A Novel Class of Inhibitors of Peptide Deformylase Discovered Through High Throughput Screening and Virtual Ligand Screening

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JM049222O

SUPPORTING INFORMATION

General Procedures. Common reagents were purchased from commercial suppliers. 4-oxo-2-thioxo-3-thiazolidineacetic acid was purchased from Aldrich (Milwaukee, WI) and 4-oxo-2-thioxo-3-thiazolidinepropanoic acid was purchased from TCI-America (Portland, OR). All reactions were carried out under an inert nitrogen atmosphere with dry solvents, under anhydrous conditions, unless otherwise stated. Organic solutions were dried over anhydrous Na_2SO_4 before being concentrated. TLC was performed on silica gel 60 F254 (Merck) with detection by UV light. Column chromatography was performed on silica gel (60-120 micron). Analytical reversed-phase HPLC was performed on a BDS C-18 column (250mm x 4.6 mm) eluted with a gradient of MeCN in H_2O containing 0.1% TFA (flow rate of 0.50 mL/min, with a detection range of 200-400 nm using maximum chromatogram). ^1H and ^{13}C spectra were recorded at 300 or at 400 MHz and 75 or at 100 MHz, respectively, for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26 ppm) or $CDCl_3$ (δ_C 77.0 ppm) as internal standard.

4-Oxo-2-thioxo-3-thiazolidinehexanoic acid (**17e**) was prepared according to the literature procedure.[1] The corresponding butanoic (**17c**), pentanoic (**17d**), heptanoic (**17f**) and octanoic acids (**17g**) were prepared in the same manner from the appropriate amino acids and their characterization data is provided, below.

4-Oxo-2-thioxo-3-thiazolidinebutanoic acid (17c): 4-aminobutanoic acid (10.31 g, 100 mmol), 22% aq. KOH (50 mL), carbon disulfide (9.7 mL, 100 mmol), chloroacetic acid (9.45 g, 100 mmol), and conc. H_2SO_4 (32 mL, 180 mmol) afforded the corresponding butanoic acid (14.78 g, 67 %) as an off-white solid: mp 124-127 °C; ^1H NMR (300

MHz, $CDCl_3$) δ 1.99-2.06 (m, 2H), 2.45 (t, 2H, *J* = 7.2), 3.98 (s, 2H), 4.09 (t, 2H, *J* = 7.2); MS AP(−) *m/z* 218.01 $(M-H)^-$. Anal. Calcd for $C_7H_9NO_3S_2$: C, 38.34; H, 4.14; N, 6.39. Found: C, 38.64; H, 3.98; N, 6.32.

4-Oxo-2-thioxo-3-thiazolidinepentanoic acid (17d): 5-Aminopentanoic acid (15.36 g, 100 mmol), 22% aq. KOH (50 mL), carbon disulfide (9.7 mL, 100 mmol), chloroacetic acid (9.45 g, 100 mmol) and conc. H_2SO_4 (32 mL, 180 mmol) afforded the corresponding pentanoic acid (9.44 g, 41 %) as an off white solid: mp 117-119 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62-1.81 (m, 4H), 2.41 (t, 2H, *J* = 6.9), 3.93-4.08 (m, 4H); MS AP(−) *m/z* 232.02 $(M-H)^-$. Anal. Calcd for $C_8H_{11}NO_3S_2$: C, 41.19; H, 4.75; N, 6.00; S, 27.49. Found: C, 40.98; H, 4.65; N, 5.90; S, 27.70.

4-Oxo-2-thioxo-3-thiazolidineheptanoic acid (17f): 7-aminoheptanoic acid (1.0 g, 6.9 mmol), aq. KOH (0.78 g, 13.7 mmol), carbon disulfide (0.53 g, 6.9 mmol), chloroacetic acid (0.65 g, 6.9 mmol) and conc. H_2SO_4 (1.22 g, 12.4 mmol) afforded the corresponding heptanoic acid (0.55 g, 30%) as an off-white solid: mp 74-76 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.27-1.46 (m, 4H), 1.56-1.72 (m, 4H), 2.35 (t, 2H, *J* = 7.4), 3.91-4.03 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.4, 26.3, 26.5, 28.5, 33.8, 35.3, 44.6, 173.9, 179.7, 201.2; MS AP(−) *m/z* 260.11 $(M-H)^-$.

4-Oxo-2-thioxo-3-thiazolidineoctanoic acid (17g): 8-Aminooctanoic acid (1.0 g, 6.3 mmol), aq. KOH (0.71g, 12.5 mmol), carbon disulfide (0.48 g, 6.3 mmol), chloroacetic acid (0.59 g, 6.3 mmol) and conc. H_2SO_4 (1.2 g, 11.3 mmol) afforded the corresponding

octanoic acid (0.50 g, 29%) as a yellow solid: mp 82-84 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.29-139 (m, 6H), 1.56-1.70 (m, 4H), 2.35 (t, 2H, *J* = 7.3), 3.93-4.00 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 24.5, 26.5, 26.6, 28.7, 28.8, 33.9, 35.3, 44.7, 173.9, 179.7, 201.2; MS AP(−) *m/z* 274.11 $(M-H)^-$. Anal. Calcd for $C_{11}H_{17}NO_3S_2$: C, 47.98; H, 6.22; N, 5.09; S, 23.29. Found: C, 48.17; H, 6.06; N, 4.93; S, 22.94.

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (2). Ethylenediamine diacetate (2.18 g, 12.0 mmol) and 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (2.41 g, 12.0 mmol), was added to stirring solution of 4-oxo-2-thioxo-3-thiazolidinehexanoic acid (**17e**) (3.00 g, 12.0 mmol) in methanol (45 mL) at rt. After 1 h, a yellow precipitate formed. Stirring was continued overnight at room temperature. The precipitate was filtered, washed with water and dried under high vacuum to give **2** (4.12 g, 79%) as a bright yellow solid: mp 184-186 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35-1.52 (m, 2H), 1.64-1.83 (m, 4H), 2.03 (s, 3H), 2.18 (s, 3H), 2.38 (t, 2H, *J* = 7.5), 4.12 (t, 2H, *J* = 7.6), 6.20 (s, 1H), 7.18-7.21 (m, 2H), 7.45-7.56 (m, 3H), 7.75 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.1, 12.8, 24.2, 26.2, 26.6, 33.8, 44.1, 105.8, 114.8, 116.4, 127.8, 127.8, 128.9, 129.6, 132.6, 137.2, 137.6, 168.0, 179.3, 193.5; MS AP(−) *m/z* 427.12 $(M-H)^-$. Anal. Calcd for $C_{22}H_{24}N_2O_3S_2$: C, 61.66; H, 5.64; N, 6.54; S, 14.96. Found: C, 61.30; H, 5.43; N, 6.48; S, 15.13.

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinebutanoic acid (**18).** Following the same procedure as for **2**, ethylenediamine diacetate (0.252 g, 1.14 mmol), 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (0.227 g, 1.14 mmol) and 4-oxo-2-thioxo-3-thiazolidinebutanoic acid (0.250 g, 1.14 mmol)

afforded **18** (290 mg, 63%) as a yellow solid: mp 87-89 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.04-2.15 (m, 2H), 2.18 (s, 3H), 2.46 (t, 2H, *J* = 7.5), 4.22 (t, 2H, *J* = 6.8), 6.20 (s, 1H), 7.16-7.23 (m, 2H), 7.45-7.57 (m, 3H), 7.76 (s, 1H); MS AP(+) *m/z* 401.03 $(M+H)^+$. Anal. Calcd for $C_{20}H_{20}N_2O_3S_2$: C, 59.98; H, 5.03; N, 6.99; S, 16.01. Found: C, 60.11; H, 5.02; N, 6.83; S, 15.85.

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinepentanoic acid (**19).** Following the same procedure as for **2**, ethylenediamine diacetate (2.31 g, 12.8 mmol), 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (2.56 g, 12.8 mmol) and 4-oxo-2-thioxo-3-thiazolidinepentanoic acid (3.00 g, 12.8 mmol) afforded **19** (3.55 g, 67%) as an orange solid: mp 88-90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.67-1.87 (m, 4H), 2.03 (s, 3H), 2.18 (s, 3H), 2.44 (t, 2H, *J* = 7.2), 4.15 (t, 2H, *J* = 7.2), 6.20 (s, 1H), 7.18-7.23 (m, 2H), 7.47-7.53 (m, 3H), 7.75 (s, 1H); ^{13}C NMR δ 24.2, 25.2 (2C), 25.6, 26.2, 26.6, 31.2(2C), 33.8, 41.6, 44.2, 125.0, 142.9, 166.6, 179.7, 194.0; MS AP(+) *m/z* 415.15 $(M+H)^+$. Anal. Calcd for $C_{21}H_{22}N_2O_3S_2$: C, 60.85; H, 5.35; N, 6.76; S, 15.47. Found: C, 60.90; H, 5.31; N, 6.76; S, 15.50.

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidineheptanoic acid (**20**)**:** Following the same procedure as for **2**, ethylenediamine diacetate (1.09 g, 6.7 mmol), 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (1.33 g, 6.7 mmol) and 4-oxo-2-thioxo-3-thiazolidineheptanoic acid (1.75 g, 6.7 mmol) afforded **20** (2.1 g, 71%) as an orange solid: mp 162-163 °C; ^{1}H

NMR (300 MHz, $CDCl_3$) δ 1.35-1.47 (m, 4H), 1.64-1.74 (m, 4H), 2.03 (s, 3H), 2.18 (s, 3H), 2.36 (t, 2H, *J* = 7.4), 4.12 (t, 2H, *J* = 7.6), 6.21 (s, 1H), 7.20 (m, 2H), 7.51 (m, 3H) and 7.75 (s, 1H); MS (+ ve mode): m/z 443.2 $(M+H)^+$; HPLC purity 91% (BDS).

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidineoctanoic acid (**21**)**:** Following the same procedure as for **2**, ethylenediamine diacetate (590 mg, 3.6 mmol), 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (720 mg, 3.6 mmol) and 4-oxo-2-thioxo-3-thiazolidineoctanoic acid (1.0 g, 3.6 mmol) afforded **21** (600 mg, 38%) as an orange solid: mp 128-129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38 (m, 4H), 1.64 (m, 6H), 2.03 (s, 3H), 2.18 (s, 3H), 2.36 (t, 2H, *J* = 7.4), 4.12 (t, 2H, *J* = 7.6), 6.21 (s, 1H), 7.20 (m, 2H), 7.51 (m, 3H) and 7.75 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.1, 12.7, 24.5, 26.5, 26.8, 28.8 (2C), 33.9, 44.4, 105.8, 115.0, 116.4, 127.8 (2C), 128.9, 129.6, 137.3, 137.5, 168.0, 179.5 and 193.5; MS (+ ve mode): m/z 457.2 $(M+H)^+$; HPLC purity 85%. Anal. Calcd for $C_{24}H_{28}N_2O_3S_2$: C, 63.13; H, 6.18; N, 6.13; S, 14.04. Found: C, 62.98; H, 6.03; N, 6.21; S, 14.05.

N-Hydroxy-5-[(2,5-dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexamide (22). *O*-(tetrahydro-2*H*-pyran-2-yl)hydroxylamine (160 mg, 1.4 mmol) was added to a solution of the acid **2** (500 mg, 1.2 mmol) in dry dichloromethane (10 mL) at rt and cooled it to 0 ^{o}C. To this cold solution was added 1-hydroxybenzotriazole hydrate (190 mg, 1.4 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (270 mg, 1.4 mmol) sequentially. The mixture was then allowed to warm to rt and stir overnight. The reaction mixture was diluted with

dichloromethane (20 mL) and the dichloromethane layer was washed with water (2 x 10 mL), brine (10 mL) and dried (Na_2SO_4), filtered and concentrated. The crude material so obtained was taken directly to the next step without further purification.

p-Toluenesulfonic acid (20 mg, 0.12 mmol) was added to a solution of the crude *O*-THP hydroxamate in dry MeOH (10 mL) and stirred at rt for 2 h, at which point the reaction was judged to be complete as indicated by formation of an $FeCl_3$ active spot on TLC. The reaction was quenched by adding water (30 mL) and extracted with ethyl acetate (3 x 15 mL). Ethyl acetate layer was washed with water (2 x 15 mL) and brine (15 mL), then dried (Na_2SO_4), filtered and concentrated. The crude compound was purified by column chromatography (silica gel, $CHCl_3$ / MeOH) to obtain **22** in pure form as yellow solid (310 mg, 60%). mp 110 – 113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (m, 2H), 1.77 (m, 4H), 2.04 (s, 3H), 2.19 (s, 3H), 2.23 (m, 2H), 4.12 (t, 2H, *J* = 7.4), 6.21 (s, 1H), 7.20 (m, 2H), 7.52 (m, 3H) and 7.76 (s, 1H); ^{13}C NMR (75 MHz) δ 11.1, 12.7, 24.5, 25.8, 26.2, 32.4, 43.8, 105.7, 114.6, 116.4, 127.7, 128.0, 128.8, 129.5, 132.6, 137.2, 137.8, 168.1, 170.9, 193.4; MS (+ ve mode): m/z 444 $(M+H)^+$; HPLC purity 92% (BDS).

5-Cyclohexylmethylene-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (23): Following the same procedure as for **2**, ethylenediamine diacetate (95.5 mg, 0.53 mmol) and cyclohexanecarboxaldehyde (0.0554 mL, 0.53 mmol), afforded **23** (75 mg, 42%) as a yellow solid: mp 130-133 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21-1.47 (m, 6H), 1.63-1.87 (m, 10 H), 2.07-2.21 (m, 1H), 2.37 (t, 2H, *J* = 7.4), 4.06 (t, 2H, *J* = 7.6), 6.83 (d, 1H, *J* = 9.4); MS AP(−) *m/z* 340.10 $(M-H)^-$. Anal. Calcd for $C_{16}H_{23}NO_3S_2$: C, 56.28; H, 6.79; N, 4.10; S, 18.78. Found: C, 55.95; H, 6.56; N, 4.02; S, 18.46.

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid methyl ester (24). Iodomethane (0.168 mL, 0.52 mmol) was added to stirring mixture of **2** (200 mg, 0.47 mmol) and potassium carbonate (193 mg, 1.4 mmol) in acetonitrile (15 mL) and allowed to react at rt overnight. The reaction mixture was filtered and the filtrate was diluted with ethyl acetate (20 mL). The organic phase was washed with 1 N NaOH (3 x 20 mL) and brine (1 x 10mL), dried over $MgSO_4$, filtered and concentrated to an orange oil. This oil was then purified by silica gel chromatography (10-40% EtOAc /hexane gradient over 20 min.) and dried under high vacuum to afford the methyl ester **24** (120 mg, 58%) as a yellow solid: mp 124-126 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33-1.49 (m, 2H), 1.61-1.85 (m, 4H), 2.03 (s, 3H), 2.18 (s, 3H), 2.33 (t, 2H, *J* = 7.8), 4.12 (t, 2H, *J* = 7.6), 6.21 (s, 1H), 7.17-7.21 (m, 2H), 7.44-7.56 (m, 3H), 7.47 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.0, 12.7, 24.3, 26.1, 26.4, 33.7, 44.0, 51.3, 76.6, 76.9, 77.4, 105.6, 114.7, 116.3, 127.6 (2C), 128.8, 129.5, 132.5, 137.1, 137.4, 167.8, 173.8, 193.3; MS AP(+) *m/z* 443.04 $(M+H)^+$. Anal. Calcd for $C_{23}H_{26}N_2O_3S_2$: C, 62.42; H, 5.92; N, 6.38; S, 14.49. Found: C, 62.25; H, 5.70; N, 6.20; S, 14.40.

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidineacetic acid (25). 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (1.99 g, 10.0 mmol) and 4-oxo-2-thioxo-3-thiazolidineacetic acid (1.91 g, 10.0 mmol) was allowed to stir in 15 mL ethanol under reflux for 18 h. The resulting precipitate was then filtered, washed with water and dried under high vacuum to afford **25** (2.49 g, 67%) as an

orange solid: mp 248-250 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (s, 3H), 2.17 (s, 3H), 4.83 (s, 2H), 6.21 (s, 1H), 7.20 (d, 2H, *J* = 8.1), 7.50-7.53 (m, 3H), 7.8 (s, 1H); MS AP (+) *m/z* 373.02 $(M+H)^+$, MS AP(−) *m/z* 371.04 $(M-H)^-$.

5-[(2,5-Dimethyl-1-phenyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinepropionic acid (**26).** Following the same procedure as for **25**, 2,5-dimethyl-1-phenylpyrrole-3-carboxaldehyde (1.99 g, 10.0 mmol) and 4-oxo-2-thioxo-3-thiazolidinepropanoic acid (2.05 g, 10.0 mmol) afforded **26** (2.21 g, 57%) as an orange solid: mp 242-244 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.02 (s, 3H), 2.18 (s, 3H), 2.84 (t, 2H, *J* = 7.8), 4.44 (t, 2H, *J* = 7.8), 6.20 (s, 1H), 7.20 (d, 2H, *J* = 8.1), 7.50-7.53 (m, 3H), 7.78 (s, 1H); MS AP(+) *m/z* 387.02 $(M+H)^+$.

The other active compounds presented in Tables 1 and 2, from our corporate compound collection and from commercial sources (for the virtual ligand screening experiment), were characterized as follows:

5-[(1,3-Diphenyl-1H-pyrazol-4-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (1). mp 174-176 °C; ^{1}H NMR (300 MHz, DMSO) δ 1.25-1.38 (m, 2H), 1.47-1.70 (m, 4H), 2.19 (t, 2H, *J* = 7.3), 4.03 (t, 2H, *J* = 7.3), 7.42 (t, 1H, *J* = 7.8), 7.54-7.61 (m, 6H), 7.64-7.68 (m, 2H), 8.06 (d, 2H, *J* = 7.7), 8.81 (s, 1H); MS AP(−) *m/z* 476.01 $(M-H)^-$.

5-[(1-(4-Bromophenyl)-2,5-dimethyl-1H-pyrrol-3-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (3). mp 163-165 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.38-1.50 (m, 2H), 1.63-1.80 (m, 4H), 2.03 (s, 3H), 2.18 (s, 3H), 2.30 (t, 2H, *J* = 7.4), 4.11 (t, 2H, *J* = 7.8), 6.19 (s, 1H), 7.09 (d, 2H, *J* = 8.6), 7.64 (d, 2H, *J* = 8.3), 7.72 (s, 1H); MS AP(+) *m/z* 508.88 $(M+H)^+$.

5-[(5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (4). mp 115-116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39-1.50 (m, 2H), 1.64-1.83 (m, 4H), 2.39 (t, 2H, *J* = 7.5), 2.44 (s, 3H), 4.12 (t, 2H, *J* = 7.5), 7.45-7.58 (m, 5H), 7.61 (s, 1H); MS AP(+) *m/z* 451.94 $(M+H)^+$.

5-[(3-(5-Methylfuran-2-yl)-1-phenyl-1H-pyrazol-4-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (5). mp 154-156°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39-

1.54 (m, 2H), 1.83-1.66 (m, 4H), 2.38 (t, 2H, *J* = 7.5), 2.45 (s, 3H), 4.13 (t, 2H, *J* = 7.5), 6.16 (d, 1H, *J* = 3.6), 6.82 (d, 1H, *J* = 3.3), 7.35-7.42 (m, 1H), 7.48-7.55 (m, 2H), 7.77 (d, 2H, *J* = 8.7), 8.07 (s, 1H), 8.18 (s, 1H); MS AP(+) *m/z* 482.00 $(M+H)^+$, MS AP(−) *m/z* 480.02 $(M-H)^-$.

5-[(1-(2-Cyanoethyl)-3-methyl-1H-pyrazol-4-y)lmethylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (6). mp 171-173 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33-1.52 (m, 2H), 1.59-1.83 (m, 4H), 2.33 (t, 2H, *J* = 7.7), 2.39 (s, 3H), 3.00 (t, 2H, *J* = 7.1), 4.10 (t, 2H, *J* = 7.6), 4.41 (t, 2H, *J* = 6.5), 7.53 (s, 1H), 7.72 (s, 1H); MS AP(−) *m/z* 391.04 $(M-H)^-$.

4-Oxo-5-[[1-phenyl-3-(2-thienyl)-1H-pyrazol-4-yl]methylene]-2-thioxo-3-thiazolidinehexanoic acid (7). mp 163-164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37-1.50 (m, 2H), 1.63-1.82 (m, 4H), 2.31 (t, 2H, *J* = 7.4), 4.12 (t, 2H, *J* = 7.3), 7.19 (dd, 1H, *J* = 3.6, 5.1), 7.37-7.56 (m, 5H), 7.81 (d, 2H, *J* = 7.5), 7.90 (s, 1H), 8.16 (s, 1H); MS AP(−) *m/z* 481.96 $(M-H)^-$.

5-[(1,3-Diphenyl-1H-pyrazol-4-yl)methylene]-4-oxo-2-thioxo-3-thiazolidinepropanoic acid (8). mp 232-234 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.49 (t, 2H, *J* = 8.1), 4.17 (t, 2H, *J* = 7.8), 7.16-7.22 (m, 1H), 7.26-7.36 (m, 5H), 7.44 (d, 2H, *J* = 7.5), 7.51 (s, 1H), 7.52 (d, 2H, *J* = 1.2), 8.06 (s, 1H); MS AP(−) *m/z* 434.00 $(M-H)^-$.

5-[[3-(4-Methylphenyl)-1-phenyl-1H-pyrazol-4-yl]methylene]-4-oxo-2-thioxo-3-thiazolidineacetic acid (9). mp 271-272 °C; ^{1}H NMR (300 MHz, DMSO) δ 2.41 (s, 3H), 4.51 (s, 2H), 7.34-7.47 (m, 3H), 7.64-7.49 (m, 5H), 8.07 (d, 2H, *J* = 8.4), 8.81 (s, 1H); MS AP(−) *m/z* 435.01 $(M-H)^-$.

5-[[5-(3,4-Dichlorophenyl)-2-furanyl]methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (10). mp 182-188 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94-1.07 (m, 2H), 1.19-1.36 (m, 4H), 1.85 (t, 2H, *J* = 7.6), 3.68 (t, 2H, *J* = 7.5), 6.63-6.67 (m , 2H), 7.10 (s, 1H), 7.16-7.28 (m, 2H,), 7.45 (d, 1H, *J* = 2.2); MS AP(−) *m/z* 468.70 $(M-H)^-$.

5-(1H-Indol-3-ylmethylene)-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (11). mp 203-205 °C; ^{1}H NMR (300 MHz, DMSO) δ 1.26-1.38 (m, 2H), 1.48-1.59 (m, 2H), 1.60-1.67 (m, 2H), 2.22 (t, 2H, *J* = 7.3), 4.02 (t, 2H, *J* = 7.4), 7.20-7.31 (m, 2H), 7.52 (d, 1H, *J* = 7.1), 7.89 (s, 1H), 7.96 (d, 1H, *J* = 7.3), 8.08 (s, 1H); MS AP(+) *m/z* 374.91 $(M+H)^+$, MS AP(−) *m/z* 373.97 $(M-H)^-$.

5-[(3-Ethoxy-4-hydroxyphenyl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (12). mp 140-143 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.11-1.82 (m, 2H), 1.2 (t, 3H, *J* = 7.2), 1.34-1.45 (m, 4H), 1.98 (t, 2H, *J* = 7.5), 3.78-3.89 (m, 4H), 6.67-6.74 (m, 3H), 7.35 (s, 1H); MS AP(−) *m/z* 394.02 $(M-H)^-$.

5-[(2-Methoxyphenyl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (13). mp 114-116 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36-1.52 (m, 2H), 1.64-1.82 (m, 4H), 2.37 (t, 2H, *J* = 7.3), 3.92 (s, 3H), 4.12 (t, 2H, *J* = 7.3), 6.95 (d, 1H, *J* = 7.8), 7.00-7.08 (m, 1H), 7.37-7.47 (m, 2H), 8.09 (s, 1H); MS AP(+) *m/z* 366.03 $(M+H)^+$.

5-[(4-Fluorophenyl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (14). mp 160-161 °C; ^{1}H NMR (300 MHz, DMSO) δ 1.23-1.34 (m, 2H), 1.43-1.72 (m, 4H), 2.18 (t, 2H, *J* = 7.2), 3.98 (t, 2H, *J* = 7.1), 7.33-7.41 (m, 2H), 7.64-7.72 (m, 2H), 7.79 (s, 1H); MS AP(−) *m/z* 352.98 $(M-H)^-$.

5-[(3-Bromo-4-methoxyphenyl)methylene]-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (15). mp 179-181 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36-1.48 (m, 2H), 1.62-1.79 (m, 4H), 2.30 (t, 2H, *J* = 8.1), 3.97 (s, 3H), 4.11 (t, 2H, *J* = 7.7), 7.02 (d, 1H, *J* = 8.6), 7.44-7.48 (m, 1H), 7.60 (s, 1H), 7.70 (s, 1H); MS AP(−) *m/z* 444.52 $(M-H)^-$.

5-(2-Methyl-3-phenyl-2-propenylidene)-4-oxo-2-thioxo-3-thiazolidinehexanoic acid (16). mp 155-157 °C; ^{1}H NMR (300 MHz, DMSO) δ 1.23-1.37 (m, 2H), 1.46-1.70 (m, 4H), 2.18-2.29 (m, 5H), 4.00 (t, 2H, *J* = 7.5), 7.30-7.48 (m, 6H), 7.55 (s, 1H); MS AP(−) *m/z* 375.01 $(M-H)^-$.

Soybean PDF2 cloning, expression, and purification. Based on the sequence similarity to *Arabidopsis* PDF2 gene, we identified soybean expressed-sequence-tagged (EST) clones from the corporate DNA database. Soybean PDF2 gene (matured form) was amplified from an EST cDNA clone with two PCR primers: 5'-CCGTAAACCCTCCTCGAACCG-3' the sequence just preceding a *NcoI* site in the soybean PDF2 gene and a primer corresponding to the vector sequence with a *BamHI* site. The PCR product was inserted into the bacterial expression vector pBX3 (a modified form of pET vector) using *NcoI* and *BamHI* sites. The PDF2 gene sequence was confirmed by the corporate DNA sequencing facility. Soybean PDF2 genes were expressed and purified as described previously.[2]

PDF Inhibition Assay. Enzyme activity was measured in half-area 96-well microtiter plates using the PDF/FDH coupled spectrophotometric assay.[3] The reaction mixture contained 50 mM Hepes buffer (pH 7.2), 0.5 unit/ml FDH, 4 mM NAD^+, and PDF. Test compounds in DMSO (final concentration of 4%) were pre-incubated with the enzyme in reaction mix for 10 min prior to adding the substrate, for-Met-Ala-Ser (final concentration of 2 mM). The reaction was monitored by following the conversion of NAD^+ to NADH at 340 nm. Inhibition was determined based on the reaction rates obtained over a 10 min period.

The effect of varying substrate and inhibitor concentration on enzyme kinetics was monitored to determine the type of inhibition. The results were analyzed using the GraFit software package (Erithacus Software Limited). The lines in figure 2 were generated using a K_m value of 6.4 mM.

Virtual Ligand Screening: additional details. The database used in our virtual screening was compiled from the "off the shelf" compounds from various vendors. Structures of those compounds were cleaned and tautomer state checked. A set of structure exclusion queries were applied to remove reactive and potentially toxic compounds and well investigated areas of chemistry. The remaining structures were combined into a single database.

Two independent ICM VLS runs were performed and lowest score of the two runs assigned to each compound. To explore the feasibility of discovering non-chelating inhibitors, an additional VLS was carried out with two crystallographic water molecules present (a metal bound water and a nearby water) in the active site.

To remove some of the artifacts of VLS, two simple filters were applied: (1) only compounds with at least one heavy atom within 3.5Å from the metal ion were retained, since an interaction of the ligand with the active site metal was expected; and (2) compounds which had heavy atom clashes with receptor shorter than 0.8 of the sum of the Van der Waals radii were removed. After filtering, a list of 4526 candidate compounds was selected.

References for Supporting Information

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(3) Lazennec, C. and Meinnel, T. Formate Dehydrogenase-Coupled Spectrophotometric Assay of Peptide Deformylase. *Anal. Biochem.* **1997**, *244*, 180-182.