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Authors

Tjin, Caroline Chandra
Wissner, Rebecca F
Jamali, Haya
et al.

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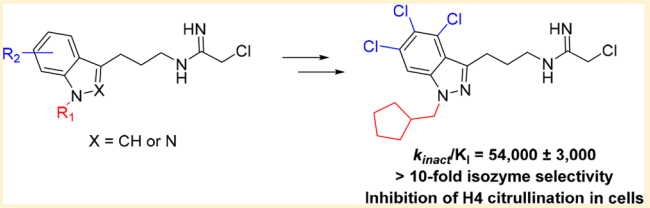
Synthesis and Biological Evaluation of an Indazole-Based Selective Protein Arginine Deiminase 4 (PAD4) Inhibitor

Caroline Chandra Tjin,[†] Rebecca F. Wissner,[†] Haya Jamali,[†] Alanna Schepartz,[†,‡] and Jonathan A. Ellman*[,†]

[†]Department of Chemistry and [‡]Department of Molecular, Cellular, and Developmental Biology, Yale University, New Haven, Connecticut 06520, United States

Supporting Information

ABSTRACT: Protein arginine deiminase 4 (PAD4) is a calcium-dependent enzyme that catalyzes the conversion of arginine to citrulline within target proteins. Dysregulation of PAD4 has been implicated in a number of human diseases, including rheumatoid arthritis and other inflammatory diseases as well as cancer. In this study, we report on the design, synthesis, and evaluation of a new class of haloacetamidine-based compounds as potential PAD4 inhibitors. Specifically, we describe the identification of 4,5,6-trichloroindazole **24** as a highly potent PAD4 inhibitor that displays >10-fold selectivity for PAD4 over PAD3 and >50-fold over PAD1 and PAD2. The efficacy of this compound in cells was determined by measuring the inhibition of PAD4-mediated H4 citrullination in HL-60 granulocytes.



KEYWORDS: *Protein arginine deiminase, mechanism-based inhibitor, rheumatoid arthritis, inflammatory disease, citrullination*

Protein arginine deiminases (PADs) are a family of Ca^{2+}-dependent enzymes that catalyze the conversion of arginines to citrullines within proteins, a phenomenon termed citrullination (Figure 1). Citrullination is a post-translational

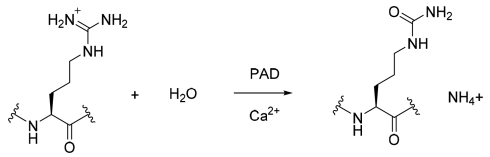


Figure 1. PAD-catalyzed conversion of arginine to citrulline.

event involved in many cellular processes such as gene regulation and cell differentiation.[1,2] This post-translational modification results in the loss of the net positive charge on the target protein, which leads to structural changes of the protein and consequently alters the protein function and its interaction with other biomolecules. Five human PADs (1, 2, 3, 4, and 6) have been identified and characterized.[3−5] PAD1 to PAD4 are catalytically active, but PAD6 has no detectable activity.[2,5,6] Although these isozymes share greater than 50% interisozyme sequence identity,[1,5] they display unique tissue expression patterns.[4,7] Significantly, dysregulation of PAD activity has been linked to the pathogenesis of many diseases.[4,7,8]

Among the four PAD isozymes, PAD4 is of great interest due to its relevance to the progression of rheumatoid arthritis (RA).[9] In the sera of RA patients, anticitrullinated protein antibodies (ACPA) that specifically recognize PAD4-mediated citrullination are found at high levels.[4,9] Because PAD4 is primarily expressed in granulocytes, overexpression of PAD4 also facilitates the formation of NETs (Neutrophil Extracellular Traps), and the resultant aberrant level of NETs is associated with inflammatory disorders such as sepsis,[10] lupus,[11] vasculitis,[12] and thrombosis.[13] In addition to promoting NETosis, citrullination by PAD4 critically regulates the expression of inflammatory cytokines in neutrophils such as TNF and IL-1β, which also actively contribute to the pathogenesis of RA.[14] In cancer, PAD4 not only acts as a transcriptional corepressor of the tumor suppressor protein p53[15] but also is involved in mediating malignancy.[16] Due to its regulatory roles in cell signaling pathways and disease pathogenesis, PAD4 has emerged as a potential therapeutic target for a range of disease states, and the development of inhibitors toward PAD4 as biological tools has become of high importance.

In recent years, numerous attempts have been carried out to identify potent and selective inactivators of PAD4. GSK implemented DNA encoded library screening to identify reversible inhibitors of PAD4.[17] The Thompson group has also developed a fluorescence polarization-activity based protein-profiling assay that enabled the discovery of streptonigrin, a natural product, as a potent and selective inhibitor of PAD4.[18] Unfortunately, off-target effects exhibited by streptonigrin complicate its use as a PAD4 pharmacological tool. Haloacetamidine mechanism-based inhibitors such as Cl-amidine (Figure 2) and BB-Cl-amidine, a second generation derivative of Cl-amidine with enhanced cell permeability and similar potency, have provided many key insights into PAD biology.[14,19,21] To date, selective

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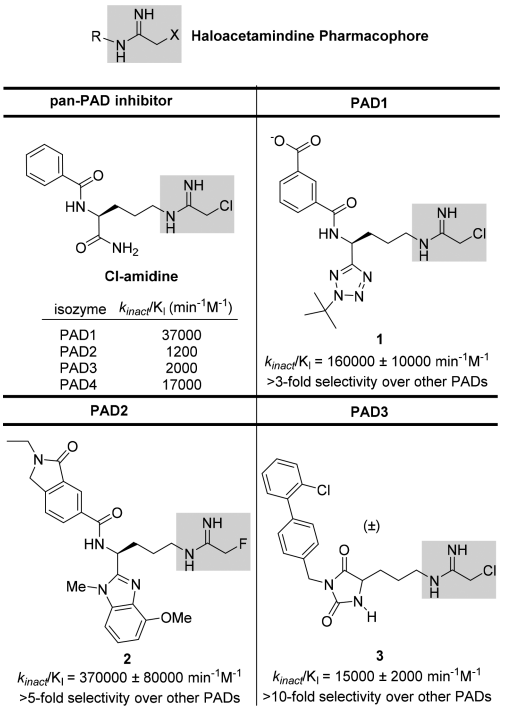


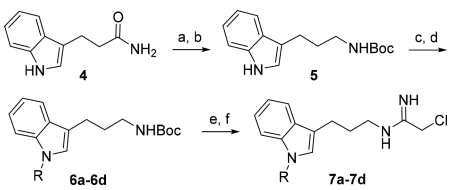
Figure 2. Potent and selective haloacetamidine-based inhibitors of PAD1, PAD2, and PAD3.

inhibitors containing the haloacetamidine warhead have been developed for PAD1,[20] PAD2,[21] and PAD3[22,23] with representative inhibitors shown in Figure 2.

Potent and selective mechanism-based haloacetamidine inhibitors against PAD4 have not yet been developed. Here, we report a new class of selective haloacetamidine-based nonpeptidic inhibitors against PAD4 bearing an indazole core, a heterocycle motif that has been incorporated into many drugs.[24] Moreover, we demonstrate that our most potent and selective indazole inhibitor prevents PAD4-mediated histone citrullination in cell culture.

We previously reported a substrate-based fragment screening approach to identify low molecular weight nonpeptidic guanidine substrates of PADs and identified an *N*-substituted indole appended to guanidine as an effective PAD4 substrate.[23] To capitalize on this nonpeptidic substrate, we prepared a series of analogous indole-derived chloracetamidine mechanism-based inhibitors **7a**−**7d** (Scheme 1). Commercially available amide **4**

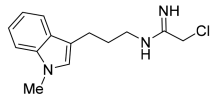
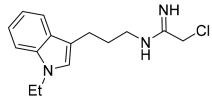
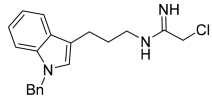
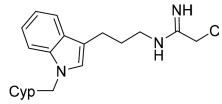
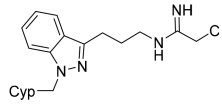
Scheme 1. Synthesis of Alkylated Indole Inhibitors[a]



[a]Reagents and conditions: (a) $LiAlH_4$, THF, reflux, 2 h; (b) Et_3N, Boc_2O, CH_2Cl_2, 0 °C to rt, 1 h; (c) NaH, DMF, 0 °C, 30 min to 1 h; (d) RX, DMF, 0 °C to rt, 1 to 2 h; (e) 33% CF_3COOH in CH_2Cl_2, 1 h; (f) ethyl-2-chloroacetamidate·HCl, Et_3N, CH_3OH, rt, 1 h.

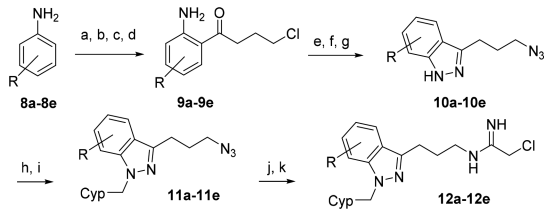
was reduced followed by Boc protection of the resulting free amine to give intermediate **5**.[25] Indole *N*-alkylation with several different alkyl halides generated **6a**−**6d**. Cleavage of the Boc group followed by addition of ethyl-2-chloroacetimidate

Table 1. IC_{50} Values for Compounds 7a−d and 12a[a]

Cmpd	Structures	PAD4 IC_{50} (μM)
7a		190 ± 5
7b		36 ± 0.5
7c		29 ± 0.6
7d		21 ± 0.9
12a		16 ± 3

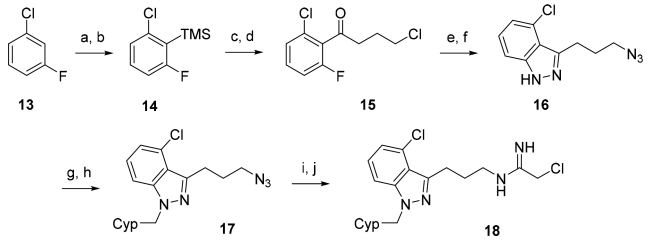
[a]IC_{50} values are reported as mean ± SD and were run in duplicate. Cyp = cyclopentyl. See Supporting Information for further assay details.

Scheme 2. Synthesis of Alkylated Indazole Inhibitors[a]



[a]Reagents and conditions: (a) $Me_2S·BCl_3$, 1,2-dichloroethane (DCE), 0 °C; (b) 4-chlorobutanenitrile, 0 °C to rt; (c) $AlCl_3$, reflux, overnight; (d) 2 M HCl, reflux, 30 min; (e) $NaNO_2$, H_2O, HCl, 0 °C, 1 h; (f) $SnCl_2$, HCl, 0 °C, 1 h; (g) NaN_3, DMF, 50 °C, overnight; (h) NaH, DMF, 0 °C, 30 min to 1 h; (i) cyclopentylmethyl bromide, DMF, 0 °C to rt, 1 to 2 h; (j) $SnCl_2$, CH_3OH, 2−4 h; (k) ethyl-2-chloroacetamidate·HCl, Et_3N, CH_3OH, rt, 1−2 h.

Scheme 3. Synthesis of 4-Chloroindazole Inhibitor 18[a]



[a]Reagents and conditions: (a) tetramethylethylenediamine (TMEDA), *n*-BuLi, THF, −75 °C, 2 h; (b) Me_3SiCl, THF, −75 °C, overnight; (c) $AlCl_3$, CH_2Cl_2, 0 °C; (d) 4-chlorobutanoyl chloride, −80 to −10 °C, 4 h; (e) NaN_3, DMF, 50 °C, 16 h; (f) hydrazine monohydrate, dimethoxyethane (DME), reflux, 17 h; (g) NaH, DMF, 0 °C, 30 min to 1 h; (h) cyclopentylmethyl bromide, DMF, 0 °C to rt, 1 to 2 h; (i) $SnCl_2$, CH_3OH, 2.5 h; (j) ethyl-2-chloroacetamidate·HCl, Et_3N, CH_3OH, rt, 1 to 2 h.

provided inhibitors **7a**−**7d**. We evaluated these inhibitors against PAD4 and observed that increasing the size of the *N*-alkyl group significantly improved the inhibitory potency against PAD4 with the cyclopentylmethyl substituent in **7d**

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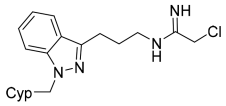
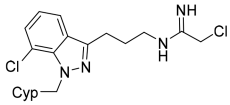
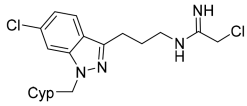
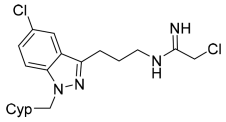
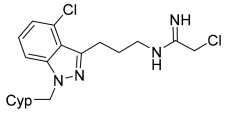
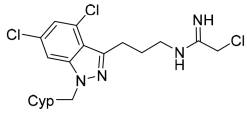
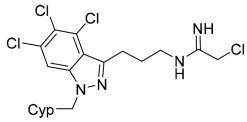
Table 2. k_{inact}/K_I Values for Compounds 12a−e, 18, and 24[a]

Cmpd	Structure	k_{inact}/K_I ($M^{-1}min^{-1}$) (fold selectivity over PAD4)			
		PAD4	PAD3	PAD2	PAD1
12a		3000 ± 30	3700 ± 100 (not selective)	160 ± 9 (19-fold selective)	20 ± 3 (150-fold selective)
12b		5300 ± 70	5400 ± 200 (not selective)	-	-
12c		5600 ± 90	2800 ± 200 (2.0-fold selective)	-	-
12d		11800 ± 300	7300 ± 700 (1.6-fold selective)	-	-
18		6700 ± 400	5200 ± 300 (1.3-fold selective)	-	-
12e		50000 ± 5000	6600 ± 700 (7.5-fold selective)	690 ± 60 (72-fold selective)	15 ± 3 (3300-fold selective)
24		54000 ± 3000[b]	5100 ± 400 (11-fold selective)	940 ± 240 (58-fold selective)	20 ± 5 (2700-fold selective)

[a] k_{inact}/K_I was determined using six concentrations of inhibitor at five different time points. k_{inact}/K_I was calculated from linear fit of k_{obs} obtained from two replicates. [b] k_{inact}/K_I was calculated from nonlinear regression of k_{obs} obtained from two replicates. $k_{obs} = k_{inact}/K_I$ because $[I] \ll K_I$. Cl-amidine under the assay conditions reported here gave the following k_{inact}/K_I values: PAD1, 4550 ± 860; PAD2, 520 ± 50; PAD3, 2340 ± 80; PAD4, 1770 ± 470.[23] See Supporting Information for further assay details.

providing the greatest inhibitory activity (Table 1). A number of additional *N*-alkyl derivatives were prepared and evaluated, but none gave any improvement in inhibitory activity over the *N*-cyclopentylmethyl derivative **7d** (data not shown).

Indazole is isosteric with indole, and therefore, the indazole analog **12a** (R = H) corresponding to indole inhibitor **7d** was synthesized (Scheme 2). Lewis acid-catalyzed addition of aniline (**8a**) to 4-chlorobutanenitrile provided the 2-aminophenyl ketone **9a**.[26] Diazotization and then reduction to the hydrazine with *in situ* cyclization to give the indazole was followed by displacement of the bromide with azide to afford indazole **10a**. *N*-Alkylation of the indazole with cyclopentylmethyl bromide yielded intermediate **11a**, which, after reduction to the amine followed by addition of ethyl-2-chloroacetimidate, furnished inhibitor **12a**. Evaluation of **12a** against PAD4 established that replacing the indole heterocycle core in **7d** with indazole resulted in a modest boost in potency (Table 1).

Given that indazole **12a** is slightly more potent than indole **7d**, we next proceeded to functionalize the ring to further enhance PAD4 potency and selectivity. We decided to install chloro substituents around the indole ring because the chloro substituent is relatively small and metabolically stable and also could provide a convenient handle for further synthetic elaboration.

The preparation of the chloro substituted inhibitors **12b−12d** and the dichloro-substituted inhibitor **12e** was accomplished according to the same sequence used to prepare the parent inhibitor **12a** employing chloro-substituted anilines **8b−8e** starting materials (Scheme 2).

To complete the SAR of all possible monochloro derivatives, we also sought to install the chloro group at position 4 of the indazole ring. However, the route used to prepare the other chloro derivatives is not appropriate for this substitution pattern because Friedel−Crafts acylation does not occur at the more sterically hindered ortho-site of 3-chloroaniline. We therefore developed a different route (Scheme 3). Directed ortho-metalation of commercially available aryl halide **13** resulted in lithiation at the most hindered 2-position, which after addition of trimethylsilyl chloride (TMSCl) afforded **14**. The silyl substituent in **14** then served to both activate and direct Lewis acid-catalyzed acylation with 4-chlorobutanenitrile at the silyl-substituted site to give ketone **15**.[27] Azide displacement of the alkyl chloride followed by hydrazone formation with concomitant S_NAr-mediated cyclization then generated indazole **16**. Next, *N*-alkylation with cyclopentylmethyl bromide provided **17**. Finally, reduction of the azide and subsequent addition of ethyl chloroacetimidate furnished inhibitor **18**.

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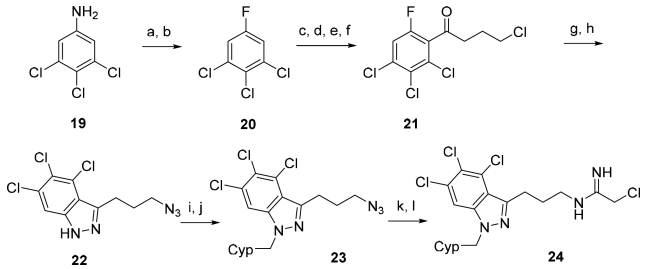
Because we desired both potency against PAD4 and selectivity over all of the other PAD isozymes, we carried out more rigorous determination of k_{inact}/K_I for the indazole inhibitors (Table 2). The parent indazole inhibitor **12a** had excellent selectivity over PAD1 and PAD2. However, selectivity for PAD4 over PAD3 was not obtained. Therefore, all of the monochloro-substituted derivatives, **12b−d** and **18**, were evaluated for inhibitory activity against both PAD4 and PAD3 (Table 2). Inhibitor **12b**, with the chloro substituent installed on position 7 of the indazole ring, resulted in modestly greater inhibitory activity against both PAD4 and PAD3. In contrast, inhibitor **12c** with the chloro substitution at position 6 resulted in both increased potency against PAD4 and 2-fold selectivity for PAD4 over PAD3. Inhibitors **18** and **12d** with the chloro substituent at positions 4 and 5 of the ring, respectively, both provided significant improvements in inhibitory activity against PAD4 and with relatively modest improvements in selectivity over PAD3. We next explored the summation of the effects of the chloro substitution in **12c** and **18** by evaluating the dichloro indazole inhibitor **12e**. Interestingly, inhibitor **12e** resulted in considerable improvement in PAD4 inhibitory activity while at the same time providing 7-fold selectivity over PAD3. Moreover, a high level of selectivity over PAD1 and PAD2 was maintained.

Based on the observation that placement of a chloro substituent at position 5 in inhibitor **12c** resulted in the greatest improvement in PAD4 potency and also improved selectivity, we hypothesized that the trichloro analog **24** might result in further enhancement (Scheme 4). The preparation of **24** was synthetically

Scheme 4. Synthesis of 4,5,6-Trichloroindole Inhibitor 24[a]



[a]Reagents and conditions: (a) $NaNO_2$, H_2O, HCl, 30 min; (b) 48% aq. HBF_4, 1.5 h; (c) lithium diisopropylamide (LDA), THF, −78 °C, 2 h; (d) $ZnCl_2$, THF, −78 to −10 °C, 4 h; (e) CuCl, THF; (f) 4-chlorobutanoyl chloride, overnight; (g) NaN_3, DMF, 50 °C, overnight; (h) hydrazine monohydrate, DME, reflux, overnight; (i) NaH, DMF, 0 °C, 30 min to 1 h; (j) cyclopentylmethyl bromide, DMF, 0 °C to rt, 1 to 2 h; (k) $SnCl_2$, CH_3OH, overnight; (l) ethyl-2-chloroacetamidate·HCl, Et_3N, CH_3OH, rt, 1 to 2 h.

more challenging and lengthier than the other chloro derivatives. Aniline **19** was first converted to aryl fluoride **20** by diazotization and treatment with HBF_4.[28] Ortho-directed metalation followed by addition of 4-chlorobutanenitrile then provided ketone **21**.[29] Azide displacement of the alkyl chloride followed by hydrazone condensation and S_NAr-mediated cyclization generated azido 4,5,6-trichloroindazole intermediate **22**. *N*-Alkylation with cyclopentylmethyl bromide provided **23**. Finally, reduction of the azide to the amine group followed by addition of ethyl-2-chloroacetimidate furnished inhibitor **24**. Indeed, the trichloroindazole inhibitor **24** further enhanced PAD4 potency and selectivity, making it the best inhibitor in this series (Table 2).

We next evaluated the ability of **24** to inhibit PAD4 activity in cells by monitoring its effect on the level of histone H4 citrullination in differentiated HL-60 cells. PAD4 is naturally overexpressed in mature HL-60 derived granulocytes and is also known to citrullinate H4 upon stimulation with calcium ionophores.[30] Western blots of cell lysates visualized with an anticitrulline histone H4 antibody showed that citrullination of H4 was significantly attenuated in cells treated with as little as 5 μM **24**. The reduced levels of citrullinated H4 induced by 10 μM or 5 μM **24** were also compared to those induced by Cl-amidine, the most extensively used pan-PAD inhibitor (Figure 3).

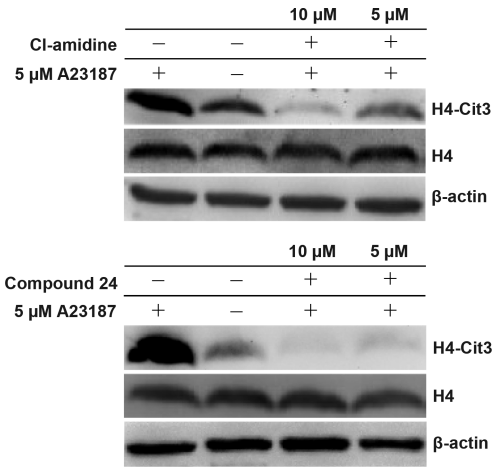


Figure 3. Evaluation of Cl-amidine and **24** in HL-60 granulocytes after stimulation with the calcium ionophore A23187.

Qualitative analysis of the Western blot indicated that the PAD4 selective inhibitor **24** is more potent than pan-PAD inhibitor Cl-amidine.

In summary, we synthesized and evaluated a series of *N*-alkylated indole chloroacetamidine inhibitors as potential PAD4 inactivators. Replacement of the indole heterocycle core with indazole and derivatization around the indazole ring with chloro substituents then led to the identification of trichloroindazole inhibitor **24** with high inhibitory activity and greater than 10-fold selectivity for PAD4 over the other three PAD isozymes. Inhibitor **24** was also evaluated for activity in cells and inhibited PAD4-mediated H4 citrullination at low micromolar concentrations. Exploration of other substituents about the indazole ring might be expected to result in further increases in inhibitor potency and PAD selectivity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00283.

Complete experimental procedures, characterization data for all compounds, IC_{50} and k_{inact}/K_I data for inhibitors, HL-60 cell culture and differentiation, and PAD4 inhibition assays and analysis (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jonathan.ellman@yale.edu.

ORCID

Alanna Schepartz: 0000-0003-2127-3932

Jonathan A. Ellman: 0000-0001-9320-5512

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

PAD, protein arginine deiminase; RA, rheumatoid arthritis; ACPA, anticitrullinated protein antibodies; NET, neutrophil extracellular trap; TNF, tumor necrosis factor alpha; IL-1β, interleukin 1 beta; H4, histone 4

■ REFERENCES

(1) Fuhrmann, J.; Thompson, P. R. Protein arginine methylation and citrullination in epigenetic regulation. *ACS Chem. Biol.* **2016**, *11*, 654−668.

(2) Fuhrmann, J.; Clancy, K. W.; Thompson, P. R. Chemical biology of protein arginine modifications in epigenetic regulation. *Chem. Rev.* **2015**, *115*, 5413−5461.

(3) Kearney, P. L.; Bhatia, M.; Jones, N. G.; Yuan, L.; Glascock, M. C.; Catchings, K. L.; Yamada, M.; Thompson, P. R. Kinetic characterization of protein arginine deiminase 4: a transcriptional corepressor Implicated in the onset and progression of rheumatoid arthritis. *Biochemistry* **2005**, *44*, 10570−10582.

(4) Jones, J. E.; Causey, C. P.; Knuckley, B.; Slack-Noyes, J. L.; Thompson, P. R. Protein arginine deiminase 4 (PAD4): current understanding and future therapeutic potential. *Curr. Opin. Drug Discov. Devel.* **2009**, *12*, 616−627.

(5) Bicker, K. L.; Thompson, P. R. The protein arginine deiminases: structure, function, inhibition, and disease. *Biopolymers* **2013**, *99*, 155−163.

(6) Taki, H.; Tomoharu, G.; Bryan, K.; Paul, R. T.; Oliver, V.; Kazuya, H.; Tatsurou, M.; Kouichiro, S.; Hiroyuki, H.; Eiji, S. Purification of enzymatically inactive peptidylarginine deiminase type 6 from mouse ovary that reveals hexameric structure different from other dimeric isoforms. *Adv. Biosci. Biotechnol.* **2011**, *2*, 304−310.

(7) Arita, K.; Hashimoto, H.; Shimizu, T.; Nakashima, K.; Yamada, M.; Sato, M. Structural basis for Ca2+-induced activation of human PAD4. *Nat. Struct. Mol. Biol.* **2004**, *11*, 777−783.

(8) Witalison, E. E.; Thompson, P. R.; Hofseth, L. J. Protein arginine deiminases and associated citrullination: physiological functions and diseases associated with dysregulation. *Curr. Drug Targets* **2015**, *16*, 700−710.

(9) Koushik, S.; Joshi, N.; Nagaraju, S.; Mahmood, S.; Mudeenahally, K.; Padmavathy, R.; Jegatheesan, S. K.; Mullangi, R.; Rajagopal, S. PAD4: pathophysiology, current therapeutics and future perspective in rheumatoid arthritis. *Expert Opin. Ther. Targets* **2017**, *21*, 433−447.

(10) Clark, S. R.; Ma, A. C.; Tavener, S. A.; McDonald, B.; Goodarzi, Z.; Kelly, M. M.; Patel, K. D.; Chakrabarti, S.; McAvoy, E.; Sinclair, G. D.; Keys, E. M.; Allen-Vercoe, E.; DeVinney, R.; Doig, C. J.; Green, F. H. Y.; Kubes, P. Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. *Nat. Med.* **2007**, *13*, 463−469.

(11) Villanueva, E.; Yalavarthi, S.; Berthier, C. C.; Hodgin, J. B.; Khandpur, R.; Lin, A. M.; Rubin, C. J.; Zhao, W.; Olsen, S. H.; Klinker, M.; Shealy, D.; Denny, M. F.; Plumas, J.; Chaperot, L.; Kretzler, M.; Bruce, A. T.; Kaplan, M. J. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. *J. Immunol.* **2011**, *187*, 538−552.

(12) Ohlsson, S. M.; Ohlsson, S.; Soderberg, D.; Gunnarsson, L.; Pettersson, A.; Segelmark, M.; Hellmark, T. Neutrophils from vasculitis patients exhibit an increased propensity for activation by anti-neutrophil cytoplasmic antibodies. *Clin. Exp. Immunol.* **2014**, *176*, 363−372.

(13) Martinod, K.; Demers, M.; Fuchs, T. A.; Wong, S. L.; Brill, A.; Gallant, M.; Hu, J.; Wang, Y. M.; Wagner, D. D. Neutrophil histone modification by peptidylarginine deiminase 4 is critical for deep vein thrombosis in mice. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 8674−8679.

(14) Sun, B.; Dwivedi, N.; Bechtel, T. J.; Paulsen, J. L.; Muth, A.; Bawadekar, M.; Li, G.; Thompson, P. R.; Shelef, M. A.; Schiffer, C. A.; Weerapana, E.; Ho, I. C. Citrullination of NF- κB p65 promotes its nuclear localization and TLR-induced expression of IL-1β and TNFα. *Sci. Immunol.* **2017**, *2*, eaal3062.

(15) Li, P.; Yao, H.; Zhang, Z.; Li, M.; Luo, Y.; Thompson, P. R.; Gilmour, D. S.; Wang, Y. M. Regulation of p53 target gene expression by peptidylarginine deiminase 4. *Mol. Cell. Biol.* **2008**, *28*, 4745−4758.

(16) Chang, X.; Han, J.; Pang, L.; Zhao, Y.; Yang, Y.; Shen, Z. Increased PADI4 expression in blood and tissues of patients with malignant tumors. *BMC Cancer* **2009**, *9*, 40.

(17) Lewis, H. D.; Liddle, J.; Coote, J. E.; Atkinson, S. J.; Barker, M. D.; Bax, B. D.; Bicker, K. L.; Bingham, R. P.; Campbell, M.; Chen, Y. H.; Chung, C. W.; Craggs, P. D.; Davis, R. P.; Eberhard, D.; Joberty, G.; Lind, K. E.; Locke, K.; Maller, C.; Martinod, K.; Patten, C.; Polyakova, O.; Rise, C. E.; Ruediger, M.; Sheppard, R. J.; Slade, D. J.; Thomas, P.; Thorpe, J.; Yao, G.; Drewes, G.; Wagner, D. D.; Thompson, P. R.; Prinjha, R. K.; Wilson, D. M. Inhibition of PAD4 activity is sufficient to disrupt mouse and human NET formation. *Nat. Chem. Biol.* **2015**, *11*, 189−191.

(18) Knuckley, B.; Jones, J. E.; Bachovchin, D. A.; Slack, J.; Causey, C. P.; Brown, S. J.; Rosen, H.; Cravatt, B. F.; Thompson, P. R. A fluopol-ABPP HTS assay to identify PAD inhibitors. *Chem. Commun.* **2010**, *46*, 7175−7177.

(19) Willis, V. C.; Gizinski, A. M.; Banda, N. K.; Causey, C. P.; Knuckley, B.; Cordova, K. N.; Luo, Y.; Levitt, B.; Glogowska, M.; Chandra, P.; Kulik, L.; Robinson, W. H.; Arend, W. P.; Thompson, P. R.; Holers, V. M. N-α-benzoyl-N5-(2-chloro-1-iminoethyl)-L-ornithine amide, a protein arginine deiminase inhibitor, reduces the severity of murine collagen-induced arthritis. *J. Immunol.* **2011**, *186*, 4396−4404.

(20) Subramanian, V.; Knight, J. S.; Parelkar, S.; Anguish, L.; Coonrod, S. A.; Kaplan, M. J.; Thompson, P. R. Design, synthesis, and biological evaluation of tetrazole analogs of CI-Amidine as protein arginine deiminase inhibitors. *J. Med. Chem.* **2015**, *58*, 1337−1344.

(21) Muth, A.; Subramanian, V.; Beaumont, E.; Nagar, M.; Kerry, P.; McEwan, P.; Srinath, H.; Clancy, K.; Parelkar, S.; Thompson, P. R. Development of a selective inhibitor of protein arginine deiminase 2. *J. Med. Chem.* **2017**, *60*, 3198−3211.

(22) Jamali, H.; Khan, H. A.; Tjin, C. C.; Ellman, J. A. Cellular activity of new small molecule protein arginine deiminase 3 (PAD3) inhibitors. *ACS Med. Chem. Lett.* **2016**, *7*, 847−851.

(23) Jamali, H.; Khan, H. A.; Stringer, J. R.; Chowdhury, S.; Ellman, J. A. Identification of multiple structurally distinct, nonpeptidic small molecule inhibitors of protein arginine deiminase 3 using a substrate-based fragment method. *J. Am. Chem. Soc.* **2015**, *137*, 3616−3621.

(24) Bendazac, niraparib, granisetron, pazopanib, axitinib, lonidamine, and lificiguat are approved drugs and drug candidates containing the indazole heterocycle. Detailed information on these drugs including compound structure, bioactivity, published studies, clinical trials, applications, and usage can be obtained at PubChem (http://pubchem.ncbi.nlm.nih.gov/).

(25) Mewshaw, R. E.; Zhou, D.; Zhou, P.; Shi, X. J.; Hornby, G.; Spangler, T.; Scerni, R.; Smith, D.; Schechter, L. E.; Andree, T. H. Studies toward the discovery of the next generation of antidepressants. 3. Dual 5-HT_{1A} and serotonin transporter affinity within a Class of *N*-aryloxyethylindolylalkylamines. *J. Med. Chem.* **2004**, *47*, 3823−3842.

(26) Cinelli, M. A.; Morrell, A.; Dexheimer, T. S.; Scher, E. S.; Pommier, Y.; Cushman, M. Design, synthesis, and biological evaluation of 14-substituted aromathecins as topoisomerase I inhibitors. *J. Med. Chem.* **2008**, *51*, 4609−4619.

(27) Bennetau, B.; Rajarison, F.; Dunoguès, J.; Babin, P. Fonctionnalisation régiosélective en position 2 de benzènes 1,3-disubstitués. *Tetrahedron* **1993**, *49*, 10843−10854.

(28) Mahiou, B.; Deinzer, M. L. Synthetic strategies in the preparation of regiospecifically chlorine-37 labeled polychlorinated dibenzo-*p*-diozins. *J. Labelled Compd. Radiopharm.* **1991**, *31*, 261−287.

DOI: 10.1021/acsmedchemlett.8b00283
ACS Med. Chem. Lett. 2018, 9, 1013−1018

(29) Rosen, J.; Steinhuebel, D.; Palucki, M.; Davies, I. One-step synthesis of α-chloro acetophenones from acid chlorides and aryl precursors. *Org. Lett.* **2007**, *9*, 667−9.
(30) Wang, Y.; Wysocka, J.; Sayegh, J.; Lee, Y.; Perlin, J. R.; Leonelli, L.; Sonbuchner, L. S.; McDonald, C. H.; Cook, R. G.; Dou, Y.; Roeder, R. G.; Clarke, S.; Stallcup, M. R.; Allis, C. D.; Coonrod, S. A. Human PAD4 regulates histone arginine methylation levels via demethylimination. *Science* **2004**, *306*, 279−283.

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