Supplementary Information

More than just GPCR Ligand: Structure-based Discovery of Thioridazine Derivatives as Pim-1 Kinase Inhibitors†‡

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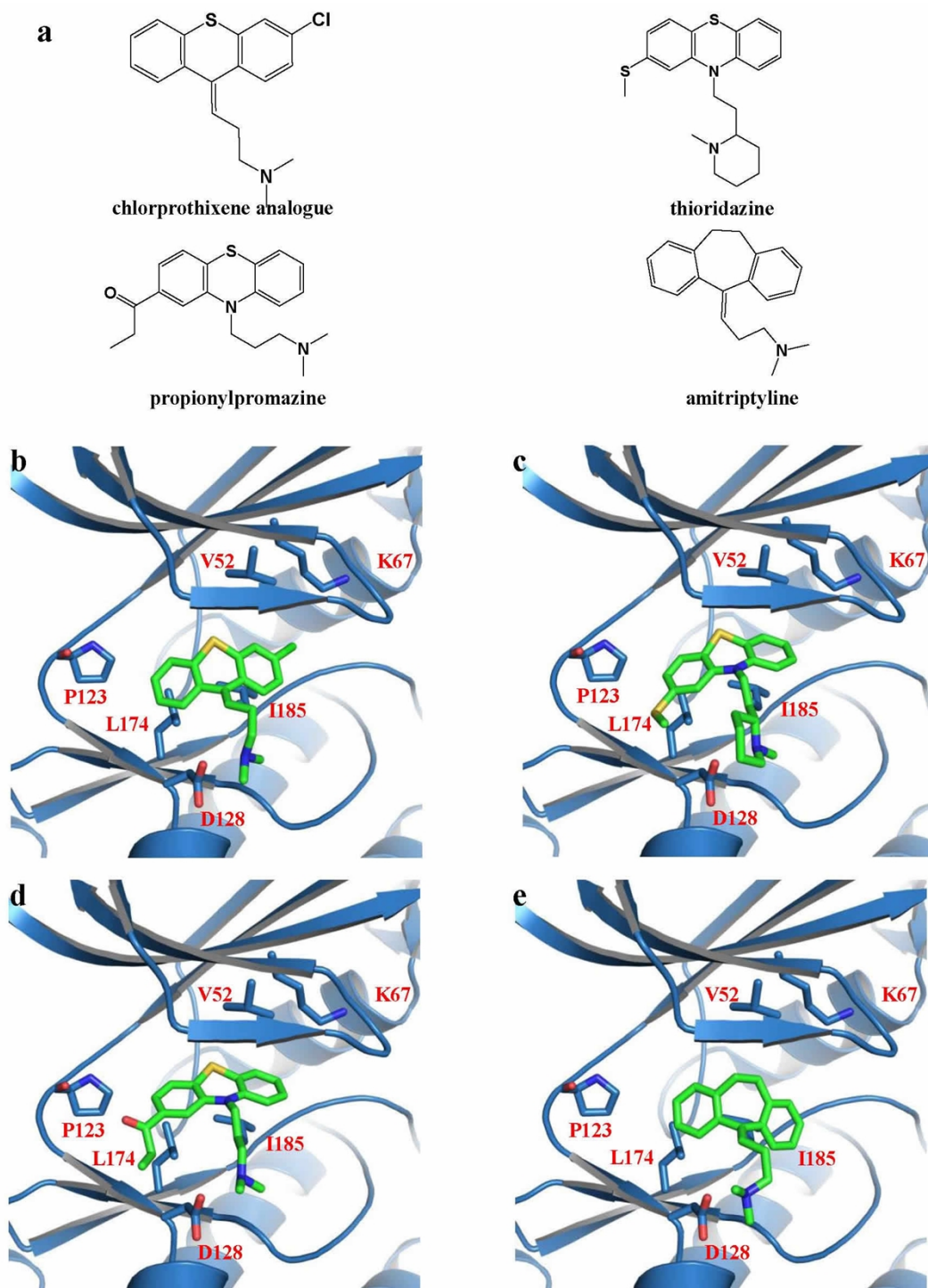Beijing 102206, China.

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Fig. S1 (a) Four representative compounds in structure-based virtual screening. (b) Modelled complex structure of chlorprothixene analogue and Pim-1 kinase domain. The score of MM-GB/SA is -37.056 kcal/mol. (c) Modelled complex structure of (+) form of thioridazine and Pim-1 kinase domain. The score of MM-GB/SA is -36.985 kcal/mol. (d) Modelled complex structure of propionylpromazine and Pim-1 kinase domain. The score of MM-GB/SA is -33.191 kcal/mol (e) Modelled complex structure of amitriptyline and Pim-1 kinase domain. The score of MM-GB/SA is -27.191 kcal/mol. The compound was shown in a green stick representation.
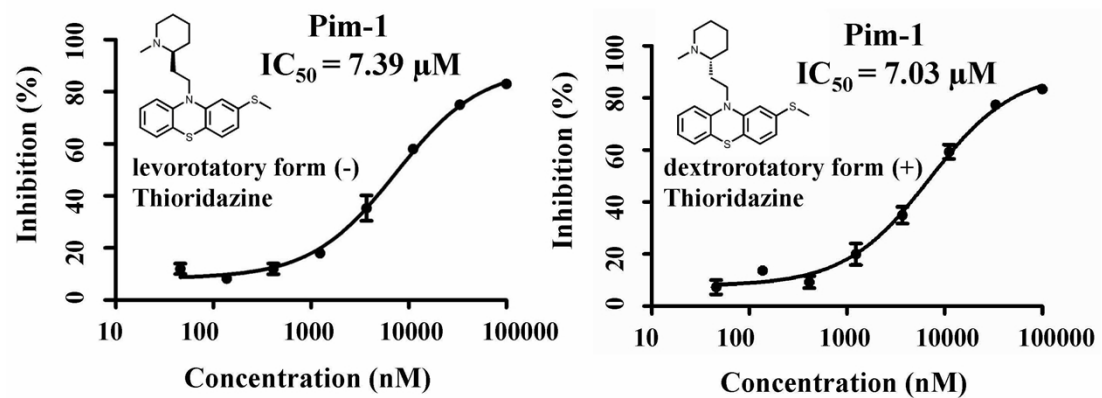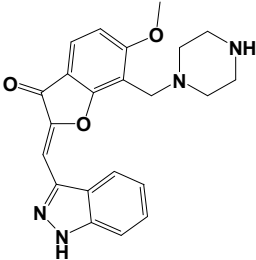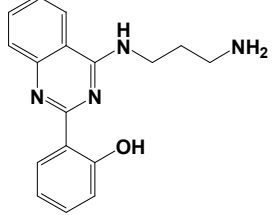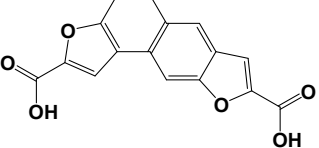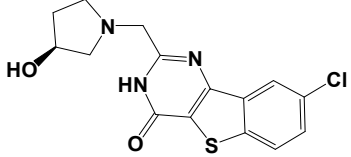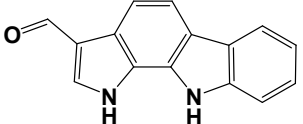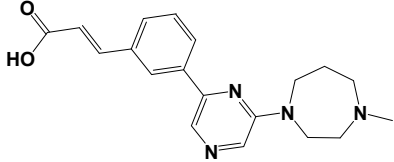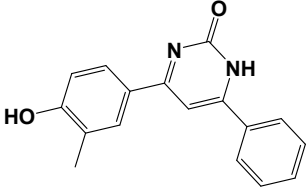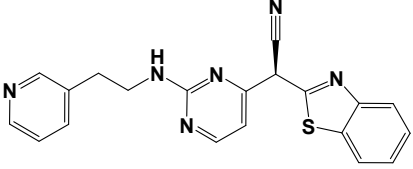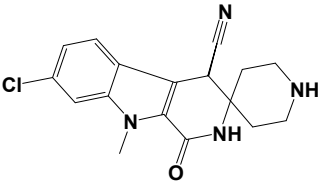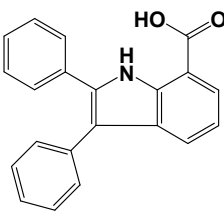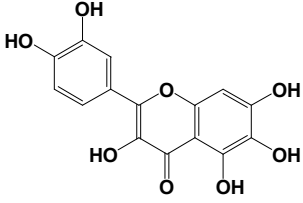
Fig. S2 Dose response inhibition of Pim-1 by two enantiomers of thioridazine. The IC_{50} value was determined from three independent tests.

Table S1. Structural comparison of thioridazine with representative Pim-1 kinase inhibitor co-crystallized with protein.

Pim-1 Inhibitors	Calculated Tanimoto Coefficient comparing with thioridazine
3umw_596	0.234
3uix_Q17	0.232
3ma3_01I	0.113
3jy0_LYG	0.243
3jpv_1DR	0.162

3f2a_985	0.342
3dcv_55E	0.164
3cy3_JN5	0.146
3cy2_MB9	0.184
3bgz_VX3	0.129
2o64_MYU	0.102
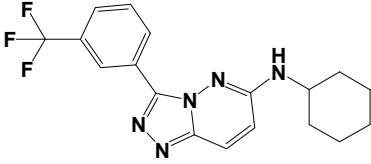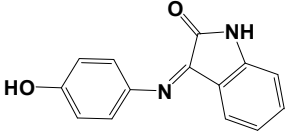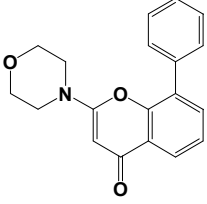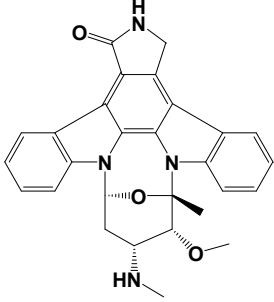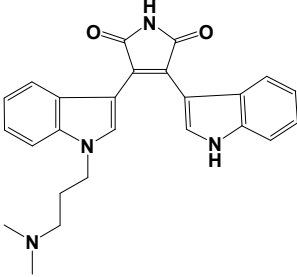
3bgq_VX2	0.289
1yxx_LI7	0.156
1yi3_LY2	0.323
1yhs_STU	0.197
1xws_BI1	0.264

Table S2. Statistics on data collection and structure refinement.

Data collection	4IAA	4MED
Space group	P65	P65
Cell dimensions		
a, b, c (Å)	98.817,98.817,80.507	98.938,98.938,80.831
α, β, γ (°)	90,90,120	90,90,120
Wavelength (Å)	1.5418	1.5418
Resolution range (Å)[a]	20.00-2.85(2.90-2.85)	20.00-2.85(2.80-2.85)
Unique reflections	10412	11198
Redundancy	12.4(5.8)	11.9(5.0)
I/σ	28.0(2.4)	30.0(2.66)
Completeness (%)	99.2(93.2)	99.9(98.9)
R_{merge}[b]	0.104(0.647)	0.122(0.638)
Structure refinement		
Resolution range (Å)	19.55-2.85(2.93-2.85)	19.67-2.80(2.87-2.80)
No. reflections	9905	10591
No. heavy atoms	2248	2242
R_{work}[c]	0.187(0.426)	0.178(0.370)
R_{free}[d]	0.231(0.488)	0.242(0.379)
Average B factor (Å2)	58.8	52.6
Rmsd bond length (Å)	0.011	0.015
Rmsd bond angles (°)	1.425	1.866
PROCHECK statistics[e]		
Core (%)	90.7	97.4
Allowed (%)	9.3	1.9
Generally Allowed (%)	0	2
Disallowed (%)	0	0

[a] Values in parentheses are for the data in the highest resolution shell. [b] $R_{merge} = \sum|I_i - I_m|/\sum I_i$, where I_i is the intensity of the measured reflection and I_m is the mean intensity of all symmetry related reflections. [c] $R_{work} = \sum|F_o - F_c|/\sum F_o$, where F_o and F_c are the observed and calculated structure factor amplitudes. [d] R_{free} is the same as Rwork, but calculated on 5% reflections not used in refinement. [e] Analyzed by PROCHECK.

Synthetic details for the preparation of compounds
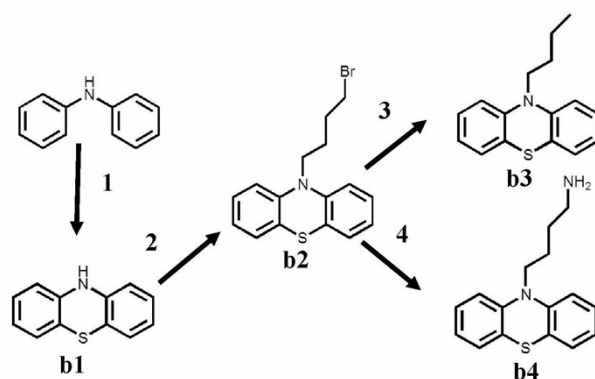
All solvents and chemicals used were reagent grade. Flash column chromatography was carried out using prepacked silica cartridges (from 4 g up to 330 g) from Redisep, Biotage, or Crawford and eluted using an Isco Companion system. The characterization of compounds was established by a combination of liquid chromatography-mass spectroscopy (LC-MS) and NMR analytical techniques. All compounds with a measured initial purity of *>95%*. ^{1}H NMR were recorded on a VARIAN VnmrJ22C (400 MHz) and were determined in CDCl3 or DMSO-d6. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference and coupling constant (J) values are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F254, 0.25 mm, art. 5715) were used for TLC analysis. Solutions were dried over anhydrous magnesium sulphate, and solvent was removed by rotary evaporation under reduced pressure.
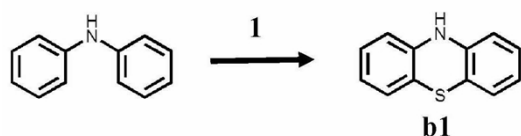
Diphenylamine (2g, 11.8mmol), sulfur (0.76g, 23.6mmol), iodine (0.3g, 1.2mmol) were heated at 170°C for 2 hours. The reaction mixture was diluted with EtOAc (200 mL), and washed with brine, dried (MgSO4) and evaporated in vacuo to a residue which was chromatographed on silica with petroleum ether and ethyl acetate (5:1) as eluantto give10H-phenothiazine (1.42g, 60%).^{1}H NMR (400MHz, DMSO-d_6) δ 6.65 (d, J = 7.6 Hz, 2H), 6.72 (t, J = 7.6 Hz, 2H), 6.88 (d, J = 7.6 Hz, 2H), 6.96 (t, J = 7.6 Hz, 2H). m/z (ES[+]) (M+H)[+] = 200.05.

b1 → **b2**
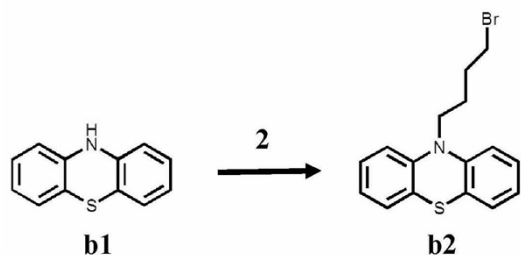
To a solution of compound b1 (1.42g, 7.13mmol) in DMF (10 ml), NaH (256.5 mg, 10.7mmol) was added at 0°C. The reaction mixture was stirred at 25°C for 1 hour, then 1,4-dibromobutane (1.54g,7.13mmol) was added, the reaction mixture was stirred at 25°C for overnight. The DMF was evaporated in vacuo to a residue which was taken up in ethyl acetate (200 mL), washed with brine, dried (MgSO4) and evaporated in vacuo to a residue which was chromatographed on silica with petroleum ether and ethyl acetate (5:1) as eluantto give10-(4-bromobutyl)-10H-phenothiazine (1.86g,78%). ^{1}H NMR (400MHz, CDCl$_3$) δ 1.97-1.99 (m,4H), 3.41 (t, J = 5.6 Hz, 2H), 3.91 (t, J = 5.6 Hz, 2H), 6.87 (d, J = 8.4 Hz, 2H), 6.93 (t, J = 7.6 Hz, 2H), 7.15-7.19 (m, 4H). m/z (ES$^+$) (M+H)$^+$ = 334.02.

b2 → **b3**
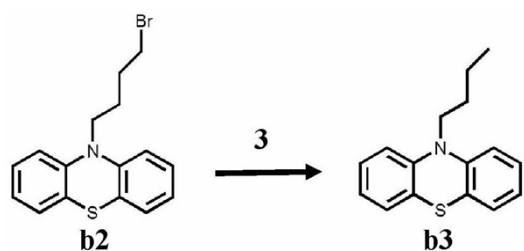
To a solution of compound b2 (100.0 mg, 0.3mmol) in DMSO (2.5ml), H$_2$O (0.5ml), NaN$_3$ (84.5mg) was added, and then the reaction mixture was stirred at 25°C for 48 hours. The DMSO was evaporated in vacuo to a residue, and then dissolved in MeOH (3ml), Pd/C (5.0 mg, 0.15mmol) was added. The reaction mixture was stirred at 50°C under an atmosphere of hydrogen for overnight. The catalyst was filtered off, washed with Methanol (20 mL) and the combined filtrates evaporated in vacuo to a residue which was filtered through a silica column to remove a trace of colloidal palladium to give10-butyl-10H-phenothiazine (38.0mg, 50%). ^{1}H NMR (400MHz, CDCl$_3$) δ 0.94 (t, J = 7.6 Hz, 3H), 1.41-1.50 (m, 2H), 1.75-1.83 (m, 2H), 3.85 (t, J = 7.2 Hz, 2H), 6.86 (d, J = 8.0 Hz, 2H), 6.88-6.92 (m, 2H), 7.12-7.16 (m, 4H). m/z (ES$^+$) (M+H)$^+$ = 256.12.

b2 → **b4**
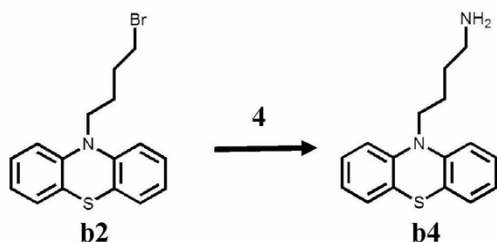
To a solution of compound b2 (50.0 mg, 0.15mmol) in DMSO (0.5ml), H$_2$O (0.2ml), NaI (45.0 mg, 0.3mmol), the reaction mixture was stirred at 25°C for 48 hours. The DMSO was evaporated

in vacuo to a residue, and then dissolved in DMF (3ml), $NH_3 \cdot H_2O$ (0.22ml) , $CsCO_3$ (97.7mg, 0.3mmol) were added. The reaction mixture was stirred at 80°C for 2 hours. The reaction mixture was diluted with EtOAc (200 mL), and washedwith brine, dried (MgSO4) and evaporated in vacuo to a residue which was chromatographed on silica with dichloromethane and methanol (10:1) as eluantto give3-(10H-phenothiazin-10-yl)propan-1-amine (30.7mg, 80%). ^{1}H NMR (400MHz, DMSO-d_6) δ 1.72-1.75 (m, 2H), 2.78 (t, J = 7.2 Hz, 2H), 3.38 (bar, 2H), 3.89 (t, J = 7.6 Hz, 2H), 6.95 (t, J = 7.6 Hz, 2H), 7.04 (d, J = 8.0 Hz, 2H), 7.15-7.23 (m, 4H). m/z (ES[+]) (M+H)[+] = 257.37.