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Synthesis and Evaluation of Thiazoloquinolinones with Linkers To Enable Targeting of CD38

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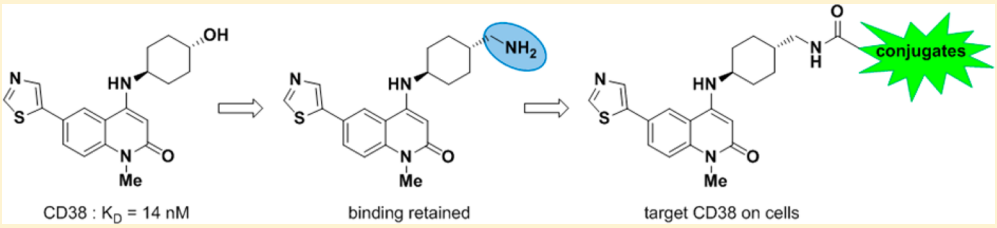
Supporting Information



ABSTRACT: Several monoclonal antibodies and inhibitors targeting CD38, an ectoenzyme overexpressed on malignant plasma cells, have previously been discovered. Herein, we expand structure−activity relationships of reported small-molecule thiazoloquinolinones and show that several 4-cyclohexylamino analogues have potent binding affinity for CD38 using surface plasmon resonance. Moreover, active amine analogues could be acylated and functionalized with alkyne and fluorescein groups. Fluorescein analogue **21** bound selectively to CD38 overexpressing cells, demonstrating the potential utility of thiazoloquinolinones as small-molecule conjugates for the delivery of therapeutic and imaging agents.

KEYWORDS: *targeting, small-molecule conjugates, CD38, oncology, quinolinones*

CD38 is a type II transmembrane glycoprotein, having both receptor and enzymatic functions.[1] As an ectoenzyme, CD38 catalyzes the net hydrolysis of nicotinamide adenine dinucleotide (NAD) to adenosine diphosphate ribose (ADP ribose). NAD is an important cofactor for several enzymes involved in normal cellular processes, and increased levels are associated with protective effects against various metabolic disorders such as obesity[2] and type-2 diabetes.[3] Additionally, CD38 catalyzes the cyclization of NAD to cyclic ADP ribose (cADPR), a potent second messenger that initiates intracellular Ca2+ signaling. CD38 therefore represents a promising pharmacological target for regulating NAD metabolism. Accordingly, several inhibitors of CD38 have been reported including the hydantoin,[4] thiazoloquinolinone,[5] and quinoline carboxamide[6] classes (e.g., **1**, **2**, and **3**, respectively, Figure 1) of small molecules that exhibit nanomolar inhibition.

More recently, CD38 gained significant attention for being targeted by therapies for hematological malignancies.[7] CD38 being found highly expressed on malignant plasma cells in patients with multiple myeloma[8] has led to the development of several CD38 monoclonal antibodies such as daratumumab.[9] Although found to be an allosteric enzyme inhibitor, daratumumab binds a nonactive site epitope of CD38 and likely induces tumor cell death through several mechanisms including antibody-dependent cell-mediated toxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). In phase II clinical trials, daratumumab demonstrated a 36% overall response rate in multiple myeloma patients who had received a median of four lines of prior therapy.[10] Sixty-five percent of responded patients remained disease free after 12 months. As a result of this promising data and its favorable safety profile, daratumumab (trademarked Darzelex) received accelerated approval as part of the FDA's Breakthrough Therapy Designation program in 2015.

Having been validated by monoclonal antibody therapy, CD38 may be a suitable target for drug delivery and imaging. Our interests in targeted nanomedicines have led us to evaluate molecules that bind CD38 as potential targeting ligands to enhance the delivery of polymeric nanoparticles termed ACCURINS[11,12] containing drug payloads to diseased tissues. Antibodies have recently been employed for the delivery of cytotoxic agents to tumors resulting in several FDA-approved antibody−drug conjugates (ADCs). Alternatively, small-molecules as targeting agents[13] may have advantages over antibodies,

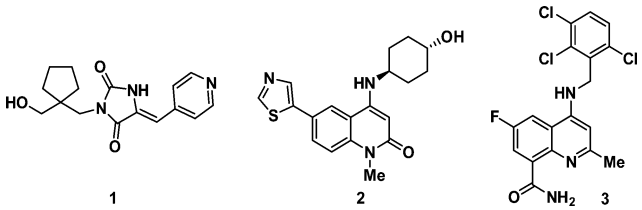


Figure 1. Representative reported CD38 inhibitors.

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which include lower production costs, and, in our interests, greater compatibility in nanoemulsion processes of polymers covalently modified with the ligands. In this regard, we have evaluated **1**−**3** as small-molecule targeting ligands for CD38. We were particularly interested in the thiazoloquinolinone compounds (e.g., **2**), given they were previously shown to be active in an in vivo setting to increase NAD levels by presumably binding and inhibiting CD38.[5] Herein, we describe our initial efforts in identifying active thiazoloquinolinone analogues with sites that can be readily modified with linkers for the potential delivery of therapeutic and imaging agents to CD38-overexpressing cancer cells.

In the original report, Haffner and co-workers suggested that thiazoloquinolinones interact with NAD reaction intermediates generated by CD38 catalysis (Figure 2).[5] The first enzymatic

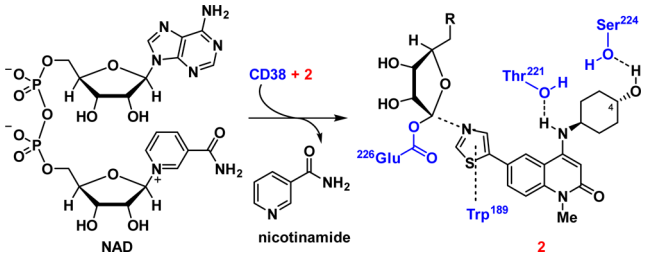
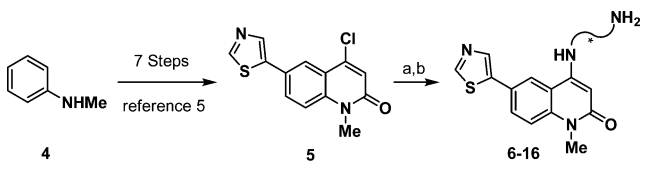


Figure 2. Proposed ternary binding complex of NAD, **2**, and CD38.

step by CD38 involves an S_N2 displacement of nicotinamide by Glu226. At this stage, it is hypothesized that the nitrogen-atom of the thiazolo moiety may form a transient, reversible adduct with the NAD fragment by displacing Glu226.[14] With this ternary complex in mind, Haffner et al. computationally docked thiazoloquinolinones with an X-ray crystal structure of CD38 covalently bound to the ribose intermediate of nicotinamide mononucleotide (NMN).[5] These molecular modeling experiments show that the thiazolo group has a pi-pi interaction with Trp189, mimicking the nicotinamide from the natural substrates. The close proximity of the aniline nitrogen and substituents on the 4-position of the cyclohexyl group to Thr221 and Ser224, respectively, suggests that favorable hydrogen bonding interactions may be present. Furthermore, their model shows that substituents at this 4-position may be solvent-exposed, which may account for their observations that several corresponding cyclohexyl analogues had similar biological activity. With this knowledge, we rationalized that the 4-position may be a suitable site for conjugation and therefore evaluated additional cyclohexyl derivatives.

To access 4-cyclohexyl analogues, we synthesized thiazoloquinolinones **6**−**16** containing primary amines in place of the hydroxyl group to serve as points of attachment for linkers and payloads (Scheme 1). The synthesis was initiated by

Scheme 1. Synthesis of Primary Amine Analogs 6−16[a]



[a]Reagents and conditions: (a) Pd_2dba_3, BrettPhos, NaO*t*-Bu, 1,4-dioxane, microwave, 125 °C; (b) TFA, CH_2Cl_2, rt.

elaboration of *N*-methylaniline **4** to chloride **5** in seven steps.[5] Aryl C−N bond coupling of **5** with various commercially available mono-Boc-protected diamines under Buchwald−Hartwig conditions (Pd_2dba_3, BrettPhos, NaO*t*-Bu) and subsequent Boc cleavage with trifluoroacetic acid (TFA) provided **6**−**16**. In addition, **2** was prepared as previously described[5] to provide a positive control for biological experiments.

Surface plasmon resonance (SPR) was utilized to evaluate binding of thiazoloquinolinone **2** and amine analogues **6**−**16** to recombinant human CD38. Benefits of this biophysical method include mimicking the cell surface environment and receptor density. Compound **2** initially gave very weak responses by SPR (Figure 3A). However, coadministration with NAD (5 μM)

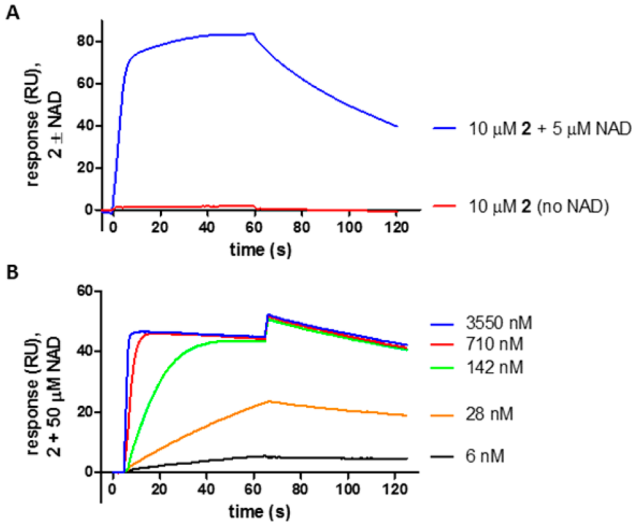


Figure 3. Binding of **2** to CD38 demonstrated by SPR in the (A) absence or presence of NAD (5 μM) and (B) dose-titration in the presence of 50 μM NAD.

increased binding responses dramatically in which the dissociation constant for **2** was determined to be 280 nM. These observations support the proposed ternary binding model depicted in Figure 2. The assay was further optimized using 50 μM NAD, a concentration slightly higher than reported physiological levels (24−29 μM)[15] and similar to the Michaelis constant (K_M = 48 μM) for hydrolysis of NAD by CD38.[16] Under these conditions, **2** demonstrated dose-dependent binding to CD38 with high affinity at 14 nM, matching the reported IC_{50} value using an enzymatic assay (Figure 3B).[5] These results exemplified SPR as a viable method to examine binding of additional thiazoloquinolinone analogues.

Structure−activity relationships (SAR) of amines **6**−**16** binding CD38 were determined using SPR in the presence of 50 μM NAD as described for **2** (Table 1). *trans*-Cyclohexyl-4-amine **6**, directly substituting the alcohol with an amino group, showed moderate affinity (200 nM), albeit 14-fold lower than **2**. In efforts to discover analogues with increased affinities, we explored different orientations of the amine on the cyclohexyl ring. Demonstrating stereochemical SAR, *cis*-cyclohexyl-4-amine **7** did not bind CD38. In addition, no activity was observed with amine substitution at the 2- and 3-positions (**8**/**9** and **10**, respectively). These results suggest that proper orientation of the amine at the 4-position may be required to

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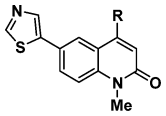
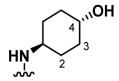
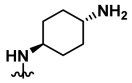
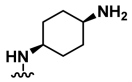
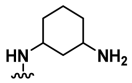
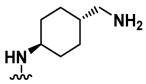
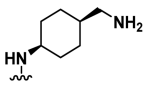
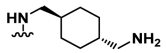
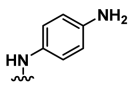
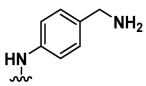
Table 1. SAR of Cyclohexyl and Phenyl Analogues



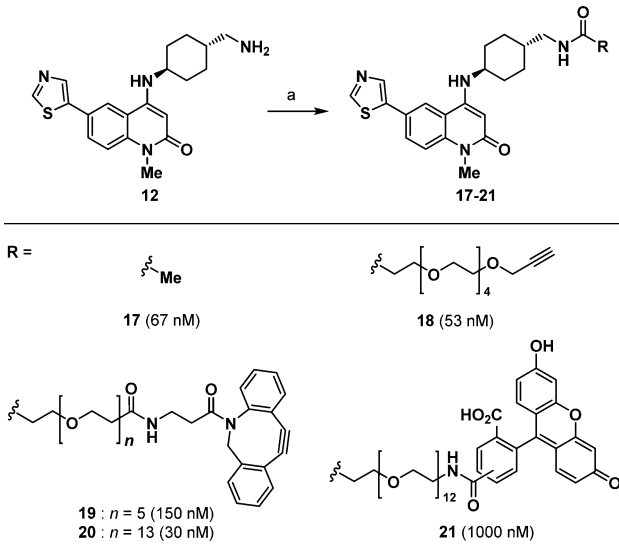
Cmpd	R	K_D (nM)[a]
2		14
6		200
7		>10,000
8		>10,000
9		>10,000
10		>10,000
11		>10,000
12		93
13		>10,000
14		390
15		85
16		46

[a]K_D values for binding to CD38 were measured using SPR in the presence of 50 μM NAD.

maintain a hydrogen bonding interaction to Ser224 similarly to **2**. Further supporting this hypothesis, placing the amine into the cyclohexyl ring (**11**) led to no binding, in which the *N*-hydrogen may not reach Ser224, whereas placing a methylene spacer ($-CH_2-$) between the amine and cyclohexyl group (**12**) may bring the *N*-hydrogen closer to Ser224 and thereby enhance hydrogen bonding. Indeed, **12** bound CD38 strongly (93 nM) with more than 2-fold increase in affinity from **6**. Placing an additional methylene spacer between the aniline nitrogen and 1-position of the cyclohexyl group led to **14** that did bind CD38 (390 nM) but not as strongly as **6** and **12**. Aryl variants **15** and **16** showed an approximate 2-fold increase in activity (85 and 46 nM, respectively) from the corresponding cyclohexyl parent compounds. This increase in affinity may be attributed to a pi−pi interaction with the adenine moiety of NAD remnant in the ternary binding complex.[6]

With amine analogues with favorable potency in hand, the synthesis of acylated conjugates was pursued to access potential CD38 targeting ligands (Scheme 2). Although aryl analogues

Scheme 2. Synthesis and Evaluation of Binding Affinities of Acylated Analogues of 12[a,b]



[a]Reagents and conditions; (a) Ac_2O, NEt_3, and DMF for **17**; NHS-ester, NEt_3, and DMF for **18**−**21**. [b]K_D values for binding to CD38 are shown in parentheses and were measured using SPR in the presence of 50 μM NAD.

15 and **16** had superior binding affinity, we focused on the derivatization of **12** in which the sp^3-hybridized cyclohexyl group may impart favorable physicochemical properties (e.g., solubility).[17] Treatment of **12** with acetic anhydride or NHS-esters provided acylated analogues **17** and **18**−**21**, respectively. Acetate **17** had slightly improved binding (67 nM), indicating that acylation may not disrupt the putative hydrogen bonding interaction with Ser224. Acylated derivatives **18**−**21** containing a PEG chain maintained activity in which the PEG_{13}-dibenzocyclooctyne (DBCO) analogue **20** bound CD38 with an affinity of 30 nM. Analogues **18**−**20** may be particularly important for targeting CD38 as they have an alkyne moiety that can undergo a Huisgen [3 + 2] cycloaddition with azide-functionalized molecules such as nanoparticles, therapeutic agents, and fluorescent probes. Finally, fluorescein analogue **21** provides a direct example of a thiazoloquinolinone conjugate for biological evaluation.

To assess the ability of thiazoloquinolinone conjugates to target CD38 in a biological setting, fluorescein analogue **21** was evaluated by flow cytometry for binding to rat C6 cell lines stably transfected to overexpress human CD38 (Figure 4). As anticipated, **21** bound to cells only in the presence of NAD in which the mean fluorescence intensity increased with higher concentrations of **21**. Fluorescein-PEG_{12}-CO_2H (see Support-

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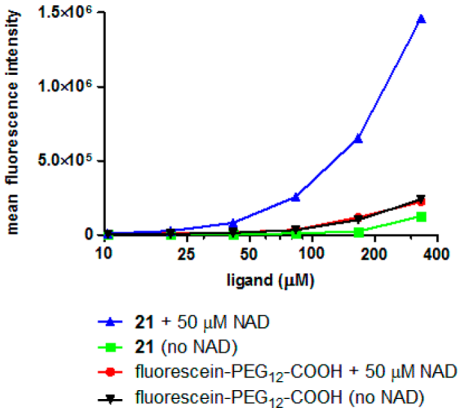

Figure 4. Binding of **21** to CD38-overexpressing C6 cells demonstrated by flow cytometry in the presence of 50 μM NAD.

ing Information for chemical structure), a negative control, bound cells weakly. These results demonstrate that the targeting specificity of **21** is likely due to the thiazoloquinolinone core scaffold inhibiting the enzyme active site of CD38.

In summary, we have developed a series of thiazoloquinolinone amine analogues that bind CD38. SAR studies demonstrated a preference for the amino group to be appended to the 4-position of cyclohexyl or phenyl moieties of thiazoloquinolinones, which may allow a favorable orientation for a hydrogen bonding interaction with Ser224 of CD38. Amine analogue **12** could be acylated to retain activity and provide linkers with alkynes for potential conjugation to functional molecules. In a pivotal experiment, fluorescein analogue **21** was shown to bind cells overexpressing CD38, warranting further exploration of thiazoloquinolinones as small-molecule conjugates targeting CD38. Furthermore, our findings that the presence of NAD is required to facilitate binding suggests that thiazoloquinolinone conjugates (e.g., **21**) may be useful as mechanism-based probes for labeling CD38 on cells.[18,19]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.6b00409.

Synthetic procedures and characterization of compounds, characterization of C6 cells, and experimental protocols for surface plasmon resonance and flow cytometry experiments (PDF)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ADC, antibody−drug conjugates; ADP ribose, adenosine diphosphate ribose; BrettPhos, 2-(dicyclohexylphosphino)3,6-dimethoxy-2′,4′,6′-triisopropyl-1,1′-biphenyl; cADPR, cyclic adenosine diphosphate ribose; CD38, cluster of differentiation 38; dba, dibenzylideneacetone; DBCO, dibenzocyclooctyne; NAD, nicotinamide adenine diphosphate; NMN, nicotinamide mononucleotide; SPR, surface plasmon resonance; TFA, trifluoroacetic acid

■ REFERENCES

(1) Malavasi, F.; Deaglio, S.; Funaro, A.; Ferrero, E.; Horenstein, A. L.; Ortolan, E.; Vaisitti, T.; Aydin, S. Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology. *Physiol. Rev.* **2008**, *88*, 841−886.

(2) Barbosa, M. T.; Soares, S. M.; Novak, C. M.; Sinclair, D.; Levine, J. A.; Aksoy, P.; Chini, E. N. The enzyme CD38 (a NAD glycohydrolase, EC 3.2.2.5) is necessary for the development of diet-induced obesity. *FASEB J.* **2007**, *21*, 3629−3639.

(3) Yoshino, J.; Mills, K. F.; Yoon, M. J.; Imai, S.-I. Nicotinamide mononucleotide, a key NAD^+ intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab.* **2011**, *14*, 528−536.

(4) Kapui, Z.; Vasas, A.; Buzder-Lantos, P.; Bátori, S.; Szabó, T.; Ferenczy, G.; Balogh, M.; Urbán- Szabó, K. Hydantoine derivatives as CD38 inhibitors. WO 2014/180544 A1, November 13, 2014.

(5) Haffner, C. D.; Becherer, J. D.; Boros, E. E.; Cadilla, R.; Carpenter, T.; Cowan, D.; Deaton, D. N.; Guo, Y.; Harrington, W.; Henke, B. R.; Jeune, M. R.; Kaldor, I.; Milliken, N.; Petrov, K. G.; Preugschat, F.; Schulte, C.; Shearer, B. G.; Shearer, T.; Smalley, T. L., Jr.; Stewart, E. L.; Stuart, J. D.; Ulrich, J. C. Discovery, synthesis, and biological evaluation of thiazoloquin(az)olin(on)es as potent CD38 inhibitors. *J. Med. Chem.* **2015**, *58*, 3548−3571.

(6) Becherer, J. D.; Boros, E. E.; Carpenter, T. Y.; Cowan, D. J.; Deaton, D. N.; Haffner, C. D.; Jeune, M. R.; Kaldor, I. W.; Poole, J. C.; Preugschat, F.; Rheault, T. R.; Schulte, C. A.; Shearer, B. G.; Shearer, T. W.; Shewchuk, L. M.; Smalley, T. L., Jr.; Stewart, E. L.; Stuart, J. D.; Ulrich, J. C. Discovery of 4-amino-8-quinoline carboxamides as novel, submicromolar inhibitors of NAD-hydrolyzing enzyme CD38. *J. Med. Chem.* **2015**, *58*, 7021−7056.

(7) van de Donk, N. W. C. J.; Janmaat, M. L.; Mutis, T.; Lammerts van Bueren, J. J.; Ahmadi, T.; Sasser, A. K.; Lokhorst, H. M.; Parren, P. W. H. I. Monoclonal antibodies targeting CD38 in hematological malignancies and beyond. *Immunol. Rev.* **2016**, *270*, 95−112.

(8) Lin, P.; Owens, R.; Tricot, G.; Wilson, C. S. Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. *Am. J. Clin. Pathol.* **2004**, *121*, 482−488.

(9) de Weers, M.; Tai, Y.-T.; van der Veer, M. S.; Bakker, J. M.; Vink, T.; Jacobs, D. C. H.; Oomen, L. A.; Peipp, M.; Valerius, T.; Slootstra, J. W.; Mutis, T.; Bleeker, W. K.; Anderson, K. C.; Lokhorst, H. M.; van de Winkel, J. G. J.; Parren, P. W. H. I. Daratumumab, a novel

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ACS Med. Chem. Lett. 2017, 8, 196−200

therapeutic human CD38 monoclonal antibody, induces killing of multiple myeloma and other hematological tumors. *J. Immunol.* **2011**, *186*, 1840−1848.
(10) Lokhorst, H. M.; Plesner, T.; Laubach, J. P.; Nahi, H.; Gimsing, P.; Hansson, M.; Minnema, M. C.; Lassen, U.; Krejcik, J.; Palumbo, A.; van de Donk, N. W. C. J.; Ahmadi, T.; Khan, I.; Uhlar, C. M.; Wang, J.; Sasser, A. K.; Losic, N.; Lisby, S.; Basse, L.; Brun, N.; Richardson, P. G. Targeting CD38 with daratumumab monotherapy in multiple myeloma. *N. Engl. J. Med.* **2015**, *373*, 1207−1219.
(11) Hrkach, J.; Hoff, D. V.; Ali, M. M.; Andrianova, E.; Auer, J.; Campbell, T.; De Witt, D.; Figa, M.; Figueiredo, M.; Horhota, A.; Low, S.; McDonnell, K.; Peeke, E.; Retnarajan, B.; Sabnis, A.; Schnipper, E.; Song, J. J.; Song, Y. H.; Summa, J.; Tompsett, D.; Troiano, G.; Hoven, T. V. G.; Wright, J.; LoRusso, P.; Kantoff, P. W.; Bander, N. H.; Sweeney, C.; Farokhzad, O. C.; Langer, R.; Zale, S. Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile. *Sci. Transl. Med.* **2012**, *4*, 128ra39.
(12) Ashton, S.; Song, Y. H.; Nolan, J.; Cadogan, E.; Murray, J.; Odedra, R.; Foster, J.; Hall, P. A.; Low, S.; Taylor, P.; Ellston, R.; Polanska, U. M.; Wilson, J.; Howes, C.; Smith, A.; Goodwin, R. J. A.; Swales, J. G.; Strittmatter, N.; Takáts, Z.; Nilsson, A.; Andren, P.; Trueman, D.; Walker, M.; Reimer, C. L.; Troiano, G.; Parsons, D.; De Witt, D.; Ashford, M.; Hrkach, J.; Zale, S.; Jewsbury, P. J.; Barry, S. T. Aurora kinase inhibitor nanoparticles target tumors with favorable therapeutic index in vivo. *Sci. Transl. Med.* **2016**, *8*, 325ra17.
(13) Srinivasarao, M.; Galliford, C. V.; Low, P. S. Principles in the design of ligand-targeted cancer therapeutics and imaging agents. *Nat. Rev. Drug Discovery* **2015**, *14*, 203−219.
(14) Preugschat, F.; Carter, L. H.; Boros, E. E.; Porter, D. J. T.; Stewart, E. L.; Shewchuck, L. M. A pre-steady state and steady state kinetic analysis of the *N*-ribosyl hydrolase activity of hCD157. *Arch. Biochem. Biophys.* **2014**, *564*, 156−163.
(15) Creeke, P. I.; Dibari, F.; Cheung, E.; van den Briel, T.; Kyroussis, E.; Seal, A. J. Whole blood NAD and NADP concentrations are not depressed in subjects with clinical pellagra. *J. Nutr.* **2007**, 2013−2017.
(16) Graeff, R.; Munshi, C.; Aarhus, R.; Johns, M.; Lee, H. C. A single residue at the active site of CD38 determines its NAD cyclizing and hydrolyzing activities. *J. Biol. Chem.* **2001**, *276*, 12169−12173.
(17) Lovering, F.; Bikker, J.; Humblet, C. Escape from flatland: Increasing saturation as an approach to improving clinical success. *J. Med. Chem.* **2009**, *52*, 6752−6756.
(18) Jiang, H.; Congleton, J.; Liu, Q.; Merchant, P.; Malavasi, F.; Lee, H. C.; Hao, Q.; Yen, A.; Lin, H. Mechanism-based small molecule probes for labeling CD38 on live cells. *J. Am. Chem. Soc.* **2009**, *131*, 1658−1659.
(19) Shrimp, J. H.; Hu, J.; Dong, M.; Wang, B. S.; MacDonald, R.; Jiang, H.; Hao, Q.; Yen, A.; Lin, H. Revealing CD38 cellular localization using a cell permeable, mechanism-based fluorescent small-molecule probe. *J. Am. Chem. Soc.* **2014**, *136*, 5656−5663.

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