Synthesis, Cytotoxicity, and Anti-*Trypanosoma cruzi* Activity of New Chalcones

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Synthesis of a cytotoxic dihydrochalcone, first isolated from a traditional Amazonian medicinal plant *Iryanthera juruensis* Warb (Myristicaceae), followed by a comprehensive SAR analysis of saturated and unsaturated chalcone synthetic intermediates, led to the identification of analogues with selective and significant in vitro anti-*Trypanosoma cruzi* activity. Further SAR studies were undertaken with the synthesis of 21 new chalcones containing two allyloxy moieties that resulted in the discovery of 2′,4′-diallyloxy-6′-methoxy chalcones with improved selectivity against this parasite at concentrations below 25 μM, four of which exhibited a selectivity index greater than 12.

Introduction

Chalcones, or 1,3-diaryl-2-propen-1-ones, are prominent secondary metabolites precursors of flavonoids and isoflavonoids in plants. Chemically, they consist of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system. The wide variety of pharmacological activities reported for these compounds include anticancer, antiinflammatory, immunomodulatory, antibacterial, and immunosuppressive, as well as antiprotozoan activity, including trypanocidal, leishmanicidal, and antimalarial.[1−7] Chagas' disease or American trypanosomiasis, caused by the vector-borne flagellate protozoan parasite *Trypanosoma cruzi*,[a] is an endemic tropical disease that has infected 20 million people in Central and South America and approximately between 50000 and 100000 people in the United States.[8,9] Responsible for around 20000 deaths per year, Chagas' disease manifests itself as potentially fatal cardiopathy or dilations in the digestive tract.[10] Its cycle starts when the reduviid bug feeds on blood and releases an infectious metacyclic trypomastigote into the bloodstream. Trypomastigotes penetrate various tissues, where they differentiate by binary fission into amastigotes. Intracellular amastigotes transform back into trypomastigotes bursting the host cell and releasing the infectious form to the bloodstream, giving rise to the clinical manifestations and amplifying the infection.[11] With no available vaccine, the only control intervention is chemotherapy involving the use of nitroheterocycles, benznidazole, and nifurtimox; these drugs were developed for veterinary use more than 30 years ago, and are effective only in the acute phase of the disease. Their wider utilization has been hindered by lack of compliance, low effectiveness, drug resistance, high cost, and severe side effects.[12,13] Clearly, a search for new types of drugs with high selectivity, minimum side effects, and low manufacture costs is urgently needed. The economical, facile, and rapid synthesis of chalcones[14] make them attractive as potential drug candidates to fight some of the so-called neglected diseases that affect the populations of many countries in the Third World, chiefly among them leishmaniasis and Chagas disease.

As part of our ongoing search for biologically active metabolites from Peruvian medicinal plants,[15] we recently reported the isolation of two dihydrochalcones from the ethyl ether extract of *Iryanthera juruensis* Warb (Myristicaceae); one of them, 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone, was found to be a major cytotoxic metabolite when tested against a panel of cancer cell lines.[16] With the aim of confirming its structure, we synthesized the natural product and three series of chalcones used as intermediates in the synthesis, namely 2′,4′-dihydroxy-6′-methoxychalcones (2′,4′-HC), 2′-hydroxy-4′,6′-dimethoxychalcones (4′,6′-MC), and 2′,4′-diallyloxy-6′-methoxychalcones (2′,4′-AC) (**4**−**9** in Scheme 1). During this study, we screened the in vitro antiproliferative and anti-*T. cruzi* activity of the synthetic compounds (Tables 1 and 2). Motivated by the promising anti-*T. cruzi* results found for the 2′,4′-AC series, we synthesized and evaluated 19 new analogues, making different substitutions on their B ring, to test whether their selectivity could be further improved and their cytotoxicity diminished (Table 3).

Chemistry

The synthesis of these compounds started with the preparation of different series of acetophenones, as summarized in Scheme 1. Initially, 2,4,6-trihydroxyacetophenone is transformed into 2,4-dihydroxy-6-methoxyacetophenone using dimethyl sulfate as methylating agent. Then, the methyl group on the *para*-methoxy position of the acetophenone is cleaved using $AlCl_3$ to obtain 2,4-dihydroxy-6-methoxyaceptophenone,[17] which is protected using allyl bromide to give 2,4-allyloxy-6-methoxy-

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[a] Abbreviations: *T. cruzi, Trypanosoma cruzi*; 2′,4′-HDC, 2′,4′-dihydroxy-6′-methoxydihydrochalcones; 4′,6′-MDC, 2′-hydroxy-4′,6′-dimethoxydihydrochalcones; 2′,4′-HC, 2′,4′-dihydroxy-6′-methoxychalcones; 4′,6′-MC, 2′-hydroxy-4′,6′-dimethoxychalcones; 2′,4′-AC, 2′,4′-diallyloxy-6′-methoxychalcones; TPSA, topological polar surface area; %ABS, percentage of absorption; *n*-ROTB, number of rotatable bonds; miLog*P*, logarithm of compound partition coefficient between *n*-octanol and water; *n*-OHNH, number of hydrogen bond donors; *n*-ON, number of hydrogen bond acceptors.

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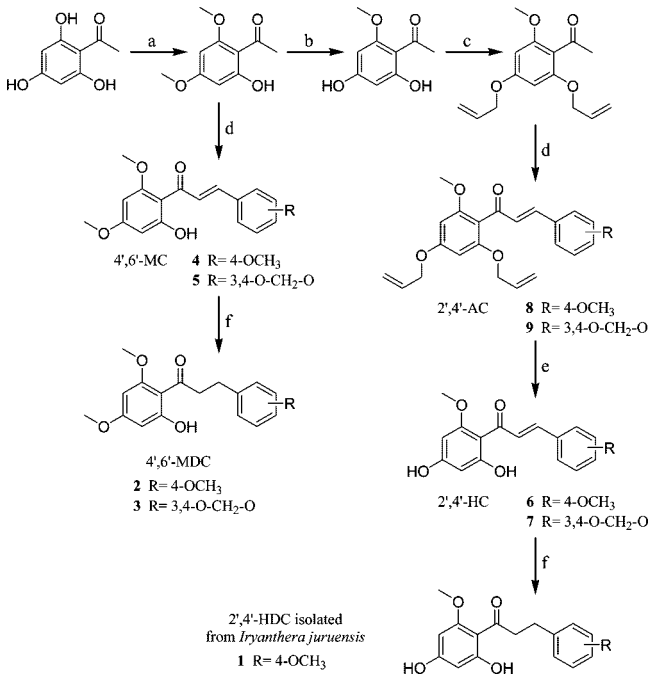
Scheme 1. Strategy for the Synthesis of Compounds **1**−**9**[a]



[a] Reagents and conditions: (a) K_2CO_3, $(CH_3)_2SO_4$, $(CH_3)_2CO$, 65 °C, 6 h. (b) $AlCl_3$, benzene, reflux, 1 h. (c) K_2CO_3, allyl bromide, DMF, rt, overnight. (d) Claisen−Schmidt aldol condensation of an acetophenone with an aromatic aldehyde, KOH, H_2O, CH_3OH, rt, 1−48 h. (e) K_2CO_3, catalytic $Pd(PPh_3)_4$, MeOH, 60 °C, 1 h. (f) catalytic Pd/C 5%, H_2 gas, 250 psi, EtOAc, rt, 1.5 h.

Table 1. Cytotoxicity of Compounds **1**−**9**

	GI_{50} (μM) in indicated cell line[a]							
compd	3T3	H460	DU145	MCF-7	M-14	HT-29	PC3	K562
1	>206	128.0	>206	65.5	109.2	>206	59.9	22.8
2	>197	>197	>197	>197	>197	>197	>197	>197
3	21.5	105.3	60.2	42.4	82.0	144.7	39.1	28.2
4	17.2	24.1	30.8	17.5	22.5	45.3	15.2	20.5
5	39.0	>190	>190	140.1	>190	>190	118.5	18.9
6	6.7	14.0	9.0	17.3	19.6	21.6	19.3	17.0
7	5.1	15.6	12.4	20.4	22.6	20.7	18.5	16.2
8	41.0	20.0	21.0	19.5	27.6	28.4	18.1	16.6
9	31.7	14.5	17.5	10.9	17.2	24.1	9.6	12.2
5FU[b]	<1.54	4.1	15.4	6.5	22.4	8.9	14.6	16.7

[a] 3T3, BALB/3T3 clone A31 embryonic mouse fibroblast cells; H460, human large cell lung cancer; DU145, human prostate carcinoma; MCF-7, human breast adenocarcinoma; M-14, human melanoma; HT-29, human colon adenocarcinoma; PC3, human prostate adenocarcinoma; K562, human chronic myelogenous leukemia cells. [b] 5FU, 5-Fluoruracil.

acetophenone.[18] Claisen−Schmidt aldol condensation of acetophenone with the corresponding aromatic aldehyde in the presence of aqueous KOH gives the chalcone product.[2,3] After a mild deprotection procedure to remove the allyl-protecting groups, using $Pd(PPh_3)_4$ and K_2CO_3, the resulting 2′4′-dihydroxy-6-methoxy chalcones[19] were finally reduced to produce the corresponding dihydrochalcones.[20]

Results

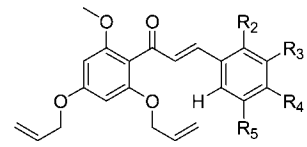
In the present study, three dihydrochalcones and six chalcones were tested for their antiproliferative activity against a panel of seven cancer cell lines and one nontumorogenic cell line (Table 1). Those initial nine compounds, plus 19 synthetic chalcones analogues, were also evaluated for their in vitro anti-*T. cruzi* activity. Their structures and bioactivities are presented in Tables 2 and 3. The cytotoxic assays showed that dihydrochalcones **1** and **2** were less active than their α,β unsaturated counterparts, namely chalcones **6** and **4** respectively. Interestingly, dihydrochalcone **3** showed greater cytotoxicity than its corresponding parent chalcone, **5**. 2′,4′-HC series was more active than the other two series of chalcones, 4′,6′-MC and 2′,4′-AC. However, when assayed for in vitro anti-*T. cruzi* activity, only chalcones **7**−**9** showed activity. We were pleased to find a moderate degree

Table 2. In Vitro anti-*T. cruzi* Activity of Compounds **1**−**9**

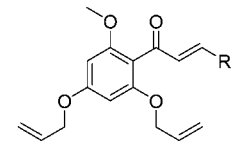
	IC_{50} (μM)[a]		
compd	*T. cruzi*	VERO[b]	SI[c]
1	>25	ND[d]	
2	>100	ND	
3	>100	ND	
4	>25	ND	
5	>25	ND	
6	>25	ND	
7	9.4	25.5	2.7
8	21.4	99.9	4.7
9	13.6	73.5	5.4
Nifurtimox	0.52	80.1	154

[a] IC_{50}: concentration that produces 50% inhibitory effect. [b] VERO, normal African green monkey kidney epithelial cells. [c] SI: Selectivity index = $IC_{50,VERO}/IC_{50,T.\ cruzi}$. [d] Not determined.

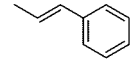
Table 3. In vitro anti-*T. cruzi* Activity of Compounds **10**−**28**



10-23



24-28

		IC_{50} (μM)[a]		
cmpd	R	*T. cruzi*	VERO[b]	SI[c]
10	R_2, R_3, R_4, R_5= H	17.1	17.1	1.0
11	R_2, R_3, R_5= H, R_4= CH_3	17.2	211.3	12.3
12	R_2, R_4, R_5= H, R_3= OCH_3	14.2	141.9	9.9
13	R_2, R_3, R_5= H, R_4= OH	20.3	76.4	3.8
14	R_2, R_5= H, R_3= OCH_3, R_4= OH	>25	ND[d]	-
15	R_3, R_5= H, R_2, R_4= OCH_3	13.1	92.6	7.1
16	R_2= H, R_3, R_4, R_5= OCH_3	3.4	40.9	12.0
17	R_2, R_3, R_5= H, R_4= CF_3	15.6	7.2	0.5
18	R_2, R_3, R_5= H, R_4= Cl	8.6	10.4	1.2
19	R_2, R_3, R_5= H, R_4= F	14.3	13.6	0.9
20	R_3, R_4, R_5= H, R_2= F	6.2	16.3	2.6
21	R_3, R_4, R_5= H, R_2= Br	13.9	13.9	1.0
22	R_2, R_3, R_5= H, R_4= NO_2	4.1	12.6	3.1
23	R_2= H, R_3, R_5= Allyloxy, R_4= Br	6.9	96.3	13.9
24		>25	ND	-
25		1.5	2.8	1.9
26		1.9	2.8	1.5
27		>100	ND	-
28		12.2	190.9	15.6
Nifurtimox		0.52	80.1	154.0

[a] IC_{50}: concentration that produces 50% inhibitory effect. [b] VERO, normal African green monkey kidney epithelial cells. [c] SI: Selectivity index = $IC_{50,VERO}/IC_{50,T.\ cruzi}$. [d] Not determined.

of selectivity for the two 2′,4′-diallyloxy chalcones when compared with compound **7**. Thus, we halted our anticancer screening due to the lack of selectivity observed with the cell lines assayed and instead directed our efforts to investigate the more promising antitrypanosomal activity. As shown in Table 3, 16 of the new synthetic **8** and **9** derivatives (compounds **10**−**13**, **15**−**23**, **25**, **16**, and **28**) showed activity against *T. cruzi* at concentrations below 25 μM, and four of them exhibited a selectivity index greater than 12.

Discussion

The bioassay-guided fractionation of the ethyl ether extract of *I. juruensis* Warb (Myristicaceae), a medicinal plant used by the Aguaruna community living in the Peruvian rainforest, led to the isolation of 2′,4′-dihydroxy-4,6′-dimethoxydihydrochalcone (**1**) as one of its main cytotoxic metabolites against a panel of cancer cell lines.[16] To synthesize the natural compound, we prepared three series of chalcones (4′,6′-MC, 2′,4′-HC, and 2′,4′-AC), which were used as chemical intermediates. The synthesis of this natural compound proved to be not an easy task due to the presence of the dihydroxy phenolic groups. When 2,4-dihydroxy-6-methoxyacetophenone was subjected to a Claisen−Schmidt condensation, no chalcone product was obtained using regular conditions because of the low pK_a (∼7) of the resorcinol. Protection of these two positions in the acetophenone was needed to proceed with the synthesis. We chose the allyloxy group as a protecting agent, mainly because it would be a natural-like moiety compared to silicon-based protecting groups and that it would resist acidic pH when compared to acetal protecting groups. Also, chalcones bearing a benzyl ether protecting group have proved to be inactive as antitumoral agents.[3] In addition, the allyloxy moiety would increase lipophilicity. To the best of our knowledge, this group has not been used before as protecting group in the synthesis of chalcones.

Extensive studies on the antimitotic and antiproliferative effects of chalcones possessing hydroxyl and methoxyl groups at the different positions of the A-ring and B-ring of chalcones have been reported.[2−5] Herein, we present, for the first time, the antiproliferative effect of 2′,4′-HC and 2′,4′-AC chalcones. In general, chalcones' cytotoxicities were higher than their dihydro counterparts lacking the α,β unsaturated carbonyl group, which can react with nucleophiles such as glutathione (GSH). Among chalcones, 2′,4′-HC chalcones (**6**, **7**) were more active than 4′,6′-MC (**4**, **5**) and 2′,4′-AC chalcones (**8**, **9**). The antiproliferative activity of 2′,4′-HC chalcones is in agreement with the results reported by Rao and co-workers,[5] which showed that the activity of 2′-hydroxychalcone derivatives was due to an inhibition of cell cycle progression rather than a direct cytotoxic effect, using the trypan blue exclusion test on Jurkat and U937 cancer cells. The lower cytotoxic activity of 4′,6′-MC and 2′,4′-AC chalcones may be explained by the substitutions on the *ortho* position of the ring A, which could affect the planarity of the molecule. Compound **8** is slightly less active than its structurally related **4**, which indicates that the cytotoxicity decreases as the size of the *ortho* substituent on ring A increases; however, it also demonstrates that some variations are possible in the substitution pattern before the activity is lost. Although the antiprotozoal activity of chalcones have been proven against leishmaniasis and malaria,[1,6,21] their activity against *T. cruzi* have been scarcely reported.[7] The use of an in vitro assay using a *T. cruzi* strain with an enzyme insert represents a major advance because the method allows for high throughput screening for assessing multiple chemical agents for their anti *T. cruzi* efficacy.[22,23] Our laboratory is now making new inserts for other strains because there is significant strain-to-strain variation in sensitivity. In addition, it is essential to have toxicity assays performed because highly toxic compounds are not useful for treatment of this disease. Drug candidates that have high efficacy and low toxicity will need further testing in animal models. In the present study, the anti-*T. cruzi* activity of 2′,4′-AC is reported for the first time. Although active, it can be observed that 2′,4′-AC bearing electron withdrawing groups on ring B (**17**−**23**, **25**, **26**) are highly toxic to VERO cells. On the other hand, 2′,4′-AC having electron donating groups on ring B (**11**, **12**, **15**, **16**, **28**) showed selectivity indexes greater than 7. As can be seen, when comparing compounds **1** and **24**, the addition of a double bond on the α-β unsaturated bridge results on the loss of the activity. The high activity of pyridinium chalcones (**25**, **26**) could not be maintained by the pyrrole analogue **27**; however, when ring B was replaced by furan (**28**), we obtained the highest selectivity index of the series. Although the selectivity indexes of our synthetic compounds did not match the positive control (Nifurtimox), it is known that this drug is only efficient during the acute phase of the disease and is also responsible for severe side effects such as anorexia, vomiting, and peripheral polyneuropathy, among others.[12] Chalcones are known to inhibit mitochondrial *Leishmania* parasite proteins such fumarate reductase, succinate dehydrogenase, NADH dehydrogenase, and NADH-cytochrome *c* reductase.[21]

Table 4. Physical Chemical Properties of Compounds **1**−**28**[a]

ID	%ABS	TPSA (Å^2)	*n*-ROTB	molecular weight	miLog*P*	*n*-OHNH donors	*n*-ON acceptors	Lipinski's violations
rule				<500	≤5	<5	<10	≤1
1	82.8	76.0	6	302.3	3.26	2	5	0
2	86.6	65.0	7	316.4	3.79	1	5	0
3	83.4	74.2	6	330.3	3.63	1	6	0
4	86.6	65.0	6	314.3	3.83	1	5	0
5	83.4	74.3	5	328.3	3.66	1	6	0
6	82.8	76.0	5	300.3	3.29	2	5	0
7	79.6	85.2	4	314.3	3.12	2	6	0
8	90.4	54.0	11	380.4	5.18	0	5	1
9	87.2	63.2	10	394.4	5.02	0	6	1
10	93.5	44.8	10	350.4	5.13	0	4	1
11	93.5	44.8	10	364.4	5.58	0	4	1
12	90.4	54.0	11	380.4	5.16	0	5	1
13	82.8	76.0	9	352.4	4.58	2	5	0
14	83.4	74.2	11	396.4	4.47	1	6	0
15	87.2	63.2	12	410.5	4.99	0	6	0
16	84.0	72.5	13	440.5	4.76	0	7	0
17	93.5	44.8	11	418.4	6.02	0	4	1
18	93.5	44.8	10	384.9	5.80	0	4	1
19	93.5	44.8	10	368.4	5.29	0	4	1
20	93.5	44.8	10	368.4	5.27	0	4	1
21	93.5	44.8	10	429.3	5.91	0	4	1
22	77.9	90.6	11	395.4	5.09	0	7	1
23	87.2	63.2	16	541.4	7.19	0	6	2
24	93.5	44.8	11	376.5	5.65	0	4	1
25	89.1	57.7	10	351.4	3.84	0	5	0
26	89.1	57.7	10	351.4	3.78	0	5	0
27	88.1	60.6	10	339.4	4.10	1	5	0
28	89.0	57.9	10	340.4	4.20	0	5	0

[a] %ABS, percentage of absorption; TPSA, topological polar surface area; *n*-ROTB, number of rotatable bonds; miLog*P*, logarithm of compound partition coefficient between *n*-octanol and water; *n*-OHNH, number of hydrogen bond donors; *n*-ON, number of hydrogen bond acceptors.

A computational study for prediction of ADME properties of all molecules was performed and is presented in Table 4. Topological polar surface area (TPSA) is a good indicator of drug absorbance in the intestines, Caco-2 monolayers penetration, and blood−brain barrier crossing.[25] TPSA was used to

calculate the percentage of absorption (%ABS) according to the equation: %ABS = 109 − 0.345 × TPSA, as reported by Zhao et al.[26] In addition, the number of rotatable bonds (*n*-ROTB) and Lipinski's rule of five[27] were also calculated. From all these parameters, it can be observed that although the oral bioavailability of compounds with selectivity indexes greater than 12 (**11**, **16**, **23**, **28**) could be affected (*n*-ROTB ranged from 10 to 16), they exhibited a great %ABS ranging from 84 to 94%. Furthermore, **11**, **16**, and **28** violate one or none of Lipinski's parameters, making them potentially promising agents for antitrypanosomal therapy.

Conclusion

The screening of synthetic intermediates against a panel of cancer cell lines and *T. cruzi* parasite during the synthesis of a natural product led to the identification of new chalcones with antiprotozoan activity and low toxicity. These series of compounds showed not only promising drug-like properties but they were also easy and economical to prepare, important characteristics to support further in vivo studies and development for possible use in developing countries, where the cost of drug therapies is a major factor.

Experimental Section

^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, using $CDCl_3$ or $CDCl_3/CD_3OD$ as a solvent on a Varian Inova 500. The chemical shifts are reported in ppm values relative to $CHCl_3$ (7.27 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C NMR). Coupling constants (*J*) are reported in hertz (Hz). Melting points were measured on a Thomas−Hoover capillary melting point apparatus and are uncorrected. All air and/or moisture sensitive reactions were carried out under argon atmosphere. Elemental analysis was performed at Atlantic Microlab, Inc., Norcross, GA. Column chromatography (CC) was carried out over Silicycle silica gel (230−400 mesh). Reactions and fractions obtained from CC were monitored on Merck silica gel 60 F254 aluminum sheets. TLC spots were visualized by inspection of plates under UV light (254 and 365 nm) and after submersion in 5% sulfuric acid or in 4% phosphomolybdic acid and heating (110 °C). All commercial reagents were obtained either from Aldrich, Acros, or Alfa Aesar and used without any further purification. 3,5-Bisallyloxy-4-bromobenzaldehyde was available from our laboratory.[18]

Synthesis of Acetophenones. To a refluxing solution of 2,4,6-trihydroxyacetophenone-monohydrate (10 g, 53.7 mmol) and K_2CO_3 (15 g, 108.7 mmol) in acetone (150 mL), $(CH_3)_2SO_4$ was added at three-hour intervals (3 × 3.5 mL, 40.0 mmol). The solution was filtered and the solvent was evaporated to afford 2,4-dimethoxy-6-hydroxyacetophenone as a yellow solid (98%). To obtain 2,4-dihydroxy-6-methoxyacetophenone, anhydrous $AlCl_3$ (11.0 g, 82.5 mmol) and 2,4-dimethoxy-6-hydroxyacetophenone (11.0 g, 56.1 mmol) were suspended in chlorobenzene (133 mL) and heated at reflux for 1 h. After cooling and evaporating of the solvent, an ice-cold H_2O-HCl (1:1, 290 mL) solution was added to the residue and sonicated until the white precipitate seemed homogeneous. The solution was filtered, and the solid was dissolved in EtOAc (200 mL) and extracted with an aqueous solution of NaOH (10%, 3 × 200 mL). The aqueous portions were mixed and neutralized with HCl (conc, 40 mL) to finally be extracted with EtOAc (2 × 250 mL) and recrystallized from the same solvent (44.1%).[18] 2,4-Diallyloxy-6-methoxyacetophenone was prepared by mixing 2,4-dihydroxy-6-methoxyacetophenone (5.8 g, 10.0 mmol), K_2CO_3 (21.8 g, 50.0 mmol), and allylbromide (11.0 mL, 40 mmol) in DMF (100 mL). After stirring for 18 h, the mixture was dissolved in deionized water (100 mL) and extracted with diethyl ether (3 × 75 mL). The organic layers were pooled and extracted with deionized water (3 × 50 mL). Finally, the organic phases were combined and dried to be subjected to column chromatography using hexanes:EtOAc step gradient (40:1 to 5:1, colorless oil, 85.0%).[18]

Synthesis of Chalcones and Dihydrochalcones. To prepare chalcones, the corresponding acetophenone (1.2 mmol), aromatic aldehyde (1.4 mmol), KOH (1.5 g, 26.7 mmol), H_2O (1.5 mL), and CH_3OH (3.0 mL) were stirred at rt for 1 to 48 h. Deionized water (50 mL) was added, and the solution was extracted with EtOAc (2 × 30 mL) and the organic layer was dried over $MgSO_4$ and evaporated. The crude extract was subjected to column chromatography using hexanes:EtOAc gradient (10:1 to 1:1).[2] To obtain 2,4-dihydroxy-6-methoxychalcone, the appropriate 2,4-diallyloxy-6-methoxychalcone (0.25 mmol) and $Pd(Ph_3P)_4$ (1 mmol%) were dissolved in CH_3OH (3 mL); after 1 min of sonication, K_2CO_3 (6 equiv) was added to the mixture and flushed with argon gas for 3 min. The solution was stirred for 1 h at 60 °C, and then it was poured over a solution of HCl (2N, 20 mL). The aqueous solution was extracted with EtOAc (2 × 20 mL), and the organic phase was dried over $MgSO_4$ and evaporated. The orange residue was purified by CC using hexanes:EtOAc step gradients (6:1 to 1:2).[19] 2,4-Dihydroxy-6-methoxydihydrochalcones were obtained by mixing the appropriate chalcone (1.5 mmol) with Pd/C 5% (0.1 equiv) in EtOAc (10 mL) and stirring the solution in a Parr flask under 250 psi of H_2 gas at rt for 1.5 h. After this, the solvent was evaporated and the residue purified by CC using hexanes:EtOAc step gradients (20:1 to 4:1).[20]

Cell Growth Inhibition Bioassay. The cytotoxicity of compounds was assayed using previously described methodology.[16]

The Parasites. *Trypanosoma cruzi* (Tulahuen C4) transfected with β-galactosidase (Lac Z) gene was obtained from Instituto de Investigaciones Cientificas Avanzadas y Servicios de Alta Tecnología−Panamá (AIP). This strain permits high throughput screening of drugs using a colorimetric enzyme assay. Drugs that inhibit the growth of *T. cruzi* (Tulahuen C4) will have no or little color, while those that do not inhibit growth will permit the strain to grow as determined by a purple color change.[22−24] The strain was maintained in monolayer VERO cells (African Green Monkey kidney epithelial cells) in complete RPMI 1640 medium without phenol red (Sigma company, St. Louis MO), supplemented with 10% heat inactivated fetal bovine serum. All cultures and assays are conducted at 37 °C under an atmosphere of 5% CO_2/95% air mixture.

In Vitro Anti-*T. cruzi* Activity. The antitrypanosome activity of chalcones and dihydrochalcones was evaluated by the colorimetric method based on the reduction of the substrate chlorophenol red β-D-galactopyranoside (CPRG) by β-galactosidase resulting from the expression of the gene for *T. cruzi* Tulahuen C4.[25] The assay was realized in 96 well plates containing monolayer VERO cells, which were infected with 5×10^4 trypomastigotes (Tulahuen C4) per well; 24 h later, 10 μg/mL of each compound were added and incubated at 37 °C. After 120 h, 25 μL of 900 μM CPRG substrate (Roche) solution were added to each well to see the antitrypanoside activity of the compound. Then they were incubated at 37 °C for 4−5 h until color developed. The compounds that had antitrypanoside activity (<50% growing inhibition) passed through a second test for determining the inhibitory concentration for 50% growth of the parasites (IC_{50}). These compounds were evaluated at 10, 2, 0.4, 0.8, and 0.16 μg/mL. Each compound and concentration was made in duplicate. The intensity of color resulting from the cleavage of CPRG by β-galactosidase was measured at 570 nm using a VersaMax Micro microplate reader. The IC_{50} of the compound were calculated by logarithmic regression of the OD values obtained compared with the untreated control. All active compounds also went through an evaluation of the cytotoxicity using Thiozol Blue (MTT; 3-[4.5 dimethylthiazol-2-y1]-2,5-diphenyl tetrazolium bromide) (Aldrich, St. Louis MO). This is specially important because compounds that may inhibit the parasite may also be highly toxic and thus not useful as future drug candidates. This reaction was measured at 570 nm using a VersaMax Micro microplate reader. Nifurtimox (Bayer) was used as positive control at concentrations of 0.1, 1, and 10 μg/mL. Negative control was comprised of a media containing 0.1% DMSO.

Physicochemical Parameters Calculation. Absorption (%ABS) was calculated by: %ABS = 109 − (0.345 × TPSA).[26] Polar

surface area (TPSA),[24] miLog*P*, number of rotable bonds, and violations of Lipinski's rule of five[27] were calculated using Molinspiration online property calculation toolkit,[28] according to previously reported literature.[25−28]

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Supporting Information Available: Melting points, isolated yields, spectral data (^{1}H and ^{13}C NMR), elemental analysis, and different Log*P* calculations of compounds **1**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

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