Supporting information for:

Synthesis and screening of a haloacetamidine containing library to identify PAD4 selective inhibitors

Justin E. Jones,[ab] Jessica L. Slack,[ab] Xuesen Zhang, Venkataraman Subramanian,[b] Corey P. Causey,[b] Scott A. Coonrod,[c] and Paul R. Thompson*[ab]

[a] Department of Chemistry, The Scripps Research Institute, Scripps Florida, 120 Scripps Way, Jupiter, Florida, USA 33458. Fax: (561)-228-3050; Tel: (561)-228-2860; E-mail: Pthompso@scripps.edu.

[b] Department of Chemistry & Biochemistry, University of South Carolina, 631 Sumter Street, Columbia, SC, USA 29208

[c] Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University 122 Hungerford Hill Road, Ithaca, NY 14853

Running Title: Haloacetamidine Library of PAD Inhibitors

Materials and Methods

Chemicals and proteins. Fmoc protected amino acids and resins were purchased from EMD (Gibbstown, NJ). PADs 1, 2, and 3 were purified by previously established methods (*1*). PAD4 was purified as previously described (*2*) The peptide Ac-TDR-NH_2 was synthesized using the Fmoc approach and purified by reverse phase HPLC.

Synthesis of 2-acetamido-5-(2-fluoroacetimidamino)-N-(2-mercaptoethyl)pentanamide (FASH). Boc-cystamine dihydrochloride (17 mg, 0.06 mmol, 4 eq.) was added to TentaGel S COOH resin (130 micron) (50 mg, 0.015 mmol, 1 eq.), HOBt (92 mg, 0.06 mmol, 4 eq.), PyBOP (31 mg, 0.06 mmol, 4eq.) and 0.4 M *N*-methylmorpholine in 4 mL of DMF and allowed to react overnight at rt. The Boc protecting group was removed with TFA:DCM (1:1). Fmoc-Orn(Dde)-OH (34 mg, 0.06 mmol, 4 eq.) was coupled to the cystamine modified TentaGel. The Fmoc group was removed with 20% piperidine in DMF and the N-terminus was acetylated. The Dde protecting group on ornithine was removed with 2% hydrazine in DMF for 2 h. Ethyl-2-fluoroacetimidate-HCl (1.7 mg, 0.06 mmol, 4 eq.) was reacted overnight at rt with the free amine of ornithine in a mixture of DMF and triethylamine (1.5 mg, 0.075 mmol, 5 eq.). The resin was washed extensively with DMF, ethanol, DCM, and 50 mM Tris-HCl pH 7.6. The compound was cleaved from the resin with 10 mM DTT in 50 mM Tris-HCl pH 7.6 for 3 h at rt. The compound was purified by HLPC and the product was confirmed by ESI-MS (Expected = 293.14, Observed = 293.0) and ^{1}HNMR (300 MHz, D_2O) δ(ppm): 5.14-5.04 (d, $^2J_{H-F}$ = 30 Hz, 2H), 4.10-4.03 (m, 2H), 3.24-3.20 (m, 2H), 2.52-2.43 (m, 2H), 1.90-1.82 (s, 3H), 1.71-1.63 (m, 2H), 1.61-1.52 (m, 2H).

Library Synthesis. Boc-cystamine dihydrochloride (694 mg, 2.4 mmol, 4 eq.) was added to TentaGel S COOH resin (130 micron) (2 g, 0.6 mmol, 1 eq.) in the presence of HOBt (367 mg, 2.4 mmol, 4eq.), PyBOP (1.25 g, 2.4 mmol, 4 eq.), and 0.4 M *N*-methylmorpholine in 20 mL of DMF. This solution was allowed to react overnight at rt. TFA/DCM (1:1) was then added to remove the Boc protecting group. Fmoc-Orn(Dde)-OH (1.3 g, 2.4 mmol, 4 eq.) was coupled to the cystamine modified TentaGel using standard Fmoc solid phase synthesis. The Fmoc protecting group was removed with 20% piperidine. The resin was divided evenly into 24 tubes. One of the 24 R-1 functionality groups (2.4 mmol, 4 eq.) was added to each tube and coupled using standard Fmoc solid phase synthesis. Upon removal of the Fmoc protecting group, the resin in each tube was evenly placed in 11 wells of a 96-well solvent resistant filter plate. One of the 11 R-2 functionality groups (2.4 mmol, 4 eq.) was added to each well. The N-terminus was acetylated after removal of the Fmoc protecting group. The Dde protecting group on ornithine was removed with 2% hydrazine in DMF for 2 h. The ethyl-2-fluoroacetimidate warhead (252 mg, 2.4 mmol, 4 eq.) was coupled to the free amine of ornithine in a mixture of DMF and triethylamine (303 mg, 3 mmol, 5 eq.) and allowed to react overnight at rt. The protecting groups were removed in TFA/DCM (1:1). The resin was washed extensively with DMF, ethanol, DCM, and 50 mM Tris-HCl pH 7.6. The resin was cleaved with 10 mM DTT in 50 mM Tris-HCl pH 7.6 for 3 h at rt. After cleavage, the solution was filtered into a 96-well microtiter plate for screening.

Synthesis of TDFA and TDCA. Fmoc-Orn(Dde)-OH (6.3 mg, 0.012 mmol, 4 eq.) was coupled to the Knorr Amide resin (50 mg, 0.003 mmol, 1 eq.). The Fmoc group was removed with 20 %

piperidine in DMF and Fmoc-Asp(tBu)-OH (4.9 mg, 0.012 mmol, 4 eq.) was coupled to the modified resin. The Fmoc group was removed with 20 % piperidine in DMF and Fmoc-Thr(tBu)-OH (4.8 mg, 0.012 mmol, 4 eq.). The Fmoc group was removed with 20% piperidine in DMF and the N-terminus was acetylated. The Dde protecting group on ornithine was removed with 2% hydrazine in DMF for 2 h. Either ethyl-2-fluoroacetimidate (1.7 mg, 0.012 mmol, 4 eq.) or ethyl-2-chloroacetimidate (1.5 mg, 0.012 mmol, 4 eq.) reacted overnight at rt with the free amine of ornithine in a mixture of DMF and triethylamine (1.5 mg, 0.015 mmol, 5 eq.). The resin was washed extensively with DMF, ethanol, and DCM. The compound was cleaved from the resin with TFA:DCM (1:1). The compounds were purified by HLPC using a C18 prep column and eluted with acetnotrile (0.05 % TFA). Compounds were monitored at 210 nm and eluted at the following retention times, TDCA (9.341 min) and TDFA (7.853 min). Purity was greater than 95% as assessed by analytical HPLC. HRMS (ESI) *m/z* calculated for TDFA [$C_{17}H_{29}FN_6O_7$], 449.2160; observed, 449.2158. HRMS (ESI) *m/z* calculated for TDCA [$C_{17}H_{29}ClN_6O_7$], 465.1864; observed, 465.1857.

Synthesis of 6-azido-TDFA. Fmoc-Orn(Dde)-OH (6.3 mg, 0.012 mmol, 4 eq.) was coupled to the Knorr Amide resin (50 mg, 0.003 mmol, 1 eq.). The Fmoc group was removed with 20 % piperidine in DMF and Fmoc-Asp(tBu)-OH (4.9 mg, 0.012 mmol, 4 eq.) was coupled to the modified resin. The Fmoc group was removed with 20 % piperidine in DMF and Fmoc-Thr(tBu)-OH (4.8 mg, 0.012 mmol, 4 eq.). The Fmoc group was removed with 20% piperidine in DMF and 6-azidohexanoic acid (1.9 mg, 0.012 mmol, 4 eq.) was coupled to the modified resin. 6-azidohexanoic acid was prepared from 6-bromobenzoic acid according to previously established methods (*3*). The Dde protecting group on ornithine was removed with 2% hydrazine in DMF for 2 h. Ethyl-2-fluoroacetimidate (1.7 mg, 0.012 mmol, 4 eq.) reacted overnight at rt

with the free amine of ornithine in a mixture of DMF and triethylamine (1.5 mg, 0.015 mmol, 5 eq.). The resin was washed extensively with DMF, ethanol, and DCM. The compound was cleaved from the resin with TFA:DCM (1:1). The compounds were purified by HLPC and the product was confirmed by MALDI-TOF-TOF (Expected = 545.57, Observed = 545.3).

Synthesis of N-[2-(D-Biotinylamino)-ethyl] -(hex-5-ynoic)-amide (Biotin-yne). To a stirred solution of pentafluorophenyl trifluoroacetate (106 µL, 0.61 mmol, 1.5 eq) in DMF (2.0 mL) was added triethylamine (0.62 mmol, 86 µL, 1.5 eq) at 0 °C. D-biotin (100 mg, 0.41 mmol, 1.0 eq) was added to this solution and the reaction was continued at room temperature for 2 h. Ether (10.0 mL) was added and the precipitated white solid was filtered, washed with ether (10.0 mL) and dried to give the PFP-biotin (134 mg, 80%). The product was confirmed by LCMS (Expected: 411.07; Observed: 411.10). To a stirred solution of PFP-biotin (80 mg, 0.20 mmol, 1.0 eq) in DMF (2.0 mL) was added ethylenediamine (135 mg, 2.0 mmol, 10.0 eq) at 0 °C. The reaction was continued for 2 h. Ether (10.0 mL) was added and the precipitated white solid was filtered, washed with ether (10.0 mL) and dried to give the biotin-NH_2 (39.0 mg, 70%). The product was confirmed by LCMS (Expected: 287.14; Observed: 287.10). To a stirred solution of Biotin-NH_2 (30 mg, 0.10 mmol, 1.0 eq) in DMF (0.5 mL) was added *N*,*N*-diisopropylethylamine (0.31 mmol, 55 µL, 3.0 eq), 5-Hexynoic acid (0.13 mmol, 14 mg, 1.3 eq) followed by HBTU (0.15 mmol, 57 mg, 1.5 eq) at 0 °C under N_2. The reaction was continued at room temperature for 12 h. Ether (10.0 mL) was added and the precipitated white solid was filtered, washed with ether (10.0 mL) and dried to give *N*-[2-(D-Biotinylamino)-ethyl]-(hex-5-ynoic)-amide (28.0 mg, 70%). The product was confirmed by LCMS (Expected: 380.18; Observed: 380.20).

Synthesis of Biotin conjugated TDFA. To a reaction mixture containing biotin-yne (0.684 mg, 0.0018 mmmol, 1 eq.), CuSO4●H_2O (0.225 mg, 0.0009 mmol, 0.5 eq.), ascorbic acid (0.5 mg, 0.0027 mmol, 1.5 eq.), in THF:H_2O (1:1), 6-azido-TDFA (1 mg, 0.0018 mmol, 1 eq.) was added. The compounds were purified by HLPC and the product was confirmed by MALDI-TOF-TOF (Expected = 926.07, Observed = 926.8589).

Library Screening. The library was screened by pre-incubating 6 μL (~ 10 μM) of each compound in Reaction Buffer (100 mM Tris-HCl pH 7.6, 10 mM $CaCl_2$, 2 mM DTT, 50 mM NaCl) with PAD4 (0.2 μM) for 15 min. The reaction was then initiated by the addition of 10 mM BAEE and reacted for a further 15 min. Reactions were quenched with liquid nitrogen and 200 μL of freshly prepared COLDER solution was added (*4*). This mixture was incubated for 30 min at 95 °C at which point the absorbance was measured at 540 nm. Each compound was assayed in duplicate and product levels were determined by comparison to a standard curve of known citrulline concnetrations.

Determination of IC_{50} Values. IC_{50} values for PADs 1, 2, 3, and 4 were determined by pre-incubating enzyme with varying concentrations of inhibitor in Reaction Buffer for 15 min at 37 °C. BAEE (10 mM) was added and the reaction was allowed to proceed for 15 min at 37 °C. Once the reaction was quenched in liquid nitrogen, product was measured as outlined above and fit to Equation 1 using GraFit (version 5.0.11) (*5*),

$$\text{Fractional Activity} = 1/(1 + ([I]/IC_{50})) \quad (1),$$

where [I] is the concentration of inhibitor, and IC_{50} is the concentration of inhibitor that yields half-maximal activity, all experiments were performed at least in duplicate and the standard errors were typically less than 20%.

Inactivation kinetics of TDFA and TDCA. PADs 1, 2, 3, or 4 were incubated in Inactivation Buffer containing 10 mM $CaCl_2$, 2 mM DTT, and 100 mM Tris-HCl pH 7.6 for 10 min at 37 °C. At various times, from 0-30 min, aliquots were removed from the Inactivation Buffer and added to a Reaction Buffer (100 mM Tris-HCl pH 7.6, 10 mM $CaCl_2$, 2 mM DTT, 50 mM NaCl, and 10 mM BAEE) in order to measure residual activity. This reaction proceeded for 15 min and was quenched by flash freezing in liquid nitrogen. Activity was measured as described above and for every concentration of inactivator, data were fit to Equation 2,

$$v = v_o e^{-kt} \quad (2),$$

using GraFit version 5.0.11 (*5*), where v is the velocity, v_o is the initial velocity, k is the pseudo-first order rate constant of inactivation (i.e., k_{obs}), and t is time. Since saturation was achieved in the plots of k_{obs} versus [I] for PADs 1, 3, and 4, the data were fit to equation 3 using GraFit version 5.0.11 (*5*),

$$k_{obs} = k_{inact}[I]/(K_I + [I]) \quad (3),$$

where k_{inact} corresponds to the maximal rate of inactivation and K_I is the concentration of I that yields half-maximal inactivation. For PAD2, saturation kinetics were not observed, and thus the resulting plots of k_{obs} versus [I] were linear and the slope of this line corresponds to the k_{inact}/K_I.

Substrate protection experiments. Progress curves were generated in the presence and absence of inactivator (250 μM) and two different concentrations of BAEE (2 and 10 mM) in order to

determine if substrate protects against PAD4 inactivation. Reaction Buffer was incubated at 37 °C for 10 min before PAD4 was added to initiate the reaction. Aliquots were taken out at 0, 2, 4, 6, 8, and 10 min and quenched by flash freezing in liquid nitrogen. Progress curves were fit to equation 4 using GraFit (version 5.0.11) (*5*),

$$[\text{Cit}] = v_i(1-e^{-k_{obs.app}*t})/k_{obs.app} \quad (4),$$

where v_i is initial velocity, $k_{obs.app}$ is the apparent pseudo first order rate constant for inactivation , t is time, [Cit] is the concentration of citrulline produced during the reaction.

Inactivator Irreversibility. In order to determine whether TDFA and TDCA irreversibly inactivate PAD4, a reaction was carried out with PAD4 and excess inactivator (1 mM) at 37 °C for 1 h. The reaction was then dialyzed against long term storage buffer (20 mM Tris-HCl pH 8, 1 mM EDTA, 2 mM DTT, 500 mM NaCl, and 10 % glycerol) for 20 h. Residual activity was measured using the assay described above.

Kinetic Assays. Kinetic assays were performed as previously described (*1*). Briefly, a reaction buffer contained 100 mM Tris-HCl pH 7.6, 2 mM DTT, 10 mM $CaCl_2$, and 50 mM NaCl and was pre-incubated with varying concentrations of Ac-TDR-NH_2 (0, 0.05, 0.25, 0.52, 1.29, 2.58, and 5.15 mM) for 10 min at 37 °C. The reaction was initiated by the addition of enzyme (PAD1 = 0.2 μM, PAD2 = 0.5 μM, PAD3 = 0.5 μM, PAD4 = 0.2 μM). After 6 min, reactions were quenched by flash freezing in liquid nitrogen and 200 μL of COLDER solution was added to each tube. The absorbance at 540 nm was quantified and product was measured based on a standard curve. The data were fit to equation 5,

$$v = V_{max}[S]/(K_m+[S]) \quad (5),$$

using the GraFit™ version 5.0.11 software package (*5*).

Cellular activity studies. HL-60 cells (1 x 10^6 ml/cell) were treated with all-trans retinoic acid (1 μM) for 48 hours at 37 °C, 5% CO_2. Cells were split into 12 well plates and treated with 2 mM $CaCl_2$ and either Cl-amidine (100 μM) or with TDXA (1, 10 and 100 nM). After 15 min at 37 °C, 5% CO_2, cells were treated with LPS (100 ng/mL). Cells were harvested after 30 min, rinsed with cold PBS and lysed with SDS lysis buffer (2% SDS, 62.5 mM Tris-HCl pH 6.8, 10% glycerol). Proteins were separated by 12% SDS-PAGE and transferred to a nitrocellulose membrane for western blot analysis. Membranes were blocked with 5% Nonfat dry milk in TBST for 1 h at room temperature. Membranes were probed with polyclonal anti-Citrulline H3 antibody (Abcam, ab5103) or polyclonal anti-Histone H3 (Abcam, ab1791).

Chromatin Immunoprecipitation experiment. MCF-7 cells were maintained in DMEM supplemented with 5% fetal bovine serum. Where indicated, Cl-amidine was diluted in cell culture medium at the final concentration of 10 and 100 μM; TDFA or TDCA was diluted at the final concentration of 1 and 10 μM. PBS was used as a control. All the drugs were incubated with cells for 48 hours prior to harvest for Chromatin Immunoprecipitation (ChIP) experiment. ChIP was performed essentially as described previously (*6*). MCF-7 cell lysates were sonicated under conditions yielding fragments ranging from 200bp to 800bp. The material was clarified by centrifugation, diluted 10-fold in dilution buffer, and pre-cleared with protein A-agarose beads. The pre-cleared, chromatin-containing supernatant was used in immunoprecipitation reactions with antibody against Histone H3 Citrulline 2/8/17 (Abcam 5103), or normal rabbit IgG (Upstate 12-370) as a control. Ten percent of the supernatant was saved as reference control. Both the immunoprecipitated genomic DNA and the control DNA were cleared of protein and residual

RNA by digestion with proteinase K and RNase (Roche), respectively. The DNA was then extracted with phenol:chloroform:isoamyl alcohol and precipitated with ethanol. Quantitative real-time PCR (qPCR) using the Power SYBR Green (Applied Biosystems 4367659) was used to determine the enrichment of immunoprecipitated DNA relative to the input DNA to the specified regions. The qPCR primers to detect H3Cit2/8/17 enrichment on the *p21* gene promoter (PBS2, p53 binding site 2, where PADI4 has been shown to bind to this site too) were forward 5'-CTGTCCTCCCCGAGGTCA-3' and reverse 5'-ACARCRCAGGCTGCTCAGAGTCT-3'; and the primers to detect H3Cit2/8/17 enrichment on the *OKL38* gene promoter (PADI4 was also shown to bind to this region) were forward 5'-CTTTTGCCTGCCCTTAACAG-3'; and reverse 5'-TCCCTGCCATCTTGTTTACC-3'. Each ChIP experiment was conducted a minimum of three times with independent chromatin isolates to ensure reproducibility.

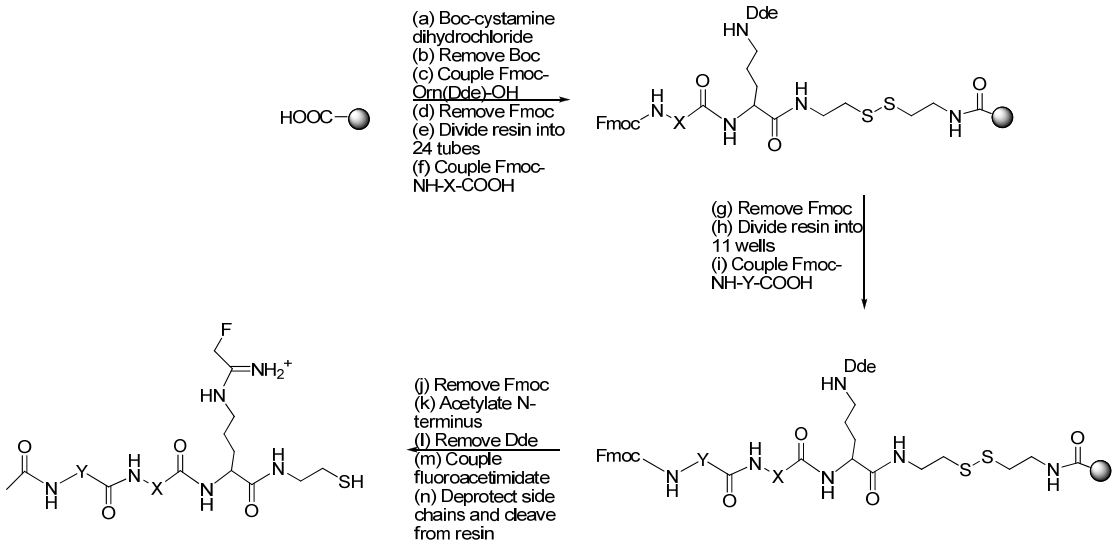
Biotin-TDFA selectivity. Reactions were carried out in 30 μL volumes in a buffer containing1 μM Biotin-TDFA, 100 mM Tris-HCl pH 7.6, 2 mM DTT, and 10 mM $CaCl_2$. Reactions were initiated by the addition of either PAD2 (1 μM), PAD4 (1 μM), or both PAD2 and PAD4 (1 μM each). Negative controls were performed where Biotin-TDFA not added to the reaction. After 1 h. of incubation, the reactions were quenched with 6x loading dye and separated by SDS-PAGE. Separated proteins were then transferred to a nitroceullose membrane at 80 V for 60 min in a transfer buffer containing 50 mM Tris, 40 mM glycine, and 200 mL methanol in 1 L. 5% BSA in PBS was used to block for 1h. The membrane was then incubated overnight with streptavadin-HRP (Invitrogen, 1:10,000). The membrane was washed 4 times in PBS. The HRP signal was detected by Pierce ECL Western Blotting substrate.

Protein purification and crystallization. GST-tagged Human PAD4 was purified and the GST-tag was cleaved as previously described (*7*). The purified PAD4 protein was concentrated in 10 mM Tris-HCl pH 8.5, 500 mM NaCl, 1 mM DTT and 1 mM EDTA to 10 mg/mL. Crystallization was performed by the sitting-drop vapour-diffusion method at 18 ºC. Preliminary crystallization condition were described as 0.1 M imidazole pH 8.0, 0.2 M lithium sulfate and 8% (w/w) PEGMME 2000 (*7*). After optimization, PAD4 (without ligand) was crystallized in the final crystallization solution containing 0.1 M imidazole pH 8.0, 0.2 M lithium sulfate and 10% (w/v) polyethylene glycol 1500. These crystals were then soaked with 5 mM $CaCl_2$ and 40 mM TDFA in the crystallization solution for 2-4 days. The soaked crystals were transferred to cryoprotectant solution (with extra 20% ethylene glycol) and flash frozen in liquid nitrogen.

Data collection and processing. X-ray diffraction data were collected at beamline LS-CAT station 21-ID-G at the Advanced Photo Source (Argonne National Laboratory). Data were processed and scaled using HKL2000 (*8*). The human PAD4 structure (PDB 1WDA) was used as a search model for molecular replacement using Molrep (*9*). The atomic model was refined with the phenix suite (*10*). Between each round of refinement, the model was fitted to the 2Fo–Fc electron-density map with the program Coot (*11*). TDFA ligand was added to the model after R-factor dropped to 0.30 based on unbiased omit density map. The final structure was refined to 2.98 Å with all residues in good geometry. Statistics for data collection, processing and refinement are shown in Table S3.

Supplemental Figures

Scheme 1



Scheme 1. Synthesis of fluoroacetamidine containing library.

Figure S1

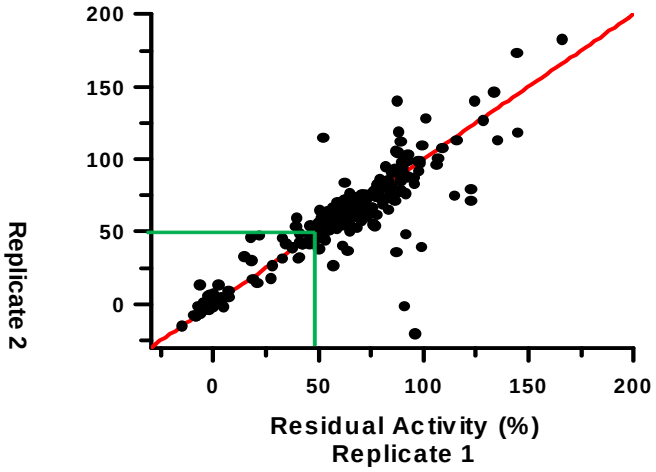


Figure S1. Plot of residual activity of one replicate versus residual activity of the second replicate for each member of the library. The green line represents 50% activity and everything inside the box displays better inhibition than the parent compound FASH.

Figure S2

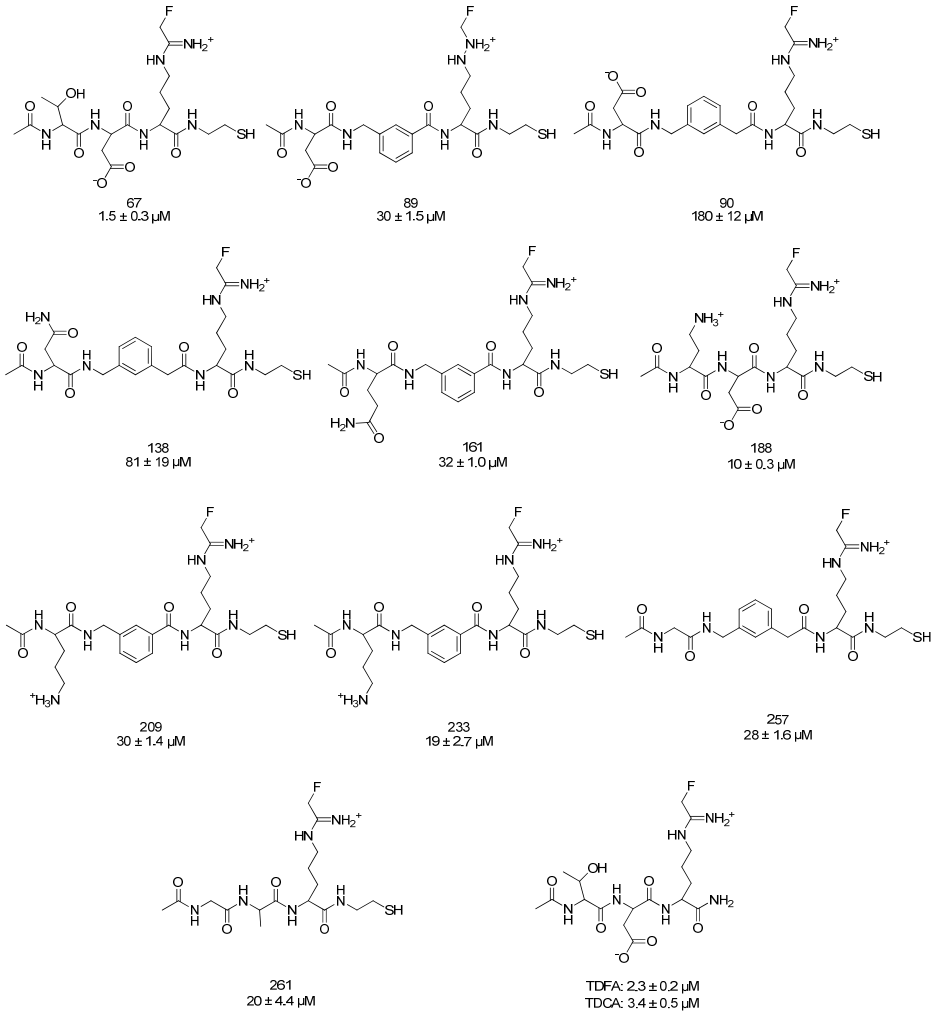


Figure S2. Structures and IC_{50} values of the top 10 hits.

Figure S3

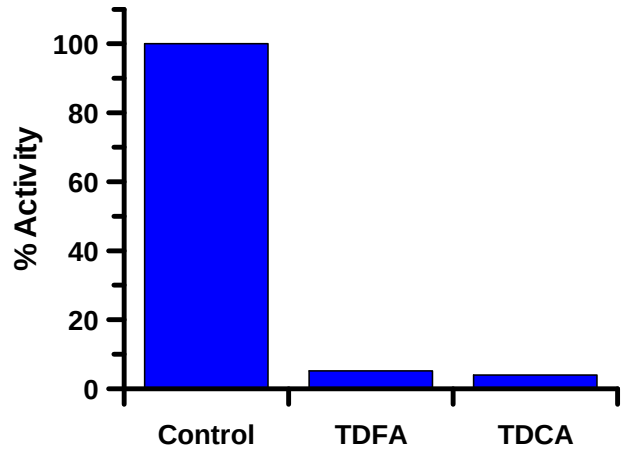


Figure S3. Plot of percent activity remaining after 20 h dialysis of pre-formed PAD4•inactivator complexes.

Figure S4

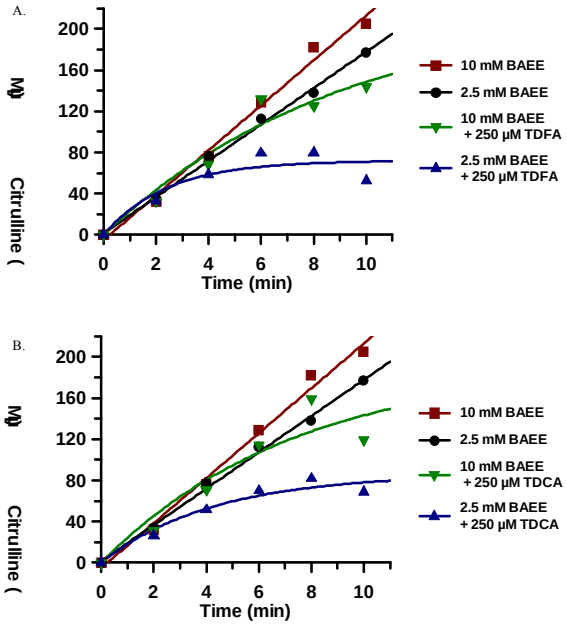


Figure S4. Substrate protection experiment with TDFA (A) and TDCA (B). Plots show the amount of product formed versus time at two different concentrations of substrate with and without inactivator.

Figure S5

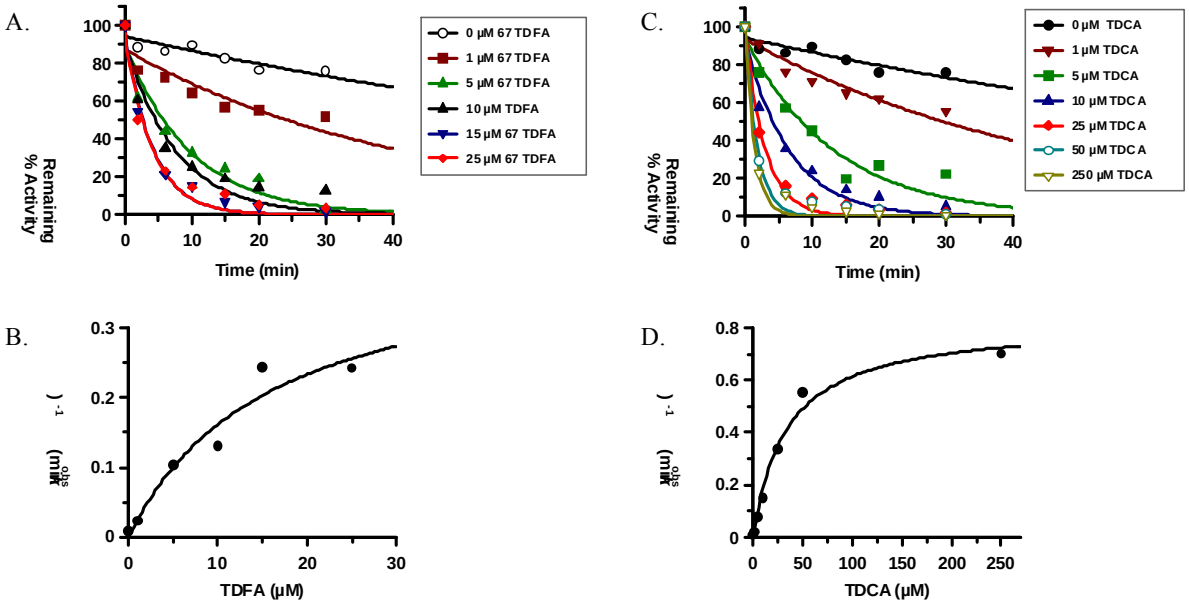


Figure S5. Inactivation of PAD4 with TDFA (A and B) and TDCA (C and D). Plots A and C show the percent activity remaining versus time with increasing concentrations of inactivator. Plots B and D show k_{obs} versus inactivator concentration.

Table S1

Table S1. Rates of catalysis for TDR substrate.

Substrate	k_{cat} (s^{-1})	K_m (mM)	k_{cat}/K_m (M^{-1} s^{-1})
PAD1			
TDR	2.4 ± 0.2	0.07 ± 0.04	34,000
BAEE[a]	0.3 ± 0.02	0.2 ± 0.1	1,500
PAD2			
TDR	0.6 ± 0.05	0.4 ± 0.1	1,500
BAEE	1.1 ± 0.1	0.1 ± 0.03	11,000
PAD3			
TDR	ND	ND	24
BAEE[a]	ND	ND	25
PAD4			
TDR	10 ± 0.6	0.3 ± 0.07	33,000
BAEE[b]	5.9 ± 0.3	1.4 ± 0.2	4,400

ND = Not Determined

[a]values from Knuckley et al. (*1*)

[b]values from Kearney et al. (*12*)

Table S2

PAD4	IC_{50} (μM)
Wt	2.3 ± 0.2
R639Q	6.6 ± 0.8
Q346A	4.4 ± 1.7
R374A	12 ± 2.0

Table S3. Data collection and refinement statistics

Table S3. Data collection and refinement statistics	
Data collection	
Space group	C2
Cell dimensions	
a, b, c [Å]	a=147.654, b=61.079, c=115.382
α, β, γ [º]	α=γ=90, β=124.543
Wavelength [Å]	0.97856
Resolution [Å]	50-2.98 (3.09-2.98)
R_{sym} or R_{merge}[b] [%]	8.0(55.5)
$I/\sigma I$	26.7(4.5)
Completeness [%]	99.9(100.0)
Redundancy	7.6(7.5)
Refinement	
Resolution [Å]	50-2.98(3.17-2.98)
No. reflections	16858(2245)
R_{work}[c]/ R_{free}[d]	19.1/23.0
No. atoms	
Protein	4996
TDFA	20
Ca^{2+}	5
Solvent	55
B-factors [Å^2]	
Protein	44.43
TDFA	62.00
Ca^{2+}	58.06
Solvent	36.56
R.m.s deviations	
Bond lengths [Å]	0.006
Bond angles [º]	1.049
Ramachandran plot	
Most favored [%]	97.3%
Additional allowed	2.7%

[a] Values in parentheses are for the highest resolution shell.

[b] $R_{merge}=\sum_h\sum_l|I(h)_l-\langle I(h)\rangle| / \sum_h\sum_l I(h)_l$, where $I(h)_l$ is the l th observation of the reflection h and $\langle I(h)\rangle$ is the weighted average intensity for all observations l of reflection h.

[c] $R_{work}=\sum_h||F_{obs}(h)|-|F_{cal}(h)|| / \sum_h|F_{obs}(h)|$, where $F_{obs}(h)$ and $F_{cal}(h)$ are the observed and calculated structure factors for reflection h respectively.

[d] R_{free} was calculated as R_{work} using the 5% of reflections which were selected randomly and omitted from refinement.

References

1. Knuckley, B., Causey, C. P., Jones, J. E., Bhatia, M., Dreyton, C. J., Osborne, T. C., Takahara, H., and Thompson, P. R. (2010) Substrate specificity and kinetic studies of PADs 1, 3, and 4 identify potent and selective inhibitors of protein arginine deiminase 3, *Biochemistry 49*, 4852-4863.
2. Knuckley, B., Bhatia, M., and Thompson, P. R. (2007) Protein arginine deiminase 4: evidence for a reverse protonation mechanism, *Biochemistry 46*, 6578-6587.
3. Grandjean, C., Boutonnier, A., Guerreiro, C., Fournier, J. M., and Mulard, L. A. (2005) On the preparation of carbohydrate-protein conjugates using the traceless Staudinger ligation, *J Org Chem 70*, 7123-7132.
4. Knipp, M., and Vasak, M. (2000) A colorimetric 96-well microtiter plate assay for the determination of enzymatically formed citrulline, *Anal Biochem 286*, 257-264.
5. Leatherbarrow, R. J. (2004) Grafit Ver 5.0, Erathicus Software, Staines, UK.
6. Zhang, X., Gamble, M. J., Stadler, S., Cherrington, B. D., Causey, C. P., Thompson, P. R., Roberson, M. S., Kraus, W. L., and Coonrod, S. A. (2011) Genome-Wide Analysis Reveals PADI4 Cooperates with Elk-1 to Activate c-Fos Expression in Breast Cancer Cells, *PLoS Genet 7*, e1002112.
7. Arita, K., Hashimoto, H., Shimizu, T., Yamada, M., and Sato, M. (2003) Crystallization and preliminary X-ray crystallographic analysis of human peptidylarginine deiminase V, *Acta Crystallogr D Biol Crystallogr 59*, 2332-2333.
8. Otwinowski, Z., and Minor, W. (1997) Processing of X-ray diffraction data collected in oscillation mode, *Macromolecular Crystallography, Pt A 276*, 307-326.
9. Vagin, A., and Teplyakov, A. (1997) MOLREP: an automated program for molecular replacement, *Journal of Applied Crystallography 30*, 1022-1025.
10. Adams, P. D., Afonine, P. V., Bunkoczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. J., Richardson, D. C., Richardson, J. S., Terwilliger, T. C., and Zwart, P. H. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution, *Acta Crystallogr D Biol Crystallogr 66*, 213-221.
11. Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics, *Acta Crystallographica Section D-Biological Crystallography 60*, 2126-2132.
12. Kearney, P. L., Bhatia, M., Jones, N. G., Yuan, L., Glascock, M. C., Catchings, K. L., Yamada, M., and Thompson, P. R. (2005) Kinetic characterization of protein arginine deiminase 4: a transcriptional corepressor implicated in the onset and progression of rheumatoid arthritis, *Biochemistry 44*, 10570-10582.