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PAPER

Direct sequential C-H iodination/organoyl-thiolation for benzenoid A-ring modification of quinonoid deactivated systems: A new protocol for potent trypanocidal quinones

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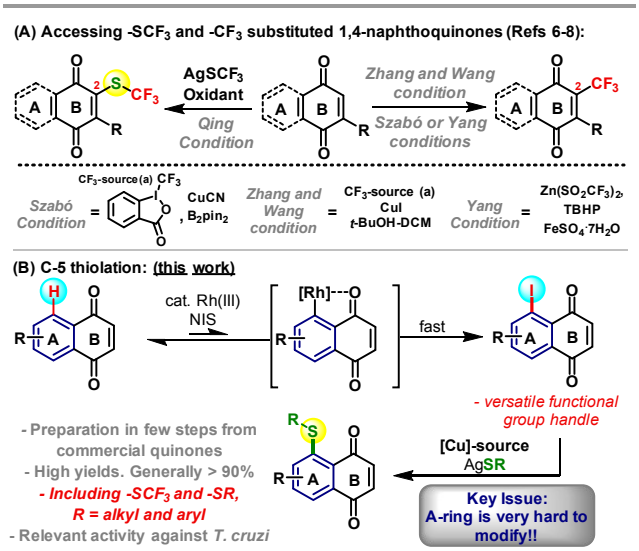
We report a sequential C-H iodination/organoyl-thiolation of naphthoquinones and their relevant trypanocidal activity. Under a combination of AgSR with a copper source, sulfur-substituted benzenoid quinones were prepared in high yields (generally > 90%). This provides an efficient and general method for preparing A-ring modified naphthoquinoidal systems, recognized as a challenge in quinone chemistry.

1,4-Naphthoquinones (1,4-NQs) are considered privileged structures implicated in important biological processes, as, for instance, electron carriers in the electron transport chain involved in cellular respiration and photosynthesis.[1] The notable bioactivity of naphthoquinones is intrinsically related with redox mechanisms, generation of reactive oxygen species, and more recently also associated to inhibition of deubiquitinases[2] and other enzymatic targets. Illustrious 1,4-NQs include atovaquone, the lapachones and the juglomycins, all of them possessing important biological activities.[2,3] Due of their biological importance, scientific endeavor has been devoted aiming at new synthetic methods for preparing 1,4-NQs with potential applicability against illness such as Chagas disease.[4]

Among the well-known functional groups in chemistry, -SCF_3 and -CF_3 groups have attracted attention because of their importance in pharmaceutical and agrochemical industries.[5] For example, incorporation of the trifluoromethylthio (-SCF_3) group into drug candidates often brings beneficial effects on the drug's metabolic stability and bioavailability, as discussed by Xue and co-workers.[5a] The strong electronegativity and high lipophilicity of this group may be associated with an improvement in biological activity. Moved by this information, recently Szabó,[6a] Zhang and Wang,[6b] Qing[7] and Yang[8] groups have developed synthetic methods by using C-H functionalization to introduce fluorinated groups in the quinoidal system (Scheme 1A). All conditions lead to the insertion of fluorinated groups at the position C-2, exemplifying well-established methods for B-ring substitution. As previously discussed by us,[9] flexible protocols for direct functionalization of the benzenoid A-ring are rare and limited to a few recently reported examples.[10] The development of new strategies/methodologies for preparing benzenoid A-ring modified 1,4-NQs represents a challenging task.



Scheme 1

The lack of general methods to promote these modifications is associated with deactivated quinoidal systems due to low nucleophilicity of the benzenoid A-ring and weak coordinating ability of the B-ring carbonyls in the case of metal catalysis. To the best of our knowledge, there are only two protocols that enable catalytic carbonyl directed C-H functionalization in 1,4-NQs.[9,10] The first example was reported by Zhang group, where rhodium catalysis was used to perform mono alkenylations with amino-naphthoquinones.[9] One drawback of the method described by Zhang is the necessity of an amino group at C-2 to compensate the electron deficiency of the quinoidal system and increase the catalyst substrate coordination allowing an efficient catalytic process. In this context, recently the da Silva Júnior and Bower groups have demonstrated the effectiveness of $[RhCp^*Cl_2]_2$ for the first catalytic directed *ortho*-functionalizations of simple (non-bias)

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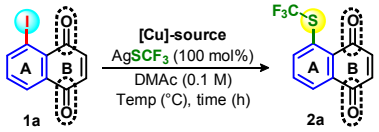
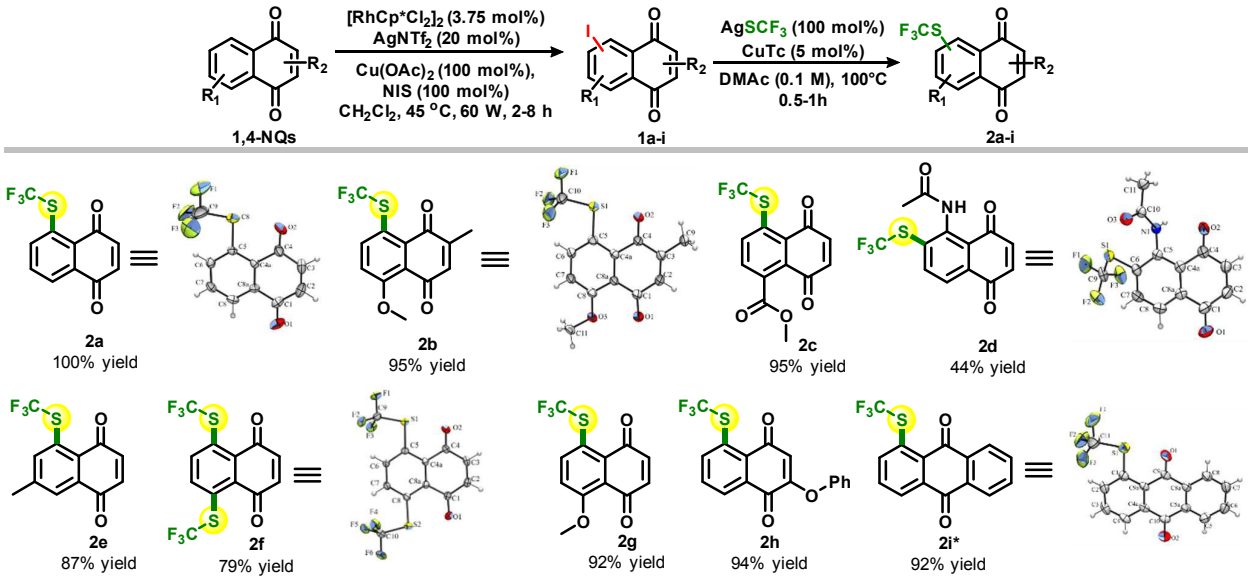
1,4-NQs by using different electrophilic iodine or bromine sources.[10] This innovative method has encouraged us to devote efforts in the development of new synthetic methodologies aiming at the modification of A-ring of the naphthoquinoidal scaffold.

Herein our strategy was based on the general and powerful method to install iodine at C-5 with high yield and regioselectivity and subsequently to combine $AgSCF_3$ with a copper source, achieving the thiolated adducts in only two steps via a direct sequential C-H iodination/organoyl-thiolation protocol (Scheme 1B). Preliminary studies involved the reaction of 5-iodo-1,4-naphthoquinone **1a** with $AgSCF_3$ in room temperature and only trace quantities (≤5%) of the product **2a** was observed (entry 1). The use of high temperature (100 ºC) produced the desired product in 39% yield (entry 2). Based on the methodology previously described by Szabó[6] and Qing,[7] we decided to investigate the effectiveness of a copper source in this reaction. Pleasingly, the experiments revealed impressive results. $AgSCF_3$/CuI resulted in the formation of product **2a** in 100% yield (entry 3). CuBr as cooper source was less effective and product **2a** was obtained in 81% yield. Further refinement aiming to minimize the amount of CuI and reaction time affords **2a** in 90% yield when 5 mol % of copper and 0.5 h was used (entry 5). Despite the effectiveness of the system (entry 5), we have continued our investigation looking for alternative Cu-salt for a quantitative preparation of **2a**. Copper(I)-thiophene-2-carboxylate (CuTc) is a well-known agent able to promote diverse reactions, for instance, Ullmann reaction.[11] As discussed by Liebeskind and co-workers,[11] CuTc is a powerful salt for this reaction, probably due to its ability to allow internal coordination from the sulfur atom to the metal and/or due to an inherent ability of the carboxylate to work as a ligand to stabilize the oxidative addition product. In this context, we have considered the use of this Cu-salt to improve the reactional conditions. As expected, CuTc was very valuable and **2a** was obtained in 100% yield in all cases (entries 7-10), except at room temperature (entry 6). The scope of this new method is outlined in Scheme 2. Iodinated naphthoquinones possessing different pattern of substitution underwent trifluoromethylthiolation to furnish **2b**, **2c** and **2e**-**i** in excellent yields. Only compound **2d** was prepared in moderate 44% yield, probably due to a larger steric hindrance caused by the bulky amide group at C-5. Structural assignments were based on detailed NMR analysis (DEPT, COSY, HSQC, HMBC) and X-ray structures of **2a**, **2b**, **2f**, **2d** and **2i**.

Table 1 Selected optimization results.



Entry	Cu-source (mol%)	Temp/°C	Time/h	Yield (%)
1	none	r.t.	24	trace
2	none	100	24	39
3	CuI (10)	100	0.5	100
4	CuBr (10)	100	0.5	81
5	CuI (5)	100	0.5	90
6	CuTc (10)	r.t.	24	24
7	CuTc (10)	50	24	100
8	CuTc (10)	100	1	100
9	CuTc (10)	100	0.5	100
10	**CuTc (5)**	**100**	**0.5**	**100**



Scheme 2 Scope of the selective trifluoromethylthiolation protocol.*Iodinated derivative **1i** used to prepare compound **2i** was not prepared by C-H activation reaction.

The potential of the trifluoromethylthiolation method herein described for preparing benzenoid substituted quinonoid compounds was also investigated with the derivatizations of 5-trifluoromethylthio-1,4-naphthoquinone **2a** and anthraquinone **2i**. Reactions of **2a** and **2i** with MCPBA in CH_2Cl_2 afforded the respective quinones-based trifluoromethyl sulfoxide **3a** and **3b** in moderate 74% and 70% yields, respectively. The structure of **3a** was also solved by crystallographic methods, reconfirming the obtention of the product already determined by NMR and HRMS analysis. Ruthenium trichloride-sodium periodate system described by Su[12]

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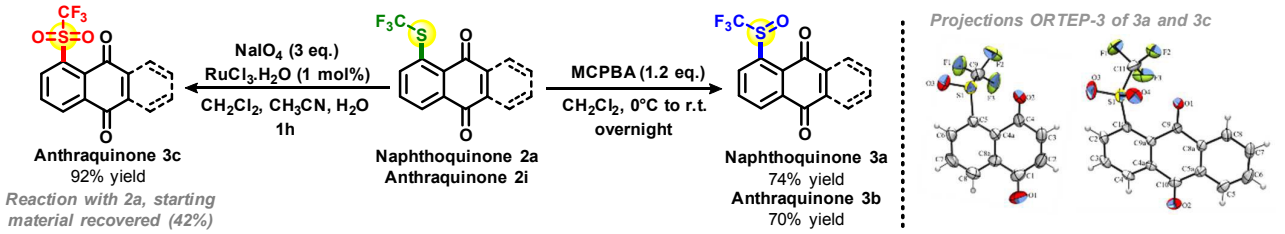
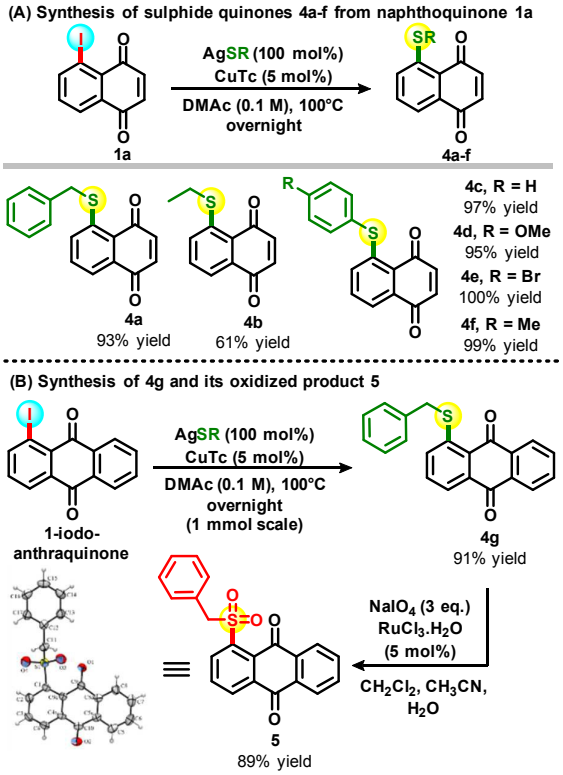
was used to perform the oxidation of the -SCF_3 group to the respective sulfone which afforded **3c** in excellent 92% yield and the structure was also confirmed by X-ray studies. The same procedure has been attempted by using **2a** as substrate. In this case, we have recovered the starting material in 42% yield and the product of oxidation was not observed (Scheme 3).



Scheme 3 Derivatizations based on the oxidation of **2a** and **2i**.

The versatility of the sequential C-H iodination/organoyl-thiolation process was also studied by using different AgSR-salts for preparing new sulfur-containing quinones **4a**-**f**. With the iodinated quinone **1a** in hands the respective sulphides **4a**-**f** were prepared in excellent yields with exception of compound **4b** synthesized in 61% yield (Scheme 4A). Compounds **4d**, **4e** and **4g** were also solved by X-ray crystallography. Benzyl, alkyl and aryl derivatives were easily prepared showing clearly the potential of this methodology for the preparation of A-ring substituted quinones with different substitution patterns. These important findings open new avenues, for example, related to Medicinal Chemistry, where in general it is necessary the synthesis of a family of compounds aiming SAR investigation.



Scheme 4 (A) Synthesis of sulphide quinones **4a**-**f**. (B) Synthesis of the sulphide quinone **4g** and the respective oxidized product **5**.

1-Iodo-anthraquinone was used to prepare **4g** in 91% yield (Scheme 4B). Subsequently, **4g** was oxidized via Su procedure[12] and the product **5** was prepared in 89% yield. The structure of **5** was also determined by X-ray analysis in addition to conventional methods of analysis.

Exploratory reactions aiming to propose a mechanism for the organoyl-thiolation process were conducted. As suggested by Szabó group,[6] CF_3 radicals may be involved in the reaction of C-H trifluoromethylation of quinones. TEMPO, which is a well-known radical scavenger, was used to evaluate the presence of radicals in those reactions. Thus, we have accomplished inhibition experiments with TEMPO, 4-oxo-TEMPO and 4-hydroxy-TEMPO (Scheme 5A). In all cases, quinone **2a** was obtained in 100% yield indicating that the reaction does not proceed via a radical mechanism. Subsequently, we have considered three experiments: (a) Presence and absence of Cu-source, (b) Absence of Ag-source and (c) Absence of AgSR (Schemes 5B for **2a** and SI for **4a**). These experiments have indicated that CuTc and AgSR are essential for forming **2a** and **4a** since the absence of Cu-source affords **2a** and **4a** in only 39% and 21% yields, respectively. Without Ag-salt, no reaction was observed in relation to **2a** and in the case of **4a**, a complex mixture of products was observed. Finally, the use of different Ag- and S-salts led to a complex mixture of products in both cases. Based on our observations and data already reported in the literature, a proposed mechanism was formulated and described in Scheme 5C. The mechanism starts with complexation between the readily available organosulfur anion and the copper complex to generate active species $TcCu^{I}SR$ plus a free Ag^{+} cation, similarly to a Liebeskind–Srogl coupling.[13a] Another -SR source (Me_4NSCF_3 and $PhCH_2SNa$) led to a complex mixture of products, even with the prior addition of another silver salt (AgOAc), which leads us to believe that ion exchange between the thio-silver salts and the $Cu^{I}Tc$ catalytic species is fundamental. In fact, air-stable bonded copper-thiolate complexes like $(bpy)Cu^{I}SCF3$ were broadly described.[13b] Finally, an oxidative addition event (**A**), generates the metalated species (**B**) and silver iodide. Reductive elimination (**C**) of the Cu^{III} complex affords the desired functionalized naphthoquinone and regenerates the active Cu^{I} species for the next catalytic cycle.

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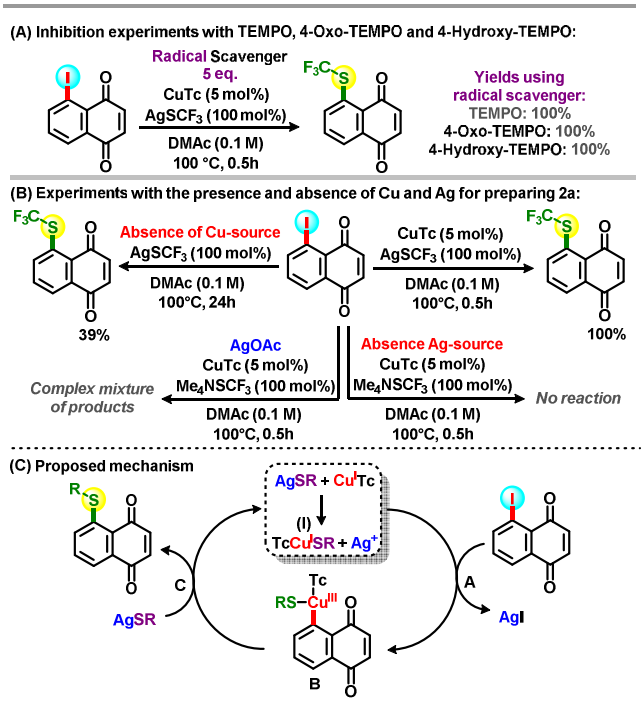

Scheme 5 Mechanistic investigation for the synthesis of sulphide quinones.

The potential of the sulfur-substituted benzenoid 1,4-NQs **2a**-**2i**, **3a**-**3c**, **4a**-**4g** and **5** as trypanocidal compounds have been investigated by screening them against trypomastigote forms of *Trypanosoma cruzi* (Tables 2). Benznidazole (Bz) and nifurtimox (Nif) are the current drugs used to treat Chagas disease. The efficiency of these drugs is related to the phase of the disease and their effectiveness is reduced in advanced stages of the infection. In this sense, the limited curative capacity in chronic phases, and high toxic effects make necessary the development of new trypanocidal drugs.[14] To our delight, only compounds **2b**, **2i** and **3b**-**c** were not active against the parasite with IC_{50}/24h >1000.0 μM. Trifluoromethylated NQs **2a**, **2c**-**e**, **2g** and **2h** have shown significant activity against *T. cruzi* with IC_{50} in the range of 67.3 to 32.7 μM, with special attention to compounds **2c** (IC_{50} = 34.5 μM) and **2d** (IC_{50} = 32.7 μM). In comparison to the standard drug, Bz (IC_{50} = 103.6 μM), **2c** and **2d** are 3.0 and 3.1-fold, respectively, more active. Finally, **4a**-**g** and **5** were also evaluated against the parasite. Compound **4f** with IC_{50} = 2.5 μM was almost 42 times more active than Bz. *This is a promising result, demonstrating the potential of A-ring substituted quinones as trypanocidal drugs*. Most active quinones evaluated against bloodstream forms of *T. cruzi* (IC_{50}/24 h) were also assayed against mammalian cells (LC_{50}/24 h), allowing the determination of the selectivity index (SI) derived from the ratio of LC_{50}/IC_{50} (Table 3). Relevant biological aspects are being currently investigated in our laboratories and will be reported in due course.

Table 2. Activity of napthoquinones on trypomastigote forms of *T. cruzi*.

Compounds	IC_{50}/24 h[a] (μM)
2a	41.2 (± 3.5)
2b	>1000.0
2c	34.5 (± 6.3)
2d	32.7 (± 4.1)
2e	67.3 (± 5.5)
2f	240.1 (± 14.8)
2g	41.9 (± 5.8)
2h	54.4 (± 5.5)
2i	>1000.0
3a	67.3 (± 6.1)
3b	>1000.0
3c	>1000.0
4a	14.3 (± 0.9)
4b	31.3 (± 2.4)
4c	12.4 (± 2.2)
4d	7.6 (± 0.3)
4e	6.6 (± 0.8)
4f	2.5 (± 0.3)
4g	64.9 (± 5.9)
5	36.3 (± 2.2)
Benznidazole	103.6 ± 0.6[4a]

[a]Mean ± SD of at least three independent experiments.

Table 3. Most active naphthoquinones selected to trypanocidal (in different conditions) and cytotoxicity assays.

	IC_{50}/24 h (μM)		LC_{50}/24 h (μM)	
Compd	**5% blood, 4 °C**	**0% blood, 37 °C**	**0% blood, 37 °C**	**SI**
4a	14.3 (± 0.9)	1.1 (± 0.2)	10.5 (± 0.2)	9.5
4c	12.4 (± 2.2)	0.3 (± 0.04)	3.6 (± 0.7)	12.0
4d	7.6 (± 0.3)	1.3 (± 0.03)	3.3 (± 0.3)	2.5
4e	6.6 (± 0.8)	1.9 (± 0.03)	6.0 (± 1.0)	3.2
4f	2.5 (± 0.3)	0.3 (± 0.06)	3.7 (± 1.4)	12.3

As the trypanocidal activity of naphthoquinones is intrinsically related to their ability to generate reactive oxygen species in a redox mechanism, we associated the biological activity of selected compounds with electrochemical studies. Cyclic voltammograms (CV) of the sulphide naphthoquinones display the common quinone behaviour, represented by two redox systems (*E*pIc/*E*pIa and *E*pIIc/*E*pIIa), as exemplified in Figure 1 for compounds **2a** and **2d**, in several scan rates. The main electrochemical parameters and CVs for **2a**, **2b**, **2d**, **2f** and **2h** are listed in Table S2 and shown in Figure S1. Considering the ease of the reduction related to *E*pIc, the compounds can be ranked as shown in the Figure 1. Redox potentials in the range of -0.336 V and -0.440 V correspond to the most active quinones.

In conclusion, we have reported efficient and reliable methodologies for preparing sulfur-substituted benzenoid quinones in high yields. The chemistry opens up new avenues for the synthesis of potent trypanocidal drugs. This manuscript represents successful example of methodologies developed for the preparation of A-ring substituted quinones. A complex task that requires the use of powerful but simple strategies based on C-H metalation arsenal.

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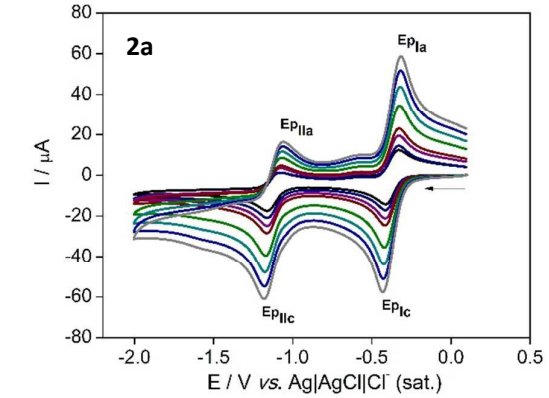

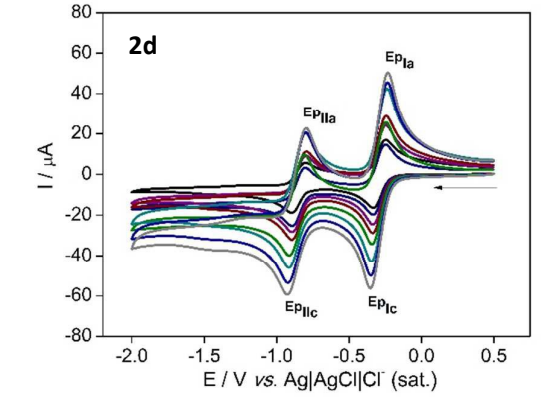

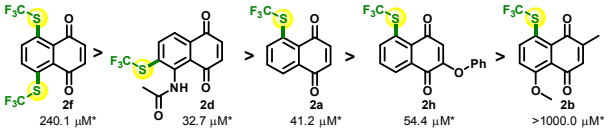

Fig. 1 Cyclic voltammetry for **2a** and **2d** (1.0 mM) at GC electrode (3 mm in diameter) in DMF containing 0.1 M $TBAPF_6$ at different scan rates of 35, 50, 75, 100, 200, 300, 400 and 500 mV s^{-1}. Structures of the trifluoromethylated 1,4-NQs ranked according to data obtained through electrochemical studies. *IC_{50}/24 h values for the lytic activity on bloodstream trypomastigotes.

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