Title

Synthesis and biological evaluation of quinones derived from natural product komaroviquinone as anti-*Trypanosoma cruzi* agents

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Abstract

Current chemotherapy drugs for Chagas' disease are insufficient due to their limited efficacy; however, anti-trypanosomal agents have recently shown promise. As such, synthetic intermediates of komaroviquinone were evaluated for anti-trypanosomal activity. Based on the results, a series of novel quinone derivatives were screened for anti-trypanosomal activity and mammalian cytotoxicity. Several quinone derivatives displayed higher antiprotozoal activity against *Trypanosoma cruzi* trypomastigotes than the reference drug benznidazole, without concomitant toxicity toward the host cell.

Keywords

Trypanosoma cruzi

Chagas' disease

Quinone

Chagas' disease continues to pose a health threat for people primarily in Latin America.¹ It is also becoming a health problem in non-endemic areas, such as Europe² and the United States,³ due to the growing number of infected people coming to these regions. The World Health Organization estimates that 7 to 8 million people worldwide are infected by the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease.⁴

Trypanosoma cruzi is most commonly transmitted to humans through the feces of the triatomine bug, or "kissing bug." Other routes of transmission, such as blood transfusion, transplacental transport, organ transplantation, and ingestion of contaminated food, also exist.⁵

Chagas' disease can be in either the acute or chronic phase. In adults, the acute phase is characterized by mild symptoms, such as fever, malaise, and facial edema, and lasts from a few weeks to months. The chronic phase typically occurs years or decades after the initial infection, causing serious heart problems due to the infection of the heart tissues.⁶

Two nitro heterocycles, benznidazole and nifurtimox, are the only antiparasitic drugs approved for Chagas' disease. However, their use is problematic⁷ as both require long-course treatment, are highly toxic, and have poor efficacy for the established chronic forms of the disease. These issues can lead to discontinuation of treatment and development of resistance. Although the disease was discovered over 100 years ago, a valid chemotherapy treatment for Chagas' disease is still unknown. However, recent advances in the development of new classes of anti-trypanosomal agents are encouraging. The representative potential targets are *T. cruzi* cysteine protease⁸ and sterol 14 α -demethylase,⁹ which are both crucial for the parasite life cycle. The more approaches taken to overcome the unfulfilled need for the treatment of Chagas' disease from different sources, the better the chances of new chemotherapy drugs becoming available to patients in the near future.

Natural products are an important resource for the discovery of novel, pharmacologically active compounds for the treatment of parasitic diseases.¹⁰ In an *in vitro* assay, Komaroviquinone,¹¹ isolated from *Dracocephalum komarovii*, inhibited the growth of trypomastigotes of *T. cruzi*, which is a known infective agent in mammalian hosts. It was experimentally proposed that the trypanocidal action of komaroviquinone was due to the generation of reactive oxygen species by the reduction-oxidation cycle of komaroviquinone,¹² mediated by *T. cruzi* old yellow enzyme (TcOYE).^{12, 13} Old yellow enzymes have been identified in yeasts, plants, and bacteria, but not in animals, making komaroviquinone an attractive candidate for the development of new anti-chagastic drugs.

Recently, we developed an efficient asymmetric total synthesis of komaroviquinone, as shown Scheme 1.¹⁴ Herein, we study the importance of the quinone moiety and fused cyclic structure of komaroviquinone for its activity against *T. cruzi*. This was done by evaluating the anti-trypanosomal

activities of synthetic intermediates of komaroviquinone (**3–6**) and a quinone analogue of compound **4**. The results verified that the quinone moiety was largely involved in the expression of anti-trypanosomal activities. This activity was maintained with simplified compounds containing the quinone moiety, but lacking a complex structure with chiral centers. From these results, a series of quinone derivatives were synthesized and their anti-trypanosomal activities were evaluated.

Komaroviquinone and its intermediates were prepared following a previously reported method,¹⁴ as shown in Scheme 1. The synthesis of komaroviquinone related compounds **8** and **12** is shown in Scheme 2. Compound **8** was synthesized by oxidation of intermediate **4**, using silver (II) oxide as an oxidant. Compound **12** was a simplified version of komaroviquinone, but still contained a tertiary hydroxyl group next to the quinone moiety. Compound **9** was iodinated with *N*-Iodosuccinimide (NIS) in the presence of *p*-toluenesulfonic acid (TsOH). An aryl magnesium halide was generated via halogen exchange and reacted with acetone to afford compound **11**, which was finally oxidized to quinone **12**.

Scheme 3 outlines the preparation of quinones **14a–c**, **17a–d**, **19a–d**, **21a–e**, and **23a–c**, which do not have a complex condensed ring structure, as in komaroviquinone. In all cases, hydroquinone methyl ether (**13a–c**, **16a–d**, **18a–d**, **20a–e**, or **22a–c**) was oxidized to quinone in the final step. Benzyl bromide **2** was reacted with a Grignard reagent to obtain the hydroquinone with an alkyl chain or cyclic hydrocarbon (**13a–c**). Nucleophilic attack of benzyl bromide **2** with an amine led to benzyl amine **15a–c**, which was converted to the amide (**16a–d**) by acylation with an acid chloride. Reaction of benzyl bromide **2** with an appropriate nucleophile in the presence of a base afforded the amide, heterocycle, and ether containing compounds (**18a–d**, **20a–e**). In the case of tertiary alcohol derivative **22a–c**, benzyl magnesium halide, generated *in situ* from benzyl bromide **2** at low temperature, was reacted with the appropriate ketone. Compounds were prepared as racemic mixtures.

As described above, it was suggested that the quinone moiety of komaroviquinone participates in the generation of reactive oxygen species within the pathogen. However, it is unknown whether the complex, fused cyclic structure of komaroviquinone is essential for the expression of anti-trypanosomal activity. As such, the anti-trypanosomal activities of intermediates **3–6** were examined (Fig. 1). Although these compounds had all the carbons required to form the structure of komaroviquinone, and compound **6** had the same configuration as komaroviquinone, with the exception of a quinone moiety, all compounds did not show activity against *T. cruzi* at 10 μ M. On the other hand, compound **8**, which was synthesized from the oxidation of intermediate **4** and compound **12**, which had a simplified structure, strongly inhibited the growth of *T. cruzi* equal to that of komaroviquinone at 10 μ M. Furthermore, Komaroviquinone compound **8** and **12** exhibited anti-trypanosomal activity even at 1 μ M. These results confirm that the quinone structure

significantly contributes to the anti-trypanosomal action of komaroviquinone.

It is important to note that compound **12**, without the fused cyclic structure and stereocenters characteristic of komaroviquinone, significantly decreased the number of trypomastigotes in the culture. The asymmetric total synthesis of komaroviquinone that we previously reported requires 14 steps, including one catalytic asymmetric reaction from commercially available compounds. This is the shortest synthetic route of optically active komaroviquinone that has been reported.¹⁵ Simplified compounds with anti-trypanosomal activity can be synthesized in fewer steps and have equal or greater activity than komaroviquinone. As such, these have the potential to be promising drug candidates, compared to komaroviquinone itself.

For this reason, we synthesized a series of quinone compounds from benzyl bromide **2** in 2 to 4 steps, and evaluated their activity *in vitro* against *T. cruzi* trypomastigotes. Although these quinone derivatives do not have the same complex, fused structure as komaroviquinone, most of them significantly decreased the number of trypomastigotes at 10 μ M (see supplementary data), and several exhibited anti-trypanosomal activity even at 1 μ M (Fig. 2).

We then assessed the half maximal inhibitory concentration (IC₅₀) values of the synthesized quinone derivatives (Table 1), and found that compounds **17a**, **21e**, **23b**, and **23c** were the most potent, with comparable or higher activity than komaroviquinone and benznidazole, an anti-trypanosomal drug currently used in the treatment of Chagas' disease. The molecular mechanism of komaroviquinone against *T. cruzi* is considered to be related to the production of reactive oxygen species (ROS). To know whether synthesized quinones produce ROS, the intracellular ROS levels were measured using an intracellular ROS assay kit¹⁸ in host HT1080 cells (Figure 3). Although the ROS production was detected by the addition of **23c**, the ROS level was less than that induced by komaroviquinone (**7**) and BZL, indicating that molecular mechanism other than generation of ROS could be exist. Compounds **21e** and **23c** have a 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethyl moiety, which is a common structure between *T. cruzi* ergosterol biosynthesis inhibitors, such as ravuconazole and posaconazole. It was assumed that the generation of reactive oxygen species was associated with the anti-trypanosomal activity of the newly synthesized quinone compounds. Compounds **21e** and **23c** were designed and synthesized on the basis of an expected improvement as ergosterol biosynthesis inhibitors. Currently, it is not clear whether the high anti-trypanosomal activity of these compounds is caused by the expected synergistic effect or by another reason; thus, a future study is necessary.

These compounds were also tested for cytotoxicity against Swiss3T3 fibroblasts (Table 1), following an established procedure, and LD₅₀ values were determined to all be greater than 100 μ M.

It was determined that the quinone moiety of komaroviquinone plays an important role in its anti-trypanosomal activity, but the complex, fused cyclic structure is not always necessarily.

Accordingly, a series of quinone derivatives were synthesized and their anti-trypanosomal activity was evaluated. Compounds **17a**, **21e**, **23b**, and **23c** showed considerable *in vitro* potency against *T. cruzi* and had low cytotoxicity. Further studies are in progress to explore their efficacy in a Chagas' disease mouse model.

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Figure 1. Effects of BZL, komaroviquinone (7), and quinone derivatives (3–6, 8, and 12) on *T. cruzi* trypomastigotes.

T. cruzi trypomastigotes were suspended in 0.1 mL of Dulbecco's modified Eagle's medium (DMEM) and incubated for 24 h in the presence of a vehicle (0.1% ethanol: *Control*), benznidazole (BZL), or one of the quinone derivatives, at a concentration of 10 μM or 1 μM. To evaluate anti-trypanosomal activity, the number of live parasites was quantified microscopically.¹⁷ Data are presented as the mean, with error bars, of four independent experiments. ** $P < 0.01$ vs. control (by Student's *t*-test; two-tailed, unpaired).

Figure 2. Effects of synthetic quinone derivatives on *T. cruzi* trypomastigotes.

T. cruzi trypomastigotes were suspended in 0.1 mL of DMEM and incubated for 24 h in the presence of vehicle (0.1% ethanol: *Control*), BZL, or one of the quinone derivatives, at a concentration of 1 μ M. To evaluate anti-trypansomal activity, the number of live parasites was quantified microscopically.¹⁷ Data are presented as the mean, with error bars, of four independent experiments. * $P < 0.05$; ** $P < 0.01$ vs. control (by Student's *t*-test; two-tailed, unpaired).

Figure 3. Reactive oxygen species production by the addition of BZL, komaroviquinone (**7**), or **23c**¹⁸.

Scheme 1. Catalytic asymmetric total synthesis of komaroviquinone. Reagents and conditions: (a) (1) Zn, THF, 0 °C to rt, (2) PdCl₂ (2.5 mol%), DMF/THF, rt; (b) 3 N HCl, AcOH/H₂O, 70 °C; (c) NIS, TsOH, 1,2-dichloroethane, 45 °C; (d) ⁴PrMgCl, Et₂O, -40 °C; (e) AgO, HNO₃, dioxane/H₂O, 10 °C.

Scheme 2. Synthesis of komaroviquinone related quinone compounds. Reagents and conditions: (a) AgO, HNO₃, dioxane/H₂O, 10 °C; (b) NIS, TsOH·H₂O, 1,2-dichloroethane, 45 °C; (c) (1) *i*-PrMgCl, Et₂O, -20 °C, (2) acetone, Et₂O, -20 °C.

Supplementary data

Fig. S1 Effect of synthetic quinone derivatives on *T. cruzi* trypomastigotes at 10 μ M.

T. cruzi trypomastigotes were suspended in 0.1 mL of Dulbecco's modified Eagle's medium (DMEM) and incubated for 24 h in the presence of a vehicle (0.1% ethanol: Control), benznidazole (BZL), or one of the quinone derivatives, at a concentration of 10 μ M. To evaluate anti-trypansomal activity, the number of live parasites was quantified microscopically.

Chemistry

General: NMR spectra were recorded on a JEOL ECA500 spectrometer, operating at 500 MHz for ¹H NMR and 125.65 MHz for ¹³C NMR. Chemical shifts were reported in the scale relative to the solvent used as an internal reference. IR spectra were measured on a shimadzu FTIR-8400S. HRMS spectra were measured on Shimadzu LCMS-IT-TOF. Column chromatographies were performed with silica gel 60 (Kanto, 63-210 μ m) or on a Biotage Isorela system using SNAP cartridges. In general, reactions were carried out in dry solvents under an argon atmosphere, unless noted otherwise. Normal glassware can be used to conduct the reactions in this manuscript.

Preparation of 5-(((3a*S*,7a*S*)-4,4-dimethyl-2-oxohexahydrobenzofuran-7a(2*H*)-yl)methyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (8**).**

To a stirred solution of **4** (50 mg, 0.13 mmol) and silver (II) oxide (63 mg, 0.51 mmol) in 1,4-dioxane (2.6 ml) was added 6 M HNO₃ (1.3 ml) at 10 °C. After the mixture was stirred for 5 h at 5 °C, another portion of silver (II) oxide (63 mg, 0.51 mmol) was added and the stirring continued for 3 h at the same temperature. The mixture was diluted with water and extracted with Et₂O. Combined organic layers were washed with brine and dried over Na₂SO₄. Filtration, evaporation and purification by silica gel column chromatography (Hexane/AcOEt = 5/1) gave the product **8** in 95% yield (44 mg).

¹H NMR (CDCl₃, 500 MHz) δ 6.61 (s, 1H), 3.91 (s, 3H), 3.20 (sept, *J* = 7.1 Hz, 1H), 3.11 (d, *J* = 14.0 Hz, 1H), 2.77 (d, *J* = 14.0 Hz, 1H), 2.51-2.46 (m, 2H), 2.15 (t, *J* = 10.3 Hz, 1H), 1.84-1.76 (m, 1H), 1.60-1.52 (m, 2H), 1.43-1.32 (m, 3H), 1.21 (d, *J* = 7.1 Hz, 3H), 1.19 (d, *J* = 7.1 Hz, 3H), 1.11 (s, 3H), 0.92 (s, 3H).

¹³C NMR (CDCl₃, 125 MHz) δ 187.71, 183.82, 175.11, 156.50, 141.51, 137.77, 136.48, 86.70, 61.05, 51.06, 36.10, 33.76, 32.81, 32.51, 32.39, 30.03, 28.90, 24.65, 20.55, 18.45.

IR (neat, cm⁻¹) 2943, 1778, 1770, 1652.

HRMS calcd for C₂₁H₂₈NaO₅ (M + Na⁺): 383.1829, found 383.1829.

Preparation of 2-(2-hydroxypropan-2-yl)-6-isopropyl-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dion (12**).**

1-iodo-3-isopropyl-2,4,5-trimethoxy-6-methylbenzene (10**).**

2-isopropyl-1,3,4-trimethoxy-5-methylbenzen **9** (690 mg, 3.1 mmol) was dissolved in 1,2-dichloroethane (3.0 ml) and TsOH·H₂O (59 mg, 0.31 mmol) and *N*-iodosuccinimide (1.0 g, 4.5 mmol) were added. The reaction mixture was allowed to stir at 45 °C over night. The reaction was quenched with the addition of aqueous Na₂S₂O₃ and NaHCO₃. The mixture was extracted with AcOEt. The combined organic layers were dried over

Na₂SO₄, and concentrated under reduced pressure. Silica gel column chromatography (Hexane/AcOEt = 99/1 to 95/5) provided **10** in 71% yield (770 mg).

2-(3-isopropyl-2,4,5-trimethoxy-6-methylphenyl)propan-2-ol (11).

To a solution of compound **10** (225 mg, 0.64 mmol) in Et₂O (2.5 ml) was added isopropylmagnesium chloride (2.0 M in Et₂O) (800 μ l, 1.6 mmol) at -20 °C. The solution was stirred for 1 h at -20 °C and acetone (120 μ l, 1.6 mmol) was added dropwise at the same temperature. The reaction mixture was quenched with saturated NH₄Cl and extracted with EtOAc three times. The organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. Silica gel column chromatography (Hexane/AcOEt = 98/2 to 85/15) provided **11** in 50% yield (90 mg).

2-(2-hydroxypropan-2-yl)-6-isopropyl-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (12).

To a stirred solution of **11** (90 mg, 0.32 mmol) in 1,4-dioxane (6.4 ml) and 6N HNO₃ (2.3 ml) silver(II) oxide (164 mg, 1.27 mmol) was added at 10 °C. After the mixture was stirred for 3 h at 5~10 °C diluted with water and extracted with AcOEt. Combined organic layers were washed with brine and dried over Na₂SO₄. Filtration, evaporation and purification by silica gel column chromatography (Hexane/AcOEt = 19/1 to 85/15) gave the product **12** in 27% yield (22 mg).

¹H NMR (CDCl₃, 500 MHz) δ 4.47 (s, 1H), 3.93 (s, 3H), 3.26 (sept, *J* = 6.7 Hz, 1H), 2.12 (s, 3H), 1.56 (s, 6H), 1.20 (d, *J* = 6.7 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 191.77, 184.55, 154.95, 149.57, 138.17, 137.39, 73.50, 60.85, 30.32, 25.18, 20.57, 13.80.

IR (neat, cm⁻¹) 2964, 1655, 1637, 1242.

HRMS calcd for C₁₆H₂₄NO₄ (M + CH₃CN + H⁺): 294.1700, found 294.1708.

Preparation of 5-heptyl-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (14a).

1-heptyl-4-isopropyl-2,3,5-trimethoxybenzene (13a).

To a stirred solution of benzylbromide **2** (150 mg, 0.50 mmol) in anhydrous THF (500 μ L) was added *n*HexMgBr (2.0 M in Et₂O) (500 μ l, 1.0 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature gradually and stirred overnight. The

reaction mixture was quenched with 1 M HCl and extracted with AcOEt. The organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. Silica gel column chromatography (Hexane/Et₂O = 50/1) provided **13a** in 16% yield (24 mg).

5-heptyl-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (14a).

To a stirred solution of **13a** (20 mg, 0.065 mmol) in acetonitrile (2.6 ml) was added cerium ammonium nitrate (0.20 M in water) (780 μ l, 0.156 mmol) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 20/1) provided **14a** in 76% yield (14mg).

¹H NMR (CDCl₃, 500 MHz) δ 6.40 (s, 1H), 3.94 (s, 3H), 3.21 (sept, *J* = 7.3 Hz, 1H), 2.35 (t, *J* = 7.8 Hz, 2H), 1.51-1.44 (m, 2H), 1.37-1.22 (m, 8H), 1.20 (d, *J* = 7.3 Hz, 6H), 0.87 (t, *J* = 7.5 Hz, 3H).

¹³C NMR (CDCl₃, 125 MHz) δ 188.40, 184.31, 156.31, 147.36, 137.66, 132.78, 61.05, 31.78, 29.37, 29.08, 28.54, 27.83, 24.60, 22.70, 20.59, 14.15.

IR (neat, cm⁻¹) 2930, 1653, 1271.

HRMS calcd for C₁₉H₃₀NO₃ (M + CH₃CN + H⁺): 320.2220, found 320.2229.

Preparation of 5-(cyclohexylmethyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (14b).

1-(cyclohexylmethyl)-4-isopropyl-2,3,5-trimethoxybenzene (13b).

To a stirred solution of benzylbromide **2** (150 mg, 0.50 mmol) in anhydrous THF (500 μ L) was added *c*HexMgCl (2.0 M in Et₂O) (500 μ l, 1.0 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature gradually and stirred overnight. The reaction mixture was quenched with 1 M HCl and extracted with AcOEt. The organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. Silica gel column chromatography (Hexane/Et₂O = 50/1) provided **13b** in 24% yield (36 mg).

5-(cyclohexylmethyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (14b).

To a stirred solution of **13b** (30 mg, 0.098 mmol) in acetonitrile (3.9 ml) was added cerium ammonium nitrate (0.20 M in water) (1.15 ml, 0.23 mmol) at 0 °C. The reaction

mixture was stirred for 1 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 20/1) provided **14b** in 78% yield (21mg).

¹H NMR (CDCl₃, 500 MHz) δ 6.38 (s, 1H), 3.94 (s, 3H), 3.21 (sept, J = 7.3 Hz, 1H), 2.25 (d, J = 7.0 Hz, 2H), 1.61-1.72 (m, 5H), 1.43-1.53 (m, 1H), 1.25-1.11 (m, 3H), 1.20 (d, J = 7.3 Hz, 6H), 0.97-0.87 (m, 2H).

¹³C NMR (CDCl₃, 125 MHz) δ 188.32, 184.40, 156.30, 145.77, 137.63, 133.90, 61.07, 37.01, 36.47, 33.33, 26.36, 26.20, 24.61, 20.60.

IR (neat, cm⁻¹) 2924, 1653, 1649, 1273.

HRMS calcd for C₁₉H₂₈NO₃ (M + CH₃CN + H⁺): 318.2064, found 318.2067.

Preparation of 5-benzyl-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (**14c**).

1-benzyl-4-isopropyl-2,3,5-trimethoxybenzene (**13c**).

To a stirred solution of benzylbromide **2** (150 mg, 0.50 mmol) in anhydrous THF (500 μ L) was added PhMgBr (1.0 M in THF) (1.0 ml, 1.0 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature gradually and stirred overnight. The reaction mixture was quenched with 1 M HCl and extracted with AcOEt. The organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. Silica gel column chromatography (Hexane/Et₂O = 30/1) provided **13c** in 8% yield (12 mg).

5-benzyl-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (**14c**).

To a stirred solution of **13c** (11 mg, 0.037 mmol) in acetonitrile (1.5 ml) was added cerium ammonium nitrate (0.20 M in water) (440 μ L, 0.088 mmol) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 20/1 to 15/1) provided **14c** in 74% yield (7 mg).

¹H NMR (CDCl₃, 500 MHz) δ 7.32 (t, J = 7.0 Hz, 2H), 7.27-7.24 (m, 1H), 7.18 (d, J = 7.0 Hz, 2H), 6.21 (s, 1H), 3.95 (s, 3H), 3.70 (s, 2H), 3.19 (sept, J = 7.0 Hz, 1H), 1.19 (d, J = 7.0 Hz, 6H).

^{13}C NMR (CDCl_3 , 125 MHz) δ 188.20, 183.99, 156.23, 146.38, 137.82, 136.69, 133.80, 129.44, 128.91, 127.03, 61.09, 34.81, 24.63, 20.56.

IR (neat, cm^{-1}) 2963, 1653, 1273.

HRMS calcd for $\text{C}_{19}\text{H}_{22}\text{NO}_3$ ($\text{M} + \text{CH}_3\text{CN} + \text{H}^+$): 312.1594, found 312.1597.

Preparation of *N*'((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)cyclohexanecarboxamide (17a).

***N*'(4-isopropyl-2,3,5-trimethoxybenzyl)cyclohexanecarboxamide (16a).**

Benzylbromide **2** (150 mg, 0.50 mmol) was dissolved in 2.0 M NH_3 in EtOH (2.5 ml) and the resulting solution was stirred at room temperature. After 4 h the solution was evaporated. The residue was dissolved in Et₂O (2.0 ml) and pyridine (120 μl , 1.5 mmol) and cyclohexanecarbonyl chloride (135 μl , 1.0 mmol) were added to this solution. The reaction mixture was stirred for 1 h at room temperature and quenched with 1 M HCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 85/15 to 45/55) provided **16a** in 20% yield (35 mg).

***N*'((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)cyclohexanecarboxamide (17a).**

To a stirred solution of **16a** (20 mg, 0.057 mmol) in acetonitrile (2.9 ml) was added cerium ammonium nitrate (0.20 M in water) (710 μl , 0.142 mmol) at 0 °C. The reaction mixture was stirred for 2 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 85/15 to 25/75) provided **17a** in 74% yield (14 mg).

^1H NMR (CDCl_3 , 500 MHz) δ 6.43 (t, J = 1.8 Hz, 1H), 5.84 (brs, 1H), 4.20 (dd, J = 1.8 Hz,

$J = 6.5$ Hz, 2H), 3.94 (s, 3H), 3.20 (sept, $J = 7.3$ Hz, 1H), 2.16-1.99 (m, 1H), 1.91-1.75 (m, 4H), 1.70-1.64 (m, 1H), 1.48-1.38 (m, 2H), 1.32-1.20 (m, 3H), 1.19 (d, $J = 7.0$ Hz, 6H).
 ^{13}C NMR (CDCl_3 , 125 MHz) δ 187.78, 184.17, 176.34, 156.10, 142.32, 138.37, 132.61, 61.10, 45.45, 37.57, 29.76, 25.74, 24.71, 20.53.
 IR (neat, cm^{-1}) 3293, 2932, 1653, 1647, 1635, 1269.
 HRMS calcd for $\text{C}_{18}\text{H}_{25}\text{NNaO}_4$ ($\text{M} + \text{Na}^+$): 342.1681, found 342.1675.

Preparation of *N*((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)-*N*-methylcyclohexanecarboxamide (17b).

***N*(4-isopropyl-2,3,5-trimethoxybenzyl)-*N*-methylcyclohexanecarboxamide (16b).**

Benzylbromide **2** (150 mg, 0.50 mmol) was dissolved in 40% methylamine in MeOH (1.0 ml) and the resulting solution was stirred at room temperature. After 1 h the solution was evaporated. The residue was dissolved in Et₂O (2.0 ml) and pyridine (120 μl , 1.5 mmol) and cyclohexanecarbonyl chloride (135 μl , 1.0 mmol) were added to this solution. The reaction mixture was stirred for 1 h at room temperature and quenched with 1 M HCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 85/15 to 45/55) provided **16b** in 39% yield (70 mg).

***N*((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)-*N*-methylcyclohexanecarboxamide (17b).**

To a stirred solution of **16b** (36 mg, 0.099 mmol) in acetonitrile (5.0 ml) was added cerium ammonium nitrate (0.20 M in water) (1.2 ml, 0.24 mmol) at 0 °C. The reaction mixture was stirred for 2 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 85/15 to 45/55) provided **17b** in 67% yield (22 mg).

^1H NMR (CDCl_3 , 500 MHz) δ 6.18 (t, J = 2.0 Hz, 1H), 4.32 (d, J = 2.0 Hz, 2H), 3.93 (s, 3H), 3.20 (sept, J = 6.8 Hz, 1H), 3.07 (s, 3H), 2.59-2.50 (m, 1H), 1.86-1.61 (m, 5H), 1.58-1.40 (m, 2H), 1.32-1.24 (m, 3H), 1.18 (d, J = 6.8 Hz, 6H).

^{13}C NMR (CDCl_3 , 125 MHz) δ 187.74, 184.00, 176.68, 156.27, 141.50, 138.07, 131.52, 61.07, 45.98, 40.82, 36.19, 29.28, 25.84, 24.67, 20.52.

IR (neat, cm^{-1}) 2932, 1653, 1647, 1635, 1267.

HRMS calcd for $\text{C}_{19}\text{H}_{28}\text{NO}_4$ ($\text{M} + \text{H}^+$): 334.2013, found 334.2011.

Preparation of *N*-((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)-*N*-methylbenzamide (17c).

***N*-((4-isopropyl-2,3,5-trimethoxybenzyl)-*N*-methylbenzamide (16c)**

To a solution of 40% methylamine in MeOH (2.0 ml) a solution of benzylbromide **2** (500 mg, 1.65 mmol) in MeOH (1.0 ml) was added and the resulting solution was stirred at room temperature. After 1 h the solution was evaporated and 440 mg of crude product was obtained. 100 mg of crude product was dissolved in CH_2Cl_2 (2.0 ml) and triethylamine (110 μl , 0.79 mmol) and benzenecarbonyl chloride (70 μl , 0.60 mmol) were added to this solution. The reaction mixture was stirred for 1 h at room temperature and quenched with water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 90/10 to 50/50) provided **16c** in 62% yield (83 mg).

***N*-((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)-*N*-methylbenzamide (17c)**

To a stirred solution of **16c** (74 mg, 0.21 mmol) in acetonitrile (8.5 ml) was added cerium ammonium nitrate (0.20 M in water) (2.3 ml, 0.46 mmol) at 0 $^\circ\text{C}$. The reaction mixture was stirred for 1 h at the same temperature before the addition of saturated NaCl. The mixture was extracted with AcOEt and the combined organic layers were dried over

Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 90/10 to 50/50) provided **17c** in 89% yield (60 mg).

¹H NMR (CDCl₃, 500 MHz) δ 7.48-7.32 (m, 5H), 6.40 (s, 1H), 4.50 (s, 2H), 3.95 (s, 3H), 3.22 (sept, *J* = 7.0 Hz, 1H), 3.00 (s, 3H), 1.20 (d, *J* = 7.0 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 187.68, 183.95, 171.99, 156.30, 140.84, 138.25, 135.37, 131.92, 130.19, 128.64, 127.22, 61.14, 45.83, 38.52, 24.74, 20.54.

IR (neat, cm⁻¹) 2960, 1653, 1647, 1635, 1267.

HRMS calcd for C₁₉H₂₁NNaO₄ (M + Na⁺): 350.1363, found 350.1361.

Preparation of *N*-cyclohexyl-*N*-((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)cyclohexanecarboxamide (17d).

***N*-cyclohexyl-*N*-(4-isopropyl-2,3,5-trimethoxybenzyl)cyclohexanecarboxamide (16d).**

To a solution of cyclohexylamine (170 μ l, 1.5 mmol) in THF (1.0 ml) a solution of benzylbromide **2** (150 mg, 0.5 mmol) in THF (1.0 ml) was added and the resulting solution was stirred at room temperature. After 5 h the solution was evaporated and 160 mg of crude product was obtained. 135 mg of the crude product was dissolved in THF (1.0 ml) and triethylamine (120 μ l, 0.86 mmol) and cyclohexanecarbonyl chloride (84 μ l, 0.63 mmol) were added to this solution. The reaction mixture was stirred for 1 h at room temperature and quenched with 1 M NaOH. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 95/5 to 70/30) provided **16d** in 87% yield (151 mg).

***N*-cyclohexyl-*N*-((4-isopropyl-5-methoxy-3,6-dioxocyclohexa-1,4-dien-1-yl)methyl)cyclohexanecarboxamide (17d).**

To a stirred solution of **16d** (120 mg, 0.28 mmol) in acetonitrile (11 ml) was added cerium

ammonium nitrate (0.20 M in water) (3.0 ml, 0.60 mmol) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature before the addition of saturated NaCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 85/15 to 60/40) provided **17d** in 62% yield (69 mg).

¹H NMR (CDCl₃, 500 MHz) δ 6.09 (t, J = 1.8 Hz, 1H), 4.19 (d, J = 1.8 Hz, 2H), 3.93 (s, 3H), 3.72-3.69 (m, 1H), 3.17 (sept, J = 7.2 Hz, 1H), 2.56-2.48 (m, 1H), 1.85-1.49 (m, 10H), 1.41-1.00 (m, 10H), 1.16 (d, J = 7.2 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 187.87, 183.95, 176.27, 156.14, 143.22, 137.79, 131.40, 61.03, 56.96, 41.10, 39.26, 31.94, 29.91, 25.89, 25.84, 25.72, 25.15, 24.63, 20.53.

IR (neat, cm⁻¹) 2932, 1653, 1647, 1635, 1269.

HRMS calcd for C₂₄H₃₆NO₄ (M + H⁺): 402.2639, found 402.2635.

Preparation of 2-isopropyl-3-methoxy-5-((2-oxopyrrolidin-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (19a).

1-(4-isopropyl-2,3,5-trimethoxybenzyl)pyrrolidin-2-one (18a).

To a solution of benzylbromide **2** (150 mg, 0.5 mmol) and pyrrolidone (115 μ l, 1.5 mmol) in THF (2.0 ml) sodium hydride (60%, 60 mg, 1.5 mmol) was added at -10 °C. The mixture was stirred for 10 min at the same temperature and DMF (200 μ l) was added to the mixture. The reaction mixture was stirred for 5.5 h at room temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 60/40 to 20/80) provided **18a** in 75% yield (113 mg).

2-isopropyl-3-methoxy-5-((2-oxopyrrolidin-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (19a).

To a stirred solution of **18a** (49 mg, 0.16 mmol) in acetonitrile (8.0 ml) was added cerium ammonium nitrate (0.20 M in water) (2.0 ml, 0.40 mmol) at 0 °C. The reaction mixture was stirred for 2 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 60/40 to 20/80) provided **19a** in 84% yield (37 mg).

^1H NMR (CDCl_3 , 500 MHz) δ 6.30 (t, $J = 1.5$ Hz, 1H), 4.22 (d, $J = 1.5$ Hz, 2H), 3.92 (s, 3H), 3.38 (t, $J = 7.0$ Hz, 2H), 3.18 (sept, $J = 6.8$ Hz, 1H), 2.42 (t, $J = 8.3$ Hz, 2H), 2.11-2.04 (m, 2H), 1.17 (d, $J = 6.8$ Hz, 6H).

^{13}C NMR (CDCl_3 , 125 MHz) δ 187.58, 183.71, 175.72, 156.22, 140.74, 138.22, 132.22, 61.10, 47.85, 40.71, 30.48, 24.69, 20.49, 18.10.

IR (KBr, cm^{-1}) 2951, 1697, 1655, 1630, 1603, 1269.

HRMS calcd for $\text{C}_{15}\text{H}_{19}\text{NNaO}_4$ ($M + \text{Na}^+$): 300.1206, found 300.1206.

Preparation of 2-isopropyl-3-methoxy-5-((2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)methyl)cyclohexa-2,5-diene-1,4-dione (19b).

1-(4-isopropyl-2,3,5-trimethoxybenzyl)-3,4-dihydroquinolin-2(1*H*)-one (18b).

To a solution of quinolone (74 mg, 0.5 mmol) in THF (1.0 ml) sodium hydride (60%, 19 mg, 0.48 mmol) was added at 0 °C. The mixture was stirred for 10 min at the same temperature and benzylbromide **2** (100 mg, 0.33 mmol) in THF (1.0 ml) and DMF (200 μl) was added to the mixture. After the mixture was stirred over night, another portion of quinolone (74 mg, 0.5 mmol) and sodium hydride (60%, 19 mg, 0.48 mmol) were added and the stirring continued for 3 h at the same temperature. The mixture was diluted with water and extracted with AcOEt. The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt 80/20 to 40/60) provided **18b** in 68% yield (83 mg).

2-isopropyl-3-methoxy-5-((2-oxo-3,4-dihydroquinolin-1(2*H*)-yl)methyl)cyclohexa-2,5-diene-1,4-dione (19b).

To a stirred solution of **18b** (85 mg, 0.23 mmol) in acetonitrile (9.0 ml) was added cerium ammonium nitrate (0.20 M in water) (2.5 ml, 0.50 mmol) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature before the addition of saturated NaCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 80/20 to 40/60) provided **19b** in 58% yield (45 mg).

^1H NMR (CDCl_3 , 500 MHz) δ 7.20-7.14 (m, 2H), 7.01 (t, $J = 7.3$ Hz, 1H), 6.68 (d, $J = 8.0$ Hz, 1H), 6.10 (t, $J = 2.2$ Hz, 1H), 4.90 (d, $J = 2.2$ Hz, 2H), 3.99 (s, 3H), 3.19 (sept, $J = 7.2$ Hz, 1H), 2.97 (t, $J = 7.5$ Hz, 2H), 2.74 (t, $J = 7.5$ Hz, 2H), 1.18 (d, $J = 7.2$ Hz, 6H).

^{13}C NMR (CDCl_3 , 125 MHz) δ 187.36, 183.68, 170.40, 156.24, 140.81, 139.03, 138.16, 131.58, 128.31, 127.83, 126.22, 123.59, 114.71, 61.16, 40.92, 31.72, 25.48, 24.69, 20.56, 20.49.

IR (KBr, cm^{-1}) 2961, 1674, 1654, 1629, 1600, 1271.

HRMS calcd for $\text{C}_{20}\text{H}_{22}\text{NO}_4$ ($\text{M} + \text{H}^+$): 340.1543, found 340.1547.

Preparation of 5-((1*H*-1,2,4-triazol-1-yl)methyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (19c**).**

1-(4-isopropyl-2,3,5-trimethoxybenzyl)-1*H*-1,2,4-triazole (18c**).**

To a stirred solution of benzylbromide **2** (150 mg, 0.50 mmol) and triazole (104 mg, 1.5 mmol) in THF (2.0 ml) were added K_2CO_3 (207 mg, 1.5 mmol) and DMF (200 μl) at 0 $^\circ\text{C}$. The reaction mixture was stirred for 2 h at room temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 67/33 to 50/50) provided **18c** in 68% yield (98 mg).

5-((1*H*-1,2,4-triazol-1-yl)methyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (19c**).**

To a stirred solution of **18c** (50 mg, 0.17 mmol) in acetonitrile (7.0 ml) was added cerium ammonium nitrate (0.20 M in water) (2.05 ml, 0.41 mmol) at 0 $^\circ\text{C}$. The reaction mixture was stirred for 1 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 80/20 to 40/60) provided **19c** in 79% yield (35 mg).

^1H NMR (CDCl_3 , 500 MHz) δ 8.20 (s, 1H), 7.97 (s, 1H), 6.30 (s, 1H), 5.15 (s, 2H), 3.94 (s, 3H), 3.19 (sept, $J = 7.3$ Hz, 1H), 1.19 (d, $J = 7.3$ Hz, 6H).

^{13}C NMR (CDCl_3 , 125 MHz) δ 186.97, 182.78, 155.89, 152.69, 144.37, 139.49, 138.78, 134.46, 61.20, 46.87, 24.80, 20.43.

IR (neat, cm^{-1}) 2963, 1653, 1647, 1636, 1273.

HRMS calcd for $\text{C}_{13}\text{H}_{16}\text{N}_3\text{O}_3$ ($\text{M} + \text{H}^+$): 262.1186, found 262.1190.

Preparation of 2-isopropyl-3-methoxy-5-((4-nitro-1*H*-imidazol-1-yl)methyl)cyclohexa-2,

5-diene-1,4-dione (19d)

1-(4-isopropyl-2,3,5-trimethoxybenzyl)-4-nitro-1H-imidazole (18d)

To a stirred solution of benzylbromide **2** (150 mg, 0.50 mmol) and nitroimidazole (170 mg, 1.5 mmol) in THF (2.0 ml) were added K₂CO₃ (207 mg, 1.5 mmol) and DMF (200 μl) at 0 °C. The reaction mixture was stirred for 7.5 h at room temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 70/30 to 30/70) provided **18d** in 72% yield (119 mg).

2-isopropyl-3-methoxy-5-((4-nitro-1H-imidazol-1-yl)methyl)cyclohexa-2,5-diene-1,4-dione (19d)

To a stirred solution of **18d** (70 mg, 0.21 mmol) in acetonitrile (8.4 ml) was added cerium ammonium nitrate (0.20 M in water) (2.5 ml, 0.50 mmol) at 0 °C. The reaction mixture was stirred for 1.5 h at the same temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 80/20 to 40/60) provided **19d** in 75% yield (48 mg).

¹H NMR (CDCl₃, 500 MHz) δ 7.82 (d, J = 1.5 Hz, 1H), 7.53 (d, J = 1.5 Hz, 1H), 6.43 (s, 1H), 4.98 (s, 2H), 3.97 (s, 3H), 3.21 (sept, J = 7.3 Hz, 1H), 1.19 (d, J = 6.5 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 186.56, 182.50, 155.93, 139.15, 139.02, 136.67, 134.70, 119.63, 61.35, 45.84, 24.90, 20.39.

IR (KBr, cm⁻¹) 3142, 3107, 2960, 1654, 1631, 1606, 1284.

HRMS calcd for C₁₄H₁₅N₃NaO₅ (M + Na⁺): 328.0904, found 328.0903.

Preparation of 5-(isopropoxymethyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (21a).

1-(isopropoxymethyl)-4-isopropyl-2,3,5-trimethoxybenzene (20a).

To a stirred solution of benzylbromide **2** (150 mg, 0.50 mmol) and 2-propanol (115 μl, 1.5

mmol) in THF (2.0 ml) were added sodium hydride (60%, 60 mg, 1.5 mmol) and DMF (200 μ l) at 0 °C. The reaction mixture was stirred for 2 h at room temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/Et₂O = 95/5 to 90/10) provided **20a** in 78% yield (109 mg).

5-(isopropoxymethyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (21a).

To a stirred solution of **20a** (61 mg, 0.22 mmol) in acetonitrile (9.0 ml) was added cerium ammonium nitrate (0.20 M in water) (2.6 ml, 0.52 mmol) at 0 °C. The reaction mixture was stirred for 1.5 h at the same temperature before the addition of saturated NaCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 95/5) provided **21a** in 70% yield (38 mg).

¹H NMR (CDCl₃, 500 MHz) δ 6.68 (t, J = 2.0 Hz, 1H), 4.30 (d, J = 2.0 Hz, 2H), 3.93 (s, 3H), 3.67 (sept, J = 6.0 Hz, 1H), 3.22 (sept, J = 7.0 Hz, 1H), 1.20 (d, J = 7.0 Hz, 6H), 1.19 (d, J = 6.0 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 188.12, 183.83, 156.06, 144.18, 138.06, 132.12, 72.56, 63.21, 61.07, 24.66, 22.08, 20.57.

IR (neat, cm⁻¹) 2972, 1653, 1267.

HRMS calcd for C₁₆H₂₄NO₄ (M + CH₃CN + H⁺): 294.1700, found 294.1698.

Preparation of 5-((cyclohexyloxy)methyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (21b).

1-((cyclohexyloxy)methyl)-4-isopropyl-2,3,5-trimethoxybenzene (20b).

To a stirred solution of benzylbromide **2** (200 mg, 0.66 mmol) and cyclohexanol (210 μ l, 2.0 mmol) in DMF (1.0 ml) was added sodium hydride (60%, 53 mg, 1.3 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature before the addition of water. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 95/5) provided **20b** in 87% yield (185 mg).

5-((cyclohexyloxy)methyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (21b).

Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 95/5 to 85/15) provided **21c** in 75% yield (23 mg).

¹H NMR (CDCl₃, 500 MHz) δ 7.30 (t, J = 7.5 Hz, 2H), 6.99 (t, J = 7.5 Hz, 1H), 6.95 (d, J = 7.5 Hz, 2H), 6.81 (t, J = 2.2 Hz, 1H), 4.89 (d, J = 2.2 Hz, 2H), 3.98 (s, 3H), 3.25 (sept, J = 7.0 Hz, 1H), 1.22 (d, J = 7.0 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 187.73, 183.48, 157.90, 156.02, 141.88, 138.32, 132.55, 129.76, 121.76, 114.74, 63.05, 61.17, 24.74, 20.57.

IR (KBr, cm⁻¹) 2963, 1651, 1635, 1599, 1500, 1448, 1258.

HRMS calcd for C₁₉H₂₂NO₄ (M + CH₃CN + H⁺): 328.1546, found 328.1543.

Preparation of 2-isopropyl-3-methoxy-5-((pyridin-3-yloxy)methyl)cyclohexa-2,5-diene-1,4-dione (21d).

3-((4-isopropyl-2,3,5-trimethoxybenzyl)oxy)pyridine (20d).

To a stirred solution of benzylbromide **2** (200 mg, 0.66 mmol) and pyridinol (188 mg, 2.0 mmol) in DMF (1.0 ml) was added sodium hydride (60%, 53 mg, 1.3 mmol) at 0 °C. The reaction mixture was stirred for 1 h at room temperature before the addition of 1 M NaOH. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 70/30 to 30/70) provided **20d** in 10% yield (21 mg).

2-isopropyl-3-methoxy-5-((pyridin-3-yloxy)methyl)cyclohexa-2,5-diene-1,4-dione (21d).

To a stirred solution of **20d** (21 mg, 0.066 mmol) in acetonitrile (2.6 ml) was added cerium ammonium nitrate (0.20 M in water) (660 μ l, 0.13 mmol) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature before the addition of saturated NaCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 70/30 to 30/70) provided **21d** in 86% yield (16 mg).

¹H NMR (CDCl₃, 500 MHz) δ 8.37 (s, 1H), 8.27 (t, J = 3.0 Hz, 1H), 7.25 (s, 2H), 6.78 (t, J = 2.2 Hz, 1H), 4.93 (d, J = 2.2 Hz, 2H), 3.98 (s, 3H), 3.25 (sept, J = 7.0 Hz, 1H), 1.22 (d, J = 7.0 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 187.49, 183.23, 156.00, 154.15, 143.22, 140.99, 138.49, 138.09, 132.73, 124.08, 121.51, 63.44, 61.21, 24.78, 20.54.

IR (KBr, cm^{-1}) 2964, 1651, 1633, 1603, 1577, 1269.

HRMS calcd for $\text{C}_{16}\text{H}_{18}\text{NO}_4$ ($\text{M} + \text{H}^+$): 288.1230, found 288.1236.

Preparation of 5-((1-(2,4-difluorophenyl)-2-(1*H*1,2,4-triazol-1-yl)ethoxy)methyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (21e).

1-(2-(2,4-difluorophenyl)-2-((4-isopropyl-2,3,5-trimethoxybenzyl)oxy)ethyl)-1*H*1,2,4-triazole (20e).

To a solution of 1-(2,4-difluorophenyl)-2-(1*H*1,2,4-triazol-1-yl)ethan-1-ol (170 mg, 0.75 mmol) in THF (1.0 ml) sodium hydride (60%, 30 mg, 0.75 mmol) was added at 0 °C. The mixture was stirred for 30 min at the same temperature and benzylbromide **2** (150 mg, 0.50 mmol) in THF (1.0 ml) and DMF (100 μl) was added to the mixture. After the reaction mixture was stirred for 1 h at 0 °C, the mixture was diluted with water and extracted with AcOEt. The combined organic layers were dried over Na_2SO_4 , filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 70/30 to 20/80) provided **20e** in 98% yield (224 mg).

^1H NMR (CDCl_3 , 500 MHz) δ 8.11 (s, 1H), 7.85 (s, 1H), 7.39-7.34 (m, 1H), 6.90-6.79 (m, 2H), 6.33 (s, 1H), 5.09 (t, J = 6.3 Hz, 1H), 4.41 (d, J = 11.5 Hz, 1H), 4.38 (d, J = 6.3 Hz, 2H), 4.23 (d, J = 11.5 Hz, 1H), 3.74 (s, 3H), 3.70 (s, 3H), 3.61 (s, 3H), 3.43 (sept, J = 7.1 Hz, 1H), 1.24 (d, J = 7.1 Hz, 3H), 1.24 (d, J = 7.1 Hz, 3H).

^{13}C NMR (CDCl_3 , 125 MHz) δ 163.03 (dd, J = 11.9 Hz, J = 249.5 Hz), 160.07 (dd, J = 11.9 Hz, J = 249.5 Hz), 154.67, 151.63 (d, J = 8.4 Hz), 145.40, 144.21, 130.90, 129.15 (dd, J = 6.0 Hz, J = 9.5 Hz), 127.55, 121.12 (d, J = 9.5 Hz), 111.92 (d, J = 17.9 Hz), 107.10, 104.26 (t, J = 25.6 Hz), 73.32, 66.61, 60.77, 60.74, 55.75, 54.41, 25.22, 21.14.

IR (neat, cm^{-1}) 2960, 1502, 1384, 1263.

HRMS calcd for $\text{C}_{23}\text{H}_{27}\text{F}_2\text{N}_3\text{NaO}_4$ ($\text{M} + \text{Na}^+$): 470.1862, found 470.1866.

5-((1-(2,4-difluorophenyl)-2-(1*H*1,2,4-triazol-1-yl)ethoxy)methyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (21e).

To a stirred solution of **20e** (100 mg, 0.22 mmol) in acetonitrile (9.0 ml) was added cerium ammonium nitrate (0.20 M in water) (2.4 ml, 0.48 mmol) at 0 °C. The reaction

mixture was stirred for 1 h at the same temperature before the addition of saturated NaCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/AcOEt = 70/30 to 20/80) provided **21e** in quantitative yield (94 mg).

¹H NMR (CDCl₃, 500 MHz) δ 8.08 (s, 1H), 7.91 (s, 1H), 7.31-7.27 (m, 1H), 6.91-6.82 (m, 2H), 6.47 (t, J = 1.8 Hz, 1H), 5.09 (t, J = 6.2 Hz, 1H), 4.42 (d, J = 6.2 Hz, 2H), 4.20 (dd, J = 1.8 Hz, J = 16.0 Hz, 1H), 4.10 (dd, J = 1.8 Hz, J = 16.0 Hz, 1H), 3.87 (s, 3H), 3.18 (sept, J = 7.3 Hz, 1H), 1.16 (d, J = 7.3 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) δ 187.57, 183.08, 163.32 (dd, J = 11.4 Hz, J = 250.0 Hz), 160.75 (dd, J = 11.9 Hz, J = 249.0 Hz), 155.92, 152.19, 144.20, 141.91, 138.23, 132.33, 128.86 (dd, J = 5.9 Hz, J = 10.8 Hz), 120.16 (dd, 3.6 Hz, 14.4 Hz), 112.32 (d, J = 25.0 Hz), 104.66 (t, J = 25.1 Hz), 74.45, 64.73, 61.09, 54.09, 24.70, 20.51.

IR (KBr, cm⁻¹) 2943, 1655, 1604, 1508, 1275.

HRMS calcd for C₂₁H₂₁F₂N₃NaO₄ (M + Na⁺): 440.1392, found 440.1387.

Preparation of 5-(2-hydroxy-2-methylpropyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (**23a**).

1-(4-isopropyl-2,3,5-trimethoxyphenyl)-2-methylpropan-2-ol (**22a**).

ⁱPrMgCl (2.0 M in Et₂O, 330 μ l, 0.66 mmol) was added to a stirred solution of benzyl bromide **2** (100 mg, 0.33 mmol) in Et₂O (1.0 ml) at -20 °C. After the resulting solution was stirred at the same temperature for 5 h, acetone (50 μ l, 0.68 mmol) was added. The reaction mixture was allowed to warm to room temperature gradually and stirred for 3 h. The reaction mixture was quenched with 1 M HCl and extracted with AcOEt. The organic phases were dried over Na₂SO₄, filtered and evaporated to dryness. Silica gel column chromatography (Hexane/AcOEt = 90/10 to 50/50) provided **22a** in 47% yield (44 mg).

5-(2-hydroxy-2-methylpropyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (**23a**).

To a stirred solution of **22a** (36 mg, 0.13 mmol) in acetonitrile (5.1 ml) was added cerium ammonium nitrate (0.20 M in water) (1.5 ml, 0.30 mmol) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature before the addition of saturated NaCl. The

^{13}C NMR (CDCl_3 , 125 MHz) δ 187.92, 185.33, 156.37, 143.07, 137.97, 136.00, 71.50, 61.06, 37.95, 25.64, 24.67, 22.22, 20.57.

IR (neat, cm^{-1}) 3462, 2934, 1651, 1385, 1273.

HRMS calcd for $\text{C}_{19}\text{H}_{28}\text{NO}_4$ ($\text{M} + \text{CH}_3\text{CN} + \text{H}^+$): 334.2013, found 334.2017.

Preparation of 5-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (23c).

2-(2,4-difluorophenyl)-1-(4-isopropyl-2,3,5-trimethoxyphenyl)-3-(1*H*-1,2,4-triazol-1-yl)propan-2-ol (22c)

PrMgCl (2.0 M in Et_2O , 3.3 ml, 6.6 mmol) was added to a stirred solution of benzylbromide **2** (1.0 g, 3.3 mmol) in Et_2O (16 ml) at -20°C . After the resulting solution was stirred at the same temperature for 5 h, 1-(2,4-difluorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethan-1-one (1.5 g, 6.6 mmol) in THF (16 ml) was added. The reaction mixture was allowed to warm to room temperature gradually and stirred over night. The reaction mixture was quenched with saturated NH_4Cl and extracted with AcOEt . The organic phases were dried over Na_2SO_4 , filtered and evaporated to dryness. Silica gel column chromatography (Hexane/Acetone = 80/20 to 40/60) provided **22c** in 35% yield.

^1H NMR (CDCl_3 , 500 MHz) δ 8.12 (s, 1H), 7.79 (s, 1H), 7.36-7.29 (m, 1H), 6.80-6.64 (m, 2H), 6.17 (s, 1H), 4.74 (d, $J = 13.8$ Hz, 1H), 4.53 (d, $J = 13.8$ Hz, 1H), 3.76 (s, 3H), 3.74 (s, 3H), 3.60 (s, 3H), 3.39 (sept, $J = 7.0$ Hz, 1H), 3.20 (d, $J = 14.3$ Hz, 1H), 3.15 (d, $J = 14.3$ Hz, 1H), 1.22 (d, $J = 7.0$ Hz, 3H), 1.21 (d, $J = 7.0$ Hz, 3H).

^{13}C NMR (CDCl_3 , 125 MHz) δ 162.64 (dd, $J = 12.5$ Hz, $J = 247.4$ Hz), 158.81 (dd, $J = 11.9$ Hz, $J = 244.4$ Hz), 154.74, 151.54, 151.08, 144.88, 130.77, 130.10, 130.01 (dd, $J = 6.0$ Hz, $J = 9.6$ Hz), 126.04, 125.59 (d, $J = 13.1$ Hz), 111.27 (dd, $J = 1.8$ Hz, $J = 20.3$ Hz), 109.67, 103.80 (t, $J = 26.8$ Hz), 60.96, 60.55, 55.72, 39.58, 25.17, 21.11, 21.14.

IR (neat, cm^{-1}) 3393, 2958, 1499, 1273.

HRMS calcd for $\text{C}_{23}\text{H}_{27}\text{F}_2\text{N}_3\text{NaO}_4$ ($\text{M} + \text{Na}^+$): 470.1862, found 470.1863.

5-(2-(2,4-difluorophenyl)-2-hydroxy-3-(1*H*-1,2,4-triazol-1-yl)propyl)-2-isopropyl-3-methoxycyclohexa-2,5-diene-1,4-dione (23c)

To a stirred solution of **22c** (72 mg, 0.14 mmol) in acetonitrile (5.5 ml) was added cerium ammonium nitrate (0.20 M in water) (1.5 ml, 0.30 mmol) at 0 °C. The reaction mixture was stirred for 1 h at the same temperature before the addition of saturated NaCl. The mixture was extracted with AcOEt and the combined organic layers were dried over Na₂SO₄, filtered, and concentrated. Silica gel column chromatography (Hexane/Acetone = 80/20 to 40/60) provided **23c** in 84% yield (48 mg).

¹H NMR (CDCl₃, 500 MHz) δ 8.08 (s, 1H), 7.83 (s, 1H), 7.42-7.35 (m, 1H), 6.80-6.72 (m, 2H), 6.41 (s, 1H), 5.13 (brs, 1H), 4.71 (d, J = 14.5 Hz, 1H), 4.54 (d, J = 14.5 Hz, 1H), 3.78 (s, 3H), 3.16-3.08 (m, 2H), 2.86 (d, J = 13.5 Hz, 1H), 1.14 (d, J = 7.5 Hz, 6H).

¹³C NMR (CDCl₃, 125 MHz) 187.36, 185.16, 162.96 (dd, J = 12.6 Hz, J = 248.6 Hz), 158.61 (dd, J = 11.4 Hz, J = 245.0 Hz), 156.08, 151.80, 144.53, 141.00, 138.43, 137.11, 129.82 (dd, J = 5.9 Hz, J = 9.6 Hz), 124.12 (d, J = 13.1 Hz), 111.73 (d, J = 17.9 Hz), 104.36 (t, J = 26.3 Hz), 74.97, 61.00, 57.36, 37.48, 24.67, 20.50, 20.44.

IR (KBr, cm⁻¹) 3399, 2963, 1651, 1616, 1599, 1500, 1273.

HRMS calcd for C₂₁H₂₁F₂N₃NaO₄ (M + Na⁺): 440.1392, found 440.1399.

Assay of the anti-trypanosomal activity of quinone derivatives on *T. cruzi* trypomastigotes

Human fibrosarcoma HT1080 cells were infected with *T. cruzi* trypomastigotes.¹⁶ The parasites were collected from the culture medium by centrifugation at 1600 × g for 10 min at room temperature.¹⁷ The trypomastigotes (5 × 10⁶) were suspended in 0.1 mL of DMEM (Sigma-Aldrich), supplemented with 10% fetal bovine serum (FBS, Sigma), and incubated for 24 h in the presence of 0.1% ethanol or various concentrations of quinone derivatives. To evaluate the anti-trypanosomal effect of the quinone derivatives, live parasites were directly quantified by using a Neubauer chamber. More than three independent experiments were performed.

Assay of cytotoxicity for quinone derivatives in Swiss3T3 cells

Exponentially growing Swiss3T3 cells were inoculated at 1 × 10⁴ cells/well, in 96-well culture plates, and incubated for 24 h in 0.2 mL of DMEM supplemented with 10% FBS. The cells were cultured for 24 h in the presence of a vehicle or various concentrations of

one of the quinone derivatives. Cell death was evaluated using a CytoTox-One Homogeneous Membrane Integrity Assay kit (Promega), according to the manufacturer's instructions.

Table 1. Effects of quinone derivatives on *T. cruzi* trypomastigotes and cytotoxicity.

Compounds	IC50 (μM) <i>vs. T. cruzi</i> trypomastigotes ^a	LD50 (μM) <i>vs. Swiss3T3</i> cells ^b
BZL	6.8	>100
Komaroviquinone	0.25	91
14a	>10	>100
14b	>10	>100
14c	>10	>100
17a	0.33	>100
17b	1.1	>100
17c	>10	>100
17d	2.4	>100
19a	>10	>100
19b	2.0	>100
19c	9.8	>100
19d	>10	>100
21a	>10	>100
21b	>10	>100
21c	>10	>100
21d	>10	>100
21e	0.3	>100
23a	>10	>100
23b	0.59	>100
23c	0.54	>100

^a IC50 values for trypanocidal activity were determined by assessing the effects of various concentrations of quinone derivatives.

^b LD50 values for Swiss3T3 cells were determined by assessing lactate dehydrogenase activity in the Swiss3T3 cell culture media, in the presence of various concentrations of quinone derivatives.

Graphical abstract