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The identification and optimization of 2,4-diketobutyric acids as flap endonuclease 1 inhibitors

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Abstract—There have been several recent reports of chemopotentiation via inhibition of DNA repair processes. Flap endonuclease 1 (FEN1) is a key enzyme involved in base excision repair (BER), a primary pathway utilized by mammalian cells to repair DNA damage. In this report, we describe the identification and SAR of a series of 2,4-diketobutyric acid FEN1 inhibitors.

Flap endonuclease-1 (FEN1) is a 43 kDa metal-dependent nuclear enzyme that exhibits both DNA structure-specific endonuclease activity and 5′ exonuclease activity. Arguably, the most well studied aspect of FEN1 function is its role in the cleavage of Okazaki fragments during DNA replication.[1] However, FEN1 also acts to cleave 5′ DNA flaps generated during a variety of other cellular processes including double-strand break repair,[2] homologous recombination,[3] and base excision repair (BER).[4] FEN1 is necessary for normal embryonic development and homozygous mouse knockouts of FEN1 are lethal.[5,6] Interestingly, however, yeast knockouts of FEN1 are viable, albeit at a slower growth rate.[7]

BER is an important cellular mechanism for the repair of DNA damage caused by alkylating agents.[8] The role of FEN1 in BER is clearly exemplified in a recent report that shows nuclease-defective FEN1 results in increased cellular sensitivity to methylmethane sulfonate (MMS), a potent DNA alkylating agent.[9] Sensitization to DNA damaging agents may improve the therapeutic window of classical chemotherapeutics by lowering the minimum effective dose.[10] Recent reports describe several small molecule inhibitors of DNA repair proteins including poly(ADP-ribose) polymerase-1 (PARP)[11] and O^6-alkylguanine–DNA alkyltransferase (ATase or MGMT).[12] These inhibitors are reported to potentiate the activity of various chemotherapeutic agents including temozolamide[13] and topotecan.[11] In light of this recent work, we embarked on a strategy to identify selective small-molecule inhibitors of FEN1 for use as chemopotentiating agents.

FEN1 is highly homologous to a related endonuclease, xeroderma pigmentosum G (XPG).[14] XPG is part of a repair pathway that excises DNA containing pyrimidine dimers, a common form of damage caused by exposure to UV light. Defects in XPG are known to cause hypersensitivity to UV light, resulting in light-induced skin lesions and carcinoma.[15] Therefore, selective inhibition of FEN1 over XPG was a key goal of this program.

A high-throughput screen was performed with our compound library in order to identify compounds with inhibitory activity against FEN1. Selected compounds were subsequently screened against XPG. FEN1 and XPG inhibition assays were performed using a fluorogenic substrate consisting of a triple-labeled double-stranded DNA molecule containing an internal 2-nucleotide gap.[16] Several classes of compounds were identified from this screen, the most prominent of which contained a 2,4-diketobutyric acid functionality (Fig. 1, compounds **1**–**3**). Generally, these compounds were low micromolar inhibitors of FEN1 (Table 1).

Keywords: Endonuclease; Cancer; FEN1.
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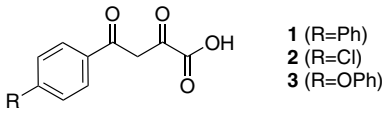
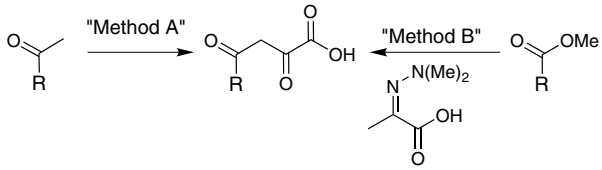


Figure 1. 2,4-Diketobutyrate hits from high-throughput screening.



Scheme 1. Reagents and conditions: Method A: (1) diethyl oxalate, NaOMe; (2) NaOH. Method B: (1) MeLi, HMPA/THF; (2) HCl.

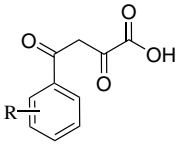
Compound **1** has been previously reported to inhibit the cap (m^7GpppXm)-dependent endonuclease of influenza virus. This endonuclease cleaves host-cell RNA in order to provide primers for viral mRNA synthesis. The blockage of this viral endonuclease by compounds related to **1** has been shown to block in vitro viral replication.[17] Similar diketobutyrates have been reported to inhibit HIV integrase[18] and glycolic acid oxidase.[19]

The synthesis of 2,4-diketobutyrates is generally accomplished through a base-promoted Claisen condensation of the appropriate methyl ketone and a dialkyl oxalate (Scheme 1, Method A). Saponification gives the requisite 2,4-diketobutyrate in good overall yield.[20] While this two-step procedure works well with a variety of substrates, the lack of availability of appropriately substituted methyl ketones greatly limited its usefulness.

A conceptually more attractive route involves the addition of the dianion of a pyruvic acid equivalent to an ester.[21] The dimethyl hydrazone of pyruvic acid was used as the nucleophile (Scheme 1, Method B). The hydrazone was easily prepared on a 60 mmol scale and was stable for at least 1 month at 0 °C. Addition of the dianion of the hydrazone to esters required methyl lithium (4 equiv) and HMPA (12 equiv). Fewer equivalents of either generally results in decreased yield and purity. While the reaction requires somewhat harsh conditions, it allows the synthesis of 3,4-diketobutyrates from a wide variety of carboxylic acids that are commercially available or readily synthesized. This method was readily adapted to a parallel synthesis format using a Bohdan mini-block system, which allowed the expeditious synthesis of a large variety of 2,4-diketobutyrates for biological evaluation.[22]

Comparison of compounds **1**–**4** (Table 1) clearly illustrate that hydrophobic substitution at the *para* position increased activity ~10-fold against both FEN1 and XPG. Substitution at the *ortho* position (**5**–**7**) slightly increased FEN1 activity, but more importantly decreases the activity against XPG. In particular, an *ortho* Cl increased FEN1 selectivity to over 10-fold. *meta* Substitution (**8**–**11**) resulted in increased activity, but fairly poor selectivity. *meta* And *para*-biaryl substitution resulted in especially potent compounds (**2**, **9**, **10**). The combination of an *ortho*-Cl with a hydrophobic group at the *meta* or

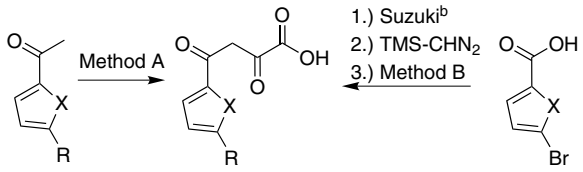
Table 1. FEN1 and XPG activity for compounds **1**–**18**



Compound[a]	Synthetic method	R	IC_{50} (μM)	
			FEN1	XPG
1	A	4-Cl	19.3	12.7
2	A	4-Ph	3.50	1.60
3	A	4-OPh	1.75	1.87
4	A	H	24.9	75.4
5	A	2-Cl	3.51	38.1
6	A	2-F	14.5	78.5
7	A	2-CH_3	12.9	54.5
8	A	3-Cl	6.96	13.1
9	B	3-Ph	3.62	3.24
10	B	3-(4-OMe-Ph)	1.36	1.59
11	A	3-OPh	7.31	5.44
12	A	2,6-DiCl	37.9	>100
13	A	2,5-DiCl	1.82	7.00
14	A	2,4-DiCl	5.53	9.85
15	A	2,3-DiCl	17.7	22.1
16	A	2-Cl, 4-(4-Cl-Ph)	0.41	1.77
17	B	2-Cl, 5-Ph	0.33	1.13
18	B	2-Cl, 5-(4-OMe-Ph)	0.19	0.57

[a] Compound identity and purity were established by ^{1}H NMR and analytical HPLC–MS.

Table 2. FEN1 and XPG activity for compounds **19–29**



Compound[a]	Synthetic method	X	R	IC_{50} (μM)	
				FEN1	XPG
19	A	O	H	64.2	41.0
20	B	O	Ph	1.66	1.81
21	B	O	4-*t*-Bu-Ph	13.5	3.13
22	B	O	4-Cl-Ph	10.8	0.98
23	B	O	4-OEt-Ph	11.3	1.40
24	B	O	2-Cl-Ph	12.2	1.85
25	A	S	H	10.1	38.7
26	B	S	Ph	7.65	1.85
27	B	S	3-CF_3	1.52	2.32
28	B	S	4-Cl, 3-F-Ph	0.57	0.90
29	B	S	4-OCF_3-Ph	0.44	0.31

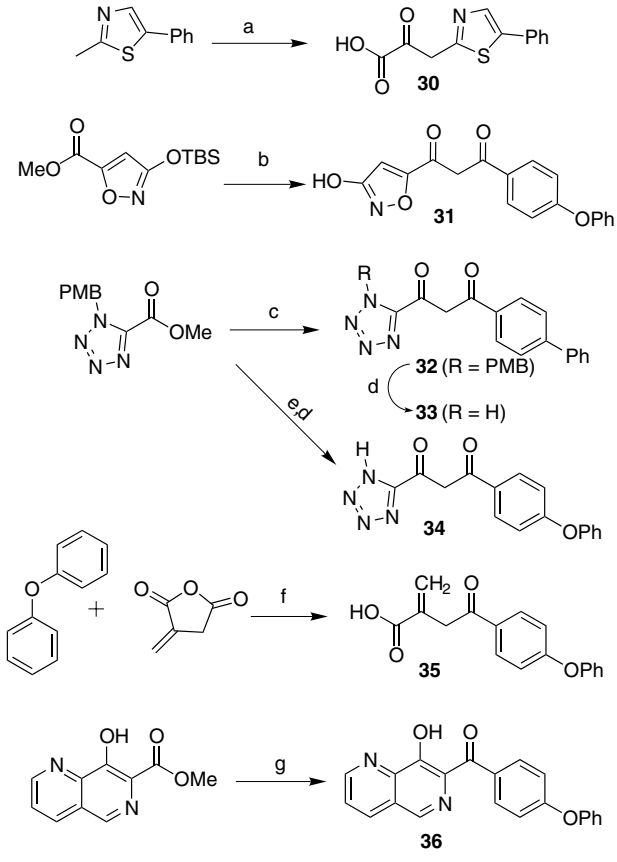
[a] Compound identity and purity were established by ^{1}H NMR and analytical HPLC–MS.
[b] $ArB(OH)_2$, 2 M Na_2CO_3, $Pd(PPh_3)_4$, NMP, heat.

para position resulted in several sub-micromolar compounds (**16–18**). Interestingly, comparisons of compounds **9** and **10** to compounds **17** and **18** show that the *ortho* chloro consistently gave a 3- to 5-fold improvement in selectivity against XPG.

Heterocyclic diketobutyrates were also explored (Table 2). These compounds were generally made through a Suzuki coupling to the heterocyclic carboxylic acid as shown in Table 2.[23] In general, unsubstituted heterocycles showed poor activity (**19**, **25**). 5-Substituted furans were generally more potent but were quite selective for XPG (**21–24**). 5-Substituted thiophenes were quite potent against FEN1, but showed little of the desired selectivity (**26–29**).

It has been reported that 2,4-diketobutyrates suffer from metabolic and toxicity problems due to the electrophilic nature of the α-keto acid.[24] In response, several groups have reported alternatives including 8-hydroxy-[1,6] naphthyridines[24] and 5-keto-tetrazoles.[25] Based on this literature, we designed several molecules to mimic the 2,4-diketobutyric acid moiety (Scheme 2). Interestingly, the N-substituted tetrazole (**32**, Table 3) proved to be one of the most active isosteres. Unsubstituted tetrazoles were largely inactive (**33–34**). The 8-hydroxy-[1,6] naphthyridine (**36**) also showed low micromolar activity. Unfortunately, none of the isosteres were sufficiently active to warrant further investigation.

In conclusion, we have identified a sub-micromolar series of 2,4-diketobutyrates as inhibitors of the DNA repair protein FEN1. These inhibitors are highly related to a series of HIV integrase inhibitors that have shown efficacy in cell culture. Several compounds showed greater than 10-fold selectivity over the highly related endonuclease, XPG. Further studies have been



Scheme 2. Reagents and conditions: (a) NaHMDS, diethyl oxalate; (b) (1) LiHMDS, THF, 4-phenoxy acetophenone; (2) TFA; (c) 4-phenyl acetophenone, diethyl oxalate, NaOMe; (d) TFA; (e) 4-phenoxy acetophenone, diethyl oxalate, NaOMe; (f) $AlCl_3$, DCE; (g) 4-phenoxy phenyl lithium, THF.

Table 3. FEN1 and XPG activity for compounds **30–36** (Scheme 2)

Compound	IC_{50} (μM)	
	FEN1	XPG
30	46.4	31.7
31	>100	>100
32	34.3	52.4
33	>100	>100
34	63.2	97
35	>100	>100
36	29.0	>100

undertaken to determine the clinical usefulness of compounds in this series as chemopotentiating agents.

References and notes

1. Badmbara, R. A.; Murante, R. S.; Henricksen, L. A. *J. Biol. Chem.* **1997**, *272*, 4647–4650.
2. Harrington, J. J.; Lieber, M. R. *EMBO J.* **1994**, *13*, 1235–1246.
3. Pont, K. G.; Dawson, R. J.; Carroll, D. *EMBO J.* **1993**, *12*, 23–24.
4. Harrington, J. J.; Lieber, M. R. *Genes Dev.* **1994**, *8*, 1344–1355.
5. Kucherlapati, M.; Yang, K.; Kuraguchi, M.; Zhao, J. Lia, M.; Heyer, J.; Kane, M.; Kunhua, F.; Russell, R.; Brown, A. M. C.; Kneitz, B.; Edelmann, W.; Kolodner, R. D.; Lipkin, M.; Kucherlapati, R. *PNAS* **2002**, *99*, 9924–9929.
6. Larsen, E.; Gran, C.; Saether, B. E.; Seeberg, E.; Klungland, A. *Mol. Cell. Biol.* **2003**, *23*, 5346–5353.
7. Moreau, S.; Morgan, E. A.; Symington, L. S. *Genetics* **2001**, *159*, 1423–1433.
8. Parikh, S. S.; Mol, C. D.; Hosfield, D. J.; Tainer, J. A. *Curr. Opin. Struct. Biol.* **1999**, *9*, 37–47.
9. Shibata, Y.; Nakamura, T. J. *Biol. Chem.* **2002**, *277*, 746–754.
10. For a review of chemosensitization: Gesner, T. G.; Harrison, S. D. *Annu. Rep. Med. Chem.* **2002**, *37*, 115–124.
11. White, A. W.; Almassy, R.; Calvert, A. H.; Curtin, N. J.; Griffin, R. J.; Hostomsky, Z.; Maegley, K.; Newell, D. R.; Srinivasan, S.; Golding, B. T. *J. Med. Chem.* **2000**, *43*, 4084–4097.
12. McElhinney, R. S.; Donnelly, D. J.; McCormick, J. E.; Kelly, J.; Watson, A. J.; Rafferty, J. A.; Elder, R. H.; Middleton, M. R.; Willington, M. A.; McMurry, B. H.; Margison, G. P. *J. Med. Chem.* **1998**, *41*, 5265–5271.
13. Middleton, M. R.; Kelly, J.; Thatcher, N.; Donnelly, D. J.; McElhinney, R. S.; McMurry, B. H.; McCormick, J. E.; Margison, G. P. *Int. J. Cancer* **2000**, *85*, 248–252.
14. Gary, R.; Ludwig, D. L.; Cornelius, H. L.; MacInnes, M. A.; Park, M. S. *J. Biol. Chem.* **1997**, *272*, 24522–24529.
15. Berneburg, M.; Lehmann, A. R. *Adv. Genet.* **2001**, *43*, 71–102.
16. This molecule, referred to as BVT substrate, was prepared by annealing oligonucleotide VT (5′-VIC-ccctccgccgtcgcgttt-TAMRA; Applied BioSystems) with oligonucleotide B (5′-aaacgcgacggcggagggtcttgctcagtgtc gtctccgacactgagcaa-Black Hole Quencher; Integrated DNA Technologies). High-throughput enzyme inhibition assays were performed using 60 μL reaction mixes (50 mM Tris–HCl; pH 8.0, 10 mM $MgCl_2$, 0.5 mM 2-ME, 6 μg BSA, 2.5 μg circular plasmid, 180 U FEN1 or 50 ng XPG, 25 μM test compound, and 5 pmol BVT substrate) contained in black 96-well plates. Reactions were incubated at room temperature for 90 min, stopped through the addition of 40 μL stop buffer (0.025% SDS, 12.5 mM EDTA) and fluorescence was measured using a Fluoroscan plate-reading fluorometer fitted with 485 nm excitation/538 nm emission filters.
17. Tomassini, J.; Selnick, H.; Davies, M. E.; Armstrong, M. E.; Baldwin, J.; Bourgeois, M.; Hastings, J.; Hazuda, D.; Lewis, J.; McClements, W.; Ponticello, G.; Radzilowski, E.; Smith, G.; Tebben, A.; Wolfe, A. *Antimicrob. Agents Chemother.* **1994**, *38*, 2827–2837.
18. Hazuda, D. J.; Felock, P.; Witmer, M.; Wolfe, A.; Stillmock, K.; Grobler, J. A.; Espeseth, A.; Gabryelski, L.; Schleif, W.; Blau, C.; Miller, M. D. *Science* **2000**, *287*, 646–650.
19. Williams, H. W. R.; Eichler, E.; Randall, W. C.; Rooney, C. S.; Cragoe, E. J.; Streeter, K. B.; Schwam, H.; Michelson, S. R.; Patchett, A. A.; Taub, D. *J. Med. Chem.* **1983**, *26*, 1196–1200.
20. General procedure A: The methyl ketone was stirred in dry THF (0.25 M). Dimethyl oxalate was added (2 equiv) followed by NaOMe (3 equiv, 1 M in MeOH). The solution was stirred 2 h at rt. Aqueous NaOH (3 equiv, 1 M) was added and the solution was stirred for 4 h. The reaction was washed with EtOAc then acidified with HCl (1 M). The desired product was extracted into EtOAc. Evaporation generally gave a crude material with adequate purity for biological evaluation.
21. Tapia, I.; Alcazar, V.; Moran, J. R.; Caballero, C.; Grande, M. *Chem. Lett.* **1990**, 697.
22. General procedure B: The pyruvic hydrazone (0.25 mmol) was dissolved in THF (1 mL) and HMPA (3.0 mmol), and cooled to 0 °C. Methyl lithium (1.0 mmol, 1.6 M in hexanes) and a solution of the methyl ester in THF (0.25 mmol in 1 mL) were subsequently added. The reaction was stirred for 2 h at 0 °C, quenched with water (5 mL), and washed with ether (10 mL). The aqueous solution was acidified to a pH = 2 with 1 M HCl and stirred for 10 min at room temperature. The acidified aqueous solution was extracted with ethyl acetate (3 × 25 mL), dried over $MgSO_4$, and concentrated. The crude product was generally purified by preparative LC–MS.
23. Suzuki coupling to the free 2,4-diketobutyrate was generally unsuccessful.
24. Zhuang, L.; Wai, J.; Embrey, M. W.; Fisher, T. E.; Egbertson, M. S.; Payne, L. S.; Guare, J. P.; Vacca, J. P.; Hazuda, D. J.; Felock, P. J.; Wolfe, A. L.; Stillmock, K. A.; Witmer, M. V.; Moyer, G.; Schleif, W. A.; Gabryelski, L. J.; Leonard, Y. M.; Lynch, J. J.; Michelson, S. R.; Young, S. D. *J. Med. Chem.* **2003**, *46*, 453–456.
25. Pais, G. C. G.; Zhang, X.; Marchand, C.; Neamati, N.; Cowansage, K.; Svarovskaia, E. S.; Pathak, V. K.; Tang, Y.; Nicklaus, M.; Pommier, Y.; Burke, T. R. *J. Med. Chem.* **2002**, *45*, 3184–3194.