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Isoquinoline-based analogs of the cancer drug clinical candidate tipifarnib as anti-*Trypanosoma cruzi* agents

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ABSTRACT

We developed a synthetic route to prepare isoquinoline analogs of the cancer drug clinical candidate tipifarnib. We show that these compounds kill *Trypanosoma cruzi* amastigotes grown in mammalian host cells at concentrations in the low nanomolar range. These isoquinolines represent new leads for the development of drugs to treat Chagas disease.

Chagas disease is caused by infection with the protozoan parasite *Trypanosoma cruzi* (*T. cruzi*). Approximately 8–11 million people in Latin America are infected with this parasite, and 30% of those can be expected to develop complications ranging from mild to terminal.[1] There is no effective treatment for Chagas disease at this time.

We have previously shown that the protein farnesyltransferase inhibitor tipifarnib (compound **1**, Fig. 1) kills *T. cruzi* parasites by binding to lanosterol 14α-demethylase.[2] This enzyme is required for production of ergosterol, a required component of the parasite's membranes, which cannot be replaced by mammalian host cell derived cholesterol.[3] Using the X-ray structure of tipifarnib bound to mammalian protein farnesyltransferase and a homology model of *T. cruzi* lanosterol 14α-demethylase with tipifarnib docked into the active site[2], we designed compound **2** (Fig. 1) which no longer inhibits protein farnesyltransferase (IC_{50} >5000 nM vs ~1 nM for tipifarnib) and is about 10-fold more potent at killing *T. cruzi* amastigotes (clinically relevant parasite life cycle stage that grows in mammalian host cells) (EC_{50} = 0.6 nM) than is our lead compound tipifarnib (EC_{50} = 4 nM).[4] The extra methyl group present in **2** clashes with the surface of protein farnesyltransferase. In addition, the amino group of tipifarnib is part of a hydrogen bond network in the active site of protein farnesyltransferase, which does not exist in the sterol demethylase.[4] Compound **2** contains a OMe group in place of this amino group. We are hopeful that these tipifarnib analogs can be taken into clinical development for Chagas disease because they are highly potent at killing the parasite, and they are expected to have the desirable oral bioavailability and pharmacokinetics that tipifarnib has.

Our modeling studies of tipifarnib docked into the active site of *T. cruzi* lanosterol 14α-demethylase shows that the carbonyl and *N*-Methyl portions of the amide of the quinoline ring are in contact with enzyme surface residues only (Fig. 2). Since such interactions are usually not strong because of the inherent flexibility of protein surface residues, we considered making a new series of analogs in which the quinolone ring is replaced with a quinoline ring. However, quinolines are well known to be susceptible to oxidative metabolism in which carbon-2 is hydroxylated leading to the quinolone ring after tautomerization of the 2-hydroxyquinoline (Fig. 3). Thus, we decided to make isoquinoline analogs of the general core structure **3** (Fig. 3).

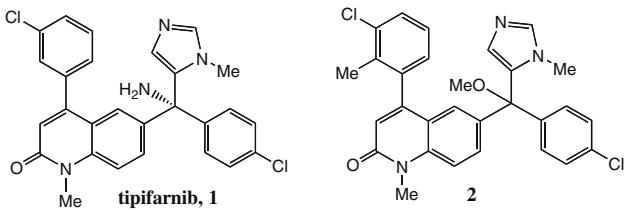

Figure 1. Structure of tipifarnib **1** and analog **2**.

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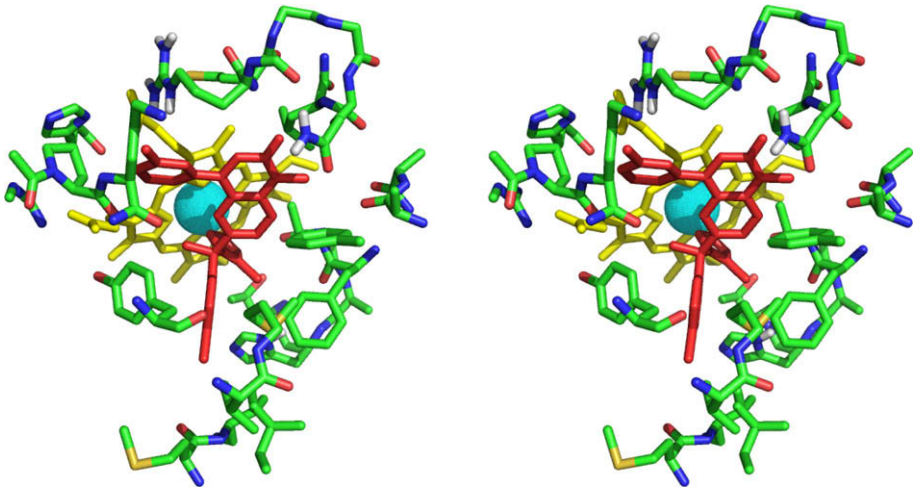


Figure 2. Stereo diagram of tipifarnib docked into the active site of the homology model of *T. cruzi* lanosterol 14α-demethylase. Tipifarnib is in red, the heme is in yellow, and the heme-iron is in cyan.

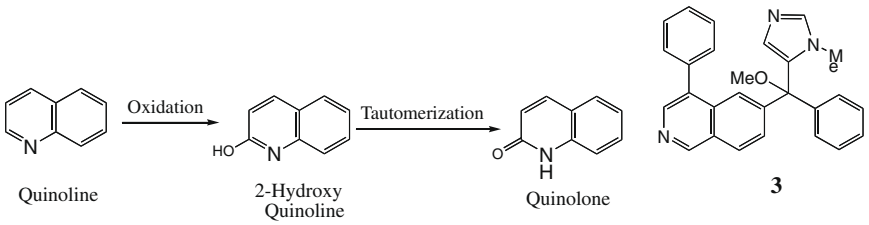


Figure 3. Metabolic oxidation of a quinoline to a quinolone. Compound **3** is the core structure of isoquinoline-based tipifarnib analogs prepared in this study.

The synthesis of the two fragments to make the isoquinoline tipifarnib analogs is shown in Figure 4. Reaction of substituted benzaldehyde **4** with trimethylsilyl cyanide followed by reduction with $LiAlH_4$ gives hydroxyamine **5**. Reductive amination with *p*-bromobenzaldehyde gives secondary amine **6**. Friedel–Crafts alkylation leads to ring closure, and the compound is tritylated on the nitrogen to give **7** in preparation for the next step. The synthesis of the second fragment, methanone **11**, is shown in Figure 4 (panel b). These are made by reacting the appropriate Weinreb amide **9** with a silylated and lithiated *N*-methylimidazole. Figure 5 shows the joining of the two fragments to give the target compounds. Compound **7** is converted to the aryl lithium by lithium–halide exchange, and treatment with methanone **11** gives tertiary alcohol **12**. Alcohol **12** is detritylated and oxidized to isoquinoline **13** with MnO_2. Conversion of the tertiary alcohol to the chloride and then to the target compound **14** is carried out with $SOCl_2$ followed by treatment with methanol. It should also be possible to heat **13** in the presence of tosic acid and methanol to give **14** in one step[5], but this was not attempted. Compound **15** was made by the same route. To make analog **16**, which lacks the phenyl substituent at the 4-position of the quinoline ring, we converted the commercially available 6-bromoisoquinoline to the aryl lithium and carried through the same sequence shown in Figure 5.

We tested the isoquinolines for inhibition of growth of *T. cruzi* amastigotes (Tulahuen strain) inside of mammalian cells (3T3 cells). The parasites stably express the β-galactosidase from *Escherichia coli*, and this enzyme converts yellow colored chlorophenol red β-D-galactopyranoside into a red colored product, which is readily measured by spectrophotometry.[5] This allows the number of parasites in the culture to be readily quantified without resorting to tedious parasite cell counting using microscopy. Data in Table 1 are provided as values of EC_{50}, the concentration of com-

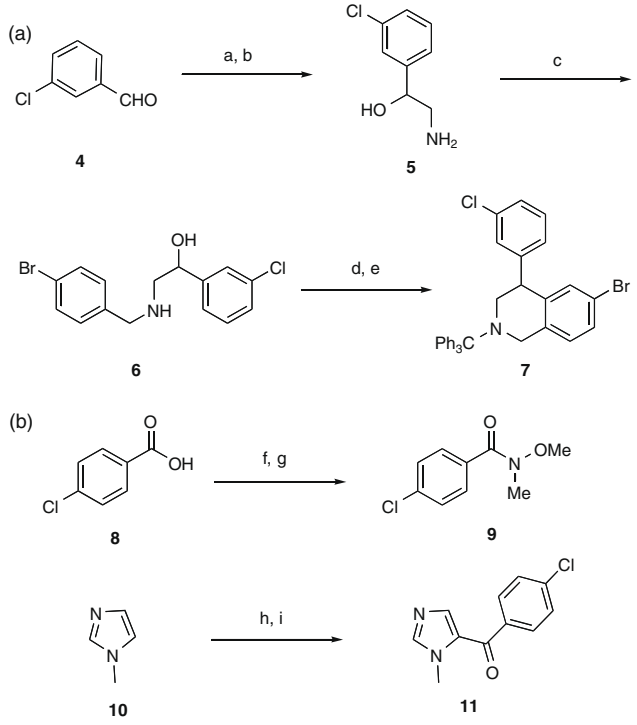


Figure 4. Synthesis of two fragments for isoquinoline tipifarnib analogs. Panel a: (a) TMSCN, ZnI_2, CH_2Cl_2, 0 °C to rt; (b) $LiAlH_4$, THF, 0 °C to rt, 70% (two steps); (c) *p*-bromobenzaldehyde, Et_3N, MeOH, then $NaBH_4$, 75%; (d) $AlCl_3$, CH_2Cl_2, 70 °C, 90%; (e) Ph_3CCl, Et_3N, DMAP, CH_2Cl_2, 93%. Panel b: (f) $SOCl_2$, 90 °C; (g) CH_3ONHCH_3, pyridine, CH_2Cl_2, rt, 90% (two steps); (h) *n*-BuLi, Et_3SiCl, THF, −78 °C; (i) **9**, *n*-BuLi, −78 °C, 75%.

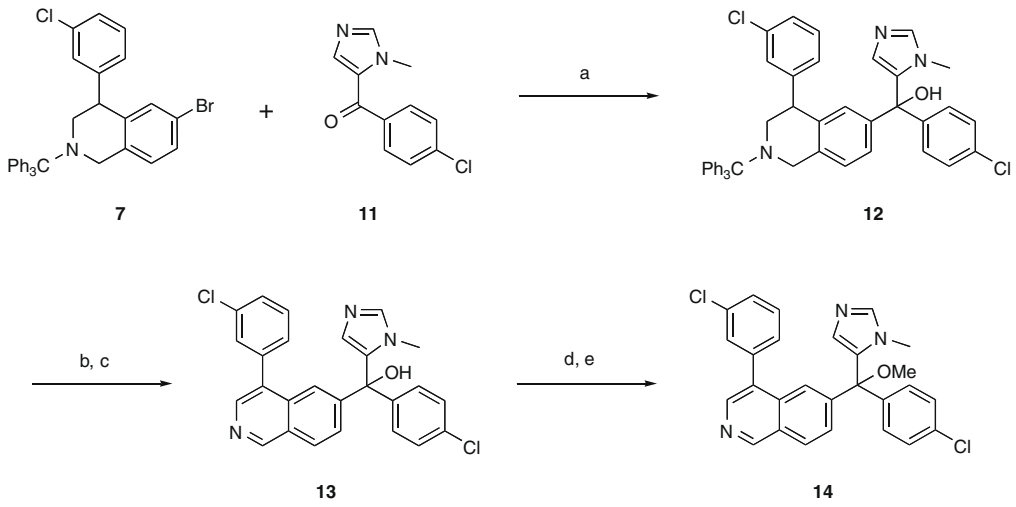
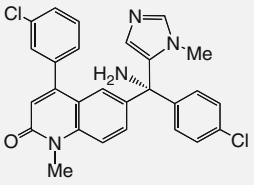
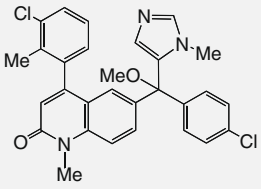
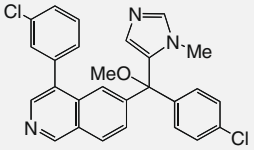
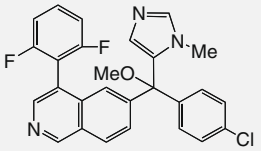
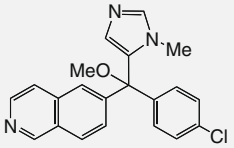


Figure 5. Synthesis of isoquinoline tipifarnib analogs. (a) *n*-BuLi, THF, −78 °C, 56%; (b) TFA, CH_2Cl_2, 85%; (c) MnO_2, dioxane, 90 °C, 64%; (d) $SOCl_2$, rt; (e) MeOH, 90 °C, 80% (two steps).

Table 1
Growth arrest of *T. cruzi* amastigotes by isoquinoline-based tipifarnib analogs[a]

Compd	Structure	EC_{50} (nM)
Tipifarnib		4 (Ref. 2)
2		0.6 (Ref. 4)
14		0.5, 0.9, 1.1[b]
15		0.9, 1.3
16		120
Posaconazole		0.3

[a] EC_{50}s were determined as described.[5]
[b] The multiple numbers represent independent determination of EC_{50}.

pound that reduces parasite growth by 50%. It can be seen that compound **14** is as potent as the corresponding quinolone **2**. Thus, the carbonyl and *N*-methyl groups of **2** are not required for efficacy in this parasite killing assay. Compound **15** with a 2,6-difluorophenyl replacing the 3-chlorophenyl substituent is also potent in this assay. Compound **16** lacks this aryl substituent and is not active against *T. cruzi*. This is consistent with our modeling showing that the phenyl substituent packs well into the active site of *T. cruzi* lanosterol 14α-demethylase. We also studied the lanosterol 14α-demethylase inhibitor posaconazole as a comparator compound. The data in Table 1 show that the new isoquinolines reported in this study are nearly as potent as posaconazole. The latter has been shown to cure mice suffering from a chronic infection with *T. cruzi*.[3] Posaconaozle is an approved drug for treatment of fungal infections. There is now discussion of the use of this agent to treat Chagas disease. The new isoquinolines reported here offer the advantage that manufacturing cost is expected to be well below that of posaconazole. Given the outstanding potency of these compounds against *T. cruzi* growth, detailed pharmacokinetic and Chagas disease efficacy studies in rodents are warranted.

Acknowledgment

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Supplementary data

Supplementary data (full details for the synthesis and characterization of the isoquinolines) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.029.

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