

Supporting Information

Characterization Data

1.1.1. (*E*)-*N*-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3*H*)-ylidene)butyramide (6a)

Light yellow crystalline; M.P = 173 °C; yield = 77%; R_f = 0.40 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3108 (H-C, Ar), 3064 (H-C, thiazoline), 2952, 2868 (H-C, alkyl asymmetric and symmetric), 1738 (C=O), 1588 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.95 (d, 1H, J = 2.1 Hz, quinolinyl-H), 8.33 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.21 (d, 1H, J = 2.4 Hz, quinolinyl-H), 7.93 (m, 2H, quinolinyl-H), 7.83 (d, 2H, J = 8.4 Hz, phenyl-H), 7.72 (m, 1H, quinolinyl-H), 7.39 (d, 2H, J = 8.4 Hz, phenyl-H), 6.48 (s, 1H, thiazoline-H), 2.36 (d, 2H, J = 3.6, aliphatic-H), 1.55 (sx, 2H, J = 7.2 Hz, aliphatic-H), 0.89 (t, 3H, J = 7.2 Hz, aliphatic-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 173.5 (C=O), 170.8 (imine, C), 149.4, 147.2, 138.3, 135.3, 134.9, 131.1, 130.8, 130.7, 129.3, 128.2, 128.0, 127.5, 126.4, 104.6 (thiazoline, C), 30.5, 20.3, 13.5. Anal. Calc. for $\text{C}_{22}\text{H}_{18}\text{BrN}_3\text{OS}$: C, 58.41; H, 4.01; N, 9.29; Found C, 58.38; H, 4.00; N, 9.25. HRMS Calcd for $\text{C}_{22}\text{H}_{18}\text{BrN}_3\text{OS} + \text{H}$: 451.0354. Found 451.0351

1.1.2. (*E*)-*N*-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3*H*)-ylidene)pentanamide (6b)

Brown crystalline; M.P = 173 °C; yield = 75%; R_f = 0.45 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3059 (H-C, Ar), 2957 (H-C, thiazoline), 2928, 2858 (H-C, alkyl asymmetric and symmetric), 1738 (C=O), 1589 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.93 (d, 1H, J = 2.1 Hz, quinolinyl-H), 8.32 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.23 (d, 1H, J = 2.4 Hz, quinolinyl-H), 7.91 (m, 2H, quinolinyl-H), 7.82 (d, 2H, J = 8.4 Hz, phenyl-H), 7.70 (m, 1H, quinolinyl-H), 7.41 (d, 2H, J = 8.4 Hz, phenyl-H), 6.45 (s, 1H, thiazoline-H), 2.36 (t, 2H, J = 7.2 Hz, aliphatic-H), 1.53 (m, 2H, aliphatic-H), 1.2 (m, 2H, aliphatic-H), 0.90 (t, 3H, J = 7.5 Hz, aliphatic-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 172.5 (C=O), 171.0 (imine, C), 149.2, 148.1, 137.3, 134.6, 133.8, 130.9, 129.8, 129.4, 128.5, 127.5, 127.0, 126.9, 126.2, 105.0 (thiazoline, C), 31.2, 22.5, 19.2, 13.2. Anal. Calc. for $\text{C}_{23}\text{H}_{20}\text{BrN}_3\text{OS}$: C, 59.23; H, 4.32; N, 9.01; Found: C, 59.21; H, 4.31; N, 9.00. HRMS Calcd for $\text{C}_{23}\text{H}_{20}\text{BrN}_3\text{OS} + \text{H}$: 465.0510. Found 465.0507

1.1.3. (*E*)-*N*-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3*H*)-ylidene)hexanamide (6c)

Off-white powder; M.P = 181-182 °C; yield = 68%; R_f = 0.48 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3102 (H-C, Ar), 3050 (H-C, thiazoline), 2913, 2850 (H-C, alkyl asymmetric and symmetric), 1736 (C=O), 1588 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.94 (d, 1H, J = 2.1 Hz, quinolinyl-H), 8.30 (d, 1H, J = 8.4 Hz quinolinyl-H), 8.19 (d, 1H, J = 2.1 Hz, quinolinyl-H), 7.93 (m, 2H, quinolinyl-H), 7.84 (d, 2H, J = 8.4 Hz, phenyl-H), 7.72 (m, 1H, quinolinyl-H), 7.40 (d, 2H, J = 8.4 Hz, phenyl-H), 6.49 (s, 1H, thiazoline-H), 2.39 (m, 2H, aliphatic-H), 1.53 (m, 2H, aliphatic-H), 1.22 (m, 4H, aliphatic-H), 0.82 (t, 3H, J = 7.5 Hz aliphatic-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 173.6 (C=O), 170.8 (imine, C), 149.6, 147.6, 138.6, 135.3, 134.5, 131.1, 131.0, 130.8, 130.7, 129.6, 128.1, 127.9, 127.5, 126.4, 104.5 (thiazoline, C), 31.0, 28.5, 26.6, 22.2, 13.7. Anal. Calc. for $\text{C}_{24}\text{H}_{22}\text{BrN}_3\text{OS}$: C, 60.00; H, 4.62; N, 8.75; Found: C, 59.98; H, 4.59; N, 8.71. . HRMS Calcd for $\text{C}_{24}\text{H}_{22}\text{BrN}_3\text{OS} +\text{H}$: 479.0667. Found 479.0664

1.1.4. (*E*)-*N*-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3*H*)-ylidene)heptanamide (6d)

Light yellow powder; M.P = 188-190 °C; yield = 70%; R_f = 0.50 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3098 (H-C, Ar), 3012 (H-C, thiazoline), 2958, 2860 (H-C, alkyl asymmetric and symmetric), 1715 (C=O), 1580 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.89 (d, 1H, J = 2.4 Hz, quinolinyl-H), 8.27 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.19 (d, 1H, J = 2.4 Hz, quinolinyl-H), 7.88 (m, 2H, quinolinyl-H), 7.80 (d, 2H, J = 8.4 Hz, phenyl-H), 7.67 (m, 1H, quinolinyl-H), 7.40 (d, 2H, J = 8.4 Hz, phenyl-H), 6.43 (s, 1H, thiazoline-H), 2.38 (m, 2H, aliphatic-H), 1.55 (m, 2H, aliphatic-H), 1.30 (m, 6H, aliphatic-H), 0.92 (t, 3H, J = 7.2 Hz, aliphatic-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 172.3 (C=O), 170.8 (imine, C), 149.0, 147.4, 137.1, 134.2, 132.8, 130.4, 129.3, 128.6, 128.2, 127.9, 127.1, 126.5, 126.0, 104.3 (thiazoline, C), 31.2, 29.7, 28.2, 24.6, 22.2, 13.5. Anal. Calc. for $\text{C}_{25}\text{H}_{24}\text{BrN}_3\text{OS}$: C, 60.73; H, 4.89; N, 8.50; Found: C, 60.69; H, 4.87; N, 8.47. HRMS Calcd for $\text{C}_{25}\text{H}_{24}\text{BrN}_3\text{OS} +\text{H}$: 493.0823. Found 493.0823

**1.1.5. (E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)octanamide
(6e)**

Light yellow powder; M.P = 192 °C; yield = 71%; R_f = 0.52 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3112 (H-C, Ar), 3055 (H-C, thiazoline), 2920, 2843 (H-C, alkyl asymmetric and symmetric), 1702 (C=O), 1592 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.93 (d, 1H, J = 2.1 Hz, quinolinyl-H), 8.31 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.20 (d, 1H, J = 2.1 Hz, quinolinyl-H), 7.94 (m, 2H, quinolinyl-H), 7.86 (d, 2H, J = 8.4 Hz, phenyl-H), 7.74 (m, 1H, quinolinyl-H), 7.42 (d, 2H, J = 8.4 Hz, phenyl-H), 6.51 (s, 1H, thiazoline-H), 2.63 (m, 2H, aliphatic-H), 1.72 (m, 2H, aliphatic-H), 1.35 (m, 8H, aliphatic-H), 0.89 (t, 3H, J = 7.5 Hz, aliphatic-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 172.0 (C=O), 170.7 (imine, C), 148.4, 147.3, 138.1, 134.7, 134.0, 130.9, 130.3, 129.9, 129.6, 128.0, 127.8, 127.4, 126.3, 104.9 (thiazoline, C), 31.0, 29.7, 28.5, 26.6, 24.5, 22.2, 13.7. Anal. Calc. for $\text{C}_{26}\text{H}_{26}\text{BrN}_3\text{OS}$: C, 61.41; H, 5.15; N, 8.26; Found: C, 61.39; H, 5.12; N, 8.25. HRMS Calcd for $\text{C}_{26}\text{H}_{26}\text{BrN}_3\text{OS} + \text{H}$: 507.0980 Found 507.0980

**1.1.6. (E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)decanamide
(6f)**

Yellow powder; M.P = 180 °C; yield = 73%; R_f = 0.53 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3105 (H-C, Ar), 3038 (H-C, thiazoline), 2930, 2865 (H-C, alkyl asymmetric and symmetric), 1705 (C=O), 1583 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.96 (d, 1H, J = 2.4 Hz, quinolinyl-H), 8.33 (d, 1H, J = 2.4 Hz, quinolinyl-H), 8.21 (d, 1H, J = 8.4 Hz, quinolinyl-H), 7.95 (m, 2H, quinolinyl-H), 7.87 (d, 2H, J = 8.4 Hz, phenyl-H), 7.76 (m, 1H, quinolinyl-H), 7.43 (d, 2H, J = 8.4 Hz, phenyl-H), 6.53 (s, 1H, thiazoline-H), 2.44 (m, 2H, aliphatic-H), 1.73 (m, 2H, aliphatic-H), 1.34 (m, 12H, aliphatic-H), 0.89 (t, 3H, J = 7.2 Hz, aliphatic-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 172.6 (C=O), 170.7 (imine, C), 148.9, 147.6, 137.4, 135.1, 133.7, 131.5, 131.1, 130.8, 130.5, 129.2, 128.1, 127.8, 127.6, 125.4, 105.3 (thiazoline, C), 37.3, 31.8, 29.4, 29.3, 29.2, 29.0, 24.7, 22.6, 14.1. Anal. Calc. for $\text{C}_{28}\text{H}_{30}\text{BrN}_3\text{OS}$: C, 62.68; H, 5.64; N, 7.83; Found: C, 62.65; H, 5.62; N, 7.80. HRMS Calcd for $\text{C}_{28}\text{H}_{30}\text{BrN}_3\text{OS} + \text{H}$: 535.1293 Found 535.1290

1.1.7. (E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)benzamide (6g)

Yellow powder; M.P = 217-220 °C; yield = 69%; R_f = 0.43 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3112 (H-C, Ar), 3062 (H-C, thiazoline), 1701 (C=O), 1588 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.84 (d, 1H, J = 2.4 Hz, quinolinyl-H), 8.27 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.17 (d, 1H, J = 2.4 Hz, quinolinyl-H), 7.94 (m, 2H, quinolinyl-H), 7.89 (m, 2H, Ar-H) 7.84 (d, 2H, J = 8.4 Hz, phenyl-H), 7.79 (m, 2H, Ar-H), 7.70 (m, 1H, quinolinyl-H), 7.61 (m, 1H, Ar-H), 7.39 (d, 2H, J = 8.4 Hz, phenyl-H), 6.47 (s, 1H, thiazoline-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 172.6 (C=O), 169.8 (imine, C), 149.9, 146.7, 137.5, 136.1, 135.2, 134.3, 134.1, 131.1, 131.0, 130.8, 130.7, 129.9, 129.6, 129.2, 128.1, 127.9, 127.3, 126.2, 105.5 (thiazoline, C). Anal. Calc. for $\text{C}_{25}\text{H}_{16}\text{BrN}_3\text{OS}$: C, 61.73; H, 3.32; N, 8.64; Found: C, 61.71; H, 3.29; N, 8.61. HRMS Calcd for $\text{C}_{25}\text{H}_{16}\text{BrN}_3\text{OS}+\text{H}$: 485.0197 Found 485.0194

1.1.8. (E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)-4-methylbenzamide (6h)

Yellow powder; M.P = 103-105 °C; yield = 68%; R_f = 0.45 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3114 (H-C, Ar), 3041 (H-C, thiazoline), 2913, 2850 (H-C, alkyl asymmetric and symmetric), 1709 (C=O), 1570 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.86 (d, 1H, J = 2.4 Hz, quinolinyl-H), 8.23 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.14 (d, 1H, J = 2.4 Hz, quinolinyl-H), 7.92 (m, 2H, quinolinyl-H), 7.81 (d, 2H, J = 8.4 Hz, phenyl-H), 7.77 (d, 2H, J = 7.8 Hz, Ar-H) 7.70 (m, 1H, quinolinyl-H), 7.48 (d, 2H, J = 7.8 Hz, Ar-H), 7.39 (d, 2H, J = 8.4 Hz, phenyl-H), 6.39 (s, 1H, thiazoline-H), 2.19 (s, 3H, aliphatic-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 171.2 (C=O), 168.9 (imine, C), 150.1, 147.7, 144.1, 137.5, 135.3, 134.5, 132.3, 131.1, 130.9, 130.6, 130.2, 130.0, 129.7, 129.0, 128.3, 126.9, 126.3, 125.4, 106.2 (thiazoline, C), 22.2. Anal. Calc. for $\text{C}_{26}\text{H}_{18}\text{BrN}_3\text{OS}$: C, 62.40; H, 3.63; N, 8.40; Found: C, 62.38; H, 3.60; N, 8.37. HRMS Calcd for $\text{C}_{26}\text{H}_{18}\text{BrN}_3\text{OS} +\text{H}$: 499.0354 Found 499.0351

1.1.9. (E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)-2,4-dichlorobenzamide (6i)

Off-white powder; M.P = 198 °C; yield = 64%; R_f = 0.32 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3115 (H-C, Ar), 3038 (H-C, thiazoline), 1679 (C=O), 1590 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.79 (d, 1H, J = 2.4 Hz, quinolinyl-H), 8.29 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.19 (d, 1H, J = 2.4 Hz, quinolinyl-H), 7.93 (m, 2H, quinolinyl-H), 7.88 (m, 2H, Ar-H) 7.82 (d, 2H, J = 8.4 Hz, phenyl-H), 7.79 (m, 1H, Ar-H), 7.69 (m, 2H, quinolinyl, Ar-H), 7.61 (d, 1H, Ar-H), 7.41 (d, 2H, J = 8.4 Hz, phenyl-H), 6.39 (s, 1H, thiazoline-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 173.4 (C=O), 171.3 (imine, C), 148.9, 147.6, 137.6, 136.3, 135.5 134.7, 134.2, 132.8, 131.9, 131.4, 130.7, 130.2, 129.9, 129.6, 128.7, 128.1, 127.9, 127.5, 126.2, 104.3 (thiazoline, C). Anal. Calc. for $\text{C}_{25}\text{H}_{14}\text{BrCl}_2\text{N}_3\text{OS}$: C, 54.08; H, 2.54; N, 7.57; Found: C, 54.05; H, 2.52; N, 7.55. HRMS Calcd for $\text{C}_{25}\text{H}_{14}\text{BrCl}_2\text{N}_3\text{OS} +\text{H}$: 552.9418 Found 552.9415

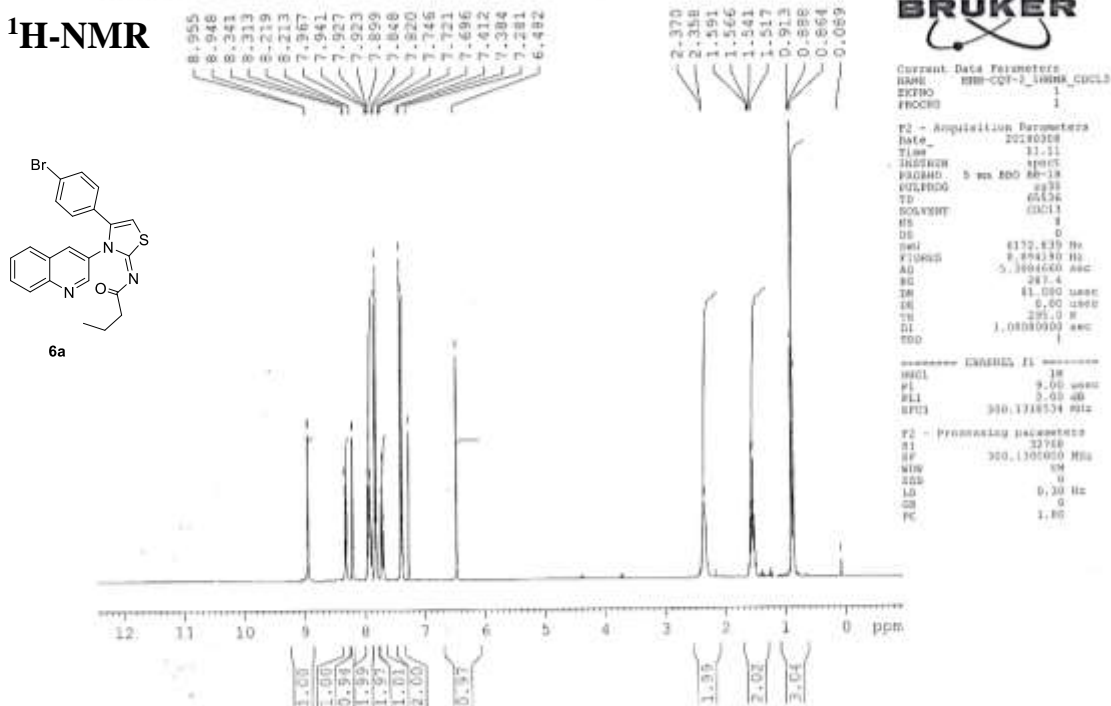
(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)-3,5-dinitrobenzamide (6j)

Orange powder; M.P = 205 °C; yield = 62%; R_f = 0.27 (EtOAc: n-hexane, 2:8); FT-IR (ATR) in cm^{-1} , 3080 (H-C, Ar), 3004 (H-C, thiazoline), 1680 (C=O), 1584 (C=C, Ar); $^1\text{H-NMR}$ (300 MHz, CDCl_3); in δ (ppm), 8.81 (s, 1H, Ar-H), 8.77 (d, 1H, J = 2.1 Hz, quinolinyl-H), 8.60 (s, 2H, Ar-H), 8.19 (d, 1H, J = 8.4 Hz, quinolinyl-H), 8.07 (d, 1H, J = 2.1 Hz, quinolinyl-H), 7.96 (m, 2H, quinolinyl-H), 7.85 (d, 2H, J = 8.4 Hz, phenyl-H), 7.73(m, 1H, quinolinyl-H), 7.37 (d, 2H, J = 8.4 Hz, phenyl-H), 6.51 (s, 1H, thiazoline-H); $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) in δ (ppm), 173.6 (C=O), 170.8 (imine, C), 151.1, 148.4, 147.9, 138.5, 137.2, 135.4, 134.9, 131.9, 130.7, 129.0, 128.7, 128.0, 126.4, 123.3, 103.6 (thiazoline, C). Anal. Calc. for $\text{C}_{25}\text{H}_{14}\text{BrN}_5\text{O}_5\text{S}$: C, 52.10; H, 2.45; N, 12.15; Found: C, 52.07; H, 2.42; N, 12.13. HRMS Calcd for $\text{C}_{25}\text{H}_{14}\text{BrN}_5\text{O}_5\text{S} +\text{H}$ 574.9899 Found 574.9895

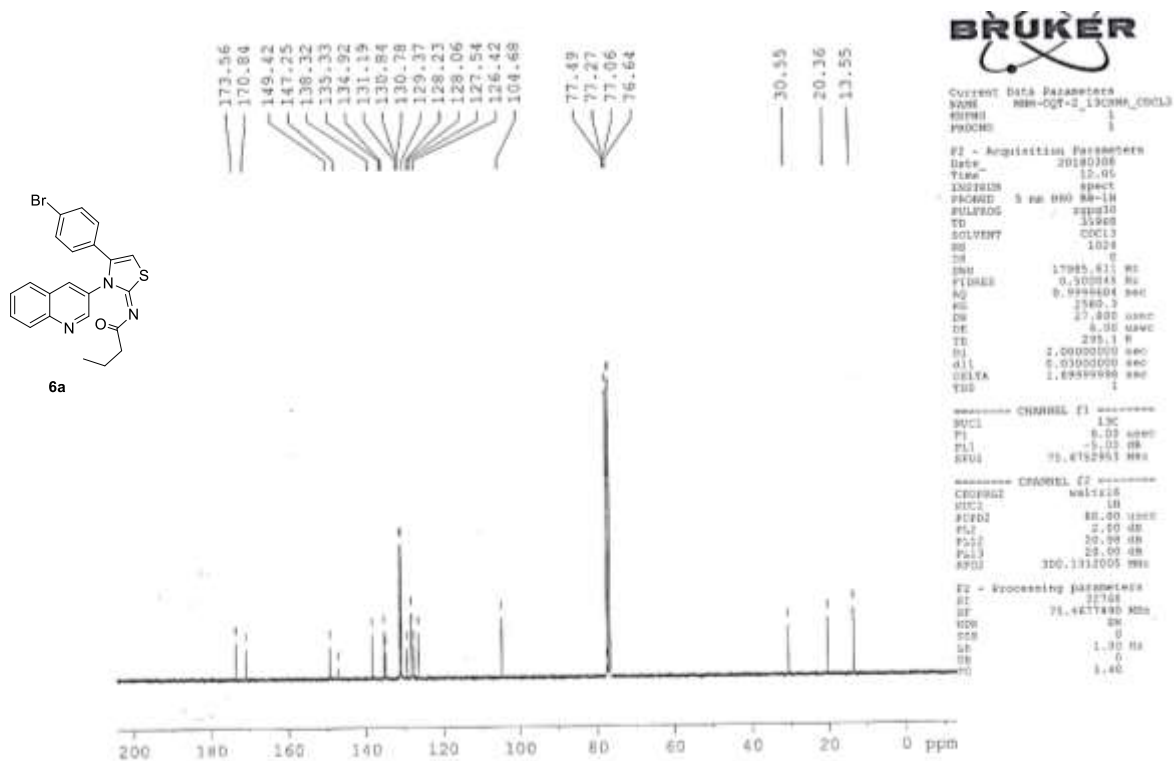
NMR Spectra

(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)butyramide (6a)

¹H-NMR

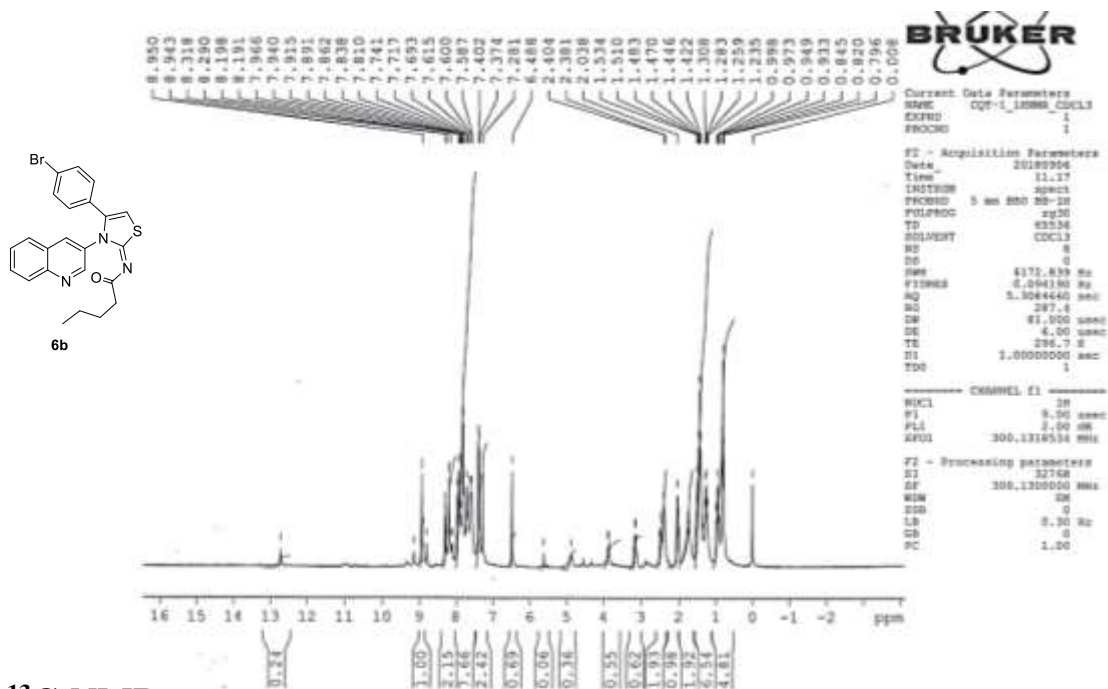


¹³C-NMR

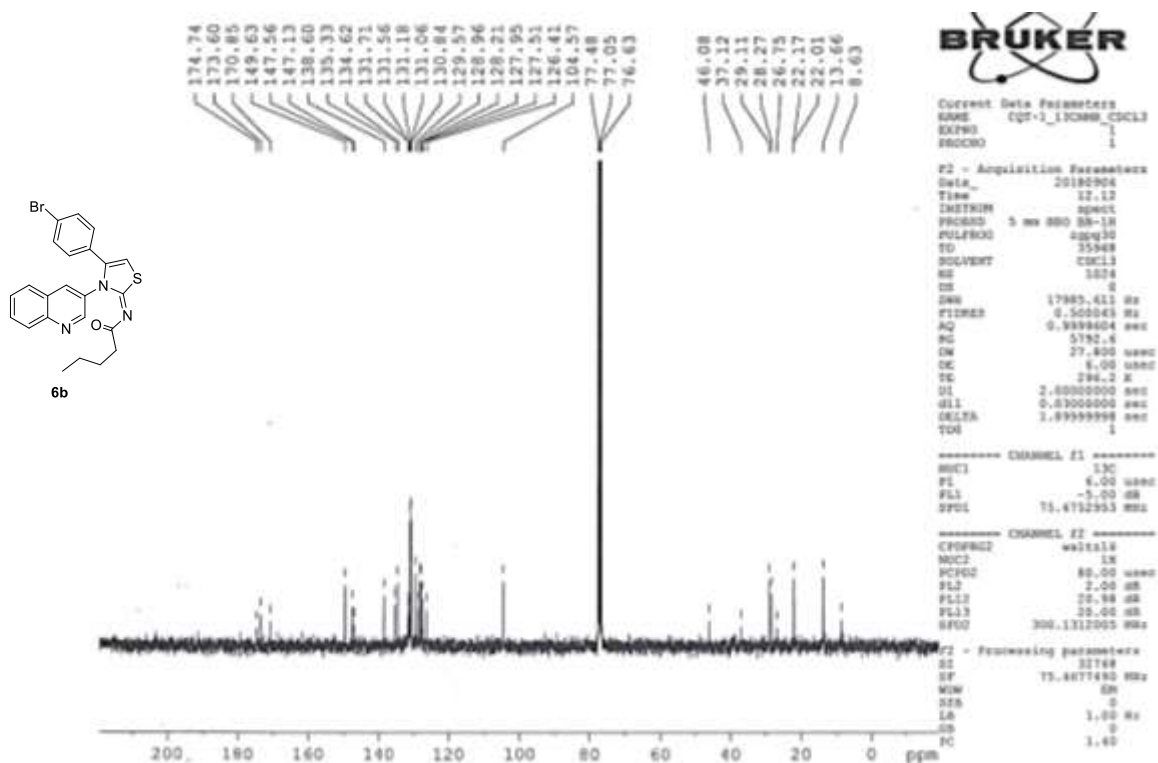


(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)pentanamide (6b)

¹H-NMR

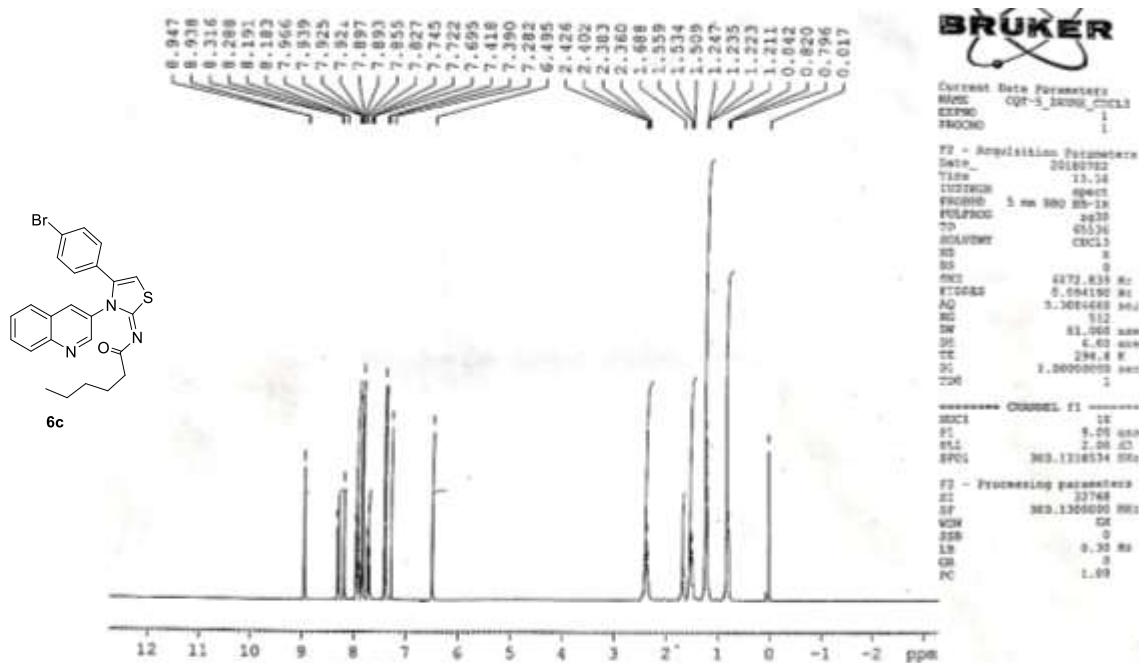


¹³C-NMR

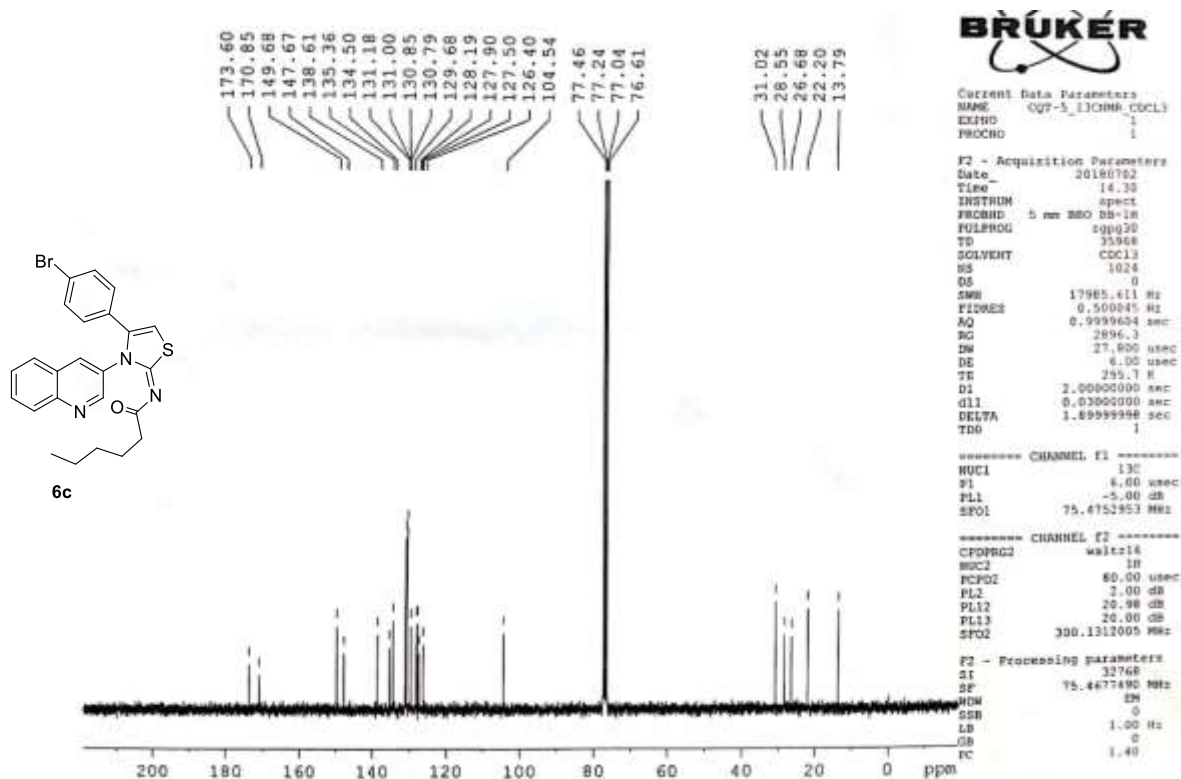


(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)hexanamide (6c)

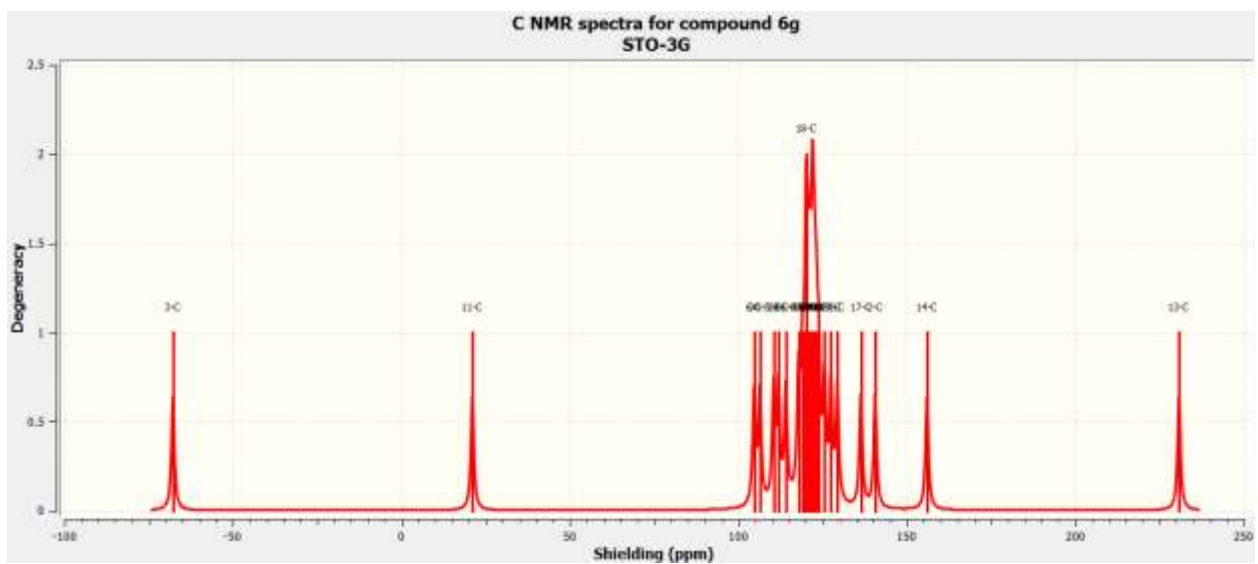
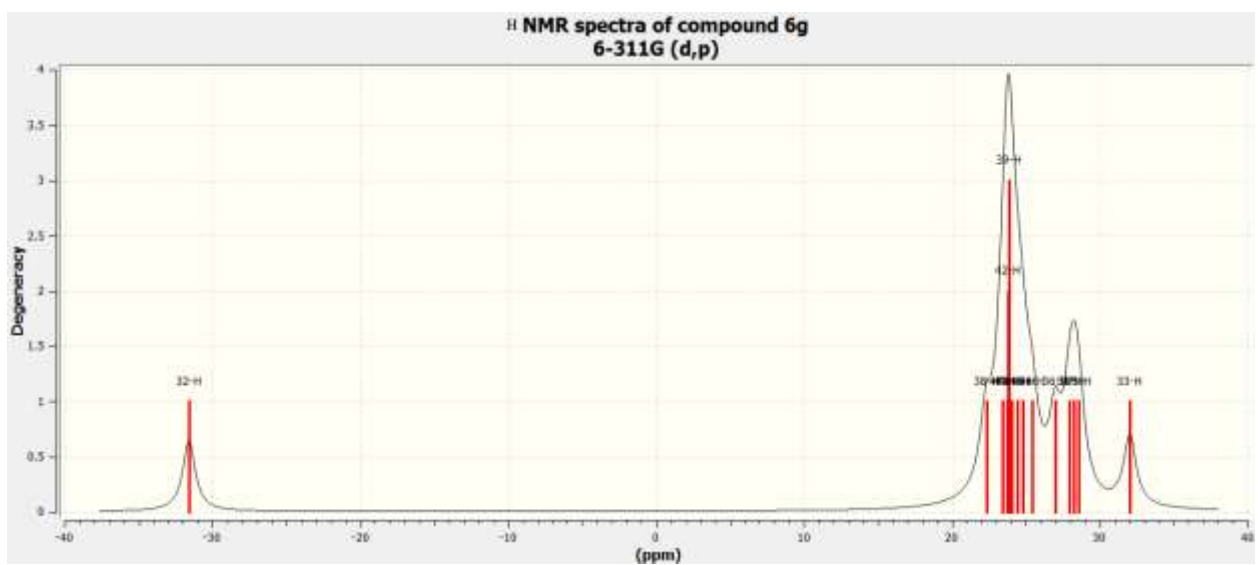
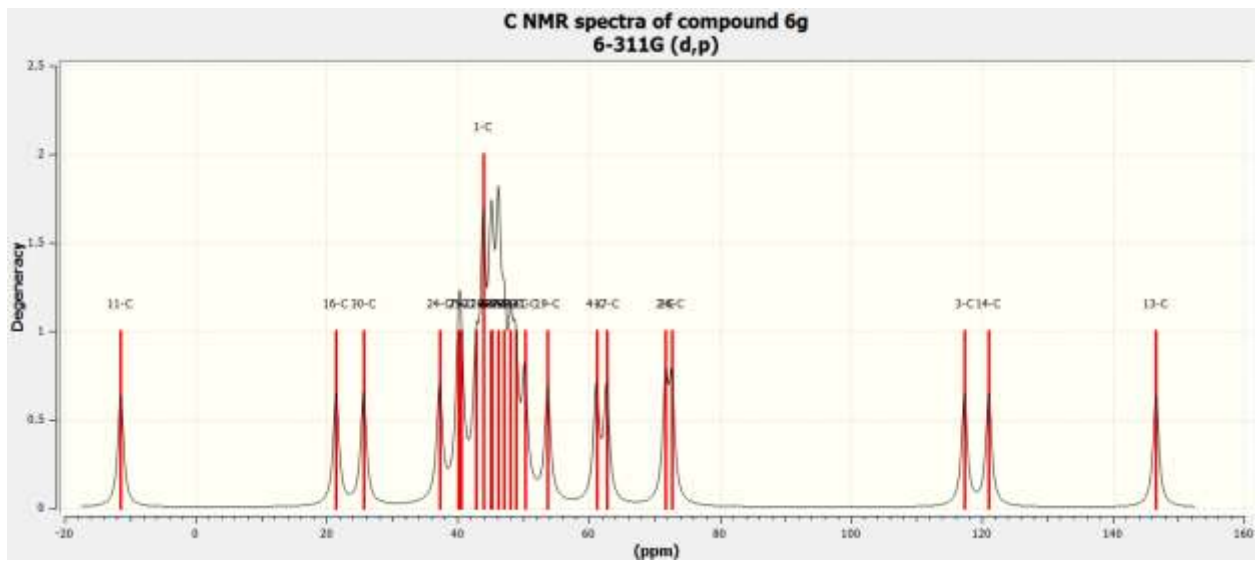
¹H-NMR

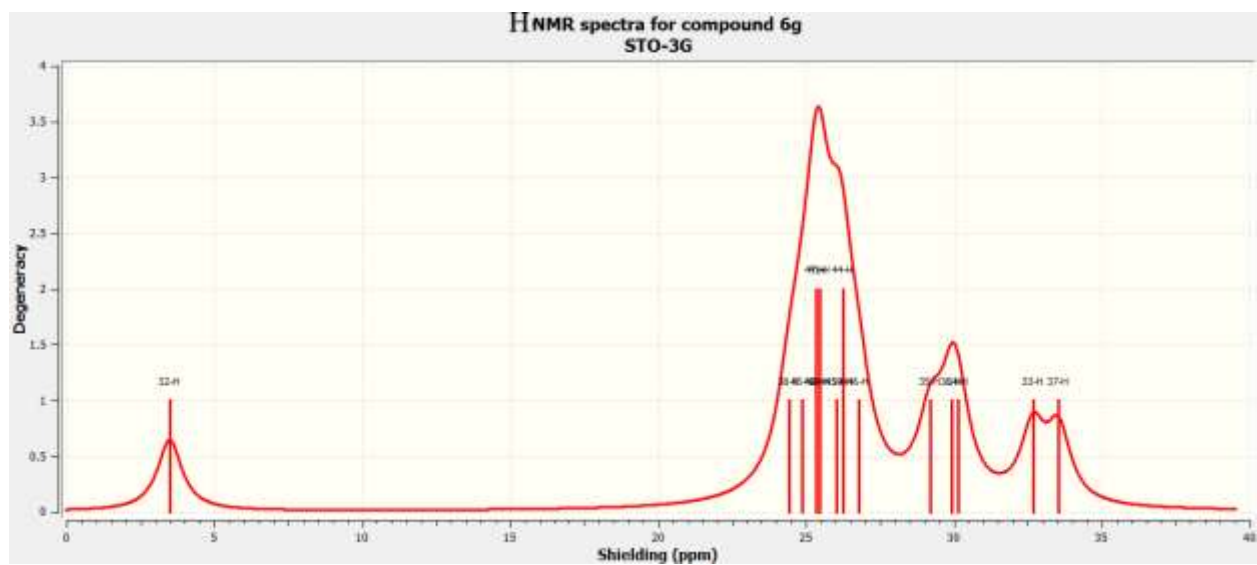


¹³C-NMR

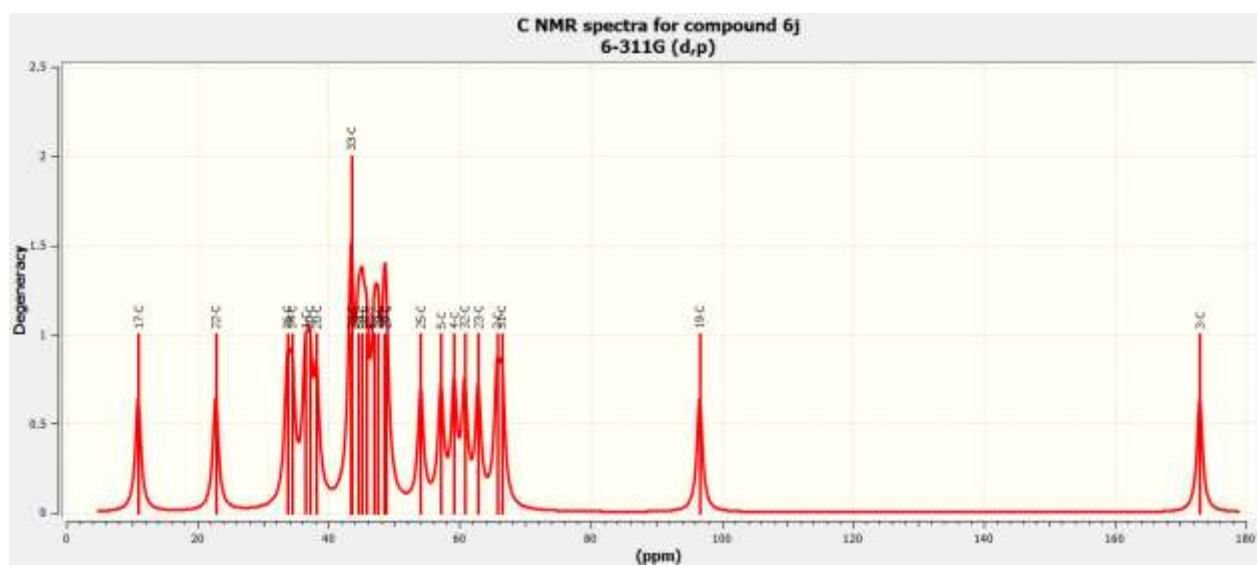


1.1.10. (*E*)-*N*-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3*H*)-ylidene)benzamide (6g)



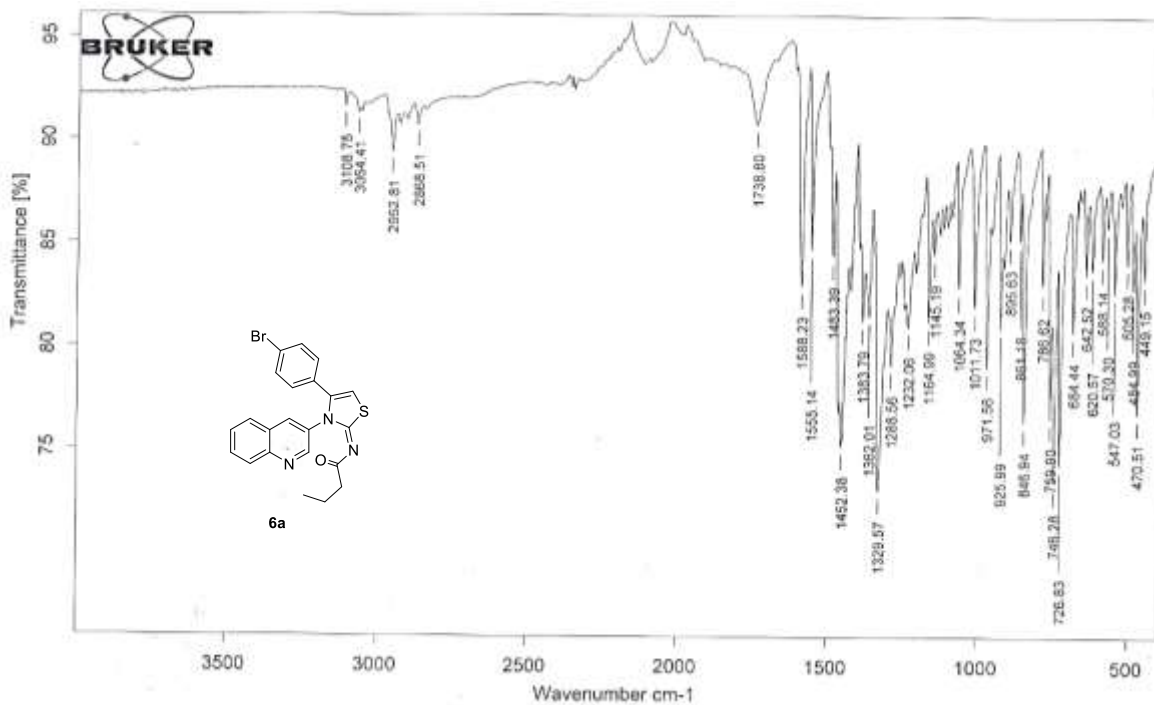


(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)-3,5-dinitrobenzamide (6j)

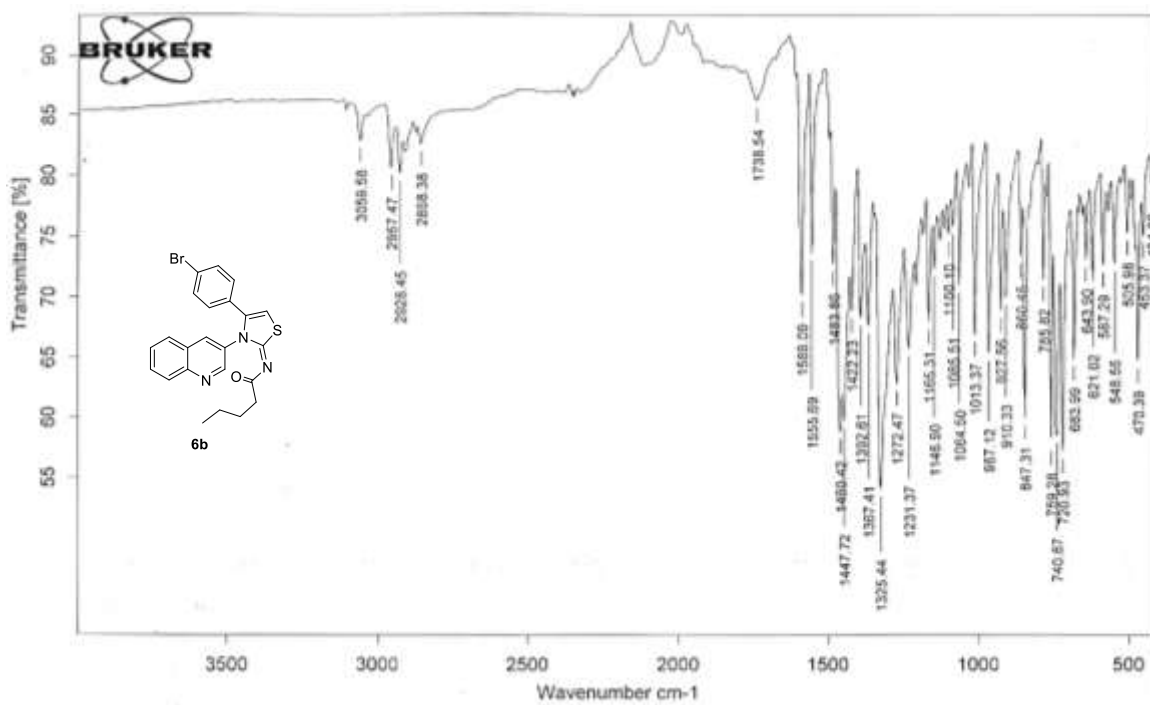


FT-IR

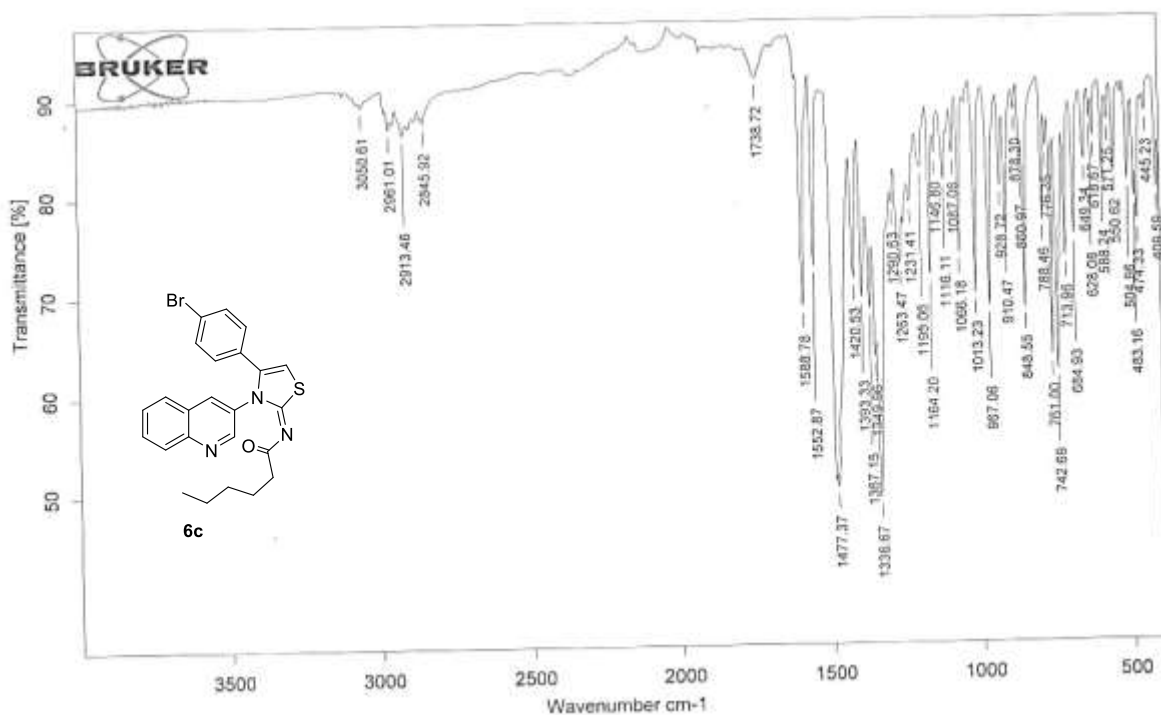
(*E*)-*N*-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3*H*)-ylidene)butyramide (6a)



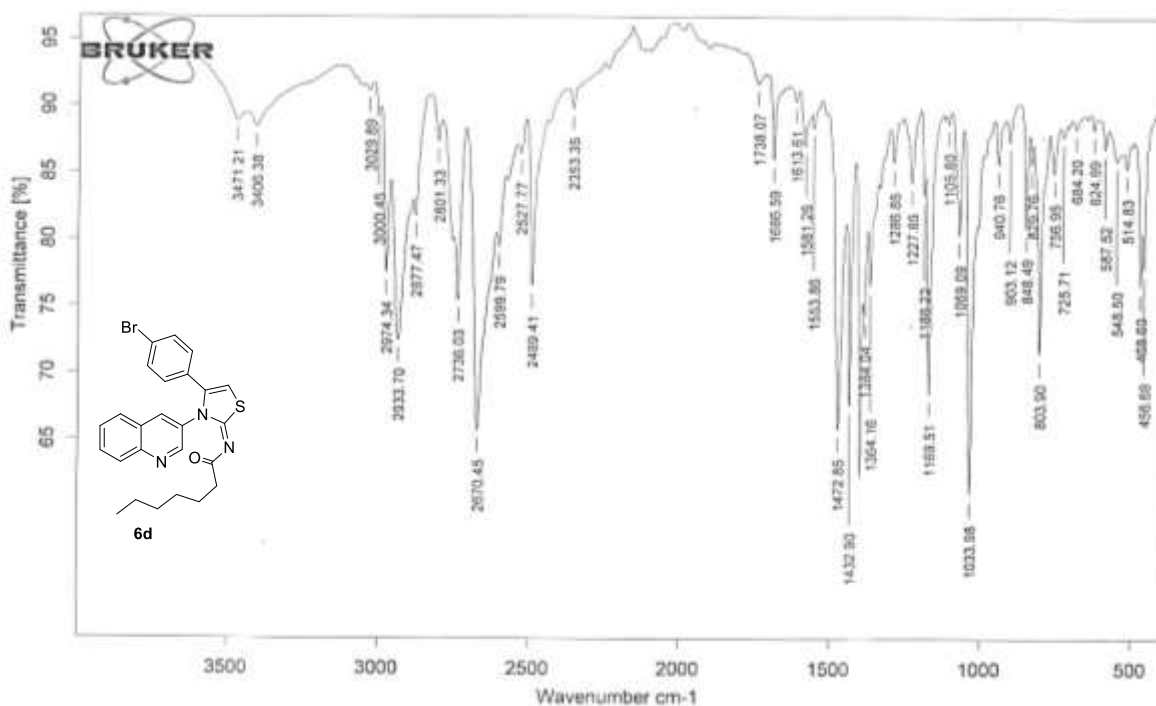
(*E*)-*N*-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3*H*)-ylidene)pentanamide (6b)



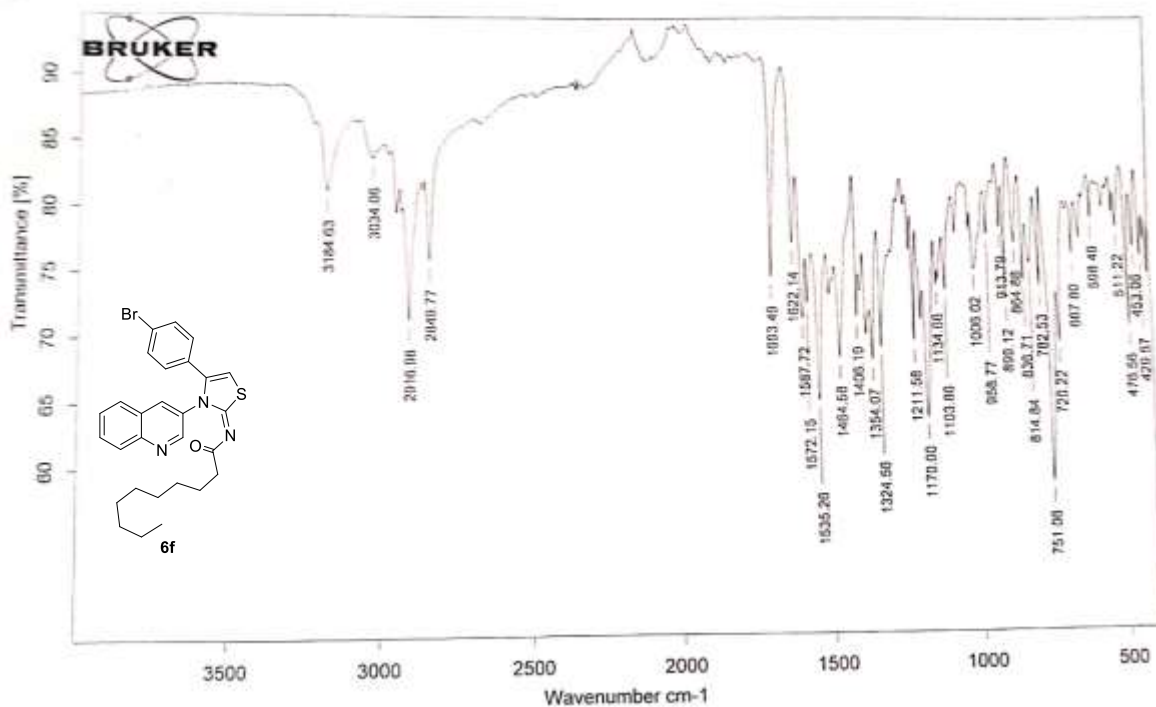
(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)hexanamide (6c)



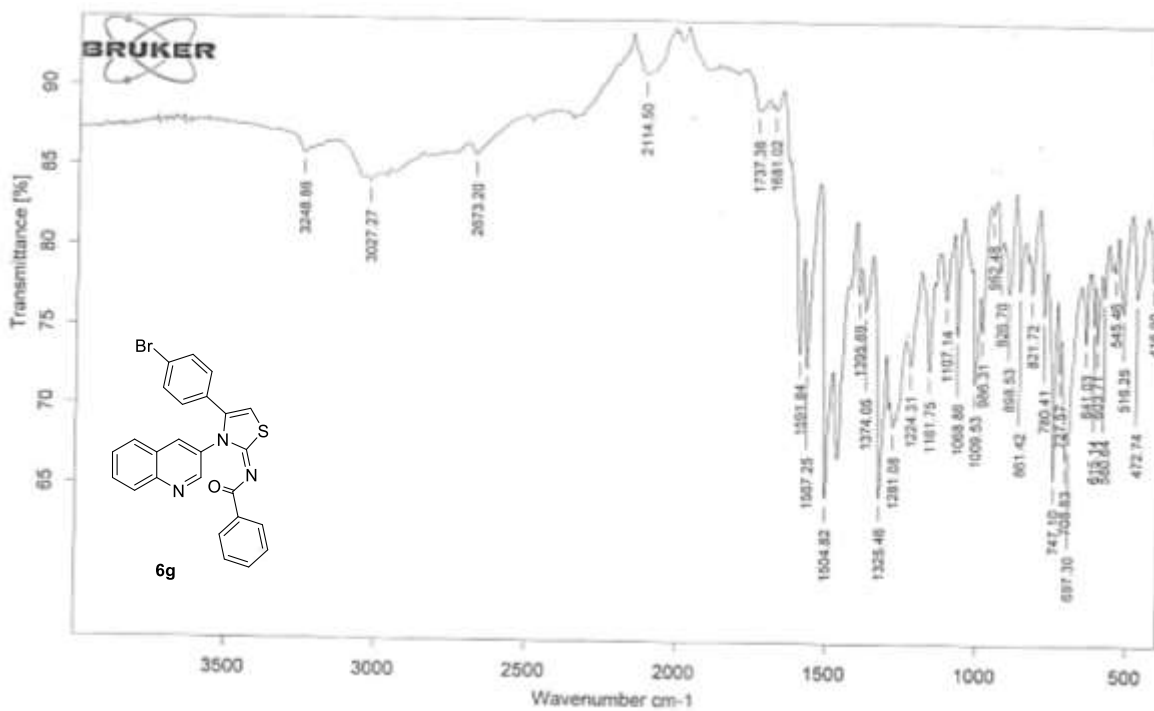
(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)heptanamide (6d)



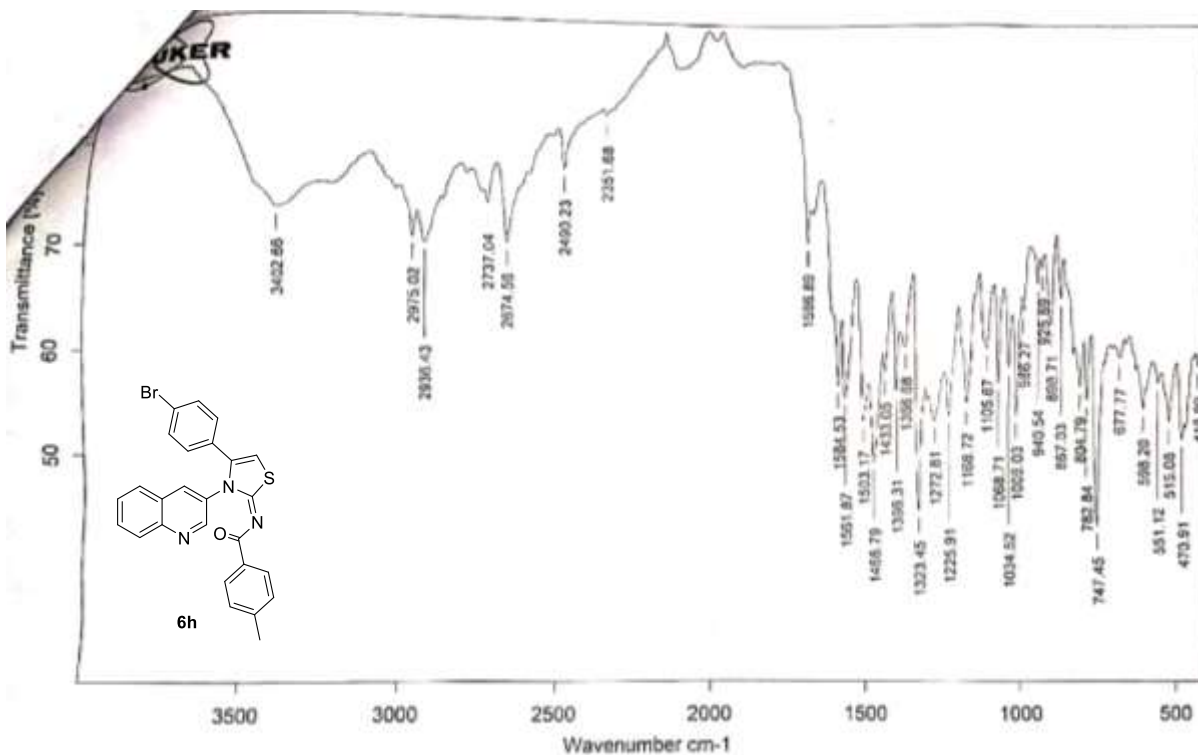
(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)decanamide (6f)



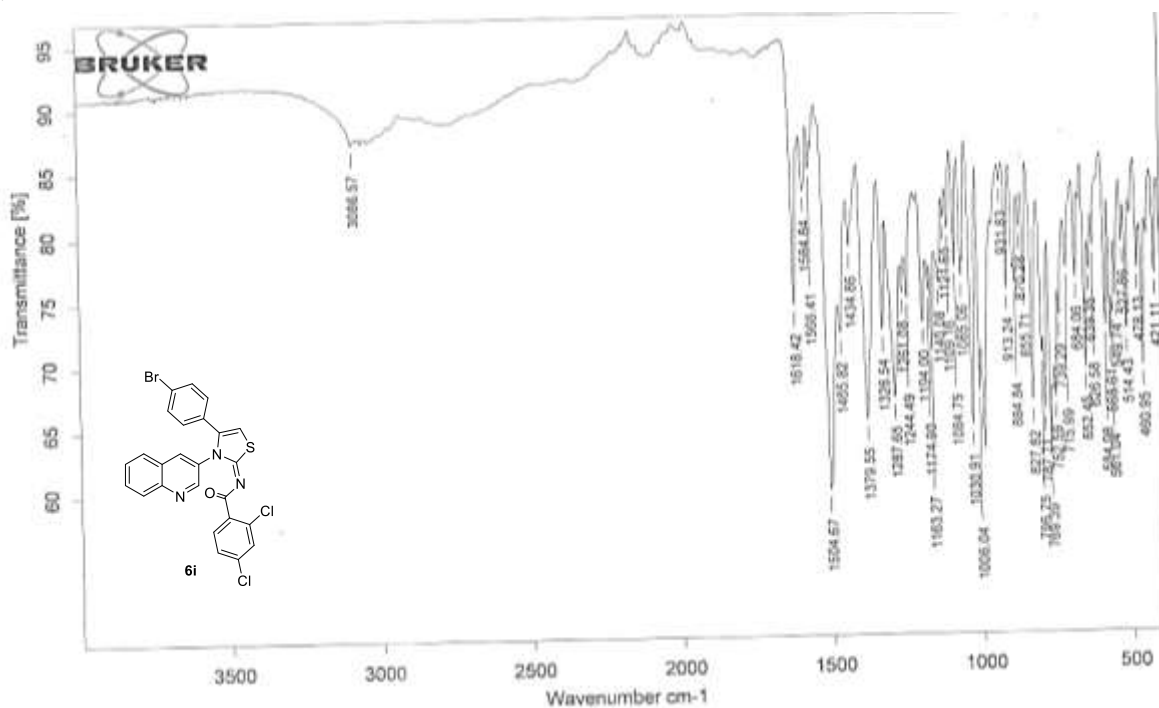
(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)benzamide (6g)



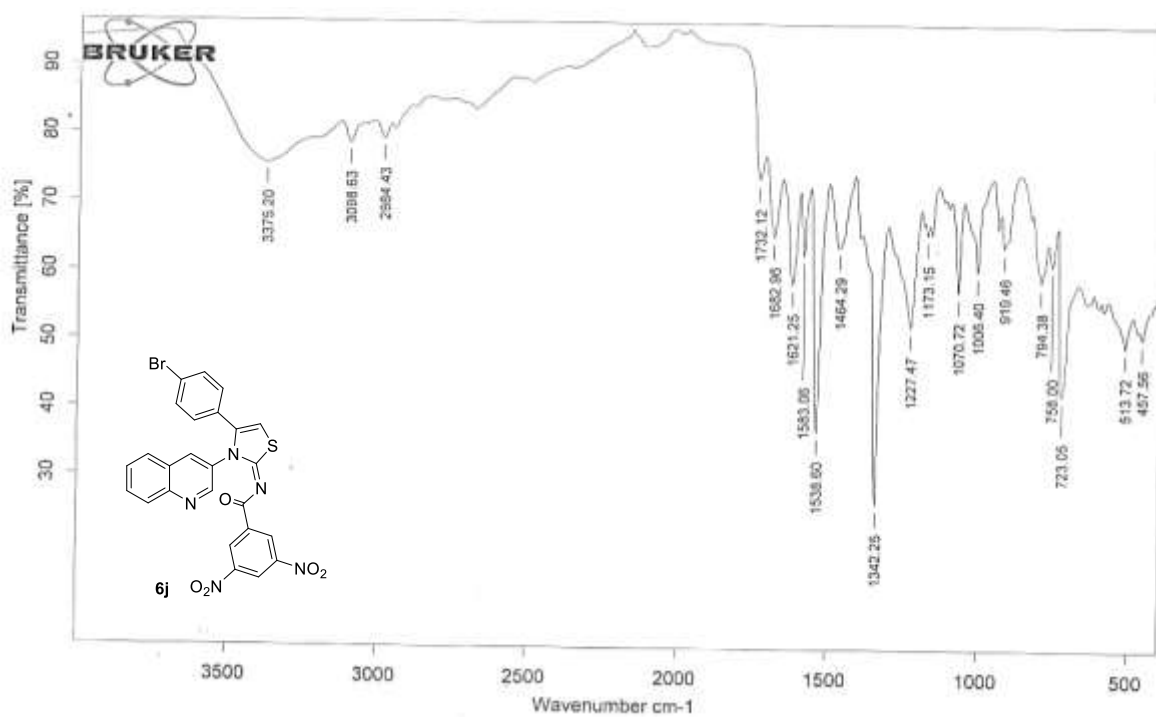
(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)-4-methylbenzamide (6h)



(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)-2,4-dichlorobenzamide (6i)



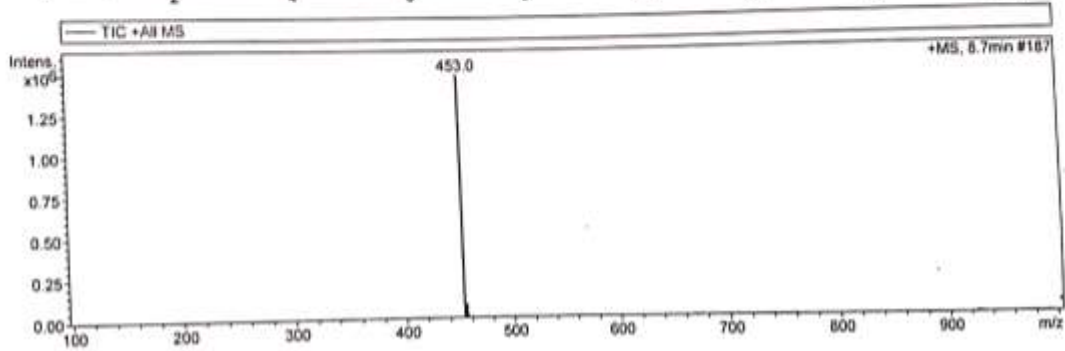
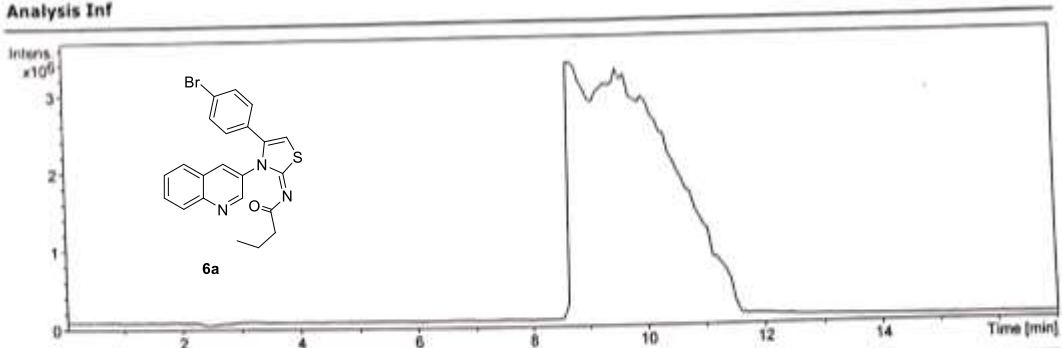
**(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)-3,5-dinitrobenzamide
(6j)**



HPLC-MS

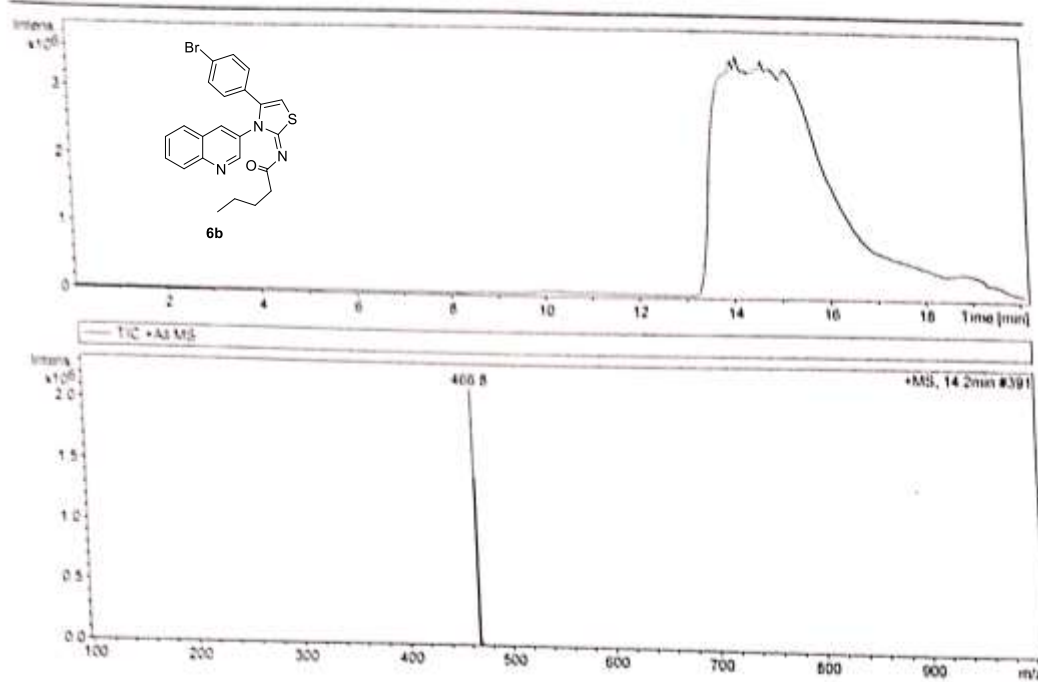
(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)butyramide (6a)

Analysis Name: MNM-CQT-2.D Instrume Agilent 6310 Ion Trap Print Dat 4/3/2018 9:13:42 AM
Method: ISOCRATIVE MODE Operator: Saqib Yasin Acq. Date 3/29/2018 1:00:56 PM
Sample Na 80204544.M
Analysis Inf

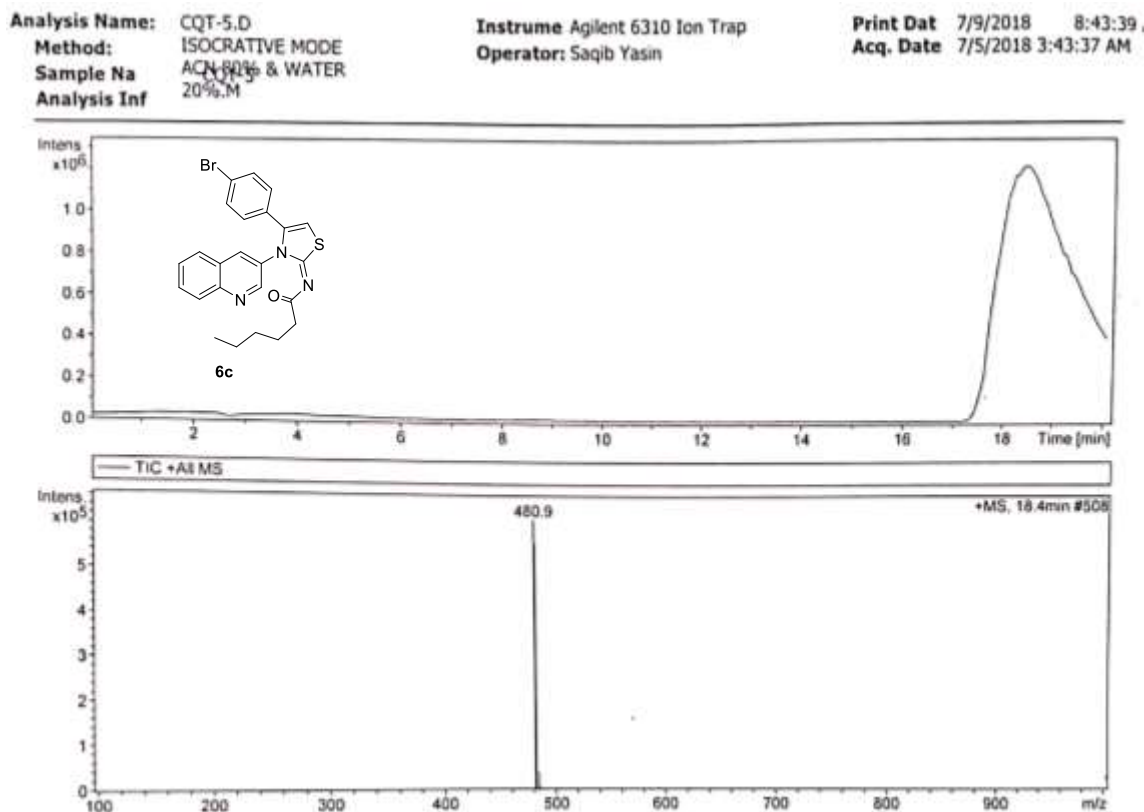


(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)pentanamide (6b)

Analysis Name: QQT-1.D Instrument: Agilent 6310 Ion Trap Print Date: 8/9/2018 12:50:50 PM
Method: ISOCRATIVE MODE Operator: Saqib Yasin Acq. Date: 8/7/2018 4:17:16 PM
Sample Name: ACN 80% & WATER 20% MeOH



(E)-N-(4-(4-Bromophenyl)-3-(quinolin-3-yl)thiazol-2(3H)-ylidene)hexanamide (6c)



Alkaline phosphatase inhibition assay and kinetic mechanism analysis

The reaction mixture comprised of 50 mM Tris-HCl buffer (5 mM MgCl₂, 0.1 mM ZnCl₂ pH 9.5), the compound (0.1 mM with final DMSO 1% (v/v) and mixture was pre-incubated for 10 min by adding 5 μL of CIALP (0.025 U/mL). Then, 10 μL of substrate (0.5 mM *p*-NPP (para nitrophenyl phosphate disodium salt) was added to initiate the reaction and the assay mixture was incubated again for 30 min at 37 °C. The change in absorbance of released *p*-nitrophenolate was monitored at 405 nm, using a 96-well microplate reader (SpectraMax ABS, USA). All the experiments were repeated three times in a triplicate manner. KH₂PO₄ was used as the reference inhibitor of CIALP. The Alkaline Phosphatase activities were calculated according to the following formula:

$$\text{Alkaline Phosphatase activity (\%)} = (\text{OD}_{\text{control}} - \text{OD}_{\text{sample}} \times 100) / \text{OD}_{\text{control}}$$

Where OD_{control} and OD_{sample} represents the optical v densities in the absence and presence of sample, respectively.

The inhibitor **6g** concentrations were used 0.00, 0.169, 0.337 and 0.674 μM , Substrate *p*-NPP concentrations were 10, 5, 2.5, 1.25, 0.625 and 0.3125 mM. Pre-incubation time and other conditions were same as described in alkaline phosphatase inhibition assay section. Maximal initial velocities were determined from initial linear portion of absorbances up to 10 minutes after addition of enzyme at per minute's interval. The inhibition type on the enzyme was assayed by Lineweaver-Burk plot of inverse of velocities ($1/V$) versus inverse of substrate concentration $1/[S]$ mM^{-1} . The EI dissociation constant K_i was determined by secondary plot of $1/V$ versus inhibitor concentration