Electronic Supplementary Material (ESI) for MedChemComm.

Electronic Supplementary Information

A novel allosteric inhibitor that prevents IKKβ activation

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Content:

Supplementary Results

I. Cell assay results

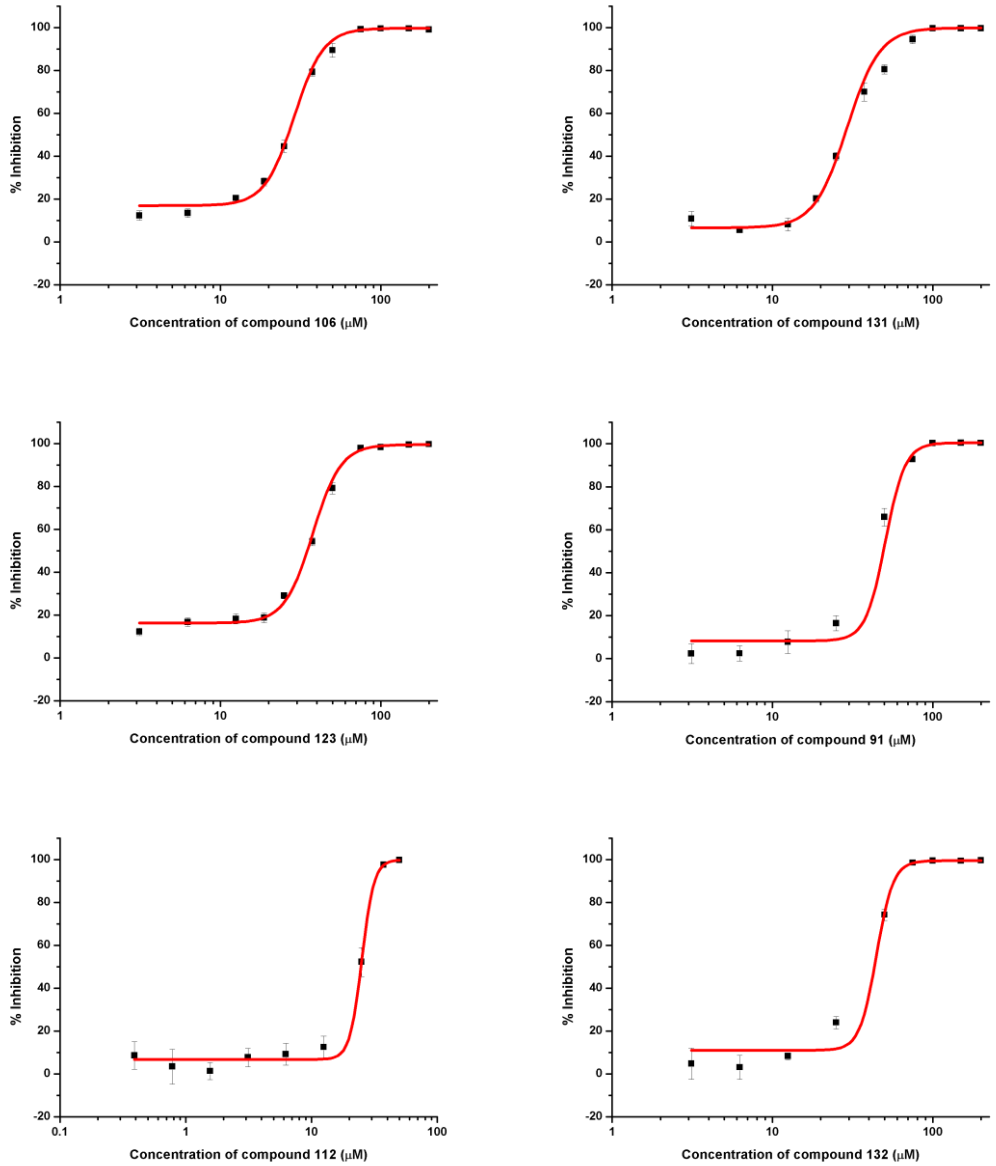
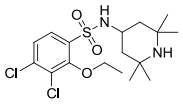
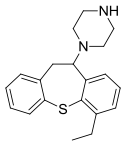
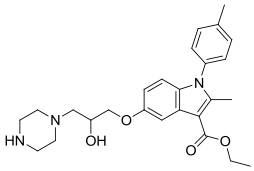
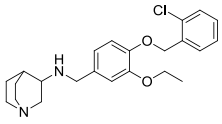
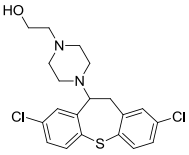
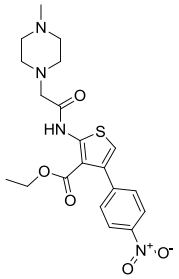
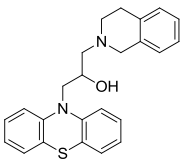


Fig. S1 Dose-response curves of other 6 compounds from virtual screen in luciferase reporter assay against HEK 293T cells.

Table S1 Compounds showed dose-dependent inhibition against HEK 293T cells.

ID	Structure	Luciferase reporter assay IC_{50}[a] (μM)	MTT cytotoxicity assay LC_{50}[b] (μM)
124		35.4 ± 5.3	149 ± 23
106		28.8 ± 1.0	33.6 ± 5.6
131		29.1 ± 1.4	29.5 ± 4.1
123		37.6 ± 1.5	82.4 ± 5.7
91		50.6 ± 4.2	45.8 ± 5.3
112 (PAINS)		25.1 ± 0.7	30.2 ± 1.7
132 (PAINS)		44.5 ± 2.6	68.3 ± 2.6

[a] IC_{50}: concentration that inhibits 50% of luciferase reporter expression.

[b] LC_{50}: concentration that causes 50% of cell death.

[a,b] The data represent the average and standard deviation (mean±SD) of at least triplicate measurements.

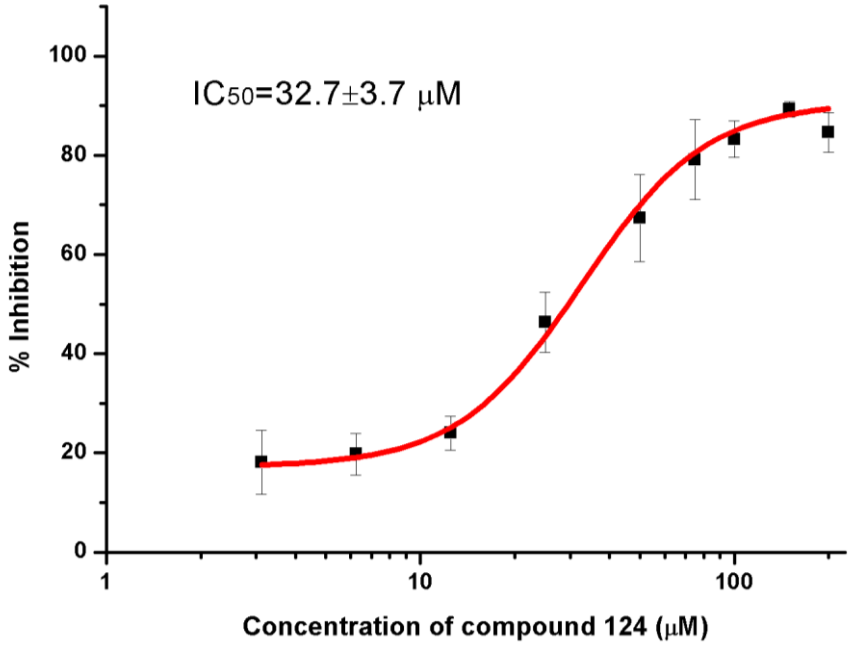


Fig. S2 Compound **124** Inhibits the phosphorylation of IκBα triggered by TNFα in Hela cells.

II. Molecular dynamics (MD) simulations

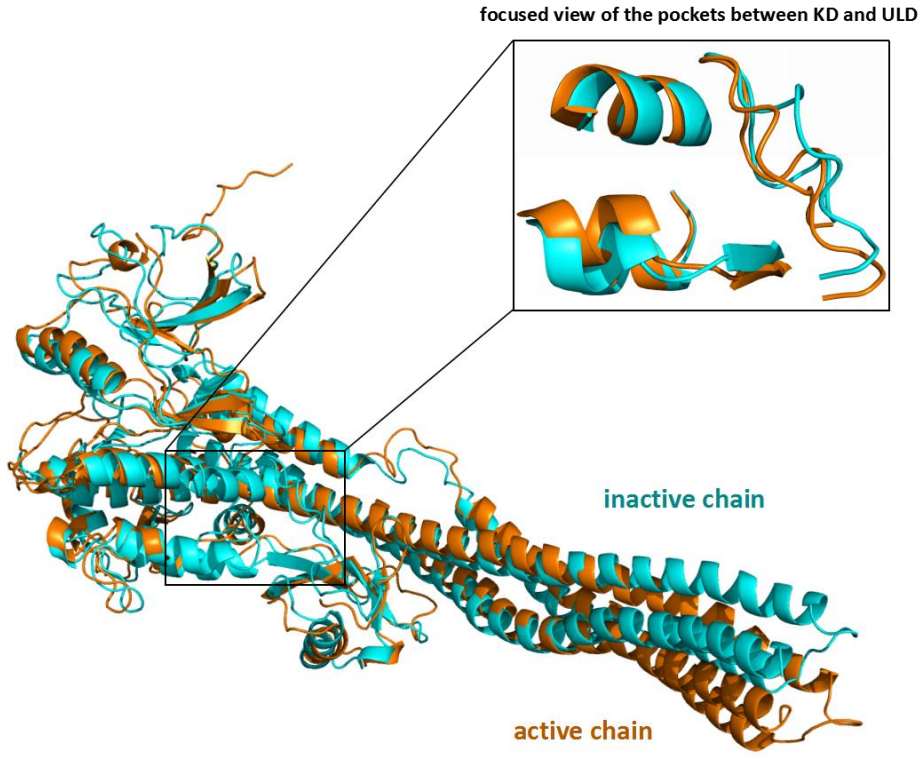


Fig. S3 Pairwise comparison of theoptimized models for the active and inactive chains in the asymmetrical IKKβ dimer structure.

Table S2. Clustering analysis of conformations from MD simulations of the apo IKKβ structure.

Chain	Cluster	Frames	Percentage
Inactive	1	2267	75.60%
	2	287	9.60%
Active	1	1666	55.50%
	2	538	17.90%
	3	451	15.00%

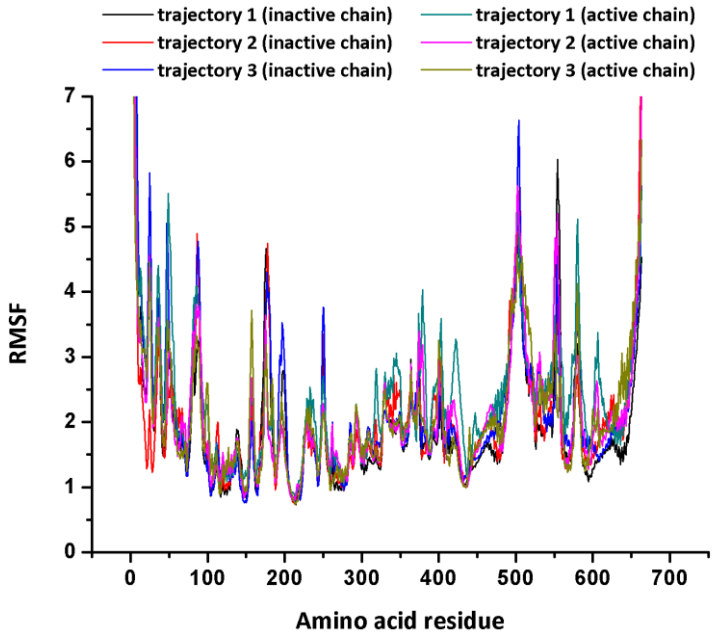


Fig. S4a Root mean square fluctuation (RMSF) of residues in the MD simulations of the apo structure.

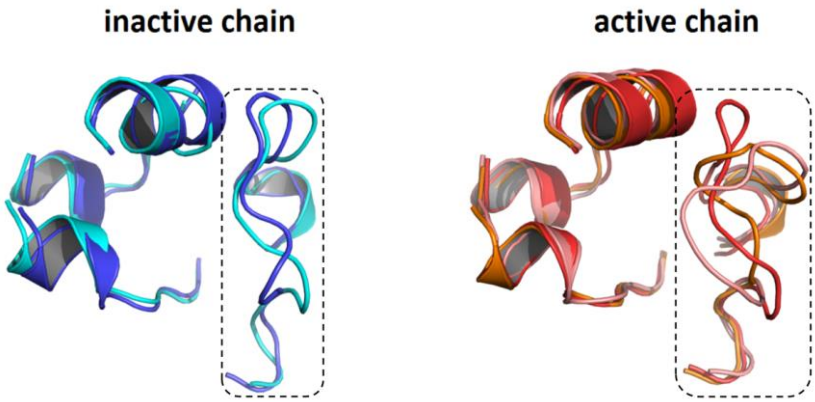


Fig. S4b. Structures of the allosteric pocket in different conformation clusters of apo IKKβ. Left: Structural comparison of the inactive chain binding pocket in the first (blue) and second cluster (cyan). Right: Structural comparison of the active chain binding pocket in the first (red), second (orange) and third cluster (pink). The Thr368-Leu386 loop (as shown in the black dashed box) exhibits more flexibility in the active chain than in the inactive chain.

a. **124** in the inactive conformation of IKKβ.

b. **124** in the active conformation of IKKβ.

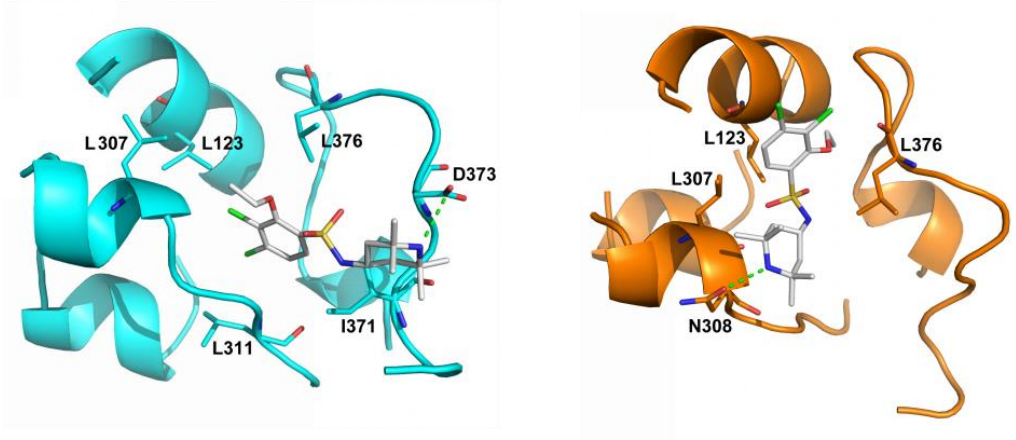


Fig. S5 The initial docking poses of **124** in the inactive and active conformations of IKKβ for MD simulations. The pockets in the inactive and active conformation are shown as cyan and orange cartoon, respectively. The key residues from IKKβ are shown as cyan (inactive conformation) and orange (active conformation) sticks. **124** are shown as white sticks. In inactive conformation, the 3,4-dichloro-2-ethoxy-phenyl forms hydrophobic interactions with L123, L307, L311, I371 and L376. A hydrogen bond is found between side-chain carboxyl oxygen atom of D373 and amide hydrogen atom of **124** pyridine ring. In active conformation, slightly different hydrophobic interactions are observed between 3,4-dichloro-2-ethoxy-phenyl and L123, L307 and L376. In addition, amide hydrogen atom of **124** pyridine ring forms hydrogen bond with side-chain acylamino oxygen atom of N308.

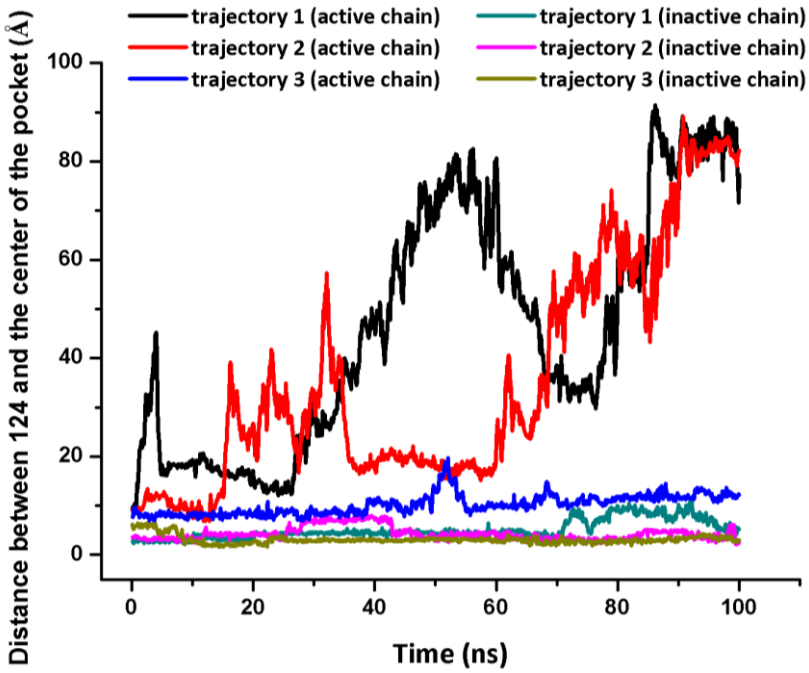


Fig. S6 The distance between **124** and the center of the binding pocket during MD simulation. The pocket was composed of R118-S127, L265-L273, L303-H313 and T368-L386.

Table S3 Predicted H-bond interactions of compound **124** with residues in the inactive IKKβ structure.

Trajectory	Compound 124	Amino acid of inactive chain	Frames at atom level	Percentage
traj1. chainA	124O1	L311N	285	28.50%
	124N1	C370O	167	16.70%
	124N1	D385OD1	150	15.00%
	124N1	G374O	89	8.90%
	124N1	D385OD2	86	8.60%
	124N1	S372O	73	7.30%
	124N1	S372O	65	6.50%
traj2. chainA	124O1	L311N	533	53.30%
	124N1	G374O	395	39.50%
	124N1	H380NE2	212	21.20%
	124N1	G374O	118	11.80%
	124N1	S372O	103	10.30%
traj3. chainA	124O1	L311N	526	52.60%
	124N2	L309O	353	35.30%
	124N1	S372O	296	29.60%
	124N1	G374O	187	18.70%
	124N1	H380NE2	46	4.60%

III. The in vitro kinase assay results

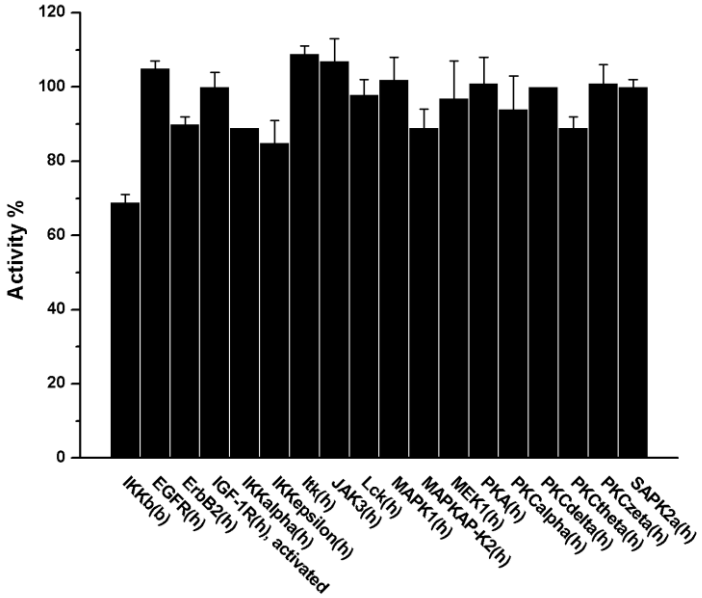


Fig. S7 The kinase selectivity results of compound **124**.

IV. Purity and structure information for the tested compounds

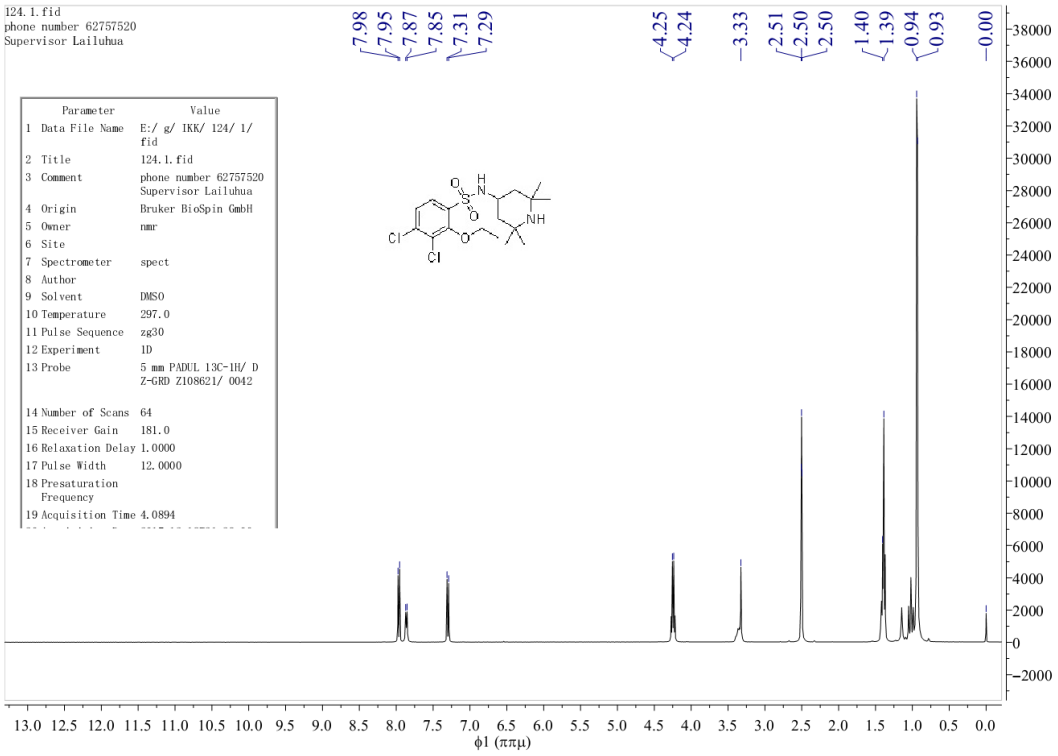


Fig. S8 ^{1}H-NMR spectra for compound **124**.

^{1}H spectra were recorded using 400 MHz on a Bruker spectrometer. The chemical shift values (δ) were reported in parts per million (ppm) relative to tetramethylsilane in DMSO-d_6. ^{1}H NMR spectra are represented as follows: chemical shift, multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, br=broad, m=multiplet), coupling constant (J values) in Hz and integration. Compound **124**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, J = 9.0 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.30 (d, J = 9.0 Hz, 1H), 4.25 (d, J = 7.0 Hz, 2H), 3.39 – 3.33 (m, 1H), 1.43 – 1.37 (m, 2H), 1.39 (d, J = 7.0 Hz, 3H), 1.14 (s, 1H), 1.02 (t, J = 12.3 Hz, 2H), 0.94 (s, 6H), 0.93 (s, 6H).

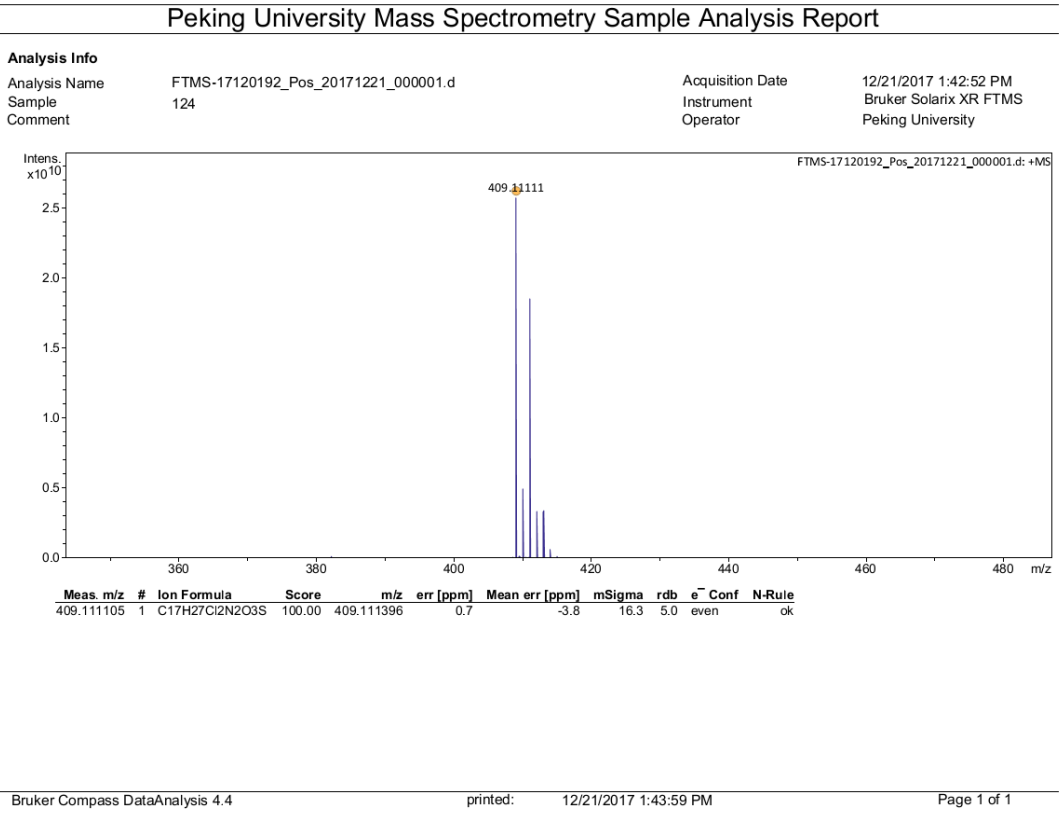


Fig. S9 Mass spectra for compound **124**.

High resolution mass spectra were recorded on a Bruker Apex IV FTMS mass spectrometer using ESI (electrospray ionization). HRMS (ESI): calcd for $C_{17}H_{27}Cl_2N_2O_3S$, [$(M+H)^+$], 409.1119, found 409.1114.

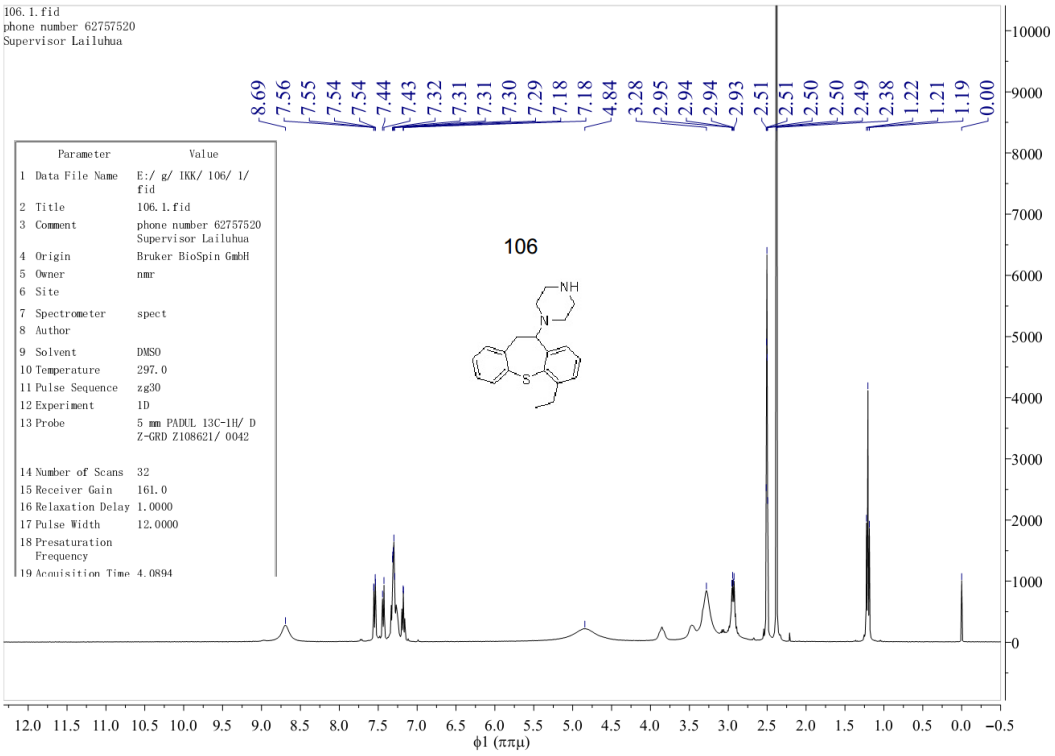


Fig. S10 ^{1}H-NMR spectra for compound **106**.

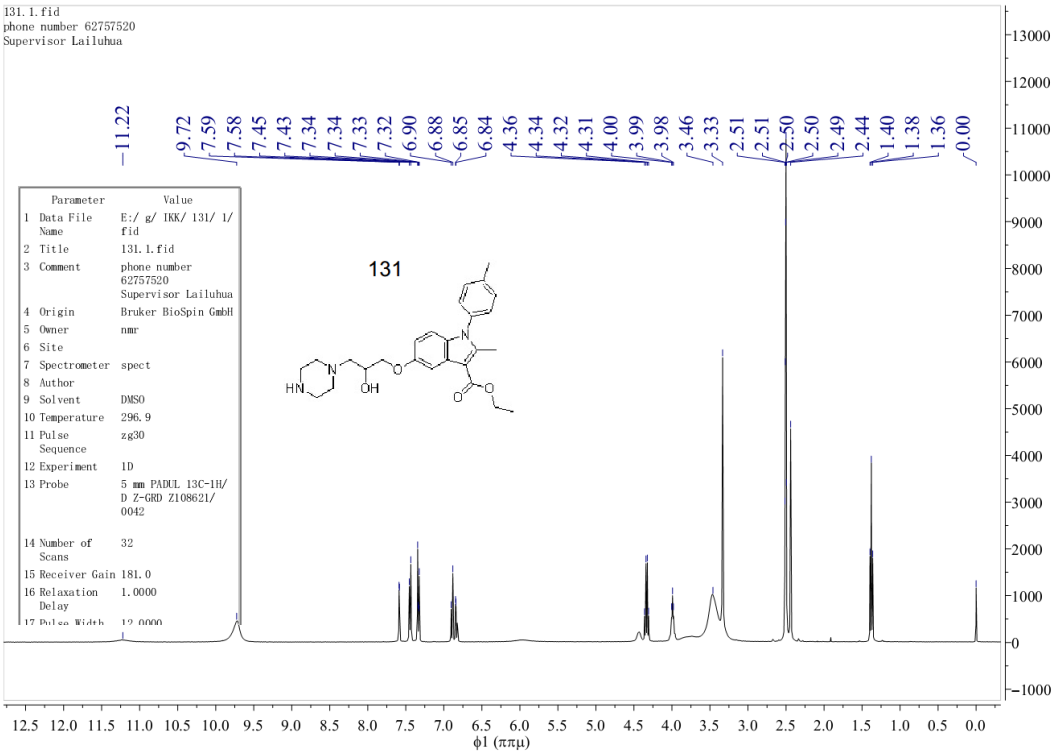


Fig. S11 ^{1}H-NMR spectra for compound **131**.

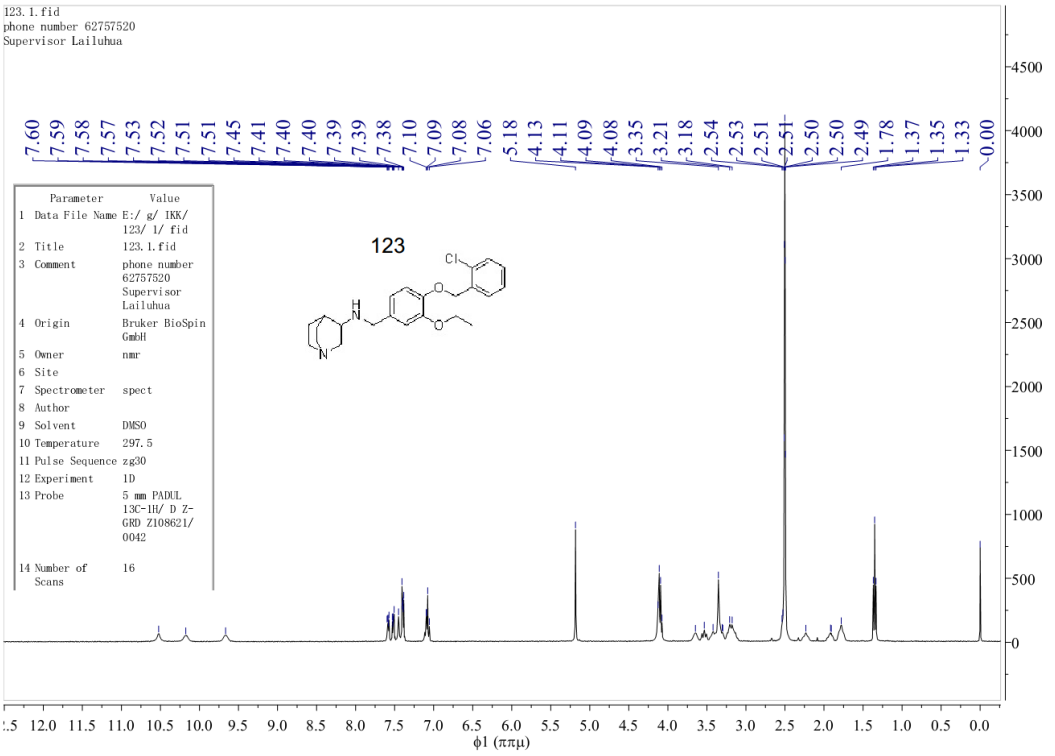


Fig. S12 ^{1}H-NMR spectra for compound **123**.

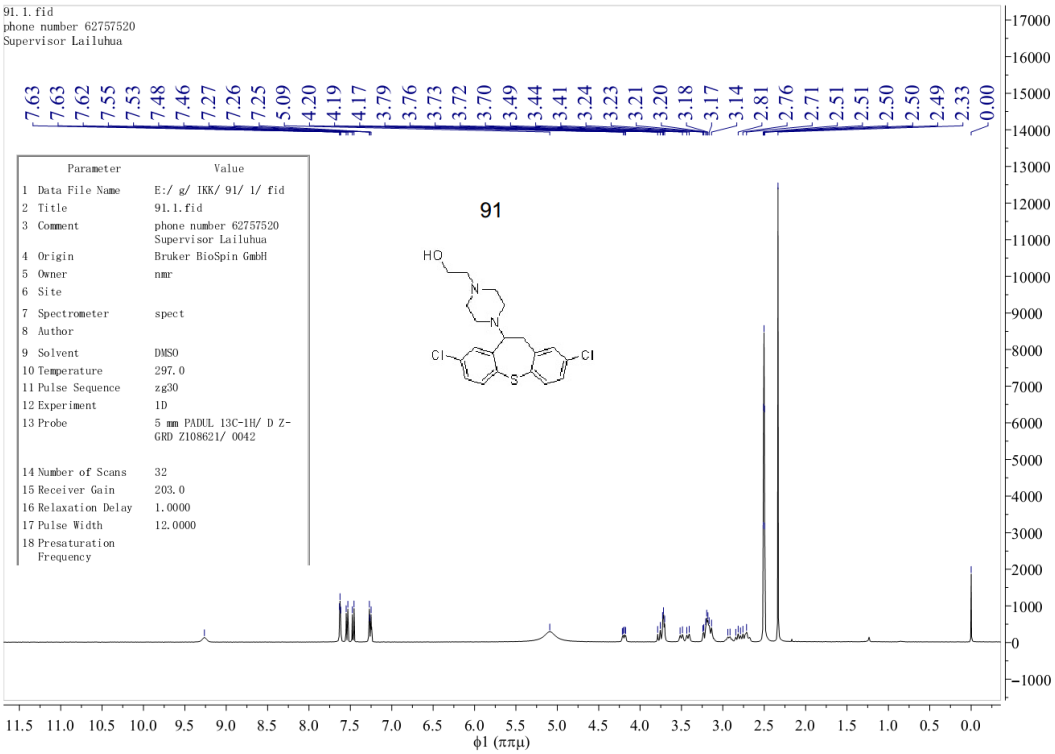


Fig. S13 ^{1}H-NMR spectra for compound **91**.

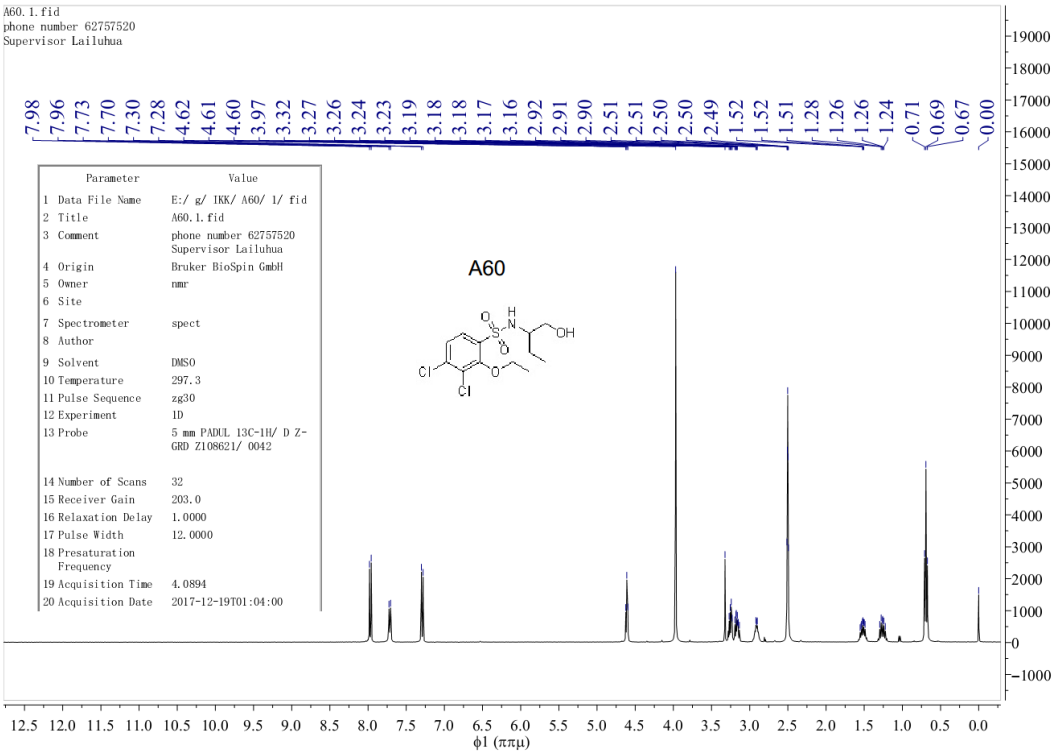


Fig. S14 ^{1}H-NMR spectra for compound **A60**.

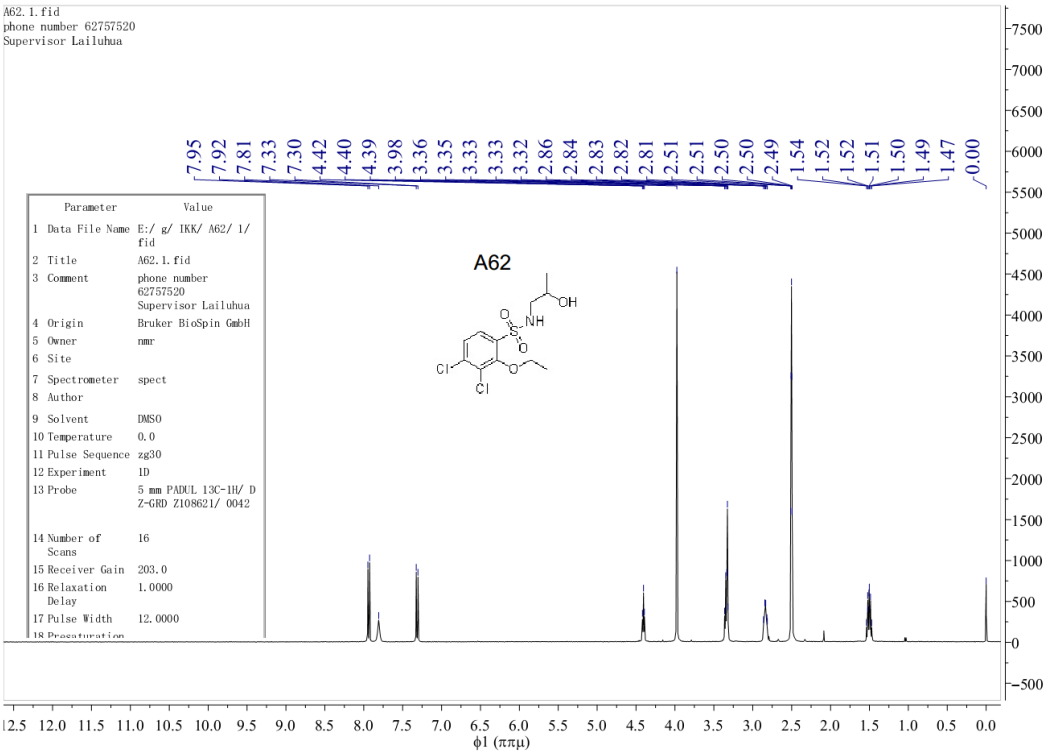


Fig. S15 ^{1}H-NMR spectra for compound **A62**.

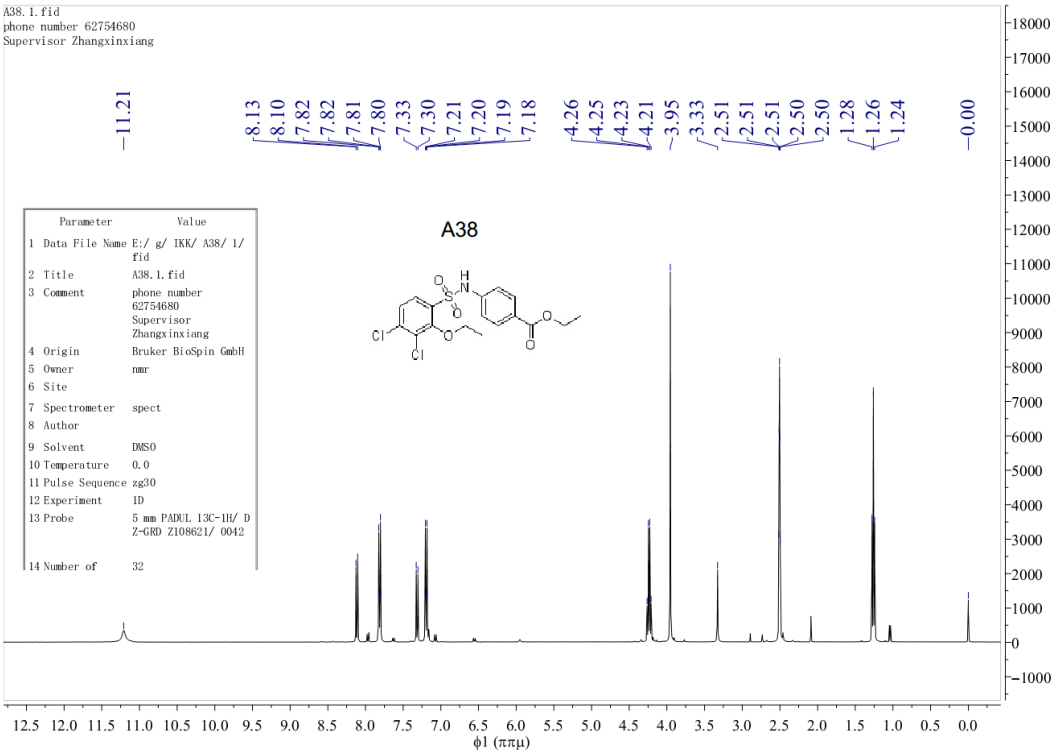


Fig. S16 ^{1}H-NMR spectra for compound **A38**.

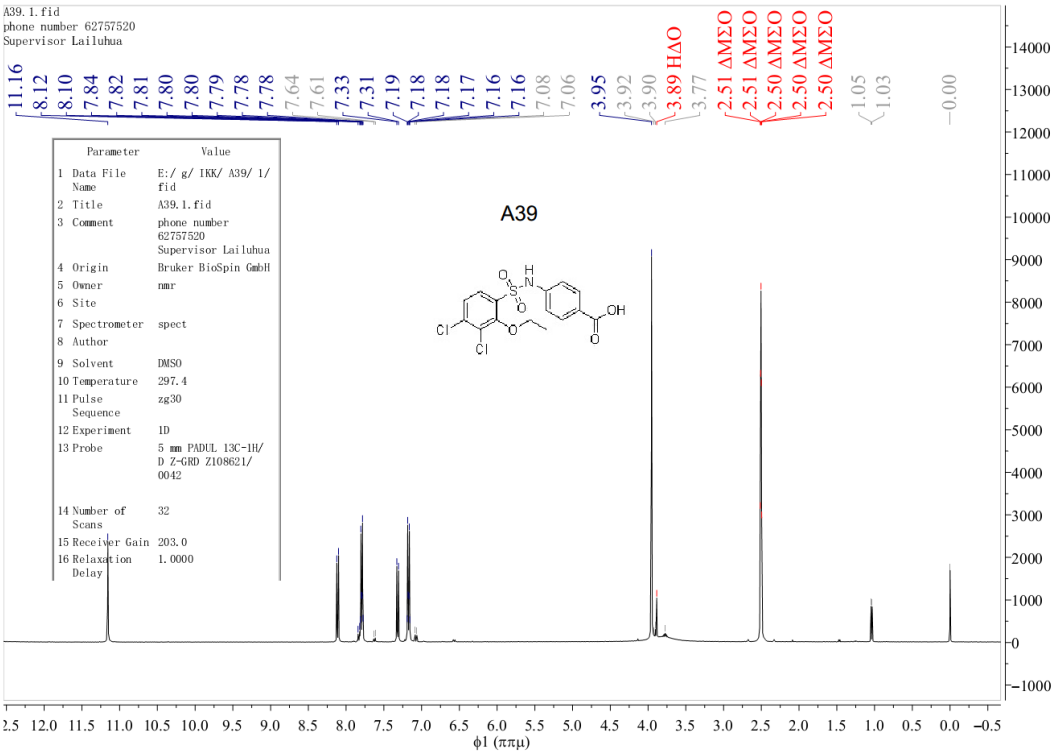


Fig. S17 ^{1}H-NMR spectra for compound **A39**.

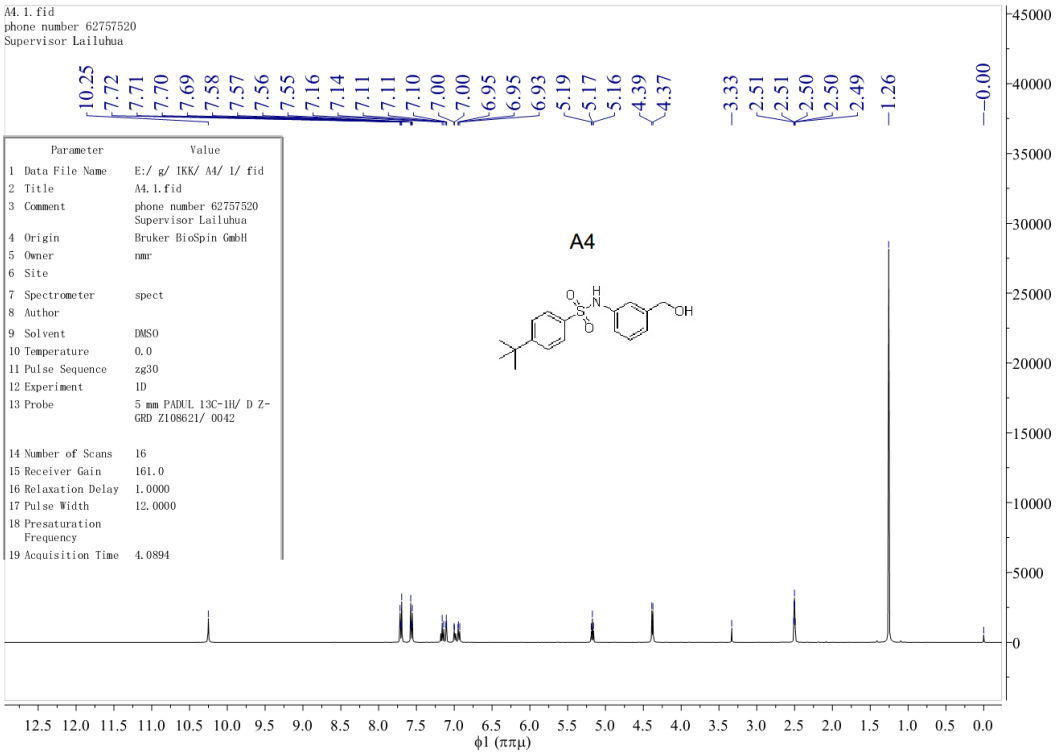


Fig. S18 ^{1}H-NMR spectra for compound **A4**.

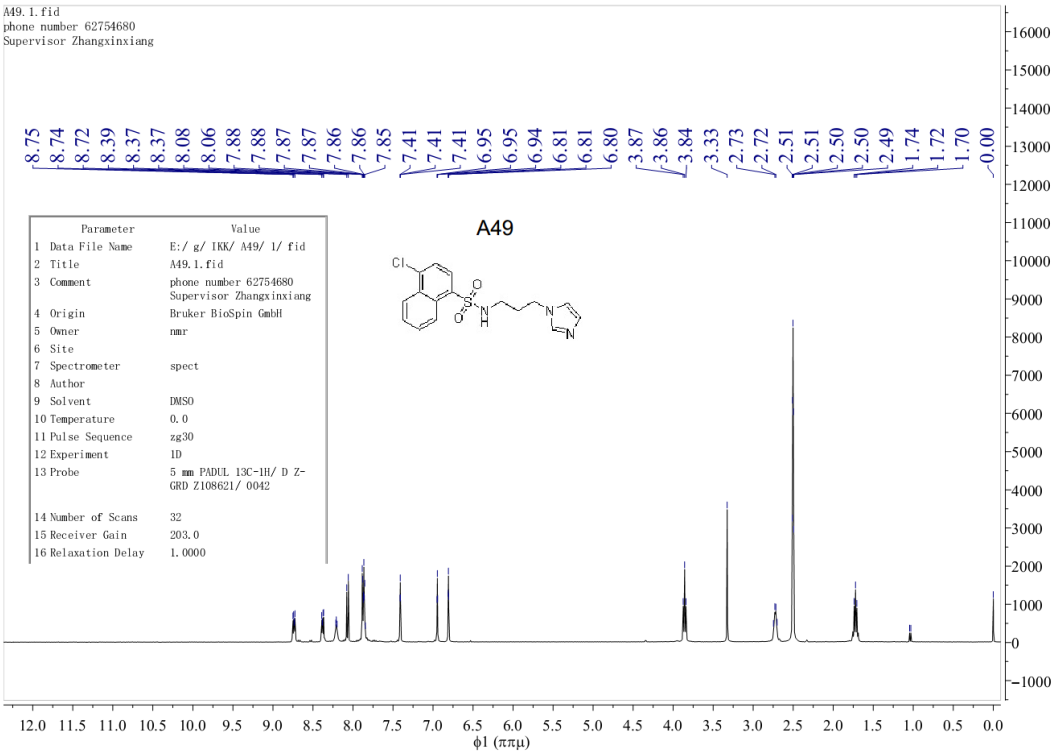


Fig. S19 ^{1}H-NMR spectra for compound **A49**.

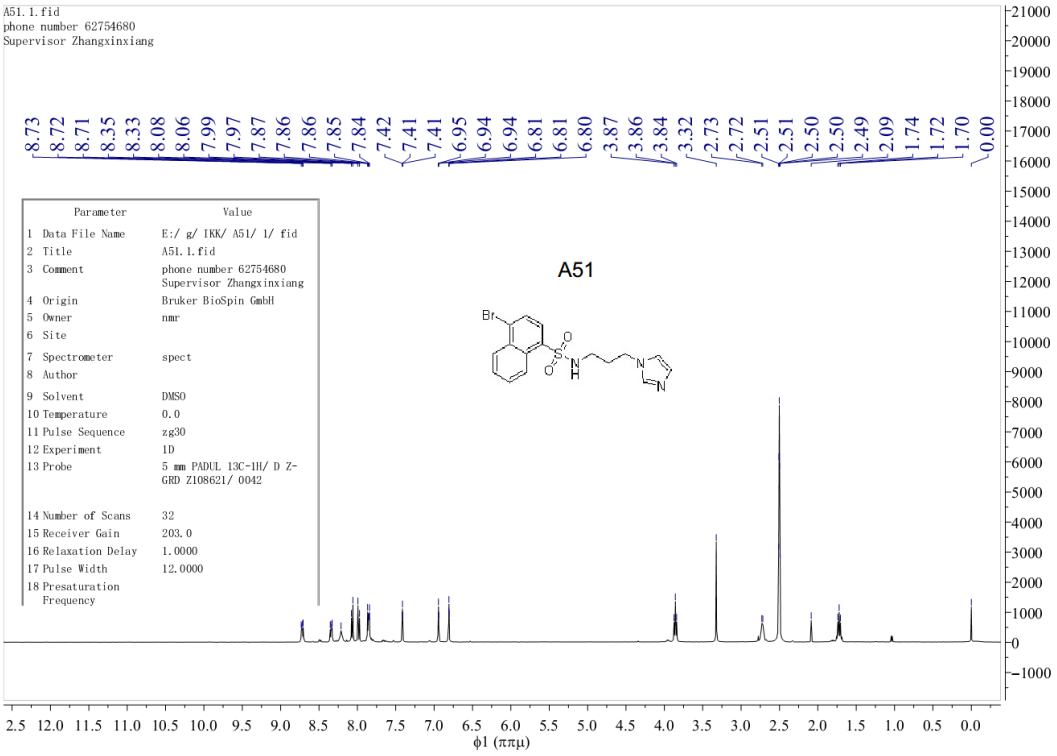


Fig. S20 ^{1}H-NMR spectra for compound **A51**.

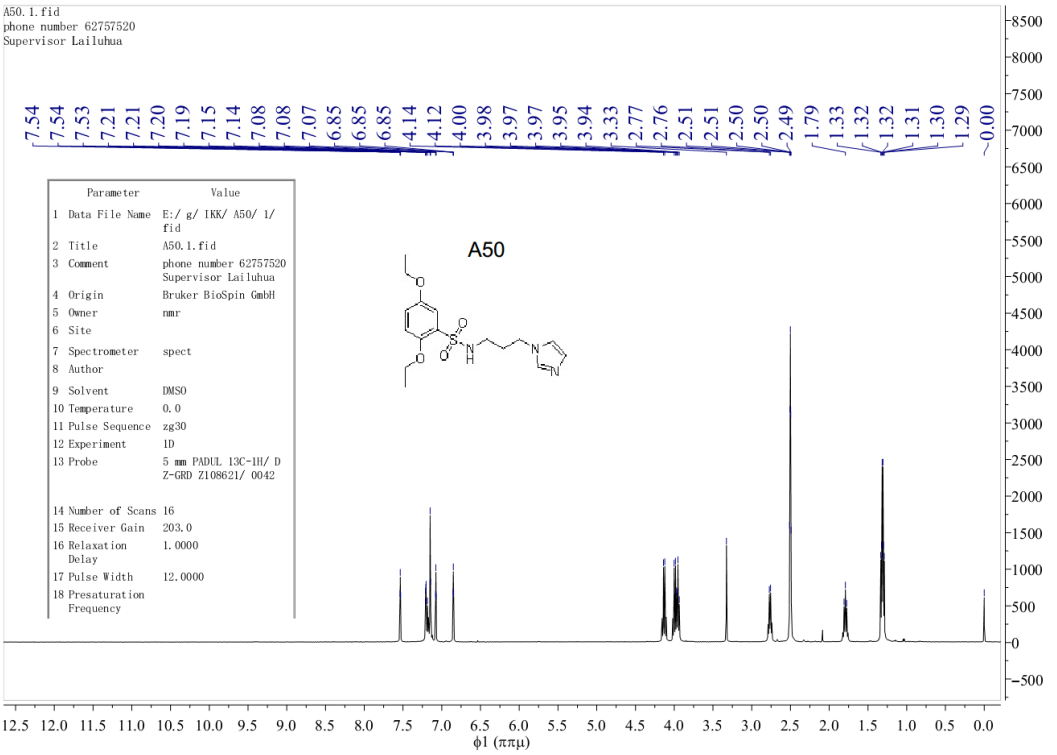


Fig. S21 ^{1}H-NMR spectra for compound **A50**.

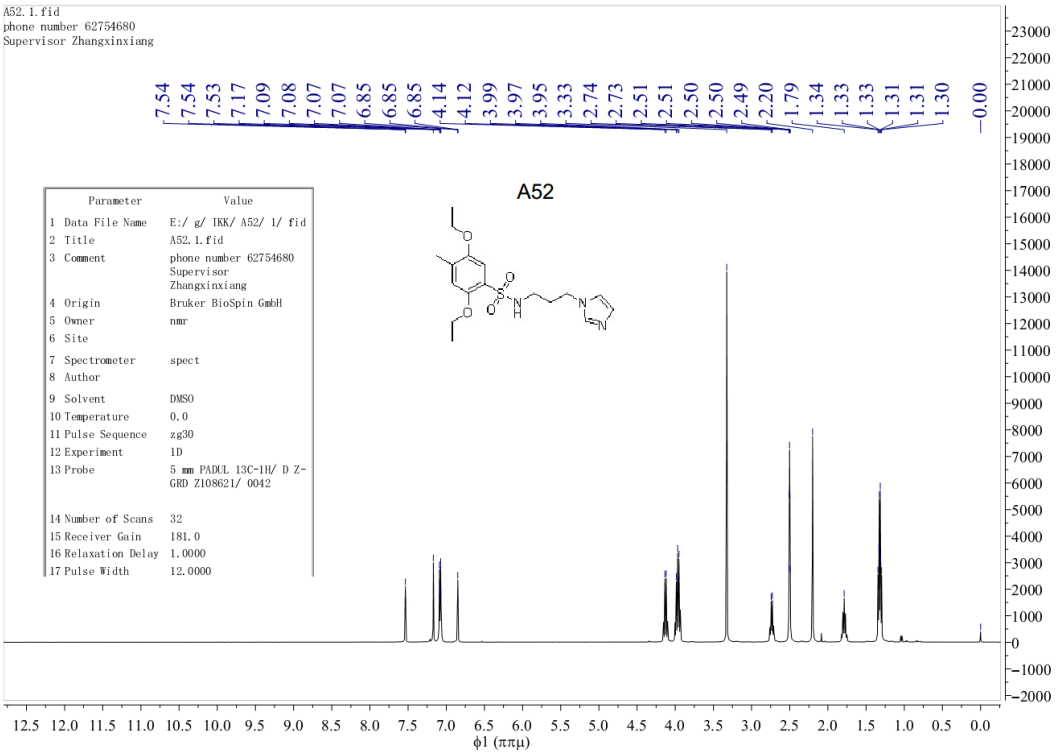


Fig. S22 ^{1}H-NMR spectra for compound **A52**.

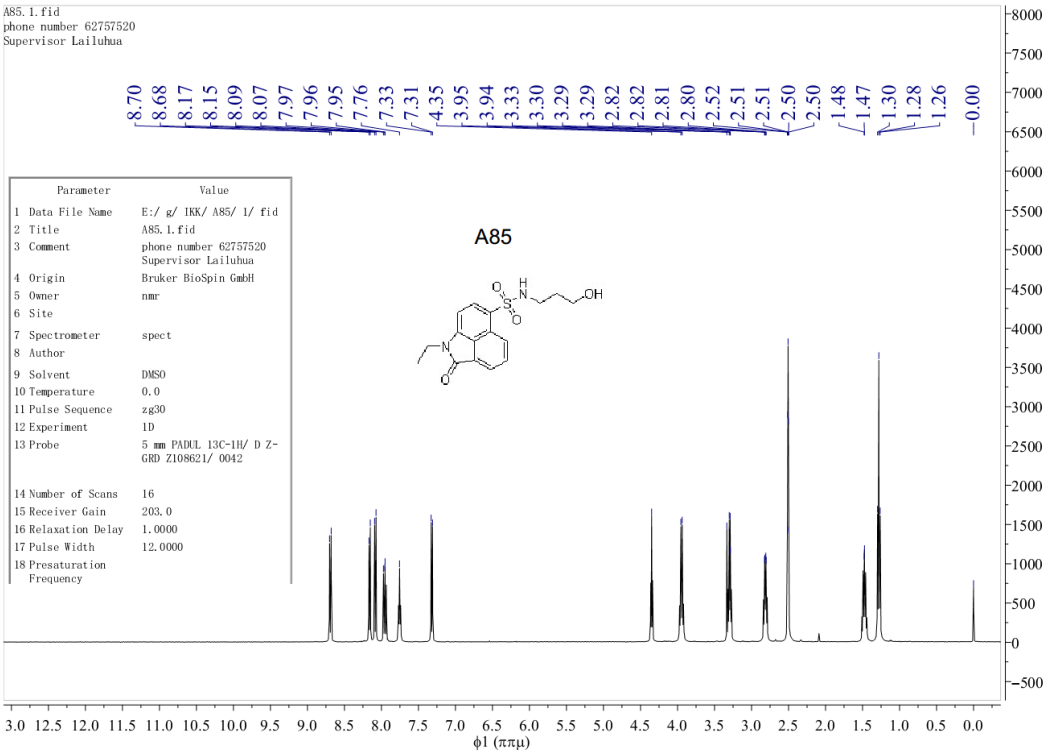


Fig. S23 ^{1}H-NMR spectra for compound **A85**.

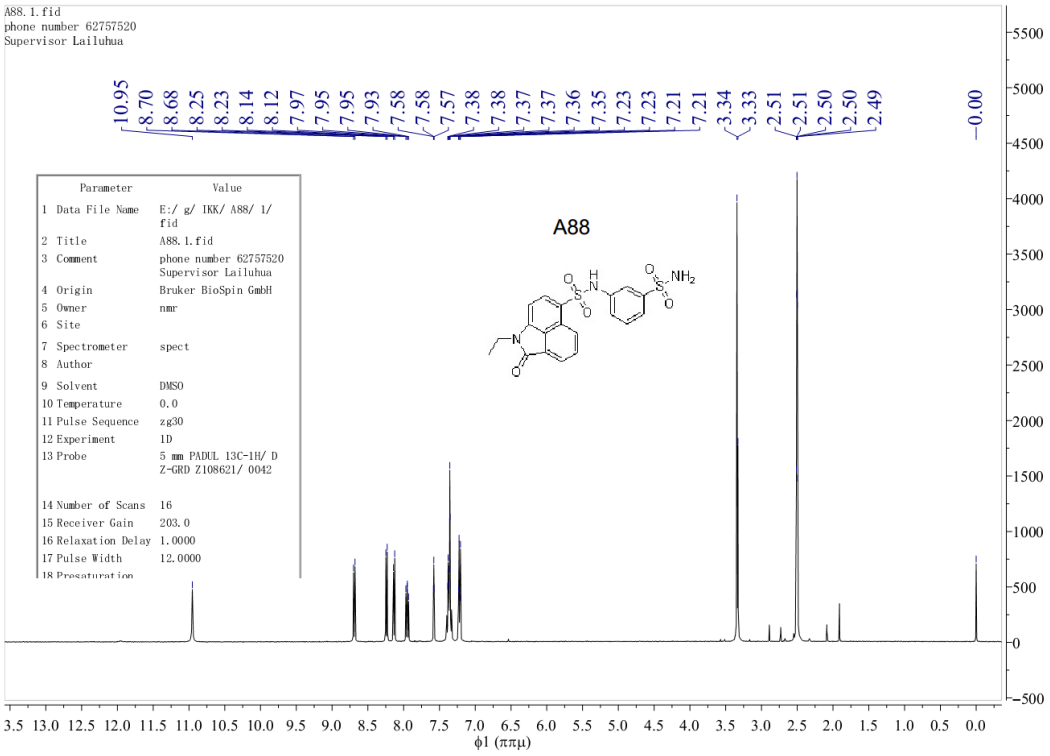


Fig. S24 ^{1}H-NMR spectra for compound **A88**.

Supplementary Methods

I. Optimization of the IKKβ crystal structure

Though the human IKKβ structures have been solved (PDB ID: 4KIK, 4E3C), the available structures are incomplete with several loops missing. So we first built the complete IKKβ structure, then used it to perform molecular dynamics (MD) simulations to sample appropriate conformations for ligand binding pocket prediction. We chose the structure of 4KIK as template for its high resolution, and the missing residues or loops were added using PRIME (Schrodinger 2016-4) with 4E3C as a reference. The loop from Y169 to T180 in the inactive chain was automatically generated and refined by PRIME. And the inhibitor KSA was removed. MD simulations for the optimization of unliganded IKKβ crystal structure were performed with Desmond (Schrodinger 2014-2) using the OPLS-AA/SPC force field. The default settings in the Solvation interface were adopted and NaCl was added to a concentration of 0.05 M to neutralize the protein. Then the system was minimized with 2,000 iterations and a convergence threshold of 50.0 kcal/mol/Å^2. The minimized system was equilibrated with a series of span simulations.

a. NVT ensemble for a simulation time of 12 ps at 10 K restraining all non-hydrogen solute atoms, with bonded time step of 1 fs, near time step of 1 fs and far time step of 3 fs in Berendsen Thermostat with relaxation time of 0.1 ps.

b. NPT ensemble for a simulation time of 12 ps at 10 K restraining all non-hydrogen solute atoms in Berendsen thermostat with relaxation time of 0.1 ps and Berendsen barostat with relaxation time of 50.0 ps. Time steps were consistent with NVT ensemble. Then continual NPT ensemble for a simulation time of 12 ps at 10 K with bonded time step of 2 fs, near time step of 2 fs and far time step of 6 fs followed by another NPT ensemble for a simulation time of 34 ps at 300 K with the same parameters.

c. Without restraints, for a simulation time of 24 ps at 300 K in Berendsen thermostat with relaxation time of 0.1 ps and Berendsen barostat with relaxation time of 2.0 ps.

Finally, the prepared model was simulated for 8 ns at 300 K and 1 atm using Martyna-Tobias-Klein integrator with bonded time step of 2 fs, near time step of 2 fs, far time step of 6 fs and other default parameters.

II. Virtual screen

Two rounds of virtual screen were performed. In the first round, rigid body docking by DOCK6 with default parameter was performed and more than 200,000 SPECS compounds were ranked by grid-based energy score. Top 20,000 compounds were subjected to the second round screen by semi-flexible docking of AutoDock 4. A grid box of 40 X 40 X 40 was created to encompass the binding pocket. Gasteiger partial charges and polar hydrogens were added to protein and ligand by AutoDockTools. The protein and ligand conformations were set to rigid and flexible, respectively. Compounds were docked to the binding pocket by genetic algorithm and ranked by empirical free energy function. The number of runs was set to 10. The maximal number of energy evaluations and generations were 10000000 and 27000, respectively.

III. NF-κB transcriptional activity assays

We utilized Dual-Glo luciferase assay system (Promega). Two plasmid vectors were transiently co-transfected into HEK 293T cells, Hela cells or SK-N-AS cells. The vector pGL4.32[*luc2p*/NF-kB-RE/Hygro] works as NF-κB Signal Reportor. It contains NF-κB response element which can drive the expression of downstream firefly luciferase upon TNFα induction. The vector pGL4.74[*hRluc*/TK]

encode renilla luciferase which express constitutively to work as control reportor to normalize the nonspecific factors. HEK 293T cells or Hela cells were seed in DMEM (10% fetal bovine serum) or SK-N-AS were seeded in DMEM (10% fetal bovine serum and 1% non-essential amino acids) at a density of 6×10^5 cells/60 mm dish, incubated for 12h to allow adherence, and then transfected with 6.5 μg pGL4.32 and 6.5 μg pGL4.74 using ViaFect™ transfection reagent (Promega). Ten hours later the cells were harvested and re-seeded in respective medium in 96-well plates (2×10^4 cells/well in 100 μl medium) and incubated overnight at 37℃and 5% CO_2. Next day, the cells were treated with or without compounds (90 μl) in triplicate for 30 min prior to stimulation with final concentration 10 ng/ml human recombinant TNFα (10 μl) expressed and purified as the previously description[1] for 6-8h. And then, the luminescence from the firefly luciferase and the renilla luciferase were measured and analyzed according to the instrucitons using a BioTek Synergy4 Microplate Reader.

IV. MTT assays

HEK293T cells were co-transfected with two plasmid vectors, treated with or without compound and stimulated with TNFα in exactly the same way to the NF-κB transcriptional activity assays. Instead of luminescence detection, 20 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, 5 mg/ml in PBS) was added to the cells in each well followed by culture in 37℃ and 5% CO_2 for 4 h. Then the medium containing MTT was aspirated thoroughly and 200 μl DMSO was added in each well to dissolve the formazan and read at 490 nm.

V. MD simulations of the apo and 124-bound IKKβ structure

We used AutoDock 4 to predict the possible binding mode of **124** to IKKβ. A grid box of 40 X 40 X 40 was created to encompass the binding pocket in active and inactive conformation chain, respectively. Gasteiger partial charges and polar hydrogens were added to protein and ligand by AutoDockTools. The protein and ligand conformations were set to rigid and flexible, respectively. **124** was docked to the binding pocket in each chain by genetic algorithm and ranked the docking results by empirical free energy function. For each chain, the molecular docking was repeated for five times and 10 posed were generated and clustered for each docking. The largest cluster in active

and inactive chain contains 23 and 20 poses, respectively. Then, the poses from the largest cluster was chosen for display and molecular dynamics simulation.

Before MD simulations were performed, AMBER99SB force field[2] and general AMBER force field (GAFF)[3] were assigned to protein and compound, respectively. After that, apo state IKKβ and IKKβ-**124** complex structure were solvated in a octahedron TIP3P water box with the 10 Å distance away from the boundary of any atoms, respectively. For each system, the water box was neutralized by adding appropriate counterions. The particle-mesh-Ewald (PME) method[4] was used to deal with long-range electrostatic interactions and the SHAKE constraint algorithm[5] was applied to all bonds involving hydrogen atoms. The non-bond interaction cutoff was set to 8.0 Å to avoid unexpected interactions under periodic box condition. Each simulation system was minimized using steepest descent algorithm for 5000 steps and conjugate gradient algorithm for 2000 steps with harmonic restraint (10 kcal/Å) applied on all atoms of protein. Then, the protein was minimized for 10,000 steps and 5000 steps with no restraint by steepest descent and conjugate gradient algorithm, respectively. After energy minimization, each system was heated from 0 K to 310 K for 50 ps, and another 500 ps equilibration was performed. For the production procedure, three trajectories of each system were conducted for at least 100 ns. All the simulations were carried out by Amber14 package.[6]

We combined all the three trajectories into a 300 ns trajectory. Then, clustering was separately performed for the active and inactive chains using the backbone atoms of residues comprising the **124** binding pocket (R118-S127, L265-L273, L303-H313 and T368-L386) in the trajectory. Hieragglo algorithm was used and epsilon was set to 4. A total of 10 clusters were generated and the structures of centroid frame were extracted. The clustering analysis was done by cpptraj implemented in Amber14 package.[6] The frame number and proportion of each cluster were shown in Table S2. We compared and displayed the major clusters structures of active and inactive chain in Fig S4b.

Western blot analysis:

1×10^7 Hela cells in DMEM (10% fetal bovine serum) were plated in each 10 cm culture plate and allowed to adhere overnight. The cells were incubated with or without compound for 30 min prior to stimulation with 10 ng/ml TNFα. After 10 min, the cells were digested by 0.05% trypsin

and collected in PBS by centrifugation. The harvested cells were washed twice with cold PBS, removed the supernatant and collect the cell pellet. The pelleted cells were suspended in 100 μl NP40 Cell Lysis Buffer (Invitrogen) plus with protease inhibitor cocktail (Roche) and phosphatase inhibitor (Sigma) and incubated on ice for 30 min, with vortexing at 10 min intervals. The lysate were centrifuged at 13,000 rpm for 10 min at 4℃ and the supernatants were collected and loaded onto 10% SDS-PAGE gels followed by transferred to polyvinylidene difluoride membranes. The membranes were block in 5% skimmed milk in Wash buffer (Tris buffer with 0.5% Tween 20) at 37 ℃ for 1h and incubated with anti-phospho-IKKβ (Ser177/181) (Cell Signaling Technology, 2697S) 1:500 or anti-β-actin (Cell Signaling Technology, 3700S) 1:1000. Horseradish peroxidase (HRP)-linked anti-rabbit antibody (Cell Signaling Technology, 7074S) 1:1000 and HRP-linked anti-mouse antibody (Cell Signaling Technology, 7076S) 1:1000 were used as secondary antibody respectively. Chemiluminescent signals were detected with the Immobilon™ Western HRP Substrate (Millipore, WBKLS0100) and imaged using the Fusion FX7 (Spectra) system (VILBER).

The in vitro kinase activity and selectivity assay

The effect of **124** on the activity of IKKβ and other 17 kinases were tested using the in vitro [γ-^{33}P-ATP] radiometric assay provided by Eurofins Pharma Discovery Services UK Limited.

Protocols provided by Eurofins are as below:

Kinases are diluted in the buffer shown below prior to addition to the reaction mix.

Buffer Composition	Protein Kinase(s)
20 mM MOPS, 1 mM EDTA, 0.01% Brij-35, 5% Glycerol, 0.1% β-mercaptoethanol, 1 mg/mL BSA	EGFR(h),ErbB2(h), IGF-1R activated(h), IKKα(h), IKKβ(h), IKKε(h), Itk(h), JAK3(h), Lck(h), MAPKAP-K2(h), PKA(h), PKCθ, PKCζ(h),
20 mM HEPES, 0.03% Triton X-100	PKCα(h), PKCδ(h)
25 mM TRIS, 0.1 mM EGTA, 0.1% β-mercaptoethanol, 1 mg/mL BSA	MEK1 (h)
50 mM TRIS, 0.1 mM EGTA, 0.1 mM Na_3VO_4, 0.1% β-mercaptoethanol, 1 mg/mL BSA	MAPK1 (h), SAPK2a (h)

(h) = Human, (m) = Mouse

All compounds are prepared to 50x final assay concentration in 100% DMSO. This working stock of the compound is added to the assay well as the first component in the reaction, followed by the remaining components as detailed in the general assay protocols below. Our positive control wells

contain all components of the reaction, except the compound of interest; however, DMSO (at a final concentration of 2%) is included in these wells to control for solvent effects. Our blank wells contain all components of the reaction, with a reference inhibitor replacing the compound of interest. This abolishes kinase activity and establishesthe base-line (0% kinase activity remaining). A table showing the reference inhibitor used to generate the blank signal for each kinase is given at the end of the protocol.

IKKβ(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 100 μM LDDRHDSGLDSMKDEEY, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 10 μM).

EGFR (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 10 mM $MnCl_2$, 0.1 mg/mL poly(Glu, Tyr) 4:1, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 10 μM).

ErbB2 (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.1 mg/mL poly(Glu-Tyr), 5 mM $MnCl_2$, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 10 μM).

IGF-1R, activated(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 1 mM Na_3VO_4, 5 mM Na-β-glycerophosphate, 500 μM KKKSPGEYVNIEFG, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 45 μM).

IKKα(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 200 μM LDDRHDSGLDSMKDEEY, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 10 μM).

IKKε(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 2 mg/mL casein, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 15 μM).

Itk(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.33 mg/mL myelin basic protein, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 200 μM).

JAK3(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 500 μM GGEEEEYFELVKKKK, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 10 μM).

Lck(h) is incubated with 50 mM Tris pH 7.5, 0.1 mM EGTA, 0.1 mM Na_3VO_4, 250 μM

KVEKIGEGTYGVVYK (Cdc2 peptide), 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 90 μM).

MAPKAP-K2(h) is incubated with 50 mM Na-β-glycerophosphate pH 7.5, 0.1 mM EGTA, 30 μM KKLNRTLSVA, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 90 μM).

MEK1(h) is incubated with 50 mM Tris pH 7.5, 0.2 mM EGTA, 0.1% β-mercaptoethanol, 0.01% Brij-35, 1 μM unactive MAPK2 (m), 10 mM MgAcetate and cold ATP (ATP concentration is 10 μM). The reaction is initiated by the addition of the MgATP. After incubation for 40 minutes at room temperature, 5 μL of this incubation mix is used to initiate a MAPK2 (m) assay. MAPK2 (m) is incubated with 25 mM Tris pH 7.5, 0.02mM EGTA, 0.33 mg/mL myelin basic protein, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 155 μM).

MAPK1(h) is incubated with 25 mM Tris pH 7.5, 0.02mM EGTA, 250 μM peptide, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 70 μM).

PKA (h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 30 μM LRRASLG (Kemptide), 10 mM MgAcetate and[γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 10 μM).

PKCα(h) is incubated with 20 mM HEPES pH 7.4, 0.03% Triton X-100, 0.1 mM $CaCl_2$, 0.1 mg/mL phosphatidylserine, 10 μg/mL diacylglycerol, 0.1 mg/mL histone H1, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 45 μM).

PKCδ(h) is incubated with 20 mM HEPES pH 7.4, 0.03% Triton X-100, 0.1 mg/mL phosphatidylserine, 10 μg/mL diacylglycerol, 50 μM ERMRPRKRQGSVRRRV, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 15 μM).

PKCθ(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 0.1 mg/mL histone H1, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 15 μM).

PKCζ(h) is incubated with 8 mM MOPS pH 7.0, 0.2 mM EDTA, 50 μM ERMRPRKRQGSVRRRV, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 15 μM).

SAPK2a(h) is incubated with 25 mM Tris pH 7.5, 0.02 mM EGTA, 0.33 mg/mL myelin basic protein, 10 mM MgAcetate and [γ-^{33}P-ATP] (specific activity approx. 500 cpm/pmol, ATP concentration is 90 μM).

Each of the reaction above is initiated by the addition of the MgATP mix. After incubation for 40 minutes at room temperature, the reaction is stopped by the addition of 3% phosphoric acid solution. 10 μL of the reaction is then spotted onto a P30 filtermat and washed three times for 5 minutes in 75 mM phosphoric acid and once in methanol prior to drying and scintillation counting.

Reference Inhibitor Information:

Reference Inhibitor	Protein Kinase(s)
Staurosporine	EGFR(h), ErbB2(h), IGF-1R(h) activated, IKKα(h), IKKβ(h), IKKε(h), Itk(h), JAK3(h), Lck(h), MAPKAP-K2(h), MEK1(h), PKA(h), PKCα(h), PKCδ(h), PKCζ(h), PKCθ(h)
PKR Inhibitor	MAPK1(h)
PP1 Analog II. 1NM-PP1	SAPK2a(h)

Supplementary References

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