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Synthesis, in vitro antitrypanosomal and antibacterial activity of phenoxy, phenylthio or benzyloxy substituted quinolones

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ABSTRACT

Chagas' disease, caused by *Trypanosoma cruzi (T. cruzi)*, is one of the most serious parasitic diseases in Latin America. The currently available chemotherapy, based on nifurtimox or benznidazole, is unsatisfactory due to the limited efficacy in the prevalent chronic stage of the disease and toxic side effects. In order to address these deficiencies, a series of quinolones based novel molecules have been synthesized and evaluated as potential antitrypanosomal agents. The most active analogue **10** inhibited *T. cruzi* with an IC_{50} of 1.3 μg/mL. The results of this study have implications in the development of novel quinolone's antitrypanosomal agents.

Chagas' disease, caused by *Trypanosoma cruzi (T. cruzi)*, is one of the most serious parasitic diseases in Latin America. It is a potentially fatal, chronic illness that currently affects about 18–20 million people and another 100 million people are at risk of acquiring the disease.[1,2] Although sequencing of *T. cruzi* genome was completed,[3] neither prospect of antitrypanosomal vaccine nor a new drug has been developed yet to prevent or treat Chagas' disease. The medication for Chagas' disease has been made entirely by chemotherapy.[4,5] The currently available chemotherapy is based on two agents introduced in the market in the 1970s: nifurtimox (4-(5-nitrofurfurylindenamino)-3-methylthiomorpholine 1,1-dioxide, Lampit, discontinued by Bayer), a nitrofuran derivative; and benznidazole (*N*-benzyl-2-(2-nitro-1*H*-imidazol-1-yl)acetamide, Rochagan, Roche), a nitroimidazole derivative.[5] Due to limited efficacy in the prevalent chronic stage of the disease and toxic side effects, the need for the discovery of new therapeutic agents to treat Chagas' disease is clear and urgent.

The quinolones and fluoroquinolones constitute a major class of antibacterial chemotherapeutic agents, which have a broad spectrum of activity against bacteria, mycobacteria, parasites, and other diseases.[6] The history of quinolones began in the late 1950s, when Lesher accidentally discovered nalidixic acid, 1-ethyl-1,4-dihydro-7-methyl-1,8-naphthylidine-3-carboxylic acid, as a by-product in the synthesis of an antimalarial agent chloroquine.[7] In the 1980s, medicinal chemists discovered that specific chemical modifications of nalidixic acid dramatically improved the antibacterial spectrum of activity. One of these modifications was the addition of fluorine (the source of the name 'fluoroquinolone'). In 1986, norfloxacin became the first fluoroquinolone marketed for human use, closely followed by ciprofloxacin in 1987. Since 1986, more than 20 fluoroquinolones have been approved by FDA and most of those remain on the market.[8]

The antibacterial activity generated by fluoroquinolones is caused by the inhibition of two bacterial enzymes: DNA gyrase (a topoisomerase enzyme in bacteria) and topoisomerase IV enzyme.[8] The general function of topoisomerases is to facilitate the uncoiling of DNA during DNA replication, as well as to facilitate the recoiling and packaging of DNA once DNA replication has occurred.[9] Like bacteria, kinetoplastid protozoa have unique and complex types of mitochondrial DNA, composed of thousands of topologically interlocked circular DNA molecules, creating a high demand for topoisomerase enzymes activity.[10] Targeting topoisomerases may be a way to follow in searching for new chemotherapeutic agents against the diseases caused by trypanosoma, such as Chagas' disease, where a high rate of *T. cruzi* proliferation and unique aspects of the kDNA replication were involved.[11]

A number of studies reported that fluoroquinolones have activity in vitro or in vivo against trypanosomes or Leishmania (a genus of trypanosome protozoa).[12–21] However, only limited in vitro activity against *T. cruzi* was observed for the fluoroquinolones and quinolones in clinical use.[12–15] The investigators found that

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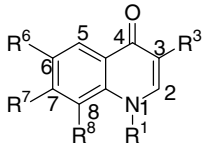
the presence of nalidixic acid at the concentration of 200 μg/mL resulted in 60% inhibition of *T. cruzi* epimastigote proliferation,[15] and another study showed that ciprofloxacin exhibited limited activity against *T. cruzi*, clearing amastigotes from 26% of macrophages at the concentration of 250 μM.[14] A combination of a fluorine atom at position 6 (see Table 1 for fluoroquinolone numbering) and a 5- or 6-membered heterocycle that contains peripheral nitrogens at position 7 in quinolones often resulted in a broad and potent antimicrobial activity. However, this combination may not be necessary for their antitrypanosomal activity against *T. cruzi*. There were no reports for this class of compounds using non-nitrogen containing heterocyclic substitutions for their activity against *T. cruzi*. Herein, as a part of our ongoing research program to discover new classes of antitrypanosomal agents, we developed a small library of quinolone derivatives which did not contain nitrogen heterocyclic substitution at position 7 of quinolone moiety for their in vitro antitrypanosomal activity against *T. cruzi*. In this letter, the potential of quinolones substituted with a non-nitrogen group such as phenoxy, phenylthio or benzyloxy at position 6, 7 or 8 as antitrypanosomal agents against *T. cruzi* was investigated. And antibacterial activity was also tested to determine the selectivity between the trypanosoma and bacterial species.

A series of phenoxy, benzyloxy and phenylthio quinolones analogues **1**–**28** listed in Table 1 were synthesized as outlined in Scheme 1. 6,7,8-Trifluoro-1,4-dihydroquinoline-3-carboxylic acid (**Ia**, **Ib** or **Ic**),[22] a crucial intermediate for the synthesis of antibacterial quinolones, was treated with excess phenol or thiophenol with NaH in DMSO to give 7,8-disubstituted derivatives **1**–**9**. Using benzyl alcohol as a nucleophile, the substitution on **Ia** was taken place under aqueous alkali condition (8 N KOH) to give **10**.[23] Under the same condition, reaction with 1-fluoroethyl quinolone **Ic** gave N^1 dehydrofluoride 7,8-dibenzyloxy derivative **11**.[24] However, when this condition was applied to **Ib**, a mixture was formed and the attempts of isolation were failed. Two sets of ^{1}H NMR spectral data from the mixture with different H–F coupling constants on 5-H of the quinolone suggest the formation of a nearly equal amount of 7,8-disubstituted and 6,7-disubstituted products. This could be due to the steric effect of *N*-cyclopropyl group which hindered the leaving of fluoride at position 8. Some of these derivatives were further elaborated: esterification of carboxylic acids **4**, **5** and **10** was accomplished by the addition of $SOCl_2$ in methanol or ethanol to afford methyl or ethyl ester **12**–**17**; and in the other way, treatment of compound **4** or **5** with 1,1-carbonyldiimidazole (CDI), followed by reacting with NH_3, gave **18** or **20**. Further dehydration of the amide **18** by $POCl_3$ gave 3-cyanoquinolone **19**.

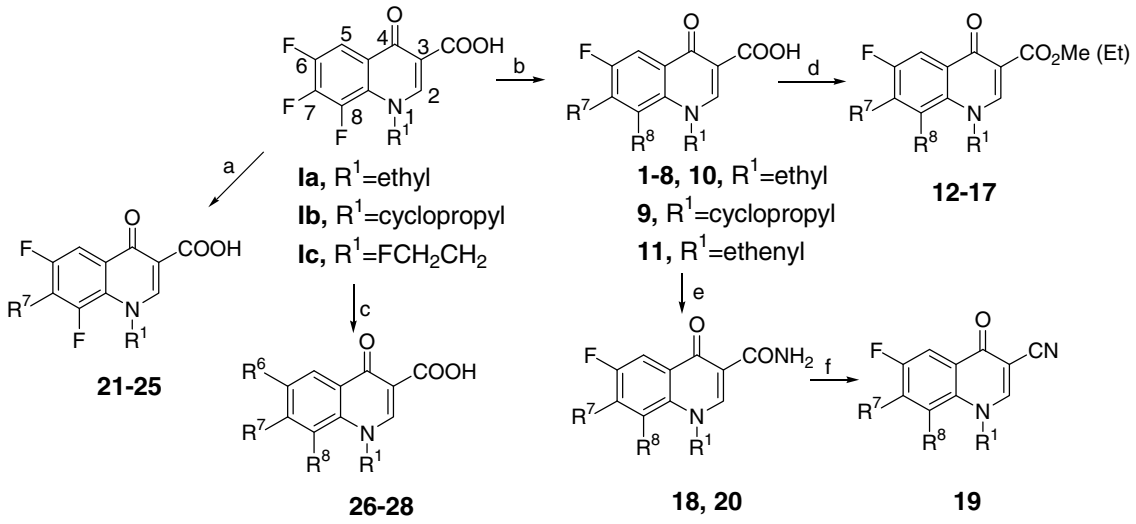
During the preparation of 7,8-disubstituted derivatives, some mono or tri-substituted by-products were also formed and perked our interests, since some antibacterial quinolones are substituted only at position 7 and with fluorines at position 6 and 8. This phenomenon prompted us to synthesize 7-monosubstituted quinolones to explore how such modifications would affect the antitrypanosomal activity. The 7-substitution of **Ia** or **Ib** by phenol in the presence of NaH/DMSO at room temperature provided **21** and **22.** In addition, the monosubstitution at 7-position of **Ia** or **Ib** by thiophenol or benzyl alcohol was achieved with 3 N KOH at 50 °C to give **23**–**25**. Furthermore, displacement of all the fluorine atoms at a higher temperature by above three nucleophiles gave tri-substituted derivatives **26**–**28**.

Compounds **1**–**28** were characterized for the antitrypanosomal activity against *T. cruzi*,[25] and the results are summarized in Table

Table 1
In vitro antitrypanosomal activity against *T. cruzi* of quinolone derivatives



Compound	R^1	R^3	R^6	R^7	R^8	Yield (%)	IC_{50} (μg/mL)
1	C_2H_5	COOH	F	4-CH_3-PhO	4-CH_3-PhO	50.8	19.4
2	C_2H_5	COOH	F	4-OH-PhO	4-OH-PhO	23.9	57.1
3	C_2H_5	COOH	F	2,4-Dichloro-PhO	2,4-Dichloro-PhO	43.3	12.7
4	C_2H_5	COOH	F	PhO	PhO	56.7	>90
5	C_2H_5	COOH	F	PhS	PhS	62.6	19.3
6	C_2H_5	COOH	F	4-F-PhS	4-F-PhS	59.0	6.2
7	C_2H_5	COOH	F	4-Cl-PhS	4-Cl-PhS	52.1	16.3
8	C_2H_5	COOH	F	4-CH_3-PhS	4-CH_3-PhS	43.7	52.7
9	*c*-Pr	COOH	F	PhS	PhS	36.6	17.8
10	C_2H_5	COOH	F	$PhCH_2O$	$PhCH_2O$	31.6	1.3
11	CH=CH_2	COOH	F	$PhCH_2O$	$PhCH_2O$	33.7	6.2
12	C_2H_5	COOMe	F	PhO	PhO	82.3	>90
13	C_2H_5	COOEt	F	PhO	PhO	43.7	1.7
14	C_2H_5	COOMe	F	PhS	PhS	66.1	2.4
15	C_2H_5	COOEt	F	PhS	PhS	75.0	6.0
16	C_2H_5	COOMe	F	$PhCH_2O$	$PhCH_2O$	38.3	3.8
17	C_2H_5	COOEt	F	$PhCH_2O$	$PhCH_2O$	31.6	1.7
18	C_2H_5	$CONH_2$	F	PhO	PhO	77.9	4.0
19	C_2H_5	CN	F	PhO	PhO	60.0	>90
20	C_2H_5	$CONH_2$	F	PhS	PhS	86.4	>90
21	C_2H_5	COOH	F	4-CH_3-PhO	F	63.2	18.3
22	*c*-Pr	COOH	F	PhO	F	63.8	>90
23	C_2H_5	COOH	F	PhS	F	76.4	34.6
24	C_2H_5	COOH	F	$PhCH_2O$	F	74.7	18.3
25	*c*-Pr	COOH	F	$PhCH_2O$	F	84.2	>90
26	C_2H_5	COOH	4-CH_3-PhO	4-CH_3-PhO	4-CH_3-PhO	28.3	>90
27	C_2H_5	COOH	PhS	PhS	PhS	35.5	39.9
28	C_2H_5	COOH	$PhCH_2O$	$PhCH_2O$	$PhCH_2O$	28.3	2.5
Benznidazole							0.83



Scheme 1. Reagents and conditions: (a) BnOH, 3 N KOH, 50 °C, or ArOH, NaH/DMSO, rt, or ArSH, 2 N KOH, 50 °C; (b) BnOH, 8 N KOH, 80 °C, or ArOH, NaH/DMSO, 60 °C, or ArSH, NaH/DMSO, rt; (c) BnOH, 12 N KOH, reflux, or ArOH, NaH/DMSO, 140 °C, or ArSH, NaH/DMSO, 50 °C; (d) $SOCl_2$, MeOH or EtOH, reflux; (e) i—1,1′-carbonyldimidazole (CDI), DMF, 100 °C; ii—NH_3, methyl-2-pyrrolidinone, rt; (f) i—$POCl_3$, methyl-2-pyrrolidinone, rt; ii—$NaHCO_3$, pH = 8.

1. Most 7,8-disubstituted quinolones exhibited significant inhibitory activity against *T. cruzi* as exemplified by **10**, **13** and **17**. Among them, the 7,8-dibenzyloxy fluoroquinolone **10** exhibited the most potent activity with an IC_{50} value of 1.3 μg/mL. Replacement of the *N*-ethyl group of **10** with ethenyl resulted in **11** with a decreased activity (IC_{50} = 6.2 μg/mL). Esterification of 3-carboxylic group (compound **16** or **17**) also resulted in a decreased potency. The 7,8-diphenoxy compound **4** was less potent than the analogues **1**–**3** in which the phenyl group was substituted with methyl, hydroxyl or chloro group. When **4** was esterified with ethanol, the activity of **13** increased greatly with an IC_{50} value of 1.7 μg/mL, and a surprising difference was found for the methyl ester **12** (IC_{50} > 90 μg/mL). An increase in inhibitory activity was also found on 3-carboxylic amide derivative **18**. However, when the amide was transformed into nitrile, compound **19** showed no improvement in potency over the acid **4**. For the phenylthio analogues, incorporation of a fluoro atom at 4-position of phenyl ring as in compound **6** increased activity by nearly 3-fold when compared to unsubstituted derivative **5**. It suggests that the electro-withdrawing property of the 4-substituent in the phenyl ring is important, which is corroborated by increased activity of **7** with a chloro group and decreased activity of **8** with a methyl group in the 4-position of phenyl ring. Esterification of **5** enhanced the inhibition, and the activity of methyl ester **14** with IC_{50} value of 2.4 μg/mL was more potent than ethyl ester **15** (6.0 μg/mL). However, modification of 3-carboxylic acid into amide **20** resulted in the depletion of activity. Good inhibitory activity was also seen in 6,7,8-tribenzyloxy substituted derivatives (**28**, IC_{50} = 2.5 μg/mL), although the 6,7,8-trisubstitutions were less favorable in other substituted patterns.

The minimum inhibitory concentrations (MICs) of the quinolones **1**–**28** against several representative Gram-negative and Gram-positive organisms are summarized in Table 2, along with data of lomefloxacin for comparison. MICs were determined by an agar dilution method as outlined by the Clinical and Laboratory Standards Institute (CLSI).[26] The MIC was defined as the lowest concentration resulting in inhibition of visible bacterial growth after incubation at 37 °C for 24 h. Among all the 28 tested compounds, only those 7-monosubstituted compounds **21**–**25** showed significant antibacterial activities (MIC < 25 μg/mL), and the activities against Gram-positive organisms of these compounds were better than that against Gram-negative organisms. Compound **22** with a cyclopropyl group at 1-position was more active than the reference agent lomefloxacin against *Staphylococcus aureus*, or *Streptococcus pneumoniae*. Compound **21** exhibited potent activity against Gram-positive organisms *S. aureus* and *S. pneumoniae* comparable to lomefloxacin. The antibacterial property of these compounds seems to show no correlation to the antitrypanosomal activity, and a Spearman's correlation showed that the correlation between the IC_{50} against *T. cruzi* and MICs against *S. aureus* (ρ = −0.263, p = 0.195) and *S. pneumoniae* (ρ = −0.253, p = 0.176) was statistically not significant.[27] Since none of these compounds showed good antitrypanosomal activity against *T. cruzi*, this small library of quinolones exhibited great selectivity on trypanosoma.

Table 2
In vitro antibacterial activity of some 7-(substituted) quinolone derivatives

Strain	MIC (μg/mL)					
	21	22	23	24	25	Lomefloxacin
S. aureus	0.78	0.39	3.13	6.25	1.56	0.78
S. pneumoniae	0.39	0.098	0.78	1.56	1.56	0.195
S. albus	12.5	1.56	>25	>25	6.25	3.13
S. epidermidis	6.25	1.56	3.13	25	1.56	0.78
E. faecalis	>25	12.5	>25	>25	12.5	25
γ-Streptococcus	>25	>25	>25	>25	6.25	6.25
E. coli	25	3.13	>25	>25	1.56	0.098
S. sonnei	12.5	1.56	12.5	12.5	1.56	0.098
S. boydii	6.25	1.56	12.5	25	1.56	0.098
S.flexneri	>25	>25	>25	>25	>25	0.78
P. mirabilis	25	6.25	>25	>25	6.25	0.39
P. vulgaris	>25	6.25	>25	>25	6.25	0.78
P. aeruginosa	>25	12.5	>25	>25	6.25	3.13
M. morganii	25	3.13	>25	>25	6.25	0.195
K. pneumoniae	6.25	1.56	12.5	6.25	0.78	0.098
S. enteritidis	25	3.13	>25	>25	3.13	0.098
S. typhimurium	25	3.13	>25	25	1.56	0.195
C. freundii	12.5	1.56	12.5	25	1.56	0.098
A. hydrophila	25	6.25	>25	>25	3.13	6.25
S. marcescens	>25	12.5	>25	>25	6.25	0.195

In summary, a series of phenoxy, phenylthio and benzyloxy substituted quinolones were synthesized and evaluated for the inhibitory activity against *T. cruzi* and antibacterial activity. Most compounds exhibited moderate to high inhibitory activity in vitro against *T. cruzi*. Among them, the 7,8-dibenzyloxy quinolone **10** demonstrated the most significant antitrypanosomal activ-

ity against *T. cruzi*, which was comparable to the positive control, benznidazole. On the other hand, compounds **21**–**25** also showed some antibacterial activity, and inhibitory activity of **22** against two Gram-positive bacteria was even higher than the reference lomefloxacin. However, the inhibitory activities of these compounds against *T. cruzi* and bacteria were unrelated. Except compounds **21**–**25**, most compounds in this library showed great selectivity on *T. cruzi* to test bacteria. Hence, these compounds are promising candidates for further efficacy evaluation against *T. cruzi*. Besides that, this work should also provide significant opportunity for the discovery of novel quinolones as antitrypanosomal agents.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.11.078.

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23. *7,8-Bis-benzyloxy-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid* (***10***)*:* Yield 270 mg (31.6%) of a white solid. Mp: 161–163 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (3H, t, J = 6.8 Hz, CH_3), 4.50 (2H, q, J = 6.8 Hz, CH_2), 5.22 (2H, s, CH_2), 5.37 (2H, s, CH_2), 7.38 (10H, m, Ph), 8.08 (1H, d, J_{HF} = 10.0 Hz, 5-H), 8.56 (1H, s, 2-H), 14.70 (1H, br s, COOH). IR cm^{-1}: 3040 (υ_{ArC-H}), 2223 ($\upsilon_{C=N}$), 1050 (υ_{C-F}); EI-MS *m*/*z*: 447 (M^+), 403 (M^+–CO_2); Anal. Calcd for $C_{26}H_{22}FNO_5$: C, 69.79; H, 4.96; N, 3.13. Found: C, 69.55; H, 4.97; N, 3.14.
24. *7,8-Bis-benzyloxy-6-fluoro-4-oxo-1-vinyl-1,4-dihydro-quinoline-3-carboxylic acid* (***11***)*:* Yield 287 mg (33.7%) of a white solid. Mp: 144–146 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.98 (2H, s, CH_2), 5.19 (1H, dd, J = 1.6 Hz, J = 7.6 Hz, =CH_2), 5.33 (2H, s, CH_2), 5.47 (1H, dd, J = 1.6 Hz, J = 15.2 Hz, =CH_2), 7.36 (10H, m, Ph), 7.53 (1H, dd, J = 7.6 Hz, J = 15.2 Hz, —CH=), 8.04 (1H, d, J_{HF} = 11.2 Hz, 5-H), 8.68 (1H, s, 2-H), 14.49 (1H, br s, COOH). Ana; Calcd for $C_{26}H_{20}FNO_5$: C, 70.11; H, 4.53; N, 3.14. Found: C, 69.86; H, 4.43; N, 3.03.
25. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells per well per 100 μL in RMPI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h, 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4) containing the β-galactosidase (Lac Z) gene were added in 100 μL per well with 2× of a serial drug dilution. The plates incubated for 4 days. For IC_{50} value determination, the substrate CPRG/Nonidet was added to the wells and the color reaction which developed during the following 2–4 h was read photometrically at 540 nm. From the sigmoidal inhibition curves IC_{50} values were calculated. The experiment was carried out in triplicate.
26. MICs were determined as described by the NCCLS (see National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing: 11th Informational Supplement*, National Committee for Clinical Laboratory Standards: Wayne, PA, 2001; Vol. 21, M100-S11.
27. Statistical analysis was performed with the Spearman test (two tails) using SPSS version 16.0.