nature synthesis

Article

https://doi.org/10.1038/s44160-023-00351-1

Miniaturization of popular reactions from the medicinal chemists' toolbox for ultrahigh-throughput experimentation

In the format provided by the authors and unedited

Content

1. General Information 2
   1.1. Materials, Reagents and Analytical 2
   1.2. High-Throughput Experimentation 3
2. Substrate Synthesis 6
3. Nanomole-Scale Suzuki Cross-Coupling 12x10 Informer Library Screen 10
4. Automation-friendly Suzuki Coupling Using 3-Boronopyridine 13
5. Nanomole-Scale Reductive Amination with Staurosporine 17
   5.1. Nanomole-Scale Staurosporine Reductive Amination Optimization 17
   5.2. Nanomole-Scale Staurosporine Reductive Amination Library Synthesis 20
   5.3. General Procedure for Reductive Amination on Staurosporine and Scale Up: 23
   5.4. Derivatization of Drug Molecules 27
6. Nanomole-Scale Amide *N*-Alkylation and Boc-Deprotection 29
   6.1. Amide *N*-Alkylation Base Optimization 29
   6.2. Amide *N*-Alkylation Electrophile Study 30
   6.3. Boc Deprotection Optimization 30
   6.4. Nanomole-Scale *N*-Alkylation/Deprotection Library Synthesis 32
   6.5. *N*-Alkylation/Deprotection Library General Procedure and Scale Up 33
7. NMR Spectra 42
8. References 75

1. General Information

1.1. Materials, Reagents and Analytical

All reagents used in this disclosure were purchased from commercial suppliers (when possible) and used as-is. Reagents and intermediates that were not commercially available were prepared as described in this manuscript or prepared according to published procedures. Anhydrous solvents were purchased from Sigma-Aldrich or Acros, in sealed bottles and were either degassed before use or moved to a nitrogen atmosphere glove box and used without further purification. Reactions, screens, and libraries were typically set up in an MBraun glove box under an N_2 atmosphere (oxygen and water levels usually < 1 ppm). Scaled up bench-scale reactions were set up in the glove box in 1-dram vials, then sealed and moved to a fume hood and stirred for 16 to 24 hours. Products were purified (when necessary) using silica gel column chromatography (Isco RediSep® normal-phase chromatography) or by preparative-scale reverse-phase HPLC.

Product isolation from a reaction mixture by preparative-scale reverse-phase HPLC was carried out using an Agilent 1200 HPLC-MSD system consisting of a 6130B single quadrupole mass-selective detector (MSD), G1315B diode array detector (DAD), G2258A autosampler, two G1361A preparative pumps, one G1379A quaternary pump with degasser, one G1312A binary pump, and three G1364B fraction collectors from Agilent Technologies (Agilent Technologies, Palo Alto, CA). System control and data analysis was performed using Agilent's ChemStation software, revision B.03.01-SR.1. A Waters SunFire C18 OBD Prep Column, 100Å, 5 µm, 19 mm X 150 mm column was used as the stationary phase (Waters Corporation, Milford, MA, USA). Gradient elution was carried out using water with 0.1% trifluoroacetic acid (solvent A) and acetonitrile with 0.1% trifluoroacetic acid (solvent B) as a mobile phase. Purification under basic conditions employed water with 0.1% NH_4OAc (solvent A) and acetonitrile with 0.1% NH_4OAc (solvent B) as the mobile phase. Electrospray (ESI) mass-triggered fraction collection was employed using positive ion polarity scanning to monitor for the target mass. Products with basic functionality that were purified under acidic conditions were assumed to be TFA salts when calculating yield.

Time (min)	% MeCN (solvent B)	Flow rate (mL/min)
0.0	2	25
3.0	2	35
33.0	95	35
33.1	100	40
36.1	100	50
36.8/end	2	25

NMR spectra were acquired on a Bruker Avance 600 MHz spectrometer equipped with a sample changer. Chemical shifts are reported as parts per million (ppm) downfield from tetramethylsilane and referenced to solvent (e.g. DMSO-d_6). NMR data are represented as follows: chemical shift, multiplicity (s = singlet, br s = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet, etc.), coupling constant, and integration. Coupling constants (*J*) are given in Hertz (Hz). For products isolated as TFA salts the ^{13}C NMR signals for trifluoroacetate are sometimes, but not always visible in product ^{13}C NMR spectra. High-resolution mass spectrometry for characterization and affinity-based ranking were acquired using a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap high-resolution, accurate-mass (HRAM) Mass Spectrometer. A Waters ACQUITY QDa mass detector was used (ESI positive ionization mode, looking for $[M+H]^+$) for product detection in high-throughput LC-MS reaction screening (details below).

1.2. High-Throughput Experimentation

For nano-scale HTE all reactions were prepared at the 1 µL scale (0.1 M, 100 nmol) or 1.2 µL scale (0.1 M, 120 nmol) in a 1,536-well microplate using the SPT Labtech Mosquito®HTS liquid handling robot inside an N_2 atmosphere glove box. In all cases, a 384-well source plate was created containing all required substrates, reagents, catalysts, and building blocks (Greiner polypropylene deepwell 384-well plate, cat. no. 784201). The SPT LabTech Mosquito® HTS was used to dispense from the source plate into the 1,536-well reaction plate (Analytical Sales and Services, 20 µL polypropylene plate, cat. no. 15020), mixing upon dispense, 3×750 nL. After dispensing the reaction plate was sealed, centrifuged, and allowed to sit at ambient temperature in the glove box for 18 to 24 hours. The next morning the reactions were quenched by the addition of 3×1 µL 0.5 M AcOH in DMSO (containing PhPh internal standard) to each reaction well, mixing upon dispense. After the nano-scale reactions were quenched, 1 µL of each quenched reaction was transferred, using the Mosquito®, from the 1,536-well reaction plate to a 384-well analytical plate (Greiner polypropylene deepwell 384-well plate, cat. no. 784201) and diluted with 60 µL DMSO. The plate was sealed, shaken, then analyzed by LC-MS. For screens where the total number of reactions was greater than 384, multiple 384-well analytical plates were prepared. For example, a 1,536 reaction screen requires four 384-well analytical plates. Images are shown below of key components of this miniaturized synthesis and screening platform for reference. For a detailed step-by-step reaction setup guide to nanomole-scale HTE using the SPT Labtech Mosquito®, including images, see the preceding publication (Santanilla, A. B. et al. *Science* **2015**, *347*, 49–53).[1]

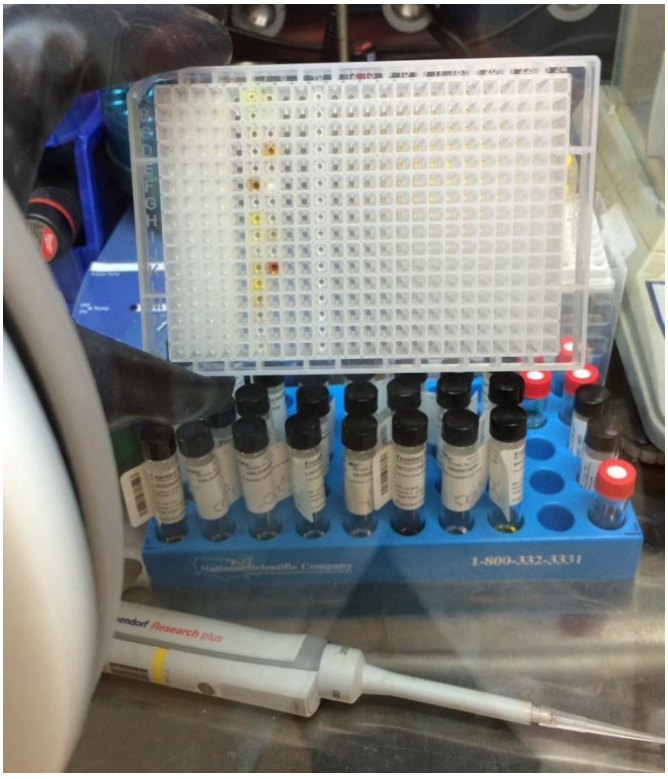


Figure S1. A source plate containing reagent stock solutions. Stock solutions were dispensed into a Greiner polypropylene deepwell 384-well plate in a predetermined order for reaction assembly using Mosquito® HTS

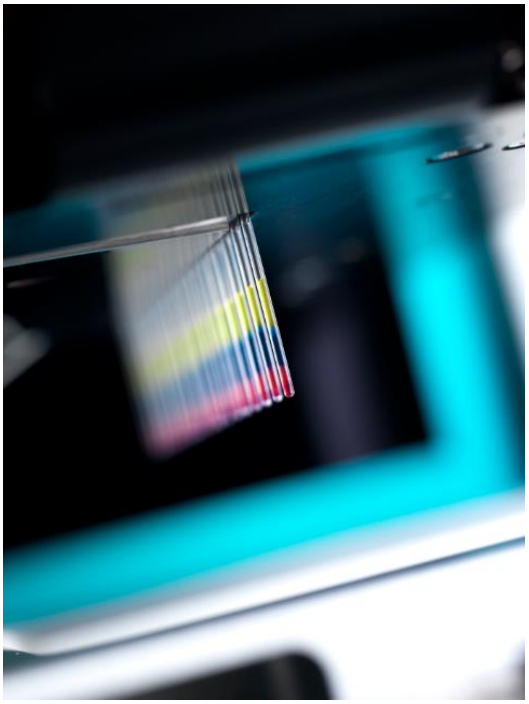


Figure S2. SPT Labtech Mosquito® HTS liquid handling robot, equipped with 1200 nL tips, utilizing the multi-aspiration feature to prepare reactions from reagent stock solutions (Image credit: SPT Labtech).

High-throughput analytical reaction screening was performed with a Waters *I*-class ACQUITY UPLC-MS (Waters Corporation, Milford, MA, USA) equipped with in-line photodiode array detector (PDA) and QDa mass detector (ESI positive ionization mode). One µL sample injections were taken from 384-well plates (samples diluted in DMSO, 1 µL quenched reaction into 60 µL DMSO). A partial loop injection mode was used with the needle placement at 1.0 mm from bottom of the wells and a 0.2 µL air gap at pre-aspiration and post-aspiration. Column used: Waters Cortecs UPLC C18+ column, 2.1mm x 50 mm with (Waters #186007114) with Waters Cortecs UPLC C18+ VanGuard Pre-column 2.1mm x 5 mm (Waters #186007125), Mobile Phase A: 0.1 % formic acid in Optima LC/MS-grade water, Mobile Phase B: 0.1% formic acid in Optima LC/MS-grade MeCN. Flow rate: 1 mL/min. Column temperature: 45 ˚C. The PDA sampling rate was 20 points/sec. The QDa detector monitored *m/z* 150-750 with a scan time of 0.06 seconds and a cone voltage of 30 V. 1 minute and 2 minute methods were used when possible, but the method was tailored for improved resolution when necessary. Sample method gradients:

1 minute method.

Time (min)	% MeCN (solvent B)	Flow rate (mL/min)
0.0	5	1
0.75	100	1
0.98	100	1
0.99/end	5	1

2 minute method.

Time (min)	% MeCN (solvent B)	Flow rate (mL/min)
0.0	5	1
1.5	100	1
1.96	100	1
1.98/end	5	1

Sample custom method.

Time (min)	% MeCN (solvent B)	Flow rate (mL/min)
0.0	5	1
0.55	40	1
1.02	100	1
1.35	100	1
1.37/end	5	1

After LC-MS data acquisition, sample data were processed using Virscidian Analytical Studio software (version 4.8). Product UV peak area (254 nm) was used to judge reaction efficiency (relative to remaining starting material, relative to an internal standard, or by comparison to a standard curve). This wavelength was chosen to minimize overlap of the DMSO solvent from with any compound peaks with a small retention time.

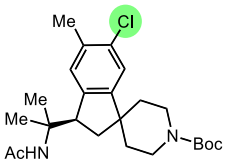
All heat maps, scatter plots, bar charts, and other visualizations presented herein were compiled and constructed in python (version 3.9.7) using matplotlib (version 3.4.3).

All raw high throughput data can be accessed at https://github.com/cernaklab/medchem-reaction-miniaturization/tree/main.

2. Substrate Synthesis

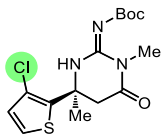
The synthesis for substrates **11**–**22**, **36**, **54** and **55** are either previously reported[2–13] or are commercially available as indicated. Since this study, many of the Informer Halides used are now commercially available from MilliporeSigma and the part numbers are given below.

(R)-tert-butyl 3-(2-acetamidopropan-2-yl)-6-chloro-5-methyl-2,3-dihydrospiro[indene-1,4'-piperidine]-1'-carboxylate (11)



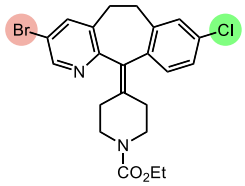
(R)-tert-butyl 3-(2-acetamidopropan-2-yl)-6-chloro-5-methyl-2,3-dihydrospiro[indene-1,4'-piperidine]-1'-carboxylate was prepared according to the published procedure from the literature.[2] Use of this core in a chemistry informer library was demonstrated by Kutchukian and Dreher.[14] The compound is now commercially available from MilliporeSigma (#901782).

(S,Z)-tert-butyl (4-(3-chlorothiophen-2-yl)-1,4-dimethyl-6-oxotetrahydropyrimidin-2(1H)-ylidene)carbamate (12)



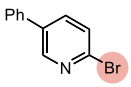
(S,Z)-tert-butyl (4-(3-chlorothiophen-2-yl)-1,4-dimethyl-6-oxotetrahydropyrimidin-2(1H)-ylidene)carbamate was prepared according to the published procedure from the literature.[3]

Ethyl 4-(3-bromo-8-chloro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11(6H)-ylidene)piperidine-1-carboxylate (13)



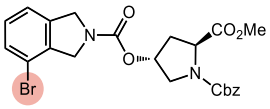
Ethyl 4-(3-bromo-8-chloro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11(6H)-ylidene)piperidine-1-carboxylate was prepared according to published procedures from the literature.[4] Use of this core in a chemistry informer library was demonstrated by Kutchukian and Dreher.[14] The compound is now commercially available from MilliporeSigma (#901729).

2-bromo-5-phenylpyridine (14)



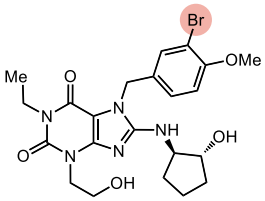
2-Bromo-5-phenylpyridine is commercially available and was purchased from an external vendor.

(2S,4R)-1-benzyl 2-methyl 4-((4-bromoisoindoline-2-carbonyl)oxy)pyrrolidine-1,2-dicarboxylate (15)



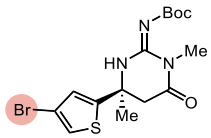
(2S,4R)-1-benzyl 2-methyl 4-((4-bromoisoindoline-2-carbonyl)oxy)pyrrolidine-1,2-dicarboxylate was prepared according to the published procedure from the literature.[5] Use of this core in a chemistry informer library was demonstrated by Kutchukian and Dreher.[14] The compound is now commercially available from MilliporeSigma (#901735).

7-(3-bromo-4-methoxybenzyl)-1-ethyl-8-(((1R,2R)-2-hydroxycyclopentyl)amino)-3-(2-hydroxyethyl)-1H-purine-2,6(3H,7H)-dione (16)



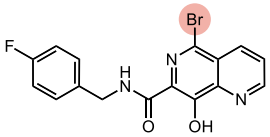
7-(3-bromo-4-methoxybenzyl)-1-ethyl-8-(((1R,2R)-2-hydroxycyclopentyl)amino)-3-(2-hydroxyethyl)-1H-purine-2,6(3H,7H)-dione was prepared according to the published procedure from the literature.[6] Use of this core in a chemistry informer library was demonstrated by Kutchukian and Dreher.[14] The compound is now commercially available from MilliporeSigma (#901747).

tert-butyl (S,Z)-(4-(4-bromothiophen-2-yl)-1,4-dimethyl-6-oxotetrahydropyrimidin-2(1H)-ylidene)carbamate (17)



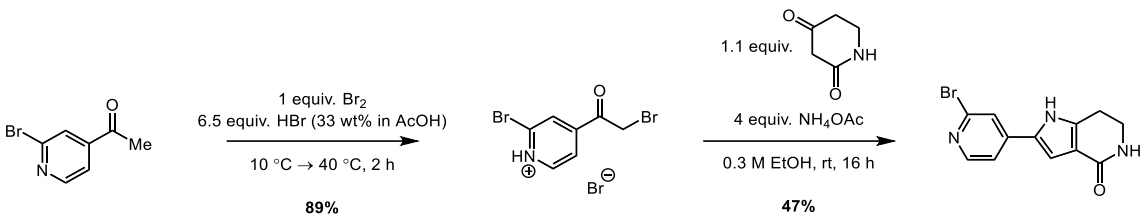
tert-butyl (S,Z)-(4-(4-bromothiophen-2-yl)-1,4-dimethyl-6-oxotetrahydropyrimidin-2(1H)-ylidene)carbamate was prepared according to the published procedure from the literature.[3] Use of this core in a chemistry informer library was demonstrated by Kutchukian and Dreher.[14] The compound is now commercially available from MilliporeSigma (#901781).

5-bromo-N-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (18)



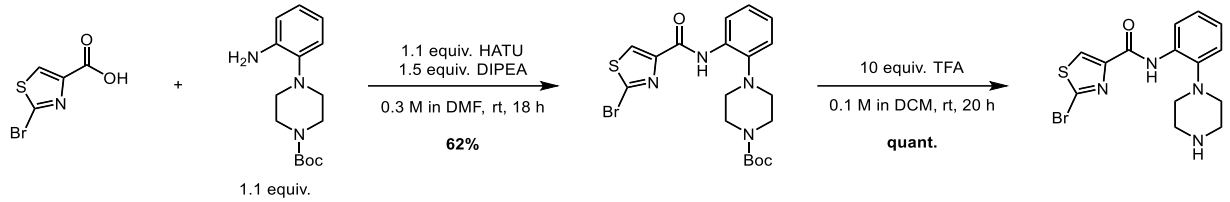
5-bromo-N-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide was prepared according to published procedures from the literature.[7] Use of this core in a chemistry informer library was demonstrated by Kutchukian and Dreher.[14] The compound is now commercially available from MilliporeSigma (#901745).

2-(2-bromopyridin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one (19):



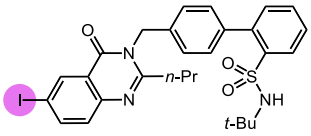
2-(2-bromopyridin-4-yl)-1,5,6,7-tetrahydro-4H-pyrrolo[3,2-c]pyridin-4-one was prepared according to published literature procedures[8,9] and spectral data for the final product were in agreement with reported literature values.[9]

2-bromo-N-(2-(piperazin-1-yl)phenyl)thiazole-4-carboxamide (20):



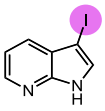
2-bromo-N-(2-(piperazin-1-yl)phenyl)thiazole-4-carboxamide was prepared according to published literature procedures and spectral data for the final product were in agreement with reported literature values.[9,10]

N-(tert-butyl)-4'-((6-iodo-4-oxo-2-propylquinazolin-3(4H)-yl)methyl)-[1,1'-biphenyl]-2-sulfonamide (21)



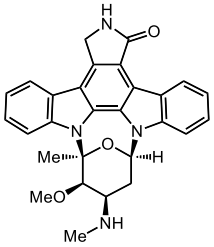
N-(tert-butyl)-4'-((6-iodo-4-oxo-2-propylquinazolin-3(4H)-yl)methyl)-[1,1'-biphenyl]-2-sulfonamide was prepared according to published procedures from the literature.[11] Use of this core in a chemistry informer library was demonstrated by Kutchukian and Dreher.[14] The compound is now commercially available from MilliporeSigma (#901752).

3-iodo-1H-pyrrolo[2,3-b]pyridine (22)



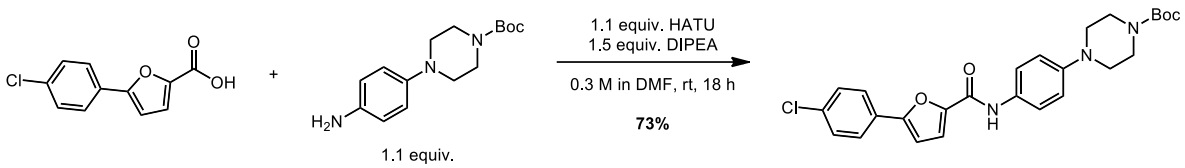
3-Iodo-1H-pyrrolo[2,3-b]pyridine is commercially available and was purchased from an external vendor.

Staurosporine (36)



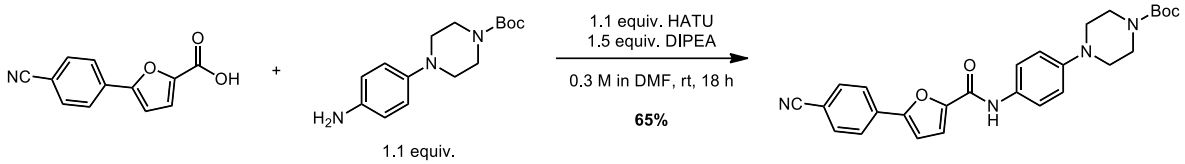
The natural product staurosporine is commercially available and was purchased from an external vendor.

***tert*-butyl 4-(4-(5-(4-chlorophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate (54)**



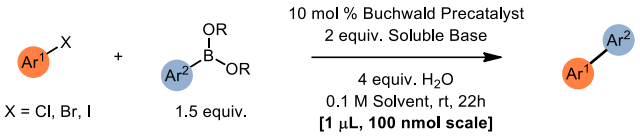
tert-butyl 4-(4-(5-(4-chlorophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate was prepared according to the published literature procedure and spectral data for the final product were in agreement with reported literature values.[12]

***tert*-butyl 4-(4-(5-(4-cyanophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate (55)**



*t*ert-butyl 4-(4-(5-(4-cyanophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate was prepared according to the published literature procedure and spectral data for the final product were in agreement with reported literature values.[13]

3. Nanomole-Scale Suzuki Cross-Coupling 12x10 Informer Library Screen



Conditions tested:

10 mol % *t*-BuXPhos G3 / 4 equiv. H2O / 2 equiv. P_2-Et / DMSO (0.1 M)

10 mol % *t*-BuXPhos G3 / 4 equiv. H2O / 2 equiv. BTMG / DMSO (0.1 M)

10 mol % APhos G3 / 4 equiv. H2O / 2 equiv. P_2-Et / DMSO (0.1 M)

10 mol % APhos G3 / 4 equiv. H2O / 2 equiv. BTMG / DMSO (0.1 M)

10 mol % APhos G3 / 4 equiv. H2O / 2 equiv. BTTP / 25% *t*-AmOH/DMSO (0.1 M)

10 mol % APhos G3 / 4 equiv. H2O / 2 equiv. BTMG / 25% *t*-AmOH /DMSO (0.1 M)

10 mol % *t*-Bu_3P G2 / 4 equiv. H2O / 2 equiv. BTTP / 25% *t*-AmOH/DMSO (0.1 M)

10 mol % *t*-Bu_3P G2 / 4 equiv. H2O / 2 equiv. BTMG / 25% *t*-AmOH /DMSO (0.1 M)

10 mol % XPhos G3 / 2 equiv. aq. K3PO4 / 25% H_2O/DMSO (0.1 M)

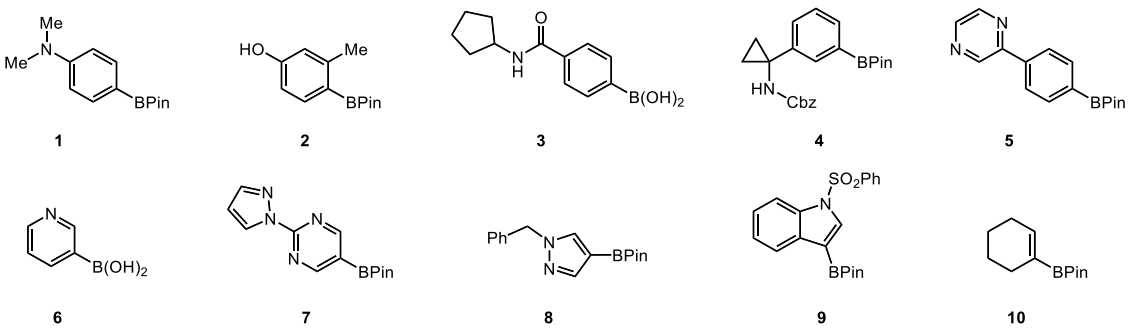
10 mol % XPhos G3 / 2 equiv. aq. BTMG / 25% H_2O/DMSO (0.1 M)

10 mol % RuPhos G3 / 2 equiv. aq. K3PO4 / 25% H_2O/DMSO (0.1 M)

10 mol % RuPhos G3 / 2 equiv. aq. BTMG / 25% H_2O/DMSO (0.1 M)

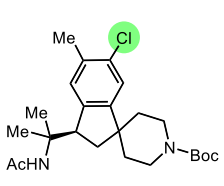
10 aryl (or vinyl) boronic acids/esters were tested against each core (1.5 equiv., 0.6 M stock in DMSO):

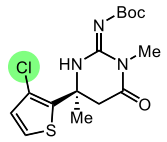
1. N,N-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**1**, MFCD05663854)
2. 3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (**2**, MFCD16994280)
3. (4-(cyclopentylcarbamoyl)phenyl)boronic acid (**3**, MFCD03788418)
4. benzyl (1-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)cyclopropyl)carbamate (**4**, MFCD19439767)
5. 2-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)pyrazine (**5**, MFCD18383786)
6. pyridin-3-ylboronic acid (**6**, MFCD00674177)
7. 2-(1H-pyrazol-1-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (**7**, MFCD11878349)
8. 1-benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (**8**, MFCD03789252)
9. 1-(phenylsulfonyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole (**9**, MFCD09999194)
10. 2-(cyclohex-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (**10**, MFCD05663845)

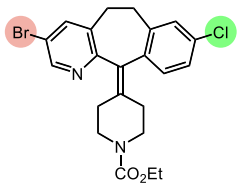


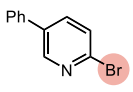
12 aryl halide cores examined (0.4 M stock in DMSO. 4 equiv. H_2O added to each core stock solution in advance of source plate preparation):

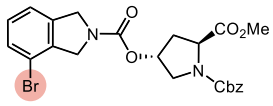
1. (R)-tert-butyl 3-(2-acetamidopropan-2-yl)-6-chloro-5-methyl-2,3-dihydrospiro[indene-1,4'-piperidine]-1'-carboxylate (**11, Core_01**)
2. (S,Z)-tert-butyl (4-(3-chlorothiophen-2-yl)-1,4-dimethyl-6-oxotetrahydropyrimidin-2(1H)-ylidene)carbamate (**12, Core_02**)
3. ethyl 4-(3-bromo-8-chloro-5H-benzo[5,6]cyclohepta[1,2-b]pyridin-11(6H)-ylidene)piperidine-1-carboxylate (**13, Core_03**)
4. 2-bromo-5-phenylpyridine (**14, Core_04**)
5. (2S,4R)-1-benzyl 2-methyl 4-((4-bromoisoindoline-2-carbonyl)oxy)pyrrolidine-1,2-dicarboxylate (**15, Core_05**)
6. 7-(3-bromo-4-methoxybenzyl)-1-ethyl-8-(((1R,2R)-2-hydroxycyclopentyl)amino)-3-(2-hydroxyethyl)-1H-purine-2,6(3H,7H)-dione (**16, Core_06**)
7. (S,Z)-tert-butyl (4-(4-bromothiophen-2-yl)-1,4-dimethyl-6-oxotetrahydropyrimidin-2(1H)-ylidene)carbamate (**17, Core_07**)
8. 5-bromo-N-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (**18, Core_08**)
9. 2-(2-bromopyridin-4-yl)-6,7-dihydro-1H-pyrrolo[3,2-c]pyridin-4(5H)-one (**19, Core_09**)
10. 2-bromo-N-(2-(piperazin-1-yl)phenyl)thiazole-4-carboxamide (**20, Core_10**)
11. N-(tert-butyl)-4'-((6-iodo-4-oxo-2-propylquinazolin-3(4H)-yl)methyl)-[1,1'-biphenyl]-2-sulfonamide (**21, Core_11**)
12. 3-iodo-1H-pyrrolo[2,3-b]pyridine (**22, Core_12**)

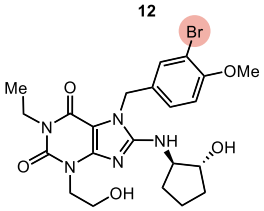

11

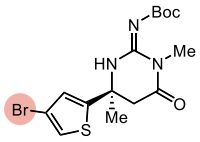

12

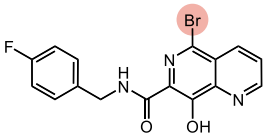

13

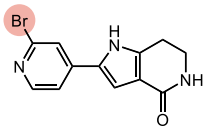

14

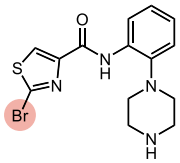

15

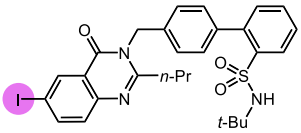

16

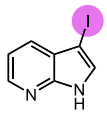

17


18


19


20

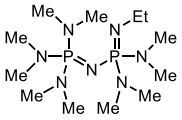

21

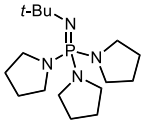

22

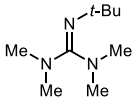
Five palladium Buchwald precatalysts tested for each reaction, two per solvent/cosolvent/base combination (10 mol % loading, 0.04 M stock in DMSO): XPhos G3, *t*-BuXphos G3, *t*-Bu_3P G2, RuPhos G3, and APhos G3

Six solvent / cosolvent / base combinations tested per reaction (2 equiv., 0.8 M stock in solvent shown):

1.	P_2-Et dispensed in DMSO	(column 22)	Final reaction solvent: DMSO
2.	BTMG dispensed in DMSO	(column 22)	Final reaction solvent: DMSO
3.	BTTP dispensed in *t*-AmOH	(column 23)	Final reaction solvent: 25% *t*-AmOH/DMSO
4.	BTMG dispensed in *t*-AmOH	(column 23)	Final reaction solvent: 25% *t*-AmOH/DMSO
5.	K_3PO_4 dispensed in H_2O	(column 24)	Final reaction solvent: 25% H_2O/DMSO
6.	BTMG dispensed in H_2O	(column 24)	Final reaction solvent: 25% H_2O/DMSO


P_2-Et


BTTP


BTMG

P_2-Et = 1-Ethyl-2,2,4,4,4-pentakis(dimethylamino)-2λ5,4λ5-catenadi(phosphazene)

BTTP = *tert*-Butylimino-tri(pyrrolidino)phosphorene

BTMG = 2-*tert*-Butyl-1,1,3,3-tetramethylguanidine

Source plate arrayed according to Figure S3 below.

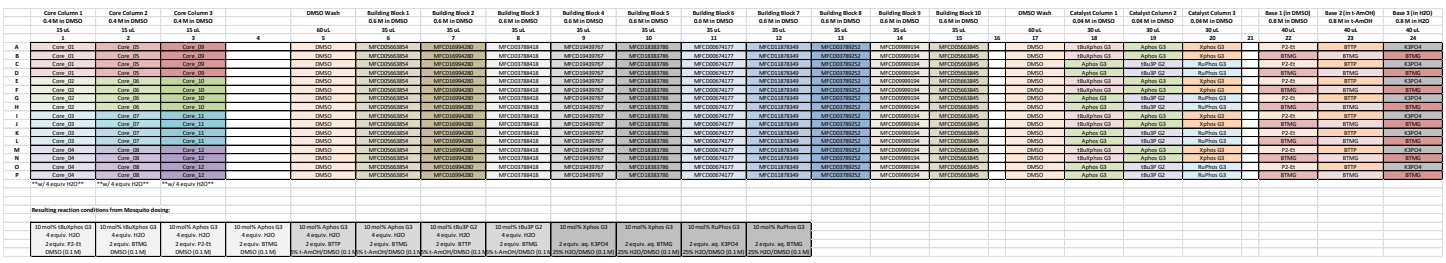


Figure S3. Suzuki Reaction Screen Source Plate

SPT Labtech Mosquito®HTS liquid handling robot used to generate reaction plate containing the 12 reaction conditions described above per aryl halide core/boronic acid building block combination. 12 reaction conditions were tested to produce a maximum of 120 possible products, 12 cores × 10 boronate building blocks. 12 cores × 10 boronate building blocks × 12 conditions = 1,440 reaction screen. Results of this screen are presented in Figure 2 as a heatmap arrayed by reaction conditions. Reactions are represented by product peak area versus internal standard (PhPh) peak area ratio.

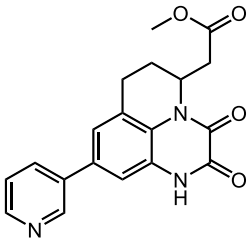
1. 250 nL was drawn up from the desired core column (core columns 1-3)
2. Tip wash (DMSO column 5)
3. 250 nL was drawn from the desired boronic acid/ester building block column (columns 6-15)
4. Tip wash (DMSO column 17)
5. 250 nL was drawn up from the desired catalyst stock solution column (columns 18-20)
6. 250 nL was drawn up from the desired base stock solution column (columns 22-24)
7. This was immediately followed by dispense mixing (3×700 nL) into the 1,536-well reaction plate to ensure all reagents are adequately combined.
8. This process was repeated for all cores (columns 1-3), all boronic acid/ester building blocks (columns 6-15) and all 12 desired reaction conditions.
9. The 1,536-well plate was sealed and briefly centrifuged and allowed to sit at room temperature in a glove box for 22 hours.
10. After 22 hours the crude reactions were diluted with 3×1 µL 0.5 M AcOH in DMSO (containing PhPh as an internal standard). The reactions were mixed after each dispense operation.
11. Mosquito® was used to transfer 1 µL of each quenched reaction from the 1,536-well reaction plate to 384-well analytical plates containing 60 µL DMSO for analysis.

4. Automation-friendly Suzuki Coupling Using 3-Boronopyridine

General procedure for Suzuki Coupling of 3-boronopyridine (23):

In a nitrogen filled glovebox, pyridin-3-ylboronic acid **23** (0.060 mmol) and aryl informer (0.050 mmol) were combined with palladium Xphos-G3 precatalyst (4.23 mg, 0.0050 mmol, 10 mol%) in anhydrous DMSO (0.1 M overall conc.) in 2 mL vials equipped with stir bars. To these mixtures, 50 uL of aq. K_3PO_4 (1.0 M aqueous solution, 0.05 mmol, 1 equiv.) was added and the mixtures were stirred at 23 °C overnight. The mixtures were quenched with 1% HOAc in DMSO (1.5 mL). The mixtures were then filtered through a filter plate (0.45 micron) and purified by RP HPLC (Waters SunfireTM 5 micron RP C-18, 30x 100 mm column), eluents: MeCN-H_2O containing 0.05% TFA.

Methyl 2-(2,3-dioxo-9-(pyridin-3-yl)-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-5-yl)acetate (24)

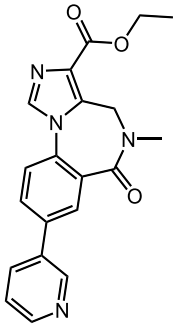


The title compound was prepared using the standard conditions described above with methyl 2-(9-bromo-2,3-dioxo-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-5-yl)acetate (aryl halide core) and 3-pyridineboronic acid pinacol

ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 5.7 mg (25% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **24**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 12.17 (s, 1H), 8.99 (d, *J* = 2.3 Hz, 1H), 8.73 (dd, *J* = 5.1, 1.5 Hz, 1H), 8.33 (dt, *J* = 8.1, 1.9 Hz, 1H), 7.78 (dd, *J* = 8.1, 5.1 Hz, 1H), 7.47 (d, *J* = 2.0 Hz, 1H), 7.34 (d, *J* = 2.0 Hz, 1H), 5.20 – 5.08 (m, 1H), 3.65 (s, 3H), 3.04 (ddd, *J* = 18.4, 13.8, 5.1 Hz, 1H), 2.94 – 2.87 (m, 1H), 2.67 (t, *J* = 7.3 Hz, 2H), 2.22 – 2.14 (m, 1H), 1.96 (tt, *J* = 13.9, 4.8 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 171.22, 154.65, 154.09, 145.79, 144.72, 138.00, 136.54, 130.86, 126.93, 125.92, 125.88, 123.73, 122.49, 112.08, 52.19, 47.62, 35.44, 23.17, 21.72; **MS** (ESI): *m/z* calculated for $C_{19}H_{18}N_3O_4$ [M+H]+ 352.1292, found 352.1278.

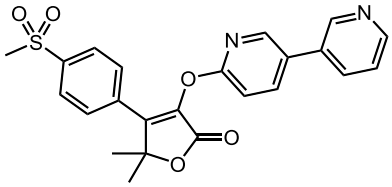
Ethyl 5-methyl-6-oxo-8-(pyridin-3-yl)-5,6-dihydro-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (25)



The title compound was prepared using the standard conditions described above with ethyl 8-bromo-5-methyl-6-oxo-5,6-dihydro-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (aryl halide core) and 3-pyridineboronic acid pinacol ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 23.5 mg (99% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **25**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.25 (d, *J* = 2.2 Hz, 1H), 8.84 (dd, *J* = 5.3, 1.5 Hz, 1H), 8.68 (ddd, *J* = 8.1, 2.3, 1.4 Hz, 1H), 8.49 (s, 1H), 8.32 (d, *J* = 2.3 Hz, 1H), 8.22 (dd, *J* = 8.4, 2.3 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 1H), 7.93 (dd, *J* = 8.1, 5.3 Hz, 1H), 5.04 (s, 1H), 4.57 (s, 1H), 4.34 (s, 2H), 3.16 (s, 3H), 1.36 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 165.83, 162.73, 145.16, 144.11, 140.11, 137.06, 135.97, 135.82, 135.33, 132.73, 131.62, 130.91, 129.85, 128.21, 126.40, 124.35, 60.67, 42.47, 35.61, 14.66; **MS** (ESI): *m/z* calculated for $C_{20}H_{19}N_4O_3$ [M+H]+ 363.1452, found 363.1436.

3-([3,3'-bipyridin]-6-yloxy)-5,5-dimethyl-4-(4-(methylsulfonyl)phenyl)furan-2(5H)-one (26)

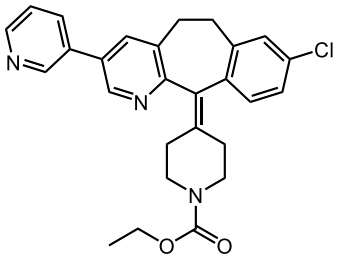


The title compound was prepared using the standard conditions described above with 3-((5-bromopyridin-2-yl)oxy)-5,5-dimethyl-4-(4-(methylsulfonyl)phenyl)furan-2(5H)-one (aryl halide core) and 3-pyridineboronic acid pinacol ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 7.5 mg (27% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **26**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.22 – 8.97 (m, 1H), 8.77 (dd, *J* = 5.2, 1.5 Hz, 1H), 8.66 (d, *J* = 2.5 Hz, 1H), 8.51 (dt, *J* = 8.2, 1.8 Hz, 1H), 8.35 (dd, *J* = 8.6, 2.6 Hz, 1H), 8.05 (d, *J* = 8.6 Hz, 2H), 7.90 (d, *J* = 8.6 Hz, 1H), 7.82 (dd, *J* = 8.1, 5.2 Hz, 1H), 7.38 (d, *J* = 8.6 Hz, 1H), 3.26 (s, 3H), 1.75 (s, 6H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 165.66, 161.68, 149.80, 146.47, 145.83,

144.70, 142.30, 139.97, 138.77, 136.92, 134.04, 133.88, 129.39, 128.49, 128.18, 125.92, 111.75, 85.14, 43.72, 26.30; **MS** (ESI): *m/z* calculated for $C_{23}H_{21}N_2O_5S$ [M+H]+ 437.1166, found 437.1148.

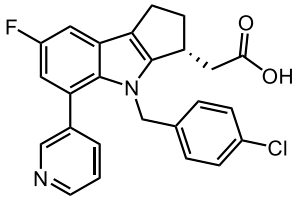
Ethyl 4-(8-chloro-3-(pyridin-3-yl)-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)piperidine-1-carboxylate (27)



The title compound was prepared using the standard conditions described above with ethyl 4-(3-bromo-8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)piperidine-1-carboxylate (aryl halide core) and 3-pyridineboronic acid pinacol ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 16.2 mg (57% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **27**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.21 (d, *J* = 2.3 Hz, 1H), 8.96 (d, *J* = 2.2 Hz, 1H), 8.85 (d, *J* = 5.3 Hz, 1H), 8.63 (d, *J* = 8.3 Hz, 1H), 8.39 (d, *J* = 2.2 Hz, 1H), 7.91 (dd, *J* = 8.1, 5.3 Hz, 1H), 7.41 (d, *J* = 2.2 Hz, 1H), 7.29 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.17 (d, *J* = 8.2 Hz, 1H), 4.06 (q, *J* = 7.0 Hz, 2H), 3.71 (ddt, *J* = 18.4, 13.0, 5.1 Hz, 2H), 3.48 (ddd, *J* = 16.3, 7.1, 4.8 Hz, 1H), 3.41 (ddd, *J* = 14.9, 9.8, 4.8 Hz, 1H), 3.18 (s, 2H), 3.03 (ddd, *J* = 16.3, 9.9, 4.7 Hz, 1H), 2.93 (ddd, *J* = 15.3, 7.1, 4.8 Hz, 1H), 2.42 (ddd, *J* = 13.9, 9.3, 4.6 Hz, 1H), 2.32 (ddd, *J* = 13.8, 9.0, 4.7 Hz, 1H), 2.26 (dq, *J* = 14.3, 4.9 Hz, 2H), 1.18 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 155.04, 145.84, 144.42, 142.68, 140.86, 140.63, 140.53, 139.89, 137.47, 135.87, 133.70, 132.79, 131.39, 131.07, 130.20, 129.48, 126.60, 126.23, 61.23, 44.51, 31.05, 30.84, 30.67, 15.01; **MS** (ESI): *m/z* calculated for $C_{27}H_{27}ClN_3O_2$ [M+H]+ 460.1786, found 460.1770.

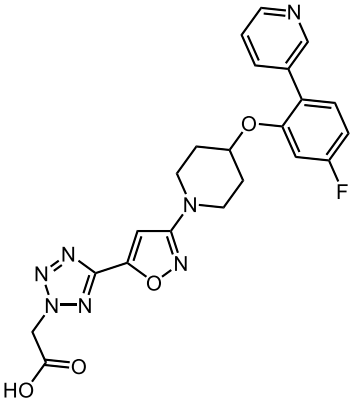
(R)-2-(4-(4-chlorobenzyl)-7-fluoro-5-(pyridin-3-yl)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid (28)



The title compound was prepared using the standard conditions described above with (R)-2-(5-bromo-4-(4-chlorobenzyl)-7-fluoro-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid (aryl halide core) and 3-pyridineboronic acid pinacol ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 9.3 mg (34% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **28**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.65 (dd, *J* = 5.2, 1.6 Hz, 1H), 8.39 (d, *J* = 2.3 Hz, 1H), 7.82 (dt, *J* = 7.8, 1.9 Hz, 1H), 7.53 – 7.50 (m, 1H), 7.32 (dd, *J* = 9.1, 2.6 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 2H), 6.72 (dd, *J* = 9.7, 2.6 Hz, 1H), 6.24 (d, *J* = 8.0 Hz, 2H), 4.95 (s, 2H), 3.48 (ddt, *J* = 11.0, 7.2, 3.6 Hz, 1H), 2.97 – 2.83 (m, 1H), 2.82 – 2.69 (m, 2H), 2.61 – 2.53 (m, 1H), 2.31 (dd, *J* = 16.1, 10.3 Hz, 1H), 2.20 (ddt, *J* = 10.8, 5.9, 2.8 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 173.38, 156.7 ($^1J_{CF}$ = 234.1 Hz), 151.94, 147.17, 146.66, 139.88, 137.34, 135.32, 134.31, 131.95, 128.80, 127.00, 126.56, 124.15, 123.60, 119.17, 111.75 ($^2J_{CF}$ = 25.3 Hz) , 104.53 ($^2J_{CF}$ = 23.4 Hz) , 49.14, 39.02, 35.79, 35.23, 23.12; **MS** (ESI): *m/z* calculated for $C_{25}H_{21}ClFN_2O_2$ [M+H]+ 435.1270, found 435.1200.

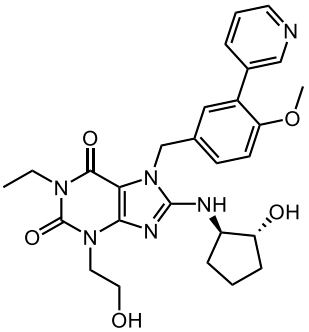
2-(5-(3-(4-(5-fluoro-2-(pyridin-3-yl)phenoxy)piperidin-1-yl)isoxazol-5-yl)-2H-tetrazol-2-yl)acetic acid (29)



The title compound was prepared using the standard conditions described above with 2-(5-(3-(4-(2-bromo-5-fluorophenoxy)piperidin-1-yl)isoxazol-5-yl)-2H-tetrazol-2-yl)acetic acid (aryl halide core) and 3-pyridineboronic acid pinacol ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 16.7 mg (58% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **29**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (d, *J* = 2.1 Hz, 1H), 8.76 (dd, *J* = 5.4, 1.5 Hz, 1H), 8.46 (dt, *J* = 8.1, 1.8 Hz, 1H), 7.89 (dd, *J* = 8.1, 5.3 Hz, 1H), 7.56 (dd, *J* = 8.5, 6.8 Hz, 1H), 7.29 (dd, *J* = 11.4, 2.5 Hz, 1H), 7.22 (s, 1H), 6.98 (td, *J* = 8.3, 2.4 Hz, 1H), 5.84 (s, 2H), 4.81 (tt, *J* = 7.5, 3.6 Hz, 1H), 3.45 (ddd, *J* = 12.9, 7.1, 3.8 Hz, 2H), 3.30 (ddd, *J* = 12.5, 8.0, 3.7 Hz, 2H), 2.03 (ddt, *J* = 13.4, 6.9, 3.5 Hz, 2H), 1.71 (dtd, *J* = 11.9, 7.7, 3.8 Hz, 2H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 167.62, 167.10, 164.07 ($^1J_{CH}$ = 244.7 Hz), 157.78, 155.77, 145.07, 143.39, 143.21, 135.45, 132.70, 132.63, 125.88, 121.73, 108.38 ($^2J_{CH}$ = 21.5 Hz), 102.57 ($^2J_{CH}$ = 25.8 Hz) , 97.42, 73.26, 54.47, 44.47, 29.41; **MS** (ESI): *m/z* calculated for $C_{22}H_{21}FN_7O_4$[M+H]+ 466.1634, found 466.1619.

1-ethyl-8-(((1R,2R)-2-hydroxycyclopentyl)amino)-3-(2-hydroxyethyl)-7-(4-methoxy-3-(pyridin-3-yl)benzyl)-3,7-dihydro-1H-purine-2,6-dione (30)

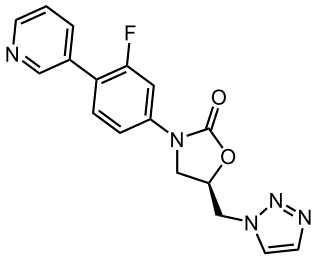


The title compound was prepared using the standard conditions described above with 7-(3-bromo-4-methoxybenzyl)-1-ethyl-8-(((1R,2R)-2-hydroxycyclopentyl)amino)-3-(2-hydroxyethyl)-3,7-dihydro-1H-purine-2,6-dione (aryl halide core) and 3-pyridineboronic acid pinacol ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 20.9 mg (66% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **30**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.97 (d, *J* = 2.0 Hz, 1H), 8.84 (dd, *J* = 5.5, 1.4 Hz, 1H), 8.51 (dt, *J* = 8.1, 1.8 Hz, 1H), 8.01 (dd, *J* = 8.1, 5.5 Hz, 1H), 7.57 (d, *J* = 2.3 Hz, 1H), 7.43 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.20 (d, *J* = 8.7 Hz, 1H), 7.11 (d, *J* = 6.8 Hz, 1H), 5.35 (s, 2H), 4.00 (m, 3H), 3.89 (m, 3H), 3.81 (s, 3H), 3.64 (t, *J* = 6.5 Hz, 2H), 2.07 (dtd, *J* = 13.2, 8.1, 5.3 Hz, 1H), 1.85 (ddt, *J* = 12.8, 8.6, 6.4 Hz, 1H), 1.72 – 1.56 (m, 2H), 1.48 (dddd, *J* = 18.6, 12.1, 8.7, 6.5 Hz, 2H), 1.08 (t, *J* = 7.0 Hz, 3H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 156.09, 153.97, 153.13, 150.91, 149.29, 144.29, 143.72, 142.38, 136.49, 130.88, 130.77, 130.54,

126.60, 123.67, 112.74, 101.62, 76.53, 61.95, 58.28, 56.36, 45.11, 44.96, 35.64, 32.80, 30.27, 20.82, 13.74; **MS** (ESI): *m/z* calculated for $C_{27}H_{33}N_6O_5$ [M+H]+ 521.2507, found 521.2495.

(R)-5-((1H-1,2,3-triazol-1-yl)methyl)-3-(3-fluoro-4-(pyridin-3-yl)phenyl)oxazolidin-2-one (31)

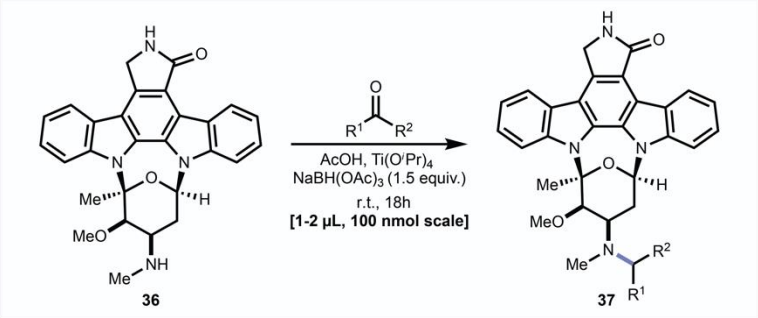


The title compound was prepared using the standard conditions described above with (R)-5-((1H-1,2,3-triazol-1-yl)methyl)-3-(3-fluoro-4-iodophenyl)oxazolidin-2-one (aryl halide core) and 3-pyridineboronic acid pinacol ester. The desired product was prepared at 0.05 mmol scale and purified by RP-HPLC to yield 16.7 mg (74% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **31**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.19 – 8.89 (m, 1H), 8.83 (dd, *J* = 5.3, 1.4 Hz, 1H), 8.50 (ddt, *J* = 8.2, 2.5, 1.4 Hz, 1H), 8.20 (d, *J* = 1.0 Hz, 1H), 7.94 (dd, *J* = 8.1, 5.3 Hz, 1H), 7.78 (d, *J* = 1.0 Hz, 1H), 7.75 (t, *J* = 8.8 Hz, 1H), 7.64 (dd, *J* = 13.5, 2.3 Hz, 1H), 7.46 (dd, *J* = 8.6, 2.3 Hz, 1H), 5.22 (dq, *J* = 9.1, 5.3 Hz, 1H), 4.89 (d, *J* = 5.0 Hz, 2H), 4.33 (t, *J* = 9.2 Hz, 1H), 3.99 (dd, *J* = 9.5, 5.6 Hz, 1H); **^{13}C NMR** (151 MHz, DMSO-*d*6) δ 159.6 ($^1J_{CH}$ = 246.2 Hz), 153.90, 144.63, 144.19, 142.32, 141.11, 141.04, 133.89, 132.96, 131.61, 126.40, 126.37, 118.16 ($^2J_{CH}$ = 13.4 Hz), 114.75, 106.06 ($^2J_{CH}$ = 27.9 Hz), 71.56, 52.16, 47.46**; MS** (ESI): *m/z* calculated for $C_{17}H_{15}FN_5O_2$ [M+H]+ 340.1204, found 340.1193.

5. Nanomole-Scale Reductive Amination with Staurosporine

5.1. Nanomole-Scale Staurosporine Reductive Amination Optimization



Two solvents tested: NMP and DMA **Two concentrations tested:** 0.1 M and 0.05 M

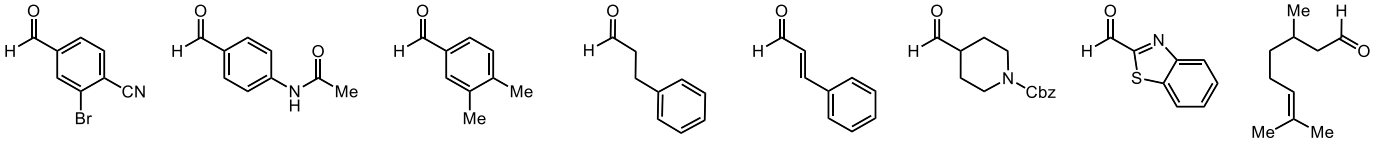
Three AcOH loadings tested: 1 equiv., 3 equiv., and 5 equiv.

Four Ti(O*i*-Pr)$_4$ [TTIP] loadings tested: 1 equiv., 2 equiv., 3 equiv., 5 equiv.

Previous studies focused on the reductive amination of staurosporine using aldehydes and ketones revealed that titanium tetraisopropoxide (TTIP) was necessary to achieve high conversions for nanomole-scale reductive amination. Stepwise reductive amination protocols using titanium tetraisopropoxide have been previously shown.[15–17]

Eight aldehyde building blocks tested (1.2 equiv., 0.6 M stock in solvent)

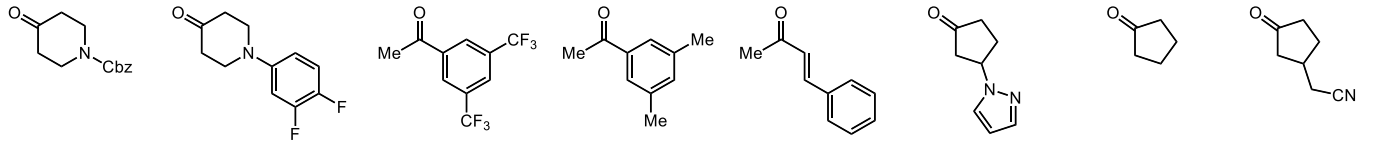
1. 2-bromo-4-formylbenzonitrile MFCD09261041
2. *N*-(4-formylphenyl)acetamide MFCD00003380
3. 3,4-dimethylbenzaldehyd MFCD00016612
4. 3-phenylpropanal MFCD00007021
5. cinnamaldehyde MFCD00007000
6. benzyl 4-formylpiperidine-1-carboxylate MFCD01317806
7. benzo[d]thiazole-2-carbaldehyde MFCD00526215
8. 3,7-dimethyloct-6-enal MFCD00038090



MFCD09261041 **A** | **MFCD00003380** **B** | **MFCD00016612** **C** | **MFCD00007021** **D** | **MFCD00007000** **E** | **MFCD01317806** **F** | **MFCD00526215** **G** | **MFCD00038090** **H**

Eight ketone building blocks tested (1.2 equiv., 0.6 M stock in solvent)

1. benzyl 4-oxopiperidine-1-carboxylate MFCD00673144
2. 1-(3,4-difluorophenyl)piperidin-4-one MFCD06739003
3. 1-(3,5-bis(trifluoromethyl)phenyl)ethanone MFCD00009910
4. 1-(3,5-dimethylphenyl)ethanone MFCD01075693
5. (E)-4-phenylbut-3-en-2-one MFCD00008779
6. 3-(1H-pyrazol-1-yl)cyclopentanone
7. Cyclopentanone MFCD00001409
8. 2-(3-oxocyclopentyl)acetonitrile



MFCD00673144 **I** | **MFCD06739003** **J** | **MFCD00009910** **K** | **MFCD01075693** **L** | **MFCD00008779** **M** | **L004984120000T** **N** | **MFCD00001409** **O** | **L005373664000F** **P**

2 solvents × 2 concentrations × 3 AcOH loadings × 4 TTIP loadings = 48 reaction conditions per building block. 48 reaction conditions × 8 building blocks = 384 reactions. Source plate arrayed as shown below. 1.2 equiv aldehyde building block used, 1.5 equiv. sodium triacetoxyborohydride used.

		Staurosporine	Dilute Staorosporine	Aldehydes and Ketones		AcOH		Wash Solvent	Dilution Solvent		Ti(OiPr)4	Dilution Solvent		STAB										
		0.5 M	0.083 M	0.6 M		2.5 M			Draw to volume		2.5 M	Draw to volume		0.75 M										
	Solvent	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD09261041		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
B	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00003380		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
C	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00016612		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
D	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00007021		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
E	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00007000		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
F	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD01317806		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
G	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00526215		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
H	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00038090		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
I	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00673144		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
J	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD06739003		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
K	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00009910		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
L	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD01075693		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
M	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00008779		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
N	DMA	Staurosporine [0.5]	Staurosporine [0.083]	L004984120000T		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
O	DMA	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00001409		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											
P	DMA	Staurosporine [0.5]	Staurosporine [0.083]	L005373664000F		AcOH in DMA		DMA	DMA		Ti(OiPr)4 in DMA	DMA		STAB in DMA											

		Staurosporine	Dilute Staorosporine	Aldehydes and Ketones		AcOH		Wash Solvent	Dilution Solvent		Ti(OiPr)4	Dilution Solvent		STAB										
		0.5 M	0.083 M	0.6 M		2.5 M			Draw to volume		2.5 M	Draw to volume		0.75 M										
	Solvent	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD09261041		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
B	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00003380		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
C	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00016612		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
D	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00007021		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
E	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00007000		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
F	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD01317806		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
G	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00526215		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
H	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00038090		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
I	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00673144		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
J	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD06739003		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
K	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00009910		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
L	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD01075693		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
M	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00008779		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
N	NMP	Staurosporine [0.5]	Staurosporine [0.083]	L004984120000T		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
O	NMP	Staurosporine [0.5]	Staurosporine [0.083]	MFCD00001409		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											
P	NMP	Staurosporine [0.5]	Staurosporine [0.083]	L005373664000F		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP											

Figure S4. Optimization Source Plate

SPT Labtech Mosquito®HTS liquid handling robot was used to generate a reaction plate containing all possible reaction conditions per aldehyde building block. 2 solvents × 2 concentrations × 3 AcOH loadings × 4 TTIP loadings = 48 reaction conditions per aldehyde building block. 48 reaction conditions × 16 building blocks = 768 reactions. The reactions were mixed (core, aldehyde/ketone, acetic acid, titanium tetraisopropoxide) and allowed to sit for 4 hours before addition of STAB to the reaction mixtures. After 18 hours, reactions were quenched and examined. 3×1 µL 0.5 M AcOH in DMSO (containing PhPh internal standard) quench solution was added to each reaction well. 1 µL of each quenched reaction (4 µL total volume) was transferred from the 1,536 plate reaction well to a 384-well analytical plate containing 60 µL DMSO per well. Results of this screen are presented in Figure 3 as a heat map arrayed by reaction conditions after quenching. Reactions are represented by relative percent conversion, calculated from UV peak area of product and staurosporine core.

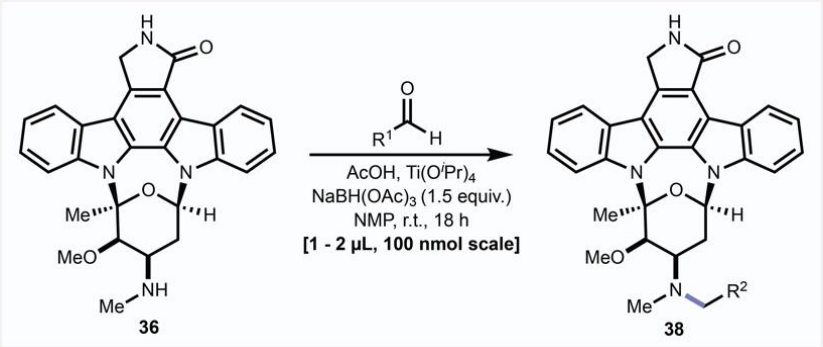
1. 1000 nL was drawn up from core column 2 column (DMA plate, dilute concentration: 2) and dispensed into wells for 50 mM reactions.
2. 200 nL was drawn up from core column 1 (DMA plate: column 1, for 100 mM reactions) or 200 nL was drawn up from the dilute core column (DMA, column 2, <u>for dilute 50 mM reactions only</u>)
3. 200 nL was drawn from the aldehyde/ketone building block column (DMA, column 3)
4. 40, 120, or 200 nL was drawn up from the AcOH column (column 5):
    a. 1 equiv., 3 equiv., 5 equiv. AcOH respectively
5. Tip wash (DMA column 7)
6. 160, 80, or 5 nL was drawn up from the dilution solvent column (DMA, column 8)
7. 40, 120, 133 or 200 nL was drawn up from the Ti(O*i*-Pr)$_4$ column (in DMA, column 10):
    a. 1 equiv., 2 equiv., 3 equiv., 5 equiv. Ti(O*i*-Pr)$_4$ respectively
8. 160, 80, 67 or 5 nL was drawn up from the dilution solvent column (DMA, column 11)
9. This was immediately followed by dispense mixing (3×700 nL) into the 1,536-well reaction plate to ensure all reagents are adequately combined.
10. This process was then repeated using the source plate containing NMP as the solvent.
11. The 1,536-well plate was sealed and briefly centrifuged and allowed to sit for 4 hours.
12. After 4 hours, a 0.75 M stock solution of STAB was prepared in DMA and NMP and dispensed into column 13 of the correct reaction plate (matching solvent).
13. Mosquito® then dispensed 200 nL STAB solution into each reaction well, followed again by dispense mixing (3×700 nL). The 1,536 well plate was centrifuged and allowed to sit at ambient temperature for 18 hours.

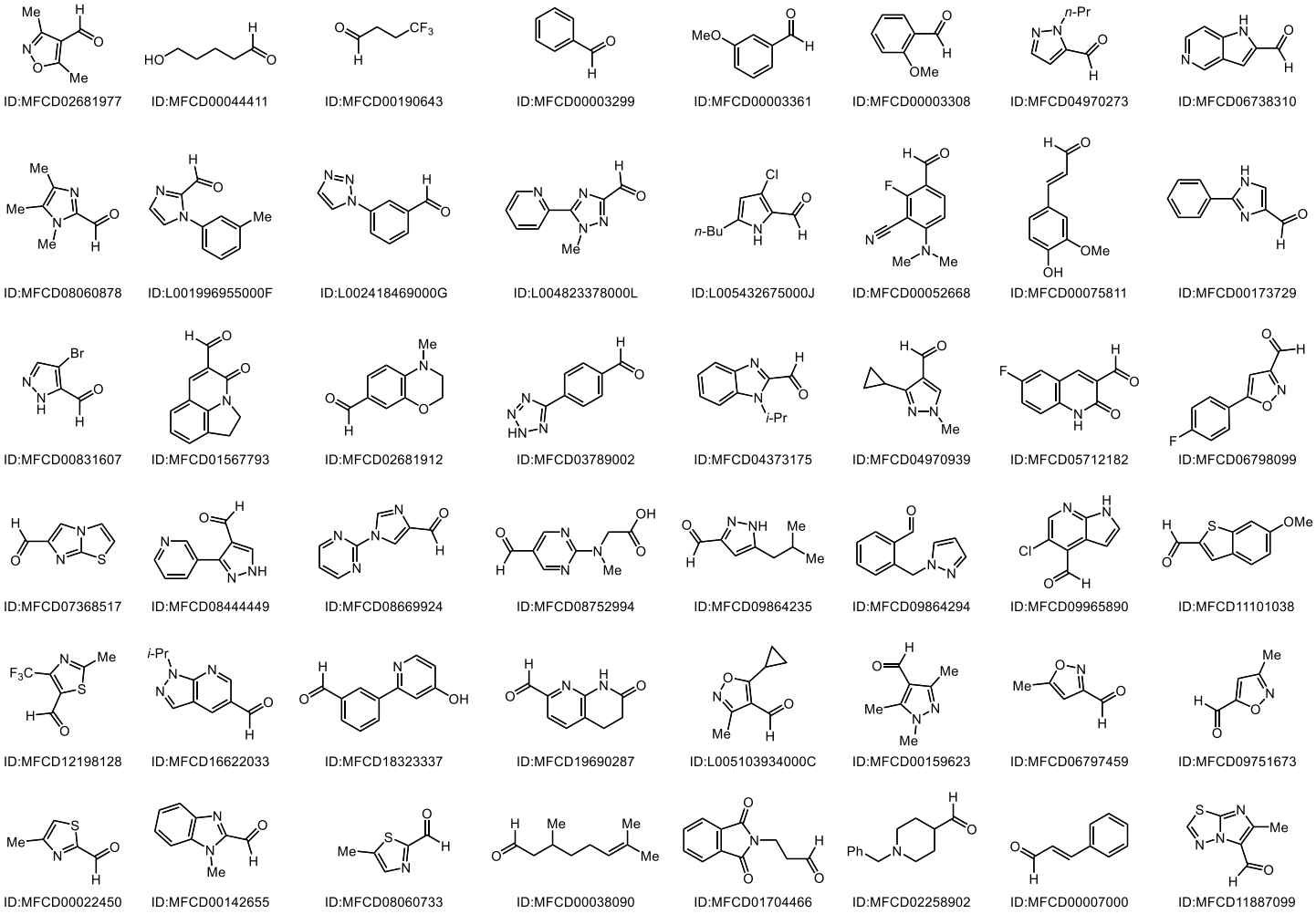
14. The next morning, the Mosquito® was used to dilute the 100 mM reactions to 50 mM via addition of 1 µL DMSO to each reaction well, followed by dispense mixing.
15. The reactions were diluted with 3×1 µL DMSO (containing PhPh as an internal standard) using the Mosquito®. The reactions were mixed after each dispense operation.
16. Mosquito® was used to transfer 1 µL of each quenched reaction from the 1,536-well reaction plate to 384-well analytical plates containing 60 µL DMSO for analysis by UPLC.

5.2. Nanomole-Scale Staurosporine Reductive Amination Library Synthesis

8 Reaction conditions identified from reaction screening for reductive amination library synthesis:

1 solvent (NMP) × 2 concentrations (0.1 M and 0.05 M) × 2 AcOH loadings (3 equiv. and 5 equiv.) × 2 Ti(O*i*-Pr)$_4$ loadings (2 equiv. and 3 equiv.). Reaction components were arrayed with 48 aldehyde building blocks and allowed to sit for 5 hours. After 5 hours, sodium triacetoxyborohydride (STAB, 0.75 M in NMP, 1.5 equiv.) added to all reaction wells. Reactions were allowed to sit at room temperature for 18 hours before quenching.





All chemistry and nanoliter pipetting was performed in an N_2 atmosphere glove box. Stock solutions of staurosporine (0.5 M in NMP and 0.083 M in NMP, 1 equiv. per reaction), aldehyde building blocks (0.6 M in NMP, 1.2 equiv. per reaction), AcOH (2.5 M in NMP, 3-5 equiv.), Ti(O*i*-Pr)$_4$ (1.5 M in NMP, 2-3 equiv.), and sodium triacetoxyborohydride (STAB, 0.75 M, 1.5 equiv.) were prepared were prepared in the glove box. A source plate was constructed using the reagent stock solutions, according to Figure S5, by pipetting 15-20 µL of the correct component stock into the wells shown. The SPT Labtech Mosquito® was used to assemble the reaction mixtures, utilizing the multi-aspiration feature and dispense mixing.

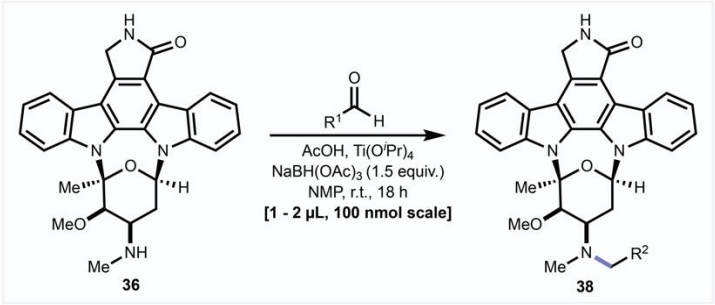
	Staurosporine 0.5 M in NMP	Dilute Staorosporine 0.083 M in NMP		0.6 M Aldehydes	0.6 M Aldehydes	0.6 M Aldehydes			Wash Solvent		2.5 M AcOH		Wash Solvent	Dilution Solvent Draw to volume		1.5 M Ti(OiPr)4	Dilution Solvent Draw to volume		0.75 M STAB					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Stauro [0.5]	Stauro [0.083]		MFCD02681977	MFCD00831607	MFCD12198128			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
B	Stauro [0.5]	Stauro [0.083]		MFCD00044411	MFCD01567793	MFCD16622033			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
C	Stauro [0.5]	Stauro [0.083]		MFCD00190643	MFCD02681912	MFCD18323337			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
D	Stauro [0.5]	Stauro [0.083]		MFCD00003299	MFCD03789002	MFCD19690287			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
E	Stauro [0.5]	Stauro [0.083]		MFCD00003361	MFCD04373175	L005103934000C			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
F	Stauro [0.5]	Stauro [0.083]		MFCD00003308	MFCD04970939	MFCD00159623			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
G	Stauro [0.5]	Stauro [0.083]		MFCD04970273	MFCD05712182	MFCD06797459			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
H	Stauro [0.5]	Stauro [0.083]		MFCD06738310	MFCD06798099	MFCD09751673			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
I	Stauro [0.5]	Stauro [0.083]		MFCD08060878	MFCD07368517	MFCD00022450			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
J	Stauro [0.5]	Stauro [0.083]		L001996955000F	MFCD08444449	MFCD00142655			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
K	Stauro [0.5]	Stauro [0.083]		L002418469000G	MFCD08669924	MFCD08060733			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
L	Stauro [0.5]	Stauro [0.083]		L004823378000L	MFCD08752994	MFCD00038090			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
M	Stauro [0.5]	Stauro [0.083]		L005432675000J	MFCD09864235	MFCD01704466			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
N	Stauro [0.5]	Stauro [0.083]		MFCD00052668	MFCD09864294	MFCD02258902			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
O	Stauro [0.5]	Stauro [0.083]		MFCD00075811	MFCD09965890	MFCD00007000			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					
P	Stauro [0.5]	Stauro [0.083]		MFCD00173729	MFCD11101038	MFCD11887099			NMP		AcOH in NMP		NMP	NMP		Ti(OiPr)4 in NMP	NMP		STAB in NMP					

Figure S5. Reductive Amination Source Plate

1. 200 nL was drawn up from core column 1 (NMP: 1) or 1000 nL was first dispensed from dilute core column (NMP dilute: 2) before drawing up 200 nL (column 2, for dilute 0.05 M reactions only)
2. 200 nL was drawn from the desired aldehyde building block column (columns 4-6)

3. Tip wash (NMP column 9)
4. 120 or 200 nL was drawn up from the AcOH column (column 12)
5. Tip wash (NMP column 13)
6. 80 or 5 nL was drawn up from the dilution/solvent column (NMP, column 14)
7. 133 or 200 nL was drawn up from the Ti(O*i*-Pr)$_4$ column (in NMP, column 16)
8. 67 or 5 nL was drawn up from the dilution/solvent column (NMP, column 17)
9. This was immediately followed by dispense mixing (3×700 nL) into the 1,536-well reaction plate to ensure all reagents are adequately combined.
10. This process was done for all aldehyde building block columns (columns 4, 5, and 6) and all 8 reaction conditions.
11. The 1,536-well plate was sealed and briefly centrifuged and allowed to sit for 5 hours.
12. After 5 hours, a 0.75 M stock solution of STAB was prepared in NMP and dispensed into column 19.
13. Mosquito® then dispensed 200 nL STAB solution into each reaction well, followed again by dispense mixing (3×700 nL). The 1,536 well plate was centrifuged and allowed to sit at ambient temperature for 18 hours.

The next morning, the reactions were diluted to 2 µL (if needed) with DMSO, then quenched by addition of 1 µL sat. aq. $NaHCO_3$ and 1 µL water to each reaction well. The reactions were further diluted with DMSO (to approximately 10 mM assuming complete conversion to product) and transferred to a 384-well LDV diamond plate for affinity ranking. 1 µL of the quenched and diluted crude reaction was drawn up for analysis by LC-MS (1 µL crude ~10 mM reaction into 60 µL DMSO followed by shaking). Percent conversion for each reaction was calculated using UV product peak area and UV core peak area. Screening results are represented in Figure 4 and in Figure S6 (more detail) as a heatmap.



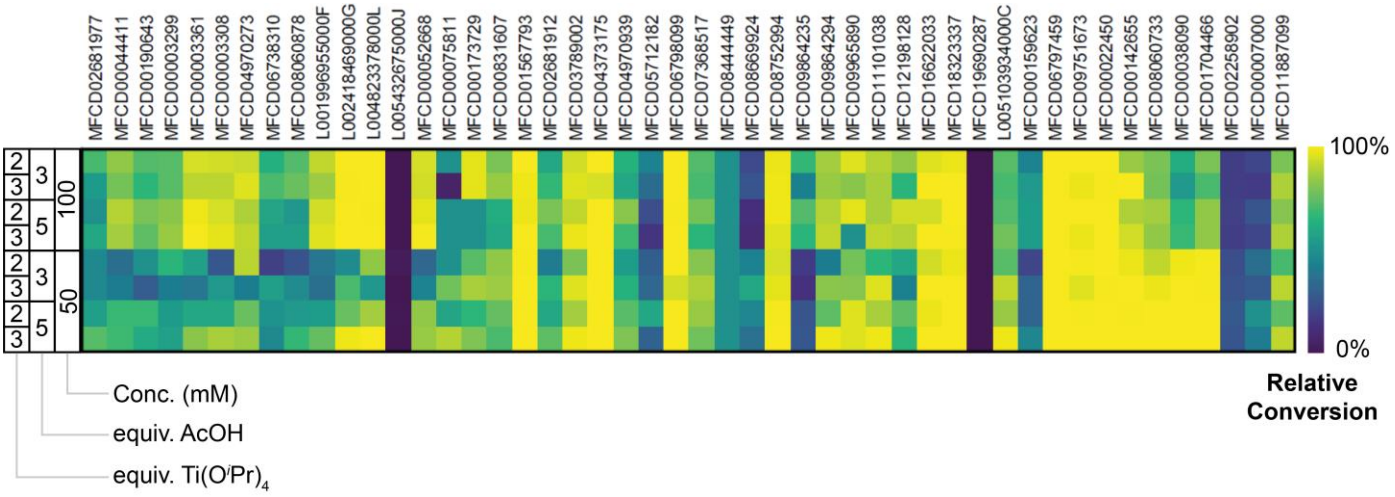
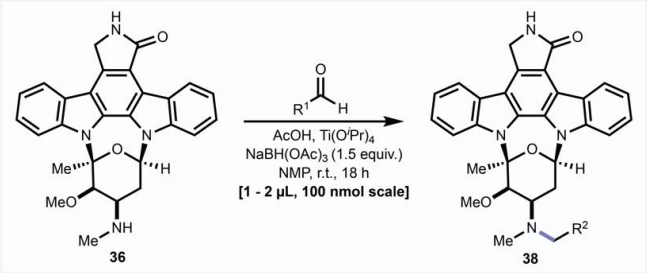


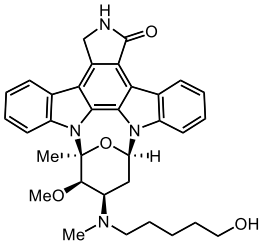
Figure S6: Reductive amination heatmap with specific aldehydes highlighted

5.3. General Procedure for Reductive Amination on Staurosporine and Scale Up:



General procedure: Staurosporine **36** (23 mg, 0.05 mmol, 1 equiv.) was weighed into a 1-dram vial equipped with a magnetic stir bar. The vial was moved to a glove box where NMP was added and the core dissolved. The desired aldehyde building block (1.2 equiv., 0.06 mmol, in NMP) added to the vial, followed by acetic acid (5 equiv., 0.25 mmol, in NMP), and finally titanium tetraisopropoxide (2 equiv., 0.1 mmol, in NMP). The mixture was stirred at ambient temperature in the glove box for 7 hours. Finally, sodium triacetoxyborohydride (STAB, 11.9 mg, 0.075 mmol, 1.5 equiv., added as stock solution in NMP) was added to the mixture, and the reaction stirred for 20 hours. The next morning, aliquots were taken of all reactions and the progress was monitored by LC-MS. Upon completion, the reactions were quenched with sat. aq. NaHCO3 (approx. 750 µL) then diluted with DI water and dichloromethane. The organic phase was separated and the aqueous phased washed three times with DCM. The combined organics were washed with brine, dried with $MgSO_4$, and concentrated. The crude residue was then taken in in 2 mL DMSO, filtered, and product submitted for purification by RP-HPLC.

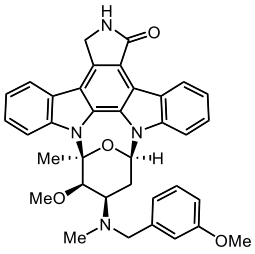
(5S,6R,7R,9R)-7-((5-hydroxypentyl)(methyl)amino)-6-methoxy-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (45)



The title compound was prepared using 5-hydroxypentanal (0.06 mmol, 1.2 equiv., MFCD00044411). The reaction was carried out at the 0.05 mmol scale and stirred for 20 hours at ambient temperature. The product was purified by RP-HPLC to yield 11.5 mg (38% yield) of the title compound as a white solid and TFA salt. Analytical data for **45**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.48 (m, 1H), 9.28 (d, *J* = 7.9 Hz, 1H), 8.62 (s, 1H), 8.06 (t, *J* = 9.4 Hz, 2H), 7.51 (m, 3H), 7.37 (t, *J* = 6.3 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 6.93 (s, 1H), 4.96 (s, 2H), 4.74 (s, 1H), 4.10 (m, 1H), 3.39 (m, 3H), 3.22 (m, 2H), 3.03 (m, 1H), 2.82 (m, 3H), 2.50 (s, 1H), 2.46 (s, 3H), 2.26 (q, *J* = 12.9, 11.4 Hz, 1H), 2.14 (m, 3H), 1.74 (m, 1H), 1.62 (m, 1H), 1.43 (m, 2H), 1.34 (m, 2H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.9, 158.2, 158.0, 137.7, 136.3, 132.3, 129.7, 126.3, 126.29, 125.7, 125.4, 125.36, 123.9, 122.7, 121.8, 120.8, 119.8, 119.7, 114.8, 114.3, 112.8, 109.3, 93.8, 93.7, 81.3, 60.4, 60.35, 59.3, 59.1, 59.0, 45.4, 37.9, 37.5, 32.0, 31.9, 27.2, 27.1, 26.8, 22.7; **HRMS** (ESI): *m/z* calculated for $C_{33}H_{37}N_4O_4$ [M+H]+ 553.28093, found 553.28134.

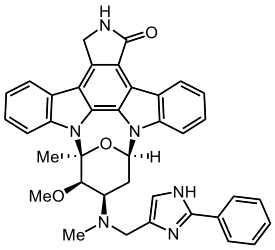
(5S,6R,7R,9R)-6-methoxy-7-((3-methoxybenzyl)(methyl)amino)-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (46)



The title compound was prepared using 3-methoxybenzaldehyde (0.06 mmol, 1.2 equiv., MFCD00003361). The reaction was carried out at the 0.05 mmol scale and stirred for 20 hours at ambient temperature. The product was purified by RP-HPLC to yield 17.8 mg (56% yield) of the title compound as a white solid and TFA salt. Analytical data for **46**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.92 (br m, 1H), 9.32 (d, *J* = 7.9 Hz, 1H), 8.65 (s, 1H), 8.10 (d, *J* = 7.7 Hz, 1H), 8.07 (d, *J* = 8.5 Hz, 1H), 7.54 (m, 3H), 7.41 (t, *J* = 7.2 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.04 (m, 4H), 5.00 (s, 2H), 4.88 (br s, 1H), 4.66 (br s, 1H), 4.23 (br m, 2H), 3.78 (s, 3H), 3.47 (br s, 1H), 2.65 (br s, 3H), 2.50 (br s, 2H) 2.41 (br s, 1H), 2.23 (br s, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.9, 159.4, 158.2 (q, J = 33.4 Hz, TFA), 137.7, 136.3, 132.3, 130.1, 129.7, 126.3, 125.8, 125.5, 125.4, 123.9, 122.7, 121.8, 120.8, 119.8, 119.7, 117.7, 114.9, 114.3, 112.7, 109.3, 81.5, 81.49, 55.2, 45.4, 37.2, 27.4; **HRMS** (ESI): *m/z* calculated for $C_{36}H_{35}N_4O_4$ [M+H]+ 587.26528, found 587.26578.

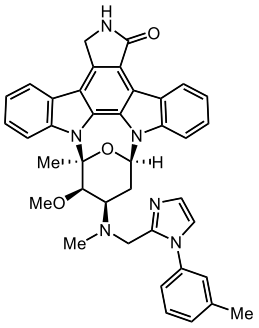
(5S,6R,7R,9R)-6-methoxy-5-methyl-7-(methyl((2-phenyl-1H-imidazol-4-yl)methyl)amino)-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (47)



The title compound was prepared using 2-phenyl-1H-imidazole-4-carbaldehyde (0.06 mmol, 1.2 equiv., MFCD00173729). The reaction was carried out at the 0.05 mmol scale and stirred for 20 hours at ambient temperature. The product was purified by RP-HPLC to yield 29.5 mg (89% yield) of the title compound as a white solid and TFA salt. Analytical data for **47**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.32 (d, *J* = 7.9 Hz, 1H), 8.66 (s, 1H), 8.09 (t, *J* = 8.5 Hz, 2H), 8.02 (d, *J* = 7.6 Hz, 2H), 7.69 (br s, 1H), 7.56 (t, *J* = 7.4 Hz, 4H), 7.49 (dt, *J* = 13.5, 7.3 Hz, 2H), 7.40 (t, *J* = 7.3 Hz, 1H), 7.32 (t, *J* = 7.4 Hz, 1H), 6.96 (d, *J* = 8.3 Hz, 1H), 5.00 (s, 2H), 4.92 (br s, 1H), 4.49 (d, *J* = 13.9 Hz, 1H), 4.41 (d, *J* = 13.5 Hz, 1H), 4.15 (br s, 1H), 3.44 (br s, 1H), 2.80 (s, 3H), 2.52 (s, 3H), 2.38 (t, *J* = 11.5 Hz, 1H), 2.29 (s, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.9, 158.5 (q, *J* = 35.3 Hz, TFA), 146.2, 137.8, 136.3, 132.3, 129.8, 129.1, 126.3, 125.8, 125.6, 125.4, 125.4, 124.0, 122.7, 121.8, 120.8, 119.8, 119.7, 117.2, 114.9, 114.8, 114.3, 112.7, 109.3, 93.8, 81.4, 78.6, 59.3, 58.3, 49.4, 45.4, 38.0, 27.5, 27.0; **HRMS** (ESI): *m/z* calculated for $C_{38}H_{35}N_6O_3$ [M+H]+ 623.27652, found 623.27667.

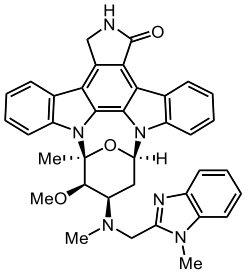
(5S,6R,7R,9R)-6-methoxy-5-methyl-7-(methyl((1-(m-tolyl)-1H-imidazol-2-yl)methyl)amino)-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (48)



The title compound was prepared using 1-(m-tolyl)-1H-imidazole-2-carbaldehyde (0.06 mmol, 1.2 equiv). The reaction was carried out at the 0.05 mmol scale and stirred for 20 hours at ambient temperature. The product was purified by RP-HPLC to yield 18.5 mg (55% yield) of the title compound as a white solid and TFA salt. Analytical data for **48**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.31 (d, *J* = 7.9 Hz, 1H), 8.60 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 1H), 8.00 (d, *J* = 8.5 Hz, 1H), 7.86 (s, 1H), 7.74 (s, 1H), 7.51 (m, 2H), 7.37 (m, 7H), 6.83 (dd, *J* = 9.3, 2.9 Hz, 1H), 4.98 (s, 2H), 4.25 (s, 1H), 4.16 (d, *J* = 15.8 Hz, 1H), 3.68 (d, *J* = 15.7 Hz, 1H), 3.39 (m, 1H), 2.52 (s, 1H), 2.39 (s, 3H), 2.31 (s, 3H), 2.27 (s, 3H), 2.12 (s, 3H), 2.06 (s, 1H), 2.02 (m, 1H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.0, 158.3, 158.0, 139.6, 137.9, 136.2, 134.7, 132.2, 130.7, 129.9, 129.5, 126.1, 126.07, 125.7, 125.2, 124.0, 123.8, 122.9, 122.7, 121.6, 120.4, 119.5, 119.4, 114.7, 114.0, 112.7, 109.1, 94.7, 82.4, 80.6, 58.4, 57.7, 46.9, 45.4, 27.7, 27.4, 20.7, 1.2; **HRMS** (ESI): *m/z* calculated for $C_{39}H_{37}N_6O_3$ [M+H]+ 637.29217, found 637.29237.

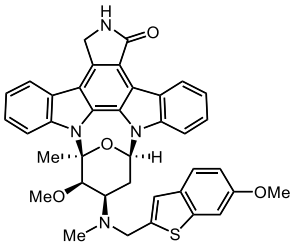
(5S,6R,7R,9R)-6-methoxy-5-methyl-7-(methyl((1-methyl-1H-benzo[d]imidazol-2-yl)methyl)amino)-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (49)



The title compound was prepared using 1-methyl-1H-benzo[d]imidazole-2-carbaldehyde (0.06 mmol, 1.2 equiv., MFCD00142655). The reaction was carried out at the 0.05 mmol scale and stirred for 20 hours at ambient temperature. The product was purified by RP-HPLC to yield 15.1 mg (46% yield) of the title compound as a white solid and TFA salt. Analytical data for **49**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.31 (d, *J* = 7.9 Hz, 1H), 8.60 (s, 1H), 8.06 (m, 2H), 7.80 (d, *J* = 7.6 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 1H), 7.56 (d, *J* = 8.1 Hz, 1H), 7.50 (m, 4H), 7.37 (t, *J* = 7.4 Hz, 1H), 7.29 (t, *J* = 7.4 Hz, 1H), 6.94 (dd, *J* = 9.0, 2.7 Hz, 1H), 4.98 (d, *J* = 17.6 Hz, 1H), 4.93 (d, *J* = 17.7 Hz, 1H), 4.51 (s, 1H), 4.41 (d, *J* = 16.1 Hz, 1H), 4.13 (d, *J* = 16.2 Hz, 1H), 3.79 (s, 3H), 3.72 – 3.58 (m, 1H), 3.05 (br s, 1H), 2.47 (s, 6H), 2.39 (t, *J* = 12.4 Hz, 1H), 2.35 (s, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.0, 158.0 (q, *J* = 32.3 Hz), 138.0, 136.3, 133.8, 132.2, 129.9, 126.2, 125.7, 125.2, 125.18, 124.6, 123.8, 122.7, 121.6, 120.4, 119.5, 119.4, 114.8, 114.0, 112.9, 111.9, 109.1, 94.6, 82.4, 81.3, 58.7, 57.6, 48.3, 45.3, 30.6, 27.8, 27.5; **HRMS** (ESI): *m/z* calculated for $C_{37}H_{34}N_6O_3$ [M+H]+ 611.27652, found 611.27705.

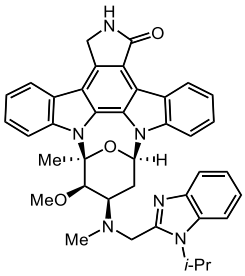
(5S,6R,7R,9R)-6-methoxy-7-(((6-methoxybenzo[b]thiophen-2-yl)methyl)(methyl)amino)-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (50)



The title compound was prepared using 6-methoxybenzo[b]thiophene-2-carbaldehyde (0.06 mmol, 1.2 equiv., MFCD11101038). The reaction was carried out at the 0.05 mmol scale and stirred for 20 hours at ambient temperature. The product was purified by RP-HPLC to yield 12.8 mg (38% yield) of the title compound as a white solid and TFA salt. Analytical data for **50**:

^{1}H NMR (600 MHz, DMSO-d_6) 10.03 (s, 1H), 9.32 (d, *J* = 7.8 Hz, 1H), 8.65 (s, 1H), 8.31 (s, 1H), 8.08 (dd, *J* = 12.5, 8.5 Hz, 2H), 7.97 (d, *J* = 8.8 Hz, 1H), 7.79 (s, 1H), 7.65 (s, 1H), 7.63 – 7.45 (m, 5H), 7.40 (s, 1H), 7.32 (t, *J* = 7.2 Hz, 1H), 7.10 (d, *J* = 8.8 Hz, 1H), 7.03 (s, 1H), 6.96 (s, 1H), 5.00 (s, 2H), 4.83 (br m, 4H), 4.16 (br m, 2H), 4.00 (br m, 2H), 3.86 (s, 3H), 3.82 (s, 3H), 3.40 (br s, 1H), 2.74 (br m, 3H), 2.49 (br s, 3H), 2.31 (br s, 3H); δ **^{13}C NMR** (126 MHz, DMSO-d_6) δ 185.5, 171.9, 160.1, 158.2, 158.0, 144.1, 140.4, 136.3, 136.2, 132.5, 132.3, 129.8, 127.6, 126.2, 125.8, 125.3, 123.9, 122.7, 121.8, 119.6, 116.3, 114.8, 114.242 109.2, 105.3, 105.0, 55.7, 55.5, 45.4, 27.6; **HRMS** (ESI): *m/z* calculated for $C_{38}H_{34}N_4O_4$ [M+H]+ 643.23735, found 643.23764.

(5S,6R,7R,9R)-7-(((1-isopropyl-1H-benzo[d]imidazol-2-yl)methyl)(methyl)amino)-6-methoxy-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (S1)



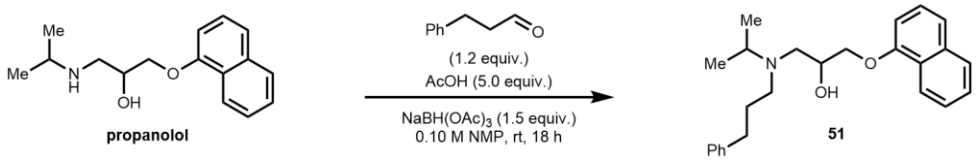
The title compound was prepared using 1-isopropyl-1H-benzo[d]imidazole-2-carbaldehyde (0.06 mmol, 1.2 equiv., MFCD04373175). The reaction was carried out at the 0.05 mmol scale and stirred for 20 hours at ambient temperature. The product was purified by RP-HPLC to yield 22.2 mg (66% yield) of the title compound as a white solid and TFA salt. Analytical data for **S1**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 9.33 (d, *J* = 7.9 Hz, 1H), 8.63 (s, 1H), 8.09 (d, *J* = 8.2 Hz, 2H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.83 (d, *J* = 7.6 Hz, 1H), 7.58 (d, *J* = 8.1 Hz, 1H), 7.51 (m, 4H), 7.39 (t, *J* = 7.4 Hz, 1H), 7.31 (t, *J* = 7.4 Hz, 1H), 6.96 (dd, *J* = 9.2, 2.9 Hz, 1H), 5.00 (s, 2H), 4.95 (m, 1H), 4.52 (s, 1H), 4.45 (d, *J* = 16.0 Hz, 1H), 4.29 (d, *J* = 16.0 Hz, 1H), 3.73 – 3.60 (m, 1H), 3.07 (d, *J* = 13.0 Hz, 1H), 2.49 (s, 3H), 2.45 (s, 3H), 2.43 – 2.35 (m, 1H), 2.29 (s, 3H), 1.68 – 1.52 (m, 6H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.0, 158.3 (q, *J* = 33.9 Hz, TFA), 150.8, 138.0, 136.3, 132.3, 131.2, 130.0, 126.2, 125.7, 125.2, 124.9, 124.9, 123.8,

122.7, 121.7, 120.5, 119.5, 119.45, 117.5, 115.6, 115.2, 114.8, 114.3, 114.0, 112.7, 109.2, 94.8, 82.6, 81.3, 58.7, 57.7, 49.7, 49.0, 45.4, 27.8, 27.3, 20.4, 20.4; **HRMS** (ESI): *m/z* calculated for $C_{39}H_{39}N_6O_3$ [M+H]+ 639.30782, found 639.30782.

5.4. Derivatization of Drug Molecules

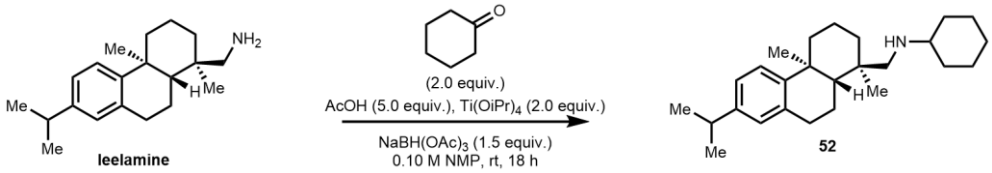
1-(isopropyl(3-phenylpropyl)amino)-3-(naphthalen-1-yloxy)propan-2-ol (51)



Propranolol (89 mg, 1.0 equiv., 0.3 mmol) was weighed into a 1-dram vial equipped with a magnetic stir bar, NMP was added under nitrogen. 3-Phenylpropionaldehyde (47 μL, 1.2 equiv., 0.36 mmol, in NMP) was added to the vial, followed by acetic acid (86 μL, 5 equiv., 1.5 mmol, in NMP). The mixture was stirred at ambient temperature for 1 h. Finally, sodium triacetoxyborohydride (STAB, 95 mg, 1.5 equiv., 0.45 mmol, in NMP) was added to the mixture, and the reaction stirred for 18 hours. Aliquots were taken and the progress was monitored by LC-MS. Upon completion, the reaction was quenched with sat. aq. $NaHCO_3$ (approx. 5 mL) then diluted with DI water and ethyl acetate. The organic phase was separated and the aqueous phased washed three times with ethyl acetate. The combined organics were washed with brine, dried with $MgSO_4$, filtered and concentrated. The crude residue was purified by silica flash column chromatography to yield 43.1 mg (38% yield) of the title compound as a pale yellow solid.
Analytical data for **51**:

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.30 (d, *J* = 8.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 1H), 7.53 – 7.47 (m, 2H), 7.45 (d, *J* = 8.1 Hz, 1H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.31 (t, *J* = 7.6 Hz, 2H), 7.25 – 7.17 (m, 3H), 6.85 (d, *J* = 7.5 Hz, 1H), 4.24 (dd, *J* = 9.1, 4.6 Hz, 1H), 4.18 – 4.08 (m, 2H), 3.80 (s, br, 1H), 3.07 (hept, *J* = 6.7 Hz, 1H), 2.75 – 2.52 (m, 6H), 1.94 – 1.79 (m, 2H), 1.11 (d, *J* = 6.7 Hz, 3H), 1.02 (d, *J* = 6.5 Hz, 3H); **^{13}C NMR** (126 MHz, $CDCl_3$) δ 154.7, 142.1, 134.6, 128.5, 128.4, 127.6, 126.5, 126.0, 125.9, 125.7, 125.3, 122.1, 120.6, 104.9, 70.7, 66.0, 52.7, 50.7, 50.2, 33.8, 30.9, 20.4, 16.4; **MS** (ESI): m/z calculated for $C_{25}H_{32}NO_2$ [M+H]+ 378.2428, found 378.2429.

N-(((1R,4aS,10aR)-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl)methyl)cyclohexanamine (52)

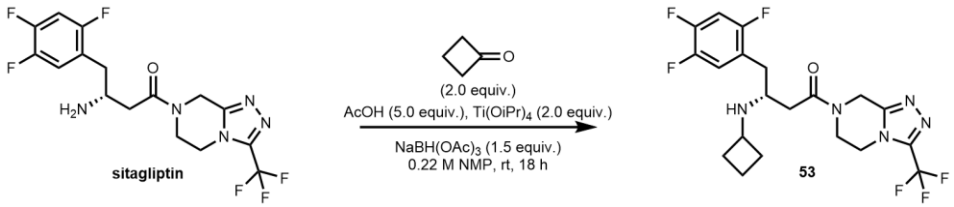


Leelamine (86 mg, 1 equiv., 0.3 mmol) was weighed into a 2-dram vial equipped with a magnetic stir bar, NMP was added under nitrogen. Cyclohexanone (37 mL, 1.2 equiv., 0.36 mmol, in NMP) was added to the vial, followed by acetic acid (86 mL, 5 equiv., 1.5 mmol, in NMP) and Ti(O^iPr)$_4$ (0.18 mL, 2 equiv., 0.6 mmol, in NMP). The mixture was stirred at ambient temperature for 1 h. Finally, sodium triacetoxyborohydride (STAB, 95 mg, 1.5 equiv., 0.45 mmol, in NMP) was added to the mixture, and the reaction stirred for 18 hours. Aliquots were taken and the progress was monitored by LC-MS. Upon completion, the reaction was quenched with sat. aq. $NaHCO_3$ (approx. 5 mL) then diluted with DI water and ethyl acetate. The organic phase was separated and the aqueous phase washed three times with ethyl acetate. The combined organics

were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was then purified by RP-HPLC to yield 60.5 mg (42% yield) of the TFA salt of the title compound as a white solid.
Analytical data for **52**:

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.89 (br, s, 2H), 7.16 (d, *J* = 8.2 Hz, 1H), 6.96 (dd, *J* = 8.2, 2.0 Hz, 1H), 6.87 (d, *J* = 2.0 Hz, 1H), 3.01 – 2.89 (m, 2H), 2.89 – 2.72 (m, 4H), 2.27 (d, *J* = 12.7 Hz, 1H), 2.11 – 2.02 (m, 2H), 1.79 – 1.62 (m, 7H), 1.54 – 1.46 (m, 2H), 1.39 – 1.19 (m, 7H), 1.15 (d, *J* = 7.1 Hz, 6H), 1.14 (s, 3H), 0.98 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 146.8, 145.2, 134.3, 126.4, 123.9, 123.6, 58.0, 54.7, 44.1, 37.4, 37.0, 35.8, 34.5, 32.9, 28.9, 28.1, 28.0, 24.9, 24.7, 24.2, 24.0, 23.9, 18.2, 18.0, 17.8; **MS** (ESI): *m/z* calculated for $C_{26}H_{42}N$ [M+H]+ 368.3312, found 368.3312.

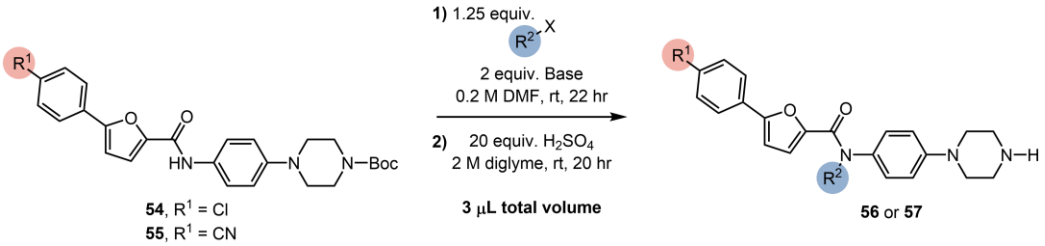
(*R*)-3-(cyclobutylamino)-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8*H*)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (53)



Sitagliptin (120 mg, 1 equiv., 0.3 mmol) was weighed into a 1-dram vial equipped with a magnetic stir bar, NMP was added and the core dissolved. Cyclobutanone (45 μL, 2.0 equiv., 0.6 mmol, in NMP) was added to the vial, followed by acetic acid (86 μL, 5 equiv., 1.5 mmol, in NMP) and $Ti(O^iPr)_4$ (0.18 mL, 2 equiv., 0.6 mmol, in NMP). The mixture was stirred at ambient temperature for 1 h. Finally, sodium triacetoxyborohydride (STAB, 95 mg, 1.5 equiv., 0.45 mmol, in NMP) was added to the mixture, and the reaction stirred for 18 h. Aliquots were taken and the progress was monitored by LC-MS. Upon completion, the reaction was quenched with sat. aq. $NaHCO_3$ (approx. 2 mL) then diluted with DI water and ethyl acetate. The organic phase was separated and the aqueous phased washed three times with ethyl acetate. The combined organics were washed with brine, dried with $MgSO_4$, filtered, and concentrated. The crude residue was purified by RP-HPLC with 0.1% formic acid additive, to give the formic acid salt of the title compound as a white solid, 54.2 mg (39%).
Analytical data for **53** (the compound appears as a 58:42 (Rot. A: Rot. B) mixture of rotamers on the NMR timescale at 298 K as indicated):

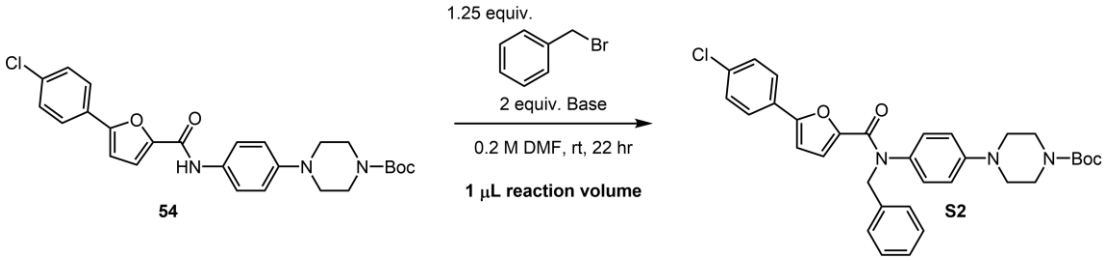
^{1}H NMR (500 MHz, CD_3OD) δ 8.42 (s, 1H, formate **H**CO_2^-), 7.41 – 7.30 (m, 1H), 7.24 – 7.12 (m, 1H), 5.06 (d, *J* = 17.6 Hz, 1H, Rot. A), 4.99 – 4.88 (m, 2H, Rot. B), 4.90 (d, *J* = 17.6 Hz, 1H, Rot. A), 4.34 – 4.17 (m, 2H), 4.16 – 4.03 (m, 1H), 4.03 – 3.84 (m, 2H), 3.78 – 3.69 (m, 1H), 3.27 – 3.17 (m, 1H), 3.06 – 2.96 (m, 1H), 2.90 – 2.80 (m, 2H), 2.41 –2.29 (m, 3H), 2.25 (quintet, *J* = 9.8 Hz, 1H), 1.97 – 1.84 (m, 2H); **^{13}C NMR** (126 MHz, CD_3OD) δ 170.7 (Rot. A), 170.5 (Rot. B), 169.1 (formate H**C**O_2^-), 157.9 (ddd, $^1J_{C\text{-}F}$ = 243.8 Hz, $^3J_{C\text{-}F}$ = 9.0 Hz, $^4J_{C\text{-}F}$ = 7.5 Hz), 152.5 (Rot. A), 152.0 (Rot. B), 150.9 (dddd, $J_{C\text{-}F}$ = 250.2 Hz, 14.5 Hz, 13.0 Hz, 3.0 Hz), 148.2 (ddd, $^1J_{C\text{-}F}$ = 243.1 Hz, $^2J_{C\text{-}F}$ = 13.2 Hz, $^4J_{C\text{-}F}$ = 3.1 Hz), 144.7 (q, $^2J_{C\text{-}F}$ = 40.0 Hz, Rot. B), 144.7 (q, $^2J_{C\text{-}F}$ = 40.5 Hz, Rot. A), 121.0 (ddd, $^2J_{C\text{-}F}$ = 18.0 Hz, $^3J_{C\text{-}F}$ = 10.8 Hz, $^4J_{C\text{-}F}$ = 5.3 Hz), 120.6 (dt, $^2J_{C\text{-}F}$ = 19.9 Hz, $^3J_{C\text{-}F}$ = 5.0 Hz), 119.6 (q, $^1J_{C\text{-}F}$ = 269.5 Hz), 106.9 (ddd, $^2J_{C\text{-}F}$ = 29.0 Hz, $^2J_{C\text{-}F}$ = 21.5 Hz, $^3J_{C\text{-}F}$ = 5.6 Hz), 54.5 (Rot. B), 54.4 (Rot. A), 51.6 (Rot. A), 51.6 (Rot. B), 44.9 (Rot. A), 44.5 (Rot. B), 43.0 (Rot. B), 42.3 (Rot. A), 39.8 (Rot. A), 39.1 (Rot. B), 33.7 (Rot. B), 33.7 (Rot. A), 30.9, 28.4, 28.1 (Rot. B), 28.0 (Rot. A), 15.8; **^{19}F NMR** (500 MHz, CD_3OD) δ -64.6 (Rot. A), -64.6 (Rot. B), -119.8 – -120.0 (m, Rot. A), -120.1 – 120.3 (m, Rot. B), -136.6 – -136.8, -144.3 – -144.6; **MS (ESI)**: m/z calculated for $C_{20}H_{22}F_6N_5O$ [M+H]+ 462.1723, found 462.1715.

6. Nanomole-Scale Amide *N*-Alkylation and Boc-Deprotection



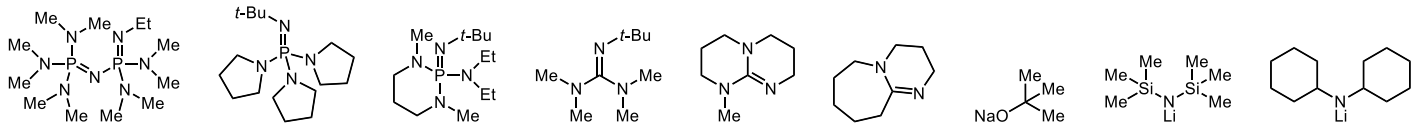
Automation-friendly amide *N*-alkylation and Boc-deprotection was explored at the nanomole-scale. Optimization of both reactions was done at the nanomole-scale in a 1,536-well microplate, seeking productive homogenous reaction conditions (utilizing soluble organic superbases in place of conventional bases such as NaH). Reaction preparation and all reagent dispensing was performed using the SPT Labtech Mosquito®HTS liquid handling robot and reagent stock solutions. An amide *N*-alkylation library was then constructed around the core shown above (R^1 = Cl and CN) at both the 240 nanomole-scale at the 40 micromole-scale to show reproducibility and scalability of the automation-friendly conditions. Alkylation of this core using conventional methodology was disclosed for the generation of MK-2 inhibitors and for this reason it was selected as a good starting point for the development of automation-friendly alkylation conditions.

6.1. Amide *N*-Alkylation Base Optimization



Tert-butyl 4-(4-(5-(4-chlorophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate (**54**) (200 nmol, 1 equiv.) was combined with base (400 nmol, 2 equiv.) in DMF in a microplate using the Mosquito®HTS. The reagents were mixed using Mosquito®HTS and sat at ambient temperature for 1 hour. After 1 hour a stock solution of benzyl bromide in DMF (250 nmol, 1.25 equiv.) was added to each reaction using the Mosquito®HTS and the reactions were again mixed to give a total reaction volume of 1 µL at 200 nmol scale. The next morning the reactions were quenched and diluted via addition of 3 µL DMSO solution containing AcOH and PhPh as an internal standard. 1 µL of each quenched and diluted reaction was then transferred to 60 µL DMSO for analysis by UPLC. Product **S2** was detected by mass spectrometry and reaction efficiency judged by an approximate conversion calculation: UV_{PDT} / (UV_{PDT} + UV_{CORE}) * 100 and/or UV_{PDT} / UV_{IS}

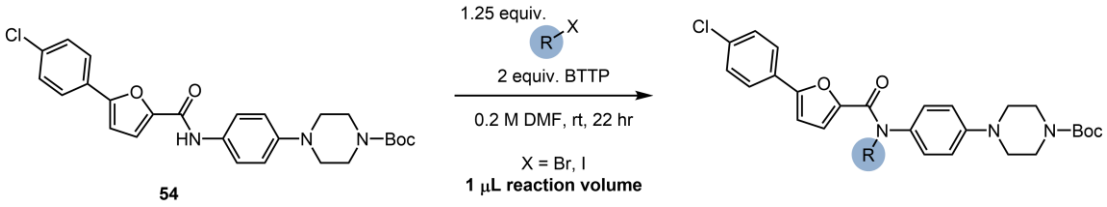
Nine bases were tested in the nano-scale amide alkylation reaction, the results are shown below:



P_2-Et	BTTP	BEMP	BTMG	MTBD	DBU	NaO*t*-Bu	LiHMDS	Cy_2NLi
76% conv.	**100% conv.**	**100% conv.**	**47% conv.**	**10% conv.**	**0% conv.**	**94% conv.**	**61% conv.**	**49% conv.**

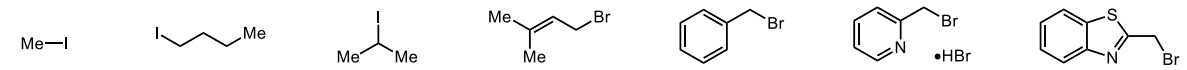
Phosphazene base such as P_2-Et, BTTP, and BEMP, along with certain organometallic bases were most effective in the nano-scale alkylation. Less basic guanidine (e.g. BTMG or MTBD) or amidine bases (DBU) were not effective in this model system.

6.2. Amide *N*-Alkylation Electrophile Study



Tert-butyl 4-(4-(5-(4-chlorophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate (**54**) (200 nmol, 1 equiv) was combined with BTTP (base, 400 nmol, 2 equiv.) in DMF in a microplate using the Mosquito®HTS. The reagents were mixed using Mosquito®HTS and sat at ambient temperature for 1 hour. After 1 hour a stock solution of alkyl halide electophile in DMF (250 nmol, 1.25 equiv.) was added to each reaction using the Mosquito®HTS and the reactions were again mixed to give a total reaction volume of 1 µL at 200 nmol scale. The next morning the reactions were quenched and diluted via addition of 3 µL DMSO solution containing AcOH and PhPh as an internal standard. 1 µL of each quenched and diluted reaction was then transferred to 60 µL DMSO for analysis by UPLC. Product was detected by mass spectrometry and reaction efficiency judged by an approximate conversion calculation: UV_{PDT} / (UV_{PDT} + UV_{CORE}) * 100 and/or UV_{PDT} / UV_{IS}

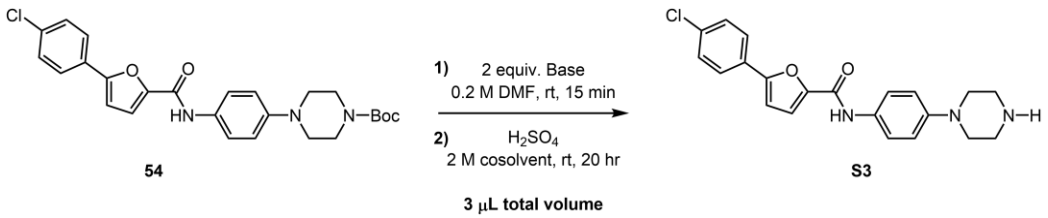
Seven electrophiles were tested in the nano-scale amide alkylation reaction, employing conditions discovered in the base screen above. The results of this study are shown below:



55% conv. **83% conv.** **<5% conv.** **96% conv.** **100% conv.** **74% conv.** (free based with 1 equiv. BTTP prior to addition) **94% conv.**

The alkylation protocol developed here is effective for the miniaturized alkylation of amides with primary alkyl iodides and allylic/benzylic bromides. Conversion from starting material to product was lower than expected with MeI (relative to BuI) but this may be due to the high volatility of methyl iodide at such a small scale. Isopropyl iodide did not deliver any product and 2-(bromomethyl)pyridine delivered alkylation product with high conversion, but only if the electrophile was free-based first before addition (HBr salt was used as shown).

6.3. Boc Deprotection Optimization



Base and core were first combined using Mosquito®HTS to mimic the conditions for nano-scale alkylation. Sulfuric acid, in a variety of cosolvents, was then added to the reactions and mixed. The reactions were monitored for Boc-

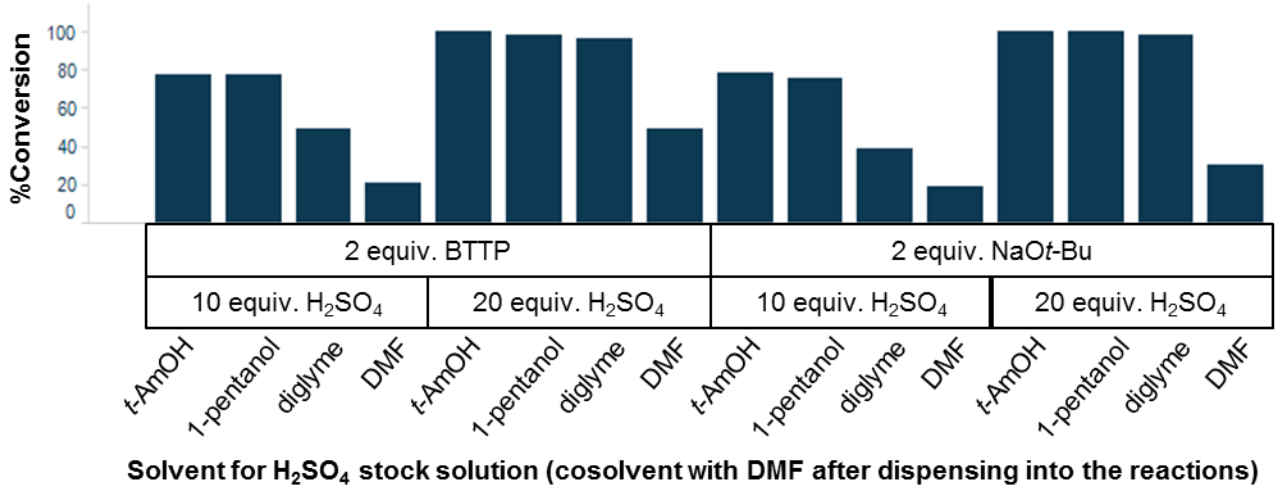
deprotection product by LC-MS. A variety of acids were screened including arylsulfonic acids, methanesulfonic acid, sulfuric acid, trichloroacetic acid, and phosphoric acid (screening not shown). Through this screening sulfuric acid was identified as a nano-scale compatible acid for Boc-deprotection. Traditional acids to effect deprotection such as HCl or TFA were avoided due to volatility concerns and possible incompatibility to the Mosquito®HTS (arising from volatility). Likewise, traditional deprotection solvents such as dioxane or DCM were excluded due to volatility concerns at the nanomole-scale.

Four H_2SO_4 stock solution solvents were tested: *t*-AmOH, 1-pentanol, diglyme, and DMF. After dispense mixing these are Boc-deprotection reaction cosolvents (with the exception of DMF as DMF is the alkylation reaction solvent).

Two loadings of H_2SO_4 were tested: 10 equiv. and 20 equiv.

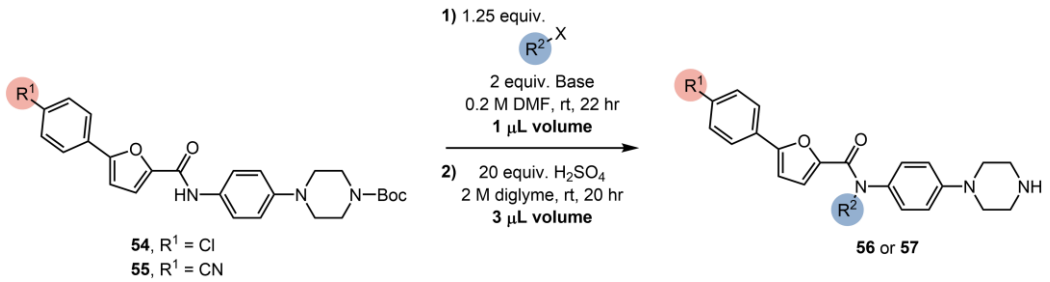
Two bases identified in the base screen were mixed with the amide core to mimic the alkylation reaction conditions: BTTP and NaO*t*-Bu

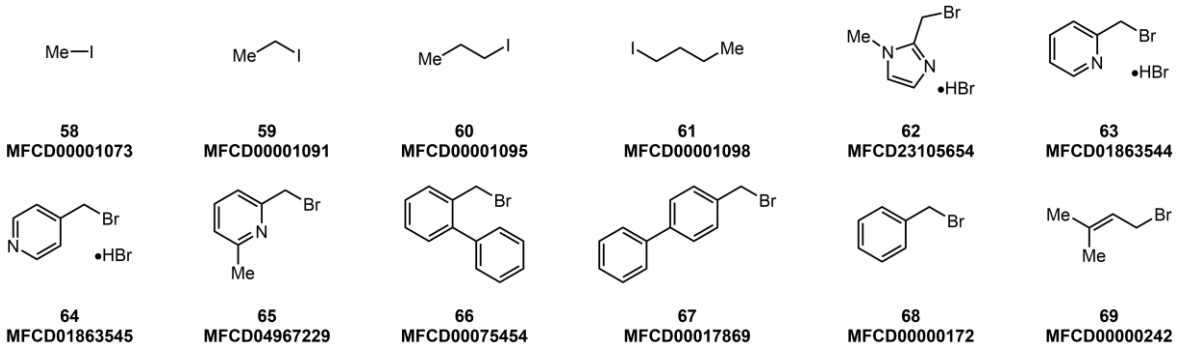
Procedure: Tert-butyl 4-(4-(5-(4-chlorophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate (**45-Cl**) (200 nmol, 1 equiv) was combined with base (BTTP or NaO*t*-Bu, 400 nmol, 2 equiv.) in DMF in a microplate using the Mosquito®HTS. The reagents were mixed using Mosquito®HTS and sat at ambient temperature for 15 minutes. 2 µL of a stock solution of sulfuric acid in the desired cosolvent (2 µmol or 4 µmol, 10 or 20 equiv. respectively) was then added to each reaction using the Mosquito®HTS and the reactions were again mixed to give a total reaction volume of 3 µL at 200 nmol scale (1 µL DMF + 2 µL deprotection cosolvent). The next morning the reactions were quenched with 2 µL sat. aq. $NaHCO_3$ and diluted via addition of 2 µL DMSO solution containing PhPh as an internal standard. 1.2 µL of each quenched and diluted reaction was then transferred to 63 µL DMSO for analysis by UPLC. Product **S3** was detected by mass spectrometry and reaction efficiency judged by an approximate conversion calculation: UV_{PDT} / (UV_{PDT} + UV_{CORE}) * 100 and/or UV_{PDT} / UV_{IS}



From this screening 20 equiv. H_2SO_4 in diglyme were advanced as nano-scale compatible Boc deprotection conditions.

6.4. Nanomole-Scale *N*-Alkylation/Deprotection Library Synthesis





Two cores were tested in nanoscale alkylation-deprotection (0.4 M stock solutions in DMF):

tert-butyl 4-(4-(5-(4-chlorophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate, **54**

tert-butyl 4-(4-(5-(4-cyanophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate , **55**

List of twelve alkyl, allylic, and benzylic halides used in this study (MDL numbers, 1.0 M stock solutions in DMF): MFCD00001073, MFCD00001091, MFCD00001095, MFCD00001098, MFCD23105654, MFCD01863544, MFCD01863545, MFCD04967229, MFCD00075454, MFCD00017869, MFCD00000172, and MFCD00000242

NOTE: any electrophiles which were delivered as HBr salts were freebased before use in the alkylation.

Four unique alkylation conditions were screened in the synthesis of the above-depicted library (1 solvent, 4 bases). One Boc deprotection condition (20 equiv. H_2SO_4 in diglyme). Alkylation conditions:

DMF – P_2-Et

DMF – BTTP

DMF – NaO*t*-Bu

DMF – LiHMDS

All chemistry and nanoliter pipetting was done under an N_2 atmosphere in a glove box. Stock solutions of the cores (0.4 M in DMF), electrophile building blocks (1 M in DMF), and bases (1.6 M in DMF), and deprotection acid (H_2SO_4 in diglyme, 2 M) were prepared in the glove box. A source plate was constructed using the reagent stock solutions, according to the diagram below, by pipetting 16-60 μL of the correct component stock into the wells shown in Figure S7. The SPT Labtech Mosquito® was used to assemble the reaction mixtures, utilizing the multi-aspiration feature and dispense mixing.

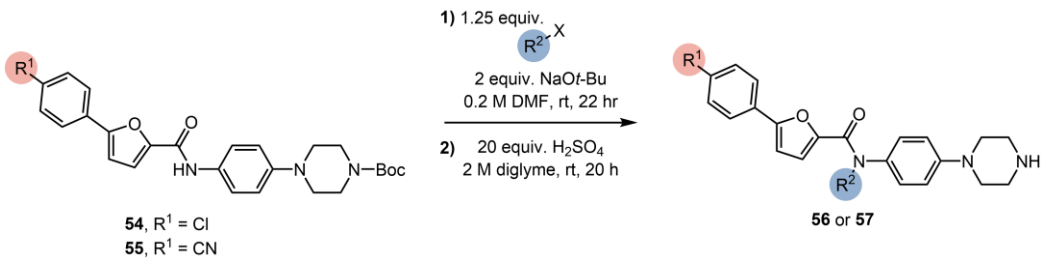
	Core Column 1 0.4 M in DMF 16 uL	Core Column 2 0.4 M in DMF 16 uL	DMF Wash 60 uL	Electrophile 1.0 M in DMF 20 uL	DMF Wash 60 uL		Base 1 1.6 M in DMF 30 uL	Base 2 1.6 M in DMF 30 uL	Base 3 1.6 M in DMF 30 uL	Base 4 1.6 M in DMF 30 uL			Acid Stock for Boc Deprotection 2.0 M in Diglyme 30 uL										
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00001073	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
B	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00001091	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
C	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00001095	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
D	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00001098	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
E	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD23105654	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
F	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD01863544	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
G	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD01863545	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
H	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD04967229	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
I	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00075454	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
J	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00017869	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
K	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00000172	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
L	Core p-Cl in DMF	Core p-CN in DMF	DMF	MFCD00000242	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
M	Core p-Cl in DMF	Core p-CN in DMF	DMF	BLANK	DMF		P2-Et in DMF	BTTP in DMF	NaOtBu in DMF	LiHMDS in DMF			H2SO4 in Diglyme											
N																								
O																								
P																								

Figure S7. Amide Alkylation-Boc Deprotection Source Plate Map

1. 500 nL was drawn up from the desired core column (column 1 or column 2)
2. 5 nL TIP wash (column 3, DMF)
3. 250 nL was drawn up from the desired base column (column 7: P2-Et, column 8: BTTP, column 9: NaOtBu, column 10: LiHMDS)
4. This was immediately followed by dispense mixing (3×500 nL) into the 1,536-well reaction plate to ensure all reagents were adequately combined. This process was repeated 4 times to cover all possible reaction conditions (4 total: 4 bases). The core and base were aged for 1 hour.
5. 250 nL was drawn from the electrophile column (column 4)
6. 5 nL Tip wash (column 5, DMF)
7. This was immediately followed by dispense mixing (3×800 nL) into the 1,536-well reaction plate to ensure all reagents were adequately combined. This was repeated for all reactions.
8. The 1,536-plate was sealed and briefly centrifuged before aging at ambient temperature for 21 hours.
9. 1000 nL was drawn up from the acid column (column 13) and dispensed into each reaction well with dispense mixing (2×800 nL).
10. 1000 nL was drawn up from the acid column (column 13) and dispensed into each reaction well with dispense mixing (3×1000 nL).
11. The 1,536-plate was sealed and briefly centrifuged before aging at ambient temperature for 20 hours.

The next morning, the reactions were quenched by addition of saturated aqueous $NaHCO_3$ (2 µL) and diluted with water (1 µL) and DMSO (2 µL). The reactions were further diluted to 10 mM using DMSO and liquid handling. 1 µL of the quenched and diluted crude reactions were drawn up for analysis by LC-MS (1 µL crude ~10 mM reaction into 60 µL DMSO followed by shaking). Screening results are represented as a heat map of percent yield, where values were calculated using standard curves created from isolated products. The results are presented in Figure 5B of the manuscript. NOTE: elevated yields (above 100% as determined by standard curves) shown for some reactions in may be due to inaccuracies in liquid handling, causing more of the core and reagents to be charged into the reaction drop than expected.

6.5. *N*-Alkylation/Deprotection Library General Procedure and Scale Up



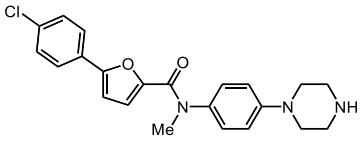
54, R^1 = Cl
55, R^1 = CN

56 or **57**

The amide core shown above (tert-butyl 4-(4-(5-(4-chlorophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate **45** or tert-butyl 4-(4-(5-(4-cyanophenyl)furan-2-carboxamido)phenyl)piperazine-1-carboxylate **46**) was weighed into a 1-dram vial equipped with a magnetic stir bar (19.25 mg or 18.89 mg respectively, 40 µmol, 1 equiv.). The core was then dissolved in DMF and then NaO*t*-Bu (80 µmol, 2 equiv.) was added as a slurry/solution in DMF. The reaction was stirred at room temperature for 30 minutes. Finally, the alkyl/allylic/benzylic halide electrophile was added (50 µmol, 1.25 equiv.) and the alkylation was allowed to stir at room temperature overnight.

The next morning, a 2 M stock solution of H_2SO_4 in diglyme was prepared and 400 µL was dispensed into the reaction vessel (800 µmol, 20 equiv.) and the reaction was stirred overnight at room temperature. The next morning, the reaction was quenched with 500 µL sat. aq. $NaHCO_3$. The reaction was diluted with DMSO, stirred, and further quenched with carbonate base on a solid support. The reaction was filtered and submitted for purification by RP-HPLC. Products were returned as TFA salts unless otherwise noted. Many members of this library were previously disclosed through publication as MK-2 inhibitors.[18,19]

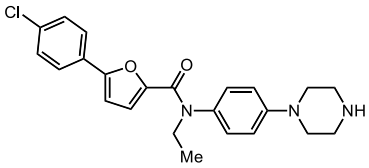
5-(4-chlorophenyl)-N-methyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S4)



The title compound was prepared using the standard conditions described above and iodomethane **58** (MFCD00001073) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 7 mg (34% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S4** matches that previously reported[18]:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.84 (s, 2H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.27 – 7.19 (m, 2H), 7.06 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 3.7 Hz, 1H), 6.52 (d, *J* = 3.8 Hz, 1H), 3.36 (t, *J* = 5.1 Hz, 4H), 3.28 (s, 3H), 3.25 (d, *J* = 7.1 Hz, 4H); **MS** (ESI): *m/z* calculated for $C_{22}H_{23}ClN_3O_2$ [M+H]+ 396.1473, found 396.1705.

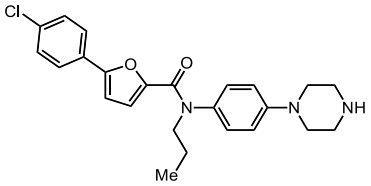
5-(4-chlorophenyl)-N-ethyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (70)



The title compound was prepared using the standard conditions described above and iodoethane **59** (MFCD00001091) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 7.9 mg (38% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **70**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.26 – 7.11 (m, 3H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.94 (d, *J* = 3.6 Hz, 1H), 6.48 (d, *J* = 3.8 Hz, 1H), 3.74 (q, *J* = 7.1 Hz, 2H), 3.43 – 3.31 (m, 4H), 3.26 (s, 4H), 1.09 (t, *J* = 7.1 Hz, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 158.7 ($^2J_{C\text{-}F}$ = 34.7 Hz), 157.6, 153.0, 149.7, 147.2, 134.2, 133.1, 129.0, 129.0, 128.2, 125.7, 118.8, 116.6, 116.2 ($^2J_{C\text{-}F}$ = 296.6 Hz), 107.9, 45.6, 45.0, 43.0, 12.6; **MS** (ESI): *m/z* calculated for $C_{23}H_{25}ClN_3O_2$ [M+H]+ 410.1630, found 410.1658.

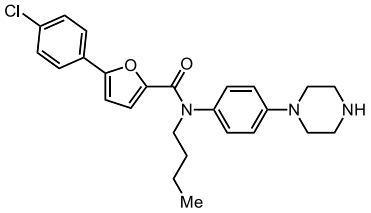
5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-propylfuran-2-carboxamide (S5)



The title compound was prepared using the standard conditions described above and 1-iodopropane **60** (MFCD00001095) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 2.8 mg (17% yield) of the title compound as an off-white residue (not TFA Salt). Analytical data for **S5**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.39 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.4 Hz, 2H), 7.11 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.92 (d, *J* = 3.7 Hz, 1H), 6.45 – 6.33 (m, 1H), 3.66 (t, *J* = 7.5 Hz, 2H), 3.09 (t, *J* = 4.9 Hz, 4H), 2.86 (t, *J* = 4.9 Hz, 4H), 1.51 (q, *J* = 7.5 Hz, 2H), 0.86 (t, *J* = 7.4 Hz, 3H); **MS** (ESI): *m/z* calculated for $C_{24}H_{27}ClN_3O_2$ [M+H]+ 424.1786, found 424.1820.

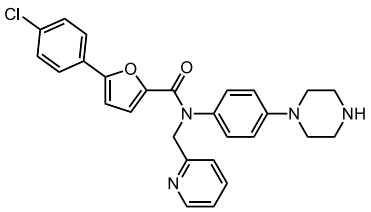
N-butyl-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S6)



The title compound was prepared using the standard conditions described above and 1-iodobutane **61** (MFCD00001098) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 7.9 mg (36% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S6**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.85 (s, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.18 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 3.7 Hz, 1H), 6.52 – 6.38 (m, 1H), 3.71 (t, *J* = 7.5 Hz, 2H), 3.37 (t, *J* = 5.1 Hz, 5H), 3.26 (s, 5H), 1.47 (p, *J* = 7.5 Hz, 2H), 1.29 (q, *J* = 7.5 Hz, 2H), 0.86 (t, *J* = 7.3 Hz, 3H); **MS** (ESI): *m/z* calculated for $C_{25}H_{29}ClN_3O_2$ [M+H]+ 438.1943, found 438.1938.

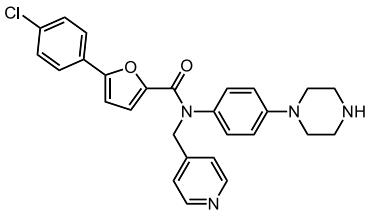
5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-(pyridin-2-ylmethyl)furan-2-carboxamide (S7)



The title compound was prepared using the standard conditions described above and 2-(bromomethyl)pyridine hydrobromide **63** (MFCD01863544) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 11.4 mg (49% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S7**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 2H), 8.53 (d, *J* = 4.8 Hz, 1H), 7.86 (t, *J* = 7.7 Hz, 1H), 7.52 (d, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 3H), 7.29 – 7.18 (m, 2H), 7.03 – 6.93 (m, 3H), 6.53 (s, 1H), 5.08 (s, 2H), 3.34 (t, *J* = 5.0 Hz, 4H), 3.23 (p, *J* = 4.7 Hz, 4H)**; MS** (ESI): *m/z* calculated for $C_{27}H_{26}ClN_4O_2$ [M+H]+ 473.1739, found 473.1761.

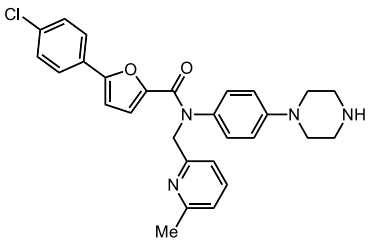
5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-(pyridin-4-ylmethyl)furan-2-carboxamide (S8)



The title compound was prepared using the standard conditions described above and 4-(bromomethyl)pyridine hydrobromide **64** (MFCD01863545) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 2.9 mg (12% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S8**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.84 (s, 2H), 8.64 (d, *J* = 5.4 Hz, 2H), 7.61 (d, *J* = 5.4 Hz, 2H), 7.42 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.2 Hz, 2H), 7.24 – 7.21 (m, 2H), 7.03 – 6.93 (m, 3H), 6.58 (d, *J* = 3.6 Hz, 1H), 5.10 (s, 2H), 3.34 (t, *J* = 5.1 Hz, 4H), 3.23 (s, 4H); **MS** (ESI): *m/z* calculated for $C_{27}H_{26}ClN_4O_2$ [M+H]+ 473.1739, found 473.1761.

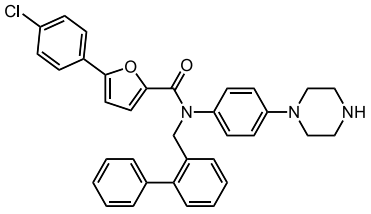
5-(4-chlorophenyl)-N-((6-methylpyridin-2-yl)methyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S9)



The title compound was prepared using the standard conditions described above and 2-(bromomethyl)-6-methylpyridine **65** (MFCD04967229) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 8.3 mg (35% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S9**

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 2H), 7.83 (t, *J* = 7.8 Hz, 1H), 7.41 (dd, *J* = 13.3, 8.0 Hz, 3H), 7.35 (d, *J* = 8.3 Hz, 2H), 7.27 (d, *J* = 8.2 Hz, 3H), 7.03 – 6.94 (m, 3H), 6.54 (s, 1H), 5.07 (s, 2H), 3.34 (t, *J* = 5.0 Hz, 4H), 3.23 (p, *J* = 4.5 Hz, 4H), 2.48 (s, 3H); **MS** (ESI): *m/z* calculated for $C_{28}H_{28}ClN_4O_2$ [M+H]+ 487.1895, found 487.1892.

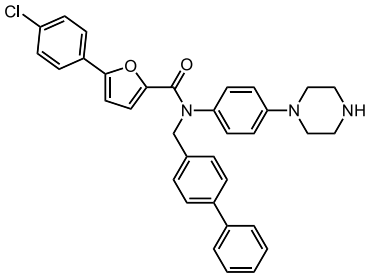
N-([1,1'-biphenyl]-2-ylmethyl)-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S10)



The title compound was prepared using the standard conditions described above and 2-(bromomethyl)-1,1'-biphenyl **68** (MFCD00075454) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 16.7 mg (63% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S10**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (s, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.46 – 7.36 (m, 3H), 7.32 (dq, *J* = 12.4, 7.4, 5.8 Hz, 5H), 7.13 (d, *J* = 7.6 Hz, 1H), 7.09 – 6.98 (m, 2H), 6.95 (d, *J* = 3.7 Hz, 1H), 6.92 (s, 4H), 6.61 – 6.42 (m, 1H), 4.92 (s, 2H), 3.38 – 3.28 (m, 4H), 3.24 (s, 4H); **MS** (ESI): *m/z* calculated for $C_{34}H_{31}ClN_3O_2$ [M+H]+ 548.2099, found 548.2123.

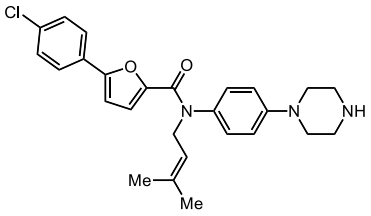
N-([1,1'-biphenyl]-4-ylmethyl)-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (71)



The title compound was prepared using the standard conditions described above and 4-(bromomethyl)-1,1'-biphenyl **69** (MFCD00017869) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 19.3 mg (73% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **71**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (s, 2H), 7.62 (dd, *J* = 15.1, 7.8 Hz, 4H), 7.50 – 7.24 (m, 8H), 7.11 (d, *J* = 8.4 Hz, 2H), 7.04 – 6.90 (m, 3H), 6.56 (d, *J* = 3.8 Hz, 1H), 5.02 (s, 2H), 3.33 (t, *J* = 5.1 Hz, 4H), 3.23 (t, *J* = 5.2 Hz, 4H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 158.9 ($^2J_{C-F}$ = 33.6 Hz), 158.2, 153.2, 149.4, 146.8, 139.8, 139.0, 136.6, 134.2, 133.1, 128.9, 128.9, 128.8, 128.7, 128.1, 127.4, 126.6, 126.6, 125.7, 119.3, 116.4 ($^1J_{C-F}$ = 296.0 Hz), 116.2, 107.9, 53.2, 45.3, 42.7; **MS** (ESI): *m/z* calculated for $C_{34}H_{31}ClN_3O_2$ [M+H]+ 548.2099, found 548.2123.

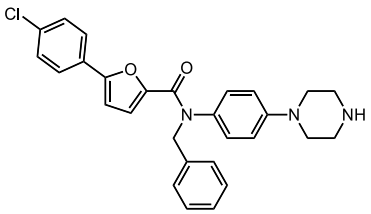
5-(4-chlorophenyl)-N-(3-methylbut-2-en-1-yl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S11)



The title compound was prepared using the standard conditions described above and 1-bromo-3-methylbut-2-ene **67** (MFCD00000242) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 9.5 mg (42% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S11**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.85 (s, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.32 (d, *J* = 8.2 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 3.7 Hz, 1H), 6.50 (d, *J* = 3.8 Hz, 1H), 5.24 (t, *J* = 7.1 Hz, 1H), 4.31 (d, *J* = 6.9 Hz, 2H), 3.36 (t, *J* = 5.0 Hz, 4H), 3.26 (s, 4H), 1.64 (s, 3H), 1.46 (s, 3H); **MS** (ESI): *m/z* calculated for $C_{26}H_{29}ClN_3O_2$ [M+H]+ 450.1943, found 450.1937.

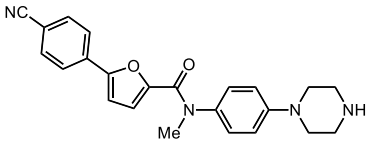
N-benzyl-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S12)



The title compound was prepared using the standard conditions described above and (bromomethyl)benzene **66** (MFCD00000172) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 8.2 mg (35% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S12**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.83 (s, 2H), 7.41 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.29 (t, *J* = 7.5 Hz, 2H), 7.24 (dd, *J* = 13.6, 7.3 Hz, 3H), 7.06 (d, *J* = 8.4 Hz, 2H), 7.02 – 6.90 (m, 3H), 6.54 (d, *J* = 3.9 Hz, 1H), 4.97 (s, 2H), 3.37 – 3.28 (m, 4H), 3.23 (s, 4H); **MS** (ESI): *m/z* calculated for $C_{28}H_{27}ClN_3O_2$ [M+H]+ 472.1786, found 472.1773.

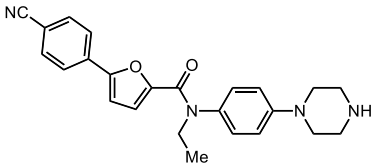
5-(4-cyanophenyl)-N-methyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S13)



The title compound was prepared using the standard conditions described above and iodomethane **58** (MFCD00001073) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 9.5 mg (47% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S13**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.87 (s, 2H), 7.80 (d, *J* = 8.2 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.6 Hz, 2H), 7.19 – 7.09 (m, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.64 (d, *J* = 3.9 Hz, 1H), 3.41 – 3.32 (m, 4H), 3.29 (s, 3H), 3.26 (d, *J* = 6.7 Hz, 4H); **MS** (ESI): *m/z* calculated for $C_{23}H_{23}N_4O_2$ [M+H]+ 387.1816, found 387.1810.

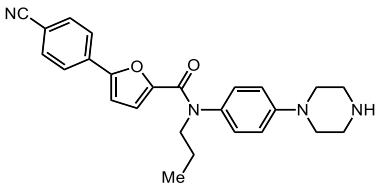
5-(4-cyanophenyl)-N-ethyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S14)



The title compound was prepared using the standard conditions described above and iodoethane **59** (MFCD00001091) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 6.3 mg (39% yield) of the title compound as an off-white residue. Analytical data for **S14**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 7.79 (d, *J* = 8.1 Hz, 2H), 7.49 (d, *J* = 8.0 Hz, 2H), 7.21 – 7.03 (m, 3H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.47 (s, 1H), 3.74 (q, *J* = 7.1 Hz, 2H), 3.07 (t, *J* = 4.9 Hz, 5H), 2.83 (t, *J* = 4.8 Hz, 4H), 1.09 (t, *J* = 7.1 Hz, 3H); **MS** (ESI): *m/z* calculated for $C_{24}H_{25}N_4O_2$ [M+H]+ 401.1972, found 401.1976.

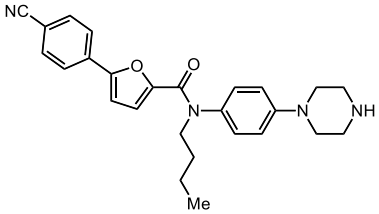
5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-propylfuran-2-carboxamide (S15)



The title compound was prepared using the standard conditions described above and 1-iodopropane **60** (MFCD00001095) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 10.8 mg (51% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S15**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.89 (s, 2H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.20 (d, *J* = 8.3 Hz, 2H), 7.15 (d, *J* = 3.1 Hz, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.58 (d, *J* = 3.9 Hz, 1H), 3.69 (t, *J* = 7.5 Hz, 2H), 3.43 – 3.30 (m, 4H), 3.26 (s, 4H), 1.51 (q, *J* = 7.5 Hz, 2H), 0.86 (t, *J* = 7.4 Hz, 3H); **MS** (ESI): *m/z* calculated for $C_{25}H_{27}N_4O_2$ [M+H]+ 415.2129, found 415.2101.

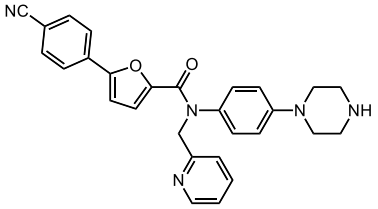
N-butyl-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S16)



The title compound was prepared using the standard conditions described above and 1-iodobutane **61** (MFCD00001098) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 9.6 mg (44% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S16**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.87 (s, 2H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 2H), 7.16 – 7.11 (m, 1H), 7.06 (d, *J* = 8.6 Hz, 2H), 6.58 (d, *J* = 3.9 Hz, 1H), 3.72 (t, *J* = 7.5 Hz, 2H), 3.43 – 3.31 (m, 4H), 3.26 (d, *J* = 6.9 Hz, 4H), 1.48 (p, *J* = 7.5 Hz, 2H), 1.29 (h, *J* = 7.4 Hz, 2H), 0.86 (t, *J* = 7.3 Hz, 3H); **MS** (ESI): *m/z* calculated for $C_{26}H_{29}N_4O_2$ [M+H]+ 429.2285, found 429.2276.

5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-(pyridin-2-ylmethyl)furan-2-carboxamide (72)

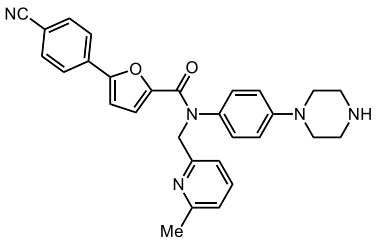


The title compound was prepared using the standard conditions described above and 2-(bromomethyl)pyridine hydrobromide **63** (MFCD01863544) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 11.2 mg (49% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **72**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.86 (s, 2H), 8.52 (d, *J* = 4.8 Hz, 1H), 7.84 (t, *J* = 7.6 Hz, 1H), 7.80 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 7.9 Hz, 1H), 7.47 (d, *J* = 8.1 Hz, 2H), 7.34 (dd, *J* = 7.5, 5.0 Hz, 1H), 7.24 (d, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 3.7 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 2H), 6.66 (s, 1H), 5.09 (s, 2H), 3.42 – 3.28 (m, 4H), 3.23 (p, *J* = 4.7 Hz, 4H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 157.9,

156.6, 152.3, 149.5, 148.8, 147.7, 137.0, 134.6, 133.1, 132.8, 128.6, 124.4, 122.4, 122.2, 119.4, 118.6, 116.2, 110.5, 110.3, 55.4, 45.4, 42.7; **MS** (ESI): *m/z* calculated for $C_{28}H_{26}N_5O_2$ [M+H]+ 464.2081, found 464.2082.

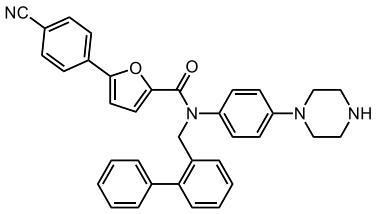
5-(4-cyanophenyl)-N-((6-methylpyridin-2-yl)methyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S17)



The title compound was prepared using the standard conditions described above and 2-(bromomethyl)-6-methylpyridine **65** (MFCD04967229) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 2.5 mg (11% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S17**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.80 (s, 2H), 7.81 (d, *J* = 8.1 Hz, 2H), 7.75 (t, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 7.8 Hz, 1H), 7.27 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 12.4 Hz, 2H), 7.00 (d, *J* = 8.3 Hz, 2H), 6.67 (s, 1H), 5.05 (s, 2H), 3.33 (t, *J* = 5.3 Hz, 4H), 3.24 (s, 4H), 2.45 (s, 3H); **MS** (ESI): *m/z* calculated for $C_{29}H_{28}N_5O_2$ [M+H]+ 478.2238, found 478.2226.

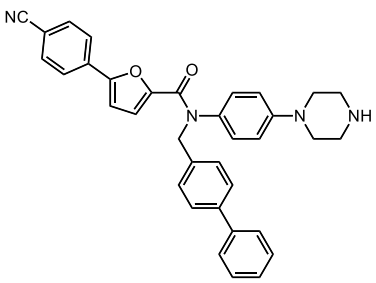
N-([1,1'-biphenyl]-2-ylmethyl)-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S18)



The title compound was prepared using the standard conditions described above and 2-(bromomethyl)-1,1'-biphenyl **68** (MFCD00075454) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 7.7 mg (30% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S18**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.81 (s, 2H), 7.84 (d, *J* = 7.9 Hz, 1H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.73 (d, *J* = 7.7 Hz, 1H), 7.69 (d, *J* = 7.9 Hz, 1H), 7.62 (dd, *J* = 14.1, 7.8 Hz, 3H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.47 – 7.42 (m, 3H), 7.38 – 7.34 (m, 2H), 7.20 (d, *J* = 3.7 Hz, 1H), 7.12 (d, *J* = 8.5 Hz, 2H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 3.8 Hz, 1H), 5.03 (s, 2H), 4.66 (s, 1H), 3.34 – 3.29 (m, 4H), 3.23 (s, 4H), 2.93 (s, 1H); **MS** (ESI): *m/z* calculated for $C_{35}H_{31}N_4O_2$ [M+H]+ 539.2442, found 539.2430.

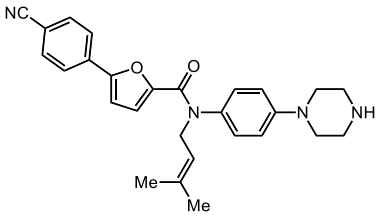
N-([1,1'-biphenyl]-4-ylmethyl)-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S19)



The title compound was prepared using the standard conditions described above and 4-(bromomethyl)-1,1'-biphenyl **69** (MFCD00017869) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 5 mg (19% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S19**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.77 (s, 2H), 7.79 (d, *J* = 8.2 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 2H), 7.39 (t, *J* = 7.5 Hz, 1H), 7.36 – 7.27 (m, 4H), 7.17 (d, *J* = 3.7 Hz, 1H), 7.13 (d, *J* = 7.5 Hz, 1H), 7.07 – 6.99 (m, 2H), 6.92 (t, *J* = 6.9 Hz, 4H), 6.71 – 6.57 (m, 1H), 4.93 (s, 2H), 3.31 (d, *J* = 5.0 Hz, 4H), 3.24 (t, *J* = 5.0 Hz, 4H); **MS** (ESI): *m/z* calculated for $C_{35}H_{31}N_4O_2$ [M+H]+ 539.2442, found 539.2462.

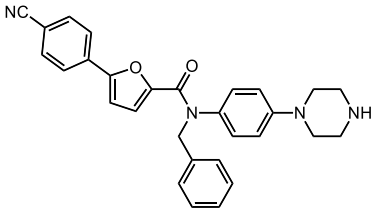
5-(4-cyanophenyl)-N-(3-methylbut-2-en-1-yl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (73)



The title compound was prepared using the standard conditions described above and 1-bromo-3-methylbut-2-ene **67** (MFCD00000242) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 11.6 mg (52% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **73**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.88 (s, 2H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 2H), 7.16 (d, *J* = 8.9 Hz, 3H), 7.04 (d, *J* = 8.5 Hz, 2H), 6.62 (d, *J* = 3.8 Hz, 1H), 5.24 (t, *J* = 7.2 Hz, 1H), 4.33 (d, *J* = 6.9 Hz, 2H), 3.41 – 3.30 (m, 4H), 3.26 (s, 4H), 1.64 (s, 3H), 1.46 (s, 3H); **^{13}C NMR** (126 MHz, DMSO-d_6) δ 157.3, 152.1, 149.5, 148.1, 135.6, 134.2, 133.1, 132.7, 128.8, 124.3, 119.3, 118.9, 118.6, 116.2, 110.4, 110.2, 47.7, 45.5, 42.7, 25.4, 17.6; **MS** (ESI): *m/z* calculated for $C_{27}H_{29}N_4O_2$ [M+H]+ 441.2285, found 441.2281.

N-benzyl-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S20)

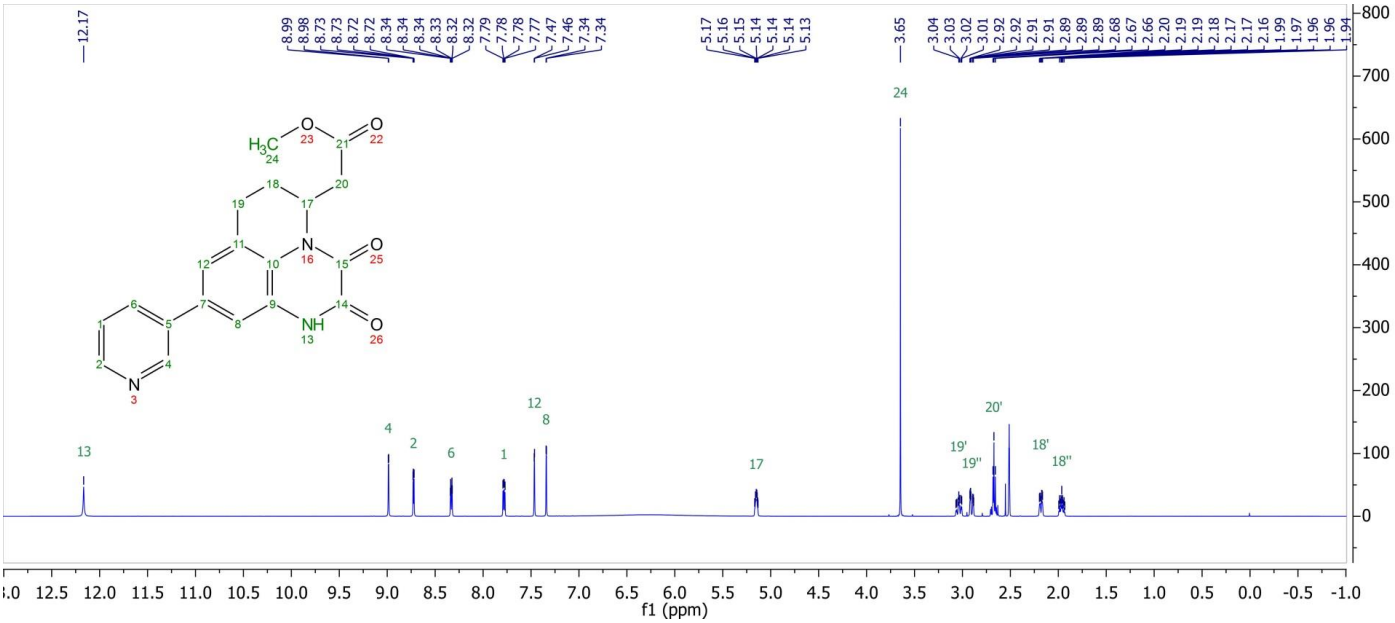


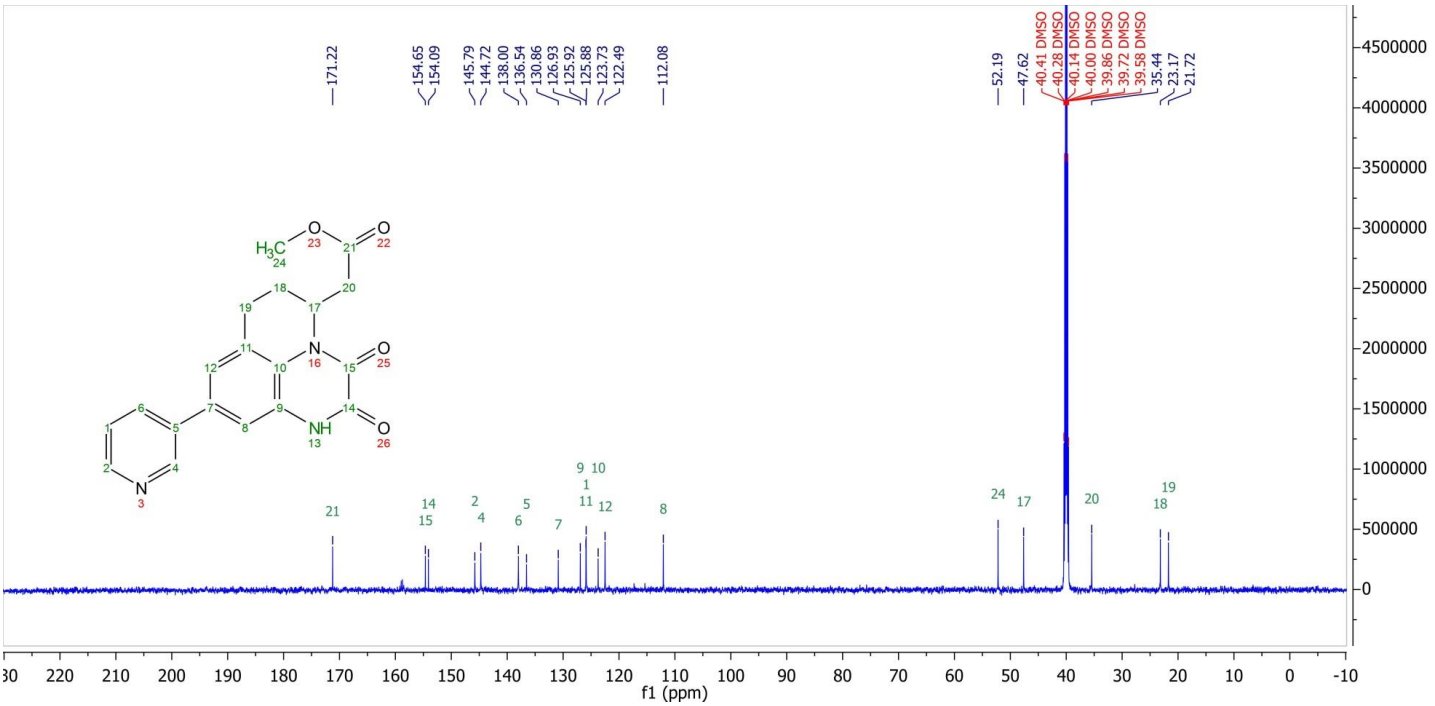
The title compound was prepared using the standard conditions described above and (bromomethyl)benzene **66** (MFCD00000172) as the electrophile. The desired product was prepared at the 0.04 mmol scale and purified by RP-HPLC to yield 6.8 mg (52% yield) of the title compound as an off-white residue (TFA salt). Analytical data for **S20**:

^{1}H NMR (600 MHz, DMSO-d_6) δ 8.79 (s, 2H), 7.80 (d, *J* = 8.1 Hz, 2H), 7.45 (d, *J* = 8.2 Hz, 2H), 7.30 (t, *J* = 7.5 Hz, 2H), 7.24 (dd, *J* = 13.1, 7.1 Hz, 3H), 7.18 (d, *J* = 3.7 Hz, 1H), 7.07 (d, *J* = 8.4 Hz, 2H), 6.97 (d, *J* = 8.5 Hz, 2H), 6.68 (d, *J* = 3.8 Hz, 1H), 4.98 (s, 2H), 3.31 (t, *J* = 5.0 Hz, 4H), 3.23 (s, 4H); **MS** (ESI): *m/z* calculated for $C_{29}H_{27}N_4O_2$ [M+H]+ 463.2129, found 463.2121.

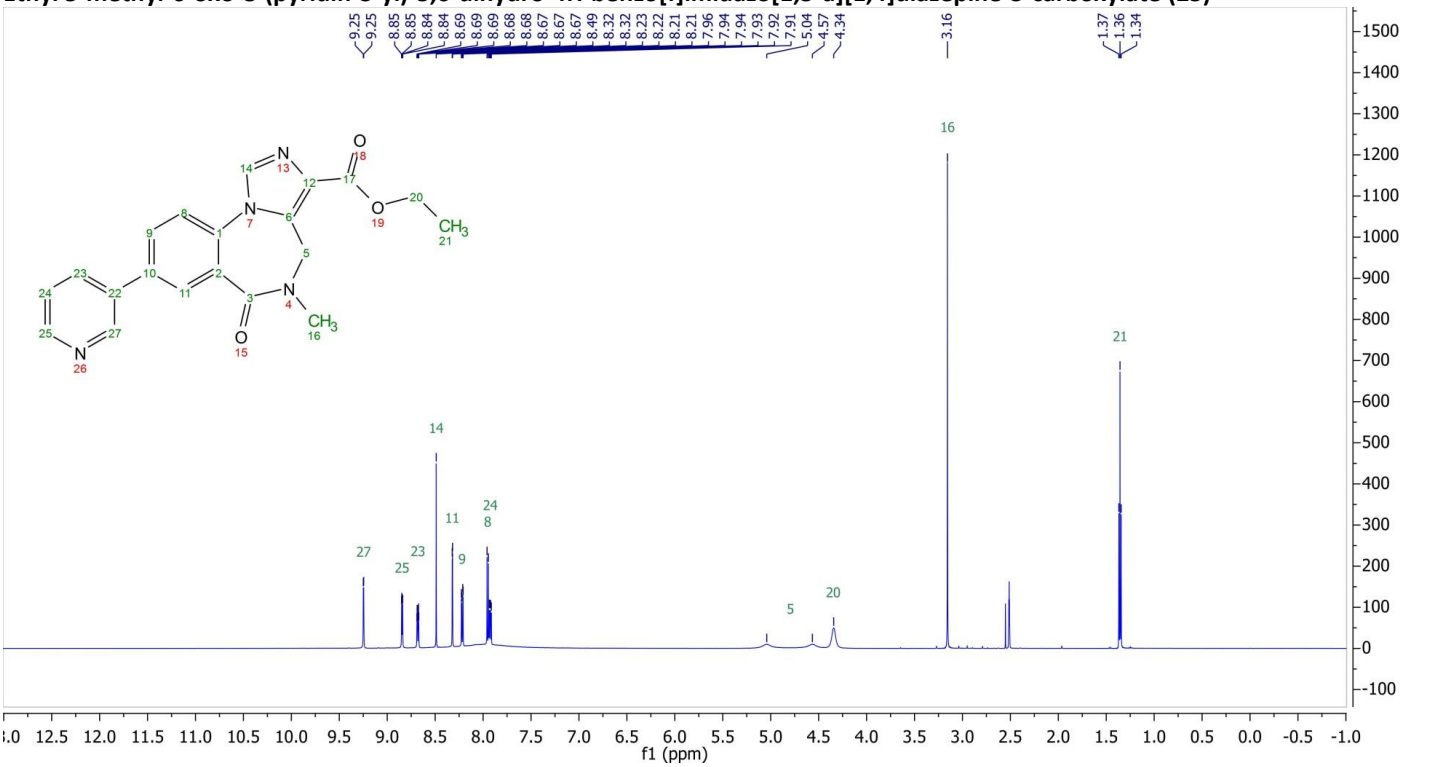
7. NMR Spectra

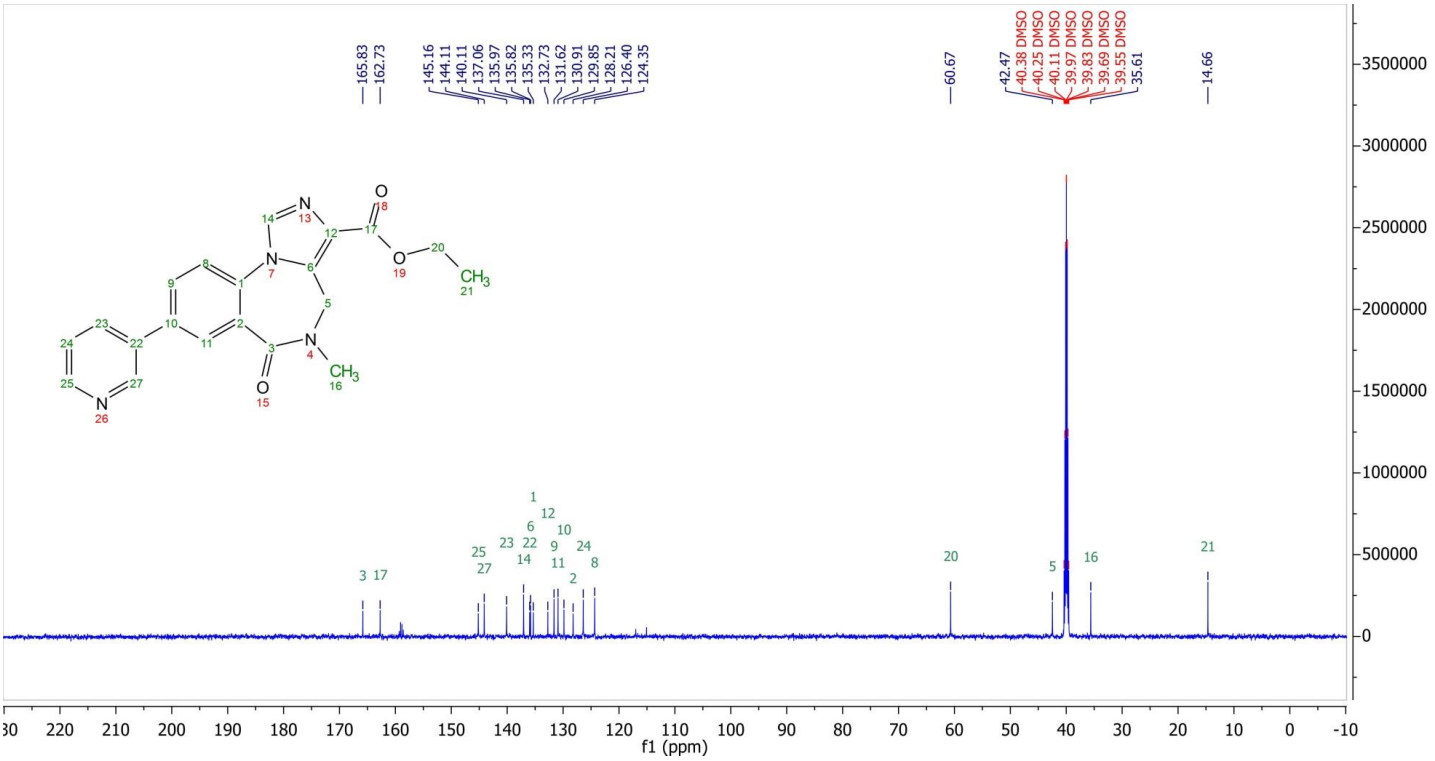
Methyl 2-(2,3-dioxo-9-(pyridin-3-yl)-2,3,6,7-tetrahydro-1H,5H-pyrido[1,2,3-de]quinoxalin-5-yl)acetate (24)



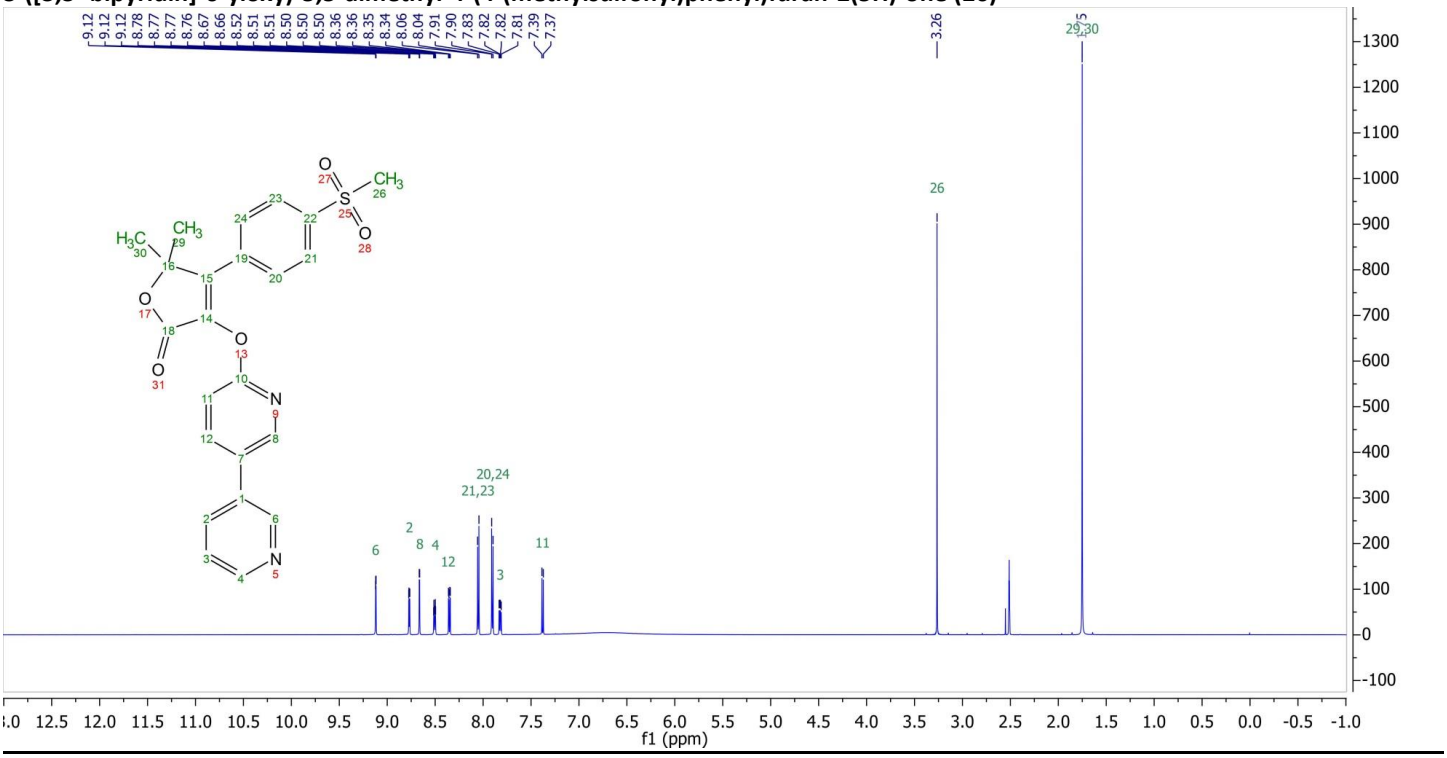


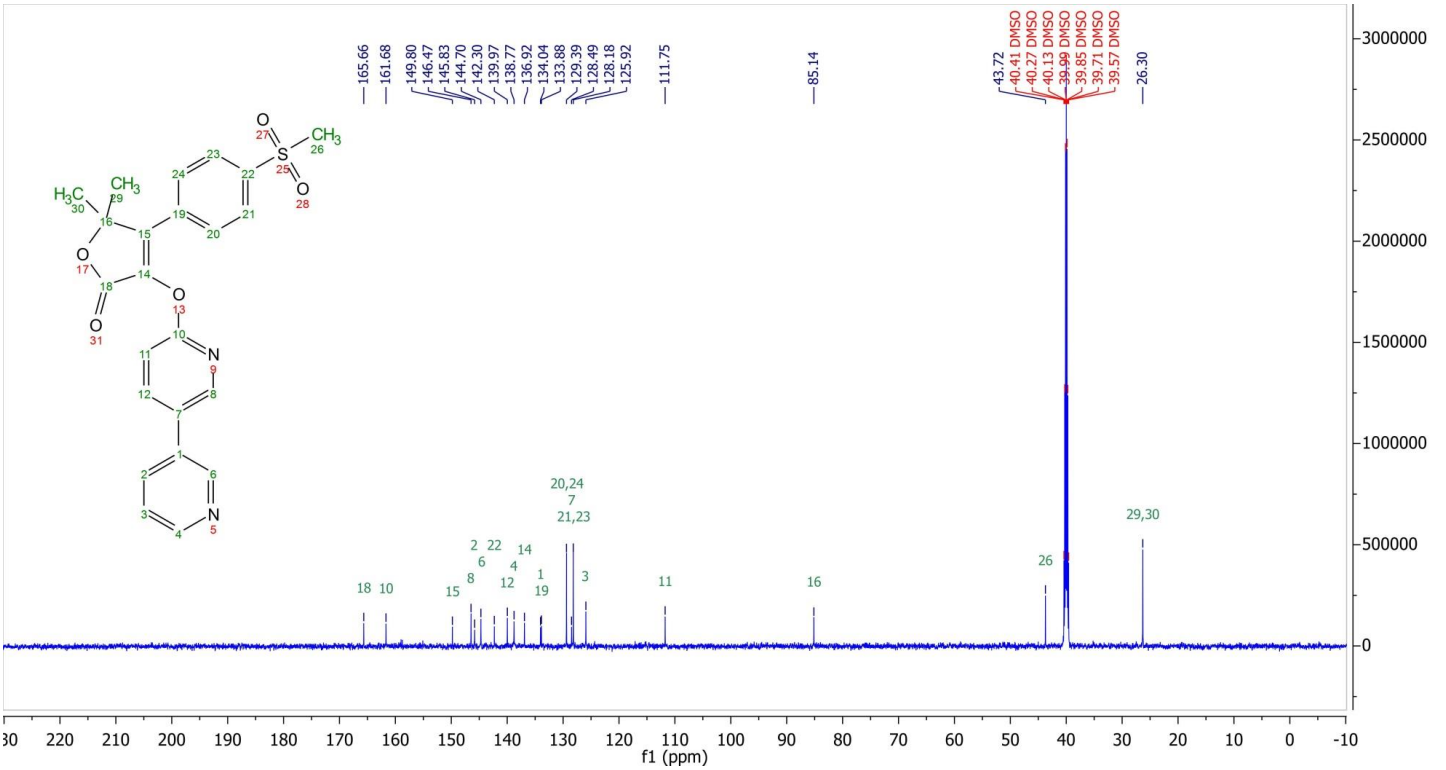
Ethyl 5-methyl-6-oxo-8-(pyridin-3-yl)-5,6-dihydro-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate (25)



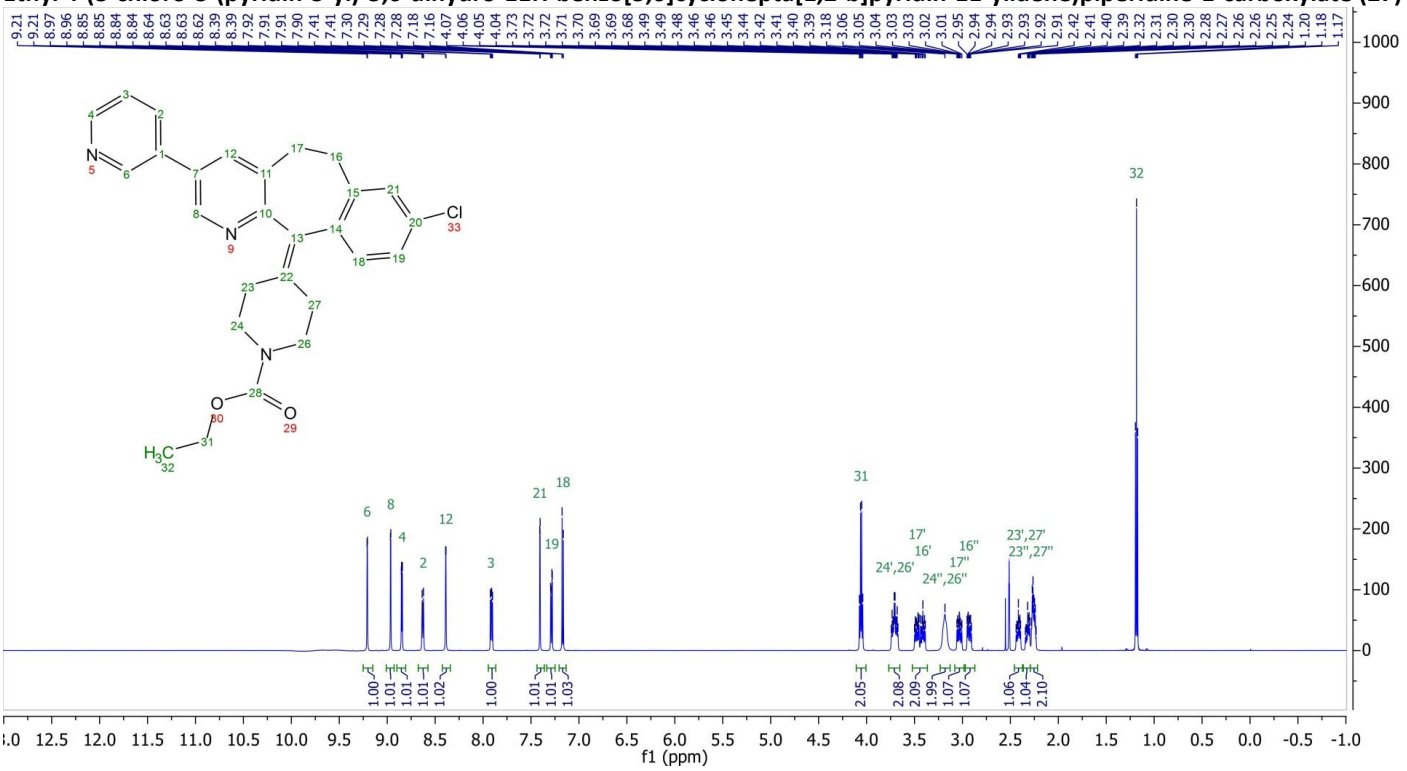


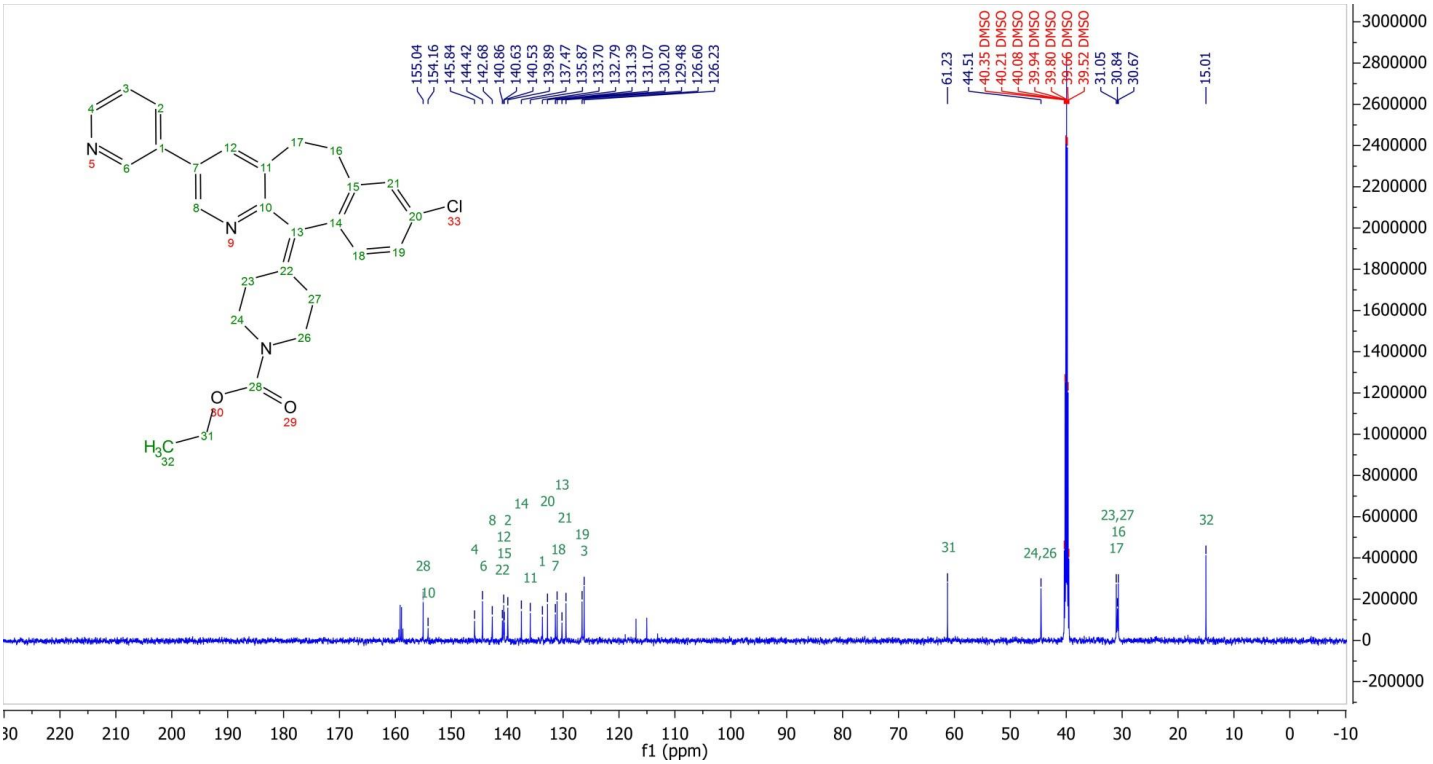
3-([3,3'-bipyridin]-6-yloxy)-5,5-dimethyl-4-(4-(methylsulfonyl)phenyl)furan-2(5H)-one (26)



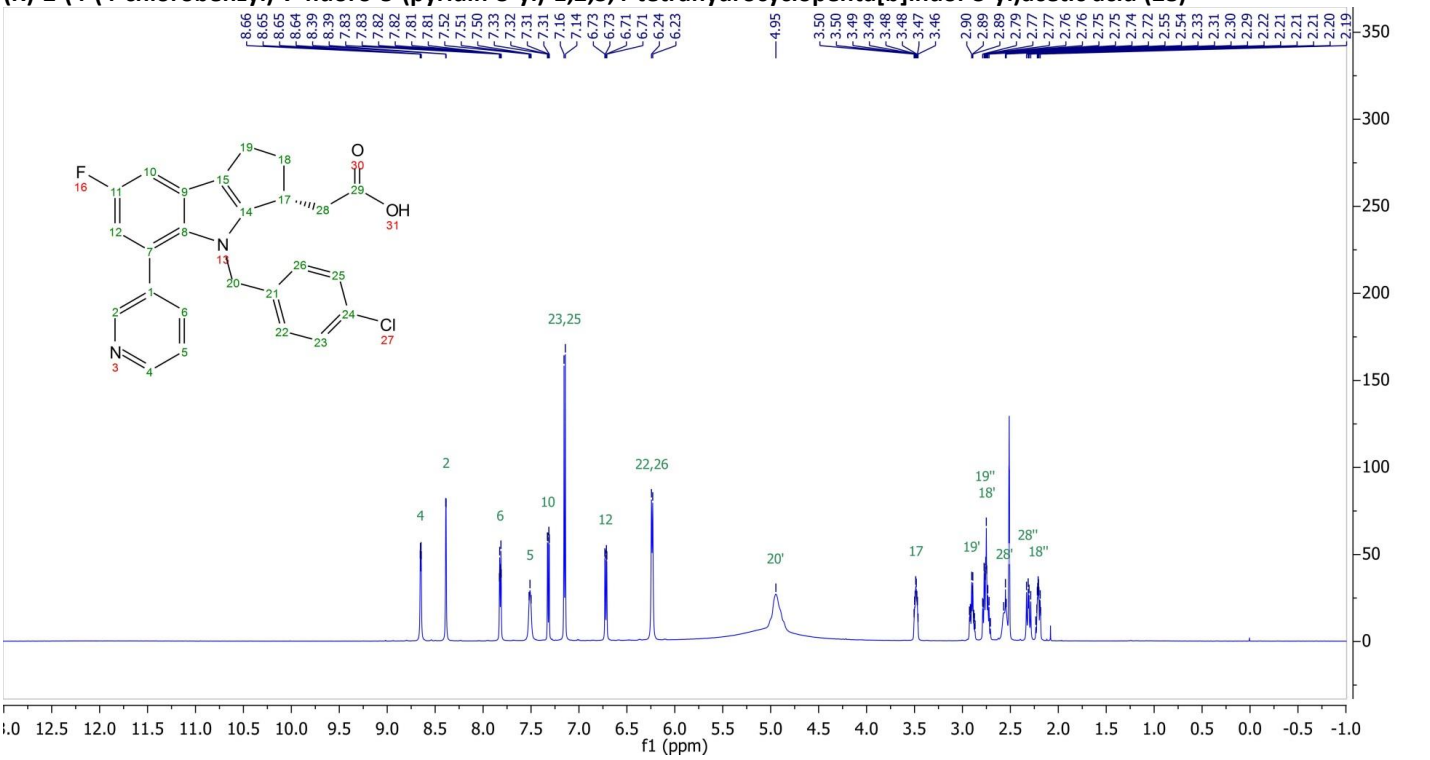


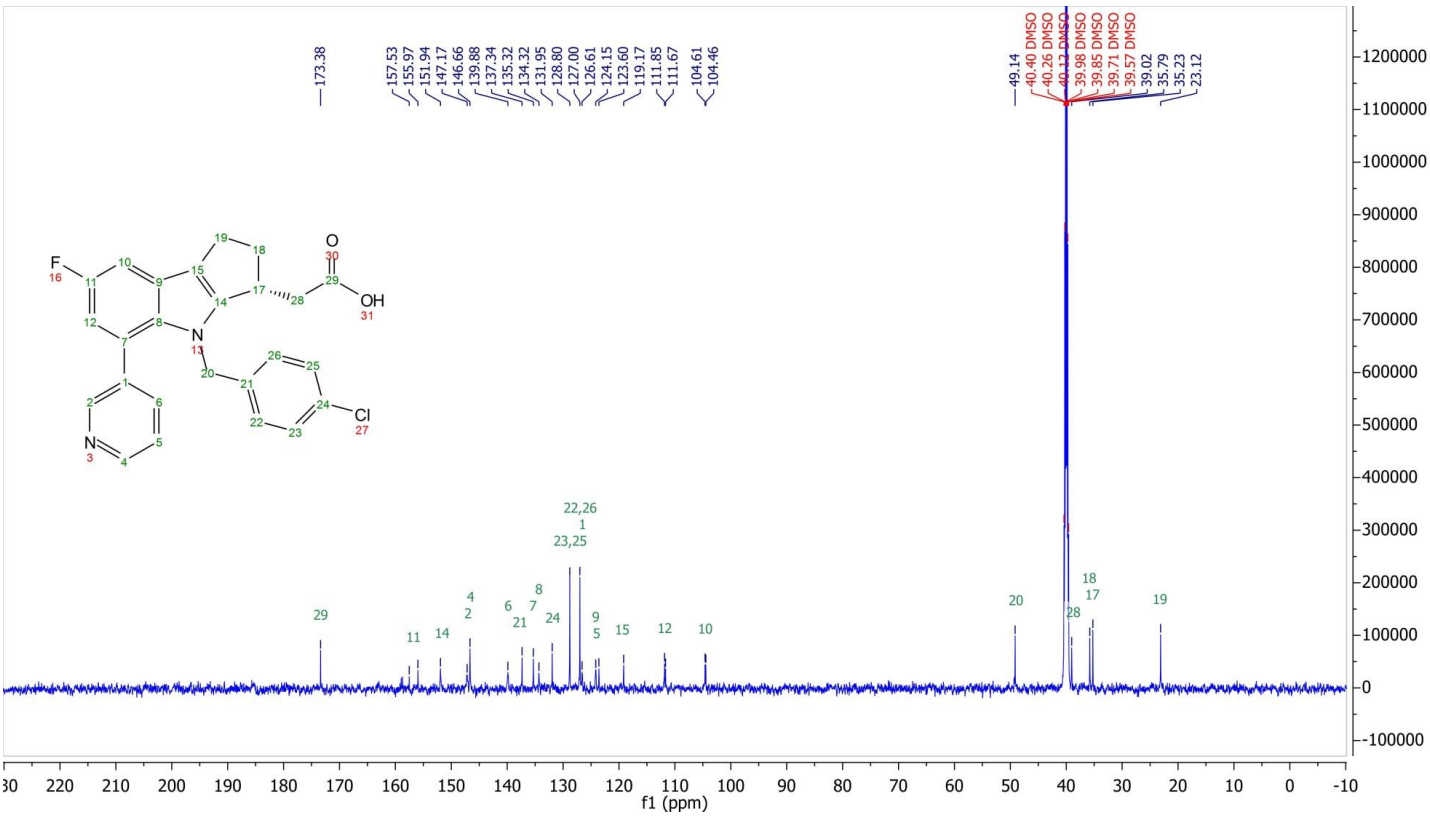
Ethyl 4-(8-chloro-3-(pyridin-3-yl)-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)piperidine-1-carboxylate (27)



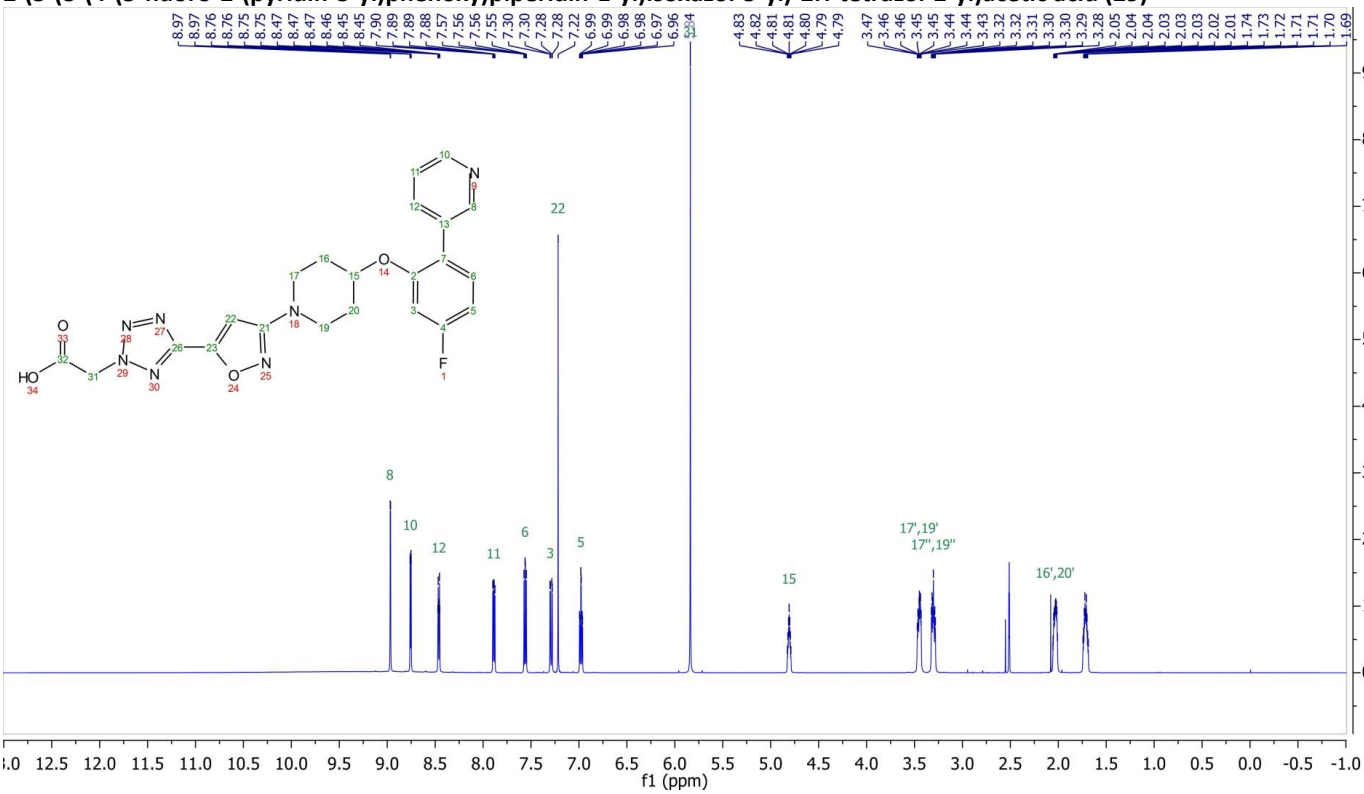


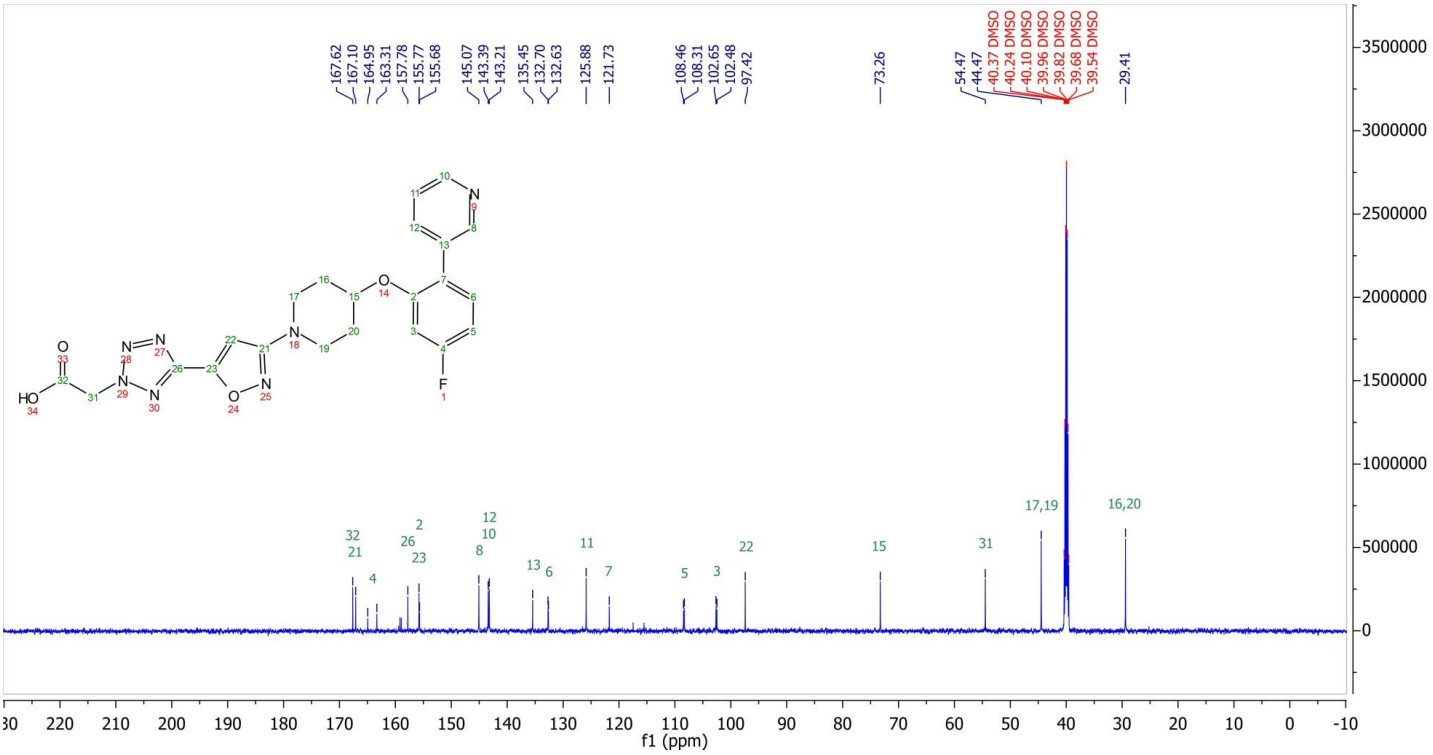
(R)-2-(4-(4-chlorobenzyl)-7-fluoro-5-(pyridin-3-yl)-1,2,3,4-tetrahydrocyclopenta[b]indol-3-yl)acetic acid (28)



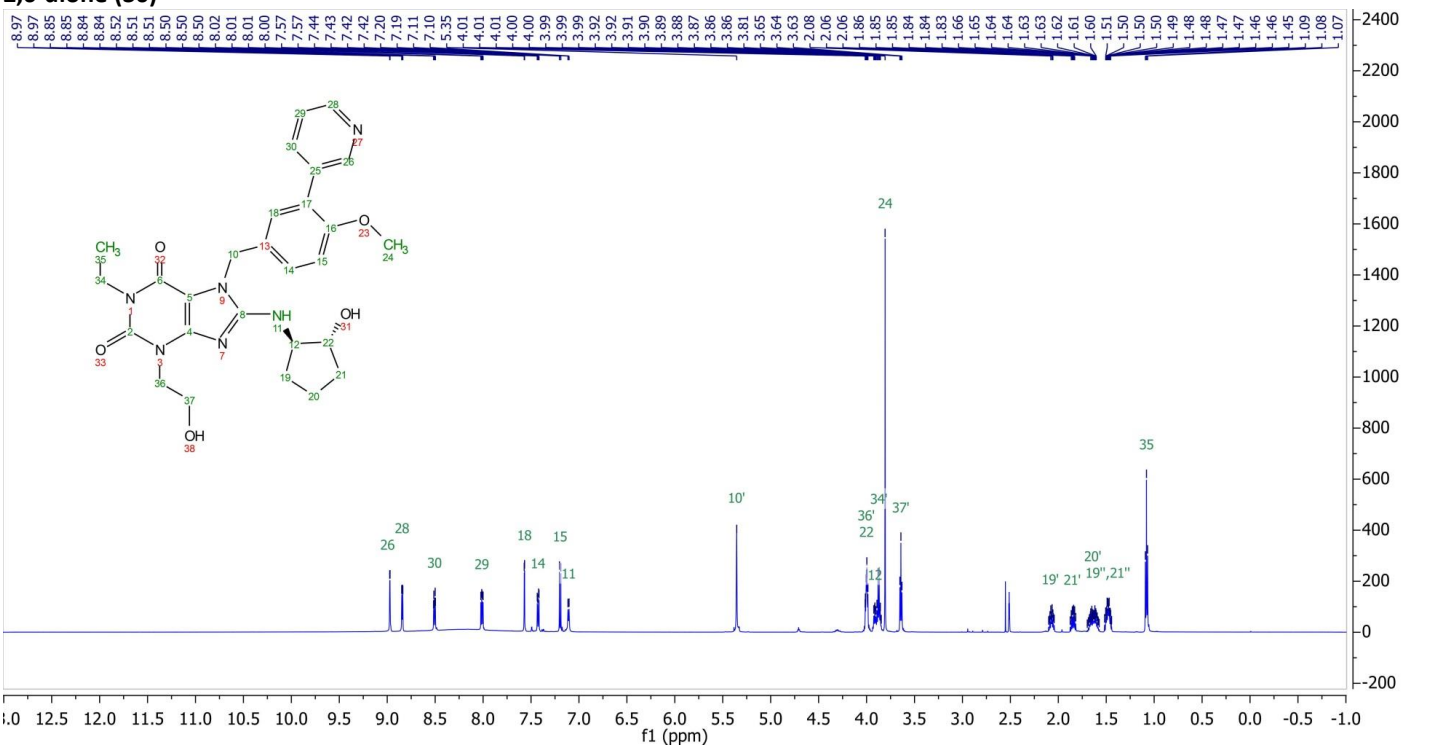


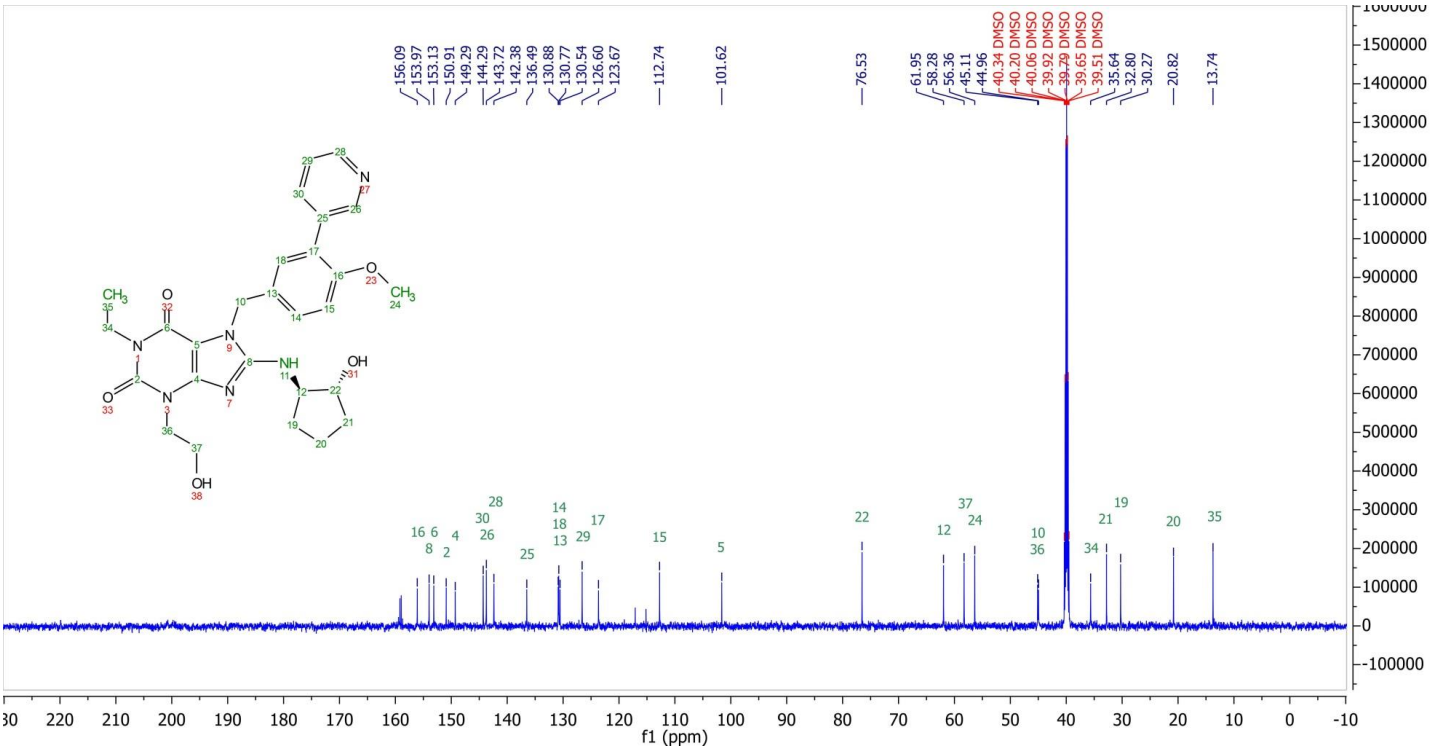
2-(5-(3-(4-(5-fluoro-2-(pyridin-3-yl)phenoxy)piperidin-1-yl)isoxazol-5-yl)-2H-tetrazol-2-yl)acetic acid (29)



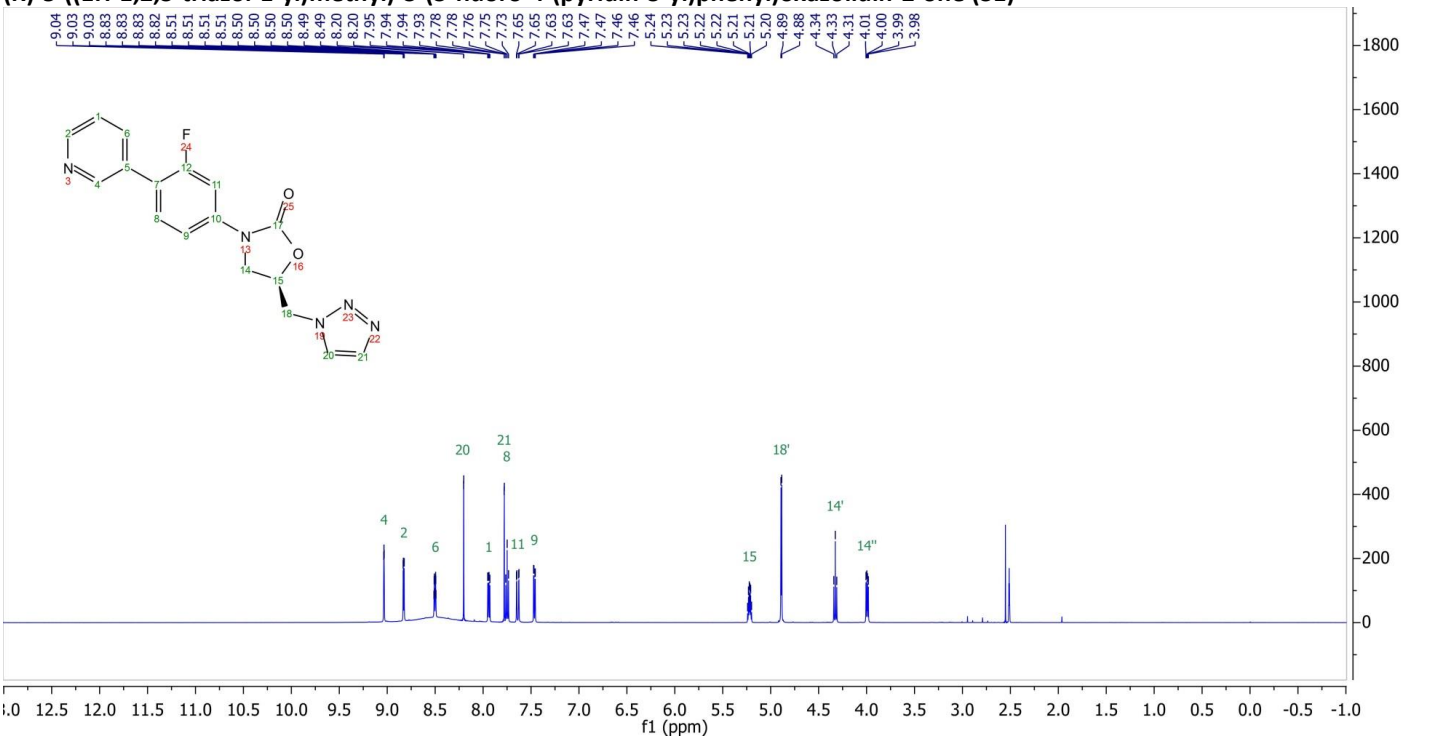


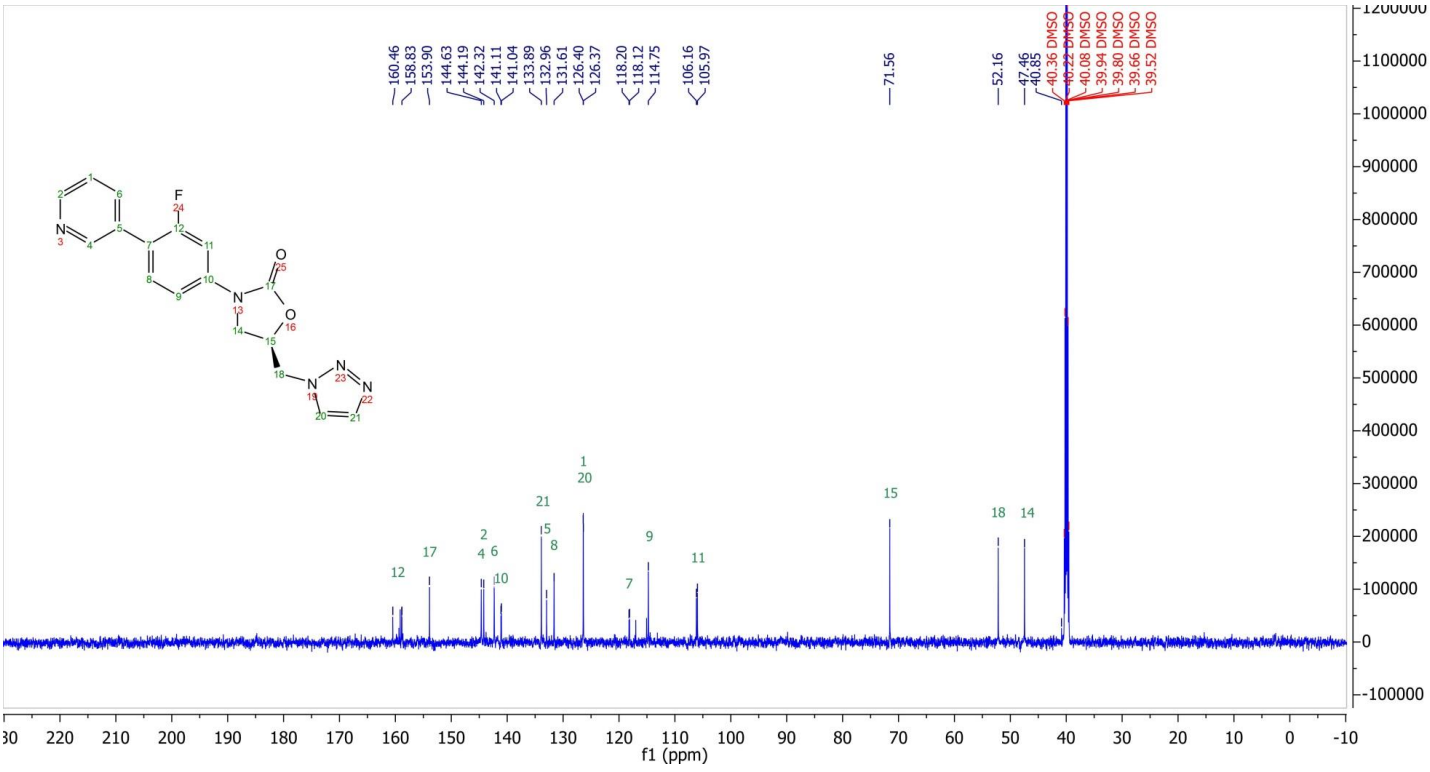
1-ethyl-8-(((1R,2R)-2-hydroxycyclopentyl)amino)-3-(2-hydroxyethyl)-7-(4-methoxy-3-(pyridin-3-yl)benzyl)-3,7-dihydro-1H-purine-2,6-dione (30)



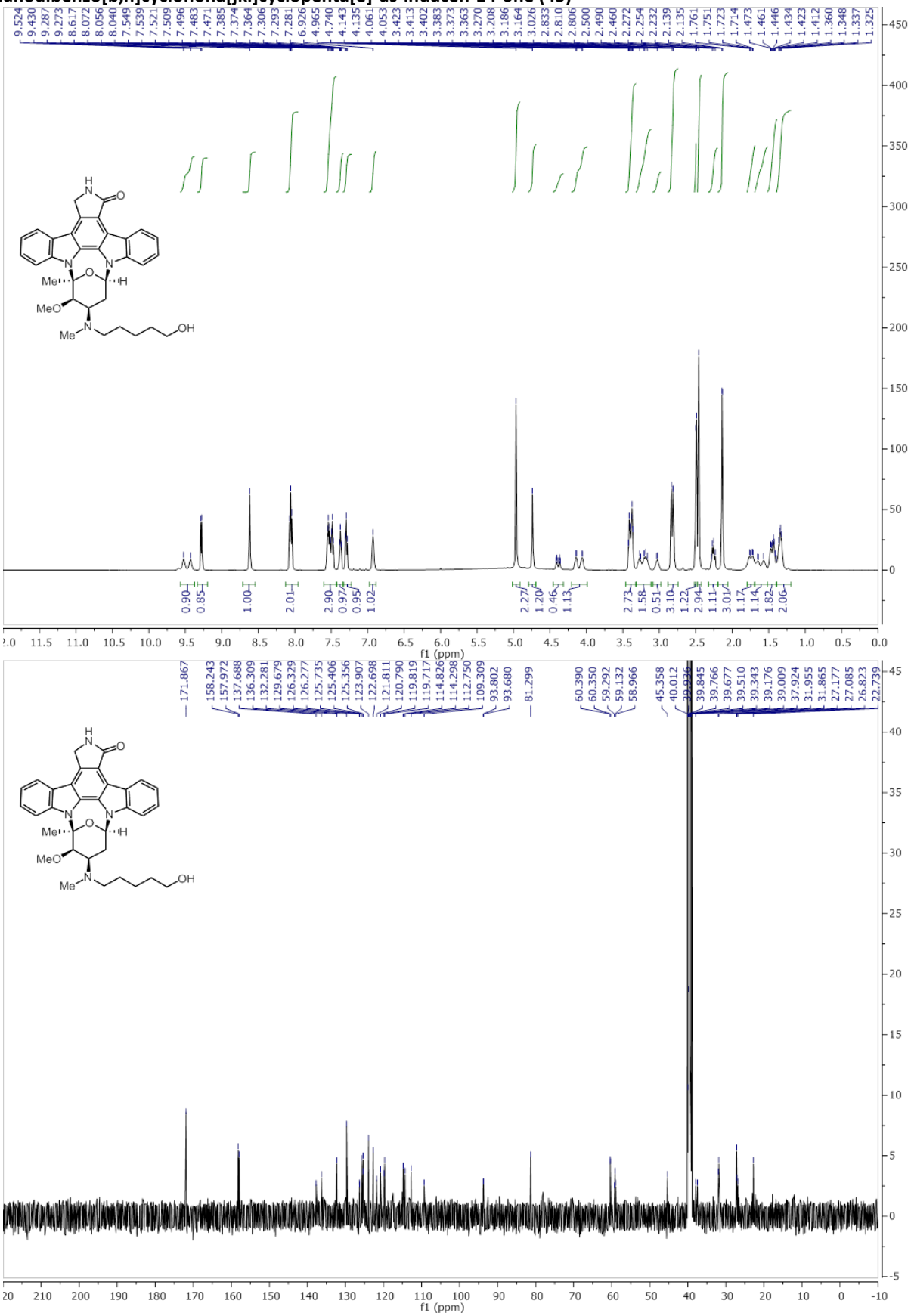


(R)-5-((1H-1,2,3-triazol-1-yl)methyl)-3-(3-fluoro-4-(pyridin-3-yl)phenyl)oxazolidin-2-one (31)

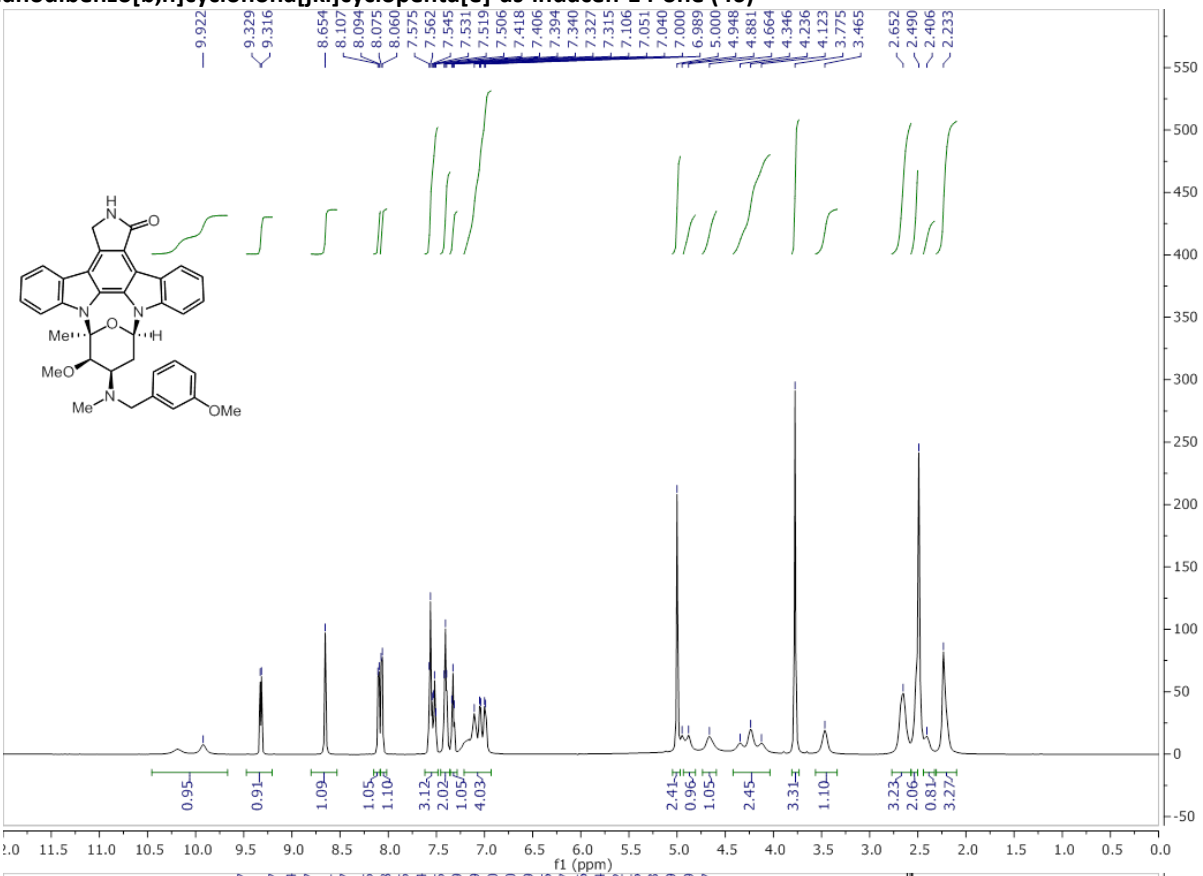


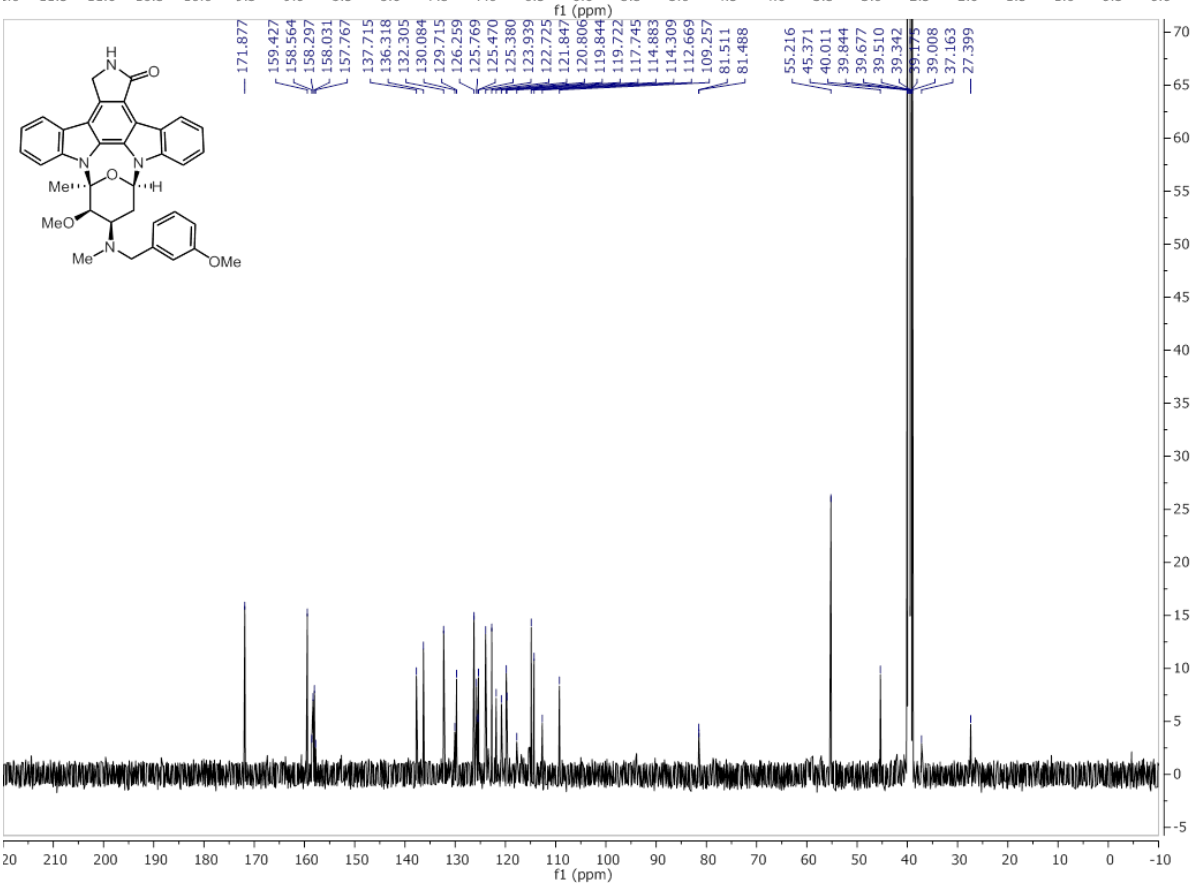


(5S,6R,7R,9R)-7-((5-hydroxypentyl)(methyl)amino)-6-methoxy-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (45)

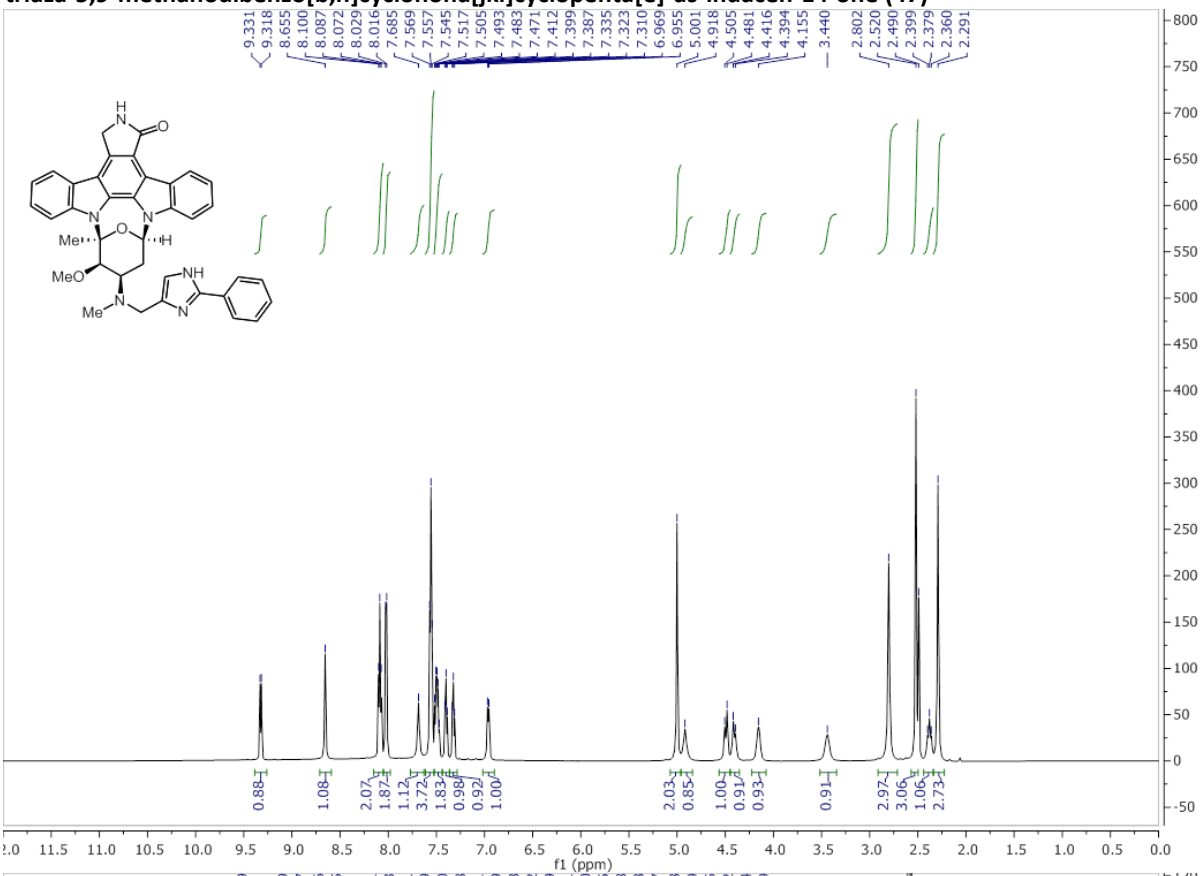


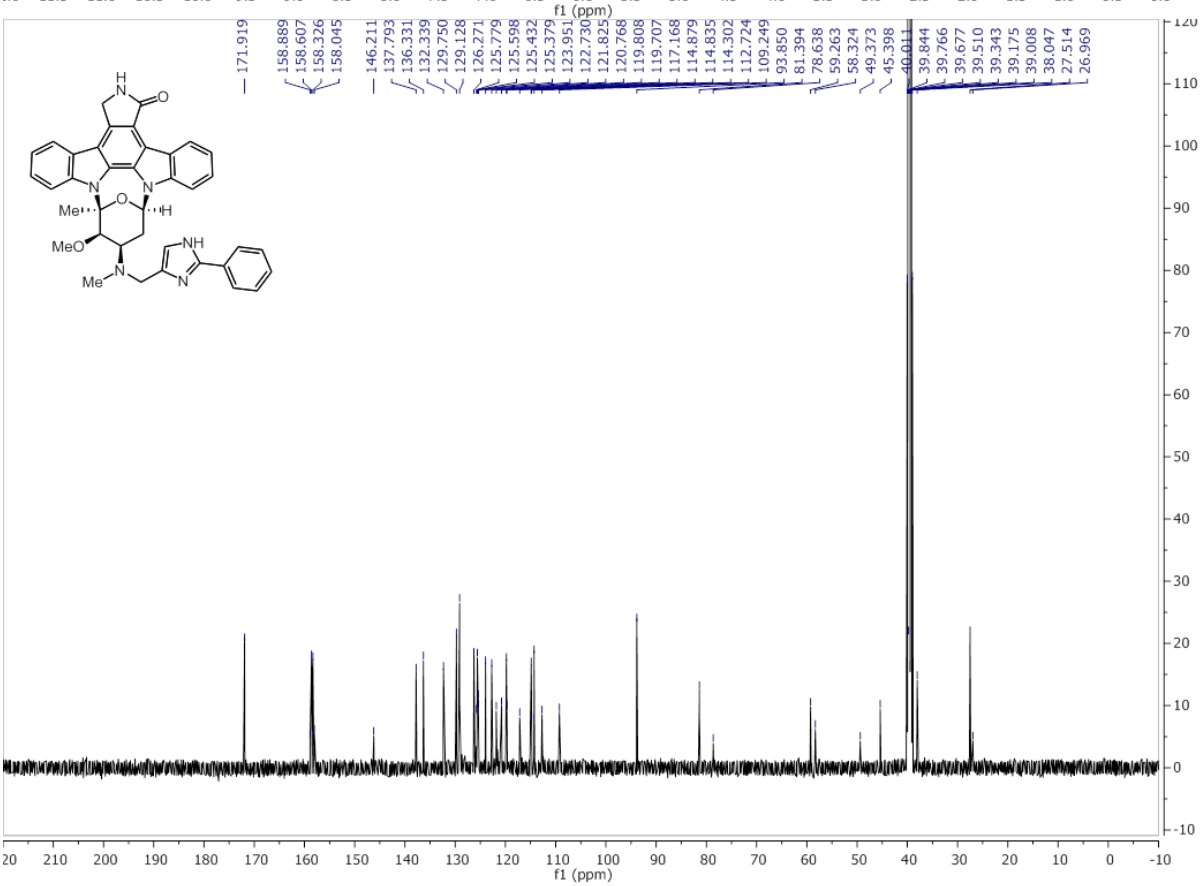
(5S,6R,7R,9R)-6-methoxy-7-((3-methoxybenzyl)(methyl)amino)-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (46)



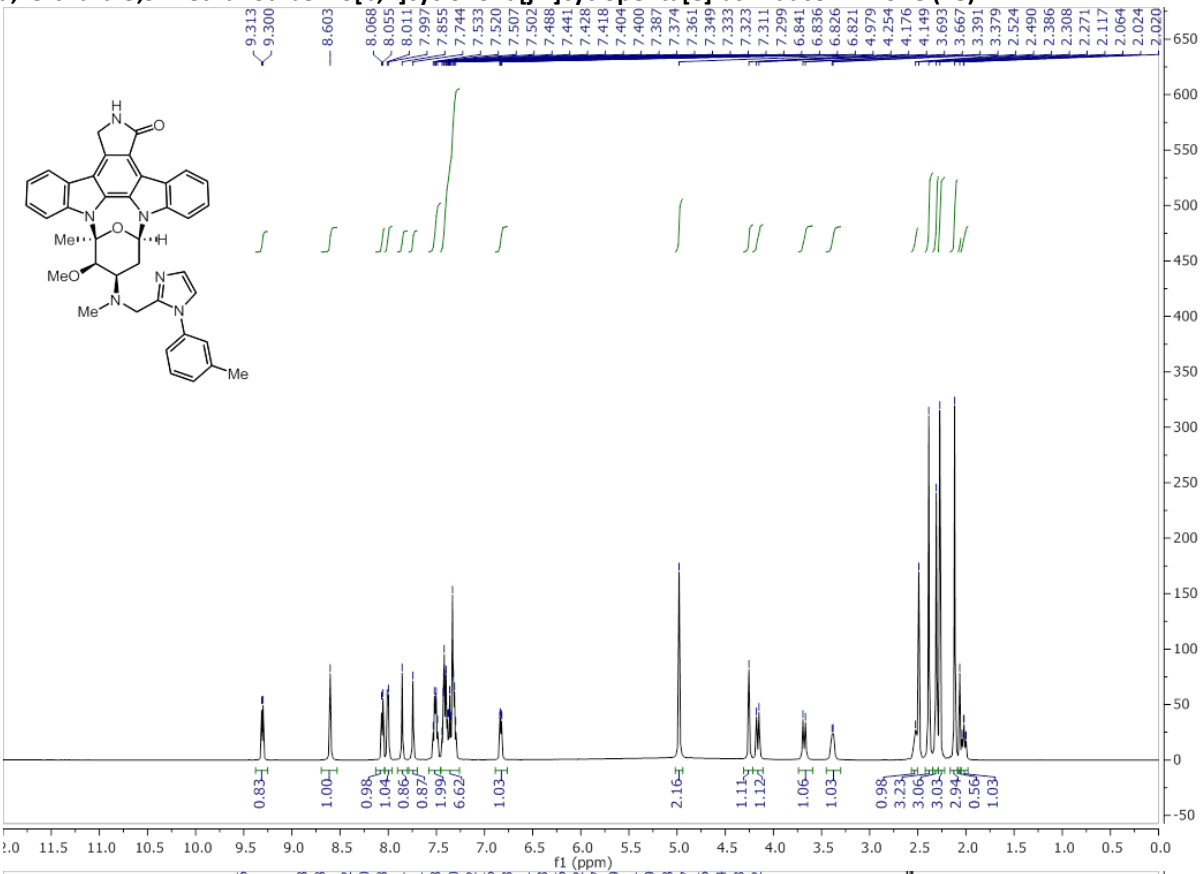


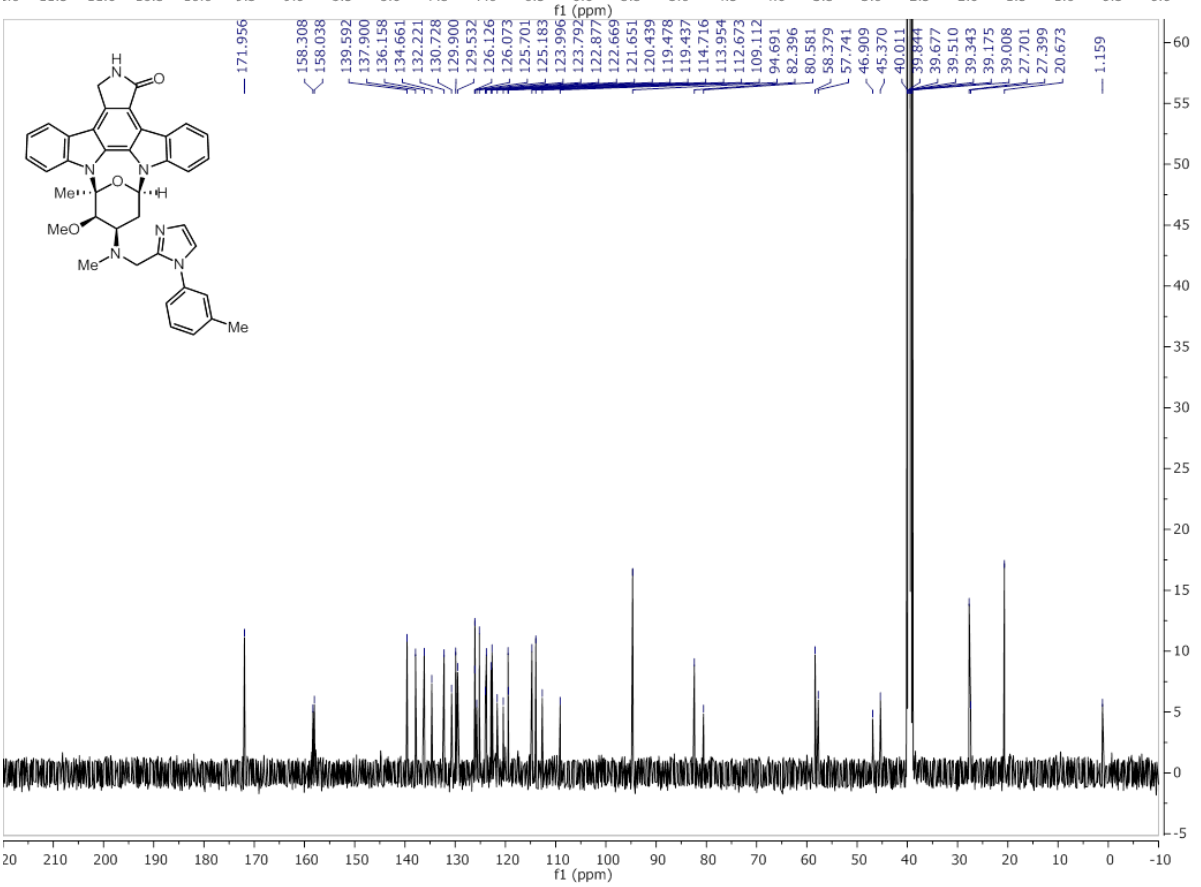
(5S,6R,7R,9R)-6-methoxy-5-methyl-7-(methyl((2-phenyl-1H-imidazol-4-yl)methyl)amino)-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (47)



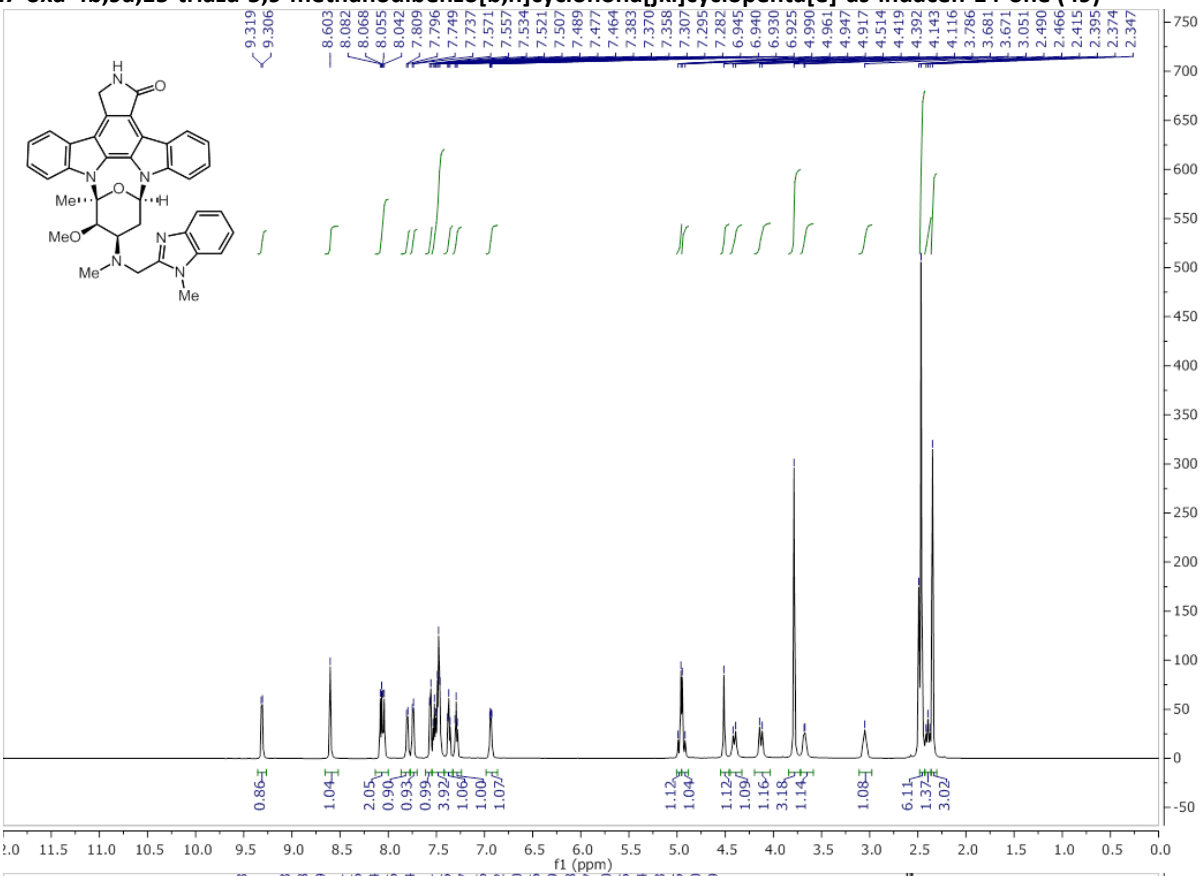


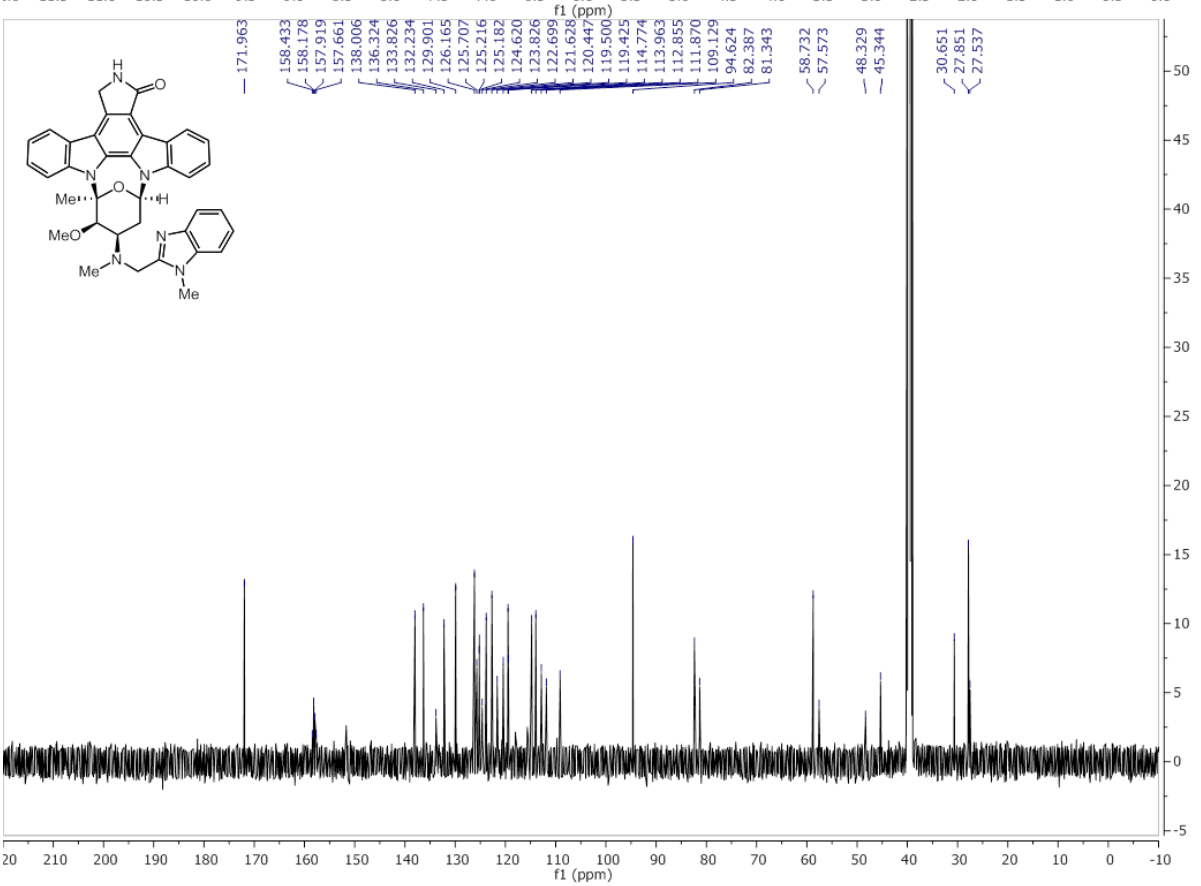
(5S,6R,7R,9R)-6-methoxy-5-methyl-7-(methyl((1-(m-tolyl)-1H-imidazol-2-yl)methyl)amino)-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (48)



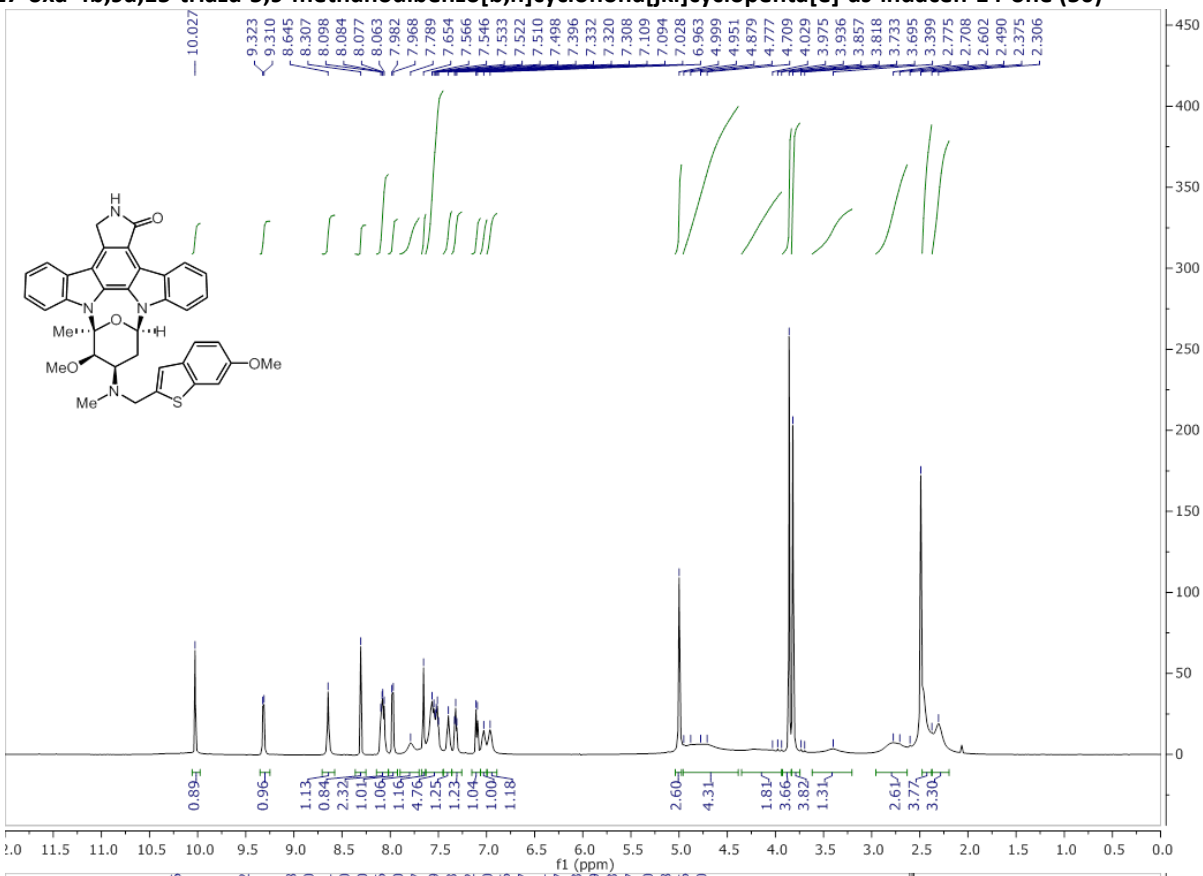


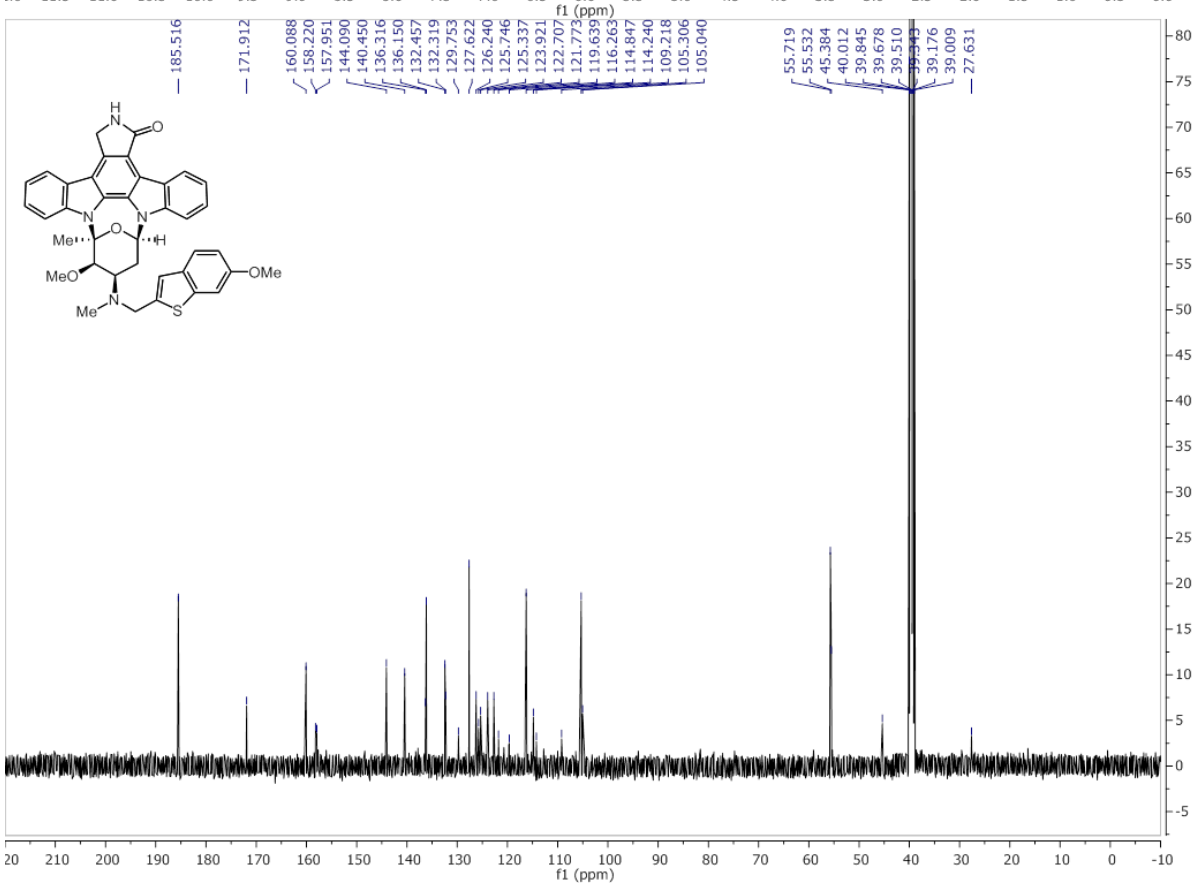
(5S,6R,7R,9R)-6-methoxy-5-methyl-7-(methyl((1-methyl-1H-benzo[d]imidazol-2-yl)methyl)amino)-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (49)



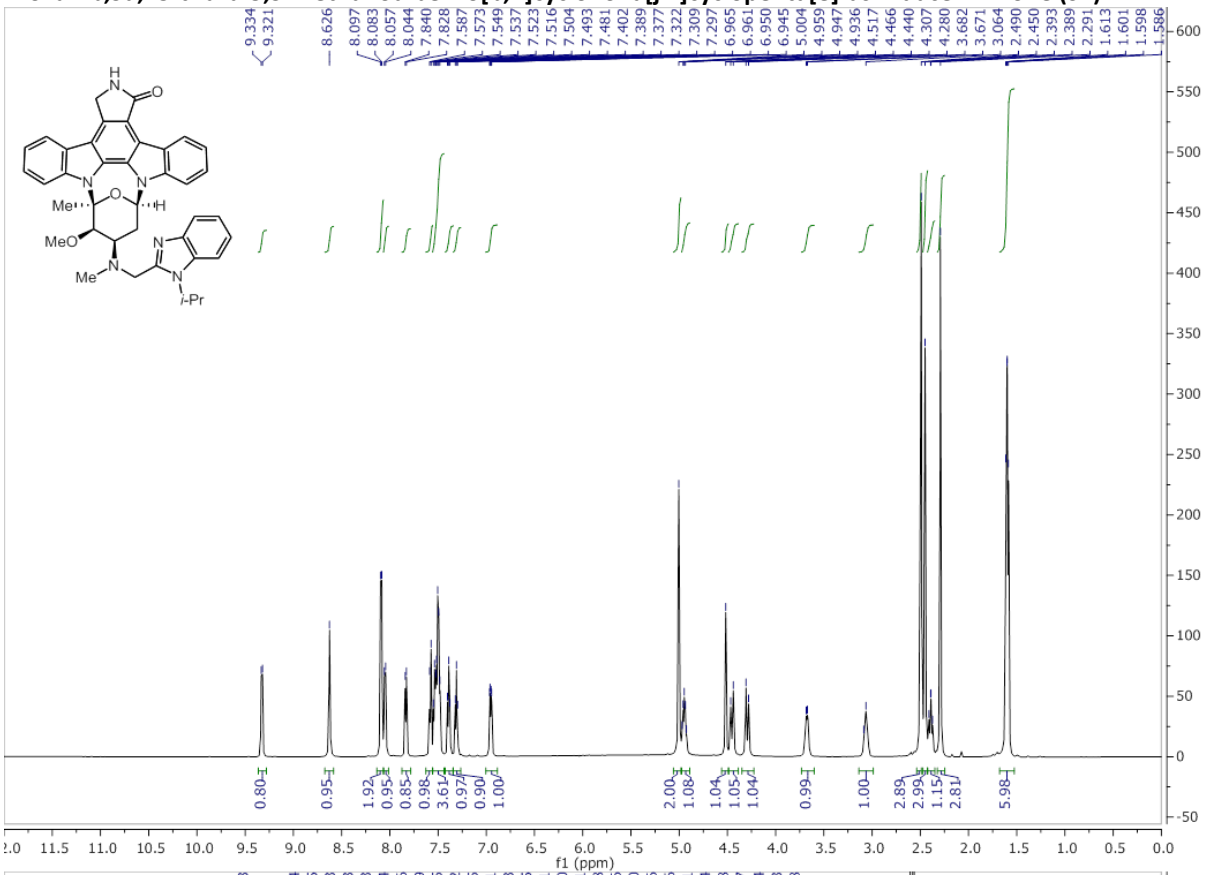


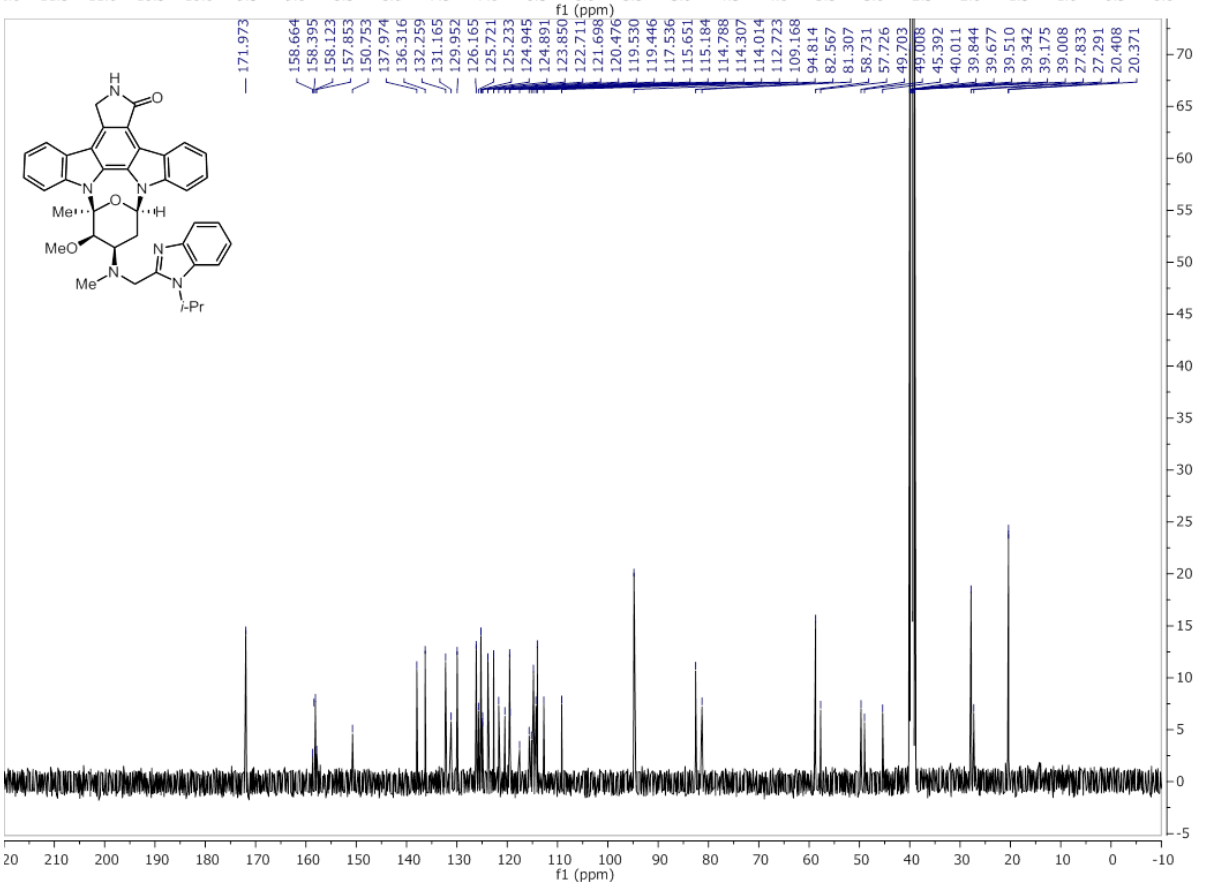
(5S,6R,7R,9R)-6-methoxy-7-(((6-methoxybenzo[b]thiophen-2-yl)methyl)(methyl)amino)-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (50)



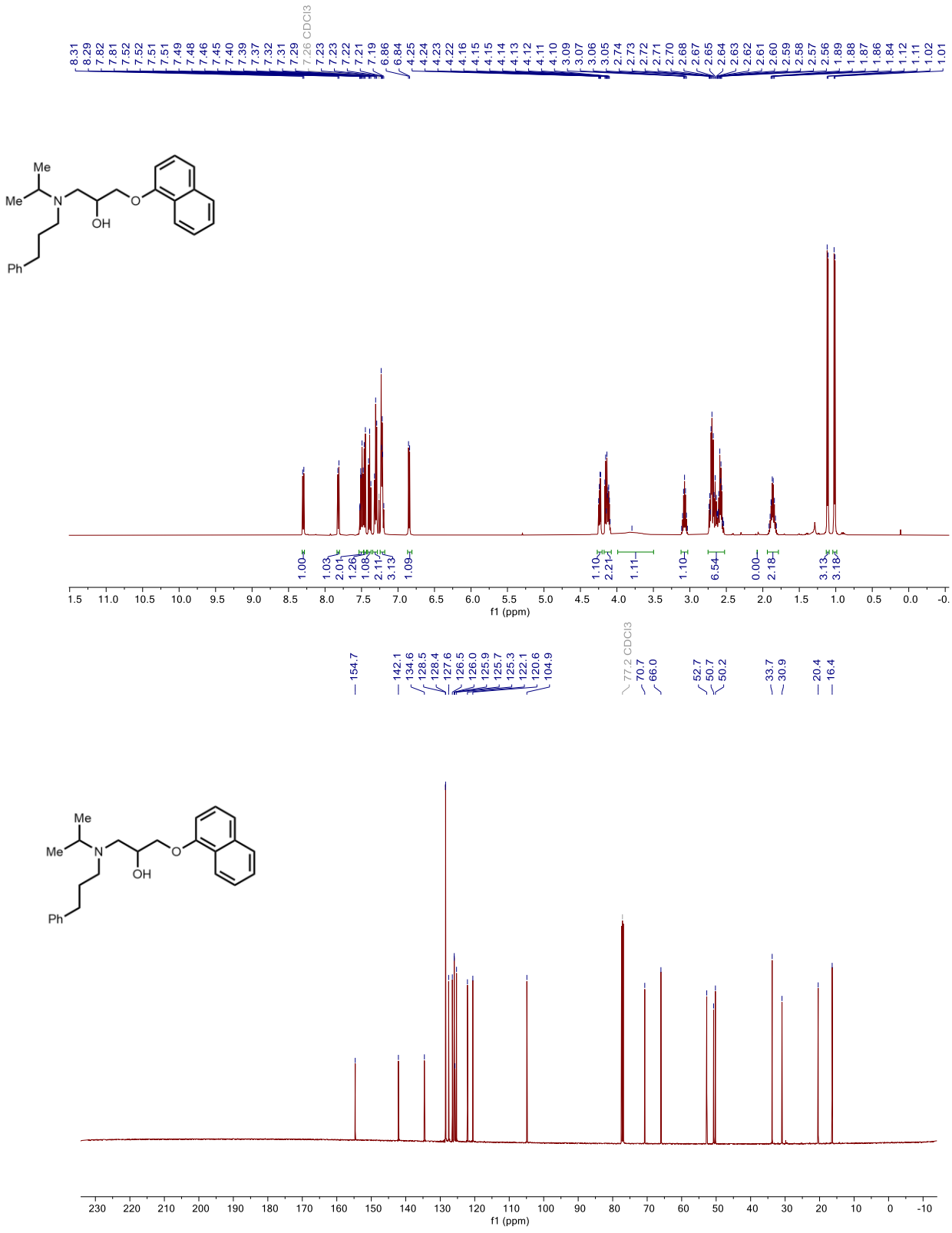


(5S,6R,7R,9R)-7-(((1-isopropyl-1H-benzo[d]imidazol-2-yl)methyl)(methyl)amino)-6-methoxy-5-methyl-6,7,8,9,15,16-hexahydro-5H,14H-17-oxa-4b,9a,15-triaza-5,9-methanodibenzo[b,h]cyclonona[jkl]cyclopenta[e]-as-indacen-14-one (S1)

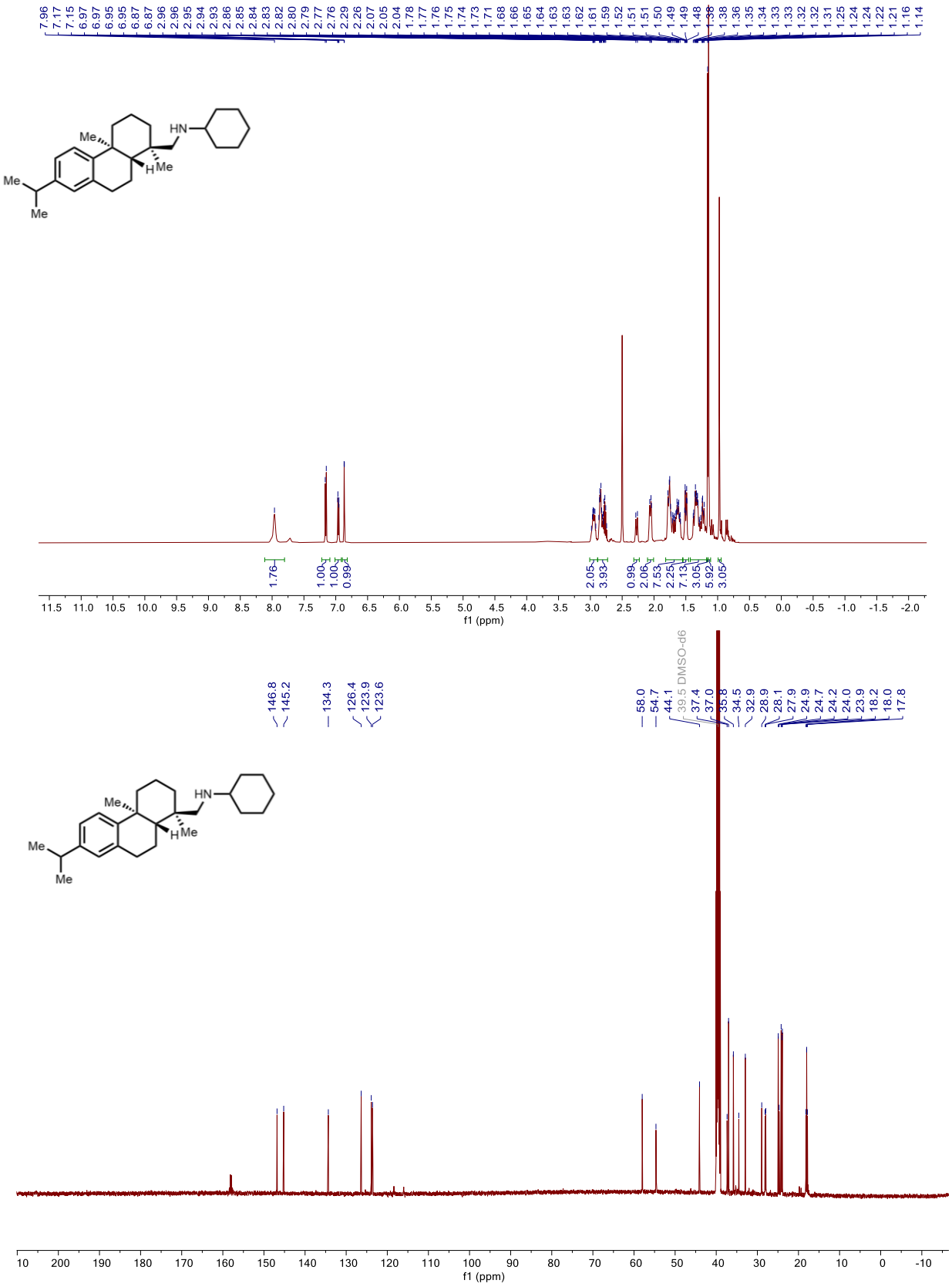




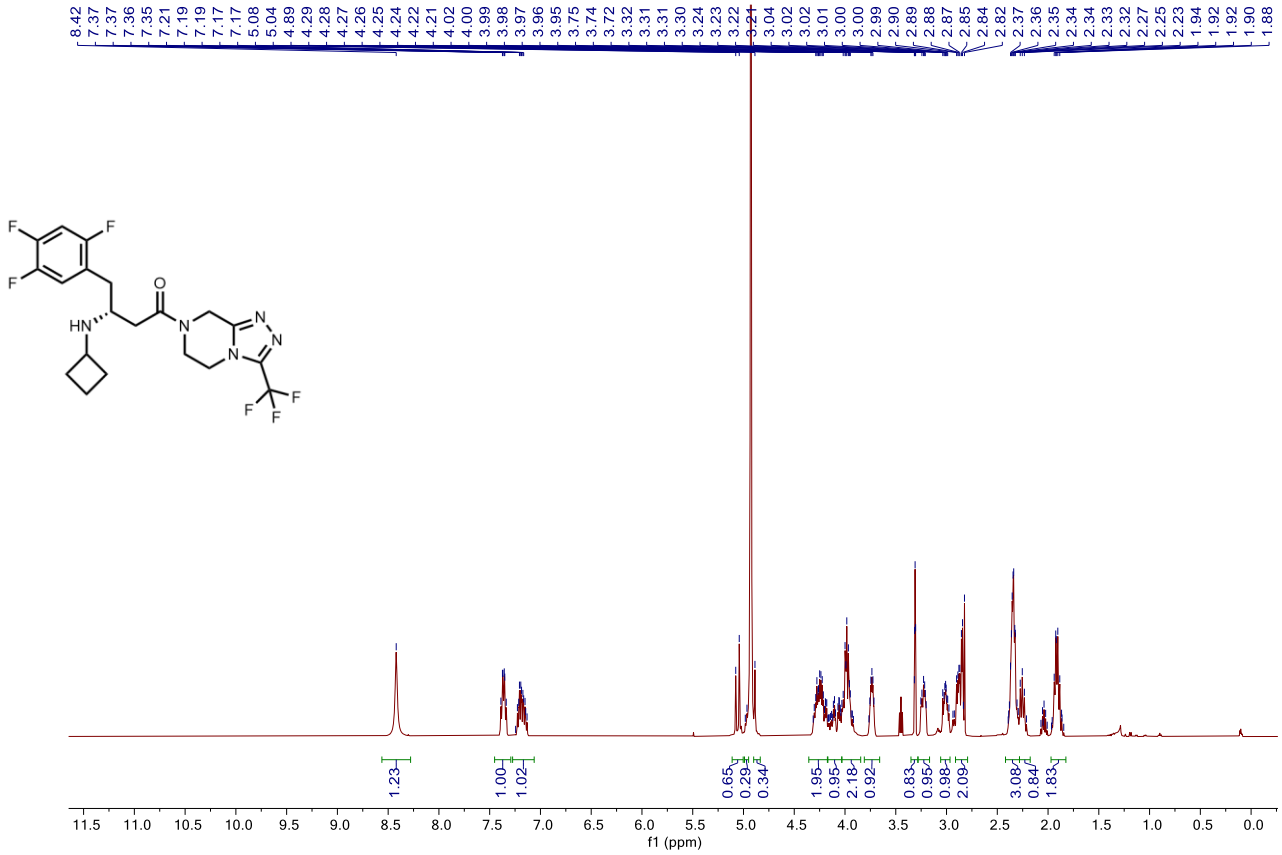
1-(isopropyl(3-phenylpropyl)amino)-3-(naphthalen-1-yloxy)propan-2-ol (51)

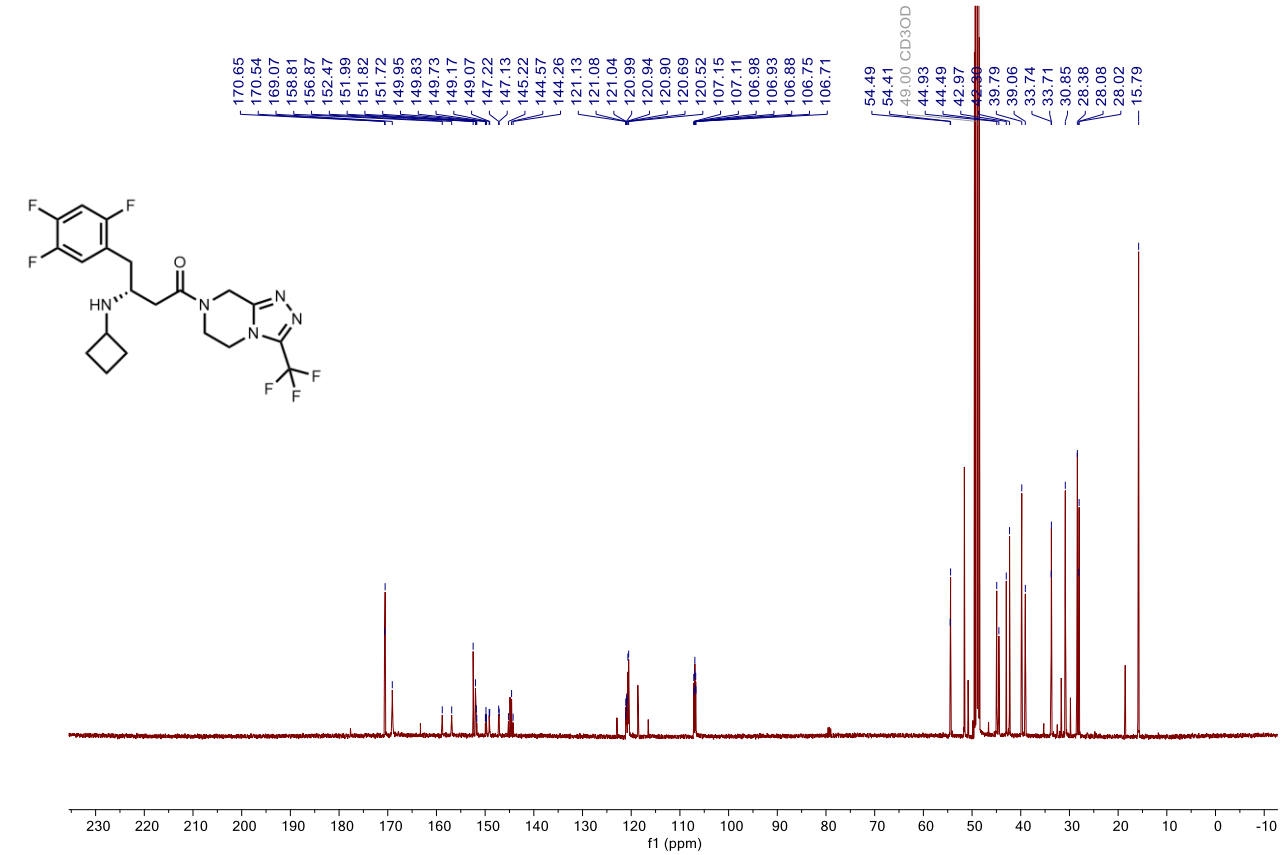


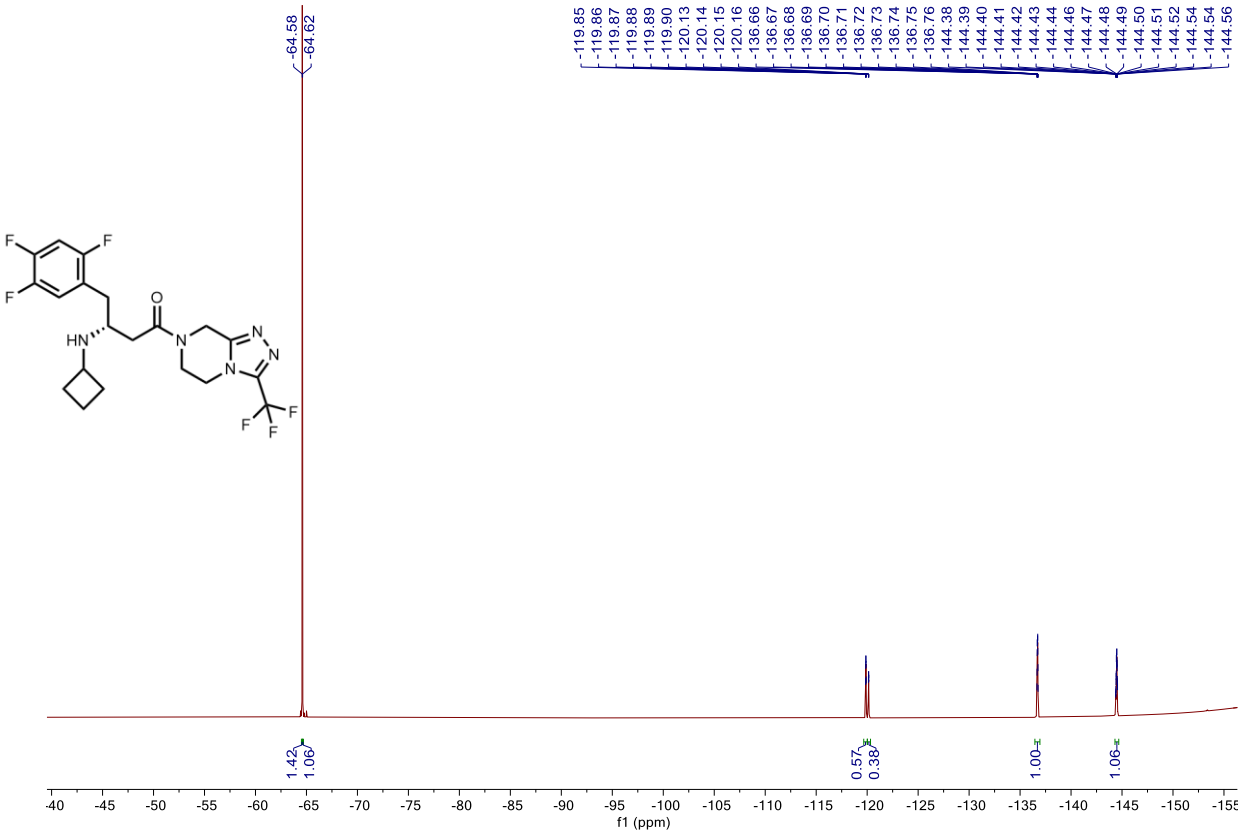
N-(((1R,4aS,10aR)-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthren-1-yl)methyl)cyclohexanamine (52)



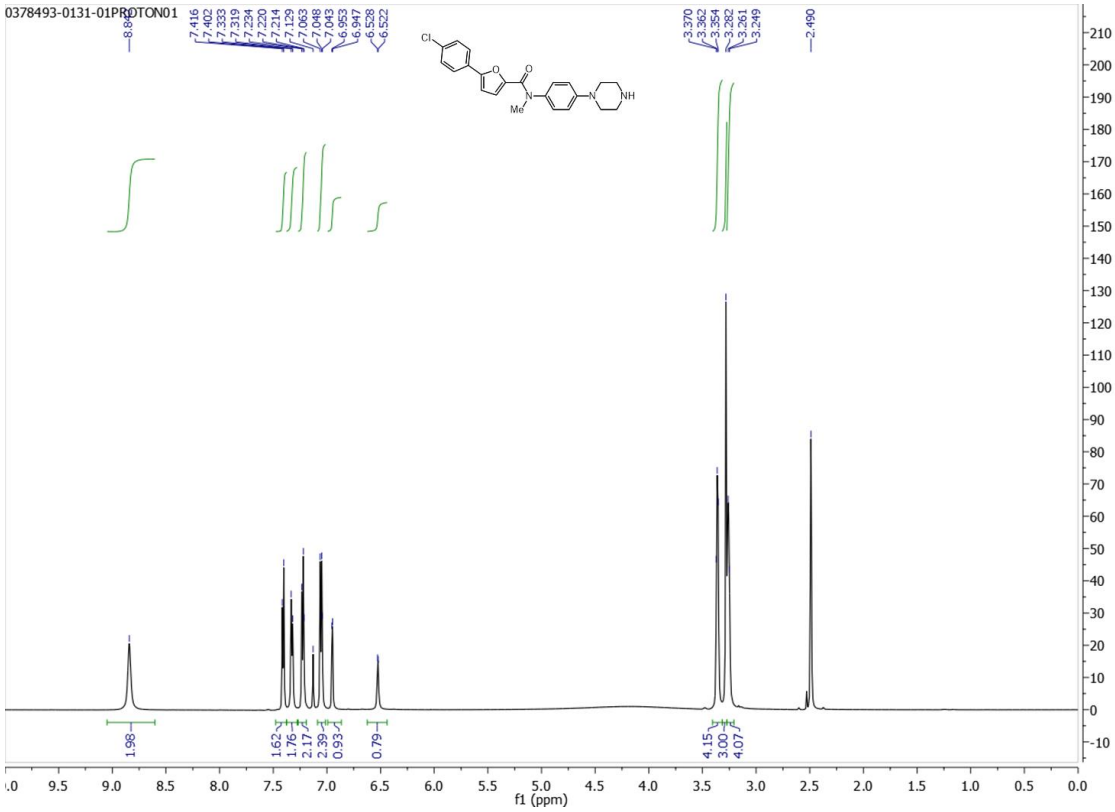
(*R*)-3-(cyclobutylamino)-1-(3-(trifluoromethyl)-5,6-dihydro-[1,2,4]triazolo[4,3-a]pyrazin-7(8*H*)-yl)-4-(2,4,5-trifluorophenyl)butan-1-one (53)



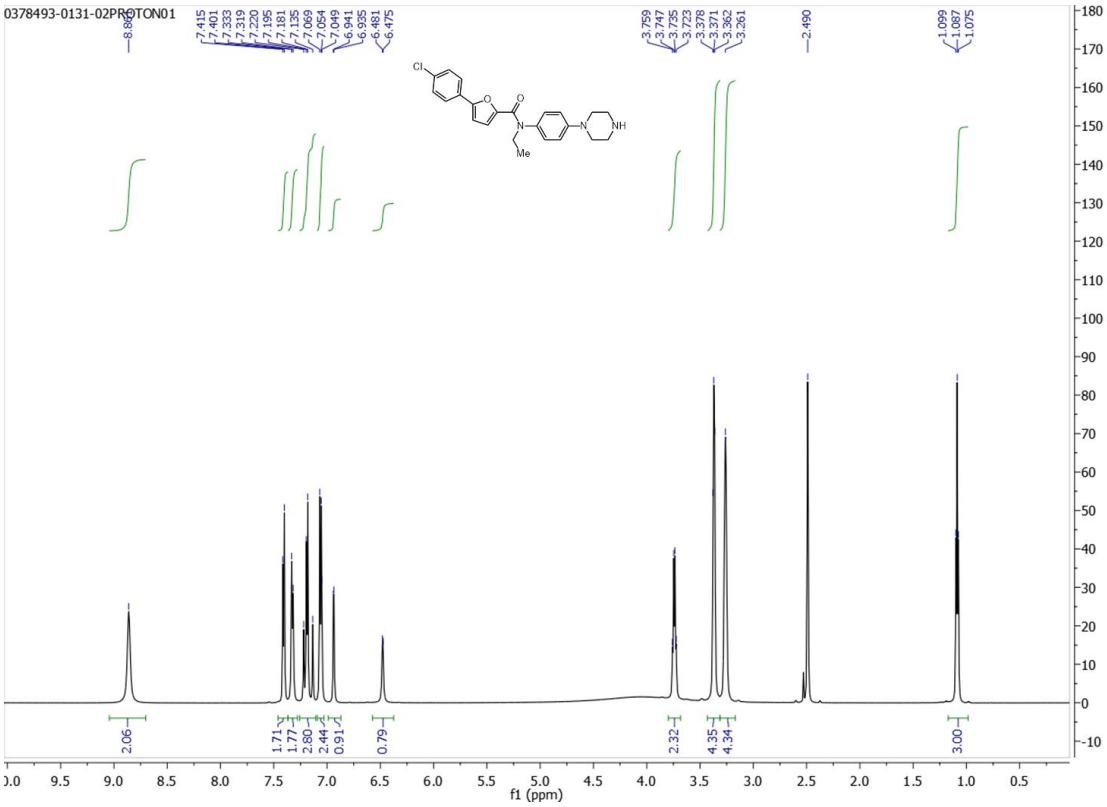


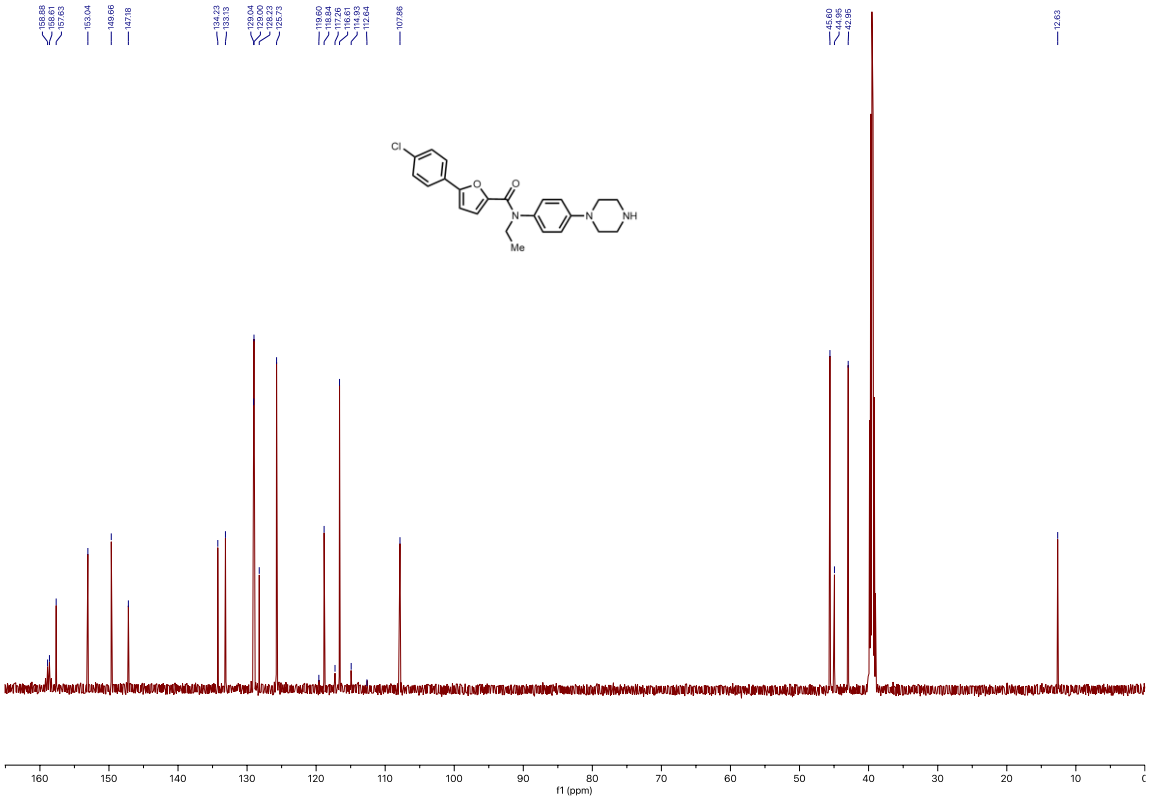

-64.58
-64.62
-119.85
-119.86
-119.87
-119.88
-119.89
-119.90
-120.13
-120.14
-120.15
-120.16
-136.66
-136.67
-136.68
-136.69
-136.70
-136.71
-136.72
-136.73
-136.74
-136.75
-136.76
-144.38
-144.39
-144.40
-144.41
-144.42
-144.43
-144.44
-144.46
-144.47
-144.48
-144.49
-144.50
-144.51
-144.52
-144.54
-144.54
-144.56
F
F
F
O
HN
N
N
N
F
F
F
1.42
1.06
0.57
0.38
1.00
1.06
-40
-45
-50
-55
-60
-65
-70
-75
-80
-85
-90
-95
-100
-105
-110
-115
-120
-125
-130
-135
-140
-145
-150
f1 (ppm)

5-(4-chlorophenyl)-N-methyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S4)

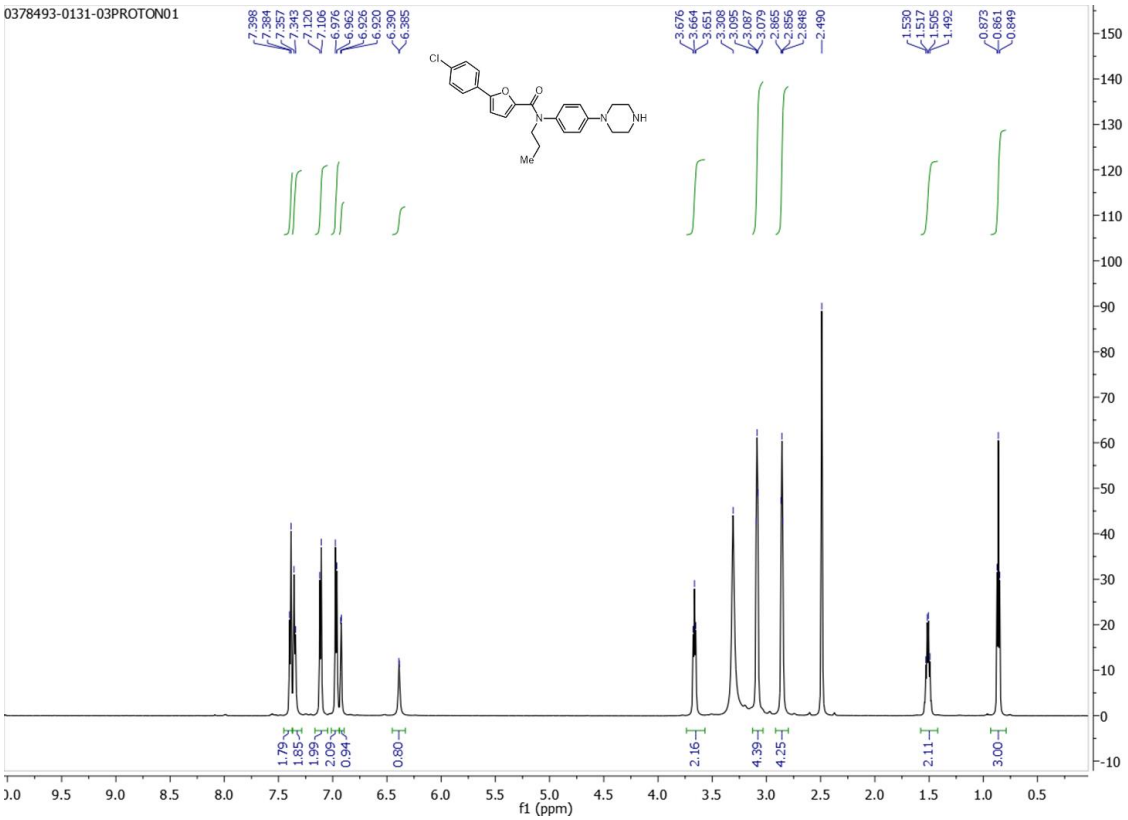


5-(4-chlorophenyl)-N-ethyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (61)

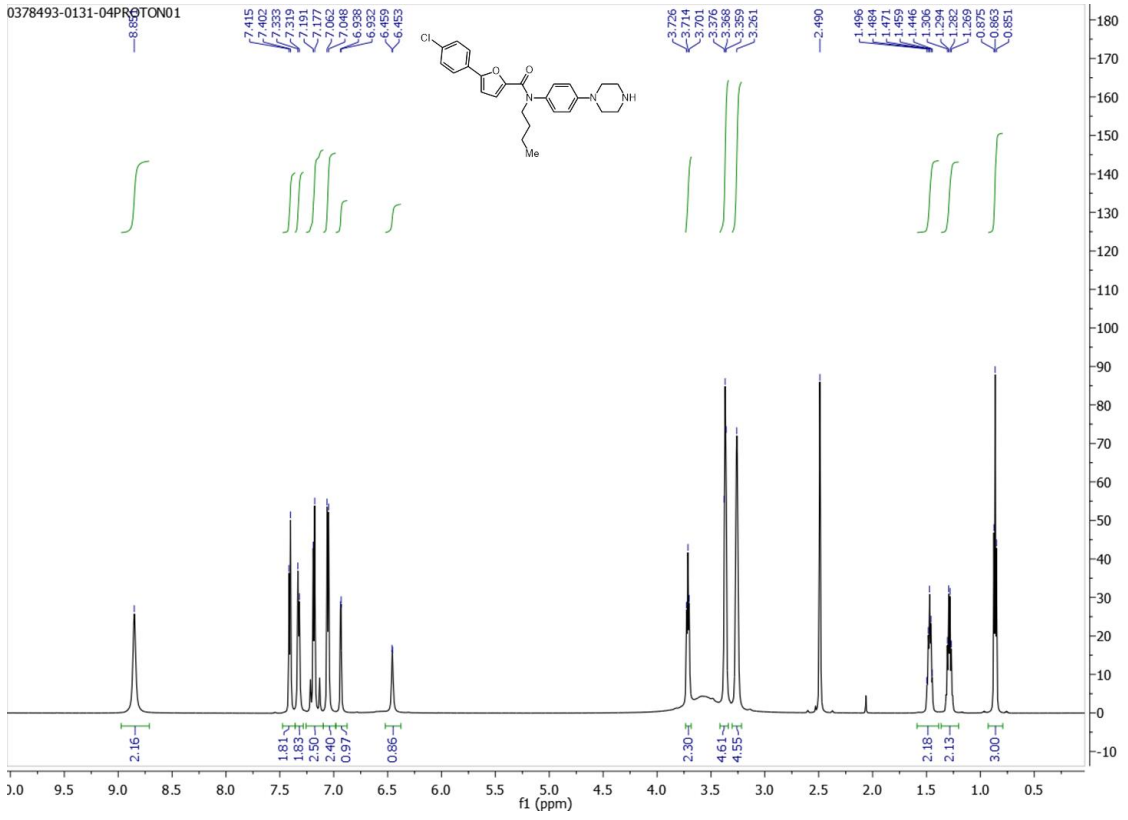




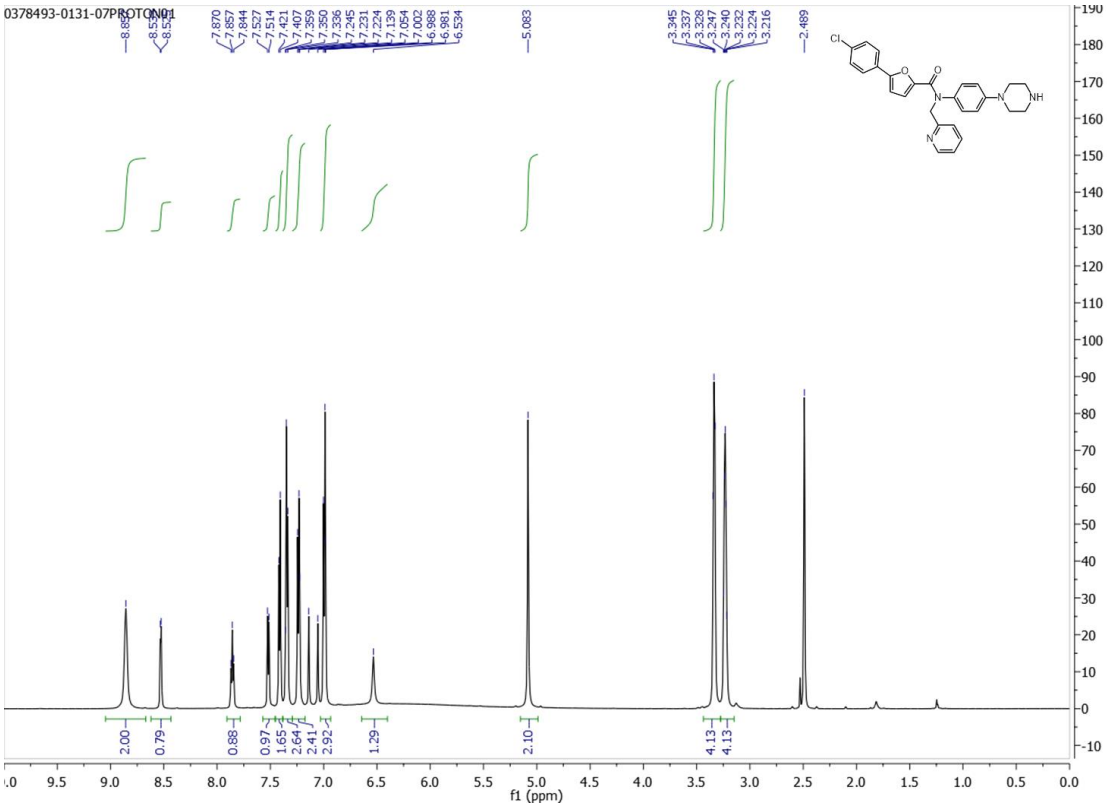
5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-propylfuran-2-carboxamide (S5)



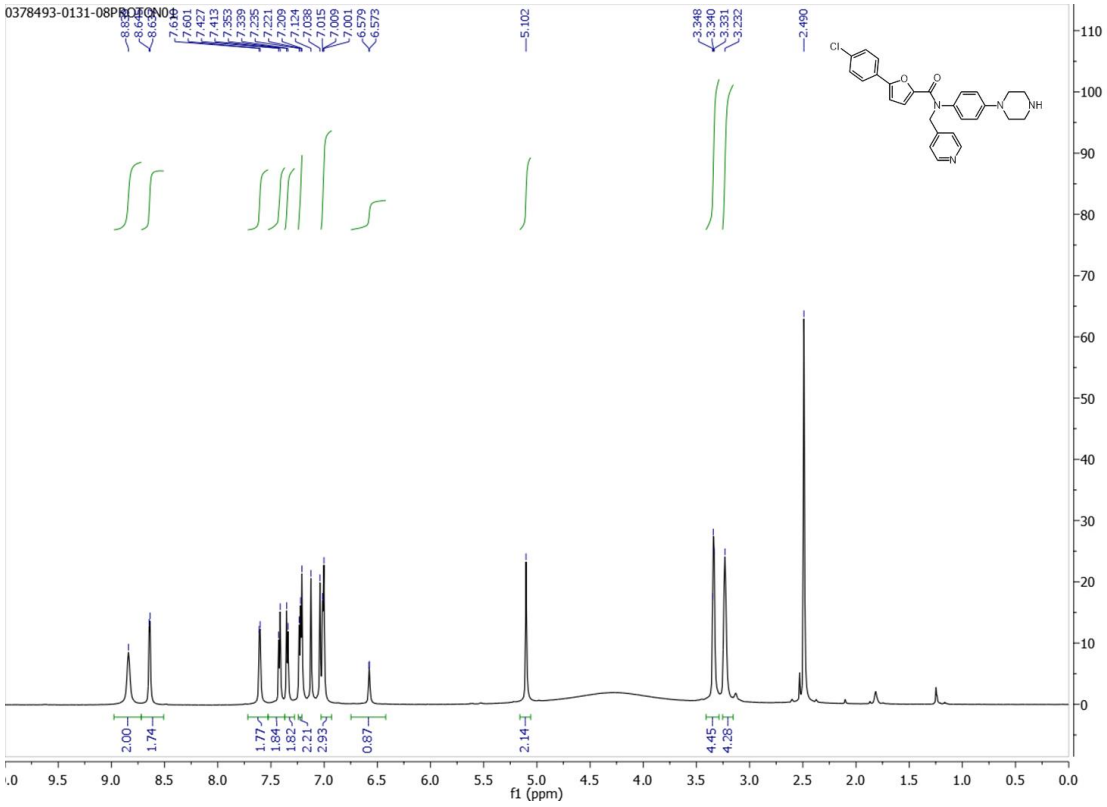
N-butyl-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S6)



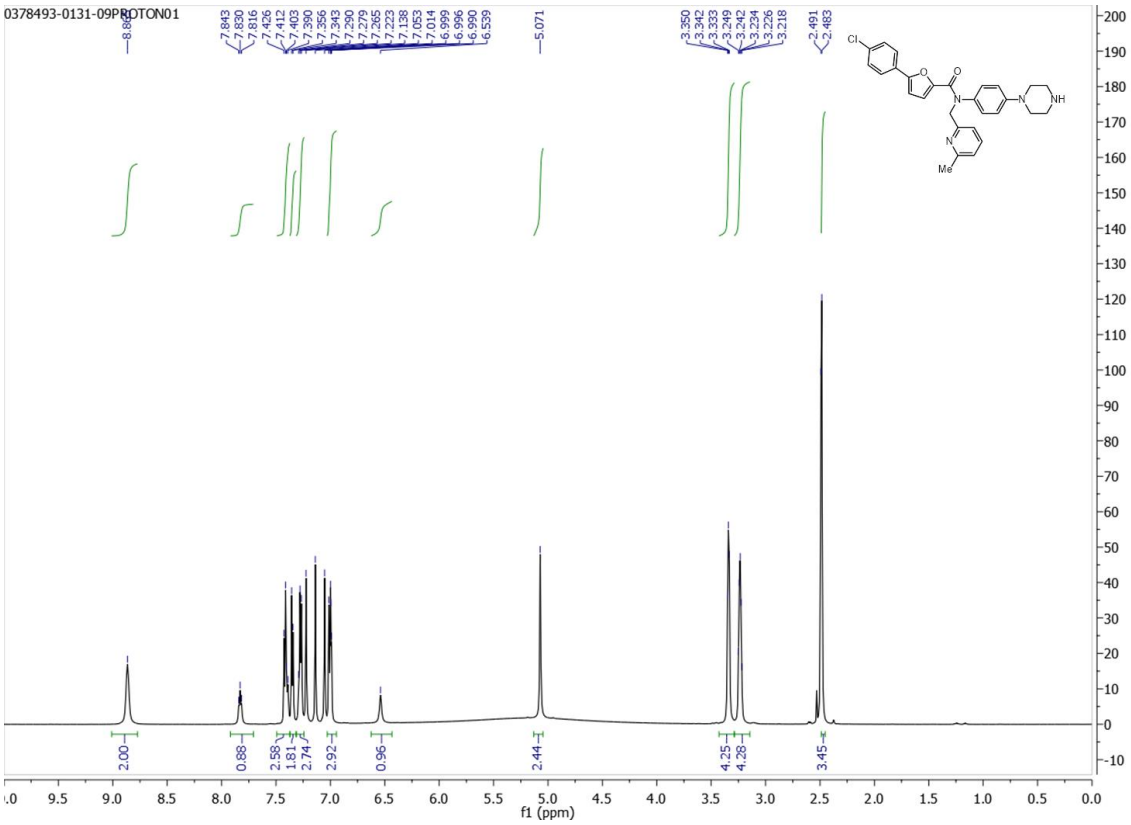
5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-(pyridin-2-ylmethyl)furan-2-carboxamide (S7)



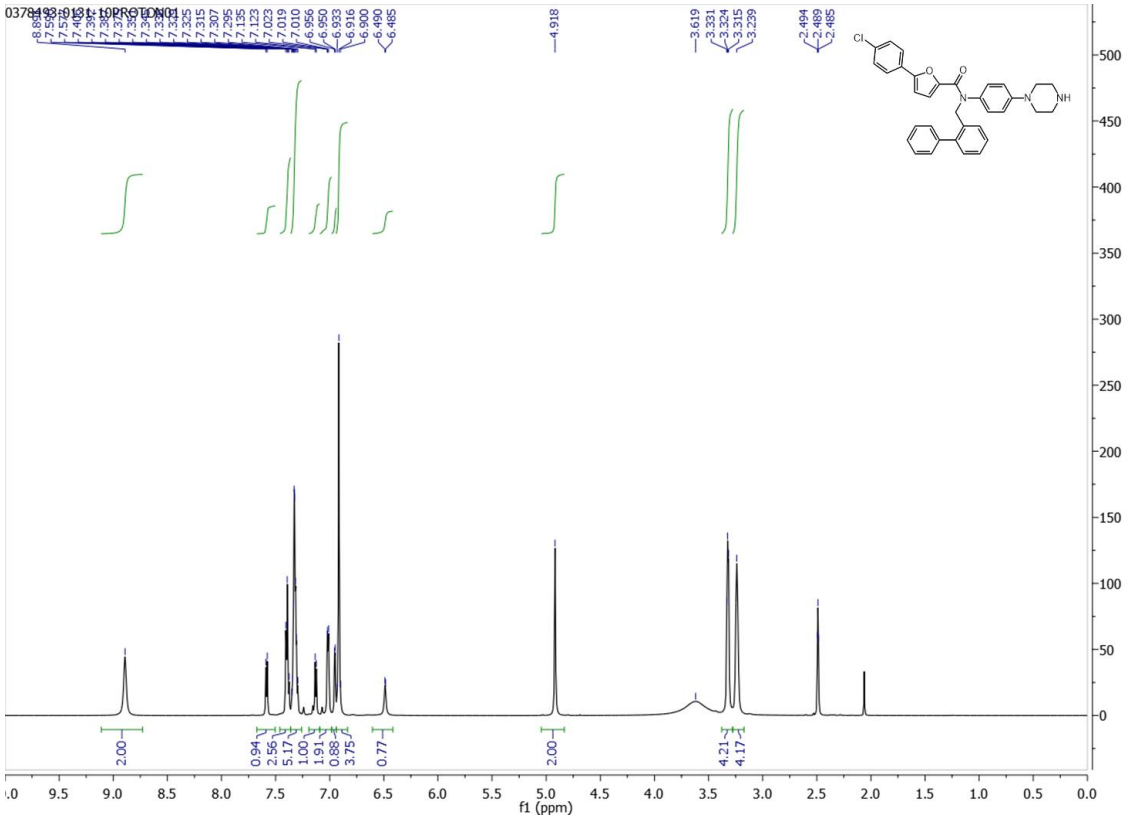
5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-(pyridin-4-ylmethyl)furan-2-carboxamide (S8)



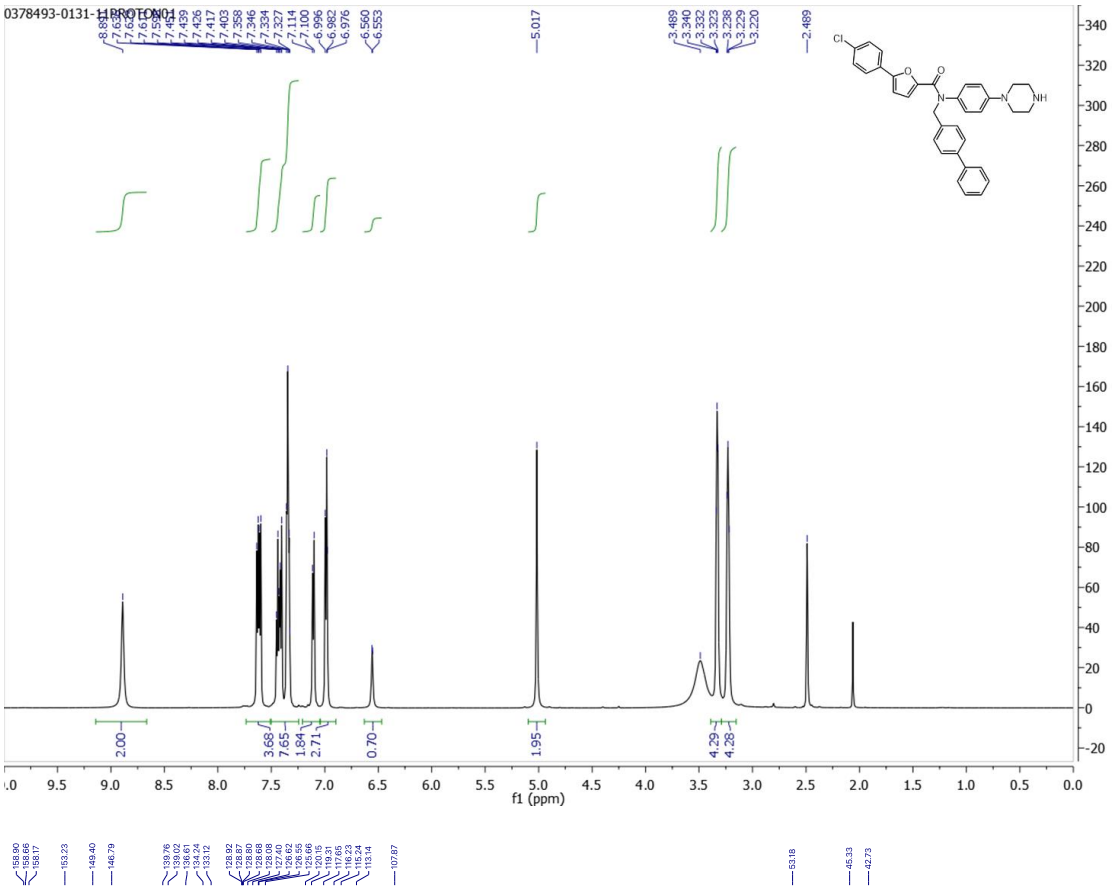
5-(4-chlorophenyl)-N-((6-methylpyridin-2-yl)methyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S9)

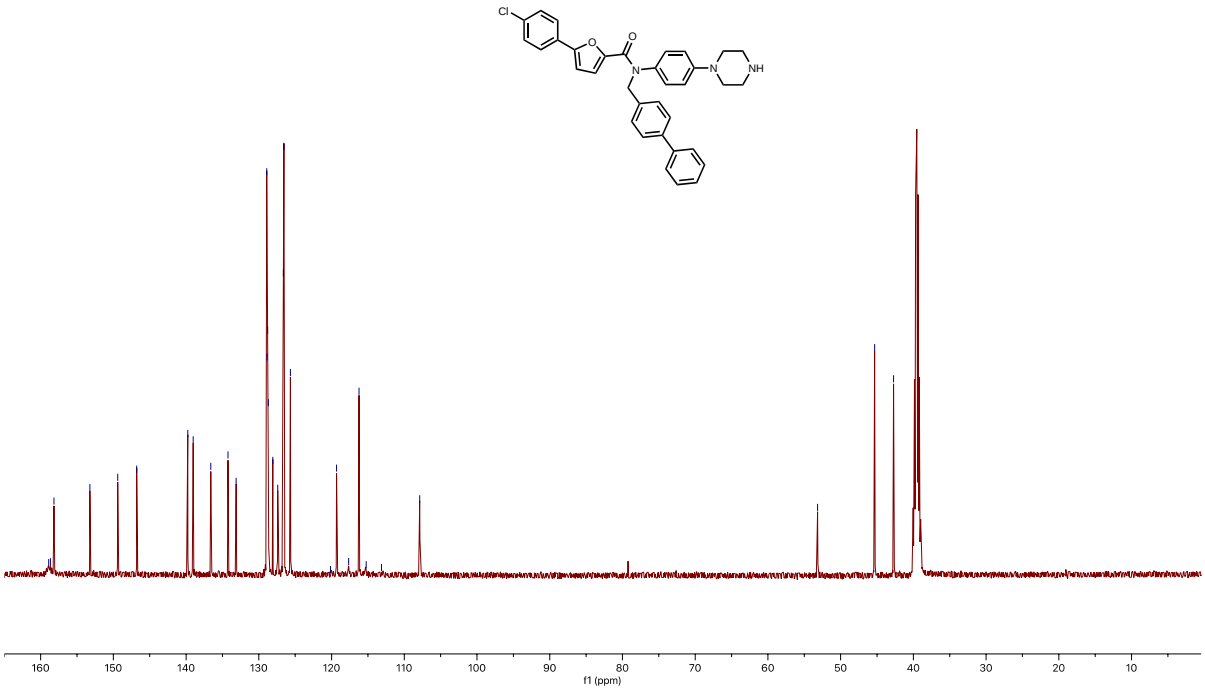


N-([1,1'-biphenyl]-2-ylmethyl)-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S10)

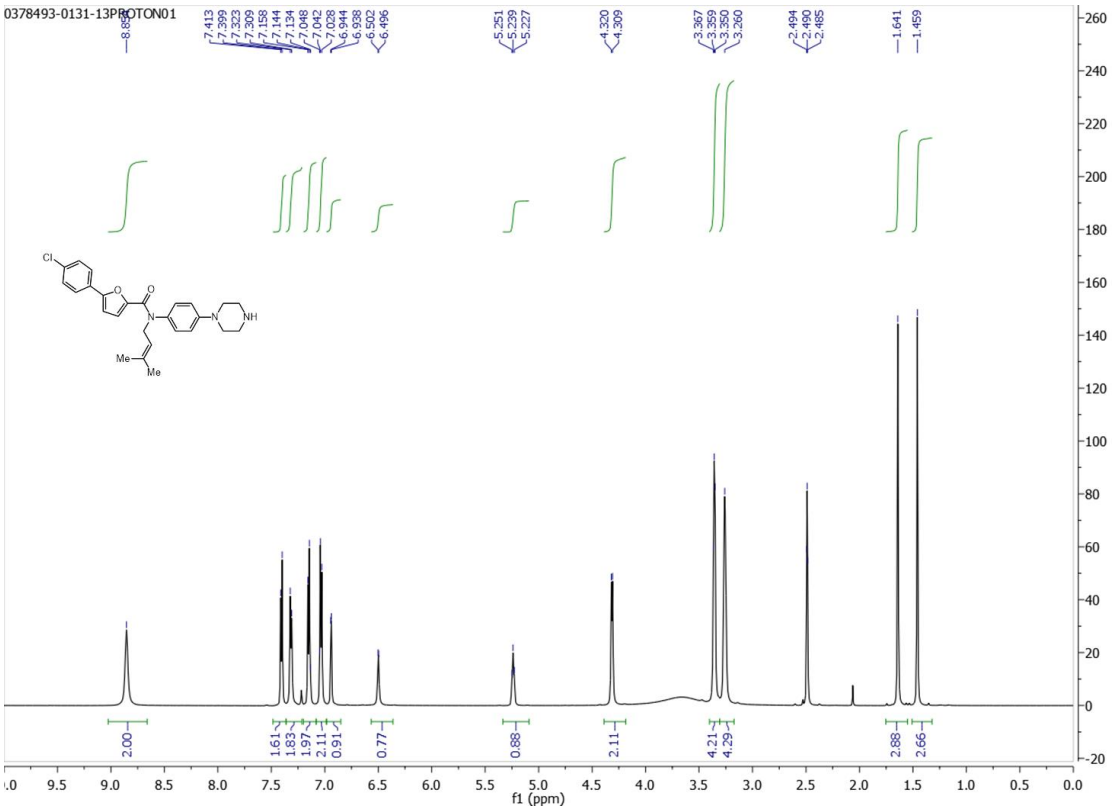


N-([1,1'-biphenyl]-4-ylmethyl)-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (62)

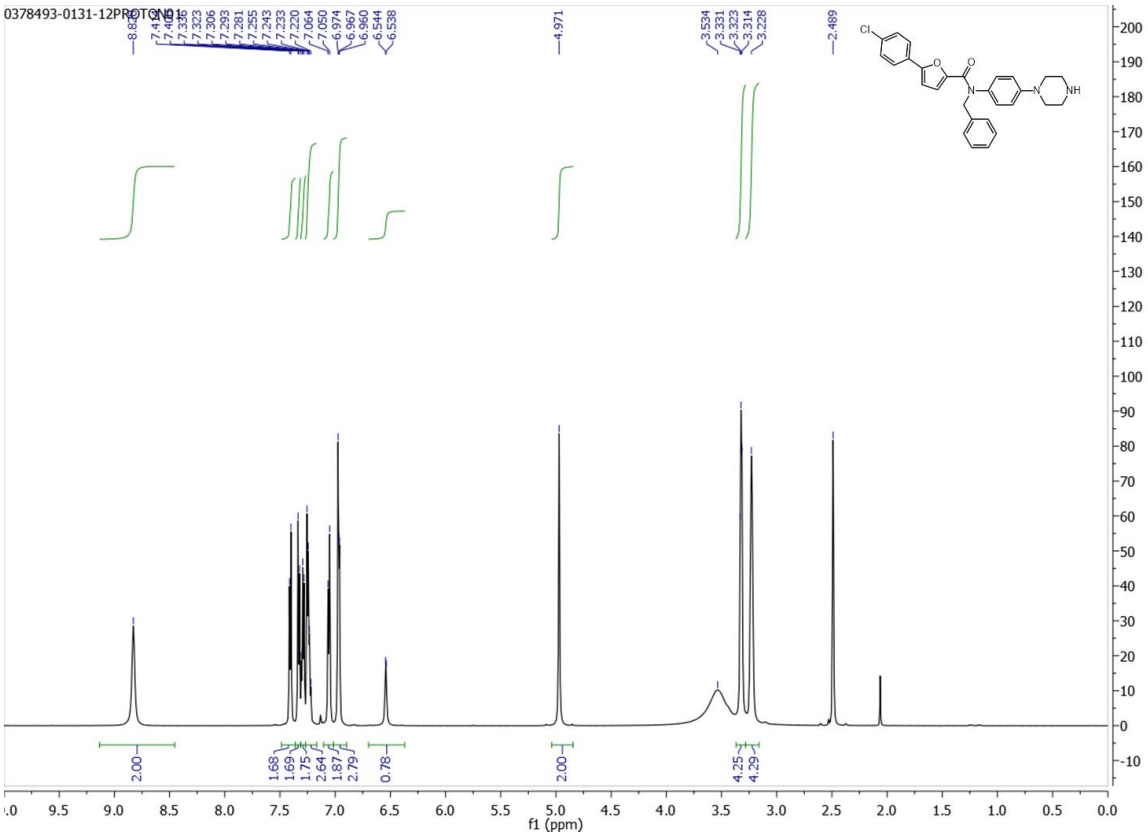




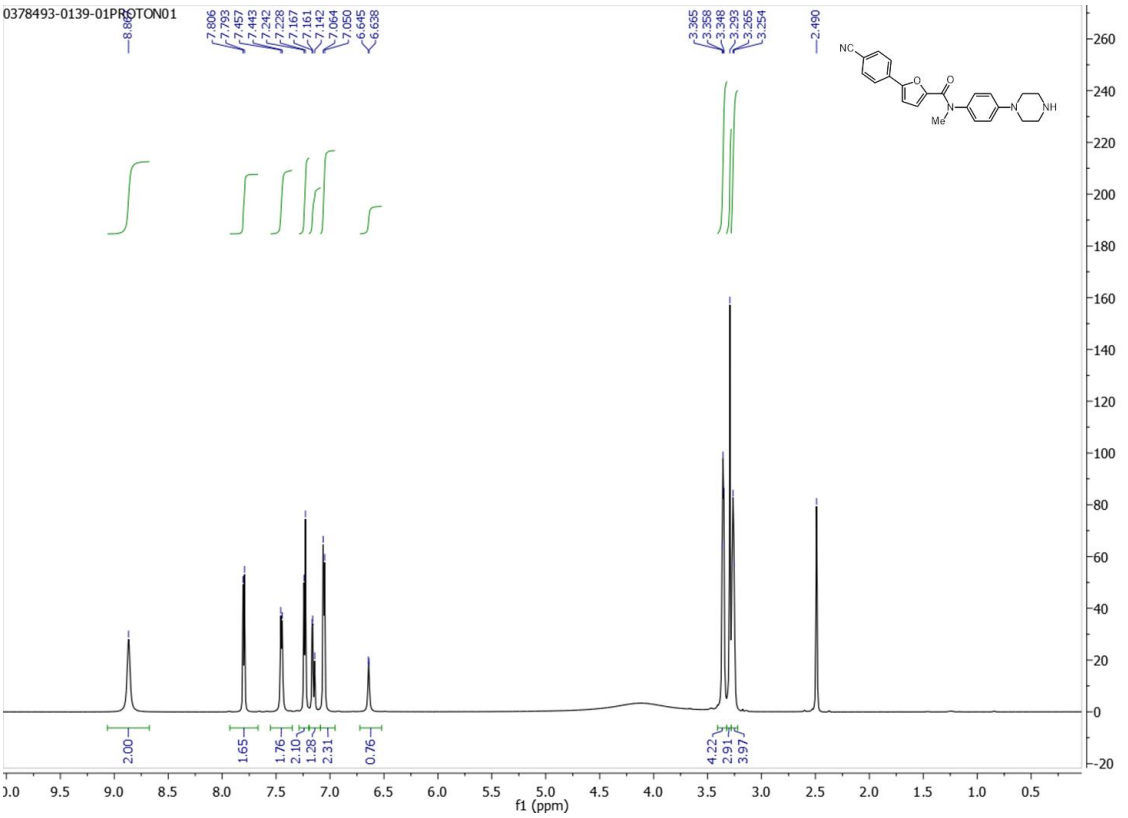
5-(4-chlorophenyl)-N-(3-methylbut-2-en-1-yl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S11)



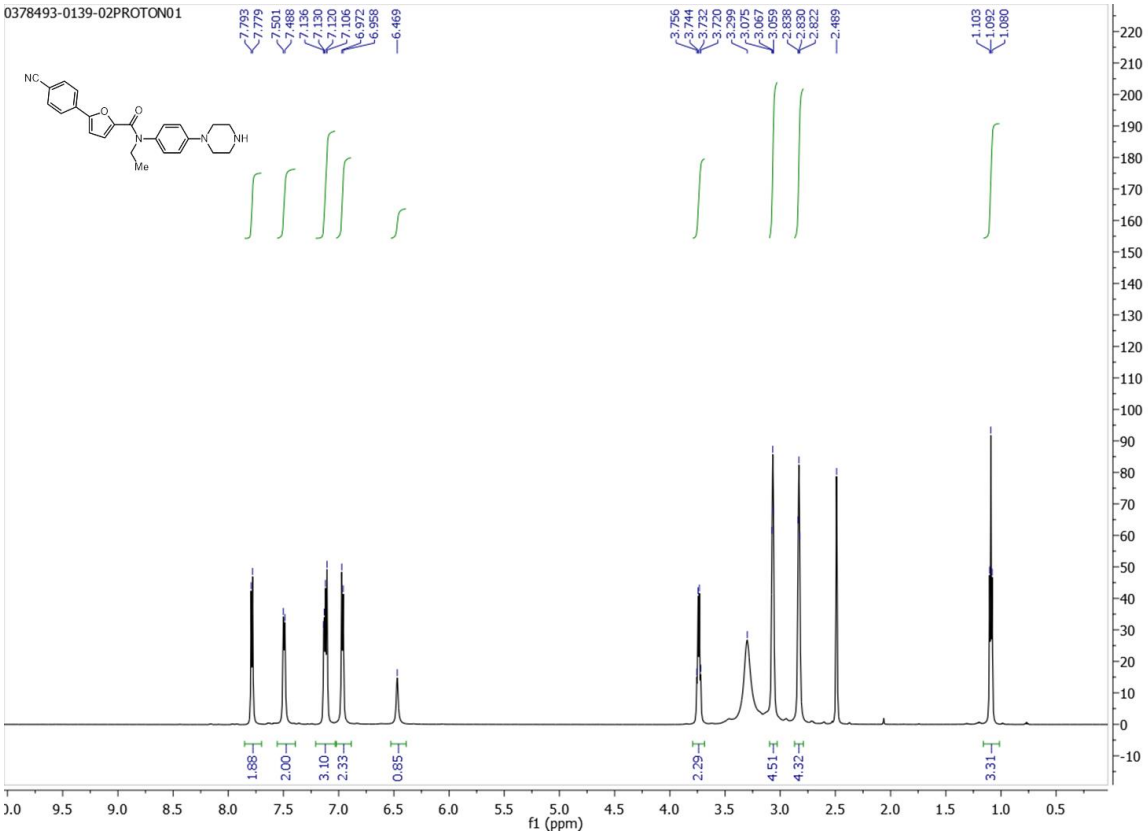
N-benzyl-5-(4-chlorophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S12)



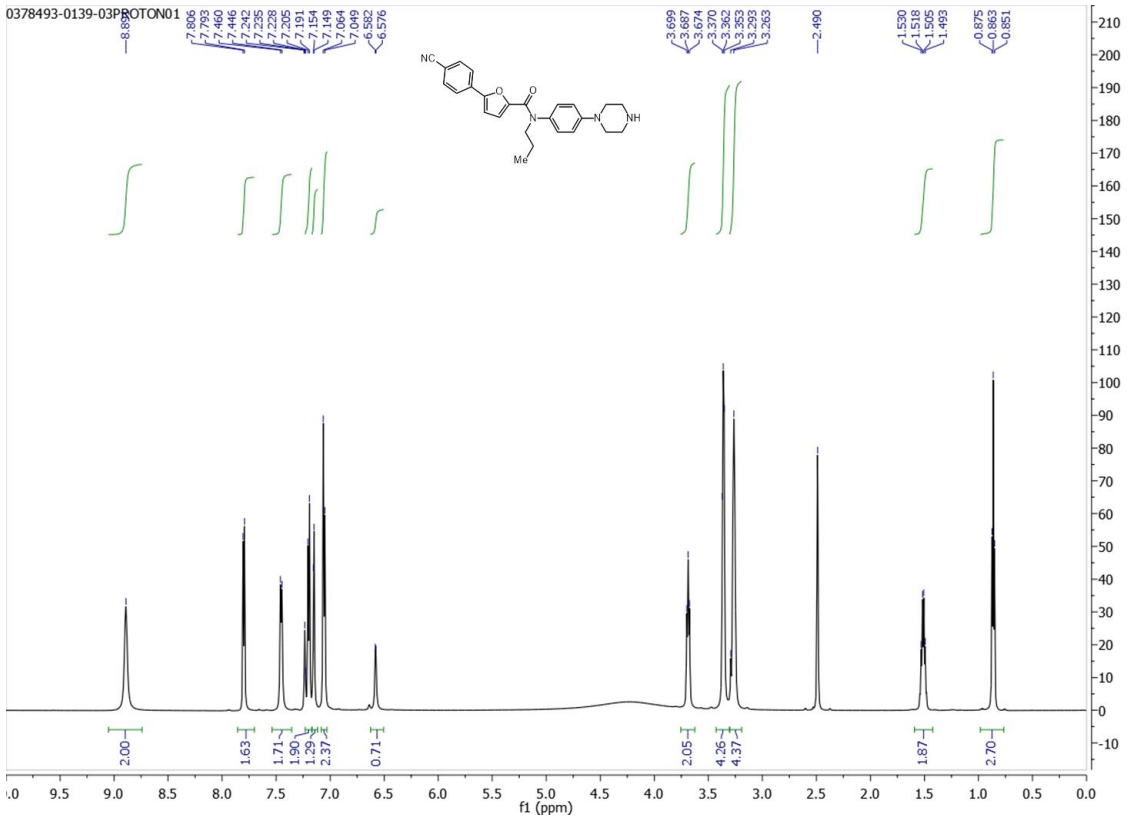
5-(4-cyanophenyl)-N-methyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S13)



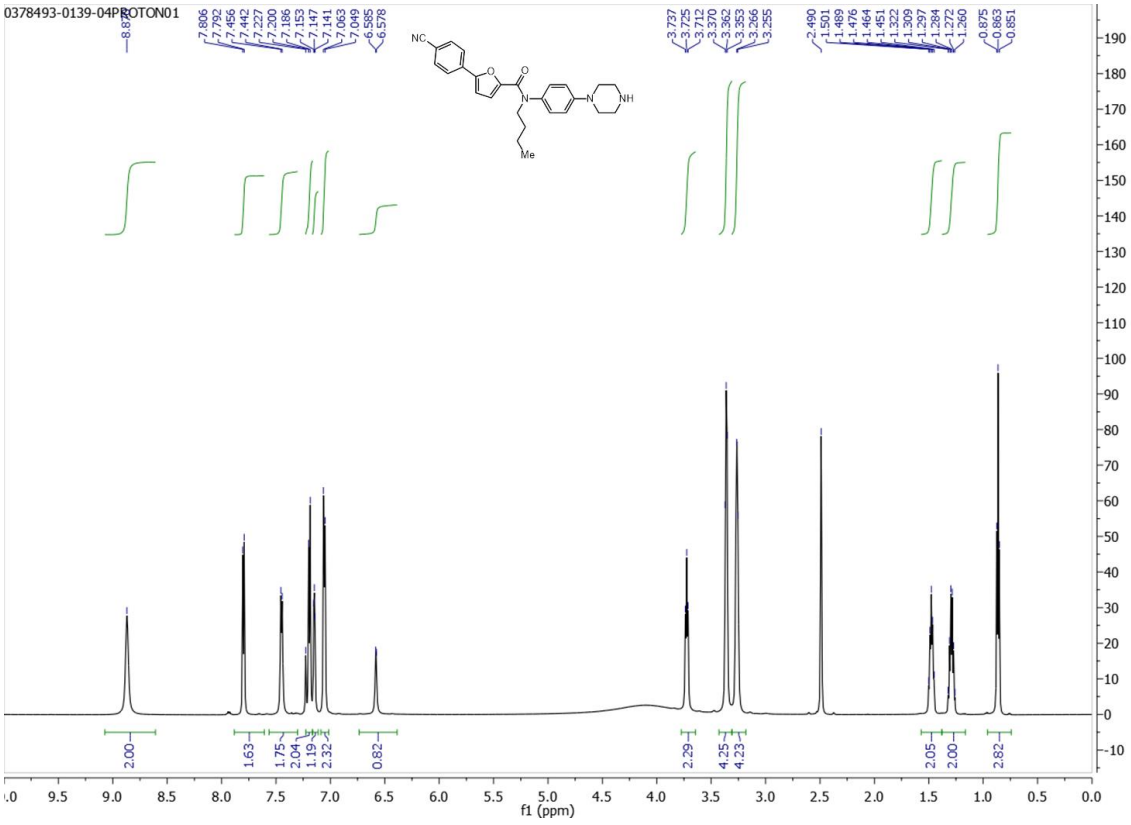
5-(4-cyanophenyl)-N-ethyl-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S14)



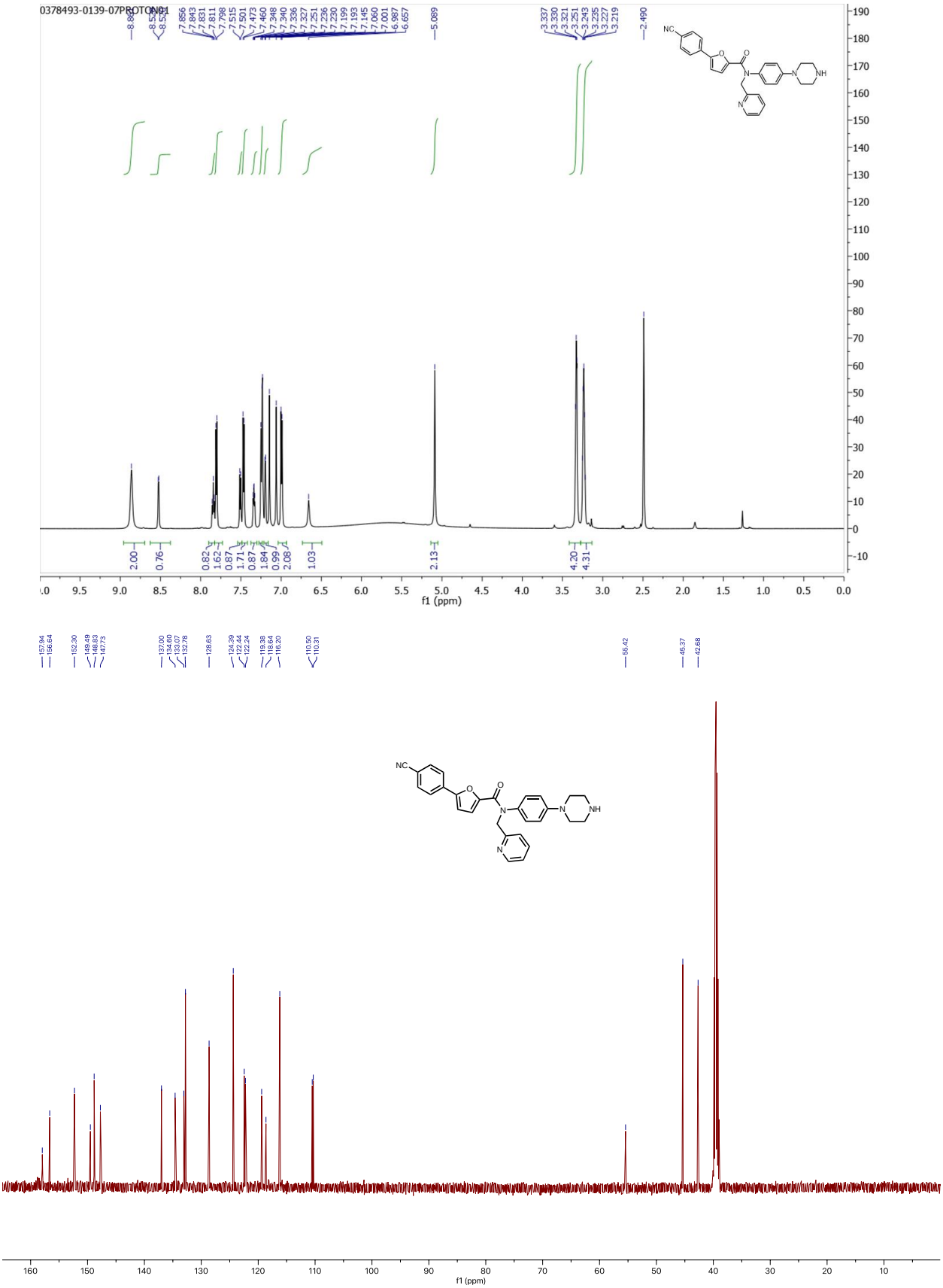
5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-propylfuran-2-carboxamide (S15)



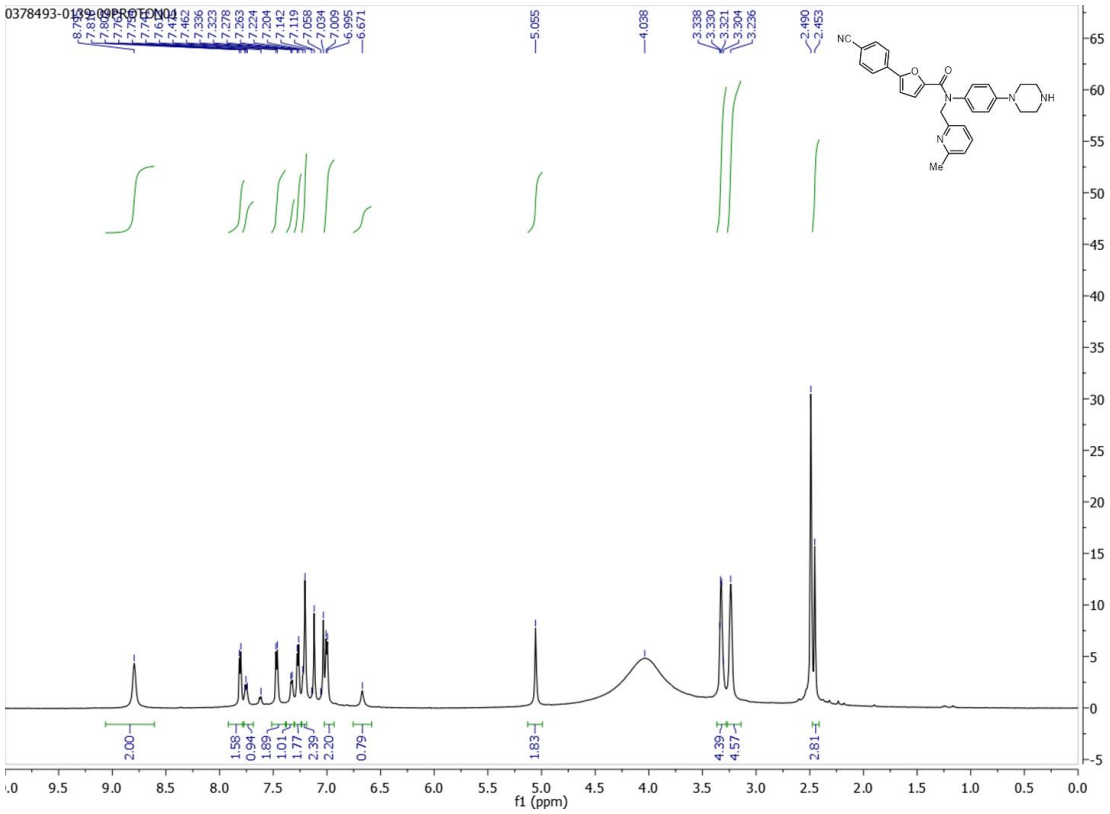
N-butyl-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S16)



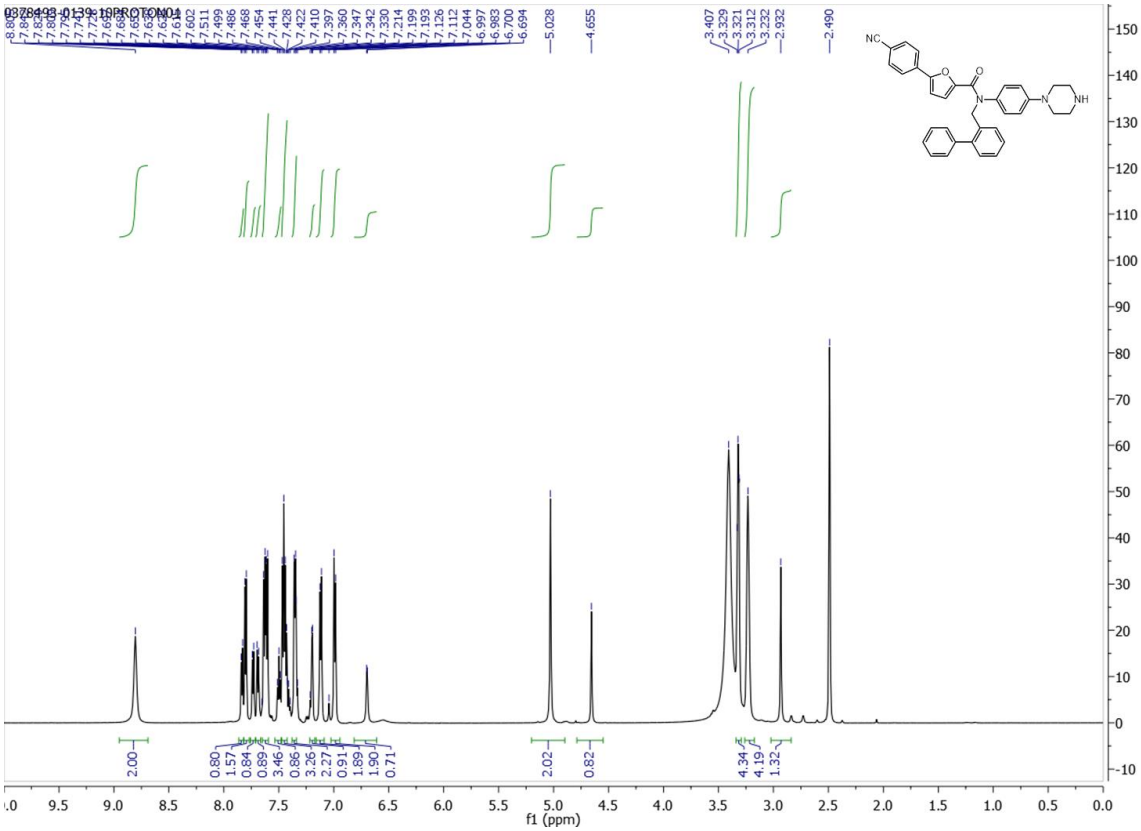
5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)-N-(pyridin-2-ylmethyl)furan-2-carboxamide (63)



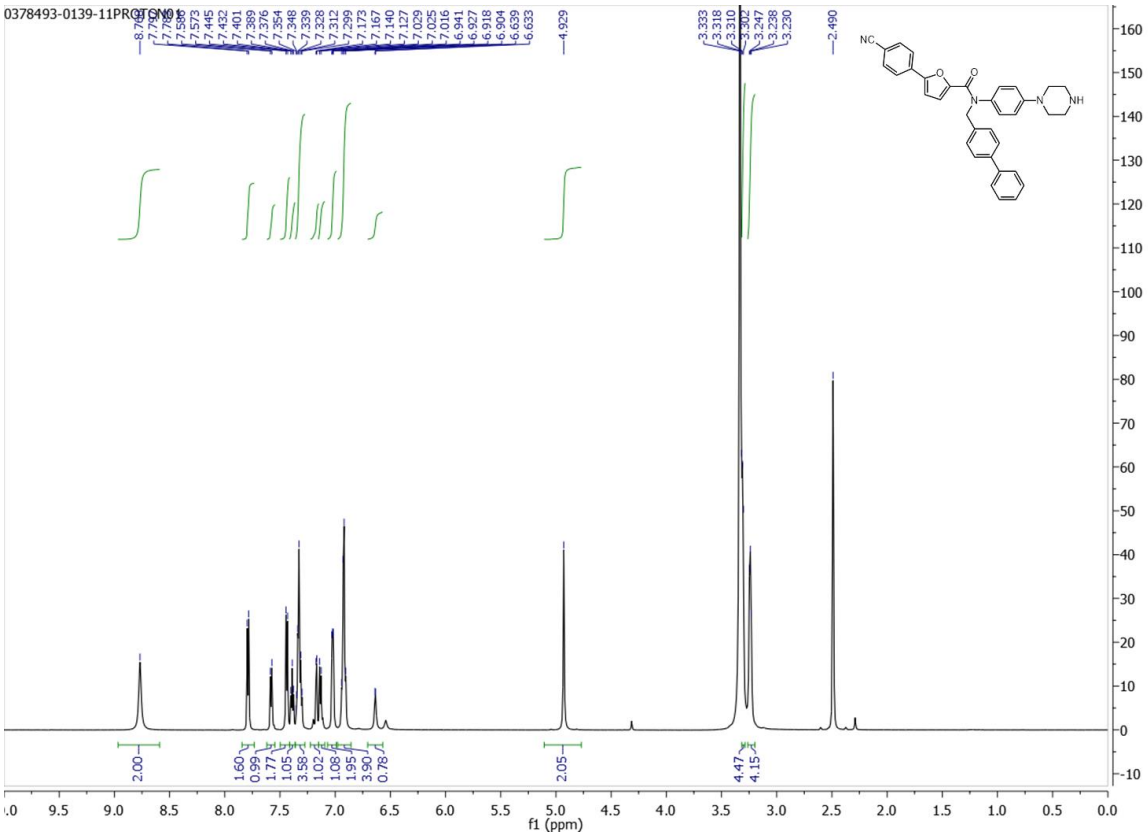
5-(4-cyanophenyl)-N-((6-methylpyridin-2-yl)methyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S17)



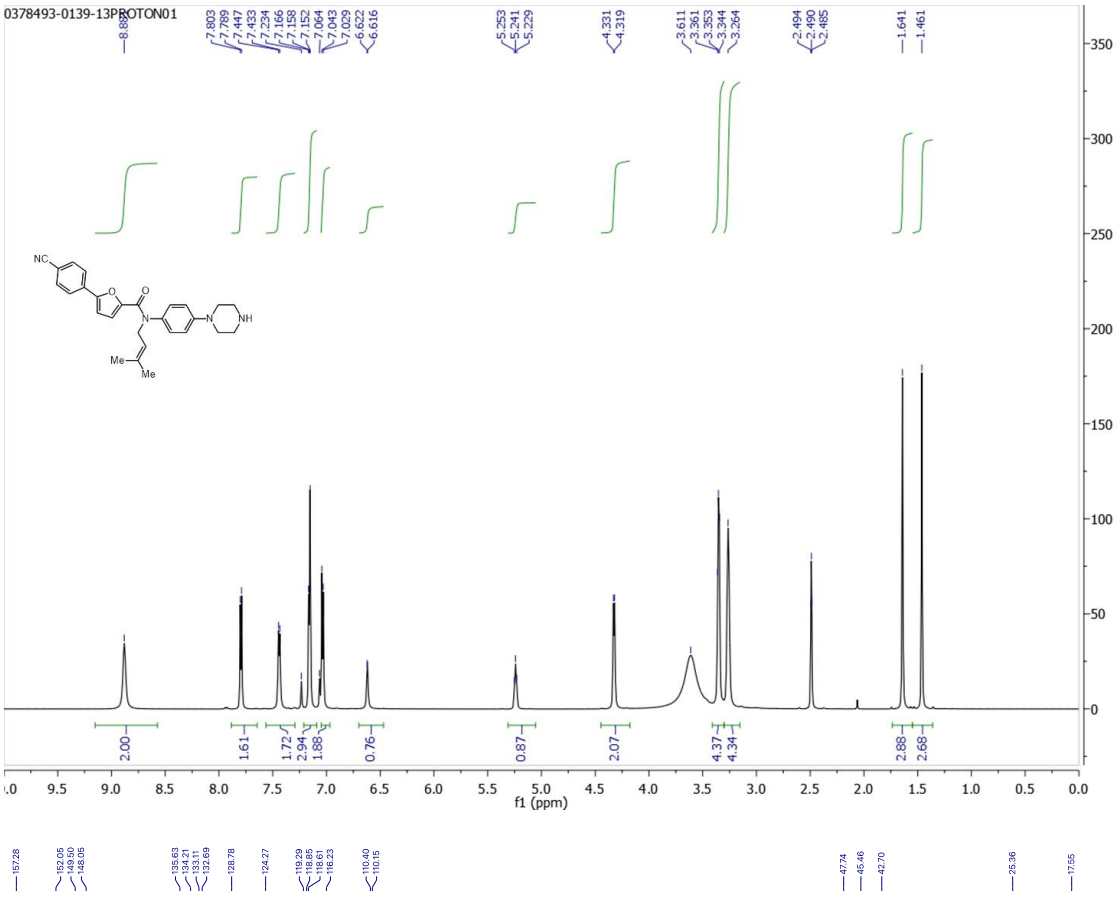
N-([1,1'-biphenyl]-2-ylmethyl)-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S18)

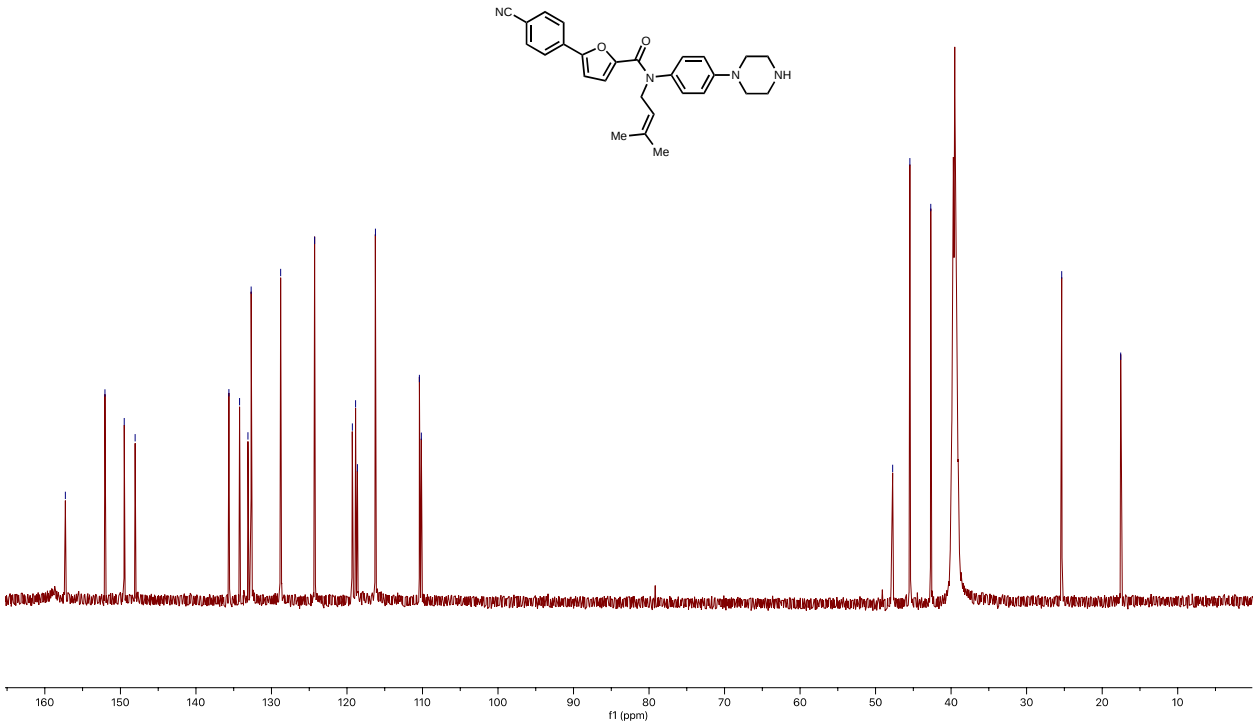


N-([1,1'-biphenyl]-4-ylmethyl)-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S19)

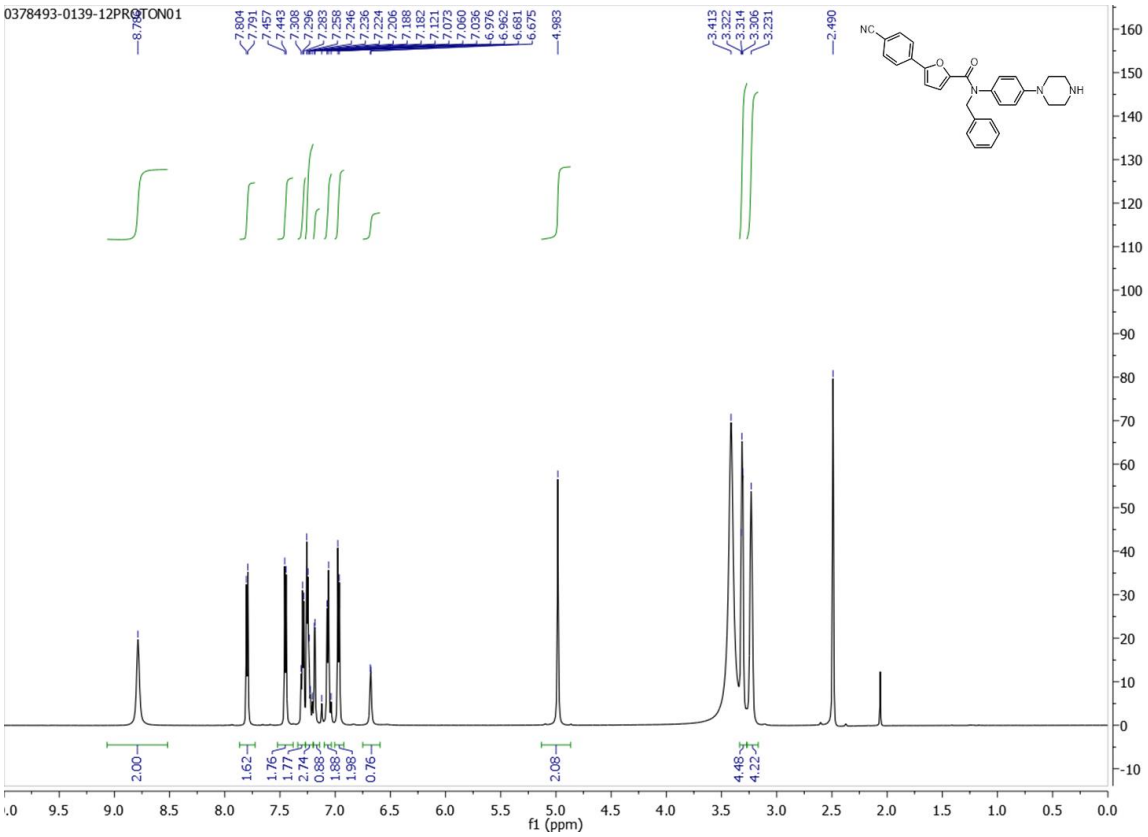


5-(4-cyanophenyl)-N-(3-methylbut-2-en-1-yl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (64)





N-benzyl-5-(4-cyanophenyl)-N-(4-(piperazin-1-yl)phenyl)furan-2-carboxamide (S20)



8. References

1. Santanilla, A. B. *et al.* Nanomole-scale high-throughput chemistry for the synthesis of complex molecules. *Science* **347**, 49–53 (2015).

2. Limanto, J. *et al.* Synthesis of a tertiary carbinamide via a novel Rh-catalyzed asymmetric hydrogenation. *J. Org. Chem.* **73**, 1639–1642 (2008).

3. Stamford, A. W. *et al.* Discovery of an orally available, brain penetrant BACE1 inhibitor that affords robust CNS Aβ reduction. *ACS Med. Chem. Lett.* **3**, 897–902 (2012).

4. George Njoroge, F. *et al.* Structure-activity relationship of 3-substituted N-(pyridinylacetyl)-4- (8-chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)- piperidine inhibitors of farnesyl-protein transferase: Design and synthesis of in vivo active antitumor compo. *J. Med. Chem.* **40**, 4290–4301 (1997).

5. Song, Z. J. *et al.* Synthesis of vaniprevir (MK-7009): Lactamization to prepare a 22-membered macrocycle. *J. Org. Chem.* **76**, 7804–7815 (2011).

6. Tong, W., Chowdhury, S. K., Su, A. D. & Alton, K. B. Quantitation of parent drug and its unstable metabolites by in situ coulometric oxidation and liquid chromatography-tandem mass spectrometry. *Anal. Chem.* **82**, 10251–10257 (2010).

7. Zeng, L. F. *et al.* Repositioning HIV-1 integrase inhibitors for cancer therapeutics: 1,6-naphthyridine-7-carboxamide as a promising scaffold with drug-like properties. *J. Med. Chem.* **55**, 9492–9509 (2012).

8. Anderson, D. R. *et al.* Pyrrolopyridine inhibitors of mitogen-activated protein kinase-activated protein kinase 2 (MK-2). *J. Med. Chem.* **50**, 2647–2654 (2007).

9. Gesmundo, N. J. *et al.* Nanoscale synthesis and affinity ranking. *Nature* **557**, 228–232 (2018).

10. Huang, X. *et al.* Discovery of a novel series of Chk1 kinase inhibitors with a distinctive hinge binding mode. *ACS Med. Chem. Lett.* **3**, 123–128 (2012).

11. De Laszlo, S. E. *et al.* The design, binding affinity prediction and synthesis of macrocyclic angiotensin II AT1 and AT2 receptor antagonists. *Bioorganic Med. Chem. Lett.* **6**, 923–928 (1996).

12. Huang, X. *et al.* Discovery and hit-to-lead optimization of non-ATP competitive MK2 (MAPKAPK2) inhibitors. *ACS Med. Chem. Lett.* **2**, 632–637 (2011).

13. Huang, X. *et al.* A three-step protocol for lead optimization: Quick identification of key conformational features and functional groups in the SAR studies of non-ATP competitive MK2 (MAPKAPK2) inhibitors. *Bioorg. Med. Chem. Lett.* **22**, 65–70 (2012).

14. Kutchukian, P. S. *et al.* Chemistry informer libraries: A chemoinformatics enabled approach to evaluate and advance synthetic methods. *Chem. Sci.* **7**, 2604–2613 (2016).

15. Bhattacharyya, S. Titanium(IV) isopropoxide and sodium borohydride : A reagent of choice for reductive amination. *Tetrahedron Lett.* **35**, 2401–2404 (1994).

16. Mattson, R. J., Pham, K. M., Leuck, D. J. & Cowen, K. A. An Improved Method for Reductive Alkylation of Amines Using Titanium(IV) Isopropoxide and Sodium Cyanoborohydride1. *J. Org. Chem.* **55**, 2552–2554 (1990).

17. Neidigh, K. A., Avery, M. A., Williamson, J. S. & Bhattacharyya, S. Facile preparation of N-methyl secondary amines by titanium(IV) isopropoxide-mediated reductive animation of carbonyl compounds. *J. Chem. Soc. - Perkin Trans. 1* 2527–2531 (1998) doi:10.1039/a803703e.

18. Huang, X. *et al.* Discovery and Hit-to-Lead Optimization of Non-ATP Competitive MK2 (MAPKAPK2) Inhibitors. *ACS Med. Chem. Lett.* **2**, 632–637 (2011).

19. Huang, X. *et al.* A three-step protocol for lead optimization: Quick identification of key conformational features and functional groups in the SAR studies of non-ATP competitive MK2 (MAPKAPK2) inhibitors. *Bioorg. Med. Chem. Lett.* **22**, 65–70 (2012).