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Synthesis and Evaluation of Quinone Derivatives for Activity against *Trypanosoma cruzi*Yutaka Suto,^{*a} Tatiana Ascencio,^b Tomoya Nobuta,^a Noriyuki Yamagiwa,^a Yoko Onizuka,^b Mayumi Ishii,^c Kayoko Kanemitsu,^c and Junko Nakajima-Shimada^{*b}^aFaculty of Pharmacy, Takasaki University of Health and Welfare, Takasaki, Gunma 370–0033, Japan; ^bDepartment of Molecular and Cellular Parasitology, Graduate School of Health Sciences, Gunma University, Maebashi, Gunma 371–8514, Japan; and ^cDrug Discovery Initiative, The University of Tokyo, Bunkyo-ku, Tokyo 113–0033, Japan.

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A series of quinone derivatives with a variety of side chains were synthesized. These synthetic quinone compounds were evaluated for *in vitro* antitrypanosomal activity against trypomastigotes and amastigotes of *Trypanosoma cruzi*, the causative agent of Chagas disease. Measurement of solubility of quinones and their ability to permeate cell membranes were assessed to address their possible use as oral drugs. Some synthesized compounds exhibited potent antitrypanosomal activity. However, most compounds with a promising activity showed poor solubility that did not seem suitable for oral usage. Meanwhile, compound 5a, an *N*-*tert*-butoxycarbonylpiperidine derivative, exhibited good antitrypanosomal activity, ability to permeate membranes, and good solubility.

Key words *Trypanosoma cruzi*; Chagas disease; quinone

Introduction

Chagas disease is a neglected tropical disease caused by the protozoan parasite, *Trypanosoma cruzi* (*T. cruzi*).¹⁾ The parasite is primarily transmitted *via* bites of the carrier vector, the blood-sucking “kissing bug.” Blood transfusion, organ transplants, congenital transmission, and ingestion of contaminated food have been documented as other routes of infection.^{2–4)} In rural areas of 21 Latin American countries where *T. cruzi* is endemic, Chagas disease is an important health problem. The disease affects 5–8 million people and causes approximately 10000 deaths per year. Almost 70 million people are at risk of infection.⁵⁾ Recently, the disease has become a public health concern in nonendemic countries in North America, Europe, Australia, and Japan as a result of immigration from Latin America.^{6–9)}

Chagas disease has two distinct phases. The acute phase is often asymptomatic, or patients develop nonspecific symptoms, such as fever and general malaise. In most cases, these symptoms decline spontaneously after 1–2 months.^{10,11)} After activation of the host immune system, parasitemia fades and becomes undetectable. This indeterminate state can last for years and even decades. The majority of infected individuals will continue their life unaffected. However, between 30 and 40% of infected individuals will progress to chronic disease. Such patients may develop severe cardiac and digestive pathologies leading to severe disability or death.¹²⁾

Only two drugs, benznidazole and nifurtimox developed more than 40 years ago, are currently in use.^{13,14)} Both drugs cause significant side effects, require long courses of treatment—up to 60d, and are effective only in the acute phase of infection. Efficacy is limited in the chronic phase. The emergence of drug resistance is also problematic. CYP51 inhibitors, such as posaconazole and E1224 (prodrug of ravuconazole), recently demonstrated encouraging results in *in vitro* and *in vivo* models and were tested in clinical trials.^{15,16)} Unfortunately, these azoles were ineffective as compared with benznidazole. Thus, an urgent need exists for safe drugs with high clinical efficacy in both phases of the disease.

Komaroviquinone, a quinone-type diterpene isolated from *Dracocephalum komarovii*, inhibits the growth of trypomastigotes of *T. cruzi*.^{17,18)} We developed an asymmetric total synthesis of komaroviquinone and studied the importance of the quinone moiety and fused cyclic structure for activity against *T. cruzi*. We thus evaluated the antitrypanosomal activity of synthetic intermediates of komaroviquinone and related quinone compounds.^{19,20)} The quinone moiety of komaroviquinone was crucial for expression of antitrypanosomal activity but the complex structure with chiral centers was not. Further, we identified that some quinones with simplified organic syntheses showed antitrypanosomal activity similar to komaroviquinone. A representative is compound **2** in Fig. 1. We present a structure–activity relationship study of compound **2**. We synthesized compound **2**-related quinone compounds and evaluated their *in vitro* antitrypanosomal activity and assessed their solubility and ability to permeate cell membranes.

Chemistry

An overview of analogs is provided in Fig. 2. We synthesized compounds **3a–k** where the other substituent was replaced by an isopropyl group in compound **2**. These analogs were used to examine whether lipophilicity, size, and electron-

Fig. 1. Chemical Structure of Komaroviquinone (**1**) and Related Compound **2**

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ic character of substituents (electron donating or accepting) on the quinone structure affected antitrypanosomal activity. Compounds **4a–h**, **5a**, and **5b** with acyl groups consisting of a substituted phenyl ring or *N*-substituted piperidyl group instead of the cyclohexyl group were synthesized to investigate the importance of the acyl group for antiparasitic activity.

A series of quinone derivatives was synthesized (Chart 1 or 2). Aldehydes **6a–k** were reacted with hydroxylamine to form corresponding oximes, which were reduced with zinc to amines **7a–k** under acidic conditions. These amines were further reacted with cyclohexanecarbonyl chloride and final oxidation using cerium ammonium nitrate (CAN) to produce quinone analogs **3a–k**.

For the synthesis of quinone derivatives **4a–h**, **5a**, and **5b** we first prepared compound **9** under previously reported conditions²⁰ (Chart 2). Carboxylic acids used as start-

ing materials were commercially available. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl) mediated coupling of amine **9**, and various carboxylic acids gave amides **10a–h**, **11a**, and **11b**. Quinone derivatives **4a–h**, **5a**, and **5b** were obtained by oxidation of amides **10a–h**, **11a**, and **11b** with CAN.

Biological Evaluation

We used the above compounds to evaluate antitrypanosomal activity. Three forms of *T. cruzi* are known: epimastigotes, trypomastigotes, and amastigotes. Trypomastigotes and amastigotes are present in the human body during infection. Trypomastigotes are the form of the parasite found in the human bloodstream. *T. cruzi* trypomastigotes invade tissues and undergo a morphological change to amastigotes. This form proliferates within cells. E1224 and posaconazole both showed inadequate efficacy in Phase 2 studies, with a high rate of protozoan relapse after the end of the treatment period.^{15,16} Posaconazole was reported to exhibit potent antiprotozoal activity against amastigotes, except for a few strains, but not against trypomastigotes.²¹ Posaconazole activity may require the parasite to undergo a certain number of replications. We assessed antiprotozoal activity of the quinone derivatives against both amastigotes and trypomastigotes to confirm activity throughout the life cycle of the parasite. We evaluated the viability inhibitory activity of quinone derivatives against trypomastigotes, their inhibitory activity against trypomastigotes to invade host cells, and their viability inhibitory activity against amastigotes. IC₅₀ values against trypomastigotes and amastigotes and cytotoxicity of compounds are provided in Table 1.

“Infection rate” is the concentration of a drug at which the percentage of host cells infected with *T. cruzi* is halved. “Amastigotes/infected cell” is the concentration of drug at which the number of parasites in the host cell is halved compared with the absence of the drug. Antiprotozoal activity of most compounds against trypomastigotes is greater than the

Fig. 2. Synthesized Quinone Compounds

Reagents and Conditions: (a) (1) HONH₂·HCl, C₂H₅OH, room temperature (r.t.), (2) AcCl, C₂H₅OH, 0 °C, (3) Zn, C₂H₅OH, 50 °C; (b) ^cHexCOCl, ^tPr₂NEt, CH₂Cl₂, 0 °C; (c) CAN, CH₃CN/H₂O, 0 °C.

Chart 1. Synthesis of Compounds **3a–k**

Reagents and Conditions: (a) Carboxylic Acid, EDCI·HCl, CH₂Cl₂, r.t.; (b) CAN, CH₃CN/H₂O, 0 °C.

Chart 2. Synthesis of Compound **4a–h**, **5a**, and **5b**

Table 1. Antitrypanosomal Activity and Cytotoxicity to Mammalian Cells of Quinone Compounds

Compound	<i>T. cruzi</i> IC ₅₀ (μM)			Swiss 3T3 CC ₅₀
	Trypomastigotes	Amastigotes		
		Infection rate	Amastigotes/infected cell	
Benznidazole	6.4	5.6	1.9	>100
2	6.8	>1	0.9	>100
3a	2.2	0.9	>1	>100
3b	2.9	0.34	0.009	>100
3c	0.1	0.5	0.17	>100
3d	0.3	4.6	2.8	>100
3e	0.38	0.72	0.08	>100
3f	N.D.	0.6	0.08	>100
3g	0.14	3	2.9	>100
3h	0.16	3.4	3.2	>100
3i	1.5	>1	0.32	>100
3j	1.2	>1	>1	>100
3k	1.8	>1	>1	>100
4a	0.35	N.D.	N.D.	>100
4b	1.2	3.9	1.8	>100
4c	0.15	2.7	1.4	>100
4d	0.2	3.8	1.6	>100
4e	0.17	7.1	3.6	>100
4f	0.14	3.8	3.2	>100
4g	0.12	1.1	2.4	>100
4h	0.1	0.17	0.13	>100
5a	2.3	0.54	0.068	>100
5b	1.6	0.25	0.058	>100

antiprotozoal activity of original compound **2**. The antiprotozoal activity of compounds **3b**, **3c**, **3e**, **3f**, **3i**, **4h**, **5a**, and **5b** against amastigotes was also greater than the activity of compound **2**. Replacement of the isopropyl group on the quinone with a linear alkyl group (compounds **3b** and **3c**) or with ring structures (compounds **3e** and **3f**) showed high antiprotozoal activity against amastigotes. Replacement of the isopropyl group with a methoxy group (compound **3i**) showed higher antiprotozoal activity against trypomastigotes and amastigotes than observed for compound **2**. In contrast, replacement with halogens (compounds **3j** and **3k**) did not show any improvement in activity against amastigotes. All compounds with a monosubstituted phenyl group instead of the cyclohexyl group on the amide side of compound **2** showed good antiprotozoal activity against trypomastigotes. However, except for compound **4h**, with a methoxy group, all compounds showed lower antiprotozoal activity against amastigotes.

Compounds with an *N*-alkoxycarbonylpiperidine instead of the cyclohexyl group (compounds **5a** and **5b**) showed high antiprotozoal activity against amastigotes, with IC₅₀ values among the highest for compounds synthesized for this study. Antiprotozoal activity against trypomastigotes was modest compared with other compounds, but 3- to 4-fold greater compared with compound **2**. No clear correlation was found between the antiprotozoal activity against trypomastigotes and amastigotes. For example, compound **4e** showed seven times higher antiprotozoal activity against trypomastigotes compared with compound **4b**; however, the activity against amastigotes (as amastigotes/infection cell) was half that of compound **4b**. Similar results were observed between compounds **3b**, **3g**, and **3h**. High antiprotozoal activity against one form

of *T. cruzi* does not necessarily indicate high activity against another form. Compounds with low activity in inhibition of survival against trypomastigotes but high activity in inhibition of infection, such as compounds **3b**, **5a**, and **5b**, may inhibit the adhesion of trypomastigotes to host cells and the invasion process. On the other hand, compounds with high activity in inhibition of survival against trypomastigotes but low inhibition of infection, such as compounds **3d**, **3g**, **3h**, and **4c–4f**, may take longer to inhibit the activity of trypomastigotes and allow them to invade and proliferate in host cells. In order to evaluate the overall antiprotozoal activity of a compound, the antiprotozoal activity against both forms should be evaluated from several perspectives.

Physicochemical Properties

Drugs for Neglected Diseases initiative (DNDi) indicates in its target product profile for Chagas disease that an ideal drug formulation is compatible with oral administration.²²⁾ Absorption of orally administered compounds depends on dissolution in gastrointestinal fluid and permeation through the small intestinal mucosa during gastrointestinal transit. For a drug to be absorbed orally, it must have adequate solubility and the ability to cross cell membranes.²³⁾ We evaluated these properties by measuring solubility at pH 6.8 and membrane permeability using Parallel Artificial Membrane Permeability Assay (PAMPA)²⁴⁾ (Table 2).

Compound **2** showed higher membrane permeability than caffeine. Caffeine displays high membrane permeability. The solubility of compound **2** at pH 6.8 was 34 μg/mL. Compounds **3a** and **3i**, with the isopropyl group replaced by the methyl and methoxy groups, respectively, showed good permeability

Table 2. Solubility and Membrane Permeability of Quinone Compounds

Compound	Solubility ^{a)}		Permeability ^{b)} (nm/s)	Compound	Solubility ^{a)}		Permeability ^{b)} (nm/s)
	(μ M)	(μ g/mL)			(μ M)	(μ g/mL)	
2	107	34	354	4a	121	38	320
3a	462	135	264	4b	70	23	290
3b	33	10	307	4c	8	3	N. D.
3c	<1	<1	N. D.	4d	3	1	N. D.
3d	6	2	N. D.	4e	59	21	210
3e	<1	<1	N. D.	4f	6	2	N. D.
3f	12	4	276	4g	9	3	N. D.
3g	3	1	N. D.	4h	19	5	275
3h	11	4	N. D.	5a	373	177	217
3i	1132	348	129	5b	38	17	186
3j	Decomp.	Decomp	N. D.	Caffeine	N. D.	N. D.	120
3k	920	272	N. D.	Sulfasalazine	N. D.	N. D.	1.1

a) Solubility at pH 6.8. b) Corning® BioCoat™ Pre-coated PAMPA Plate System was used.

Table 3. Metabolic Stability, Plasma Protein Binding, and Light Stability of Compound **5a**

Metabolic stability ^{a)} (mL/min/kg)		Plasma protein binding (%)		Light stability ^{b)} (%)	
Human	Mouse	Human	Mouse	Light, 0 °C	Dark, 37 °C
343	1849	96	93	45	>90

a) Clearance in human and mouse liver microsomes. b) % of remaining after 3 h.

and improved solubility. Compounds **3a** and **3i** exhibited approximately 4- and 10-fold increased solubility, respectively, compared with compound **2**. However, compound **3b**, with an *n*-propyl group instead of the isopropyl group, showed approximately a three-fold decrease in solubility. Further, compounds **3c–h** had longer alkyl chains or cyclic alkyl groups and displayed poor solubility, showing that oral administration would be impractical. Compounds **4a–h**, with a phenyl ring substituted for the cyclohexyl ring, displayed lower solubility than compound **2**. Compound **4h**, with promising antitrypanosomal activity, also showed problematic aqueous solubility as low as compounds with monosubstituted phenyl rings. Compounds **5a** and **5b**, both with an *N*-alkoxycarbonylpiperidine group, showed good permeability but varied substantially in solubility. Compound **5a**, with a *tert*-butoxycarbonyl group at the nitrogen of piperidine ring, showed 4.6-fold increased solubility compared with compound **2**; however, a benzyloxycarbonyl group in compound **5b** yielded half this solubility. Adequate antitrypanosomal activity and permeability combined with contrasting aqueous solubility of compounds **5a** and **5b** suggest that solubility can be improved while maintaining desirable biological activity and membrane permeability by exploring the alkoxy group of compounds with a carbamate structure as contained in compounds **5a** and **5b**.

Compound **5a** was predicted to be efficiently absorbed by the gastrointestinal tract using Swiss ADME²⁵⁾ to estimate various parameters of the compound. This prediction probably reflected very high membrane permeability and good solubility of compound **5a**. The calculated log P value of compound **5a** was 2.17, suggesting an adequate balance between lipophilicity and hydrophilicity. IC₅₀ values of compound **5a** against trypanomastigotes and amastigotes were 2.3 and 0.068 μ M, respectively. Lipophilic ligand efficacies (LLE)²⁶⁾ calculated from the log P value were 3.46 and 5.01. An LLE value should

be greater than 5. Compound **5a** meets this requirement on the basis of its antiprotozoal activity against amastigotes.

Metabolic stability in mouse and human liver microsomes, plasma protein binding (PPB), and photostability of compound **5a** are summarized in Table 3.

The rate of metabolism of compound **5a** was high in both mouse and human liver microsomes, as were PPB values. These properties may pose a problem for the evaluation of efficacy and safety using model animals and are issues that must be resolved in the future. Instability in light was also identified as a problem for compound **5a**. This compound was stable at 37 °C in a buffer solution in the dark, but considerable decomposition was observed even at 0 °C under fluorescent light irradiation. This problem is not critical for evaluating drug efficacy at the laboratory level, but care must be taken when handling solutions. Komaroviquinone, the starting point for quinone synthesis is converted to komarovispirone by light irradiation,²⁷⁾ and photodegradation of compound **5a** may occur by a similar mechanism. Photoisomerization of komaroviquinone likely involves radical dehydrogenation of the hydroxyl group at position 7 adjacent to the quinone structure as a starting point for subsequent structural transformation. Therefore, adjusting chemical moieties around the quinone structure of compound **5a** may improve its stability in light.

Conclusion

We synthesized quinones in which the isopropyl group on the quinone ring of compound **2** was replaced by other groups. Also, we synthesized quinones in which the cyclohexyl ring on the amide side of compound **2** was replaced by other cyclic structures. These compounds were evaluated for their antiprotozoal activity against trypanomastigotes and amastigotes of *T. cruzi*. In addition, solubility and membrane permeability were measured to confirm whether compounds with promising

antiprotozoal activity had physicochemical properties compatible with oral administration. Data were also used to assess relationships between chemical structure and physicochemical properties. Most compounds with good antiprotozoal activity showed poor solubility; however, compound **5a** showed adequate antiprotozoal activity, solubility, and high membrane permeability. We are planning to evaluate the *in vivo* efficacy of compound **5a** using acute and chronic phase mouse models. Compound **5a** has issues with metabolic stability and photostability. We hope to solve these problems with an additional study of structure–activity and structure–property relationships around the amide nitrogen and carbamoyl groups.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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