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Synthesis and in vitro protozoocidal activity of diazabicyclic benzotropolone derivatives

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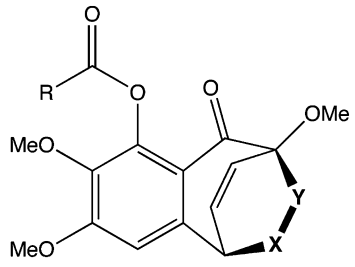
Abstract—We describe the synthesis and protozoocidal evaluation of a series of diazabicycles based on benzotropolone ethers. Several of the compounds, which can be obtained through a high-yielding hetero Diels–Alder reaction using simple and readily available starting materials, have in vitro activities against *Trypanosoma cruzi* and *Leishmania donovani* that are comparable to, and in some cases better than, those of currently used chemotherapies.

Despite continued efforts from local governments, international NGOs, and philanthropic foundations, parasitoses such as malaria, sleeping sickness, Chagas disease, and Leishmaniasis are still endemic in tropical and sub-tropical underdeveloped regions. As a matter of fact, these ailments represent a threat to nearly half of the World population, making them a public health concern of global proportions.[1,2] Attempts to control transmission vectors have been largely unsuccessful, and the complex biology and remarkable adaptability of the causative protozoa have thwarted the development of effective vaccines.[3] As a result, chemotherapy continues to be the mainstay of treatment against these parasitic infections. However, most of the drugs currently available for this purpose have severe side-effects and are significantly toxic, and their intensive and extensive use has induced selection of drug-resistant parasites.[4,5] Consequently, the search for readily accessible novel compounds with protozoocidal activity and improved pharmacological characteristics is of primary importance.[6]

In recent reports, we have described the synthesis and antiparasitic evaluation of a series of endocyclic oxazine and hydrazine esters (Fig. 1).[7,8] The rationale behind the design of these compounds was the incorporation of fragments prone to homolytic cleavage into simple molecular frameworks through the use of high-yielding hetero Diels–Alder cycloadditions. Molecules bearing –N–O– or –N–N– bonds, whose standard dissociation energies (BDEs) are between 35 and 38 kcal/mol,[9] could easily lead to the generation of free radicals. Given the high susceptibility to oxidative stress shown by the protozoa,[10,11] this could make these compounds effective in the treatment of parasitic diseases.



a) **X** = N-Ph, **Y** = O
b) **X** = **Y** = N-CO_2Et

Figure 1. General structure of previously reported benzotropolone-based oxazine (**a**) and hydrazine (**b**) esters with antiparasitic activity.[7,8]

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Indeed, several of the esters showed modest to significant activity against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense*, and *Trypanosoma cruzi*, and while most of the oxazines were somewhat cytotoxic,[7] the hydrazines displayed low to negligible toxicities.[8] These exploratory studies revealed that a new class of molecules with antiparasitic potential and completely novel structures could be obtained from commercially available starting materials using simple and well-established chemistries.

One of the disadvantages of the compounds shown in Figure 1 is the susceptibility to hydrolysis of the ester functionality. Although this behavior could, in some cases, confer pro-drug characteristics to the molecules,[7,8] premature hydrolysis would lead to lipophobic phenols with reduced membrane permeability. A simple approach to circumvent this potential problem, while at the same time conserving the original molecular framework, involves substituting the labile aliphatic and aromatic esters with alkyl or benzyl ethers. We thus decided to explore the synthesis and in vitro protozoocidal activity of a series of endocyclic hydrazines based on benzotropolone ethers (Fig. 2), and our results are discussed in this letter.

The preparation of the ethers involved the derivatization of the C4′ phenolic position of trimethylpurpurogallin (**1**) through standard Williamson etherification conditions (Fig. 2). Briefly, **1** was dissolved in DMSO and treated with a 3- to 5-fold excess of the appropriate alkyl or benzyl halide in the presence of solid NaOH to give ethers **2c**–**2j** in yields ranging from 40% to 81%. The selection of the halides employed in the preparation of the ethers was guided by several factors. Namely, they are all readily available, they provide a small but representative series of alkyl and aryl substitutions at the C4′ position of the benzotropolone, and their structure corresponds with that of the esters studied in our earlier work.[7,8]

As discussed above, the incorporation of the bridged hydrazine moiety relies on an hetero Diels–Alder reaction on the electron-rich dienes of the benzotropolone ethers. The dienophiles employed in the reaction contained electron-deficient azo functional groups, and included dimethyl, diethyl, and diisopropyl azodicarboxylates (DMAD, DEAD, and DIAD, respectively). These reagents were chosen because they are commercially available, their use in cycloaddition reactions is well documented,[8,12] and they allow for a relatively large library of compounds based on a common molecular framework to be rapidly prepared. Treatment of **1**, tetramethylpurpurogallin (**2b**), and ethers **2c**–**2j** with two equivalents of the appropriate dienophile in refluxing toluene afforded exclusively products **3a**–**3j**, **4a**–**4j**, and **5a**–**5j** in 55% to quantitative yields (Fig. 2). In all cases, the reactions proceeded smoothly, and no starting materials were detectable by TLC after 2–10 h. The success of the Diels–Alder reactions was conveniently confirmed by inspection of the ^{1}H NMR spectra. Apart from the new signals corresponding to the methyl, ethyl, and isopropyl carbamates observed in the spectra of the adducts, the chemical shifts for protons in the seven-membered ring change considerably after the cycloaddition. While protons on carbons C6 and C7 in the starting materials display signals at approximately 6.5 and 6.9 ppm, respectively, their resonances shift to roughly 7.3 and 5.7 ppm in the products. These changes are consistent with the conversion of the pseudo-aromatic tropolone ring into a diazabicyclic framework in which the C5–C6 double bond is not co-planar with the remaining aromatic ring. It is also worth noting that slow conformational equilibria of the carbamate moieties leads to substantial broadening of the bridged ring system ^{1}H and ^{13}C NMR signals.[8] To overcome this, NMR spectra for all products were recorded at 90 °C.

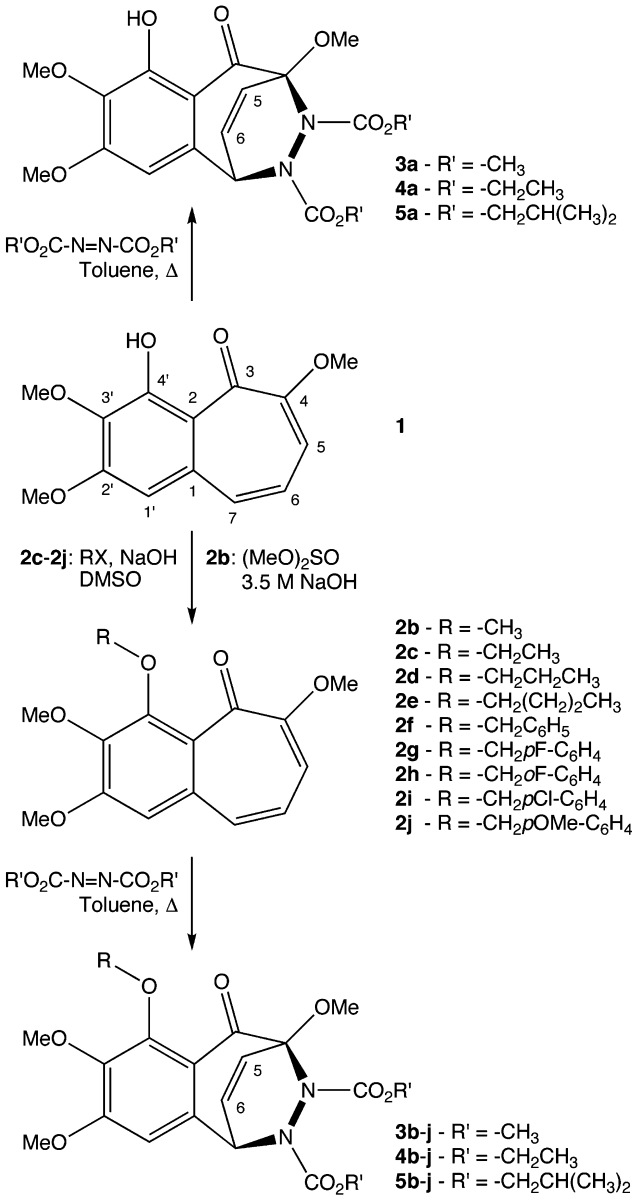

Figure 2. Synthetic route to diazabicycles **3a**–**j**, **4a**–**j**, and **5a**–**j**, and numbering scheme used in the text. Complete experimental details are available as Supplementary data.

The compounds were assayed against *P. falciparum*, *T. brucei rhodesiense*, *T. cruzi*, and *Leishmania donovani*. None of the hydrazines were significantly active against

the former two parasites, with IC_{50}s at least three orders of magnitude larger than those determined for the respective controls, and only results for the latter two are presented in Table 1. Of these, *T. cruzi* was the most susceptible to this series of compounds. The IC_{50}s of **3e**, **3i**, **4f**–**4i**, and **5d**–**5j** against this trypanosomatid ranged from 1.9 to 83.8 μM. This makes them comparable to the control, benznidazole, which had an IC_{50} of 8.7 μM in the same assay. Compounds **5f**, **5h**, and **5j** have virtually the same activity than the control, and **5i**, the most active member of the series, is nearly five times more active with an IC_{50} below 1.9 μM. Further analysis of the data presented in Table 1 leads to a loose correlation between the structure of the C4′ substituent and the activity against *T. cruzi*. With the exception of **3e**, **4b**, **5d**, and **5e**, which have IC_{50}s in the 23.4–57.4 μM range and contain aliphatic ethers at C4′, the majority of the active compounds bear substituted benzyl groups at this position. On the other hand, the activities of our previous series of ester derivatives correlated with the presence of aliphatic substituents at the C4′ position.[8] As suggested above, this could be a reflection of the different propensity to hydrolysis, and thus bioavailability, of aromatic vis-à-vis aliphatic esters. Finally, it is also evident that the number of active hydrazine ethers increases and their activities improve as the carbamate groups become larger and more lipophilic. While not presented here, these trends were observed in the *P. falciparum* and *T. brucei rhodesiense* assays as well.

In the *L. donovani* assays, all but two of the compounds had IC_{50}s of 47.4 μM or higher, or roughly 6-fold less active than stibogluconate (pentostam), which had an IC_{50} of 7.8 μM. However, hydrazines **4h** and **4j** showed activities nearly identical to that of the control. Although the results for these two are clear exceptions, they are consistent with the trend seen in all the assays, and confirm that compounds bearing substituted benzyl ethers at C4′ are in general the most active.

Cytotoxicity against KB cells is also reported in Table 1. In general, only inactive compounds could be considered nontoxic to cells. The exception is hydrazine **5g**, which is only 2 times less active than benznidazole in the *T. cruzi* assay and nontoxic in concentrations of up to 300 μg/mL. Compounds **4f**–**4j**, **5e**, **5f**, and **5h**–**5j**, which have the highest activities against the trypanosomatids, are fairly toxic, with ED_{50}s ranging from 42.0 to 215.4 μg/mL. However, the therapeutic indexes (TIs) of the hydrazines with the highest activities against *T. cruzi* and *L. donovani* are 37.6 (compound **5i**) and 21.3 (compound **4h**), respectively. These are higher than the TIs obtained for our earlier series of hydrazine esters,[8] and indicate that the compounds discussed herein have slightly improved pharmacological properties.

In summary, 28 novel diazabicycles based on simple benzotropolone ether frameworks were prepared, and their in vitro antiprotozoocidal properties determined. As detailed above, *T. cruzi* is the most susceptible to these new Diels–Alder adducts, with ten of the compounds being active against this parasite. From this subgroup, three had activities comparable to that of benznidazole, and one was nearly 5-fold more active. Additionally, two of the compounds displayed activity against *L. donovani* similar to that of pentostam. Cursory analysis of our results reveals that the activity increases with benzylic substituents at the C4′ position and bulkier aliphatic carbamates on the hydrazine. This suggests that membrane permeability is an important factor in the activity of these compounds, and would explain why phenols **3a**, **4a**, and **5a** are inactive. The mode of action of these molecules is still unknown, but it is likely related to the presence of the bridged hydrazine group. As mentioned earlier, the low BDE of the single –N–N– bond in this moiety could lead to free radicals with the potential of disrupting oxygen metabolism processes specific to trypanosomatids in general,[10] and *T. cruzi* in particular.[13,14] This hypothesis is supported not only by our earlier results,[7,8] but also by a recent report from Gamenara and co-workers on the protozoocidal activity of eucarvone-based oxazines.[15]

Our efforts are now focused on evaluating the effects on biological activity associated with structural variations to the frameworks of the hetero Diels–Alder adducts. In order to maximize the availability and minimize the costs of potential drug candidates, simple and easily

Table 1. In vitro activity of hydrazines **3a**–**3j**, **4a**–**4j**, and **5a**–**5j** against trypanosomatids

Compound	IC_{50}[a] (μM)						Toxicity (ED_{50} μg/mL)[a]		
	T. cruzi			*L. donovani*					
	3	**4**	**5**	**3**	**4**	**5**	**3**	**4**	**5**
a	>73.5	>66.7[b]	>64.6	>73.5	>66.7[b]	>64.6	>300	>300[b]	>300
b	>71.0	37.4[b]	>62.7	>71.0	>68.8[b]	>62.7	>300	>300[b]	>300
c	>68.8	>64.6	>60.9	>68.8	>64.6	>60.9	>300	>300	>300
d	>66.6	>62.7	42.3	>66.6	>62.7	>59.2	>300	>300	298.8
e	57.4	>60.9	23.4	>64.6	>60.9	>57.6	>300	76.0	22.1
f	>60.2	54.1	12.1	>60.2	>57.0	>54.1	>300	215.4	94.0
g	>58.1	51.3	18.0	>58.1	>55.1	>52.4	56.6	51.8	>300
h	>58.1	54.1	9.5	>58.1	8.6	>52.4	137.2	99.7	109.0
i	50.9	83.8	<1.9	>56.3	>53.5	47.4	55.9	99.9	42.0
j	>56.8	>53.9	9.8	>56.8	9.9	>51.3	>300	63.1	47.9

[a] Benznidazole (IC_{50} 8.7 μM, *T. cruzi*) and pentostam (IC_{50} 7.8 μMSb^V, *L. donovani*) were used as controls. Toxicity was assayed against KB cells using podophyllotoxin (ED_{50} 0.006 μg/mL) as standard. All reported values are averages of three independent repeats.

[b] The activity and cytotoxicity of **4a** and **4b** was reported in a previous communication.[8]

accessible scaffolds, such as tropane and tropolone derivatives, are currently being considered. Work with these frameworks is well underway and our results will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.05.044.

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