Evaluation of substituted ebselen derivatives as potential trypanocidal agents

Heeren M. Gordhan, Stephen L. Patrick, Maria I. Swasy, Amber L. Hackler, Mark Anayee, Jennifer E. Golden, James C. Morris, Daniel C. Whitehead

PII: S0960-894X(16)31288-4
DOI: http://dx.doi.org/10.1016/j.bmcl.2016.12.021
Reference: BMCL 24510

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 11 November 2016
Revised Date: 5 December 2016
Accepted Date: 7 December 2016

Please cite this article as: Gordhan, H.M., Patrick, S.L., Swasy, M.I., Hackler, A.L., Anayee, M., Golden, J.E., Morris, J.C., Whitehead, D.C., Evaluation of substituted ebselen derivatives as potential trypanocidal agents, *Bioorganic & Medicinal Chemistry Letters* (2016), doi: http://dx.doi.org/10.1016/j.bmcl.2016.12.021

Evaluation of substituted ebselen derivatives as potential trypanocidal agents

Heeren M. Gordhan[a]; Stephen L. Patrick[b]; Maria I. Swasy[a]; Amber L. Hackler[b]; Mark Anayee[a]; Jennifer E. Golden,[c] James C. Morris[b]*; Daniel C. Whitehead[a]*

[a]Department of Chemistry, 467 Hunter Laboratories, Clemson University, Clemson, SC, 29634, USA

[b]Eukaryotic Pathogens Innovation Center, Department of Genetics and Biochemistry, 249 Life Sciences Building, Clemson University, Clemson, SC, 29634, USA

[c]Department of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin, 777 Highland Ave., Madison, WI 53705-2222, USA

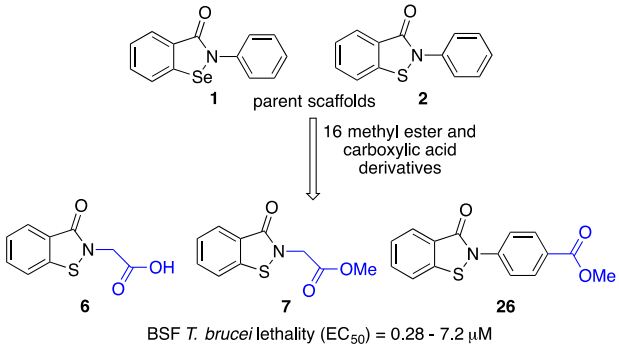
Abstract

Human African trypanosomiasis is a disease of sub-Saharan Africa, where millions are at risk for the illness. The disease, commonly referred to as African sleeping sickness, is caused by an infection by the eukaryotic pathogen, *Trypanosoma brucei*. Previously, a target-based high throughput screen revealed ebselen (*EbSe*), and its sulfur analog, *EbS,* to be potent *in vitro* inhibitors of the *T. brucei* hexokinase 1 (TbHK1).

These molecules also exhibited potent trypanocidal activity *in vivo*. In this manuscript, we synthesized a series of sixteen *EbSe* and *EbS* derivatives bearing electron-withdrawing carboxylic acid and methyl ester functional groups, and evaluated the influence of these substituents on the biological efficacy of the parent scaffold. With the exception of one methyl ester derivative, these modifications ablated or blunted the potent TbHK1 inhibition of the parent scaffold. Nonetheless, a few of the methyl ester derivatives still exhibited trypanocidal effects with single-digit micromolar or high nanomolar EC_{50} values.

Keywords: ebselen; trypanosomes; hexokinases

Graphical Abstract

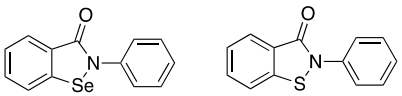


Human African trypanosomiasis (HAT), or African Sleeping Sickness, is caused by the bloodstream form (BSF) of the eukaryotic pathogen *Trypanosoma brucei* and is transmitted by the tsetse fly.[1] Two subspecies of *T. brucei* cause human disease: *T. b. gambiense*, responsible for West African sleeping sickness, and *T. b. rhodesiense*, responsible for the more acute East African sleeping sickness.[1-3] Although treatments for HAT have existed for decades, these therapeutics are marked by serious adverse side-effects, such as acute toxicity, neural disorders, and in some cases death.[4] In other cases, the therapeutics currently available are ineffective against the late stages of the disease once the trypanosomes cross the blood-brain barrier.[1,4]

BSF trypanosomes generate energy currency, in the form of adenosine triphosphate (ATP), through glycolysis, which occurs within a specialized organelle called the glycosome.[1,3,4] The first step of glycolysis involves the phosphorylation of glucose by enzymes known as hexokinases (HKs).[4] In trypanosomes, the HKs involved in the first step of glycolysis are called TbHK1 and TbHK2, with the former having been genetically and chemically validated for therapeutic design.[5] Several known inhibitors of mammalian hexokinases have demonstrated activity toward the inhibition of TbHK1 *in vitro* and exhibit trypanocidal effects in whole cell assays.[5] One such agent is lonidamine exhibiting an IC_{50} value (half-maximal inhibitory concentration) of 850 μM toward hexokinases in recombinant TbHK1 (*r*TbHK1), expressed and purified as a transgene in *Escherichia coli* or yeast.[2]

Through a high-throughput screening (HTS) effort conducted by some of us, two related groups of novel inhibitors, the isobenzothiazolinone and isobenzoselenazolinone classes, were discovered as inhibitors of TbHK1. One of six isobenzoselenazolinone

candidates was ebselen (*EbSe*, **1**, Scheme 1), a drug that is non-toxic to humans and has been evaluated in clinical trials in ischemic stroke patients.[3,5] Ebselen demonstrated high potency against *r*TbHK1 with an IC_{50} of 0.05 ± 0.03 μM.[3,5] Similarly, *EbS* (*i.e.* 2-phenyl-1,2-benzisothiazol-3(*2H*)-one, **2**, Scheme 1), which replaces the selenium in ebselen with sulfur, had an IC_{50} value of 2.0 ± 0.5 μM against *r*TbHK1.[3,5] Subsequently, we demonstrated that incubation of TbHK1 with *EbSe* and *EbS* inhibitors led to a five-fold decrease in hexokinase activity that remained irreversible despite a 200–fold dilution of enzyme and inhibitor.[3] One hypothesis for the efficacy of *EbSe* toward the inhibition of the trypanosome enzyme is the formation of selenyl-sulfide (Se-S) adducts with Cys residues within the hexokinase, leading to the eventual oxidation of certain Cys residues to the corresponding sulfonic acid.[3] Indeed, we demonstrated that *EbSe* induced the oxidation of two Cys residues in TbHK1, C327 and C369, resulting in significantly reduced enzymatic activity.[3]



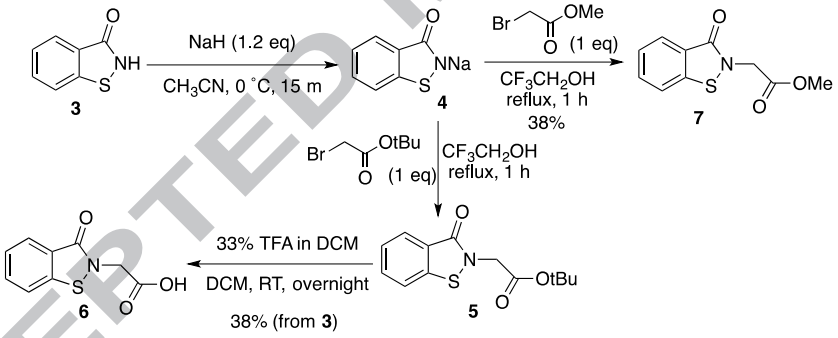
EbSe; **1** EbS; **2**

Scheme 1. *EbSe* (**1**) and *EbS* (**2**) discovered through HTS and further evaluated by Joice *et al*.

In this manuscript, we sought to expand upon this initial discovery by exploring the synthesis of a series of *EbSe*/*EbS* derivatives modified with electron-withdrawing carboxylic acid and methyl ester substituents. Our motivation to pursue these derivatives was three-fold: 1.) we hypothesized that the incorporation of electron-withdrawing substituents onto the scaffold might enhance the electrophilicity of the chalcogen in the core scaffold, thus facilitating the covalent modification of TbHK1 and

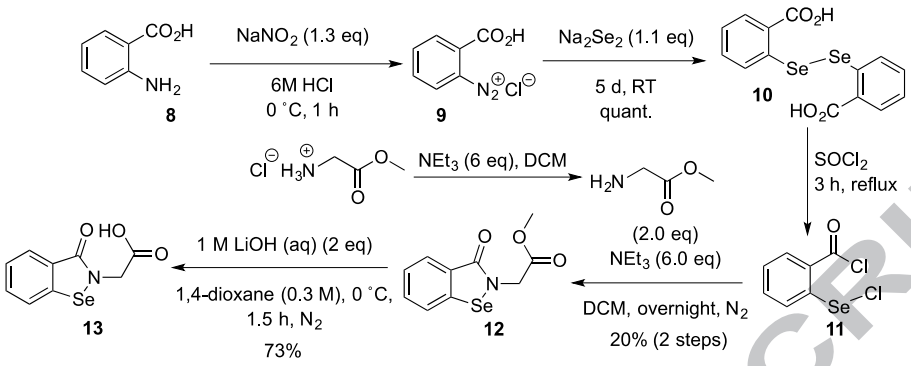
enhance *in vivo* potency[3], 2.) the carboxylic acid moiety, if tolerated, would offer a convenient synthetic handle for further exploration of derivative structures, and 3.) the carboxylic acid moiety would provide a simple handle for the incorporation of the drug onto a peptidic targeting sequence in order to investigate a novel drug-delivery strategy.[6]

Our study commenced with the synthesis of a series of *EbSe*/*EbS* derivatives bearing a carboxymethyl moiety on the nitrogen atom of the heterocycle. The first *EbS* derivative **6** was synthesized according to a one-pot protocol described by Dou, *et al*.[11] Thus, the corresponding methyl ester **7** was isolated by alkylation of the nitrogen atom of **3** in 38% yield (Scheme 2).[7] Similarly, **3** was alkylated to give **5**, followed by *t*-Bu ester cleavage to give acid **6**.



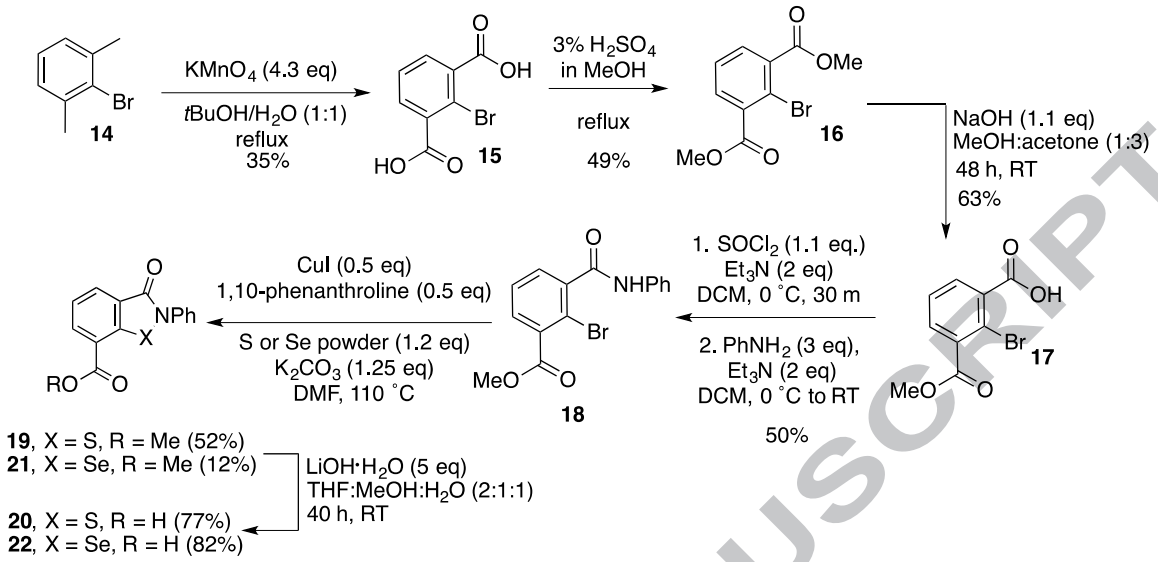
Scheme 2: Preparation of *EbS* derivatives **6** and **7**.

The synthesis of the analogous *EbSe* derivative **13** (Scheme 3) initiated with the diazotization of anthranilic acid **8**.[8-10] The diazonium salt **9** was treated with disodium diselenide to provide diselenide **10,** which was subsequently treated with thionyl chloride under reflux to afford selenyl chloride **11**. That compound was then acylated with the methyl ester of glycine to afford **12** in a modest 20% yield.[8,11] Hydrolysis of the methyl ester in **12** with lithium hydroxide provided *EbSe* derivative **13** in 73% yield.[7]



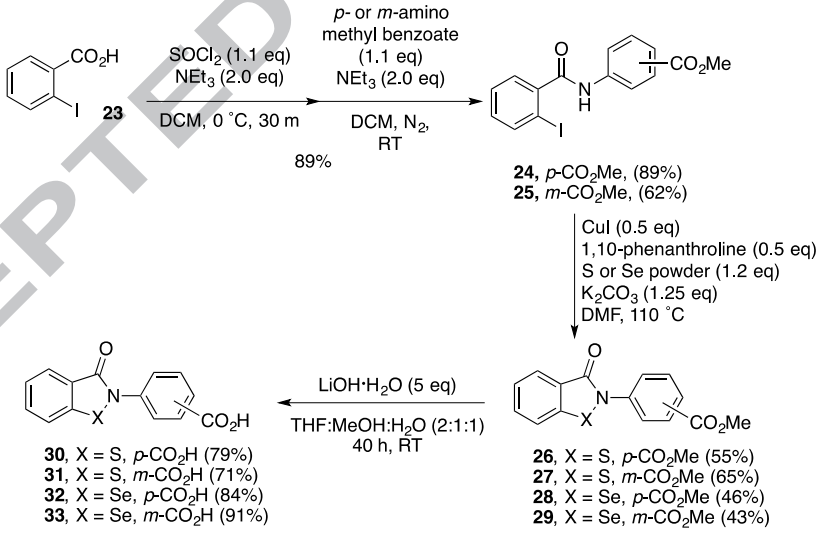
Scheme 3: Preparation of *EbSe* derivatives **12** and **13**.

Synthetic protocols for the second *EbS* and *EbSe* derivatives, bearing a carboxylic acid at the *ortho*-position relative to the chalcogen, are shown in Scheme 4. The symmetrical, commercially available, 2-bromo-1,3-dimethylbenzene **14** was oxidized to the isophthalic acid **15** in 35% yield by action of potassium permanganate.[12] Fischer esterification of **15** in the presence of methanol and sulfuric acid returned the dimethyl ester **16** in 49% yield.[12] The symmetrical dimethyl ester was then hydrolyzed with sodium hydroxide to afford monoacid **17**.[13] Monoacid **17** was converted to the corresponding amide **18** in 50% yield by a two-step process involving conversion to the acid chloride followed by amidation with aniline. Amide **18** was then cyclized by a convenient copper (I) iodide protocol in the presence of sulfur powder to afford **19** in 52% yield.[14,15] The resulting *EbS* methyl ester was hydrolyzed with lithium hydroxide monohydrate to provide carboxylic acid **20** in 77% yield.[16] In a similar fashion, the *EbSe* methyl ester **21** was obtained, albeit in a poor 12% yield, and was subsequently hydrolyzed to afford **22** in 82% yield.[12-16]



Scheme 4. Synthesis of EbS derivatives **19** and **20** and EbSe derivatives **21** and **22**.

Further varieties of *EbS*/*EbSe* derivatives were obtained by incorporating the carboxylic acid functionality in either the *para* or *meta* positions of the *N*-phenyl ring. The synthesis of these derivatives are depicted in general in Scheme 5. Iodobenzoic



Scheme 5. Synthesis of ester derivatives **26**-**29** and carboxylic acid derivatives **30**-**33**.

acid **23** was converted to its corresponding acid chloride by action of thionyl chloride followed by amidation with either the *para* or *meta* isomer of amino methylbenzoate to afford amides **24** (89% yield) and **25** (62% yield), respectively. Amides **24** and **25** were

each reacted in the presence of either sulfur or selenium powder under the copper(I) iodide/1,10-phenanthroline conditions described above.[14,15] In this manner, methyl ester analogs **26** (55% yield), **27** (65% yield), **28** (46% yield), and **29** (43% yield) were prepared. This suite of methyl esters was then subjected to standard saponification conditions[16] to afford acid analogs **30** (79% yield), **31** (79% yield), **32** (84% yield), and **33** (91% yield).

Next, we evaluated the carboxylic acid and methyl ester derivatives of *EbSe* and *EbS* against both TbHK1 *in vitro* and against BSF trypanosomes in whole-cell assays (conducted in triplicate). This biological screening yielded four results: percent TbHK1 inhibition, IC_{50} values against TbHK1, percent BSF growth inhibition, and BSF LD_{50}. The derivatives were screened against TbHK1 and BSF *T. brucei* at a standard concentration of 10 μM. Enzyme activity of *r*TbHK1 was scored after incubation with the drug with the percent inhibition of the enzyme being recorded. The reduction in trypanosome growth in a cell culture, caused by incubation with the drug, is reflected by the BSF growth inhibition value. BSF EC_{50} values refer to the dose of compound necessary to reduce cell number by one-half *in vitro* and was pursued for those compounds that demonstrated promising activity at 10 μM. **Figure 2** shows the *EbSe/EbS* derivatives that were screened against TbHK1 and whole cell trypanosomes (*i.e.* **6**, **7**, **12**, **13**, **19**-**22**, and **26-33**). The biological data resulting from the evaluation of these molecules is assembled in Table 1.

Recall that percent TbHK1 inhibition (determined at 10 μM drug concentration) refers to the amount of rTbHK1 enzyme inhibition. Referring to **Table 1**, only a few of the *EbSe/EbSe* derivatives exhibited any activity against TbHK1 in preliminary trials.

EbS ester **7** was weakly active against TbHK1 showing 12.8 ± 7.5% inhibition of the enzyme. EbS derivative **26**, bearing a *para*-methyl ester on the *N*-phenyl substituent exhibited a 22.8 ± 3.8% inhibition of TbHK1, but its corresponding carboxylic acid **30** exhibited no activity. The most potent molecule in this assay was *EbSe* ester **28**, which demonstrated a 79.0 ± 2.8% TbHK1 inhibition. Thus, the IC_{50} value for TbHK1 inhibition by *EbSe* ester **28** was determined to be 3.2 ± 0.01 μM. Interestingly, positioning the methyl ester in the *meta* position (*i.e.* **27**) instead of *para* to the chalcogen completely ablated the TbHK1 inhibitory activity observed for the latter compound. Finally, the only free carboxylic acid that elicited only mild TbHK1 inhibition was *EbSe* acid **32**, bearing a carboxylic acid at the *meta* position on the *N*-aryl ring (18.5 ± 2.8% TbHK1 inhibition). The relatively poor performance of the carboxylic acid derivatives as compared to the corresponding methyl esters **26** and **28**, may suggest that the anionic carboxylate may stymie interactions with the enzyme.

The percent BSF growth inhibition (determined at 10 μM) refers to the reduction in trypanosome growth in a whole cell assay (conducted in triplicate) by the non-conjugated *EbSe/EbS* analogues. Of the *N*-carboxymethylated *EbSe/EbS* derivatives, *EbS* methyl ester **7** revealed a potent BSF growth inhibition of 98.3 ± 1.3%, and its corresponding acid **6** effected a BSF growth inhibition of 77.6 ± 4.3%. Of the *ortho*-substituted (*i.e.* relative to the chalcogen) analogues, the esters **19** and **21** exhibited 52.5 ± 4.9% and 55.6 ± 7.5% BSF growth inhibition, respectively. Further, the corresponding acids **21** and **22** were ineffective toward promoting trypanosome growth inhibition. The *para*-substituted *N*-aryl methyl ester **26** exhibited 66.6 ± 10.9% BSF

growth inhibition, while its corresponding acid **30** was ineffective against trypanosome growth. The *meta*-substituted analogs were not effective at inhibiting trypanosome growth. The *para*-substituted N-aryl methyl ester derivative of *EbSe* (*i.e.* **28**) showed 38.6 ± 3.8% BSF growth inhibition, while its corresponding acid **32** was not active. Similar to the *meta*-substituted *N*-aryl esters and carboxylic acids of *EbS*, the corresponding *meta*-substituted *EbSe* ester **29** and acid **33** did not promote effective growth inhibition against trypanosomes. Thus, the most active analog from the collection of small molecule *EbSe/EbS* esters and carboxylic acids was *N*-carboxymethylated ester of *EbS* (*i.e.* **7**).

It is evident that the methyl ester derivatives (*i.e.* **7**, **19**, **21**, **26**, and **28**) were more effective at inhibiting the growth of BSF trypanosomes than their corresponding carboxylic acid derivatives (with the notable exception of carboxylic acid **6**). We attribute the greater efficacy of the charge-neutral ester derivatives to their presumed ability to more readily cross the cell membrane than their anionic carboxylic acid counterparts.[4,17] Further, it is curious that these molecules are relatively adept at inhibiting BSF parasite growth despite their lackluster performance as *in vitro* TbHK1 inhibitors.

Four of the *EbSe*/*EbS* methyl esters yielded reasonably potent BSF EC_{50} values. The *N*-carboxymethyl *EbS* acid **6** and ester **7** exhibited a BSF EC_{50} values of 7.24 ± 0.5 μM and 0.28 ± 0.1 μM, respectively. *EbSe* ester **28**, with *para N*-phenyl functionalization, revealed a higher BSF EC_{50} value of 13.0 ± 1.5 μM. The *para*-substituted *N*-phenyl *EbS* methyl ester **26** exhibited BSF EC_{50} value of 3.8 ± 0.1 μM.

Table 1: Biological data on *EbS* and *EbSe* derivatives.[a]

STRUCTURE	TbHK1% Inhibition (10 μM)	TbHK1 IC_{50} (μM)	% BSF Growth Inhibition (10 μM)	BSF EC_{50} (μM)
1[b]	-	0.05 ± 0.03	-	2.9 ± 0.28
2[b]	-	2.0 ± 0.5	-	0.03 ± 0.07
6	0.87 ± 7.9%	>10	77.6 ± 4.3%	7.2 ± 0.5
7	12.8 ± 7.6%	>10	98.3 ± 1.3%	0.28 ± 0.1
12	0%	>10	0%	>10
13	0%	>10	0%	>10
19	18.0 ± 0.4%	>10	52.5 ± 4.9%	~10
20	0%	>10	10.9 ± 10.9%	>10
21	2.7 ± 5.1%	>10	55.6 ± 7.5%	~10
22	0%	>10	0%	>10
26	22.8 ± 3.8%	>10	66.5 ± 10.9%	3.8 ± 0.1
27	1.7 ± 0.3%	>10	8.8 ± 22.1%	>10
28	79.0 ± 2.8%	3.16 ± 0.06	38.6 ± 3.8%	13.0 ± 1.5
29	0%	>10	18.0 ± 20.8%	>10
30	0%	>10	0%	>10
31	0%	>10	15.8 ± 47.3%	>10
32	17.2 ± 14.6%	>10	12.0 ± 17.4%	>10
33	18.5 ± 2.8%	>10	0%	>10

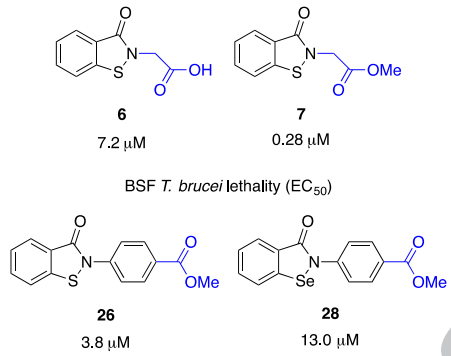
[a]Trypanosome cultures were assayed in triplicate in a total volume of 40 μL, and the DMSO vehicle was used as a negative control. Percent inhibition was calculated by comparison to the growth of parasites grown with the DMSO controls from each plate. EbS (10 μM) was included in each assay as a positive control. Dose-response curves performed in triplicate for compounds that elicited > 50% growth inhibition at 10 μM were pursued in a 384-well plate format, with 50% effective concentrations (*i.e.* EC_{50}) determined using GraphPad Prism (version 6.0) software (GraphPad Software, Inc., La Jolla, CA). [b]data taken from Ref. 5.

In summary, most of the carboxylic acid derivatives of **1** and **2** (*i.e.* **6**, **13**, **20**, **22**, **30**, and **31**) were ineffective against the TbHK1 enzyme *in vitro*, while *EbSe* acids **32** and **33** were modestly active, demonstrating ~17-18% inhibition. The corresponding esters (**7**, **12**, **19**, **21**, **26**, and **27**) showed improved activity against TbHK1 compared to the acid derivatives, with *EbSe* ester **28** showing the greatest enzyme inhibition (*ca.* 79%).

With respect to growth inhibition of whole-cell BSF parasites, some of the ester derivatives exhibited moderate to potent inhibitory activity while the corresponding carboxylic acids (with the notable exception of **6**) were poor inhibitors. Indeed, *EbS* ester **7** demonstrated almost complete inhibition of BSF growth.

Ultimately, four of the sixteen derivatives prepared in this study exhibited similar trypanocidal effects as compared to the parent compounds **1** and **2** (Scheme 6). The most potent compound arising from our study was methyl ester **7** exhibiting an EC_{50} value of 0.28 ± 0.1 μM, placing it in between EbS **2** (EC_{50} = 0.030 ± 0.067 μM)[5] and EbSe **1** (EC_{50} = 2.9 ± 0.28 μM)[5] in terms of trypanocidal potency. Further, compounds **6** and **26** returned EC_{50} values below 10 μM. It is particularly interesting that compound **28** was the only molecule in our study whose trypanocidal activity was harbingered by potent *in vitro* TbHK1 inhibition (~80 % inhibition). Indeed, the most potent compound in terms of trypanocidal activity (*i.e.* **7**) arising from our study exhibited a relatively unimpressive ~13% inhibition of TbHK1 *in vitro*. Thus, this study may suggest that the potent trypanocidal activity of the parent molecules, EbSe (**1**) and EbS (**2**) may only be partly explained by their potent TbHK1 inhibition.[3,5] Rather, this class of molecules may disrupt multiple vital functions within the BSF parasite in order elicit their potent

trypanocidal properties. Future studies in our laboratories will focus in part on understanding the mechanism of action of his class of potent trypanocides.



Scheme 6. Most potent EbSe/EbS derivatives.

Acknowledgements

The authors would like to thank Clemson University and the Department of Chemistry for providing funding. Work in the JCM laboratory was partially supported by a Center for Biomedical Excellence (COBRE) grant of the National Institutes of Health under award number P20GM109094 and by an award from the National Institute of Allergy and Infectious Diseases of the National Institutes of Health (R21AI105656). Additionally, the Clemson University Honors College and the Clemson University Creative Inquiry Program are thanked for their support. The supplementary information associated with this article can be found in the online version at [enter URL here].

References:

(1). Hide, G. *Clin. Microbiol. Rev*. **1999**, *12* (1), 112-125.
(2). Chambers, J.W.; Fowler, M.L.; Morris, M.T.; Morris, J.C. *Molecular & Biochemical Parasitology*. **2008**, *158*, 202-207.
(3). Joice, A.C.; Harris, M.T.; Kahney, E.W.; Dodson, H.C.; Maselli, A.G.; Whitehead, D.C.; Morris, J.C. *International Journal for Parasitology: Drugs and Drug Resistance*. **2013**, *3*, 154-160.

(4). Nwagwu, M.; Opperdoes, F.R. *Acta Trop*. **1982**, *39* (1), 61-72.
(5). Sharlow, E.R.; Lyda, T.A.; Dodson, H.C.; Mustata, G.; Morris, M.T.; Leimgruber, S.S.; Lee, KH.; Kashiwada, Y.; Close, D.; Lazo, J.S.; Morris, J.C. *PLoS Neglected Tropical Diseases*. **2010**, *4* (4), e659.
(6). We briefly evaluated a strategy that tagged the EbSe/EbS carboxylic acid derivatives with a peptidic peroxisome targeting sequence that has previously been shown to deliver molecular cargo to glycosome of BSF *T. brucei* parasites (See: Lin, S.; Morris, M.T.; Ackroyd, P.C.; Morris, J.C.; Christensen, K.A. *Biochemistry*. **2013**, *52*, 3629-3637). While this study did not yield promising results in terms of trypanocidal activity, our efforts toward that end are described in the Electronic Supplementary Information.
(7). Dou, D.; Alex, D.; Du, B.; Tiew, KC. Aravapalli, S.; Mandadapu, S.R.; Calderone, R.; Groutas, W.C. *Bioorg. Med. Chem*. **2011**, *19* (19), 5782-5787.
(8). Kamigata, N.; Iizuka, H.; Izuoka, A.; Kobayashi, M. *Bull. Chem. Soc. Jpn*. **1986**, *59* (7), 2179-83.
(9). Selvakumar, K.; Shah, P.; Singh, H.B.; Butcher, R.J. *Chem. Eur. J.* **2011**, *17* (45), 12741-12755.
(10). Bhakuni, B.S.; Balkrishna, S.J.; Kumar, A.; Kumar, S. *Tetrahedron Lett*. **2012**, *53*, 1354-1357.
(11). Stoyanovsky, D.A.; Jiang, J.; Murphy, M.P.; Epperly, M.; Zhang, X.; Li, S.; Greenberger, J.; Kagan, V.; Bayir, H. *ACS Med. Chem. Lett*. **2014**, *5* (12), 1304-1307.
(12). Bhakuni, B.S.; Balkrishna, S.J.; Kumar, A.; Kumar, S. *Tetrahedron Lett*. **2012**, *53*, 1354-1357.
(13). Dahl, B.J.; Branchaud, B.P. *Org. Lett*. **2006**, *8* (25), 5841-5844.
(14). Bhayana, B.; Wilcox, C.S.; *Angew. Chem. Int. Ed*. **2007**, *46*, 6833-6836.
(15). Krasikova, V.; Katkevics, M. *Chem. Heterocycl. Compd*. **2013**, *48* (11), 1684-1690.
(16). Wehlan, H.; Jezek, E.; Lebrasseur, N.; Pavé, G.; Roulland, E.; White, A.J.P.; Burrows, J.N.; Barrett, A.G.M. *J. Org. Chem*. **2006**, *71*, 8151-8158.
(17). Silverman, R.B. *The Organic Chemistry of Drug Design and Drug Action*, 2nd ed.; Elsevier: Burlington, 2004.