ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

Letter

Targeting NOX2 via p47/phox-p22/phox Inhibition with Novel Triproline Mimetics

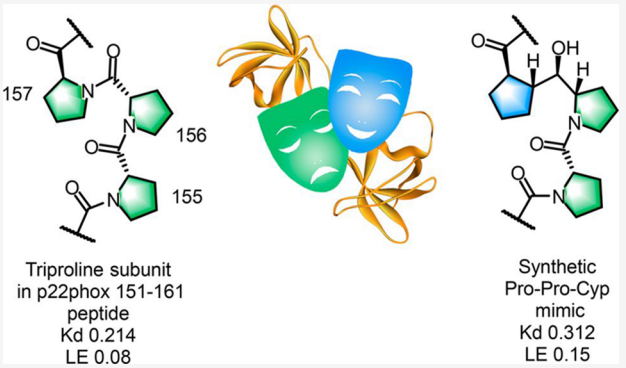
Jean-Baptiste Garsi, Balázs Komjáti, Gregorio Cullia, Imre Fejes, Melinda Sipos, Zoltán Sipos, Eszter Fördős, Piroska Markacz, Barbara Balázs, Nathalie Lancelot, Sylvie Berger, Eric Raimbaud, David Brown, Laurent-Michel Vuillard, Laure Haberkorn, Cyprian Cukier, Zoltán Szlávik, and Stephen Hanessian*

Cite This: *ACS Med. Chem. Lett.* 2022, 13, 949−954

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: On the basis of the knowledge that the proline-rich hot spot **PPP**RPP region of P(151)PSNPPPRPP(160), an oligopeptide derived from the cytosolic portion of $p22^{phox}$ (p22), binds to the single functional bis-SH3 domain of the regulatory protein $p47^{phox}$ (p47), we designed a mimetic of the tripeptide **PPP** based on NMR and X-ray crystallographic data for the p22(151−161) peptide PPSN**PPP**RPPA with a peptide construct. Incorporation of the synthetic pseudo-triproline mimetic Pro-Pro-Cyp in a molecule derived from molecular modeling studies led to only a 7-fold diminution in activity in a surface plasmon resonance assay relative to the same molecule containing the natural Pro-Pro-Pro tripeptide. The alternative sequence corresponding to a Pro-Cyp-Pro insertion was inactive. This is a first example of the use of a triproline mimetic to interfere with the formation of the p47−p22 complex, which is critical for the activation of NOX, leading to the production of reactive oxygen species as superoxide anions.



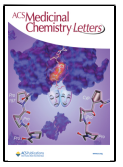
KEYWORDS: *Triproline mimetic, protein−protein interaction, cytosolic proteins, hot spots*

The NADPH oxidases (reduced forms of nicotinamide adenine dinucleotide phosphate oxidases) are a family of membrane-associated multicomponent enzymes present in phagocytes and macrophages.[1−5] Also known as NOX, they are widely distributed in eukaryotic organisms and play a crucial role in maintaining the delicate balance of physiological redox processes responsible for the maintenance of host defense mechanisms against invading pathogens as well as in recruiting inflammatory cells to the site of infection.[6−8] This is accomplished through reduction of molecular oxygen across membranes and release of reactive oxygen species (ROS) in the form of superoxide anions ($O_2^{\bullet-}$) that destroy the biochemical machinery of invading microorganisms.[9] Among the seven isoforms of these enzymes, NOX2 (NADPH 2, or $gp91^{phox}$), a phagocystic oxidase, has garnered much attention because of its ubiquitous presence in tissues and organs, where ROS production can trigger physiological responses that ultimately lead to innate and adaptive immunity.[10]

NADPH oxidase comprises six subunits, including the trans membrane p22 and NOX2, which in combination with flavocytochrome becomes the catalytic site of the oxidase complex. Regulatory cytosolic proteins such as p47 undergo a series of phosphorylations, eventually forming reversible contacts with other multidomain proteins.[11]

NOX2 becomes catalytically active in vivo when trans-membrane protein p22 binds to the single functional bis-SH3 domain of the regulatory protein p47, leading to an enzyme complex that enhances the production of ROS.[12−14] Under normal physiological conditions, ROS production mediated by NOX2 is highly regulated to defend against invading pathogens without causing damage to host tissue. Upon the action of external stimuli, the dormant or resting-state form of NADPH oxidase is activated through a series of reversible protein−protein interactions (PPIs) among its regulatory proteins to produce ROS in such a manner that host tissue is not damaged.[15] However, the beneficial gatekeeper role of NOX2 toward external harmful stimuli can be jeopardized by the overproduction of ROS under certain pathological conditions, causing oxidative stress and a cascade of physiological events resulting in serious diseases such as cancer, cystic fibrosis,

Received: March 3, 2022
Accepted: April 26, 2022
Published: May 10, 2022



https://doi.org/10.1021/acsmedchemlett.2c00094
ACS Med. Chem. Lett. 2022, 13, 949−954

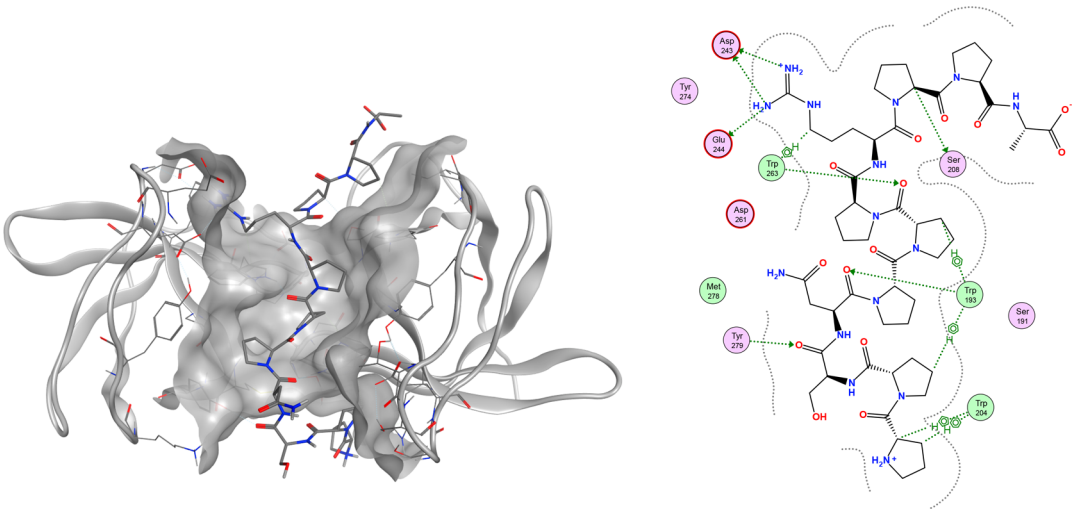


Figure 1. Structure of p22(151−161) peptide PPSN**PPP**RPPA (**1**) cocrystallized with a tandem protein construct.

schizophrenia, Alzheimer's disease, and a host of cardiac- and lung-related diseases.[1,16−19] Since the activation of the catalytic site of the NOX2 enzyme complex involves the interaction of the essential regulatory p47 and p22 proteins to produce ROS in an inducible manner, it has been suggested that interfering with this specific PPI can be exploited to render NOX2 as a druggable target.[1,20,21]

Developing small-molecule inhibitors of p47 presents obvious challenges because of the relatively large surfaces of the interacting proteins at the site of contact.[22] Interestingly, a number of natural products such as apocynin, celastrol, and glyotoxin have been reported as NOX2 inhibitors.[1,21] Among the synthetic compounds, ebselen, a selenoindazole discovered by high-throughput screening, continues to attract attention.[20] PR-39, a peptide of bacterial origin, has been reported to inhibit NADPH oxidase activity by binding to the Src homology domains of p47.[23] A series of pyrazolopyridinediones showed selective NOX4 activity but were inert toward NOX2.[24] Recently, dimeric 2-aminoquinolines bridged by a ethylenedioxa ether linker exhibiting in vitro K_i activity in the 20 μM range were obtained using a fragment-based approach.[25]

Interfering with PPIs through the use of small peptides or peptidomimetics has been a successful strategy in drug discovery.[26] In such cases, identifying so-called "hot spots" in protein partners engaged in PPIs has been the norm. Given the available structural information related to NOX, we considered a peptidomimetic approach to interfere with the PPI of p47 with p22 as a proof of concept.

The structure of p47 has been investigated by elegant X-ray crystallographic studies[27] and in solution by NMR spectroscopy.[22] Valuable insights into the conformation of p47 have identified critical regions for recognition, and relevant amino acid sequences, including proline-rich domains (PRDs), have been proposed. The structure of a p47−p22 complex comprising the conserved tandem SH3 domain of p47 bound to an oligopeptide P(151)PSN**PPP**RPP(160) derived from the cytosolic portion of p22 shows that binding involves the proline-rich sequence **PPP**RPP of p22.[28]

The prospects of developing a small-molecule inhibitor of the PPIs between p47 and the hotspots in p22 to control overproduction of ROS represents a major challenge because of the enormous conformational change of the cytosolic p47 component. In fact, p47 needs to adopt an activated conformation by phosphorylation of serine residues in order to bind to p22 prior to translocation of the complex from the cytosol to the membrane.[28] As a rule, developing peptidomimetics of peptides with fewer than six residues is a good starting point, whereas peptide sequences consisting of 6−15 residues are considered challenging.[29,30]

To optimize the bioactive peptide sequence, the hot spots in the 16 amino acid sequence in p22(151−166) that would potentially interact with regions of p47 were identified using X-ray crystallography, surface plasmon resonance (SPR), and an Ala scan. A cocrystal structure of the extended conformation of p47 with the truncated undecapeptide PPSN**PPP**RPPA (**1**) is shown in Figure 1 (PDB: 7YXW).

To improve the crystallizability of the protein and to avoid the domain swap observed by Rittinger,[28] a single A200G mutation was introduced. The N-terminal Pro-Pro (151−152) is involved in a hydrophobic interaction with Trp(204) and Trp(193), while the Ser-Asn (153−154) residue acts more like a linker to the central triproline unit. Of particular interest is the spatial alignment of the **Pro-Pro-Pro** (155−157) triad, which is engaged in hydrophobic and hydrogen-bonding interactions with Trp(193) and Trp(263), respectively.

To determine the individual roles of the nine central amino acids in the 16 amino acid sequence PPSN**PPP**RPPAEARKK, we conducted an Ala scan from Ser(153) to Glu(162) in conjunction with binding affinity and free enthalpy changes. We were pleased to find that the **PPP**R(155−158) sequence was the hot spot of p22, and hence, it was considered as a suitable candidate for peptidomimetic design. Furthermore, the spatial disposition of the central Pro(156) within the triproline triad and the ionic interaction of Arg(158) with Asp(243) and Glu(244) were critical in the conceptual design of the intended triproline mimetic. The Ala scan revealed that replacement of Pro(159) in the C-terminal part of PPSN**PPP**RPPAEARKK has no impact on binding, while Pro(160) has an important role in occupying a hydrophobic region. Taken together, these studies indicated that the pentapeptide EARKK could be removed, leaving the proline-rich undecapeptide PPSN**PPP**RPPA (**1**) representing p22(151−161) as a truncated construct. Having garnered sufficient structural information from the X-ray and Ala scan studies, we considered undecapeptide **1** to be a starting point in search

https://doi.org/10.1021/acsmedchemlett.2c00094
ACS Med. Chem. Lett. 2022, 13, 949−954

of truncated analogues and potential small-molecule peptidomimetics. The main challenge was to replace the natural amino acid sequence of the **PPP**R(155−158) hot spot involved in most of the essential interactions with p47 with a mimetic molecule that would adopt a well-defined geometry in the binding groove comprising two SH3 domains.

On the basis of these results and our understanding of the role of each amino acid, the following structural optimizations were performed to design a truncated peptidomimetic (data not shown): Pro(151) and Pro(152) were replaced by lipophilic groups; Ser(153) and Asp(154) were replaced by a suitable linker; Arg(158) was truncated or left unmodified; and Pro(159) and Pro(160) were replaced by lipophilic side chains. Two of the optimized triproline peptidomimetics are presented as **I** and **II** in Figure 2. The substantial reduction of

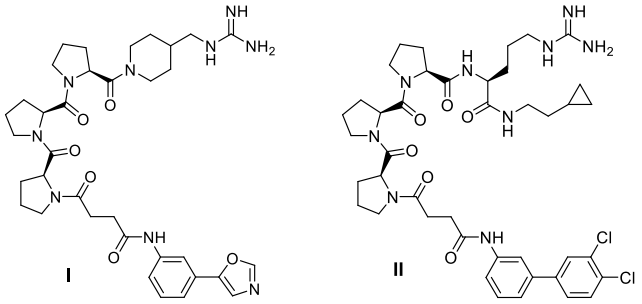


Figure 2. Structures of triproline mimetics **I** and **II**.

molecular weight and the maintained affinity of **II** resulted in a significantly better ligand efficiency compared with the p22(151−166) peptide (vide infra). Compound **II** was chosen as a surrogate for further modifications.

As part of our ongoing interest in the design and synthesis of proline-derived motifs and peptidomimetics,[31−35] we recently reported the stereocontrolled synthesis of a series of diastereomeric diproline (Pro-Pro) mimetics represented by a generic pseudo-diproline dimeric Pro-Cyp structure (Figure 3).[36] The conceptual design element considered replacing the

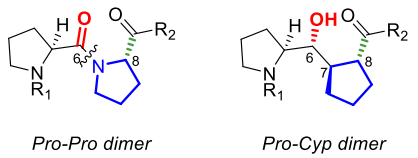


Figure 3. Pro-Pro and pseudo-diproline Pro-Cyp motifs.

second Pro in the Pro-Pro dimer with a cyclopentanecarboxylic acid (Cyp) in which the stereochemical combinations of three contiguous stereogenic centers were unambiguously established. The use of Cyp as a proline surrogate has been previously reported sporadically as an effort to simulate a diproline motif, but seldom in the context of a medicinally relevant target.[37−44] For the purposes of a triproline mimetic for the **Pro-Pro-Pro** (155−157) sequence in undecapeptide **1** representing p22 (Figure 1), we envisaged two variants, one in which the Cyp unit would replace the C-terminal Pro(157) (**B**) and another in which the Cyp unit would replace the central Pro(156) (**C**) (Figure 4A). Superposition of the Pro-Pro-Pro subunit (**A**) representing the natural **PPP** segment with the two pseudo-triproline motifs **B** and **C** showed excellent overlap (Figure 4B).

The appropriate stereoisomer of Cyp was selected on the basis of docking the candidate compounds into p47 protein. The Cyp diproline unit was placed in the two possible positions corresponding to the triproline core of compound **II**. All possible diastereomers were modeled, and thus, a total of 16 isomers were docked. Compound **II** maintains all of the specific and necessary hydrogens bonds and salt bridges as p22(151−161). The cyclopropylethyl chain in **II** replacing the last proline in undecapeptide **1** sits on a hydrophobic surface. The succinamide moiety takes the place of the first four amino acids of the undecapeptide, while the appended 3,4-dichlorobiphenyl ring is well-positioned relative to the hydrophobic surface formed by Trp(204) and Trp(193). The Pro-Pro-Cyp 2*S*,6*R*,7*R*,8*R* isomer **III**, replacing both **Pro-Pro**(155−156) and **Pro-Pro**(156−157), maintained the helical structure and most of the specific interactions with the protein. It was therefore chosen for synthesis and evaluation. When **Pro-Pro**(155−156) was replaced, rotation of the amide bond between Cyp and the linker was observed in some of the docking poses as a result of the subtle conformation change of Cyp compared with diproline, causing strain on the linker. In either case, the Cyp-containing pseudo-triproline unit would expose a hydrophobic surface similarly to proline and provide possible polar interactions with the stereodefined OH group replacing an amide carbonyl. Preliminary data on the two pseudo-triproline analogues **I** and **II** as internal reference compounds revealed that the latter would be a better analogue to use as a control. Pro-Pro-Cyp **B** and Pro-Cyp-Pro **C** motifs were elaborated to include an N-terminal 3,4-dichlorobiphenyl succinamide and a C-terminal hydrophobic cyclopropylethylarginine amide, leading to the fully decorated analogues **III** and **IV**, respectively (Figure 5), each having a 6*R*,7*R*,8*R* stereochemistry comprising three contiguous stereogenic centers.

We were pleased to find that analogue **III**, in which **Pro**(157) was replaced by a cyclopentanecarboxamide, was only 7 times less active in the SPR assay relative to triproline

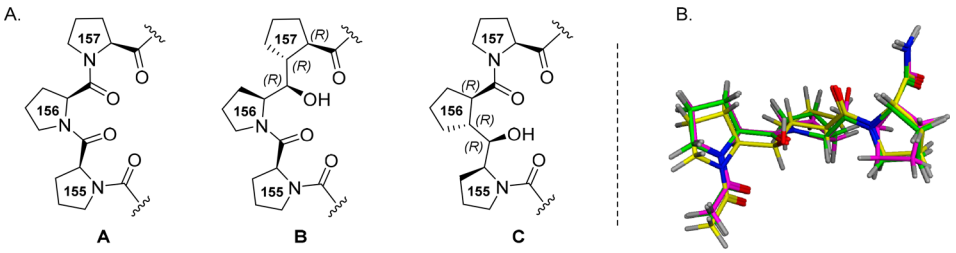


Figure 4. (A) Structures of Pro-Pro-Pro trimer **A**, Pro-Pro-Cyp trimer **B**, and Pro-Cyp-Pro trimer **C**. (B) Gas-phase optimized geometries of **A** (green), **B** (magenta), and **C** (yellow). Oxygen atoms ares shown in red and nitrogen atoms in blue.

https://doi.org/10.1021/acsmedchemlett.2c00094
ACS Med. Chem. Lett. 2022, 13, 949−954

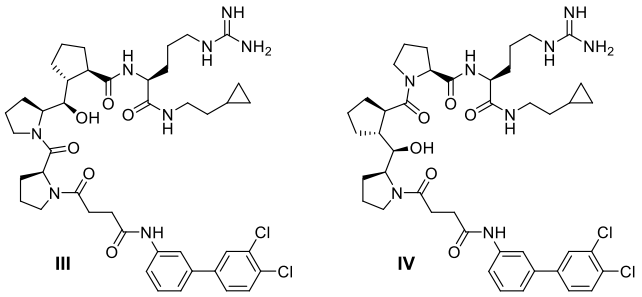


Figure 5. Structures of triproline mimetics **III** and **IV**.

analogue **II**. The SPR ligand efficiencies (SPR LEs) of control analogue **II** and triproline mimetic analogue **III** were comparable to that of p22(151−166). In contrast, analogue **IV** was totally inactive. Thus, replacement of the pivotal Pro(157) in undecapeptide model **1**, a surrogate for p22, with a cyclopentanecarboxamide in the form of synthetic mimetic **III** does not negatively affect the PPI with p47. Moreover, it appears that the presence of the middle Pro(156) in the Pro-Pro-Cyp triad in **III** is essential for activity (Figures 5, 6 and

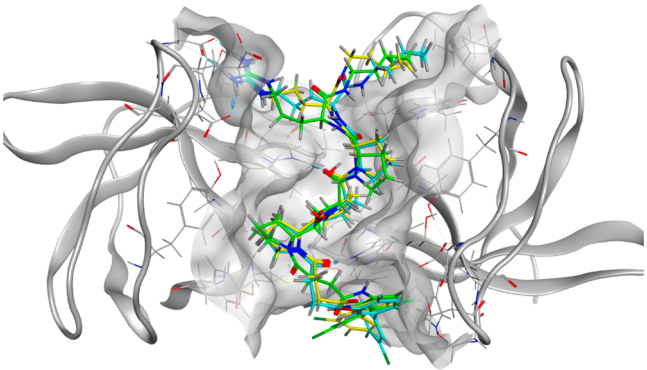


Figure 6. Superimposition of pseudopeptides **II**−**IV** in the p47 SH3 domain.

Table 1. Experimental Affinities of Triproline Mimetic Analogues

compound	SPR K_d (μM)	SPR LE
p22(151−166)	0.214	0.08
I	5.71	0.15
II	0.043	0.18
III	0.312	0.15
IV	>30	NA

Table 1). The replacement of the Pro(156) amide carbonyl in the **PPPR** segment of undecapeptide **1** with an *R*-configured secondary alcohol facing the solvent in the active analogue **III** is beneficial, since it does not disrupt the specific hydrogen-bonding interactions in the complex.

The side chains of peptidomimetic **III** could potentially be further optimized to improve the affinity and drug-likeness. The arginine amino acid or the guanidine derivatives seem to be a bottleneck for cellular penetration. Because of their strong basic character, they are invariably in a protonated state under physiological conditions, which may disfavor membrane penetration. The lipophilic side chain and linker connected to the N-terminal unit in **III** could be optimized to reduce the flexibility.[45]

Although the proline side chains in undecapeptide **1** are only capable of hydrophobic interactions and the backbone forms two hydrogen bonds with p47, the essential helical structure is difficult to mimic perfectly. Further studies are required to develop better di- or triproline mimetics in proline-rich polypeptides to achieve higher affinity while improving drug-likeness.

In conclusion, inasmuch as the design and synthesis of pseudo-triproline mimetic **III** was part of a proof-of-concept study, the result represents a first example of a rationally designed and novel synthetic triproline mimetic to exhibit submicromolar in vitro activity against the binding of p47 with p22, two critical proteins involved in the activation of NOX1 and NOX2 in relation to regulation of ROS overproduction. Large numbers of biologically relevant PPIs in eukaryotic cells[28] involve linear motifs[46] in which proline-rich domains encompassed within structurally distinct polyproline type II helices play critical roles in recognition and specificity with other proteins acting as partners.[47,48] Incorporation of our Pro-Cyp dipeptide mimetic as a pseudo-diproline or extended versions of it with Pro-Cyp combinations with optimized N- and C-terminal residues to simulate the action of natural oligoproline sequences in designated proteins would augur well for the development of new drug prototypes as modulators of PPIs in medicinally relevant projects.[49−51]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00094.

Synthetic schemes; general information; experimental procedures; ^{1}H NMR and HRMS characterization for all compounds; additional ^{13}C NMR description, NMR spectra, and HPLC for **I**−**IV**; general procedures for crystallography, modeling, and surface plasmon resonance; ala-scan raw data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Stephen Hanessian − *Department of Chemistry, Université de Montréal, Montréal, QC H2V 0B3, Canada; Department of Pharmaceutical Sciences, University of California Irvine, Irvine, CA 92617, USA;* orcid.org/0000-0003-3582-6972; Email: stephen.hanessian@umontreal.ca

Authors

Jean-Baptiste Garsi − *Department of Chemistry, Université de Montréal, Montréal, QC H2V 0B3, Canada*

Balázs Komjáti − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*

Gregorio Cullia − *Department of Chemistry, Université de Montréal, Montréal, QC H2V 0B3, Canada*

Imre Fejes − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*

Melinda Sipos − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*

Zoltán Sipos − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*

Eszter Fördős − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*

Piroska Markacz − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*
Barbara Balázs − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*
Nathalie Lancelot − *Institut de Recherche Servier, 78290 Croissy, France*
Sylvie Berger − *Institut de Recherche Servier, 78290 Croissy, France*
Eric Raimbaud − *Institut de Recherche Servier, 78290 Croissy, France*
David Brown − *Institut de Recherche Servier, 78290 Croissy, France*
Laurent-Michel Vuillard − *Institut de Recherche Servier, 78290 Croissy, France*
Laure Haberkorn − *Institut de Recherche Servier, 78290 Croissy, France*
Cyprian Cukier − *Selvita S.A., 30-348 Kraków, Poland*
Zoltán Szlávik − *Servier Research Institute of Medicinal Chemistry, Budapest 1031, Hungary*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.2c00094

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Generous financial assistance from the NSERC/Servier Senior Industrial Research Chair Program is gratefully acknowledged. We thank Dr. Sofiane Hocine for assistance with the artwork for the supplementary cover and the graphical abstract.

■ ABBREVIATIONS

Cyp, cyclopentane carboxylic acid; NADPH, nicotinamide adenine dinucleotide phosphate; NOX, NADPH oxidase; ROS, reactive oxygen species; PPI, protein−protein interaction; PRD, proline-rich sequence recognition domain; SH3, SRC homology 3; SPR, surface plasmon resonance

■ REFERENCES

(1) Diebold, B. A.; Smith, S. M. E.; Li, Y.; Lambeth, J. D. NOX2 as a Target for Drug Development: Indications, Possible Complications, and Progress. *Antioxid. Redox Signaling* **2015**, *23*, 375−405.
(2) Babior, B. M.; Lambeth, J. D.; Nauseef, W. The Neutrophil NADPH Oxidase. *Arch. Biochem. Biophys.* **2002**, *397*, 342−344.
(3) Vignais, P. V. The Superoxide-Generating NADPH Oxidase: Structural Aspects and Activation Mechanism. *Cell. Mol. Life Sci.* **2002**, *59*, 1428−1459.
(4) Babior, B. M. NADPH Oxidase: An Update. *Blood* **1999**, *93*, 1464−1476.
(5) Groemping, Y.; Rittinger, K. Activation and Assembly of the NADPH Oxidase: A Structural Perspective. *Biochem. J.* **2005**, *386*, 401−416.
(6) Bedard, K.; Krause, K. H. The NOX Family of ROS-Generating NADPH Oxidases: Physiology and Pathophysiology. *Physiol. Rev.* **2007**, *87*, 245−313.
(7) Lambeth, J. D. NOX Enzymes and the Biology of Reactive Oxygen. *Nat. Rev. Immunol.* **2004**, *4*, 181−189.
(8) Sumimoto, H. Structure, Regulation and Evolution of Nox-Family NADPH Oxidases That Produce Reactive Oxygen Species. *FEBS J.* **2008**, *275*, 3249−3277.
(9) Rada, B.; Leto, T. L. Oxidative Innate Immune Defenses by Nox/Duox Family NADPH Oxidases. *Contrib Microbiol.* **2008**, *15*, 164−187.
(10) Augsburger, F.; Filippova, A.; Rasti, D.; Seredenina, T.; Lam, M.; Maghzal, G.; Mahiout, Z.; Jansen-Dürr, P.; Knaus, U. G.; Doroshow, J.; Stocker, R.; Krause, K. H.; Jaquet, V. Pharmacological Characterization of the Seven Human NOX Isoforms and Their Inhibitors. *Redox Biol.* **2019**, *26* (May), 101272.
(11) Rastogi, R.; Geng, X.; Li, F.; Ding, Y. NOX Activation by Subunit Interaction and Underlying Mechanisms in Disease. *Front. Cell. Neurosci.* **2017**, *10*, 1−13.
(12) Sumimoto, H.; Kage, Y.; Nunoi, H.; Sasaki, H.; Nose, T.; Fukumaki, Y.; Ohno, M.; Minakami, S.; Takeshige, K. Role of Src Homology 3 Domains in Assembly and Activation of the Phagocyte NADPH Oxidase. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 5345−5349.
(13) Leto, T. L.; Adams, A. G.; De Mendez, I. Assembly of the Phagocyte NADPH Oxidase: Binding of Src Homology 3 Domains to Proline-Rich Targets. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 10650−10654.
(14) Sumimoto, H.; Hata, K.; Mizuki, K.; Ito, T.; Kage, Y.; Sakaki, Y.; Fukumaki, Y.; Nakamura, M.; Takeshige, K. Assembly and Activation of the Phagocyte NADPH Oxidase. *J. Biol. Chem.* **1996**, *271*, 22152−22158.
(15) Dröge, W. Free Radicals in the Physiological Control of Cell Function. *Physiol. Rev.* **2002**, *82*, 47−95.
(16) Quinn, M. T.; Ammons, M. C. B.; DeLeo, F. R. The Expanding Role of NADPH Oxidases in Health and Disease: No Longer Just Agents of Death and Destruction. *Clin. Sci.* **2006**, *111*, 1−20.
(17) Cave, A. C.; Brewer, A. C.; Narayanapanicker, A.; Ray, R.; Grieve, D. J.; Walker, S.; Shah, A. M. NADPH Oxidases in Cardiovascular Health and Disease. *Antioxid. Redox Signaling* **2006**, *8*, 691−728.
(18) Hernandes, M. S.; D'Avila, J. C.; Trevelin, S. C.; Reis, P. A.; Kinjo, E. R.; Lopes, L. R.; Castro-Faria-Neto, H. C.; Cunha, F. Q.; Britto, L. R. G.; Bozza, F. A. The Role of Nox2-Derived ROS in the Development of Cognitive Impairment after Sepsis. *J. Neuroinflammation* **2014**, *11*, 36.
(19) Costa, A.; Scholer-Dahirel, A.; Mechta-Grigoriou, F. The Role of Reactive Oxygen Species and Metabolism on Cancer Cells and Their Microenvironment. *Semin. Cancer Biol.* **2014**, *25*, 23−32.
(20) Smith, S. M. E.; Min, J.; Ganesh, T.; Diebold, B.; Kawahara, T.; Zhu, Y.; McCoy, J.; Sun, A.; Snyder, J. P.; Fu, H.; Du, Y.; Lewis, I.; Lambeth, J. D. Ebselen and Congeners Inhibit NADPH Oxidase 2-Dependent Superoxide Generation by Interrupting the Binding of Regulatory Subunits. *Chem. Biol.* **2012**, *19*, 752−763.
(21) Kim, J. A.; Neupane, G. P.; Lee, E. S.; Jeong, B. S.; Park, B. C.; Thapa, P. NADPH Oxidase Inhibitors: A Patent Review. *Expert Opin. Ther. Pat.* **2011**, *21*, 1147−1158.
(22) Ogura, K.; Nobuhisa, I.; Yuzawa, S.; Takeya, R.; Torikai, S.; Saikawa, K.; Sumimoto, H.; Inagaki, F. NMR Solution Structure of the Tandem Src Homology 3 Domains of P47 Phox Complexed with a P22phox-Derived Proline-Rich Peptide. *J. Biol. Chem.* **2006**, *281* (6), 3660−3668.
(23) Shi, J.; Ross, C. R.; Leto, T. L.; Blecha, F. PR-39, a Proline-Rich Antibacterial Peptide That Inhibits Phagocyte NADPH Oxidase Activity by Binding to Src Homology 3 Domains of P47phox. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 6014−6018.
(24) Laleu, B.; Gaggini, F.; Orchard, M.; Fioraso-Cartier, L.; Cagnon, L.; Houngninou-Molango, S.; Gradia, A.; Duboux, G.; Merlot, C.; Heitz, F.; Szyndralewiez, C.; Page, P. First in Class, Potent, and Orally Bioavailable NADPH Oxidase Isoform 4 (Nox4) Inhibitors for the Treatment of Idiopathic Pulmonary Fibrosis. *J. Med. Chem.* **2010**, *53*, 7715−7730.
(25) Solbak, S. M. Ø.; Zang, J.; Narayanan, D.; Høj, L. J.; Bucciarelli, S.; Softley, C.; Meier, S.; Langkilde, A. E.; Gotfredsen, C. H.; Sattler, M.; Bach, A. Developing Inhibitors of the P47phox-P22phox Protein-Protein Interaction by Fragment-Based Drug Discovery. *J. Med. Chem.* **2020**, *63* (3), 1156−1177.
(26) Sillerud, L. O.; Larson, R. S. Design and Structure of Peptide and Peptidomimetic Antagonists of Protein-Protein Interaction. *Curr. Protein Pept. Sci.* **2005**, *6* (2), 151−169.

(27) Yuzawa, S.; Suzuki, N. N.; Fujioka, Y.; Ogura, K.; Sumimoto, H.; Inagaki, F. Crystallization and Preliminary Crystallographic Analysis of the Autoinhibited Form of the Tandem SH3 Domain of P47phox. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2003**, *59* (8), 1479−1480.
(28) Groemping, Y.; Lapouge, K.; Smerdon, S. J.; Rittinger, K. Molecular Basis of Phosphorylation-Induced Activation of the NADPH Oxidase. *Cell* **2003**, *113*, 343−355.
(29) Lau, J. L.; Dunn, M. K. Therapeutic Peptides: Historical Perspectives, Current Development Trends, and Future Directions. *Bioorg. Med. Chem.* **2018**, *26*, 2700−2707.
(30) Hummel, G.; Reineke, U.; Reimer, U. Translating Peptides into Small Molecules. *Mol. BioSyst.* **2006**, *2*, 499−508.
(31) Hocine, S.; Berger, G.; Hanessian, S. Design and Synthesis of Backbone-Fused, Conformationally Constrained Morpholine-Proline Chimeras. *J. Org. Chem.* **2020**, *85*, 4237−4247.
(32) Vilchis-Reyes, M. A.; Hanessian, S. Proline Methanologues: Design, Synthesis, Structural Properties, and Applications in Medicinal Chemistry. *Top Heterocycl Chem.* **2015**, *48*, 51−96.
(33) Berger, G.; Vilchis-Reyes, M.; Hanessian, S. Structural Properties and Stereochemically Distinct Folding Preferences of 4,5-*cis* and *trans*-Methano-L-proline Oligomers: The Shortest Crystalline PPII-Type Helical Proline-Derived Tetramer. *Angew. Chem., Int. Ed.* **2015**, *54*, 13268−13272.
(34) Hanessian, S.; Auzzas, L. The Practice of Ring Constraint in Peptidomimetics Using Bicyclic and Polycyclic Amino Acids. *Acc. Chem. Res.* **2008**, *41*, 1241−1251.
(35) Hanessian, S. Structure-Based Organic Synthesis of Drug Prototypes: A Personal Odyssey. *ChemMedChem* **2006**, *1*, 1300−1330.
(36) Garsi, J.-B.; Aguiar, P. M.; Hanessian, S. Design of Pseudodiproline Dimers as Mimetics of Pro-Pro Units: Stereocontrolled Synthesis, Configurational Relevance, and Structural Properties. *J. Org. Chem.* **2021**, *86*, 16834−16847.
(37) Hanko, R.; Rabe, K.; Dally, R.; Hoppe, D. Stereoselektive Synthese von Cyclischen Hydroxyalkyl-Dipeptidisosteren über Metallierte *N*,*N*-Dialkylcarbamidsäure-2-alkenylester. *Angew. Chem.* **1991**, *103*, 1725−1727.
(38) Jarho, E. M.; Venäläinen, J. I.; Huuskonen, J.; Christiaans, J. A. M.; Garcia-Horsman, J. A.; Forsberg, M. M.; Järvinen, T.; Gynther, J.; Männistö, P. T.; Wallén, E. A. A. A Cyclopent-2-Enecarbonyl Group Mimics Proline at the P2 Position of Prolyl Oligopeptidase Inhibitors. *J. Med. Chem.* **2004**, *47*, 5605−5607.
(39) Dai, N.; Wang, X. J.; Etzkorn, F. A. The Effect of a Trans-Locked Gly-Pro Alkene Isostere on Collagen Triple Helix Stability. *J. Am. Chem. Soc.* **2008**, *130*, 5396−5397.
(40) Wang, X. J.; Xu, B.; Mullins, A. B.; Neiler, F. K.; Etzkorn, F. A. Conformationally Locked Isostere of PhosphoSer-Cis-Pro Inhibits Pin1 23-Fold Better than PhosphoSer-Trans-Pro Isostere. *J. Am. Chem. Soc.* **2004**, *126*, 15533−15542.
(41) Wang, X. J.; Hart, S. A.; Xu, B.; Mason, M. D.; Goodell, J. R.; Etzkorn, F. A. Serine-Cis-Proline and Serine-Trans-Proline Isosteres: Stereoselective Synthesis of (*Z*)- and (*E*)-Alkene Mimics by Still-Wittig and Ireland-Claisen Rearrangements. *J. Org. Chem.* **2003**, *68*, 2343−2349.
(42) Maassen, A.; Gebauer, J. M.; Theres Abraham, E.; Grimm, I.; Neudörfl, J.-M.; Kühne, R.; Neundorf, I.; Baumann, U.; Schmalz, H.-G. Triple-Helix-Stabilizing Effects in Collagen Model Peptides Containing PPII-Helix-Preorganized Diproline Modules. *Angew. Chem., Int. Ed.* **2020**, *59*, 5747−5755.
(43) Vasta, J. D.; Choudhary, A.; Jensen, K. H.; McGrath, N. A.; Raines, R. T. Prolyl 4-Hydroxylase: Substrate Isosteres in Which an (*E*)- or (*Z*)-Alkene Replaces the Prolyl Peptide Bond. *Biochemistry* **2017**, *56*, 219−227.
(44) Bandur, N. G.; Harms, K.; Koert, U. First Stereoselective Synthesis of a Pro-Pro *E*-Alkene Dipeptide Isostere. *Synlett* **2005**, *5*, 773−776.
(45) Flaten, G. E.; Kottra, G.; Stensen, W.; Isaksen, G.; Karstad, R.; Svendsen, J. S.; Daniel, H.; Svenson, J. In Vitro Characterization of Human Peptide Transporter HPEPT1 Interactions and Passive Permeation Studies of Short Cationic Antimicrobial Peptides. *J. Med. Chem.* **2011**, *54*, 2422−2432.
(46) Davey, N. E.; Van Roey, K.; Weatheritt, R. J.; Toedt, G.; Uyar, B.; Altenberg, B.; Budd, A.; Diella, F.; Dinkel, H.; Gibson, T. J. Attributes of Short Linear Motifs. *Mol. BioSyst.* **2012**, *8*, 268−281.
(47) Freund, C.; Schmalz, H. G.; Sticht, J.; Kuhne, R. Proline Rich Sequence Recognition Domain (PRD): Ligands, Function and Inhibition. *Handb. Exp. Pharmacol.* **2008**, *186*, 407−429.
(48) Zarrinpar, A.; Bhattacharyya, R. P.; Lim, W. A. The Structure and Function of Proline Recognition Domains. *Sci. STKE* **2003**, *2003*, re8.
(49) Kay, B. K.; Williamson, M. P.; Sudol, M. The Importance of Being Proline: The Interaction of Proline-Rich Motifs in Signaling Proteins with Their Cognate Domains. *FASEB J.* **2000**, *14*, 231−241.
(50) Opitz, R.; Müller, M.; Reuter, C.; Barone, M.; Soicke, A.; Roske, Y.; Piotukh, K.; Huy, P.; Beerbaum, M.; Wiesner, B.; Beyermann, M.; Schmieder, P.; Freund, C.; Volkmer, R.; Oschkinat, H.; Schmalz, H.-G.; Kühne, R. A Modular Toolkit to Inhibit Proline-Rich Motif−Mediated Protein−Protein Interactions. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 5011−5016.
(51) Lu, H.; Zhou, Q.; He, J.; Jiang, Z.; Peng, C.; Tong, R.; Shi, J. Recent Advances in the Development of Protein−Protein Interactions Modulators: Mechanisms and Clinical Trials. *Signal Transduction Targeted Ther.* **2020**, *5*, 1−23.

https://doi.org/10.1021/acsmedchemlett.2c00094
ACS Med. Chem. Lett. 2022, 13, 949−954