Synthesis of Analogs of Waltheriones S and T and Their Activity Against *Trypanosoma cruzi*

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Abstract

Several alkaloids of the waltherione family exhibit antitrypanosomal activity in the sub-micromolar or nanomolar range. While the overwhelming majority of waltheriones are based on a quinoline core structure, two structurally simpler pyridone-based congeners, waltheriones S and T, have recently been isolated and found to inhibit *Trypanosoma cruzi* with single-digit micromolar potency. Here, we report on the synthesis of a series of analogs of waltheriones S and T based on pyridone ring formation *via* cyclization of an appropriate triketone precursor with ammonia and the assessment of their activity against *Trypanosoma cruzi*. The data show that the methoxy group at the C(3)-position of the pyridone ring can be removed without significant loss in potency. Further modification of 3-desmethoxy waltherione T through methoxylation at the C(1') position of the C(6)-side chain or double methoxylation at the C(1')-position and the pyridone nitrogen had no significant impact on antitrypanosomal activity. These findings contrast with the activity differences between the corresponding quinoline-based natural waltheriones M, Q, and H, where the methoxy-bearing waltheriones Q and H are one order of magnitude more potent than the unsubstituted parent compound waltherione M. Our data indicate that the SAR for monocyclic waltheriones S and T does not simply parallel that of the quinoline-based congeners and they point to the importance of a rigid quinoline core for potent activity against *T. cruzi*.

Introduction

Chagas disease, also known as American trypanosomiasis, is a devastating infectious disease that is estimated to affect 6-7 Mio individuals globally and that is responsible for *ca*. 12 000 deaths every year.[1] Chagas is a protozoal disease that is caused by the trypanosomatid *Trypanosoma cruzi*, which is mostly transmitted by blood-sucking triatomine insects, although other routes of transmission are also possible.[2,3] Only two old drugs, benznidazole and nifurtimox, are currently available for the treatment of Chagas disease;[1,4] while these drugs can be curative when administered shortly after the infection, their efficacy diminishes at later stages of the disease. In addition, both drugs are associated with significant side effects, especially in older patients. While significant progress has been made in the development of new drugs against other trypanosomatidic diseases, this is not the case for Chagas disease, where a preclinical pipeline is virtually absent and clinical studies are currently performed only with drugs approved/in development for other indications.[5,6]

Among compounds that have been reported to inhibit intracellular *T. cruzi* amastigotes *in vitro* with high potency and selectivity[6] are a number of quinoline alkaloids that were isolated from the roots of *Waltheria indica*.[7,8] This includes, for example, waltheriones F (**1**), M (**2**), G (**3**), Q (**4**), H (**5**), and J (**6**) (Figure 1), which exhibit IC_{50} values against *T. cruzi* of 0.02 - 1.1 μM; **1**, **3**, and **4** show selectivity ratios *vs*. non-infected L6 rat skeletal muscle myoblasts cells of >10.[7]

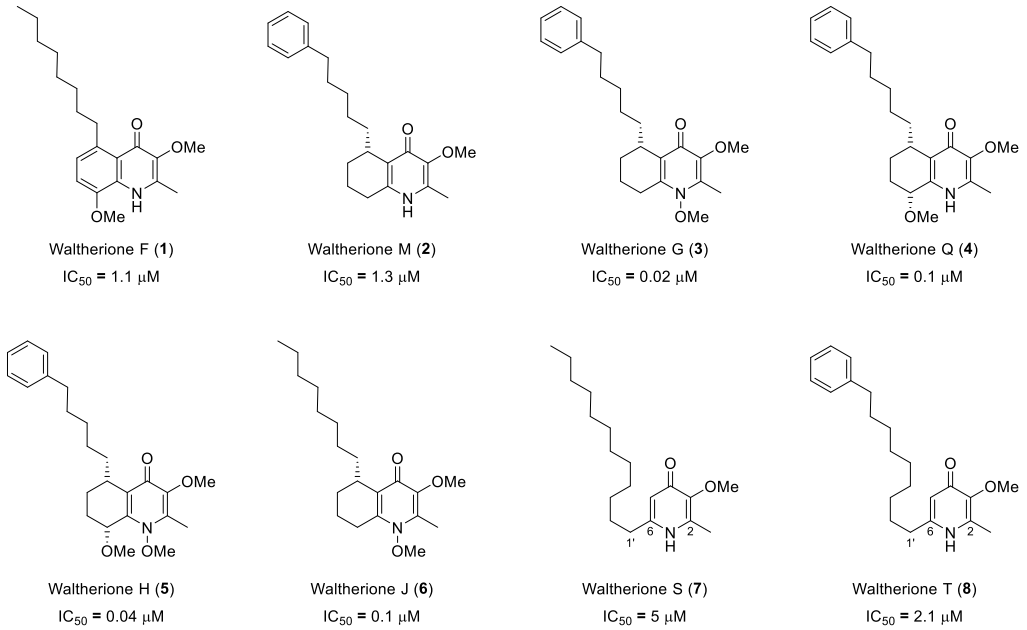


Figure 1. Selected waltheriones from *Waltheria indica* and their IC_{50} values against *T. cruzi* amastigotes in L6 rat skeletal muscle myoblast cells. Data for **1**, **2**, **3**, **5**, and **6** are from ref. [7], those for **4**, **7**, and **8** are from ref. [8].

More recently, the continued phytochemical exploration of *Waltheria indica* has also yielded monocyclic pyridone-derived waltheriones S (**7**) and T (**8**) (Figure 1), which were found to be similarly effective *T. cruzi* inhibitors as waltheriones F, I, and L[7] (IC_{50} values of 5.0 and 2.1 μM, respectively; selectivity ratios of >6 and >13).[8] In particular, waltherione T (**8**) is essentially equipotent with waltherione M (**2**) (Figure 1)[7] but shows a higher selectivity ratio (>13 *vs*. 5). The molecular target(s) of waltheriones has (have) not been elucidated. Likewise, no SAR studies either on bicyclic waltheriones or the pyridone-derived variants **7** and **8** have been reported to date that would have assessed structural modifications other than those present in the different natural product congeners. While the synthesis of a series of waltherione F analogs has been reported,[9] along with the total synthesis of the natural product itself, no biological data were disclosed for these compounds. Apart from waltherione F,[9-11] the only other waltherione for which a total synthesis has been completed is waltherione A,[12] which is only a weak inhibitor of *T. cruzi*.[13]

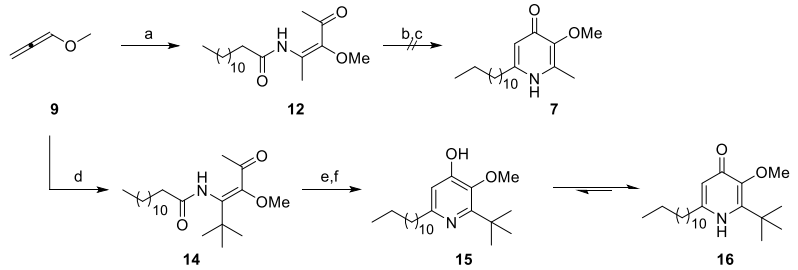
In this communication, we describe the synthesis of a series of analogs of waltheriones S (**7**) and T (**8**) and the assessment of their activity against *T. cruzi* amastigotes in L6 cells. In particular, we were interested if oxidative modifications of these monocyclic waltheriones at C(6) and C(1'), in analogy to the quinoline-derived congeners, would lead to enhanced antitrypanosomal activity. The SAR data accrued in our study indicate that the 3-methoxy group in **7** and **8** is not essential for anti-trypanosomal activity; at the same time, no modifications could be identified that would have led to a significant increase in potency, indicating that the exceptional antitrypanosomal activity of waltheriones G, H, J, and Q is linked to their bicyclic structure.[14]

Results and Discussion

In an initial phase, we aimed to explore the effects of replacing the natural C(2)-methyl substituent in waltheriones S and T by groups that would be more bulky and/or more lipophilic. Reissig and co-workers have reported the synthesis of 2,6-disubstituted 3-methoxy-4-hydroxypyridines (which are tautomeric with the corresponding 4-pyridones)[15] *via* the acid-catalyzed cyclization of β-methoxy-β-keto-enamides. The latter were obtained in a three-component reaction between methoxyallene (**9**), a nitrile (delivering the C(2)-substituent) and a carboxylic acid (the source of the C(6)-substituent).[16-20] Following this approach, and in order to establish a basis for analog synthesis, we first investigated the synthesis of natural waltherione S (**7**) *via* the three-component reaction between methoxyallene (**9**), acetonitrile (**10**) and an excess of tridecanoic acid (**11**) (Scheme 1). However, the reaction gave only very low conversion to the desired enamide intermediate **12** (according to ^{1}H-NMR analysis of the crude reaction product); in addition, **12**

could not be separated from excess tridecanoic acid (**11**). The material isolated after flash chromatography was a 14:1 mixture of **11** and **12**, respectively, corresponding to a chemical yield of **12** of only 3.5%. Treatment of this mixture with Et_3N and TMSOTf according to Reissig[16-20] gave none of the desired waltherione S (**7**). The reasons for this outcome are unclear, although we note that only few examples of the above multicomponent reaction have been described with acetonitrile (**10**) as the nitrile component. With trifluoroacetic acid, the corresponding 3-methoxy-4-hydroxypyridine was obtained in 37% overall yield, without purification of the intermediate β-methoxy-β-keto-enamide.[17,20] Reactions with pyridine-2-carboxylic acid[19] or benzoic acid[19] afforded the corresponding enamides in low isolated yields of 22% and 13%, respectively, and it has been suggested that competing reaction pathways may be operating for reactions involving α-acidic nitriles.[20] Indeed, when pivalonitrile (**13**) was substituted for acetonitrile in the reaction with methoxyallene (**9**) and tridecanoic acid (**11**), enamide **14** could be isolated in 56% yield. When treated with Et_3N and TMSOTf, the latter underwent smooth cyclization, affording pyridone **16** in 76% yield (Scheme 1). As the use of nitriles with α-hydrogens in the three-component reaction appeared to be problematic, we chose to investigate an alternative approach towards the targeted 3-methoxy pyridones.

Scheme 1. Attempted synthesis of waltherione S *via* a three-component reaction.[a]

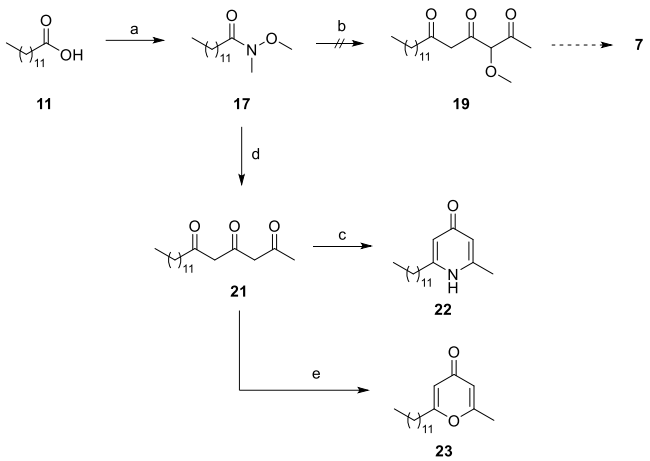


[a]Reagents and conditions. (a) *i*) *n*-BuLi, Et_2O, – 40 °C, 30 min; *ii*) acetonitrile (**10**), – 78 °C, 4h; *iii*) tridecanoic acid (**11**), –78 °C to r.t., 16h, 3.5% based on ^{1}H-NMR analysis; (b) TMSOTf, Et_3N, CH_2Cl_2, 0 °C to reflux, 3 days; (c) AcOH, r.t., 1h; (d) *i*) *n*-BuLi, Et_2O, –40 °C, 30 min; *ii*) pivalonitrile (**13**), –78 °C, 4h; *iii*) tridecanoic acid (**11**), –78 °C to r.t., 56%, 16h; (e) TMSOTf, Et_3N, CH_2Cl_2, 0 °C to reflux, 3 days; (f) AcOH, r.t., 1h, 76%.

To this end, tridecanoic acid (**11**) was converted into its Weinreb amide (**17**), which was submitted to reaction with 3-methoxypentane-2,4-dione (**18**) in the presence of LiHMDS to produce triketone **19** as a precursor for a Paal-Knorr-type cyclization (Scheme 2). These conditions had been identified in previous optimization work with **17** and acetylacetone (**20**) to deliver triketone **21** in 72% yield (Scheme 2). Unfortunately, none of the desired **19** was observed in the reaction of **17** and **18**, either with LiHMDS or with LDA as the base. In light of the difficulties to access waltherione S (and, by inference, 3-methoxy substituted analogs thereof), we chose to determine

if a 3-methoxy group was in fact required for antitrypanosomal activity. We would thereby capitalize on the ready accessibility of triketone **21**, which could indeed be converted into 3-desmethoxy waltherione S (**22**) by reaction with aqueous ammonia in excellent yield (94%) (Scheme 2). Treatment of **21** with PPh_3 and CCl_4[22] also gave pyranone **23** in 65% yield.

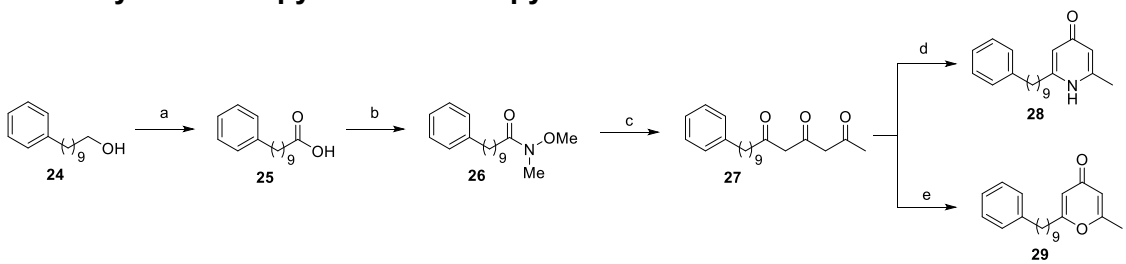
Scheme 2. Synthesis of pyridone 22 and pyranone 23.[a]



[a]Reagents and conditions (a) *N,O*-Dimethyl hydroxylamine hydrochloride, DCC, DMAP, CH_2Cl_2, 0 °C to r.t., 2h, 58%; (b) 3-methoxypentane-2,4-dione (**18**), LiHMDS or LDA, THF, 0 °C to r.t., 1h; (c) acetylacetone (**20**), LiHMDS, THF, 0 °C to r.t., 1h, 72%; (d) NH_4OH (aq. 25%), 100 °C, 3h, 94%; (e) PPh_3, CCl_4, THF, r.t., 4d, 65%.

Interestingly, pyridone **22** was found to be virtually equipotent with waltherione S (**7**) against *T. cruzi* amastigotes in L6 rat skeletal muscle myoblast cells (Table 1), thus indicating that the 3-methoxy group in waltherione S (**7**) was not required for antitrypanosomal activity. To consolidate this finding, we also prepared 3-desmethoxy-waltherione T (**28**) *via* 10-phenyldecanoic acid (**27**), following the same overall approach as for **22** (Scheme 3).

Scheme 3. Synthesis of pyridone 28 and pyranone 29.[a]



[a]Reagents and conditions. (a) Oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, – 55 °C to r.t., 1h; (b) $NaClO_2$, NaH_2PO_4, H_2O, 2-methyl-2-butene/*t*-BuOH (1:1), r.t., 1h, 81% over two steps; (c) *N,O*-dimethyl hydroxylamine hydrochloride, DCC, DMAP, CH_2Cl_2, 0 °C to r.t., 2h, 86%; (d) acetylacetone (**20**), LiHMDS, THF, 0 °C to r.t., 78%, 1h; (e) NH_4OH (aq. 25%), 100 °C, 3h, quant.; (e) PPh_3, CCl_4, THF, r.t., 4d, 51%.

Pyridone **28** was thus obtained in 5 steps and 56% overall yield from commercial 10-phenyl-1-decanol (**24**). In addition, triketone **27** was also transformed into pyranone **29** in 51% yield.[22]

As illustrated by the data in Table 1, 3-desmethoxy-waltherione T (**28**), like **22**, was found to be an equipotent inhibitor of intracellular *T. cruzi* amastigote growth in L6 cells as the parent natural product. Accordingly, **28** exhibits the same *in vitro* activity as the reference drug benznidazole, while **22** appears to be somewhat less potent. Both compounds showed good selectivity *vs*. uninfected host cells, with SI values of 18 and 15.5, respectively (Table 1). Compared to the removal of the 3-methoxy group, the replacement of the 2-methyl substituent in waltherione S (**7**) by a *tert*-butyl group in **16** causes a further reduction in potency; more importantly, **16** shows virtually no selectivity *vs*. non-infected cells (Table 1). A pronounced loss in activity was also observed upon replacement of the pyridone nitrogen by oxygen, with both pyranones **23** and **29** also being less selective than the corresponding parent pyridones **22** and **28**.

Table 1. Antitrypanosomal activity and selectivity of waltherione analogs 16, 22, 28, 23, and 29.

Cpd[a]	IC_{50} *T. cruzi* [μM][b]	Rel. IC_{50}[c]	IC_{50} L6 [μM][d]	SI[e]
16	12.5	4.50	15.6	1.25
22	5.5	1.96	98.0	18.0
23	28.0	10.0	58.4	2.1
28	2.3	0.82	35.5	15.5
29	29.0	10.36	57.0	1.96
Waltherione S (**7**)	5.0[f]	1.78	>32	>6
Waltherione T (**8**)	2.1[f]	0.75	>29	>13
Benznidazole[f]	2.9[f]/2.8	1	>345[g]	>139[g]

[a]For cpd structures, see Schemes 1-3. [b]Inhibition of intracellular *T. cruzi* amastigote growth in L6 rat skeletal muscle myoblast cells. Data are average values of two independent experiments. For *T. cruzi* inhibition, deviations of individual IC_{50} values from the average were < 20%. [c]Relative IC_{50} values: IC_{50} compound/IC_{50} benznidazole control in the same set of experiments. [d]Inhibition of proliferation of uninfected L6 cells. Data are from two independent experiments. Deviations of individual IC_{50} from the average were < 20%, except for **22**, where the individual IC_{50} values were 161.5 μM and 35.1 μM. This is due to solubility problems. [e]Selectivity index = IC_{50} cytotoxicity/IC_{50} antitrypanosomal activity. [f]Positive control. [g]Data from ref. [8].

Based on these findings, our focus shifted towards the evaluation of oxidative modifications of 3-desmethoxy-waltherione T (**28**) at the benzylic position of the C(6)-side chain and the pyridone nitrogen. This line of investigation was driven by the previous observation in the literature that the C(1')- and/or N-methoxylated waltheriones G, H, J, and Q were significantly more potent inhibitors of *T. cruzi* than the unsubstituted parent waltherione M (*cf*. Figure 1).[7,8] Specifically, our primary targets for synthesis were C(1')-methoxy-3-desmethoxy waltherione T (**30**) and its N-methoxy

derivative (**31**) (Figure 2), which are related to waltheriones Q and H (Figure 1), respectively. The synthesis of both compounds was planned to proceed through an appropriately protected derivative of C(1')-hydroxy-3-desmethoxy waltherione T; this would allow not only the introduction of a methoxy group at C(1') but also of functionalized handles for target identification studies.

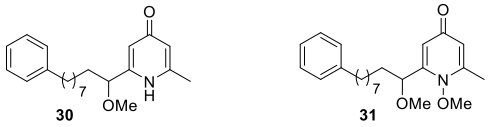
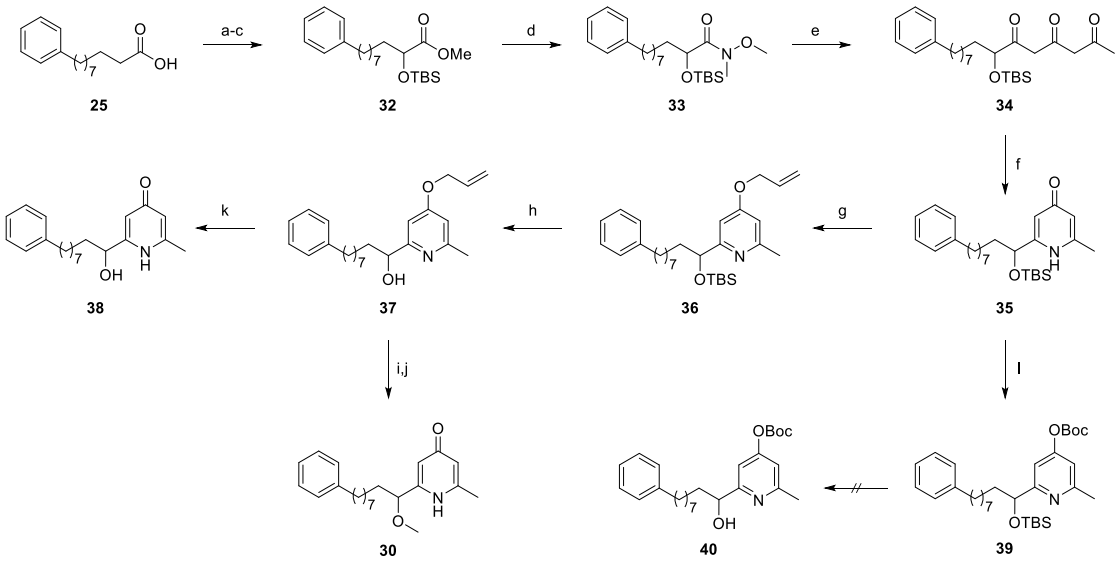


Figure 2. Methoxylated analogs of 3-desmethoxy-waltherione T (**28**) targeted for synthesis.

The synthesis of **30** and **31** commenced with the conversion of 10-phenyldecanoic acid (**25**) into methyl ester **32** *via* a sequence of Fischer esterification, α-hydroxylation of the resulting methyl ester with Davis' oxaziridine,[23] and TBS-ether formation in 65% overall yield (Scheme 4). Methyl ester **32** was then transformed into the corresponding Weinreb amide **33** by treatment with *N,O*-dimethyl hydroxylamine hydrochloride and *i*-PrMgCl; reaction of **33** with acetylacetone (**20**) in the presence of LiHMDS gave triketone **34** (Scheme 4).

Scheme 4. Synthesis of pyridones 30 and 38.[a]

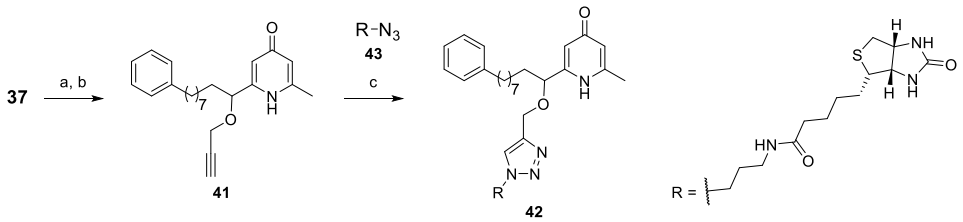


[a]Reagents and conditions. (a) H_2SO4 (64 mol%), MeOH, r.t., 24h; (b) KHMDS, Davis' oxaziridine, THF, – 78 °C to r.t., 1.5h; (c) TBSCl, imidazole, CH_2Cl_2, r.t., 20h, 65% over three steps; (d) *N,O*-dimethyl hydroxylamine hydrochloride, *i*PrMgCl, THF, – 30 °C to – 20 °C, 2h, 85%; (e) acetylacetone (**20**), LiHMDS, THF, 0 °C to r.t., 1h, 76%; (f) NH_4OAc, (±)-CSA (10 mol%), 3Å MS, MeOH, 50 °C, 2.5h, 91%; (g) allyl bromide, K_2CO_3, DMF, 50 °C, 16h, 96%; (h) TBAF (1M THF), THF, 50 °C, 2h, 60%; (i) MeI, NaH, DMF, 0 °C to r.t., 12h; (j) $Pd(PPh_3)_4$ (5 mol%), $NaBH_4$, MeOH/THF (4:1), 0 °C to r.t., 1.5h, 90% over two steps; (k) $Pd(PPh_3)_4$ (5 mol%), $NaBH_4$, MeOH/THF (4:1), 0 °C to r.t., 1h, quant.; (l) NaHMDS, Boc_2O, THF, 80 °C, 1.5h, 74%.

When **34** was subjected to the previously established cyclization conditions (aq. 25% NH_4OH, reflux), pyridone **35** was isolated in 67% yield. In addition, the corresponding free alcohol resulting from concomitant TBS-removal was obtained in 31% yield. Conditions screening eventually identified a combination of NH_4OAc, catalytic (±)-camphorsulfonic acid, and molecular sieves as a superior reagent system for the cyclization reaction, which gave **35** in 91% yield without any detectable loss of the TBS group (Scheme 4). In order to enable the selective alkylation of the benzylic hydroxy group after TBS removal, **35** was then converted into 4-allyloxy-pyridine **36** in 96% yield by alkylation with allyl bromide in the presence of K_2CO_3 as base**.** TBS-removal from **36** furnished alcohol **37**, which was elaborated into C(1')-methoxy-3-desmethoxy waltherione T (**30**) by alkylation with methyl iodide and subsequent Pd-catalyzed allyl ether cleavage in excellent overall yield (90%) (Scheme 4). Direct de-allylation of **37** gave C(1')-hydroxy-3-desmethoxy waltherione T (**38**). It should be noted here that our initial approach towards **30** had involved the protection of **35** as a *tert*-butyl carbonate rather than an allyl ether. However, exposure of the corresponding Boc-protected hydroxy pyridine **39** to TBAF, either unbuffered or buffered with AcOH, at varying temperatures (0 °C to 50 °C) always led to simultaneous cleavage of the Boc- and the TBS-group.[24]

In analogy to the synthesis of **30**, benzylic alcohol **37** was also elaborated into propargyl ether **41**, which underwent 1,3-dipolar cycloaddition with azide **43** in the presence of $CuSO_4$ and sodium ascorbate[25] to furnish probe **42** (Scheme 5).

Scheme 5. Synthesis of waltherione derivatives 41 and 42.[a]

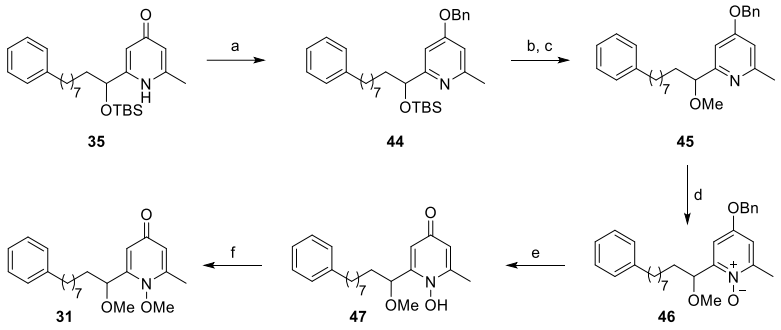


[a]Reagents and conditions. (a) Propargyl bromide, NaH, DMF, 0 °C to r.t., 16h; (b) $Pd(PPh_3)_4$ (5 mol%), $NaBH_4$, MeOH/THF (4:1), 0 °C, 1h, quant. over two steps; (c) $CuSO_4 \cdot 5H_2O$ (1 mol-%), sodium ascorbate (5 mol-%), H2O/*t*BuOH (1:1), r.t., 16h, 50%.

The synthesis of N, C(1')-dimethoxy-3-desmethoxy waltherione T (**31**) proceeded through O-benzyl protected hydroxy pyridine **44**, which was obtained from **35** by reaction with benzyl bromide, followed by TBS-removal with TBAF and methylation of the ensuing free alcohol with methyl iodide in 28% overall yield. Compared to the synthesis of C(1')-methoxy-3-desmethoxy waltherione T (**30**), protection of **35** as an allyl ether was not a viable option, due to the eventual need for nitrogen oxidation with *m*-CPBA. In the event, treatment of **45** with *m*-CPBA gave N-

oxide **46** in 83% yield; hydrogenolytic benzyl ether cleavage then furnished N-hydroxy pyridone **47** (84% yield). Methylation of **47** proved to be difficult and could only be accomplished in 22% yield, due to concomitant C(4)-O-methylation resulting in the formation a doubly methylated product.

Scheme 6. Synthesis of waltherione T analog 31.[a]



[a]Reagents and conditions (a) Benzyl bromide, K_2CO_3, DMF, 50 °C, 2h 45 min, 80% yield; (b) TBAF (1M THF), THF, 1h 45 min; (c) MeI, NaH, DMF, 0 °C to r.t., 20h, 35% over two steps; (d) *m*-CPBA, CH_2Cl_2, 0 °C to r.t., 2h, 83% yield; (e) H_2 (1 atm), Pd/C (10 mol%), MeOH, 4h, 84% yield; (f) MeI, NaH, DMF, r.t., 25h, 22% yield.

As can be seen from the data in Table 2, the oxygenated 3-desmethoxy waltherione T derivatives **30**, **31**, **38**, **41**, **42**, **46**, and **47** in general were found to be less potent inhibitors of *T. Cruzi* amastigotes than the parent compound **28**; as the only exception, the C(1')-methoxy derivative **30** exhibits similar activity. Even if the racemic nature of the compounds is taken into account, it is clear that none of the modifications investigated led to a significant increase in activity. In addition, analogs **38**, **41**, **42**, and **47** all show low selectivity *vs*. uninfected S6 cells.

These findings are somewhat surprising, given the fact that C(1')-methoxylation and C(1'), C(6)-bis-methoxylation of waltherione M results in a potency increase against *T. cruzi* of 13-fold (waltherione Q (**4**) and >30-fold (waltherione **J** (**6**)), respectively (see Figure 1). While our data set is limited, it does indicate that the SAR for pyridone-based waltheriones does not simply parallel that for the quinolone-derived congeners, thus highlighting the importance of a bicyclic core structure for sub-μM antitrypanosomal activity. The most pronounced drop in antitrypanosomal activity is observed for biotin conjugate **42**. In the absence of knowledge on the molecular target of waltheriones it is unclear whether this is due to impaired interactions with (a) target protein(s) or reduced cellular uptake (or both). It remains to be determined if **42** is a suitable probe for target identification, given its moderate potency and limited selectivity. Experiments to this end are planned for the immediate future.

Table 2. Antitrypanosomal activity and selectivity of oxygenated analogs of 22.

Cpd[a]	IC_{50} *T. cruzi* [μM][b]	Rel. IC_{50}[c]	IC_{50} L6 [μM][d]	SI[e]
38	8.9	3.56	43	4.83
30	2.42	0.97	42	17.4
47	4.87	2.85	95.9	19.7
31	3.62	2.11	47.7	13.2
41	16	6.4	29	1.81
42	85.55	34.22	115.9	1.35
46	16.75	9.8	36.4	2.17
Benznidazole[f]	2.5/1.7[c]	1	>345[g]	>139[g]

[a]For cpd structures, see Schemes 4 - 6. [b]Inhibition of intracellular *T. cruzi* amastigote growth in L6 rat skeletal muscle myoblast cells. Data are average values of two independent experiments. Deviations between individual IC_{50} values were < 20%. [c]Relative IC_{50} values: IC_{50} compound/IC_{50} benznidazole control in the same set of experiments. IC_{50}'s of benznidazole used for the calculation of SI values were 2.5 μM (cpds. **30**, **38**, **41**, **42**) or 1.7 μM (cpds. **31**, **46, 47**). [d]Inhibition of proliferation of uninfected L6 cells. Data are average values of two independent experiments. Deviations between individual IC_{50} values were < 20%. [e]Selectivity index = IC_{50} cytotoxicity/IC_{50} antitrypanosomal activity. [f]Positive control. [g]Data from ref. [8].

Conclusions

We have prepared a series of analogs of the pyridine-based antitrypanosomal alkaloids waltherione S (**7**) and T (**8**) and we have assessed their activity against *T. cruzi* amastigotes in L6 rat skeletal muscle myoblast cells. Our data show that the C(3)-methoxy group in **7** and **8** is not essential for their antitrypanosomal activity. At the same time, oxidative modifications at the C(1') benzylic position in the C(6) side chain as they are found in the most active quinoline-based waltheriones do not lead to a significant increase in antitrypanosomal activity, at least in the absence of the C(3)-methoxy group. It remains to be explored if other modifications of waltheriones S (**7**) and T (**8**), for example the incorporation of substituents at position C(4) of the pyridone ring or substitution of the terminal phenyl moiety in the C(6) side chain of waltherione T (**8**), could lead to more potent monocyclic analogs. Given the non-essentiality of the C(3)-methoxy group in waltheriones S (**7**) and T (**8**) future research should also investigate the importance of this group for antitrypanosomal activity in the quinoline-based waltherione variants.

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