 1H), 7.63 (t,  $J$  16.0 Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.6, 166.4, 151.3, 144.6, 142.6, 141.4, 133.0, 123.6, 123.1, 121.2, 117.6, 111.1, 57.6, 55.8, 50.4, 31.7, 30.1, 29.2, 28.8, 26.3, 22.5, 20.5, 14.0; ESI-MS:  $m/z$  452  $[\text{M}+\text{Na}]^+$ ; HRMS (ESI)  $m/z$ : calcd for  $\text{C}_{23}\text{H}_{31}\text{O}_5\text{N}_3\text{Na}^+$  452.2155, found 452.2143.

**1-(1-Decyl)-(1H-1,2,3-triazol-4-yl)methyl (E)-3-(4-acetoxy-3-methoxyphenyl) acrylate (5b).** Compound **5b** was prepared in 92% yield from decyl azide (**4b**), alkyne **3**; viscous liquid; IR (neat,  $\nu$ ,  $\text{cm}^{-1}$ ): 2920, 2855, 1765, 1714, 1640;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.20-1.40 (m, 14H), 1.82-2.98 (m, 2H), 2.17 (s, 3H), 3.91 (s, 3H), 4.34 (t,  $J$  7.35 Hz, 2H), 5.34 (s, 2H), 6.29 (d,  $J$  16.0 Hz, 1H), 6.86-7.09 (m, 3H), 7.63 (t,  $J$  16.0 Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.6, 166.4, 151.3, 144.6, 142.6, 141.4, 133.0, 123.6, 123.1, 121.2, 117.6, 111.1, 57.6, 55.8, 50.4, 31.7, 30.1, 29.3, 29.2, 29.1, 28.8, 26.3, 22.5, 20.5, 14.0; ESI-MS:  $m/z$  480  $[\text{M}+\text{Na}]^+$ ; HRMS (ESI): calcd for  $\text{C}_{25}\text{H}_{35}\text{O}_5\text{N}_3\text{Na}^+$   $m/z$  480.2468, found 480.2455.

**1-(1-Dodecyl)-(1H-1,2,3-triazol-4-yl)methyl (E)-3-(4-acetoxy-3-methoxyphenyl) acrylate (5c).** Compound **5c** was prepared in 95% yield from dodecyl azide (**4c**), alkyne **3**; viscous liquid; IR (neat,  $\nu$ ,  $\text{cm}^{-1}$ ): 2923, 2854, 1766, 1714, 1639;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.20-1.42 (m, 18H), 1.86-1.97 (m, 2H), 2.17 (s, 3H), 3.85 (s, 3H), 4.34 (t,  $J$  7.35 Hz, 2H), 5.36 (s, 2H), 6.39 (d,  $J$  16.0 Hz, 1H), 7.01-7.15 (m, 3H), 7.62 (s, 1H), 7.67 (d,  $J$  16.0 Hz, 2H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.7, 166.5, 151.3, 144.7, 142.7, 141.5, 133.1, 123.5, 123.2, 121.2, 117.7, 111.1, 57.7, 55.8, 50.4, 31.8, 30.2, 29.5, 29.4, 29.3, 29.2, 29.1, 28.5, 26.4, 22.6, 20.6, 14.0; ESI-MS:  $m/z$  508  $[\text{M}+\text{Na}]^+$ ; HRMS (ESI): calcd for  $\text{C}_{27}\text{H}_{39}\text{N}_3\text{O}_5\text{Na}^+$   $m/z$  508.2781, found 508.2782.

**1-(1-Tetradecyl)-(1H-1,2,3-triazol-4-yl)methyl-3-(4-acetoxy-3-methoxyphenyl) acrylate (5d).** Compound **5d** was prepared in 98% yield from tetradecyl azide (**4d**), alkyne **3**; viscous liquid; IR (neat,  $\nu$ ,  $\text{cm}^{-1}$ ): 2927, 2851, 1763, 1718, 1638.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.18-1.37 (m, 22H), 1.80-1.98 (m, 2H), 2.32 (s, 3H), 3.85 (s, 3H), 4.34 (t,  $J$  7.35 Hz, 2H), 5.35 (s, 2H), 6.39 (d,  $J$  16.0 Hz, 1H), 7.00-7.17 (m, 3H), 7.63 (s, 1H), 7.68 (d,  $J$  16.0 Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  168.7, 166.5, 151.3, 144.7, 142.7, 141.5, 133.1, 123.5, 123.2, 121.2, 117.7, 111.1, 57.7, 55.8, 50.4, 31.8, 30.2, 29.5, 29.5, 29.5, 29.4, 29.4, 29.3, 29.1, 28.5, 26.4, 22.6, 20.6, 14.0; ESI-MS:  $m/z$  536  $[\text{M}+\text{Na}]^+$ ; HRMS (ESI): calcd for  $\text{C}_{29}\text{H}_{43}\text{O}_5\text{N}_3\text{Na}^+$   $m/z$  536.3094, found 536.3089.

**General procedure for the synthesis of alkyl-1,2,3-triazole ferulates (6a-d).** To the suspension of compound **5** (0.25 g) in  $\text{MeOH}:\text{CHCl}_3$  (1:1, v/v),  $\text{K}_2\text{CO}_3$  (1.2 mol eq.) was added and stirred for 4 h at ambient temperature. Progress of the reaction was monitored by TLC (on pre-coated glass plate with silica) using hexane/EtOAc (9:1) as the eluting system. After completion of the reaction,  $\text{K}_2\text{CO}_3$  was filtered off and the solvent was evaporated under vacuum followed by extraction into EtOAc. The organic layer was washed with brine solution and further dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Solvent was removed under vacuum and the crude product obtained was further purified by silica gel column chromatography. Finally, the products were verified using the ESI-MS and HRMS methods. The analytical and spectroscopic data for the synthesized alkyl-1,2,3-triazole ferulates (**6a-d**) are given below.

**1-(1-Octyl)-(1H-1,2,3-triazol-4-yl)methyl-3-(4-hydroxy-3-methoxyphenyl) acrylate (6a):** Compound **6a** was prepared in 87% yield; solid; MP: 88-90 °; IR (KBr,  $\nu$ ,  $\text{cm}^{-1}$ ): 3374, 3011, 2922, 2859, 1714, 1629;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.20-1.40 (m, 10H), 1.84-1.98 (m, 2H), 3.91 (s, 3H), 4.34 (t,  $J$  7.35 Hz, 2H), 5.35 (s, 2H), 6.29 (d,  $J$  16.0 Hz, 1H), 6.88-7.09 (m, 3H), 7.63 (s, 1H), 7.63 (d,  $J$  16.0 Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ ):  $\delta$  167.0, 148.2, 146.8, 145.5, 142.8, 126.6, 123.5, 123.0, 114.7, 114.6, 109.4, 57.3, 55.8, 50.3, 31.7, 30.1,



29.3, 28.8, 26.4, 22.5, 14.0; ESI-MS:  $m/z$  386  $[M-H]^-$ ; HRMS (ESI): calcd for  $C_{21}H_{28}N_3O_4$   $m/z$  386.2074, found 386.2090.

**1-(1-Decyl)-(1H-1,2,3-triazol-4-yl)methyl)-3-(4-hydroxy-3-methoxyphenyl) acrylate (6b):** Compound **6b** was prepared in 82% yield; solid; MP: 72-74 °C; IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3428, 3076, 2925, 2853, 1715, 1628;  $^1H$  NMR (300 MHz,  $CDCl_3$ ):  $\delta$  H ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.20-1.40 (m, 14H), 1.84-1.98 (m, 2H), 3.91 (s, 3H), 4.34 (t,  $J$  7.35 Hz, 2H), 5.35 (s, 2H), 6.30 (d,  $J$  16.0 Hz, 1H), 6.81-7.15 (m, 3H), 7.63 (s, 1H), 7.64 (d,  $J$  16.0 Hz, 1H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ ):  $\delta$  167.0, 148.2, 146.8, 145.5, 142.8, 126.6, 123.5, 123.1, 114.7, 114.6, 109.3, 57.4, 55.8, 50.3, 31.7, 30.1, 29.3, 29.2, 29.1, 28.8, 26.4, 22.5, 14.0; ESI-MS:  $m/z$  438  $[M+Na]^+$ . HRMS (ESI): calcd for  $C_{23}H_{33}O_4N_3Na^+$   $m/z$  438.2363, found 438.2353.

**1-(1-Dodecyl)-(1H-1,2,3-triazol-4-yl)methyl)-3-(4-hydroxy-3-methoxyphenyl) acrylate (6c):** Compound **6c** was prepared in 81% yield; solid; MP: 64-65 °C; IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3420, 3031, 2953, 2851, 1713, 1626;  $^1H$  NMR (300 MHz,  $CDCl_3$ ):  $\delta$  H ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.19-1.438 (m, 18H), 1.85-1.97 (m, 2H), 3.91 (s, 3H), 4.34 (t,  $J$  7.35 Hz, 2H), 5.34 (s, 2H), 6.29 (d,  $J$  16.0 Hz, 1H), 6.88-7.09 (m, 3H), 7.62 (s, 1H), 7.63 (d,  $J$  16.0 Hz, 1H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ ):  $\delta$  166.9, 148.3, 146.8, 145.5, 142.8, 126.4, 123.5, 123.0, 114.8, 114.4, 109.3, 57.3, 55.7, 50.3, 31.7, 30.1, 29.4, 29.4, 29.3, 29.2, 29.1, 28.8, 26.3, 22.5, 13.9. ESI-MS:  $m/z$  442  $[M-H]^-$ . HRMS (ESI): calcd for  $C_{25}H_{36}N_3O_4$   $m/z$  442.2700, found: 442.2715.

**1-(1-Tetradecyl)-(1H-1,2,3-triazol-4-yl)methyl)-3-(4-hydroxy-3-methoxyphenyl) acrylate (6d):** Compound **6d** was prepared in 85% yield; solid; MP: 62-64 °C; IR (KBr,  $\nu$ ,  $cm^{-1}$ ): 3435, 3083, 2925, 2850, 1714, 1626;  $^1H$  NMR (300 MHz,  $CDCl_3$ ):  $\delta$  H ppm 0.87 (t,  $J$  6.5 Hz, 3H), 1.20-1.36 (m, 22H), 1.86-1.94 (m, 2H), 3.91 (s, 3H), 4.33 (t,  $J$  7.35 Hz, 2H), 5.34 (s, 2H), 6.91 (d,  $J$  16.0 Hz, 1H), 6.89-7.07 (m, 3H), 7.62 (s, 1H), 7.64 (d,  $J$  16.0 Hz, 1H);  $^{13}C$  NMR (75 MHz,  $CDCl_3$ ):  $\delta$  166.9, 148.3, 146.9, 145.5, 142.7, 126.4, 123.5, 123.0, 114.8, 114.4, 109.4, 57.3, 55.7, 50.3, 31.7, 30.0, 29.4, 29.4, 29.4, 29.3, 29.3, 29.2, 29.2, 28.8, 26.3, 22.5, 13.9; ESI-MS:  $m/z$  470  $[M-H]^-$ ; HRMS (ESI): calcd for  $C_{27}H_{40}N_3O_4$   $m/z$  470.3013, found 470.3033.

## Biological Studies

**Antioxidant/free-radical scavenging activity assay.** The antioxidant activity of prepared compounds **6(a-d)** were determined by measuring their free radical scavenging activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) according to reported method.<sup>26</sup> DPPH solution (0.1 mM) was prepared by dissolving 3.94 mg in 100 mL methanol, kept in a cool dark place, and used fresh. 0.1 mM DPPH solution (2 mL) was added to methanol (1 mL) and absorbance was taken immediately at 517 nm for control reading. Seven different concentrations of 0.25, 0.50, 1.0, 1.5, 2.0, 3.0 and 4 mM test compounds **6(a-d)** as well as standard compound (BHT) were taken and the volume was made uniformly to 3 mL using methanol and DPPH (2 mL) solutions. After 40 min incubation at 30 °C in the dark, the absorbance was measured at 517 nm against methanol as blank using Perkin-Elmer Lambda-35 spectrophotometer.

The free radical scavenging activity (FRSA in %) of the test samples and BHT was calculated using the formula:

$$FRSA \% = [(A_c - A_s)/A_c] \times 100$$

where, " $A_c$ " is the absorbance of the control and " $A_s$ " is the absorbance of the tested sample after 40 min.

**Anticancer activity.** Cellular viability was assessed using an MTT assay with minor modifications as described previously.<sup>27</sup> Briefly, four different cell lines were seeded into 96-well plates (10,000 cells/well in 100  $\mu$ L of culture medium supplemented with 10% serum) and incubated for 18–24 h in humid incubator with constant supply of 5%  $CO_2$ . The compounds **6(a-d)** were dissolved in DMSO to prepare stock solutions ranging from 0.5 to 5000  $\mu$ M in 10 fold increments. From working stock, 2  $\mu$ L of test compound and doxorubicin (as a standard control anticancer drug prepared in DMSO), were added to the culture media to achieve a final concentration of 0 to 100  $\mu$ M. Cells were further incubated to grow for 48 hrs under the standard cell culture conditions. At the end of the incubation period, filter-sterilized 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

in PBS (5 mg/mL; 10  $\mu$ L per well) was added to each well. After 2 hrs of incubation, the medium was removed and 100  $\mu$ L of DMSO was added to each well to solubilise the MTT-formazone complex giving purple colour. Absorbance of the complex, which is directly proportional to cell viability was measured at 562 nm using multimode micro plate reader (TecanGENios; Tecan AG, Mannedorf, Switzerland). The IC<sub>50</sub> values were determined based on the observed cell growth in the presence and absence of the test compounds. The results presented are the average three independent experiments, each performed in triplicate.

**Drug-likeness property:** The parameters for drug-likeness were evaluated according to the Lipinski's 'rule-of-five', using the Molinspiration WebME Editor 1.16. [<http://www.molinspiration.com>].

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## Supplementary Material

Supplementary information contains <sup>1</sup>H & <sup>13</sup>C-NMR spectra of the compounds **2**, **3**, **5(b-d)** and **6(b-d)** [Figure S1-S10].

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