Supporting Information

Discovery, synthesis, and structure-based optimization of a series of *N*-(*tert*-butyl)-2-(*N*-arylamido)-2-(pyridin-3-yl) acetamides (ML188) as potent non-covalent small molecule inhibitors of the severe acute respiratory syndrome coronavirus (SARS-CoV) 3CL protease

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General. All NMR spectra were recorded on a Bruker 400 or 600 MHz instrument. ^{1}H chemical shifts are reported in δ values in ppm. Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet, dd = doublet of doublets, m = multiplet), coupling constant (Hz). Low resolution mass spectra were obtained on an Agilent 1200 series 6130 mass spectrometer. High resolution mass spectra were recorded on a Waters Q-TOF API-US. Analytical thin layer chromatography was performed on Analtech silica gel GF 250 micron plates. Analytical HPLC was performed on an HP1100 with UV detection at 214 and 254 nm along with ELSD detection, LC/MS (J-Sphere80-C18, 3.0 x 50 mm, 4.1 min gradient, 5%[0.05%TFA/CH_3CN] : 95%[0.05%TFA/H_2O] to 100%[0.05%TFA/CH_3CN]. Preparative RP-HPLC purification was performed on a custom HP1100 automated purification system with collection triggered by mass detection or using a Gilson Inc. preparative UV-based system using a Phenomenex Luna C18 column (50 x 30 mm I.D., 5 μm) with an acetonitrile (unmodified)-water (0.1% TFA) custom gradient. Normal-phase silica gel preparative purification was performed using an automated Combi-flash companion from ISCO. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification. All polymer-supported reagents were purchased from Argonaut Technologies and Biotage.

LC-MS General Procedures and Conditions

The HPLC measurement was performed using an Agilent 1200 system comprising a binary pump with degasser, an autosampler, a column oven, a diode-array detector (DAD) and a column as specified in the respective methods below. Flow from the column was split to a SQ mass

spectrometer and Polymer Labs ELSD. The MS detector was configured with an ES ionization source. Nitrogen was used as the nebulizer gas. The source temperature was maintained at 350 °C. Data acquisition was performed with Agilent Chemstation software. Reversed phase HPLC was carried out on a Kinetex C18 column (2.6 μm, 2.1 x 30 μm) from Phenomenex, with a flow rate of 1.5 mL/min, at 45 ºC. The gradient conditions used were either: method A) 93% A (water + 0.1% TFA), 7% B (acetonitrile), to 95% B in 1.1 minutes, returning to initial conditions at 1.11 minutes or method B) 93% A (water + 0.1% TFA), 7% B (acetonitrile), to 95% B in 3.1 minutes, returning to initial conditions at 3.2 minutes using an injection volume of 1 μL. Low-resolution mass spectra (single quadrupole MSD detector) were acquired in electrospray mode by scanning from 100 to 700 in 0.25 seconds, step size of 0.1 and peak width of 0.03 minutes. The capillary needle voltage was 3.0 kV and the fragmentor voltage was 100V.

Solubility Assay

Kinetic solubility was independently measured at pH 7.4 using 0.2 M Sodium Phosphate, pH 7.4. Each compound is made to 100 μM in triplicate in each buffer by adding 1μL of the 10 mM compound solution to 99 μL of buffer in 96 well round bottom plate (Thermo-Fisher #4917). The plate is sealed and shaken for 4 hours at room temperature. After 4 hours, the plate is centrifuged at 1150 x g for 10 minutes (3000 rpm in a Jouan E4 rotor) and standard solutions are made to 200 μM by adding 2 μL 10 mM compound solution to 98 μL DMSO in the last column of the plate (in the same row as the corresponding samples for each compound). Samples and standards are prepared for analysis by HPLC by adding an equal amount of sample supernatant and standard solution to the HPLC prep solution (1:1 ACN/0.1 N HCL) in a plate compatible with the HPLC auto injector. The plate is sealed with a seal or cap mat and samples are analyzed immediately after preparation. Samples are analyzed on an HPLC with UV detection (214 nm and 254 nm). The plate nest is cooled to 15 ^{o}C and the column temperature is set to 45 ^{o}C. A 0.75 min. gradient/run is applied from t=0 at 85/15 (water/acetonitrile) to t=0.6 min at 5/95 (water/acetonitrile). Volumes of 2, 4, and 6 μL of the standard solutions are injected and 10 μL of each sample is injected. Peak areas are measured and samples are quantified using the standard curve generated from the standard solutions. Solubility is reported as concentration (in μM) or μg/mL remaining of the sample (ML188, solubility in PBS buffer 95 μg/mL (219 μM)).

General Procedure for 4CC-Ugi Reaction in Library Format.

To a series of 13 x 100 mm screw top glass tubes fitted with a magnetic stir bar equimolar amounts (0.08 mmol) of carboxaldehyde, amine, carboxylic acid were combined in methanol (0.2 M, 1.5 mL) and subsequently treated with *tert*-butylisocyanide (0.08 mmol). The mixture was stirred for 16h at 50 °C, and then concentrated under a stream of nitrogen in a well ventilated hood. The crude mixtures were reconstituted in MeOH, treated with Argoresin MP-Trisamine (Biotage Inc.) scavenger for 2h and applied to a Celite pad using a manifold fitted with polypropylene filter tubes capable of filtering 24 samples in parallel. The filtrates were purified directly using an automated mass-guided RP-HPLC and product containing fractions were concentrated to give final products >95% purity as judged by LC-MS (215 nm and ELSD).

Full characterization for **16-(*R*)-ML188**, **17-20** can be found below**.** Final library array products as described in the text in Scheme 1 using components **2A-B, a-e, 1-8** can be found below (Tables 1 and 2). Final product LC-MS [M+H] and SARS-3CLpro % inhibition at 100 μM are indicated.

Representative NMR spectra of 10% of the library found in Tables 1 and 2 have been provided and can be found in the characterization section. Analogues **21** – **48** (text Figure 8) and **49 – 61** (text Table 1) were prepared in an analogous manner and purified by Gilson Inc. preparative UV-based RP-HPLC. Chromatographic, LC-MS, and representative ^{1}H NMR data for these remaining analogues (≥20%) can be found below and in Table 3.

Table 1. Library 2A: nicotinaldehyde array % inhibition (100 μM) and [M+H].

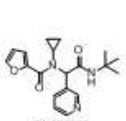
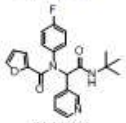
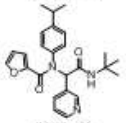
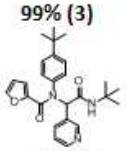
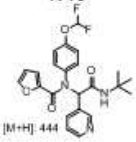
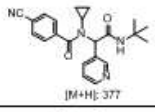
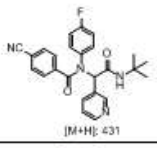
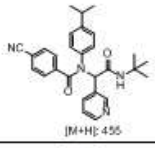
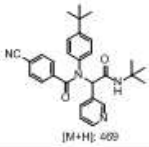
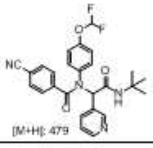
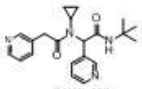
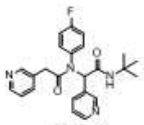
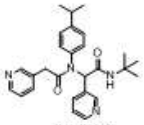
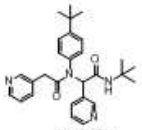
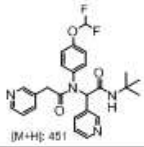
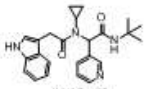
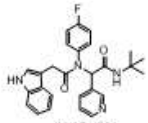
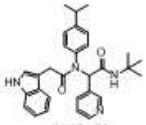
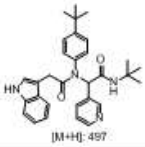
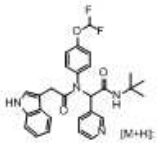
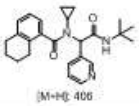
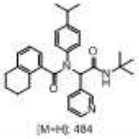
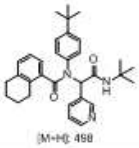
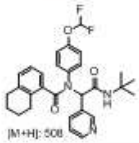
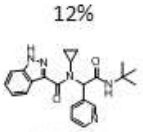
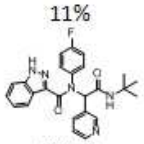
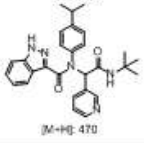
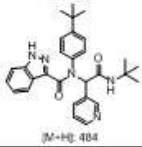
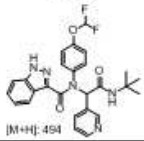
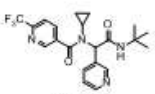
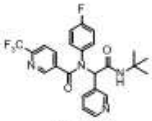
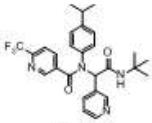
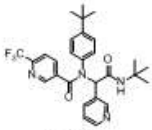
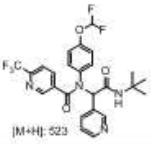
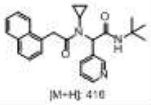
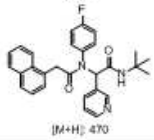
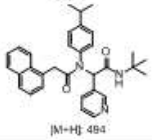
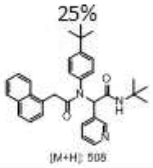
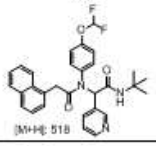
R1/R2	a.	b.	c.	d.	e.
1.	0% (5)  [M+H]: 342	1% (6)  [M+H]: 396	**69% (4)**  [M+H]: 420	**99% (3)**  [M+H]: 435	47%  [M+H]: 444
2.	2%  [M+H]: 377	0%  [M+H]: 431	2%  [M+H]: 455	0%  [M+H]: 469	4%  [M+H]: 479
3.	16%  [M+H]: 367	12%  [M+H]: 421	10%  [M+H]: 445	23%  [M+H]: 459	25%  [M+H]: 451
4.	15%  [M+H]: 406	11%  [M+H]: 459	9%  [M+H]: 483	15%  [M+H]: 497	39%  [M+H]: 507
5.	19%  [M+H]: 406	17%  [M+H]: 460	0%  [M+H]: 484	17%  [M+H]: 498	20%  [M+H]: 508
6.	12%  [M+H]: 392	11%  [M+H]: 446	19%  [M+H]: 470	22%  [M+H]: 484	26%  [M+H]: 494
7.	12%  [M+H]: 421	12%  [M+H]: 475	0%  [M+H]: 499	13%  [M+H]: 513	19%  [M+H]: 523
8.	8%  [M+H]: 416	0%  [M+H]: 470	5%  [M+H]: 484	25%  [M+H]: 508	8%  [M+H]: 518

Table 2. Library 2B: thiophene-3-carbaldehyde array % inhibition (100 μM) and [M+H].

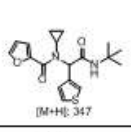
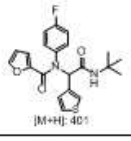
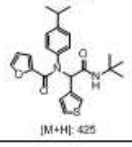
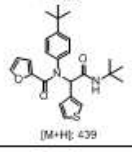
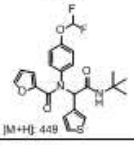
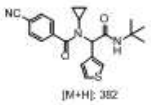
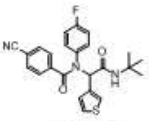
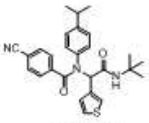
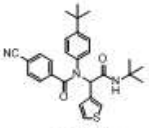
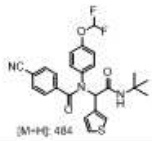
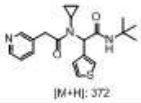
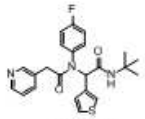
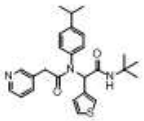
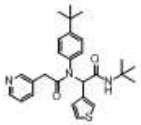
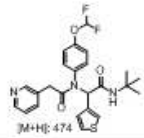
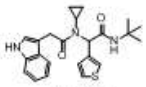
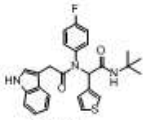
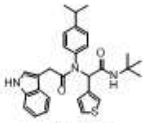
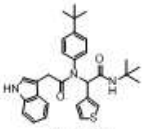
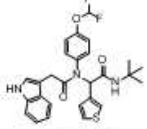
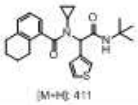
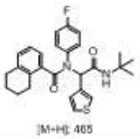
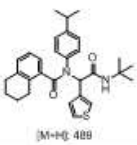
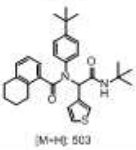
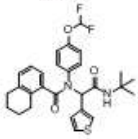
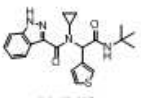
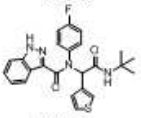
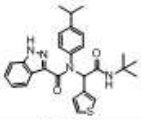
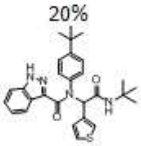
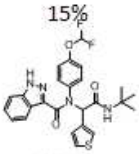
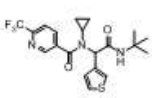
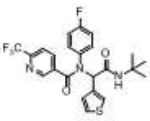
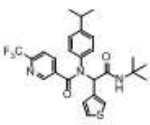
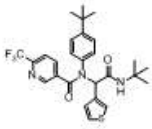
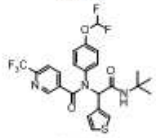
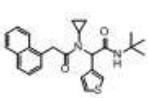
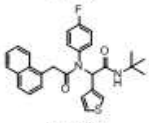
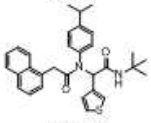
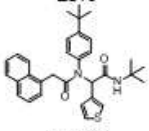
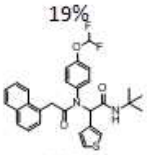
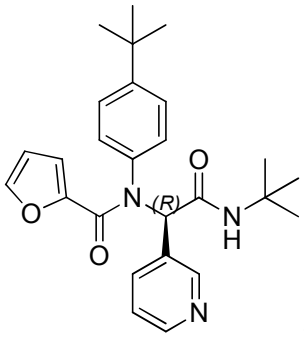
R1/R2	a.	b.	c.	d.	e.
1.	11%  [M+H]: 347	14%  [M+H]: 401	11%  [M+H]: 425	23%  [M+H]: 439	29%  [M+H]: 449
2.	13%  [M+H]: 382	19%  [M+H]: 436	2%  [M+H]: 460	25%  [M+H]: 474	19%  [M+H]: 484
3.	29%  [M+H]: 372	22%  [M+H]: 426	10%  [M+H]: 450	38%  [M+H]: 464	**54%**  [M+H]: 474
4.	48%  [M+H]: 410	20%  [M+H]: 464	11%  [M+H]: 488	29%  [M+H]: 502	38%  [M+H]: 512
5.	34%  [M+H]: 411	17%  [M+H]: 465	10%  [M+H]: 489	15%  [M+H]: 503	16%  [M+H]: 513
6.	15%  [M+H]: 397	18%  [M+H]: 451	12%  [M+H]: 475	20%  [M+H]: 489	15%  [M+H]: 499
7.	14%  [M+H]: 426	14%  [M+H]: 480	10%  [M+H]: 504	21%  [M+H]: 513	23%  [M+H]: 516
8.	19%  [M+H]: 421	22%  [M+H]: 475	10%  [M+H]: 499	23%  [M+H]: 513	19%  [M+H]: 523

Table 3. LC-MS characterization data for 21 – 61.

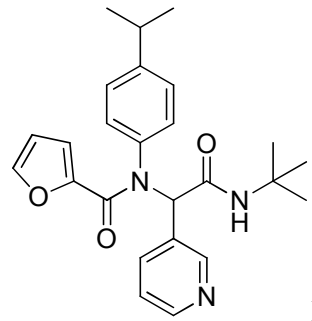
Compound	[M+H]	HPLC Rt	Method
21	448.3	2.334	B
22	435.2	2.023	B
23	450.2	2.322	B
24	462.2	2.352	B
25	445.2	2.077	B
26	448.2	2.345	B
27	450.2	2.300	B
28	458.3	2.433	B
29	478.2	2.489	B
30	445.2	1.864	B
31	434.2	1.66	B
32	438.2	2.098	B
33	474.2	2.278	B
34	460.2	2.106	B
35	445.2	1.755	B
36	434.2	1.739	B
37	433.2	2.258	B
38	434.2	1.997	B
39	412.3	2.007	B
40	398.2	1.912	B
41	474.3	2.314	B
42	460.2	2.074	B
43	446.3	1.995	B
44	434.2	1.87	B
45	424.3	2.429	B

46	474.3	2.303	B
47	460.2	2.061	B
48	446.3	2.002	B
49	435.1	0.729	A
50	468.2	0.789	A
51	448.3	0.653	A
52	448.2	0.589	A
53	435.2	0.772	A
54	435.2	0.859	A
55	448.3	0.732	A
56	434.2	0.71	A
57	434.2	0.794	A
58	423.3	0.753	A
59	423.3	0.822	A
60	424.2	0.765	A
61	423.2	0.68	A

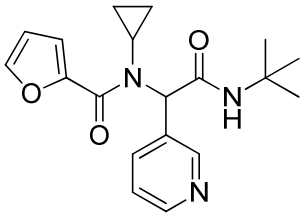
Characterization Data for Compounds 16-(*R*), 17-20, 22, 27, and 31.



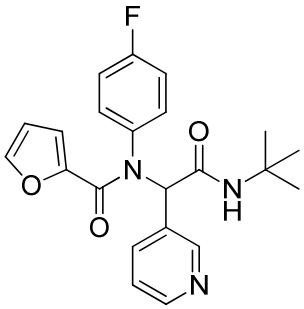
(*R*)-N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)furan-2-carboxamide, 16-(*R*). $[\alpha]_D$ = +38.9 (c = 1.0, $CHCl_3$); ^{1}H NMR (400MHz, $CDCl_3$) δ 8.46 (2H, d, *J* = 5.4 Hz), 7.50 (1H, d, *J* = 8.0 Hz), 7.37 (1H, d, *J* = 1.1 Hz), 7.24 (2H, d, *J* = 8.6 Hz), 7.05 (1H, dd, *J* = 7.8, 4.8 Hz), 6.98 (2H, bs), 6.21 (1H, s), 6.13 (1H, dd, *J* = 3.6, 1.7 Hz), 6.10 (1H, s), 5.37 (1H, d, *J* = 3.6 Hz), 1.36 (9H, s), 1.26 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.7, 159.5, 152.4, 151.3, 149.4, 146.1, 144.9, 138.1, 136.4, 130.4, 130.1, 126.0, 122.7, 117.0, 111.1, 63.6, 51.7, 34.6, 31.2, 28.6; HRMS (ES+, M+H) calcd for $C_{26}H_{32}N_3O_3$: 434.2440, found 434.2444.



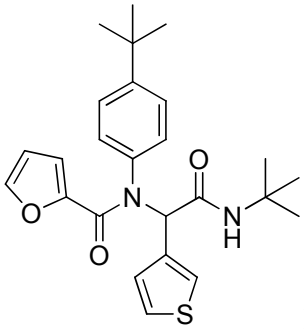
N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-N-(4-isopropylphenyl)furan-2-carboxamide, 17. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.46 (2H, m), 8.49 (1H, d, *J* = 7.9 Hz), 7.38 (1H, s), 7.07 (3H, m), 6.98 (2H, bs), 6.15 (2H, m), 6.10 (1H, s), 5.38 (1H, d, *J* = 3.6 Hz), 2.87 (1 H, p, *J* = 6.9 Hz), 1.37 (9H, s), 1.20 (6H, d); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 159.5, 151.4, 150.0, 149.4, 146.1, 144.8, 138.0, 136.6, 130.5, 130.4, 127.0, 122.7, 117.0, 111.1, 63.5, 51.6, 33.6, 28.5, 23.8, 23.7; HRMS (ES+, M+H) calcd for $C_{25}H_{30}N_3O_3$: 420.2287, found 420.2290.



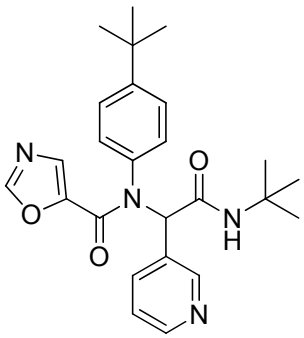
N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-N-cyclopropylfuran-2-carboxamide, 18. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.68 (1H, s), 8.57 (1H, d, *J* = 4.0 Hz), 7.97 (1H, d, *J* = 7.9), 7.53 (1H, s), 7.32 (1H, dd, *J* = 7.7, 4.8), 7.10 (1H, d, *J* = 3.4 Hz), 6.57 (1H, s), 6.51 (1H, dd, *J* = 3.4, 1.6 Hz), 5.66 (1H, s), 2.90 (1H, m), 1.36 (9H, s), 0.99 (1H, m), 0.80 (m, 1H), 0.68 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.3, 162.4, 150.5, 148.9, 147.2, 144.5, 137.1, 131.1, 122.8, 117.1, 111.2, 64.7, 51.1, 31.2, 28.2, 10.4, 9.5; HRMS (ES+, M+H) calcd for $C_{19}H_{24}N_3O_3$: 342.1818, found 342.1815.



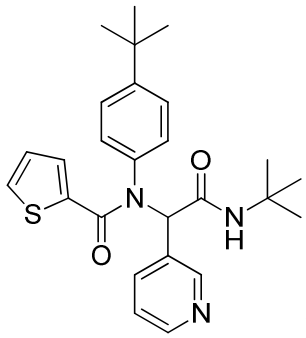
N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-N-(4-fluorophenyl)furan-2-carboxamide, 19. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.41 (1H, d, *J* = 4.0 Hz), 8.37 (1H, s), 7.43 (1H, d, *J* = 8.0), 7.29 (1H, s), 7.05 (2H, dd, *J* = 7.8, 4.8), 6.85 (2H, m), 6.29 (1H, s), 6.15 (1H, s), 6.13 (1H , dd, *J* = 3.5, 1.6 Hz), 5.55 (1H, d, *J* = 3.6 Hz), 5.24 (s, 1H), 1.30 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.8, 162.5 (d, *J* = 248), 159.3, 151.3, 149.5, 146.1, 144.9, 137.8, 134.9 (d, *J* = 3.1 Hz), 132.9 (d, *J* = 8.6 Hz), 130.3, 123.0, 117.2, 115.8 (d, *J* = 22.4 Hz), 111.1, 62.8, 51.7, 28.5; HRMS (ES+, M+H) calcd for $C_{22}H_{23}N_3O_3F$: 396.1723, found 396.1725.



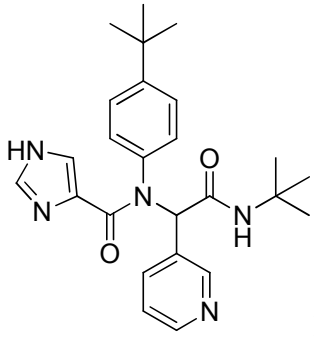
N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(thiophen-3-yl)ethyl)furan-2-carboxamide, 20. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.37 (1H, s), 7.26 (2H, s), 7.24 (1H, s), 7.13 (1H, m), 6.99 (2H, bs), 6.89 (1H, d, *J* = 5.0 Hz), 6.13 (1H , m), 6.10 (1H, s), 6.06 (1H, s) 5.33 (1H, d, *J* = 3.6Hz), 1.35 (9H, s), 1.29 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.1, 159.3, 152.0, 146.4, 144.6, 137.2, 134.9, 129.6, 129.0, 126.7, 125.8, 125.1, 116.7, 111.1, 61.6, 51.4, 34.6, 31.3, 28.6; HRMS (ES+, M+H) calcd for $C_{25}H_{31}N_2O_3S$: 439.2055, found 439.2057.



N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)oxazole-5-carboxamide, 22. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.48 (2H, s), 7.84 (1H, s), 7.48 (1H, d, *J* = 7.9 Hz), 7.28 (2H, m), 7.08 (1H, m), 6.06 (1H, s), 5.85 (1H , bs), 5.70 (1H, s), 5.30 (1H, s) 1.37 (9H, s), 1.27 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.3, 157.7, 152.8, 152.1, 151.2, 149.3, 144.1, 137.7, 134.9, 131.1, 130.3, 129.9, 125.9, 122.7, 62.9, 51.5, 34.4, 30.9, 28.3; HRMS (ES+, M+H) calcd for $C_{25}H_{31}N_4O_3$: 435.2396, found 435.2397.

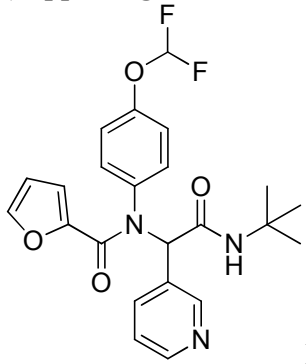


N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)thiophene-2-carboxamide, 27. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.47 (1H, s), 8.44 (1H, d, *J* = 4.0 Hz), 7.48 (1H, d, *J* = 8.0 Hz), 7.29 (1H, dd, *J* = 4.8, 0.9 Hz), 7.23 (2H, d, *J* = 8.3 Hz), 7.05 (1H, dd, *J* = 7.9, 4.8 Hz), 7.01 (1H, bs), 6.75 (2H, m), 6.29 (1H , bs), 6.14 (1H, s), 1.36 (9H, s), 1.26 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.9, 162.9, 152.6, 151.4, 149.4, 138.0, 137.5, 136.5, 133.1, 131.6, 130.6, 130.5, 126.7, 126.1, 122.7, 64.1, 51.7, 34.6, 31.2, 28.6; HRMS (ES+, M+H) calcd for $C_{26}H_{32}N_3O_2S$: 450.2215, found 450.2213.

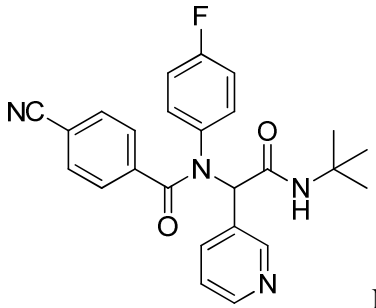


N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-1H-imidazole-4-carboxamide, 31. ^{1}H NMR (600MHz, $CDCl_3$) δ 11.66 (1H, bs), 8.47 (1H, d, *J* = 1.9 Hz), 8.41 (1H, dd, *J* = 3.2, 1.4 Hz), 7.59 (2H, s), 7.39 (1H, d, *J* = 5.3 Hz), 7.22 (3H, bs), 7.01 (1H, dd, *J* = 5.3, 4.8 Hz), 6.32 (1H, bs), 6.20 (1H, s), 5.43 (1H, bs), 1.24 (9H, s), 1.23 (9H, s). ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.8, 161.1, 152.9, 151.4, 149.5, 137.9, 137.3, 135.6, 133.2, 130.5, 126.2, 125.0, 122.8, 63.5, 51.6, 34.6, 31.2, 28.5; HRMS (ES+, M+H) calcd for $C_{25}H_{32}N_5O_2$: 434.2556, found 434.2555.

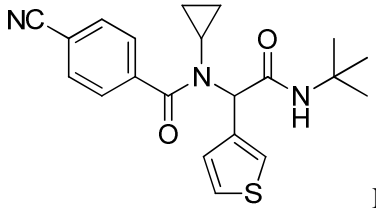
Characterization Data of Representative Compounds from Scheme 1 (text)/Tables 1 and 2 (Supporting Information).



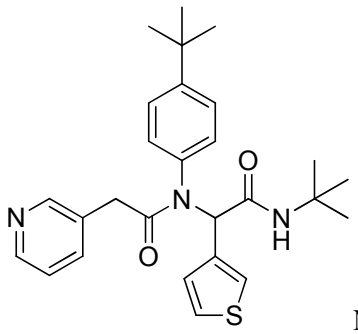
N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-N-(4-(difluoromethoxy)phenyl)furan-2-carboxamide, 2A-1e. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.69 (1H, s), 8.65 (1H, dd, *J* = 5.3, 1.1 Hz), 8.07 (1H, d, *J* = 8.1 Hz), 7.55 (1H, dd, *J* = 8.0, 5.4 Hz), 7.37 (1H, d, *J* = 1.0 Hz), 7.06 (3H, s), 6.58 (1H, t, *J* = 73.0 Hz), 6.44 (1H, s), 6.3 (1H, s), 6.26 (1H, dd, *J* = 3.6, 1.7 Hz), 5.87 (1H, d, *J* = 3.6 Hz), 1.39 (9H, s).



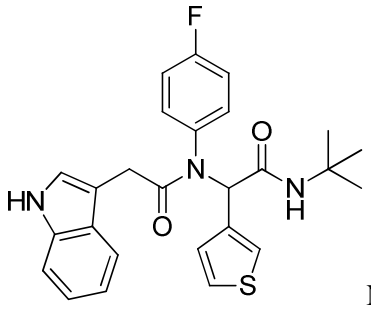
N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-4-cyano-N-(4-fluorophenyl)benzamide, 2A-2b. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.92 (1H, s), 8.65 (1H, d, *J* = 4.8 Hz), 8.07 (1H, d, *J* = 8.1 Hz), 7.56 (1H, dd, *J* = 8.0, 5.4 Hz), 7.49 (2H, d, *J* = 8.4 Hz), 7.37 (2H, d, *J* = 8.4 Hz), 7.00 (2H, bs), 6.82 (2H, t, *J* = 8.4 Hz), 6.29 (1H, s), 6.25 (1H, bs), 1.39 (9H, s).



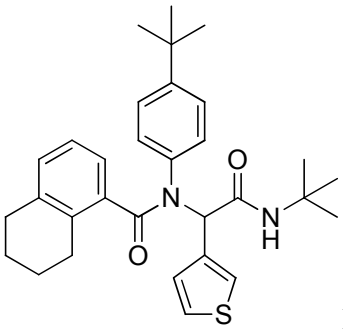
N-(2-(tert-butylamino)-2-oxo-1-(thiophen-3-yl)ethyl)-4-cyano-N-cyclopropylbenzamide, 2B-2a. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.68 (4H, m), 7.5 1 (1H, d, *J* = 2.7 Hz), 7.38 (1H, m), 7.24 (1H, d, *J* = 5.1 Hz), 5.80 (1H, s), 5.71 (1H, s), 2.61 (1H, m), 1.34 (9H, s), 0.71 (1H, m), 0.42 (2H, m), 0.25 (1H, m).



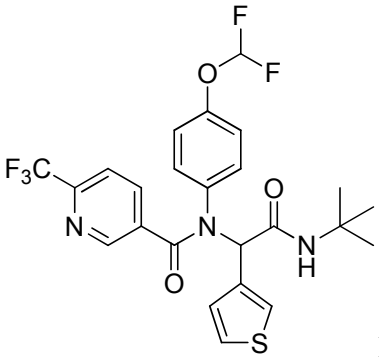
N-(tert-butyl)-2-(N-(4-(tert-butyl)phenyl)-2-(pyridin-3-yl)acetamido)-2-(thiophen-3-yl)acetamide, 2B-3d. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.60 (1H, d, *J* = 5.3 Hz), 8.43 (1H, s), 8.10 (1H, d, *J* = 8.1 Hz), 7.62 (1H, dd, *J* = 7.8, 5.4 Hz), 7.27 (3H, bs), 7.14 (2H, m), 6.74 (1H, dd, *J* = 4.6, 1.4 Hz), 5.91 (1H, s), 5.56 (1H, s), 3.56 (2H, s), 1.32 (9H, s), 1.29 (9H, s).



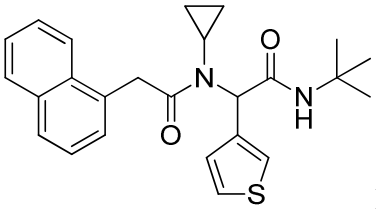
N-(tert-butyl)-2-(N-(4-fluorophenyl)-2-(1H-indol-3-yl)acetamido)-2-(thiophen-3-yl)acetamide, 2B-4b. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.99 (1H, s), 7.36 (1H, d, *J* = 7.9 Hz), 7.31 (1H, d, *J* = 8.1 Hz), 7.15 (2H, m), 7.10 (1H, m), 7.05 (1H, m), 6.95 (1H, m), 6.85 (2H, bs), 6.75 (1H, d, *J* = 4.9 Hz), 6.10 (1H, s), 5.84 (1H, bs), 3.57 (2H, s), 1.30 (9H, s).



N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(thiophen-3-yl)ethyl)-5,6,7,8-tetrahydronaphthalene-1-carboxamide, 2B-5d. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.31 (1H, d, *J* = 2.6 Hz), 7.17 (1H, dd, *J* = 4.9, 3.0 Hz), 7.08 (2H, d, *J* = 8.6 Hz), 7.00 (1H, s), 6.97(2H, m), 6.86 (2H, d, *J* = 8.2 Hz), 6.77 (1H, d, *J* = 7.8 Hz), 6.14 (1H, s), 6.08 (1H, s), 2.63 (2H, m), 2.52 (2H, m), 1.68 (4H, m), 1.37 (9H, s), 1.21 (9H, s).

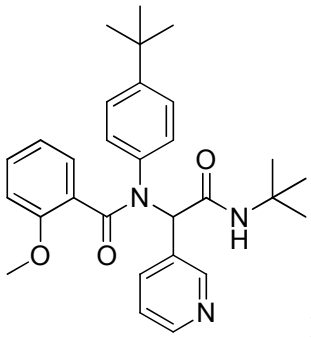


N-(2-(tert-butylamino)-2-oxo-1-(thiophen-3-yl)ethyl)-N-(4-(difluoromethoxy)phenyl)-6-(trifluoromethyl)nicotinamide, 2B-7e. ^{1}H NMR (400MHz, $CDCl_3$) δ 8.60 (1H, s), 7.78 (1H, d, *J* = 8.1 Hz), 7.49 (1H, d, *J* = 8.2 Hz), 7.21 (1H, m), 7.04 (2H, bs), 6.82 (3H, m), 6.39 (1H, t, *J* = 73.2 Hz), 6.17 (1H, s), 5.58 (1H, s), 1.37 (9H, s).

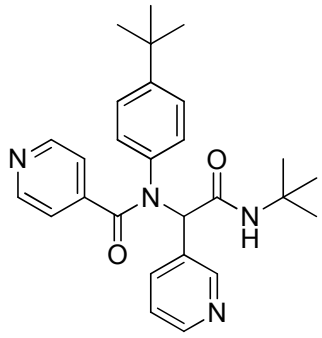


N-(tert-butyl)-2-(N-cyclopropyl-2-(naphthalen-1-yl)acetamido)-2-(thiophen-3-yl)acetamide, 2B-8a. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.79 (3H, m), 7.69 (1H, s), 7.46 (2H, m), 7.40 (2H, m), 7.24 (1H, m), 7.05 (1H, dd, *J* = 4.1, 0.8 Hz) 5.95 (1H, bs), 5.65 (1H, s), 4.14 (2H, s), 2.47 (1H, m), 1.31 (9H, s), 1.14 (1H, m), 0.86 (2H, m), 0.68 (1H, m).

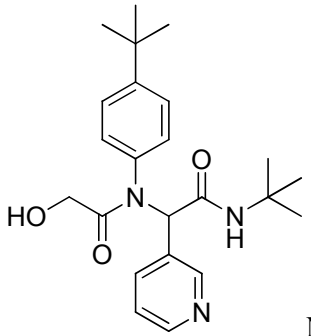
Characterization Data of Representative Compounds from Figure 8 (text) and Table 1 (text).



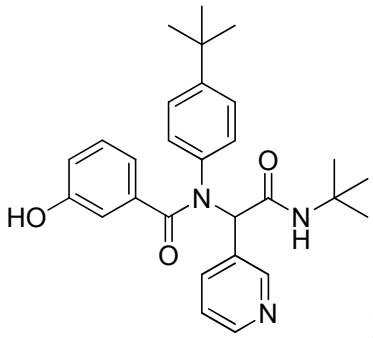
N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-2-methoxybenzamide, 33. ^{1}H NMR (400MHz, CD_3OD) δ 8.72 (1H, bs), 8.60 (1H, bs), 8.24 (1H, bs), 7.73 (2H, bs), 7.30 (1H, d, *J* = 7.4 Hz), 7.22 (1H, t, *J* = 7.4 Hz), 7.11 (2H, d, *J* = 8.4 Hz), 7.00 (2H, d, *J* = 8.3 Hz), 8.87 (1H, t, *J* = 7.4 Hz), 6.76 (1H, d, *J* = 8.2 Hz), 6.25 (1H, s), 3.68 (3H, s), 1.29 (9H, s), 1.15 (9H, s).



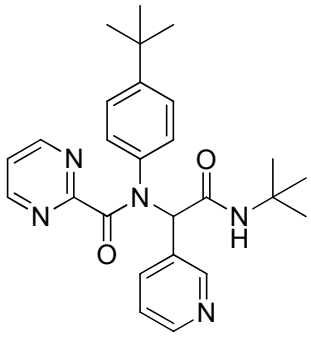
N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)isonicotinamide, 35. ^{1}H NMR (400MHz, CD_3OD) δ 8.57 (1H, s), 8.50 (3H, m), 7.98 (1H, m), 7.57 (2H, m), 7.51 (1H, m), 7.12 (4H, m), 6.28 (1H, s), 1.34 (9H, s), 1.14 (9H, s).



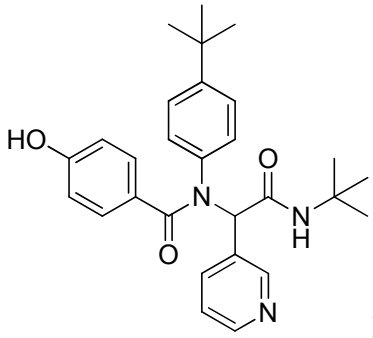
N-(tert-butyl)-2-(N-(4-(tert-butyl)phenyl)-2-hydroxyacetamido)-2-(pyridin-3-yl)acetamide, 40. ^{1}H NMR (400MHz, CD_3OD) δ 8.50 (2H, m), 7.99 (1H, d, *J* = 6.5 Hz), 7.55 (1H, m), 7.33 (2H, d, *J* = 9.1 Hz), 7.15 (2H, bs), 6.12 (1H, s), 3.83 (1H, d, *J* = 3.0 Hz), 1.29 (9H, s), 1.26 (9H, s).



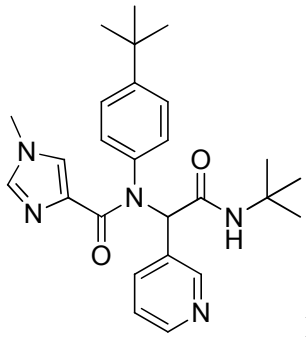
N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-3-hydroxybenzamide, 44. ^{1}H NMR (400MHz, CD_3OD) δ 8.63 (1H, bs), 8.56 (1H, bs), 8.16 (1H, bs), 7.66 (1H, m), 7.14 (2H, d, *J* = 8.8 Hz), 7.02 (2H, d, *J* = 8.1 Hz), 6.97 (1H, t, *J* = 7.9 Hz), 6.78 (1H, s), 6.74 (1H, d, *J* = 7.7 Hz), 6.66 (1H, dd, *J* = 8.2, 1.5 Hz), 6.26 (1H, s), 1.29 (9H, s), 1.17 (9H, s).



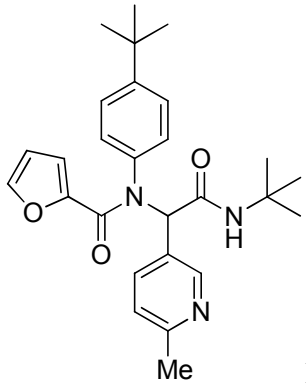
N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)pyrimidine-2-carboxamide, 45. ^{1}H NMR (400MHz, CD_3OD) δ 8.65 (1H, d, *J* = 11.8 Hz), 8.54 (1H, d, *J* = 5.2 Hz), 8.43 (1H, s), 8.38 (1H, s), 8.10 (1H, d, *J* = 6.8 Hz), 7.60 (1H, m), 7.13 (1H, d, *J* = 8.6 Hz), 7.06 (1H, d, *J* = 7.9 Hz), 6.32 (1H, s), 1.31 (9H, s), 1.15 (9H, s).



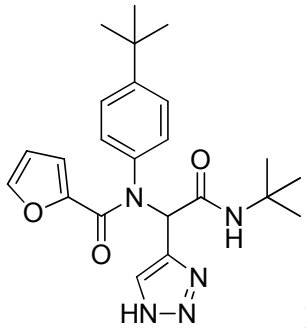
N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-4-hydroxybenzamide, 47. ^{1}H NMR (400MHz, CD_3OD) δ 8.61 (1H, s), 8.54 (1H, d, *J* = 5.0 Hz), 8.12 (1H, d, *J* = 6.4 Hz), 7.63 (1H, m), 7.19 (4H, m), 7.02 (2H, d, *J* = 8.2 Hz), 6.54 (2H, d, *J* = 8.7 Hz), 6.24 (1H, s), 1.29 (9H, s), 1.20 (9H, s).



N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)-1-methyl-1H-imidazole-4-carboxamide, 52. ^{1}H NMR (400MHz, CD_3OD) δ 8.81 (1H, s), 8.46 (1H, s), 8.43 (1H, d, *J* = 4.76 Hz), 7.78 (1H, d, *J* = 8.0 Hz), 7.37 (5H, m), 6.23 (1H, s), 5.44 (1H, s), 3.63 (3H, s), 1.33 (9H, s), 1.29 (9H, s).



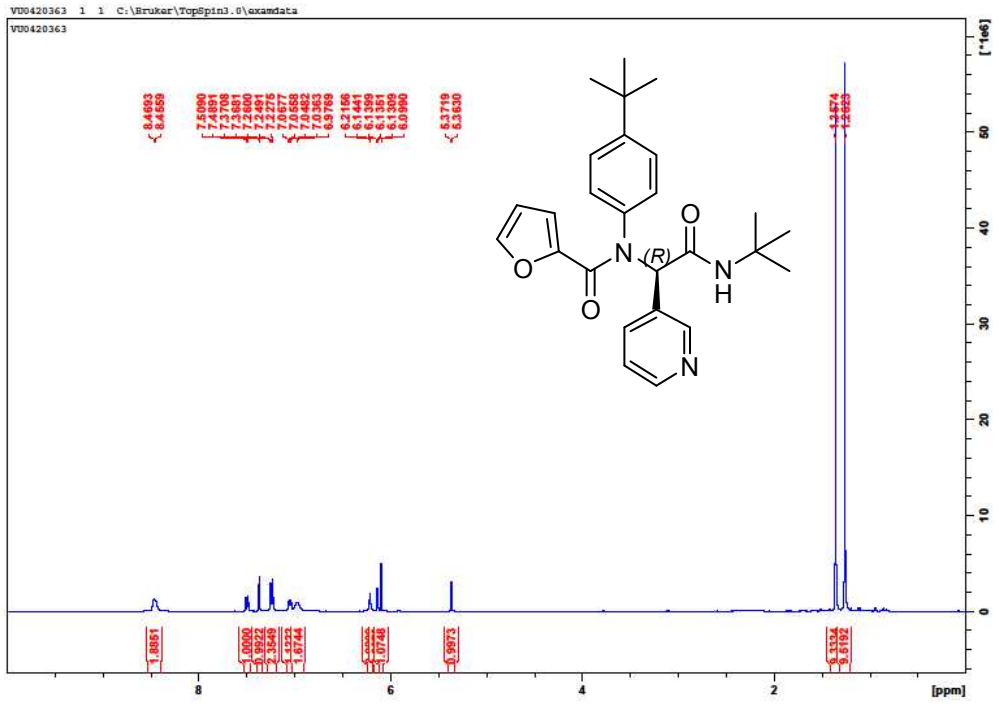
N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-1-(6-methylpyridin-3-yl)-2-oxoethyl)furan-2-carboxamide, 55. ^{1}H NMR (400MHz, CD_3OD) δ 8.56 (1H, s), 8.24 (1H, d, *J* = 8.3 Hz), 7.71 (1H, d, *J* = 8.3 Hz), 7.52 (1H, s), 7.41 (2H, d, *J* = 8.8 Hz), 7.22 (2H, m), 6.28 (1H, dd, *J* = 3.6, 3.3 Hz), 6.23 (1H, s), 5.62 (1H, d, *J* = 3.5 Hz), 2.69 (3H, s), 1.31 (9H, s), 1.27 (9H, s).

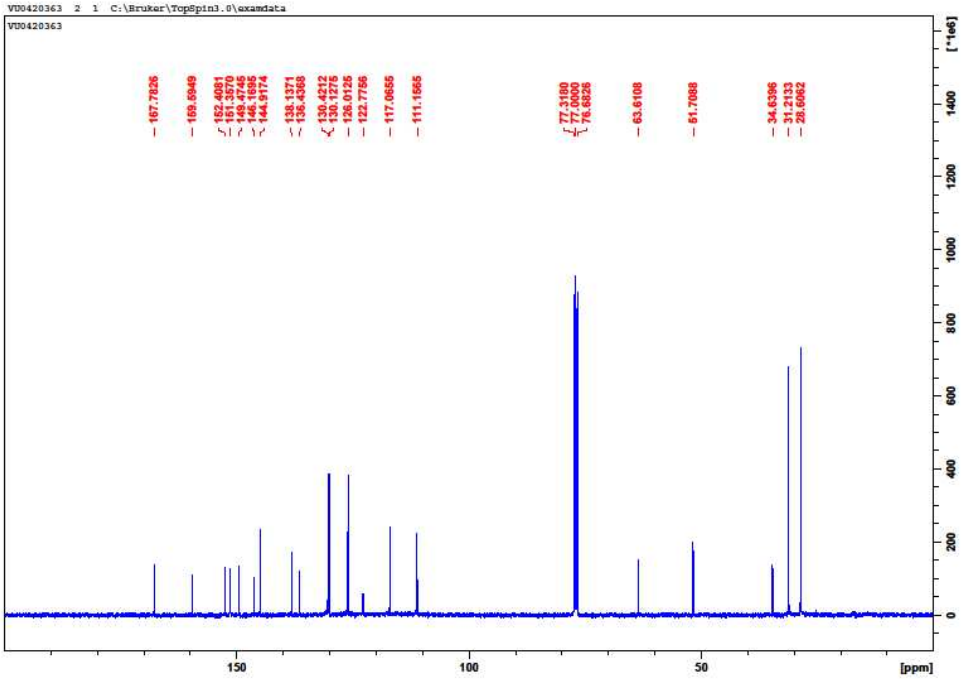


N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(1H-1,2,3-triazol-4-yl)ethyl)furan-2-carboxamide, 60. ^{1}H NMR (400MHz, CD_3OD) δ 7.52 (1H, d, *J* = 1.2 Hz), 7.44 (1H, s), 7.34 (2H, d, *J* = 8.6 Hz), 7.15 (2H, bs), 6.30 (1H, s), 6.25 (1H, dd, *J* = 3.6, 1.6 Hz), 5.46 (1H, d, *J* = 3.5 Hz), 4.58 (1H, s), 1.34 (9H, s), 1.29 (9H, s).

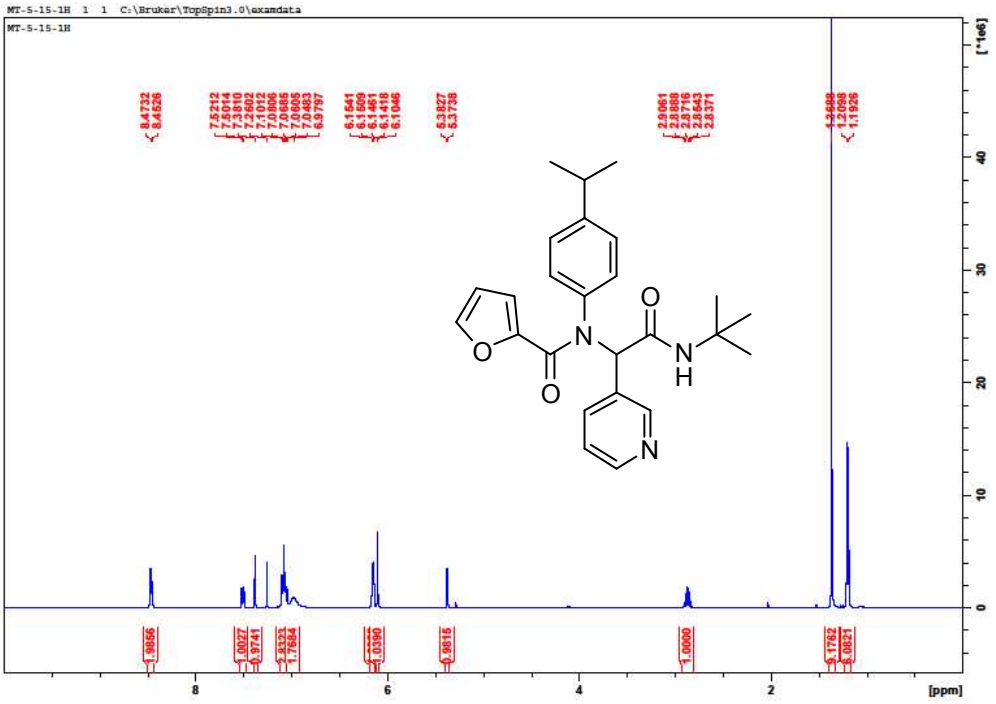
Representative ^{1}H and ^{13}C NMR Spectra.

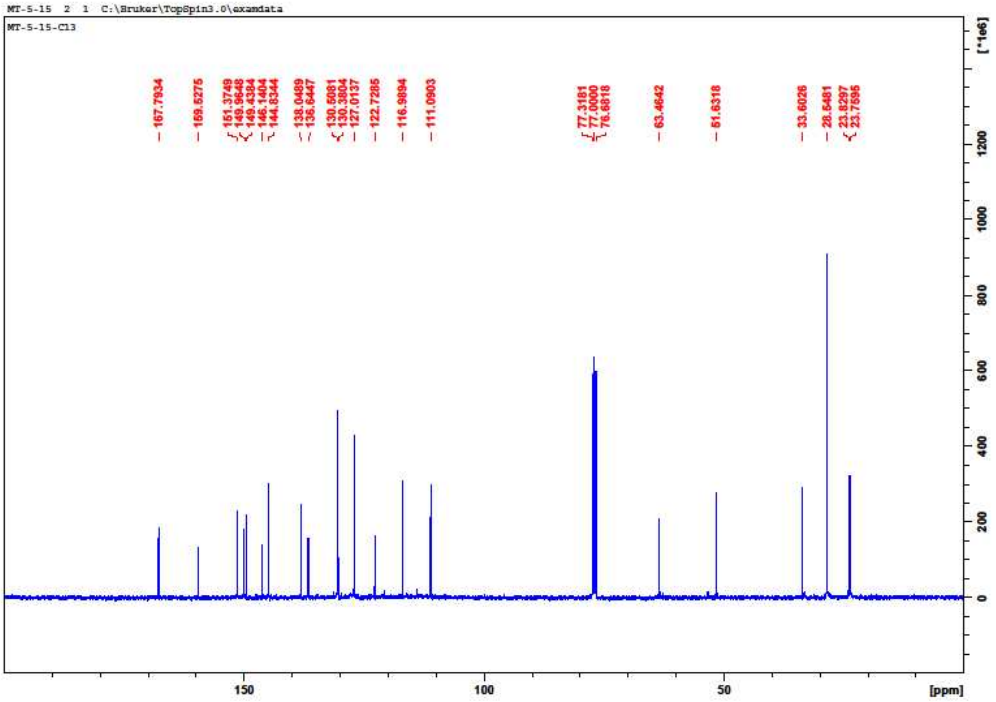
^{1}H and ^{13}C NMR spectra 16-(*R*) ML188.



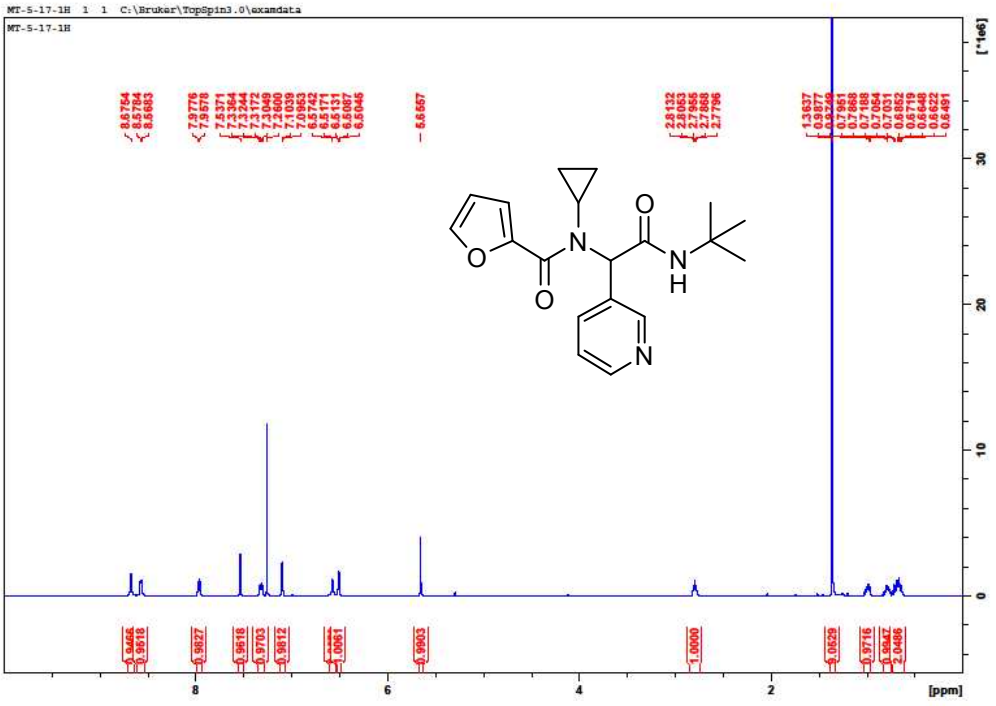


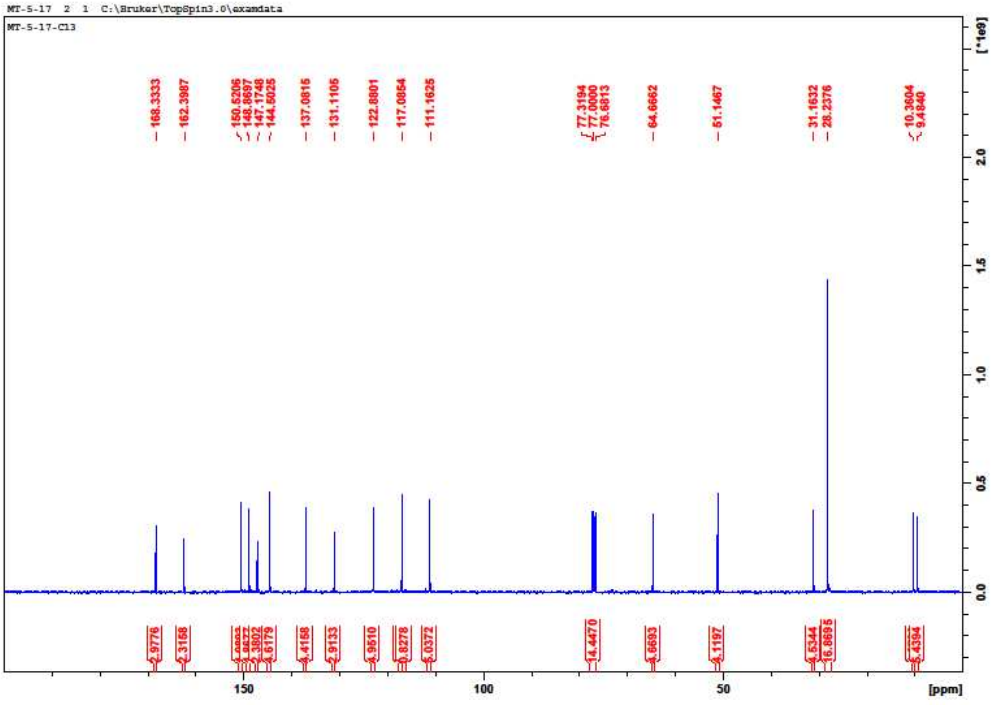
^{1}H and ^{13}C NMR spectra 17.



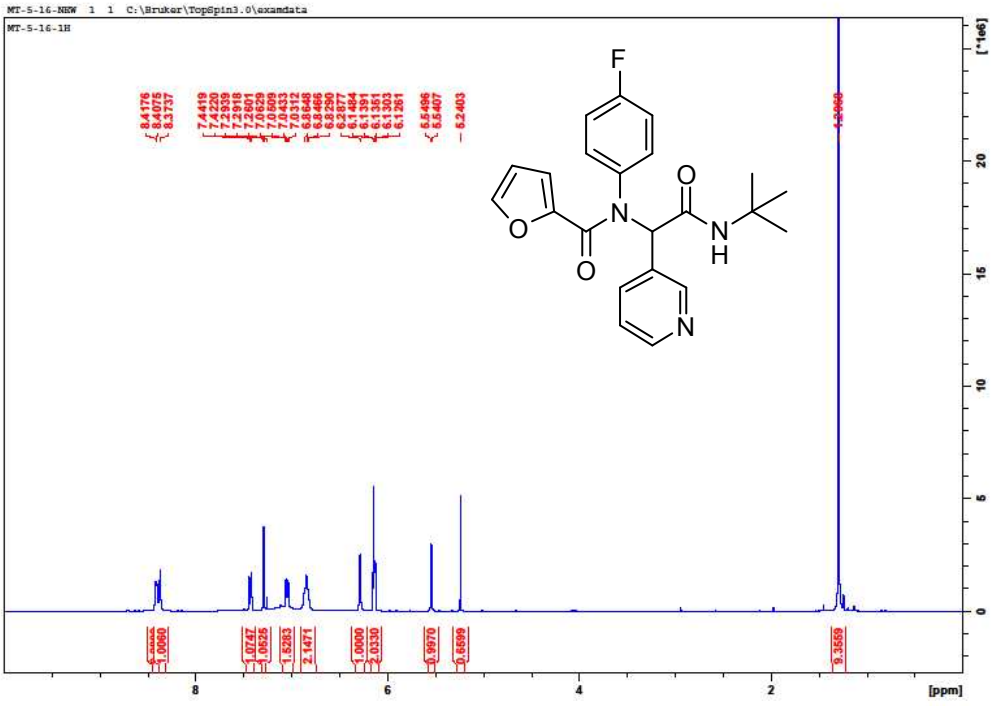


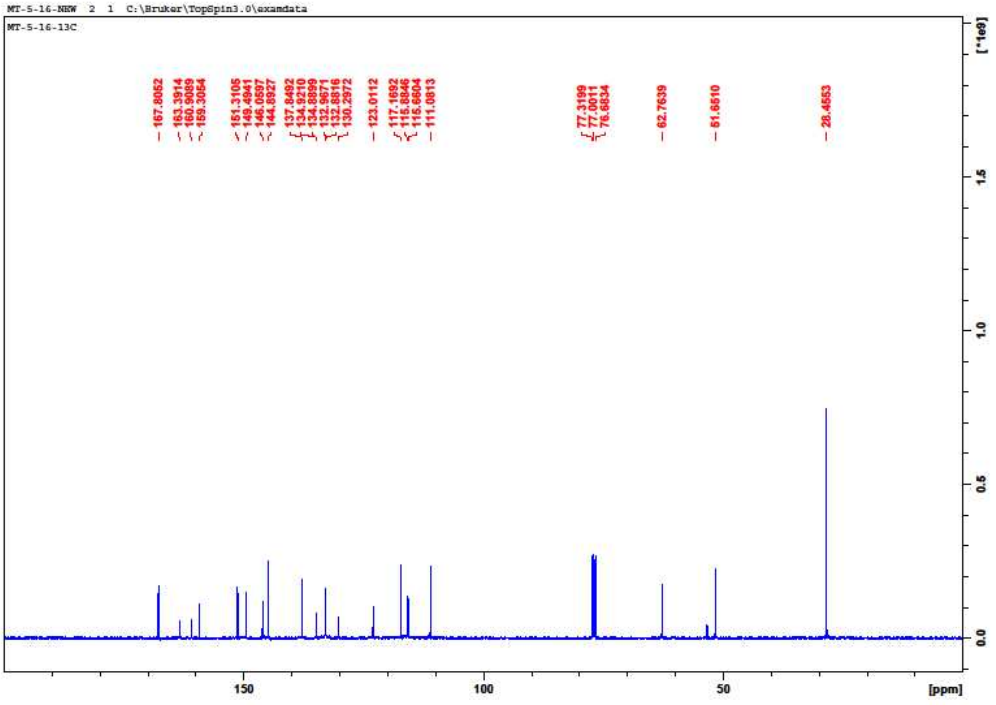
^{1}H and ^{13}C NMR spectra 18.



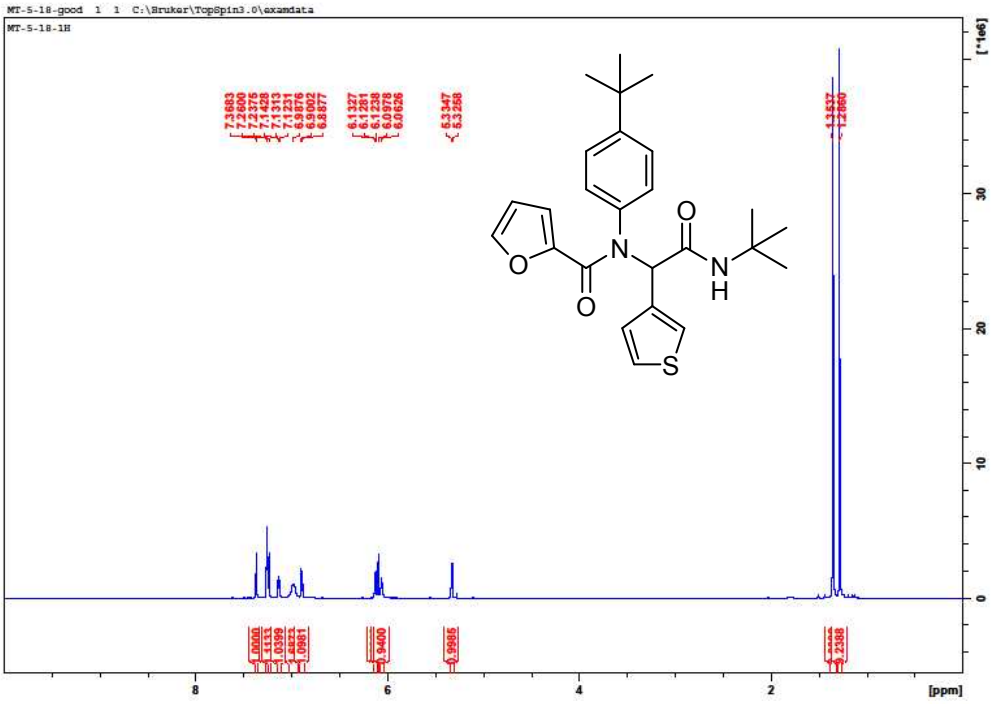


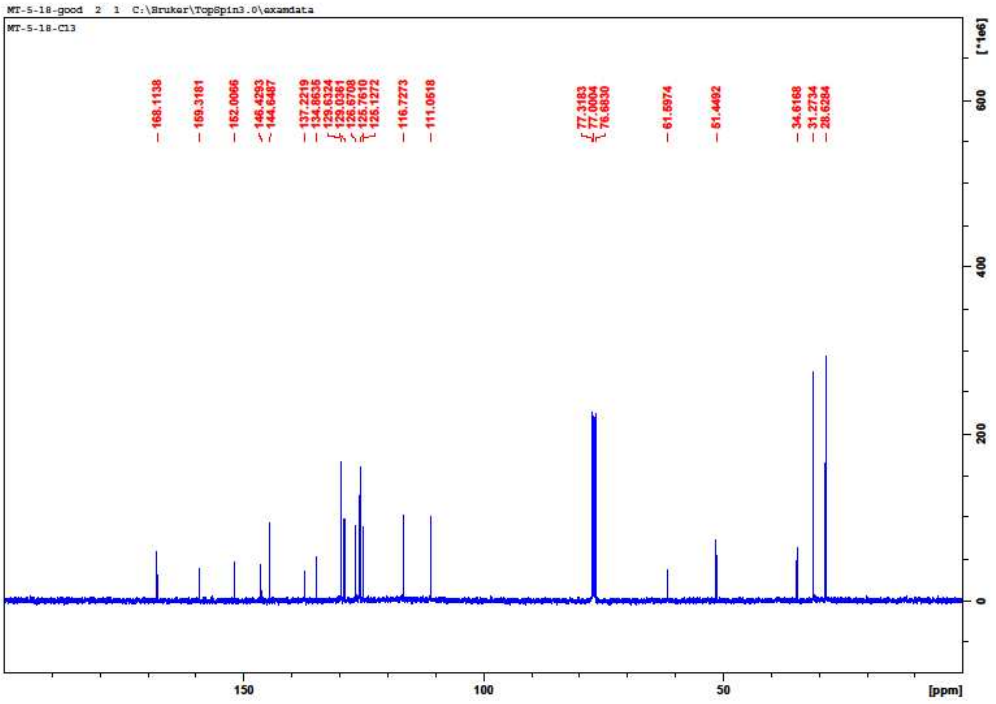
^{1}H and ^{13}C NMR spectra 19.



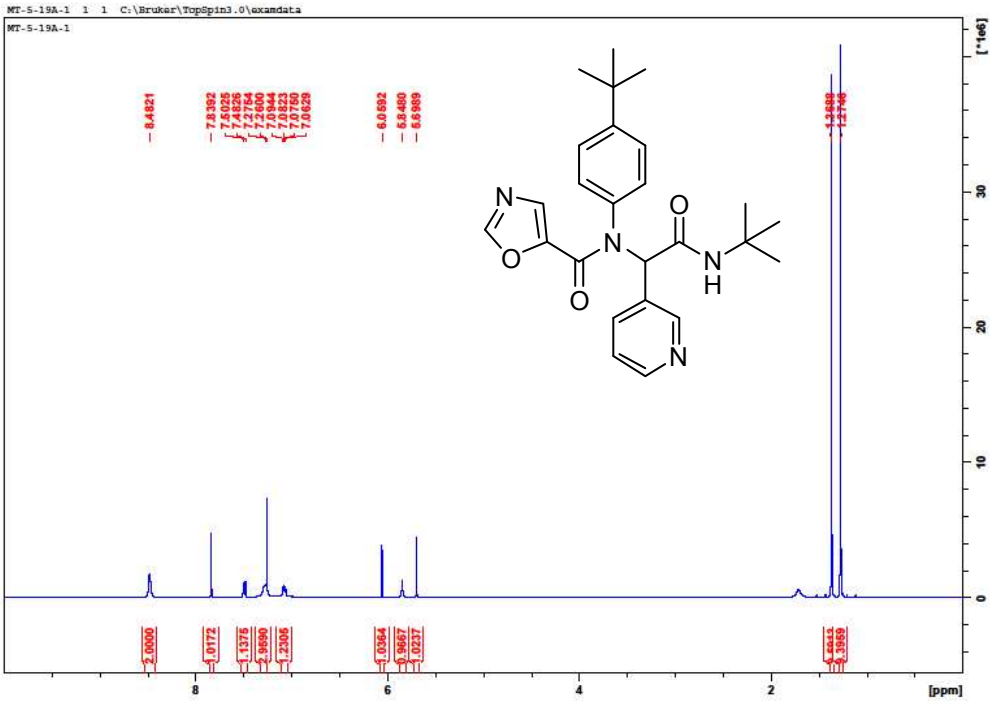


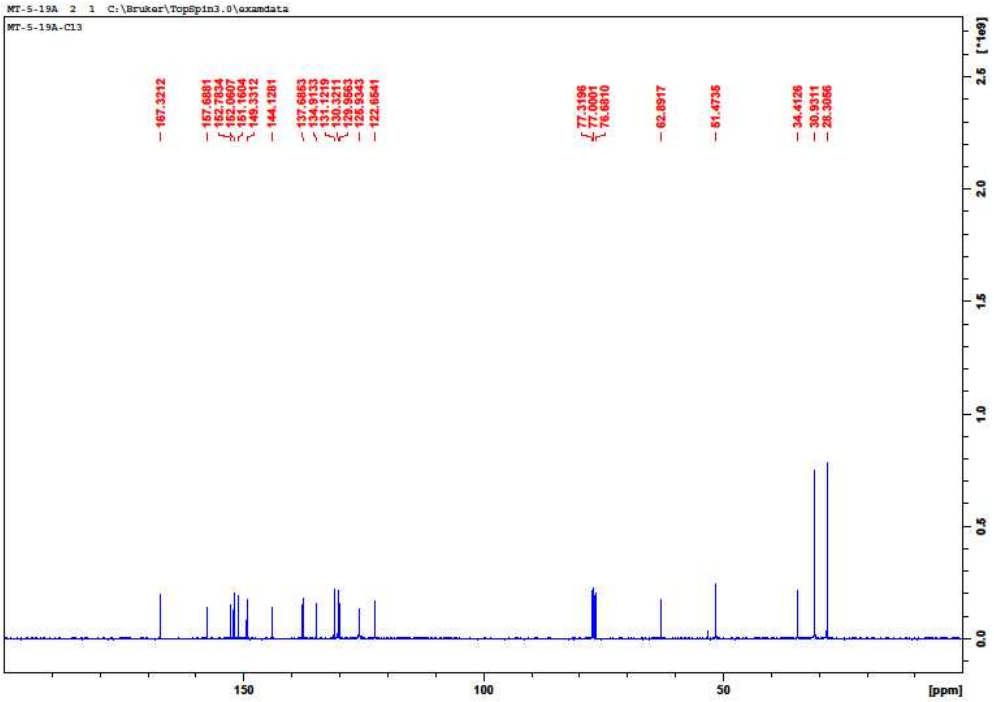
^{1}H and ^{13}C NMR spectra 20.



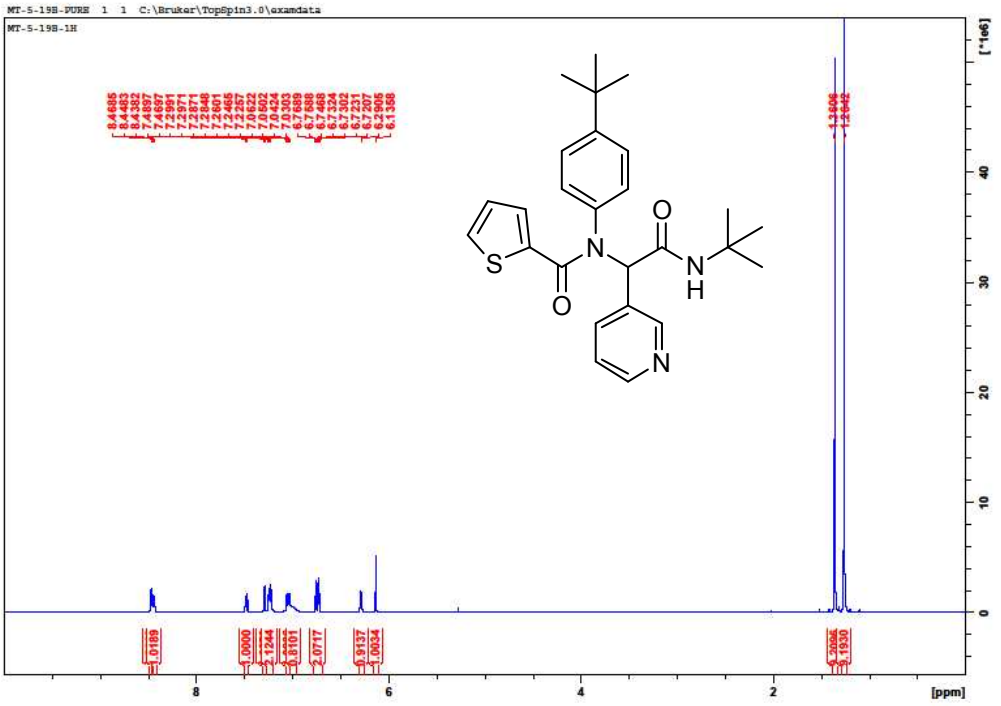


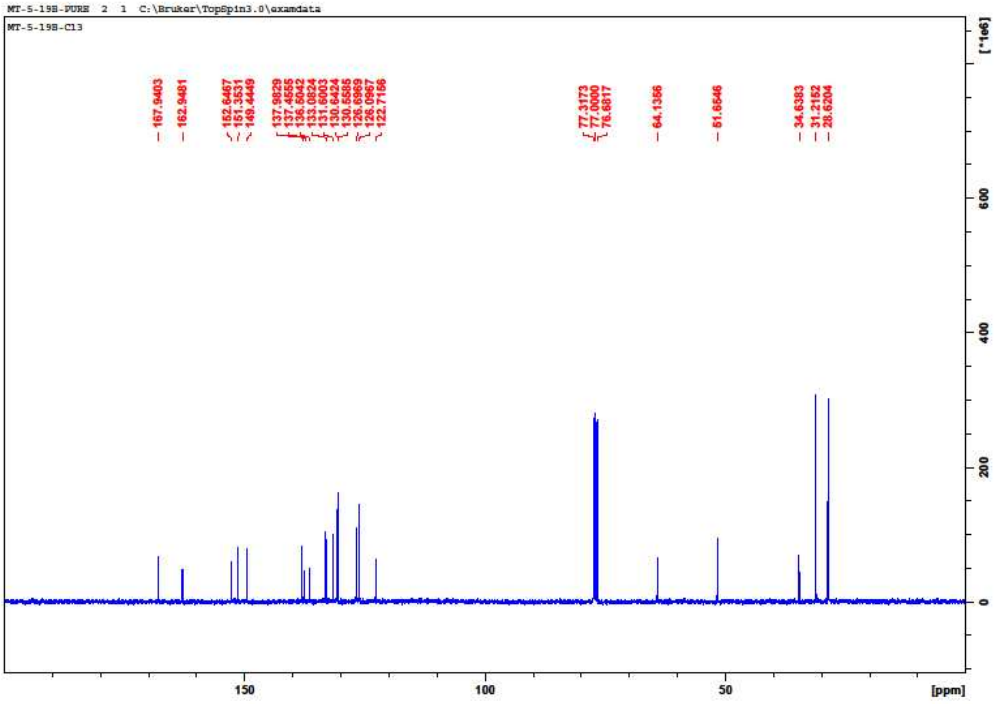
^{1}H and ^{13}C NMR spectra 22.



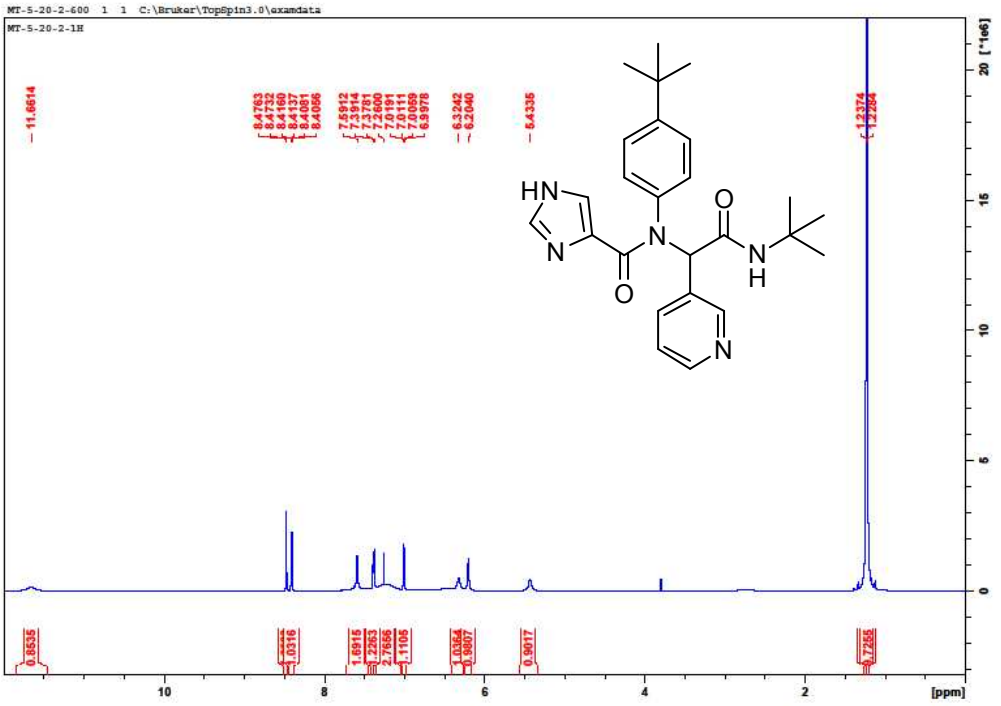


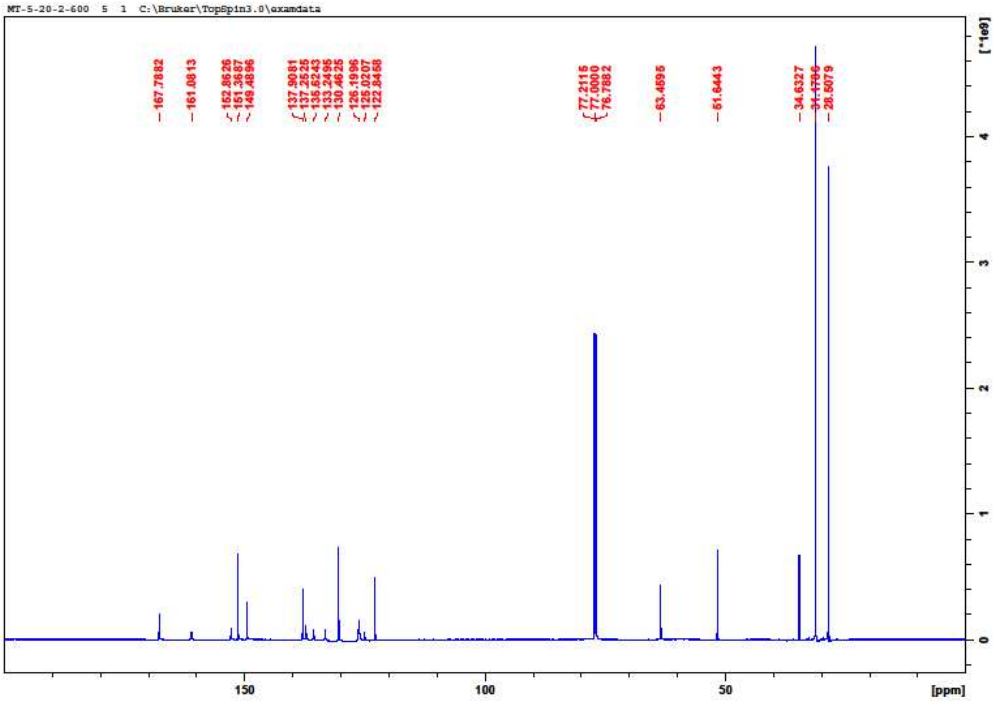
^{1}H and ^{13}C NMR spectra 27.



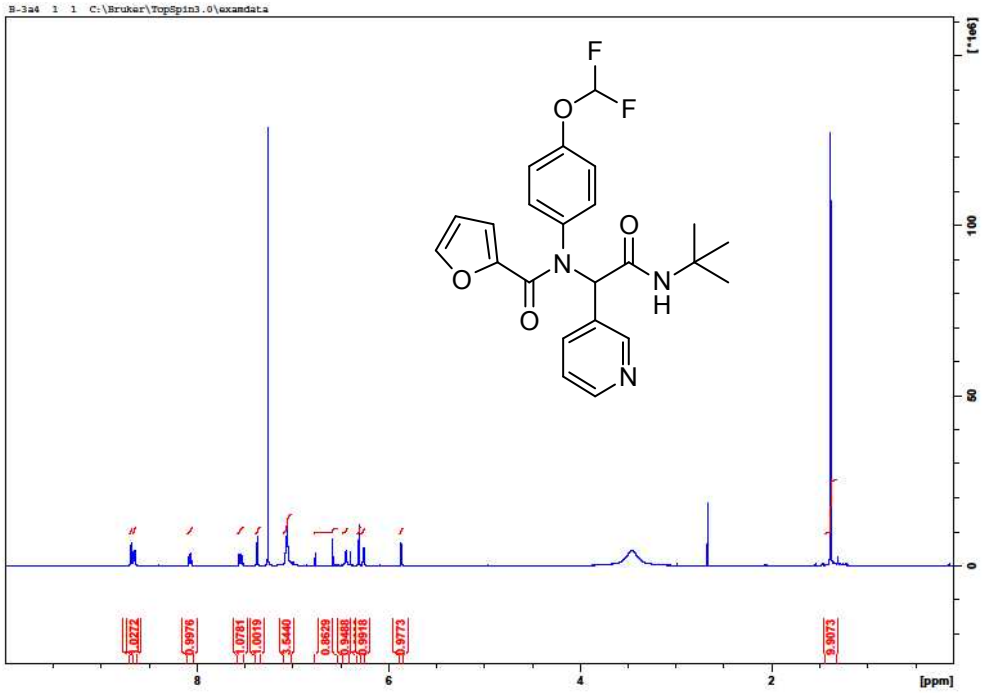


^{1}H and ^{13}C NMR spectra 31.

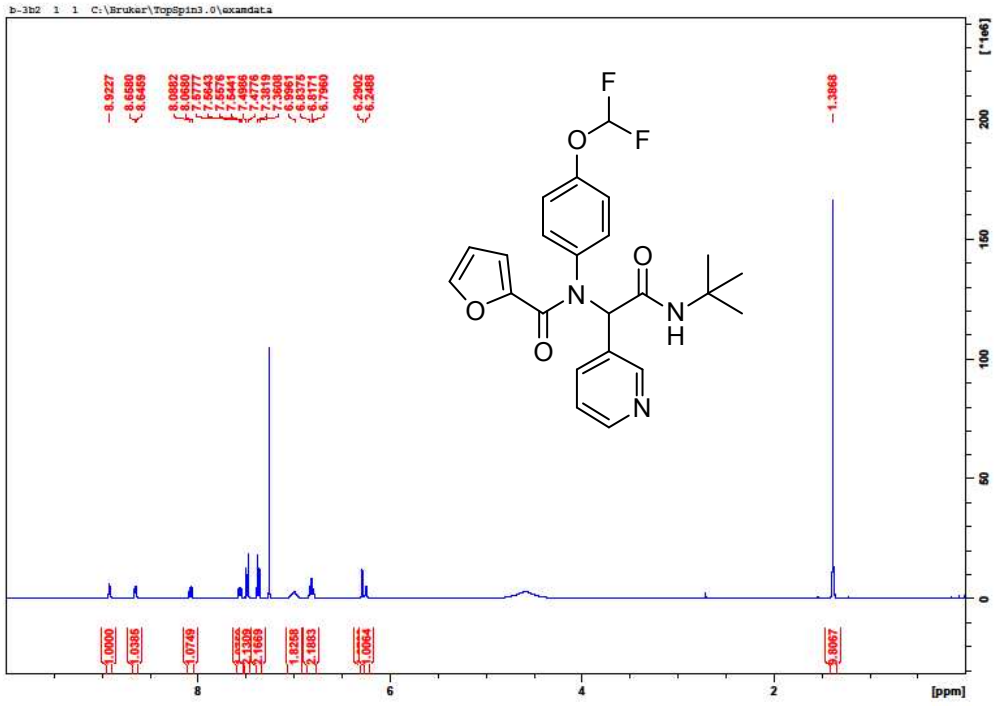




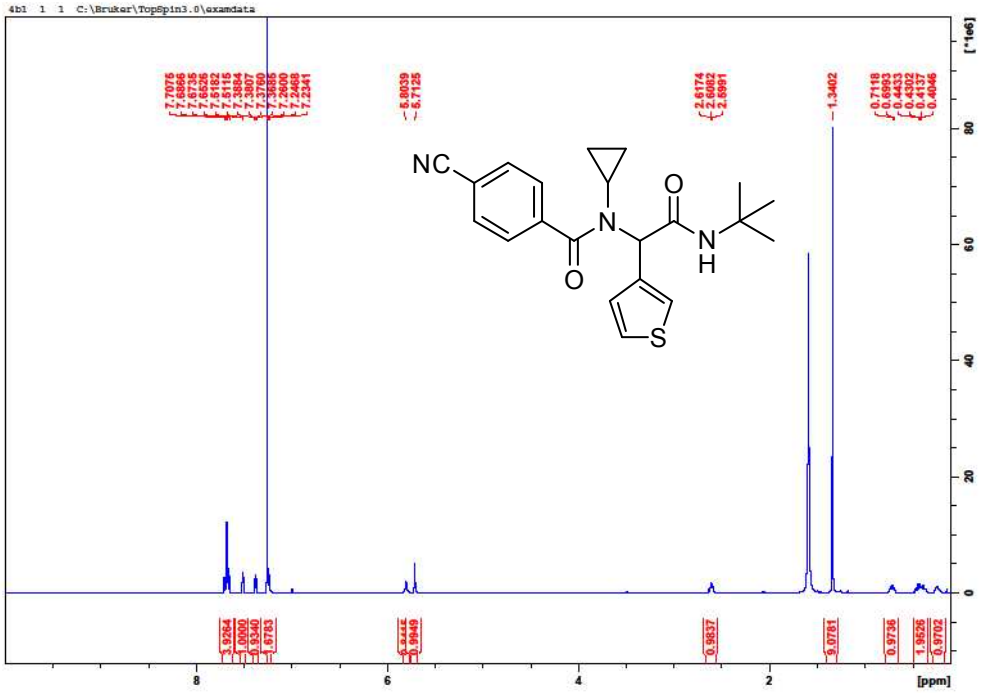
[1]H NMR spectra 2A-1e.



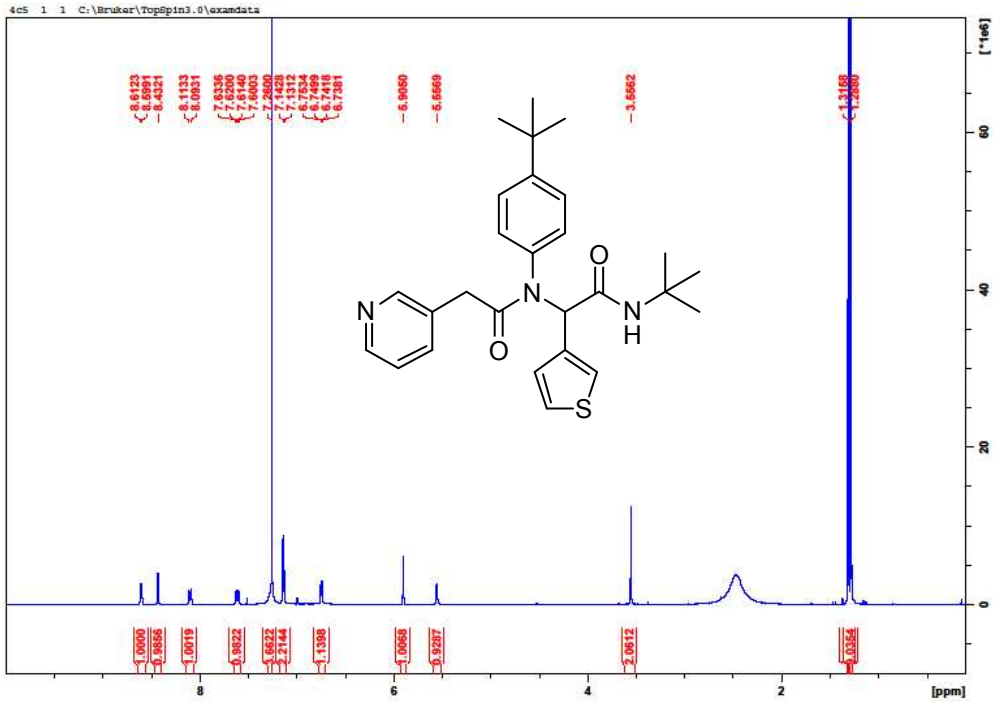
[1]H NMR spectra 2A-2b.



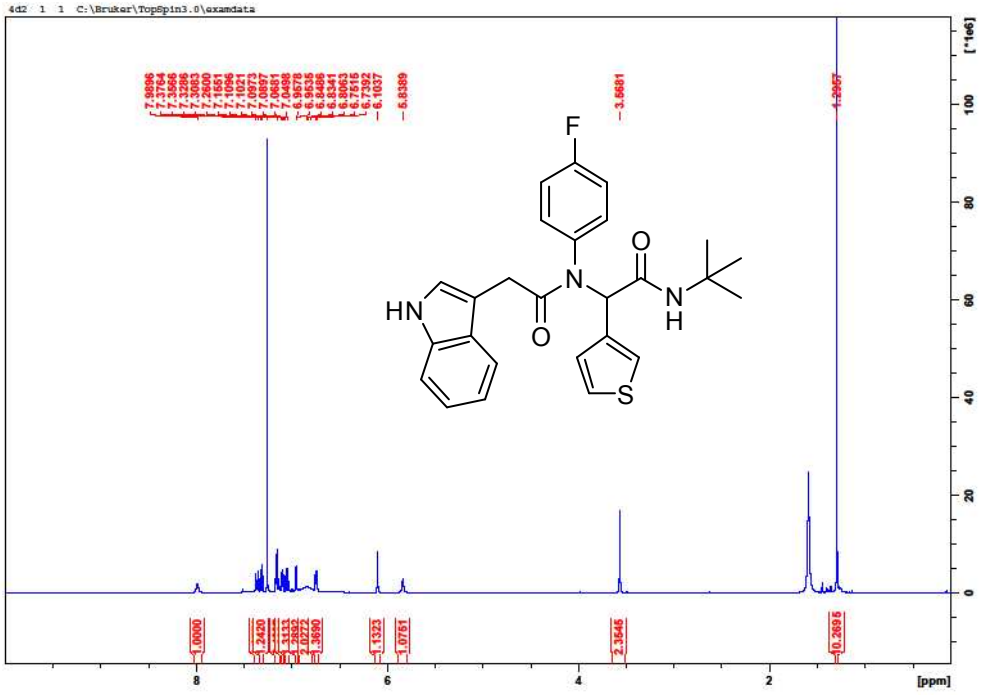
^{1}H NMR spectra 2B-2a.



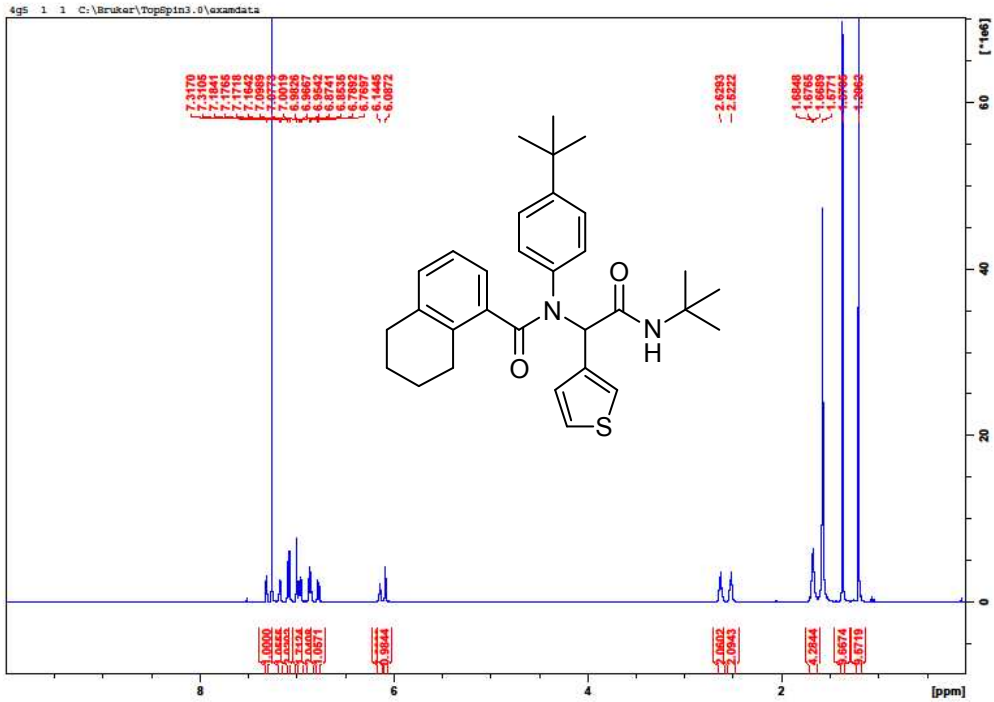
^{1}H NMR spectra 2B-3d.



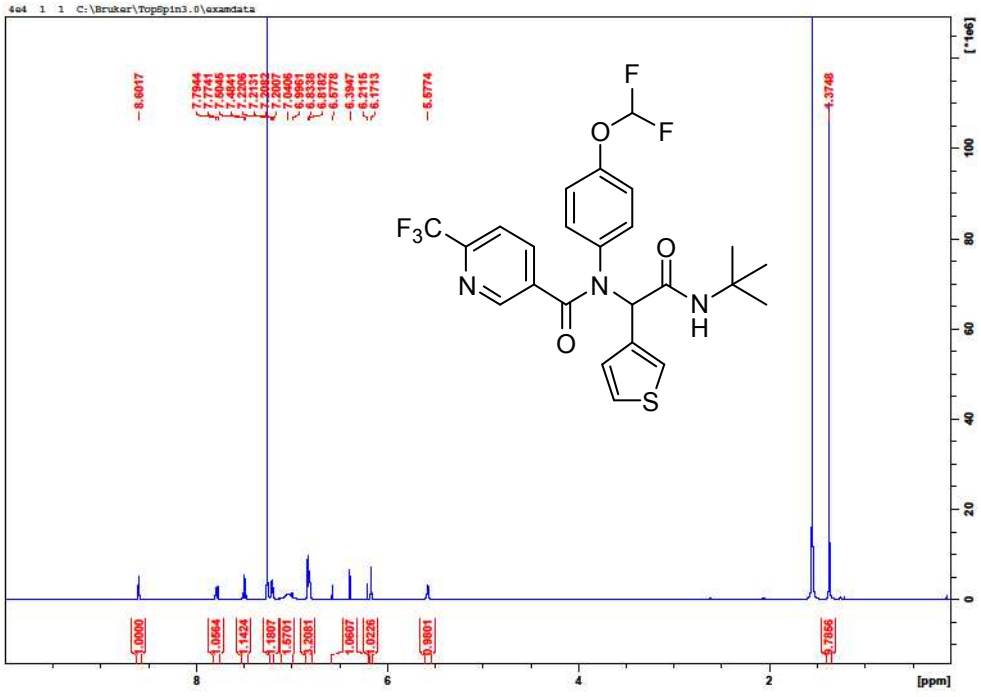
^{1}H NMR spectra 2B-4b.



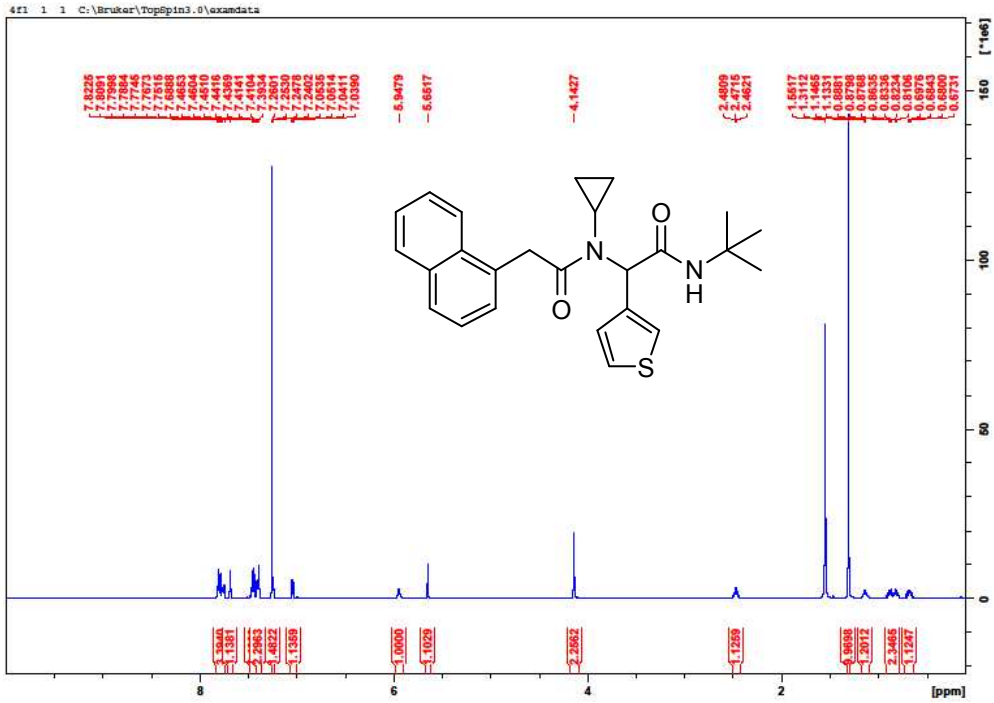
^{1}H NMR spectra 2B-5d.



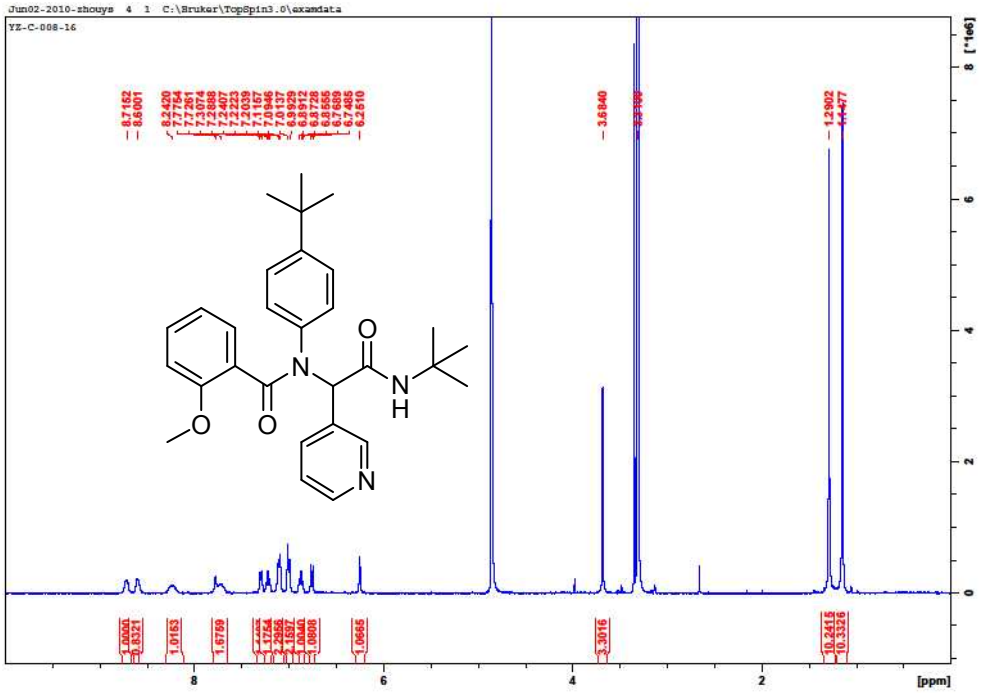
^{1}H NMR spectra 2B-7e.



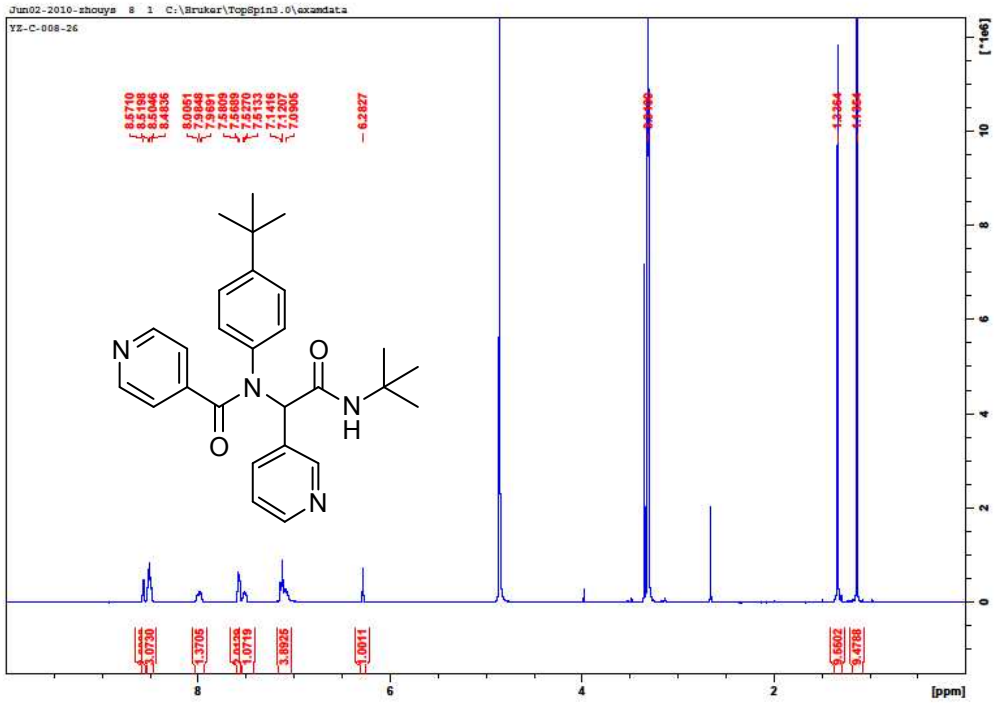
^{1}H NMR spectra 2B-8a.



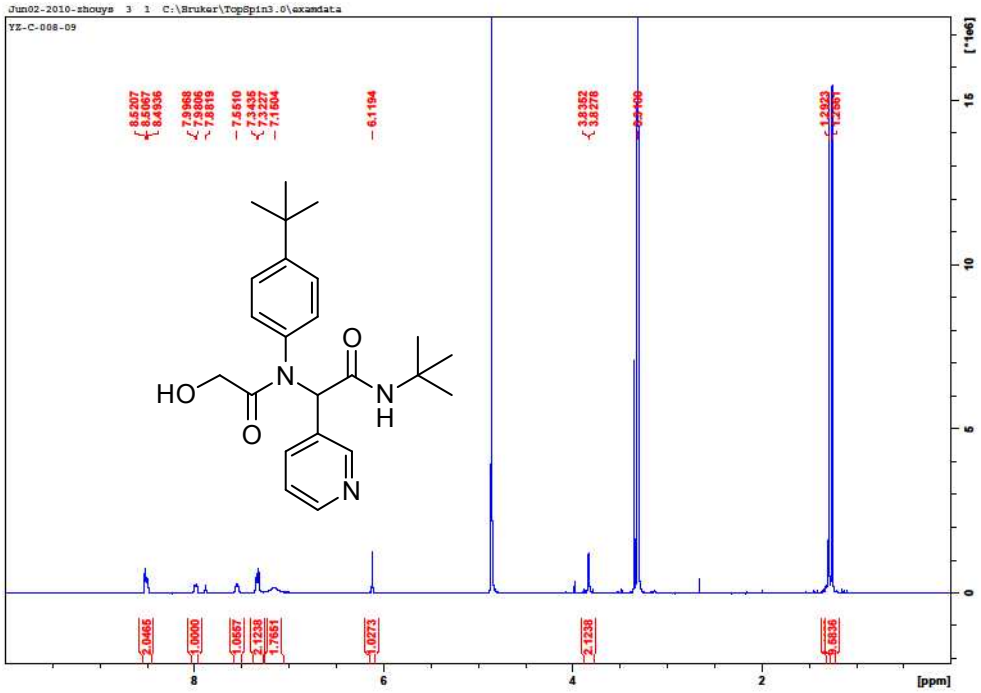
[1]H NMR spectra 33.



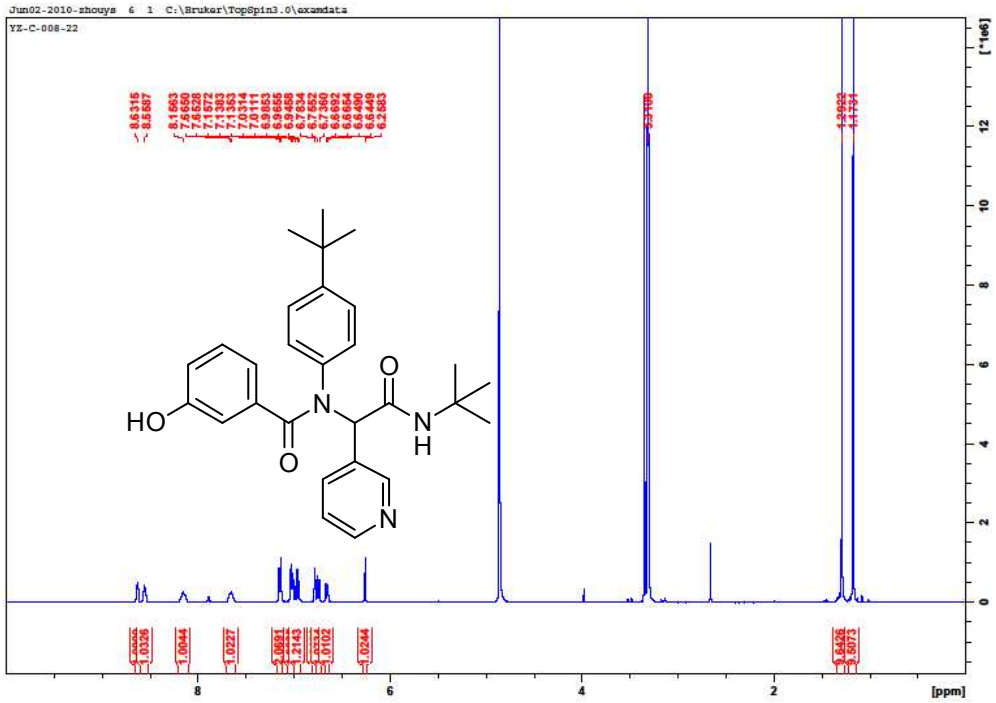
[1]H NMR spectra 35.



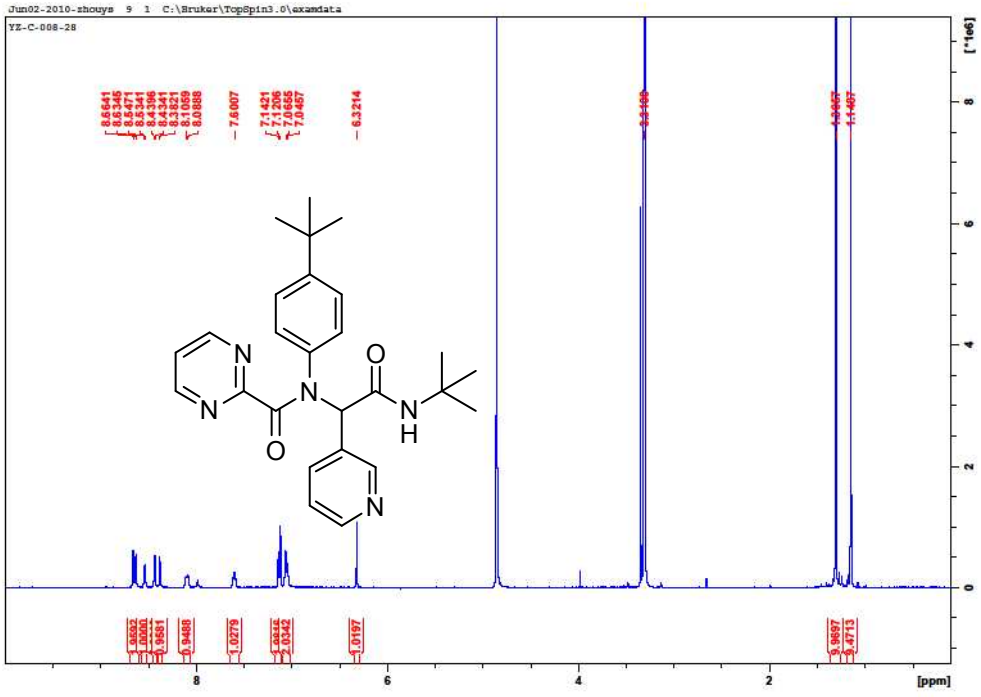
^{1}H NMR spectra 40.



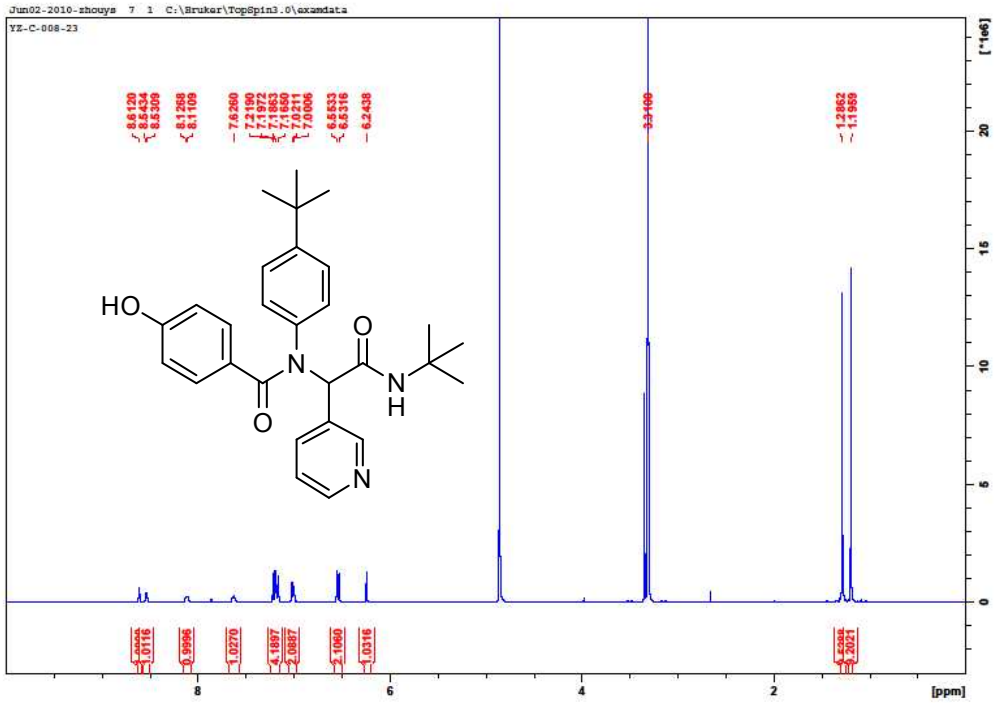
^{1}H NMR spectra 44.



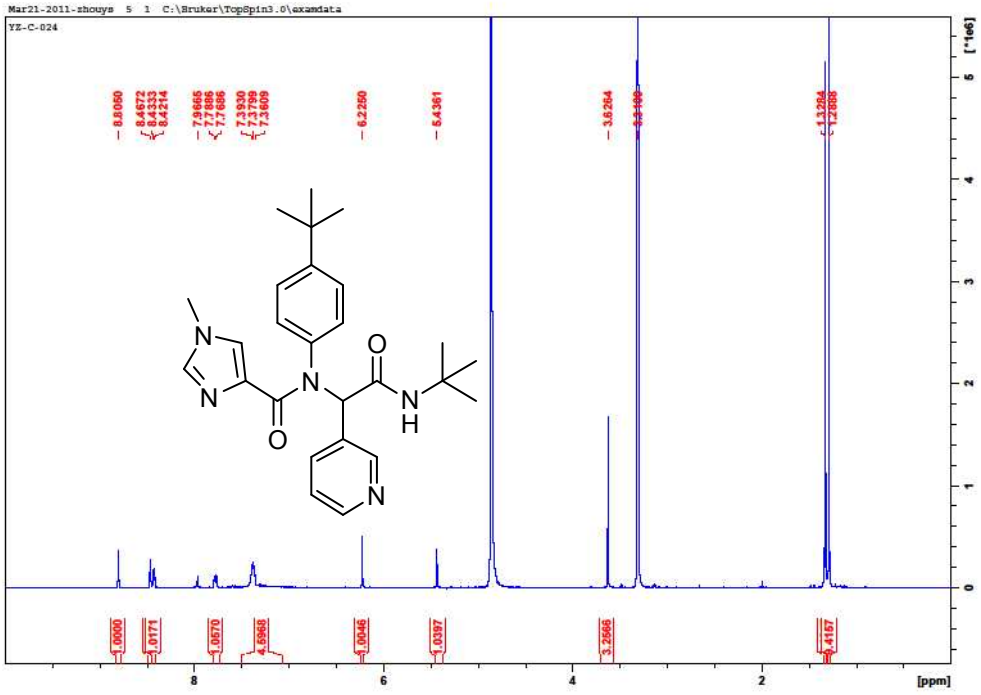
^{1}H NMR spectra 45.



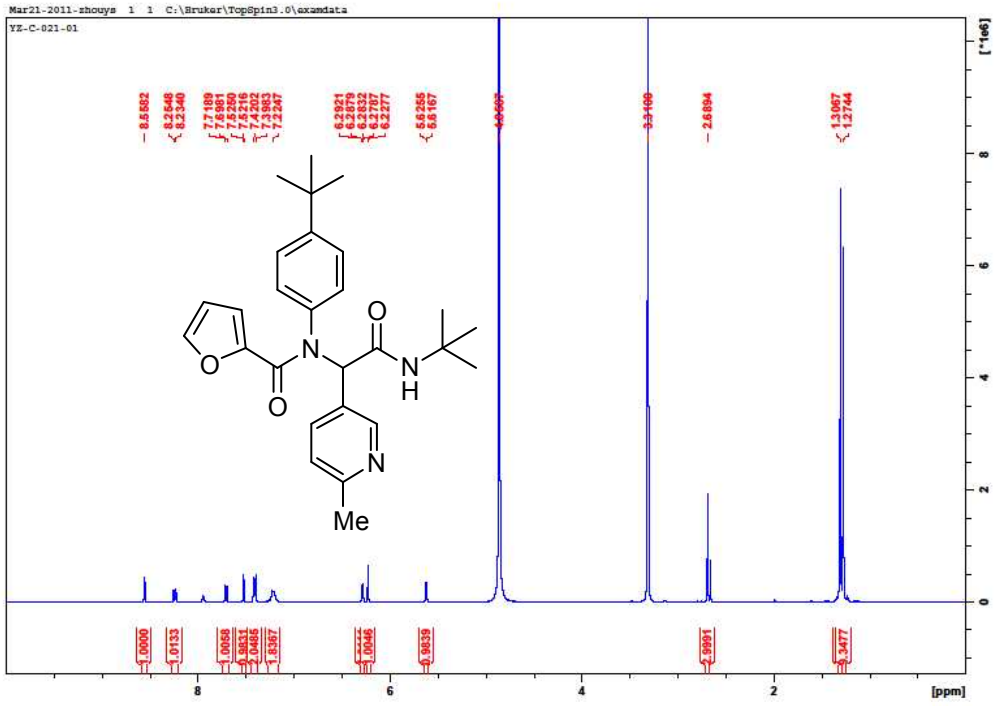
^{1}H NMR spectra 47.



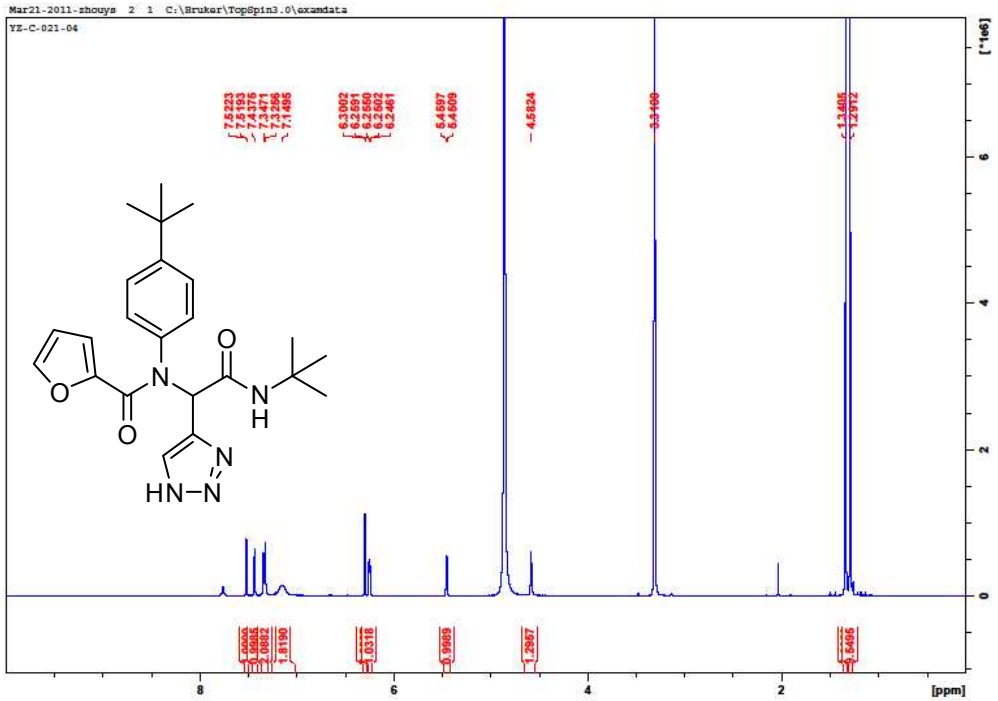
^{1}H NMR spectra 52.



^{1}H NMR spectra 55.



^{1}H NMR spectra 60.



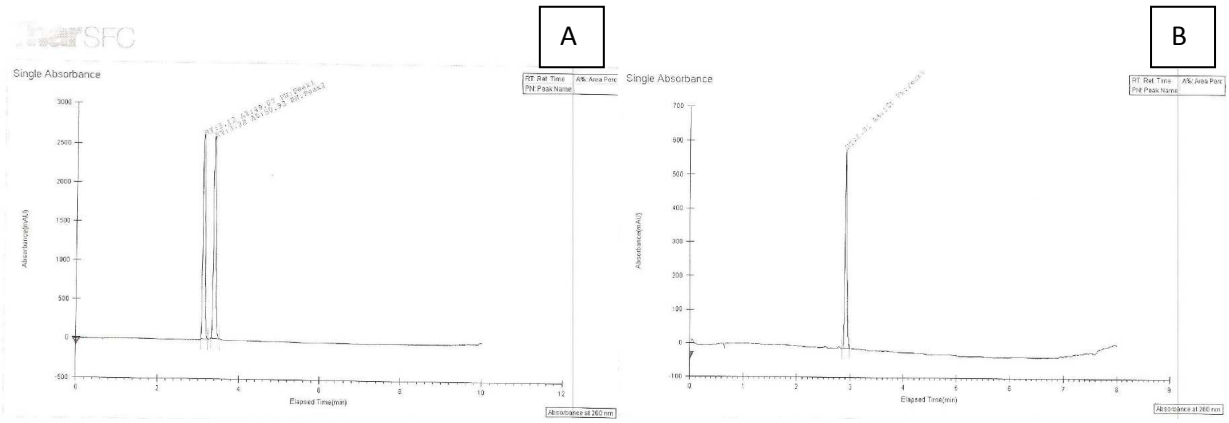


Figure 1. Analytical SFC chromatogram of racemic mixture **3** (left panel A) and purified eluting isomer/peak A retention time = 2.91 min- (*R*)-N-(4-(tert-butyl)phenyl)-N-(2-(tert-butylamino)-2-oxo-1-(pyridin-3-yl)ethyl)furan-2-carboxamide (**ML188**) (right panel B).

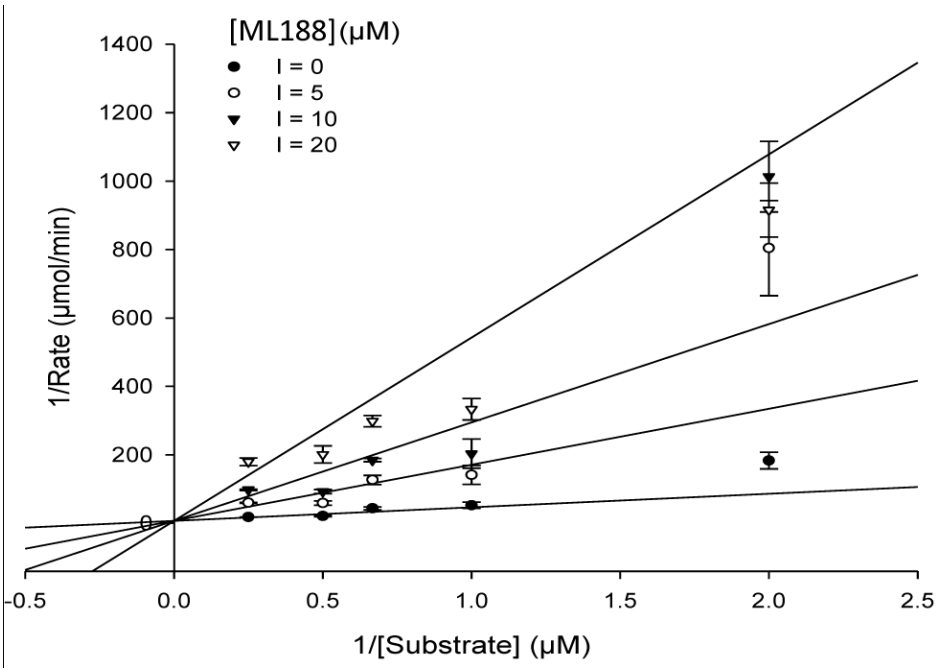


Figure 2. Inhibition of SARS 3CLpro by **ML188**. The initial rate of the SARS 3CLpro catalyzed reaction as a function of substrate concentration at fixed, variable concentrations of ML188 was determined in triplicate and plotted in double-reciprocal format. The error bars represent the standard deviation of the triplicate rate data. The data were fit to the equation describing full competitive inhibition and the resulting Ki value was determined to be 1.6 ± 0.26 uM.

Table 4. Data Collection and Refinement Statistics for SARS-CoV in Complex with ML-188

Data Collection Parameters	
Crystal Conditions	Flash-Cooled at 100 ^{o}K
X-ray Source and Detector	LS-CAT 21-ID-G, Rayonix-300
Wavelength (Å)	0.979
Resolution Limit (Å)	1.96
Space Group	C2
Unit Cell Dimensions (Å) a, b, c	106.73, 82.67, 53.12, β=106.0^{o}
Data Processing Statistics	**Overall (Last Shell)**
Data Resolution Range (Å)	50.0 – 1.95 Å (1.98 – 1.95]
Reflections	
Total Recorded (n)	426,716
Averaged (n)	31,309

Completeness (%)[a]	97.8(88.8)
Average Redundancy	7.2
R_{merge} (%)	6.4 (17.0)
Average I / σ I	53.5 (7.0)
Refinement Statistics	**Overall (Last Shell)**
Data Resolution Range (Å)	50.0 – 1.96 Å (2.00 – 1.95)
Reflections in Working Set (n)	29,719 (1,946)
Reflections in Test Set (n)	1,588 (92)
R_{cryst} (%)[b]	18.7(20.9)
R_{free} (%)[c]	23.5(24.7)
Figure of merit (FOM)[d]	0.85
RMS Deviations Bond length (Å)	0.012
RMS Deviation Bond angles (^{o})	1.39
Estimated overall coordinate error based on Maximum Liklihood (Å)	0.098
Molecules in Final Model (#)	**Average B-factor (Chain A) (Å^2)**
Protein Chains (1)	35
Inhibitor **ML-188** (1)	45
DMSO (2)	59, 86
Solvent H_2O (467)	< 80.0[e]

[a] Completeness for $I/\sigma(I) > 1.0$.
[b] $R_{work} = \sum ||F_o|-|F_c|| / \sum |F_o|$
[c] R_{free} was calculated against 5% of the reflections removed at random.
[d] Figure of Merit = ($|\sum P(\alpha)e^{i\alpha} / \sum P(\alpha)|$), where α is the phase and $P(\alpha)$ is the phase probability distribution.
[e] Cutoff criteria on solvent B-factors.