Understanding the Mechanism of Action of Pyrrolo[3,2-b]quinoxaline-derivatives as Kinase Inhibitors

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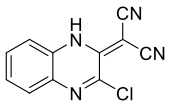
1. Chemistry

All reactions, unless otherwise stated, were carried out under inert gas atmosphere using standard Schlenk-techniques. All reagents were used as received unless otherwise noted. Solvents were purchased in the best quality available, degassed by purging thoroughly with nitrogen and dried over activated molecular sieves of appropriate size. Alternatively, they were purged with argon and passed through alumina columns in a solvent purification system (Innovative Technology). Reactions were monitored by thin layer chromatography (TLC) using Merck TLC silica gel 60 F_{254}. Flash column chromatography was performed over silica gel (230-400 mesh). NMR spectra were recorded on AV 300, AV2 400 or AV2 500 MHz Bruker spectrometers. Chemical shifts are given in ppm. The spectra are calibrated to the residual ^{1}H and ^{13}C signals of the solvents. Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), doublet-doublet (dd), quintet (quint), septet (sept), multiplet (m), and broad (br). Melting points were determined on a Mettler Toledo MP70 melting point instrument. High-resolution electrospray ionization mass spectrometry was performed on a Finnigan MAT 900 (Thermo Finnigan, San Jose, CA, USA) double-focusing magnetic sector mass spectrometer. Ten spectra were acquired. A mass accuracy ≤2 ppm was obtained in the peak matching acquisition mode by using a solution containing 2 µL PEG200, 2 µL PPG450, and 1.5 mg NaOAc (all obtained from Sigma-Aldrich, Buchs, Switzerland) dissolved in 100 mL MeOH (HPLC Supra grade, Scharlau, E-Barcelona) as internal standard.

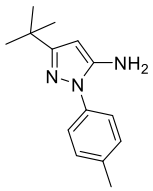
The following compounds were prepared according to previously reported procedures: **5**,[1] **9**,[2] **14**,[3] **15**,[4] **16**,[4] **19**[5] and **20**.[5]

2-(3-Chloroquinoxalin-2(1*H*)-ylidene)malononitrile (**5**)[1]



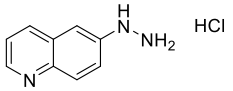
74% yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.67-7.62 (m, 2H), 7.57 (ddd, *J* = 8.4 Hz, *J* = 6.8 Hz, *J* = 1.1 Hz, 1H), 7.36 (ddd, *J* = 8.1 Hz, *J* = 6.8 Hz, *J* = 1.2 Hz, 1H), NH-not observed; ^{13}C NMR (100 MHz, DMSO-d_6): δ = 149.3, 141.9, 134.8, 134.1, 131.3, 127.5, 125.5, 120.2, 118.6, 44.5, one carbon is missing due to overlapping; IR (film): ϋ = 3235, 3203, 3100, 3056, 3013, 2979, 2220, 2208, 1616, 1577, 1488, 1410, 1096, 969, 799, 764, 594 cm^{-1}; MS (ESI): *m/z*: calcd for $C_{11}H_5ClN_4Na^+$: 251.0, found: 250.9.

3-Amino-5-*tert*-butyl-2-*p*-tolyl-2*H*-pyrazole **(14)**[3]



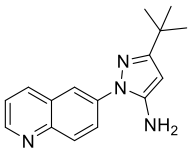
Brown solid; Yield: 98%; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 7.43 (d, *J* = 8.3 Hz, 2H), 7.24 (d, *J* = 8.3 Hz, 2H), 5.36 (s, 1H), 5.10 (s, 2H), 2.33 (s, 3H), 1.21 (s, 9H); ^{13}C NMR (75 MHz, DMSO): δ=160.4, 146.8, 137.2, 134.8, 129.3, 122.5, 86.7, 31.8, 30.2, 20.5; IR (neat): ϋ = 3467, 3272, 3133, 2955, 2859, 1633, 1583, 1557, 1516, 1490, 1460, 1382, 1357, 1245, 1129, 1108, 1076, 992, 947, 819, 753, 728, 662, 616, 502, 424, 404 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{14}H_{20}N_3^+$ 230.2; found, 230.0.

1-(Quinolin-6-yl)hydrazine hydrochloride **(15)**[4]



Brown solid; Yield: 58%; ^{1}H NMR (500 MHz, DMSO-d_6): δ= 8.71 (dd, *J* = 4.2, 1.4 Hz, 1H), 8.19 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 9.1 Hz, 1H), 7.52 – 7.42 (m, 2H), 7.30 (d, *J* = 2.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ=^{13}C NMR (126 MHz, DMSO) δ 147.2, 144.7, 143.0, 135.2, 129.1, 128.8, 122.1, 120.6, 106.6; IR (neat): ϋ = 3573, 3198, 3069, 2971, 2580, 2433, 1619, 1557, 1519, 1486, 1419, 1388, 1372, 1321, 1250, 1194, 1151, 1078, 1029, 990, 959, 927, 865, 822, 804, 755, 635, 611, 476, 460, 419 cm^{-1}; MS (ESI), *m/z*: calcd for $C_9H_{10}N_3$ 160.1; found, 159.9.

3-*Tert*-butyl-1-(quinolin-6-yl)-l*H*-pyrazol-5-amine **(16)**[4]



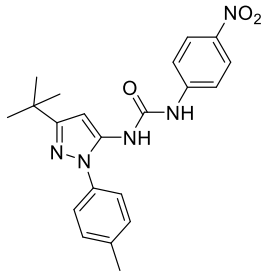
Yellow solid; Yield: 42%; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.88 (dd, *J* = 4.0, 1.5 Hz, 1H), 8.40 (d, *J* = 8.3 Hz, 1H), 8.14 (s, 1H), 8.08 (s, 2H), 7.55 (dd, *J* = 8.3, 4.2 Hz, 1H), 5.47 (s, 1H), 5.42 (s, 2H), 1.26 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6): δ= 161.4, 150.1, 147.6, 145.6, 137.4, 136.0, 129.7, 128.0, 125.4, 121.9, 118.9, 87.3, 31.9, 30.1; IR (neat): ῡ = 3306, 3188, 2957, 1625, 1595, 1558, 1505, 1461, 1387, 1361, 1243, 1121, 984, 882, 837, 794, 766, 755, 720, 628, 601, 475, 416 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{16}H_{18}N_4$ 267.2; found, 267.0.

General procedure for the synthesis of ureas

To a mixture of aniline (1.0 eq) in CH_2Cl_2 (0.3 M) was added the isocyanate (1.0 eq). The reaction mixture was stirred at 25 °C for 24 hours, and the resulting precipitate was filtered off and washed with DCM and hexane affording the desired compounds in pure form.

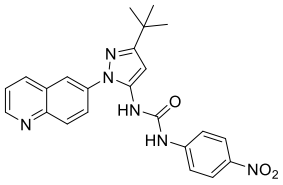
This method was used to obtain intermediates **17** and **18**.

1-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-3-(4-nitrophenyl)urea (**17**)



Yellow solid; Yield: 97%; mp 190-193 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 9.72 (s, 1H), 8.57 (s, 1H), 8.17 (d, *J* = 9.2 Hz, 2H), 7.64 (d, *J* = 9.3 Hz, 2H), 7.42 – 7.32 (m, 4H), 6.39 (s, 1H), 2.37 (s, 3H), 1.28 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ= 160.6, 151.2, 146.0, 141.2, 136.9, 136.5, 136.0, 129.7, 125.1, 124.3, 117.5, 95.6, 32.0, 30.2, 20.6; IR (neat): ῡ = 3339, 2960, 1732, 1543, 1498, 1328, 1302, 1269, 1188, 1174, 1137, 1110, 1014, 996, 852, 822, 750, 689, 499, 440 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{21}H_{24}N_5O_3^+$ 394.1874; found, 394.1874.

1-(3-(*tert*-Butyl)-1-(quinolin-6-yl)-1*H*-pyrazol-5-yl)-3-(4-nitrophenyl)urea (**18**)

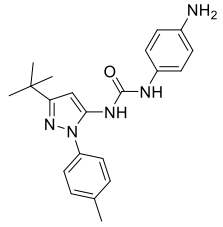


Yellow solid; Yield: %99; mp 162-165 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 9.72 (s, 1H), 8.96 (dd, *J* = 4.2, 1.6 Hz, 1H), 8.82 (s, 1H), 8.47 (d, *J* = 9.3 Hz, 1H), 8.20 – 8.15 (m, 4H), 7.96 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.66 – 7.59 (m, 3H), 6.49 (s, 1H), 1.32 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6): δ= 161.5, 151.2, 151.0, 146.4, 145.9, 141.3, 137.1, 136.4, 136.1, 130.2, 127.9, 126.4, 125.1, 122.2, 122.1, 118.0, 117.6, 96.2, 32.1, 30.1 one carbon is missing due to overlapping; IR (neat): ῡ = 3356, 2960, 1732, 1539, 1499, 1379, 1329, 1301, 1272, 1241, 1175, 1112, 1020, 985, 850, 832, 792, 751, 689, 630, 604, 497, 425 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{23}H_{23}N_6O_3^+$ 431.1826; found, 431.1825.

General procedure B for the reduction of nitro phenyls

To a solution of nitrophenyl (1.0 eq) in MeOH (0.3 M) was added 10% Pd/C (10% wt). The reaction mixture was stirred at 25 °C under a hydrogen balloon for 4-12 hours.The reaction mixture was filtered through a pad of celite, washed with MeOH and concentrated under reduced pressure obtaining the corresponding anilines in pure form. This method was used to obtain intermediates **6a**, **6b** and **11**. Compound **11** was further purified by flash column chromatography (toluene: acetone, 3:1).

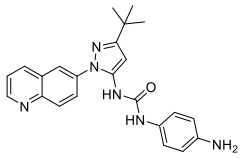
1-(4-Aminophenyl)-3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)urea (**6a**)



Yellow solid; Yield: 98%; mp 127-130 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.49 (s, 1H), 8.13 (s, 1H), 7.48 – 7.26 (m, 4H), 7.02 (d, *J* = 8.6 Hz, 2H), 6.48 (d, *J* = 8.6 Hz, 2H), 6.31 (s, 1H), 4.77 (s, 2H), 2.37 (s, 3H), 1.26 (s, 9H);

^{13}C NMR (75 MHz, DMSO-d_6): δ= 160.5, 151.7, 144.2, 137.7, 136.7, 136.1, 129.7, 128.3, 124.4, 120.5, 114.1, 94.4, 32.0, 30.2, 20.6; IR (neat): ῡ = 3349, 3276, 2959, 1643, 1614, 1591, 1552, 1513, 1370, 1295, 1215, 1170, 1119, 1014, 989, 817, 801, 654, 570, 522, 501, 414 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{21}H_{26}N_5O^+$ 364.2132; found, 364.2132.

1-(4-Aminophenyl)-3-(3-(*tert*-butyl)-1-(quinolin-6-yl)-1*H*-pyrazol-5-yl)urea (**6b**)

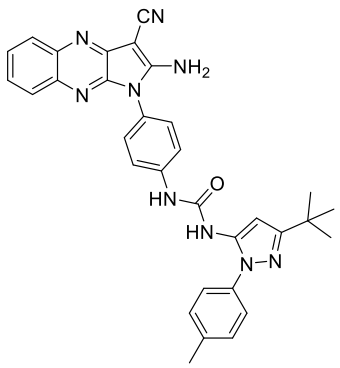


Yellow solid; Yield: 89%; mp 139-142 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ= 8.95 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.48 – 8.45 (m, 2H), 8.37 (s, 1H), 8.17 – 8.13 (m, 2H), 7.94 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.61 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.01 (d, *J* = 8.7 Hz, 2H), 6.48 (d, *J* = 8.7 Hz, 2H), 6.41 (s, 1H), 4.77 (br, 2H), 1.31 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6): δ= 161.3, 151.8, 150.9, 146.3, 144.3, 138.3, 136.3, 130.1, 128.1, 127.9, 126.5, 122.1, 121.9, 120.7, 114.1, 95.0, 32.1, 30.2, one carbon is missing due to overlapping; IR (neat): ῡ = 3331, 2959, 1716, 1627, 1599, 1546, 1512, 1428, 1376, 1314, 1239, 1195, 1174, 1133, 987, 904, 829, 799, 781, 635, 612, 523, 481, 473 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{23}H_{25}N_6O^+$ 401.2084; found, 401.2089.

General procedure B for the cyclization of 2-(3-chloroquinoxalin-2-yl)malononitrile

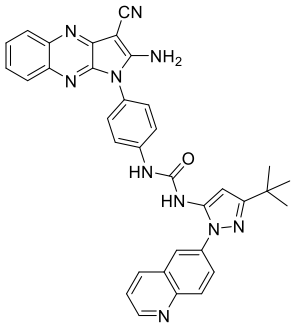
To a mixture of 2-(3-chloroquinoxalin-2-yl)malononitrile (1.0 eq) in DMF (0.1 M) was added the primary amine (1.2 eq). The reaction mixture was heated at 80 °C for 12 h, concentrated and purified by flash column chromatography (hexane/EtOAc, 1:1 to EtOAc/MeOH/Et_3N, 90:9:1). This method was used to obtain intermediates **7a** and **7b**. In the case of **10**, the reaction mixture was heated at 80 °C for 4.5 h. The reaction mixture was concentrated under reduced pressure and EtOAc was added. The obtained precipitate was filtered off and washed with EtOH, obtaining the desired product in pure form.

1-(4-(2-Amino-3-cyano-1*H*-pyrrolo[*2,3-b*]quinoxalin-1-yl)phenyl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**7a**)



Yellow solid; Yield: 55%; mp 200-205 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 9.33 (s, 1H), 8.44 (s, 1H), 8.26 (s, 2H), 7.75 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.93 (dd, *J* = 8.4, 1.2 Hz, 1H), 7.64 (d, *J* = 8.9 Hz, 2H), 7.57 (ddd, *J* = 8.4, 7.0, 1.5 Hz, 1H), 7.48 (ddd, *J* = 8.3, 7.0, 1.5 Hz, 1H), 7.46 – 7.41 (m, 4H), 7.36 – 7.34 (m, 2H), 2.39 (s, 3H), 1.29 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ= 160.5, 160.0, 151.6, 143.7, 143.0, 140.6, 140.4, 137.3, 136.9, 136.8, 136.1, 129.7, 129.6, 127.6, 127.1, 126.7, 125.6, 125.4, 124.4, 119.0, 115.2, 95.4, 60.4, 32.0, 30.2, 20.6; IR (neat): ῡ = 3275, 2959, 2863, 1642, 1552, 1514, 1441, 1369, 1295, 1210, 1172, 1120, 1034, 1014, 990, 817, 800, 756, 666, 598, 570, 527, 520, 504, 449, 404 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{32}H_{30}N_9O^+$ 556.2568; found, 556.2568.

1-(4-(2-Amino-3-cyano-1*H*-pyrrolo[2,3-b]quinoxalin-1-yl)phenyl)-3-(3-(*tert*-butyl)-1-(quinolin-6-yl)-1*H*-pyrazol-5-yl)urea (**7b**)

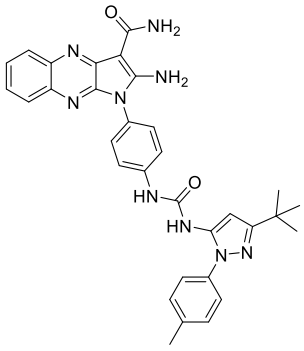


Brown solid; Yield: 62%; mp 200-203 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 9.38 (s, 1H), 8.97 (dd, *J* = 4.1, 1.7 Hz, 1H), 8.73 (s, 1H), 8.49 (d, *J* = 8.2 Hz, 1H), 8.25 (br, 1H), 8.16 – 8.19 (m, 2H), 7.98 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.92 (dd, *J* = 8.2, 1.2 Hz, 1H), 7.74 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.64 –7.61 (m, 3H), 7.57 (ddd, *J* = 8.4, 7.0, 1.4 Hz, 1H), 7.48 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 2H), 6.49 (s, 1H), 1.33 (s, 9H); ^{1}H NMR (125 MHz, DMSO-d_6): δ= 161.4, 160.0, 151.9, 150.9, 146.3, 143.7, 143.0, 140.7, 140.4, 137.5, 137.3, 136.4, 136.3, 130.1, 129.6, 128.0, 127.6, 127.1, 126.8, 126.3, 125.7, 125.4, 122.2, 121.8, 119.0, 115.3, 96.6, 60.4, 32.2, 30.2; IR (neat): ῡ = 3312, 3169, 2957, 2923, 2856, 2208, 1712, 1653, 1548, 1506, 1438, 1377, 1361, 1309, 1263, 1241, 1201, 1137, 1123, 1022, 986, 899, 885, 836, 756, 599, 529, 417 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{34}H_{29}N_{10}O^+$ 593.2520; found, 593.2521.

General procedure for the hydrolysis of carbonitriles

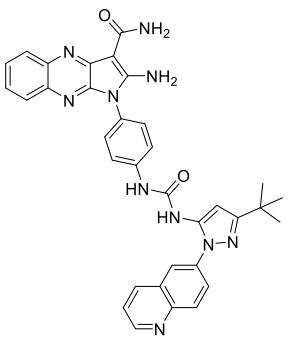
A solution of the carbonitrile (1.0 eq) in concentrated sulfuric acid (0.15 M) was stirred for 30 min at 25 °C. The reaction was poured into ice cold water, basified with a solution of ammonium hydroxide (25%) and the formed solid was filtered off and washed with cold water. In the case of **13**, the obtained residue was purified by flash column chromatography (Acetone:MeOH:Et_3N, 90:9:1).

2-Amino-1-(4-(3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)phenyl)-1*H*-pyrrolo[2,3-b]quinoxaline-3-carboxamide (**8a**)



Yellow solid; Yield 90%; mp 214-216 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ= 9.36 (s, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.77 – 7.76 (m, 2H), 7.66 (d, *J* = 9.1 Hz, 2H), 7.57 (t, *J* = 6.7 Hz, 1H), 7.47-7.42 (m, 5H), 7.36 – 7.33 (m, 3H), 6.39 (s, 1H), 2.39 (s, 3H), 1.29 (s, 9H); ^{13}C NMR (125 MHz, DMSO-d_6): δ= 166.6, 160.6, 159.3, 151.6, 143.7, 141.5, 140.3, 139.6, 137.0, 136.8, 136.1, 129.7, 129.3, 127.6, 126.8, 126.6, 125.5, 125.1, 124.3, 119.0, 95.5, 82.5, 32.0, 30.2, 20.6; IR (neat): ῡ = 3220, 2959, 1643, 1515, 1413, 1367, 1313, 1194, 1087, 987, 823, 756, 613, 598, 524, 475, 459, 436 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{32}H_{32}N_9O_2^+$ 574.2674; found, 574.2673.

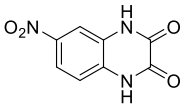
2-Amino-1-(4-(3-(3-(*tert*-butyl)-1-(quinolin-6-yl)-1*H*-pyrazol-5-yl)ureido)phenyl)-1*H*-pyrrolo[2,3-b]quinoxaline-3-carboxamide (**8b**)



Yellow solid; Yield 90%; mp 258-260 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ= 9.32 (s, 1H), 8.97 (dd, *J* = 4.1, 1.3 Hz, 1H), 8.68 (s, 1H), 8.51 – 8.47 (m, 1H), 8.22 – 8.15 (m, 2H), 7.98 (dd, *J* = 9.0, 2.2 Hz, 2H), 7.93 (dd, *J* = 8.3, 1.1 Hz, 1H), 7.78 – 7.72 (m, 2H), 7.69 – 7.60 (m, 3H), 7.60 – 7.53 (m, 1H), 7.50 – 7.41 (m, 3H), 7.32, (br, 1H), 6.49 (s, 1H), 1.34 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6): δ= 166.7, 166.7, 161.4, 159.3, 159.2, 152.6, 151.7, 151.6, 151.0, 148.5, 146.4, 144.2, 143.8, 143.7, 141.5, 140.5, 140.3, 139.8, 137.9, 137.6, 136.9, 136.8, 136.4, 136.3, 135.1, 130.2, 129.6, 129.3, 128.6, 128.0, 127.6, 126.8, 126.6, 126.5, 126.5, 125.6, 125.5, 125.1, 123.0, 122.2, 122.1, 121.7, 119.2, 119.1, 118.9, 113.2, 96.0, 82.6, 32.1, 30.2; IR (neat): ῡ = 3203,

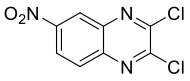
2958, 1706, 1644, 1506, 1463, 1413, 1363, 1312, 1193, 1080, 1029, 986, 835, 758, 614, 597, 573, 527, 515, 478, 471, 447, 436, 418, 405 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{34}H_{31}N_{10}O_2^+$ 611.2626; found, 611.2624.

6-Nitro-1,4-dihydroquinoxaline-2,3-dione (**19**)[5]



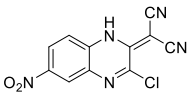
Off-white solid; 89% yield; ^{1}H-NMR (400 MHz, DMSO-d_6): δ= 12.33 (s, 1H), 12.13 (s, 1H), 7.95-7.09 (m, 2H), 7.22 (d, *J* = 8.7 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ= 155.1, 154.7, 142.1, 131.6, 126.0, 118.5, 115.4, 110.3; IR (neat): ῡ = 3122, 3060, 2941, 2838, 1686, 1610, 1534, 1497, 1401, 1335, 1286, 1093, 891, 833, 807, 742, 662, 594, 532, 468 cm^{-1}; MS (ESI), *m/z*: calcd for $C_8H_5N_3NaO_4^+$ 230.0; found, 230.0.

2,3-Dichloro-6-nitroquinoxaline (**20**)[5]



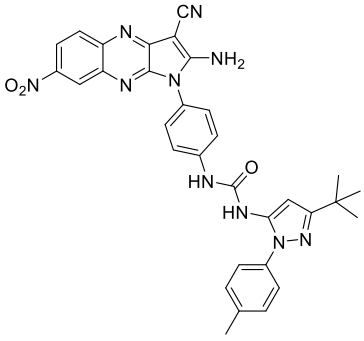
Off-white solid; 90% yield; ^{1}H-NMR (400 MHz, DMSO-d_6): δ= 8.91 (dd, *J* = 2.5, 0.4 Hz, 1H), 8.61 (dd, *J* = 9.2, 2.5 Hz, 1H), 8.32 (dd, *J* = 9.2, 0.4 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ= 155.1, 154.7, 142.1, 131.6, 126.0, 118.5, 115.4, 110.3; IR (neat): ῡ = 3090, 3056, 3041, 1612, 1574, 1563, 1524, 1353, 1275, 1162, 1125, 1073, 1013, 916, 854, 831, 741, 455, 436 cm^{-1}; MS (ESI), *m/z*: calcd for $C_8H_4Cl_2N_3O_2^+$ 244.0; found, 244.2.

2-(3-Chloro-6-nitroquinoxalin-2(1*H*)-ylidene)malononitrile (**9**)[2]



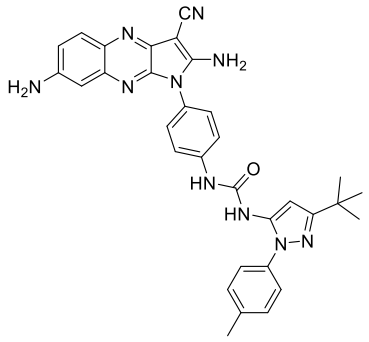
Orange solid; 89% yield; ^{1}H-NMR (400 MHz, DMSO-d_6): δ= 8.39 (d, *J* = 2.4 Hz, 1H), 8.22 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.55 (d, *J* = 9.2 Hz, 1H); IR (neat): ῡ = 3246, 3133, 2212, 1629, 1586, 1508, 1460, 1399, 1337, 1287, 1249, 1102, 1079, 914, 847, 743, 648, 603, 576, 460 cm^{-1}; MS (ESI), *m/z*: calcd for $C_{11}H_5ClN_5O_2^+$ 274.0; found, 274.0.

1-(4-(2-Amino-3-cyano-7-nitro-1*H*-pyrrolo[*2,3-b*]quinoxalin-1-yl)phenyl)-3-(3-(*tert*-butyl)-1-(p-tolyl)-1H-pyrazol-5-yl)urea (**10**)



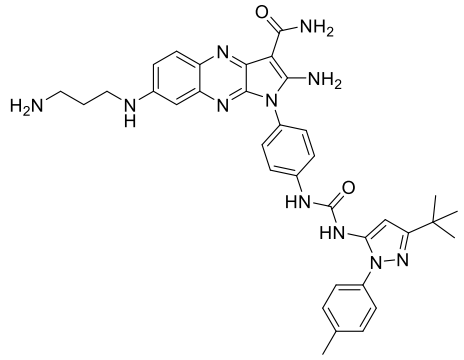
Orange solid; Yield: 71%; mp 326-329 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 9.37 (d, *J* = 5.3 Hz, 1H), 8.72 (br, 2H), 8.54 (d, *J* = 2.7 Hz, 1H), 8.46 (s, 1H), 8.31 (dd, *J* = 9.1, 2.7 Hz, 1H), 8.06 (d, *J* = 9.2 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.49 – 7.40 (m, 4H), 7.35 (d, *J* = 8.2 Hz, 2H), 6.40 (s, 1H), 2.39 (s, 3H), 1.29 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6): δ= 161.2, 160.5, 151.7, 146.3, 144.8, 144.2, 143.7, 140.9, 136.9, 136.8, 136.1, 135.9, 129.7, 129.6, 128.0, 124.8, 124.3, 123.6, 120.5, 119.0, 114.4, 95.6, 62.3, 32.0, 30.2, 20.6; IR (neat): ῡ = 3438, 3177, 2958, 2224, 1660, 1563, 1505, 1436, 1335, 1258, 821, 501 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{32}H_{29}N_{10}O_3^+$ 601.2419; found, 601.2418.

1-(3-(*Tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-3-(4-(2,7-diamino-3-cyano-1*H*-pyrrolo[2,3-b]quinoxalin-1-yl)phenyl)urea (**11**)



Orange solid; Yield: 71%; mp 293-295 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ= 9.38 (s, 1H), 8.72 (br, 2H), 8.54 (d, *J* = 2.6 Hz, 1H), 8.47 (s, 1H), 8.31 (dd, *J* = 9.2, 2.6 Hz, 1H), 8.06 (d, *J* = 9.2 Hz, 1H), 7.66 (d, *J* = 8.9 Hz, 2H), 7.47 (d, *J* = 8.9 Hz, 2H), 7.43 (d, *J* = 8.4 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 6.40 (s, 1H), 2.39 (s, 3H), 1.29 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6): δ= 161.2, 160.5, 151.6, 146.3, 144.8, 144.2, 143.7, 140.9, 136.9, 136.8, 136.1, 135.9, 129.7, 129.7, 129.6, 128.1, 124.8, 124.3, 123.6, 120.5, 119.0, 114.4, 95.5, 62.3, 32.0, 30.2, 20.6; IR (neat): ῡ = 3319, 3165, 2957, 2201, 1650, 1634, 1547, 1512, 1471, 1435, 1311, 1203, 822, 662, 512 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{32}H_{31}N_{10}O^{+}$ 571.2677; found, 571.2675.

2-Amino-7-((3-aminopropyl)amino)-1-(4-(3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)phenyl)-1*H*-pyrrolo[*2,3-b*]quinoxaline-3-carboxamide (**13**)



To a solution of **11** (100 mg, 0.175 mmol) in DMF (0.84 mL) KI (58 mg, 0.351 mmol), DIPEA (61 µL, 0.351 mmol) and *tert*-butyl (3-bromopropyl)carbamate (85 mg, 0.351 mmol) were added. The reaction mixture was heated at 80 °C for 8 hours. The reaction mixture was concentrated under reduced pressure and the residue purified by flash column chromatography obtaining the product that was used without further purification. Yellow solid; Orange solid; 29% ; ^{1}H NMR (500 MHz, DMSO-d_6): δ= 9.43 (s, 1H), 7.85 – 7.56 (m, 8H), 7.48 – 7.38 (m, 5H), 7.38 – 7.30 (m, 3H), 7.20 (br, 1H), 7.01 (dd, *J* = 10.5, 2.1 Hz, 1H), 6.69 (d, *J* = 1.9 Hz, 1H), 6.37 (s, 1H), 3.21 – 3.11 (m, 2H), 2.96 – 2.82 (m, 2H), 2.38 (s, 3H), 1.92 – 1.78 (m, 2H), 1.29 (s, 9H); ^{13}C NMR (126 MHz, DMSO-d_6): δ= 166.7, 160.4, 157.1, 151.7, 146.6, 143.2, 140.0, 139.1, 137.8, 136.8, 136.6, 136.1, 133.0, 129.5, 129.0, 127.2, 125.9, 124.1, 119.0, 117.9, 103.3, 95.6, 82.1, 37.2, 31.9, 30.1, 28.8, 26.6, 20.4; IR (neat): ῡ = 3222, 3034, 2954, 1630, 1514, 1410, 1064, 612, 452, 418, 409 cm^{-1}; HRMS (ESI), *m/z*: calcd for $C_{35}H_{41}N_{11}O_2^{2+}$ 323.6717; found, 323.6716.

2. Selectivity profiles of compounds 1, 8a and 8b

The selectivity profiling was performed at DiscoveRx in a library of 456 kinases at a concentration of 1 µM. Briefly, kinases were mostly expressed as fusion proteins to T7 phase and grown in 24-well blocks in *E. Coli* (derived from the BL21 strain). The rest of the kinases were expressed in HEK-293 cells and tagged with DNA for qPCR detection. Affinity resins were generated by mixing streptavidin-coated magnetic beads with biotinylated small molecule ligands for 30 minutes at 25 °C, followed by biotin and blocking buffer addition (SeaBlock (Pierce), 1 % BSA, 0.05 % Tween 20, 1 mM DTT). Inhibitors were kept at 40x stocks in 100 % DMSO and added to the corresponding 384-well plates (40 µL). After 1 hour of incubation at 25 °C while shacking, affinity beads were washed with 0.05% Tween 20 in PBS. The beads were re-suspended in PBS buffer containing 0.05 % Tween 20 and 0.5 µM non-biotinylated affinity ligand, to then be incubated at 25 °C for 30 minutes while shacking. The kinase concentration present in the eluate was determined by qPCR.[6]

3. Evaluation of efficacy in cell-based models

Lymphoma cell lines were obtained as previously reported,[7] and chronic lymphocytic leukemia cell lines were kindly provided by Cristina Scielzo (Milan, IT). Cell lines identity was validated by STR DNA fingerprinting.[8] The anti-proliferative activity in 96-well plates, the IC_{50} values estimate and drug combinations screening were performed as previously described.[9] Combination were defined: synergistic if the Chou- Talalay Combination Index (CI) was < 0.9; additive if CI between 0.9 and 1.1; antagonistic if CI>1.1.[10] Birabresib was acquired from Selleck Chemicals (Houston, TX).

4. Gene expression profiling

K562 cells obtained from Dr. Silvio Hemmi (Institute of Molecular Life Sciences, UZH) were cultured using RPMI medium supplemented with 10 % (v/v) fetal bovine serum. The media was additionally supplemented with 100 units/mL of penicillin, 100 μg/mL of streptomycin, 4.5 g/L glucose, 0.11 g/L sodium pyruvate and 2 mM glutamine and the cells were grown at 37 ºC in 5 % CO_2 atmosphere with 80 % relative humidity.

1 mL solution of 5.5 x 10^5 K562 cells/mL were added per well in a 6 -plate. The cells were diluted 1:1 by adding 1 mL of a two-fold concentrated compound solution in media. The final compound concentrations were 1 μM for dasatinib and **8b** and 10 μM for **1**. In parallel, a DMSO control was prepared. The cells were then incubated for 6 hours.

The cell suspensions were then centrifuged for 5 min at 4 °C at 500 rpm and the supernatant was discarded. The cells were resuspended in 5 mL cold PBS and centrifuged for 5 min at 4 °C at 500 rpm. The supernatant was discarded and the cell pellets were frozen in liquid nitrogen and stored in the freezer at -20 °C. The RNA was then isolated using the RNeasy Plus Mini Kit (Qiagen) following the instructions provided by the manufacturer.

Illumina RNA sequencing

Library preparation

The quantity and quality of the isolated RNA was determined with a Qubit® (1.0) Fluorometer (Life Technologies, California, USA) and a Tapestation (Agilent, Waldbronn, Germany). The TruSeq Stranded mRNA Sample Prep Kit (Illumina, Inc, California, USA) was used in the succeeding steps. Briefly, total RNA samples (100 ng) were ribosome depleted and reverse-transcribed into double-stranded cDNA with Actinomycin added during first-strand synthesis. The cDNA was fragmented, end-repaired and polyadenylated before ligation of TruSeq adapters. The adapters contain the index for multiplexing. Fragments containing TruSeq adapters on both ends were selectively enriched with PCR. The quality and quantity of the enriched libraries were validated using Qubit® (1.0) Fluorometer and the Tapestation (Agilent, Waldbronn, Germany). The product is a smear with an average fragment size of approximately 360 bp. The libraries were normalized to 10 nM in Tris-Cl 10 mM, pH 8.5 with 0.1% Tween 20.

Cluster Generation and Sequencing

The TruSeq SR Cluster Kit v4-cBot-HS or TruSeq PE Cluster Kit v4-cBot-HS (Illumina, Inc, California, USA) was used for cluster generation using 8 pM of pooled normalized libraries on the cBOT. Sequencing was performed on the Illumina HiSeq 2500 SIngle read at 1 X125 bp using the TruSeq SBS Kit v4-HS (Illumina, Inc, California, USA).

Adapter Sequences

Oligonucleotide sequences for TruSeq™ RNA and DNA Sample Prep Kits

TruSeq Universal Adapter

5’ AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT

TruSeq™ Adapters

TruSeq Adapter, Index 1

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACATCACGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 2

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGATGTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 3

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTTAGGCATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 4

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTGACCAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 5

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACACAGTGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 6

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGCCAATATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 7

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCAGATCATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 8

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTTGAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 9

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGATCAGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 10

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACTAGCTTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 11

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGGCTACATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 12

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCTTGTAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 13

5’GATCGGAAGAGCACACGTCTGAACTCCAGTCACAGTCAACAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 14

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACAGTTCCGTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 15

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACATGTCAGAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 16

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCCGTCCCGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 18 4

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTCCGCACATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 19

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTGAAACGATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 20

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTGGCCTTATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 21

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGTTTCGGAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 22

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACCGTACGTAATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 23

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACGAGTGGATATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 25

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACACTGATATATCTCGTATGCCGTCTTCTGCTTG

TruSeq Adapter, Index 27

5’ GATCGGAAGAGCACACGTCTGAACTCCAGTCACATTCCTTTATCTCGTATGCCGTCTTCTGCTTG

Data analysis

Data analysis was done using SUSHI,[11] an NGS data analysis workflow management system developed by the FGCZ bioinformatics team, which supports selected open source NGS data analysis packages. In

detail, reads were aligned with the STAR[12] aligner with the additional options (--outFilterMatchNmin 30 --outFilterMismatchNmax 10 --outFilterMismatchNoverLmax 0.05 --outFilterMultimapNmax 50) which means that at least 30 bp matching were required, and at most 10 or 5% mismatches were accepted. Read alignments were only reported for reads with less than 50 valid alignments. The human genome build and annotation from Ensembl (GRCh37) were used as the reference. Expression counts were computed using the Bioconductor package GenomicRanges.[13] Differential expression was computed using the DESeq2 package.[14]

5. Chemical proteomics

The chemical proteomics experiments were performed as previously described.[15]

Compounds and immobilization

The corresponding coupable molecules were stored as 10 mM solutions in DMSO. To 100 µL of the 10 mM compound solution, 900 µL of a 100 mM Et_3N solution was added, affording a final compound concentration of 1 mM.

100 µL of NHS-Activated Sepharose 4 Fast Flow beeds (GE Healthcare Life Sciences) were centrifuged at 13 000 rpm for 1 min. The beads were then washed with 500 µL DMSO and centrifuged at 13 000 rpm for 1 min. The process was repeated twice.

The beads were then incubated with 100 µL of the 1 mM compound solution described above overnight in an overhead rotator. As a control experiment the beads were also incubated with 100 µL of a 100 mM Et_3N solution.

500 µL of DMSO was then added to the beads, centrifuged at 13 000 rpm for 1 min and the supernatant was discarded. The affinity matrix was then blocked with 100 µL of a 0.8 M ethanolamine solution in DMSO during four hours in a spinner. The beads were centrifuged at 13 000 rpm for 1 min and the supernatant was discarded. They were then washed with 500 µL DMSO, centrifuged at 13 000 rpm for 1 min, and finally with 500 µL of isopropanol, centrifuged at 13 000 rpm for 1 min and stored at 4 °C until the next day.

Affinity purification method

K562 cell pellets were collected by centrifuging 410 million cells at 500 g for 3 minutes. The cell pellet was washed with 5 mL ice-cold PBS and centrifuged at 500 g for 3 minutes. The cell pellet was resuspended in 5.5 mL of lysis buffer composed of 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.2% NP-40, 0.5% N-dodecyl-β-D-maltoside, 5% glycerol, 1.5 mM $MgCl_2$, 25mM NaF, 1 mM Na_3VO_4, 1 mM dithiothreitol (DTT) and one protease inhibitor cocktail tablet (cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack, Roche) per every 50 mL buffer. The mixture was incubated on ice for 20 minutes, centrifuged at 4 °C for 15 min at 15 000 g and the supernatant (the cell lysate) was kept.

The beads were then washed with 500 µL of lysis buffer, centrifuged at 11 000 rpm for 2 minutes and the supernatant was discarded. The drug-coupled affinity matrices or empty beads were then incubated in 500 µL of cell lysate at 4 °C for 2 hours in a spinner.

The cell lysates were transferred to spin columns (Mobicol ‘F’, product code M105035F, MoBiTec Molecular Biotechnology) and the columns were washed six times with a buffer composed of 50 mM HEPES-NaOH at pH 8.0, 0.5 mM EDTA and one protease inhibitor cocktail tablet (cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack, Roche) per every 50 mL of buffer. Retained proteins were eluted

with 700 µL of 100 mM formic acid solution, lyophilized and stored at -80 °C. 460 µL of the eluted formic acid were then used for further analysis.

Tryptic digestion method, Liquid Chromatography and Mass Spectrometry

The lyophilized samples were dissolved in 48 µl of 6 M Urea and 20 mM Tris pH 8.2 and transferred into Eppendorf tubes. 1 µL DTT (0.5 M in water) to an end concentration of 10 mM was added and incubated during 2 h at 37 °C. 2.5 uL IAA (1 M in water) to an end concentration of 50 mM was added and incubated during 30 min at room temperature in the dark. The samples were then diluted to 195 µL end volume with digest buffer and 5 µL of trypsin (100 ng/µl in 10 mM HCl) was added. The samples were submitted to the microwave for 30 min at 60°C.

Samples were then desalted with ZipTip C18 and eluted in 20 µL 50% ACN, 0.1% TFA. Samples were dried, dissolved in 20 µL 0.1% formic acid and transferred to autosampler vials for LC/MS/MS analysis. 3 µL were injected.

Database searches were performed by using the Mascot (SwissProt, all species, carboxyamidomethyl as fixed Cys modification; SwissProt, human, carboxyamidomethyl as fixed Cys modification) search program. Mascot was searched with a fragment ion mass tolerance of 0.030 Da and a parent ion tolerance of 10.0 PPM. Carbamidomethyl of cysteine was specified in Mascot as a fixed modification. Oxidation of methionine was specified in Mascot as a variable modification.

Criteria for protein identification

Scaffold (version Scaffold_4.6.1, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they could be established at greater than 5.0 % probability to achieve an FDR less than 1.0 % by the Scaffold Local FDR algorithm. Protein identifications were accepted if they could be established at greater than 99.0 % probability and contained at least 2 identified peptides. Protein probabilities were assigned by the Protein Prophet algorithm.[16] Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony. Proteins sharing significant peptide evidence were grouped into clusters. Proteins were annotated with GO terms from goa_human.gaf (downloaded 02.07.2016).[17] Only human proteins showing a peptide count difference equal or larger than three where considered for further analysis.

6. Angiogenesis assays

6.1 Cell Proliferation

HUVEC (human umbilical vein endothelial cells) were purchased from Promocell (Heidelberg, Germany) and cultivated in endothelial cell growth medium (Promocell) for two passages. 1.5 x 10^3 cells/well were seeded in a 96-well plate. After 24 h, cells were treated with the indicated concentrations of compounds and incubated for 72 h. Finally, cells were washed with PBS, incubated with 100 µL/well crystal violet solution (0.5% crystal violet, 20% methanol in H_2O) for 10 min, washed and dried. For solvation of crystal violet, 100 µl/well ethanol/Na-citrate solution (50% ethanol, 50% 0.1 M Na-Citrate in H_2O) were added, incubated for 5 min and measured at 540 nm using a microplate reader (Sunrise, Tecan, Männedorf, Switzerland). Proliferation is indicated as % of untreated controls. No anti-proliferative effects were obtained for any of the inhibitors tested (data not shown). N = 6-9.

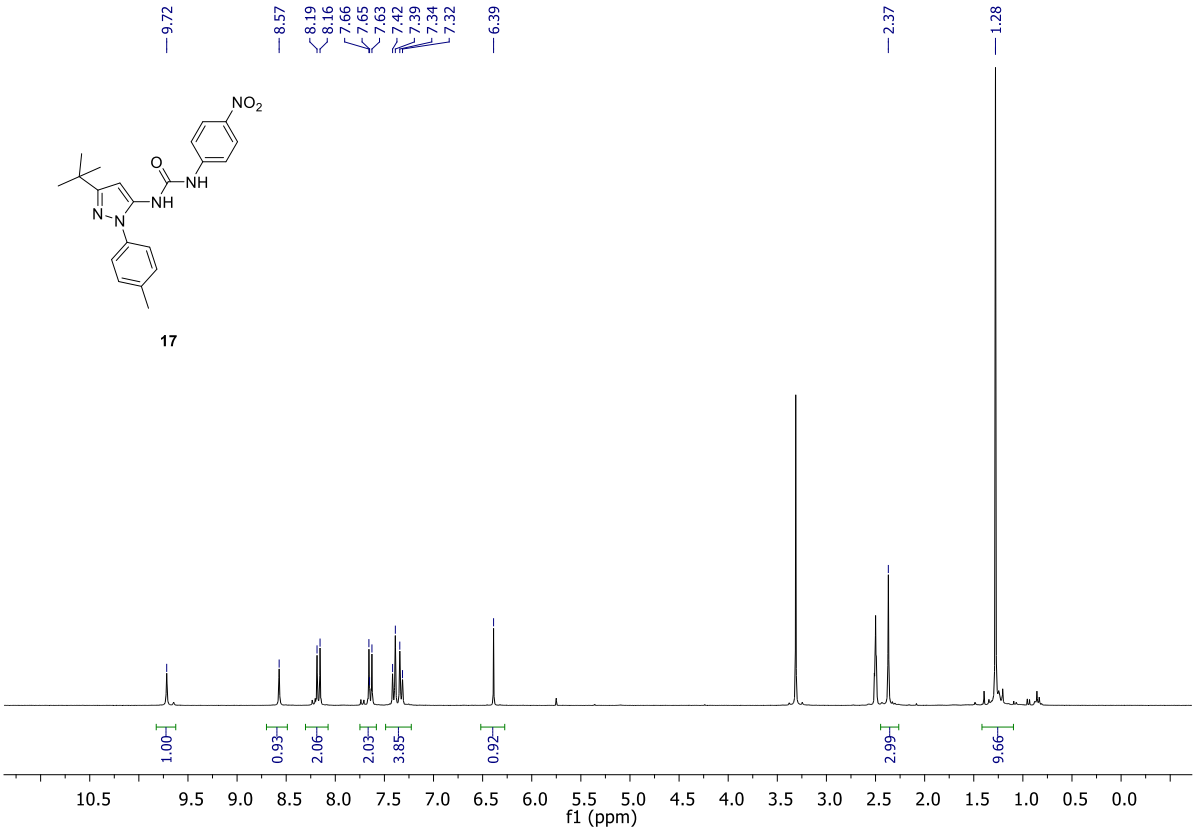
6.2 Tube formation

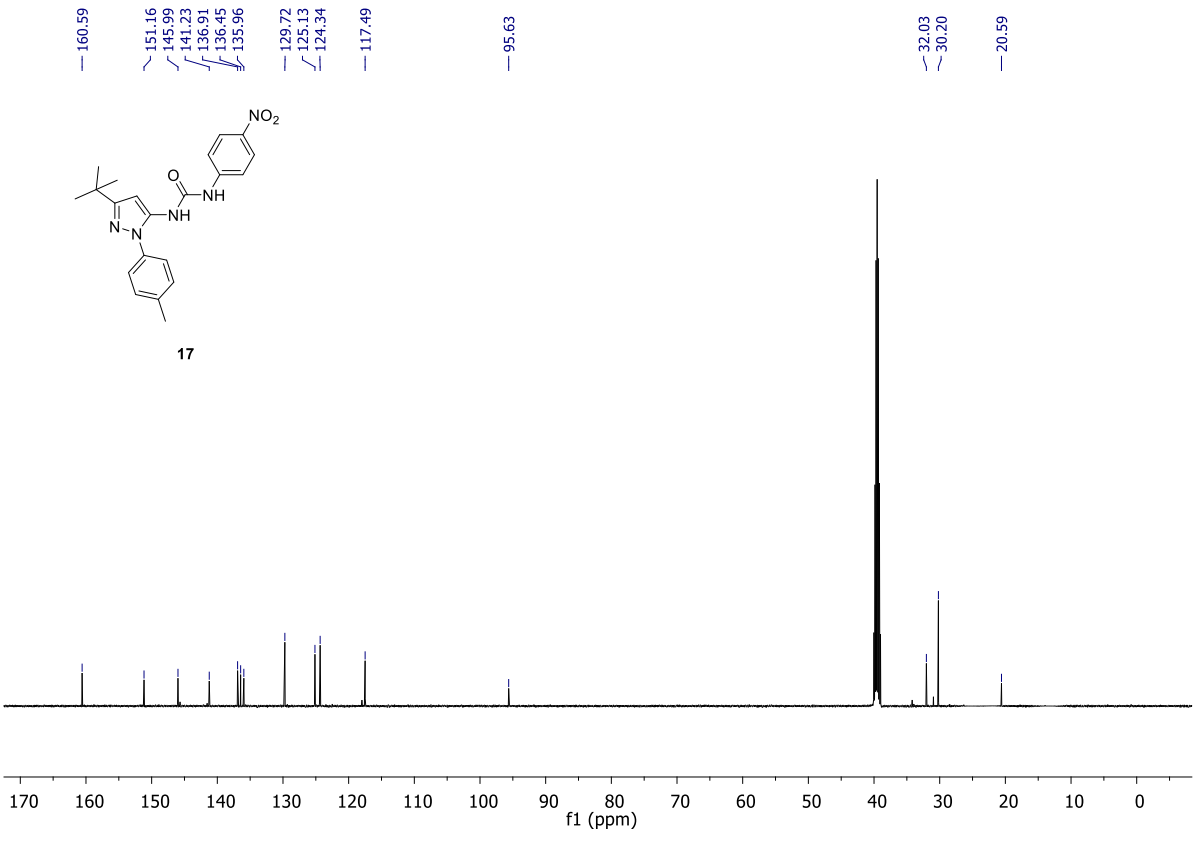
11 x 10^3 HUVEC were seeded on matrigel (Matrigel™, Schubert&Weiss-OMNILAB, Münich, Germany) in an angiogenesis slide from ibidi (Munich, Germany), treated as indicated and incubated for 15 h. Images were taken using the TILLvisION system. Analysis of images was performed by Wimasis GmbH (Munich, Germany). As parameters of tube formation, tube length, number of branching points and number of loops were analyzed. N = 6-9

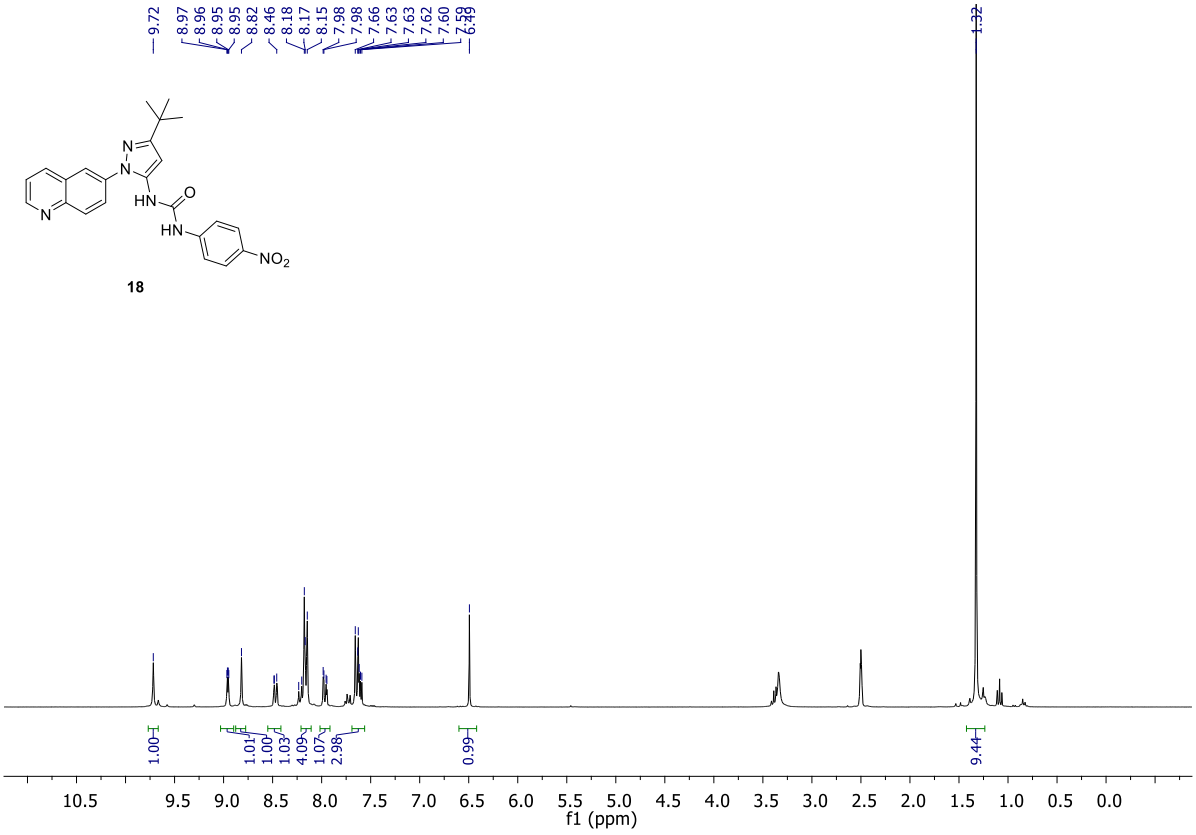
7. *In vivo* assays

NOD-Scid (NOD.CB17-Prkdcscid/NCrHsd) mice were subcutaneously inoculated with diffuse large B-cell lymphoma xenograft model subcutaneously injecting the RI-1 cell line (15 x 10^6 cells/mouse, 200 µL of PBS). Mice maintenance and animal experiments were performed under institutional guidelines established for the Animal Facility at The Institute of Research in Biomedicine (IRB, Bellinzona, CH). and with study protocols approved by the local Cantonal Veterinary Authority (No. TI-20-2015). Treatments were started with tumors of approximately 100 mm^3 volume. Tumor size was measured using a digital caliper [tumor volume (mm3) =$D \times d^2/2$], in which the width and the length are the shortest and the longest diameters of each tumor, respectively. Mice were divided in three groups: 1) vehicle (controls); 2) **8a** (100 mg/kg, i.p.); 3) **8b** (100 mg/kg, i.p.). Drug powders were prepared in 5% DMSO and 5% mannitol in 90% of water. 100 microliters of volume were i.p. injected in a mouse of 20-22 gr of body weight in order to provide a dose of 100mg/kg. Differences in tumor volumes were calculated using the Wilcoxon rank-sum test (Stata/SE 12.1 for Mac, Stata Corporation, College Station, TX). The P-value (P) for significance was < 0.05.

8. NMR traces of selected compounds



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7.98
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7.63
7.63
7.62
7.60
1.32
HN
O
HN
NO2
18
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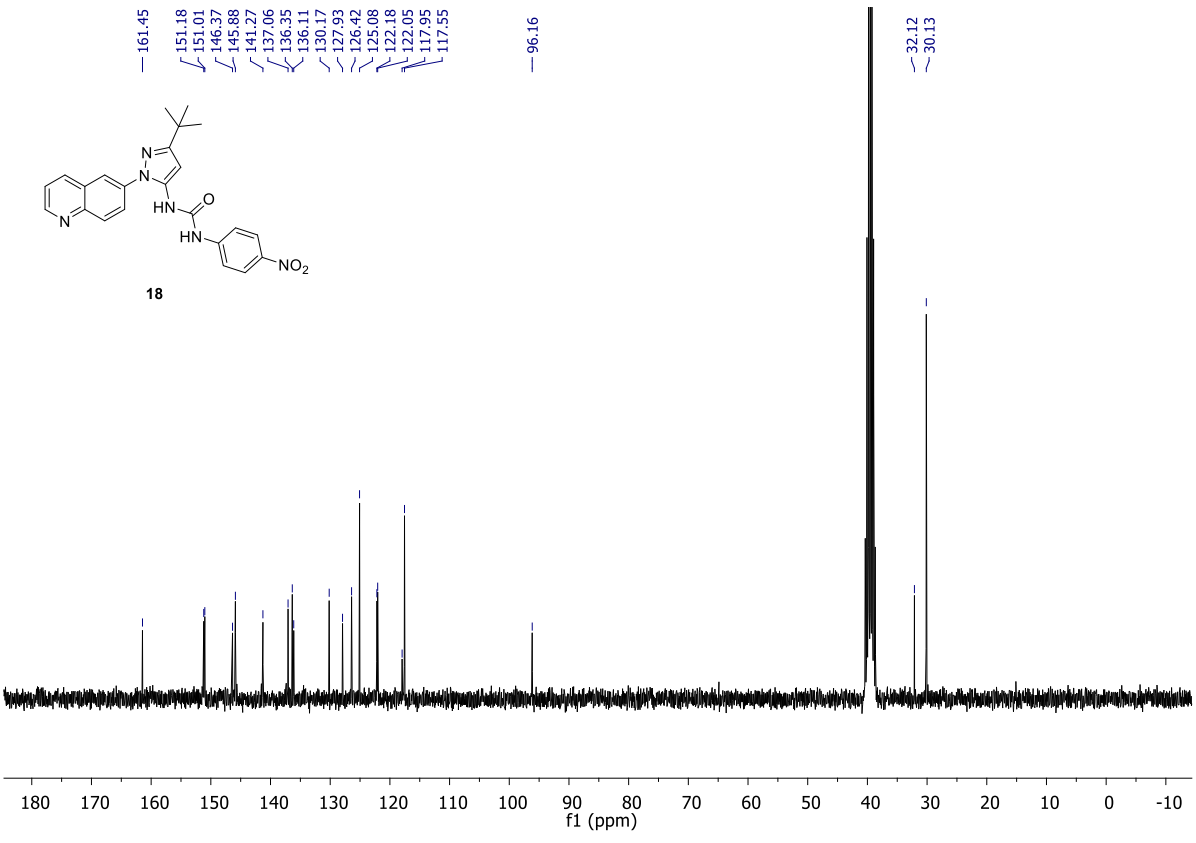
1.01
1.00
1.03
4.09
1.07
2.98
0.99
9.44
f1 (ppm)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146.37
145.88
141.27
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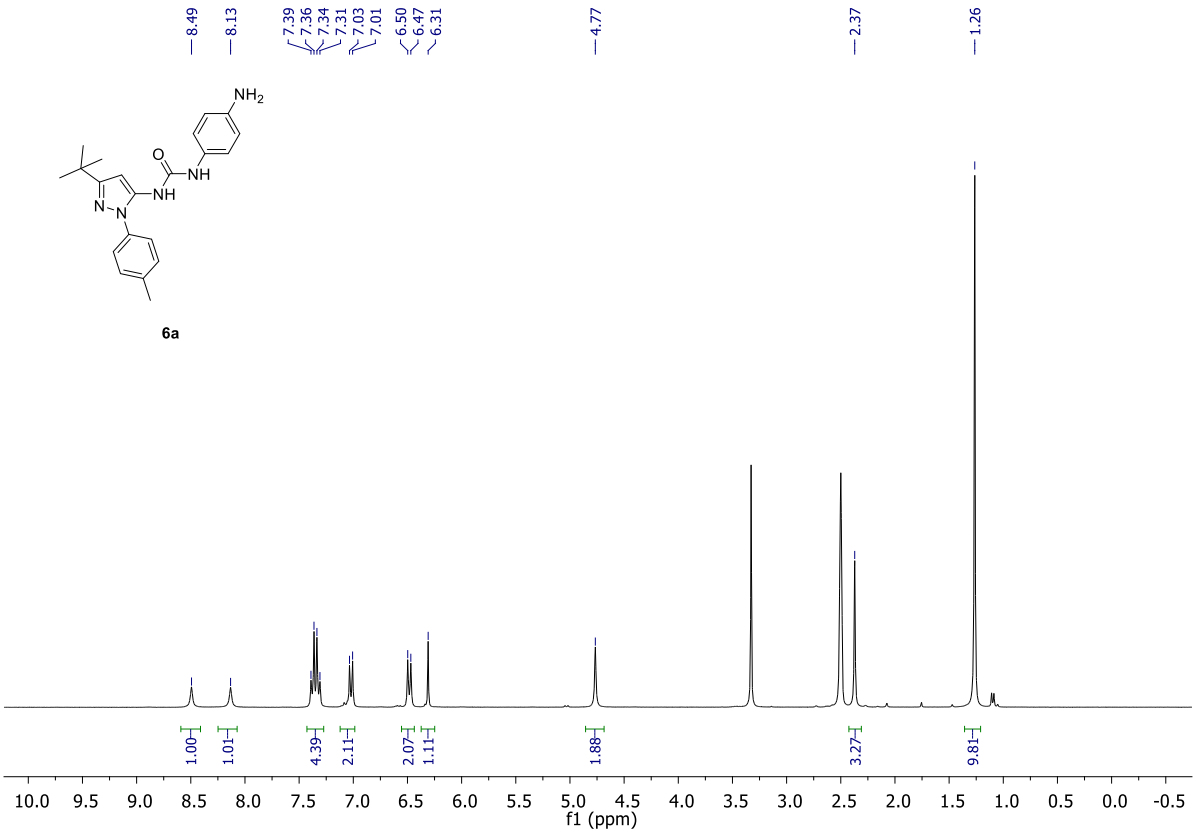
136.35
136.11
130.17
127.93
126.42
125.08
122.18
122.05
117.95
117.55
96.16
32.12
30.13
HN
O
HN
NO2
18
f1 (ppm)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7.31
7.03
7.01
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6.47
6.31
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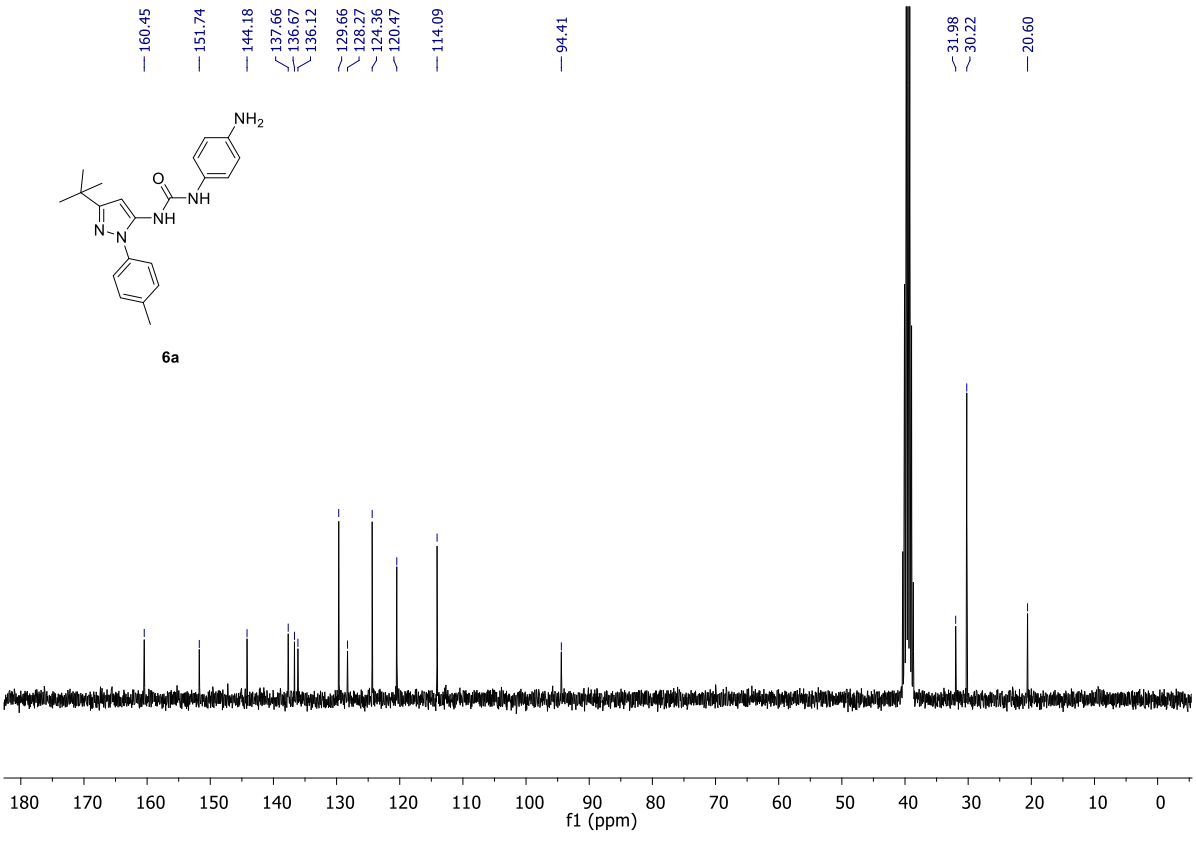
2.37
1.26
NH2
O
NH
NH
N
N
1.00
1.01
4.39
2.11
2.07
1.11
1.88
3.27
9.81
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

6a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120.47
114.09
94.41
31.98
30.22
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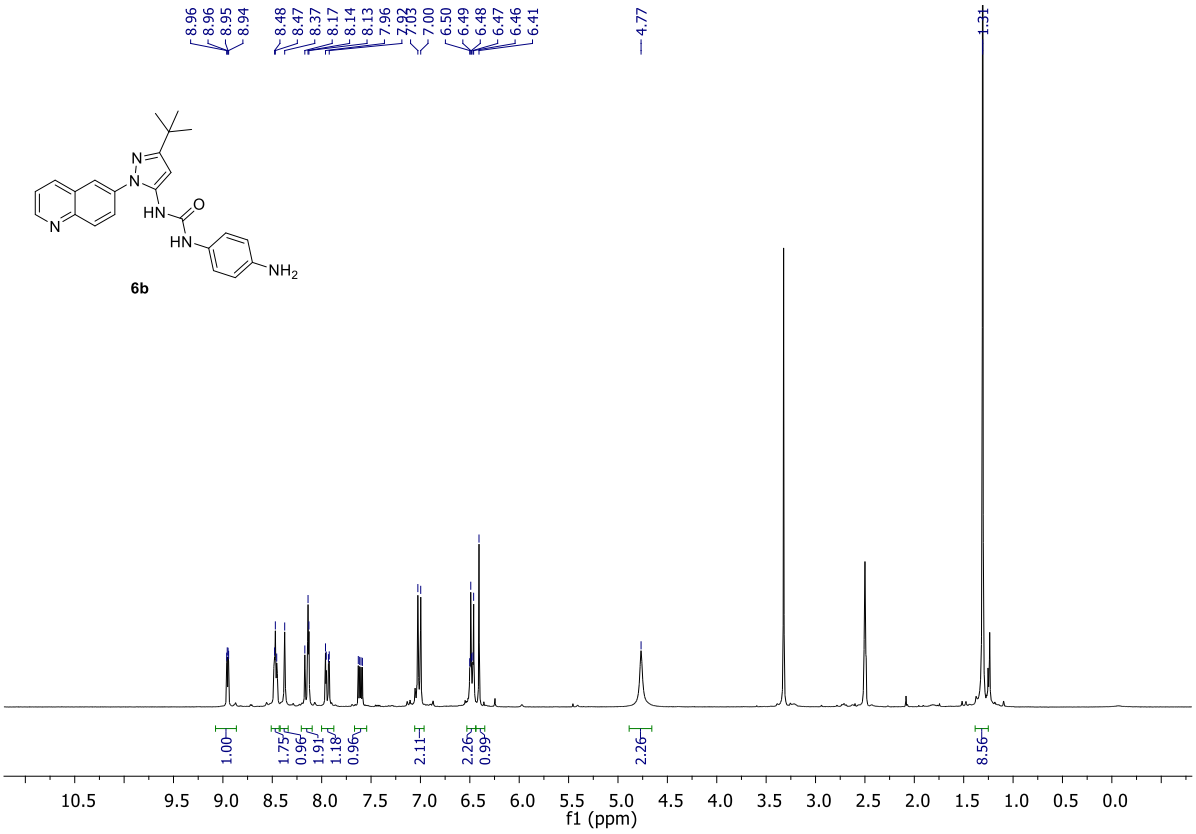
NH2
O
NH
NH
N
N
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

6a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8.96
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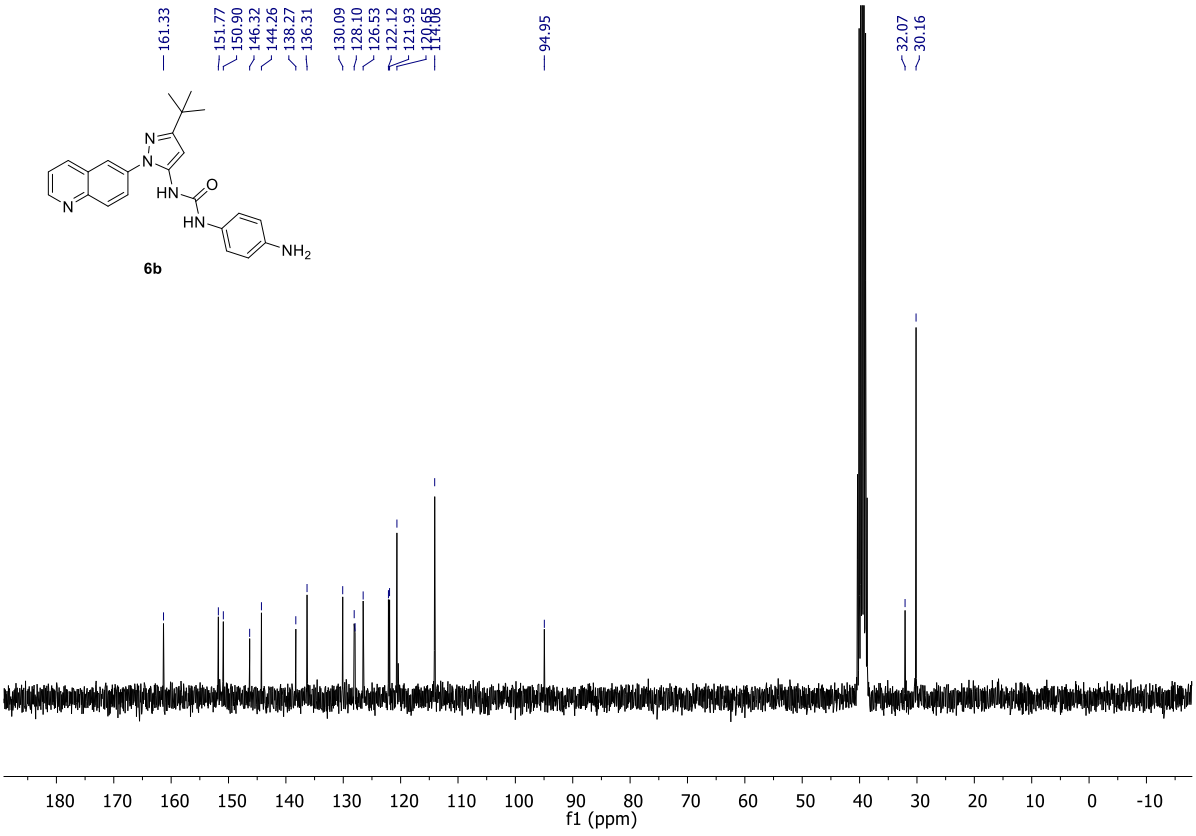
7.96
7.03
7.00
6.50
6.49
6.48
6.47
6.46
6.41
4.77
1.31
1.00
1.75
0.96
1.91
1.18
0.96
2.11
2.26
0.99
2.26
8.56
10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)
HN
O
HN
NH2
N
N
6b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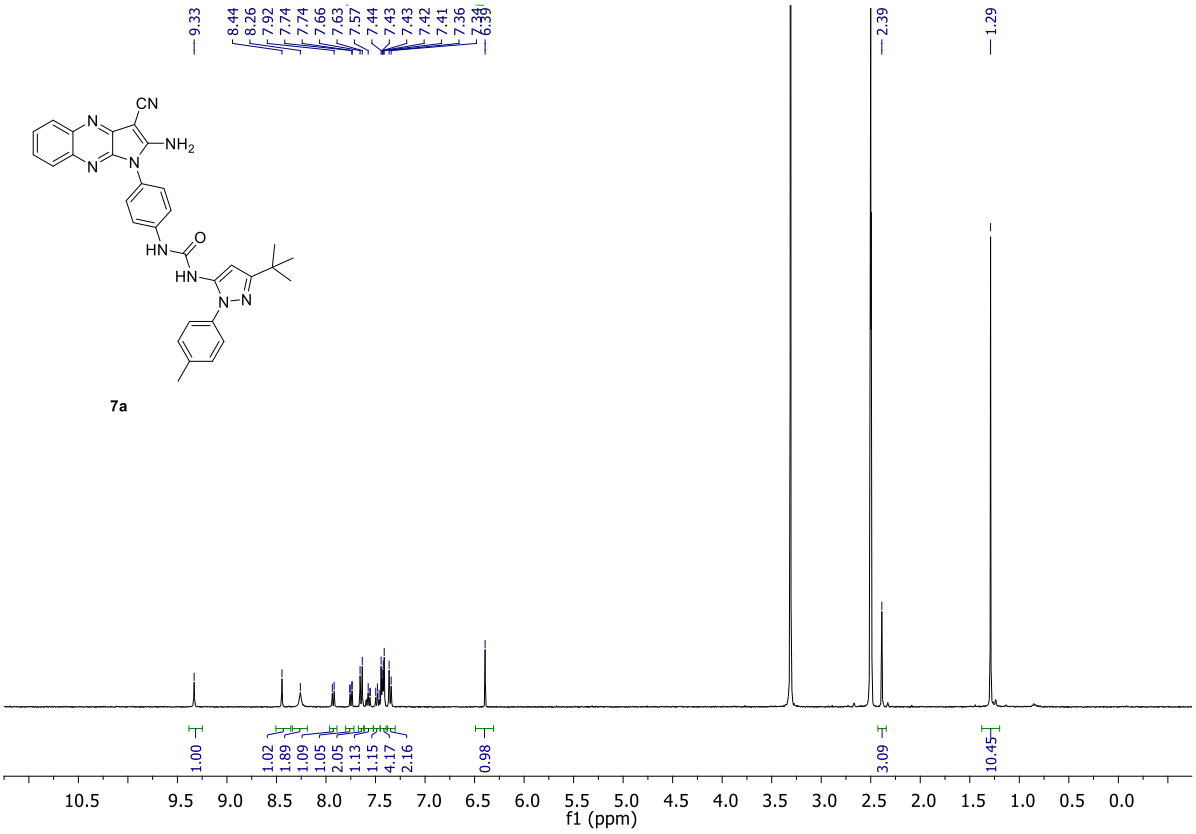
122.12
121.93
94.95
32.07
30.16
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)
HN
O
HN
NH2
N
N
6b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7.63
7.57
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7.43
7.43
7.42
7.41
7.36
7.34
6.39
2.39
1.29
CN
NH2
N
N
N
HN
O
HN
N
N
7a
1.00
1.02
1.89
1.09
1.05
2.05
1.13
1.15
4.17
2.16
0.98
3.09
10.45
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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2.5
2.0
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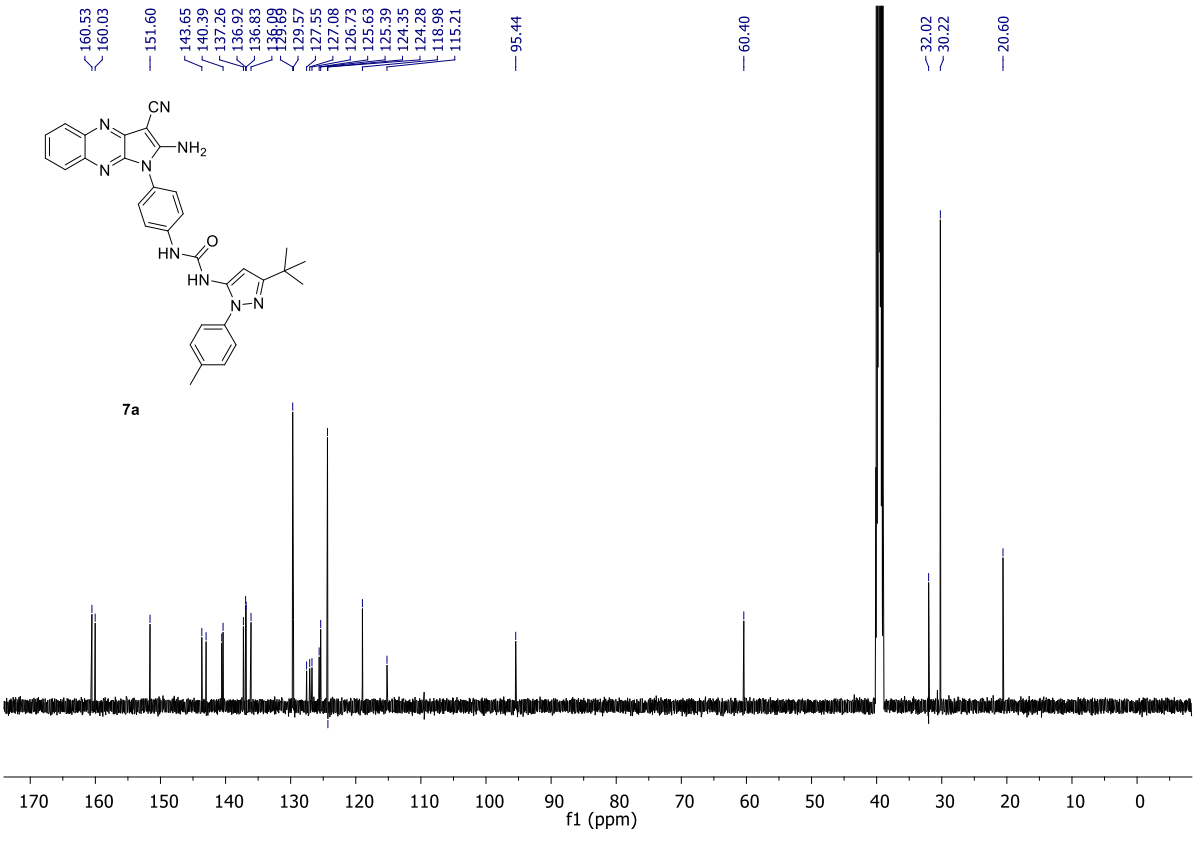
0.0
f1 (ppm)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127.08
126.73
125.63
125.39
124.35
124.28
118.98
115.21
95.44
60.40
32.02
30.22
20.60
CN
NH2
N
N
N
HN
O
HN
N
N
7a
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130
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110
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70
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50
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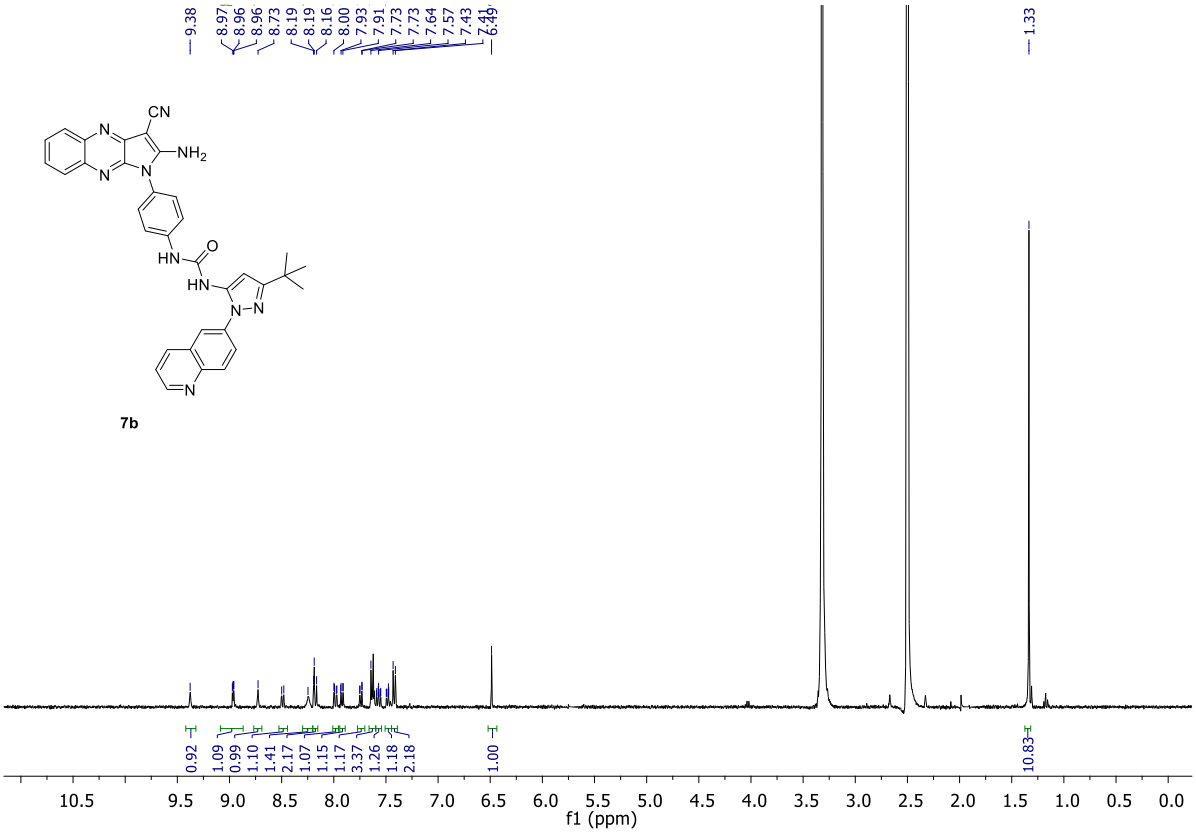
30
20
10
0
f1 (ppm)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8.16
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7.91
7.73
7.73
7.64
7.57
7.43
7.41
1.33
CN
NH2
HN
O
HN
N
N
N
7b
0.92
1.09
0.99
1.10
1.41
2.17
1.07
1.15
1.17
3.37
1.26
1.18
2.18
1.00
10.83
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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3.0
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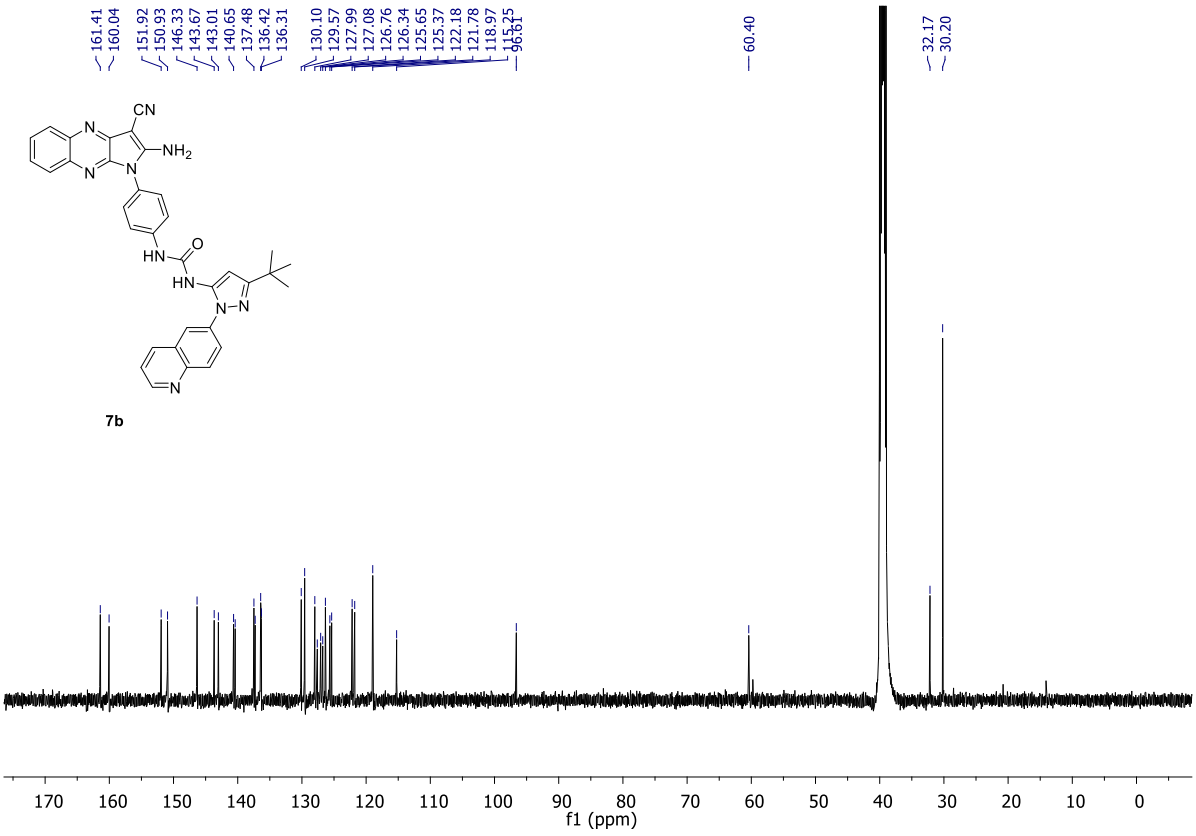
f1 (ppm)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130.10
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127.08
126.76
126.34
125.65
125.37
122.18
121.78
118.97
115.25
96.61
60.40
32.17
30.20
CN
NH2
HN
O
HN
N
N
N
7b
170
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150
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130
120
110
100
90
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70
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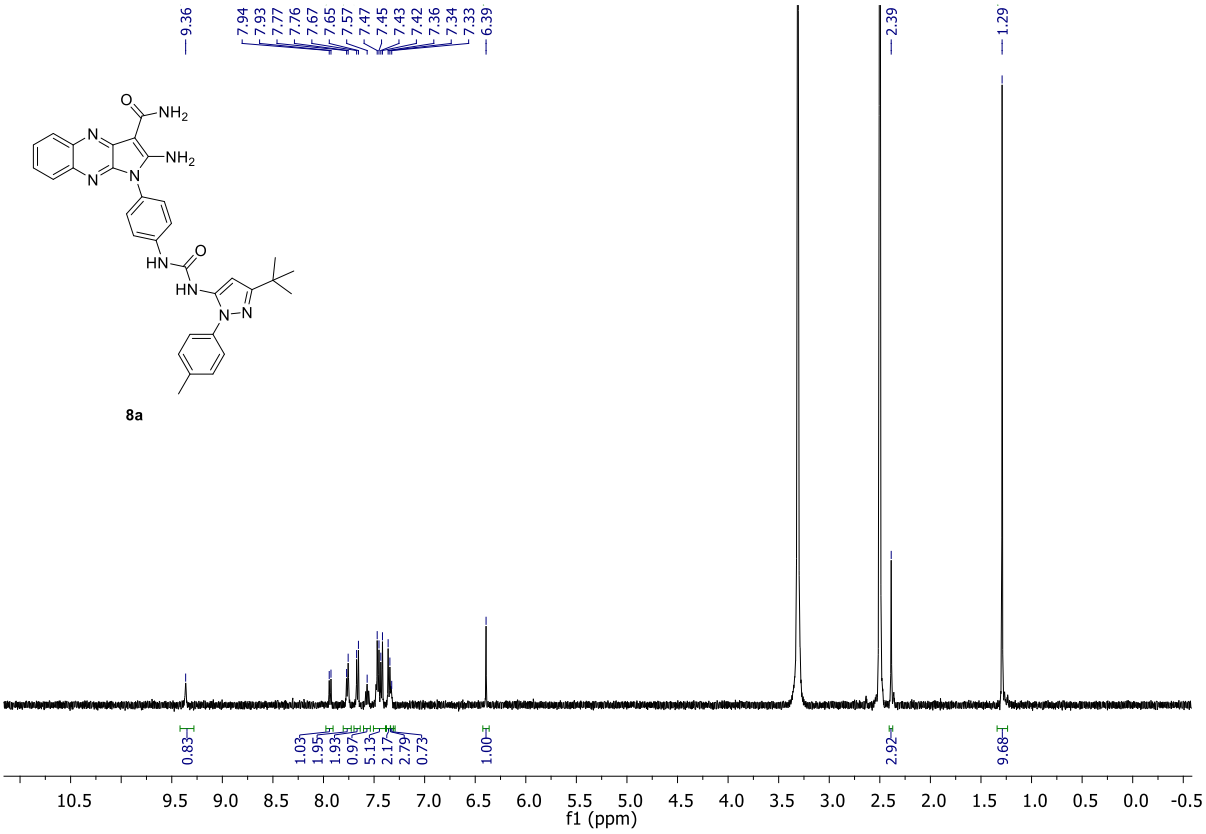
50
40
30
20
10
0
f1 (ppm)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7.57
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7.42
7.36
7.34
7.33
6.39
2.39
1.29
0.83
1.03
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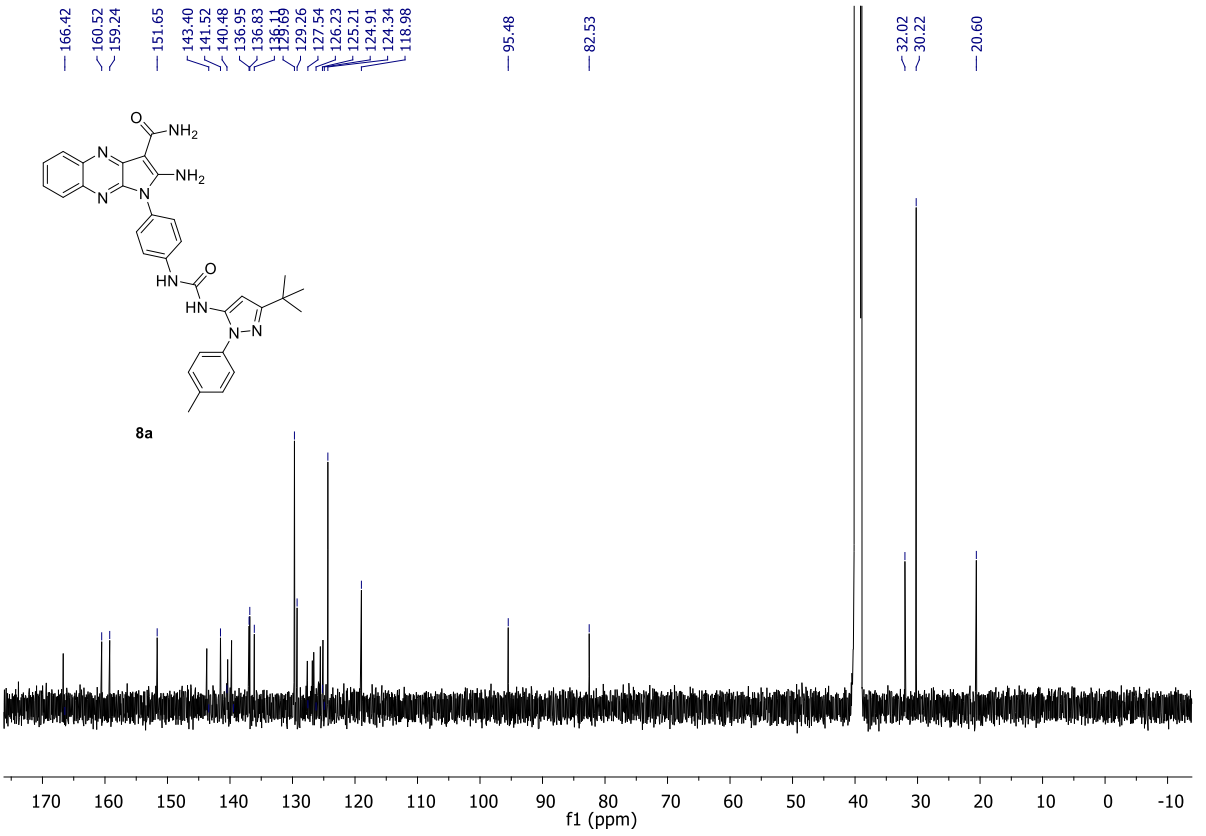
9.68
f1 (ppm)

8a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136.83
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127.54
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124.34
118.98
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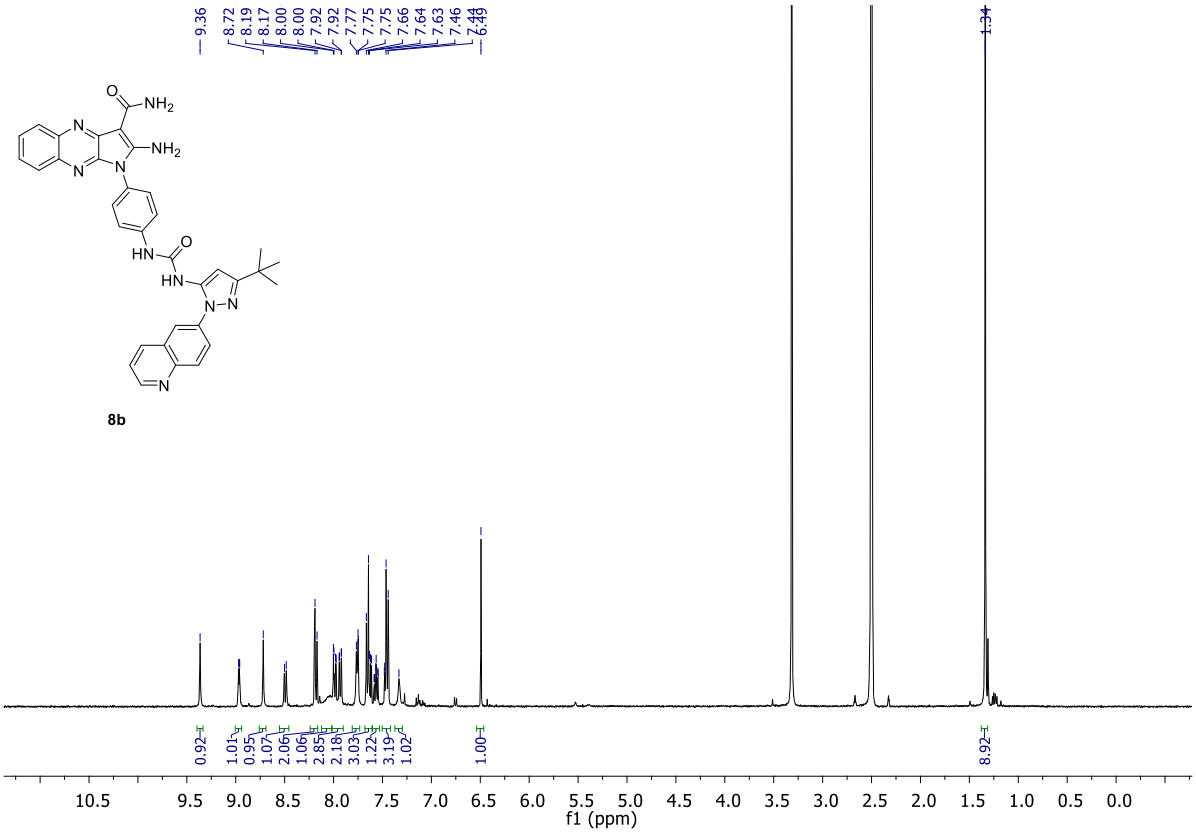
30.22
20.60
f1 (ppm)

8a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7.75
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7.63
7.46
7.44
7.43
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0.92
1.01
0.95
1.07
2.06
1.06
2.85
2.18
3.03
1.22
3.19
1.02
1.00
8.92
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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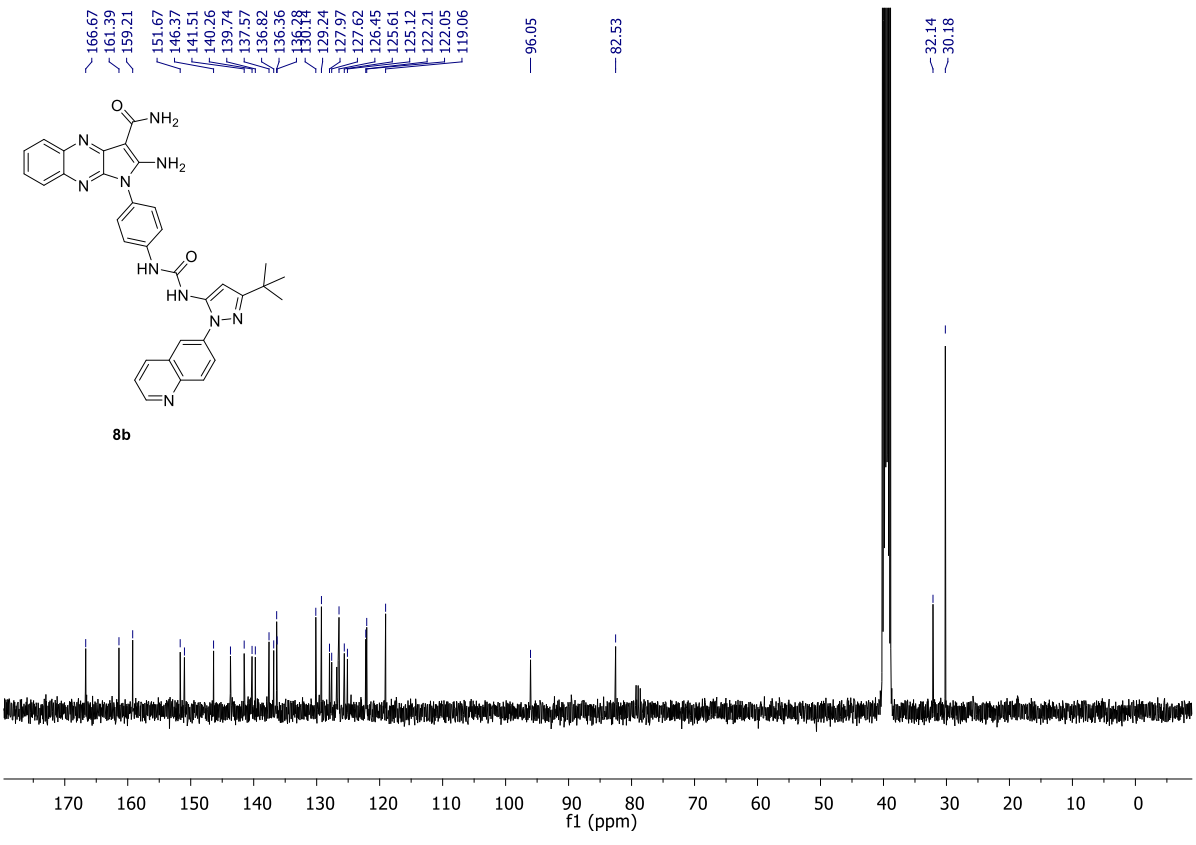
f1 (ppm)

8b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130.14
129.24
127.97
127.62
126.45
125.61
125.12
122.21
122.05
119.06
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82.53
32.14
30.18
170
160
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100
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80
70
60
50
40
30
20
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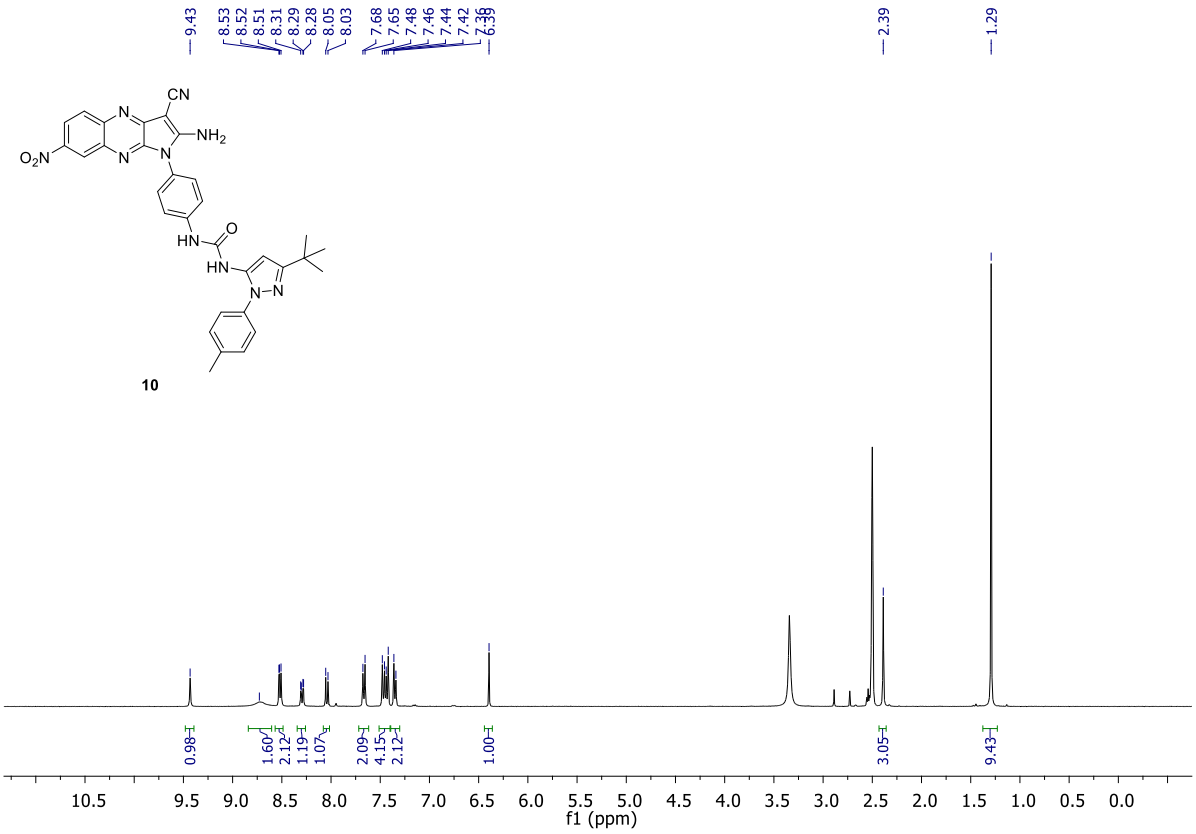
0
f1 (ppm)

8b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7.44
7.42
7.36
7.35
2.39
1.29
CN
NH2
O2N
N
HN
O
N-N
10
0.98
1.60
2.12
1.19
1.07
2.09
4.15
2.12
1.00
3.05
9.43
10.5
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
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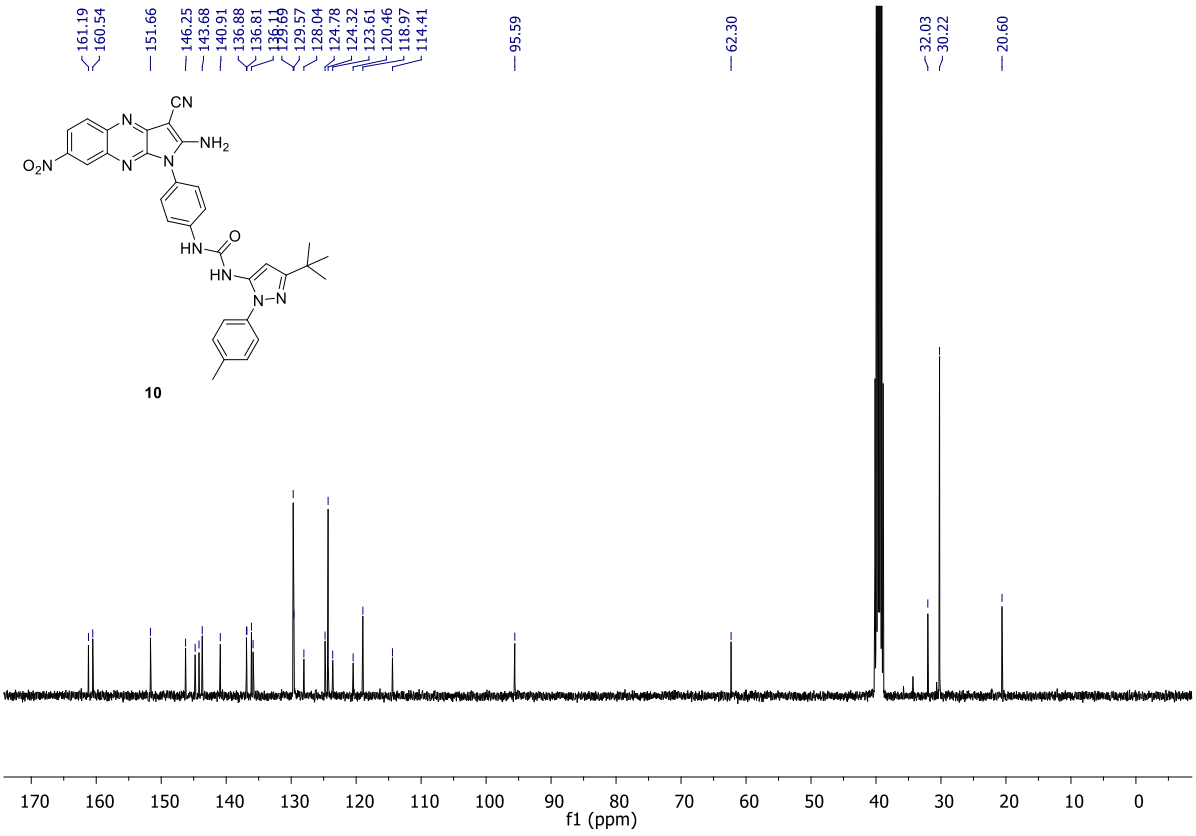
f1 (ppm)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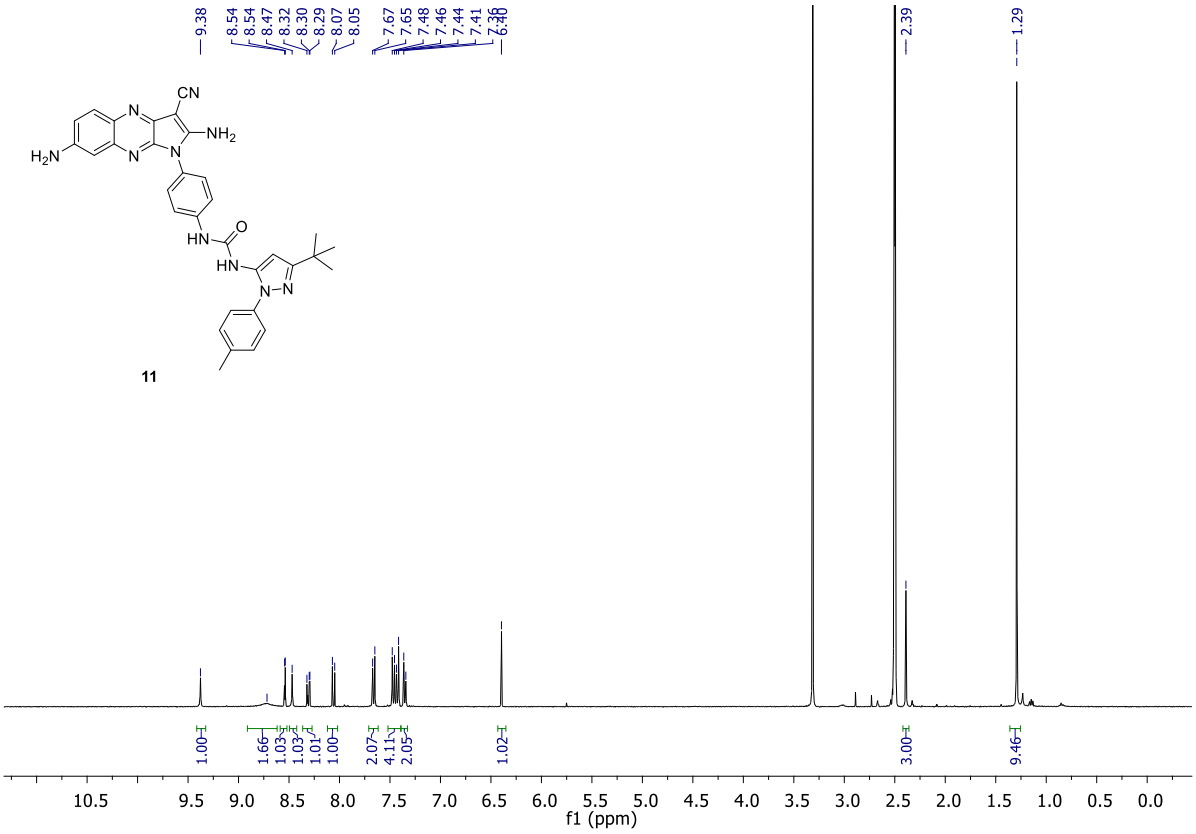
129.57
128.04
124.78
124.32
123.61
120.46
118.97
114.41
95.59
62.30
32.03
30.22
20.60
CN
NH2
O2N
N
HN
O
N-N
10
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)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8.05
7.67
7.65
7.48
7.46
7.44
7.41
7.36
7.30
6.40
2.39
1.29
CN
NH2
H2N
N
N
N
HN
O
HN
N-N
11
1.00
1.66
1.03
1.03
1.01
1.00
2.07
4.11
2.05
1.02
3.00
9.46
10.5
9.5
9.0
8.5
8.0
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7.0
6.5
6.0
5.5
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3.5
3.0
2.5
2.0
1.5
1.0
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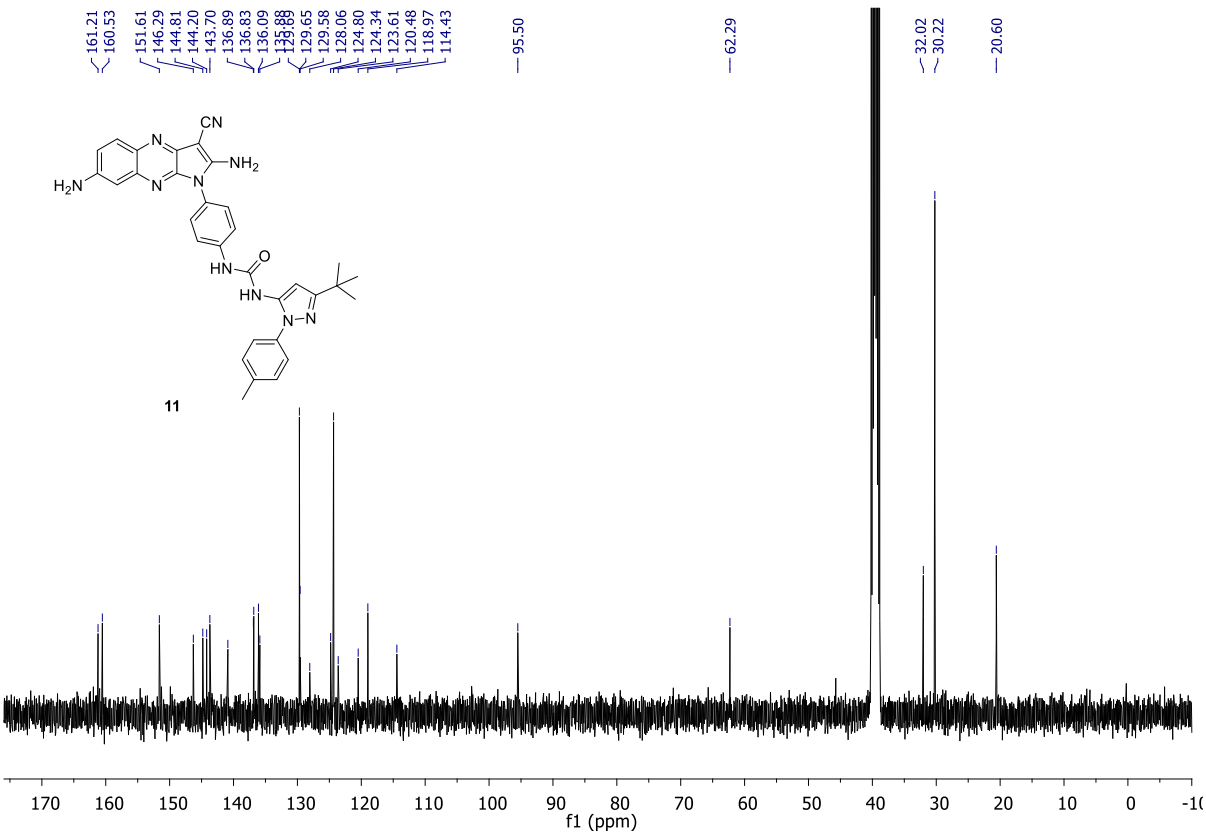
0.0
f1 (ppm)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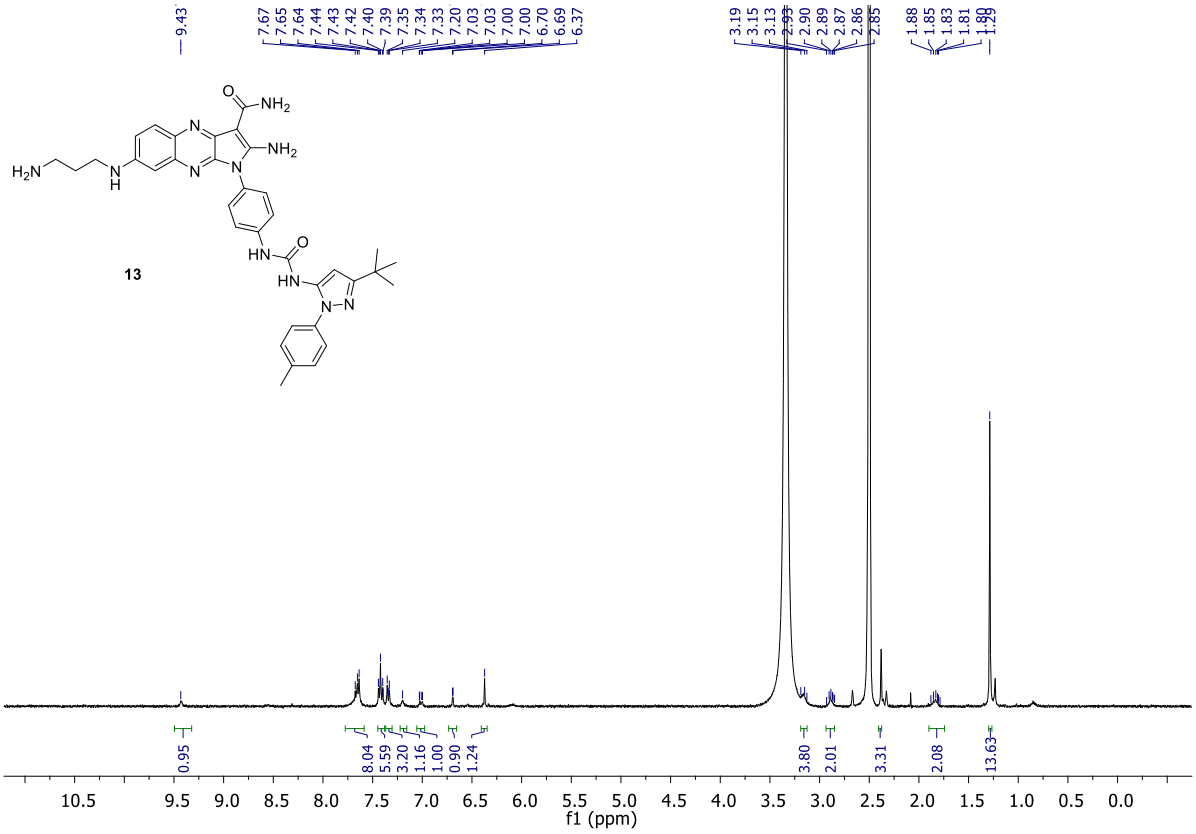
129.58
128.06
124.80
124.34
123.61
120.48
118.97
114.43
95.50
62.29
32.02
30.22
20.60
CN
NH2
H2N
N
N
N
HN
O
HN
N-N
11
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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7.33
7.20
7.03
7.03
7.00
7.00
6.70
6.69
6.37
3.19
3.15
3.13
2.93
2.90
2.89
2.87
2.86
2.85
1.88
1.85
1.83
1.81
1.80
1.29
13
0.95
8.04
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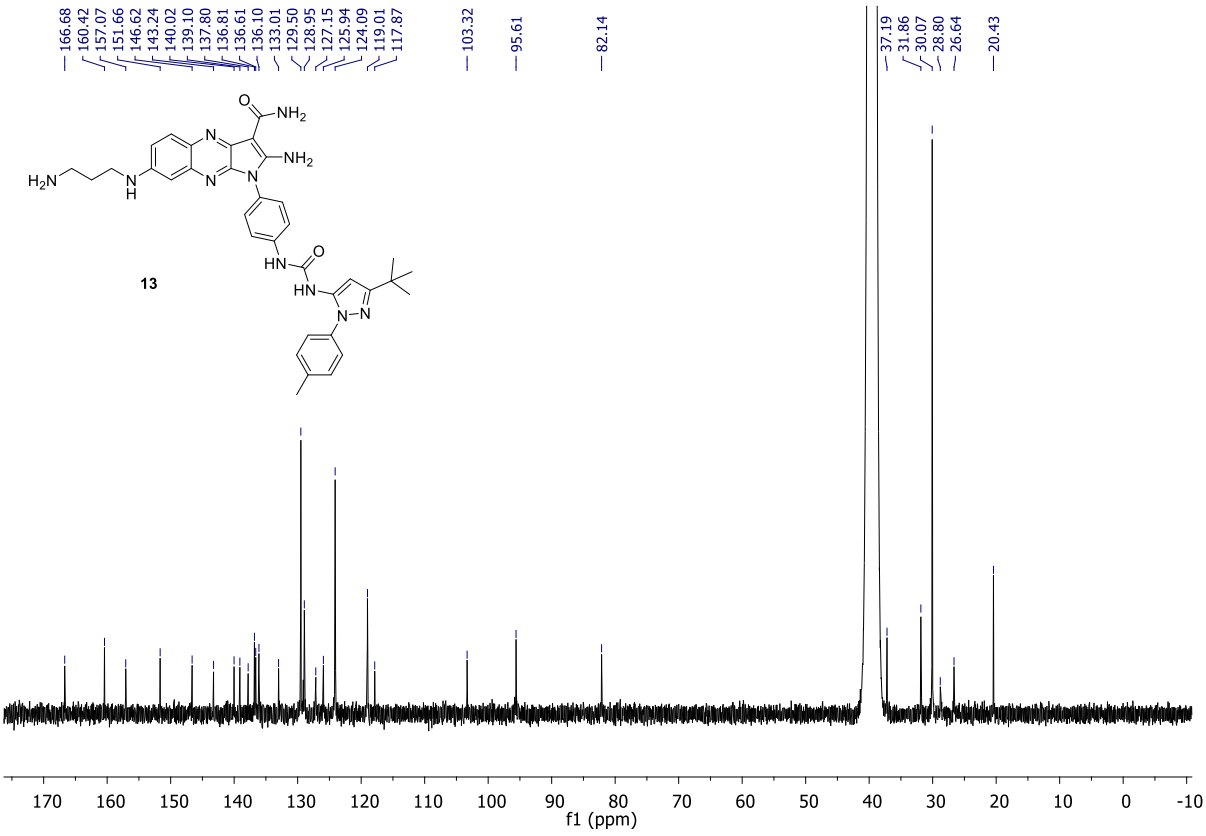
13.63
f1 (ppm)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133.01
129.50
128.95
127.15
125.94
124.09
119.01
117.87
103.32
95.61
82.14
37.19
31.86
30.07
28.80
26.64
20.43
13
f1 (ppm)

9. References

1. Pratt, E. F.; Kereszte.Jc, *J. Org. Chem.* **1967**, *32*, 49.
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