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Original article

Synthesis, biological evaluation and SAR of sulfonamide 4-methoxychalcone derivatives with potential antileishmanial activity

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Abstract

Despite clinical importance of leishmaniasis, an infectious disease that affects 12 thousand million people in 88 countries, the treatment is still unsatisfactory due to its limited efficacy, cost expensive and undesirable side effects. Aiming to develop new antileishmanial lead compounds, we used a rational approach to synthesize a new set of sulfonamide 4-methoxychalcone derivatives (**3a**–**3i**) and evaluate the sulfonamide and methoxy moieties as promising adding-groups to chalcones. For that purpose we tested this new set against *Leishmania braziliensis* promastigotes and intracellular amastigotes and determined its cell toxicity profile. Interestingly all compounds presented a concentration-dependent antileishmanial profile and the benzylamino derivative (**3i**) showed a biological activity better than pentamidine. None of these compounds affected *Trypanosoma cruzi* epimastigotes, which suggests a specific antileishmanial profile. The structure–activity analysis of these sulfonamide 4-methoxychalcone derivatives pointed the molecular volume, the HOMO density concentrated in the chalcone moiety and the conformational structure of the compounds as important structural and stereoelectronic features for the antileishmanial activity. In addition, these compounds also fulfilled Lipinski rule of 5 and presented druglikeness similar to antileishmanial drugs. Altogether these results point the sulfonamide 4-methoxychalcone derivatives as potential lead compounds for designing new candidates for leishmaniasis treatment.

Keywords: Leishmaniasis; SAR; Sulfonamide 4-methoxychalcones; Antiparasite; Promastigote; Amastigote

1. Introduction

Disseminated leishmaniasis is an emerging infectious disease, mostly due to *Leishmania braziliensis*. Importantly the disease presents clinical and histopathological features where *L. braziliensis* is responsible for both cutaneous and mucocutaneous leishmaniasis in several Latin American countries [1]. Therefore, currently these parasitic diseases

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cause significant morbidity and mortality, mainly in the developing world [2].

Despite the progress in important fundamental knowledge about *L. braziliensis*, the current chemotherapy for leishmaniasis is still unsatisfactory due to the limited efficacy, long-term treatment, cost expensive and undesirable side effects [3–5]. The development of drug resistance by the pathogens, especially in HIV–*Leishmania* co-infected patients, has also aggravated the health problem [6]. Thus, there is an urgent need for the development of new, efficient and safe drugs against leishmaniasis.

Natural and synthetic chalcones are described presenting a wide range of potential pharmacological profiles [7], such as anti-inflammatory [8], trypanocidal [9], antibacterial [10], antiviral [11,12], antitumoral [13], antimalarial [14], and antileishmanial [15–18] activities. Recently a series of substitution-containing chalcone derivatives (**1**) have been reported as antileishmanial agents (Scheme 1) [9]. In addition, sulfonamide analogues based on the herbicide oryzalin (**2**), presented antiparasitic activity including an antileishmanial profile (Scheme 1) [19].

In this work we used a rational approach and synthesized a new set of sulfonamide 4-methoxychalcone derivatives (**3a–3i**) to identify the sulfonamide and methoxy as promising adding-groups to chalcones and develop new lead antiparasitic compounds. In addition we focused on the structure–activity relationships (SAR) of these sulfonamide 4-methoxychalcones series to determine structural and stereoelectronic features that could lead to the antileishmanial profile (Scheme 1). For that purpose we analyzed the role of groups with different electronic and volume properties in the sulfonamide moiety of the 4-methoxychalcones' B ring.

To compare with other chalcone series described in the literature [9], the new 3-sulfonamide 4-methoxychalcone series were evaluated for its antiparasitic activity against *L. braziliensis* promastigotes and *Trypanosoma cruzi* epimastigotes. We also tested the most active compound against *L. braziliensis* intracellular amastigotes to evaluate its ability for decreasing the parasite burden in infected host cells. In addition the cytotoxicity profile in mouse peritoneal macrophages was evaluated, which allowed the determination of their selectivity index. Finally, these biological effects were analyzed with the compounds' different theoretical parameters (dipole, E_{Homo}, E_{Lumo}, cLogP, molecular weight and volume, higher HOMO coefficient orbital and density) calculated using a molecular modeling approach in the structure–activity relationship (SAR) study [20].

2. Results and discussion

2.1. Design and synthesis

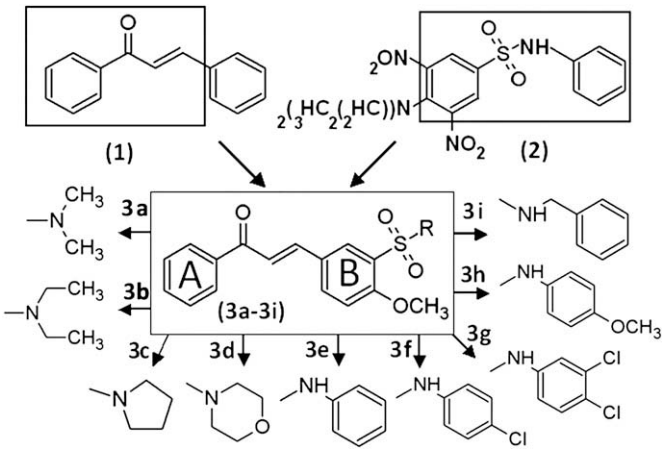
The sulfonamide 4-methoxychalcone derivatives are prepared as shown in Scheme 2. The 4-methoxychalcone (**3**) was obtained by aldolic condensation of 4-methoxybenzaldehyde and acetophenone according to the general literature procedure [19] (Scheme 2).

Treatment of 4-methoxychalcone (**3**) with chlorosulfonic acid at room temperature for one week [21] afforded the sulfonyl chloride derivative. Preparation of sulfonamides **3a–3i** was accomplished by reaction of sulfonyl chloride derivative with different amines in the presence of methanol at room temperature. We characterized all the synthesized compounds by spectroscopic data such as IR NMR and elemental analysis [22]. The chalcones **3a**, **3b** and **3e** were prepared as previously described [22,23].

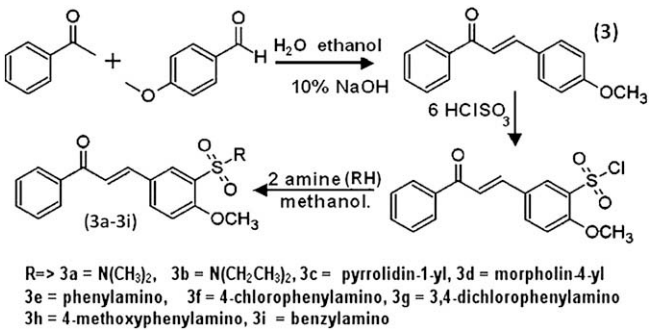
2.2. Biological evaluation

In this work we evaluated the inhibitory profile of the 4-methoxychalcone (**3**) and sulfonamide 4-methoxychalcone derivatives (**3a–3i**) against *L. braziliensis* promastigote forms (Fig. 1A). Interestingly all the chalcones tested showed a concentration-dependent inhibitory effect on the *in vitro Leishmania* growth assays (Fig. 1A).

Interestingly, the addition of sulfonamide generated derivatives with a better inhibitory profile against *L. braziliensis* than the 4-methoxychalcone (**3**), except for the aniline substituted sulfonamide 4-methoxychalcone (**3e**) (Fig. 1A and Table 1). According to our results, most of the sulfonamide 4-methoxychalcone derivatives showed a more potential inhibitory



Scheme 1. Rational approach to design the sulfonamide 4-methoxychalcone derivatives.



Scheme 2. Synthetic approach used to obtain the sulfonamide 4-methoxychalcone derivatives.

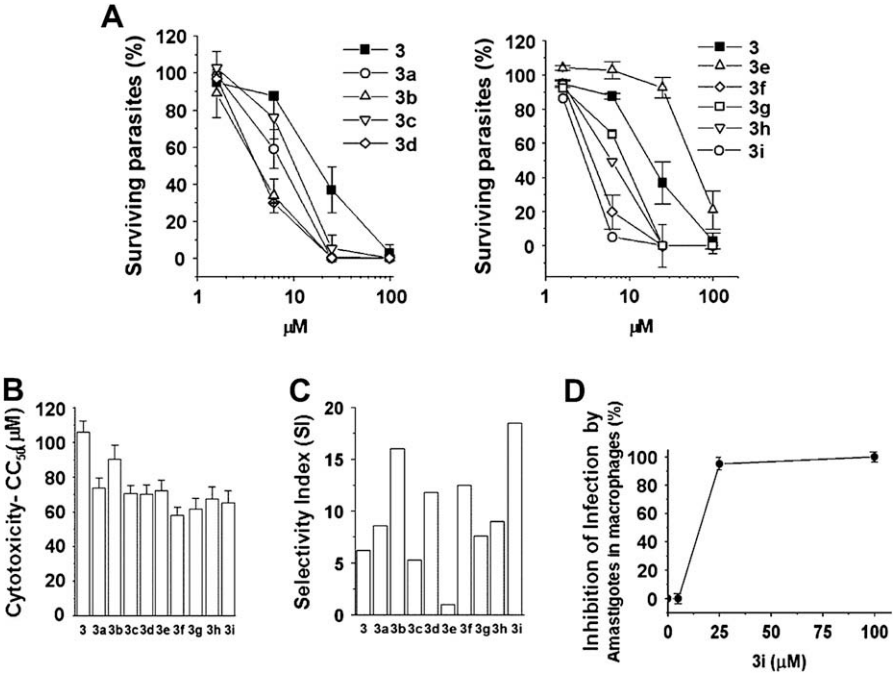


Fig. 1. Biological evaluation of 4-methoxychalcone (**3**) and sulfonamide 4-methoxychalcone derivatives (**3a**–**3i**). Inhibitory effects on *Leishmania braziliensis in vitro* proliferation (A), cytotoxicity profile on mouse macrophages (B), selectivity index (SI) calculated as described by Ferreira et al. [24] (C) and the inhibition of the amastigotes infection on macrophages by the most active compound (**3i**) (D).

activity ($IC_{50} = 3.5 \pm 0.6$ to 8.6 ± 0.4 μM) than that of 4-methoxychalcone (**3**) ($IC_{50} = 16.6 \pm 1.6$ μM) and other chalcones without substituent groups such as those described by Lunardi and co-workers [9] ($IC_{50} = 13.7$–182.3 μM) (Table 1). Although our compounds were less active than amphotericin B ($IC_{50} = 0.3 \pm 0.02$ μM), they were more active than pentamidine isothionate ($IC_{50} = 19.6$ μM) [25], one current drug used for leishmaniasis treatment.

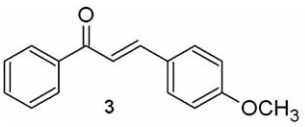
The addition of the aniline on the substituent group in the compound **3e** was the only one that negatively affected the inhibitory activity on the growth of *L. braziliensis* promastigotes forms ($IC_{50} = 69 \pm 3.7$ μM) compared to **3**. Importantly, all substitutions (electron-acceptor or electron-donor substituents) at the sulfonamide moiety or at *para*-position of aniline aromatic ring maintained the antileishmanial profile (compounds **3a**–**3d** and **3f**–**3h**) suggesting the feasibility of new interactions on these positions (Fig. 1 and Table 1).

The compound **3i** showed the best profile against *L. braziliensis* promastigotes ($IC_{50} = 3.5 \pm 0.6$ μM). Apparently the benzylamino group significantly contributes to this activity, since this compound was about 20-fold more potent than the compound **3e** ($IC_{50} = 69.0 \pm 3.7$ μM) (Fig. 1 and Table 1). Notably the addition of the carbon spacer on the substituent (**3i**) was enough to avoid the deleterious effect of the aniline group (**3e**), probably allowing hydrophobic interactions with the parasite target. We also carried out amastigotes assays with the most active compound (**3i**) to evaluate its ability for reducing the parasite load in infected host cells. In the untreated controls we obtained an infection rate of 30.1% (mean = 2.9 parasites/infected cell). The results showed that **3i** is able to reduce significantly the number of intracellular amastigotes suggesting its potential as a lead antileishmanial compound (Fig. 1D).

In this work we experimentally evaluated the cytotoxic activity of 4-methoxychalcone (**3**) and sulfonamide 4-methoxychalcone derivatives (**3a**–**3i**) against mouse peritoneal macrophages. Our results showed CC_{50} values ranging from 57.8 ± 4.8 to 105.7 ± 6.5 μM (Fig. 1B). Interestingly the presence of the substituents of the sulfonamide group seems to play a more important role for the antileishmanial activity than for the cytotoxicity profile (Fig. 1 and Table 1). The CC_{50} values were used to calculate the selectivity index (SI) for these derivatives, which were higher than the non-substituted 4-methoxychalcone, except for **3c** and **3e**. This result showed the improvement of the selectivity index of the derivatives compared to the leading compound (Fig. 1C).

Finally, we also tested the sulfonamide 4-methoxychalcone derivatives' effects against *T. cruzi* epimastigotes forms. However, different from the chalcone series described by Lunardi et al. that was active on both *L. braziliensis* (21.9–182.3 μM) and *T. cruzi* strains (24.8–126.4 μM), this new series showed no activity in concentrations up to 500 μM (not shown). These data suggest that the sulfonamide 4-methoxychalcone series target is probably different from that of Lunardi series [9], due to its specificity for Leishmania. Previous studies showed that chalcone derivatives might act in the parasite mitochondria by the inhibition of fumarate reductase, succinate dehydrogenase, NADH dehydrogenase, or succinate- and NADH-cytochrome *c* reductase activity [26,27]. However, as these enzymes may be present in *T. cruzi* and the series described herein does not affect this parasite, further investigation about its mechanism of action should be performed.

Table 1
Comparison of the antileishmanial activity (IC_{50}) of 4-methoxychalcone (**3**) and sulfonamide 4-methoxychalcone derivatives (**3a–3i**) with their theoretical molecular electronic properties (dipole, E_{Homo}, E_{Lumo}, cLog P, volume, molecular weight (MW)) and Lipinski profile, including the number of hydrogen bond donor and acceptor groups (HDG and HAG, respectively)



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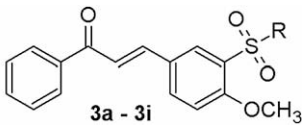
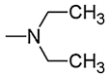
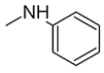
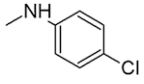
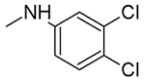
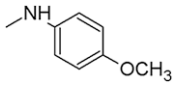
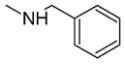


3a - 3i

C	R	IC_{50} (μM)	Dipole (Debye)	HOMO (eV)	LUMO (eV)	Lipinski rule of 5				
						Volume (Å^3)	HDG	HAG	clogP	MW
3	–	16.6 ± 1.6	3.39	−8.43	2.01	263.80	0	2	3.63	238.29
3a		8.6 ± 0.4	7.32	−8.38	2.04	346.07	0	5	2.61	345.42
3b		5.6 ± 0.4	8.95	−8.40	2.09	382.91	0	5	3.29	373.47
3c		13.0 ± 1.3	7.23	−8.76	0.95	371.83	0	5	2.93	371.46
3d		5.9 ± 1.3	7.31	−8.59	1.87	379.89	0	6	2.21	387.46
3e		69.0 ± 3.7	8.67	−8.57	1.87	391.84	1	5	4.04	393.46
3f		4.6 ± 1.3	8.20	−8.19	2.09	405.13	1	5	4.60	427.91
3g		8.1 ± 1.6	7.84	−8.89	0.951	418.27	1	5	5.16	462.35
3h		7.5 ± 0.8	7.56	−8.27	2.06	419.38	1	6	3.91	423.49
3i		3.5 ± 0.6	7.17	−8.59	1.87	410.52	1	5	4.11	407.49

2.3. Molecular modeling and SAR studies

In this study we compared the biological profile of this new series with its structural features to establish a structure–activity relationship. All calculations were performed using SPARTAN'06 (Wavefunction Inc., Irvine, CA, 2000). The structures were minimized and the equilibrium geometry was obtained in vacuum using a semi-empirical AM1 module. To evaluate the electronic properties of the AM1 minimal energy conformations, they were submitted to a Density Functional Theory (DFT) calculation with a 6-31G* basis set of the SPARTAN'06 package. The electronic properties (HOMO and LUMO energy, HOMO density and dipole moment) were calculated for all compounds. Theoretical logP (clogP) was calculated at AM1 semi-empirical level using the Villar method, included in Spartan [24,28–30].

An overall analysis of the activity of the compounds showed that the addition of sulfonamide substituent increased the activity of these compounds in comparison to compound **3**. Interestingly the increase of molecular volume, weight and dipole moment of these derivatives seem to improve the activity compared to **3** (Table 1) except for **3e**.

The conformational analysis of these compounds inferred that the enhancement of the biological activity is probably due to the new interactions in a new plane of these molecules caused by the addition of the sulfonamide group (Fig. 2). The HOMO (Highest Occupied Molecular Orbital) density of the most active compounds is concentrated in the center of the chalcone moiety (the carbonyl group and unsaturated linker between A and B rings – Scheme 1) thus suggesting a role for these regions in the antileishmanial activity (Fig. 2).

The aniline **3e** derivative presents a substituent that significantly compromised the biological activity, compared to **3**, and that reoriented the HOMO density to the substituent (Fig. 2). Importantly, the addition of chlorine, an electron-acceptor atom, in **3f** restored the activity to a significant level. However, the volume, the steric and electronic features are probably important to the antileishmanial profile of this series as the addition of two chlorines (**3g**) or a methoxy (**3h**) group, which possess a bigger volume and different electrostatic features, decreased the activity in more than 2-fold compared to **3f**. However, it should be considered that these substituents still contributed for the activity as these derivatives were more potent than **3e** (Table 1).

Interestingly the addition of a spacer (C1) in the **3i** structure, the most active compound, led to the best activity and reoriented the HOMO density to the chalcone moiety, which apparently contributed to the antileishmanial profile compared to **3e**. The most stable conformation of this derivative revealed a π–π stacking interaction between the benzyl and the chalcone ring, which may be important for the interaction with the target and for avoiding the predicted prohibitive areas (Figs. 2 and 3).

We also evaluated some electronic properties of the sulfonamide 4-methoxychalcone chalcone derivatives including HOMO and LUMO (Highest and Lowest Unoccupied Molecular Orbitals) energy values, HOMO and LUMO orbital coefficients distribution, and molecular electrostatic potential (MEP). Our results showed that sulfonamide 4-methoxychalcone derivatives led to different HOMO and LUMO energy values, and MEPs that alone did not present any direct correlation with the antileishmanial activity (Table 1 and Fig. 2).

In the effort to study the hydrophobic pattern, we calculated the theoretical parameters related to the oral bioavailability, according to Lipinski rule of 5 [31] (Table 1). Our results revealed that the sulfonamide 4-methoxychalcone lipophilicity ($2.21 > \text{cLogP} < 4.60$) is not greater than 5.0, which, according to Lipinski, is an important feature for good drug absorption and permeation [32–34](Table 1).

In addition the molecular volume and weight of derivatives ($346.07\ \text{Å}^3 > \text{MV} < 419.38\ \text{Å}^3$ and $345.42 > \text{MW} < 462.35$) are similar to more than 80% of all Fluka traded drugs ($\text{MW} < 450$ Da) and to that determined by Lipinski "Rule of 5" [31–34]. In fact, according to our research, most of the sulfonamide 4-methoxychalcone derivatives evaluated ($2.21 > \text{Log P} < 4.60$, $345.42\ \text{Da} > \text{MW} < 423.49\ \text{Da}$, hydrogen bond acceptors $=2–5$ and donors $= 0–1$) fulfilled all Lipinski rules, which states that most "drug-like" molecules have $\log \text{P} \leq 5$, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10, and donors ≤ 5 (Table 1). These data may suggest the potentiality of these derivatives as new candidate to antileishmanial agents.

Currently, there are many approaches that assess druglikeness of compounds based on topological descriptors, fingerprints of molecular druglikeness, structural keys or other properties as clogP and molecular weights [33]. In this work we used the Osiris program (www.organic-chemistry.org/prog/peo) for calculating the fragment-based druglikeness of all compounds and other antileishmanial drugs including pentamidine and glucantime (Fig. 3). Our theoretical data showed that the most active compounds presented a druglikeness value higher than the compounds currently used in therapy and evaluated in this work (Fig. 3). In this study we also verified the drug score, which combines druglikeness,

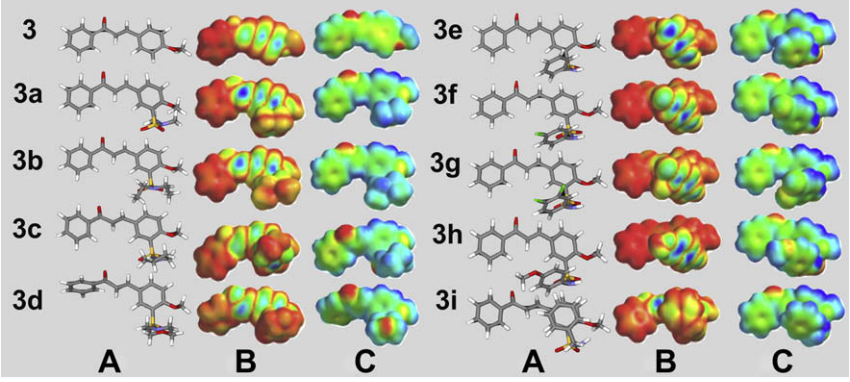


Fig. 2. Minimal energy conformation (A), HOMO density encoded onto a van der Waals surface (B) and electrostatic potential map (C) of 4-methoxychalcone (**3**) and sulfonamide 4-methoxychalcone derivatives (**3a–3i**). HOMO absolute density coefficient (isodensity 0.002 e/au^3) mapped from deepest red (0.00) to deepest blue (0.02). The chalcone moiety of all molecules is turned to the front in (A), (B) and (C). The position is slightly altered in (B) and (C) to allow a better view of the parameters analyzed. (For interpretation of the references to color in figure legends, the reader is referred to the web version of this article.)

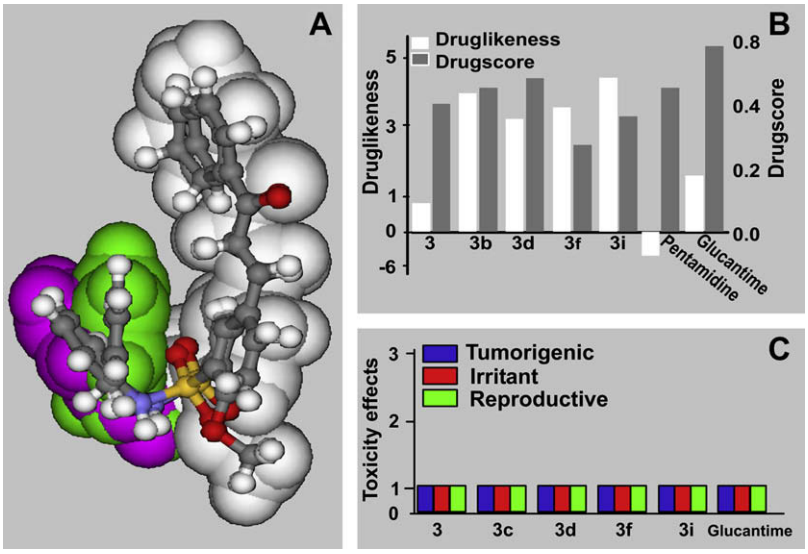


Fig. 3. Structural alignment of the most (**3i**) and the less active (**3e**) derivatives of the sulfonamide 4-methoxychalcone series (A), and the druglikeness and drug score values (B) and the theoretical toxicity evaluation (C) of the most active compounds (**3b**, **3d**, **3f** and **3i**) compared to the non-substituted 4-methoxychalcone and antileishmanial drugs. (A) The most (**3i**) and the less active (**3e**) compounds are represented in stick and ball colored by element (Grey = carbon, white = hydrogen, blue = nitrogen, red = oxygen and orange = sulfur). The white color in the CPK structure shows the chalcone and sulfonamide parts in these compounds (**3i** and **3e**) whereas green represents the contributing region of the most active compound (**3i**) and purple indicates the prohibitive region of the less active derivative (**3e**). (For interpretation of the references to color in figure legends, the reader is referred to the web version of this article.)

clogP, logS, molecular weight, and toxicity risks in one value and that may be used to consider the compound overall potential to qualify for a drug. Our data showed that compound **3i** presented a close value to pentamidine and a good profile based on the program values (Fig. 3) [24,28–30].

According to our theoretical toxicity evaluation of the tumorigenic, irritant and reproductive profile of the sulfonamide 4-methoxychalcone derivatives (**3a–3i**), they show a low profile for these toxicity effects similar to glucantime and the non-substituted methoxychalcone (Fig. 3), except for **3h** that presented a medium tumorigenic profile (not shown). It is important to note that the toxicity predicted herein neither is a fully reliable toxicity prediction nor guarantees that these compounds are completely free of any toxic effect. However, it reinforced the promising profile of these compounds for further experimental investigation [24,28–30].

3. Conclusion

In conclusion, our data show that the combination of two different pharmacophoric groups (*i.e.* chalcone and sulfonamide) enhanced the derivatives' antileishmanial activity at least 2-fold compared to **3** and other chalcones [9]. The sulfonamide 4-methoxychalcone derivatives exhibited a potential antileishmanial activity *in vitro* where compound **3i** presented the highest activity against *L. braziliensis*. The structure–function analysis revealed that volume, the HOMO density concentrated in the chalcone moiety, and the conformational structure of the compounds are important structural and stereoelectronic features for the antileishmanial activity. The theoretical study of these molecules also showed that they fulfilled Lipinski rule of 5 and present druglikeness similar to antileishmanial drugs. These results point the sulfonamide 4-methoxychalcone derivatives as lead compounds for designing new candidates for leishmaniasis treatment.

4. Experimental protocols

4.1. Chemistry

All reagents and solvents used were of analytical grade. TLC was carried out using silica gel F-254 Glass Plate (20 × 20 cm). The ^{1}H NMR (200–400 MHz) and ^{13}C NMR (50–100 MHz) spectra were obtained from Bruker AC-200F and Varian model Unity (Varian Oxford AS-400 spectrometer) using tetramethylsilane as internal standard. The values of the coupling constants (J) are given in hertz. Fourier transform infrared (FT-IR) absorption spectra were recorded in a Perkin–Elmer 16PC spectrophotometer. The solid samples were measured using potassium bromide pellets. Microanalyses were carried out on a Carlo Erba EA 1110 instrument.

4.1.1. General procedure for the preparation of compounds *3a–3i*

The sulfonyl chloride was treated with benzylamine in methanol at 0 °C. The mixture was reacted at room temperature for 2 h and then poured into iced-water. The precipitate was collected by filtration, washed with iced-methanol, dried and recrystallized in ethanol. *Compound **3a***: yield 43%, m.p. 140–144 °C (lit. [22] 140–141 °C). *Compound **3b***: yield 40%, m.p. 149–150 °C. IR (KBr, cm^{-1}) ν_{max}: 1659, 1595, 1323, 1217, 1144, 700. ^{1}H NMR (200 MHz, $CDCl_3$, ppm) δ: 8.30 (s, 1H, Ar-H), 8.05 (d, 2H, Ar-H), 7.76 (d, J = 15.7 Hz, 1H, olefinic H), 7.48 (m, 5H, Ar-H), 7.04 (d, 1H, Ar-H),

3.98 (s, 3H, OCH_3), 3.35 (q, 4H, CH_2), 1.13 (t, 6H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$, ppm) δ: 190.10, 157.99, 142.75, 137.97, 134.75, 132.83, 130.48, 129.88, 128.60, 128.46, 127.35, 121.46, 112.41, 56.13, 41.78, 14.24. Anal. Calcd for $C_{20}H_{23}NO_4S$: C, 64.32; H, 6.21; N, 3.75; S, 8.59. Found: C, 64.00; H, 6.11; N, 3.73; S, 8.03%. *Compound* ***3c***: yield 51%, m.p. 162–166 °C. IR (KBr, cm^{-1}) ν_{max}: 1661, 1594, 1328, 1217, 1148, 701. ^1H NMR (200 MHz, $CDCl_3$, ppm) δ: 8.3 (s, 1H, Ar-H), 8.01 (d, 2H, Ar-H), 7.81 (d, $J = 14.3$ Hz, 1H, olefinic H), 7.77 (d, 1H, Ar-H), 7.47 (m, 3H, Ar-H and 1H, olefinic H), 7.08 (d, 1H, Ar-H), 3.99 (s, 3H, OCH_3), 3.38 (t, 4H, N–CH_2), 1.83 (q, 4H, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$, ppm) δ: 191.05, 157.36, 142.94, 137.97, 135.20, 133.13, 131.40, 128.90, 128.37, 128.11, 121.85, 112.76, 57.20, 48.05, 26.09. Anal. Calcd for $C_{20}H_{21}NO_4S$: C, 64.67; H, 5.70; N, 3.77; S, 8.63. Found: C, 64.22; H, 5.62; N, 3.83; S, 8.49%. *Compound* ***3d***: yield 38%, m.p. 158–158 °C. IR (KBr, cm^{-1}) ν_{max}: 1661, 1596, 1152, 1109, 699. ^1H NMR (200 MHz, $CDCl_3$, ppm) δ: 8.23 (d, 1H, Ar-H), 8.03 (d, 2H, Ar-H), 7.77 (d, $J = 15.7$ Hz, 1H, olefinic H), 7.74 (d, 1H, Ar-H), 7.45 (m, 3H, Ar-H and 1H, olefinic H), 7.08 (d, 1H, Ar-H), 3.99 (s, 3H, OCH_3), 3.74 (t, 4H, O–CH_2), 3.27 (t, 4H, N–CH_2). ^{13}C NMR (50 MHz, $CDCl_3$, ppm) δ: 190.06, 158.25, 142.46, 137.95, 135.18, 132.94, 131.12, 128.66, 128.50, 127.72, 126.92, 121.81, 112.79, 66.71, 56.32, 46.01. Anal. Calcd for $C_{20}H_{21}NO_5S$: C, 62.00; H, 5.46; N, 3.62; S, 8.28. Found: C, 61.70; H, 5.37; N, 3.73; S, 8.05%. *Compound* ***3e***: yield 47%, m.p. 200–202 °C. IR (KBr, cm^{-1}) ν_{max}: 3329, 1661, 1597, 1158, 693. ^1H NMR (200 MHz, DMSO-d_6, ppm) δ: 8.20 (s, 1H, Ar-H), 7.91 (d, 2H, Ar-H), 7.50 (m, 6H, Ar-H), 7.44 (d, $J = 15.7$ Hz, 1H, olefinic H), 7.02 (m, 5H, Ar-H), 4.10 (s, 3H, OCH_3). ^{13}C NMR (50 MHz, DMSO-d_6, ppm) δ: 189.61, 157.49, 142.24, 137.53, 134.64, 132.50, 130.00, 128.65, 128.25, 127.24, 128.04, 126.93, 124.07, 120.17, 115.42, 56.05. Anal. Calcd for $C_{22}H_{19}NO_4S$: C, 67.16; H, 4.87; N, 3.56; S, 8.15. Found: C, 66.82; H, 4.77; N, 3.50; S, 7.80%. *Compound* ***3f***: yield 51%, m.p. 205–207 °C. IR (KBr, cm^{-1}) ν_{max}: 3249, 1660, 1598, 1496, 1147, 689. ^1H NMR (200 MHz, DMSO-d_6, ppm) δ: 8.15 (d, 1H, Ar-H), 8.01 (d, 2H, Ar-H), 7.65 (m, 5H, Ar-H), 7.60 (d, $J = 14.0$ Hz, 1H, olefinic H), 7.56 (d, 1H, Ar-H), 7.26 (d, $J = 14.0$ Hz, 1H, olefinic H), 7.03 (d, 2H, Ar-H), 7.47 (s, 1H, Ar-H), 4.09 (s, 3H, OCH_3). ^{13}C NMR (50 MHz, DMSO-d_6, ppm) δ: 190.05, 157.90, 142.42, 138.00, 135.88, 135.83, 133.22, 131.57, 130.41, 128.67, 128.92, 128.76, 128.53, 127.70, 122.96, 122.36, 112.88, 57.04. Anal. Calcd for $C_{22}H_{18}ClNO_4S$: C, 61.75; H, 4.24; Cl, 8.29; N, 3.27; S, 7.49. Found: C, 62.03; H, 4.22; N, 3.43; S, 7.42%. *Compound* ***3g***: yield 48%, m.p. 218–219 °C. IR (KBr, cm^{-1}) ν_{max}: 3238, 1652, 1590, 1495, 1160, 654. ^1H NMR (200 MHz, $CDCl_3$, ppm) δ: 8.15 (s, 1H, Ar-H), 8.01 (d, 2H, Ar-H), 7.78 (d, $J = 14.8$ Hz, 1H, olefinic H), 7.67 (s, 1H, Ar-H), 7.55 (m, 4H, Ar-H), 7.01 (dd, 1H, Ar-H), 7.09 (d, $J = 14.8$ Hz, 1H, olefinic H), 7.24 (d, 1H, Ar-H), 4.09 (s, 3H, OCH_3). ^{13}C NMR (50 MHz, $CDCl_3$, ppm) δ: 190.04, 157.35, 142.09, 137.86, 136.03, 135.79, 133.00, 130.92, 130.14, 129.14, 128.68, 128.53, 128.16, 126.69, 122.78, 122.23, 120.26, 112.76, 56.83. Anal. Calcd for $C_{22}H_{17}Cl_2NO_4S$: C, 57.15; H, 3.71; Cl, 15.34; N, 3.03; S, 6.94. Found: C, 56.93; H, 3.71; N, 3.09; S, 6.50%. *Compound* ***3h***: yield 60%, m.p. 183–185 °C. IR (KBr, cm^{-1}) ν_{max}: 3242, 1659, 1595, 1154, 643. ^1H NMR (200 MHz, DMSO-d_6) δ: 8.1 (d, 2H, Ar-H), 8.09 (d, 2H, Ar-H), 7.73 (d, $J = 15.7$ Hz, 1H, olefinic H), 7.69 (d, $J = 15.7$ Hz, 1H, olefinic H), 7.50 (m, 3H, Ar-H), 7.02 (m, 5H, Ar-H), 4.09 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3). ^{13}C NMR (50 MHz, DMSO-d_6, ppm) δ: 189.68, 158.52, 157.00, 143.10, 138.23, 135.83, 133.78, 131.44, 130.77, 129.44, 129.22, 127.76, 127.55, 122.05, 115.42, 114.87, 57.25, 55.76. Anal. Calcd for $C_{23}H_{21}NO_5S$: C, 65.23; H, 5.00; N, 3.31; S, 7.57. Found: C, 65.58; H, 4.92; N, 3.41; S, 7.40%. *Compound* ***3i***: yield 42%, m.p. 167–169 °C. IR (KBr, cm^{-1}) ν_{max}: 3210, 1655, 1591, 1162, 693. ^1H NMR (200 MHz, $CDCl_3$, ppm) δ: 8.25 (s, 1H, Ar-H), 8.04 (d, 2H, Ar-H), 7.73 (d, $J = 15.8$ Hz, 1H, olefinic H) 7.63 (d, 1H, Ar-H), 7.42 (m, 4H, Ar-H), 7.14 (d, $J = 15.8$ Hz, 1H, olefinic H), 7.03 (m, 4H, Ar-H), 6.94 (d, 1H, Ar-H), 5.2 (t, 1H, NH), 4.14 (d, 2H, CH_2), 4.09 (s, 3H, OCH_3). ^{13}C NMR (50 MHz, $CDCl_3$, ppm) δ: 190.73, 157.99, 143.12, 138.63, 136.75, 135.96, 133.65, 129.99, 129.37, 129.21, 129.09, 128.57, 122.51, 113.10, 57.17, 48.43. Anal. Calcd for $C_{23}H_{21}NO_4S$: C, 67.79; H, 5.19; N, 3.44; S, 7.87. Found: C, 67.52; H, 5.11; N, 3.50; S, 7.62%.

4.2. Biology

4.2.1. Drugs

All sulfonamide 4-methoxychalcone derivatives (**3**, **3a–3i**) were added into the cultures as a dimethyl sulfoxide (DMSO) solution (50 μM). The final solvent (DMSO) concentrations 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 grown at 28 °C in Schneider's supplemented with 5% of heat inactivated fetal bovine serum (FBS). *T. cruzi* (Y strain) epimastigotes were grown at 28 °C in LIT medium supplemented with 10% of FBS. The J774.A1 macrophage cell line was cultivated in RPMI 1640 (Gibco BRL) medium supplemented with 2 g/L of sodium bicarbonate and 10% of FBS without Hepes.

4.2.3. Antiparasitic assays

4.2.3.1. Promastigotes. For the parasite growth inhibition assays, *Leishmania* promastigotes were harvested on the exponential phase of growth and the concentration was adjusted to 10×10^6 parasites/ml in Schneider's medium plus 5% FBS. The compounds solubilized in DMSO were diluted to appropriate concentrations ranging from 100 to 1.6 μM in culture medium. One hundred microliters of the parasite suspension was added to 96-well plates and incubated at 28 °C for 72 h in the presence of different compounds' concentrations. Amphotericin B (ranging from 31.25 to 1000 nM) was used as positive

control and DMSO 1% was used as negative controls. Three experiments were carried out in triplicate, and the number of live parasites was determined by counting in Neubauer chamber. The 50% inhibitory concentrations (IC_{50}) were determined by linear regression analysis, and represented the mean ± standard error of three independent experiments (GraphPad Software, San Diego, CA). The IC_{50} for pentamidine isothionate was obtained from Lima et al. [25].

T. cruzi epimastigotes were harvested at the exponential phase of growth and the concentration adjusted to 5×10^6 parasites/ml in LIT medium plus 10% FBS. One hundred microliters of the parasite suspension was added to 96-well plates and incubated at 28 °C for 72 h in the presence of different compounds' concentrations (ranging from 100 to 1.6 μM). Benzonidazole (10 μM) was used as positive control and DMSO 1% was used as negative control. Experiments were carried out in triplicate, and the number of live parasites was determined by counting in Neubauer chamber.

4.2.3.2. Amastigotes. L. braziliensis axenic amastigotes were obtained by differentiation of promastigotes from the stationary phase in Schneider medium plus 20% FBS pH 6.3 at 34 °C for 72 h in 25 cm^2 tissue culture flasks [34]. The J774.A1 cells were removed by scrapping and incubated at 34 °C with axenic amastigotes at a 10:1 parasite:cell ratio in RPMI medium supplemented with 20% FBS and maintained under gentle shaking overnight. One hundred microliters of the cell suspension was seeded on 13 mm glass coverslips in 24-well plates, and incubated with different **3i** concentrations (1–100 μM) for 48 h at 34 °C/5% CO_2. After that, coverslips were air-dried, methanol fixed, Giemsa stained. The rate of cell infection as well as the number of amastigotes per cell was microscopically evaluated (1000×) by counting 200 random cells in at least of two independent experiments in duplicate. The percentage of inhibition (PI) was calculated according to Guru et al. and values higher than 25% were considered significative. Amphotericin B was used as positive control, and DMSO 1% as negative control.

4.2.4. Cytotoxic assays

Mouse peritoneal macrophages were harvested from the peritonea of healthy mice after injection of 5 ml sterile phosphate saline buffer (PBS) containing 0.5% EDTA. Cells (5×10^5 cells/ml) were cultivated for 72 h at 37 °C in 96-well microplates in DMEM medium supplemented with 10% FBS in the presence of different compounds' concentrations. The cytotoxic effect of chalcones was assessed by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay [35,36]. The 50% cytotoxicity concentrations (CC_{50}) were determined by linear regression analysis and represent the mean ± standard error of three independent experiments (GraphPad Software, San Diego, CA).

4.3. Molecular modeling methodology

Molecular modeling was performed using SPARTAN'04 (Wavefunction Inc. Irvine, CA, 2000) and Osiris programs (http://www.organic-chemistry.org/prog/peo/druglikeness.html) as described elsewhere [19,24,28–30]. Structures were minimized and the equilibrium geometry was obtained in vacuum using a semi-empirical AM1 module. In order to evaluate the electronic properties of the AM1 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'06 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). They were generated in a range from −65 to +23 kcal/mol. These color-coded isosurface values provide an indication of the overall molecular size and location of negative (red) or positive (blue) electrostatic potentials. The electronic properties (HOMO energy, HOMO orbital coefficients distribution, LUMO density, dipole moment, dipole moment vector and lipophilicity-cLogP) were calculated for all compounds. Hydrogen bond acceptor and donor, molecular weight and volume and the theoretical toxicity properties were calculated in the Osiris Property Explorer (http://www.organic-chemistry.org/) and in the *in silico* screening program (http://www.molinspiration.com/cgi-bin/properties).

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