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Quinoxaline *N*,*N*′-dioxide derivatives and related compounds as growth inhibitors of *Trypanosoma cruzi*. Structure–activity relationships

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Abstract—Quinoxaline derivatives presented good inhibitor activity of growth of *Trypanosoma cruzi* in in vitro assays. The 50% inhibitory doses were of the same order of that of Nifurtimox. Derivative **13**, a quinoxaline *N*,*N*′-dioxide derivative, and the reduced derivatives **19** and **20** were the most cytotoxic compounds against the protozoan. Structural requirements for optimal activity were studied by computational methods. From statistical analysis we could establish a multiple correlation between activity and lipophilic properties and LUMO energy.

1. Introduction

Chagas' disease or American trypanosomiasis is an important health problem that affects around 20 million people in Central and South America.[1] Around 2–3 million individuals develop the typical symptoms of this disease that results in 50,000 yearly deaths.[2] The causative agent of this disease is the haemoflagellate protozoan *Trypanosoma cruzi* (*T. cruzi*), which is transmitted in rural areas to humans and other mammals by reduviid bugs such as *Rhodnius prolixus* and *Triatoma infestans*.[3] The main route of transmission is the result of blood-sucking activity of Chagas' disease vectors on mammals when feeding in a cyclic process. The parasite presents three main morphological forms in a complex life cycle. It replicates within the crop and midgut of Chagas' disease vectors as the epimastigote form, it is released with the insect excrements as the nondividing highly infective metacyclic trypomastigotes that invade mammalian tissues via wounds provoked by blood-sucking action. The parasite multiplies intra-cellularly as amastigote form, which is released as the nondividing bloodstream trypomastigote form that invades other tissues. The existence of the epimastigote form as an obligate mammalian intracellular stage has been revisited[4] and confirmed recently.[5] Despite the progress made in the study of *T. cruzi* biochemistry and physiology,[3] in which several crucial enzymes for parasite survival, absent in the host, have been identified as potential targets for the design of new drugs,[6] the chemotherapy to control this parasitic infection remains underdeveloped. The pharmacology is based on old and quite unspecific drugs associated with long term treatments that rise to severe side effects.[7] In fact, although Nifurtimox (4-([5-nitrofurfurylidene]-amino)-3-methylthio morpholine-1,1-dioxide, Nfx) and Benznidazole (*N*-benzyl-2-nitro-1-imidazoleacetamide), the only two drugs currently in use for clinical treatment of this disease, are able to wipe out parasitemia and reduce

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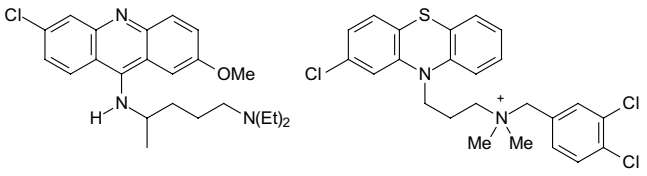
serological titres, they are not specific enough to all *T. cruzi* strains to guarantee complete cure.[8]

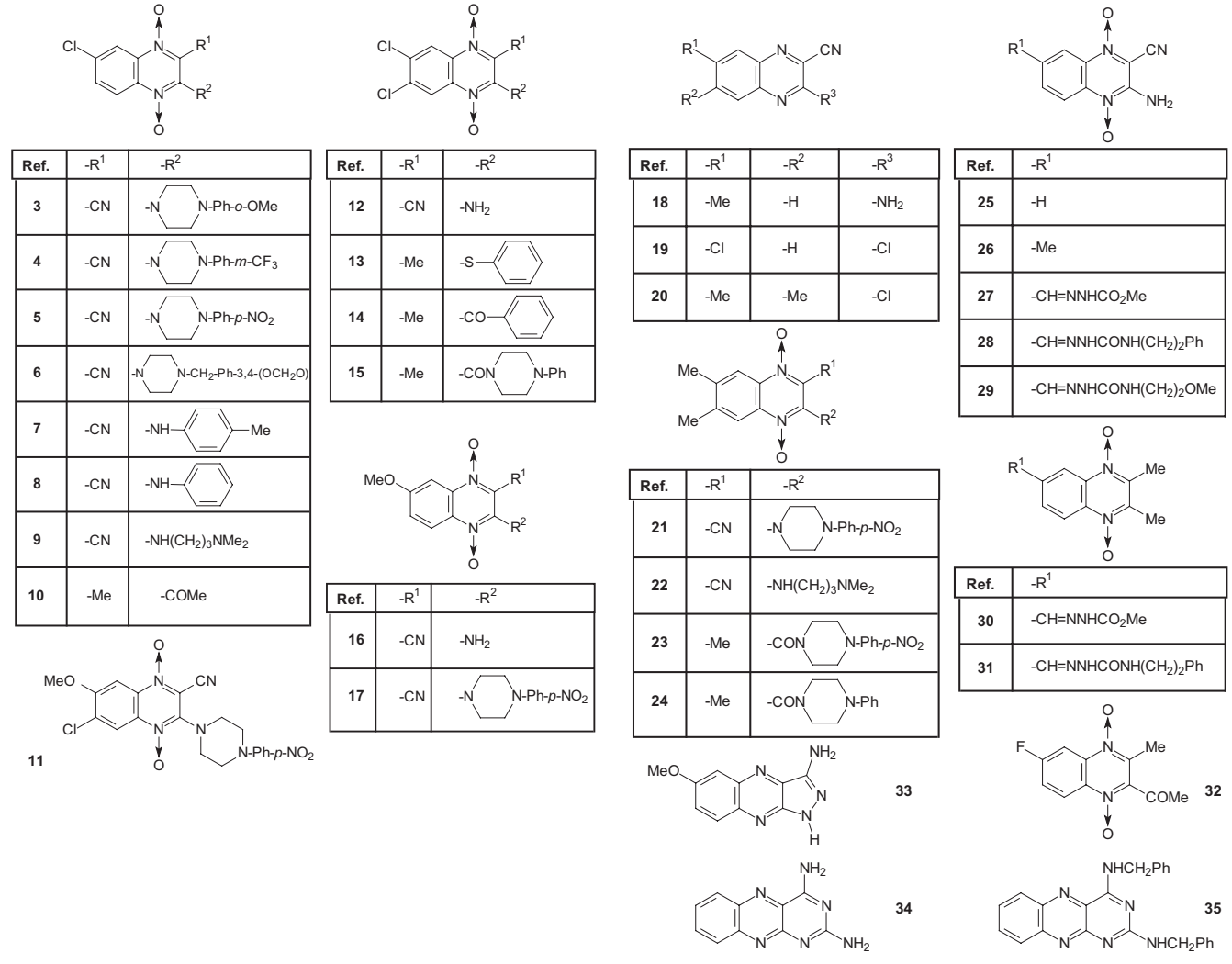
Trypanothione reductase (TR) is the most thoroughly studied enzyme of the trypanothione redox metabolism, it is a key enzyme of the parasite anti-oxidant defence system, and it does not occur in the mammalian host. The main difference between TR and glutathione reductase (GR) is the mutually exclusive specificity towards their respective disulfide substrate (trypanothione and glutathione, respectively).[9] So, TR is a highly attractive target for a structure-based drug design against trypanosomatids. The three-dimensional structure of TR has been solved in free form, in complex with the substrates NADPH, glutathionylspermidine and trypanothione as well as the competitive inhibitor mepacrine (**1**, Fig. 1).[10] Mepacrine was the first tricyclic compound identified as a competitive inhibitor of *T. cruzi* TR but not of human GR.[11] Rational drug design approaches on TR led to the discovery of phenothiazines and other tricyclic drugs as specific competitive inhibitors of the parasite enzyme over host GR with low micromolar inhibitor constants.[12] Based on modelling studies, a series of phenothiazines (i.e., **2**, Fig. 1) were obtained as competitive inhibitors.



Mepacrine, 1 **2**

Figure 1. Inhibitors of *T. cruzi* trypanothione reductase.

Some quinoxaline derivatives developed by us resembled, by the planar structure and by the kind of substituents, the skeleton of these inhibitors. On the other hand, when these quinoxalines exist as *N*,*N*′-dioxide the resulted compounds are capable to act as substrate of reductive enzymes, where the reduction-products reacting with oxygen, produce reactive oxygen species.[13] These two facts and the capacity of quinoxaline *N*,*N*′-dioxide to act as anti-infective agents towards a great number of microorganisms[14] conducted us to evaluate some selected quinoxaline derivatives as anti-trypanosomal drugs (Fig. 2). The 33 derivatives described in the



Ref.	-R1	-R2
3	-CN	-N N-Ph-*o*-OMe
4	-CN	-N N-Ph-*m*-CF_3
5	-CN	-N N-Ph-*p*-NO_2
6	-CN	-N N-CH_2-Ph-3,4-(OCH_2O)
7	-CN	-NH Me
8	-CN	-NH
9	-CN	-$NH(CH_2)_3NMe_2$
10	-Me	-COMe

Ref.	-R1	-R2
12	-CN	-NH_2
13	-Me	-S
14	-Me	-CO
15	-Me	-CON N-Ph

Ref.	-R1	-R2
16	-CN	-NH_2
17	-CN	-N N-Ph-*p*-NO_2

Ref.	-R1	-R2	-R3
18	-Me	-H	-NH_2
19	-Cl	-H	-Cl
20	-Me	-Me	-Cl

Ref.	-R1	-R2
21	-CN	-N N-Ph-*p*-NO_2
22	-CN	-$NH(CH_2)_3NMe_2$
23	-Me	-CON N-Ph-*p*-NO_2
24	-Me	-CON N-Ph

Ref.	-R1
25	-H
26	-Me
27	-CH=NNHCO_2Me
28	-CH=NNHCONH$(CH_2)_2$Ph
29	-CH=NNHCONH$(CH_2)_2$OMe

Ref.	-R1
30	-CH=NNHCO_2Me
31	-CH=NNHCONH$(CH_2)_2$Ph

Figure 2. Chemical structures of quinoxaline *N*,*N*′-dioxide and related derivatives studied as anti-trypanosomal compounds.

present paper were carefully selected from our more than one hundred-quinoxaline-library in order to cover a wide structural spectrum. Reduced and di-*N*-oxide quinoxalines bearing moieties with different volume, polarity, lipophilicity and donor/acceptor-hydrogen bond capacity were included.

In order to gain insight the mechanism of action of these series of derivatives some physicochemical descriptors were studied theoretically and analysed the relationships between them and the activity.

Chemistry.[15] The studied compounds were prepared following synthetic procedures previously reported.[16] Derivatives **3**–**11**, **16**–**19**, **25**–**29**, **32** and **33** were obtained as mixture of inseparable 6- and 7-isomers, which were evaluated without further separation. For simplicity in Figure 2 is shown only one of them.

Biology.[17] All the compounds were tested in vitro against *T. cruzi*, as previously described. The compounds were incorporated into the media at 25 μM and their ability to inhibit growth of the parasite was evaluated in comparison to the control (no drug added to the media).

Nfx was used as the reference trypanocidal drug. The percentage of growth inhibition (PGI) was calculated as follows: $\text{PGI}\ (\%) = \{1 - [(A_p - A_{0p})/(A_c - A_{0c})]\} \times 100$.[18] The ID_{50} concentration (50% inhibitory dose) was assessed for compounds presenting higher trypanocidal activity (**3**, **4**, **13**, **14**, **19**, **20** and Nfx). Readings were done on day 5 of growth, and were determined as the drug concentration required to reduce the absorbance to one half of that of the control (without drug).

Theoretical studies.[19] Total, HOMO's and LUMO's energy, nitrogen N–O's Mulliken charge, dipolar moments and ClogP were determined and analysed.[20]

2. Results and discussion

In the present paper, we report the biological activity of 33 quinoxaline *N*,*N*′-dioxide derivatives and related compounds, against epimastigote form of two strain of *T. cruzi*. The inhibitory action at 25 μM on the Tulahuen strain is shown in Table 1.

Derivatives **3**, **4**, **13**, **14**, **19** and **20** resulted the most active compounds against this strain. So, these were selected to study on Brener strain (Table 2). All of selected derivatives presented similar bio-activities in both strain of *T. cruzi* as the reference drug, Nfx.

Derivatives **19** and **20**, which displayed good ID_{50} in both strains, present an excellent electrophile centre on the heterocycle's carbon 3 that could unselectively react with biological nucleophile (amines and phosphates from DNA, thiols, alcohols and amines from proteins) (Fig. 3). So we assumed that the selective parasitic cell cytotoxicity could be low.

Nevertheless, compounds **3**, **4**, **13** and **14** together with the other *N*-oxide derivatives could act in a different manner. In order to investigate the mechanism of action of these drugs some physicochemical properties were theoretically determined, in terms of semiempirical

Table 1. Biological and physiochemical characterization of quinoxaline *N*,*N*′-dioxide and related derivatives

Ref.	PGI[a] (%)[b,c]	ELUMO[d]	CLogP	qN[e]
3	100	−41.75	5.30	0.304
4	94	−45.15	5.94	0.341
5[f]	3	−46.79	6.31	0.305
6	33	−41.35	4.04	0.287
7	19	−43.30	6.27	0.301
8	63	−43.64	6.01	0.301
9	16	−40.23	4.69	0.206
10	51	−38.32	4.98	0.328
11[f]	6	−46.85	5.89	0.315
12	25	−47.17	4.93	0.253
13	93	−34.72	7.65	0.318
14	91	−38.49	7.01	0.328
15	47	−40.99	5.86	0.328
16	25	−40.11	3.55	0.269
17[f]	19	−43.83	5.16	0.311
18	0	−25.56	1.13	−0.035
19	100	−37.47	2.56	−0.049
20	81	−31.78	2.30	−0.051
21[f]	0	−41.34	6.14	0.305
22	1	−37.41	4.29	0.286
23[f]	1	−35.40	6.17	0.330
24	0	−31.79	5.34	0.328
25	25	−40.11	3.60	0.256
26	5	−39.00	3.89	0.259
27	19	−45.05	2.67	0.251
28	11	−38.58	3.77	0.258
29	20	−45.06	1.86	0.252
30	28	−35.11	3.72	0.330
31	17	−33.77	4.85	0.330
32	22	−38.36	4.41	0.332
33	0	−25.61	−0.58	−0.055
34	0	−27.73	−1.08	−0.072
35	31	−25.87	2.49	−0.072

[a] Percentage of growth inhibition.
[b] Inhibition of epimastigotes growth of Tulahuen strain, dose = 25 μM.
[c] The results are the means of three different experiments with a SD less than 10% in all cases.
[d] kcal/mol.
[e] Mulliken charge of the *N*-oxide nitrogen less negative.
[f] The compounds presented low solubility in the bio-assay.

Table 2. ID_{50} (μM) values for the most active compounds against both studied strains

Ref.	ID_{50}, Tulahuen (μM)[a]	ID_{50}, Brener (μM)[a]
3	6.5	9.5
4	6.7	7.0
13	6.5	4.5
14	11.8	12.5
19	4.5	3.0
20	19.6	3.0
Nfx	7.7	3.4

[a] The results are the means of three different experiments with a SD less than 10% in all cases.

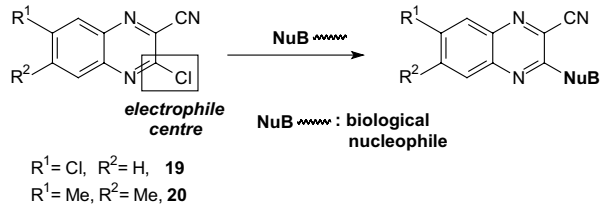


Figure 3. Speculative mechanism of action for the most active derivatives **19** and **20**.

methods (AM1). The physicochemical descriptors related to activity are summarized in Table 1. Molecular modelling of compounds allowed the evaluation of the electronic and lipophilic characteristic.

When we considered only the *N*-oxide derivatives, excluding the derivatives that presented low solubility in the bio-assay experiments (**5**, **11**, **17**, **21** and **23**), it was obtained a good multiple correlation between electronic, expressed as LUMO energies and Mulliken charge of the *N*-oxide nitrogen less positive, and lipophilic–hydrophilic properties, expressed as $(CLogP)^2$ values, and the anti-trypanosomal activities observed against Tulahuen strain (Eq. 1[21]). When the correlation matrix for the used physicochemical properties descriptors was performed[22] an important cross-correlation between qN and the other descriptors was observed. Specially, a significative correlation was observed between $(CLogP)^2$ and Mulliken charge in the *N*-oxide nitrogen atom, this fact was previously reported by Gasco et al. referring to oxygen atom of this moiety.[23] So, this descriptor was not considered in the multilinear regression.[24] LUMO energy and $(CLogP)^2$ were reasonably independent vectors that allowed to safely use these factors in the multilinear regression. Statistically significant correlation was obtained, see Eq. 2.

$$\begin{aligned} \mathrm{PGI}_{25\,\mu\mathrm{M,Tulahuen}} &= -129.0(\pm 47.0)3.0(\pm 1.1)E_{\mathrm{LUMO}} \\ &\quad + 1.97(\pm 0.33)(\mathrm{CLogP})^2 \\ r = 0.821,\ \ q^2 = 0.638,\ \ n = 11 & \qquad (2) \end{aligned}$$

According to Eq. 2 and by observation of the different families of compounds some structural conclusions could be remarked: (i) the presence of an halo-substituent at benzo ring of quinoxaline produce more active compounds (for example, compare the activity of the dichloro **15** with activity of the dimethyl-analogue **24** or activities of chloro-derivative **9** and dimethyl-analogue **22**), the electron-withdrawing substituent promotes an adequate LUMO energy in the compounds; (ii) compounds more hydrophilic decrease the anti-trypanosomal activity (compare activity of derivatives **13** and **14** with **15**, or activity of **35** with **34**). These electronic properties indicate that a reductive metabolism could be implicated in the mechanism of action, the exigency in the lipophilic properties could be indicating the capacity of the compounds' entry on the parasitic cells and/or the requirements in the interaction compound–target enzyme.

Table 3. Physiochemical properties and Lipinski's 'rule of 5'

Ref.	ClogP	H-bond donors	H-bond acceptors	Molecular weight	'Rule of 5' criteria met
3	5.30	0	6	412	3
4	5.94	0	5	450	3
13	7.65	0	3	353	3
14	7.01	0	3	349	3
19	2.56	0	3	224	All
20	2.30	0	3	218	All

Besides, Lipinsky has described desired ranges for certain properties thought to be important for pharmacokinetics and drugs development. They are CLopP <5, number of hydrogen bond donors $\leqslant$5, number of hydrogen bond acceptors $\leqslant$10 and molecular weight <500.[25] A compound that fulfils at least three out of the four criteria is said to adhere to Lipinski's 'rule of 5'. Table 3 lists the values of these properties for the best derivatives and suggests that these compounds are reasonable starting points for a drug discovery effort. This information allow us to re-design new structures with increased anti-chagasic activity.

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18. $A_p = A_{600}$ of the culture containing the drug at day 5; $A_{0p} = A_{600}$ of the culture containing the drug right after addition of the inocula (day 0); $A_c = A_{600}$ of the culture in the absence of any drug (control) at day 5; $A_{0c} = A_{600}$ in the absence of the drug at day 0.
19. Firstly the compounds were built with standard bond lengths and angles using the PC SPARTAN *Pro* molecular modeling program.[20] Compounds were constructed and studied as 7-substituted derivatives. The energy of each compound was minimised by molecular mechanics methods (using SYBYL molecular mechanics force fields implemented in PC SPARTAN *Pro* package) and then by semiempirical methods (AM1). With the structure of minimum energy a single point calculation were performed using AM1 semiempirical method implemented in the package. LogP calculated (CLogP) by Villar algorithm implemented in PC SPARTAN *Pro* package was used.
20. (a) Wavefunction Inc., 18401 Von Karman Avenue, Suite 370. Irvine, California 92612 USA; (b) PC SPARTAN *Pro* User's Guide, Wavefunction Inc., California, 1999.
21. $$\mathrm{PGI}_{25\,\mu\mathrm{M,Tulahuen}} = -228.7(\pm67.8) - 3.8(\pm1.1)E_{\mathrm{LUMO}} + 259.5(\pm135.4)qN + 1.6(\pm0.4) \times (\mathrm{CLogP})^{2};$$
$$r = 0.855,\ q^{2} = 0.685,\ n = 11. \quad (1)$$
22. Correlation matrix for the used physicochemical descriptors.

	$(\mathrm{CLogP})^2$	QN	E_{LUMO}
E_{LUMO}	0.226	0.428	1
qN	0.539	1	
$(\mathrm{CLogP})^2$	1		

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