Synthesis of trypanocidal tetrahydrofuran lignans

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In honor of Professor Otto R. Gottlieb

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Abstract

Several tetra-substituted 2,5-dihydrofuran lignans have been prepared using a sequential Michael addition-carbocyclization with palladium as the catalyst. The synthetic compounds were evaluated against trypomastigote forms of *Trypanossoma cruzi* and the higher activity for diastereoisomeric compounds could be correlated to the *trans* configuration of the aromatic rings. The highest activity was observed for compound **14b** in which IC_{50} was 1.5µM.

Keywords: Tetrahydrofuran lignans, Chagas disease, trypanocidal

Introduction

Chagas disease is an important tropical disease caused by the protozoan *Trypanosoma cruzi* which has the hematophagous reduviid bug *Rhodnius prolixus* (Hemiptera) as major parasite vector. It is endemic to 21 countries with 16-18 million already infected in Latin America and with approximately 100 million of the people living at risk. [1-3] Approximately 30-40% of the patients are affected by irreversible heart and gastrointestinal tract lesions.[4,5] Thus, such disease has caused approximately 3 million disabilities each year. Therefore, it represents a very serious public health and economic problem in these countries.

The search for solutions has been attempted in several different directions. In particular, an important approach has been addressed to the development of new drugs, vaccine therapy, besides the controlling of the specific vector.[1-3] To date there have been only two trypanocidal drugs, Nifurtimox (4-[(5-nitrofurfurylidene)amino]-3-(methylthio)morpholine 1,1-dioxide) and benznidazole (N-benzyl-2-nitro-1-imidazoleacetamide). The daily administration of

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benznidazole to the early chronic patients for two months has diminished the risk of developing cardiomyopathy.[1] However, the side effect to overdose for a long term of the treatment and the emergence of the drug resistant strain has been the major challenges.[6-8]

Due to the increasing influx of people into urban area and the transfusion of contaminated blood, the disease characteristics has changed from endemic to epidemic.[1, 9] Between 1960 and 1989, the prevalence of infected blood in blood banks in selected cities of South America ranged from 1.7 % in São Paulo, Brazil to 53.0 % in Santa Cruz, Bolivia, a percentage far higher than that observed for hepatitis or HIV infection. In this regard, an approach to eliminate the parasite in blood involve the chemoprophylactic agent, crystal violet (N-{4-bis[[4-(dimethylamino)-phenyl]methylene]-2,5-cyclohexadien-1-ylidene}-N-methylammonium chloride), a dye discovered to be effective many years ago.[10] Nevertheless, such treatment has not been well accepted by patients due to the bluish colour it confers to the blood.

A number of investigations dealing with effective trypanocidals described the isolation of diterpene,[11] naphtoquinones,[12] and lignans[13-15] as active compounds. Our research group started with the investigation of natural products as a potential source of trypanocidal drugs since 1998[13] when the lignans (-)-grandisin (**1**) and (+)-veraguensin (**2**) were discovered as the most active natural products against trypomatigote forms of *Trypanosoma cruzi*. These structurally simple lignans were formerly isolated from twigs of *Virola surinamensis*, a common woody species from Amazon Forest. Since both compounds showed potency about forty times higher than crystal violet (Figure 1), we envisaged further investigations of synthetic analogs in order to better understand the structure-activity relationship.

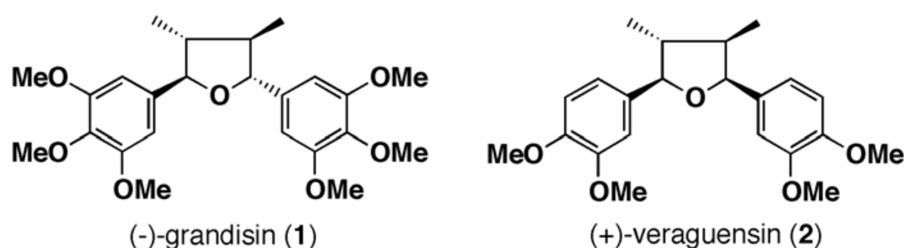


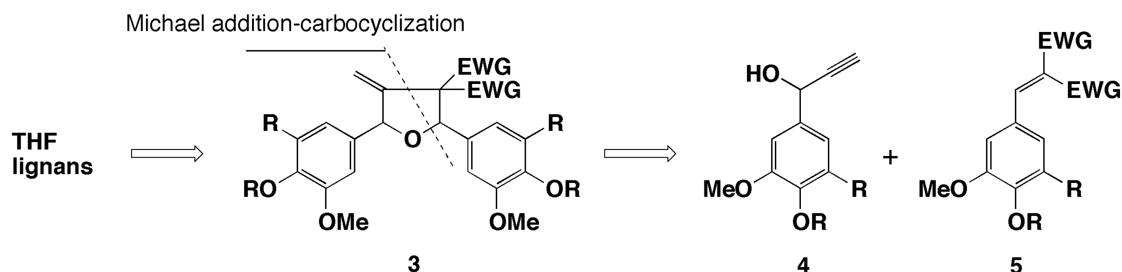
Figure 1

A drug candidate with simple structure would be desirable, because it would allow the access of a large number of derivatives and then studies involving structure-activity relationship would be carried out.

Such considerations prompted us to synthesize several derivatives of **1** and **2**, via a short and simple convergent route. Our general strategy was based on the construction of a tetrahydrofuran (THF) ring by using sequential Michael addition-carbocyclization reaction with palladium catalyst.[16] We report herein, the synthesis of several THF lignan derivatives and their trypanocidal activity.

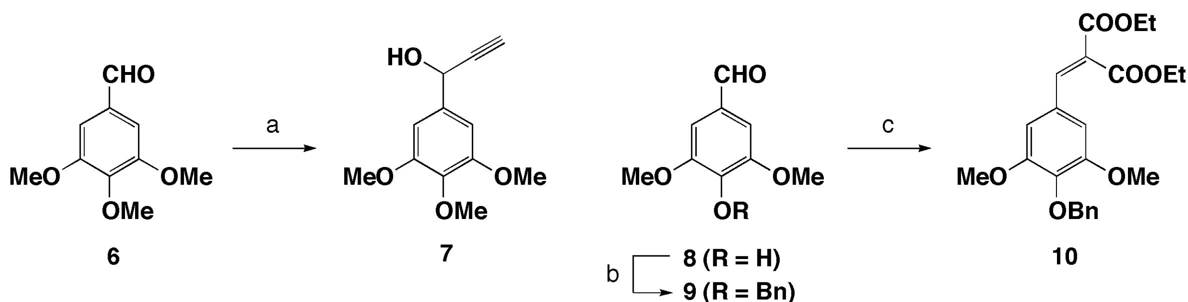
Results and Discussion

In the strategic analysis to synthesize THF lignans and its derivatives, methylenetetrahydrofuran **3** was chosen as a viable intermediate for the easy introduction of several functional groups at the THF ring (Scheme 1). The key step in the proposed sequence, a Michael addition-carbocyclization, would provide **3** from 1.5 equivalent (eq) of propargyl alcohol **4** as a donor and arylidenemalonate **5** with vicinal electron withdrawing groups as a acceptor (EWG) by using substoichiometric amounts of palladium reagent and base.[16] Moreover, this reaction could be carried out in hindered substrates at the aromatic moieties such as **4** and **5**.



Scheme 1

The first objective in the synthesis was the development of workable synthesis of propargyl alcohol **7** and arylidenemalonate **10** (Scheme 2). The 3,4,5- trimethoxybenzaldehyde (**6**) was converted to **7** via addition of ethynylmagnesium bromide in high yield. On the other hand, preparation of **10** started with syringaldehyde (**8**), which was converted to the corresponding benzyl ether **9** using K_2CO_3 in dimethylformamide (DMF). In this step, the typical reaction conditions using NaH in THF or DMF, and K_2CO_3 in acetone, were conducted to the formation of a complex mixture. Knoevenagel reaction with diethyl malonate and **9** by using catalytic amount of pyrrolidinium acetate gave rise to **10** in high yield but without decarboxylation.[17, 18]



Reagens and conditions: (a) HCCHMgBr, THF, 0 °C, 83%; (b) BnBr, K_2CO_3, 75 °C, 97%; (c) diethylmalonate, AcOH, pyrrolidine, toluene, Δ, 80%

Scheme 2

The formation of the methylenetetrahydrofuran **11** from **7** and **10** was accomplished through a sequential Michael addition-carbocyclization protocol using improved Balme's palladium-mediated approach (Figure 2).[16] Treatment of **7** with catalytic amount of n-butyl lithium (n-BuLi) generated the corresponding alkoxide in few minutes. After sequential addition of catalytic amount of $Pd(AcOH)_2$, triphenylphosphine (TPP), and **10**, the substrates were smoothly converted to a diastereomeric mixture **11a** and **11b** after 12 h in excellent yield (entry 2). The low diastereoselectivity was interesting in this case since many types of compounds could be generated. This 'one pot reaction' to couple tertiary alcohol and arylidenemalonate in high yield could not be promoted by adding over 0.1 eq of n-BuLi, as shown in the original reference (entry 3 and 4)[16] but using a reaction time of 12 hr (entry 1). These results paved a way to the possibility of coupling between several hindered Michael acceptor and donor substituted at the aromatic moiety. Since the mixture of diastereomeric compounds was quite difficult to separate by silica gel column, the following reaction was then performed with the diastereomeric mixture.

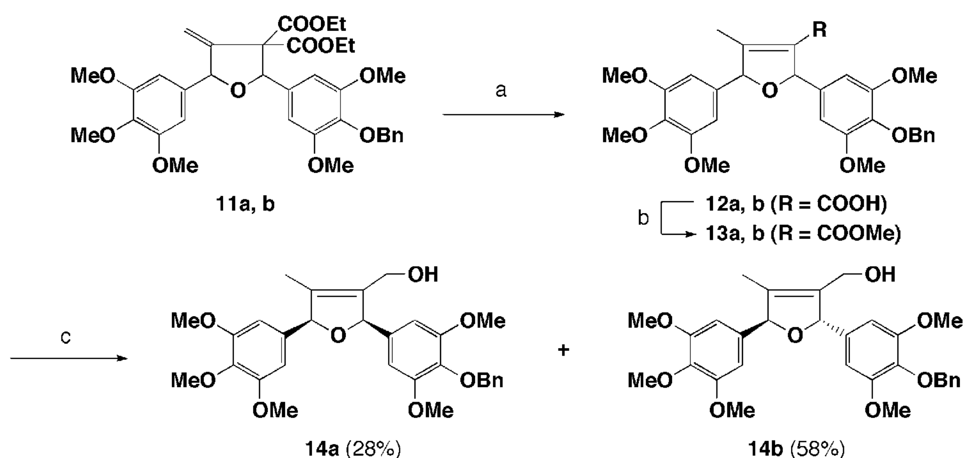
At the next step, although several conditions mentioned in Krapcho's report were tried, simply mono-decarboethoxylated THF compound from **11** could be obtained only in low yield.[24] For example, the treatment of KCN in dimethylsulfoxide (DMSO) under 150 ^{o}C was obtained to corresponding mono-decarboethoxylated furan in 26% yield. The desired compound could be one of the precursors to synthesize THF lignan derivatives, but due to the low yield, the optimization of the next reaction step was further investigated. Treatment of **11** with an alkaline aqueous solution followed by acidification provided monocarboxylic acid **12** via sequential reactions of hydrolysis of ester, decarboxylation, and isomerization of olefin in high yield (Scheme 3). Since it was difficult to separate the mixture of each diastereomer by silica gel chromatography, the mixture **12a/12b** was treated with excess of $LiAlH_4$. However, complete transformation to the corresponding allyl alcohol **14 did not occur**. Accordingly, **12a/12b** was treated with MeI and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to yield methyl ester **13a/13b**[25], which was then converted to **14a/14b** by using $LiAlH_4$ in high yield without 1,4-reduction products. The allyl alcohols **14a** and **14b** were easily separated using silica gel column chromatography.

Entry	Reagents and conditions [a]		Diasteromeric ratio [b]	Yield (%)
	n-BuLi (eq.)	Time (h)	**11a:11b**	
1	0.10	6	0.46:1	76
2	0.10	12	0.48:1	86
3	0.15	6	0.45:1	45
4	0.30	6	-[c]	-[c]

[a] n-BuLi, THF, 0°C-rt, then $Pd(OAc)_2$ (0.05 eq.),TPP (0.05 eq.), **10** (1.5 eq.), THF. Rt. [b] Determined by ^{1}H-NMR spectroscopy. [c] Complex mixture.

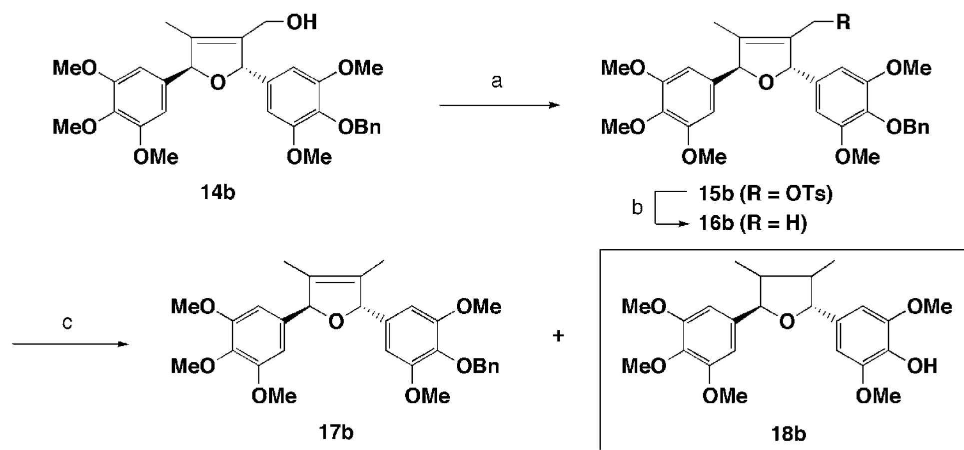
Figure 2

Allyl alcohol **14b,** which was the major diastereoisomer of **14**, was transformed in a two-steps sequence using tosylation with tosyl chloride (TsCl) and 4-(dimethylamino)pyridine (DMAP), followed by reduction with excess of $LiAlH_4$ to yield 7,7′-dihydrofuran **16b** (Scheme 4.). Catalytic hydrogenation of **16b** with $Pd(OH)_2$/C gave the phenol **17b** by hydrogenolysis. In this step, we attempted to transform **16b** to the desired THF lignan **18b** directly, but this conversion was not possible under normal hydrogenation conditions due to the low reactivity at the hindered tetrasubstituted olefin. For example, the reaction with **16b** by using $Pd(OH)_2$ on carbon at room temperature (rt) for 48 h led to the formation of complex mixture as indicated by TLC.



Reagents and conditions: (a) 1.0 M NaOH, 0°C-rt, then 1.0 M HCl, 90%; (b) MeI, DBU, MeCN, rt, quant.; (c) $LiAlH_4$, THF, 0°C-rt, then separation.

Scheme 3

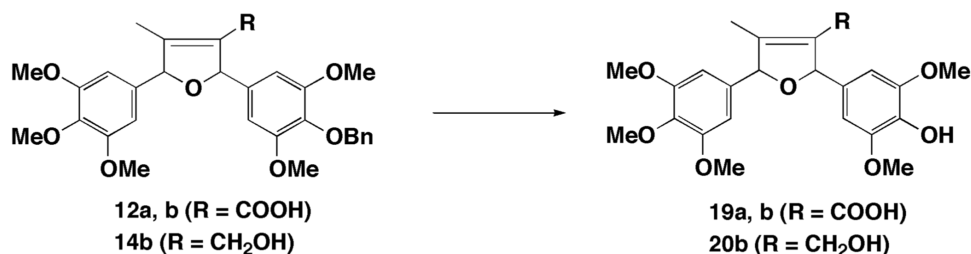


Reagents and conditions: (a) TsCl, DMAP, 0°C,-rt; (b) $LiAlH_4$, THF, 0°C-rt, 52% (2steps); (c) H2 (1atm), $Pd(OH)_2$, AcOH-MeOH, rt, 51%

Scheme 4

The hydrogenation carried out on **16b** indicated that the hydrogenolysis at the benzylic position is preferred over olefin reduction in the 7′,7-dehydrofuran ring. In order to obtain original phenol derivatives, the synthetic intermediates **12** and **14b** were evaluated in this reaction (Scheme 5.) and the hydrogenolysis using with $Pd(OH)_2/C$ at 0 °C, yielded the phenols **19** and **20b**, respectively, in excellent yields.

Several conditions and reagents including PtO_2, Pd/C, and Wilkinson's catalyst were attempted to reduce the tetra-substituted olefin. But it seems to be difficult to distinguish between hydrogenolysis at the benzyl group as a protective group and reduction of the tetra-substituted olefin. Moreover, the obtained phenols (**17b**, **19a**, **19b**, and **20b**) were quite labile under several hydrogenation conditions to produce tetrahydrofuran derivatives. In view of this drawback, the only alternative pathway to avoid hydrogenolysis at the secondary benzylic position involved the use of aldehyde **6** in the same sequence.



Reagents and conditions: H_2 (1atm), $Pd(OH)_2$, AcOH-MeOH, 0°C, 84-95%

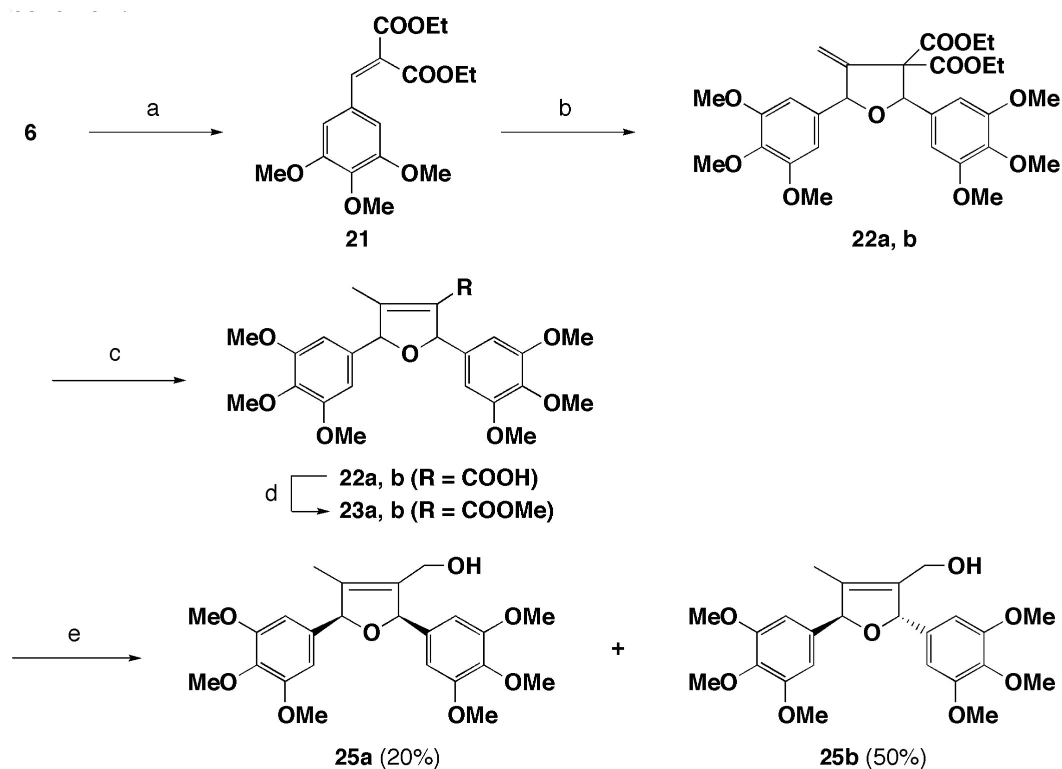
Scheme 5

In short steps, the allyl alcohol **25a** and **25b** was obtained in high yield via the same steps (Scheme 6.). Although it was difficult to separate most of isomers of intermediates by using silica gel column chromatography, **25a** and **25b** exhibited excellent resolution without the application of preparative TLC.

Table 1. Tripomastigote lysis potency of the synthetic tetrahydrofuran lignans, intermediates and natural lignans

Compounds	Concentration (μg/mL) x % of lysis (±SD)				IC_{50} (μM)
	5.0	50.0	100.0	500.0	
7	12.5 ± 8	47.1 ± 7	53.3 ± 8	64.3 ± 8	429.3
10a	33.9 ± 8	48.2 ± 11	62.5 ± 6	87.9 ± 8	75.0
11a	0.0 ±	20.1 ± 9	31.9 ± 6	40.9 ± 5	1242.2
11b	16.1 ± 3	35.7 ± 0	45.5 ± 5	48.2 ± 8	580.0
12a	0.0	16.0 ± 6	21.4 ± 3	25.7 ± 7	9622.0
12b	21.4 ± 3	24.1 ± 4	25.0 ± 5	63.4 ± 9	580.0
13a, b	14.3 ± 5	36.1 ± 2	42.9 ± 1	56.5 ± 7	68.7
14a	20.9 ± 4	31.1 ± 2	41.2 ± 2	39.5 ± 1	51.2
14b	57.1 ± 8	66.6 ± 6	67.2 ± 10	74.0 ± 2	1.5
16a, b	19.8 ± 5	37.8 ± 5	45.2 ± 11	50.3 ± 2	84.2
18	12.5 ± 8	47.3 ± 7	53.3 ± 8	64.3 ± 8	439.3
19a	46.9 ± 13	48.0 ± 2	68.4 ± 12	77.4 ± 4	10.4
19b	5.1 ± 3	31.1 ± 2	34.5 ± 4	39.5 ± 1	160.6
20b	16.4 ± 5	40.7 ± 5	40.7 ± 7	44.15 ± 4	127.0
21	33.9 ± 8	48.2 ± 11	62.5 ± 5	87.5 ± 8	75.0
23a/23b	46.9 ± 12	55.9 ± 6	61.6 ± 5	74.0 ± 8	9.0
24a/24b	16.4 ± 5	7.8 ± 8	30.0 ± 4	41.2 ±5	278.0
25a/25b	11.9 ± 9	24.9 ± 3	37.8 ± 7	52.6 ±0	93.0

Positive control – Violet Gentian (250 μ/mL), negative control – infected blood + 5 % DMSO.



Reagents and conditions: (a) diethyl malonate, AcOH, pyrrolidine, toluene, Δ, 75%; (b) **7**, n-BuLi, THF, 0°C-rt then **21**, $Pd(OAc)_2$ TPP, rt, 92% (**22a:22b**=1:2); (c) 1.0 M NaOH, 0°C-rt, 85%; (d) MeI, DBU, MeCN, rt, quant.; (c) $LiAlH_4$, THF, 0°C-rt,then separation.

Scheme 6

The first synthesis of 7,7′-dehydrofuran **17b** was accomplished by using a convergent strategy via nine steps in 9% total yield from syringaldehyde (**8**). Then, from aldehyde **6**, allyl alcohol **25a** and **25b** were obtained in 41% total yield. The advantage of the reaction sequence can be summarized by two points. One is the facile induction of several moieties at THF ring. The second is the possibility to scale up this synthesis at inexpensive cost using a catalytic amount and easily available reagents.

The evaluation of trypanocidal activity was carried out according to the procedure described in the literature (Table 1)[21]. In comparison with grandisin (**1**) and veraguensin (**2**) which exhibited total tripomastigote lysis at the concentration of 3.7 and 2.3 µM, all synthetic lignans showed lower activities, except for the lignan **14b** in which a IC_{50} of 1.5µM was determined. The *trans* configuration between aromatic ring seems to be an important requirement for activity since **11b** and **14b** were more active than the corresponding **11a** and **14a,** although for veraguensin (*cis*) and grandisin (*trans*) no significant difference could be observed.

Since the tetrahydrofuran lignans have showed promising trypanocidal activity[13-15], further systematic investigations are required for a better evaluation of this potential.

Experimental Section

General Procedures. EI-MS were measured at 70 eV on a HP 5990/5988 A spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker DPX-300 (300 and 75 MHz) while 2D experiments (HMQC and HMBC) were recorded in a Bruker DRX-500 (500 and 125 MHz) using $CDCl_3$ (Aldrich) as solvent and TMS as int. standard. Chemical shifts were reported in units (ppm) and coupling constants (*J*) in Hz. ESI analysis were performed on a triple quadrupole (Quattro-LC, Micromass, UK). Dilute standards (10mg ml-1) were prepared daily in 80% v/v (methanol/water) with 0.25mg ml-1 as the final concentration. Routine TLC analysis were performed Silica gel (Merck, 70-230 mesh) was used for CC and silica-gel 60 PF_{254} Merck (0.50 mm and 1 mm) for anal. and prep. TLC. Spots on chromatograms were detected under UV light (254 and 365 nm) and by spraying H_2SO_4 60% and ceric sulphate solutions followed by heating.

Propargyl alcohol (7). To a cold (0 ^{o}C) and stirred solution of 3,4,5-trimethoxybenzaldehyde (**6**, 1.0 g, 5.10 mmol) in THF (20 mL) was added dropwise over a period of 10 min, ethynylmagnesium bromide (15.0 mL, 0.5 M solution in THF, 7.50 mmol). After 10 min of stirring at 0 ^{o}C, the resultant solution was allowed to warm to room temperature and stirred for another 2 h. The mixture was poured into AcOEt (200 mL) and washed with a saturated aqueous NH_4Cl solution (3 x 25 mL) and brine (3 x 25 mL). The aqueous layers were extracted with AcOEt (3 x 50 mL), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (20-40% AcOEt in hexane) gave 0.94 g (83%) of propargyl alcohol **7** as a colorless solid: 128-133°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.68 (d, 1H, *J* = 2.1 Hz), 2.75 (d, 1H, *J* = 5.7 Hz), 3.82 (s, 3H), 3.87 (s, 6H), 5.40 (dd, 1H, *J* = 2.1, 5.7 Hz), and 6.87 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 5.60, 60.7, 64.3, 74.7, 83.5, 103.6, 135.7, 137.9, 153.2.

Benzyl ether (9). To a stirred solution of syringaldehyde (**8**, 1.0 g, 5.49 mmol) in DMF (10 mL) at rt was added benzyl bromide (1.41 g, 8.24 mmol) and K_2CO_3 (0.84 g, 6.08 mmol). The resultant suspension was warmed to 75 ^{o}C, and stirring was continued for 10 h. The mixture was cooled to rt and then poured into AcOEt (300 mL), and washed with water (3 x 50 mL), and brine (3 x 50 mL). The aqueous layers were extracted with AcOEt (3 x 50 mL), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (5-15% AcOEt in hexane) gave 1.45 g (97%) of benzyl ether **9** as colorless oil. The analytical data were consistent with those in the previous report[23].

Arylidenemalonate (10). To a solution of benzyl ether **9** (0.50 g, 1.84 mmol) in toluene (10 mL) at rt was added ethyl malonate (0.30 g, 1.87 mmol), AcOH (30 mg, 0.50 mmol) and pyrrolidine (15 mg, 0.21 mmol). The resultant solution was reflux for 4 h, and cooled to rt. The mixture was poured into AcOEt (200 mL), and washed with water (3 x 50 mL), and brine (3 x 50 mL). The aqueous layers were extracted with AcOEt (3 x 50 mL), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (50% CH_2Cl_2 in hexane-100% CH_2Cl_2-2% AcOEt in CH_2Cl_2) gave 0.61 g (80%) of arylidenemalonate

10 as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.30 (t, 3H, *J* = 6.9 Hz), 1.33 (t, 3H, *J* = 6.9 Hz), 3.81 (s, 6H), 4.30 (q, 2H, *J* = 6.9 Hz), 4.33 (q, 2H, *J* = 6.9 Hz), 5.05 (s, 2H), 6.72 (s, 2H), 7.31 (m, 3H), 7.45 (m, 2H), and 7.64 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 14.1, 56.0, 61.5, 61.6, 75.0, 106.9, 125.3, 127.9, 128.1, 128.3, 128.4, 137.4, 139.1, 142.0, 153.5, 164.1, 166.9. ESI $[M+1]^+$ 421.

Methylenetetrahydrofurans (11a and 11b). *n*-BuLi (58 µL, 1.6 M solution in hexane, 93 µmol) was added dropwise to a cooled (0 ^{o}C) and stirred solution of propargyl alcohol **7** (311 mg, 1.40 mmol) in THF (2.5 mL) and then the reaction mixture was allowed to reach rt for 15 min. The solution of arylidenemalonate **10** (387 mg, 0.93 mmol) in THF (3.0 mL) was then added followed by both $Pd(OAc)_2$ (10.4 mg, 46.3 µmol) and TPP (12.1 mg, 46.1 µmol). Stirring was continued for 12 h and poured into AcOEt (100 mL), and washed with saturated aqueous NH_4Cl solution (3 x 20 mL), and brine (3 x 20 mL). The aqueous layers were extracted with AcOEt (3 x 20 mL), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (20-30% AcOEt in hexane) gave 0.51 g (86%) of methylenetetrahydrofurans **11a** and **11b** (diastereomeric ratio was 0.48:1 which was determined by ^{1}H-NMR experiment) as a colorless oil: Analytical and bioassay sample was purified by using preparative TLC.

11a. R_f 0.53 (40% AcOEt in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.82 (t, 3H, *J* = 6.9 Hz), 1.31 (t, 3H, *J* = 6.9 Hz), 3.43 (dq, 1H, *J* = 6.9, 10.8 Hz), 3.81 (s, 6H), 3.82 (dq, 1H, 6.9, 10.8 Hz), 3.88 (s, 3H), 3.90 (s, 6H), 4.31 (q, 2H, *J* = 6.9 Hz), 4.99 (s, 2H), 5.03 (d, 1H, *J* = 2.7 Hz), 5.30 (d, 1H, *J* = 2.7 Hz), 5.62 (t, 1H, *J* = 2.7 Hz), 5.76 (s, 1H), 6.79 (s, 2H), 6.93 (s, 2H), 7.30 (m, 3H), and 7.70 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.6, 14.0, 56.0, 56.1, 60.8, 61.4, 61.9, 68.9, 75.0, 83.7, 84.0, 104.0, 106.0, 113.6, 127.8, 128.1, 128.6, 132.6, 135.1, 136.6, 137.7, 138.2, 149.3, 153.0, 153.2, 168.1, 168.4. ESI $[M+1]^+$ 637.

11b. R_f 0.52 (40% AcOEt in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.85 (t, 3H, *J* = 7.2 Hz), 1.30 (t, 3H, *J* = 7.2 Hz), 3.54 (dq, 1H, *J* = 7.2, 10.5 Hz), 3.82 (s, 6H), 3.84 (dq, 1H, *J* = 7.2, 10.5 Hz), 3.85 (s, 3H), 3.87 (s, 6H), 4.31 (q, 2H, *J* = 7.2 Hz), 4.98 (s, 2H), 5.25 (d, 1H, *J* = 2.1 Hz), 5.69 (d, 1H, *J* = 2.1 Hz), 5.86 (t, 1H, *J* = 2.1 Hz), 5.92 (s, 1H), 6.62 (s, 2H), 6.73 (s, 2H), 7.30 (m, 3H), and 7.70 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6, 14.0, 56.1, 60.8, 61.6, 62.0, 69.0, 75.0, 83.8, 83.9, 104.0, 104.5, 113.8, 127.8, 128.1, 128.6, 133.1, 136.1, 136.6, 137.7, 137.8, 147.8, 153.1, 153.3, 167.6, 167.8. ESI $[M+1]^+$ 637.

Carboxylic acids (12a and 12b). To a cold (0 ^{o}C) and stirred solution of methylenetetrahydrofurans **11a** and **11b** (200 mg, 0.31 mmol) in MeOH (8 mL) was added dropwise 5.0 M aqueous NaOH solution (2.0 mL, 10 mmol), and the resultant solution was stirred for 12 h at rt. After cooling to 0 ^{o}C and treating 1.0 M aqueous HCl solution until pH 3, the reaction mixture was concentrated *in vacuo*. The crude was dissolved with AcOEt (50 mL), and washed with 1% aqueous HCl solution (3 x 10 mL) and brine (3 x 10 mL). The aqueous layer was extracted with AcOEt (3 x 10 mL), and the combined organic layer was dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (1-6% MeOH in CH_2Cl_2) gave 152 mg (90%) of carboxylic acids **12a** and **12b**. The sample for analysis and

bioassay was purified by preparative TLC.

12a. Colorless solid, mp 130-134°C (MeOH); R_f 0.55-0.40 (70% AcOEt in hexane), R_f 0.47 (5% MeOH in CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.07 (dd, 3H, J = 0.6, 2.1 Hz), 3.77 (s, 6H), 3.79 (s, 6H), 3.81 (s, 3H), 4.97 (s, 2H), 5.62 (dd, 1H, J = 0.6, 3.9 Hz), 5.98 (dd, 1H, J = 2.1, 3.9 Hz), 6.51 (s, 2H), 6.65 (s, 2H), 7.31 (m, 3H), and 7.47 (m, 2H).

12b. Colorless solid, mp 129-132°C (MeOH); R_f 0.67-0.55 (70% AcOEt in hexane), R_f 0.48 (5% MeOH in CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.05 (dd, 3H, J = 0.9, 1.5 Hz), 3.82 (s, 6H), 3.85 (s, 3H), 3.87 (s, 6H), 4.99 (s, 2H), 5.80 (dd, 1H, J = 0.9, 5.4 Hz), 6.12 (dd, 1H, J = 1.5, 5.4 Hz), 6.52 (s, 2H), 6.60 (s, 2H), 7.30 (m, 3H), and 7.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.9 (CH_3), 56.2 (CH_3), 60.8 (CH_3), 75.0 (CH_2), 88.1 (CH), 92.1 (CH), 104.2 (CH), 104.5 (CH), 126.9 (C), 127.8 (CH), 128.1 (CH), 128.4 (CH), 134.7 (C), 136.3 (C), 137.2 (C), 137.8 (C), 138.3 (C), 153.5 (C), 153.6 (C), 155.4 (C), and 168.1 (C); ESI $[M-1]^-$ 635.

Esters (13a and 13b). To a solution of carboxylic acids **12a** and **12b** (0.10 g, 0.19 mmol) in MeCN (2 mL) at rt was added MeI (109 mg, 0.77 mmol) and DBU (58 mg, 0.38 mmol). After stirring for 1 h, the reaction mixture was concentrated *in vacuo*. The crude was dissolved with AcOEt (50 mL), and washed with water (3 x 10 mL) and brine (3 x 10 mL). The aqueous layer was extracted with AcOEt (3 x 10 mL), and the combined organic layer was dried over Na_2SO_4. Filtration and concentration followed by silica gel chromatography (20-40% AcOEt in hexane) gave 103 mg (quant.) of esters **13a** and **13b** as a colorless oil. The samples for analysis were purified by preparative TLC.

13a. R_f 0.46 (40% AcOEt in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (dd, 3H, J = 0.6, 2.1 Hz), 3.67 (s, 3H), 3.77 (s, 6H), 3.79 (s, 6H), 3.81 (s, 3H), 4.98 (s, 2H), 5.60 (dq, 1H, J = 0.6, 4.5 Hz), 5.99 (dq, 1H, J = 2.1, 4.5 Hz), 6.51 (s, 2H), 6.62 (s, 2H), 7.31 (m, 3H), and 7.47 (m, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.1, 51.7, 56.4, 56.5, 60.7, 75.2, 87.8, 92.0, 105.1, 105.4, 127.5, 128.1, 128.4, 128.7, 135.0, 136.9, 138.1, 138.2, 138.5, 153.1, 153.7, 153.8 and 164.1. ESI $[M+1]^+$ 551.

13b. R_f 0.47 (40% AcOEt in hexane; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.04 (dd, 3H, J = 0.6, 1.8 Hz), 3.70 (s, 3H), 3.84 (s, 6H), 3.85 (s, 3H), 3.88 (s, 6H), 5.00 (s, 2H), 5.79 (dq, 1H, J = 0.6, 5.4 Hz), 6.14 (dq, 1H, J = 1.8, 5.4 Hz), 6.54 (s, 2H), 6.59 (s, 2H), 7.32 (m, 3H), and 7.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.7, 51.5, 56.3, 60.9, 75.0, 88.4, 92.2, 104.3, 104.4, 127.5, 127.8, 128.2, 128.5, 135.1, 136.7, 137.4, 138.0, 138.4, 152.5, 153.6, 153.7, and 164.1. ESI $[M+1]^+$ 551.

Allyl alcohol (14a and 14b). To a cold (0 °C) and stirred solution of esters **13a** and **13b** (72 mg, 0.13 mmol) in THF (2 mL) was slowly added $LiAlH_4$ (30 mg, 0.79 mmol). After 10 min of stirring at 0 °C, the resultant solution was allowed to warm to room temperature and stirred for another 2 h. The mixture was treated with AcOEt (1 ml) at 0 °C and filtered through a pad of celite. The filtration was concentrated *in vacuo*, and then chromatographed on silica gel (20-40% AcOEt in hexane) gave allyl alcohol **14a** (19 mg, 28%) and **14b** (40 mg, 58%) as a colorless oil.

14[a]. R_f 0.40 (60% AcOEt in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.71 (s, 3H), 3.78 (s, 6H), 3.82 (s, 6H), 3.83 (s, 3H), 4.08 (dd, 1H, J = 2.1, 12.6 Hz), 4.35 (d, 1H, J = 12.6 Hz), 4.99 (s, 2H), 5.57 (d, 1H, J = 2.1 Hz), 5.83 (m, 1H), 6.57 (s, 2H), 6.64 (s, 2H), 7.31 (m, 3H), and 7.47 (m,

2H). ESI $[M+1]^+$ 523.

14b. R_f 0.50 (60% AcOEt in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.67 (d, 3H, *J* = 2.1 Hz), 3.84 (s, 6H), 3.85 (s, 3H), 3.88 (s, 6H), 4.01 (dd, 1H, *J* = 1.5, 12.3 Hz), 4.32 (d, 1H, *J* = 12.3 Hz), 5.01 (s, 2H), 5.74 (dd, 1H, *J* = 1.5, 5.4 Hz), 6.00 (dd, 1H, *J* = 2.1, 5.4 Hz), 6.57 (s, 2H), 6.60 (s, 2H), 7.31 (m, 3H), and 7.48 (m, 2H); ^{13}C NMR (75 MHz, CDCl3): δ 10.6, 56.2, 56.3, 60.8, 75.0, 89.3, 91.8, 103.8, 103.9, 127.8, 128.1, 128.5, 134.4, 135.0, 136.3, 136.8, 137.0, 137.7, 137.9, 153.4, and 153.8. ESI $[M+1]^+$ 523.

7,7′-Dehydrofuran (16b). To a solution of allyl alcohol **14b** (50 mg, 95.7 μmol) in CH_2Cl_2 (1.0 mL) was added DMAP (23 mg, 0.19 mmol). To the cold (0 ^{o}C) and vigorously stirred solution was slowly added TsCl (36 mg, 0.19 mmol). After 12 h of stirring at rt, the reaction mixture was poured into AcOEt (20 mL), and washed water (3 x 5 mL) and brine (3 x 5 mL). The aqueous layers were extracted with AcOEt (3 x 10 mL), and the combined organic layers were dried over Na_2SO_4. Filtration and concentration gave the desired crude sulfonate ester **15b** as a colorless oil, which was used in the next step without further purification.

To a cold (0 ^{o}C) and stirred solution of crude sulfonate ester in THF (2.0 mL) was slowly added $LiAlH_4$ (22 mg, 1.58 mmol). After 10 min of stirring at 0 ^{o}C, the reaction mixture was allowed to warm to room temperature and stirred for another 5 h. The mixture was treated with AcOEt (1 ml) at 0 ^{o}C and filtered through a pad of celite. The filtration was concentrated *in vacuo*, and then chromatographed on silica gel (10-30% AcOEt in hexane) gave 25 mg (52%, 2 steps) of 7,7′-dehydrofuran **16b** as a colorless oil: R_f 0.57 (40% AcOEt in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.61 (s, 6H), 3.83 (s, 6H), 3.84 (s, 3H), 3.88 (s, 6H), 5.00 (s, 2H), 5.69 (s, 2H), 6.53 (s, 2H), 6.54 (s, 2H), 7.32 (m, 3H), and 7.48 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 10.5, 56.1, 56.2, 60.8, 75.0, 91.7, 104.0, 104.1, 127.8, 128.1, 128.5, 130.9, 131.1, 136.7, 137.1, 137.2, 137.7, 137.8, 153.4, and 153.7. ESI $[M+1]^+$ 507.

Phenol (17b). To a suspension of $Pd(OH)_2$ on carbon (20%, 3.6 mg) and 7,7′-dehydrofuran **16b** (3.6 mg, 7.1 μmol) in 5% AcOH in MeOH (1 mL) was stirred for 24 h under H_2 atmosphere at rt. The mixture was filtered through a pad of celite. The filtration was concentrated *in vacuo*, and then preparative silica gel TLC (40% AcOEt in hexane) gave 1.5 mg (51%) of phenol **17b** as a colorless oil: R_f 0.40 (40% AcOEt in hexane); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.60 (s, 6H), 3.84 (s, 3H), 3.88 (s, 6H), 3.91 (s, 6H), 5.51 (s, 1H), 5.67 (s, 2H), 6.53 (s, 2H), and 6.54 (s, 2H). ESI $[M+1]^+$ 417.

Phenols (19a and **19b).** To a suspension of $Pd(OH)_2$ on carbon (20%, 4.0 mg) and carboxylic acids **12a** and **12b** (12.0 mg, 22.4 μmol) in 5% AcOH in MeOH (1 mL) was strring for 30 min under H_2 atmosphere at 0 ^{o}C. The mixture was filtered through a pad of celite. The filtration was concentrated *in vacuo*, and then preparative silica gel TLC (5% EtOH in CH_2Cl_2) gave phenols **19a** (R_f 0.50, 3.8 mg, 38%) and **19b** (R_f 0.52, 5.7 mg, 57%).

19^a. Colorless solid, mp 122-124°C (MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.07 (dd, 3H, *J* = 0.6, 2.1 Hz), 3.78 (s, 6H), 3.81 (s, 3H), 3.85 (s, 6H), 5.61 (dd, 1H, *J* = 0.6, 4.2 Hz), 5.97 (dd, 1H, *J* = 2.1, 4.2 Hz), 6.51 (s, 2H), and 6.65 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.1, 56.1, 56.3,

60.8, 87.5, 91.7, 104.8, 104.9, 126.6, 131.9, 134.6, 135.0, 138.2, 147.0, 153.5, 155.5 and 167.2. ESI $[M-1]^-$ 445.

19b. Colorless solid, mp 123-125°C (MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.05 (dd, 3H, *J* = 0.9, 1.8 Hz), 3.85 (s, 3H), 3.87 (s, 6H), 3.89 (s, 6H), 5.78 (dd, 1H, *J* = 0.9, 5.7 Hz), 6.11 (dd, 1H, *J* = 1.8, 5.7 Hz), 6.52 (s, 2H), and 6.60 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 12.9, 56.2, 56.3, 60.8, 88.3, 92.0, 104.1, 104.3, 126.9, 131.8, 134.8, 135.0, 138.4, 147.1, 153.6, 155.3 and 167.7. ESI $[M-1]^-$ 445.

Phenol (20b). To a suspension of $Pd(OH)_2$ on carbon (20%, 3.6 mg) and allyl alcohol **14b** (10.0 mg, 19.2 μmol) in 5% AcOH in MeOH (1 mL) was stirred for 30 min under a H_2 atmosphere at 0 °C. The mixture was filtered through a pad of celite. The filtrate was concentrated *in vacuo*, and then preparative silica gel TLC (100% AcOEt) gave 7.0 mg (84%) of phenol **20b** as a colorless solid: R_f 0.53 (100% AcOEt); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.61 (bs, 1H), 1.68 (d, 3H, *J* = 0.9 Hz), 3.84 (s, 3H), 3.88 (s, 6H), 3.91 (s, 6H), 4.02 (d, 1H, *J* = 12.6 Hz), 4.32 (d, 1H, *J* = 12.6 Hz), 5.55 (s, 1H), 5.72 (d, 1H, *J* = 5.4 Hz), 5.99 (dd, 1H, *J* = 0.9, 5.4 Hz), 6.75 (s, 2H), and 6.61 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.7, 56.2, 56.4, 56.5, 60.8, 89.5, 91.7, 103.7, 104.0, 132.3, 134.5, 134.7, 135.0, 136.7, and 137.9. ESI $[M+1]^+$ 433.

Arylidenemalonate (21). 3,4,6-Trimethoxybenzaldehyde (**6**, 1.00 g, 5.10 mmol) was converted to arylidenemalonate **21** (1.30 g, 75%) according to the procedure described above for **10** from **9.** Yellowish oil; ESI $[M+1]^+$ 353.

Methylenetetrahydrofurans (22a and **22b).** Arilidenemalonate **21** (338 mg, 1.00 mmol) was converted to methylenetetrahydrofurans **22a** and **22b** (1:2, 515 mg, 92%) according to the procedure described above for **11** from **10.**

22a. Gummy; R_f 0.52 (40% AcOEt in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.84 (t, 3H, *J* = 6.9 Hz), 1.31 (t, 3H, *J* = 6.9 Hz), 3.51 (m, 1H), 3.80 (m, 1H), 3.82 (s, 3H), 3.85 (s, 6H), 3.88 (s, 3H), 3.91 (s, 6H), 4.32 (m, 2H), 5.03 (d, 1H, *J* = 2.7 Hz), 5.30 (d, 1H, *J* = 2.7 Hz), 5.62 (t, 1H, *J* = 2.7 Hz), 5.76 (s, 1H), 6.80 (s, 2H), 6.93 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.5, 13.9, 55.1, 55.9, 56.0, 61.4, 61.9, 68.9, 83.6, 83.9, 103.9, 106.0, 132.5, 135.1, 137.7, 149.3, 152.7, 153.1, 168.1, 168.3. ESI $[M+1]^+$ 561.

22b. Gummy; R_f 0.51 (40% AcOEt in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.86 (t, 3H, *J* = 6.3 Hz), 1.30 (t, 3H, *J* = 6.3 Hz), 3.54 (m, 1H), 3.82 (s, 3H), 3.85 (m, 9H), 3.87 (s, 6H), 3.88 (s, 1H), 4.32 (m, 2H), 5.26 (d, 1H, *J* = 2.1 Hz), 5.70 (d, 1H, *J* = 2.1 Hz), 5.86 (t, 1H, *J* = 2.1 Hz), 5.91 (s, 1H), 6.62 (s, 2H), 6.74 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ 13.4, 14.0, 55.0, 60.7, 60.8, 61.6, 61.2, 69.0, 83.7, 83.8, 103.9, 104.4, 113.7, 133.7, 136.1, 137.8, 147.7, 152.8, 153.2, 167.6, 167.8. ESI $[M+1]^+$ 561.

Carboxylic acids (23a and **23b).** methylenetetrahydrofurans **22a** and **22b** (100 mg, 0.18 mmol) was converted to carboxylic acids **23a** and **23b** (72 mg, 89%) according to the procedure described above for **12** from **11.**

23a. Colorless solid, Mp 134-137°C (MeOH); R_f 0.55-0.40 (70% AcOEt in hexane), R_f 0.48 (5% MeOH in CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.08 (bs, 3H), 3.77 (s, 6H), 3.84 (s, 6H), 3.85 (s, 6H), 5.62 (d, 1H, *J* = 4.2 Hz), 5.98 (dd, 1H, *J* = 2.1, 4.2 Hz), 6.50 (s, 2H), 6.65 (s, 2H). ESI

$[M+1]^+$ 459.

23b. Colorless solid, Mp 134-138°C (MeOH); R_f 0.67-0.55 (70% AcOEt in hexane), R_f 0.49 (5% MeOH in CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.06 (bs, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 3.87 (s, 6H), 3.89 (s, 6H), 5.79 (d, 1H, *J* = 5.4 Hz), 6.13 (dd, 1H, *J* = 1.5, 5.4 Hz), 6.52 (s, 2H), 6.61 (s, 2H). ESI $[M+1]^+$ 459.

Esters (24a and 24b). Carboxylic acid **23a** and **23b** (60 mg, 0.13 mmol) was converted to ethers **24a** and **24b** (61 mg, quant.) according to the procedure described above for **13** from **12**.

24a. Colorless oil; R_f 0.44 (40% AcOEt in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.05 (bs, 3H), 3.69 (s, 3H), 3.77 (s, 6H), 3.84 (s, 6H), 3.85 (s, 3H), 5.61 (d, 1H, *J* = 4.8 Hz), 5.97 (dd, 1H, *J* = 2.1, 4.8 Hz), 6.50 (s, 2H), 6.63 (s, 2H). ESI $[M+1]^+$ 475.

24b. Colorless oil; R_f 0.46 (40% AcOEt in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 2.03 (bs, 3H), 3.71 (s, 3H), 3.81 (s, 3H), 3.82 (s, 3H), 3.87 (s, 6H), 3.88 (s, 6H), 5.79 (dd, 1H, *J* = 0.9, 5.1 Hz), 6.14 (dd, 1H, *J* = 1.8, 5.1 Hz), 6.53 (s, 2H), 6.60 (s, 2H). ESI $[M+1]^+$ 475.

Allyl alcohol (25a and 25b). Ethers **24a** and **24b** (30 mg, 0.06 mmol) was converted to ethers **25a** (6.0 mg, 20%) and **25b** (13 mg, 50%) according to the procedure above described for **14** from **13**.

25a. Colorless oil; R_f 0.37 (60% AcOEt in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.70 (bs, 3H), 3.81 (s, 6H), 3.83 (s, 6H), 3.85 (s, 6H), 4.12 (d, 1H, *J* = 16.8 Hz), 4.35 (d, 1H, *J* = 16.8 Hz), 5.55 (d, 1H, *J* = 3.9 Hz), 5.83 (m, 1H), 6.57 (s, 2H), 6.65 (s, 2H). ESI $[M+1]^+$ 447.

25b. Colorless oil; R_f 0.46 (60% AcOEt in hexane); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.67 (bs, 3H), 3.82 (s, 6H), 3.87 (s, 6H), 3.88 (s, 6H), 4.02 (d, 1H, *J* = 17.1 Hz), 4.34 (d, 1H, *J* = 17.1 Hz), 5.74 (d, 1H, J = 5.7 Hz), 6.02 (d, 1H, *J* = 5.7 Hz), 6.58 (s, 2H), 6.62 (s, 2H). ESI $[M+1]^+$ 447.

In vitro bioassay: the bioassays were carried out using blood collected by cardiac puncture of Swiss albino mice in the parasitemy peak (7th day) after infection with the Y strain of *T. cruzi.* The infected blood was diluted with blood of healthy mice to achieve a concentration of 10^6 trypomastigote forms/mL. The standard solutions (in DMSO) were added into the infected blood to provide concentrations of 5.0, 25.0 and 50.0 μg/mL, respectively. The plates were incubated at 4°C during 24 hours and the number of parasites determined according to method described [5]. The bioassays were performed in triplicate on microtiter plates (96 wells), which contained 200 μL of mixture/well. Negative and positive controls containing either DMSO or gentian violet were carried out in parallel.

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