ACS Medicinal Chemistry Letters

pubs.acs.org/acsmedchemlett

Letter

Development of Pyrazolopyrimidine Anti-*Wolbachia* Agents for the Treatment of Filariasis

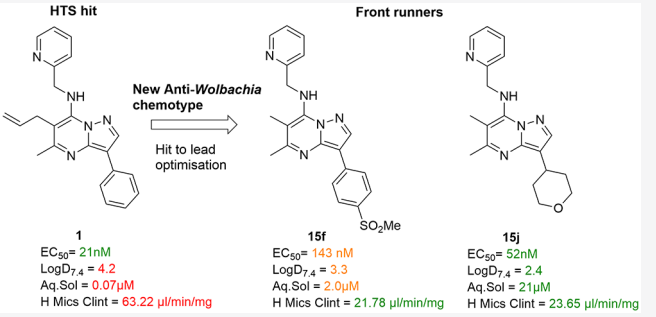
Paul McGillan, Neil G. Berry, Gemma L. Nixon, Suet C. Leung, Peter J. H. Webborn, Mark C. Wenlock, Stefan Kavanagh, Andrew Cassidy, Rachel H. Clare, Darren A. Cook, Kelly L. Johnston, Louise Ford, Stephen A. Ward, Mark J. Taylor, W. David Hong,* and Paul M. O'Neill*

Cite This: https://doi.org/10.1021/acsmedchemlett.1c00216

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Anti-*Wolbachia* therapy has been identified as a viable treatment for combating filarial diseases. Phenotypic screening revealed a series of pyrazolopyrimidine hits with potent anti-*Wolbachia* activity. This paper focuses on the exploration of the SAR for this chemotype, with improvement of metabolic stability and solubility profiles using medicinal chemistry approaches. Organic synthesis has enabled functionalization of the pyrazolopyrimidine core at multiple positions, generating a library of compounds of which many analogues possess nanomolar activity against *Wolbachia in vitro* with improved DMPK parameters. A lead compound, **15f**, was selected for *in vivo* pharmacokinetics (PK) profiling in mice. The combination of potent anti-*Wolbachia* activity in two *in vitro* assessments plus the exceptional oral PK profiles in mice puts this lead compound in a strong position for *in vivo* proof-of-concept pharmacodynamics studies and demonstrates the strong potential for further optimization and development of this series for treatment of filariasis in the future.



KEYWORDS: *Wolbachia, Pyrazolopyrimidine, Onchocerciasis, Filariasis*

Filarial nematodes are the causative pathogens of the neglected tropical diseases lymphatic filariasis (LF) and onchocerciasis that affect tens of millions people throughout the tropics and contribute to serious public health and socio-economic problems. Onchocerciasis (the cause of river blindness) is the second leading infectious cause of blindness. These diseases combined are one of the leading causes of morbidity worldwide. The main causative agents for these conditions are the nematodes *Onchocerca volvulus* (onchocerciasis), *Wuchereria bancrofti*, *Brugia malayi*, and *Brugia timori* (LF).[1] The latest recommended treatment for LF in areas which are not coendemic for onchocerciasis or other filarial disease, loiasis, is a triple combination of ivermectin, diethylcarbamazine plus albendazole.[2] Ivermectin is the recommended treatment for onchocerciasis; however, it cannot be used in areas co-endemic for loiasis due to potentially fatal adverse effects. These direct acting antifilarial drugs primarily target microfilariae, the immature worm stage, and thus can prevent transmission, but they have little macrofilaricidal activity against the adult worms. Hence, these drugs require lengthy treatments that can be as long as 15 years.[3,4] The association of current direct acting antifilarial agents with undesired adverse effects, contraindicated patient groups combined with a growing concern of resistance development, is driving current research efforts to identify and generate safe therapeutic alternatives.[5,6]

The nematodes which are responsible for causing these two filarial diseases share an essential endosymbiotic relationship with the bacterium *Wolbachia*.[7,8] Although the exact nature of this relationship is not yet fully understood, anti-*Wolbachia* therapy has been proven clinically by an existing antibiotic, doxycycline, which delivers safe macrofilaricidal activity with superior therapeutic outcomes compared to current antifilarial drugs.[9−11]

Pyrazolopyrimidine compounds have frequently appeared in the literature with a variety of different pharmacological activities such as kinase inhibitors,[12,13] antituberculosis,[14] antimalarial[15] and antiviral[16] agents, and antidepressants.[17] The original hit for this chemotype (**1**) which resulted from a phenotypic high-throughput screen (HTS) of a divergent chemical library donated by the Medicines for Malaria Venture

Received: April 15, 2021
Accepted: August 16, 2021

(MMV) is displayed in Figure 1 with measured *in vitro* activity (EC_{50}) against *Wolbachia* (*w*AlbB) infected insect cells (*Aedes*

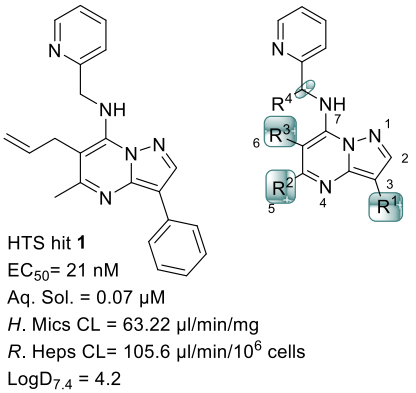


Figure 1. Structure of the HTS hit **1** and the pyrazolopyrimidine scaffold with the areas for SAR studies highlighted.

albopictus, C6/36) and drug metabolism/pharmacokinetic (DMPK) properties. According to the HTS data of other close pyrazolopyrimidine analogues screened in the same campaign (data not shown), some preliminary indication of structure−activity relationship (SAR) was observed and the areas of focus for SAR studies and optimization are highlighted in Figure 1. The aim of the work described here was to develop pyrazolopyrimidine leads with potent anti-*Wolbachia* activity and desired DMPK properties that could be further developed as oral drugs for the treatment of filariasis.

The cascade depicted in Figure 2 was followed for the optimization of the compounds described in this work. *In vitro* anti-*Wolbachia* potency was assessed in parallel with *in vitro*

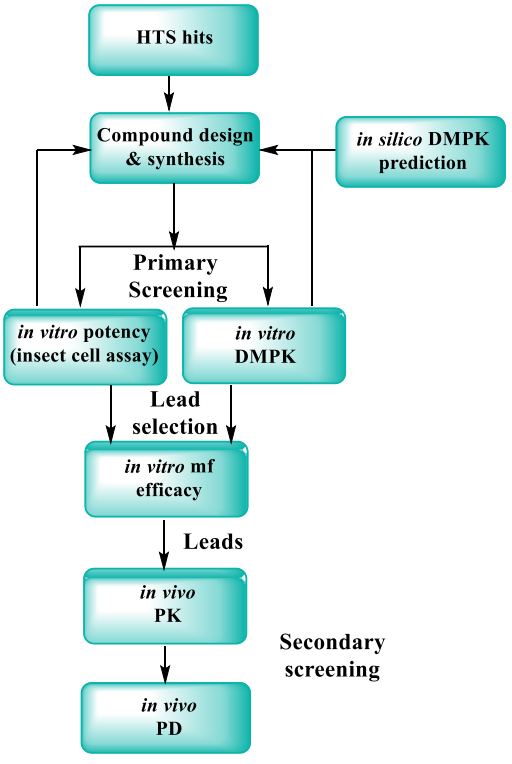


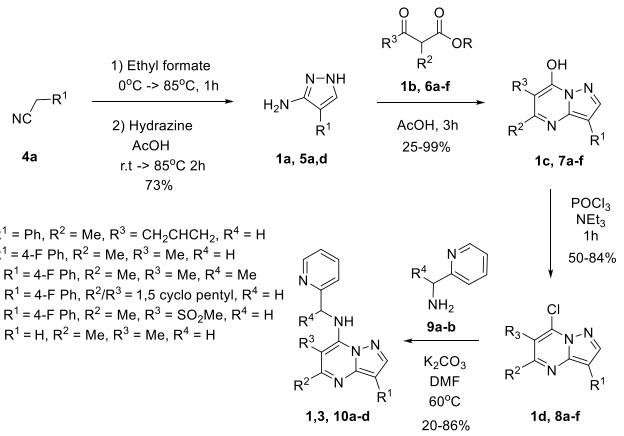
Figure 2. Screening cascade for the design, synthesis and testing of anti-*Wolbachia* compounds.

DMPK screening.[18] Compounds showing a good balance of potency and metabolic stability were then selected to test their anti-*Wolbachia* activity against *Brugia malayi* microfilariae (mf) *in vitro* and for *in vivo* PK profiling in mice. This secondary *in vitro* assay was developed to provide a link between the *in vitro* insect cell-based assay and the *in vivo B. malayi* SCID mouse model. Although the throughput of the mf assay is limited due to the availability of the *B. malayi* mf, it enables the assessment of the anti-*Wolbachia* activity in one of the targeted human parasites, providing important evidence of translation between different species of *Wolbachia* and hosts. Finally, front runners identified from the two above tests would be selected for the *in vivo* PD study where SCID mice are infected with the human parasite *B. malayi*.[19]

Despite the high potency (EC_{50} = 21 nM) of the original hit **1**, DMPK assessments highlighted poor metabolic stability and low aqueous solubility. (Figure 1) The phenyl ring, allyl substitution, and methylene linker were some of the positions within the hit molecule that were predicted to be susceptible to oxidative CYP metabolism. Hence, the early hit to lead optimization was focused on enhancing the metabolic stability while maintaining potency.

The initial synthetic route (Scheme 1) was developed to allow for the synthesis of analogues with modifications at the

Scheme 1. Synthetic Route for Analogues in the Pyrazolopyrimidine Template



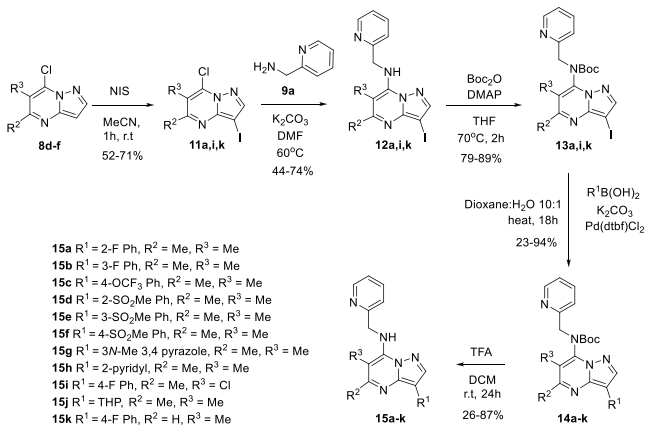
R^2-, R^3-, and 7-positions of the pyrazolopyrimidine ring. While many necessary 5-amino pyrazoles (**1a**, **5d**, R^1 = Ph, H respectively) are commercially available, it was also possible to synthesize them in a two-step reaction from the corresponding nitrile (**4a**, R^1 = 4-F-Ph) using base (NaOEt or LDA) and ethyl formate followed by hydrazine mediated cyclization. This was followed by an acid-catalyzed pyrimidine ring formation using the pyrazoles **1a**, **5a,d** and *β*-keto esters **1b**, **6a−f**.[20]

When the 5-position of the pyrazolopyrimidine is unsubstituted (R^2 = H), acid catalyzed cyclization is not suitable for the initial imine formation step. For these analogues, the pyrimidine ring was formed by reaction of the appropriate pyrazole and aldehyde to give the imine intermediate which cyclizes upon addition of KO^{t}Bu. Chlorination of the 7-hydroxy pyrimidine intermediates **1c**, **7a−f** was achieved using phosphoryl chloride and subsequent substitution of intermediates **1d**, **8a−f** was achieved by aromatic substitution with the appropriate amines, **9a,b**, to produce the pyrazolopyrimidines, **1**, **3**, and **10a−d**.

https://doi.org/10.1021/acsmedchemlett.1c00216
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

While it is possible to use the appropriate nitriles as starting material for the synthesis of target compounds with different groups at the R^1-position, a divergent orientated synthesis at this position was desirable for the production of a number of target analogues from a common intermediate. (Scheme 2)

Scheme 2. Optimized Route for the Synthesis of Analogues with Modified 3-Positions[a]



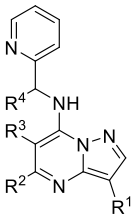
[a]Note: THP = Tetrahydropyran.

Functionalization at the R^1-position can also be achieved *via* iodination of the pyrazolopyrimidines **8d−f** (R^1 = H) using *N*-iodosuccinimide (NIS).[2] This pathway requires Boc-protection of the free amines **12** to allow for Suzuki-mediated coupling of intermediates **13** with the corresponding boronic acid. Finally TFA-mediated Boc-deprotection of compounds **14a−k** afforded the desired targets **15a−k**, which contained a variety of monosubstituted phenyl rings and nitrogen heterocycles.

Substitutions at the R^3-Position. Replacement of the allyl group at this position in the original hit **1** with a methyl substituent resulted in excellent potency (compound **2**, Table 1, EC_{50} = 19 nM) and improved metabolic stability as shown by rat hepatocyte clearance of compound **2** (Table 2). The analogue with ring-fusion of a lipophilic cyclopentyl ring at R^2 and R^3 (**10b**) showed good anti-*Wolbachia* activity (EC_{50} = 79 nM) and demonstrated that modifications at both positions could be tolerated. This modification did not improve aqueous solubility, but the cyclopentyl ring connecting these two positions increased metabolic stability. Chlorine was also tolerated at the R^3-position in terms of potency and has a positive effect on metabolic stability in comparison to the corresponding 6-methyl analogues (**15i** vs **3**). On the other hand, SO_2Me group at the R^3-position (**10c**) reduced anti-*Wolbachia* activity noticeably.

Substitutions at the R^4-Position. In an effort to reduce the potential metabolism of the methylene linker, an analogue with a methyl substitution at this position (**10a**) was investigated. Methylation of the linker imparts a 2-fold increase in human microsomal stability, but this modification leads to a 4-fold loss in activity (compounds **10a** vs **3**). According to HTS results (data not shown here), we found that the SAR was restricted at the 2′-pyridyl side-chain. Any minor modifications to this ring significantly reduced compound potency. For this reason, we focused more on the R^1-position of the pyrazolopyrimidine core since the HTS data suggested that a wider range of modifications might be tolerated at this position.

Table 1. *In Vitro* Potency Data of Key Analogues



	R^1	R^2	R^3	R^4	Anti-*Wolbachia* EC_{50} (nM)[a]
1	Ph	Me	$CH_2CH{=}CH_2$	H	21
2	Ph	Me	Me	H	19
3	4-F Ph	Me	Me	H	17
10a	4-F Ph	Me	Me	Me	93
10b	4-F Ph	1-cyclopentyl	5-cyclopentyl	H	79
10c	4-F Ph	Me	SO_2Me	H	704
10d	H	Me	Me	H	105
15a	2-F Ph	Me	Me	H	11
15b	3-F Ph	Me	Me	H	1000
15c	4-OCF_3 Ph	Me	Me	H	664
15d	2-SO_2Me Ph	Me	Me	H	>2500
15e	3-SO_2Me Ph	Me	Me	H	>2500
15f	4-SO_2Me Ph	Me	Me	H	143
15g		Me	Me	H	119
15h		Me	Me	H	43
15i	4-F Ph	Me	Cl	H	51
15j		Me	Me	H	52
15k	4-F Ph	H	Me	H	38

[a]Note: All tested compounds showed no cytotoxicity against the insect cells at the top concentration (5 μM) in the assay.

Table 2. *In Vitro* DMPK Data of Pyrazolopyrimidine Analogues[a]

	$LogD_{7.4}$	Aq. Sol. (μM)	H. Mics. CL. (μL/min/mg)	R. Hep. CL (μL/min/10^6 cells)	H. PPB (%)
1	4.2	0.07	63.22	105.60	99.9
2	4.0	0.40	ND	59.03	99.80
3	4.3	0.90	49.19	67.66	99.8
10a	4.3	0.30	24.49	75.43	ND
10b	3.3	0.50	48.86	41.25	99.0
10c	3.7	0.01	8.76	46.48	99.7
10d	2.0	561	261.80	109.60	77.0
15a	4.0	0.60	83.97	113.20	99.9
15b	4.1	1.00	59.33	86.16	99.9
15c	4.5	5.00	8.76	156.60	97.9
15d	2.3	11.0	74.15	>300.00	88.0
15e	3.0	2.00	12.08	147.80	96.6
15f	3.3	2.00	21.78	27.58	99.8
15g	2.7	43.0	44.83	10.31	91.8
15h	2.3	30.0	243.60	144.90	97.4
15i	4.3	2.0	44.62	26.37	99.5
15j	2.4	21.0	23.65	71.84	ND
15k	4.0	6.0	37.48	45.53	99.6

[a]Notes: Aq. Sol. = aqueous solubility in pH7.4 PBS; H. Mics. CL = intrinsic human microsomal clearance measured *in vitro*; R. Hep. CL = intrinsic Rat hepatocyte clearance measured *in vitro*; ND = not determined.

Substitutions at the R^1-Position. The R^1-aryl side chain is not essential for anti-*Wolbachia* activity; however, removal of

https://doi.org/10.1021/acsmedchemlett.1c00216
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

the aromatic ring resulted in some reduction in potency (**10d** EC_{50} = 105 nM) coupled with a significant increase in aqueous solubility. A range of small substitutions at the *para*-position of the phenyl ring are generally tolerated, while a 4-fluoro substitution (**3**) appears to be optimal for activity (EC_{50} = 17 nM). Substitution of the polar SO_2Me group at the *para*-position (**15f**) resulted in reducing LogD, increasing aqueous solubility and improving metabolic stability. However, similar substitution with the SO_2Me group at the *ortho*- and *meta*-positions of the phenyl ring is not tolerated, as shown by compounds **15d** and **15e**, where substitution results in a significant reduction in potency. On the other hand, smaller substituents, such as fluorine are tolerated in the *ortho*-position although these small substitutions offered no significant improvements to DMPK properties. While compound **3** displayed improved *in vitro* metabolic stability comparing with HTS hit **1**, it still suffered from poor aqueous solubility.

In an attempt to reduce LogD further and to improve aqueous solubility, analogues **15g** and **15h** containing the heterocyclic aromatic 1-methyl-1*H*-pyrazolyl and 2-pyridyl groups were synthesized as more polar/LogD reducing phenyl ring replacements. These modifications both greatly enhanced the aqueous solubility and lowered LogD with the 2-pyridyl analogue **15h** maintaining the majority of its potency.

Compound **15g** proved to be reasonably stable metabolically; however, a near 10-fold drop in activity supported further investigation into other areas of the scaffold to improve overall properties. Exploration of saturated heterocyclic ring systems led to the synthesis of compound **15j** containing the tetrahydropyran (THP) moiety. The THP side-chain is well tolerated for potency (EC_{50} = 52 nM) while improving DMPK properties comparing with HTS hit **1**.

Substitutions at the R^2-Position. Substitution at this position is not essential for anti-*Wolbachia* activity. It was demonstrated that the removal of the methyl substituent from the R^2-position (**15k**) was tolerated in terms of potency and resulted in some improvement in DMPK properties when compared to compound **3**. This result suggests the R^2-position was another area that could be further explored for optimization of potency and DMPK in future work.

Anti-*Wolbachia* Activity Assessment in *B. malayi* mf Assay. The mf assay is an orthogonal *in vitro* assay that uses *B. malayi* microfilariae to confirm the anti-*Wolbachia* activity of tested compounds against the human parasitic nematode.[21,22] After being screened for potency and DMPK properties *in vitro*, a number of selected analogues were tested at 5 μM alongside the gold-standard doxycycline for comparison of anti-*Wolbachia* activity in the mf assay. The majority of tested compounds demonstrated good activity, comparable to doxycycline, in this assay except for **15i** (Table 3). The secondary readout of the mf assay is the motility of the mf by the tested compounds comparing with vehicle control. For anti-*Wolbachia* drugs, such as doxycycline, they should not affect the motility of the parasitic worm since this indicates off-target effects. For this reason, the chloro-substituted analogue **15i** was considered unsuitable for further development as an anti-*Wolbachia* drug as it had significant effects on worm motility in the assay.

***In Vivo* Pharmacokinetic (PK) Profiling in Mice.** Taking all the *in vitro* results into consideration, compounds **15f** and **15j** possessed a suitable balance of high potency, good DMPK properties and acceptable preliminary safety profiles (e.g., cytotoxicity and hERG inhibition[23]), and **15f** was chosen for *in vivo* PK evaluation. Compound **15f** was dosed orally to SCID mice at 50 and 100 mg/kg using a standard suspended vehicle (SSV); results from the study are shown in Chart 1 and Table 4. Despite limited aqueous solubility, this compound demonstrates good tolerability, excellent *in vivo* PK profiles with high exposure, reasonable half-life and dose-proportional AUC. Based on this data **15f** has been selected as a lead for an *in vivo* proof-of-concept pharmacodynamics study and further optimization.

Table 3. *In Vitro* Potency of Key Analogues in the *B. malayi* mf Assay

molecule	anti-*Wolbachia* potency from cell assay EC_{50} (nM)	anti-*Wolbachia* potency from mf assay (6 days at 5 μM % *Wolbachia* reduction in wsp:gst[a])
DOX	17	86.5
1	21	75.60
3	17	83.20
10d	105	77.10
15f	143	80.40
15i	51	toxic[b]
15j	52	85

[a]wsp, *Wolbachia* surface protein copy number, median % reduction cf. vehicle (DMSO) control; gst, GST copy number (single copy gene, worm size biomarker). [b]Significantly reduced motility of the mf after 6 days incubation comparing with vehicle (DMSO) control.

Chart 1. Mean Plasma Concentration of Compound 15f Following Dosing to SCID Mice with SSV

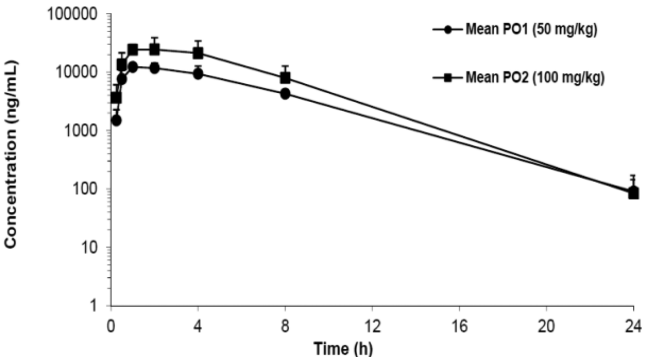


Table 4. *In Vivo* PK Profile of Compound 15f, Dosing to SCID Mice Using SSV

dosage (oral)	50 mg/kg	100 mg/kg
$T_{1/2}$ (h)	2.9	2.4
C_{max} (μg/L)	13 067	27 800
T_{max} (h)	1.33	1.67
AUC_{0-t} (μg·h/L)	82 209	162 617

In summary, the initial potent pyrazolopyrimidine hit **1**, which has poor metabolic stability and inadequate aqueous solubility parameters, has been optimized to provide a number of highly potent compounds with enhanced DMPK properties as represented by lead molecules **15f** and **15j**. A summary of the key SAR is highlighted in Figure 3.

More explorations of the R^1-position and potential functionalization of the 2-position of the pyrazolopyrimidine core could further improve the anti-*Wolbachia* potency and the

https://doi.org/10.1021/acsmedchemlett.1c00216
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

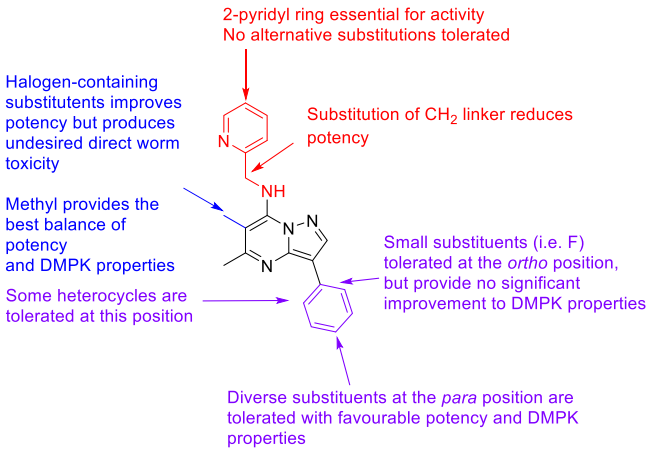


Figure 3. Summary of the SAR of anti-*Wolbachia* pyrazolopyrimidines.

overall DMPK properties. These future directions will be determined by the results of *in vivo* efficacy studies of **15f** which will be reported in due course. In addition, the *in vivo* PK and PD studies of the other lead, **15j** will be triggered if the proof-of-concept *in vivo* efficacy study of **15f** is positive.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.1c00216.

In vitro biological testing methods, experimental procedure of the synthesis, and characterization of the reported compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Paul M. O'Neill − *Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, United Kingdom;* orcid.org/0000-0003-4338-0317; Email: pmoneill@liverpool.ac.uk

W. David Hong − *Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, United Kingdom; Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom;* orcid.org/0000-0002-0030-3007; Email: davidhwq@liverpool.ac.uk

Authors

Paul McGillan − *Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, United Kingdom*

Neil G. Berry − *Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, United Kingdom*

Gemma L. Nixon − *Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, United Kingdom;* orcid.org/0000-0002-9730-0960

Suet C. Leung − *Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, United Kingdom*

Peter J. H. Webborn − *Drug Safety & Metabolism, IMED Biotech Unit, AstraZeneca U.K., Cambridge CB2 0AA, United Kingdom;* Present Address: PJHW Consultancy Ltd., Macclesfield, Cheshire SK11 9HD, United Kingdom

Mark C. Wenlock − *Drug Safety & Metabolism, IMED Biotech Unit, AstraZeneca U.K., Cambridge CB2 0AA, United Kingdom;* Present Address: Cyprotex Discovery Limited, No. 24 Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG, United Kingdom

Stefan Kavanagh − *Oncology Safety Sciences, Clinical Pharmacology & Safety Sciences, R&D, AstraZeneca, Cambridge CB2 0AA, United Kingdom*

Andrew Cassidy − *Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom*

Rachel H. Clare − *Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom;* orcid.org/0000-0002-3945-0530

Darren A. Cook − *Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom*

Kelly L. Johnston − *Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom; Institute of Systems, Molecular & Integrative Biology, School of Life Sciences, University of Liverpool, Liverpool L69 7ZB, United Kingdom*

Louise Ford − *Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom*

Stephen A. Ward − *Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom*

Mark J. Taylor − *Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmedchemlett.1c00216

Author Contributions

The manuscript was written through contributions of all authors. W.D.H., M.J.T., S.A.W., and P.M.O. designed research; P.McG. performed synthesis; P.J.H.W., M.C.W., and S.K. performed *in silico* and *in vitro* DMPK and safety studies; A.C., R.H.C., D.A.C., and K.L.J. performed parasitology studies; W.D.H., N.G.B. G.L.N., L.F., K.L.J., and P.M.O. analyzed data.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The A·WOL consortium is supported by grants from the Bill & Melinda Gates Foundation awarded to the Liverpool School of Tropical Medicine. This work was also supported by a GHIT grant awarded to M.J.T., S.A.W., and P.O.N. Experiments, including synthesis and animal PK studies, performed at Wuxi AppTec, led by Nigel Liverton and Chengkun Huang, were supported by the Bill & Melinda Gates Foundation financially. We would also like to thank Mark Timms in AstraZeneca Compound Management in Alderley Park for the logistics of arranging the *in vitro* DMPK and safety studies.

■ ABBREVIATIONS

LF, lymphatic filariasis; DEC, diethylcarbamazine; HTS, high-throughput screening; DMPK, drug metabolism pharmacokinetics; DOX, doxycycline; PD, Pharmacodynamics; PK, pharmacokinetics; SAR, structure−activity relationships; *B.malayi*, *Brugia malayi*; mf, microfilariae; SCID, severe combined immunodeficient

https://doi.org/10.1021/acsmedchemlett.1c00216
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX

REFERENCES

(1) Taylor, M. J.; Hoerauf, A.; Bockarie, M. Lymphatic filariasis and onchocerciasis. *Lancet* **2010**, *376* (9747), 1175−85.

(2) Weil, G. J.; Jacobson, J. A.; King, J. D. A triple-drug treatment regimen to accelerate elimination of lymphatic filariasis: From conception to delivery. *International Health* **2020**, *13* (Supplement_1), S60−S64.

(3) Bockarie, M. J.; Deb, R. M. Elimination of lymphatic filariasis: do we have the drugs to complete the job? *Curr. Opin. Infect. Dis.* **2010**, *23* (6), 617−20.

(4) Mackenzie, C. D.; Homeida, M. M.; Hopkins, A. D.; Lawrence, J. C. Elimination of onchocerciasis from Africa: possible? *Trends Parasitol.* **2012**, *28* (1), 16−22.

(5) Boussinesq, M.; Fobi, G.; Kuesel, A. C. Alternative treatment strategies to accelerate the elimination of onchocerciasis. *Int. Health* **2018**, *10* (suppl_1), i40−i48.

(6) Johnston, K. L.; Ford, L.; Taylor, M. J. Overcoming the challenges of drug discovery for neglected tropical diseases: the A.WOL experience. *J. Biomol. Screening* **2014**, *19* (3), 335−43.

(7) Taylor, M. J.; Bandi, C.; Hoerauf, A. Wolbachia bacterial endosymbionts of filarial nematodes. *Adv. Parasitol.* **2005**, *60*, 245−84.

(8) Saint Andre, A.; Blackwell, N. M.; Hall, L. R.; Hoerauf, A.; Brattig, N. W.; Volkmann, L.; Taylor, M. J.; Ford, L.; Hise, A. G.; Lass, J. H.; Diaconu, E.; Pearlman, E. The role of endosymbiotic Wolbachia bacteria in the pathogenesis of river blindness. *Science* **2002**, *295* (5561), 1892−5.

(9) Taylor, M. J.; Makunde, W. H.; McGarry, H. F.; Turner, J. D.; Mand, S.; Hoerauf, A. Macrofilaricidal activity after doxycycline treatment of Wuchereria bancrofti: a double-blind, randomised placebo-controlled trial. *Lancet* **2005**, *365* (9477), 2116−21.

(10) Hoerauf, A.; Specht, S.; Buttner, M.; Pfarr, K.; Mand, S.; Fimmers, R.; Marfo-Debrekyei, Y.; Konadu, P.; Debrah, A. Y.; Bandi, C.; Brattig, N.; Albers, A.; Larbi, J.; Batsa, L.; Taylor, M. J.; Adjei, O.; Buttner, D. W. Wolbachia endobacteria depletion by doxycycline as antifilarial therapy has macrofilaricidal activity in onchocerciasis: a randomized placebo-controlled study. *Med. Microbiol. Immunol.* **2008**, *197* (3), 295−311.

(11) Turner, J. D.; Tendongfor, N.; Esum, M.; Johnston, K. L.; Langley, R. S.; Ford, L.; Faragher, B.; Specht, S.; Mand, S.; Hoerauf, A.; Enyong, P.; Wanji, S.; Taylor, M. J. Macrofilaricidal activity after doxycycline only treatment of Onchocerca volvulus in an area of Loa loa co-endemicity: a randomized controlled trial. *PLoS Neglected Trop. Dis.* **2010**, *4* (4), e660.

(12) Ali, S.; Heathcote, D. A.; Kroll, S. H.; Jogalekar, A. S.; Scheiper, B.; Patel, H.; Brackow, J.; Siwicka, A.; Fuchter, M. J.; Periyasamy, M.; Tolhurst, R. S.; Kanneganti, S. K.; Snyder, J. P.; Liotta, D. C.; Aboagye, E. O.; Barrett, A. G.; Coombes, R. C. The development of a selective cyclin-dependent kinase inhibitor that shows antitumor activity. *Cancer Res.* **2009**, *69* (15), 6208−15.

(13) Patel, H.; Periyasamy, M.; Sava, G. P.; Bondke, A.; Slafer, B. W.; Kroll, S. H. B.; Barbazanges, M.; Starkey, R.; Ottaviani, S.; Harrod, A.; Aboagye, E. O.; Buluwela, L.; Fuchter, M. J.; Barrett, A. G. M.; Coombes, R. C.; Ali, S. ICEC0942, an Orally Bioavailable Selective Inhibitor of CDK7 for Cancer Treatment. *Mol. Cancer Ther.* **2018**, *17* (6), 1156−1166.

(14) Soares de Melo, C.; Feng, T. S.; van der Westhuyzen, R.; Gessner, R. K.; Street, L. J.; Morgans, G. L.; Warner, D. F.; Moosa, A.; Naran, K.; Lawrence, N.; Boshoff, H. I.; Barry, C. E., 3rd; Harris, C. J.; Gordon, R.; Chibale, K. Aminopyrazolo[1,5-a]pyrimidines as potential inhibitors of Mycobacterium tuberculosis: Structure activity relationships and ADME characterization. *Bioorg. Med. Chem.* **2015**, *23* (22), 7240−50.

(15) Azeredo, L.; Coutinho, J. P.; Jabor, V. A. P.; Feliciano, P. R.; Nonato, M. C.; Kaiser, C. R.; Menezes, C. M. S.; Hammes, A. S. O.; Caffarena, E. R.; Hoelz, L. V. B.; de Souza, N. B.; Pereira, G. A. N.; Ceravolo, I. P.; Krettli, A. U.; Boechat, N. Evaluation of 7-arylaminopyrazolo[1,5-a]pyrimidines as anti-Plasmodium falciparum, antimalarial, and Pf-dihydroorotate dehydrogenase inhibitors. *Eur. J. Med. Chem.* **2017**, *126*, 72−83.

(16) Hwang, J. Y.; Windisch, M. P.; Jo, S.; Kim, K.; Kong, S.; Kim, H. C.; Kim, S.; Kim, H.; Lee, M. E.; Kim, Y.; Choi, J.; Park, D. S.; Park, E.; Kwon, J.; Nam, J.; Ahn, S.; Cechetto, J.; Kim, J.; Liuzzi, M.; No, Z.; Lee, J. Discovery and characterization of a novel 7-aminopyrazolo[1,5-a]pyrimidine analog as a potent hepatitis C virus inhibitor. *Bioorg. Med. Chem. Lett.* **2012**, *22* (24), 7297−301.

(17) Wustrow, D. J.; Capiris, T.; Rubin, R.; Knobelsdorf, J. A.; Akunne, H.; Davis, M. D.; MacKenzie, R.; Pugsley, T. A.; Zoski, K. T.; Heffner, T. G.; Wise, L. D. Pyrazolo[1,5-a]pyrimidine CRF-1 receptor antagonists. *Bioorg. Med. Chem. Lett.* **1998**, *8* (16), 2067−70.

(18) Clare, R. H.; Cook, D. A.; Johnston, K. L.; Ford, L.; Ward, S. A.; Taylor, M. J. Development and validation of a high-throughput anti-Wolbachia whole-cell screen: a route to macrofilaricidal drugs against onchocerciasis and lymphatic filariasis. *J. Biomol. Screening* **2015**, *20* (1), 64−9.

(19) Halliday, A.; Guimaraes, A. F.; Tyrer, H. E.; Metuge, H. M.; Patrick, C. N.; Arnaud, K. O.; Kwenti, T. D.; Forsbrook, G.; Steven, A.; Cook, D.; Enyong, P.; Wanji, S.; Taylor, M. J.; Turner, J. D. A murine macrofilaricide pre-clinical screening model for onchocerciasis and lymphatic filariasis. *Parasites Vectors* **2014**, *7*, 472.

(20) Dillard, L. W.; Dwyer, M. P.; Girijavallabhan, V. M.; Guzi, T. J.; He, Z. M.; James, R. A.; Park, H.; Paruch, K., Tran, V. D. Pyrazolopyrimidines as Cyclin Dependent Kinase Inhibitors. Patent WO2004022560 A1, 2004.

(21) Hong, W. D.; Benayoud, F.; Nixon, G. L.; Ford, L.; Johnston, K. L.; Clare, R. H.; Cassidy, A.; Cook, D. A. N.; Siu, A.; Shiotani, M.; Webborn, P. J. H.; Kavanagh, S.; Aljayyoussi, G.; Murphy, E.; Steven, A.; Archer, J.; Struever, D.; Frohberger, S. J.; Ehrens, A.; Hubner, M. P.; Hoerauf, A.; Roberts, A. P.; Hubbard, A. T. M.; Tate, E. W.; Serwa, R. A.; Leung, S. C.; Qie, L.; Berry, N. G.; Gusovsky, F.; Hemingway, J.; Turner, J. D.; Taylor, M. J.; Ward, S. A.; O'Neill, P. M. AWZ1066S, a highly specific anti-Wolbachia drug candidate for a short-course treatment of filariasis. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (4), 1414−1419.

(22) Clare, R. H.; Bardelle, C.; Harper, P.; Hong, W. D.; Borjesson, U.; Johnston, K. L.; Collier, M.; Myhill, L.; Cassidy, A.; Plant, D.; Plant, H.; Clark, R.; Cook, D. A. N.; Steven, A.; Archer, J.; McGillan, P.; Charoensutthivarakul, S.; Bibby, J.; Sharma, R.; Nixon, G. L.; Slatko, B. E.; Cantin, L.; Wu, B.; Turner, J.; Ford, L.; Rich, K.; Wigglesworth, M.; Berry, N. G.; O'Neill, P. M.; Taylor, M. J.; Ward, S. A. Industrial scale high-throughput screening delivers multiple fast acting macrofilaricides. *Nat. Commun.* **2019**, *10* (1), 11.

(23) hERG inhibition, IC_{50} = 24.9 μM (**15f**) and > 33.3 μM (**15j**); cyctotoxicity (against THP-1), IC_{50} > 50 μM (**15f** and **15j**).

https://doi.org/10.1021/acsmedchemlett.1c00216
ACS Med. Chem. Lett. XXXX, XXX, XXX−XXX