SUPPORTING INFORMATION

Discovery of a novel benzimidazole conjugated quinazolinone derivative as promising SARS-CoV-2 3CL protease inhibitor†

Bui Thi Buu Hue,*[a] Huynh Nguyet Huong Giang,[b] Cuong Quoc Nguyen,[a] Feng-Pai Chou,[bd] Danh La Duc Thanh,[ac] Quang De Tran,[a] Vo Trung Hieu,[a] Lam Hoang Phuong Mai,[a] Hong-Cheu Lin,*[cd] and Tung-Kung Wu*[bd]

[a] Department of Chemistry, College of Natural Sciences, Can Tho University, Can Tho City 94000, Vietnam

[b] Department of Biological Science and Technology, National Yang Ming Chiao Tung University, Hsinchu 30010, Taiwan

[c] Department of Material Science, National Yang Ming Chiao Tung University, 1001 Ta-Hsueh Rd., Hsinchu 30010, Taiwan

[d] Center for Emergent Functional Matter Science, National Yang Ming Chiao Tung University, 1001 Ta-Hsueh Rd., Hsinchu 30010, Taiwan.

* Correspondence:
btbhue@ctu.edu.vn (B.T.B.H.)
linhc@nycu.edu.tw (H.-C.L.)
tkwmll@nycu.edu.tw (T.-K.W.)

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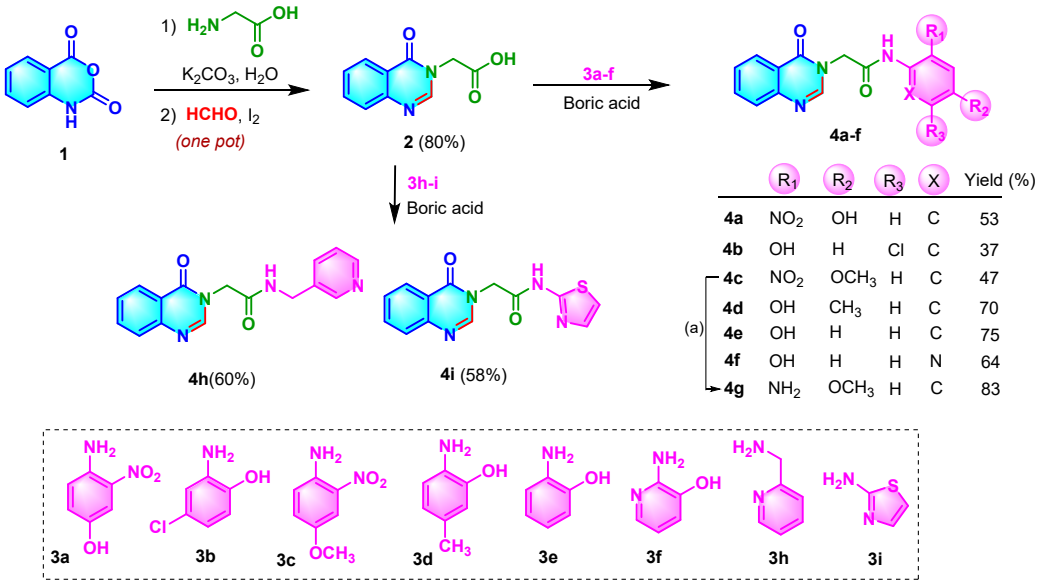
S1. Chemical syntheses

All reagents were purchased from commercial sources and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on 0.2 mm precoated silica gel 60 F254 plates (Merck), and compounds were visualized on TLC with UV light. The derivatives were synthesized and then purified by flash column chromatography using silica gel 45−63 μm (230−400 mesh) and 60 pore size. The NMR spectra were obtained from a Varian 300 MHz or Bruker 400 MHz spectrometer. The chemical shifts (δ) were reported in parts per million (ppm) relative to tetramethylsilane (TMS) or the internal solvent signal of deuterated solvents. Multiplicities were indicated by *s* (singlet), *d* (doublet), *t* (triplet), and *m* (multiplet).



Scheme S1. Synthesis of compounds 4a-i

Synthetic procedure of compound (2)

A mixture of isatoic anhydride **1** (163.1 mg, 1 mmol), glycine (82.5 mg, 1.1 mmol), and K_2CO_3 (304.0 mg, 2.2 mmol) in H_2O (5 mL) was stirred at room temperature for 30 minutes then formaldehyde (120.1 mg, 4 mmol), I_2 (381.0 mg, 1.5 mmol), and KI (23.0 mg, 0.15 mmol) were added. The resulting mixture was refluxed at 100°C for 5h. The reaction mixture was quenched by saturated aqueous solution of $Na_2S_2O_3$ (5 mL), neutralized by aqueous solution of 10% HCl to pH ~3. The aqueous layer was extracted with ethyl acetate, EtOAc (3 × 10 mL). The combined organic layers were washed with saturated aqueous solution of NaCl (30 mL), dired over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure to obtain the crude product (**2**) which was used for the next step without any further purification (132.6 mg, 80%). R_f= 0.15 (EtOAc:MeOH: AcOH= 65:30:5).

Synthetic procedure of compounds 4a-f

A mixture of (**2**) (0.5 mmol) and boric acid (0.1 mmol) in toluene (5 mL) was stirred at room temperature for 30 minutes and then amines (**3a-f**) (0.5 mmol) was added. The resulting mixture was refluxed at 120°C for 12h. At the end of the reaction, solvent was evaporated under reduced pressure. Column chromatography of the crude product provided the desired **4a-f**.

Synthetic procedure of compound 4g

To a mixture of compound **4c** (0.15 mmol) in methanol (5 mL) was added CH_3COONH_4 (0.75 mmol) and Zn (0.75 mmol). The resulting mixture was stirred at room temperature for 20 minutes. The reaction mixture was then filtered and the exccess of methanol was evaporated under reduced pressure. The residue was disolved in water (10 mL) and the water phase was extracted with EtOAc (3×30 mL). The combined organic layers were washed with aqueous saturated solution of NaCl (30 mL), dried over Na_2SO_4, filtered and solvent was evaporated under reduced pressure. Column chromatography of the crude product afforded the desired compound **4g** as a white solid (40.3 mg - 83%), R_f = 0.42 (Hex:EtOAc = 1:4).

***N*-(4-Hydroxy-2-nitrophenyl)-2-(4-oxoquinazolin-3(4*H*)-yl)acetamide (4a)**: Yield 42% as yellow solid. R_f = 0.30 (Hex:EtOAc = 1:2). Mp 297-299°C. FT-IR (KBr) ν_{max} (cm^{-1}): 3285, 1655, 1683, 1615, 1557, 1540, 1521, 1360, 1292, 1280, 1234, 1217, 1168, 969, 777. HR-ESI-MS found *m/z* 339.0731 $[M-H]^-$ (calcd. 339.0729, $C_{16}H_{12}N_4O_5$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 10.37 (*s*, 1H), 8.32 (*s*, 1H), 8.15 (*dd*, J_1 = 7.5, J_2 = 1.0, 1H), 7.85 (*m*, 1H), 7.71 (*d*, J = 8.0, 1H), 7.57 (*t*, J =7.7, 1H), 7.43 (*d*, J = 8.5, 1H), 7.30 (*d*, J = 3.0, 1H), 7.12 (*dd*, J_1 = 9.0, J_2 = 3.0, 1H), 4.87 (*s*, 2H). ^{13}C-NMR (125MHz, DMSO-d_6, δ ppm): 165.9, 160.1, 155.0, 148.3, 148.0, 134.5, 127.2, 127.1, 126, 121.8, 121.4, 121.1, 110.6, 48.3.

***N*-(5-Chloro-2-hydroxyphenyl)-2-(4-oxoquinazolin-3(4*H*)-yl)acetamide (4b)**: Yield 37% as white solid. R_f = 0.23 (Hex:EtOAc = 1:1). Mp 284-286°C. FT-IR (KBr) ν_{max} (cm^{-1}): 3247, 3222, 3049, 2957, 2923, 2854, 1694, 1673, 1614, 1544, 1477, 1408, 1373, 1293, 1171, 1118, 969, 917, 892, 813, 774. HR-ESI-MS found *m/z* 330.0643 $[M+H]^+$ (calcd. 330.0645, $C_{16}H_{12}ClN_3O_3$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 10.28 (*s*, 1H), 9.86 (*s*, 1H), 8.35 (*s*, 1H), 8.15 (*dd*, J_1 = 8.0, J_2 = 1.5, 1H), 7.98 (*d*, J = 2.5 Hz, 1H), 7.89-7.84 (*m*, 1H), 7.72 (*d*, J = 7.5, 1H), 7.59-7.55 (*m*, 1H), 6.97 (*dd*, J_1 = 8.5, J_2 = 2.5, 1H), 6.90 (*d*, J = 8.5, 1H), 4.97 (*s,* 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 166.0, 160.22, 148.5, 148.0, 146.2, 134.4, 127.2, 127.0, 125.9, 123.7, 122.0, 121.4, 120.8, 116.2, 48.7.

***N*-(4-methoxy-2-nitrophenyl)-2-(4-oxoquinazolin-3(4H)-yl)acetamide (4c)**: Yield 38% as yellow solid. R_f = 0.31 (Hex:EtOAc = 1:1). Mp 215-217°C. FT-IR (KBr) ν_{max} (cm^{-1}): 3313, 3001, 2944, 1678, 1613, 1588, 1466, 1365, 1307, 1280, 1253, 777. HR-ESI-MS found *m/z* 353.0611 $[M-H]^-$ (calcd. 353.0886,

$C_{17}H_{15}N_4O_5$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm)*:* 10.43 (*s*, 1H), 8.33 (*s*, 1H), 8.16 (*dd*, J_1 = 1.5, J_2 = 9.5, 1H), 7.87-7.84 (*m*, 1H), 7.71 (*d*, *J* = 8.5, 1H), 7.58-7.55 (*m*, 2H), 7.49 (*d*, *J* = 3.0, 1H), 7.31 (*dd*, J_1 = 9.0, J_2 = 3.0, 1H), 4.89 (*s*, 2H), 3.84 (*s*, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm)*:* 166.0, 160.1, 156.4, 148.3, 147.9, 143.4, 134.5, 127.5, 127.2, 127.1, 126.0, 123.2, 121.4, 120.2, 109.1, 56.0, 48.3.

***N*-(2-Hydroxy-4-methylphenyl)-2-(4-oxoquinazolin-3(4*H*)-yl)acetamide (4d)**: Yield 38% as white solid. R_f = 0.36 (Hex:EtOAc = 1:2). Mp 242-244°C. FT-IR (KBr) ν_{max} (cm^{-1}): 3250, 3228, 3054, 1698, 1687, 1670, 1614, 1549, 1519, 1478, 1375, 1322, 1294, 1204, 1172, 1120, 968, 774. HR-ESI-MS found *m/z* 310.1194 $[M-H]^-$ (calcd. 310.1192, $C_{17}H_{15}N_3O_3$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 9.64 (*s*, 1H), 9.57 (*s*, 1H), 8.35 (*s*, 1H), 8.15 (*dd*, J_1 = 7.5, J_2 = 1.0, 1H), 7.87-7.84 (*m*, 1H), 7.71 (*d*, *J* = 8.0, 1H), 7.64 (*s*, 1H), 7.58-7.55 (*m*, 1H), 6.73-6.78 (*m*, 2H), 4.94 (*s*, 2H), 3.31 (*s*, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 165.5, 160.2, 148.6, 148.1, 145.3, 134.4, 127.4, 127.2, 127.0, 126.0, 125.5, 124.9, 122.5, 121.4, 115.1, 48.7, 20.3.

***N*-(2-Hydroxyphenyl)-2-(4-oxoquinazolin-3(4*H*)-yl)acetamide (4e)**: Yield 76% as white solid. R_f = 0.15 (Hex:EtOAc = 1:1). FT-IR (KBr) ν_{max} (cm^{-1}): 3264, 3061, 1697, 1677, 1617, 1541, 1476, 1455, 1374, 1300, 1258, 1222, 965, 756. ESI-MS found *m/z* 295.9 $[M+H]^+$ (calcd. 296.1, $C_{16}H_{14}N_4O_2$) and *m/z* 293.8 $[M+H]^+$ (calcd. 294.1, $C_{16}H_{13}N_3O_3$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 9.83 (*s*, 1H), 9.69 (*s*, 1H), 8.36 (*s*, 1H), 8.16 (*d*, *J* = 8.0, 1H), 7.87-7.80 (*m*, 2H), 7.71 (*d*, *J* = 8.0, 1H), 7.58-7.55 (*m*, 1H), 6.95-6.92 (*m*, 1H), 6.90-6.88 (*m*, 1H), 6.76-6.73 (*m*, 1H), 4.96 (*s*, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 165.6, 160.2, 148.6, 148.1, 147.6, 134.4, 127.2, 127.0, 126.0, 125.8, 124.6, 121.9, 121.4, 118.9, 115.3, 48.7.

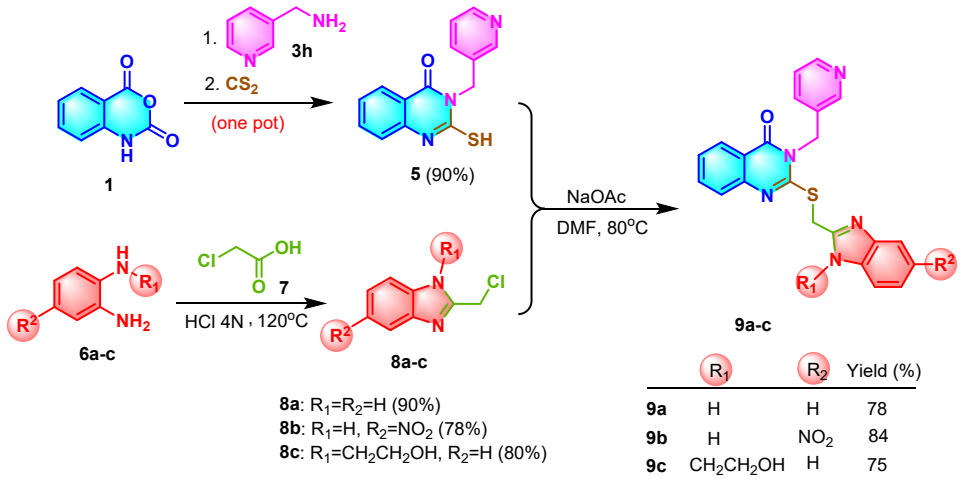
***N*-(3-Hydroxypyridin-2-yl)-2-(4-oxoquinazolin-3(4*H*)-yl)acetamide (4f)**: Yield 41% as white solid. R_f = 0.24 (Hex:EtOAc = 3:1). Mp 215-217°C. HR-ESI-MS found *m/z* 297.0983 $[M+H]^+$ (calcd. 297.0988, $C_{15}H_{12}N_4O_3$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 8.36 (*s*, 1H), 8.15 (*dd*, J_1 = 8.0, J_2 = 1.5, 1H), 7.90 (*dd*, J_1 = 4.5, J_2 = 1.5, 1H), 7.87-7.84 (*m*, 1H), 7.41 (*d*, *J* = 7.5, 1H), 7.58-7.55 (*m*, 1H),7.28 (*dd*, J_1 = 7.5, J_2 = 1.5, 1H), 7.14 (*dd*, J_1 = 8.0, J_2 = 4.5, 1H), 5.03 (*s*, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 167.1, 160.2, 148.5, 148.0, 145.1, 145.0, 139.8, 138.4, 134.6, 127.2, 127.1, 126.0, 124.7, 122.2, 121.4, 48.5.

***N*-(2-Amino-4-methoxyphenyl)-2-(4-oxoquinazolin-3(4*H*)-yl)acetamide (4g)**: Yield 83% as white solid. R_f = 0.42 (Hex:EtOAc = 1:4). Mp 219-221°C. FT-IR (KBr) ν_{max} (cm^{-1}): 3455, 3359, 3254, 2923, 2853, 1660, 1611, 1542, 1514, 1468, 1378, 1325, 1294, 1262, 1212, 1173, 1105, 1033, 777, 700. HR-ESI-MS found *m/z* 323.1141 $[M-H]^-$ (calcd. 323.1144, $C_{17}H_{16}N_4O_3$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 9.43 (*s*, 1H), 8.35 (*s*, 1H), 8.22-8.16 (*m*, 1H), 7.87-7.84 (*m*, 1H), 7.71 (*d*, *J* = 8.0, 1H). 7.57 (*t*, *J* = 7.5, 1H),

6.96 (*d*, *J* = 8.5, 1H), 6.29 (*d*, *J* = 2.5, 1H), 6.12 (*dd*, J_1 = 11.0, J_2 = 2.5, 1H), 4.90 (*s*, 2H), 4.84 (*s*, 2H), 3.66 (*s*, 3H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 165.7, 160.4, 158.2, 148.6, 148.1, 144.1, 134.4, 127.2, 127.1, 126.0, 121.5, 115.5, 101.7, 100.3, 54.8, 48.6.

2-(4-Oxoquinazolin-3(4*H*)-yl)-*N*-(pyridin-3-ylmethyl)acetamide (4h): Yield 80% as white solid. R_f = 0.13 (EtOAc:MeOH = 4:1). Mp 215-217°C. FT-IR (KBr) ν_{max} (cm^{-1}): 3279, 3070, 2991, 2956, 1685, 1658, 1608, 1559, 1470, 1367, 1259, 783, 755. ESI-MS found *m/z* 294.9 $[M+H]^+$ (calcd. 295.1, $C_{16}H_{14}N_4O_2$) and *m/z* 292.8 $[M+H]^+$ (calcd. 293.1, $C_{16}H_{14}N_4O_2$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 8.88 (*t*, *J* = 6.0, 1H), 8.51 (*d*, *J* = 2.0, 1H), 8.47 (*dd*, J_1 = 4.5, J_2 = 1.5, 1H), 8.33 (*s*, 1H), 8.15 (*dd*, J_1 = 8.0, J_2 = 1.5, 1H), 7.86-7.83 (*m*, 1H), 7.71-7.68 (*m*, 2H), 7.58-7.54 (*m*, 1H), 7.36 (*dd*, J_1 = 8.0, J_2 = 5.0, 1H), 4.72 (*s*, 2H), 4.36 (*d*, *J* = 6.0, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 166.9, 160.3, 148.7, 148.5, 148.1, 148.1, 135.0, 134.5, 134.4, 127.2, 127.0, 126.0, 123.4, 121.5, 48.2.

2-(4-Oxoquinazolin-3(4*H*)-yl)-*N*-(thiazol-2-yl)acetamide (4i): Yield 32% as white solid. R_f = 0.33 (Hex:EtOAc = 1:1). Mp 215-217°C. HR-ESI-MS found *m/z* 287.0599 $[M+H]^+$ (calcd. 287.0603, $C_{13}H_{10}N_4O_2S$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 12.61 (*s*, 1H), 8.37 (*s*, 1H), 8.15 (*dd*, J_1 = 8.0, J_2 = 1.0, 1H), 7.89-7.86 (*m*, 1H), 7.73 (*d*, *J* = 7.5, 1H), 7.60-7.56 (*m*, 1H), 7.50 (*d*, *J* = 3.5 Hz, 1H), 7.25 (*d*, *J* = 3.5 Hz, 1H), 4.98 (*s*, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 165.7, 160.2, 157.5, 148.3, 148.0, 137.8, 134.6, 127.2, 125.9, 121.3, 113.9, 48.2.



Scheme S2. Synthesis of compounds 5, 9a-c

Synthetic procedure of compound 5

A mixture of isatoic anhydride **1** (1.0 mmol) and amine **3h** (1.1 mmol) in EtOH (5 mL) was stirred at room temperature for 15 minutes and then KOH (1.0 mmol) was added. The resulting mixture was continued to stirr for another 15 minutes and then CS_2 (0.25 mL) was slowly added. The reaction mixture was refluxed

at 80ºC for 3h. At the end of the reaction, the excess EtOH was evaporated under reduced pressure. The obtained residue was suspended in water (20 mL) followed by adding an aqueous solution of 10% HCl until pH ~ 7. The precipitated solid was filtered and dried at 50ºC. Column chromatography of the crude product obtained the compound **5**.

Synthetic procedure of compound (8a-c)

To a mixture of *N*-substituted *o*-phenylenediamine **6a-c** (1.0 mmol) in aqueous solution of 4 N HCl (2 mL) was added 2-chloroacetic acid **7**. The resulting mixture was refluxed at 120°C for 2h. The reaction mixture was neutralized by aqueous saturated solution of $NaHCO_3$. The equeous layer was extracted with EtOAc (5×20 mL). The combined organic layers were washed with aqueous saturated solution of NaCl, dried over Na_2SO_4, filtered and solvent was evaporated under reduced pressure. Column chromatography of the crude product obtained compounds **8a-c**.

Synthetic procedure of compound 9a-c

A mixture of **5** (0.2 mmol), **8a-c** (0.2 mmol) and NaOAc (0.6 mmol) in DMF (0.5 mL) was stirred at 80°C for 30 minutes. At the end of the reaction, water (50 mL) was added. The equeous layer was extracted with EtOAc (5×5 mL). The combined organic layers were washed with aqueous saturated solution of NaCl, dried over Na_2SO_4, filtered and solvent was evaporated under reduced pressure. Column chromatography of the crude product obtained the compounds **9a-c**.

2-Mercapto-3-(pyridin-3-ylmethyl)quinazolin-4(3*H*)-one (5): Yield 90% as white solid. ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 8.62 (*s*, 1H), 8.45 (*d*, J = 4.0, 1H), 7.97 (*dd*, J_1 = 8.0, J_2 = 1.0, 1H), 7.78-7.75 (*m*, 1H), 7.43 (*d*, *J* = 8.0, 1H), 7.35 (*td*, J_1 = 7.5, J_2 = 1.0, 1H), 7.32 (*dd*, J_1 = 8.0, J_2 = 5.0, 1H), 5.68 (*s*, 2H).

3-(((1*H*-Benzo[*d*]imidazol-2-yl)methyl)thio)-2-(pyridin-3-ylmethyl)isoquinolin-1(2*H*)-one (9a): Yield 78% as white solid. FT-IR (KBr) ν_{max} (cm^{-1}): 3633, 3199, 1684, 1553, 1431, 1159, 745. HR-ESI-MS found *m/z* 400.1232 $[M+H]^+$ (calcd. 400.1232, $C_{22}H_{17}N_5OS$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 8.61 (*d*, *J* = 2.0, 1H), 8.49 (*dd*, J_1 = 4.5, J_2 = 1.5, 1H), 8.12 (*dd*, J_1 = 8.0, J_2 = 1.0, 1H), 7.85-7.82 (*m*, 1H), 7.72 (*dt*, J_1 = 8.0, J_2 = 2.0, 1H), 7.66 (*d*, *J* = 8.0, 1H), 7.52-7.48 (*m*, 3H), 7.35 (*ddd*, J_1 = 8.0, J_2 = 5.0, J_3 = 1.0, 1H), 7.18-7.15 (*m*, 1H), 5.28 (*s*, 2H), 4.82 (*s*, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 160.9, 155.6, 149.6, 148.7, 148.6, 146.7, 135.0, 134.8, 131.4, 126.5, 126.3, 123.6, 121.9, 118.8, 44.9, 29.5.

3-(((5-Nitro-1*H*-benzo[*d*]imidazol-2-yl)methyl)thio)-2-(pyridin-3-ylmethyl)isoquinolin-1(2*H*)-one (9b) . Yield 84% as white solid. FT-IR (KBr) ν_{max} (cm^{-1}): 3438, 2994, 1695, 1686, 1548, 1472, 1336, 1167, 670. HR-ESI-MS found *m/z* 445.1081 $[M+H]^+$ (calcd. 445.1083, $C_{22}H_{16}N_6O_3S$). ^{1}H-NMR (500 MHz,

DMSO-d_6, δ ppm): 13.08 (*s*, 1H), 8.62 (*d*, J = 1.5, 1H), 8.49 (*dd*, J_1 = 4.5, J_2 = 1.5, 1H), 8.42 (*d*, J = 1.5, 1H), 8.11 (*dd*, J_2 = 8.0, J_2 = 1.5, 1H), 8.08 (*dd*, J_1 = 9.0, J_2 = 2.0, 1H), 7.84-7.81 (*m*, 1H), 7.72 (*dt*, J_1 = 8.0, J_2 = 2.0, 1H), 7.67 (*d*, J = 9.0, 1H), 7.63 (*d*, J = 8.0, 1H), 7.49 (*td*, J_1 = 7.5, J_2 = 1.0, 1H), 7.36 (*ddd*, J_1 = 8.0, J_2 = 5.0, J_3 = 1.0, 1H), 5.39 (*s*, 2H), 5.87 (*s*, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 160.9, 155.4, 148.8, 148.6, 146.6, 142.5, 134.9, 134.7, 131.3, 126.5, 126.3, 126.1, 123.6, 118.8, 44.9, 29.4.

3-(((1-(2-Hydroxyethyl)-5-nitro-1*H*-benzo[d]imidazol-2-yl)methyl)thio)-2-(pyridin-3-ylmethyl)isoquinolin-1(2*H*)-one (9c): Yield 75% as white solid. FT-IR (KBr) ν_{max} (cm^{-1}): 3449, 2947, 1675, 1551, 1414, 721. HR-ESI-MS found *m/z* 444.1493 $[M+H]^+$ (calcd. 444.1494, $C_{24}H_{21}N_5O_2S$). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 8.61 (*d*, J = 2.0, 1H), 8.49 (*dd*, J_1 = 4.5, J_2 = 1.5, 1H), 8.11 (*dd*, J_1 = 8.0, J_2 = 1.5, 1H), 7.85-7.82 (*m*, 1H), 7.73 (*dt*, J_1 = 8.0, J_2 = 2.0, 1H), 7.63 (*d*, J = 8.0, 1H), 7.57 (*d*, J = 9.0, 1H), 7.49 (*td*, J_1 = 8.0, J_2 = 1.0, 1H), 7.35 (*ddd*, J_1 = 7.5, J_2 = 4.5, J_3 = 0.5, 1H), 7.21 (*td*, J_1 = 7.5, J_2 = 1.0, 1H), 7.16 (*td*, J_1 = 7.5, J_2 = 1.0, 1H), 5.38 (*s*, 2H), 4.92 (*s*, 2H), 4.47 (*t*, J = 5.5, 2H), 3.75 (*t*, J = 5.5, 2H). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 170.4, 160.9, 155.8, 150.2, 148.7, 148.6, 146.7, 142.1, 135.2, 135.0, 134.7, 131.4, 126.6, 126.3, 126.0, 123.6, 122.1, 121.6, 118.7, 118.6, 110.5, 59.7, 44.9, 28.5, 25.3.

S2. Spectrometry data

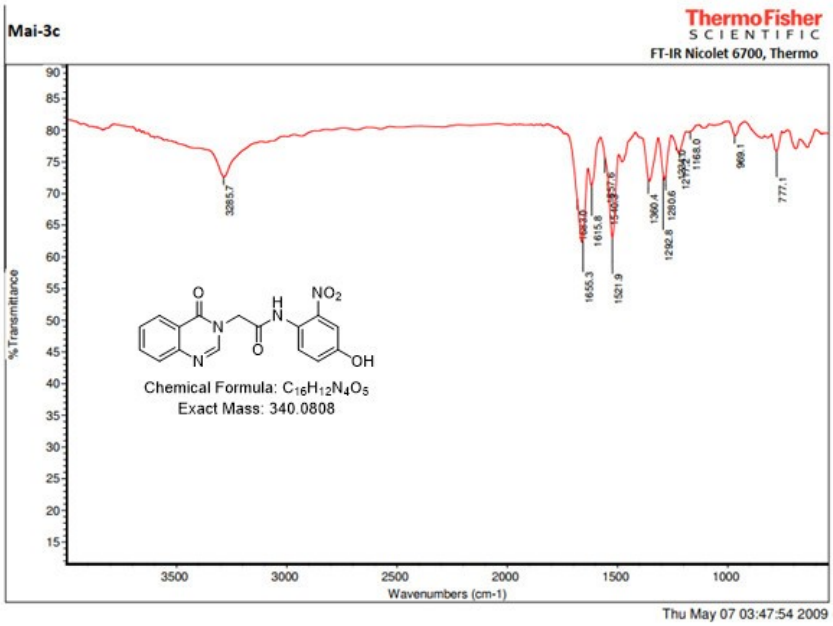


Figure S1. IR spectrum of compound **4a**

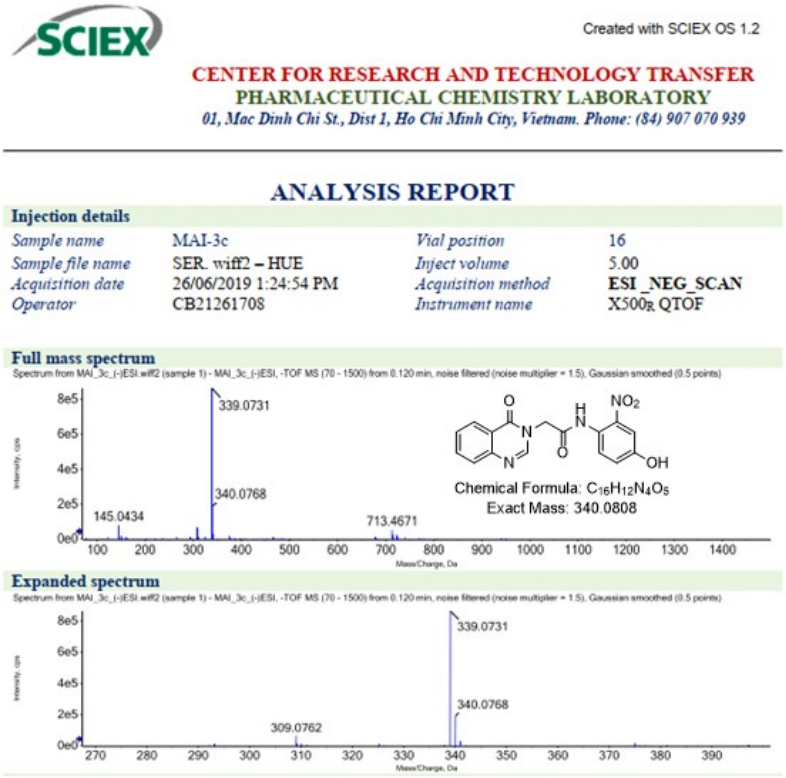


Figure S2. MS spectrum of compound **4a**

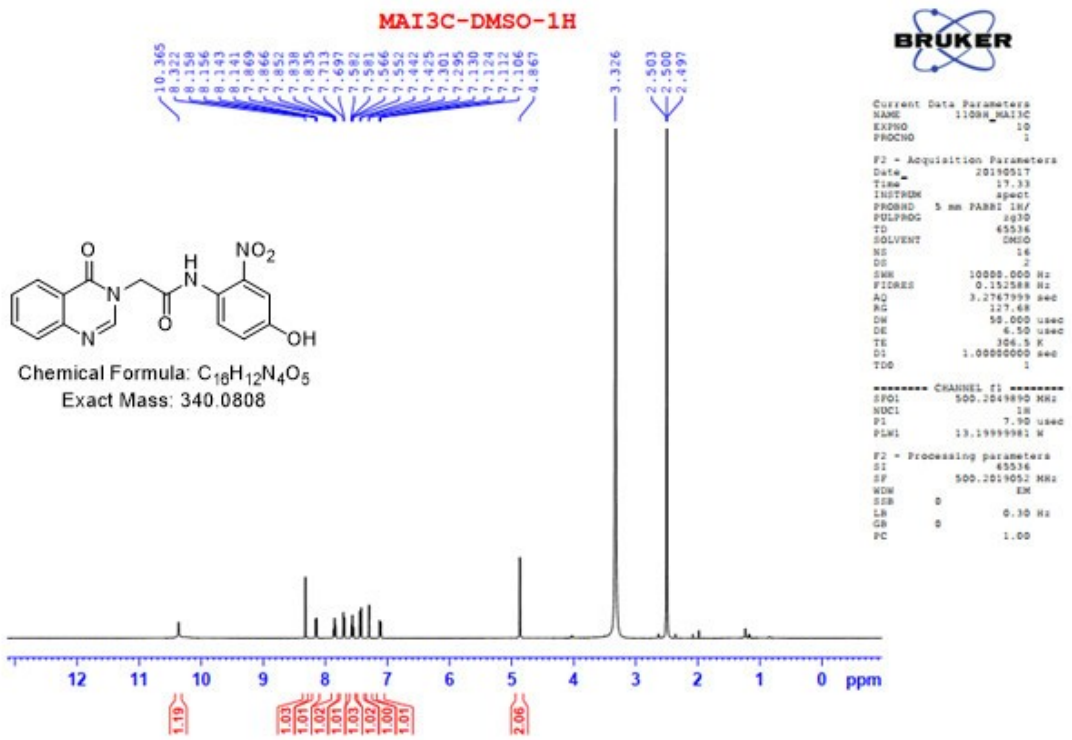


Figure S3. ^{1}H-NMR spectrum of compound **4a**

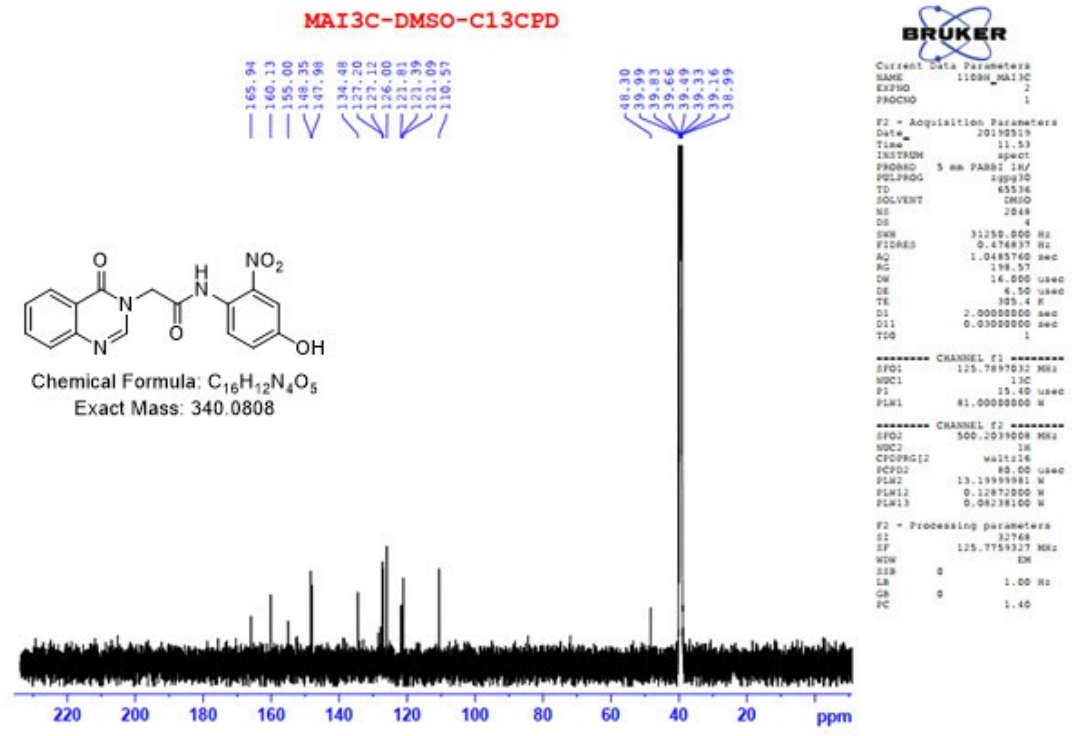


Figure S4. ^{13}C-NMR spectrum of compound **4a**

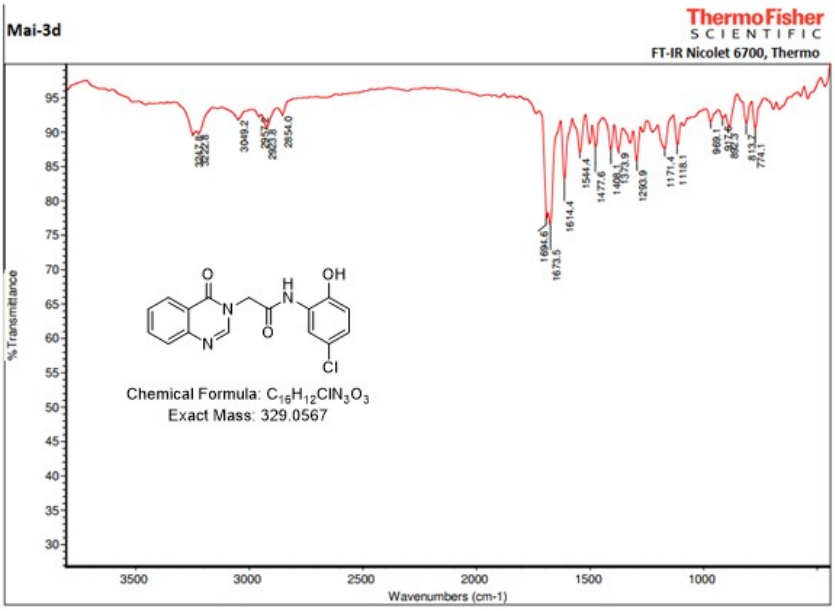


Figure S5. IR spectrum of compound **4b**

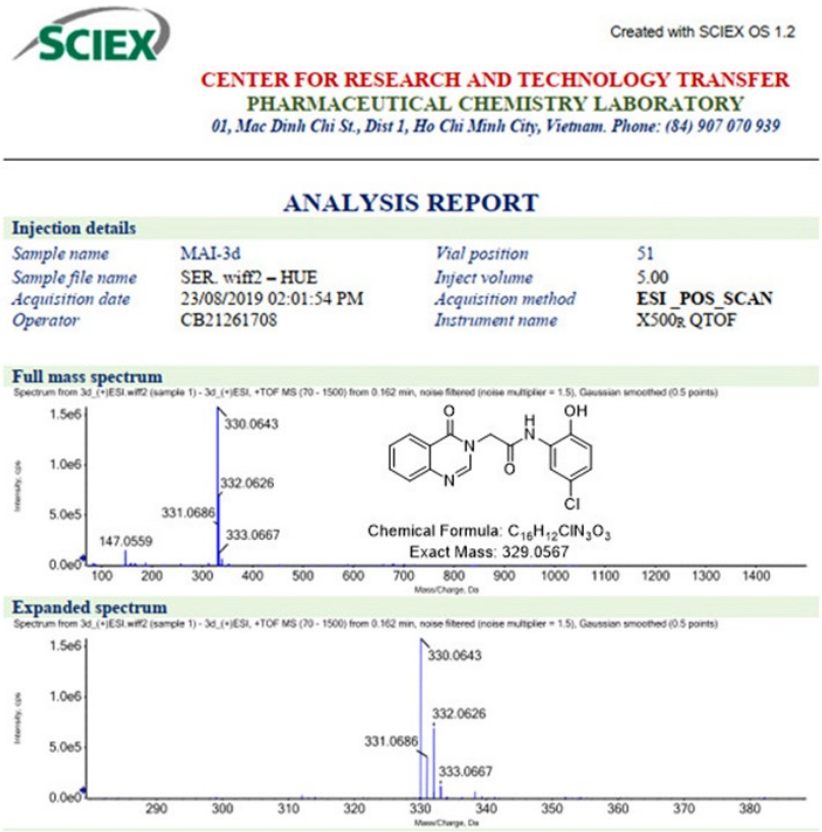


Figure S6. MS spectrum of compound **4b**

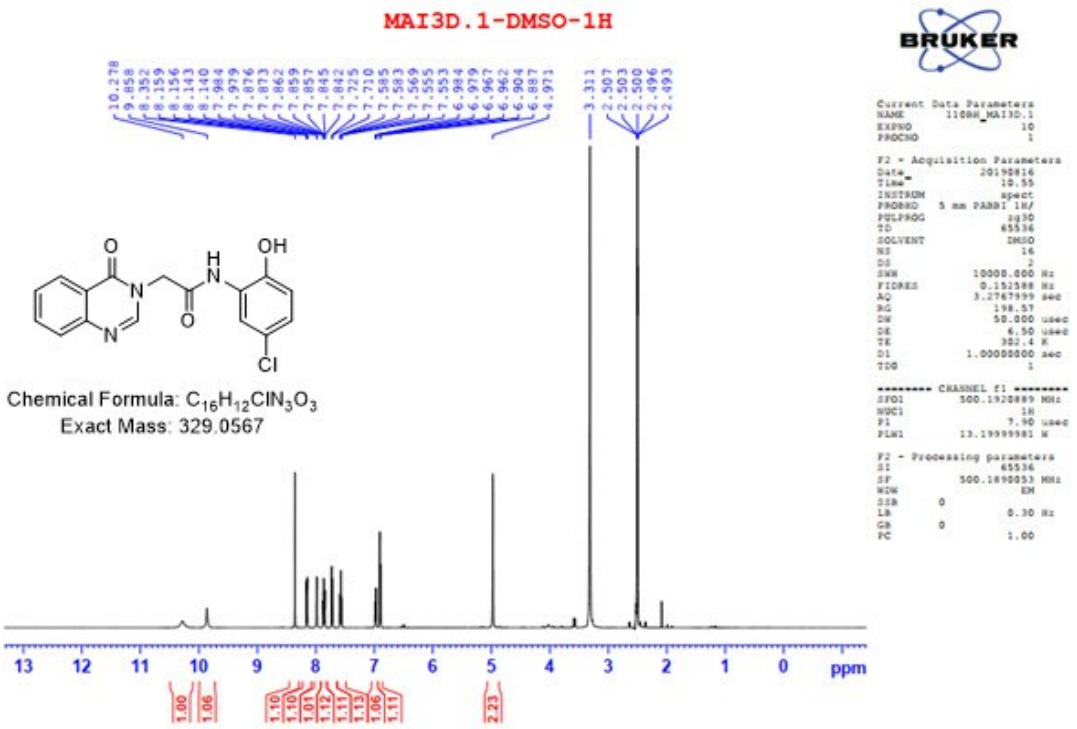


Figure S7. [1]H-NMR spectrum of compound **4b**

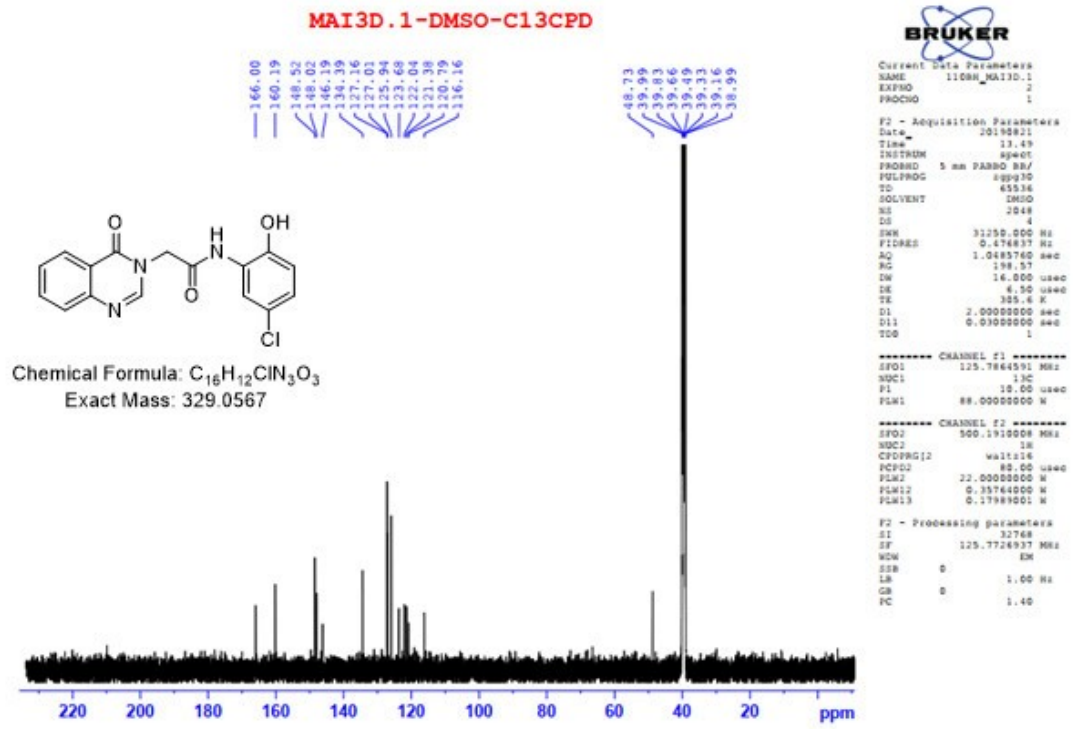


Figure S8. [13]C-NMR spectrum of compound **4b**

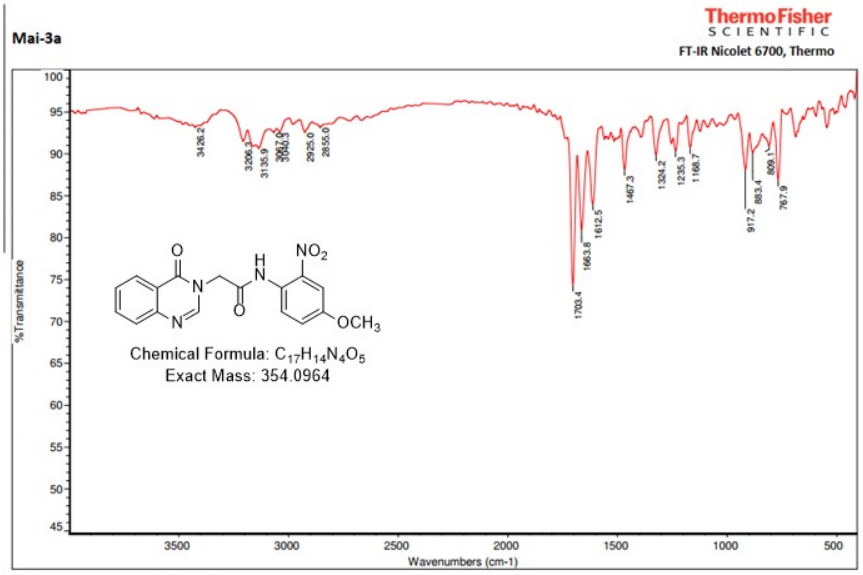


Figure S9. IR spectrum of compound **4c**

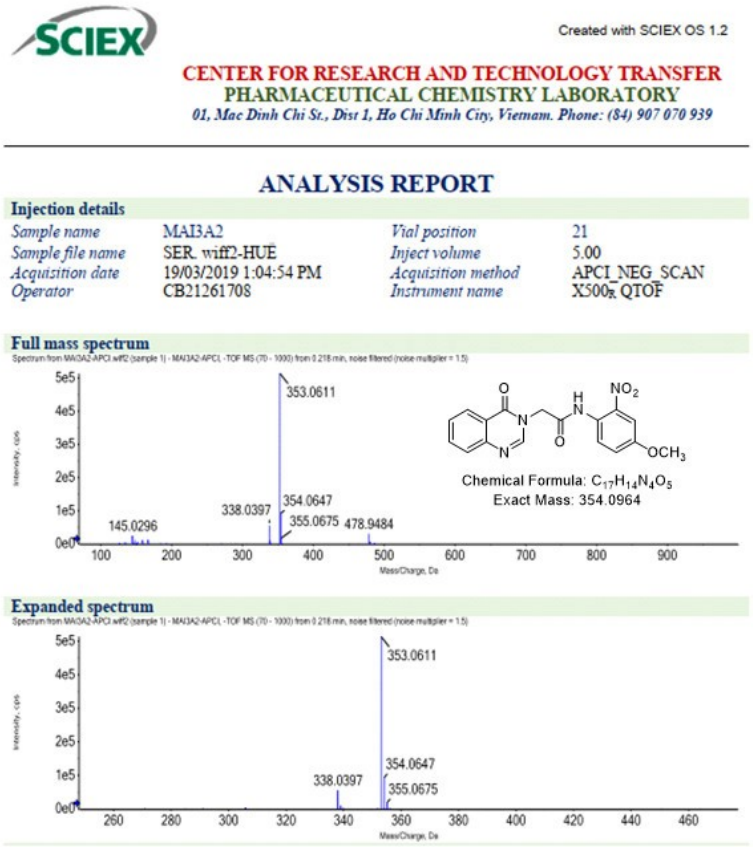


Figure S10. MS spectrum of compound **4c**

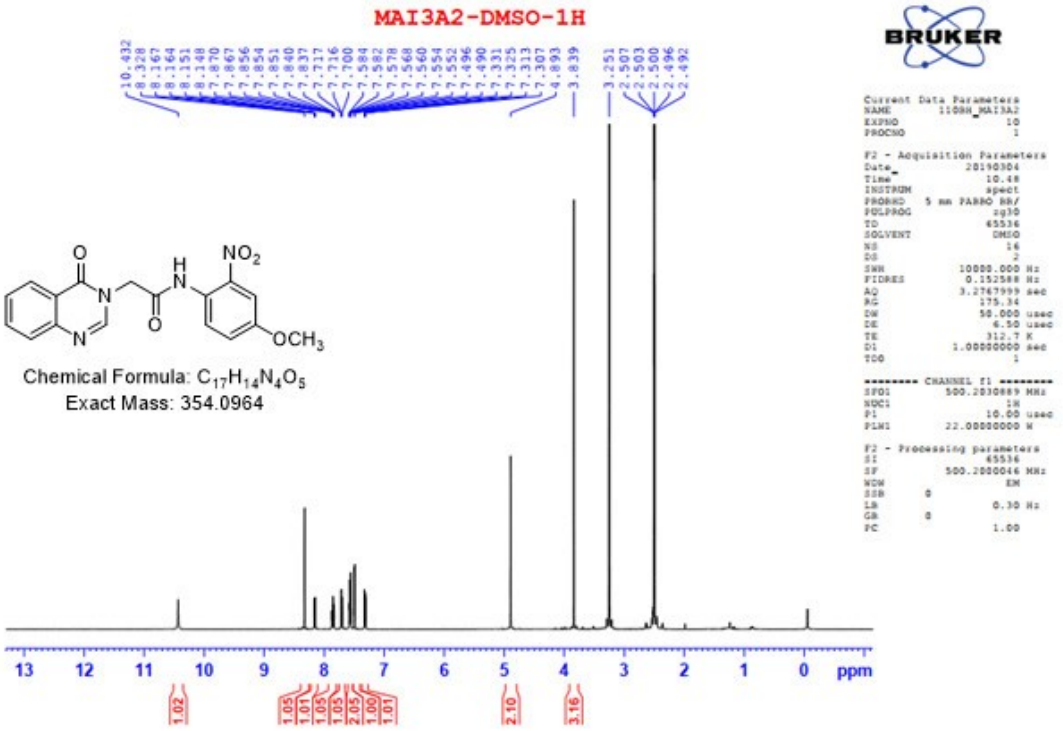


Figure S11. ^{1}H-NMR spectrum of compound **4c**

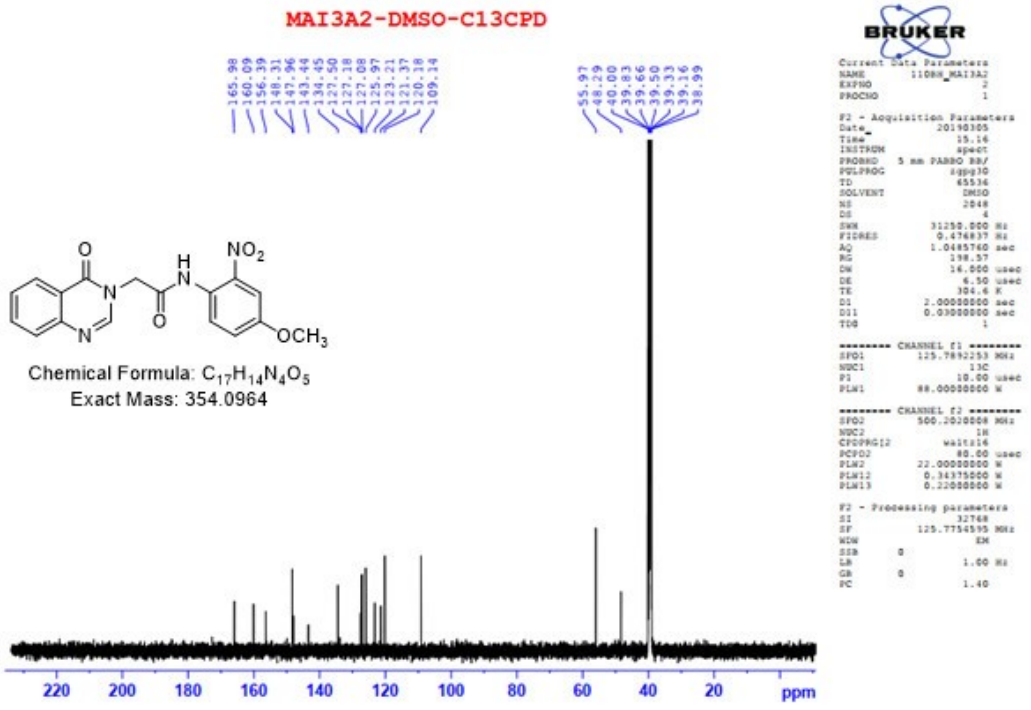


Figure S12. ^{13}C-NMR spectrum of compound **4c**

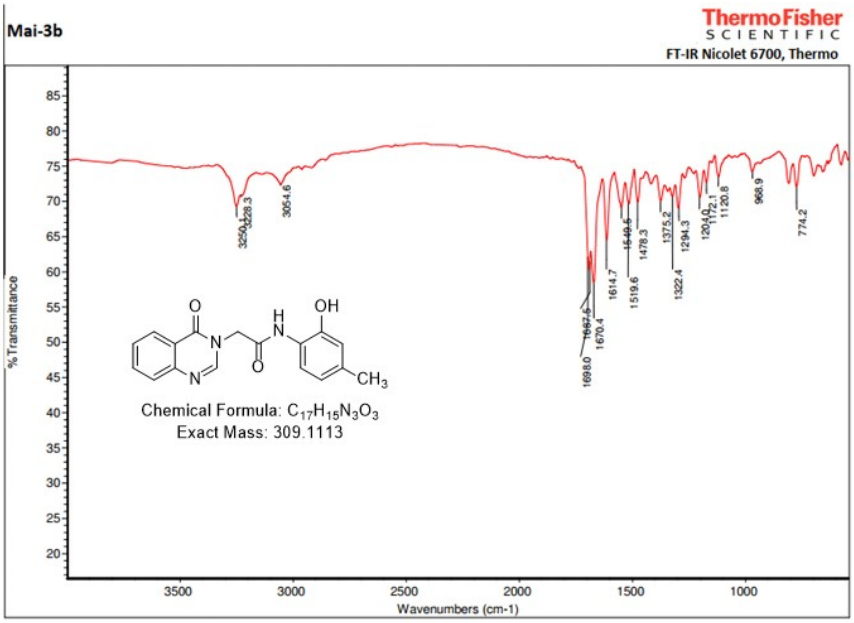


Figure S13. IR spectrum of compound **4d**

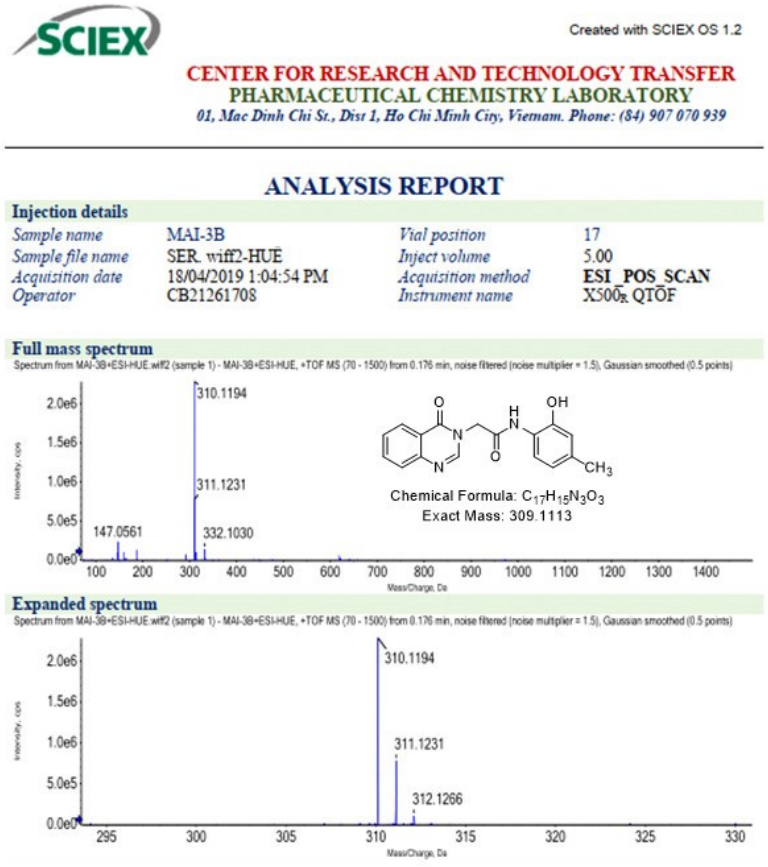


Figure S14. MS spectrum of compound **4d**

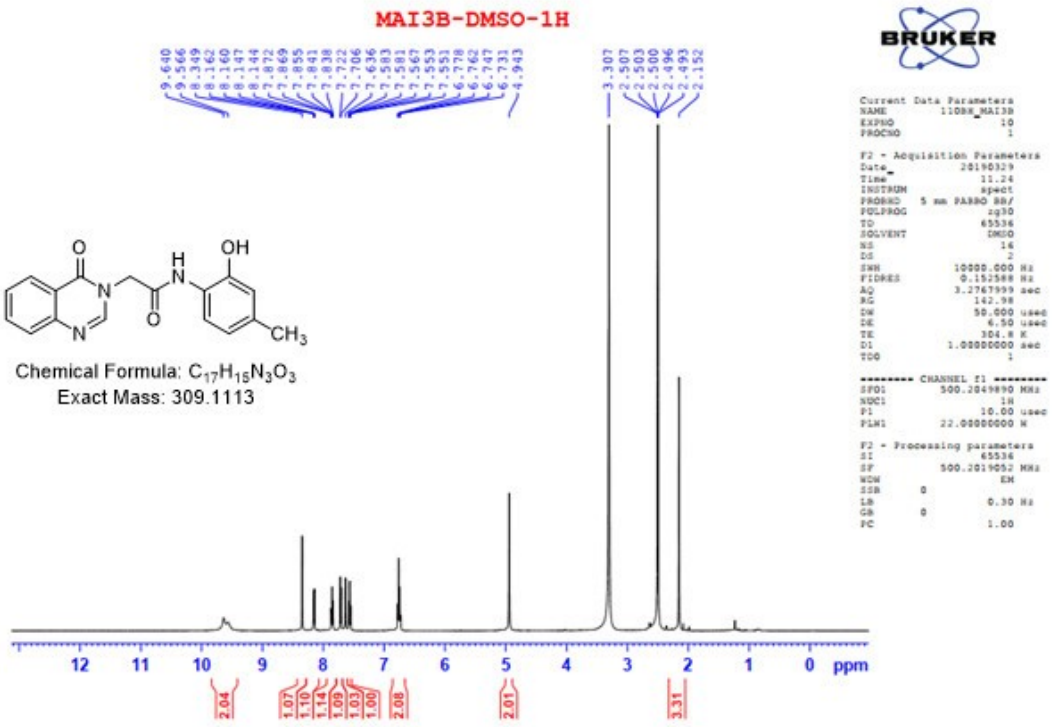


Figure S15. ^{1}H-NMR spectrum of compound **4d**

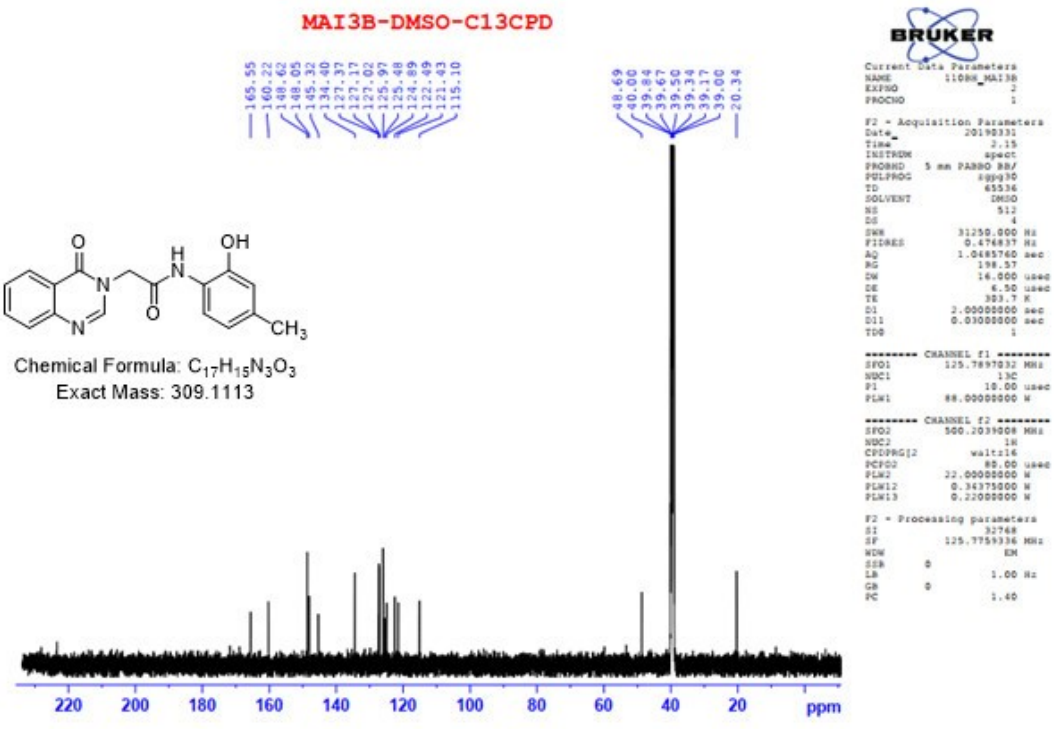


Figure S16. ^{13}C-NMR spectrum of compound **4d**

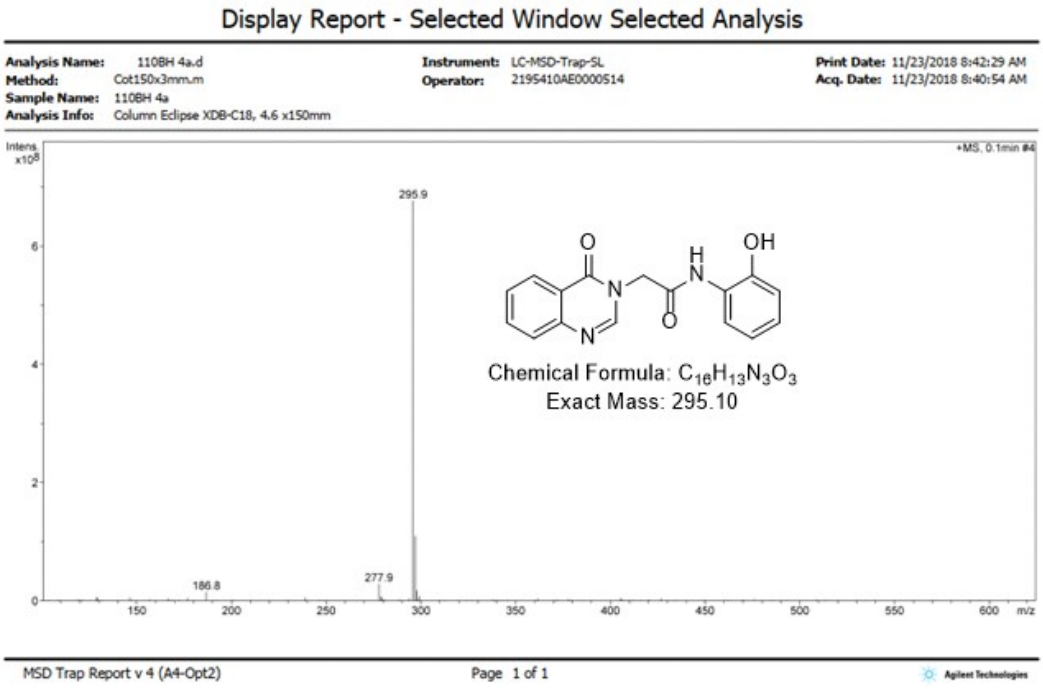


Figure S17. MS $[M+H]^+$ spectrum of compound **4e**

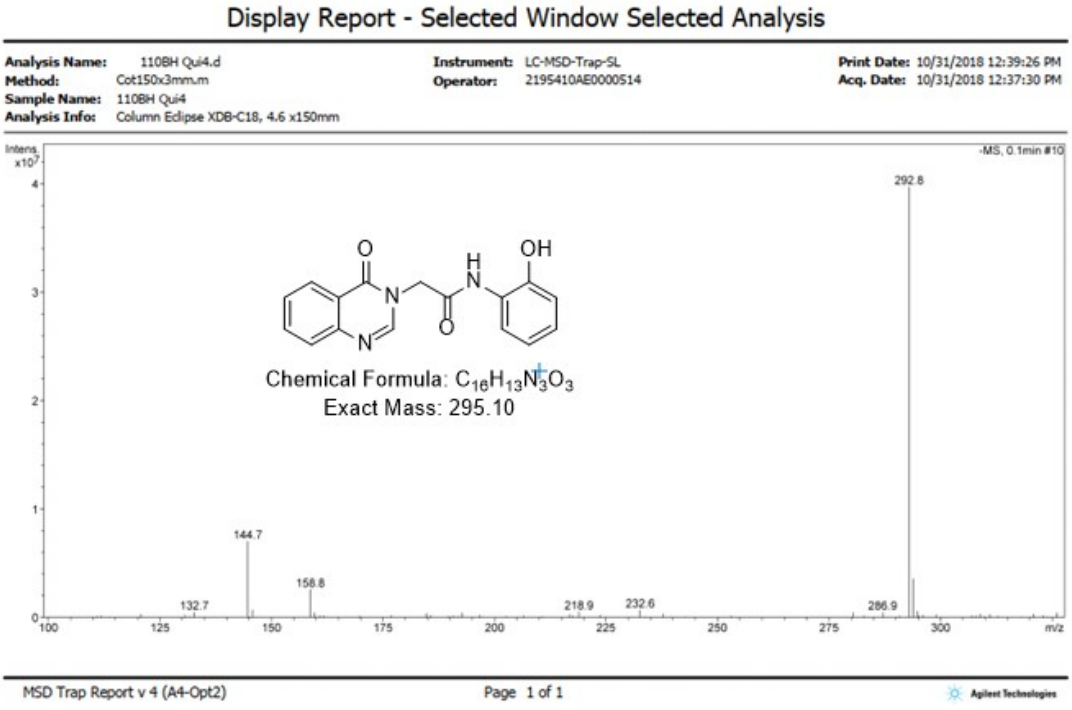


Figure S18. MS $[M-H]^-$ spectrum of compound **4e**

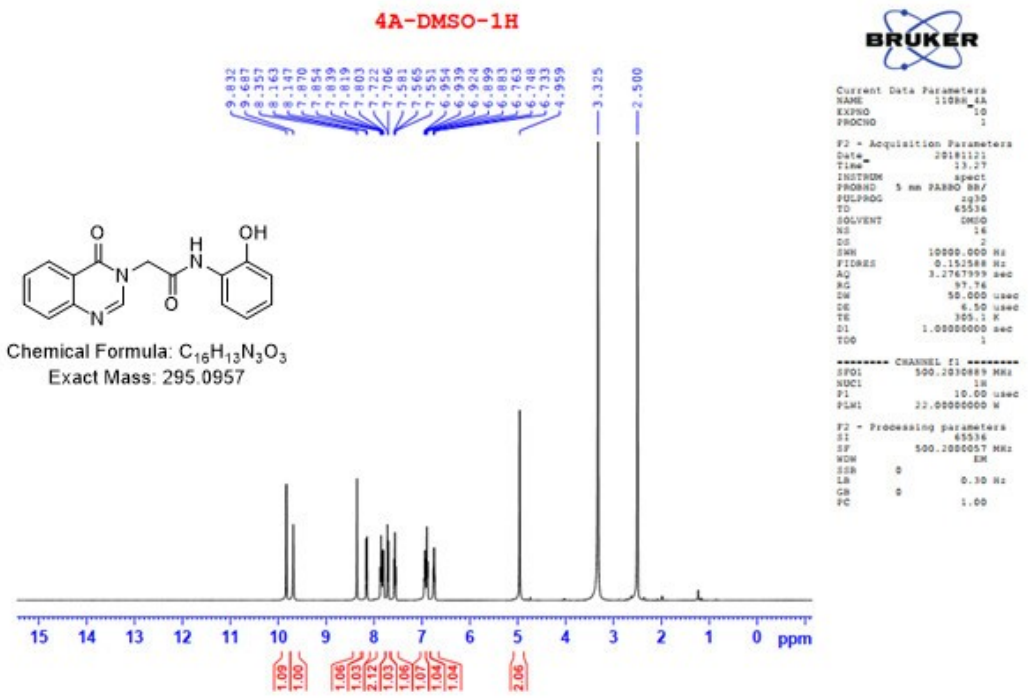


Figure S19. ^{1}H-NMR spectrum of compound **4e**

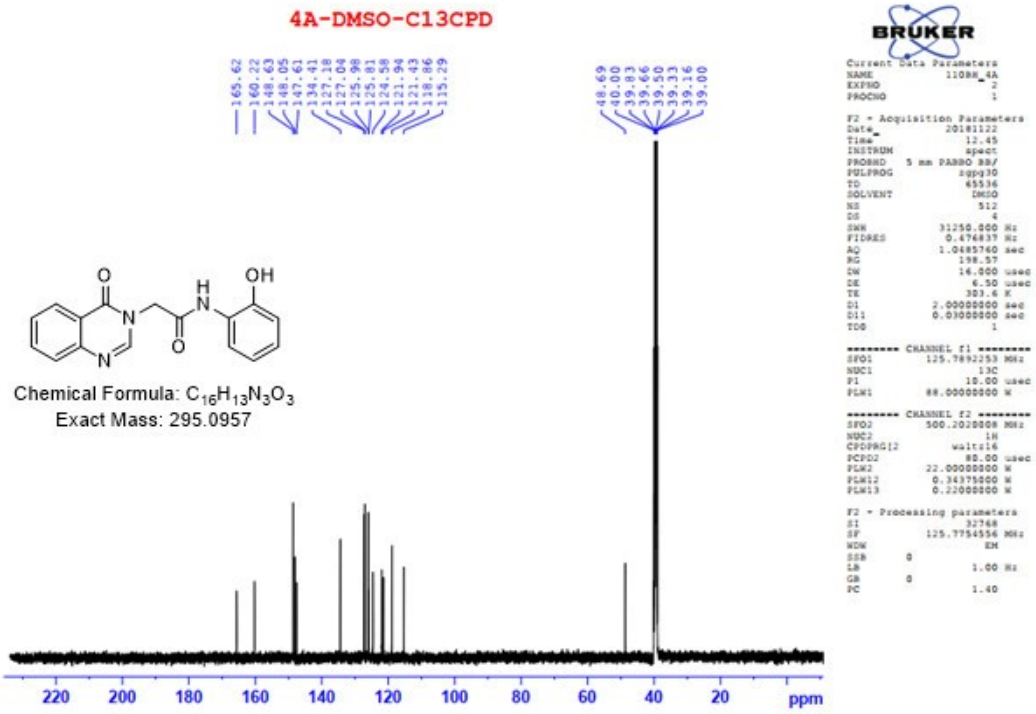


Figure S20. ^{13}C-NMR spectrum of compound **4e**

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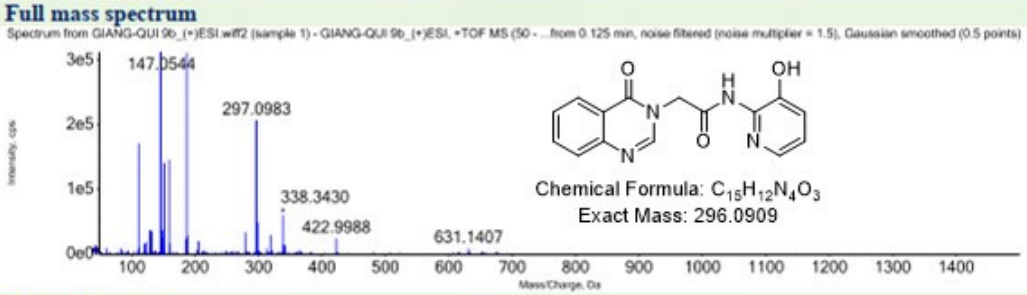
PHARMACEUTICAL CHEMISTRY LABORATORY
01, Mac Dinh Chi St., Dist 1, Ho Chi Minh City, Vietnam. Phone: (84) 907 070 939

ANALYSIS REPORT

Injection details

Sample name	GIANG-QUI 9b	*Vial position*	35
Sample file name	SER. wiff2 – HUE	*Inject volume*	5.00
Acquisition date	10/01/2020 3:25:14 PM	*Acquisition method*	**ESI _POS_SCAN**
Operator	CB21261708	*Instrument name*	X500R QTOF

Full mass spectrum



Expanded spectrum

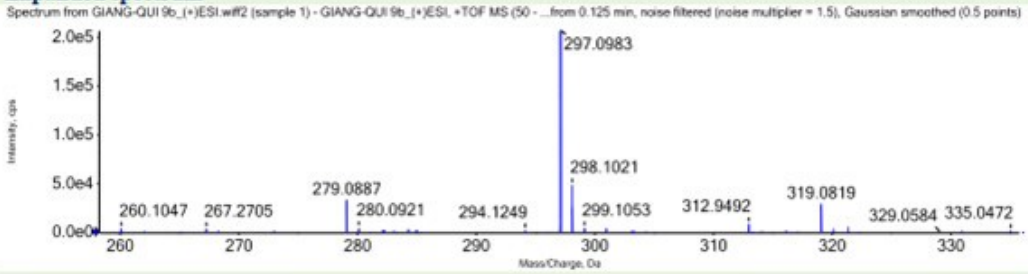


Figure S21. MS spectrum of compound **4f**

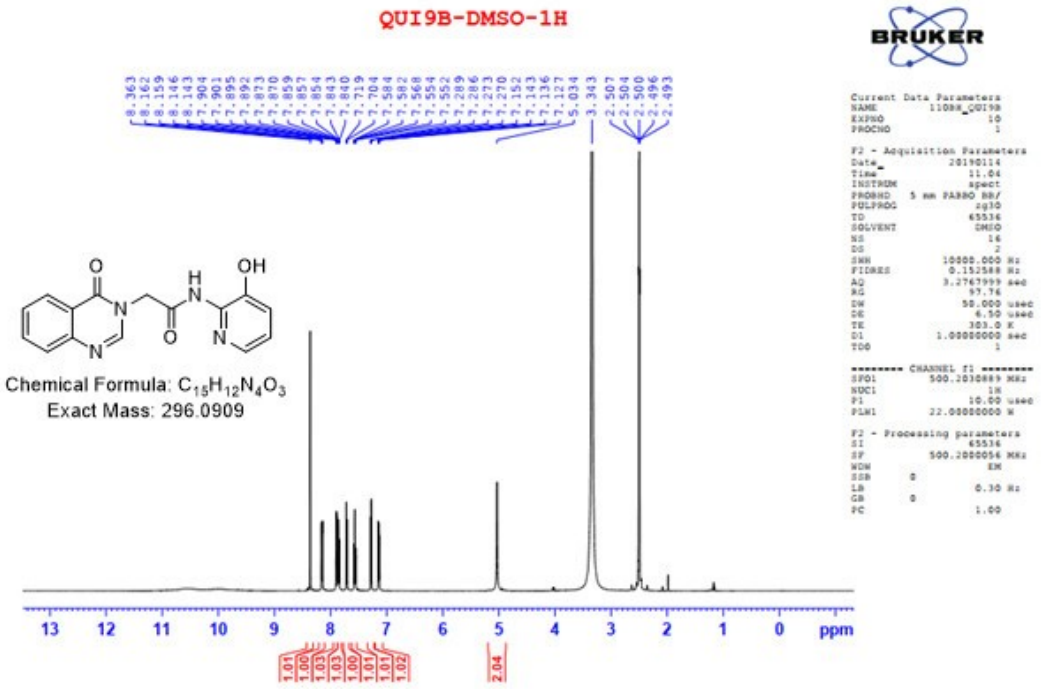


Figure S22. ^{1}H-NMR spectrum of compound **4f**

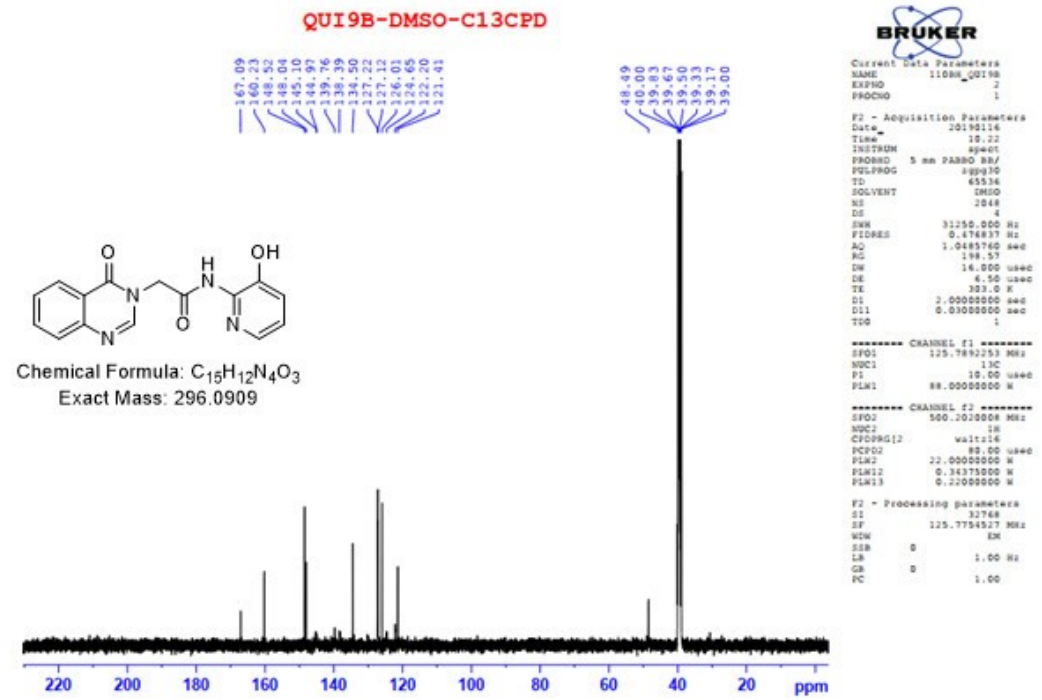


Figure S23. ^{13}C-NMR spectrum of compound **4f**

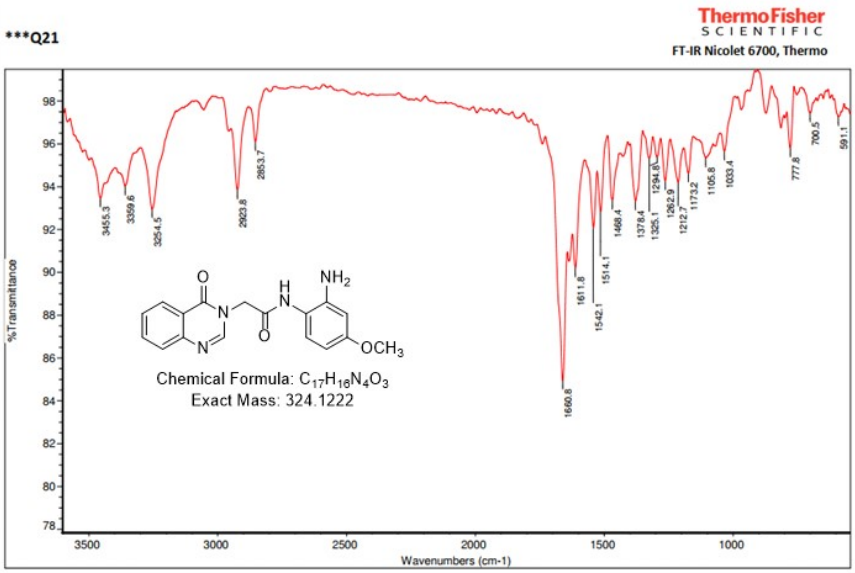


Figure S24. MS spectrum of compound **4g**

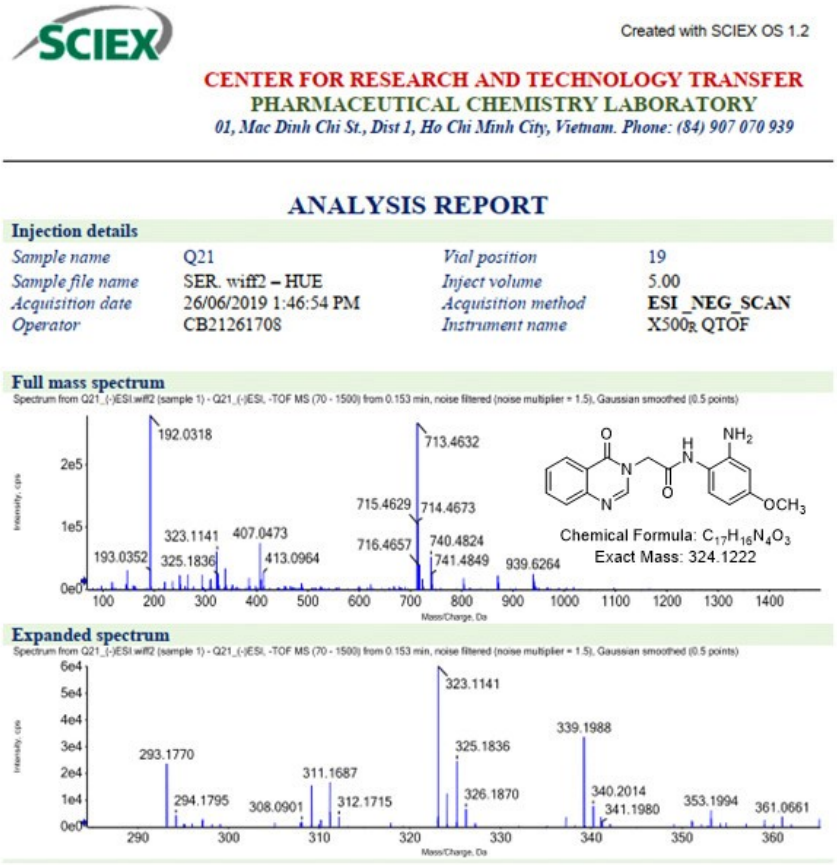


Figure S25. MS spectrum of compound **4g**

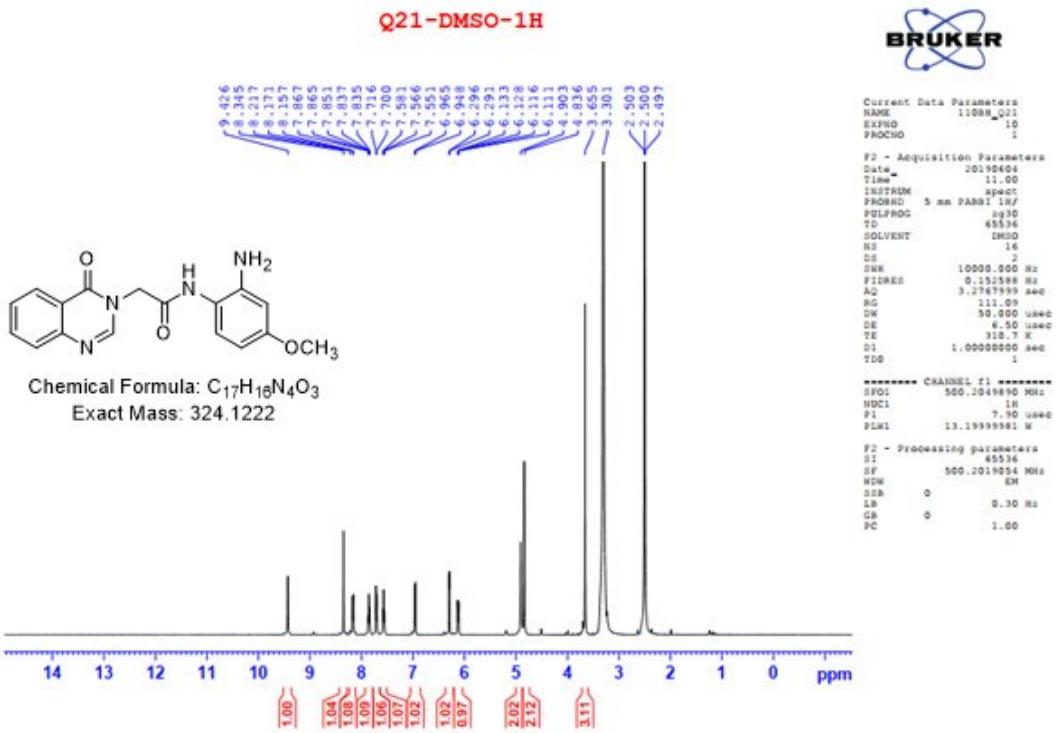


Figure S26. [1]H-NMR spectrum of compound **4g**

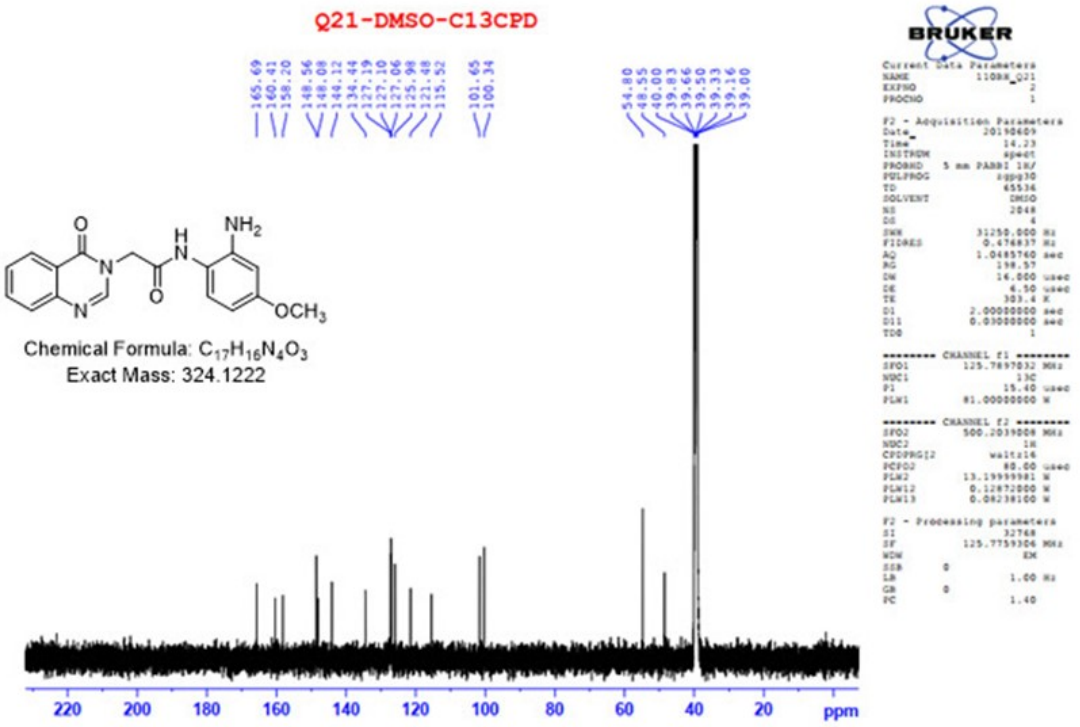


Figure S27. [13]C-NMR spectrum of compound **4g**

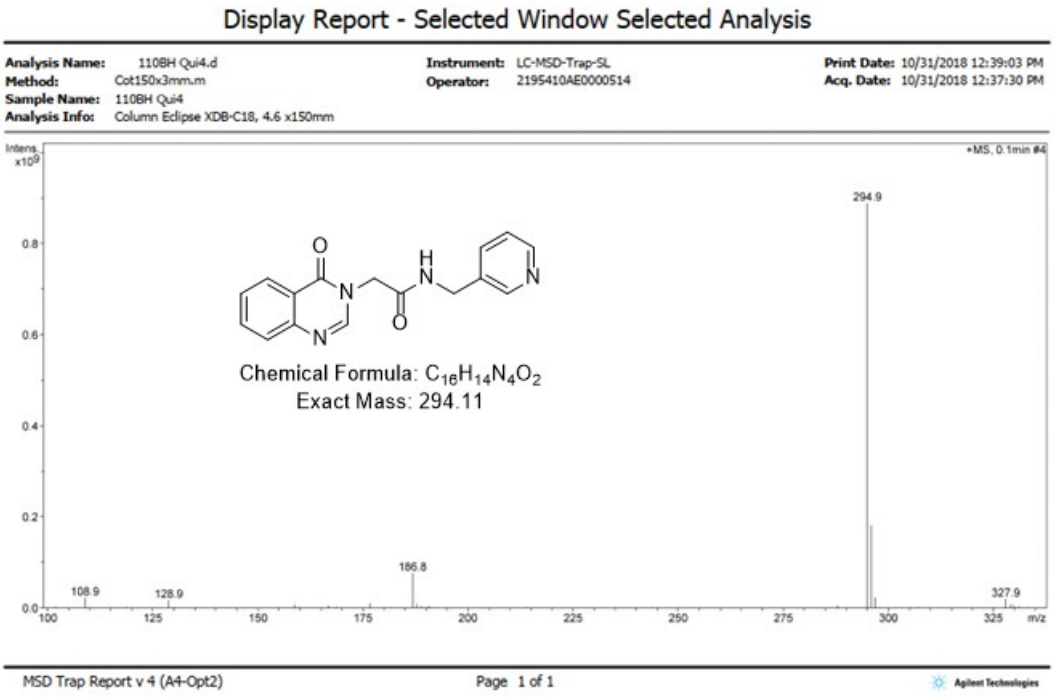


Figure S28. MS $[M+H]^+$ spectrum of compound **4h**

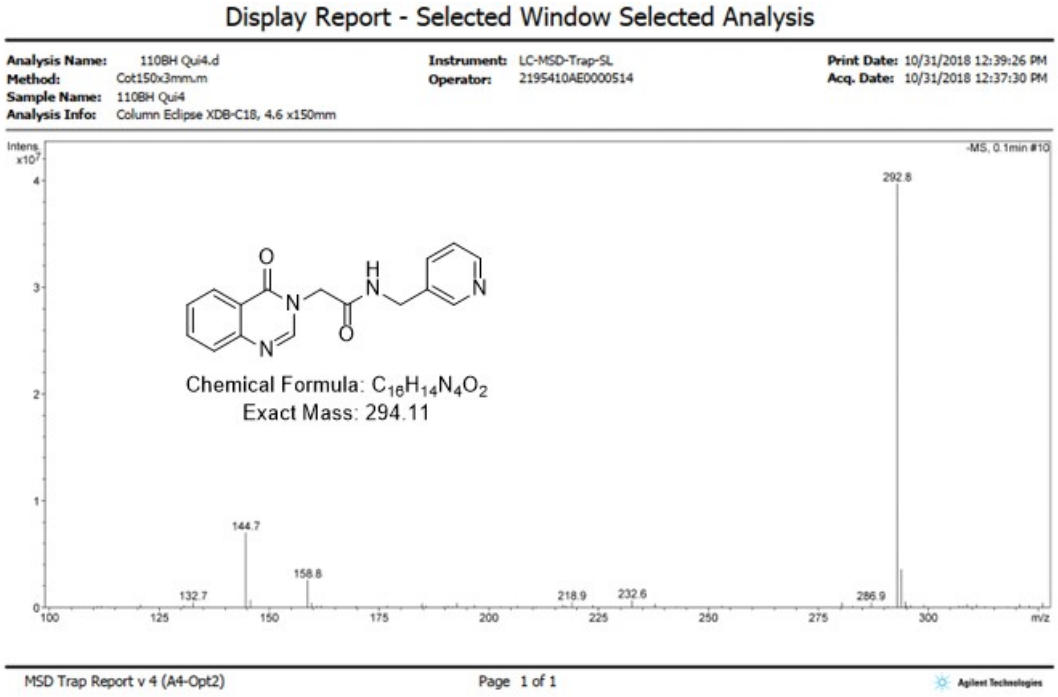


Figure S29. MS $[M-H]^-$ spectrum of compound **4h**

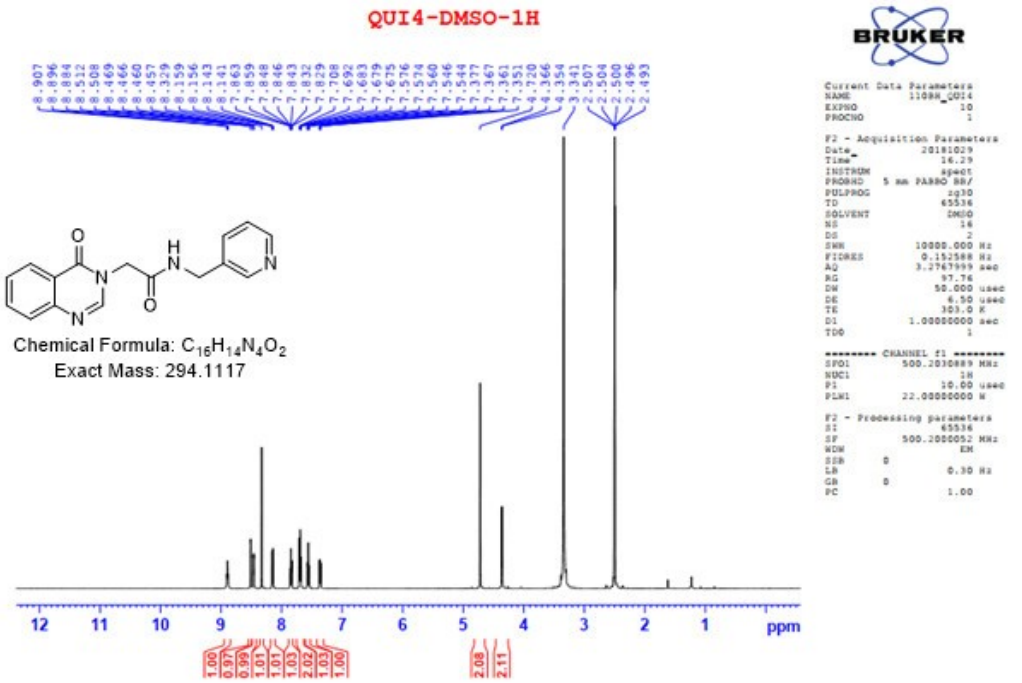


Figure S30. [1]H-NMR spectrum of compound **4h**

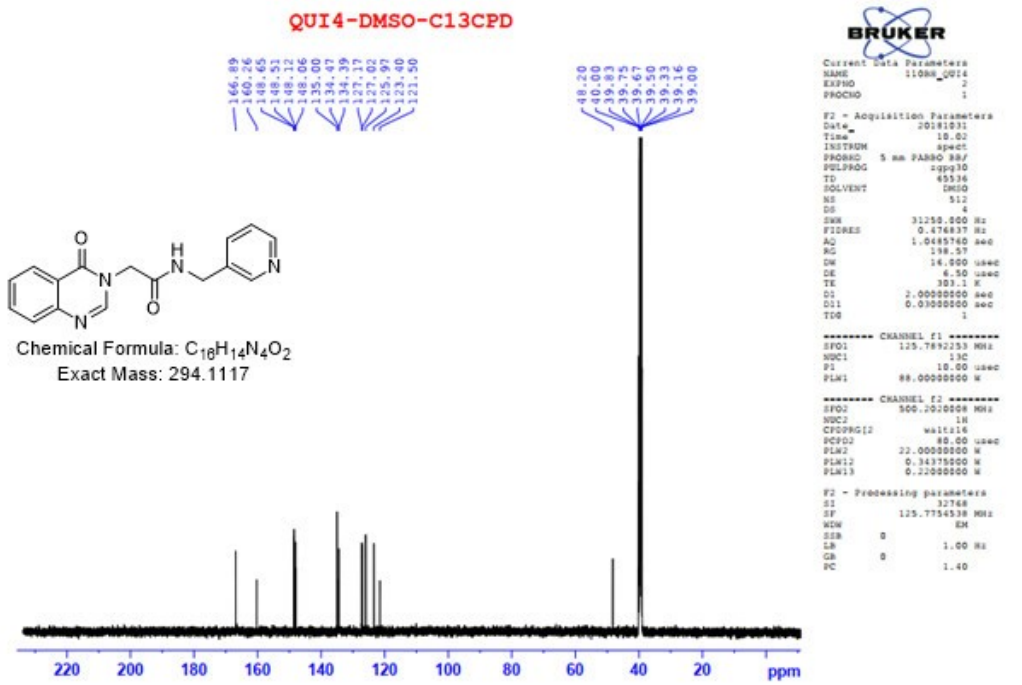


Figure S31. [13]C-NMR spectrum of compound **4h**

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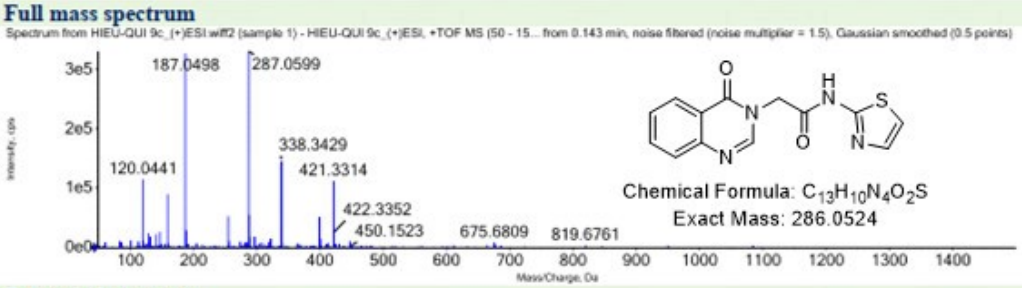
PHARMACEUTICAL CHEMISTRY LABORATORY
01, Mac Dinh Chi St., Dist 1, Ho Chi Minh City, Vietnam. Phone: (84) 907 070 939

ANALYSIS REPORT

Injection details

Sample name	HIEU-QUI 9c	*Vial position*	36
Sample file name	SER. wiff2 – HUE	*Inject volume*	5.00
Acquisition date	10/01/2020 3:27:14 PM	*Acquisition method*	**ESI_POS_SCAN**
Operator	CB21261708	*Instrument name*	X500$_R$ QTOF

Full mass spectrum



Expanded spectrum

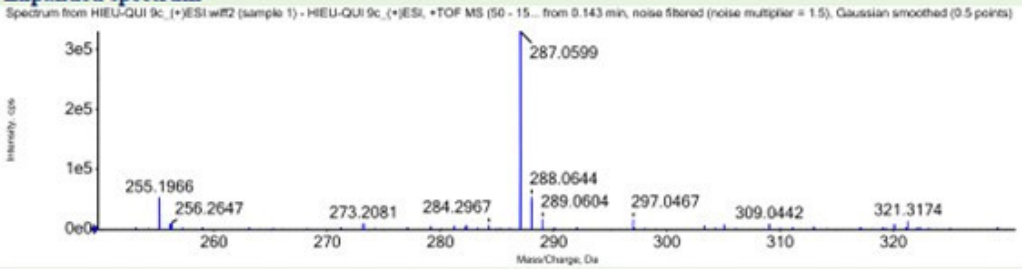


Figure S32. MS spectrum of compound **4i**

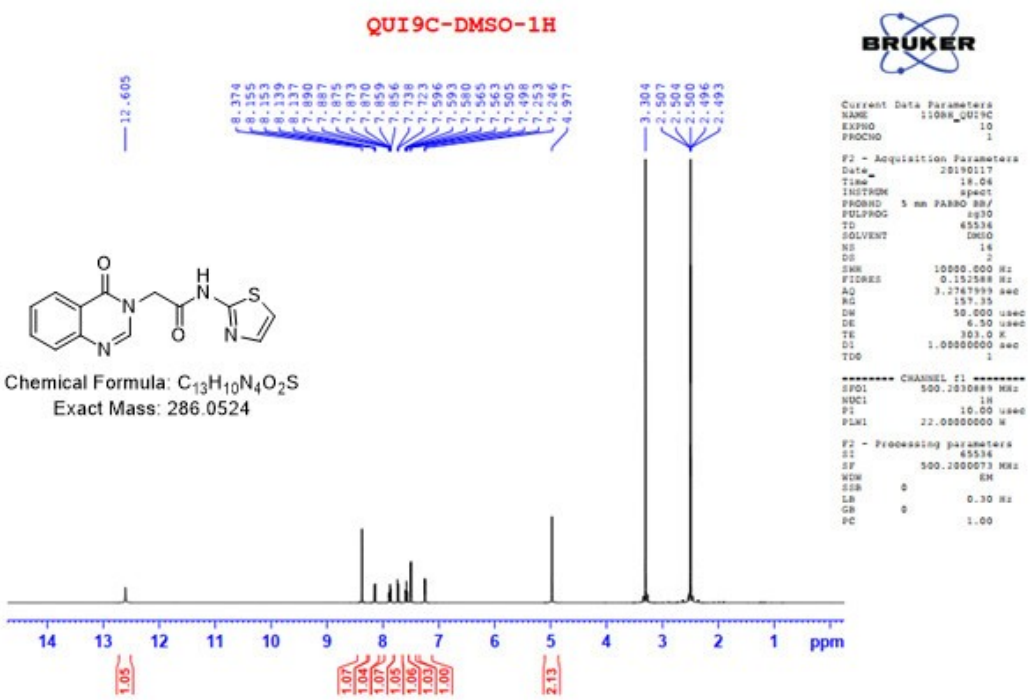


Figure S33. ^{1}H-NMR spectrum of compound **4i**

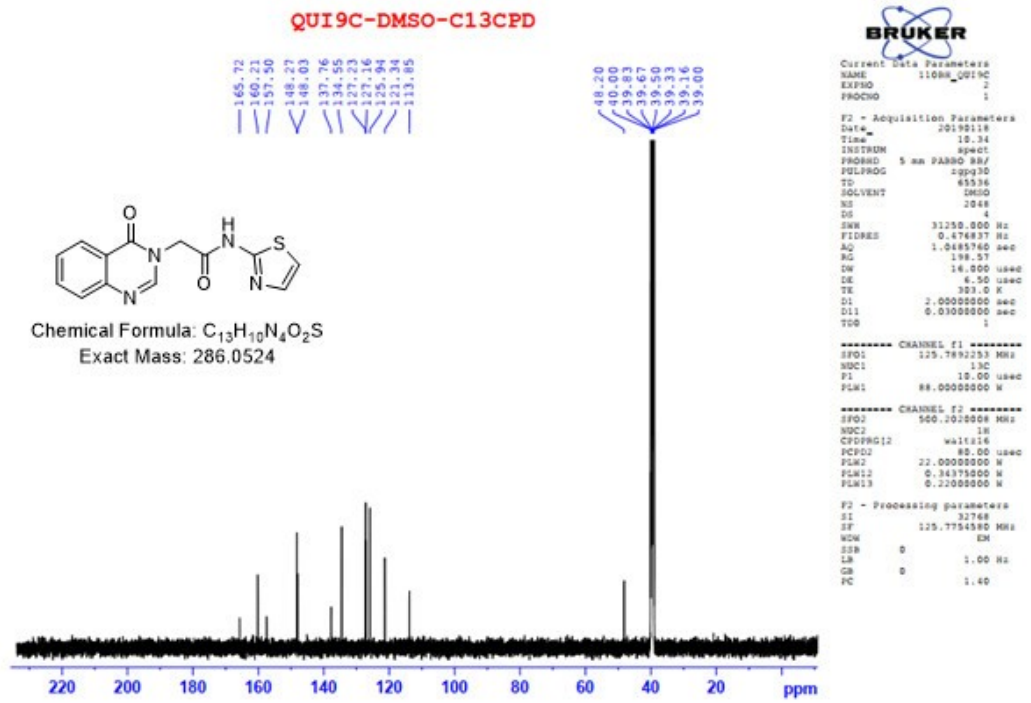


Figure S34. ^{13}C-NMR spectrum of compound **4i**

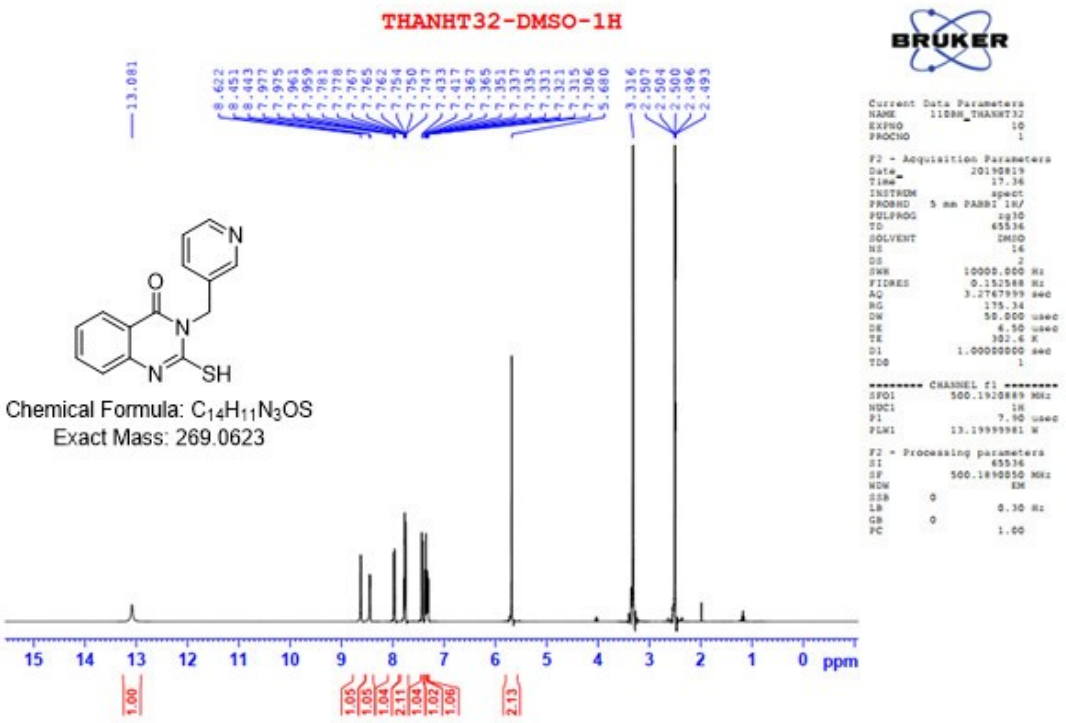


Figure S35. [1]H-NMR spectrum of compound **5**

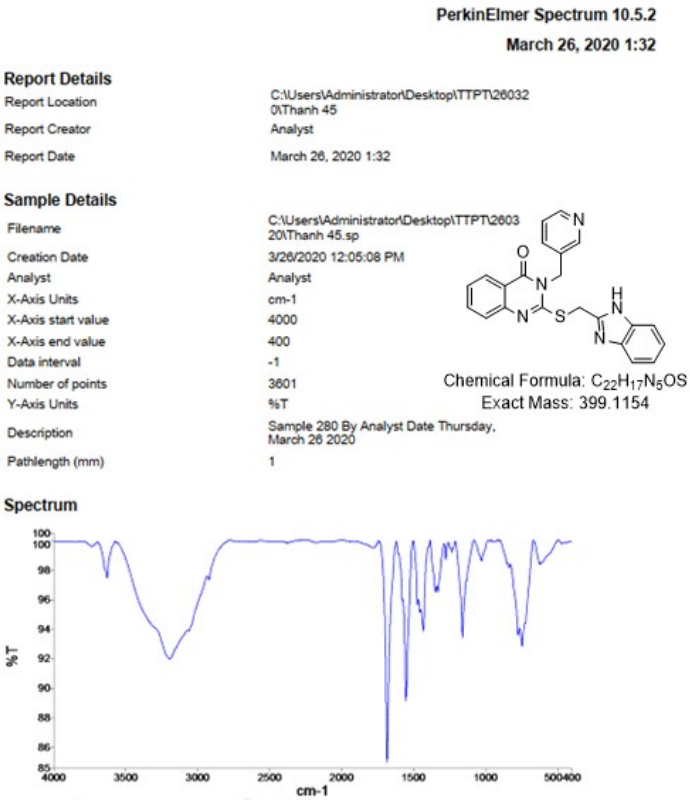


Figure S36. IR spectrum of compound **9a**

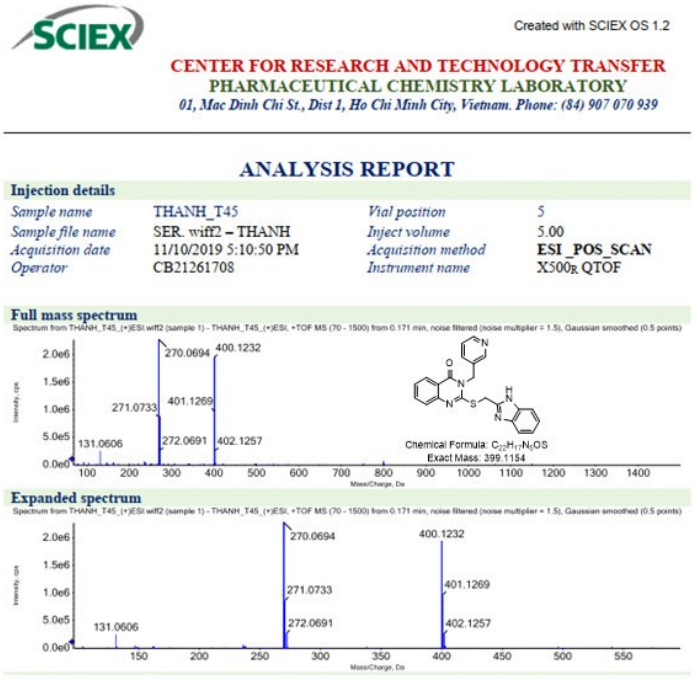


Figure S37. MS spectrum of compound **9a**

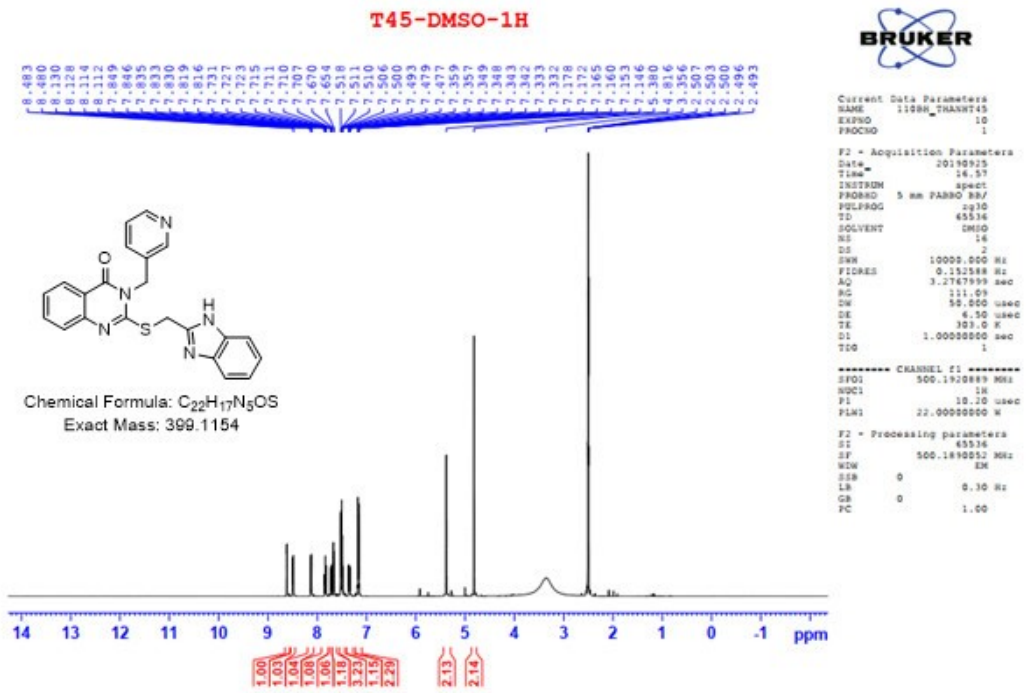


Figure S38. [1]H-NMR spectrum of compound **9a**

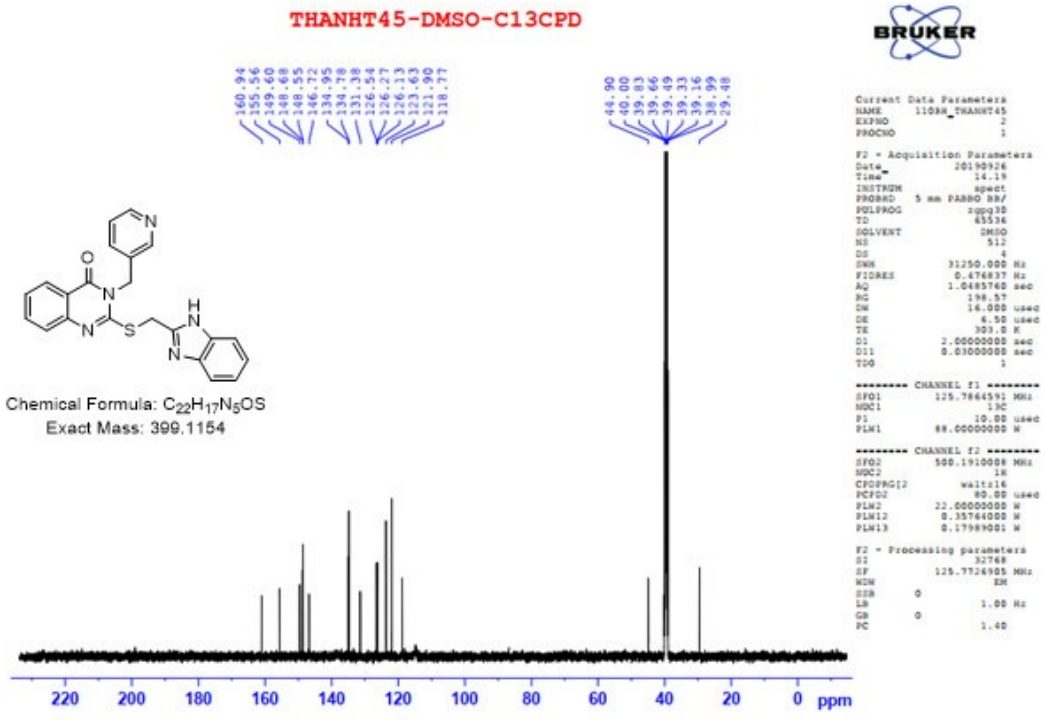


Figure S39. [13]C-NMR spectrum of compound **9a**

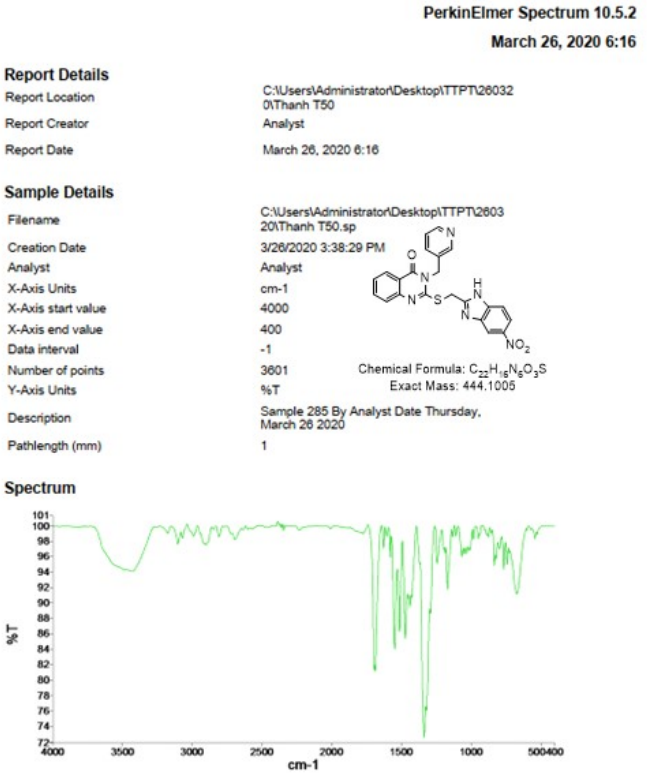


Figure S40. MS spectrum of compound **9b**

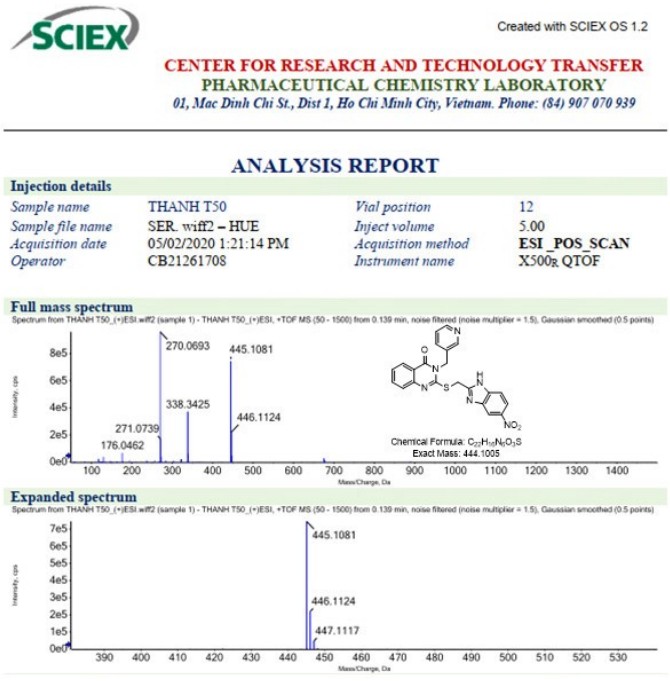


Figure S41. MS spectrum of compound **9b**

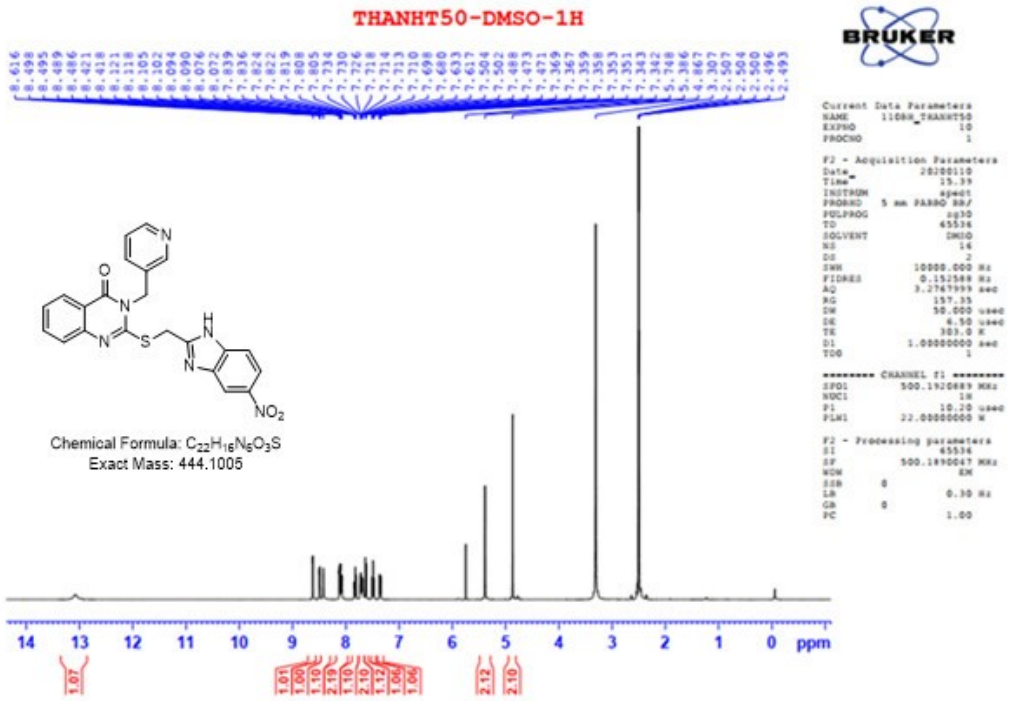


Figure S42. [1]H-NMR spectrum of compound **9b**

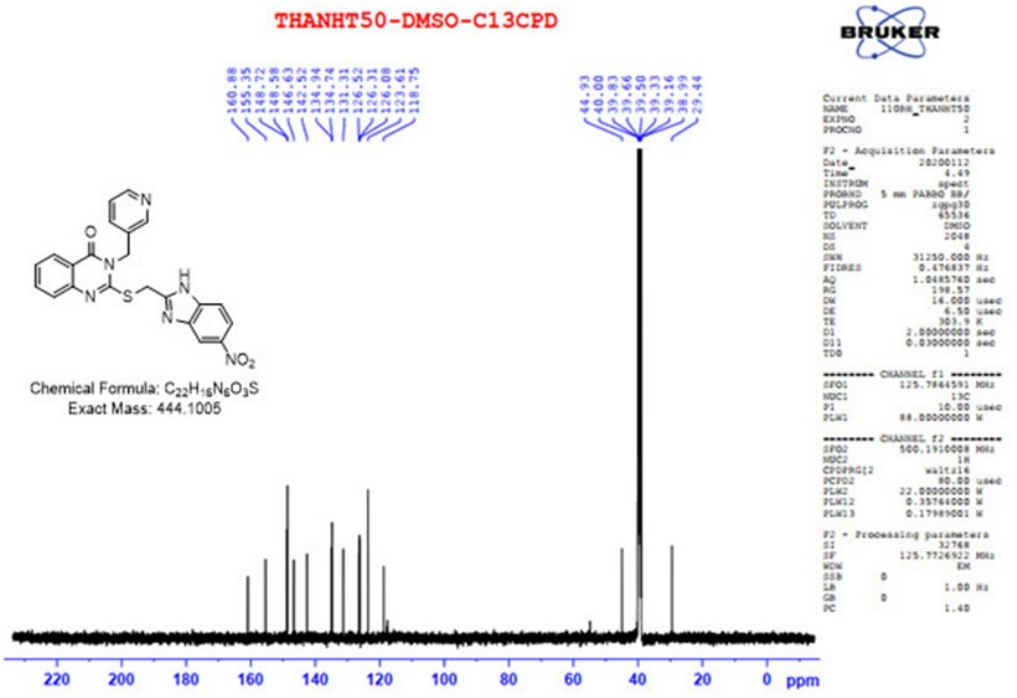


Figure S43. [13]C-NMR spectrum of compound **9b**

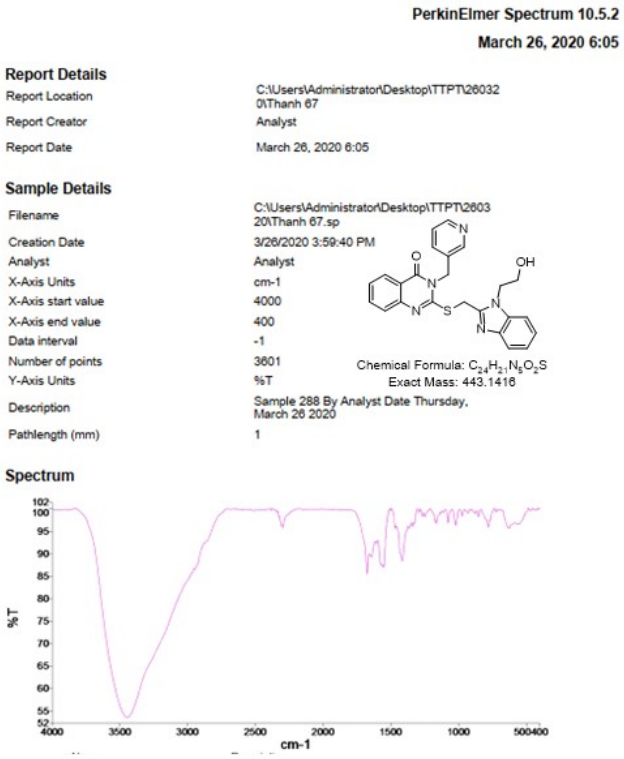


Figure S44. IR spectrum of compound **9c**

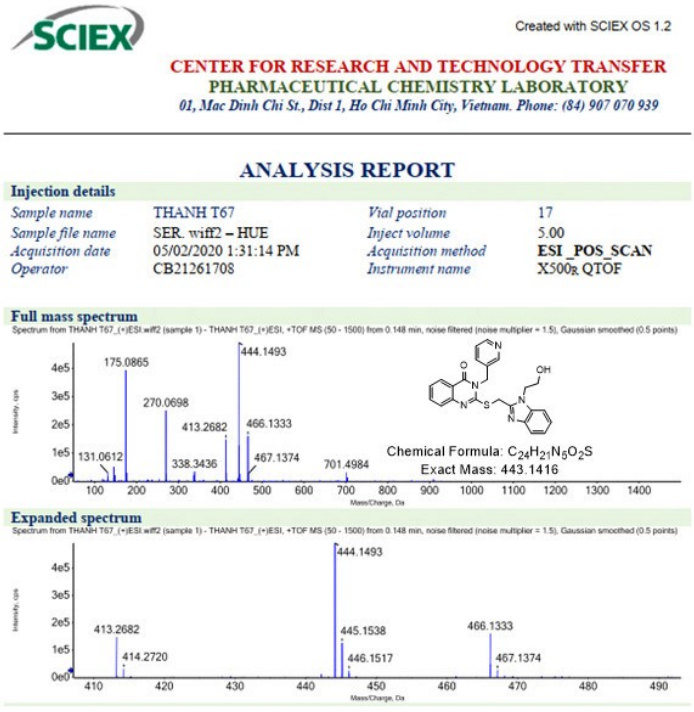


Figure S45. MS spectrum of compound **9c**

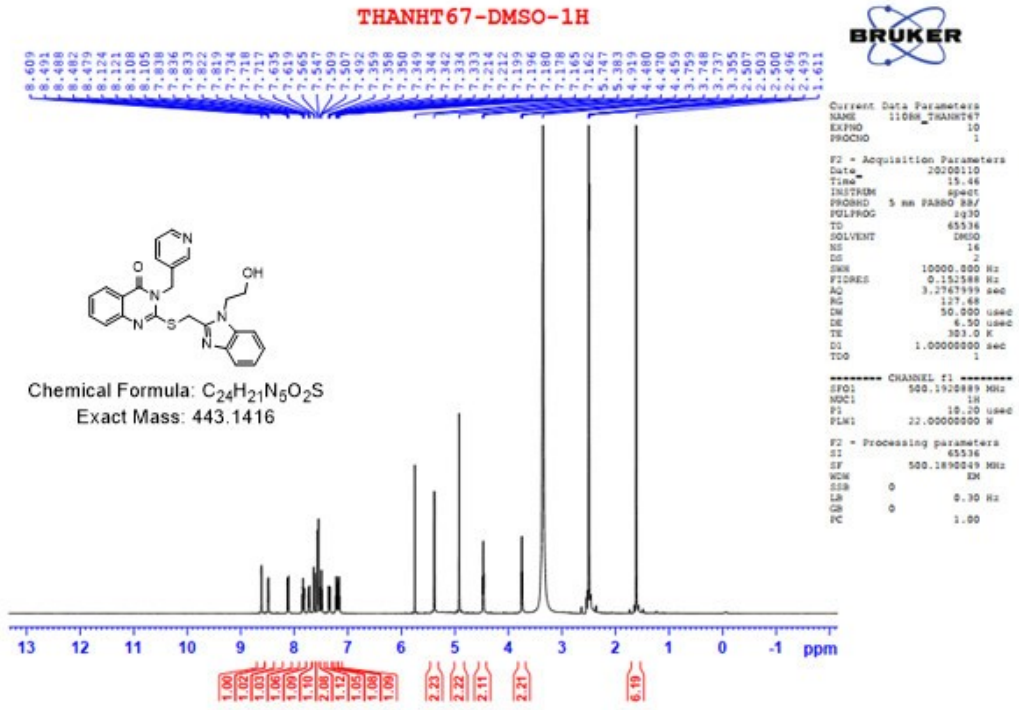


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Figure S46. ^{1}H-NMR spectrum of compound **9c**

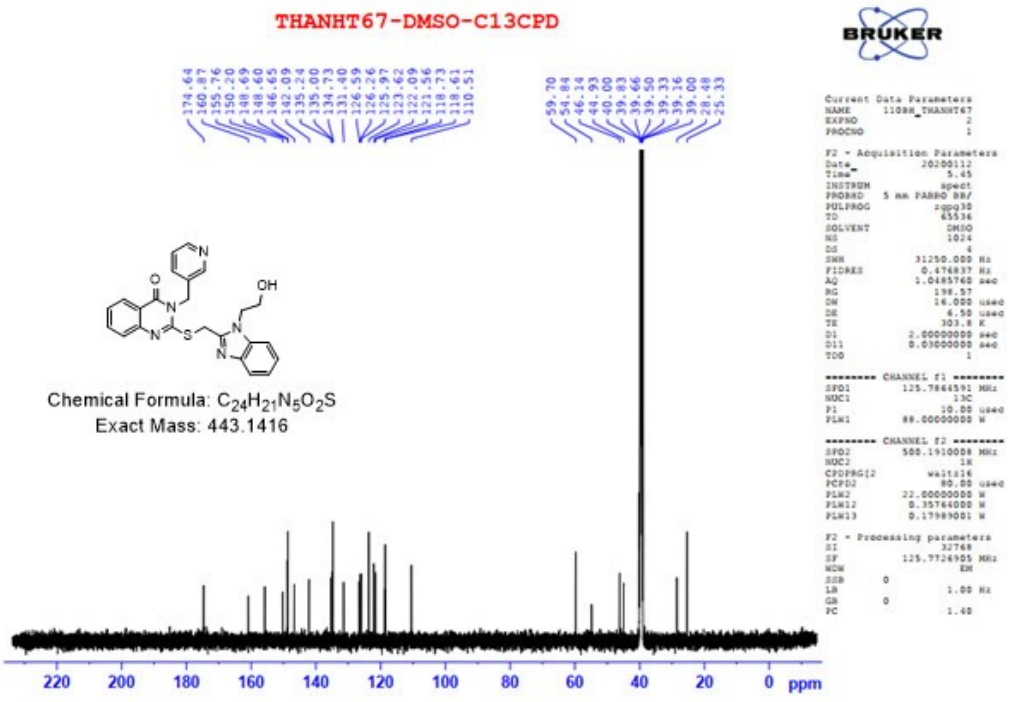

Figure S47. ^{13}C-NMR spectrum of compound **9c**

S3. Molecular Cloning and Protein Expression of M^{pro}

The SARS-CoV-2 Mpro gene was obtained from Addgene and cloned into the pGEX-5X vector, which has a glutathione S-transferase (GST) tag attached to the N-terminus of the recombinant M^{pro} protein as previously described.[30] After the recombinant plasmid was transformed into E. coli BL21 (DE3) competent cells, isopropyl thio-β-D-thiogalactopyranoside (IPTG) (0.5 mM) was added to induce recombinant Mpro protein expression for 10 hours at 16°C and 180 rpm. The expressed proteins were purified using Glutathione Sepharose 4 Fast Flow resin, followed by cleavage with Factor Xa and analysis of protein purity by SDS-PAGE. The protein appears as a homogeneous band on SDS-PAGE. Protein concentration was determined by Bradford assay, aliquoted into vials, and stored at -80°C until use. To confirm enzyme activity and stability, GC376 (a preclinical cysteine protease inhibitor that binds to Mpro of feline coronavirus (FCoV)) was used as a positive control in each experiment to verify the activity of the enzyme in that assay.37,38 Purified proteins are stable at -80°C for at least six months.

S4. In vitro inhibitory activity assay of 3CL protease

Protease activity assays were performed in a 384-well black flat-bottomed microtiter plate (Thermo Scientific™ Nunc™). In a final volume of 25 μL, the recombinant SARS-CoV-2 3CL protease was added at a final concentration of 50 nM and mixed with different concentrations of compounds in assay buffer (final concentration: 20 mM Tris pH 7.3, 100 mM NaCl, 1% DTT, 1% EDTA, 1% DMSO) and pre-incubated at 37ºC for 30 min. The FRET substrate, DABCYL-KTSAVLQSGFRKME-EDANS, was then added to a final concentration of 50 μM. The completed reaction was incubated at 37 ºC for 1 h. First, the activity of the purified recombinant protein was tested against the positive control substrate GC-376 and an IC_{50} similar to that reported in the literature to confirm that the activity of the enzyme was close to that reported in the literature.37,38 This enzyme activity was assumed to be 100% for subsequent calculation of inhibitory activity. Blank wells contain the same compound concentration as substrate but without 3CL protease. Inhibition rates were then calculated by comparison with control wells without added inhibitor. The fluorescence signal (excitation/emission, 355 nm/460 nm) of released EDANS was measured using a fluorometer (Fluoroskan Ascent FL). IC50 values were determined by nonlinear regression (GraphPad Prism 8.0.1).

S5. Theoretical studies

Table S1: Molecular descriptor and drug-likeness calculation of compound **9b**

Parameter	Value
Molecular weight (g/mol)	444.47
HBA	6
HBD	1
RP	6
TPSA (Å^2)	147.58
Molar refractivity	124.23
Solubility (mg/mL)	7.46e-03 (moderately soluble)
Lipinski's rule	Yes
Veber's rule	No (TPSA > 140 Å^2)
Egan's rule	No (TPSA >131.6 Å^2)
Ghose's rule	Yes
Muegge's rule	Yes
GI absorption	Low
BBB permeant	No
P-gp substrate	No
Log Kp (skin permeation) (cm/s)	-6.76
Bioavailability score	0.55

HBA: hydrogen bond acceptor, HBD: hydrogen bond donor, RB: rotatable bonds, TPSA: topological polar surface area, BBB: blood-brain barrier, GI: gastrointestinal