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Design, Synthesis, and Biological Activity of Coniferyl Aldehyde Derivatives as Potential Anticancer and Antioxidant Agents

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ABSTRACT

Natural products are known to exhibit antimicrobial, anticancer, and antioxidant activities. Among these natural products is cinnamon which contains cinnamaldehyde. Cinnamaldehyde and its derivatives have been reported to have anticancer and antioxidant activities. Coniferyl aldehyde, a non-cytotoxic compound and a cinnamaldehyde derivative, has also been shown to have anticancer activity. In this study, several derivatives of coniferyl aldehyde were synthesized and evaluated for their anticancer and antioxidant activities. Compounds 1, 2, 4, and 8-11 showed cytotoxic activity against H1299 cell line, a non-small cell lung cancer cells, with 4 being the most potent with IC_{50} value of 6.7 μM. The antioxidant assay experiment showed that compounds 1, 2, and 4 resulted in half the scavenging activity of vitamin C at all tested concentrations. The coniferyl aldehyde itself showed dose-dependent antioxidant activity, with a proposed free radical stabilization mechanism. Thus, our study showed that the synthesized coniferyl aldehyde derivatives exhibit anticancer and antioxidant activities, which might act as potential therapeutic agents.
Keywords: Coniferyl aldehyde, Anticancer, Antioxidant, DPPH.

1. INTRODUCTION

The use of natural products for the treatment of several conditions, such as cancer, bacterial infections, fungal infections, and as antioxidants is widely reported[1-11]. Among these natural The use of natural products for the treatment of several conditions, such as cancer, bacterial infections, fungal infections, and as antioxidants is widely reported[1-11]. Among these natural products is the cinnamon spice. Cinnamon spice is obtained from the inner bark of several *Cinnamomum* trees[12,13]. A major constituent of cinnamon spice is cinnamaldehyde[12].

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Cinnamaldehyde and its derivatives have been reported to show several pharmacological properties. Among the commonly studied properties are the anticancer[14-17] and antioxidant[18-21] activities. The anticancer activity of cinnamaldehyde has been observed in several cancer cell lines, such as leukemia (K562) and breast cancer (MCF7)[22,23]. It has also shown to enhance anticancer effects in combination treatments[24-26]. Moreover, cinnamaldehyde has shown to have a glucolipid lowering effects in diabetic animals leading to enhancement of glucose and lipid homeostasis[27]. Cinnamaldehyde has also shown to be involved in fatty acid metabolism and has alleviated steatosis, *in vivo*[28]. It has also shown to possess antibacterial[29-31] and antifungal[32] activities. Cinnamaldehyde also has an emerging role in the treatment of inflammatory bowel diseases[33].

Cinnamaldehyde (**Figure 1**) has conjugated systems of benzene ring and α,β-unsaturated carbonyl system. Similarly, coniferyl aldehyde (**Figure 1**) has also the same systems along with the presence of the hydroxyl group. The ability of these conjugated systems to be stabilized by resonance[34] upon accepting electrons give rise for their biological activity, such as anticancer and/or antioxidant.

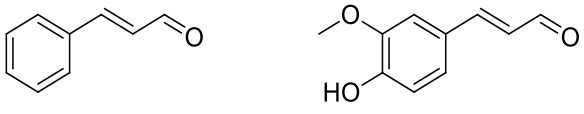


Figure 1: Chemical structure of cinnamaldehyde and coniferyl aldehyde

Coniferyl aldehyde is a derivative of cinnamaldehyde and its derivatives have shown to exhibit anticancer activity[35, 36] variably. The anticancer activity of coniferyl aldehyde derivatives has been reported in non-small cell lung cancer (NSCLC) cell line (H1299)[35], but was non-toxic on breast (MCF7) and small cell lung cancer (NCI-H187) cell lines[36].

As mentioned above, cinnamaldehyde and coniferyl aldehyde along with their derivatives possess multiple pharmacological activities. Hence, further development of derivatives of these compounds would be needed to explore their pharmacological activities. Along with the reported anticancer and antioxidant activities of cinnamaldehyde and its derivatives, further investigation of different coniferyl aldehyde derivatives is worth studying. Herein, we aimed to synthesize different coniferyl aldehyde and cinnamaldehyde derivatives to investigate their potential anticancer and antioxidant activity.

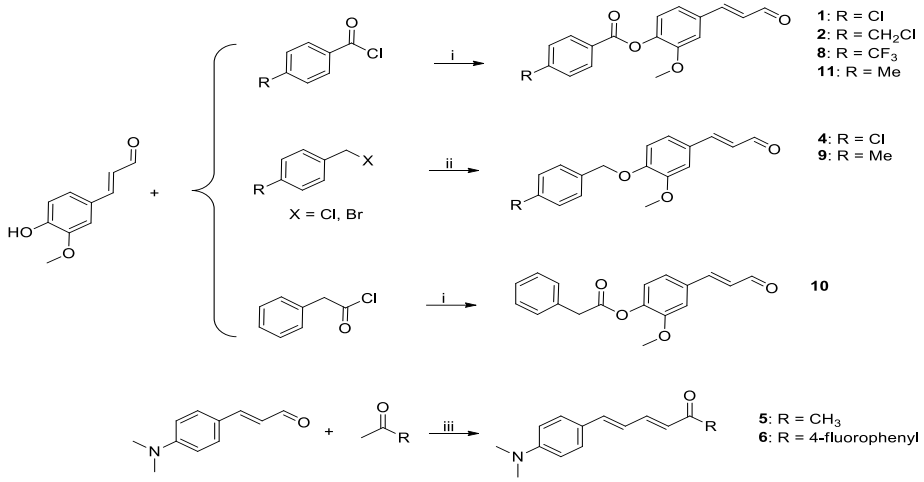
2. RESULTS AND DISCUSSION

As cinnamaldehyde and its derivative coniferyl aldehyde considered among the components of natural products that possess therapeutic uses, several derivatives were synthesized to investigate further anticancer and antioxidant effects.

2.1 Chemistry

The synthesis of coniferyl aldehyde derivatives (compounds **1**, **2**, **4**, and **8-11**) and 4-(Dimethylamino) cinnamaldehyde (DA) derivatives **(**compounds **5** and **6)** were achieved using 4-hydroxy-3-methoxy cinnamic aldehyde (coniferyl aldehyde) and DA as starting materials as presented in **Scheme 1**.

Briefly, **1**, **2**, **8**, and **11** were synthesized via acetylation reactions by reacting coniferyl aldehyde with various benzoyl chloride derivatives under basic conditions, using trimethylamine (TEA), at room temperature (RT). These reactions afforded the desired products with 33, 32, 30 and 57 % yield, respectively. Similar procedure was carried out for the synthesis of **10** but using phenyl acetyl chloride with a yield of 56 %. Compounds **4** and **9** were synthesized via alkylating the phenolic oxygen of coniferyl aldehyde by reacting it with various benzyl halides under reflux conditions, which provided 84 and 40 % yield of the target compounds, respectively. It was noticed that the % yields of the alkylation reactions were generally higher than acetylation reactions, probably due to the high incidence of acid chlorides' hydrolysis during the reaction, which can be optimized by using anhydrous conditions. Compounds **5** and **6** were formed via crossed aldol condensation reactions of DA with acetone and 4′-fluoroacetophenone, respectively, under basic conditions using sodium hydroxide, at RT. It was also noticed that the % yield for compound **5** was 15 %, while for **6** was 70 %, which was likely related to both the volatility of the ketone used and the electron withdrawing power to stabilize the anionic intermediate.



Scheme 1: The synthetic pathways for coniferyl aldehyde and 4-(Dimethylamino) cinnamaldehyde derivatives; i: TEA, DMF, RT, overnight; ii: K_2CO_3, DMF, reflux; iii: NaOH, RT, overnight**.**

All compounds were structurally characterized by ^{1}H and ^{13} C NMR, with aid of melting points, as described at the experimental section. **Figure2** shows the ^{1}H NMR spectrum for compound **2** as an example to generally show the logical assignment of the peaks to their corresponding atoms.

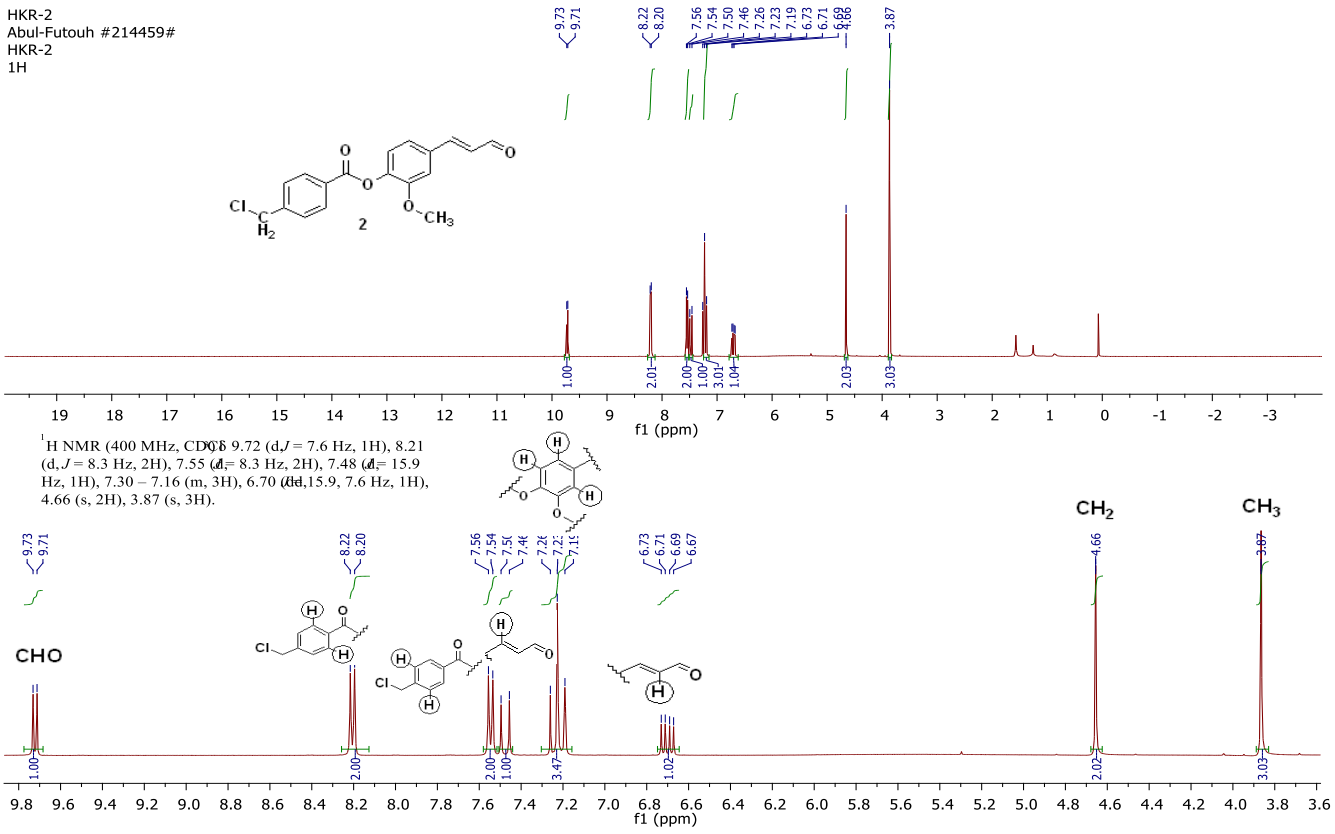


Figure 2: ^{1}H NMR spectrum for compound 2.

2.2 Cytotoxicity Assays

The antiproliferative activity of the synthesized compounds was investigated on two NSCLC cell lines; A549 and H1299. In previous work, we have found that coniferyl aldehyde derivatives that contain cinnamaldehyde nucleus has shown to have promising cytotoxic activity against H1299 cell line, but insignificantly against A549 cell line[35]. Hence, the cytotoxicity of the new derivatives was also investigated on these cell lines.

In our previous work[35], coniferyl aldehyde derivatives were synthesized to evaluate their inhibitory effect against aldehyde dehydrogenase enzyme (ALDH) isoforms (ALDH1A1, ALDH1A3, and ALDH3A1), which are expressed variably in cancer cell lines and are known to be involved in cancer proliferation. Although H1299 cell line lacks these ALDH isoforms in contrast to A549 cell line, coniferyl aldehyde derivatives were cytotoxic against them. It has been suggested that these derivatives might be detoxified in A549 cells via these isoforms, thus preventing their cytotoxic effect[35]. Hence, more coniferyl aldehyde derivatives were synthesized to check their cytotoxicity against these cell lines that variably express ALDH isoforms. This is also to check whether structure changes might interfere with activity on both cell lines.

Table 1 represents the IC_{50} values for the synthesized compounds on A549 and H1299 cell lines after being treated for 96 hours. Compounds **2** and **8** showed cytotoxic activity on A549 cell line, with IC_{50} values of 32.5 and 58.0 μM, respectively. On H1299 cell line, except for compounds **5** and **6**, all other compounds showed relative cytotoxicity with compounds **2** and **4** having the lowest IC_{50} values (9.6 and 6.7 μM, respectively). These results generally were found aligned with our previous work, in which A549 cells were considered as resistant cells, and with the opposite for H1299 cell line[35].

Table 1: Cytotoxicity (IC_{50} ± SEM) of **1**, **2**, **4-6** and **8-11** on A549 and H1299 cell lines after treatment for 96 h; n = 3.

Compound	**CytotoxicityIC_{50} ± SEM (μM)**	
	A549	**H1299**
1	>60	**18 ± 4**
2	**32.5 ± 2.5**	**9.6 ± 0.4**
4	>60	**6.7 ± 1.2**
5	>60	>60
6	>60	>60
8	**58 ± 1.5**	**12.5 ± 0.5**
9	>60	**30.5 ± 1.5**
10	>60	**58.3 ± 1.7**
11	>60	**48.3 ± 2.8**

The main nucleus for these derivatives is cinnamaldehyde. Cinnamaldehyde has shown to be cytotoxic on a number of cancer cell lines (K562[22] and MCF7[23]) as discussed above. In addition, cinnamaldehyde has shown to be a promising adjuvant compound to be combined with 5-fluorouracil and oxaliplatin against colorectal carcinoma[24]. Moreover, cinnamaldehyde has also shown to enhance apoptosis induced by hyperthermia and doxorubicin on A549 and U87MG (glioblastoma) cell lines, respectively[25, 26]. The presence of α,β-unsaturated aldehyde (electrophilic Michael acceptor) pharmacophore on cinnamaldehyde has shown to negatively affect the proliferation and tumor growth of melanoma cells[37]. Hence, the cytotoxicity of the synthesized compounds on A549 and H1299 cell line may be due to the presence of this pharmacophore as they are considered also cinnamaldehyde derivatives. In addition to that our compounds are cinnamaldehyde derivatives, compound **2** has a benzyl chloride moiety (as shown in **Scheme 1**), which could act as an alkylating agent and perhaps explain why it showed the highest cytotoxic activity on A549 cells. Furthermore, compound **8** showed also a considerable activity on both cell lines, which may be due to the presence of CF_3 moiety, whereby numerous

pharmacologically active compounds have been found to have flourin-containing functional groups[38].

Moreover, most of the newly synthesized coniferyl aldehyde derivatives were cytotoxic on H1299 cell line while non-cytotoxic on A549 cell line, which is similar with our previous results indicating possible detoxification of compounds via ALDH isoforms in A549 cell line[35]. It may be concluded that coniferyl aldehyde derivatives may be effective against ALDH non-expressing cells and they might act as substrates for ALDH enzymes in line with our previous work[35].

2.3 Antioxidant activity

Nucleophiles, such as free radicals, do exist inside our body. The balance between their production and elimination is essential to maintain normal physiologic functions[39]. When the body becomes unable to control these free radicals, state of oxidative stress occurs, causing the spreading of various diseases[39]. The synthesized compounds exhibit the electrophilic Michael acceptor pharmacophore (α,β-unsaturated carbonyl system) that is susceptible to nucleophilic attack at its β-carbon. Hence, the investigation of the synthesized compounds as antioxidants was performed.

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay[40] is a common antioxidant assay that works by the reduction of DPPH radical, in its stable form (DPPH*) (purple-colored), to DPPH-H (yellow-colored) through the presence of radical species or antioxidants. DPPH* absorbs light at 515-517 nm[41] and absorption disappears upon reduction. Therefore, free radical scavenging activity testing was performed to test the antioxidant activity of the synthesized compounds.

Vitamin C is known to be a potent antioxidant[42]. The antioxidant activity for the synthesized compounds was measured in relative to vitamin C as a reference along with the use of coniferyl aldehyde (the starting material). **Figure 3** represents the scavenging activity of vitamin C, coniferyl aldehyde, and the new compounds as referenced to vitamin C.

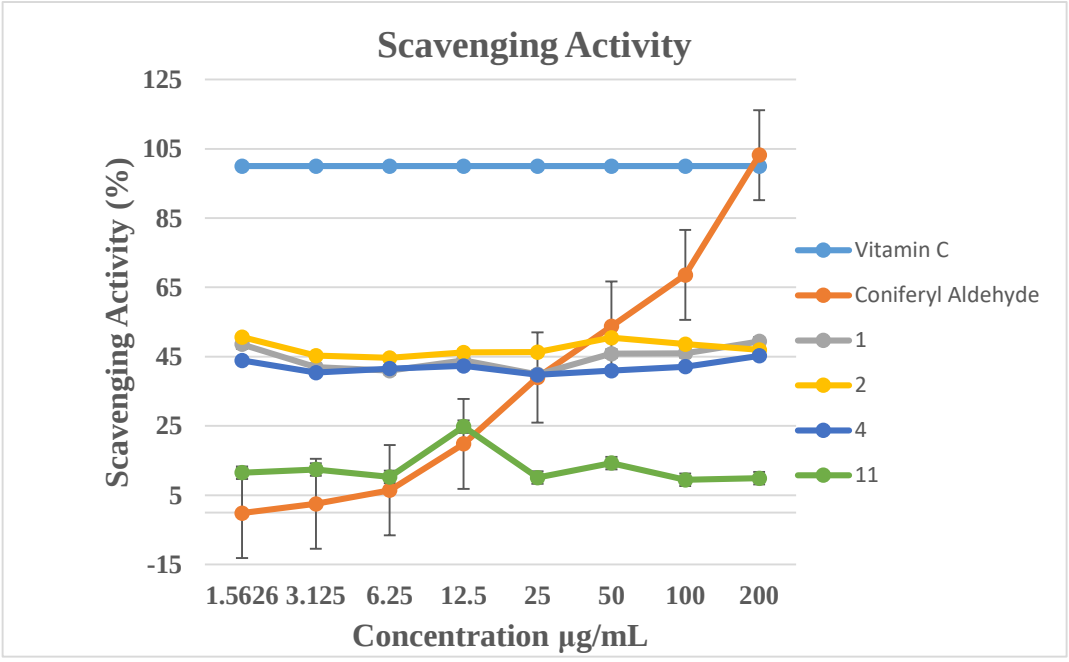


Figure 3: The scavenging activity of vitamin C, coniferyl aldehyde, 1, 2, 4 and 11 as referenced to vitamin C; n = 3.

The scavenging activity of vitamin C was found to be ~ 40% in our experiments. For the purpose of this study, the scavenging activity of the tested compounds was referenced to vitamin C, which was considered to have 100% scavenging activity, as presented in **Figure 3**. The scavenging activity of coniferyl aldehyde increased by increasing its concentration to finally meet the scavenging activity of vitamin C around the concentration of 200 µg/mL, showing a dose-dependent scavenging activity (**Figure 3**). This may be justified due to the presence of the conjugated systems; the α,β-unsaturated carbonyl system and the aromatic ring in coniferyl aldehyde and the tested compounds, along with the presence of hydroxyl group in coniferyl aldehyde, in which accepting the free electron of DPPH* cause resonance stabilization within the structure[34] (**Figure 4**), in a similar pattern to how vitamin C reduces the free radicals.

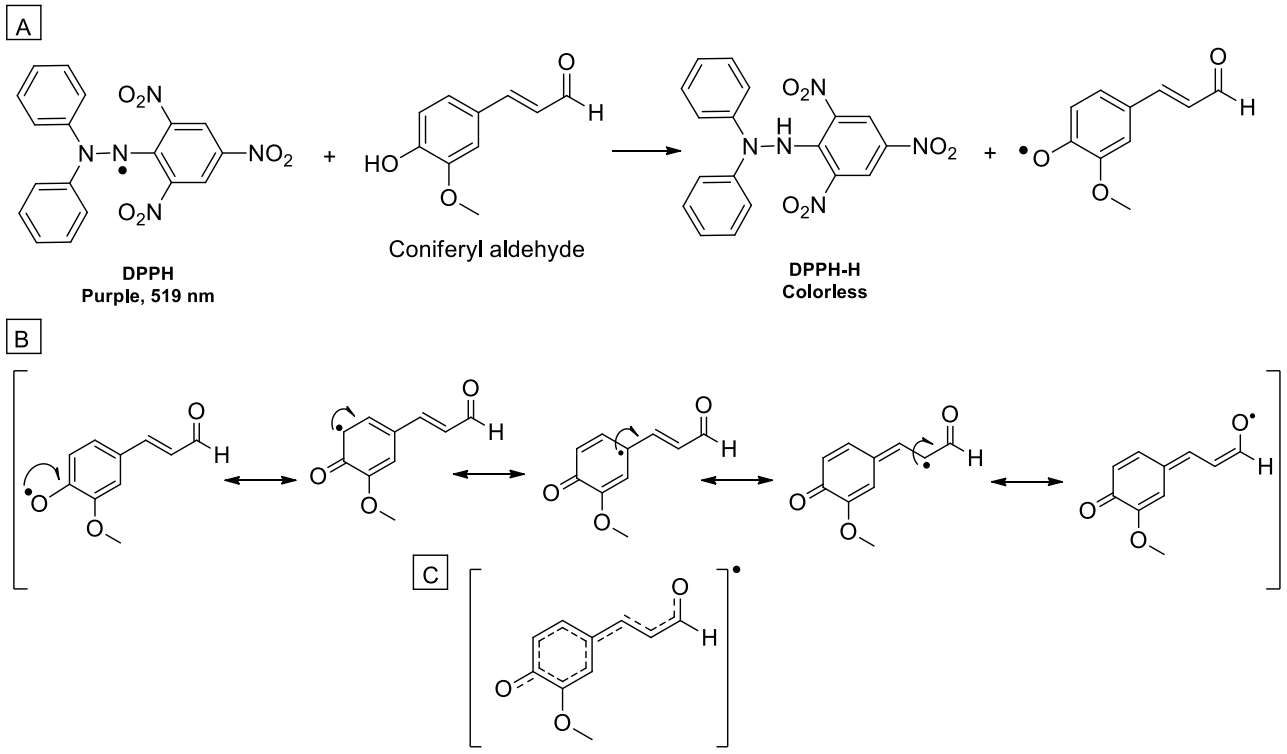


Figure 4: **The proposed mechanistic antioxidant activity of coniferyl aldehyde (panel A), with electronic delocalization of the formed free radical (panel B) and the hybrid form (panel C).**

Compounds **1**, **2**, and **4** have around the half scavenging activity of vitamin C as presented in **Figure 3**, at all tested concentrations resulting in almost consistent effect regardless of the concentration used. Interestingly, it can be realized that all those compounds contain chlorine, either aliphatic or aromatic, which may provide a structurally related clue for biological activity. Although, more analogues with variable substitutions should be synthesized and investigated to prove this conclusion and optimize the structure-activity relationship. Compound **11** showed the lowest scavenging activity with a value of ~ 5% at 200 µg/mL. Compounds **5**, **6**, and **8-10** showed no scavenging activity.

Although there are variations in the scavenging activity among the synthesized compounds, these results indicated that coniferyl aldehyde derivatives could exhibit potential

scavenging activity, which may be justified due to the presence of the α,β-unsaturated carbonyl system and the aromatic ring on them.

Compounds **1**, **2**, **8**, **10**, and **11** has an ester within their structure as presented in **Scheme 1**. Esterases, which break ester bonds, are expressed in human tissues and perform various functions, such as the metabolism of endogenous molecules and drugs[43]. Theoretically, breaking the ester bond in **1**, **2**, **8**, **10**, and **11** will release a metabolite like coniferyl aldehyde that might have similar scavenging activity to coniferyl aldehyde. Thus, compounds **1**, **2**, **8**, **10**, and **11** may act as prodrugs and be metabolized to deliver an effective antioxidant. Although only compounds **1**, **2**, and **11** (to a lower extent) showed antioxidant activity via DPPH assay *in vitro*, further *in vivo* studies are required to validate the esterases' effect hypothesis. *In vivo* studies might show that compounds **8**, **10**, and **11** possess antioxidant activity in contrast to the DPPH *in vitro* assay.

3. EXPERIMENTAL

3.1 Materials

Coniferyl aldehyde, 4-(Dimethylamino) cinnamaldehyde, 4-hydroxy-3-methoxy cinnamic aldehyde, 4-chlorobenzoyl chloride, 4-(Chloromethyl) benzoyl chloride, 4-Chlorobenzyl chloride, 4′-Fluoroacetophenone, 4-(Trifluoromethyl) benzoyl chloride, 4-Methylbenzyl bromide, phenyl acetyl chloride, p-toluoyl chloride, acetone, hexane, ethyl acetate, methanol, magnesium sulfate ($MgSO_4$), dichloromethane (DCM), triethylamine (TEA), potassium carbonate (K_2CO_3), dimethylformamide (DMF), sodium hydroxide (NaOH), (3-4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT), vitamin C (ascorbic acid), and DPPH were purchased from Sigma (Dorset, UK); A549 [A549 (ATCC CCL-185)] and H1299 [NCI-H1299 (ATCC CRL-5803)] human NSCLC cell lines were purchased from ATCC (USA); PBS, RPMI-1640 medium, fetal bovine serum (FBS) and L-glutamine were purchased from Euroclone (Pero, Italy); DMSO was purchased from TEDIA (USA). Chemical reactions were monitored by analytical thin layer chromatography (TLC) using Merck 9385 silica gel 60 F254 aluminum-backed plates through visualizing the spotted plates under ultraviolet (UV) at 254 and 366 nm. Intermediates and final products were purified by column chromatography using silica gel (pore size 60 Å, 40–63 µm particle size). Proton and carbon NMR were analyzed for all intermediates and final products on Bruker AMX400 (400 MHz) nuclear magnetic resonance spectrometer. Chemical shifts were reported in parts per million (δ, ppm) downfield from internal TMS. Coupling constants (*J*) were expressed in Hertz (Hz). Melting points were measured with a Gallenkamp melting point apparatus.

3.2 Chemistry

3.2.1 (E)-2-methoxy-4-(3-oxoprop-1-en-1-yl)phenyl 4-chlorobenzoate (1)

To 530 mg (1.68 mmol, 1.0 eq) 4-hydroxy-3-methoxy cinnamic aldehyde in 4 mL DMF was added 585 mg (3.36 mmol, 2.0 eq) 4-chlorobenzoyl chloride and (2.52 mmol,1.5 eq) TEA. The reaction was stirred at RT for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added to quench the reaction. The precipitate was then filtered, washed frequently with distilled water and dried. The crude was then purified by column chromatography using 70:30 (hexane:ethyl acetate) affording 33% of the titled product as a white solid, melting point 134-136 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (d, *J* = 7.6 Hz, 1H, C*H*O), 8.25 – 8.08 (m, 2H, Ar*H*), 7.57 – 7.47 (m, 2H, Ar*H*), 7.30 – 7.13 (m, 3H, Ar*H* & C*H*=CH-CHO), 6.70 (dd, *J* = 15.9, 7.6 Hz, 1H, CH=C*H*-CHO), 3.87 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.54, 163.73, 151.92, 151.90, 142.42, 140.47, 133.28, 131.88, 129.14, 128.99, 127.60, 123.72, 122.05, 111.64, 56.16.

3.2.2 (E)-2-methoxy-4-(3-oxoprop-1-en-1-yl)phenyl 4-(chloromethyl)benzoate (2)

To 500 mg (2.8 mmol, 1.0 eq) 4-hydroxy-3-methoxy

cinnamic aldehyde in 4 mL DMF was added 1.12 g (5.6 mmol, 2.0 eq) 4-(Chloromethyl) benzoyl chloride and (4.2 mmol,1.5 eq) TEA. The reaction was stirred at RT for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added to quench the reaction. The precipitate was then filtered, washed frequently with distilled water, and dried. The crude was then purified by column chromatography using 100% DCM affording 32% of the titled product as an off-white solid, melting point 125-127 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.70 (d, *J* = 7.6 Hz, 1H, C*H*O), 7.36 (q, *J* = 8.5 Hz, 4H, Ar*H*), 6.98 (d, *J* = 2.1 Hz, 1H, Ar*H*), 6.87 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar*H*), 6.80 (d, *J* = 8.2 Hz, 1H, Ar*H*), 6.55 (d, *J* = 15.8 Hz, 1H, C*H*=CH-CHO), 6.26 (dt, *J* = 15.9, 5.9 Hz, 1H, CH=C*H*-CHO), 5.12 (s, 2H, CH_2), 3.91 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 194.16, 173.69, 149.89, 147.87, 135.70, 133.79, 131.11, 130.72, 128.86, 128.75, 127.08, 119.65, 114.23, 109.68, 63.91, 56.07.

3.2.3(E)-3-(4-((4-chlorobenzyl)oxy)-3-methoxyphenyl) acrylaldehyde (4)

To 500 mg (2.8mmol, 1.0 eq) 4-hydroxy-3-methoxy cinnamic aldehyde in 4 mL DMF was added 1.12 g (5.6 mmol, 2.0 eq) 4-Chlorobenzyl chloride and (4.2mmol, 1.5 eq) K_2CO_3. The reaction was stirred at reflux for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added and a precipitate was formed. The precipitate was then filtered, washed frequently with distilled water, and dried. The crude was then purified by column chromatography using 100% DCM affording 83.88% of the titled product as a white solid, melting point 110-112 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (d, *J* = 7.4 Hz, 1H, C*H*O), 7.43 – 7.18 (m, 3H, Ar*H*), 7.00 – 7.15 (m, 4H, Ar*H*), 6.86 (d, *J* = 15.3 Hz, 1H, C*H*=CH-CHO), 6.59 (dd, *J* = 15.3, 7.3 Hz, 1H, CH=C*H*-CHO), 5.15 (d, *J* = 6.0 Hz, 2H, CH_2), 3.94 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.55, 152.65, 150.79, 149.95, 134.88, 134.00, 128.92, 128.62, 127.67, 127.01, 123.13, 113.49, 110.52, 70.17, 56.06.

3.2.4 (3E,5E)-6-(4-(dimethylamino)phenyl)hexa-3,5-dien-2-one (5)

To 300 mg (1.7 mmol, 1.0 eq) 4-(Di methylamino) cinnamaldehyde in 10 mL acetone was added (3.4 mmol, 2.0 eq) NaOH. The reaction was stirred under N_2 for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added and then extracted three times with DCM (25 mL), the organic layer was dried over anhydrous $MgSO_4$ then the organic solvent was evaporated under reduced pressure to obtain the product. The crude was then purified by column chromatography using 100% DCM affording 15% of the titled product as a bright yellow solid, melting point 110-112 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 8.6 Hz, 2H, Ar*H*), 6.89 (d, *J* = 8.6 Hz, 2H), 6.68 (dt, *J* = 14.2, 6.1 Hz, 3H, CH=C*H*-CO), 6.17 (t, *J* = 14.2 Hz, 1H, C*H*=CH-CO), 3.02 (s, 6H, NCH_3), 2.30 (s, 3H, COCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.61, 151.22, 145.10, 142.35, 128.94, 128.08, 122.20, 112.16, 40.33, 27.27.

3.2.5 (2E,4E)-5-(4-(dimethylamino)phenyl)-1-(4-fluorophenyl)penta-2,4-dien-1-one (6)

To 300 mg (1.7 mmol, 1.0 eq) 4-(Dimethylamino)cinnamaldehyde in 10 mL methanol was added (3.4 mmol, 2.0 eq) NaOH, and (1.7 mmol, 1.0 eq) 4′-Fluoroacetophenone. The reaction was stirred under N_2 for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added and then extracted with DCM. The organic layer was dried over anhydrous $MgSO_4$ then the organic solvent was evaporated under reduced pressure to obtain the product. The crude was then purified by column chromatography using 50:50 (DCM : hexane) affording 70% of the titled product as a red solid, melting point 128-130 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (dd, *J* = 8.5, 5.6 Hz, 2H, Ar*H*), 7.62 (dd, *J* = 14.8, 11.0 Hz, 1H, HC=C*H*-CH=CH), 7.48 – 7.28 (m, 2H, ArH & *H*C=CH-CH=CH), 7.13 (t, *J* = 8.5 Hz, 2H, Ar*H*), 7.07 – 6.86 (m, 1H, HC=CH-CH=C*H*), 6.82 (dd, *J* = 14.8, 5.6 Hz, 1H, HC=CH-C*H*=CH), 6.67 (d, *J* = 8.6 Hz, 2H, Ar*H*), 3.02

(s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.00, 165.5 (d, *J* = 254.5 Hz, 1C), 151.31, 146.75, 143.54, 135.19, 130.97, 130.78 (d, *J* = 24.24, 1C), 129.10, 124.26, 122.42, 122.20, 115.77, 115.55, 112.16, 111.89, 77.41, 40.33, 40.21.

3.2.6 (E)-2-methoxy-4-(3-oxoprop-1-en-1-yl)phenyl 4-(trifluoromethyl)benzoate (8)

To 250 mg (1.4 mmol, 1.0 eq) 4-hydroxy-3-methoxy cinnamic aldehyde in 4 mL DMF was added (2.8 mmol, 2.0 eq) 4-(Trifluoromethyl)benzoyl chloride and (2.1 mmol, 1.5 eq) TEA. The reaction was stirred at RT for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added to quench the reaction then extracted with DCM, the organic layer was dried over anhydrous $MgSO_4$ then the organic solvent was evaporated under reduced pressure to obtain the product. The crude was then purified by column chromatography using 100% DCM affording 30% of the titled product as a yellow solid, melting point 78-80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (d, *J* = 7.8 Hz, 1H, C*H*O), 7.62 (d, *J* = 8.1 Hz, 2H, Ar*H*), 7.53 (d, *J* = 8.0 Hz, 2H, Ar*H*), 7.39 (dd, *J* = 14.6, 11.7 Hz, 1H, CH=C*H*-CHO), 7.07 (d, *J* = 9.3 Hz, 2H, Ar*H*), 6.85 (d, *J* = 8.1 Hz, 1H, Ar*H*), 6.59 (dd, *J* = 15.8, 7.7 Hz, 1H, C*H*=CH-CHO), 3.92 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.29, 157.31, 155.38, 154.73, 145.23, 132.62, 132.01, 131.88, 131.22, 130.48, 130.44, 127.85, 127.46, 118.23, 117.22, 115.33, 60.83.

3.2.7(E)-3-(3-methoxy-4-((4-methylbenzyl) oxy)phenyl) acrylaldehyde (9)

To 250 mg (1.4 mmol, 1.0 eq) 4-hydroxy-3-methoxy cinnamic aldehyde in 4 mL DMF was added (2.8 mmol, 2.0 eq) 4-Methylbenzyl bromide and (2.1 mmol, 1.5 eq) K_2CO_3. The reaction was stirred at reflux for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added and a precipitate was formed. The precipitate was then filtered, washed frequently with distilled water and dried. The crude was then purified by column chromatography using 100% DCM affording 40% of the titled product as a yellow solid, melting point 98-100 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.64 (d, *J* = 7.8 Hz, 1H, C*H*O), 7.38 (d, *J* = 15.8 Hz, 1H, C*H*=CH-CHO), 7.31 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.17 (d, *J* = 7.6 Hz, 2H, Ar*H*), 7.00 – 7.15 (m, 2H, Ar*H*), 6.89 (d, *J* = 8.5 Hz, 1H, Ar*H*), 6.59 (dd, *J* = 15.6, 7.8 Hz, 1H, CH=C*H*-CHO), 5.16 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3), 2.34 (s, 3H, ArCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.19, 147.47, 144.50, 132.52, 127.90, 123.98, 121.92, 121.35, 117.84, 107.99, 105.05, 71.96, 71.88, 71.64, 71.32, 65.39, 50.64, 15.81.

3.2.8 (E)-2-methoxy-4-(3-oxoprop-1-en-1-yl)phenyl 2-phenylacetate (10)

To 530 mg (1.68 mmol, 1.0 eq) 4-hydroxy-3-methoxy cinnamic aldehyde in 4 mL DMF was added 585 mg (1.68 mmol, 1.0 eq) phenyl acetyl chloride and (2.52 mmol,1.5 eq) TEA. The reaction was stirred at RT for overnight. The reaction was followed up by TLC, and when was completed, 25 mL water was added to quench the reaction. The precipitate was then filtered, washed frequently with distilled water and dried. The crude was then purified by column chromatography using 100% DCM affording 56 % of the titled product as a white solid, melting point 112-114 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (d, *J* = 7.6 Hz, 1H, C*H*O), 7.46 – 7.33 (m, 5H, Ar*H*), 7.33 – 7.26 (m, 1H, Ar*H*), 7.16 – 7.08 (m, 2H, Ar*H* & C*H*=CH-CHO), 7.06 (d, *J* = 7.9 Hz, 1H, Ar*H*), 6.64 (dd, *J* = 15.8, 7.6 Hz, 1H, CH=C*H*-CHO), 3.89 (s, 2H, CH_2), 3.79 (s, 3H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 188.10, 163.92, 146.52, 146.21, 136.94, 127.94, 127.56, 124.01, 123.33, 123.27, 121.98, 118.02, 116.48, 106.00, 50.53, 35.59.

3.2.9 (E)-2-methoxy-4-(3-oxoprop-1-en-1-yl)phenyl 4-methylbenzoate (11)

To 530 mg (1.68 mmol, 1.0 eq) 4-hydroxy-3-methoxy cinnamic aldehyde in 4 mL DMF was added 585 mg (1.68 mmol, 1.0 eq) p-toluoyl chloride and (2.52 mmol,1.5 eq) TEA. The reaction was stirred at RT for overnight. The

reaction was followed up by TLC, and when was completed, 25 mL water was added to quench the reaction. The precipitate was then filtered, washed frequently with distilled water and dried. The crude was then purified by recrystallization using 1:1 methanol:water affording 57% of the titled product as an off-white solid, melting point 118-120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.69 (d, *J* = 7.1 Hz, 1H, C*H*O), 8.07 (d, *J* = 6.9 Hz, 2H, Ar*H*), 7.35 – 7.51 (m, 1H, Ar*H*), 7.29 (d, *J* = 7.0 Hz, 2H, Ar*H*), 7.12 – 7.25 (m, Ar*H* & C*H*=CH-CHO), 6.67 (dt, *J* = 14.7, 6.9 Hz, 1H, CH=C*H*-CHO), 3.84 (s, 3H, OCH_3), 2.44 (s, 3H, ArCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.50, 164.51, 152.03, 151.94, 144.66, 142.68, 132.92, 130.44, 129.36, 128.72, 126.26, 123.76, 121.97, 111.54, 56.05, 21.81.

3.3 Cytotoxicity Assays

Cytotoxicity assays was performed as described before[35]. Briefly, A549 and H1299 were cultured in RPMI-1640 medium being supplemented with 10% (v/v) heat inactivated fetal bovine. The cells were maintained in 5% CO_2 humidified incubator at 37 °C. A549 and H1299 cells were seeded at 750 and 1000 cells per well, respectively, into 96-well plates and left for next day for attachment. Media was changed the next day and treatment using the new compounds were added at various concentrations ranging between 0.01 and 60 μM for 96 h. Cell viability was then measured using MTT assay. Optical densities were then measured by the BioTeK SYNERGY HTX multi-mode plate reader at 540 nm. Dose–response curves were generated using the GraphPad Prism 9 Software, and nonlinear regression analysis was used to fit the data. IC_{50} values were determined from these curves.

3.4 Antioxidant Activity

Antioxidant activity was performed as described before[44]. A serial dilution of the synthesized compounds and vitamin C dissolved in DMSO is prepared to give a range of final well concentrations as follows: 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.5625 μg/mL. A stock solution of DPPH in ethanol (1 mg/mL) is prepared and diluted to 40 μg/mL. To a 96-well plate, 20 μL of each sample is added then followed by 280 μL of the 40 μg/mL DPPH. The 96-well plate was incubated for 30 minutes in dark at RT for 30 minutes. The absorbance was read at 517 nm by the BioTeK SYNERGY HTX multi-mode plate reader. The scavenging activity is calculated according to the equation: Antioxidant activity % = ((Absorbance of DPPH* − Absorbance of the sample) / (Absorbance of DPPH*)) × 100%.

Informed Consent: Not Applicable

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تصميم وتصنيع وفحص النشاط البيولوجي لمشتقات مركب كونيفيريل ألدهيد كمركبات محتملة مضادة للسرطان والأكسدة

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ملخص

من المعروف أن النواتج الطبيعية تملك أنشطة مضادة للميكروبات والسرطان والأكسدة. من بين هذه النواتج الطبيعية هي القرفة التي تحتوي على مركب سينامالديهيد. وجدت الدراسات أن مركب السينامالديهايد ومشتقاته لهم أنشطة مضادة للسرطان والأكسدة. كما أظهر مركب كونيفيريل ألدهيد، وهو مركب غير سام للخلايا ومشتق من مركب سينامالديهايد، أن له نشاطا مضادا للسرطان. في هذه الدراسة، تم تصنيع عدد من مشتقات مركب كونيفيريل ألدهيد وتقييم أنشطتها المضادة للسرطان والأكسدة. أظهرت المركبات 1 و2 و4 و8–11 نشاطا ساما للخلايا ضد خلايا H1299، وهي خلايا سرطان الرئة ذات الخلايا غير الصغيرة، وأظهر مركب 4 أعلى فعالية لقتل الخلايا حيث بلغت قيمة نصف التركيز المثبط الأقصى 6.7 ميكرومولار. كما أظهرت مركبات 1 و2 و4 نشاطا مضاد للأكسدة بالمقارنة مع فيتامين سي، حيث وصلت نسبة الكسح للمركبات نصف نسبة الكسح لفيتامين سي في جميع التركيزات التي تم اختبارها للمركبات، وذلك من خلال تجربة فحص مضادات الأكسدة باستخدام مركب DPPH. أظهر مركب كونيفيريل ألدهيد نفسه نشاطا مضادا للأكسدة يعتمد على الجرعة، حيث من المقترح أن ذلك تم عن طريق آلية تثبيت الشوارد الحرة في المركب. وهكذا، أظهرت دراستنا أن مشتقات مركب كونيفيريل ألدهيد تظهر أنشطة مضادة للسرطان والأكسدة، والتي قد تمثل مركبات علاجية محتملة.

الكلمات الدالة: كونيفيريل ألدهيد، مضاد للسرطان، مضاد للأكسدة، DPPH.

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