Published in final edited form as:

ACS Comb Sci. 2017 December 11; 19(12): 748–754. doi:10.1021/acscombsci.7b00119.

Efficient synthesis of 1,9-substituted benzo[*h*] [1,6]naphthyridin-2(1*H*)-ones and evaluation of their *Plasmodium* *falciparum* gametocytocidal activities

Hao Li^a, Wei Sun^a, Xiuli Huang^a, Xiao Lu^a, Paresma R. Patel^{a,#}, Myunghoon Kim^a, Meghan J. Orr^a, Richard M. Fisher^a, Takeshi Q Tanaka^{b,⊥}, John C. McKew^{a,+}, Anton Simeonov^a, Philip E. Sanderson^a, Wei Zheng^a, Kim C. Williamson^{c,d}, and Wenwei Huang^{a,*}

^aNational Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD, 20892, United States

^bLaboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, United States

^cDepartment of Biology, Loyola University Chicago, Chicago, IL 60660, United States

^dMicrobiology and Immunology Department, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814

Abstract

A novel three-component, two-step, one-pot nucleophilic aromatic substitution (S_NAr)–intramolecular cyclization–Suzuki coupling reaction was developed for the synthesis of benzo[*h*][1,6]naphthyridin-2(1*H*)-ones (Torins). Based on the new efficiently convergent synthetic route, a library of Torin analogs was synthesized. The antimalarial activities of these compounds were evaluated against asexual parasites using a growth inhibition assay and gametocytes using a viability assay.

Keywords

quinoline; benzo[*h*][1; 6]naphthyridin-2(1*H*)-ones; Torin; one-pot reaction; malaria; gametocytocidal activity

*Corresponding Author: Telephone: 301-217-5740; huangwe@mail.nih.gov.

#US Food & Drug Administration, 10903 New Hampshire Ave, Silver Spring, MD 20993

⊥Faculty of Medicine/Graduate School of Medicine, Kagawa University, 761-0793 Japan

+Lumos Pharma 4200 Marathon Drive, Suite 200, Austin, TX 78756

ORCID

Wenwei Huang: 0000-0002-7727-9287

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Supporting Information. Experimental procedures, ¹H NMR, HRMS, and LC-MS spectra for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

INTRODUCTION

Quinoline-based heterocycles have attracted much attention from both synthetic chemists and medicinal chemists due to their interesting anticancer,¹ antimalarial,² antibacterial,³ antimycobacterial,⁴ anti-inflammatory,⁵ antihypertensive,⁶ antiviral,⁷ and anti-oxidant activities.⁸ Several marketed drugs such as quinine and cabozantinib contain the quinoline scaffold.^{2a, 2b, 9, 10} Structurally complex tricyclic benzo[*h*][1,6]naphthyridin-2(1*H*)-ones (Torins) constitute a new family of quinolines which were not characterized until recently.¹¹ Torin compounds have been reported as potent inhibitors of mammalian target of rapamycin (mTOR),¹² bone marrow kinase in the X chromosome (BMX),¹³ and Bruton's tyrosine kinase (BTK)¹⁴ for the treatment of cancers. Torin 2 (**1**) (Figure 1) was identified as a promising antimalarial agent effective against multiple life-cycle stages of the *Plasmodium* parasite.^{11b, 15} In a screen of a collection of 5520 known drugs and biological active compounds, Torin 2 showed an EC₅₀ of 8 nM against stages III-V gametocytes and the gametocytocidal activity was decoupled from its mTOR activity.^{15a} In an *in vivo* mouse malaria transmission model, we found that Torin 2 completely blocked oocyst formation in mosquitoes by a single IV dose of 4 mg/kg. Thus, a medicinal chemistry program was initiated to develop Torin compounds as drug candidates to block malaria transmission.

Two approaches to the synthesis of the tricyclic lactam-fused quinoline core of Torins have been reported in the literature. A Povarov-type multicomponent reaction was developed to afford tetrahydrobenzonaphthyridinones which were converted to the corresponding dihydrobenzonaphthyridinones by DDQ oxidation.¹⁶ Gray and coworkers reported a five-step sequence for the synthesis of Torin compounds starting from either 4,6-dichloroquinoline^{11a, 12b} or 4-chloro-6-bromoquinoline.^{12a} While these methods provide elegant approaches to Torin derivatives, a synthetic route which diverges at the last two steps would enable the facile assembly of a structurally diverse compound library for structure-activity relationship (SAR) studies. In this paper, we report an efficient synthesis of Torin analogs using a novel one-pot nucleophilic aromatic substitution (S_NAr)–intramolecular cyclization-Suzuki coupling reaction (Scheme 1b) and the antimalarial activities of a small library of Torin compounds.

RESULTS AND DISCUSSION

Chemistry

The five-step synthesis of Torin compounds reported by Gray and coworkers is shown in Scheme 1a.^{12a} Reaction of 4-chloro-6-bromoquinoline **2** with anilines gave **3**, which were reduced to alcohols **4** by NaBH₄. Oxidation with MnO₂ followed by Horner-Wadsworth-Emmons olefination and *in situ* ring closure afforded intermediates **6**. Suzuki reaction of **6** with boronic acids produced the desired compounds **7**. Since it is laborious to introduce diversity at the R² position using this five-step reaction sequence, we proposed an alternative route as described in Scheme 1b. Using *cis*- α,β -unsaturated ester **10** as the point of divergence, we envisaged Torin analogs **7** could be synthesized by a facile procedure consisting of a nucleophilic aromatic substitution (S_NAr)–intramolecular cyclization-Suzuki coupling sequence. This new procedure would enable the facile assembly of a library of Torin 2 analogs with diverse substituents at regions A and B (Figure 1). Compound **10** was

prepared from commercially available 1-(2-amino-5-bromophenyl)ethan-1-one **8** in two steps with 46% overall yield through a Vilsmeier-Haack reaction¹⁷ followed by a Still-Gennari olefination to selectively form *cis*- α,β -unsaturated esters.¹⁸

The reaction conditions of the one-pot synthesis were initially explored using 3-(trifluoromethyl)aniline (**11**) and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-amine (**12**) as the model substrates. The results were summarized in Table 1. (*Z*)-Ethyl 3-(6-bromo-4-chloroquinolin-3-yl)acrylate (**10**) was first treated with aniline **11** at 180 °C for 15 min under microwave irradiation using 20 mol% HCl as a catalyst followed by a Pd(PPh₃)₄-catalyzed Suzuki coupling with boronic ester **12** at 150 °C for 10 min. Different solvents were screened (entries 1–5), and the combination of DMF, *i*-PrOH, and H₂O (2: 1: 1) provided the highest yield of **1** (89%). In the nucleophilic aromatic substitution (S_NAr)-intramolecular cyclization cascade, a catalytic amount of HCl was required for an efficient reaction in the first S_NAr step (entry 5 vs entry 6). When K₃PO₄ instead of NaHCO₃ was used as the base for the Suzuki coupling, the product yields were further improved (entry 6 vs entry 7) and the use of K₃PO₄ can prevent overpressure due to the formation of CO₂ under microwave heating. Finally, two equivalents of K₃PO₄ were sufficient for the coupling reaction (entry 8) while excess K₃PO₄ led to a lower product yield (entry 9). Therefore, the one-pot reaction used 0.2 equivalents of HCl as the acid catalyst and DMF as the solvent for the nucleophilic aromatic substitution (S_NAr)-intramolecular cyclization step, and the Suzuki coupling was carried out with 2.0 equivalents of K₃PO₄ as the base and DMF, *i*-PrOH, and H₂O (2: 1: 1) as the solvent of choice (entry 8).

With the optimized conditions in hand, the substrate scope of the one-pot synthesis was then investigated. A wide variety of Torin compounds were successfully prepared as shown in Table 2. This new method has a broad substrate scope and excellent functional group compatibility. Functional groups including amine, amide, pyridine, phenol, aryl halides, ketone, and free carboxylic acid were well-tolerated. Both electron donating and electron withdrawing substituents on the aromatic rings in region A and B worked well in the reaction.

SAR studies

Preliminary SAR studies on both inhibition of asexual parasite growth and gametocytocidal activity were carried out and results are summarized in Table 2. The gametocytocidal activity of Torin analogs are generally 2- to 5-fold weaker than their corresponding activities in inhibiting growth of asexual parasites. Since a key objective of this program was to develop malaria transmission blocking agents, our SAR discussion will focus on the gametocytocidal activity of these Torin 2 analogs. We first explored the SAR in region A of Torin 2 by holding the 3-trifluoromethylphenyl group constant at the R² position. A series of pyridyl derivatives (**13**–**25**) with different substituents at the R¹ position were synthesized. We found that the 6-amino-3-pyridyl at the R¹ position is optimal for the gametocytocidal activity. Capping the amino group with an acetyl (**13**) resulted in 2.9-fold loss of potency, while replacement of the amino group with piperidine (**14**) led to 159-fold decrease in potency. Shifting the 6-amino-3-pyridyl (**1**) to 5-amino-3-pyridyl (**15**) led to a 10-fold reduction in potency, and removal of the 2-amino substitution (**16**) resulted in a 6-fold loss

of activity. Exchanging the pyridyl moiety of the 6-amino-3-pyridyl with a phenyl (**17**) caused a 2-fold reduction in potency. Unsubstituted 3-pyridyl and 4-pyridyl had similar activity (**16** vs **18**). 5-(2-pyridonyl) (**19**) and 6-trifluoromethyl-3-pyridyl (**20**) resulted in 7- to 21-fold loss of activity, respectively. Consistent with our previous observation^{15d}, the 6-methyl-3-pyridyl (**21**) showed good potency with an EC₅₀ of 73 nM. Shifting the 6-methyl-3-pyridyl to 5-methyl-3-pyridyl (**22**) led to a 45-fold reduction in potency. Both 4-trifluoromethyl (**24**) and 4-methyl (**25**) derivatives had weak or no activity in the gametocyte assay, suggesting that substituents are not tolerated at the 4 position. Additionally, substitution of 6-amino-3-pyridyl with 2-amino-5-pyrimidinyl (**26**) gave similar potency, while *N,N*-dimethyl-2-amino-5-pyrimidinyl (**27**), and simple 5-pyrimidinyl (**29**) resulted in loss of activity. Five-membered heteroaryls (**30–32**), bicyclic heteroaryls (**33–36**), substituted phenyls (**37–44**), and 1,2,3,6-tetrahydropyridinyl (**45**) at the R¹ position all led to less active compounds.

Having established an SAR profile of region A, we next turned our attention to region B and kept the 6-amino-3-pyridyl group constant at the R¹ position. Substituted aryls were investigated for their structure-activity relationship (**46–55**). Removal of the 3-trifluoromethyl led to a 2-fold loss of potency (**1** vs **46**). 3-Methyl (**49**) was slightly more potent than Torin 2 with an EC₅₀ of 20 nM. Sterically bulky 3-isopropyl substitution (**51**) resulted in a 7-fold loss of activity suggesting large substituents are not tolerated at the 3 position of the phenyl group in region B. 2-Methyl (**47**) was about 7-fold less potent than 3-methyl analog (**49**). Both 2- and 3-methoxy substituted analogs (**48** and **50**) are less active than Torin 2. Interestingly, 3-carboxyl acid (**52**) and 3-tetrazole (**53**) had weak or no gametocytocidal activity. Finally, hydroxyl substitution at both 3 and 4 positions (**54** and **55**) could be tolerated, but less active than Torin 2.

CONCLUSION

A novel one-pot nucleophilic aromatic substitution (S_NAr)–intramolecular cyclization–Suzuki coupling was developed for an efficient synthesis of Torin derivatives. Using this new method, diverse substitution patterns can be introduced to both region A and region B in a one-pot procedure based on a common intermediate **10**, which greatly facilitates compound library synthesis. The newly developed synthetic route toward Torin derivatives offers several advantages over current methods such as shorter reaction time, more general substrate scope, broader functional group compatibility, and higher efficiency. This new method provides an important synthetic tool for optimizing Torin compounds for the treatment of malaria and its transmission. A preliminary SAR study showed that Torin analogs have promising gametocytocidal activities which could lead to the discovery of novel compounds as malaria transmission blocking agents.

EXPERIMENTAL PROCEDURES

General experimental details

All commercially available reagents, compounds, and solvents were purchased and used without further purification. Column chromatography on silica gel was performed on RediSep column using the Teledyne Isco CombiFlash Rf system. Preparative purification

was performed on a Waters semi-preparative HPLC. The column used was a Phenomenex Luna C18 (5 micron, 30 × 75 mm) at a flow rate of 45 mL/min. The mobile phase consisted of acetonitrile and water (each containing 0.1% trifluoroacetic acid). A gradient of 10% to 50% acetonitrile over 8 minutes was used during the purification. Fraction collection was triggered by UV detection (220 nm).

¹H spectra were recorded using an INOVA 400 MHz spectrometer (Varian). Samples were analyzed on an Agilent 1200 series LC/MS using a Zorbax Eclipse XDB-C18 reverse phase (5 micron, 4.6 × 150 mm) column and a flow rate of 1.1 mL/min. The mobile phase was a mixture of acetonitrile and H₂O each containing 0.05% trifluoroacetic acid. LC Method A, a gradient of 4% to 100% acetonitrile over 7 minutes was used during analytical analysis. LC Method B, a gradient of 4% to 100% acetonitrile over 3 minutes was used during analytical analysis. High resolution mass spectrometry was recorded on Agilent 6210 Time-of-Flight LC/MS system.

General procedure for synthesis of Torin derivatives—To a 2 mL microwave tube (sharp bottom) charged with ethyl (Z)-3-(6-bromo-4-chloroquinolin-3-yl)acrylate **10** (68 mg, 0.2 mmol) in DMF (0.5 mL) was added a substituted aniline (0.3 mmol, 1.5 eq) and HCl (4M in dioxane, 0.01 mL, 0.2 eq). The reaction was heated under microwave irradiation at 100 °C for 15 min. After cooling down, DMF (0.5 mL) and *i*-PrOH (0.5 mL) were added followed by aq. K₃PO₄ solution (0.8 M, 0.5 mL, 2.0 eq). The mixture was stirred for 5 min. Nitrogen gas was bubbled through the reaction solution for 1–2 min, and then Pd(PPh₃)₄ (11.5 mg, 0.01 mmol, 0.05 eq) and a boronic acid (0.4 mmol, 2.0 eq) were added. The reaction was heated under microwave irradiation at 150 °C for 10 min. The mixture was filtered through Celite and the filtrate was purified by reverse phase HPLC. The pure fractions were dried down by a lyophilizer to afford the desired Torin derivatives **7**.

Biology—Stage III–V gametocytes were enriched using Percoll density gradient centrifugation as described previously¹⁹. Briefly, 2.5 µL/well incomplete medium was dispensed into each well of 1,536-well plates using the Multidrop Combi followed by 23 nL compound transfer using the NX-TR Pintool (WAKO Scientific Solutions, San Diego, CA). Then, 2.5 µL/well of gametocytes was dispensed with a seeding density of 20,000 cells/well using the Multidrop Combi. The assay plates were incubated for 72 h at 37 °C with 5% CO₂. After addition of 5 µL/well of 2X AlamarBlue dye (Life Technologies, cat. no. DAL1100), the plates were incubated for 24 h at 37 °C with 5% CO₂ and then were read in a fluorescence detection mode (Ex = 525 nm, Em = 598 nm) on a ViewLux plate reader (PerkinElmer).

Asexual parasites of *P. falciparum* strain 3D7 were cultured as described previously.²⁰ Drug activity in inhibiting growth of asexual stage parasites was tested using a SYBR Green assay as described previously.²¹ Briefly, parasites were diluted to 0.5% parasitemia in complete culture medium with 2% hematocrit and drugs were diluted in DMSO (0.5%). The pre-diluted parasites were dispensed into a 1536-well plate (2.5 µL/well). After 72 h incubation under the standard culture condition, 5 µL/well of lysis buffer containing SYBR Green I was added to the parasite culture and incubated for 30 min at room temperature. The

fluorescence of each well was measured at 520 nm following excitation at 490 nm using a ViewLux platereader (PerkinElmer).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources

The Intramural Research Programs of the National Center for Advancing Translational Sciences and National Institute of Allergy and Infectious Diseases, and Public Health Service grants AI101396 and AI114761 (KW) from the National Institute of Allergy and Infectious Diseases.

This work was supported by the Intramural Research Programs of the National Center for Advancing Translational Sciences and National Institute of Allergy and Infectious Diseases, and Public Health Service grants AI101396 and AI114761 (KW) from the National Institute of Allergy and Infectious Diseases. We thank Paul Shinn, Danielle VanLeer, Misha Itkin, Zina Itkin, and Crystal McKnight from the National Center for Advancing Translational Sciences for preparing compound plates. We also thank the analytical chemistry group from the National Center for Advancing Translational Sciences for analytical supports. TQT is a JSPS Research Fellow in Biomedical and Behavioral Research at the NIH.

References

1. (a) Solomon VR, Lee H. Quinoline as a privileged scaffold in cancer drug discovery. *Curr Med Chem.* 2011; 18:1488–1508. [PubMed: 21428893] (b) Kumar S, Bawa S, Gupta H. Biological activities of quinoline derivatives. *Mini Rev Med Chem.* 2009; 9:1648–1654. [PubMed: 20088783] (c) Kumar EV, Etukala JR, Ablordeppey SY. Indolo[3,2-b]quinolines: synthesis, biological evaluation and structure activity-relationships. *Mini Rev Med Chem.* 2008; 8:538–554. [PubMed: 18537709]
2. (a) Anthony MP, Burrows JN, Duparc S, Moehrle JJ, Wells TN. The global pipeline of new medicines for the control and elimination of malaria. *Malar J.* 2012; 11:316. [PubMed: 22958514] (b) Barnett DS, Guy RK. Antimalarials in Development in. *Chem Rev* 2014. 2014;11221–11241.(c) Biamonte MA, Wanner J, Le Roch KG. Recent advances in malaria drug discovery. *Bioorg Med Chem Lett.* 2013; 23:2829–2843. [PubMed: 23587422] (d) Schrader FC, Barho M, Steiner I, Ortmann R, Schlitzer M. The antimalarial pipeline--an update. *Int J Med Microbiol.* 2012; 302:165–171. [PubMed: 22898490] (e) Leven M, Held J, Duffy S, Tschan S, Sax S, Kamber J, Frank W, Kuna K, Geffken D, Siethoff C, Barth S, Avery VM, Wittlin S, Mordmuller B, Kurz T. Blood schizontocidal and gametocytocidal activity of 3-hydroxy-N'-arylidene propanehydrazonamides: a new class of antiplasmodial compounds. *J Med Chem.* 2014; 57:7971–7976. [PubMed: 25195945] (f) Bray PG, Ward SA, O'Neill PM. Quinolines and artemisinin: chemistry, biology and history. *Curr Top Microbiol Immunol.* 2005; 295:3–38. [PubMed: 16265885]
3. (a) Eswaran S, Adhikari AV, Shetty NS. Synthesis and antimicrobial activities of novel quinoline derivatives carrying 1,2,4-triazole moiety. *Eur J Med Chem.* 2009; 44:4637–4647. [PubMed: 19647905] (b) Mahamoud A, Chevalier J, Davin-Regli A, Barbe J, Pages JM. Quinoline derivatives as promising inhibitors of antibiotic efflux pump in multidrug resistant *Enterobacter aerogenes* isolates. *Curr Drug Targets.* 2006; 7:843–847. [PubMed: 16842215]
4. Keri RS, Patil SA. Quinoline: a promising antitubercular target. *Biomed Pharmacother.* 2014; 68:1161–1175. [PubMed: 25458785]
5. (a) Ko TC, Hour MJ, Lien JC, Teng CM, Lee KH, Kuo SC, Huang LJ. Synthesis of 4-alkoxy-2-phenylquinoline derivatives as potent antiplatelet agents. *Bioorg Med Chem Lett.* 2001; 11:279–282. [PubMed: 11212091] (b) Bekhit AA, El-Sayed OA, Aboulmagd E, Park JY. Tetrazolo[1,5-a]quinoline as a potential promising new scaffold for the synthesis of novel anti-inflammatory and antibacterial agents. *Eur J Med Chem.* 2004; 39:249–255. [PubMed: 15051173] (c) Lavrado J,

- Moreira R, Paulo A. Indoloquinolines as scaffolds for drug discovery. *Curr Med Chem*. 2010; 17:2348–2370. [PubMed: 20491639]
6. (a) Zhang CB, Cui X, Hong L, Quan ZS, Piao HR. Synthesis and positive inotropic activity of N-(4,5-dihydro-[1,2,4]triazolo[4,3-a]quinolin-7-yl)-2-(piperazin-1-yl)acetamide derivatives. *Bioorg Med Chem Lett*. 2008; 18:4606–4609. [PubMed: 18657421] (b) Ferlin MG, Chiarello G, Antonucci F, Caparrotta L, Frolidi G. Mannich bases of 3H-pyrrolo[3,2-f]quinoline having vasorelaxing activity. *Eur J Med Chem*. 2002; 37:427–434. [PubMed: 12008057]
7. Streckowski L, Mokrosz JL, Honkan VA, Czarny A, Cegla MT, Wydra RL, Patterson SE, Schinazi RF. Synthesis and quantitative structure-activity relationship analysis of 2-(aryl or heteroaryl)quinolin-4-amines, a new class of anti-HIV-1 agents. *J Med Chem*. 1991; 34:1739–1746. [PubMed: 2033597]
8. Orhan Puskullu M, Tekiner B, Suzen S. Recent studies of antioxidant quinoline derivatives. *Mini Rev Med Chem*. 2013; 13:365–372. [PubMed: 23190035]
9. Kurzrock R, Sherman SI, Ball DW, Forastiere AA, Cohen RB, Mehra R, Pfister DG, Cohen EEW, Janisch L, Nauling F, Hong DS, Ng CS, Ye L, Gagel RF, Frye J, Muller T, Ratain MJ, Salgia R. Activity of XL184 (Cabozantinib), an Oral Tyrosine Kinase Inhibitor, in Patients With Medullary Thyroid Cancer. *J Clin Oncol*. 2011; 29:2660–2666. [PubMed: 21606412]
10. (a) Leroy D, Campo B, Ding XC, Burrows JN, Cherbuin S. Defining the biology component of the drug discovery strategy for malaria eradication. *Trends Parasitol*. 2014; 30:478–490. [PubMed: 25131411] (b) Baumann M, Baxendale IR. An overview of the synthetic routes to the best selling drugs containing 6-membered heterocycles. *Beilstein J Org Chem*. 2013; 9:2265–2319. [PubMed: 24204439] (c) Dechy-Cabaret O, Benoit-Vical F. Effects of antimalarial molecules on the gametocyte stage of *Plasmodium falciparum*: the debate. *J Med Chem*. 2012; 55:10328–10344. [PubMed: 23075290] (d) Burrows JN, van Huijsduijnen RH, Mohrle JJ, Oeuvray C, Wells TN. Designing the next generation of medicines for malaria control and eradication. *Malar J*. 2013; 12:187. [PubMed: 23742293]
11. (a) Liu Q, Chang JW, Wang J, Kang SA, Thoreen CC, Markhard A, Hur W, Zhang J, Sim T, Sabatini DM, Gray NS. Discovery of 1-(4-(4-propionylpiperazin-1-yl)-3-(trifluoromethyl)phenyl)-9-(quinolin-3-yl)benzo[h][1,6]naphthyridin-2(1H)-one as a highly potent, selective mammalian target of rapamycin (mTOR) inhibitor for the treatment of cancer. *J Med Chem*. 2010; 53:7146–7155. [PubMed: 20860370] (b) Hanson KK, Ressurreicao AS, Buchholz K, Prudencio M, Herman-Ornelas JD, Rebelo M, Beatty WL, Wirth DF, Hanscheid T, Moreira R, Marti M, Mota MM. Torins are potent antimalarials that block replenishment of *Plasmodium* liver stage parasitophorous vacuole membrane proteins. *Proc Natl Acad Sci U S A*. 2013; 110:E2838–2847. [PubMed: 23836641]
12. (a) Liu Q, Wang J, Kang SA, Thoreen CC, Hur W, Ahmed T, Sabatini DM, Gray NS. Discovery of 9-(6-aminopyridin-3-yl)-1-(3-(trifluoromethyl)phenyl)benzo[h][1,6]naphthyridin-2(1H)-one (Torin2) as a potent, selective, and orally available mammalian target of rapamycin (mTOR) inhibitor for treatment of cancer. *J Med Chem*. 2011; 54:1473–1480. [PubMed: 21322566] (b) Liu Q, Wang J, Kang SA, Thoreen CC, Hur W, Choi HG, Waller DL, Sim T, Sabatini DM, Gray NS. Discovery and optimization of potent and selective benzonaphthyridinone analogs as small molecule mTOR inhibitors with improved mouse microsome stability. *Bioorg Med Chem Lett*. 2011; 21:4036–4040. [PubMed: 21621413]
13. Liu F, Zhang X, Weisberg E, Chen S, Hur W, Wu H, Zhao Z, Wang W, Mao M, Cai C, Simon NI, Sanda T, Wang J, Look AT, Griffin JD, Balk SP, Liu Q, Gray NS. Discovery of a selective irreversible BMX inhibitor for prostate cancer. *ACS Chem Biol*. 2013; 8:1423–1428. [PubMed: 23594111]
14. Wu H, Wang W, Liu F, Weisberg EL, Tian B, Chen Y, Li B, Wang A, Wang B, Zhao Z, McMillin DW, Hu C, Li H, Wang J, Liang Y, Buhrlage SJ, Liang J, Liu J, Yang G, Brown JR, Treon SP, Mitsiades CS, Griffin JD, Liu Q, Gray NS. Discovery of a potent, covalent BTK inhibitor for B-cell lymphoma. *ACS Chem Biol*. 2014; 9:1086–1091. [PubMed: 24556163]
15. (a) Sun W, Tanaka TQ, Magle CT, Huang W, Southall N, Huang R, Dehdashti SJ, McKew JC, Williamson KC, Zheng W. Chemical signatures and new drug targets for gametocytocidal drug development. *Sci Rep*. 2014; 4:3743. [PubMed: 24434750] (b) Derbyshire ER, Zuzarte-Luis V, Magalhaes AD, Kato N, Sanschagrin PC, Wang J, Zhou W, Miduturu CV, Mazitschek R, Sliz P, Mota MM, Gray NS, Clardy J. Chemical interrogation of the malaria kinome. *Chembiochem*.

- 2014; 15:1920–1930. [PubMed: 25111632] (c) Mott BT, Eastman RT, Guha R, Sherlach KS, Siriwardana A, Shinn P, McKnight C, Michael S, Lacerda-Queiroz N, Patel PR, Khine P, Sun H, Kasbekar M, Aghdam N, Fontaine SD, Liu D, Mierzwa T, Mathews-Griner LA, Ferrer M, Renslo AR, Inglese J, Yuan J, Roepe PD, Su XZ, Thomas CJ. High-throughput matrix screening identifies synergistic and antagonistic antimalarial drug combinations. *Sci Rep*. 2015; 5:13891. [PubMed: 26403635] (d) Patel PR, Sun W, Kim M, Huang X, Sanderson PE, Tanaka TQ, McKew JC, Simeonov A, Williamson KC, Zheng W, Huang W. In vitro evaluation of imidazo[4,5-c]quinolin-2-ones as gametocytocidal antimalarial agents. *Bioorg Med Chem Lett*. 2016; 26:2907–2911. [PubMed: 27156776]
16. (a) Vicente-Garcia E, Catti F, Ramon R, Lavilla R. Unsaturated lactams: new inputs for povarov-type multicomponent reactions. *Org Lett*. 2010; 12:860–863. [PubMed: 20092279] (b) Di Pietro O, Viayna E, Vicente-Garcia E, Bartolini M, Ramon R, Juarez-Jimenez J, Clos MV, Perez B, Andrisano V, Luque FJ, Lavilla R, Munoz-Torrero D. 1,2,3,4-Tetrahydrobenzo[h][1,6]naphthyridines as a new family of potent peripheral-to-midgorge-site inhibitors of acetylcholinesterase: synthesis, pharmacological evaluation and mechanistic studies. *Eur J Med Chem*. 2014; 73:141–152. [PubMed: 24389509]
17. Maluleka MM, Mphahlele MJ. 6,8-Dibromo-4-chloroquinoline-3-carbaldehyde as a synthon in the development of novel 1,6,8-triaryl-1H-pyrazolo[4,3-c]quinolines. *Tetrahedron*. 2013; 69:699–704.
18. Still WC, Gennari C. Direct Synthesis of Z-Unsaturated Esters - a Useful Modification of the Horner-Emmons Olefination. *Tetrahedron Lett*. 1983; 24:4405–4408.
19. Tanaka TQ, Williamson KC. A malaria gametocytocidal assay using oxidoreduction indicator, alamarBlue. *Mol Biochem Parasitol*. 2011; 177:160–163. [PubMed: 21316401]
20. Sun W, Huang X, Li H, Tawa G, Fisher E, Tanaka TQ, Shinn P, Huang W, Williamson KC, Zheng W. Novel lead structures with both *Plasmodium falciparum* gametocytocidal and asexual blood stage activity identified from high throughput compound screening. *Malar J*. 2017; 16:147. [PubMed: 28407766]
21. (a) Eastman RT, Pattaradilokrat S, Raj DK, Dixit S, Deng B, Miura K, Yuan J, Tanaka TQ, Johnson RL, Jiang H, Huang R, Williamson KC, Lambert LE, Long C, Austin CP, Wu Y, Su XZ. A class of tricyclic compounds blocking malaria parasite oocyst development and transmission. *Antimicrob Agents Chemother*. 2013; 57:425–435. [PubMed: 23129054] (b) Smilkstein M, Sriwilaijaroen N, Kelly JX, Wilairat P, Riscoe M. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. *Antimicrob Agents Chemother*. 2004; 48:1803–1806. [PubMed: 15105138]

Torin 2 (**1**)

Figure 1.
Structure of Torin 2 and two regions proposed for SAR studies

a. previous work

Reagents and conditions: (i) R^2NH_2 , 1,4-dioxane, 90 °C, 4-12 h; (ii) $NaBH_4$, EtOH; (iii) MnO_2 , r.t.; (iv) triethyl phosphonoacetate, K_2CO_3 , EtOH, 100 °C, 12 h; (v) $R^1B(OH)_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3 , 1,4-dioxane, 80 °C, 4-12 h.

b. this work

Reagents and conditions: (i) $POCl_3$, DMF, 60 °C, 4 h, 61%; (ii) ethyl bis(2,2,2-trifluoroethoxy)phosphine, K_2CO_3 , 18-Crown-6, THF, 0 °C, 1 h, 76% (iii) R^2NH_2 , cat. HCl, DMF, MW, 100 °C, 15 min; (iv) $R^1B(OH)_2$, $Pd(PPh_3)_4$, K_3PO_4 , DMF/-PrOH/water, MW, 150 °C, 10 min.

Scheme 1.

(a) Literature procedure for the synthesis of Torin 2 analogs; (b) New route for Torin 2 analogs

Table 1Optimization of the reaction conditions for the two-step one-pot reaction^a

entry	solvent (step 1)	MW temp (oC)	HCl (equiv)	solvent (step 2)	base (equiv)	yield ^b (%)
1	<i>i</i> -PrOH	180	0.2	DMF + H ₂ O	NaHCO ₃ (2.0)	31
2	DMF	180	0.2	DMF + H ₂ O	NaHCO ₃ (2.0)	28
3	CH ₃ CN	180	0.2	<i>i</i> -PrOH + H ₂ O	NaHCO ₃ (2.0)	81
4	<i>i</i> -PrOH	180	0.2	<i>i</i> -PrOH + H ₂ O	NaHCO ₃ (2.0)	50
5	DMF	180	0.2	<i>i</i> -PrOH + H ₂ O	NaHCO ₃ (2.0)	89
6	DMF	180	0	<i>i</i> -PrOH + H ₂ O	NaHCO ₃ (2.0)	53
7	DMF	180	0	<i>i</i> -PrOH + H ₂ O	K ₃ PO ₄ (2.0)	78
8	DMF	100	0.2	<i>i</i> -PrOH + H ₂ O	K ₃ PO ₄ (2.0)	90
9	DMF	100	0.2	<i>i</i> -PrOH + H ₂ O	K ₃ PO ₄ (3.0)	83

^aReactions were carried out with **10** (0.2 mmol), **11** (0.3 mmol), and **12** (0.4 mmol) in a mixed solvent using Pd(PPh₃)₄ (0.01 mmol) as the catalyst.^bIsolated yields.

Table 2

Torin derivatives and their biological activities

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
1			12	35
13			55	103
14			5215	5564
15			155	357
16			72	216
17			43	78

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
18			92	246
19			128	246
20			155	754
21			17	73
22			1962	3338
23			3989	7772

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
24			26430	inactive
25			5096	10760
26			16	33
27			1856	3318
28			748	1404
29			359	452

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
30			80	169
31			286	765
32			326	1037
33			1020	3667
34			21	99
35			148	252

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
36			1134	1813
37			229	657
38			2310	5066
39			292	392
40			392	547
41			18700	inactive

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
42			15680	19960
43			1734	3358
44			736	3769
45			15720	18390
46			24	63
47			50	145
48			69	200

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
49			8	20
50			63	126
51			98	247
52			6513	8485
53			26560	inactive
54			39	111

Compound	R ¹	R ²	asexual EC ₅₀ (nM) ^{a, b}	gametocytocidal EC ₅₀ (nM) ^{a, c}
55			24	43

^a Average EC₅₀ from triplicated measurements;

^b Chloroquine (EC₅₀ = 16 nM) was used as a control for the asexual parasites growth inhibition assay;

^c Both methylene blue (EC₅₀ = 256 nM) and primaquine (EC₅₀ = 1288 nM) were used as controls for the gametocyte viability assay.