Supplementary data

Microwave-assisted synthesis, molecular docking and antiproliferative activity of (3/5-aryl-1,2,4-oxadiazole-5/3-yl)(3,4,5-trimethoxyphenyl)methanone oxime derivatives

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Contents:

Chemistry

Unless otherwise noted, all materials were obtained from commercially available sources and were used without purification. The reaction process was monitored by TLC with silica gel plates (thickness 250μm, Indicator F-254) under UV light. The products were purified using column chromatography (60 Å, 200-300 mesh, Qingdao Ocean Chemicals) or silica gel plates (0.25 mm layer, Qingdao Ocean Chemicals) with the designated solvents. The melting points were measured on a hot-stage microscope (X-4, Beijing Taike Ltd.) and are uncorrected. Mass spectra (MS) were measured on an Agilent 1100-sl mass spectrometer with an electrospray ionization source from Agilent Co. Ltd. High resolution accurate mass determinations (HRMS) for some target compounds were obtained on a Bruker Micromass Time of Flight mass spectrometer equipped with electrospray ionization (ESI). NMR spectra were obtained on a Bruker AVANCE 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) and Bruker AVANCE 600 (^{1}H, 600 MHz; ^{13}C, 150 MHz) in $CDCl_3$ or DMSO-d_6 (internal standard tetramethylsilane).

General synthetic procedures for amidoxime (18a–h)

The stirring solution of appropriate benzonitrile (3.6 mmol) and hydroxylamine hydrochloride (25.2 mmol) in water was alkalized with a 1 M NaOH solution to pH 10. The mixture was stirred at 30 ℃ for 1 hour, then under reflux for another 2 hours to full conversion and checked by TLC. After the reaction was completed, the mixture was cooled to RT. The precipitate was filtered and washed with water, which was used for the next step without further purification.

General synthetic procedures for (3-aryl-1,2,4-oxadiazole-5-yl)(3,4,5-trimethoxyphenyl)methanol (20a–h)

The 3,4,5-trimethoxymandelic acid **19** (0.94 mmol), appropriate amidoxime (0.94 mmol), HOBt (1.04 mmol) and EDC·HCl (0.94 mmol) in dry acetonitrile (0.5 mL) were stirred together for 30 minutes in a microwave cavity (closed vessel mode). After adding the dry toluene (3 mL), microwave irradiation at 150 W was used and the mixture was held at 120 ℃ for 30 minutes. After the reaction was completed, the mixture was poured into water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined organic layers were washed with water, dried over Na_2SO_4, and the solvent was then evaporated under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc = 2:1 as eluent) on silica gel to afford pure product.

(3-phenyl-1,2,4-oxadiazole-5-yl)(3,4,5-trimethoxyphenyl)methanol (20a)

Colorless oil (210 mg, 56%); ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.07 (d, J=6.2 Hz, 2H, Ar-H), 7.48 (d, J=7.1 Hz, 3H, Ar-H), 6.75 (s, 2H, Ar-H), 6.00 (s, 1H, CH), 3.89 (s, 6H, OCH_3), 3.82 (s, 3H, OCH_3); MS (ESI) *m/z*: 343.1 $[M+H]^+$, 365.1 $[M+Na]^+$, 381.1 $[M+Na]^+$.

[3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (20b)

White solid (194 mg, 63%); mp: 120–122 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.92 (s, 2H, Ar-H), 7.25 (s, 2H, Ar-H), 6.74 (s, 2H, Ar-H), 6.00 (s, 1H, CH), 3.81 (s, 9H, OCH_3), 2.38 (s, 3H, CH_3); MS (ESI) *m/z*: 357.0 $[M+H]^+$, 379.0 $[M+Na]^+$, 355.0 $[M–H]^–$.

[3-(4-methoxyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (20c)

White solid (224 mg, 67%); mp: 100–101 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.00 (d, J=8.9 Hz, 2H, Ar-H), 6.97 (d, J=8.9 Hz, 2H, Ar-H), 6.75 (s, 2H, Ar-H), 6.00 (s, 1H, CH), 3.86 (s, 9H, OCH_3), 3.82 (s, 3H, OCH_3); MS (ESI) *m/z*: 374.1 $[M+H]^+$, 396.1 $[M+Na]^+$, 372.0 $[M–H]^–$.

[3-(2-chloropyridin-4-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (20f)

White solid (168 mg, 41%); mp: 90–92 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.52 (d, J=5.1 Hz, 1H, Ar-H), 8.00 (s, 1H, Ar-H), 7.87 (d, J=3.9 Hz, 1H, Ar-H), 6.74 (s, 2H, Ar-H), 6.05 (s, 1H, Ar-H), 3.88 (s, 6H, OCH_3), 3.83 (s, 3H, OCH_3); MS (ESI) *m/z*: 378.0 $[M+H]^+$.

[3-(thiophen-2-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (20g)

White solid (185 mg, 54%); mp: 79–80 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.79 (dd, J_1=1.1 Hz, J_2=3.7 Hz, 1H, C_4H_3S), 7.50 (dd, J_1=1.1 Hz, J_2=5.0 Hz, 1H, C_4H_3S), 7.13 (dd, J_1=3.7 Hz, J_2=5.0 Hz, 1H, C_4H_3S), 6.75 (s, 2H, Ar-H), 6.02 (s, 1H, CH), 3.86 (s, 6H, OCH_3), 3.83 (s, 3H, OCH_3); MS (ESI) *m/z*: 370.9 $[M+Na]^+$, 386.9 $[M+K]^+$.

General synthetic procedures for 3-aryl-5-benzoyl-1,2,4-oxadiazole (11a–h)

To a solution of compound **20a–h** (1 mmol) in CH_2Cl_2 (5 mL) was added PCC (2 mmol), then the mixture was stirred for 2-3 hours. When the reaction was completed (TLC control), the mixture was poured into water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined organic layers were washed with water, dried over Na_2SO_4, and the solvent was then evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2 as eluent) on silica gel to afford pure product.

3-phenyl-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11a)

Yellow solid (197 mg, 95%); mp: 133–135 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.22 (d, *J*=5.8 Hz, 2H, Ar-H), 7.85 (s, 2H, Ar-H), 7.55 (d, *J*=6.8 Hz, 3H, Ar-H), 4.02 (s, 3H, O$\underline{CH_3}$), 3.96 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ=175.7, 169.2, 167.8, 152.1 (×2), 143.8, 130.8, 128.1 (×2), 127.8, 126.6 (×2), 125.0, 107.5 (×2), 60.2, 55.4 (×2); MS (ESI) *m/z*: 341.1 $[M+H]^+$, 363.1 $[M+Na]^+$, 379.0 $[M+K]^+$.

3-(4-methyphenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11b)

Yellow solid (178 mg, 93%); mp: 138–139 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.06 (d, *J*=8.2 Hz, 2H, Ar-H), 7.83 (s, 2H, Ar-H), 7.34 (d, *J*=7.9 Hz, 2H, Ar-H), 4.01 (s, 3H, O$\underline{CH_3}$), 3.97 (s, 6H, O$\underline{CH_3}$), 2.44 (s, 3H, $\underline{CH_3}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ=175.8, 169.0, 167.7, 152.1 (×2), 143.7, 141.3, 128.7 (×2), 127.8, 126.4 (×2), 122.1, 107.5 (×2), 60.1, 55.3 (×2), 20.6; MS (ESI) *m/z*: 355.0 $[M+H]^+$, 377.0 $[M+Na]^+$.

3-(4-methoxyphenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11c)

Yellow solid (201 mg, 92%); mp: 152–154 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.09 (d, *J*=8.5 Hz, 2H, Ar-H), 7.82 (s, 2H, Ar-H), 6.99 (d, *J*=8.5 Hz, 2H, Ar-H), 4.01 (s, 3H, O$\underline{CH_3}$), 3.96 (s, 6H, O$\underline{CH_3}$), 3.88 (s, 3H, O$\underline{CH_3}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ=175.8, 169.0, 167.5, 161.4, 152.1 (×2), 143.7, 128.2 (×2), 127.8, 117.4, 113.5 (×2), 107.5 (×2), 60.1, 55.3 (×2), 54.4; MS (ESI) *m/z*: 371.0 $[M+H]^+$, 393.1 $[M+Na]^+$.

3-(4-bromophenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11d)

Yellow solid (126 mg, 89%); mp: 155–157 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.00 (d, *J*=8.3 Hz, 2H, Ar-H), 7.78 (s, 2H, Ar-H), 6.66 (d, *J*=8.3 Hz, 2H, Ar-H), 4.02 (s, 3H, O$\underline{CH_3}$), 3.95 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.4, 170.4, 168.1, 153.1 (×2), 144.9, 132.4 (×2), 129.0 (×2), 128.6, 126.5, 124.9, 108.5 (×2), 61.1, 56.4 (×2); MS (ESI) *m/z*: 419.1 $[M+H]^+$, 441.0 $[M+Na]^+$, 457.0 $[M+K]^+$.

3-(2-fluorophenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11e)

Yellow solid (144 mg, 87%); mp: 153–155 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.13 (m, 1H, Ar-H), 7.85 (s, 2H, Ar-H), 7.57 (m, 1H, Ar-H), 7.31 (m, 2H, Ar-H), 4.02 (s, 3H, O$\underline{CH_3}$), 3.97 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.4, 169.8, 165.7, 162.1-159.5, 153.1 (×2), 144.8, 133.5-133.4, 130.5, 128.7, 124.7-124.6, 117.1-116.9, 114.4-114.3, 108.5 (×2), 61.1, 56.3 (×2); MS (ESI) *m/z*: 359.1 $[M+H]^+$, 381.1 $[M+Na]^+$, 397.1 $[M+K]^+$.

3-(2-chloropyridin-4-yl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11f)

Yellow solid (145 mg, 84%); mp: 129–130 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.61 (d, J=5.1 Hz, 1H, Ar-H), 8.07 (s, 1H, Ar-H), 7.96 (dd, J_1=1.3 Hz, J_2=5.1 Hz,1H, Ar-H), 7.75 (s, 2H, Ar-H), 4.03 (s, 3H, O$\underline{CH_3}$), 3.97 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ=175.0, 170.0, 165.3, 152.2 (×2), 151.8, 149.9, 144.2, 135.2, 127.3, 121.1, 118.9, 107.5 (×2), 60.1, 55.4 (×2); MS (ESI) *m/z*: 376.0 $[M+H]^+$, 397.9 $[M+Na]^+$.

3-(thiophen-2-yl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11g)

Yellow solid (152 mg, 88%); mp: 143–145 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.89 (dd, J_1=1.1 Hz, J_2=3.7 Hz, 1H, C_4H_3S), 7.80 (s, 2H, Ar-H), 7.57 (dd, J_1=1.1 Hz, J_2=5.0 Hz, 1H, C_4H_3S), 7.20 (m, J_1=3.7 Hz, J_2=5.0 Hz,1H, C_4H_3S), 4.01 (s, 3H, O$\underline{CH_3}$) , 3.96 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (150 MHz, $CDCl_3$): δ=175.4, 168.9, 163.8, 152.1 (×2), 143.8, 129.2 (×2), 127.6, 127.3, 126.4, 107.5 (×2), 60.1, 55.3 (×2); MS (ESI) *m/z*: 346.9 $[M+H]^+$, 368.9 $[M+Na]^+$.

3-(thiophen-3-yl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (11h)

Yellow solid (166 mg, 85%); mp: 127–129 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.13 (d, J=1.4 Hz, 1H, C_4H_3S), 7.78 (s, 2H, Ar-H), 7.66 (d, J=4.8 Hz, 1H, C_4H_3S), 7.47 (t, J_1=3.0 Hz, J_2=7.2 Hz, 1H, C_4H_3S), 4.00 (s, 3H, O$\underline{CH_3}$), 3.94 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=176.4, 169.9, 165.1, 153.0 (×2), 144.7, 128.7, 128.4, 127.6, 127.5, 126.0, 108.5 (×2), 61.1, 56.3 (×2); MS (ESI) *m/z*: 347.1 $[M+H]^+$, 369.1 $[M+Na]^+$, 385.1 $[M+K]^+$.

General synthetic procedures for compounds (12a–h)

An excess of hydroxylamine hydrochloride was added to a solution of compound **11a–h** (1 mmol) and sodium acetate (10 mmol) in absolute ethanol (5 mL). The mixture was heated at 80 ℃ for 5 hours. When the reaction was completed (TLC control), the mixture was poured into water (20 mL) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with water, dried over Na_2SO_4, and the solvent was then evaporated under reduced pressure. The residue was purified by column chromatography (methanol: CH_2Cl_2 = 1:50 as eluent) on silica gel to afford pure product.

(*Z*+*E*)-(3-phenyl-1,2,4-oxadiazole-5-yl)(3,4,5-trimethoxyphenyl)methanone oxime (12a)

White solid (58 mg, 81%) as a 2:1 mixture of *Z* and *E* isomers; mp: 147–148 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=10.50 (s, 1H, N-OH), 8.17 (dd, J_1=1.6 Hz, J_2=8.4 Hz, 2H, Ar-H), 7.54 (m, 3H, Ar-H), 6.88 (s, 2H, Ar-H), 3.90 (s, 3H, O$\underline{CH_3}$), 3.84 (s, 6H, O$\underline{CH_3}$); (*E* isomer) δ=9.57 (s, 1H, N-OH), 8.12 (dd, J_1=1.4 Hz, J_2=8.4 Hz, 2H, Ar-H), 7.50 (m, 3H, Ar-H), 6.95 (s, 2H, Ar-H), 3.95 (s, 3H, O$\underline{CH_3}$),

3.89 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6): (*Z* isomer) δ=169.8, 168.2, 153.6 (×2), 142.7, 139.9, 132.3, 129.8 (×2), 127.2 (×2), 127.5, 126.2, 104.4 (×2), 60.6, 56.5 (×2); (*E* isomer) δ=173.4, 168.4, 152.9 (×2), 144.0, 139.0, 132.1, 129.7 (×2), 127.8 (×2), 126.5, 124.8, 107.7 (×2), 60.5, 56.5 (×2); MS (ESI) *m/z*: 356.1 $[M+H]^+$, 733.2 $[2M+Na]^+$.

(*Z*+*E*)-[3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (12b)

White solid (65 mg, 86%) as a 3:2 mixture of *Z* and *E* isomers; mp: 131–133 ℃; ^{1}H NMR (300 MHz, DMSO-d_6): (*Z* isomer) δ=13.04 (s, 1H, N-OH), 8.00 (d, *J*=8.0 Hz, 2H, Ar-H), 7.43 (d, *J*=7.9 Hz, 3H, Ar-H), 6.85 (s, 2H, Ar-H), 3.77 (s, 6H, O$\underline{CH_3}$), 3.71 (s, 3H, O$\underline{CH_3}$), 2.40 (s, 3H, $\underline{CH_3}$); (*E* isomer) δ=12.91 (s, 1H, N-OH), 7.89 (d, *J*=8.0 Hz, 2H, Ar-H), 7.36 (d, *J*=7.9 Hz, 3H, Ar-H), 6.98 (s, 2H, Ar-H), 3.78 (s, 6H, O$\underline{CH_3}$), 3.75 (s, 3H, O$\underline{CH_3}$), 2.38 (s, 3H, $\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6): (*Z* isomer) δ=169.6, 168.1 153.6 (×2), 142.7, 142.4, 139.9, 130.4 (×2), 127.6 (×2), 127.8, 123.4, 104.3 (×2), 60.6, 56.4 (×2), 21.6; (*E* isomer) δ=173.2, 168.3, 152.9 (×2), 144.0, 142.1, 139.0, 130.3 (×2), 127.5 (×2), 124.9, 123.7, 107.7 (×2), 60.5, 56.5 (×2), 21.5; MS (ESI) *m/z*: 370.1 $[M+H]^+$, 392.0 $[M+Na]^+$.

(*Z*+*E*)-[3-(4-methoxyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (12c)

White solid (74 mg, 83%) as a 3:1 mixture of *Z* and *E* isomers; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=10.63 (s, 1H, N-OH), 8.10 (d, *J*=8.7 Hz, 2H, Ar-H), 7.04 (d, *J*=8.8 Hz, 2H, Ar-H), 6.91 (s, 2H, Ar-H), 3.90 (s, 3H, O$\underline{CH_3}$), 3.89 (s, 3H, O$\underline{CH_3}$), 3.87 (s, 6H, O$\underline{CH_3}$); (*E* isomer) δ=10.09 (s, 1H, N-OH), 8.05 (d, *J*=8.7 Hz, 2H, Ar-H), 6.98 (d, *J*=8.8 Hz, 2H, Ar-H), 6.94 (s, 2H, Ar-H), 3.94 (s, 3H, O$\underline{CH_3}$), 3.87 (s, 6H, O$\underline{CH_3}$), 3.87 (s, 3H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): (*Z* isomer) δ=168.1, 162.5, 153.4 (×2), 143.3, 140.3, 129.4 (×2), 126.9, 123.0, 118.0, 114.5 (×2), 104.9 (×2), 61.0, 56.3 (×2), 55.5; (*E* isomer) δ=167.6, 162.2, 153.0 (×2), 145.1, 139.8, 129.9 (×2), 126.0, 123.0, 116.7, 114.3 (×2), 107.2 (×2), 61.0, 56.3 (×2), 55.4; MS (ESI) *m/z*: 384.1 $[M-H]^-$.

(*Z*+*E*)-[3-(4-bromophenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (12d)

White solid (54 mg, 84%) as a 3:1 mixture of *Z* and *E* isomers; mp: 103–105 ℃; ^{1}H NMR (600 MHz, $CDCl_3$): (*Z* isomer) δ=10.71 (s, 1H, N-OH), 8.04 (d, *J*=8.5 Hz, 2H, Ar-H), 7.66 (d, *J*=8.5 Hz, 2H, Ar-H), 6.84 (s, 2H, Ar-H), 3.89 (s, 3H, O$\underline{CH_3}$), 3.84 (s, 6H, O$\underline{CH_3}$); (*E* isomer) δ=8.69 (s, 1H, N-OH), 7.98 (d, *J*=8.5 Hz, 2H, Ar-H), 7.60 (d, *J*=8.5 Hz, 2H, Ar-H), 6.91 (s, 2H, Ar-H), 3.94 (s, 3H, O$\underline{CH_3}$) , 3.87 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6): (*Z* isomer) δ=169.6, 167.2, 153.2 (×2), 142.2,

139.6, 132.6 (×2), 129.3 (×2), 127.4, 125.6, 125.1, 104.1 (×2), 60.3, 56.1 (×2); (*E* isomer) δ=173.2, 167.4, 152.6 (×2), 143.6, 138.7, 132.5 (×2), 129.1 (×2), 127.4, 125.4, 124.5, 107.4 (×2), 60.2, 56.2 (×2); MS (ESI) *m/z*: 434.1 $[M+H]^+$, 456.0 $[M+Na]^+$, 431.9 $[M-H]^-$; HRMS *m/z* $[M+H]^+$ calcd for $C_{18}H_{17}BrN_3O_5$: 434.0352, found: 434.0345.

(*Z*+*E*)-[3-(2-fluorophenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (12e)

White solid (69 mg, 82%) as a 4:1 mixture of *Z* and *E* isomers; mp: 149–151 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): (*Z* isomer) δ=13.36 (s, 1H, N-OH), 8.10 (td, J_1=1.4 Hz, J_2=7.6 Hz, 1H, Ar-H), 7.68 (m, 1H, Ar-H), 7.45 (m, 2H, Ar-H), 6.87 (s, 2H, Ar-H), 3.77 (s, 6H, O$\underline{CH_3}$), 3.71 (s, 3H, O$\underline{CH_3}$); (*E* isomer) δ=8.48 (s, 1H, N-OH), 7.98 (td, J_1=1.4 Hz, J_2=7.6 Hz, 1H, Ar-H), 7.67 (m, 1H, Ar-H), 7.63 (m, 1H, Ar-H), 7.00 (s, 2H, Ar-H), 3.78 (s, 6H, O$\underline{CH_3}$), 3.74 (s, 3H, O$\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6): (*Z* isomer) δ=169.4, 165.0 (d, *J*=5.3), 161.6, 159.1, 153.6 (×2), 134.3 (d, *J*=8.4), 131.2, 127.9, 125.7 (d, *J*=3.4), 117.3 (d, *J*=20.0), 114.4 (d, *J*=12.2), 104.3 (×2), 60.6, 56.4 (×2); (*E* isomer) δ=169.4, 165.2 (d, *J*=5.8), 161.6, 159.0, 152.9 (×2), 141.3 (d, *J*=469.5), 134.1 (d, *J*=8.7), 131.1, 125.6 (d, *J*=3.7), 124.9 (d, *J*=4.2), 117.3 (d, *J*=20.5), 114.6 (d, *J*=12.2), 107.8 (×2), 60.5, 56.5 (×2); MS (ESI) *m/z*: 374.2 $[M+H]^+$, 396.1 $[M+Na]^+$, 412.1 $[M+K]^+$ 371.9 $[M-H]^-$; HRMS *m/z* $[M+H]^+$ calcd for $C_{18}H_{17}FN_3O_5$: 374.1152, found: 374.1147.

(*Z*+*E*)-[3-(2-chloropyridin-4-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (12f)

White solid (44 mg, 86%) as a 3:1 mixture of *Z* and *E* isomers; mp: 213–214 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=9.61 (s, 1H, N-OH), 8.57 (s, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 6.89 (s, 2H, Ar-H), 3.95 (s, 3H, O$\underline{CH_3}$), 3.90 (s, 6H, O$\underline{CH_3}$); (*E* isomer) δ=8.97 (s, 1H, N-OH), 8.61 (s, 1H, Ar-H), 8.10 (s, 1H, Ar-H), 7.97 (s, 1H, Ar-H), 6.82 (s, 2H, Ar-H), 3.90 (s, 3H, O$\underline{CH_3}$), 3.86 (s, 6H, O$\underline{CH_3}$); MS (ESI) *m/z*: 391.0 $[M+H]^+$.

(*Z*+*E*)-[3-(thiophen-2-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (12g)

White solid (71 mg, 80%) as a 4:1 mixture of *Z* and *E* isomers; mp: 116–118 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=10.40 (s, 1H, N-OH), 7.91 (d, *J*=3.6 Hz,1H, Ar-H), 7.57 (d, *J*=5.0 Hz,1H, Ar-H), 7.20 (t, 1H, Ar-H), 6.86 (s, 2H, Ar-H), 3.89 (s, 3H, O$\underline{CH_3}$), 3.85 (s, 6H, O$\underline{CH_3}$); (*E* isomer) δ=9.34 (s, 1H, N-OH), 7.85 (dd, J_1=0.9 Hz, J_2=3.6 Hz, 1H, Ar-H), 7.53 (dd, J_1=0.9 Hz, J_2=5.0 Hz, 1H, Ar-H),

7.16 (dd, J_1=3.7 Hz, J_2=4.9 Hz, 1H, Ar-H), 6.93 (s, 2H, Ar-H), 3.94 (s, 3H, O$\underline{CH_3}$), 3.89 (s, 6H, O$\underline{CH_3}$); MS (ESI) *m/z*: 362.0 $[M+H]^+$, 745.1 $[2M+Na]^+$.

(*Z*+*E*)-[3-(thiophen-3-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (12h)

White solid (194 mg, 82%) as a 5:1 mixture of *Z* and *E* isomers; mp: 95–97 ℃; ^{1}H NMR (600 MHz, DMSO-d_6): (*Z* isomer) δ=12.89 (s, 1H, N-OH), 8.38 (d, *J*=2.58 Hz, 1H, Ar-H), 7.80 (q, 1H, Ar-H), 7.66 (d, *J*=4.92 Hz, 1H, Ar-H), 6.84 (s, 2H, Ar-H), 3.77 (s, 6H, O$\underline{CH_3}$), 3.72 (s, 3H, O$\underline{CH_3}$); (*E* isomer) δ=13.05 (s, 1H, N-OH), 8.27 (d, *J*=2.58 Hz, 1H, Ar-H), 7.74 (q, 1H, Ar-H), 7.59 (d, *J*=4.92 Hz, 1H, Ar-H), 6.98 (s, 2H, Ar-H), 3.78 (s, 6H, O$\underline{CH_3}$), 3.74 (s, 3H, O$\underline{CH_3}$); ^{13}C NMR (75 MHz, DMSO-d_6): (*Z* isomer) δ=173.1, 169.5, 165.0, 153.0 (×2), 144.0, 139.0, 129.5, 129.1, 127.7, 126.0, 107.7 (×2), 60.5, 56.5 (×2); (*E* isomer) δ=173.1, 169.5, 164.8, 153.6 (×2), 142.7, 139.9, 129.8, 129.3, 127.8, 126.2, 104.3 (×2), 60.6, 56.4 (×2); MS (ESI) *m/z*: 362.1 $[M+H]^+$, 384.1 $[M+Na]^+$, 359.8 $[M-H]^-$; HRMS *m/z* $[M+H]^+$ calcd for $C_{16}H_{16}N_3O_5S$: 362.0811, found: 362.0804.

(*Z*+*E*)-[3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone methoxime (13b)

To a solution of anhydrous K_2CO_3 (2 mmol) and dimethyl sulfate (1 mmol) in DMF (5 mL) was added dropwise the solution of compound **12b** (1 mmol) in DMF (0.5 mL) and the mixture was heated under stirring at 50 ℃ for 3h. The reaction was diluted with H_2O (100 mL) then extracted with EtOAc (3×100 mL) and the combined organic phases were washed with H_2O (3×100 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (*n*-hexane:EtOAc = 2:1 as eluent) on silica gel to afford pure product. White solid (94 mg, 85%) as a 3:1 mixture of *Z* and *E* isomers; mp: 306–308 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=8.00 (d, *J*=7.8 Hz, 2H, Ar-H), 7.29 (d, *J*=7.9 Hz, 2H, Ar-H), 6.84 (s, 2H, Ar-H), 4.19 (s, 3H, O$\underline{CH_3}$), 3.93 (s, 3H, O$\underline{CH_3}$), 3.87 (s, 6H, O$\underline{CH_3}$), 2.42 (s, 3H, $\underline{CH_3}$); (*E* isomer) δ=8.06 (d, *J*=8.1 Hz, 2H, Ar-H), 7.32 (d, *J*=8.1 Hz, 2H, Ar-H), 6.82 (s, 2H, Ar-H), 4.10 (s, 3H, O$\underline{CH_3}$), 3.89 (s, 3H, O$\underline{CH_3}$), 3.84 (s, 6H, O$\underline{CH_3}$), 2.43 (s, 3H, $\underline{CH_3}$); MS (ESI) *m/z*: 384.1 $[M+H]^+$, 406.0 $[M+Na]^+$.

(*Z*+*E*)-[3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone ethyloxime (14b)

To a solution of anhydrous K_2CO_3 (2 mmol) and dimethyl sulfate (1 mmol) in DMF (5 mL) was added dropwise the solution of compound **12b** (1 mmol) in DMF (0.5 mL) and the mixture was heated under

stirring at 50 ℃ for 3h. The reaction was diluted with H_2O (100 mL) then extracted with EtOAc (3 × 100 mL) and the combined organic phases were washed with H_2O (3 × 100 mL) and brine (50 mL), dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by column chromatography (*n*-hexane:EtOAc = 2:1 as eluent) on silica gel to afford pure product. White solid (85 mg, 74%) as a 3:2 mixture of *Z* and *E* isomers; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=8.01 (d, *J*=8.1 Hz, 2H, Ar-H), 7.29 (d, *J*=7.9 Hz, 2H, Ar-H), 6.90 (s, 2H, Ar-H), 4.47 (dd, J_1=7.1 Hz, J_2=14.2 Hz, 2H, O$\underline{CH_2}CH_3$), 3.93 (s, 3H, O$\underline{CH_3}$), 3.87 (s, 6H, O$\underline{CH_3}$), 2.41 (s, 3H, $\underline{CH_3}$), 1.40 (t, J_1=7.1 Hz, J_2=14.1 Hz, 3H, OCH$_2\underline{CH_3}$); (*E* isomer) δ=8.06 (d, *J*=8.1 Hz, 2H, Ar-H), 7.32 (d, *J*=8.1 Hz, 2H, Ar-H), 6.83 (s, 2H, Ar-H), 4.37 (dd, J_1=7.1 Hz, J_2=14.1 Hz, 2H, O$\underline{CH_2}CH_3$), 3.89 (s, 3H, O$\underline{CH_3}$), 3.84 (s, 6H, O$\underline{CH_3}$), 2.43 (s, 3H, $\underline{CH_3}$), 1.34 (t, J_1=7.1 Hz, J_2=14.2 Hz, 3H, OCH$_2\underline{CH_3}$); MS (ESI) *m/z*: 398.1 $[M+H]^+$, 420.1 $[M+Na]^+$.

2-hydroxy-2-(3,4,5-trimethoxyphenyl)acetonitrile (22)

Sodium cyanide (2.5 g, 51 mmol) was dissolved in water (100 mL). 3,4,5-Trimethoxylbenzaldehyde (10 g, 51 mmol) was added, and the mixture was stirred for 5 minutes at 0 ℃. Then, a solution of sodium disulfite (9.69 g, 51 mmol) in water (150 mL) was added, and the mixture stirred for 1 hour at RT. The white solid product was filtered off and used in the next step without purification.

2-[(tetrahydro-2H-pyran-2-yl)oxy]-2-(3,4,5-trimethoxyphenyl)acetonitrile (23)

In a round-bottomed flask maintained under N_2, appropriate amounts of compound **22** (4 mmol) and 3,4-dihydro-2*H*-pyran (10 mmol) as well as 1.5 mol% of *p*-TsOH·H_2O were dissolved in dry toluene (30 mL). The resulting mixture was heated to 55 ℃ for 2 hours. The solvent was removed under reduced pressure and the residue was extracted with EtOAc (20 mL × 3) and washed with water, dried over anhydrous sodium sulfate and concentrated under vacuum to afford crude product which was used for the next step without further purification.

N-hydroxy-2-[(tetrahydro-2H-pyran-2-yl)oxy]-2-(3,4,5-trimethoxyphenyl)acetimidamide (24)

To a solution of sodium (9.8 mmol) in absolute methanol (10 mL) was added hydroxylamine hydrochloride (9.8 mmmol) followed by compound **23** (6.5 mmol). After stirring for 5 hours, the mixture was poured into water (20 mL) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with water, dried over Na_2SO_4, and the solvent was then evaporated under reduced

pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc = 1:1 as eluent) on silica gel to afford pure product. Brown oil (3.64 g, 79%).

General synthetic procedure for compounds (26a–c)

Sodium metal (1.0 mmol) was added into absolute ethanol (6 mL) stirred under N_2. After 10 minutes at RT, corresponding methyl benzoate **23a–c** (1.0 mmol) and compound **24** were added to the mixture, which was then heated under reflux for 3 hours. When the reaction was completed (TLC control), the mixture was cooled to RT. The residue was dissolved in a mixture of 10 mL of water and HCl aq. (3 mL, 12 M) and stirred at RT for 1 h. When the reaction was completed (TLC control), the mixture was poured into water (20 mL) and extracted with EtOAc (20 mL × 3). The combined organic layers were washed with water, dried over Na_2SO_4, and the solvent was then evaporated under reduced pressure. The residue was purified by column chromatography (*n*-hexane:EtOAc = 1:1 as eluent) on silica gel to afford pure product.

[5-(4-methyphenyl)-1,2,4-oxadiazole-3-yl](3,4,5-trimethoxyphenyl)methanol (26b)

White solid (121 mg, 78%); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.95 (d, J=7.96 Hz, 2H, Ar-H), 7.26 (d, J=7.88 Hz, 2H, Ar-H), 5.95 (s, 1H, C<u>H</u>), 3.83 (s, 6H, OC<u>H</u>$_3$), 3.81 (s, 3H, OC<u>H</u>$_3$).

General synthetic procedures for 3-aryl-5-benzoyl-1,2,4-oxadiazole (15a–c)

To a solution of compound **24a–c** (1 mmol) in CH_2Cl_2 (5 mL) was added PCC (2 mmol), then the mixture was stirred for 2-3 hours. When the reaction was completed (TLC control), the mixture was poured into water (20 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined organic layers were washed with water, dried over Na_2SO_4, and the solvent was then evaporated under reduced pressure. The residue was purified by column chromatography (CH_2Cl_2 as eluent) on silica gel to afford pure product.

3-(3,4,5-trimethoxybenzoyl)-5-phenyl-1,2,4-oxadiazole (15a)

White solid (85 mg, 91%); mp: 107–109 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.23 (d, J=7.3 Hz, 2H, Ar-H), 7.67 (s, 2H, Ar-H), 7.62 (d, J=7.3 Hz, 1H, Ar-H), 7.56 (t, J_1=7.7 Hz, J_2=15.0 Hz, 2H, Ar-H), 3.98 (s, 3H, OC<u>H</u>$_3$), 3.94 (s, 6H, OC<u>H</u>$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=180.4, 175.4, 165.3, 152.0 (×2), 143.2, 132.4, 128.9, 128.2 (×2), 127.4 (×2), 122.3, 107.3 (×2), 60.0, 55.3 (×2); MS (ESI) *m/z*: 341.1 $[M+H]^+$, 363.1 $[M+Na]^+$.

3-(3,4,5-trimethoxybenzoyl)-5-(4-methyphenyl)-1,2,4-oxadiazole (15b)

White solid (74 mg, 89%); mp: 115–117 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.14 (d, J=8.2 Hz, 2H, Ar-H), 7.68 (s, 2H, Ar-H), 7.37 (d, J=8.0 Hz, 2H, Ar-H), 3.99 (s, 3H, O$\underline{CH_3}$), 3.96 (s, 6H, O$\underline{CH_3}$), 2.46 (s, 3H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=181.6, 176.7, 166.3, 153.7, 153.1 (×2), 144.5, 144.2, 130.1 (×2), 128.5 (×2), 120.7, 108.4 (×2), 61.1, 56.4 (×2), 21.9; (ESI) *m/z*: 355.1 $[M+H]^+$, 377.1 $[M+Na]^+$; HRMS *m/z* $[M+H]^+$ calcd for $C_{19}H_{19}N_2O_5$: 355.1294, found: 355.1290.

3-(3,4,5-trimethoxybenzoyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (15c)

White solid (81 mg, 91%); mp: 161–163 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.29 (dd, J_1=5.3 Hz, J_2=8.5 Hz, 1H, Ar-H), 8.19 (d, J=8.7 Hz, 1H, Ar-H), 7.68 (s, 2H, Ar-H), 7.28 (t, J_1=8.7 Hz, J_2=11.4 Hz, 1H, Ar-H), 7.04 (d, J=8.7 Hz, 1H, Ar-H), 4.01 (s, 3H, O$\underline{CH_3}$), 3.93 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=181.3, 175.5, 166.2, 165.9 (d, J=254.6), 153.1 (×2), 144.2, 131.0 (×2, d, J=9.5), 129.8, 119.7 (d, J=30.0), 116.7 (×2, d, J=22.3), 108.3 (×2), 61.0, 56.3 (×2); MS (ESI) *m/z*: 381.3 $[M+Na]^+$, 407.3 $[M+K]^+$.

General synthetic procedure for compounds (16a–c)

The synthetic procedures for compounds **16a–c** used the same method of that for compounds **12a–h**.

(*Z*)-(5-phenyl-1,2,4-oxadiazole-3-yl)(3,4,5-trimethoxyphenyl)methanone oxime (16a)

White solid (75 mg, 83%); mp: 147–149 ℃; ^{1}H NMR (300 MHz, DMSO-d_6): δ=11.31 (s, 1H, N-OH), 8.01 (d, J=7.4 Hz, 2H, Ar-H), 7.67 (t, J_1=7.4 Hz, J_2=14.7 Hz, 1H, Ar-H), 7.57 (t, J_1=7.5 Hz, J_2=15.2 Hz, 2H, Ar-H), 7.07 (s, 2H, Ar-H), 3.70 (s, 3H, O$\underline{CH_3}$), 3.68 (s, 6H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): δ=164.7, 152.8 (×2), 149.0, 147.7, 139.0, 132.3, 131.0, 128.1 (×2), 126.6 (×2), 119.2, 103.9 (×2), 59.9, 55.1 (×2); MS (ESI) *m/z*: 356.2 $[M+H]^+$, 378.2 $[M+Na]^+$, 425.4 $[M+K]^+$, 353.9 $[M–H]^–$.

(*Z*+*E*)-[5-(4-methyphenyl)-1,2,4-oxadiazole-3-yl](3,4,5-trimethoxyphenyl)methanone oxime (16b)

White solid (68 mg, 86%) as a 8:1 mixture of *Z* and *E* isomers; mp: 152–154 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=11.23 (s, 1H, N-OH), 7.91 (d, J=8.1 Hz, 2H, Ar-H), 7.38 (d, J=8.0 Hz, 2H, Ar-H), 7.08 (s, 2H, Ar-H), 3.70 (s, 3H, O$\underline{CH_3}$), 3.69 (s, 6H, O$\underline{CH_3}$), 2.40 (s, 3H, $\underline{CH_3}$); (*E* isomer) δ=12.48 (s, 1H, N-OH), 8.03 (d, J=8.1 Hz, 2H, Ar-H), 7.45 (d, J=8.0 Hz, 2H, Ar-H), 6.88 (s, 2H, Ar-H), 3.77 (s, 6H, O$\underline{CH_3}$), 3.73 (s, 3H, O$\underline{CH_3}$), 2.42 (s, 3H, $\underline{CH_3}$); ^{13}C NMR (100 MHz, $CDCl_3$): (*Z* isomer) δ=164.8, 152.7 (×2), 149.0, 147.8, 143.1, 138.8, 127.8 (×2), 128.1, 126.7 (×2), 119.3, 103.8 (×2), 59.9, 55.1 (×2), 20.6; MS (ESI) *m/z*: 370.3 $[M+H]^+$, 392.2 $[M+Na]^+$, 368.0 $[M–H]^–$.

(*Z*+*E*)-[5-(4-fluorophenyl)-1,2,4-oxadiazole-3-yl](3,4,5-trimethoxyphenyl)methanone oxime (16c)

White solid (81 mg, 85%) as a 4:1 mixture of *Z* and *E* isomers; mp: 134–136 ℃; ^{1}H NMR (300 MHz, $CDCl_3$): (*Z* isomer) δ=12.55 (s, 1H, N-OH), 8.13 (q, *J*=5.4 Hz, 2H, Ar-H), 7.39 (t, *J*=8.6 Hz, 2H, Ar-H), 7.12 (s, 2H, Ar-H), 3.70 (s, 9H, O$\underline{CH_3}$); (*E* isomer) δ=11.96 (s, 1H, N-OH), 8.20 (q, *J*=5.4 Hz, 2H, Ar-H), 7.47 (t, *J*=8.6 Hz, 2H, Ar-H), 6.89 (s, 2H, Ar-H), 3.77 (s, 9H, O$\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6): (*Z* isomer) δ=174.6, 166.3, 163.9, 153.7 (×2), 151.6, 139.8, 131.4 (×2, d, *J*=9.3), 129.6, 120.8, 116.2 (×2, d, *J*=21.9), 105.2 (×2), 60.5, 56.3 (×2); (*E* isomer) δ=167.7, 166.4, 152.9 (×2), 151.0, 145.2, 138.9, 131.3 (×2, d, *J*=9.4), 129.1, 120.5, 117.3 (×2, d, *J*=22.4), 107.5 (×2), 60.5, 56.5 (×2); MS (ESI) *m/z*: 374.2 $[M+H]^+$, 396.2 $[M+Na]^+$, 372.0 $[M–H]^–$; HRMS *m/z* $[M+H]^+$ calcd for $C_{18}H_{17}FN_3O_5$: 374.1152, found: 374.1146.

Biology

Cell culture: The human gastric adenocarcinoma cell line SGC-7901, the human fibrosarcoma cell line HT-1080 and the human pulmonary carcinoma cell line A-549 were cultured in RPMI-1640 medium containing 10% FBS, 100 U/mL streptomycin and 100 U/mL penicillin at 37 ℃ in a humidified atmosphere containing 5% CO_2. All cell lines were purchased from the American Type Culture Collection (ATCC, Manassas, VA).

MTT assay: The in vitro antiproliferative activities of CA-4 (**2**), Nocodazole (**4**) and all of the target compounds were determined with the MTT (Sigma) assay. Briefly, cells were seeded into 96-well plates at a density of 1-3 × 10^4/well (depending on the cell growth rate). Twenty-four hours later, triplicate wells were treated with media and the agents. After 72 h of incubation at 37 ℃ in 5% CO_2, the drug-containing medium was removed and replaced with 100 mL of fresh medium containing 5 mg/mL MTT solution. After 4 h of incubation, the medium with MTT was removed, and 100 mL of dimethyl sulphoxide (DMSO) was added to each well. The plates were gently agitated until the purple formazan crystals were dissolved, and the OD_{490} was determined using a microplate reader (MK3, Thermo, Germany). The data were calculated and plotted as the per cent viability compared to the control. The 50% inhibitory concentration (IC_{50}) was defined as the concentration that reduced the absorbance of the untreated wells by 50% of the vehicle in the MTT assay.

Immunofluorescence assay: Immunostaining was carried out to detect the microtubule-associated tubulin protein after exposure to CA-4 (**2**) and the investigated compound **16b**. A549 cells were seeded at 1 × 10^4 cells per well on a 24-well plate and grown for 24 h. The cells were treated with the vehicle

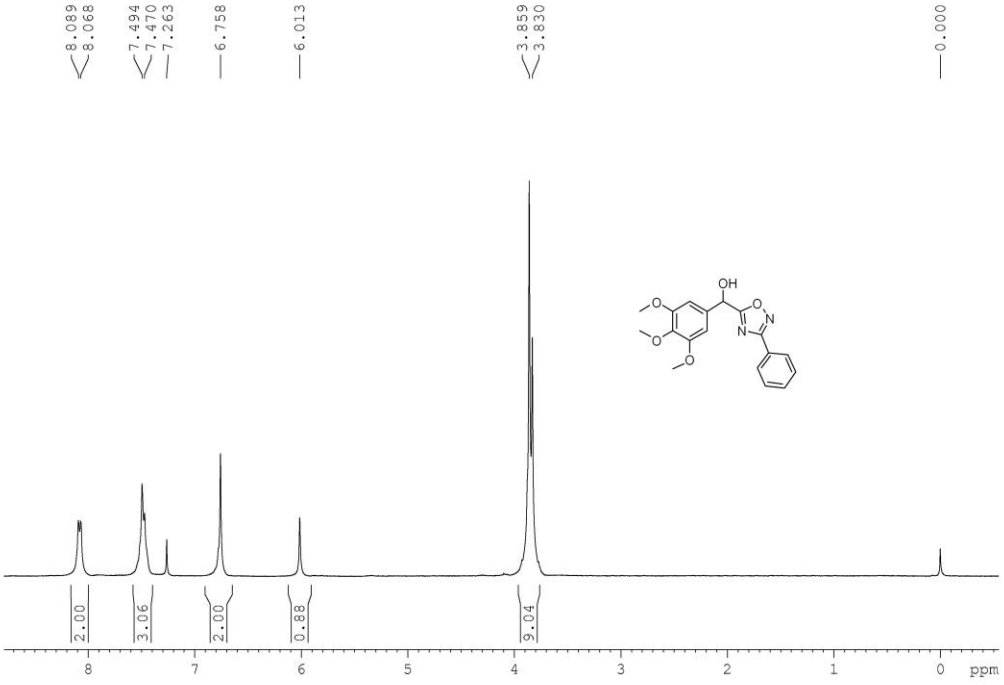
or twice the IC_{50} concentration of CA-4 (**2**) or **16b** for 12 h. The control and treated cells were fixed with 4% formaldehyde in PBS for 30 min at -20 ℃, washed twice with PBS and permeabilized with 0.1% (*v*/*v*) Triton X-100 in PBS for 5 min. The cells were then blocked with 5% bovine serum albumin (BSA) in PBS for 10 min. The primary α-tubulin antibody (Santa Cruz, CA) was diluted (1:100) with 2% BSA in PBS and incubated overnight at 4 ℃. The cells were washed with PBS to remove unbound primary antibody, and the cells were then incubated with FITC-conjugated antimouse secondary antibody and diluted (1:1000) with 2% BSA in PBS for 3 h at 37 ℃. The cells were washed with PBS to remove unbound secondary antibody, the nuclei were stained with 4,6-diamino-2-phenolindol dihydrochloride (DAPI) and immunofluorescence was then detected using a fluorescence microscope (Olympus, Tokyo, Japan).

Cell cycle analysis: A549 cells (8×10^4 cells) were incubated with various concentrations of CA-4 (**2**), **16b** or 0.05% DMSO for the indicated times. The cells were collected by centrifugation, washed with PBS and fixed in ice-cold 70% ethanol. The fixed cells were harvested by centrifugation and resuspended in 500 μl of PBS containing 1 mg/mL RNase. After 30 min of incubation at 37 ℃, the cells were stained with 50 mg/mL propidium iodide (PI) at 4 ℃ in the dark for 30 min. The samples were then analyzed by FACScan flow cytometry (Becton-Dickinson, Franklin Lakes, NJ, USA). The experiments were repeated at least three times.

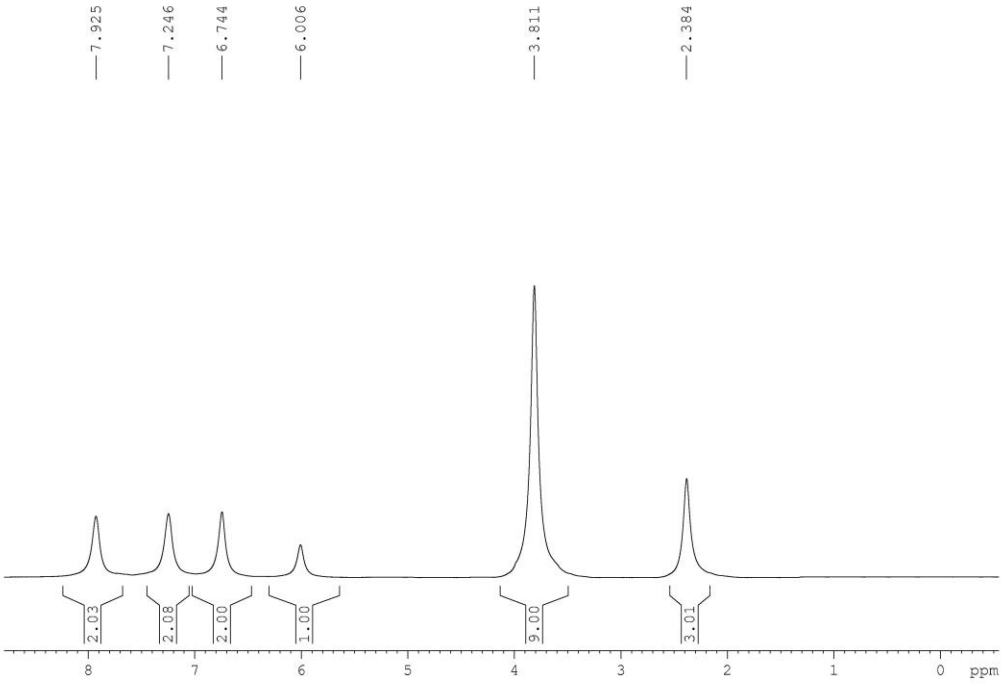
Molecular modelling

The molecular modelling studies were performed with Accelrys Discovery Studio 3.0. The crystal structures of tubulin complexed with DAMA-colchicine (PDB: 1SA0) and TN16 (PDB: 3HKD) were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb). In the docking process, the protein protocol was prepared via several operations, including the standardization of atom names, insertion of missing atoms in residues and removal of alternate conformations, insertion of missing loop regions based on SEQRES data, optimization of short and medium sized loop regions with the Looper Algorithm, minimization of remaining loop regions, calculation of pK, and protonation of the structure. The receptor model was then typed with the CHARMm force field, and a binding sphere with radius of 9.0 Å was defined with the original ligand (DAMA-colchicine or TN16) as the binding site. The **16b** was drawn with Chemdraw and fully minimized using the CHARMm force field. Finally, **16b** was docked into the binding site using the CDOCKER protocol with the default settings.

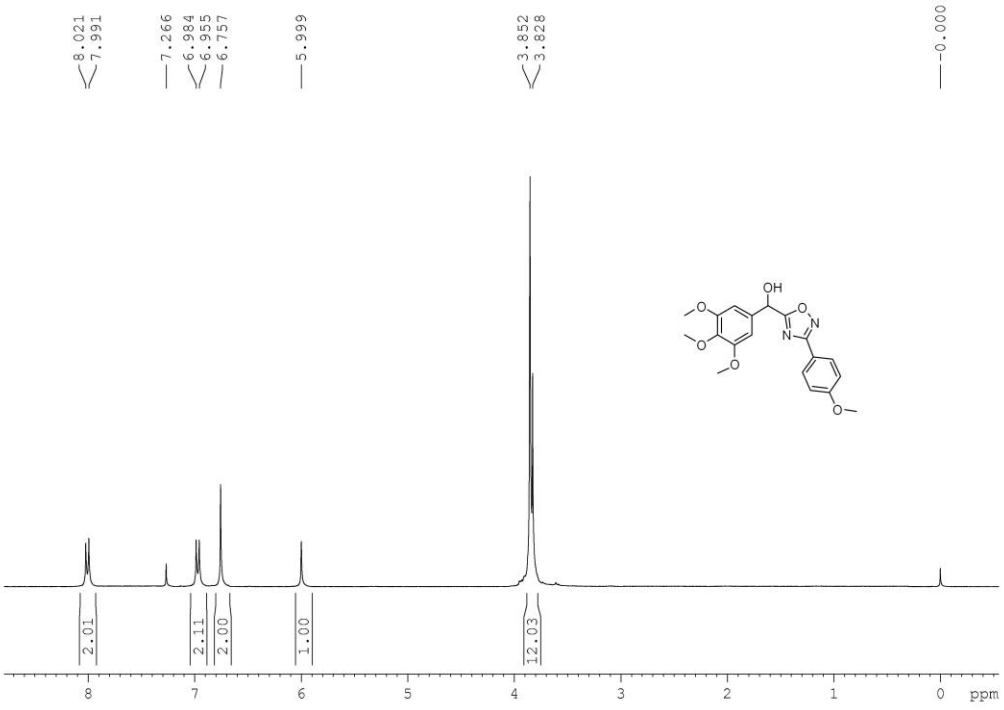
1. (3-phenyl-1,2,4-oxadiazole-5-yl)(3,4,5-trimethoxyphenyl)methanol (**20a**).



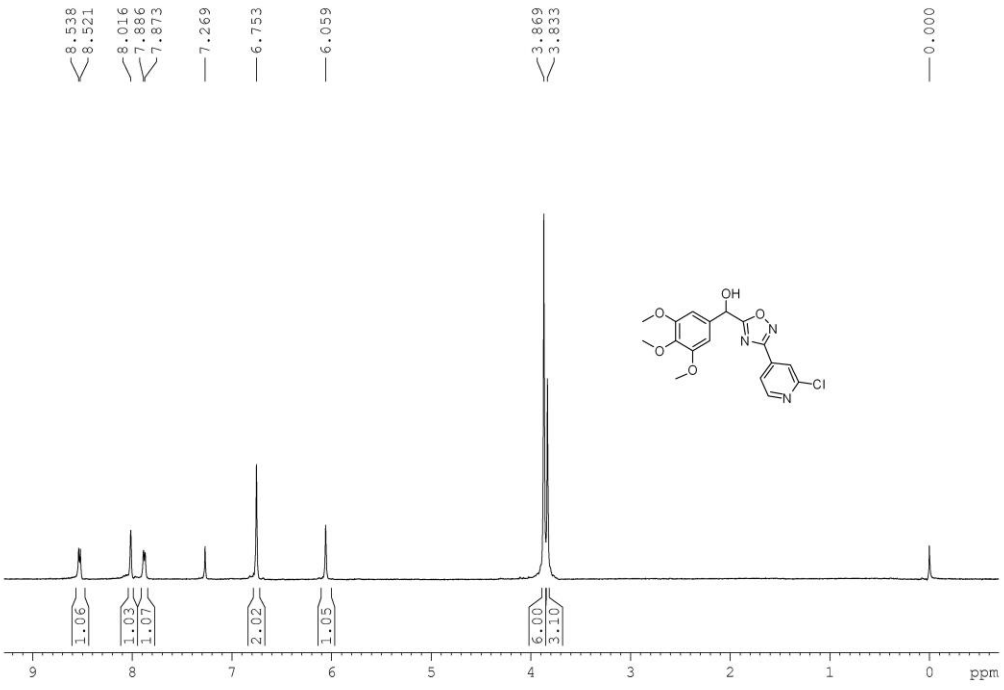
2. [3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (**20b**).



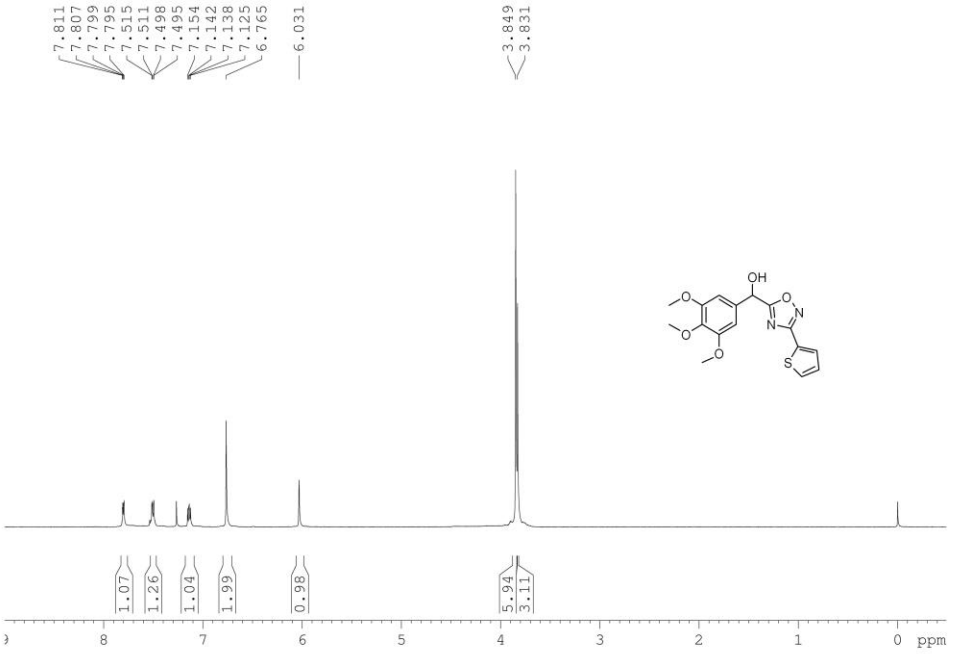
3. [3-(4-methoxyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (**20c**).



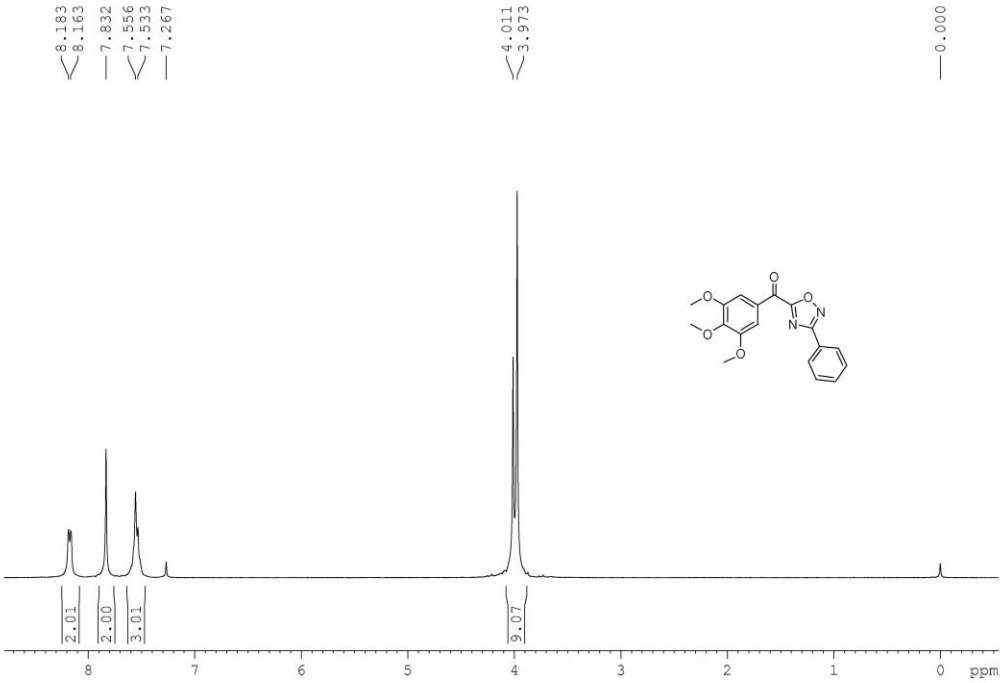
4. [3-(2-chloropyridin-4-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (**20f**).

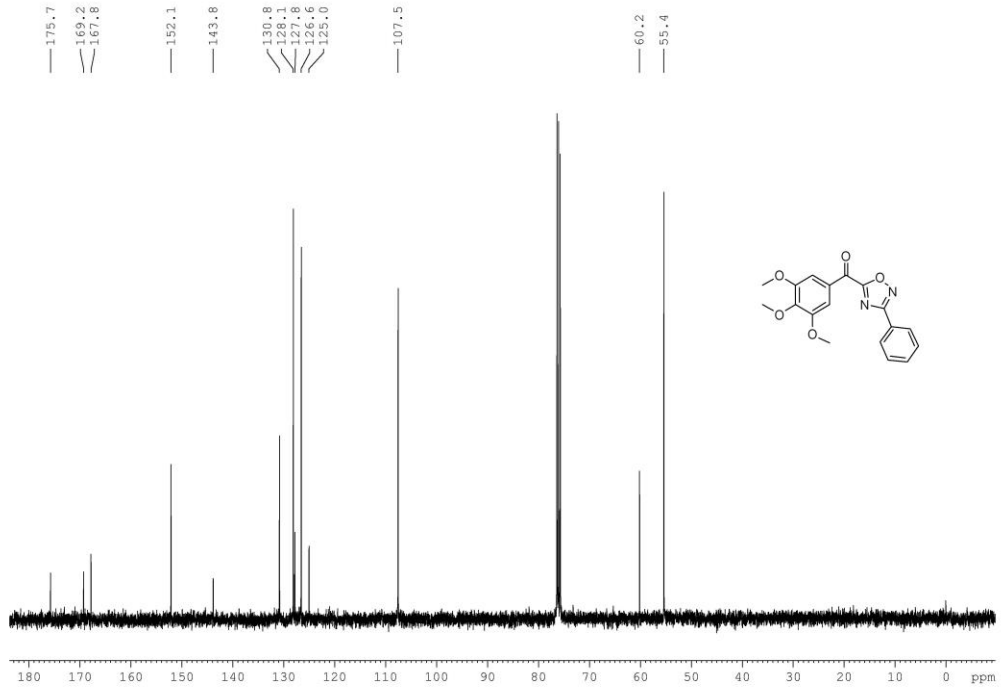


5. [3-(thiophen-2-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanol (**20g**).

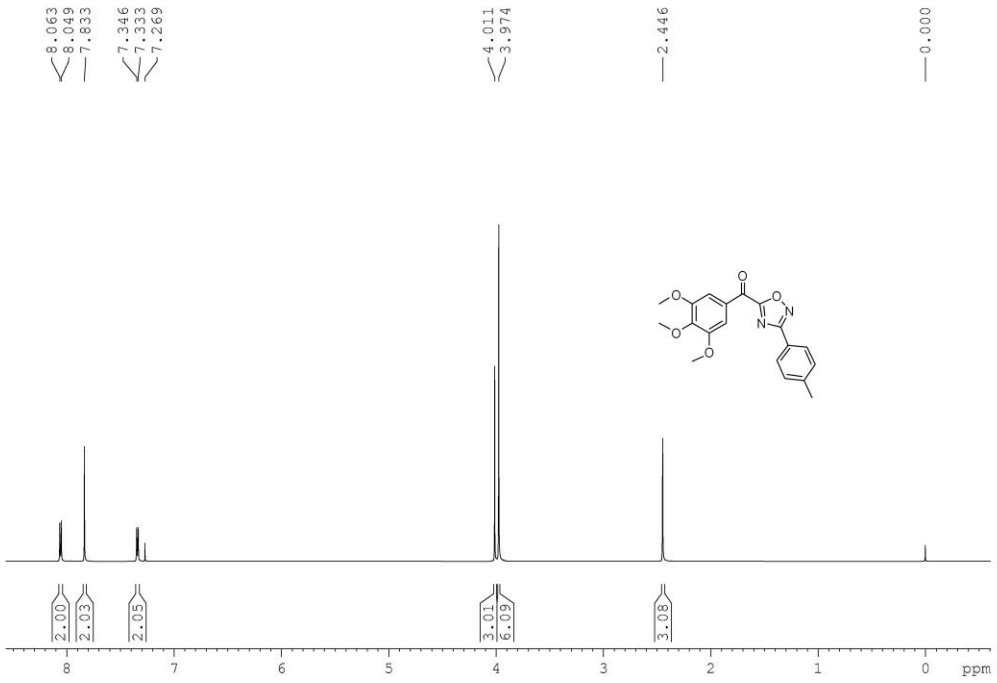


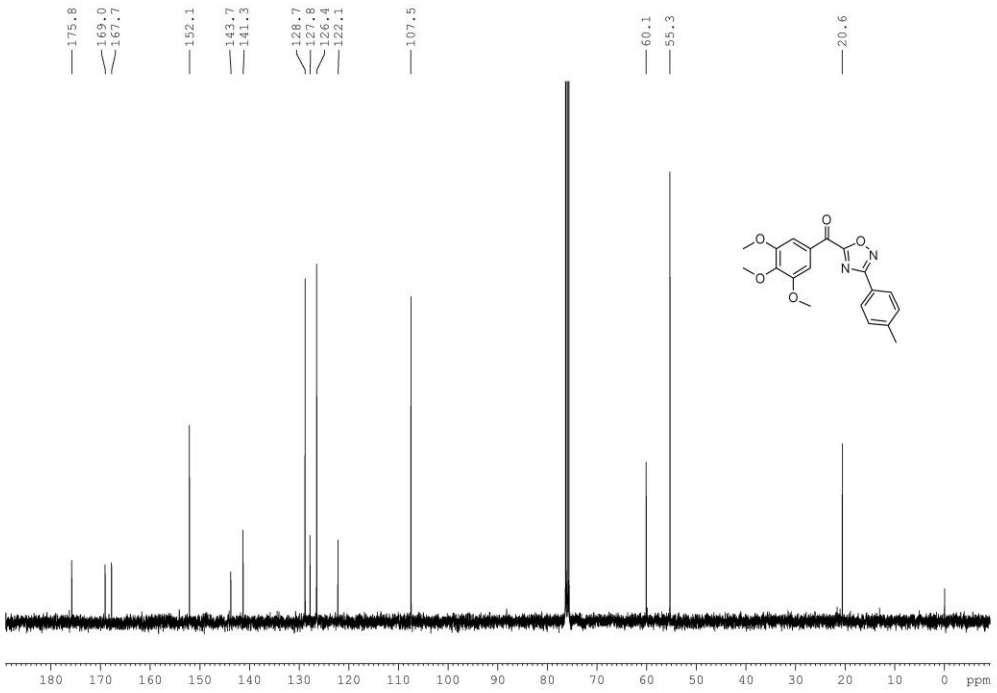
6. 3-phenyl-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11a**).



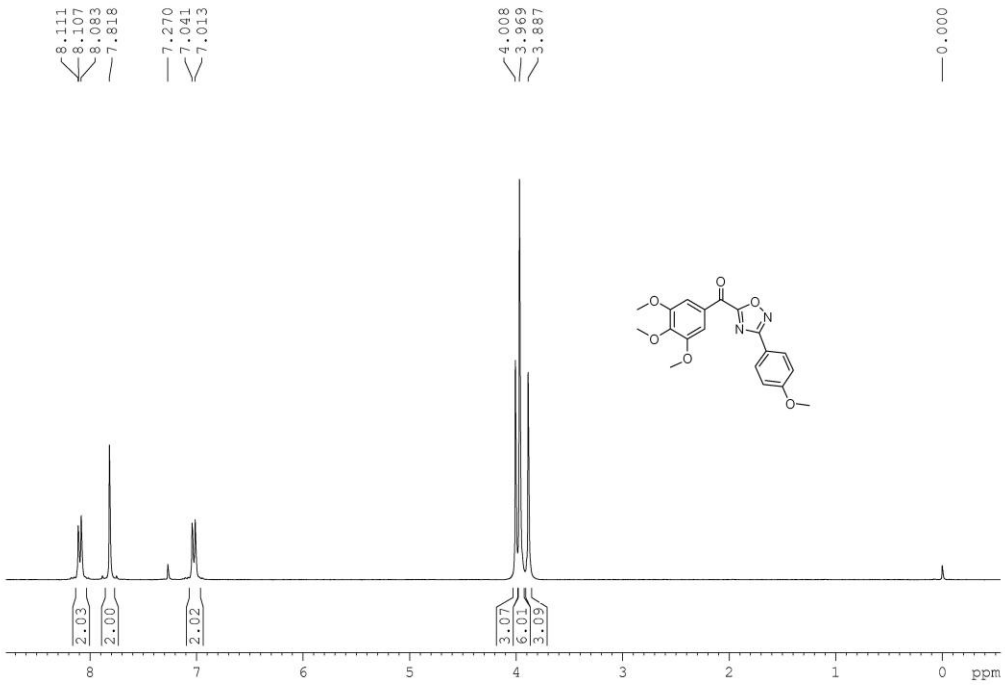


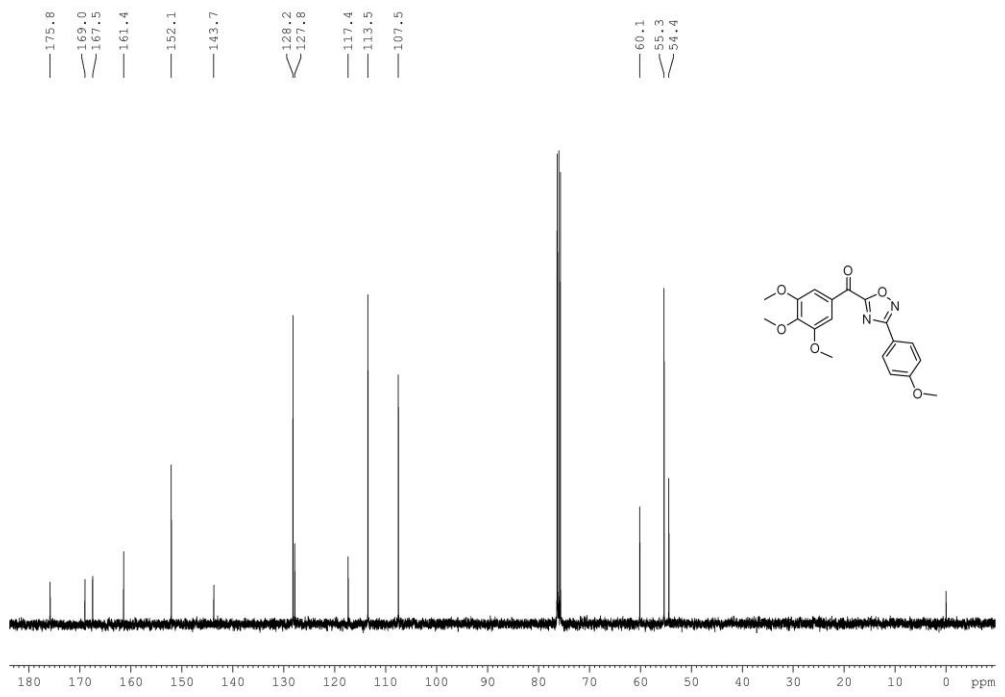
7. 3-(4-methyphenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11b**).



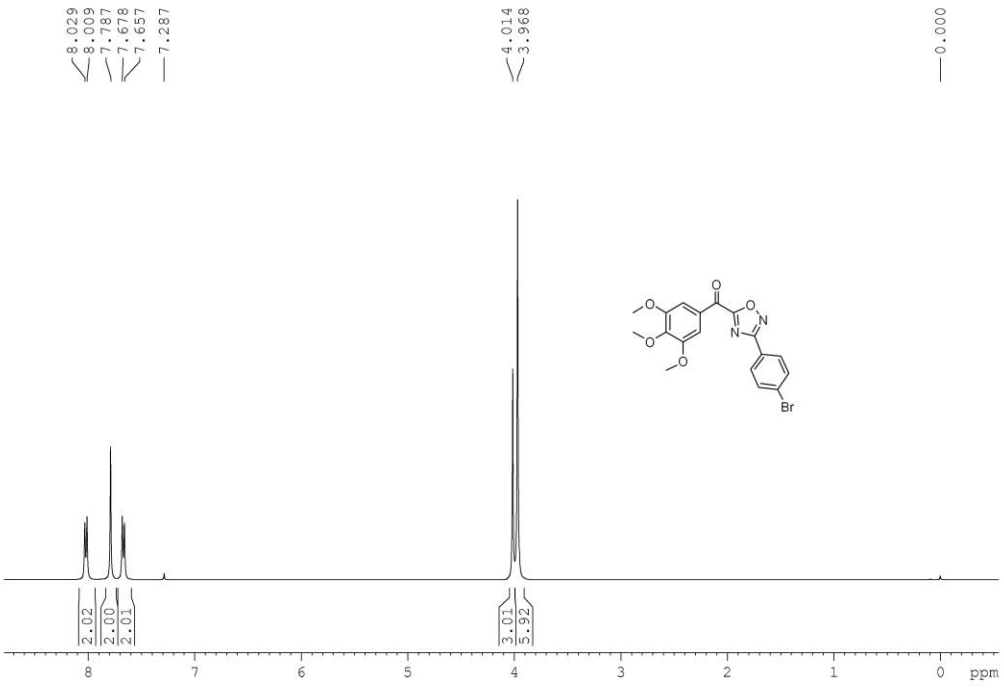


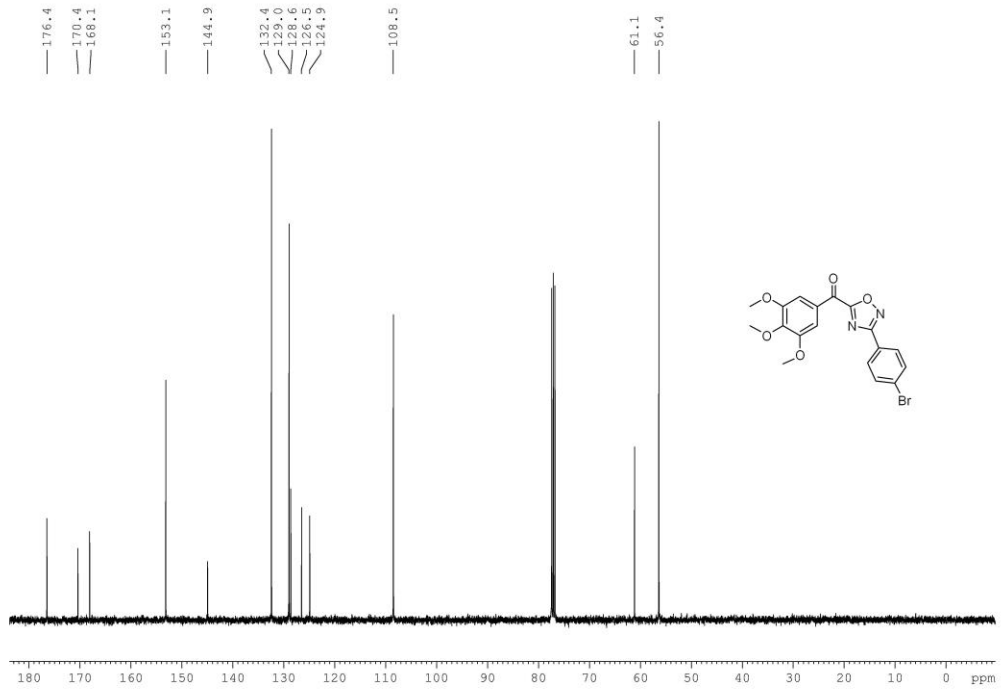
8. 3-(4-methoxyphenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11c**).



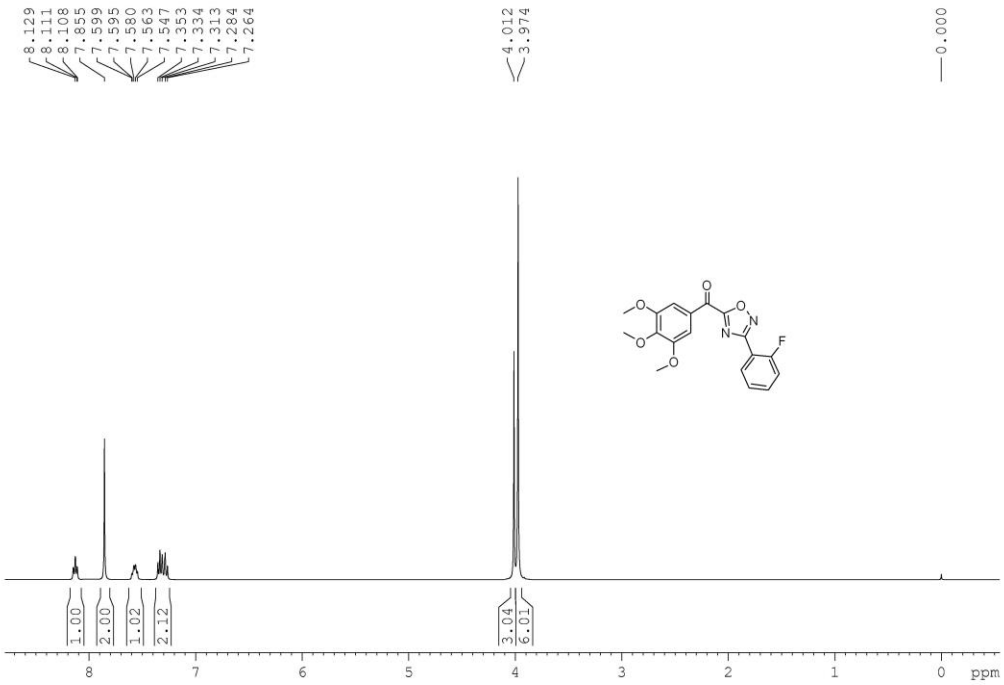


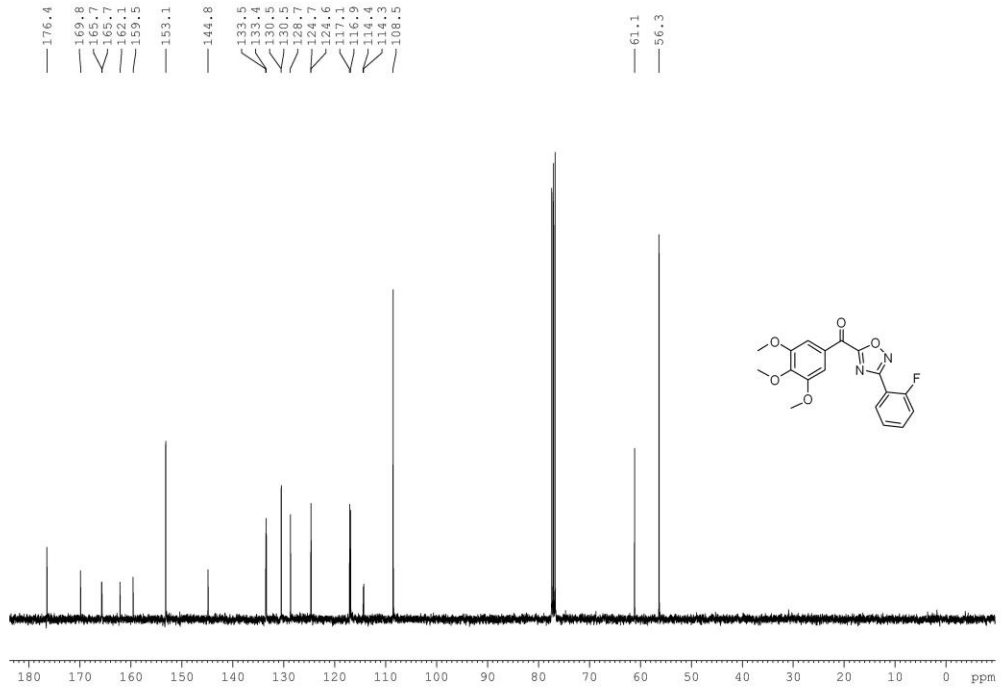
9. 3-(4-bromophenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11d**).



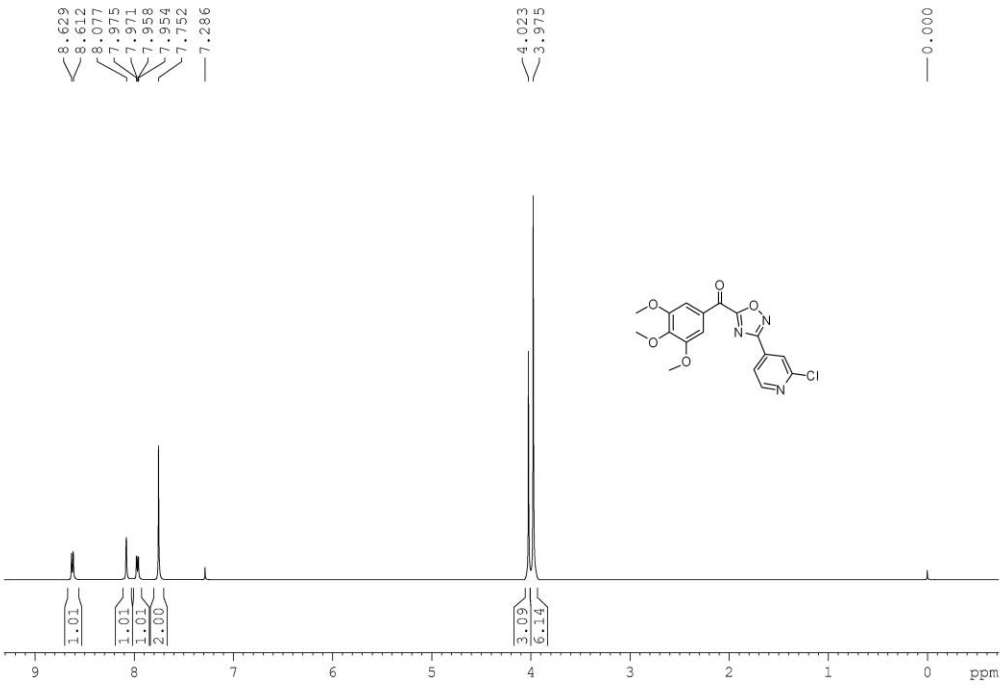


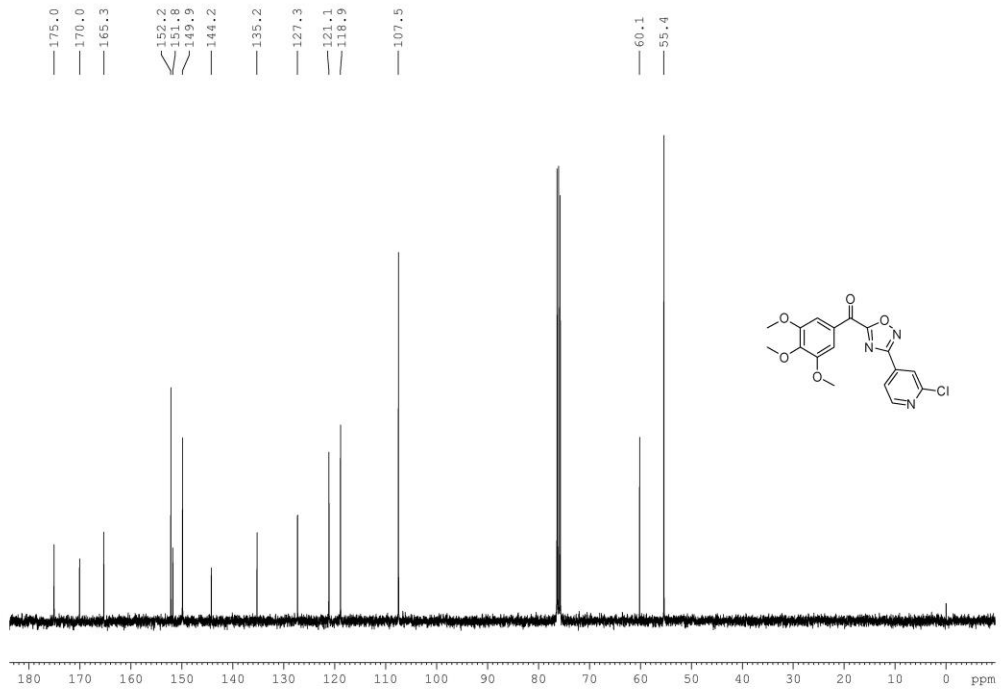
10. 3-(2-fluorophenyl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11e**).



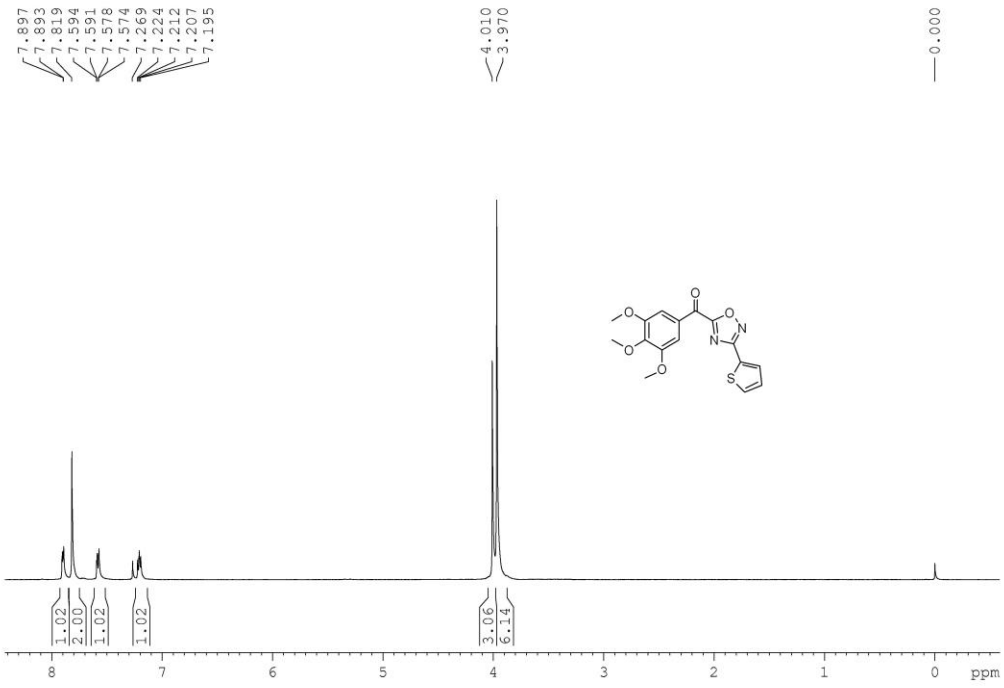


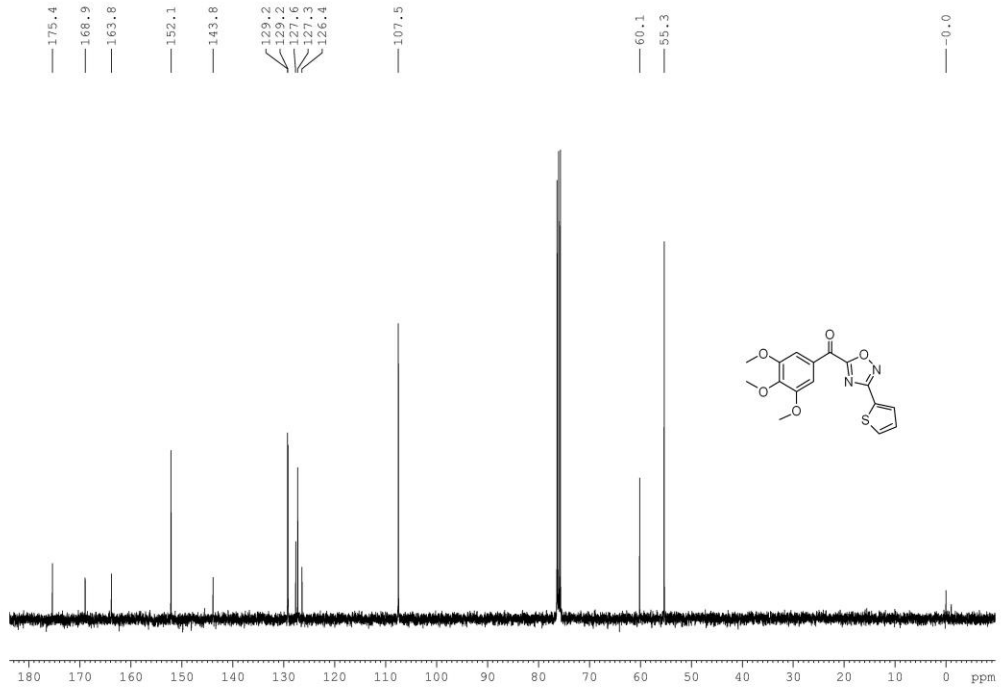
11. 3-(2-chloropyridin-4-yl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11f**).



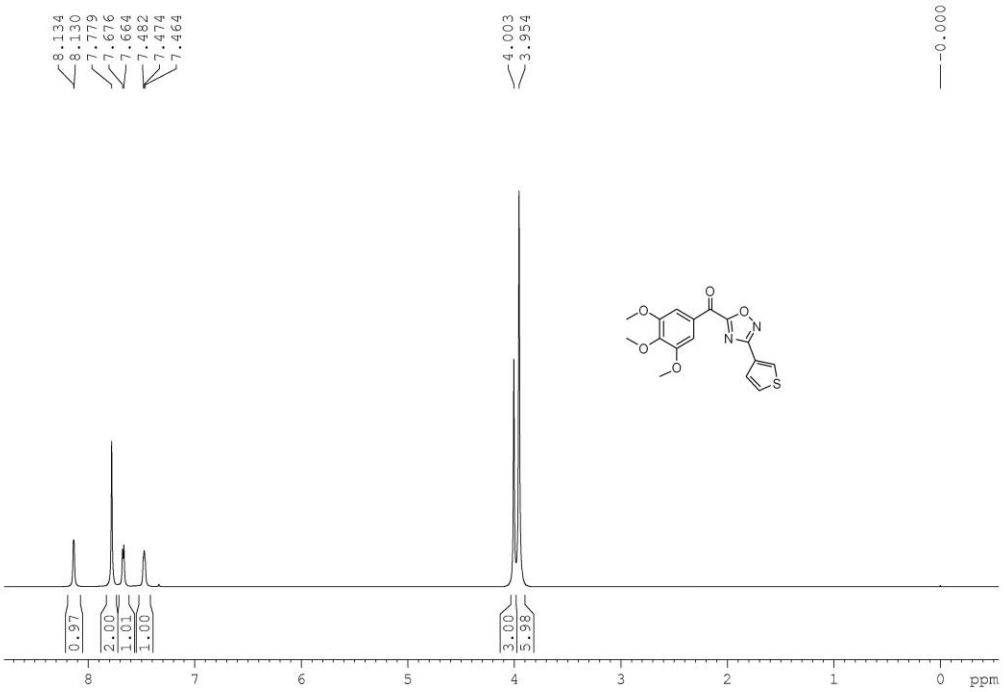


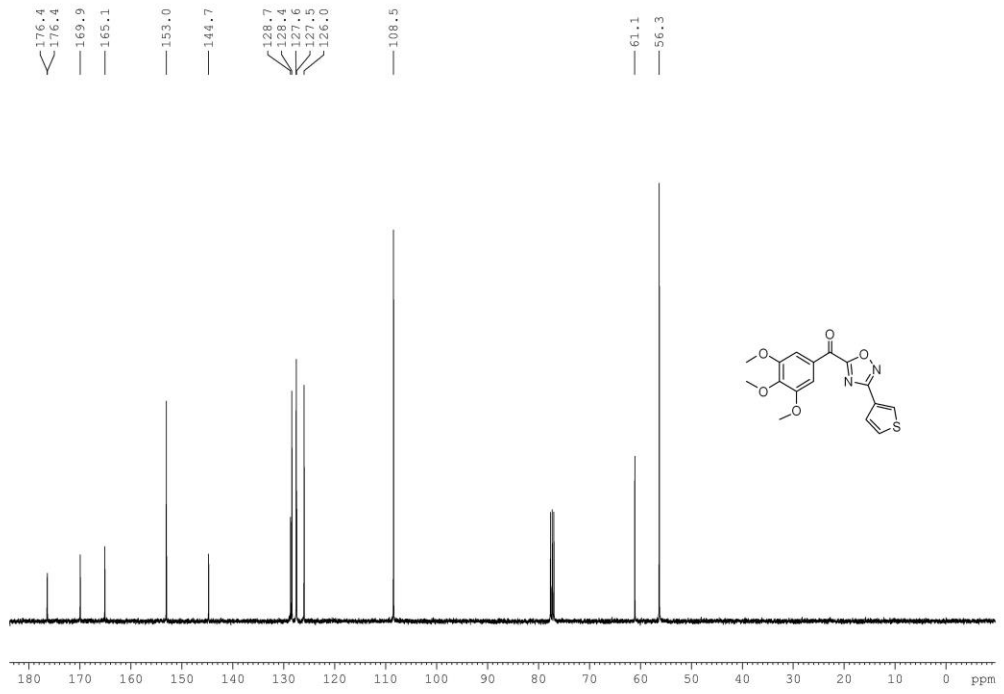
12. 3-(thiophen-2-yl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11g**).



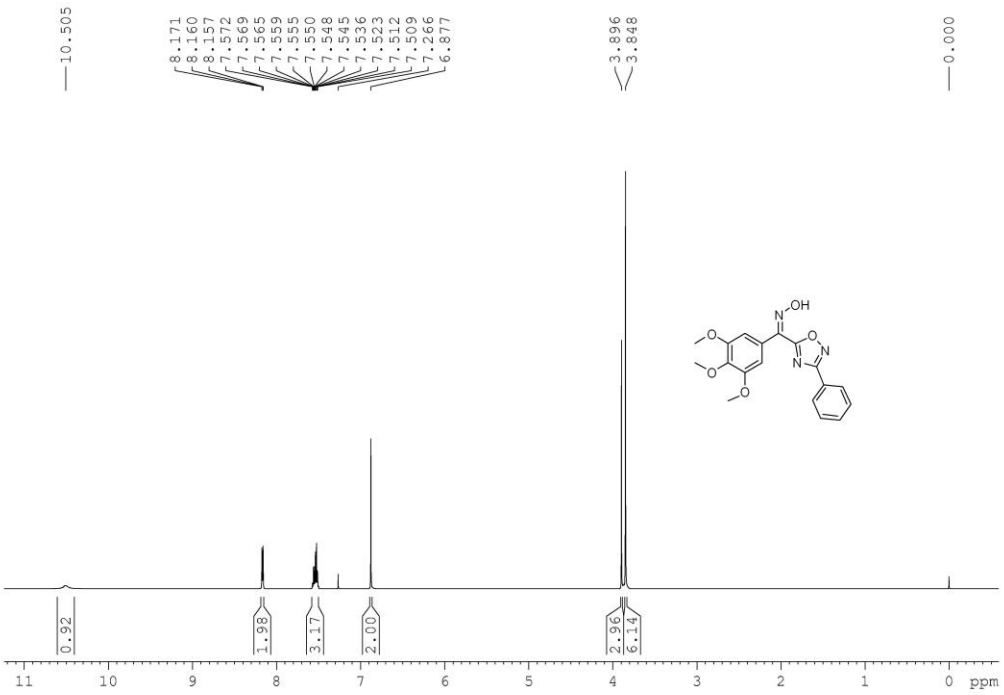


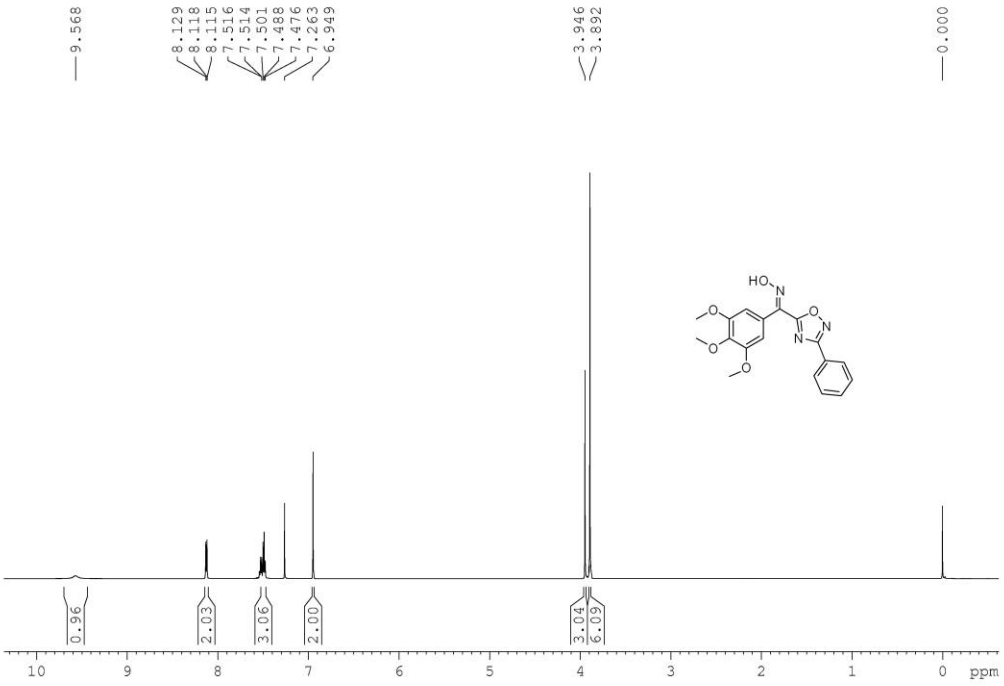
13. 3-(thiophen-3-yl)-5-(3,4,5-trimethoxybenzoyl)-1,2,4-oxadiazole (**11h**).

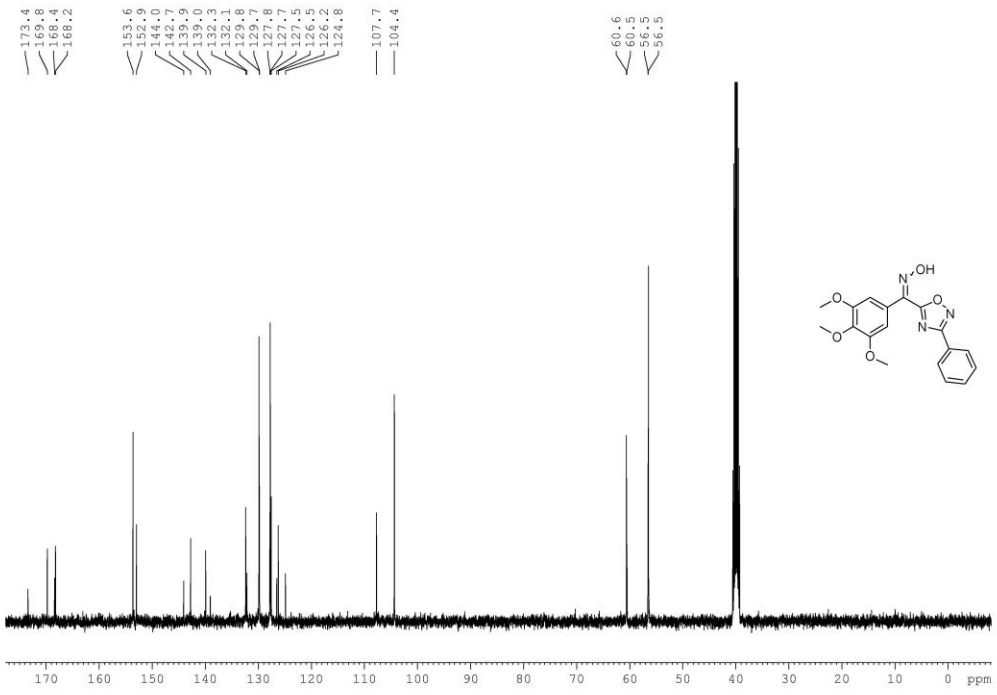




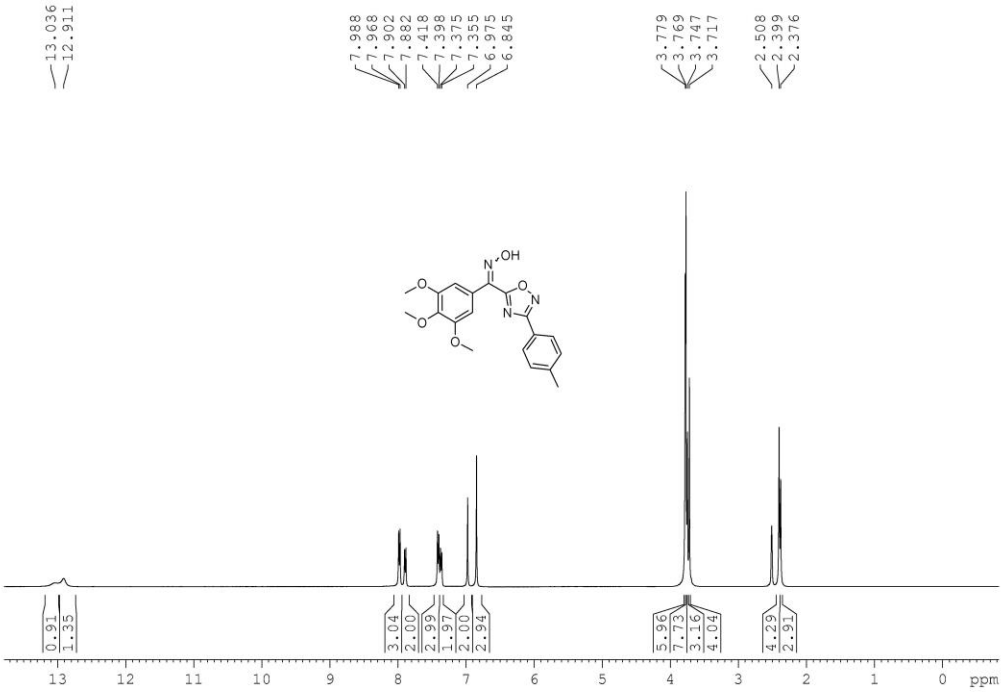
14. (*Z*+*E*)-(3-phenyl-1,2,4-oxadiazole-5-yl)(3,4,5-trimethoxyphenyl)methanone oxime (**12a**).

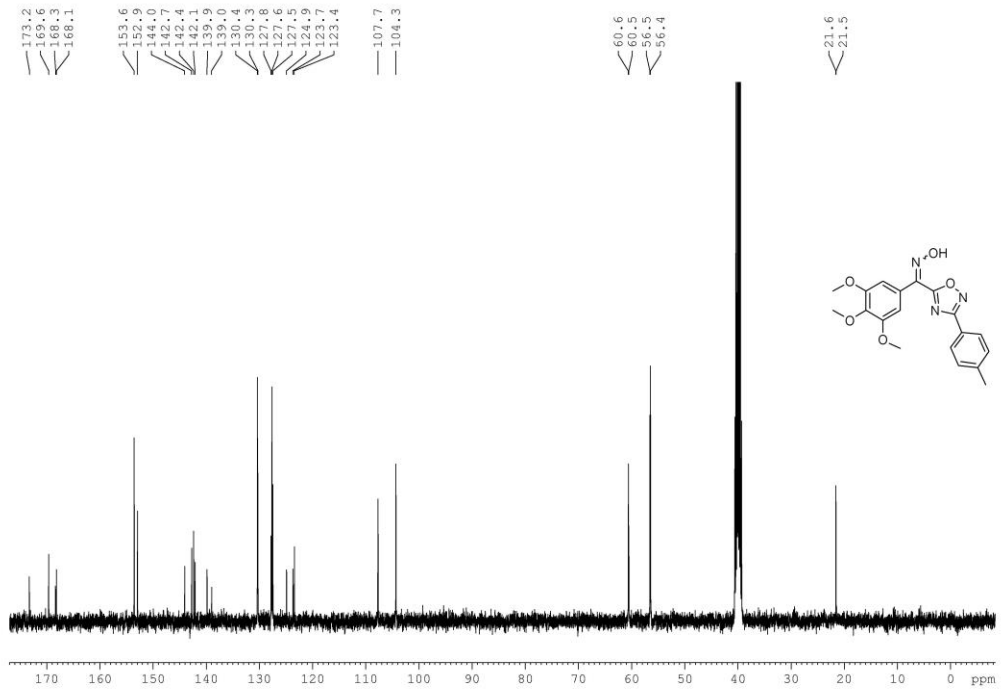




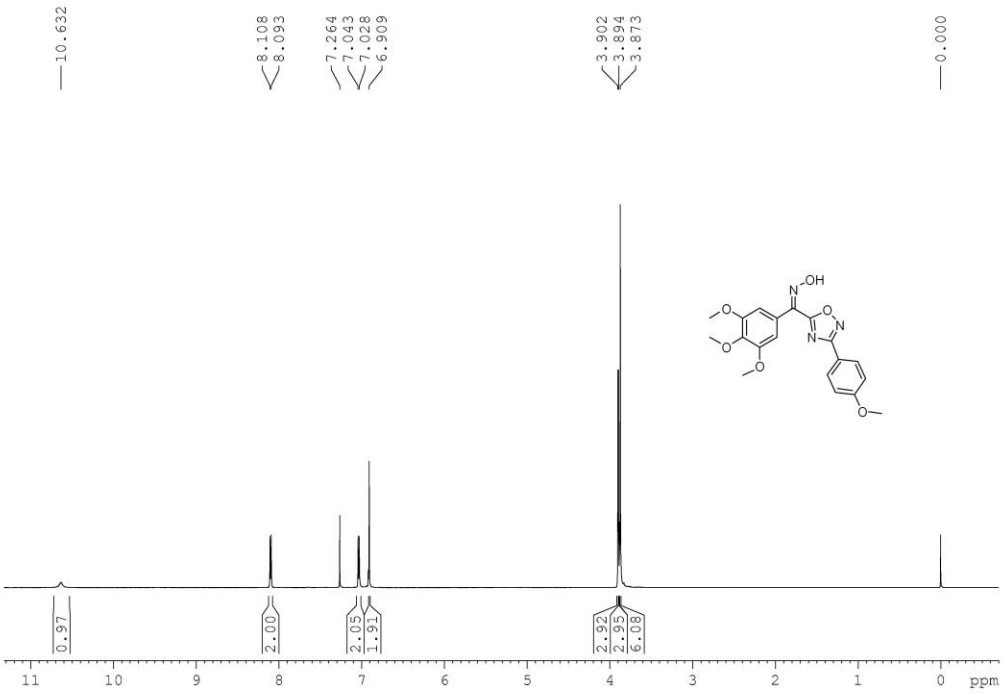

173.4
169.8
168.4
168.2
153.6
152.9
144.0
142.7
139.9
139.0
132.3
132.1
129.8
129.7
127.8
127.7
127.5
126.5
126.2
124.8
107.7
104.4
60.6
60.5
56.5
56.5
N
OH
O
O
O
N
O
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

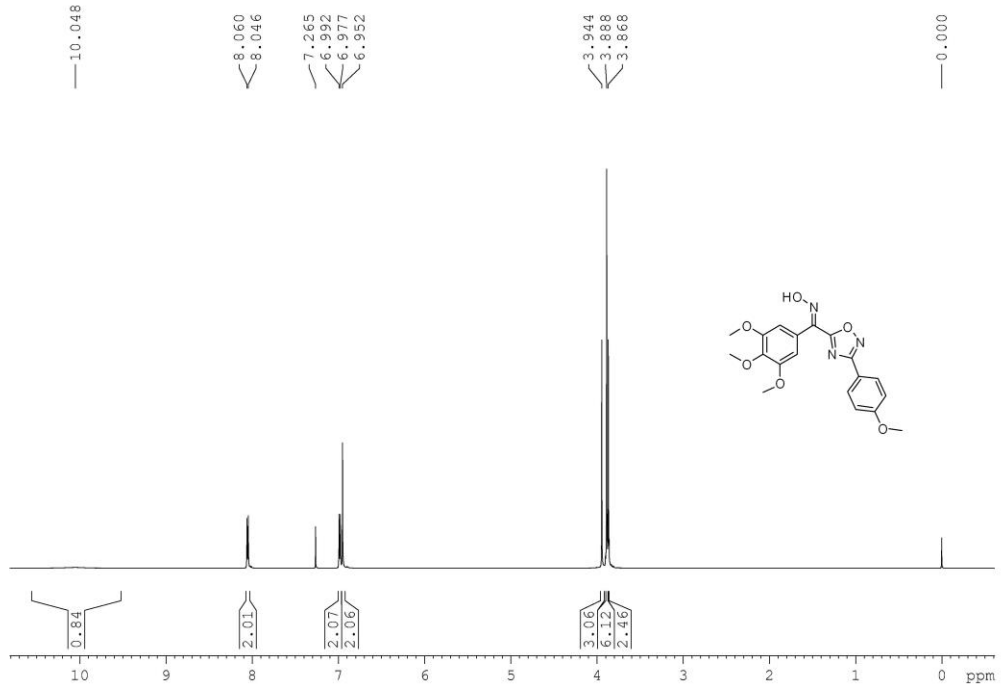
15. (*Z*+*E*)-[3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (**12b**).

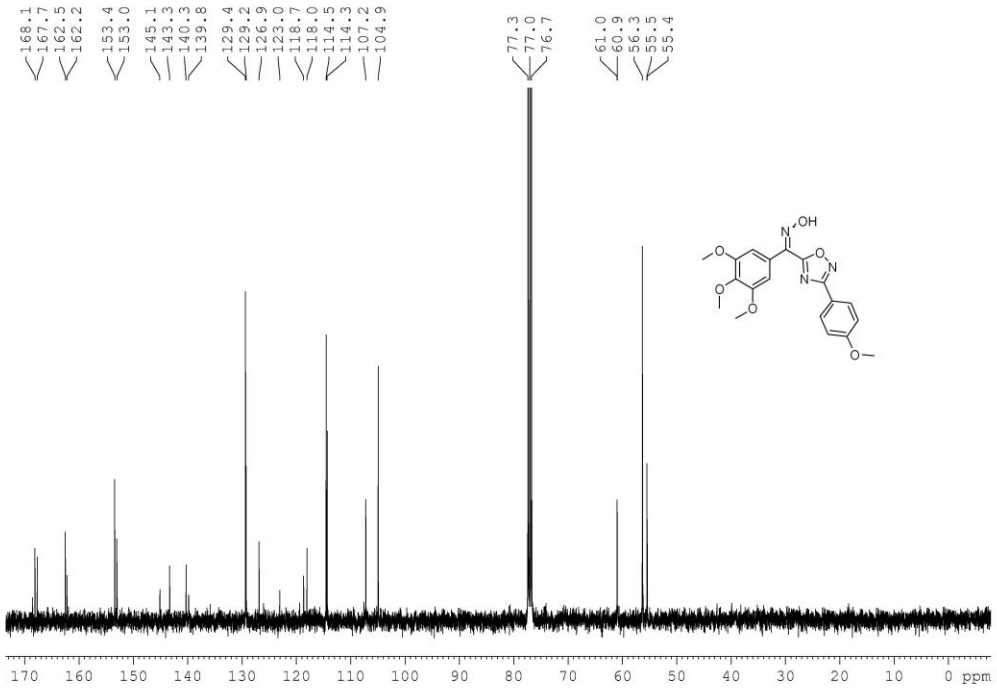




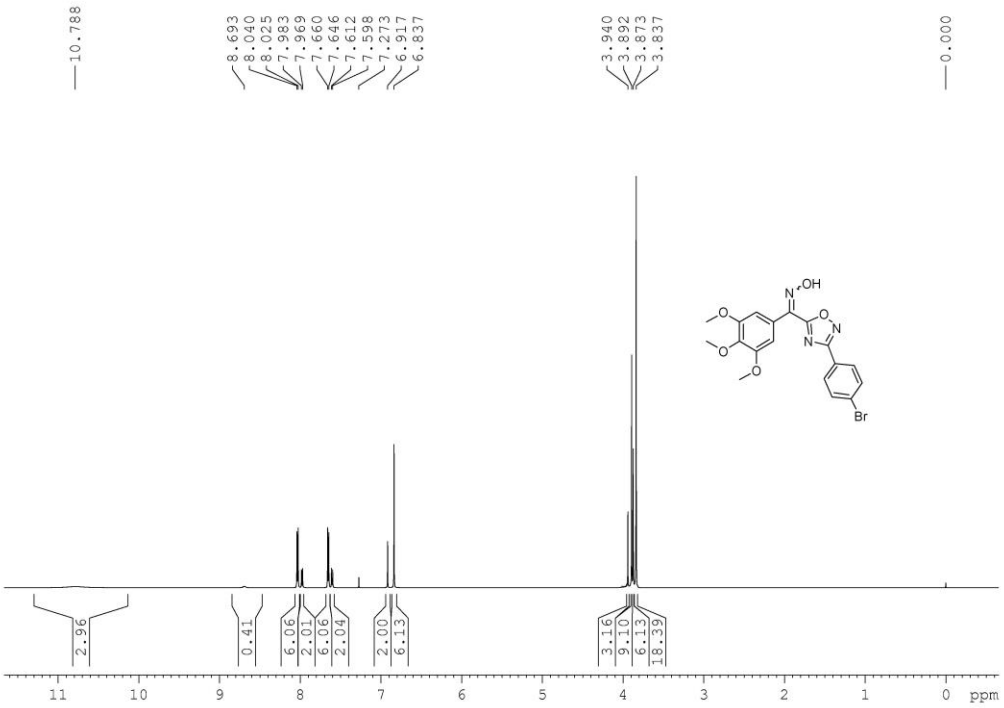
16. (*Z*+*E*)-[3-(4-methoxyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (**12c**).

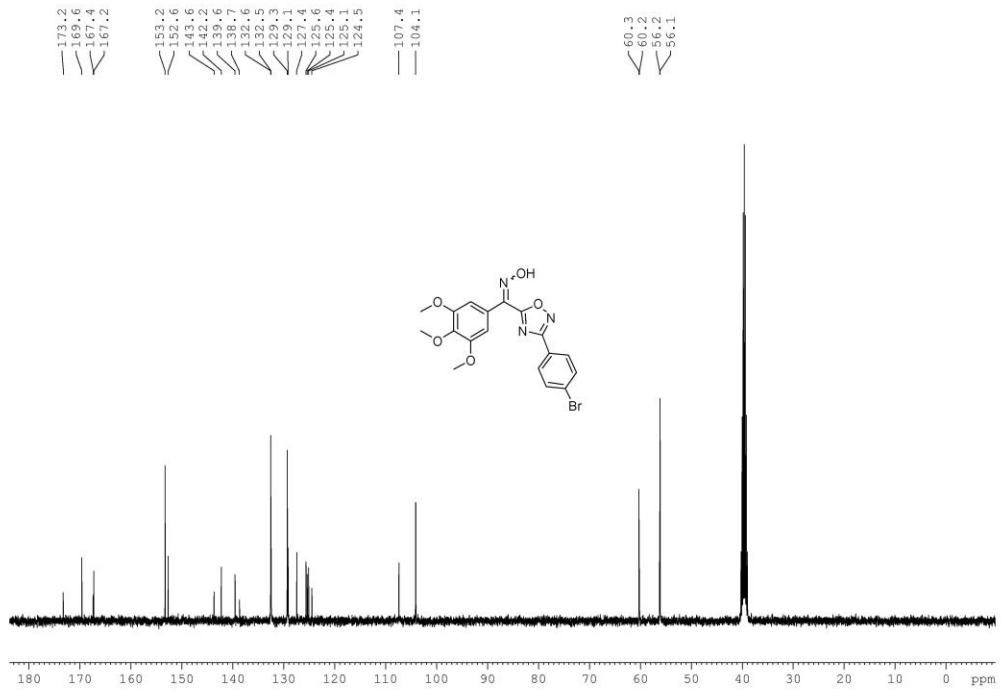




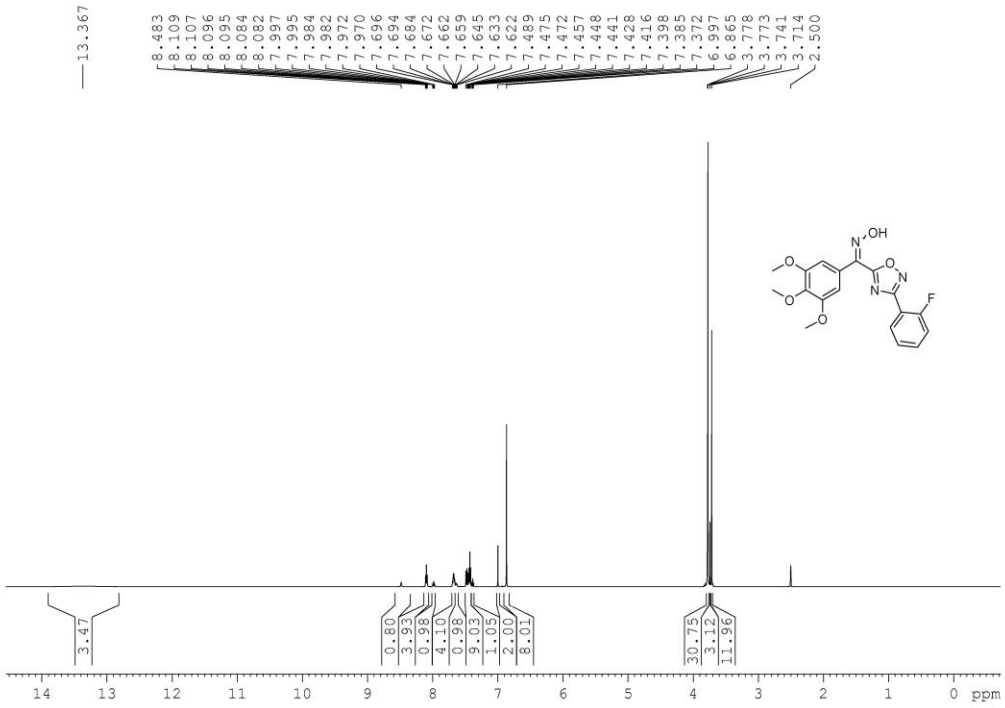

168.1
167.7
162.5
162.2
153.4
153.0
145.1
143.3
140.3
139.8
129.4
129.2
126.9
123.0
118.7
118.0
114.5
114.3
107.2
104.9
77.3
77.0
76.7
61.0
60.9
56.3
55.5
55.4
OH
N
O
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0 ppm

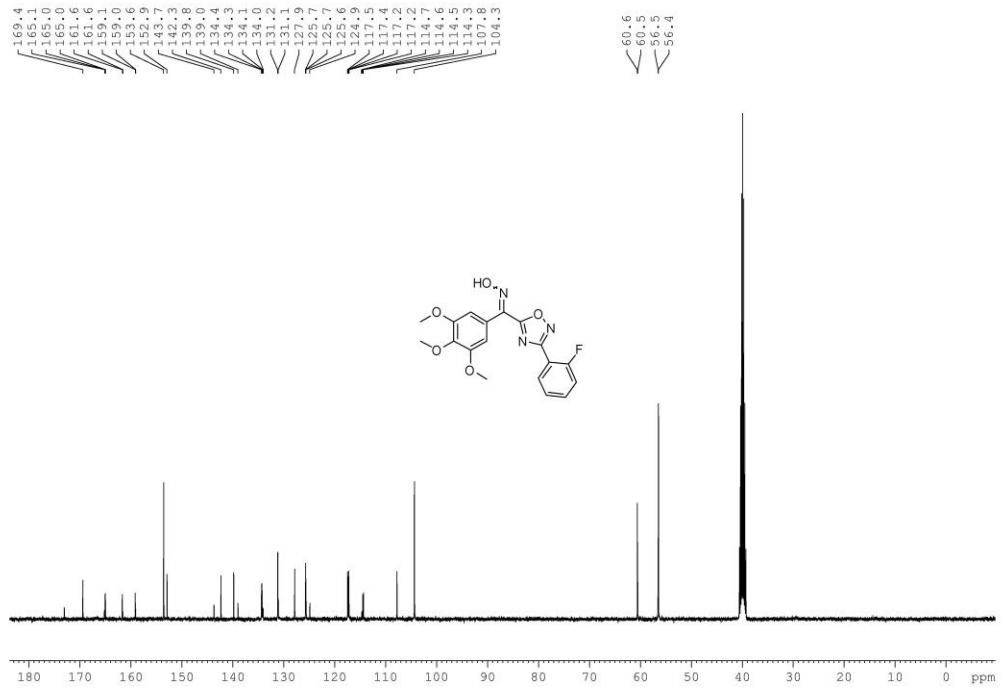
17. (*Z*+*E*)-[3-(4-bromophenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (**12d**).



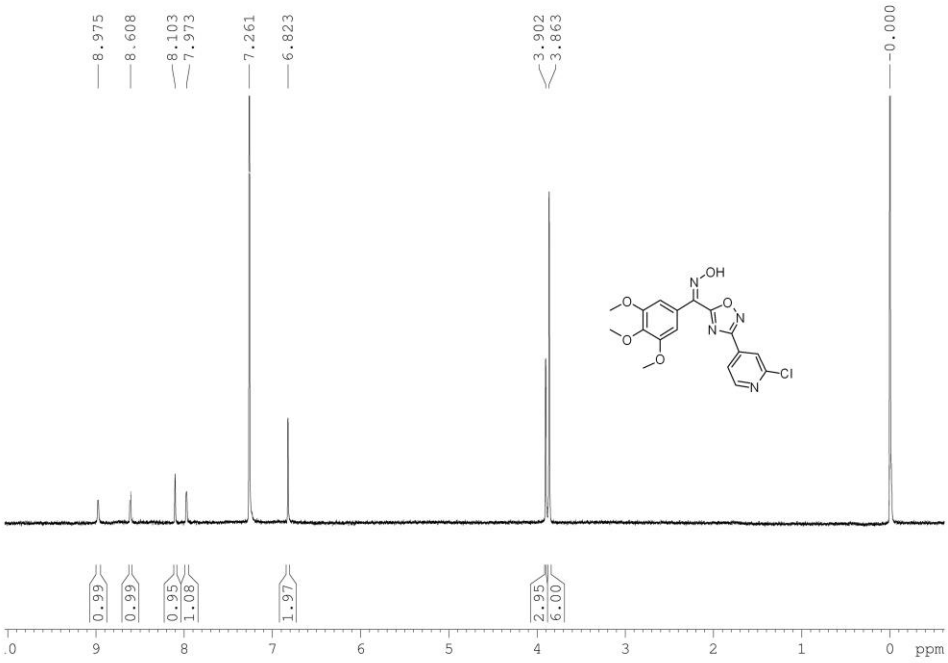


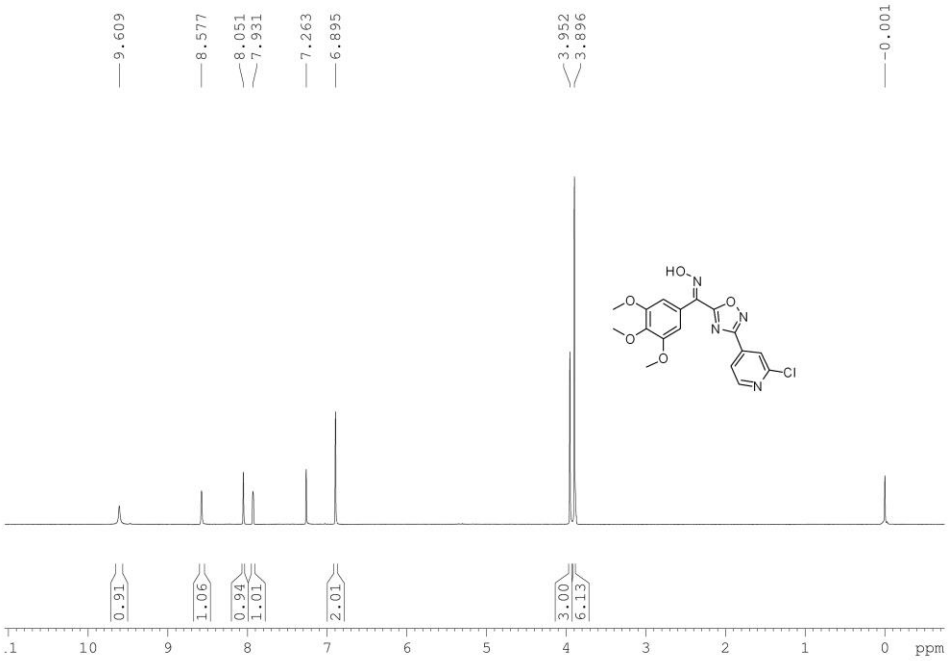
18. (*Z*+*E*)-[3-(2-fluorophenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (**12e**).



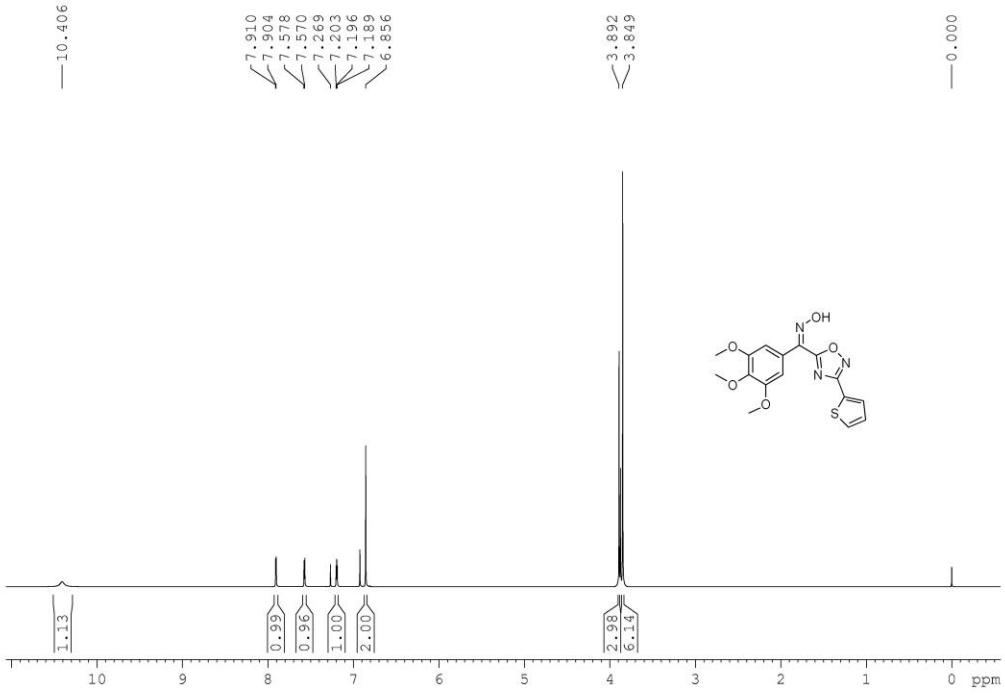


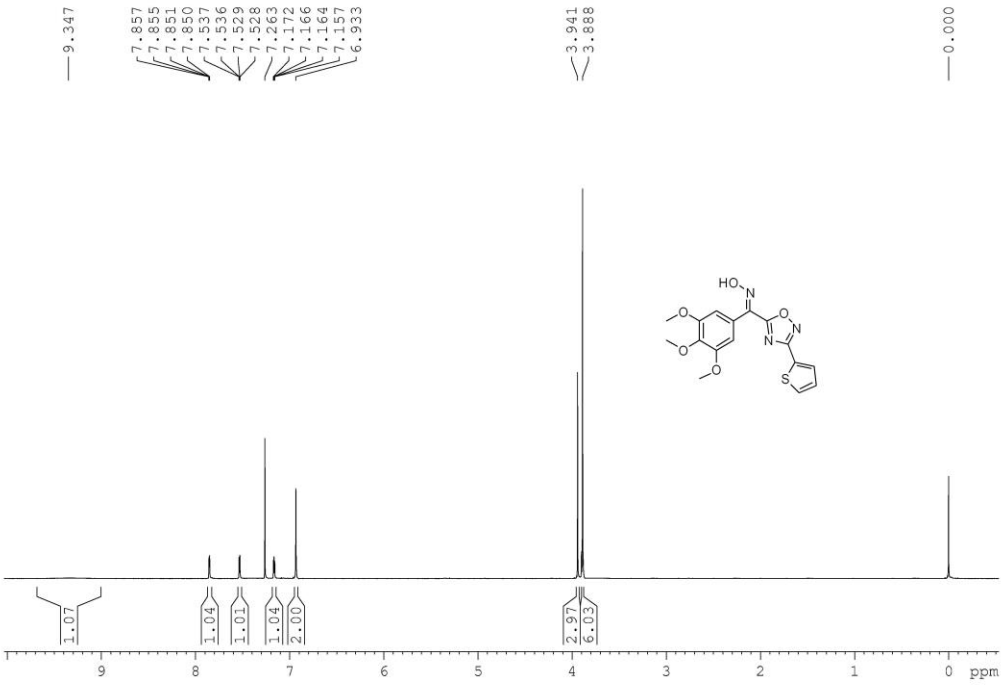
19. (*Z*+*E*)-[3-(2-chloropyridin-4-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (**12f**).



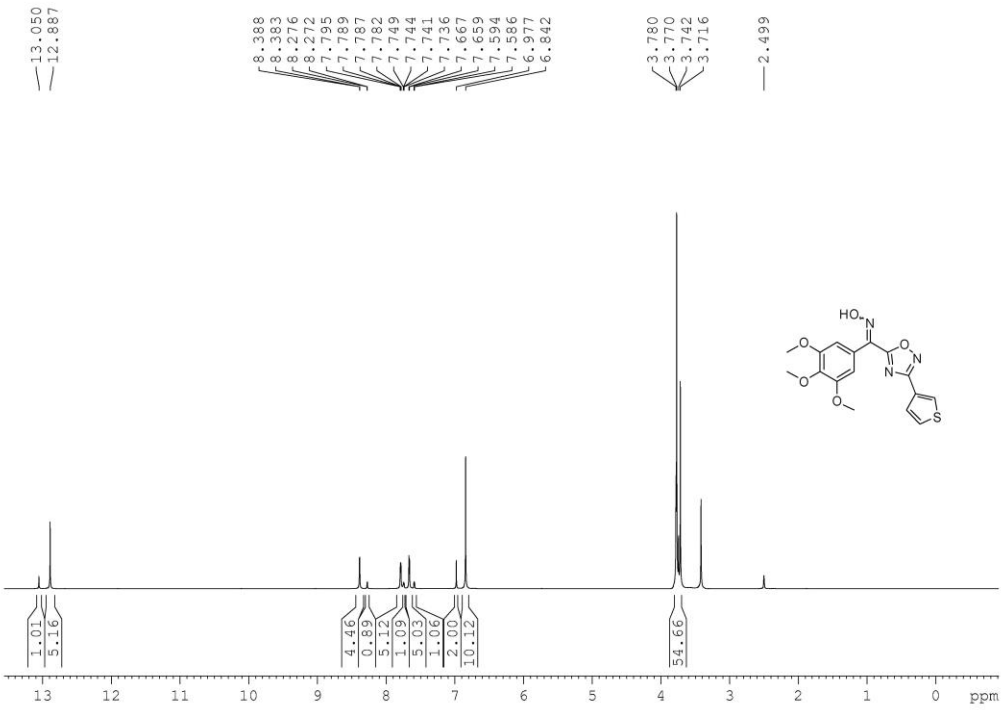


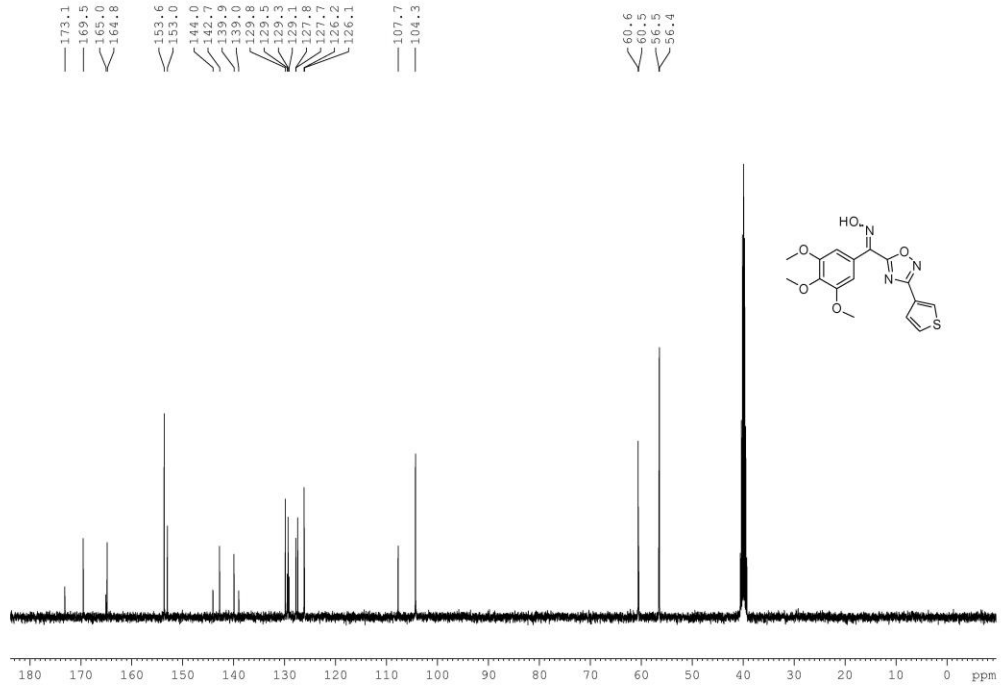
20. (Z+E)-[3-(thiophen-2-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (**12g**).



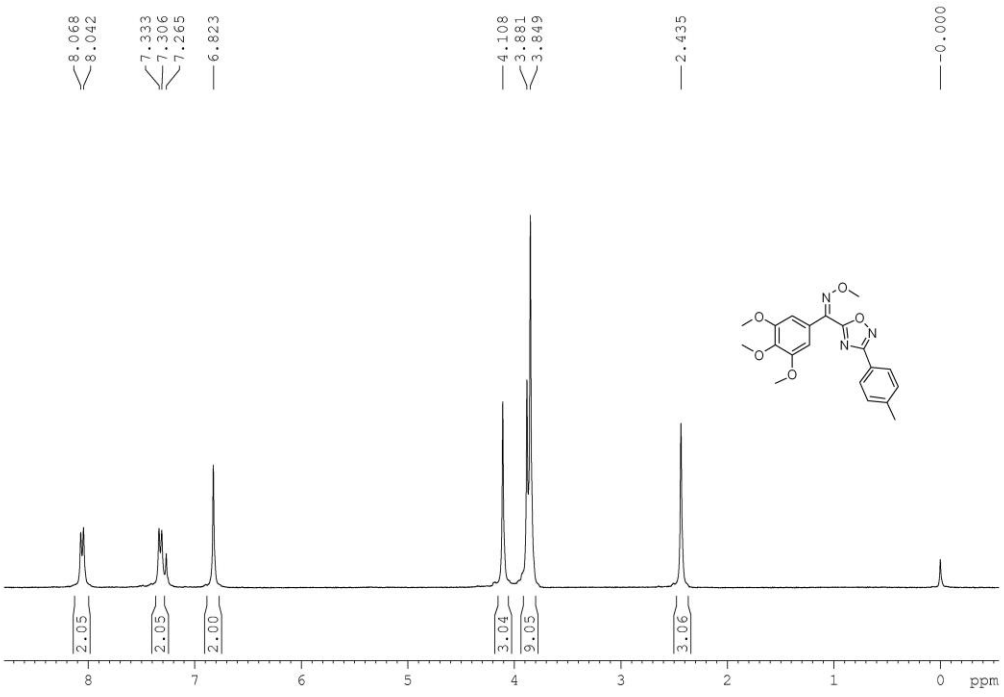


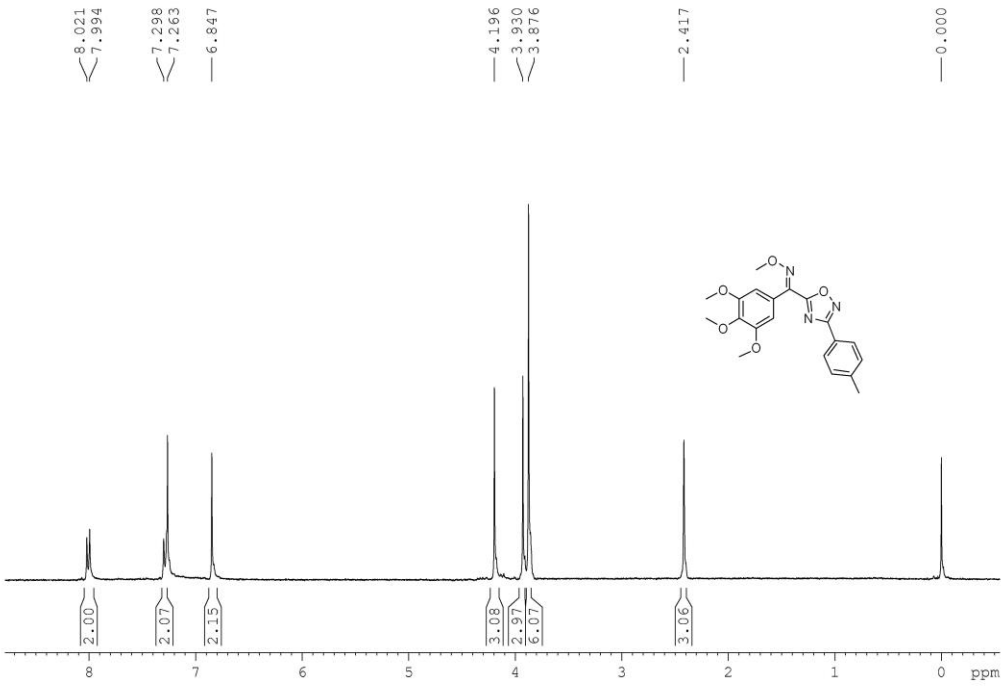
21. (*Z*+*E*)-[3-(thiophen-3-yl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone oxime (**12h**).



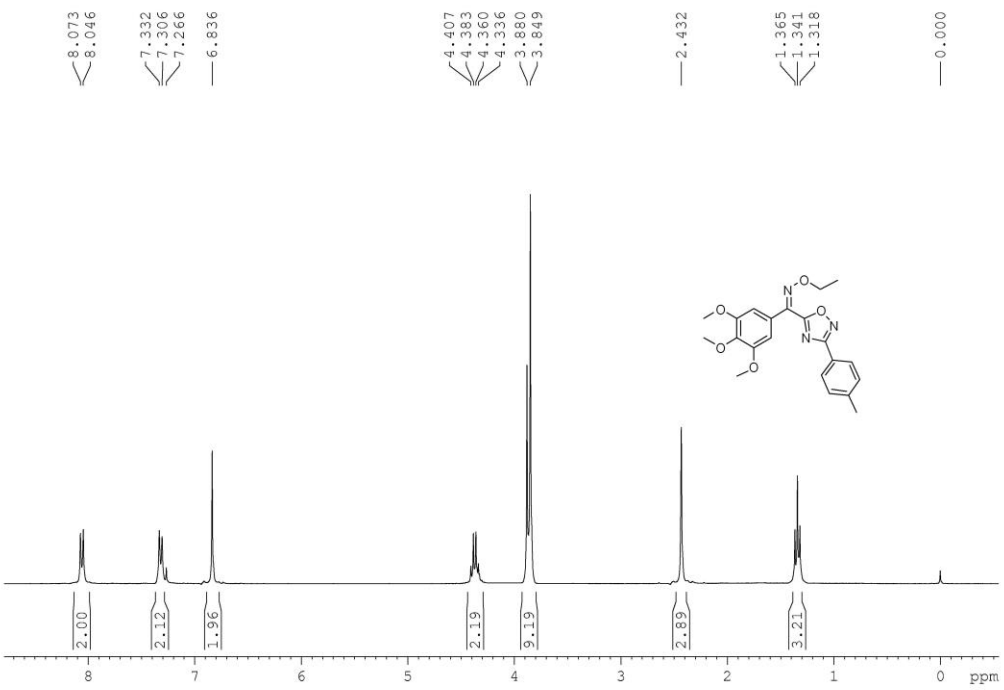


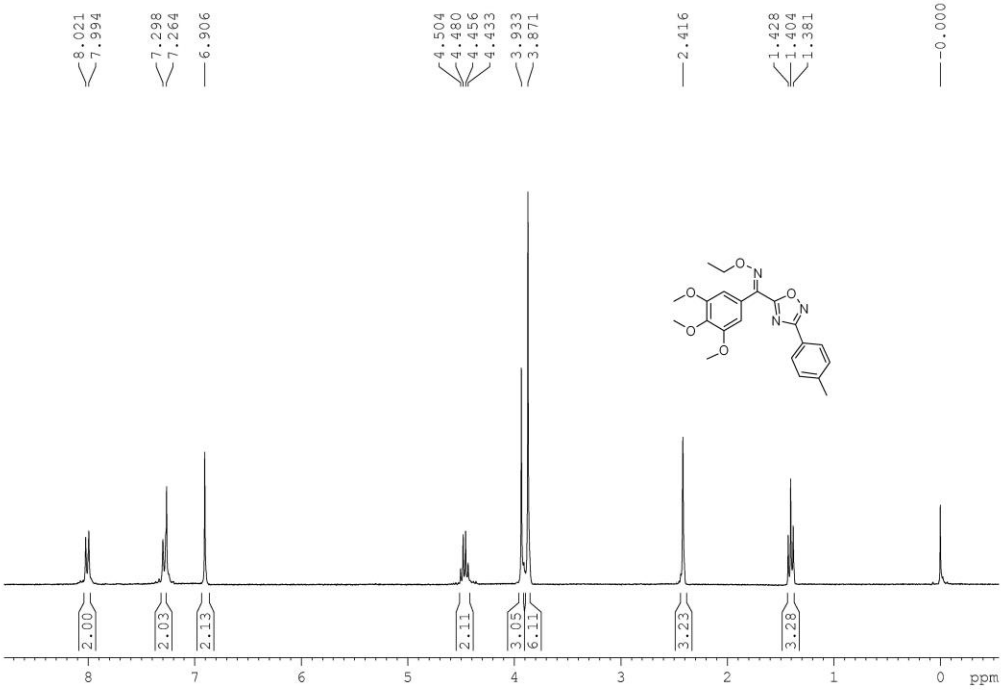
22. (*Z*+*E*)-[3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone methoxime (**13b**).



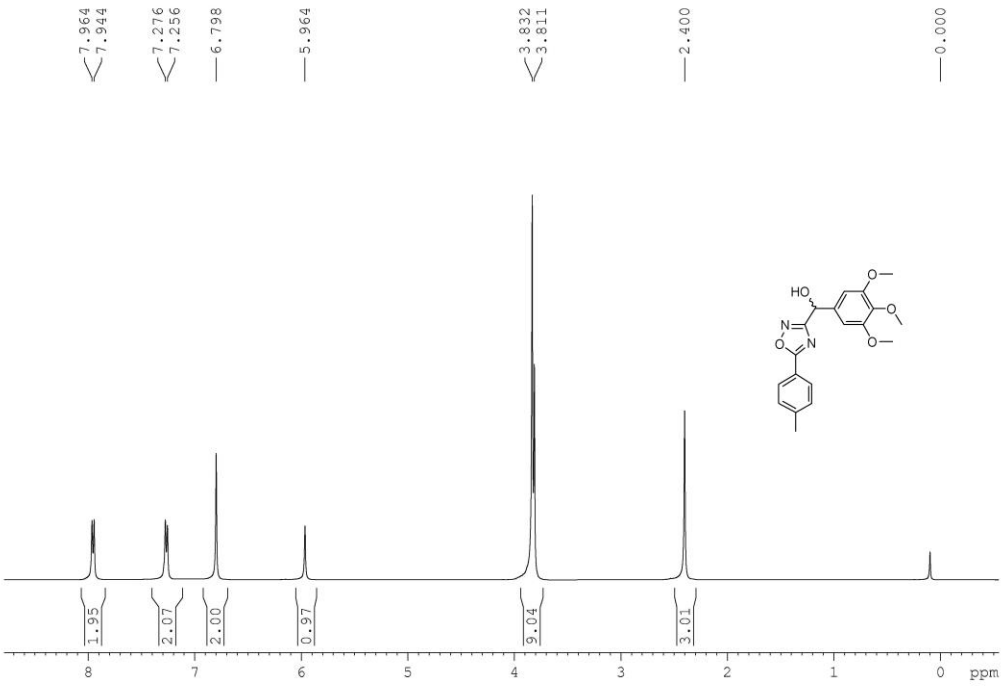


23. (*Z*+*E*)-[3-(4-methyphenyl)-1,2,4-oxadiazole-5-yl](3,4,5-trimethoxyphenyl)methanone ethyloxime (**14b**).

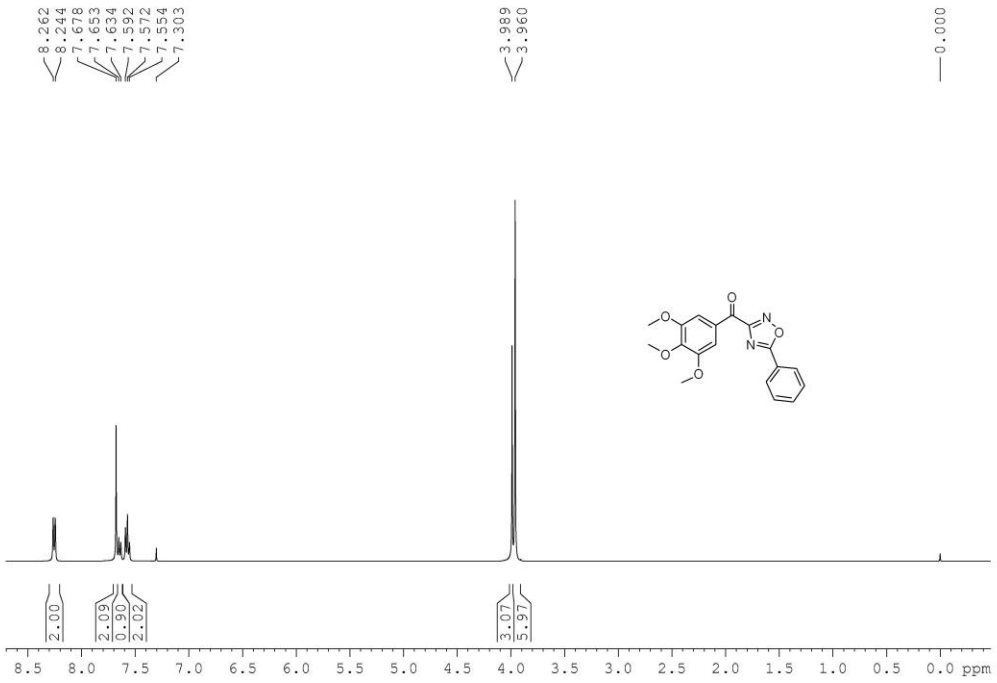


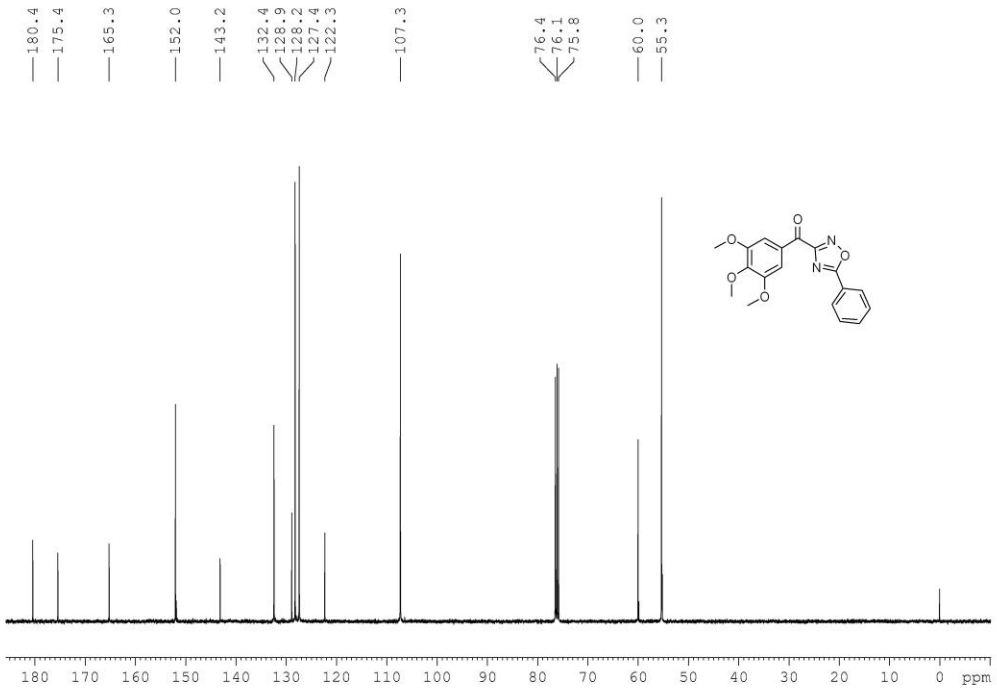


24. [5-(4-methyphenyl)-1,2,4-oxadiazole-3-yl](3,4,5-trimethoxyphenyl)methanol (**26b**).

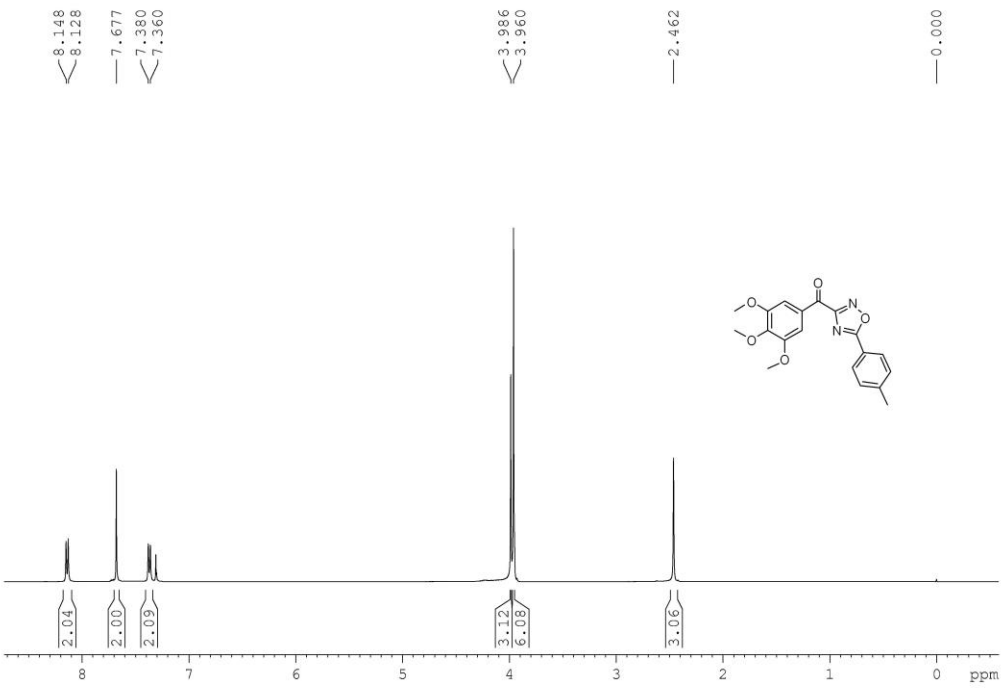


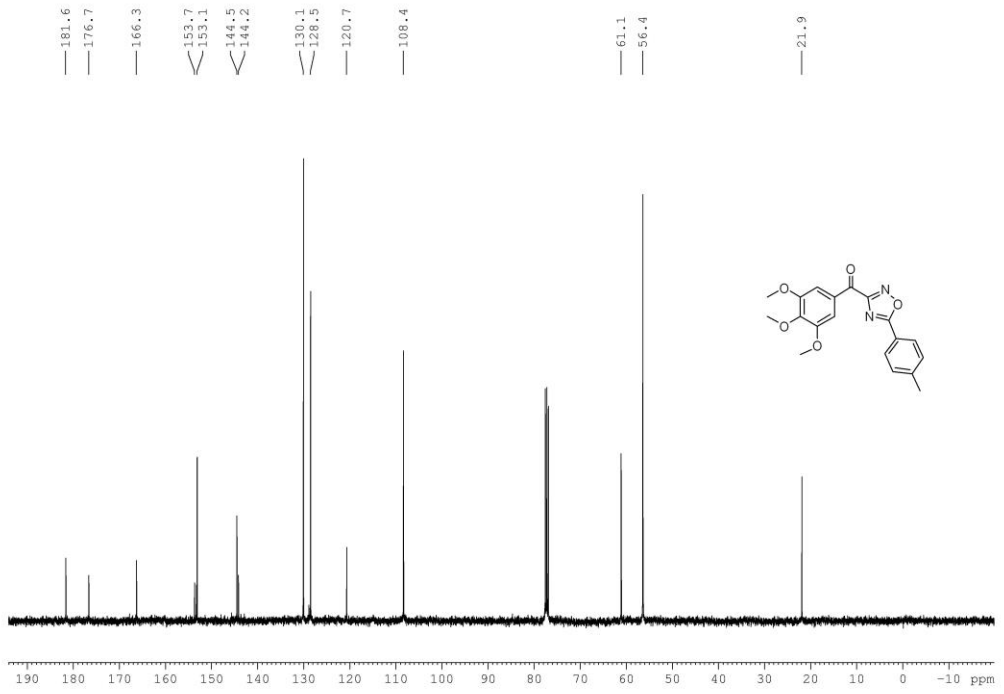
25. 3-(3,4,5-trimethoxybenzoyl)-5-phenyl-1,2,4-oxadiazole (**15a**).



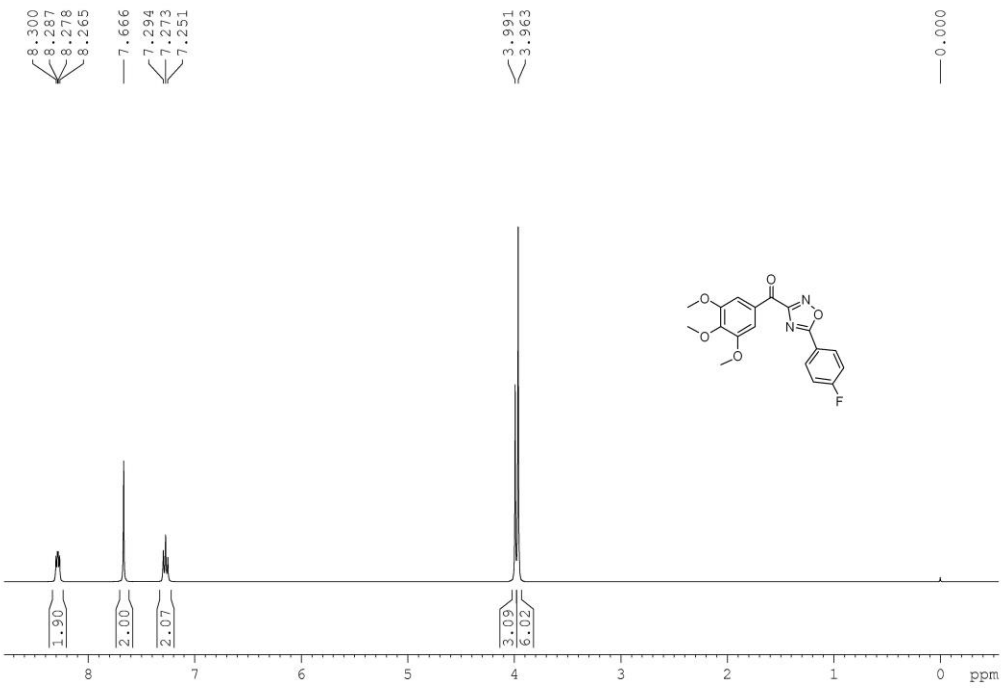


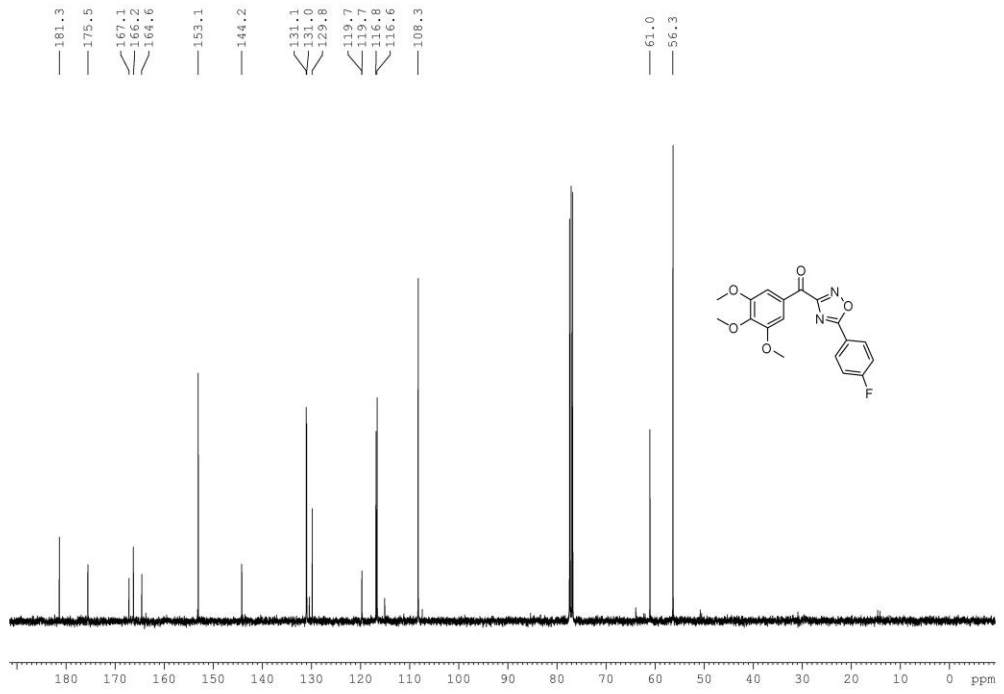
26. 3-(3,4,5-trimethoxybenzoyl)-5-(4-methyphenyl)-1,2,4-oxadiazole (**15b**).



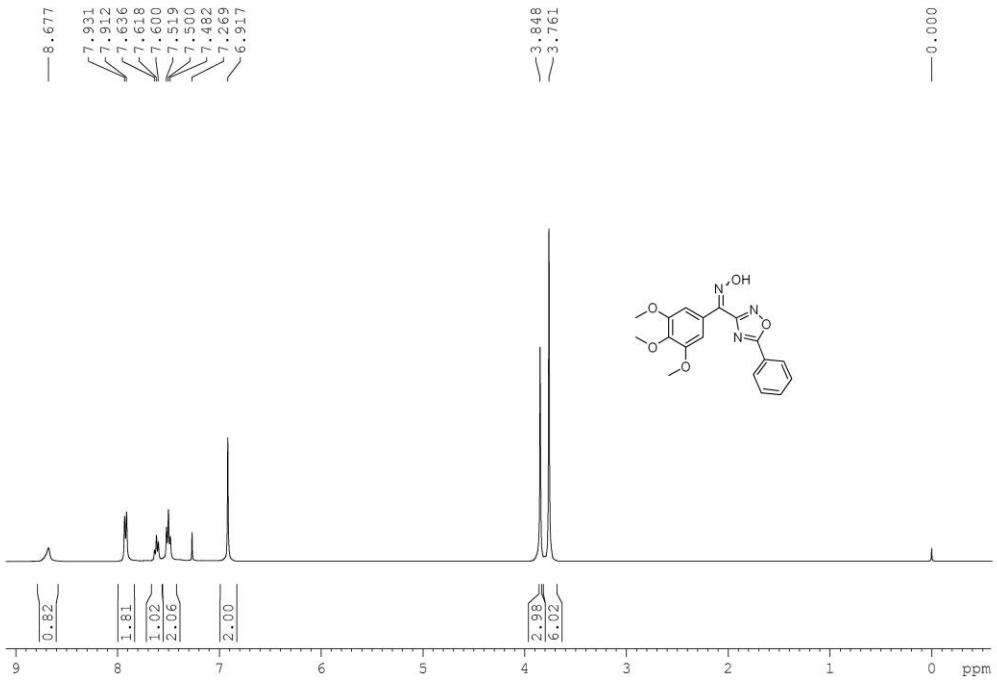


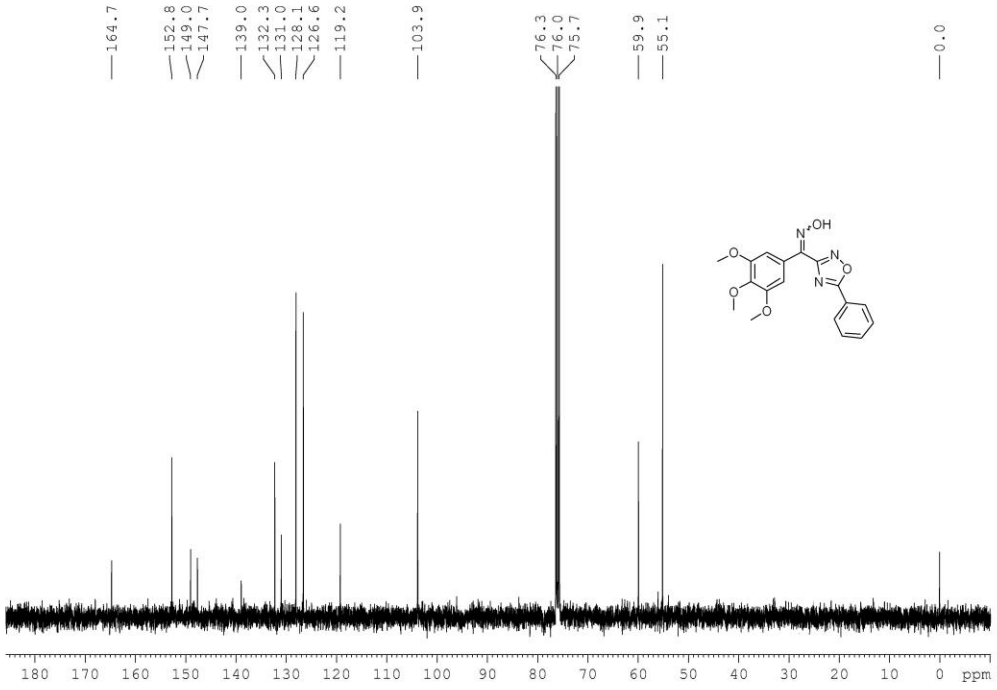
27. 3-(3,4,5-trimethoxybenzoyl)-5-(4-fluorophenyl)-1,2,4-oxadiazole (**15c**).



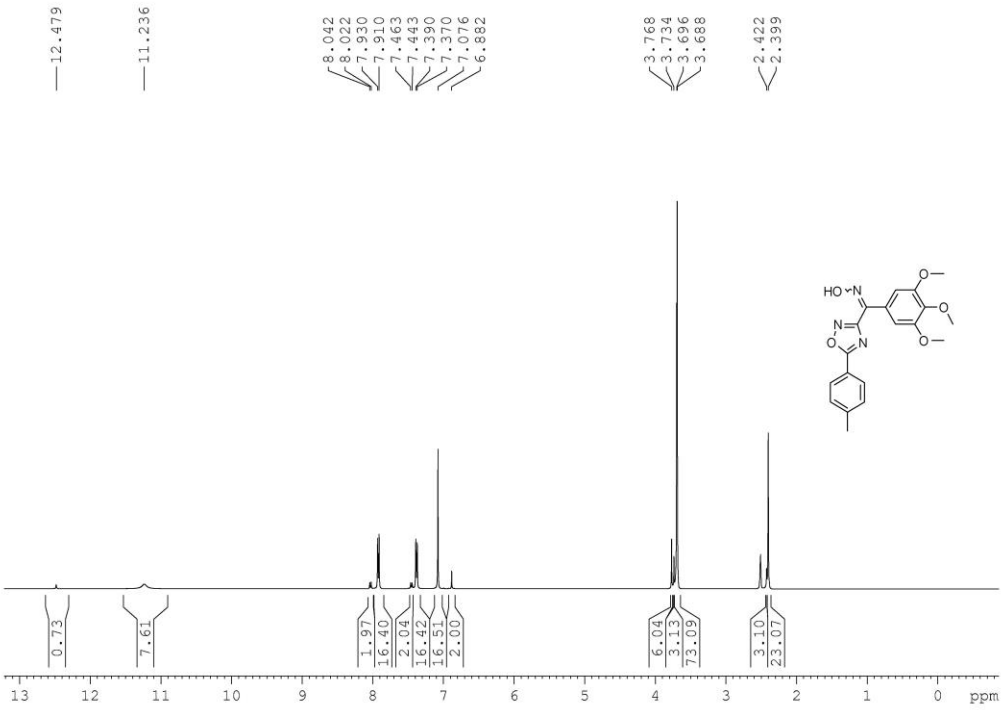


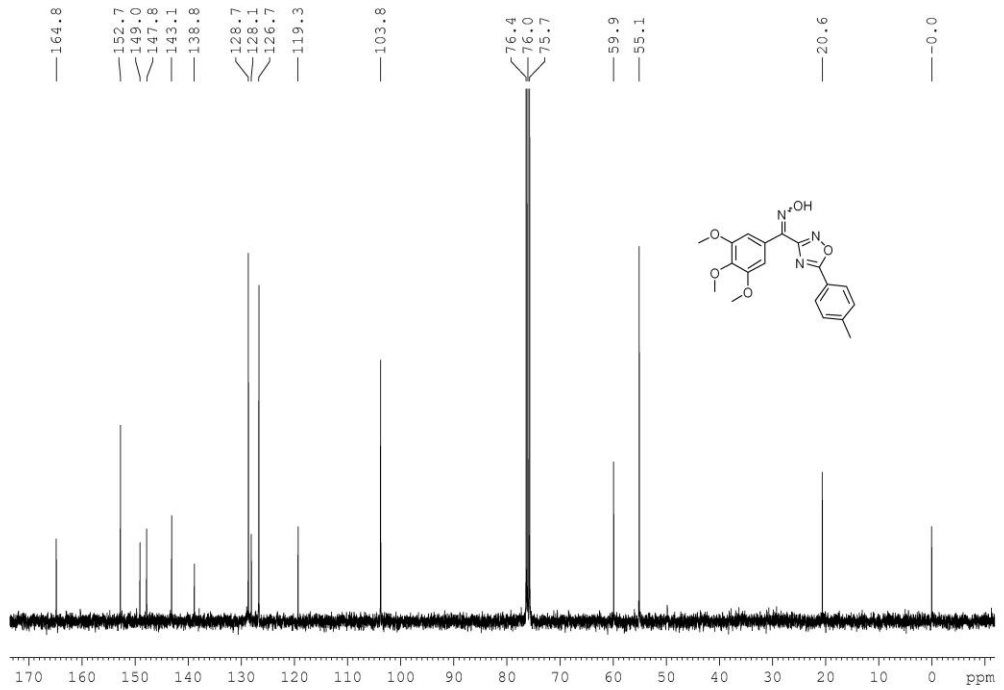
28. (Z)-(5-phenyl-1,2,4-oxadiazole-3-yl)(3,4,5-trimethoxyphenyl)methanone oxime (**16a**).





29. (Z+E)-[5-(4-methyphenyl)-1,2,4-oxadiazole-3-yl](3,4,5-trimethoxyphenyl)methanone oxime (**16b**).





30. (Z+E)-[5-(4-fluorophenyl)-1,2,4-oxadiazole-3-yl](3,4,5-trimethoxyphenyl)methanone oxime (**16c**).