Novel Aminoquinoline Derivatives Significantly Reduce Parasite Load in *Leishmania infantum* Infected Mice

Jelena Konstantinović,[†] Milica Videnović,[‡] Stefania Orsini,[▽] Katarina Bogojević,[†] Sarah D'Alessandro,[⊥] Diletta Scaccabarozzi,[#] Nataša Terzić Jovanović,[§] Luigi Gradoni,[▽] Nicoletta Basilico,^{*,†} and Bogdan A. Šolaja^{*,†,||}

[†]University of Belgrade, Faculty of Chemistry, Studentski trg 12-16, P.O. Box 51, 11158 Belgrade, Serbia

[‡]University of Belgrade, Faculty of Chemistry Innovative Centre, Studentski trg 12-16, 11158 Belgrade, Serbia

[§]University of Belgrade, Institute of Chemistry, Technology, and Metallurgy, Njegoševa 12, 11000 Belgrade, Serbia

^{||}Serbian Academy of Sciences and Arts, Knez Mihailova 35, 11158 Belgrade, Serbia

[⊥]Dipartimento di Scienze Biomediche, Chirurgiche e Odontoiatriche, Università degli Studi di Milano, Milan, Italy

[#]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy

[▽]Unit of Vector-borne Diseases, Istituto Superiore di Sanità, Rome, Italy

Supporting Information

ABSTRACT: In this Letter, a detailed analysis of 30 4-aminoquinoline-based compounds with regard to their potential as antileishmanial drugs has been carried out. Ten compounds demonstrated $IC_{50} < 1 \mu M$ against promastigote stages of *L. infantum* and *L. tropica*, and five compounds showed $IC_{50} < 1 \mu M$ against intramacrophage *L. infantum* amastigotes. Two compounds showed dose-dependent enhancement of NO and ROS production by bone marrow-derived macrophages and remarkable reduction of parasite load *in vivo*, with advantage of being short-term and orally active. To the best of our knowledge, this is the first example of 4-amino-7-chloroquinoline derivatives active in *Leishmania infantum* infected mice.

KEYWORDS: *Leishmania infantum*, promastigote, amastigote, mice model, aminoquinoline

Leishmaniasis is a neglected parasitic disease transmitted by more than 90 sand fly species. The disease may occur in humans and animals, including dogs and rodents. Human infection is caused by about 21 of the 30 species of *Leishmania* parasites that infect mammals.^{1,2} Currently, 2 million people are infected every year, and more than 350 million people are at risk mostly in tropical and subtropical areas.³ The life cycle of *Leishmania* parasite includes sand fly and vertebrate host stages. After an infected sand fly deposits metacyclic promastigotes into the host's skin during blood feeding, they are phagocytized by macrophages and then transformed into aflagellated amastigotes. In macrophages, amastigotes multiply, and after being released, they infect new macrophages. The sand fly ingests infected macrophages during a blood meal, and the life cycle continues within the sand fly gut.² Treatment of leishmaniasis varies and

adapts to the severity of the disease and species of the parasite.¹ Antileishmanial drugs that are presently in use are pentavalent antimonials, amphotericin B, pentamidine, miltefosine, and others, with AmBisome and sodium stibogluconate–paromomycin combination therapy being the first choice for fighting leishmania infection (Chart 1).^{3,4} All current therapies present serious side effects, including toxicity, and for some of them, resistance development is emerging.⁵ In addition, the vaccine for preventing human leishmaniasis, which could have an immense influence on suppression of the disease, is still not available.⁶ Thus, the development of potent small molecule inhibitors of

Received: February 1, 2018

Accepted: May 4, 2018

Published: May 4, 2018

Chart 1. Examples of Current Antileishmanial Medicines and New Potent Drug Candidate

Leishmania parasites and clinical trials of new drugs are the priorities (DNDI-0690, Chart 1).^{4,7}

Beside their antimalarial activity, derivatives of 4-aminoquinoline also demonstrated antibacterial, antifungal, antitumor, and antileishmanial activity.⁸ 4-Amino-7-chloroquinoline analogues and their Pt(II) complexes were shown to be active against promastigotes of different *Leishmania* species.⁸ Another study investigated the *in vitro* activity of a series of 4-amino-7-chloroquinolines conjugated to sulfonamide, hydrazide, and hydrazine against *L. amazonensis* promastigotes and amastigotes, revealing the ability of these compounds to induce depolarization of the mitochondrial membrane potential in promastigotes and infected macrophages *in vitro*.^{9,10} Steroid linked aminoquinolines also proved to have significant activity against both promastigote and amastigote forms of *L. major* *in vitro*.¹¹

Here, we report on the synthesis and antileishmanial potential of novel 4-aminoquinoline derivatives and some aminoquinoline-based drugs previously published by our group (Chart 2).^{12–16}

Chart 2. Structures of Examined Compounds

The introduction of different substituents at C(3) of quinoline moiety was of interest since it is expected to influence the electronic density to a substantial extent. Sontochin-like compounds, as well as our previously published aminoquinoline derivatives with fluorine atom at the same position proved to be of significant antiplasmodial potential.¹² Here, we explore the effect of nitro and amino substituents on antileishmanial activity

(Scheme 1). Nitro substituent in **31**¹² enabled nucleophilic substitution under mild conditions,¹⁷ resulting in compounds **1**

Scheme 1. Synthesis of Substituted Chloroquine-Like Compounds 1–4^a

^aReagents and conditions: (i) amine, Et₃N, CH₂Cl₂, 0 °C to reflux; (ii) SnCl₂, EtOH.

and **2** in reasonably good yield. Reduction of nitro group to amino using tin(II)-chloride¹² afforded compounds **3** and **4**.

Aminoquinolines with adamantane carrier were synthesized as presented in Scheme 2. Commercially available 1-adamantane-

Scheme 2. Synthesis of Adamantane Derivatives 5 and 6^a

^aReagents and conditions: (i) (1) PCC, DCM, (2) *tert*-butyl (3-aminopropyl)carbamate, MeOH/DCM, AcOHglac, (3) NaBH₄; (ii) TFA, DCM; (iii) **31**, DCM, 0 °C to reflux; (iv) SnCl₂, EtOH.

methanol was transformed into mono-Boc protected amine **32** in moderate yield using the procedure we established earlier.¹² Removal of the protecting group under standard conditions gave amine **33** in good yield. Dichloride **31** was submitted to nucleophilic substitution with amine **33** affording aminoquinoline **5** in good yield. The nitro group in **5** was further reduced to amine **6** using SnCl₂.

Compound **10** with piperazine moiety in linker was synthesized in several reaction steps, starting from commercially available 3,3'-piperazine-1,4-diylidipropan-1-amine **34** (Scheme 3). After protection of amino groups and nucleophilic substitution with 4,7-dichloroquinoline, compound **36** was obtained in moderate yield. Removal of protecting group under standard conditions gave amine **37** in high yield. Reductive amination of the obtained amine with adamantane-1-acetalde-

Scheme 3. Synthesis of Compound 10^a

^aReagents and conditions: (i) Boc₂O, DCM, r.t.; (ii) 4,7-dichloroquinoline, 80 °C, 1 h; 125 °C, 6–8 h; (iii) TFA/DCM (v/v 1:10), r.t., 24 h; 2.5 M NaOH; (iv) aldehyde, NaBH(OAc)₃, DCM, r.t., 24 h; (v) MeOH/HCl.

hyde furnished the compound 38. Compound 10 was obtained as HCl salt of amine 38 (confirmed by elemental analysis).

Benzothiophene derivative 15 was obtained by reductive amination starting from aldehyde 39¹³ in good yield. Compound 21 was obtained in rather low yield by Sonogashira coupling reaction of 40¹³ and *N*-(prop-2-yn-1-yl)-*N'*-(quinolin-4-yl)-butane-1,4-diamine 41 (Scheme 4).

Scheme 4. Synthesis of Novel Benzothiophene Derivatives 15 and 21^a

^aReagents and conditions: (i) (1) AQ3, AcOHglac, MeOH/DCM, (2) NaBH₄; (ii) 41, PdCl₂(PPh₃)₂, PPh₃, CuI, Et₃NH, DMF, MW, 120 °C, 25 min.

Reduction of the quinoline core was designed in order to explore the effect of resulting deviation from planarity on inhibition of *Leishmania* proliferation (Scheme 5). Compound

Scheme 5. Synthesis of Tetrahydroquinoline Core^a

^aReagents and conditions: (i) PtO₂, H₂, AcOH, HClO₄; (ii) 2 M HCl, 70 °C; (iii) (1) AcOHglac, 28% HCl, NaNO₂(aq), 0 °C, (2) CuCl, 28% HCl, 0 °C to r.t.

42, synthesized according to the procedure described in the literature,¹⁸ was transformed into 43 in acceptable yield. Selective reduction of benzene core of quinoline and hydrogenolysis of chlorine were performed by hydrogenation method using platinum(IV) oxide in acetic acid as solvent in the presence of perchloric acid. Deacetylation of compound 43 in 2 M HCl gave compound 44 in high yield. Using Sandmeyer reaction conditions (NaNO₂, AcOH, HCl, CuCl), compound 44 was transformed into 45.

Syntheses of thiophene-based tetrahydroquinoline compounds are presented on Scheme 6. Compound 45 was submitted to Buchwald–Hartwig amination affording the amines 22, 23, and 46 in low to moderate yield. Compound 46 with eight

Scheme 6. Synthesis of Compounds 22–25^a

^aReagents and conditions: (i) amine, Pd(OAc)₂, SPhos, K₃PO₄, dioxane, 85 °C, 24h; (ii) for compound 46, (1) 4-[5-(4-formylphenyl)thiophen-2-yl]benzonitrile, AcOHglac, MeOH/DCM, (2) NaBH₄; (iii) 37% HCHO, ZnCl₂, NaBH₃CN, MeOH.

methylene groups in the linker was subjected to reductive amination with 4-[5-(4-formylphenyl)thiophen-2-yl]-benzonitrile¹³ to obtain 24. Methylation of secondary nitrogen using 37% formaldehyde gave compound 25 in high yield.

The syntheses of other compounds are presented in our previous papers.^{12–16} Full details of synthetic procedures, NMR spectra, and HPLC purities are given in the Supporting Information.

Thirty compounds presented on Chart 2 were first examined for their activity against *L. infantum* and *L. tropica* promastigote stage using standard MTT assay (Table 1, Table S1). Ten

Table 1. *In Vitro* Activities against *L. infantum* and *L. tropica* Promastigotes and Cytotoxicity against THP-1 Human Cells^a

compd	<i>L. infantum</i> IC ₅₀ (μM) ^b	<i>L. tropica</i> IC ₅₀ (μM) ^b	THP-1 IC ₅₀ (μM) ^c	SI (THP/ <i>L.i.</i>) ^d	SI (THP/ <i>L.t.</i>) ^d
1	8.67	2.77	23.66	2.7	8.5
2	6.49	2.96	>109.6	>16.9	>37.0
3	16.60	9.35	>65.2	>3.9	>7.0
4	16.60	6.63	>59.7	>3.6	>9.0
5	1.91	2.24	12.59	6.6	5.6
6d	1.77	1.30	6.39	3.6	4.9
7	0.73	0.66	1.81	2.5	2.7
8	2.46	1.84	3.29	1.3	1.8
9	2.40	2.35	4.90	2.0	2.1
10	0.52	0.51	1.00	1.9	2.0
11	1.14	1.31	2.96	2.6	2.3
12	0.64	0.68	3.76	5.8	5.5
13	1.23	1.24	4.25	3.4	3.4
14	0.51	0.50	1.91	3.8	3.8
15	0.48	0.43	4.73	9.9	10.9
16	1.03	0.81	2.31	2.2	2.8
17	1.02	0.85	4.28	4.2	5.0
18	1.24	1.02	2.35	1.9	2.3
19	0.98	0.91	2.44	2.5	2.7
20	1.55	1.22	2.79	1.8	2.3
21	1.02	1.37	7.11	7.0	5.2
22	>76.5	>76.5	>76.5	>1	>1
23	>69.1	>69.1	>69.1	>1	>1
24	0.72	0.75	2.31	3.2	3.1
25	0.83	0.80	3.68	4.4	4.6
26	2.30	1.94	5.01	2.2	2.6
27	1.22	1.54	2.80	2.3	1.8
28	5.42	7.11	8.10	1.5	1.1
29	0.35	0.30	1.38	4.0	4.6
30	0.80	1.06	3.85	4.8	3.6
control ^c	0.13	0.14	>10.8	>83.1	>77.1

^aAntileishmanial IC₅₀ values against promastigote stages (μM), MTT assay. ^bAll *in vitro* experiments were performed in duplicate, mean values are given. ^cCytotoxicity against differentiated THP-1, human monocytic cell line derived from an acute monocytic leukemia patient. ^dSelectivity index. ^eControl drug: amphotericin B.

compounds showed IC₅₀ values of the same order of magnitude as amphotericin B (IC₅₀ < 1 μM), which was used as a positive control. C(3)-Substituted chloroquine-like compounds (1–4) displayed poor antileishmanial activity against both promastigote species. However, hybrids of such compounds with adamantane carrier resulted in more active derivatives 5 and 6. Among adamantane derivatives without substituent at C(3), compound

7 showed clear improvement of potency. The most potent compound was **10**, with a piperazine moiety in the linker.

Benzothiophene compounds **15** and **17** with a chlorine atom at the C(7) position of the quinoline moiety were more potent than their des-chloro analogues **16** and **18**, suggesting that chlorine atom would be important for the activity. Replacing the phenyl group with a C≡C bond did not produce any significant effect on the activity (**18** vs **21**).

While chloroquine-like compounds, tetrahydroquinolines **22** and **23**, were completely inactive, hybrids with thiophene carrier **24** and **25** showed >100-fold increase in activity. Among other thiophenes, compound **29**, with eight methylene groups between two nitrogens, demonstrated the highest potency against both *Leishmania* promastigote species. All compounds were also checked for cytotoxicity against differentiated THP-1 cells. For both species, moderate selectivity indices were obtained ($SI_{\text{THP-1/L.i.}} = 1.3\text{--}9.9$; $SI_{\text{THP-1/L.i.}} = 1.1\text{--}10.9$, Table 1).

All compounds were tested against intramacrophage amastigotes of *L. infantum* at 0.5 μM , nontoxic concentration on human cells (Table S2). Compounds that showed more than 25% inhibition were tested in dose–response experiments, and IC_{50} were calculated. Five compounds showed IC_{50} less than 1 μM . Among them, compounds **10**, **15**, and **18** were the most active, while compound **15** was the least toxic with good selectivity index (Table 2).

Table 2. *In Vitro* Activities against Intramacrophage *L. infantum* Amastigotes

compd	in vitro antiamastigote activity at 0.5 μM ^a	in vitro antiamastigote activity IC_{50} (μM) ^b	THP-1 IC_{50} (μM) ^c	SI (THP/IPT) ^d
8	29.6	1.91	3.29	1.72
10	72.2	0.31	1.00	3.22
11	26.4	1.85	2.96	1.60
13	38.9	1.29	4.25	3.29
14	26.4	>1	1.91	<1.91
15	47.6	0.58	4.73	8.15
18	42.7	0.65	2.35	3.61
19	36.9	0.73	2.44	3.34
20	42.2	0.79	2.79	3.51
24	29.6	>1	2.31	<2.31
control ^e	95.5	0.21	>10.8	>51.4

^aMean value of two or three experiments. ^bMean value of two experiments. ^cCytotoxicity against differentiated THP-1, human monocytic cell line derived from an acute monocytic leukemia patient. ^dSelectivity index (IC_{50} against THP-1/ IC_{50} against intracellular amastigotes). ^eControl drug: amphotericin B.

Three compounds with good antileishmanial potential (**10**, **15**, and **18**) were subjected to *in vivo* tolerability evaluation in a mice model. All compounds were tested orally at 300 mg/kg (single dose) as a suspension in 0.1% Tween/0.5% HEC in water. Compound **15** was also tested at a lower dose, 50 mg/kg, by subcutaneous route of administration (in sunflower oil). Compound **18** showed toxic effects when given orally with 3/5 mice alive at the end of the experiment, while compounds **10** and **15** given either p.o. or s.c. proved to be tolerable since all mice survived 30 days after administration and showed normal appearance and behavior.

Compounds **10** and **15** were further evaluated for reduction of liver parasite load in a mouse model of visceral leishmaniasis (Balb/c mice infected intravenously with *L. infantum* amasti-

gotes). Results presented in Figure 1 are given as % of reduction relative to control (untreated infected mice, Tables S3 and S4).

Figure 1. Reduction of parasite load in a mouse model by compounds **10** and **15**.

Compound **15** was tested per os at two different doses 50 mg/kg $\times$ 4 days and 100 mg/kg $\times$ 4 days and showed significant reduction of parasites in the liver, 95% and 99%, respectively. Compound **10** was also tested per os at 60 mg/kg $\times$ 4 days and 100 mg/kg $\times$ 4 days. At lower applied dose, it reduced parasite load by 96% compared to control. Although at higher dose complete clearance was achieved, it showed signs of toxicity since one mouse died on day 10 and one mouse died on day 12. Both compounds were also subjected to s.c. administration at lower doses (Figure 1). Compound **10** administered at dose 10 mg/kg $\times$ 4 days reduced parasite load by 81%. At 5 mg/kg $\times$ 5 days and lower, both compounds exhibited poorer activities (57% and 18–48% for **10** and **15**, respectively). However, these results are extremely important, giving us the essential information about dose-dependent behavior of selected 4-aminoquinolines.

In order to investigate the putative mechanism of action (MOA),⁹ we examined the production of nitric oxide and ROS by IFN γ primed murine bone marrow-derived macrophages (BMDM) treated with **10** or **15**. Experiments were performed at several nontoxic BMDM concentrations. Nontoxic concentrations were determined by MTT assay (data not shown). The concentration of nitrite and ROS was determined using Griess reagent and H₂DCFDA, respectively. Results revealed that compounds **10** and **15** increased the production of nitric oxide by IFN γ -primed macrophages only at the highest dose used (Figure S1). Both compounds induced a persistent increase of ROS at all the doses tested (Figure S2).

Currently, several noteworthy *in vivo* studies have been published.^{19–21} Nitroimidazo-oxazole compound DNDI-VL-2098 previously identified as a favorable candidate did not proceed to the clinical study.^{20,22} However, its oxazine derivative DNDI-0690 was very recently recognized as a new promising candidate for a Phase I trial for VL.^{4,7} In the last ten years, only a few 4-amino- and 2-alkenylquinolines with modest activity against *Leishmania* parasites *in vivo* were also identified.^{23–25} 8-Aminoquinoline derivative sitamaquine appeared to be orally active against visceral leishmaniasis and is presently under clinical investigation.²⁶

Here, we identified two 4-amino-7-chloroquinoline compounds bearing benzothiophene or adamantane moieties as potent antileishmanial candidates with significant *in vivo* efficacy. The importance of this work lies in discovery of highly active

short-term compounds, tolerable in mice, being the first example of a 4-amino-7-chloroquinoline active in *L. infantum* mice model of visceral leishmaniasis. Although a certain dose-dependent enhancement of NO and ROS production (which can contribute to the amastigotes killing) was observed in the presence of **10** and **15**, MOA still remains unclear. Further studies will be focused on discovering a specific target in order to elucidate the mechanism of action. Based on compounds' tolerability in mice model and noteworthy *in vivo* activity, future efforts will be focused on improvement of pharmacokinetic profile and enhancement of the antileishmanial activity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.8b00053.

Full details of synthetic procedures, biological assays, procedures for the determination of the HPLC purity, *in vitro* activities on promastigote stages, *in vitro* activities against intramacrophages *L. infantum* amastigotes, *in vivo* antileishmanial activity, nitric oxide and ROS production; NMR spectra and HPLC purity spectra of all tested compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Phone: +381-11-263-86-06. Fax: +381-11-263-60-61. E-mail: bsolaja@chem.bg.ac.rs or bogdan.solaja@sanu.ac.rs.

*Phone: +39-02 5031-5069. Fax: +39-02-5031-5093. E-mail: nicoletta.basilico@unimi.it.

ORCID

Bogdan A. Šolaja: 0000-0002-9975-2725

Author Contributions

B.Š. and N.B. designed the research. The manuscript was written by J.K. with contributions of all authors. All authors have given approval to the final version of the manuscript.

Funding

This work was supported by the Ministry of Science and Technological Development of Serbia (Grant 172008), Serbian Academy of Sciences and Arts, Executive Programme of Scientific and Technological Cooperation between the Italian Republic and the Republic of Serbia for the years 2016–2018, and by “Ministero dell’Istruzione, dell’Università e della Ricerca (PRIN) Project: 20154JRJPP_004”.

Notes

The study followed the International Guiding Principles for biomedical research involving animals (European Directive 2010/63/UE), and it was reviewed by a local Ethics Committees. The study was approved by the Veterinary Directorate at the Ministry of Agriculture and Environmental Protection of Serbia (decision no. 323–07–02444/2014–05/1) and by the Directorate of Animal Health and Veterinary Drugs at the Ministry of Health of Italy (authorization no. 120/2015-PR). The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Olgica Djurković-Djaković and MSc Jelena Srbljanović (Institute for Medical Research, University of Belgrade) for performing *in vivo* toxicity studies and Loredana Cavicchini for assistance in culturing leishmania *in vitro*. We also

thank Prof. Donatella Taramelli (Dipartimento di Scienze Farmacologiche e Biomolecolari, University of Milan) for helpful discussion, and COST Action CM1307 for support.

■ ABBREVIATIONS

BMDM, murine bone marrow-derived macrophages; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Tween 80, Polysorbate 80; HEC, hydroxyethyl cellulose; ROS, reactive oxygen species; MOA, mechanism of action; AQ3, N-(7-chloroquinolin-4-yl)propane-1,3-diamine.

■ REFERENCES

- (1) Leishmaniasis Fact Sheet N°375, February 2015. <http://www.who.int/mediacentre/factsheets/fs375/en/> (accessed October 16, 2017).
- (2) Centers for Disease Control and Prevention, Parasites - Leishmaniasis, January 2013. <http://www.cdc.gov/parasites/leishmaniasis/> (accessed October 16, 2017).
- (3) WHO Technical Report Series no. 949. Report of a Meeting of the WHO Expert Committee on the Control of Leishmaniases, Geneva, 22–26 March 2010. http://www.who.int/neglected_diseases/resources/who_trs_949/en/ (accessed October 16, 2017).
- (4) DNDi Portfolio December 2017. <https://www.dndi.org/diseases-projects/portfolio/> (accessed March 22, 2018).
- (5) Sangshetti, J. N.; Kalam Khan, F. A.; Kulkarni, A. A.; Arote, R.; Patil, R. H. Antileishmanial drug discovery: comprehensive review of the last 10 years. *RSC Adv.* **2015**, *5*, 32376–32415.
- (6) Gillespie, P. M.; Beaumier, C. M.; Strych, U.; Hayward, T.; Hotez, P. J.; Bottazzi, M. E. Status of Vaccine Research and Development of Vaccines for Leishmaniasis. *Vaccine* **2016**, *34*, 2992–2995.
- (7) Thompson, A. M.; O'Connor, P. D.; Marshall, A. J.; Yardley, V.; Maes, L.; Gupta, S.; Launay, D.; Brailard, S.; Chatelain, E.; Franzblau, S. G.; Wan, B.; Wang, Y.; Ma, Z.; Cooper, C. B.; Denny, W. A. 7-Substituted 2-Nitro-5,6-dihydroimidazo[2,1-b][1,3]oxazines: Novel Antitubercular Agents Lead to a New Preclinical Candidate for Visceral Leishmaniasis. *J. Med. Chem.* **2017**, *60*, 4212–4233.
- (8) Carmo, A. M. L.; Silva, F. M. C.; Machado, P. A.; Fontes, A. P. S.; Pavan, F. R.; Leite, C. Q. F.; Leite, S. R.; Coimbra, E. S.; Da Silva, A. D. Synthesis of 4-Aminoquinoline Analogues and Their Platinum(II) Complexes as New Antileishmanial and Antitubercular agents. *Biomed. Pharmacother.* **2011**, *65*, 204–209 and refs therein.
- (9) Antinarelli, L. M. R.; Dias, R. M. P.; Souza, I. O.; Lima, W. P.; Gameiro, J.; da Silva, A. D.; Coimbra, E. S. 4-Aminoquinoline Derivatives as Potential Antileishmanial Agents. *Chem. Biol. Drug Des.* **2015**, *86*, 704–714.
- (10) Soares, R. R.; Antinarelli, L. M. R.; Souza, I. O.; Lopes, F. V.; Scopel, K. K. G.; Coimbra, E. S.; da Silva, A. D.; Abramo, C. In Vivo Antimalarial and In Vitro Antileishmanial Activity of 4-Aminoquinoline Derivatives Hybridized to Isoniazid or Sulfa or Hydrazine Groups. *Lett. Drug Des. Discovery* **2017**, *14*, 597–604.
- (11) Antinarelli, L. M. R.; Carmo, A. M. L.; Pavan, F. R.; Leite, C. Q. F.; Da Silva, A. D.; Coimbra, E. S.; Salunke, D. B. Increase of Leishmanicidal and Tubercular Activities Using Steroids Linked to Aminoquinoline. *Org. Med. Chem. Lett.* **2012**, *2*, 16.
- (12) Terzić, N.; Konstantinović, J.; Tot, M.; Burojević, J.; Djurković-Djaković, O.; Srbljanović, J.; Štajner, T.; Verbić, T.; Zlatović, M.; Machado, M.; Albuquerque, I. S.; Prudêncio, M.; Sciotti, R. J.; Pecic, S.; D'Alessandro, S.; Taramelli, D.; Šolaja, B. A. Reinvestigating Old Pharmacophores: Are 4-Aminoquinolines and Tetraoxanes Potential Two-Stage Antimalarials? *J. Med. Chem.* **2016**, *59*, 264–281.
- (13) Konstantinović, J.; Videnović, M.; Srbljanović, J.; Djurković-Djaković, O.; Bogojević, K.; Sciotti, R.; Šolaja, B. Antimalarials With Benzothioether Moieties as Aminoquinoline Partners. *Molecules* **2017**, *22*, 343.
- (14) Konstantinović, J.; Kiriš, E.; Kota, K.; Kugelman-Tonos, J.; Videnović, M.; Cazares, L. H.; Terzić, N.; Verbić, T. Ž.; Andjelković, B.; Duplantier, A. J.; Bavari, S.; Šolaja, B. A. New Steroidal 4-Aminoquinolines Antagonize Botulinum Neurotoxin Serotype A in Mouse

Embryonic Stem Cell Derived Motor Neurons in Post-intoxication Model. *J. Med. Chem.* **2018**, *61*, 1595–1608.

(15) Marković, O. S.; Cvijetić, I. N.; Zlatović, M. V.; Opsenica, I. M.; Konstantinović, J. M.; Terzić Jovanović, N. V.; Šolaja, B. A.; Verbić, T. Ž. Human Serum Albumin Binding of Certain Antimalarials. *Spectrochim. Acta, Part A* **2018**, *192*, 128–139.

(16) Šolaja, B. A.; Opsenica, D.; Smith, K. S.; Milhous, W. K.; Terzić, N.; Opsenica, I.; Burnett, J. C.; Nuss, J.; Gussio, R.; Bavari, S. Novel 4-Aminoquinolines Active against Chloroquine-Resistant and Sensitive *P. falciparum* Strains that also Inhibit Botulinum Serotype A. *J. Med. Chem.* **2008**, *51*, 4388–4391.

(17) Gerster, J. F.; Lindstrom, K. J.; Miller, R. L.; Tomai, M. A.; Birmachu, W.; Bomersine, S. N.; Gibson, S. J.; Imbertson, L. M.; Jacobson, J. R.; Knafla, R. T.; Maye, P. V.; Nikolaides, N.; Oneyemi, F. Y.; Parkhurst, G. J.; Pecore, S. E.; Reiter, M. J.; Scribner, L. S.; Testerman, T. L.; Thompson, N. J.; Wagner, T. L.; Weeks, C. E.; Andre, J.-D.; Lagain, D.; Bastard, Y.; Lupu, M. Synthesis and Structure–Activity-Relationships of 1H-Imidazo[4,5-c]quinolines That Induce Interferon Production. *J. Med. Chem.* **2005**, *48*, 3481–3491.

(18) Korotchenko, V.; Sathunuru, R.; Gerena, L.; Caridha, D.; Li, Q.; Kreishman-Deitrick, M.; Smith, P. L.; Lin, A. J. Antimalarial Activity of 4-Amidinoquinoline and 10-Amidinobenzonaphthyridine Derivatives. *J. Med. Chem.* **2015**, *58*, 3411–3431.

(19) Dea-Ayuela, M. A.; Castillo, E.; Gonzalez-Alvarez, M.; Vega, C.; Rolón, M.; Bolás-Fernández, F.; Borrás, J.; González-Rosende, M. E. *Vivo* and *in Vitro* Anti-leishmanial Activities of 4-Nitro-N-pyrimidin and N-Pyrazin-2-ylbenzenesulfonamides, and N2-(4-Nitrophenyl)-N1-propylglycinamide. *Bioorg. Med. Chem.* **2009**, *17*, 7449–7456.

(20) Gupta, S.; Yardley, V.; Vishwakarma, P.; Shivahare, R.; Sharma, B.; Launay, D.; Martin, D.; Puri, S. K. Nitroimidazo-oxazole Compound DNDI-VL-2098: An Orally Effective Preclinical Drug Candidate for the Treatment of Visceral Leishmaniasis. *J. Antimicrob. Chemother.* **2015**, *70*, 518–527.

(21) Galiana-Roselló, C.; Bilbao-Ramos, P.; Dea-Ayuela, M. A.; Rolón, M.; Vega, C.; Bolás-Fernández, F.; García-España, E.; Alfonso, J.; Coronel, C.; González-Rosende, M. E. *In Vitro* and *In Vivo* Antileishmanial and Trypanocidal Studies of New N-Benzene- and N-Naphthalenesulfonamide Derivatives. *J. Med. Chem.* **2013**, *56*, 8984–8998.

(22) VL-2098. <https://www.dndi.org/diseases-projects/portfolio/completed-projects/vl-2098/> (accessed March 22, 2018).

(23) Nakayama, H.; Desrivot, J.; Bories, C.; Franck, X.; Figadère, B.; Hocquemiller, R.; Fournet, A.; Loiseau, P. M. *In Vitro* and *In Vivo* Antileishmanial Efficacy of a New Nitrilquinoline against *Leishmania donovani*. *Biomed. Pharmacother.* **2007**, *61*, 186–188.

(24) Gopinath, V. S.; Pinjari, J.; Dere, R. T.; Verma, A.; Vishwakarma, P.; Shivahare, R.; Moger, M.; Kumar Goud, P. S.; Ramanathan, V.; Bose, P.; Rao, M. V.; Gupta, S.; Puri, S. K.; Launay, D.; Martin, D. Design, Synthesis and Biological Evaluation of 2-Substituted Quinolines as Potential Antileishmanial Agents. *Eur. J. Med. Chem.* **2013**, *69*, 527–536.

(25) Deshpande, S.; Shivahare, R.; Debnath, U.; Sane, S. A.; Gupta, S.; Katti, S. B. Synthesis and Bio-evaluation of 7-trifluoromethyl Substituted 4-aminoquinoline Derivatives as Antileishmanial Agents. *Nat. Prod. J.* **2017**, *7*, 137–143.

(26) Loiseau, P. M.; Cojean, S.; Schrével, J. Sitamaquine as a Putative Antileishmanial Drug Candidate: From the Mechanism of Action to the Risk of Drug Resistance. *Parasite* **2011**, *18*, 115–119.