Small-Molecule Covalent Bond Formation at Tyrosine Creates a Binding Site and Inhibits Activation of Ral GTPases

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RGL2 (50-514). BL-21 (DE3) *E. coli* cells containing RGL2 (50-514) in pGEX-6P-1 plasmid was grown in Terrific Broth (TB) at 37 °C until OD_{600} reached 0.6. Protein expression was induced with 0.5 mM IPTG at 16 °C for 16-20 h. Cells were harvested by centrifugation and lysed by passing multiple times through a microfluidizer in a buffer containing 400 mM NaCl, 50 mM Tris pH 8.0, 10% glycerol and 8 mM β-mercaptoethanol. The sample was clarified by centrifugation at 35,000 x g for 1 h at 4 °C, prior to being loaded onto a 5 mL GSTrap HP column (GE, Boston, MA, Catalog Number: 17528202). The column was then washed with buffer containing 200 mM NaCl, 20 mM Tris pH 8.0, 10% glycerol and 1 mM TCEP, prior to being eluted with the same buffer supplemented with 10 mM glutathione. The GST tag was cleaved by adding 1:100 w/w HRV-3C enzyme (ThermoFisher, Waltham, MA, Catalog Number: 88946) to the eluted protein and dialyzing against 200 mM NaCl, 20 mM Tris pH 8.0, 1 mM TCEP for 48 h at 4 °C. The sample was re-purified on the GSTrap HP column to remove the cleaved GST tag. Finally, the protein was further purified on a HiLoad 26/600 Superdex 200 pg SEC column (GE, Boston, MA, Catalog Number: 28989336) with 200 mM NaCl, 20 mM Tris pH 8.0 and 1 mM TCEP as buffer.

HIS-RalA (1-178). The plasmid of pET21a(+)-RalA was transformed into competent *E. coli* BL21(DE3) strain. Bacterial culture was grown in LB medium at 37 °C to an OD_{600} of approximately 0.6 and then induced with 0.5 mM IPTG at 32 °C for 5 h. Cells were collected by centrifugation and the pellet was lysed by micro-fluidizer in lysis buffer (phosphate buffer, pH 7.6, 2 mM $MgCl_2$). The His-RalA protein was purified at 4 °C using Ni-IMAC chromatography (HisTrap HP, GE Healthcare) and eluted with 500 mM imidazole in lysis buffer with a gradient method. After the fractions consisting of His-RalA were combined and concentrated, the protein was further purified using size exclusion chromatography (Superdex 200 pg, GE Healthcare) in 10 mM HEPES (pH 7.5), 10 mM NaCl, 5 mM $MgCl_2$, 1 mM DTE, 1 µM GDP. After purification protein HIS-RalA was concentrated to 25 mg/mL for crystallization.

HIS-RalB (12-185). The plasmid of pHIS-RalB was transformed into competent *E. coli* BL21(DE3) strain. Bacterial culture was grown in TB medium at 37 °C to an OD_{600} of approximately 0.6 and then induced with 0.5 mM IPTG at 25 °C for 16 h. Cells were collected by centrifugation and the pellet was lysed by micro-fluidizer in lysis buffer (phosphate buffer, pH 7.6, 2 mM $MgCl_2$). The His-RalB protein was purified at 4 °C using Ni-IMAC chromatography (HisTrap HP, GE Healthcare) and eluted with 500 mM imidazole in lysis buffer with a gradient method. After the fractions consisting of His-RalB was combined and concentrated, the protein was further purified using size exclusion chromatography (Superdex 200 pg, GE Healthcare) in 50 mM sodium phosphate buffer pH 7.6, 100 mM NaCl, 1 mM $MgCl_2$. The plasmids of pHIS-RalBS50A, pHIS-

RalBT69A, pHIS-RalBY82F and pHIS-RalBS85A mutants were generated using site-directed mutagenesis. The corresponding proteins were expressed and purified following the same protocol as HIS-RalB.

HIS-H-Ras (1-189). The plasmid of preceiver-B01.2x-HRas was transformed into competent *E. coli* BL21(DE3) strain. Bacterial culture was grown in TB medium at 37 °C to an OD_{600} of approximately 0.6 and then induced with 0.5 mM IPTG at 25 °C for 16 h. Cells were collected by centrifugation and the pellet was lysed by micro-fluidizer in lysis buffer (phosphate buffer, pH 7.6, 2 mM $MgCl_2$). The His-H-Ras protein was purified at 4 °C using Ni-IMAC chromatography (HisTrap HP, GE Healthcare) and eluted with 500 mM imidazole in lysis buffer with a gradient method. After the fractions consisting of His-Ras were combined and concentrated, the protein was further purified using size exclusion chromatography (Superdex 200 pg, GE Healthcare) in 20 mM Tris, pH 8.0, 100 mM NaCl, 2 mM $MgCl_2$. Then the protein was concentrated and stored at -80 °C for further experiments.

K-RAS (1-169). The plasmid of pet21a(+)-MBP-TEV-KRAS(1-169) was transformed into competent E. coli BL21(DE3) strain. Bacterial culture was grown in LB medium at 37 °C to an OD600 of approximately 0.6 and then induced with 0.5 mM IPTG at 37 °C for 3.5 h. Cells were collected by centrifugation and the pellet was lysed by micro-fluidizer in lysis buffer (phosphate buffer, pH 7.6, 2 mM MgCl2). The His-MBP-TEV-KRAS(1-169) protein was purified at 4 °C using Ni-IMAC chromatography (HisTrap HP, GE Healthcare) and eluted with 500 mM imidazole in lysis buffer with a gradient method. After the fractions consist of His-MBP-TEV-KRAS(1-169) was combined and concentrated, the protein was further purified using size exclusion chromatography (Superdex 200 pg, GE Healthcare) in 20 mM Tris, PH=8.0, 100 mM NaCl. After purification, the protein was concentrated to about 3.6 mg/mL. DTT and EDTA were supplemented to the protein solution to give a final concentration of 1 mM and 0.5 mM respectively. TEV protease in 20 mM Tris, PH=8.0, 100 mM NaCl was mixed with HIS-MBP-TEV-KRAS(1-169) at a molar ratio of 1:5. Then the mixture was incubated at 4 oC for 72 h. A SDS-page gel was run to confirm the cleavage. Then 81 mL of buffer (20 mM sodium phosphate, 0.3 M NaCl, PH=7.7, 4 mM MgCl2) was mixed with the cleavage reaction to give about 100 mL of solution, which decrease DTT and EDTA concentration to 0.2 and 0.1 mM respectively. The solution was load to Ni-IMAC column (HisTrap HP, GE Healthcare) at 4 °C and washed with 50 mL of buffer (20 mM sodium phosphate, 0.3 M NaCl, PH=7.7, 4 mM MgCl2). After the flow through and washing solution consist of KRAS(1-169) was combined and concentrated, the protein was further purified using size exclusion chromatography (Superdex 200 pg, GE Healthcare) in 20 mM Tris, PH=8.0, 100 mM NaCl.2 mM

MgCl2. After purification, the protein was concentrated and stored at -80 oC for further experiments.

HIS-SOS-cat (564-1049). The plasmid of ProEX HTb-SOScat was transformed into competent *E. coli* BL21(DE3) strain. Bacteria culture was grown in LB medium at 37 °C to an OD_{600} of approximately 0.6 and then induced with 0.5 mM IPTG at 16 °C for 16 h. Cells were collected by centrifugation and the pellet was lysed by micro-fluidizer in lysis buffer (phosphate buffer, pH 7.6, 2 mM $MgCl_2$). The His-SOS-cat protein was purified at 4 °C using Ni-IMAC chromatography (HisTrap HP, GE Healthcare) and eluted with 500 mM imidazole in lysis buffer with a gradient method. After the fractions consisting of His-RalB was combined and concentrated, the protein was further purified using size exclusion chromatography (Superdex 200 pg, GE Healthcare) in 20 mM Tris, pH 8.0, 100 mM NaCl, 2 mM $MgCl_2$. Then the protein was concentrated and stored at -80 °C for further experiments.

Computational Analysis of Binding Sites. Identification of druggable binding sites on the crystal structures was carried out using the Schrödinger Software Suite (Small-Molecule Drug Discovery Suite 2019-1, Schrödinger, LLC, New York, NY, 2019). Structures were retrieved from the Protein Data Bank (PDB)(1) and prepared using the Protein Preparation Wizard workflow in Maestro. Missing side chains and loops were added with the Prime (2) module. Protein and ligand structures were protonated at pH 7.0 using PROPKA (3) and Epik (4), respectively. The SiteMap (5) module was used to evaluate the binding site of the compound on the prepared structure. The covalent bond between compound **1** and Tyr-82 in the RalA-**1** structure was removed. The region around the compound plus an additional 6 Å buffer was evaluated for potential binding sites. All other parameters were left to their default setting. Binding sites are identified in SiteMap by overlaying a three-dimensional grid around the region. Each point of the grid (site point) is evaluated using van der Waals energies. Points are linked together to form the putative binding site. Each site is evaluated based on its ability to bind a ligand (SiteScore) and its druggability (DrugScore). Both SiteScore and DrugScore use the weighted sums of three parameters, namely the (i) number of site points in the binding site; (ii) enclosure score that is a measure of how open the binding site is to solvents; and (iii) hydrophilic character of the binding site (hydrophilic score). Unlike DrugScore, SiteScore limits the impact of hydrophilicity in charged and highly polar sites. A binding site with SiteScore and DrugScore of 0.8 is considered to be able to fit a small molecule ligand. SiteScore and DrugScore values closer to 0.8 are considered ‘difficult’ to drug, while binding sites with SiteScore and DrugScore closer to 1.1 are classified as highly ‘druggable’ (6).

Table S1. X-ray data-collection and refinement statistics

	RalA.GDP – **1**	RalA.GDP – **2**	RalA.GDP – **4**	RalA.GDP – **5**	RalA.GDP – **6**
Data collection					
Wavelength (Å)	1.00003	0.97625	0.97625	0.97625	0.97625
Space group	C 2 2 21	C 2 2 21	C 2 2 21	C 2 2 21	C 2 2 21
Cell dimensions					
a, b, c (Å)	64.85 104.33 55.24	65.11 104.38 55.59	65.10 104.37 55.49	65.281 104.924 55.409	65.12 104.75 55.67
α, β, γ (°)	90.00 90.00 90.00	90.00 90.00 90.00	90.00 90.00 90.00	90.00 90.00 90.00	90.00 90.00 90.00
Resolution (Å)	39.00 – 1.18	38.05 – 1.30	39.15 – 1.50	39.19 – 1.49	55.31 – 1.63
R_{sym}	0.045 (0.807)	0.086 (1.224)	0.165 (0.943)	0.082 (1.487)	0.166 (1.567)
R_{meas}	0.048 (0.909)	0.093 (1.331)	0.179 (1.033)	0.088 (1.602)	0.179 (1.689)
R_{pim}	0.015 (0.419)	0.035 (0.519)	0.068 (0.416)	0.033 (0.593)	0.066 (0.626)
CC1/2	1.000 (0.681)	0.999 (0.658)	0.996 (0.676)	0.999 (0.728)	0.996 (0.621)
I/σ(I)	22.7 (1.6)	15.2 (1.5)	14.6 (1.8)	16.3 (1.5)	11.6 (1.5)
Completeness (%)	100.0 (100.0)	99.9 (99.6)	99.7 (99.8)	100.0 (100.0)	98.7 (100.0)
Multiplicity	8.1 (4.7)	6.9 (6.4)	6.8 (6.2)	7.2 (7.1)	7.2 (7.1)
Refinement					
Resolution (Å)	39.02 – 1.18 (1.20 – 1.18)	38.07 – 1.30 (1.33 – 1.30)	39.16 - 1.50 (1.55 – 1.50)	39.20 – 1.49 (1.54 – 1.49)	55.34 – 1.63 (1.70 – 1.63)
No. unique reflections	61827	46861	30567	31546	23868
R_{work}	0.1253	0.1341	0.1327	0.1275	0.1366
R_{free}	0.1472	0.1671	0.1688	0.1641	0.1857
R.m.s.d values					
Bond lengths (Å)	0.013	0.010	0.016	0.009	0.014
Bond angles (°)	1.181	1.055	1.267	1.038	1.195
No. atoms					
Protein	1409	1350	1368	1350	1350
Ligand/ions	71	51	50	50	54
B-factors (Å^2)					
Protein	14.64	13.67	11.96	17.19	12.99
ligand/ions	22.42	16.64	17.27	19.93	15.54
Ramachandran plot					
Favored (%)	98.2	98.2	97.0	97.00	97.6

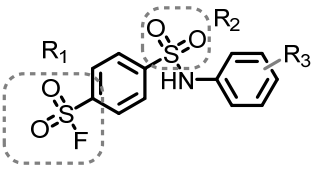
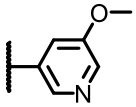
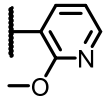
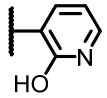
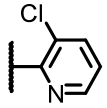
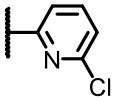
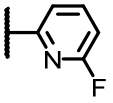
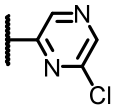
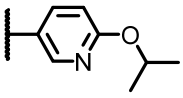
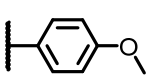
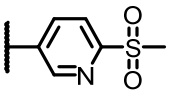
Allowed (%)	1.8	1.8	3.00	3.00	1.8
Outliers (%)	0.0	0.0	0.0	0.0	0.0
Rotamer outliers (%)	0.0	0.0	0.0	0.0	0.0
PDB code	6P0I	6P0L	6P0M	6P0K	6P0N

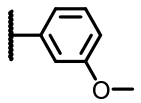
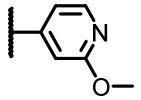
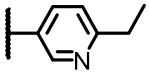
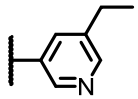
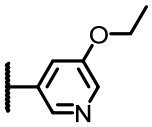
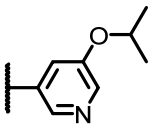
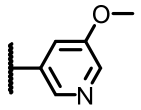
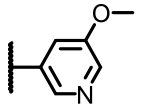
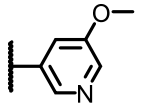
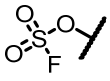
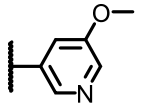
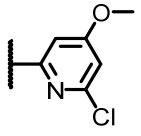
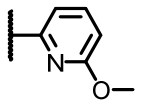
	RalA.GDP – pocket_open	RalA.GDP – pocket_closed			
Data collection					
Wavelength (Å)	1.00003	0.97625			
Space group	C 2 2 21	C 2 2 21			
Cell dimensions					
a, b, c (Å)	64.96 104.88 55.55	64.95 104.81 55.22			
α, β, γ (°)	90.00 90.00 90.00	90.00 90.00 90.00			
Resolution (Å)	39.16 – 1.54	30.78 – 1.31			
R_{sym}	0.113 (0.725)	0.072 (1.004)			
R_{meas}	0.122 (0.816)	0.078 (1.094)			
R_{pim}	0.047 (0.393)	0.030 (0.429)			
CC1/2	0.998 (0.643)	0.999 (0.684)			
I/σ(I)	12.9 (1.6)	17.3 (1.6)			
Completeness (%)	99.5 (93.9)	99.7 (98.9)			
Multiplicity	6.6 (4.1)	6.9 (6.3)			
Refinement					
Resolution (Å)	39.17 – 1.54 (1.60 – 1.54)	30.78 – 1.31 (1.34 – 1.31)			
No. unique reflections	28327	45413			
R_{work}	0.1365	0.1201			
R_{free}	0.1750	0.1560			
R.m.s.d values					
Bond lengths (Å)	0.008	0.009			
Bond angles (°)	1.083	1.075			

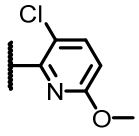
No. atoms					
Protein	1350	1350			
Ligand/ions	30	30			
B-factors (Å^2)					
Protein	12.30	15.10			
ligand/ions	10.28	11.35			
Ramachandran plot					
Favored (%)	97.0	98.2			
Allowed (%)	3.0	1.8			
Outliers (%)	0.0	0.0			
Rotamer outliers (%)	0.0	0.0			
PDB code	6P0O	6P0J			

*Highest-resolution shell values are shown in parentheses.

Table S2. Compound Chemical Structures

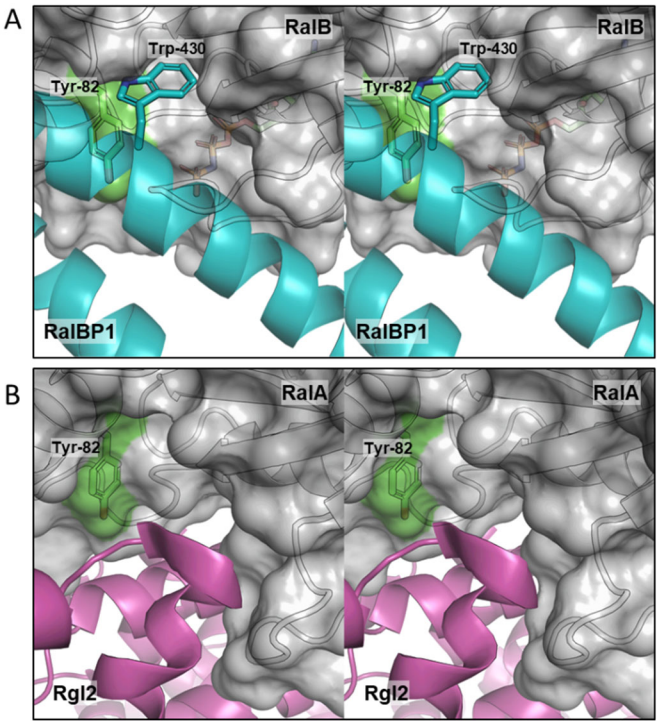
Compound				IC_{50} at 24 h (μM)	
	R_1	R_2	R_3	RalB WT	RalB Tyr82Phe
1				49.5 ± 2.3	NI*
2				23.1 ± 6.8	NI
3				17.0 ± 7.7	79.3 ± 19.0
4				41.7 ± 8.5	215 ± 84.5
5				24.4 ± 4.9	NI
6				51.5 ± 5.2	NI
7				49.4 ± 3.5	NI
8				ND†	ND
9				60.3 ± 3.0	100 ± 11.1
10				147 ± 42.0	238 ± 55.4

11				26.0 ± 4.6	164 ± 42.3
12				146 ± 14.2	NI
13				164 ± 26.0	NI
14				91.4 ± 2.9	167 ± 8.7
15				85.5 ± 7.9	235 ± 167
16				79.5 ± 2.3	333 ± 31.0
17				ND	ND
18				ND	ND
19				ND	ND
20				ND	ND
21				24.0 ± 1.0	138 ± 34.2
22				64.0 ± 4.1	NI

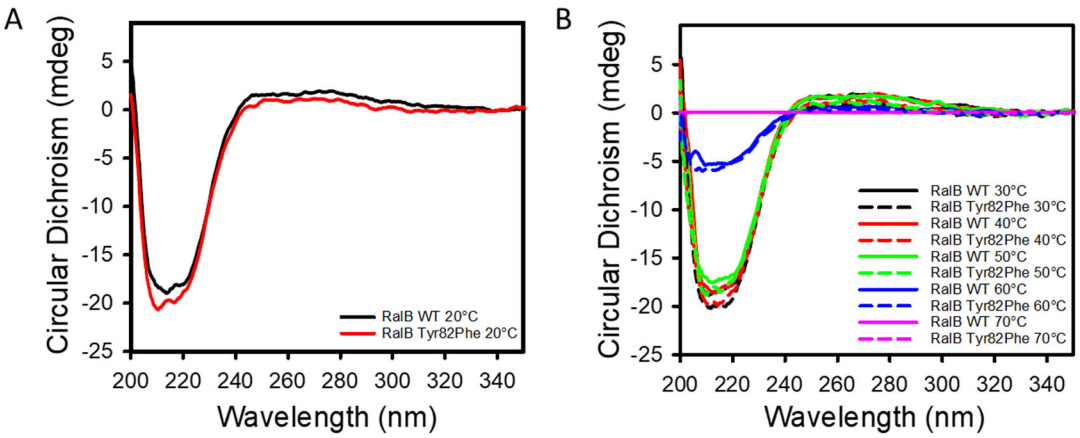
23				127 ± 11.7	NI

*No Inhibition

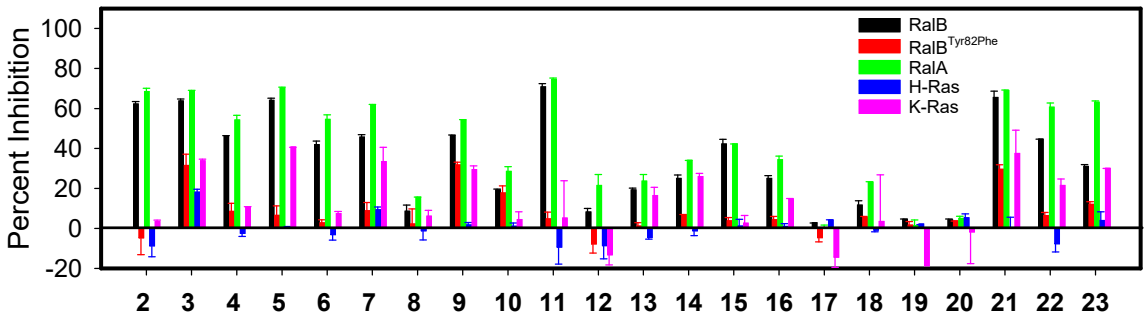
†Not Determined



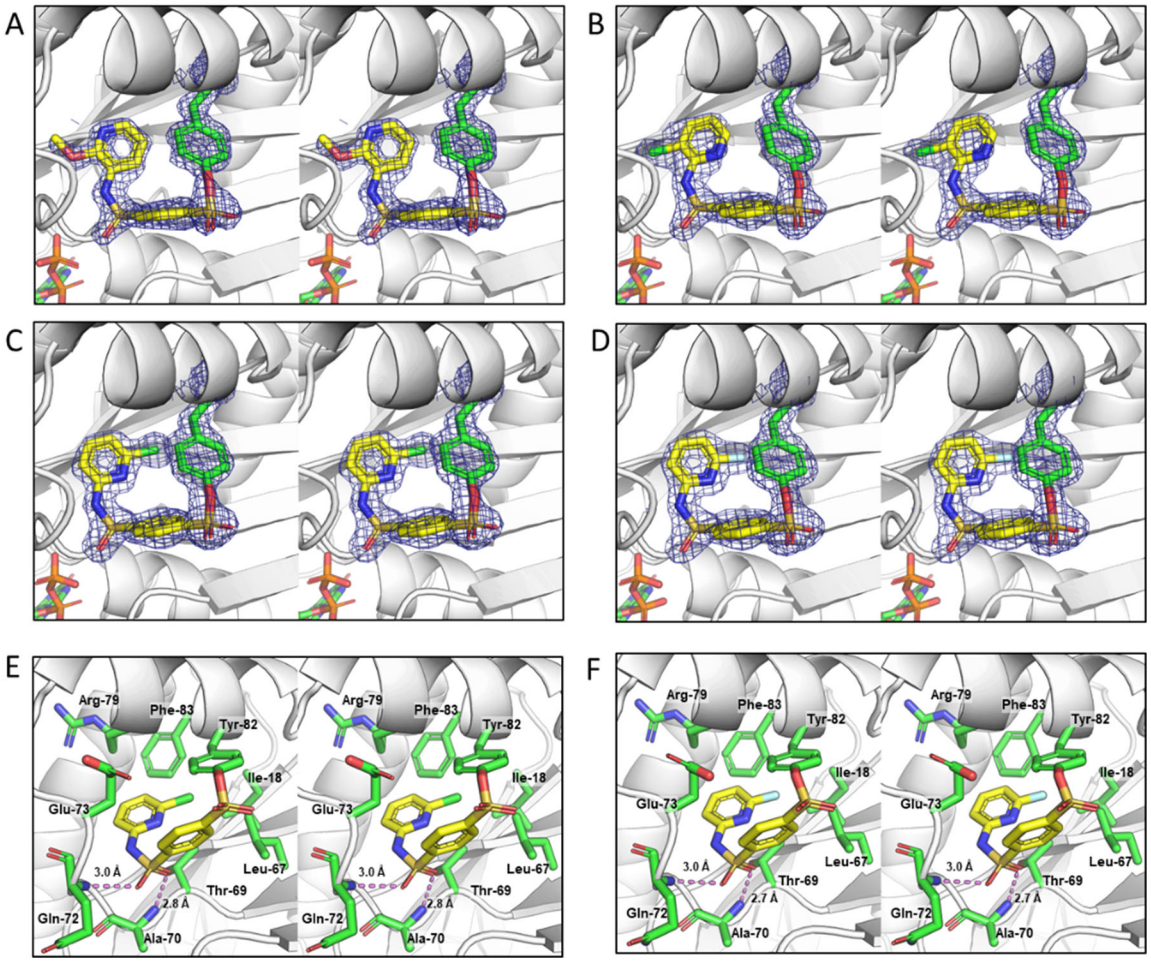
Supplementary Figure 1. (A) Solution NMR structure of RalB (gray surface) in complex with RalBP1 (cyan cartoon; PDB: 2KWI). **(B)** X-ray crystal structure of RalA (gray surface) in complex with Rgl2 (pink cartoon) (PDB: 5CM8). The sidechains of $Ral^{Tyr\text{-}82}$ and $RalBP1^{Trp\text{-}430}$ are highlighted as sticks.



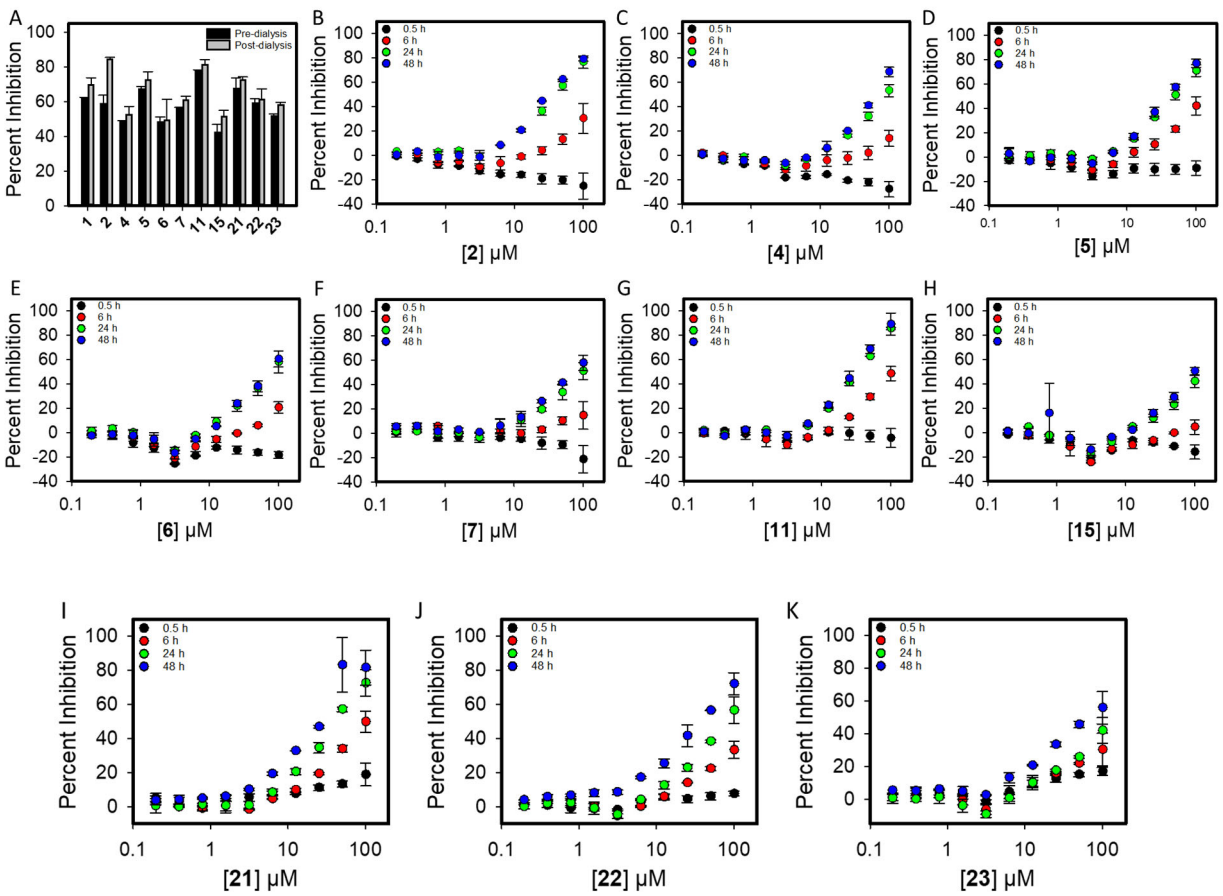
Supplementary Figure 2. (A) Circular Dichroism (CD) spectrum of RalB WT and Tyr82Phe in assay buffer (20 mM Tris pH 8.0, 100 mM NaCl, 10 mM $MgCl_2$) at 20°C. **(B)** Temperature stability of RalB WT and Tyr82Phe mutant were analyzed by CD as in **A**.



Supplementary Figure 3. Percent inhibition of Rgl2-mediated guanine nucleotide exchange of RalB, RalB Tyr82Phe mutant and RalA and SoS-mediated guanine nucleotide exchange of H-Ras and K-Ras by 50 μM compounds after 24 h incubation at 4°C (mean ± SD, n = 2).



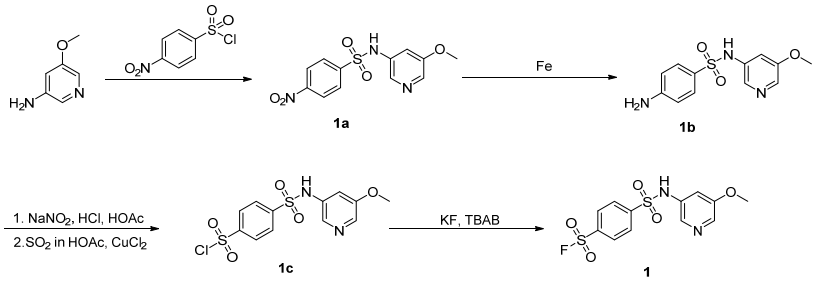
Supplementary Figure 4. A) Stereo image of the composite omit electron density map (blue mesh) of compound **2** (sticks with yellow carbon, red oxygen, blue nitrogen and orange sulfur) covalently bound to RalA Tyr-82 (sticks with green carbon) [PDB code: 6P0L]. **B)** Stereo image of the composite omit electron density map of compound **4** covalently bound to RalA Tyr-82 (colored as in **A**) [PDB code: 6P0M]. **C)** Stereo image of the composite omit electron density map of compound **5** covalently bound to RalA Tyr-82 (colored as in **A**) [PDB code: 6P0K]. **D)** Stereo image of the composite omit electron density map of compound **6** covalently bound to RalA Tyr-82 (colored as in **A**) [PDB code: 6P0N) **E**) Stereo image of RalA-**5** complex, highlighting the binding interactions between **5** (sticks with yellow carbons, blue nitrogens, red oxygens, orange sulfurs, green chlorine) and the RalA pocket (stick with green carbons). Hydrogen-bonds are displayed in dashes and labeled with distance (PDB code: 6P0K). **F**) Stereo image of RalA-**6** complex, highlighting the binding interactions between **6** (sticks with yellow carbons, blue nitrogens, red oxygens, orange sulfurs, cyan fluorine) and the RalA pocket (stick with green carbons). Hydrogen-bonds are displayed in dashes and labeled with distance (PDB code: 6P0N).



Supplementary Figure 5. A) Percent inhibition of Rgl2-mediated guanine nucleotide exchange of RalB by 100 µM compounds after 24 h incubation at 4°C followed by 24 h dialysis against assay buffer at 4°C. Concentration-dependent percent inhibition of Rgl2-mediated guanine nucleotide exchange of RalB after 0.5, 6, 24 and 48 h incubation at 4°C with **B) 2**, **C) 4**, **D) 5**, **E) 6**, **F) 7**, **G) 11**, **H) 15**, **I) 21, J) 22 and K) 23** (mean ± SD, n = 2).

General Synthesis Methods. All chemicals were purchased from either Sigma-Aldrich or Acros Organics and used as received. Column chromatography was carried out with silica gel (60A, 40-63 μm). 1H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm using either residual $CHCl_3$ or DMSO as internal references. Anhydrous solvent and reagents were all analytically pure and dried through routine protocols. Compounds **2**-**9** were synthesized at Enamine.

Synthesis scheme of compound **1**



N-(5-methoxypyridin-3-yl)-4-nitrobenzenesulfonamide (**1a**): A mixture of 3-amine-5-methoxy-pyridine (0.596 g, 4.8 mmol) and 4-nitrobenzene-1-sulfonyl chloride (1.07 g, 4.8 mmol) in pyridine (15 mL) was stirred at room temperature 1 h. The mixture was concentrated and the residue was purification by column chromatography to give 1a (1.158 g, 78% yield). ESI-MS (m/z) for $C_{12}H_{12}N_3O_5S^+$ $[M+H]^+$: calculated 310.0, found 310.0.

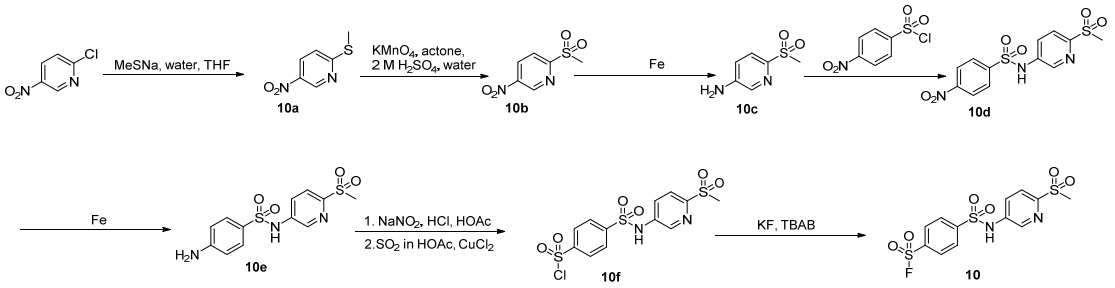
4-amino-N-(5-methoxypyridin-3-yl)benzenesulfonamide (**1b**): To a mixture of **1a** (1.766 g, 5.71 mmol) in EtOH (20 mL) and NH_4Cl (aq, 4 mL) was added iron (1.62 g, 29.0 mmol). The resulting mixture was refluxed for 2 h under stirring. The reaction mixture was filtered with Celite and the filtrate was concentrated. The residue was purified by column chromatography to give **1b** (1.116 g, 70% yield) was prepared from 1a. ^{1}H NMR (400MHz, d_6-DMSO): δ 10.14 (s, 1H), 7.93 (d, *J* = 2.8 Hz, 1H), 7.88-7.87 (d, *J* = 2.0 Hz, 1H), 7.42-7.40 (d, *J* = 8.8 Hz, 2H), 7.02-7.01 (t, *J* = 2.4 Hz, 1H), 6.56-6.54 (d, *J* = 8.8 Hz, 2H), 6.03 (s, 2H), 3.75 (s, 3H). ESI-MS (m/z) for $C_{12}H_{14}N_3O_3S^+$ $[M+H]^+$: calculated 280.1, found 280.0.

4-(N-(5-methoxypyridin-3-yl)sulfamoyl)benzene-1-sulfonyl chloride (**1c**): A 20-mL scintillation vial was charged with **1b** (363 mg, 1.0 mmol). Concentrated hydrochloric acid (1 mL) and water (1 mL) was added, and the suspension was cooled to 0 °C. At the same temperature, sodium nitrite solution of ($NaNO_2$, 40% wt, 0.5 mL) was added dropwise via a syringe. The diazotization process took 30min to complete at 0 °C, in another 20-mL scintillation vial, copper (II) chloride ($CuCl_2$ $2H_2O$, 80 mg) was added a saturated solution of SO_2 in AcOH at 0 °C to make a light green

suspension. The diazonium solution was added to the SO_2 solution at 0 °C. The resulting mixture was allowed to be warmed to 5-10 °C and was stirred for additional 1h. The mixture was poured into ice-water and extracted with EA (20 mL). The organic phase was washed with water, brine, dried over Na_2SO_4 and filtered. The crude product of **1c** was used in the next step without further purification. ESI-MS (m/z) for $C_{12}H_{12}ClN_2O_5S_2^+$ $[M+H]^+$: calculated 363.0, found 363.0.

4-(N-(5-methoxypyridin-3-yl)sulfamoyl)benzene-1-sulfonyl fluoride (**1**): A mixture of crude sulfonyl chloride of **1c**, KF (230 mg, 4mmol) and TBAB (30 mg, 0.1 mmol) in THF (15 mL) and water (3mL) was stirred at room temperature for 4 h. The mixture was extracted with EA (20 mLX2). The organic phase was washed with water, brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by Prep-HPLC to give **1** (163 mg, 47% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 8.36-8.33 (d, *J* = 8.4 Hz, 2H), 8.13-8.11 (d, *J* = 8.0 Hz, 2H), 8.04-8.03 (d, *J* = 2.4 Hz, 1H), 7.90 (d, *J* = 1.6 Hz, 1H), 7.08 (s, 1H), 3.77 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 155.99, 146.89, 134.59, 133.59, 133.62, 130.33, 128.93, 113.23, 56.13. ESI-MS (m/z) for $C_{12}H_{12}FN_2O_5S_2^+$ $[M+H]^+$: calculated 347.0, found 347.0.

Synthesis scheme of compound **10**



2-(methylthio)-5-nitropyridine (**10a**): Sodium methanethiolate (10 mL, 30% wt) was added to a solution of 2-chloro-5-nitropyridine (1.9 g, 12.0 mmol) in THF (40 mL) and water (10 mL) at 0°C. Then the mixture was allowed to warm to room temperature and stirred for 2h. The mixture was extracted with EA (20 mLX2). The organic phase was washed with water, brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by column chromatography (PE/EA= 30:1) to give **10a** (1.1 g, 53.7% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ 9.26-9.25 (d, *J* = 2.4 Hz, 1H), 8.24-8.21 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 1H), 7.31-7.29 (d, *J* = 8.8 Hz, 1H), 2.65 (s, 3H). ESIMS (m/z) for $C_6H_7N_2O_2S^+$ $[M+H]^+$: calculated 171.0, found 171.0.

2-(methylsulfonyl)-5-nitropyridine (**10b**): To a solution of **10a** (1.106 g, 6.5 mmol) in 20 mL of acetone was added 20 mL of H_2SO_4 (1 M, aq) slowly. After that, a solution of $KMnO_4$ (1.4 g, 8.9 mmol) in water (24 mL) was added dropwise to the mixture and then stirred overnight at room

temperature. The suspension was filtered and washed with a mixture of EtOH and MeOH (10:1, v/v). The filtrate was concentrated and the residue was purification was purified by column chromatography to give **10b** (1.002 g, 76% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.52-9.51 (d, *J* = 2.0 Hz, 1H), 8.78-8.75 (dd, *J* = 2.0 Hz, *J* = 8.8 Hz, 1H), 8.33-8.31 (d, *J* = 8.8 Hz, 1H), 3.33 (s, 3H). ESI-MS (m/z) for $C_6H_7N_2O_4S^+$ $[M+H]^+$: calculated 203.0, found 203.0.

6-(methylsulfonyl)pyridin-3-amine (**10c**): To a mixture of **10b** (1.154 g, 5.71 mmol) in EtOH (20 mL) and NH_4Cl (aq, 4 mL) was added iron (1.62 g, 29.0 mmol). The resulting mixture was refluxed for 2 h under stirring. The reaction mixture was filtered with Celite and the filtrate was concentrated. The residue was purified by column chromatography to give **10c** (0.559 g, 57% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ 8.08-8.07 (d, *J* = 2.4 Hz, 1H), 7.82-7.80 (d, *J* = 8.4 Hz, 1H), 7.05-7.03 (dd, *J* = 2.4 Hz, *J* = 8.4 Hz, 1H), 3.13 (s, 3H). ESI-MS (m/z) for $C_6H_9N_2O_2S^+$ $[M+H]^+$: calculated 173.0, found 173.0.

N-(6-(methylsulfonyl)pyridin-3-yl)-4-nitrobenzenesulfonamide (**10d**): A mixture of **10c** (0.827 g, 4.8 mmol) and 4-nitrobenzene-1-sulfonyl chloride (1.07 g, 4.8 mmol) in pyridine (15 mL) was stirred at room temperature 1 h. The mixture was concentrated and the residue was purification by column chromatography to give **10d** (0.793 g, 46% yield). ESI-MS (m/z) for $C_{12}H_{12}N_3O_6S_2^+$ $[M+H]^+$: calculated 358.0, found 358.0.

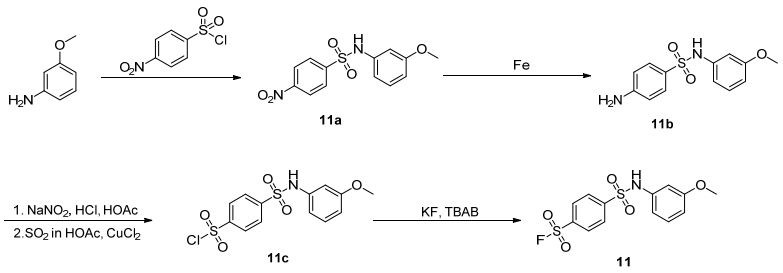
4-amino-N-(6-(methylsulfonyl)pyridin-3-yl)benzenesulfonamide (**10e**): The same procedure was same as **10c**. **10e** (0.32 g, 58.2% yield) was prepared from 10d. ^{1}H NMR (400MHz, d_4-MeOH): δ 8.40-8.39 (d, *J* = 2.4 Hz, 1H), 7.94-7.92 (d, *J* = 8.8 Hz, 1H), 7.80-7.77 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 1H), 7.56-7.54 (d, *J* = 8.8 Hz, 2H), 6.64-6.62 (d, *J* = 8.8 Hz, 2H), 3.14 (s, 3H). ESI-MS (m/z) for $C_{12}H_{14}N_3O_4S_2^+$ $[M+H]^+$: calculated 328.0, found 328.0.

4-(N-(6-(methylsulfonyl)pyridin-3-yl)sulfamoyl)benzene-1-sulfonyl chloride (**10f**): A 20-mL scintillation vial was charged with **10e** (327 mg, 1.0 mmol). Concentrated hydrochloric acid (1 mL) and water (1 mL) was added, and the suspension was cooled to 0 °C. At the same temperature, sodium nitrite solution of ($NaNO_2$, 40% wt, 0.5 mL) was added dropwise via a syringe. The diazotization process took 30min to complete at 0 °C, in another 20-mL scintillation vial, copper (II) chloride ($CuCl_2$ $2H_2O$, 80 mg) was added a saturated solution of SO_2 in AcOH at 0 °C to make a light green suspension. The diazonium solution was added to the SO_2 solution at 0 °C. The resulting mixture was allowed to be warmed to 5-10 °C and was stirred for additional 1h. The mixture was poured into ice-water and extracted with EA (20 mL). The organic phase was washed with water, brine, dried over Na_2SO_4 and filtered. The crude product of **10f** was used for next step

without further purification. ESI-MS (m/z) for $C_{12}H_{12}ClN_2O_6S_3^+$ $[M+H]^+$: calculated 411.0, found 411.0.

4-(N-(6-(methylsulfonyl)pyridin-3-yl)sulfamoyl)benzene-1-sulfonyl fluoride (**10**): A mixture of crude sulfonyl chloride of **10f**, KF (230 mg, 4mmol) and TBAB (30 mg, 0.1 mmol) in THF (15 mL) and water (3mL) was stirred at room temperature for 4 h. The mixture was extracted with EA (20 mLX2). The organic phase was washed with water, brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated and the residue was purified by Prep-HPLC to give **10** (113 mg, 28.6% yield in two steps). ^{1}H NMR (400MHz, d_6-DMSO): δ 11.72 (br, 1H), 8.49 (d, *J* = 2.0 Hz, 1H), 8.39-8.37 (d, *J* = 8.4 Hz, 2H), 8.23-8.21 (d, *J* = 8.4 Hz, 2H), 7.99-7.97 (d, *J* = 8.8 Hz, 1H), 7.85-7.82 (dd, *J* = 2.4 Hz, *J* = 8.8 Hz, 1H), 3.20 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 153.13, 146.23, 141.28, 137.78, 136.32, 136.07, 130.57, 128.98, 127.95, 122.67, 40.56. ESI-MS (m/z) for $C_{12}H_{12}FN_2O_6S_3^+$ $[M+H]^+$: calculated 395.0, found 395.0.

Synthesis scheme of compound **11**



N-(3-methoxyphenyl)-4-nitrobenzenesulfonamide (**11a**): Following the same procedure as **10c**, **11a** (750 mg, 67% yield) was prepared. ESI-MS (m/z) for $C_{13}H_{13}N_2O_5S^+$ $[M+H]^+$: calculated 309.0, found 309.0.

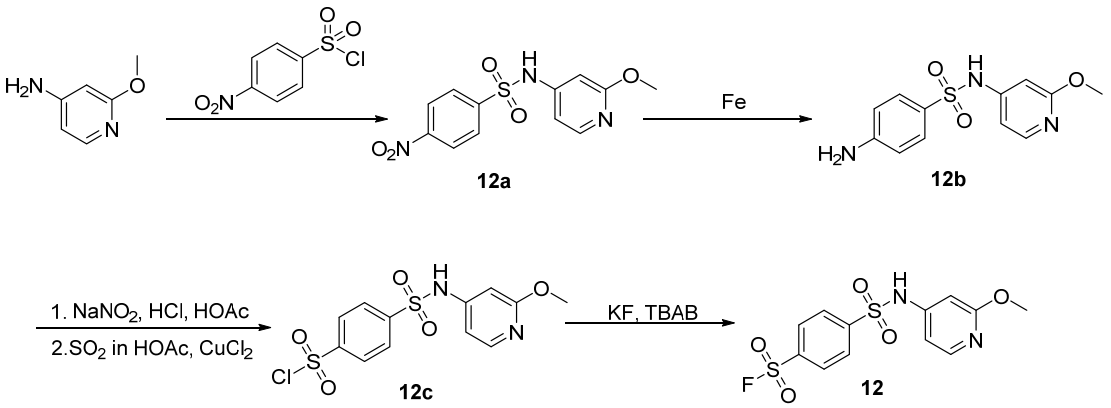
4-amino-N-(3-methoxyphenyl)benzenesulfonamide (**11b**): Following the same procedure as **10b**, **11b** (200 mg, 72% yield) was prepared. ^{1}H NMR (400MHz, d_6-DMSO): δ 9.85 (s, 1H), 7.41-7.39 (d, *J* = 8.4 Hz, 2H), 7.11-7.07 (t, *J* = 8.4 Hz, 1H), 6.65-6.64 (m, 2H), 6.55-6.53 (d, *J* = 8.8 Hz, 3H), 5.95 (s, 2H), 3.65 (s, 3H). ESI-MS (m/z) for $C_{13}H_{15}N_2O_3S^+$ $[M+H]^+$: calculated 279.0, found 279.0.

4-(N-(3-methoxyphenyl)sulfamoyl)benzenesulfonyl chloride (**11c**): Following the same procedure as **10e**, **11c** was prepared and used in the next step without further purification. ESI-MS (m/z) for $C_{13}H_{13}ClNO_5S_2^+$ $[M+H]^+$: calculated 362.0, found 362.0.

4-(N-(3-methoxyphenyl)sulfamoyl)benzene-1-sulfonyl fluoride (**11**): Following the same procedure as **10**, compound **11** was prepared. ^{1}H NMR (400MHz, d_6-DMSO): δ 10.66 (s, 1H),

8.36-8.34 (d, *J* = 8.4 Hz, 2H), 8.11-8.09 (d, *J* = 8.4 Hz, 2H), 7.16 (m, 1H), 6.68-6.66 (m, 3H), 3.67 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 160.28, 146.85, 138.39, 135.78, 130.74, 130.21, 128.94, 113.74, 110.45, 106.94, 55.51. ESI-MS (m/z) for $C_{13}H_{13}FNO_5S_2^+$ $[M+H]^+$: calculated 346.0, found 346.0.

Synthesis scheme of compound **12**



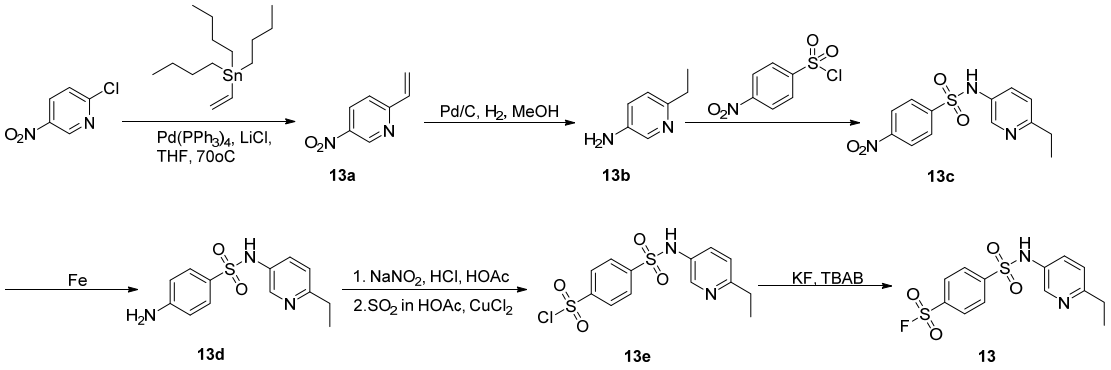
N-(2-methoxypyridin-4-yl)-4-nitrobenzenesulfonamide (**12a**): Following the same procedure as **12c**, **12a** (510 mg, 78% yield) was prepared.

4-amino-N-(2-methoxypyridin-4-yl)benzenesulfonamide (**12b**): Following the same procedure as **10b**, **12b** (325 mg, 70% yield) was prepared. ^{1}H NMR (400MHz, d_4-MeOH): δ 8.17-8.15 (m, 2H), 7.91-7.89 (d, *J* = 7.6 Hz, 1H), 7.77-7.73 (d, *J* = 8.4 Hz, 1H), 7.16-7.15 (d, *J* = 4.0 Hz, 1H), 7.10-7.09 (d, *J* = 4.0 Hz, 1H), 6.87 (s, 1H), 3.35 (s, 3H).

4-(N-(2-methoxypyridin-4-yl)sulfamoyl)benzenesulfonyl chloride (**12c**): Following the same procedure as **10f**, **12c** was prepared and used in the next step without further purification.

4-(N-(2-methoxypyridin-4-yl)sulfamoyl)benzenesulfonyl fluoride (**12**): Following the same procedure as **10**, **12** was prepared. ^{1}H NMR (400MHz, d_6-DMSO): δ 8.39-8.37 (d, *J* = 8.0 Hz, 2H), 8.22-8.20 (d, *J* = 8.0 Hz, 2H), 7.97-7.96 (d, *J* = 5.6 Hz, 1H), 6.75-6.73(dd, *J* = 1.6 Hz, *J* = 5.6 Hz, 1H), 6.47-6.46 (d, *J* = 1.6 Hz, 1H), 3.78 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 164.59, 158.99, 158.61, 148.53, 147.60, 146.69, 136.13, 135.88, 130.46, 128.90, 107.90, 97.90, 54.04. ESI-MS (m/z) for $C_{12}H_{12}FN_2O_5S_2^+$ $[M+H]^+$: calculated 347.0, found 347.0.

Synthesis scheme of compound **13**



5-nitro-2-vinylpyridine (**13a**): A mixture of 2-chloro-5-nitropyridine (1.5 g, 9.5 mmol), tributyl(vinyl)stannane (5.5 ml, 18.9 mmol), $Pd(PPh_3)_4$ (500 mg, 0.43 mmol) and LiCl (2.8 g, 66.2 mmol) in dry THF (40 mL) was stirred at 70 °C overnight under N_2. The mixture was concentrated and the residue was purified by column chromatography (PE/EA= 10:1) to give **13a** as a white solid (1.0 g, 71% yield). ^{1}H NMR (400MHz, d_4-MeOH): δ 9.32-9.31 (d, *J* = 2.4 Hz, 1H), 8.55-8.53 (dd, *J* = 2.8 Hz, *J* = 8.4 Hz, 1H), 7.71-7.69 (d, *J* = 8.4 Hz, 1H), 8.99-8.97 (dd, *J* = 8.0 Hz, *J* = 17.2 Hz, 1H), 6.49-6.44 (dd, *J* = 0.8 Hz, *J* = 17.2 Hz, 1H), 5.75-5.72 (dd, *J* = 0.8 Hz, *J* = 10.8 Hz, 1H). ESI-MS (m/z) for $C_7H_7N_2O_2^+$ $[M+H]^+$: calculated 151.0, found 151.0.

6-ethylpyridin-3-amine (**13b**): A mixture of **13a** (1.0 g, 6.7 mmol) and palladium on carbon (200 mg, 10% wt) in EtOH was stirred overnight under H_2 (2 atm). The mixture was filtered and the filtrated was concentrated to give **13b** as light-yellow oil (740 mg, 91% yield). ^{1}H NMR (400MHz, d_4-MeOH): δ 7.88-7.87 (d, *J* = 2.4 Hz, 1H), 7.09-7.06 (dd, *J* = 2.8 Hz, *J* = 8.4 Hz, 1H), 7.03-7.01 (d, *J* = 8.4 Hz, 1H), 2.68-2.62 (q, *J* = 7.6 Hz, 2H), 1.23-1.19 (t, *J* = 7.6 Hz, 3H). ESI-MS (m/z) for $C_7H_{11}N_2^+$ $[M+H]^+$: calculated 123.1, found 123.1.

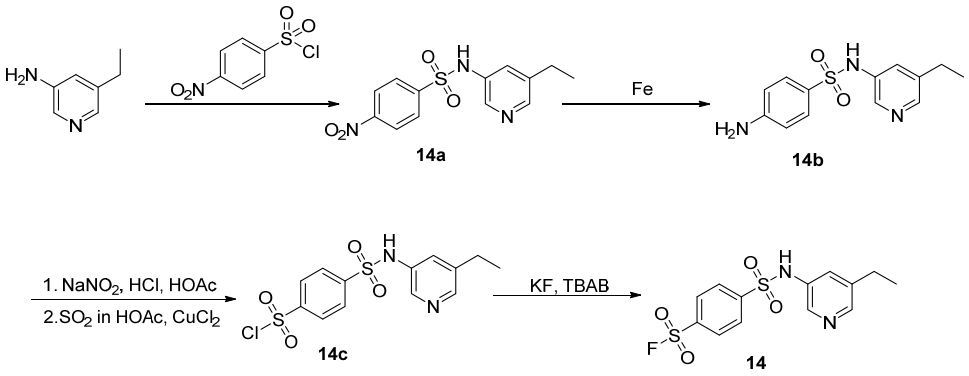
N-(6-ethylpyridin-3-yl)-4-nitrobenzenesulfonamide (**13c**): Following the same procedure as **10c**, **13c** was prepared as a yellow solid (1.67 g, 91% yield). ESI-MS (m/z) for $C_{13}H_{14}N_3O_4S^+$ $[M+H]^+$: calculated 308.1, found 308.0.

4-amino-N-(6-ethylpyridin-3-yl)benzenesulfonamide (**13d**): Following the same procedure as **10b**, **13d** was prepared as a yellow solid (1.36 g, 90% yield). ^{1}H NMR (400MHz, d_4-MeOH): δ 8.07-8.08 (d, *J* = 2.4 Hz, 1H), 7.52-7.49 (dd, *J* = 2.8 Hz, *J* = 8.4 Hz, 1H), 7.41-7.39 (d, *J* = 8.8 Hz, 2H), 7.03-7.01 (d, *J* = 8.4 Hz, 1H), 6.60-6.59 (d, *J* = 8.8 Hz, 2H), 2.74-2.68 (q, *J* = 7.6 Hz, 2H), 1.24-1.20 (t, *J* = 7.6 Hz, 3H). ESI-MS (m/z) for $C_{13}H_{16}N_3O_2S^+$ $[M+H]^+$: calculated 278.1, found 278.1.

4-(N-(6-ethylpyridin-3-yl)sulfamoyl)benzene-1-sulfonyl chloride (**13e**): Following the same procedure as **10e**, **13e** was prepared and used in the next step without further purification. ESI-MS (m/z) for$C_{13}H_{14}ClN_2O_4S_2^+$ $[M+H]^+$: calculated 361.0, found 361.0.

4-(N-(6-ethylpyridin-3-yl)sulfamoyl)benzene-1-sulfonyl fluoride (**13**): Following the same procedure as **10**, **13** was prepared as a white solid (270 mg, 54% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 8.36-8.34 (d, *J* = 8.4 Hz, 2H), 8.22-8.21 (d, *J* = 2.4 Hz, 1H), 8.10-8.08 (d, *J* = 8.4 Hz, 2H), 7.53-7.50 (m, 1H), 7.29-7.27 (d, *J* = 8.4 Hz, 2H), 2.73-2.67 (q, *J* = 7.6 Hz, 2H), 2.18-2.14 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 159.04, 158.87, 158.68, 146.51, 140.52, 135.96, 135.73, 132.35, 131.91, 130.35, 128.95, 123.97, 29.26, 13.77. ESI-MS (m/z) for $C_{13}H_{14}FN_2O_4S_2^+$ $[M+H]^+$: calculated 345.0, found 345.0.

Synthesis scheme of compound **14**



N-(5-ethylpyridin-3-yl)-4-nitrobenzenesulfonamide (**14a**): Following the same procedure as **10c**, **14a** (470 mg, 75% yield) was prepared. ESI-MS (m/z) for $C_{13}H_{14}N_3O_4S^+$ $[M+H]^+$: calculated 308.1, found 308.0.

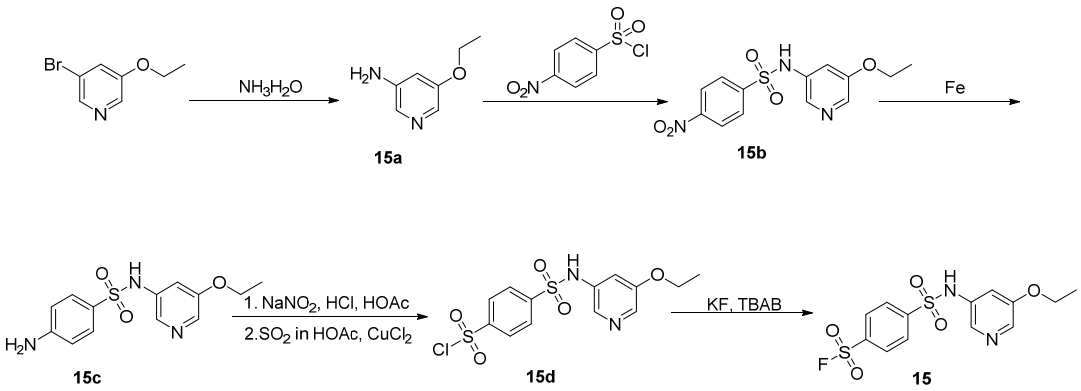
4-amino-N-(5-ethylpyridin-3-yl)benzenesulfonamide (**14b**): Following the same procedure as **10b**, **14b** was prepared. ESI-MS (m/z) for $C_{13}H_{16}N_3O_2S^+$ $[M+H]^+$: calculated 278.1, found 278.1.

4-(N-(5-ethylpyridin-3-yl)sulfamoyl)benzenesulfonyl chloride (**14c**): Following the same procedure as **10e**, **14c** was prepared and used in the next step without further purification. ESI-MS (m/z) for $C_{13}H_{14}ClN_2O_4S_2^+$ $[M+H]^+$: calculated 361.0, found 361.0.

4-(N-(5-ethylpyridin-3-yl)sulfamoyl)benzenesulfonyl fluoride (**14**): Following the same procedure as **10**, **14** was prepared. ^{1}H NMR (400MHz, d_6-DMSO): δ 8.36-8.34 (d, *J* = 8.4 Hz, 2H), 8.25 (s, 1H), 8.16 (s, 1H), 8.12-8.09 (d, *J* = 8.4 Hz, 2H), 7.41 (s, 1H), 2.61-2.55 (q, *J* = 7.6 Hz, 2H), 1.12-1.09 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 146.45, 145.10, 140.84, 139.21, 135.97,

135.73, 134.30, 130.33, 129.11, 128.98, 25.40, 15.37. ESI-MS (m/z) for $C_{13}H_{14}FN_2O_4S_2^+$ $[M+H]^+$: calculated 345.0, found 345.0.

Synthesis scheme of compound **15**



5-ethoxypyridin-3-amine (**15a**): A mixture of 3-bromo-5-ethoxypyridine (2.1 g, 10.0 mmol) and $CuSO_4$ $5H_2O$ (0.5 g, 2.0 mmol) in Ammonium hydroxide (40 mL, 25%) was stirred at 140 °C overnight in seal tube. The mixture was cooled to room temperature and added 1N NaOH (5 mL, aq.). The mixture was concentrated and the residue was purified by column chromatography (PE/EA=1:1) to give **15a** as a yellow oil (138 mg, 10% yield). ESI-MS (m/z) for C7H11NO $^+$ $[M+H]^+$: calculated 345.0, found 345.0.

N-(5-ethoxypyridin-3-yl)-4-nitrobenzenesulfonamide (**15b**): Following the same procedure as **10c**, **15b** was prepared as a yellow solid (210 mg, 91% yield). ESI-MS (m/z) for $C_{13}H_{14}N_3O_5S^+$ $[M+H]^+$: calculated 324.1, found 324.0.

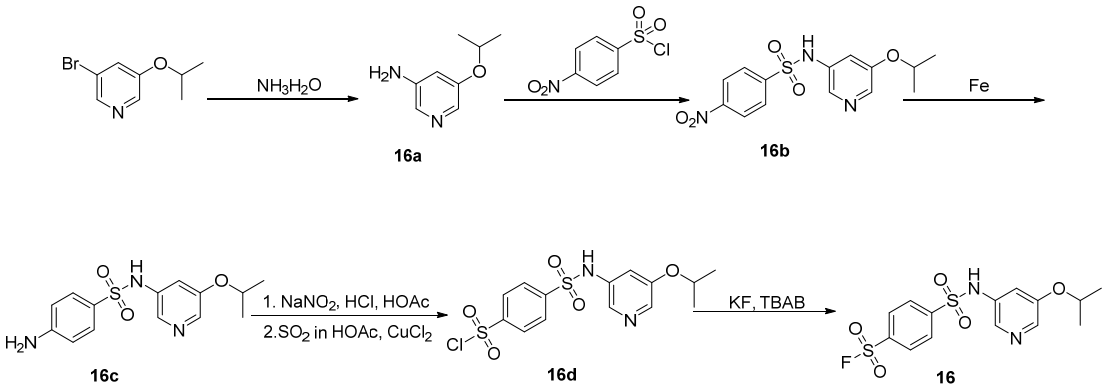
4-amino-N-(5-ethoxypyridin-3-yl)benzenesulfonamide (**15c**): Following the same procedure as **10b**, **15c** was prepared as a white solid (177 mg, 93% yield). ^{1}H NMR (400MHz, d_4-MeOH): δ 7.88-7.87 (d, *J* = 2.4 Hz, 1H), 7.79 (d, *J* = 2.4 Hz, 1H), 7.46-7.44 (d, *J* = 8.8 Hz, 2H), 7.15-7.14 (t, *J* = 2.4 Hz, 1H), 6.62-6.60 (d, *J* = 8.8 Hz, 2H), 4.06-4.01 (q, *J* =6.8 Hz, 2H), 1.39-1.36 (t, *J* = 6.8 Hz, 3H). ESI-MS (m/z) for $C_{13}H_{16}N_3O_3S^+$ $[M+H]^+$: calculated 294.1, found 294.1.

4-(N-(5-ethoxypyridin-3-yl)sulfamoyl)benzenesulfonyl chloride (**15d**): Following the same procedure as **10e**, **15d** was prepared and used in the next step without further purification. ESI-MS (m/z) for $C_{13}H_{14}ClN_2O_5S_2^+$ $[M+H]^+$: calculated 377.0, found 377.0.

4-(N-(5-ethoxypyridin-3-yl)sulfamoyl)benzenesulfonyl fluoride (**15**): Following the same procedure as **10**, **15** was prepared as a white solid (100 mg, 46% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 10.95 (s, 1H), 8.37-8.34 (d, *J* = 8.8 Hz, 2H), 8.13-8.11 (d, *J* = 8.8 Hz, 2H), 8.05 (d, *J* =

2.4 Hz, 1H), 7.90 (d, *J* = 2.4 Hz, 1H), 7.07 (s, 1H), 4.07-4.01 (q, *J* = 7.2 Hz, 2H), 1.32-1.28 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 155.23, 146.43, 136.00, 135.76, 134.64, 134.37, 130.37, 128.95, 113.74, 64.30, 14.79. ESI-MS (m/z) for $C_{13}H_{14}FN_2O_5S_2^+$ $[M+H]^+$: calculated 361.0, found 361.0.

Synthesis scheme of compound **16**



5-isopropoxypyridin-3-amine (**16a**): Following the same procedure as **15a**, **16a** (390 mg, 21% yield) was prepared. ^{1}H NMR (400MHz, $CDCl_3$): δ 7.72 (d, *J* = 2.4 Hz, 1H), 7.70-7.69 (d, *J* = 2.4 Hz, 1H), 6.51-6.50 (t, *J* = 2.4 Hz, 1H), 4.55-4.49 (m, 1H), 1.34-1.32 (d, *J* = 6.0 Hz, 6H). ESI-MS (m/z) for $C_8H_{13}N_2O^+$ $[M+H]^+$: calculated 153.1, found 153.1.

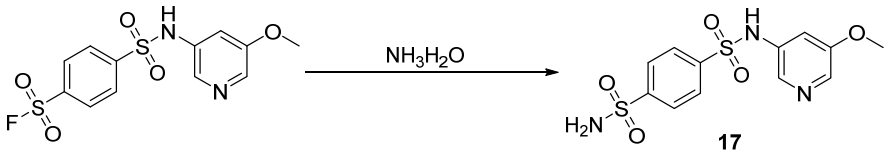
N-(5-isopropoxypyridin-3-yl)-4-nitrobenzenesulfonamide (**16b**): Following the same procedure as **10c**, **16b** was prepared. ESI-MS (m/z) for $C_{14}H_{16}N_3O_5S^+$ $[M+H]^+$: calculated 337.1, found 337.1.

4-amino-N-(5-isopropoxypyridin-3-yl)benzenesulfonamide (**16c**): Following the same procedure as **10b**, **16c** was prepared. ESI-MS (m/z) for $C_{14}H_{18}N_3O_3S^+$ $[M+H]^+$: calculated 308.1, found 308.1;

4-(N-(5-isopropoxypyridin-3-yl)sulfamoyl)benzenesulfonyl chloride (**16d**): Following the same procedure as **10e**, **16d** was prepared and used for next step without further purification. ESI-MS (m/z) for $C_{14}H_{16}ClN_2O_5S_2^+$ $[M+H]^+$: calculated 391.0, found 391.0.

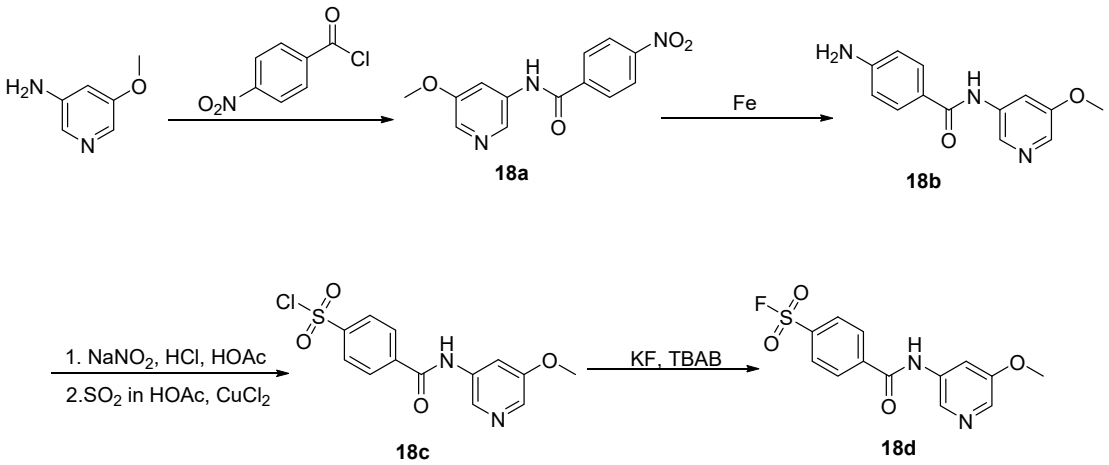
4-(N-(5-isopropoxypyridin-3-yl)sulfamoyl)benzenesulfonyl fluoride (**16**): Following the same procedure as **10e**, **16** (14 mg, 15% yield) was prepared. ^{1}H NMR (400MHz, d_6-DMSO): δ 10.92 (s, 1H), 8.37-8.35 (d, *J* = 8.4 Hz, 2H), 8.12-8.10 (d, *J* = 8.4 Hz, 2H), 8.03 (d, *J* = 2.4 Hz, 2H), 7.89 (d, *J* = 2.4 Hz, 2H), 7.03-7.02 (t, *J* = 2.4 Hz, 2H), 4.60-4.54 (m, 1H), 1.23-1.21 (d, *J* = 6.0 Hz, 6H). ^{13}C NMR (400MHz, d_6-DMSO): δ 154.26, 146.40, 136.01, 135.76, 135.48, 134.70, 130.36, 130.09, 129.09, 128.96, 115.14, 70.91, 21.86. ESI-MS (m/z) for $C_{14}H_{16}FN_2O_5S_2^+$ $[M+H]^+$: calculated 375.0, found375.0.

Synthesis scheme of compound **17**



N-(5-methoxypyridin-3-yl)benzene-1,4-disulfonamide (**17**): compound **1** (30 mg, 0.1 mmol) in a mixture of ammonium hydroxide (5 mL, 28% wt.) and dioxane (3 mL) was stirred for 2 h in a seal tube. The mixture was concentrated and the residue was purified by prep-HPLC to give **17** (11 mg, 37% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 8.06-7.93 (m, 5H), 7.60 (s, 1H), 7.12 (s, 1H), 3.78 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 146.48, 142.22, 133.09, 132.65, 128.11, 127.35, 113.57, 56.27. ESI-MS (m/z) for $C_{12}H_{14}N_3O_5S_2^+$ $[M+H]^+$: calculated 344.0, found 344.0.

Synthesis scheme of compound **18**



N-(5-methoxypyridin-3-yl)-4-nitrobenzamide (**18a**): A mixture of 5-methoxypyridin-3-amine (300 mg, 2.4 mmol), 4-nitrobenzoyl chloride (445 mg, 2.4 mmol) and Et_3N (485 mg, 4.8 mmol) in DCM was stirred for 30 min. The mixture was concentrated and the residue was purified by column chromatography to give **18a** (500 mg, 76% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 10.85 (s, 1H), 8.61-8.60 (d, *J* = 2.0 Hz, 1H), 8.40-8.38 (m, 2H), 8.24-8.22 (m, 2H), 8.10 (d, *J* = 1.6 Hz, 1H), 7.91-7.90 (t, *J* = 2.0 Hz, 1H), 3.85 (s, 3H). ESI-MS (m/z) for $C_{13}H_{12}N_3O_4^+$ $[M+H]^+$: calculated 274.1, found 274.0.

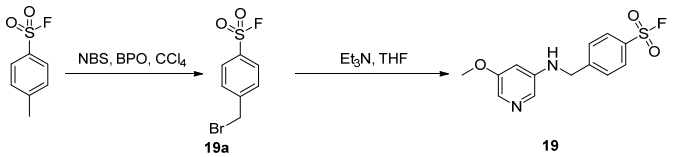
4-amino-N-(5-methoxypyridin-3-yl)benzamide (**18b**): Following the same procedure as **10b**, **18b** (405 mg, 91% yield) was prepared. ^{1}H NMR (400MHz, $CDCl_3$): δ 8.43 (s, 1H), 8.20 (d, *J* = 1.6 Hz,

1H), 8.05-8.02 (m, 2H), 7.73-7.71 (d, *J* = 8.8 Hz, 2H), 6.66-6.64 (d, *J* = 8.8 Hz, 2H), 3.83 (s, 3H). ESI-MS (m/z) for $C_{13}H_{14}N_3O_2^+$ $[M+H]^+$: calculated 244.1, found 244.1.

4-((5-methoxypyridin-3-yl)carbamoyl)benzenesulfonyl chloride (**18c**): Following the same procedure as **10e**, **18c** was prepared and used in the next step without further purification. ESI-MS (m/z) for $C_{13}H_{12}ClN_2O_4S^+$ $[M+H]^+$: calculated 327.0, found 327.0.

4-((5-methoxypyridin-3-yl)carbamoyl)benzenesulfonyl fluoride (**18**): Following the same procedure as **10**, **18** (67 mg, 34% yield) was prepared. ^{1}H NMR (400MHz, $CDCl_3$): δ 10.94 (s, 1H), 9.02 (s, 1H), 8.93 (s, 1H), 8.34-8.32 (d, *J* = 8.4 Hz, 2H), 8.15-8.13 (d, *J* = 8.4 Hz, 2H), 7.97 (s, 1H), 4.04 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 164.78, 156.33, 141.52, 141.45, 137.15, 134.75, 134.51, 132.76, 132.32, 131.49, 130.10, 129.20, 114.59, 56.43. ESI-MS (m/z) for $C_{13}H_{12}FN_2O_4S^+$ $[M+H]^+$: calculated 311.0, found 311.0.

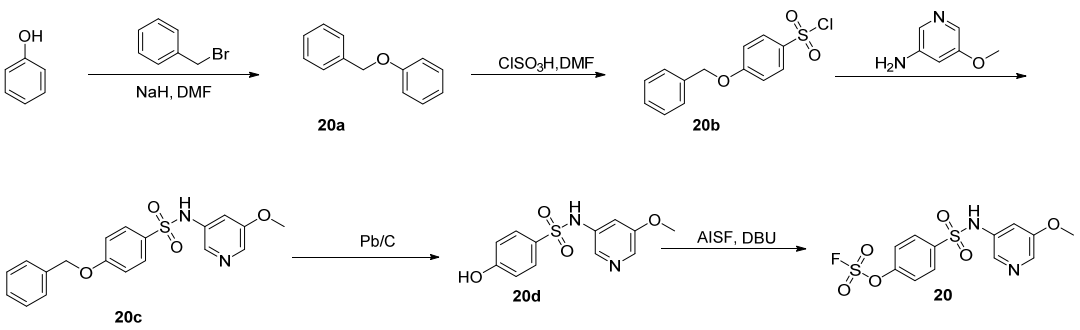
Synthesis scheme of compound **19**



4-(bromomethyl)benzenesulfonyl fluoride (**19a**): To a mixture of 4-methylbenzenesulfonyl fluoride (500 mg, 2.8 mmol) and NBS (920 mg, 1.8 mmol) in CCl_4 (20 mL) was added BPO (39 mg, 0.14 mmol) at 80 °C. After the reaction completed, the mixture was cooled to room temperature and concentrated. The residue was column chromatography (PE/EA=4:1) to give crude **19a** (510 mg , 72% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 7.99-7.97 (d, *J* = 8.0 Hz, 2H), 7.67-7.66 (d, *J* = 8.0 Hz, 2H), 4.54 (s, 2H).

4-(((5-methoxypyridin-3-yl)amino)methyl)benzenesulfonyl fluoride (**19**): To a solution of **19a** (510 mg , 2.0 mmol), Et_3N (0.8 g, 4.0 mmol) and 5-methoxypyridin-3-amine (250 mg, 2.0 mmol) In THF (20 mL) was refluxed for 4h. The mixture was concentrated and the residue was purified by prep-HPLC to give **19** (4.3 mg, 1.0% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 8.27 (s, 1H), 8.24-8.22 (d, *J* = 8.0 Hz, 2H), 7.89 (s, 1H), 7.84-7.82 (d, *J* = 8.0 Hz, 2H), 7.23 (s, 1H), 6.79 (br, 1H), 5.80 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 159.14, 150.13, 143.51, 132.59, 132.35, 130.54, 129.62, 126.57, 126.31, 122.86, 121.56, 111.32, 62.74, 57.22. ESI-MS (m/z) for $C_{13}H_{14}FN_2O_3S^+$ $[M+H]^+$: calculated 297.1, found 297.1.

Synthesis scheme of compound **20**



Benzyloxy-benzene (**20a**): To a solution of phenol (5.0 g, 53.1 mmol) in DMF (40 mL) was added 60% of NaH (5.3 g, 132.8 mmol) at 0 °C under N_2. The resulting mixture was warmed to room temperature and stirred for 30 min. BnBr (9.8 g, 55.8 mmol) was added at 0 °C. The mixture was stirred at room temperature overnight. The reaction mixture was quenched with water (150 mL). The mixture was extracted with EA (20 mLX3). The organic phase was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated. The residue was purified by column chromatography (PE-PE/EA=20/1) to give **20a** (3.0 g, 31% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ 7.39-7.38 (m, 2H), 7.35-7.33 (m, 2H), 7.28-7.22 (m, 3H), 5.95-5.92 (m, 3H), 4.99 (s, 2 H).

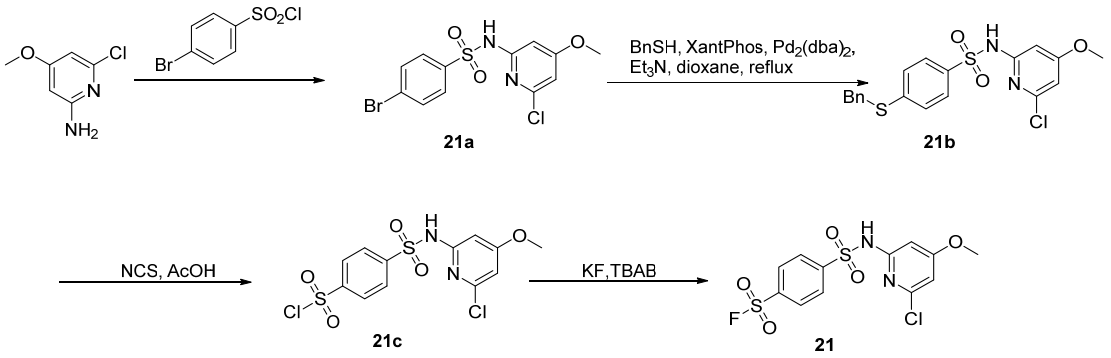
4-(benzyloxy)benzenesulfonyl chloride (**20b**): A mixture of **20a** (3.0 g, 16.3 mmol) and sulfurochloridic acid (5.7 g, 48.9 mmol) in DMF (10 mL) was heated to 100 °C and stirred for 4h. The mixture was cooled to room temperature and poured into ice-water. The mixture was extracted with EA (20 mLX3). The organic phase was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated to give crude **20b**, which was used for next step without further purification.

4-(benzyloxy)-N-(5-methoxypyridin-3-yl)benzenesulfonamide (**20c**): Following the same procedure as **10c**, **20c** (310 mg, 64% yield) was prepared. ESI-MS (m/z) for $C_{19}H_{19}N_2O_4S^+$ $[M+H]^+$: calculated 371.1, found 371.1.

4-hydroxy-N-(5-methoxypyridin-3-yl)benzenesulfonamide (**20d**): A solution of **20c** (310 mg, 0.84 mmol) and 10% of Pd/C (50 mg) in MeOH (50 mL) was stirred for 3 h under H_2. The mixture as filtered and the filtrate was concentrated to give **20d** (0.2 g, 84% yield), which was used in the next step without further purification. ^{1}H NMR (400MHz, d_6-DMSO): δ 7.95-7.94 (d, *J* = 2.4 Hz, 1H), 7.87-7.86 (d, *J* = 2.4 Hz, 1H), 7.62-7.60 (d, *J* = 8.8 Hz, 2H), 7.03-7.02 (t, *J* = 2.4 Hz, 1H), 6.87-6.85 (d, *J* = 8.8 Hz, 2H), 3.75 (s, 3H). ESI-MS (m/z) for $C_{12}H_{13}N_2O_4S^+$ $[M+H]^+$: calculated 281.0, found 281.0.

4-(N-(5-methoxypyridin-3-yl)sulfamoyl)phenyl sulfurofluoridate (**20**): To a solution of **20d** (200 mg, 0.7 mmol) and (4-acetamidophenyl)-(fluorosulfonyl)-sulfamoyl fluoride (AISF) (224 mg, 0.71 mmol) in THF (10 mL) was added DBU (215 mg, 1.42 mmol) slowly (over 30s). The mixture was stirred at room temperature for 10 min. The mixture was diluted with EA, then washed with 0.3 N HCl, water and brine. The organic phase was dried over Na_2SO_4 and filtered. The filtrate was concentrated and residue was purified by prep-HPLC to give **20** (14 mg, 5.4% yield). ^{1}H NMR (400MHz, d_6-DMSO): δ 8.04-8.02 (m, 1H), 8.01-7.99 (m, 2H), 7.92-7.91 (d, *J* = 1.6 Hz, 1H), 7.86-7.84 (d, *J* = 8.8 Hz, 1H), 7.08-7.06 (t, *J* = 2.4 Hz, 1H), 3.77 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 163.45, 155.93, 140.25, 134.97, 134.24, 133.74, 130.19, 123.08, 112.89, 56.08. ESI-MS (m/z) for $C_{12}H_{12}FN_2O_6S_2^+$ $[M+H]^+$: calculated 363.0, found 363.0.

Synthesis scheme of compound **21**



4-bromo-N-(6-chloro-4-methoxypyridin-2-yl)benzenesulfonamide (**21a**): Following the same procedure as **10c**, **21a** (130 mg, 84% yield) was prepared. ESI-MS (m/z) for $C_{12}H_{11}BrClN_2O_3S^+$ $[M+H]^+$: calculated 376.9, 378.9, found 377.0, 379.0.

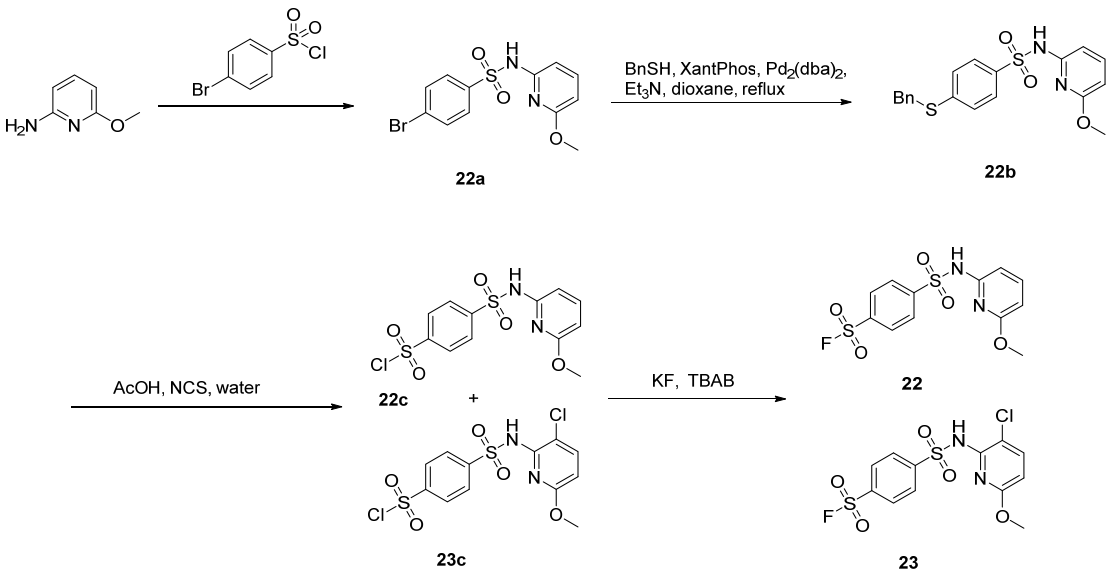
4-(benzylthio)-N-(6-chloro-4-methoxypyridin-2-yl)benzenesulfonamide (**21b**): A mixture of **21a** (415 mg, 1.1 mmol), BnSH (171 mg, 1.4 mmol), $Pd_2(dba)_3$ (101 mg, 0.11 mmol) , xantphos (104 mg, 0.22 mmol), and DIEA (284 mg, 2.2 mmol) in dioxane (10 mL) was heated to 100 °C under N_2, and stirred overnight. The reaction mixture was cooled to room temperature, and filtered over Celite. The filtrate was concentrated. The residue was dissolved in EA (40 mL), washed with brine, and dried over Na_2SO_4. The solution was filtered, and the filtrate was concentrated. The residue was purified by column chromatography (PE-PE/EA=1/1) to give **21b** (357 mg, 77% yield). ESI-MS (m/z) for $C_{19}H_{18}ClN_2O_3S_2^+$ $[M+H]^+$: calculated 421.0, found 421.0.

4-(N-(6-chloro-4-methoxypyridin-2-yl)sulfamoyl)benzenesulfonyl chloride (**21c**): following To a solution of **21b** (190 mg, 0.68 mmol) in AcOH (3 mL) and water (0.3 mL) was added to NCS (275 mg, 2.0 mmol) at 0 °C. The mixture was stirred 1h and then warmed to room temperature. After

2h, the mixture was diluted with EA, dried over Na_2SO_4 and filtered. The filtrated was concentrated to give 6-hydroxypyridine-3-sulfonyl chloride (crude), which was used for next step without further purification. ESI-MS (m/z) for $C_{12}H_{11}Cl_2N_2O_5S_2^+$ $[M+H]^+$: calculated 396.9, found 397.0.

4-(N-(6-chloro-4-methoxypyridin-2-yl)sulfamoyl)benzenesulfonyl fluoride (**21**): Following the same procedure as **10**, **21** (5.4 mg, 5.4% yield) was prepared. ^{1}H NMR (400MHz, $CDCl_3$): δ 8.21-8.13 (m, 4H), 6.76 (d, *J* = 2.0 Hz, 1H), 6.60 (d, *J* = 2.4 Hz, 1H), 3.86 (s, 3H). ESI-MS (m/z) for $C_{12}H_{11}ClFN_2O_5S_2^+$ $[M+H]^+$: calculated 381.0, found 381.0.

Synthesis scheme of compound **22 and 23**



4-bromo-N-(6-methoxypyridin-2-yl)benzenesulfonamide (**22a**): Following the same procedure as **10c**, **22a** was prepared as white solid (350 mg, 88% yield). ESI-MS (m/z) for $C_{12}H_{12}BrN_2O_3S^+$ $[M+H]^+$: calculated 343.0, 345.0, found 343.0, 345.0.

4-(benzylthio)-N-(6-methoxypyridin-2-yl)benzenesulfonamide (**22b**): Following the same procedure as **21b**, **22b** was prepared as a yellow solid (240 mg, 61% yield). ESI-MS (m/z) for $C_{19}H_{19}N_2O_3S_2^+$ $[M+H]^+$: calculated 387.1, found 387.0.

Synthesis of **22c** and **23c**: Following the same procedure was same as **21c**, **22c** was prepared and used in the next step without further purification.

4-(N-(6-methoxypyridin-2-yl)sulfamoyl)benzenesulfonyl fluoride (**22**): Following the same procedure as **10**, **22** was prepared as white solid (4.4 mg, 5% yield) from **22c**. ^{1}H NMR (400MHz, $CDCl_3$): δ 8.22-8.20 (d, *J* = 8.4 Hz, 2H), 8.14-8.12 (d, *J* = 8.4 Hz, 2H), 7.54-8.50 (t, *J* = 8.0 Hz,

3H), 6.78-6.76 (d, *J* = 8.0 Hz, 1H), 6.47-6.45 (d, *J* = 8.0 Hz, 1H), 3.72 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 162.95, 149.48, 148.52, 141.66, 135.59, 135.35, 130.09, 129.24, 105.06, 104.48, 53.78. ESI-MS (m/z) for $C_{12}H_{12}FN_2O_5S_2^+$ $[M+H]^+$: calculated 347.0, found 347.0.

4-(N-(3-chloro-6-methoxypyridin-2-yl)sulfamoyl)benzenesulfonyl fluoride (**23**): Following the same procedure as **10**, **23** was prepared as white solid (11 mg, 12% yield) from **23c**. ^{1}H NMR (400MHz, $CDCl_3$): δ 8.38-8.36 (d, *J* = 8.4 Hz, 2H), 8.19-8.17 (d, *J* = 8.4 Hz, 2H), 7.67 (s, 1H), 7.50-7.48 (d, *J* = 8.8 Hz, 1H), 6.42-6.40 (d, *J* = 8.8 Hz, 1H), 3.65 (s, 3H). ^{13}C NMR (400MHz, d_6-DMSO): δ 161.15, 149.81, 145.74, 141.64, 135.19, 129.98, 129.01, 112.10, 107.53, 54.22. ESI-MS (m/z) for $C_{12}H_{11}ClFN_2O_5S_2^+$ $[M+H]^+$: calculated 381.0, found 381.0.

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