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Trimethoxy-chalcone derivatives inhibit growth of *Leishmania braziliensis*: Synthesis, biological evaluation, molecular modeling and structure–activity relationship (SAR)

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ABSTRACT

In this work we described the synthesis, the antileishmanial activity and the molecular modeling and structure–activity relationship (SAR) evaluations of a series of chalcone derivatives. Among these compounds, the methoxychalcones **2h**, **2i**, **2j**, **2k** and **2l** showed significant antileishmanial activity (IC_{50} <10 μM). Interestingly **2i** (IC_{50} = 2.7 μM), **2j** (IC_{50} = 3.9 μM) and **2k** (IC_{50} = 4.6 μM) derivatives presented better antileishmanial activity than the control drug pentamidine (IC_{50} = 6.0 μM). Our SAR study showed the importance of methoxy di-*ortho* substitution at phenyl ring A and the relationship between the frontier orbital HOMO coefficients distribution of these molecules and their activity. The most active compounds **2h**, **2i**, **2j**, **2k**, and **2l** fulfilled the Lipinski rule-of-five which theoretically is important for good drug absorption and permeation through biological membranes. The potential profile of **2j** (IC_{50} = 3.9 μM and CC_{50} = 216 μM) pointed this chalcone derivative as a hit compound to be further explored in antileishmanial drug design.

1. Introduction

Neglected tropical diseases are an uncontrolled public health problem in development countries. They currently threaten more than one billion people and cause around 500.000 deaths every year.[1] Some of these diseases are caused by parasitic protozoan with distinct morphological stages during development in both vertebrate and invertebrate hosts.[2–4]

Leishmaniasis is an infectious disease caused by at least 20 different species of parasites of genus *Leishmania*. They are responsible for different clinic manifestations that classify this disease (e.g., cutaneous or visceral leishmaniasis).[5] The life cycle of this parasite includes two forms: (i) the flagellated promastigote, which colonizes the gut of the sandfly vector and is the infective form, and (ii) the obligatory intracellular amastigote in the mammalian macrophage.[3,5–7] The transmission of promastigote forms in old world occurs by the bite of the female sandfly of the *Phlebotomus* sp. and in the new world by *Lutzomyia* sp.[3,6,7]

Despite the progress in basic knowledge on the parasite, current therapy for leishmaniasis is still unsatisfactory, due to the limited effectiveness, long term treatment, high cost and undesirable secondary effects like hepatic, heart, pancreas and kidney toxicity.[8–10] Pentavalent antimonials (SbV) such as sodium stibogluconate (Pentostam®) and meglumine antimoniate (Glucantime®) are the first choice for treatment. These drugs only exist in their parenteral forms and currently second-line compounds are necessary including pentamidine (Pentacarinat®) and amphotericin B (AmBisome®).[5,9–12] The development of drug resistance by the pathogens, especially in HIV-*Leishmania* co-infected patients increased this health problem.[5,13]

Flavonoids are an extensive group of compounds occurring in plants. Chalcones are open chain flavonoids with two aromatic rings linked by a carbonyl group and two α,β-insatured carbon atoms.[14–16] Chalcones are natural-like compounds, and as such they can show multitarget profile similar to other compounds described in the literature.[17] Thus natural and synthetic chalcones are described in the literature with different pharmacological profiles,

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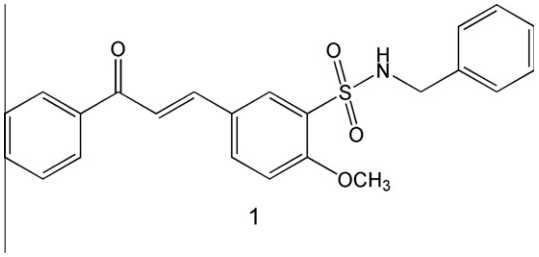

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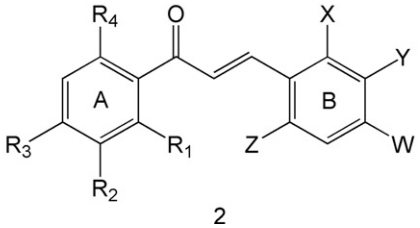

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Figure 1. Sulfonamide 4-methoxychalcone (**1**) and chalcone set studied in this work (**2**).

such as anti-inflammatory,[18] trypanocidal,[19] antibacterial,[20] antiviral,[21,22] antitumoral,[15] antimalarial,[23] and antileishmanial activities.[24–27] Recently, we described the effects of a new set of sulfonamide 4-methoxychalcones against *L. braziliensis* promastigote forms.[24] Compound **1** (Fig. 1) presented the best profile against *L. braziliensis* promastigote forms (IC_{50} = 3.5 ± 0.6 μM).

In this work we synthesized novel chalcones (**2**) based on a 1,3-diacetyl biphenyl nucleus without sulfonamide group (Fig. 1) and evaluated their antileishmanial profile against *L. braziliensis* promastigote forms. Since changes in the 3-propenone moiety causes the lost of the activity, all modifications were performed in both aromatic rings. On that purpose we focused on the structure–activity relationship (SAR) of these new chalcone analogues to determine structural and stereoeletronic features that can improve the antileishmanial profile by introducing substituent with different electronic and volume properties in both phenyl rings moiety of the chalcone (**2**).

2. Results and discussion

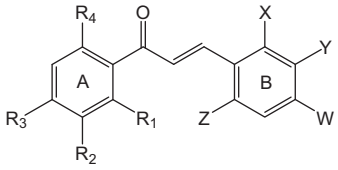
2.1. Chemistry

The chalcone derivatives new set **2a**–**2t** (Table 1) was prepared by aldol condensation between aromatic acetophenones and corresponding aldehydes in methanol, KOH (50% w/v), at room temperature and magnetic agitation for 24 h (Fig. 2).[16] After precipitation and recrystallization, the purified chalcones were obtained in yields from 36% to 97% as described in Section 4.

2.2. Biological results

The compounds (**2a–2t**) were evaluated against *L. braziliensis* promastigote forms and most of them showed some level of activity (Table 1). Among the active derivatives, the analogues **2h**, **2i**, **2j**, **2k** and **2l** presented the best antileishmanial profile, with values (IC_{50}) smaller than 10 μM. Among them, **2i** (IC_{50} = 2.70 μM), **2j** (IC_{50} = 3.94 μM) and **2k** (IC_{50} = 4.62 μM) derivatives were more active than standard antileishmanial agent pentamidine (IC_{50} = 6.0 μM), used as positive control in this study. Out of these three antileishmanial analogues, compound **2j** showed the lowest

Table 1
Comparison of antileishmanial activity in promastigote forms (IC_{50}), cytotoxicity profile in VERO cells (CC_{50}) and selective index (SI) of chalcone derivatives (**2a**–**2t**)



Chalcone	R^1	R^2	R^3	R^4	X	Y	W	Z	IC_{50} (μM)	CC_{50} (μM)	SI
2a	H	OCH_3	OCH_3	OCH_3	H	H	H	H	12.8 ± 4	392	30.6
2b	H	OCH_3	OCH_3	OCH_3	H	H	Cl	H	29.7 ± 1	279	9.4
2c	H	OCH_3	OCH_3	OCH_3	H	Cl	H	H	15.4 ± 5	382	24.8
2d	H	OCH_3	OCH_3	OCH_3	Cl	H	H	H	Nd	Nd	—
2e	H	OCH_3	OCH_3	OCH_3	H	Cl	Cl	H	147.8 ± 16	906	6.1
2f	H	OCH_3	OCH_3	OCH_3	H	OCH_3	H	H	14.0 ± 4	295	21.1
2g	OCH_3	H	OCH_3	OCH_3	H	H	OCH_3	H	Nd	Nd	-
2h	OCH_3	H	OCH_3	OCH_3	H	H	Cl	H	8.2 ± 2	147	17.9
2i	OCH_3	H	OCH_3	OCH_3	H	Cl	H	H	2.7 ± 1	9	3.3
2j	OCH_3	H	OCH_3	OCH_3	Cl	H	H	H	3.9 ± 1	216	55.4
2k	OCH_3	H	OCH_3	OCH_3	H	Cl	Cl	H	4.6 ± 1	109	23.7
2l	OCH_3	H	OCH_3	OCH_3	Cl	H	H	Cl	7.4 ± 1	135	18.2
2m	OCH_3	H	OCH_3	OCH_3	H	NO_2	H	H	20.0 ± 2	84	4.2
2n	OCH_3	H	OCH_3	OCH_3	NO_2	H	H	H	29.7 ± 2	3350	112.8
2o	OCH_3	H	OCH_3	OCH_3	H	H	NO_2	H	11.7 ± 1	124	10.6
2p	OCH_3	H	OCH_3	OCH_3	$C_{10}H_8$*				Nd	Nd	-
2q	OCH_3	H	OCH_3	OCH_3	$C_{10}H_8$**				Nd	Nd	-
2r	OCH_3	H	OCH_3	OCH_3	H	Y-OCH_2O-W***		H	Nd	Nd	-
2s	OCH_3	H	OCH_3	OCH_3	H	H	$N(CH_3)_2$	H	Nd	Nd	-
2t	OCH_3	H	OCH_3	OH	H	Cl	H	H	194.1 ± 30	341	1.8
Pentamidine									6.0		
Amphotericine B									0.3		

SI (Selectivity index) = CC_{50}/IC_{50}.
Nd = not detected at concentration of 50 μM.
* **B** aromatic ring exchange by 1-naphthyl ring.
** **B** aromatic ring exchange by 2-naphthyl ring.
*** Methylenedioxy functional group as substituent at Y and W **B** aromatic ring positions.

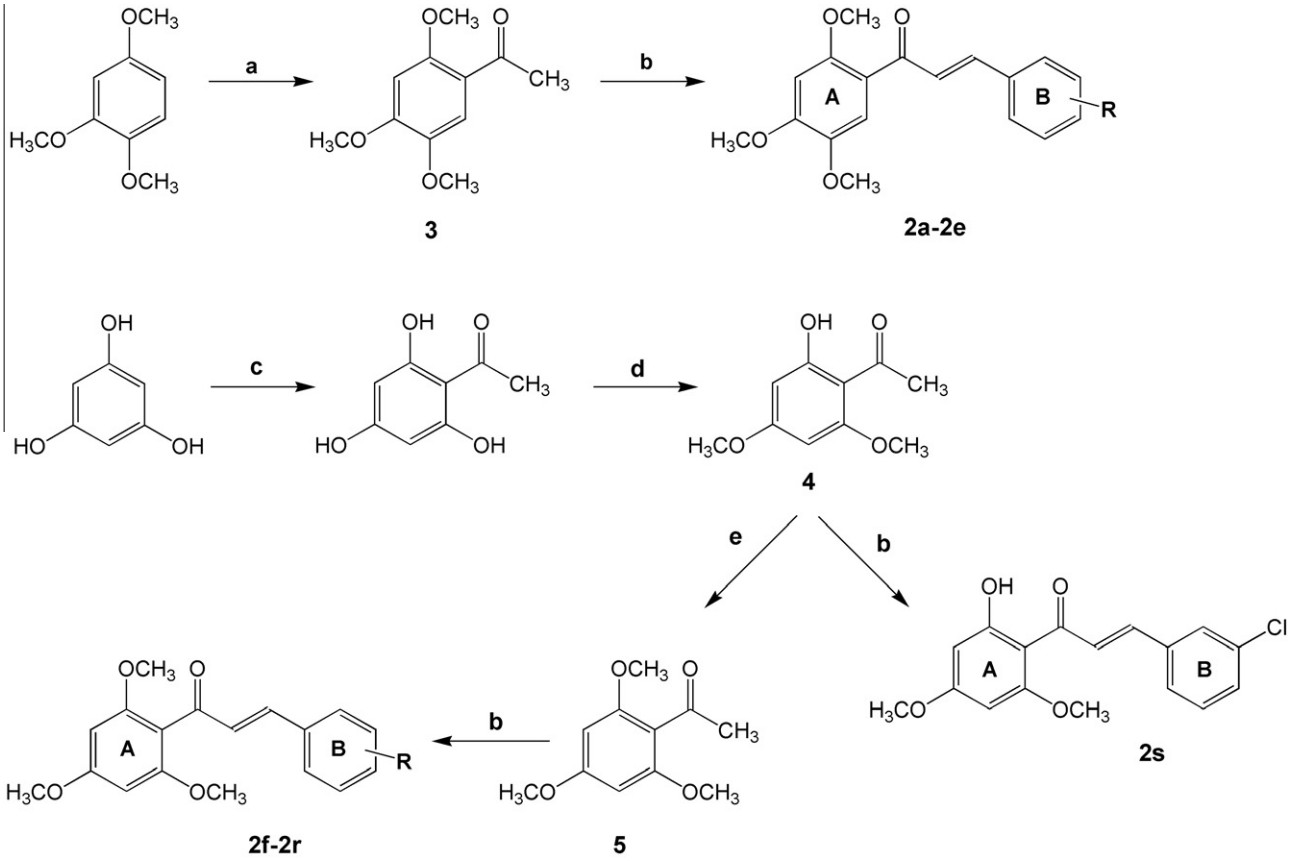


Figure 2. Synthesis of 2,4,5-trimethoxyacetophenone (**3**), 2,4,6-trimethoxyacetophenone (**4**) and xanthoxyline (**5**), and corresponding chalcones (**2a**–**2t**). (a) acetyl chloride, $AlCl_3$, dichloromethane, 10 °C, 1 h; (b) corresponding aldehyde, KOH 50% w/v, methanol, rt, 24 h; (c) (i) CH_3CN, $ZnCl_2$, ether, HCl (g), 0–5 °C; (ii) H_2O, reflux; (d) Me_2SO_4, K_2CO_3, acetone; (e) Me_2SO_4, NaOH/acetone, reflux, 24 h.

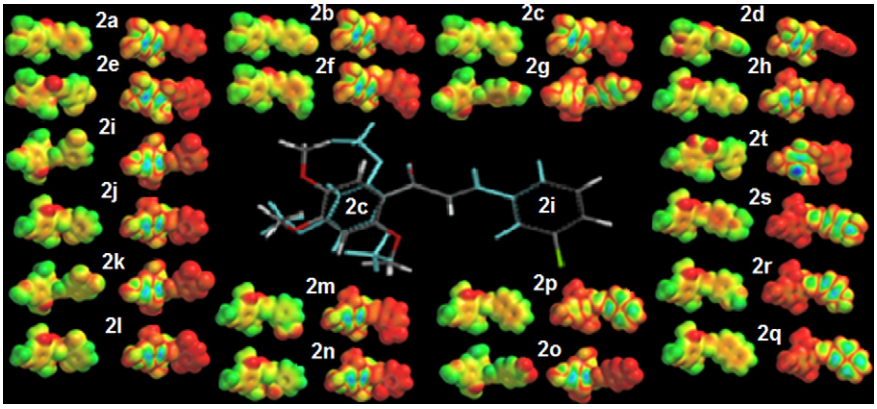


Figure 3. Electrostatic potential map and HOMO density encoded onto a van der Waals surface of chalcone derivatives (**2a**–**2t**), and structural alignment of most active derivative (**2i**) and its isomer (**2c**) (inset). Molecular electrostatic potential in a 0.002 eV/au^3 surface and energy range varying from deepest red to deepest blue.

toxicity level in Vero cells assays and the best selectivity index against promastigote forms of *L. braziliensis*. Interestingly, **2j** was able to inhibit iNOS, an enzyme involved in inflammatory process, with no toxicity on macrophages at IC_{50} .[16]

2.3. Molecular modeling and structure–activity relationship (SAR) studies

In order to provide useful guidelines for designing new more potent antileishmanial agents, we used a molecular modeling approach and theoretically evaluated the chalcone derivatives. Interestingly, an overall look into the structure-activity relationship data revealed that most chalcones analogues with methoxy substituent at different positions on chalcone phenyl ring **A** (**R^1**–**R^4**) and other substituents at chalcone phenyl ring **B** (**X**, **Y**, **W** and **X**) introduced diversified stereoelectronic effects and modulated the antileishmanial profile against *Leishmania braziliensis* promastigote form.

The theoretical analysis of chlorine atoms substituent at phenyl ring **B** (**2h**, **2i**, **2j**, **2k** and **2l**) pointed to an intricate relationship be-

Table 2
Theoretical molecular electronic properties (E_{HOMO}, E_{LUMO} and dipole moment) and Lipinski parameters (volume, hydrogen bond donor and acceptor groups, *c* log P and molecular weight (MW)) of chalcone derivatives **2a**–**2t**

Chalcone	Dipole moment (debye)	E_{homo} (eV)	E_{lumo} (eV)	Lipinski rule of 5				
				Volume (Å^3)	HBD	HBA	*c* log *P*	MW (Da)
2a	2.64	−5.43	−1.61	317.3	0.0	4.0	3.37	298.3
2b	2.96	−5.51	−1.78	330.8	0.0	4.0	3.93	332.7
2c	2.10	−5.50	−1.80	330.8	0.0	4.0	3.93	332.7
2d	2.15	−5.45	−1.67	328.8	0.0	4.0	3.94	332.7
2e	3.07	−5.56	−1.93	344.5	0.0	4.0	4.49	367.2
2f	3.27	−5.39	−1.57	344.5	0.0	5.0	3.25	328.3
2g	1.85	−5.68	−1.41	344.7	0.0	5.0	3.25	328.3
2h	3.61	−5.91	−1.75	331.1	0.0	4.0	3.93	332.7
2i	2.23	−5.91	−1.78	331.0	0.0	4.0	3.93	332.7
2j	2.49	−5.87	−1.54	331.1	0.0	4.0	3.93	332.7
2k	3.59	−5.98	−1.92	344.7	0.0	4.0	4.49	367.2
2l	3.28	−5.90	−1.60	344.6	0.0	4.0	4.49	367.2
2m	3.66	−6.01	−2.54	339.5	0.0	7.0	3.41	343.3
2n	2.62	−5.90	−2.16	339.6	0.0	7.0	3.41	343.3
2o	6.76	−6.09	−2.70	339.6	0.0	7.0	3.41	343.3
2p	3.63	−5.62	−1.50	367.2	0.0	4.0	4.37	348,3
2q	2.87	−5.63	−1.71	368.5	0.0	4.0	4.37	348.3
2r	2.96	−5.53	−1.50	341.8	0.0	6.0	3.15	342.3
2s	3.52	−5.11	−1.25	367.1	0.0	5.0	3.66	341.4
2t	1.98	−5.91	−2.0	309.8	1.0	4.0	3.67	318.7

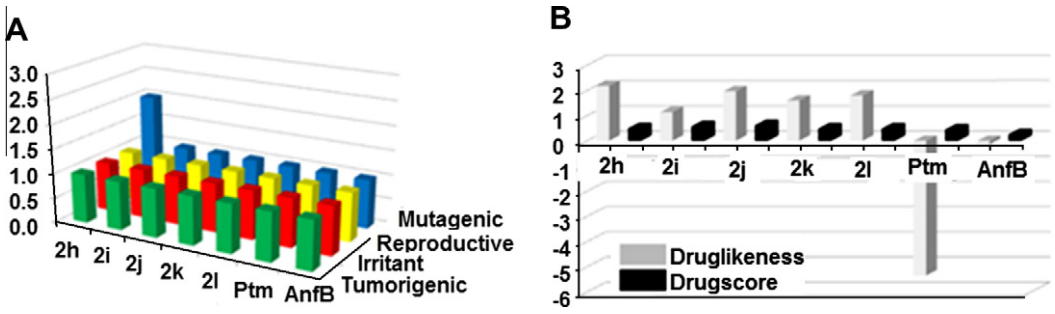


Figure 4. Theoretical toxicity evaluation (A), and the druglikeness and drugscore (B) values of the most active compounds (**2h**, **2i**, **2j**, **2k**, and **2l**) compared to the antileishmanial drugs pentamidine and amphotericin B. The scale of toxicity risk ranges from low (0–1), medium (1–2) and high (2–3) calculated by using Osiris program.[39]

tween steric bulk and electronic properties of chlorine atom (Table 1). Besides electronegative, halogens such as chlorine are also lipophilic, which may allow the permeability through the cell memb rane.

Interestingly our data showed a higher activity profile for the methoxy group substituent in the phenyl rings **A** and **B** compared to the chlorine atom substitution probably due to the nature of the methoxy position. In fact, the insertion of di-*ortho*- methoxy substituent at aromatic ring **A** (**R^1** and **R^4**) imposes a structural rigidity and reduces conformational freedom of the phenyl ring that apparently improved the antileishmanial activity. The structural rigidity imposed by methoxyl substituents at di-*ortho*- positions on the aromatic ring is related to the volume of methoxy, which prevents bond rotation due to the steric hindrance.

The comparison of methoxy group at the phenyl ring **A** in *meta*- (**R^2**) or di-*ortho*- (**R^1**) (**2c** and **2i** respectively) positions revealed an improvement over seven orders of magnitude in the antileishmanial profile (Table 1). The structural alignment of these derivatives showed a clear conformational difference (Fig. 3). Importantly the torsion dihedral angles for all chalcone derivatives are defined as the angle difference between the plane of the carbonyl group and phenyl ring **A**. The derivatives with significant antileishmanial activity have a larger variation of the torsion dihedral angles, which are influenced by the methoxy group at *ortho*- position (**R^1** and **R^4**). The compounds **2h** and **2i** have higher activity (Table 1) and torsion dihedral angle (−64.01° and 63.12° respectively) compared to **2b** and **2c** (−38.75° and −38.25° respectively). These data pointed to the steric importance of the carbonyl group and the phenyl ring **A** moiety to the antileishmanial activity.

The theoretical analysis of some electronic properties of these derivatives, including HOMO and LUMO energies and distribution and dipole moment values (Table 2) showed no clear direct correlation with the antileishmanial activity. Nevertheless, the electrostatic potential map of the derivatives (Fig. 3) revealed that the electronic density is located on the carbonyl group. Derivatives that presented better antileishmanial activity (**2h**, **2i**, **2j**, **2k** and **2l**) possess the electronic density HOMO at phenyl ring **A**, whereas **2p**, **2q**, **2r**, and **2s** with no antileishmanial activity presented the electronic density HOMO at phenyl ring **B** far from carbonyl group (Fig. 3). A similar result was observed for the series **1** synthesized by Fröhner et al. since these derivatives also presented the electronic density HOMO on the carbonyl group.[24] Therefore our results reinforced that the carbonyl group contributes not only with a steric perspective but also with an important electronic contribution to antileishmanial activity on both chalcone derivatives series (**1** and **2**).

Currently, many theoretical approaches such as the Lipinski rule of five assess the physical-chemical theoretical parameters related to the oral bioavailability (Table 2). Our results revealed that **2a**–**2t** derivatives lipophilicity ($3.93 > c\log P < 4.49$) is not greater than 5.0, which, according to Lipinski, is an important feature for good drug absorption and permeation.[24,29–36]

In addition the molecular volume and weight of the active derivatives (331.1(Å^3 > MV < 344.7 Å^3 and 332.78 > MW < 367.22) are

similar to the predicted by Lipinski 'Rule of five' (Table 2).[33,36,37] In fact, according to our research, all chalcone derivatives with antileishmanial activity (**2h**, **2i**, **2j**, **2k,** and **2l**) (3.93 > *c* log *P* < 4.49, 332.78 Da > MW < 367.22 Da, hydrogen bond acceptors = 4 and donors = 0) fulfilled all Lipinski rules, which states that most 'drug-like' molecules have log $P \leqslant 5$, molecular weight $\leqslant 500$, number of hydrogen bond acceptors $\leqslant 10$, and donors $\leqslant 5$ (Table 1). These data may suggest the potentiality of these derivatives as new candidates to antileishmanial agents.

Currently, there are also many approaches that assess druglikeness of compounds based on topological descriptors, fingerprints of molecular druglikeness, structural keys or other properties as *c* log *P* and molecular weight.[24,31,35,38] In this work we used the Osiris software[39] for calculating the fragment-based druglikeness of all compounds also comparing with other antileishmanial drugs including pentamidine and amphotericin B (Fig. 4). Our in silico results showed that the active compounds **2h**, **2i**, **2j**, **2k**, and **2l** presented the druglikeness value better than pentamidine and amphotericin B currently used in therapy (Fig. 4). We also analyzed the drugscore, which evaluate druglikeness, *c* log *P*, cLogS, molecular weight, toxicity risks in one value and that may be used to consider the compound overall potential as a drug.[24,40–43] Our data showed that all active compounds presented drugscore values greater than the drugs pentamidine and amphotericin B (Fig. 4).

According to our theoretical toxicity evaluation of the tumorigenic, irritant, reproductive and mutagenic profiles of **2h**, **2i**, **2j**, **2k**, and **2l**, all showed a low theoretical toxicity profile similar to pentamidine and amphotericin B, except for **2h** (Fig. 4). Far from establishing that these compounds are completely free of any toxicity effect, this theoretical result reinforced the promising profile of these derivatives for further experimental investigation.[24,38,40–43]

3. Conclusion

According to our results, the position of methoxy group in phenyl ring **A**, in special at di-*ortho*- (**R**$^{\mathbf{1}}$ and **R**$^{\mathbf{4}}$) substituents and chlorine atom at phenyl ring **B** seems to be important to antileishmanial activity. Among the chalcone series (**2a**–**2t**), **2j** derivative showed the most promising results including low IC_{50} against *L. braziliensis* (3.9 μM), good in silico and in vitro toxicological profile (CC_{50} = 216 μM) and with theoretical physical-chemical indexes (druglikeness and drugscore) better than pentamidine and amphotericin B. The theoretical study of **2j** also showed that it fulfilled Lipinski rule of five pointing it as a hit compound for further exploring and to help on designing new candidates for leishmaniasis treatment.

4. Materials and methods

4.1. Chemistry

All reagents were obtained commercially, except for the acetophenones 2,4,5-trimethoxyacetophenone (**3**), xanthoxyline (**4**) and 2,4,6-trimethoxyacetophenone (**5**), which were prepared as previously described, with yield of 81%, 70% and 84%, respectively.[16,25,28] All structures were confirmed by melting points and by chemical identification data: infrared spectroscopy (IR), ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) and elementary analysis. ^{1}H NMR spectra revealed that all structures were configured *E* ($J_{H\alpha\text{-}H\beta}$ = 16.0 Hz).

All chalcones were prepared by aldol condensation between aromatic acetophenones and corresponding aldehydes, in methanol, KOH (50% w/v), at room temperature and magnetic agitation for 24 h.[16] Distilled water and 10% hydrochloric acid were added to the reaction for total precipitation of the compounds, which were then obtained by vacuum filtration and later recrystallized in dichloromethane and hexane. All solvents used were of analytical grade. The purified chalcones were obtained in yields between 36% and 97%, and are soluble in dimethylsulfoxide, ethyl acetate, acetone, chloroform and dichloromethane.

The chemical characterization of chalcones **2g**–**2t** was previously described by our group[16,44] whereas we described that of **2a**–**2f** below. Chalcones **2b**, **2c, 2d** and **2e** are new compounds whereas chalcones **2a** and **2f** were previously described by Anjaneyulu and col.[45] The data of **2a** and **2f** are according with the literature. Melting points were determined with a Microquimica MGAPF-301 apparatus and are uncorrected. Elementary analysis was carried out using a CHNS EA 1110; percentages of C and H agreed with the product formula (within ±0.4% of theoretical values for C). IR spectra were recorded with Abb Bomen FTLA 2000 spectrometer on KBr disks. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained from Varian Oxford AS-400 spectrometer, using tetramethylsilane as internal standard. The values of the coupling constants (J) are given in Hertz. The delta value for each methoxy group was determined as previously described using sign integration (2,4,6-triOCH_3 derivatives) and RMN based calculations (2,4,5-triOCH_3 derivatives).[46] The small difference in the delta values between Hα and Hβ can be explained by the presence of methoxy groups at position *ortho* and *para* at A-ring, which reduces the withdrawing function of carbonyl group.

4.1.1. (2*E*)-1-(2′,4′,5′-Trimethoxyphenyl)-3-phenyl-2-propen-1-one (2a)

Yellow solid; mp 112–114 °C; Yield: 71%; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H, *o*-OCH_3), 3.94 (s, 3H, *m*-OCH_3), 3.97 (s, 3H, *p*-OCH_3), 6.55 (s, 1H, H3′), 7.40 (s, 1H, H6′), 7.37–7.43 (m, 3H, H3, H4, H5), 7.60–7.64 (m, 2H, H2, H6), 7.68 (d, 1H, *J* = 16.0 Hz, Hα), 7.72 (d, 1H, *J* = 16.0 Hz, Hβ). ^{13}C NMR ($CDCl_3$) δ 56.40 (*o*-OCH_3), 56.58 (*m*-OCH_3), 57.02 (*p*-OCH_3), 97.24 (C3′), 113.39 (C6′), 120.679 (Cα), 127.39 (C4), 128.54 (C2, C6), 129.06 (C3, C5), 130.17 (C1′), 135.78 (C1), 142.10 (Cβ), 143.63 (C5′), 153.83 (C2′), 155.09 (C4′), 190.08 (C=O); IR ν_{max}/cm^{-1} 16450, 1217 (C=O), 1582 (C=C), 1265, 1031 (C–O), 2993, 2941, 2830, 1517, 1412, 1148, 980, 769 (Ar) (KBr); Anal. Calcd for $C_{18}H_{18}O_4$: C 72.47; H 6.08. Found: C 72.65; H 6.81.

4.1.2. (2*E*)-1-(2′,4′,5′-Trimethoxyphenyl)-3-(4-chlorophenyl)-2-propen-1-one (2b)

Light yellow solid; mp 129–130 °C; Yield: 70%; ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H, *o*-OCH_3), 3.91 (s, 3H, *m*-OCH_3), 3.97 (s, 3H, *p*-OCH_3), 6.35 (s, 1H, H3′), 7.10 (d, 2H, *J* = 8.0 Hz, H2, H6), 7.13 (d, 2H, *J* = 8.0 Hz, H3, H5), 7.46 (s, 1H, H6′), 7.65 (d, 1H, *J* = 16.0 Hz, Hα), 7.73 (d, 1H, *J* = 16.0 Hz, Hβ). ^{13}C NMR ($CDCl_3$) δ 56.31 (*o*-OCH_3), 56.37 (*m*-OCH_3), 56.85 (*p*-OCH_3), 97.87 (C3′), 112.53 (C6′), 119.03 (Cα), 128.26 (C2, C6), 129.54 (C3, C5, C1′), 132.18 (C1, C4), 139.43 (Cβ), 143.16 (C5′), 154.22 (C2′), 155.08 (C4′), 191.89 (C=O); IR ν_{max}/cm^{-1} 1660, 1217 (C=O), 1606 (C=C), 1275, 1026 (C–O), 2953, 2827, 1513, 1501, 1148, 821 (Ar) (KBr); Anal. Calcd for $C_{18}H_{17}ClO_4$: C 64.97; H 5.15. Found: C 64.98; H 5.91.

4.1.3. (2*E*)-1-(2′,4′,5′-Trimethoxyphenyl)-3-(3-chlorophenyl)-2-propen-1-one (2c)

Yellow solid; mp 126–127 °C; Yield: 62%; ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H, *o*-OCH_3), 3.93 (s, 3H, *m*-OCH_3), 3.98 (s, 3H, *p*-OCH_3), 6.34 (s, 1H, H3′), 7.04–7.07 (m, 2H, H5, H6), 7.26 (s, 1H, H2), 7.33–7.35 (m, 1H, H4), 7.40 (s, 1H, H6′), 7.64 (d, 1H, *J* = 16.0 Hz, Hα), 7.70 (d, 1H, *J* = 16.0 Hz, Hβ). ^{13}C NMR ($CDCl_3$) δ 55.80 (*o*-OCH_3), 56.37 (*m*-OCH_3), 56.90 (*p*-OCH_3), 97.83 (C3′), 112.94 (C6′), 118.24 (Cα), 126.31 (C6), 126.56 (C2), 128.13 (C4), 128.65 (C1′), 129.36 (C5), 134.01 (C3), 140.19 (C1), 142.91 (Cβ), 143.37 (C5′), 153.94 (C2′), 154.82 (C4′), 190.93 (C=O); IR ν_{max}/cm^{-1} 1661,

1221 (C=O), 1609 (C=C), 1273, 1032 (C–O), 2934, 2838, 1517, 1472, 1409, 1158, 817 (Ar) (KBr); Anal. Calcd for $C_{18}H_{17}ClO_4$: C 64.97; H 5.15. Found: C 64.80; H 5.87.

4.1.4. (2*E*)-1-(2′,4′,5′-Trimethoxyphenyl)-3-(2-chlorophenyl)-2-propen-1-one (2d)

Yellow solid; mp 141–143 °C; Yield: 90%; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H, *o*-OCH_3), 3.92 (s, 3H, *m*-OCH_3), 3.97 (s, 3H, *p*-OCH_3), 6.54 (s, 1H, H3′), 7.28–7.30 (m, 2H, H4, H5), 7.39 (s, 1H, H6′), 7.41–7.44 (m, 1H, H3), 7.60 (d, 1H, J = 16.0 Hz, Hα), 7.70–7.72 (m, 1H, H6), 8.08 (d, 1H, J = 16.0 Hz, Hβ). ^{13}C NMR ($CDCl_3$) δ 56.15 (*o*-OCH_3), 56.30 (*m*-OCH_3), 56.74 (*p*-OCH_3), 96.87 (C3′), 113.13 (C6′), 120.09 (C1′), 126.94 (Cα), 127.73 (C5), 129.63 (C6), 130.19 (C3), 130.58 (C4), 133.85 (C2), 135.33 (C1), 137.60 (Cβ), 143.37 (C5′), 153.74 (C2′), 154.92 (C4′), 189.59 (C=O); IR ν_{max}/cm^{-1} 1648, 1217 (C=O), 1588 (C=C), 1267, 1024 (C–O), 1149 (C-Cl), 2935, 2834, 1616, 1517, 1483, 1408, 1358, 1198, 965, 819, 751 (Ar) (KBr); Anal. Calcd for $C_{18}H_{17}ClO_4$: C 64.97; H 5.15. Found: C 64.75; H 5.81.

4.1.5. (2*E*)-1-(2′,4′,5′-Trimethoxyphenyl)-3-(3,4-dichlorophenyl)-2-propen-1-one (2e)

Yellow solid; mp 136–137 °C; Yield: 43%; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H, *o*-OCH_3), 3.95 (s, 3H, *m*-OCH_3), 3.98 (s, 3H, *p*-OCH_3), 6.54 (s, 1H, H3′), 7.46 (s, 1H, H6′), 7.42 (d, 1H, J = 16.0 Hz, Hα), 7.46 (d, 1H, J = 16.0 Hz, Hβ), 7.59–7.62 (m, 2H, H5, H6), 7.68 (s, 1H, H2). ^{13}C NMR ($CDCl_3$) δ 56.44 (*o*-OCH_3), 56.59 (*m*-OCH_3), 57.04 (*p*-OCH_3), 97.02 (C3′), 113.32 (C6′), 120.17 (Cα), 127.58 (C6), 129.00 (C2), 129.88 (C1′), 131.04 (C5), 133.31 (C4), 133.85 (C3), 135.97 (Cβ), 138,99 (C1), 143.71 (C5′), 152.92 (C2′), 154.24 (C4′), 190.35 (C=O); IR ν_{max}/cm^{-1} 1654, 1222 (C=O), 1591 (C=C), 1268, 1025 (C–O), 2941, 2838, 1524, 1476, 1413, 1354, 1147, 969, 821 (Ar) (KBr); Anal. Calcd for $C_{18}H_{16}Cl_2O_4$: C 58.87; H 4.39. Found: C 58.68; H 5.00.

4.1.6. (2E)-1-(2′,4′,5′-Trimethoxyphenyl)-3-(3-methoxyphenyl)-2-propen-1-one (2f)

Yellow solid; mp 97–99 °C; Yield: 52%; ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H, *o*-OCH_3), 3.90 (s, 3H, *m*-OCH_3), 3.93 (s, 3H, *p*-OCH_3), 3.97 (s, 3H, OCH_3), 6.55 (s, 1H, H3′), 6.94 (d, 1H, J = 8.0 Hz, H4), 7.14 (s, 1H, H2), 7.22 (d, 1H, J = 8.0 Hz, H6), 7.32 (t, 1H, J = 8.0 Hz, H5), 7.39 (s, 1H, H6′), 7.61 (d, 1H, J = 16.0 Hz, Hα), 7.68 (d, 1H, J = 16.0 Hz, Hβ). ^{13}C NMR ($CDCl_3$) δ 55.49 (*o*-OCH_3), 56.34 (*m*-OCH_3), 56.53 (*p*-OCH_3), 57.00 (OCH_3), 97.21 (C3′), 113.37 (C6′), 11.71 (C2), 115.77 (C4), 120.62 (C6), 121.15 (Cα), 127.73 (C1′), 130.05 (C5), 137.19 (C1), 141.97 (Cβ), 143.62 (C5′), 153.86 (C2′), 155.10 (C4′), 160.03 (C3), 190.07 (C=O); IR ν_{max}/cm^{-1} 1646, 1219 (C=O), 1586 (C=C), 1266, 1026 (C–O), 2938, 2834, 1517, 1480, 1409, 1144, 847, 792 (Ar) (KBr); Anal. Calcd for $C_{19}H_{20}O_5$: C 69.50; H 6.14. Found: C 69.82; H 6.41.

4.2. Biology

4.2.1. Chemicals

Serum was purchased from GIBCO (Grand Island, NY) and all other reagents were purchased from Sigma Chemical Co. All chalcones (**2a**–**2t**) were solubilized in DMSO 0.3 M and kept at 4 °C. The final concentration of DMSO into the cultures never exceeded 1% (v/v) and had no effect on the parasites proliferation or morphology.

4.2.2. Parasites and cell line

L. braziliensis (MHOM/BR/75/M-2904) promastigotes were obtained in the exponential stage of growth in Schneider's medium at 28 °C supplemented with 5% of BSF (Bovine Serum Fetal) and washed two times in PBS (Phosphate Buffer Saline) pH 7.2, at 4 °C, at 1,500×g/10 min. The number of protozoa was determined in Neubauer chamber and they were kept in Schneider medium with 5% of BSF at 4 °C.

4.2.3. Antileishmanial and cytotoxic assays[24]

The assays were made in three independent experiments, in plates of 96 wells. Initially we performed a screening in concentrations of 500 and 50 μM of each chalcone derivative (20 μL), in presence of 180 μL of suspension of 5.0×10^6 promastigotes/mL in Schneider's medium supplemented with 5% of BSF. The plates were incubated at 28 °C for 72 hours and visually evaluated in inverted microscope to check the mobility of the protozoa. The activity of the compounds was determined through the total number counting of living protozoa in Neubauer chamber. The protozoa were incubated also without chalcones, in presence of 1% of DMSO, as the negative control, and with pentamidine and amphotericin B, for positive control. The compounds that inhibited the mobility of the protozoa with 50 μM were selected to determine the IC_{50} values by evaluation in the concentrations of 500, 100, 50, 25, 10, 5 and 1 μM, in three independent experiments (like description above). The IC_{50} values were determined by linear regression analysis, and represented the mean ± standard error of three independent experiments (GraphPad Software). The cytotoxicity of the compounds was evaluated in Vero cells, in the same conditions.[24]

4.3. Molecular modeling methodology

Molecular modeling was performed using SPARTAN′08 (Wavefunction Inc., Irvine, CA, 2000) and Osiris programs.[24,39–43] Structures were minimized with mechanical molecular force field MMFF and the equilibrium geometry was obtained in vacuum using a semi-empirical RM1 module. In order to evaluate the electronic properties of the RM1 minimal energy conformations, they were submitted to a single-point calculation using DFT (Density Functional Theory) method with a 6-31G* basis set of the SPARTAN′08 package. The three-dimensional isosurfaces of the molecular electrostatic potential maps (MEPs) at the van der Waals contact surface represented electrostatic potentials superimposed onto a surface of constant electron density (0.002 e/au^3).[24,40–43] The color-coded isosurface values provide an indication of the overall molecular size and location of negative (red) or positive (blue) electrostatic potentials.[24,40–43] The electronic properties (HOMO energy, HOMO orbital coefficients distribution, LUMO density, dipole moment and lipophilicity-*c* log *P*) were calculated for all compounds.

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