

Tetrahydroisoquinoline-7-carboxamide Derivatives as New Selective Discoidin Domain Receptor 1 (DDR1) Inhibitors

Zhen Wang,^{†,‡,§} Yali Zhang,^{§,‡} Sergio G. Bartual,^{||} Jinfeng Luo,[#] Tingting Xu,^Ψ Wenting Du,^φ Qiuju Xun,[#] Zhengchao Tu,[#] Rolf A. Brekken,^φ Xiaomei Ren,[#] Alex N. Bullock,^{*,||} Guang Liang,^{*,§} Xiaoyun Lu,^{*,†} and Ke Ding^{*,†,§}

[†]School of Pharmacy, Jinan University, 601 Huangpu Avenue West, Guangzhou 510632, China

[#]State Key Laboratory of Respiratory Diseases, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences, 190 Kaiyuan Avenue, Guangzhou 510530, China

[§]Chemical Biology Research Center, School of Pharmaceutical Sciences, Wenzhou Medical University, Wenzhou, People's Republic of China

^ΨDepartment of Pulmonary Medicine, The Second Affiliated Hospital, Wenzhou Medical University, Wenzhou, People's Republic of China

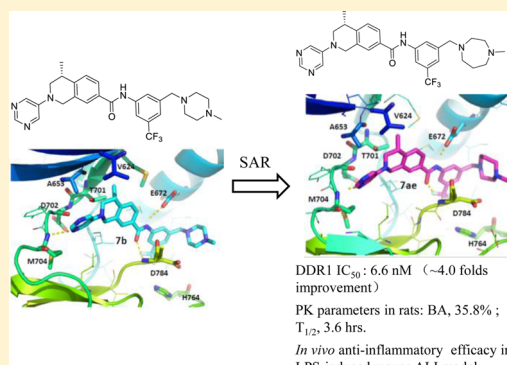
^{||}Structural Genomics Consortium, University of Oxford, Old Road Campus, Roosevelt Drive, Oxford OX3 7DQ, U.K.

^φDivision of Surgical Oncology, Department of Surgery and the Hamon Center for Therapeutic Oncology Research, UT Southwestern Medical Center, Dallas, Texas 75390-8593, United States

Supporting Information

ABSTRACT: Acute lung injury (ALI) is a deadly symptom for serious lung inflammation. Discoidin Domain Receptor 1 (DDR1) is a new potential target for anti-inflammatory drug discovery. A new selective tetrahydroisoquinoline-7-carboxamide based DDR1 inhibitor **7ae** was discovered to tightly bind the DDR1 protein and potently inhibit its kinase function with a K_d value of 2.2 nM and an IC_{50} value of 6.6 nM, respectively. The compound dose-dependently inhibited lipopolysaccharide (LPS)-induced interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) release in mouse primary peritoneal macrophages (MPMs). In addition, **7ae** also exhibited promising *in vivo* anti-inflammatory effects in a LPS-induced mouse ALI model. To the best of our knowledge, this is the first "proof of concept" investigation on the potential application of a small molecule DDR1 inhibitor to treat ALI.

KEYWORDS: DDR1, inhibitor, structure–activity relationship (SAR), inflammation, acute lung injury (ALI)



Acute lung injury (ALI) is an inflammatory condition, which is characterized by accumulation of neutrophils in lung tissue.^{1–3} ALI has clinically proven to be a leading cause of acute respiratory failure in critically ill patients for which standard treatment is mainly supportive due to lack of effective therapies.⁴ Collective investigations demonstrate that various inflammatory cytokines, e.g., interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), play crucial roles in mediating, amplifying, and perpetuating ALI processes.^{3,5,6} Suppressing the overproduction of cytokines becomes a new promising strategy for the clinical management of ALI.

Discoidin domain receptors (DDR), i.e., DDR1 and DDR2, are transmembrane receptor tyrosine kinases (RTKs), which were activated by triple-helical collagens.^{7–9} DDR1 and DDR2 have been demonstrated to play critical roles in regulation of cellular morphogenesis, differentiation, proliferation, adhesion, migration, and invasion.^{10–14} Increasing evidence has suggested the potential role of DDRs in regulation of secretion of

inflammatory factors in atherosclerosis, organ fibrosis, and many other inflammatory disorders.^{15,16} DDR1 not only induced secretion of inflammatory factors, but more importantly amplified the effects by other stimuli such as pro-inflammatory cytokines or bacterial products.¹⁷ Direct activation of DDR1 with agonistic anti-DDR1 Ab augmented lipopolysaccharide (LPS)-induced interleukin-1 β (IL-1 β), interleukin-8 (IL-8), macrophage inflammatory protein-1 α (MIP-1 α), and monocyte chemoattractant protein-1 (MCP-1) release from granulocyte-macrophage colony-stimulating factor (GM-CSF)-induced human monocyte-derived primary macrophages (GM macrophages), although it also induced low-level release of these proteins without LPS activation.¹⁷ Besides, it was also reported renal cortical slices of DDR1 null mice

Received: December 7, 2016

Accepted: February 9, 2017

Published: February 9, 2017

showed a blunted response of chemokines to LPS that was accompanied by a considerable protection against the LPS-induced mortality, further suggesting the importance of DDR1 in mediating inflammation and fibrosis.¹⁸ Additionally, genetic inhibition of DDR1 has been reported to alleviate bleomycin-induced lung fibrosis by blocking p38 mitogen-activated protein kinase (p38 MAPK) activation.¹⁹ These results were further pharmacologically validated by utilizing our recently disclosed selective DDR1 inhibitors in a bleomycin-induced mouse model of lung fibrosis.²⁰ Thus, DDR1 may serve as a novel potential molecular target for anti-inflammatory drug discovery.

A number of well-characterized kinase inhibitors were reported to potently suppress the functions of DDR1 and DDR2.¹⁴ However, few of them were developed by using DDR1 or DDR2 as the primary target. Only recently, several selective DDR1/DDR2 inhibitors were disclosed with different selectivity profiles (Figure 1).^{20–26} In 2016, we reported

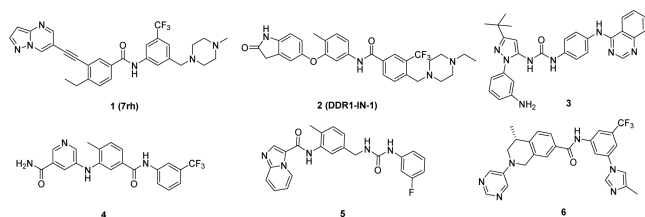


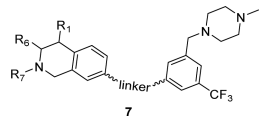
Figure 1. Chemical structures of reported selective DDR1/DDR2 inhibitors.

tetrahydroisoquinoline-7-carboxamide derivatives as new selective DDR1 inhibitors and the arguably first “proof of concept” investigation on their potential application to treat inflammation mediated pulmonary fibrosis.²⁰ Herein, we would like to describe the structural optimization of this class of compounds and the efforts yielded **7ae** as a highly specific DDR1 inhibitor with promising *in vivo* therapeutic effect in a LPS-induced mouse ALI model.

Tetrahydroisoquinoline-7-carboxamides **7a** and **7b** have been identified as highly selective DDR1 inhibitors with IC₅₀ values of 442 and 24.3 nM, respectively, representing promising lead molecules for optimization (Table 1).²⁰ To minimize our synthetic burden, *R/S* racemic molecules were first utilized for the structure–activity relationship (SAR) exploration. *R/S*-Racemic mixture (**7c**) of **7b** displayed an IC₅₀ value of 38.3 nM under the experimental conditions. The other new molecules were synthesized by using previously reported protocols (Schemes S1–S3).²⁰

Our previous investigation suggested that a hydrogen bond between the pyrimidinyl moiety of **7a** or **7b** with the NH of Met704 in the hinge region of DDR1 was critical for the compounds to exhibit strong DDR1 inhibition.²⁰ Not surprisingly, replacement of the hinge binding 5-pyrimidinyl group (**7a**) by a 4-pyrimidinyl (**7d**) or a 5-pyrimidinylmethyl moiety (**7e**) caused total loss of the DDR1 inhibitory activity, which likely results from unfavorable orientations of the heterocyclic heads that prevent the formation of critical hydrogen bonds. Our previous study also revealed that the (*R*)-methyl substituent at R₁ position in **7b** occupied a small hydrophobic recess formed by Val624, Ala653, and Met699 of DDR1 to achieve potency enhancement.²⁰ However, a dimethyl substitution at R₁ position (**7g**) caused 9-fold potency loss. When the methyl group was merged to R₆ position, the resulting compound (**7f**) demonstrated 14-fold less potency

Table 1. *In Vitro* Inhibitory Activities of Compounds **7a–7j** against DDR1, DDR2, Bcr-Abl, and c-Kit^a



Cpds	R ₇	R ₁	R ₆	linker	Kinase inhibition (IC ₅₀ , nM)			
					DDR1	DDR2	Bcr-Abl	c-Kit
1					13.1 ± 3.3	203 ± 36	414 ± 50	2500 ± 820
7a		H	H		442 ± 69	8000 ± 1440	>10000	>10000
7b		<i>R</i> -Me	H		24.3 ± 4.1	514 ± 32	>10000	>10000
7c		(<i>R/S</i>)-Me	H		38.3 ± 0.8	1800 ± 351	2100 ± 120	>10000
7d		H	H		>10000	>10000	>10000	>10000
7e		H	H		>10000	>10000	>10000	>10000
7f		H	(<i>R/S</i>)-Me		545 ± 101	7600 ± 1422	>10000	>10000
7g		Me, Me	H		223 ± 15	4500 ± 710	>10000	>10000
7h		(<i>R</i>)-Me	H		75.7 ± 5.4	1400 ± 215	>10000	>10000
7i		(<i>S</i>)-Me	H		614 ± 33	>10000	>10000	>10000
7j		(<i>R/S</i>)-Me	H		>10000	>10000	>10000	>10000

^aDDR1 and DDR2 experiments were performed using LANCE ULTRA kinase assay according to the manufacturer's instructions. Bcr-Abl and c-Kit activity experiments were performed using the Förster resonance energy transfer (FRET)-based Z'-Lyte assay according to the manufacturer's instructions. All the data are mean values from at least three independent experiments.

than the corresponding racemic compound **7c**. Nevertheless, all the compounds exhibited excellent DDR1 selectivity over the structurally related DDR2, Bcr-Abl, and c-Kit kinases. For instance, compound **7c** exhibited 47-, 55-, and 261-fold less potency against the mentioned kinases, respectively.

A computational docking study further suggested that the linker amide in **7b** formed two pairs of strong hydrogen bonds with Glu672 in the C-helix and Asp784 in the DFG motif of the protein, respectively. In order to validate the contribution of these hydrogen bonds to DDR1 inhibition, compounds with a reversed amide (**7h**) or a urea linker (**7j**) were designed and synthesized. It was evident that compound **7h** exhibited 3-fold less potency than that of **7b**, while **7j** totally abolished the DDR1 inhibitory activity. Further cocrystal structure determination confirmed that compound **7h** bound into the ATP binding pocket of DDR1 (PDB ID: SFDX) with a similar conformation to that of **7b**.²⁰ The methyl group in R₁ position of **7h** was also nicely accommodated in the small hydrophobic recess formed by Val624, Ala653, and Thr701 of DDR1. However, the reversed amide linker forced the hydrophobic trifluoromethylphenyl group to moderately rotate away from the C-helix, and accordingly induced longer distances between amide moiety and the corresponding Glu672 and Asp784 residues with values of 2.5 and 2.3 Å (Figure 2A). Thus, **7h**

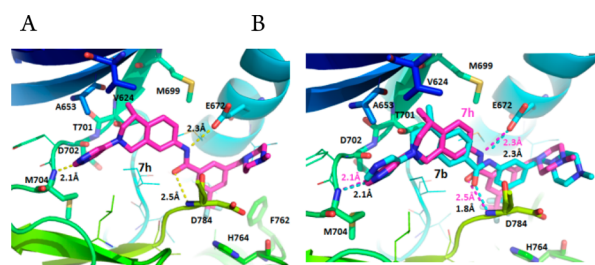


Figure 2. ((A) Cocystal structure of **7h** (colored by purple) with DDR1 (PDB ID: 5FDX). Hydrogen bonds (H-bonds) are indicated by yellow dashed lines. (B) Superposition of the docking conformation of **7b** (colored by cyan) into the X-ray complex of **7h** with DDR1. The H-bonds are indicated by purple and cyan dashed lines for **7h** and **7b**, respectively, and the distances of H-bonds are given by purple and black, respectively.

might form relatively weaker hydrogen bond networks with the corresponding amino acids than that of **7b** (Figure 2B). This structural information provides a plausible explanation for the potency loss of compound **7h**. Not surprisingly, the *S*-isomer **7i** was 8-fold less potent than compound **7h** because the methyl group was out of the small hydrophobic cavity.

Structural investigation also revealed that the trifluoromethylphenyl group in **7b** bound deeply into a hydrophobic pocket formed by the DFG-out conformation, while the hydrophilic 1-(4-methyl)piperazinyl moiety was exposed to a solvent-accessible pocket. We first examined the contribution of substituents at R_4 position to the DDR1 inhibitory activity by introducing various hydrophobic groups in this position. It was found that this position well tolerated a variety of hydrophobic substituents with different sizes. For instance, racemic compounds harboring ethyl (**7l**), isopropyl (**7m**), tertiary butyl (**7n**), and phenyl (**7q**) groups, exhibited IC_{50} values of 50.5, 49.9, 66.6, and 44.6 nM respectively, against DDR1 kinase, which were almost equipotent to **7c**, whereas the cyclopropyl (**7o**) or cyclohexyl (**7p**) substituted compounds were obviously less potent with IC_{50} values of 89.0 and 123 nM, respectively (Table 2). The results also suggested that a lipophilic R_4 -substitution is crucial for the compounds to maintain their strong inhibition against DDR1. When the CF_3 group in **7c** was removed, the resulting compound **7k** totally abolished its activity against the kinase.

Further investigation demonstrated that a hydrophilic group at R_2 also contributed greatly to the DDR1 kinase inhibition. When the 1-(4-methyl)piperazinylmethyl moiety was removed, the resulting compound **7r** exhibited an IC_{50} value of 191 nM against DDR1, which was approximately 5-fold less potent than the original compound **7c**. When 1-(4-methyl)piperazinylmethyl moiety was moved at R^5 position, the resulting compound **7s** totally abolished the kinase activity. A change of hydrophilic 1-(4-methyl)piperazinylmethyl moiety from R_2 to R_3 position (**7t**) obviously improved the DDR1 inhibitory activity with an IC_{50} value of 19.9 nM, but the selectivity over DDR2 and Bcr-Abl was significantly decreased. It was also found that the 1-(4-methyl)piperazinyl group at R_2 position could be replaced by a 1-(4-methyl)piperazinyl (**7u**), 1-(4-methyl)piperazinylethyl (**7v**), 1-(4-ethyl)piperazinylmethyl (**7w**), or 1-(4-cyclohexyl)piperazinylmethyl (**7x**) to maintain strong DDR1 inhibitory activities with IC_{50} values ranging from 71.1 to 132 nM. However, when the 1-(4-methyl)piperazinylmethyl group was replaced by a morpholinomethyl (**7y**), thiomorpholinomethyl (**7z**), piperidin-1-

Table 2. *In Vitro* Inhibitory Activities of Compounds **7k**–**7af** against DDR1, DDR2, Bcr-Abl, and c-Kit^a

7

Cpd s	R_2	R_3	R_4	R_5	Kinase inhibition (IC_{50} , nM)			
					DDR1	DDR2	Bcr-Abl	c-Kit
7k		H	H	H	>10000	>10000	>1000 0	>1000 0
7l		H	Et	H	50.5 ± 6.7	1400 ± 163	>1000 0	>1000 0
7m		H	iPr	H	49.9 ± 8.1	682 ± 73	8700 ± 905	>1000 0
7n		H	tBu	H	66.6 ± 12.4	940 ± 40.5	4300 ± 920	>1000 0
7o		H		H	89.0 ± 14	1100 ± 131	9900 ± 115	>1000 0
7p		H		H	123 ± 19	462 ± 59	>1000 0	>1000 0
7q		H		H	44.6 ± 6.6	1400 ± 105	10000	>1000 0
7r	H	H	CF_3	H	191 ± 20.2	10000	>1000 0	>1000 0
7s	H	H	CF_3		10000	>10000	>1000 0	>1000 0
7t	H		CF_3	H	19.9 ± 0.9	354 ± 20	547 ± 13	>1000 0
7u		H	CF_3	H	132 ± 24	2100 ± 120	>1000 0	>1000 0
7v		H	CF_3	H	80.7 ± 12	1500 ± 210	>1000 0	>1000 0
7w		H	CF_3	H	71.1 ± 10.5	944 ± 126	>1000 0	>1000 0
7x		H	CF_3	H	79.9 ± 7.0	846 ± 99	>1000 0	>1000 0
7y		H	CF_3	H	193 ± 64	4500 ± 712	>1000 0	>1000 0
7z		H	CF_3	H	209 ± 16	3700 ± 500	>1000 0	>1000 0
7aa		H	CF_3	H	167 ± 36	2200 ± 387	>1000 0	>1000 0
7ab		H	CF_3	H	222 ± 50	2600 ± 300	>1000 0	>1000 0
7ac		H	CF_3	H	254 ± 11	6700 ± 730	>1000 0	>1000 0
7ad		H	CF_3	H	25.6 ± 1.3	604 ± 109	>1000 0	>1000 0
7ae					6.6 ± 0.5	255 ± 32	8100 ± 1130	>1000 0
7af					167 ± 26	2600 ± 370	>1000 0	>1000 0

^aDDR1 and DDR2 experiments were performed using LANCE ULTRA kinase assay according to the manufacturer's instructions. Bcr-Abl and c-Kit activity experiments were performed using the Förster resonance energy transfer (FRET)-based Z'-Lyte assay according to the manufacturer's instructions. All the data are mean values from at least three independent experiments.

ylmethyl (**7aa**), pyrrolidin-1-ylmethyl (**7ab**), or dimethylaminomethyl (**7ac**) substituent, the resulting compounds were 4.4–6.6-fold less potent than **7c**. This might be rationalized by the fact that all of the new molecules lacked a solvent-exposing N-atom, which had been shown to form a favorable interaction with the protein as determined by a 2.3 Å cocystal structure.²⁰

Encouragingly, 1-methylhomopiperazinemethyl substituted compound **7ad** displayed a similar DDR1 inhibitory potency to that of **7b**. Further evaluation also revealed the *R*-isomer (**7ae**) was 25-fold more potent than the corresponding *S*-isomer (**7af**), with an IC_{50} value of 6.6 nM. Additionally, compound **7ae** also exhibited target selectivity over DDR2, Bcr-Abl, and c-Kit with factors of 38-, 1227-, and 1515-fold, respectively.

The binding affinity of compound **7ae** for the DDR1 kinase was further determined by using an active-site-dependent competition binding assay (conducted by DiscoverX Corporation, San Diego, USA).²⁷ It was shown that compound **7ae** tightly bound to the ATP-binding site of the kinase with a binding constant (K_d) value of 2.2 nM, validating its strong kinase inhibition against DDR1. We further profiled the target specificity of this compound against a panel of 468 kinases (including 403 nonmutated kinases) using the DiscoverX screening platform at a concentration of 1.0 μ M, which was about 450 times higher than its K_d value with DDR1. The results revealed that **7ae** demonstrated great target specificity with S(1) and S(10) scores of 0.015 and 0.022, respectively (Table S1). For instance, **7ae** showed almost 100% competition rate (99.9% inhibition, ctrl% = 0.1) with DDR1 at 1.0 μ M, and it only showed obvious binding to a small portion of the kinases investigated. The major “off targets” included abelson murine leukemia viral oncogene (Abl), cyclin-dependent-kinase 11 (CDK 11), DDR2, Ephrin type-A receptor 8 (EPHA8), hormonally up-regulated Neu-associated kinase (HUNK), and nerve growth factor receptors A (TrkA), B (TrkB), and C (TrkC). The binding affinities (K_d) or kinase inhibitory activities (IC_{50}) of compound **7ae** against these “off targets” were further determined by using DiscoverX’s platform or our in-house kinase assays (Table S2). It was shown that compound **7ae** was approximately 16–1227-fold less potent against the majority of these kinases, although it demonstrated similar binding affinities with TrkB and TrkC to that of DDR1 kinase. However, the compound exhibited 2.7–8.0-fold less potency against TrkB and TrkC, respectively, in the biochemical assays of kinase function.

The inhibitory effect of compound **7ae** on the activation of DDR1 and downstream p38 signal in primary human lung fibroblasts was also investigated to confirm its strong DDR1 kinase inhibition (Figure 3). The results revealed that **7ae** dose-dependently inhibited the phosphorylation of DDR1 and the downstream p38 protein. It was also noteworthy that compound **7ae** displayed obviously stronger signal inhibition than that of our previously reported DDR1 inhibitor **1**.

DDR1 has been implicated as a critical regulator of inflammation.^{14,19} We therefore determined the potential

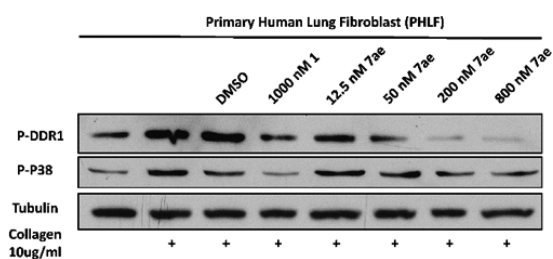


Figure 3. Inhibitor **7ae** inhibited DDR1-mediated signaling in a concentration-dependent manner in primary human lung fibroblasts. Lysates were probed for the indicated targets by Western blot analysis.

anti-inflammatory effect of **7ae** by measuring its capability to suppress the LPS-induced release of cytokines. It was found that compound **7ae** dose-dependently suppressed the LPS-induced production of interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) in mouse primary peritoneal macrophages (MPMs) as determined by enzyme-linked immunosorbent assays (ELISA) (Figure 4), suggesting its promising *in vitro* anti-inflammatory activity.

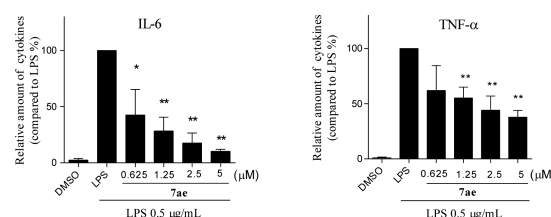


Figure 4. Compound **7ae** inhibited LPS-induced IL-6 and TNF- α release in a dose-dependent manner in MPMs. Each bar represents mean \pm SE of 3–5 independent experiments. Statistical significance relative to LPS is indicated: * p < 0.05, ** p < 0.01.

The therapeutic potential of **7ae** was further studied in a LPS-induced ALI model.²⁸ Compound **7ae** was orally administered at 20 or 40 mg/kg twice daily (BID) based on its pharmacokinetics (PK) parameters (Table S3) for 7 days prior to the administration of LPS (20 μ L, 5 mg/kg). It was evident that pretreatment with compound **7ae** markedly reduced the LPS-induced pulmonary edema as determined by lung wet/dry (W/D) ratio (Figure 5A). Meanwhile, the total

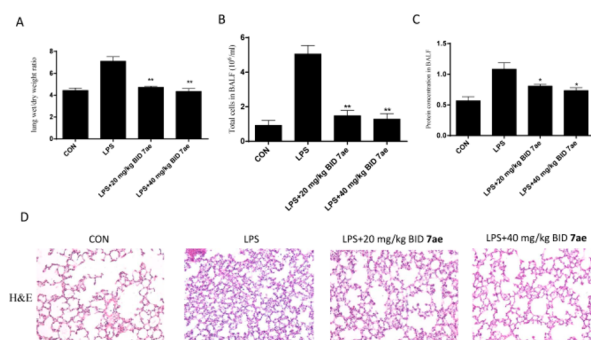


Figure 5. Compound **7ae** attenuated LPS induced ALI in mice. (A) Lung W/D ratio. (B) Total amount of cells in BALF. (C) Protein concentration in BALF. (D) Hematoxylin and eosin staining. Statistical significance relative to LPS group was indicated: * p < 0.05, ** p < 0.01.

cell number and total protein concentration in bronchial alveolar lavage fluid (BALF) were increased remarkably after LPS administration compared to the control group (Figure 5B,C). Administration of **7ae** dose-dependently inhibited the LPS-induced increase in total cell number and total protein concentration in BALF (Figure 5B,C). LPS treatment also resulted in significant pulmonary congestion, thickening of alveolar wall, and interstitial edema (Figure 5D). These pathological changes were also markedly reduced by the administration of **7ae** (Figure 5D).

In summary, an extensive SAR investigation was conducted based on our recently disclosed tetrahydroisoquinoline-7-carboxamide based DDR1 inhibitors. The effort yielded a highly promising candidate **7ae**, which tightly bound the DDR1

protein with a K_d value of 2.2 nM and potently inhibited its kinase function with an IC_{50} value of 6.6 nM. Furthermore, the compound was notably less potent against most of the 403 nonmutated kinases when tested at 1000 nM (which is approximately 450 times higher than its K_d value with DDR1), indicating its great target specificity. In addition, **7ae** demonstrated reasonable pharmacokinetic properties in rats and exhibited a promising anti-inflammatory effect *in vivo* using a LPS-induced mouse ALI model. Compound **7ae** may serve as a new lead compound for anti-inflammatory drug discovery.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acsmedchemlett.6b00497](https://doi.org/10.1021/acsmedchemlett.6b00497).

Synthetic procedures for compounds **7d–7af**, the results of the kinase selectivity profiling study of compound **7ae**, procedures for Kinome^{scan} screening, *in vitro* kinase assay, Western blot analysis, *in vitro* anti-inflammatory activity, determination of pharmacokinetic parameters in rats, *in vivo* anti-inflammatory experiments protein expression and purification, crystallization and structure determination, computational study and the 1H and ^{13}C NMR spectra of compounds **7d–7af** (PDF)

Accession Codes

Atomic coordinates and experimental data for the cocrystal structure of **7h** with DDR1 (PDB ID: SFDX).

■ AUTHOR INFORMATION

Corresponding Authors

*Tel: +86-20-85228025. Fax: +86-20-85224766. E-mail: luxy2016@jnu.edu.cn (X.L.).

*E-mail: alex.bullock@sgc.ox.ac.uk (A.N.B.).

*E-mail: wzmcliangguang@163.com (G.L.).

*E-mail: dingke@jnu.edu.cn (K.D.).

ORCID

Wenting Du: 0000-0001-6765-0129

Ke Ding: 0000-0001-9016-812X

Author Contributions

‡These authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors appreciate the financial support from National Natural Science Foundation of China (21572230 and 81425021), Guangdong Province (2013A022100038, 2015A030312014 and 2015A030306042, 2016A050502041), Guangzhou City (201508030036), and Jinan University. We also thank Diamond Light Source for beam time (proposal mx10619) as well as the staff of beamlines I04 and I04-1 for their assistance with crystal testing and data collection. The SGC is a registered charity (number 1097737) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada, Innovative Medicines Initiative (EU/EFPIA) [ULTRA-DD grant no. 115766], Janssen, Merck & Co., Novartis Pharma AG, Ontario Ministry of Economic Development and Innovation, Pfizer, São Paulo Research

Foundation-FAPESP, Takeda, and Wellcome Trust [106169/ZZ14/Z].

■ ABBREVIATIONS

DDR, discoidin domain receptor; SAR, structure–activity relationship; K_d , binding constant; IC_{50} , half maximal (50%) inhibitory concentration (IC) of a substance; LPS, lipopolysaccharide; IL-6, interleukin-6; TNF- α , tumor necrosis factor- α ; MPMs, mouse primary peritoneal macrophages; ALI, acute lung injury; RTKs, receptor tyrosine kinases; aa, amino acid; IL-1 β , interleukin-1 β ; IL-8, interleukin-8; MIP-1 α , macrophage inflammatory protein-1 α ; MCP-1, monocyte chemoattractant protein-1; GM-CSF, granulocyte-macrophage colony-stimulating factor; p38 MAPK, P38 mitogen-activated protein kinase; Val, valine; Ala, alanine; Met, methionine; PDB, Protein Data Bank; DFG, Asp-Phe-Gly; Abl, abelson murine leukemia viral oncogene; CDK 11, cyclin-dependent-kinase 11; EPHA8, Ephrin type-A receptor 8; HUNK, hormonally up-regulated Neu-associated kinase; TrkA, nerve growth factor receptor A; ELISA, enzyme linked immunosorbent assays; PK, pharmacokinetic; BID, twice daily; W/D, wet/dry; BALF, bronchial alveolar lavage fluid; IL-1 β , interleukin-1 β ; IL-12, interleukin-12; ICAM-1, intercellular cell adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1

■ REFERENCES

- (1) Johnson, J. D.; Edman, J. C.; Rutter, W. J. A receptor tyrosine kinase found in breast carcinoma cells has an extracellular discoidin I-like domain. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 5677–5681.
- (2) Alves, F.; Vogel, W.; Mossie, K.; Millauer, B.; Hofler, H.; Ullrich, A. Distinct structural characteristics of discoidin I subfamily receptor tyrosine kinases and complementary expression in human cancer. *Oncogene* **1995**, *10*, 609–618.
- (3) Vogel, W. Discoidin domain receptors: structural relations and functional implication. *FASEB J.* **1999**, *13*, S77–S82.
- (4) Shrivastava, A.; Radziejewski, C.; Campbell, E.; Kovac, L.; McGlynn, M.; Ryan, T. E.; Davis, S.; Goldfarb, M. P.; Glass, D. J.; Lemke, G.; Yancopoulos, G. D. An orphan receptor tyrosine kinase family whose members serve as nonintegrin collagen receptors. *Mol. Cell* **1997**, *1*, 25–34.
- (5) Vogel, W.; Gish, G. D.; Alves, F.; Pawson, T. The discoidin domain receptor tyrosine kinases are activated by collagen. *Mol. Cell* **1997**, *1*, 13–23.
- (6) Leitinger, B. Molecular analysis of collagen binding by the human discoidin domain receptors, DDR1 and DDR2. Identification of collagen binding sites in DDR2. *J. Biol. Chem.* **2003**, *278*, 16761–16769.
- (7) Leitinger, B.; Steplewski, A.; Fertala, A. The D2 period of collagen II contains a specific binding site for the human discoidin domain receptor, DDR2. *J. Mol. Biol.* **2004**, *344*, 993–1003.
- (8) Leitinger, B.; Kwan, A. P. The discoidin domain receptor DDR2 is a receptor for type X collagen. *Matrix Biol.* **2006**, *25*, 355–364.
- (9) Agarwal, G.; Mihai, C.; Iscru, D. F. Interaction of discoidin domain receptor 1 with collagen type I. *J. Mol. Biol.* **2007**, *367*, 443–455.
- (10) Valiathan, R. R.; Marco, M.; Leitinger, B.; Kleer, C. G.; Fridman, R. Discoidin domain receptor tyrosine kinases: new players in cancer progression. *Cancer Metastasis Rev.* **2012**, *31*, 295–321.
- (11) Vogel, W. F.; Abdulhussein, R.; Ford, C. E. Sensing extracellular matrix: an update on discoidin domain receptor function. *Cell. Signalling* **2006**, *18*, 1108–1116.
- (12) Leitinger, B. Discoidin domain receptor functions in physiological and pathological conditions. *Int. Rev. Cell Mol. Biol.* **2014**, *310*, 39–87.

- (13) Iwai, L. K.; Luczynski, M. T.; Huang, P. H. Discoidin domain receptors: a proteomic portrait. *Cell. Mol. Life Sci.* **2014**, *71*, 3269–3279.
- (14) Li, Y.; Lu, X.; Ren, X.; Ding, K. Small molecule discoidin domain receptor kinase inhibitors and potential medical applications. *J. Med. Chem.* **2015**, *58*, 3287–3301.
- (15) Ju, G. X.; Hu, Y. B.; Du, M. R.; Jiang, J. L. Discoidin domain receptors (DDRs): potential implications in atherosclerosis. *Eur. J. Pharmacol.* **2015**, *751*, 28–33.
- (16) Borza, C. M.; Pozzi, A. Discoidin domain receptors in disease. *Matrix Biol.* **2014**, *34*, 185–192.
- (17) Matsuyama, W.; Wang, L.; Farrar, W. L.; Faure, M.; Yoshimura, T. Activation of discoidin domain receptor 1 isoform b with collagen up-regulates chemokine production in human macrophages: role of p38 mitogen-activated protein kinase and NF-kappa B. *J. Immunol.* **2004**, *172*, 2332–2340.
- (18) Flamant, M.; Placier, S.; Rodenas, A.; Curat, C. A.; Vogel, W. F.; Chatziantoniou, C.; Dussaule, J. C. Discoidin domain receptor 1 null mice are protected against hypertension-induced renal disease. *J. Am. Soc. Nephrol.* **2006**, *17*, 3374–3381.
- (19) Avivi-Green, C.; Singal, M.; Vogel, W. F. Discoidin domain receptor 1-deficient mice are resistant to bleomycin-induced lung fibrosis. *Am. J. Respir. Crit. Care Med.* **2006**, *174*, 420–427.
- (20) Wang, Z.; Bian, H.; Bartual, S. G.; Du, W.; Luo, J.; Zhao, H.; Zhang, S.; Mo, C.; Zhou, Y.; Xu, Y.; Tu, Z.; Ren, X.; Lu, X.; Brekken, R. A.; Yao, L.; Bullock, A. N.; Su, J.; Ding, K. Structure-based design of tetrahydroisoquinoline-7-carboxamides as selective discoidin domain receptor 1 (DDR1) inhibitors. *J. Med. Chem.* **2016**, *59*, 5911–5916.
- (21) Gao, M.; Duan, L.; Luo, J.; Zhang, L.; Lu, X.; Zhang, Y.; Zhang, Z.; Tu, Z.; Xu, Y.; Ren, X.; Ding, K. Discovery and optimization of 3-(2-(Pyrzolo[1,5-a]pyrimidin-6-yl)ethyn yl)benzamides as novel selective and orally bioavailable discoidin domain receptor 1 (DDR1) inhibitors. *J. Med. Chem.* **2013**, *56*, 3281–3295.
- (22) Kim, H. G.; Tan, L.; Weisberg, E. L.; Liu, F.; Canning, P.; Choi, H. G.; Ezell, S. A.; Wu, H.; Zhao, Z.; Wang, J.; Mandinova, A.; Griffin, J. D.; Bullock, A. N.; Liu, Q.; Lee, S. W.; Gray, N. S. Discovery of a potent and selective DDR1 receptor tyrosine kinase inhibitor. *ACS Chem. Biol.* **2013**, *8*, 2145–2150.
- (23) Elkamhawry, A.; Park, J. E.; Cho, N. C.; Sim, T.; Pae, A. N.; Roh, E. J. Discovery of a broad spectrum antiproliferative agent with selectivity for DDR1 kinase: cell line-based assay, kinase panel, molecular docking, and toxicity studies. *J. Enzyme Inhib. Med. Chem.* **2016**, *31*, 158.
- (24) Richters, A.; Nguyen, H. D.; Phan, T.; Simard, J. R.; Grutter, C.; Engel, J.; Rauh, D. Identification of type II and III DDR2 inhibitors. *J. Med. Chem.* **2014**, *57*, 4252–62.
- (25) Terai, H.; Tan, L.; Beauchamp, E. M.; Hatcher, J. M.; Liu, Q.; Meyerson, M.; Gray, N. S.; Hammerman, P. S. Characterization of DDR2 inhibitors for the treatment of DDR2 mutated non-small cell lung cancer. *ACS Chem. Biol.* **2015**, *10*, 2687.
- (26) Murray, C. W.; Berdini, V.; Buck, I. M.; Carr, M. E.; Cleasby, A.; Coyle, J. E.; Curry, J. E.; Day, J. E.; Day, P. J.; Hearn, K.; Iqbal, A.; Lee, L. Y.; Martins, V.; Mortenson, P. N.; Munck, J. M.; Page, L. W.; Patel, S.; Roomans, S.; Smith, K.; Tamanini, E.; Saxty, G. Fragment-based discovery of potent and selective DDR1/2 Inhibitors. *ACS Med. Chem. Lett.* **2015**, *6*, 798–803.
- (27) Fabian, M. A.; Biggs, W. H., 3rd; Treiber, D. K.; Atteridge, C. E.; Azimioara, M. D.; Benedetti, M. G.; Carter, T. A.; Ciceri, P.; Edeen, P. T.; Floyd, M.; Ford, J. M.; Galvin, M.; Gerlach, J. L.; Grotzfeld, R. M.; Herrgard, S.; Insko, D. E.; Insko, M. A.; Lai, A. G.; Lelias, J. M.; Mehta, S. A.; Milanov, Z. V.; Velasco, A. M.; Wodicka, L. M.; Patel, H. K.; Zarrinkar, P. P.; Lockhart, D. J. A small molecule-kinase interaction map for clinical kinase inhibitors. *Nat. Biotechnol.* **2005**, *23*, 329–336.
- (28) Chen, G.; Zhang, Y.; Liu, X.; Fang, Q.; Wang, Z.; Fu, L.; Liu, Z.; Wang, Y.; Zhao, Y.; Li, X.; Liang, G. Discovery of a new inhibitor of myeloid differentiation 2 from cinnamamide derivatives with anti-inflammatory activity in sepsis and acute lung injury. *J. Med. Chem.* **2016**, *59*, 2436–2451.