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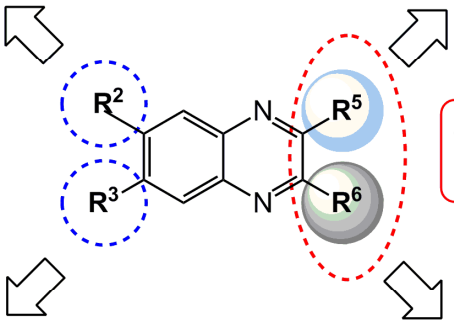
Hidrogen replacement by **halogen elements** (Cl or Br) increase activity while by **methoxyl** (OMe) group slightly decrease

Aryl at R^5 combined with **amine** (NHR^6) or **methylthio** (SMe) on R^6 reduced acitivity **Diaryl** substitution on R^5 and R^6 increase potency and reduce toxicity



Changes on R^5 and R^6 position interfere directly on the activity

Hidrogen substitution by **methoxyl** (OMe), **OCH_2**, and **Cl** are tolerated and not showed great interference on the activity

Methylsulfoxyl (SOMe), **methylsulfonyl** (SO_2Me), and **Aryl** groups are the main responsible for the acitvity
Their substitution by **amine** (NHR^6) and methylthio (SMe) reduced potency

Synthesis and biological evaluation of novel 2,3-disubstituted quinoxaline derivatives as antileishmanial and antitrypanosomal agents

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Abbreviations

T. cruzi, Trypanosoma cruzi; L. amazonensis, Leishmania amazonensis; IC, inhibitory concentration; EC, effetive concentration; SI, selective index; CC, cytotoxic concentration; $LLCMK_2$, Epithelial cells from the kidney from *Macaca mulatta*.

ABSTRACT

Quinoxalines belong to the *N*-containing heterocyclic compounds that stand out as having promising biological activity due to their privileged scaffold. In this work, we report the synthesis, antileishmanial, and antitrypanosomal properties of 46 new 2,3-disubstituted quinoxaline and 40 previously reported derivatives. Among all of the compounds screened for *in vitro* activity against epimastigotes and trypomastigotes of *T. cruzi* and promastigotes of *L. amazonensis* as well as mammalian toxicity on $LLCMK_2$ cells and J774 macrophages, analogues from series **5**, **6**, **7**, **9**, **12**, and **13** displayed high activity at micromolar IC_{50} and EC_{50} concentrations. Sixteen quinoxaline derivatives were selected and evaluated on *T. cruzi* and/or *L. amazonensis* amastigotes. The most active compounds were **6a-b** and **7d-e**, on all evolutive forms of *L. amazonensis* and *T. cruzi* evaluated with IC_{50} values 0.1-0.8 μM on promastigotes and epimastigotes 1.4-8.6 on amastigotes. Compounds **5k**, **12b** and **13a** were the most selective (SI = 19.5-38.4) on amastigotes of *T. cruzi*. In general their activity was directly related to the methylsulfoxyl, methylsulfonyl, and amine groups as well as the presence of chorine or bromine in the molecules. The current results indicate that these quinoxaline derivatives are novel and promising agents for further development towards a treatment for Chagas' disease and leishmaniasis.

KEYWORDS: Quinoxaline derivatives; *Leishmania amazonensis; Trypanosoma cruzi;* Anti-trypanosomatid agents

Introduction

Neglected tropical diseases are significant public health problems and have been attracting increasing worldwide attention [1]. Chagas' disease is caused by the protozoan *Trypanosoma cruzi*, which is found in 21 countries and affects approximately 8 million people, with approximately 50,000 new cases per year [2]. Latin America has most of the cases of Chagas' disease, which has become a global health problem as a result of migration to non-endemic regions, such as Australia, Europe, the United States, and Canada, resulting in annual treatment costs of approximately USD$ 600 million [3,4]. Its pathogenesis is subdivided into an acute phase characterized by nonspecific inflammation, an asymptomatic indeterminate phase, and a chronic phase, during which approximately 30-40% of the cases develop irreversible cardiovascular, gastrointestinal, and neurological lesions [5,6]. The transmission of Chagas' disease generally occurs through the bite and infection with contaminated feces of insects of the subfamily Triatominae (Hemiptera, Reduviidae). It may also be transmitted through blood transfusion or congenitally and orally, such as through contaminated food [7,8].

Leishmaniasis is endemic in 98 countries worldwide, with approximately 350 million people at risk of infection and 12 million currently infected. The disease may be caused by more than 20 different species of *Leishamania* sp, which are responsible for clinical manifestations that can be classified as cutaneous, mucocutaneous, and visceral. Cutaneous leishmaniasis is the most common clinical form of the disease, with 1.5 million new cases per year. The species that stand out in the New World are *L. amazonensis*, *L. braziliensis*, and *L. guyanensis* [2,9,10]. Among the cutaneous leishmaniasis cases worldwide, 70-75% occur in just 10 countries, including Brazil, which experienced approximately 30,000 new cases in 2010 [11,12]. Transmission occurs through the bite of the female phlebotomine sandfly. The vector in the New World is *Lutzomyia*,

and the vector in the Old World is *Phlebotomus* [13]. Clinical manifestations are characterized by single or multiple lesions that are usually located on the legs, arms, and head. They initially appear as papules and progress into nodules and finally ulcerative lesions [14,15].

Nitroderivative compounds, such as benznidazole and nifurtimox, are currently the primary treatment options for Chagas' disease, despite their reduced efficacy in the chronic phase and adverse reactions in approximately 40% of patients [16-18]. For antileishmania chemotherapy, pentavalent antimonials, such as meglumine antimoniate and sodium stibogluconate, are the first-line treatment, whereas amphotericin B, pentamidine, paromomycin, and miltefosine are the second-line treatment [19-21]. However, the available drugs for both diseases have severe side effects, long-term treatment, and variable efficacy, factors that encourage the search for new therapeutic alternatives [22].

Quinoxalines belong to the *N*-containing heterocyclic compounds that stand out as having promising biological activity because of their privileged scaffold [23,24]. They have numerous reported biological activities, including anticancer [25-27], beneficial effects for sleep disorders [28], antimycobacterial [29-30], antibacterial [31], antifungal [32], antiviral [33], anti-inflammatory, and antioxidant activities [34-35]. The antiprotozoal activity of quinoxalines is relevant, especially their antitrypanosomatid activity, which has been reported for quinoxaline 1,4-di-*N*-oxide [36,37], 3-trifluoromethylquinoxaline *N*,*N*'-dioxide [38], and 3-aminoquinoxaline-2-carbonitrile 1,4-dioxide derivatives [39]. Quinoxalines also exhibit antileishmanial activity, which has been reported for 4-substituted pyrrolo[1,2-a]quinoxalines [40], 3-phenyl-1-(1,4-di-*N*-oxide quinoxalin-2-yl)-2-propen-1-one [41-42], and 1,4-di-*N*-oxide quinoxaline derivatives [43].

Recently, we have reported the activity of 3-chloro-7-methoxy-2-(methylsulfonyl)quinoxaline, against *T. cruzi* [44]. A synergistic effect between this quinoxaline and benznidazole was observed against epimastigotes and trypomastigotes, accompanied by an antagonistic interaction against $LLCMK_2$ cells. Based on the above considerations, novel 2,3-disubstituted quinoxaline derivatives were synthesized to evaluate their *in vitro* antitrypanosomal and antileishmanial activity.

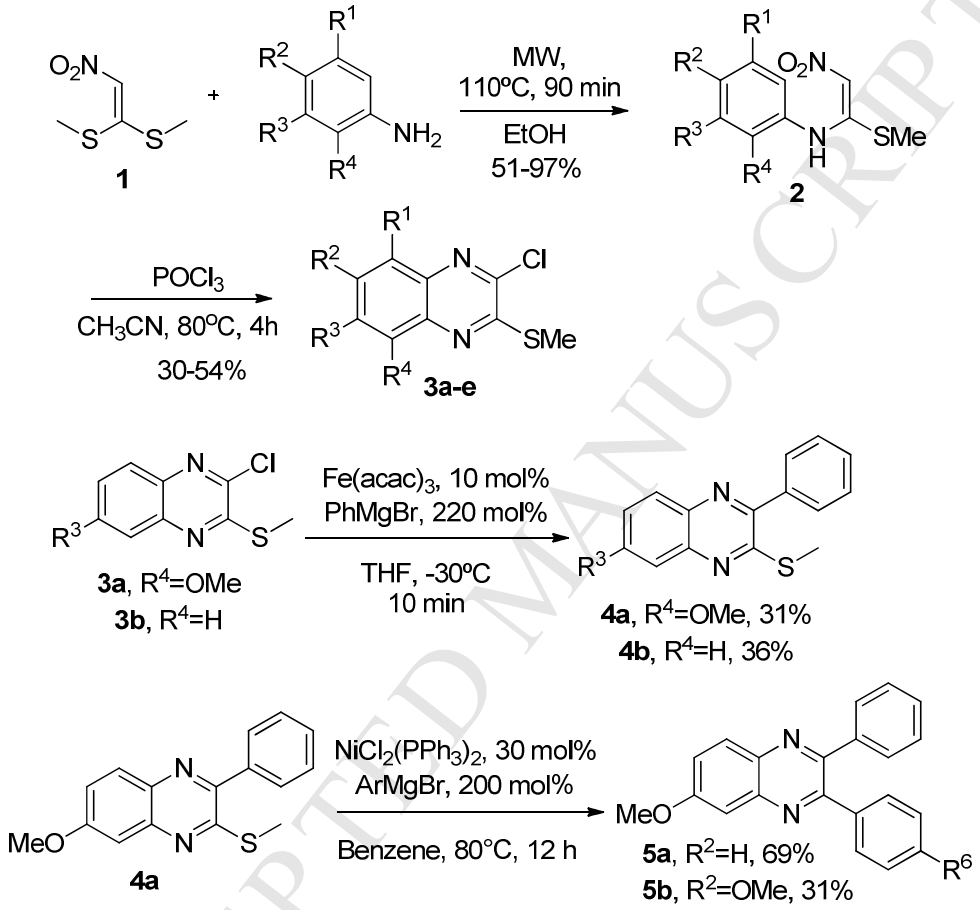
Results and Discussion

The discovery and development of new drugs for the treatment of neglected diseases, such as leishmaniasis and Chagas' disease, is necessary and urgent. Their current treatments have several limitations, including limited effectiveness, parenteral administration, long courses of treatment, severe side effects, toxicity, and high cost, making them unaffordable for most patients [22,45,46]. Many studies have reported that quinoxaline derivatives are promising chemotherapeutic agents against *Leishmania* sp and *T. cruzi* [36-43].

Several methods have been reported for the synthesis of quinoxalines [47]. In the present study, we have focused on straight forward synthetic routes, especially those based on green chemistry principles, Thus, we synthesized 46 new 2,3-disubstituted quinoxaline derivatives (**3d**; **5e**, **5fa-fb**, **5g**, **5ka**; **6a-b**; **7d**; **8a**; **10a**; **11a-d**, **11f-p**; **12a-p**; **13a-b**; **14a-c**) and 40 previously reported compounds (**1**; **2a-p**; **3a-c**, **3e**; **4a-b**; **5a-d**, **5f**, **5h-k**; **7a-c**, **7e**; **9a-c**; **10b-c**; **11e**) with the goal of discovering new drugs for the treatment of Chagas' disease and leishmaniasis.

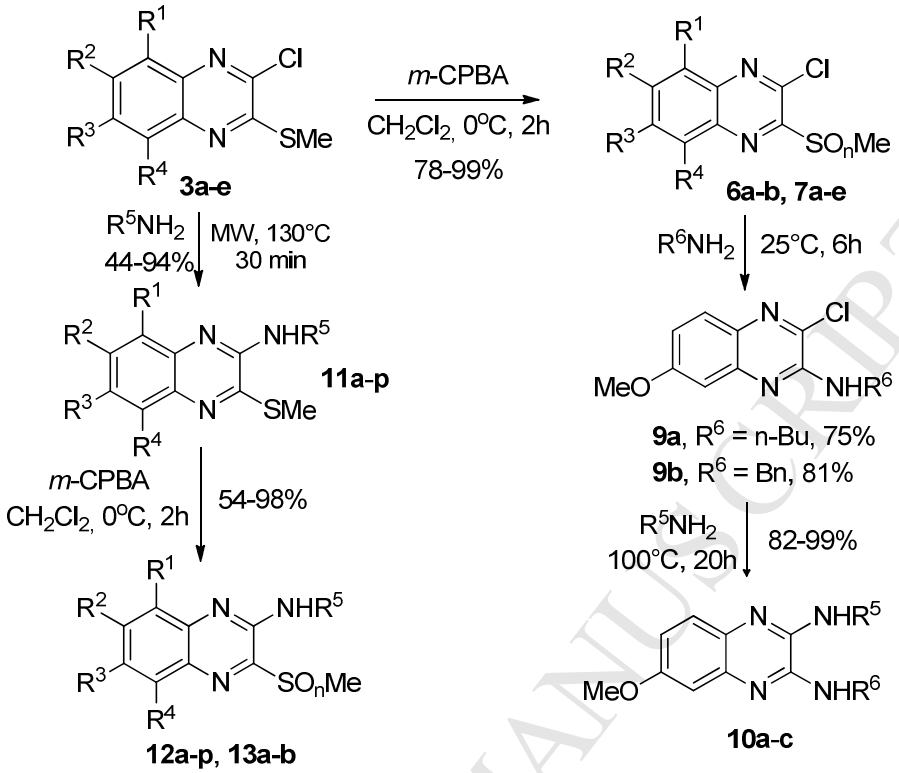
Initially, quinoxaline derivatives were prepared using a procedure described by Venkatesh et al. [48]. The first step was improved by using microwave (MW) irradiation [49] and the nitroketene

N,*S*-acetal derivatives **2**, obtained by vinylic substitution, were cyclized to produce quinoxaline **3**. By using quinoxaline **3** as starting material, we also synthesized quinoxalines **4a**-**b** and **5a**-**b** through cross-coupling reactions (Scheme 1).



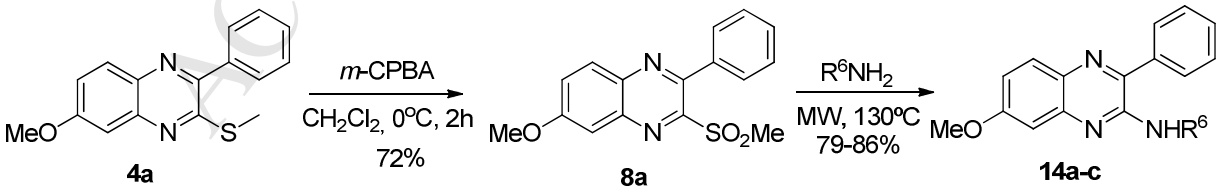
Scheme 1. Synthesis of quinoxalines **3**, **4** and **5a-b**

Employing a series of sulfur oxidations with *m*-chloroperbenzoic acid, and solvent-free nucleophilic substitutions, we synthesized quinoxalines **6**, **7**, **9**, **10**, **11**, **12** and **13**, using quinoxalines **3** as starting material (Scheme 2) [48].



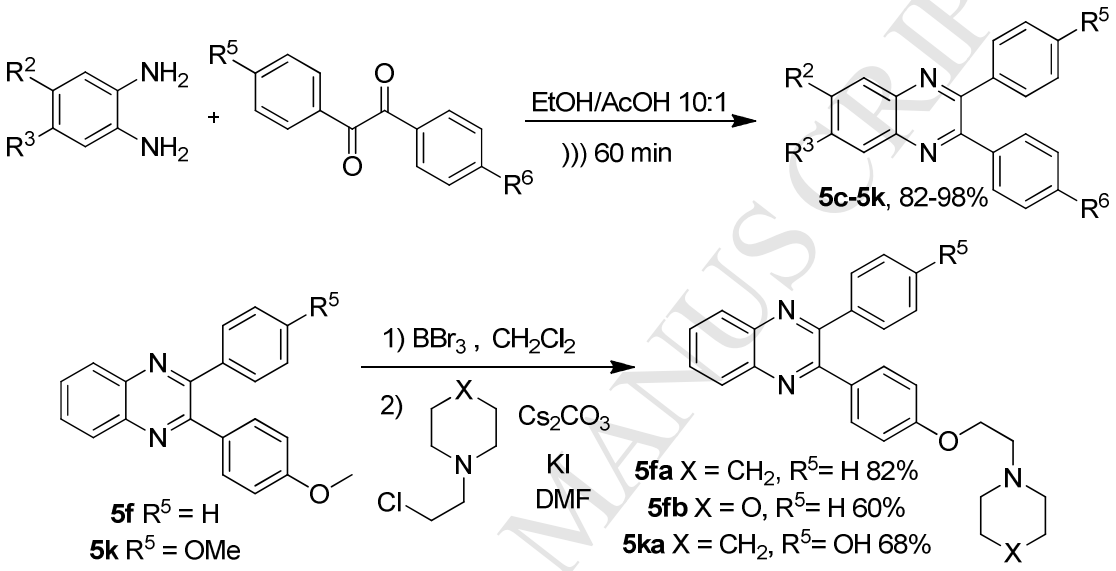
Scheme 2. Synthesis of quinoxalines **6**, **7**, **9, 10, 11, 12** and **13**

Quinoxalines **8a** and **14a-c** were synthesized using **4a** as starting material through oxidation followed by nucleophylic substitution (Scheme 3). We have tried to synthesize aryilaminoquinoxalines **14** at room temperature without success, thus microwave irradiation was applied under the same conditions used to obtain aminosulfonylquinoxalines **11** reaching good results.



Scheme 3. Synthesis of quinoxalines **8a** and **14a-c**

Quinoxalines **5c-k** were synthesized through the condensation of 1,2-diarylethanediones with *O*-phenylenediamine by using ultrasound irradiation as energy source (Scheme 4) [50]. Compounds **5fa**, **5fb**, and **5ka** were prepared from **5f** or **5k** by deprotection of the methoxyl group followed by *O*-alkylation.

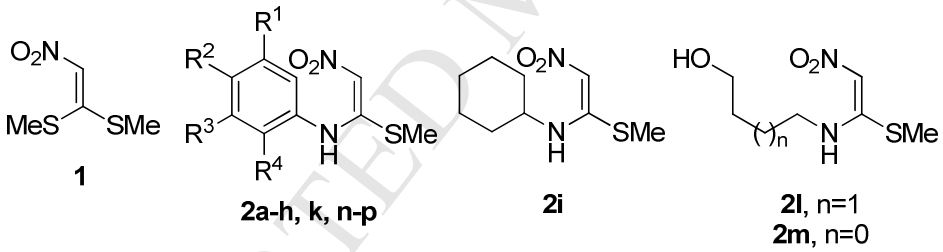


Scheme 4: Synthesis of 2,3-aryldisubstituted quinoxalines **5**

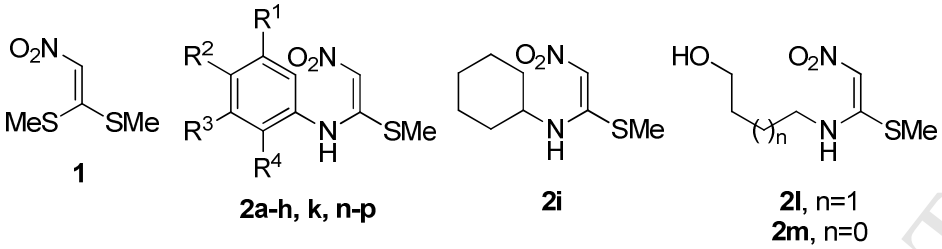
The screening for antichagasic and antileishmanial activity was performed on the epimastigote and trypomastigote forms of *T. cruzi* and promastigote form of *L. amazonensis*. Epimastigotes and promastigotes are the extracellular replicative forms inside the insect vectors of *T. cruzi* and *L. amazonensis*, respectively. The easily cultivable and drug-sensitive epimastigote and promastigote forms make these models an excellent choice for preliminary *in vitro* screening. Trypomastigotes are an extracellular non-replicative stage of *T.* cruzi found in the bloodstream of infected vertebrate hosts. Selective toxicity is an important principle of antiparasitic therapy, therefore the cell viability was also carried out to verify their cytotoxic effects on mammalian cells (LLCMK$_2$ and J774-A1 macrophages).

Compound l,l-bis(methylthio)-2-nitroethene **1** that was used in the synthesis of quinoxaline derivatives was also assayed against both protozoa (Table 1). Notably, compound **1** presented activity against the epimastigote and promastigote forms. Thirteen nitroketene *N*,*S*-acetal derivatives **2**, which were synthesized through the reaction of compound **1** with primary or secondary amines (Scheme 1) [49], showed antileishmanial and antitrypanosomal activity at different levels (Table 1). The presence of benzenamine and 2-[nitroethenyl]benzenamine rings in compounds **2b** and **2k**, respectively, and the presence of fluorine and chlorine on the benzene ring (**2o** and **2p**, respectively) caused an increase in biological activity, with IC_{50} values < 26 µM for both protozoa. The presence of the amine group in **2n** made it the only active compound in this group against trypomastigotes.

Table 1. *In vitro* antileishmanial and antitrypanosomal activities of 1,1-bis(methylthio)-2-nitroethene (**1**) and nitroketene *N*,*S*-acetals (**2**)



Comp					µM					
					Promastigote		Epimastigote		Trypomastigote	
	R^1	R^2	R^3	R^4	$IC_{50/72h}$	SI	$IC_{50/96h}$	SI	$EC_{50/96h}$	SI
1					29.6 ± 2.1	6.0	10.8 ± 0.6	18.3	>50.0	ND
2a	H	H	OMe	H	27.9 ± 5.9	ND	32.0 ± 8.3	ND	NT	ND
2b	H	H	H	H	24.1 ± 0.6	ND	11.4 ± 6.6	ND	NT	ND
2c	H	OMe	H	H	28.7 ± 3.5	ND	39.0 ± 12.4	ND	NT	ND
2d	H	OCH_2	CH_2O	H	42.3 ± 0.8	ND	61.9 ± 11.0	ND	NT	ND
2f	H	OH	H	H	41.5 ± 7.3	ND	67.3 ± 15.6	ND	NT	ND
2h	H	Br	H	H	33.0 ± 3.2	ND	20.4 ± 4.3	ND	NT	ND
2i					21.9 ± 8.2	ND	70.1 ± 13.6	ND	NT	ND
2k	H	F	H	H	19.7 ± 1.2	ND	19.7 ± 6.6	ND	NT	ND

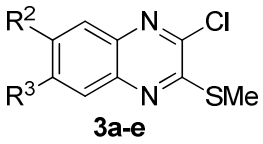


Comp					μM					
					Promastigote		Epimastigote		Trypomastigote	
	R^1	R^2	R^3	R^4	$IC_{50/72h}$	SI	$IC_{50/96h}$	SI	$EC_{50/96h}$	SI
2l					73.6 ± 4.8	11.1	>100.0	ND	>50.0	ND
2m					>100.0	ND	90.1 ± 3.1	9.6	>50.0	ND
2n	H	NMe_2	H	H	52.8 ± 4.2	18.9	31.8 ± 3.8	6.6	35.0 ± 6.1	6.0
2o	H	F	H	H	17.2 ± 0.4	8.4	18.1 ± 1.4	10.5	>50.0	ND
2p	H	Cl	H	H	21.3 ± 0.2	6.9	25.8 ± 1.8	7.3	>50.0	ND

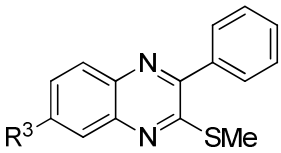
IC: inhibitory concentration; EC: effective concentration; SI: selective index; NT: not tested; ND: not determined

Using nitroketene *N,S*-acetals **2**, five 3-chloro-2-methylthioquinoxalines **3** were synthesized (Scheme 1)**.** The changes in the pyrazine ring were not able to increase the activity levels in relation to compound **2** but made it more selective against protozoa than against mammalian cells (Table 2). Compound **3a** was 19.3-times more selective for epimastigotes, whereas **3e** was 22.1- and 16.1-times more selective for promastigotes and epimastigotes, respectively.

Table 2. *In vitro* antileishmanial and antitrypanosomal activities of 3-chloro-2-methylthioquinoxalies (**3**) and 3-aryl-2-methylthioquinoxalines (**4**)



3a-e



4a-b

Comp			µM					
			Promastigote		**Epimastigote**		**Trypomastigote**	
	R^2	R^3	**$IC_{50/72h}$**	**SI**	**$IC_{50/96h}$**	**SI**	**$EC_{50/96h}$**	**SI**
3a	H	OMe	93.6 ±2.6	9.8	38.7 ± 2.9	19.3	>50.0	ND
3b	H	H	74.1 ± 7.2	13.5	52.9 ± 5.4	8.1	>50.0	ND
3c	OMe	H	>100.0	ND	>100.0	ND	>50.0	ND
3d*	Br	H	57.9 ± 10.6	7.4	47.8 ± 2.6	9.2	>50.0	ND
3e	Cl	H	45.3 ± 1.0	22.1	39.4 ± 1.6	16.1	>50.0	ND
4a		OMe	>100.0	ND	>100.0	ND	NT	ND
4b		H	>100.0	ND	>100.0	ND	NT	ND

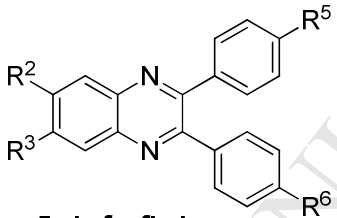
IC: inhibitory concentration; EC: effective concentration; SI: selective index; NT: not tested; ND: not determined. *Gray highlights the newly synthesized quinoxaline derivatives

Two 3-aryl-2-methylthioquinoxalines **4** were prepared from compound **3a** (Scheme 2). The chloride replacement by phenyl group in the pyrazine ring in compounds **4a** and **4b** caused a reduction of potency, with no activity at the highest concentration evaluated against all evolutive forms (Table 2).

Fourteen 2,3-diarylsubstituted quinoxalines **5** were obtained as described in Schemes 2 and 3. The addition of an extra phenyl group in the pyrazine ring in these compounds improved the biological activity against *L. amazonensis* and *T. cruzi* (Table 3) in relation to quinoxaline derivatives **4a** and **4b**. The additional methoxyl groups in the phenyl rings in compound **5c** gave rise to compound **5k**, and this change was responsible for an increase in the activity against epimastigotes. Although **5k** was moderately active against promastigotes and epimastigotes, its low toxicity to host cells revealed selectivity indices of 19.7 and 9.0, respectively. Structural

changes in **5k** gave rise to compound **5ka**, which showed an increase in activity (IC_{50} = 5.7 µM) against the promastigote form of *L. amazonensis*. However, higher toxicity against the host cell was observed compared with its precursor **5k**, thereby causing a reduction of the selectivity index to 8.2. Compound **5f**, which has only one methoxyphenyl group on R^5, was more active against both protozoa, especially against promastigotes, with an IC_{50} of 12.8 µM.

Table 3. *In vitro* antileishmanial and antitrypanosomal activities of 2,3-diarylsubstituted quinoxalines (**5**)



5c-k, fa, fb, ka

Comp					µM					
					Promastigote		**Epimastigote**		**Trypomastigote**	
	R^2	R^3	R^5	R^6	**$IC_{50/72h}$**	**SI**	**$IC_{50/96h}$**	**SI**	**$EC_{50/96h}$**	**SI**
5a	H	OMe	H	H	NT	ND	NT	ND	NT	ND
5b	H	OMe	H	OMe	40.5 ± 17.7	ND	>100.0	ND	NT	ND
5c	H	H	H	H	21.1 ± 0.3	ND	>100.0	ND	NT	ND
5d	H	H	Me	H	8.9 ± 1.2	ND	35.7 ± 17.6	ND	NT	ND
5e*	Cl	H	Me	H	24.3 ± 1.9	ND	36.0 ± 11.1	ND	NT	ND
5f	H	H	OMe	H	12.8 ± 0.0	ND	21.5 ± 0.8	ND	NT	ND
5fa*	H	H	H	$OCH_2CH_2NC_5H_{10}$	1.9 ± 0.2	29.6	21.4 ± 1.1	1.8	20.3 ± 2.3	1.8
5fb*	H	H	H	$OCH_2CH_2NC_4H_8O$	6.2 ± 0.6	8.7	40.3 ± 4.5	4.6	>50.0	ND
5g*	Cl	H	H	OMe	31.5 ± 1.6	9.5	43.8 ± 3.0	6.5	>50.0	ND
5h	H	Cl	H	H	28.1 ± 1.4	6.6	42.7 ± 2.2	6.1	>50.0	ND
5i	Cl	Cl	H	H	5.3 ± 0.7	38.5	54.0 ± 1.8	8.7	>50.0	ND
5j	Cl	Cl	H	Me	22.2 ± 1.0	3.2	83.4 ± 6.2	1.3	>50.0	ND
5k	H	H	OMe	OMe	30.0 ± 0.6	19.7	36.6 ± 3.0	9.0	>100.0	ND
5ka*	H	H	OH	$OCH_2CH_2NC_5H_{10}$	5.7 ± 0.4	8.2	39.2 ± 4.7	1.0	>50.0	ND

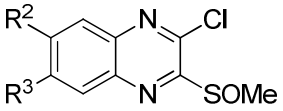
IC: inhibitory concentration; EC: effective concentration; SI: selective index; NT: not tested; ND: not determined. *Gray highlights the newly synthesized quinoxaline derivatives

Piperidine and morpholine derivatives **5fa** and **5fb**, respectively, were synthesized. Similar to compound **5ka**, these structural changes led to a significant increase in activity, mainly against promastigotes, with IC_{50} values of 1.9 µM for **5fa** and 6.2 µM for **5fb**. Such changes increased their potency, resulting in increased selectivity of 29.6 and 8.7, respectively.

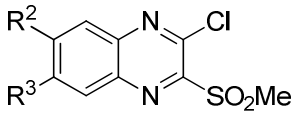
Furthermore, compound **5fa** was the only one that showed activity against trypomastigotes of *T. cruzi*, with an EC_{50} of 20.3 µM. All of the chlorine containing compounds in this group were active against both protozoa. In quinoxaline **5i**, two chlorines at the benzene ring were responsible for increased activity against the promastigote form (IC_{50} = 5.3 µM), with low levels of toxicity and a high selectivity index of 38.5. Halogeneted molecules have been described by other researchers to improve the antiprotozoal properties of compounds [51-52].

To investigate the influence of methylsulfoxyl and methylsulfonyl groups on biological activity, 3-chloro-2-methylthioquinoxalines **3** underwent oxidation reactions led to a series of 3-chloro-2-methylsulfoxylquinoxalines **6** and 3-chloro-2-methylsulfonylquinoxalines **7** (Scheme 1). The assays performed with these two groups of quinoxaline derivatives confirmed the influence of halogens on activity (Table 4). Compound **6b**, which has two chlorines in its structure, was the most potent quinoxaline derivative against all evolutive forms, with an IC_{50} of 0.1 µM for promastigotes and epimastigotes and an EC_{50} of 1.7 µM for trypomastigotes. Moreover, it was the most selective for *L. amazonensis* (selectivity index = 107.6). Compound **6a** was among the most active (IC_{50} > 0.8 µM) and selective (selectivity index = 39.2 and 27.8) compounds against *T. cruzi* and *L. amazonensis.*

Table 4. *In vitro* antileishmanial and antitrypanosomal activities of 3-chloro-2-methylsulfoxylquinoxalines (**6**) and 3-chloro-2-methylsulfonylquinoxalines **(7**)



6a-b



7a-e

Comp			µM					
			Promastigote		**Epimastigote**		**Trypomastigote**	
	R^2	R^3	**$IC_{50/72h}$**	**SI**	**$IC_{50/96h}$**	**SI**	**$EC_{50/96h}$**	**SI**
6a*	H	OMe	0.8 ± 0.2	27.8	0.5 ± 0.1	39.2	4.2 ± 1.2	4.4
6b*	Cl	H	0.1 ± 0.0	107.6	0.1 ± 0.0	54.2	1.7 ± 0.1	2.9
7b	H	H	1.6 ± 0.6	14.4	0.6 ± 0.1	70.1	6.4 ± 0.3	7.3
7c	OMe	H	2.9 ± 0.7	7.6	3.1 ± 0.4	7.5	9.8 ± 1.4	2.4
7d*	Br	H	0.2 ± 0.1	71.8	0.3 ± 0.1	49.6	1.8 ± 0.1	7.0
7e	Cl	H	0.2 ± 0.1	66.0	0.3 ± 0.0	25.9	6.9 ± 1.0	4.1

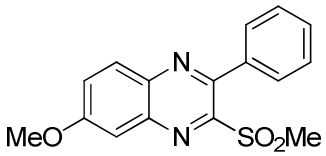
IC: inhibitory concentration; EC: effective concentration; SI: selective index; *Gray highlights the newly synthesized quinoxaline derivatives

Compounds **7** with a methylsulfonyl group showed excellent activity, similar to compounds **6**. The most active (IC_{50} > 0.3 µM) and selective (SI = 25.9-71.8) were compounds **7d** and **7e**, which have bromine and fluorine in the structure, respectively. The addition of a methoxyl group in the benzene ring in compound **7c** resulted in decreased activity and selectivity against *T. cruzi* and *L. amazonensis*. Despite the cytotoxicity observed in host cells, high levels of selectivity were obtained. This increase in the antiprotozoal activity of compounds in groups **6** and **7** was directly linked to the introduction of methylsulfoxyl and methylsulfonyl groups in the quinoxaline ring.

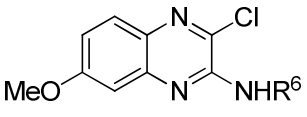
Compound **4a** oxidation gave rise to compound 2-phenyl-3-methylsulfonyl-6-methoxyquinoxaline (**8a**). Chlorine replacement by phenyl ring resulted in decreased activity and selectivity against *T. cruzi* and *L. amazonensis*. It showed an IC_{50} values of 24.7 and 28.4 µM and

an EC_{50} value of 48.1 µM for the promastigote, epimastigote, and trypomastigote forms, respectively (Table 5).

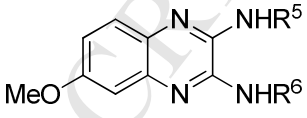
Table 5. *In vitro* antileishmanial and antitrypanosomal activities of 2-phenyl-3-methylsulfonyl-6-methoxyquinoxaline (**8**), 3-chloro-2-aminoquinoxalines (**9**) and 2,3-diaminoquinoxalines (**10**)



8a



9a-b



10a-b

Comp			µM					
			Promastigote		**Epimastigote**		**Trypomastigote**	
	R^5	R^6	**$IC_{50/72h}$**	**SI**	**$IC_{50/96h}$**	**SI**	**$EC_{50/96h}$**	**SI**
8a			24.7 ± 2.3	4.1	28.4 ± 1.9	9.3	48.1 ± 0.9	5.5
9a		^{n}Bu	36.2 ± 1.0	4.4	49.5 ± 6.4	7.9	> 50.0	ND
9b		Bn	24.5 ± 1.7	5.4	15.9 ± 1.6	22.2	>50.0	ND
10a*	^{n}Bu	^{n}Bu	20.9 ± 0.9	4.3	29.4 ± 5.0	3.2	> 50.0	ND
10b	^{n}Bu	Bn	13.6 ± 0.8	3.6	38.4 ± 2.9	0.6	> 50.0	ND
10c	Ph	Ph	6.5 ± 0.0	4.4	45.6 ± 3.4	1.0	37.9 ± 2.3	1.2

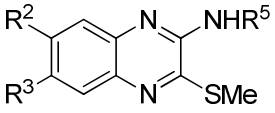
IC: inhibitory concentration; EC: effective concentration; SI: selective index; ND: not determined. *Gray highlights the newly synthesized quinoxaline derivatives

3-Chloro-2-methylsulfonylquinoxalines **7** were submitted to nucleophilic substitution reactions that provided 3-chloro-2-aminoquinoxalines **9** and 2,3-diaminoquinoxalines **10** (Scheme 1). Phenyl ring addition in the amine group made compound **9b** more active and selective than compound **9a**. Compound **9b** had IC_{50} values of 24.5 and 15.9 µM against the promastigote and epimastigote forms, respectively, which was up to 22-times more selective for the protozoa than for the host cells (Table 5). As well as other quinoxaline derivatives, 2,3-diaminoquinoxalines **10** were considered more active against the promastigote form of *L. amazonensis* (IC_{50} = 6.5-20.9 µM) than for *T. cruzi* epimastigotes (IC_{50} = 29.4-45.6 µM, EC_{50} > 50 µM) (Table 5). However,

when evaluated against the trypomastigote form of *T. cruzi*, these two classes of compounds showed no activity, with the exception of compound **10c** that had an EC_{50} value of 37.9 µM. This loss of antiprotozoal activity was further enhanced by the substitution of the methylsulfoxyl and methylsulfonyl groups for an amine group in the quinoxaline ring.

A series with sixteen 3-amino-2-methylthioquinoxalines **11** was synthesized using 3-chloroquinoxalines **3** as starting material through substitution reactions (Scheme 1). These compounds showed different levels of activity (IC_{50} between 21.5 and > 100 µM) (Table 6). Despite the large number of compounds obtained and evaluated, no high selectivity index was found. The best selectivity was obtained with compound **11f** against the epimastigote form of *T. cruzi*, which was 8.5-times more selective for protozoa than for host cells.

Table 6. *In vitro* antileishmanial and antitrypanosomal activities of 3-amino-2-methylthioquinoxalines (**11**)



11a-p

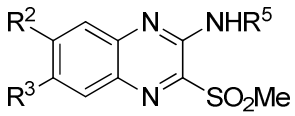
Comp				µM					
				Promastigote		Epimastigote		Trypomastigote	
	R^2	R^3	R^5	$IC_{50/72h}$	SI	$IC_{50/96h}$	SI	$EC_{50/96h}$	SI
11a*	H	OMe	Me_2	42.8 ± 1.3	5.9	83.6 ± 1.5	6.2	>50.0	ND
11b	H	OMe	^{n}Bu	35.2 ± 4.0	2.1	86.7 ± 1.5	3.1	>50.0	ND
11c*	H	OMe	CH_2CH_2OH	82.9 ± 1.4	5.0	>100.0	ND	>50.0	ND
11d*	H	H	^{n}Bu	75.9 ± 1.7	1.9	93.1 ± 1.0	1.2	>50.0	ND
11e	H	H	EtOH	96.2 ± 2.2	2.9	>100.0	ND	>50.0	ND
11f*	H	OMe	Cyclohexyl	29.8 ± 4.4	1.2	30.5 ± 5.8	8.5	>50.0	ND
11g*	OMe	H	^{n}Bu	30.2 ± 1.7	3.5	69.2 ± 2.1	1.1	>50.0	ND
11h*	OMe	H	CH_2CH_2OH	>100.0	ND	>100.0	ND	>50.0	ND
11i*	OMe	H	Cyclohexyl	21.5 ± 1.3	2.4	41.0 ± 8.7	1.8	>50.0	ND
11j*	OMe	H	^{i}Bu	26.9 ± 0.5	4.7	95.7 ± 1.0	0.7	>50.0	ND
11k*	OMe	H	Isopentyl	27.1 ± 2.1	3.3	79.6 ± 1.7	0.7	>50.0	ND
11l*	Br	H	^{n}Bu	25.2 ± 2.7	5.1	41.8 ± 3.5	0.9	>50.0	ND
11m*	Br	H	CH_2CH_2OH	>100.0	ND	>100.0	ND	>50.0	ND
11n*	H	OMe	^{i}Bu	27.6 ± 4.2	6.7	69.9 ± 2.7	1.0	>50.0	ND
11o*	Cl	H	^{n}Bu	24.4 ± 2.5	4.6	63.6 ± 4.5	1.0	>50.0	ND
11p*	Cl	H	Cyclohexyl	>100.0	ND	52.3 ± 2.3	4.8	>50.0	ND

IC: inhibitory concentration; EC: effective concentration; SI: selective index; ND: not determined. *Gray highlights the newly synthesized quinoxaline derivatives

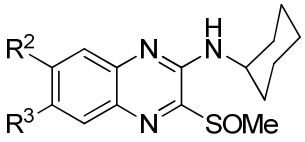
The methylthio groups in quinoxaline derivatives **11** were subsequently oxidized to sulfone and sulfoxide, giving rise to 3-amino-2-methylsulfonylquinoxalines **12** and 3-amino-2-methylsulfoxylquinoxalines **13**, respectively (Scheme 1).

Sixteen 3-amino-2-methylsulfonylquinoxalines **12** were synthesized. The replacement for a methylsulfonyl group was responsible for an increase in the antileishmanial and antitrypanosomal activity, similar to the observations with the compounds **7** (Table 7). Among the products of this synthesis, derivatives **12b**, **12c**, and **12f** differ only in the amine group. The similarities between these compounds were also seen with regard to biological activity, with high activity against the promastigote and epimastigote forms (IC_{50} = 2.2-3.6 µM) and moderate activity against the trypomastigote form (EC_{50} = 27.7 and 39.2 µM). Notably, the butylamine group in **12b** caused a significant reduction of toxicity in host cells compared with the other compounds, with selectivity index ratios of 108.5 and 10.0 for epimastigotes and trypomastigotes, respectively.

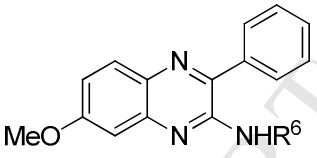
Table 7. *In vitro* antileishmanial and antitrypanosomal activities of 3-amino-2-methylsulfonylquinoxalines (**12**), 3-amino-2-methylsulfoxylquinoxalines (**13**) and 3-aryl-2-aminoquinoxalines (**14**)



12 a-p



13 a-b



14 a-c

Comp					µM					
					Promastigote		**Epimastigote**		**Trypomastigote**	
	R^2	R^3	R^5	R^6	**$IC_{50/72h}$**	**SI**	**$IC_{50/96h}$**	**SI**	**$EC_{50/96h}$**	**SI**
12a*	H	OMe	Me_2		35.9 ± 1.0	4.5	25.2 ± 3.7	6.8	>50.0	ND
12b*	H	OMe	^{n}Bu		2.5 ± 0.3	17.1	3.6 ± 0.3	108.5	39.2 ± 1.8	10.0
12c*	H	OMe	C_2H_4OH		2.9 ±0.4	13.3	2.9 ± 0.8	25.2	35.7 ± 4.1	2.0
12d*	H	H	^{n}Bu		2.9 ± 0.5	11.4	4.4 ± 1.0	5.5	15.7 ± 1.2	1.5
12e*	H	H	C_2H_4OH		2.9 ± 0.8	13.0	4.2 ± 1.0	4.4	22.6 ± 3.9	0.8
12f*	H	OMe	Cyclohexyl		2.9 ± 0.1	18.1	2.2 ± 0.4	20.8	27.7 ± 2.5	1.6
12g*	OMe	H	^{n}Bu		32.2 ± 5.7	5.5	55.3 ± 1.0	1.6	41.2 ± 1.2	2.1
12h*	OMe	H	C_2H_4OH		69.2 ± 6.1	3.7	90.9 ± 4.7	2.6	>50.0	ND
12i*	OMe	H	Cyclohexyl		14.7 ± 1.5	6.5	29.1 ± 4.8	2.7	23.6 ± 2.6	3.3
12j*	OMe	H	^{i}Bu		27.8 ± 2.4	3.6	55.4 ± 1.7	1.5	24.8 ± 4.1	3.3
12k*	OMe	H	Isopentyl		21.2 ± 2.1	5.0	38.0 ± 2.5	2.6	22.9 ± 1.9	4.2
12l*	Br	H	^{n}Bu		1.6 ± 0.5	28.3	2.3 ± 0.1	43.7	5.6 ± 0.5	17.8
12m*	Br	H	C_2H_4OH		0.8 ± 0.4	40.3	1.6 ± 0.0	3.4	8.2 ± 2.7	0.7
12n*	H	OMe	^{i}Bu		2.6 ± 0.3	22.8	3.1 ± 0.3	22.6	11.7 ± 1.7	6.0
12o*	Cl	H	^{n}Bu		1.4 ± 0.3	50.6	2.3 ± 0.1	93.4	9.0 ± 1.8	23.8
12p*	Cl	H	Cyclohexyl		2.2 ± 0.1	15.0	2.0 ± 0.0	38.3	3.9 ± 1.4	19.6
13a*	H	OMe			2.5 ± 0.4	8.0	2.5 ± 0.3	72.8	25.4 ± 1.5	7.1
13b*	Cl	H			1.9 ± 0.0	33.8	1.8 ± 0.0	21.8	6.3 ± 1.4	6.2
14a*				^{n}Bu	>100.0	ND	66.8 ± 5.0	12.5	>50.0	ND
14b*				C_2H_4OH	72.8 ± 4.3	4.8	>100.0	ND	>50.0	ND
14c*				$C_2H_4NC_5H_{10}$	50.3 ± 1.9	0.9	47.6 ± 2.4	3.8	>50.0	ND

IC: inhibitory concentration; EC: effective concentration; SI: selective index; ND: not determined. *Gray highlights the newly synthesized quinoxaline derivatives

The presence of bromine or chlorine in compounds **12l**, **12m**, **12o**, and **12p** caused a significant increase activity compared with the other compounds that belong to this group, especially against trypomastigotes, with EC_{50} < 9.0 µM. The introduction of a halogen and a butylamine group in **12l** and **12o** was responsible for the improvement in activity and consequently more selectivity, which was 17.8- to 92.0-times more selective for the protozoa.

3-amino-2-methylsulfoxylquinoxalines **13** were obtained through oxidation of amino-methylthioquinoxalines **11**. Compounds **13a** and **13b** showed high activity against promastigotes and epimastigotes, with IC_{50} values < 2.5 µM and low levels of cytotoxicity (Table 7). Furthermore, **13a** was almost 73-times more selective for epimastigotes, whereas **13b** was 33.8- and 21.8-times more selective for promastigotes and epimastigotes, respectively. Compound **13b** was more active against the trypomastigote form than **13a**, with an EC_{50} of 6.3 and 25.4, respectively, and a selectivity index > 7.

From a mixture of 3-phenyl-7-methylsulfonyl-2-methoxyquinoxaline (**8a**) and amine derivatives, three 3-aryl-2-aminoquinoxalines **14** were obtained. The changes in the quinoxaline ring, such as the addition of aryl and an amine group, were responsible for a decrease in the antiprotozoal activity (Table 7). This low activity against these protozoa was also seen in analogues **4** and **8**, which have an aryl group at the same position and an amine group in compounds **9a** and **9b**.

The results obtained with the quinoxaline derivatives were compared to the ones obtained with amphotericin B and benznidazol, antileishmanial and antitrypanosomal reference-drugs, respectively. Benznidazole exhibited an IC_{50} of 8.1 µM on epimastigotes and an EC_{50} 3.4 µM on trypomastigotes [53] while amphotericin B showed an IC_{50} 0,75 µM on promastigotes.

Analogues from series **5**, **6**, **7**, **9**, **12,** and **13** with high activity and selectivity on promastigotes, and/or epimastigotes and trypomastigotes may be considered equally or more potent than the reference-drugs and were selected to be evaluated on intracellular amastigotes.

T. cruzi and *L. amazonensis* are obligate intracellular protozoan parasites. Amastigotes are the replicative stage inside mammalian host cells and are the clinically important stage of these parasites. The *in vitro* antiproliferative activity on intracellular amastigotes were performed on *L. amazonensis* amastigotes infecting macrophages and *T. cruzi* amastigotes infecting $LLCMK_2$ cells. The results are presented in Table 8.

Table 8. *In vitro* activity of quinoxaline derivatives against intracellular amastigotes of *L. amazoznensis* and *T. cruzi*

Comp	**Amastigote** ***L. amazonensis***		**Amastigote** ***T. cruzi***	
	$IC_{50/72h}$ (µM)	**SI**	**$IC_{50/96h}$ (µM)**	**SI**
5k	NT	ND	8.6 ± 1.9	38.4
6a*	4.6 ± 1.0	4.9	NT	ND
6b*	1.5 ± 0.1	9.0	NT	ND
7b	NT	ND	> 15.0 ± 0.0	ND
7d*	1.4 ± 0.0	9.9	13.5 ± 1,8	1.1
7e	3.1 ± 0.6	4.3	8.6 ± 3.2	0.9
9b	25.0 ± 0.0	5.3	>20.0 ± 0.0	ND
12b*	12.9 ± 2.5	3.3	14.3 ± 1.4	27.3
12c*	17.3 ± 1.4	2.2	40.5 ± 1.0	1.8
12f*	20.2 ± 2.3	2.6	>25.0 ± 0.0	ND
12l*	NT	ND	>25.0 ± 0.0	ND
12m*	NT	ND	7.4 ± 0.3	0.8
12n*	NT	ND	14.9 ± 0.4	4.7
12p*	NT	ND	9.6 ± 0.7	7.9
13a*	7.3 ± 0.3	2.8	9.3 ± 0.6	19.5
13b*	NT	ND	7.7 ± 0.2	5.1

IC: inhibitory concentration; SI: selective index; NT: not tested; ND: not determined: *Gray highlights the newly synthesized quinoxaline derivatives

Although all compounds evaluated on *L. amazonensis* amastigotes showed some level of antiparasite activity, with IC_{50} lower than 25 µM the most active were **6a, 6b, 7d** and **7e** with IC_{50} values less than 4.6 µM. As in the case of antiproliferative activity on promastigotes, 2,7-dichloro-3-(methylsulfinyl)quinoxaline (**6b**) and 6-bromo-3-chloro-2-(methylsulfonyl) quinoxaline (**7d**) were the most potent (IC_{50} values of 1.5 and 1.4 µM, respectively) and selective (SI = 9.0 and 9.9, respectively) compounds on amastigotes of *L. amazonensis* values very similar to obtained with Amphotericin B (IC_{50} of 0.4 µM).

On amastigotes of *T. cruzi* the most active compounds were **5k**, **7e**, **12m**, **12p**, **13a** and **13b** with IC_{50} values less than 9.6 µM while benznidazole presents IC_{50} of 26.1 µM [54]. Despite the slight decrease in the activity on *T. cruzi* amastigotes, some of them showed a high selective index. We have to highlight compound **5k**, a quinoxaline 2,3-diarylsubstituted, although not the most active (IC_{50}: 8.6 µM), it becomes the most selective (SI: 38.4) against the amastigotes of *T. cruzi* due to its low cytotoxicity on mammalian cells ($LLCMK_2$ cells).

Other compounds that deserve attention for its high activity and selectivity on *T. cruzi* amastigotes are 2-butylamino-3-methylsulfonyl-6-methoxyquinoxaline **(12b)** and 2-cycloexyl-3-methylsulfinyl-6-methoxyquinoxaline **(13a)**, with IC_{50} values of 14.3 and 9.3 µM an SI ratio of 27.3 and 19.5 respectively.

Evaluation of the antileishmanial and antitrypanosomal activity led to the identification of a number of structure activity relationships, which showed that the new compounds can be equally or more potent than reference-drugs.

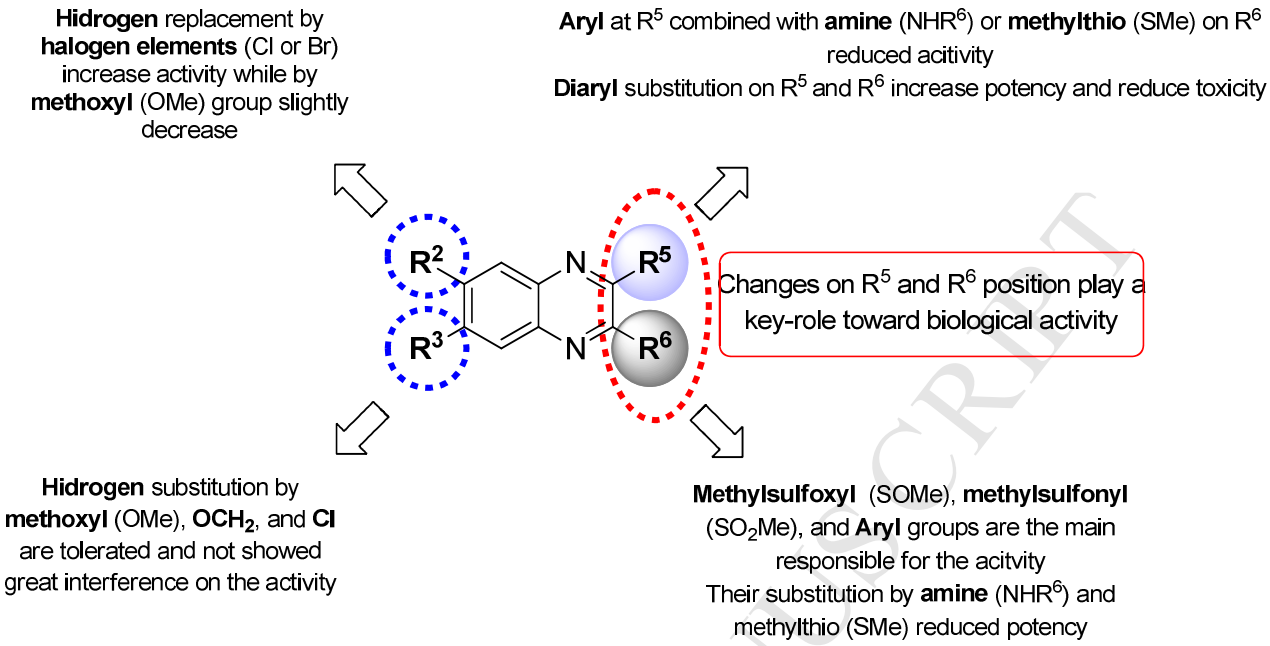


Fig. 1 General SAR scheme of quinoxaline derivatives against *T. cruzi* and *L. amazonensis*

Although clearly defining structure-activity relationships (SAR) is difficult, the comparisons of the activities of quinoxaline derivatives against *T. cruzi* and *L. amazonensis* allowed us to conclude that the methylsulfoxyl and methylsulfonyl groups at the R^6 in the quinoxaline ring (compounds **6**, **7**, **12**, and **13**) were mainly responsible for the antileishmanial and antitrypanosomal activity (Fig. 1). These characteristics of compounds **6** and **7**, coupled with halogens at R^5 position, were generally responsible for the increase in activity. Unfortunately, however, they were also responsible for high cytotoxicity and consequently low selectivity. Replacement by an amino group at the same position in compounds **12** and **13** caused a reduction of cytotoxicity in mammalian cells and an increase in the selective index. The methylthio groups at the R^6 position of the quinoxalines (compounds **2**, **3**, **4**, and **11)** resulted in dramatically reduced activity. Importantly, the addition of two aryl groups in the quinoxaline ring in analogues **5** was responsible for higher activity against *L. amazonensis* than against *T. cruzi* with low toxicity levels.

The 2,3-diarylsubstituted quinoxalines (**5**) presented a moderate to high antitrypanosomal and antileishmanial activity with low levels of toxicity and appeared to be more selective than the other quinoxalines derivatives evaluated. The analogs **4a-b**, **8a**, and **14a-c** including only an aryl group at R^5 did not displayed significant activity and indicate that this group alone was not sufficient for providing antitrypanosomal and antileishmanial activity. Recently, 4-trichloromethylpyrrolo[1,2-a]quinoxalines where evaluated against *Plasmodium falciparum,* aryl addition on the same position increase the potency and was able to reduced the toxicity [55].

Compounds with halogen elements at positions R^2 in the quinoxaline ring, resulted in improved activity while methoxyl group slightly decrease it. Its improvements in the activity against *L. infantum, L. amazonensis*, and *P. falciparum* by the halogens and methoxyl groups have been described [43]. Hidrogen substitution by methoxyl, OCH_2 and chloride at R^3 are tolered and not showed great activity interference.

Conclusion

In conclusion, among the various quinoxaline derivatives synthesized and evaluated in the present study, series **5**, **6**, **7**, **12**, and **13** exhibited potent antileishmanial and antitrypanosomal activity. Methylsulfoxyl, methylsulfonyl, and amine were the main groups responsible for this activity. In summary, the present results revealed high *in vitro* antiprotozoal activity against *T. cruzi* and *L. amazonensis*, which encourages further investigations to identify potential targets and delineate putative mechanisms of action involved the antileishmanial and antitrypanosomal properties of these compounds.

Experimental section

Unless otherwise noted, all commercially available reagents were purchased from Aldrich Chemical Co. Reagents and solvents were purified when necessary according to the usual procedures described in the literature. The IR spectra refer to films and were measured on a Bomem M102 spectrometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX-400 (400 and 100 MHz, respectively). Mass spectra were recorded on a Shimadzu GCMS-QP5000. Direct-infusion Ultrahigh Resolution and Accurate Mass Spectrometry (orbitrap ESI-FT-MS) was performed with an LTQ Orbitrap Velos FT-MS instrument (Thermo Fischer Scientific, Bremen, Germany) equipped with an electrospray source (HESI-II) that operated in full-scan negative-ionization mode. The elemental analyses were performed on a Fisons EA 1108 CHNS-O. Analytical thin-layer chromatography (TLC) was performed on a 0.25 µm film of silica gel containing fluorescent indicator UV_{254} supported on an aluminum sheet (Sigma-Aldrich). Flash column chromatography was performed using silica gel (Kieselgel 60, 230-400 mesh, E. Merck). Reactions were conducted in an ultrasound bath Branson mod. 1510 or in a CEM Discovery focused microwave oven. The synthetic compounds showed purity rates above to 99% in gas chromatography.

General procedure to synthesize 2-chloro-3-methylthioquinoxalines 3a-e [48]: To a suspension of *N*,*S*-acetals **2o-p** [49] (0.208 mmol) in CH_3CN (1 mL), $POCl_3$ (0.625 mmol) was added dropwise at 0°C over a period of 15 min with constant stirring. After completion of the addition, the reaction mixture was heated at 80°C for 3-4 h and monitored by TLC. It was then cooled and neutralized with ice-cold saturated $NaHCO_3$ solution (2.5 mL), extracted with $CHCl_3$

(3 × 2 mL), washed with H_2O (2 × 2 mL) followed by brine (1 × 2 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to give quinoxalines **3a-k** and **5**, which were purified by column chromatography over silica gel using hexane:EtOAc (9:1) as eluent.

2-Chloro-6-methoxy-3-methylthioquinoxaline (3a) [48]: 54% yield. MP : 109 – 111 ºC. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.83 - 7.80 (m, 1H), 7.28 – 7.26 (m, 2H), 3.96 (s, 3H), 2.67 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 161.08, 157.10, 142.94, 134.68, 129.08, 121.18, 105.95, 99.99, 55.80, 13.76. IR (ν_{max}, KBr): 3006, 1616, 1494, 1213 cm^{-1}. MS (*m/z*) : 240 (M^+, 100), 205 (88), 190 (35), 159 (40), 63 (29).

2-Chloro-3-methylthioquinoxaline (3b) [48]: 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ :7.99 – 7.93 (m, 2H), 7.73 -7.69 (m,1H), 7.66 – 7.62 (m, 1H), 2.69 (s, 3H). IR (ν_{max}, KBr): 2925, 2850, 1527, 1267, 1118, 999, 765 cm^{-1}. MS (*m/z*): 210 (M^+, 100), 177 (47), 175 (80), 160 (52), 129 (55), 102 (60), 75 (35), 50 (30).

2-Chloro-7-methoxy-3-methylthioquinoxaline (3c) [48]: 31% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.84 (d, *J* = 9.18 Hz, 1H), 7.33 (dd, *J* = 9.18, 2.84 Hz,1H), 7.23 (d, *J* = 2.84 Hz, 1H), 3.93 (s, 3H), 2.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.59, 152.58, 145.50, 140.44, 137.45, 128.41, 122.68, 106.32, 55.79, 13.76. MS (*m/z*): 240 (M^+, 88), 207 (100), 190 (25), 63 (20).

6-Bromo-3-chloro-2-methylthioquinoxaline (3d): 30% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 (d, *J* = 2.00 Hz, 1H), 7.83 (d, *J* = 9.07, 1H), 7.77 (dd, *J* = 9.07, 2.00 Hz, 1H), 2.66 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.71, 146.23, 140.13, 139.53, 133.68, 130.53, 128.74, 122.23, 13.95. MS (*m/z*): 290 (M^+, 100), 255 (80), 100 (60), 75 (65).

2,7-Dichloro-3-methylthioquinoxaline (3e) [48]: 32% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.91 (d, *J* = 2.19 Hz, 1H), 7.88 (d, *J* = 8.98, 1H), 7.63 (dd, *J* = 8.98, 2.19 Hz, 1H), 2.66 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.50, 146.32, 139.21, 134.29, 131.07, 128.60, 127.21, 123.41, 13.95. MS (*m/z*): 244 (M^+, 100), 211 (88), 194 (60), 100 (51), 75 (39).

General procedure for iron catalyzed cross-coupling reactions: synthesis of 4a-b [48]**:** A flame-dried 100 mL two-necked flask was charged with quinoxalines **3a** or **3b** (1.0 mmol), $Fe(acac)_3$ (0.10 mmol), and THF (5 mL), and the mixture was cooled to -30°C. A solution of phenylmagnesium bromide or 4-methoxyphenylmagnesium bromide (2.2 mmol) was added via syringe to the resulting red solution, causing an immediate color change to dark brown-black. The reaction mixture was further stirred for 10-15 min and quenched with brine solution. It was then extracted with $CHCl_3$ (3 × 5 mL), washed with H_2O (2 × 10 mL) followed by brine (20 mL), and dried over anhydrous Na_2SO_4. Standard purification by column chromatography using hexane:EtOAc (9:1) as eluent provided the products **4a** and **4b**.

3-Phenyl-7-methoxy-2-methylthioquinoxaline (4a) [48]: 31% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93 (d, *J* = 8.81Hz, 1H), 7.76 – 7.74 (m, 2H), 7.51 – 7.48 (m, 3H), 7.30 – 7.24 (m, 2H), 3.98 (s, 3H), 2.63 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.86, 155.89, 150.93, 143.13, 137.57, 135.06, 130.19, 129.40, 129.02, 128.39, 120.65, 105.83, 55.75, 13.70. IR (ν_{max}, KBr): 2952, 2923, 2852, 1616, 1222, 1097, 829, 692 cm^{-1}. MS (*m/z*) : 282 (M^+, 100), 267 (32), 249 (50), 235 (18), 192 (15), 141 (18), 77 (35), 63 (30).

3-Phenyl-2-methylthioquinoxaline (4b) [56]: 36% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.06 – 8.05 (m, 1H), 7.99 - 7.97 (m, 1H), 7.79 – 7.76 (m, 2H), 7.70 – 7.66 (m, 1H), 7.64 – 7.59 (m, 1H),

7.52 – 7.51 (m, 3H), 2.64 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 155.94, 153.50, 141.68, 139.32, 137.45, 129.75, 129.70, 129.27, 129.01, 128.46, 128.10, 127.49, 13.77. IR (ν_{max}, KBr): 2920, 2852, 1328, 1272, 1128, 1089, 759, 690 cm^{-1}. MS (*m/z*): 252 (M+, 100), 237 (75), 219 (68), 205 (25), 134 (20), 102 (23), 77 (50), 51 (38).

General procedure for nickel-catalyzed cross-coupling reactions of 4a: synthesis of 5a and 5b quinoxalines [48]**:** A solution of the respective Grignard reagent (0.018 mmol) in Et_2O was added dropwise to a stirring suspension of $(PPh_3P)_2NiCl_2$ (30 mol%, 0.027 mmol) in dry benzene (3 mL) in an argon atmosphere, and the mixture was refluxed for 15 min. After the catalyst reduction, the Grignard reagent (PhMgBr or 4-$MeOC_6H_4MgBr$; 0.16 mmol) and a solution of 2-methylthio-3-phenylquinoxaline **4a** (0.089 mmol) in dry benzene (2 mL) were added to the reaction mixture and refluxed for 12 h. It was then cooled, poured into a saturated solution of NH_4Cl (5 mL), and extracted with $CHCl_3$ (3 × 50 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to give the crude products **5a** or **5b**, which were purified by column chromatography using hexane:EtOAc (19:1) as the eluent.

2,3-diphenyl-7-methoxyquinoxaline (5a) [57]: 69% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.05 (d, *J* = 9.16 Hz, 1H), 7.53 – 7.47 (m, 5H), 7.42 (dd, *J* = 9.16 , 2.85 Hz, 1H), 7.35-7.29 (m, 6H), 3.99 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.91, 152.17, 150.95, 139.24, 137.41, 130.16, 129.80, 128.65, 128.44, 128.21, 123.32, 106.52, 55.84. MS (*m/z*): 312 (M^+, 100), 297 (25), 269 (23), 209 (10), 156 (20), 134 (30), 106 (57), 63 (64).

3-phenyl-7-methoxy-2-(4-methoxyphenyl)quinoxaline (5b) [48]: 31% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.03 (d, *J* = 9.28 Hz, 1H), 7.52 – 7.33 (m, 9H), 6.86 – 6.84 (m, 1H), 3.98 (s,

3H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.87, 160.15, 152.96, 150.92, 142.85, 139.59, 137.16, 131.62, 131.32, 130.14, 129.73, 128.40, 128.30, 122.95, 113.73, 106.46, 55.83, 55.31.

General experimental procedure for the synthesis of 2,3-diarylquinoxalines 5c-5k [50]**:** A mixture of 1,2-diketone (1.0 mmol), 1,2-diamine (1.0 mmol), and absolute ethanol (4 mL) or absolute ethanol/acetic acid (4 mL/0.4 mL) was irradiated under ultrasound in an open glass at room temperature (22-25°C) until completion of the reaction. The progress of the reaction was monitored by TLC. After the reaction was completed, the mixture was concentrated under vacuum, and the residue was purified by a flash chromatography column in silica gel using EtOAc:hexane (7:3) as the eluent to provide the desired product **5**.

2,3-diphenylquinoxaline (5c) [57]: 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19 – 8.17 (m, 2H), 7.78 – 7.75 (m, 2H), 7.53 – 7.51 (m, 4H) 7.36 – 7.31 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.43, 141.20,139.06, 129.91, 129.80, 129.17, 128.75, 128.23. GC-MS (70 eV) *m/z* (%): 282 (M^+, 100), 281 (85), 179 (46), 76 (36).

2-(methylphenyl)-3-phenylquinoxaline (5d) [57]: 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.18 – 8.15 (m, 2H), 7.76 – 7.74 (m, 2H), 7.55 – 7.52 (m, 2H) 7.42 (d, 2H, *J* = 8.21 Hz), 7.38 – 7.34 (m, 3H), 7.13 (d, 2H, *J* = 8.21 Hz), 2.36 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.46, 141.29, 141.09, 139.31, 138.82, 136.18, 129.82, 129.79, 129.71, 129.16, 128.95, 128.71, 128.24, 21.28. GC-MS (70 eV) *m/z* (%): 296 (M^+, 100), 295 (68), 147 (28), 76(24).

6-chloro-2-phenyl-3-(p-tolyl)quinoxaline and 6-chloro-3-phenyl-2-(p-tolyl)quinoxaline (5e): 87% yield.^{1}H NMR (400 MHz, CDCl3) δ: 8.15 (d, 1H, *J* = 2.32 Hz), 8.09 (d, 1H, *J* = 8.98 Hz),

7.71 – 7.67 (m, 1H), 7.54 – 7.49 (m, 2H), 7.42 – 7.32 (m, 5H), 7.14 (d, *J* = 8.26 Hz, 2H), 2.36 (s, 3H). IR (KBr) ν_{max}:3043, 2918, 1597, 1466, 1340, 1066 cm^{-1} . GC-MS (70 eV) *m/z* (%): 330 (M^+, 100), 329 (65), 315 (40), 165 (27), 75 (23). Anal. Calcd. For $C_{21}H_{15}N_2Cl$: C 76.24%, H 4.57%, N 8.47%, Found: C 76.49%, H 4.61%, N 8.47%.

2-(4-methoxyphenyl)-3-phenylquinoxaline (5f) [58]: 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.16 – 8.14 (m, 2H), 7.76 – 7.72 (m, 2H), 7.55 – 7.53 (m, 2H), 7.48 (d, 2H, *J* = 8.96 Hz), 7.38 – 7.34 (m, 2H), 6.85 (d, 2H, *J* = 8.96 Hz), 3.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.20, 153.39, 153.00, 141.29, 140.97,139.42, 131.33, 129.80, 129.71, 129.55, 129.13, 129.04, 128.69, 128.29, 113.70, 55.26. GC-MS (70 eV) *m/z* (%): 312 (M^+, 100), 311 (57), 297 (35), 179 (23).

6-chloro-2-(methoxyphenyl)-3-phenylquinoxaline and 6-chloro-3-(methoxyphenyl)-2-phenylquinoxaline (5g): 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.16 – 8.13 (m, 1H), 8.07 (d, 2H, *J* = 8.90 Hz), 7.69 – 7.66 (m, 1H), 7.54 – 7.51 (m, 2H), 7.48 – 7.45 (m, 2H), 7.39 – 7.33 (m, 3H), 6.85 (d, 2H, *J* = 8.90 Hz), 3.82 (s, 3H). IR (dichloromethane) ν_{max}: 3061, 2933, 1608, 1514, 1466, 1342, 1252, 1175 cm^{-1}. GC-MS (70 eV) *m/z* (%): 346 (M^+, 100), 345 (49), 331 (27), 178 (24), 75 (25). Anal. Calcd. For $C_{21}H_{15}N_2OCl$: C 72.73%, H 4.36%, N 8.08%, found: C 72.75%, H 4.61%, N 7.72%.

6-chloro-2,3-diphenylquinoxaline (5h) [59]: 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.15 (d, 1H, *J* = 2.22 Hz), 8.08 (d, 1H, *J* = 8.94 Hz), 7.67 (dd, 1H, *J* = 8.94, 2.22 Hz), 7.51 – 7.49 (m, 4H), 7.37 – 7.29 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.17, 153.51, 141.40, 139.63, 138.66, 138.59, 135.55, 130.84, 130.34, 129.96, 129.77, 129.74, 129.01, 128.93, 128.21, 127.99. GC-MS (70 eV) *m/z* (%): 316 (M^+, 100), 315 (80), 178 (40), 75 (35).

6,7-dichloro-2,3-diphenylquinoxaline (5i) [60]: 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.28 (s, 2H), 7.51 – 7.49 (m, 4H), 7.41 – 7.32 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.50, 139.95, 138.39, 134.43, 129.80, 129.29, 128.37. GC-MS (70 eV) *m/z* (%): 350 (M^+, 100), 349 (80), 315 (10), 247 (20), 212 (30), 177 (57), 109 (34).

6,7-dichloro-2-(4-methylphenyl)-3-phenylquinoxaline (5j) [61]: 96% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.23 (s, 2H), 7.52 – 7.48 (m, 2H), 7.41 – 7.32 (m, 5H), 7.14 – 7.10 (m, 2H), 2.35 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.49, 140.02, 139.82, 139.48, 138.65, 135.53, 134.28, 134.15, 130.06, 129.93, 129.81, 129.24, 129.09, 128.37, 21.40. IR (dichloromethane) ν_{max}: 3055, 2920, 1609, 1450, 1439, 1337, 1107, 879 cm^{-1}. GC-MS (70 eV) *m/z* (%): 364 (M^+, 100), 363 (60), 349 (45), 177 (25), 109 (26). Anal. Calcd. for $C_{21}H_{14}N_2Cl_2$: C 69.06%, H 3.86%, N 7.67%, Found: C 69.28%, H 3.97%, N 7.16%.

2,3-di-(4-methoxyphenyl)-quinoxaline (5k) [58]: 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12 (dd, 2H, *J* = 6.29, 23.51 Hz), 7.72 (dd, 2H, *J* = 6.29, 3.51 Hz), 7.49 (d, 4H, *J* = 8.89 Hz), 6.87 (d, 4H, *J* = 8.89 Hz), 3.83 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 160.15, 153.01, 141.04, 131.67, 131.22, 129.51, 128.96, 113.75, 55.29. GC-MS (70 eV) *m/z* (%): 342 (M^+, 100), 341 (32), 311 (34), 209 (12), 166 (48), 133 (54), 103 (45).

Experimental procedure for 2,3-diarylquinoxalines 5fa and 5fb: To a solution of quinoxaline **5f** (1.50 mmol) in anhydrous CH_2Cl_2 (20 mL) was added a 1.0 M solution of BBr_3 in CH_2Cl_2 (6.0 mL or 12.0 mL to produce **5ka**) at 0°C over 10 min under an argon atmosphere, and the mixture was stirred for 15 min at the same temperature. The mixture was allowed to warm to room temperature, and stirring continued for 21 h. To this was added an aqueous saturated solution of

$NaHCO_3$ (100 mL), and the mixture was concentrated under reduced pressure to remove CH_2Cl_2. To a solution of the crude product in DMF (20 mL), 1-(2-chloroethyl)-piperidine monohydrochloride (1.75 g, 1.65 mmol), potassium carbonate (3.3 mmol), and KI (0.075 mmol) were added, and the resulting mixture was stirred at 25°C for 20 h. The solution was then heated for 45 min at 50°C, after which it was cooled, and the solvent was removed under reduced pressure. The residue was resuspended in CH_2Cl_2 (30 mL). The organic layers were washed with H_2O (30 mL) and dried over anhydrous $MgSO_4$, and the solvent was removed under reduced pressure. The products were purified by a chromatography column in silica gel using dichloromethane:methanol (8:2) as eluent.

2-[4-(2-piperidine)ethoxyphenyl]-3-phenylquinoxaline (5fa): 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.16 – 8.13 (m, 2H), 7.76 – 7.72 (m, 2H), 7.55 – 7.52 (m, 2H), 7.46 (d, 2H, *J* = 8.90 Hz), 7.37 – 7.34 (m, 3H), 6.85 (d, 2H, *J* = 8.90 Hz), 4.13 (t, 2H, J 6.05 Hz), 2.78 (t, 2H, *J* = 6.05 Hz), 2.55 – 2.49 (m, 4H), 1.62 (quint, 4H, *J* = 6.05 Hz), 1.49 -1.41 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.46, 153.43, 153.04,141.31, 141.00, 139.42, 131.46, 131.35, 129.85, 129.75, 129.59, 129.16, 129.06, 128.74, 128.33, 114.41, 65.99, 57.83, 55.08, 25.85, 24.13. IR (dichloromethane) ν_{max}: 3057, 2932, 1605, 1512, 1466, 1344, 1250 cm^{-1}. GC-MS (70 eV) *m/z* (%): 409 (M^+, 1), 98 (100), 96 (5), 70 (4). Anal. Calcd. for $C_{27}H_{27}N_3O$: C 79.19%, H 6.65%, N 10.26%, Found: C 78.96%, H 6.91%, N 10.00%.

2-[4-(2-morpholine)ethoxyphenyl]-3-phenylquinoxaline (5fb): 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.17 – 8.13 (m, 2H), 7.76 – 7.69 (m, 2H), 7.55 – 7.52 (m, 2H), 7.46 (d, 2H, *J* =8.75 Hz), 7.39 – 7.32 (m, 3H), 6.85 (d, 2H, *J* = 8.75 Hz), 4.11 (t, 2H, *J* = 5.65 Hz), 3.73 (t, 4H, *J* = 4.76 Hz), 2.79 (t, 2H, *J* =5.65 Hz), 2.57 (t, 4H, *J* = 4.76 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.21, 153.24, 152.80, 141.15, 140.85, 139.27, 131.45, 131.24, 129.74, 129.62, 129.50, 129.01,

128.90, 128.60, 128.18, 114.24, 67.77, 65.72, 57.44, 53.98. IR (dichloromethane) ν_{max}: 3059, 2922, 1637, 1607, 1250, 1117 cm^{-1}. GC-MS (70 eV) *m/z* (%): 411 (M^+, 1), 100 (100), 70 (5), 56 (10). Anal. Calcd. for $C_{26}H_{25}N_3O_2$: C 75.89%, H 6.12%, N 10.21%, Found : C 75.22%, H 5.91%, N 10.08%.

4-(3-(4-(2-(piperidin-1-yl)ethoxy)phenyl)quinoxalin-2-yl)phenol (5ka): 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 – 8.07 (m, 2H), 7.70 – 7.68 (m, 2H), 7.51 – 7.43 (m, 2H), 7.32 (d, *J* = 8.54 Hz, 2H), 6.89 – 6.79 (m, 4H), 4.46 – 4.44 (m, 2H), 3.35 – 3.32 (m, 2H), 3.20 – 3.10 (m, 4H), 2.05 – 1.95 (m, 2H), 1.70 – 1.62 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.94, 157.46, 153.24, 152.76, 140.94, 139.27, 131.46, 131.36, 129.80, 129.65, 128.91, 128.80, 115.66, 114.34, 63.00, 56.49, 54.23, 23.16, 22.09. MS (eletrospray / ion trap) *m/z*: 426.3.

General procedure for oxidation with mCPBA: A solution of *m*CPBA (0.11 mmol) in CH_2Cl_2 (1 mL) was added dropwise to a stirred solution of the quinoxaline (0.11 mmol) in CH_2Cl_2 (1 mL) at 0°C over a period of 30 min. The reaction mixture was further stirred at room temperature for 1-2 h and monitored by TLC. It was then poured into ice-cold H_2O, washed with 10% $NaHCO_3$ solution (2 × 2 mL) and H_2O (2 mL) followed by brine (2 mL), and dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to give crude products that were purified over silica gel using hexane:EtOAc (2:1) as the eluent to provide the desired product.

2-chloro-6-methoxy-3-(methylsulfinyl)quinoxaline (6a): 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 9.07 Hz, 1H), 7.66 (d, *J* = 2.71 Hz, 1H), 7.55 (dd, *J* = 9.07, 2.71 Hz, 1H), 3.99 (s,3H), 3.03 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ : 161.93, 156.71, 143.42, 139.86, 139.11, 129.20, 126.36, 107.08, 56.18, 39.71.

2,7-dichloro-3-(methylsulfinyl)quinoxaline (6b): 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.31 (d, *J* = 9.15 Hz, 1H), 7.66 (d, *J* = 2.24 Hz, 1H), 7.55 (dd, *J* = 9.15, 2.24 Hz, 1H), 3.06 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 157.28, 143.16, 139.98, 139.18, 133.24, 132.69, 130.86, 127.43, 39.71.

2-chloro-6-methoxy-3-methylsulfonylquinoxaline (7a) [48]: 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.98 (d, *J* = 9.26 Hz, 1H), 7.59 (dd, *J* = 9.26, 2.85 Hz, 1H), 7.40 (d, *J* = 2.85 Hz, 1H), 4.01 (s,3H), 3.53 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 162.19, 150.00, 140.33, 139.07, 138.67, 129.23, 127.39, 106.68, 56.17, 40.27. MS (*m/z*): 272 (M^+, 63), 210 (68), 193 (90), 158 (100), 117 (50), 77 (36).

2-chloro-3-methylsulfonylquinoxaline (7b) [62]: 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.19 – 8.13 (m, 2H), 8.01 – 7.91 (m, 2H), 3.57 (s, 3H). ^{13}C NMR (400 MHz, CDCl3) δ : 150.16, 142.75, 141.40, 138.25, 133.86, 131.87, 129.61, 128.51, 40.16. MS (*m/z*): 242(M^+, 20), 180 (45), 163 (58),102 (100), 75 (40), 51 (22).

3-chloro-6-methoxy-2-(methylsulfonyl)quinoxaline (7c) [48]: 99% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 8.02 (d, *J* = 9.27 Hz, 1H), 7.53 (dd, *J* = 9.27, 2.78 Hz,1H), 7.37 (d, *J* = 2.78 Hz, 1H), 4.02 (s, 3H), 3.53 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 164.06, 147.25, 145.09, 142.00, 130.58, 130.26, 125.53, 105.85, 56.30, 40.35. MS (*m/z*): 272 (M^+, 70), 193 (85), 181 (100), 117 (88), 77 (75).

6-bromo-3-chloro-2-(methylsulfonyl)quinoxaline (7d): 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.31 (d, *J* =1.95 Hz, 1H), 8.03 (d, *J* = 8.90 Hz, 1H), 8.00 (dd, *J* = 8.90, 1.95 Hz, 1H), 3.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 150.42, 143.07, 142.62, 137.00, 135.65, 130.91, 130.62, 128.75, 40.17.

3,6-dichloro2-(methylsulfonyl)quinoxaline (7e) [62]: 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12 – 8.08 (m, 2H), 7.86 (dd, *J* = 9.10, 2.27 Hz, 1H), 3.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 150.30, 142.97, 133.81, 133.08, 130.67, 130.25, 128.32, 127.52, 40.19. MS (*m/z*): 276 (M^+, 30), 214 (70), 197 (78), 136 (100), 100 (84).

2-phenyl-3-methylsulfonyl-6-methoxyquinoxaline (8a) [48]: 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.10 (d, *J* = 9.14 Hz, 1H), 7.87 – 7.85 (m, 2H), 7.58 (d, *J* = 9.14, 2.83 Hz, 1H), 7.54 – 7.53 (m, 3H), 7.41 (d, *J* = 2.83 Hz, 1H), 4.02 (s, 3H), 3.43 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.95, 152.13, 148.79, 140.41, 139.08, 136.51, 130.31, 130.21, 129.83, 129.73, 126.74, 106.19, 56.12, 40.72. MS (*m/z*): 314 (M^+, 28), 235 (100), 192 (19), 77 (30).

2-dimethylamino-3-methylsulfonyl-6-methoxyquinoxaline (12a): 15% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, *J* = 9.23 Hz, 1H), 7.38 (dd, *J* = 9.23, 2.91 Hz, 1H), 7.19 (d, *J* = 2.91 Hz, 1H), 3.93 (s, 3H), 3.42 (s, 3H), 3.26 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 158.26, 149.71, 144.21, 138.37, 138.28, 127.55, 125.77, 106.33, 55.76, 42.01, 41.95. MS (*m/z*): 281 (M^+, 72), 252 (28), 202 (70), 159 (100), 219 (20), 117 (47).

2-butylamino-3-methylsulfonyl-6-methoxyquinoxaline (12b): 63% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.63 (d, *J* = 9.26 Hz, 1H), 7.36 (dd, *J* = 9.26, 2.90 Hz, 1H), 7.20 (d, *J* = 2.90 Hz, 1H), 3.90 (s, 3H), 3.58 – 3.53 (m, 2H), 3.38 (s, 3H), 1.72 - 1.65 (m, 2H), 1.47 (sext, *J* = 7.35 Hz, 2H), 0.98 (t, *J* = 7.35 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.44, 147.42, 140.22, 140.10, 135.55, 127.24, 125.96, 107.09, 55.70, 40.81, 40.66, 31.14, 20.26, 13.85. MS (*m/z*): 309 (M^+, 42), 266 (100), 230 (62), 159 (90), 147 (30).

2-(2-hydroxyethanolamina)-3-methylsulfonyl-6-methoxyquinoxaline (12c): 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61 - 7.59 (m, 1H), 7.39 – 7.36 (m, 1H), 7.12 - 7.08 (m, 1H), 3.95 –

3.88 (m, 5H), 3.80 – 3.75 (m, 2H), 3.41 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.81, 140.68, 138.98, 135.95, 133.34, 126.82, 126.32, 107.09, 62.78, 55.70, 44.35, 40.59.

2-butylamino-3-methylsulfonylquinoxaline (12d): 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.87 - 7.84 (m,1H), 7.73 – 7.65 (m, 2H), 7.43 - 7.39 (m, 1H), 3.62 - 3.57 (m, 2H), 3.42 (s, 3H), 1.74 - 1.66 (m, 2H), 1.47 (sext, *J* = 7.68 Hz, 2H), 0.98 (t, *J* = 7.68 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.08, 144.06, 141.07, 134.62, 132.89, 129.50, 126.32, 125.31, 40.82, 40.48, 31.02, 20.24, 13.85. MS (*m/z*): 279 (M^+, 30), 236 (100), 200 (75), 129 (95), 102 (48).

2-(2-hydroxyethanolamino)-3-methylsulfonylquinoxaline (12e): 91% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 – 7.86 (m, 1H), 7.71 – 7.69 (m, 2H), 7.48 - 7.44 (m, 1H), 3.94 - 3.92 (m, 2H), 3.83 – 3.79 (m, 2H), 3.45 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 143.06, 141.51, 139.94, 133.29, 130.12, 129.53, 125.97, 125.93, 62.72, 44.41, 40.46. MS (*m/z*): 267 (M^+, 35), 236 (100), 129 (100), 102 (47).

2-cycloexylamino-3-methylsulfonyl-6-methoxyquinoxaline (12f): 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.61 (d, *J* = 9.37 Hz, 1H), 7.35 (dd, *J* = 9.37, 2.86 Hz, 1H), 7.18 (d, *J* = 2.86 Hz, 1H), 4.14 – 4.08 (m, 1H), 3.89 (s, 3H), 3.38 (s, 3H), 2.10 – 2.05 (m, 2H), 1.80 - 1.62 (m, 4H), 1.50 – 1.32 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.34, 146.68, 140.16, 140.04, 135.46, 127.21, 125.93, 107.03, 106.85, 55.70, 49.11, 40.72, 32.50, 25.80, 24.66. MS (*m/z*): 335 (M^+, 50), 278 (68), 253 (100), 174 (32), 55 (20).

2-butylamino-3-methylsulfonyl-7-methoxyquinoxaline (12g): 71% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.73 (d, *J* = 9.03 Hz, 1H), 7.05 (dd, *J* = 9.03, 2.86 Hz, 1H), 7.02 (d, *J* = 2.86 Hz, 1H), 3.95 (s, 3H), 3.60 – 3.56 (m, 2H), 3.38 (s, 3H), 1.73 - 1.68 (m, 2H), 1.48 (sext, *J* = 7.85 Hz, 2H), 1.00 (t, *J* = 7.85 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.58, 148.61, 146.20, 137.67,

130.61, 130.41, 118.52, 104.38, 55.83, 40.79, 40.77, 31.08, 20.26, 13.87. MS (*m/z*): 309 (M^+, 32), 266 (99), 230 (80), 159 (100), 147 (28), 77 (37).

2-(2-hydroxyethanolamino)-3-methylsulfonyl-7-methoxyquinoxaline (12h): 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.74 (d, *J* = 9.02 Hz, 1H), 7.08 (dd, *J* = 9.02, 2.61 Hz, 1H), 6.97 (d, *J* = 2.61 Hz, 1H), 3.94 – 3.90 (m, 5H), 3.80 – 3.76 (m, 2H), 3.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.91, 149.03, 145.29, 137.91, 130.80, 130.67, 119.16, 104.04, 63.90, 55.94, 44.42, 40.78.

2-cycloexylamino-3-methylsulfonyl-7-methoxyquinoxaline (12i): 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 9.08 Hz, 1H), 7.03 (dd, *J* = 9.08, 2.71 Hz, 1H), 6.99 (d, *J* = 2.71 Hz, 1H), 4.18 – 4.11 (m, 1H), 3.95 (s, 3H), 3.36 (s, 3H), 2.10 – 2.05 (m, 2H), 1.80 - 1.75 (m, 2H), 1.67 - 1.64 (m, 2H), 1.52 – 1.28 (m, 4H). ^{13}C NMR (100 MHz, CDCl3) δ: 163.55, 147.89, 146.31, 137.51, 130.59, 122.99, 118.44, 104.34, 55.81, 49.09, 40.86, 32.45, 25.77, 24.65. MS (*m/z*): 335 (M^+, 37), 278 (62), 253 (100), 174 (38), 162 (40).

2-isobutylamino-3-methylsulfonyl-7-methoxyquinoxaline (12j): 62% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.72 (d, *J* = 9.08 Hz, 1H), 7.04 (dd, *J* = 9.08, 2.72 Hz, 1H), 7.00 (d, *J* = 2.76 Hz, 1H), 3.94 (s, 3H), 3.43 – 3.40 (m, 2H), 3.37 (s, 3H), 2.07 – 1.97 (m, 1H), 1.04 (d, *J* = 6.59 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.61, 148.78, 146.20, 137.64, 130.62, 130.46, 118.55, 104.37, 55.84, 48.43, 40.81, 27.94, 20.40. MS (*m/z*): 209 (M^+, 18), 266 (100), 253 (60), 159 (68).

2-isopentylamino-3-methylsulfonyl-7-methoxyquinoxaline (12k): 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.72 (d, *J* = 8.89 Hz, 1H), 7.04 (dd, *J* = 8.89, 2.65 Hz, 1H), 7.01 (d, *J* = 2.65 Hz, 1H), 3.95 (s, 3H), 3.61 – 3.56 (m, 2H), 3.37 (s, 3H), 1.81 – 1.71 (m, 1H), 1.63 – 1.58 (m,

2H), 0.98 (d, *J* = 6.81 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 163.61, 148.61, 146.25, 137.64, 130.64, 130.43, 118.55, 104.40, 55.84, 40.80, 39.32, 37.93, 26.00, 22.57. MS (*m/z*): 323 (M^+, 15), 267 (100), 159 (62), 77 (12).

7-bromo-2-butylamino-3-methylsulfonylquinoxaline (12l): 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 2.04 Hz, 1H), 7.69 (d, *J* = 9.02 Hz, 1H), 7.48 (dd, *J* = 9.02, 2.04 Hz, 1H), 3.59 – 3.54 (m, 2H), 3.41 (s, 3H), 1.72 - 1.65 (m, 2H), 1.46 (sext, *J* = 7.56 Hz, 2H), 0.98 (t, *J* = 7.56 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.34, 144.68, 141.32, 133.26, 130.55, 128.87, 128.77, 127.51, 40.90, 40.47, 30.91, 20.22, 13.83. MS (*m/z*): 359 (M^{+2}, 20), 357 (M^+, 18), 316 (100), 314 (97), 280 (80), 278 (84), 209 (80), 207 (78).

7-bromo-2-(2-hydroxyethanolamino)-3-methylsulfonylquinoxaline (12m): 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.88 (d, *J* = 2.04 Hz, 1H), 7.72 (d, *J* = 8.56 Hz, 1H), 7.52 (dd, *J* = 8.56, 2.04 Hz, 1H), 3.93 – 3.91 (m, 2H), 3.81 - 3.77 (m, 2H), 3.43 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 143.84, 133.54, 130.58, 130.16, 129.78, 129.46, 128.51, 127.91, 62.14, 44.11, 40.45.

2-isobutylamino-3-methylsulfonyl-6-methoxyquinoxaline (12n): 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.62 (d, *J* = 9.41 Hz, 1H), 7.36 (dd, *J* = 9.41, 2.84 Hz, 1H), 7.20 (d, *J* = 2.84 Hz, 1H), 3.90 (s, 3H), 3.42 – 3.38 (m, 5H), 2.06 – 1.96 (m, 1H), 1.03 (d, *J* = 6.65 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.44, 147.56, 140.19, 129.84, 127.23, 126.01, 111.47, 107.07, 55.72, 48.51, 40.69, 27.94, 20.40. MS (*m/z*): 309 (M^+, 23), 266 (100), 253 (43), 176 (22), 159 (65).

7-chloro-2-butylamino-3-methylsulfonylquinoxaline (12o): 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.77 (d, *J* = 8.96 Hz, 1H), 7.71 (d, *J* = 2.24 Hz, 1H), 7.35 (dd, *J* = 8.96, 2.24 Hz, 1H),

3.60 – 3.55 (m, 2H), 3.41 (s, 3H), 1.73 - 1.65 (m, 2H), 1.46 (sext, *J* = 7.85 Hz, 2H), 0.98 (t, J - 7.85 Hz, 3H). MS (*m/z*): 313 (M^+, 23), 270 (100), 234 (78), 163 (82), 136 (31).

2-cycloexylamino-3-methylsulfonyl-7-chloroquinoxaline (12p): 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.76 (d, *J* =8.79 Hz, 1H), 7.70 (d, *J* =1.96 Hz, 1H), 7.33 (dd, *J* = 8.79,1.96 Hz, 1H), 4.15 – 4.11 (m, 1H), 3.40 (s, 3H), 2.09 – 2.05 (m, 2H), 1.80 - 1.75 (m, 2H), 1.49 - 1.25 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 147.64, 141.08, 138.93, 135.82, 132.97, 130.49, 126.15, 125.37, 49.39, 40.54, 32.25, 25.71, 24.59. MS (*m/z*): 339 (M^+, 65), 282 (98), 257 (100), 178 (55), 55 (53).

2-cycloexyl-3-methylsulfinyl-6-methoxyquinoxaline (13a): 54% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.59 (d, *J* = 8.92 Hz, 1H), 7.28 (dd, *J* = 8.92, 2.98 Hz, 1H), 7.12 (d, *J* = 2.98 Hz, 1H), 4.13 - 4.09 (m, 1H), 3.88 (s, 3H), 3.01 (s, 3H), 2.10 - 2.05 (m, 2H), 1.78 – 1.74 (m, 2H), 1.48 - 1.24 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.90, 149.64, 145.81, 138.24, 136.07, 127.12, 123.87, 107.08, 55.64, 48.61, 39.09, 32.72, 32.34, 25.88, 24.70. MS (*m/z*): 319 (M^+, 23), 302 (100), 147 (23), 55 (30).

2-cycloexyl-7-chloro-3-methylsulfinylquinoxaline (13b): 87% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 – 7.94 (m, 1H), 7.66 – 7.64 (m, 1H), 7.29 – 7.25 (m, 1H), 4.16 - 4.07 (m, 1H), 3.02 (s, 3H), 2.08 - 2.05 (m, 2H), 1.78 – 1.74 (m, 2H), 1.52 - 1.26 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 150.77, 146.59, 143.19, 137.31, 133.85, 129.84, 125.31, 125.25, 48.81, 39.45, 32.43, 32.12, 25.77, 24.61. MS (*m/z*): 323 (M^+, 12), 306 (100), 178 (38), 55 (65).

General procedure to synthesize 2-chloro-3-amino-quinoxalines 9a and 9b [48]: A mixture of quinoxaline **7a** (1.00 mmol) and *N*-butyl amine or benzyl amine (2.00 mmol) in DMF (2 mL) was

stirred at 25°C for 6 h and monitored by TLC. The reaction mixture was then diluted with $CHCl_3$ (20 mL) and washed with H_2O (2 × 20 mL) followed by brine (20 mL), and the organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum to give the crude products **9a** and **9b**, which were purified by column chromatography using hexane:EtOAc (1:1) as the eluent.

2-chloro-7-methoxy-3-butylaminoquinoxaline (9a) [48]: 75% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 8.99 Hz, 1H), 7.07 (d, *J* = 2.72 Hz, 1H), 7.01 (dd, *J* = 8.99, 2.72 Hz, 1H), 3.91 (s, 3H), 3.60 - 3.55 (m, 2H), 1.74 – 1.67 (m, 2H), 1.48 (sext, *J* = 7.56 Hz , 2H), 1.00 (t, *J* = 7.56 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.14, 148.43, 143.05, 134.97, 131.63, 128.79, 116.69, 105.05, 55.65, 41.26, 31.30, 20.23, 13.88. MS (*m/z*): 265 (M+, 28), 230 (50), 222 (51), 209 (72), 159 (100), 77 (17).

2-chloro-7-methoxy-3-benzylaminoquinoxaline (9b) [48]: 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 (d, *J* = 9.07 Hz, 1H), 7.43 - 7,30 (m, 5H), 7.08 (d, *J* = 2.69 Hz, 1H), 7,.04 (dd, *J* = 9.07, *J* = 2.69 Hz, 1H), 4.79 (d, *J* = 5.56 Hz, 2H), 3.91 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.23, 148.15, 134.77, 132.00, 129.01, 128.82, 128.50, 128.29, 127.96, 127.71, 117.11, 105.16, 55.68, 45.55. MS (m/z) :299 (M+, 37),106 (100), 91 (63), 65 (20).

Synthesis of 2,3-diaminoquinoxalines 10a-b: A mixture of quinoxalines **9a** or **9b** (1 mmol) and *N*-butylamine (1 mL) was heated at 100°C for 20 h in a pressure tube with constant stirring and monitored by TLC. The *N*-butylamine was evaporated under vacuum to give the products **10a** and **10b**, which were isolated by column chromatography over silica gel using hexane:EtOAc (2:1).

2,3-dibutylamino-6-methoxyquinoxaline (10a): 98% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.57 (d, *J* = 9.22 Hz, 1H), 7.11 (d, *J* = 2.94 Hz, 1H), 6.96 (dd, *J* = 9.22, 2.94 Hz, 1H), 3.88 (s, 3H), 3.55 - 3.48 (m, 4H), 1.67 – 1.59 (m, 4H), 1.48 – 1.38 (m, 4H), 0.97 – 0.93 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 157.11, 145.02, 143.32, 137.99, 131.67, 126.32, 114.75, 106.23, 55.66, 41.64, 41.51, 31.50, 31.44, 20.39, 13.91. MS (m/z) :302 (M^+, 80),259 (78), 246 (38), 229 (53),203 (100), 147 (22).

3-benzilamino-2-butylamino-6-methoxyquinoxaline (10b) [48]: 82% yield. MS (*m/z*): 336 (M+, 80), 280 (35), 245 (72), 202 (38), 91 (100).

General procedure for the synthesis of 2-amino-quinoxalines 10c, 11a-p and 14a-c: A mixture of 2-chloro-quinoxalines (1 mmol) and the appropriate amines (1 mL) was placed in sealed glass and irradiated for 30 min in a microwave oven at 130°C. The 2-aminoquinoxalines were purified by flash chromatography using hexane:EtOAc (2:1).as eluent.

2,3-dianilino-6-methoxyquinoxaline (10c) [63]: 93% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.90 – 6.45 (m, 13H), 3.81(s, 3H). MS (*m/z*): 342 (M^+, 80), 341 (100), 298 (12), 224 (20), 77 (31).

2-dimethylamino-3-methylthio-6-methoxyquinoxaline (11a): 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.68 (d, *J* = 8.93 Hz, 1H), 7.20 (d, *J* = 2.82 Hz, 1H), 7.15 (dd, *J* = 8.93, 2.82 Hz, 1H), 3.90 (s, 3H), 3.00 (s, 6H), 2.63 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 158.46, 153.45, 152.27, 140.14, 133.71, 127.98, 119.42, 106.25, 55.59, 41.58, 13.35. MS (*m/z*): 249 (M^+, 39), 234 (100), 219 (20), 117 (21).

2-butylamino-3-methylthio-6-methoxyquinoxaline (11b): 67% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.59 (d, *J* = 8.87 Hz, 1H), 7.19 (d, *J* = 2.95 Hz, 1H), 7.12 (dd, *J* = 8.87, 2.95 Hz, 1H),

3.89 (s, 3H), 3.57 – 3.53 (m, 2H), 2.72 (s, 3H), 1.71 - 1.64 (m, 2H), 1.46 (sext, *J* = 7.79 Hz, 2H), 0.98 (t, *J* = 7.79 Hz, 3H). ^{13}C NMR (100 MHz, CDCl3) δ: 156.71, 148.11, 146.73, 137.97, 134.74, 126.87, 119.12, 106.92, 55.59, 41.24, 31.50, 20.29, 13.91, 12.70. MS (*m/z*): 277 (M^+, 100), 262 (38), 234 (70), 221 (95), 188 (48).

2-(2-hydroxyethanolamino)-3-methylthio-6-methoxyiquinoxaline (11c): 44% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.54 (d, *J* = 8.87 Hz, 1H), 7.19 – 7.11 (m, 2H), 3.91 – 3.85 (m, 5H), 3.75 – 3.70 (m, 2H), 2.72 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 157.11, 148.32, 146.99, 138.25, 133.37, 126.34, 119.44, 106.90, 63.56, 55.59, 45.27, 12.79. MS (*m/z*): 265 (M^+, 80), 247 (38), 234 (95), 221 (100), 159 (49).

2-butylamino-3-methylthioquinoxaline (11d): 85% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.77 (dd, *J* = 8.36, 1.49 Hz, 1H), 7.68 (dd, *J* = 8.36, 1.49 Hz, 1H), 7.47 - 7.43 (m, 1H), 7.34 – 7.30 (m, 1H), 3.62 - 3.57 (m, 2H), 2.73 (s, 3H), 1.73 - 1.65 (m, 2H), 1.47 (sext, *J* = 7.71 Hz, 2H), 0.99 (t, *J* = 7.71 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ : 146.71, 139.71, 137.29, 128.99, 127.96, 127.09, 125.97, 124.13, 41.20, 31.42, 20.27, 13.93, 12.73. MS (*m/z*): 247 (M^+, 50), 232 (51), 200 (68), 191 (100), 129 (45).

2-(2-hydroxyethanolamino)-3-methylthioquinoxaline (11e) [64]: 49% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (dd, *J* = 8.12, 1.43 Hz, 1H), 7.64 (dd, *J* = 8.12, 1.43 Hz, 1H), 7.49 - 7.45 (m, 1H), 7.39 – 7.34 (m, 1H), 3.92 - 3.90 (m, 2H), 3.79 – 3.76 (m, 2H), 2.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 138.39, 137.51, 128.29, 127.92, 127.47, 127.14, 125.49, 124.77, 63.57, 45.30, 12.85. MS (*m/z*): 235 (M^+, 32),204 (65), 191 (100), 129 (38).

2-cycloexylamino-3-methylthio-6-methoxyquinoxaline (11f): 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 (d, *J* = 8.91 Hz, 1H), 7.18 (d, *J* = 2.93 Hz, 1H), 7.12 (dd, *J* = 8.91, 2.93 Hz, 1H),

4.13 – 4.06 (m, 1H), 3.90 (s, 3H), 2.72 (s, 3H), 2.15 – 2.09 (m, 2H), 1.80 - 1.73 (m, 2H), 1.79 – 1.62 (m, 3H), 1.53 - 1.43 (m, 2H), 1.33 – 1.24 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 156.61, 147.31, 146.73, 137.84, 134.77, 126.85, 119.09, 106.85, 55.59, 49.46, 33.05, 25.89, 24.88, 12.75. MS (*m/z*): 303 (M^+, 48), 288 (20), 221 (100), 188 (43), 55 (14).

2-butylamino-3-methylthio-7-methoxyquinoxaline (11g): 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 8.88 Hz, 1H), 7.06 (d, *J* = 2.74 Hz, 1H), 6.97 (dd, *J* = 8.88, 2.74 Hz, 1H), 3.90 (s, 3H), 3.60 – 3.56 (m, 2H), 2.71 (s, 3H), 1.72 - 1.67 (m, 2H), 1.47 (sext, *J* = 7.69 Hz, 2H), 0.99 (t, *J* = 7.69 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.63, 149.46, 143.37, 141.08, 132.82, 128.09, 115.43, 105.50, 55.59, 41.20, 31.43, 20.30, 13.94, 12.83. MS (*m/z*): 277 (M^+, 85), 230 (84), 221 (100), 188 (90).

2-(2-hydroxyethanolamino)-3-methylthio-7-methoxyquinoxaline (11h): 80% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67 (d, *J* = 9.72 Hz, 1H), 7.01 – 6.98 (m, 2H), 3.92 – 3.87 (m, 5H), 3.77 – 3.73 (m, 2H), 2.71 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.82, 149.81, 143.54, 139.81, 133.09, 128.14, 116.11, 105.00, 63.61, 55.63, 45.30, 12.93.

2-cycloexylamino-3-methylthio-7-methoxyquinoxaline (11i): 94% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.65 (d, *J* = 8.75 Hz, 1H), 7.05 (d, *J* = 2.40 Hz, 1H), 6.96 (dd, *J* = 8.75, 2.40 Hz, 1H), 4.15 – 4.12 (m, 1H), 3.90 (s, 3H), 2.70 (s, 3H), 2.15 – 2.10 (m, 2H), 1.80 - 1.64 (m, 3H), 1.53 - 1.43 (m, 2H), 1.34 – 1.26 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.58, 148.64, 143.37, 141.14, 132.73, 128.05, 115.30, 105.48, 55.56, 49.42, 32.99, 25.86, 24.88, 12.86. MS (*m/z*): 303 (M^+, 35), 288 (22), 221 (100), 188 (56), 55 (18).

2-isobutylamino-3-methylthio-7-methoxyquinoxaline (11j): 89% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 9.05 Hz, 1H), 7.06 (d, *J* = 2.76 Hz, 1H), 6.96 (dd, *J* = 9.05, 2.76 Hz, 1H),

3.90 (s, 3H), 3.43 – 3.40 (m, 2H), 2.71 (s, 3H), 2.06 – 1.96 (m, 1H), 1.03 (d, *J* = 6.65 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.63, 149.55, 143.39, 141.07, 132.84, 128.08, 115.42, 105.50, 55.59, 48.82, 28.12, 20.40, 12.83. MS (*m/z*): 277 (M^+, 40), 262 (18), 234 (55), 221 (100), 188 (50).

2-isopentylamino-3-methylthio-7-methoxyquinoxaline (11k): 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 8.90 Hz, 1H), 7.07 (d, *J* = 2.85 Hz, 1H), 6.97 (dd, *J* = 8.90, 2.85 Hz, 1H), 3.90 (s, 3H), 3.62 – 3.57 (m, 2H), 2.70 (s, 3H), 1.81 – 1.69 (m, 1H), 1.63 – 1.58 (m, 2H), 0.99 (d, *J* = 6.48 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 159.63, 149.43, 143.37, 141.10, 132.82, 128.09, 115.42, 105.53, 55.59, 39.77, 38.31, 26.07, 22.65, 12.81. MS (*m/z*): 291 (M^+, 50), 244 (30), 235 (35), 220 (100), 188 (52).

7-bromo-2-butylamino-3-methylthioquinoxaline (11l): 65% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.83 (d, *J* = 2.17 Hz, 1H), 7.60 (d, *J* = 8.67 Hz, 1H), 7.38 (dd, *J* = 8.67, 2.17 Hz, 1H), 3.59 – 3.54 (m, 2H), 2.71 (s, 3H), 1.71 - 1.64 (m, 2H), 1.45 (sext, *J* = 7.53 Hz, 2H), 0.98 (t, *J* = 7.53 Hz, 3H). MS (*m/z*): 327 (M+2, 40), 325 (M^+, 38), 312 (48), 310 (45), 269 (100), 238 (45).

7-bromo-2-(2-hydroxyethanolamina)-3-methylthioquinoxaline (11m): 46% yield. ^{13}C NMR (100 MHz, DMSO-d_6) δ: 149.11, 147.67, 140.29, 135.12, 128.46, 127.29, 126.59, 120.33, 58.87, 43.37, 12.30.

2-isobutylamino-3-methylthio-6-methoxyquinoxaline (11n): 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ : 7.59 (d, *J* = 8.84 Hz, 1H), 7.20 (d, *J* = 2.94 Hz, 1H), 7.12 (dd, *J* = 8.84, 2.94 Hz, 1H), 3.89 (s, 3H), 3.41 – 3.48 (m, 2H), 2.73 (s, 3H), 2.04 – 1.95 (m, 1H), 1.02 (d, *J* = 6.78 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.21, 146.77, 137.97, 134.71, 130.91, 126.87, 119.14, 106.89,

55.60, 48.90, 28.11, 20.42, 12.72. MS (*m/z*): 277 (M^+, 47), 262 (15), 234 (78), 221 (100), 188 (30).

7-chloro-2-butylamino-3-methylthioquinoxaline (11o): 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.69 - 7.66 (m, 2H), 7.26 (dd, *J* = 7.11, 2.42 Hz, 1H), 3.60 – 3.55 (m, 2H), 2.72 (s, 3H), 1.72 - 1.65 (m, 2H), 1.46 (sext, *J* = 7.50 Hz, 2H), 0.99 (t, *J* = 7.50 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.30, 147.05, 140.39, 135.74, 133.23, 128.11, 125.17, 124.62, 41.19, 31.33, 20.24, 13.88, 12.72. MS (*m/z*): 281 (M^+, 53), 266 (60), 234 (70), 225 (100), 192 (40).

2-cycloexylamino-3-methylthio-7-chloroquinoxaline (11p): 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.67 – 7.65 (m, 2H), 7.24 (dd, *J* = 8.69, 2.40 Hz, 1H), 4.16 – 4.07 (m, 1H), 2.71 (s, 3H), 2.13 – 2.09 (m, 2H), 1.80 - 1.65 (m, 3H), 1.53 - 1.43 (m, 2H), 1.34 – 1.23 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 148.49, 147.06, 140.45, 135.66, 133.19, 128.09, 125.16, 124.50, 49.63, 32.87, 25.82, 24.85, 12.78. MS (*m/z*): 307 (M^+, 28), 292 (18), 225 (100), 192 (30), 55 (25).

2-butylamino-3-phenyl-7-methoxyquinoxaline (14a): 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (d, *J* = 9.06 Hz, 1H), 7.70 – 7.65 (m, 2H), 7.57 – 7.48 (m, 3H), 7.10 (d, *J* = 2.63 Hz, 1H), 7.01 (dd, *J* = 9.06, 2.63 Hz, 1H), 3.94 (s, 3H), 3.56 -3.51 (m, 2H), 1.65 – 1.58 (m, 2H), 1.41 (sext, *J* = 7.51 Hz , 2H), 0.96 (t, *J* = 7.51 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.19, 150.85, 148.10, 144.12, 137.37, 132.68, 130.16, 129.65, 129.59, 128.73, 116.37, 105.40, 55.93, 41.37, 31.69, 20.61, 14.19. MS (*m/z*): 307 (M^+, 48), 264 (90), 250 (100), 131 (27), 77 (34).

2-(2-hydroxyethanamino)-3-phenyl-7-methoxyquinoxaline (14b): 82% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.79 (d, *J* = 9.61 Hz, 1H), 7.69 – 7.66 (m, 2H), 7.56 – 7.49 (m, 3H), 7.06 – 7.03 (m, 2H), 3.92 (s, 3H), 3.88 - 3.86 (m, 2H), 3.70 – 3.67 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ:

161.23, 150.99, 143.90, 142.00, 136.64, 132.75, 129.89, 129.59, 129.38, 128.47, 116.85, 104.55, 63.86, 55.72, 45.32.

2-(2-piperidinylethylamino)-3-phenyl-7-methoxyquinoxaline (14c): 86% yield. ^{1}H NMR (400 MHz, CDCl3) δ: 7.78 (d, *J* = 9.06 Hz, 1H), 7.72 – 7.70 (m, 2H), 7.56 – 7.45 (m, 3H), 7.09 (d, *J* = 2.88 Hz, 1H), 7.00 (dd, *J* = 9.06, 2.88 Hz, 1H), 3.93 (s, 3H), 3.60 -3.56 (m, 2H), 2.57 (t, *J* = 6.19 Hz, 2H), 2.42 – 2.34 (m, 4H), 1.49 – 1.41 (m , 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 161.19, 150.85, 148.10, 144.12, 137.37, 132.68, 130.16, 129.65, 129.59, 128.73, 116.37, 105.40, 55.93, 41.37, 31.69, 20.61, 14.19. MS (*m/z*): 111 (52), 98 (100).

Preparation of the compound solutions: The compounds were dissolved in dimethylsulfoxide (DMSO) and finally diluted in culture medium prior to the assay. The DMSO concentration never exceeded 1% in the *in vitro* assays. Benznidazole (Laboratório Central de Medicamentos, Pernambuco, Brazil) and amphotericin B (Cristalia Ltda, Sao Paulo, Brazil)) were used in all of the experiments as positive controls for *T. cruzi* and *L. amazonensis*, respectively.

Parasites and cell culture: The epimastigote forms of *T. cruzi* (Y strain) were maintained in culture at 28°C with weekly transfers in liver infusion tryptose (LIT) medium supplemented with 10% fetal bovine serum (FBS; Gibco Invitrogen, Grand Island, NY, USA). Four-day-old cultured forms (exponential growth phase) were used for all of the experiments.

The promastigote forms of *L. amazonensis* (WHOM/BR/75/JOSEFA strain) were maintained in culture at 25°C with weekly transfers to fresh Warren's medium supplemented with 10% FBS. Three-day-old cultured forms (exponential growth phase) were used for all of the experiments.

$LLCMK_2$ cells (epithelial cells from the kidney of the monkey *Macaca mulatta*) were cultured and maintained in Dulbecco's modified Eagle medium (DMEM; Gibco Invitrogen, Grand Island, NY, USA) supplemented with 2 mM L-glutamine and 10% FBS at 37°C in a humidified 5% CO_2 atmosphere.

JJ74-A1 macrophages were cultured and maintained in Roswell Park Memorial Institute medium (RPMI-1640; Gibco Invitrogen, Grand Island, NY, USA) supplemented with 2 mM L-glutamine and 10% FBS at 37°C in a humidified 5% CO_2 atmosphere.

The trypomastigote forms of *T. cruzi* were obtained from the supernatant of a monolayer of infected $LLCMK_2$ cells in DMEM supplemented with 10% FBS at 37°C in a humidified 5% CO_2 atmosphere.

***In vitro* growth inhibition assay for the epimastigote and promastigote forms:** Epimastigote or promastigote (1×10^6 cells/mL) cultures were inoculated in a 24-well plate in the absence or presence of different concentrations of quinoxaline derivatives (0.1-100 μM). Activity against the epimastigote and promastigote forms was evaluated after 96 and 72 h, respectively [53,65]. The cell density for each concentration was determined by counting in a hemocytometer (Improved Double Neubauer). The concentration that inhibited cell growth in 50% (IC_{50}) was determined by nonlinear regression analysis.

Effect on viability of the trypomastigote forms: The tissue culture-derived trypomastigotes (1×10^7 cells/mL) were added in 96-well microplates in the absence or presence of different concentrations of quinoxaline derivatives (0.1-50 μM). The parasites were incubated for 24 h at 37°C in a 5% CO_2 atmosphere. The results were obtained by observing motility, which allowed the determination of the viability of the parasites using the Pizzi–Brener method [66]. The effective concentration of the drug to reduce parasite viability by 50% (EC_{50}) was calculated by nonlinear regression analysis.

In vitro **cytotoxicity in cellular lines:** $LLCMK_2$ cells and J774-A1 macrophages (2.5×10^5 and 5×10^5 cells/mL, respectively) were seeded in 96-well microplates. The cells were allowed to attach for 24 h at 37°C in a 5% CO_2 atmosphere. The medium was then replaced by different concentrations of quinoxaline derivatives (1-1000 μM). Cytotoxicity in $LLCMK_2$ cells and J774-A1 macrophages was evaluated after 96 and 48 h, respectively, using the standard MTT colorimetric assay [67]. The cytotoxic concentration that reduced cell viability by 50% (CC_{50}) was estimated by nonlinear regression analysis. The selectivity index was used to compare cytotoxicity between mammalian cells and protozoa (ratio: CC_{50} divided by IC_{50} or EC_{50} of the compound in the protozoa).

In vitro **activity on intracellular amastigote form of *T. cruzi:*** $LLCMK_2$ cells (2.5×10^5 cells/mL) were seeded in 24-well plates with round coverslips and then maintained at 37 ◦C in a

5% CO_2 for 24h until confluent monolayer was obtained. Trypomastigotes were added to the wells at a concentration of 10 parasites per host cell. After 24 h, non-internalized parasites were removed by washing, and the infected $LLCMK_2$ cells were treated with different concentrations of quinoxaline derivatives (0.1 to 50 μM). After 96 h the cells were fixed with methanol and stained with Giemsa, and the coverslips were permanently prepared with Entellan (Merck). By counting 200 cells under a light microscope (Olympus CX 31), we estimated the percentage of infected cells and number of intracellular amastigotes. The survival index (percentage of infected cells x number of amastigotes per cell) was calculated and IC_{50} values were then determined by nonlinear regression analysis.

In vitro* activity on intracellular amastigote form of *L. amazonensis

Peritoneal macrophages from healthy BALB/c mice were harvested and plated (3×10^5 cells/mL) in a 24-well plate with round coverslips using RPMI medium supplemented with 10% FBS and allowed to adhere for 2 h at 37 °C in 5% CO_2. Adhered macrophages were then infected with promastigotes in the stationary growth phase using a ratio 1:7 at 34 °C for 4 h. Afterwards, non-interiorized parasites were removed by washing and the infected culture was incubated with different concentrations of quinoxaline derivatives (0.1 to 50 μM) for 48 h at 34 °C. The cells were fixed, stained and prepared as described above for amastigotes of *T. cruzi.* The survival index was calculated and IC_{50} values were then determined by nonlinear regression analysis.

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Author Contributions

Performed the experiments: JC, VK and DPS. Contributed reagents/materials/analysis tools: AGC, TUN and CVN. Analyzed the data: JC, VK and CVN. Wrote the paper: JC, VK, DPS, TUN, AGC and CVN.

Notes

The authors declare that there are no conflicts of interest.

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Synthesized 46 new 2,3-disubstituted quinoxaline derivatives and 40 previously reported.

Evaluated cytotoxicity and activity on all evolutive forms of *T. cruzi* and *L. amazonensis*.

Analogs from groups 5, 6, 7, 12 and 13 exhibited promising activity and selectivity.

Activity related to the methylsulfoxyl, methylsulfonyl, and amine groups.