Synthesis and Antibacterial Activity of Novel Caffeic Acid Hybrid Derivatives

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ABSTRACT

Several novel esters / hybrid derivatives of caffeic acid were synthesized and tested for potential antibacterial activity. This combinatorial synthesis of novel caffeic ester / hybrid derivatives can be a useful approach to generate potent chemotherapeutic agents in developing new drug candidates.

Keywords: Caffeic acid, CAPE, NF-kB, Fungicidal, ¹HNMR, TOF MS, DCC, DMAP, Antibacterial, Ditch-plate method.

1. INTRODUCTION

Phenolic phytochemicals are known to exhibit anti-inflammatory, antioxidant, anticarcinogenic, antidiabetic, antiatherosclerosis and immunomodulatory activities in animals\textsuperscript{1,2}. These are mostly polyphenols known as secondary plant metabolites\textsuperscript{3}, present in plants and trees. Polyphenols are commonly divided into flavonoids and the hydroxyl cinnamic acids. The 3,4-dihydroxy cinnamic acid derivatives are known as caffeic acid derivatives and are widely distributed in the plant kingdom. The most common caffeic acid derivatives are esters of caffeic acid with quinic acid and the caffeic acid phenylethyl ester (CAPE). CAPE is an active component of propolis from honeybee hives and is widely known for its antiviral, anti-inflammatory, and immunomodulatory properties\textsuperscript{4}. Caffeic acid esters also have the ability to alter the redox state and induce apoptosis\textsuperscript{5-9}. Since CAPE, an ester derivative of Caffeic acid or esterification of Caffeic acid with phenyl ethyl groups is a naturally occurring active compound having antimicrobial, anti-inflammatory and antioxidant / anticancer properties, we thought of synthesizing compounds with novel ether, ester and hybrid derivatives of Caffeic acid wherein Caffeic acid would be etherified, esterified and hybridized with various other compounds and to check whether these compounds possess above biological activities. The objective of this study is to condense two molecules of the same disease domain to produce more potent candidate in the same disease domain or to condense two molecules of different disease domain to produce mixed variety of those disease domain or to have drug candidate with entirely different biological activity.

2. MATERIALS AND METHODS

2.1 Materials

Chemicals used were of a laboratory grade. The reactions were monitored by TLC on aluminium-backed silica plate visualized by UV-light.

2.2 Experimental work

Melting points were determined on a Thomas Hoover capillary melting point apparatus using digital thermometer. IR spectra were recorded on a Shimadzu FTIR Prestige model as KBr pellet.
**1H NMR spectra were recorded on a Varian 400 MHz spectrometer in CDCl₃. Chemical shifts were recorded in parts per million down field from tetramethyl silane. Mass spectra were recorded on a TOF MS ES mass spectrometer. Elemental analysis were carried out as a percentage on a Thermo finnigan, Flash EA 1112 series, Italy.**

### 3. RESULTS AND DISCUSSION

Preparation of 3,4-dialkoxy caffeic acids: - Caffeic acid was subjected to esterification (MeOH / EtOH, Conc. H₂SO₄) followed by etherification (K₂CO₃ / Acetone / Alkyl – Aryl halide) to yield crude ether derivatives which were purified by column chromatography. These purified ether derivatives were subjected to hydrolysis (Aq. KOH / EtOH and then Conc. HCl) to yield 3,4-dialkoxy caffeic acids respectively.

**Compound 1: (E)-3-(3, 4-dimethoxyphenyl) prop-2-enoic acid**

1H NMR (CDCl₃, ϰϬϬ MHz, δ ppm): 3.93 (s, 3H, 2 x Ar–OCH₃), 6.30 (d, J = 16.0 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 3H, ArH), 7.74 (d, J = 16.0 Hz, 1H, Trans double bond); TOF MS ES: 231 (M + Na); Molecular Formula C₁₁H₁₂O₄; Melting range 162 – 165°C; Elemental Analysis, Calcd.: C 63.45 %, H 5.81 %, O 30.74 %. Found C 63.48 %, H 5.85 %, O 30.70 %;

**Compound 2: (E)-3-(3, 4-diethoxyphenyl) prop-2-enoic acid**

1H NMR (CDCl₃, ϰϬϬ MHz, δ ppm): 4.13 (q, J = 3.3, 6.9 Hz, 4H, 2 x –OCH₂CH₃ group), 6.30 (d, J = 16.0 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 3H, ArH), 7.74 (d, J = 16.0 Hz, 1H, Trans double bond); TOF MS ES: 259 (M + Na); Molecular Formula C₁₃H₁₆O₄; Melting range 144 – 147°C; Elemental Analysis, Calcd.: C 66.09 %, H 6.83 %, O 27.09 %. Found C 66.12 %, H 6.87 %, O 27.05 %;

**Synthesis of Fused Molecules using compound (1) and (2)**

These were prepared by following general method as depicted below.

To a stirred solution of eugenol [A] (1 eq.) in dichloromethane (30 ml) was added pyridine [E] (0.5 eq.), DMAP [D] (0.05 eq.), DCC [C] (1.3 eq.) and aromatic / substituted aromatic acid [B] (1.3 eq.) respectively at room temperature and stir it for next 24 hrs. As the reaction proceeds the by-product urea derivative precipitates out of the reaction mixture and floats on the surface (TLC). The organic layer was concentrated under reduced pressure to minimum, preadsorbed on silica gel and purified by column chromatography (SiO₂, 100 – 200 mesh) with increase in concentration of ethyl acetate in petroleum ether to yield pure compound. The purified compounds were unambiguously characterized by 1H NMR, IR and Mass spectroscopy.

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**Reaction Scheme:**

![Reaction Scheme](image-url)

Probable mechanism for fused / hybrid molecules:

Ar. acid + DCC

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<th>R</th>
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<td>3,4-Dimethoxy cinnamic acid</td>
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<tr>
<td>4</td>
<td>Isoeugenol</td>
<td>3,4-Dimethoxy cinnamic acid</td>
<td>-CH3</td>
</tr>
<tr>
<td>5</td>
<td>Methyl Paraben</td>
<td>3,4-Dimethoxy cinnamic acid</td>
<td>-CH3</td>
</tr>
<tr>
<td>6</td>
<td>Methyl Ferulate</td>
<td>3,4-Dimethoxy cinnamic acid</td>
<td>-CH3</td>
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<td>11</td>
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<td>-C2H5</td>
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<td>Ethyl Paraben</td>
<td>3,4-Diethoxy cinnamic acid</td>
<td>-C2H5</td>
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**Compound 3: (4-allyl-2-methoxy-phenyl) (E)-3-{3, 4-dimethoxyphenyl} prop-2-enolate**

\[^1^H\] NMR (CDCl\textsubscript{3}, 400 MHz) δ ppm : 3.39 (d, J = 6.6 Hz, 2H, Benzylic proton), 3.83 (s, 3H, 1 x Ar–OCH\textsubscript{3}), 3.92 (s, 3H, 1 x Ar–OCH\textsubscript{3}), 3.93 (s, 3H, 1 x Ar–OCH\textsubscript{3}), 5.0 – 5.2 (m, 2H, olefinic proton ‘a’), 5.9 – 6.1 (m, 1H, olefinic proton ‘b’), 6.55 (d, J = 15.8 Hz, 1H, Trans double bond), 6.7 – 7.2 (m, 6H, ArH), 7.8 (d, J = 15.8 Hz, 1H, Trans double bond); TOF MS ES: 377 (M + Na); Molecular Formula C\textsubscript{21}H\textsubscript{22}O\textsubscript{5}; Melting range 112 – 115\degree C; Elemental Analysis, Calcd.: C 71.17 %, H 6.26 %, O 22.57 %; Found C 71.20 %, H 6.30 %, O 22.54 %.

**Compound 4: [2-methoxy-4-[(E)-prop-1-enyl] phenyl] (E)-3-{3, 4-dimethoxyphenyl} prop-2-enolate**

\[^1^H\] NMR (CDCl\textsubscript{3}, 400 MHz) δ ppm : 1.85 (d, J = 8.5 Hz, 3H, Terminal –CH\textsubscript{3} from isoeugenol moiety), 3.85 (s, 3H, 1 x Ar–OCH\textsubscript{3}), 3.92 (s, 6H, 2 x Ar–OCH\textsubscript{3}), 6.1 – 6.3 (m, 1H, olefinic proton ‘a’), 6.38 (d, J = 15.6 Hz, olefinic proton ‘b’), 6.52 (d, J = 15.8 Hz, 1H, Trans double bond), 6.8 – 7.22 (m, 6H, ArH), 7.79 (d, J = 15.8 Hz, 1H, Trans double bond); TOF MS ES: 377 (M + Na); Molecular Formula C\textsubscript{21}H\textsubscript{22}O\textsubscript{5}; Melting range 110 – 112\degree C; Elemental Analysis, Calcd.: C 71.17 %, H 6.26 %, O 22.57 %. Found C 71.21 %, H 6.29 %, O 22.53 %.

**Compound 5: methyl 4-[(E)-3-{3,4-dimethoxyphenyl}prop-2-enoyl]oxybenzoate**

\[^1^H\] NMR (CDCl\textsubscript{3}, 400 MHz) δ ppm : 3.93 (s, 3H, 1 x –OCH\textsubscript{3} from – COOCH\textsubscript{3} group), 3.94 (s, 6H, 2 x Ar–OCH\textsubscript{3}), 6.50 (d, J = 15.9 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 3H, ArH from 3,4-dimethoxy cinnamic acid moiety), 7.25 (d, J = 8.8 Hz, 2H, ArH from methyl paraben moiety), 7.83 (d, J = 15.8 Hz, 1H, Trans double bond), 8.1 (d, J = 8.8 Hz, 2H, ArH from methyl paraben moiety); TOF MS ES: 365 (M + Na); Molecular Formula C\textsubscript{20}H\textsubscript{19}O\textsubscript{5}; Melting range 103 – 105\degree C; Elemental Analysis, Calcd.: C 66.66 %, H 5.30 %, O 28.04 %. Found C 66.70 %, H 5.33 %, O 28.00 %.

**Compound 6: methyl (E)-3-{4-[[E]-3-{3,4-dimethoxyphenyl}prop-2-enoyl]oxy-3-methoxy-phenyl]prop-2-enolate**

\[^1^H\] NMR (CDCl\textsubscript{3}, 400 MHz) δ ppm : 3.82 (s, 3H, Ar x –OCH\textsubscript{3}), 3.88 (s, 3H, Ar x –OCH\textsubscript{3}), 3.93 (s, 3H, Ar x –OCH\textsubscript{3}), 3.94 (s, 1H, 1 x –OCH\textsubscript{3} from –COOCH\textsubscript{3} group), 6.41 (d, J = 16.1 Hz, 1H, Trans double bond), 6.54 (d, J = 15.7 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 6H, ArH), 7.67 (d, J = 15.8 Hz, 1H, Trans double bond), 7.83 (d, J = 15.8 Hz, 1H, Trans double bond); TOF MS ES: 421 (M + Na); Molecular Formula C\textsubscript{22}H\textsubscript{22}O\textsubscript{5}; Melting range 133 – 135\degree C; Elemental Analysis, Calcd.: C 66.32 %, H 5.57 %, O 28.11 %. Found C 66.35 %, H 5.60 %, O 28.08 %.

**Compound 7: ethyl 4-[(E)-3-{3,4-dimethoxyphenyl}prop-2-enoyl]oxybenzoate**

\[^1^H\] NMR (CDCl\textsubscript{3}, 400 MHz) δ ppm : 1.40 (t, J = 7.0 Hz, 3H, –CH\textsubscript{3} from -COOCH\textsubscript{3}CH\textsubscript{3} group), 3.94 (s, 6H, 2 x Ar–OCH\textsubscript{3}), 4.38 (q, J = 7.3, 14.2 Hz, 2H, -OCH\textsubscript{2} from –COOCH\textsubscript{3}CH\textsubscript{3} group), 6.50 (d, J = 16.1 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 3H, ArH from 3,4-dimethoxy cinnamic acid moiety), 7.25 (d, J = 8.8 Hz, 2H, ArH from ethyl paraben moiety), 7.83 (d, J = 15.8 Hz, 1H, Trans double bond), 8.1 (d, J = 8.8 Hz, 2H, ArH from ethyl paraben moiety); TOF MS ES: 379 (M + Na); Molecular Formula C\textsubscript{20}H\textsubscript{20}O\textsubscript{5}; Melting range 85 – 87\degree C; Elemental Analysis, Calcd.: C 67.41 %, H 5.66 %, O 26.94 %. Found C 67.45 %, H 5.70 %, O 26.90 %.

**Compound 8: (4-allyl-2-methoxy-phenyl) (E)-3-{3,4-dieth oxyphenyl}prop-2-enolate**

\[^1^H\] NMR (CDCl\textsubscript{3}, 400 MHz) δ ppm : 1.49 (t, J = 7.0 Hz, 6H, 2 x –CH\textsubscript{3}, Terminal –CH\textsubscript{3} from 3,4-dieth oxy cinnamic acid moiety), 3.39 (d, J = 6.6 Hz, 2H, Benzylic proton), 3.83 (s, 3H, 1 x Ar–
OCH₃), 4.13 (q, J = 3.0, 6.5 Hz, 4H, 2 x -OCH₃ from –OCH₂CH₃ moiety), 5.0 – 5.2 (m, 2H, olefinic proton ‘a’), 5.9 – 6.1 (m, 1H, olefinic proton ‘b’), 6.5 (d, J = 15.8 Hz, 1H, Trans double bond), 6.7 – 7.2 (m, 6H, ArH), 7.8 (d, J = 15.8 Hz, 1H, Trans double bond); TOF MS ES: 449 (M + Na); Molecular Formula C₁₅H₁₀O₃; Melting range 128 – 130°C; Elemental Analysis, Calcd.: C 67.59 %, H 6.15 %, O 26.26 %. Found C 67.62 %, H 6.18 %, O 26.22 %;

**Compound 12: ethyl 4-[(E)-3-(3,4-diethoxyphenyl)prop-2-enoyl]oxybenzoate**

1H NMR (CDCl₃, 400 MHz) δ ppm : 1.38 (t, J = 7.0 Hz, 3H, –CH₃), 1.85 (d, J = 8.5 Hz, 3H, Terminal –CH₃ from isoeugenol moiety), 3.85 (s, 3H, 1 x –OCH₃ from isoeugenol moiety), 4.13 (q, J = 3.0, 6.5 Hz, 4H, 2 x OCH₂ from –OCH₂CH₃ moiety), 6.1 – 6.3 (m, 1H, olefinic proton ‘a’), 6.38 (d, J = 15.6 Hz, olefinic proton ‘b’), 6.51 (d, J = 15.8 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 6H, ArH), 7.79 (d, J = 15.8 Hz, 1H, Trans double bond); TOF MS ES: 407 (M + Na); Molecular Formula C₁₅H₁₂O₃; Melting range 143 – 145°C; Elemental Analysis, Calcd.: C 72.23 %, H 6.85 %, O 20.92 %. Found C 72.26 %, H 6.89 %, O 20.88 %;

**Compound 9: [2-methoxy-4-{[E]-prop-1-enyl}phenyl] (E)-3-(3,4-diethoxyphenyl)prop-2-enoate**

1H NMR (CDCl₃, 400 MHz) δ ppm : 1.49 (t, J = 7.0 Hz, 6H, 2 x –OCH₃CH₃, Terminal –CH₃ from 3,4-diethoxy cinnamic acid moiety), 1.85 (d, J = 8.5 Hz, 3H, Terminal –CH₃ from isoeugenol moiety), 3.85 (s, 3H, 1 x –OCH₃ from isoeugenol moiety), 4.13 (q, J = 3.0, 6.5 Hz, 4H, 2 x OCH₂ from –OCH₂CH₃ moiety), 6.1 – 6.3 (m, 1H, olefinic proton ‘a’), 6.38 (d, J = 15.6 Hz, olefinic proton ‘b’), 6.51 (d, J = 15.8 Hz, 1H, Trans double bond), 6.8 – 7.2 (m, 6H, ArH), 7.79 (d, J = 15.8 Hz, 1H, Trans double bond); TOF MS ES: 405 (M + Na); Molecular Formula C₁₅H₁₀O₃; Melting range 72 – 75°C; Elemental Analysis, Calcd.: C 72.23 %, H 6.85 %, O 20.92 %. Found C 72.26 %, H 6.89 %, O 20.88 %;
3.2 Biological activity

Antibacterial Activity using ditch plate method

The synthesized molecules were screened for their antibacterial activity at 100 μg/ml concentration using ditch plate method against Gram + ve (Staphylococcus aureus, Corynebacterium diphtheriae) and Gram negative (Escherichia coli, Klebsiella pneumoniae, Salmonella typhi) bacterial species qualitatively. The results of the antibacterial activities are summarized in Table 1.

Ditch plate method is the method of chosen to test the antibacterial activity of compounds\(^1\). It is a preliminary method to screen the anti-microbial potential of compounds / drugs, which are insoluble or partially soluble in aqueous phase. In this method, the test compound is seeded in an agar plate and the test organisms are streaked across to test the inhibition of the growth as a marker of anti-microbial activity.

**Procedure:** A ditch (10 mm x 70 mm) is cut into sterile MH agar plate. The test drug / compound is added to 5 ml molten MH agar butt at 40\(^\circ\)C and this mixture is poured into the ditch and allowed to solidify. The ditch should be made in level with the rest of the agar by pouring the mixture. The different bacterial cultures are streaked perpendicular to the ditch using nichrome wire loop. The plate is then incubated at 37\(^\circ\)C for 24 hours. The results are observed as inhibition of bacterial growth on the ditch as well as adjacent to the ditch.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Active against micro-organisms</th>
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| 1          | Staphylococcus aureus [Gram positive]  
             Salmonella typhi [Gram negative]  
             Klebsiella pneumoniae [Gram negative]  
             Corynebacterium diphtheriae [Gram positive]  
             Escherichia coli [Gram negative] |
| 2          | Staphylococcus aureus [Gram positive]  
             Salmonella typhi [Gram negative]  
             Klebsiella pneumoniae [Gram negative]  
             Corynebacterium diphtheriae [Gram positive]  
             Escherichia coli [Gram negative] |
| 5          | Staphylococcus aureus [Gram positive]  
             Escherichia coli [Gram negative] |
| 7          | Staphylococcus aureus [Gram positive]  
             Escherichia coli [Gram negative] |
| 10         | Staphylococcus aureus [Gram positive]  
             Escherichia coli [Gram negative] |
| 12         | Staphylococcus aureus [Gram positive]  
             Escherichia coli [Gram negative] |

4. CONCLUSION

The novel hybrid derivatives of 3,4-Dimethoxy cinnamic acid and 3,4-Diethoxy cinnamic acid were synthesized by cost effective industry viable process following the principle of green chemistry. The synthesis of hybrid derivatives is another way to prepare ester derivatives using DCC as dehydrating agent in a reasonably good yield. The probable mechanism for the formation of hybrid derivative was also discussed.

The biological activity suggest that the base molecule 3,4-Dimethoxy cinnamic acid and 3,4-Diethoxy cinnamic acid have anti-bacterial activity against both the bacterial cultures. Its derivatives viz. 5, 7, 10, 12 were also active against certain Gram + ve and Gram – ve cultures. Thus, fused molecules of 3,4-Dimethoxy cinnamic acid (5, 7) and 3,4-Diethoxy cinnamic acid (10, 12) were potential antibacterial candidates. In depth analysis of these compounds through structure activity relationship studies would provide further insight and can be an interesting topic of future studies.

The structural diversity and the pronounced biological activities encountered in the 3,4-Dimethoxy cinnamic acid and 3,4-Diethoxy cinnamic acid derivatives suggests that this class of compounds is worthy for further studies that may lead to derivatives by using combinatorial chemistry approach is an alternative strategy to new therapeutic discovery. In other words the generation of diverse cinnamic acid derivatives develops new therapeutic molecules that might result in candidates having better activity.

5. ACKNOWLEDGEMENT

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