Supporting Information

Discovery of Potent and Selective Inhibitors of Cdc2-like Kinase 1 (CLK1) as a New Class of Autophagy Inducers

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Table S1. Kinase inhibition profile of compound **25**[a]

Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
Abl(h)	>10000[b]	MARK4(h)	>10000[b]
Abl (H396P) (h)	>10000[b]	MEKK2(h)	>10000[b]
Abl (M351T)(h)	>10000[b]	MELK(h)	>10000[b]
Abl (Q252H) (h)	>10000[b]	Mer(h)	2972
Abl(T315I)(h)	>10000[b]	Met(h)	>10000[b]
Abl(Y253F)(h)	>10000[b]	Met(D1246H)(h)	>10000[b]
ACK1(h)	>10000[b]	Met(D1246N)(h)	>10000[b]
ALK(h)	>10000[b]	Met(M1268T)(h)	>10000[b]
ALK1(h)	>10000[b]	Met(Y1248C)(h)	>10000[b]
ALK2(h)	>10000[b]	Met(Y1248D)(h)	>10000[b]
ALK4(h)	>10000[b]	Met(Y1248H)(h)	>10000[b]
ALK6(h)	>10000[b]	MINK(h)	3623
Arg(h)	>10000[b]	MKK6(h)	>10000[b]
AMPKα1(h)	>10000[b]	MKK7β(h)	>10000[b]
AMPKα2(h)	>10000[b]	MLCK(h)	>10000[b]
A-Raf(h)	>10000[b]	MLK1(h)	7196
ARK5(h)	>10000[b]	MLK2(h)	1260
ASK1(h)	>10000[b]	Mnk2(h)	>10000[b]
Aurora-A(h)	>10000[b]	MOK(h)	>10000[b]
Aurora-B(h)	>10000[b]	MRCKα(h)	>10000[b]
Aurora-C(h)	>10000[b]	MRCKβ(h)	>10000[b]
Axl(h)	>10000[b]	MSK1(h)	1430
Blk(h)	9621	MSK2(h)	266
Bmx(h)	>10000[b]	MSSK1(h)	>10000[b]
BRK(h)	>10000[b]	MST1(h)	>10000[b]
BrSK1(h)	>10000[b]	MST2(h)	1265
BrSK2(h)	>10000[b]	MST3(h)	>10000[b]
BTK(h)	>10000[b]	MST4(h)	>10000[b]
BTK(R28H)(h)	>10000[b]	mTOR(h)	>10000[b]
B-Raf(h)	>10000[b]	mTOR/FKBP12(h)	>10000[b]
B-Raf(V599E)(h)	>10000[b]	MuSK(h)	>10000[b]
CaMKI(h)	>10000[b]	MYLK2(h)	>10000[b]
CaMKIß(h)	>10000[b]	MYO3B(h)	>10000[b]
CaMKIγ(h)	>10000[b]	NEK1(h)	449
CaMKIIα(h)	>10000[b]	NEK2(h)	>10000[b]
CaMKIIβ(h)	>10000[b]	NEK3(h)	>10000[b]
CaMKIIγ(h)	>10000[b]	NEK6(h)	>10000[b]
CaMKIδ(h)	>10000[b]	NEK7(h)	>10000[b]
CaMKIIδ(h)	>10000[b]	NEK9(h)	>10000[b]
CaMKIV(h)	>10000[b]	NIM1(h)	>10000[b]
CaMKK1(h)	>10000[b]	NEK11(h)	2357
CaMKK2(h)	>10000[b]	NLK(h)	3273

CDK1/cyclinB(h)	>10000[b]	NUAK2(h)	>10000[b]
CDK2/cyclinA(h)	>10000[b]	p70S6K(h)	546
CDK2/cyclinE(h)	>10000[b]	PAK1(h)	>10000[b]
CDK3/cyclinE(h)	>10000[b]	PAK2(h)	>10000[b]
CDK4/cyclinD3(h)	>10000[b]	PAK4(h)	>10000[b]
CDK5/p25(h)	>10000[b]	PAK3(h)	>10000[b]
CDK5/p35(h)	>10000[b]	PAK5(h)	>10000[b]
CDK6/cyclinD3(h)	>10000[b]	PAK6(h)	>10000[b]
CDK7/cyclinH/MAT1(h)	>10000[b]	PAR-1Bα(h)	>10000[b]
CDK9/cyclin T1(h)	1428	PASK(h)	154
ChaK1(h)	>10000[b]	PEK(h)	>10000[b]
CHK1(h)	>10000[b]	PDGFRα(h)	>10000[b]
CHK2(h)	>10000[b]	PDGFRα(D842V)(h)	5342
CHK2(I157T)(h)	>10000[b]	PDGFRα(V561D)(h)	>10000[b]
CHK2(R145W)(h)	>10000[b]	PDGFRβ(h)	>10000[b]
CK1γ1(h)	>10000[b]	PDHK4(h)	>10000[b]
CK1γ2(h)	>10000[b]	PDK1(h)	>10000[b]
CK1γ3(h)	>10000[b]	PhKγ2(h)	>10000[b]
CK1δ(h)	>10000[b]	Pim-1(h)	3505
CK2(h)	>10000[b]	Pim-2(h)	>10000[b]
CK2α1(h)	>10000[b]	Pim-3(h)	>10000[b]
CK2α2(h)	>10000[b]	PKA(h)	549
CLIK1(h)	1164	PKAcβ(h)	1153
CLK1(h)	2	PKBα(h)	4132
CLK2(h)	31	PKBβ(h)	>10000[b]
CLK3(h)	5590	PKBγ(h)	1908
CLK4(h)	8	PKCα(h)	6565
cKit(h)	>10000[b]	PKCβI(h)	1343
cKit(D816V)(h)	>10000[b]	PKCβII(h)	>10000[b]
cKit(D816H)(h)	>10000[b]	PKCγ(h)	>10000[b]
cKit(V560G)(h)	>10000[b]	PKCδ(h)	>10000[b]
cKit(V654A)(h)	>10000[b]	PKCε(h)	>10000[b]
CSK(h)	>10000[b]	PKCη(h)	>10000[b]
c-RAF(h)	>10000[b]	PKCι(h)	>10000[b]
cSRC(h)	>10000[b]	PKCμ(h)	>10000[b]
DAPK1(h)	>10000[b]	PKCθ(h)	2629
DAPK2(h)	>10000[b]	PKCζ(h)	>10000[b]
DCAMKL2(h)	>10000[b]	PKD2(h)	>10000[b]
DCAMKL3(h)	>10000[b]	PKD3(h)	>10000[b]
DDR1(h)	>10000[b]	PKG1α(h)	807
DDR2(h)	>10000[b]	PKG1β(h)	248
DMPK(h)	>10000[b]	PKR(h)	>10000[b]
DRAK1(h)	>10000[b]	Plk1(h)	>10000[b]
DYRK1A(h)	138	Plk3(h)	>10000[b]

DYRK1B(h)	690	PRAK(h)	>10000[b]
DYRK2(h)	>10000[b]	PRKG2(h)	344
DYRK3(h)	4976	PRK2(h)	229
eEF-2K(h)	>10000[b]	PrKX(h)	>10000[b]
EGFR(h)	>10000[b]	PTK5(h)	>10000[b]
EGFR(L858R)(h)	>10000[b]	Pyk2(h)	>10000[b]
EGFR(L861Q)(h)	>10000[b]	Ret(h)	>10000[b]
EGFR(T790M)(h)	>10000[b]	Ret (V804L)(h)	>10000[b]
EGFR(T790M,L858R)(h)	4818	Ret(V804M)(h)	>10000[b]
EphA1(h)	>10000[b]	RIPK2(h)	3571
EphA2(h)	>10000[b]	ROCK-I(h)	>10000[b]
EphA3(h)	>10000[b]	ROCK-II(h)	>10000[b]
EphA4(h)	>10000[b]	Ron(h)	>10000[b]
EphA5(h)	>10000[b]	Ros(h)	1071
EphA7(h)	>10000[b]	Rse(h)	>10000[b]
EphA8(h)	>10000[b]	Rsk1(h)	>10000[b]
EphB2(h)	>10000[b]	Rsk2(h)	>10000[b]
EphB1(h)	>10000[b]	Rsk3(h)	>10000[b]
EphB3(h)	>10000[b]	Rsk4(h)	>10000[b]
EphB4(h)	>10000[b]	SAPK2a(h)	>10000[b]
ErbB2(h)	>10000[b]	SAPK2a(T106M)(h)	>10000[b]
ErbB4(h)	>10000[b]	SAPK2b(h)	>10000[b]
FAK(h)	>10000[b]	SAPK3(h)	>10000[b]
Fer(h)	>10000[b]	SAPK4(h)	>10000[b]
Fes(h)	>10000[b]	SGK(h)	>10000[b]
FGFR1(h)	>10000[b]	SGK2(h)	>10000[b]
FGFR1(V561M)(h)	>10000[b]	SGK3(h)	>10000[b]
FGFR2(h)	>10000[b]	SIK(h)	>10000[b]
FGFR2(N549H)(h)	>10000[b]	SIK2(h)	>10000[b]
FGFR3(h)	>10000[b]	SIK3(h)	>10000[b]
FGFR4(h)	>10000[b]	SLK(h)	>10000[b]
Fgr(h)	>10000[b]	Snk(h)	>10000[b]
Flt1(h)	>10000[b]	SNRK(h)	>10000[b]
Flt3(D835Y)(h)	>10000[b]	Src(1-530)(h)	>10000[b]
Flt3(h)	5283	Src(T341M)(h)	>10000[b]
Flt4(h)	>10000[b]	SRPK1(h)	>10000[b]
Fms(h)	>10000[b]	SRPK2(h)	>10000[b]
Fms(Y969C)(h)	>10000[b]	STK25(h)	>10000[b]
Fyn(h)	>10000[b]	STK33(h)	>10000[b]
GCK(h)	>10000[b]	Syk(h)	>10000[b]
GCN2(h)	>10000[b]	TAK1(h)	>10000[b]
GRK1(h)	>10000[b]	TAO1(h)	1154
GRK2(h)	>10000[b]	TAO2(h)	1689
GRK3(h)	>10000[b]	TAO3(h)	1205

GRK5(h)	>10000[b]	TBK1(h)	>10000[b]
GRK6(h)	>10000[b]	Tec(h) activated	>10000[b]
GRK7(h)	>10000[b]	TGFBR1(h)	>10000[b]
GSK3α(h)	418	Tie2 (h)	>10000[b]
GSK3β(h)	1594	Tie2(R849W)(h)	>10000[b]
Hck(h)	>10000[b]	Tie2(Y897S)(h)	>10000[b]
Hck(h) activated	>10000[b]	TLK1(h)	>10000[b]
HIPK1(h)	>10000[b]	TLK2(h)	>10000[b]
HIPK2(h)	>10000[b]	TNIK(h)	1969
HIPK3(h)	>10000[b]	TrkA(h)	179
HIPK4(h)	748	TrkB(h)	457
HPK1(h)	>10000[b]	TrkC(h)	526
ICK(h)	>10000[b]	TSSK1(h)	>10000[b]
IGF-1R(h)	>10000[b]	TSSK2(h)	>10000[b]
IGF-1R(h), activated	>10000[b]	TSSK3(h)	>10000[b]
IKKα(h)	>10000[b]	TSSK4(h)	>10000[b]
IKKβ(h)	>10000[b]	TTBK1(h)	>10000[b]
IKKε(h)	>10000[b]	TTBK2(h)	>10000[b]
IR(h)	>10000[b]	TTK(h)	>10000[b]
IR(h), activated	>10000[b]	Txk(h)	8500
IRE1(h)	>10000[b]	TYK2(h)	3700
IRR(h)	220	ULK1(h)	>10000[b]
IRAK1(h)	4450	ULK2(h)	>10000[b]
IRAK4(h)	1516	ULK3(h)	>10000[b]
Itk(h)	4582	Wee1(h)	>10000[b]
JAK1(h)	>10000[b]	WNK2(h)	>10000[b]
JAK2(h)	>10000[b]	WNK3(h)	>10000[b]
JAK3(h)	>10000[b]	VRK2(h)	>10000[b]
JNK1α1(h)	>10000[b]	Yes(h)	>10000[b]
JNK2α2(h)	>10000[b]	ZAK(h)	>10000[b]
JNK3(h)	>10000[b]	ZAP-70(h)	>10000[b]
KDR(h)	>10000[b]	ZIPK(h)	>10000[b]
Lck(h)	>10000[b]	ATM(h)	279
Lck(h) activated	>10000[b]	ATR/ATRIP(h)	>10000[b]
LIMK1(h)	>10000[b]	DNA-PK(h)	412
LKB1(h)	>10000[b]	PI3 Kinase (p110β/p85α)(h)	1606
LOK(h)	>10000[b]	PI3 Kinase (p120γ)(h)	1745
Lyn(h)	>10000[b]	PI3 Kinase (p110δ/p85α)(h)	1212
LRRK2(h)	>10000[b]	PI3 Kinase (p110α/p85α)(h)	2023
LTK(h)	>10000[b]	PI3 Kinase (p110α(E542K)/p85α)(h)	6569
MAPK1(h)	>10000[b]	PI3 Kinase (p110α(H1047R)/p85α)(h)	1026
MAPK2(h)	>10000[b]	PI3 Kinase (p110α(E545K)/p85α)(h)	2793
MAP4K4(h)	>10000[b]	PI3 Kinase (p110α/p65α)(h)	2541
MAP4K5(h)	>10000[b]	PI3KC2α(h)	>10000[b]

MAPKAP-K2(h)	>10000[b]	PI3KC2γ(h)	362
MAPKAP-K3(h)	>10000[b]	PIP4K2α(h)	>10000[b]
MEK1(h)	>10000[b]	PIP5K1α(h)	>10000[b]
MEK2(h)	>10000[b]	PIP5K1γ(h)	>10000[b]
MARK1(h)	>10000[b]		

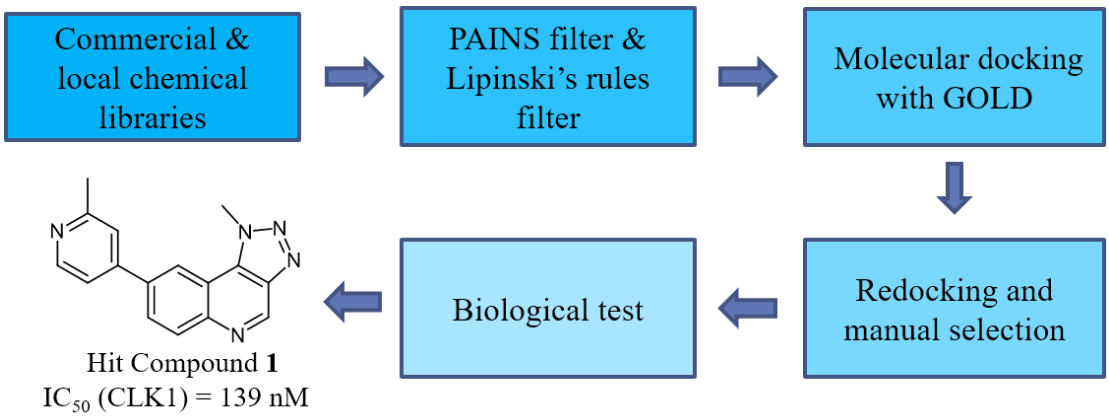
[a]IC_{50} values were determined using the KinaseProfiler of Eurofins. In case of CLKs and DYRKs, the data represent the mean values of two independent experiments. In other cases, the data represent the results of a single experiment. [ATP] = 10 μM. [b]Inhibition% @ 10 μM was lower than 50%. [ATP] = 10 μM. For detailed protocols, see http://www.eurofins.com/pharmadiscovery.

Table S2. Crystallographic data collection and refinement statistics[a]

CLK1 + compound **25**	
PDB ID	5X8I
Data collection	
Space group	P65
Cell dimension	
a, *b*, *c*(Å)	68.33, 68.33, 285.72
α, β, γ (°)	90, 90, 120
Resolution (Å)[a]	50.00 -1.90 (1.97-1.90)
R_{merge}	0.086 (0.431)
I/δ^{Ia}	30.72 (8.07)
Completeness (%)[a]	99.7 (100.0)
Redundancy[a]	15.9 (15.9)
Refinement	
Resolution (Å)	33.60-1.90
No. reflections	58718
R_{work}/ R_{free}	0.166/0.206
No. atoms	
Protein	5386
Ligand/ion	62
Water	630
B-factors	
Protein	23.96
Ligand/ion	18.37
Water	35.03
R.m.s. deviations	
Bond lengths (Å)	0.007
Bond angles (°)	1.129
Ramachandran analysis	
Favored (%)	97.4
Allowed (%)	2.6
Outliers(%)	0.0

[a] Values for the outmost resolution shell are given in parentheses.

Figure S1. Virtual screening led to the discovery of hit compound[a]



[a]To identify new CLK1 inhibitors as autophagy inducers, we first of all performed a virtual screening against various commercial chemical libraries including Chemdiv, Specs, Enamine, Pharmakon, Selleck, and MCE, as well as our in-house chemical library. Before the virtual screening, all the compound databases were filtered by Lipinski's rules of five, and the "pan-assay interference compounds" (PAINS) were also removed. The receptor structure was taken from the crystal structure of CLK1 (PDB ID 1Z57). The preparation and pre-process of receptor and ligand were made on the platform of Discovery Studio 3.1 (Accelrys Inc., San Diego, CA, USA). The CHARMm force field was used. The binding pocket was defined as a sphere with a radius of 9 Å, an area large enough to cover the ATP-binding region at the catalytic site. The GOLD version 5.1 program (CCDC, Cambridge, UK) was adopted for the molecular docking. GoldScore incorporated into the GOLD program package was employed to evaluate and rank the binding poses. After finishing the first round of docking, top 1% of the docking-ranked molecules were re-docked on a more flexible condition. Finally, high-ranking candidates were carefully selected for biological assays, which led to the discovery of the hit compound **1**.

Figure S2. Dendrogram representation of the kinase selectivity profile of **25**. The figure was generated using KinomeRender 1.4 (http://bcb.med.usherbrooke.ca/kinomerenderLig.php).

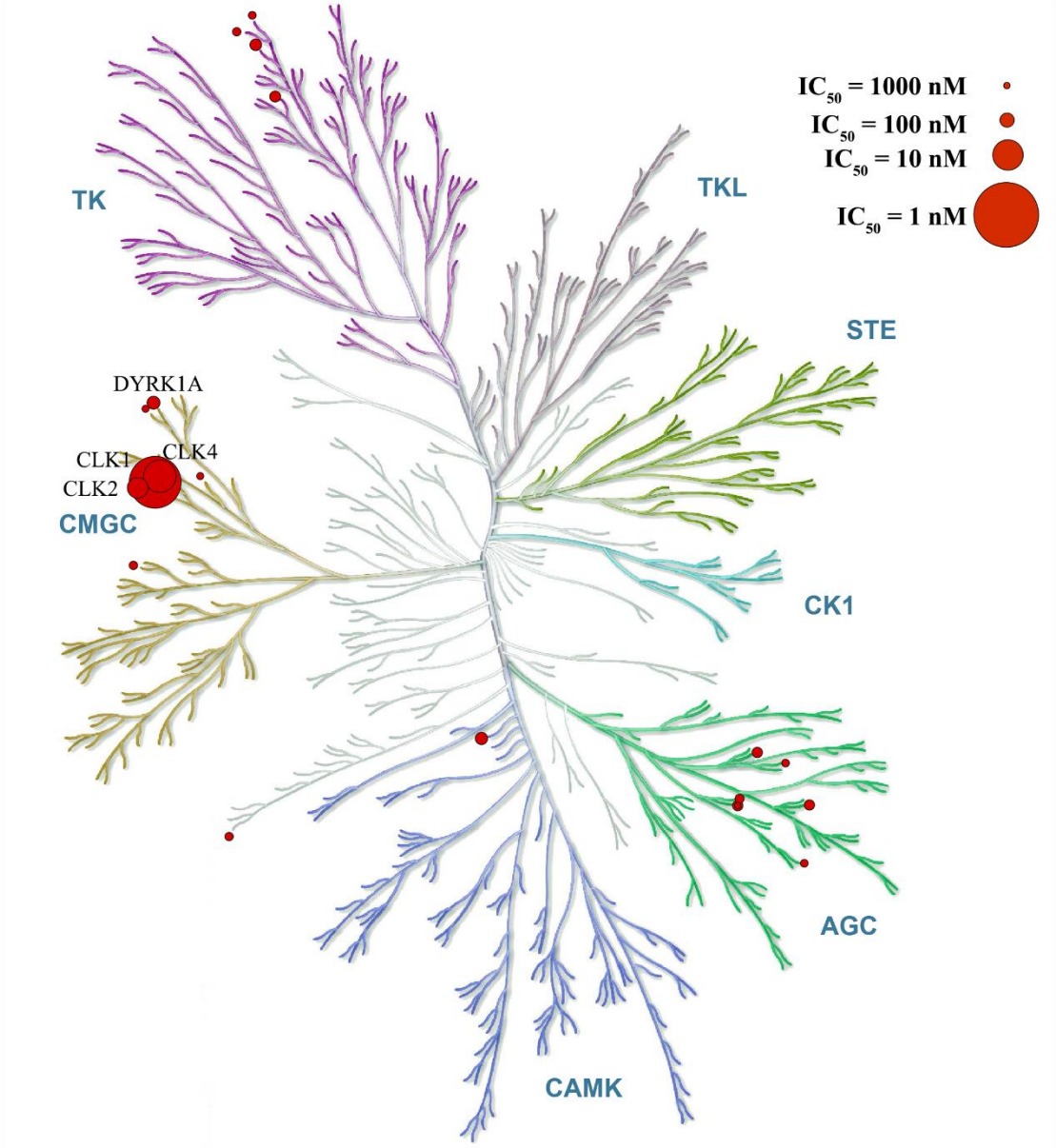


Figure S3. (A) Overview of X-ray co-crystal structure of CLK1 in complex with **25**. (B) A 2D ligand-interaction diagram for **25**. The ligand-interaction plot was generated using maestro 10.

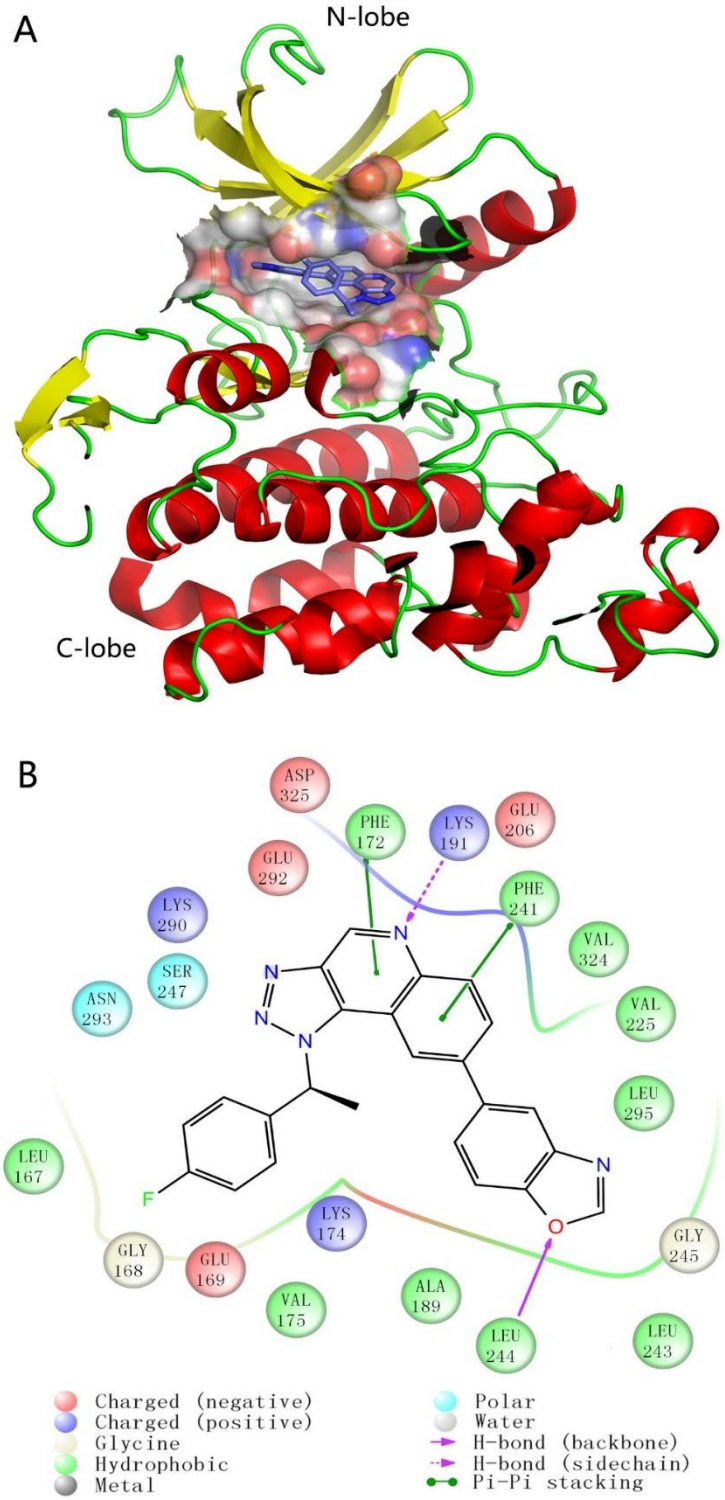


Figure S4. Effects of **25** (20 nM and 100 nM) on the location and redistribution of SR proteins. BNL CL.2 cells treated with DMSO (0.1%) or **25** (20 nM and 100 nM) for 24 h were fixed and probed with anti-SR proteins antibody (mAb1H4G7). Diffuse staining and typical speckles demonstrated by mAb1H4G7 represent active and stored forms of SR proteins respectively. DAPI was used to dye the nucleus. Scale bar: 10 μm.

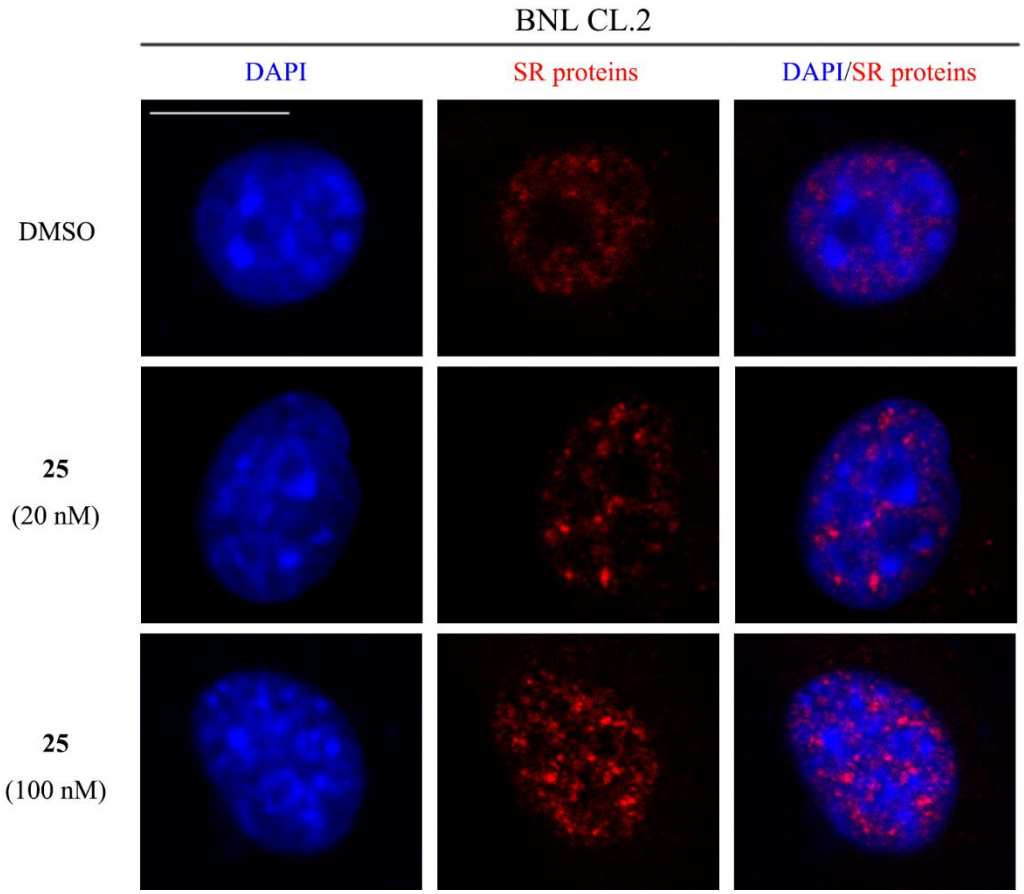
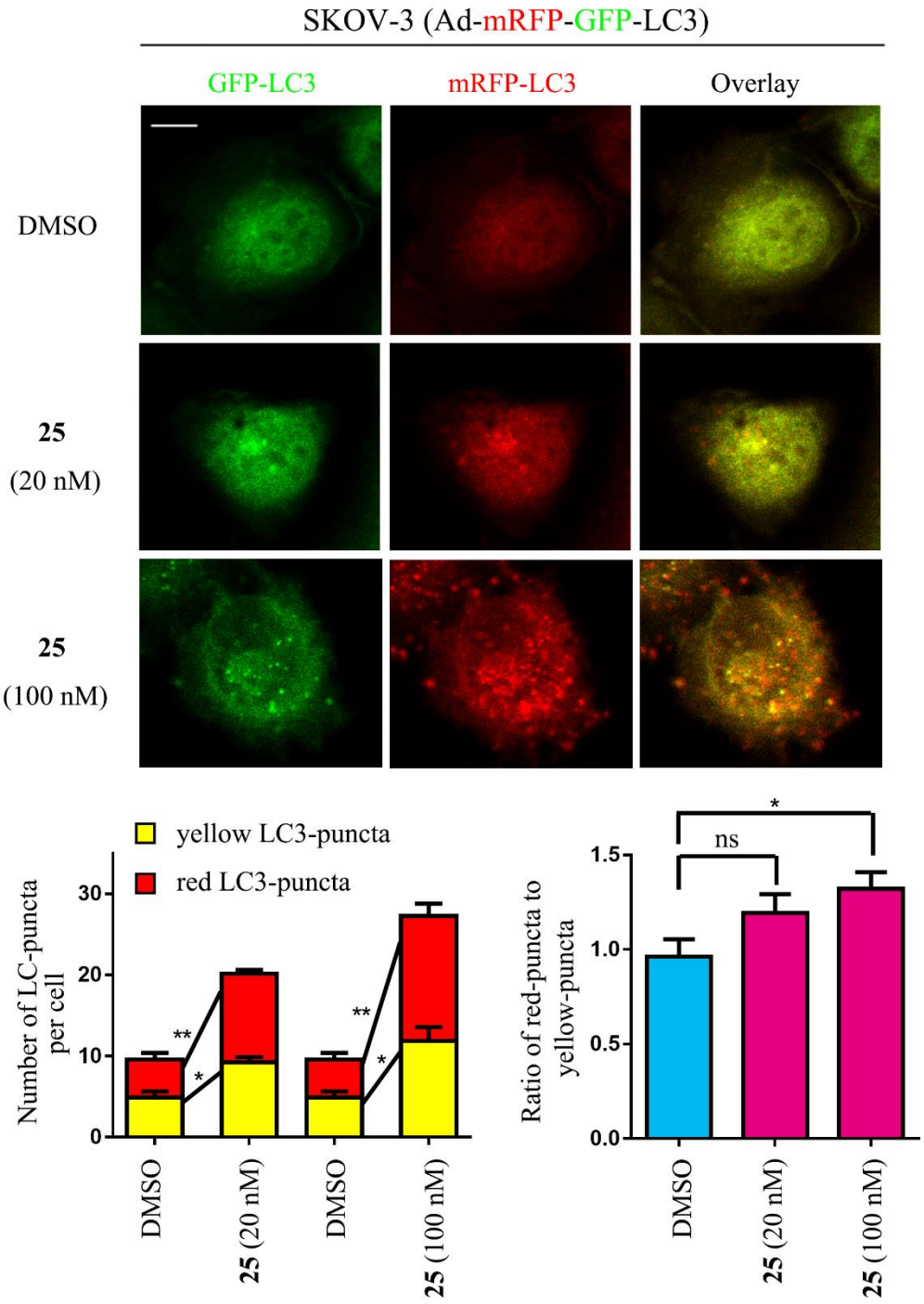


Figure S5. Detection of autophagy and autophagic flux induced by **25** (20 nM and 100 nM) in SKOV-3 cells[a]



[a]Ad-mRFP-GFP-LC3-infected SKOV-3 cells were treated with DMSO or **25** (20 nM and 100 nM) for 24 h and fixed before examination by confocal microscopy. Representative photographs are presented. Scale bar: 10 μm. Alignment of green and red signals appears yellow. The number of LC3-puncta (mean ± SEM) in overlays was quantified and is shown below. More than 90 cells were counted in each individual experiment (n = 3). * $P < 0.05$, ** $P < 0.01$; ns, no statistical significance.

Figure S6. *In vitro* effects of **18** on LC3. SKOV-3 cells were treated with DMSO, **25** (the positive control, 10 μM) or **18** (10 μM) for 24 h. Then whole cell lysates were subjected to immunoblot assay to detect LC3.

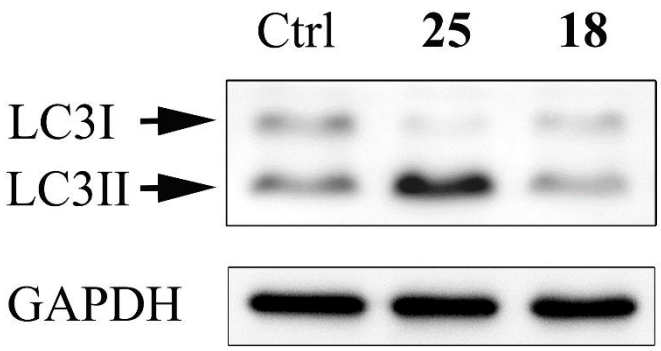
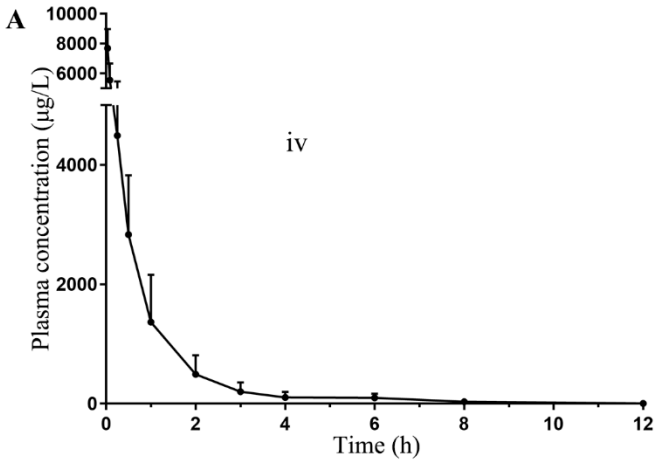
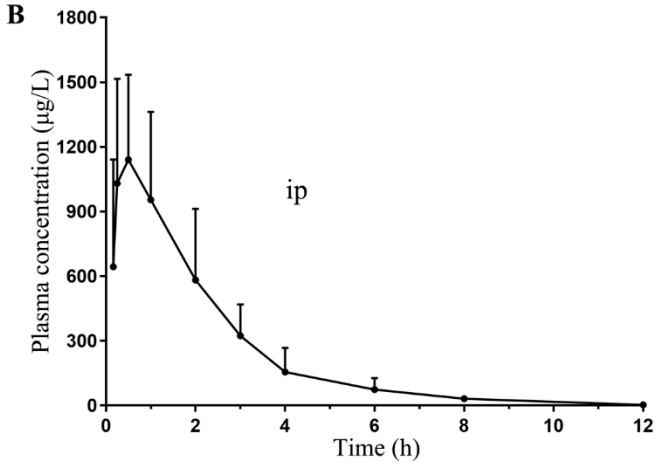


Figure S7. Pharmacokinetic characteristics of compound **25**. (A) Plasma concentration−time curve of **25** in SD rats after a single intravenous dose of 10 mg/kg. Blood was collected at indicated time points (0.03, 0.08, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 h), and the plasma concentrations were determined by LC-MS. Points, mean; bars, SEM; n = 6. Pharmacokinetic parameters of **25** (iv) is shown in the right panel. (B) Plasma concentration−time curve of **25** in SD rats after a single intraperitoneal dose of 10 mg/kg. Blood was collected at indicated time points (0.16, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 h), and the plasma concentrations were determined by LC-MS. Points, mean; bars, SEM; n = 6. Pharmacokinetic parameters of **25** (ip) is shown in the right panel.

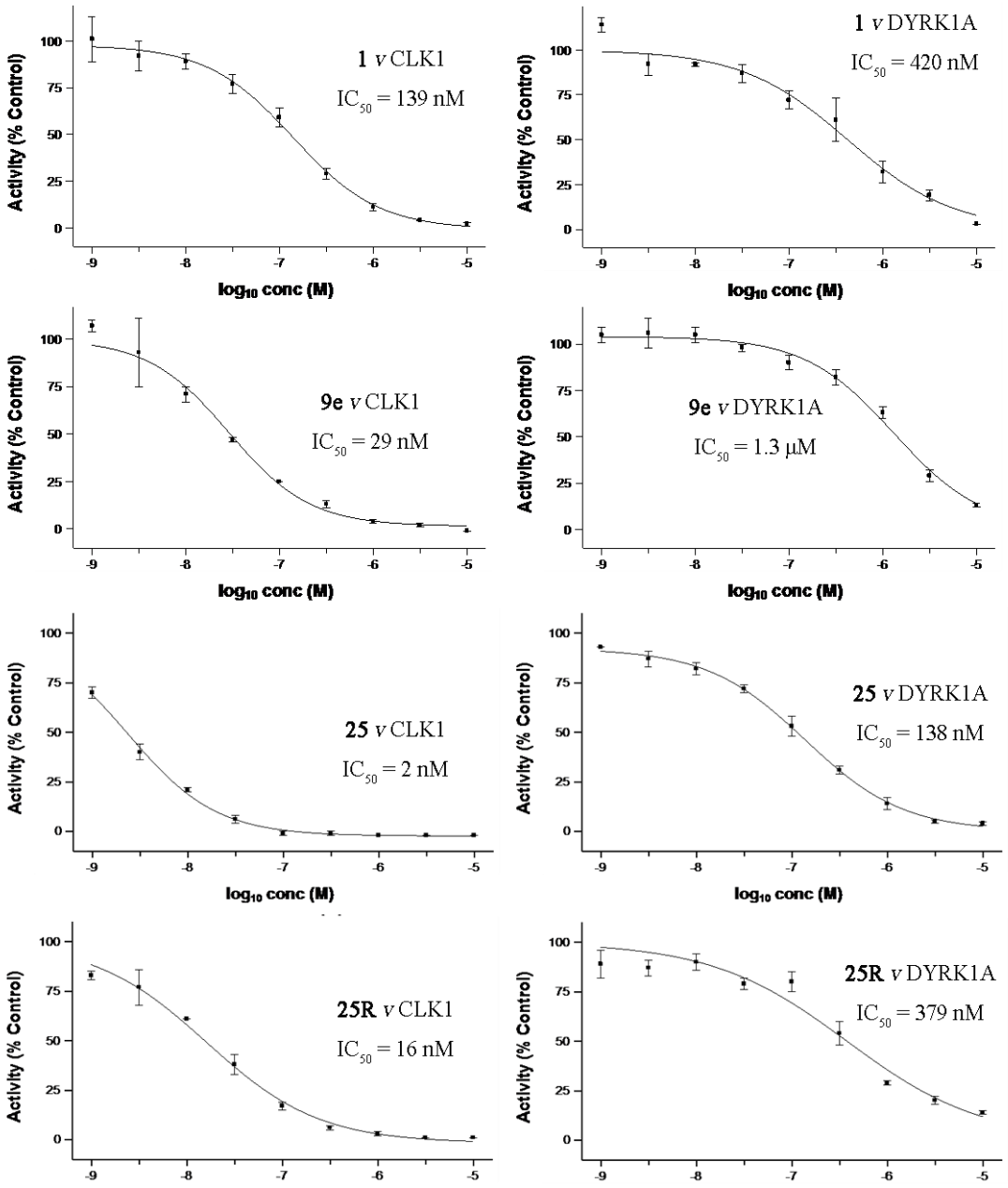


parameter	iv (10 mg/kg)
$AUC_{0-\infty}$ (μg·h/L)	5222.86
C_{max} (μg/L)	7698.75
$MRT_{0-\infty}$ (h)	1.20
Vss (L/kg)	3.59
CL (L/h/kg)	1.92
$t_{1/2}$ (h)	1.30



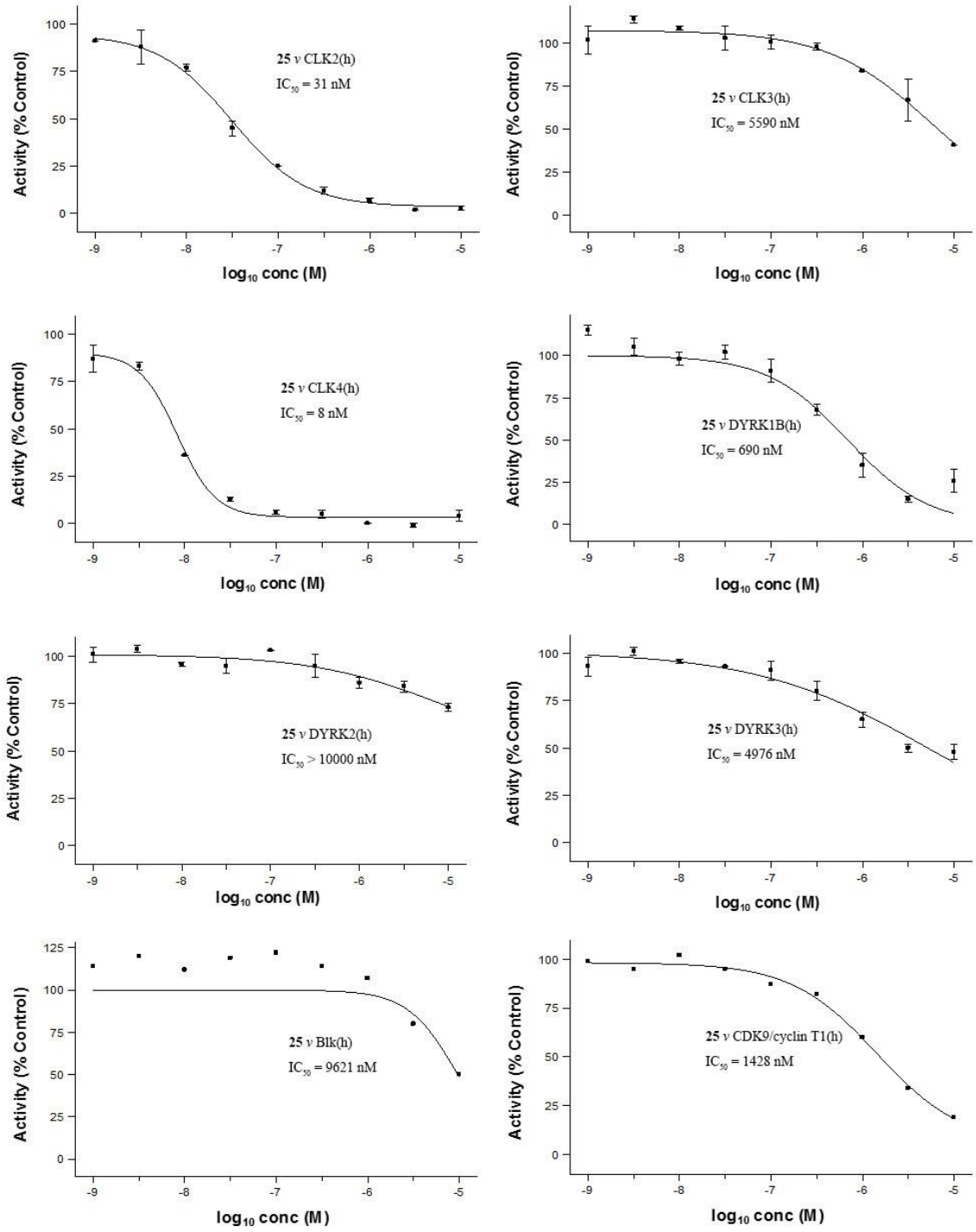
parameter	ip (10 mg/kg)
$AUC_{0-\infty}$ (μg·h/L)	2760.58
C_{max} (μg/L)	1140.37
T_{max} (h)	0.5
F (%)	52.86
$MRT_{0-\infty}$ (h)	2.12
Vss (L/kg)	13.13
CL (L/h/kg)	6.85
$t_{1/2}$ (h)	1.33

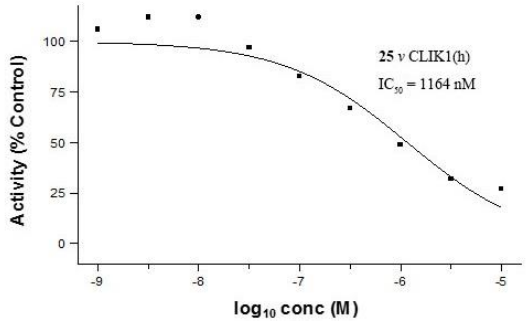
Figure S8. IC_{50} curves of compounds **1**, **9e**, **25**, and **25R**[a] on CLK1 and DYRK1A. The values were determined using the KinaseProfiler of Eurofins, in duplicates at 10 different concentrations with an ATP concentration of 10 μM. For detailed protocols, see http://www.eurofins.com/pharmadiscovery.

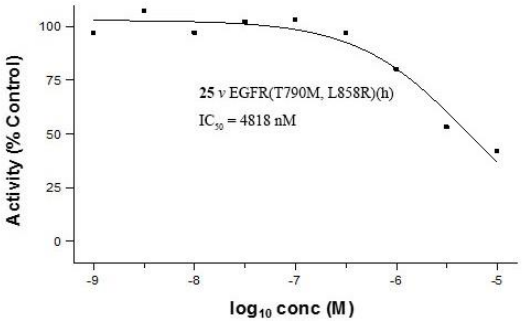


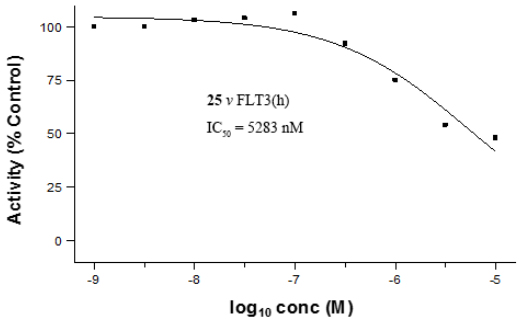
[a]**25R** is the enantiomer of **25**.

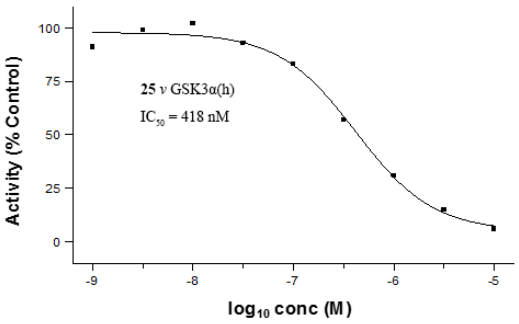
Figure S9. IC_{50} curves of compound **25** on remaining kinases whose activity% @ 10 μM is lower than 50%. In cases of CLKs and DYRKs, IC_{50} values were determined in duplicates at 10 different concentrations. In other cases, IC_{50} values were determined in singlicate at 10 different concentrations. [ATP] = 10 μM. For detailed protocols, see http://www.eurofins.com/pharmadiscovery.

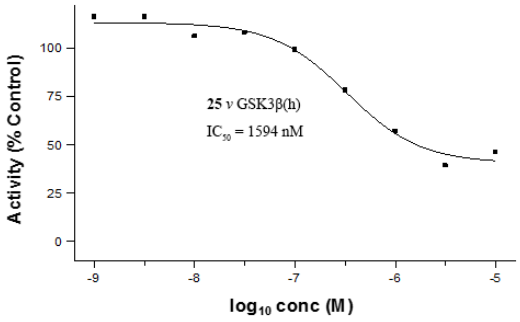


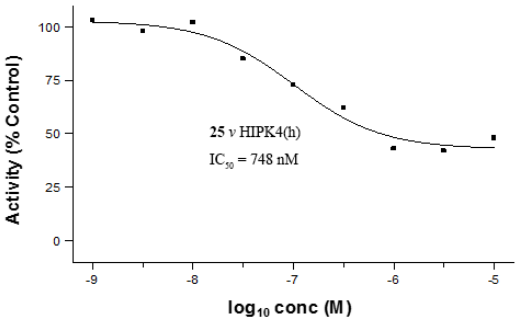

25 v CLIK1(h)
IC50 = 1164 nM
Activity (% Control)
log10 conc (M)

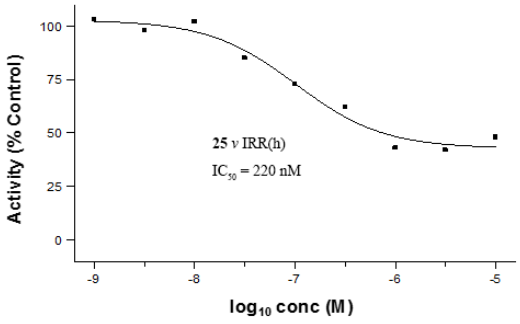

25 v EGFR(T790M, L858R)(h)
IC50 = 4818 nM
Activity (% Control)
log10 conc (M)

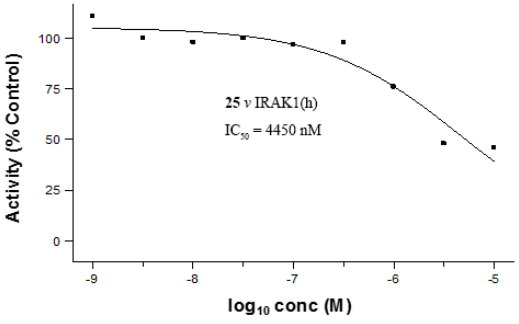

25 v FLT3(h)
IC50 = 5283 nM
Activity (% Control)
log10 conc (M)

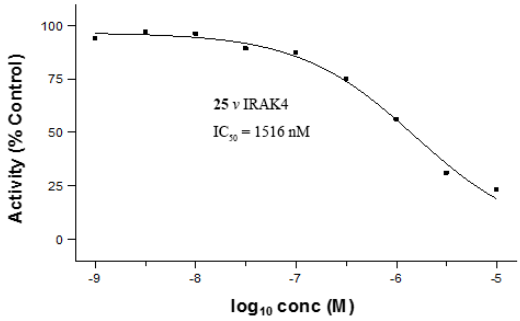

25 v GSK3α(h)
IC50 = 418 nM
Activity (% Control)
log10 conc (M)


25 v GSK3β(h)
IC50 = 1594 nM
Activity (% Control)
log10 conc (M)


25 v HIPK4(h)
IC50 = 748 nM
Activity (% Control)
log10 conc (M)


25 v IRR(h)
IC50 = 220 nM
Activity (% Control)
log10 conc (M)


25 v IRAK1(h)
IC50 = 4450 nM
Activity (% Control)
log10 conc (M)


25 v IRAK4
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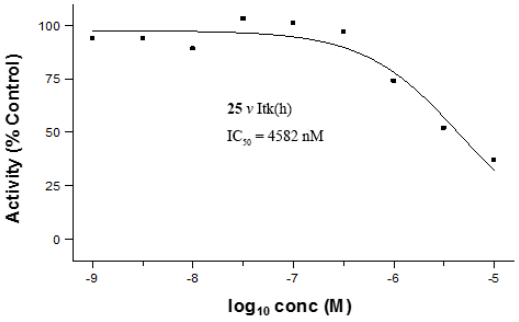
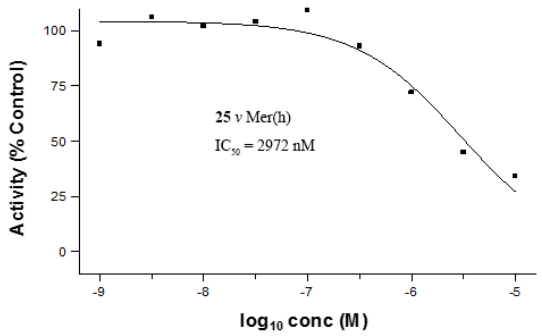
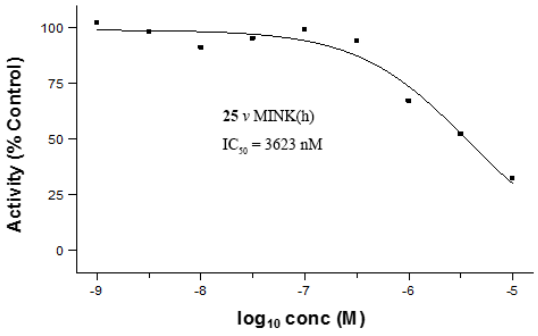
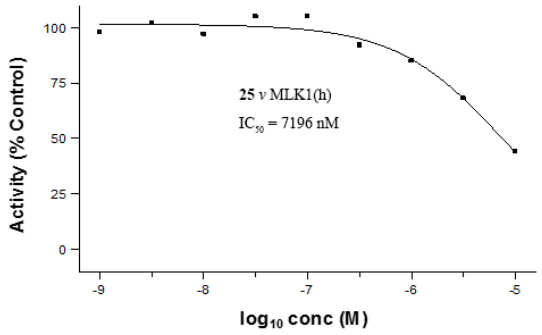
log10 conc (M)


25 v Itk(h)
IC50 = 4582 nM
Activity (% Control)
log10 conc (M)


Activity (% Control)
log10 conc (M)
25 v Mer(h)
IC50 = 2972 nM


Activity (% Control)
log10 conc (M)
25 v MINK(h)
IC50 = 3623 nM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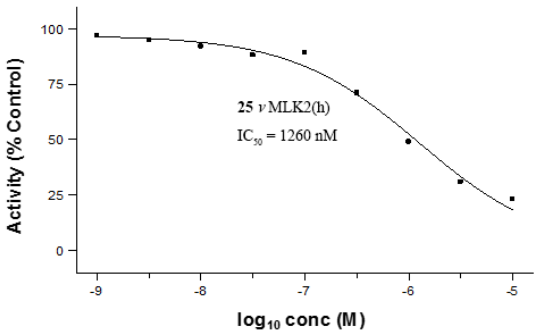
log10 conc (M)
25 v MLK1(h)
IC50 = 7196 nM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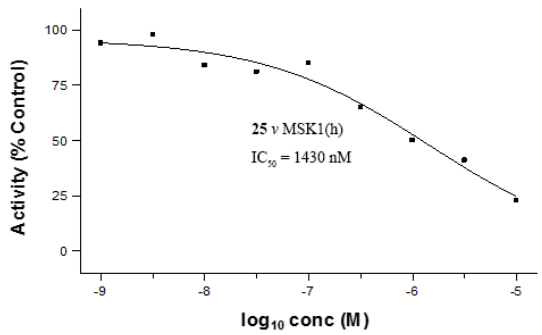
25 v MLK2(h)
IC50 = 1260 nM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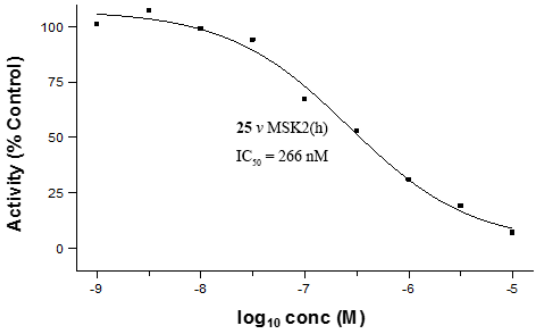
25 v MSK1(h)
IC50 = 1430 nM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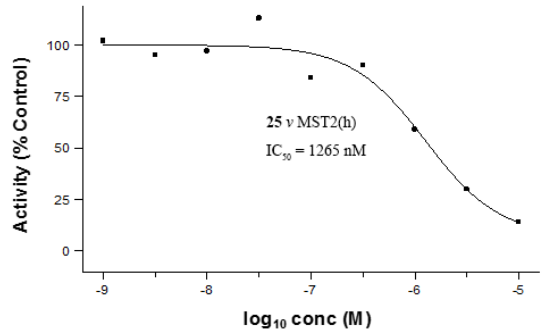
25 v MSK2(h)
IC50 = 266 nM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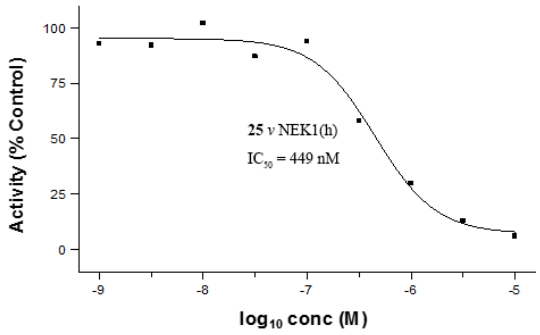
log10 conc (M)
25 v MST2(h)
IC50 = 1265 nM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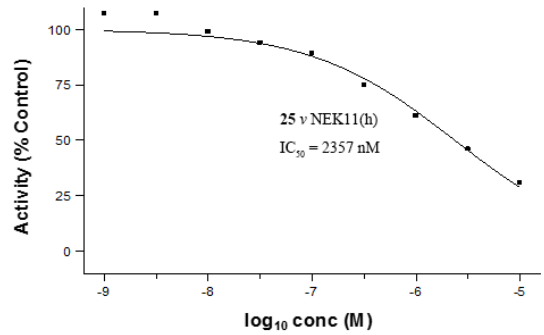
log10 conc (M)
25 v NEK1(h)
IC50 = 449 nM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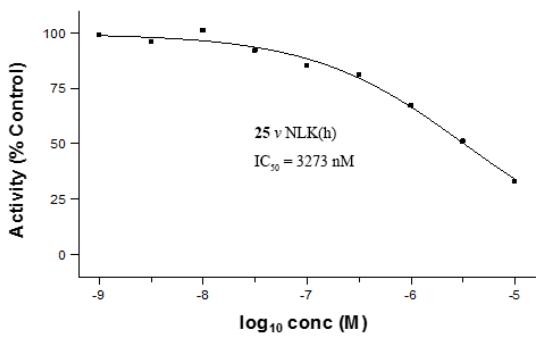
25 v NEK11(h)
IC50 = 2357 nM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25 v NLK(h)
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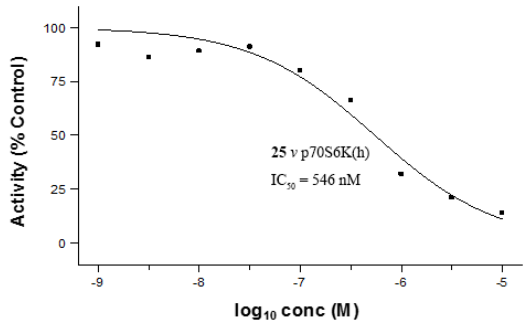
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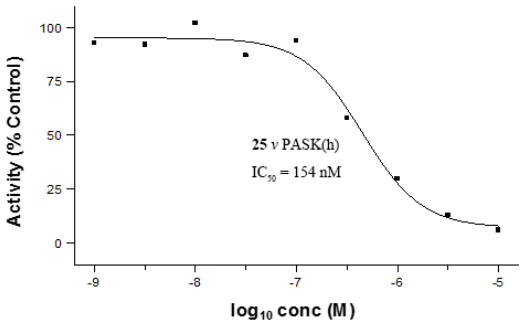
25 v p70S6K(h)
IC50 = 546 nM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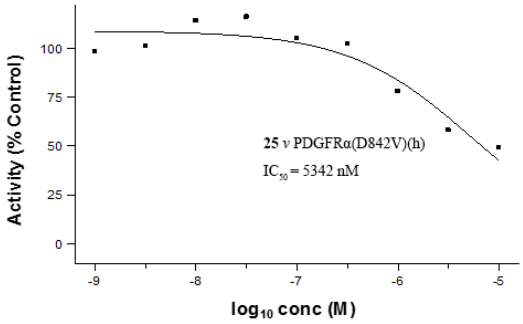
log10 conc (M)
25 v PASK(h)
IC50 = 154 nM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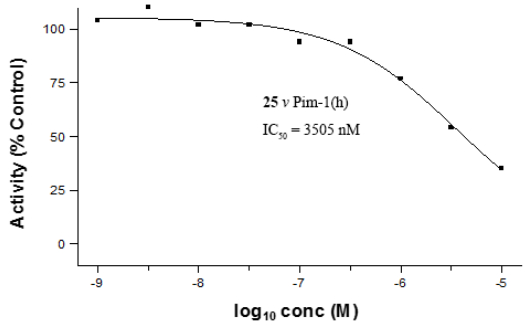
log10 conc (M)
25 v PDGFRα(D842V)(h)
IC50 = 5342 nM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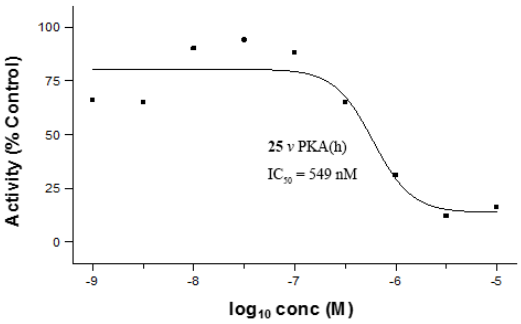
log10 conc (M)
25 v Pim-1(h)
IC50 = 3505 nM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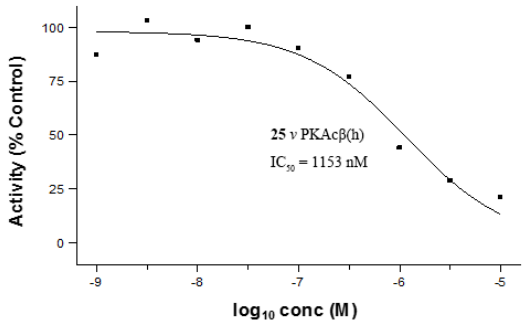
log10 conc (M)
25 v PKA(h)
IC50 = 549 nM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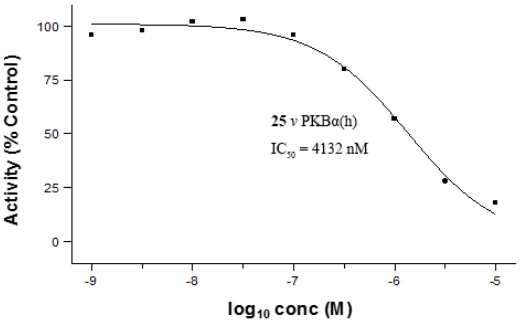
log10 conc (M)
25 v PKAcβ(h)
IC50 = 1153 nM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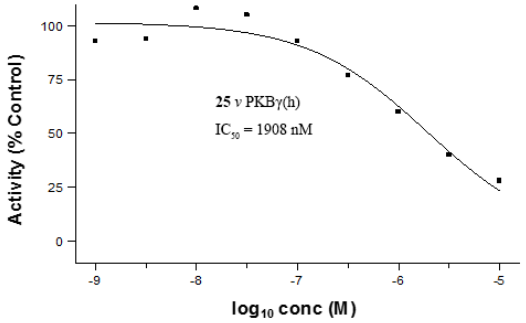
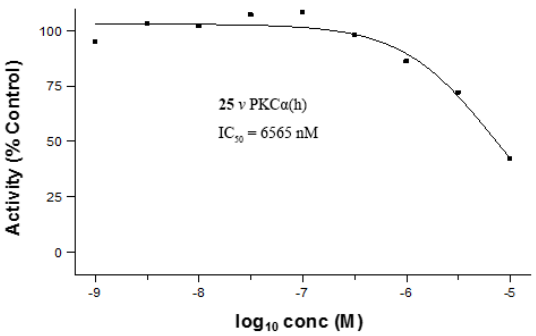
log10 conc (M)
25 v PKBα(h)
IC50 = 4132 nM


Activity (% Control)
log10 conc (M)
25 v PKBγ(h)
IC50 = 1908 nM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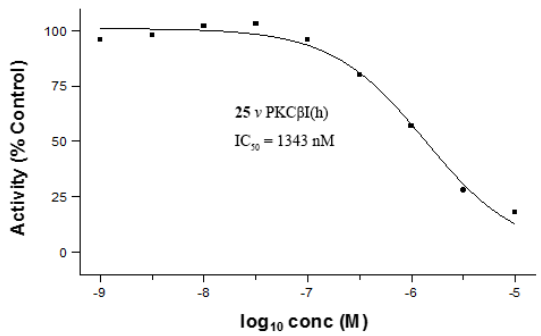
25 v PKCα(h)
IC50 = 6565 nM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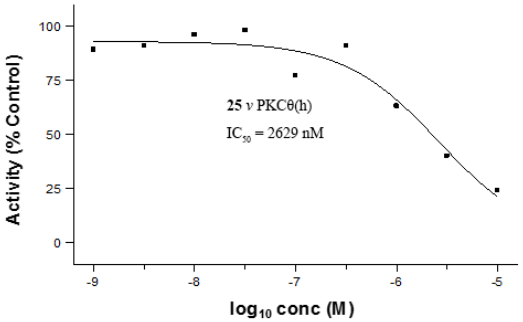
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25 v PKCθ(h)
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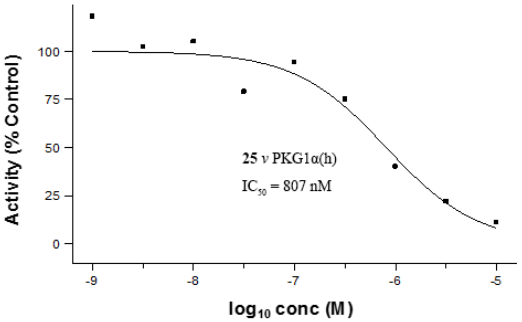
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25 v PKG1α(h)
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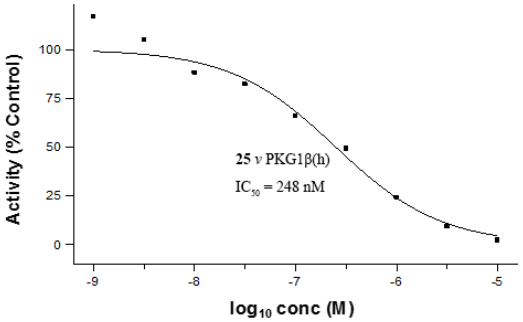
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25 v PKG1β(h)
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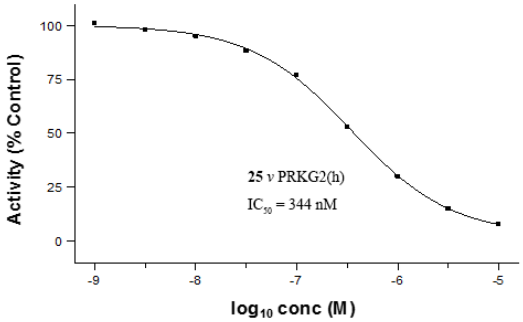
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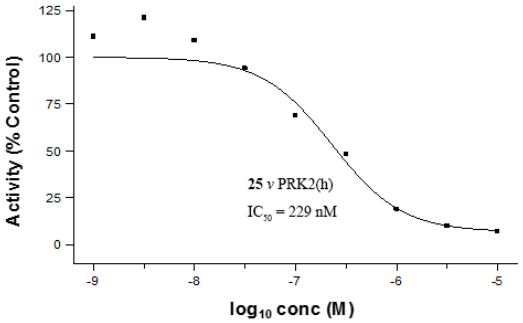
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25 v PRK2(h)
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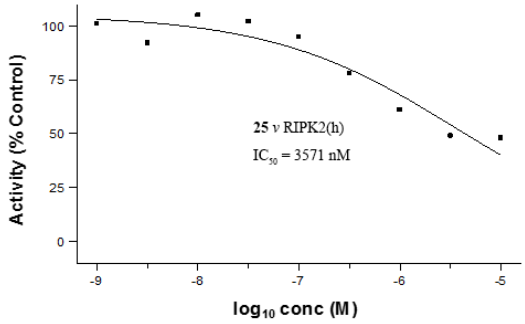
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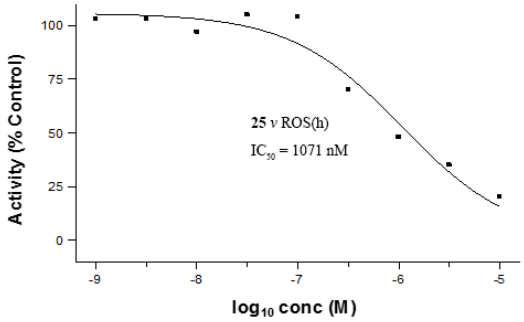
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25 v ROS(h)
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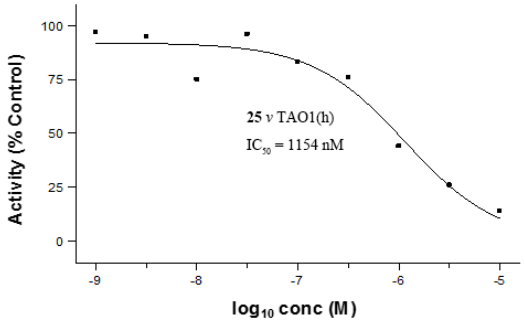
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log10 conc (M)
25 v TAO1(h)
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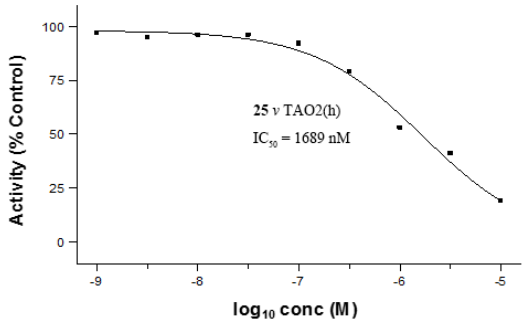
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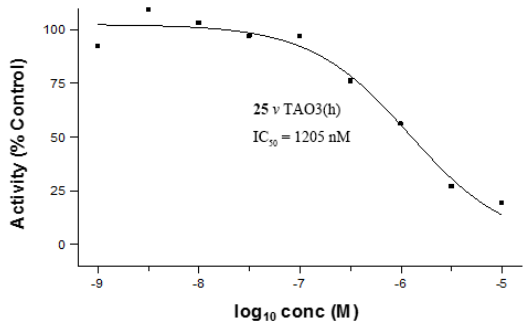
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log10 conc (M)
25 v TAO3(h)
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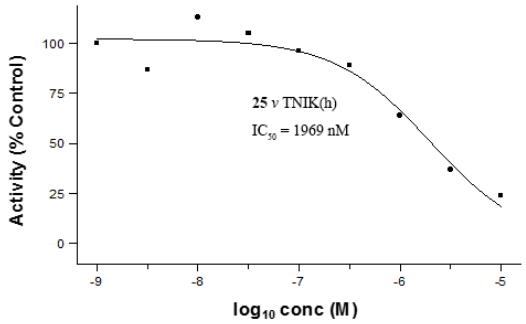
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25 v TNIK(h)
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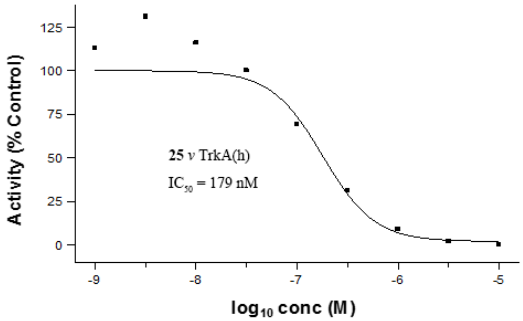
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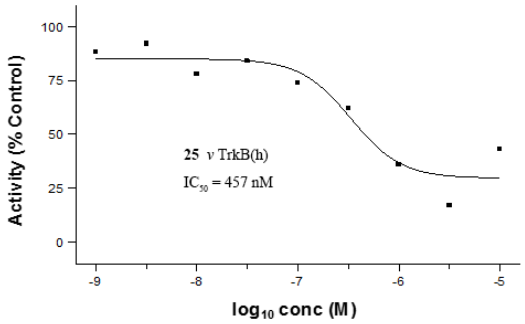
log10 conc (M)
25 v TrkA(h)
IC50 = 179 nM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25 v TrkB(h)
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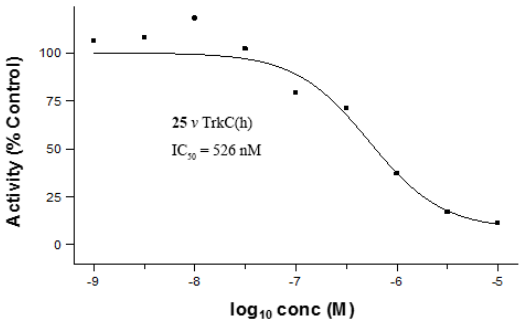
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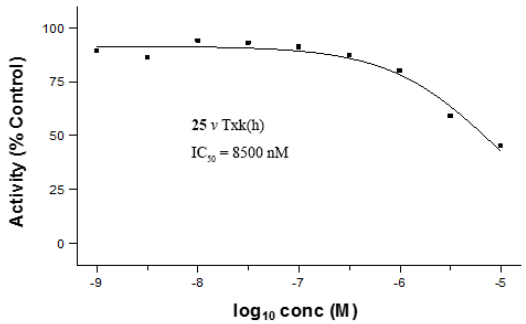
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25 v Txk(h)
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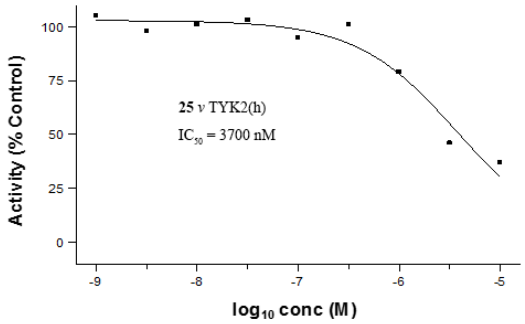
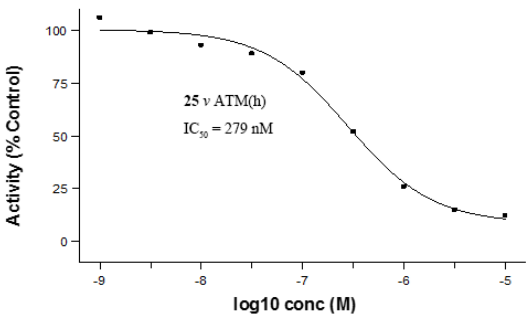
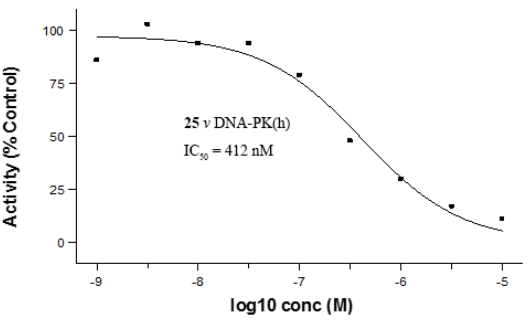
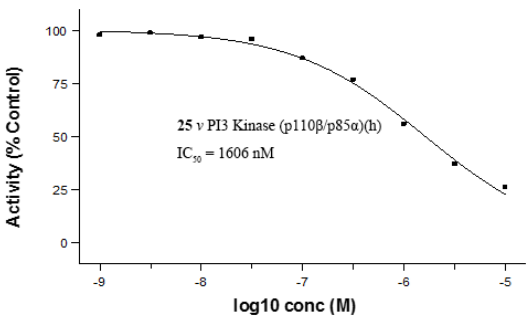
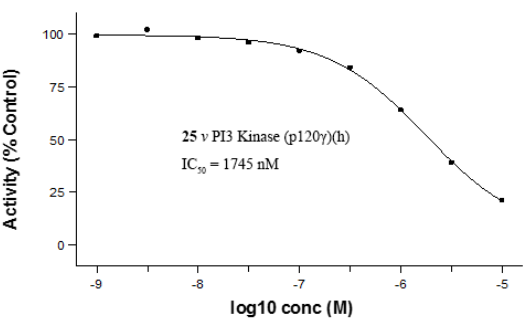

Activity (% Control)
log10 conc (M)
25 v TYK2(h)
IC50 = 3700 nM


Activity (% Control)
log10 conc (M)
25 v ATM(h)
IC50 = 279 nM


Activity (% Control)
log10 conc (M)
25 v DNA-PK(h)
IC50 = 412 nM


Activity (% Control)
log10 conc (M)
25 v PI3 Kinase (p110β/p85α)(h)
IC50 = 1606 nM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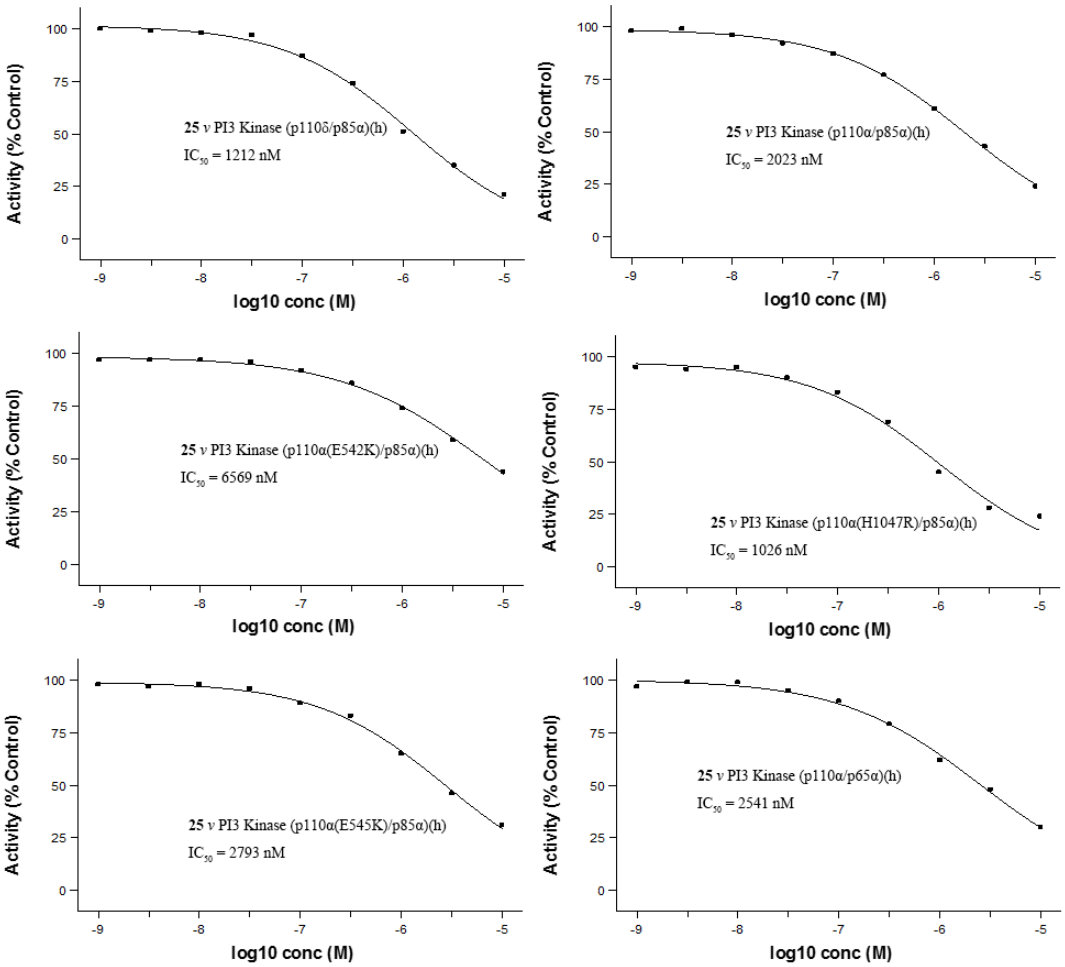
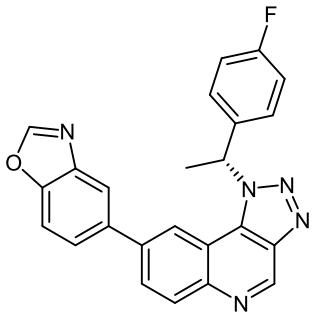
log10 conc (M)
25 v PI3 Kinase (p120γ)(h)
IC50 = 1745 nM


Activity (% Control)
log10 conc (M)
25 v PI3 Kinase (p110δ/p85α)(h)
IC50 = 1212 nM
25 v PI3 Kinase (p110α/p85α)(h)
IC50 = 2023 nM
25 v PI3 Kinase (p110α(E542K)/p85α)(h)
IC50 = 6569 nM
25 v PI3 Kinase (p110α(H1047R)/p85α)(h)
IC50 = 1026 nM
25 v PI3 Kinase (p110α(E545K)/p85α)(h)
IC50 = 2793 nM
25 v PI3 Kinase (p110α/p65α)(h)
IC50 = 2541 nM

Synthesis and Structure Confirmation of Compound 25R (the Enantiomer of Compound 25)



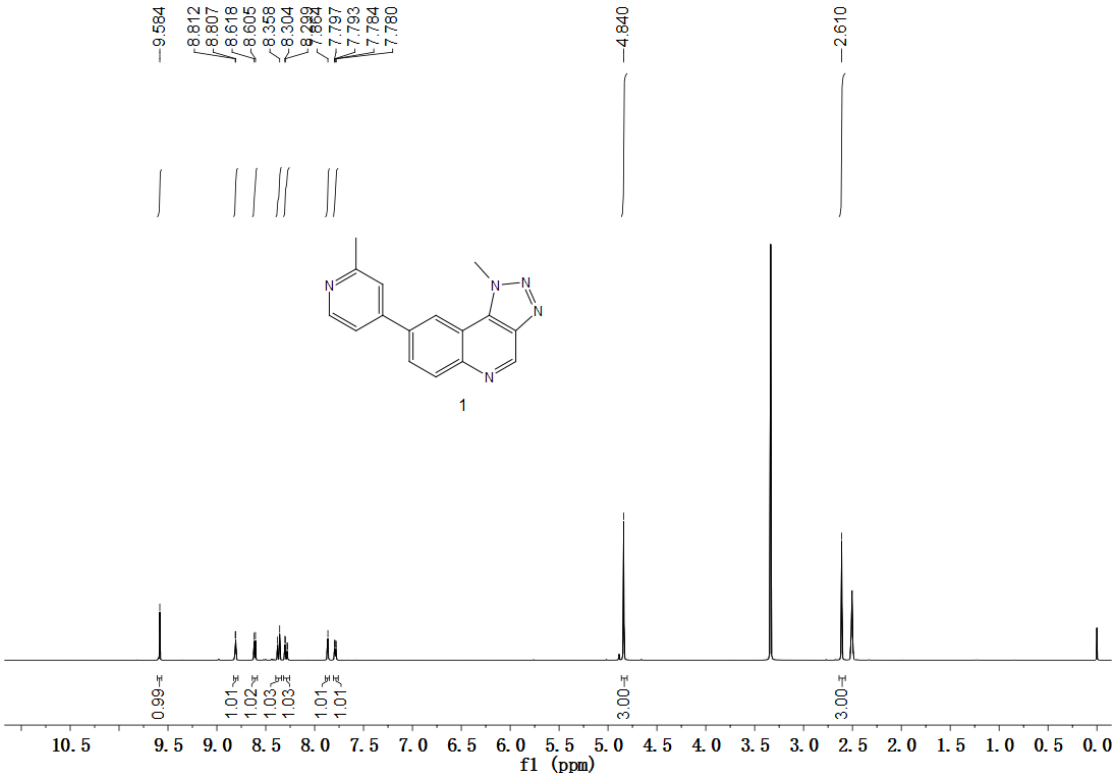
25R

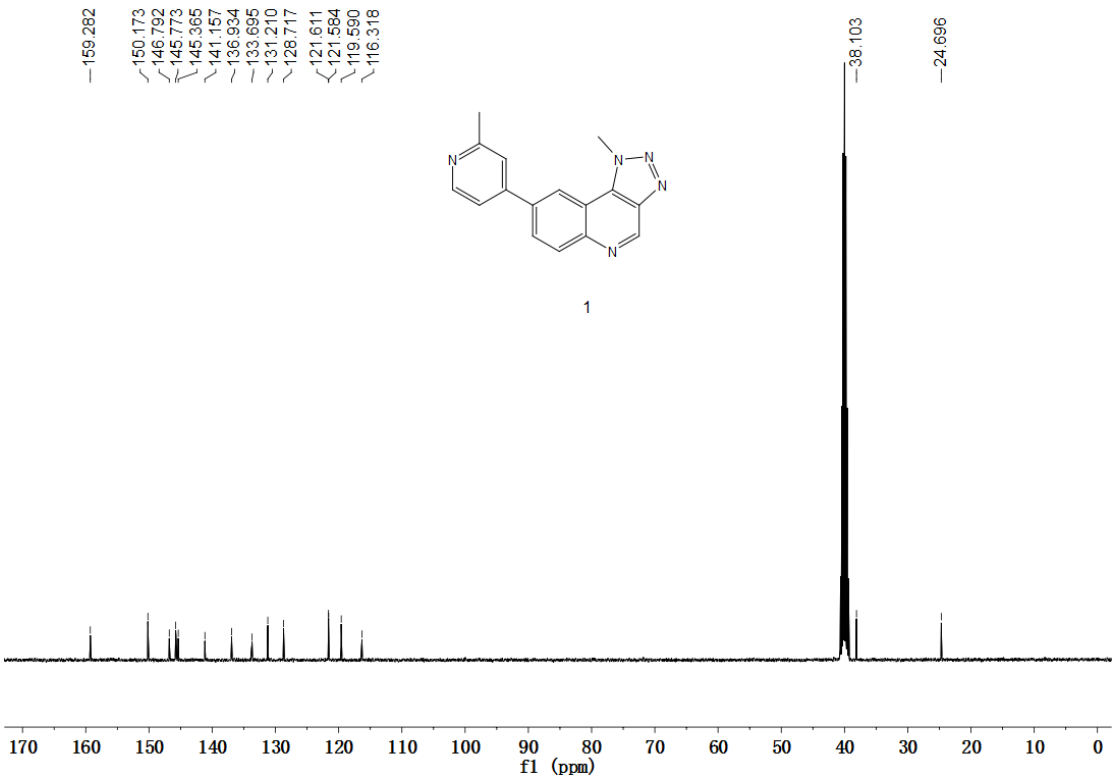
(*R*)-(+)-5-(1-(1-(4-Fluorophenyl)ethyl)-1*H*-[1,2,3]triazolo[4,5-*c*]quinolin-8-yl)benzo[d]oxazole (25R). The title compound was prepared from **8f** (50 mg, 135 μmol) and self-prepared **28** (33 mg, 135 μmol) using the procedure described for compound **1** in 84.3% yield as a white powder. $[\alpha]_D^{25}$ = +238.74 (c = 0.191, CH_3Cl, ee 100%). ^1H NMR (400 MHz, DMSO) δ 9.63 (s, 1H), 8.87 (s, 1H), 8.52 (d, *J* = 1.6 Hz, 1H), 8.32 (d, *J* = 8.7 Hz, 1H), 8.24 (dd, *J* = 8.7, 1.9 Hz, 1H), 8.18 (d, *J* = 1.2 Hz, 1H), 8.00 (d, *J* = 8.3 Hz, 1H), 7.77 (dd, *J* = 8.3, 1.6 Hz, 1H), 7.32 (dd, *J* = 8.7, 5.4 Hz, 2H), 7.19 (t, *J* = 8.8 Hz, 2H), 7.06 (q, *J* = 6.7 Hz, 1H), 2.22 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, DMSO) δ 163.26, 160.83, 155.64, 150.71, 145.43, 144.87, 141.37, 140.21, 139.22, 138.00, 137.97, 137.35, 133.14, 131.26, 129.35, 128.48, 128.39, 124.82, 121.53, 120.99, 116.59, 116.38, 115.70, 110.48, 60.08, 23.55. ESI-MS m/z 410.1 $[M + H]^+$. HRMS m/z (ESI) calcd for $C_{24}H_{17}FN_5O$ $[M + H]^+$ 410.1412; found, 410.1406. Chiral HPLC analysis (mobile phase: 2-propanol/n-hexane = 30/70, flow rate = 1.0 mL/min, λ = 255.9 nm, t_R = 45.89 min).

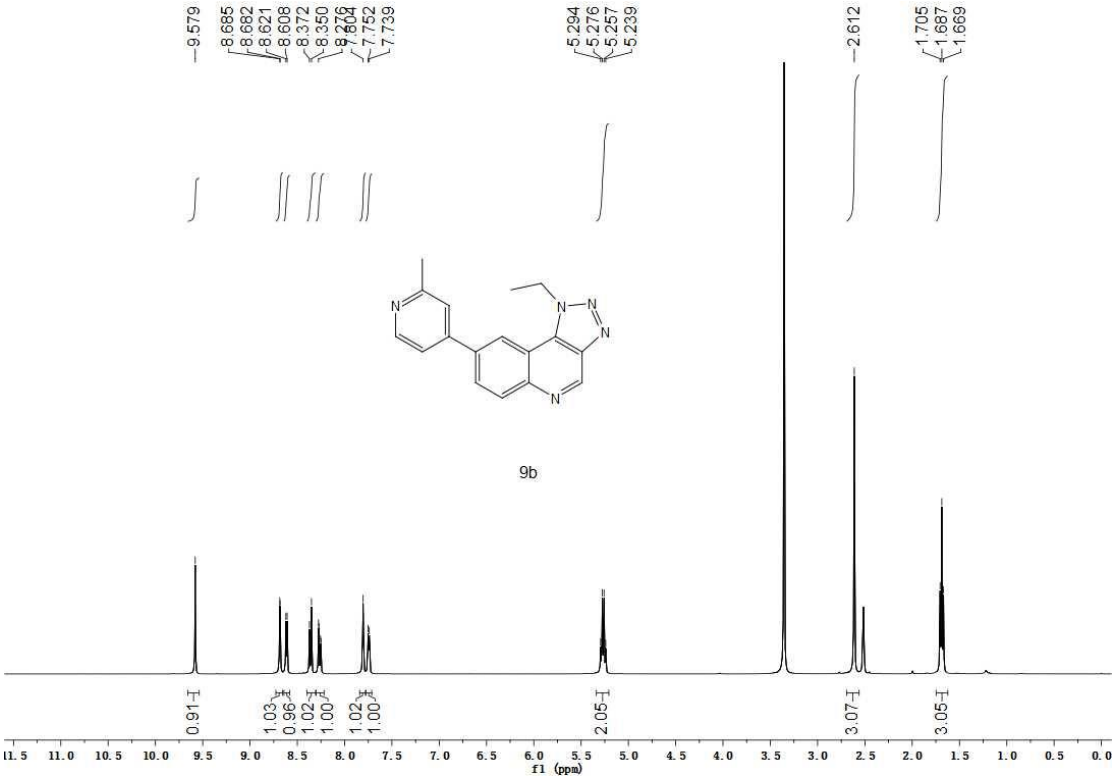
Pharmacokinetic Assessments of Compound 25.

Catheters were surgically placed into the jugular veins of male Sprague−Dawley rats (Chinese Academy of Medical Science, Beijing, China) to collect serial blood samples. The animals were fasted overnight prior to dosing, and food was withheld until 4 h after dosing. Compound **25** was dissolved in 2.5% ethanol, 2.5% castor oil, and 95% saline for a concentration of 1 mg/mL. The rats (200 ± 10 g) were administered with a single dose of compound **25** (10 mg/kg) by intraperitoneal injection (n = 6) or intravenous injection (n = 6). Blood was collected in heparin-containing tubes at indicated time points and centrifuged at 4 °C immediately to obtain plasma. The plasma concentrations of **25** were measured by high performance liquid chromatography (HPLC) with tandem mass spectrometric detection (3200 QTRAP system, Applied Biosystems). Non-compartmental pharmacokinetic parameters were obtained from the plasma concentration−time profiles using DAS software (Enterprise, version 2.0, Mathematical Pharmacology Professional Committee of China). At last, all surviving animals were transferred to the repository or euthanized after completing the test.

Copies of ^{1}H- and ^{13}C-NMR Spectra



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5.276
5.257
5.239
2.612
1.705
1.687
1.669
9b
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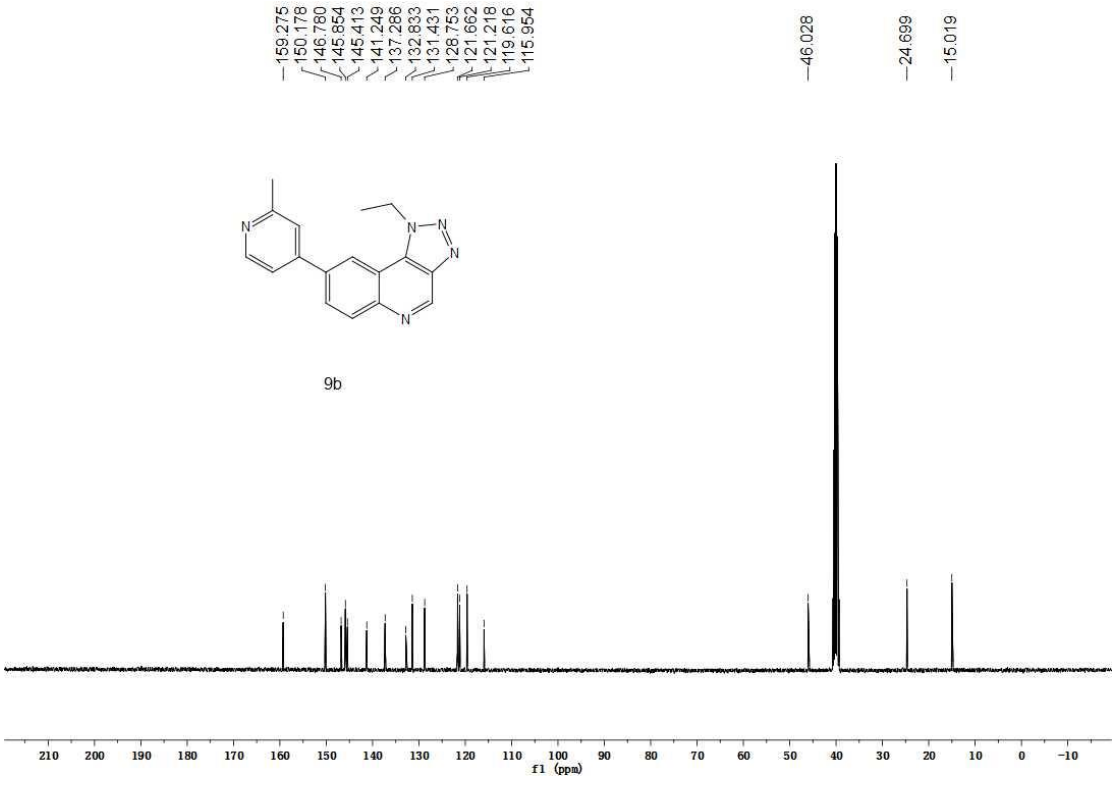
0.96
1.02
1.00
1.02
1.00
2.05
3.07
3.05
f1 (ppm)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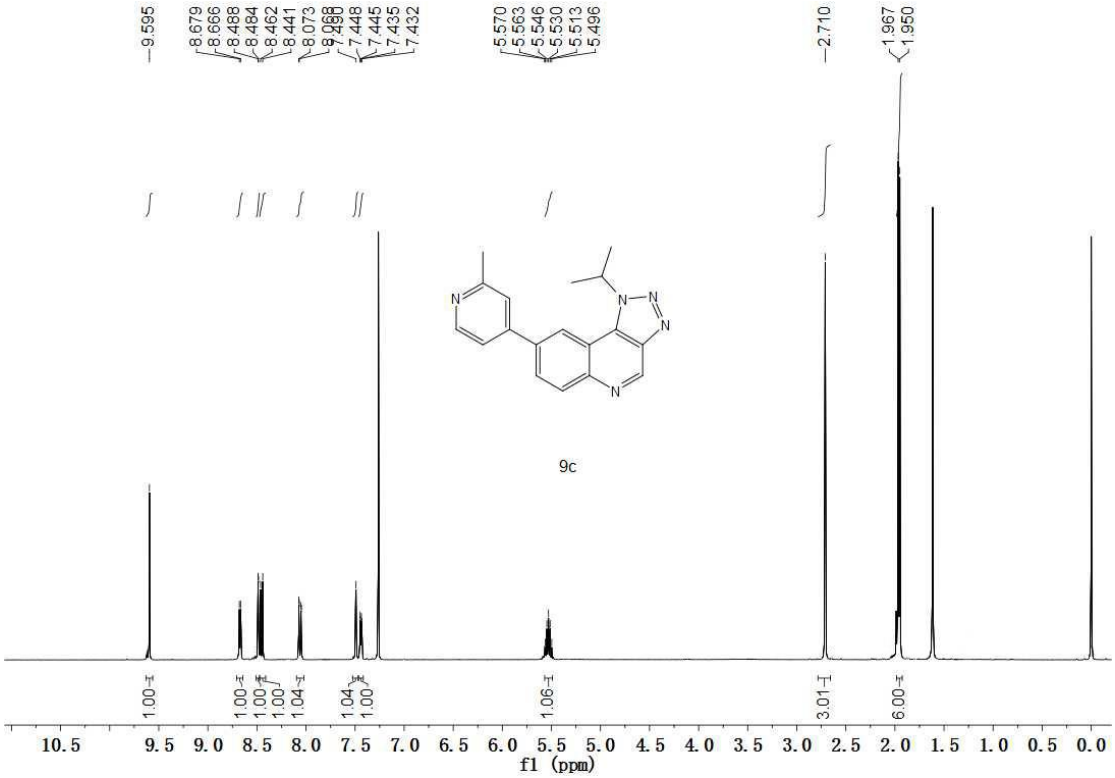
15.019
9b
f1 (ppm)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5.563
5.546
5.530
5.513
5.496
2.710
1.967
1.950
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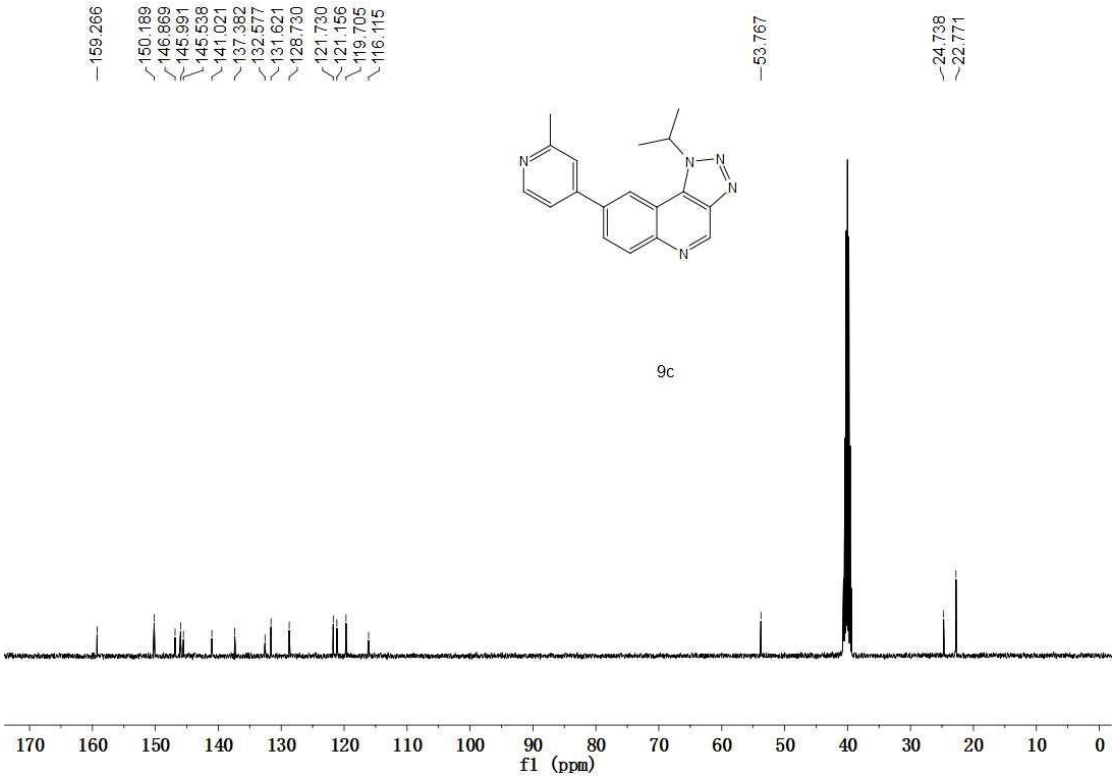
1.00
1.00
1.04
1.04
1.00
1.06
3.01
6.00
f1 (ppm)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24.738
22.771
9c
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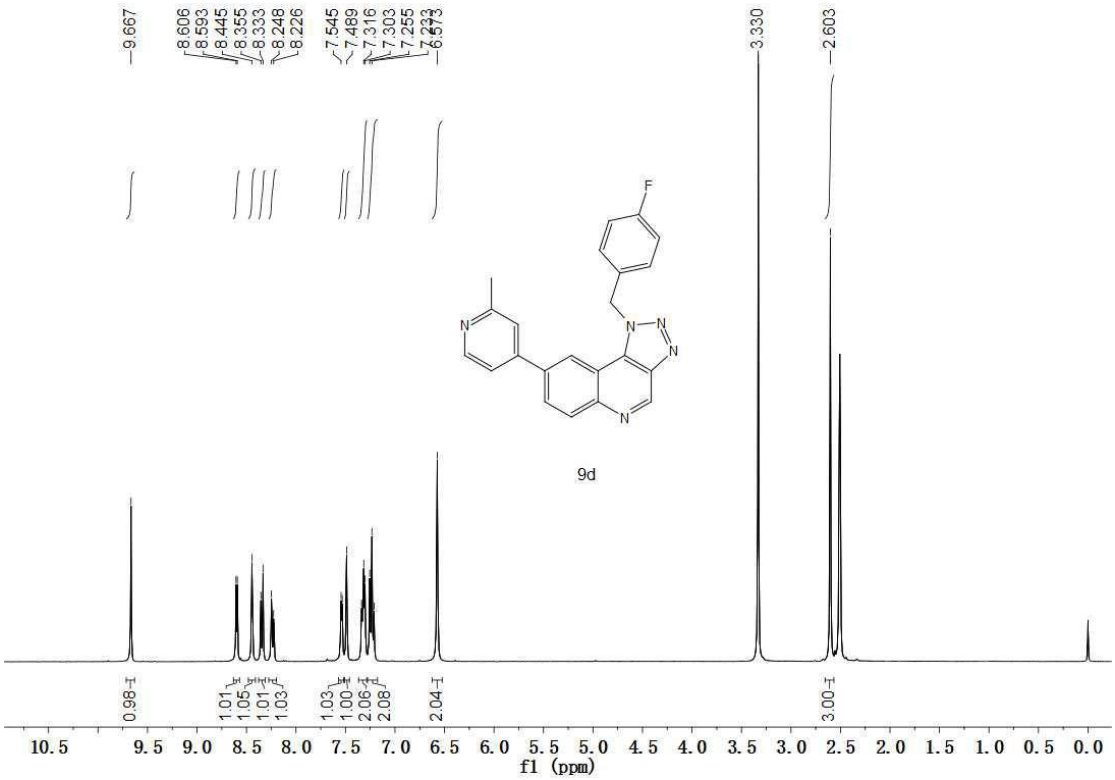
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7.303
7.255
6.573
3.330
2.603
F
N
N
N
N
N
9d
0.98
1.01
1.05
1.01
1.03
1.03
1.00
2.06
2.08
2.04
3.00
10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
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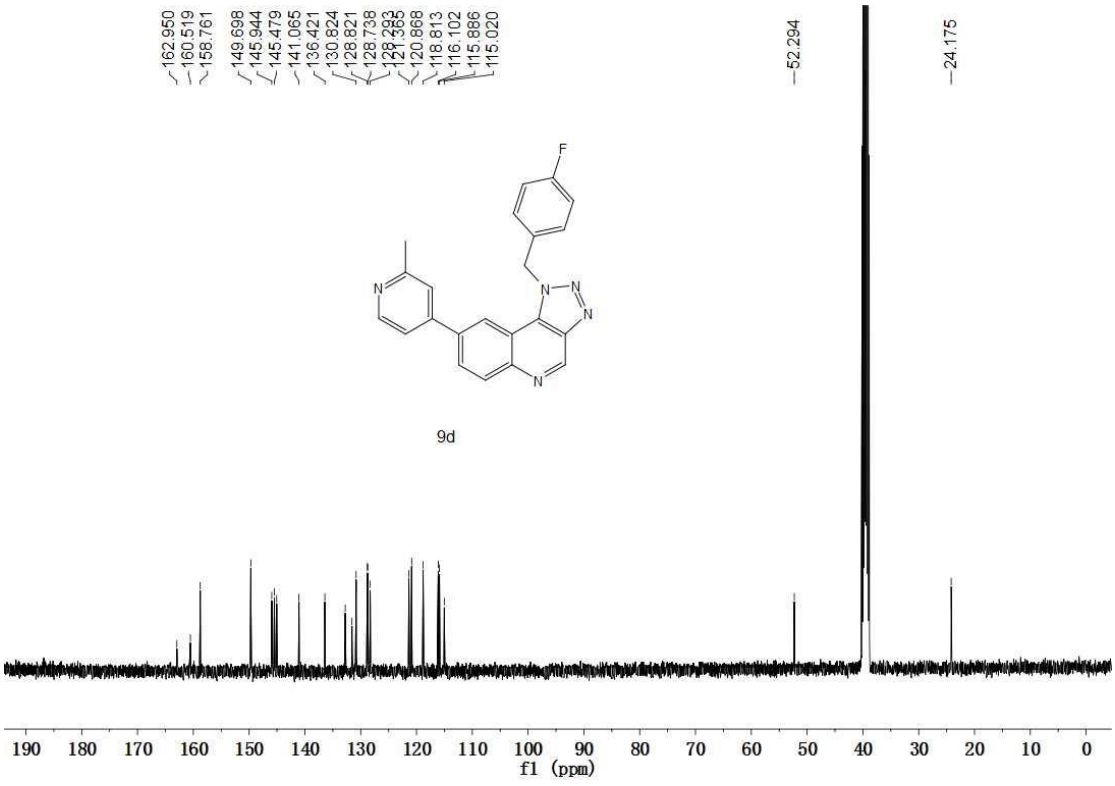
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121.365
120.868
118.813
116.102
115.886
115.020
52.294
24.175
F
N
N
N
N
N
9d
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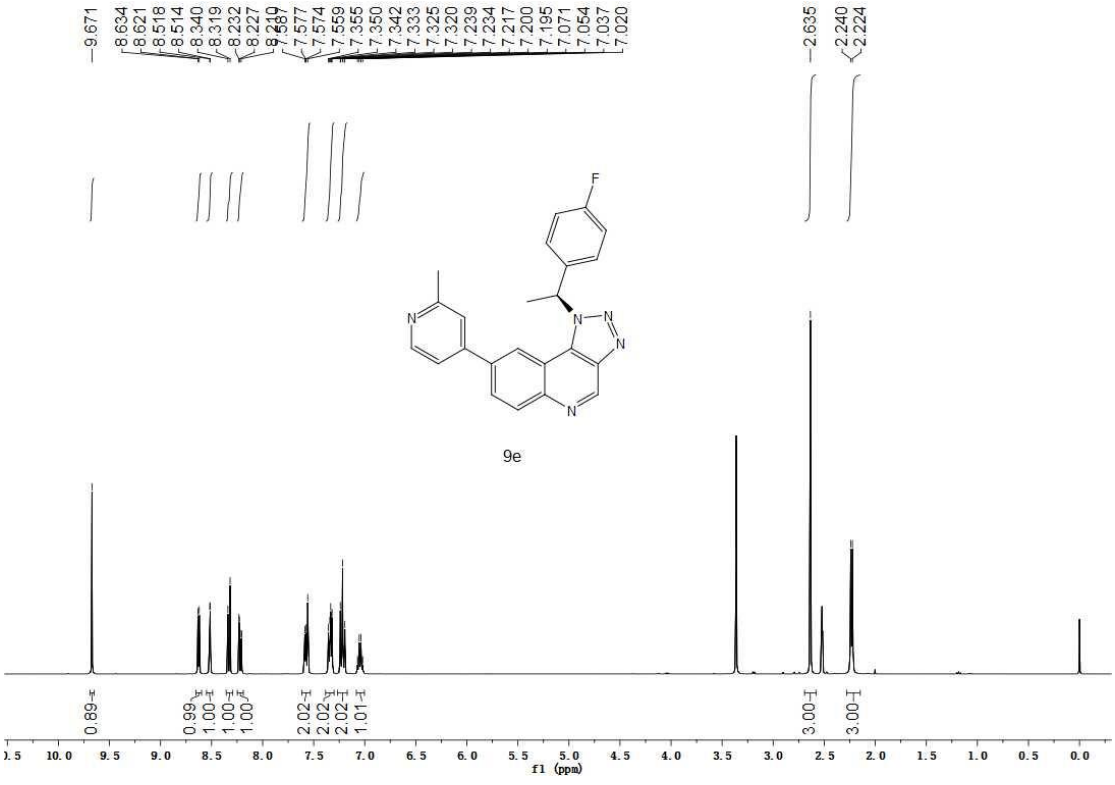
f1 (ppm)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8.232
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7.574
7.559
7.355
7.350
7.342
7.333
7.325
7.320
7.239
7.234
7.217
7.200
7.195
7.071
7.054
7.037
7.020
2.635
2.240
2.224
F
N
N
N
N
N
9e
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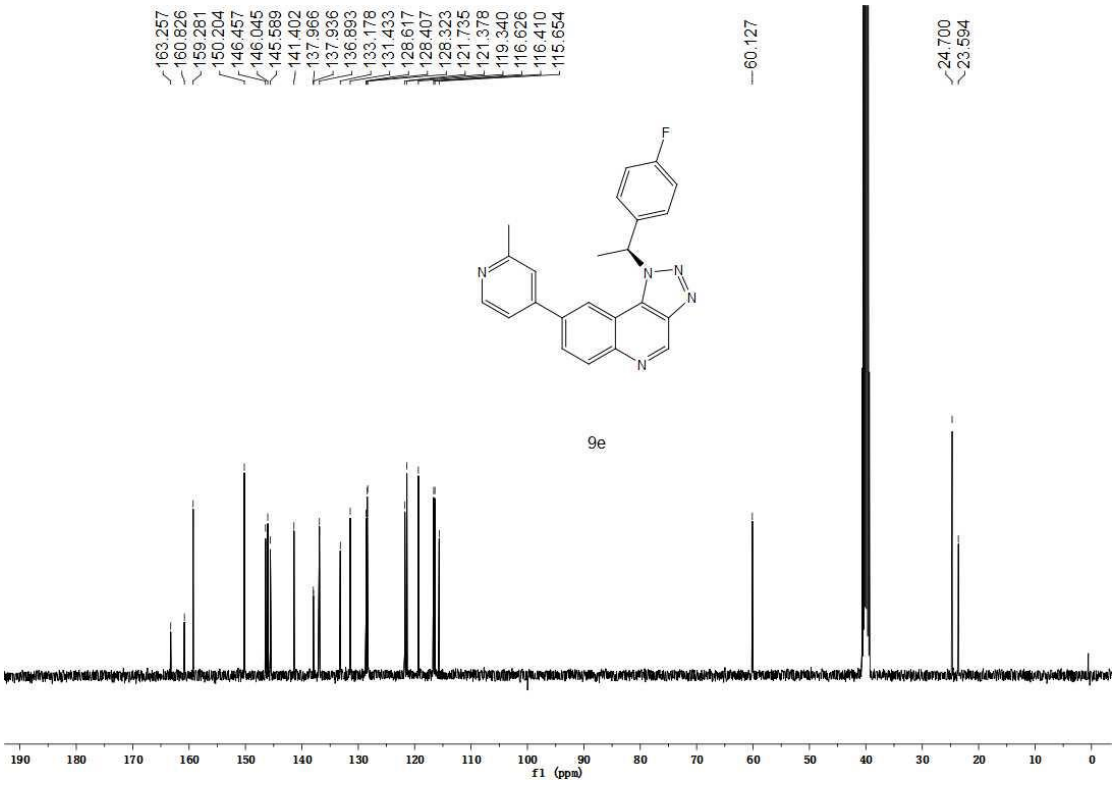
3.00
f1 (ppm)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128.323
121.735
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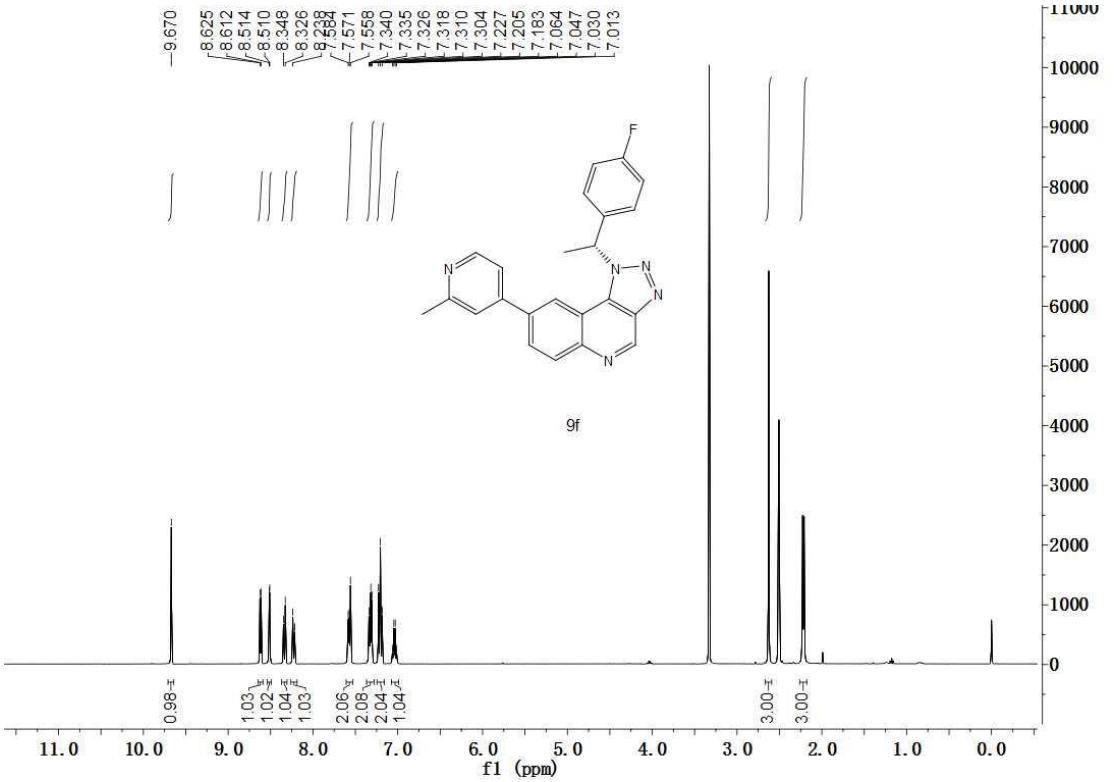
F
N
N
N
N
N
9e
f1 (ppm)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7.064
7.047
7.030
7.013
9f
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1.04
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2.06
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1.04
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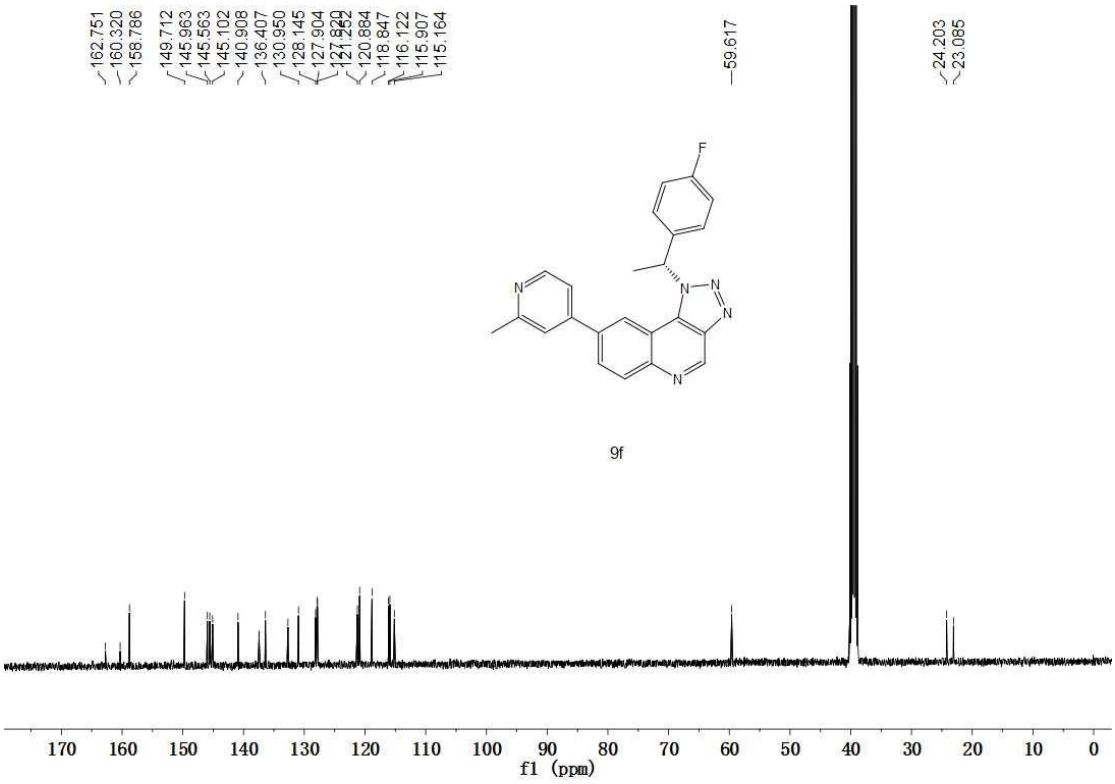
3.00
f1 (ppm)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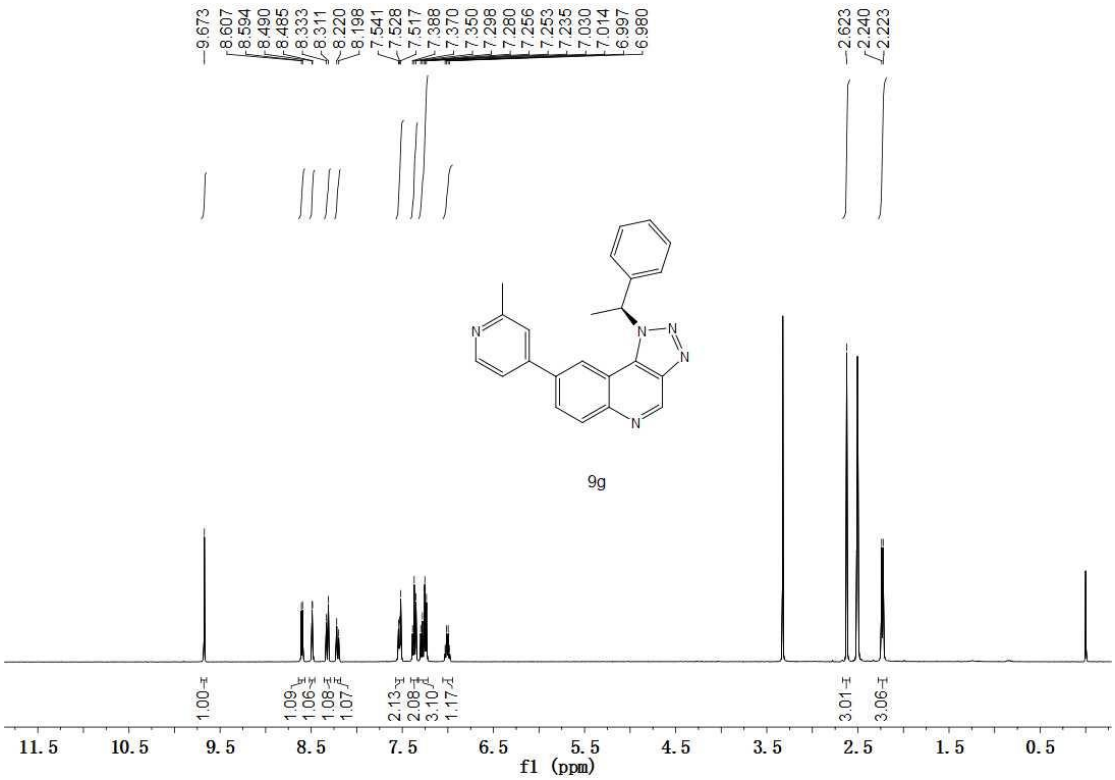
9f
f1 (ppm)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7.370
7.350
7.298
7.280
7.256
7.253
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7.030
7.014
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6.980
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2.240
2.223
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1.09
1.06
1.08
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3.06
11.5
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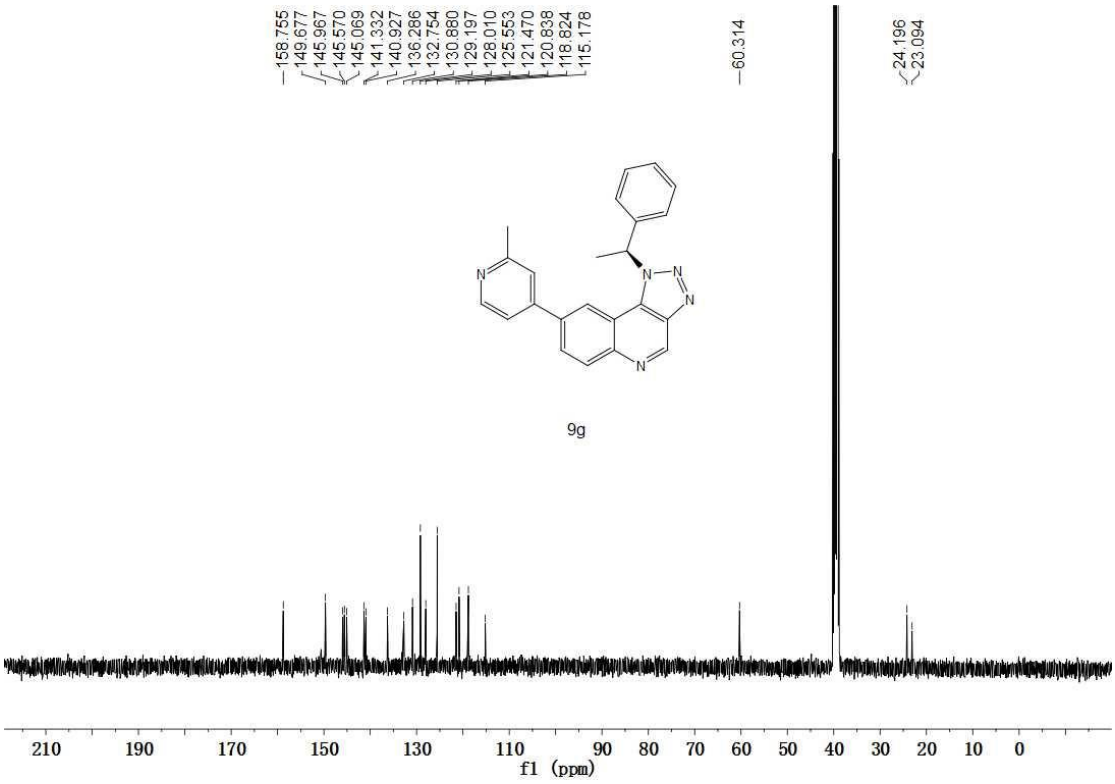
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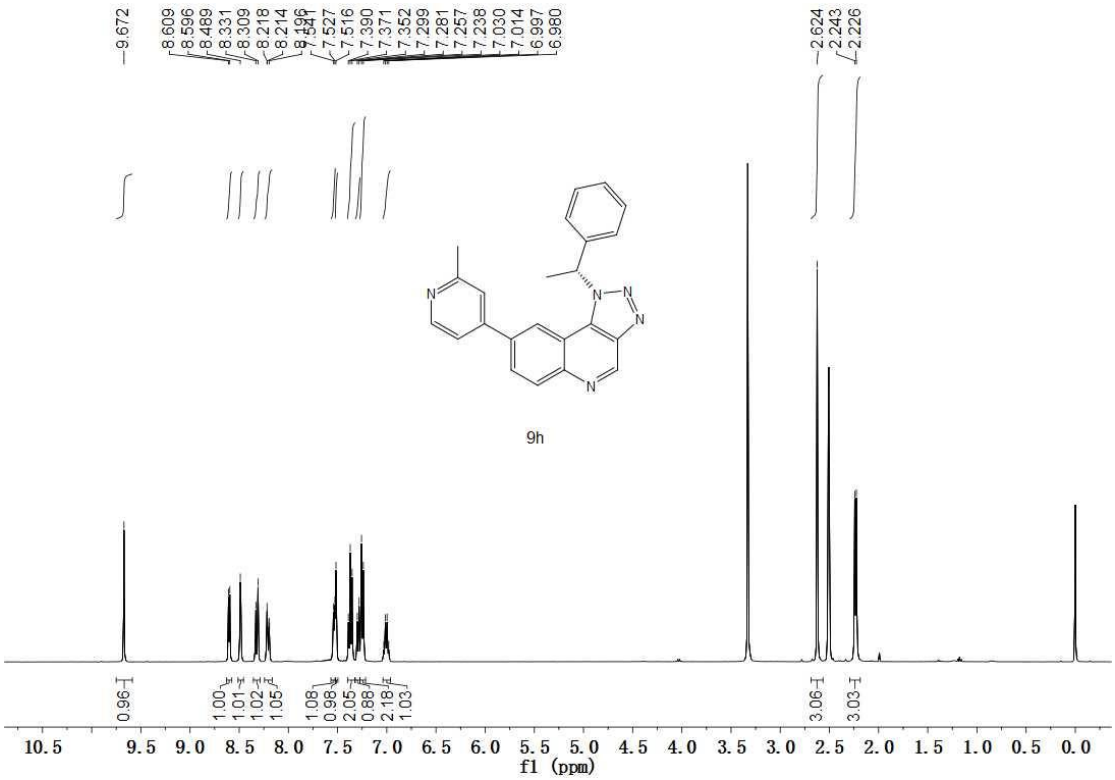
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6.980
2.624
2.243
2.226
9h
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1.01
1.02
1.05
1.08
0.98
2.05
0.88
2.18
1.03
3.06
3.03
10.5 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
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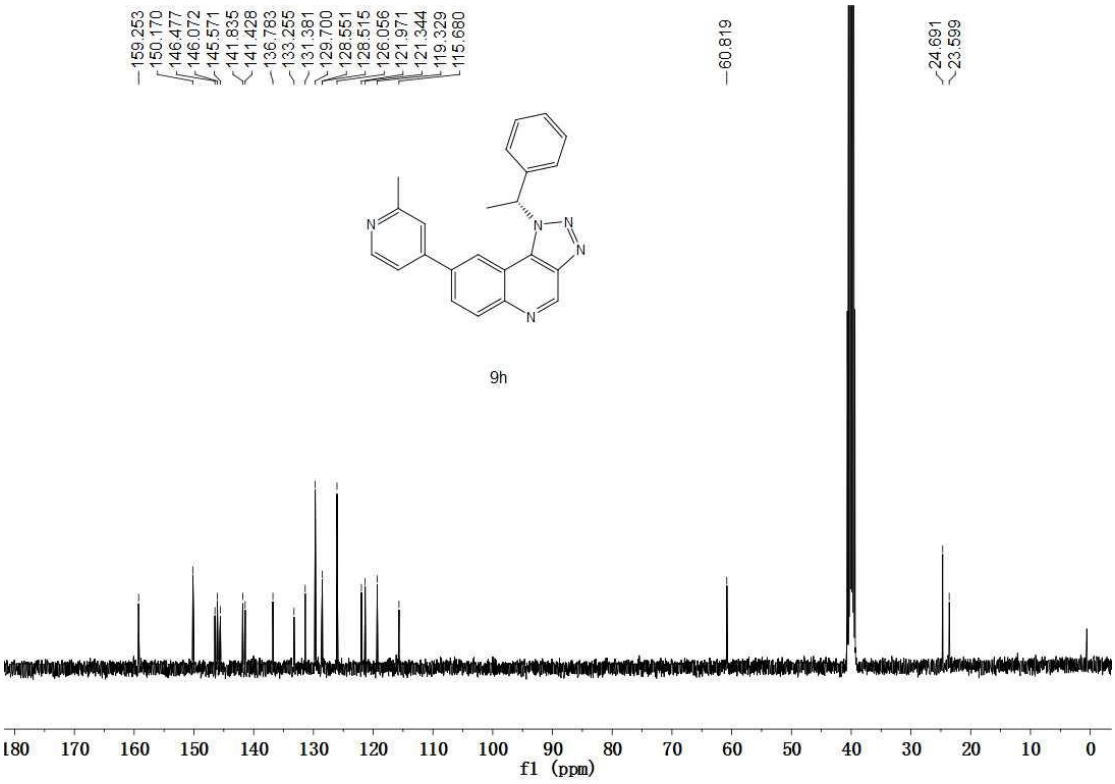
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126.056
121.971
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60.819
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23.599
9h
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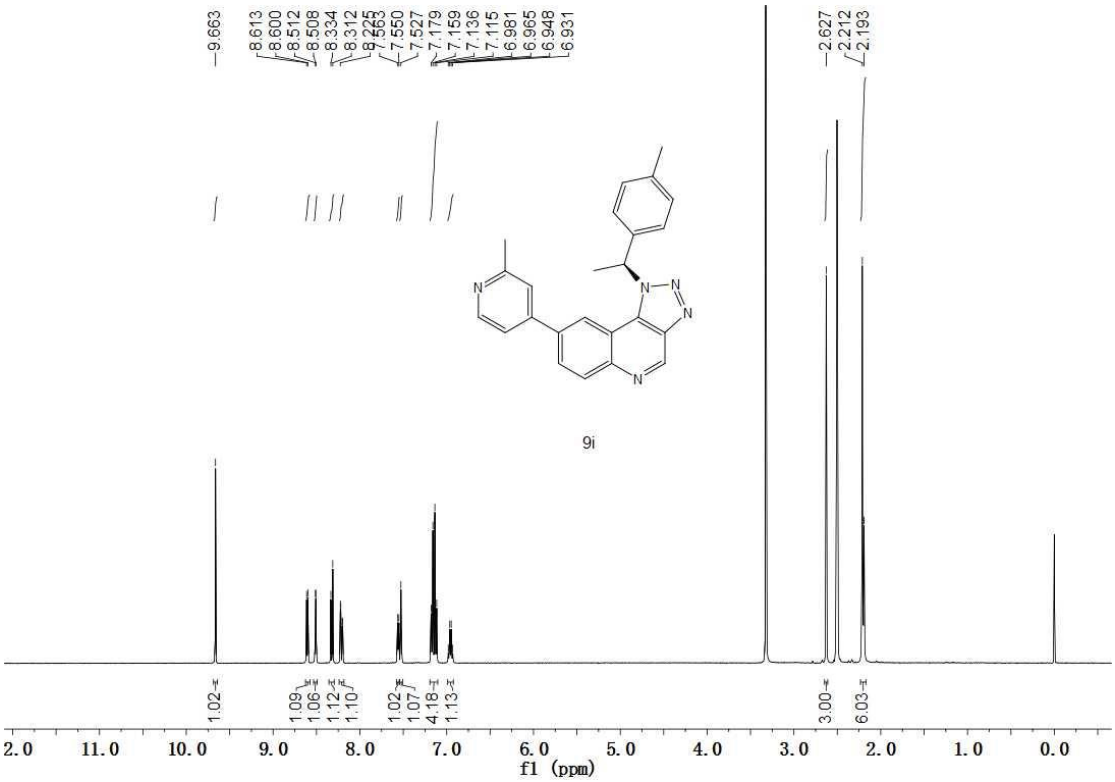
f1 (ppm)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7.136
7.115
6.981
6.965
6.948
6.931
2.627
2.212
2.193
1.02
1.09
1.06
1.12
1.10
1.02
1.07
4.18
1.13
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6.03
2.0
11.0
10.0
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8.0
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6.0
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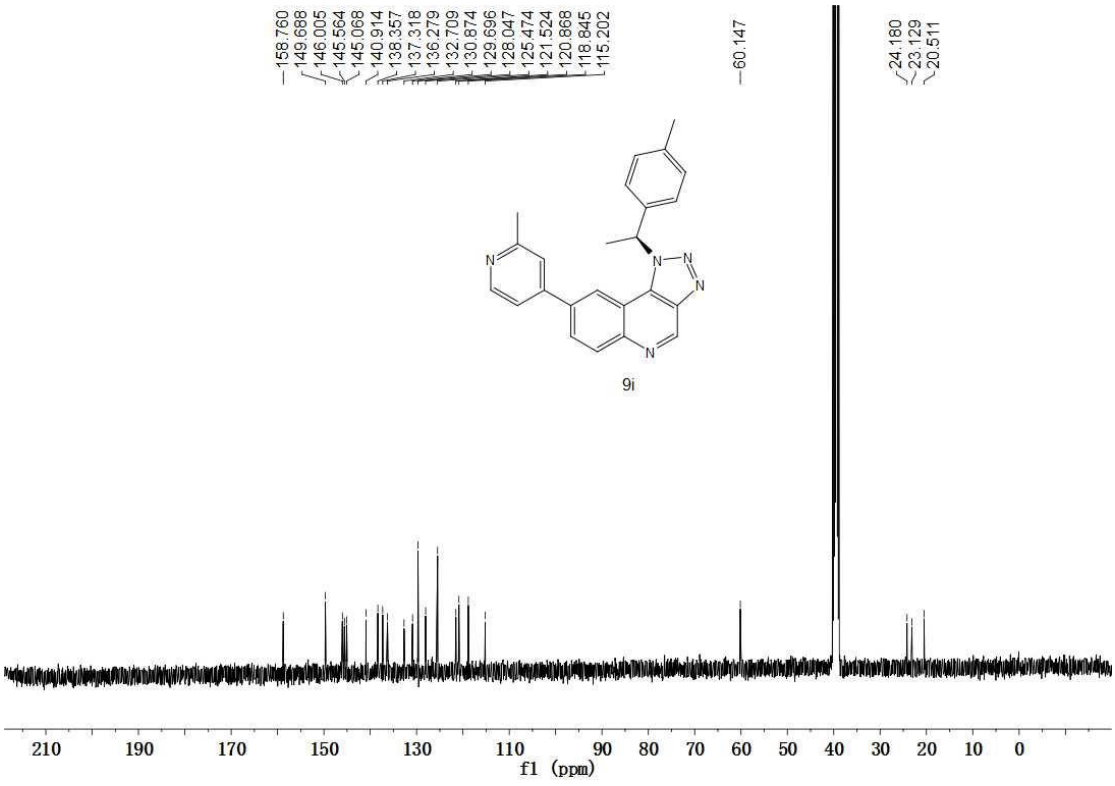
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

9i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125.474
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118.845
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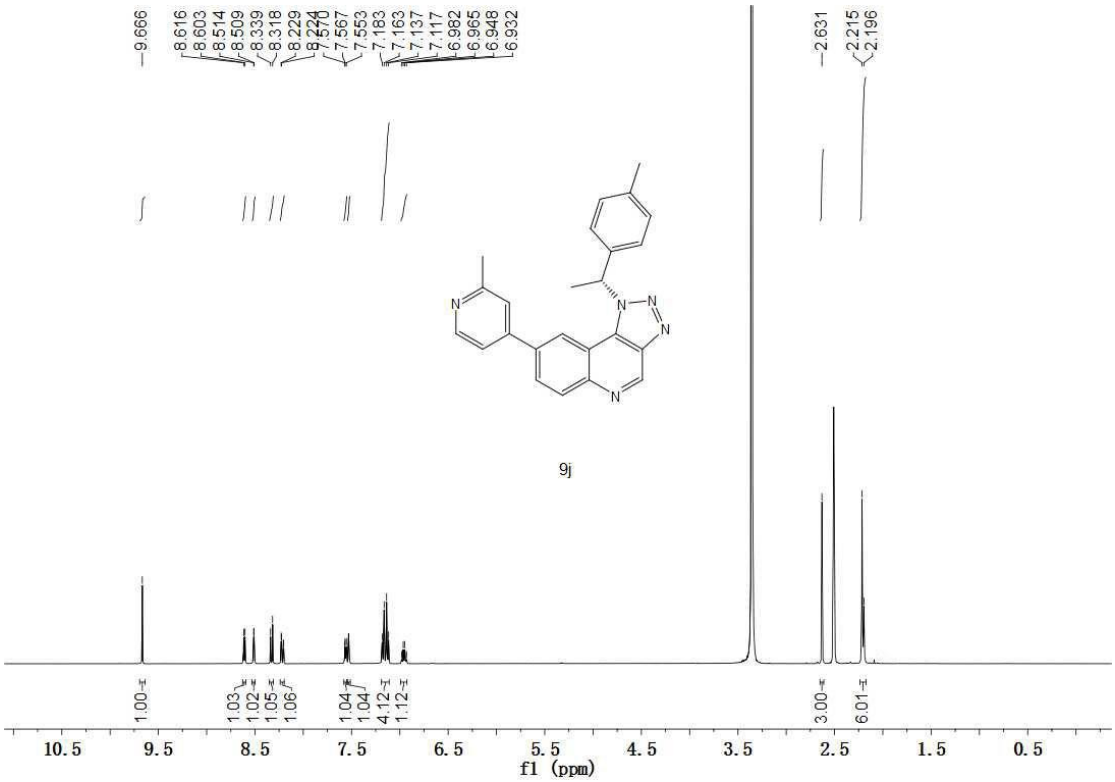
9i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6.965
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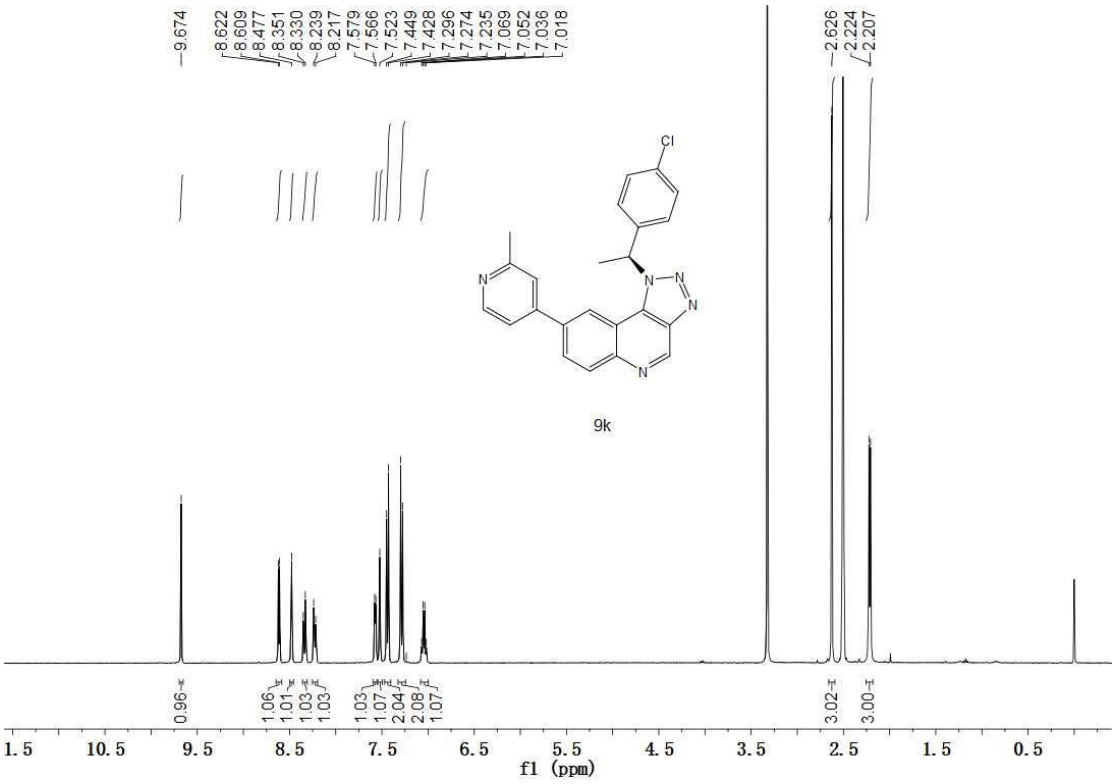
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7.274
7.235
7.069
7.052
7.036
7.018
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2.224
2.207
Cl
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1.06
1.01
1.03
1.03
1.03
1.07
2.04
2.08
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3.00
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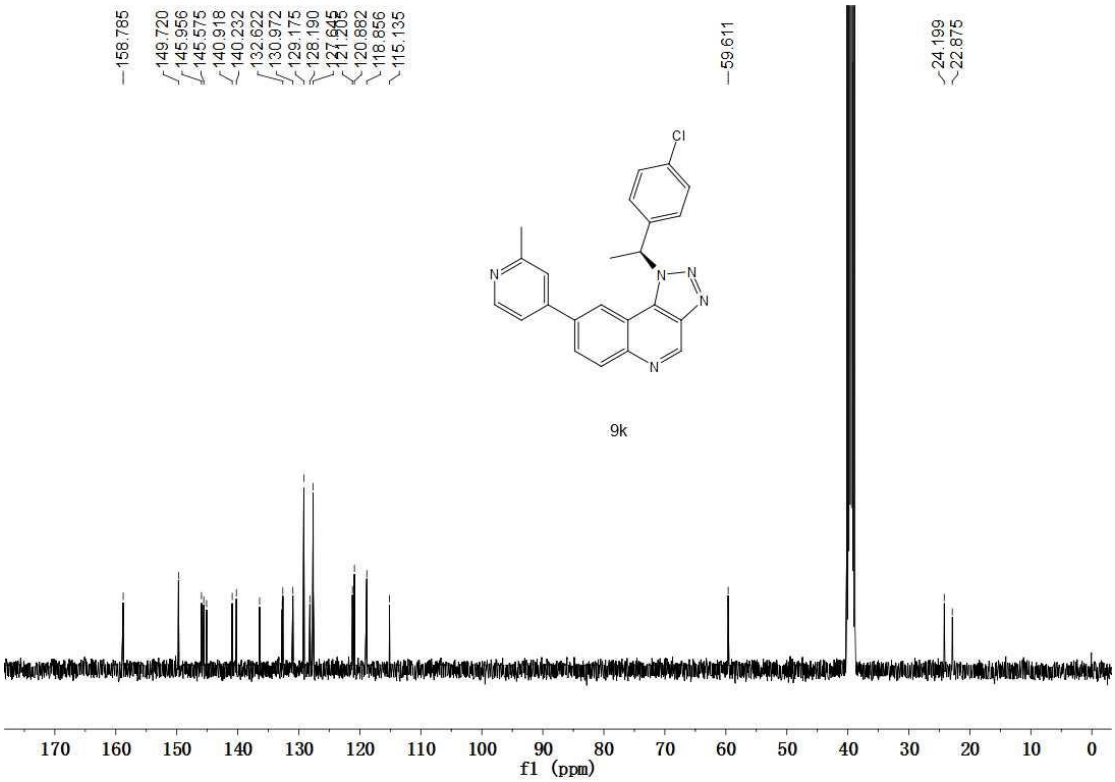
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118.856
115.135
59.611
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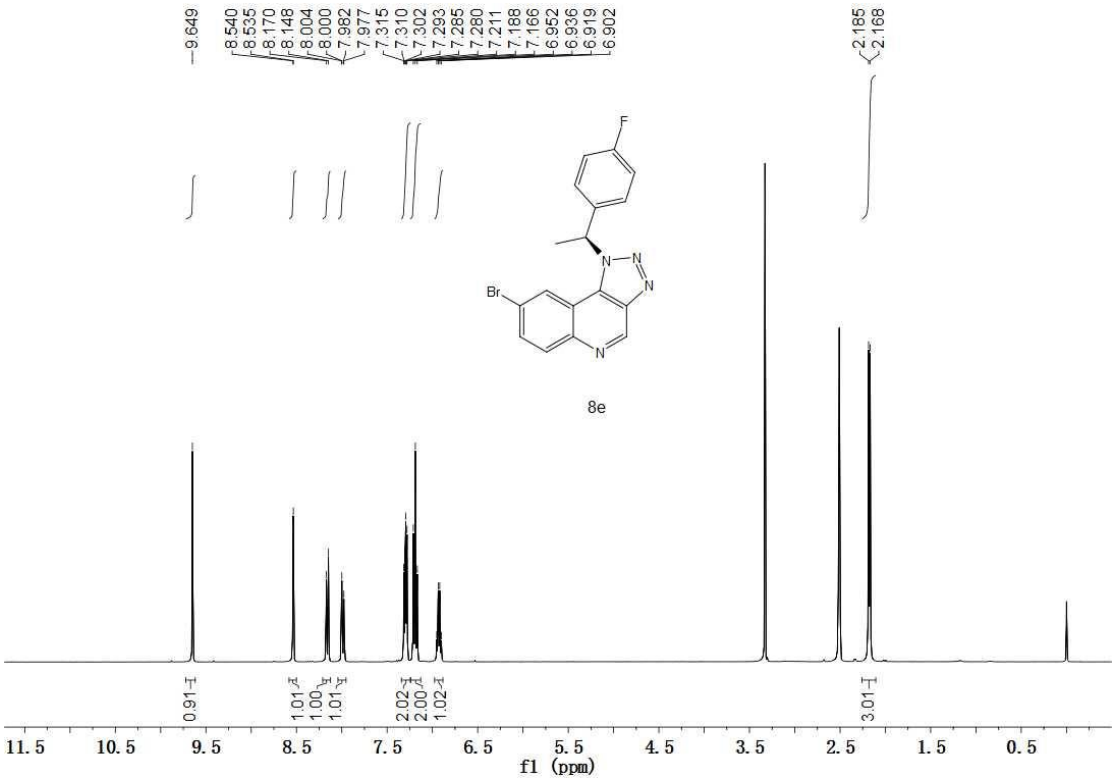
f1 (ppm)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7.293
7.285
7.280
7.211
7.188
7.166
6.952
6.936
6.919
6.902
2.185
2.168
F
Br
N
N
N
N
8e
0.91
1.01
1.00
1.01
2.02
2.00
1.02
3.01
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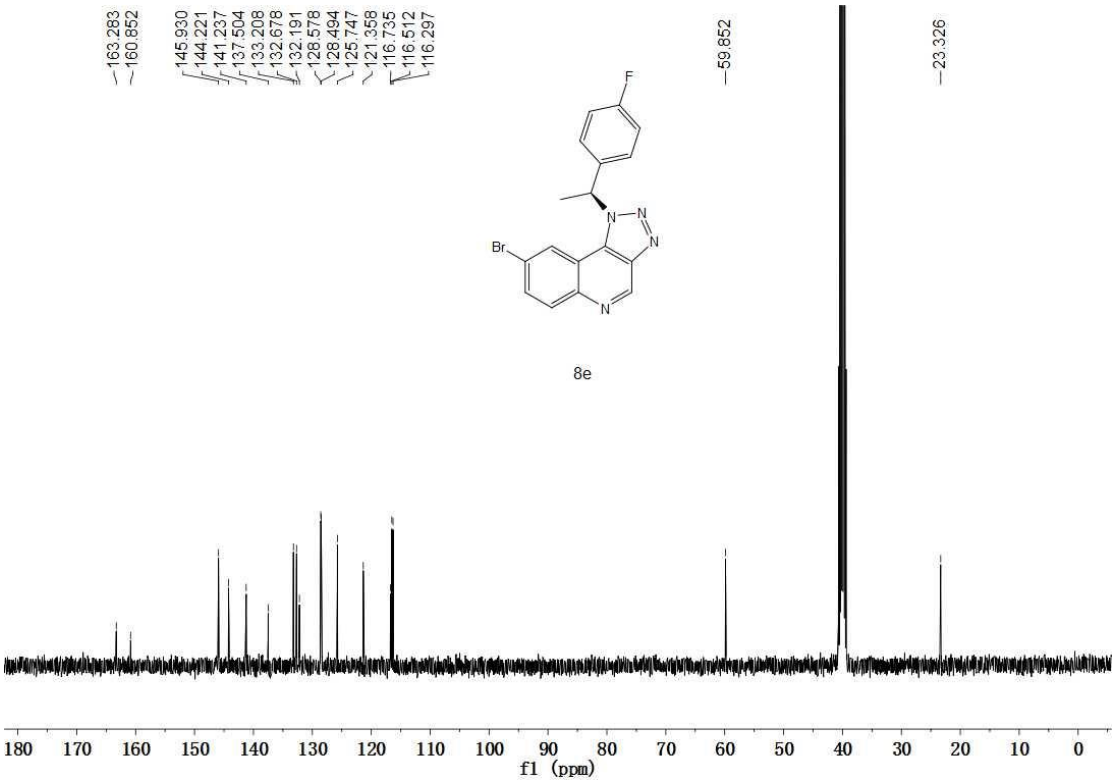
f1 (ppm)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116.512
116.297
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8e
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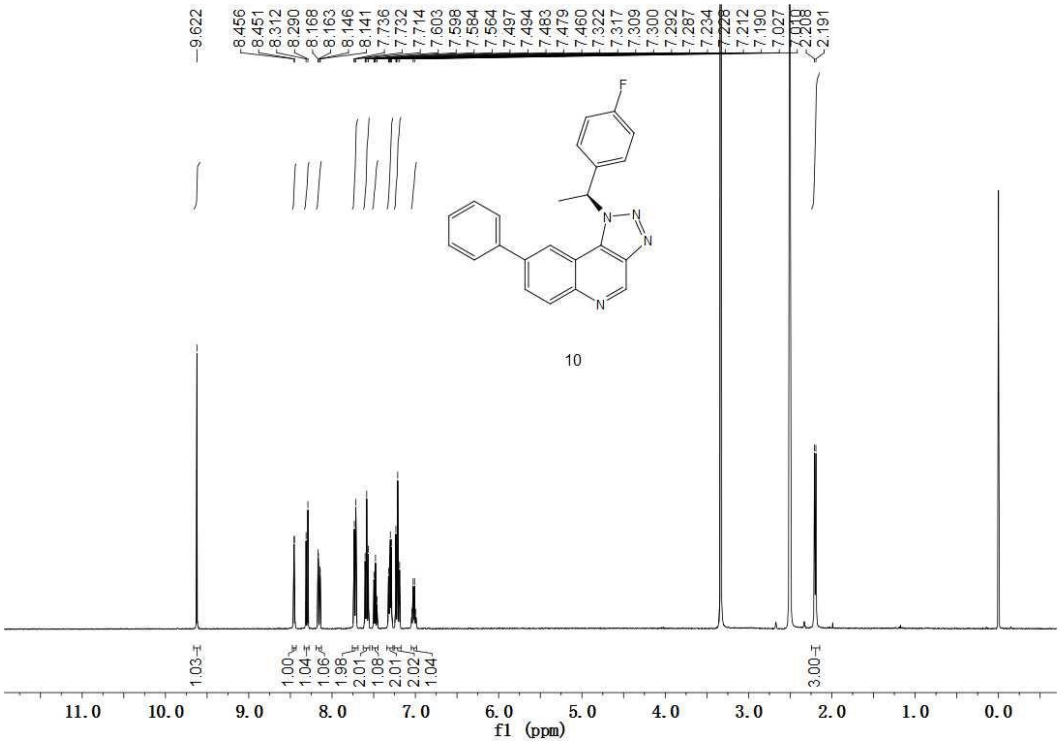
f1 (ppm)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7.598
7.584
7.564
7.497
7.494
7.483
7.479
7.460
7.322
7.317
7.309
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7.292
7.287
7.234
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7.190
7.027
2.208
2.191
F
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N
N
10
1.03
1.00
1.04
1.06
1.98
2.01
1.08
2.01
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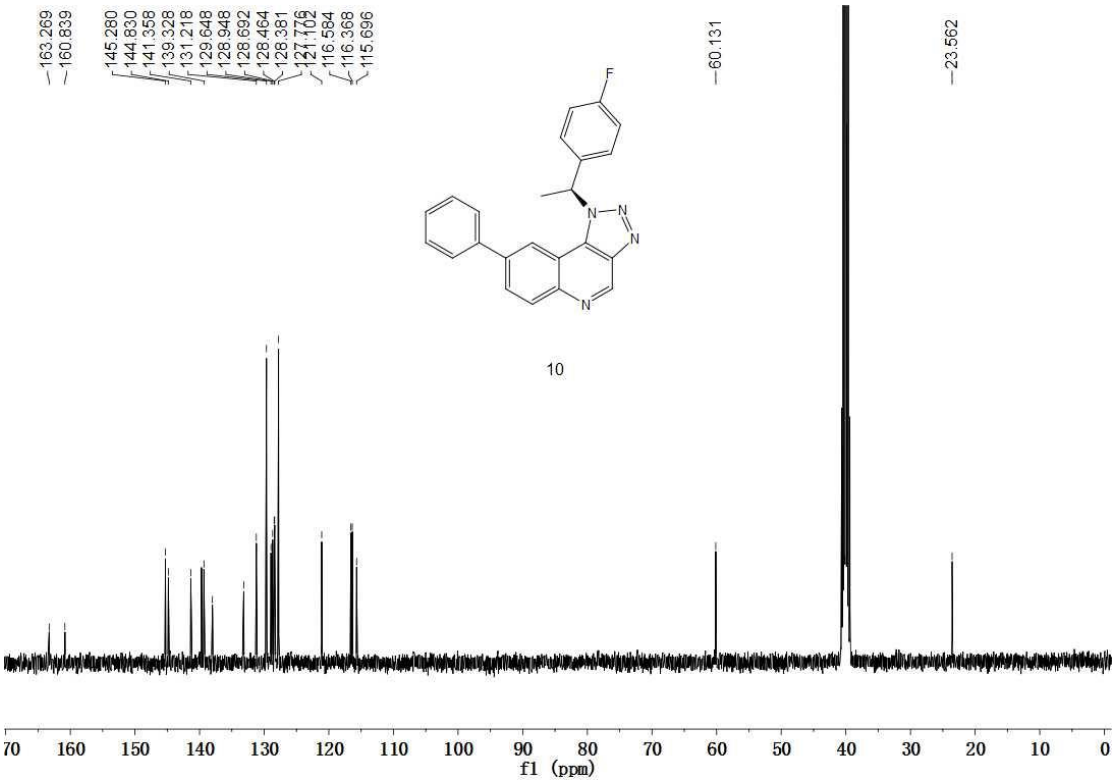
f1 (ppm)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116.584
116.368
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N
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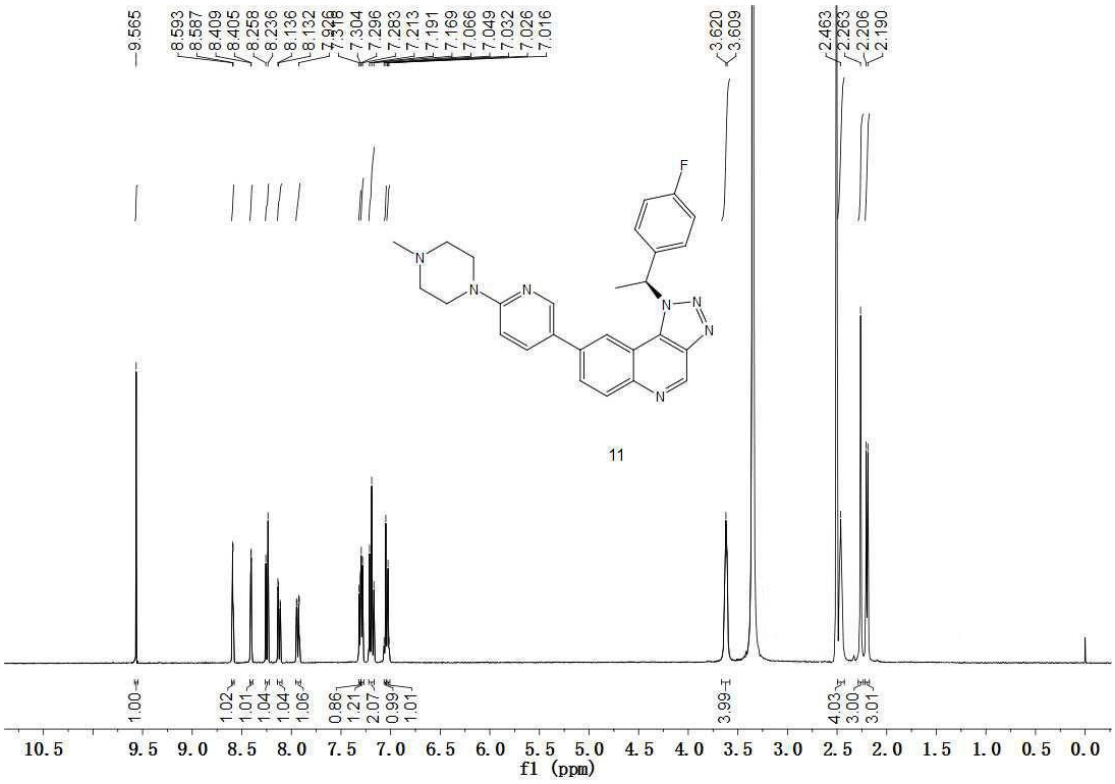
f1 (ppm)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7.032
7.026
7.016
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3.609
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2.263
2.206
2.190
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N
N
N
N
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1.01
1.04
1.04
1.06
0.86
1.21
2.07
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1.01
3.99
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3.00
3.01
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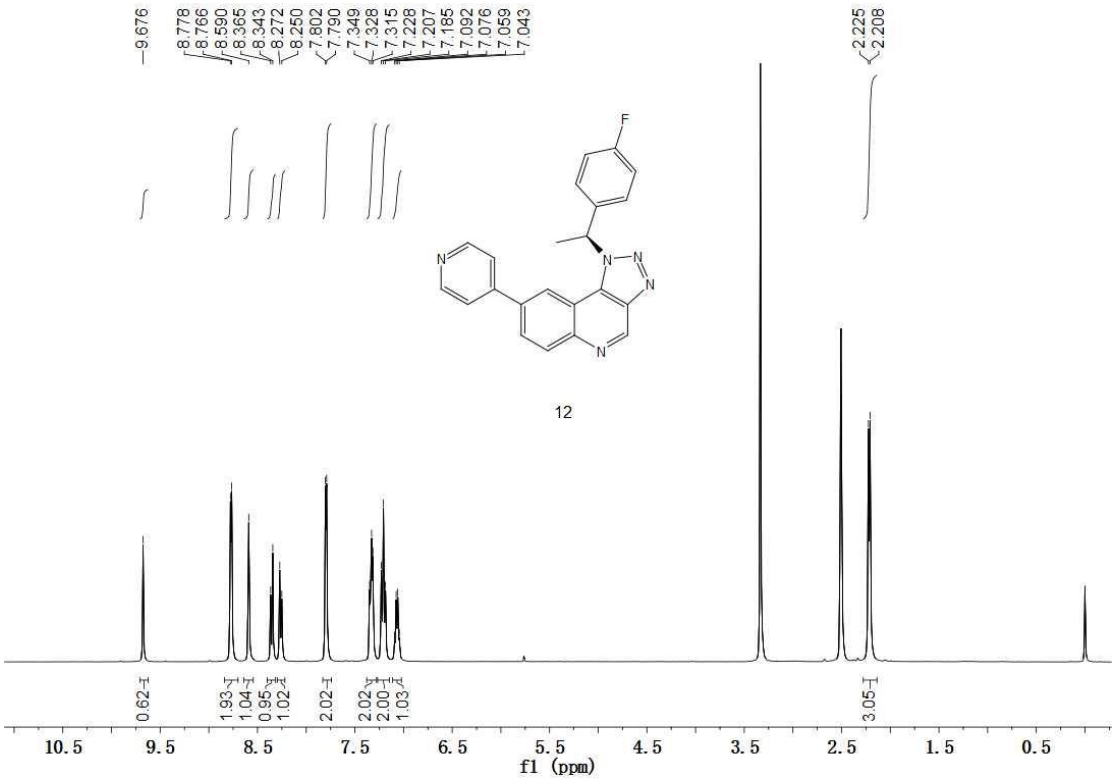
f1 (ppm)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7.228
7.207
7.185
7.092
7.076
7.059
7.043
2.225
2.208
F
N
N
N
N
N
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1.93
1.04
0.95
1.02
2.02
2.02
2.00
1.03
3.05
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1.5
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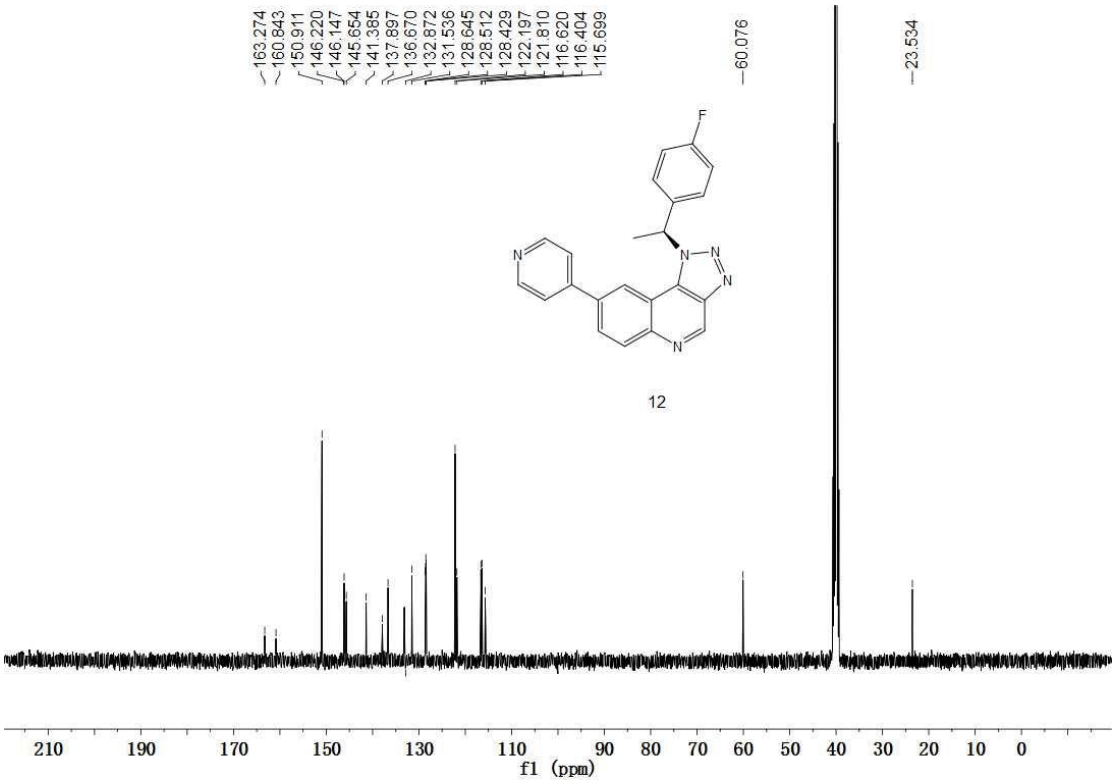
f1 (ppm)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128.429
122.197
121.810
116.620
116.404
115.699
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23.534
F
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N
N
N
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90
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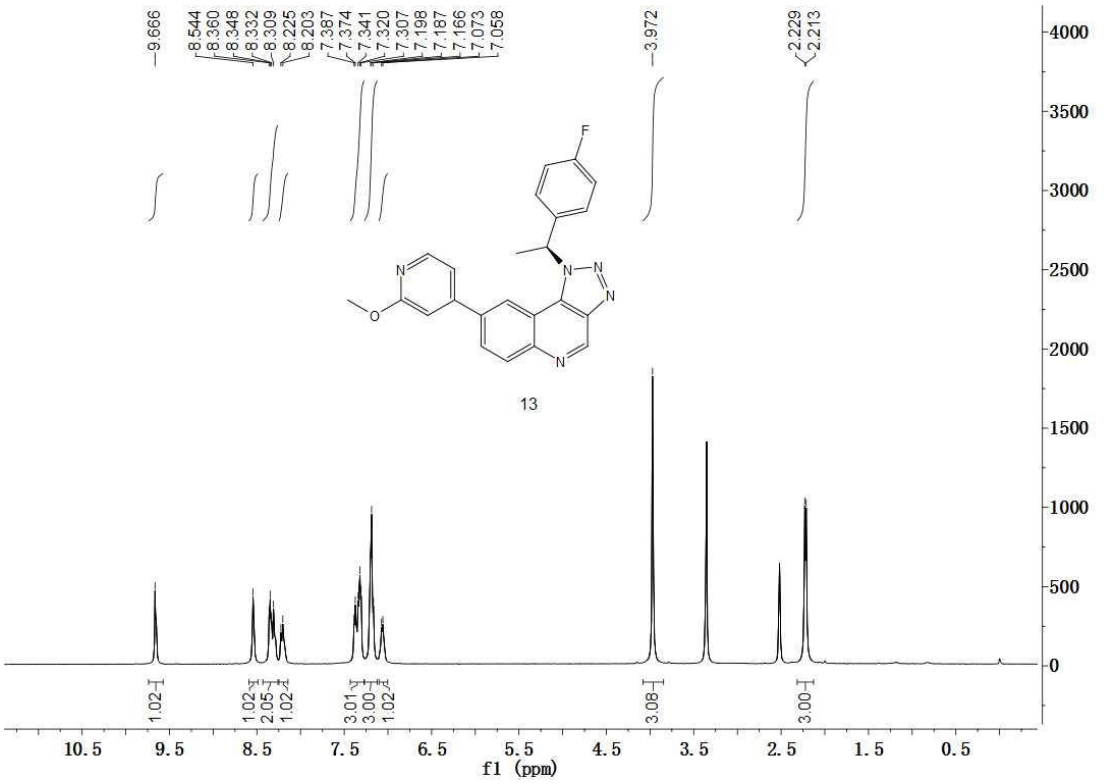
f1 (ppm)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7.187
7.166
7.073
7.058
3.972
2.229
2.213
F
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N
N
O
N
13
4000
3500
3000
2500
2000
1500
1000
500
0
1.02
1.02
2.05
1.02
3.01
3.00
1.02
3.08
3.00
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7.5
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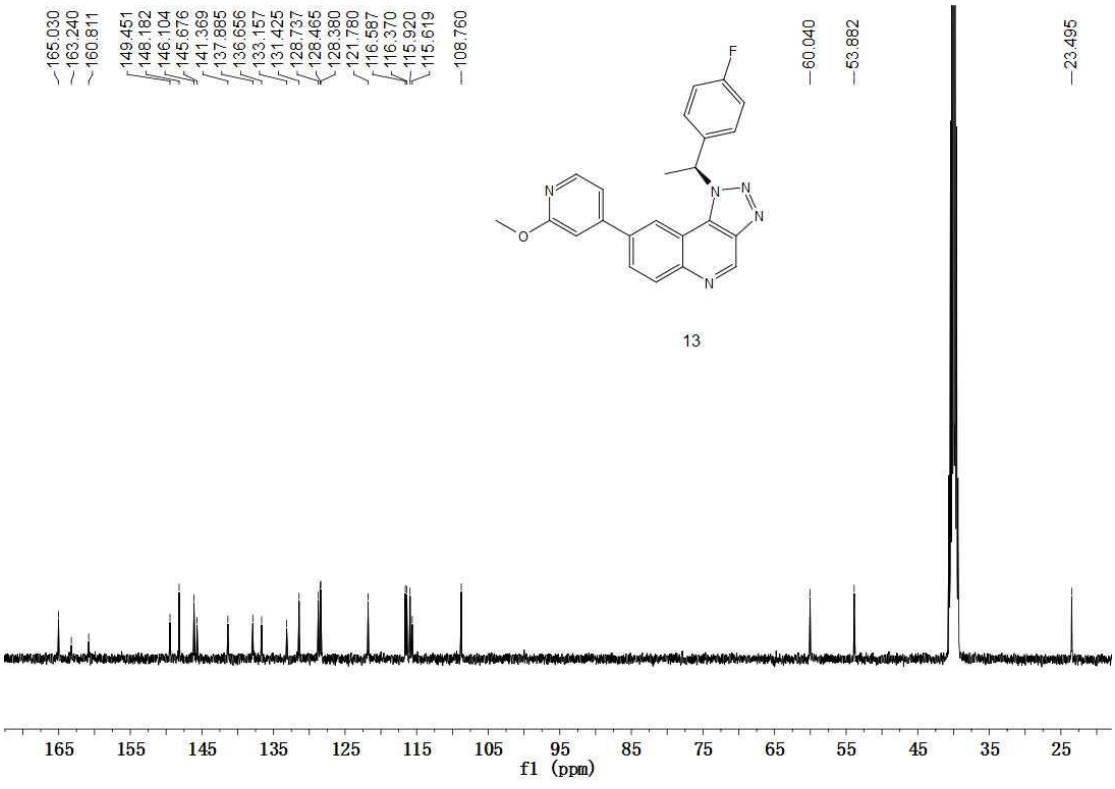
f1 (ppm)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128.737
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128.380
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116.587
116.370
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60.040
53.882
23.495
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N
N
N
N
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155
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135
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115
105
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85
75
65
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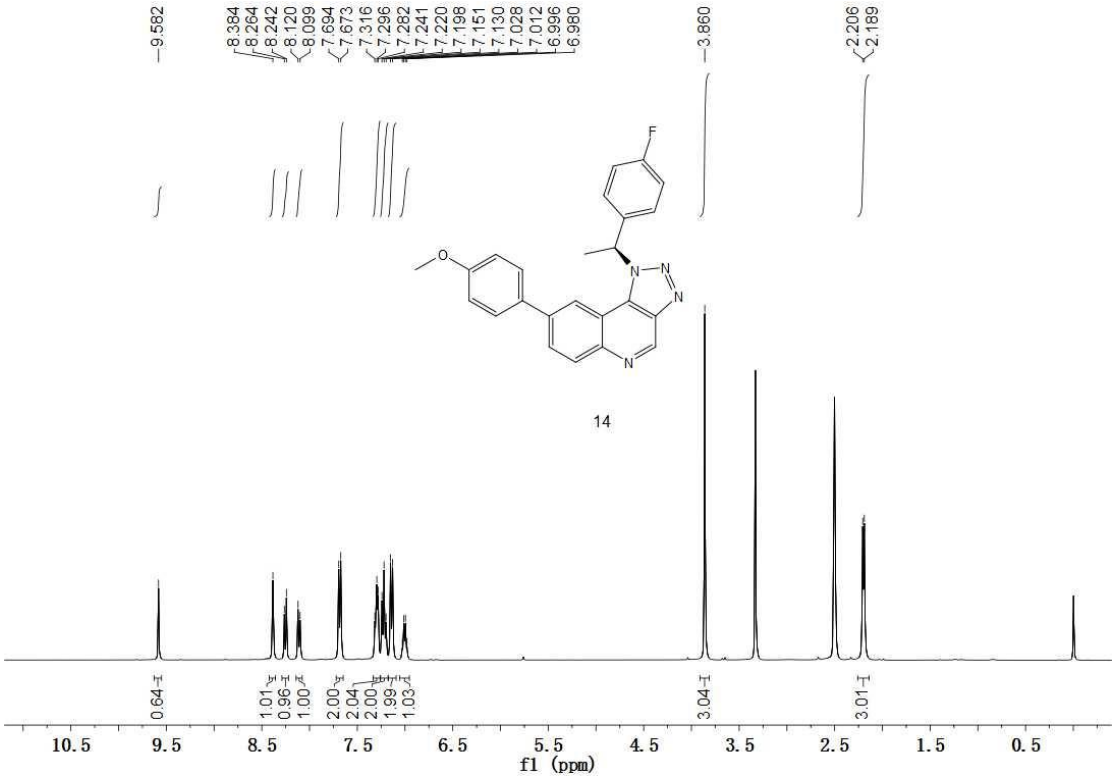
35
25
f1 (ppm)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7.198
7.151
7.130
7.028
7.012
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6.980
3.860
2.206
2.189
F
O
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N
N
N
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0.96
1.00
2.00
2.04
2.00
1.99
1.03
3.04
3.01
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7.5
6.5
5.5
4.5
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2.5
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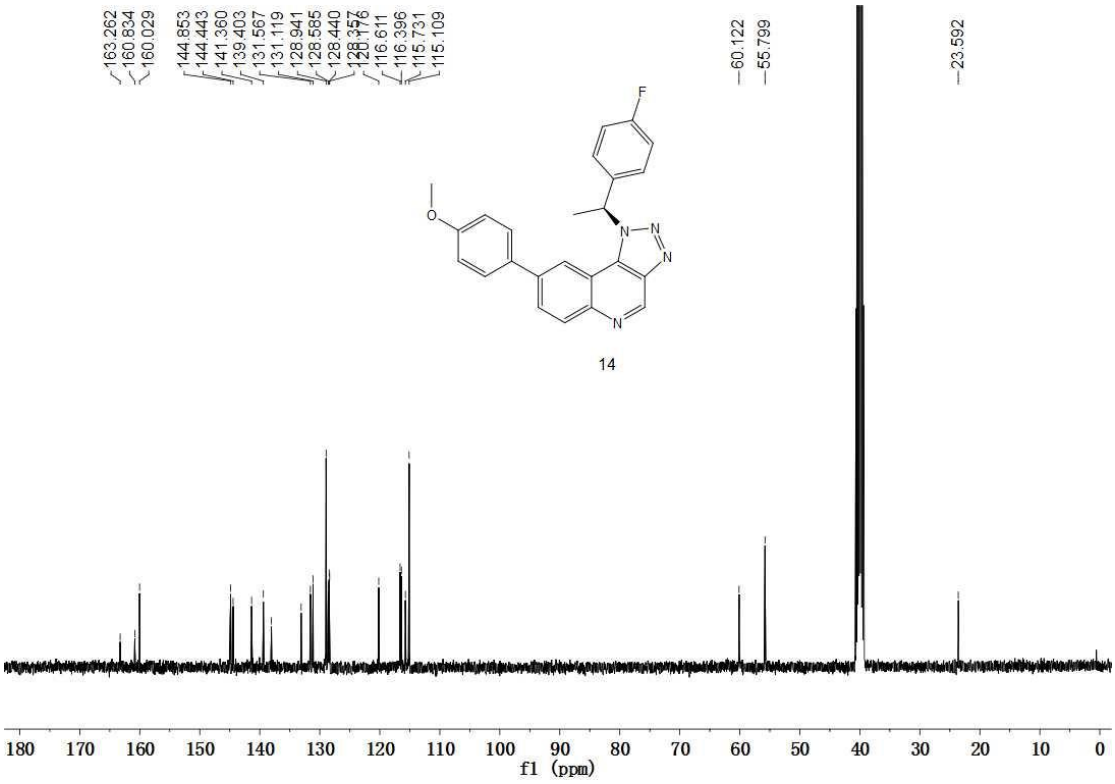
f1 (ppm)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116.396
115.731
115.109
60.122
55.799
23.592
F
O
N
N
N
N
14
180
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130
120
110
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80
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60
50
40
30
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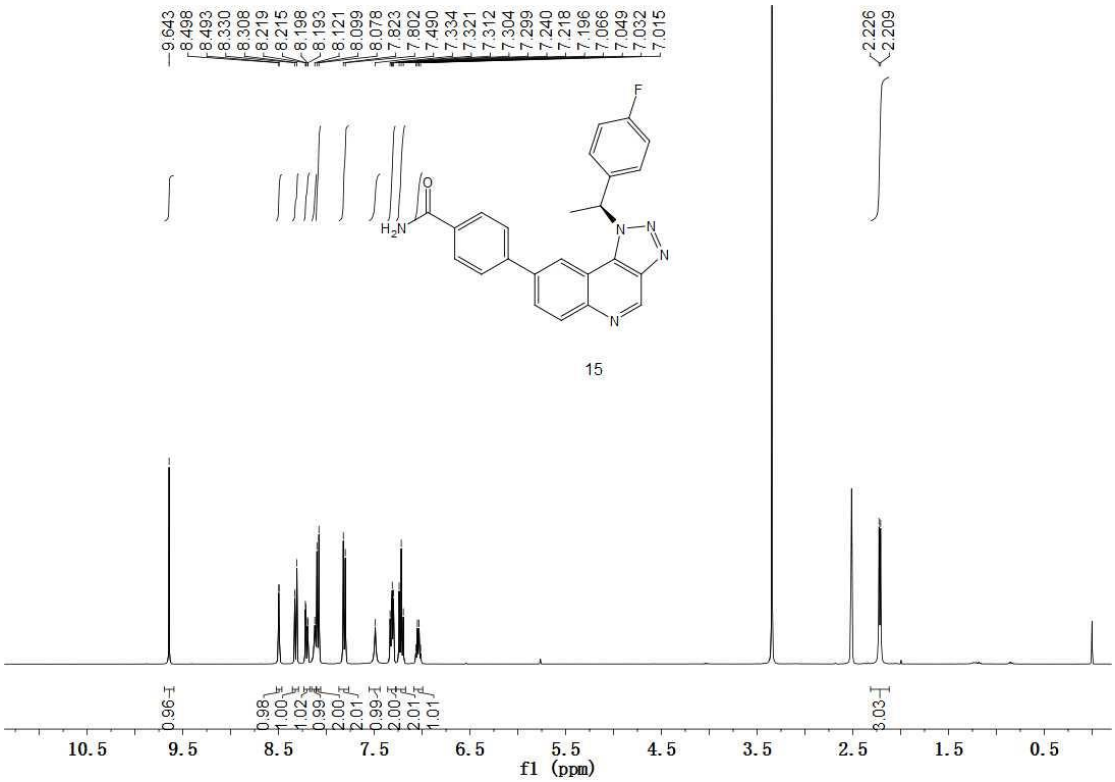
10
0
f1 (ppm)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7.490
7.334
7.321
7.312
7.304
7.299
7.240
7.218
7.196
7.066
7.049
7.032
7.015
2.226
2.209
F
O
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N
N
N
N
15
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0.98
1.00
1.02
0.99
2.00
2.01
0.99
2.00
2.01
1.01
3.03
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7.5
6.5
5.5
4.5
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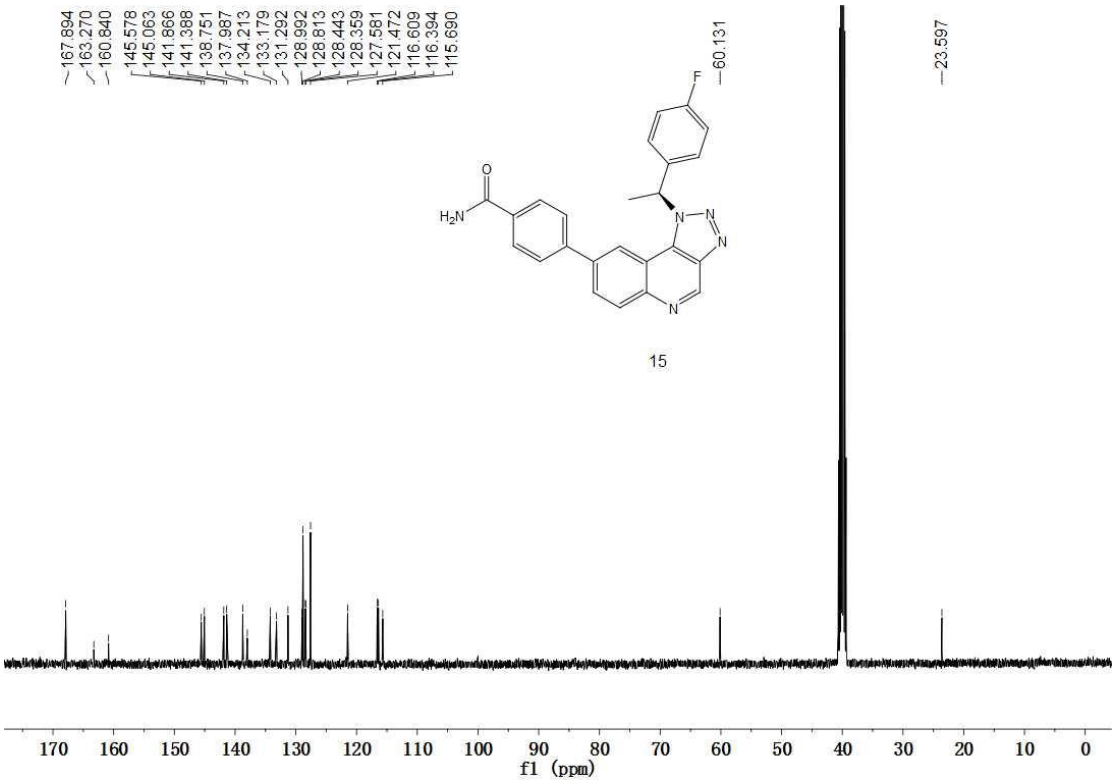
f1 (ppm)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23.597
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O
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N
N
N
N
15
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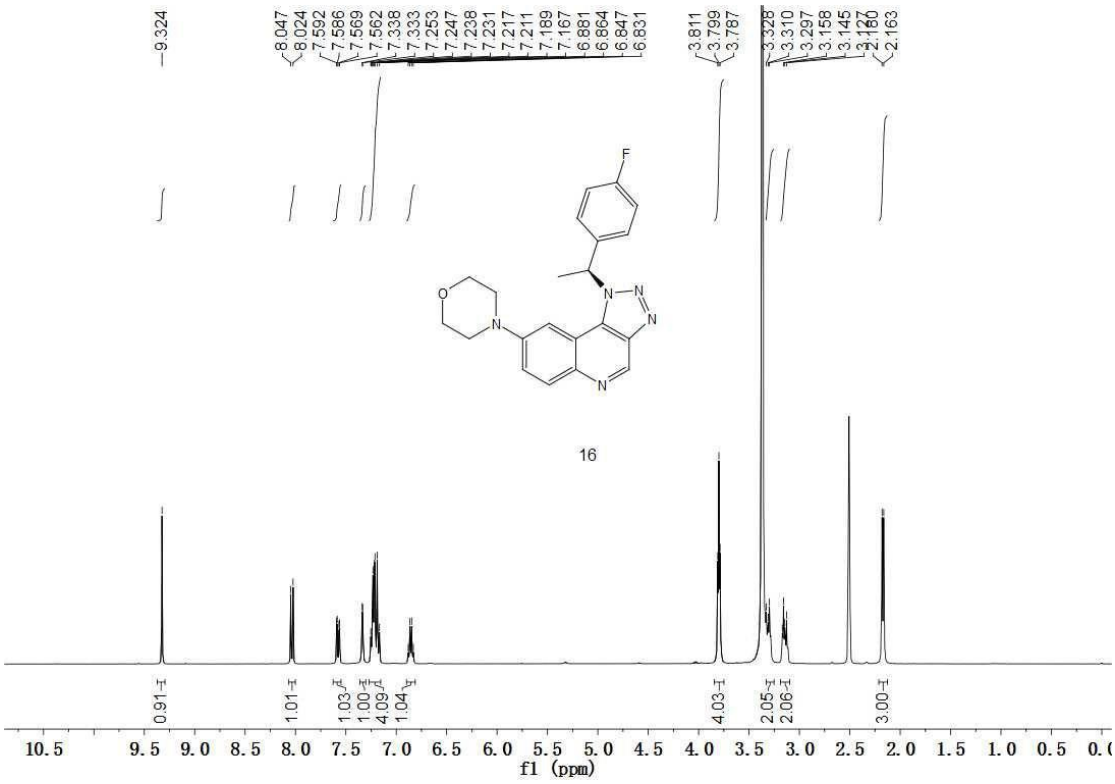
f1 (ppm)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6.831
3.811
3.799
3.787
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3.297
3.158
3.145
2.180
2.163
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1.03
1.00
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1.04
4.03
2.05
2.06
3.00
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6.0
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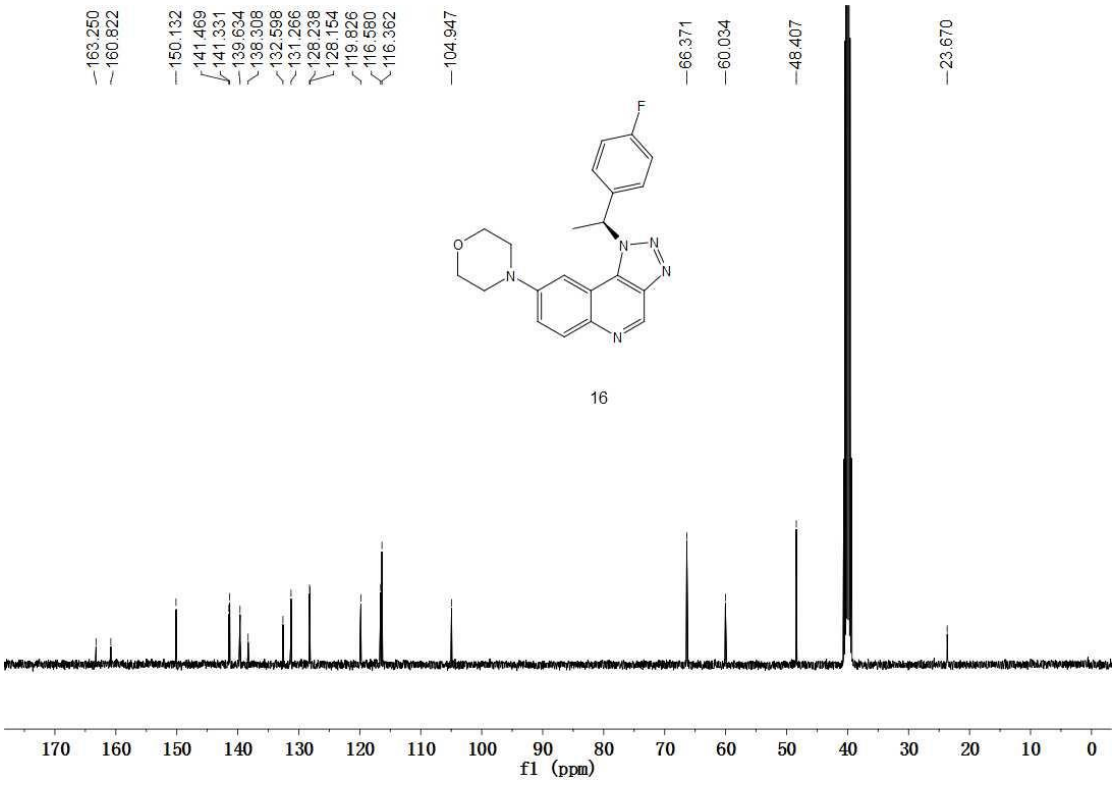
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116.362
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60.034
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23.670
F
O
N
N
N
N
16
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50
40
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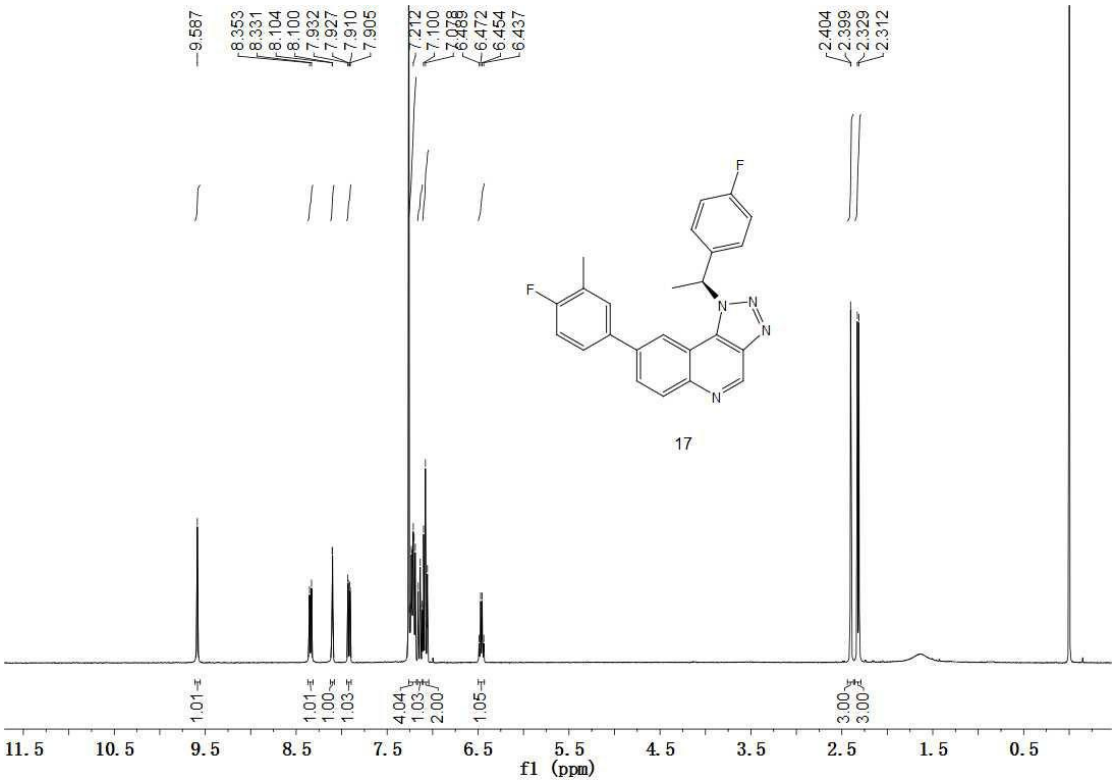
f1 (ppm)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N
N
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1.01
1.00
1.03
4.04
1.03
2.00
1.05
3.00
3.00
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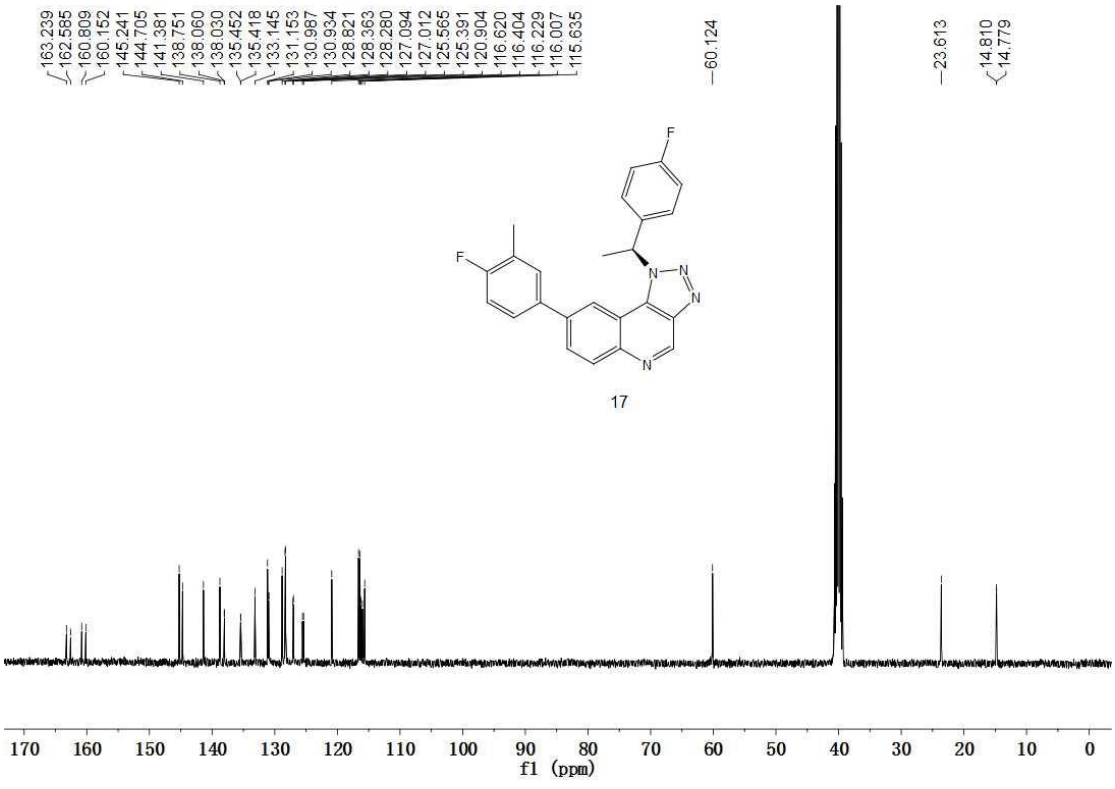
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127.094
127.012
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125.391
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17
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50
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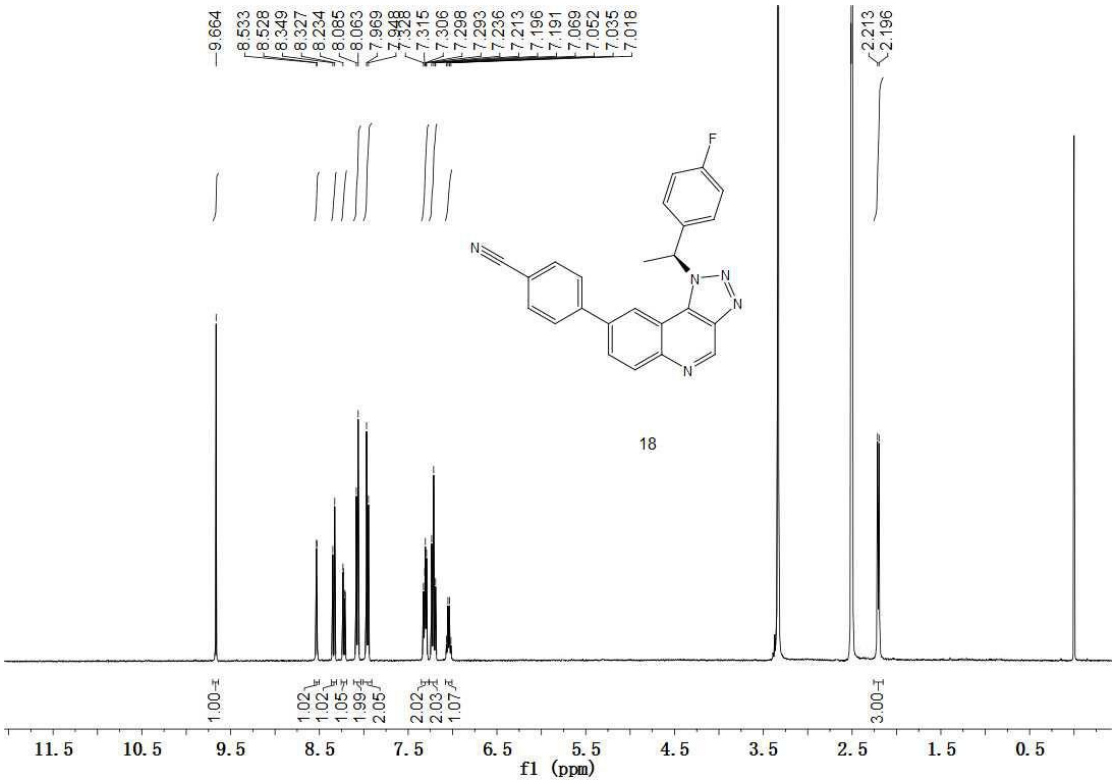
f1 (ppm)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7.191
7.069
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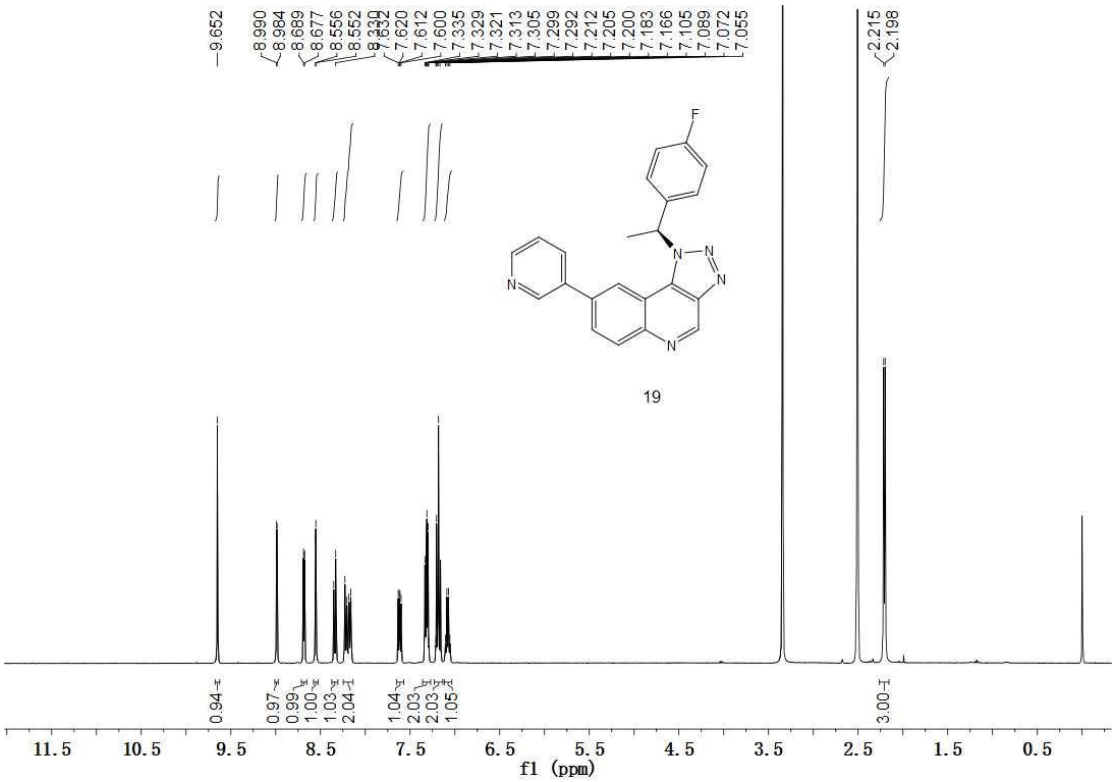
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7.072
7.055
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2.04
1.04
2.03
2.03
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3.00
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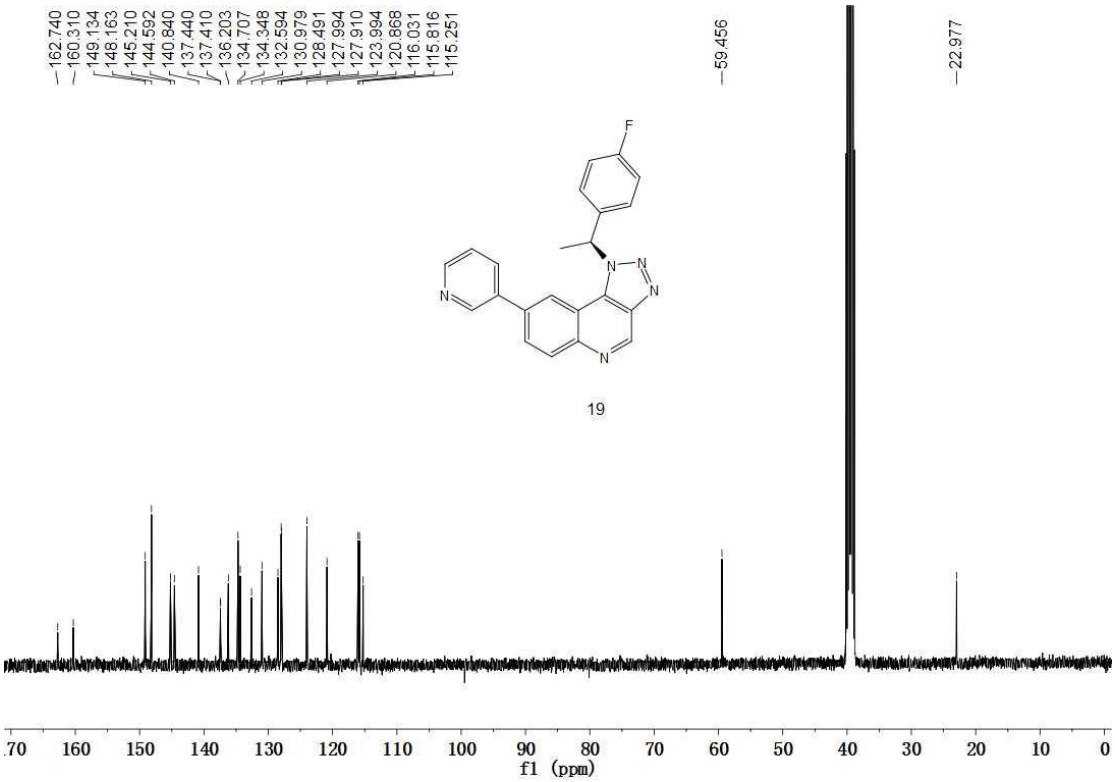
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127.910
123.994
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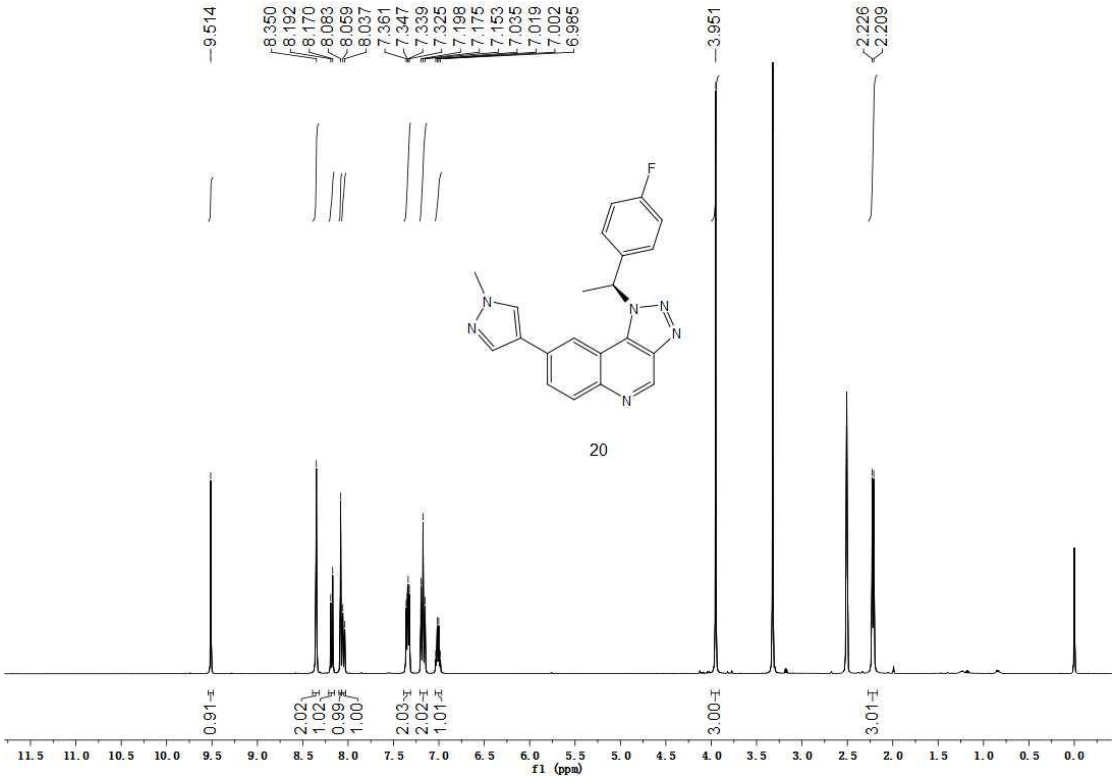
19
f1 (ppm)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3.01
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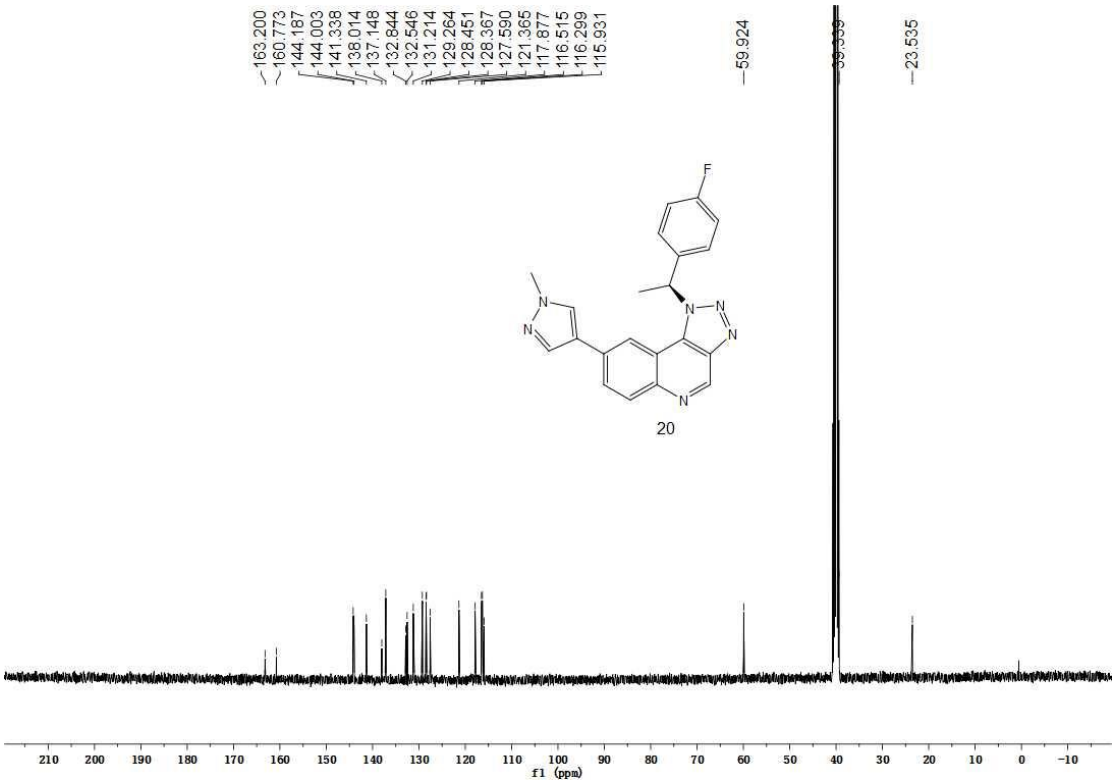
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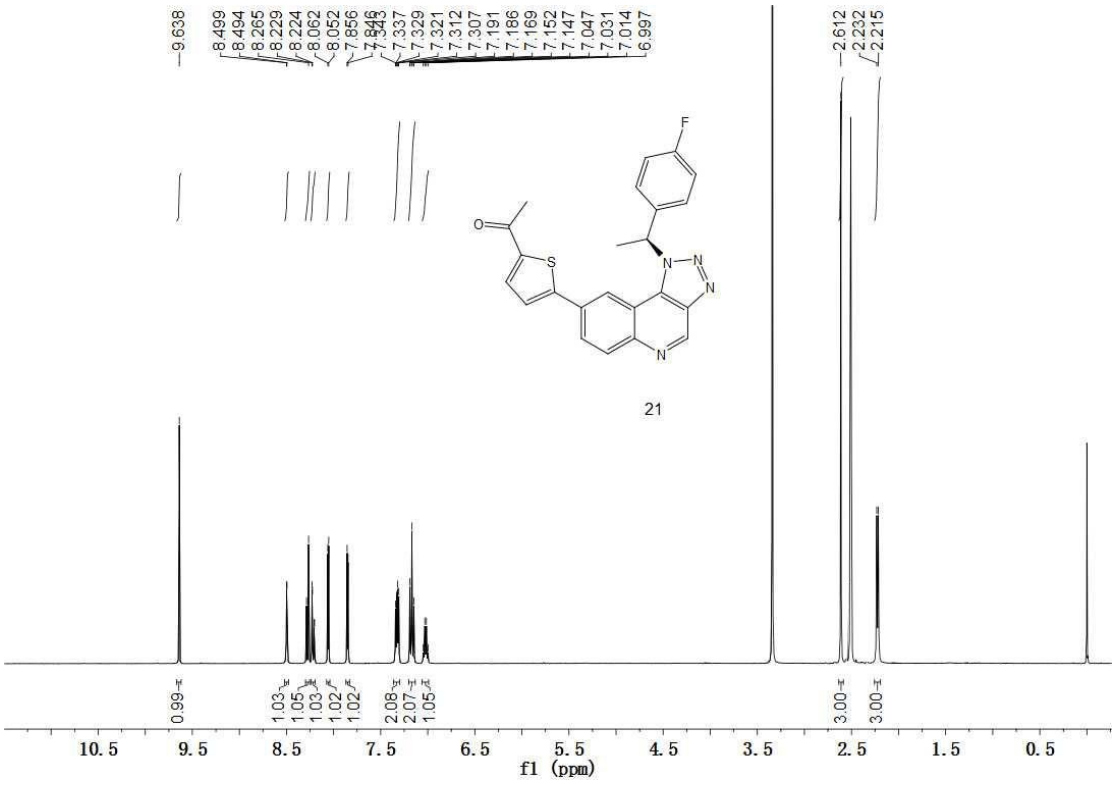
f1 (ppm)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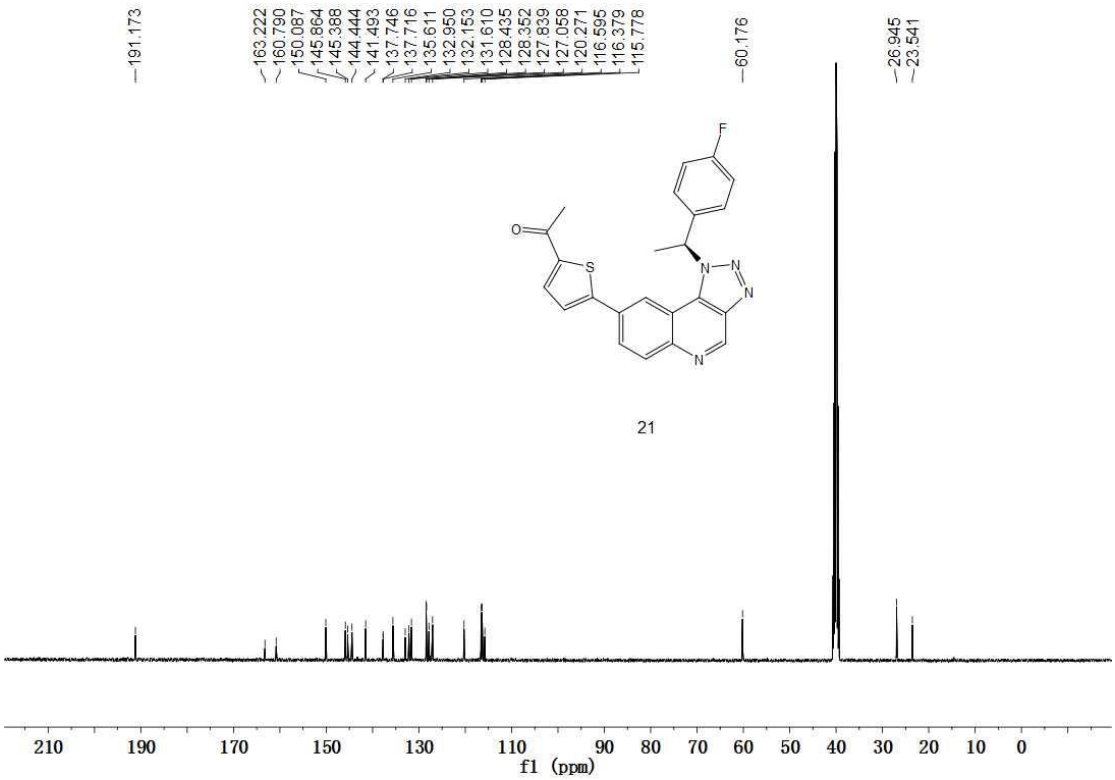
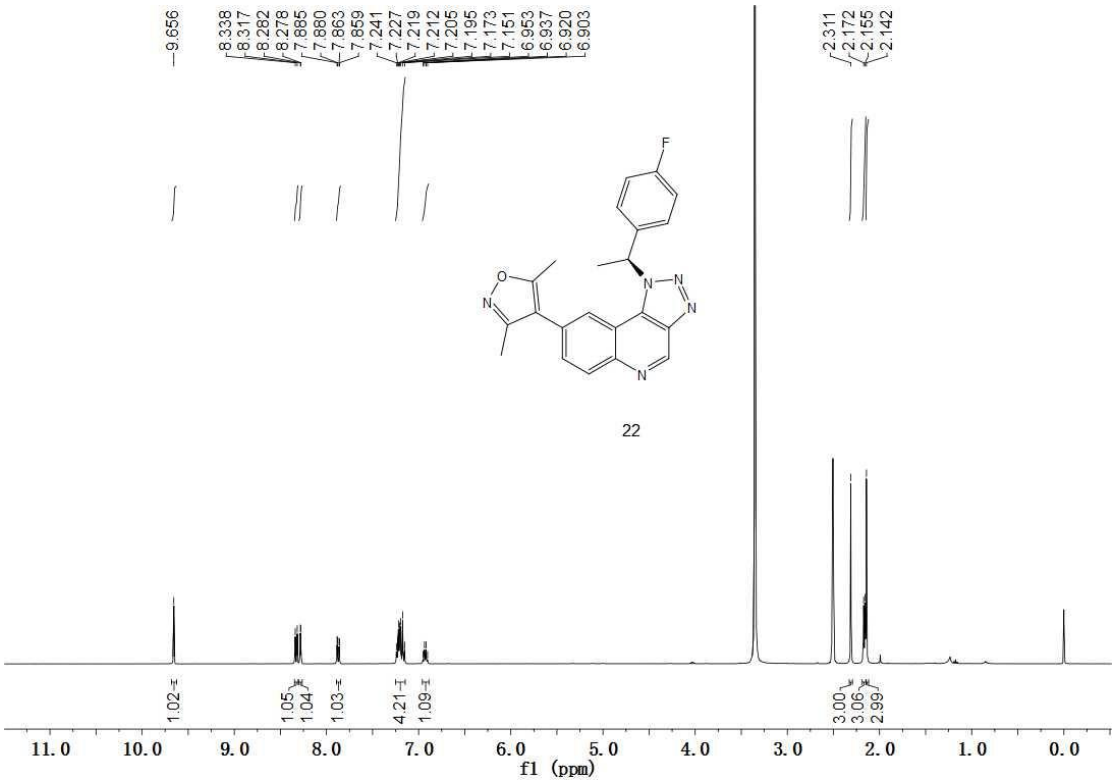
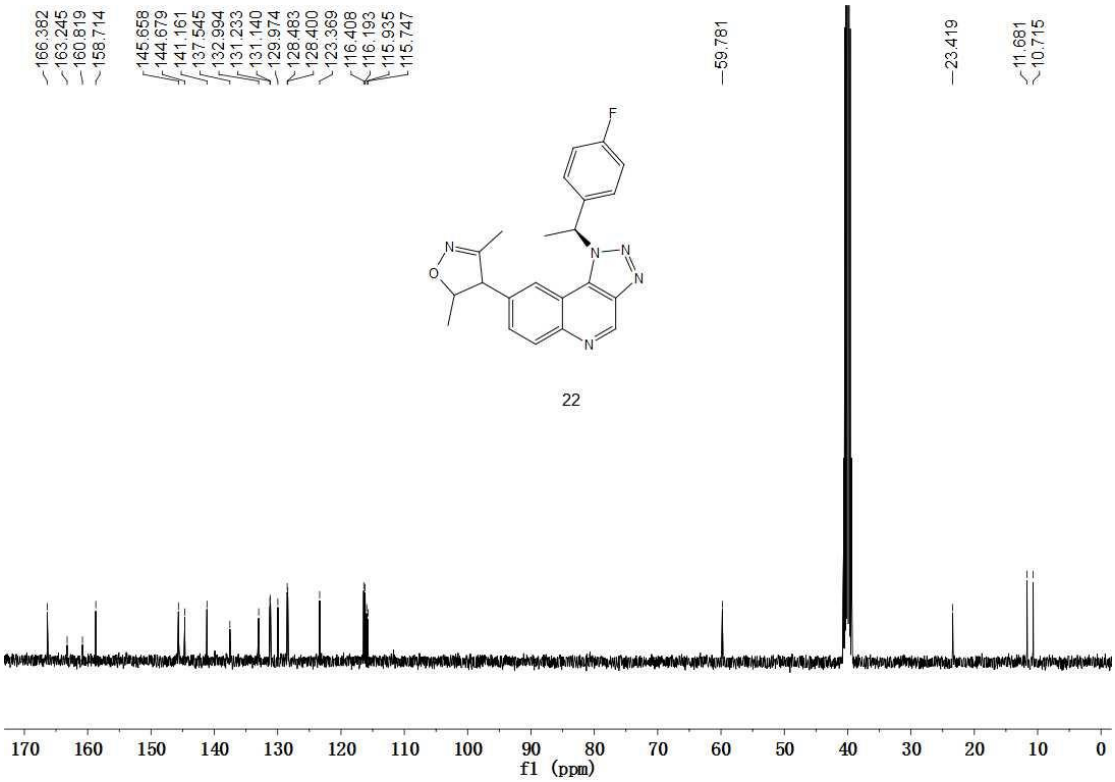
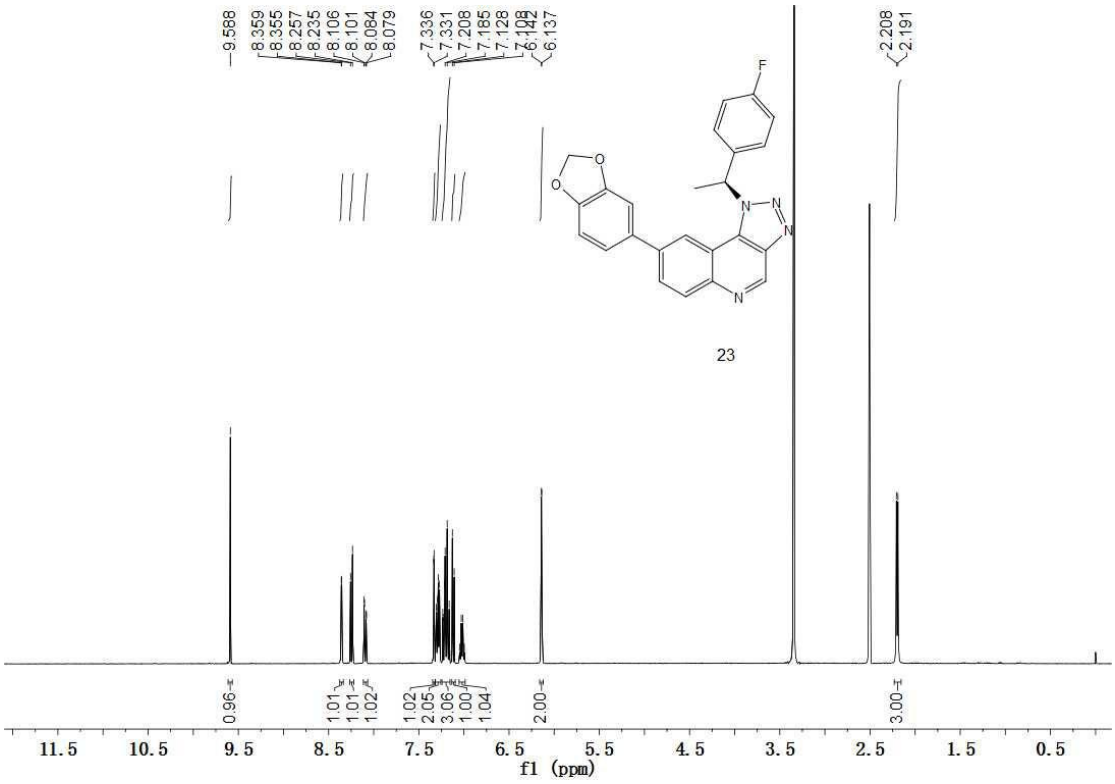
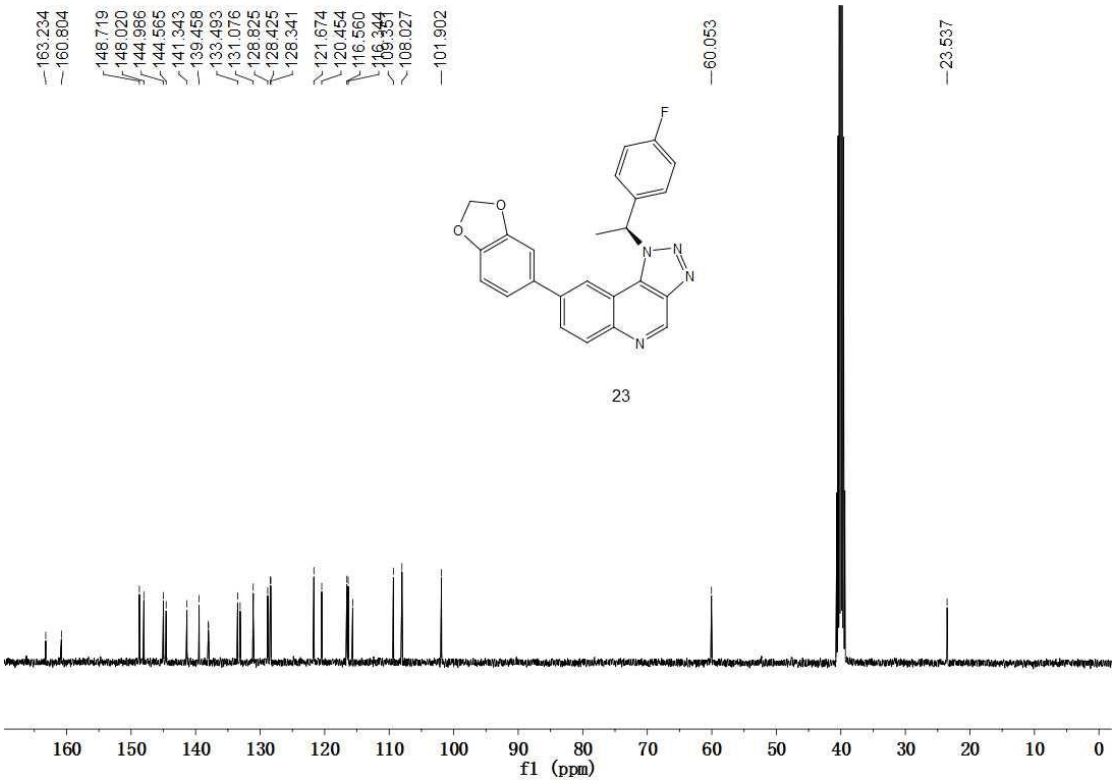
7.047
7.031
7.014
6.997
2.612
2.232
2.215
F
O
S
N
N
N
N
21
0.99
1.03
1.05
1.03
1.02
1.02
2.08
2.07
1.05
3.00
3.00
10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
f1 (ppm)

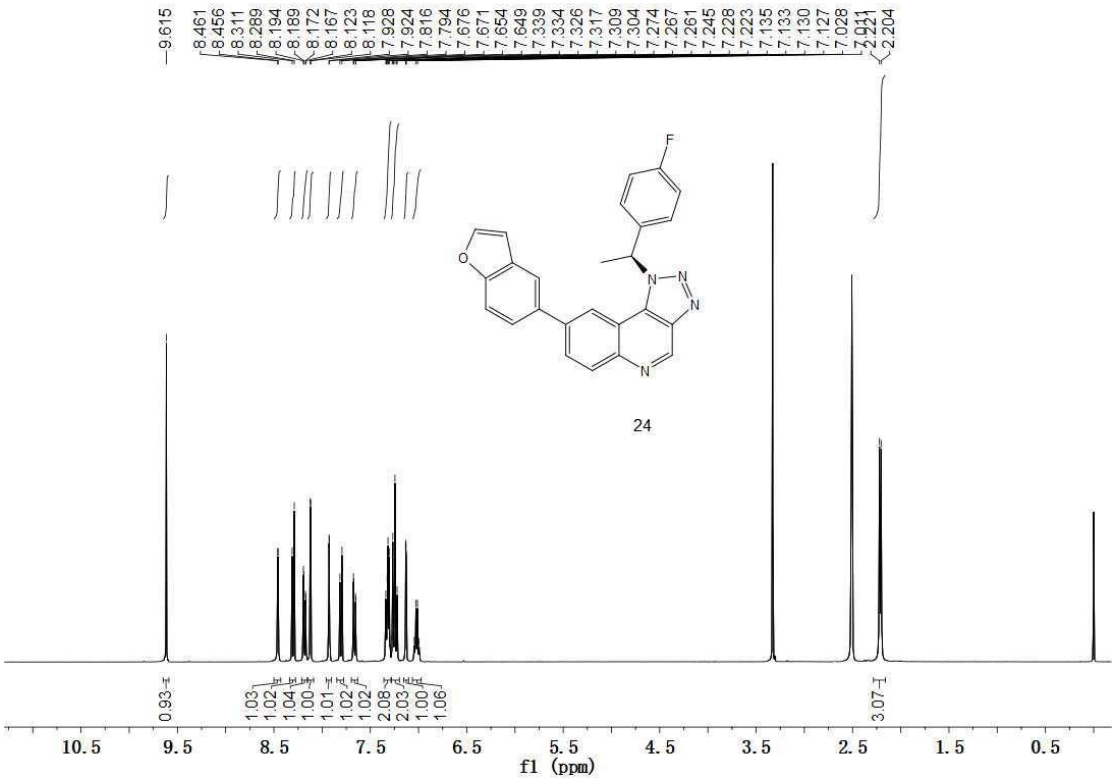

191.173
163.222
160.790
150.087
145.864
145.388
144.444
141.493
137.746
137.716
135.611
132.950
132.153
131.610
128.435
128.352
127.839
127.058
120.271
116.595
116.379
115.778
60.176
26.945
23.541
F
O
S
N
N
N
N
21
210
190
170
150
130
110
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

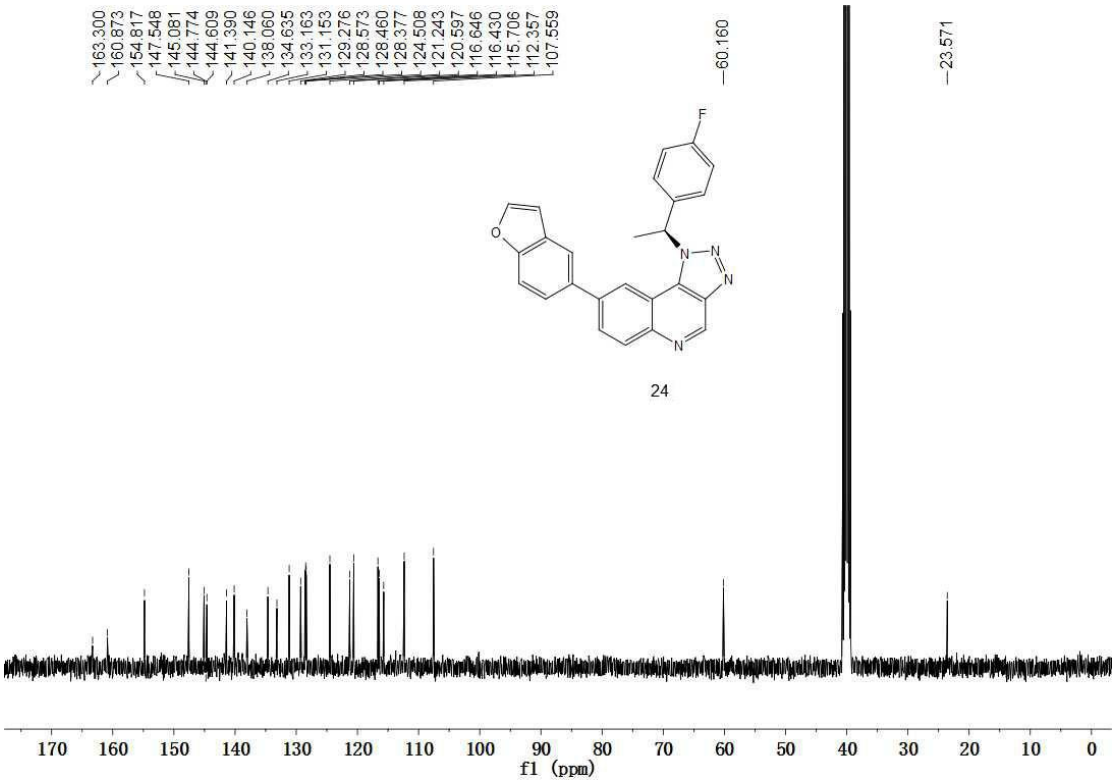

9.656
8.338
8.317
8.282
8.278
7.885
7.880
7.863
7.859
7.241
7.227
7.219
7.212
7.205
7.195
7.173
7.151
6.953
6.937
6.920
6.903
2.311
2.172
2.155
2.142
F
O
N
N
N
N
N
22
1.02
1.05
1.04
1.03
4.21
1.09
3.00
3.06
2.99
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

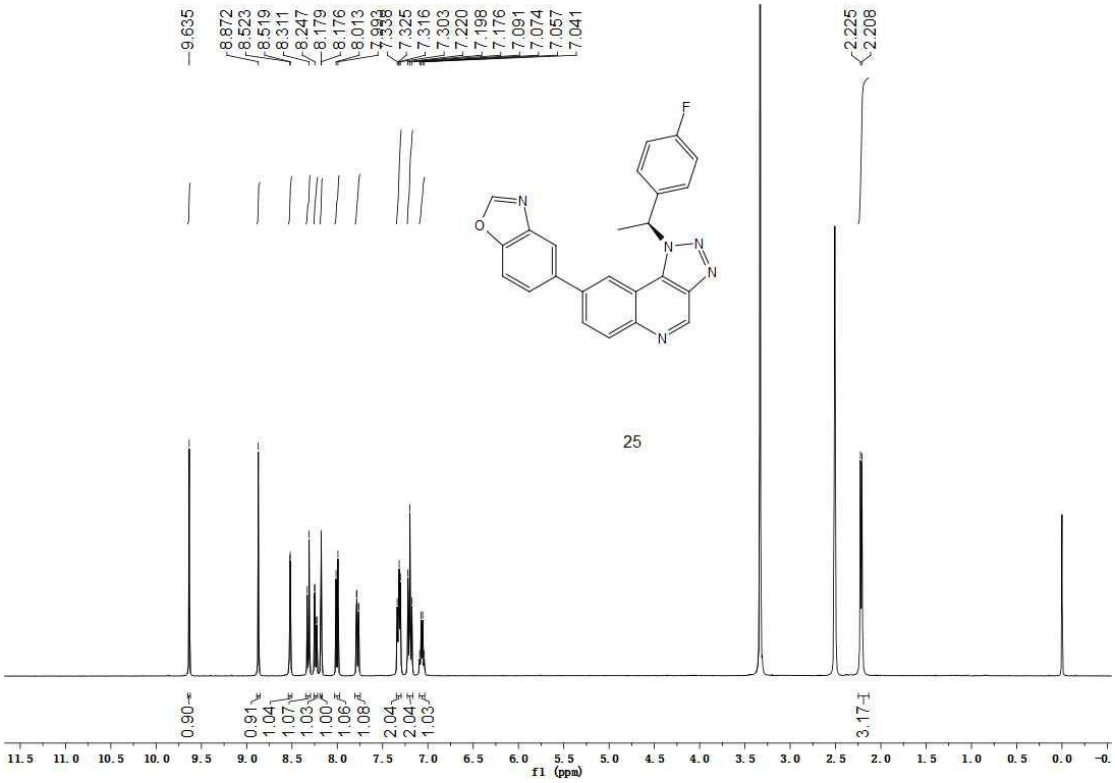

166.382
163.245
160.819
158.714
145.658
144.679
141.161
137.545
132.994
131.233
131.140
129.974
128.483
128.400
123.369
116.408
116.193
115.935
115.747
59.781
23.419
11.681
10.715
F
N
O
N
N
N
N
22
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


9.588
8.359
8.355
8.257
8.235
8.106
8.101
8.084
8.079
7.336
7.331
7.208
7.185
7.128
6.137
2.208
2.191
F
O
O
N
N
N
23
0.96
1.01
1.01
1.02
1.02
2.05
3.06
1.00
1.04
2.00
3.00
11.5
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)


163.234
160.804
148.719
148.020
144.986
144.565
141.343
139.458
133.493
131.076
128.825
128.425
128.341
121.674
120.454
116.560
116.344
109.351
108.027
101.902
60.053
23.537
F
O
O
N
N
N
N
23
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


9.615
8.461
8.456
8.311
8.289
8.194
8.189
8.172
8.167
8.123
8.118
7.928
7.924
7.816
7.794
7.676
7.671
7.654
7.649
7.339
7.334
7.326
7.317
7.309
7.304
7.274
7.267
7.261
7.245
7.228
7.223
7.135
7.133
7.130
7.127
7.028
2.221
2.204
F
O
N
N
N
N
24
0.93
1.03
1.02
1.04
1.00
1.01
1.02
1.02
2.08
2.03
1.00
1.06
3.07
10.5
9.5
8.5
7.5
6.5
5.5
4.5
3.5
2.5
1.5
0.5
f1 (ppm)


163.300
160.873
154.817
147.548
145.081
144.774
144.609
141.390
140.146
138.060
134.635
133.163
131.153
129.276
128.573
128.460
128.377
124.508
121.243
120.597
116.646
116.430
115.706
112.357
107.559
60.160
23.571
F
O
N
N
N
N
24
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


9.635
8.872
8.523
8.519
8.311
8.247
8.179
8.176
8.013
7.993
7.338
7.325
7.316
7.303
7.220
7.198
7.176
7.091
7.074
7.057
7.041
2.225
2.208
F
O
N
N
N
N
25
0.90
0.91
1.04
1.07
1.03
1.00
1.06
1.08
2.04
2.04
1.03
3.17
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

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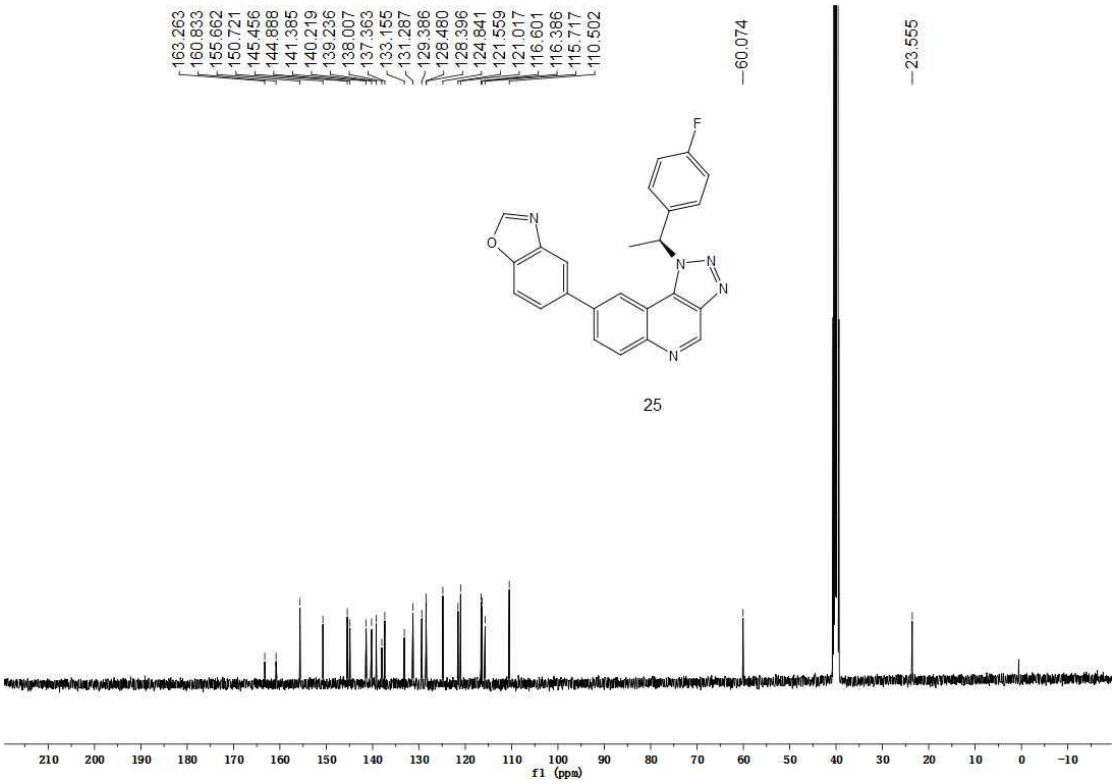
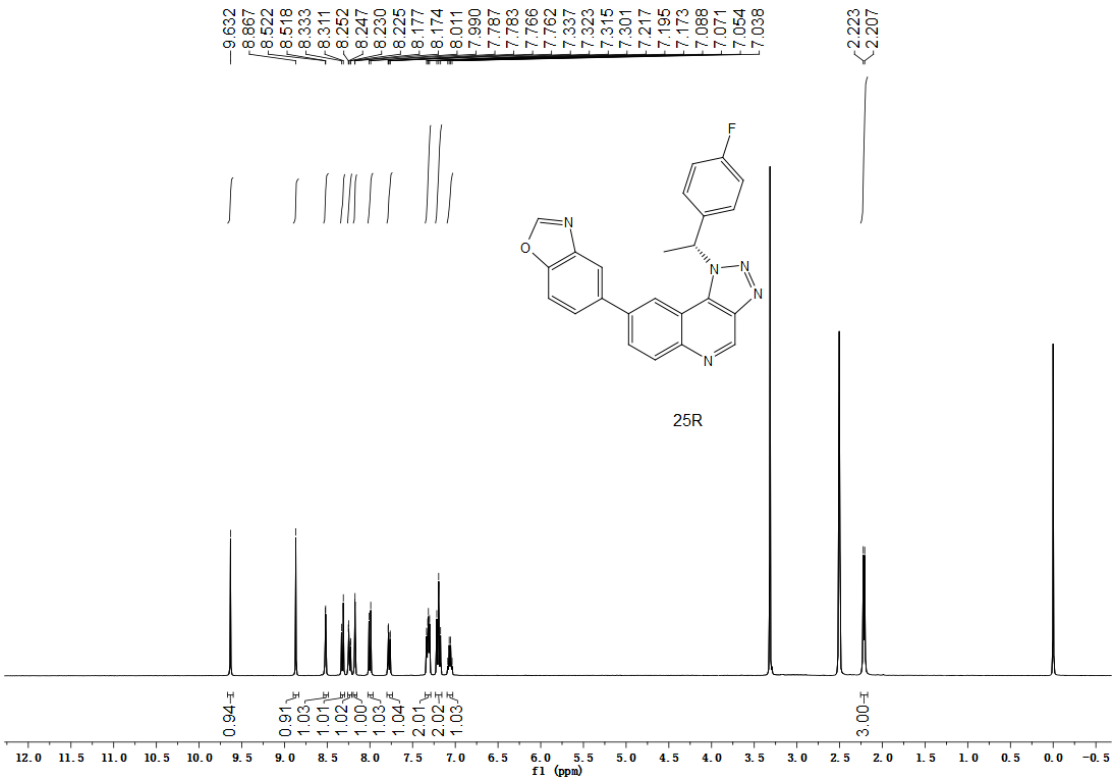
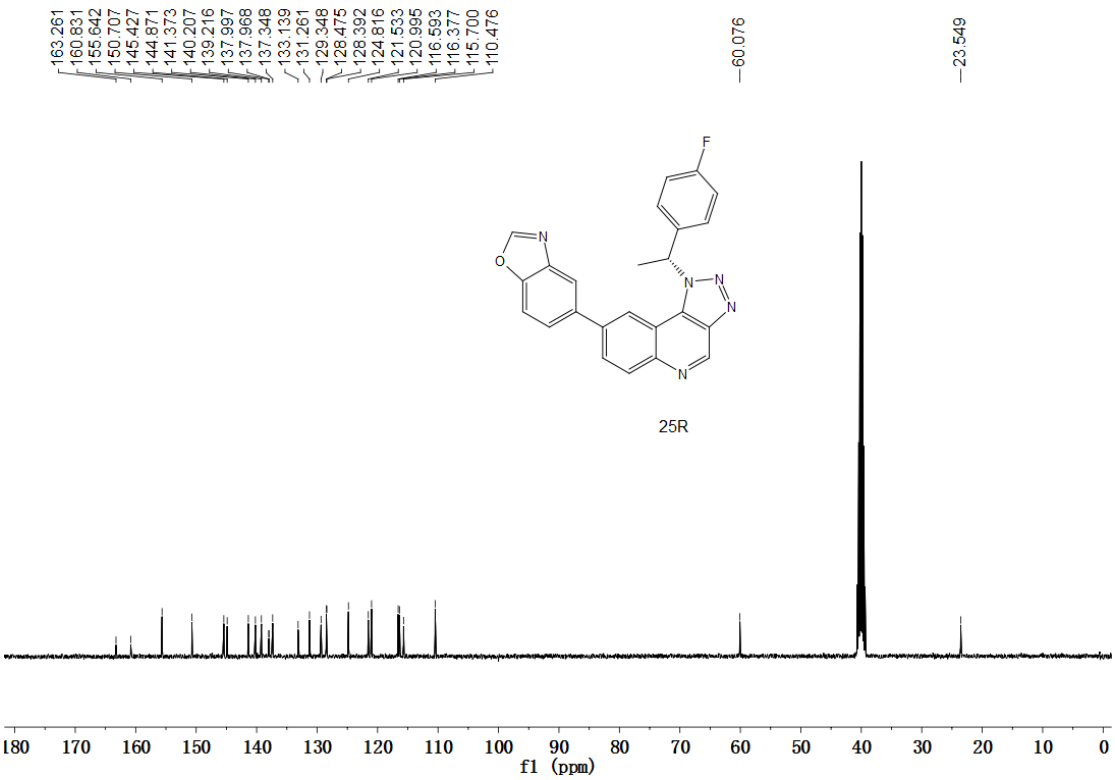
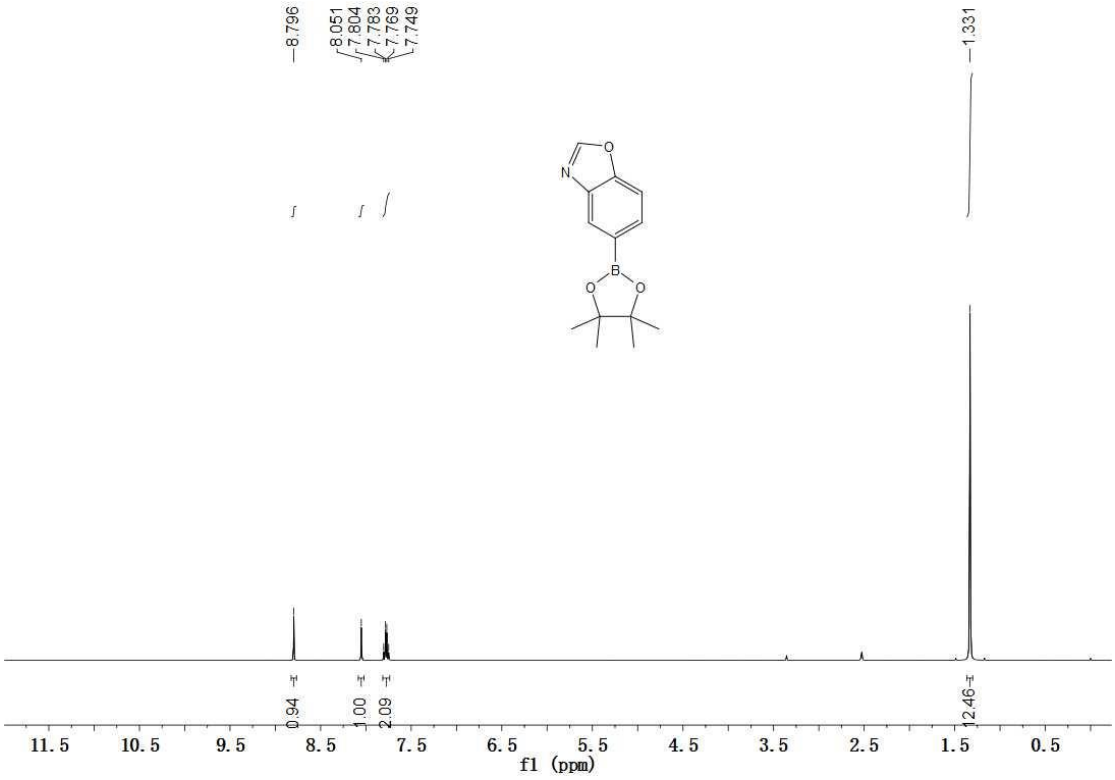
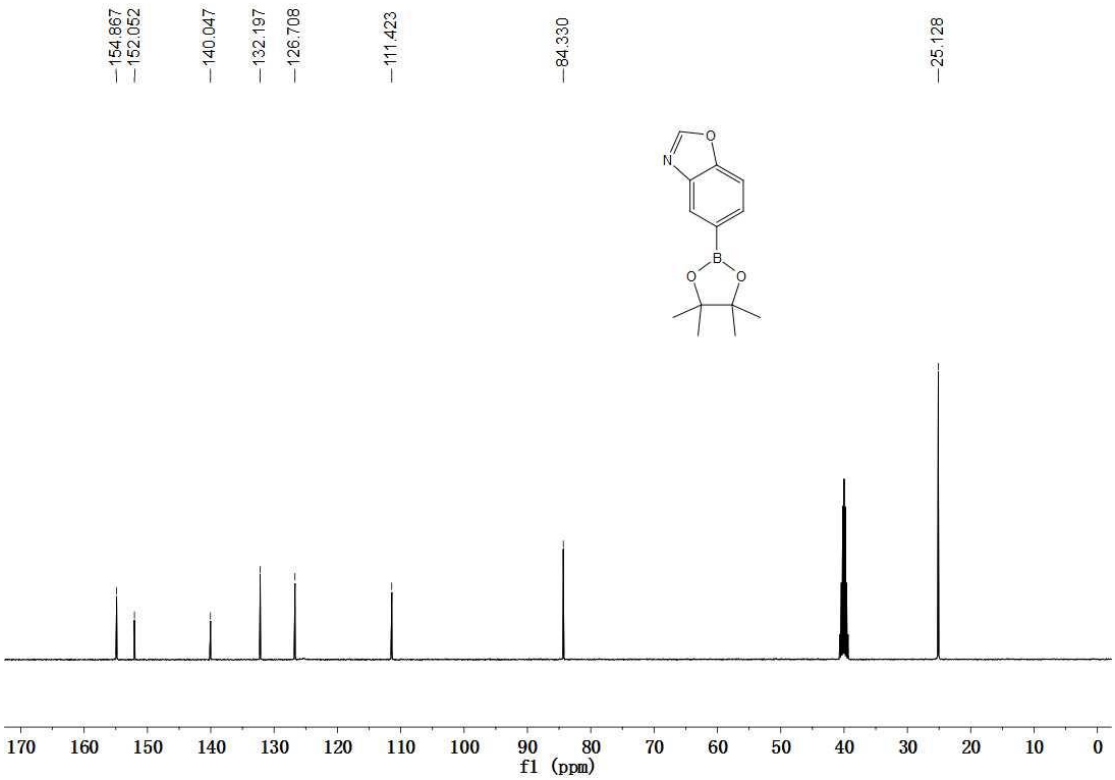

163.263
160.833
155.662
150.721
145.456
144.888
141.385
140.219
139.236
138.007
137.363
133.155
131.287
129.386
128.480
128.396
124.841
121.559
121.017
116.601
116.386
115.717
110.502
60.074
23.555
F
N
O
N
N
N
N
25
f1 (ppm)

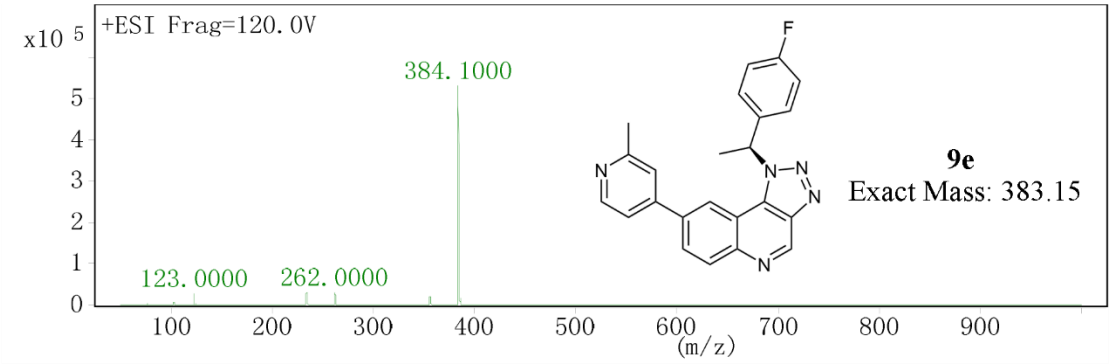

9.632
8.867
8.522
8.518
8.333
8.311
8.252
8.247
8.230
8.225
8.177
8.174
8.011
7.990
7.787
7.783
7.766
7.762
7.337
7.323
7.315
7.301
7.217
7.195
7.173
7.088
7.071
7.054
7.038
2.223
2.207
F
N
O
N
N
N
N
25R
0.94
0.91
1.03
1.01
1.02
1.00
1.03
1.04
2.01
2.02
1.03
3.00
f1 (ppm)

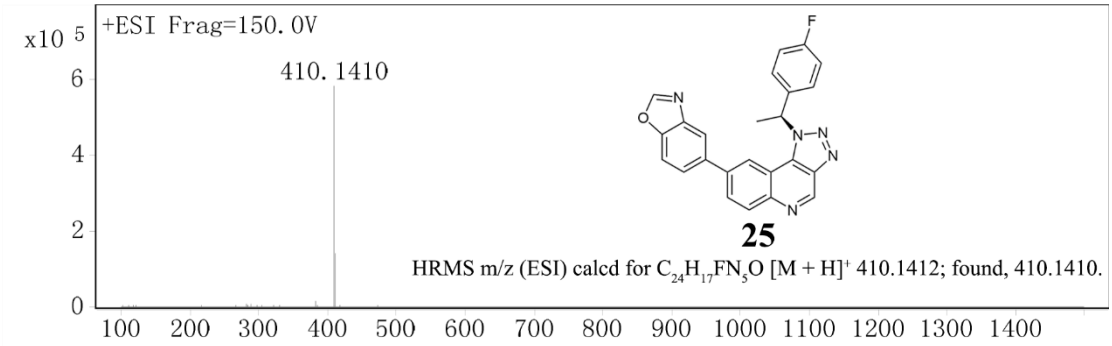

163.261
160.831
155.642
150.707
145.427
144.871
141.373
140.207
139.216
137.997
137.968
137.348
133.139
131.261
129.348
128.475
128.392
124.816
121.533
120.995
116.593
116.377
115.700
110.476
60.076
23.549
F
O
N
N
N
N
N
25R
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

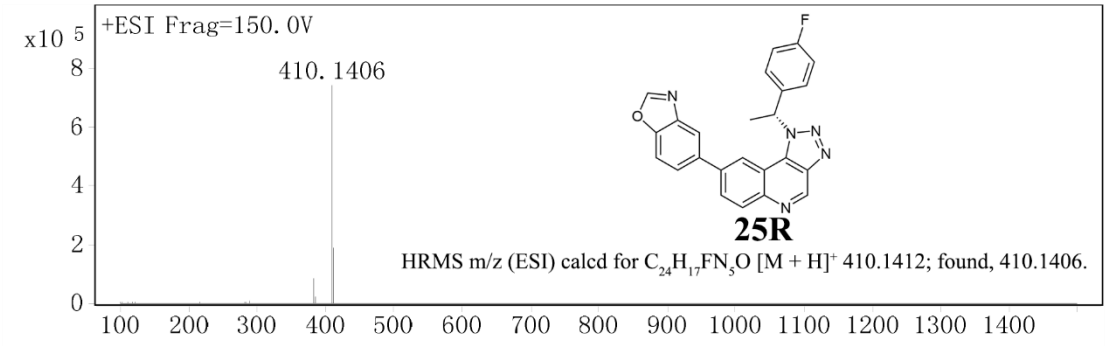

8.796
8.051
7.804
7.783
7.769
7.749
1.331
O
N
B
O
O
0.94
1.00
2.09
12.46
11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5
f1 (ppm)


154.867
152.052
140.047
132.197
126.708
111.423
84.330
25.128
N
O
B
O
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

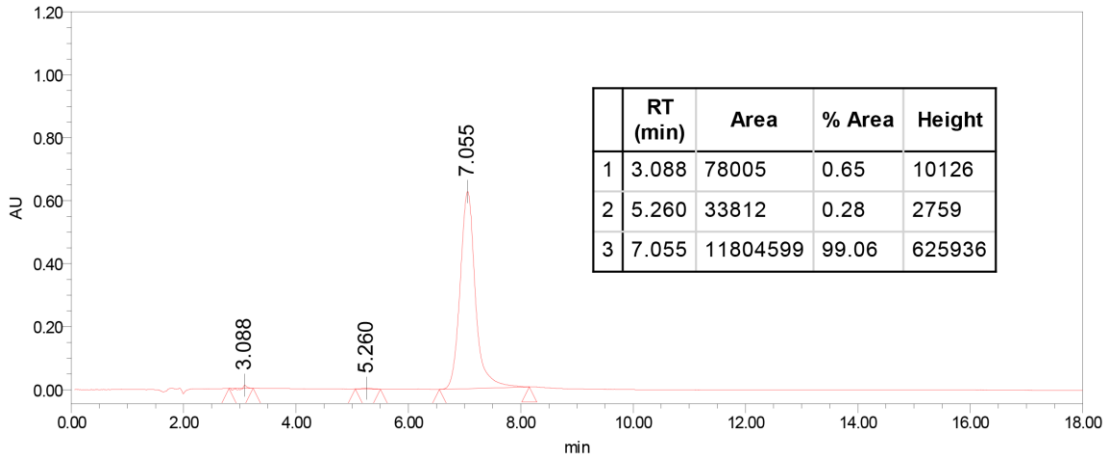
Copies of MS Spectra







HPLC Purity Analysis for Compound 25



	RT (min)	Area	% Area	Height
1	3.088	78005	0.65	10126
2	5.260	33812	0.28	2759
3	7.055	11804599	99.06	625936

Confirmation of Enantiomeric Purity of Compound 25