

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Ashton, TD;Calic, PPS;Dans, MG;Ooi, ZK;Zhou, Q;Palandri, J;Loi, K;Jarman, KE;Qiu, D;Lehane, AM;Maity, BC;De, N;Giannangelo, C;MacRaild, CA;Creek, DJ;Mao, EY;Gancheva, MR;Wilson, DW;Chowdury, M;de Koning-Ward, TF;Famodimu, MT;Delves, MJ;Pollard, H;Sutherland, CJ;Baud, D;Brand, S;Jackson, PF;Cowman, AF;Sleebs, BE

Title:

Property and Activity Refinement of Dihydroquinazolinone-3-carboxamides as Orally Efficacious Antimalarials that Target PfATP4

Date:

2024-08-22

Citation:

Ashton, T. D., Calic, P. P. S., Dans, M. G., Ooi, Z. K., Zhou, Q., Palandri, J., Loi, K., Jarman, K. E., Qiu, D., Lehane, A. M., Maity, B. C., De, N., Giannangelo, C., MacRaild, C. A., Creek, D. J., Mao, E. Y., Gancheva, M. R., Wilson, D. W., Chowdury, M., ... Sleebs, B. E. (2024). Property and Activity Refinement of Dihydroquinazolinone-3-carboxamides as Orally Efficacious Antimalarials that Target PfATP4. *Journal of Medicinal Chemistry*, 67 (16), pp.14493-14523. <https://doi.org/10.1021/acs.jmedchem.4c01241>.

Persistent Link:

<https://hdl.handle.net/11343/358358>

License:

CC BY-NC-ND

Property and Activity Refinement of Dihydroquinazolinone-3-carboxamides as Orally Efficacious Antimalarials that Target PfATP4

Trent D. Ashton, Petar P. S. Calic, Madeline G. Dans, Zi Kang Ooi, Qingmiao Zhou, Josephine Palandri, Katie Loi, Kate E. Jarman, Deyun Qiu, Adele M. Lehane, Bikash Chandra Maity, Nirupam De, Carlo Giannangelo, Christopher A. MacRaid, Darren J. Creek, Emma Y. Mao, Maria R. Gancheva, Danny W. Wilson, Mrityika Chowdury, Tania F. de Koning-Ward, Mufuliat T. Famodimu, Michael J. Delves, Harry Pollard, Colin J. Sutherland, Delphine Baud, Stephen Brand, Paul F. Jackson, Alan F. Cowman, and Brad E. Sleebs*



Cite This: *J. Med. Chem.* 2024, 67, 14493–14523



Read Online

ACCESS |



Metrics & More

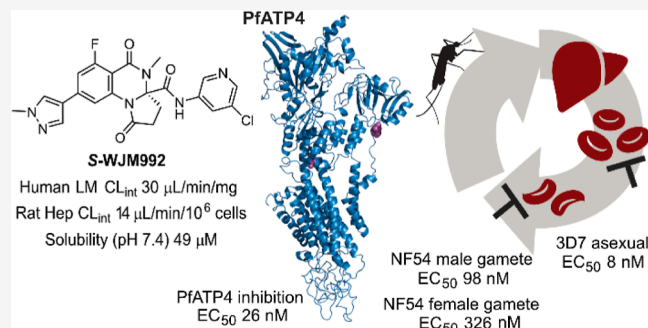


Article Recommendations



Supporting Information

ABSTRACT: To contribute to the global effort to develop new antimalarial therapies, we previously disclosed initial findings on the optimization of the dihydroquinazolinone-3-carboxamide class that targets PfATP4. Here we report on refining the aqueous solubility and metabolic stability to improve the pharmacokinetic profile and consequently in vivo efficacy. We show that the incorporation of heterocycle systems in the 8-position of the scaffold was found to provide the greatest attainable balance between parasite activity, aqueous solubility, and metabolic stability. Optimized analogs, including the frontrunner compound S-WJM992, were shown to inhibit PfATP4-associated Na⁺-ATPase activity, gave rise to a metabolic signature consistent with PfATP4 inhibition, and displayed altered activities against parasites with mutations in PfATP4. Finally, S-WJM992 showed appreciable efficacy in a malaria mouse model and blocked gamete development preventing transmission to mosquitoes. Importantly, further optimization of the dihydroquinazolinone class is required to deliver a candidate with improved pharmacokinetic and risk of resistance profiles.



INTRODUCTION

Malaria is a devastating disease that causes significant morbidity and mortality. There were 233 million cases of malaria worldwide in 2019 resulting in 576,000 deaths.¹ The number of annual deaths increased by 32,000 in the past 2 years in part due to the COVID-19 pandemic impacting access to medical resources and antimalarial therapies.¹ Malaria is caused by *Plasmodium* parasites of which five species are known to infect humans. *Plasmodium falciparum* is the deadliest accounting for 70% of cases worldwide and is most prevalent in sub-Saharan Africa.¹ *Plasmodium vivax* is largely endemic to South-East Asia and the Americas and is responsible for disease relapse after treatment because of a dormant liver stage form.² *Plasmodium knowlesi*, *Plasmodium malariae*, and *Plasmodium ovale* are emerging pathogens that rarely cause significant disease burden.^{3,4}

Malaria is curbed and treated by chemotherapies that are generally classified as chemo-preventative and curative therapies. Chemoprevention of malaria is achieved through the use of causal chemoprophylaxis agents that include Malarone (atovaquone and proguanil) and sulfadoxine-

pyrimethamine + amodiaquine which prevent the initial liver stage infection but also the asexual blood stage infection, while suppressive chemoprophylaxis prevents and treats the asexual blood stage infection, and includes chloroquine, mefloquine, and doxycycline. Therapies are still in development that are mass administered as a single low dose in malaria endemic regions and prevent the transmission of the malaria parasite from the infected human host to the *Anopheles* mosquito by way of a blood meal. This strategy is currently reliant on antimalarials such as primaquine and artemisinin that have transmission-blocking properties, therefore uncovering novel antimalarials with transmission-blocking capabilities is highly desired.

Received: June 3, 2024

Revised: July 24, 2024

Accepted: July 25, 2024

Published: August 12, 2024



ACS Publications

© 2024 The Authors. Published by
American Chemical Society

14493

<https://doi.org/10.1021/acs.jmedchem.4c01241>
J. Med. Chem. 2024, 67, 14493–14523

Curative treatment of malaria involves the use of antimalarial agents such as chloroquine, mefloquine, and amodiaquine, however, these agents have become less effective because of widespread resistance.¹ Artemisinin-based combination therapies (ACTs) are now the frontline treatment for malaria but concerning resistance has emerged in Southeast Asia and more recently in sub-Saharan Africa.^{5,6} In the last 20 years, mass high throughput screening efforts against the malaria parasite have uncovered a multitude of new chemotypes and resulted in the development of new antimalarial structural classes with novel mechanisms of action that have entered clinical trials,⁷ including the DHODH inhibitor, DMS265⁸ and the PfATP4 inhibitor KAE609 (cipargamin) **1**.^{9,10} Unfortunately, the analysis of recrudescence parasites from patients treated with these candidates in clinical trials uncovered resistance mutations in DHODH¹¹ and PfATP4,¹² raising concerns about their future clinical development. Due to the high attrition rate of clinical candidates, there is a perpetual need to develop new antimalarial chemotypes with a high barrier to resistance, and ideally, target multiple stages of the parasite's lifecycle.¹³

To contribute to the global effort to develop new antimalarials we recently conducted a high throughput screen against the *P. falciparum* asexual blood stage parasite using the Janssen Jumpstarter library containing 80,000 compounds with diverse structures. This screen uncovered several new chemotypes with moderate antimalarial activity,^{14–16} including the dihydroquinazolinone-3-carboxamide scaffold (**4**) (Figure 1).¹⁷ A forward genetic resistance study and phenotypic

characterization uncovered that the dihydroquinazolinone scaffold targets PfATP4.¹⁷ PfATP4 belongs to the P-type ATPase superfamily and is required for the transport of Na⁺ across the parasite plasma membrane out of the parasite cytosol.^{10,18} PfATP4 is believed to import H⁺ while effluxing Na⁺, and disruption of its activity by small molecule inhibitors results in an increase in cytosolic [Na⁺], an increase in cytosolic pH, and parasite death.^{10,19} PfATP4 is a known and clinically validated antimalarial target and several inhibitors have been developed including KAE609 **1**, SJ733 **2**, and PA21A050 **3** (Figure 1).^{9,20,21} A limitation with these PfATP4 inhibitors is that they have a moderate risk of resistance,²² and therefore the development of a PfATP4 inhibitor that has a high barrier to resistance and retains its activity against PfATP4 mutant parasites is highly desirable.²² Although the dihydroquinazolinone scaffold (represented by WJM921 **4** in Figure 1) was shown to have a moderate risk of resistance [minimum inoculum of resistance (MIR) of 9.6×10^5], the slightly reduced activity against the PfATP4^{G358S} clinical drug-resistant strain relative to the activity against PfATP4^{WT} parasites,⁹ made it worthy of further investigation.

Initial optimization of the dihydroquinazolinone hit scaffold led to the frontrunner compound WJM921 **4** which has potent activity against asexual stage parasites (Figure 1) and moderate activity against transmission-stage male and female gametes.¹⁷ In a four-day *P. berghei* mouse model, modest efficacy was observed with **4** (30% reduction of parasitemia at 40 mg/kg), although its systemic plasma exposure and half-life were limited by its moderate metabolic stability (mouse CL_{int} 55 μ L/mg/min) and low aqueous solubility (5 μ M at pH 7.4).¹⁷

Here, we report on further optimization of the dihydroquinazolinone scaffold which was focused on enhancing in vitro metabolic stability and aqueous solubility while maintaining in vitro antiparasitic activity to improve efficacy in a mouse model of malaria. We show that the incorporation of polar functionality improved aqueous solubility, but antiparasitic activity and metabolic stability were finitely balanced by overall lipophilicity. Frontrunner compounds were then evaluated against parasites with resistance-associated PfATP4 mutations and in assays of PfATP4-associated Na⁺-ATPase activity to examine whether or not the structural modifications applied had altered the mechanism of action. We also evaluated the activity of frontrunner compounds against male and female gametes and in a mosquito transmission model to investigate the potential of this chemical series in a transmission-blocking therapy. Finally, we evaluated the optimized compounds in asexual blood stage mouse models to test their oral efficacy.

RESULTS AND DISCUSSION

Synthesis. The general synthesis of dihydroquinazolinone analogs began with commercially available substituted anthranilic acids **5**. These anthranilic acids **5** were converted

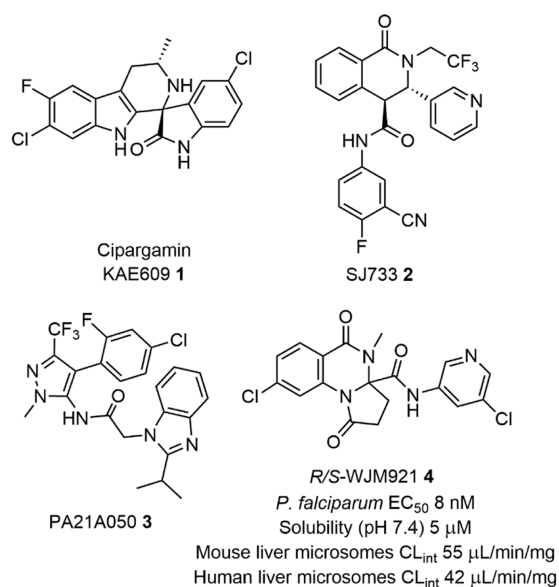
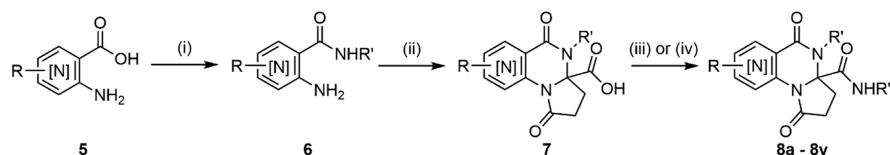


Figure 1. Structures of the antimalarial clinical candidates and the dihydroquinazolinone antimalarial class which target PfATP4.

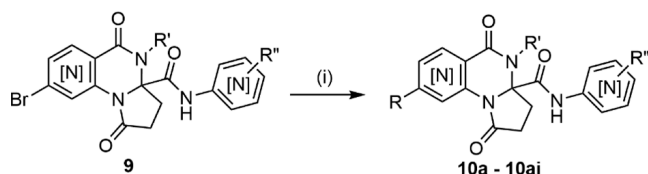
Scheme 1. General Synthesis of the Dihydroquinazolinone-3 Carboxamide Analogs^a



^aReagents and Conditions: (i) R'NH₂, TCFH, NMI, MeCN; (ii) α -ketoglutaric acid, AcOH, 80 or 135 °C; (iii) R''NH₂, HBTU, DIPEA, THF, 21 or 60 °C; (iv) R''NH₂, TCFH, NMI, MeCN

to N-substituted anthranilamides **6** using the appropriate aliphatic amine and chloro-*N,N,N',N'*-tetramethylformamidium hexafluorophosphate (TCFH) under basic conditions (Scheme 1). The anthranilamides **6** were then reacted with α -ketoglutaric acid in acetic acid at an elevated temperature to afford the carboxylic acid intermediates **7**. **7** was then reacted with a substituted aniline using either HBTU and Huning's base or TCFH and *N*-methyl imidazole (NMI) to afford the carboxamide products **8**. To install a variety of functionalized aryl groups and heterocycles at the 8-position, a Suzuki reaction was employed using a suitable boronic acid or pinacol ester, catalytic Pd(dppf)Cl₂ and K₃PO₄ in dioxane and water at reflux to afford a range of 8-*N*-substituted analogs **10** (Scheme 2). To install aliphatic amines or N-linked heterocycles in the

Scheme 2. Synthesis of 8-Substituted Analogs^a



^aReagents and Conditions: (i) R'B(OH)₂, Pd(dppf)Cl₂ (5–10 mol %), K₃PO₄, dioxane/H₂O, 110 °C, 1 h; Or aliphatic or aryl amine (R'), XantPhos-Pd-G3, Cs₂CO₃, dioxane 120 °C, 1 h.

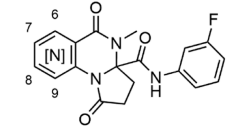
8-position, Buchwald conditions were applied using an amine, catalytic XPhos-Pd-G3, and Cs₂CO₃ in dioxane at an elevated temperature to afford a series of 8-functionalized analogs **10**.

Property-Activity Refinement. To achieve the ideal target metabolic stability and aqueous solubility parameters (human liver microsome CL_{int} <10 μ L/min/mg; rat hepatocytes <10 μ L/min/10⁶ cells and >50 μ M at pH 7.4), we investigated the effects of adding polar groups into various positions of the scaffold and the inclusion of functionality that would mitigate metabolic soft spots while ensuring that the activity against asexual parasites was maintained or enhanced as measured by lactate dehydrogenase (LDH) assay over 72 h.²³ Human HepG2 cell cytotoxicity was also monitored over 48 h using Cell Titer Glo as a measure of cell growth.²⁴

We first investigated the inclusion of an endocyclic nitrogen into the scaffold. We previously established that the 3'-position of the exocyclic aryl ring tolerated an endocyclic nitrogen (WJM921 **4** in Figure 1),¹⁷ but incorporation of an endocyclic nitrogen into the aryl ring of the tricyclic core had not been explored. An endocyclic nitrogen was inserted into each position of the aryl ring, and it was observed that an endocyclic nitrogen in either the 7- or 9- position (**8c** and **8e**) was not tolerated (EC₅₀s > 10 μ M) (Table 1). An endocyclic nitrogen in either the 6- or 8- position (**8b** or **8d**) led to an approximately 10-fold decrease in activity (EC₅₀ 3.55 and 2.55 μ M) relative to the carbocyclic analog **8a** (EC₅₀ 0.23 μ M).

It was previously shown that an 8-methoxy substituent in the 8-position (**8f**) or 6-fluoro substituent (**8j**) improved antiparasitic activity by 20-fold and fivefold, respectively.¹⁷ Accordingly, the addition of an 8-methoxy substituent in combination with a 6-endocyclic nitrogen (**8g**) enhanced activity (EC₅₀ 0.048 μ M) by approximately 70-fold compared to the unsubstituted equivalent (**8b**) (EC₅₀ 3.55 μ M), but was 6-fold less active than the carbocyclic comparator (**8f**) (EC₅₀ 0.008 μ M) (Table 2). The 8-methoxy substituent in combination with 9-endocyclic nitrogen (**8i**) also enhanced

Table 1. Activities of Endocyclic Nitrogen Analogs



compound	[N]	Pf parasite EC ₅₀ (SD) μ M ^a	HepG2 EC ₅₀ μ M ^b	PSA (Å ²) ^c	CLogD ^c
8a	none	0.23 (0.01)	>40	70	1.6
8b	6	3.55 (0.64)	>40	83	0.9
8c	7	>10		83	1.0
8d	8	2.25 (0.17)	>40	83	0.6
8e	9	>10		83	1.0

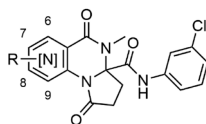
^aEC₅₀ data represent means and SDs for *n* = 4 or more experiments performed in duplicate measuring LDH activity of *P. falciparum* 3D7 asexual parasites for 72 h. ^bEC₅₀ data represent means for *n* = 2 experiments performed in duplicate measuring Cell Titer Glo growth inhibition of HepG2 cells over 48 h. ^cCalculated using Biovia software.

activity (EC₅₀ 0.660 μ M) compared to its unsubstituted counterpart (**8e**), while 7-endocyclic iteration (**8h**) was significantly less active (EC₅₀ > 1 μ M). A combination of the 6-fluoro and 8-endocyclic nitrogen (**8k**) resulted in a 5-fold increase in activity (EC₅₀ 0.395 μ M) compared to the analog without a methoxy group (**8d**). Notably, the addition of an endocyclic nitrogen into the dihydroquinazolinone scaffold did not introduce human HepG2 cell cytotoxicity.

The aqueous solubility at pH 7.4 was slightly enhanced (13 μ M) by the addition of a 6-endocyclic nitrogen (**8g**) while the incorporation of the 9-endocyclic nitrogen (**8i**) did not improve solubility (4.8 μ M) compared to the carbocycle equivalent (**8f**) (3.4 μ M) (Table 2). The integration of the 6-endocyclic nitrogen was accompanied by a decrease in the eLogD (1.8) and markedly enhanced the metabolic stability in human microsomes (CL_{int} 27 μ L/min/mg) and rat hepatocytes (CL_{int} 41 μ L/min/10⁶ cells) relative to the carbocyclic analog (**8f**). A decrease in eLogD (2.6) and an increase in metabolic stability (CL_{int} 58 μ L/min/mg and 80 μ L/min/10⁶ cells) was less pronounced with the addition of the 9-endocyclic nitrogen (**8i**). This was consistent with previous findings that metabolic stability was intrinsically linked to the overall lipophilicity of the scaffold.¹⁷ This study set indicated the need to introduce polar functionality elsewhere on the dihydroquinazolinone scaffold to improve solubility and metabolic stability.

We previously established that the inclusion of a variety of substituents including an aryl group (**10a**) in the 8-position enhanced antiparasitic activity (EC₅₀ 0.010 μ M), while substitution at other positions on the scaffold largely impacted activity.¹⁷ We reasoned that the 8-position would be suitable for the introduction of heteroaromatic systems with an ionizable nitrogen to enhance solubility and metabolic stability. To investigate this concept, heteroaryl groups were installed on the 8-position on the scaffold with a 3'-fluoro substitution on the exocyclic aryl group. It was observed that 2-, 3- and 4-pyridyl systems in the 8-position (**10b**, **10c**, **10d**) were tolerated, but were approximately five to 8-fold less active (EC₅₀ 0.062, 0.047, and 0.082 μ M) than the 8-carbocycle counterpart (**10a**) (Table 3). The inclusion of an 8-pyrimidine ring (**10e**) led to a further 10-fold decrease in activity (EC₅₀ 0.632 μ M), suggesting an additional polarity of the endocycle nitrogen was not tolerated. Accordingly, an unsubstituted 4-

Table 2. Activity of Endocyclic Nitrogen Analogs



compound	[N]	R	Pf parasite EC ₅₀ (SD) μM ^a	HepG2 EC ₅₀ μM ^b	PSA (Å ²) ^c	eLogD ^d	human LM CL _{int} (μL/min/mg) ^e	rat Hep CL _{int} (μL/min/10 ⁶ cells) ^e	solubility (pH 7.4) μM
8f	none	8-OMe	0.008 (0.001)	>40	79	2.9	107	158	3.4
8g	6	8-OMe	0.048 (0.010)	>40	92	1.8	27	41	13
8h	7	8-OMe	>1.0		92				
8i	9	8-OMe	0.660 (0.040)	>40	92	2.6	58	80	4.8
8j	none	6-F	0.069 (0.007)	>40	70				
8k	8	6-F	0.395 (0.045)	>40	83				

^aEC₅₀ data represent means and SDs for $n = 4$ or more experiments performed in duplicate measuring LDH activity of *P. falciparum* 3D7 asexual parasites for 72 h. ^bEC₅₀ data represent means for $n = 2$ experiments performed in duplicate measuring Cell Titer Glo growth inhibition of HepG2 cells over 48 h. ^cCalculated using Bivia software. ^dShake-flask method. ^eT1/2 values are located in Table S2. LM = liver microsomes; Hep = hepatocytes.

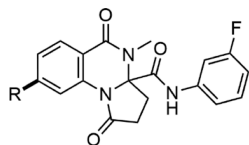
pyrazole (**10f**) also exhibited decreased activity (EC₅₀ 0.577 μM) compared to **10a**. The inclusion of *N*-methyl substitution on the 4-pyrazole (**10g**) markedly increased the potency (EC₅₀ 0.064 μM) while the *N*-methyl 3-pyrazole (**10h**) was twofold less active (EC₅₀ 0.115 μM). Introduction of a *N*-methyl-4-imidazole or a *N*-substituted imidazole in the 8-position (**10i** and **10j**) also led to a decrease in antiparasitic activity (EC₅₀ 0.845 and 0.822 μM), but *N*-substituted pyrazole (**10k**) exhibited similar potency (EC₅₀ 0.025 μM) to the carbocyclic analog (**10a**). An amine-linked aryl group in the 8-position (**10l**) was 9-fold less potent (EC₅₀ 0.090 μM) than **10a**, while amine-linked heteroaryl groups (**10m** and **10n**) were significantly less potent (EC₅₀ 1.52 and 0.243 μM). Polar *N*-substituted aliphatic groups (**10o**, **10p**, and **10q**) were also not tolerated (EC₅₀s > 0.502 μM). In general, these data signified that the positioning of an endocyclic nitrogen in the 8-position of the heterocycle influenced overall the polarity and that analogs with a calculated LogD of ≤1.8 (except for those with amine-linked aryl groups) displayed a significant decrease in antiparasitic activity.

We next incorporated the most potent 8-heteroaryl substituents (from Table 3) in combination with an exocyclic aryl ring with a 3-endocyclic nitrogen and a 5-chloro substituent from **4** (Figure 1) to assess parasite activity and in vitro ADME. These analogs were benchmarked against derivatives with a chloro, methoxy, and trifluoromethoxy group in the 8-position (**8o**, **8p**, and **8q**) that were shown to exhibit potent parasite activity (EC₅₀ 0.019, 0.016, and 0.037 μM), moderate in vitro metabolism (human CL_{int} 42, 36, and 46 μL/min/mg) and low aqueous solubility (5.2–14 μM) (Table 4). Incorporation of 2-pyridyl and 3-pyridyl in the 8-position (**10r** and **10s**) was shown to decrease parasite potency by 2- and threefold (EC₅₀ 0.038 and 0.067 μM), while the 4-pyridyl variant (**10t**) was approximately ninefold less active (EC₅₀ 0.177 μM) than the benchmark compounds. Although the activity was largely maintained and aqueous solubility was improved (EC₅₀ 31 and 90 μM) with **10r** and **10s**, the incorporation of a pyridyl group led to a decrease in metabolic stability in human liver microsomes (human CL_{int} 81 and 101 μL/min/mg), presumably due to *N*-oxidation of the pyridyl nitrogen. To mitigate *N*-oxidation, functional groups were added adjacent to the 3-pyridyl nitrogen. It was observed that the derivative with a methoxy group (**10u**) retained activity (EC₅₀ 0.048 μM) whereas the analog with trifluoromethyl

group (**10v**) had 4-fold greater activity (EC₅₀ 0.010 μM) and the analog with a nitrile (**10w**) had significantly lower activity (EC₅₀ 0.66 μM). The metabolic stability in human liver microsomes of **10u** was slightly improved (CL_{int} 70 μL/min/mg) relative to naked pyridyl analog **10s** but was decreased in rat hepatocytes (CL_{int} 35 μL/min/10⁶ cells). The aqueous solubility of **10u** was also reduced (15 μM) relative to the 3-pyridyl progenitor **10s**, and therefore pyridyl analogs were not further explored. Inclusion of either a 4-(*N*-methyl)-pyrazole or a *N*-pyrazole in the 8-position (**10x** and **10y**) resulted in an approximately twofold decrease in activity (EC₅₀ 0.037 and 0.031 μM) compared to **8p** but significantly increased aqueous solubility (189 and 102 μM), although there was no appreciable improvement in metabolic stability (human CL_{int} 49 and 53 μL/min/mg). Interestingly, the inclusion of an *N*-piperidine and a 4-pyran (**10z** and **10aa**) decreased parasite activity (EC₅₀ 0.23 and 0.13 μM), while analogs **10ab** and **10ac** and with 4-piperidine or 4-(*N*-methyl) piperidine were significantly less active (EC₅₀ > 1.0 and 0.63 μM). These data reaffirmed the previous observation (from Table 3) that analogs with low eLogD (<1.8) were associated with decreased parasite activity. Conversely, the addition of the polar functionality, such as the 4-(*N*-methyl) piperidine, significantly improved metabolic stability (human CL_{int} 23 μL/min/mg; rat CL_{int} 1.9 μL/min/10⁶ cells) and enhanced aqueous solubility (189 μM), signifying that there is a finite balance between polarity and lipophilicity that governs metabolic stability and parasite activity.

We next explored whether the addition of a fluorine to the 6-, 7- and 9- positions on the tricyclic scaffold in combination with an 8-chloro or 8-methoxy substituent would assist in enhancing both parasite activity and metabolic stability. It was evident that a fluorine in the 6-position with an 8-methoxy substituent (**8r**) improved parasite activity by 4-fold (EC₅₀ 0.004 μM) while a 6-fluoro combined with an 8-chloro substituent (**8s**) maintained activity (EC₅₀ 0.030 μM) relative to the benchmark analogs **8p** and **8o** (Table 5). A fluorine in the 7-position (**8t**) resulted in a 4-fold decrease in activity (EC₅₀ 0.086 μM) whereas a fluorine in the 9-position (**8u**) decreased activity by 20-fold (EC₅₀ 0.43 μM). The addition of the fluoro in the 6-position with both analog **8r** and **8s** did not improve metabolic stability (human CL_{int} 27 and 40 μL/min/mg; rat CL_{int} 19 and 9 μL/min/10⁶ cells) and marginally decreased aqueous solubility (3.9 and 1.7 μM) which was

Table 3. Activity of 8-Heterocyclic Analogs



Compound	R	Pf parasite EC ₅₀ (SD) μM ^a	HepG2 EC ₅₀ μM ^b	PSA (Å ²) ^c	cLogD ^c
8l	Cl	0.017 (0.004)	>40	68	2.3
8m	OMe	0.014 (0.002)	>40	79	1.6
10a	Ph	0.010 (0.001)	>40	70	3.1
10b		0.062 (0.012)	>40	83	2.4
10c		0.047 (0.003)	>40	83	2.0
10d		0.082 (0.012)	>40	83	2.0
10e		0.632 (0.073)	>40	96	1.4
10f		0.577 (0.053)	>40	98	1.6
10g		0.064 (0.010)	>40	88	1.8
10h		0.115 (0.014)	>40	88	2.2
10i		0.845 (0.199)	>40	88	1.7
10j		0.822 (0.072)	>40	88	1.3
10k		0.025 (0.005)	>40	88	1.9
10l		0.090 (0.010)	>40	82	3.2
10m		1.52 (0.39)	>40	95	2.0
10n		0.243 (0.030)	>40	95	2.6
10o		0.502 (0.066)	>40	82	1.5
10p		>10	-	85	1.2
10q		>10	-	76	1.7

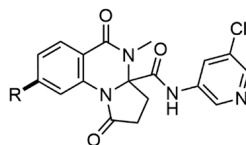
^aEC₅₀ data represent means and SDs for $n = 4$ or more experiments performed in duplicate measuring LDH activity of *P. falciparum* 3D7 asexual parasites for 72 h. ^bEC₅₀ data represent means for $n = 2$ experiments performed in duplicate measuring Cell Titer Glo growth inhibition of HepG2 cells over 48 h. ^cCalculated using Biovia software.

likely a consequence of the added lipophilicity (e Log D 2.7) contributed by the fluorine. To understand the basis for the metabolic degradation, metabolite identification using human liver microsomes was undertaken on analog 8r. This study revealed that the major metabolic transformation was N-demethylation, while the minor metabolites detected were indicative of oxidation of the exocyclic pyridine ring and O-methylation of the methoxy group (Figure S5). Evidence from our previous study¹⁷ has shown the N-methyl substituent is required for antiparasitic activity and therefore we replaced the N-methyl group with substituents of a similar size that may

mitigate metabolic N-demethylation. It was shown that the inclusion of the N-cyclopropyl group (8v and 8w) maintained parasite activity (EC₅₀ 0.023 and 0.011 μM) whereas the N-trifluoroethyl group (8x) decreased activity (EC₅₀ 0.18 μM). Larger substituents including an N-isopropyl and N-propyl were previously found to significantly impact activity,¹⁷ so were not included in this study. The addition of the N-cyclopropyl group (8w) did not enhance metabolic stability but was found to exacerbate metabolic degradation (human CL_{int} 157 μL/min/mg; rat CL_{int} 31 μL/min/10⁶ cells). A metabolite identification study using human liver microsomes on analog 8w revealed the major metabolic transformation was the oxidation of the N-cyclopropyl group (Figure S6). We then considered whether an N-trideuteromethyl group would mitigate metabolism as was found in other studies.²⁵ Expectedly, the parasite activity (EC₅₀ 0.18 μM) of the analog 8y with an N-trideuteromethyl group mirrored that of the N-methyl derivative 8p, and although metabolic stability of 8y in human liver microsomes was enhanced 2-fold (CL_{int} 16 μL/min/mg), the metabolic stability was unchanged on incubation with rat hepatocytes (CL_{int} 28 μL/min/10⁶ cells).

We reasoned that combining functionalities with suitable properties from study sets in Tables 1–5 may provide an avenue to reduce metabolic degradation and improve aqueous solubility while maintaining parasite activity. The amalgamation of a 6-endocyclic nitrogen with a 4-(N-methyl)pyrazole in the 8-position and an exocyclic pyridine ring in analog 10ad significantly impacted parasite activity (EC₅₀ 0.51 μM), but markedly enhanced metabolic stability (human CL_{int} 5.4 μL/min/mg; rat CL_{int} 2.1 μL/min/10⁶ cells) (Table 6). Consistent with the previous observations analog 10ad has a Log $D < 1.8$ which appears to be the threshold for desirable parasite potency and metabolic stability for this antimalarial scaffold. A combination of the N-trideuteromethyl group with 4-(N-methyl)pyrazole (10ae) resulted in comparable parasite activity (EC₅₀ 0.051 μM) relative to 10x, although metabolic stability was not significantly improved. A N-difluoromethyl group was then introduced to replace the N-methyl group on the 4-pyrazole as a measure to reduce the possibility of metabolic N-demethylation. The inclusion of the N-difluoromethyl group (10af) enhanced parasite activity (EC₅₀ 0.008 μM) by 5-fold compared to 10ae but did not improve metabolic stability (human CL_{int} 63 μL/min/mg; rat CL_{int} 23 μL/min/10⁶ cells). The combination of 4-(N-difluoromethyl) pyrazole and the N-trideuteromethyl group (10ag) also did not significantly enhance metabolic stability. A 6-fluoro substituent in combination with a 4-(N-methyl) pyrazole gave analog 10ah that had twofold improved parasite activity (EC₅₀ 0.014 μM) compared to 10x, but the addition of the 6-fluorine did not appreciably improve metabolic stability (human CL_{int} 52 μL/min/mg; rat CL_{int} 8.8 μL/min/10⁶ cells), while the N-trideuteromethyl variant 10ai had slightly improved metabolic stability (human CL_{int} 31 μL/min/mg; rat CL_{int} 15 μL/min/10⁶ cells). Overall, the metabolic stability in rat hepatocytes of analogs in this set of analogs was desirable (CL_{int} 8 to 20 μL/min/10⁶ cells) while stability in human liver microsomes could not be improved beyond 30 μL/min/mg while preserving parasite potency. It was noted there was only a slight improvement in mouse liver microsome stability between the N-methyl and N-trideuteromethyl iterations (for example, 10ah versus 10ai: CL_{int} of 52 versus 33 μL/min/mg) but not with rat hepatocyte stability (10ah versus 10ai: CL_{int} of 8.8 versus 15 μL/min/mg) (Table 6). Given these data, the N-

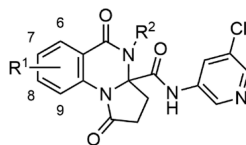
Table 4. Activity of 8-Substituted Analogs



Compound	R	Pf parasite EC ₅₀ (SD) μM ^a	HepG2 EC ₅₀ μM ^b	PSA (Å ²) ^c	eLogD _d	Human LM CL _{int} (μL/min/ mg) ^e	Rat Hep CL _{int} (μL/min/ 10 ⁶ cells) ^e	Solubility (pH 7.4) (μM)
8o	Cl	0.019 (0.003)	>40	83	2.8	42	10	5.2
8p	OMe	0.016 (0.001)	>40	92	2.2	31	24	11
8q	OCF ₃	0.037 (0.021)	>40	92	3.5	46	20	14
10r		0.038 (0.01)	>40	96	2.8	81	28	31
10s		0.067 (0.012)	>40	96	2.7	101	9.9	90
10t		0.177 (0.050)	>40	96	2.7	105	12	136
10u		0.048 (0.014)	>40	105	3.4	70	35	15
10v		0.010 (0.005)	>40	96	3.4	62	35	<2.5
10w		0.660 (0.370)	>40	119	-	-	-	-
10x		0.037 (0.003)	>40	100	2.4	49	17	189
10y		0.031 (0.001)	>40	100	2.6	53	21	102
10z		0.23 (0.033)	>40	86	-	-	-	-
10aa		0.13 (0.010)	>40	92	-	-	-	-
10ab		>1.0	-	86	-	-	-	-
10ac		0.630 (0.049)	>40	86	0.8	23	1.9	189

^aEC₅₀ data represent means and SDs for $n = 4$ or more experiments performed in duplicate measuring LDH activity of *P. falciparum* 3D7 asexual parasites for 72 h. ^bEC₅₀ data represent means for $n = 2$ experiments performed in duplicate measuring Cell Titer Glo growth inhibition of HepG2 cells over 48 h. ^cCalculated using Biovia software. ^dShake-flask method. ^e $T_{1/2}$ values are located in Table S2. LM = liver microsomes; Hep = hepatocytes.

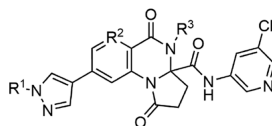
Table 5. Activities of Substituted Fluorine and N-Substituted Analogs



compound	R ¹	R ²	Pf parasite EC ₅₀ (SD) μM ^a	HepG2 EC ₅₀ μM ^b	PSA (Å ²) ^c	eLogD ^d	human LM CL _{int} (μL/min/mg) ^e	rat Hep CL _{int} (μL/min/10 ⁶ cells) ^e	solubility (pH 7.4) (μM)
8p	8-OMe	Me	0.016 (0.001)	>40	92	2.2	31	24	12
8o	8-Cl	Me	0.019 (0.003)	>40	83	2.8	42	10	5.2
8r	8-OMe, 6-F	Me	0.004 (0.001)	>40	92	2.1	27	19	3.9
8s	8-Cl, 6-F	Me	0.030 (0.007)	>40	83	2.7	40	9	1.7
8t	8-Cl, 7-F	Me	0.086 (0.022)	>40	83				
8u	8-OMe, 9-F	Me	0.43 (0.10)	>40	92				
8v	8-OMe	CyPr	0.023 (0.010)	>40	92				
8w	8-Cl	CyPr	0.011 (0.004)	>40	83	3.1	157	31	9.9
8x	8-OMe	CH ₂ CF ₃	0.18 (0.06)	>40	92	3.4	41	42	<2.5
8y	8-OMe	CD ₃	0.016 (0.003)	>40	92	2.2	16	28	9.9

^aEC₅₀ data represent means and SDs for $n = 4$ or more experiments performed in duplicate measuring LDH activity of *P. falciparum* 3D7 asexual parasites for 72 h. ^bEC₅₀ data represent means for $n = 2$ experiments performed in duplicate measuring Cell Titer Glo growth inhibition of HepG2 cells over 48 h. ^cCalculated using Biovia software. ^dShake-flask method. ^eT_{1/2} values are located in Table S2. LM = liver microsomes; Hep = hepatocytes.

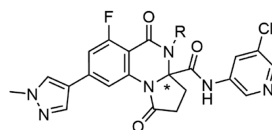
Table 6. Activities of 8-Pyrazole Analogs



compound	R ¹	R ²	R ³	Pf parasite EC ₅₀ (SD) μM ^a	HepG2 EC ₅₀ (SD) μM ^b	PSA (Å ²) ^c	eLogD ^d	human LM CL _{int} (μL/min/- mg) ^e	rat Hep CL _{int} (μL/min/10 ⁶ cells) ^e	solubility (pH 7.4) (μM)
10x	4-pyrazole (N-Me)	CH	CH ₃	0.037 (0.003)	>40	100	2.4	49	17	189
10ad	4-pyrazole (N-Me)	N	CH ₃	0.51 (0.043)	>40	113	1.6	5.4	2.1	
10ae	4-pyrazole (N-Me)	CH	CD ₃	0.051 (0.005)	>40	100	2.4	41	8.7	132
10af	4-pyrazole (N-CHF ₂)	CH	CH ₃	0.008 (0.001)	>40	100	2.9	63	23	
10ag	4-pyrazole (N-CHF ₂)	CH	CD ₃	0.007 (0.001)	>40	100	2.9	36	19	52
10ah	4-pyrazole (N-Me)	CF	CH ₃	0.014 (0.001)	>40	100	2.3	52	8.8	167
10ai	4-pyrazole (N-Me)	CF	CD ₃	0.016 (0.001)		100	2.3	31	15	109

^aEC₅₀ data represent means and SDs for $n = 4$ or more experiments performed in duplicate measuring LDH activity of *P. falciparum* 3D7 asexual parasites for 72 h. ^bEC₅₀ data represent means for $n = 2$ experiments performed in duplicate measuring Cell Titer Glo growth inhibition of HepG2 cells over 48 h. ^cCalculated using Biovia software. ^dShake-flask method. ^eT_{1/2} values are located in Table S2. LM = liver microsomes; Hep = hepatocytes.

Table 7. Activities of Chiral 8-Pyrazole Analogs



compound	R	*chirality	Pf parasite EC ₅₀ (SD) μM ^a	eLogD ^b	human LM CL _{int} (μL/min/mg) ^c	mouse LM CL _{int} (μL/min/mg)	rat Hep CL _{int} (μL/min/10 ⁶ cells) ^c	solubility (pH 7.4) (μM)
10ah WJM992	CH ₃	R/S	0.014 (0.001)	2.3	52	29	8.8	167
10aha	CH ₃	R	>1.0	2.4	125		28	49
10ahb	CH ₃	S	0.008 (<0.001)	2.3	30	51	14	49

^aEC₅₀ data represent means and SDs for $n = 4$ or more experiments performed in duplicate measuring LDH activity of *P. falciparum* 3D7 asexual parasites for 72 h. ^bShake-flask method. ^cT_{1/2} values are located in Table S2. LM = liver microsomes; Hep = hepatocytes.

trideuteromethyl substituent was not further considered. The combination analogs in this cohort did however have desirable aqueous solubility (~100 μM).

It was reasoned that **10ah** has suitable parasite activity and properties for frontrunner compounds and therefore the stereoisomers at the 3a-position were separated by chiral

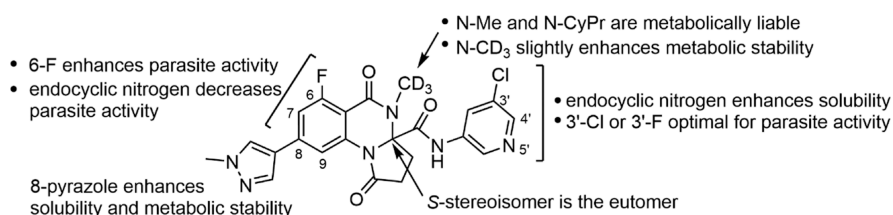


Figure 2. Summary of the structure activity-property relationship.

Table 8. Evaluation of Selected Compounds against PfATP4 Drug-Resistant *P. falciparum* Strains^a

compound	Dd2 EC ₅₀ (SD) μ M	Dd2 PfATP4 drug-resistant EC ₅₀ (SD) μ M ^{a,b}		Dd2 PfATP4 “49” resistant EC ₅₀ (SD) μ M ^{a,c}		
		PfATP4 ^{I398F,P990R,D1247Y}	PfATP4 ^{G358S}	PfATP4 ^{F156L}	PfATP4 ^{D425E}	PfATP4 ^{2.8xCNV}
10x	0.043 (0.005)	0.672 (0.190)	0.706 (0.313)	0.102 (0.010)	0.077 (0.017)	0.093 (0.018)
10y	0.161 (0.013)	1.25 (0.27)	1.19 (0.43)	0.332 (0.390)	0.230 (0.072)	0.327 (0.010)
10ag	0.007 (0.001)	0.119 (0.038)	0.182 (0.058)	0.011 (0.001)	0.014 (0.001)	0.011 (0.001)
10ahb	0.005 (<0.001)	0.067 (0.034)	0.088 (0.009)	0.009 (0.002)	0.007 (<0.001)	0.012 (0.002)
“49” ^d	0.015 (0.004)	0.142	0.028	0.025 (0.001)	0.030 (0.007)	0.030 (0.005)
KAE609 1 ^d	0.002 (<0.001)	0.009	1.34	0.003 (<0.001)	0.002 (<0.001)	0.004 (0.005)
SJ733 2 ^d	0.054 (0.007)	0.085	>10	0.112 (0.023)	0.079 (0.003)	0.136 (0.017)

^aEC₅₀ values represent means of 3 independent experiments against PfATP4 drug-resistant *P. falciparum* Dd2 strains in a 72 h LDH assay format.

^bDd2 PfATP4^{I398F,P990R,D1247Y} and PfATP4^{G358S} resistant strains. ^cCompound “49” resistant populations. ^dReference data taken from Ashton, et al.¹⁷ Dose response curves are shown in Figure S4 and S5.

Table 9. Evaluation of Selected Compounds against *P. knowlesi* Parasites and *P. falciparum* Multidrug-Resistant Strains^a

compound	wildtype parasites EC ₅₀ (SD) μ M		<i>P. falciparum</i> multidrug resistant parasites EC ₅₀ (SD) μ M			
	<i>P. falciparum</i> 3D7	<i>P. knowlesi</i> YH1	Dd2	W2 ^{me}	7G8	CAM3.1
10c	0.053 (0.010)		0.052 (0.008)	0.062 (0.005)		0.040 (0.010)
10x	0.019 (0.002)	0.173 (0.046)	0.020 (0.004)	0.017 (0.004)	0.041 (0.009)	0.023 (0.003)
10y	0.024 (0.004)	0.099 (0.041)	0.023 (0.002)	0.020 (<0.001)	0.041 (0.009)	0.027 (0.005)
10ag	0.004 (0.001)	0.060 (0.020)	0.003 (0.001)	0.003 (<0.001)	0.006 (0.001)	0.005 (0.001)
10ah	0.005 (0.001)	0.037 (0.033)	0.004 (0.002)	0.004 (0.001)	0.008 (0.001)	0.004 (0.001)
SJ733 2	0.043 (0.027)	0.23 (0.02)				

^aEC₅₀ values represent the means of 3 independent experiments against *P. falciparum* asexual parasites for 72 h and *P. knowlesi* asexual parasites for 48 h quantified using a fluorometer and an SYBR-green stain. See Figure S6 and S7 for dose response curves.

chromatography for further biological characterization. Previous crystallography and parasite activity data on **4** (Figure 1) established the *S*-isomer was the active enantiomer,¹⁷ and therefore the stereoisomers of **10ah** were assigned based on their asexual parasite activity. Accordingly, the *S*-isomer of **10ahb** was approximately 2-fold more potent (EC₅₀ 0.008 and 0.016 μ M) than the *R/S*-racemate, while the *R*-isomers were >250-fold less potent (EC₅₀ > 1.0 μ M) (Table 7). Fortuitously, it was revealed that active *S*-isomer **10ahb** has increased metabolic stability in human liver microsomes relative to the *R*-isomer (**10aha**) stability (human CL_{int} 30 versus 125 μ L/min/mg). Despite the relatively moderate metabolic stability in mouse microsomes, **10ah** had suitable aqueous solubility (>49 μ M at pH 7.4) for further phenotypic profiling and assessment in mouse models.

Overall, a desire to refine the activity-property profile prompted the exploration of additional structural motifs and polar functionality on the dihydroquinazolinone framework which resulted in improved solubility and metabolism while maintaining parasitic activity with no human HepG2 cytotoxicity (Figure 2).

Activity against PfATP4 Drug-Resistant Parasites. To investigate whether the antiparasitic activity of the optimized dihydroquinazolinone analogs resulted from the inhibition of PfATP4 and whether the analogs had the same resistance

profile as WJM921 **4**, we evaluated a representative set of analogs against PfATP4 inhibitor-resistant parasite lines. The parasite lines profiled include the lab-generated PfATP4^{I398F,P990R,D1247Y} strain, the clinically relevant PfATP4^{G358S} parasite strain,^{9,20} and the PfATP4^{F156L}, PfATP4^{D425E} and PfATP4^{2.8xCNV} parasite populations that were selected using the dihydroquinazolinone analog, “compound 49”.¹⁷ The results show that all dihydroquinazolinone derivatives tested (**10x**, **10y**, **10ag** and **10ah**) were between 7- and 25-fold less active (EC₅₀ 0.088–1.19 μ M) against the PfATP4^{G358S} parasite strain than against the Dd2 parental line (EC₅₀ 0.005–0.161 μ M), whereas KAE609 **1** and SJ733 **2** were greater than 500-fold less active against this PfATP4 mutant strain (Table 8 and Figure S4). Dihydroquinazolinone derivatives **10x**, **10y**, **10ag** and **10ah** were also 20- to 50-fold less active (EC₅₀ 0.067–1.25 μ M) against PfATP4^{I398F,P990R,D1247Y} parasites. In comparison, KAE609 **1** and SJ733 **2** were 3- and 1.5-fold less active (EC₅₀ 0.009–0.085 μ M) against the PfATP4^{I398F,P990R,D1247Y} strain. Analogs **10x**, **10y**, and **10ag** were between 1.5- and 2-fold less active (EC₅₀ 0.007–0.327 μ M) against the compound “49” resistant strains, PfATP4^{F156L}, PfATP4^{D425E}, and PfATP4^{2.8xCNV} compared to the Dd2 parental strain, whereas compound **10ah** exhibited similar potency (EC₅₀ 0.009 and 0.007 μ M) against the PfATP4^{F156L} and PfATP4^{D425E} mutant strains and was 2-fold less active (EC₅₀ 0.012 μ M) against the PfATP4^{2.8xCNV}

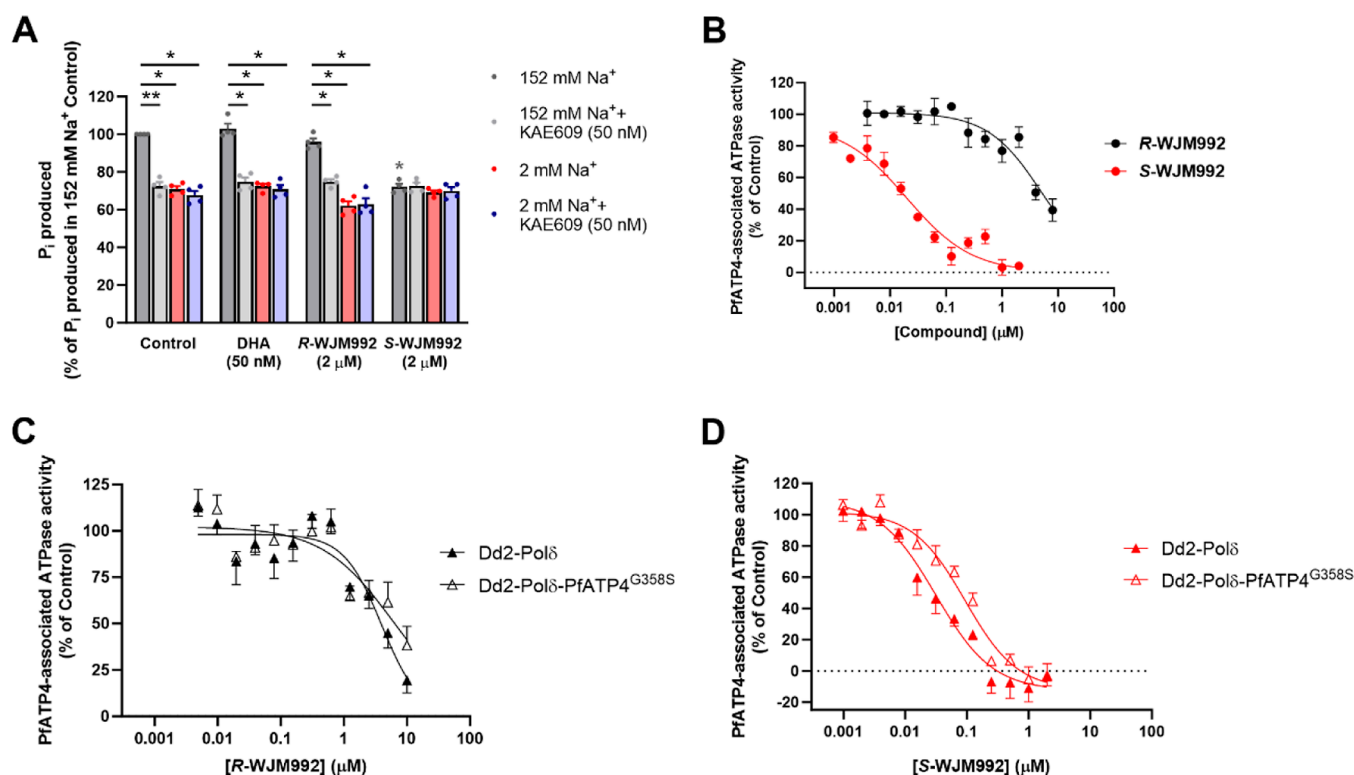


Figure 3. Analog S-WJM992 **10ahb** inhibits Na^+ -ATPase activity in membranes prepared from isolated *P. falciparum* parasites more potently than the less active isomer R-WJM992 **10aha**. (A) The effects of analogs **10aha** ($2\ \mu\text{M}$) and **10ahb** ($2\ \mu\text{M}$), DHA ($50\ \text{nM}$; negative control) and 0.2% v/v DMSO (solvent-only control) on ATPase activity in membranes prepared from 3D7 parasites, under high- $[\text{Na}^+]$ conditions ($152\ \text{mM Na}^+$) and low- $[\text{Na}^+]$ conditions ($2\ \text{mM Na}^+$; arising from the addition of $1\ \text{mM Na}_2\text{ATP}$) in the presence and absence of KAE609 **1** ($50\ \text{nM}$). The symbols show the data from individual experiments and the bars show the mean ($\pm\text{SEM}$) from 4 independent experiments performed with different membrane preparations. The data show the P_i produced as a percentage of that measured in the $152\ \text{mM Na}^+$ control. In individual experiments, the P_i produced in the $152\ \text{mM Na}^+$ control varied from 72 to 139 nmol per mg (total) protein per min. The (prenormalized) data for different compounds and conditions were compared using repeated measures one-way ANOVA with a Geisser-Greenhouse correction and *post hoc* Tukey test. For comparisons between different conditions for the same test compound (or for the control), significant differences are shown with black asterisks. For comparisons between the control and a compound under the same test condition, a significant difference is shown with a colored asterisk (in this case, a gray asterisk, as the only significant difference was between the control and analog **10ahb** in the $152\ \text{mM Na}^+$ condition). * $P < 0.05$, ** $P < 0.01$. (B) Potency of analog **10aha** (black circles) and **10ahb** (red circles) against PfATP4-associated ATPase activity in membranes prepared from 3D7 parasites. The data are the mean ($\pm\text{SEM}$) obtained from four independent experiments performed with different membrane preparations, with the exception of the highest two concentrations of **10aha**, for which data are from three independent experiments. (C,D) Potency of analogs **10aha** (black; C) and **10ahb** (red; D) against PfATP4-associated ATPase activity in membranes prepared from Dd2-Pol δ parasites (closed triangles) and Dd2-Pol δ -PfATP4^{G358S} parasites (open triangles). The data are the mean (shown $\pm$ or $-$ SEM) from four (C) or three (D) independent experiments. In (B–D), PfATP4-associated ATPase activity was calculated by subtracting the P_i production measured in the low- $[\text{Na}^+]$ ($2\ \text{mM}$) condition from that measured in high- $[\text{Na}^+]$ ($152\ \text{mM}$) in the presence of each of the different concentrations of analogs **10aha** and **10ahb** and is expressed as a percentage of that obtained for the high- $[\text{Na}^+]$ ($152\ \text{mM}$) control.

strain. These fold differences between the PfATP4 mutant and parental strains were similar to the 2-fold differences shown with compound “49”. These data confirm that the dihydroquinazolinone analogs retain their PfATP4 resistance profile suggesting that alterations to the scaffold during the optimization process have not impacted the on-target activity against PfATP4. However, the reduced activity against the clinically relevant PfATP4^{G358S} parasite strain raises concern for the future development of the dihydroquinazolinone class due to the impact on the efficacious dose required to clear resistant mutants.

Activity against Multidrug-Resistant Parasites. In our preliminary SAR study, we established that the dihydroquinazolinone scaffold was not vulnerable to the mechanisms that confer resistance to various (non-PfATP4 targeting) antimalarials.¹⁷ To assess whether the changes incorporated in the optimization of the dihydroquinazolinone scaffold altered their susceptibility to resistance mechanisms of antimalarials in the

field, we evaluated a selection of frontrunner compounds against chloroquine, mefloquine, and artemisinin-resistant parasite lines. It was found that all the dihydroquinazolinone analogs tested (**10x**, **10y**, **10ag** and **10ah**) had similar potency against wildtype and drug-resistant parasite strains (Table 9 and Figure S6), indicating that the modifications installed in the optimization process had not altered their susceptibility to resistance mechanisms of clinically used antimalarials.

Activity against *P. knowlesi* Parasites. It is known that there are significant differences in the ATP4 protein sequence between *Plasmodium* species and this has resulted in PfATP4 inhibitors having variable activity between *P. falciparum* and *P. knowlesi* parasites.²⁶ To determine whether there is a disparity between *P. falciparum* and *P. knowlesi* activity, representative dihydroquinazolinone compounds were evaluated against *P. knowlesi* YH1 asexual parasites using a SYBR DNA-staining assay. It was shown that analogs **10ag**, **10ah**, **10x**, and **10y** were 4- to 15-fold less active against *P. knowlesi* parasites (EC_{50}

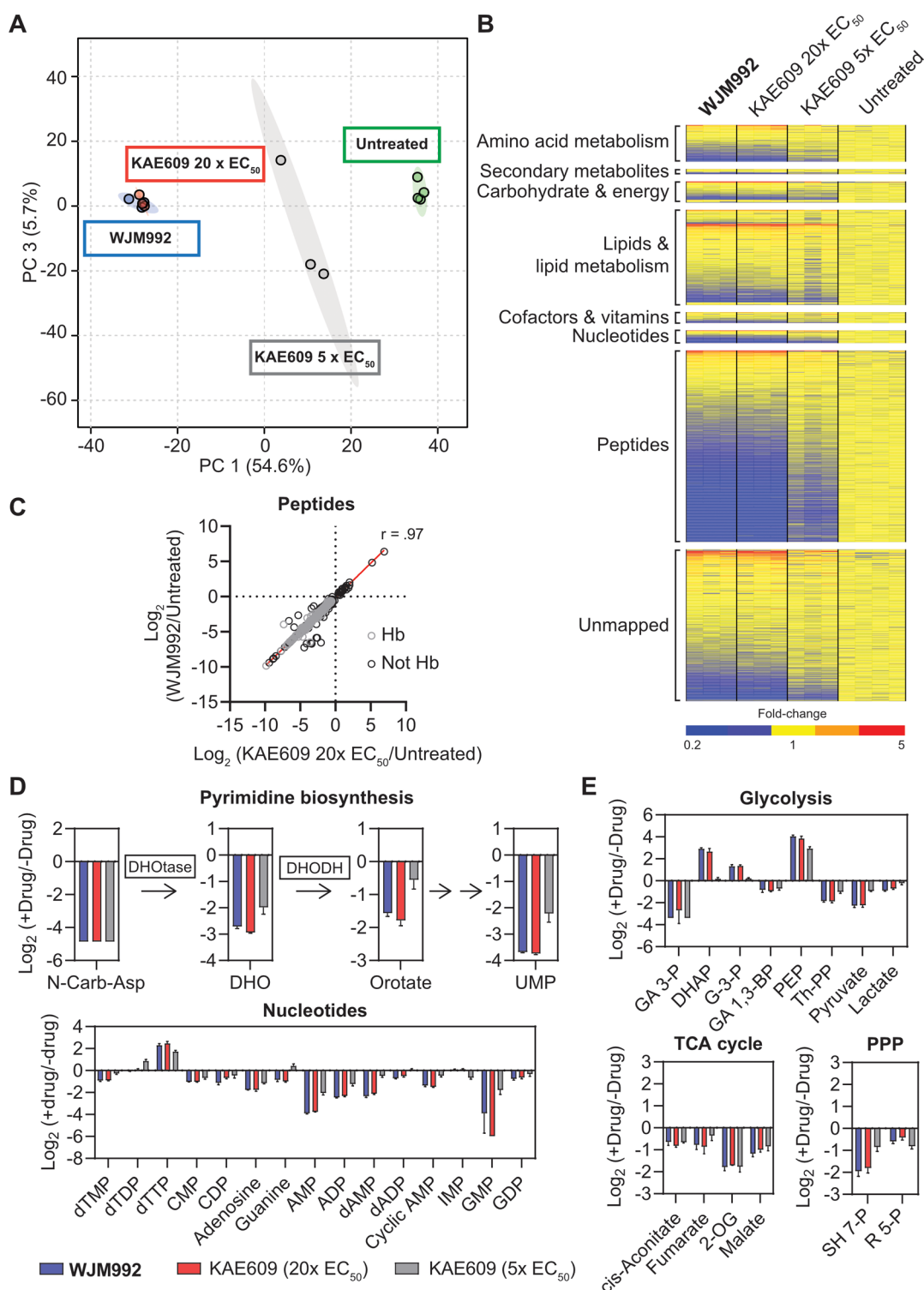


Figure 4. Metabolomic signature of RS-WJM992 **10ah** reflects that of KAE609 **1**. (A) Principal component analysis of the global metabolite profiles showing overlap of **10ah** with KAE609 **1**. (B) Heatmap analysis of the relative abundance of all metabolites revealed that both **10ah** and KAE609 induce widespread metabolic disruption. (C) Pearson correlation of the average log₂ fold-change for all peptides significantly perturbed by **10ah** and KAE609 ($p < 0.05$ and fold-change ≥ 1.5 or ≤ 0.67). Approximately 43% of perturbed peptides were mapped to the sequence of hemoglobin. (D) Significant perturbations to pyrimidine biosynthesis and nucleotide metabolites ($p < 0.05$ and fold-change ≥ 1.5 or ≤ 0.67). (E) Significant perturbations to central carbon metabolites ($p < 0.05$ and fold-change ≥ 1.5 or ≤ 0.67). For (D,E) data represents the log₂ fold-change of treated samples expressed relative to the average of the untreated control ($n = 3-4$).

0.037–0.173 μM) compared to *P. falciparum* 3D7 parasites (EC₅₀ 0.004–0.024 μM) (Table 9 and Figure S7). The PfATP4 inhibitor SJ733 **2** was also 5-fold less active against *P. knowlesi* (EC₅₀ 0.23 μM) than against *P. falciparum* (EC₅₀ 0.043

μM), consistent with the previous findings.²⁶ It is unknown whether other dihydroquinazolinone analogs synthesized follow the same activity trend, but frequent screening against *P. knowlesi* and *P. vivax* during optimization of PfATP4

inhibitors may ensure cross-species activity is maintained. These data have important ramifications for which malaria endemic region of the world a PfATP4-targeted antimalarial therapy would be distributed. Furthermore, the cross-species phenomena may have implications in testing PfATP4 inhibitors in mouse models using murine-specific *P. berghei*.

Activity against PfATP4. The most direct assay available for measuring PfATP4 activity entails measuring Na⁺-dependent ATPase activity [inorganic phosphate (P_i) production from ATP] in membranes prepared from isolated parasites. Na⁺-ATPase activity, which accounts for approximately one-third of total membrane ATPase activity, has been shown to be inhibited by numerous PfATP4-associated compounds including KAE609 1,^{10,27–30} consistent with it corresponding to PfATP4 activity. Furthermore, certain PfATP4 mutations conferring resistance to growth inhibition by PfATP4-associated compounds have been shown to reduce the sensitivity of Na⁺-ATPase activity to inhibition by the compounds^{10,28–30} and alter the kinetic properties (*K_m* for Na⁺) of Na⁺-ATPase activity.^{29,30}

Using this assay, we tested the effects of *R*-WJM992 (**10aha**) and *S*-WJM992 (**10ahb**) on *P. falciparum* membrane ATPase activity under high-[Na⁺] (152 mM) and low-[Na⁺] (2 mM) conditions and in the presence and absence of KAE609 1 at a concentration (50 nM) that completely inhibits the Na⁺-ATPase activity. When tested at 2 μM, **10ahb**, but not **10aha**, inhibited ATPase activity in the high-[Na⁺] condition when KAE609 1 was not present (Figure 3A). Neither compound affected ATPase activity in the presence of KAE609 1 or under low-[Na⁺] conditions. These data suggest that 2 μM of the *S*-isomer **10ahb** completely inhibited PfATP4 activity whereas 2 μM of the *R*-isomer **10aha** did not significantly inhibit PfATP4 activity. The antimalarial drug dihydroartemisinin (DHA) used at a concentration >10 × EC₅₀ for growth inhibition of 3D7 parasites (50 nM)³¹ did not affect membrane ATPase activity under any of the conditions tested (Figure 3A). We investigated the potency by which **10ahb** and **10aha** inhibited Na⁺-ATPase activity (Figure 3B). For **10aha**, we tested some concentrations higher than 2 μM to determine whether the compound might be a weak inhibitor of PfATP4. These experiments yielded EC₅₀ values for the inhibition of Na⁺-ATPase activity of 18.2 ± 7.1 nM for **10ahb** and 4.6 ± 0.9 μM for **10aha** (mean ± 7.1 SEM).

A G358S mutation in PfATP4 confers high grade resistance to the most clinically advanced PfATP4 inhibitors, KAE609 1 and SJ733 2.²⁹ We investigated whether this mutation affected the potency by which **10aha** (Figure 3C) or **10ahb** (Figure 3D) inhibited PfATP4-associated ATPase activity. Membranes were prepared from the Dd2-Polδ-PfATP4^{G358S} line generated previously²⁹ and its Dd2-Polδ parent.³² It should be noted that the PfATP4 protein expressed by Dd2 parasites has a G1128R mutation relative to the 3D7 PfATP4 protein for which data are shown in Figure 3B. The *R*-isomer **10aha** inhibited the Na⁺-ATPase activity of Dd2-PfATP4^{WT} and Dd2-PfATP4^{G358S} with EC₅₀ values of 4.1 ± 0.5 and 7.5 ± 2.0 μM, respectively (*P* = 0.13, paired *t*-test). For *S*-isomer **10ahb**, the EC₅₀ values were 26.4 ± 4.5 nM for Dd2-PfATP4^{WT} and 81.3 ± 10.0 nM for Dd2-PfATP4^{G358S} (*P* = 0.03, paired *t*-test). The G358S mutation rendered PfATP4 3.2-fold less sensitive to inhibition by compound **10ahb**.

Metabolomics. To further confirm the optimized dihydroquinazolinone series targets PfATP4, we profiled the cellular metabolomic response to *RS*-WJM992 **10ah** compared

to the known PfATP4 inhibitor, KAE609 1. Previous metabolomics studies have identified that antimalarials sharing a common mode of action induce similar metabolic signatures.^{33,34} Magnetically purified *P. falciparum* 3D7 infected red blood cells were exposed to 70 nM of **10ah** (5 × EC₅₀), 5 nM (5 × EC₅₀) or 20 nM (20 × EC₅₀) of KAE609 1, or vehicle (DMSO) for 5 h, followed by metabolite extraction and untargeted LC–MS analysis.

Principal component analysis of the global metabolite profiles demonstrated that the metabolic response to **10ah** overlaps with KAE609 1 (Figure 4A) suggesting these compounds share a similar mode of action. Heatmap analysis revealed that both **10ah** and KAE609 1 induce widespread metabolic disruption (Figure 4B and Supporting File S1), consistent with metabolic collapse due to loss of the ionic gradient generated by PfATP4. The most prominent metabolic signature induced by **10ah** and KAE609 1 was perturbation in the abundance of a subset of putative peptides (Figure S8A). Among the top 50 significantly perturbed metabolites, all except two are depleted peptides (Figure S8B). A targeted analysis of all 578 putative peptides detected in this study revealed that 390 were significantly perturbed by **10ah** (*p* < 0.05 and fold-change ≥1.5 or ≤0.67) and 96% of these were decreased in abundance compared to the untreated control (Figure 4B–C and Supporting file S1). This profile of perturbed putative peptides was also observed with KAE609 1 treatment (*r* = 0.996, *p* < 0.0001), and the vast majority (91%) of the overall 418 peptides significantly perturbed in this study were dysregulated by both **10ah** and KAE609 1 (Figures 4B,C, S8 and Supporting file S1). Accurate mass and isomer analysis of these significantly perturbed peptides further revealed that ~43% could be mapped to the sequence of hemoglobin and all of these putative hemoglobin-derived peptides were decreased in abundance compared to the untreated control (Figure 4C).

Disrupted nucleotide metabolism was the other major metabolic impact in cells treated with **10ah** or KAE609 1 (Figure S8A). This response included depletion of pyrimidine biosynthesis pathway metabolites and decreased general nucleotide levels, except for the pyrimidine deoxyribonucleotides, deoxythymidine triphosphate, and deoxythymidine diphosphate, which were increased (Figure 4D). Further to inducing a peptide and nucleotide signature, treatment with these compounds also results in significant perturbations in central carbon metabolites (Figure 4E), cofactors and vitamins, amino acid derivatives, and lipids (Figures 4B and S9–S11). This pleiotropic metabolic profile has previously been identified as a signature of PfATP4 inhibition in metabolomic studies of other PfATP4 inhibitors.^{34–37} Combined with our biochemical and genetic studies, this metabolomics data supports PfATP4 being the target of **10ah**.

Transmission Stage Activity. PfATP4 inhibitors are known to block transmission of gametocytes to the mosquito host in laboratory experiments^{17,20,38} and it was also shown that KAE609 1 cleared gametocytes from infected patients in clinical trials.³⁹ PfATP4 inhibitors block transmission by killing both gametocytes and female and male gametes, presumably due to the disruption of the Na⁺ and H⁺ gradients across the parasite plasma membrane and the dysregulation of parasite [Na⁺], pH and osmotic homeostasis. To determine the effect of the optimized dihydroquinazolinone scaffold on the transmission stage of the parasite lifecycle we assessed a

cohort of analogs in a dual gamete formation assay and a standard membrane feeding assay.

In the dual gamete formation assay, NF54 *P. falciparum* stage V gametocytes were treated with compound and then gametocytogenesis was induced by the addition of xanthurenic acid simulating the mosquito midgut environment.^{40,41} After 20 min the effect on male gamete development was then quantified using automated microscopy to measure exflagellation, while female gamete viability was determined at 24 h by measuring the fluorescence of an α Pfs25 antibody. It was determined that analog RS-WJM992 **10ah** potently inhibited both male and female gametocyte development (EC_{50} 0.098 and 0.361 μ M), while the effect of analog **10y** was more modest (EC_{50} 0.326 and 0.953 μ M) (Table 10 and Figure

Table 10. *P. falciparum* NF54 Male and Female Gamete Activity of Selected Compounds^a

compound	male EC_{50} μ M	female EC_{50} μ M
10y	0.326	0.953
10ah RS-WJM992	0.098	0.361

^a EC_{50} data represents the means of 3 replicate experiments against *P. falciparum* NF54 male and female gametes quantified by measuring exflagellation at 30 min by microscopy or by measuring fluorescence at 24 h using an α Pfs25 antibody, respectively. See Figure S12 for error values.

S12). This activity follows the trend of the asexual stage activity, with **10ah** being more potent. The activity of **10ah** against male and female gametes is comparable to the activity reported on the PfATP4 clinical candidate KAE609 **1**.^{17,20,38}

On demonstrating that S-WJM992 **10ah** potently inhibited gamete development, we then assessed compound **10ahb** in a standard membrane-feeding assay that measures the capacity of compounds to block the transmission of parasites to mosquitoes by way of a blood meal. In this model, blood infected with stage V gametocytes are treated with **10ahb** (at 1, 0.25, 0.1, and 0.05 μ M on independent occasions) and R-WJM992 **10aha** (less active control) (at 1 μ M) and then the blood is fed to *Anopheles stephensi* mosquitoes via a membrane feeding reservoir. Seven days after the infected blood meal, the mosquito midguts are dissected and oocyst numbers on the midgut hemolymph are counted by visualization under a microscope. At 1 and 0.25 μ M of **10ahb**, a near complete reduction in oocyst numbers (0 and 2.4%) was observed relative to the vehicle control and the less active R-isomer **10aha** (54 and 63%) (Figure 5). Moreover, no infected mosquitoes were observed on treatment with 1 and 0.25 μ M of **10ahb**, whereas approximately 50–60% were infected in the vehicle and **10aha** control treatment groups. At lower treatment doses (0.1 and 0.05 μ M) of **10ahb**, the oocyst count and the number of infected mosquitoes increased (9.5 and 26%) compared to the higher treatment doses (1 and 0.25 μ M) but remained significantly lower than the vehicle (60%) (Figure S13). The higher oocyst and infected mosquito count at 0.05 than at 0.1 μ M is attributed to experimental error. The reduction in oocyst numbers and infected mosquitoes is aligned with transmission data on KAE609 **1** from a previous study.³⁸ Collectively these data showed that the dihydroquinazolinone scaffold has potential as a transmission blocking agent.

Efficacy in a 4-Day *P. berghei* Mouse Model. Several optimized analogs have in vitro ADME properties that were

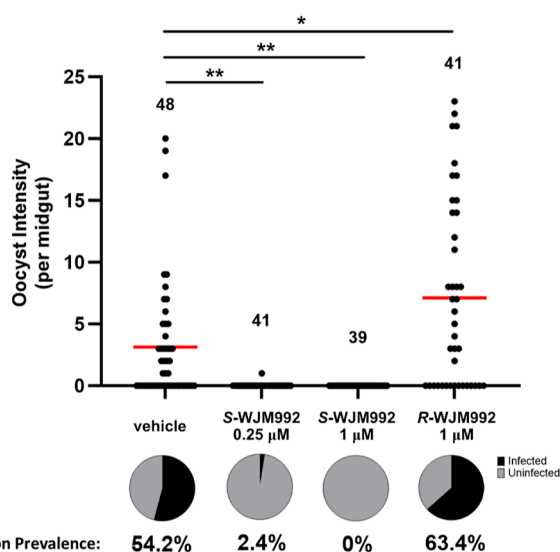


Figure 5. Activity of R-WJM992 **10aha** and S-WJM992 **10ahb** in a standard membrane feeding assay. Oocyst counts and intensity per each midgut dissected from *Anopheles stephensi* mosquitoes 7 days post the blood meal infected with *P. falciparum* NF54 stage V gametocytes treated with compound at the indicated concentration. Red bars indicate average oocyst intensity. Numbers indicate the total number of mosquito midguts dissected per treatment group. Infection prevalence indicates the percentage of mosquitoes that were infected. Transmission intensity (oocyst burden) was compared between each group and the vehicle control in a pairwise fashion and significance was tested using the Wilcoxon rank sum test. ** $P < 0.001$; * $P < 0.05$. Repeat experiments are shown in Figure S13.

deemed suitable for evaluation in a 4-day *P. berghei* mouse model. In this model, mice are infected with *P. berghei* ANKA, a species that naturally infects mice. Two hours postinfection mice are administered the compound by oral gavage, and then dosed compound again at 24, 48, and 72 h. Blood samples are taken on days 2, 3, and 4 and parasitemia is quantified. It was shown that compounds **10x** and **10y** at 20 mg/kg moderately reduced parasitemia from 21 to 53% on day 4 (Table 11 and

Table 11. Efficacy of Selected Compounds in a *P. berghei* 4 Day Mouse Model^a

compound	% reduction in parasitemia
8y	78.6
10x	34.2
10y	53.5
10ahb S-WJM992	62.7
CQ	99.9

^aCompounds were administered q.d. 20 mg/kg by p.o. 4 h after infection with *P. berghei* ANKA parasites (day 0) and then on days 1, 2, and 3. Data are average % parasitemia for $n = 4$ mice on day 4. Chloroquine (CQ) (10 mg/kg) was used as a positive control. Error values are shown in Figure S14.

Figure S14) and derivatives **8y** and **10ahb** (S-WJM992) at 20 mg/kg significantly reduced parasitemia by 79 and 63% on day 4. Compounds **8y** and **10ahb** did not completely clear parasitemia and this may be attributed to the sequence variation in ATP4 between *P. falciparum* and *P. berghei* that may have reduced activity against *P. berghei* parasites compared to *P. falciparum*. The species differentiation in ATP4 has also been attributed to the lower efficacy of SJ733 **2** in a *P. berghei*

mouse model in comparison to the efficacy displayed in a *P. falciparum* humanized mouse model.²⁰ Furthermore, it was recently found that a variety of PfATP4 inhibitors all showed varying degrees of reduced activity against *P. knowlesi* parasites engineered to express *P. berghei* ATP4.⁴² For this reason, we assessed the efficacy of **10ah** in a *P. falciparum* humanized mouse model.

Efficacy in a 4-Day *P. falciparum* SCID Mouse Model.

A humanized *P. falciparum* SCID mouse model⁴³ was used to evaluate the racemic frontrunner analog RS-WJM992 **10ah** (a sample of the S-WJM992 was not available at the time of the study). In this model using NOD-SCID IL-2R γ^{null} mice,⁴⁴ human erythrocytes are continually engrafted into the mice to reach a minimum of 40% human circulating erythrocytes before the introduction of *P. falciparum* 3D7^{0087/N9} infected blood. At approximately 1–2% parasitemia, **10ah** was administered to mice at 25 mg/kg orally on day 1 and then again at 24, 48, and 72 h. Parasitemia is quantified every day using a TER-119-Phycoerythrin antibody (a marker of murine erythrocytes) and SYTO-16 (a nucleic acid dye) measured by flow cytometry and microscopy,⁴⁵ and upon clearance of parasitemia on day 5, parasite recrudescence is monitored every third subsequent day until 5% parasitemia is measured or cure at day 30. Bioanalysis of the blood from compound **10ah** treated mice showed that the C_{max} of **10ah** was relatively low (<30 ng/mL) indicating the compound was poorly absorbed (Figure S16). This is consistent with the low permeability, that is, apical A to B value (1.5×10^{-6} cm/s) and high B to A efflux ratio (24.3×10^{-6} cm/s) in Caco-2 cells (Table S1). Despite the limited systemic exposure, **10ah** reduced parasitemia to levels below quantification on day 5 (Figure 6 and S15),

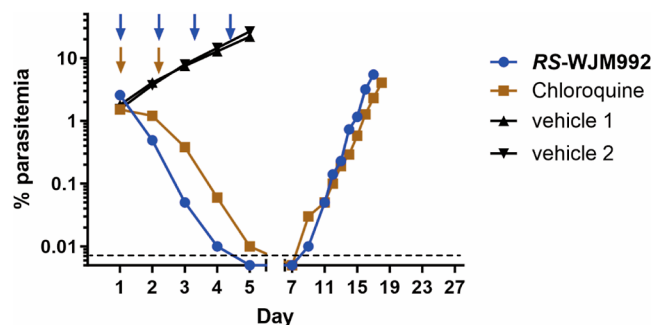


Figure 6. Activity of RS-WJM992 **10ah** in a *P. falciparum* 4 day humanized NOD-scid IL-2R γ^{null} mouse model. **10ah** was dosed orally at 25 mg/kg q.d. for 4 days (indicated by blue arrows) while chloroquine was dosed orally at 10 mg/kg q.d. for 2 days (indicated by brown arrows). Parasitemia is expressed as the % of *P. falciparum*-infected human erythrocytes and was measured at each time point by flow cytometry using TER-119-phycoerythrin and SYTO-16 staining. Parasitemia below 0.005% could not be quantified, indicated by the dotted line.

consistent with the rate of parasite clearance shown by KAE609 **1**⁹ whereas the rate of parasite clearance by chloroquine (two 10 mg/kg p.o. doses) was slightly slower. Although parasitemia was reduced to undetectable levels on day 5, parasite recrudescence was observed between days 7 and 9, and 5% parasitemia was reached on day 17 (Figure 6). The model showed that a higher dose of **10ah**, improved formulation or further optimization of the dihydroquinazolinone scaffold to improve absorption and lower clearance

would be required to achieve sterilizing cure in this mouse model.

CONCLUSIONS

We previously reported on preliminary optimization of the dihydroquinazolinone-3-carboxamide antimalarial class and showed that it targets PfATP4.¹⁷ The frontrunner analog WJM921 **4** (Figure 1) from this study exhibited potent in vitro asexual activity but only showed modest efficacy in a 4-day *P. berghei* mouse model (30% reduction of parasitemia at 40 mg/kg). The modest efficacy was attributed to the low aqueous solubility (5 μM at pH 7.4) and modest metabolic stability (mouse CL_{int} 55 $\mu\text{L}/\text{mg}/\text{min}$). To improve on these properties to enhance in vivo efficacy, we investigated different structural modifications to the dihydroquinazolinone scaffold. We found that the inclusion of an endocyclic nitrogen in the scaffold largely decreased parasite activity, although an endocyclic nitrogen at the 6-position markedly improved human and rat metabolic stability ($\text{CL}_{\text{int}} \sim 10 \mu\text{L}/\text{mg}/\text{min}$), and modestly improved aqueous solubility (13 μM) (Table 2). Metabolism was found to be intrinsically linked to the lipophilicity of the scaffold, and metabolite identification showed that N-demethylation was the major metabolite. This metabolic soft spot could not be suppressed without affecting parasite activity with a variety of structural changes. A N-cyclopropyl group was found to be equipotent to the N-methyl orthologues but was also prone to high metabolic turnover which we reasoned was due to the lipophilic contribution of the cyclopropyl group (Table 5). Deuteration of the methyl group is a strategy that has been used in the past to mitigate metabolism while not impacting biological activity or overall lipophilicity.²⁵ Accordingly the inclusion of an N-trideuteromethyl group resulted in a slight improvement in metabolic stability (Tables 5–7). The 8-position on the scaffold was tolerant of functional groups including several heterocycles. We reasoned that the 8-position could be a site for introducing functionality to lower the overall lipophilicity of the scaffold and improve solubility and metabolic stability. It was found that the 4-substituted N-methyl pyrazole and N-substituted pyrazole markedly increased aqueous solubility while maintaining parasite activity and modestly improving metabolic stability (Tables 3, 4, and 6). The 8-pyrazole modification and 6-fluoro were incorporated to produce frontrunner analog, WJM992 **10ah** of which the active S-isomer showed enhanced human metabolic stability relative to the less active R-isomer (Table 7).

The frontrunner analog S-WJM992 **10ahb** mediated a 63% reduction in parasitemia at 20 mg/kg p.o. in the *P. berghei* 4-day mouse model. It was noted that the systemic exposure of **10ahb** in the mouse model was low, and we suspected that this was related to the pyrazole group that contributed to the modest permeability and high efflux ratio measured in a Caco-2 cell permeability assay. It was also noted that there are noticeable amino acid differences in the orthologues of ATP4 between *Plasmodium* species and that this could be a reason for the modest reduction in parasitemia observed in the *P. berghei* mouse model. Indeed, analogs were between 4 and 15-fold less active against *P. knowlesi* parasites compared to *P. falciparum* (Table 9). Accordingly, RS-WJM992 **10ah** dosed at 25 mg/kg b.i.d. for 4 days in a *P. falciparum* humanized mouse model reduced parasitemia by >99%, although recrudescence was observed 4 to 5 days after the last drug treatment. This study highlights the need for genetically engineered *P. falciparum*

parasite lines expressing ATP4 from other *Plasmodium* species to account for species differentiation between ATP4 orthologs.

To confirm the mechanism of action of the new dihydroquinazolinone analogs we profiled analogs against PfATP4 resistant lines and found the new analogs displayed slightly reduced potency against compound “49” resistant parasites¹⁷ and significantly reduced activity against the lab generated PfATP4^{I398F,P990R,D1247Y} strain²⁰ and the clinically relevant PfATP4^{G358S} parasite strain⁹ compared to wildtype *P. falciparum* parasites (Table 8). However, it was revealed that analogs retained their potency against common multidrug-resistant parasite lines (Table 8). The frontrunner analog S-WJM992 10ahb was shown to inhibit PfATP4-associated membrane ATPase activity and gave rise to a metabolic signature that was equivalent to that of KAE609 1, signifying that alteration of the dihydroquinazolinone scaffold had not affected the PfATP4 mechanism of action. Furthermore, compounds were shown to block male and female gamete development in vitro and correspondingly block transmission of parasites from a blood meal to the mosquito, reminiscent of the transmission-blocking phenotype observed with KAE609 1. Overall, modification of the dihydroquinazolinone has improved the physicochemical traits and led to improved efficacy in mouse models of malaria, although further optimization is required to increase potency against the parasite and improve absorption and half-life and most critically further work is required to improve the risk of resistance profile of this scaffold if it is to join the armamentarium of antimalarials in development to combat malaria.

EXPERIMENTAL SECTION

General Chemistry Methods. Solvents and reagents were obtained commercially and used without further purification. NMR spectra were recorded on either a Bruker Ascend 300 or a Bruker Ultrashield 400. Spectra were processed using MestReNova 14.3 software. Chemical shifts (δ) are recorded in parts per million (ppm) and referenced to the corresponding solvent signal. Coupling constants (J) are recorded in Hertz (Hz) and multiplicities are described by singlet (s), broad singlet (br s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt), doublet of doublet of doublets (ddd) and multiplet (m). Column chromatography was conducted with silica gel using prepacked Phenomenex (particle size 40–60 μ m) in combination with a CombiFlash NextGen or with prepacked SepaFlash columns (particle size 40–63 μ m) in combination with a CombiFlash Rf 200, CombiFlash NextGen or Biotage Selekt. LCMS was recorded on an Agilent LCMS system composed of an Agilent G6120B Mass Detector, 1260 Infinity G1312B Binary pump, 1260 Infinity G1367E HiPALS autosampler, and 1260 Infinity G4212B Diode Array Detector. Final compounds were determined to be >95% pure using this method, unless stated otherwise. High-resolution mass spectrometry (HRMS) was acquired through the Bio21 Mass Spectrometry and Proteomics Facility using a Thermo Scientific nano-LC Q Exactive plus mass spectrometer. Compounds 8a–e and 8j were commercially sourced and used without further purification.

Chemistry Procedures. *General Procedure A: Methyl Carboxamide Formation.* To a stirring solution of the appropriate anthranilic acid (1 equiv) in tetrahydrofuran (THF) or dimethylformamide (DMF) at 0 °C was added either CDI (1.1 equiv) or EDCI-HCl (2 equiv), HOBt (1.2 equiv) and DIPEA (5 equiv), followed by methylamine hydrochloride (2.5 equiv). The reaction was then stirred at rt until complete conversion was observed via LC–MS. The reaction mixture was then diluted with EtOAc, washed with cold water and brine, then dried over anhyd. MgSO₄ filtered and

concentrated in vacuo. The crude product was then isolated using column chromatography to afford the corresponding amide.

General Procedure B: HATU Mediated Amide Coupling. To a stirred solution of the appropriate carboxylic acid (1 equiv) in THF under N₂ was added HATU (1.5 equiv) and DIPEA (5 equiv). The mixture was stirred at rt for 15 min before the appropriate amine (1.2 equiv) was added and the reaction was then stirred for an additional 16 h. Upon completion, the reaction mixture was diluted with EtOAc washed with water and brine, then dried over anhyd. MgSO₄ filtered and concentrated in vacuo. The crude product was then isolated using column chromatography to afford the corresponding amide.

General procedure C: TCFH-NMI mediated amide coupling. To a stirred solution of the appropriate carboxylic acid (1 equiv), amine (1.3 equiv) and TCFH (1.4 equiv) in MeCN was added NMI (2.5 equiv). The reaction was stirred at rt for 1.5 h before being diluted with EtOAc (30 mL), washed with H₂O and brine, then dried over MgSO₄, filtered and concentrated in vacuo. The crude product was then purified using column chromatography to yield the corresponding amide.

General Procedure D: Cyclisation of Aminobenzamides with α -Ketoglutaric Acid. To a stirred solution of the relevant aminobenzamide (1 equiv) in AcOH was added α -ketoglutaric acid (1.3 equiv). The reaction was then refluxed for 4 h. Upon completion, the reaction mixture was cooled and then concentrated to remove excess AcOH. The resulting residue was then diluted with water and stirred for 1 h, resulting in a precipitate. The precipitate was isolated via vacuum filtration to afford the corresponding dihydroquinazolinone, which was then used without further purification.

General Procedure E: Buchwald–Hartwig Cross Coupling of Aryl Bromides. A solution of the appropriate aryl bromide (1 equiv), appropriate amine (3.5 equiv), Cs₂CO₃ (1.5 equiv) and XPhos-Pd-G3 (2 mol %) in 1,4-dioxane was heated at 120 °C under microwave irradiation for 30 min. The reaction mixture was then filtered through Celite and the filtrate concentrated in vacuo. The desired product was then isolated via reverse phase preparative high-performance liquid chromatography (HPLC).

General Procedure F: Suzuki-Miyaura-Type Cross Coupling of Aryl Bromides. A solution of the appropriate aryl bromide (1 equiv), appropriate boronic acid or ester (1.5 equiv), K₃PO₄ (2.5 equiv) and Pd(dppf)Cl₂ (0.1 equiv) in 1,4-dioxane and water (9:1) was heated at 130 °C under microwave radiation for 20 min. The crude mixture was diluted with EtOAc, washed with water and brine, then dried over anhyd. MgSO₄, filtered and concentrated in vacuo. The desired product was then isolated via reverse phase preparative HPLC.

2-Amino-4-methoxy-N-methyl-benzamide (6f). 2-Amino-4-methoxy-benzoic acid (1.80 g, 10.8 mmol) was reacted according to General procedure A using CDI to give 6f as a light-brown powder (1.35 g, 70%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.96 (d, J = 4.0 Hz, 1H), 7.41 (d, J = 8.8 Hz, 1H), 6.59 (s, 2H), 6.20 (d, J = 2.4 Hz, 1H), 6.08 (dd, J = 8.8, 2.4 Hz, 1H), 3.69 (s, 3H), 2.69 (d, J = 4.4 Hz, 3H). LC–MS (m/z): 181.2 [M + H]⁺.

8-Methoxy-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxylic Acid (7f). Compound 6f (1.35 g, 7.51 mmol) was reacted according to General procedure D to afford compound 7f as a white powder (1.97 g, 90%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.88–7.77 (m, 2H), 6.86 (dd, J = 8.8, 2.4 Hz, 1H), 3.82 (s, 3H), 3.04 (s, 3H), 2.81–2.55 (m, 4H). LC–MS (m/z): 289.2 [M – H]⁺.

***N*-(3-Chlorophenyl)-8-methoxy-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (8f).** Compound 7f (71 mg, 0.245 mmol) was reacted with 3-chloroaniline according to General procedure B to afford compound 8f as a white solid (11 mg, 11%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.97 (s, 1H), 7.82 (d, J = 8.7 Hz, 1H), 7.64 (s, 1H), 7.46 (d, J = 8.3 Hz, 1H), 7.42 (s, 1H), 7.29 (app t, J = 8.1 Hz, 1H), 7.13 (d, J = 7.5 Hz, 1H), 6.89 (d, J = 6.6 Hz, 1H), 3.82 (s, 3H), 3.19 (s, 3H), 2.90–2.79 (m, 2H), 2.77–2.67 (m, 1H), 2.64–2.54 (m, 1H). ¹³C NMR (75 MHz, CDCl₃): δ 172.8, 168.2, 164.4, 162.1, 137.4, 137.3, 135.0, 130.8, 130.2, 125.8, 120.8, 118.6, 112.8, 111.6, 103.9, 81.0, 55.9, 30.7, 30.2, 29.6. HRMS (m/z): [M + H]⁺ calcd for C₂₀H₁₈ClN₃O₄, 400.1059; found, 400.1057.

3-Amino-5-bromo-N-methylpicolinamide (6a). 3-Amino-5-bromopicolinic acid (500 mg, 2.30 mmol) was reacted according to General procedure A with EDCI to afford compound **6a** as an off-white solid (530 mg, 85%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.53 (d, *J* = 4.8 Hz, 1H), 7.80 (d, *J* = 2.0 Hz, 1H), 7.39 (d, *J* = 2.0 Hz, 1H), 7.02 (br s, 2H), 2.74 (d, *J* = 4.8 Hz, 3H). LC–MS (*m/z*): 230.0 [M + H]⁺.

3-Amino-5-methoxy-N-methylpicolinamide (6g). To a stirred solution of 3-amino-5-bromo-N-methylpicolinamide (0.1 g, 4.35 mmol) in dioxane (5.0 mL) were added MeOH (0.695 mL, 21.73 mmol) and NaOtBu (0.858 g, 6.085 mmol) and the mixture was degassed by N₂ for 10 min before the addition of Pd₂(dba)₃ (0.08 g, 0.087 mmol) and *t*BuBrettPhos (0.211 g, 0.435 mmol) successively. The reaction mixture was heated at 100 °C for 16 h. On completion, the reaction mixture was cooled to room temperature, filtered through Celite and concentrated in vacuo to afford a crude residue which was purified using column chromatography to give 3-amino-5-methoxy-N-methylpicolinamide **6g** as an off-white solid (0.600 g, 76%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.27 (d, *J* = 4.8 Hz, 1H), 7.47 (d, *J* = 2.4 Hz, 1H), 6.89 (br s, 2H), 6.64 (d, *J* = 2.4 Hz, 1H), 3.77 (s, 3H), 2.73 (d, *J* = 4.8 Hz, 3H). LC–MS (*m/z*): 182.0 [M + H]⁺.

8-Methoxy-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrimidine-3a(1H)-carboxylic Acid (7g). Compound **6g** (304 mg, 2.08 mmol) was reacted according to General procedure D to afford compound **7g** as a gray solid (305 mg, 65%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.23 (s, 1H), 8.16 (s, 1H), 3.90 (s, 3H), 3.06 (s, 3H), 2.79–2.58 (m, 4H). LC–MS (*m/z*): 292.2 [M + H]⁺.

N-(3-Chlorophenyl)-8-methoxy-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrido[2,3-*e*]pyrrolo[1,2-*a*]pyrimidine-3a(1H)-carboxamide (8g). To a stirred solution of compound **7g** (100 mg, 0.343 mmol) in DMF (3 mL) was added PyBOP (357 mg, 0.687 mmol) and DIPEA (299 μL, 1.72 mmol). After 10 min, 3-chloroaniline (237 μL, 2.23 mmol) was added and the reaction mixture was heated for 16 h at 60 °C. Upon completion, the mixture was cooled to room temperature, water was added, and the resultant precipitate was collected by filtration. The residue was triturated with EtOH, DCM and *n*-pentane (2:2:6) to afford compound **8g** as a white solid (43 mg, 31%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.00 (s, 1H), 8.27 (d, *J* = 2.4 Hz, 1H), 7.84 (d, *J* = 2.4 Hz, 1H), 7.63 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.30 (t, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 3.91 (s, 3H), 3.21 (s, 3H), 2.92–2.81 (m, 2H), 2.80–2.64 (m, 2H). ¹³C NMR (75 MHz, DMSO): δ 173.7, 168.8, 160.2, 157.3, 139.1, 136.0, 133.8, 132.7, 130.1, 129.8, 124.3, 120.9, 119.7, 111.1, 80.3, 56.1, 30.4, 29.7, 27.5. HRMS *m/z*: [M + H]⁺ calcd for C₁₉H₁₇ClN₄O₄, 401.1011; found, 401.1010.

2-Amino-6-chloro-N-methylnicotinamide (6ha). To a solution of 2-amino-6-chloronicotinic acid (2.0 g, 10.4 mmol) in CHCl₃ (15 mL) was added 2 drops of dry DMF followed by oxalyl chloride (4.44 g, 23.2 mmol), dropwise at 0 °C. The temperature of the reaction mixture was maintained at 0 °C for 1 h and then concentrated in vacuo. The residue was coevaporated two times with CHCl₃ to give a crude oil. The oil was dissolved again in CHCl₃ (25 mL) and cooled to 0 °C and to this solution was gradually added 2 M solution of methylamine in THF (1.88 g, 23.2 mmol). The reaction mixture was allowed to warm to room temperature over 2 h and stirred for 16 h. Upon completion, cold water was added to the reaction mixture and extracted with CHCl₃ (3 × 30 mL). The organic layer was washed with brine, and then dried over anhyd. Na₂SO₄, filtered and concentrated in vacuo. The residue was purified using column chromatography to afford compound **6ha** as an off-white solid (1.70 g, 79%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.60 (d, *J* = 4.0 Hz, 1H), 8.48 (s, 1H), 7.90 (s, 1H), 2.78 (d, *J* = 4.0 Hz, 3H). LC–MS (*m/z*): 205.0 [M + H]⁺.

6-Chloro-4-((2,4-dimethoxybenzyl)amino)-N-methylnicotinamide (6hb). To a stirred solution of **6ha** (1.70 g, 8.29 mmol) in DMF (920 mL) was added Et₃N (3.46 mL, 24.9 mmol) followed by (2,4-dimethoxyphenyl)methanamine (1.37 mL, 9.12 mmol) at 0 °C. The reaction mixture was allowed to warm slowly to rt and stirred for 16 h. Upon completion, the reaction mixture was diluted with EtOAc (60 mL) and washed with water (3 × 25 mL). The combined organic

phase was washed with brine, and then dried over anhyd. Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified using column chromatography to afford compound **6hb** (1.60 g, 57%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.73 (br s, 1H), 8.51 (br s, 1H), 8.29 (s, 1H), 7.16 (d, *J* = 8.0 Hz, 1H), 6.69 (s, 1H), 6.59 (s, 1H), 6.51–6.47 (m, 1H), 4.27 (d, *J* = 4.8 Hz, 2H), 3.82 (s, 3H), 3.75 (s, 3H), 2.72 (d, *J* = 4.0 Hz, 3H). LC–MS (*m/z*): 336.1 [M + H]⁺.

4-((2,4-Dimethoxybenzyl)amino)-6-methoxy-N-methylnicotinamide (6hc). To a stirred solution of **6hb** (750 mg, 2.23 mmol) in DMSO (20 mL) was added NaOMe (25% solution in MeOH, 4.8 mL, 22.3 mmol) and the mixture was heated at 80 °C for 48 h. Upon completion, the reaction mixture was cooled to rt, cold water (25 mL) was added to the reaction and extracted with EtOAc (3 × 15 mL). The combined organic phase was washed with brine, and then dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified using column chromatography to afford compound **6hc** as an off white solid (280 mg, 38%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.49 (br s, 1H), 8.30 (br s, 1H), 8.21 (s, 1H), 7.12 (d, *J* = 8.0 Hz, 1H), 6.58 (s, 1H), 6.51–6.47 (m, 1H), 5.85 (s, 1H), 4.20 (d, *J* = 4.8 Hz, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.74 (s, 3H), 2.71 (d, *J* = 4.0 Hz, 3H). LC–MS (*m/z*): 332.0 [M + H]⁺.

4-Amino-6-methoxy-N-methylnicotinamide (6h). To a stirred solution of 4-((2,4-dimethoxybenzyl)amino)-6-methoxy-N-methylnicotinamide (500 mg, 1.51 mmol) in DCM (18 mL) was added trifluoroacetyl (TFA) (3.46 mL, 45.3 mmol) and the mixture was refluxed for 2 h. On completion, the reaction mixture was cooled to rt and then concentrated under reduced pressure to remove volatiles. The residue was neutralized by slow addition of 20% aqueous NaOH solution at 0 °C and extracted with EtOAc (3 × 10 mL). The combined organic phase was washed with brine, and then dried over anhyd. Na₂SO₄, filtered and concentrated under reduced pressure to afford compound **6h** as brown sticky oil (225 mg, 82%) which was used for the next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.20 (br s, 1H), 8.19 (s, 1H), 7.01 (s, 2H), 5.92 (s, 1H), 3.76 (s, 3H), 2.71 (d, *J* = 4.0 Hz, 3H). LC–MS (*m/z*): 182.0 [M + H]⁺.

2-Methoxy-6-methyl-5,9-dioxo-5,6,8,9-tetrahydropyrido[3,4-*e*]pyrrolo[1,2-*a*]pyrimidine-6a(7H)-carboxylic Acid (7h). Compound **6h** (200 mg, 1.10 mmol) was reacted according to General procedure D and triturated with an EtOH and Et₂O mixture (1:4) to afford compound **7h** (110 mg, 34%). LC–MS (*m/z*): 292.2 [M + H]⁺.

N-(3-Chlorophenyl)-2-methoxy-6-methyl-5,9-dioxo-5,6,8,9-tetrahydropyrido[3,4-*e*]pyrrolo[1,2-*a*]pyrimidine-6a(7H)-carboxamide (8h). To a stirred solution of **7h** (500 mg, 1.72 mmol) in DMF (7 mL) under N₂ was added 3-chloroaniline (363 μL, 3.43 mmol). The reaction mixture was cooled to 0 °C and DMTM (950 mg, 3.43 mmol) was added portionwise. The mixture was allowed to warm slowly to rt and stirred for an additional 12 h. On completion, the reaction mixture was diluted with EtOAc (30 mL). The organic layer was washed with cold water (3 × 25 mL) and brine, and then dried over anhyd. Na₂SO₄, filtered and concentrated in vacuo. The residue was purified using column chromatography followed by trituration with ethanol and diethyl ether (1:3) to afford compound **8h** as a white solid (25 mg, 4%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.93 (br s, 1H), 8.64 (s, 1H), 7.64 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.33–7.28 (m, 2H), 7.16 (d, *J* = 8.0 Hz, 1H), 3.91 (s, 3H), 3.19 (s, 3H), 2.93–2.67 (m, 4H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.4, 168.2, 166.4, 160.8, 148.8, 143.6, 138.9, 130.1, 121.2, 120.0, 110.5, 99.0, 80.5, 54.1, 29.8, 27.6. HRMS *m/z*: [M + H]⁺ calcd for C₁₉H₁₇ClN₄O₄, 401.1011; found, 401.1010.

2-Amino-6-chloro-N-methylnicotinamide (6ia). To a solution of 2-amino-6-chloronicotinic acid (2.0 g, 11.6 mmol) in DMF (15 mL) at 0 °C were successively added EDC·HCl (4.4 g, 23.2 mmol), HOBt (1.9 g, 23.2 mmol) and DIPEA (10.1 mL, 57.9 mmol). The mixture was stirred for 15 min under N₂ and thereafter methylamine hydrochloride (1.6 g, 23.2 mmol) was added. The reaction mixture was warmed to room temperature and stirred for 16 h. On completion, the mixture was diluted with EtOAc (50 mL). The organic layer was washed with cold water (3 × 50 mL) and brine, and

then dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was purified using column chromatography to afford compound **6ia** as an off-white solid (1.66 g, 77%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.42 (d, J = 4.0 Hz, 1H), 7.87 (d, J = 8.0 Hz, 1H), 7.49 (br s, 2H), 6.62 (d, J = 8.0 Hz, 1H), 2.72 (d, J = 4.0 Hz, 3H). LC–MS (m/z): 186.0 $[\text{M} + \text{H}]^+$.

2-((2,4-Dimethoxybenzyl)amino)-6-methoxy-N-methylnicotinamide (6ib). To a solution of **6ia** (500 mg, 2.69 mmol) in toluene (15 mL) were added 2,4-dimethoxybenzaldehyde (895 mg, 5.39 mmol) and AcOH (0.19 mL, 3.23 mmol) and the mixture was stirred at room temperature for 4 h. Thereafter, $\text{Na}(\text{OAc})_3\text{BH}$ (1.71 g, 8.08 mmol) was added to the reaction mixture and the mixture stirred overnight. On completion, the reaction mixture was basified by cold sat. NaHCO_3 (aq) solution and extracted with EtOAc (3×10 mL). The combined organic phase was washed with brine and then dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure to afford 6-chloro-2-((2,4-dimethoxybenzyl)amino)-N-methylnicotinamide as a brown solid which was used directly in the next step without further purification. To a stirred solution of 6-chloro-2-((2,4-dimethoxybenzyl)amino)-N-methylnicotinamide (500 mg, 1.49 mmol) in DMSO was added NaOMe (25% solution in MeOH, 3.21 mL, 14.8 mmol) and the mixture was heated at 130 °C for 3 h. On completion, the reaction mixture was cooled to room temperature, and water (25 mL) was added to the reaction and extracted with EtOAc (3×15 mL). The combined organic phase was washed with brine and then dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was then purified using column chromatography to afford compound **6ib** as a light yellow solid (114 mg, 23%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 9.10 (t, J = 5.2 Hz, 1H), 8.10 (br s, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.21 (d, J = 8.0 Hz, 1H), 6.53–6.45 (m, 2H), 5.90 (d, J = 8.0 Hz, 1H), 4.35 (d, J = 5.2 Hz, 2H), 3.78 (s, 3H), 3.74 (s, 6H), 2.70 (d, J = 6.0 Hz, 3H). LC–MS (m/z): 332.0 $[\text{M} + \text{H}]^+$.

2-Amino-6-methoxy-N-methylnicotinamide (6i). To a stirred solution of **6ib** (140 mg, 0.422 mmol) in DCM (8 mL) was added TFA (0.97 mL, 12.7 mmol) and the mixture was refluxed for 2 h. On completion, the reaction mixture was cooled to room temperature and concentrated under reduced pressure to remove volatiles. The residue was neutralized by slow addition of 20% aqueous NaOH at 0 °C and extracted with EtOAc (3×10 mL). The combined organic phase was washed with brine and then dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure to afford compound **6i** as an off-white solid (75 mg, 98%) which was used in the next step without further purification. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.08 (d, J = 4.0 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.33 (br s, 2H), 5.95 (d, J = 8.4 Hz, 1H), 3.78 (s, 3H), 2.70 (d, J = 4.0 Hz, 3H). LC–MS (m/z): 182.2 $[\text{M} + \text{H}]^+$.

2-Methoxy-6-methyl-5,9-dioxo-5,6,8,9-tetrahydropyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrimidine-6a(7H)-carboxylic Acid (7i). Compound **6i** (310 mg, 1.71 mmol) was reacted according to General procedure D and triturated with Et_2O and filtered off to afford compound **7i** as an off-white powder (280 mg, 56%). LC–MS (m/z): 292.3 $[\text{M} + \text{H}]^+$.

N-(3-Chlorophenyl)-2-methoxy-6-methyl-5,9-dioxo-5,6,8,9-tetrahydropyrido[3,2-*e*]pyrrolo[1,2-*a*]pyrimidine-6a(7H)-carboxamide (8i). To a stirred solution of compound **7i** (180 mg, 0.618 mmol) in DMF (4.0 mL) under N_2 was added 3-chloroaniline (131 μL , 1.24 mmol). The reaction mixture was cooled to 0 °C and DMTMM (342 mg, 1.24 mmol) was added portionwise. The mixture was allowed to warm slowly to room temperature and stirred for 12 h. On completion, the reaction mixture was diluted with EtOAc (15 mL). The organic layer was washed with cold water (3×15 mL) and brine, and then dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was purified using column chromatography followed by reverse phase preparative HPLC to afford compound **8i** as a white solid (23 mg, 9%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.29 (br s, 1H), 8.13 (d, J = 8.0 Hz, 1H), 7.65 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.31 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 8.0 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 3.92 (s, 3H), 3.16 (s, 3H), 2.95–2.82 (m, 2H), 2.80–2.59 (m, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.2, 168.5, 165.4, 162.1, 147.0, 139.6, 139.4, 132.9,

130.4, 124.0, 120.1, 119.0, 110.0, 108.8, 81.3, 54.3, 30.0, 29.8, 26.4. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{17}\text{ClN}_4\text{O}_4$, 401.1011; found, 401.1010.

Ethyl 3,5-Difluoroisonicotinate (5ka). To a solution of diisopropylamine (1.59 mL, 11.3 mmol) in THF (8 mL) was added *n*-BuLi (4.52 mL, 11.3 mmol), 2.5 M in hexane at –78 °C. The mixture was warmed to room temperature and stirred for 30 min. The mixture was again cooled to –78 °C, and a solution of 3,5-difluoropyridine (1.0 g, 8.69 mmol) in THF (1 mL) was added and stirred for 1.5 h. Thereafter, a solution of ethyl chloroformate (0.91 mL, 9.56 mmol) in THF (1.0 mL) was added dropwise at –78 °C and stirred for another 1 h at the same temperature. The reaction mixture was then quenched with aq sat. NaHCO_3 solution and extracted with EtOAc (3×20 mL). The organic layer was washed with brine and then dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was then purified using column chromatography to afford compound **5ka** as an off-white gum (570 mg, 35%). ^1H NMR (400 MHz, CDCl_3): δ 8.43 (s, 2H), 4.45 (q, J = 7.2 Hz, 2H), 1.40 (t, J = 7.2 Hz, 3H). GC–MS, (m/z): 187.0 $[\text{M}]^+$.

Ethyl 3-Azido-5-fluoroisonicotinate (5kb). To a stirred solution of ethyl 3,5-difluoroisonicotinate **5ka** (1.4 g, 7.48 mmol) in DMSO (10 mL) was added NaN_3 (4380 mg, 6.73 mmol) and the mixture was heated at 70 °C for 1.5 h. On completion, the reaction mixture was cooled to room temperature and water (25 mL) was added, then extracted with EtOAc (3×15 mL). The combined organic phase was washed with brine (15 mL) and then dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified using column chromatography to afford compound **5kb** as a light yellow-white solid (882 mg, 56%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.40 (s, 1H), 8.32 (s, 1H), 4.43 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H). LC–MS (m/z): 211.2 $[\text{M} + \text{H}]^+$.

3-Azido-5-fluoroisonicotinic Acid (5kc). To a stirred solution of ethyl 3-azido-5-fluoroisonicotinate **5kb** (2.2 g, 10.5 mmol) in 5:1 MeOH/ H_2O (24.0 mL) was added NaOH (1.68 g, 41.9 mmol) and the mixture was stirred at room temperature for 16 h. On completion, the reaction mixture was concentrated and diluted with water (5 mL), then acidified with 1 N HCl. The mixture was then concentrated under vacuo, and the resultant residue was suspended in EtOH (10 mL) and filtered through Celite. The filtrate was then concentrated in vacuo to afford compound **5kc** (1.61 g, 84%) which was used without further purification. ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.58 (s, 1H), 8.50 (s, 1H). LC–MS (m/z): 183.5 $[\text{M} + \text{H}]^+$.

3-Amino-5-fluoroisonicotinic Acid (5k). To a stirred solution of 3-azido-5-fluoroisonicotinic acid **5kc** (1.8 g, 9.89 mmol) in MeOH (15 mL) was added Pd/C (10% on carbon, 2.10 g, 1.98 mmol, 20 mol %). The mixture was then stirred under H_2 for 3 h. Upon completion, the reaction mixture was filtered through Celite and the filtrate was concentrated in vacuo to afford compound **5k** (1.54 g, quant.), which was used without further purification. LC–MS (m/z): 155.0 $[\text{M} + \text{H}]^+$.

3-Amino-5-fluoro-N-methylisonicotinamide (6k). Compound **5k** (620 mg, 3.97 mmol) was reacted according to General procedure A with HATU (3.02 g, 7.94 mmol) to afford compound **6k** as a brown gum (503 mg, 75%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.36 (br s, 1H), 7.93 (s, 1H), 7.70 (s, 1H), 6.04 (br s, 2H), 2.71 (s, 3H). LC–MS (m/z): 170.1 $[\text{M} + \text{H}]^+$.

4-Fluoro-6-methyl-5,9-dioxo-5,6,8,9-tetrahydropyrido[4,3-*e*]pyrrolo[1,2-*a*]pyrimidine-6a(7H)-carboxylic Acid (7k). Compound **6k** (600 mg, 3.55 mmol) was reacted according to General procedure D, to afford compound **7k** (305 mg, 65%). LC–MS (m/z): 280.1 $[\text{M} + \text{H}]^+$.

N-(3-Chlorophenyl)-4-fluoro-6-methyl-5,9-dioxo-5,6,8,9-tetrahydropyrido[4,3-*e*]pyrrolo[1,2-*a*]pyrimidine-6a(7H)-carboxamide (8k). To a stirred solution of **7k** (100 mg, 0.343 mmol) in DCM (5 mL) was added DIPEA (312 μL , 1.79 mmol). The reaction mixture was then cooled to 0 °C and POCl_3 (357 mg, 0.687 mmol) was added dropwise. The mixture was allowed to warm to room temperature over 30 min and stirred for an additional 2 h. On completion, the mixture was diluted with water (10 mL) and extracted with 5% MeOH in DCM (3×10 mL). The combined

organic phase was dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure. The residue was purified by reverse phase preparative HPLC to afford compound **8k** as a white solid (43 mg). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.15 (br s, 1H), 9.03 (s, 1H), 8.56 (d, J = 2.4 Hz, 1H), 7.62 (s, 1H), 7.44 (d, J = 8.0 Hz, 1H), 7.30 (t, J = 8.0 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 3.19 (s, 3H), 2.91–2.87 (m, 2H), 2.79–2.71 (m, 2H). LC–MS (m/z): 389.2 $[\text{M} + \text{H}]^+$. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{14}\text{ClFN}_4\text{O}_3$, 389.0811; found, 389.0811.

2-Amino-4-chloro-*N*-methyl-benzamide (6l). 2-Amino-4-chlorobenzoic acid (3.44 g, 20.0 mmol) was reacted according to General procedure A with CDI, to afford compound **6l** as an off-white powder (3.38 g, 91%). LC–MS (m/z): 185.0 $[\text{M} + \text{H}]^+$.

8-Chloro-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxylic Acid (7l). Compound **6l** (2.09, 11.3 mmol) was reacted according to General procedure D, to afford compound **7l** as a white solid (2.98 g, 90%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.28 (d, J = 2.0 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.37 (dd, J = 8.4, 2.1 Hz, 1H), 3.07 (s, 3H), 2.86–2.56 (m, 4H). LC–MS (m/z): 293.2 $[\text{M} - \text{H}]^-$.

8-Chloro-*N*-(3-fluorophenyl)-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (8l). Compound **7l** (881 mg, 2.99 mmol) was reacted with 3-fluoroaniline according to General procedure D to afford compound **8l** as an off-white powder (1.16 g, quant.). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.04 (s, 1H), 7.95–7.86 (m, 2H), 7.47–7.37 (m, 2H), 7.37–7.26 (m, 2H), 6.98–6.86 (m, 1H), 3.21 (s, 3H), 2.97–2.61 (m, 4H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.5, 168.6, 161.8 (d, J = 240.2), 161.1, 139.4 (d, J = 10.8), 137.2, 135.9, 130.2 (d, J = 9.3), 129.9, 125.8, 120.5, 119.5, 116.9 (d, J = 2.8), 111.1, (d, J = 20.9), 108.0 (d, J = 25.9), 80.7, 30.4, 29.5, 27.2. LC–MS (m/z): 386.2 $[\text{M} - \text{H}]^-$. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{15}\text{ClFN}_3\text{O}_3$, 388.0859; found, 388.0857.

***N*-(3-Fluorophenyl)-8-methoxy-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (8m).** Compound **7f** (74 mg, 0.255 mmol) was reacted with 3-fluoroaniline according to General procedure B, to afford compound **8m** as a white solid (17 mg, 17%). ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ 9.99 (s, 1H), 7.81 (d, J = 8.6 Hz, 1H), 7.44–7.41 (m, 2H), 7.31–7.29 (m, 2H), 6.90–6.89 (m, 2H), 3.82 (s, 3H), 3.19 (s, 3H), 2.91–2.79 (m, 2H), 2.77–2.65 (m, 1H), 2.63–2.54 (m, 1H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.4, 169.1, 162.5, 161.9, 161.8 (d, J = 241.7 Hz), 139.5 (d, J = 10.9 Hz), 136.4, 130.1 (d, J = 9.3 Hz), 129.7, 116.7 (d, J = 2.8 Hz), 113.7, 111.6, 110.9 (d, J = 20.9 Hz), 107.8 (d, J = 26.1 Hz), 106.0, 80.9, 55.6, 30.2, 29.6, 27.0. LC–MS (m/z): 382.2 $[\text{M} - \text{H}]^-$. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{20}\text{H}_{18}\text{FN}_3\text{O}_4$, 384.1354; found, 384.1352.

2-Amino-4-bromo-*N*-methyl-benzamide (6b). 2-Amino-4-bromobenzoic acid (2.16 g, 9.98 mmol) was reacted according to General procedure A with CDI to afford compound **6b** as a tan solid (1.94 g, 85%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 8.23 (d, J = 4.8 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 6.90 (d, J = 2.0 Hz, 1H), 6.79–6.18 (m, 3H), 2.71 (d, J = 4.5 Hz, 3H). LC–MS (m/z): 230.2 $[\text{M} + \text{H}]^+$.

8-Bromo-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxylic Acid (7b). Compound **6b** (1.87 g, 8.16 mmol) was reacted according to General procedure D to afford compound **7b** as an off-white powder (2.51 g, 91%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.43 (d, J = 1.7 Hz, 1H), 7.84 (d, J = 8.4 Hz, 1H), 7.50 (dd, J = 8.4, 1.8 Hz, 1H), 3.06 (s, 3H), 2.84–2.54 (m, 4H). ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$): δ 172.91, 170.90, 161.11, 136.64, 130.14, 127.85, 126.72, 120.31, 117.86, 79.39, 39.52, 30.05, 29.14, 28.03. LC–MS (m/z): 340.0 $[\text{M} + \text{H}]^+$.

8-Bromo-*N*-(3-fluorophenyl)-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (9a). Compound **7b** (679 mg, 2.00 mmol) was reacted according to General procedure B to afford compound **9a** as a gray powder (857 mg, 99%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.03 (s, 1H), 8.07 (d, J = 1.9 Hz, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.53 (dd, J = 8.4, 1.9 Hz, 1H), 7.42 (ddd, J = 11.9, 2.9, 1.5 Hz, 1H), 7.37–7.21 (m, 2H), 7.13–6.80 (m, 1H), 3.20 (s, 3H), 3.05–2.55 (m, 3H).

***N*-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-phenyl-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (10a).** Compound **9a** (48 mg, 0.111 mmol) was reacted with phenylboronic acid (23 mg, 0.189 mmol) according to General procedure F, to afford compound **10a** as a white solid (9 mg, 19%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.1 (s, 1H), 8.2 (d, J = 1.7 Hz, 1H), 8.0 (d, J = 8.2 Hz, 1H), 7.7–7.6 (m, 3H), 7.5 (t, J = 7.3 Hz, 2H), 7.5–7.4 (m, 2H), 7.3 (q, J = 7.3 Hz, 2H), 6.9 (t, J = 8.0 Hz, 1H), 3.2 (s, 3H), 3.0–2.5 (m, 3H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.7, 169.0, 162.0, 161.9 (d, J = 241.8 Hz), 144.6, 139.5 (d, J = 9.8 Hz), 138.8, 135.5, 130.2 (d, J = 9.2 Hz), 129.3, 128.7, 127.0, 124.1, 119.8, 116.8 (d, J = 2.2 Hz), 111.0 (d, J = 20.8 Hz), 107.8 (d, J = 25.9 Hz), 80.9, 66.5, 30.5, 29.6, 27.3. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{25}\text{H}_{20}\text{FN}_3\text{O}_3$, 430.1563; found, 430.1563.

***N*-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (9b).** A solution of compound **9a** (434 mg, 1.00 mmol), bis(pinacolato)diboron, (260 mg, 1.02 mmol), KOAc (292 mg, 2.98 mmol) and Pd(dppf) $\text{Cl}_2 \cdot \text{CH}_2\text{Cl}_2$ (47 mg, 0.0576 mmol, 5 mol %) in 1,4-dioxane (4 mL) was degassed for 30 min. The reaction was then heated to 100 °C for 6 h. The mixture was then cooled to rt and suspended in EtOAc, then washed with water. The organic phase was then washed with brine and then dried over anhyd. MgSO_4 , filtered and concentrated in vacuo. The crude product was then purified using column chromatography to afford compound **9b** as a white solid (119 mg, 25%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 9.99 (s, 1H), 8.22 (s, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.58 (dd, J = 7.7, 1.1 Hz, 1H), 7.50–7.35 (m, 1H), 7.35–7.25 (m, 2H), 7.02–6.74 (m, 1H), 3.21 (s, 3H), 3.05–2.57 (m, 4H), 1.30 (s, 12H). LC–MS (m/z): 480.0 $[\text{M} + \text{H}]^+$.

***N*-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(2-pyridyl)-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (10b).** Compound **9b** (40 mg, 0.0835 mmol) was reacted with 2-bromopyridine (17 mg, 0.105 mmol) according to General procedure F to afford compound **10b** as a white powder (119 mg, 25%). ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.07 (s, 1H), 8.78–8.69 (m, 1H), 8.65 (dd, J = 1.5, 0.8 Hz, 1H), 8.11–7.87 (m, 4H), 7.56–7.37 (m, 2H), 7.33–7.19 (m, 2H), 6.99–6.81 (m, 1H), 3.24 (s, 3H), 3.09–2.57 (m, 4H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.4, 168.9, 161.8, 161.7 (d, J = 240.1 Hz), 158.1, 154.3, 149.8, 142.7, 139.5 (d, J = 12.1 Hz), 137.6, 135.3, 130.1 (d, J = 9.0 Hz), 128.4, 123.6, 123.5, 121.0, 120.9, 119.2, 116.7 (d, J = 2.3 Hz), 110.9 (d, J = 21.6 Hz), 107.8 (d, J = 26.4 Hz), 80.8, 30.5, 29.5, 27.1. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{19}\text{FN}_4\text{O}_3$, 431.1514; found, 431.1513.

***N*-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(3-pyridyl)-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (10c).** Compound **9a** (83 mg, 0.192 mmol) was reacted with 3-pyridylboronic acid (39 mg, 0.317 mmol) according to General procedure F to afford compound **10c** as an off-white solid (57 mg, 82%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.07 (s, 1H), 8.89 (dd, J = 2.5, 0.9 Hz, 1H), 8.65 (dd, J = 4.8, 1.6 Hz, 1H), 8.19 (s, 1H), 8.13–8.04 (m, 1H), 8.00 (d, J = 8.1 Hz, 1H), 7.70 (dd, J = 8.1, 1.8 Hz, 1H), 7.55 (ddd, J = 8.0, 4.8, 0.9 Hz, 1H), 7.49–7.39 (m, 1H), 7.37–7.22 (m, 2H), 6.96–6.82 (m, 1H), 3.24 (s, 3H), 2.99–2.62 (m, 4H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.6, 168.8, 161.8 (d, J = 241.8 Hz), 161.6, 149.5, 147.7, 141.4, 139.5 (d, J = 11.0 Hz), 135.5, 134.4, 134.3, 130.1 (d, J = 9.3 Hz), 128.8, 124.2, 124.1, 120.2, 119.2, 116.8 (d, J = 2.8 Hz), 111.0 (d, J = 20.8 Hz), 107.9 (d, J = 26.2 Hz), 80.8, 30.5, 29.6, 27.2. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{19}\text{FN}_4\text{O}_3$, 431.1514; found, 431.1513.

***N*-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(4-pyridyl)-2,3-dihydropyrrolo[1,2-*a*]-quinazoline-3a-carboxamide (10d).** Compound **9a** (81 mg, 0.187 mmol) was reacted with 4-pyridylboronic acid (43 mg, 0.350 mmol) according to General procedure F to afford compound **10d** as a white powder (43 mg, 87%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.07 (s, 1H), 8.75–8.66 (m, 2H), 8.25 (d, J = 1.7 Hz, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.75 (dd, J = 8.2, 1.8 Hz, 1H), 7.71–7.63 (m, 2H), 7.49–7.38 (m, 1H), 7.37–7.21 (m, 2H), 6.90 (dt, J = 8.5, 5.1 Hz, 1H), 4.09 (q, J = 5.2 Hz, 1H), 3.24 (s, 3H), 3.17 (d, J = 5.2 Hz, 3H), 2.98–2.61 (m, 4H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.6, 161.8 (d, J = 241.9 Hz), 161.5, 150.5, 145.6, 141.5, 139.4 (d, J = 10.9 Hz), 135.5, 130.1 (d, J = 9.3 Hz), 128.8, 124.2, 121.4,

121.0, 119.2, 116.8 (d, $J = 2.8$ Hz), 111.0 (d, $J = 21.0$ Hz), 107.9 (d, $J = 26.1$ Hz), 80.7, 30.5, 29.6, 27.2. HRMS m/z : $[M + H]^+$ calcd for $C_{24}H_{19}FN_4O_3$, 431.1514; found, 431.1511.

N-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-pyrimidin-5-yl-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (10e). Compound **9a** (81 mg, 0.187 mmol) was reacted with pyrimidin-5-ylboronic acid (35 mg, 0.283 mmol) according to General procedure F to afford compound **10e** as a white solid (11 mg, 14%). 1H NMR (400 MHz, DMSO- d_6): δ 10.08 (s, 1H), 9.26 (s, 1H), 9.12 (s, 2H), 8.21 (s, 1H), 8.04 (d, $J = 8.1$ Hz, 1H), 7.78 (dd, $J = 8.1, 1.8$ Hz, 1H), 7.44 (dd, $J = 11.8, 2.5$ Hz, 1H), 7.31 (q, $J = 7.3$ Hz, 2H), 6.98–6.83 (m, 1H), 3.24 (s, 3H), 3.00–2.61 (m, 4H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.6, 168.7, 161.8 (d, $J = 241.7$ Hz), 161.5, 158.1, 155.0, 139.4 (d, $J = 10.9$ Hz), 138.1, 135.6, 130.1 (d, $J = 9.3$ Hz), 128.9, 124.3, 120.8, 119.2, 116.9 (d, $J = 2.8$ Hz), 111.0 (d, $J = 20.8$ Hz), 107.9 (d, $J = 26.1$ Hz), 80.7, 30.5, 29.5, 27.3. HRMS m/z : $[M + H]^+$ calcd for $C_{23}H_{18}FN_5O_3$, 432.1467; found, 432.1465.

N-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(1H-pyrazol-4-yl)-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (10f). Compound **9a** (79 mg, 0.183 mmol) was reacted with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (53 mg, 0.273 mmol) according to General procedure F to afford compound **10f** as a white powder (9 mg, 12%). 1H NMR (300 MHz, DMSO- d_6): δ 3.14 (s, 1H), 10.06 (s, 1H), 8.10 (br s, 2H), 8.01 (s, 1H), 7.85 (d, $J = 8.2$ Hz, 1H), 7.57 (dd, $J = 8.2, 1.6$ Hz, 1H), 7.49–7.37 (m, 1H), 7.36–7.21 (m, 2H), 6.97–6.82 (m, 1H), 3.21 (s, 3H), 2.95–2.55 (m, 4H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.5, 169.0, 161.9, 161.8 (d, $J = 241.7$ Hz), 139.5 (d, $J = 11.0$ Hz), 137.5, 135.5, 130.1 (d, $J = 9.5$ Hz), 128.5, 122.4, 120.1, 118.3, 116.9, 116.7 (d, $J = 2.8$ Hz), 110.9 (d, $J = 20.8$ Hz), 107.7 (d, $J = 26.0$ Hz), 80.8, 30.3, 29.6, 27.1. HRMS m/z : $[M + H]^+$ calcd for $C_{22}H_{18}FN_5O_3$, 420.1467; found, 420.1465.

N-(3-Fluorophenyl)-4-methyl-8-(1-methylpyrazol-4-yl)-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (10g). Compound **9a** (82 mg, 0.190 mmol) was reacted with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazole (58 mg, 0.279 mmol) according to General procedure F to afford compound **10g** as a white solid (10 mg, 12%). 1H NMR (300 MHz, DMSO- d_6): δ 10.07 (s, 1H), 8.18 (s, 1H), 7.97 (d, $J = 1.7$ Hz, 1H), 7.89–7.79 (m, 2H), 7.52 (dd, $J = 8.1, 1.7$ Hz, 1H), 7.40 (d, $J = 11.5$ Hz, 1H), 7.33–7.20 (m, 2H), 6.93–6.83 (m, 1H), 3.87 (s, 3H), 3.20 (s, 3H), 3.04–2.56 (m, 4H). HRMS m/z : $[M + H]^+$ calcd for $C_{23}H_{20}FN_5O_3$, 434.1623; found, 434.1622.

N-(3-Fluorophenyl)-4-methyl-8-(1-methylpyrazol-3-yl)-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (10h). Compound **9a** (75 mg, 0.174 mmol) was reacted with 1-methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrazole (62 mg, 0.298 mmol) according to General procedure F to afford compound **10h** as a brown oil (7 mg, 9%). 1H NMR (300 MHz, $CDCl_3$): δ 8.41 (s, 1H), 8.10 (d, $J = 7.9$ Hz, 1H), 8.03 (s, 1H), 7.57 (s, 1H), 7.40 (d, $J = 10.5$ Hz, 1H), 7.31 (d, $J = 8.0$ Hz, 1H), 7.20 (app t, $J_{app} = 7.3$ Hz, 1H), 7.08 (d, $J = 8.1$ Hz, 1H), 6.85–6.79 (m, 1H), 6.42 (s, 1H), 3.99 (s, 3H), 3.41 (s, 3H), 3.17–3.07 (m, 1H), 2.87–2.60 (m, 3H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.6, 168.8, 161.8 (d, $J = 241.7$ Hz), 161.5, 141.3, 139.5 (d, $J = 10.9$ Hz), 138.3, 135.0, 134.1, 130.1 (d, $J = 9.3$ Hz), 128.5, 125.5, 120.5, 120.2, 116.7 (d, $J = 2.9$ Hz), 111.0 (d, $J = 21.0$ Hz), 107.8 (d, $J = 26.2$ Hz), 106.6, 80.7, 37.8, 30.5, 29.5, 27.1. HRMS m/z : $[M + H]^+$ calcd for $C_{23}H_{20}FN_5O_3$, 434.1623; found, 434.1622.

N-(3-Fluorophenyl)-4-methyl-8-(1-methylimidazol-4-yl)-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (10i). Compound **9b** (40 mg, 0.0835 mmol) was reacted with 4-bromo-1-methyl-imidazole (16 mg, 0.100 mmol) according to General procedure F to afford compound **10i** as a white solid (2 mg, 6%). 1H NMR (300 MHz, DMSO- d_6): δ 10.06 (s, 1H), 8.26 (d, $J = 1.6$ Hz, 1H), 7.83 (d, $J = 8.2$ Hz, 1H), 7.75–7.68 (m, 2H), 7.63 (dd, $J = 8.2, 1.6$ Hz, 1H), 7.41 (dt, $J = 12.0, 1.9$ Hz, 1H), 7.35–7.23 (m, 2H), 6.98–6.81 (m, 1H), 3.70 (s, 3H), 3.21 (s, 3H), 2.95–2.53 (m, 4H). HRMS m/z : $[M + H]^+$ calcd for $C_{23}H_{20}FN_5O_3$, 434.1623; found, 434.1622.

N-(3-Fluorophenyl)-8-(1H-imidazole-1-yl)-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide

(10j). Compound **9a** (200 mg, 0.462 mmol) was reacted with imidazole (63 mg, 0.926 mmol) according to General procedure E using $Me_4tButylXphos$ (4.4 mg, 2 mol %) to afford compound **10j** as a white solid (50 mg, 25%). 1H NMR (400 MHz, DMSO- d_6): δ 10.06 (s, 1H), 8.30 (s, 1H), 8.04 (d, $J = 2.0$ Hz, 1H), 8.01 (d, $J = 8.0$ Hz, 1H), 7.74 (s, 1H), 7.64 (dd, $J = 8.4$ Hz, 2.0 Hz, 1H), 7.45–7.41 (m, 1H), 7.35–7.26 (m, 2H), 7.18 (s, 1H), 6.93–6.88 (m, 1H), 3.23 (s, 3H), 2.95–2.86 (m, 2H), 2.81–2.67 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.6, 168.7, 161.8 (d, $J = 241.8$ Hz), 161.2, 139.9, 139.4 (d, $J = 11.0$ Hz), 136.2, 135.7, 130.5, 130.1 (d, $J = 9.4$ Hz), 129.9, 118.9, 117.9, 117.5, 117.0 (d, $J = 2.8$ Hz), 112.1, 111.1 (d, $J = 21.0$ Hz), 108.1 (d, $J = 26.0$ Hz), 80.8, 30.4, 29.6, 27.3. ^{19}F NMR (282 MHz, DMSO): δ –112.3. HRMS m/z : $[M + H]^+$ calcd for $C_{22}H_{18}FN_5O_3$, 420.1467; found, 420.1466.

N-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(1H-pyrazol-1-yl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10k). Compound **9a** (200 mg, 0.462 mmol) was reacted with pyrazole (63 mg, 0.926 mmol) according to General procedure E with $Me_4tButylXphos$ (4.4 mg, 2 mol %) to afford compound **10k** as a white solid (45 mg, 23%). 1H NMR (400 MHz, DMSO- d_6): δ 10.07 (s, 1H), 8.85 (d, $J = 2.0$ Hz, 1H), 8.42 (s, 1H), 7.97 (d, $J = 8.4$ Hz, 1H), 7.83 (s, 1H), 7.79 (d, $J = 8.4$ Hz, 1H), 7.44–7.41 (m, 1H), 7.32–7.26 (m, 2H), 6.94–6.85 (m, 1H), 6.61 (s, 1H), 3.23 (s, 1H), 2.96–2.85 (m, 2H), 2.84–2.55 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.4, 168.8, 161.8 (d, $J = 241.9$ Hz), 161.5, 142.5, 142.1, 139.4 (d, $J = 11.0$ Hz), 136.1, 130.1 (d, $J = 9.3$ Hz), 129.5, 128.3, 118.1, 116.8 (d, $J = 2.8$ Hz), 114.8, 111.0 (d, $J = 20.9$ Hz), 110.6, 108.8, 107.9 (d, $J = 26.0$ Hz), 80.8, 30.3, 29.6, 27.1. ^{19}F NMR (282 MHz, DMSO): δ –112.3. HRMS m/z : $[M + H]^+$ calcd for $C_{22}H_{18}FN_5O_3$, 420.1467; found, 420.1465.

8-Anilino-N-(3-fluorophenyl)-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (10l). Compound **9a** (41 mg, 0.0949 mmol) was reacted with aniline (30 μ L, 0.329 mmol) according to General procedure E to afford compound **10l** as a tan powder (22 mg, 52%). 1H NMR (300 MHz, DMSO- d_6): δ 9.99 (s, 1H), 8.86 (s, 1H), 7.68 (d, $J = 8.6$ Hz, 1H), 7.52 (d, $J = 2.2$ Hz, 1H), 7.45 (dt, $J = 12.5, 1.9$ Hz, 1H), 7.38–7.25 (m, 4H), 7.23–7.15 (m, 2H), 6.99 (t, $J = 7.3$ Hz, 1H), 6.90 (s, 1H), 6.85 (dd, $J = 8.7, 2.3$ Hz, 1H), 3.18 (s, 3H), 2.92–2.53 (m, 4H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.4, 169.3, 162.3, 161.8 (d, $J = 241.7$ Hz), 148.0, 141.2, 139.7 (d, $J = 10.9$ Hz), 136.4, 130.1 (d, $J = 9.3$ Hz), 129.4, 129.3, 121.9, 119.3, 116.5 (d, $J = 2.8$ Hz), 112.6, 111.3, 110.8 (d, $J = 20.9$ Hz), 107.5 (d, $J = 26.2$ Hz), 105.3, 80.9, 30.1, 29.6, 26.8. HRMS m/z : $[M + H]^+$ calcd for $C_{25}H_{21}FN_4O_3$, 445.1671; found, 445.1669.

N-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(pyridin-3-ylamino)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10m). Compound **9a** (400 mg, 0.925 mmol) was reacted with 3-aminopyridine (305 mg, 3.24 mmol) according to General procedure E to afford compound **10m** as a white solid (26 mg, 6%). 1H NMR (400 MHz, DMSO- d_6): δ 10.00 (s, 1H), 9.03 (s, 1H), 8.48 (s, 1H), 8.19 (d, $J = 4.0$ Hz, 1H), 7.72 (d, $J = 8.4$ Hz, 1H), 7.59 (d, $J = 8.0$ Hz, 1H), 7.55 (s, 1H), 7.45 (d, $J = 10.0$ Hz, 1H), 7.39–7.27 (m, 3H), 6.94–6.85 (m, 2H), 3.19 (s, 1H), 2.89–2.65 (m, 3H), 2.60–2.52 (m, 1H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.4, 169.3, 162.1, 161.8 (d, $J = 241.7$ Hz), 147.2, 142.7, 141.2, 139.6 (d, $J = 11.0$ Hz), 137.9, 136.4, 130.1 (d, $J = 9.3$ Hz), 129.6, 125.5, 116.6 (d, $J = 2.8$ Hz), 112.9, 112.1, 110.8 (d, $J = 21.2$ Hz), 107.6 (d, $J = 26.1$ Hz), 105.7, 80.8, 30.1, 29.6, 26.9. ^{19}F NMR (282 MHz, DMSO): δ –112.3. HRMS m/z : $[M + H]^+$ calcd for $C_{24}H_{20}FN_5O_3$, 446.1623; found, 446.1623.

N-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(pyridin-2-ylamino)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10n). Compound **9a** (400 mg, 0.925 mmol) was reacted with 2-aminopyridine (305 mg, 3.24 mmol) according to General procedure E to afford compound **(10n)** as an off-white solid (20 mg, 5%). 1H NMR (400 MHz, DMSO- d_6): δ 10.08 (br s, 1H), 9.58 (br s, 1H), 8.26 (d, $J = 1.6$ Hz, 1H), 8.22–8.18 (m, 1H), 7.74 (d, $J = 8.0$ Hz, 1H), 7.66–7.57 (m, 2H), 7.49–7.41 (m, 1H), 7.32–7.27 (m, 2H), 6.93 (d, $J = 8.4$ Hz, 1H), 6.91–6.83 (m, 2H), 3.18 (s, 3H), 2.91–2.79 (m, 2H), 2.75–2.65 (m, 1H), 2.62–2.55 (m, 1H). ^{13}C NMR (75

MHz, DMSO- d_6): δ 173.5, 169.2, 162.4, 155.0, 147.2, 145.8, 139.7 (d, J = 10.9 Hz), 137.6, 135.9, 130.2 (d, J = 9.3 Hz), 128.6, 116.5 (d, J = 2.8 Hz), 115.7, 114.6, 112.8, 111.9, 110.8 (d, J = 21.1 Hz), 108.6, 107.5 (d, J = 26.3 Hz), 81.2, 30.0, 29.7, 26.8. ^{19}F NMR (282 MHz, DMSO): δ -112.3. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{20}\text{FN}_5\text{O}_3$, 446.1623; found, 446.1623.

N-(3-Fluorophenyl)-4-methyl-8-morpholino-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10o). Compound **9a** (100 mg, 0.231 mmol) was reacted with morpholine (69 μL , 0.809 mmol) according to General procedure E to afford compound **10o** as a white solid (14 mg, 13%). ^1H NMR (400 MHz, DMSO- d_6): δ 9.93 (s, 1H), 7.69 (d, J = 8.4 Hz, 1H), 7.44 (d, J = 11.6 Hz, 1H), 7.66–7.27 (m, 3H), 6.95–6.87 (m, 2H), 3.73 (s, 4H), 3.23 (s, 4H), 3.17 (s, 3H), 2.87–2.75 (m, 2H), 2.74–2.63 (m, 1H), 2.61–2.50 (m, 1H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.4, 169.3, 162.2, 161.8 (d, J = 241.6 Hz), 153.7, 139.6 (d, J = 10.7 Hz), 136.3, 130.1 (d, J = 9.4 Hz), 129.0, 116.7 (d, J = 2.9 Hz), 111.3, 110.9, 110.8 (d, J = 21.1 Hz), 107.7 (d, J = 26.2 Hz), 104.8, 80.9, 65.8, 46.9, 30.0, 29.7, 27.0. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{23}\text{FN}_4\text{O}_4$, 439.1776; found, 439.1775.

N-(3-Fluorophenyl)-4-methyl-1,5-dioxo-8-(piperazin-1-yl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10p). Compound **9a** (300 mg, 0.694 mmol) was reacted with *tert*-butyl piperazine-1-carboxylate (453 mg, 2.43 mmol) according to General procedure E to afford 90 mg (24%) of *tert*-butyl 4-(3a-((3-fluorophenyl)carbamoyl)-4-methyl-1,5-dioxo-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinazolin-8-yl)piperazine-1-carboxylate as a brown solid. *tert*-Butyl 4-(3a-((3-fluorophenyl)carbamoyl)-4-methyl-1,5-dioxo-1,2,3,3a,4,5-hexahydropyrrolo[1,2-*a*]quinazolin-8-yl)piperazine-1-carboxylate (185 mg, 0.344 mmol) was then dissolved in DCM (4 mL), cooled to 0 °C and TFA (790 μL , 10.3 mmol) was added. The reaction was stirred at 0 °C for 2 h, then was concentrated in vacuo. The crude product was then purified via reverse phase preparative HPLC to afford compound **10p** as a white solid (22 mg, 15%). ^1H NMR (400 MHz, DMSO- d_6): δ 10.07 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H), 7.63–7.57 (m, 2H), 7.41 (d, J = 8.0 Hz, 1H), 7.37–7.25 (m, 2H), 7.11 (d, J = 8.0 Hz, 1H), 4.00–3.93 (m, 1H), 3.17–3.09 (m, 1H), 2.90–2.70 (m, 3H), 2.69–2.60 (m, 1H), 1.75–1.66 (m, 1H), 1.65–1.47 (m, 1H), 0.91 (t, J = 7.2 Hz, 3H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.9, 169.9, 162.8, 162.3 (d, J = 241.7 Hz), 154.5, 140.1 (d, J = 10.9 Hz), 136.8, 130.6 (d, J = 9.3 Hz), 129.5, 117.1 (d, J = 3.0 Hz), 111.8, 111.3 (d, J = 20.8 Hz), 110.7, 108.2 (d, J = 26.1 Hz), 105.3, 81.4, 48.4, 45.7, 30.5, 30.2, 27.5. ^{19}F NMR (282 MHz, DMSO- d_6): δ -112.4. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{24}\text{FN}_5\text{O}_3$, 438.1936; found, 438.1935.

N-(3-Fluorophenyl)-4-methyl-8-(4-methylpiperazin-1-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10q). Compound **9a** (400 mg, 0.925 mmol) was reacted with 1-methylpiperazine (325 mg, 3.24 mmol) according to General procedure E to afford compound **10q** as a white solid (26 mg, 6%). ^1H NMR (400 MHz, DMSO- d_6): δ 9.91 (s, 1H), 7.67 (d, J = 8.8 Hz, 1H), 7.44 (d, J = 9.6 Hz, 1H), 7.36–7.25 (m, 3H), 6.94–6.85 (m, 2H), 3.29 (m, 4H), 3.17 (s, 3H), 2.88–2.79 (m, 2H), 2.78–2.59 (m, 2H), 2.58–2.52 (m, 4H), 2.27 (s, 3H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.4, 169.3, 162.2, 161.8 (d, J = 241.6 Hz), 153.4, 139.6 (d, J = 11.0 Hz), 136.3, 130.1 (d, J = 9.4 Hz), 129.0, 116.7 (d, J = 2.9 Hz), 111.6, 110.8 (d, J = 21.0 Hz), 110.6, 107.7 (d, J = 26.1 Hz), 105.0, 80.9, 53.9, 46.4, 45.2, 30.0, 29.7, 27.0. ^{19}F NMR (282 MHz, DMSO- d_6): δ -112.4. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{26}\text{FN}_5\text{O}_3$, 452.2093; found, 452.2093.

8-Chloro-N-(5-chloro-3-pyridyl)-4-methyl-1,5-dioxo-2,3-dihydropyrrolo[1,2-*a*]quinazoline-3a-carboxamide (8o). Compound **7i** (77 mg, 0.261 mmol) was reacted with 5-chloropyridin-3-amine (49 mg, 0.381 mmol) according to General procedure B to afford compound **7l** as a tan powder (12 mg, 11%). ^1H NMR (300 MHz, DMSO- d_6): δ 10.24 (s, 1H), 8.64 (s, 1H), 8.36 (s, 1H), 8.08 (app t, J_{app} = 2.2 Hz, 1H), 7.95 (d, J = 2.0 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.41 (dd, J = 8.4, 2.1 Hz, 1H), 3.21 (s, 3H), 3.00–2.64 (m, 4H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.4, 169.2, 161.0, 143.6, 140.7, 137.3, 135.8, 135.2, 130.3, 129.8, 127.8, 125.7, 120.3, 119.2,

80.6, 30.3, 29.5, 27.3. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{18}\text{H}_{14}\text{Cl}_2\text{N}_4\text{O}_3$, 405.0517; found, 405.0518.

N-(5-Chloropyridin-3-yl)-8-methoxy-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (8p). Compound **7f** (250 mg, 0.862 mmol) was reacted with 5-chloropyridin-3-amine (200 mg, 1.55 mmol) according to General procedure B and the crude product was purified via reverse phase preparative HPLC to afford compound **8p** as a white solid (49 mg, 14%). ^1H NMR (400 MHz, DMSO- d_6): δ 10.20 (br s, 1H), 8.65 (d, J = 1.6 Hz, 1H), 8.34 (d, J = 2.0 Hz, 1H), 8.08 (t, J = 2.0 Hz, 1H), 7.82 (d, J = 8.8 Hz, 1H), 7.44 (d, J = 2.4 Hz, 1H), 6.90 (dd, J = 8.8 Hz, 2.4 Hz, 1H), 3.82 (s, 3H), 3.19 (s, 3H), 2.92–2.82 (m, 2H), 2.78–2.60 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.3, 169.7, 162.5, 161.8, 143.4, 140.6, 136.4, 135.5, 130.3, 129.8, 127.5, 113.4, 111.7, 105.8, 80.8, 55.6, 30.1, 29.6, 27.1. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{19}\text{H}_{17}\text{ClN}_4\text{O}_4$, 401.1011; found, 401.1010.

2-Nitro-4-(trifluoromethoxy)benzonitrile (6qa). To a stirred solution of 1-bromo-2-nitro-4-(trifluoromethoxy)benzene (2.0 g, 6.99 mmol) in DMF (3.5 mL) was added CuCN (626 mg, 6.99 mmol) and the mixture was stirred at 150 °C for 1 h. The reaction mixture was then cooled, toluene (19 mL) was added and then the resulting mixture was refluxed for an additional 1 h. On completion of the reaction, the mixture was diluted with EtOAc (40 mL) and washed with cold water (2 $\times$ 40 mL). The organic layer was dried over anhyd. Na_2SO_4 , filtered and concentrated under reduced pressure. The crude product was purified using column chromatography to afford compound **6qa** as a light yellow solid (1.20 g, 74%). ^1H NMR (400 MHz, DMSO- d_6): δ 8.39–8.34 (m, 2H), 8.04 (d, J = 8.0 Hz, 1H). GC–MS (m/z): 232.0 $[\text{M}]^+$.

2-Nitro-4-(trifluoromethoxy)benzoic Acid (6qb). 55% H_2SO_4 (20.0 mL) was added to **6qa** (1.5 g, 6.46 mmol) and the resulting suspension was refluxed for 16 h. Upon completion, the reaction mixture was poured on to ice–water (30 mL) and extracted with EtOAc (3 $\times$ 25 mL). The organic layers were combined and extracted with 1 M NaOH (3 $\times$ 15 mL). The aqueous layers were combined, acidified with 10% HCl solution and again extracted with EtOAc (3 $\times$ 25 mL). The organic layers were then combined, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford compound **6qb** as an off-white solid (798 mg, 49%). ^1H NMR (400 MHz, DMSO- d_6): δ 14.00 (br s, 1H), 8.12 (s, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.81 (d, J = 8.0 Hz, 1H). LC–MS (m/z): 250.14 $[\text{M} - \text{H}]^+$.

N-Methyl-2-nitro-4-(trifluoromethoxy)benzamide (6qc). Compound **6qb** (600 mg, 2.39 mmol) was reacted according to General procedure A to afford 348 mg (55%) of compound **6qc** as a light-yellow solid. ^1H NMR (400 MHz, DMSO- d_6): δ 8.68 (br s, 1H), 8.11 (s, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 8.0 Hz, 1H), 2.76 (d, J = 4.4 Hz, 3H). LC–MS (m/z): 265.2 $[\text{M} + \text{H}]^+$.

2-Amino-N-methyl-4-(trifluoromethoxy)benzamide (6q). To a stirred solution of **6qc** (350 mg, 1.33 mmol) in mixed solvent $[\text{MeOH}/\text{H}_2\text{O}, (4:1)]$ (8.0 mL) was added Fe powder (370 mg, 6.63 mmol) followed by NH_4Cl (709 mg, 13.3 mmol) and the resulting mixture was refluxed for 2 h. On completion, the reaction mixture was cooled to rt, filtered through Celite and the filtrate was concentrated. The resultant residue was diluted with water and extracted with DCM (3 $\times$ 15 mL). The organic layers were combined, washed with brine and then dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified using column chromatography to afford compound **6q** as an off-white solid (265 mg, 85%). ^1H NMR (400 MHz, DMSO- d_6): δ 8.25 (br s, 1H), 7.54 (d, J = 8.0 Hz, 1H), 6.75 (br s, 2H), 6.63 (s, 1H), 6.41 (d, J = 8.0 Hz, 1H), 2.72 (d, J = 4.4 Hz, 3H). LC–MS (m/z): 235.0 $[\text{M} + \text{H}]^+$.

4-Methyl-1,5-dioxo-8-(trifluoromethoxy)-1H,2H,3H,3aH,4H,5H-pyrrolo[1,2-*a*]quinazoline-3a-carboxylic Acid (7q). Compound **6q** (305 mg, 1.30 mmol) was reacted according to General procedure D to afford compound **7q** as an off-white solid (296 mg, 66%). ^1H NMR (400 MHz, DMSO- d_6): δ 14.23 (br s, 1H), 8.22 (s, 1H), 8.04 (d, J = 8.4 Hz, 1H), 7.28 (d, J = 8.4 Hz, 1H), 3.08 (s, 3H), 2.91–2.58 (m, 4H). LC–MS (m/z): 345.29 $[\text{M} + \text{H}]^+$.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(trifluoromethoxy)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**8q**). To a stirred solution of compound **7q** (250 mg, 0.726 mmol) in pyridine (4.0 mL) under N₂ was added T₃P (2.31 mL, 3.63 mmol, 50% solution in EtOAc). The mixture was stirred for 10 min and thereafter 5-chloropyridin-3-amine (187 mg, 1.45 mmol) was added, and the mixture was heated at 50 °C for 16 h. On completion, the reaction mixture was cooled to room temperature, and concentrated in vacuo. Water was added to the residue and the aqueous layer was extracted by 10% MeOH in DCM. The combined organic layers were washed with water (2 × 20 mL) and brine and then dried over anhyd. Na₂SO₄, filtered and concentrated in vacuo. The residue was purified using column chromatography, followed by reverse phase preparative HPLC to afford compound **8q** as an off-white solid (46 mg, 14%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.27 (s, 1H), 8.63 (s, 1H), 8.36 (s, 1H), 8.07 (s, 1H), 8.02 (d, *J* = 8.8 Hz, 1H), 7.91 (s, 1H), 7.31 (d, *J* = 8.8 Hz, 1H), 3.23 (s, 1H), 2.92–2.87 (m, 2H), 2.78–2.67 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.4, 169.0, 160.9, 150.9, 143.5, 140.9, 136.5, 135.4, 130.4, 130.2, 127.8, 119.9 (d, *J* = 258.2 Hz), 119.2, 117.4, 112.3, 80.8, 30.3, 29.5, 27.3. ¹⁹F NMR (282 MHz, DMSO-*d*₆): δ –56.7. HRMS *m/z*: [M + H]⁺ calcd for C₁₉H₁₄ClF₃N₄O₄, 455.0729; found, 455.0728.

8-Bromo-*N*-(5-chloropyridin-3-yl)-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**9r**). Compound **7b** (800 mg, 2.36 mmol) was reacted with 5-chloropyridin-3-amine (364 mg, 2.81 mmol) according to General procedure B to afford compound **9r** as an off-white solid (797 mg, 75%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.20 (s, 1H), 8.64 (d, *J* = 2.0 Hz, 1H), 8.35 (d, *J* = 2.0 Hz, 1H), 8.11–8.06 (m, 2H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.54 (dd, *J* = 8.4 Hz, 2.0 Hz, 1H), 3.21 (s, 3H), 2.93–2.82 (m, 2H), 2.79–2.67 (m, 2H). LC–MS (*m/z*): 447.0 [M – H][–].

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(pyridin-2-yl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10r**). To a solution of compound **9r** (190 mg, 0.423 mmol) in DMF (5 mL) was added 2-(tributylstannyl)pyridine (778 mg, 2.11 mmol) and the mixture was degassed with N₂ for 10 min before the addition of Pd₂(dba)₃ (38 mg, 0.042 mmol, 10 mol %) followed by P(*o*-tol)₃ (257 mg, 0.085 mmol, 20 mol %). The mixture was then heated at 80 °C for 8 h. Upon completion, the reaction mixture was diluted with EtOAc (25 mL), filtered through Celite and the filtrate was washed with water (2 × 15 mL), and then dried over anhyd. Na₂SO₄, concentrated under reduced pressure and purified using column chromatography to afford compound **10r** as a white solid (70 mg, 37%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.25 (s, 1H), 8.72 (d, *J* = 4.8 Hz, 1H), 8.69 (s, 1H), 8.63 (d, *J* = 2.0 Hz, 1H), 8.32 (d, *J* = 2.0 Hz, 1H), 8.07 (s, 1H), 8.03–7.93 (m, 4H), 7.45–7.42 (m, 1H), 3.25 (s, 3H), 2.96–2.85 (m, 2H), 2.80–2.67 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.4, 169.6, 161.6, 154.3, 149.8, 143.5, 142.8, 140.5, 137.5, 135.4, 135.3, 130.3, 128.4, 127.5, 123.6, 123.5, 120.9, 120.8, 119.1, 80.7, 30.4, 29.5, 27.2. HRMS *m/z*: [M + H]⁺ calcd for C₂₃H₁₈ClN₅O₃, 448.1171; found, 448.1169.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(pyridin-3-yl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10s**). Compound **9r** (100 mg, 0.222 mmol) was reacted with pyridin-3-ylboronic acid (49 mg, 0.40 mmol) according to General procedure F to afford compound **10s** as a white solid (40 mg, 40%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.29 (s, 1H), 8.90 (d, *J* = 2.0 Hz, 1H), 8.66–8.63 (m, 2H), 8.32 (d, *J* = 2.0 Hz, 1H), 8.21 (d, *J* = 2.0 Hz, 1H), 8.11–8.07 (m, 2H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.71 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 7.57–7.53 (m, 1H), 3.25 (s, 3H), 2.98–2.86 (m, 2H), 2.82–2.69 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.5, 169.5, 161.5, 149.5, 147.7, 143.5, 141.4, 140.7, 135.4, 134.4, 134.3, 130.2, 128.8, 127.6, 124.2, 124.1, 120.0, 119.1, 30.4, 29.5, 27.3. HRMS *m/z*: [M + H]⁺ calcd for C₂₃H₁₈ClN₅O₃, 448.1171; found, 448.1171.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(pyridin-4-yl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10t**). Compound **9r** (100 mg, 0.222 mmol) was reacted with pyridin-4-ylboronic acid (49 mg, 0.40 mmol) according to General procedure F to afford compound **10t** as white solid (19 mg, 19%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.29 (s, 1H), 8.71 (d, *J* = 5.6 Hz, 2H),

8.62 (s, 1H), 8.35–8.27 (m, 2H), 8.09 (s, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.69 (d, *J* = 5.6 Hz, 2H), 3.24 (s, 3H), 3.00–2.85 (m, 2H), 2.84–2.65 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.5, 169.5, 161.4, 150.5, 145.7, 143.2, 141.5, 140.8, 135.5, 130.2, 128.8, 127.6, 124.1, 121.4, 120.8, 119.0, 80.7, 30.4, 29.6, 27.4. HRMS *m/z*: [M + H]⁺ calcd for C₂₃H₁₈ClN₅O₃, 448.1171; found, 448.1170.

N-(5-Chloropyridin-3-yl)-8-(6-methoxypyridin-3-yl)-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10u**). Compound **9r** (160 mg, 0.356 mmol) was reacted with (6-methoxypyridin-3-yl)boronic acid (65 mg, 0.427 mmol) according to General procedure F to afford compound **10u** as white solid (34 mg, 20%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.25 (s, 1H), 8.66 (s, 1H), 8.51 (s, 1H), 8.33 (s, 1H), 8.14 (s, 1H), 8.10 (s, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.64 (d, *J* = 8.4 Hz, 1H), 6.97 (d, *J* = 8.4 Hz, 1H), 3.91 (s, 3H), 3.24 (s, 3H), 2.92–2.88 (m, 2H), 2.79–2.67 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.5, 169.5, 163.8, 161.6, 145.1, 143.5, 141.4, 140.6, 137.7, 135.4, 130.3, 128.7, 127.9, 127.6, 123.6, 119.4, 118.3, 111.0, 80.7, 53.5, 30.4, 29.5, 27.3. HRMS *m/z*: [M + H]⁺ calcd for C₂₄H₂₀ClN₅O₄, 478.1277; found, 478.1277.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(6-(trifluoromethyl)pyridin-3-yl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10v**). Compound **9r** (125 mg, 0.28 mmol) was reacted with (6-(trifluoromethyl)pyridin-3-yl)boronic acid (64 mg, 0.336 mmol) according to General procedure F to afford compound **10v** as an off-white solid (76 mg, 53%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.27 (br s, 1H), 9.09 (s, 1H), 8.65 (s, 1H), 8.39–8.32 (m, 2H), 8.28 (s, 1H), 8.10 (s, 1H), 8.09–8.04 (m, 2H), 7.79 (d, *J* = 7.6 Hz), 3.25 (s, 1H), 2.94–2.90 (m, 2H), 2.80–2.74 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.57, 169.46, 161.38, 148.37, 146.01 (d, *J* = 34.2 Hz), 143.35, 140.83, 139.87, 137.65, 136.67, 135.52, 130.26, 128.90, 127.69, 124.68, 123.50, 121.16 (d, *J* = 2.9 Hz), 120.74, 119.55, 80.72, 30.45, 29.59, 27.40. ¹⁹F NMR (282 MHz, DMSO-*d*₆): δ –66.3. HRMS *m/z*: [M + H]⁺ calcd for C₂₄H₁₇ClF₃N₅O₃, 516.1045; found, 516.1044.

N-(5-Chloropyridin-3-yl)-8-(6-cyanopyridin-3-yl)-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10w**). Compound **9r** (120 mg, 0.257 mmol) was reacted with (6-(trifluoromethyl)pyridin-3-yl)boronic acid (64 mg, 0.336 mmol) according to General procedure F to afford compound **10w** as a white solid (60 mg, 49%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 9.08 (s, 1H), 8.65 (s, 1H), 8.35 (s, 1H), 8.33 (s, 1H), 8.26 (s, 1H), 8.19 (d, *J* = 8.1 Hz, 1H), 8.09 (s, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.79 (d, *J* = 8.3 Hz, 1H), 3.25 (s, 3H), 2.95–2.88 (m, 2H), 2.80–2.73 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.5, 169.4, 161.3, 149.4, 143.6, 140.7, 139.6, 137.7, 136.0, 135.5, 135.3, 132.1, 130.3, 129.3, 128.9, 127.7, 124.8, 120.9, 119.6, 117.4, 80.6, 30.5, 29.5, 27.4. HRMS *m/z*: [M + H]⁺ calcd for C₂₄H₁₇ClN₆O₃, 473.1124; found, 473.1122.

N-(5-Chloropyridin-3-yl)-4-methyl-8-(1-methyl-1H-pyrazol-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10x**). Compound **9r** (100 mg, 0.222 mmol) was reacted with (1-methyl-1H-pyrazol-4-yl)boronic acid (50 mg, 0.40 mmol) according to General procedure F to afford compound **10x** as a white solid (37 mg, 37%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.25 (br s, 1H), 8.63 (s, 1H), 8.32 (s, 1H), 8.19 (s, 1H), 7.95–8.71 (m, 1H), 8.02 (s, 1H), 7.87–7.84 (m, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 3.88 (s, 3H), 3.21 (s, 3H), 2.95–2.82 (m, 2H), 2.79–2.62 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.4, 169.7, 161.7, 137.2, 136.4, 130.3, 128.8, 128.6, 127.5, 122.1, 120.7, 118.1, 116.5, 80.7, 38.8, 30.2, 29.6, 27.2. HRMS *m/z*: [M + H]⁺ calcd for C₂₂H₁₉ClN₆O₃, 451.1280; found, 451.1281.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(1H-pyrazol-1-yl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (**10y**). Compound **9r** (300 mg, 0.667 mmol) was reacted with pyrazole (113 mg, 1.67 mmol) according to General procedure E using Me₄tButylXphos (6.4 mg, 2 mol %) to afford compound **10y** as a white solid (35 mg, 12%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.24 (br s, 1H), 8.63 (s, 1H), 8.55 (s, 1H), 8.47 (s, 1H), 8.32 (s, 1H), 8.08 (s, 1H), 7.98 (d, *J* = 8.4 Hz, 1H), 7.83 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 6.61 (s, 1H), 3.29 (s, 3H), 2.97–2.90 (m, 2H), 2.79–2.67 (m,

2H). HRMS m/z : $[M + H]^+$ calcd for $C_{21}H_{17}ClN_6O_3$, 437.1125; found, 437.1127.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(piperidin-1-yl)-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (10z). Compound **9r** (300 mg, 0.667 mmol) was reacted with piperidine (114 mg, 1.33 mmol) according to General procedure E using SPhos (1.4 mg, 5 mol %) and $Pd_2(dba)_3$ (3.1 g, 5 mol %) to afford compound **10z** as a white solid (25 mg, 8%). 1H NMR (400 MHz, DMSO- d_6): δ 10.11 (br s, 1H), 8.64 (s, 1H), 8.32 (s, 1H), 8.10 (t, J = 2.0 Hz, 1H), 7.64 (d, J = 884 Hz, 1H), 7.35 (s, 1H), 6.82 (dd, J = 8.8 Hz, 2.0 Hz, 1H), 3.17 (s, 3H), 2.87–2.78 (m, 2H), 2.74–2.54 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.3, 170.3, 162.2, 153.6, 142.9, 140.8, 136.4, 130.2, 129.1, 127.4, 111.3, 109.5, 104.6, 80.9, 48.0, 29.9, 29.8, 27.2, 24.8, 23.8. HRMS m/z : $[M + H]^+$ calcd for $C_{23}H_{24}ClN_5O_3$, 454.1642; found, 454.1645.

N-(5-Chloropyridin-3-yl)-8-(3,6-dihydro-2H-pyran-4-yl)-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (9aa). Compound **9r** (250 mg, 0.556 mmol) was reacted with 2-(3,6-dihydro-2H-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (140 mg, 0.667 mmol) according to General procedure F to afford compound **9r** as a white solid (177 mg, 71%). 1H NMR (400 MHz, DMSO- d_6): δ 10.22 (s, 1H), 8.63 (s, 1H), 8.33 (s, 1H), 8.08 (s, 1H), 7.96 (s, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 Hz, 1H), 6.40 (s, 1H), 4.29–4.25 (t, 1H), 3.83 (t, J = 5.2 Hz, 1H), 3.22 (s, 3H), 2.95–2.82 (m, 2H), 2.78–2.64 (m, 2H), 2.50–2.41 (m, 2H). LC–MS (m/z): 453.2 $[M + H]^+$.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(tetrahydro-2H-pyran-4-yl)-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (10aa). A solution of **9aa** (200 mg, 0.442 mmol) in MeOH/EtOH (10 mL, 1:1) was degassed with N_2 for 10 min and then PtO_2 (1.0 mg, 0.044 mmol, 10 mol %) was added and the mixture was stirred under H_2 for 4h. Upon completion, the reaction mixture was filtered through Celite, and then the filtrate was concentrated in vacuo and the residue was purified by reverse phase preparative HPLC to afford compound **9aa** as a white solid (42.0 mg, 21%). 1H NMR (400 MHz, DMSO- d_6): δ 10.18 (br s, 1H), 8.60 (s, 1H), 8.33 (s, 1H), 8.07 (s, 1H), 7.83–7.81 (m, 2H), 7.23 (d, J = 8.0 Hz, 1H), 3.94 (dd, J = 7.2 Hz, 3.6 Hz, 2H), 3.44 (t, J = 7.2 Hz, 2H), 3.21 (s, 3H), 2.93–2.79 (m, 3H), 2.75–2.63 (m, 2H), 1.77–1.56 (m, 4H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.3, 169.7, 161.8, 151.2, 143.4, 140.6, 135.5, 135.1, 130.3, 128.0, 127.5, 124.2, 118.8, 118.7, 80.7, 67.1, 40.6, 33.1, 30.2, 29.6, 27.3. HRMS m/z : $[M + NH_4]^+$ calcd for $C_{23}H_{23}ClN_4O_4$, 472.1746; found, 472.1743.

tert-Butyl 4-(3a-((5-chloropyridin-3-yl)carbamoyl)-4-methyl-1,5-dioxo-1,2,3,4a,4,5-hexahydropyrrolo[1,2-a]quinazolin-8-yl)-3,6-dihydropyridine-1(2H)-carboxylate (9ra). Compound **9r** (200 mg, 0.445 mmol) was reacted with *tert*-butyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,6-dihydropyridine-1(2H)-carboxylate (165 mg, 0.534 mmol) according to General procedure F to afford compound **9ra** as a brown solid (170 mg, 69%). 1H NMR (400 MHz, DMSO- d_6): δ 10.23 (s, 1H), 8.65–8.63 (m, 1H), 8.33 (d, J = 2.0 Hz, 1H), 8.09–8.07 (m, 1H), 7.94 (s, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.42 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.29 (s, 1H), 4.04 (s, 2H), 3.54 (s, 2H), 3.21 (s, 3H), 2.95–2.83 (m, 2H), 2.77–2.62 (m, 2H), 1.43 (s, 9H). LC–MS (m/z): 552.4 $[M + H]^+$.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(1,2,3,6-tetrahydropyridin-4-yl)-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (9ab). To a stirred suspension of *tert*-butyl 4-(3a-((5-chloropyridin-3-yl)carbamoyl)-4-methyl-1,5-dioxo-1,2,3,4a,4,5-hexahydropyrrolo[1,2-a]quinazolin-8-yl)-3,6-dihydropyridine-1(2H)-carboxylate **9ra** (250 mg, 0.453 mmol) in EtOH (5 mL) was added a solution of 4 N HCl in dioxane (2.84 mL, 11.3 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. On completion, the mixture was concentrated under reduced pressure and the residue was triturated with diethyl ether to afford compound **9ab** as a HCl salt, which was used for the next step without further purification. 1H NMR (400 MHz, DMSO- d_6): δ 10.35 (br s, 1H), 9.28 (br s, 2H), 8.69 (s, 1H), 8.36 (s, 1H), 8.12 (s, 1H), 7.99 (s, 1H), 7.88 (d, J = 8.0 Hz, 1H), 7.45 (d, J = 8.0 Hz, 1H), 6.30 (s, 1H), 3.83–3.69 (m, 2H), 3.51–3.23 (m, 2H), 3.22

(s, 3H), 2.97–2.81 (m, 2H), 2.80–2.61 (m, 3H), 2.48–2.50 (m, 1H). LC–MS (m/z): 452.3 $[M + H]^+$.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(piperidin-4-yl)-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (10ab). A solution of **9ab** (190 mg, 0.382 mmol) as a HCl salt in MeOH/EtOH (10 mL, 1:1) was neutralized by slow addition of Et_3N . The mixture was then degassed with N_2 for 10 min before the addition of PtO_2 (1.9 mg, 0.0764 mmol, 20 mol %) and the mixture was then stirred under an atmosphere of H_2 for 4 h. On completion, the reaction mixture was filtered through Celite and then the filtrate was concentrated under reduced pressure and the resultant residue was purified by reverse phase preparative HPLC to afford compound **9ab** as a white solid (17 mg, 10%). 1H NMR (400 MHz, DMSO- d_6): δ 8.43 (s, 1H), 8.21 (s, 1H), 8.00 (s, 1H), 7.82 (s, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.08 (d, J = 8.0 Hz, 1H), 3.16 (s, 3H), 3.12–3.03 (m, 2H), 2.90–2.71 (m, 2H), 2.70–2.60 (m, 5H), 1.79–1.72 (m, 2H), 1.60–1.48 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.3, 170.0, 162.0, 157.6, 145.9, 142.1, 141.4, 139.6, 135.4, 130.2, 127.9, 127.6, 123.9, 118.6, 118.3, 81.0, 45.6, 41.8, 32.7, 30.1, 29.9, 27.7. HRMS m/z : $[M + H]^+$ calcd for $C_{23}H_{24}ClN_5O_3$, 454.1641; found, 454.1639.

N-(5-Chloropyridin-3-yl)-4-methyl-8-(1-methylpiperidin-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (10ac). Compound **9r** (250 mg, 0.556 mmol) was reacted with 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,6-tetrahydropyridine (149 mg, 0.667 mmol) according to General procedure F. The resultant product was then dissolved in MeOH/EtOH (10 mL, 1:1) and was then degassed with N_2 for 10 min before the addition of PtO_2 (7.8 mg, 0.0343 mmol, 10 mol %). The mixture was then stirred under H_2 for 4 h. Upon completion, the reaction mixture was then filtered through Celite and the filtrate was concentrated in vacuo. The crude product was then purified via reverse phase preparative HPLC to afford compound **10ac** as an off-white solid (16 mg, 6%). 1H NMR (400 MHz, DMSO- d_6): δ 10.19 (s, 1H), 8.61 (s, 1H), 8.34 (s, 1H), 8.07 (s, 1H), 7.81–7.79 (m, 2H), 7.21 (d, J = 8.0 Hz, 1H), 3.20 (s, 3H), 2.93–2.79 (m, 4H), 2.76–2.60 (m, 2H), 2.58–2.53 (m, 1H), 2.19 (s, 3H), 1.99–1.93 (m, 2H), 1.77–1.73 (m, 2H), 1.64–1.60 (m, 2H). ^{13}C NMR (75 MHz, DMSO- d_6): δ 173.3, 169.7, 161.8, 151.7, 143.4, 140.6, 135.0, 130.3, 127.9, 127.6, 124.3, 118.8, 118.6, 80.7, 55.5, 46.1, 41.3, 32.6, 32.6, 30.2, 29.6, 27.2. HRMS m/z : $[M + H]^+$ calcd for $C_{24}H_{26}ClN_5O_3$, 468.1798; found, 468.1802.

2-Amino-6-fluoro-4-methoxybenzonitrile (5ra). Dry ammonia gas was purged for 5 min through DMSO (10 mL) in a sealed tube. 2,6-Difluoro-4-methoxybenzonitrile (1.0 g, 5.91 mmol) was added then added to the tube and the mixture was heated at 90 °C for 20 h. On completion, the mixture was cooled to rt and excess NH_3 was removed by purging the mixture with N_2 for 10 min. The mixture was then diluted with EtOAc (25 mL) and the organic layer was washed with cold water (25 mL) and brine and then dried over anhyd. Na_2SO_4 , filtered and concentrated in vacuo to afford compound **5ra** as an off-white solid (882 mg, 90%), which was used in the next step without further purification. 1H NMR (400 MHz, DMSO): δ 6.37 (br s, 2H), 6.17–6.12 (m, 2H), 3.71 (s, 3H). LC–MS (m/z): 167.1 $[M + H]^+$.

2-Amino-6-fluoro-4-methoxybenzoic Acid (5r). A suspension of compound **5ra** (1.1 g, 6.62 mmol) in 10% aqueous KOH solution (50 mL) was refluxed for 6 h. On completion, the reaction mixture was cooled to 0 °C, acidified by portionwise addition of solid citric acid. The mixture was stirred at 0 °C for another 30 min, and the resultant precipitate was isolated via vacuum filtration, washed with cold water and dried in vacuo to afford compound **5r** (788 mg, 64%), which was used without further purification. LC–MS (m/z): 186.1 $[M + H]^+$.

2-Amino-6-fluoro-4-methoxy-N-methylbenzamide (6r). Compound **5r** (790 mg, 4.27 mmol) was reacted according to General procedure A to afford compound **6r** as an off-white solid (518 mg, 61%). 1H NMR (400 MHz, DMSO- d_6): δ 7.80 (br s, 1H), 6.30 (br s, 2H), 6.07 (d, J = 2.0 Hz, 1H), 5.97 (dd, J = 13.6 Hz, 2.0 Hz, 1H), 6.69 (s, 3H), 2.72 (d, J = 4.4 Hz, 3H). LC–MS (m/z): 199.0 $[M + H]^+$.

6-Fluoro-8-methoxy-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxylic Acid (7r). Compound **6r** (520 mg, 2.62 mmol) was reacted according to General procedure D to afford compound **7r** (710 mg, 88%), which was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆): δ 14.06 (br s, 1H), 7.69 (s, 1H), 6.75 (d, *J* = 12.0 Hz, 1H), 3.88 (s, 3H), 3.02 (s, 1H), 2.79–2.51 (m, 4H). LC–MS (*m/z*): 305.2 [M + H]⁺.

N-(5-Chloropyridin-3-yl)-6-fluoro-8-methoxy-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (8r). Compound **6r** (100 mg, 0.324 mmol) was reacted with 5-chloropyridin-3-amine (83 mg, 0.649 mmol) according to General procedure B to afford compound **8r** as a white solid (30 mg, 22%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.21 (s, 1H), 8.65 (d, *J* = 2.0 Hz, 1H), 8.35 (d, *J* = 2.0 Hz, 1H), 8.09 (d, *J* = 2.0 Hz, 1H), 7.31 (s, 1H), 6.79 (dd, *J* = 12.8 Hz, 2.0 Hz, 1H), 3.83 (s, 3H), 3.17 (s, 3H), 2.88–2.78 (m, 2H), 2.77–2.55 (m, 2H), 2.91–2.78 (m, 2H), 2.75–2.61 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.3, 169.5, 163.2, 163.1, 162.2 (d, *J* = 259.3 Hz), 158.8, 158.7, 143.5, 140.6, 137.3, 137.2, 135.3, 130.3, 127.6, 102.7 (d, *J* = 3.3 Hz), 102.5 (d, *J* = 8.9 Hz), 100.1 (d, *J* = 25.3 Hz), 80.4, 56.1, 29.9, 29.7, 27.1. ¹⁹F NMR (282 MHz, DMSO-*d*₆): δ –108.4. HRMS *m/z*: [M + H]⁺ calcd for C₁₉H₁₆ClFN₄O₄, 419.0918; found, 419.0918.

Methyl 4-Chloro-2,6-difluorobenzoate (5sa). To a suspension solution of 4-chloro-2,6-difluorobenzoic acid (1.0 g, 5.91 mmol) in DMF (10 mL) was added Cs₂CO₃ (2.54 g, 7.79 mmol) at 0 °C. To this mixture MeI (650 μL, 10.4 mmol) was added dropwise and then reaction mixture was allowed to warm slowly to room temperature and stirred for 16 h. On completion, the mixture was filtered, and the filtrate was diluted with EtOAc (25 mL), washed with cold water (3 × 25 mL) and brine and then dried over anhyd. Na₂SO₄, filtered and concentrated in vacuo to afford compound **5sa** as a white solid (1.0 g, 93%), which was used for the next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.57–7.55 (m, 2H), 3.89 (s, 3H); LC–MS (*m/z*): 207.09 [M + H]⁺.

Methyl 2-Amino-4-chloro-6-fluorobenzoate (5s). Dry ammonia gas was purged for 5 min through DMSO (15 mL) in a sealed tube, followed by the addition of compound **5sa** (1.0 g, 4.84 mmol). The mixture was then heated at 90 °C for 20 h. On completion, the mixture was cooled to rt, excess NH₃ was removed by purging N₂ through the mixture for 10 min. The mixture was then diluted with EtOAc (25 mL) and the organic layer was washed with cold water (25 mL) and brine, and then dried over anhyd. Na₂SO₄, filtered and concentrated in vacuo to afford compound **5s** as an off-white solid (970 mg, 98%), which was used for the next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆): δ 6.87 (br s, 2H), 6.67 (s, 1H), 6.46 (dd, *J* = 11.2 Hz, 2.0 Hz, 1H), 3.78 (s, 3H); LC–MS (*m/z*): 204.13 [M + H]⁺.

2-Amino-4-chloro-6-fluoro-N-methylbenzamide (6s). To a solution of compound **5s** (970 mg, 4.76 mmol) in THF (5.0 mL) was added 40% aqueous methylamine solution (10 mL) slowly and stirred at rt for 16 h. On completion, the mixture was diluted with EtOAc (50 mL), washed with water (3 × 30 mL) and brine and then dried over anhyd. Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified by trituration with diethyl ether and *n*-pentane (4:1) to afford compound **6s** as a brown solid (959 mg, 99%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.15 (br s, 1H), 7.72 (br s, 2H), 6.57 (s, 1H), 6.47 (d, *J* = 10.4 Hz, 1H), 2.72 (d, *J* = 4.4 Hz, 3H). LC–MS (*m/z*): 203.18 [M + H]⁺.

8-Chloro-6-fluoro-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxylic Acid (7s). Compound **6s** (1.2 g, 5.92 mmol) was reacted according to General procedure D to afford compound **7s** (1.2 g, 65%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 14.17 (br s, 1H), 8.16 (s, 1H), 7.38 (d, *J* = 10.8 Hz, 1H), 3.04 (s, 3H), 2.81–2.55 (m, 4H). LC–MS (*m/z*): 313.07 [M + H]⁺.

8-Chloro-N-(5-chloropyridin-3-yl)-6-fluoro-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (8s). Compound **7s** (200 mg, 0.64 mmol) was reacted with 5-chloropyridin-3-amine (165 mg, 1.28 mmol) according to General procedure B to afford compound **8s** as a gray solid (41 mg, 15%). ¹H

NMR (400 MHz, DMSO-*d*₆): δ 10.23 (s, 1H), 8.65 (s, 1H), 8.37 (s, 1H), 8.09 (s, 1H), 7.84 (s, 1H), 7.41 (d, *J* = 11.2 Hz, 1H), 3.18 (s, 3H), 2.93–2.81 (m, 2H), 2.80–2.62 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.4, 169.0, 160.9 (d, *J* = 264.1 Hz), 157.9 (d, *J* = 3.8 Hz), 143.7, 140.9, 137.8 (d, *J* = 12.9 Hz), 137.1 (d, *J* = 4.0 Hz), 135.2, 130.3, 127.9, 116.7, 116.7, 114.5 (d, *J* = 25.5 Hz), 108.3 (d, *J* = 8.3 Hz), 80.3, 30.1, 29.6, 27.4. ¹⁹F NMR (282 MHz, DMSO-*d*₆): δ –108.3. HRMS *m/z*: [M + H]⁺ calcd for C₁₈H₁₃Cl₂FN₄O₃, 423.0422; found, 423.0420.

2-Amino-4-chloro-5-fluoro-N-methylbenzamide (6t). 2-Amino-4-chloro-5-fluorobenzoic acid (500 mg, 2.65 mmol) was reacted according to General procedure A to afford compound **6t** as a white solid (425 mg, 79%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.29 (br s, 1H), 7.49 (d, *J* = 10.8 Hz, 1H), 6.85 (d, *J* = 6.8 Hz, 1H), 6.50 (br s, 2H), 2.71 (d, *J* = 4.0 Hz, 1H). LC–MS (*m/z*): 203.0 [M + H]⁺.

8-Chloro-7-fluoro-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxylic Acid (7t). Compound **6t** (350 mg, 1.73 mmol) was reacted according to General procedure D to afford compound **7t** (410 mg, 76%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 17.24 (br s, 1H), 8.39 (d, *J* = 6.4 Hz, 1H), 7.82 (d, *J* = 8.8 Hz, 3H), 3.07 (s, 3H), 2.89–2.57 (m, 4H). LC–MS (*m/z*): 313.0 [M + H]⁺.

8-Chloro-N-(5-chloropyridin-3-yl)-7-fluoro-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (8t). To a stirred solution of compound **7t** (200 mg, 0.64 mmol) in pyridine (4 mL) was added T₃P (357 mg, 0.687 mmol) at rt. After 10 min, 5-chloropyridin-3-amine (165 mg, 1.28 mmol) was added and the mixture was heated at 50 °C overnight. On completion, the mixture was cooled to room temperature, evaporated under reduced pressure to remove excess pyridine. Water (15 mL) was added to the residue and extracted with 10% MeOH in DCM (3 × 15 mL). The combined organic layers were dried over anhyd. Na₂SO₄, filtered and concentrated in vacuo. The residue was purified using column chromatography to afford compound **8t** as a white solid (54 mg, 20%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.24 (s, 1H), 8.63 (d, *J* = 2.0 Hz, 1H), 8.36 (d, *J* = 2.0 Hz, 1H), 8.08–8.05 (m, 2H), 7.82 (d, *J* = 8.8 Hz, 1H), 3.22 (s, 3H), 2.91–2.85 (m, 2H), 2.78–2.71 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.4, 169.0, 160.1 (d, *J* = 2.0 Hz), 154.6 (d, *J* = 246.6 Hz), 143.6, 140.8, 135.2, 131.6 (d, *J* = 3.0 Hz), 130.2, 127.9, 124.3 (d, *J* = 19.2 Hz), 122.9, 121.2 (d, *J* = 6.7 Hz), 115.5 (d, *J* = 23.7 Hz), 80.6, 30.5, 29.3, 27.3. ¹⁹F NMR (282 MHz, DMSO-*d*₆): δ –117.9. HRMS *m/z*: [M + H]⁺ calcd for C₁₈H₁₃Cl₂FN₄O₃, 423.0423; found, 423.0426.

(E)-N-(2-Fluoro-3-methoxyphenyl)-2-(hydroxyimino)acetamide (5ua). To a stirred solution of sodium sulfate (6.44 g, 45.3 mmol, 8 equiv) in water (10 mL) was added a solution of chloralhydrate (1.03 g, 6.24 mmol) in water (10 mL). 2-Fluoro-3-methoxyaniline (2.0 g, 10.4 mmol) was added followed by conc. HCl (3 mL). A solution of hydroxylamine (50% in water, 2.36 mL, 17.0 mmol) was then added and the reaction mixture was refluxed for 4 h. The mixture was cooled to room temperature and then extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over anhyd. Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified using column chromatography to afford compound **5ua** (250 mg, 21%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.29 (s, 1H), 9.78 (s, 1H), 7.73 (s, 1H), 7.40 (t, *J* = 7.2 Hz, 1H), 7.12–7.08 (m, 1H), 7.01–6.97 (m, 1H), 3.83 (s, 3H). LC–MS (*m/z*): 210.6 [M – H]⁺.

7-Fluoro-6-methoxyindoline-2,3-dione (5ub). Compound **5ua** (250 mg, 1.19 mmol) was added to BF₃·Et₂O (98%, 4.52 mL, 35.7 mmol) in portion at 40 °C. The mixture was heated at 90 °C for 3 h. The reaction mixture was cooled down to room temperature, poured onto ice and extracted with EtOAc (3 × 15 mL). The combined organic layers were dried over anhyd. Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure and purified using column chromatography to afford compound **5ub** (186 mg, 80%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 11.56 (s, 1H), 7.41 (d, *J* = 8.0 Hz, 1H), 6.84–6.81 (m, 1H), 3.95 (s, 3H). LC–MS (*m/z*): 196.0 [M + H]⁺.

2-Amino-3-fluoro-4-methoxybenzoic Acid (5u). A suspension of compound **5ub** (220 mg, 1.13 mmol), NaOH (45 mg, 1.13 mmol) and NaCl (132 mg, 2.26 mmol) in water (10 mL) was stirred at rt for

20 min and was then cooled to 0 °C. H₂O₂ (30% in water, 0.384 mL, 3.38 mmol, 3 equiv) was added dropwise and the mixture was stirred at 0 °C for 20 min and then at rt for 3 h. The reaction mixture was quenched with glacial AcOH, filtered then washed with water. The resultant precipitate was then dried to afford compound **5u** (146 mg, 71%) which was used for the next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆): δ 12.50 (br s, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 6.41 (t, *J* = 8.4 Hz, 1H), 3.84 (s, 3H). LC–MS (*m/z*): 184.0 [*M* – H]⁺.

2-Amino-3-fluoro-4-methoxy-*N*-methylbenzamide (6u). Compound **5u** (140 mg, 0.756 mmol) was reacted according to General procedure A to afford compound **6u** as a brown solid (90 mg, 60%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.16 (s, 1H), 7.34–7.31 (m, 1H), 6.38–6.35 (m, 3H), 3.82 (s, 3H), 2.71 (d, *J* = 4.4 Hz, 3H). LC–MS (*m/z*): 199.2 [*M* + H]⁺.

9-Fluoro-8-methoxy-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxylic Acid (7u). Compound **6u** (90 mg, 0.454 mmol) was reacted according to General procedure D to afford compound **7u** (95 mg, 68%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.95 (br s, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.22–7.17 (m, 1H), 3.92 (s, 3H), 3.06 (s, 3H), 2.83–2.71 (m, 2H), 2.70–2.58 (m, 2H). LC–MS (*m/z*): 309.0 [*M* + H]⁺.

***N*-(5-Chloropyridin-3-yl)-9-fluoro-8-methoxy-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (8u).** To a stirred solution of **7u** (70 mg, 0.227 mmol) in pyridine (3 mL) under N₂ was added T₃P (1.45 mL, 2.27 mmol, 50% solution in EtOAc). The mixture was stirred at rt for 10 min and thereafter 5-chloropyridin-3-amine (58 mg, 0.454 mmol) was added. Then the mixture was heated at 50 °C for 16 h. On completion, the reaction mixture was cooled to rt, and volatiles were removed under reduced pressure. Water was added to the resultant residue and the aqueous layer was extracted by 10% MeOH in DCM. The combined organic layers were dried over anhyd. Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure. The residue was purified using column chromatography to afford compound **8u** as a white solid (16 mg, 17%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.63 (s, 1H), 8.59 (s, 1H), 8.34 (s, 1H), 8.07 (s, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.21 (t, *J* = 8.4 Hz, 1H), 3.90 (s, 3H), 3.16 (s, 3H), 3.01–2.94 (m, 2H), 2.85–2.68 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 174.0, 168.7, 168.2 (d, *J* = 268.6 Hz), 162.0 (d, *J* = 2.5 Hz), 151.2 (d, *J* = 8.5 Hz), 145.5, 143.3, 142.1, 139.5, 135.7, 130.5, 124.0 (d, *J* = 3.9 Hz), 116.0, 111.8, 81.5, 56.4, 30.4, 28.5, 26.7. ¹⁹F NMR (282 MHz, DMSO-*d*₆): δ –141.1. HRMS *m/z*: [*M* + H]⁺ calcd for C₁₉H₁₆ClFN₄O₄, 419.0917; found, 419.0915.

2-Amino-*N*-cyclopropyl-4-methoxybenzamide (6v). 2-Amino-4-methoxybenzoic acid (1.0 g, 5.99 mmol) was reacted with cyclopropanamine (833 μL, 12.0 mmol) according to General procedure A to afford compound **6v** as an off-white solid (810 mg, 66%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.96 (s, 1H), 7.38 (d, *J* = 8.8 Hz, 1H), 6.60 (br s, 2H), 6.19 (d, *J* = 1.6 Hz, 1H), 6.05 (d, *J* = 6.8 Hz, 1H), 3.68 (s, 3H), 2.80–2.72 (m, 1H), 0.64–0.51 (m, 4H). LC–MS (*m/z*): 207.0 [*M* + H]⁺.

4-Cyclopropyl-8-methoxy-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxylic Acid (7v). Compound **6v** (410 mg, 1.99 mmol) was reacted according to General procedure D to afford compound **7v** (565 mg, 90%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.89 (br s, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.74 (d, *J* = 2.4 Hz, 1H), 6.84 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 4.08 (m, 1H), 3.81 (s, 3H), 2.97–2.88 (m, 1H), 2.79–2.62 (m, 3H), 0.96–0.78 (m, 4H). LC–MS (*m/z*): 207.0 [*M* + H]⁺.

***N*-(5-Chloropyridin-3-yl)-4-cyclopropyl-8-methoxy-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (8v).** Compound **7v** (250 mg, 0.79 mmol) was reacted with 5-chloropyridin-3-amine (203 mg, 1.58 mmol) according to General procedure B to afford compound **8v** as a white solid (101 mg, 30%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.30 (s, 1H), 8.59 (d, *J* = 2.0 Hz, 1H), 8.32 (d, *J* = 2.0 Hz, 1H), 8.04 (s, 1H), 7.79 (d, *J* = 8.8 Hz, 1H), 7.28 (d, *J* = 2.0 Hz, 1H), 6.88 (dd, *J* = 8.8 Hz, 2.0 Hz, 1H), 3.81 (s, 3H), 3.31–3.28 (m, 1H), 2.84–2.77 (m, 2H), 2.68–2.53 (m, 2H), 0.96–0.83 (m, 4H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.3, 170.5,

163.1, 162.5, 143.3, 140.1, 136.3, 135.7, 130.4, 129.9, 127.0, 114.9, 111.9, 106.2, 82.3, 55.6, 29.8, 25.9, 25.7, 8.8, 7.5. HRMS *m/z*: [*M* + H]⁺ calcd for C₂₁H₁₉ClN₄O₄, 427.1168; found, 427.1166.

2-Amino-4-chloro-*N*-cyclopropylbenzamide (6w). 2-Amino-4-chlorobenzoic acid (2.0 g, 11.7 mmol) was reacted with cyclopropanamine (1.33 g, 23.3 mmol) according to General procedure A to afford compound **6w** as an off-white solid (1.61 g, 65%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.23 (br s, 1H), 7.42 (d, *J* = 8.4 Hz, 1H), 6.73 (d, *J* = 1.6 Hz, 1H), 6.67 (br s, 2H), 6.23 (d, *J* = 2.4 Hz, 1H), 6.12 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 2.81–2.73 (m, 1H), 0.70–0.62 (m, 2H), 0.57–0.48 (m, 2H). LC–MS (*m/z*): 211.2 [*M* + H]⁺.

8-Chloro-4-cyclopropyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxylic Acid (7w). Compound **6w** was reacted according to General procedure D to afford compound **7w** (450 mg, 98%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 14.12 (br s, 1H), 8.20 (d, *J* = 1.6 Hz, 1H), 7.86 (d, *J* = 8.4 Hz, 1H), 7.35 (dd, *J* = 8.4 Hz, 1.6 Hz, 1H), 2.99–2.89 (m, 1H), 2.81–2.65 (m, 4H), 1.01–0.75 (m, 4H). LC–MS (*m/z*): 321.2 [*M* + H]⁺.

8-Chloro-*N*-(5-chloropyridin-3-yl)-4-cyclopropyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (8w). Compound **7w** (200 mg, 0.625 mmol) was reacted with 5-chloropyridin-3-amine (160 mg, 2.5 mmol) according to General procedure B to afford compound **8w** as an off-white solid (70 mg, 26%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.30 (s, 1H), 8.58 (s, 1H), 8.34 (s, 1H), 8.03 (s, 1H), 7.95 (s, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.32 (s, 1H), 3.32–3.27 (m, 1H), 2.95–2.80 (m, 2H), 2.78–2.60 (m, 2H), 1.05–0.80 (m, 4H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.3, 170.0, 162.2, 143.4, 140.3, 137.1, 135.6, 135.5, 130.3, 129.9, 127.2, 126.0, 120.9, 120.7, 82.0, 29.6, 26.0, 26.0, 8.7, 7.4. HRMS *m/z*: [*M* + H]⁺ calcd for C₂₀H₁₆Cl₂N₄O₃, 431.0672; found, 431.0671.

2-Amino-4-methoxy-*N*-(2,2,2-trifluoroethyl)benzamide (6x). 2-Amino-4-methoxybenzoic acid (500 mg, 2.99 mmol) was reacted with 2,2,2-trifluoroethan-1-amine hydrochloride (811 mg, 5.98 mmol) according to General procedure A to afford compound **6x** as an off-white solid (342 mg, 46%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.55 (t, *J* = 6.4 Hz, 1H), 7.51 (d, *J* = 8.8 Hz, 1H), 6.66 (br s, 2H), 6.23 (d, *J* = 2.4 Hz, 1H), 6.12 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 4.03–3.94 (m, 2H), 3.71 (s, 3H). LC–MS (*m/z*): 249.20 [*M* + H]⁺.

8-Methoxy-1,5-dioxo-4-(2,2,2-trifluoroethyl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxylic Acid (7x). Compound **6x** (340 mg, 1.37 mmol) was reacted according to General procedure D to afford compound **7x** (480 mg, 98%). LCMS (*m/z*): 359.22 [*M* + H]⁺.

***N*-(5-Chloropyridin-3-yl)-8-methoxy-1,5-dioxo-4-(2,2,2-trifluoroethyl)-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (8x).** Compound **7x** (325 mg, 0.907 mmol) was reacted with 5-chloropyridin-3-amine (175 mg, 1.36 mmol) according to General procedure B to afford compound **8x** as a white solid (54 mg, 13%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 10.53 (s, 1H), 8.60 (s, 1H), 8.34 (s, 1H), 8.04 (t, *J* = 2.0 Hz, 1H), 7.88 (d, *J* = 8.8 Hz, 1H), 7.32 (s, 1H), 6.95 (dd, *J* = 8.8 Hz, 2.0 Hz, 1H), 4.87–4.76 (m, 1H), 4.38–4.24 (m, 1H), 3.85 (s, 3H), 2.91–2.78 (m, 2H), 2.75–2.61 (m, 2H). ¹³C NMR (75 MHz, DMSO-*d*₆): δ 173.3, 169.5, 164.1, 163.4, 143.5, 139.9, 136.6, 135.5, 130.4, 130.4, 126.8, 124.5 (d, *J* = 279.8 Hz), 112.4, 112.3, 106.8, 82.5, 55.8, 44.4 (d, *J* = 33.7 Hz), 29.2, 27.1. ¹⁹F NMR (282 MHz, DMSO-*d*₆): δ –68.9. HRMS *m/z*: [*M* + H]⁺ calcd for C₂₀H₁₆ClF₃N₄O₄, 469.0885; found, 469.0884.

2-Amino-4-methoxy-*N*-(methyl-*d*₃)benzamide (6y). 2-Amino-4-methoxybenzoic acid (300 mg, 1.795 mmol) was reacted with thereafter methan-*d*₃-amine hydrochloride (266 mg, 3.77 mmol) according to General procedure A to afford compound **6y** as an off-white solid (202 mg, 63%). ¹H NMR (400 MHz, DMSO-*d*₆): δ 7.94 (s, 1H), 7.41 (d, *J* = 8.8 Hz, 1H), 6.60 (br s, 2H), 6.20 (d, *J* = 2.4 Hz, 1H), 6.08 (dd, *J* = 8.8 Hz, 2.4 Hz, 1H), 3.69 (s, 3H). LC–MS (*m/z*): 184.08 [*M* + H]⁺.

8-Methoxy-4-(methyl-*d*₃)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxylic Acid (7y). Compound **6y** (200 mg, 1.092 mmol) was reacted according to General procedure D to afford compound **7y** as an off-white solid (295 mg, 94%). ¹H NMR

(400 MHz, DMSO- d_6): δ 7.89–7.72 (m, 2H), 6.84 (d, J = 7.6 Hz, 1H), 3.81 (s, 3H), 2.81–2.55 (m, 4H). LC–MS (m/z): 294.19 [M + H]⁺.

N-(5-Chloropyridin-3-yl)-8-methoxy-4-(methyl- d_3)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (8y). Compound 7y (150 mg, 0.511 mmol) was reacted with 5-chloropyridin-3-amine (132 mg, 1.02 mmol) according to General procedure B to afford compound 8y as an off-white solid (85 mg, 42%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.11 (s, 1H), 8.65 (s, 1H), 8.34 (s, 1H), 8.08 (s, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.45 (s, 1H), 6.89 (d, J = 8.4 Hz, 1H), 3.83 (s, 3H), 2.91–2.58 (m, 4H). ¹³C NMR (75 MHz, DMSO- d_6): δ 173.3, 169.8, 162.6, 161.8, 143.4, 140.6, 136.4, 135.5, 130.3, 129.8, 127.5, 113.4, 111.7, 105.8, 80.7, 55.6, 29.6, 27.1. HRMS m/z : [M + H]⁺ calcd for C₁₉H₁₄D₃ClN₄O₄, 404.1200; found, 404.1197.

N-(5-Chloropyridin-3-yl)-4-methyl-1,5-dioxo-8-(pyrimidin-5-yl)oxy-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10ad). Compound 9r (160 mg, 0.355 mmol) was reacted with (1-methyl-1H-pyrazol-4-yl)boronic acid (67 mg, 0.533 mmol) according to General procedure F using Pd-PEPPSI-IPr (12 mg, 5 mol %) to afford compound 10ad as a white solid (27 mg, 17%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.26 (br s, 1H), 8.81 (s, 1H), 8.64 (s, 1H), 8.39 (s, 1H), 8.37 (s, 1H), 8.33 (s, 1H), 8.08 (s, 1H), 7.99 (s, 1H), 3.90 (s, 3H), 3.23 (s, 3H), 2.94–2.80 (m, 2H), 2.79–2.67 (m, 2H). ¹³C NMR (75 MHz, DMSO- d_6): δ 173.8, 169.4, 164.1, 150.4, 143.7, 143.7, 136.8, 134.5, 133.0, 131.6, 130.2, 129.4, 127.9, 125.2, 122.9, 117.4, 80.3, 38.8, 30.4, 29.7, 27.8. HRMS m/z : [M + H]⁺ calcd for C₂₁H₁₈ClN₇O₅, 452.1233; found, 452.1231.

2-Amino-4-bromo-N-(methyl- d_3) benzamide (6ae). 2-Amino-4-bromobenzoic acid (1.0 g, 4.63 mmol) was reacted with methan- d_3 -amine hydrochloride (392 mg, 5.56 mmol) according to General procedure A to afford compound 6ae as an off-white solid (550 mg, 53%). ¹H NMR (400 MHz, DMSO- d_6): δ 8.20 (s, 1H), 7.37 (d, J = 8.4 Hz, 1H), 6.90 (d, J = 1.2 Hz, 1H), 6.65–6.62 (m, 3H). LC–MS (m/z): 231.88 [M + H]⁺.

8-Bromo-4-(methyl- d_3)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxylic acid (7ae). Compound 6ae (550 mg, 2.37 mmol) was reacted according to General procedure D to afford compound 7ae as a gray solid (660 mg, 80%). ¹H NMR (400 MHz, DMSO- d_6): δ 14.14 (br s, 1H), 8.43 (s, 1H), 7.83 (d, J = 7.6 Hz, 1H), 7.50 (d, J = 7.6 Hz, 1H), 2.80–2.51 (m, 4H). LC–MS (m/z): 341.85 [M + H]⁺.

8-Bromo-N-(5-chloropyridin-3-yl)-4-(methyl- d_3)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (9ae). Compound 7ae (660 mg, 1.93 mmol) was reacted with 5-chloropyridin-3-amine (496 mg, 3.86 mmol) according to General procedure B to afford compound 9ae as an off-white solid (420 mg, 49%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.21 (s, 1H), 8.64 (s, 1H), 8.36 (s, 1H), 8.11–8.06 (m, 2H), 7.83 (d, J = 8.4 Hz, 1H), 7.56–7.52 (m, 1H), 2.93–2.82 (m, 2H), 2.79–2.67 (m, 2H). LC–MS (m/z): 452.2 [M + H]⁺.

N-(5-Chloropyridin-3-yl)-4-(methyl- d_3)-8-(1-methyl-1H-pyrazol-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10ae). Compound 9ae (120 mg, 0.265 mmol) was reacted with (1-methyl-1H-pyrazol-4-yl)boronic acid (40 mg, 0.318 mmol) according to General procedure F to afford compound 10ae as an off-white solid (30 mg, 25%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.24 (s, 1H), 8.64 (d, J = 1.4 Hz, 1H), 8.32 (d, J = 1.8 Hz, 1H), 8.19 (s, 1H), 8.08 (s, 1H), 8.01 (s, 1H), 7.88–7.83 (m, 2H), 7.52 (d, J = 8.0 Hz, 1H), 3.88 (s, 3H), 2.90–2.83 (m, 2H), 2.77–2.70 (m, 2H). ¹³C NMR (75 MHz, DMSO- d_6): δ 173.4, 169.7, 161.8, 143.5, 140.5, 137.2, 136.4, 135.5, 135.4, 130.3, 128.8, 128.6, 127.5, 122.2, 120.7, 118.1, 116.6, 80.7, 38.8, 29.6, 27.2. HRMS m/z : [M + H]⁺ calcd for C₂₂H₁₆D₃ClN₆O₅, 454.1468; found, 454.1467.

N-(5-Chloropyridin-3-yl)-8-(1-(difluoromethyl)-1H-pyrazol-4-yl)-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10af). Compound 9r (80 mg, 0.178 mmol) was reacted with 1-(difluoromethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (52 mg, 0.213 mmol) according to General procedure F to afford compound 10af as a white solid (25 mg, 29%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.26 (br s, 1H), 8.77

(s, 1H), 8.64 (s, 1H), 8.33 (s, 1H), 8.25 (s, 1H), 8.15–8.06 (m, 2H), 7.92 (d, J = 8.1 Hz, 1H), 7.86 (t, J = 59.0 Hz, 1H), 7.67 (d, J = 7.9 Hz, 1H), 3.22 (s, 3H), 2.95–2.87 (m, 2H), 2.80–2.70 (m, 2H). ¹³C NMR (75 MHz, DMSO- d_6): δ 173.5, 169.5, 161.6, 143.5, 140.7, 140.2, 135.5, 135.4, 130.3, 128.7, 127.6, 126.8, 123.0 (d, J = 4.4 Hz), 119.2, 117.5, 110.3 (t, J = 248.7 Hz), 80.7, 30.3, 29.6, 27.3. ¹⁹F NMR (282 MHz, DMSO- d_6): δ –94.5. HRMS m/z : [M + H]⁺ calcd for C₂₂H₁₇ClF₂N₆O₅, 487.1092; found, 487.1089.

N-(5-Chloropyridin-3-yl)-8-(1-(difluoromethyl)-1H-pyrazol-4-yl)-4-(methyl- d_3)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10ag). Compound 9ae (80 mg, 0.177 mmol) was reacted with 1-(difluoromethyl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (52 mg, 0.213 mmol) according to General procedure F to afford compound 10ag as a white solid (28 mg, 33%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.26 (br s, 1H), 8.77 (s, 1H), 8.65 (s, 1H), 8.33 (s, 1H), 8.25 (s, 1H), 8.12–8.06 (m, 2H), 7.91 (d, J = 8.1 Hz, 1H), 7.86 (t, J = 58.9 Hz, 1H), 7.67 (d, J = 8.2 Hz, 1H), 2.92–2.84 (m, 2H), 2.79–2.65 (m, 2H). ¹³C NMR (75 MHz, DMSO- d_6): δ 173.5, 169.5, 161.6, 143.5, 140.6, 140.2, 135.5, 135.4, 135.4, 130.3, 128.7, 127.6, 126.8, 123.1 (d, J = 3.1 Hz), 119.2, 117.5, 110.3 (t, J = 248.9 Hz), 80.7, 29.6, 27.3. ¹⁹F NMR (282 MHz, DMSO- d_6): δ –94.5. HRMS m/z : [M + H]⁺ calcd for C₂₂H₁₄D₃ClF₂N₆O₅, 490.1280; found, 490.1277.

2-Amino-4-Bromo-6-Fluoro-N-methylbenzamide (6ah). Methyl 2-amino-4-chloro-6-fluorobenzoate (800 mg, 3.23 mmol) was reacted according to General procedure A to afford compound 6ah as a yellow solid (680 mg, 85%). ¹H NMR (400 MHz, DMSO- d_6): δ 8.16 (s, 1H), 6.73 (s, 1H), 6.59 (d, J = 10.0 Hz, 1H), 6.17 (s, 2H), 2.73 (d, J = 4.4 Hz, 3H). LC–MS (m/z): 246.89 [M + H]⁺.

8-Bromo-6-fluoro-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxylic Acid (7ah). Compound 6ah (840 mg, 3.4 mmol) was reacted according to General procedure D to afford compound 7ah as an off-white solid (1.1 g, 91%). ¹H NMR (400 MHz, DMSO- d_6): δ 14.26 (br s, 1H), 8.24 (s, 1H), 7.50 (d, J = 9.6 Hz, 1H), 3.04 (s, 3H), 2.76–2.57 (m, 4H). LC–MS (m/z): 356.87 [M + H]⁺.

8-Bromo-N-(5-chloropyridin-3-yl)-6-fluoro-4-methyl-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (9ah). Compound 7ah (1.1 g, 3.08 mmol) was reacted with 5-chloropyridin-3-amine (792 mg, 6.16 mmol) according to General procedure B to afford compound 9ah as an off-white solid (1.01 g, 70%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.23 (s, 1H), 8.64 (d, J = 2.0 Hz, 1H), 8.36 (d, J = 2.0 Hz, 1H), 8.09 (s, 1H), 7.98 (s, 1H), 7.51 (d, J = 12.0 Hz, 1H), 3.17 (s, 3H), 2.93–2.65 (m, 4H). LCMS (ESI) m/z : 469.20 [M + H]⁺.

N-(5-Chloropyridin-3-yl)-6-fluoro-4-methyl-8-(1-methyl-1H-pyrazol-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10ah). Compound 9ah (130 mg, 0.278 mmol) was reacted with (1-methyl-1H-pyrazol-4-yl)boronic acid (53 mg, 0.417 mmol) according to General procedure F to afford compound 10ah as a brown solid (60 mg, 46%). ¹H NMR (400 MHz, DMSO- d_6): δ 10.26 (s, 1H), 8.65 (s, 1H), 8.34 (s, 1H), 8.25 (s, 1H), 8.09 (s, 1H), 7.91 (s, 1H), 7.88 (s, 1H), 7.39 (d, J = 12.4 Hz, 1H), 3.88 (s, 3H), 3.19 (s, 3H), 2.87–2.77 (m, 2H), 2.74–2.63 (m, 2H). ¹³C NMR (75 MHz, DMSO- d_6): δ 173.4, 169.4, 161.3, 143.6, 140.6, 138.7 (d, J = 11.3 Hz), 136.9 (d, J = 4.0 Hz), 136.7, 135.3, 130.3, 129.4, 127.6, 119.9 (d, J = 2.3 Hz), 112.5 (d, J = 2.9 Hz), 109.9 (d, J = 22.7 Hz), 106.8 (d, J = 8.5 Hz), 80.5, 38.8, 30.0, 29.7, 27.2. ¹⁹F NMR (282 MHz, DMSO- d_6): δ –111.0. HRMS m/z : [M + H]⁺ calcd for C₂₂H₁₈ClFN₆O₅, 469.1186; found, 469.1185.

R-N-(5-Chloropyridin-3-yl)-6-fluoro-4-methyl-8-(1-methyl-1H-pyrazol-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-*a*]quinazoline-3a(1H)-carboxamide (10aha). Compound 10ah was prepared via reverse phase chiral HPLC of racemic compound 10ah, using a Waters auto purification instrument. Column: REFLECT I-Cellulose C (250 × 20 mm, 5 μ m), operating at ambient temperature and flow rate of 16 mL/min. Diluent: DMSO, Mobile phase: A = Acetonitrile, B = 0.1% Formic acid in water; Gradient Profile: Mobile phase initial composition of 20% A and 80% B, then 40% A and 60% B in 5 min, then to 70% A and 30% B in 30 min, then to 95% A and

5% B in 31 min, held this composition up to 34 min for column washing, then returned to initial composition in 35 min and held this composition up to 38 min ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.26 (br s, 1H), 8.64 (s, 1H), 8.33 (s, 1H), 8.25 (s, 1H), 8.09 (t, J = 2.0 Hz, 1H), 7.91 (s, 1H), 7.89 (s, 1H), 7.39 (d, J = 12.0 Hz, 1H), 3.88 (s, 3H), 3.18 (s, 3H), 2.92–2.76 (m, 2H), 2.75–2.63 (m, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.4, 169.5, 161.4 (d, J = 259.0 Hz), 158.8 (d, J = 3.7 Hz), 143.4, 140.7, 138.7 (d, J = 11.3 Hz), 137.0 (d, J = 3.8 Hz), 136.8, 135.6, 130.3, 129.4, 127.6, 119.9 (d, J = 2.2 Hz), 112.5 (d, J = 3.1 Hz), 109.9 (d, J = 22.8 Hz), 106.8 (d, J = 8.7 Hz), 80.5, 38.8, 30.0, 29.8, 27.3. ^{19}F NMR (282 MHz, $\text{DMSO}-d_6$): δ –111.0. HPLC purity: 99.41%, ee 100%. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{18}\text{ClFN}_6\text{O}_3$, 469.1186; found, 469.1183.

S-N-(5-Chloropyridin-3-yl)-6-fluoro-4-methyl-8-(1-methyl-1H-pyrazol-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxamide (10ahb). Compound 10ahb was prepared via reverse phase chiral HPLC of racemic compound 10ah, using a Waters auto purification instrument. Column: REFLECT I-Cellulose C (250 $\times$ 20 mm, 5 μm), operating at ambient temperature and flow rate of 16 mL/min. Diluent: DMSO, Mobile phase: A = Acetonitrile, B = 0.1% Formic acid in water; Gradient Profile: Mobile phase initial composition of 20% A and 80% B, then 40% A and 60% B in 5 min, then to 70% A and 30% B in 30 min, then to 95% A and 5% B in 31 min, held this composition up to 34 min for column washing, then returned to initial composition in 35 min and held this composition up to 38 min ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.27 (br s, 1H), 8.64 (s, 1H), 8.33 (s, 1H), 8.26 (s, 1H), 8.10 (t, J = 2.0 Hz, 1H), 7.91 (s, 1H), 7.89 (s, 1H), 7.39 (d, J = 12.0 Hz, 1H), 3.88 (s, 3H), 3.18 (s, 3H), 2.92–2.75 (m, 2H), 2.74–2.63 (m, 2H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.4, 169.5, 161.3 (d, J = 259.0 Hz), 158.7 (d, J = 3.8 Hz), 143.4, 140.7, 138.6 (d, J = 11.4 Hz), 137.0 (d, J = 3.8 Hz), 136.7, 130.3, 129.4, 127.6, 119.9 (d, J = 2.3 Hz), 112.5 (d, J = 3.0 Hz), 109.9 (d, J = 22.8 Hz), 106.8 (d, J = 8.8 Hz), 80.5, 38.8, 30.0, 29.8, 27.3. ^{19}F NMR (282 MHz, $\text{DMSO}-d_6$): δ –111.1. HPLC purity: 99.86%; ee 100%.

2-Amino-4-bromo-6-fluorobenzoic Acid (5ai). To a stirred solution of methyl 2-amino-4-bromo-6-fluorobenzoate (0.500 g, 2.02 mmol) in THF (10 mL) was added KOSiMe_3 (0.774 g, 6.05 mmol) and the mixture was stirred at rt for 16 h. Upon completion, 10% aq citric acid solution was added to the mixture until acid, then stirred for 10 min and then extracted with EtOAc. Organic layers were then combined, washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to afford compound 5ai as an off-white solid (0.43 g, 91%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 6.80 (s, 1H), 6.54 (dd, J = 10.8 Hz, 2.0 Hz, 1H). LC–MS (m/z): 233.9 $[\text{M} + \text{H}]^+$.

2-Amino-4-bromo-6-fluoro-N-(methyl- d_3)benzamide (6ai). Compound 5ai (420 mg, 1.80 mmol) was reacted with methan- d_3 -amine hydrochloride (190 mg, 2.69 mmol) was reacted according to General procedure A to afford compound 6ai as an off-white solid (300 mg, 69%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.15 (s, 1H) 6.72 (s, 1H), 6.59 (d, J = 10.0 Hz, 1H), 6.18 (s, 2H). LC–MS (m/z): 250.2 $[\text{M} + \text{H}]^+$.

2-Amino-6-fluoro-N-(methyl- d_3)-4-(1-methyl-1H-pyrazol-4-yl)-benzamide (6aj). Compound 6ai (350 mg, 1.40 mmol) was reacted with (1-methyl-1H-pyrazol-4-yl)boronic acid (437 mg, 2.10 mmol) according to General procedure F to afford compound 6aj as a light brown solid (230 mg, 65%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 8.08 (s, 1H), 7.96 (br s, 1H), 7.77 (s, 1H), 6.68 (s, 1H), 6.58 (d, J = 11.6 Hz, 1H), 6.09 (s, 2H), 3.85 (s, 3H). LC–MS (m/z): 252.1 $[\text{M} + \text{H}]^+$.

6-Fluoro-4-(methyl- d_3)-8-(1-methyl-1H-pyrazol-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]quinazoline-3a(1H)-carboxylic Acid (7ai). Compound 6aj (200 mg, 0.979 mmol) was reacted according to General procedure D to afford compound 7ai as a gray powder (180 mg, 63%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 14.08 (br s, 1H), 8.31 (s, 1H), 8.23 (s, 1H), 7.95 (s, 1H), 7.37 (d, J = 11.6 Hz, 1H), 3.88 (s, 3H), 2.74–2.60 (m, 4H). LC–MS (m/z): 362.3 $[\text{M} + \text{H}]^+$.

N-(5-Chloropyridin-3-yl)-6-fluoro-4-(methyl- d_3)-8-(1-methyl-1H-pyrazol-4-yl)-1,5-dioxo-2,3,4,5-tetrahydropyrrolo[1,2-a]

quinazoline-3a(1H)-carboxamide (10ai). Compound 7ai (90 mg, 0.249 mmol) was reacted with 5-chloropyridin-3-amine (64 mg, 0.498 mmol) according to General procedure B to afford compound 10ai as a white solid (22 mg, 19%). ^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ 10.27 (br s, 1H), 8.64 (s, 1H), 8.33 (s, 1H), 8.26 (s, 1H), 8.09 (s, 1H), 7.91 (s, 1H), 7.88 (s, 1H), 7.38 (d, J = 12.8 Hz, 1H), 3.88 (s, 3H), 2.91–2.63 (m, 4H). ^{13}C NMR (75 MHz, $\text{DMSO}-d_6$): δ 173.4, 169.4, 161.3 (d, J = 258.9 Hz), 158.7 (d, J = 3.6 Hz), 143.4, 140.7, 138.6 (d, J = 11.2 Hz), 137.0 (d, J = 4.0 Hz), 136.7, 135.5, 130.3, 129.4, 127.6, 119.8 (d, J = 2.4 Hz), 112.4, 109.8 (d, J = 22.5 Hz), 106.8, 80.4, 38.8, 29.7, 27.3. ^{19}F NMR (282 MHz, $\text{DMSO}-d_6$): δ –111.1. HRMS m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{22}\text{H}_{15}\text{D}_3\text{ClFN}_6\text{O}_3$, 472.1374; found, 472.1373.

P. falciparum 3D7 Asexual Stage LDH Assay. This is a platform assay conducted at the Walter and Eliza Hall Institute as previously described.⁴³

HepG2 Viability Assay. This assay is a platform assay conducted at the Walter and Eliza Hall Institute as previously described.²⁴

In Vitro ADME. Liver microsome stability, hepatocyte stability, aqueous solubility, and LogD are platform assays conducted by TGC Lifesciences as previously described.¹⁵ Metabolic stability data for standards are located in Table S3.

P. falciparum LDH Growth Assay. This assay format was used to evaluate compounds against parasites with mutations in PfATP4. Early ring-stage *P. falciparum* parasites were obtained via sorbitol synchronization 2 days (or one cycle of growth) before assay setup. Compound concentrations were prepared at 2 times the desired concentration and 100 μL were added to the 96-well plates. Each compound was tested in duplicate. Complete RPMI media was added to the plate (50 μL /well). DMSO was prepared at the same concentration as the compounds and 50 μL was added to the 96-well plate. Cell suspensions were adjusted to 0.5% parasitemia and 4% hematocrit and 50 μL were added to all wells, achieving a final compound concentration of 1 $\times$ and a final 2% hematocrit of RBCs. Uninfected RBCs control (2% hematocrit) were added into 3 wells in column 12 (100 μL /well) as a control. Plates were incubated at 37 $^\circ\text{C}$ following gassing with 1% O_2 and 5% CO_2 in 94% N_2 for 72 h. After incubating for 72 h, plates were frozen at –80 $^\circ\text{C}$ and then thawed at room temperature for at least 2 h. To evaluate LDH activity, the Malstat reagent mix was added to a new 96-well flat-bottom assay plate with 75 μL in each well. The Malstat reagent preparation consisted of 10 mL of 1 M Tris (pH 9.0), 1.24 g of sodium L-lactate, 500 μL of 20% Triton X, and 25 mg of APAD was dissolved in 50 mL of distilled water. To make Malstat Reagent Mix, Nitroblue tetrazolium (NBT; 2 mg/mL) and phenazine ethosulfate (PES) were added to Malstat Reagent at a ratio of 10:1:1 (Malstat reagent/NBT:PES). Parasite culture was resuspended and 30 μL was added and mixed in plates containing the Malstat reagent. Plates were incubated in the dark for approximately 30–40 min. Absorbance was measured using a ClarioStar Microplate reader at 650 nm. Values were normalized to DMSO control as a percentage. Values were plotted using a 4-parameter log dose, nonlinear regression analysis with a sigmoidal dose–response (variable slope) curve fit in GraphPad Prism (version 10.1.0) to generate compound curve and half-maximal effective concentration (EC_{50}) values.

Measurements of P. falciparum Membrane ATPase Activity. Membranes were prepared from saponin-isolated trophozoite-stage parasites as described previously.³⁰ Membranes were prepared either from 3D7 parasites, or from Dd2-Pol δ -PfATP4^{G358S} and Dd2-Pol δ parasites,²⁹ as indicated in the relevant Figure. Protein concentrations in the membrane preparations were determined using a Bradford assay, and the ATPase assays were conducted using a PiColorLock Gold Phosphate Detection System (Innova Biosciences), as described previously.¹⁰ Membrane preparations were added to either a high- $[\text{Na}^+]$ solution (yielding 150 mM NaCl, 20 mM KCl, 2 mM MgCl_2 , and 50 mM Tris (pH 7.2) in the final reactions) or a Na^+ -free solution (yielding 150 mM choline chloride, 20 mM KCl, 2 mM MgCl_2 , and 50 mM Tris (pH 7.2) in the final reactions) containing the compound(s) of interest or solvent alone. The final concentration of DMSO in all the reactions was $\leq 0.2\%$ v/v and the concentration of (total) protein was 50 $\mu\text{g}/\text{mL}$. The reactions were commenced by the addition of

ATP ($\text{Na}_2\text{ATP} \cdot 3\text{H}_2\text{O}$; MP Biomedicals; final $[\text{ATP}] = 1 \text{ mM}$) and ran for 10 min at 37°C .

Metabolomics. Metabolomics sample preparation: *P. falciparum* cultures (3D7 strain) synchronized to the mid trophozoite stage were magnet purified to achieve a parasitemia of >90% and hematocrit of 0.5%. Infected RBCs were treated with 70 nM of compound **10ah** ($5 \times \text{EC}_{50}$), 5 nM ($5 \times \text{EC}_{50}$), or 20 nM ($20 \times \text{EC}_{50}$) of the known PfATP4 inhibitor KAE609 **1**, or an equivalent volume of vehicle (DMSO) for 5 h with a minimum of three independent incubations per condition. Following compound incubation, cultures were centrifuged at 1200g for 3 min, the media was removed, and the cell pellets were washed in 1 mL of ice-cold PBS. Samples were again centrifuged at 1200g for 3 min to remove all of the PBS and metabolites were extracted from 5×10^7 cells using 90 μL of ice-cold methanol extraction solvent. Samples were then incubated on an automatic vortex mixer at 4°C for 1 h before being centrifuged at 21,000g for 10 min. The supernatants were transferred into HPLC vials and stored at -80°C until liquid chromatography–mass spectrometry (LC–MS) analysis. A 10 μL aliquot from each sample was pooled to serve as a quality control sample for monitoring instrument reproducibility and to aid downstream metabolite identification.

Liquid chromatography–mass spectrometry: LC–MS data was acquired on an Orbitrap Exploris 120 mass spectrometer (Thermo Scientific) coupled with a Vanquish Flex HPLC system (Thermo Scientific). Analytical separation was performed with a 150 mm $\times$ 4.6 mm, 5 μm ZIC-PHILIC column (Merck) attached to a guard of the same material. The mobile phases were 20 mM ammonium carbonate (solvent A) and acetonitrile (solvent B) set at a flow rate of 0.35 mL/min. The gradient profile was as follows: 0–10 min, 80–50% B; 10–12 min, 50–5% B; 12–14 min, 5% B; 14–16 min, 5–80% B and 16–22 min, 80% B. Data were acquired as a full scan in positive and negative ionization modes with a heated electrospray source and an Orbitrap resolution of 120,000 from 70 to 1050 m/z . Ion source voltage was 3500 V in positive mode and 2500 V in negative mode. The ion transfer tube temperature was 325°C and the vaporizer temperature was 350°C . Gas mode was set to static with sheath gas, aux gas, and sweep gas at 50, 10, and 1, respectively. Samples within the LC–MS batch were sorted according to blocks of replicates and randomized. To facilitate metabolite identification, approximately 350 authentic metabolite standards were analyzed before the LC–MS batch, and their peaks and retention time were manually checked using the MZmine software. Pooled biological quality control samples and extraction solvent blanks were analyzed periodically throughout the batch to monitor LC–MS signal reproducibility and assist metabolite identification procedures.

Data analysis and metabolite identification: Raw LC–MS metabolomics data were analyzed using the open source software, IDEOM (<http://mzmatch.sourceforge.net/ideom.php>).⁴⁶ Briefly, the IDEOM workflow uses msconvert to convert raw files to mzXML format, XCMS (Centwave) to pick LC–MS peak signals, and MZmatch for alignment and annotation of related metabolite peaks. Default IDEOM parameters were used to eliminate unwanted noise and artifact peaks. Confident metabolite identification was made by matching accurate masses to the retention time of the ~350 authentic standards. When these authentic standards were unavailable, putative metabolite identification used accurate mass and predicted retention times, as previously described.⁴⁷ Metabolite abundance was represented by LC–MS peak height. For fold-change calculations, metabolites that were not detected in a sample are expressed relative to half the minimum detected intensity in the data set. Statistical significance was determined using Welch's *t*-test ($\alpha = 0.05$). Principal component analysis and hierarchical clustering analysis were performed in MetaboAnalyst (version 6.0)⁴⁸ on the log-transformed (base 10) and autoscaled (mean-centered and divided by the standard deviation of each variable) metabolite peak intensity data. To assess whether putative peptides could originate from hemoglobin, the sequences of human hemoglobin α , β , and δ chains (HBA_HUMAN P69905, HBB_HUMAN P68871, HBD_HUMAN P02042) were searched for any peptide with monoisotopic mass within 0.002 m/z of

the identified peptide using custom Python scripts, as previously described.⁴⁹

Parasite Growth Assays with Asexual Stage *P. knowlesi* and Multidrug-Resistant *P. falciparum*. *P. falciparum* 3D7 parasites and multidrug-resistant strains Dd2, W2mef, 7G8, and artemisinin-resistant Cambodian isolate (Cam3.I (2539T)), and *P. knowlesi* YH1, were cultured in human O^+ erythrocytes (RBCs) (Australian Red Cross Blood Service), in RPMI-HEPES culture medium (pH 7.4, 50 $\mu\text{g/mL}$ hypoxanthine, 25 mM NaHCO_3 , 20 $\mu\text{g/mL}$ gentamicin, 0.5% Albumax II (GibcoBRL)) (Thermo Fisher Scientific) and maintained in an atmosphere of 1% O_2 , 4% CO_2 and 95% N_2 according to established protocols (Trager, 1976). Parasites were synchronized with 5% (w/v) sorbitol (Sigma-Aldrich) treatments for ring stages. Growth assays for measuring drug inhibition of in-cycle, ring to schizont stage parasites have been described previously. Parasites were grown at 1% hematocrit, at 1 and 2% parasitemia for *P. falciparum* and *P. knowlesi* respectively. Compounds were added at ring stages, and parasite growth was measured at late trophozoite/schizont stages in the next cycle of growth (68–72 h postinvasion for *P. falciparum*; 44–48 h postinvasion for *P. knowlesi*). Parasitaemia was measured using a SYBR DNA staining assay using a fluorometer. After the incubation period, supernatants were removed and well contents were resuspended in PBS. An equal volume of SYBR Safe Stain (0.02% (v/v) in SYBR Lysis buffer (pH 7.5, 20 mM TRIS, 5 mM EDTA, 0.008% Saponin (w/v), 0.08% Triton X100 (v/v)) was added to wells and mixed. After incubating for 30 min to 1 h, fluorescence was measured on a fluorometer (BMG LabTech PHERAstar FS) (excitation, 485; emission, 520). The background (noninfected RBCs) was subtracted from all samples and the data for parasites exposed to compounds were normalized against the data for untreated parasites to calculate the percent survival of compound-treated parasites. IC_{50} s were determined for each drug using GraphPad Prism (GraphPad Software) according to the recommended protocol for nonlinear regression of a log-(inhibitor)-versus-response curve.

Dual Gamete Formation Assay. Compounds were tested in the *P. falciparum* Dual Gamete Formation Assay (DGFA) as previously described.⁴¹ Briefly, mature stage V gametocytes were exposed to compounds for 48 h at 37°C in 384 well plates in gametocyte culture medium (RPMI 1640 supplemented with 25 mM HEPES, 50 $\mu\text{g mL}^{-1}$ hypoxanthine, 4.8 g L^{-1} NaHCO_3 , 2 mM L-glutamine, 5% pooled type AB serum, 0.5% Albumax II (Gibco)) under a 1% O_2 /3% CO_2 /96% N_2 environment. Gametogenesis was then triggered by the addition of 10 μL ookinete medium (gametocyte culture medium supplemented with 100 μM xanthurenic acid and 0.27 $\mu\text{g mL}^{-1}$ Cy3-labeled anti-Pfs25 antibody) to each well at room temperature. Plates were then cooled on a metal block at 4°C for 4 min to ensure even cooling and then stabilized for a further 4 min at 28°C . At 20 min postinduction, male gametogenesis was recorded in each well by automated brightfield microscopy using a 4 $\times$ objective lens and 1.5 $\times$ magnifier (6 $\times$ effective magnification). Afterward, plates were incubated in the dark at room temperature for 24 h and then female gametogenesis was recorded in each well by automated fluorescence microscopy (anti-Pfs25-positive cells). All experiments were performed in quadruplicate with DMSO and Cabamiquine as negative and positive controls, respectively. All data were evaluated in comparison to the positive and negative controls to calculate the percentage inhibition of male and female gametocytes, and dose-response analysis and IC_{50} calculation were performed using GraphPad Prism.

Standard Membrane Feeding Assay. *P. falciparum* NF54 gametocytes were prepared for blood-feeding to mosquitoes according to the method described by Delves et al.⁴¹ with some slight modifications. Briefly, gametocyte cultures seeded at 2% overall parasitemia and 4% hematocrit were maintained at 37°C for 17 days with daily media changes. Gametocyte functional viability was assessed by ex-flagellation assay at day 14 postinduction, observed by light microscopy. *Anopheles stephensi* (SD500 strain) were reared as described previously.⁵⁰ Gametocyte aliquots from a single NF54 culture were mixed in the membrane feeder reservoir with **10ahb** at 250 or 1000 nM, the inactive stereoisomer **10aha** at 1000, 1 mM

methylene blue (used as infectivity blocking control) or culture medium with DMSO (no-drug control), then SMFA was carried out. The concentration of DMSO during experimental mosquito feeds 1 and 2 was at 1.0% v/v, except for the 250 nM solution, which was a 1:4 dilution of the 1000 nM preparation and therefore included DMSO at 0.25%. For experimental mosquito feeds 3–5 test compound dose was adjusted for **10ahb** to 50 nM or 100 nM, while DMSO concentration was adjusted to 0.25% in all treatments. All other conditions remained the same as in the first two experimental feeds. Pots of 70–80 female *An. stephensi* mosquitoes (two to 5 days old) were allowed to feed on 500 μ L of the respective culture-drug-DMSO mixture, presented to each pot of mosquitoes in a preheated 3D-printed water channel membrane feeder,⁵¹ until fully fed. Mosquitoes were placed in an incubator at standard conditions and midguts were dissected in 0.25% mercurochrome stain for oocyst counts by light microscopy 7–8 days postfeed. Statistical significance of observed differences was tested by the nonparametric Wilcoxon rank sum test.

P. berghei Mouse Model. The mouse model conducted on compounds **10x**, **10y**, and **8y** uses a method as previously described.⁵² The use of animals was approved by the Walter and Eliza Hall Institute of Medical Research Animal Ethics Committee under approval number 2020.036 and all procedures were conducted in compliance with the animal ethics guidelines to promote the wellbeing of animals used for scientific purposes, National Health and Medical Research Council 2008 (Australian code for the care and use of animals for scientific purposes, NHMRC eighth Edition 2013). ASMU/Swiss outbred (pathogen-free), females, 4 weeks, 15–18 g were kept in exhausted ventilated cages (EVC) with corn cob bedding, under standard conditions (21 °C $\pm$ 3 °C, 40–70% relative humidity, 12/12 h, 6am –6 pm light/dark cycle) with food and water ad libitum.

“Donor” female Swiss mice were infected intraperitoneally (IP) with blood-stage *P. berghei* parasites constitutively expressing GFP (*P. berghei* ANKA GFPcon 259cl2.⁵³ Three days later, groups of 3 “acceptor” Swiss mice were infected intravenously (IV) with 1×10^7 parasitized red blood cells from the “donor mice”. Test compounds **10x**, **10y**, and **8y** were prepared in a vehicle which was a partial suspension consisting of 0.5% carboxymethylcellulose/0.5% benzyl alcohol/0.4% polysorbate 80 in water. Two h postinfection, mice were treated orally once daily on 4 consecutive days (q.d. regimen, once a day) with a dose of test compounds (20 mg/kg) or chloroquine (10 mg/kg). Control mice were left untreated. Peripheral blood samples were taken 24 h after administration of the last dose, and parasitemia was measured by flow cytometry (proportion of GFP-positive cells in 100,000 recorded events using Attune Nxt, ThermoFisher) and microscopic analysis of Giemsa-stained blood smears. Parasitemia values were averages of 3 mice per group.

The *P. berghei* mouse model that was conducted on compounds **10ahb** uses a method that was previously described.¹⁶ Ethics statement: Experiments involving rodents were performed in strict accordance with the recommendations of the Australian Government and National Health and Medical Research Council Australian code of practice for the care and use of animals for scientific purposes. The protocols were approved by the Animal Welfare Committee at Deakin University (approval no. G14/2020). Procedure: A stock solution of compound (**10x**) was made by suspending the compound in 70% (v/v) Tween 80 and 30% (v/v) ethanol in distilled sterile water. Each day, a fresh aliquot of the compound stock was diluted 1/10 in water for administration to mice. Biological assessment of the in vivo antimalarial efficacy of **10ahb** was assessed using the *P. berghei* rodent malaria 4-day suppressive test in 8 week old female mice from Ozgene. Mice at day 0 were infected with 2×10^7 *P. berghei* ANKA-infected erythrocytes. At 2 h and days 1, 2, and 3 postinfection, mice were administered by oral gavage with 20 mg/kg **10ahb** (6 mice) or vehicle control (6 mice). Two mice were also dosed with artesunate using the same regime at 30 mg/kg. The parasitemia of mice was assessed by visualizing Giemsa-stained thin blood smears by microscopy and a minimum of 1000 RBCs were counted. To calculate the percent antimalarial activity the following formula was

used: $100 - (\text{mean parasitemia treated day 4 postinfection} / \text{mean parasitemia vehicle control day 4 postinfection}) \times 100$. Mice were culled the day following the last oral gavage.

P. falciparum NOD-Scid IL-2R γ ^{null} Mouse Model. This mouse model was conducted at the Art of Discovery, SL. **10ah** was administered at 25 mg/kg of mouse bodyweight for 4 days once-a-day per oral route to *P. falciparum* infected NSG mice engrafted with human erythrocytes. **10ah** was formulated in 1% methyl cellulose, 0.1% tween-80 in double distilled water. The formulation was prepared daily and administered at 10 mL/kg. The *P. falciparum* strain P3D70087/N9 was used in this model.⁵⁴

The model was performed using 22–28 gr female NOD-scid IL-2R γ ^{null} mice (NSG) from Charles River, France, and maintained for at least 1 week before entering experimental procedures. The mice were housed in The Art of Discovery animal facility at BIC Bizkaia building (Derio, Basque Country, Spain), which is equipped with HEPA filtered in/out air-conditioned with 15 air renovations per hour at 22 $\pm$ 2 °C; 40–70% relative humidity; 12 h light/dark period. The mice were accommodated in racks with ventilated disposable cages (Innovive) in groups of up to five individuals with autoclaved dust-free corn cob bedding (Innovive). The animals were fed with gamma-irradiated standard pellet (Envigo) and ultrafiltered water (Innovive) ad libitum. Studies with animals were performed at The Art of Discovery. The studies performed at TAD were approved by The Art of Discovery Institutional Animal Care and Use Committee (TAD-IACUC). This Committee is certified by the Biscay County Government (Bizkaiko Foru Aldundia, Basque Country, Spain) to evaluate animal research projects from Spanish institutions according to point 43.3 from Royal Decree 53/2013, from the first of February (BOE-A-2013-1337). All experiments were carried out in accordance with European Directive 2010/63/EU.

The model was conducted as previously described.⁴⁴ Briefly, immunodeficient female NSG mice were engrafted with human erythrocytes to have a minimum of 40% of human erythrocytes circulating in peripheral blood during the whole experiment. NSG mice engrafted with human erythrocytes were then intravenously infected with parasitized red blood cells 72 h before drug treatment inception. At this point (day 1 of the study) mice had between 1 and 2% of parasitemia on average and the mice were randomly allocated to selected treatments. The effect of treatment on parasitemia was assessed by measuring the percentage of infected erythrocytes in peripheral blood every 24 h until parasitemia was below the selected limit of quantitation (usually 0.01%). During the study, samples of peripheral blood were taken from mice to measure the concentration of drugs and/or their metabolites. The parasitemia in mice was regularly measured up to day 30 of the experiment to check the presence of circulating parasitized human erythrocytes. Parasite net growth inhibition and parasite clearance of every individual in the study were calculated from measurements of parasitemia in samples of peripheral blood of mice. Parasitemia was measured every 24 h by flow cytometry in all mice of the study until the limit of quantitation (0.01%) was reached and, when recrudescence was detected, until parasitemia at treatment inception was reached again. To measure parasitemia blood samples of peripheral blood from *P. falciparum*-infected mice were stained with TER-119-Phycoerythrin (a marker of murine erythrocytes) and SYTO-16 (nucleic acid dye) and then analyzed by flow cytometry (Attune Nxt Acoustic Focusing Flow Cytometer, InvitroGen).⁴⁵

A qualitative assessment of the PD response was performed in selected individuals of the study. First, the cellular morphology of parasites circulating in blood was analyzed by microscopy in blood samples taken at 48 h (1 parasite cycle) and/or 96 h (two parasite cycles) after treatment inception. Second, an analysis of the population distribution of the different parasite stages in peripheral blood during treatment was performed by flow cytometry using the samples of measurement of parasitemia. To do this, the blood samples were stained with TER-119-Phycoerythrin and SYTO-16 for flow cytometry analysis. The data was plotted whereby the x-axis measures DNA content in human erythrocytes infected by *P. falciparum* (SYTO-16, red rectangles) while the y-axis shows only glycophorin-A-

associated Ly-76 protein-negative events (i.e., human erythrocytes not stained by the monoclonal antibody). The microscopic analysis was done with blood films from peripheral blood samples stained with a buffered Giemsa solution.

Samples of peripheral blood (30 μ L) were taken at different time points after dosing, mixed with 30 μ L of 10.9 mM potassium oxalate, 59.5 mM sodium fluoride in H₂O Milli-Q and immediately frozen on a thermal block at -80°C . The frozen samples were stored at -80°C until analysis. Blood from *P. falciparum* Pf3D70087/N9-infected huMice harboring a parasitemia $\sim 1.5\%$ was used for preparation of standard curves, and calibration and quality control samples. The drugs were extracted from 10 μ L of lysates, obtained by protein precipitation of blood samples, by using standard liquid–liquid extraction methods. The samples were analyzed by LC–MS/MS for quantification in Waters Micromass UPLC–TQD (Waters, Manchester, UK) mass spectrometers. Blood concentration vs time was analyzed using Phoenix WinNonlin version 8.2 (Certara) from which exposure-related values (t_{max} , C_{max} , and AUC $_{0-t}$) were estimated.

■ ASSOCIATED CONTENT

Data Availability Statement

Raw metabolomic data is available at the NIH Common Fund's National Metabolomics Data Repository (NMDR) Web site, the Metabolomics Workbench, <https://www.metabolomicsworkbench.org>, where it has been assigned Study ID ST003179. The data can be accessed directly via its Project DOI: [10.21228/M8214C](https://doi.org/10.21228/M8214C).

■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jmedchem.4c01241>.

Molecular Formula Strings (CSV)

Metabolomics data (XLSX)

Metabolism identification and Caco-2 data, *P. falciparum* and *P. knowlesi* asexual (3D7 and drug-resistant lines) and gamete dose response data, metabolomic, standard membrane feeding assay and mouse model data, and compound spectra (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Brad E. Sleebs – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia; orcid.org/0000-0001-9117-1048; Phone: 61 3 9345 2718; Email: sleebs@wehi.edu.au

Authors

Trent D. Ashton – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia; orcid.org/0000-0002-6707-6064

Petar P. S. Calic – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia; orcid.org/0000-0001-6538-2394

Madeline G. Dans – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia

Zi Kang Ooi – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia

Qingmiao Zhou – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia

Josephine Palandri – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia

Katie Loi – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia

Kate E. Jarman – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia

Deyun Qiu – Research School of Biology, Australian National University, Canberra 2601, Australia

Adele M. Lehane – Research School of Biology, Australian National University, Canberra 2601, Australia; orcid.org/0000-0002-0050-9101

Bikash Chandra Maity – TCG Lifesciences, Kolkata, West Bengal 700091, India

Nirupam De – TCG Lifesciences, Kolkata, West Bengal 700091, India

Carlo Giannangelo – Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia; orcid.org/0000-0002-6654-7266

Christopher A. MacRaid – Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia; orcid.org/0000-0002-3694-3989

Darren J. Creek – Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria 3052, Australia; orcid.org/0000-0001-7497-7082

Emma Y. Mao – Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide, Adelaide 5005, Australia

Maria R. Gancheva – Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide, Adelaide 5005, Australia

Danny W. Wilson – Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide, Adelaide 5005, Australia

Mrittika Chowdury – School of Medicine, Deakin University, Waurn Ponds, Victoria 3216, Australia; Institute for Mental and Physical Health and Clinical Translation, Deakin University, Geelong, Victoria 3216, Australia

Tania F. de Koning-Ward – School of Medicine, Deakin University, Waurn Ponds, Victoria 3216, Australia; Institute for Mental and Physical Health and Clinical Translation, Deakin University, Geelong, Victoria 3216, Australia

Mufuli T. Famodimu – Department of Infection Biology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.; orcid.org/0000-0002-8852-1040

Michael J. Delves – Department of Infection Biology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.; orcid.org/0000-0001-8526-4782

Harry Pollard – Department of Infection Biology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.

Colin J. Sutherland – Department of Infection Biology, London School of Hygiene and Tropical Medicine, London WC1E 7HT, U.K.

Delphine Baud – MMV Medicines for Malaria Venture, ICC, Geneva 1215, Switzerland

Stephen Brand – MMV Medicines for Malaria Venture, ICC, Geneva 1215, Switzerland

Paul F. Jackson – Emerging Science & Innovation, Discovery Sciences, Janssen R&D LLC, La Jolla, California 92121, United States

Alan F. Cowman – The Walter and Eliza Hall Institute of Medical Research, Parkville 3052, Australia; Department of Medical Biology, The University of Melbourne, Parkville 3010, Australia

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acs.jmedchem.4c01241>

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was funded by the National Health and Medical Research Council of Australia (Development grant 1135421 to B.E.S. and A.F.C.; Synergy grant to 1185354 to T.F.dK-W. and D.J.C.), the Victorian State Government Operational Infrastructure Support and Australian Government NHMRC IRISS. B.E.S. gratefully acknowledges the support from the MMV Medicines for Malaria Venture (RD-18-0003) for the chemistry, in vitro ADME profiling, and the SCID mouse model. This work was supported by a Medical Research Council Career Development Award (MR/V010034/1) awarded to M.J.D. M.T.F. is supported by an MMV grant (RD-21-1003) awarded to M.J.D. Mosquito infection studies at LSHTM are supported by Wellcome Trust Biomedical Resources Grant 221363/Z/20/Z awarded to C.J.S. We thank and acknowledge the Australian Red Cross Lifeblood for the provision of fresh red blood cells. We thank Dr Keith Watson from the Walter and Eliza Hall Institute for the helpful advice. A.F.C. is a Howard Hughes International Scholar and an Australia Fellow of the NHMRC. B.E.S. is a Corin Centenary Fellow. Z.K.O. and Q.Z. were supported by an Alan Harris Scholarship, Q.Z. was supported by the China Scholarship Council, and E.Y.M. was supported by an ARC-RTP Scholarship.

ABBREVIATIONS

ACT, artemisinin combination therapy; CQ, chloroquine; DGFA, dual gamete formation assay; DHODH, dihydroorotate dehydrogenase; GFP, green fluorescent protein; HT, high throughput; LDH, lactate dehydrogenase; MIR, minimum inoculum of resistance; NOD-SCID, nonobese diabetic-severe combined immunodeficiency; Pb, *Plasmodium berghei*; PfATP4, *P. falciparum* non-SERCA-type Ca^{2+} -transporting P-ATPase; Pf, *Plasmodium falciparum*; PRR, parasite reduction ratio; SAR, structure activity relationship; SMFA, standard membrane feeding assay; TCP, target candidate profile; WJM, WEHI Janssen MMV

REFERENCES

- (1) World Malaria Report 2023. World Health Organisation: Geneva, 2023. <https://www.who.int/publications/i/item/world-malaria-report-2019> (accessed 12 Dec, 2022).
- (2) Mendis, K.; Marchesini, P.; Carter, R.; Sina, B. The neglected burden of *Plasmodium vivax* malaria. *Am. J. Trop. Med. Hyg.* **2001**, 64 (1 suppl), 97–106.
- (3) Millar, S. B.; Cox-Singh, J. Human infections with *Plasmodium knowlesi*-zoonotic malaria. *Clin. Microbiol. Infect.* **2015**, 21 (7), 640–648.
- (4) Sutherland, C. J. Persistent parasitism: The adaptive biology of malariae and ovale Malaria. *Trends Parasitol.* **2016**, 32 (10), 808–819.
- (5) Ashley, E. A.; Dhorda, M.; Fairhurst, R. M.; Amaratunga, C.; Lim, P.; Suon, S.; Sreng, S.; Anderson, J. M.; Mao, S.; Sam, B.; Sopha, C.; Chuor, C. M.; Nguon, C.; Sovannarothe, S.; Pukrittayakamee, S.; Jittamala, P.; Chotivanich, K.; Chutasmit, K.; Suchatsoonthorn, C.; Runchaoen, R.; Hien, T. T.; Thuy-Nhien, N. T.; Thanh, N. V.; Phu, N. H.; Htut, Y.; Han, K. T.; Aye, K. H.; Mokuolu, O. A.; Olaosebikan, R. R.; Folaranmi, O. O.; Mayxay, M.; Khanthavong, M.; Hongvanthong, B.; Newton, P. N.; Onyamboko, M. A.; Fanello, C. I.; Tshefu, A. K.; Mishra, N.; Valecha, N.; Phyto, A. P.; Nosten, F.; Yi, P.; Tripura, R.; Borrmann, S.; Bashraheil, M.; Peshu, J.; Faiz, M. A.; Ghose, A.; Hossain, M. A.; Samad, R.; Rahman, M. R.; Hasan, M. M.; Islam, A.; Miotto, O.; Amato, R.; MacInnis, B.; Stalker, J.; Kwiatkowski, D. P.; Bozdech, Z.; Jeeyapant, A.; Cheah, P. Y.; Sakulthaew, T.; Chalk, J.; Intharabut, B.; Silamut, K.; Lee, S. J.; Vihokhern, B.; Kunasol, C.; Imwong, M.; Tarning, J.; Taylor, W. J.; Yeung, S.; Woodrow, C. J.; Flegg, J. A.; Das, D.; Smith, J.; Venkatesan, M.; Plowe, C. V.; Stepniewska, K.; Guerin, P. J.; Dondorp, A. M.; Day, N. P.; White, N. J. Spread of Artemisinin resistance in *Plasmodium falciparum* Malaria. *N. Engl. J. Med.* **2014**, 371 (5), 411–423.
- (6) Uwimana, A.; Legrand, E.; Stokes, B. H.; Ndikumana, J.-L. M.; Warsame, M.; Umulisa, N.; Ngamije, D.; Munyaneza, T.; Mazarati, J.-B.; Munguti, K.; Campagne, P.; Criscuolo, A.; Arie, F.; Murindahabi, M.; Ringwald, P.; Fidock, D. A.; Mbituyumuremyi, A.; Menard, D. Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum* kelch13 R561H mutant parasites in Rwanda. *Nat. Med.* **2020**, 26, 1602–1608.
- (7) Ashton, T. D.; Devine, S. M.; Möhrle, J. J.; Laleu, B.; Burrows, J. N.; Charman, S. A.; Creek, D. J.; Sleebs, B. E. The development process for discovery and clinical advancement of modern antimalarials. *J. Med. Chem.* **2019**, 62 (23), 10526–10562.
- (8) Phillips, M. A.; Lotharius, J.; Marsh, K.; White, J.; Dayan, A.; White, K. L.; Njoroge, J. W.; El Mazouni, F.; Lao, Y.; Kokkonda, S.; Tomchick, D. R.; Deng, X.; Laird, T.; Bhatia, S. N.; March, S.; Ng, C. L.; Fidock, D. A.; Wittlin, S.; Lafuente-Monasterio, M.; Benito, F. J.; Alonso, L. M.; Martinez, M. S.; Jimenez-Diaz, M. B.; Bazaga, S. F.; Angulo-Barturen, I.; Haselden, J. N.; Louttit, J.; Cui, Y.; Sridhar, A.; Zeeman, A. M.; Kocken, C.; Sauerwein, R.; Decherling, K.; Avery, V. M.; Duffy, S.; Delves, M.; Sinden, R.; Ruecker, A.; Wickham, K. S.; Rochford, R.; Gahagen, J.; Iyer, L.; Riccio, E.; Mirsalis, J.; Bathurst, I.; Rueckle, T.; Ding, X.; Campo, B.; Leroy, D.; Rogers, M. J.; Rathod, P. K.; Burrows, J. N.; Charman, S. A. A long-duration dihydroorotate dehydrogenase inhibitor (DSM265) for prevention and treatment of malaria. *Sci. Transl. Med.* **2015**, 7 (296), 296ra111.
- (9) Rottmann, M.; McNamara, C.; Yeung, B. K.; Lee, M. C.; Zou, B.; Russell, B.; Seitz, P.; Plouffe, D. M.; Dharia, N. V.; Tan, J.; Cohen, S. B.; Spencer, K. R.; Gonzalez-Paez, G. E.; Lakshminarayana, S. B.; Goh, A.; Suwanarusk, R.; Jegla, T.; Schmitt, E. K.; Beck, H. P.; Brun, R.; Nosten, F.; Renia, L.; Dartois, V.; Keller, T. H.; Fidock, D. A.; Winzeler, E. A.; Diagana, T. T. Spiroindolones, a potent compound class for the treatment of malaria. *Science* **2010**, 329 (5996), 1175–1180.
- (10) Spillman, N. J.; Allen, R. J.; McNamara, C. W.; Yeung, B. K.; Winzeler, E. A.; Diagana, T. T.; Kirk, K. Na(+) regulation in the malaria parasite *Plasmodium falciparum* involves the cation ATPase PfATP4 and is a target of the spiroindolone antimalarials. *Cell Host Microbe* **2013**, 13 (2), 227–237.
- (11) Llanos-Cuentas, A.; Casapia, M.; Chuquiyauri, R.; Hinojosa, J. C.; Kerr, N.; Rosario, M.; Toovey, S.; Arch, R. H.; Phillips, M. A.; Rozenberg, F. D.; Bath, J.; Ng, C. L.; Cowell, A. N.; Winzeler, E. A.; Fidock, D. A.; Baker, M.; Möhrle, J. J.; Hooft van Huijsduijnen, R.; Gobeau, N.; Araeipour, N.; Andenmatten, N.; Rückle, T.; Duparc, S. Antimalarial activity of single-dose DSM265, a novel plasmodium dihydroorotate dehydrogenase inhibitor, in patients with uncomplicated *Plasmodium falciparum* or *Plasmodium vivax* malaria infection: a proof-of-concept, open-label, phase 2a study. *Lancet Infect. Dis.* **2018**, 18 (8), 874–883.

- (12) Schmitt, E. K.; Ndayisaba, G.; Yeka, A.; Asante, K. P.; Grobusch, M. P.; Karita, E.; Mugerwa, H.; Asiimwe, S.; Oduro, A.; Fofana, B.; Doumbia, S.; Su, G.; Csermak Renner, K.; Venishetty, V. K.; Sayyed, S.; Straimer, J.; Demin, I.; Barsainya, S.; Boulton, C.; Gandhi, P. Efficacy of Cipargamin (KAE609) in a Randomized, Phase II Dose-Escalation Study in Adults in Sub-Saharan Africa With Uncomplicated *Plasmodium falciparum* Malaria. *Clin. Infect. Dis.* **2022**, 74 (10), 1831–1839.
- (13) Burrows, J. N.; Duparc, S.; Gutteridge, W. E.; Hooft van Huijsduijnen, R.; Kaszubska, W.; Macintyre, F.; Mazzuri, S.; Möhrle, J. J.; Wells, T. N. C. New developments in anti-malarial target candidate and product profiles. *Malar. J.* **2017**, 16 (1), 26.
- (14) Bailey, B. L.; Nguyen, W.; Ngo, A.; Goodman, C. D.; Gancheva, M. R.; Favuzza, P.; Sanz, L. M.; Gamo, F.-J.; Lowes, K. N.; McFadden, G. I.; Wilson, D. W.; Laleu, B.; Brand, S.; Jackson, P. F.; Cowman, A. F.; Sleebs, B. E. Optimisation of 2-(N-phenyl carboxamide) triazolopyrimidine antimalarials with moderate to slow acting erythrocytic stage activity. *Bioorg. Chem.* **2021**, 115, 105244.
- (15) Nguyen, W.; Dans, M. G.; Currie, I.; Awalt, J. K.; Bailey, B. L.; Lumb, C.; Ngo, A.; Favuzza, P.; Palandri, J.; Ramesh, S.; Penington, J.; Jarman, K. E.; Mukherjee, P.; Chakraborty, A.; Maier, A.; van Dooren, G.; Papenfuss, T.; Wittlin, S.; Churchyard, A.; Baum, J.; Winzeler, E. A.; Baud, D.; Brand, S.; Jackson, P. F.; Cowman, A. F.; et al. 7-N-Substituted-3-oxadiazole quinolones with potent antimalarial activity target the cytochrome bc1 complex. *ACS Infect. Dis.* **2023**, 9 (3), 668–691.
- (16) Nguyen, W.; Boulet, C.; Dans, M. G.; Loi, K.; Jarman, K. E.; Watson, G. M.; Tham, W.-H.; Fairhurst, K. J.; Yeo, T.; Fidock, D. A.; Wittlin, S.; Chowdury, M.; de Koning-Ward, T. F.; Chen, G.; Yan, D.; Charman, S. A.; Baud, D.; Brand, S.; Jackson, P. F.; Cowman, A. F.; Gilson, P. R.; Sleebs, B. E. Activity refinement of aryl amino acetamides that target the *P. falciparum* STAR-related lipid transfer 1 protein. *Eur. J. Med. Chem.* **2024**, 270, 116354.
- (17) Ashton, T. D.; Dans, M. G.; Favuzza, P.; Ngo, A.; Lehane, A. M.; Zhang, X.; Qiu, D.; Chandra Maity, B.; De, N.; Schindler, K.; Yeo, T.; Park, H.; Uhlemann, A.-C.; Churchyard, A.; Baum, J.; Fidock, D. A.; Jarman, K. E.; Lowes, K. N.; Baud, D.; Brand, S.; Jackson, P. F.; Cowman, A. F.; Sleebs, B. E. Optimization of 2,3-dihydroquinazolinone-3-carboxamides as antimalarials targeting PfATP4. *J. Med. Chem.* **2023**, 66 (5), 3540–3565.
- (18) Spillman, N. J.; Allen, R. J.; Kirk, K. Na⁺ extrusion imposes an acid load on the intraerythrocytic malaria parasite. *Mol. Biochem. Parasitol.* **2013**, 189 (1–2), 1–4.
- (19) Spillman, N. J.; Kirk, K. The malaria parasite cation ATPase PfATP4 and its role in the mechanism of action of a new arsenal of antimalarial drugs. *Int. J. Parasitol.: Drugs Drug Resist.* **2015**, 5 (3), 149–162.
- (20) Jiménez-Díaz, M. B.; Ebert, D.; Salinas, Y.; Pradhan, A.; Lehane, A. M.; Myrand-Lapierre, M.-E.; O’Loughlin, K. G.; Shackleford, D. M.; Justino de Almeida, M.; Carrillo, A. K.; Clark, J. A.; Dennis, A. S. M.; Diep, J.; Deng, X.; Duffy, S.; Endsley, A. N.; Fedewa, G.; Guiguemde, W. A.; Gómez, M. G.; Holbrook, G.; Horst, J.; Kim, C. C.; Liu, J.; Lee, M. C. S.; Matheny, A.; Martínez, M. S.; Miller, G.; Rodríguez-Alejandre, A.; Sanz, L.; Sigal, M.; Spillman, N. J.; Stein, P. D.; Wang, Z.; Zhu, F.; Waterson, D.; Knapp, S.; Shelat, A.; Avery, V. M.; Fidock, D. A.; Gamo, F.-J.; Charman, S. A.; Mirsalis, J. C.; Ma, H.; Ferrer, S.; Kirk, K.; Angulo-Barturen, I.; Kyle, D. E.; DeRisi, J. L.; Floyd, D. M.; Guy, R. K. (+)-SJ733, a clinical candidate for malaria that acts through ATP4 to induce rapid host-mediated clearance of *Plasmodium*. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, 111 (50), No. E5455.
- (21) Vaidya, A. B.; Morrissey, J. M.; Zhang, Z.; Das, S.; Daly, T. M.; Otto, T. D.; Spillman, N. J.; Wyvrat, M.; Siegl, P.; Marfurt, J.; Wirjanata, G.; Sebayang, B. F.; Price, R. N.; Chatterjee, A.; Nagle, A.; Stasiak, M.; Charman, S. A.; Angulo-Barturen, I.; Ferrer, S.; Belén Jiménez-Díaz, M.; Martínez, M. S.; Gamo, F. J.; Avery, V. M.; Ruecker, A.; Delves, M.; Kirk, K.; Berriman, M.; Kortagere, S.; Burrows, J.; Fan, E.; Bergman, L. W. Pyrazoleamide compounds are potent antimalarials that target Na⁺ homeostasis in intraerythrocytic *Plasmodium falciparum*. *Nat. Commun.* **2014**, 5 (1), 5521.
- (22) Duffey, M.; Blasco, B.; Burrows, J. N.; Wells, T. N. C.; Fidock, D. A.; Leroy, D. Assessing risks of *Plasmodium falciparum* resistance to select next-generation antimalarials. *Trends Parasitol.* **2021**, 37 (8), 709–721.
- (23) Nguyen, W.; Hodder, A. N.; de Lezongard, R. B.; Czabotar, P. E.; Jarman, K. E.; O’Neill, M. T.; Thompson, J. K.; Jousset Sabroux, H.; Cowman, A. F.; Boddey, J. A.; Sleebs, B. E. Enhanced antimalarial activity of plasmepsin V inhibitors by modification of the P(2) position of PEXEL peptidomimetics. *Eur. J. Med. Chem.* **2018**, 154, 182–198.
- (24) Gilson, P. R.; Tan, C.; Jarman, K. E.; Lowes, K. N.; Curtis, J. M.; Nguyen, W.; Di Rago, A. E.; Bullen, H. E.; Prinz, B.; Duffy, S.; Baell, J. B.; Hutton, C. A.; Jousset Sabroux, H.; Crabb, B. S.; Avery, V. M.; Cowman, A. F.; Sleebs, B. E. Optimization of 2-anilino 4-amino substituted quinazolines into potent antimalarial agents with oral *in vivo* activity. *J. Med. Chem.* **2017**, 60 (3), 1171–1188.
- (25) Steverlyncx, J.; Sitdikov, R.; Rueping, M. The deuterated “magic methyl” group: a guide to site-selective trideuteromethyl incorporation and labeling by using CD(3) reagents. *Chemistry* **2021**, 27 (46), 11751–11772.
- (26) van Schalkwyk, D. A.; Blasco, B.; Davina Nuñez, R.; Liew, J. W. K.; Amir, A.; Lau, Y. L.; Leroy, D.; Moon, R. W.; Sutherland, C. J. *Plasmodium knowlesi* exhibits distinct *in vitro* drug susceptibility profiles from those of *Plasmodium falciparum*. *Int. J. Parasitol.: Drugs Drug Resist.* **2019**, 9, 93–99.
- (27) Dennis, A. S. M.; Rosling, J. E. O.; Lehane, A. M.; Kirk, K. Diverse antimalarials from whole-cell phenotypic screens disrupt malaria parasite ion and volume homeostasis. *Sci. Rep.* **2018**, 8 (1), 8795.
- (28) Gilson, P. R.; Kumarasingha, R.; Thompson, J.; Zhang, X.; Penington, J. S.; Kalhor, R.; Bullen, H. E.; Lehane, A. M.; Dans, M. G.; de Koning-Ward, T. F.; Holien, J. K.; Soares da Costa, T. P.; Hulett, M. D.; Buskes, M. J.; Crabb, B. S.; Kirk, K.; Papenfuss, A. T.; Cowman, A. F.; Abbott, B. M. A 4-cyano-3-methylisoquinoline inhibitor of *Plasmodium falciparum* growth targets the sodium efflux pump PfATP4. *Sci. Rep.* **2019**, 9 (1), 10292.
- (29) Qiu, D.; Pei, J. V.; Rosling, J. E. O.; Thathy, V.; Li, D.; Xue, Y.; Tanner, J. D.; Penington, J. S.; Aw, Y. T. V.; Aw, J. Y. H.; Xu, G.; Tripathi, A. K.; Gnädig, N. F.; Yeo, T.; Fairhurst, K. J.; Stokes, B. H.; Murithi, J. M.; Kimpornsin, K.; Hasemer, H.; Dennis, A. S. M.; Ridgway, M. C.; Schmitt, E. K.; Straimer, J.; Papenfuss, A. T.; Lee, M. C. S.; Corry, B.; Sinnis, P.; Fidock, D. A.; van Dooren, G. G.; Kirk, K.; Lehane, A. M. A G358S mutation in the *Plasmodium falciparum* Na⁺ pump PfATP4 confers clinically-relevant resistance to cipargamin. *Nat. Commun.* **2022**, 13 (1), 5746.
- (30) Rosling, J. E. O.; Ridgway, M. C.; Summers, R. L.; Kirk, K.; Lehane, A. M. Biochemical characterization and chemical inhibition of PfATP4-associated Na⁺-ATPase activity in *Plasmodium falciparum* membranes. *J. Biol. Chem.* **2018**, 293 (34), 13327–13337.
- (31) Cui, L.; Wang, Z.; Miao, J.; Miao, M.; Chandra, R.; Jiang, H.; Su, X. Z.; Cui, L. Mechanisms of *in vitro* resistance to dihydroartemisinin in *Plasmodium falciparum*. *Mol. Microbiol.* **2012**, 86 (1), 111–128.
- (32) Kimpornsin, K.; Kochakarn, T.; Yeo, T.; Okombo, J.; Luth, M. R.; Hoshizaki, J.; Rawat, M.; Pearson, R. D.; Schindler, K. A.; Mok, S.; Park, H.; Uhlemann, A. C.; Jana, G. P.; Maity, B. C.; Laleu, B.; Chenu, E.; Duffy, J.; Moliner Cubel, S.; Franco, V.; Gomez-Lorenzo, M. G.; Gamo, F. J.; Winzeler, E. A.; Fidock, D. A.; Chookajorn, T.; Lee, M. C. S. Generation of a mutator parasite to drive resistance discovery in *Plasmodium falciparum*. *Nat. Commun.* **2023**, 14 (1), 3059.
- (33) Cobbold, S. A.; Chua, H. H.; Nijagal, B.; Creek, D. J.; Ralph, S. A.; McConville, M. J. Metabolic dysregulation induced in *Plasmodium falciparum* by dihydroartemisinin and other front-line antimalarial drugs. *J. Infect. Dis.* **2016**, 213 (2), 276–286.
- (34) Creek, D. J.; Chua, H. H.; Cobbold, S. A.; Nijagal, B.; MacRae, J. I.; Dickerman, B. K.; Gilson, P. R.; Ralph, S. A.; McConville, M. J. Metabolomics-based screening of the Malaria Box reveals both novel

and established mechanisms of action. *Antimicrob. Agents Chemother.* **2016**, *60* (11), 6650–6663.

(35) Allman, E. L.; Painter, H. J.; Samra, J.; Carrasquilla, M.; Llinás, M. Metabolomic profiling of the Malaria Box reveals antimalarial target pathways. *Antimicrob. Agents Chemother.* **2016**, *60* (11), 6635–6649.

(36) Murithi, J. M.; Owen, E. S.; Istvan, E. S.; Lee, M. C. S.; Otilie, S.; Chibale, K.; Goldberg, D. E.; Winzeler, E. A.; Llinás, M.; Fidock, D. A.; Vanaerschot, M. Combining stage specificity and metabolomic profiling to advance antimalarial drug discovery. *Cell Chem. Biol.* **2020**, *27* (2), 158–171.e3.

(37) Simwela, N. V.; Guiguemde, W. A.; Straimer, J.; Regnault, C.; Stokes, B. H.; Tavernelli, L. E.; Yokokawa, F.; Taft, B.; Diagana, T. T.; Barrett, M. P.; Waters, A. P. A conserved metabolic signature associated with response to fast-acting anti-malarial agents. *Microbiol. Spectr.* **2023**, *11* (6), 039766-22.

(38) van Pelt-Koops, J. C.; Pett, H. E.; Graumans, W.; van der Vegte-Bolmer, M.; van Gemert, G. J.; Rottmann, M.; Yeung, B. K. S.; Diagana, T. T.; Sauerwein, R. W. The spiroindolone drug candidate NITD609 potentially inhibits gametocytogenesis and blocks *Plasmodium falciparum* transmission to Anopheles mosquito vector. *Antimicrob. Agents Chemother.* **2012**, *56* (7), 3544–3548.

(39) White, N. J.; Pukrittayakamee, S.; Phyto, A. P.; Rueangweerayut, R.; Nosten, F.; Jittamala, P.; Jeeyapant, A.; Jain, J. P.; Lefevre, G.; Li, R.; Magnusson, B.; Diagana, T. T.; Leong, F. J. Spiroindolone KAE609 for falciparum and vivax malaria. *N. Engl. J. Med.* **2014**, *371* (5), 403–410.

(40) Delves, M. J.; Miguel-Blanco, C.; Matthews, H.; Molina, I.; Ruecker, A.; Yahya, S.; Straschil, U.; Abraham, M.; León, M. L.; Fischer, O. J.; Rueda-Zubiaurre, A.; Brandt, J. R.; Cortés, Á.; Barnard, A.; Fuchter, M. J.; Calderón, F.; Winzeler, E. A.; Sinden, R. E.; Herreros, E.; Gamo, F. J.; Baum, J. A high throughput screen for next-generation leads targeting malaria parasite transmission. *Nat. Commun.* **2018**, *9* (1), 3805.

(41) Delves, M. J.; Straschil, U.; Ruecker, A.; Miguel-Blanco, C.; Marques, S.; Dufour, A. C.; Baum, J.; Sinden, R. E. Routine in vitro culture of *P. falciparum* gametocytes to evaluate novel transmission-blocking interventions. *Nat. Protoc.* **2016**, *11* (9), 1668–1680.

(42) Mohring, F.; van Schalkwyk, D. A.; Henrici, R. C.; Blasco, B.; Leroy, D.; Sutherland, C. J.; Moon, R. W. Cation ATPase (ATP4) orthologue replacement in the malaria parasite *Plasmodium knowlesi* reveals species-specific responses to ATP4-targeting drugs. *mBio* **2022**, *13*, No. e0117822.

(43) Gilson, P. R.; Nguyen, W.; Poole, W. A.; Teixeira, J. E.; Thompson, J. K.; Guo, K.; Stewart, R. J.; Ashton, T. D.; White, K. L.; Sanz, L. M.; Gamo, F. J.; Charman, S. A.; Wittlin, S.; Duffy, J.; Tonkin, C. J.; Tham, W. H.; Crabb, B. S.; Cooke, B. M.; Huston, C. D.; Cowman, A. F.; Sleebs, B. E. Evaluation of 4-amino 2-anilinoquinazolines against *Plasmodium* and other apicomplexan parasites in vitro and in a *P. falciparum* humanized NOD-scid IL2Rγ(null) mouse model of malaria. *Antimicrob. Agents Chemother.* **2019**, *63* (3), No. e01804-18.

(44) Jiménez-Díaz, M. B.; Mulet, T.; Viera, S.; Gómez, V.; Garuti, H.; Ibáñez, J.; Alvarez-Doval, A.; Shultz, L. D.; Martínez, A.; Gargallo-Viola, D.; Angulo-Barturen, I. Improved murine model of malaria using *Plasmodium falciparum* Competent strains and non-myelodepleted NOD-scid IL2Rγnull mice engrafted with human erythrocytes. *Antimicrob. Agents Chemother.* **2009**, *53* (10), 4533–4536.

(45) Jiménez-Díaz, M. B.; Mulet, T.; Gómez, V.; Viera, S.; Alvarez, A.; Garuti, H.; Vázquez, Y.; Fernández, A.; Ibáñez, J.; Jiménez, M.; Gargallo-Viola, D.; Angulo-Barturen, I. Quantitative measurement of *Plasmodium*-infected erythrocytes in murine models of malaria by flow cytometry using bidimensional assessment of SYTO-16 fluorescence. *Cytometry, Part A* **2009**, *75* (3), 225–235.

(46) Creek, D. J.; Jankevics, A.; Burgess, K. E. V.; Breitling, R.; Barrett, M. P. IDEOM: an Excel interface for analysis of LC-MS-based metabolomics data. *Bioinformatics* **2012**, *28* (7), 1048–1049.

(47) Creek, D. J.; Jankevics, A.; Breitling, R.; Watson, D. G.; Barrett, M. P.; Burgess, K. E. V. Toward global metabolomics analysis with

hydrophilic interaction liquid chromatography-mass spectrometry: improved metabolite identification by retention time prediction. *Anal. Chem.* **2011**, *83* (22), 8703–8710.

(48) Pang, Z.; Zhou, G.; Ewald, J.; Chang, L.; Hacariz, O.; Basu, N.; Xia, J. Using MetaboAnalyst 5.0 for LC-HRMS spectra processing, multi-omics integration and covariate adjustment of global metabolomics data. *Nat. Protoc.* **2022**, *17* (8), 1735–1761.

(49) Edgar, R. C. S.; Siddiqui, G.; Hjerrild, K.; Malcolm, T. R.; Vinh, N. B.; Webb, C. T.; Holmes, C.; MacRaild, C. A.; Chernih, H. C.; Suen, W. W.; Counihan, N. A.; Creek, D. J.; Scammells, P. J.; McGowan, S.; de Koning-Ward, T. F. Genetic and chemical validation of *Plasmodium falciparum* aminopeptidase PfA-M17 as a drug target in the hemoglobin digestion pathway. *eLife* **2022**, *11*, No. e80813.

(50) Kristan, M.; Thorburn, S. G.; Hafalla, J. C.; Sutherland, C. J.; Oguike, M. C. Mosquito and human hepatocyte infections with *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*. *Trans. R. Soc. Trop. Med. Hyg.* **2019**, *113* (10), 617–622.

(51) Witmer, K.; Sherrard-Smith, E.; Straschil, U.; Tunnicliffe, M.; Baum, J.; Delves, M. An inexpensive open source 3D-printed membrane feeder for human malaria transmission studies. *Malar. J.* **2018**, *17* (1), 282.

(52) Favuzza, P.; de Lera Ruiz, M.; Thompson, J. K.; Triglia, T.; Ngo, A.; Steel, R. W. J.; Vavrek, M.; Christensen, J.; Healer, J.; Boyce, C.; Guo, Z.; Hu, M.; Khan, T.; Murgolo, N.; Zhao, L.; Penington, J. S.; Reaksudsan, K.; Jarman, K.; Dietrich, M. H.; Richardson, L.; Guo, K. Y.; Lopaticki, S.; Tham, W. H.; Rottmann, M.; Papenfuss, T.; Robbins, J. A.; Boddey, J. A.; Sleebs, B. E.; Sabroux, H. J.; McCauley, J. A.; Olsen, D. B.; Cowman, A. F. Dual plasmepsin-targeting antimalarial agents disrupt multiple stages of the malaria parasite life cycle. *Cell Host Microbe* **2020**, *27* (4), 642–658.e12.

(53) Franke-Fayard, B.; Trueman, H.; Ramesar, J.; Mendoza, J.; van der Keur, M.; van der Linden, R.; Sinden, R. E.; Waters, A. P.; Janse, C. J. A *Plasmodium berghei* reference line that constitutively expresses GFP at a high level throughout the complete life cycle. *Mol. Biochem. Parasitol.* **2004**, *137* (1), 23–33.

(54) Angulo-Barturen, I.; Jiménez-Díaz, M. B.; Mulet, T.; Rullas, J.; Herreros, E.; Ferrer, S.; Jiménez, E.; Mendoza, A.; Regadera, J.; Rosenthal, P. J.; Bathurst, I.; Pompliano, D. L.; Gómez de las Heras, F.; Gargallo-Viola, D. A murine model of falciparum-malaria by in vivo selection of competent strains in non-myelodepleted mice engrafted with human erythrocytes. *PLoS One* **2008**, *3* (5), No. e2252.