Design, synthesis and evaluation of novel $^{19}\text{F}$ magnetic resonance sensitive protein tyrosine phosphatase inhibitors†‡

Yu Li,a Guiquan Xia,a Qi Guo,a Li Wu,b Shizhen Chen,c Zhigang Yang,a Wei Wang,a Zhong-Yin Zhang,b Xin Zhouc and Zhong-Xing Jiang*acde

Fluorine is a highly attractive element for both medicinal chemistry and imaging technologies. To facilitate protein tyrosine phosphatase (PTP)-targeted drug discovery and imaging-guided PTP research on fluorine, several highly potent and $^{19}\text{F}$ MR sensitive PTP inhibitors were discovered through a structure-based focused library strategy.

Introduction

PTPs play crucial roles in such fundamental cellular processes as proliferation, differentiation, survival, apoptosis, motility and adhesion.1 Abnormal PTP activity is well known to be associated with a broad spectrum of human diseases.2 As a superfamily of more than 100 signalling enzymes, many PTPs have emerged as attractive drug targets, such as mPTPB for tuberculosis, SHP2 for many types of cancers, LYP for autoimmune diseases, and PTP1B for type 2 diabetes, obesity and breast cancer.3 To this end, the discovery of highly potent and specific small-molecule PTP inhibitors and their application in probing the biological and pathological mechanisms of PTPs, especially with the aid of modern imaging and spectroscopy technologies, are the cornerstone for PTP-targeted drug discovery.

As a versatile element in biomedical research, fluorine has promising utility in PTP-targeted drug discovery. On one hand, the introduction of fluorine(s) into bioactive molecules is usually accompanied by improved pharmacokinetic properties and protein–ligand binding interactions.4 Thus, fluorination has become a routine strategy in drug discovery, and fluorinated compounds have made up over 20% of all pharmaceuticals. On the other hand, fluorinated molecules can be monitored in vivo without ionizing radiation and background signals by $^{19}\text{F}$ magnetic resonance ($^{19}\text{F}$ MR) which provides high-contrast and non-invasive spectroscopy ($^{19}\text{F}$ NMR) and images ($^{19}\text{F}$ MRI). In recent years, $^{19}\text{F}$ MRI/NMR has been widely used in tracking targets of interest5 and monitoring biological reactions.6 Therefore, the discovery of fluorinated small-molecule PTP inhibitors with high $^{19}\text{F}$ MR sensitivity may provide easy access to PTP-targeted drugs and detailed understanding of PTPs’ biological and pathological mechanisms.

A recent discovery of a $^{19}\text{F}$ MRI sensitive salinomycin derivative with specific toxicity towards cancer cells7 by this group prompted us to develop novel fluorinated PTP inhibitors. Herein, ortho-bis(trifluoromethyl)carbinol phenol was designed as a novel chemical scaffold for $^{19}\text{F}$ MRI sensitive PTP inhibitors (Scheme 1). Due to the strong electron-withdrawing ability of 2 trifluoromethyl groups, bis(trifluoromethyl)carbinol is a weak acid and is therefore a suitable substitute for the carboxylic group in salicylic acid from which a number of highly potent and selective PTP inhibitors have recently been discovered.8 Consequently, the ortho-bis(trifluoromethyl)carbinol phenols may mimic the well-established binding mode of salicylic acid-

![Scheme 1 Design of $^{19}\text{F}$ MR sensitive PTP inhibitors.](image-url)
based inhibitors at the highly positively charged active site of PTPs. It is noteworthy that the 6 symmetric fluorines in bis(trifluoromethyl)carbinol, which were recently employed in the construction of highly 19F MRI sensitive dendritic drug delivery vehicles, aggregate provide a strong 19F MR signal for conveniently probing the mode of interaction and related biological reactions using 19F NMR and 19F MRI. Moreover, cell permeability is a challenge for PTP inhibitors. Bis(trifluoromethyl)carbinol-based PTP inhibitors without a negative charge may exhibit favorable cell permeability, bioavailability and pharmacokinetic properties by the introduction of hydrophobic trifluoromethyl groups.

Materials and methods

Chemistry general information

1H, 13F and 13C NMR spectra were recorded at 400 MHz. Chemical shifts (δ) are in ppm and coupling constants (J) are in Hertz (Hz). 1H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using CDCl3, acetone-d6, or DMSO-d6 as solvents. 13C NMR spectra were referenced to solvent carbons (77.16 ppm for CDCl3, 29.84, 206.26 ppm for acetone-d6, and 39.52 ppm for DMSO-d6). 19F NMR spectra were referenced to 2% perfluorobenzene (s, −164.90 ppm). The splitting patterns for 1H NMR spectra are denoted as follows: s (singlet), d (doublet), q (quartet), m (multiplet), dd (doublet of doublets), and td (triplet of doublets). High resolution mass spectra were recorded using electron spray ionization (ESI).

Unless otherwise indicated, all reagents were obtained from a commercial supplier and used without prior purification. DCM and DMF were dried and freshly distilled prior to use. Flash chromatography was performed using silica gel (200–300 mesh) with either petroleum ether/EtOAc as eluents.

Synthesis of compounds

Phenol 1c. Hexafluoroacetone trihydrate (9.71 g, 6.1 mL, 44.1 mmol) was dried over concentrated sulfuric acid and the resulting anhydrous hexafluoroacetone was bubbled into a solution of 4-phenylphenol (5.00 g, 29.4 mmol) and aluminum chloride (0.39 g, 2.94 mmol) in 1,2-dichloroethane (250 mL) slowly. After the addition, the mixture was heated under reflux at 80 °C until 4-phenylphenol was consumed, as indicated by TLC. The reaction mixture was then cooled to rt, washed with 2 N HCl (100 mL) and extracted with DCM (50 mL × 2). The combined organic layers were dried over anhydrous Na2SO4, concentrated under vacuum and purified by flash chromatography using silica gel (5% EtOAc/petroleum ether) to give 1c as white wax (3.6 g, 85% yield).

Phenol 1a. Phenol 1a was prepared from benzene (0.80 g, 10.2 mol) by following the general procedure as clear oil (2.5 g, 30% yield). 1H NMR (CDCl3, 400 MHz) δ 7.39–7.53 (m, 3H), 7.73 (dd, J = 7.4, 0.9 Hz, 2H); 13F NMR (CDCl3, 376 MHz) δ −78.69.

Phenol 1b. Phenol 1b was prepared from p-cresol (3.0 g, 27.7 mmol) by following the general procedure as white wax (6.1 g, 80% yield). 1H NMR (CDCl3, 400 MHz) δ 2.31 (s, 3H), 6.16 (s, 1H), 6.81 (d, J = 8.3 Hz, 1H), 6.88 (s, 1H), 7.15 (dd, J = 8.3, 1.6 Hz, 1H), 7.23 (s, 1H); 13F NMR (CDCl3, 376 MHz) δ −78.64.

Phenol 1d. Phenol 1d was prepared from [1,1′-biphenyl]-3-ol (5.00 g, 29.4 mmol) in the same manner as described for 1c (8.6 g, 87% yield). 1H NMR (CDCl3, 400 MHz) δ 7.13 (d, J = 1.8 Hz, 1H), 7.19−7.26 (m, 1H), 7.35−7.47 (m, 3H), 7.47−7.59 (m, 3H); 19F NMR (CDCl3, 376 MHz) δ −78.72; 13C NMR (acetone-d6, 100 MHz) δ 79.9−81.1 (m), 114.5, 117.0, 120.2, 124.2 (q, J = 286 Hz), 128.8, 129.1, 129.9, 140.0, 145.3, 157.5; HRMS (ESI) calcd for C13H11F6O2− ([M + H]+) 337.0658, found 337.0654.

Phenol 1e. Phenol 1e was prepared from [1,1′-biphenyl]-2-ol (5.00 g, 29.4 mmol) in the same manner as described for 1c (3.6 g, 74% yield). 1H NMR (CDCl3, 400 MHz) δ 7.11 (t, J = 7.9 Hz, 1H), 7.35 (dd, J = 7.5, 1.5 Hz, 1H), 7.43−7.50 (m, 3H), 7.50−7.59 (m, 3H); 19F NMR (CDCl3, 376 MHz) δ −78.41; 13C NMR (acetone-d6, 100 MHz) δ 80.6−81.8 (m), 115.5, 121.4, 121.4 (q, J = 286 Hz), 128.2, 128.3, 129.2, 130.5, 131.1, 133.8, 134.8, 154.9; HRMS (ESI) calcd for C13H11F6O2− ([M + H]+) 337.0658, found 337.0654.

Phenol 1f. Phenol 1f was prepared from 2-naphthalenol (5.00 g, 34.7 mmol) in the same manner as described for 1c (6.2 g, 57% yield). 1H NMR (CDCl3, 400 MHz) δ 7.29 (s, 1H), 7.42 (dd, J = 11.1, 3.9 Hz, 1H), 7.52 (dd, J = 11.1, 4.0 Hz, 1H), 7.68 (d, J = 8.3 Hz, 1H), 7.82 (d, J = 8.2 Hz, 1H), 8.04 (s, 1H); 19F NMR (CDCl3, 376 MHz) δ −78.40; 13C NMR (acetone-d6, 100 MHz) δ 80.2−81.4 (m), 113.3, 117.7, 124.2 (q, J = 286 Hz), 125.5, 125.6, 128.9, 129.0, 129.5, 130.6, 135.7, 154.0; HRMS (ESI) calcd for C13H11F6O2− ([M + H]+) 311.0501, found 311.0489.

Phenol 1g. Phenol 1g was prepared from 1-naphthalenol (5.00 g, 34.7 mmol) in the same manner as described for 1c (6.5 g, 60% yield). 1H NMR (CDCl3, 400 MHz) δ 7.39 (s, 2H), 7.46−7.63 (m, 2H), 7.71−7.84 (m, 1H), 8.24−8.38 (m, 1H); 13F NMR (CDCl3, 376 MHz) δ −78.55; 13C NMR (acetone-d6, 100 MHz) δ 81.2−82.4 (m), 107.0, 120.6, 123.5, 124.18 (q, J = 287 Hz), 124.19, 126.8, 126.9, 128.2, 129.0, 135.9, 155.5; HRMS (ESI) calcd for [(M + H]+) C13H11F6O2− 311.0501, found 311.0498.

Naphthol 3. Naphthol 3 was prepared from 2,7-naphthalenediyl (30.0 g, 187.2 mmol) in the same manner as described for 1c (10.2 g, 17% yield). 1H NMR (acetone-d6, 400 MHz) δ 7.01−7.13 (m, 2H), 7.25 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 8.04 (s, 1H); 13F NMR (acetone-d6, 376 MHz) δ −76.10; 13C NMR (acetone-d6, 100 MHz) δ 80.1−81.5 (m), 107.9, 111.7, 114.2, 118.4, 124.1, 124.3 (q, J = 286 Hz), 130.3, 131.5, 137.6, 154.5, 158.2; HRMS (ESI) calcd for C13H11F6O2− ([M + H]+) 327.0450, found 327.0444.
**Naphthol 4.** To an ice-cold suspension of diol 3 (2.40 g, 7.36 mmol) in trifluoroacetic acid, acetone (2.2 mL, 29.5 mmol) was added and then TFA (10.8 mL, 145.87 mmol) was added to the mixture dropwise. The reaction mixture was warmed slowly to rt and then stirred for 48 h. After evaporation of the solvent, the residue was purified by flash chromatography using silica gel (2% EtOAc/petroleum ether) to give 4 as white wax (0.85 g, 34% yield). 1H NMR (acetone-d6, 400 MHz) δ 1.61 (s, 6H), 7.11–7.24 (m, 2H), 7.34 (s, 1H), 7.95 (d, J = 8.8 Hz, 1H), 8.09 (s, 1H); 19F NMR (acetone-d6, 376 MHz) δ −78.30; 13C NMR (acetone-d6, 100 MHz) δ 27.0, 76.6–77.8 (m), 102.8, 108.5, 110.0, 113.7, 119.2, 123.2 (q, J = 287 Hz), 125.2, 128.4, 131.6, 138.0, 150.0, 158.5; HRMS (ESI) calcd for C16H18F6O5+ ([M + H]+) 367.0763, found 367.0773.

**Ester 8.** To a solution of 4 (470.0 mg, 1.28 mmol) and methyl bromoacetate (588.7 mg, 3.85 mmol) in acetone, K2CO3 (381.5 mg, 3.85 mmol) was added and then the reaction mixture was heated under reflux until 4 was consumed, as indicated by TLC. After removal of the solvent under reduced pressure, the residue was dissolved in EtOAc (25 mL) and then washed with 2 N HCl (30 mL) and brine (30 mL × 2). The organic layer was dried over anhydrous Na2SO4, concentrated under vacuum and used without purification. The residue was dissolved in TFA/H2O (9/1, 11.3 mL); then, anisole (45 μL) was added and the resulting mixture was stirred overnight. The reaction mixture was concentrated under vacuum and then diluted with EtOAc (25 mL) and washed with brine (30 mL × 2). The organic layer was dried over anhydrous Na2SO4, concentrated under vacuum and purified by flash chromatography using silica gel (20–80% EtOAc/petroleum ether) to give 7a as clear oil (131 mg, 77% yield). 1H NMR (acetone-d6, 400 MHz) δ 0.88 (t, J = 7.4 Hz, 3H), 1.41–1.66 (m, 2H), 2.32–3.31 (m, 2H), 4.64 (s, 2H), 7.09–7.25 (m, 2H), 7.42 (s, 1H), 7.91 (d, J = 9.0 Hz, 1H), 8.09 (s, 1H); 19F NMR (acetone-d6, 376 MHz) δ −76.08; 13C NMR (acetone-d6, 100 MHz) δ 11.1, 23.0, 41.0, 67.4, 79.7–80.3 (m), 105.4, 111.9, 114.8, 117.8, 123.6 (q, J = 286 Hz), 124.1, 129.5, 130.8, 136.6, 154.4, 157.8, 168.6; HRMS (ESI) calcd for C18H16F6NO4+ ([M + H]+) 426.1135, found 426.1118.

**Amide 7b.** Amide 7b was prepared from 4 (110.0 mg, 0.30 mmol) in the same manner as described for 7a (100 mg, 78% yield). 1H NMR (acetone-d6, 400 MHz) δ 0.48–0.64 (m, 2H), 0.66–0.80 (m, 2H), 2.06 (dt, J = 4.4, 2.2 Hz, 1H), 4.61 (s, 2H), 7.08–7.23 (m, 2H), 7.42 (s, 1H), 7.90 (d, J = 9.0 Hz, 1H), 8.08 (s, 1H); 19F NMR (acetone-d6, 376 MHz) δ −76.06; 13C NMR (acetone-d6, 100 MHz) δ 5.9, 22.8, 67.6, 79.9–80.5 (m), 105.5, 112.1, 114.9, 119.7, 123.8 (q, J = 286 Hz), 124.2, 127.9, 131.0, 136.7, 154.5, 158.0; HRMS (ESI) calcd for C18H16F6NO4+ ([M + H]+) 424.0978, found 424.0976.

**Amide 7c.** Amide 7c was prepared from 4 (150.0 mg, 0.41 mmol) in the same manner as described for 7a (130 mg, 72% yield). 1H NMR (acetone-d6, 400 MHz) δ 1.12 (t, J = 7.1 Hz, 3H), 1.26 (t, J = 8.0 Hz, 3H), 3.42 (q, J = 7.0 Hz, 2H), 3.51 (q, J = 7.1 Hz, 2H), 4.95 (s, 2H), 7.02–7.18 (m, 2H), 7.30 (s, 1H), 7.85 (d, J = 8.8 Hz, 1H), 8.02 (s, 1H); 19F NMR (acetone-d6, 376 MHz) δ −76.04; 13C NMR (acetone-d6, 100 MHz) δ 12.8, 14.1, 40.6, 66.0, 78.0–79.2 (m), 104.7, 110.0, 116.5, 122.4, 123.1 (q, J = 288 Hz), 129.6, 130.2, 136.0, 153.9, 157.6, 166.0; HRMS (ESI) calcd for C18H18F6NO4+ ([M + H]+) 440.1291, found 440.1298.

**Amide 7d.** Amide 7d was prepared from 4 (150.0 mg, 0.41 mmol) in the same manner as described for 7a (90 mg, 42% yield). 1H NMR (DMSO-d6, 400 MHz) δ 1.62 (s, 6H), 2.00 (d, J = 10.7 Hz, 10H), 4.53 (s, 2H), 7.06 (d, J = 8.4 Hz, 2H), 7.18 (s, 1H), 7.44 (s, 1H), 7.85 (d, J = 8.7 Hz, 1H); 13C NMR (DMSO-d6, 100 MHz) δ −76.01; 13C NMR (DMSO-d6, 100 MHz) δ 28.7, 35.9, 40.9, 51.0, 67.0, 78.0–79.1 (m), 104.6, 110.1, 116.6, 122.5, 123.1 (q, J = 288 Hz), 129.6, 130.2, 136.0, 153.9, 157.3, 166.4; HRMS (ESI) calcd for C23H26F6NO4+ ([M + H]+) 518.1761, found 518.1766.

**Amide 7e.** Amide 7e was prepared from 4 (100.0 mg, 0.27 mmol) in the same manner as described for 7a (91 mg, 71% yield). 1H NMR (acetone-d6, 400 MHz) δ 4.51 (d, J = 8.0 Hz, 2H), 4.71 (s, 2H), 7.10–7.16 (m, 1H), 7.17–7.33 (m, 6H), 7.40 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 8.08 (s, 1H); 13C NMR (acetone-d6, 376 MHz) δ −76.03; 13C NMR (acetone-d6, 100 MHz) δ
105.7, 112.2, 115.1, 118.2, 123.9 (q, \( J = 7.4 \) Hz, 1H), 7.30–7.38 (m, 2H), 7.42 (s, 1H), 7.90 (d, \( J = 9.0 \) Hz, 1H), 8.09 (s, 1H); \(^{13}C\) NMR (acetone-d6, 376 MHz) \( \delta = 76.03; \) \(^{13}C\) NMR (acetone-d6, 100 MHz) \( \delta = 42.7, 55.4, 55.6, 67.5, 79.8–80.4 \) (m), 105.6, 112.0, 112.1, 115.0, 118.0, 120.2, 123.8 (q, \( J = 286 \) Hz), 124.2, 129.7, 130.1, 131.8, 136.7, 149.1, 149.8, 154.4, 158.0, 168.8; HRMS (ESI) calcd for C_{28}H_{20}F_{6}NO_{4}{^+} [(M + H)]^+ 534.1346, found 534.1369.

Amide 7i. Amide 7i was prepared from 4 (150.0 mg, 0.41 mmol) in the same manner as described for 7a (29 mg, 16% yield). \(^{1}H\) NMR (acetone-d6, 400 MHz) \( \delta = 4.81 \) (s, 2H), 6.26 (s, 1H), 7.20–7.29 (m, 1H), 7.29–7.35 (m, 3H), 7.51 (d, \( J = 7.5 \) Hz, 2H), 7.81 (t, \( J = 8.4 \) Hz, 3H), 8.04 (s, 1H); \(^{19}F\) NMR (acetone-d6, 376 MHz) \( \delta = 76.08; \) \(^{13}C\) NMR (acetone-d6, 100 MHz) \( \delta = 54.3, 67.3, 78.8–79.4 \) (m), 105.1, 110.6, 117.1, 120.6, 123.0, 123.7 (q, \( J = 288 \) Hz), 125.2, 128.0, 128.9, 130.1, 130.7, 136.5, 140.6, 144.9, 154.9, 157.7, 160.9; HRMS (ESI) calcd for C_{28}H_{20}F_{6}NO_{4}{^+} [(M + H)]^+ 556.0512, found 556.0510.

Amide 7j. Amide 7j was prepared from 4 (150.0 mg, 0.41 mmol) in the same manner as described for 7a (29 mg, 16% yield). \(^{1}H\) NMR (acetone-d6, 400 MHz) \( \delta = 4.81 \) (s, 2H), 7.11 (t, \( J = 7.4 \) Hz, 1H), 7.20–7.39 (m, 4H), 7.42 (s, 1H), 7.72–7.83 (m, 2H), 7.95 (d, \( J = 9.0 \) Hz, 1H), 8.12 (s, 1H); \(^{19}F\) NMR (acetone-d6, 376 MHz) \( \delta = 76.04; \) \(^{13}C\) NMR (acetone-d6, 100 MHz) \( \delta = 67.7, 79.7–80.3 \) (m), 105.6, 111.9, 114.8, 117.9, 120.5, 123.6 (q, \( J = 286 \) Hz), 124.2, 124.5, 129.0, 129.6, 130.9, 132.6, 136.6, 138.6, 154.2, 157.9, 166.8; HRMS (ESI) calcd for C_{28}H_{16}F_{6}NO_{4}{^+} [(M + H)]^+ 450.0978, found 450.0982.

Amide 7k. Amide 7k was prepared from 4 (150.0 mg, 0.41 mmol) in the same manner as described for 7a (130 mg, 63% yield). \(^{1}H\) NMR (acetone-d6, 400 MHz) \( \delta = 4.82 \) (s, 2H), 7.18–7.29 (m, 2H), 7.34 (d, \( J = 7.4 \) Hz, 1H), 7.44 (dd, \( J = 16.7, 8.8 \) Hz, 3H), 7.60–7.70 (m, 4H), 7.73–7.89 (m, 3H), 8.09 (s, 1H); \(^{19}F\) NMR (acetone-d6, 376 MHz) \( \delta = 76.04; \) \(^{13}C\) NMR (acetone-d6, 100 MHz) \( \delta = 68.0, 80.0–80.6 \) (m), 105.9, 112.2, 115.0, 118.2, 120.9, 121.0, 123.9 (q, \( J = 286 \) Hz), 124.4, 127.1, 127.6, 127.7, 127.8, 129.4, 130.0, 131.1, 136.8, 137.2, 138.3, 140.9, 154.4, 158.1, 167.0; HRMS (ESI) calcd for C_{28}H_{20}F_{6}NO_{4}{^+} [(M + H)]^+ 536.1279, found 536.1272.

Amide 7l. Amide 7l was prepared from 4 (120.0 mg, 0.33 mmol) in the same manner as described for 7a (47 mg, 27% yield). \(^{1}H\) NMR (acetone-d6, 400 MHz) \( \delta = 4.82 \) (s, 2H), 7.18–7.29 (m, 2H), 7.34 (d, \( J = 7.4 \) Hz, 1H), 7.44 (dd, \( J = 16.7, 8.8 \) Hz, 3H), 7.60–7.70 (m, 4H), 7.73–7.89 (m, 3H), 8.09 (s, 1H); \(^{19}F\) NMR (acetone-d6, 376 MHz) \( \delta = 76.04; \) \(^{13}C\) NMR (acetone-d6, 100 MHz) \( \delta = 68.0, 80.0–80.5 \) (m), 105.9, 112.2, 115.0, 118.2, 120.9, 121.0, 123.9 (q, \( J = 286 \) Hz), 124.4, 127.1, 127.6, 127.7, 127.8, 129.4, 130.0, 131.1, 136.8, 137.2, 138.3, 140.9, 154.4, 158.1, 167.0; HRMS (ESI) calcd for C_{28}H_{20}F_{6}NO_{4}{^+} [(M + H)]^+ 536.1279, found 536.1272.
Amide 7o. Amide 7o was prepared from 4 (110.0 mg, 0.30 mmol) in the same manner as described for 7a (110 mg, 68% yield). \(^1\)H NMR (acetone-\(d_6\), 400 MHz) \(\delta\) 4.79 (s, 2H), 6.98-7.11 (m, 2H), 7.39 (d, \(J = 45.1\) Hz, 11H), 7.81-7.95 (m, 1H), 8.06 (s, 1H); \(^{19}\)F NMR (acetone-\(d_6\), 376 MHz) \(\delta\) -76.07; \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz) \(\delta\) 66.03, 78.4, 80.0 (m), 105.5, 112.4, 115.3, 122.5 (q, \(J = 288\) Hz), 129.6, 130.4, 136.0, 135.7, 156.7, 166.7; HRMS (ESI) calcd for C\(_{22}\)H\(_{16}\)F\(_6\)N\(_2\)O\(_4\)S\(^+\) ([M + H]\(^+\)) 536.1291, found 536.1296.

Amide 7p. Amide 7p was prepared from 4 (150.0 mg, 0.41 mmol) in the same manner as described for 7a (53 mg, 35% yield). \(^1\)H NMR (acetone-\(d_6\), 400 MHz) \(\delta\) 5.06 (s, 2H), 6.86-7.02 (m, 2H), 7.26-7.49 (m, 4H), 7.52-7.65 (m, 2H), 7.65-7.89 (m, 4H), 8.03 (s, 1H); \(^{19}\)F NMR (acetone-\(d_6\), 376 MHz) \(\delta\) -76.12; \(^{13}\)C NMR (DMSO-\(d_6\), 100 MHz) \(\delta\) 65.5, 78.0-79.2 (m), 104.0, 104.1, 110.0, 116.4, 122.5, 123.1 (q, \(J = 286\) Hz), 126.9, 127.5, 128.1, 129.6, 130.3, 132.0, 135.76, 135.83, 136.8, 137.2, 137.5, 153.88, 153.93, 156.0, 157.0, 166.0; HRMS (ESI) calcd for C\(_{22}\)H\(_{16}\)F\(_6\)N\(_2\)O\(_4\)S\(^+\) ([M + H]\(^+\)) 536.1291, found 536.1296.

Amide 7q. Amide 7q was prepared from 9 (200.0 mg, 0.47 mmol) in the same manner as described for 7m (126 mg, 76% yield). \(^1\)H NMR (acetone-\(d_6\), 400 MHz) \(\delta\) 5.09 (s, 2H), 7.24-7.54 (m, 5H), 7.77 (d, \(J = 8.0\) Hz, 1H), 7.96 (dd, \(J = 12.5, 5.3\) Hz, 2H), 8.11 (s, 1H); \(^{19}\)F NMR (acetone-\(d_6\), 376 MHz) \(\delta\) -76.12; \(^{13}\)C NMR (acetone-\(d_6\), 100 MHz) \(\delta\) 67.5, 80.0-81.1 (m), 106.0, 112.4, 115.3, 118.3, 121.8, 122.2, 124.1 (q, \(J = 286\) Hz), 124.70, 124.74, 127.0, 130.1, 131.4, 133.0, 137.0, 149.6, 154.8, 158.1, 158.3, 168.2; HRMS (ESI) calcd for C\(_{22}\)H\(_{16}\)F\(_6\)N\(_2\)O\(_6\)S\(^+\) ([M + H]\(^+\)) 517.0651, found 517.0646.

Amide 7r. Amide 7r was prepared from 9 (120.0 mg, 0.28 mmol) in the same manner as described for 7m (95 mg, 61% yield). \(^1\)H NMR (acetone-\(d_6\), 400 MHz) \(\delta\) 2.34 (s, 3H), 4.65 (s, 2H), 7.03-7.16 (m, 2H), 7.21 (d, \(J = 8.1\) Hz, 2H), 7.37 (s, 1H), 7.67 (d, \(J = 8.3\) Hz, 2H), 7.89 (dd, \(J = 22.1, 8.7\) Hz, 1H), 8.12 (s, 1H); \(^{19}\)F NMR (acetone-\(d_6\), 376 MHz) \(\delta\) -76.03; \(^{13}\)C NMR (acetone-\(d_6\), 100 MHz) \(\delta\) 21.1, 66.7, 79.9-80.5 (m), 105.7, 112.1, 118.2, 123.8 (q, \(J = 286\) Hz), 124.4, 128.8, 130.0, 131.1, 136.1, 136.8, 144.5, 154.4, 158.0, 167.1; HRMS (ESI) calcd for C\(_{22}\)H\(_{19}\)F\(_6\)N\(_2\)O\(_6\)S\(^+\) ([M + H]\(^+\)) 553.0863, found 553.0861.

\(^{19}\)F MRI experiments

\(^{19}\)F MRI experiments were performed on a 9.4 T micro-imaging system with a 10 mm inner diameter \(^{19}\)F coil (376.4 MHz) for both radiofrequency transmission and reception. The MSME (Multi-Slice Multi-Echo) pulse sequence was employed for all MRI acquisitions with a single average. FOV = 8 × 8 mm\(^2\), SL = 40.0 mm TR = 2500 ms and TE = 7.6 ms were used. The data collection time was 160 ms.

Computational analysis

For computational analysis, PDB code 3O5X was used as a model structure. Molecular docking was carried out using AutoDock Vina. The small molecule binding mode was modelled manually using Moloc (Gerber Molecular Design, Switzerland). The image was produced by using PyMOL.

PTP activity assay

PTP activity was assayed using \(p\)-nitrophenyl phosphate (\(p\)NPP) as a substrate in 3,3-dimethylglutarate buffer (50 mM 3,3-dimethylglutarate, pH 7.0, 1 mM EDTA, 150 mM NaCl) at 25 °C. The library compounds were screened using a 96-well format. The amount of the \(p\)-nitrophenol product was determined from the absorbance at 405 nm detected using a Spectra MAX340 microplate spectrophotometer (Molecular Devices). The nonenzymatic hydrolysis of \(p\)NPP was corrected by

![Scheme 2 Synthesis of the bis(trifluoromethyl)carbinol library.](image-url)
measuring the control without the addition of an enzyme. All PTPs used in the study were recombinant proteins prepared in-house.

Results and discussion

To probe the structure–activity relationship of the ortho-bis(trifluoromethyl)carbinol phenol-based inhibitors, a structure-based focused library strategy was employed. Our initial effort involved the construction of a focused library of 7 bis(trifluoromethyl)carbinol-substituted benzene to identify the optimal relative positions for these substituents (Scheme 2). Through the Lewis acid-catalysed Friedel–Crafts reaction, the bis(trifluoromethyl)-carbinol moiety was conveniently anchored to benzene, phenols, and naphthols in good yields. Due to the strong directing effect of the phenolic hydroxyl group, the desired ortho-bis(trifluoromethyl)carbinol phenols were isolated as the major products (1b–g).

The ability of library compounds 1a–g to inhibit a selected panel of PTPs of therapeutic interest, including mPTPB, SHP2, PTP1B, CD45, LYP, and FAP-1, was assessed at pH 7 and 25 °C (Table 1). The results indicate that the phenolic hydroxyl group plays a crucial role in PTP binding through which the inhibitors may mimic the binding mode of salicylic acid-based inhibitors. No appreciable activity was found for 1a, which lacks a phenolic hydroxyl group in the scaffold. The PTP inhibitory activity is also very sensitive to the size and position of the substituent. Neither 1c with a para-phenyl group nor 1d with a meta-phenyl group has appreciable activity, while 1b with a small-sized para-methyl group has moderate activity. Among the library compounds 1a–g, 2-naphthol derived 1f is the most potent one for the selected panel of PTPs, which was then selected for further optimization.

To further improve the potency and selectivity, 1f was modified into a focused library to target both the active site and a peripheral secondary binding site of PTPs (Scheme 3). Starting from 2,7-naphthalene-diol 2, a core compound 3, with an extra 7-hydroxyl group compared to 1f, was constructed through Friedel–Crafts reaction in good yield. Then, a panel of amines 5a–r with structural diversity were selected for the construction of side chains 6a–r by reaction with bromoacetyl bromide, respectively. After protecting the 2 neighbouring hydroxyl groups in 3 with acetones, side chains 6a–r were anchored to the 7-hydroxyl group in 4 in the presence of K₂CO₃ to give ester intermediates, after which the acetonide protecting group was removed with TFA to give amides 7a–p in good yields over 2 steps. However, the preparation of 7m, 7q and 7r was unsuccessful. So, an alternative method was developed by first anchoring an acetic acid side chain to 4 and then coupling amines 5m, 5q, and 5r, respectively, to give the corresponding amides 7m, 7q, and 7r. In this way, the focused library of 18 ortho-bis(trifluoromethyl)carbinol phenols 7a–r with an amide side chain was conveniently prepared.

To illustrate the structures of ortho-bis(trifluoromethyl)carbinol phenols 7a–r, a single-crystal X-ray structure of 7c was obtained (Fig. 1). However, many attempts to prepare a single-crystal of 7p were unsuccessful.

As expected, the activities of library compounds 7a–r are much higher than those of 1f (Table 2). Compound 7r with a sulfonohyrazide side chain was identified as a highly potent and selective mPTPB inhibitor with an IC₅₀ value of 2.3 μM.
and more than 7-fold selectivity compared to SHP2, PTP1B, CD45, LYP, and FAP-1. It is interesting to point out that most of aliphatic amine derived compounds 7a–c and 7e–g show no appreciable PTP inhibitory activity, while bulky aliphatic amine derived compounds 7d, 7h, and 7i exhibit moderate activities. In contrast, most of aromatic amine derived compounds 7j–r have good activities and selectivity except for the positively charged 7m. Among them, compound 7p with a bulky aromatic group on the side chain exhibits very high activity toward the selected panel of PTPs with an IC_{50} value ranging from 2.2 μM for FAP-1 to 6.6 μM for PTP1B. Based on these observations, it is obvious that the potency of ortho-Bis(trifluoromethyl)carbinol phenol-based inhibitors can be considerably optimized up to 58-fold by tethering an amide side chain. A bulky aromatic group-containing side chain, i.e. 7l and 7p, can efficiently promote the binding affinity between PTPs and inhibitors by interacting with a peripheral pocket in the vicinity of the PTP active sites, probably through steric effects and π–π stacking.

Computational analysis of the binding activity of 7p in the highly conservative active site of PTPs provided some insight into the structure–activity relationship between these novel inhibitors and PTPs. Oncogenic SHP2 with a known complex structure (PDB ID: 3O5X) was selected as a model. Fig. 2 shows the binding mode of 7p with SHP2 compared to that of a known salicylic acid-based SHP2 inhibitor 10 which has an IC_{50} of 5.5 μM toward SHP2. As expected, the ortho-Bis(trifluoromethyl)carbinol phenol moiety can mimic the binding mode of salicylic acid by interacting with the corresponding amino acid residues Trp423, Arg465 and Gln510 (the distances between O of ortho-Bis(trifluoromethyl)carbinol and the three hydrogen bonding heavy atoms of the residues are 3.6 Å). However, due to the difference in molecular geometry, the side chains of 7p and 10 interacted with SHP2 in different ways. Instead of interacting with Arg362 and Lys364 of SHP2, the aromatic side chain in 7p has a strong π–π interaction with Tyr-279.

Finally, the 19F magnetic resonance properties of PTP inhibitors 7a–r were investigated. As designed, all 6 symmetrical fluorines in 7a–r generated a strong singlet 19F NMR signal, respectively (Fig. 3). Unified 19F signal dramatically improved the 19F NMR sensitivity of these fluorinated inhibitors for downstream applications. Then, 7p, with a high potency toward a panel of PTPs, was selected for the 19F MRI study. It was found that 7p has a very short longitudinal relaxation time T_1 of 299 ms, which could further improve its 19F MRI sensitivity by allowing the collection of more transient signals without prolonging the data acquisition time. The 19F MRI phantom experiment on an array of 7p solutions indicated that 7p could be clearly imaged by 19F MRI with a scan time of 120 seconds at a concentration of as low as 8.3 mM (or 50 mM in 19F concentration, Fig. 3). Therefore, 7p is a novel PTP inhibitor as well as a highly valuable tool molecule whose local information, such as distribution and concentration, and interactions with PTPs, such as the binding mode and affinity, can be conveniently monitored by 19F MR spectroscopy and imaging without extra modification in the absence of background signals.

### Conclusions and outlook

In summary, we have successfully demonstrated a strategy for developing novel 19F magnetic resonance sensitive small molecule PTP inhibitors for drug discovery and biomedical research through rational molecular design and symmetrical fluorination. ortho-Bis(trifluoromethyl)carbinol phenol is a valuable substitute for salicylic acid in PTP inhibitor discovery, which successfully integrates the PTP binding ability and high 19F NMR signal generating ability. As fluorinated drugs are booming in pharmaceutical industry, it is of great importance to utilize their inherent 19F magnetic resonance properties in target identification, pharmacology study, in vivo drug tracking, image/spectroscopy-guided drug therapy and beyond.

Finally, we want to point out that both 19F NMR and 19F MRI are valuable modalities for biomedical research. 19F MRI provides high-contrast images at 19F concentrations of mM and above, while 19F NMR provides sensitive spectroscopy even at sub-μM 19F concentrations. To improve the PTP inhibition potency and selectivity, studies on the 19F MRI sensitivity of these inhibitors and their application in the 19F magnetic resonance-guided PTP mechanism are currently in progress and will be published in due course.
Acknowledgements

We are thankful for financial support from the National Natural Science Foundation of China (21372181, 21402144, 21572168, and 21575157), Key Laboratory of Synthetic Chemistry of Natural Substances (Shanghai Institute of Organic Chemistry) and State Key Laboratory for Modification of Chemical Fibers and Polymer Materials (Donghua University). LW and ZYZ are supported by NIH RO1 CA69202 and P30 CA023168.

Table 2  IC50 (μM) of 7a–r for a selected panel of PTPs

<table>
<thead>
<tr>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
<th>IC50 (μM)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>18.1 ± 9.5</td>
<td></td>
<td>7e</td>
<td>6.7 ± 1.2</td>
<td></td>
<td>7g</td>
<td>13.8 ± 2.0</td>
<td></td>
<td>7i</td>
<td>6.6 ± 0.6</td>
<td></td>
<td>7k</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7l</td>
<td>10.0 ± 0.8</td>
<td></td>
<td>7m</td>
</tr>
<tr>
<td>7b</td>
<td>9.4 ± 0.4</td>
<td></td>
<td>7f</td>
<td>6.9 ± 1.4</td>
<td></td>
<td>7n</td>
<td>10.0 ± 0.8</td>
<td></td>
<td>7o</td>
<td>6.6 ± 0.6</td>
<td></td>
<td>7p</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7q</td>
<td>10.0 ± 0.8</td>
<td></td>
<td>7t</td>
</tr>
<tr>
<td>7c</td>
<td>5.1 ± 0.4</td>
<td></td>
<td>7h</td>
<td>6.8 ± 1.2</td>
<td></td>
<td>7r</td>
<td>10.0 ± 0.8</td>
<td></td>
<td>7s</td>
<td>6.6 ± 0.6</td>
<td></td>
<td>7u</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7v</td>
<td>10.0 ± 0.8</td>
<td></td>
<td>7w</td>
</tr>
<tr>
<td>7d</td>
<td>3.5 ± 0.5</td>
<td></td>
<td>7x</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7a</td>
<td>14.7 ± 3.0</td>
<td></td>
<td>7y</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7b</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7z</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7c</td>
</tr>
<tr>
<td>7e</td>
<td>4.8 ± 0.1</td>
<td></td>
<td>7i</td>
<td>7.5 ± 1.1</td>
<td></td>
<td>7r</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7m</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7n</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7o</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7p</td>
</tr>
<tr>
<td>7f</td>
<td>4.7 ± 0.2</td>
<td></td>
<td>7j</td>
<td>7.2 ± 0.5</td>
<td></td>
<td>7n</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7q</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7o</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7r</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7p</td>
</tr>
<tr>
<td>7g</td>
<td>4.7 ± 0.2</td>
<td></td>
<td>7k</td>
<td>4.8 ± 0.1</td>
<td></td>
<td>7o</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7r</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7p</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7q</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7r</td>
</tr>
<tr>
<td>7h</td>
<td>4.7 ± 0.2</td>
<td></td>
<td>7l</td>
<td>4.7 ± 0.2</td>
<td></td>
<td>7o</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7r</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7p</td>
<td>12.6 ± 4.8</td>
<td></td>
<td>7q</td>
<td>7.3 ± 0.5</td>
<td></td>
<td>7r</td>
</tr>
</tbody>
</table>

Fig. 2  The calculated structure of 7p bound to SHP2 compared with a salicylic acid-based inhibitor 10.

Fig. 3  19F NMR of selected inhibitors (upper) and 19F MRI of 7p (lower).
Notes and references