Fragment based discovery of a small molecule RhoGDI2 ligand HR3199 that inhibits cancer cell metastasis

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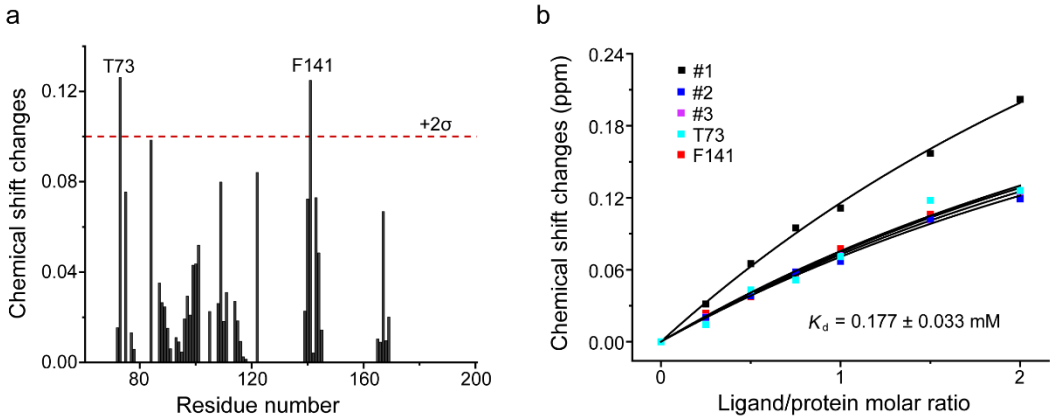
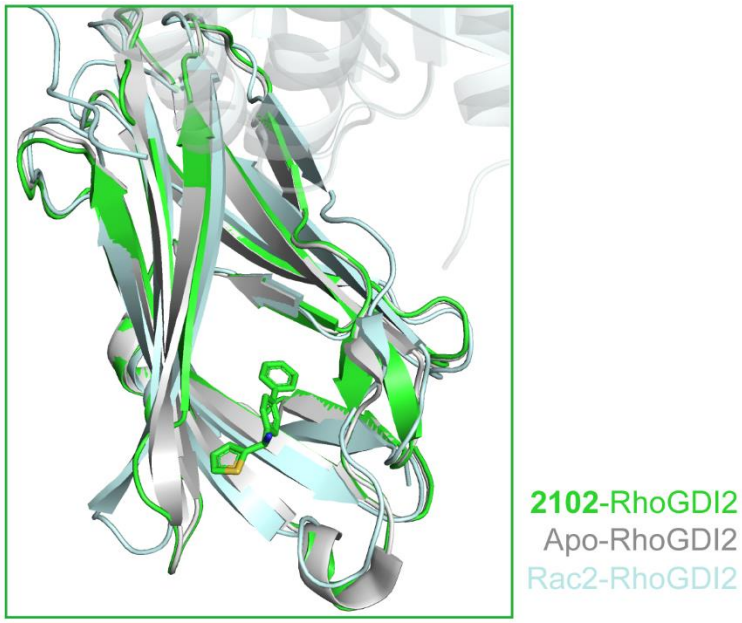
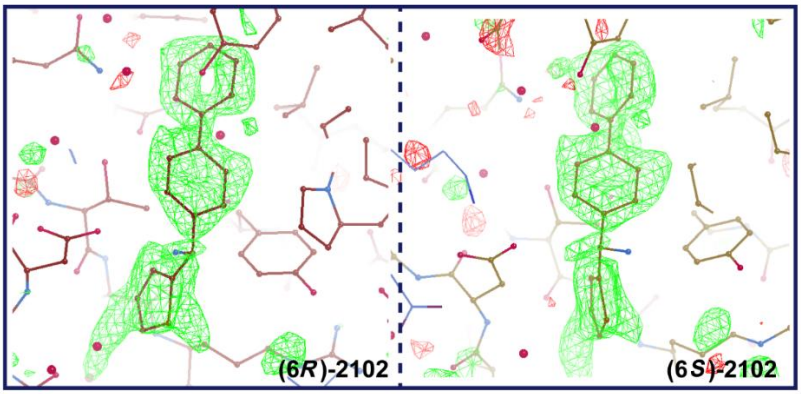


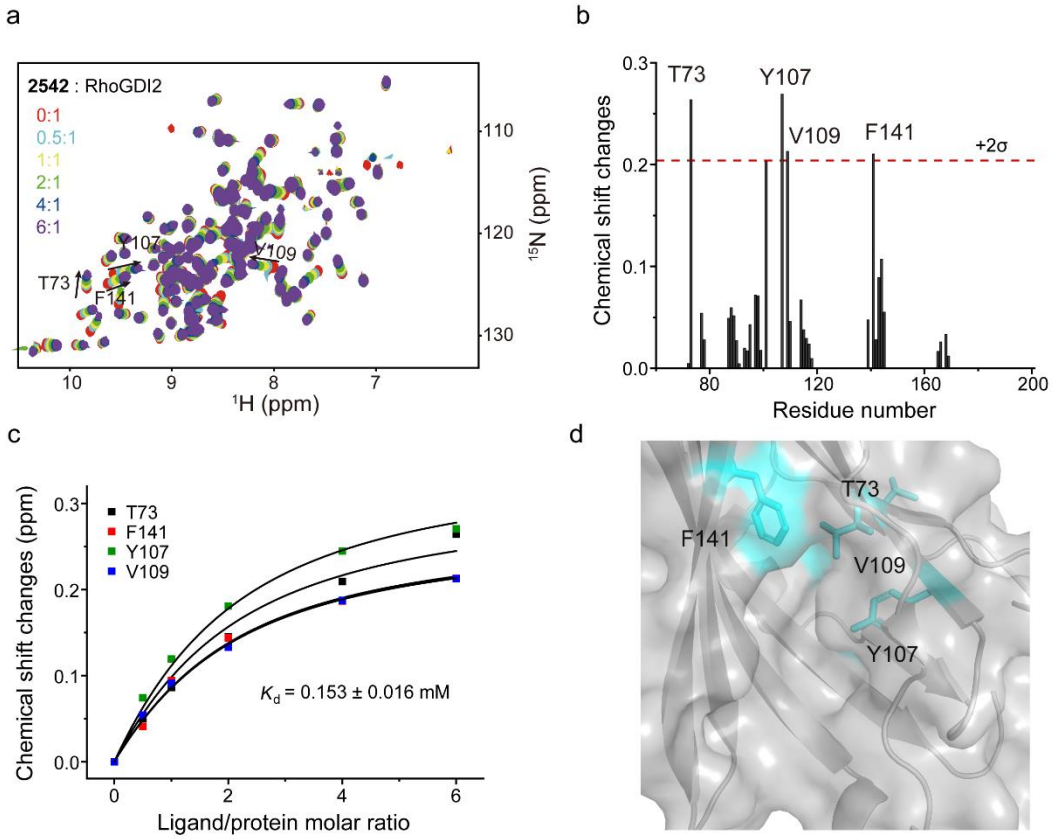
Supplementary Fig. 1. The binding affinity of **2102** determined by the dose-dependent chemical shift perturbation of RhoGDI2. **a**) The residue-by-residue chemical shift changes of RhoGDI2 perturbed by a 2-fold excess of **2102**. The red dashed line represents two standard deviations above the average of the chemical shift perturbations including unassigned residues. **b**) The binding affinity of **2102** determined by best fitting of the dose-dependent chemical shift changes of RhoGDI2. Annotated is the K_d value with a fitting error. Unassigned residues are labeled as #1/2/3.



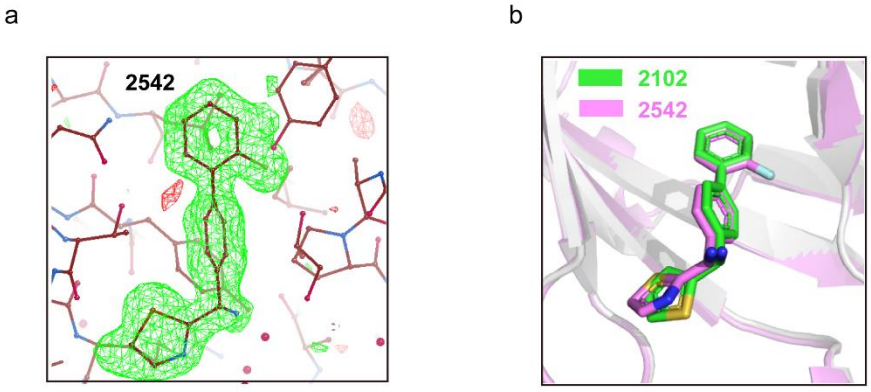
Supplementary Fig. 2. Structural comparison of RhoGDI2 in its free from or in the bound form with Rac2 or **2102**.



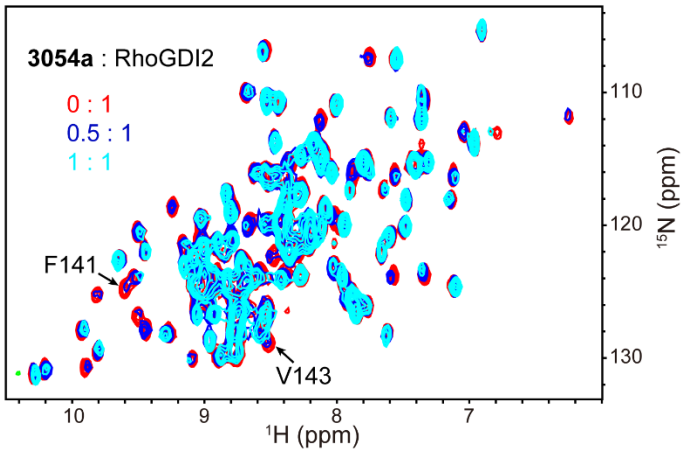
Supplementary Fig. 3. The omit map (contoured at 3σ) of RhoGDI2 in complex with **2102** enantiomers.



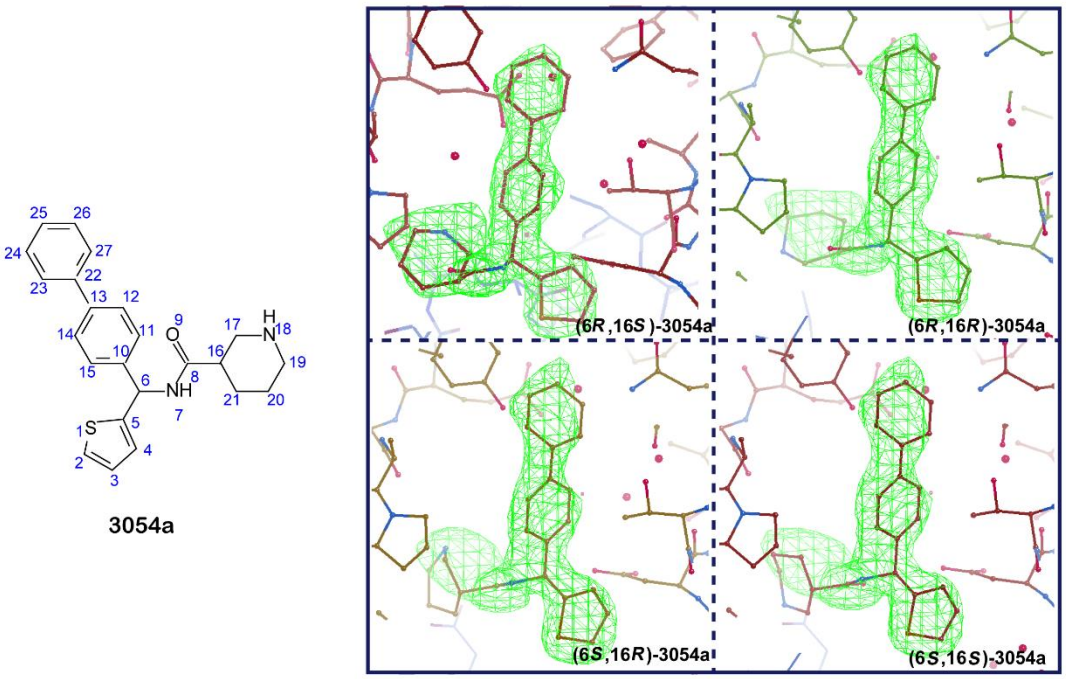
Supplementary Fig. 4. The chemical shift perturbations of ^{15}N-labeled RhoGDI2 induced by compound **2542**. **a**) Superposition of ^{1}H/^{15}N-HSQC spectra of RhoGDI2 upon titration of **2542**. The ligand/protein molar ratios are annotated. **b**) The residue-by-residue chemical shift changes of RhoGDI2 perturbed by a 6-fold excess of **2542**. The red dashed line represents two standard deviations above the average of the chemical shift perturbations (unassigned residues are included). **c**) The binding affinity of **2542** determined by the best fitting of the dose-dependent chemical shift changes of RhoGDI2. The K_d value with a fitting error is annotated. **d**) The binding topology of **2542** mapped on the surface of RhoGDI2 (PDB: 5H1D). Residues of significant chemical shift changes are colored in cyan.



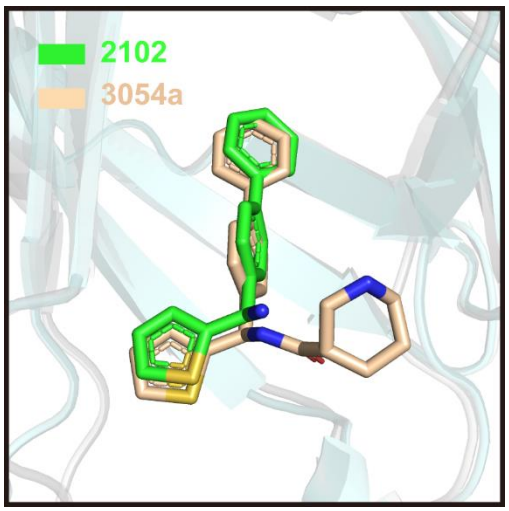
Supplementary Fig. 5. Conserved pose of compounds 2102 and 2542. a, The omit map (mFo-DFc) of compound **2542**, contoured at 3σ. **b**, Superimposition of the crystal structures of RhoGDI2 in complex with **2542** (violet) or **2102** (green)**.**



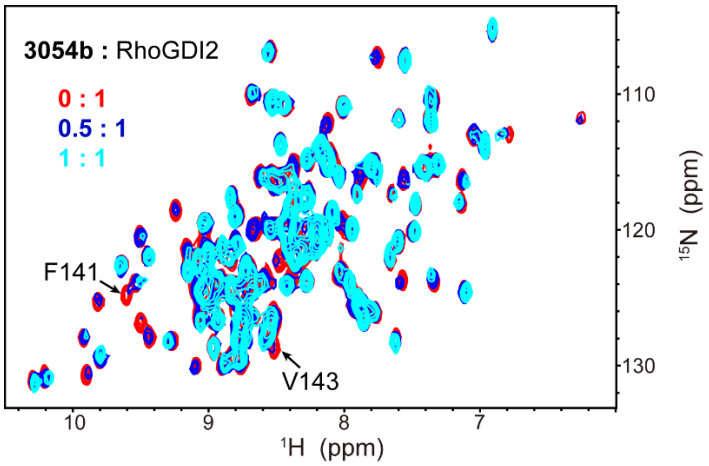
Supplementary Fig. 6. Superposition of ^{1}H/^{15}N-HSQC spectra of RhoGDI2 upon titration of **3054a**. The ligand/protein molar ratios are annotated. Residues showing disappearing peaks upon titration are indicated by arrows.



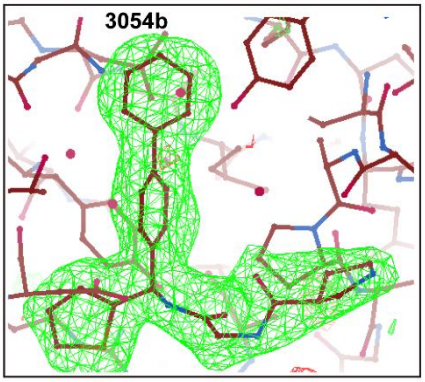
Supplementary Fig. 7. The **(*6R*)-3054a** enantiomer fits well with the omit map contoured at 4σ. The chemical structure and atom numbering of **3054a** are shown on the left.



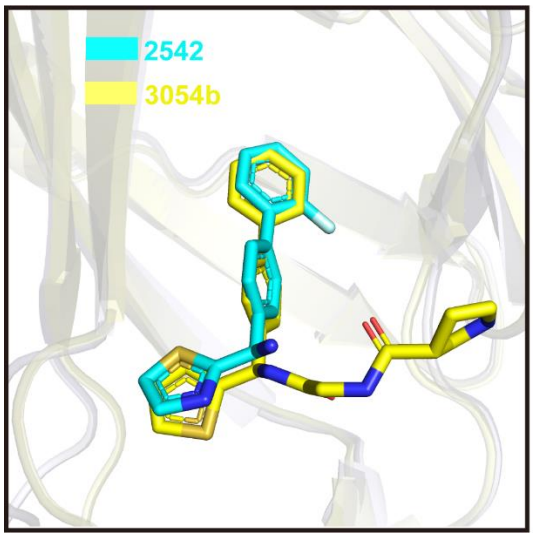
Supplementary Fig. 8. Superimposition of RhoGDI2 structures in complex with **2102** or **3054a**.



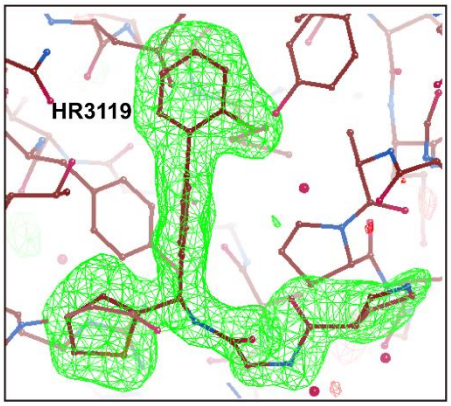
Supplementary Fig. 9. Superposition of ^{1}H/^{15}N-HSQC spectra of RhoGDI2 upon titration of **3054b**. The ligand/protein molar ratios are annotated. Residues showing disappearing signals upon titration of **3054b** are indicated by arrows.



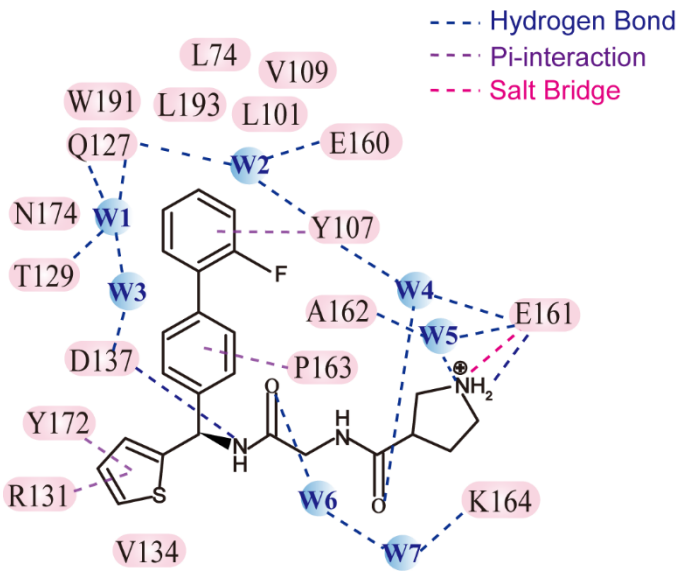
Supplementary Fig. 10. The omit map (mFo-DFc) of compound **3054b**, contoured at 3σ.



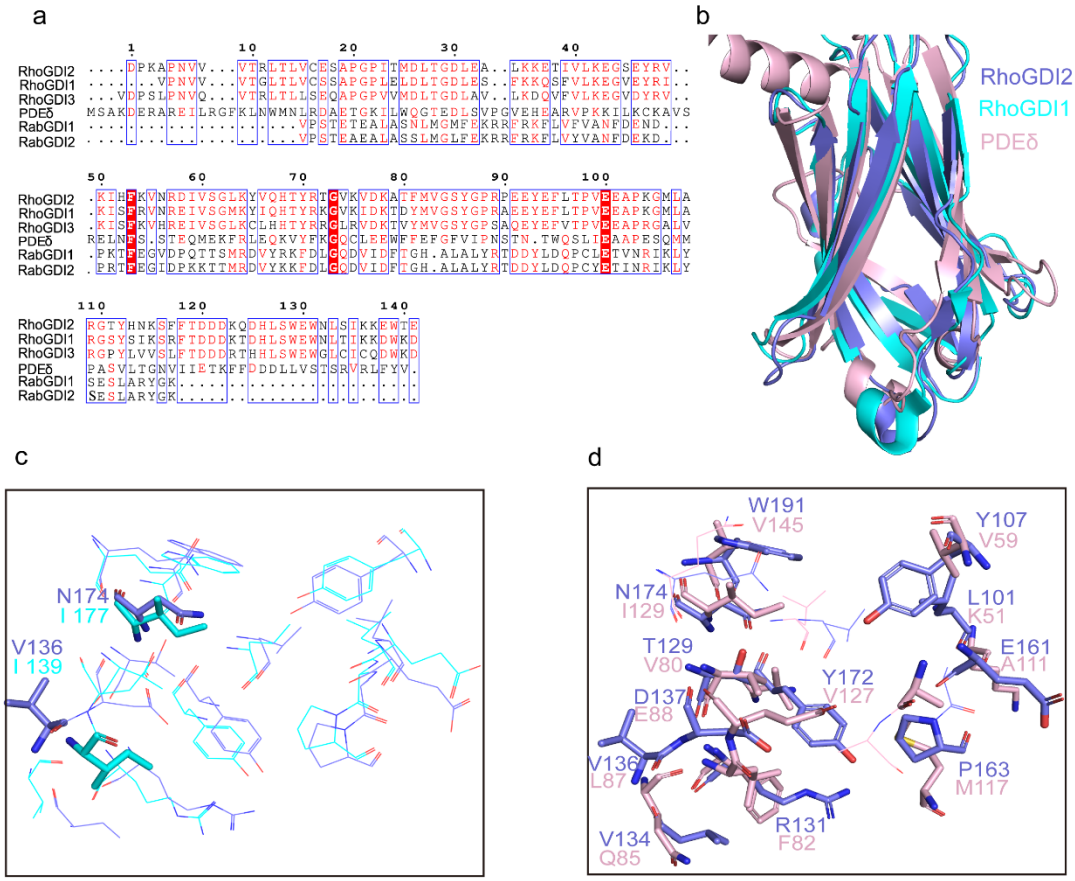
Supplementary Fig. 11. Superimposition of RhoGDI2 structures in complex with **2542** or **3054b**.



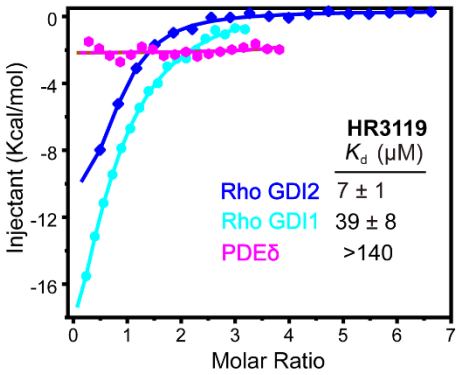
Supplementary Fig. 12. The omit map (mFo-DFc) of compound **HR3119**, contoured at 3σ.



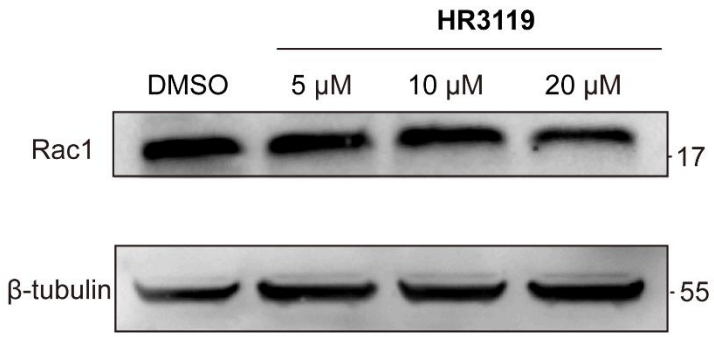
Supplementary Fig. 13. Schematic summary of interactions between RhoGDI2 and **HR3119**. The dark, magenta, and pale blue dashes denote the hydrogen bonds, salt bridge, and π interactions, respectively. Water molecules are in blue.



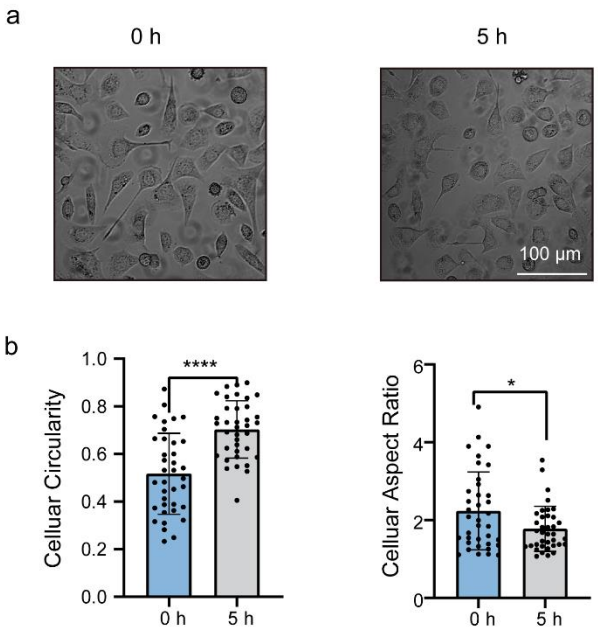
Supplementary Fig. 14. The sequence and structural analysis of RhoGDI2 and its homologs. **a**, Sequence alignment of the human GDIs. **b**,The structural comparison of RhoGDI2, RhoGDI1(PDB ID:1HH4) and PDEδ (PDB ID:7PAE). **c**, Residues proximal to the ligand-binding site of RhoGDI1/2. Conserved residues are shown in lines, while non-conserved residues are highlighted in sticks. **d**, Residues proximal to the ligand-binding site of RhoGDI2 and PDEδ.



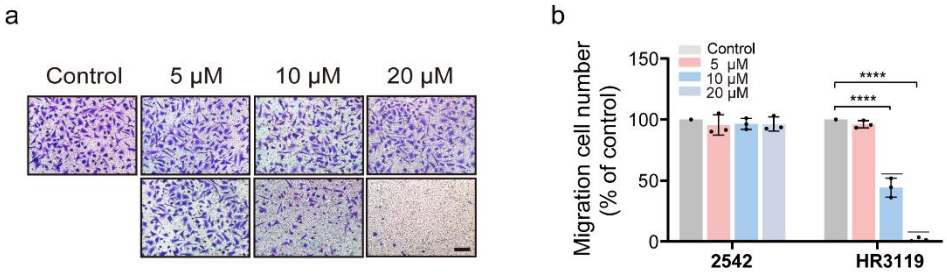
Supplementary Fig. 15. Affinity of **HR3119** to RhoGDI2 and its homologs RhoGDI1 and PDEδ.



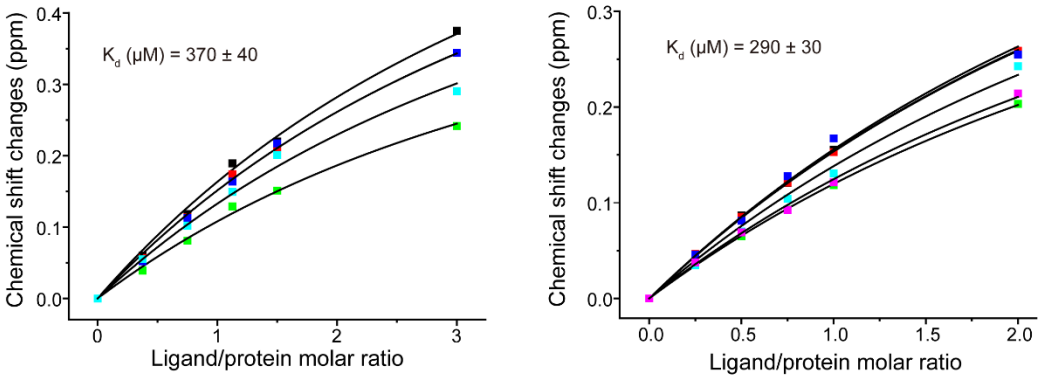
Supplementary Fig. 16. Rac1 levels in MDA-MB-231 cells are reduced by the treatment of **HR3119**.



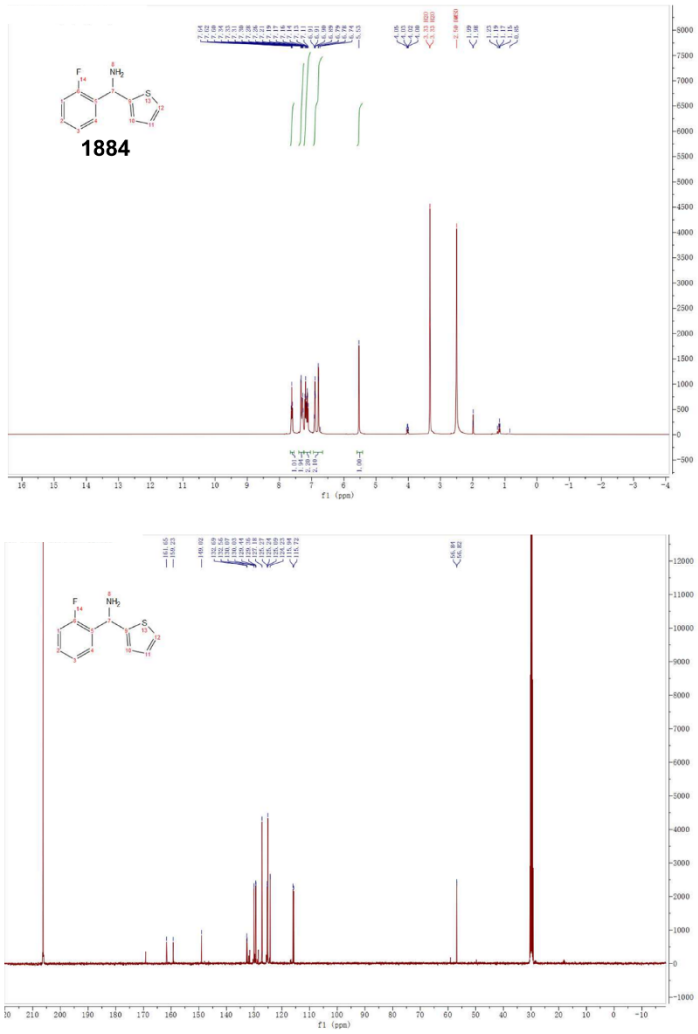
Supplementary Fig. 17. The cellular morphology of MDA-MB-231 cells altered by the treatment of **HR3119. a**, Images of MDA-MB-231 cells treated by 10 μM **HR3119** for 5 h. **b**, Statistics of cellular morphology upon **HR3119** treatment.



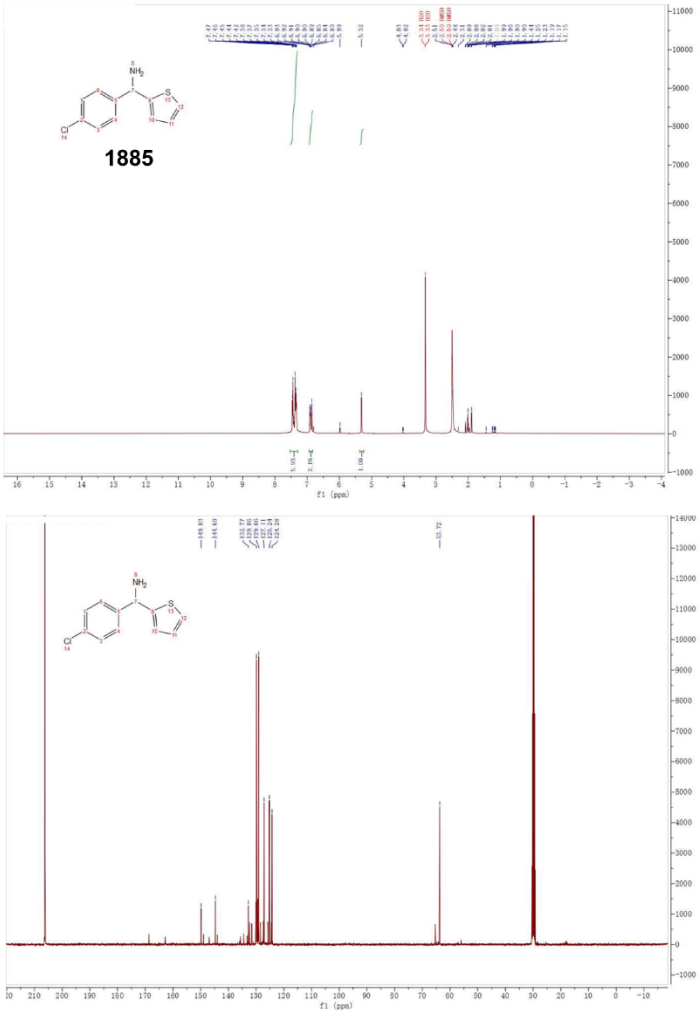
Supplementary Fig. 18. Inhibition of breast cancer migration by compound **HR3119. a**, The dose-dependent anti-invasion activity of **HR3119** assessed by the transwell migration assay. The weak binder **2542** was used as a negative control. Scale bar: 100 μm. **b**, Statistics of the anti-invasion activity of **HR3119** or **2542**. Three biological duplicates were measured to estimate the standard deviation. **** $p < 0.0001$, one-way ANOVA



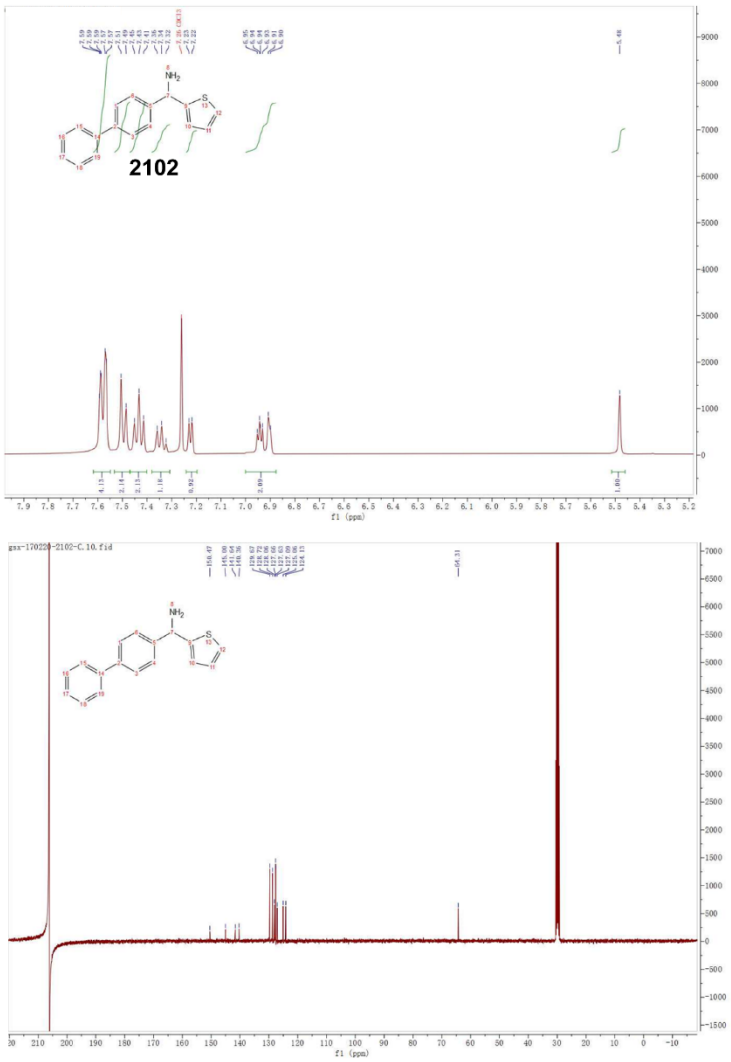
Supplementary Fig. 19. The binding affinity of **(6*S*,16*R*)-HR3119 or (6*S*,16*S*)-HR3119** to RhoGDI2 determined by the best fitting of the dose-dependent chemical shift changes of RhoGDI2. The K_d value with a fitting error is annotated.



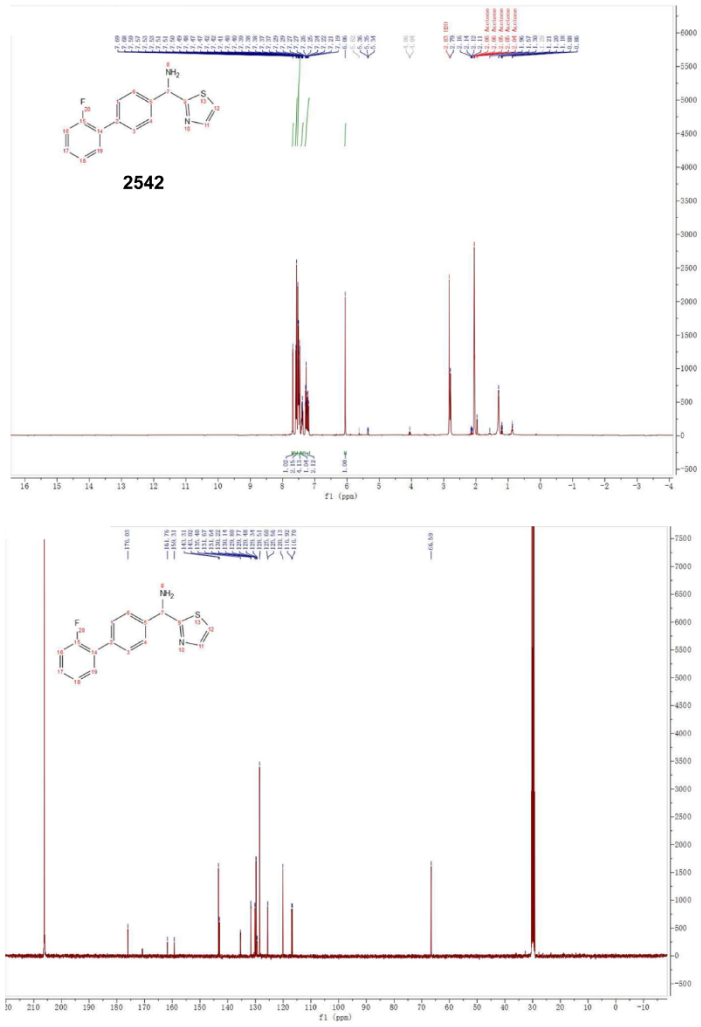
Supplementary Fig. 20. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **1884**.



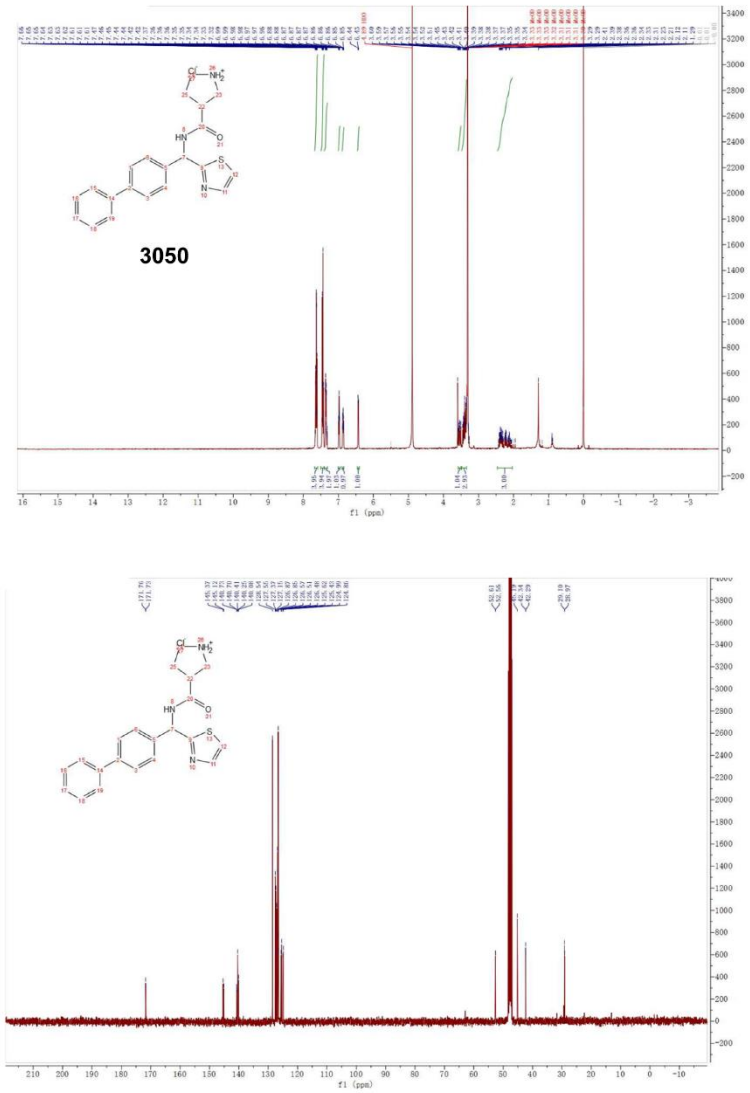
Supplementary Fig. 21. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **1885**.



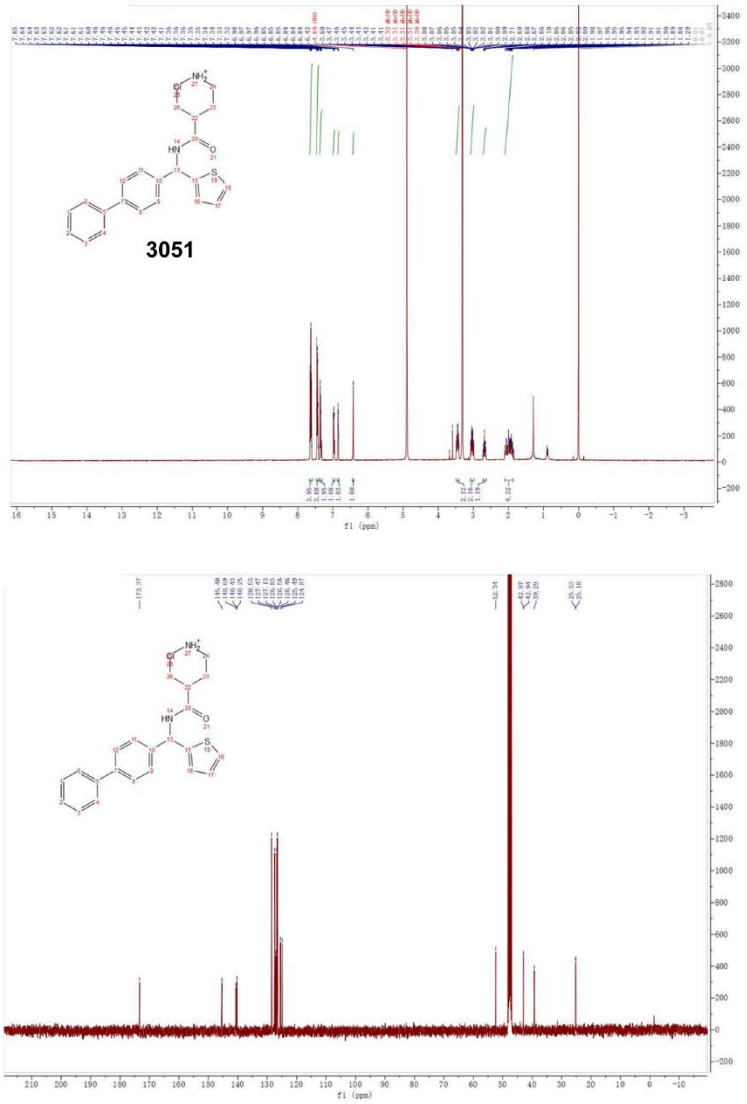
Supplementary Fig. 22. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **2102**.



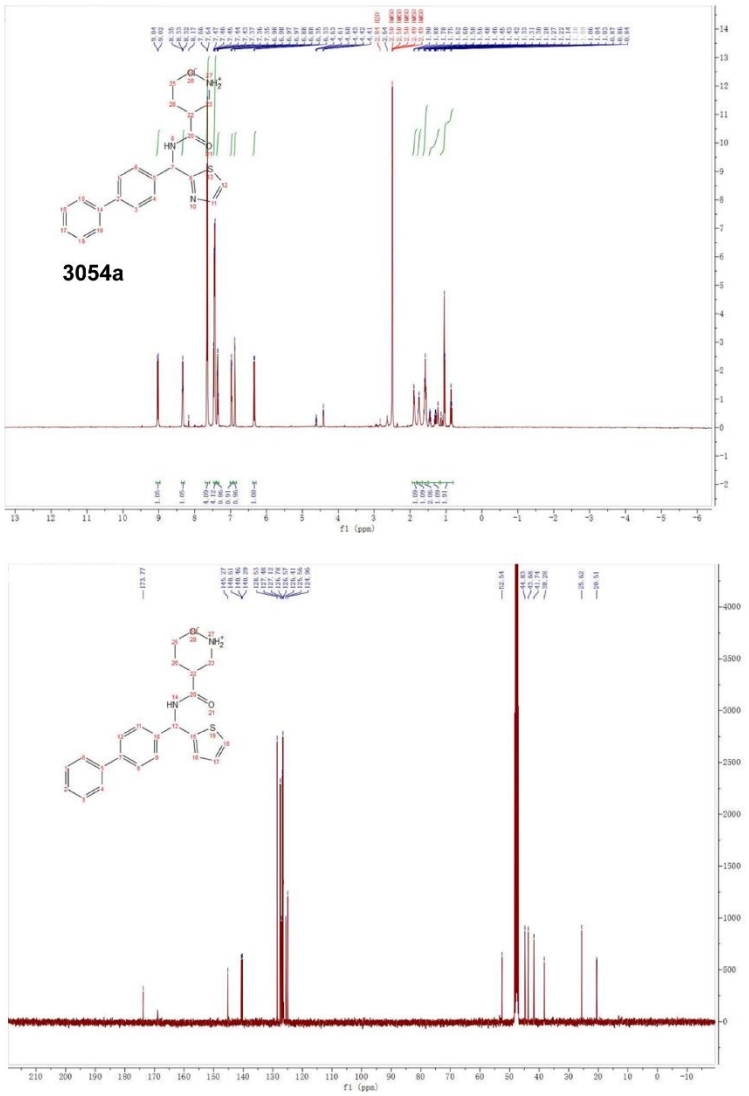
Supplementary Fig. 23. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **2542**.



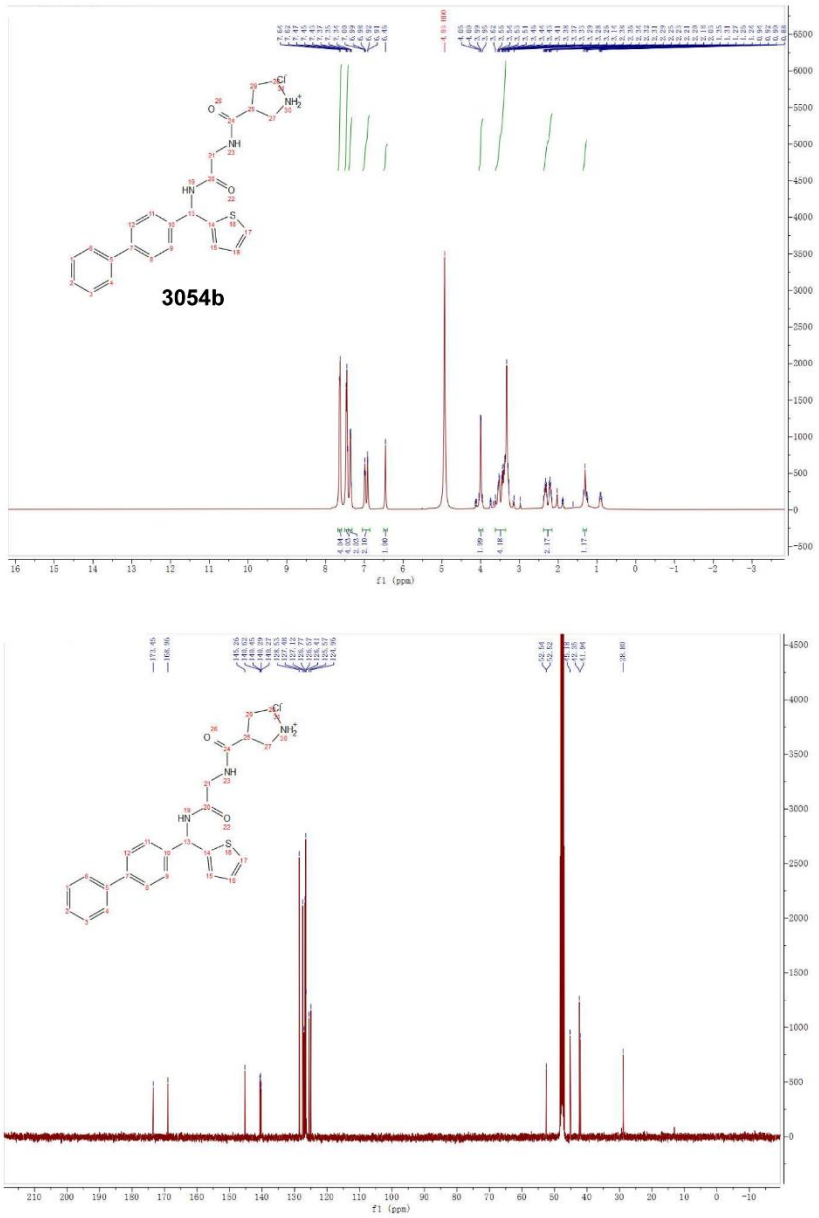
Supplementary Fig. 24. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **3050**.



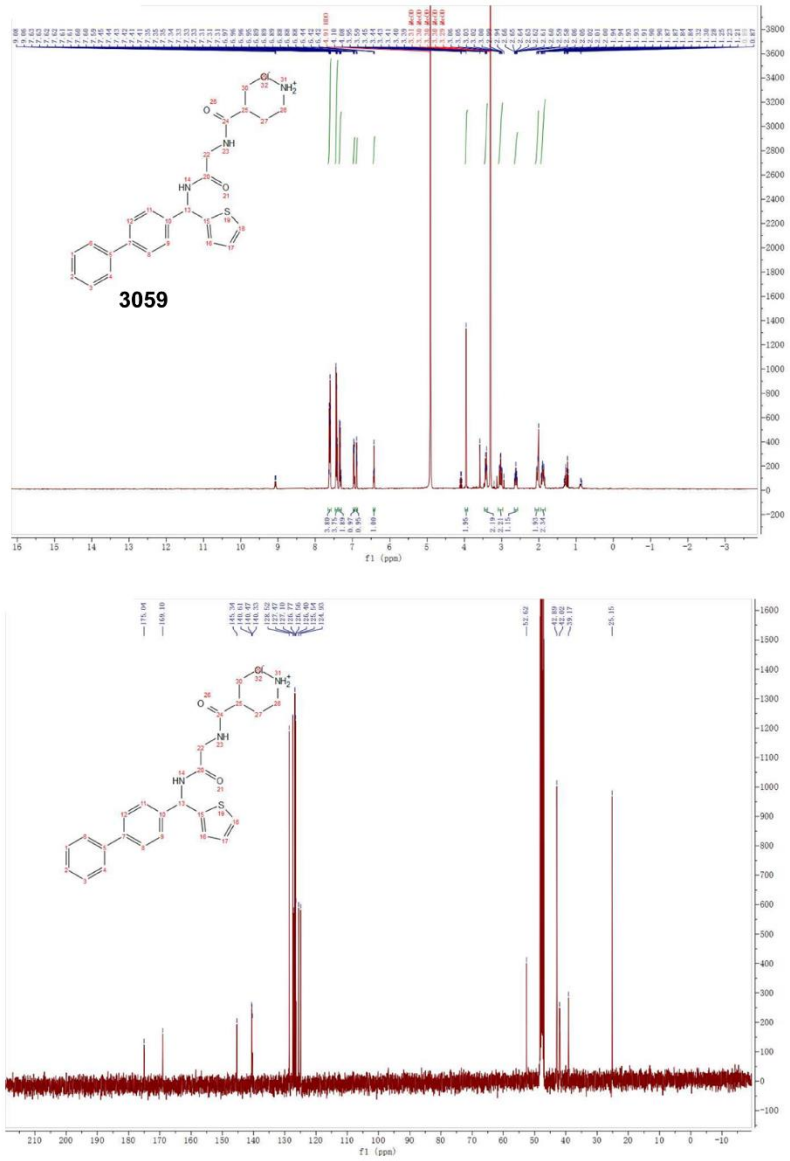
Supplementary Fig. 25. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **3051**.



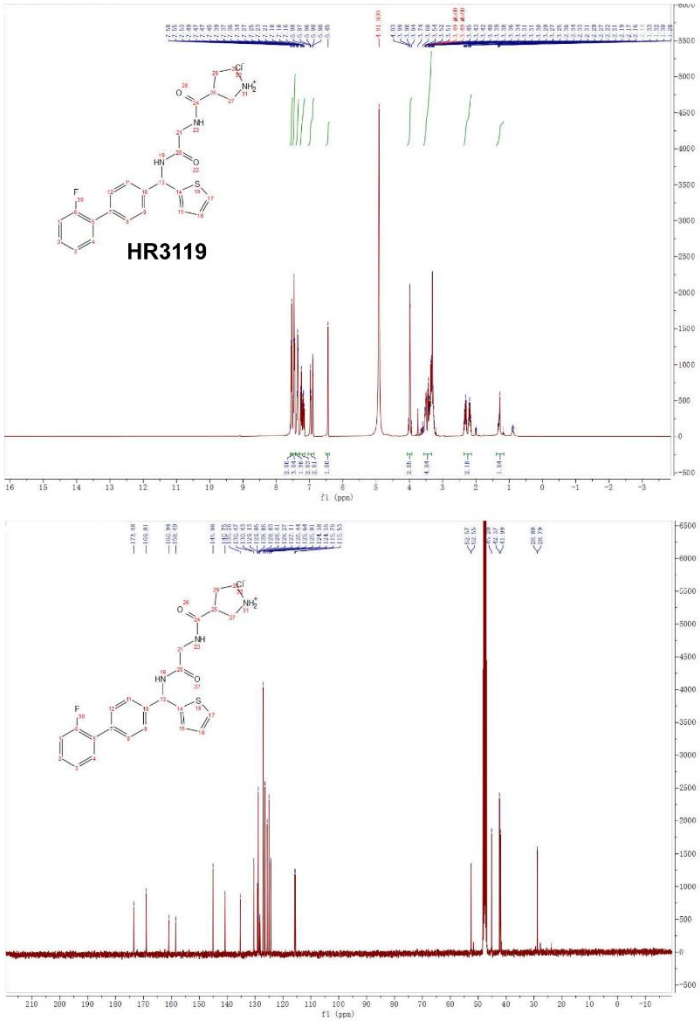
Supplementary Fig. 26. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **3054a**.



Supplementary Fig. 27. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **3054b**.



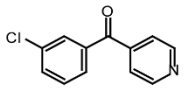
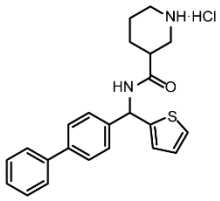
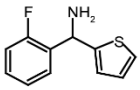
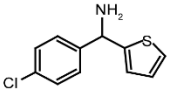
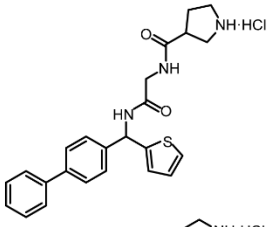
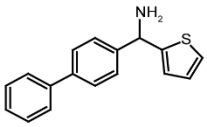
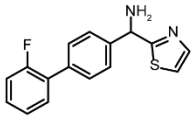
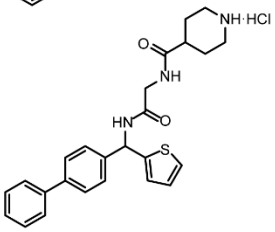
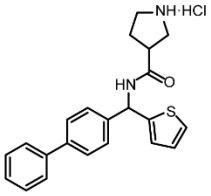
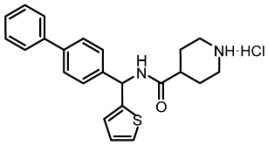
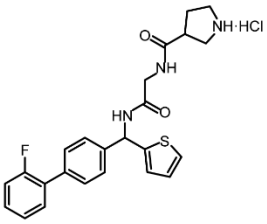
Supplementary Fig. 28. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **3059**.



Supplementary Fig. 29. ^{1}H-NMR and ^{13}C-NMR spectrum of compound **HR3119**.

Supplementary Tables

Supplementary Table 1. Structure and affinities of fragment-derived compounds of RhoGDI2

ID	Structure	K_d (μM)	ID	Structure	K_d (μM)
Hit1		714 ± 26[a]	**3054a**		35 ± 5[b]
1884		1058 ± 165[a]			
1885		363 ± 25[a]	**3054b**		20 ± 3[b]
2102		177 ± 33[a]			
2542		153 ± 16[a]	**3059**		22 ± 4[c]
3050		167 ± 4[b]			
3051		149 ± 0[b]	**3119**		7 ± 1[c]

[a] K_d values were determined from the dose-dependent chemical shift perturbations. [b] K_d values were determined from the SPR assay. [c] K_d values were determined from the ITC assay.

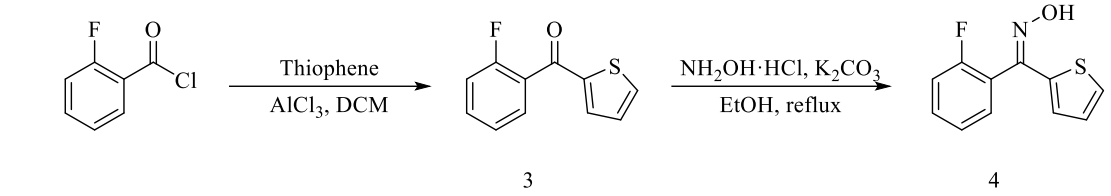
Supplementary Table 2. X-ray crystallography data collection and refinement statistics for the crystal structures of RhoGDI2-ligand complexes.

PDB ID	**8ZQ0**	**8ZQ5**	**8ZR3**	**8ZQX**	**8ZRC**
Inhibitor ID	**2102**	**2542**	**3054a**	**3054b**	**HR3119**
Data Collection					
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell dimensions					
a,b,c (Å)	51.969,51.969, 97.446	55.176,55.176, 96.006	53.958,53.958, 94.868	55.313,55.313, 95.148	55.492,55.492, 94.691
α,β,γ (˚)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength (Å)	0.9786	0.9778	0.9785	0.9785	0.9785
Resolution (Å)	40.00-1.65 (1.68-1.65)*	40.00-1.70 (1.73-1.70)*	40-2.37 (2.41-2.37)*	50-2.13 (2.17-2.13)*	39.24-1.85 (1.89-1.85)*
Completeness (%)	99.5 (100)	100.0 (100)	99.7 (100)	97.5 (96.9)	99.7 (100)
Average Redundancy	7.1 (7.5)	18.9 (19.3)	17.6 (12.5)	24.2 (20.4)	8.3 (8.4)
R_{merge} (%)	9.5 (48.1)	10.2 (58.4)	10.1 (47.6)	9.0 (73.2)	11.1 (60.9)
I/σI	15.4 (4.8)	31.8 (5.5)	26.3 (2.9)	39.1 (2.9)	10.9 (3.4)
$CC_{1/2}$	0.998 (0.902)	0.980 (0.955)	0.998 (0.990)	0.996 (0.948)	0.992 (0.923)
Refinement					
No.reflections used/free	16854/828	16970/822	4998/259	8576/418	13147/651
R_{work}/R_{free} (%)	16.38/21.23	17.39/21.56	20.51/26.03	19.26/23.37	20.11/24.52
Number of atoms					
Protein	1097	1104	1092	1103	1097
Ligand	19	20	27	30	31
Glycerol		6		12	
Ethylene glycol		4			16
Water	151	179	34	36	89
B factors (Å^2)					
Protein	14.681	16.013	36.540	46.988	30.907
Ligand	30.890	18.063	46.543	52.735	30.165
Glycerol		32.835		51.771	
Ethylene glycol		32.830			38.925
Water	29.606	28.445	36.950	46.288	36.575
R.m.s. deviations					
Bond lengths (Å)	0.006	0.007	0.003	0.008	0.007
Bond angles (˚)	0.827	0.897	0.579	0.926	0.890
Ramachandran plot					
Favored(%)	97.78	98.52	98.52	97.79	98.52
Allowed(%)	2.22	1.48	1.48	2.21	1.48
Outliers(%)	0	0	0	0	0

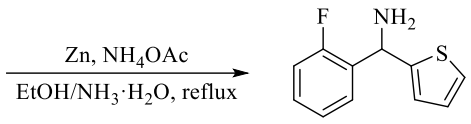
* Values in parentheses are for the highest-resolution shell.

Supplementary Methods

Preparation of compound 1884



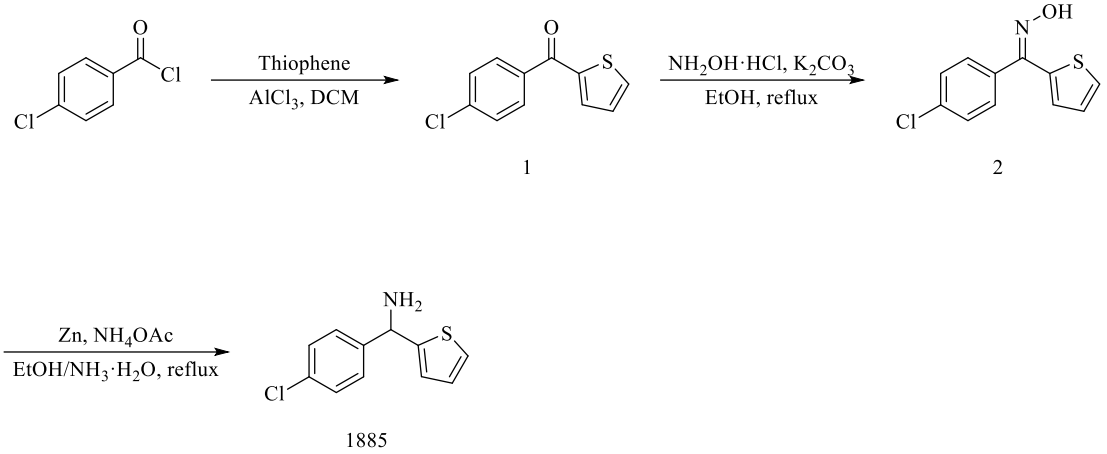
3 4



1884

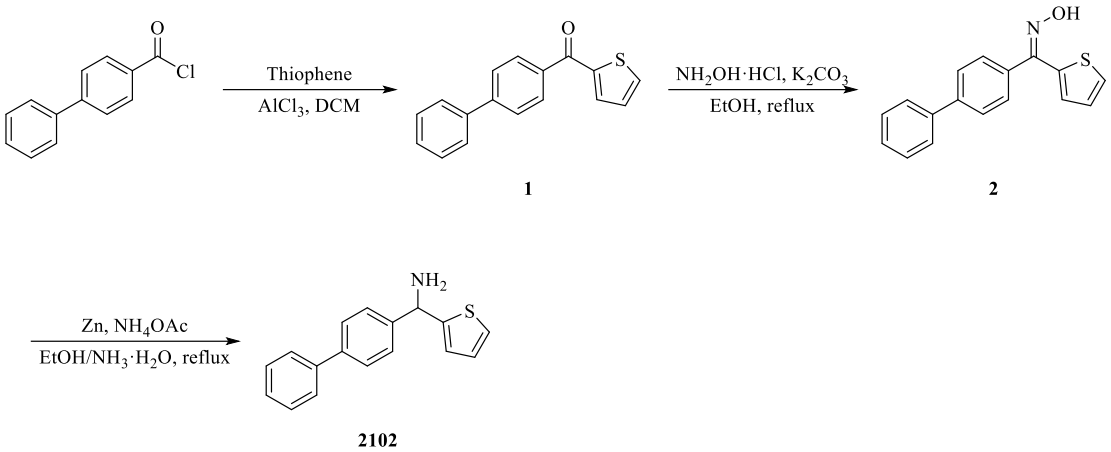
(2-fluorophenyl)(thiophen-2-yl)methanamine (1884). To a solution of aluminum chloride (3mmol, 1.5eq) in dry dichloromethane (10mL), was slowly added 2-fluorobenzoyl chloride (2mmol, 1.0eq) dissolved in dry dichloromethane (10mL), stirred in an ice bath under argon protect for 1h. Thiophene (10mmol, 5.0eq) dissolved in dry dichloromethane (4mL) was slowly added to the reaction solution. The mixture was stirred at room temperature for 2h. Ice water (20mL) and cold hydrochloride acid (2M, 10mL) were slowly added to quench the mixture. The organic layer was collected and washed with water (3 × 50 mL), saturated sodium carbonate solution (3 × 50 mL) and brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound (2-fluorophenyl)(thiophen-2-yl)methanone **(3)** was purified by column chromatography. The ketone compound **3** (1mmol, 1.0eq), hydroxylamine hydrochloride (3mmol, 3.0eq) and potassium carbonate (4mmol, 4.0eq) were dissolved in ethanol (5mL). The mixture was stirred and heated at 85℃ for 12h under argon protect, reflux. Remove the solvent by vacuum distillation and dissolve the mixture in dichloromethane. Wash the solvent with water (3 × 50 mL) and brine (50mL). Remove the solvent by vacuum distillation to obtain the crude compound (2-fluorophenyl)(thiophen-2-yl)methanone oxime **(4)**. The ketoxime compound **4** (0.5mmol, 1.0eq), zinc dust (2.5mmol, 5.0eq), and ammonium acetate (0.5mmol, 1.0eq) were dissolved in ethanol (4mL) and ammonia liquor (2mL) and stirred and heated at 90℃ for 12h under Ar protect. Filter the zinc dust and remove the solvent by vacuum distillation and dissolve the mixture in ether acerate (20mL). The solution was washed with saturated sodium bicarbonate solution (30mL) and water (2 × 50 mL). Remove the solvent by vacuum distillation and the product was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (2-fluorophenyl)(thiophen-2-yl)methanamine **(1884)** a light yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 7.62 (t, J = 7.8 Hz, 1H), 7.30 (dt, J = 20.8, 6.0 Hz, 2H), 7.16 (dt, J = 24.3, 8.8 Hz, 2H), 6.90 (t, J = 4.2 Hz, 1H), 6.79 (d, J = 3.5 Hz, 1H), 5.53 (s, 1H). ^{13}C NMR (101 MHz, Acetone) δ 160.44 (d, J = 244.0 Hz), 149.02, 132.63 (d, J = 13.2 Hz), 130.05 (d, J = 4.1 Hz), 129.40 (d, J = 8.4 Hz), 127.17, 125.25 (d, J = 3.5 Hz), 125.09, 124.23, 115.83 (d, J = 22.2 Hz), 56.83 (d, J = 2.3 Hz).

Preparation of compound 1885



(4-chlorophenyl)(thiophen-2-yl)methanamine (1885). To a solution of aluminum chloride (3mmol, 1.5eq) in dry dichloromethane (10mL), was slowly added 4-chlorobenzoyl chloride (2mmol, 1.0eq) dissolved in dry dichloromethane (10mL), stirred in an ice bath under argon protect for 1h. Thiophene (10mmol, 5.0eq) dissolved in dry dichloromethane (4mL) was slowly added to the reaction solution. The mixture was stirred at room temperature for 2h. Ice water (20mL) and cold hydrochloride acid (2M, 10mL) was slowly added to quench the mixture. The organic layer was collected and washed with water (3 × 50 mL), saturated sodium carbonate solution (3 × 50 mL) and brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound (4-chlorophenyl)(thiophen-2-yl)methanone **(1)** was purified by column chromatography. The ketone compound **1** (1mmol, 1.0eq), hydroxylamine hydrochloride (3mmol, 3.0eq) and potassium carbonate (4mmol, 4.0eq) were dissolved in ethanol (5mL). The mixture was stirred and heated at 85℃ for 12h under argon protect, reflux. Remove the solvent by vacuum distillation and dissolve the mixture in dichloromethane. Wash the solvent with water (3 × 50 mL) and brine (50mL). Remove the solvent by vacuum distillation to obtain the crude compound (4-chlorophenyl)(thiophen-2-yl)methanone oxime **(2)**. The ketoxime compound **2** (0.5mmol, 1.0eq), zinc dust (2.5mmol, 5.0eq) and ammonium acetate (0.5mmol, 1.0eq) were dissolved in ethanol (4mL) and ammonia liquor (2mL) and stirred and heated at 90℃ for 12h under Ar protect. Filter the zinc dust and remove the solvent by vacuum distillation and dissolve the mixture in ether acerate (20mL). The solution was washed by saturated sodium bicarbonate solution (30mL) and water (2 × 50 mL). Remove the solvent by vacuum distillation and the product was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (4-chlorophenyl)(thiophen-2-yl)methanamine **(1885)** as a light yellow solid. ^{1}H NMR (400 MHz, DMSO) δ 7.47 – 7.42 (m, 2H), 7.39 – 7.32 (m, 3H), 6.91 (dq, J = 5.3, 3.2 Hz, 1H), 6.85 (d, J = 3.8 Hz, 1H), 5.32 (s, 1H). ^{13}C NMR (101 MHz, Acetone) δ 149.83, 144.69, 132.77, 129.86, 129.06, 127.11, 125.24, 124.28, 63.72.

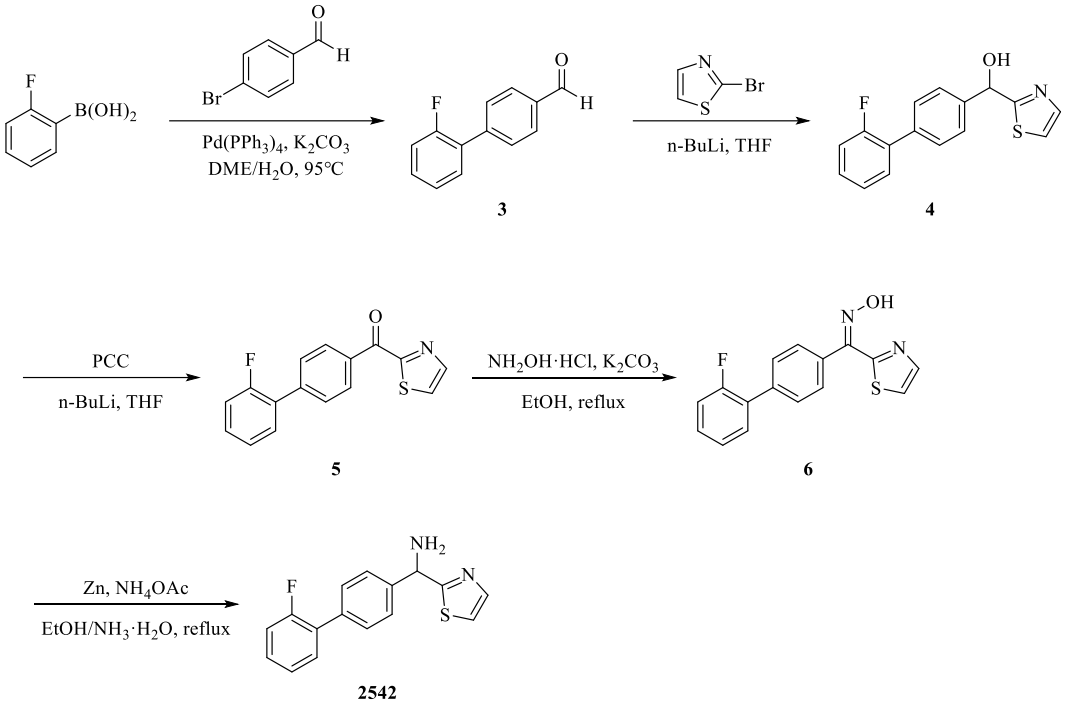
Preparation of compound 2102



1 2 2102

[1,1'-biphenyl]-4-yl(thiophen-2-yl)methanamine (2102). To a solution of aluminum chloride (3 mmol, 1.5eq) in dry dichloromethane (10 mL), was slowly added [1,1'-biphenyl]-4-carbonyl chloride (2 mmol, 1.0eq) dissolved in dry dichloromethane (10 mL), stirred in an ice bath under argon protect for 1 h. Thiophene (10 mmol, 5.0eq) dissolved in dry dichloromethane (4 mL) was slowly added to the reaction solution. The mixture was stirred at room temperature for 2 h. Ice water (20 mL) and cold hydrochloride acid (2 M, 10 mL) were slowly added to quench the mixture. The organic layer was collected and washed with water (3 × 50 mL), saturated sodium carbonate solution (3 × 50 mL) and brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound [1,1'-biphenyl]-4-yl(thiophen-2-yl)methanone **(1)** was purified by column chromatography. The ketone compound **1** (1 mmol, 1.0eq), hydroxylamine hydrochloride (3 mmol, 3.0eq), and potassium carbonate (4 mmol, 4.0eq) were dissolved in ethanol (5 mL). The mixture was stirred and heated at 85℃ for 12 h under argon protection, and reflux. Remove the solvent by vacuum distillation and dissolve the mixture in dichloromethane. Wash the solvent with water (3 × 50 mL) and brine (50 mL). Remove the solvent by vacuum distillation to obtain the crude compound [1,1'-biphenyl]-4-yl(thiophene-2-yl)methanone oxime **(2)**. The ketoxime compound **2** (0.5 mmol, 1.0eq), zinc dust (2.5 mmol, 5.0eq), and ammonium acetate (0.5 mmol, 1.0eq) were dissolved in ethanol (4 mL) and ammonia liquor (2 mL) and stirred and heated at 90℃ for 12 h under Ar protect. Filter the zinc dust remove the solvent by vacuum distillation and dissolve the mixture in ether acerate (20 mL). The solution was washed with saturated sodium bicarbonate solution (30 mL) and water (2 × 50 mL). Remove the solvent by vacuum distillation and the product was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound [1,1'-biphenyl]-4-yl(thiophene-2-yl)methanamine **(2102)** as a light yellow solid; ^{1}H NMR (400 MHz, CDCl3): δ = 7.66 – 7.54 (m, 4H), 7.50 (d, J = 8.0 Hz, 2H), 7.43 (t, J = 7.5 Hz, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.22 (d, J = 5.0 Hz, 1H), 7.04 – 6.84 (m, 2H), 5.48 (s, 1H); ^{13}C NMR (101 MHz, Acetone): δ = 150.47, 145.01, 141.64, 140.36, 129.68, 128.72, 128.06, 127.66, 127.63, 127.09, 125.06, 124.13, 64.31.

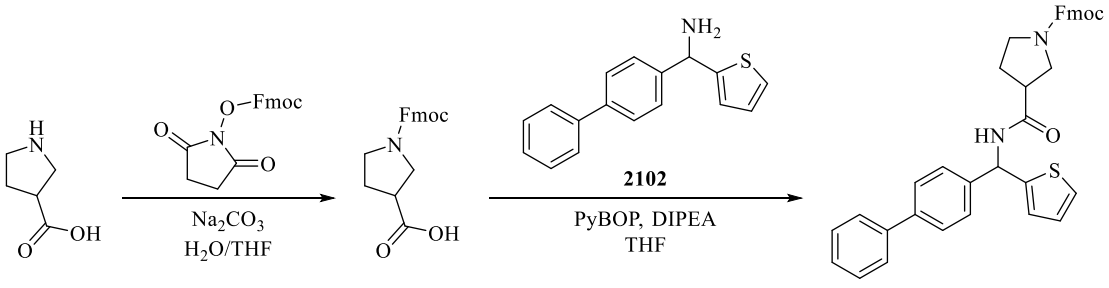
Preparation of compound 2542



(2'-fluoro-[1,1'-biphenyl]-4-yl)(thiazol-2-yl)methanamine (2542). A solution of (2-fluorophenyl) boronic acid (4 mmol, 1.0eq), 4-bromobenzaldehyde (4 mmol, 1.0eq), tetrakis (triphenylphosphine) palladium (0.4 mmol, 0.1eq) and potassium carbonate (12 mmol, 3.0eq) in 1,2-Dimethoxyethane (15 mL) and water (5 mL) was stirred and heated at 95℃ under argon protect for 12 h. Water (30 mL) and ether acerate (30 mL) were added to the mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound 2'-fluoro-[1,1'-biphenyl]-4-carbaldehyde **(3)** as a white solid. 2-bromothiazole (2 mmol, 1.0eq) was dissolved in dry tetrahydrofuran (8 mL) and stirred at -78℃ in dry ice/acetone bath under argon protect and a solution of n-BuLi (2 mmol, 1.0eq) in dry tetrahydrofuran was slowly added to the reaction mixture and stirred for another 30 min at -78℃. The aldehyde compound **3** (2 mmol, 1.0eq) dissolved in dry tetrahydrofuran (8 mL) was slowly added to the reaction mixture. The solution was warmed to room temperature and stirred overnight. Saturated ammonium chloride solution (10 mL) was added to quench the reaction and ether acerate (30 mL) was added. Collect the organic layer and wash with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (2'-fluoro-[1,1'-biphenyl]-4-yl)(thiazol-2-yl)methanol **(4)** as a white solid. The alcohol compound **4** (1 mmol, 1.0eq) and pyridinium chlorochromate (1.5 mmol, 1.5eq) were dissolved in dichloromethane (20 mL) and the mixture was stirred at room temperature for 30 min. Water (10 mL) and ether acerate (30 mL) were added to the reaction mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound

(2'-fluoro-[1,1'-biphenyl]-4-yl)(thiazol-2-yl)methanone **(5)** as a white solid. The ketone compound **5** (0.5 mmol, 1.0eq), hydroxylamine hydrochloride (1.5 mmol, 3.0eq), and potassium carbonate (2 mmol, 4.0eq) were dissolved in ethanol (5 mL). The mixture was stirred and heated at 85℃ for 12 h under argon protection, and reflux. Remove the solvent by vacuum distillation and dissolve the mixture in dichloromethane. Wash the solvent with water (3 × 50 mL) and brine (50 mL). Remove the solvent by vacuum distillation to obtain the crude compound (2'-fluoro-[1,1'-biphenyl]-4-yl)(thiazol-2-yl)methanone oxime **(6)**. The ketoxime compound **6** (0.3 mmol, 1.0eq), zinc dust (1.5mmol, 5.0eq), and ammonium acetate (0.3 mmol, 1.0eq) were dissolved in ethanol (4 mL) and ammonia liquor (2 mL) and stirred and heated at 90℃ for 12 h under Ar protect. Filter the zinc dust remove the solvent by vacuum distillation and dissolve the mixture in ether acerate (20 mL). The solution was washed with saturated sodium bicarbonate solution (30 mL) and water (2 × 50 mL). Remove the solvent by vacuum distillation and the product was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound **2542** as a light yellow solid; ^{1}H NMR (400 MHz, Acetone): δ = 7.68 (d, J = 3.3 Hz, 1H), 7.61 – 7.44 (m, 6H), 7.39 (tdd, J = 7.1, 5.0, 1.7 Hz, 1H), 7.31 – 7.17 (m, 2H), 6.06 (s, 1H); ^{13}C NMR (101 MHz, Acetone): δ = 176.03, 160.54 (d, J = 246.0 Hz), 143.31, 143.02, 135.40, 131.66 (d, J = 3.4 Hz), 130.18 (d, J = 8.4 Hz), 129.78 (d, J = 3.2 Hz), 129.41 (d, J = 13.2 Hz), 128.51, 125.58 (d, J = 3.7 Hz), 120.13, 116.81 (d, J = 22.8 Hz), 66.59.

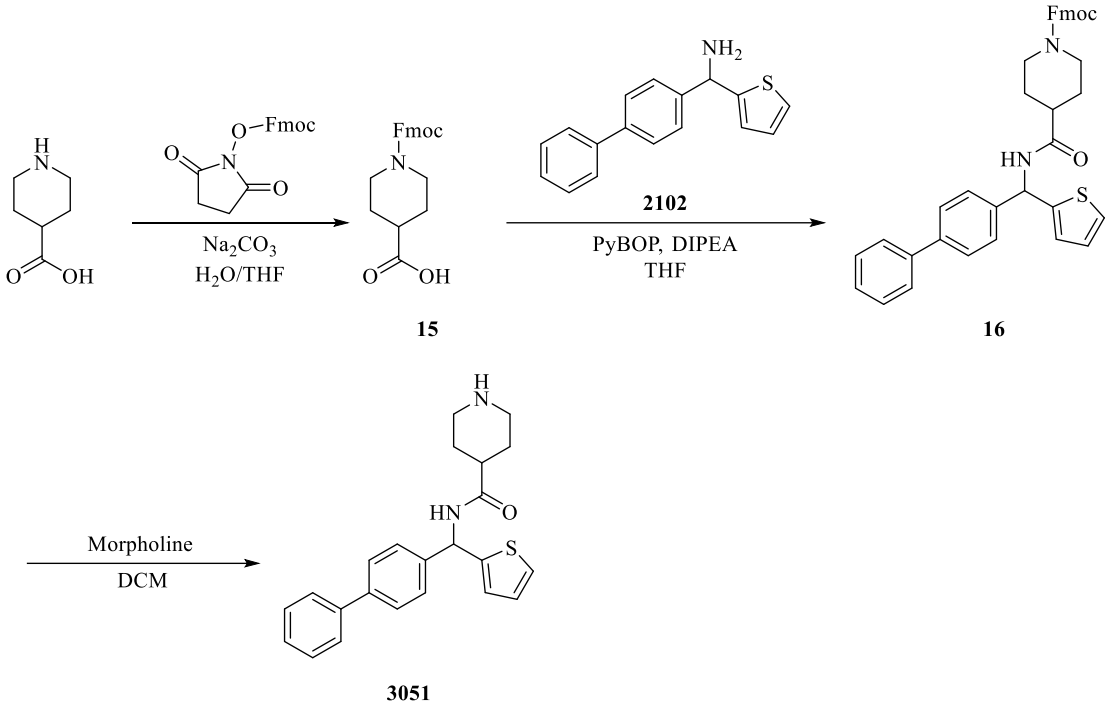
Preparation of compound 3050



N-([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)pyrrolidine-3-carboxamide (3050). A solution of pyrrolidine-3-carboxylic acid (5mmol, 1.0eq), (9H-fluoren-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (10mmol, 2.0eq) and sodium carbonate (25mmol, 5.0eq) dissolved in water (10mL) and tetrahydrofuran (15mL) was stirred at room temperature overnight. Water (30mL) and ether acerate (30mL) were added to the mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column

chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound 1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidine-3-carboxylic acid **(11)** as a white solid. A solution of compound **2102** (1mmol, 1.0eq), compound **11** (1mmol, 1.0eq), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.5mmol, 1.5eq) and N, N-diisopropylethylamine (2.5mmol, 2.5eq) dissolved in THF (5mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (30mL) and ether acerate (30mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl)methyl 3-(([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)carbamoyl)pyrrolidine-1-carboxylate **(12)** as a white to light yellow solid. Compound **12** (0.5mmol, 1.0eq) was dissolved in morpholine (5mL) and dichloromethane (5mL) at room temperature overnight. Ether acerate (30mL) was added to the mixture and the organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound N-([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)pyrrolidine-3-carboxamide **(3050)** as a white to light yellow solid. Hydrochloric acid (4M in 1,4-dioxane, 0.2mL) can be added to the dichloromethane/petroleum mixture to give the salt form of **3050** if needed. ^{1}H NMR (400 MHz, MeOD) δ 7.63 (td, J = 7.7, 7.0, 1.4 Hz, 4H), 7.44 (t, J = 8.0 Hz, 4H), 7.38 – 7.30 (m, 2H), 6.98 (ddd, J = 5.2, 3.5, 1.6 Hz, 1H), 6.87 (ddt, J = 5.8, 3.6, 1.2 Hz, 1H), 6.43 (d, J = 4.2 Hz, 1H), 3.57 – 3.50 (m, 1H), 3.48 – 3.34 (m, 3H), 2.44 – 2.06 (m, 3H). ^{13}C NMR (101 MHz, MeOD) δ 171.75 (d, J = 2.1 Hz), 145.25 (d, J = 25.3 Hz), 140.72 (d, J = 3.2 Hz), 140.41, 140.16 (d, J = 17.2 Hz), 128.54, 127.55, 127.37, 127.15, 126.86 (d, J = 2.1 Hz), 126.60 – 126.42 (m), 125.53 (d, J = 19.3 Hz), 124.93 (d, J = 13.7 Hz), 52.58 (d, J = 4.8 Hz), 45.19, 42.31 (d, J = 4.9 Hz), 29.10, 28.97.

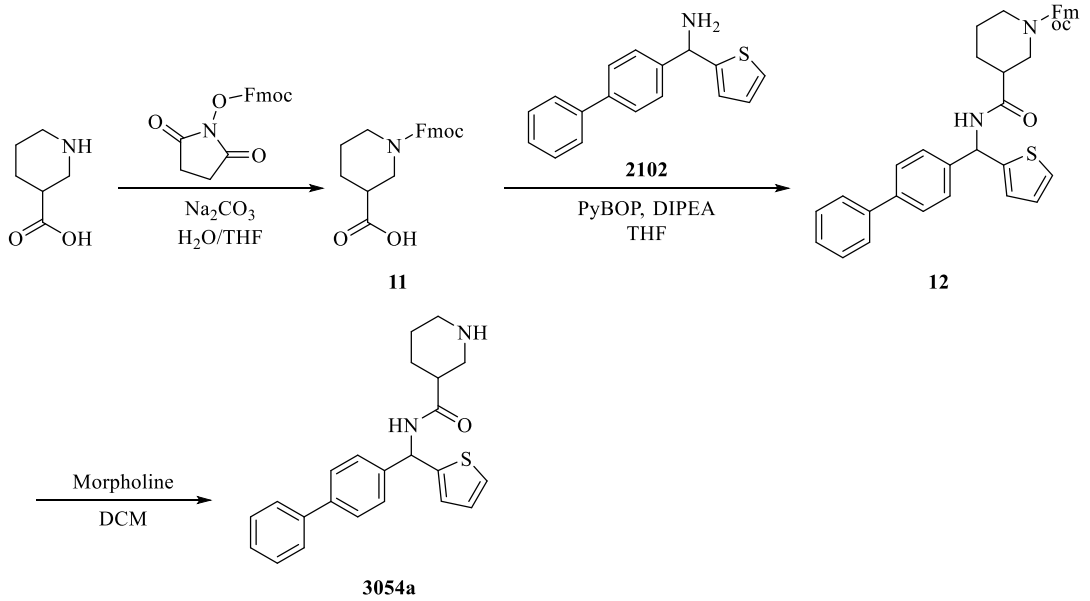
Preparation of compound 3051



N-([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)piperidine-4-carboxamide (3051). A solution of piperidine-4-carboxylic acid (5mmol, 1.0eq), (9H-fluoren-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (10mmol, 2.0eq) and sodium carbonate (25mmol, 5.0eq) dissolved in water (10mL) and tetrahydrofuran (15mL) was stirred at room temperature overnight. Water (30mL) and ether acerate (30mL) were added to the mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound 1-(((9H-fluoren-9-yl)methoxy)carbonyl)piperidine-4-carboxylic acid **(5)** as a white solid. A solution of compound **2102** (1mmol, 1.0eq), compound **15** (1mmol, 1.0eq), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.5mmol, 1.5eq) and N,N-diisopropylethylamine (2.5mmol, 2.5eq) dissolved in THF (5mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (30mL) and ether acerate (30mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl)methyl 4-(([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)carbamoyl)piperidine-1-carboxylate **(16)** as a white to light yellow solid. Compound **16** (0.5mmol, 1.0eq) was dissolved in morpholine (5mL) and dichloromethane (5mL) at room temperature overnight. Ether acerate (30mL) was added to the mixture and the organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound N-([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)piperidine-4-carboxamide **(3051)** as a white to light yellow solid.

Hydrochloric acid (4M in 1,4-dioxane, 0.2mL) can be added to the dichloromethane/petroleum mixture to give the salt form of **3051** if needed. ^{1}H NMR (400 MHz, MeOD) δ 7.68 – 7.58 (m, 4H), 7.48 – 7.40 (m, 4H), 7.38 – 7.30 (m, 2H), 6.97 (dd, J = 5.1, 3.6 Hz, 1H), 6.85 (dt, J = 3.5, 1.2 Hz, 1H), 6.42 (s, 1H), 3.44 (tt, J = 8.3, 4.0 Hz, 2H), 3.03 (tdd, J = 12.6, 7.6, 3.4 Hz, 2H), 2.68 (tt, J = 10.9, 4.0 Hz, 1H), 2.11 – 1.87 (m, 4H). ^{13}C NMR (101 MHz, MeOD) δ 173.37, 145.40, 140.69, 140.43, 140.25, 128.53, 127.47, 127.13, 126.83, 126.56, 126.46, 125.49, 124.87, 52.34, 42.95 (d, J = 2.9 Hz), 39.29, 25.25 (d, J = 14.8 Hz)

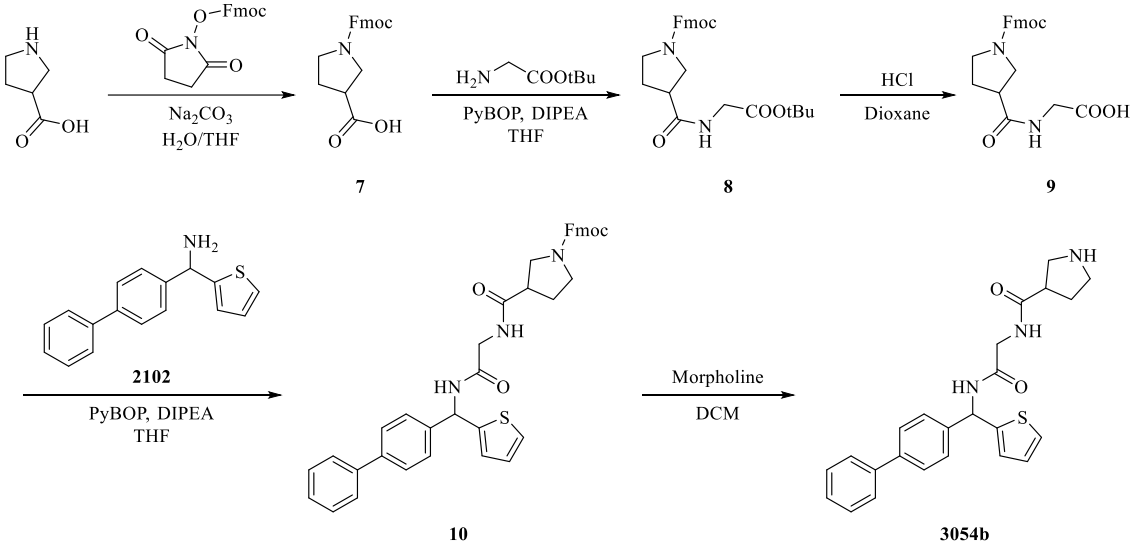
Preparation of compound 3054a



N-([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)piperidine-3-carboxamide (3054a). A solution of piperidine-3-carboxylic acid (2 mmol, 1.0eq), (9H-fluoren-9-yl)methyl(2,5-dioxopyrrolidin-1-yl) carbonate (4 mmol, 2.0eq) and sodium carbonate (10 mmol, 5.0eq) dissolved in water (4 mL) and tetrahydrofuran (6 mL) was stirred at room temperature overnight. Water (30 mL) and ether acerate (30 mL) were added to the mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound 1-(((9H-fluoren-9-yl)methoxy)carbonyl)piperidine-3-carboxylic acid **(11)** as a white solid. Compound **2102** was synthesized as described before. A solution of compound **2102** (1 mmol, 1.0eq), compound **11** (1 mmol, 1.0eq), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (1.5 mmol, 1.5eq) and N, N-diisopropylethylamine (2.5 mmol, 2.5eq) dissolved in THF (5 mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (30 mL) and ether acerate (30 mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl) methyl3 -(([1,1'-biphenyl] -4-yl (thiophene-2-yl)methyl)carbamo
yl)piperidine-1carboxylate **(12)** as a white to light yellow solid. Compound **12** (0.5 mmol, 1.0eq) was dissolved in morpholine (5 mL) and dichloromethane (5 mL) at room temperature overnight. Ether

acetate (30 mL) was added to the mixture and the organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound N-([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)piperidine-3-carboxamide **(3054a)** as a white to light yellow solid. Hydrochloric acid (4 M in 1,4-dioxane, 0.2 mL) can be added to the dichloromethane/petroleum mixture to give the salt form of **3054a** if needed. ^{1}H NMR (500 MHz, DMSO): δ = 9.03 (d, J = 9.1 Hz, 1H), 8.33 (t, J = 5.9 Hz, 1H), 7.65 (d, J = 8.0 Hz, 4H), 7.57 – 7.39 (m, 5H), 7.36 (t, J = 7.4 Hz, 1H), 6.97 (dd, J = 5.2, 3.5 Hz, 1H), 6.88 (d, J = 3.5 Hz, 1H), 6.34 (d, J = 9.0 Hz, 1H), 2.49 – -0.06 (m, 9H); ^{13}C NMR (101 MHz, MeOD): δ = 173.77, 145.27, 140.61, 140.46, 140.29, 128.53, 127.48, 127.12, 126.78, 126.57, 126.41, 125.56, 124.96, 52.53 (d, J = 1.9 Hz), 44.83, 43.68, 41.74, 38.28, 25.62, 20.52.

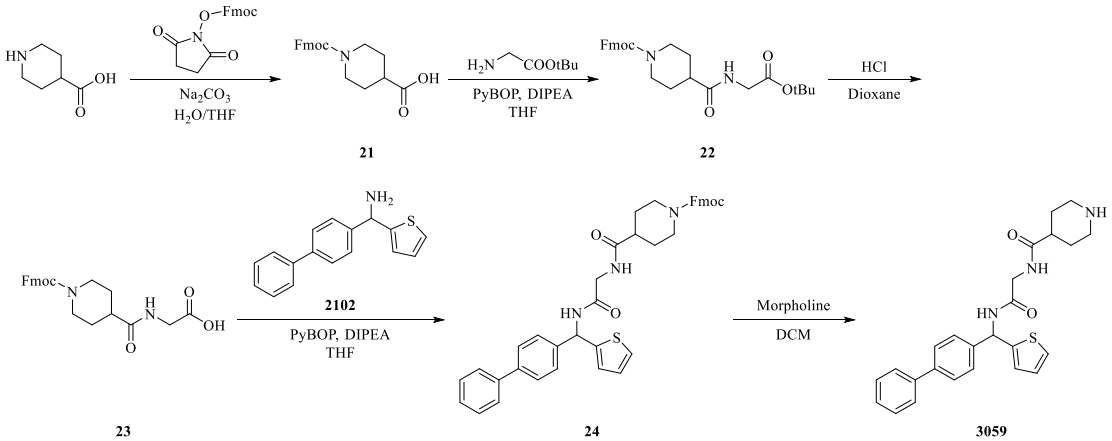
Preparation of compound 3054b



N-(2-(([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)amino)-2-oxoethyl)pyrrolidine-3-carboxamide (3054b). A solution of pyrrolidine-3-carboxylic acid (5 mmol, 1.0eq), (9H-fluoren-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (10 mmol, 2.0eq) and sodium carbonate (25 mmol, 5.0eq) dissolved in water (10 mL) and tetrahydrofuran (15 mL) was stirred at room temperature overnight. Water (30 mL) and ether acerate (30 mL) were added to the mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane /petroleum ether to give the compound1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidine-3-carboxylic acid (**7**) as a white solid. A solution of compound **7** (2 mmol, 1.0eq), tert-butyl glycinate (2 mmol, 1.0eq), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (3 mmol, 1.5eq) and N, N-diisopropylethylamine (5 mmol, 2.5eq) dissolved in THF (10 mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (30 mL) and ether acerate (30 mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture

of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl)methyl 3-((2-(tert-butoxy)-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate **(8)** as a white solid. Compound **8** was dissolved in 1,4-dioxane (5 mL) and hydrochloric acid (4 M in 1,4-dioxane, 5 mL) was added. Stir the solution at room temperature overnight. The solvent was removed by vacuum distillation to give the compound (1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidine-3-carbonyl)glycine **(9)** as a white solid. Compound **2102** was synthesized as described before. A solution of compound **2102** (1 mmol, 1.0eq), compound **9** (1 mmol, 1.0eq), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (1.5 mmol, 1.5eq) and N, N-diisopropylethylamine (2.5 mmol, 2.5eq) dissolved in THF (5 mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (30 mL) and ether acerate (30 mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl)methyl 3-((2-(([1,1'-biphenyl]-4-yl(thiophene-2-yl)methyl)amino)-2-oxoethyl)carbamoyl)pyrrolidine-1 carboxylate **(10)** as a white to light yellow solid. Compound **10** (0.5 mmol, 1.0eq) was dissolved in morpholine (5 mL) and dichloromethane (5 mL) at room temperature overnight. Ether acetate (30 mL) was added to the mixture and the organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound N-(2-(([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl) amino)-2- oxoethyl)pyrrolidine-3-carboxamide **(3054b)** as a white to light yellow solid. Hydrochloric acid (4 M in 1,4-dioxane, 0.2 mL) can be added to the dichloromethane/petroleum mixture to give the salt form of **3054b** if needed. ^{1}H NMR (400 MHz, MeOD) δ 7.63 (d, J = 7.7 Hz, 4H), 7.45 (t, J = 8.2 Hz, 4H), 7.36 (d, J = 6.1 Hz, 2H), 7.06 – 6.83 (m, 2H), 6.46 (s, 1H), 4.08 – 3.89 (m, 2H), 3.65 – 3.35 (m, 4H), 2.27 (ddq, J = 49.3, 13.2, 7.2, 6.6 Hz, 2H), 1.33 (d, J = 16.1 Hz, 1H). ^{13}C NMR (101 MHz, MeOD) δ 173.46, 168.96, 145.26, 140.28 (d, J = 1.6 Hz), 128.53, 127.48, 127.12, 126.77, 126.57, 126.41, 125.57, 124.96, 52.53 (d, J = 2.1 Hz), 45.18, 42.35, 41.94, 28.79 (d, J = 1.5 Hz).

Preparation of compound 3059

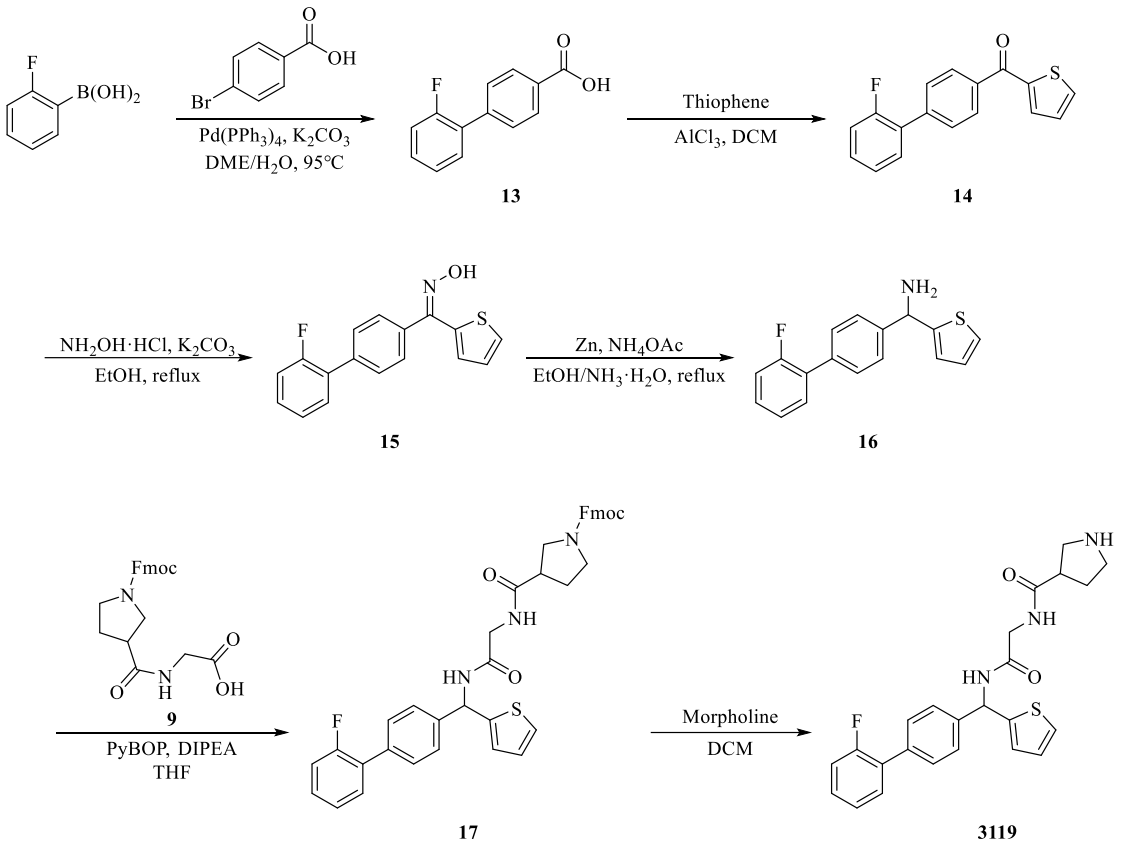


N-(2-(([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)amino)-2-oxoethyl)piperidine-4-carboxamide

(3059). A solution of piperidine-4-carboxylic acid (5mmol, 1.0eq), (9H-fluoren-9-yl)methyl (2,5-dioxopyrrolidin-1-yl) carbonate (10mmol, 2.0eq) and sodium carbonate (25mmol, 5.0eq) dissolved in water (10mL) and tetrahydrofuran (15mL) was stirred at room temperature overnight. Water (30mL) and ether acerate (30mL) were added to the mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound 1-(((9H-fluoren-9-yl)methoxy)carbonyl)piperidine-4-carboxylic acid **(21)** as a white solid. A solution of compound **21** (2mmol, 1.0eq), tert-butyl glycinate (2mmol, 1.0eq), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (3mmol, 1.5eq) and N,N-diisopropylethylamine (5mmol, 2.5eq) dissolved in THF (10mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (30mL) and ether acerate (30mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl)methyl 4-((2-(tert-butoxy)-2-oxoethyl)carbamoyl)piperidine-1-carboxylate **(22)** as a white solid. Compound **22** was dissolved in 1,4-dioxane (5mL) and hydrochloric acid (4M in 1,4-dioxane, 5mL) was added. Stir the solution at room temperature overnight. The solvent was removed by vacuum distillation to give the compound (1-(((9H-fluoren-9-yl)methoxy)carbonyl)piperidine-4-carbonyl)glycine **(23)** as a white solid. A solution of compound **2102** (1mmol, 1.0eq), compound **23** (1mmol, 1.0eq), benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate (1.5mmol, 1.5eq) and N,N-diisopropylethylamine (2.5mmol, 2.5eq) dissolved in THF (5mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (30mL) and ether acerate (30mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl)methyl 4-((2-(([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)amino)-2-oxoethyl)carbamoyl)piperidine-1-carboxylate **(24)** as a white to light yellow solid. Compound **24** (0.5mmol, 1.0eq) was dissolved in morpholine (5mL) and dichloromethane (5mL) at room temperature overnight. Ether acerate (30mL) was added to the mixture and the organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound N-(2-(([1,1'-biphenyl]-4-yl(thiophen-2-yl)methyl)amino)-2-oxoethyl)piperidine-4-carboxamide **(3059)** as a white to light yellow solid. Hydrochloric acid (4M in 1,4-dioxane, 0.2mL) can be added to the dichloromethane/petroleum mixture to give the salt form of **3059** if needed. ^{1}H NMR (400 MHz, MeOD) δ 7.65 – 7.56 (m, 4H), 7.43 (dd, J = 8.2, 7.0 Hz, 4H), 7.37 – 7.29 (m, 2H), 6.96 (dd, J = 5.1, 3.5 Hz, 1H), 6.89 (dt, J = 3.6, 1.2 Hz, 1H), 6.46 – 6.40 (m, 1H), 3.95 (s, 2H), 3.42 (dt, J = 13.0, 4.0 Hz, 2H), 3.03 (td, J = 12.4, 3.4 Hz, 2H), 2.62 (tt, J = 10.7, 3.9 Hz, 1H), 2.09 – 1.98 (m, 2H), 1.97 – 1.82 (m, 2H). ^{13}C NMR (101 MHz, MeOD) δ 175.04, 169.10, 140.61, 140.47, 140.33, 128.52, 127.47,

127.11, 126.77, 126.56, 126.40, 125.54, 124.93, 52.62, 42.89, 42.02, 39.15 (d, J = 4.5 Hz), 25.15.

Preparation of compound HR3119



N-(2-(((2'-fluoro-[1,1'-biphenyl]-4-yl)(thiophen-2-yl)methyl)amino)-2-oxoethyl)pyrrolidine-3-carboxamide (HR3119). A solution of (2-fluorophenyl)boronic acid (4 mmol, 1.0eq), 4-bromobenzoic acid (4 mmol, 1.0eq), tetrakis(triphenylphosphine)palladium (0.4 mmol, 0.1eq) and potassium carbonate (12 mmol, 3.0eq) in 1,2-Dimethoxyethane (15 mL) and water (5 mL) was stirred and heated at 95℃ under argon protect for 12h. Water (30 mL) and ether acerate (30 mL) were added to the mixture and the organic layer was collected and washed with brine (3 × 50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound 2'-fluoro-[1,1'-biphenyl]-4-carboxylic acid **(13)** as a white solid. Compound **13** (2 mmol, 1.0eq) was reacted similarly to the synthesis of compound **1** to give the compound (2'-fluoro-[1,1'-biphenyl]-4-yl)(thiophene-2-yl)methanone **(14)** as a white solid. Compound **14** (1 mmol, 1.0eq), hydroxylamine hydrochloride (3 mmol, 3.0eq), and potassium carbonate (4 mmol, 4.0eq) were reacted similarly to the synthesis of compound **2** to give to crude compound (2'-fluoro-[1,1'-biphenyl]-4-yl)(thiophene-2-yl)methanone oxime **(15)**. The ketoxime compound **15** (0.5 mmol, 1.0eq), zinc dust (2.5 mmol, 5.0eq) and ammonium acetate (0.5 mmol, 1.0eq) were reacted similarly to the synthesis of compound **2102** to give the compound (2'-fluoro-[1,1'-biphenyl]-4-yl)(thiophene-2-yl)methanamine **(16)** as a white to light yellow solid. Compound **9** was synthesized as described before. A solution of compound **16** (0.25 mmol, 1.0eq), compound **9** (0.25 mmol, 1.0eq), (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (0.38 mmol, 1.5eq) and

N, N-diisopropylethylamine (0.63 mmol, 2.5eq) dissolved in THF (1.25 mL) was stirred at room temperature overnight. Saturated ammonium chloride solution (15 mL) and ether acerate (15 mL) were added to the mixture and the organic layer was collected. The organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound (9H-fluoren-9-yl)methyl 3-((2-(((2'-fluoro-[1,1'-biphenyl]-4-yl)(thiophene-2-yl)methyl)amino)-2-oxoethyl)carbamoyl)pyrrolidine-1-carboxylate **(17)** as a white to light yellow solid. Compound **17** (0.1 mmol, 1.0eq) was dissolved in morpholine (1 mL) and dichloromethane (1 mL) at room temperature overnight. Ether acetate (15 mL) was added to the mixture and the organic layer was washed with saturated ammonium chloride solution (3 × 50 mL) and brine (50 mL). The solvent was removed by vacuum distillation and the compound was purified by column chromatography. The solution was crystallized from the mixture of dichloromethane/petroleum ether to give the compound N-(2-(((2'-fluoro-[1,1'-biphenyl]-4-yl)(thiophene-2-yl)methyl)a-mino)-2-oxoethyl)pyrrolidine-3carbox-amide **(3119)** as a white to light yellow solid. Hydrochloric acid (4 M in 1,4-dioxane, 0.2 mL) can be added to the dichloromethane/petroleum mixture to give the salt form of **3119** if needed. ^{1}H NMR (400 MHz, MeOD) δ 7.54 (d, J = 8.0 Hz, 2H), 7.51 – 7.43 (m, 3H), 7.36 (t, J = 5.8 Hz, 2H), 7.29 – 7.14 (m, 2H), 6.97 (t, J = 4.4 Hz, 1H), 6.90 (d, J = 3.5 Hz, 1H), 6.45 (s, 1H), 3.98 (d, J = 3.0 Hz, 2H), 3.53 – 3.32 (m, 4H), 2.25 (ddt, J = 46.3, 13.4, 6.7 Hz, 2H), 1.30 (d, J = 12.6 Hz, 1H).^{13}C NMR (101 MHz, MeOD) δ 173.48, 169.01, 159.71 (d, J = 246.4 Hz), 145.08, 140.75, 135.26, 130.45 (d, J = 3.3 Hz), 129.10 (d, J = 8.2 Hz), 128.85 (d, J = 2.9 Hz), 128.34 (d, J = 13.4 Hz), 127.11, 126.44, 125.64, 125.01, 124.37 (d, J = 3.7 Hz), 115.64 (d, J = 23.0 Hz), 52.56 (d, J = 2.1 Hz), 45.20, 42.37, 41.99, 28.79 (d, J = 1.8 Hz).