SUPPLEMENTARY MATERIAL

Synthesis, antibacterial evaluation and QSAR of caffeic acid derivatives

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Abstract

The present study evaluates the antibacterial effects of a set of 16 synthesized caffeic acid ester derivatives against strains of *Staphylococcus aureus* and *Escherichia coli*, as well as discussing their Structure-Activity Relationship (SAR). The antibacterial assays were performed using microdilution techniques in 96-well microplates to determine minimal inhibitory concentration (MIC). The results revealed that five of the compounds, present strong to optimum antibacterial effect. Of the sixteen esters derivatives evaluated, the products with alkyl side chains, as propyl caffeate (**3**), butyl caffeate (**6**) and pentyl caffeate (**7**), presented the best antibacterial activity with MIC values of around 0.20 µM against *Escherichia coli* and only butyl caffeate (**6**) showing the same MIC against *Staphylococcus aureus*. For products with aryl substituents, the best MIC results against the tested strain of *Escherichia coli* were 0.23 µM for (di-(4-chlorobenzyl)) caffeate (**13**) and 0.29 µM for diphenylmethyl caffeate (**10**) and all were less active against the *Staphylococcus aureus* strain. Preliminary Quantitative Structure-Activity Relationship (QSAR) analyses confirmed that certain structural characteristics, such as a median linear carbon chain and the presence of electron withdrawal substituents at the *para* position of the aromatic ring help potentiate antibacterial activity.

Keywords: caffeic ester, antimicrobial activity, Structure Activity-Relationship, *Staphylococcus*, *Escherichia*.

Supporting Information

Methyl caffeate (**1**): Amber amorphous solid, 87.77% yield; IR υmax (KBr, cm^{-1}): 3313, 3097, 2957, 1676, 1600 and 1440, 1276 and 1178; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 3.68 (3H; s), 6.27 (1H; d; *J* = 16.0 Hz), 6.76 (1H; d; *J*=8.0 Hz), 7.00 (1H; dd; *J*=8.0 Hz, 2.0 Hz), 7,06 (1H; d; *J*=2.0 Hz), 7.48 (1H; d; *J*=16.0 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 51.4, 113.8, 114.8, 115.8, 121.6, 125.6, 145.3, 145.7, 148.5, 167.2 [1].

Ethyl caffeate (**2**): Gray amorphous solid, 86.53% yield; IR υmax (KBr, cm^{-1}): 3179, 3065, 2990, 1657, 1606 and 1448, 1283 and 1220; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 1.23 (3H; t; *J*=7.0 Hz), 4.15 (2H; q; *J*=7.0 Hz), 6.25 (1H; d; *J*=16.0 Hz), 6.76 (1H; d; *J*=8.0 Hz), 7.00 (1H; dd; *J*=8.0 Hz, 2.0 Hz), 7.05 (1H; d; *J*=2.0 Hz), 7.47 (1H; d; *J*=16.0 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 14.4, 59.8, 114.1, 114.8, 115.8, 121.5, 125.6, 145.1 145.6, 148.5, 166.7 [2].

Propyl caffeate (**3**): Brown amorphous solid, 85.96% yield; IR υmax (KBr, cm^{-1}): 3459, 3085, 2970, 1663, 1606 and 1442, 1277 and 1188; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 0.91 (3H; t; *J*=7.4 Hz), 1.63 (2H; sext.; *J*=7.0 Hz), 4.06 (2H; t; *J*=6.6 Hz), 6.26 (1H; d; *J*=16.0 Hz), 6.76 (1H; d; *J*=8.0 Hz), 7.00 (1H; dd; *J*=8.0 Hz, 2.0 Hz), 7.05 (1H; d; *J*=2.0 Hz), 7.47 (1H; d; *J*=16.0 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 10.4, 21.8, 65.3, 114.1, 114.8, 115.8, 121.5, 125.6, 145.1, 145.7, 148.5, 166.8 [3].

Isopropyl caffeate (**4**): Black amorphous solid, 93.06% yield; IR υmax (KBr, cm^{-1}): 3307, 3047, 2976, 1677, 1594 and 1442, 1277 and 1188; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 1.23 (6H; d; *J*=6.2 Hz), 4.98 (1H; sept.; *J*=6.2 Hz), 6.23 (1H; d; *J*=15.8 Hz), 6.75 (1H; d; *J*=8.0 Hz), 6.99 (1H; dd; *J*=8.0, 2.0 Hz), 7,04 (1H; d; *J*=2.0 Hz), 7.44 (1H; d; *J*=16.0 Hz); ^{13}C NMR (CD_3OD, 50 MHz): δ_C 22.2, 69.0, 115.1, 115.6, 116.5, 122.9, 127.6, 146.6, 146.7, 149.4, 168.8 [2].

Methoxyethyl caffeate (**5**)

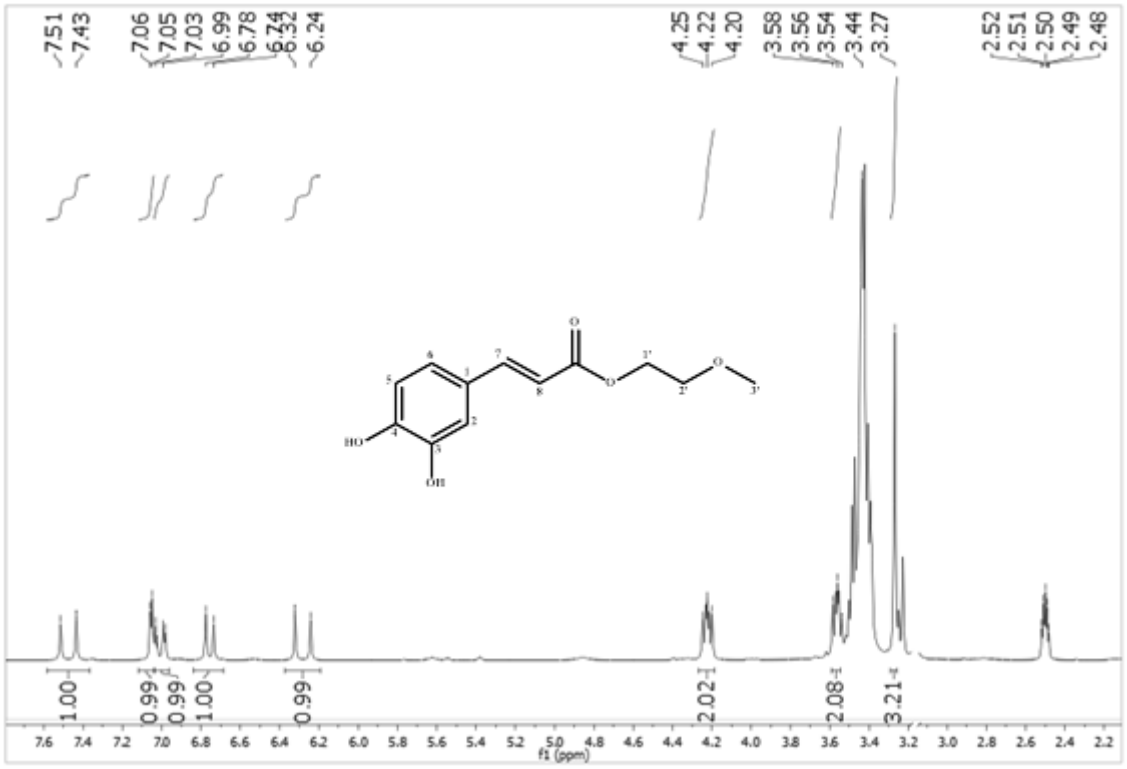


Figure 1. ^{1}H NMR spectrum of methoxyethyl caffeate (DMSO-d_6, 200 MHz).

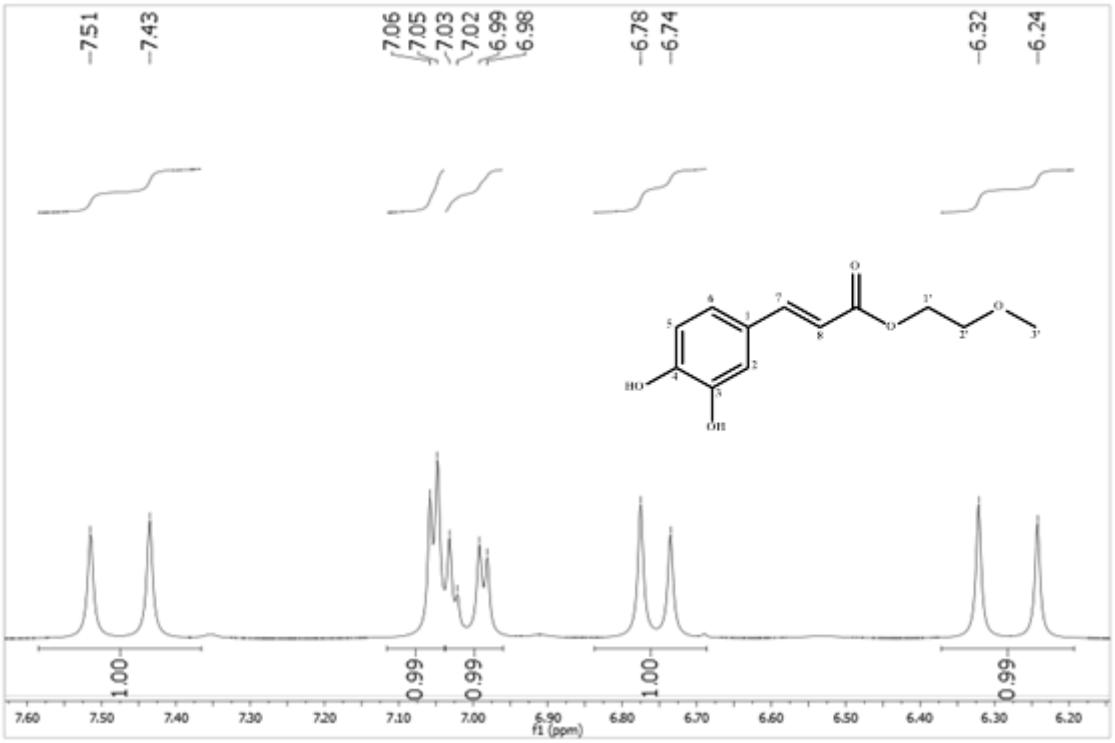


Figure 2. Expansion of the ^{1}H NMR spectrum of methoxyethyl caffeate (DMSO-d_6, 200 MHz).

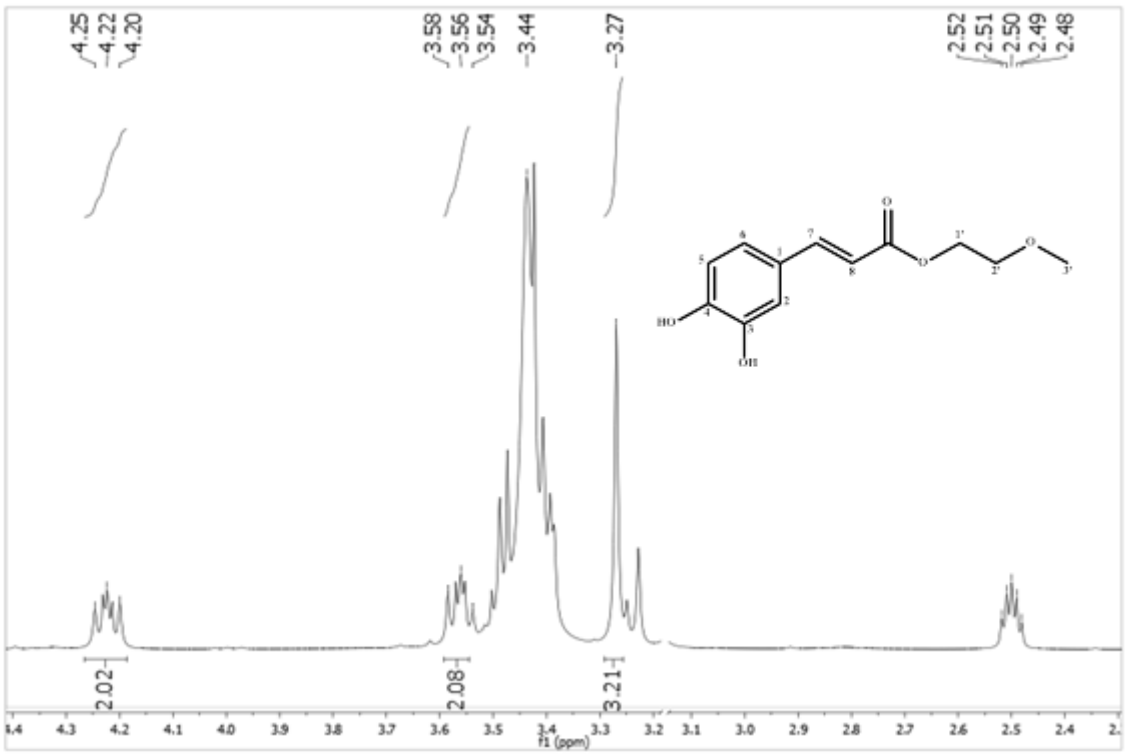


Figure 3. Expansion of the ^{1}H NMR spectrum of methoxyethyl caffeate (DMSO-d_6, 200 MHz).

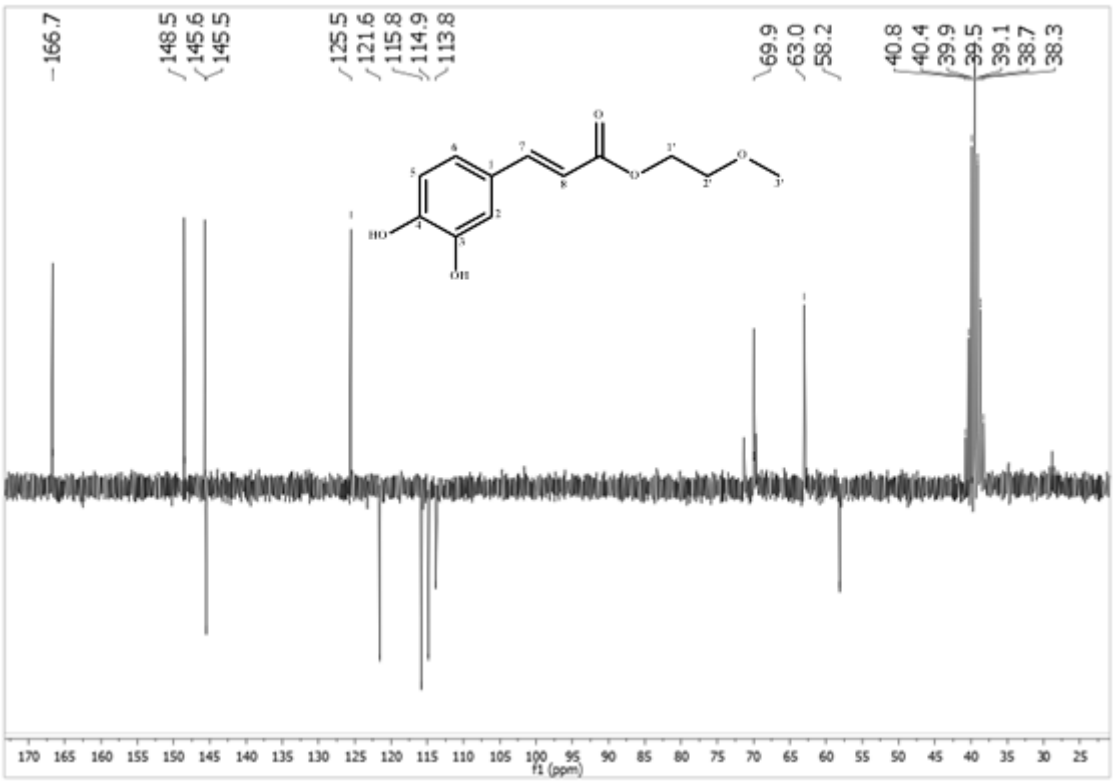


Figure 4. ^{13}C NMR spectrum of methoxyethyl caffeate (DMSO-d_6, 50 MHz).

Butyl caffeate (**6**): Black Liquid, 91.53% yield; IR υmax (KBr, cm^{-1}): 3334, 3059, 2957, 1683, 1606 and 1448, 1277 and 1181; ^{1}H NMR (DMSO-d_6, 500 MHz): δ_H 0.90 (3H; t; *J*=7.5 Hz), 1.36 (2H; sext.; *J* = 7.5 Hz 2H, H-3'), 1.60 (2H; quint; *J*=6.5 Hz), 4.11 (2H; t; *J*=6.5 Hz), 6.26 (1H; d; *J*=16.0 Hz), 6.76 (1H; d; *J*=8.0 Hz), 6.99 (1H; dd; *J*=8.5 Hz, 2.0 Hz), 7.05 (1H; d; *J*=2.5 Hz), 7.46 (1H; d; *J*=15.5 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz): δ_C 13.66, 18.75, 30.42, 63.49, 114.09, 114.80, 115.79, 121.43, 125.57, 145.05, 145.62, 148.42, 166.70 [2].

Pentyl caffeate (**7**): Brown liquid, 50.50% yield; IR υmax (KBr, cm^{-1}): 3402, 3039, 2957, 1695, 1606 and 1460, 1283 and 1175; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 0.87 (3H; t; *J*=7.0 Hz), 1.36–1.22 (4H; m), 1.62 (2H; quint; *J*=6.8 Hz), 4.10 (2H; t; *J*=6.6 Hz), 6.26 (1H; d; *J*=16.0 Hz), 6.76 (1H; d; *J*=8.0 Hz), 7.00 (1H; dd; *J*=8.0 Hz, 2.0 Hz), 7.05 (1H; d; *J*=2.0 Hz), 7.46 (1H; d; *J*=16.0 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 14.0, 21.9, 27.7, 28.1, 63.8, 114.1, 114.9, 115.8, 121.4, 125.5, 145.1, 145.6, 148.4, 166.7 [2].

Isopentyl caffeate (**8**): Gray amorphous solid, 49.00% yield; IR υmax (KBr, cm^{-1}): 3313, 3053, 2957, 1677, 1606 and 1448, 1283 and 1181; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 0.90 (6H; d; *J*=6.4 Hz), 1.51 (2H; q; *J*=6.4 Hz), 1.78–1.58 (1H; m), 4.13 (2H; t; *J*=6.6 Hz), 6.25 (1H; d; *J*=16.0 Hz), 6.76 (1H; d; *J*=8.0 Hz), 6.99 (1H; d; *J*=8.0 Hz), 7.04 (1H; s), 7.46 (1H; d; *J*=16.0 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 22.4, 24.7, 37.1, 62.2, 114.1, 114.8, 115.8, 121.4, 125.5, 145.1, 145.6, 148.4, 166.7 [2].

Decyl caffeate (**9**): Brown liquid, 47.53% yield; IR υmax (KBr, cm^{-1}): 3299, 3059, 2964, 1677, 1606 and 1442, 1277 and 1175; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 0.84 (3H; t; *J*=6.0 Hz), 1.30–1.23 (14H; m), 1.60 (2H; quint; *J*=6.2 Hz), 4.10 (2H; dd; *J*=6.2 Hz), 6.25 (1H; d; *J*=16.0 Hz), 6.75 (1H; d; *J*=8.0 Hz), 6.99 (1H; dd; *J*=8.0 Hz, 2.0 Hz), 7.04 (1H; d; *J*=2.0 Hz), 7.46 (1H; d; *J*=15.8 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 14.1, 22.2, 25.6, 28.4, 28.8, 28.8, 29.1, 29.1, 31.4, 63.7, 114.1, 114.9, 115.9, 121.5, 125.6, 145.2, 145.7, 148.5, 166.7 [2].

Diphenylmethyl caffeate (**10**)

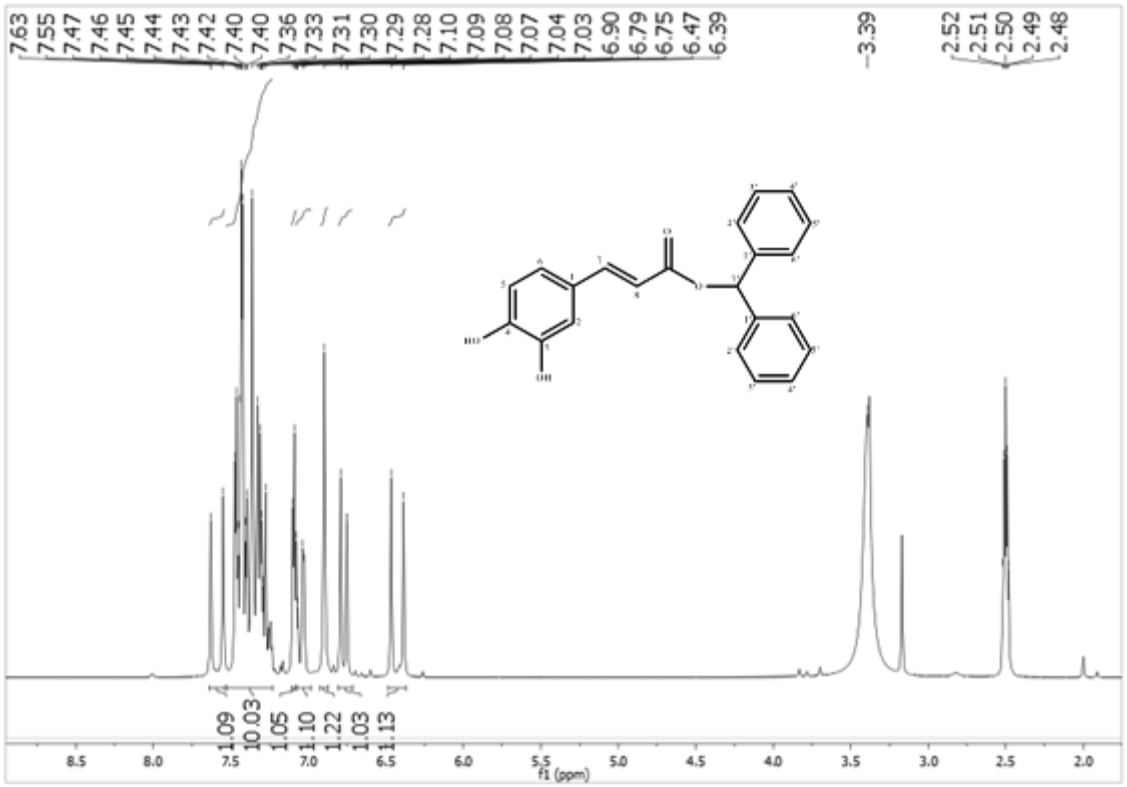


Figure 5. ^{1}H NMR spectrum of diphenylmethyl caffeate (DMSO-d_6, 200 MHz).

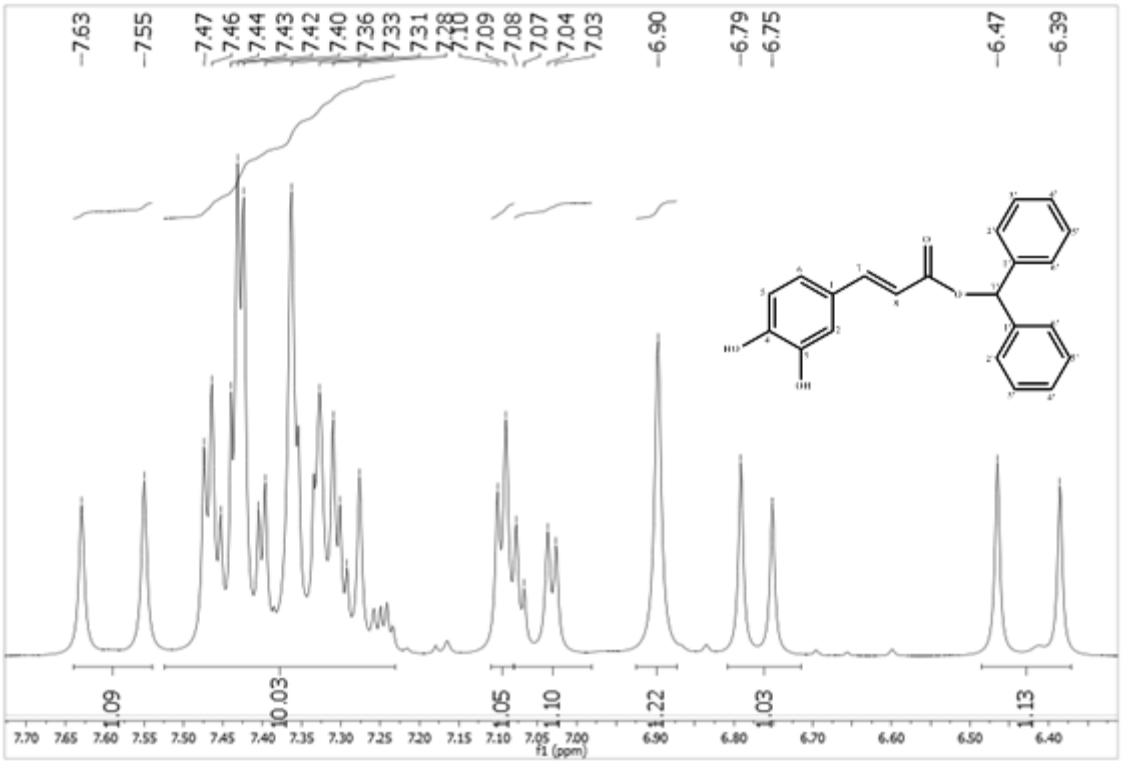


Figure 6. Expansion of the ^{1}H NMR spectrum of diphenylmethyl caffeate (DMSO-d_6, 200 MHz).

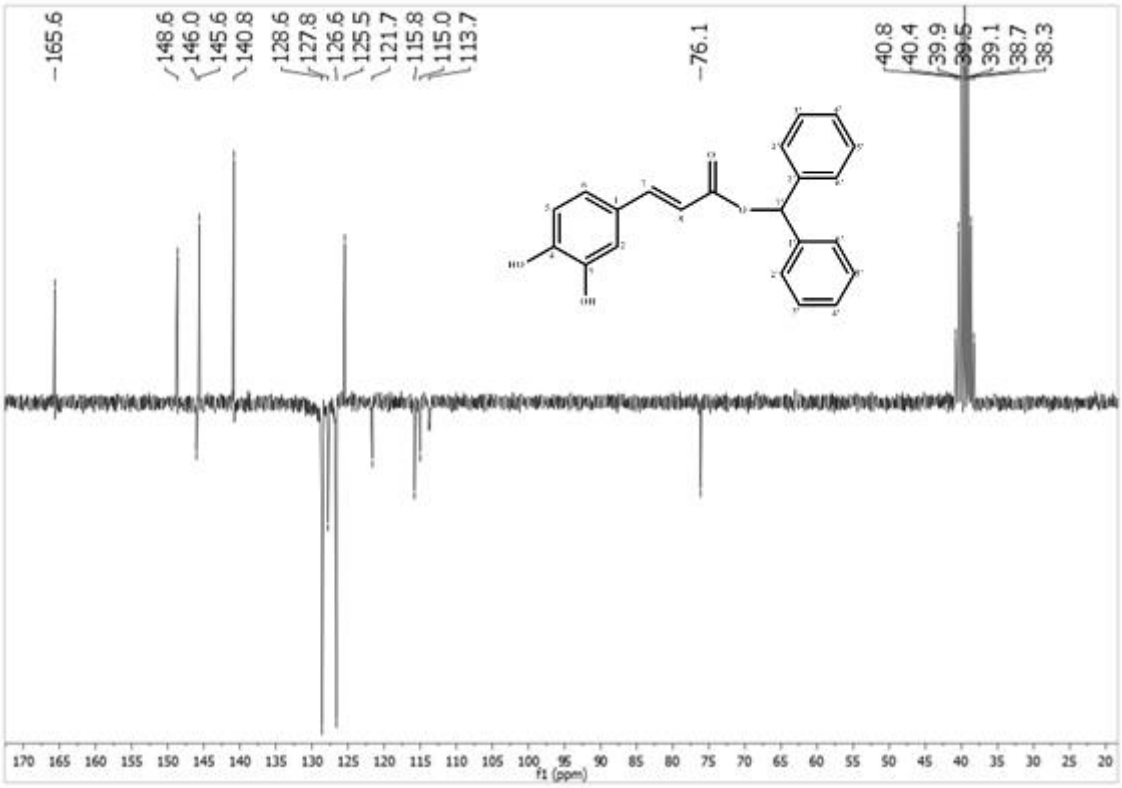


Figure 7. ^{13}C NMR spectrum of diphenylmethyl caffeate (DMSO-d_6, 50 MHz).

4-Chlorobenzyl caffeate (**11**)

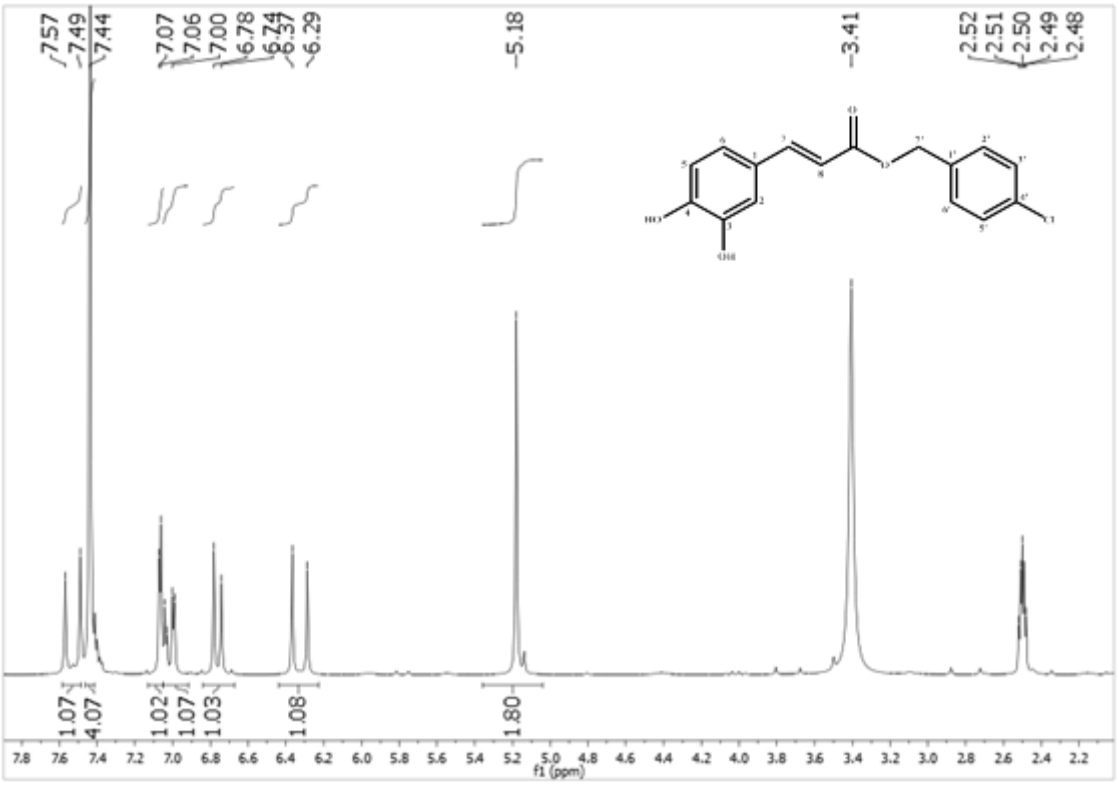


Figure 8. ^{1}H NMR spectrum of 4-chlorobenzyl caffeate (DMSO-d_6, 200 MHz).

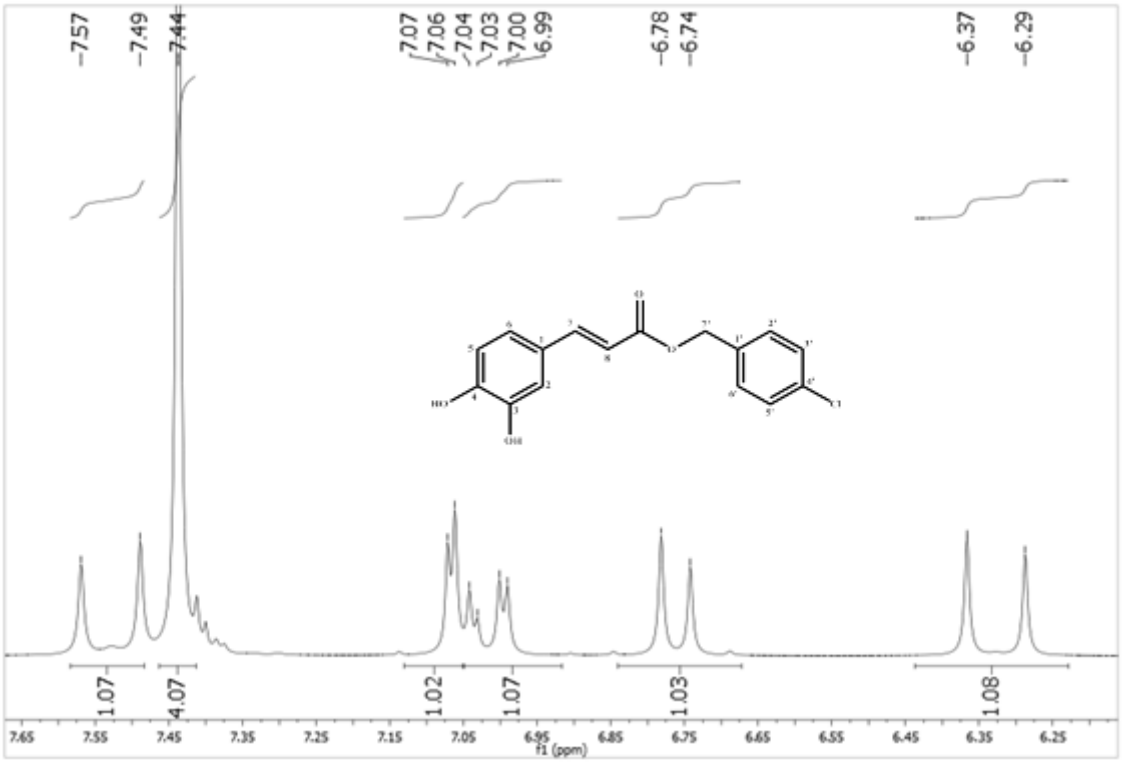


Figure 9. Expansion of the ^{1}H NMR spectrum of 4-chlorobenzyl caffeate (DMSO-d_6, 200 MHz).

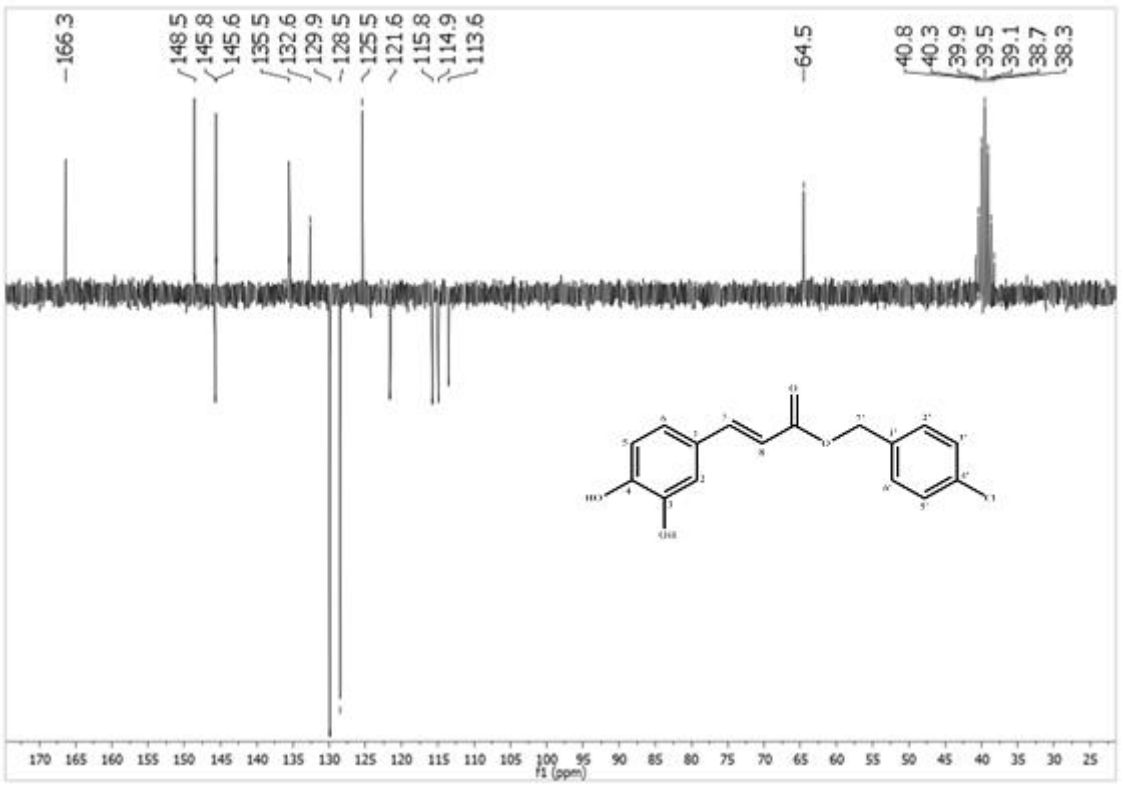


Figure 10. ^{13}C NMR spectrum of 4-chlorobenzyl caffeate (DMSO-d_6, 50 MHz).

4-Methoxybenzyl caffeate (**12**): Amber amorphous solid, 42.70% yield; IR υmax (KBr, cm^{-1}): 3338, 3059, 2945, 1702, 1614 and 1435, 1194 and 1125; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 3.75 (3H; s), 5.11 (2H; s), 6.29 (1H; d; *J*=16.0 Hz), 6.75 (1H; d; *J*=8.0 Hz), 6.93 (2H; d; *J*=8.6 Hz); 7.00 (1H; dd; *J*=8.0 Hz, 2.0 Hz), 7.05 (1H; d; *J*=2.0 Hz), 7.35 (2H; d; *J*=8.8 Hz), 7.49 (1H; d; *J*=16.0 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 55.2, 65.2, 113.6, 113.9, 114.9, 115.8, 121.5, 125.5, 128.4, 130.1, 145.4, 145.6, 148.5, 159.2, 166.6 [4].

(Di-(4-chlorobenzyl)) caffeate (**13**)

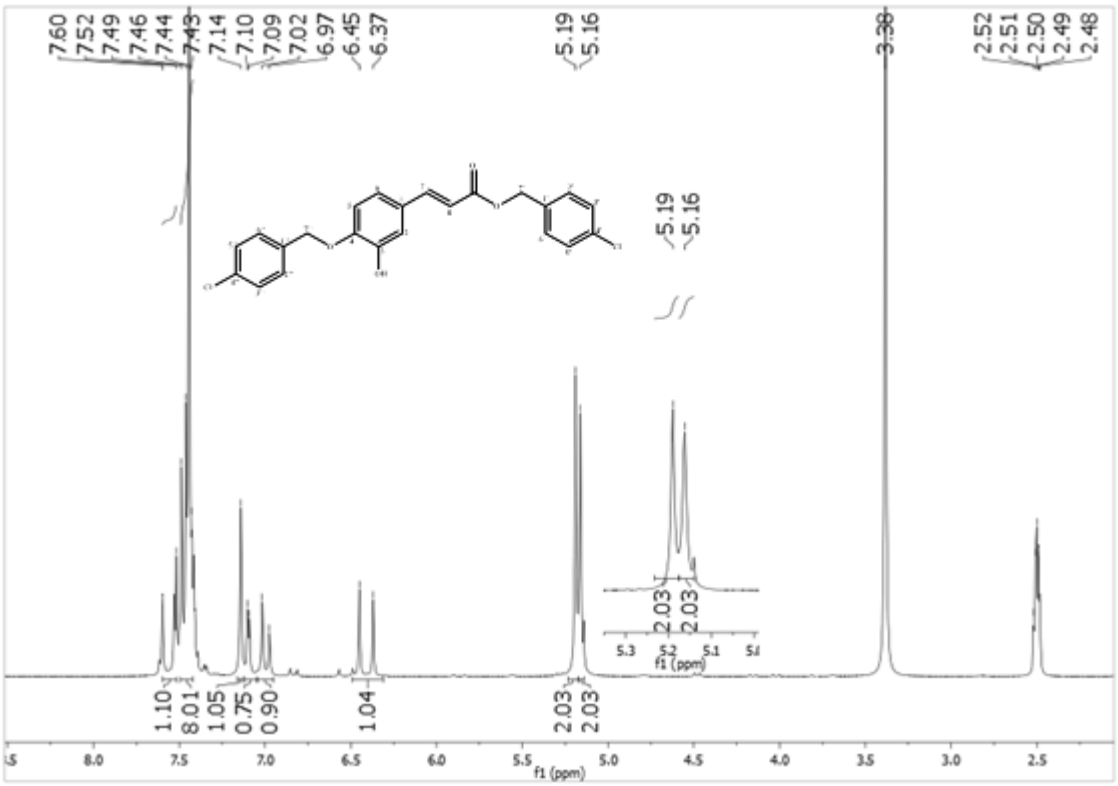


Figure 11. ^{1}H NMR spectrum of (di-(4-chlorobenzyl)) caffeate (DMSO-d_6, 200 MHz).

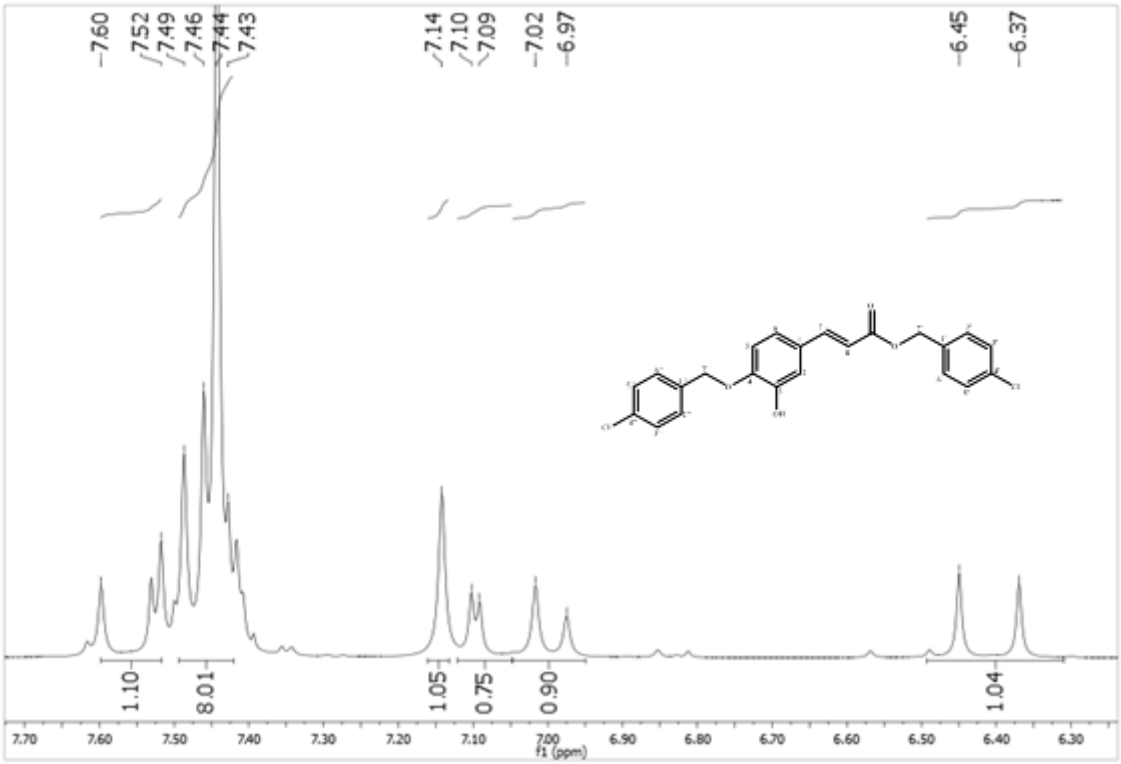


Figure 12. Expansion of the ^{1}H NMR spectrum of (di-(4-chlorobenzyl)) caffeate (DMSO-d_6, 200 MHz).

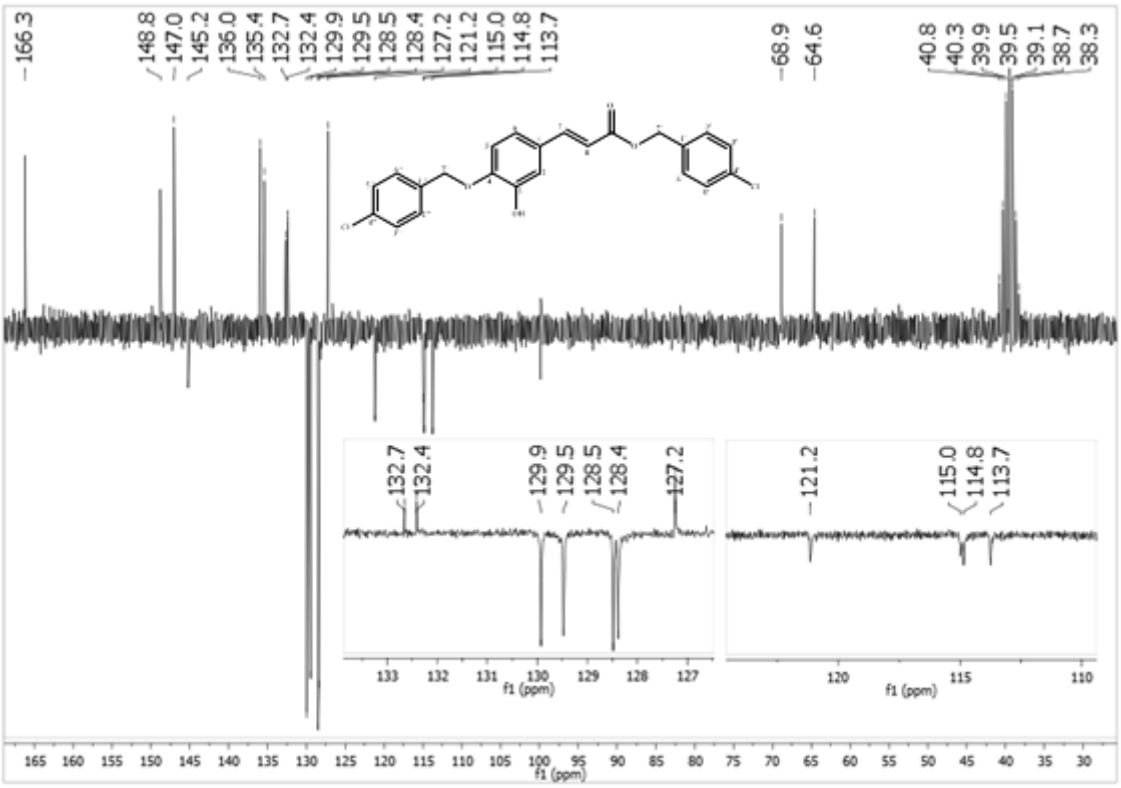


Figure 13. ^{13}C NMR spectrum of (di-(4-chlorobenzyl)) caffeate (DMSO-d_6, 50 MHz).

(Di-(4-methoxybenzyl)) caffeate (**14**)

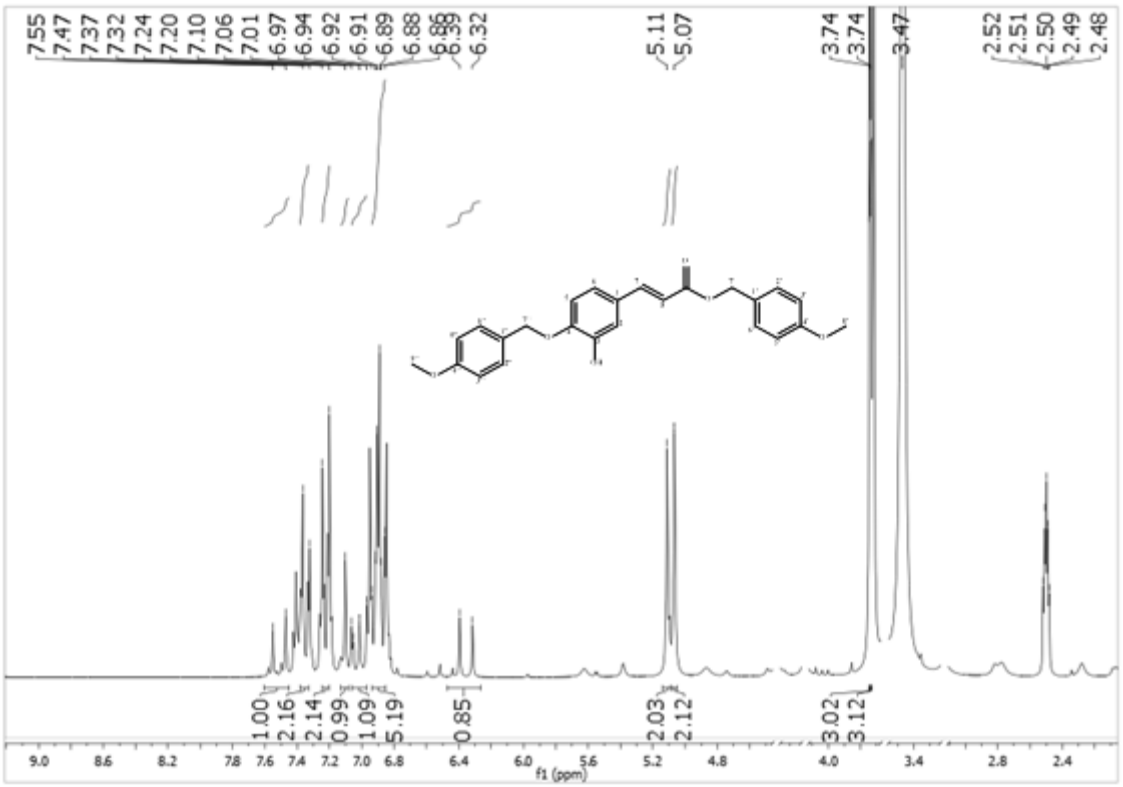
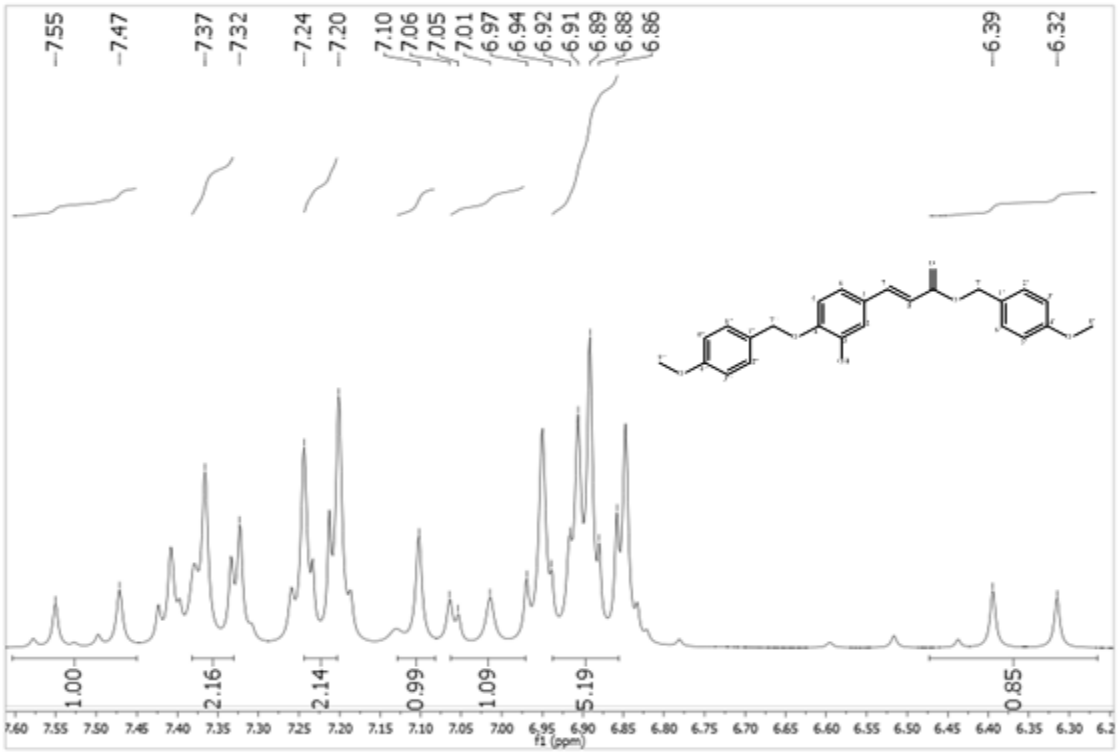


Figure 14. ^{1}H NMR spectrum of (di-(4-methoxybenzyl)) caffeate (DMSO-d_6, 200 MHz).



Spectrum 15. Expansion of the ^{1}H NMR spectrum of (di-(4-methoxybenzyl)) caffeate (DMSO-d_6, 200 MHz).

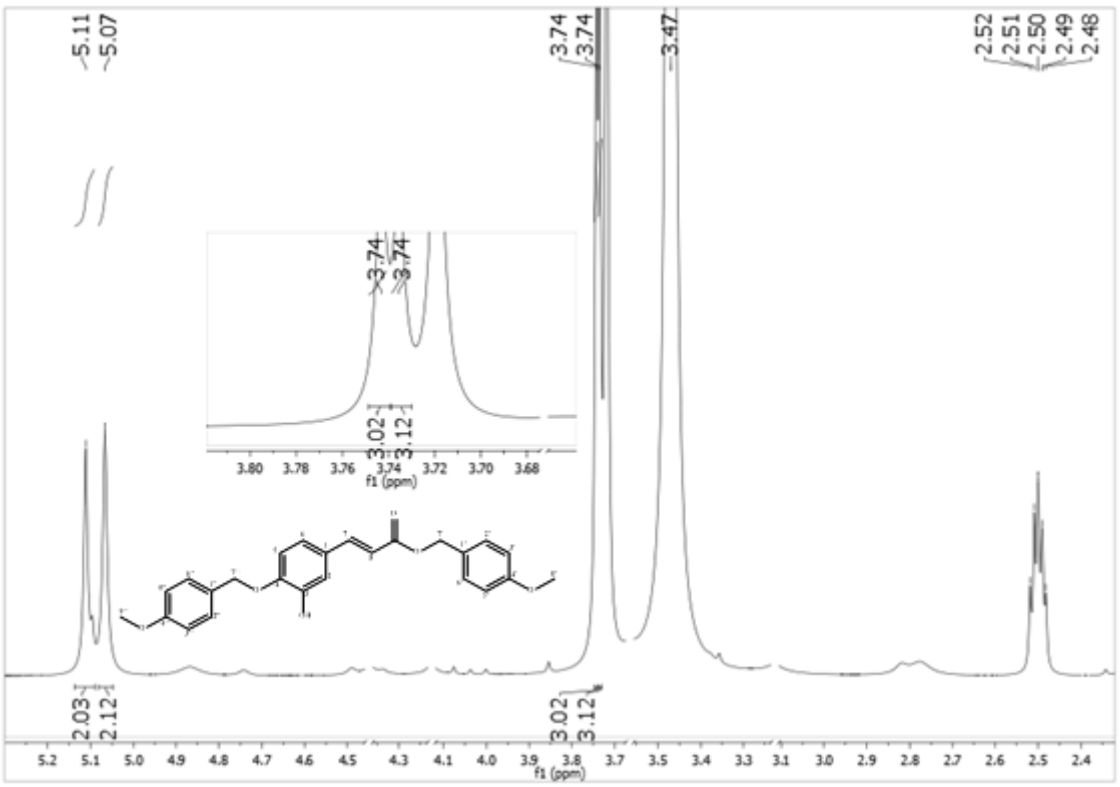
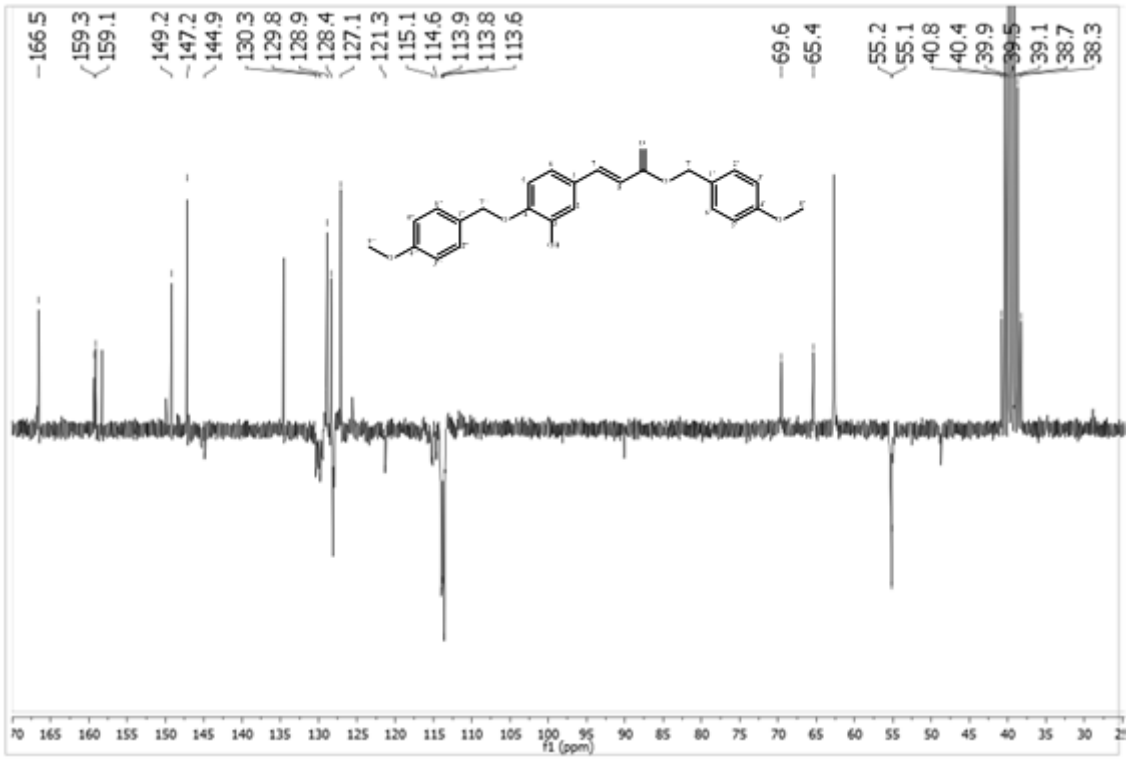


Figure 16. Expansion of the ^{1}H NMR spectrum of (di-(4-methoxybenzyl)) caffeate (DMSO-d_6, 200 MHz).



Spectrum 17. ^{13}C NMR spectrum of (di-(4-methoxybenzyl)) caffeate (DMSO-d_6, 50 MHz).

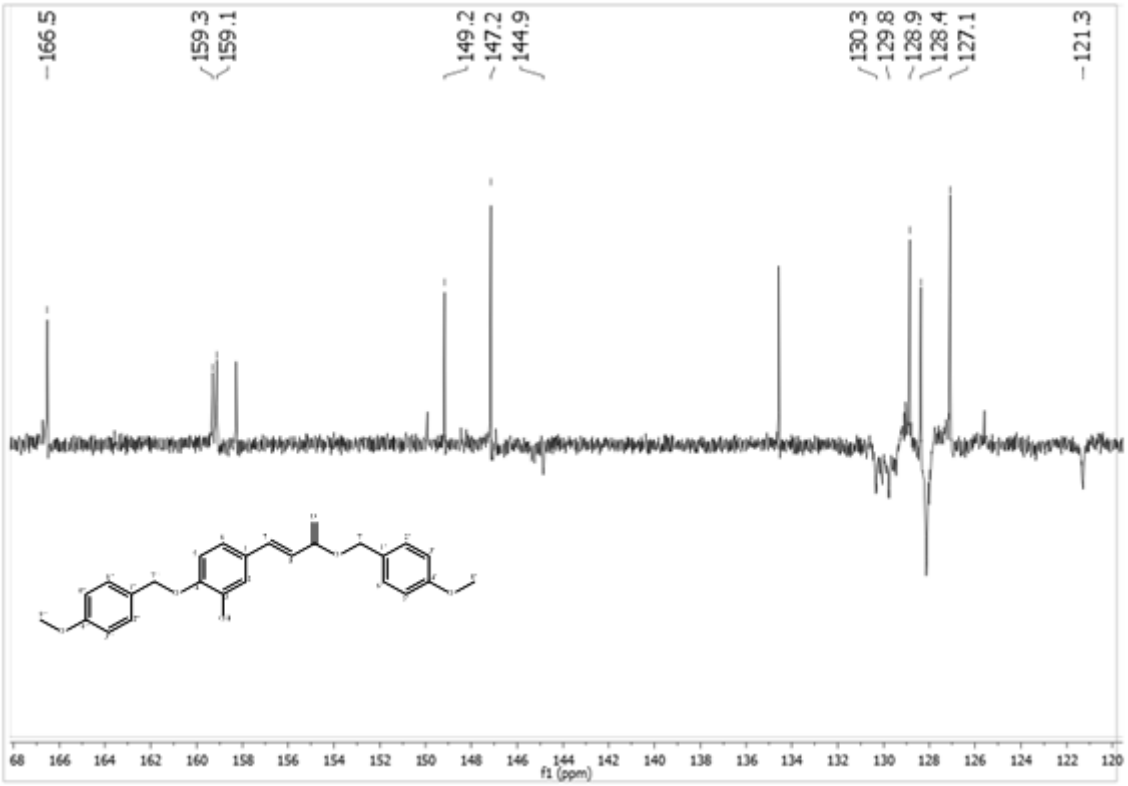


Figure 18. Expansion of the ^{13}C NMR spectrum of (di-(4-methoxybenzyl)) caffeate (DMSO-d_6, 50 MHz).

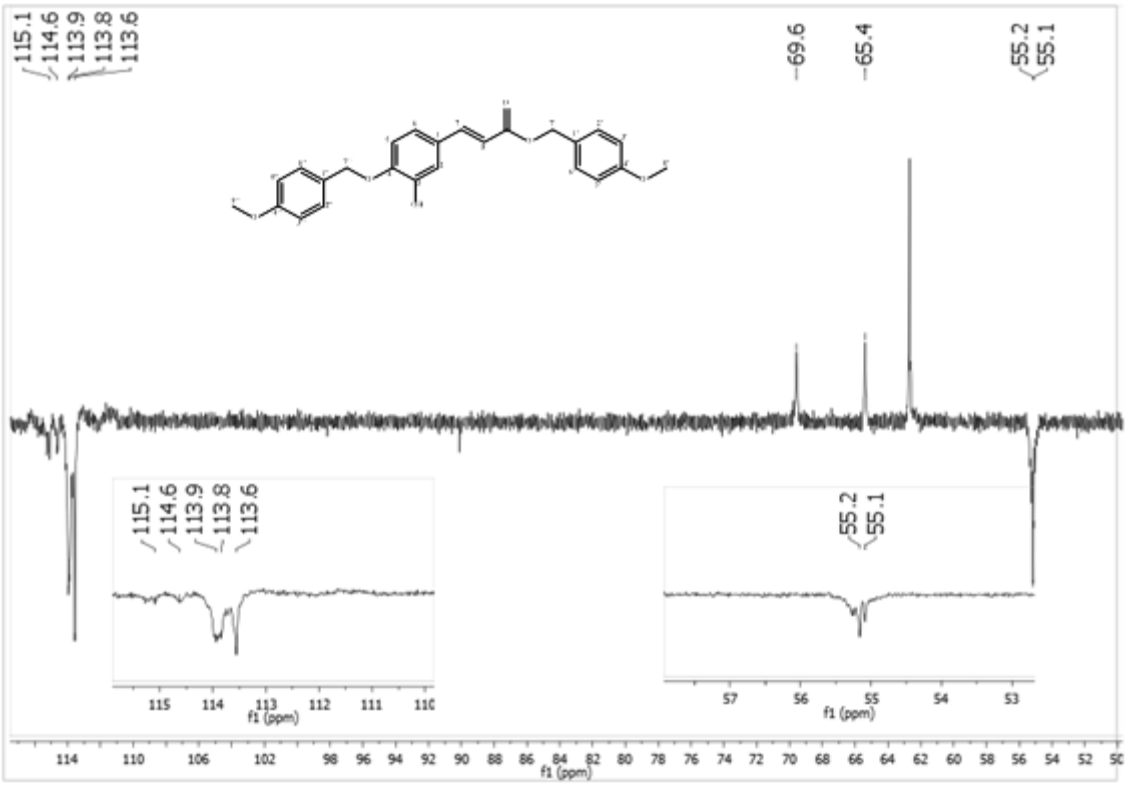


Figure 19. Expansion of the ^{13}C NMR spectrum of (di-(4-methoxybenzyl)) caffeate (DMSO-d_6, 50 MHz).

Benzyl caffeate (**15**): Yellow amorphous solid, 53.10% yield; IR υmax (KBr, cm^{-1}): 3299, 3039, 2964, 1689, 1600 and 1454, 1283 and 1181; ^{1}H NMR (DMSO-d_6, 200 MHz): δ_H 5.19 (2H; s), 6.33 (1H; d; *J*=16.0 Hz), 6.76 (1H; d; *J*=8.0 Hz), 7.02 (1H; dd; *J*=8.0 Hz, 2.0 Hz), 7.07 (1H; d; *J*=2.0 Hz), 7.41–7.32 (5H; m), 7.45 (1H; d; *J*=16.0 Hz); ^{13}C NMR (DMSO-d_6, 50 MHz): δ_C 65.4, 113.7, 114.9, 115.8, 121.7, 125.6, 128.0, 128.3, 128.7, 136.5, 145.5, 145.7, 148.6, 166.6 [2].

4-Methylbenzyl caffeate (**16**)

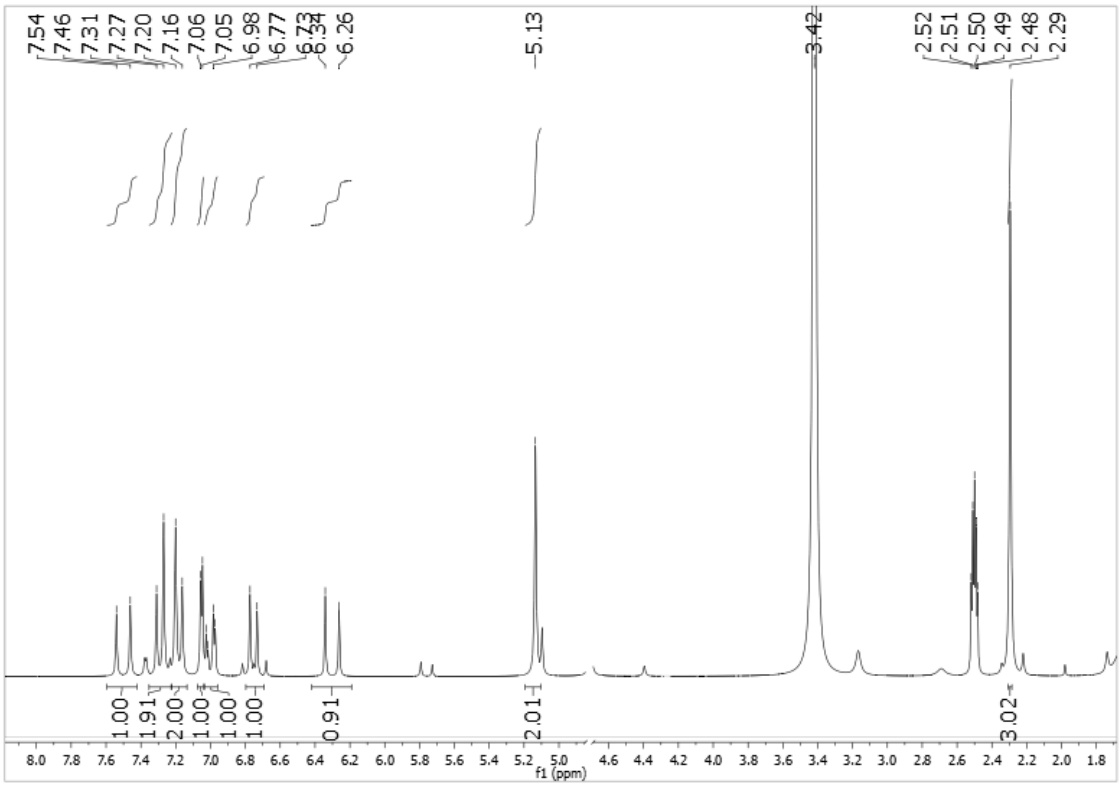


Figure 20. ^{1}H NMR spectrum of 4-methylbenzyl caffeate (DMSO-d_6, 200 MHz).

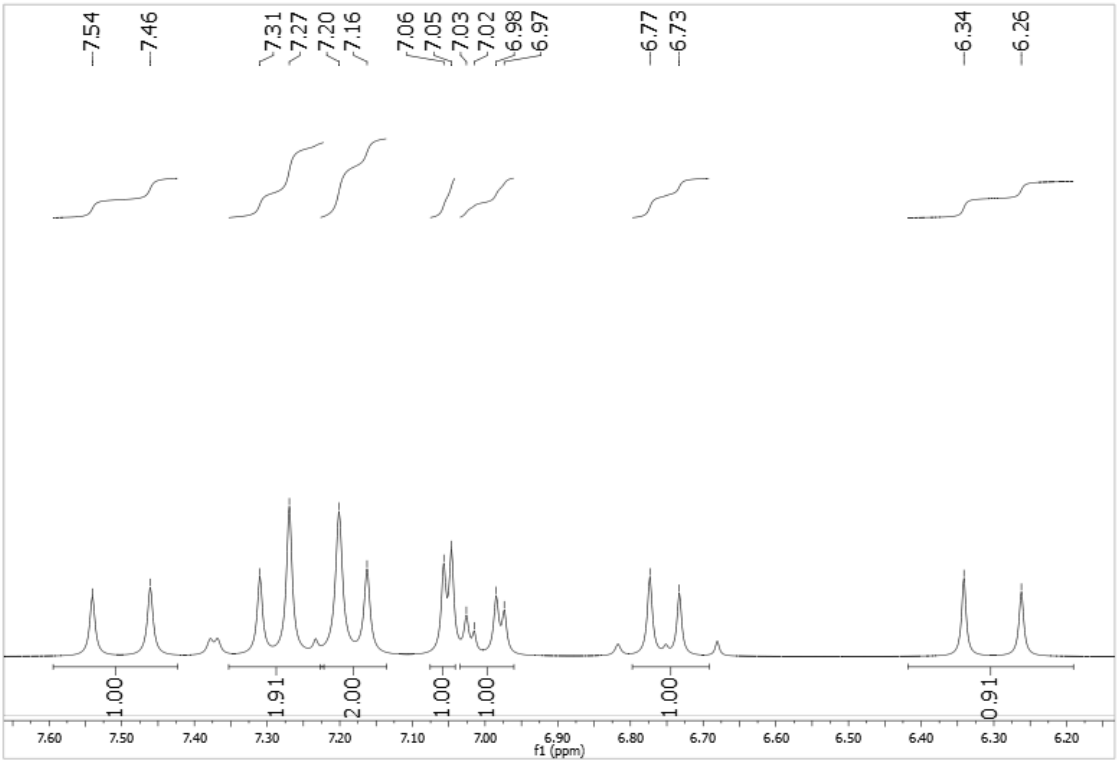


Figure 21. Expansion of the ^{1}H NMR spectrum of 4-methylbenzyl caffeate (DMSO-d_6, 200 MHz).

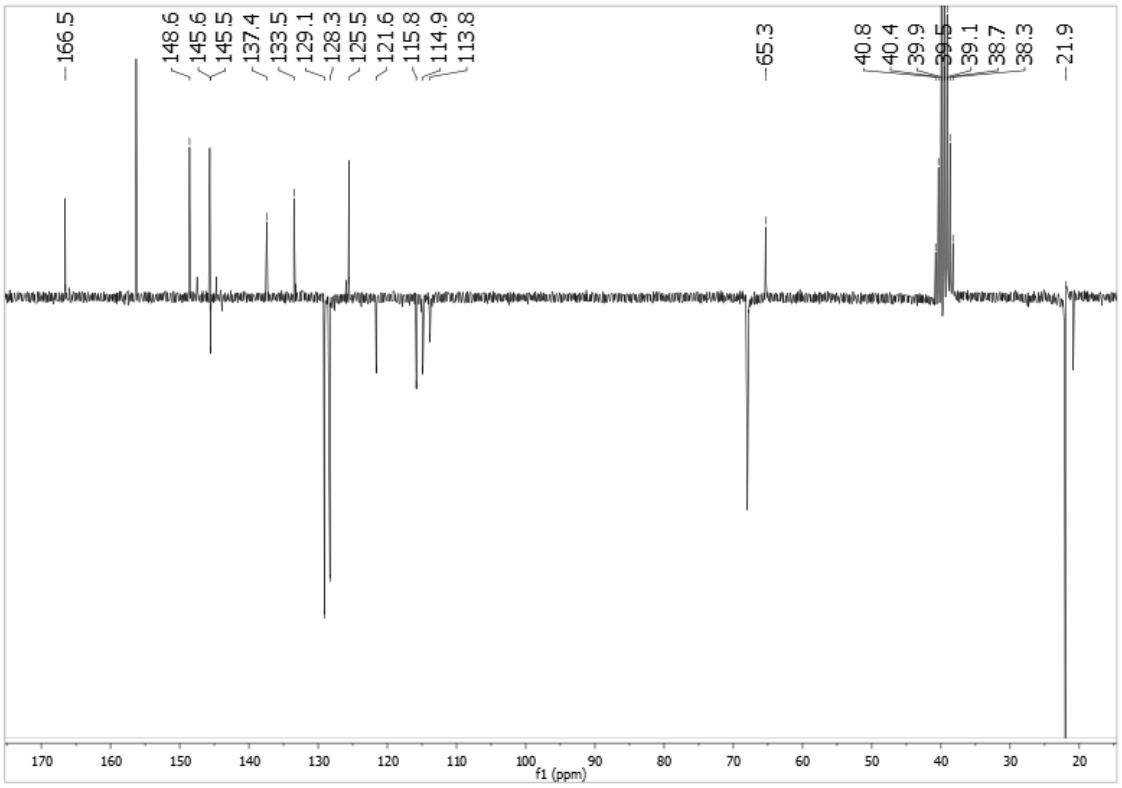


Figure 22. ^{13}C NMR spectrum of 4-methylbenzyl caffeate (DMSO-d_6, 50 MHz).

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