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Synthesis and trypanocidal activity of 1,4-bis-(3,4,5-trimethoxy-phenyl)-1,4-butanediol and 1,4-bis-(3,4-dimethoxyphenyl)-1,4-butanediol

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Abstract—Chagas' disease is endemic in Central and South American countries. Specific chemotherapy with nifurtimox or benznidazole has been recommended for treatment of recent infection but they have limited efficacy. The natural products veraguensin (**1**) and grandisin (**2**) have shown potent in vitro activity against trypomastigote parasite (Y strain) with IC_{50} 2.3 μM (**1**) and 3.7 μM (**2**). We report herein the synthesis and in vitro trypanocidal evaluation of symmetrical and unsymmetrical 1,4-diaryl-1,4-diol derivatives as potential trypanocidal analogs of natural compounds **1** and **2**. Among the synthesized products, compounds 1,4-bis-(3,4,5-trimethoxyphenyl)-1,4-butanediol (**6a**) and 1,4-bis-(3,4-dimethoxyphenyl)-1,4-butanediol (**6b**) showed better activity against *Trypanosoma cruzi* trypomastigotes with IC_{50} 100 and 105 μM (Y strain), respectively, and 110 μM (Bolivia strain) for both compounds. However, the most active compound of this series was 1,4-bis-(3,4-dimethoxyphenyl)butane-1,4-dione (**7b**) with IC_{50} 10 and 200 μM against Y and Bolivia strains, respectively.

1. Introduction

Trypanosoma cruzi is the etiological agent of Chagas' disease, a debilitating condition that affects 18–20 million people in Central and South American countries and 2–3 million have shown clinical symptoms of which 3000 evolve to deaths.[1,2] Although various attempts of treatment had been performed since its discovery, a completely efficient drug has not been found.[3] The currently available drugs, nifurtimox (4-(5-nitrofurfurylindenamino)-3-methylthiomorpholine-1,1-dioxide) (Lampit®, discontinued by Bayer) and benznidazole (*N*-benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide) (Rochagan, Roche), have shown limited efficacy, strong side-effects, and low effectiveness.[4,5] Their high toxicity may involve the generation of nitroanion radical from reduction of nitroheteroaryl drugs that further react with oxygen and give sequentially superoxide, hydrogen peroxide, and hydroxyl radical. These oxygen species are responsible for damaging parasite cellular components.[6] A further drawback of this therapy involves the drug resistance and different strains' susceptibility.

Despite being widely transmitted by triatomine insects, the blood transfusion is a relevant mechanism of the disease infection and gentian violet (*N*-[4-bis[[4-(dimethylamino)-phenyl]methylene]-2,5-cyclohexadien-1-ylidene] *N*-methylammonium chloride) has been used as a chemoprophylactic agent in blood banks of endemic areas.[7]

The comparative studies of genomes of *T. cruzi* are promising since they can identify characteristics that distinguish pathogen and host, providing information about parasite-specific targets for drug discovery.[8] Comprising the predicted 22,570 proteins encoded by genes, the *T. cruzi* genome sequence was recently deposited in GenBank/EMBL/DDBJ and it has been explored to discover new targets.[9] The elucidation of biochemical processes of this protozoan parasite has led to the identification of very promising targets for

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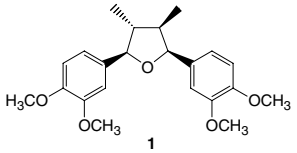
the chemotherapy of Chagas' disease, as exemplified by trypanothione reductase,[10] glutathionyl spermidine synthase,[11] dihydroorotate dehydrogenase,[12] farnesyl diphosphate synthase,[13] squalene synthase,[11] glyceraldehyde-3-phosphate dehydrogenase,[14] iron superoxide dismutase,[15] proline racemase, hexokinase,[16] dihydrofolate reductase,[17] prenyltransferases, ornithine decarboxylase, cysteine, serine, threonine, and metallo proteinases,[18] *trans*-sialidases,[19–21] glucose-6-phosphate isomerase,[22] DNA topoisomerase,[23] hypoxanthine-phosphoribosyltransferase, $C_{14}\Delta^{24,25}$-esterol methyltransferase,[24] etc. Platelet-activating factor-like activity was recently isolated from lipid extract of *T. cruzi* epimastigotes and its activity was related to modulation of cell differentiation toward the infectious stage.[25] Even though the mechanisms underlying the differentiation and proliferation of the parasite are still under investigation, Urbina et al., 1993, showed that platelet-activating factor (PAF) antagonist can inhibit the proliferation of both epimastigote and amastigote forms of *T. cruzi* probably involving alterations in phospholipids' composition.

The screening of potential trypanocidal compounds from plant extracts and natural products has provided a diverse class of compounds illustrated by naphthoquinones, terpenoids, isoflavonoids, and alkaloids, which may interfere with the redox equilibrium and cause oxidative stress.[23,26,27]

The Brazilian plants *Virola surinamensis* and *Piper solmsianum* from Amazon and Atlantica Forests, respectively, have proved to be a rich source of tetrahydrofuran neolignans such as veraguensin 2,5-bis(3,4-dimethoxyphenyl)tetrahydro-3,4-dimethylfuran (**1**) and grandisin 2,5-bis(3,4,5-trimethoxyphenyl)tetrahydro-3,4-dimethylfuran (**2**), which show potent in vitro activity against trypomastigote parasite.[28] Based on experiments involving the Y strain of *T. cruzi*, compounds **1** and **2** were able to produce lysis of parasites with IC_{50} 2.3 and 3.7 μM, respectively, while gentian violet showed a value of IC_{50} 31 μM. Although the compounds are very effective, their high lipophilicity is a serious limitation to carry on in vivo assays. Furthermore, compound **1** is a competitive PAF-receptor antagonist in experiments performed with PAF isolated from human and rabbit platelets (IC_{50} 1.1 μM).[29] This result was further investigated by Hwang et al., 1985, to synthesize new derivatives with the diaryl-tetrahydrofuran analogues without the methyl substituents. From this series, compound **3** exhibited a potent and orally active platelet-activating factor (PAF)-specific and competitive receptor antagonist (IC_{50} 20.0 nM).[30]



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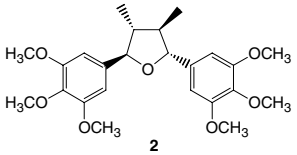

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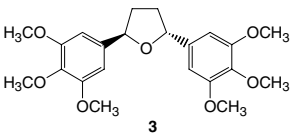

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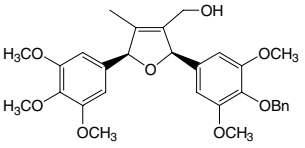
We have been interested in the synthesis of analogues of natural products **1** and **2**, containing appropriate chemical modifications to afford products with improved water solubility and biological activity.[31] Compounds **4** and **5** are promising derivatives synthesized so far as they presented IC_{50} 51.2 and 1.5 μM, respectively. Hereby, we describe our results on the synthesis and in vitro trypanocidal evaluation of symmetrical and unsymmetrical 1,4-diaryl-1,4-diol derivatives, which were designed by molecular simplification of the lead compounds **1**–**3** containing a nonrigid scaffold. This synthetic strategy also allows the use of products and intermediates in the synthesis of constrained derivatives, such as furan, pyrroles, thiophenes, and pyrrolidines.



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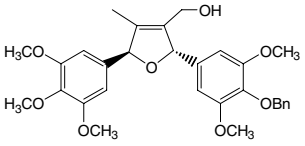

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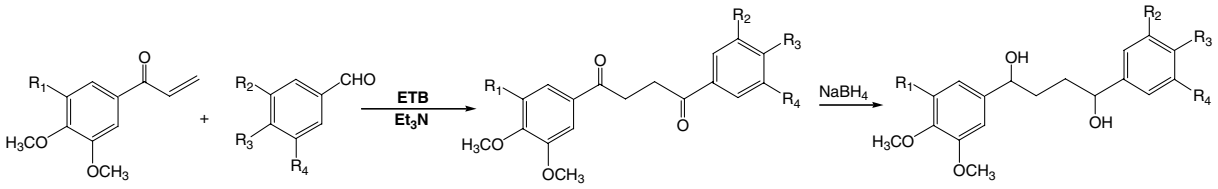
2. Results and discussion

The synthesis of 1,4-bis-(3,4,5-trimethoxyphenyl)-1,4-butanediol (**6a**) and 1,4-bis-(3,4-dimethoxyphenyl)-1,4-butanediol (**6b**) hinges on either 1,4-diaryl-1,4-diketones **7a** and **7b** or 1,4-diarylacetylenic-1,4-glycols **8a** and **8b** formation, respectively, accessible from the corresponding α,β-unsaturated arylketone[32–34] and modified arylaldehyde or arylaldehyde and arylacetylenic derivatives,[35,36] followed by reduction of carbonyl or acetylenic groups, respectively.

Preparations of **7a** and **7b** were attempted under various reaction conditions with sodium cyanide or 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (ETB) to promote the Michael conjugate addition of substituted aldehydes (**9a**–**e**) to α,β-unsaturated arylketones (**10a**,**b**, Stetter reaction).[33,37,38] Using the former approach, we were unsuccessful in attempts to accomplish this reaction under a variety of conditions. On the other hand, condensation was achieved using ETB to generate the intermediate carbanion in the presence of triethylamine, as outlined in Scheme 1. The reaction was performed with different solvents, such as dioxane/EtOH, DMF/EtOH, and EtOH, that afforded compound **7a** in 21%, 12%, and 10% yields, respectively, whereas for compound **7c** the best yield was 38% in a mixture of DMF/EtOH. Although the yields were not high, the targets diaryl-1,4 dicarbonyl **7a**–**e** were directly accessed from readily available starting materials under relatively mild conditions. The problems faced in this reaction may be due to the reduced reactivity of the aldehydes **9a** and **9b** due to the donor effect of the OMe group at *para*-position of the ring. Indeed, several works that had been reported[32,34,39,40] focus on reactions that have



10a: R_1=OCH_3
10b: R_1=H

9a: R_2=R_3=R_4=OCH_3
9b: R_2=H ,R_3=R_4=OCH_3
9c: R_2=R_4=H ,R_3=OH
9d: R_2=H ,R_3= OH, R_4=OCH_3
9e: R_2=H ,R_3=O Bn,R_4=OCH_3

7a: R_1=R_2=R_3=R_4=OCH_3
7b: R_1=R_2=H ,R_3=R_4=OCH_3
7c: R_1=R_2=R_4=H ,R_3=OH
7d: R_1=R_2=H ,R_3=O H, R_4=OCH_3
7e: R_1=R_2=R_4=O CH_3,R_3=OBn

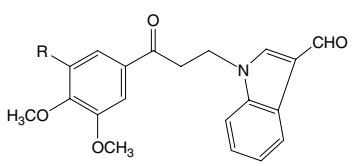
6a: R_1=R_2=R_3=R_4=OCH_3
6b: R_1=R_2=H ,R_3=R_4=OCH_3

Scheme 1. Conjugate addition of modified aldehydes **9a**–**e** to α,β-unsaturated arylketones **10a**,**b**, mediated by ETB with triethylamine as a catalyst to give diaryl-1,4 dicarbonyl **7a**–**e**, followed by reduction.

halogens (iodine or bromine) incorporated at *meta*-position to deactivate the carbonyl group of the arylaldehyde and facilitate the addition of cyanide or thiazolium ions. The ^{1}H and ^{13}C NMR spectral data of all products were in accordance with the structure, showing for compound **7a** a chemical shift at δ 7.24, for *ortho*-aromatic hydrogens, δ 3.86 and 3.87 for methoxyl groups, and δ 3.37 related to α-carbonyl methylene group.

The diketones **7a** and **7b** were reduced with sodium borohydride to give the corresponding diols **6a** and **6b** with 38 and 45% yields, respectively. The ^{1}H NMR showed two sets of nonequivalent hydrogens for both compound **6a** (δ 1.90–1.70 for the methylene group and δ 4.64–4.54 for methyne carbinols) and compound **6b** (δ 2.00–1.90 and 2.40–2.30 for the methylene group, and δ 5.13 and 4.94 for methyne carbinols), indicating the presence of diastereomeric isomers rather than the symmetrical *meso* structure. It could be argued that an intramolecular hydride transfer from an alkoxymetal hydride intermediate to the remaining carbonyl group may give a preferential *trans*-diol relationship.[41]

It is worth noting that, in preliminary experiments using different starting material with ETB, such as 3-indolcarboxaldehyde instead of substituted benzaldehyde, we found that the amino group of the heterocycle was preferentially added to the double bond of α,β-unsaturated arylketone, giving compounds **11a** and **11b** (45%). The ^{1}H NMR spectrum showed all characteristic signals, which consist of δ 8.24–6.77, related to the aromatic and heteroaromatic hydrogens, δ 4.62 and 3.45 representing the methylene groups, and the methoxyl and aldehyde hydrogen, respectively, at δ 3.87–3.84 and 9.90.

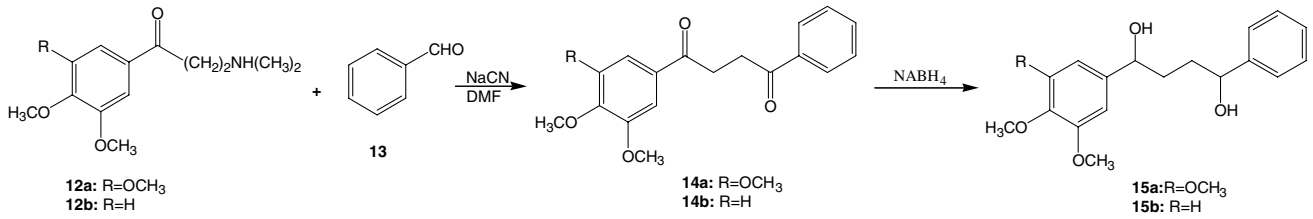


11a:R=H
11b:R=OCH_3

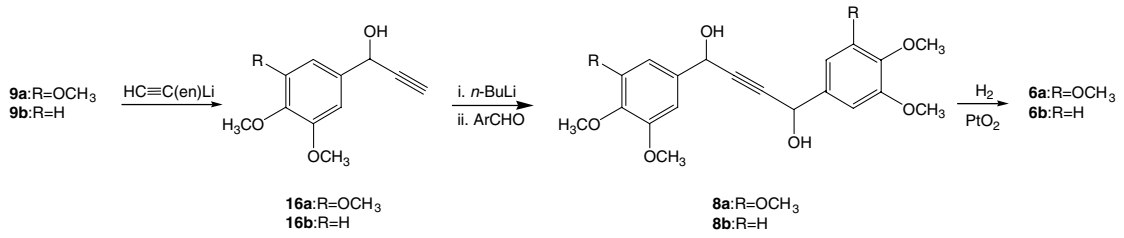
During this investigation, additional experiments were conducted on unsubstituted arylaldehydes to confirm the influence of methoxyl substituents. Since the required starting α,β-unsaturated arylketones **10a** and **10b** were easily obtained from β-amino-arylcarbonyl derivatives, which in turn involve the classical Mannich reaction,[34] we investigated, therefore, a direct approach using the readily available reagents **12a** and **12b**, Scheme 2. Thus, treatment of benzaldehyde **13** with sodium cyanide, followed by **12a** or **12b**, gave the diketones **14a** and **14b** with 36 and 48% yield, respectively, whose ^{1}H NMR data showed the same chemical shift for methylene groups at δ 3.45. Comparatively, the similar procedure attempting to convert compounds **9a** and **9b** or even *p*-hydroxybenzaldehyde into diaryl diketones was unsuccessful, as expected. Reduction of compounds **14a**,**b** afforded 1,4-diaryl-1,4-diols **15a**,**b**.

The required intermediates **8a** and **8b** were obtained by condensation of arylaldehydes with acetylide anion derived from either lithium acetylide complexed with ethylenediamine, lithium or magnesium acetylides.[35,36] In this approach, we intended to use stronger nucleophiles such as acetylide anions to convert readily available aldehydes into acetylenic carbinols, whose transformation of the *sp* terminal carbon allows generation of structural variety of symmetrical and unsymmetrical 1,4-diarylacetylenic-1,4-glycols. Thus, addition of lithium acetylide complexed with ethylenediamine to 3,4,5-trimethoxylbenzaldehyde (**9a**) or 3,4-dimethoxylbenzaldehyde (**9b**) gave acetylenic carbinols **16a** and **16b**, which were further treated with *n*-butyllithium to afford the corresponding carbanion and alkoxy dianions in THF. Condensation of these intermediates with the same aldehydes **9a** or **9b** at low temperature provided 1,4-diarylacetylenic-1,4-glycols **8a** and **8b** in 59% and 45% yield, respectively (Scheme 3). These compounds were characterized on the basis of their spectral properties, which showed two sets of signals for carbinol hydrogens of compounds **8a** and **8b**, respectively, at δ 5.38, 5.39 and δ 5.41, 5.42. These structures were further confirmed by their ^{13}C NMR spectra, with carbinol and acetylenic carbons, respectively, at δ 64.92 and 86.67, 86.73 for compound **8a**, and δ 64.80 and 86.78 for compound **8b**.

Concerning the reaction with lithium acetylide complex, several heteroarylaldehydes and arylaldehydes with different substitution patterns were investigated. However, the yields of the corresponding acetylenic carbinols were disappointing owing to the high instability



Scheme 2. Preparation of unsymmetrical diaryl diols **15a**,**b** from diaryl diketones **14a**,**b** by replacement of the amino group of compounds **12a**,**b** by aldehyde **13**, via a carbanion cyanohydrin.



Scheme 3. Preparation of symmetrical acetylenic glycols **8a**,**b**, via addition of acetylenic carbinols **16a** and **16b** to arylaldehydes.

of the acetylide reagent under storage and handling, as well self-condensation of aldehydes.

Furthermore, an attempt to repeat the reaction sequence for the preparation of **8a** and **8b** by condensation of the acetylenic carbinol monoanion, obtained from **16a** or **16b** by reaction with *tert*-butyldimethylsilyl chloride (TBSCl) followed by treatment with *n*-butyllithium, led to recovery of the starting materials.

The preparation of acetylenic derivatives was earlier discussed in several reviews pointing out the efficient use of ethylmagnesium halides or alkyl lithium to abstract ethynyl proton.[36,42] Considering that acetylene gas does not form the monolithium or mono-Grignard acetylide in ether because of their immediate conversion into acetylide dianion, we envisaged that using this solvent the acetylenic glycols **8a** and **8b** could be straightforwardly obtained. Contrary to our expectations, neither dilithium nor di-Grignard acetylide afforded satisfactory results on attempted condensation of **9a** or **9b**, giving poor yields of acetylenic carbinols (~20%) and products (~8%) besides recovery of the starting materials (~40%).

Hydrogenation of compounds **8a** and **8b** in the presence of palladium-carbon gave only by-products that comprise a butane chain bridge instead of a 1,4-butanediol between the two aryl groups.[43] On the other hand, the reduction performed with platinum oxide[35] allowed isolation of products **6a** and **6b** with 73% and 41% yield, respectively.

The trypanocidal activity of products and intermediates was evaluated against two *T. cruzi* strains, Bolivia and Y, with distinct parasitemic level. The results are depicted in Table 1 which shows IC_{50} values ranging from 10 μM to inactive compounds. Products **6a** and **6b** showed similar activity against both strains with IC_{50} 100–110 μM that represents an interesting activity for these series of compounds. On the other hand, the diaryl-1,4 dicarbonyl intermediate **7b**, with IC_{50} 10 μM, had better activity against Y strain when compared to natural products **1** and **2**. Regarding the 1,4-diarylacetylenic-1,4-glycols, compound **8a** was the most active with IC_{50} 110 and 140 μM for Y and Bolivia strains, respectively.

Table 1. In vitro activities of natural products **1** and **2**, and synthesized compounds against bloodstream trypomastigotes of *Trypanosoma cruzi* Y and Bolivia strains[a,b]

Compound	IC_{50} (μM)	
	Y strain	Bolivia strain
Gentian violet	31	33
1	2.3	—
2	3.7	—
4	51.2	—
5	1.5	—
6a	100	110
6b	105	110
7a	210	1870
7b	10	200
7e	110	140
8a	110	140
8b	520	420
14a	3.5×10^6	3.5×10^6
14b	2.1×10^6	7.4×10^5
15a	1080	1900
15b	1180	230
16a	180	450
16b	760	440

[a] Positive control, gentian violet, concentration of 250 μg/mL.
[b] Negative control, mice blood infected + 5.0% of DMSO.

In summary, we report a convenient synthesis of new analogues of natural products veraguensin (**1**) and grandisin (**2**) containing nonrigid structures. The final products **6a** and **6b**, comprising a 1,4-diaryl-1,4-diol structure with different patterns of substitution, were successfully prepared from 1,4-diaryl-1,4-diketones or 1,4-diaryl acetylenic-1,4-glycols in moderate yields in spite of the

low reactivity of the reactants. Except for the 1,4-diaryl-1,4-diketone **7b**, the target products and intermediates were less active than the previous tetrahydrofuran neolignan leads **1**–**5**, revealing that the central tetrahydrofuran ring and the methoxyl groups attached to both aromatic rings of the natural products may play a critical role toward the parasite inhibitory activity.

3. Experimental

3.1. Materials and methods

^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX-400 (400 and 100 MHz) using $CDCl_3$ (Aldrich) as solvent and TMS as internal standard. Chemical shifts were reported in δ units (ppm) and coupling constants (J) in Hz. IR spectra were recorded on a Nicolete Protege FT460 spectrophotometer. TLC was performed on silica gel (E. Merck) SIL G-25UV254 and compounds on developed plates were detected either by viewing with a UV lamp (254 nm), or by spraying with a 10% sulfuric acid, 1.5% molybdic acid, 1% ceric sulfate spray followed by heating to 150 °C. Column chromatography was performed on Kieselgel 60 (70–230 mm mesh, E. Merck).

3.2. 1,4-Bis-(3,4,5-trimethoxyphenyl)butane-1,4-dione (7a)

A mixture of compound **10a** (100.0 mg, 0.45 mmol), 3,4,5-trimethoxybenzaldehyde (**9a**) (97.0 mg, 0.5 mmol), the catalyst 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (7.0 mg, 0.03 mmol), and triethylamine (0.1 mL, 0.5 mmol) in dioxane/EtOH (1.0 mL, 8:2, v/v), under nitrogen atmosphere, was heated at reflux. Another portion (7.0 mg) of thiazolium salt was added at the end of 10 and 20 h, and the mixture refluxed for a total of 30 h. The solution was evaporated, and the residue was dissolved with dichloromethane. The organic layer was washed with saturated NaCl solution and water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The compound **7a** was purified by flash column chromatography (silica gel Merck, 230–400 mesh, 7:3 hexane/ethyl acetate) to give a yellow solid (132.2 mg, 36%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.37 (s, 4H, H-2, H-3), 3.86 (s, 6H, OCH_3), 3.87 (s, 12H, OCH_3), 7.24 (s, 4H, H-6, H-10, H-6′, H-10′); ^{13}C NMR (100 MHz): δ 32.90 (C-2, C-3), 56.71 (OCH_3), 61.37 (OCH_3), 105.98, 132.39, 143.06, 153.49, 198.03 (C–Ar).

3.3. 1,4-Bis-(3,4-dimethoxyphenyl)butane-1,4-dione (7b)

It was prepared as described above from compound **10b** (100.0 mg, 0.52 mmol), 3,4-dimethoxybenzaldehyde (**9b**) (95.0 mg, 0.57 mmol), the catalyst 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (9.0 mg, 0.035 mmol), and triethylamine (0.1 mL, 0.7 mmol) in dioxane/EtOH (1.0 mL, 8:2, v/v). Purification by chromatography (silica gel Merck, 230–400 mesh, 7:3 hexane/ethyl acetate) gave a yellow solid (54.0 mg, 29%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.36 (s, 4H, H-2 and H-3), 3.87 (s, 6H, OCH_3), 3.89 (s, 6H, OCH_3), 6.85 (d, 2H, J_{ortho} 8.3, H-9, H-9′), 7.50 (d, 2H, J_{meta} 2.0, H-6, H-6′), 7.65 (dd, 2H, J_{ortho} 8.3, J_{meta} 2.0, H-10, H-10′); ^{13}C NMR (100 MHz): δ 32.70 (C-2, C-3), 56.40 (OCH_3), 56.50 (OCH_3), 110.50, 110.60, 123.20, 130.50, 149.40, 153.70 (C–Ar).

3.4. 1-(4-Hydroxyphenyl)-4-(3,4-dimethoxyphenyl)-butane-1,4-dione (7c)

It was prepared as described above from compound **10b** (100.0 mg, 0.52 mmol), 4-hydroxybenzaldehyde (**9c**) (95.0 mg, 0.57 mmol), the catalyst 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (9.0 mg, 0.035 mmol), and triethylamine (0.1 mL, 0.7 mmol) in DMF/EtOH (1.0 mL, 1:1, v/v). Purification by chromatography (silica gel Merck, 230–400 mesh, 7:3 hexane/ethyl acetate) gave a yellow solid (62.0 mg, 38%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.29–3.39 (m, 4H, J 4.0, CH_2–CH_2), 3.86, 3.89 (s, 2× 3H, OCH_3), 6.81 (d, 2H, J_{ortho} 8.8, H–Ar), 6.85 (d, 1H, J_{ortho} 8.5, H–Ar), 7.49 (d, 1H, J_{meta} 2.0, H–Ar), 7.66 (dd, 1H, J_{ortho} 8.5, J_{meta} 2.0, H–Ar), 7.82 (d, 2H, J_{ortho} 8.8, H–Ar).

3.5. 1-(3-Methoxy-4-hydroxyphenyl)-4-(3,4-dimethoxyphenyl)butane-1,4-dione (7d)

It was prepared as described above from compound **10b** (148.0 mg, 0.77 mmol), 3-hydroxy-4-methoxybenzaldehyde (**9d**) (129.0 mg, 0.85 mmol), the catalyst 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (13.0 mg, 0.051 mmol), and triethylamine (0.12 mL, 0.85 mmol) in dioxane/EtOH (1.0 mL, 8:2, v/v). Purification by chromatography (silica gel Merck, 230–400 mesh, 7:3 hexane/ethyl acetate) gave a yellow oil (10.0 mg, 5%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.34–3.36 (s, 4H, CH_2–CH_2), 3.87 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 6.85 (d, 1H, J_{ortho} 8.3, ArH), 6.91 (d, 1H, J_{ortho} 8.3, H–Ar), 7.50 (d, 2H, J_{meta} 2.0, ArH), 7,60 (dd, 1H, J_{ortho} 8.3, J_{meta} 2.0, ArH), 7.65 (dd, 1H, J_{ortho} 8.8, J_{meta} 2.0, ArH); ^{13}C NMR (100 MHz): δ 30.10, 32.70 (C-2, C-3), 56.40, 56.50 (OCH_3), 110.25, 110.42, 110.54, 114.28, 123.22, 123.88, 130.17, 130.44, 146.95, 149.36, 150.78, 153.69, (C–Ar), 197.85, 197.93 (C═O).

3.6. 1-(3,4,5-Trimethoxyphenyl)-4-(3,5-dimethoxyphenyl-4-*O*-benzyl)butane-1,4-dione (7e)

It was prepared as described above from compound **10a** (30.0 mg, 0.14 mmol), 1-(3,5-dimethoxy-4-*O*-benzyl)benzaldehyde (**9e**) (44.0 mg, 0.16 mmol), the catalyst 3-ethyl-5-(2-hydroxyethyl)-4-methylthiazolium bromide (3.0 mg, 0.01 mmol), and triethylamine (0.2 mL, 1.0 mmol) in DMF/EtOH (1.0 mL, 1:1, v/v). Purification by chromatography (silica gel Merck, 230–400 mesh, 7:3 hexane/ethyl acetate) gave a yellow solid (62.0 mg, 38%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.36 (s, 4H, CH_2–CH_2), 3.82 (s, 6H, OCH_3), 3.86 (s, 3H, OCH_3), 3.87 (s, 6H, OCH_3), 5.05 (s, 2H, OCH_2Ph), 7.19 (s, 2H, ArH), 7.21 (s, 2H, ArH), 7.24–7.30 (m, 3H, ArH:OBn), 7.40 (dd, 2H, J_{ortho} 8.1, J_{meta} 1.5, ArH:OBn); ^{13}C NMR (100 MHz): δ 34.23, 34.28 (C-2, C-3), 58.06, 58.07, 62.72 (OCH_3), 107.36, 129.80, 129.97, 130.20, 133.89,

139.06, 143.25, 144.44, 154.85, 155.17 (C–Ar), 199.40 (C=O).

3.7. 1-(Phenyl)-4-(3,4,5-trimethoxyphenyl)butane-1,4-dione (14a)

A solution of benzaldehyde (**13**) (97.0 mg, 0.5 mmol), NaCN (60.3 mg, 1.2 mmol) in DMF (2.5 mL), under nitrogen atmosphere, was heated at 40 °C for 30 min. After that, a mixture of 3-(*N*,*N*-dimethylamine)-1-(3,4,5-trimethoxyphenyl)prop-1-one (**12a**) (130.6 mg, 1.2 mmol) in DMF (3.0 mL) was poured slowly into the solution. The reaction mixture was stirred for 24 h. The reaction mixture was cooled in an ice water and quenched with water, and the aqueous layer was extracted with ethyl ether. The organic layer was washed with brine, water, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The compound **14a** was purified by flash column chromatography (silica gel Merck, 230–400 mesh, 8:2 hexane/ethyl acetate) to give a white solid (132.2 mg, 36%); IR ν_{max} (nujol/cm^{-1}) 1675 (C=O); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.45 (t, 4H, *J* 4.0, CH_2–CH_2), 3.93 (d, 9H, *J* 1.3, OCH_3), 7.31 (s, 2H, ArH), 7.48 (t, 2H, J_{ortho} 7.0, J_{meta} 1.5, ArH), 7.59 (tt, 1H, J_{ortho} 7.0, J_{meta} 1.5, ArH), 8.05 (dd, 2H, J_{ortho} 8.0, J_{meta} 1.5, ArH).

3.8. 1-(Phenyl)-4-(3,4-dimethoxyphenyl)butane-1,4-dione (14b)

It was prepared as described above from compound **13** (148.4 mg, 1.4 mmol), NaCN (68.6 mg, 1.4 mmol), and 3-(*N*,*N*-dimethylamine)-1-(3,4-dimethoxy-phenyl)prop-1-one (**12b**) (300.0 mg, 1.3 mmol). Purification by chromatography (silica gel Merck, 230–400 mesh, 8:2 hexane/ethyl acetate) gave a white solid (181.1 mg, 48.0%); IR ν_{max} (nujol/cm^{-1}) 1684, 1667 (C=O); NMR ^{1}H (400 MHz/$CDCl_3$): δ 3.44 (t, 4H, J 4.0, CH_2–CH_2), 3.93, 3.95 (s, 2× 3H, OCH_3), 6.91 (d, 1H, J_{ortho} 8.0, ArH), 7.48 (t, 2H, J_{ortho} 7.0, J_{meta} 1.5, ArH), 7.57 (m, 2H, ArH), 7.71 (dd, 1H, J_{ortho} 8.0, J_{meta} 2.0, ArH), 8.05 (dd, 2H, J_{ortho} 8.0, J_{meta} 1.5, ArH).

3.9. 1-(3,4,5-Trimethoxyphenyl)pro-2-in-1-ol (16a)

A mixture of lithium acetylide ethylenediamine (470.0 mg, 5.1 mmol) in DMSO (4.0 mL) was added to a solution of 3,4,5-trimethoxybenzaldehyde (**9a**) (500.0 mg, 2.5 mmol) in DMSO (2.0 mL), under nitrogen atmosphere. The mixture was stirred for 1 h at room temperature when monitoring by TLC (hexane/ethyl acetate 1:1) showed completion. The reaction mixture was cooled, quenched with saturated aqueous NH_4Cl, and extracted with ethyl ether. The organic layer was dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure followed by purification on silica gel (Merck, 230–400 mesh, hexane/ethyl acetate 1:1) afforded the compound **16a** as a yellow solid (113.2 mg, 20%); IR ν_{max} (cm^{-1}): 3.242 (OH), 2.117 (C≡C); ^{1}H NMR (400 MHz/$CDCl_3$): δ 2.25 (d, 1H, *J* 6.0, OH), 2.71 (d, 1H, *J* 2.2, H-1), 3.86 (s, 6H, OCH_3), 3.91 (s, 3H, OCH_3), 5.44 (dd, 1H, *J* 2.2 and 6.0, H-3), 6.81 (s, 2H, H-5, H-9); ^{13}C NMR (100 MHz): δ 56.51, 61.24 (OCH_3), 64.89 (C-3), 75.25 (C-1), 83.80 (C-2), 103.98, 136.04, 138.37, 153.73 (C–Ar).

3.10. 1-(3,4-Dimethoxyphenyl)prop-2-in-1-ol (16b)

It was prepared as described above from lithium acetylide ethylenediamine (555.0 mg, 6.0 mmol) and compound **9b** (200.0 mg, 1.2 mmol). Purification by chromatography (silica gel Merck, 230–400 mesh, 1:1 hexane/ethyl acetate) gave a yellow solid (141.0 mg, 61%); IR ν_{max} (cm^{-1}): 3.237 (OH), 2.109 (C≡C); ^{1}H NMR (400 MHz/$CDCl_3$): δ 2.69 (d, 1H, *J* 2.3, H-1), 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.45 (s, 1H, H-3), 6.87 (d, 1H, J_{ortho} 8.6, H-8), 7.09–7.14 (m, 2H, H-9 and H-5); ^{13}C NMR (100 MHz): δ 56.28, 56.35 (OCH_3), 64.67 (C-3), 75.10 (C-1), 83.98 (C-2), 110.15, 111.30, 119.44, 133.07, 149.50, 149.63 (C–Ar).

3.11. 1,4-Bis-(3,4,5-trimethoxyphenyl)but-2-in-1,4-diol (8a)

A solution of compound **16a** (77.0 mg, 0.35 mmol) in anhydrous THF, under nitrogen atmosphere, was cooled to 0 °C and *n*-BuLi (2.1 M, 0.33 mL) was added and stirred for 40 min. After that, the temperature was lowered to −78 °C and a solution of 3,4,5-trimethoxybenzaldehyde (**9a**) (34.0 mg, 0.17 mmol) in anhydrous THF (0.5 mL) was added. The reaction mixture was stirred at −78 °C for 3 h when monitoring by TLC (hexane/ethyl acetate 1:1) showed completion. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with ethyl ether. The organic layer was dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure followed by purification on silica gel (Merck, 230–400 mesh, hexane/ethyl acetate 1:1) afforded the compound **8a** as a yellow solid (43.0 mg, 43%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.73 (s, 12H, OCH_3), 3.75 (s, 6H, OCH_3), 5.38 (d, 2H, H-1, H-4), 6.66 (d, 4H, H-6, H-10, H-6′, H-10′); ^{13}C NMR (100 MHz): δ 56.50, 61.22 (OCH_3), 64.92 (C-1, C-4), 86.67, 86.73 (C-2, C-3), 104.04, 136.48, 136.51, 138.24, 153.66 (C–Ar).

3.12. 1,4-Bis-(3,4-dimethoxyphenyl)but-2-in-1,4-diol (8b)

It was prepared as described above from compound **16b** (77.0 mg, 0.4 mmol), *n*-BuLi (2.1 M, 0.33 mL), and 2,4-dimethoxybenzaldehyde **9b** (33.3 mg, 0.2 mmol). Purification by chromatography (silica gel Merck, 230–400 mesh, 8:2 hexane/ethyl acetate) gave a yellow oil (32.0 mg, 45%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 3.77 (s, 6H, OCH_3), 3.79 (s, 6H, OCH_3), 5.41 (d, 2H, *J* 3.7, H-1, H-4), 6.75 (d, 2H, J_{ortho} 8.8, H-9, H-9′), 6.98–7.02 (m, 4H, H-6, H-10, H-6′, H-10′); ^{13}C NMR (100 MHz): δ 56.26, 56.35 (OCH_3), 64.80 (C-1, C-4), 86.78 (C-2, C-3), 110.28, 111.28, 119.43, 133.37, 133.38, 149.46, 149.55 (C–Ar).

3.13. 1,4-Bis-(3,4,5-trimethoxyphenyl)-1,4-butane diol (6a)

3.13.1. Method 1. To a stirred solution of **7a** (11.0 mg, 26 mmol) in tetrahydrofuran (0.7 mL) and methanol (1.4 mL) was added dropwise sodium borohydride

(2.0 mg, 0.05 mmol) in water (0.5 mL). The reaction mixture was stirred at room temperature for 6 h when monitoring by TLC (hexane/ethyl acetate 1:1) showed completion. The reaction mixture was cooled in an ice-water bath and quenched with water, and the aqueous layer was extracted with ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure followed by purification on silica gel (Merck, 230–400 mesh, hexane/ethyl acetate 1:1) afforded the compound **6a** as a white oil (4.2 mg, 38%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 1.70–1.90 (m, 4H, CH_2–CH_2), 3.75 (s, 3H, OCH_3), 3.78 (s, 12H, OCH_3), 4.55-4.65 (m, 2H, H-1, H-4), 6.48 (s, 4H, H-6, H-6′, H-10, H-10′); ^{13}C NMR (100 MHz): δ 38.40, 38.50 (CH_2), 58.60, 63.20 (OCH_3), 71.00, 77.20, 77.30, 106.30, 106.40, 140.10, 144.70, 144.80, 156.50 (C–Ar).

3.13.2. Method 2. A mixture of compound **8a** (32.0 mg, 0.076 mmol), PtO_2 (3.0 mg) in MeOH was stirred in the presence of H_2 (gas) at room temperature until uptake was completed (3 h). The reaction mixture was then filtered in celite pad and the solvent was removed under reduced pressure to gave the compound **6a** as a white oil (23.4 mg, 72%).

3.14. 1,4 -Bis-(3,4-dimethoxyphenyl)-1,4-butane diol (6b)

3.14.1. Method 1. It was prepared as described above from compound **7b** (42.5 mg, 0.12 mmol), sodium borohydride (6.5 mg, 0.17 mmol). Purification by chromatography (silica gel Merck, 230–400 mesh, 8:2 hexane/ethyl acetate) gave a white oil (19.3 mg, 45%); ^{1}H NMR (400 MHz/$CDCl_3$): δ 1.90–2.00 (m, 2H, CH_2), 2.30–2.40 (m, 2H, CH_2), 3.79 (s, 3H, OCH_3), 3.81 (s, 6H, OCH_3), 3.84 (s, 3H, OCH_3), 4.94 (t, 1H, J 5.0, CHOH), 5.13 (t, 1H, J 5.0, CHOH), 6.78 (d, 2H, J_{ortho} 8.0, H-9, H-9′), 6.85–6.92 (m, 4H, H-6, H-10, H-6′, H-10′); ^{13}C NMR (100 MHz): δ 34.50, 36.00 (CH_2), 56.30, 56.40 (OCH_3), 81.40, 81.50, 109.40, 110.00, 111.40, 111.50, 118.30, 118.60, 136.00, 136.60, 148.60, 148.70, 149.40, 149.50 (C–Ar).

3.14.2. Method 2. A mixture of compound **8b** (50.0 mg, 0.014 mmol), PtO_2 (3.0 mg) in MeOH was stirred in the presence of H_2 (gas) at room temperature until uptake was completed (3 h). The reaction mixture was then filtered in celite pad and the solvent was removed under reduced pressure to gave the compound **6b** as a yellow oil (20.6 mg, 41%).

3.15. Biological assays

The bioassays were carried out using blood collected by cardiac puncture of albino Swiss mice on the parasitemy peak after infection with Y strain (seventh day) and with Bolivia strain (14th day) of *T. cruzi*.[44] The infected blood was diluted to the concentration of 2×10^6 trypomastigotes per mL. The assays were performed on titration microplates (96 wells) with 190 μL of blood in triplicate. The compounds were solubilized in dimethyl sulfoxide (DMSO) and diluted in blood to give 5.0, 25.0, 50.0, and 100.0 μg/mL as final concentrations. The plates were incubated at 4 °C and the number of parasites was counted after 24 h, according to the procedure described by Brener.[45] Infected blood with the same volume of DMSO was used as control and gentian violet was used as positive control (250.0 μg/mL). After total lysis, all samples were inoculated in healthy mice in order to detect a possible parasitemy.

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