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Unsymmetrical 1,5-diaryl-3-oxo-1,4-pentadienyls and their evaluation as antiparasitic agents

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ABSTRACT

In this work the synthesis and antiparasitical activity of new 1,5-diaryl-3-oxo-1,4-pentadienyl derivatives are described. First, compounds **1a**, **1b**, **1c** and **1d** were prepared by acid-catalyzed aldol reaction between 2-butanone and benzaldehyde, anisaldehyde, *p*-*N*,*N*-dimethylaminobenzaldehyde and *p*-nitrobenzaldehyde. Reacting each of the methyl ketones **1a**, **1b**, **1c** and **1d** with the *p*-substituted benzaldehydes under basic-catalyzed aldol reaction, we further prepared compounds **2a**–**2p**. All twenty compounds were evaluated for antiproliferative activity, particularly for promastigote of *Leishmania amazonensis* and epimastigote of *Trypanosoma cruzi*. All compounds showed good activity while nitro compounds **2i** and **2k** showed inhibition activity at a few μM.

1. Introduction

The 1,5-diarylpentanoid dibenzylideneacetone (Fig. 1) is the parent of a class of compounds having an acyclic dienone attached to aryl groups in both β-positions. These structures resemble those of the curcuminoids (1,7-diarylheptanes) and the chalcones (1,3-diarylpropanes), which are very important bioactive natural compounds found in many plant species. Accompanying these structural similarities, synthetic chalcones and related compounds[1] have shown biological activities such as antitumor,[2] anticancer and antioxidant,[3] antifungal,[4,5] antimitotic,[6] chemoprotective,[7] anti-inflammatory,[8,9] antimicrobial,[10] anti-nociceptive,[11] antibacterial,[12] antimalarial.[13,14] In addition, dibenzylideneacetone potentiates TRAIL-induced apoptosis by down-regulation of cell survival proteins and up-regulation of death receptors through activation of ROS and CHOP mediated pathways.[15] The good bio-availability of some dibenzylideneacetone and their derivatives, which is required for bioactivities[16], as well as their mode of cross linking, has raised the interest of chemists in their synthesis.

Aher et al. have shown that dibenzylideneacetone have good potential to inhibit some parasites growth.[14] These findings motivated us to investigate the activity of similar compounds against *Leishmania amazonensis* and *Trypanosoma cruzi*, the etiologic agents that causes '*Leishmaniosis*' and 'Chagas disease', respectively. These two diseases affect, according to the World Health Organization, 12 million of people in 88 countries, and 350 million are at risk of acquiring this infection.[17] Additionally, Chagas disease is considered a serious public health problem that affects approximately 10 million people in Latin America. The incidence of this disease has been estimated to include 300,000 new cases per year, and approximately 10,000 people die from this infection annually.[18–20] In view of the lack of safe medication and the serious side effects caused by the use of available chemotherapy,[20] there is a need of new drugs for the treatment of these diseases. In the past decade, chalcones and related compounds emerged as a new class of antitrypanosomatids agents.[21–26] Thus, due to their structural similarities with chalcones and curcuminoids we attempted to synthesize some dibenzylidene derivatives. The search for fascinating pharmacologically active molecular building blocks, based on diverse structural features, easy synthetic routes and desired functionalities have attracted our attention to synthesize dibenzylideneacetones systems.

2. Synthesis plan

Dibenzylideneacetones (Fig. 1) can be formed by the direct reaction of benzaldehyde (A1) with acetone (K1) using basic or acid

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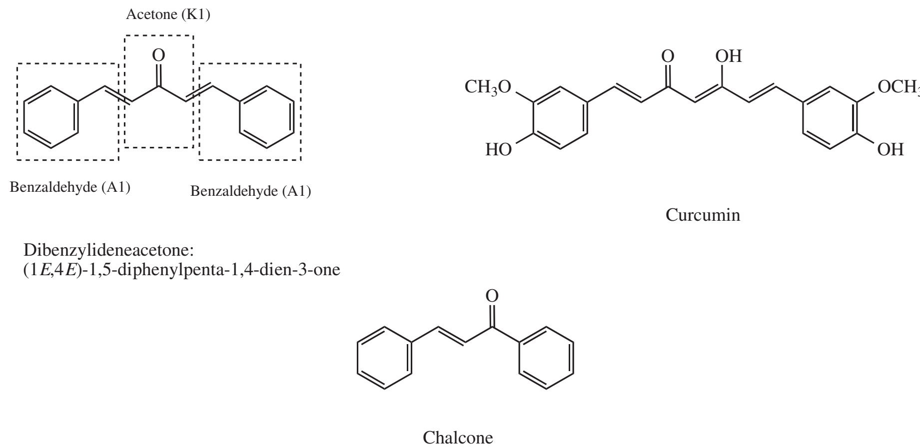


Figure 1. Basic structure of a dibenzylideneacetone and their natural congeners curcumin and chalcone.

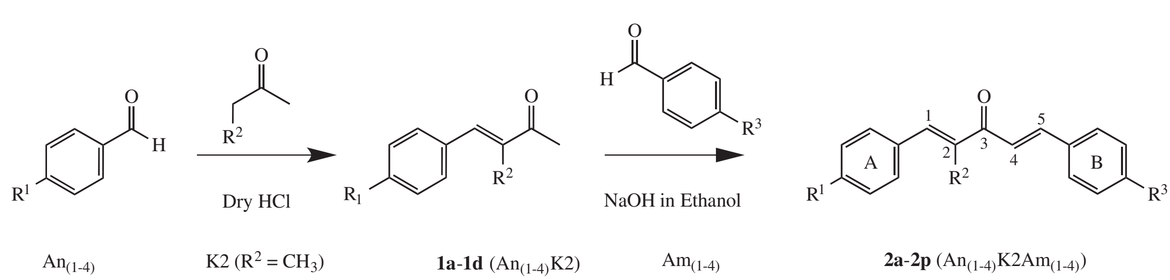
catalysis.[27] Acid catalysts used for cross–aldol condensation reaction include sulfuric, hydrochloric,[28,29] and Lewis[30–32] acids. Generally, aldol condensation can be carried at room temperature or ethanol under reflux condition.[33,34] Dibenzylideneacetones and chalcones usually are more easily synthetized in one step by aldol condensation under basic catalysis. This procedure works very well for symmetric ketones and a variety of substituted aldehydes. However, only a few studies used non-symmetric ketones and different aldehydes to be attached to the ends of the alkyl chain spacer.[16,35] These non-symmetric 1,5-diaryl-3-oxo-1,4-pentadienyls can be formed in two steps.[35] Methyl–alkyl ketones are good for aldol condensation because of the easy regiochemistry control. Therefore, these ketones react with an appropriate aldehyde in acidic medium (thermodynamic enol formation), and then the dehydrated aldol is isolated (Scheme 1). The second aldehyde is then added dropwise in cold ethanol solution, stirred, and the non-symmetric 1,5-diaryl-3-oxo-1,4-pentadienyl is formed (Scheme 1). Using this methodology[35] and combining different aldehydes with ketones, it is possible to generate a great molecular diversity, creating a library of compounds with 1,5-diarylpentane basic structures, which are still to be explored as to their bioactivities.

Thus, herein is reported a new series of dibenzylideneacetones, which have been prepared according Scheme 1, by combination of four *p*-substituted aldehydes (A1–A4) with butanone (K2) resulting in the four 4-aryl-3-methylbutenones **1a**-**1d** [R^1 = H (A1) or OCH_3 (A2) or NO_2 (A3) or $N(CH_3)_2$ (A4), respectively; R^2 = CH_3], which were individually further condensed with the same aldehydes, resulting in the sixteen compounds **2a**–**2p** ($An_{(1-4)}K2Am_{(1-4)}$). The antiproliferative activities of the twenty-synthetized compounds were evaluated, particularly in promastigotes of *L. amazonensis* and epimastigotes and trypomastigotes of *T. cruzi*.

3. Results and discussion

3.1. Chemistry

The reaction of benzaldehyde (**A1**) and three *p*-substituted benzaldehydes (**A2**–**A4**) with butanone, using gaseous HCl as catalyst as outlined in Scheme 1, produced the 4-aryl-3-methylbutenone **1a**–**1d**, which were isolated and characterized by spectroscopic data. These reactions were performed at room temperature by stirring the reaction mixture and passing dry gaseous HCl until the reaction mixture turned to red. Stirring continued until the completion of starting materials, which was monitored by TLC. The reaction was very clear giving yields of 48–61% of **1a**–**1d**, which were analyzed by NMR as follows: compound **1** showed two characteristic signals of two methyl groups in the shielded region at δ_H 2.06 and 2.47, whereas one =CHsignal was displayed at δ_H 7.39 as singlet; additionally three aromatic signals having integration for five proton were detected at δ_H 7.32, 7.41 and 7.52. ^{13}C NMR spectra of **1** showed signals for two methyl groups at δ_C 13.0 and 25.9, and six peaks from δ_C 128–139 for eight carbons, with two of these peaks having double intensity for aromatic carbons. The characteristic peak at δ_C 200.3 is due to carbonyl carbon. The ^{1}H NMR spectra of compounds **1a**, **1b** and **1c** are similar each other. Only compound **1b** showed an extra signal at δ_H 3.85 due to OCH_3 substitution on benzene, and a signal at δ_H 3.02 for compound **1d** due to $N(CH_3)_2$ substitution in benzene ring. By reacting each of the intermediary compounds **1a**–**1d** systematically with benzaldehyde (**A1**), anisaldehyde (**A2**), *N*,*N*-dimethylaminobenzaldehyde (**A3**) and nitrobenzaldehyde (**A4**) the respective sixteen dibenzylidene ketones **2a**–**2p** were produced in 45–93% yield after re-crystallization from ethanol. All these reactions were carried in basic medium in ethanol, and their



Scheme 1. Reaction conditions for the synthesis of the assayed compounds, using *p*-substituted aldehydes A1 (R^1,R^3 = H), A2 (R^1,R^3 = OCH_3), A3 (R^1,R^3 = NO_2) and A4 (R^1,R^3 = $N(CH_3)_2$) and butanone (K2, R^2 = CH_3) resulting in **1a**–**1d**, respectivelly; and further reaction of each enone **1a**–**1d** with same aldehydes to give the collection $An_{(1-4)}K2Am_{(1-4)}$ (**2a**–**2p**).

progress was monitored by TLC. The reaction time varied for each AnK2Am product and depended on the nature and reactivity of the aldehyde used. Thus, as expected for aldol condensations, electron donators (amine and methyl–ether) at *para*-position in the benzene rings decreases the reaction time, while nitrobenzaldehyde reacted very fast. The aldol-dehydrated products are preferably *E* regeoisomers but *Z* isomers were also obtained in 1–5%. The structures of these new compounds were determined by ^{1}H and ^{13}C NMR, HMQC and HMBC spectra. Compounds **2a**–**2d** were synthesized from **1a**, by treating it with benzaldehyde, anisaldehyde, nitrobenzaldehyde and *N*,*N*-dimethylaminobenzaldehyde, respectivelly, in basic conditions in ethanol. The presence of a pair of doublets at δ_H 7.53 and 7.69, with a coupling constant of c.a. 16 Hz, typical for a *trans* two spins system, clearly confirms that the aldol condensation with the second aldehyde did occur. Also, extra signals in the aromatic region were noted in the ^{1}H spectra and the signal for methyl ketone group disappeared, showing that an aromatic ring had been added and the methyl group had been substituted to a significant extent. The major peaks in the NMR spectra of these compounds are almost the same except from an additional signal in compounds **2a**, **2b** and **2d**. Compound **2a** has benzene ring with no substituent, while in compound **2b** the *para* hydrogen of benzene is substituted by a OCH_3 group, showing a singlet at δ_H 3.85 ppm in ^{1}H NMR, and a peak at δ_C 55.4 ppm in ^{13}C NMR. Compound **2d** shows an extra signal at δ_H 3.06 due to $N(CH_3)_2$, and **2c** is prompt recognized by the strong deshielding effect caused by NO_2 group to the *ortho* hydrogen's (δ_H 8.26). Compounds **2e**–**2p** were similarly synthesized from compound **1b**–**1d**, so the spectroscopic study is comparable to that of compounds **2a**–**2d**, showing approximately the same signals shifts as shown in the compounds derived from **1**. The structures of all compounds were further identified using mass spectrometry and UV–vis analysis.

3.2. Biological evaluation

The synthesized compounds **1a**–**1d** and **2a–2p** were evaluated against promastigote forms of *L. amazonensis* and epimastigote and trypomastigote forms of *T. cruzi*. The intermediate compounds AnK2 (**1a**–**1d**) were found inactive in the bioassays, indicating that the second aldol reaction is important since it introduces groups that contribute to bioactivity. The more active compounds against epimastigotes of *T. cruzi* were **2d**, **2i**, **2j**, and **2k**, exhibiting an IC_{50} value (inhibitory concentration for 50% of the parasites) of 13.3, 3.5, 10.0, and 1.8 μM, respectively (Table 1). It is important to note that, for both forms epimastigote and trypomastigote, the activities of compounds **2k** and **2i** (EC_{50}, effective concentration for 50% of the parasites, 15.4 and 17.5 μM, respectively) were found greater than the positive control benznidazole (IC_{50} of 6.5 μM and EC_{50} 34.5).[36] Considering the evaluation of activity against promastigotes of *L. amazonensis* it was observed that substances **2c**, **2g**, **2i**, **2l** and **2k** are the most active, with IC_{50} values of 11.6, 14.8, 13.4, 12.0 and 3.4 μM, respectively. Amphotericin B, the reference drug for *L. amazonensis*, has an IC_{50} of 0.06 μM, which is better than our compounds, although its toxicity is high (3.74 μM).[37]

The literature concerning antiparasitary activities of curcuminoids and chalcones reports results with IC_{50} values around those we found in the present work.[26,38] Symmetrical dibenzylideneketones made using cyclic ketones gave antileishmanial activity with lower IC_{50} values.[39] A recent study showed that both electron withdraw or donator groups at the aryl part of symmetrical dibenzylideneacetones, affect positively the bioactivity compared with nonsubstituted compounds.[40] Our results, using the series of asymmetrical dibenzylydeneketone compounds (Table 1), shows that the strong electron-attractor NO_2 substituent in A3 is the most responsible for the great activity observed. Compounds **2c** (A1K2A3) and **2i** (A3K2A1) differ only in the aromatic ring where the NO_2 is attached, but their bioactivity is significantly different. This result indicates that introducing the electron-attracting group during the first reaction results in better activity of the final compounds. Thus, nitro compounds **2i** (A3K2A1) and **2k** (A3K2A3) were found to be the most active antiparasitics in the three assays performed (Table 1), and indeed compound **2k**, with two NO_2 groups, is the most active amongst the twenty tested substances. These results parallels those recently published by others researchers, which found that nitroreductase enzymes, present in *Leishmania danovani* but not in the mammal cells, may activate NO_2-containing drugs into a more active form.[41]

The mechanism by which drugs act against parasites have been studied by many groups around the world.[41,42] Many of these studies suggest that drugs can bind to nucleic acids and cell wall components, or inhibit enzymes activity in the parasite. Along with the presence of *p*-NO_2 group in our compounds, that putatively can be

Table 1
Antiparasitary and citotoxicity activities measured for of compounds **1a**–**1d** and **2a**–**2p**

Compounds	Epimastigote IC_{50} (μM) Average ± SD	Trypomastigote EC_{50} (μM) Average ± SD	Promastigote IC_{50} (μM) Average ± SD	$LLCMK_2$ CC_{50} (μM) Average ± SD	M. J774A1 CC_{50} (μM) Average ± SD
1a (A1K2)	>100	>100	>100	687.5 ± 17.68	742.5 ± 81.32
1b (A2K2)	>100	>100	>100	280.0 ± 28.28	240.0 ± 14.14
1c (A3K2)	82.2 ± 7.42	>100	>100	266.0 ± ± 12.73	70.5 ± 16.26
1d (A4K2)	>100	>100	>100	150.0 ± 0.00	160.0 ± 14.14
2a (A1K2A1)	15.9 ± 1.34	71.7 ± 2.33	15.3 ± 0.35	33.6 ± 5.28	34.5 ± 7.78
2b (A1K2A2)	14.3 ± 5.13	>100	20.4 ± 3.32	93.0 ± 9.90	51.0 ± 11.31
2c (A1K2A3)	16.9 ± 0.00	65.0 ± 7.07	11.6 ± 1.70	43.3 ± 4.16	26.0 ± 1.41
2d (A1K2A4)	13.3 ± 2.90	>100	20.0 ± 2.83	53.0 ± 4.24	20.2 ± 3.96
2e (A2K2A1)	16.7 ± 1.41	73.3 ± 4.67	22.3 ± 1.77	44.0 ± 0.00	54.0 ± 8.49
2f (A2K2A2)	23.6 ± 2.33	>100	21.8 ± 0.28	75.0 ± 7.07	34.1 ± 1.75
2g (A2K2A3)	25.2 ± 4.60	>100	14.8 ± 1.77	20.1 ± 6.80	30.0 ± 6.56
2h (A2K2A4)	21.3 ± 0.00	>100	18.0 ± 0.00	411.0 ± 55.15	36.2 ± 4.35
2i (A3K2A1)	3.5 ± 0.35	17.5 ± 2.05	13.4 ± 1.98	264.3 ± 3.25	47.3 ± 4.16
2j (A3K2A2)	10.0 ± 0.00	73.3 ± 0.00	15.5 ± 1.70	172.1 ± 15.77	36.6 ± 1.15
2k (A3K2A3)	1.8 ± 0.21	15.4 ± 4.10	3.4 ± 0.07	32.5 ± 1.20	43.0 ± 4.24
2l (A3K2A4)	14.0 ± 0.00	>100	12.0 ± 0.71	25.9 ± 9.50	24.7 ± 2.52
2m (A4K2A1)	24.1 ± 2.62	78.3 ± 2.40	21.5 ± 0.71	191.0 ± 12.73	28.5 ± 0.71
2n (A4K2A2)	>100	>100	20.1 ± 1.48	285.1 ± 81.81	34.3 ± 5.13
2o (A4K2A3)	>100	>100	>100	167.0 ± 8.49	966.6 ± 57.74
2p (A4K2A4)	25.1 ± 1.27	>100	20.5 ± 2.12	314.3 ± 0.00	10.0 ± 0.00
Benznidazole	6.5 ± 0.7	34.5 ± 7.6		614.7 ± 115.2	
Amphotericin B			0.06 ± 0.00		3.7 ± 0.31

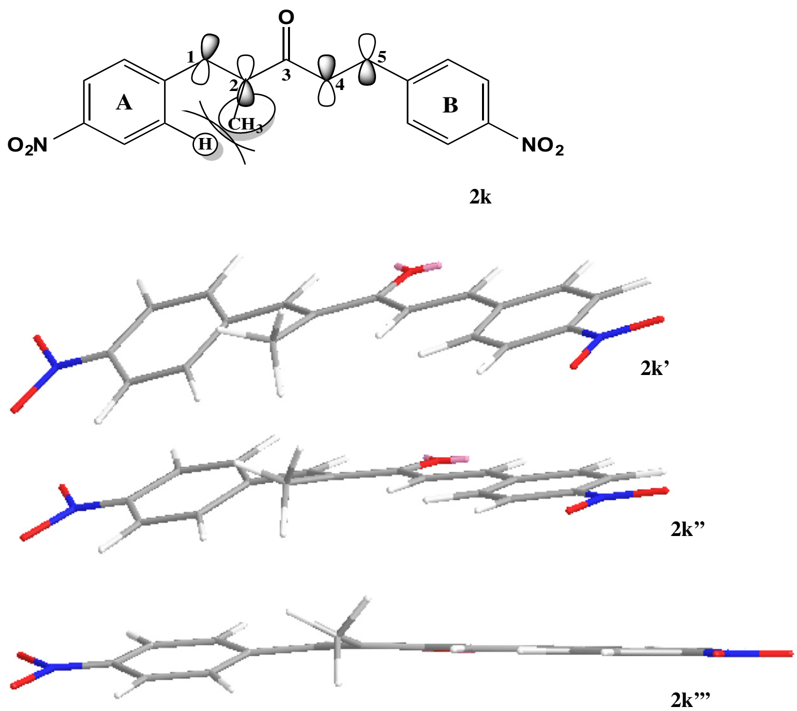


Figure 2. Structural features of compound **2k**. **2k**′-**2k**‴ are conformers generated from geometry computational optimization, showing that ring-A gets out of the plane formed of ring-B and C-3 - C-5, due steric hindrance caused by the presence CH_3 group close to Ar-H in ring-A.

reduced/activated by parasite reductases, our nitrobenzylideneketones **2i** and **2k** may be good electrophiles to bind some parasite cells components. These molecules were studied using the semi empirical quantum-mechanical AM1 parameterization,[43] in order to see possible binding points. The molecular geometries that came out from these calculations (see **2k**′ to **2k**‴ in Fig. 2) shows that the presence of the methyl group at C-2 of the 1,4-pentadienyl-3-one chain disturbs the first enone π system (1-en-3-one), while the second part, formed by the ring-B bound to C-5 and the 4-en-3-one, is planar with an almost perfect parallelism of the *p*-orbitals. As a result, carbon C-1 contains only small electron density compared with C-5. These observations may have some relationship with our bioactivity results, which showed that electron attractor group present in the ring-A connected to C-1 contributes for greater bioactivity. Probably C-1 is a good electrophilic center to bind parasite cell components.

In addition, we also evaluated the cytotoxicity of the compounds against $LLCMK_2$ cells (kidney epithelial cells from *Macaca mulatta*) and macrophages J774A1. The results showed that the active substances were more toxic against the parasites than for the cell lines tested. Finally, our results demonstrated that compounds **2i** and **2k** proved to be the most active against this trypanosomatids and may become promising compounds for treatment of leishmaniasis and Chagas disease.

4. Conclusions

The reaction of 2-butanone with benzaldehyde, anisaldehyde, *N*,*N*-dimethylaminobenzaldehyde and nitrobenzaldehyde gave **1a**, **1b**, **1c** and **1d**, which on subsequent coupling with the same aldehydes yielded the respective benzylidenedienones **2a**–**2p**. These compounds were investigated for antiproliferative against promastigotes of *L. amazonensis* and epimastigotes and tripomastigotes of *T. cruzi*, and some compounds were shown to have good reproducible activity, while compounds **2i** and **2k** showed enhanced potency. It is clear that the presence of the *p*-nitrobenzene group at the methyl side strongly contributes to antiparasitic activity.

5. Experimental

5.1. Chemistry

All chemicals were purchased from Organics, Sigma–Aldrich, Acros Chemicals and Fisher Scientific Ltd and used without further purification. The deuterated solvents from Apolo were used for the NMR analysis. Thin layer chromatography (TLC) was performed with precoated silica gel G-25-UV254 plates and was carried out at 254 nm under UV, and by ceric sulphate in 10% H_2SO_4 solution. High-resolution mass spectral data (ESI-HRMS) were acquired using a Thermo Scientific LTQ Orbitrap XL spectrometer. ^{1}H NMR, ^{13}C NMR and 2D experiments (*g*HSQC (^{1}H/^{1}H), gHMBC (^{1}H/^{13}C) and NOESY) were performed on a Brüker AVANCE 400 operating at 400.15 MHz and 100.62 MHz, respectively. $CDCl_3$ was used as solvent and tetramethylsilane (TMS) as internal reference. Compounds **1a**–**1d** and **2a**–**2p** were dissolved in organic solvents at about 10 mg mL^{-1} each and transferred into a 5-mm NMR tube. Chemical shifts (δ ppm) were measured with accuracy of 0.01 (^{1}H) and 0.1 ppm (^{13}C). The UV–vis spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer using 1 cm optical length quartz cuvettes at 25 °C and chloroform as solvent.

5.1.1. (*E*)-3-Methyl-4-phenylbut-3-en-2-one (1a)

Benzaldehyde (10 g, 90 mmol) and 2-butanone (1.36 g, 1.69 mL, 18.9 mmol) were taken in a 50 mL two–necked round bottom flask. Dry HCl gas was passed in the content of the flask until it was saturated and red coloration appeared. The reaction mixture was stirred for 8 h. The crude product was diluted with toluene and washed with $NaHSO_3$ solution. The organic layer was separated, dried with anhydrous Na_2SO_4 and evaporated in vacuum. The residue was distilled under reduced pressure to give pure compound, which was solidified by keeping in a refrigerator for 2 days.

Percent yield: 52%; mp: 34–35 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.52 (d, 1H, J = 4 Hz, ArH), 7.41 (m, 3H, Ar H), 7.39 (m, 1H, CH=CCH_3), 7.32 (m, 1H, ArH), 2.47 (s, 3H, $COCH_3$), 2.06 (d, J = 1.6 Hz, 3H, CH=CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 129.7, 200.3, 139.7, 137.8, 135.9, 128.6, 128.5, 25.9, 12.9; HRMS ESI(+): calcd for $C_{11}H_{13}O$ $(M+H)^+$ 161.0961, found 161.0962; UV–vis λ_{max} (nm) in CH_2Cl_2: 278.

5.1.2. (*E*)-4-(4-Methoxyphenyl)-3-methylbut-3-en-2-one (1b)

Percent yield: 67%; mp: Oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.47 (s, 1H, =CH), 7.40 (d, J = 12 Hz, 2H, ArH), 6.93 (d, J = 12 Hz, 2H, ArH), 3.85 (s, 3H, OCH_3), 2.45 (s, 3H, =CCH_3), 2.07 (d, J = 1.6 Hz, 3H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.6, 200.2, 159.9, 139.6, 135.8, 128.4, 114.0, 55.3, 25.8, 12.9; HRMS ESI(+): calcd for $C_{12}H_{15}O_2$ $(M+H)^+$ 191.1067, found 191.1072; UV–vis λ_{max} (nm) in CH_2Cl_2: 308.

5.1.3. (*E*)-3-Methyl-4-(4-nitrophenyl)but-3-en-2-one (1c)

Percent yield: 61%; mp: 84–85 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.27 (d, J = 8 Hz, 2H, ArH), 7.55 (d, J = 8 Hz, 2H, ArH), 7.52 (s, 1H, =CH), 2.49 (s, 3H, $COCH_3$), 2.06 (d, J = 1.6 Hz, 3H, =CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 199.6, 147.3, 142.5, 140.6, 136.5, 130.2, 123.7, 26.0, 13.2; HRMS ESI(+): calcd for $C_{11}H_{11}NO_3$ $(M+H)^+$ 206.0812, found 206.0825; UV–vis λ_{max} (nm) in CH_2Cl_2: 300.

5.1.4. (*E*)-4-(Dimethylamino)phenyl)-3-methylbut-3-en-2-one (1d)

Percent yield: 48%; mp: 122–123 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 (s, 1H, =CH), 7.41 (d, J = 8Hz, 2H, ArH), 6.71 (d, J = 12Hz, 2H, ArH), 3.02 (s, 6H, $N(CH_3)_2$, 2.44 (s, 3H, $COCH_3$), 2.10 (d, J = 4 Hz, 3H, =CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 200.2, 150.5, 140.7, 133.4, 131.9, 123.6, 111.7, 40.2, 25.71, 13.0; HRMS ESI(+): calcd for $C_{13}H_{17}NO$ $(M+H)^+$ 204.1383, found 204.1385; UV–vis λ_{max} (nm) in CH_2Cl_2: 366.

5.1.5. (1*E*,4*E*)-2-Methyl-1,5-diphenylpenta-1,4-dien-3-one (2a)

A Solution of A1K2 (**1a**, 50 mg, 3.13 mmole), and benzaldehyde (39.8 mg, 3.75 mmole) in ethanol (5 mL) was stirred for 5 min at room temperature and a sodium hydroxide solution in ethanol (4 mL, 50 mmole) was added stirring continued for seven hours; the solvent evaporated in vacuum and the residue was dissolved in ethyl acetate. Extraction with a $NaHSO_3$ solution, drying with Na_2SO_4, and concentration in vacuum, the crude product, which was collected as yellow precipitate and further purified by column chromatography and recrystallized from ethanol, gave pure compound **2a**. Percent yield: 51.4%; mp: 52–53 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.67 (d, J = 16 Hz, 1H, Ar-CH), 7.59 (m, 1H, ArH), 7.56 (m, 2H, ArH), 7.40 (m, 4H, ArH), 7.34 (s, 1H, Ar-CH), 7.28 (d, J = 8 Hz, 1H, COCH), 6.92 (d, J = 8 Hz, 2H, ArH), 3.85 (s, 3H, OCH_3), 2.19 (d, J = 1.6 Hz, 3H, =C–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.9, 161.4, 143.4, 138.7, 138.1, 133.3, 130.0, 129.7, 128.4, 127.9, 119.7, 114.4, 55.41, 13.9; HRMS ESI(+): calcd for $C_{18}H_{17}O$ $(M+H)^+$ 249.1274, found 249.1283; UV–vis λ_{max} (nm) in CH_2Cl_2: 313.

5.1.6. (1*E*,4*E*)-5-(4-Methoxyphenyl)-2-methyl-1-phenylpenta-1,4-dien-3-one (2b)

Percent yield: 57%; mp: 115–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.67 (d, J = 16 Hz, 1H, CH_3OAr–CH), 7.59 (m, 1H, ArH), 7.56 (m, 2H, ArH), 7.40 (m, 4H, ArH), 7.34 (s, 1H, Ar-CH), 7.28 (d, J = 8 Hz, 1H, COCH), 6.92 (d, J = 8 Hz, 2H, ArH), 3.85 (s, 3H, OCH_3), 2.19 (d, J = 1.6 Hz, 3H, =C–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.9, 161.4, 143.38, 138.7, 138.1, 133.3, 130.0, 129.7, 128.4, 127.9, 119.7, 114.4, 55.4, 13.9; HRMS ESI(+): calcd for $C_{19}H_{18}O_2$ $(M+H)^+$ 279.1380, found 279.1388; UV–vis λ_{max} (nm) in CH_2Cl_2: 340.

5.1.7. (1*E*,4*E*)-2-Methyl-5-(4-nitrophenyl)-1-phenylpenta-1,4-dien-3-one (2c)

Percent yield: 50.5%; mp: 138–140 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.26 (d, J = 8 Hz, 2H, ArH), 7.75 (d, J = 12 Hz, 2H, ArH), 7.69 (d, J = 16 Hz, 1H, =CH), 7.62 (s, 1H, =CH), 7.53 (d, J = 16 Hz, 1H, =CH), 7.43–7.50 (m, 5H, ArH), 7.34 (t, 1H, ArH), 2.21 (d, J = 1.6 Hz, 3H, =CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 191.8, 148.4, 141.4, 1403.2, 139.9, 138.4, 135.6, 134.63, 129.8, 128.8, 125.7, 124.2, 124.0, 13.7; HRMS ESI(+): calcd for $C_{18}H_{16}NO_3$ $(M+H)^+$ 294.1125, found 294.1138; UV–vis λ_{max} (nm) in CH_2Cl_2: 300.

5.1.8. (1*E*,4*E*)-5-(4-Dimethylamino)phenyl)-2-methyl-1-phenylpenta-1,4-dien-3-one (2d)

Percent yield: 68%; mp: 91–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.68 (d, J = 16Hz, 1H, =CH), 7.52 (d, J = 8Hz, 2H, ArH, 7.40 (m, 4H, ArH), 7.31 (m, 1H, =CH), 7.19 (d, J = 16Hz, 1H, =CH), 6.68 (d, J = 8Hz, 2H, ArH), 3.04 (s, 6H, $N(CH_3)_2$, 2.19 (d, J = 1.6 Hz, 2H, =CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 193.1, 151.9, 144.55, 138.9, 137.0, 136.4, 130.1, 129.7, 128.4, 128.2, 122.9, 116.9, 111.7, 40.17, 14.1; HRMS ESI(+): calcd for $C_{20}H_{22}NO$ $(M+H)^+$ 292.1696, found 292.1710; UV–vis λ_{max} (nm) in CH_2Cl_2: 414.

5.1.9. (1*E*,4*E*)-1-(4-Methoxyphenyl)-2-methyl-5-phenylpenta-1,4-dien-3-one (2e)

Percent yield: 51%; mp: 57–58 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.67 (d, J = 12 Hz, 1H, =CH), 7.61 (m, 2H, ArH), 7.56 (s, 1H, CH_3-C=CH), 7.45 (m, 3H, ArH), 7.39 (m, 3H, =CHand ArH), 6.95 (d, J = 8 Hz, 2H, ArH), 3.85 (s, 3H, OCH_3), 2.21 (d, J = 1.6 Hz, 3H, =C–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.6, 159.9, 143.0, 138.8, 136.6, 135.3, 131.7, 130.08, 128.9, 128.5, 128.2, 122.1, 114.0, 55.4, 13.8; HRMS ESI(+): calcd for $C_{19}H_{19}O_2$ $(M+H)^+$ 279.1380, found 279.1388; UV–vis λ_{max} (nm) in CH_2Cl_2: 335.

5.1.10. (1*E*,4*E*)-1,5-Bis(4-methoxyphenyl)-2-methypenta-1,4-dien-3-one (2f)

Percent yield: 91%; mp: 86–88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.65 (d, J = 16, 1H, =CH), 7.56 (d, J = 12 Hz, 2H, ArH), 7.53 (s, 1H, CH_3C=CH), 7.44 (d, J = 8Hz, 2H, ArH), 7.30 (d, J = 12 Hz, 1H, =CH), 6.95 (d, J = 8 Hz, 2H, ArH), 6.92 (d, J = 8 Hz, 2H, ArH), 3.85 (d, J = 4 Hz, 6H, OCH_3), 2.20 (d, J = 1.6 Hz, 3H, =C–CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 13.9, 55.4, 114.0, 114.3, 119.8, 128.0, 128.7, 129.9, 131.6, 136.8, 138.1 (1C, =C), 159.8, 161.3, 192.7; HRMS ESI(+): calcd for $C_{20}H_{21}O_3$ $(M+H)^+$ 309.1485, found 309.1494; UV–vis λ_{max} (nm) in CH_2Cl_2: 348.

5.1.11. (1*E*,4*E*)-1-(4-Methoxyphenyl)-2-methyl-5-(4-nitrophenyl)penta-1,4-dien-3-one (2g)

Percent yield: 69.3%; mp: 145–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.25 (d, J = 12 Hz, 2H, ArH), 7.74 (d, J = 12 Hz, 2H, ArH), 7.66 (d, J = 16 Hz, 1H, =CH), 7.59 (s, 1H, =CH), 7.54 (d, J = 16 Hz, 1H, =CH), 7.47 (d, J = 8 Hz, 2H, ArH), 6.97 (d, J = 8Hz, 2H, ArH), 3.87 (s, 3H, OCH_3), 2.22 (d, J = 1.6 Hz, 3H, =CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 191.6, 160.2, 148.3, 141.5, 140.0, 139.8, 136.4, 131.83, 128.7, 128.2, 125.8, 124.2, 114.1, 55.4, 13.3; HRMS ESI(+): calcd for $C_{19}H_{18}NO_4$ $(M+H)^+$ 324.1230, found 324.12449; UV–vis λ_{max} (nm) in CH_2Cl_2: 348.

5.1.12. (1*E*,4*E*)-5-(4-(Dimethylamino)phenyl)-1-(4-methoxyphenyl)-2-methypenta-1,4-dien-3-one (2h)

Percent yield: 93%; mp: 68–70 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.66 (d, J = 12 Hz, 1H, =CH), 7.50 (s, 1H, =CH), 7.49 (d, J = 8 Hz, 2H, ArH), 7.41 (d, J = 12 Hz, 2H, ArH), 7.20 (d, J = 16 Hz, 1H, =CH), 6.92 (d, J = 8 Hz, 2H, =CH), 6.65 (d, J = 8Hz, 2H, ArH), 3.82 (s, 3H, OCH_3), 2.99 (s, 6H, $N(CH_3)_2$, 2.19 (d, J = 1.6 Hz, 3H, =CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.9, 159.7, 151.8, 144.1, 137.0, 137.0, 131.52, 130.1, 128.9, 123.0, 116.9, 113.9, 111.7, 55.33, 40.1, 14.1; HRMS ESI(+): calcd for $C_{21}H_{23}NO_2$ $(M+H)^+$ 322.1802, found 322.1811; UV–vis λ_{max} (nm) in CH_2Cl_2: 413.

5.1.13. (1*E*,4*E*)-2-Methyl-1-(4-nitrophenyl)-5-phenylpenta-1,4-dien-3-one (2i)

Percent yield: 92%; mp: 100–101 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.28 (d, *J* = 8 Hz, 2H, ArH), 7.73 (d, *J* = 16 Hz, 2H, ArH), 7.62 (m, 2H, ArH), 7.58 (d, *J* = 12 Hz, 2H, =CH), 7.55 (s, 1H, CH_3C=CH), 7.42 (m, 3H, ArH), 7.36 (d, *J* = 16 Hz, 2H, =CH), 2.19 (d, *J* = 4 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.2, 147.3, 144.6, 142.6, 141.6, 135.3, 134.8, 130.6, 130.3, 129.0, 128.4, 123.7, 121.4, 14.1; HRMS ESI(+): calcd for $C_{18}H_{16}NO_3$ $(M+H)^+$ 294.1125, found 294.1134; UV–vis λ_{max} (nm) in CH_2Cl_2: 308.

5.1.14. (1*E*,4*E*)-5-(4-Methoxyphenyl)-2-methyl-1-(4-nitrophenyl)penta-1,4-dien-3-one (2j)

Percent yield: 63%; mp: 74–76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.27 (d, *J* = 8 Hz, 2H, ArH), 7.71 (d, *J* = 16 Hz, 1H, =CH), 7.58 (d, 4H, ArH), 7.52 (s, 1H, =CH), 7.26 (s, 1H, CH_3C=CH), 6.93 (d, *J* = 8 Hz, 2H, ArH), 3.87 (s, 3H, OCH3), 2.19 (d, *J* = 1.6 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.2, 161.7, 147.2, 144.5, 142.8, 141.7, 134.6, 130.2, 130.2, 127.5, 123.3, 119.1, 114.5, 55.5, 14.2; HRMS ESI(+): calcd for $C_{19}H_{18}NO_4$ $(M+H)^+$ 324.1230, found 322.1250; UV–vis λ_{max} (nm) in CH_2Cl_2: 316.

5.1.15. (1*E*,4*E*)-2-Methyl-1,5-bis(4-nitrophenyl)penta-1,4-dien-3-one (2k)

Percent yield: 65%; mp: 188–189 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.31 (t, *J* = 0 Hz, *J* = 4 Hz, 1H, ArH), 8.29 (t, *J* = 0 Hz, *J* = 4 Hz, 2H, ArH), 8.23 (t, *J* = 0 Hz, *J* = 4 Hz, 1H, ArH), 7.74 (m, 3H, ArH and CH_3-C=CH), 7.60 (d, *J* = 8 Hz, 3H, =CHand ArH), 7.49 (d, *J* = 16 Hz, 1H, =CH), 2.21 (d, *J* = 1.6 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 191.2, 148.6, 147.4, 142.1, 141.3, 141.3, 141.0, 136.4, 130.3, 128.9, 125.0, 124.3, 123.8, 14.0; HRMS ESI(+): calcd for $C_{18}H_{15}N_2O_5$ $(M+H)^+$ 339.0910, found 339.0910; UV–vis λ_{max} (nm) in CH_2Cl_2: 304.

5.1.16. (1*E*,4*E*)-5-(4-Dimethylamino)phenyl-2-methyl-1-(4-nitrophenyl)penta-1,4-dien-3-one (2l)

Percent yield: 61%; mp: 129–131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.26 (d, *J* = 8 Hz, 2H, ArH), 7.71 (d, *J* = 16 Hz, 1H, =CH), 7.56 (d, *J* = 12 Hz, 2H, ArH), 7.52 (d, *J* = 8 Hz, 2H, ArH), 7.48 (s, 1H, =CH), 7.12 (d, *J* = 16 Hz, 1H, =CH), 3.68 (d, *J* = 8 Hz, 2H, ArH), 3.05 (s, 6H, $N(CH_3)_2$), 2.19 (d, *J* = 1.6 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.4, 152.1, 147.0, 145.8, 143.1, 142.1, 133.6, 130.4, 130.2, 123.7, 122.5, 116.2, 111.8, 40.1, 14.4; HRMS ESI(+): calcd for $C_{20}H_{21}N_2O_3$ $(M+H)^+$ 337.1547, found 337.1568; UV–vis λ_{max} (nm) in CH_2Cl_2: 415.

5.1.17. (1*E*,4*E*)-1-(4-Dimethylamino)phenyl)-2-methyl-5-phenylpenta-1,4-dien-3-one (2m)

Percent yield: 67%; mp: 60–62 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.68 (s, 1H, =CH), 7.61 (m, 2H, ArH), 7.56 (s, 1H, CH_3–C=CH), 7.47 (d, *J* = 12Hz, 2H, =CH), 7.46 (d, *J* = 0Hz, 2H, ArH), 7.39 (m, 2H, ArH), 6.73 (d, *J* = 8 Hz, 2H, ArH), 3.04 (s, 6H, $N(CH_3)_2$), 2.24 (d, *J* = 1.6 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 150.6, 142.2, 142.1, 140.1, 135.6, 134.2, 132.0, 129.8, 128.8, 128.1, 123.7, 122.4, 111.7, 40.12, 13.9; HRMS ESI(+): calcd for $C_{20}H_{22}NO$ $(M+H)^+$ 292.1610, found 292.1705; UV–vis λ_{max} (nm) in CH_2Cl_2: 401.

5.1.18. (1*E*,4*E*)-1-(4-Dimethylamino)phenyl-5-(4-methoxyphenyl)-2-methylepenta-1,4-dien-3-one (2n)

Percent yield: 79%; mp: 110–112 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.62 (d, *J* = 16 Hz, 1H, =CH), 7.56 (d, *J* = 8 Hz, 2H, ArH), 7.54 (brs, 1H, CH_3–C=CH), 7.45 (d, *J* = 8 Hz, 2H, ArH), 7.34 (s, *J* = 16 Hz, 1H, =CH), 6.91 (d, *J* = 8 Hz, 2H, ArH), 6.73 (d, *J* = 8 Hz, 2H, ArH), 3.85(s, 3H, OCH_3), 3.03 (s, 6H, $N(CH_3)_2$), 2.23 (d, *J* = 4 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.5, 161.1, 150.5, 142.1, 139.5, 134.6, 131.9, 129.8, 128.3, 123.9, 120.1, 114.3, 111.7, 55.4, 40.2, 14.0; HRMS ESI(+): calcd for $C_{21}H_{24}NO_2$ $(M+H)^+$ 322.1802, found 322.1812; UV–vis λ_{max} (nm) in CH_2Cl_2: 401.

5.1.19. (1*E*,4*E*)-1-(4-Dimethylamino)phenyl-2-methyl-5-(4-nitrophenyl)penta-1,4-dien-3-one (2o)

Percent yield: 57.6%; mp: 212–215 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8 Hz, 2H, =CH), 7.74 (d, *J* = 8 Hz, 2H, ArH), 7.62 (d, *J* = 8 Hz, 2H, ArH), 7.58 (s, 1H, CH_3-C=CH), 7.48 (d, *J* = 8 Hz, 2H, ArH), 6.73 (d, *J* = 12 Hz, 2H, ArH), 3.05 (s, 6H, $N(CH_3)_2$), 2.25 (d, *J* = 1.6 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 191.3, 150.6, 148.1, 141.9, 141.4, 139.0, 133.8, 132.3, 128.6, 126.2, 124.1, 123.4, 111.7, 40.1, 13.8; HRMS ESI(+): calcd for $C_{20}H_{21}N_2O_3$ $(M+H)^+$ 337.1547, found 337.1562; UV–vis λ_{max} (nm) in CH_2Cl_2: 418.

5.1.20. (1*E*,4*E*)-1,5-Bis(4-dimethylamino)phenyl-2-methylepenta-1,4-dien-3-one (2p)

Percent yield: 60%; mp: 146–147 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.63 (d, *J* = 16 Hz, 2H, =CH), 7.63 (d, *J* = 8 Hz, 2H, ArH), 7.44 (d, *J* = 8 Hz, 2H, ArH), 7.30 (s, 1H, CH_3–C=CH), 6.73 (d, *J* = 8 Hz, 2H, ArH), 6.68 (d, *J* = 8 Hz, 2H, ArH), 3.03 (d, *J* = 0 Hz, 12H, $N(CH_3)_2$), 2.23 (d, *J* = 1.6 Hz, 3H, $=CCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 192.8, 151.6, 150.3, 143.3, 138.5, 134.7, 131.8, 129.90, 124.2, 123.4, 117.4, 111.9, 111.8, 40.2, 14.1; HRMS ESI(+): calcd for $C_{22}H_{27}N_2O$ $(M+H)^+$ 335.2118, found 335.2124. UV–vis λ_{max} (nm) in CH_2Cl_2: 422.

5.2. Biological activity

5.2.1. Parasites and cells

Leishmania amazonensis promastigote forms (MHOM/BR/Josefa) were maintained at 25 °C in Warren's medium (brain heart infusion plus haemin and folic acid) pH 7.0, supplemented with 10% Fetal Bovine Serum (FBS, Gibco Invitrogen, Grand Island, NY, USA). Epimastigote forms of *T. cruzi* (Y strain) were maintained at 28 °C in liver infusion tryptose medium (LIT) supplemented with 10% inactivated FBS and trypomastigote forms were obtained from the supernatant of a monolayer of infected $LLCMK_2$ cells (epithelial cells from the kidney of the monkey *Macaca mulatta*) in DMEM supplemented with 10% FBS at 37 °C in a humidified 5% CO_2 atmosphere. J774A1 murine macrophages were maintained in tissue flasks with RPMI 1640 medium (Gibco Invitrogen Corporation, New York, USA) pH 7.6, with sodium bicarbonate and L-glutamine added, and supplemented with 10% FBS at 37 °C in a 5% CO_2–air mixture.

5.2.2. Antiproliferative assay

The effects of synthesized compounds were evaluated in promastigotes of *L. amazonensis* and epimastigotes of *T. cruzi*. The inoculum (1×10^6 cells/mL) was introduced into 24-well plate containing the compounds dissolved in dimethylsulfoxide (DMSO) and Warren's medium or LIT in several concentrations (1.0–100.0 μM). The final concentration of DMSO did not exceed 1%. Cell grown was determined by counting the parasites with a Neubauer hemocytometer after incubation for 72 h at 25 °C for *L. amazonensis* or for 96 h at 28 °C for *T. cruzi*. The results were expressed as percentage of inhibition in relation to the control cultured. The 50% inhibitory concentration (IC_{50}) was determined by logarithm regression analysis of the data obtained.

5.2.3. Viability of trypomastigote forms of *T. cruzi*

The tissue-culture-derived trypomastigote forms (1×10^7 cells/mL) were resuspended in DMEM and added in duplicate to 96-well microplates in presence of different concentrations of the compounds (1.0–100.0 μM). Parasites were incubated for 24 h at 37 °C in a 5% CO_2 atmosphere. The results were obtained by observing motility, allowing the determination of the viability of the parasites, using the Pizzi–Brener method.[44] The EC_{50} value (i.e., the concentration that lyses 50% of the parasites) was then calculated.

5.2.4. Cytotoxicity assay

The cytotoxicity was evaluated in J774A1 macrophages and $LLCMK_2$ cells. For macrophages, a suspension of 5×10^5 cells/mL was cultured in RPMI 1640 medium supplemented with 10% FBS and added to each well in 96-well micro plates. For $LLCMK_2$ cells, a suspension of 2.5×10^5 cells/mL was cultured in DMEM. After 24 h, the different compounds were added to each well (10.0–1000.0 μM) and the plates were incubated for 48 h for macrophages or 96 h for $LLCMK_2$ cells in a 5% CO_2–air mixture at 37 °C. Following incubation, MTT assay was performed (2 mg/mL stock solution, 50 μL/well). After 4 h of incubation, the MTT processing was stopped, and the formazan crystals were solubilized by adding DMSO (150 μL/well).[45] The relative amount of formazan/well produced by viable cells was determined spectrophotometrically at 570 nm by blanking against an appropriate control. The CC_{50} values (50% cytotoxic concentration) were estimated and the selectivity index (SI) was used to compare cytotoxicity between cells and protozoa (ratio: CC_{50} of cells divided by CC_{50} of the compound in the protozoa).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2013.12.020.

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