ACS Medicinal Chemistry Letters

Letter

Cite This: ACS Med. Chem. Lett. 2019, 10, 34−39

pubs.acs.org/acsmedchemlett

Nongenotoxic 3-Nitroimidazo[1,2-*a*]pyridines Are NTR1 Substrates That Display Potent *in Vitro* Antileishmanial Activity

Cyril Fersing,[†] Louise Basmaciyan,[‡] Clotilde Boudot,[§] Julien Pedron,[∥] Sébastien Hutter,[‡] Anita Cohen,[‡] Caroline Castera-Ducros,[†] Nicolas Primas,[†] Michèle Laget,[◊] Magali Casanova,[‡] Sandra Bourgeade-Delmas,[⊥] Mélanie Piednoel,[†] Alix Sournia-Saquet,[∥] Valère Belle Mbou,[¶] Bertrand Courtioux,[§] Élisa Boutet-Robinet,[#] Marc Since,[▽] Rachel Milne,[○] Susan Wyllie,[○] Alan H. Fairlamb,[○] Alexis Valentin,[⊥] Pascal Rathelot,[†] Pierre Verhaeghe,*[,∥] Patrice Vanelle,[†] and Nadine Azas[‡]

[†]Aix Marseille Univ, CNRS, ICR UMR 7273, Équipe Pharmaco-Chimie Radicalaire, Faculté de Pharmacie, 27 Boulevard Jean Moulin, 13385 Marseille, France

[‡]Aix Marseille Univ, IRD, AP-HM, SSA, VITROME, Marseille, France

[§]Université de Limoges, UMR INSERM 1094, Neuroépidémiologie Tropicale, Faculté de Pharmacie, 2 rue du Dr Marcland, 87025 Limoges, France

[∥]LCC−CNRS Université de Toulouse, CNRS, UPS, Toulouse, France

[¶]CHU de Limoges, Service d'anatomopathologie, 2 avenue Martin Luther King, 87042 Limoges, France

[◊]Aix Marseille Univ, INSERM, UMR MD1, U1261, SSA, MCT, Marseille, France

[⊥]UMR 152 PharmaDev, Université de Toulouse, IRD, UPS, Toulouse, France

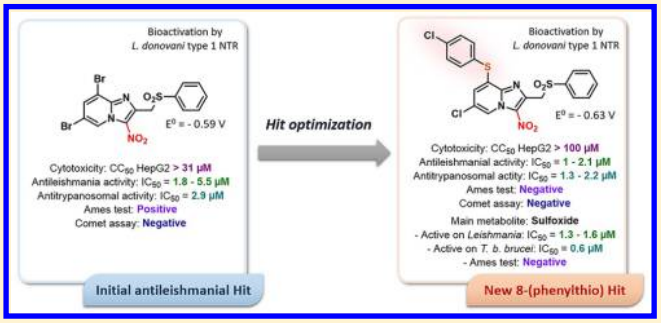
[#]Toxalim (Research Centre in Food Toxicology), Université de Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France

[▽]Centre d'Etudes et de Recherche sur le Médicament de Normandie, Normandie Univ., UNICAEN, CERMN, 14000 Caen, France

[○]University of Dundee, School of Life Sciences, Division of Biological Chemistry and Drug Discovery, Dow Street, Dundee DD1 5EH, Scotland, United Kingdom

Supporting Information

ABSTRACT: Twenty nine original 3-nitroimidazo[1,2-*a*]-pyridine derivatives, bearing a phenylthio (or benzylthio) moiety at position 8 of the scaffold, were synthesized. *In vitro* evaluation highlighted compound **5** as an antiparasitic hit molecule displaying low cytotoxicity for the human HepG2 cell line (CC_{50} > 100 μM) alongside good antileishmanial activities (IC_{50} = 1−2.1 μM) against *L. donovani*, *L. infantum*, and *L. major*; and good antitrypanosomal activities (IC_{50} = 1.3−2.2 μM) against *T. brucei brucei* and *T. cruzi*, in comparison to several reference drugs such as miltefosine, fexinidazole, eflornithine, and benznidazole (IC_{50} = 0.6 to 13.3 μM). Molecule **5**, presenting a low reduction potential ($E°$ = −0.63 V), was shown to be selectively bioactivated by the *L. donovani* type 1 nitroreductase (NTR1). Importantly, molecule **5** was neither mutagenic (negative Ames test), nor genotoxic (negative comet assay), in contrast to many other nitroaromatics. Molecule **5** showed poor microsomal stability; however, its main metabolite (sulfoxide) remained both active and nonmutagenic, making **5** a good candidate for further *in vivo* studies.



KEYWORDS: *Leishmania ssp, imidazopyridine, nitroaromatic, nitroreductases, Ames test, comet assay*

Trypanosomatids are flagellated protozoan parasites that belong to the Kinetoplastid order. These unicellular parasites are responsible for several vector borne diseases in mammals. Among the trypanosomatids, *Leishmania spp* and *Trypanosoma spp* are the two main parasites encountered in human pathology and responsible for lethal infections: visceral leishmaniasis (VL),[1] human African trypanosomiasis (HAT),[2] or Chagas disease (CD).[3] These neglected tropical diseases threaten millions of people worldwide with current total deaths estimated at 30,000 per annum, mainly in tropical and

Received: July 31, 2018
Accepted: December 19, 2018
Published: December 19, 2018

DOI: 10.1021/acsmedchemlett.8b00347
ACS Med. Chem. Lett. 2019, 10, 34−39

subtropical areas.[4] New efficient, safe, and cheap oral anti-Kinetoplastid agents are urgently needed due to the major limitations of the few currently available drugs (i.e., toxicity, mode of administration, cost) and the emergence of resistant parasites (mainly toward antimony derivatives and miltefosine).[5] In this context, small azaheterocyclic molecules, including imidazo[1,2-*a*]pyridine derivatives,[6] are studied to enrich the antitrypanosomatid drug discovery pipeline.[7,8]

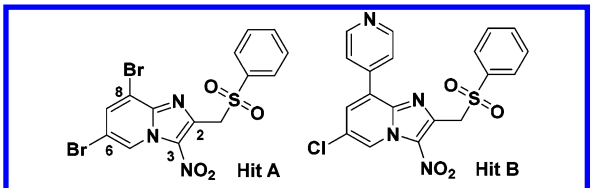
Recently, nitroaromatic molecules, such as fexinidazole[9] and delamanid,[10] showed a renewed interest against infectious diseases such as HAT, VL, CD, and even tuberculosis.[11] Indeed, these compounds act as prodrugs, requiring the bioactivation of their nitro group into reduced reactive intermediates, in order to display their antiparasitic properties. This bioactivation step is catalyzed by nitroreductases (NTR), enzymes found in some bacteria and parasites, but not in mammalian cells. Thus, the concept of exploiting NTR to activate prodrugs is an attractive strategy to target selectively pathogen parasites and bacteria.

Two NTRs have been identified and characterized in *Leishmania spp*: (i) an essential mitochondrial type 1 NTR (NTR1)[12] catalyzing a two-electron reduction reaction and that bioactivates 5-nitroimidazoles like fexinidazole[13,14] and (ii) a cytosolic type 2 NTR (NTR2)[15] catalyzing a monoelectronic reduction reaction. NTR2 more particularly activates bicyclic nitroheterocycles containing a 4-nitroimidazole moiety, such as delamanid and pretomanid.[16] In *Trypanosoma brucei spp*, only the type 1 NTR was discovered, mediating the bioactivation of the nitroaromatic drugs benznidazole[17] and nifurtimox.[18] To date, the structures of parasitic NTRs remain unknown, as no X-ray diffraction data is available. Thus, drug design techniques such as docking cannot be applied for designing new NTR substrates.

Previously, we reported the antileishmanial activity of 3-nitroimidazo[1,2-*a*]pyridine derivatives. After identifying a hit molecule bearing bromine atoms at positions 6 and 8 of the imidazopyridine ring (hit **A**),[19] a pharmacomodulation study led to a series of 8-aryl-6-chloro-3-nitroimidazo[1,2-*a*]-pyridines that displayed an improved activity against *Leishmania spp* and *Trypanosoma b. brucei*,[20] suggesting the key role of the substituent at position 8 of the scaffold (Figure 1).

In a continuation of this project, we wanted to explore the influence of the insertion of a heteroatomic bridge between the imidazopyridine ring and an aryl group at position 8, using a synthetic route to functionalize antiparasitic azaheterocycles by a phenylthio moiety.[21,22] We applied this synthetic procedure to a previously described substrate: 8-bromo-6-chloro-3-nitro-2-(phenylsulfonylmethyl)imidazo[1,2-*a*]pyridine.[20] Twenty-six original nitroaromatic derivatives bearing a sulfur group at position 8 were then synthesized, using various thiophenol or benzylthiol reagents. The majority of these compounds were prepared with moderate to good yields (58−95%). The use of disubstituted thiophenols (compounds **20**−**22**) led to lower yields (31−38%).

All synthesized molecules were first screened *in vitro* against the promastigote form of *L. donovani* and potencies were compared to those achieved with commercial reference drugs (i.e., amphotericin B, miltefosine, and pentamidine) along with drug-candidate fexinidazole (Table 1). To assess antiparasitic selectivity, the cytotoxicity of all compounds was measured against the HepG2 human cell line, using doxorubicin as control.



	Hit A	Hit B
IC_{50} *L. donovani* promastigotes (μM)	1.8	1.2
IC_{50} *L. donovani* Intramacrophage amastigotes (μM)	5.5	2.3
IC_{50} *L. infantum* axenic amastigotes (μM)	4.4	3.0
IC_{50} *T. brucei brucei* trypomastigotes (μM)	2.9	0.25
CC_{50} *HepG2* / CC_{50} *THP1* (μM)	>31 / >25	>50 / >25

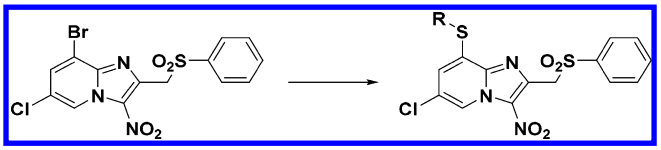
Figure 1. Structure of previously reported hit molecules **A** and **B** in 3-nitroimidazo[1,2-*a*]pyridine series.

About half of the tested molecules showed poor solubility in the culture medium and could not be tested against the HepG2 cell line above 4−6 μM. This is particularly noted with the four benzylthioether derivatives **23** to **26**, which could not be tested. Three compounds (**10**, **18**, and **20**) showed moderate solubility in the culture medium, ranging from 15.6 to 25 μM. Finally, four molecules, all presenting a *para*-substituted thiophenyl moiety, showed satisfactory CC_{50} values, above 50 μM, indicating both good solubility in the culture medium and a low cytotoxicity on the tested cell line. Among the 22 molecules evaluated on the promastigote stage of *L. donovani*, only compound **19** was found to be devoid of antileishmanial activity, perhaps due to its charged carboxylate group. The active molecules display IC_{50} values ranging between 1.0 and 2.3 μM, slightly lower than miltefosine and comparable to fexinidazole. Two compounds (**2** and **5**) functionalized with a *p*-halophenylsulfane group are particularly promising and can be considered as hit compounds, with respective selectivity index of >62.5 and >100 (2 to 4 times better than miltefosine). The 3D structure of hit compound **5** was confirmed by X-ray diffraction (see Supporting Information).

A secondary screening was conducted on hit molecules **2** and **5**, against the intramacrophage amastigote stage of *L. donovani* and *L. major*, on the promastigote stage of *L. major* and on *L. infantum* axenic amastigotes. The CC_{50} values for these molecules (Table 2) were also determined against THP1 monocyte cell line. None of these compounds showed cytotoxic effects on THP1 cells up to 62.5 μM. Molecule **5** showed the best activities against intracellular *L. donovani* and *L. major* amastigotes (IC_{50} values of 1.3 and 2.1 μM, respectively), three times better than miltefosine against *L. donovani*. Compound **5** also returned an IC_{50} value of 1.7 μM against *L. infantum* axenic amastigotes, about two times lower than fexinidazole. This compound also demonstrated promising activity against the promastigote stage of *L. major* (IC_{50} = 1.3 μM). Moreover, molecules **2** and **5** also demonstrated *in vitro* activity against *Trichomonas vaginalis* (IC_{50} = 4.6−5.0 μM), comparable to that of the reference drug metronidazole (IC_{50} = 4.2 μM). This efficacy against *T. vaginalis*, also found with fexinidazole,[23] is probably due to similar modes of bioactivation by parasitic nitroreductases.[24] Nevertheless, compared with atovaquone (IC_{50} = 1 nM), the activity of

DOI: 10.1021/acsmedchemlett.8b00347
ACS Med. Chem. Lett. 2019, 10, 34−39

Table 1. Synthesis, *in Vitro* Antileishmanial Activity, and Cytotoxicity of 3-Nitroimidazopyridine Derivatives 1−26[a]



Cmpd	R-	*L. donovani* promastigotes IC_{50} (μM)	cytotoxicity HepG2 CC_{50} (μM)	selectivity index[f]
1	C_6H_5-	1.7 ± 0.1	>3.9[d]	>2.3
2	**4-Br-C_6H_4-**	**1.0 ± 0.3**	**>62.5**[d]	>62.5
3	2-Cl-C_6H_4-	1.4 ± 0.3	>5[d]	>3.6
4	3-Cl-C_6H_4-	0.7 ± 0.1	>3.9[d]	>3.6
5	**4-Cl-C_6H_4-**	**1.0 ± 0.3**	**>100**[d]	>100
6	2-F-C_6H_4-	1.2 ± 0.3	>5[d]	>4.2
7	3-F-C_6H_4-	1.2 ± 0.2	>3.9[d]	>2.1
8	4-F-C_6H_4-	1.3 ± 0.3	>5[d]	>3.8
9	2-CH_3O-C_6H_4-	2.3 ± 0.2	>50[d]	>21.7
10	3-CH_3O-C_6H_4-	2.3 ± 0.4	>15.6[d]	>6.8
11	4-CH_3O-C_6H_4-	1.5 ± 0.3	>62.5[d]	>41.7
12	2-CF_3-C_6H_4-	1.5 ± 0.3	>4.1[d]	>2.7
13	3-CF_3-C_6H_4-	1.3 ± 0.2	>3.9[d]	>2.4
14	4-CF_3-C_6H_4-	2.1 ± 0.3	>3.9[d]	>1.5
15	2-CH_3-C_6H_4-	1.7 ± 0.3	>3.9[d]	>1.5
16	3-CH_3-C_6H_4-	1.4 ± 0.2	>5.6[d]	>4
17	4-CH_3-C_6H_4-	1.4 ± 0.2	>3.9[d]	>2.2
18	4-*i*prop-C_6H_4-	1.6 ± 0.1	>25[d]	>15.6
19	4-(CH_2COOH)-C_6H_4-	65.6 ± 1.3	>62.5[d]	>1
20	2,3-di-Cl-C_6H_3-	2.0 ± 0.1	>25[d]	>12.5
21	2,4-di-Cl-C_6H_3-	1.7 ± 0.1	>3.9[d]	>2.2
22	2,5-di-Cl-C_6H_3-	1.3 ± 0.1	>3.9[d]	>1.5
23	C_6H_5-CH_2-	NS[g]	NS[g]	-
24	4-Br-C_6H_4−CH_2-	NS[g]	NS[g]	-
25	4-Cl-C_6H_4−CH_2-	NS[g]	NS[g]	-
26	4-CH_3O-C_6H_4-CH_2-	NS[g]	NS[g]	-
amphotericin B[c]		0.07 ± 0.01	8.8 ± 0.2	125.7
miltefosine[c]		3.1 ± 0.2	85 ± 8.8	27.4
pentamidine[c]		6.0 ± 0.8	2.3 ± 0.5	0.4
fexinidazole[c]		1.2 ± 0.20	>200[e]	>166.7
doxorubicin[b]		-	0.2 ± 0.05	-

[a]Reagents and conditions: thiophenol or benzylthiol reagent (1 equiv), NaH (1 equiv), DMSO, N_2, RT, 0.5−2 h. [b]Doxorubicin was used as a cytotoxic reference drug. [c]Amphotericin B, Miltefosine, Pentamidine, and Fexinidazole were used as antileishmanial reference drugs. [d]The product could not be tested at higher concentrations in the culture medium. [e]The CC_{50} value was not reached at the highest tested concentration. [f]SI = CC_{50} HepG2/IC_{50} *L. donovani* promastigote. [g]NS = nonsoluble in the culture medium. In bold: hit molecules.

compound **5** against *Plasmodium falciparum* (K1 strain) is negligible (IC_{50} = 19 μM), demonstrating its selectivity for the parasites expressing nitroreductases. Finally, the *in vitro* antitrypanosomal activity of compounds **2** and **5** were evaluated against the bloodstream form of *T. b. brucei* and the epimastigote form of *T. cruzi* and compared to reference drugs (i.e., eflornithine, suramin, benznidazole, and nifurtimox) and to drug candidate fexinidazole (Table 2). With IC_{50} values ranging from 1.3 to 6.2 μM, these molecules showed a better activity than eflornithine against *T. b. brucei* and a better activity than fexinidazole against *T. cruzi*, while molecule **5** was two times less active than drug candidate fexinidazole on *T. b. brucei* and two times less active than benznidazole on *T. cruzi*. Finally, the amino derivative **27** of hit-compound **5**, prepared by reduction of the nitro group, was tested *in vitro* (Table 3). As expected, **27** did not show antileishmanial activity (IC_{50} = 32 to 124 μM) and presented a limited antitrypanosomal activity (IC_{50} = 11 μM), in accordance with the hypothesis that these nitroaromatic molecules require bioactivation by parasitic NTRs, making the nitro group a key element of the pharmacophore.

To investigate the pharmaceutical potential of compound **5** (best molecule in the series) further, its microsomal stability was measured after incubation with hepatic microsomes isolated from female mice. Molecule **5** exhibited a poor stability with a half-life ($T_{1/2}$ of propranolol control = 26 min) of 3 min. An LC/MS/MS study (see Supporting Information) enabled us to identify that the primary metabolite of **5** was a sulfoxide derivative. This sulfoxide metabolite (**28**) was then synthesized from **5**, along with a probable secondary sulfone metabolite (**29**), respectively, by partial or complete oxidation of the sulfur atom, using *m*-CPBA (Table 3). The activities of metabolite **28** and molecule **29** were evaluated *in vitro* against *Leishmania spp* and *T. b. brucei*. Both molecules demonstrated promising activity against the promastigote stage of *L. donovani* and of *L. infantum* axenic amastigotes. Likewise, both molecules also demonstrated antitrypanosomal activity, thus compound **5** appears to resemble fexinidazole in that it is similarly metabolized into bioactive sulfoxide and sulfone derivatives.[13] These results suggest that the poor microsomal stability of **5** should not be a barrier to further *in vivo* evaluation.

The mutagenicity of many nitroaromatic molecules has been known for many years.[25] Nevertheless, considering the therapeutic potential of these molecules, many attempts have been made to dissociate their anti-infective activity from their mutagenic character.[26] However, the difficulty remains that the anti-infective mechanism(s) of these compounds involves the formation of mutagenic derivatives within microorganisms.[27] The *Salmonella typhimurium* strains used in the Ames test, the most common method for assessing the mutagenicity of a substance, also express nitroreductases,[28] particularly type 1. This may explain why nitrated molecules are generally identified as mutagens in this test. The mutagenic properties of hit **A**, molecule **5**, and sulfoxide metabolite **28** were then evaluated by the Ames test, using four classical strains of *S. typhimurium* expressing NTRs, both with and without addition of the metabolizing S9 mixture. Hit **A** shows a mutagenic character in the absence of S9 mix on each strain of *S. typhimurium*, as well as on the TA97a strain in metabolizing conditions. Interestingly, the Ames test was negative on all four strains of *Salmonella* exposed to molecules **5** or **28**, with or without S9 mix (Table 4). Molecules **5** and its principal metabolite **28** therefore appeared as nonmutagenic nitroaromatic derivatives in the Ames test. Thus, the introduction of a *p*-chlorophenylthioether group at position 8 of the imidazo[1,2-*a*]pyridine ring, along with the oxidation of the sulfur atom, seem to favor selectivity for parasitic NTRs.

To complete the toxicological profile of hit compound **5**, a comet assay using a HepG2 human hepatic cell line, which does not express any NTR, was carried out on hit **A** and molecule **5**. HepG2 cells were incubated with the drugs for 2

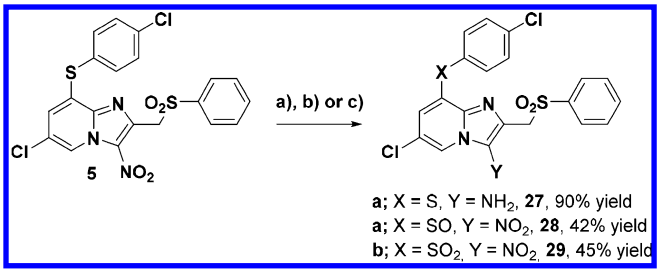
DOI: 10.1021/acsmedchemlett.8b00347
ACS Med. Chem. Lett. 2019, 10, 34−39

Table 2. *In Vitro* Antiparasitic Profile of Compounds 2 and 5 toward Kinetoplastids

Cmpd	*L. donovan* intramacrophage amastigotes IC_{50} (μM)	*L. infantum* axenic amastigotes IC_{50} (μM)	*L. major* promastigotes IC_{50} (μM)	*L. major* intramacrophage amastigotes IC_{50} (μM)	*T. brucei brucei* trypanosomes IC_{50} (μM)	*T. cruzi* epimastigotes IC_{50} (μM)	THP1 CC_{50} (μM)
2	3.5 ± 0.3	9.0 ± 1.4	1.3 ± 0.2	5	3.8 ± 0.45	6.2 ± 1.9	>62.5[e]
5	**1.3 ± 0.1**	**1.7 ± 0.3**	**1.3 ± 0.2**	**2.1 ± 0.1**	**1.3 ± 0.11**	**2.2 ± 0.08**	**>62.5**[e]
amphotericin B[a]	0.4 ± 0.01	0.06 ± 0.001	0.6 ± 0.08	0.2 ± 0.07	-	-	3.6 ± 0.7
miltefosine[a]	4.3 ± 1.7	0.8 ± 0.2	-	-	-	-	>40[e]
fexinidazole[a,b,c]	>50[d]	3.4 ± 0.8	-	-	0.6 ± 0.05	8.9 ± 0.7	>62.5[d]
eflornithine[b]	-	-	-	-	13.3 ± 2.1	-	-
suramin[b]	-	-	-	-	0.03 ± 0.004	-	-
benznidazole[c]	-	-	-	-	-	1.2 ± 0.1	-
nifurtimox[c]	-	-	-	-	-	0.3 ± 0.02	-

[a]Amphotericin B, miltefosine, and fexinidazole were used as antileishmanial reference drugs. [b]Eflornithine, suramin, and fexinidazole were used as anti-*T. b. brucei* reference drugs. [c]Fexinidazole, benznidazole, and nifurtimox were used as anti-*Trypanosoma cruzi* reference drugs. [d]Molecule was not tested at higher concentrations. [e]Molecules could not be tested at higher concentrations because of a lack of solubility in the culture media.

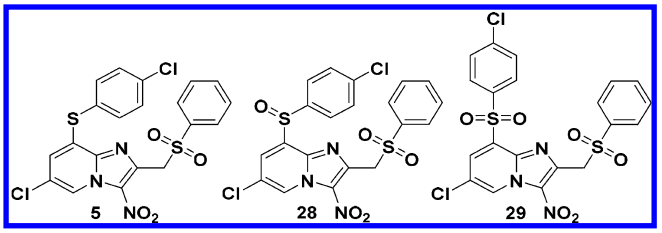
Table 3. Synthesis of Imidazopyridine Derivatives 27, 28 (Metabolite), and 29 and Their Biological Activities[a]



Cmpd	*L. donovani* pro. IC_{50} (μM)	*L. infantum* axe. ama. IC_{50} (μM)	*T. brucei brucei* trypo. IC_{50} (μM)	HepG2 CC_{50} (μM)
5	1.0 ± 0.3	1.7 ± 0.3	1.3 ± 0.45	>100
27	124.0 ± 1.1	31.6 ± 2.7	10.6 ± 1.2	>62.5[b]
28	1.3 ± 0.3	1.6 ± 0.4	0.6 ± 0.11	>12.5[b]
29	1.3 ± 0.3	3.7 ± 0.5	1.0 ± 0.05	>12.5[b]

[a]Reagents and conditions: (a) Fe (10 equiv), AcOH, reflux, 40 min; (b) *m*CPBA (1 equiv), DCM, 0 °C, 3 h; (c) *m*CPBA (2 equiv), DCM, RT, 24 h. [b]Compounds could not be tested at higher concentrations due to a poor aqueous solubility.

Table 4. Complementary *in Vitro* Biological Evaluations for Molecules 5, 28, and 29



molecule 5	
Ames test (at 250 μM and 2.5 mM ± S9 mix)	negative
Comet assay (at 2 and 72 h; 1, 10, and 20 μM)	negative
CHI $LogD_{7.4}$	4.26
thermodynamic aqueous solubility (μM)	1.4
binding % to human albumin	99.95
microsomal stability: $t_{1/2}$ (min)	3
IC_{50} *L. donovani* promastigote wild-type strain (μM)	0.26
IC_{50} *L. donovani* promastigote NTR1 overexpressing strain (μM)	0.033
IC_{50} *L. donovani* promastigote NTR2 overexpressing strain (μM)	0.3
molecule 28	
Ames test (at 62.5 and 625 μM ± S9 mix)	negative
molecule 29	
IC_{50} *L. donovani* promastigote wild-type strain (μM)	0.6
IC_{50} *L. donovani* promastigote NTR1 overexpressing strain (μM)	0.08
IC_{50} *L. donovani* promastigote NTR2 overexpressing strain (μM)	0.7

or 72 h (Table 4). No increase in the percentage of DNA laddering was observed for these two compounds at the three tested concentrations: 1, 10, and 20 μM (see Supporting Information). These molecules are therefore not genotoxic according to this test. This assay reveals, for hit **A**, a profile identical to fexinidazole or metronidazole: a mutagenic character shown by the Ames test and no genotoxic properties objectified on mammalian cells (micronucleus test or comet assay).[29] However, molecule **5** was neither mutagenic nor genotoxic.

Among the accepted criteria for the selection of antiparasitic candidate for preclinical evaluation,[30] the percentage of albumin binding may affect both the distribution and the elimination of the molecule under consideration. The binding percentage to human albumin of compound **5** was measured by LC/MS, after incubation in the presence of human plasma, and compared with four reference drugs. With a bound fraction of 99.95% ($\log(f_u) = -3.28$), this molecule is therefore strongly bound to albumin (Table 4). The chromatographic hydrophobicity index (CHI) of molecule **5** was also determined and provides a $logD_{7.4}$ value of 4.26, making this molecule very lipophilic, in accordance with a high albumin binding (Table 4). However, the binding of a drug to plasma proteins was shown to have little effect on the *in vivo* efficacy of this drug when its mechanism of action results in a covalent binding to its target,[31] which is the likely mode of action proposed for anti-infective nitroaromatics. Moreover, the high lipophilicity of **5** may facilitate its activity against the amastigote stage, by increasing its penetration into both macrophages and parasites.

In order to check that compounds **5** and **29** were substrates of parasitic NTRs, their IC_{50} values were determined on three strains of *L. donovani* promastigotes: a wild type strain, a strain overexpressing NTR1, and a strain overexpressing NTR2. The IC_{50} of **5** and **29** are about eight times lower in the strain overexpressing NTR1 than the two other strains (Table 4). These data demonstrate that these compounds are selectively bioactivated by NTR1 in *L. donovani*, the same type 1

DOI: 10.1021/acsmedchemlett.8b00347
ACS Med. Chem. Lett. 2019, 10, 34−39

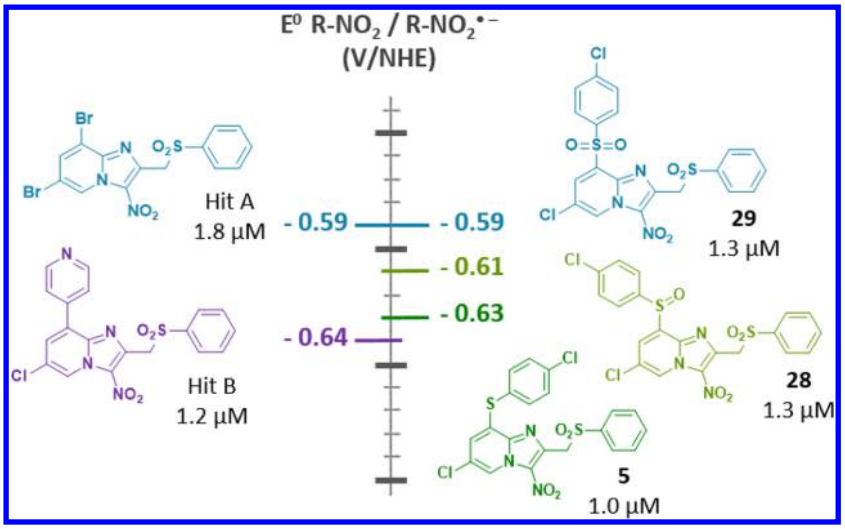


Figure 2. Reduction potentials measured for hit **A**, hit **B**, hit compound **5**, metabolite **28**, and derivative **29**.

nitroreductase that is responsible for the bioactivation of fexinidazole and its associated metabolites.

On the basis that molecules **5**, **28**, and **29** could be substrates of parasitic nitroreductases, we determined their reduction potential to examine whether the absence of mutagenicity of molecules **5** and **28** (and probable selectivity for *L. donovani* NTR1 *versus S. typhimurium* NTRs) was associated with a lower reduction potential. For the three tested molecules (as for the two hit molecules **A** and **B**), the cyclic voltammogram showed a reversible single electron reduction for a 1 $V \cdot s^{-1}$ scan rate, corresponding to the formation of a nitro radical anion. The measured reduction potentials of compounds **5**, **28**, and **29** ranged from −0.63 to −0.59 V/NHE. As expected, the more oxidized the sulfur atom in position 8, the higher the reduction potential (Figure 2). Nonmutagenic molecules **5** and **28** presented a lower reduction potential than mutagenic Hit **A**, but the difference in $E°$ values was not significant. Thus, steric parameters should be explored in order to explain the atypical behavior of the current series that is selectively bioactivated by *L. donovani* NTR1 and not by *S. typhimurium* type 1 NTR. These data suggest that the introduction of a thiophenyl moiety at position 8 of the scaffold maintains affinity for the parasitic type 1 NTR while decreasing the affinity for the bacterial counterpart.

Finally, a preliminary *in vivo* toxicity study was conducted on swiss mice (three mice per group) that were treated by intraperitoneal administration (50 μL) of molecule **5** at either 1 (group 1) or 10 (group 2) mg/kg for 5 days. There was no sign of acute toxicity noted on living mice. In each group, a mouse was sacrificed at D7, D15, or D30 and autopsied. Histology analysis (brain, liver, kidney, spleen, heart, lung, adipose tissues, and muscles) did not reveal any sign of chronic toxicity in group 1. In group 2, only a discrete inflammatory infiltrate was detected in the liver, classified as nonspecific, and a hypodermic lymph node was observed in the skin in addition to some fibrosis and inflammation at the injection site (see Supporting Information).

In summary, introducing a *para*-chlorophenylthioether moiety at position 8 of the antileishmanial 3-nitroimidazo-[1,2-*a*]pyridine pharmacophore led to hit molecule **5** that displayed improved *in vitro* antikinetoplastid activity against both *Leishmania* (*donovani* and *infantum*) and *Trypanosoma* (*brucei brucei* and *cruzi*) parasites, in comparison with the previously identified hit molecules in the series. Moreover, **5** was the first compound in the series that was neither mutagenic nor genotoxic. It presented a short microsomal half-life due to transformation into the sulfoxide metabolite (**28**) that remains active and nonmutagenic. Hit compound **5** presents a low reduction potential and is selectively bioactivated by *L. donovani* NTR1. These results make molecule **5** a good candidate for a hit to lead program focused on developing novel and safe nitroaromatic drug candidates that selectively target flagellated protozoans.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00347.

Experimental details and characterization (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pierre.verhaeghe@univ-tlse3.fr.

ORCID

Élisa Boutet-Robinet: 0000-0002-5223-4568
Susan Wyllie: 0000-0001-8810-5605
Alan H. Fairlamb: 0000-0001-5134-0329
Pierre Verhaeghe: 0000-0003-0238-2447

Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by Aix-Marseille Université, the Université de Toulouse,and the CNRS. A.H.F. and S.W. are supported by funding from the Wellcome Trust (WT105021). C.F. thanks the Assistance Publique-Hôpitaux de Marseille for hospital appointment. J.P. thanks the Université Paul Sabatier and the Conseil Régional Occitanie for PhD funding. C.P. and A.B.(Institut Pasteur de Lille) are acknowledged for their contribution in determining PK parameters.

DOI: 10.1021/acsmedchemlett.8b00347
ACS Med. Chem. Lett. 2019, 10, 34−39

■ ABBREVIATIONS

CHI, chromatographic hydrophobicity index; CD, Chagas disease; HAT, human African trypanosomiasis; NTR, nitroreductase; VL, visceral leishmaniasis

■ REFERENCES

(1) Burza, S.; Croft, S. L.; Boelaert, M. Leishmaniasis. *Lancet* **2018**, *392*, 951−970.

(2) Büscher, P.; Cecchi, G.; Jamonneau, V.; Priotto, G. Human African trypanosomiasis. *Lancet* **2017**, *390* (10110), 2397−2409.

(3) Pérez-Molina, J. A.; Molina, I. Chagas Disease. *Lancet* **2018**, *391*, 82−94.

(4) (a) World Health Organization (WHO). http://www.who.int/neglected_diseases/diseases/en/. (b) http://www.who.int/healthinfo/global_burden_disease/en/. (c) http://www.who.int/chagas/en/.

(5) Bilbe, G. Overcoming neglect of kinetoplastid diseases. *Science* **2015**, *348* (6238), 974−976.

(6) Silva, D. G.; Gillespie, J. R.; Ranade, R. M.; Herbst, Z. M.; Nguyen, U. T. T.; Buckner, F. S.; Montanari, C. A.; Gelb, M. H. New class of antitrypanosomal agents based on imidazopyridines. *ACS Med. Chem. Lett.* **2017**, *8* (7), 766−770.

(7) Konstantinović, J.; Videnović, M.; Orsini, S.; Bogojević, K.; D'Alessandro, S.; Scaccabarozzi, D.; Jovanović, N. T.; Gradoni, L.; Basilico, N.; Šolaja, B. A. Novel aminoquinoline derivatives significantly reduce parasite load in *Leishmania infantum* infected mice. *ACS Med. Chem. Lett.* **2018**, *9* (7), 629−634.

(8) Devine, W.; Thomas, S. M.; Erath, J.; Bachovchin, K. A.; Lee, P. J.; Leed, S. E.; Rodriguez, A.; Sciotti, R. J.; Mensa-Wilmot, K.; Pollastri, M. P. Antiparasitic lead discovery: toward optimization of a chemotype with activity against multiple protozoan parasites. *ACS Med. Chem. Lett.* **2017**, *8* (3), 350−354.

(9) Chappuis, F. Oral fexinidazole for human African trypanosomiasis. *Lancet* **2018**, *391* (10116), 100−102.

(10) Patterson, S.; Wyllie, S.; Norval, S.; Stojanovski, L.; Simeons, F. R.; Auer, J. L.; Osuna-Cabello, M.; Read, K. D.; Fairlamb, A. H. The anti-tubercular drug delamanid as a potential oral treatment for visceral leishmaniasis. *eLife* **2016**, *5*, No. e09744.

(11) Fairlamb, A. H.; Patterson, S. Current and future prospects of nitro-compounds as drugs for trypanosomiasis and leishmaniasis. *Curr. Med. Chem.* **2018**, *25*, DOI: 10.2174/0929867325666180426164352.

(12) Voak, A. A.; Gobalakrishnapillai, V.; Seifert, K.; Balczo, E.; Hu, L.; Hall, B. S.; Wilkinson, S. R. An essential type I nitroreductase from *Leishmania major* can be used to activate leishmanicidal prodrugs. *J. Biol. Chem.* **2013**, *288* (40), 28466−28476.

(13) Wyllie, S.; Patterson, S.; Stojanovski, L.; Simeons, F. R.; Norval, S.; Kime, R.; Read, K. D.; Fairlamb, A. H. The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis. *Sci. Transl. Med.* **2012**, *4* (119), 119re1.

(14) Wyllie, S.; Patterson, S.; Fairlamb, A. H. Assessing the essentiality of *Leishmania donovani* nitroreductase and its role in nitro drug activation. *Antimicrob. Agents Chemother.* **2013**, *57* (2), 901−906.

(15) Wyllie, S.; Roberts, A. J.; Norval, S.; Patterson, S.; Foth, B. J.; Berriman, M.; Read, K. D.; Fairlamb, A. H. Activation of bicyclic nitro-drugs by a novel nitroreductase (NTR2) in *Leishmania*. *PLoS Pathog.* **2016**, *12* (11), No. e1005971.

(16) Patterson, S.; Wyllie, S.; Stojanovski, L.; Perry, M. R.; Simeons, F. R.; Norval, S.; Osuna-Cabello, M.; De Rycker, M.; Read, K. D.; Fairlamb, A. H. The *R* enantiomer of the antitubercular drug PA-824 as a potential oral treatment for visceral leishmaniasis. *Antimicrob. Agents Chemother.* **2013**, *57* (10), 4699−4706.

(17) Hall, B. S.; Wilkinson, S. R. Activation of benznidazole by trypanosomal type I nitroreductases results in glyoxal formation. *Antimicrob. Agents Chemother.* **2012**, *56* (1), 115−123.

(18) Hall, B. S.; Bot, C.; Wilkinson, S. R. Nifurtimox activation by trypanosomal type I nitroreductases generates cytotoxic nitrile metabolites. *J. Biol. Chem.* **2011**, *286* (15), 13088−13095.

(19) Castera-Ducros, C.; Paloque, L.; Verhaeghe, P.; Casanova, M.; Cantelli, C.; Hutter, S.; Tanguy, F.; Laget, M.; Remusat, V.; Cohen, A.; Crozet, M. D.; Rathelot, P.; Azas, N.; Vanelle, P. Targeting the human parasite *Leishmania donovani*: Discovery of a new promising anti-infectious pharmacophore in 3-nitroimidazo[1,2-*a*]pyridine series. *Bioorg. Med. Chem.* **2013**, *21* (22), 7155−7164.

(20) Fersing, C.; Boudot, C.; Pedron, J.; Hutter, S.; Primas, N.; Castera-Ducros, C.; Bourgeade-Delmas, S.; Sournia-Saquet, A.; Moreau, A.; Cohen, A.; Pratviel, G.; Crozet, M. D.; Wyllie, S.; Fairlamb, A.; Valentin, A.; Rathelot, P.; Azas, N.; Courtioux, B.; Verhaeghe, P.; Vanelle, P. 8-Aryl-6-chloro-3-nitro-2-(phenylsulfonylmethyl)imidazo[1,2-*a*]pyridines as potent antitrypanosomatid molecules bioactivated by type 1 nitroreductases. *Eur. J. Med. Chem.* **2018**, *157*, 115−126.

(21) Verhaeghe, P.; Dumètre, A.; Castera-Ducros, C.; Hutter, S.; Laget, M.; Fersing, C.; Prieri, M.; Yzombard, J.; Sifredi, F.; Rault, S.; Rathelot, P.; Vanelle, P.; Azas, N. 4-Thiophenoxy-2-trichloromethyquinazolines display *in vitro* selective antiplasmodial activity against the human malaria parasite *Plasmodium falciparum*. *Bioorg. Med. Chem. Lett.* **2011**, *21* (19), 6003−6006.

(22) Kieffer, C.; Cohen, A.; Verhaeghe, P.; Hutter, S.; Castera-Ducros, C.; Laget, M.; Remusat, V.; M'Rabet, M. K.; Rault, S.; Rathelot, P.; Azas, N.; Vanelle, P. Looking for new antileishmanial derivatives in 8-nitroquinolin-2(1*H*)-one series. *Eur. J. Med. Chem.* **2015**, *92*, 282−294.

(23) Raether, W.; Seidenath, H. The activity of fexinidazole (HOE 239) against experimental infections with *Trypanosoma cruzi*, trichomonads and *Entamoeba histolytica*. *Ann. Trop. Med. Parasitol.* **1983**, *77* (1), 13−26.

(24) Pal, D.; Banerjee, S.; Cui, J.; Schwartz, A.; Ghosh, S. K.; Samuelson, J. *Giardia, Entamoeba* and *Trichomonas* enzymes activate metronidazole (nitroreductases) and inactivate metronidazole (nitroimidazole reductases). *Antimicrob. Agents Chemother.* **2009**, *53* (2), 458−464.

(25) McCann, J.; Spingarn, N. E.; Kobori, J.; Ames, B. N. Detection of carcinogens as mutagens: bacterial tester strains with R factor plasmids. *Proc. Natl. Acad. Sci. U. S. A.* **1975**, *72* (3), 979−983.

(26) Walsh, J. S.; Wang, R.; Bagan, E.; Wang, C. C.; Wislocki, P.; Miwa, G. T. Structural alterations that differentially affect the mutagenic and antitrichomonal activities of 5-nitroimidazoles. *J. Med. Chem.* **1987**, *30* (1), 150−156.

(27) Blumer, J. L.; Friedman, A.; Meyer, L. W.; Fairchild, E.; Webster, L. T., Jr.; Speck, W. T. Relative importance of bacterial and mammalian nitroreductases for niridazole mutagenesis. *Cancer Res.* **1980**, *40* (12), 4599−4605.

(28) Yanto, Y.; Hall, M.; Bommarius, A. S. Nitroreductase from *Salmonella typhimurium*: characterization and catalytic activity. *Org. Biomol. Chem.* **2010**, *8* (8), 1826−1832.

(29) Tweats, D.; Bourdin Trunz, B.; Torreele, E. Genotoxicity profile of fexinidazole, a drug candidate in clinical development for human African trypanomiasis (sleeping sickness). *Mutagenesis* **2012**, *27* (5), 523−532.

(30) Katsuno, K.; Burrows, J. N.; Duncan, K.; Hooft van Huijsduijnen, R.; Kaneko, T.; Kita, K.; Mowbray, C. E.; Schmatz, D.; Warner, P.; Slingsby, B. T. Hit and lead criteria in drug discovery for infectious diseases of the developing world. *Nat. Rev. Drug Discovery* **2015**, *14* (11), 751−758.

(31) Smith, D. A.; Di, L.; Kerns, E. H. The effect of plasma protein binding on *in vivo* efficacy: misconceptions in drug discovery. *Nat. Rev. Drug Discovery* **2010**, *9* (12), 929−939.

DOI: 10.1021/acsmedchemlett.8b00347
ACS Med. Chem. Lett. 2019, 10, 34−39