Electronic Supplementary Information (ESI)

For

Discovery and structural insight of a highly selective protein kinase inhibitor hit through click chemistry

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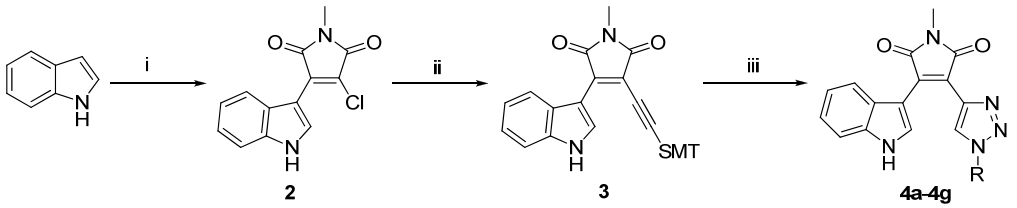
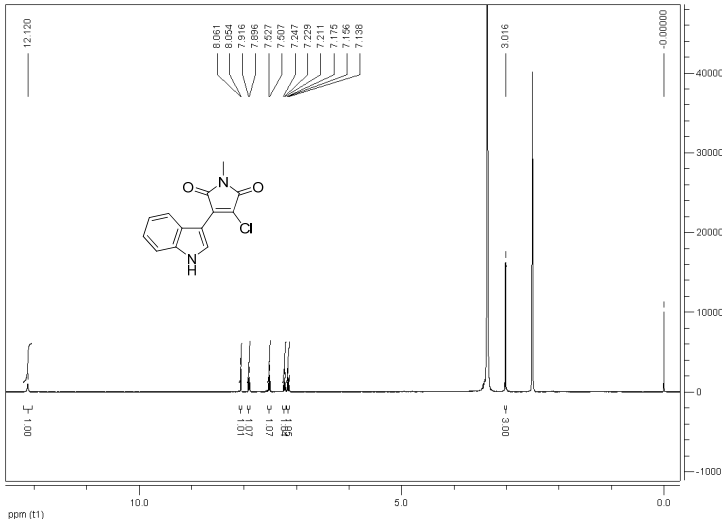
The synthesis of 4a-4g



4a R = $CH_2CH_2CH_2OH$
4b R = CH_2CH_2OH
4c R = $CH_2CH_2CH_2Ph$
4d R = CH_2CH_2Ph
4e R = CH_2CH_2OPh
4f R = CH_2Ph
4g R = $CH_2CH{=}CH_2Ph$

2-Chloro-3-(1*H*-indol-3-yl)-N-methylmaleimide (2):

To a solution of indole (5.5 mmol, 665 mg) in THF (20 mL) was added EtMgBr (1.0 M in THF, 5.5 mL). After heating to 60℃ for 1h a solution of 2,3-dichloro-*N*- methylmaleimide (5.5 mmol, 1g) in THF(20 mL) was added. The mixture was heated at 65℃ and the reaction was followed by TLC. After 1h the mixture was cooled to room temperature and poured into ice water, then extracted with EtOAc. The organic layer was washed with sat. aq NH_4Cl, brine and dried with Na_2SO_4. The solution was removed *in vacuo* and purified by flash chromatography to afford a yellow solid **2**, 752 mg, yield 52%. **^{1}H NMR** (400 MHz, DMSO-d_6, δ: ppm) 12.12 (s, 1H), 8.05 (d, 1H, *J* = 2.8 Hz), 7.90 (d, 1H, *J* = 8.0Hz), 7.51 (d, 1H, *J* = 8.0 Hz), 7.23 (t, 1H, *J* = 7.2 Hz), 7.16 (t, 1H, *J* = 7.2 Hz), 3.02 (s, 3H).



3-(1*H*-indol-3-yl)-1-methyl-4-((trimethylsilyl)ethynyl)-1*H*-pyrrole-2,5-dione (3):

To a solution of the **2** (450 mg, 1.71 mmol) in Et_3N (40 mL) & THF (10 mL) was added $Pd(PPh_3)_4$ (110 mg, 0.09 mmol) and CuI (326 mg, 0.17 mmol), and the solution was degassed with argon. Trimethylsilylacetylene (0.5 mL, 3.5 mmol) was then added and the solution stirred overnight. The mixture was poured onto sat. aq NH_4Cl (70 mL) and extracted with ether (3 x 20 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was removed in vacuo and the product purified by flash chromatography to yield a red solid **3**, 411 mg, yield 75%. **^{1}H NMR** (400 MHz, DMSO-d_6, δ: ppm) 12.30 (s, 1H), 8.33 (s, 1H), 8.31 (s, 1H), 7.52 (d, 1H, *J* = 8.0 Hz), 7.26 (t, 1H, *J* = 7.6 Hz), 7.16 (t, 1H, *J* = 7.6 Hz), 2.97 (s, 3H), 0.24 (s, 9H). **^{13}C NMR** (75 MHz, DMSO-d_6, δ: ppm) 170.39, 168.63, 139.44, 136.77, 132.82, 125.01, 123.21, 122.97, 120.40, 112.49, 110.95, 109.50, 106.31, 98.06, 24.23, -0.47. **HRMS** (ESI, m/z): calcd for $C_{18}H_{18}N_2O_2Si$ $(M+Na)^+$, 345.1030; found, 345.1030.

General procedure for the preparation of (4):

To the solution of the **3** (1 equiv) in THF & MeOH (1:1), tetrabutylammonium fluoride (1 equiv.) was added. The reaction was monitored by TLC analysis. Upon the disappearance of the reactant, azides (1 equiv.) was added, followed by sodium ascorbate (0.2 equiv.) and copper (□) sulfate pentahydrate (0.02 equiv.). The mixture was stirred overnight, at which point it cleared and TLC analysis indicated complete consumption of the reactants. The reaction mixture was poured onto ice water, and extracted with ethyl acetate, dried over Na_2SO_4. The solvent was removed *in vacuo* and the residue was purified by column chromatography to afford **4**.

3-(1*H*-indol-3-yl)-4-(1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)-*N*-methylmaleimide (4a):

Yield 45%. **^{1}H NMR**(400 MHz, DMSO-d_6, δ: ppm) 1.974 (2H, t, *J* = 6.4 Hz), 3.036 (3H, s), 3.398-3.411 (2H, m), 4.496 (2H, t, *J* = 6.4 Hz), 4.678 (1H, t, *J* = 4.0 Hz), 6.877-6.911 (2H, m), 7.125 (1H, t, *J* = 6.4 Hz), 7.461 (1H, d, *J* = 8.0 Hz), 8.209 (1H, s), 8.473 (1H, s), 11.918 (1H, s). **^{13}C NMR**(100 MHz, DMSO-d_6, δ: ppm) 24.033, 32.942, 46.698, 57.327, 104.720, 111.994, 119.810, 119.981, 121.567, 121.950, 125.017, 125.832, 131.768, 132.056, 136.421, 136.707, 170.776, 170.926. **HRMS** (ESI, m/z) calcd for $C_{18}H_{17}N_5O_3Na$ $(M+Na)^+$ 374.1226, found 374.1224.

3-(1*H*-indol-3-yl)-4-(1-(2-hydroxyethyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4b):

Yield 55%. **^{1}H NMR**(400 MHz, DMSO-d_6, δ: ppm) 3.038 (3H, s), 3.796 (2H, q, $^1J = {}^2J$ =5.2 Hz), 4.491 (2H, t, *J* = 5.2Hz), 5.065 (1H, t, *J* = 5.2Hz), 6.916 (1H, t, *J* = 7.6 Hz), 6.995 (1H, d, *J* = 8.0 Hz), 7.128 (1H, t, *J* = 7.6 Hz), 7.460 (1H, d, *J* = 8.4 Hz), 8.227 (1H, d, *J* = 2.4 Hz), 8.479 (1H, s), 11.905 (1H, s). **^{13}C NMR**(100 MHz, DMSO-d_6, δ: ppm) 24.034, 52.209, 59.811, 104.736, 111.914, 119.837, 120.164, 121.749, 121.899, 125.179, 126.142, 131.744, 131.788, 136.377, 136,616, 170.830, 170.959. **HRMS** (ESI, m/z) calcd for $C_{17}H_{15}N_5O_3Na$ $(M+Na)^+$ 360.1062, found 360.1067.

3-(1*H*-indol-3-yl)-4-(1-(3-phenylpropyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4c):

Yield 42%. ^{1}H NMR(400 MHz, DMSO-d6, δ: ppm) 2.152 (2H, penta, *J* = 7.2 Hz), 2.556 (2H, t, *J* = 7.6 Hz), 3.039 (3H, s), 4.455 (2H, t, *J* = 6.8 Hz), 6.824-6.878 (2H, m), 7.104 (1H, t, *J* = 6.4 Hz), 7.194-7.314 (5H, m), 7.461 (1H, d, *J* = 8.0 Hz), 8.202 (1H, s), 8.523 (1H, s), 11.930 (1H, s). ^{13}C NMR(100 MHz, DMSO-d6, δ: ppm) 24.032, 31.352, 31.723, 48.955, 104.739, 112.016, 119.752, 119.987, 121.532, 121.938, 125.006, 125.725, 125.982, 128.294, 128.373, 131.735, 132.082, 136.450, 136.854, 140.689, 170.760, 170.928. HRMS (ESI, m/z) calcd for $C_{24}H_{21}N_5O_2Na$ $(M+Na)^+$ 434.1593, found 434.1587.

3-(1*H*-indol-3-yl)-4-(1-(3-phenylethyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4d):

Yield 47%. **^{1}H NMR**(400 MHz, DMSO-d_6, δ: ppm) 3.020 (3H, s), 3.190 (2H, t, *J* = 7.2 Hz), 4.714 (2H, t, *J* = 7.2 Hz), 6.823-6.897 (2H, m), 7.127 (2H, t, *J* = 7.6 Hz), 7.202-7.235 (3H, m), 7.286 (2H, t, *J* = 7.6 Hz), 7.461 (1H, d, *J* = 8.0 Hz), 8.158 (1H, d, *J* = 2.4 Hz), 8.433 (1H, s), 11.906 (1H, s). **^{13}C NMR**(100 MHz, DMSO-d_6, δ: ppm) 24.027, 35.644, 50.478, 104.770, 111.954, 119.766, 119.838, 121.693, 121.899, 125.049, 125.734, 126.586, 128.363, 128.699, 131.615, 131.661, 136.404, 136.574, 137.509, 170.779, 170.943. **HRMS** (ESI, m/z) calcd for $C_{23}H_{19}N_5O_2Na$ $(M+Na)^+$ 420.1431, found 420.1431.

3-(1*H*-indol-3-yl)-4-(1-(2-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4e):

Yield 22%. **^{1}H NMR**(400 MHz, DMSO-d_6, δ: ppm) 3.035 (3H, s), 4.416 (2H, t, *J* = 4.8 Hz), 4.861 (2H, t, *J* = 4.8 Hz), 6.749 (1H, t, *J* = 7.6 Hz), 6.865 (1H, d, *J* = 8.0 Hz), 6.911-6.969 (3H, m), 7.073 (1H, t, *J* = 7.6 Hz), 7.286 (2H, t, *J* = 7.6 Hz), 7.440 (1H, d, *J* = 8.4 Hz), 8.191 (1H, d, *J* = 2.4 Hz), 8.587 (1H, s), 11.908 (1H, s). **^{13}C NMR**(100 MHz, DMSO-d_6, δ: ppm) 24.045, 49.115, 66.043, 104.721, 111.940, 114.557, 119.787, 119.865, 121.084, 121.605, 121.889, 125.054, 126.275, 129.544, 131.754, 131.990, 136.399,

136.835, 157.829, 170.797.170.932. **HRMS** (ESI, m/z) calcd for $C_{23}H_{19}N_5O_3Na$ $(M+Na)^+$ 436.1373, found 436.1380.

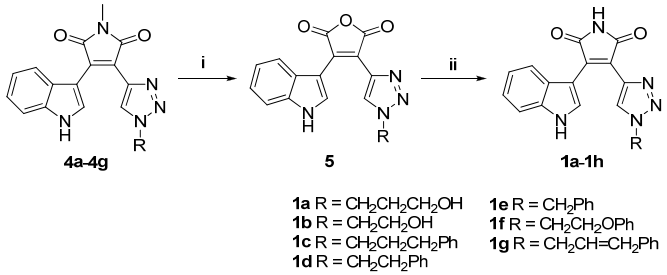
3-(1*H*-indol-3-yl)-4-(1-(3-phenylmethyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4f):

Yeild 53%. **^{1}H NMR** (400 MHz, DMSO-d6, δ: ppm) 3.038 (s, 3H), 5.712 (s, 2H), 6.812-6.844 (m, 2H), 7.130 (t, 1H, *J* = 6.8 Hz), 7.309-7.479 (m, 6H), 8.209 (s, 1H), 8.574 (s, 1H), 11.935 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d6, δ: ppm) 24.043, 52.719, 104.677, 112.016, 119.765, 119.811, 121.531, 121.955, 124.961, 125.983, 127.699, 128.112, 128.752, 131.797, 132.187, 136.007, 136.414, 170.753, 170.900. **HRMS** (ESI, m/z) calcd for $C_{22}H_{17}N_5O_2Na$ $(M+Na)^+$ 406.1281, found 406.1275.

3-(1*H*-indol-3-yl)-4-(1-(3-cinnamyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4g):

Yeild 71%. **^{1}H NMR**(400 MHz, DMSO-d_6, δ: ppm) 3.047 (s, 3H), 5.283 (d, 2H, *J* = 6.0 Hz), 6.493-6.563 (m,1H), 6.666 (d, 1H, *J* = 8.0 Hz), 6.874-6.953 (m, 2H), 7.120 (t, 1H, *J* = 7.6 Hz), 7.299 (t, 1H, *J* = 7.2 Hz), 7.371 (t, 2H, *J* = 7.2 Hz), 7.472 (d, 3H, *J* = 8.0 Hz), 8.224 (d, 1H, *J* = 2.4 Hz), 8.532 (s, 1H), 11.942 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6, δ: ppm) 24.037, 51.315, 104.730, 112.003, 119.838, 119.910, 121.631, 121.935, 123.627, 125.038, 125.696, 126.545, 128.139, 128.684, 131.801, 132.060, 133.608, 135.696, 136.439, 170.768, 170.917. **HRMS** (ESI, m/z) calcd for $C_{26}H_{22}N_3O_2$ $(M+H)^+$ 408.1460, found 408.1466.

The synthesis of 1a-1g



General procedure for the preparation of (1a-1g): To a solution of **4** (0.1 mM) in EtOH (5 mL) was added KOH aqueous solution (2 mL, 1M) and the reaction was stirred for 2 h. Water (5 mL) was added and the mixture was acidified to *p*H 1 with 2M HCl . After extraction with ethyl actate (three times), the organic layers were dried and concentrated to give the crude anhydride **5**. To this crude anhydride in DMF (1.5 mL) was added ammonium acetate (0.5 mM). The reaction mixture was heated at 140℃ for 2 h and then cooled down slowly. The reaction mixture was poured onto ice water, and extracted with ethyl acetate, dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography to give compound **1a-1g**.

3-(1*H*-indol-3-yl)-4-(1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1a):

Yield 74%. **^{1}H NMR** (400 MHz, DMSO-d_6, δ: ppm) 1.973 (penta, 2H, *J* = 6.8 Hz), 3.383-3.427 (m, 2H), 4.486 (t, 2H, *J* = 6.8 Hz), 4.659 (t, 1H, *J* = 4.8 Hz), 6.850-6.906 (m, 2H), 7.095-7.135 (m, 1H), 7.450 (d, 1H, *J* = 8.0 Hz), 8.169 (d, 1H, *J* = 2.8 Hz), 8.450 (s, 1H), 11.097 (s, 1H), 11.875 (d, 1H, *J* = 2.0 Hz). **^{13}C NMR** (100 MHz, DMSO-d_6, δ: ppm) 32.927, 46.665, 57.331, 104.632, 111.931, 119.751, 120. 671, 121.530, 121.864, 125.077, 125.831, 131.662, 132.511, 136.382, 136.670, 171.939, 172.122. **HRMS** (ESI, m/z) calcd for $C_{17}H_{15}N_5O_3$ $(M+Na)^+$ 360.1070. found 360.1067.

3-(1*H*-indol-3-yl)-4-(1-(2-hydroxyethyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1b):

Yield 62%. **^{1}H NMR** (400 MHz, DMSO-d_6, δ: ppm) 3.798 (apparent s, 2H), 4.486 (t, 2H, *J* = 5.2 Hz), 5.075

(s, 1H), 6.911 (t, 1H, *J* = 7.6 Hz), 6.979 (d, 1H, *J* = 8.0 Hz), 7.123 (t, 1H, *J* = 7.6 Hz), 7.455 (d, 1H, *J* = 8.4 Hz), 8.193 (s, 1H), 8.468 (s, 1H), 11.124 (s, 1H), 11.892 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6, δ: ppm) 52.186, 59.797, 104.648, 111.855, 119.775, 120.824, 121.710, 121.812, 125.230, 126.134, 131.685, 132.197, 136.342, 136.579, 162.868, 172.002,172.155. **HRMS** (ESI, m/z) calcd for $C_{16}H_{13}N_5O_3Na$ $(M+Na)^+$ 346.0916, found 346.0911.

3-(1*H*-indol-3-yl)-4-(1-(3-phenylpropyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1c):

Yield 81%. **^{1}H NMR** (400 MHz, DMSO-d_6, δ: ppm) 2.151 (penta, 2H, *J* = 6.8 Hz), 2.559 (t, 2H, *J* = 7.6 Hz), 4.445 (t, 2H, *J* = 7.2 Hz), 6.818-6.872 (m, 2H), 7.075-7.116 (m, 1H), 7.176-7.211 (m, 3H), 7.275-7.312 (m, 2H). 7.450 (d, 1H, *J* = 8.0 Hz), 8.162 (d, 1H, *J* = 2.8 Hz), 8.499 (s, 1H), 11.103 (s, 1H), 11.885 (d, 1H, *J* = 2.0 Hz). **^{13}C NMR** (100 MHz, DMSO-d_6, δ: ppm) 31.356, 31.722, 48.925, 104.644, 111.963, 119.690, 120.649, 121.502, 121.857, 125.052, 125.730, 125.977, 128.300, 128.367, 131.640, 132.511, 136.410, 136.821, 140.698, 171.928, 172.129. **HRMS** (ESI, m/z) calcd for $C_{23}H_{19}N_5O_3Na$ $(M+Na)^+$ 420.1427. found 420.1431.

3-(1*H*-indol-3-yl)-4-(1-(2-phenylethyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1d):

Yield 53%. **^{1}H NMR** (400 MHz, DMSO-d_6, δ: ppm) 3.188 (t, 2H, *J* = 7.2 Hz), 4.702 (t, 2H, *J* = 7.2 Hz), 6.811 (d, 1H, *J* = 8.0 Hz), 6.868 (t, 1H, *J* = 8.0 Hz), 7.120 (t, 1H, *J* = 7.6 Hz), 7.208-7.310 (m, 5H), 7.453 (d, 1H, *J* = 8.0 Hz), 8.124 (d, 1H, J = 2.8 Hz), 8.438 (s, 1H), 11.108 (s, 1H), 11.893 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6, δ: ppm) 35.657, 50.466, 104.683, 111.907, 119.720, 120.499, 121.658, 121.825, 125.105, 125.760, 126.582, 128.367, 128.711, 131.577, 132.067, 136.378, 136.544, 137.529, 171.957, 172.150. **HRMS** (ESI, m/z) calcd for $C_{22}H_{17}N_5O_2Na$ $(M+Na)^+$ 406.1277, found 406.1275.

3-(1*H*-indol-3-yl)-4-(1-benzyl-1*H*-1,2,3-triazol-4-yl)-maleimide (1e):

Yield 43%. **^{1}H NMR** (400 MHz, DMSO-d6, δ: ppm) 5.695 (s, 2H), 6.820-6.832 (m, 2H), 7.095-7.136 (m, 1H), 7.307-7.463 (m, 6H), 8.166 (d, 1H, *J* = 2.8 Hz), 8.549 (s, 1H), 11.117 (s, 1H), 11.903 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d6, δ: ppm) 52.734, 104.611, 111.937, 119.736, 120.503, 121.503, 121.856, 125.057, 125.922, 127.727, 128.096, 128.729, 131.404, 131.677, 135.977, 136.396, 171.885, 172.074. **HRMS** (ESI, m/z) calcd for $C_{21}H_{15}N_5O_2Na$ $(M+Na)^+$ 392.1110, found 392.1118.

3-(1*H*-indol-3-yl)-4-(1-(2-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1f):

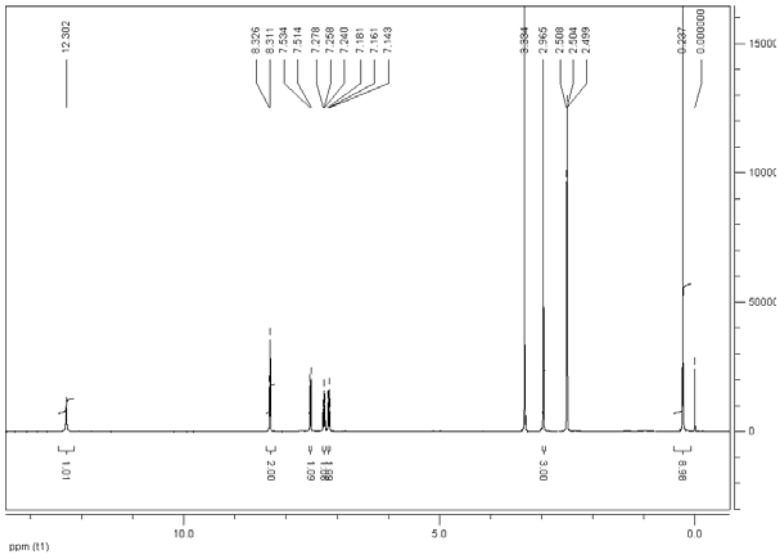
Yield 28%. **^{1}H NMR** (400 MHz, Acetone-d_6, δ: ppm) 4.503 (t, 2H, *J* = 5.2 Hz), 4.934 (t, 2H, *J* = 5.2 Hz), 6.839 (t, 1H, J = 7.6 Hz), 6.931-6.970 (m, 3H), 7.071-7.141 (m, 2H), 7.282 (t, 1H, *J* = 7.6 Hz), 7.460 (d, 1H, *J* = 8.0 Hz), 8.354 (s, 1H), 8.513 (s, 1H), 9.891 (s, 1H), 10.986 (s, 1H). **^{13}C NMR** (100 MHz, Acetone-d_6, δ: ppm) 50.396, 67.281, 106.501, 112.540, 115.569, 120.849, 122.091, 122.432, 122.921, 123.080, 126.693, 127.115, 130.385, 132.533, 132.698, 137.704, 138.392, 159.274, 172.351, 172.539. **HRMS** (ESI, m/z) calcd for $C_{22}H_{17}N_5O_3Na$ $(M+Na)^+$ 422.1222, found 422.1224.

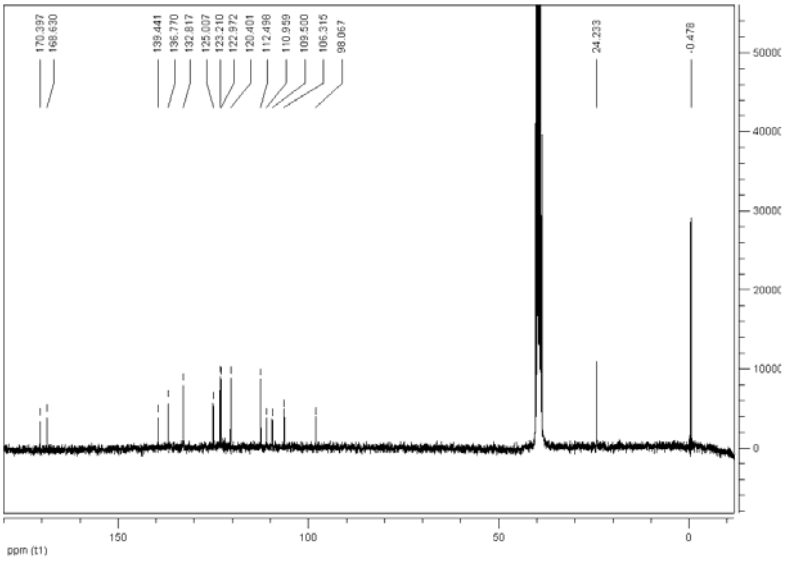
3-(1*H*-indol-3-yl)-4-(1-(3-cinnamyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1g):

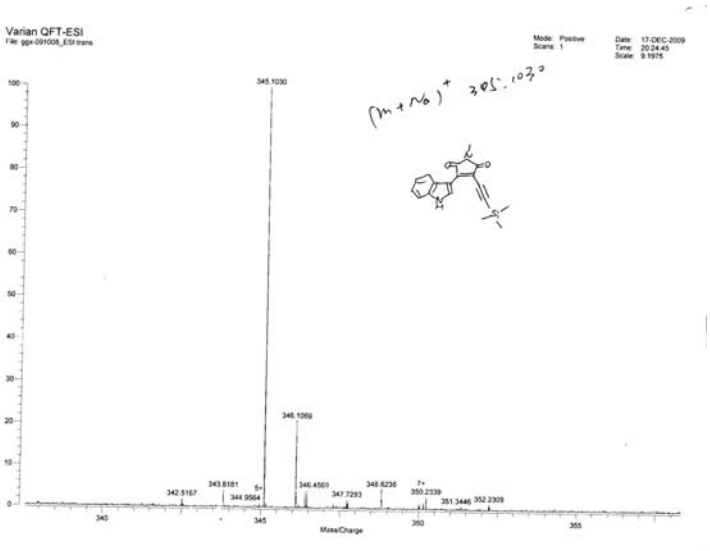
Yield 39%. **^{1}H NMR** (400 MHz, DMSO-d_6, δ: ppm) 5.320 (d, 2H, *J* = 6.0 Hz), 6.539-6.610 (dt, 1H, J_1 = 16.0 Hz, J_2 = J_3 = 6.0 Hz), 6.720 (d, 1H, *J* = 15.6 Hz), 6.912-6.986 (m, 2H), 7.159 (dt, 1H, J_1 = J_2 = 8.0 Hz, J_3 = 1.2Hz), 7.346(t, 1H, *J* = 7.6 Hz), 7.417 (t, 2H, *J* = 7.6 Hz), 7.498-7.530 (m, 3H), 8.230 (d, 1H, *J* = 2.8 Hz), 8.561 (s, 1H), 11.183 (s, 1H), 11.956 (s, 1H). **^{13}C NMR** (100 MHz, DMSO-d_6, δ: ppm) 51.297, 104.640, 111.936, 119.768, 120.600, 121.592, 121.846, 123.611, 125.105, 125.676, 126.550, 128.130, 128.675, 131.689, 132.508, 133.639, 135.704, 136.401, 136.977, 171.928, 172.101. **HRMS** (ESI, m/z) calcd for $C_{21}H_{15}N_5O_2Na$ $(M+Na)^+$ 418.1270, found 418.1275.

^{1}H NMR, ^{13}C NMR, HRMS spectrum of

3-(1*H*-indol-3-yl)-1-methyl-4-((trimethylsilyl) ethynyl)-1*H*-pyrrole-2,5-dione (3):

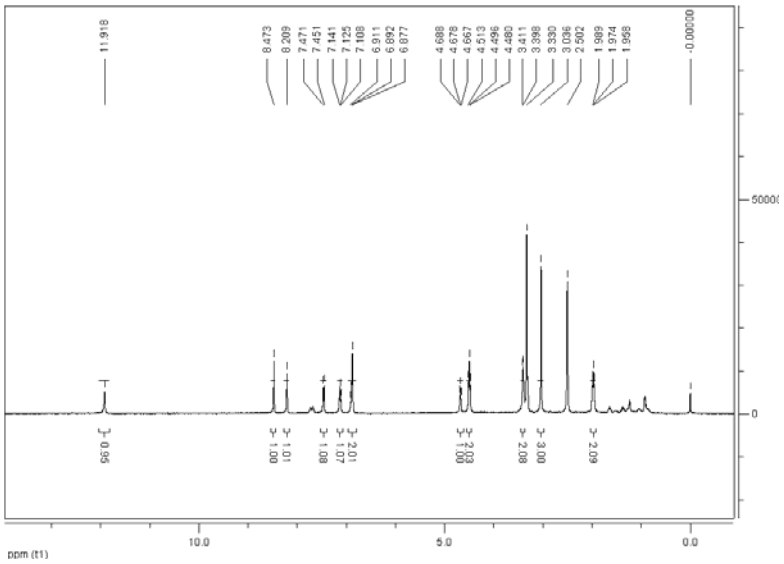


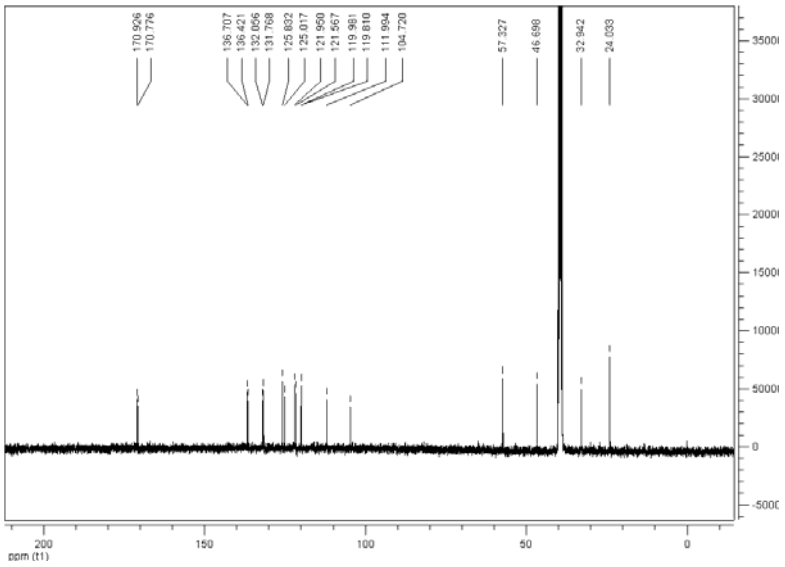


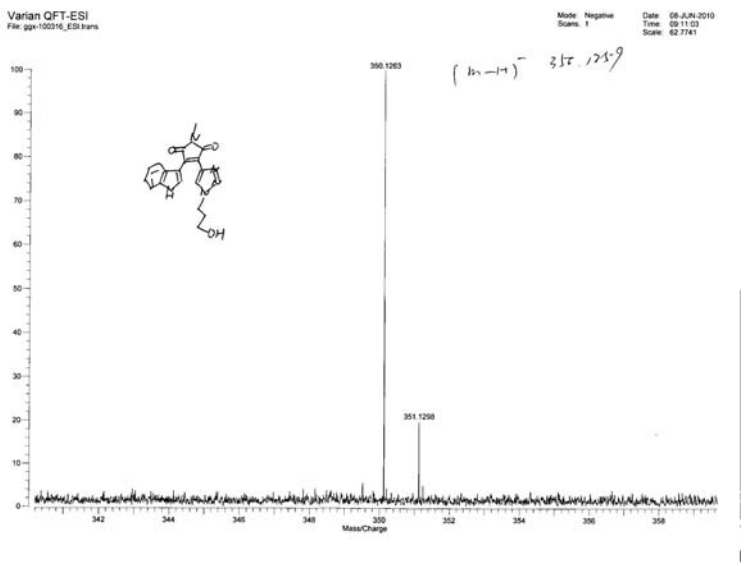


^{1}H NMR, ^{13}C NMR, HRMS spectrum of

3-(1*H*-indol-3-yl)-4-(1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)-*N*-methylmaleimide (4a):

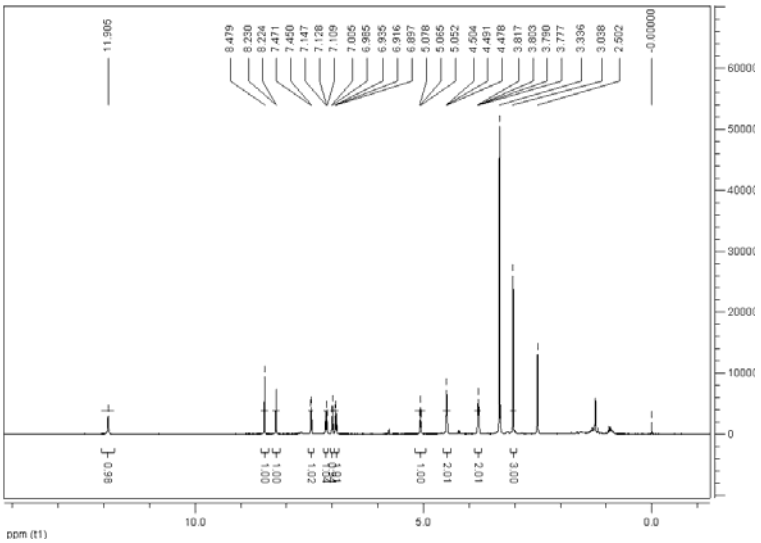


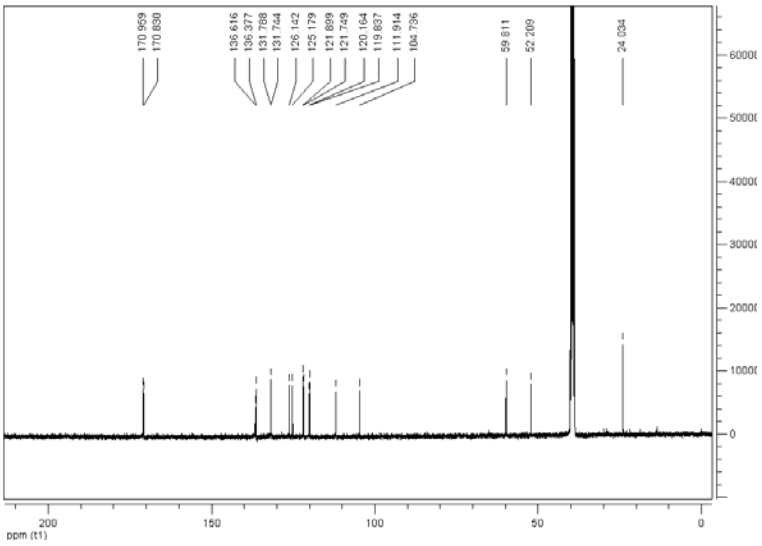


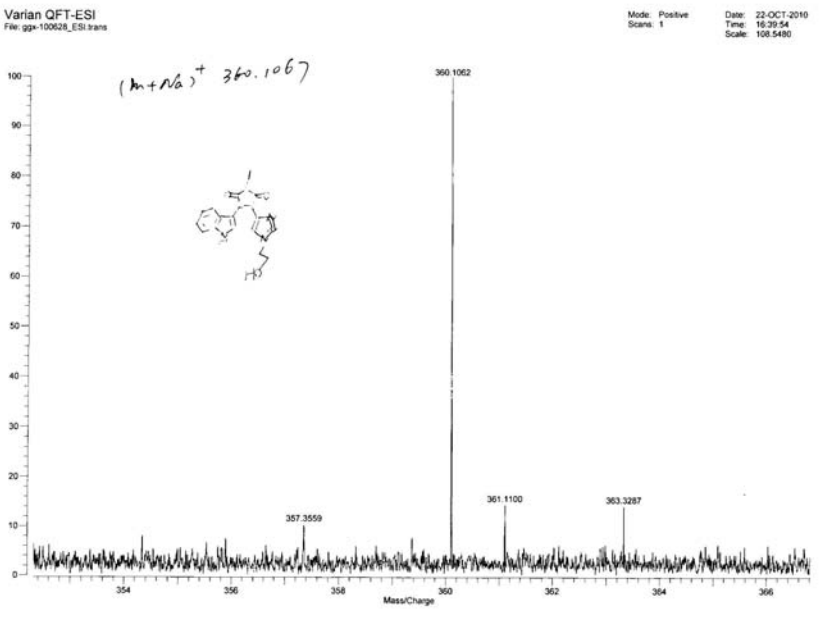


^{1}H NMR, ^{13}C NMR, HRMS spectrum of

3-(1*H*-indol-3-yl)-4-(1-(2-hydroxyethyl)-1*H*-1,2,3-triazol-4-yl)-*N*-methylmaleimide (4b):

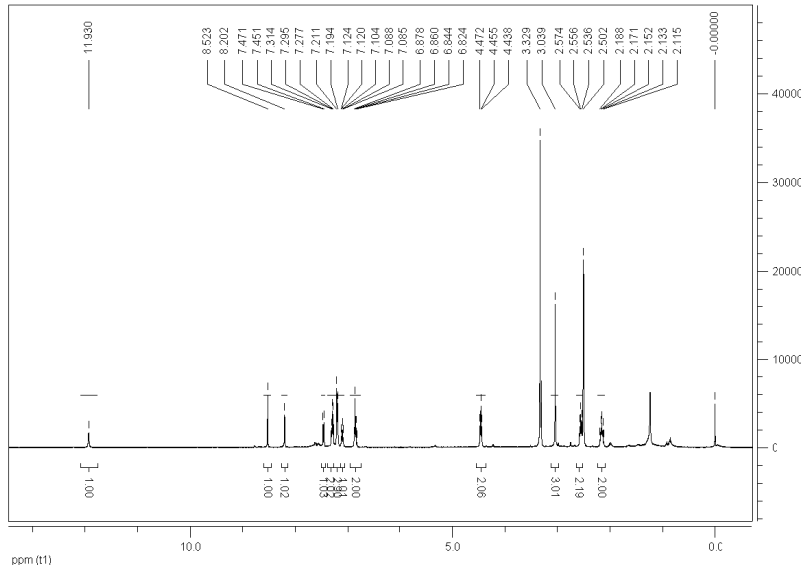


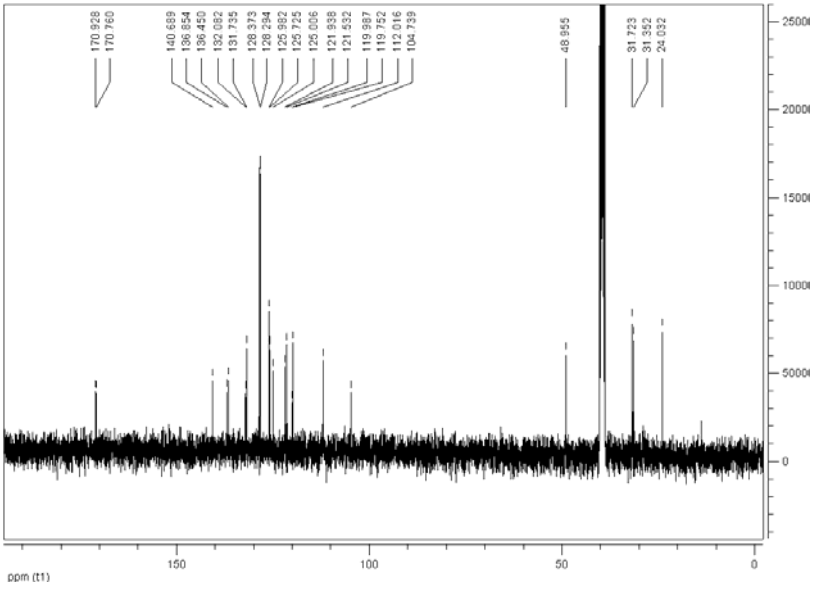


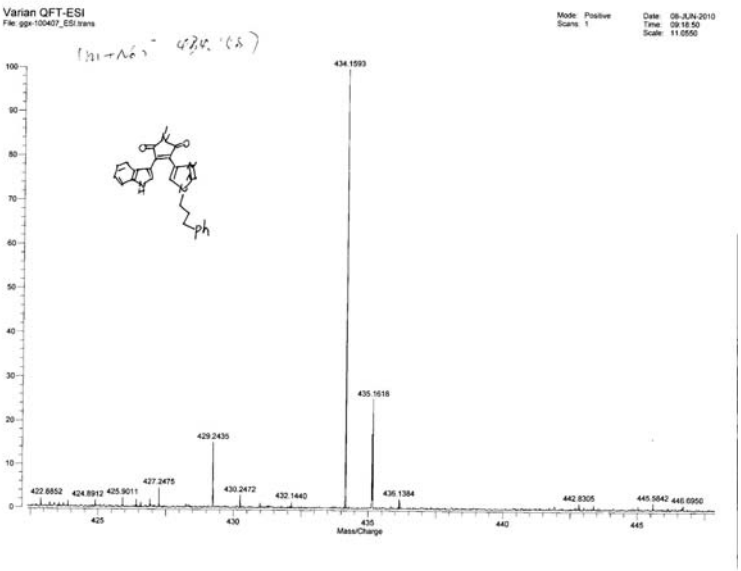


^{1}H NMR, ^{13}C NMR, HRMS spectrum of

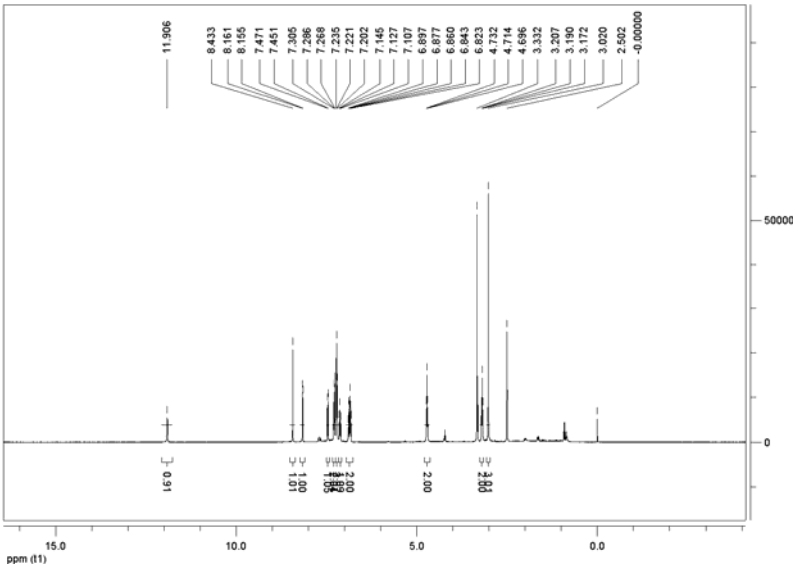
3-(1*H*-indol-3-yl)-4-(1-(3-phenylpropyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4c):

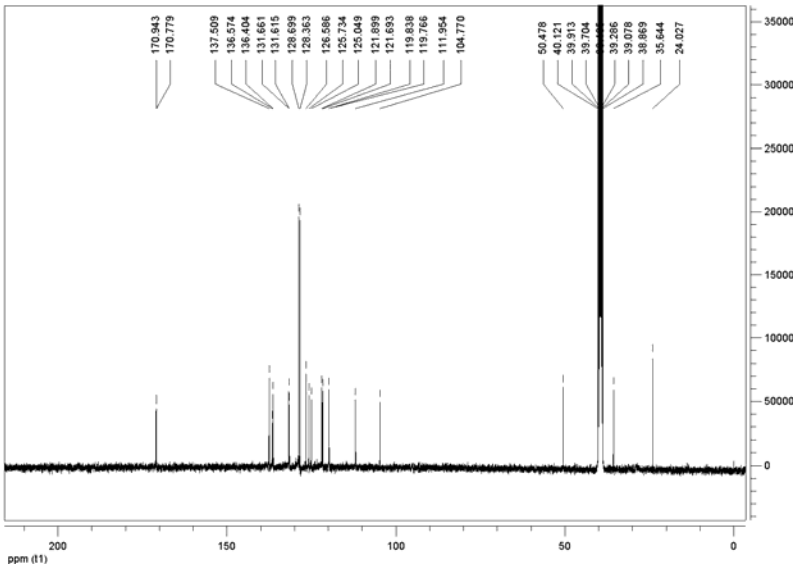


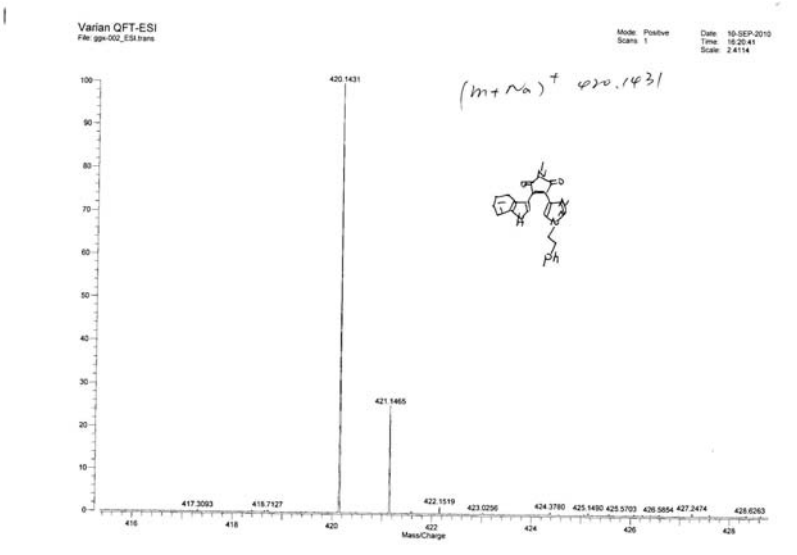




^{1}H NMR, ^{13}C NMR, HRMS spectrum of
3-(1*H*-indol-3-yl)-4-(1-(3-phenylethyl)-1*H*-1,2,3-triazol-4-yl)-*N*-methylmaleimide (4d):

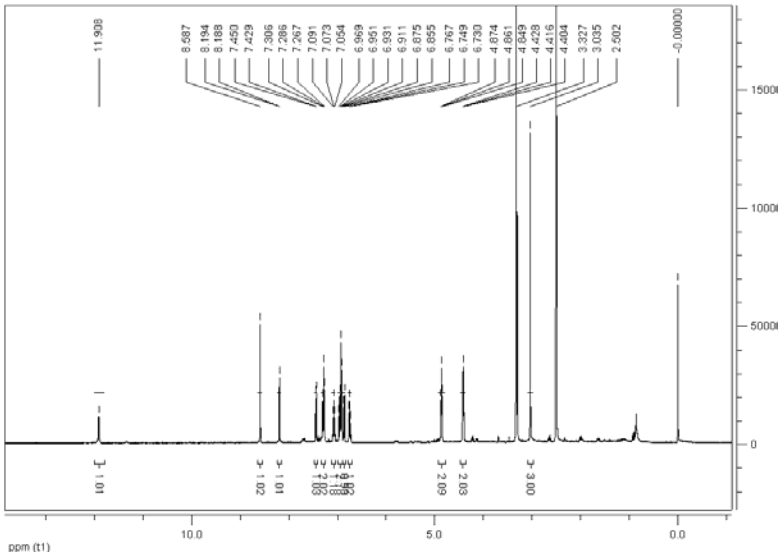


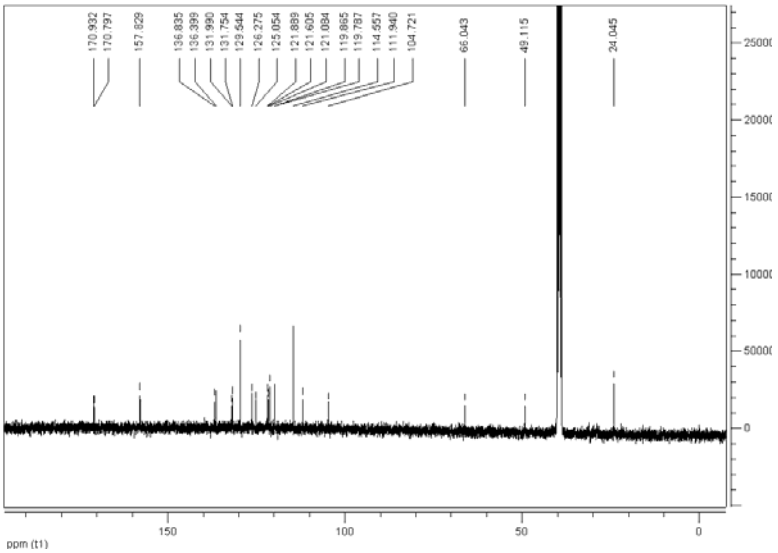


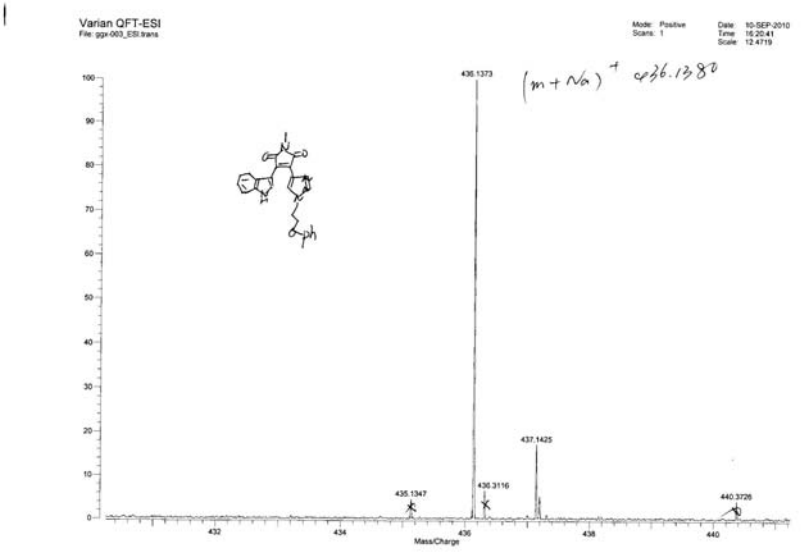


^{1}H NMR, ^{13}CNMR, HRMS spectrum of

3-(1*H*-indol-3-yl)-4-(1-(2-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl)-*N*-methylmaleimide (4e):

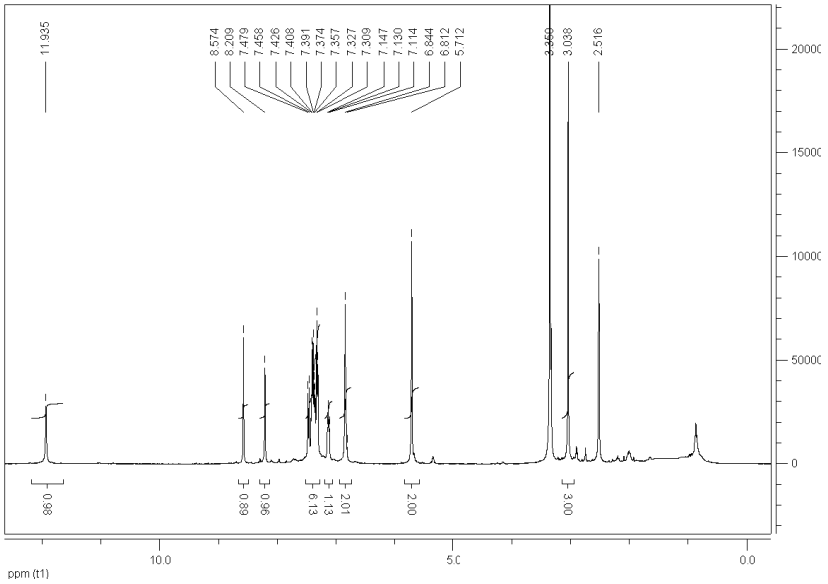


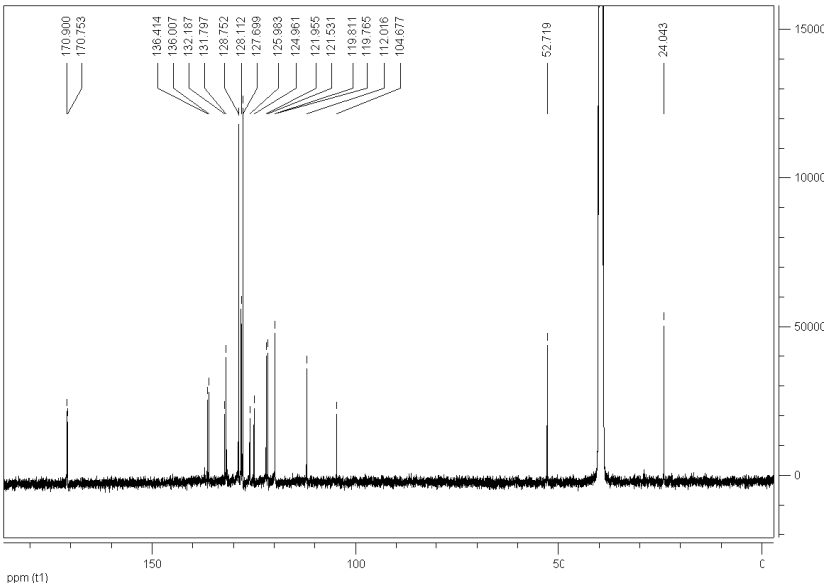


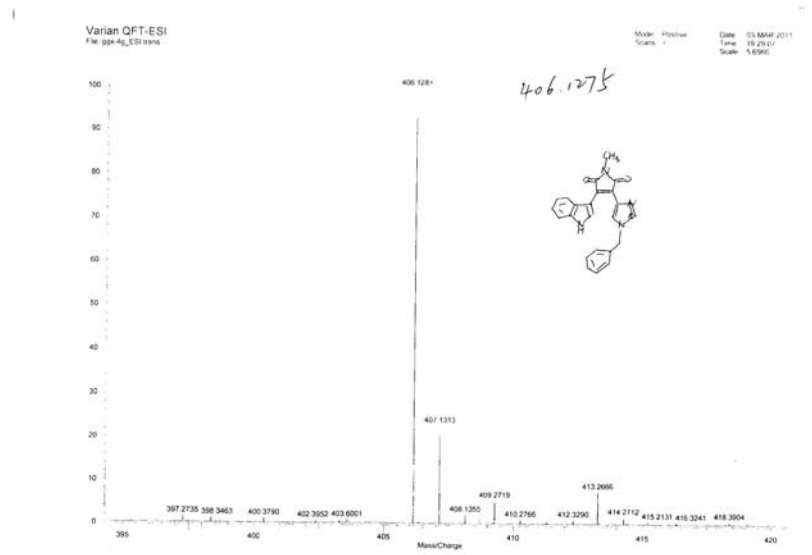


^{1}H NMR, ^{13}C NMR, HRMS spectrum of

3-(1*H*-indol-3-yl)-4-(1-(3-phenylmethyl)-1*H*-1,2,3-triazol-4-yl)-N-methylmaleimide (4f):

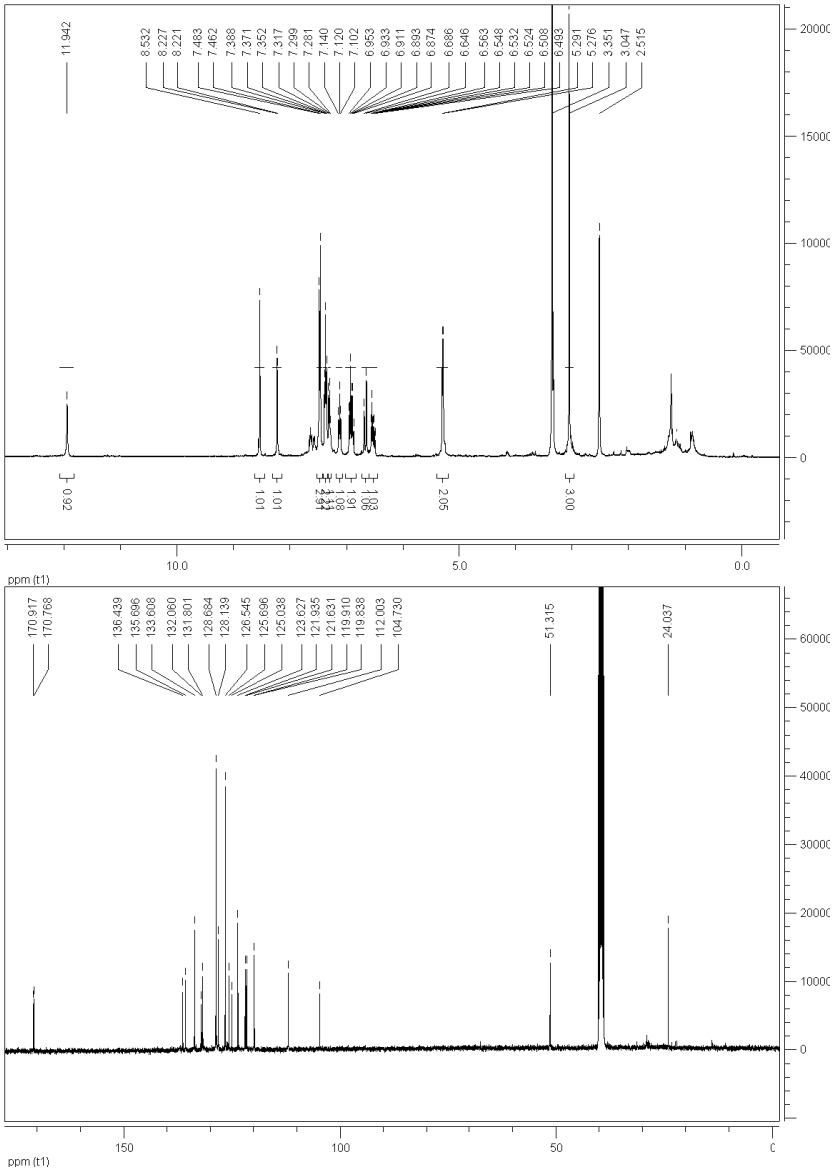


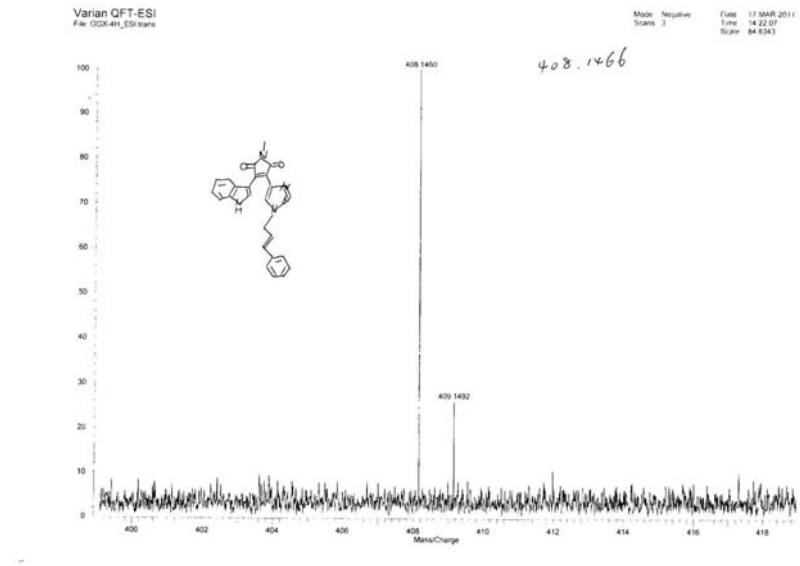




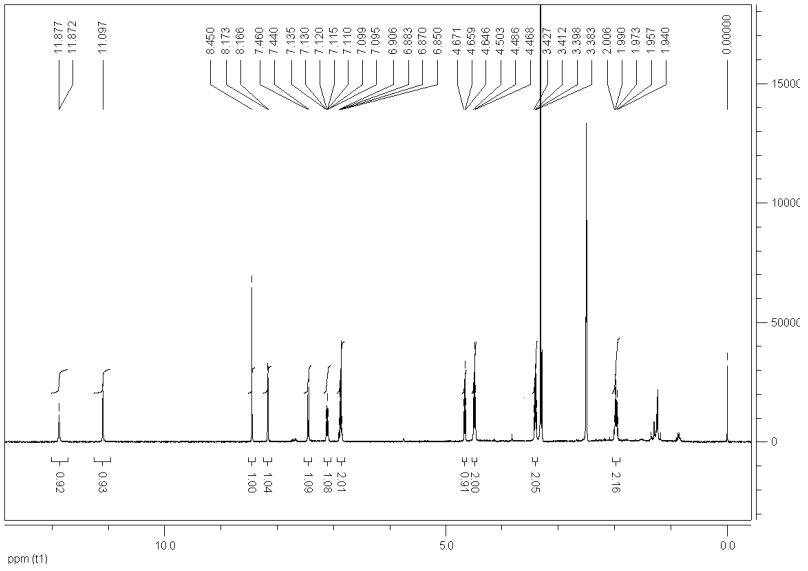
^{1}H NMR, ^{13}C NMR, HRMS spectrum of

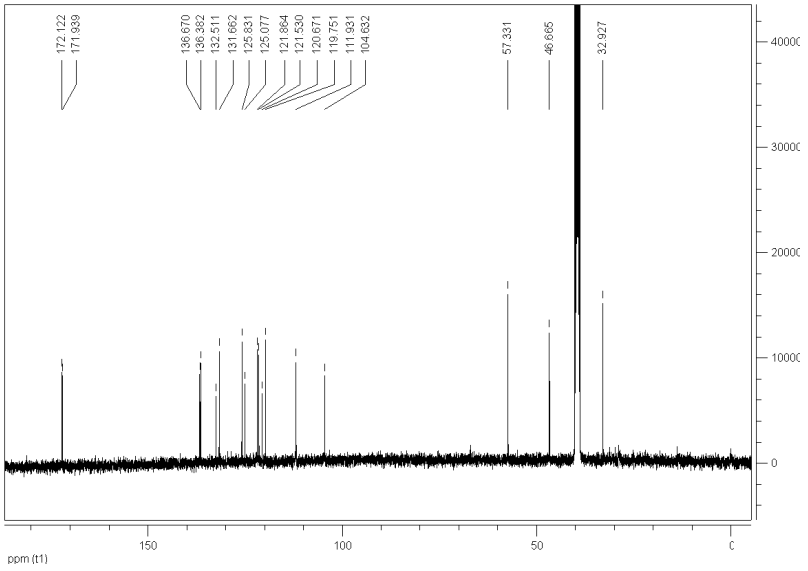
3-(1*H*-indol-3-yl)-4-(1-(3-cinnamyl)-1*H*-1,2,3-triazol-4-yl)-*N*-methylmaleimide (4g):

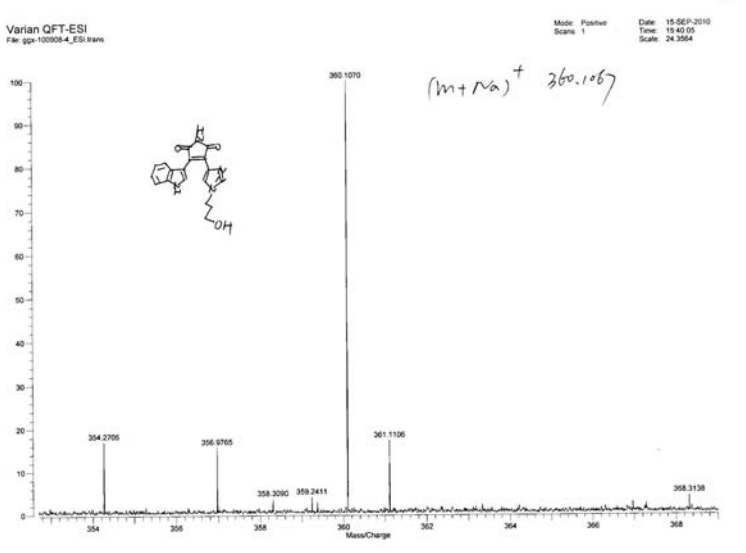




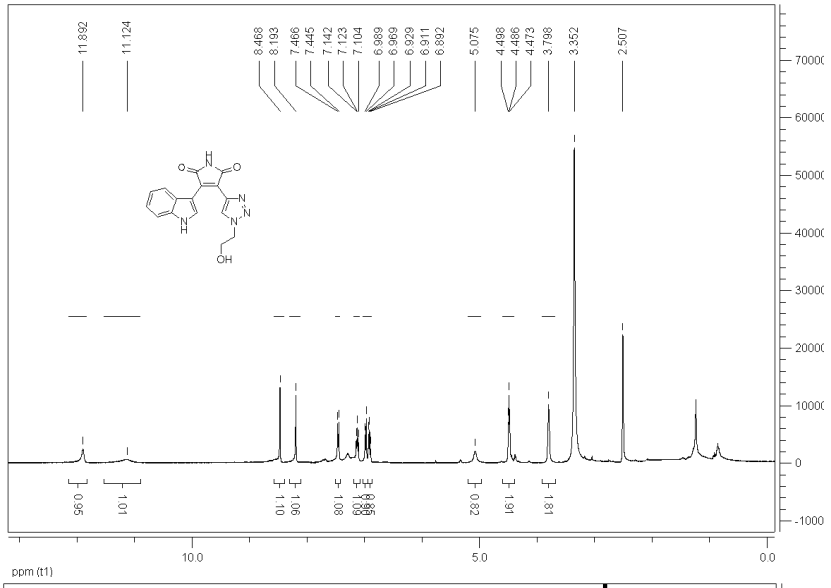
^{1}H NMR, ^{13}C NMR, HRMS spectrum of 3-(1*H*-indol-3-yl)-4-(1-(3-hydroxypropyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1a):

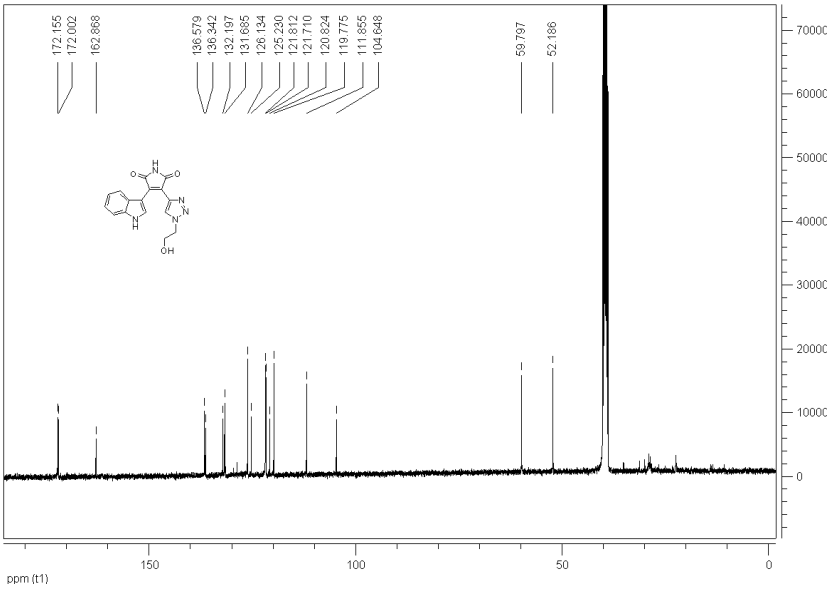


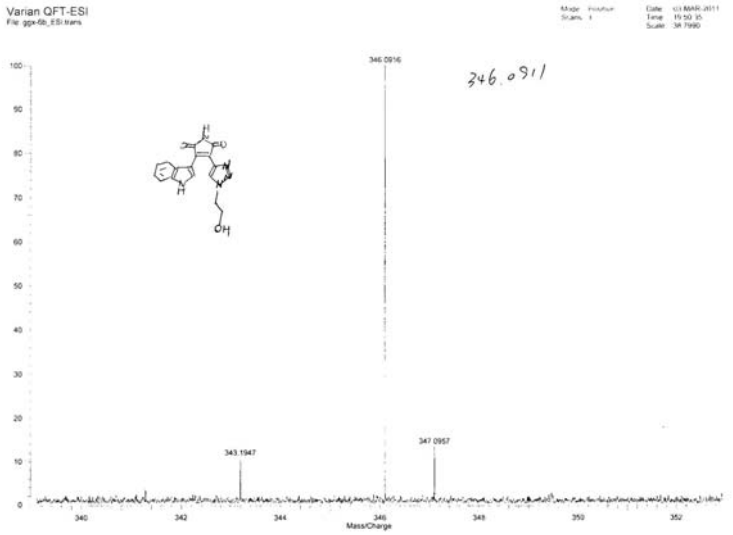




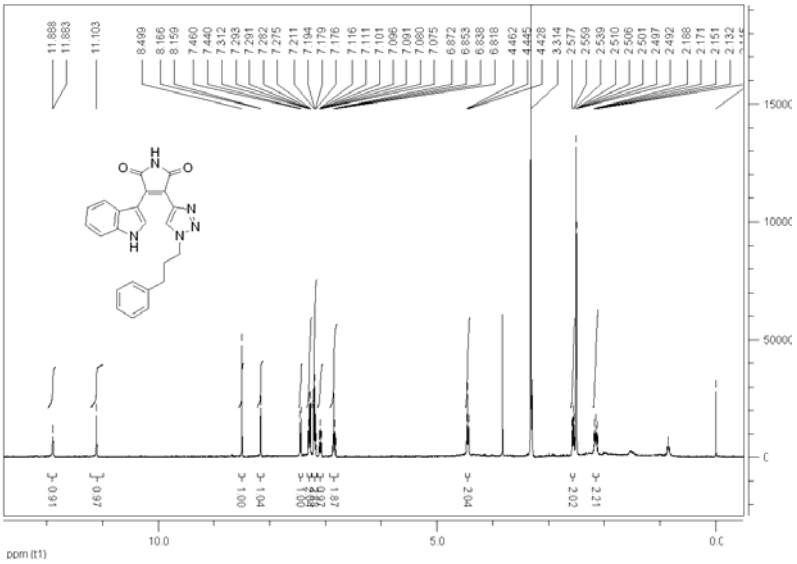
^{1}H NMR, ^{13}C NMR, HRMS spectrum of
3-(1*H*-indol-3-yl)-4-(1-(3-hydroxyethyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1b):

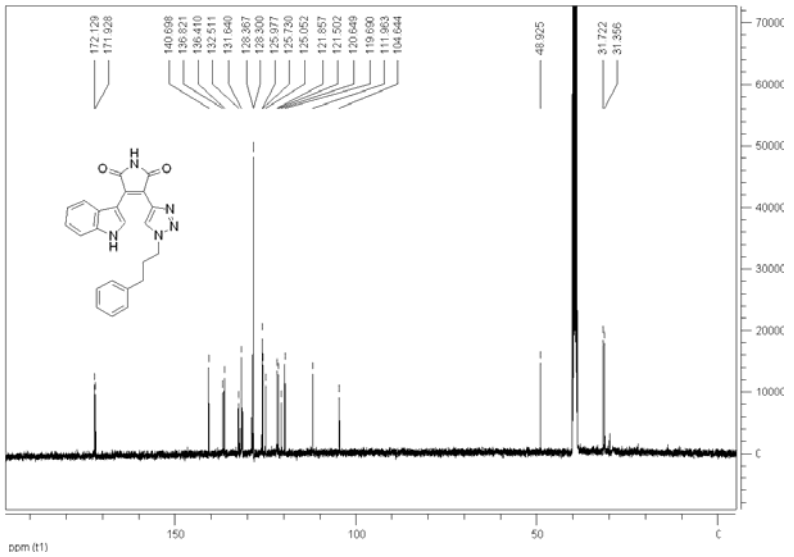


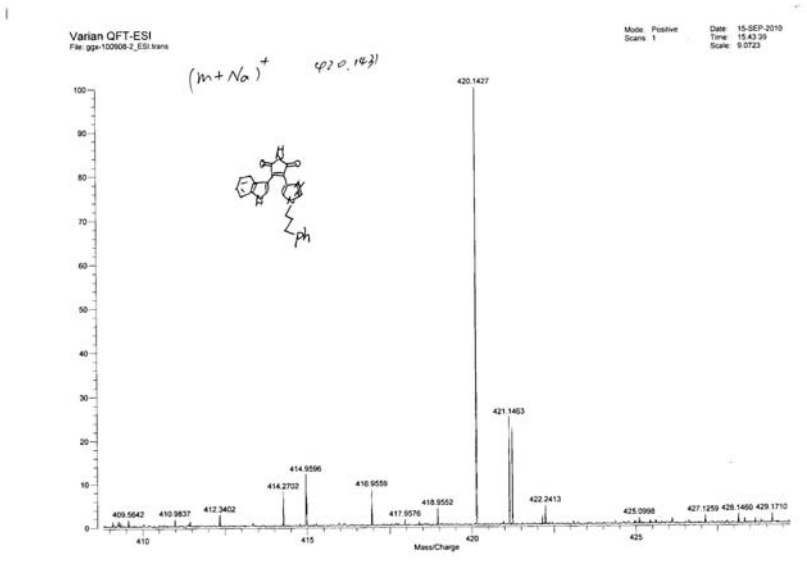




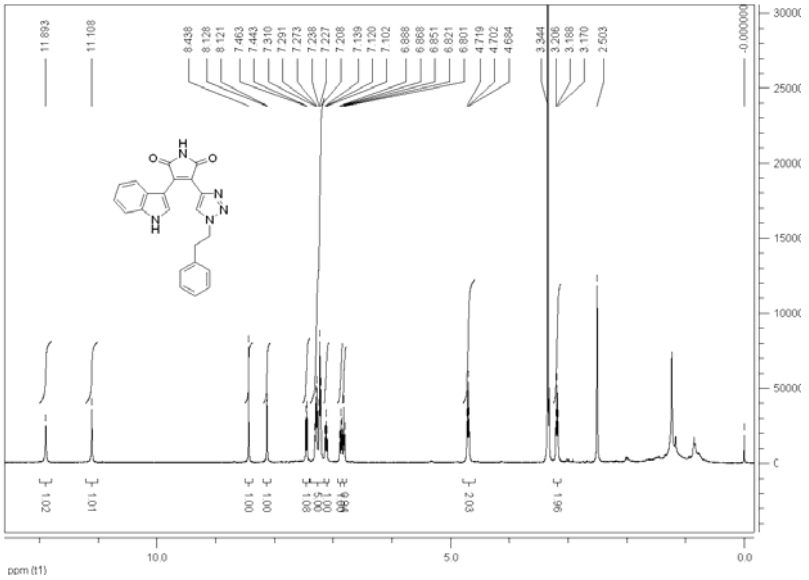
^{1}H NMR, ^{13}C NMR, HRMS spectrum of
3-(1*H*-indol-3-yl)-4-(1-(3-phenylpropyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1c):

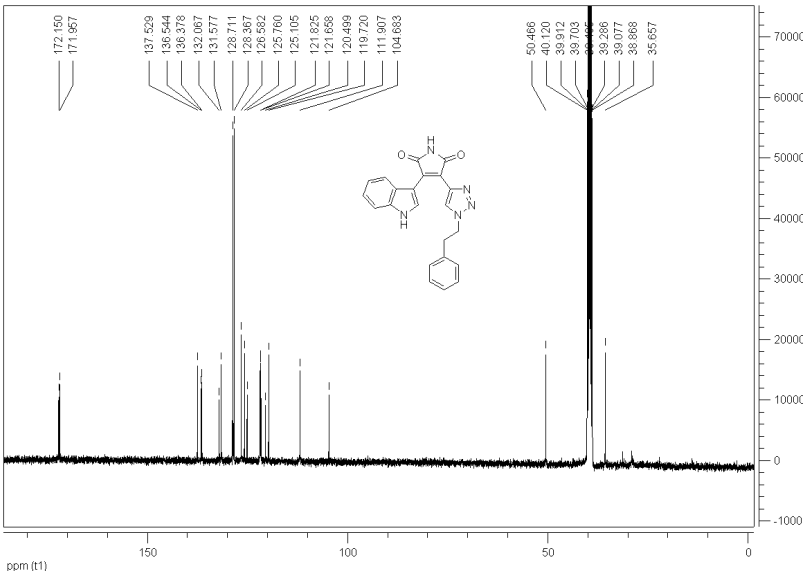


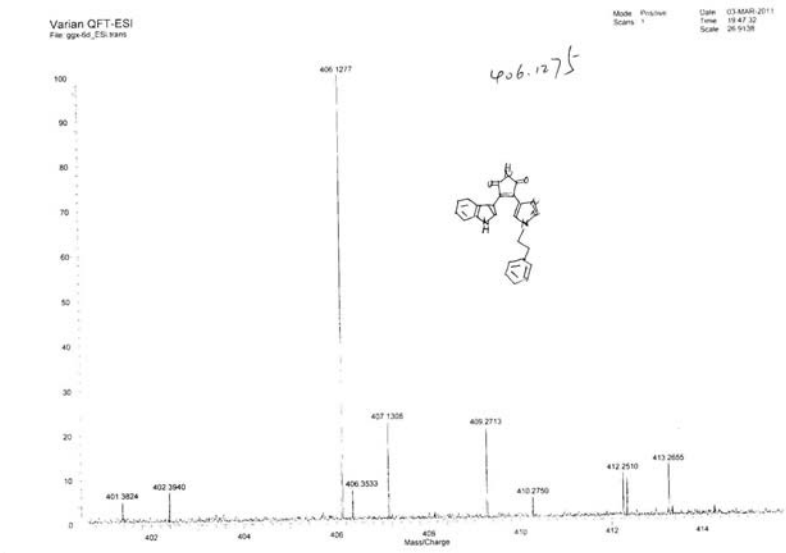




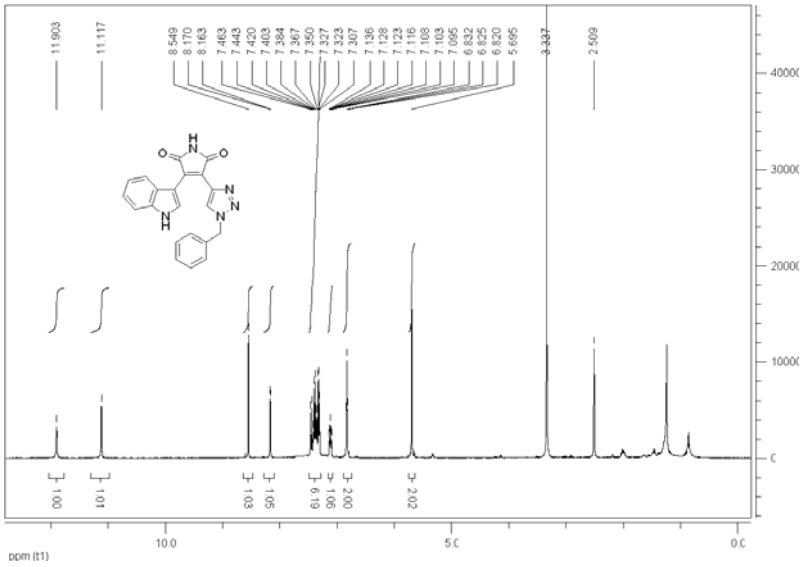
^{1}H NMR, ^{13}C NMR, HRMS spectrum of
3-(1*H*-indol-3-yl)-4-(1-(3-phenylethyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1d):

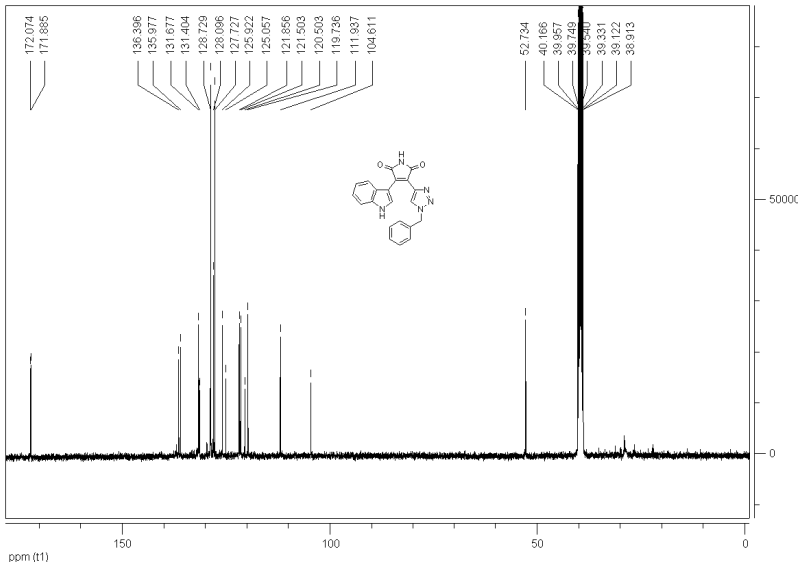


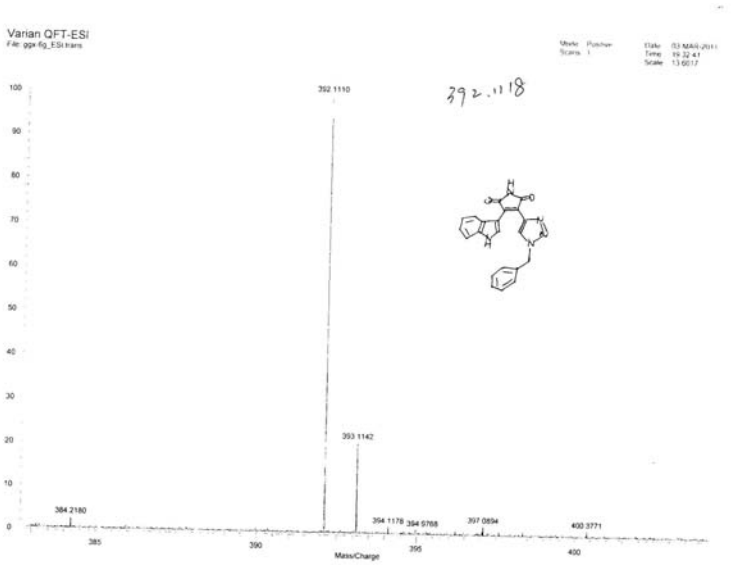




^{1}H NMR, ^{13}C NMR, HRMS spectrum of
3-(1*H*-indol-3-yl)-4-(1-(3-phenylmethyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1e):

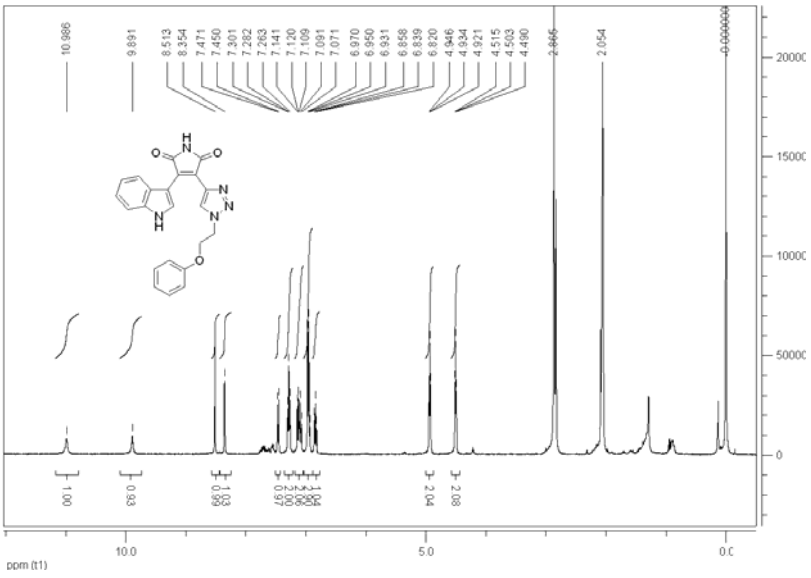


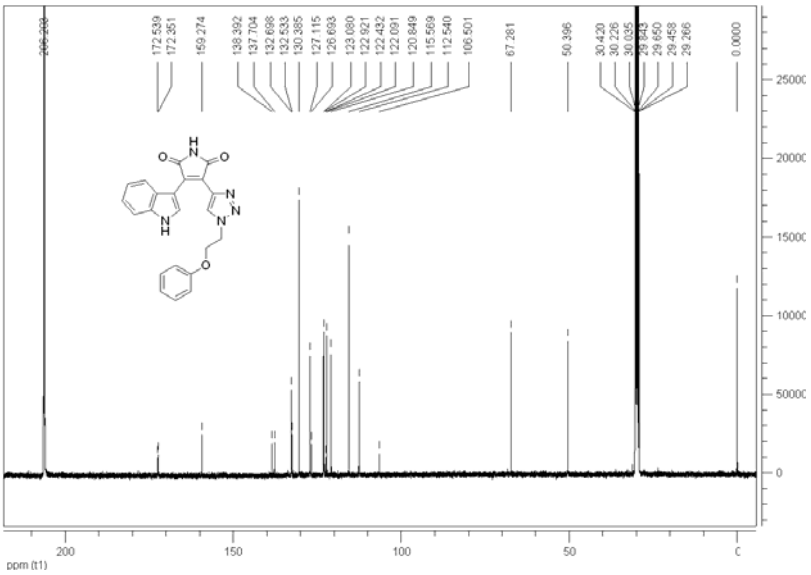


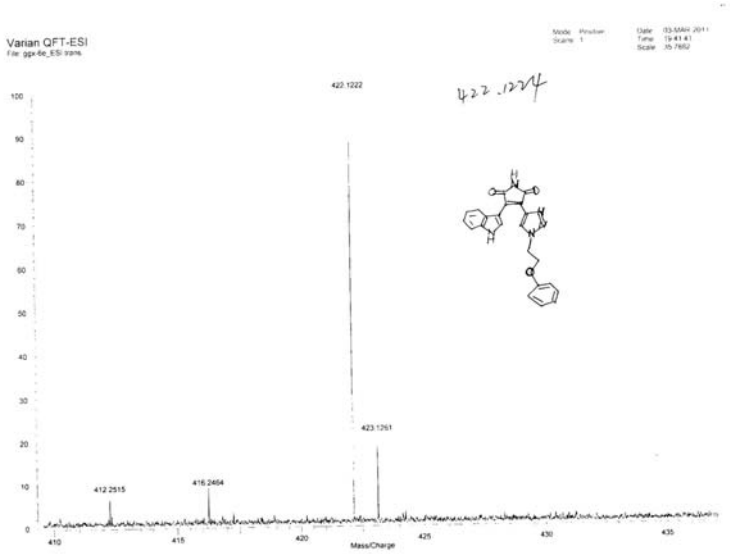


^{1}H NMR, ^{13}C NMR, HRMS spectrum of

3-(1*H*-indol-3-yl)-4-(1-(3-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1f):

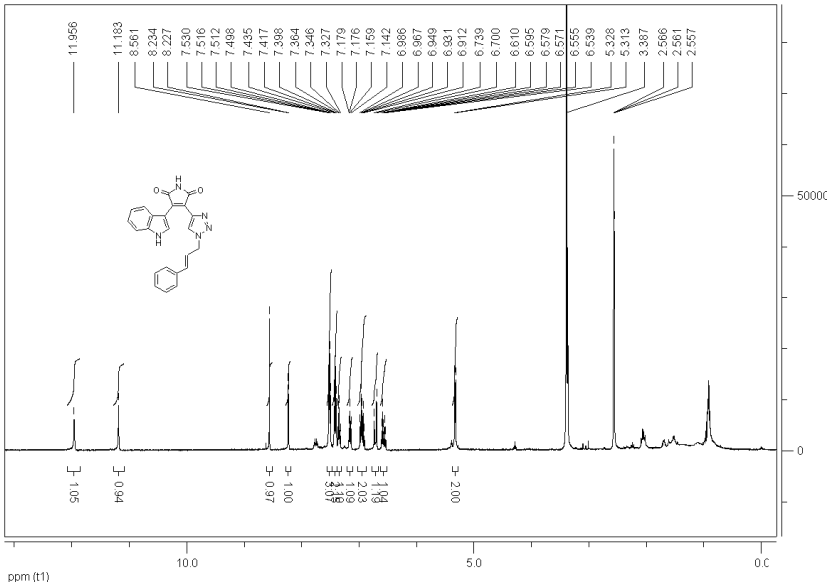


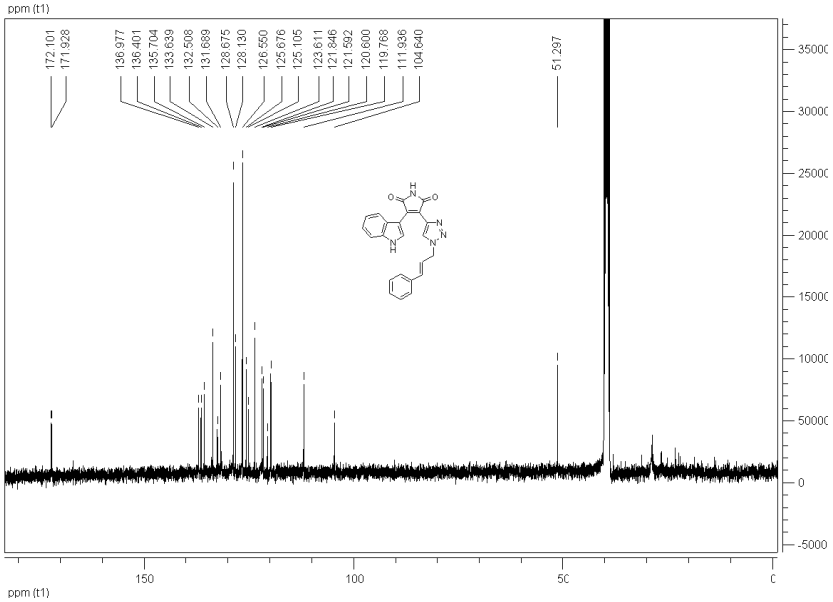


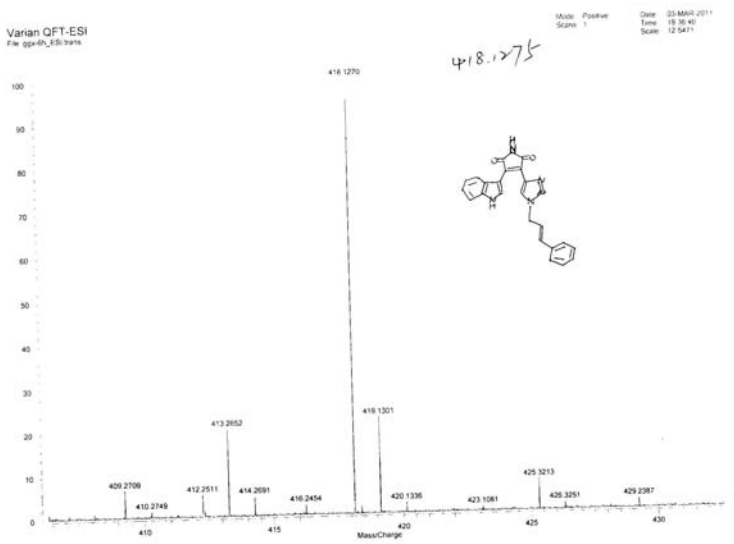


^{1}H NMR, ^{13}C NMR, HRMS spectrum of

3-(1*H*-indol-3-yl)-4-(1-(3-cinnamyl)-1*H*-1,2,3-triazol-4-yl)-maleimide (1g):







A panel of 124-kinase assay for 1c at 500 nM.

The kinases represent for the following abbreviations can be found on http://www.kinase-screen.mrc.ac.uk/index.htm.

general assay procedure:

All assays (25.5 μL volume) are carried out robotically at room temperature (21℃) and are linear with respect to time and enzyme concentration under the conditions used. Assays are performed for 30 min using Multidrop Micro reagent dispensers in a 96-well format. To plates containing 0.5 μL of compounds, 15 μL of an enzyme mix containing enzyme and peptide/protein substrate in buffer is added. Compounds are pre-incubated in the presence of the enzyme and peptide/protein substrate for 5 minutes before initiation of the reaction by addition of 10 μL of ATP .Assays are incubated at room temperature for 30mins before termination by the addition of 5 μL orthophosphoric acid. The assay plates are then harvester onto P81 Unifilter Plates by a Perkin Elmer Harvester and dried in air. The dry Unifilter plates are then sealed on the addition of MicroScint O and are counted in Packard Topcount NXT scintillation counters.

Kinaes	MKK1	MKK2	MKK6	ERK1	ERK2	JNK1	JNK2	JNK3	p38a MAPK	p38b MAPK	p38g MAPK	p38d MAPK
Mean %activity	95±10	107±22	93±7	91±9	111±9	94±2	100±18	115±6	93±3	110±6	119±5	120±7

Kinaes	ERK8	RSK1	RSK2	PDK1	PKBα	PKBβ	SGK1	S6K1	PKA	ROCK2	PRK2	PKCα
Mean %activity	71±9	108±2	89±7	109±8	120±13	128±24	100±1	122±23	101±8	111±9	125±7	110±4

Kinaes	PKCγ	PKCz	PKD1	STK33	MSK1	MNK1	MNK2	MAPKAP-K2	MAPKAP-K3	PRAK	CAMKKb	CAMK1
Mean %activity	122±6	108±10	87±4	100±2	93±4	94±12	108±4	100±5	110±9	112±11	82±23	92±3

Kinaes	SmMLCK	PHK	DAPK1	CHK1	CHK2	GSK3b	CDK2-CyclinA	PLK1	Aurora A	Aurora B	TLK1	LKB1
Mean %activity	94±20	108±4	104±14	100±16	104±2	32±0	95±2	91±1	89±3	101±4	109±5	108±8

Kinaes	AMPK	MARK1	MARK2	MARK3	MARK4	BRSK1	BRSK2	MELK	NUAK1	TSSK1	CK1	CK2
Mean %activity	111±5	107±6	100±1	97±15	94±2	95±3	104±10	95±18	94±7	84±13	103±12	94±4

Kinaes	ERK	RSK	RSK	PDK	PKBα	PKBβ	SGK	S6K1	PKA	ROCK	PRK	PKC

	8	1	2	1			1			2	2	α
Mean %activity	71±9	108±2	89±7	109±8	120±13	128±24	100±1	122±23	101±8	111±9	125±7	110±4

Kinaes	TTBK1	DYRK1A	DYRK2	DYRK3	NEK2a	NEK6	IKKb	IKKe	TBK1	PIM1	PIM2	PIM3
Mean %activity	102±1	88±18	120±6	106±21	127±8	102±18	91±20	90±9	94±7	84±5	100±8	82±4

Kinaes	SRPK1	EF2K	EIF2AK3	HIPK1	HIPK2	HIPK3	CLK2	PAK2	PAK4	PAK5	PAK6	MST2
Mean %activity	120±5	95±3	101±12	111±4	103±1	105±14	52±1	114±8	108±14	106±6	112±6	126±12

Kinaes	MST4	GCK	MINK1	MEKK1	MLK1	MLK3	TESK1	TAO1	ASK1	TAK1	IRAK1	IRAK4
Mean %activity	94±5	94±8	104±1	116±2	90±4	101±4	113±19	129±11	112±4	104±13	99±10	93±6
Kinaes	RIPK2	OSR1	TTK	MPSK1	Src	Lck	CSK	YES1	ABL	BTK	JAK2	SYK
Mean %activity	84±12	115±3	108±13	90±1	91±10	99±5	100±11	87±25	105±3	106±11	111±4	89±3

Kinaes	ZAP70	TIE2	BRK	EPH-A2	EPH-A4	EPH-B1	EPH-B2	EPH-B3	EPH-B4	FGF-R1	HER4	IGF-1R
Mean %activity	24±32	102±4	101±15	138±2	113±8	113±22	133±11	92±3	114±17	84±14	130±15	102±8

Kinaes	IR	IRR	TrkA	VEGFR2
Mean %activity	101±4	106±6	116±11	52±1

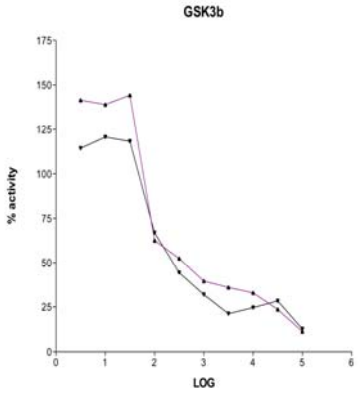
Compound 1c IC50

GSK3b assay

GSK3b (5–20 mU diluted in 20 mM MOPS pH 7.5, 1 mM EDTA, 0.01% Brij35, 5% glycerol, 0.1% b-mercaptoethanol, 1 mg/ml BSA) is assayed against Phospho-GS2 peptide (YRRAAVPPSPSLSRHSSPHQS(PO4)EDEEE) in a final volume of 25.5 µl containing 8 mM MOPS pH 7.0, 0.2 mM EDTA, 20 µM Phospho GS2 peptide, 10 mM magnesium acetate and 0.005 mM [33P-g-ATP] (50-1000 cpm/pmole) and incubated for 30 min at room temperature. Assays are stopped by addition of 5 µl of 0.5 M (3%) orthophosphoric acid and then harvested onto P81 Unifilter plates with a wash buffer of 50 mM orthophosphoric acid.

% activity

micromolar	**100.000**	**30.000**	**10.000**	**3.000**	**1.000**	**0.300**	**0.100**	**0.030**	**0.010**	**0.003**	nM	micromolar
1c	12.6	28.4	24.6	21.2	32.0	44.5	66.9	118.3	120.7	114.4	108.8	0.11
1c	11.1	23.6	33.0	36.1	39.7	52.4	62.5	144.2	139.0	141.4	83.89	0.08

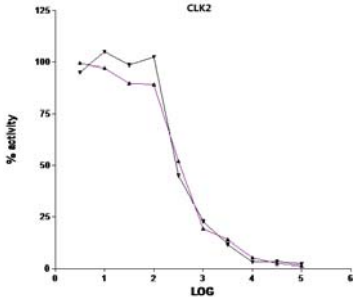


CLK2 assay

CLK2 (5-20mU diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/ml BSA) is assayed against a substrate peptide (RNRYRDVSPFDHSR) in a final volume of 25.5 µl containing 50mM Tris pH 7.5, 0.3mM peptide, 10mM DTT, 10 mM magnesium acetate and 0.005 mM [33P-γ-ATP] (50-1000 cpm/pmole) and incubated for 30 min at room temperature. Assays are stopped by addition of 5 µl of 0.5 M (3%) orthophosphoric acid and then harvested onto P81 Unifilter plates with a wash buffer of 50 mM orthophosphoric acid.

% activity

micromolar	**100.000**	**30.000**	**10.000**	**3.000**	**1.000**	**0.300**	**0.100**	**0.030**	**0.010**	**0.003**	nM	micromolar
1c	2.1	3.2	3.0	11.6	22.8	45.0	102.3	98.4	104.9	94.9	345.2	0.3452
1c	1.2	2.3	5.1	13.9	19.2	51.9	88.9	89.5	97.0	99.3	346.3	0.3463

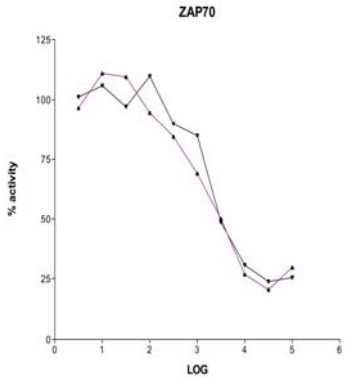


ZAP70 assay

ZAP70 (5-20mU diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/ml BSA0.1%,β-mercaptoethanol) is assayed against a substrate peptide (Poly Glut Tyr) in a final volume of 25.5 µl containing 50mM Tris pH 7.5, 0.1mMEGTA, 10mM MnCl,1mg/ml substrate peptide, 10 mM magnesium acetate 0.005 mM [33P-γ-ATP] (50-1000cpm/pmole) and incubated for 30 min at room temperature. Assays are stopped by addition of 5 µl of0.5 M (3%) orthophosphoric acid and then harvested onto P81 Unifilter plates with a wash buffer of 50mM orthophosphoric acid.

% activity

micromolar	100.000	30.000	10.000	3.000	1.000	0.300	0.100	0.030	0.010	0.003	nM	micromolar
1c	25.4	23.7	30.6	48.7	84.9	89.9	109.8	97.2	105.9	101.2	2089	2.09
1c	29.6	20.4	26.7	50.1	69.1	84.5	94.6	109.5	111.0	96.6	1229	1.23

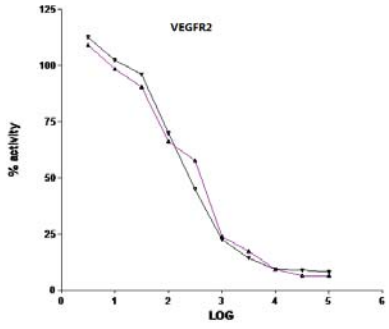


VEGFR2 assay

VEGFR2 (5-20mU diluted in 50 mM Tris pH 7.5, 0.1 mM EGTA, 1 mg/ml BSA) is assayed against a substrate peptide (KKKSPGEYVNIEFG) in a final volume of 25.5 µl containing 50mM Tris pH 7.5, 300µM substrate peptide, 10 mM magnesium acetate and 0.02 mM [33P-g-ATP] (50-1000 cpm/pmole) and incubated for 30 min at room temperature. Assays are stopped by addition of 5 µl of 0.5 M (3%) orthophosphoric acid and then harvested onto P81 Unifilter plates with a wash buffer of 50 mM orthophosphoric acid.

% activity

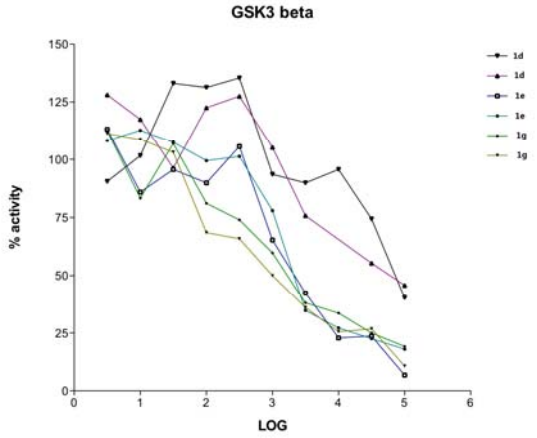
micromolar	100.000	30.000	10.000	3.000	1.000	0.300	0.100	0.030	0.010	0.003	nM	micromolar
1c	8.0	8.9	9.2	14.3	22.5	44.8	69.9	96.0	102.3	112.5	159.1	0.16
1c	6.4	6.5	9.2	17.4	23.8	57.6	66.1	90.4	98.4	109.0	232.7	0.23



Compound 1d, 1e and 1g IC50

% activity

micromolar	100.000	30.000	10.000	3.000	1.000	0.300	0.100	0.030	0.010	0.003	nM	micromolar
1d	47.5	77.4	96.2	91.1	94.3	131.2	127.6	129.2	101.4	91.6	39492	39.49
1d	52.2	60.7	108.7	78.7	104.6	124.2	119.8	96.7	115.3	124.7	30290	30.29
1e	18.0	32.8	32.1	49.3	69.4	105.0	91.1	96.2	87.5	111.5	2003	2.00
1e	27.8	31.8	35.9	42.5	80.6	101.2	99.6	106.5	111.2	107.3	1458	1.46
1g	28.8	33.9	41.5	45.5	64.6	77.1	83.4	106.0	85.3	111.0	1730.9	1.73
1g	21.3	35.5	34.4	43.7	56.0	69.9	72.2	102.7	107.7	110.0	1246.5	1.25



ATP-competation assay of compound 1c against GSK-3β

The ATP competitive assays followed the same assay procedure of kinase assay but different concentrations of ATP were used. We used 10 dilutions of ATP ranging from 0.5uM to 500uM.

SUMMARY mean % activity and standard deviation of compound

ATP conc. (μM)	0.5	1	2	5	10	20	50	100	200	500
mean % activity	11.8	18.1	40.0	43.3	53.5	57.6	66.8	81.2	83.9	91.1
SD	4.2	1.8	3.1	2.8	1.3	2.4	7.0	5.0	6.1	6.1