Cyclic Voltammetric Study of Some Anti-*Chagas*-Active 1,4-Dioxidoquinoxalin-2-yl Ketone Derivatives

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The electrochemical properties of 24 1,4-dioxidoquinoxalin-2-yl ketone derivatives with varying degrees of anti-*Chagas* activity were investigated in the aprotic solvent dimethylformamide (DMF) by cyclic voltammetry and first-derivative cyclic voltammetry. For this group of compounds, the first reduction in DMF was either reversible or quasireversible and consistent with reduction of the *N*-oxide functionality to form the radical anion. The second reduction process for these compounds was irreversible under the conditions used. The reduction potentials correlated well with molecular structure. Substitution in the 3-, 6-, and 7- positions of the quinoxaline ring by electron-withdrawing substituents directly affected the ease of reduction and improved the biological activities of these compounds, whereas substitution by electron-donating groups had the opposite effect. The electrochemical results, when combined with previous work on their mechanism of action against *Chagas* disease and their measured anti-*Chagas* activities, indicated that the quinoxaline 1,4-dioxide system serves as a promising starting point for chemical modifications aimed at improving the *T. cruzi* activity *via* a possible bioreduction mechanism.

Introduction. – *Chagas* disease continues to represent a health threat for an estimated 8 million people, the majority of whom live in Latin America [1]. Although more than 100 years have elapsed since the discovery of this disease, the discovery of a valid chemotherapy treatment for *Chagas* disease is still an open field and remains an unsolved problem. Early experiments carried out with one of the main drugs used for treating *Chagas* disease, nifurtimox (Nfx), suggest that intracellular reduction followed by redox cycling, yielding reactive oxygen species (ROS), may be the major mode of action of Nfx against *Trypanosoma cruzi*. ROS can cause direct cellular damage by reacting with various biological macromolecules, or indirect cellular damage by the generation of the highly reactive hydroxy radical *via Haber–Weis* and *Fenton* reactions [2][3].

It is well-known that some quinoxaline *N,N′*-dioxides are species that suffer a bioreductive process under hypoxic conditions, producing hydroxy radical and quinoxaline. Quinoxalines could produce parasitic damage through the production of radical species affecting the redox metabolism [4]. As previously observed [5], the absence of the *N*-oxide moiety produces a decrease in the antiepimastigote activity, confirming that this group plays a key role in the mechanism of quinoxaline *N,N′*-dioxide antitrypanosomal activity.

In the search for more selective and less toxic antichagasic drugs, we developed new quinoxaline derivatives possessing the *N*,*N*′-dioxide moiety as the bioreductive pharmacophore [6]. In this publication, we demonstrated that our quinoxaline derivatives affect the mitochondrial dehydrogenase activity [6]. Therefore, electrochemistry plays an important role when studying the formation of this radical species and its reactivity in one-pot systems [7]. The purpose of the present study was to investigate the electrochemical properties of a series of 1,4-dioxidoquinoxalin-2-yl ketone derivatives, and determine the possible relationship between their reduction potentials, their structure, and their antichagasic activity.

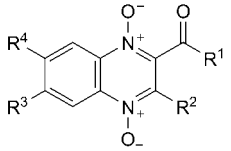
Experimental. – *Chemical Synthesis.* The compounds **1**–**24** presented in this article, along with their structures, are shown in *Table 1*. The methods for the synthesis of these 1,4-dioxidoquinoxalin-2-yl ketone derivatives were previously reported [6].

Pharmacology. The anti-*T. cruzi* activity of the 1,4-dioxidoquinoxalin-2-yl ketone derivatives presented in this study was tested *in vitro* against the epimastigote form of *T. cruzi*, Tulahuen 2 strain, as previously described [6]. The existence of the epimastigote form of *T. cruzi* as an obligate mammalian intracellular stage has been revised and confirmed [8]. The compounds were incorporated into the biological 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) at day 5. Nfx was used as the reference trypanosomicidal drug. The percentage of growth inhibition (*PGI*) and the half-maximal inhibitory concentration IC_{50} [μM] against *T. Cruzi* were calculated as indicated in *Table 1*.

Electrochemistry. Test solns. contained 1.0 mM of the quinoxaline compound and 0.10M tetrabutylammonium perchlorate ($(Bu_4N)ClO_4$). Test solns. were deaerated for 15 min prior to recording the electrochemical data by passing a gentle stream of prepurified N_2 through the soln. A N_2 atmosphere was maintained over the test soln. during all experiments. Cyclic-voltammetric measurements were carried out with a *CH-Instruments-630* voltammetric analyzer. Solution resistance was uncompensated. The electrodes consisted of a Pt-disk (1.6 mm diameter) electrode, a Pt-wire auxilliary electrode, and a $Ag/AgNO_3$ (0.1M in MeCN) reference electrode. Scan rates ranged from 0.1 V/s to 1 V/s. Half-wave potentials and the difference in peak potentials were calculated with the equations $E_{1/2} = (E_{pa} + E_{pc})/2$ and $\Delta E_p = E_{pa} - E_{pc}$, resp. [9]. For first-derivative cyclic voltammograms, E_{pc} was measured at the point where the derivative curves crossed the baseline [9]. To account for daily variations in the reference electrode and liquid-junction potentials, ferrocene (Fc) was added to each solution following measurements of the test compound, and used as an internal reference redox system [10]. All potentials are reported *vs.* the ferrocene/ferrocinium (Fc/Fc^+) redox couple, *i.e.*, $E_{pc,SRE} - E_{1/2,Fc/Fc^+}$ or $E_{1/2,SRE} - E_{1/2,Fc/Fc^+}$. Half-wave potentials ($E_{1/2}$) for ferrocene varied from −0.0095 V to 0.0485 V during the course of this study. Peak currents were measured from the extrapolated baselines for both the cathodic and anodic processes [11]. DMF (*Fisher Scientific*) was used as the solvent, and $(Bu_4N)ClO_4$ (*Aldrich Chemical Company*) served as the supporting electrolyte. Ferrocene was obtained from *Aldrich Chemical Company*. All reagents were obtained in the highest purity available and used without further purification.

Results and Discussion. – *Electrochemical Behavior.* The present study included a series of 24 1,4-dioxidoquinoxalin-2-yl ketone derivatives. The electrochemical properties of these derivatives **1**–**24** were studied by cyclic voltammetry and first-derivative cyclic voltammetry in DMF with $(Bu_4N)ClO_4$ as supporting electrolyte. The structures and summary data for these compounds are provided in *Tables 1*–*3*, and representative voltammograms are shown in *Figs. 1* and *2*. *Hammett* plots are provided in *Fig. 3*. All reductions were diffusion controlled, as indicated by fairly constant current functions at varying scan rates for each derivative [9][11].

Table 1. *Structure, Cyclic Voltammetric Data, and Anti-*Chagas *Activity of the 1,4-Dioxidoquinoxalin-2-yl Ketone Derivatives* **1**–**24**



	R^1	R^2	R^3	R^4	Cyclic voltammetry[a])			Anti-*Chagas* activity[b])	
					i_{pa}/i_{pc}	$E_{1/2}$ [V]	ΔE_p [V]	*PGI* [%][c])	IC_{50} [μM][d])
1	Me	Me	F	F	0.722	−1.418	0.072	100	0.4
2	Et	CF_3	Cl	Cl	0.901	−1.093	0.065	100	0.78
3	Me	CF_3	F	F	0.829	−1.179	0.069	100	0.032
4	^{i}Pr	CF_3	F	F	0.436	−1.181	0.076	23	>25
5	^{t}Bu	CF_3	F	F	0.916	−1.176	0.075	30	4.9
6	Ph	Me	H	H	0.672	−1.546	0.069	19	>25
7	Ph	Me	H	Me	0.724	−1.580	0.065	8	>25
8	Ph	Me	F	F	0.748	−1.442	0.071	92	4.2
9	Ph	Me	H	MeO	0.685	−1.573	0.075	9	
10	Ph	CF_3	H	H	0.926	−1.308	0.066	100	0.9
11	Ph	CF_3	H	Me	0.804	−1.344	0.065	99	1.3
12	Ph	CF_3	H	MeO	0.838	−1.366	0.081	98	
13	Ph	CF_3	F	F	0.867	−1.188	0.069	100	0.7
14	Ph	CHF_2	H	H	0.884	−1.400	0.069	36	>25
15	Ph	CHF_2	H	MeO	0.971	−1.458	0.069	100	10
16	Ph	CHF_2	H	Cl	0.922	−1.291	0.068	100	6.4
17	Ph	CHF_2	Cl	Cl	0.515	−1.189	0.072	100	4.7
18	Ph	CHF_2	H	F	0.763	−1.334	0.072	100	6.3
19	naphthalen-1-yl	CF_3	F	F	0.889	−1.196	0.073	100	3.9
20	furan-2-yl	CF_3	F	F	0.910	−1.183	0.071	100	5.2
21	thiophen-2-yl	CF_3	F	F	0.792	−1.190	0.072	100	2.8
22	4-chlorophenyl	CF_3	F	F	0.934	−1.176	0.073	100	2.5
23	4-(4-chlorophenyl)piperazin-1-yl	CF_3	H	H	0.647	−1.341	0.066	100	0.79
24	4-phenylpiperazin-1-yl	CF_3	H	H	0.846	−1.349	0.066	100	0.76
Nfx								100	7.7

[a]) Substrate, 1.0 mM; $(Bu_4N)ClO_4$, 0.10M; DMF; Pt working electrode; $Ag/AgNO_3$ reference electrode; Pt-wire counter electrode; 100 mV/s; r.t.; *E vs.* (Fc/Fc^+)/V; currents reported in μA. [b]) *T. cruzi* antiprolifertive activity of quinoxaline *N,N′*-dioxides and nifurtimox (Nfx) as reference drug. [c]) Percentage of growth inhibition; inhibition of epimastigote growth of Tulahuen 2 strain, dose = 25 μM. The results are the mean of three independent experiments with a SD less than 10% in all cases.[d]) IC_{50} = Inhibitory concentration (50%) [μM] against *T. cruzi*.

For all the quinoxaline *N,N′*-dioxide derivatives studied, the first cyclic-voltammetric wave observed during the reduction was either reversible or quasireversible (*Figs. 1,a*, and *2,a*). Values of ΔE_p (65–81 mV at 0.1 V/s) and $E_{pc} - E_{1/2}$ (−32.5 to −40.5 mV at 0.1 V/s) for this redox couple were reasonably close to the theoretical values for a reversible one-electron transfer, *i.e.*, 59 mV for ΔE_p and −28.5 mV for $E_{pc} - E_{1/2}$ [11]. In addition, i_{pa}/i_{pc} values calculated for the first voltammetric wave were

Table 2. *First-Derivative Voltammetric Data of the 1,4-Dioxidoquinoxalin-2-yl Ketone Derivatives* **1**–**24**[a])

	1st Derivative[b])					
	$E_{pc,1}$	$E_{pa,1}$	$E_{1/2,1}$	$E_{pc,2}$	$E_{pc,3}$	$E_{pc,4}$
1	– 1.466	– 1.394	– 1.430	– 2.002	– 2.264	– 2.474
2	– 1.128	– 1.058	– 1.093	– 1.861	– 2.122	– 2.329
3	– 1.213	– 1.142	– 1.178	– 1.925	– 2.090	– 2.273
4	– 1.220	– 1.139	– 1.180	– 1.907	– 2.138	–
5	– 1.212	– 1.138	– 1.175	– 1.957	– 2.222	– 2.519
6	– 1.579	– 1.508	– 1.544	– 2.020	– 2.161	– 2.193
7	– 1.614	– 1.545	– 1.580	– 2.045	– 2.180	– 2.221
8	– 1.481	– 1.405	– 1.443	– 1.921	– 2.127	– 2.267
9	– 1.611	– 1.534	– 1.573	– 2.025	– 2.235	–
10	– 1.342	– 1.270	– 1.306	– 2.083	– 2.259	–
11	– 1.379	– 1.307	– 1.343	– 2.076	– 2.154	– 2.252
12	– 1.408	– 1.326	– 1.367	– 2.386	– 2.534	–
13	– 1.227	– 1.153	– 1.190	– 1.923	– 2.068	– 2.207
14	– 1.437	– 1.364	– 1.401	– 2.003	– 2.090	– 2.197
15	– 1.493	– 1.420	– 1.457	– 2.019	– 2.095	– 2.253
16	– 1.327	– 1.254	– 1.291	– 1.978	– 2.097	–
17	– 1.226	– 1.152	– 1.189	– 1.874	– 2.073	– 2.231
18	– 1.369	– 1.295	– 1.332	– 1.976	– 2.148	– 2.457
19	– 1.232	– 1.160	– 1.196	– 1.923	–	–
20	– 1.220	– 1.144	– 1.182	– 1.924	– 2.058	–
21	– 1.228	– 1.154	– 1.191	– 1.909	–	–
22	– 1.215	– 1.139	– 1.177	– 1.921	– 2.155	– 2.330
23	– 1.376	– 1.308	– 1.342	– 2.065	– 2.382	–
24	– 1.383	– 1.313	– 1.348	– 2.118	– 2.449	–

[a]) Substrate, 1.0 mM; $(Bu_4N)ClO_4$, 0.10M; DMF; Pt working electrode; $Ag/AgNO_3$ reference electrode; Pt wire counter electrode; 100 mV/s; r.t.; *E vs.* (Fc/Fc^+)/V; currents reported in μA. [b]) E_{pc} and E_{pa} determined at the point where the derivative curve crosses the baseline [9].

close to unity ($i_{pa}/i_{pc} = 0.65$ to 0.97) at all scan rates for all derivatives but compounds **4** and **17** ($i_{pa}/i_{pc} = 0.44$ and 0.52, resp.), indicating the absence of significant kinetic or other complications in the electrode process [11]. Thus, the reduction product for this redox couple appeared fairly stable on the experimental time scale for these derivatives. This was further supported by multiple scans of the first voltammetric wave, which showed no changes in the reversibility of this electrode process upon redox cycling (*Figs. 1*,*b*, and *2*,*b*). The first voltammetric wave observed was consistent with the reversible reduction of the *N*-oxide functionality to a radical anion, in conjunction with other studies of the electrochemical behavior of quinoxaline *N*,*N*′-dioxides in DMF [12–16]. In fact, reduction of quinoxaline 1,4-dioxide in DMF has been shown *via* ESR and electrochemistry to involve a one-electron reduction of the nitrone to the radical anion [15]. A second, irreversible voltammetric wave was observed for all derivatives at potentials between – 1.7 and – 2.4 V (*Tables 2* and *3*, *Figs. 1*,*c*, and *2*,*c*), which was attributed to the formation of the dianion [17]. The irreversibility of this

Table 3. *Cyclic Voltammetric Data of the 1,4-Dioxidoquinoxalin-2-yl Ketone Derivatives*[a])

	E_{pc1} [V]	E_{pa1} [V]	$E_{1/2}$ [V]	i_{pc1} [μA]	i_{pa1} [μA]	i_{pa}/i_{pc}	ΔE_p [V]	E_{pc}-$E_{1/2}$ [V]	n[b])	$i_{pc}/v^{1/2}C$	E_{pc2}[c]) [V]	i_{pc2} [μA]	E_{pc3}[c]) [V]	i_{pc3} [μA]
1	−1.454	−1.382	−1.418	4.58	3.31	0.722	0.072	−0.036	0.792	0.01448	−1.988	4.86	−2.251	4.70
2	−1.126	−1.061	−1.093	3.60	3.25	0.903	0.065	−0.033	0.88	0.01138	−1.846	3.56	−2.109	2.68
3	−1.213	−1.144	−1.179	4.13	3.42	0.829	0.069	−0.0345	0.826	0.01305	−1.923	5.75	−2.086	2.23
4	−1.219	−1.143	−1.181	3.14	1.37	0.436	0.076	−0.038	0.750	0.00993	−1.908	3.71	−2.338	2.15
5	−1.214	−1.139	−1.176	4.05	3.71	0.916	0.075	−0.0375	0.760	0.01279	−1.960	5.06	−2.249	3.49
6	−1.581	−1.512	−1.546	4.23	2.84	0.672	0.069	−0.0345	0.826	0.01337	−2.016	4.18	−2.200	5.35
7	−1.613	−1.548	−1.580	3.91	2.83	0.724	0.065	−0.0325	0.877	0.01236	−2.039	4.27	−2.224	3.55
8	−1.478	−1.407	−1.442	3.18	2.38	0.748	0.071	−0.0355	0.803	0.01005	−1.918	3.96	−2.129	4.98
9	−1.610	−1.535	−1.573	7.77	5.32	0.685	0.075	−0.0375	0.760	0.02456	−1.952	9.72	−2.173	6.37
10	−1.341	−1.275	−1.308	4.33	4.00	0.926	0.066	−0.033	0.864	0.01368	−2.071	9.06	−2.27(sh)	–
11	−1.376	−1.311	−1.344	2.79	2.25	0.804	0.065	−0.0325	0.877	0.00883	−2.06(sh)	–	−2.154	10.59
12	−1.407	−1.326	−1.366	6.27	5.26	0.838	0.081	−0.0405	0.704	0.01984	−2.23(sh)	–	−2.533	15.53
13	−1.223	−1.154	−1.188	4.37	3.79	0.867	0.069	−0.0345	0.826	0.01382	−1.919	8.89	−2.07(sh)	–
14	−1.434	−1.365	−1.400	4.69	4.14	0.884	0.069	−0.0345	0.826	0.01482	−1.99(sh)	–	−2.090	11.70
15	−1.493	−1.424	−1.458	4.03	3.92	0.971	0.069	−0.0345	0.826	0.01275	−1.99(sh)	–	−2.095	11.75
16	−1.325	−1.257	−1.291	4.25	3.92	0.922	0.068	−0.034	0.838	0.01342	−1.975	11.04	−2.09(sh)	–
17	−1.225	−1.153	−1.189	5.18	2.66	0.515	0.072	−0.036	0.792	0.01636	−1.872	6.48	−2.01(sh)	–
18	−1.370	−1.298	−1.334	4.63	3.54	0.763	0.072	−0.036	0.792	0.01465	−1.974	8.77	−2.146	2.41
19	−1.232	−1.159	−1.196	3.97	3.53	0.889	0.073	−0.0365	0.781	0.01255	−1.923	8.62	−2.02(sh)	–
20	−1.219	−1.148	−1.183	3.91	3.56	0.910	0.071	−0.0355	0.803	0.01236	−1.921	8.28	−2.15(sh)	–
21	−1.226	−1.154	−1.190	2.83	2.24	0.792	0.072	−0.036	0.792	0.00895	−1.898	5.76	–	–
22	−1.213	−1.140	−1.176	3.16	2.95	0.934	0.073	−0.0365	0.781	0.00999	−1.915	7.08	−2.32(sh)	–
23	−1.374	−1.308	−1.341	3.88	2.51	0.647	0.066	−0.033	0.864	0.01226	−2.064	4.45	−2.382	3.99
24	−1.382	−1.316	−1.349	3.71	3.14	0.846	0.066	−0.033	0.864	0.01172	−2.113	3.73	−2.447	3.74

[a]) Substrate, 1.0 mM; $(Bu_4N)ClO_4$, 0.10M; DMF; Pt working electrode; $Ag/AgNO_3$ reference electrode; Pt wire counter electrode; 100 mV/s; r.t.; *E vs.* (Fc/Fc^+)/V; currents reported in μA. [b]) Estimated from $E_{pc} - E_{1/2}/(-0.0285)$ [9]. [c]) sh = shoulder.

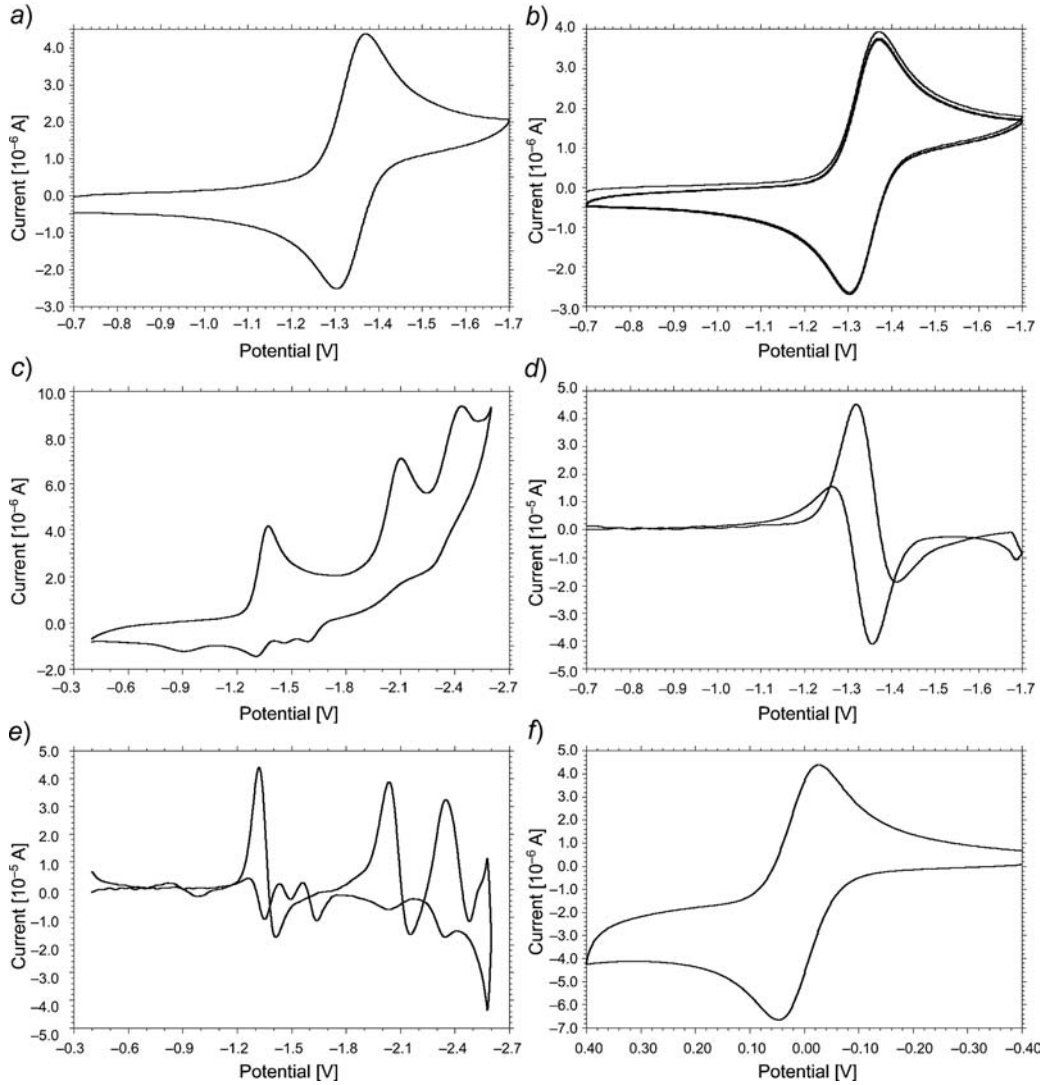


Fig. 1. *Cyclic voltammetric reduction of [1,4-dioxido-3-(trifluoromethyl)quinoxalin-2-yl](4-phenylpiperazin-1-yl)methanone* (**24**) *in DMF at 100 mV/s* (*E vs.* ($Ag/AgNO_3$)/V): *a*) single scan between −0.7 and −1.7 V, *b*) multiple scans between −0.7 and −1.7 V, *c*) single scan between −0.4 and −2.6 V, *d*) first-derivative cyclic voltammogram between −0.7 and −1.7 V, *e*) first-derivative cyclic voltammogram between −0.4 and −2.6 V, and *f*) cyclic voltammogram for the ferrocene redox couple used as a reference for peak-potential determination.

voltammetric wave might be due to a following chemical reaction, *i.e.*, to an EEC mechanism. A third irreversible voltammetric wave was observed at potentials more negative than −2.1 V for all compounds studied, with the exception of compound **21** (*Figs. 1,c*, and *2,c*). This electrode process could be due to reduction of the group at

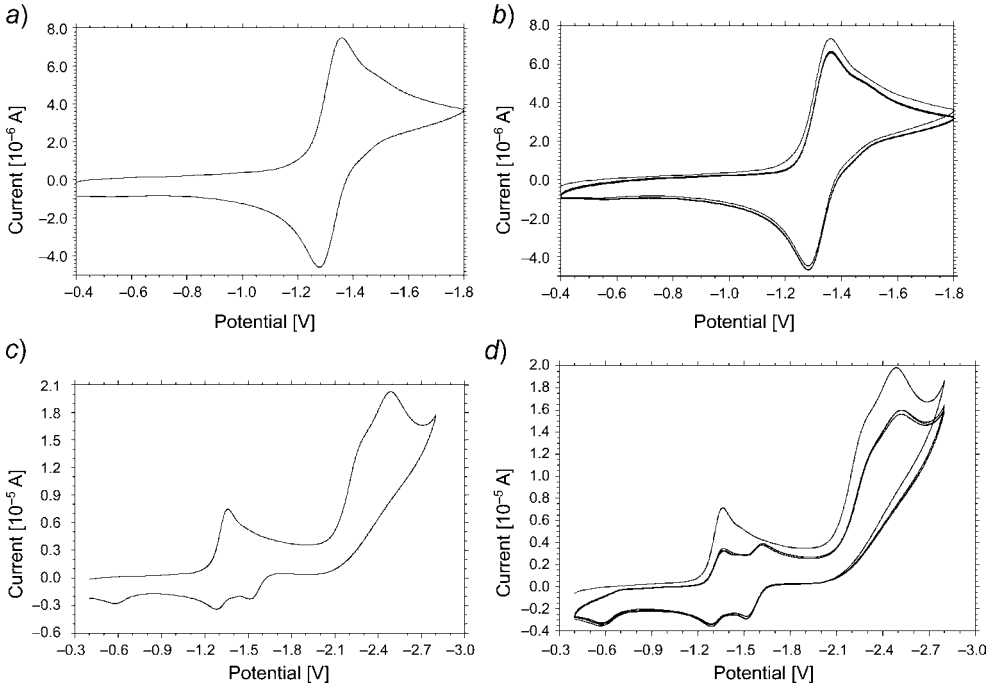


Fig. 2. *Cyclic voltammetric reduction of [7-methoxy-1,4-dioxido-3-(trifluoromethyl)quinoxalin-2-yl]phenylmethanone* (**12**) *in DMF at 100 mV/s* (*E vs.* (Ag/$AgNO_3$)/V): *a*) single scan between −0.4 and −1.8 V, *b*) multiple scans between −0.4 and −1.8 V, *c*) single scan between −0.4 and −2.8 V, and *d*) multiple scans between −0.4 and −2.8 V.

position 2 of the quinoxaline ring. In some cases, the second and third reduction waves were close together so that one of the waves appeared as a shoulder to the other (*Fig. 2,c*). For some of the compounds, a fourth voltammetric process at a more negative potential than the third reduction process was evident in the voltammograms. The second, third, and fourth reduction processes were not investigated in further detail.

For derivative **12**, multiple scans between −0.4 and −2.8 V resulted in the observation of a new redox couple at a more negative potential than the voltammetric wave for the formation of the radical anion (*Fig. 2,d*). Since this electrode process appeared between the first and third voltammetric waves following redox cycling, it is attributed mechanistically to the product formed *via* a chemical step that follows the third voltammetric wave.

Relationship Between Electrochemical Behavior and Structure. Examination of the data for the first voltammetric wave and the anti-*Chagas*-activity data indicates that structure influences both the reduction potentials and activities of these compounds (*e.g.*, *Tables 1* and *2*, *Fig. 3*). The effects of incorporating an electron-withdrawing group (*i.e.*, Cl, F, CHF_2, and CF_3) or an electron-releasing group (*i.e.*, Me and MeO) onto the conjugated quinoxaline ring have been reported in previous studies [7][12–

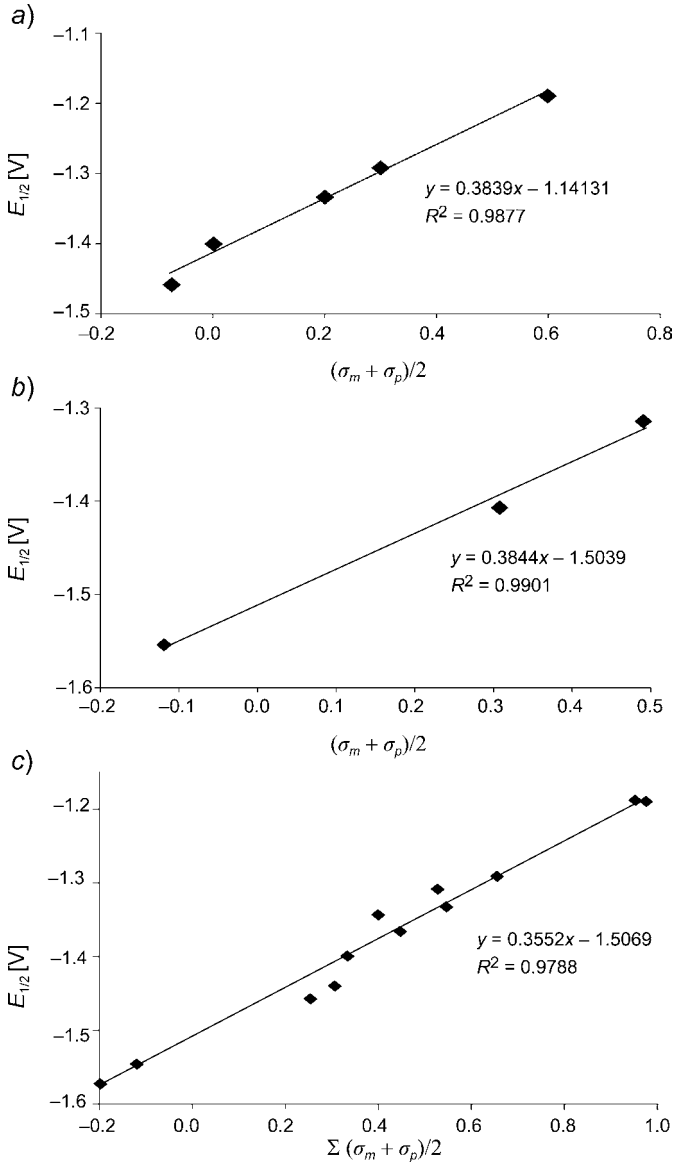


Fig. 3. *Plot of* a) *half-wave potential* ($E_{1/2}$ *vs.* Fc/Fc$^+$) vs. $(\sigma_{m\text{-}x} + \sigma_{p\text{-}x})/2$ *for substituent groups* R^3-/R^4- *of compounds* **14**–**18**; b) *half-wave potential* ($E_{1/2}$ *vs.* Fc/Fc$^+$) vs. $(\sigma_{m\text{-}x} + \sigma_{p\text{-}x})/2$ *for substituent group* R^2 *of* **6**, **10**, *and* **14**, *and* c) *half-wave potential* ($E_{1/2}$ *vs.* Fc/Fc$^+$) vs. $\Sigma(\sigma_{m\text{-}x} + \sigma_{p\text{-}x})/2$ *for substituent groups* R^2 *and* R^3/R^4 *of compounds* **6**–**18**

14][16–18]. The present study correlates well with these earlier reports on the structural effects on potential. Substitution of a single electron-withdrawing group at the quinoxaline ring system at position 3 resulted in a positive shift in reduction

potential and an increase in the activity. For example, replacing the 3-Me group in compound **6** with the CHF_2 group in compound **14** and the CF_3 group in compound **10** resulted in positive shifts in $E_{1/2}$ of 0.146 V and 0.238 V, respectively. The anti-*Chagas* activities of these three derivatives followed the same order, *i.e.*, compound **10** was the most active and compound **6** the least active. Other examples include derivatives **7** *vs.* **11**, and **8** *vs.* **13**. The same effect resulted when similar substitutions were made at positions 6 or 7 (*cf.* compounds **6** *vs.* **8**, **10** *vs.***13**, and **14** *vs.* **16** *vs.* **17** *vs.* **18**), whereas an electron-donating group in these positions resulted in a more hindered reduction and generally decreased activity. For example, replacing the H- atom at C(7) in compound **6** with the MeO group in **9** and the Me group in **7** resulted in shifts in $E_{1/2}$ of -0.027 V and -0.034 V, respectively. These decreases in potential were accompanied by concurrent decreases in the activities of these three compounds. Other examples include derivatives **10** *vs.* **11** *vs.* **12**, and **14** *vs.* **15** *vs.* **16**. In addition, substitution by a second electron-withdrawing Cl-group at the quinoxaline ring enhanced the positive shift of the potential and of the activity (*cf.* **16** *vs.* **17**), coinciding with a previously reported study [16]. These results were consistent with the facilitation of quinoxaline reduction by a positive charge at the reaction site [18]. This was further demonstrated by *Fig. 3*, which showed that the reduction potentials for the quinoxaline derivatives within various analogues generally fitted the modified *Hammett* equation, $\Delta E_{1/2} = \rho_{\pi R}\sigma_x$ (correlation coefficients ranged from 0.98 to 0.99), where σ_x is the polar inductive electronic substituent constant taken as the average of the sum of σ_m and σ_p (*Table 4*, [19]) and ρ is the reaction constant [18][20]. Comparison of the data in *Tables 1* and *2* demonstrated that the substituent group R^1 had relatively little effect on the reduction potential, presumably due to the distance from the site of reduction. $E_{1/2}$ Values for compounds **3**–**5** covered a range of only 0.005 V, and those for compounds **13** and **19**–**22** spanned a range of only 0.020 V from most positive to most negative ones.

Table 4. Hammett *Substituent Constants*[a])

	R^3/R^4	$\sigma_{p\text{-}x}$	$\sigma_{m\text{-}x}$	$(\sigma_{m\text{-}x}+\sigma_{p\text{-}x})/2$	R^2	$\sigma_{p\text{-}x}$	$\sigma_{m\text{-}x}$	$(\sigma_{m\text{-}x}+\sigma_{p\text{-}x})/2$	$\Sigma(\sigma_{m\text{-}x}+\sigma_{p\text{-}x})/2$
6	H/H	0	0	0	Me	−0.17	−0.07	−0.12	−0.12
7	Me/H	−0.17	−0.07	−0.12	Me	−0.17	−0.07	−0.12	−0.24
8	F/F	0.12	0.68	0.4	Me	−0.17	−0.07	−0.12	0.28
9	MeO/H	−0.27	0.12	−0.075	Me	−0.17	−0.07	−0.12	−0.195
10	H/H	0	0	0	CF_3	0.54	0.43	0.485	0.485
11	Me/H	−0.17	−0.07	−0.12	CF_3	0.54	0.43	0.485	0.365
12	MeO/H	−0.27	0.12	−0.075	CF_3	0.54	0.43	0.485	0.41
13	F/F	0.12	0.68	0.4	CF_3	0.54	0.43	0.485	0.885
14	H/H	0	0	0	CHF_2	0.32	0.29	0.305	0.305
15	MeO/H	−0.27	0.12	−0.075	CHF_2	0.32	0.29	0.305	0.23
16	Cl/H	0.23	0.37	0.3	CHF_2	0.32	0.29	0.305	0.605
17	Cl/Cl	0.46	0.74	0.6	CHF_2	0.32	0.29	0.305	0.905
18	F/H	0.06	0.34	0.2	CHF_2	0.32	0.29	0.305	0.505

[a]) *Hammett* substituent constant values are taken from [19].

The data suggested that a relationship between reduction potential and activity for the described quinoxaline derivatives is possible. In this case, bioreductive activation

would generally be expected to be more facile for the more easily reduced derivatives. In general, upon comparing the activity data with the reduction potential data presented in *Table 1*, it can be observed that the most active compounds, with a *PGI* of 100% and IC_{50} below that of the drug Nfx, were those that had the least negative reduction potentials. The three compounds with activities well below a *PGI* of 100%, *i.e.*, compounds **6**, **7**, and **9**, all had half-wave potentials more negative than -1.5 V (*vs.* Fc/Fc^+). Likewise, compounds **6** and **7** had IC_{50} values above 25 μM (the IC_{50} value of **9** was not measured). Most of the derivatives possessing either a CF_3 or CHF_2 group at C(3) had potentials more positive than the most easily reduced and biologically active 3-Me derivative, *i.e.*, compound **1**, and were generally much more active than 3-Me derivatives **6**–**9**. In a previous study, we were able to demonstrate that mitochondrial dehydrogenases are involved in the anti-*T. cruzi* activity of the most active derivatives [6]. Specifically, derivatives **3** and **13** decreased mitochondrial-dehydrogenases activity in the parasites and demonstrated better animal-survival percentages in the Y-infected mice than benznidazole. Thus, it is conceivable that the redox behavior of these quinoxaline derivatives is important to their mechanism of action. However, a direct correlation between anti-*Chagas* activity and potential for all the derivatives used in this study was not observed when taken together. The fact that certain exceptions exist in the data, *cf.* derivatives **4** and **5**, indicates that redox behavior alone cannot be used to explain the activities observed for these compounds. Obviously, electrochemical data must be interpreted with some degree of caution. Aside from bioreduction, factors such as lipophilicity, diffusion, configuration, solubility, metabolism, absorption or site binding, and bioactivation must also be considered when determining the *in vivo* mechanism of action of antichagasic compounds. A detailed study of the mode of action of the 1,4-dioxidoquinoxalin-2-yl ketone derivatives is beyond the scope of this article, and the exact mechanism of action remains unclear at this point. Nevertheless, the results presented above indicate that the quinoxaline *N*-oxide system constitutes a starting point for further chemical modifications to improve the *T. cruzi* activity *via* a possible bioreduction mechanism, and support further *in vivo* studies of these compounds in animal models of *Chagas* disease.

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