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

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S 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
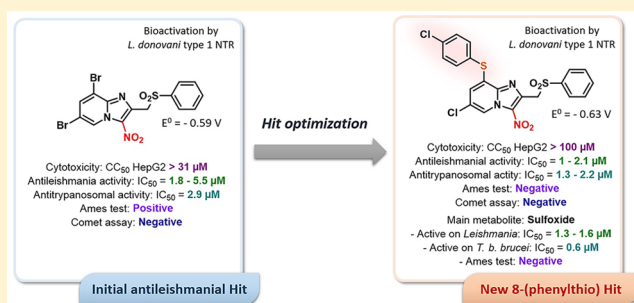
Trypanosomatids are flagellated protozoan parasites that belong to the Kinetoplastid order. These unicellular parasites are responsible for several vector borne diseases in mammals. Among the trypanosomatids, Leishmania spp and Trypanosoma spp are the two main parasites encountered in human pathology 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

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]
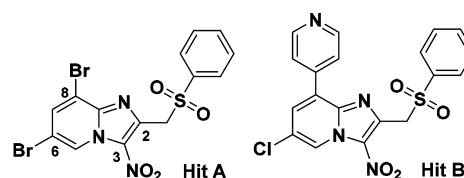
Recently, nitroaromatic molecules, such as fexinidazole[9] and delamanid,[10] showed a renewed interest against infectious diseases such as HAT, VL, CD, and even tuberculosis.[11] Indeed, these compounds act as prodrugs, requiring the bioactivation of their nitro group into reduced reactive intermediates, 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.


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

	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

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

Table 1. Synthesis, *in Vitro* Antileishmanial Activity, and Cytotoxicity of 3-Nitroimidazopyridine Derivatives 1−26[a]
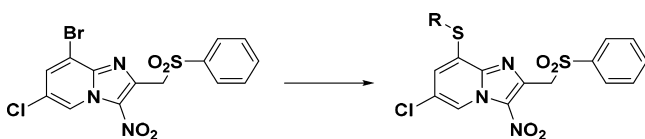
Cmpd	R-	*L. donovani* promastigotes IC_{50} (μM)	cytotoxicity HepG2 CC_{50} (μM)	selectivity index[f]
1	C_6H_5-	1.7 ± 0.1	>3.9[d]	>2.3
2	**4-Br-C_6H_4-**	**1.0 ± 0.3**	**>62.5[d]**	**>62.5**
3	2-Cl-C_6H_4-	1.4 ± 0.3	>5[d]	>3.6
4	3-Cl-C_6H_4-	0.7 ± 0.1	>3.9[d]	>3.6
5	**4-Cl-C_6H_4-**	**1.0 ± 0.3**	**>100[d]**	**>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.
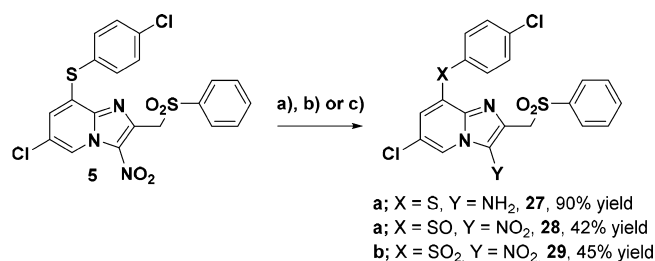
The mutagenicity of many nitroaromatic molecules has been known for many years.[25] Nevertheless, considering the therapeutic potential of these molecules, many attempts have been made to dissociate their anti-infective activity from their mutagenic character.[26] However, the difficulty remains that 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 nitro-aromatic 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

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]

a; X = S, Y = NH$_2$, **27**, 90% yield
a; X = SO, Y = NO$_2$, **28**, 42% yield
b; X = SO$_2$, Y = NO$_2$, **29**, 45% yield

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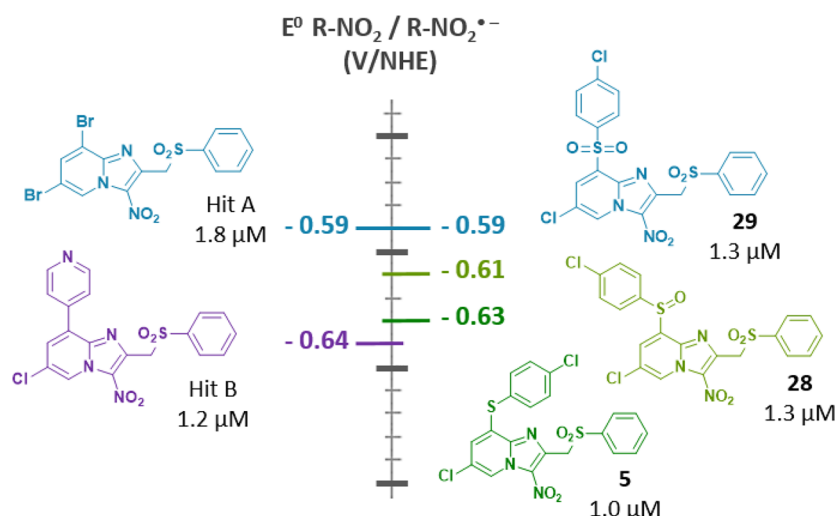
Figure 2. Reduction potentials measured for hit **A**, hit **B**, hit compound **5**, metabolite **28**, and derivative **29**.

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·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)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

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

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