

VIP Very Important Paper

Special
Collection

Targeting the IspD Enzyme in the MEP Pathway: Identification of a Novel Fragment Class

Eleonora Diamanti⁺,^[a, b] Mostafa M. Hamed⁺,^[a] Antoine Lacour,^[a, c] Patricia Bravo,^[d, e] Boris Illarionov,^[f] Markus Fischer,^[f] Matthias Rottmann,^[d, e] Matthias Witschel,^[g] and Anna K. Hirsch^{*[a, b, c]}

Dedicated to the memory of Prof. Dr François Diederich (1952–2020), who initiated our consortium.

The enzymes of the 2-C-methylerythritol-D-erythritol 4-phosphate (MEP) pathway (MEP pathway or non-mevalonate pathway) are responsible for the synthesis of universal precursors of the large and structurally diverse family of isoprenoids. This pathway is absent in humans, but present in many pathogenic organisms and plants, making it an attractive source of drug targets. Here, we present a high-throughput

screening approach that led to the discovery of a novel fragment hit active against the third enzyme of the MEP pathway, PflSpD. A systematic SAR investigation afforded a novel chemical structure with a balanced activity–stability profile (16). Using a homology model of PflSpD, we proposed a putative binding mode for our newly identified inhibitors that sets the stage for structure-guided optimization.

The 2-C-methylerythritol-D-erythritol 4-phosphate (MEP) pathway, consists of seven enzymes, and is an essential biosynthetic pathway for the production of isopentenyl diphosphate (IDP) and its isomer dimethylallyl diphosphate (DMADP) both of which are universal building blocks of isoprenoids, a large and structurally diverse group of natural products with crucial physiological functions.^[1] As the MEP pathway is absent in humans, but essential in most Gram-negative bacteria, *Mycobacterium tuberculosis* and *Plasmodium falciparum*, the parasite responsible for malaria, it is an attractive source of anti-infective drug targets.^[2] Inhibitors able to target this pathway have the advantage to exhibit a novel mechanism of action without target-based side effects. Nevertheless, despite the important functions served by the MEP pathway few inhibitors have been reported so far.^[3] Importantly, fosmidomycin,^[4] a potent inhibitor of the second enzyme of the MEP pathway, IspC or DXR, has undergone phase II clinical trials as antimalarial chemotherapeutic agent in combination with clindamycin and piper-
aquine, validating the enzymes of the MEP pathway as drug targets.^[5] In the present study, we focused our attention on IspD, alternatively known as MEP cytidyltransferase or ygbP protein, that is the third enzyme in the MEP pathway.^[6] IspD catalyzes the formation of 4-diphosphocytidyl-2-C-methylerythritol (CDP-ME) from MEP and cytidine triphosphate CTP in the presence of Mg²⁺, with the release of inorganic diphosphate (PP_i) (Figure 1).^[7]

Looking at the IspD inhibitors, to date only a few compounds have been reported.^[8] Aiming to enlarge the portfolio of IspD inhibitors and particularly of *Plasmodium* IspD (PflSpD), we performed a high-throughput screening (HTS), using the proprietary BASF library of about 100,000 diverse selected compounds. The search for novel antimalarial com-

[a] Dr. E. Diamanti,⁺ Dr. M. M. Hamed,⁺ A. Lacour, Prof. Dr. A. K. H. Hirsch
Helmholtz Institute for Pharmaceutical Research (HIPS)
Helmholtz Centre for Infection Research (HZI)
Saarland University Campus E8.1, 66123 Saarbrücken (Germany)
E-mail: Anna.Hirsch@helmholtz-hips.de

[b] Dr. E. Diamanti,⁺ Prof. Dr. A. K. H. Hirsch
Helmholtz International Lab for Anti-Infectives
Saarland University Campus E8.1, 66123 Saarbrücken (Germany)

[c] A. Lacour, Prof. Dr. A. K. H. Hirsch
Department of Pharmacy, Saarland University
Campus E8.1, 66123 Saarbrücken (Germany)

[d] P. Bravo, Dr. M. Rottmann
Swiss Tropical and Public Health Institute
Socinstrasse 57, 4002 Basel (Switzerland)

[e] P. Bravo, Dr. M. Rottmann
Universität Basel
Petersplatz 1, 4003 Basel (Switzerland)

[f] Dr. B. Illarionov, Prof. Dr. M. Fischer
Hamburg School of Food Science
University of Hamburg
Grindelallee 117, 20146 Hamburg (Germany)

[g] Dr. M. Witschel
BASF-SE
Carl-Bosch-Strasse 38, 67056 Ludwigshafen (Germany)

[†] These authors contributed equally to this work.

Supporting information for this article is available on the WWW under
<https://doi.org/10.1002/cmdc.202100679>

This article belongs to a Joint Special Collection dedicated to François Diederich.

© 2021 The Authors. ChemMedChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

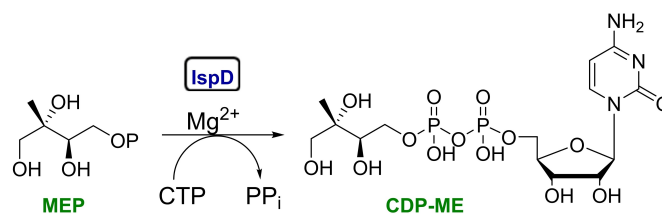


Figure 1. Reaction catalyzed by the IspD protein. MEP: 2-C-methylerythritol-D-erythritol 4-phosphate; CDP-ME: 4-diphosphocytidyl-2-C-methylerythritol.

pounds endowed with a novel mechanism of action has a continuous and long history of research. Although novel approved treatments and preventions helped to save many lives, malaria is still responsible for more than 400,000 deaths worldwide, mostly young children.^[9]

Herein we describe the identification of a fragment-like compound (**16**) able to inhibit *Pf*spD *in vitro* in the micromolar range and with a suitable physicochemical profile (Figure 2).

The screening performed against *Pf*spD led to the identification of compound **1**. Despite its moderate activity it attracted our attention due to its fragment-like size and modular structure that lends itself to chemical modification. In order to identify structural features that are critical for *Pf*spD inhibition, we followed a classical structure–activity relationship (SAR) study, as we could not rely on any structural information about the protein. High-resolution structures are only available for *Escherichia coli* lspD,^[10] besides additional structures from non-pathogenic organisms.^[11] We therefore, conducted a focused SAR study with two inter-related objectives: *i*) find a replacer for the pyrrole ring as it is known to be a structural alert,^[12] *ii*) validate the fragment hit by improving the potency and the physicochemical profile for further optimization. With these goals in mind, we initially started our exploration by keeping the core molecule constant and varying only the terminal nitro group (**1–8**); while, the second subset of molecules (**9–18**) includes modifications around the pyrrole ring.

Half-maximal inhibitory concentration (IC_{50}) values against purified *Pf*spD are reported in Table 1, while Table 2 summa-

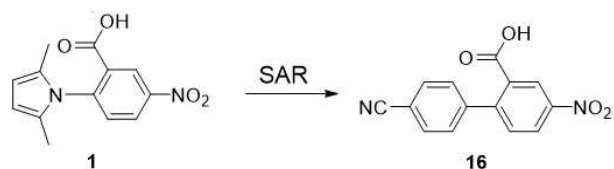


Figure 2. Chemical structures of compounds reported in the present study.

Table 1. Inhibition values by 5-substituted 2-pyrrol-1-benzoic acid derivatives (**1–8**) determined using the coupled photometric assay with purified *Pf*spD.

Compd	R	IC_{50} [μ M]*
1	NO ₂	271 ± 24
2	H	> 500
3	Cl	263 ± 35
4	Br	117 ± 20
5	I	208 ± 37
6	CH ₃	> 500
7	–OCH ₃	> 500
8	–NHCOCH ₃	> 500

Table 2. [*] IC_{50} values were obtained from two independent experiments. Table 2

Inhibition values by 2-substituted-5-nitrobenzoic acid derivatives (**9–18**) determined with the coupled photometric assay using purified *Pf*spD.

Compd	R	IC_{50} [μ M]*
9		264 ± 30
10		225 ± 27
11		> 500
12		> 500
13		> 500
14		> 500
15		277 ± 56
16		151 ± 17
17		> 500
18		280 ± 55

rizes the second set. Details about the assay are reported in the Supporting Information, section 3.

Compounds **1–8** were synthesized following the classical Paal–Knorr pyrrole condensation by refluxing 5-substituted anthranilic acid in toluene with 1.5 eq of 2,5-hexanedione in the presence of molecular sieves (Scheme S1).^[13]

Based on our previously reported discovery of azolopyrimidines^[8a] and pseudilins^[14] as halogenated and allosteric modulators of the enzyme lspD, we were intrigued to also evaluate the influence of halogens in our new scaffold, leading to derivatives **3–5**. In fact, while for the azolopyrimidine scaffold only activity against the plant *Arabidopsis thaliana* (*At*lspD) is reported, the pseudilin-type inhibitors showed potency against the malaria parasite too, *in vitro* and in cell-based assays. Specifically, in our series of compounds, the best halogen turned out to be bromine (**4**) that is slightly more potent than hit **1** and about two-fold more potent than its iodo derivative **5**. By contrast, activity is completely lost upon introduction of an electron-donating group (**6–8**) and also a short elongation with an acetamide group **8** is not tolerated. The drop in activity observed for compound **2** suggests the influence played by a substituent in position 5 of the phenyl ring within this subset of derivatives.

Next, we moved our attention to the pyrrole core. For consistency, we maintained the initial 5-nitrobenzoic acid scaffold, having the 2-position occupied by a small but diverse library of aliphatic and substituted aromatic rings (Table 2). For the synthesis of this series of compounds, we relied on the classical Suzuki cross-coupling reaction between 2-bromo-5-nitrobenzoic acid and the respective boronic acid derivatives (Scheme S2).

Interestingly, the pyrrolidine **9** and the piperidine **10** could replace the dimethyl pyrrole ring without a significant loss in activity. Introducing another heteroatom in the piperidine ring to give the morpholine **11** led to a further decrease in activity. Other 5-membered heterocyclic rings such as the furan **12** and the thiophene **13** were not beneficial for the activity. Although, the unsubstituted phenyl **14** did not show significant activity, further substitution on the ring seemed to be beneficial. Substituents of variable nature such as the methyl **15** and the nitrile **16** restored the activity of the unsubstituted phenyl, suggesting there is room for further modification on this side of the molecule. The nitrile derivative **16** showed almost two-fold higher potency than its methyl analogue **15**. Exploring other polar groups such as the hydroxy **17** was not useful, hinting that the electronic effects exerted by the substituents may affect the activity. Remarkably, further growing on the hydroxyl with an isopropyl **18** regained the activity, indicating some space that could be available to modulate the activity.

To gain further insights into this class of compounds, we built a homology model for PflspD using EclspD (PDB 1152) as a template and docked our compounds into the substrate binding pocket (Figure 3). The docked pose of hit compound **1** (Figure 3a) shows that the carboxylic group forms two H bonds with Lys207 and Ile205 in the binding pocket. Both H bonds were also seen in the case of **4** and **16** with a substituted phenyl instead of the dimethyl pyrrole. Another H bond is formed between the compounds having the terminal NO₂ group and Arg429.

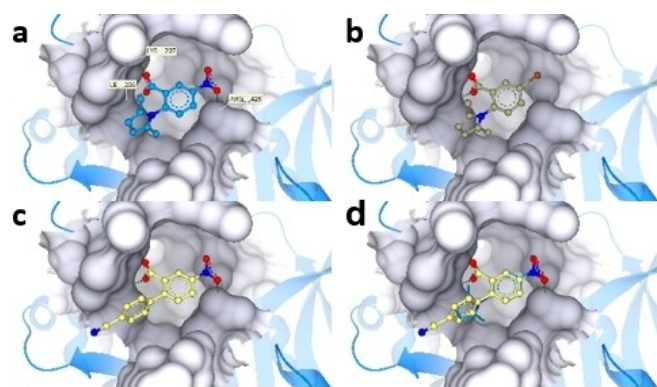


Figure 3. Docked poses of compounds **1**, **4**, and **16** in the homology model of PflspD using SeeSAR 11.0. a) The carboxylic group of **1** (blue) forms two H bonds (green dotted lines) with Lys207 and Ile205 and the NO₂ group is engaged in a H bonding interaction with Arg429. b) The carboxylic group in **4** (beige) forms the same H bonds as for compound **1**. c) Compound **16** (yellow) forms the same H bonds with Lys207, Ile205 and Arg429 as the hit compound **1**. d) Overlay of the docked poses of compounds **1** and **16**.

Table 3. [*] IC₅₀ values were obtained from two independent experiments. Table 3 Summary of ligand-efficiency scores calculated on StarDrop version: 7.0.1.29911.

Compd	1	4	16
PflspD	271 ± 24	117 ± 20	151 ± 17
IC ₅₀ [μM]			
cLogP	1.79	3.84	1.75
MW ^[a]	262.3	294.1	270.2
HA ^[b]	19	17	20
LE ^[c]	0.26	0.32	0.26
LLE ^[d]	1.77	0.085	2.072

[a] Molecular weight. [b] Non-hydrogen atom. [c] Ligand efficiency. [d] Lipophilic ligand efficiency.

Finally, as the affinity for the target is not the only aspect to be considered during fragment-based drug discovery, we also focused our attention on the physicochemical properties of our compounds (see Table 3).^[15] Despite compound **4** showing the best potency in our series of compounds, we do not consider it a suitable candidate for further fragment growing as the lipophilic ligand-efficiency (LLE) parameter is not ideal. Most probably, the better IC₅₀ value is due to the higher cLogP value. Conversely, with compound **16** we have a good balance in all the ligand efficiency scores evaluated. Of note, having an aromatic core with a nitrile substituent as in **16**, has several advantages compared to the pyrrole liability.^[16]

In conclusion, the present report describes the identification of compound **16** as an optimized fragment hit, targeting PflspD with high potential for further fragment growing and optimization.

Acknowledgements

This project received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska Curie grant agreement No 860816. A. K. H. H. acknowledges funding from the European Research Council (ERC starting grant 757913) and the Helmholtz-Association's Initiative and Networking Fund. Open Access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: MEP pathway · IspD · fragment · *Plasmodium falciparum* · drug discovery

- [1] a) J. Gershenzon, N. Dudareva, *Nat. Chem. Biol.* **2007**, *3*, 408–414; b) T. Masini, B. S. Kroezen, A. K. H. Hirsch, *Drug Discov.* **2013**, *18*, 1256–1262.
- [2] S. Heuston, M. Begley, C. G. M. Gahan, C. Hill, *Microbiology* **2012**, *158*, 1389–1401.
- [3] T. Masini, A. K. H. Hirsch, *J. Med. Chem.* **2014**, *57*, 9740–9763.
- [4] B. Lell, R. Ruangweerayut, J. Wiesner, M. A. Missinou, A. Schindler, T. Baranek, M. Hintz, D. Hutchinson, H. Jomaa, P. G. Kremsner, *Antimicrob. Agents Chemother.* **2003**, *47*, 735–738.
- [5] a) J. W. H. Jomaa, S. Sanderbrand, C. W. B. Altincicek, M. Hintz, M. E. I. Turbachova, H. K. L. J. Zeidler, E. B. D. Soldati, *Science* **1999**, *285*, 1573–1576; b) G. Mombo-Ngoma, J. Remppis, M. Sievers, R. Zoleko Manego, L. Endamne, L. Kabwende, L. Veletzky, T. T. Nguyen, M. Groger, F. Lotsch, J. Mischlinger, L. Flohr, J. Kim, C. Cattaneo, D. Hutchinson, S. Duparc, J. Moehrle, T. P. Velavan, B. Lell, M. Ramharter, A. A. Adegnika, B. Mordmuller, P. G. Kremsner, *Clin. Infect. Dis.* **2018**, *66*, 1823–1830.
- [6] J. W. F. Rohdich, M. Fellermeier, S. Sagner, S. Herz, K. Kis, W. Eisenreich, a. M. H. Z. A. Bacher, *PNAS* **1999**, *96*, 11758–11763.
- [7] A. Frank, M. Groll, *Chem. Rev.* **2016**, *117*, 5675–5703.
- [8] a) M. C. Witschel, H. W. Höffken, M. Seet, L. Parra, T. Mietzner, F. Thater, R. Niggeweg, F. Röhl, B. Illarionov, F. Rohdich, J. Kaiser, M. Fischer, A. Bacher, F. Diederich, *Angew. Chem. Int. Ed.* **2011**, *50*, 7931–7935; *Angew. Chem.* **2011**, *123*, 8077–8081; b) Z. Baatarkhuu, P. Chaignon, F. Borel, J.-L. Ferrer, A. Wagner, M. Seemann, *Sci. Rep.* **2018**, *8* 17892; c) L. S. Imlay, C. M. Armstrong, M. C. Masters, T. Li, K. E. Price, R. L. Edwards, K. M. Mann, L. X. Li, C. L. Stallings, N. G. Berry, P. M. O'Neill, A. R. Odom, *ACS Infect. Dis.* **2015**, *1*, 157–167.
- [9] https://www.cdc.gov/malaria/features/world_malaria_day_2021.html, **2021**.
- [10] a) A. M. L. Stéphane, B. Richard, D. E. Cane, M. E. Bowman, W. Kwiatkowski, J. P. Noel, I. Kang, C. Chow, *Nat. Struct. Biol.* **2001**, *8*, 641–648; b) L. E. Kemp, C. S. Bond, W. N. Hunter, *Acta Crystallogr. D Biol. Crystallogr.* **2003**, *59*, 607–610.
- [11] a) M. Gabrielsen, J. Kaiser, F. Rohdich, W. Eisenreich, R. Laupitz, A. Bacher, C. S. Bond, W. N. Hunter, *FEBS J.* **2006**, *273*, 1065–1073; b) C. Björkelid, T. Bergfors, L. M. Henriksson, A. L. Stern, T. Unge, S. L. Mowbray, T. A. Jones, *Acta Crystallogr. D Biol. Crystallogr.* **2011**, *67*, 403–414.
- [12] a) H. K. Ropponen, C. D. Bader, E. Diamanti, B. Illarionov, M. Rottmann, M. Fischer, M. Witschel, R. Müller, A. K. H. Hirsch, *ChemMedChem* **2021**, *16*, 2089–2093; b) W. Zhu, M. Groh, J. Hauptenthal, R. W. Hartmann, *Chem. Eur. J.* **2013**, *19*, 8397–8400.
- [13] J. E. Bradner, M. R. Mckeown, P. B. Rahl, R. A. Young, J. J. Marineau, Int. PCT Pub. No. WO2014071247 A1, **2014**.
- [14] A. Kunfermann, M. Witschel, B. Illarionov, R. Martin, M. Rottmann, H. W. Hoffken, M. Seet, W. Eisenreich, H. J. Knolker, M. Fischer, A. Bacher, M. Groll, F. Diederich, *Angew. Chem. Int. Ed.* **2014**, *53*, 2235–2239; *Angew. Chem.* **2014**, *126*, 2267–2272.
- [15] S. Schultes, C. de Graaf, E. E. J. Haaksma, I. J. P. de Esch, R. Leurs, O. Krämer, *Drug Discovery Today Technol.* **2010**, *7*, e157–e162.
- [16] F. F. Fleming, L. Yao, P. C. Ravikumar, L. Funk, B. C. Shook, *J. Med. Chem.* **2010**, *53*, 7902–7917.

Manuscript received: October 26, 2021

Revised manuscript received: December 14, 2021

Accepted manuscript online: December 17, 2021

Version of record online: January 11, 2022