Discovery of VU6015929: A Selective Discoidin Domain Receptor 1/2 (DDR1/2) Inhibitor to Explore the Role of DDR1 in Antifibrotic Therapy

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S Supporting Information

ABSTRACT: Herein, we report the discovery of a potent and selective dual DDR1/2 inhibitor, **7e** (VU6015929), displaying low cytotoxicity, good kinome selectivity, and possessing an acceptable in vitro DMPK profile with good rodent in vivo pharmacokinetics. VU6015929 potently blocks collagen-induced DDR1 activation and collagen-IV production, suggesting DDR1 inhibition as an exciting target for antifibrotic therapy.

KEYWORDS: Discoidin domain receptor 1 (DDR1), tyrosine receptor kinase, collagen, fibrosis

7e, VU6015929

Receptor tyrosine kinases (RTKs) are a large family of cell-surface receptors responsible for regulating cellular proliferation, differentiation, survival, metabolism, and life cycle.¹ RTKs are divided into 20 subfamilies sorted by structural similarity and associated endogenous ligand. Discoidin domain receptors 1 and 2 (DDR1 and DDR2) are RTKs, which, unlike other members of the receptor family, are activated by collagens as opposed to peptide-like growth factors.² DDRs are widely expressed in developing organs, while their expression in adult organs is low or undetectable. DDR1 expression is predominant in epithelial cells, while DDR2 is most commonly found in cells of mesenchymal origin.^{3–6} Upon ligand binding, these receptors can control various cell functions including cell adhesion, migration, survival, and collagen production. Both receptors are well documented to play critical roles during embryonic development of mice and humans, however, their role during adulthood is limited.³ Expression of DDR1 is low in regions of the adult kidney such as the glomerulus; however, its expression can increase following glomerular injury.⁴ Upregulation of DDR1 has been observed in patients with common kidney diseases such as lupus nephritis and Goodpasture's syndrome. Genetic mouse models of chronic kidney disease show that deletion of DDR1 improves survival and reduces fibrosis and inflammation.⁵ In addition to DDR1's pro-inflammatory effect, this receptor has also been implicated as having a deleterious role in kidney injury via regulation of extracellular matrix production by resident cells.⁶

Blocking DDR1 activity with a small molecule inhibitor has emerged as an attractive option to treat DDR1 related progressions of fibrotic disease.^{2,6} Several small molecule DDR1 antagonists have been reported to date (Figure 1). Inhibitor **1** was originally reported as a selective DDR2

Figure 1. Structures of reported compounds **1–5** that display inhibitory activity against DDR1.

Received: August 15, 2019

Accepted: November 25, 2019

Published: November 25, 2019

antagonist ($IC_{50} = 18.6$ nM), although it displayed similar inhibition of DDR1 ($IC_{50} = 12.4$ nM).⁷ Efforts to identify DDR1 selective antagonists have yielded compounds such as **2** (DDR1-IN-1),⁸ with approximately 4-fold selectivity for DDR1 versus DDR2 and **3** with approximately 20-fold selectivity.⁹ Allosteric (type II/III) inhibitors, i.e., **4**, of DDR1/DDR2 have also been reported,¹⁰ but the recent Roche compound **5** is the most selective to date for DDR1 (~63-fold selective, but with a MW of 565).^{11,12} While these ligands have garnered interest in DDR1 as an antifibrotic therapeutic agents, either cytotoxicity, kinase selectivity, broader selectivity, or poor DMPK profiles (or a combination of all four) have precluded more definitive proof of concept studies from being performed, and additional tools are needed.^{6–12}

While awaiting new high-throughput screening (HTS) hits, we utilized **1**, due to its clean ancillary kinase panel,⁷ and showed that inhibition of DDR1 by **1** significantly reduced DDR1-mediated collagen IV production;⁶ however, the DMPK profile of **1** proved unacceptable along with noted cytotoxicity at modest concentrations. Thus, we elected to perform a multidimensional optimization campaign focused on **1**, surveying a broad range of chemical diversity (Figure 2).

Figure 2. Multidimensional optimization strategy for **1**, exploring a wide range of structural diversity and assessing the minimum pharmacophore.

In short order, ~200 analogues were synthesized and screened against both purified DDR1 and DDR2 kinase domains using a Lanthascreen Eu kinase binding assay binding assay,^{6,13} and ~80% of these proved inactive, highlighting core elements of the essential DDR1 pharmacophore (Figure 3). While frustrating that SAR was steep, data derived from this initial effort identified a smaller range of productive targets (**7**) and focused subsequent efforts.

The synthesis of analogues **7** (Scheme 1) required only four steps from commercial starting materials. Here, either 4-methyl-3-nitrobenzoic acid or 4-fluoro-3-nitrobenzoic acid **8** underwent a HATU-mediated amide coupling with 3- or 3,4-disubstituted anilines **9** to afford, after a microwave-assisted nitro reduction with $SnCl_2$, anilino amides **10** in yields ranging from 32 to 96% for the two steps. Treatment of anilino amides **10** with 5-bromonicotinaldehyde under reductive amination conditions yields **11** in 43–77% isolated yields. Finally, a standard Suzuki coupling reaction with **11** and various heteroaryl boronic acids delivers analogues **7** in 38–63% yields.¹³

The most robust series of active compounds are highlighted in Table 1, and all active analogues were dual DDR1/DDR2 inhibitors. We were pleased that a simple methylene homologation afforded active compounds, and our medicinal chemistry strategy explored several avenues on further improving compounds; here, one motivation was to find a

Figure 3. Regions and substituents explored in analogues **6** that were inactive as DDR1/DDR2 antagonists (<30% tracer binding in the presence of 100 nM inhibitor, single point assay),⁶ leading to a basic DDR1 pharmacophore **7**.

Scheme 1. Synthesis of Analogues **7**^a

^aReagents and conditions: (a) HATU, *N,N*-diisopropylethylamine, DCM, rt, 17 h, 49–91%; (b) $SnCl_2$, EtOAc/EtOH, 110 °C (μ W), 25 min, 67–95%; (c) 5-bromonicotinaldehyde, $NaBH_3CN$, AcOH, MeOH, 50 °C, 2 h, 43–77%; (d) Aryl-B(OH)₂, Pd(dppf)Cl₂, Cs₂CO₃, 1,4-dioxanes/H₂O, 90 °C, 3 h, 38–63%.

suitable isosteric replacement for the carboxamide moiety. The matched-pair homologated analogue of **1**, the aminomethyl pyridine **7a**, proved more potent than the parent **1** (DDR1 $IC_{50} = 3.97$ nM, DDR2 $IC_{50} = 4.79$ nM). Replacement of the carboxamide with a pyrrole as in **7b**, was 4–5-fold less active (DDR1 $IC_{50} = 15.2$ nM, DDR2 $IC_{50} = 11.0$ nM), and adding an eastern 4-position substituent to **7b**, to afford **7c**, lost ~3-fold in DDR inhibitory activity (DDR1 $IC_{50} = 36.4$ nM, DDR2 $IC_{50} = 44.1$ nM). Replacement of the pyrrole with a 3-*N*-methylpyrazole, such as **7d**, showed improved activity (DDR1 $IC_{50} = 8.53$ nM, DDR2 $IC_{50} = 4.56$ nM) relative to **1**. Within the **7d** core replacement of the methyl group in the central phenyl ring with a fluorine, alongside substitution of the eastern aryl methoxy to an aryl trifluoromethyl ether, provided another very potent analogue **7e** (DDR1 $IC_{50} = 4.67$ nM, DDR2 $IC_{50} = 7.39$ nM). Removal of the western heterocycle altogether, as with the unsubstituted pyridine **7f**, only lost ~2-fold (DDR1 $IC_{50} = 10.5$ nM, DDR2 $IC_{50} = 10.6$ nM). Finally, a simple OCH₃ moiety, **7g**, was also a viable heterocyclic/carboxamide replacement (DDR1 $IC_{50} = 5.89$ nM, DDR2 $IC_{50} = 6.36$ nM). As all analogues **6** were potent inhibitors of DDR1 and DDR2, we employed a panel of in vitro DMPK assays to further triage these compounds prior to deeper profiling. As a reference, the lead **1** displayed low free fraction in plasma (rat

c1ccc(cc1NCc2cc()ccn2)C(=O)Nc3cc(*)cc(*)c3^aTR-FRET LanthaScreen Eu kinase binding assay with DDR1 and DDR2.⁶

The high affinity binding of **7e** to DDR1 kinase detected by the LanthaScreen Eu kinase binding assay (Table 1) suggested that **7e** would potentially inhibit collagen-induced DDR1 phosphorylation in cells. To analyze the ability of **7e** to inhibit DDR1 phosphorylation in cells, we incubated serum-starved HEK293 expressing full length human DDR1 cDNA (HEK293-DDR1) with various amounts of lead compound **1**

or **7e** together with collagen I. After 24 h, the levels of total and phosphorylated DDR1 were analyzed in cell lysates by Western blot analysis (**Figure 5A**). Both **1** and **7e** inhibited collagen I-

induced DDR1 phosphorylation in a dose dependent manner. However, lower doses of **7e** were required to achieve better inhibition of DDR1 phosphorylation, visible at the lowest dose of 4 nM (Figure 5A,B). To better determine the IC_{50} values, we used a capture ELISA assay using HEK293 cells expressing flagged human full length DDR1 cDNA (HEK293-DDR1-FLAG) incubated with inhibitors and collagen I for 18 h. Analysis of the phosphorylated DDR1/total DDR1 ratio revealed an IC_{50} for **7e** of 0.7078 ± 0.3533 nM, while the IC_{50} for **1** was ~ 7 -fold weaker at 4.799 ± 2.066 nM. The low IC_{50} s for **1** and **7e** confirm that both compounds are potent inhibitors of DDR1 (Figure 5C,D).

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moreover, we demonstrated that this effect can be inhibited by **1** in a DDR1 dependent manner.⁶ To determine whether **7e** can also prevent DDR1-mediated collagen IV, we treated serum-starved DDR1-null mesangial cells reconstituted with human full length DDR1 cDNA with **1** and **7e** (both at 3 μ M). After 24 h, the levels of collagen IV were analyzed in cell lysates by Western blot analysis. As shown in Figure 6A, both **1**

Figure 6. DDR1 inhibitors **1** and **7e** block collagen IV production in a DDR1 dependent manner. Serum-starved DDR1-null mesangial cells expressing human full length DDR1b cDNA were treated with DMSO, **1**, or **7e** (both at 3 μ M) for 24 h and analyzed for the levels of collagen IV (CIV) and Akt (loading control) by Western blot analysis (A). CIV and Akt bands were quantified as described in SI methods. The values are the mean $\pm$ SEM of three experiments performed at least in triplicate (* p < 0.05, ** p < 0.0001 compared to no-inhibitor cells) (B) Western blot analysis of CIV and FAK (loading control) levels in serum-starved DDR1-null mesangial cells expressing human full length DDR1b cDNA treated with DMSO or DDR1-null mesangial cells (KO) treated with DMSO or **7e** (3 μ M) for 24 h. CIV and FAK bands were quantified by densitometry analysis, and values are the mean $\pm$ SEM of one representative experiment performed in triplicate (* p < 0.05, compared to DDR1b expressing cells).

and **7e** significantly inhibited collagen IV production compared to cells treated with vehicle only; however, with a lower p value for **7e** (Figure 6B). Similar to **1**, **7e** did not decrease collagen IV production in the DDR1-null mesangial cells (data not shown). These results suggest that **7e** (VU6015929) is an improved tool compound over **1** in affecting DDR1 phosphorylation due to a combination of its enhanced DDR1 potency and more drug-like physiochemical and DMPK profiles.

In summary, we performed a multidimensional optimization effort around known DDR1/2 dual inhibitor **1**, and quickly identified key elements of a DDR1 pharmacophore. Targeted optimization then produced **7e** (VU6015929), a new dual DDR1/2 inhibitor with improved binding and cell-based potency, enhanced physiochemical and DMPK properties, and clean kinome profile to serve as a new in vitro and in vivo tool compound. Moreover, **7e** was shown to potently inhibit collagen IV production, further validating a role for DDR1 as pro-fibrotic receptor. Results from ongoing in vivo work rodent disease models will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmedchemlett.9b00382>.

General methods for the synthesis and characterization of all compounds, and methods for the in vitro and in vivo DMPK protocols and figures (PDF)

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Author Contributions

D.E.J. and C.M.B. contributed equally. C.W.L. and A.P. drafted/corrected the manuscript and directed the science. D.E.J. performed the chemical synthesis. C.W.L. and A.P. oversaw the target selection and interpreted the biological data. C.M.B. performed the in vitro molecular pharmacology studies. A.L.B. performed the in vitro and in vivo DMPK studies. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was in part supported by Veterans Affairs Merit Review 1I01BX002025 (A.P.); National Institute of Health grants R01-DK119212 (A.P., C.M.B.), P30-DK114809 (A.P.). A.P. is the recipient of a VA Senior Research Career Scientist award. We thank the William K. Warren, Jr. and the William K. Warren Foundation who funded the William K. Warren, Jr. Chair in Medicine (to C.W.L.).

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(13) See [Supporting Information \(SI\)](#) for details.