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Synthesis and biological characterization of new aryloxyindole-4,9-diones as potent trypanosomicidal agents

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Abstract

A new indole-4,9-dione and their phenoxy derivatives were synthesized and evaluated *in vitro* against the epimastigote form of *Trypanosoma cruzi*, Y strain. All of these novel compounds were found to be extremely potent and selective that the standard drug nifurtimox. Interestingly, phenoxyindole-4,9-dione **9d** displayed excellent nanomolar inhibitory activity, IC_{50}=20 nM, and high selectivity index, SI = 625. *In silico* studies using MOE program were performed to generate a preliminary pharmacophore model.

Keywords: Indolequinones, anti-*T. cruzi*, cytotoxicity, pharmacophore model.

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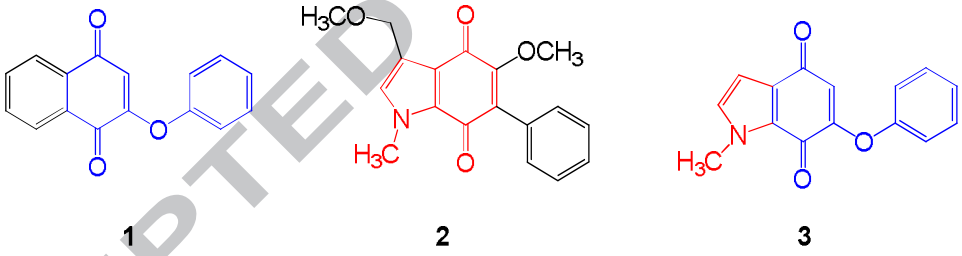
Chagas disease, or American tripanosomiasis, is a neglected tropical disease caused by the protozoan *Trypanosoma cruzi*, which affects 16-18 million people in many rural areas of Latin America.[1] There are two main drugs used for the treatment of this disease, nifurtimox (Nfx) and benznidazole, but both are toxic and present severe side effects.[2,3] Therefore, there is a need for more effective drugs and many efforts have been done in the search for new compounds with potential clinical utility.[4] Taking into account the anti-trypanosomal activity of natural naphthoquinones, the preparation of many compounds

containing the naphthoquinone pharmacophore and their activity on *Trypanosoma cruzi* were described.[5] In addition some heterocyclic quinones such as naphthopyranoquinones, naphthofuranoquinones showed interesting trypanosomicidal activities.[6-8] Furthermore, the potent trypanosomicidal activity of 2-phenoxy-1,4-naphthoquinone (**1**, Fig. 1) against the amastigote forms of *T. cruzi* with an IC_{50} of 1.70 μM but low selectivity index (SI < 5) was described.[9] Some studies suggest that the anti-trypanosomal activity of quinones is related to oxygen radical formation and consequently redox cycling.[10] Moreover, the presence of heteroatoms in the aromatic moiety contribute to an enhancement of the anti-trypanosomal activity.[8] On the other hand, the potent cytotoxic activity of some indolequinones, such as compound **2** (Fig. 1), has been described by Moody *et al.*[11,12] The proposed mechanism of action of this indolequinone implicates the induction of caspase-dependent apoptosis but does not involve redox cycling or oxidative stress in all cancer cells assay. Based on these precedents, we became interested in the synthesis of new aryloxy indolequinones (using as template compound **3**, Fig. 1) as compounds with enhanced trypanosomicidal activity and decreased cell cytotoxicity.

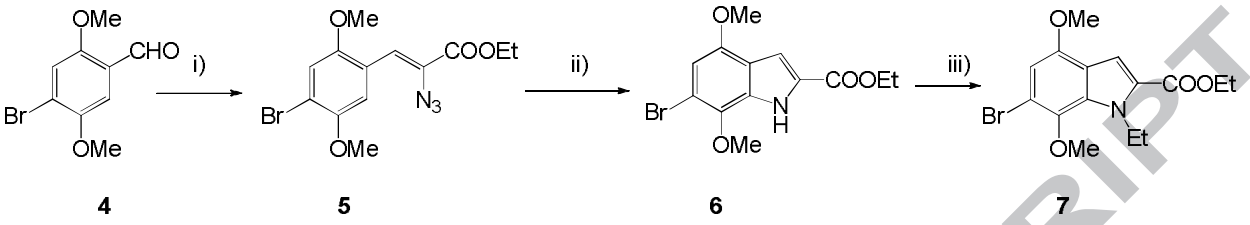


1 **2** **3**

Figure 1. Chemical structures of compounds with biological properties and designed template for new trypanosomicidal agents.

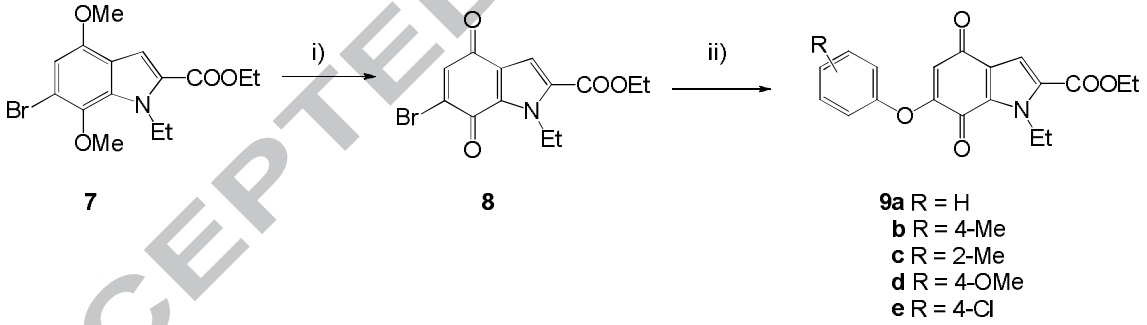
Knowing that the usual approach for the preparation of aryloxyquinones is done by nucleophilic substitution reaction of haloquinones with phenols,[13-15] we decided to synthesize the unknown bromoindol-4,7-dione **8** (Scheme 2). Moreover, considering the versatility of the Hemetsberger-Knittel reaction[16-18] for the preparation of indoles, we take advantage of this methodology to obtain bromoindol **7** (Scheme 1). Thus, reaction of 4-bromo-2,5-dimethoxybenzaldehyde (**4**)[19] with ethyl azidoacetate and subsequent thermal cyclization of 2-azidocinnamate **5** resulted in the formation of indole **6** in 52% yield (two

steps). Next, treatment of compound **6** with bromoethane in the presence of NaH in DMF afforded the *N*-alkylated indole **7** (Scheme 1).



Scheme 1. *Reagents and conditions:* i) Ethyl azidoacetate, Na, ethanol, -10 °C, 3 h, 57%; ii) toluene, reflux, 12 h, 92%; iii) NaH, bromoethane, DMF, 1 h, 98%.

Oxidative demethylation of indol **7** with excess silver (II) oxide and HNO_3 in tetrahydrofuran[20] gave bromoquinone **8**. Finally, aryloxyindole-4,9-diones **9a-e** were obtained in 36-72% yield by nucleophilic substitution reaction of **8** with phenols and sodium hydride in tetrahydrofuran (Scheme 2). Compound **1** was prepared as described,[9] by reaction of 2-bromo-1,4-naphthoquinone with phenol and potassium carbonate in dimethyl formamide (DMF).



Scheme 2. *Reagents and conditions:* i) AgO, HNO_3 6N, THF, rt, 5 min, 68%; ii) NaH, ArOH, THF, rt, 10 min, 36-72%.

The *in vitro* trypanosomicidal activity of compounds **6**-**8** and phenoxyquinones **1**, **9a-e** was initially tested against the epimastigote form of *T. cruzi*, Y strain (Tc II).[21] For each derivative a dose-response assay, between 0.01 and 50 μM, was evaluated to calculate the IC_{50} concentration (50% inhibitory concentration). Nfx (Bayer) was used as the reference trypanosomicidal drug.[11] All phenoxyquinones showed potent trypanosomicidal activity

and they are over tenfold more active that the reference drug Nfx, which has an IC_{50} of 7.0 μM (Table 1). Among them, compounds **9b** and **9d** displayed the most potent inhibitory activity (IC_{50} = 0.02 μM for epimastigotes). While it is not possible to establish a structure–relationship with these compounds, there are some preliminary analyses that we can do with these results. First, the presence of donor groups at C-4 in the phenyl ring of the phenoxyindolequinone derivatives have a remarkable effect on the trypanosomicidal activity and the most active compounds were **9b** (4-Me) and **9d** (4-OMe) with an IC_{50} of 0.02 μM. On the other hand, compounds without a phenoxy group bound to the indolequinone system, had the lowest activity (compound **8**). This fact indicates the importance of the phenoxy moiety on the eventual pharmacophore structure. Finally, a potential antichagasic drug must show low toxicity in mammalian host cells and for this reason, the cytotoxic effects in J774 murine macrophage-like cells (ATCC, USA) of compounds with the strongest trypanosomicidal effects *vs* epimastigotes were determined.[23] It is well known that naphthoquinones generate ROS (Reactive oxygen species) and, as expected several of the tested compounds were quite cytotoxic. According to the strategies for the development of novel drugs for tropical disease the selective index should be higher than 50.[22,23] Table 1 shows for some compounds with higher epimastigote toxicity with IC_{50} values for J774 murine cell proliferation and their selectivity indexes as the ratios of the IC_{50} in epimastigotes. These results indicate all of these compounds tested are more selective than Nfx (SI = 40). The most remarkable results came from compound **9d**, which exhibits greater selectivity in regard of its toxicity toward murine cells (SI = 625). This result is very promising for the developing of new selective and more potent trypanosomicidal agents.

Table 1. Effect of phenoxyindolequinones upon culture growth of *T. cruzi* and selectivity index *vs.* murine cells.

Compound	%GI[a,b]	epimastigote IC_{50} (μM)[b]	J774 IC_{50} (μM)	SI[c]
1	87.2 ± 2.1	0.05 ± 0.02	12.5	250
6	26.9 ± 7.2	-	-	-
7	2.9 ± 1.3	-	-	-
8	97.1 ± 2.2	0.53 ± 0.12	n.d.	-
9a	84.7 ± 2.4	0.22 ± 0.08	22	100
9b	92.1 ± 3.2	0.02 ± 0.01	n.d.	-
9c	100.0	0.14 ± 0.05	12.5	89.3
9d	90.5 ± 4.7	0.02 ± 0.01	12.5	625
9e	94.6 ± 2.3	0.13 ± 0.05	n.d.	-
Nfx[d]	49.9	7.00	316	40

[a] %GI = Percentage of growth inhibition on *T.cruzi* culture Y at 10 μM.

[b] The results are means of three independent experiments.

[c] Selectivity Index: expressed as the ratio of IC_{50} in J774 cells to IC_{50} in epimastigotes.

[d] 5μM was used for this assay.

n.d.: not determined.

Then, MOE program[24] was used to generate a preliminary pharmacophore model[25,26] related with *in vitro* trypanosomicidal activity (IC_{50}) of compounds **1**, **7-9a-e**. First, to determinate the essential chemical features of those molecules with more relevant (lower) IC_{50} values, compounds **9b** and **9d** were chosen to create the pharmacophore model ("Activity Pharmacophore", Figure 2). With fully optimized structures of compounds **9b** and **9d**, it was possible identify regions of pharmacophoric importance such as; aromatic ring (Aro), hydrogen bond acceptor (Acc) and hydrophobic rings (Hyd) (Figure 2b-c). Furthermore, the distance between the pharmacophoric features was measured and shown in the Figure 2d.

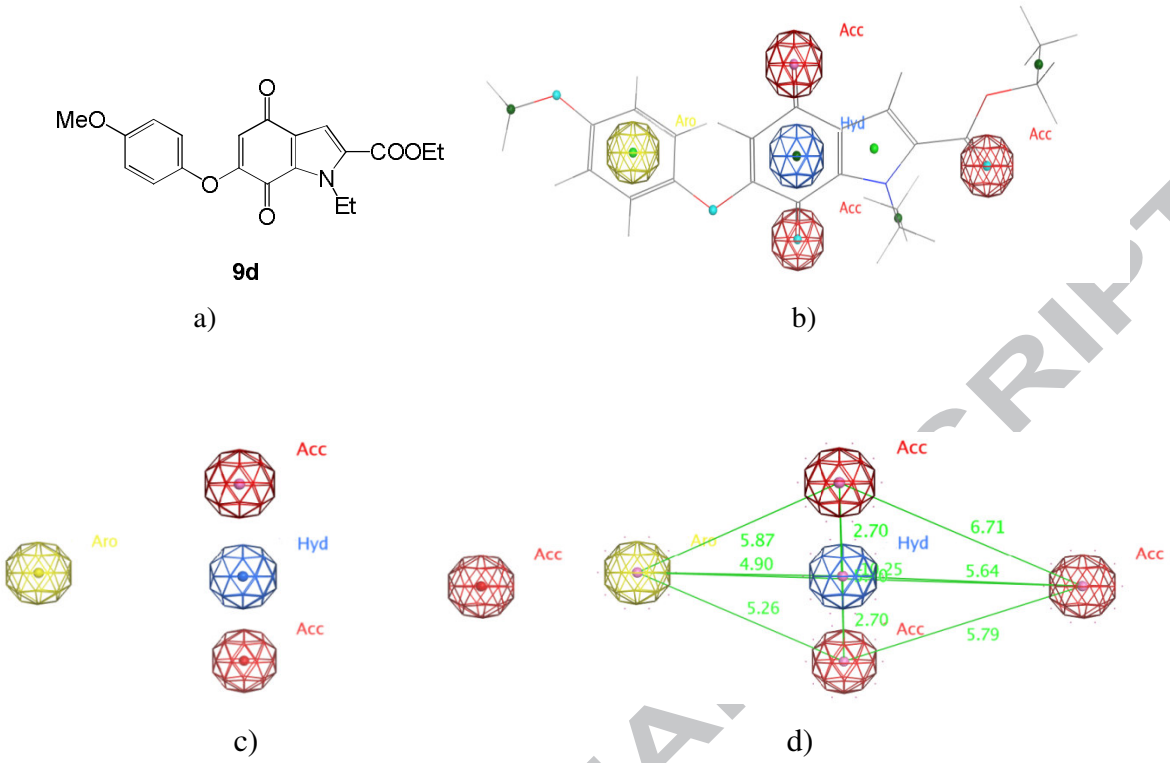
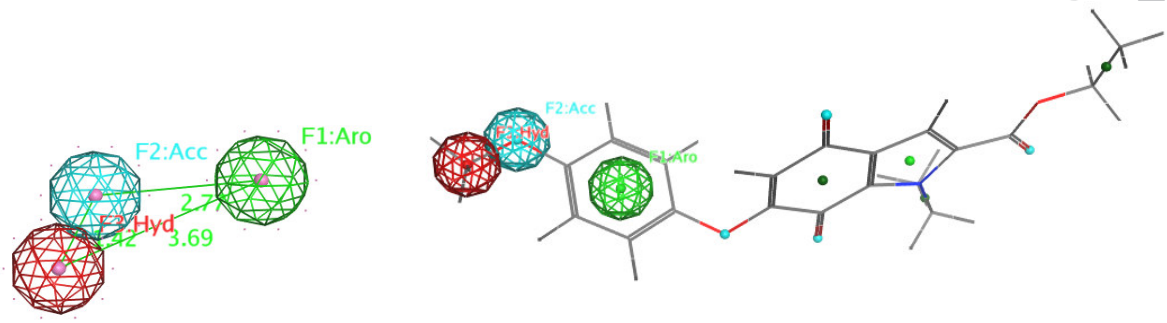


Figure 2. Graphical display of "Activity Pharmacophore". **a)** 2D picture of the modelled **9d** molecule. **b)** 3D drawing of **9d** molecule overlapped with the pharmacophore picture. **c)** 3D drawing of isolated pharmacophore. The different features are depicted in yellow (aromatic), red (H-bond acceptor) and blue (hydrophobic). **d)** Distances between the centre of pharmacophoric features for **9a** and **9d** molecules are shown in green numbers.

In summary, the pharmacophoric features found as relevant for both **9b** and **9d** molecules were: i) Aromatic (Aro); ii) Hydrogen bond Acceptor (Acc) and iii) Hydrophobic moiety (Hyd). These three essential characteristics of **9b** and **9d**, were also identified in molecules **9a**, **9c** and **9e**. It is noticeable that all these molecules having the above pharmacophoric features showed lowers IC_{50} values, when compared with the IC_{50} value of Nfx (7μM), suggesting that the generated pharmacophore model is an identifier of the essential characteristics that are responsible for biological activity.

Finally, once the pharmacophoric features that make a molecule reactive against *T. cruzi in vitro* were elucidated, a "Selectivity Pharmacophore" was designed, assessing the same three essential characteristics centred in the *O*-phenyl scaffold of the molecules.

Modifying and assigning these features in a spatial arrangement as it is presented in the Figure 3a, and applying them in the molecules database of molecules previously resulted selected by the "Activity Pharmacophore" it was possible to identify just the compound **9d** that is the one with the best IC_{50} and IS (Figure 3b).



a) b)

Figure 3. a) "Selectivity Pharmacophore" showing the spatial arrangement of three essential features Aro (green), Acc (blue) and Hyd (red). **b)** "Selectivity pharmacophore" showed as big green, blue and red spheres over the 3D image of **9d** best active and selective compound. Small spheres are features related with the main tripanosomicidal activity according to the "Activity pharmacophore" previously designed.

In conclusion, the present study provides useful information concerning the optimal structural requirements necessary for designing new, more potent and selective trypanosomicidal agents.

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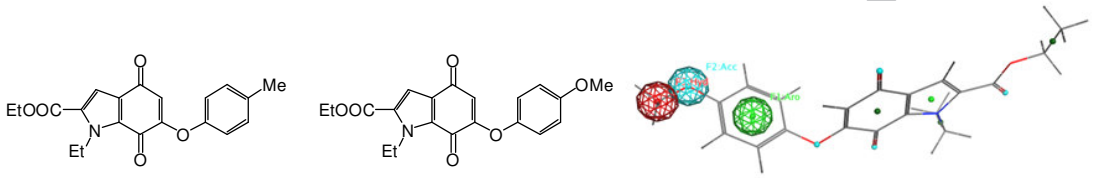

IC_{50} = 20 nM (On epimastigote culture) 20 nM