Supporting Information

Synthesis and Biochemical Evaluation of Δ^2-Isoxazoline Derivatives as DNA Methyltransferase 1 Inhibitors

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Benzaldehyde Oxime (9a).[1-3]
Pale yellow oil, 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.15 (br s, 1H), 8.20 (s, 1H), 7.63–7.54 (m, 2H), 7.45–7.35 (m, 3H). ESI-MS *m/z*: 122 $(M + H)^+$.
4-Nitrobenzaldehyde Oxime (9b).[1-3]
Yellow solid, 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25 (d, *J* = 8.7 Hz, 2H), 8.20 (s, 1H), 7.98 (s, 1H), 7.75 (d, *J* = 8.7 Hz, 2H). ESI-MS *m/z*: 167 $(M + H)^+$.
4-Methoxybenzaldehyde Oxime (9c).[1-3]
White solid, 94% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H), 8.05 (br s, 1H), 7.52 (d, *J* = 8.8 Hz, 2H), 6.91 (d, *J* = 8.8 Hz, 2H), 3.84 (s, 3H). ESI-MS *m/z*: 152 $(M + H)^+$.
(4-[[(4-Methylphenyl)sulfonyl]oxy]-benzaldehyde Oxime (9d).
White solid, 99% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.71 (d, *J* = 8.4 Hz, 2H), 7.50 (d, *J* = 8.7 Hz, 2H), 7.43 (s, 1H), 7.31 (d, *J* = 8.4 Hz, 2H), 7.01 (d, *J* = 8.7 Hz, 2H), 2.45 (s, 3H). ESI-MS *m/z*: 292 $(M + H)^+$.
2-Phenylacetaldehyde Oxime (9h).[4, 5]
Pale yellow solid, 91% yield. 55:45 mixture of geometric isomers **a** and **b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (br s, 1H, isomer **a**), 7.88 (br s, 1H, isomer **b**), 7.57 (t, *J* = 6.3, 1H, isomer **b**), 7.45–7.15 (m, 5H, isomer **a** and 5H isomer **b**), 6.93 (t, *J* = 5.3 Hz, 1H, isomer **a**), 3.75 (d, *J* = 5.3 Hz, 2H, isomer **a**), 3.57 (d, *J* = 6.2, 2H, isomer **b**). ESI-MS *m/z*: 136 $(M + H)^+$.
(*p*-Nitrophenyl)-Acetaldehyde Oxime (9i).
Yellow solid, 87% yield. 60:40 mixture of geometric isomers **a** and **b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.19 (m, 2H, isomer **a** and 2H, isomer **b**) 7.92 (br s, 1H, isomer **a**), 7.55 (t, *J* = 6.1, 1H, isomer **b**), 7.48 (br s, 1H, isomer **b**), 7.42–7.38 (m, 2H, isomer **a** and 2H, isomer **b**), 6.90 (t, *J* = 5.5 Hz, 1H, isomer **a**), 3.84 (d, *J* = 5.5 Hz, 2H, isomer **a**), 3.65 (d, *J* = 6.1, 2H, isomer **b**). ESI-MS *m/z*: 181 $(M + H)^+$.
(*p*-Methoxyphenyl)acetaldehyde Oxime (9j).
White solid, 90% yield. 55:45 mixture of geometric isomers **a** and **b**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.65 (br s, 1H, isomer **a**), 7.51 (t, *J* = 6.2, 1H, isomer **b**), 7.23 (br s, 1H, isomer **b**), 7.20–7.09 (m, 2H, isomer **a** and 2H, isomer **b**) 6.93–6.78 (m, 3H, isomer **a** and 2H, isomer **b**), 3.82 (s, 3H, isomer **a** and 3H, isomer **b**), 3.68 (d, *J* = 5.4, 2H, isomer **a**), 3.49 (d, *J* = 6.2, 2H, isomer **b**). ESI-MS *m/z*: 166 $(M + H)^+$.
4-(*tert*-Butoxycarbonyl)benzaldehyde Oxime (9l).
4-(*tert*-Butoxycarbonyl)benzaldehyde was prepared according to a described procedure[6] and the corresponding oxime **9l** was prepared according to the general procedure reported above. White solid, 70% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.23 (br s, 1H), 8.18 (s, 1H), 8.00 (d, *J* = 8.5 Hz, 2H), 7.62 (d, *J* = 8.5 Hz, 2H), 1.60 (s, 9H). ESI-MS *m/z*: 166 $(M + H - {}^tBu)^+$.

***N*-Hydroxybenzenecarboximidoyl chloride (12a).**[3, 7, 8]
Pale yellow amorphous solid, 98% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.62 (br s, 1H), 7.90–7.78 (m, 2H), 7.48–7.36 (m, 3H).
***N*-Hydroxy-4-nitro-benzenecarboximidoyl chloride (12b).**[3, 8]
Pale yellow solid, 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.31 (br s, 1H), 8.27 (d, *J* = 9.0 Hz, 2H), 8.04 (d, *J* = 9.0 Hz, 2H).
***N*-Hydroxy-4-methoxy-benzenecarboximidoyl chloride (12c).**[3, 9]
Yellow solid, 97% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.78 (d, *J* = 8.4 Hz, 2H), 7.70 (br s, 1H), 6.92 (d, *J* = 8.4 Hz, 2H), 3.85 (s, 3H).
***N*-Hydroxy-4-[[(4-methylphenyl)sulfonyl]oxy]-benzenecarboximidoyl chloride (12d).**
White solid, 99% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.97 (s, 1H), 7.78 (d, *J* = 8.4 Hz, 2H), 7.72 (d, *J* = 7.8 Hz, 2H), 7.32 (d, *J* = 7.8 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 2.45 (s, 3H).
***N*-Hydroxy-2-phenylacetimidoyl chloride (12h).**[10]
Yellow solid, 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (br s, 1H), 7.45–7.13 (m, 5H), 3.81 (s, 2H).
***N*-Hydroxy-2-(4-nitrophenyl)acetimidoyl chloride (12i).**[10]
Pale yellow solid, 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.21 (d, *J* = 8.4 Hz, 2H), 8.12 (s, 1H), 7.45 (d, *J* = 8.4 Hz, 2H), 3.91 (s, 2H).
***N*-Hydroxy-2-(4-methoxyphenyl)acetimidoyl chloride (12j).**[11]
Pale yellow solid, 96% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.29 (br s, 1H), 7.17 (d, *J* = 8.4 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 3.78 (s, 3H), 3.72 (s, 2H).
***N*-Hydroxy-2-(4-*tert*-butoxycarbonylphenyl)acetimidoyl chloride (12l).**
White solid, 80% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H), 8.05 (d, *J* = 8.8 Hz, 2H), 7.89 (d, *J* = 8.8 Hz, 2H), 1.60 (s, 9 H)

Table A. Elemental Analysis of Compounds **7a–k** and **8a–k**.

compd	Mol Formula	calcd, %			found, %		
		C	H	N	C	H	N
7a	$C_{12}H_{16}N_2O \cdot HCl$	59.87	7.12	11.64	59.99	7.10	11.61
7b	$C_{12}H_{15}N_3O_3 \cdot HCl$	50.44	5.64	14.71	50.58	5.66	14.67
7c	$C_{13}H_{18}N_2O_2$	66.64	7.74	11.96	66.48	7.76	11.99
7d	$C_{19}H_{22}N_2O_4S$	60.94	5.92	7.48	61.05	5.93	7.46
7e	$C_{13}H_{16}N_2O_3$	62.89	6.50	11.28	63.07	6.49	11.26
7f	$C_{12}H_{17}N_3O \cdot HCl$	56.36	7.09	16.43	56.51	7.07	16.39
7g	$C_{12}H_{16}N_2O_2 \cdot HCl$	56.14	6.67	10.91	56.01	6.89	10.93
7h	$C_{13}H_{18}N_2O \cdot HCl$	61.29	7.52	11.00	61.13	7.53	11.02
7i	$C_{13}H_{17}N_3O_3 \cdot HCl$	52.09	6.05	14.02	52.16	6.06	13.99
7j	$C_{14}H_{20}N_2O_2 \cdot HCl$	59.05	7.43	9.84	59.16	7.41	9.82
7k	$C_{13}H_{19}N_3O \cdot HCl$	57.88	7.47	15.58	57.77	7.49	15.61
8a	$C_{12}H_{14}N_2O$	71.26	6.98	13.85	71.46	6.96	13.82
8b	$C_{12}H_{13}N_3O_3$	58.29	5.30	16.99	58.41	5.31	16.95
8c	$C_{13}H_{16}N_2O_2$	67.22	6.94	12.06	67.33	6.93	12.03
8d	$C_{19}H_{20}N_2O_4S$	61.27	5.41	7.52	61.42	5.43	7.50
8e	$C_{13}H_{14}N_2O_3$	63.40	5.73	11.38	63.58	5.75	11.35
8f	$C_{12}H_{15}N_3O \cdot HCl$	56.80	6.36	16.56	56.66	6.37	16.59
8g	$C_{12}H_{14}N_2O_2$	66.04	6.47	12.84	66.16	6.46	12.82
8h	$C_{13}H_{16}N_2O \cdot HCl$	61.78	6.78	11.08	61.64	6.80	11.10
8i	$C_{13}H_{15}N_3O_3 \cdot HCl$	52.44	5.42	14.11	52.34	5.43	14.14
8j	$C_{14}H_{18}N_2O_2 \cdot (COOH)_2$	57.14	5.99	8.33	57.31	5.98	8.31
8k	$C_{13}H_{17}N_3O \cdot (COOH)_2$	56.07	5.96	13.08	56.23	5.95	13.05

References

1. Ramón, R. n. S.; Bosson, J.; Díez-González, S.; Marion, N.; Nolan, S. P. Au/Ag-Cocatalyzed Aldoximes to Amides Rearrangement under Solvent- and Acid-Free Conditions. *J. Org. Chem.* **2010,** *75*, 1197-1202.
2. Jain, N.; Kumar, A.; Chauhan, S. M. S. Metalloporphyrin and heteropoly acid catalyzed oxidation of CNOH bonds in an ionic liquid: biomimetic models of nitric oxide synthase. *Tetrahedron Lett.* **2005,** *46*, 2599-2602.
3. Sanders, B. C.; Friscourt, F. d. r.; Ledin, P. A.; Mbua, N. E.; Arumugam, S.; Guo, J.; Boltje, T. J.; Popik, V. V.; Boons, G.-J. Metal-Free Sequential [3 + 2]-Dipolar Cycloadditions using Cyclooctynes and 1,3-Dipoles of Different Reactivity. *J. Am. Chem. Soc.* **2010,** *133*, 949-957.
4. Rapoport, H.; Nilsson, W. The Reaction of Aldoximes with Alkali1. *J. Org. Chem.* **1962,** *27*, 629-631.
5. Ritson, D. J.; Cox, R. J.; Berge, J. Indium mediated allylation of glyoxylate oxime ethers, esters and cyanoformates. *Organic & Biomolecular Chemistry* **2004,** *2*, 1921-1933.
6. Hishikawa, K.; Nakagawa, H.; Furuta, T.; Fukuhara, K.; Tsumoto, H.; Suzuki, T.; Miyata, N. Photoinduced Nitric Oxide Release from a Hindered Nitrobenzene Derivative by Two-Photon Excitation. *J. Am. Chem. Soc.* **2009,** *131*, 7488-7489.
7. Dubrovskiy, A. V.; Larock, R. C. Synthesis of Benzisoxazoles by the [3 + 2] Cycloaddition of in situ Generated Nitrile Oxides and Arynes. *Org. Lett.* **2010,** *12*, 1180-1183.
8. Tóth, M.; Kun, S.; Bokor, É.; Benltifa, M.; Tallec, G.; Vidal, S.; Docsa, T.; Gergely, P.; Somsák, L.; Praly, J.-P. Synthesis and structure-activity relationships of C-glycosylated oxadiazoles as inhibitors of glycogen phosphorylase. *Bioorg. Med. Chem.* **2009,** *17*, 4773-4785.
9. Di Nunno, L.; Vitale, P.; Scilimati, A.; Simone, L.; Capitelli, F. Stereoselective dimerization of 3-arylisoxazoles to cage-shaped bis-[beta]-lactams syn 2,6-diaryl-3,7-diazatricyclo[4.2.0.02,5]octan-4,8-diones induced by hindered lithium amides. *Tetrahedron* **2007,** *63*, 12388-12395.
10. Kumaran, G.; Kulkarni, G. H. Synthesis of α-Functionalized and Nonfunctionalized Hydroximoyl Chlorides from Conjugated Nitroalkenes and Nitroalkanes. *J. Org. Chem.* **1997,** *62*, 1516-1520.
11. Velaparthi, S.; Brunsteiner, M.; Uddin, R.; Wan, B.; Franzblau, S. G.; Petukhov, P. A. 5-tert-Butyl-N-pyrazol-4-yl-4,5,6,7-tetrahydrobenzo[d]isoxazole-3-carboxamide Derivatives as Novel Potent Inhibitors of Mycobacterium tuberculosis Pantothenate Synthetase: Initiating a Quest for New Antitubercular Drugs. *J. Med. Chem.* **2008,** *51*, 1999-2002.