Supporting Information

Inhibition of Cancer-Associated Mutant Isocitrate Dehydrogenases by 2-thiohydantoin compounds

Fangrui Wu,[†,§] Baisong Zheng,[†,§] Hong Jiang,[†,§] Mari Kogiso,[¶] Yuan Yao,[†] Chao Zhou,[†] Xiao-Nan Li,[¶] Yongcheng Song[,†,‡]*

[†] Department of Pharmacology, [¶] Department of Pediatrics-oncology, [‡] Dan L. Duncan Cancer Center, Baylor College of Medicine, 1 Baylor Plaza, Houston, TX 77030, USA.

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Table S1. Data processing and refinement statistics

	IDH1^{R132H}:**16**	IDH1^{R132H}:**22**
A. Data processing		
Wavelength (Å)	1.542	1.542
Space group	$P4_32_12$	$P4_32_12$
Unit cell dimensions		
a, b, c (Å)	82.41, 82.41, 304.30	82.59, 82.59, 306.60
α, β, γ (°)	90.0, 90.0, 90.0	90.00, 90.00, 90.00
Resolution (Å)	46.3-3.2(3.28-3.20)	40.9-3.3(3.38-3.29)
Unique reflections	18114(894)	16627(824)
Completeness (%)	96.0(72.4)	90.6(33.0)
Redundancy	8.7(10.4)	6.2(6.9)
R_{merge} (%)	19.8(67.3)	16.9(72.0)
$I/\sigma(I)$[b]	10.1(2.6)	8.5(2.1)
B. Refinement		
Resolution (Å)	46.3-3.2(3.28-3.20)	40.9-3.3(3.38-3.29)
Number of reflections used in working set	16668(882)	14621(364)
Number of reflections for R_{free} calculation	898 (50)	784(19)
R_{work} (%)	19.1(21.5)	21.0(22.1)
R_{free} (%)[a]	27.7(28.7)	29.7(27.2)
Mean B-factor from Wilson plot (Å^2)	46.0	53.1
RMSD of bond length (Å)	0.01	0.01
RMSD of bond angle (°)	1.46	1.40
Ramachandran plot[b]		
Residues in most favored regions	90.1%	88.2%
Residues in additional allowed regions	9.9%	11.8%
Residues in generously allowed regions	0.0%	0.0%
Residues in disallowed regions	0.0%	0.0%

[a]A subset of the data (5%) was excluded from the refinement and used to calculate R_{free}.
[b]Ramachandran plot is generated by Procheck[1].

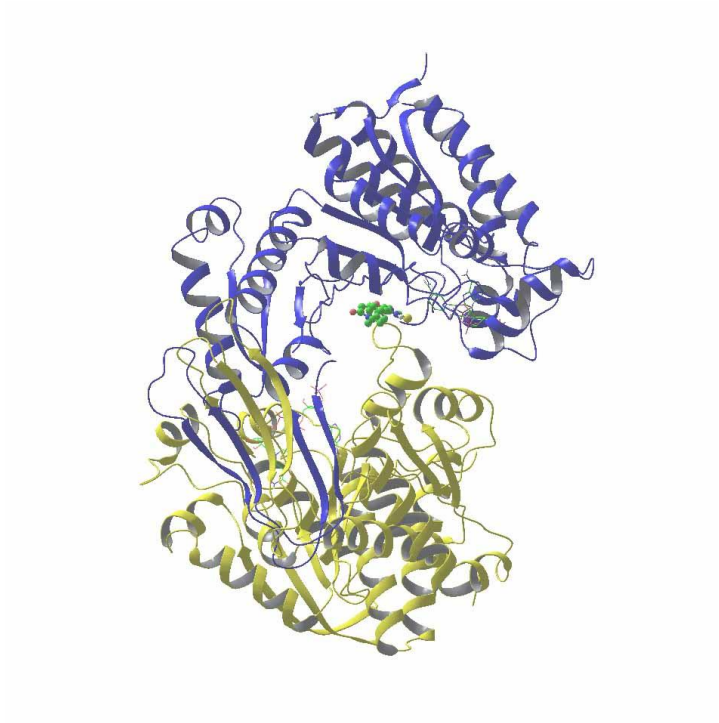


Figure S1. The overall structure of IDH1(R132H) in complex with compound **22** (ball & stick model) and NADPH.

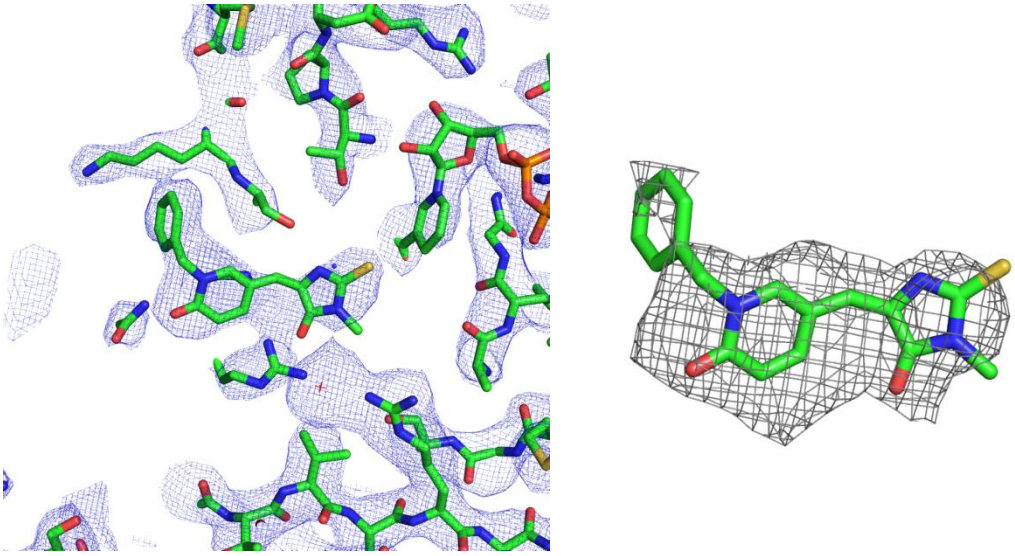
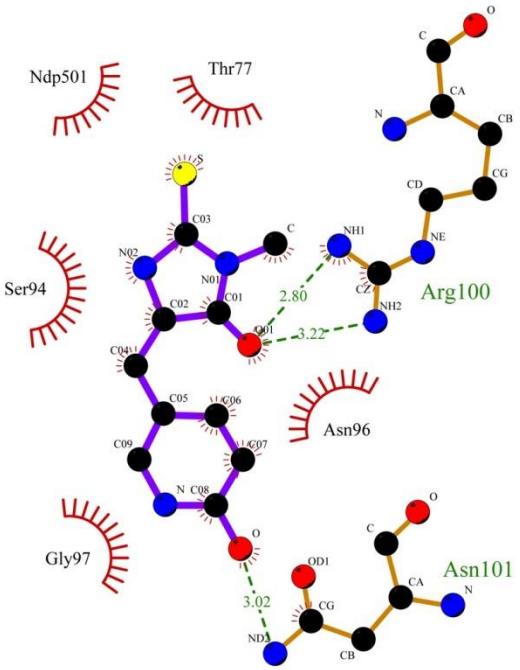


Figure S2. (Left) The $2F_o$-F_c electron density map of compound **22**, contoured at 1σ; (Right) The F_o-F_c omit map of **22** at 3σ.

A



B

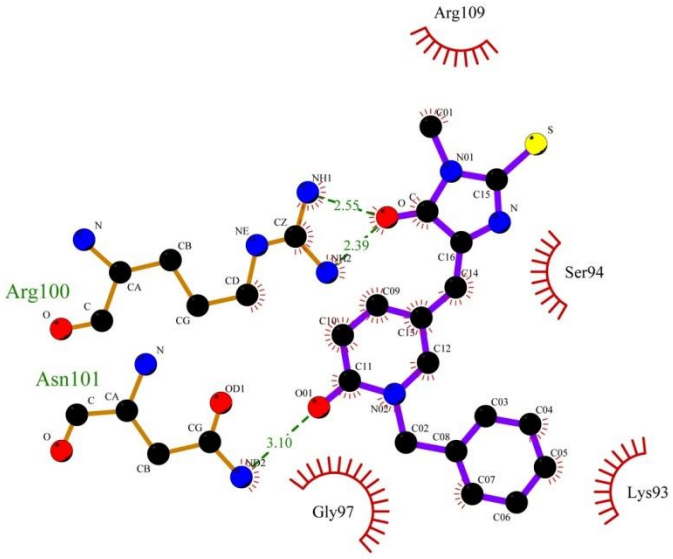


Figure S3. Ligand-protein interactions of between IDH1(R132H) and (**A**) compound **16** and (**B**) compound **22**.

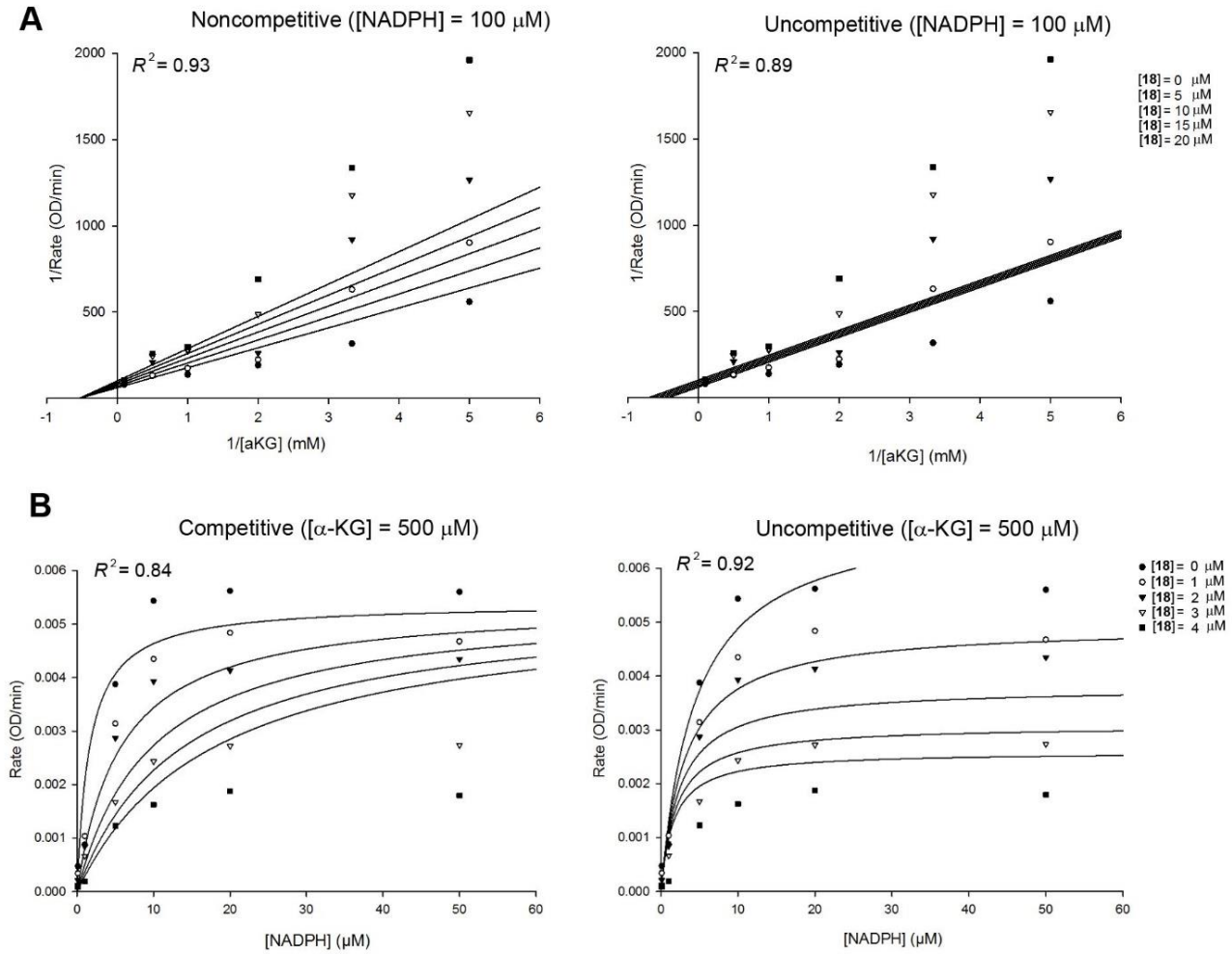


Figure S4. Unfavorable fitting models for enzyme kinetic studies of compound **18**. (**A**) Noncompetitive (left) and uncompetitive (right) inhibition models with variable concentrations of α-KG using a Lineweaver-Burk plot; and (**B**) Competitive (left) and uncompetitive (right) inhibition models with variable concentrations of NADPH using a Michaelis-Menten plot.

Experimental Section

All reagents were purchased from Alfa Aesar (Ward Hill, MA) or Aldrich (Milwaukee, WI). Compounds were characterized by ^{1}H NMR on a Varian (Palo Alto, CA) 400-MR spectrometer and HRMS on a ThermoFisher LTQ-Orbitrap mass spectrometer. Compound purities were determined by a Shimadzu Prominence HPLC with a Phenomenex C18 column (4.6 x 250 mm, Methonal:H_2O 60:40, monitored at 254 and 280 nm). The purities of all compounds were found to be >95%.

General synthetic method. Glycine (6.54g, 87 mmol) in EtOH (12 mL) containing 1 equivalent of 50% aqueous KOH (9.78 g, 87 mmol) was added R^1 isothiocyanate dropwise at 0 °C. The mixture was stirred fro 3 h, and then acidified with 1 M HCl (200 mL) to precipitate the crude α-thioureido acid product. After cooling to 0 °C, the white powder was collected by filtration, which was dissolved in acetone (250 mL) and treated with H_2SO_4 (5 mL). Upon completion of the reaction monitored by TLC, the solvent was removed by reduced pressure and saturated aqueous $NaHCO_3$ was carefully added at 0 °C. After neutralization, the resulting precipitation was filtered to give 3-R^1-substituted 2-thiohydantoin as a white powder.

A mixture of a 2-thiohydantoin (10 mmol), sodium acetate (0.8 g, 10 mmol), 6-methoxynicotinaldehyde (1.5 g, 11 mmol) or another aromatic aldehyde in 15 mL of acetic acid was refluxed for 3 h. The precipitated solid was filtered, washed successively with acetic acid, water, ethanol and diethyl ether to give (6-methoxypyridin-3-yl)methylene-2-thiohydantoin as a yellow or light brown powder, which (8.0 mmol) was refluxed overnight in 6 N HCl (10 mL). Upon cooling to room temperature, the resulting precipitate was filtered and washed with cold diethyl ether to give (2-pyridinone-3-yl)methylene-2-thiohydantoin as a brown powder. Other heterocyclic compounds in Chart 1 can be synthesized similarly with the above method.

3-benzyl-5-(3,4-dihydroxybenzylidene)-2-thioxoimidazolidin-4-one (Compound 4). Using the general method A described above, compound **4** was synthesized from 3-benzyl-5-(3,4-dimethoxy

benzylidene)-2-thioxoimidazolidin-4-one, yield = 73%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.26 (s, 1H), 8.62 (s, 1H), 8.20 (s, 1H), 7.23 (m, 2H), 7.23-7.10 (m, 4H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.49 (s, 1H), 4.99 (s, 2H).

3-Benzyl-5-(3,4-dimethoxybenzylidene)-2-thioxoimidazolidin-4-one (Compound 5). Using the general method A described above, compound **5** was synthesized from 3-benzyl-2-thioxoimidazolidin-4-one and 3,4-dimethoxybenzaldehyde, yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.79-3.87 (m, 6H), 5.02 (s, 2H), 6.65 (s, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 7.27-7.43 (m, 7H), 12.50 (s, 1H).

3,4-dimethoxybenzylidene-3-phenyl-2-thioxoimidazolidin-4-one (Compound 6). Using the general method A described above, compound **6** was synthesized from 3-phenyl-2-thioxoimidazolidin-4-one and 3,4-dimethoxybenzaldehyde, yield = 73%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.79-3.87 (m, 6H), 6.67 (s, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.33-7.54 (m, 7H), 12.60 (s, 1H).

3,4-dimethoxybenzylidene-3-(4-methoxybenzyl)-2-thioxoimidazolidin-4-one (Compound 7). Using the general method A described above, compound **7** was synthesized from 3-(4-methoxybenzyl)-2-thioxoimidazolidin-4-one and 3,4-dimethoxy-benzaldehyde, yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.71 (s, 3H), 3.79 (s, 3H), 3.874(s, 3H), 4.94 (s, 2H), 6.62 (s, 1H), 6.84-6.90 (m, 2H), 7.02 (d, *J* = 8.8 Hz, 1H), 7.24-7.33 (m, 3H), 7.39-7.42 (m, 1H), 12.45 (s, 1H).

3,4-dihydroxybenzylidene-3-phenyl-2-thioxoimidazolidin-4-one (Compound 8). Using the general method A described above, compound **8** was synthesized from compound **6**, yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.55 (s, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 7.18-7.22 (m, 2H), 7.35-7.38 (m, 2H), 7.45-7.55 (m, 5H), 12.41 (s, 1H).

3,4-dihydroxybenzylidene-3-(4-hydroxybenzyl)-2-thioxoimidazolidin-4-one (Compound 9). Using the general method A described above, compound **9** was synthesized from compound **7**, yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.87 (s, 2H), 6.49 (s, 1H), 6.68-6.72 (m, 2H), 7.79 (d, *J* = 8.4 Hz, 1H), 7.13-7.19 (m, 4H), 12.25 (s, 1H).

3-benzyl-5-(pyridin-3-ylmethylene)-2-thioxoimidazolidin-4-one (Compound 10). Using the general method A described above, compound **10** was synthesized from 3-benzyl-2-thioxoimidazolidin-4-one and nicotinaldehyde, yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.93 (s, 2H), 6.19 (s, 1H), 7.20-7.38 (m, 6H), 8.37 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.59 (d, *J* = 8.0 Hz, 1H), 8.99 (d, *J* = 2.0 Hz, 1H).

3-benzyl-5-((6-methoxypyridin-3-yl)methylene)-2-thioxoimidazolidin-4-one (Compound 11). Using the general method A described above, compound **11** was synthesized from 3-benzyl-2-thioxoimidazolidin-4-one and 6-methoxynicotinaldehyde, yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.90 (s, 3H), 5.01 (s, 2H), 6.67 (s, 1H), 6.91 (d, *J* = 8.8 Hz, 1H), 7.24-7.33 (m, 5H), 8.21 (dd, *J* = 8.8, 2.4 Hz, 1H), 8.55 (d, *J* = 2.4 Hz, 1H), 12.52 (s, 1H).

5-((1-benzyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 12). Using the general method A described above, compound **12** was synthesized from compound **11**, yield = 50%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.99 (s, 2H), 6.40 (d, *J* = 9.6 Hz, 1H), 6.55 (s, 1H), 7.25-7.33 (m, 5H), 7.97 (dd, *J* = 9.6, 2.4 Hz, 1H), 8.00 (d, *J* = 2.4 Hz, 1H), 12.34 (s, 1H).

3-ethyl-5-((6-methoxypyridin-3-yl)methylene)-2-thioxoimidazolidin-4-one (Compound 13). Using the general method A described above, compound **13** was synthesized from 3-ethyl-2-thioxo-imidazolidin-4-one and 6-methoxynicotinaldehyde. yield = 76%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.16 (t, *J* = 4.4 Hz, 3H), 3. 81-3.84 (m, 2H), 3.90 (s, 3H), 6.63 (s, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 8.20 (dd, *J* = 8.8, 2.8 Hz, 1H), 8.54 (d, *J* = 2.4 Hz, 1H), 12. 38 (s, 1H).

5-((1-ethyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 14). Using the general method A described above, compound **14** was synthesized from compound **13**. yield = 72%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 1.10-1.17 (m, 3H), 3.78-3.83 (m, 2H), 6.38 (d, *J* = 9.2 Hz, 1H), 6.51 (s, 1H), 7.92-8.01 (m, 2H), 12.20 (brs, 2H).

5-((6-methoxypyridin-3-yl)methylene)-3-methyl-2-thioxoimidazolidin-4-one (Compound 15). Using the general method A described above, compound **15** was synthesized from 3-methyl-2-thioxo-imidazolidin-4-one and 6-methoxynicotinaldehyde. yield = 77%. ^{1}H NMR (400 MHz, DMSO-d_6): δ =

3.20 (s, 3H), 3.90 (s, 3H), 6.63 (s, 1H), 6.90 (d, *J* = 8.8 Hz, 1H), 8.20 (dd, *J* = 8.8, 2.0 Hz, 1H), 8.54 (d, *J* = 2.8 Hz, 1H).

5-((1-ethyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 16). Using the general method described above, compound **16** was synthesized from 3-methyl-2-thiohydantoin (1.2g, 10 mmol) and 6-methoxynicotinaldehyde (1.5 g, 11 mmol), followed by deprotection (refluxing in 6N HCl), as a brown powder (1.3g, 65% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.18 (s, 3H), 6.38 (d, J = 9.6 Hz, 1H), 6.51 (s, 1H), 7.95 (dd, J = 9.6, 2.8 Hz, 1H), 8.00 (d, J = 2.8 Hz, 1H), 12.20 (brs, 2H); HRMS (ESI) $[M+H]^+$ Calcd for $C_{10}H_{10}N_3O_2S^+$: 236.0488, Found: 236.0493.

5-((6-methoxypyridin-3-yl)methylene)-2-thioxoimidazolidin-4-one (Compound 17). Using the general method A described above, compound **17** was synthesized from 2-thioxoimidazolidin-4-one and 6-methoxynicotinaldehyde, yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.90 (s, 3H), 6.49 (s, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 8.18 (dd, *J* = 8.8, 2.4 Hz, 1H), 8.51 (d, *J* = 2.4 Hz, 1H), 12.17 (s, 1H), 12.36 (s, 1H).

5-((5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 18). Using the general method described above, compound **18** was synthesized from 2-thiohydantoin (1.2g, 10 mmol) and 6-methoxynicotinaldehyde (1.5 g, 11 mmol), followed by deprotection (refluxing in 6N HCl), as a brown powder (1.5g, 71% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.26 (s, 1H), 6.37 (d, J = 10.8 Hz, 1H), 7.75-7.80 (m, 2H), 10.36 (s, 1H), 11.13 (s, 1H), 11.75 (s, 1H), 12.64 (s, 1H); HRMS (ESI) $[M+H]^+$ Calcd for $C_9H_8N_3O_2S^+$: 222.0332, Found: 222.0340.

2-(4-((6-methoxypyridin-3-yl)methylene)-5-oxo-2-thioxoimidazolidin-1-yl)acetic acid (Compound 19). Using the general method A described above, compound **19** was synthesized from ethyl 2-(5-oxo-2-thioxoimidazolidin-1-yl)acetate and 6-methoxynicotinaldehyde, yield = 78% based on aldehyde. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.91 (s, 3H), 4.50 (s, 2H), 6.71 (s, 1H), 6.92 (d, *J* = 8.8 Hz, 1H), 8.23 (d, *J* = 8.8 Hz, 1H), 8.57 (s, 1H), 12.57 (s, 1H), 13.24 (s, 1H).

2-(5-oxo-4-((6-oxo-1,6-dihydropyridin-3-yl)methylene)-2-thioxoimidazolidin-1-yl)acetic acid (Compound 20). Using the general method A described above, compound **20** was synthesized from compound **19**, yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.48 (s, 2H), 6.39 (s, 1H), 6.59 (s, 1H), 7.81-8.05 (m, 3H), 12.21 (s, 1H), 12.41 (s, 1H).

1-methyl-5-((1-methyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 21). Using the general method A described above, compound **21** was synthesized from 3-methyl-2-thioxo- imidazolidin-4-one and 1-methyl-6-oxo-1,6-dihydropyridine-3-carbaldehyde, yield = 71%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.19 (s, 3H), 3.51 (s, 3H), 6.42-6.46 (m, 2H), 7.87 (dd, *J* = 9.6, 2.8 Hz, 1H), 8.29 (d, *J* = 2.4 Hz, 1H).

1-benzyl-5-((1-methyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 22). Using the general method described above, compound **22** was synthesized from 3-methyl-2-thiohydantoin (0.6g, 5 mmol) and 1-benzyl-6-oxo-1,6-dihydropyridine-3-carbaldehyde (1.2 g, 6 mmol) as a yellow powder (1.3g, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.19 (s, 3H), 5.14 (s, 2H), 6.45-6.51 (m, 2H), 7.27-7.36 (m, 5H), 7.90-7.95 (m, 1H) 8.45 (d, J = 2.8 Hz, 1H), 12.20 (s, 1H); HRMS (ESI) $[M+H]^+$ Calcd for $C_{17}H_{16}N_3O_2S^+$: 326.0958, Found: 326.0966.

2-methoxy-5-((1-methyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridine 1-oxide (Compound 23). Using the general method A described above, compound **23** was synthesized from 3-methyl-2-thioxo- imidazolidin-4-one and 5-formyl-2-methoxypyridine 1-oxide, yield = 57%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.18 (s, 3H), 4.01 (s, 3H), 6.45-6.61 (m, 2H), 7.88 (d, *J* = 9.2 Hz, 1H), 8.61 (s, 1H), 12.25 (s, 1H).

1-hydroxy-5-((1-methyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 24). Using the general method A described above, compound **24** was synthesized from 3-methyl-2-thioxo-imidazolidin-4-one and 1-hydroxy-6-oxo-1,6-dihydropyridine-3-carbaldehyde, yield = 30%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.17 (s, 3H), 6.38 (s, 1H), 6.55 (d, *J* = 9.6 Hz, 1H), 7.78-7.80 (m, 1H), 8.37 (d, *J* = 2.0 Hz, 1H), 10.65 (s, 1H), 12.17 (s, 1H).

5-((2-methoxypyridin-3-yl)methylene)-3-methyl-2-thioxoimidazolidin-4-one (Compound 25). Using the general method A described above, compound **25** was synthesized from 3-methyl-2-thioxo-imidazolidin-4-one and 2-methoxynicotinaldehyde, yield = 78%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.20 (s, 3H), 3.95 (s, 3H), 6.66 (s, 1H), 7.05-7.09 (m, 1H), 8.11-8.20 (m, 2H), 12.32 (s, 1H).

3-((1-methyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 26). Using the general method A described above, compound **26** was synthesized from compound **25**, yield = 72%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.18 (s, 3H), 6.45-6.49 (m, 2H), 7.61-7.65 (m, 1H), 7.99-8.02 (m, 1H), 12.45 (brs, 1H), 12.59 (brs, 1H).

5-((6-methoxypyridin-2-yl)methylene)-3-methyl-2-thioxoimidazolidin-4-one (Compound 27). Using the general method A described above, compound **27** was synthesized from 3-methyl-2-thioxo-imidazolidin-4-one and 6-methoxypicolinaldehyde, yield = 70%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.22 (s, 3H), 3.99 (s, 3H), 6.70 (s, 1H), 6.89 (s, J = 8.0 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.82 (dd, J = 8.0, 6.8 Hz, 1H).

6-((1-methyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 28). Using the general method A described above, compound **28** was synthesized from compound **27**, yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.18 (s, 3H), 6.31 (s, 1H), 6.44 (d, J = 9.2 Hz, 1H), 6.63 (d, J = 7.2 Hz, 1H), 7.49 (dd, J = 9.2, 7.2 Hz, 1H).

3-methyl-5-(pyridin-4-ylmethylene)-2-thioxoimidazolidin-4-one (Compound 29). Using the general method A described above, compound **29** was synthesized from 3-methyl-2-thioxo-imidazolidin-4-one and isonicotinaldehyde, yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.20 (s, 3H), 6.55 (s, 1H), 7.70 (d, J = 6.4 Hz, 2H), 8.61 (d, J = 6.0 Hz, 2H).

5-((2-methoxypyridin-4-yl)methylene)-3-methyl-2-thioxoimidazolidin-4-one (Compound 30). Using the general method A described above, compound **30** was synthesized from 3-methyl-2-thioxo-imidazolidin-4-one and 2-methoxyisonicotinaldehyde, yield = 77%. ^{1}H NMR (400 MHz, DMSO-d_6): δ

= 3.18 (s, 3H), 3.87 (s, 3H), 6.50 (s, 1H), 7.15 (s, 1H), 7.29 (dd, *J* = 5.2, 1.6 Hz, 1H), 8.19 (d, *J* = 5.2 Hz, 1H).

4-((1-methyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 31). Using the general method A described above, compound **31** was synthesized from compound **30**, yield = 71%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.16 (s, 3H), 6.36 (s, 1H), 6.84 (s, 1H), 6.88 (dd, *J* = 6.8, 1.6 Hz, 1H), 7.36 (d, *J* = 6.8 Hz, 1H), 12.14 (s, 1H), 12.41 (s, 1H).

5-((2-bromopyridin-4-yl)methylene)-2-thioxoimidazolidin-4-one (Compound 32). Using the general method A described above, compound **32** was synthesized from 2-thioxoimidazolidin-4-one and 2-bromoisonicotinaldehyde, yield = 71%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.39 (s, 1H), 7.63 (d, *J* = 5.2 Hz, 1H), 7.81 (s, 1H), 8.40 (d, *J* = 5.2 Hz, 1H), 12.41 (brs, 1H), 12.59 (brs, 1H).

5-((2-phenylpyridin-4-yl)methylene)-2-thioxoimidazolidin-4-one (Compound 33). Using the general method B described above, compound **33** was synthesized from 2-thioxoimidazolidin-4-one, phenylbonic acid and 2-bromoisonicotinaldehyde, yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.49 (s, 1H), 7.43-7.64 (m, 4H), 8.08-8.13 (m, 3H), 8.66 (d, *J* = 4.8 Hz, 1H), 12.47 (brs, 1H), 12.56 (brs, 1H).

5-((5-(3,4-difluorophenyl)furan-2-yl)methylene)-3-methyl-2-thioxoimidazolidin-4-one (Compound 34). Using the general method B described above, yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.21 (s, 3H), 6.57 (s, 1H), 7.27-7.32 (m, 2H), 7.53-7.60 (m, 1H) 7.81-7.84 (m, 1H), 8.12-8.18 (m, 1H).

5-((6-methoxypyridin-3-yl)methylene)-2-thioxothiazolidin-4-one (Compound 35). Using the general method A described above, compound **35** was synthesized from 2-thioxothiazolidin-4-one and 6-methoxynicotinaldehyde, yield = 75%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.93 (s, 3H), 6.99 (d, *J* = 8.8 Hz, 1H), 7.66 (s, 1H), 7.88 (dd, *J* = 8.8, 2.8 Hz, 1H), 8.52 (d, *J* = 2.4 Hz, 1H).

5-((6-oxo-1,6-dihydropyridin-3-yl)methylene)-2-thioxothiazolidin-4-one (Compound 36). Using the general method A described above, compound **36** was synthesized from compound **35**, yield = 70%. ^{1}H

NMR (400 MHz, DMSO-d_6): δ = 6.50 (d, *J* = 10.0 Hz, 1H), 7.53 (s, 1H), 7.60 (dd, *J* = 9.6, 2.8 Hz, 1H), 8.05 (d, *J* = 2.4 Hz, 1H).

5-((6-methoxypyridin-3-yl)methylene)imidazolidine-2,4-dione (Compound 37). Using the general method A described above, compound **37** was synthesized from imidazolidine-2,4-dione and 6-methoxynicotinaldehyde, yield = 71%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.88 (s, 3H), 6.40 (s, 1H), 6.85 (d, *J* = 8.8 Hz, 1H), 8.01 (dd, *J* = 8.8, 2.4 Hz, 1H), 8.41 (d, *J* = 2.8 Hz, 1H), 10.51 (s, 1H), 11.22 (s, 1H).

5-((6-oxo-1,6-dihydropyridin-3-yl)methylene)imidazolidine-2,4-dione (Compound 38). Using the general method A described above, compound **38** was synthesized from compound **37**, yield = 72%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.26 (s, 1H), 6.36 (d, *J* = 9.6 Hz, 1H), 7.75-7.79 (m, 2H), 10.37 (s, 1H), 11.14 (s, 1H).

5-((2-amino-4-oxo-1H-imidazol-5(4H)-ylidene)methyl)pyridin-2(1H)-one (Compound 39). Using the general method A described above, compound **39** was synthesized from 2-amino-1H-imidazol-4(5H)-one and 6-hydroxynicotinaldehyde, yield = 71%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 6.11 (s, 1H), 6.35 (d, *J* = 9.6 Hz, 1H), 7.06 (brs, 2H), 8.03 (s, 1H), 8.15 (brs, 1H).

5-((2-(methylthio)-4-oxo-1H-imidazol-5(4H)-ylidene)methyl)pyridin-2(1H)-one (Compound 40). Using the general method A described above, compound **40** was synthesized from 2-(methylthio)-1H-imidazol-4(5H)-one and 6-hydroxynicotinaldehyde, yield = 72% based on aldehyde. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.61 (s, 3H), 6.40 (d, *J* = 9.6 Hz, 1H), 6.63 (s, 1H), 8.17 (d, *J* = 2.8 Hz, 1H), 8.49 (dd, *J* = 9.6, 2.8 Hz, 1H).

5-((1,3-dimethyl-5-oxo-2-thioxoimidazolidin-4-ylidene)methyl)pyridin-2(1H)-one (Compound 41). Using the general method A described above, compound **41** was synthesized from 1,3-dimethyl-2-thioxoimidazolidin-4-one and 6-hydroxynicotinaldehyde, yield = 74%. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.22 (s, 3H), 3.52 (s, 3H), 6.40 (d, *J* = 10.0 Hz, 1H), 6.75 (s, 1H), 8.35 (dd, *J* = 9.6, 2.4 Hz, 1H), 8.47 (d, *J* = 2.4 Hz, 1H).