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Structural basis of TRPV3 inhibition by an antagonist

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Supplementary Information for

Structural basis of TRPV3 inhibition by an antagonist

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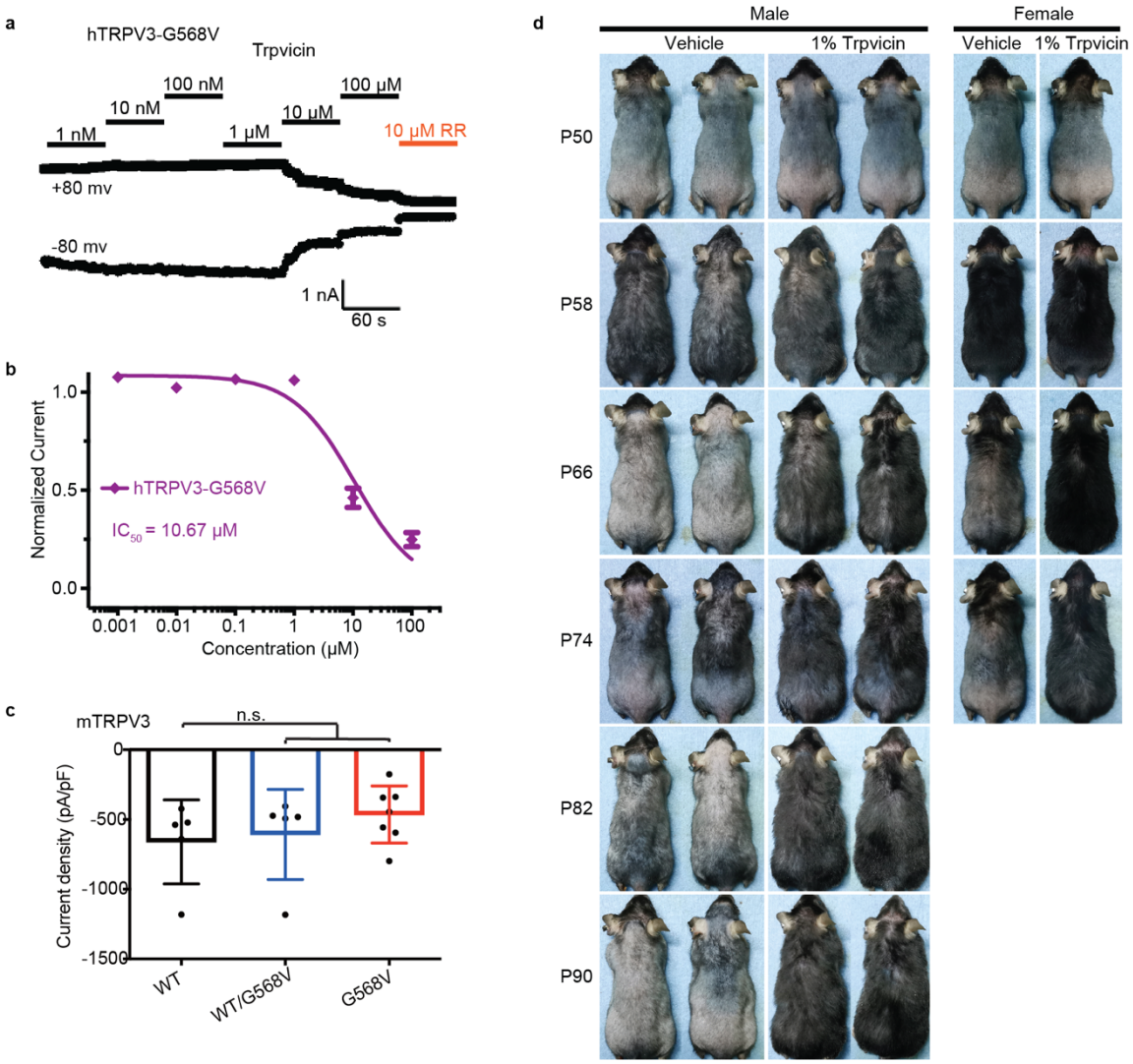
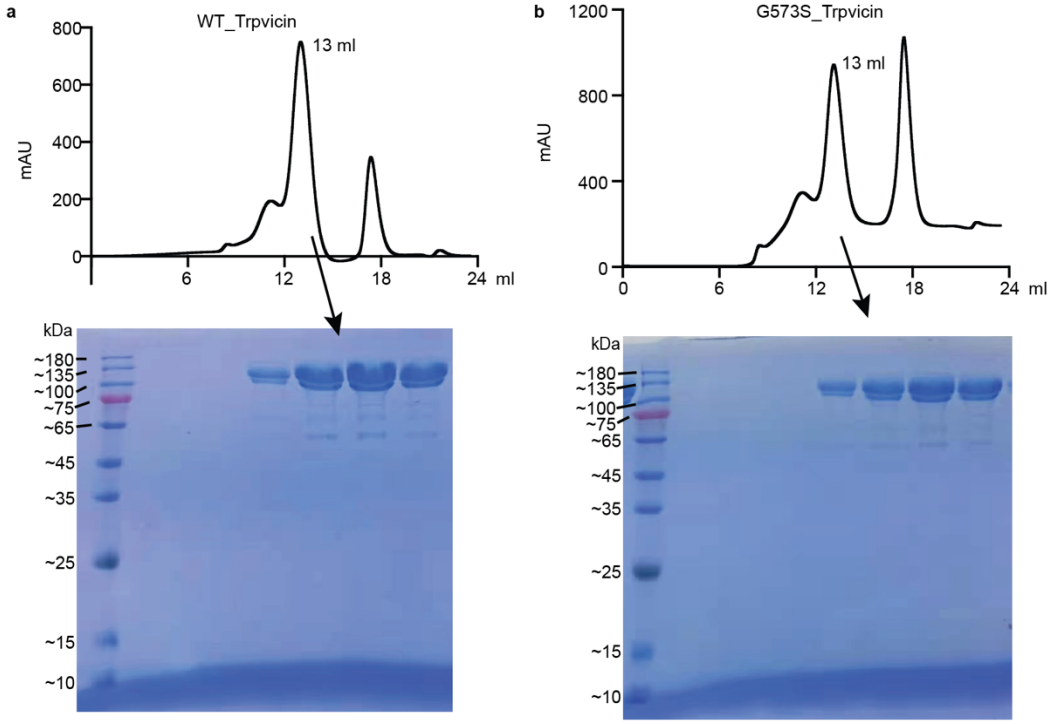
This file contains Supplementary Figure1-3, Table 1-5, and Note 1.

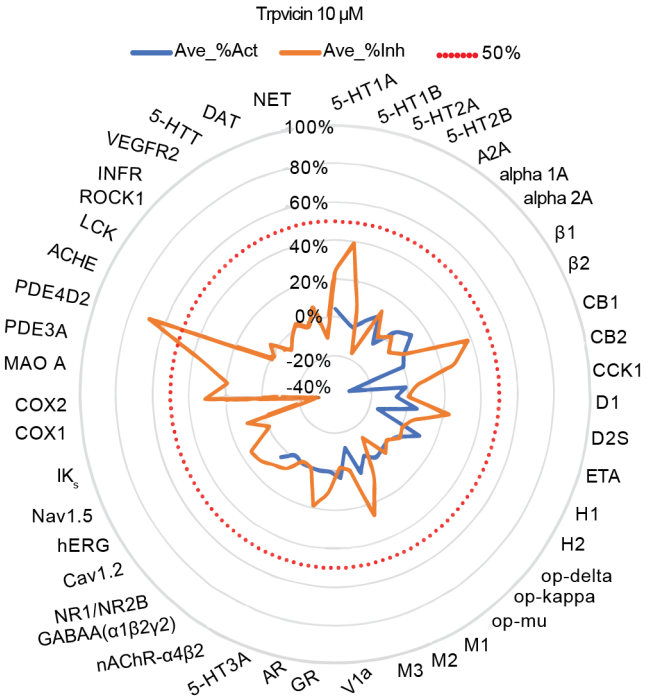


Supplementary Fig. 1 | Trpvicin relieves hair loss of TRPV3 KI mice.
a, The representative current traces of hTRPV3-G568V mutant inhibited by increasing concentrations of Trpvicin (from 1 nM to 100 μM, black bar), at ±80 mV. 10 μM RR was used to assess the whole amplitudes of leak currents. **b**, Curve fitting of dose-dependent inhibition of hTRPV3-G568V leak currents at -80 mV by Trpvicin (hTRPV3-G568V, logIC_{50} = 1.03 ± 0.08, *n* = 6 biologically independent cells). Data are presented as mean values +/- SEM. **c**, The current density at -80 mV of mTRPV3-WT/G568V and mTRPV3-G568V channels activated by 300 μM 2-APB, compared with mTRPV3-WT. One-way ANOVA followed by Bonferroni post-tests (mTRPV3-WT, *n* = 5; mTRPV3-WT/G568V, *n* = 5; mTRPV3-G568V, *n* = 7).Data are presented as mean values +/- SEM; n.s. not significant. **d**, *Trpv3*$^{+/G568V}$ littermate mice were topically treated with 1wt% Trpvicin or vehicle once per day since P50. During the following 40 days, the vehicle-treated mice developed shorter hair shafts (apparently at P58 and P74) and cyclic hair shedding (apparently at P66 and P90). In contrast, the Trpvicin-treated mice showed relatively longer hair shafts and less hair shedding. The improvement in hair loss by Trpvicin treatment occurred in both genders.



Supplementary Fig. 2 | Purification of hTRPV3-WT and hTRPV3-G573S.

a-b, Representative size-exclusion chromatography profiles of purified hTRPV3-WT (**a**) and hTRPV3-G573S (**b**) with Superose 6 Increase 10/30 column. Peaks corresponding to target proteins at 13 ml were labeled and the eluted peak was shown on SDS-PAGE. Representative figures from three independent experiments were shown.



Supplementary Fig. 3 | Summary of the Safety-Screen 47 Panel profiling.

The interference of native ligand binding of a total of 47 targets by 10 μM Trpvicin, the inhibition (or stimulation) was all below 40% except PDE4D2 which was inhibited by nearly 70%.

Supplementary Table. 1 | Results of the Safety-Screen 47 Panel profiling and summary

	Target	Agonist			Antagonist			
		Trpvicin 10 μM	Reference Compound		Trpvicin 10 μM	Reference Compound		
		Ave_%Act	EC_{50} (nM)	Compound ID	Ave_% Inh	IC_{50}(nM) or % Inh	Compound ID	Comment
GPCRs	5-HT1A	4.49%	0.56	Serotonin	24.21%	0.93	WAY-100635	
	5-HT1B	-0.34%	4.36	RU 24969	39.09%	0.42	SB-224289	
	5-HT2A	-4.12%	9.80	Serotonin	6.06%	0.47	Risperidone	
	5-HT2B	1.35%	19.88	Serotonin	-16.84%	0.23	RS-127445	
	A2A	7.48%	11.44	NECA	10.35%	231.80	ZM241385	
	alpha 1A	-6.36%	4.63	Epinephrine	-2.30%	3.73	Prazosin	
	alpha 2A	6.90%	2.15	Epinephrine	3.24%	16.94	Yohimbine	
	β1	11.79%	0.09	Isoprenaline	-3.35%	10.28	Propranolol	
	β2	2.78%	0.01	Isoprenaline	3.31%	0.47	ICI 118551	
	CB1	0.56%	0.10	CP55940	37.92%	1.23	Rimonabant	
	CB2	-32.12%	0.46	CP55940	27.14%	89.06	SR144528	
	CCK1	-0.99%	0.40	CCK-8	4.55%	653.70	Loxiglumide	
	D1	-6.53%	1.07	Dopamine	0.12%	1.39	SCH23390	

	D2S	5.70%	2.33	Dopamine	23.05%	1.15	Amisulpride	
	ETA	-15.49%	6.64	Endothelin 2	4.23%	5.89	Macitentan	
	H1	11.06%	11.16	Histamine	0.53%	0.98	Clemastine	
	H2	0.54%	0.65	Histamine	2.24%	197.00	Nizatidine	
	op-delta	-2.77%	0.72	SNC 80	-3.35%	30.69	Naloxone	
	op-kappa	-1.88%	1.07	Dynorphin A (1-10)	1.12%	6.42	LY2795050	
	op-mu	-0.09%	8.92	Endomorphin 1	-13.23%	4.77	Naloxone	
	M1	-4.61%	110.70	Acetylcholine	7.56%	1.72	Atropine	
	M2	3.15%	157.30	Acetylcholine	26.11%	2.22	Atropine	
	M3	-11.74%	7.86	Acetylcholine	-0.13%	4.73	Atropine	
	V1a	3.28%	1.36	Argipressin	-2.18%	44.17	Conivaptan	
Nuclear receptors	GR	0.09%	5.88	DHAP	11.34%	6.01	Mifepristone	
	AR	0.30%	2.13	DHT	19.07%	152.70	Enzalutamide	
Ion Channels	5-HT3A	0.00%	2921.00	Serotonin	-0.33%	99.19%	Ondansetron	1μM
	nAChRα4β2	0.29%	369.60	Acetylcholine	-1.13%	1070.00	Adiphenine	
	GABAA (α1β2γ2)	-1.92%	886.3	GABA	7.11%	4095.00	PTX	
	NR1/NR2B	3.79%	132.10	Glycine	9.19%	84.11%	D-AP5	100 μM
	Cav1.2	NA	NA	NA	13.31%	86.25%	Nifedipine	1 μM
	hERG	NA	NA	NA	14.81%	25.55	Cisapride	
	Nav1.5	NA	NA	NA	-0.78%	92.04%	TTX	50 μM
	IKS	NA	NA	NA	10.14%	90.63%	293B	50 μM
Enzymes	COX1	NA	NA	NA	-30.96%	33.90	SC-560	
	COX2	NA	NA	NA	31.01%	55.85	Valdecoxib	
	MAO A	NA	NA	NA	19.11%	1.03	Clorgyline	
	PDE3A	NA	NA	NA	34.48%	20.48	Cilostamide	
	PDE4D2	NA	NA	NA	68.97%	4.62	ML-030	
	ACHE	NA	NA	NA	-2.82%	33.52	Donepezil	
	LCK	NA	NA	NA	3.77%	0.12	TG 100572	
	ROCK1	NA	NA	NA	-6.84%	1.10	AT13148	
	INSR	NA	NA	NA	-2.27%	48.93	NVP-AEW541	
	VEGFR2	NA	NA	NA	2.25%	1.04	Nintedanib	
Transporters	5-HTT	NA	NA	NA	-3.00%	109.50	Centanafadine	
	DAT	NA	NA	NA	6.87%	103.50	Centanafadine	
	NET	NA	NA	NA	-10.55%	28.14	Centanafadine	

Ave_%Act, average_% activation; Ave_%Inh, average_% inhibition.

Supplementary Table. 2 | Trpvicin Pharmacokinetics parameters by LC-MS/MS

	$t_{1/2}$	T_{max}	C_{max}	AUC $_{0-t}$	AUC $_{0-inf}$	Cl	Vz	MRT $_{0-inf}$	F
	h	h	ng/mL	ng/mL*h	ng/mL*h	L/h/kg	L/kg	h	%
IV (2.5mpk)	8.80	-	1523	5970	6083	0.00042	0.00547	9.28	-
SD	3.33	-	236	1047	1002	0.00007	0.00289	3.13	-
PO (10mpk)	10.96	0.139	1553	11633	12146	-	-	13.61	50.15
SD	2.65	0.096	427	1597	1525	-	-	4.94	2.25

$t_{1/2}$, half-life; Tmax, time of maximum plasma concentration; Cmax, maximum plasma concentration; AUC, area under the curve (measure of exposure); Vz, volume of distribution; Cl, plasma clearance; MRT, mean residence time; F, oral bioavailability.

Supplementary Table. 3 | Primer sequences

Target genes	Forward	Reverse
hTRPV3	AAAACGGTCCGATGAAAGCCCACCCCAA GGAGATG	AAAAGCGGCCGCCACCGAGGTTTCCGGG AATTCCTC
hTRPV3-G573S	GGTTTCCAGTCCATGAGCATGTACAGCGT CATG	CATGACGCTGTACATGCTCATGGACTGGA AACC
hTRPV3-G568V	CTCTACTATACGCGGGTTTTCCAGTCCAT GGGC	GCCCATGGACTGGAAAACCCGCGTATAGT AGAG
hTRPV3-A556V	TGCCTCGTGCTGGCCATGGTGCTGGGCT GGGCGAACAT	ATGTTCGCCCAGCCCAGCACCATGGCCA GCACGAGGCA
hTRPV3-A560T	ATGGCCCTGGGCTGGACGAACATGCTCT ACTAT	ATAGTAGAGCATGTTCGTCCAGCCCAGGG CCAT
hTRPV3-F597Y	TTGTTTGTATATATCGTGTATTTGCTTGGA TTTGGAGTA	TACTCCAAATCCAAGCAAATACACGATATA TACAAACAA
hTRPV3-F601A	ATATCGTGTTTTTGCTTGGAGCGGGAGTA GCCTTGGCCT	AGGCCAAGGCTACTCCCGCTCCAAGCAA AAACACGATAT
hTRPV3-T660A	CTGTTCCTGCTCATCGCCTATGTCATCCT CACC	GGTGAGGATGACATAGGCGATGAGCAGG AACAG
hTRPV3-T665A	ATCACCTATGTCATCCTCGCCTTTGTTCTC CTCCTCAAC	GTTGAGGAGGAGAACAAAGGCGAGGATG ACATAGGTGAT
hTRPV3-F666Y	ACCTATGTCATCCTCACCTATGTTCTCCTC CTCAACATG	CATGTTGAGGAGGAGAACATAGGTGAGG ATGACATAGGT
hTRPV3-F666A	ACCTATGTCATCCTCACCGCTGTTCTCCT CCTCAACATG	CATGTTGAGGAGGAGAACAGCGGTGAGG ATGACATAGGT
mTRPV3	GCGGAATTCAAAGGCCTACGTCGACATGA ATGCCCACTCCAAGGAGATG	GAACAGAACTTCCAGTGCGGCCGCCACC GACGTTTCTGGGAATTCATC
mTRPV3-G568V	ATGCTCTACTACACGAGAGTATTCCAGTC TATGGGCATG	CATGCCCATAGACTGGAATACTCTCGTGT AGTAGAGCAT
hTRPV1	AATTCAAAGGCCTACGTCGACATGAAGAA ATGGAGCAGCACAGAC	CAGAACTTCCAGTGCGGCCGCCTTCTCCC CGGAAGCGGCAGGACT
mTRPV2	AATTCAAAGGCCTACGTCGACATGACTTC AGCCTCCAACCCCCCA	CAGAACTTCCAGTGCGGCCGCGTGGGAC TGGAGGACCTGAAGAGG
hTRPV4	AATTCAAAGGCCTACGTCGACATGGCGGA TTCCAGCGAAGGCCCC	CAGAACTTCCAGTGCGGCCGCGAGCGGG GCGTCATCAGTCCTCCA
hTRPV5	AATTCAAAGGCCTACGTCGACATGGGGG GTTTTCTACCTAAGGCA	CAGAACTTCCAGTGCGGCCGCAAAATGGT AGACCTCCTCTCCATC
hTRPV6	AATTCAAAGGCCTACGTCGACACGGGACC TCTACAGGGAGACGGT	CAGAACTTCCAGTGCGGCCGCGAGAGCT GGGAATATCAGATC
hTRPA1	CCGCTCGAGCGGATGAAGCGCAGCCTGA GGA	TCCCCGCGGGGACTAAGGCTCAAGATGG TGTGTTTTTG
hTRPM8	CCGCTCGAGCGGATGTCCTTTCGGGCAG CCA	TCCCCGCGGGGATTATTTGATTTTATTAG CAATCTCTTTCAGAAGACCC

66 **Supplementary Table .4 | Small molecule screening data**

Category	Parameter	Description
Assay	Type of assay	In vitro, cell-based assay
	Target	TRPV3 G573S mutant
	Primary measurement	Detection of cell viability
	Key reagents	CellTiter-Glo® Luminescent Cell Viability Assay (Promega)
	Assay protocol	https://www.promega.com.cn/-/media/files/resources/protocols/technical-bulletins/0/celltiter-glo-luminescent-cell-viability-assay-protocol.pdf
	Additional comments	
Library	Library size	~110,000
	Library composition	Natural products, old drugs, molecular fragments
	Source	BioBioPha, MedChemExprss, Chembridge
	Additional comments	
Screen	Format	Corning® 384-well Solid White Polystyrene Microplates with tissue culture treated
	Concentration(s) tested	10 uM compound, 0.1% DMSO
	Plate controls	0.1% DMSO
	Reagent/ compound dispensing system	CellTiter-Glo® Luminescent Cell Viability Assay (Promega)/ Echo 520 Liquid Handler (Labcyte)/ Multidrop™ Combi Reagent Dispenser (Thermo Scientific)
	Detection instrument and software	EnVision® 2105 multimode plate reader driven by EnVision Workstation software
	Assay validation/QC	Standard deviation of controls
	Correction factors	None
	Normalization	None
	Additional comments	
Post-HTS analysis	Hit criteria	Beyond three standard deviations of controls
	Hit rate	1.7%
	Additional assay(s)	Retesting of initial hits in original assay
	Confirmation of hit purity and structure	structure and purity were verified analytically
	Additional comments	

67

Supplementary Table. 5 | Cryo-EM data collection, refinement and validation statistics

	$hTRPV3_{Apo}$ (EMDB-33218) (PDB 7XJ3)	$hTRPV3_{Trpvicin}$ (EMDB-33214) (PDB 7XJ0)	$hTRPV3\text{-}G573S\text{-}C4_{Trpvicin}$ (EMDB-33217) (PDB 7XJ2)	$hTRPV3\text{-}G573S\text{-}C2_{Trpvicin}$ (EMDB-33216) (PDB 7XJ1)
Data collection and processing				
Magnification	81,000	130,000	130,000	
Voltage (kV)	300	300	300	
Electron exposure (e–/Å2)	60	60	60	
Defocus range (μm)	-1.0 ~ -2.0	-1.2 ~ -2.2	-1.2 ~ -2.2	
Pixel size (Å)	1.09	1.04	1.04	
Initial particle images (no.)	1,451,587	1,491,046	691,221	
Final particle images (no.)	347,665	503,757	48,492	152,978
Symmetry imposed	C4	C4	C4	C2
Map resolution (Å)	3.54	2.53	3.64	2.93
FSC threshold	0.143	0.143	0.143	0.143
Map resolution range (Å)	3.0-5.0	2.2-4.2	3.5-5.5	2.7-4.7
Refinement				
Initial model used (PDB code)	6MHO	This study	This study	This study
Model resolution (Å)	3.58	2.68	3.77	3.05
FSC threshold	0.5	0.5	0.5	0.5
Map sharpening B factor (Å2)	-163	-110	-144	-89
Model composition				
Non-hydrogen atoms	18,493	18,791	18,438	18,910
Protein residues	2,428	2,428	2,428	2,444
Ligands	14	24	4	20
B factors (Å2)				
Protein	64.1	60.5	80.9	54.3
Ligand	70.5	28.4	68.4	41.7
R.m.s. deviations				
Bond lengths (Å)	0.008	0.015	0.004	0.004
Bond angles (°)	0.772	1.192	0.589	0.711
Validation				
MolProbity score	2.86	2.93	2.41	2.70
Clashscore	12	18	16	12
Poor rotamers (%)	2.5	5.1	1.4	2.6
Ramachandran plot				
Favored (%)	90.7	92.3	92.8	92.4
Allowed (%)	9.3	7.7	7.2	7.6
Disallowed (%)	0	0	0	0

Supplementary Note 1

General information:

^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer at ambient temperature with $CDCl_3$ as the solvent unless otherwise stated. ^{13}C NMR spectra were recorded on a Varian 101 MHz spectrometer (with complete proton decoupling) at ambient temperature. Chemical shifts are reported in parts per million relatives to chloroform (^{1}H, δ 7.26; ^{13}C, δ 77.00). Data for ^{1}H NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet) and coupling constants. High-resolution mass spectra were obtained at Peking University Mass Spectrometry Laboratory using a Bruker APEX Flash chromatography. The samples were analyzed by HPLC/MS on a Waters Auto Purification LC/MS system (3100 Mass Detector, 2545 Binary Gradient Module, 2767 Sample Manager, and 2998 Photodiode Array (PDA) Detector). The system was equipped with a Waters C18 5μm SunFire separation column (150*4.6 mm), equilibrated with HPLC grade water (solvent A) and HPLC grade methanol (solvent B) with a flow rate of 1.0 mL/min at room temperature. Analytical thin layer chromatography was performed using 0.25 mm silica gel 60-F plates. Flash chromatography was performed using 200-400 mesh silica gel. Yields refer to chromatographically and spectroscopically pure materials, unless otherwise stated. All reagents were used as supplied by Sigma-Aldrich, J&K and Alfa Aesar Chemicals. Methylene chloride, toluene, were distilled from calcium hydride; dimethylformamide was distilled from sodium carbonate; ethanol was distilled from magnesium ethoxide. All reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise noted. The figures are prepared by ChemDraw.

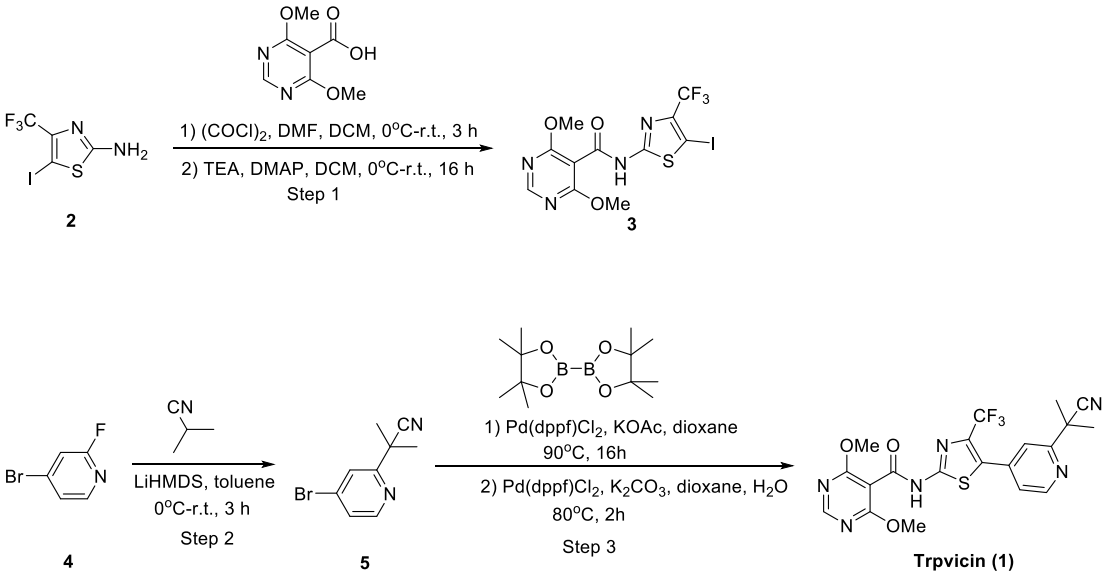
Abbreviations used

DCM	Dichloromethane
TEA	Triethylamine
DMAP	4-(Dimethylamino)pyridine
DMF	N,N-Dimethylformamide
PE	Petroleum ether
LCMS	Liquid Chromatography-Mass Spectrometry

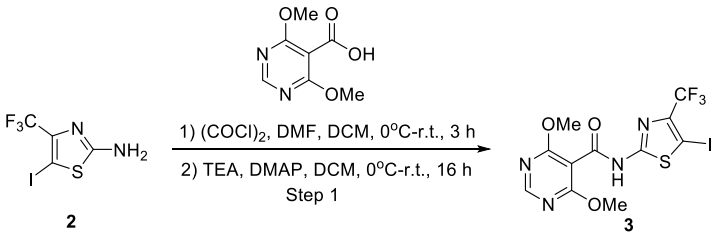
NMR Nuclear Magnetic Resonance

Detailed experimental procedures:

Syntheses of Trpvicin：



Step 1: N-(5-iodo-4-(trifluoromethyl)thiazol-2-yl)-4,6-dimethoxypyrimidine-5-carboxamide (3)



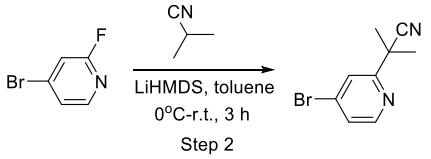
To a stirred solution of 4,6-dimethoxypyrimidine-5-carboxylic acid (2.06 g, 11.22 mmol, 1.10 equiv.) in DCM (20.00 mL) was added $(COCl)_2$ (3.89 g, 30.60 mmol, 3.00 equiv.) and DMF (10 mg) in DCM (0.1 mL) in portions at 0 °C. The resulting mixture was stirred for 3 h at room temperature under argon atmosphere. The resulting mixture was concentrated under reduced pressure and dissolved with DCM (10 mL), which was added to a stirred solution of 5-iodo-4-(trifluoromethyl)thiazol-2-amine **2** (3.00 g, 10.20 mmol, 1.00 equiv.), TEA (4.12 g, 40.80 mmol, 4.00 equiv.) and DMAP (124.00 mg, 1.02 mmol, 0.10 equiv.) in DCM (30.00 mL) in portions at 0 °C. The resulting mixture was stirred for 16 h at room temperature under argon atmosphere. The reaction was diluted with DCM (100 mL), washed with water (2x80 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (10:1-4:1) to afford **3** (3.1 g, 66% yield) as a white solid.

HRMS (ESI^+) *m/e* calcd for $[M + H]^+$ $C_{11}H_8F_3IN_4O_3S$ 459.9314, found 460.9406.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.39 (s, 1H), 8.50 (s, 1H), 4.10 (s, 6H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ 168.9, 162.4, 160.5, 159.2, 142.2, 141.8, 122.1, 119.4, 97.4, 66.6, 55.8.

Step 2: 2-(4-bromopyridin-2-yl)-2-methylpropanenitrile (5)



4 **5**

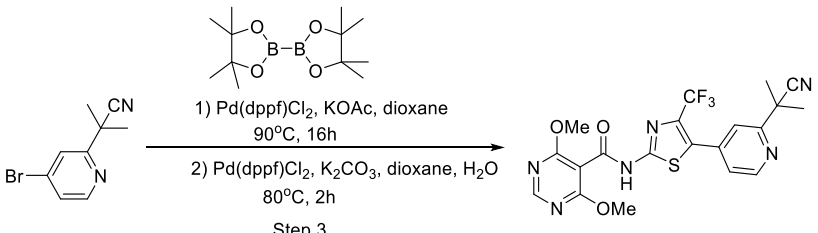
To a stirred solution of 4-bromo-2-fluoropyridine **4** (4.00 g, 22.73 mmol, 1.00 equiv.) and isobutyronitrile (1.65 g, 23.87 mmol, 1.05 equiv.) in toluene (40.00 mL) was added LiHMDS (34.1 mL, 34.10 mmol, 1.50 equiv., 1 M in *n*-hexane) dropwise at 0 °C. The resulting mixture was stirred for 3 h at 0 °C under argon atmosphere. The reaction was monitored by LCMS. Desired product could be detected by LCMS. The reaction was quenched with sat. NH_4Cl(aq.) at 0 °C. The resulting mixture was extracted with EtOAc (3 x 150 mL). The combined organic layers were washed with brine (2x100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (10:1) to afford **5** (3.2 g, 62% yield) as a white solid.

HRMS (ESI$^+$) *m/e* calcd for $[M + H]^+$ $C_9H_9BrN_2$ 223.9949, found 225.0984.

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.41 (dd, *J* = 5.2, 1.3 Hz, 1H), 7.75 (t, *J* = 1.5 Hz, 1H), 7.41 (dt, *J* = 5.2, 1.7 Hz, 1H), 1.74 (s, 6H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ161.1, 150.4, 134.0, 126.3, 123.7, 123.6, 39.5, 27.8.

Step 3: N-(5-(2-(2-cyanopropan-2-yl)pyridin-4-yl)-4-(trifluoromethyl)thiazol-2-yl)-4,6-dimethoxypyrimidine-5-carboxamide (1)



5 Trpvicin **(1)**

Into a 100 mL round-bottom flask were added 2-(4-bromopyridin-2-yl)-2-methylpropanenitrile **5** (1.47 g, 6.53 mmol, 1.00 equiv.), dioxane (20 mL), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane)

(1.82 g, 7.18 mmol, 1.10 equiv.), $Pd(dppf)Cl_2$ (477.00 mg, 0.65 mmol, 0.10 equiv.) and KOAc (1.28 g, 13.06 mmol, 2.00 equiv.) at room temperature. The resulting mixture was stirred for 16 h at 90 °C under argon atmosphere. The reaction was monitored by LCMS, and desired borate ester could be detected by LCMS. The reaction was cooled down to room temperature. To the above mixture was added N-(5-iodo-4-(trifluoromethyl)thiazol-2-yl)-4,6-dimethoxypyrimidine-5-carboxamide (3.00 g, 6.52 mmol, 1.00 equiv.), dioxane (10 mL), H_2O (6 mL), $Pd(dppf)Cl_2$ (477.00 mg, 0.65 mmol, 0.10 equiv.) and K_2CO_3 (1.80 g, 13.06 mmol, 2.00 equiv.) at room temperature. The resulting mixture was stirred for additional 2 h at 80 °C under argon atmosphere. The reaction was monitored by LCMS, and desired coupling product could be detected by LCMS. The reaction was cooled down to room temperature, then diluted with water. The resulting mixture was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine (2x80 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EtOAc (10:1-4:1) to afford **1** (2.7 g, 86% yield) as a yellow oil.

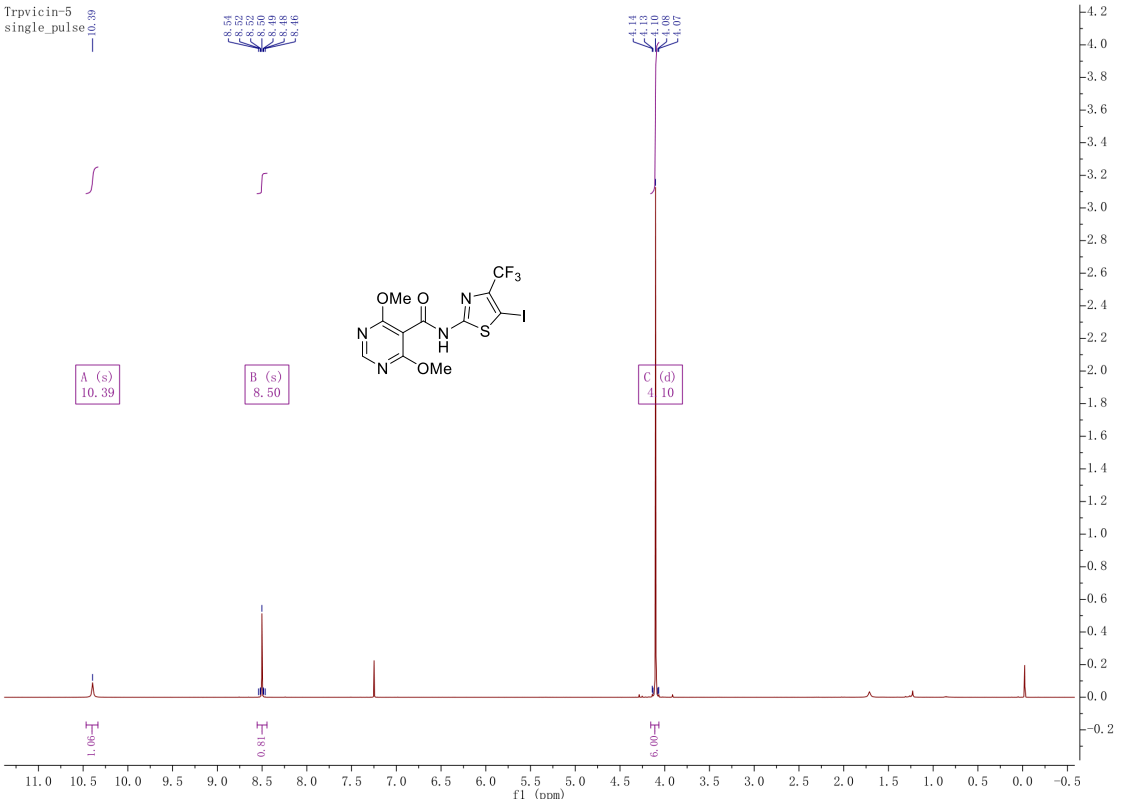
HRMS (ESI^+) *m/e* calcd for $[M + H]^+$ $C_{20}H_{17}F_3N_6O_3S$ 478.1035, found 479.1177.

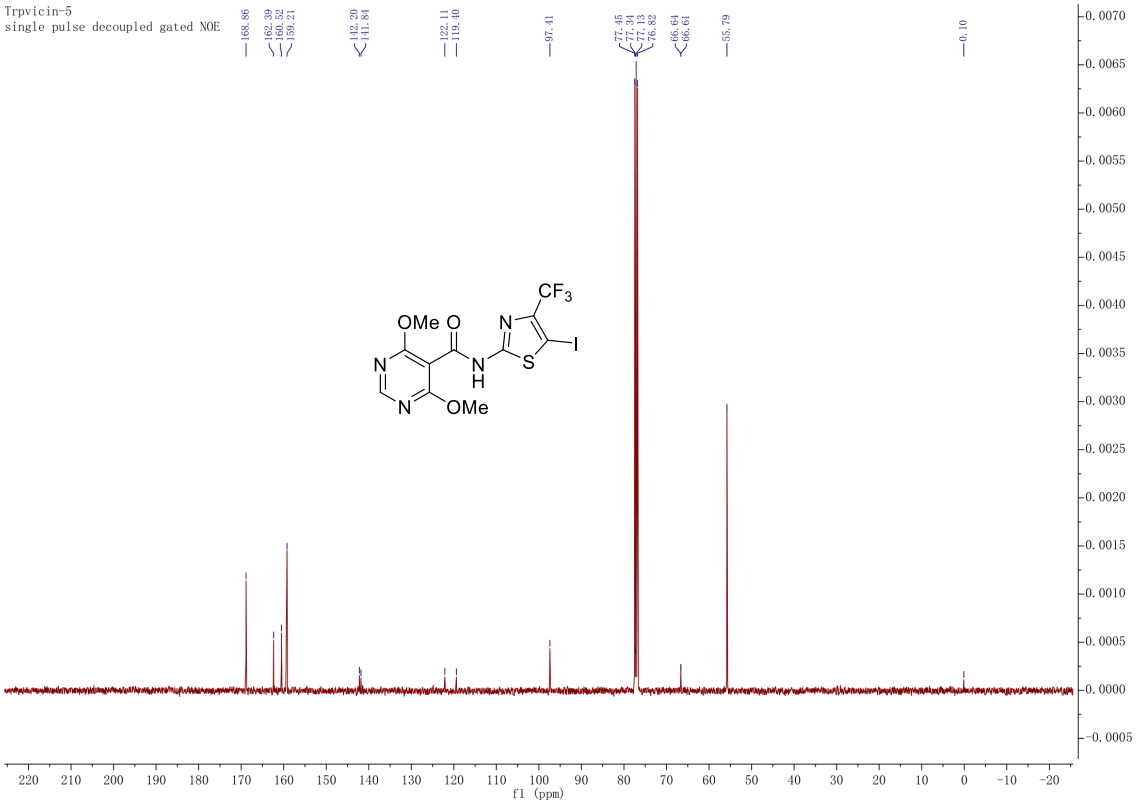
^1H NMR (400 MHz, Chloroform-*d*) δ 10.26 (s, 1H), 8.67 (dd, *J* = 5.0, 0.8 Hz, 1H), 8.53 (s, 1H), 7.69 – 7.56 (m, 1H), 7.33 (ddd, *J* = 5.1, 1.7, 0.6 Hz, 1H), 4.15 (s, 6H), 1.79 (s, 6H).

^{13}C NMR (101 MHz, Chloroform-*d*) δ168.9, 160.4, 159.3, 157.4, 150.0, 138.7, 130.9, 123.8, 123.3, 122.3, 120.3, 97.5, 55.8, 39.7, 29.8, 27.9.

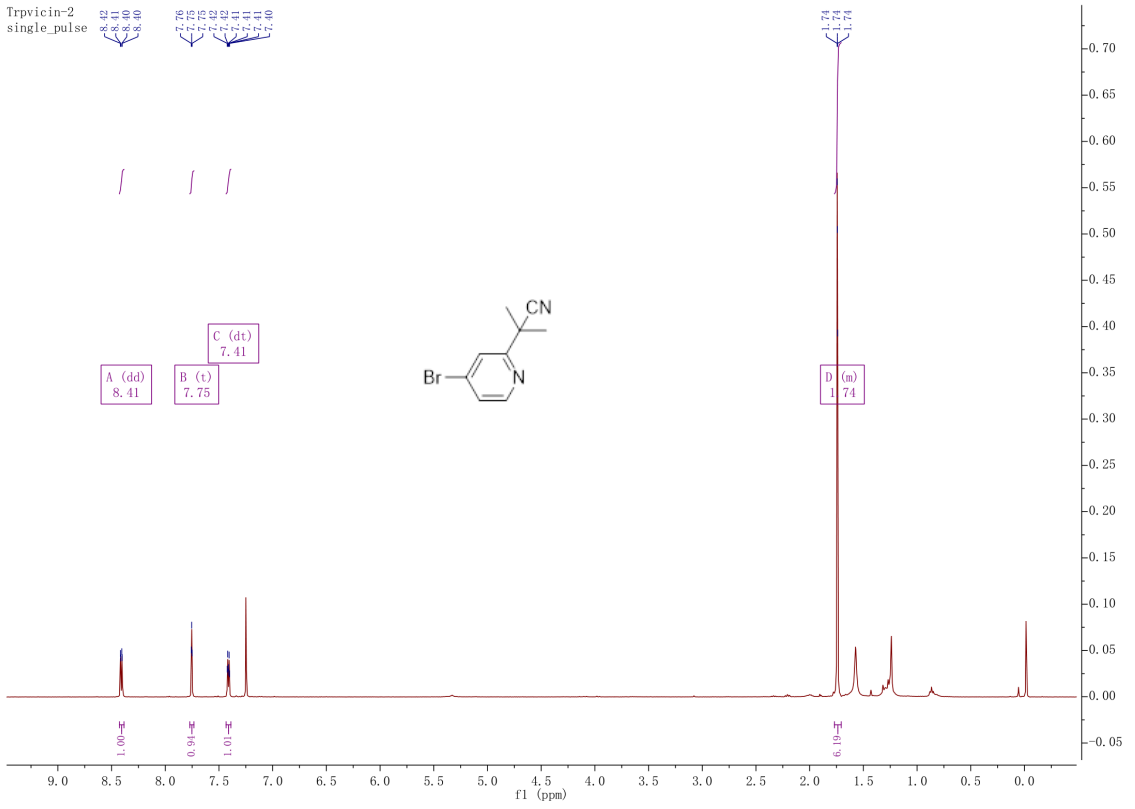
NMR spectra

^{1}H-NMR and ^{13}C-NMR in $CDCl_3$





172 ^{1}H-NMR and ^{13}C-NMR in $CDCl_3$



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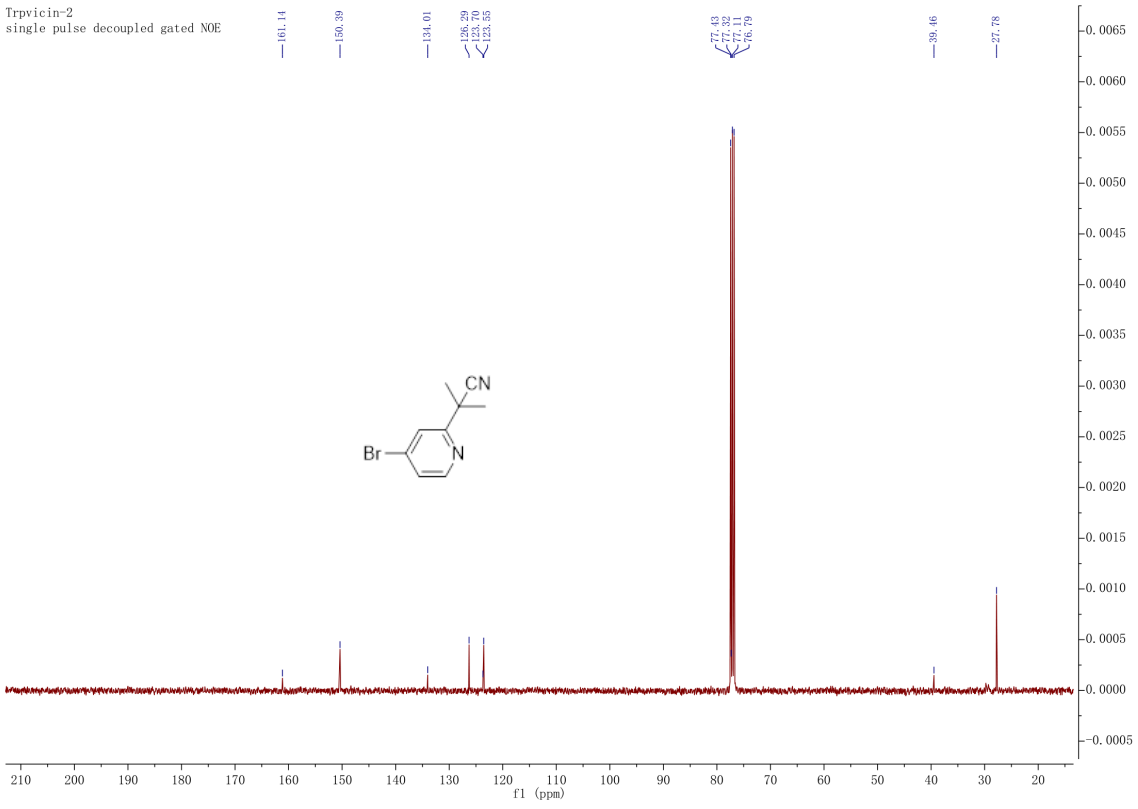

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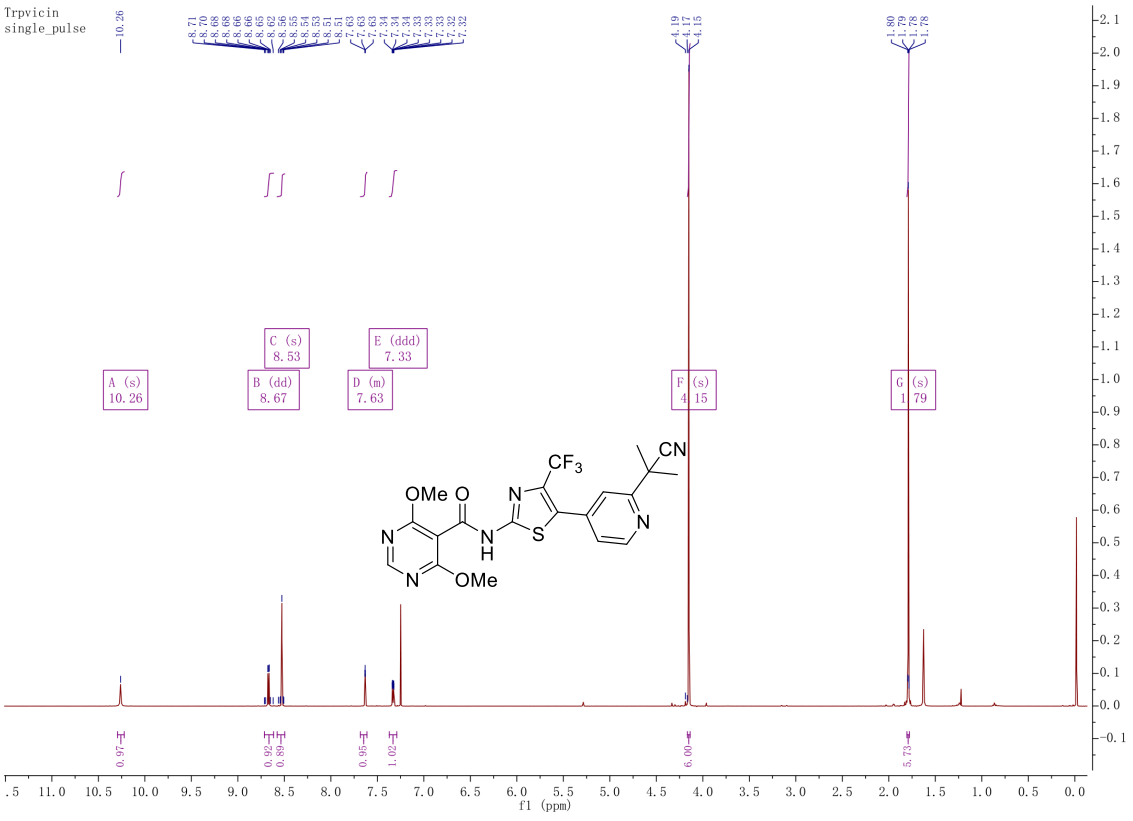
177 ^{1}H-NMR and ^{13}C-NMR in $CDCl_3$



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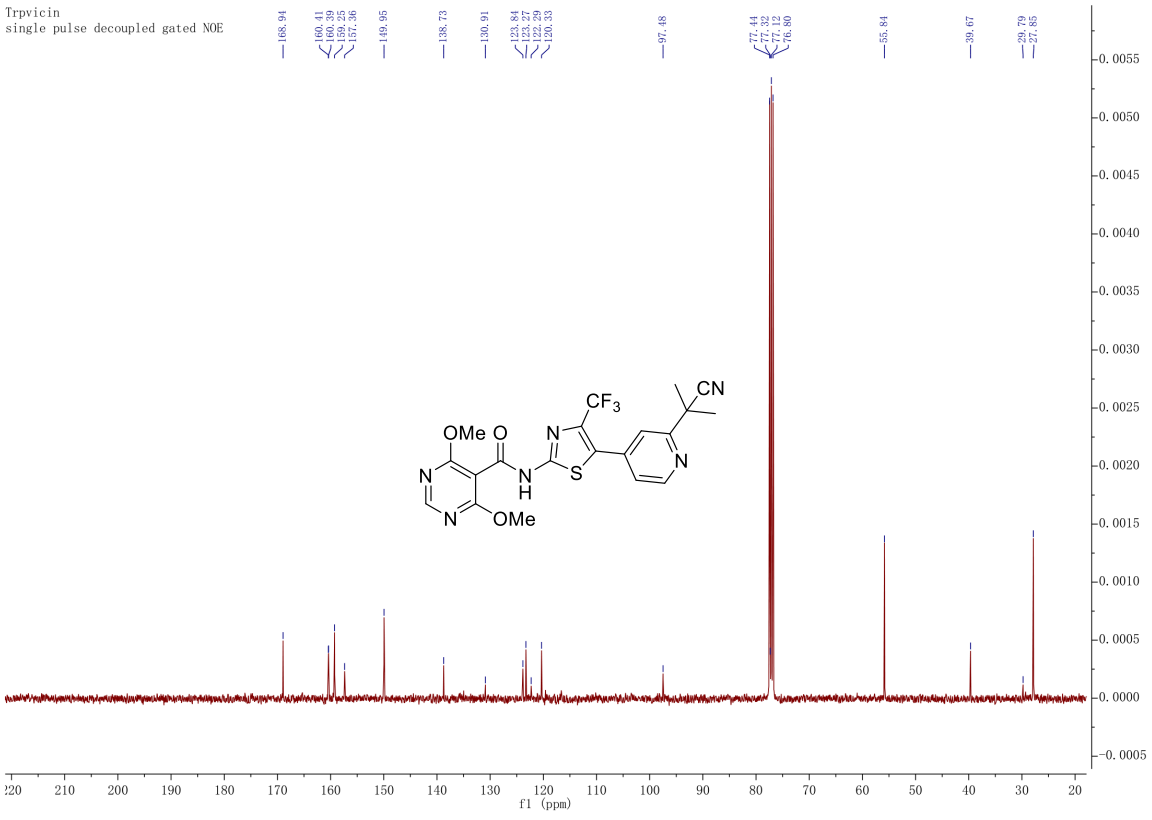

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