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2,4-Diamino-8-quinazoline carboxamides as novel, potent inhibitors of the NAD hydrolyzing enzyme CD38: Exploration of the 2-position structure-activity relationships

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ABSTRACT

Starting from 4-amino-8-quinoline carboxamide lead **1a** and scaffold hopping to the chemically more tractable quinazoline, a systematic exploration of the 2-substituents of the quinazoline ring, utilizing structure activity relationships and conformational constraint, resulted in the identification of 39 novel CD38 inhibitors. Eight of these analogs were 10–100-fold more potent human CD38 inhibitors, including the single digit nanomolar inhibitor **1am**. Several of these molecules also exhibited improved therapeutic indices relative to hERG activity. A representative analog **1r** exhibited suitable pharmacokinetic parameters for *in vivo* animal studies, including moderate clearance and good oral bioavailability. These inhibitor compounds will aid in the exploration of the enzymatic functions of CD38, as well as furthering the study of the therapeutic implications of NAD enhancement in metabolic disease models.

1. Introduction

Cluster of Differentiation 38 (CD38, EC 3.2.2.5) is a type II membrane glycoprotein, localized on chromosome 4 and expressed in immune cells, such as T cells, B cells, and dendritic cells.[1,2] CD38 has a single N-terminal transmembrane domain with multiple asparagine-linked glycosylation sites at its extracellular C-terminal end. The immunoglobulin Cluster of Differentiation 31 (CD31, PECAM-1) binds to CD38 and this interaction modulates leukocyte migration.

CD38 is overexpressed on the surface of many hematological tumor cells and significant efforts have been made to exploit the therapeutic implications of this finding for diagnosis and treatment. In 2015, the FDA approved Genmab's CD38 monoclonal antibody daratumumab (Darzalex™) for the treatment of multiple myeloma (plasma cell myeloma).[3] This therapeutic validation has intensified the interest in CD38.

Besides leukocytes, CD38 is also expressed in other tissues, including liver, intestine, kidney, pancreas, prostate, muscle, bone, and brain.[4] CD38 also has enzymatic activity in addition to its receptor functions. One of its substrates is the redox cofactor nicotinamide adenine dinucleotide (NAD), which CD38 converts to cyclic ADP-ribose (cADPR) as well as ADP-ribose.[5,6] cADPR is a second messenger that regulates cytosolic calcium fluxes. Perhaps more importantly, CD38 reduces the concentration of NAD by functioning as a NAD glycohydrolase. By decreasing the availability of this critical enzyme cofactor, CD38 modulates the activity of many NAD-dependent proteins, including oxidoreductases, sirtuins, and the poly(ADP-ribose) polymerases (PARPs).

Inhibiting degradation of NAD via inhibition of CD38 is one approach to potentially increase NAD. Activation of sirtuin1 increases insulin sensitivity, implying that elevation of NAD could have positive effects on metabolic diseases.[7] CD38 (−/−) mice have elevated NAD levels compared to wild-type littermates.[8] Furthermore, the knockout mice, when fed a high fat diet, are resistant to weight gain. They also have increased energy expenditure. Moreover, small molecule CD38 inhibitors elevate NAD levels in diet-induced obese mice.[9,10]

This murine NAD paradigm is likely operational in humans as well, because the obese have decreased NAD levels, yet exercise and caloric restriction, which both protect against metabolic diseases, elevate NAD levels. NAD concentrations are also decreased in the elderly, while type II diabetes rates increase with age, and this inverse relationship might be correlated.[11,12] Thus, treatment

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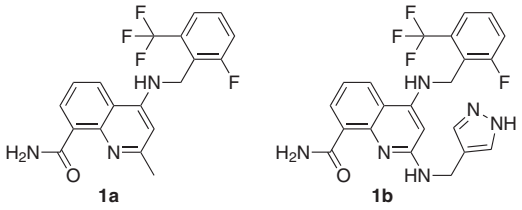
of metabolic diseases like obesity, where NAD levels are abnormally low, with a CD38 inhibitor could have therapeutic utility, by increasing this important cofactor of metabolic proteins.

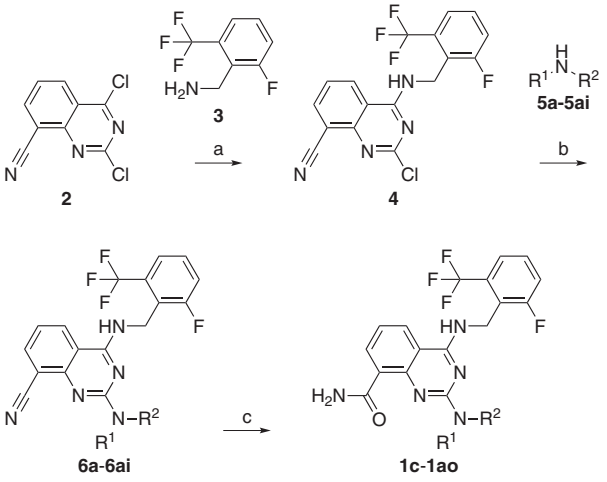
Because of the potential therapeutic benefits of elevating NAD, the discovery of CD38 inhibitors has been pursued by various researchers.[13–18] This laboratory has recently disclosed novel CD38 inhibitors, including the quinoline-8-carboxamides exemplified by **1a** (IC_{50} = 510 nM). Compound **1a** had acceptable pharmacokinetics to be utilized as a tool compound and raised NAD levels in a diet-induced obese mouse model; however, this compound had modest potency and drug development liabilities such as hERG inhibition. CD38 protein X-ray co-crystal structures of the quinoline-8-carboxamide series (e.g., PDB codes 4XJT and 4XJS) revealed additional space in the binding pocket, near the 2-position of the quinoline, that could be occupied for potential increases in potency or alteration of drug properties. Preliminary chemistry efforts at this position produced the pyrazole **1b** (IC_{50} = 72 nM) with increased CD38 inhibitory activity relative to its congener **1a**. To increase the ease of synthesis and allow for rapid structure-activity relationship (SAR) development at this position, the synthesis of quinazolines as a bioisosteric core replacement for the quinoline were undertaken. This article describes GlaxoSmithKline's detailed efforts to explore the SAR at the 2-position of that quinazoline series.



2. Chemistry

The quinazoline CD38 inhibitors **1c–1ao** were prepared as depicted in Scheme 1. Room temperature, base-promoted, regioselective, *ipso* displacement of the 4-chloro group of 2,4-dichloroquinazoline **2** by 2-fluoro-6-trifluoromethylbenzylamine **3** provided the 4-aminoquinazoline **4**. Subsequent thermal, base-promoted, *ipso* displacement of the 2-chloro moiety of the quinazoline **4** with amines **5a–5ai** afforded the quinazoline nitriles **6a–6ai**. Hydrolysis of the cyanides **6a–6ai** with basic hydrogen peroxide gave the desired quinazoline carboxamides **1c–1ao**.

Many of the amines **5a–5k** and **5m–5n** were commercially available, while some amines **5l**, **5s**, **5z**, and **5aa** were known in the literature. The rest of the amines **5o–5r**, **5t–5y**, and **5ab–5ai** were synthesized as depicted in Schemes 2 and 3. Ketones **7a–7f** were heated at reflux with *N,N*-dimethylformamide dimethyl acetal to produce β-amino-enones **8a–8f**. Subsequent Michael addition with hydrazines, followed by cyclization, and dehydration afforded the protected pyrazoles **9a–9g**. Then, removal of the various protecting groups gave the free amines **5o–5r** and **5t–5v**. Some ketones **7a** and **7c** were commercially available, but other ketones **7b** and **7d–7f** were prepared. For example, protection of the aminoesters **10a–10b** gave carbamates **11a–11b**. Subsequent Michael addition of these species **11a–11b** or commercially available compounds **10a–10f**, **11c**, and **11d** to unsaturated esters **12a–12b**, ketone **12c**, or nitrile **12d**, then *in situ* Dieckmann or Thorpe-Ziegler cyclization afforded β-ketoesters **13a**, **13d–13g**, β-diketones **13h–13i**, and β-ketonitriles **13j–13n**.[19,20] The β-ketoesters **13a-13e** could be decarboxylated under Krapcho conditions



Scheme 1. Synthesis of 2-substituted quinazoline carboxamides **1c–1ao**. Reagents and conditions: a) iPr_2NEt, **3**, THF, RT, 81%; b) iPr_2NEt, R^1R^2NH **5a–5ai**, *n*-BuOH or 1,4-dioxane or DMSO, 100 °C, 11–91%; c) NaOH or KOH, 30% H_2O_2, DMSO, 5–81%.

to provide the ketones **7b** and **7d–7f**.[21] Alternatively, the β-ketoesters **13f–13g**, β-ketoesters **13h–13i**, and β-ketonitriles **13j–13n** could also be heated with hydrazine to give the protected pyrazolone **9j**, 3-alkylpyrazoles **9l–9m**, or 3-aminopyrazoles **9n–9r**, then deprotected to give amines **5y** and **5ac–5ai**. In another method, the ketones **7d–7e** could be derived from the β-amino esters **14a–14b**. First, monoalkylation of β-amino esters **14a** or **14b** with ethyl 2-bromoacetate, followed by protection of the free secondary amines provided the carbamates **15a–15b**. Then, Dieckmann condensation provided the β-ketoesters **13b–13c**. β-Diketones **16a–16b** could be synthesized by Claisen condensation of ketone **7c** with esters or anhydrides. These diketones **16a–16b** could be converted to pyrazoles **5w–5x** as above via hydrazine addition to give pyrazoles **9h–9i**, followed by deprotection.

As shown in Scheme 3, the pyrazole aminoalcohol **5ab** was synthesized from the ketoalcohol **17a**. First, base-promoted protection of the secondary alcohol **17a** with *tert*-butyldiphenylsilyl chloride afforded the silylether **18a**. Then, Claisen condensation of the enolate of ketone **18a** with diethyl oxalate **19a** provided the diketoester **20a**. Aldol condensation of the β-diketoester **20a** with Eschenmoser's salt **21a**, followed by elimination of dimethylamine provided a methylene diketone, which was subjected *in situ* to conjugate addition by 4-methoxybenzylamine **22a** with subsequent cyclization to give the pyrrolidinone **23a**. Then, condensation of the ketone **23a** with hydrazine gave the pyrazole **24a**. Fluoride-mediated cleavage of the silyl ether protecting group of **24a** provided the alcohol **25a**. Subsequent reduction of the amide **25a** afforded the pyrrolidine **9k**. Finally, palladium hydroxide on carbon catalyzed hydrogenolysis of the benzyl group of pyrazole **9k** afforded the amine **5ab**.

3. Results and discussion

The SAR among the quinazoline analogs are depicted in Table 1. Although the quinazolines were less difficult to prepare than the corresponding quinolines, the 2-(4-aminomethylpyrazole)quinazoline carboxamide **1c** (IC_{50} = 450 nM) was ~6-fold less potent than its corresponding quinoline **1b** (IC_{50} = 72 nM), possibly due to a higher penalty to desolvate the additional nitrogen and/or alteration of the π-stacking energy of the quinazoline ring with the indole ring of ^{189}Trp, relative to the quinoline ring. Like the previously prepared quinolines, these quinazolines reversibly bind at the active site and exhibit mixed inhibition of CD38. Searching