Quinolines as Chemotherapeutic Agents for Leishmaniasis

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Abstract: The development of leishmanicidal quinolines and their *in vitro* (promastigote and amastigote) and, where applicable, *in vivo* activities are reviewed. This survey provides a direct comparison of bioactivity across different species (e.g. *L. donovani, L. amazonensis, L. chagasi, L infantum*), and in different animal models (e.g. *L. donovani* Balb/c mice and *L. donovani* infected hamsters). The progress of selected quinolines through pre-clinical development and phase I/II trials, and the lead quinoline drugs sitamaquinine and Imiquimod, are discussed in conjunction with delivery systems and combination therapies.

1. Introduction

Leishmaniasis is a group of parasitic diseases spread by two genera of sandfly and causes symptoms ranging from skin and mucous-membrane lesions (cutaneous, CL or mucocutaneous leishmaniasis, MCL) to fatal organ infestation (visceral leishmaniasis, VL). The *Leishmania* parasite exists in the promastigote form in the sandfly vector which is first ingested by neutrophils [1] and then by macrophages in the human host or animal reservoir [2]. It then transforms into the non-flagellated amastigote, multiplies, ruptures the macrophage and circulates to infect fresh cells. This infection may be localized in cutaneous tissue (CL) resulting in chronic lesions which may self-heal over time. Alternatively, infection of mucosae (MCL) can lead to severe tissue destruction and disfigurement while infection of the spleen, liver and bone marrow (VL) is usually fatal if not treated [3].

Leishmaniasis is endemic in many developing countries from Central and South

America to southern Europe, the Middle East, Africa and South-West Asia and it is likely that this range will spread with altering climate patterns [4]. The number of cases has increased sharply over the last 10 years with an estimated 12 million patients. Most of these developing countries are also struggling with endemic HIV/AIDS and there has been a sharp increase in co-infection rates [5]. Both diseases attack immune cells increasing the infection risk of the other, the probability of relapse and clinical progression in both. The availability of highly active anti-retroviral therapy (HAART) in Europe has led to far fewer new leishmaniasis infections in HIV-positive patients, but this treatment is only sparsely available in the majority of developing countries.

To date, the treatment of leishmaniasis has relied on toxic compounds and / or established drugs originally developed for other diseases. The first chemotherapy for newly diagnosed patients is still antimony (V) based drugs in most countries [6]. These include sodium stibogluconate (Pentostan®) and meglumine antimoniate (Glucantime®) which are complex mixtures of oligomeric sugar - metal complexes, rather than discreet molecules [7]. The antimony (V) is reduced to more toxic trivalent antimony in the parasite although there are competing theories concerning the leishmanicidal mechanism of these compounds [8]. The antimonials require up to 28 days of parenteral administration and significant acquired drug resistance has emerged. The more expensive pentamidine and amphotericin B is usually effective in these cases, but still require injection or infusion and are beset with side-effects [9,10]. The anti-cancer agent Miltefosine (hexadecylphosphocholine) is the first effective oral drug, although it is teratogenic, still moderately expensive and can lead to resistance if treatment is incomplete [10,11].

In the quest for less toxic and less expensive chemotherapies, a large number of plant extracts and plant-derived natural products have been tested for anti-protozoal activity [12]. Most of the major classes of natural products have representatives with *in vitro* or *in vivo* activity against *Leishmania*, including phenolic compounds, terpenoids, quinones and alkaloids. Arguably, the most promising candidates belong to the latter family which include the anti-malarial quinine, an aminoquinoline that inhibits choline transport in *L. major* promastigotes [13]. The 8-aminoquinoline sitamaquine is an orally available drug that showed considerable promise in preclinical models,

but yielded mixed results in clinical trials, exhibiting variable cure rates and nephrotoxicity [14]. Structurally simpler 2-substituted quinolines, some isolated from the Bolivian medicinal plant *Galipea longiflora* (Rutaceae), show potent oral activity in a murine model of CL [15,16] and VL [17].

Simple quinolines could be important leads in the development of affordable, orally available treatments for leishmaniasis. Current chemotherapies and recent advances have been broadly reviewed elsewhere [18,19]. Our purpose here is to survey the structure activity relationship (SAR) of leishmanicidal quinoline analogues. This task is complicated by the different ways of quantifying and reporting leishmanicidal activity. Substituted quinolines have been investigated in clinical, *in vivo* and / or *in vitro* growth inhibition or related studies using promastigote and / or amastigote forms of between one and five of the *ca* 32 species of *Leishmania* and employing a variety of measures from the commonly used IC_{50} to the esoteric G-index (G = SD_{90} for meglumine antimoniate / SD_{90} for test compound). Direct comparison between studies is therefore not always meaningful, but a qualitative meta-analysis does allow some global trends to be inferred.
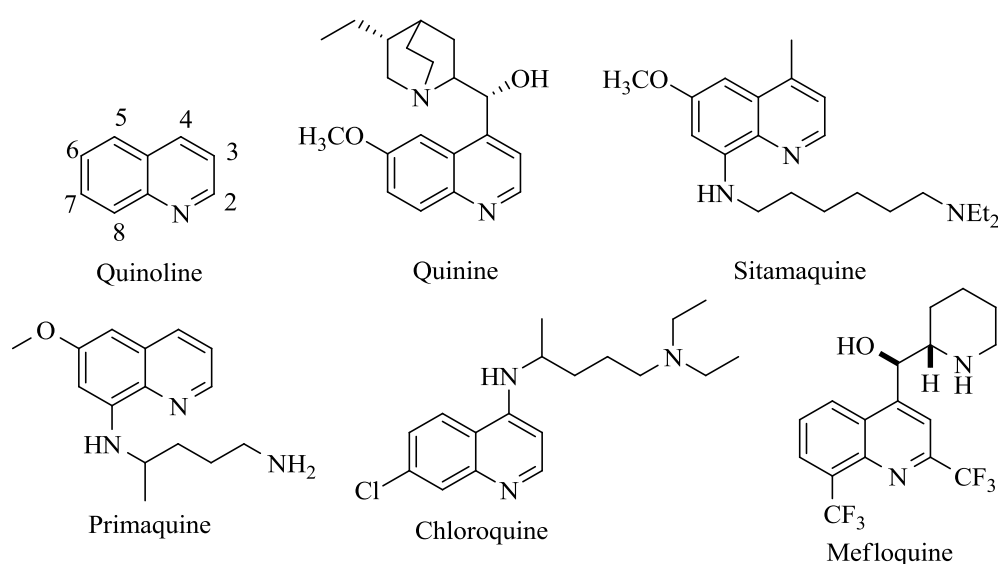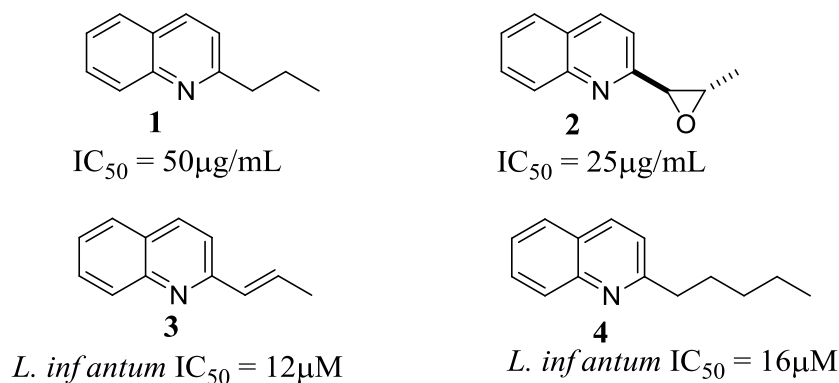

Fig. (1). Structures of quinoline and the anti-parasitic aminoquinolines, quinine, sitamaquine, primaquine, chloroquine and mifloquine.

2. Monosubstituted Quinolines

A series of 2-substituted quinoline alkaloid natural products isolated from a Bolivian medicinal plant, *G. longiflora*, used to treat CL ulcers demonstrated potent activity against a mouse model of New World CL [15]. These structurally simple quinolines show potent oral activity against both CL [15,16] and VL [17]. A variety of substitutions at the 2-position of the quinoline ring have been extensively studied because of the potent activity seen with isolated three - five carbon chain quinoline natural products (2-*n*-propylquinoline (**1**) , 2-(l, 2-*trans*-epoxypropyl) quinoline (chimanine D) (**2**) 2-(E)-prop-1-enylquinoline (chimanine B) (**3**) and 2-*n*-pentylquinoline (**4**)) which show greater potency than *N*-methylglucamine antimonate against *L. amazonensis, L. braziliensis* and *L. donovani* [20].

1

$IC_{50} = 50\mu g/mL$

2

$IC_{50} = 25\mu g/mL$

3

L. infantum $IC_{50} = 12\mu M$

4

L. infantum $IC_{50} = 16\mu M$

Substitution of an unsaturated group at the 2-position (e.g. α,β-unsaturated aldehyde or allylic alcohol (**5-14**)) increased *in vitro* activity relative to the unexpectedly potent 2-*n*-propylquinoline [21,22]. Compounds **5**, **8**, **9**, **11**, **12** showed significant inhibition of *L. amazonensis* and *L. infantum* amastigote proliferation in macrophages [23]. Analogues **5** and **9** had similar bioactivities (IC_{50} = 3.6, 3.2 μM respectively) against resistant strains of *L. donovani*. Both **5** and **9** were approximately 3 times more active than miltefosine against the HePC-R line and 40 times more active then sitamaquine on the Sita-R line and all other *Leishmania* tested cell lines [24]. Increasing the chain length of the 2-substituent resulted in a dramatic decrease in leishmanicidal activity. 2-But-2-enenitrile and 2-hex-2-ene analogs, for example exhibited *in vitro* IC_{50} = <30 μM when screened against *L. donovani, L. amazoniensis* and *L. infantum* [17,25].

Saquinavir (**15**), a 2-substituted aminoquinoline inhibits *L. major* parasite growth by 17% to 65% over 2 to 3 days in a dose-dependent manner. However, inhibition of *L.*

infantum, parasite growth was only 5% to 34% over the same period with irreversible leishmanicidal activity [26].
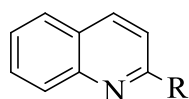
5 R = CH=CHCH$_2$OH; IC$_{50}$ = 2μM
6 R = CH=CHCH$_3$; IC$_{50}$ = 12μM
7 R = CH=CHPh; IC$_{50}$ = 11μM
8 R = CH=CHCOH; IC$_{50}$ = 2μM
9 R = CH=CHOCH$_3$; IC$_{50}$ = 3μM
10 R = C^{o}CSi(CH$_3$)$_3$; IC$_{50}$ = 8μM
11 R = CH=CHBr; IC$_{50}$ = 2μM
12 R = CH=CBr$_2$; IC$_{50}$ = 2μM
13 R = CH=CHQ; IC$_{50}$ = 8μM
**14** R = C^{o}CH; IC$_{50}$ = <5μM
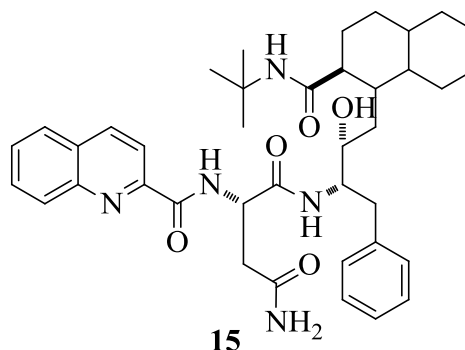
15

L. major at 50 μM 48% of control grown

By comparison, 3-substituted quinolines demonstrate limited *in vitro* activity against *Leishmania* species of all disease types investigated (*L. amazonensis*, *L. infantum*, *L. major*, *L. mexicana* and *L. donovani*) [23,27]. Investigations of 3-substituted bisquinolines or halogenated analogues indicated that these compounds were either inactive or possessed significant cytotoxicity against host cells [23,27]. The corresponding 5- and 6-substituted quinolines have received less attention. Zosuquidar, a P-glycoprotein inhibitor, is a promising reversing agent against *Leishmania* parasites. The 5-substituted quinoline exhibited 16 - 30% growth inhibition of *L. tropica* at 1 μM [28]. Similarly, the 6-substituted quinolines demonstrated some limited activity against *L. amazonensis* and *L. infantum* (IC$_{50}$ = >200 μM) [23].

Various 8-sulfonamido and boronate quinolines exhibit particularly high *in vitro* activity against *Leishmania* parasites [29,30]. The IC$_{50}$ values of 8-quinolinylsulfonamides range from 0.45 to 2.99 μM and 2.12 to 2.85 μM for *L. chagasi* and *L. amazonensis* promastigotes respectively. Compounds **16**, **17** and **18** were twice as active as the 'gold standard' amphotericin B against *L. chagasi*. Compounds **19**, **20** and **21** also demonstrated significant activity ranging from IC$_{50}$ = 6.27 to 14.47 μM and 2.18 to 13.96 μM against *L. amazonensis* and *L. chagasi*

respectively [29]. The introduction of a nitro group or fluorine atom at the 4-position of the phenyl moiety induced a loss of activity against *Leishamania* promastigotes. Cell infection by *L. amazonensis* decreasing by 67.1% at 5 µM for **16** and 82.1 % at 25 µM for **19** [29].
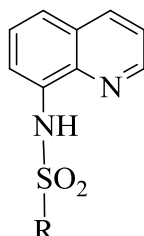
16 R = 3-bromo-2-chloropyridine; IC$_{50}$ = 2.25µM
17 R = Ph-Ph; IC$_{50}$ = 2.18µM
18 R = 4-dimethylnaphthalen-1-amine ; IC$_{50}$ = 3.53µM
19 R = *p*-propylbenzene; IC$_{50}$ = 13.96µM
20 R = 3,5-difluorobenzene; IC$_{50}$ = 0.45µM
21 R = *p*-bromobenzene; IC$_{50}$ = 2.99µM
**22** R = 2,3-dibromothiophene; IC$_{50}$ = 0.53µM

The 8-boronic ester substituted quinolines **23**-**28** contain varying side chains attached to the quinoline moiety via a boronic acid linker. These side chains consist of vinyl or aryl (phenyl, furanyl, pyridinyl or dihydrooxazolyl) substituents. Aryl components were further substituted with amines, halogens or cyano groups [30]. Compounds **23** and **24** displayed IC$_{50}$ values of 0.02 and 0.07 µg/ml respectively. Over 70 similar compounds were tested with IC$_{50}$ values ranging from 0.20-0.80 µg/ml against *L. donovani* amastigotes cultured from golden hamsters. Growth inhibition was also observed with *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense* and *T. cruzi* [30]. Bisquinoline (1,1'-Bis-[(8-quinolyl)oxy]methane) was also tested for its anti-parasitic activity against intracellular *L. donovani* promastigotes and amastigotes and displayed an IC$_{50}$ of 2.4 and 2.3 µM respectively. Intraperitoneal (IP) treatments against *L. donovani*-BALB/c mice exhibited reduced splenic and liver parasite loads of 89% and 91% respectively [31].
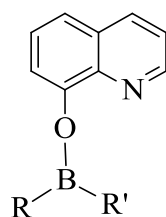
23 R = 2-F-4-Cl-Ph, R' = 3-F-Ph; IC_{50} = 0.02μg/ml
24 R = 3-Me-4-Cl-Ph, R' = 4-NC-Ph; IC_{50} = 0.07μg/ml
25 R = 3-NC-4F-Ph, R' = vinyl; IC_{50} = 0.19μg/ml
26 R = 3,5-di-F-Ph, R' = vinyl; IC_{50} = 0.21μg/ml
27 R = 3-NC-Ph, R' vinyl=; IC_{50} = 0.25μg/ml
28 R = 4-Cl-Ph, R' = 4-OMe-3-F-Ph; IC_{50} = 0.29μg/ml
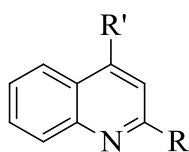
3. Disubstituted Quinolines

Some 2,3-substituted indolylquinoline derivatives exhibit significant anti-leishmanial activity. Indolylquinolines **29**, **30**, and **31** bearing a reactive α-chloroacetamide group inhibit the growth of *L. donovani* promastigotes *in vitro* and are cytotoxic to both the promastigote and amastigote forms of the parasite, while the non-chlorinated **32** was inactive [32]. Derivatives **29**, **30**, and **31** were also effective in eliminating *L. donovani* amastigotes from BALB/c mouse peritoneal macrophages *in vitro* [32]. Recently, **30** was shown to reduced the splenic and liver parasite burdens in *L. donovani*-infected hamsters by >93.0% [33]. In addition, both **30** and the less reactive **32** proved to be 'dual inhibitors' of type I and II DNA topoisomerases of *L. donovani*, suggesting the possibility of rational drug design [34]. By contrast, simple 2,3-disubstituted quinolines had no leishmanicidal activity in the assays employed. 2-Methyl-3-carboxyquinoline, for example, had an *in vitro* IC_{50} = >30 μg/mL against *L. donovani* [35].
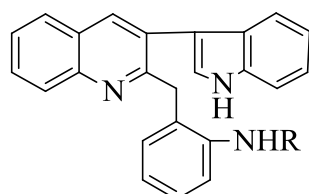
29 R = H; GI 40% (5mg/L, day 6)
30 R = $C(O)CHCl_2$; GI 40% (5mg/L, day 6)
31 R = $C(O)CH_2Cl$; GI 40% (5mg/L, day 6)
32 R = $C(O)CH_3$; NA

Studies of 2,4-disubstituted quinolines are limited to 4-methoxy or carboxy quinolines with a 2-alkyl or aryl group. These derivatives were again isolated from the stem bark of *G. longiflora,* the extract of which is used as a traditional medicine for the treatment of CL [36]. The most potent compound was **33**, which displayed *in vitro* activity against *L. donovani*, *L. amazonensis*, and *L. braziliensis*. Compounds **34**, **35** and **36** demonstrated limited *in vitro* activity against *L. donovani* and *L. infantum* [37]. The novel 2,4-disubstituted quinoline 2-(2-methylquinolin-4-ylamino)-*N*-phenylacetamide, also inhibited *L. donovani* in a dose dependent manner at a concentration of 5.0 μg/ml, inhibiting parasite growth by 66% on the 2nd day and by 95% by the 7th day of the assay [38].


33 R' = OCH$_3$, R = Ph; IC$_{50}$ = 30μg/mL
34 R' = COOH, R = *p*-PhOMe; IC$_{50}$ = 22μg/mL
35 R' = COOH, R = *p*-PhOH; IC$_{50}$ = >17μg/mL
**36** R' = COOH, R = *p*-PhCl; IC$_{50}$ = >18.2μg/mL

The 2, 6-disubstituted **37**, **38** and **39** exhibited relatively good *in vitro* activity against promastigotes of *L. chagasi.* These quinolines were more active against promastigotes than amastigotes but less active than the reference amphotericin B (IC$_{50}$ = 0.01 μM, IC$_{90}$ = 0.03 μM) [39].

2,8-Disubstituted quinolines have received limited attention. 2-Substituted quinolin-8-ols were investigated in bioassays of intracellular amastigotes of *L. amazonensis* and *L. infantum* but were cytotoxic towards the host cell macrophages [23]. 2-((Z)-Prop-1-enyl)quinolin-8-ol was active (IC$_{50}$ = 14 μM) against *L. infantum* but other 2-substituted quinolin-8-ol derivatives showed no inhibition against selected *Leishmania* strains [23].
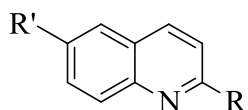
37 R' = OMe, R = 2-furanyl; IC_{50} = 60μM
38 R' = Me, R = 2-thiophene; IC_{50} = 64μM
39 R' = Cl, R = *m*-toluidine; IC_{50} = 53μM
40 R' = CH_3, R = CH=CHC(O)OCH_3; IC_{50} = 8μM

Bisquiolines **41**-**44** displayed significant activity against *L. donovani* intracellular promastigotes and amastigotes. All of the tested bisquinolines, except compound **42**, displayed activities comparable to pentamidine (IC_{50} = 2.1 and 2.8 μg/mL, promastigotes and amastigotes, respectively). These promising *in vitro* results justified a trial of IP administration (12.5 mg/kg) using *L. donovani*-BALB/c mice. Bisquinolines **43** and **44** resulted in significant reductions in parasite burden in spleen (73.5, 82%; control 57%) and liver (78, 88.5%; control 60%) relative to the control, sodium antimony gluconate [31].
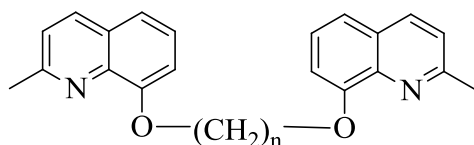
41 n=1, IC_{50} (μg/mL) = 3.4 (promastigote); 2.9 (amastigote)
42 n=3, IC_{50} (μg/mL) = 13.5 (promastigote); 10 (amastigote)
43 n=4, IC_{50} (μg/mL) = 3.2 (promastigote); 2.7 (amastigote)
44 n=6, IC_{50} (μg/mL) = 2.8 (promastigote); 2.4 (amastigote)

4,7-Disubstituted quinolines based on the anti-malarial chloroquine display potent *in vitro* anti-leishmanial activity. The cinchona alkaloid-like 7-chloro quinolines **45**-**47** inhibited *L. donovani* promastigote growth at concentrations comparable to that of the chemotherapeutic agent pentamidine (2.8 μg/mL) but were less potent than amphotericin B (IC_{50} = 0.18 μg/mL) in the same assay [40]. Similar 7-chloro quinolines **48**-**51** displayed moderate potency against *L. major* amastigotes (IC_{50} = 1.5 μg/mL) [41]. Analogue **52** exhibited a minimum effective concentration (MEC) of 10-50 and 10 μg/ml respectively against *L. mexicana* and *L. tropica major* amastigotes.
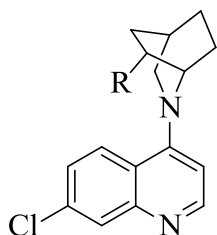
45 R = $CNH_2C_2H_6$; IC_{50} = 1.9µM
46 R = $CNH_2C_4H_{10}$; IC_{50} = 3.8µM
47 R = CH_2; IC_{50} = 3.0µM
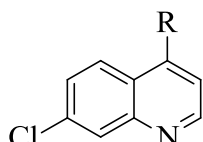
48 R = piperazin-1-yl-(*p*-tolyl)methane,
    IC_{50} = 70.6 µM
49 R = 4-(1,1'-biphenyl)-4-methylpiperazin-1-yl),
    IC_{50} = 62.3 µM
50 R = 4-benzylpiperazin-1-yl,
    IC_{50} = 62.2 µM
**51** R = 4-(t-Bu)phenyl)(piperazin-1-yl)methone,
    IC_{50} = 32.6 µM
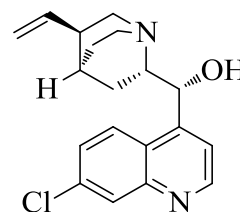

52 *L. mexicana* = 10-50µg/ml
    L. t. major = 10µg/ml

Chloroquine exhibits partial efficacy against *Leishmania* promastigotes and amastigotes of *L. mexicana* and *L. donovani*. Chloroquine inhibited *L. donovani* amastigote survival (55% at 3 µM) and *L. mexicana* promastigote and amastigote numbers were reduced during early stages of infection, but recovered after 48 h [42,43]. Of a series of novel chloroquine-ethambutol-isoxyl tripartite hybrids only one, **53** (IC_{50} = 9.90 µM), inhibited *L. mexicana* as effectively as pentamidine (IC_{50} = 13.32 µM) [44]. Amodiaquine analog **54** was evaluated against *L. donovani*, displaying an effective inhibition concentration of 2.3 µM [45]. A series of 4,7-chloroquinolinyl derivatives were condensed with tryptophan derivatives to identify activity against a range of *Leishmania* species. However, only tryptophan derivative **55** showed significant activity against *L. amazoniensis*, *L. braziliensis*, *L. chagasi* and *L. major* (IC_{50} = 30.4, 14.64, 30.96 and 22.17 µM, respectively) although none were as active as the control, amphotericin B (0.3-1.9 µM) [46].
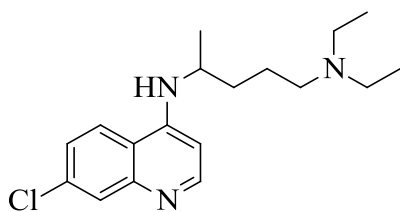
Chloroquine
amastigotes = 79 IP 10μg/mL, 48hrs
promastigotes = 5 IP 10μg/mL, 48hrs
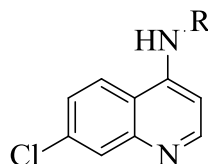
53 R = ethyl-3-(3-phenoxypropyl)urea,
IC_{50} = 9.90 μM, *L. mexicana*
54 R = 2-(piperazin-1-yl)phenol,
IC_{50} = 2.3 μM, *L. donovani*
55 R = 3-(indol-3-yl)propanoic acid,
IC_{50} = 14.64 μM, *L. braziliensis*

Bisquinoline **56** displayed an IC_{50} of 2.0 and 2.1 μM (promastigotes and amastigotes, respectively) against intracellular *L. donovani*. Significant reductions (compared to sodium antimony gluconate, 250 mg/kg dose, parasite burden were also identified in the parasite burden of the spleen (95%) and liver (98.5%) of *L. donovani*-infected BALB/c mice 30 days post IP treatment [31].
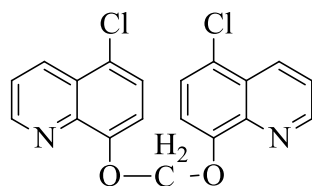
56
L. donovani
Promastigote, IC_{50} = 2.0μM
Amastigote, IC_{50} = 2.1μM

Primaquine, a 6,8-substituted aminoquinoline used for the treatment of *P. vivax* malaria, is potent against the amastigote form of *L. donovani* [47]. It also exhibits potency against axenically cultured amastigotes of three *Leishmania* species. *L. amazonensis* and *L. infantum* amastigotes and promastigotes were equally sensitive to primaquine, while *L. m. mexicana* amastigotes were about two-fold more susceptible than the corresponding promastigote form [48]. Primaquine-loaded polyisohexylcyanoacrylate nanoparticles exhibited a 21-fold improvement in anti-leishmanial activity *in vitro* against *L. donovani* infected macrophages relative to primaquine alone [14]. Analogues of primaquine exhibit improved activity against a

number of *Leishmania* species [49]. Analogue **57**, for example, has an MEC of 10 μg/ml, compared to 100 μg/ml for the parent [30].
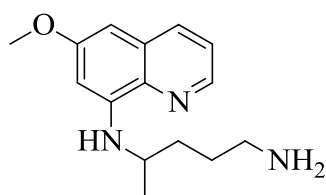
Primaquine
L. donovani IC_{50} = 19.9μg/mL
L. amazonensis IC_{50} = 53.43μM
L. infantum IC_{50} = 62.59μM
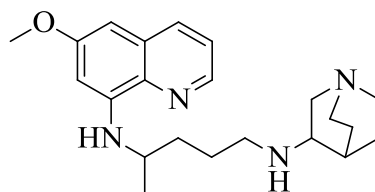*L. mexicana* IC_{50} = 22.54μM

57
L. donovani MEC = 10μg/ml
L. mexicana, MEC = 10μg/ml
L. t. major, MEC = 10μg/ml

4. Trisubstituted Quinolines

Sitamaquine (WR-6026) is the most studied example of a leishmanicidal tri-substituted quinoline with an EC_{50} of 5.09 μM and an EC_{90} of 20.33 μM *in vitro* against *L. donovani* [50]. It has also shown promising activity in Phase II trials for the oral treatment of VL [51,52]. The molecular target of this cationic molecule is not known, but it has been demonstrated to interact with mitochondrial and other lipid membranes and results in alteration of parasite morphology [53,54]. Analogues of this 4,6,8-tri-substituted quinoline bearing structural variations in the 8-[6-(diethylamino)hexyl]amine] side chain have also proved active against *L. donovani* in hamsters, although none were as potent as the parent [55]. Replacing the diethylamino moiety with di-*n*-propylamino, for example, decreased the G-index (G = SD_{90} for meglumine antimoniate / SD_{90} for test compound) from 474 to 333. Replacing this moiety with a substituted-l-piperazine or related heterocycle resulted in a greater decrease in activity in the same assay [56]. Retaining this diethylamino group, but shortening the tether from hexyl to pentyl, or lengthening it from hexyl to heptyl, also lowered the G-index (178 and 96.8 respectively). Retaining all three substituents of sitamaquine but introducing a 2-hydroxy substituent almost completely removed activity, ruling out this derivative as an active metabolite. Extending the aromatic nucleus from quinoline to acridine had a similarly deleterious effect on leishmanicidal activity [57].

Quinolines substituted at positions 2-, 3- and 4- are available by condensation of aniline with β-keto esters (Figure 2) [58]. A small library of these compounds demonstrated anti-leishmanial efficacy *in vitro* against *L. chagasi* promastigotes and one example (**58**) showed an IC_{50} of 3.55 μg/mL against amastigote-infected macrophages. This compound was about 8.3-fold more potent than the standard pentavalent antimony (IC_{50} = 29.55 μg/mL) in the same assay [58]. A 2,4,6-substituted bisquinoline (**59**) compound showed activity *in vitro* against *L. tarentolae* with an MIC of 6.5 μg/ml with a low level of cytotoxicity against macrophages [59].
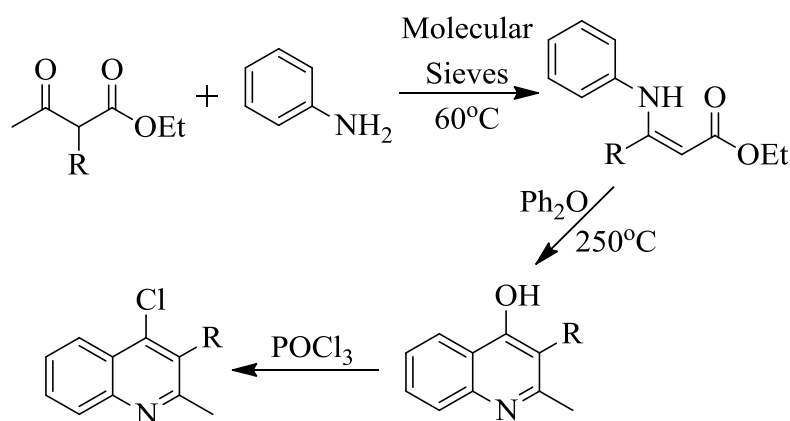
Fig. (2)
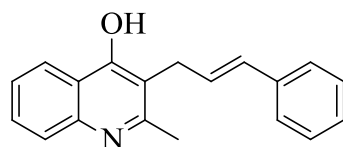
58
L. Chagasi
IC_{50} = 3.55 μg/mL
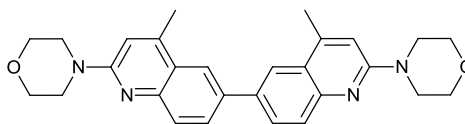
59
L. tarentolae
MEC = 6.5 μg/mL

A trisubstituted primaquine analogue bearing an additional 2-ethyl group was the most active of a small library of 2-substituted primaquines tested against *L. donovani* in hamsters with a G-index of 10, compared with 0.96 for primaquine, 2.4 for 2-methoxy primaquine and 1.6 for 2-ethenyl primaquine [60]. A 5,6,8-substituted primaquine analogue showed an MEC of 10, 5 and 10 μg/ml respectively against *Leishmania* parasites *L. mexicana*, *L. t. major* and *L. donovani*. Compound **60** exhibited a 10-fold greater MEC than that of primaquine (100 μg/ml) [61].

13
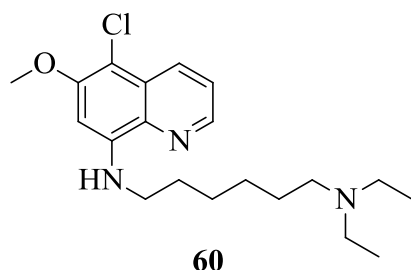
60

L. donovani MEC = 10μg/ml
L. mexicana, MEC = 10μg/ml
L. t. major, MEC = 5μg/ml

A series of boronated 5,7,8-trisubstituted quinolines proved very effective against *L. donovani* amastigotes. The boronic ester was located at the 8-position of the quinoline moiety while the 5 and 7-positions were either halogenated, methylated or aminated. Halogenated analogues **61**, **62** and **63** exhibited IC_{50} values of 0.052, 0.15 and 0.18 μg/ml, respectively. Dimethyl compound **64** and amine substituted **65** had IC_{50} values of 0.94 and 1.1 μg/ml, respectively [30].
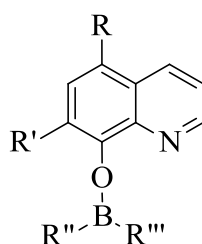
61 R, R' = Cl; R" = 4-Cl-Ph; R'" = 4-F-Ph
IC_{50} = 0.052μg/ml
62 R = Cl, R' = I; R" = 4-Cl-Ph; R'" = 4-Cl-Ph
IC_{50} = 0.15μg/ml
63 R, R' = Cl; R" = 3-F-Ph; R'" =
IC_{50} = 0.18μg/ml
64 R, R' = CH₃; R' = 4-Cl-Ph; R" = 4-F-Ph
IC_{50} = 0.94μg/ml
65 R = Cl; R' = dimethylamine; R" = 3-Cl-4-pyridyl; R'" = vinyl
IC_{50} = 1.1μg/ml

5. Polysubstituted Quinolines

The anti-leishmanial activity of tetra- and penta-substituted quinolines has not been investigated systematically. Adding additional functionality to sitamaquine generally decreases potency, although there are still a number of leishmanicidal analogues in this category and 5-methoxy sitamaquine was significantly more potent than the

parent against *L. major* and *L. m. amazonensis* in random-bred albino mice [62]. The 2,3,4,6-substituted derivatives are readily available by the Brønstedt or Lewis acid catalysed Friedländer condensation [63,64] of 2-aminoaryl aldehydes, ketones or α-hydroxy derivatives with carbonyl compounds (e.g. Figure 3).
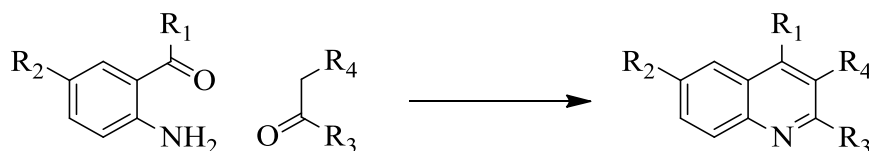
Fig. (3) Friedlander synthesis

A small library of compounds, **66**-**70**, was prepared in this way and were tested *in vitro* against *P. falciparum, L. donovani, L. infantum, T. cruzi* and *T. b. rhodesiense* [35]. Some of these compounds exhibited significant activity against *P. falciparum* and *T. cruzi*, while others displayed modest activity against *L. donovani* and *L. infantum*. One notable feature of this data is the importance of the chlorine at position 6 for leishmanicidal potency.
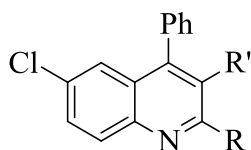
66 R = CH_2Cl, R' = CO_2Et; IC_{50} = 13.2μg/mL
67 R, R' = CH_3; IC_{50} = 12.9μg/mL
68 R = 2-methylpiperidine, R' = CO_2Et; IC_{50} = 12.2μg/mL
69 R = CH_3, R' = CO_2Et; IC_{50} = 3.4μg/mL
70 R = CH_3, R' = COMe; IC_{50} = 11.8μg/mL

The 2,4,6,8-substituted quinolines, compounds **71** and **72** were similarly substituted with a difference at the 2-position, which contained a phenyl or tricyclic ring. The phenyl substituent proved more toxic then the tricyclic ring with a MEC of 10 compared to 10-100 μg/ml *in vitro* against *L. mexicana*. Both substitutions proved more toxic then primaquine [61]. Quinolines substituted at the 2,5,7,8-position exhibited increased toxicity when substituted with a boronic ester at the 8-position. Compounds **73**, **74** and **75** exhibited significant activity against *L. donovani* [30].
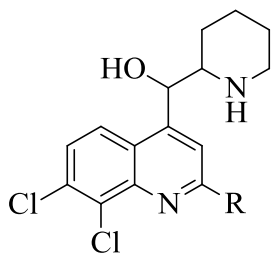
71 R = Ph, MEC = 10μg/ml

72 R = , MEC = 10-100μg/ml
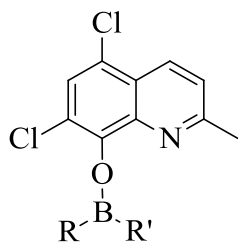
73 R = 4-Cl-Ph; R' = 4-Cl-Ph , IC_{50} = 0.12μg/ml
74 R = 3-Me$_2$N-Ph; R' = vinyl, IC_{50} = 0.16μg/ml
75 R = 3-pyridyl; R' = vinyl, IC_{50} = 0.22μg/ml

The 4,5,6,8-substituted aminoquinoline, NPC1161, is an anti-malarial drug with improved activity relative to primaquine and anti-leishmanial activity against *L. donovani* [65,66]. Attaching the water soluble copolymer *N*-(2-hydroxypropyl)methacrylamide (HPMA) to NPC1161 reduced parasite numbers *in vitro*. All ManN-containing HPMA copolymer conjugates (compounds **76**-**79**) were effective in killing all parasites at 30 μg/ml but were toxic to macrophages at the same dose [66].
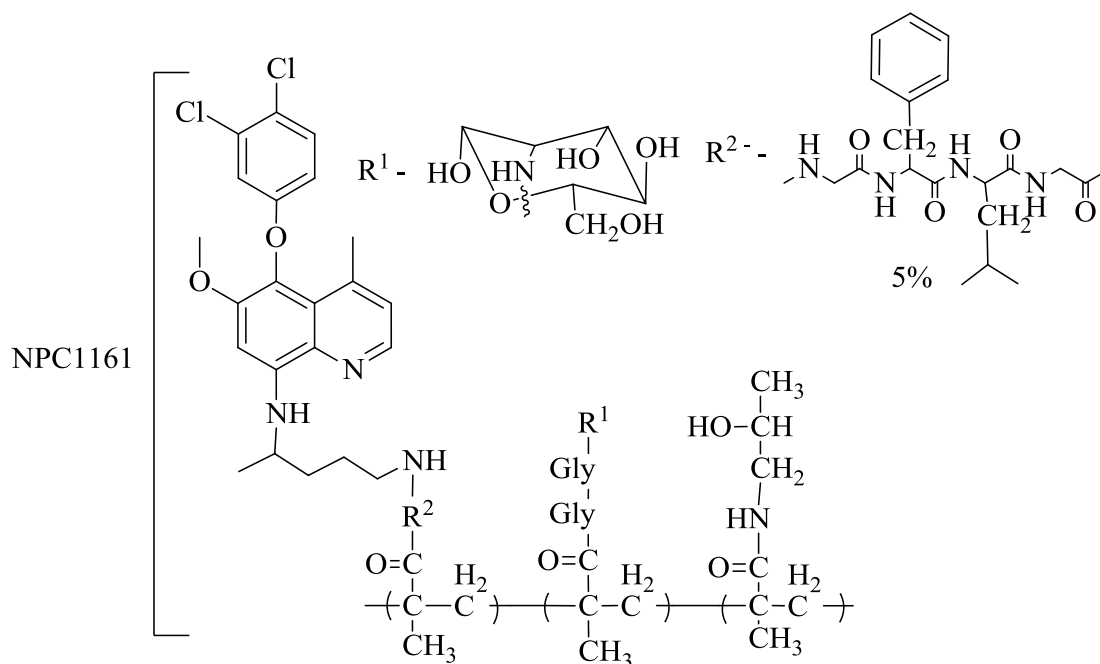
76 ManN 5%, %Inhib (10μg/ml) = 43
77 ManN 10%, %Inhib (10μg/ml) = 41
78 ManN 15%, %Inhib (10μg/ml) = 75
79 ManN 20%, %Inhib (3μg/ml) = 29

Few conclusions can be drawn from this small survey other than all the compounds examined were more active than primaquine but less active than some other simpler 8-aminoquinolines (see above). Increasing the bulk of the 2-substituent of primaquine is reported to increase blood-schizontocidal anti-malarial activity and reduce methemoglobin toxicity [67]. Additional 5-(3-trifluoromethylphenoxy) substitution provided the highly substituted tafenoquine (WR238605), an effective prophylactic drug [68] for use against *P. falciparum* and with potent *in vitro* anti-leishmanial activity against *L. donovani* promastigotes [49]. Compounds **80**, **81** and **82** have IC_{50} comparable to pentamidine (IC_{50} = 3.4 μg/mL). IC_{90} values were also obtained ranging from 6.5-6.7 μg/mL compared with 8.0 μg/mL for pentamidine. However, all compounds were less potent then amphotericin B (IC_{50} = 0.17 and IC_{90} = 1.7 μg/mL) [49]. The 2,3,4,7,8-substituted quinoline, skimmianine, exhibited limited activity against *L. braziliensis*, *L. donovani* and *L. amazonensis* (IC_{90} = 100 μg/mL) [36].

17
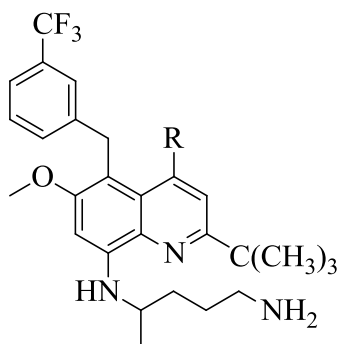
80 R = H; IC$_{50}$ = 3.0μM
81 R = CH$_3$; IC$_{50}$ = 3.4μM
82 R = C$_2$H$_5$; IC$_{50}$ = 2.9μM

Analogues with an oxygenated substituent at the 5-position were more potent than the unsubstituted or 5-methyl compounds. This study again confirmed the importance of the C-6 substituent with the 5,6-dimethoxy and certain 6-hydroxy compounds exhibiting the highest leishmanicidal activities. Minor variations in the 8-amino side-chain had little effect in this series of compounds.

6. Structure Activity Relationship (SAR)

A meta-analysis of the above data allows the formulation of a qualitative SAR. This task is complicated by: (i) the different models used to assess bioactivity *in vitro* (promastigote and amastigote) and *in vivo* (mouse, canine, rat and hamster models); (ii) the different units used to quantify bioactivity (eg IC$_{50}$ reported in either μg/mL or μM, MEC, GI, G Index) and (iii) the unknown and possibly different molecular targets for different classes of quinolines. The SAR presented in Figure 4 is therefore only a qualitative guide.
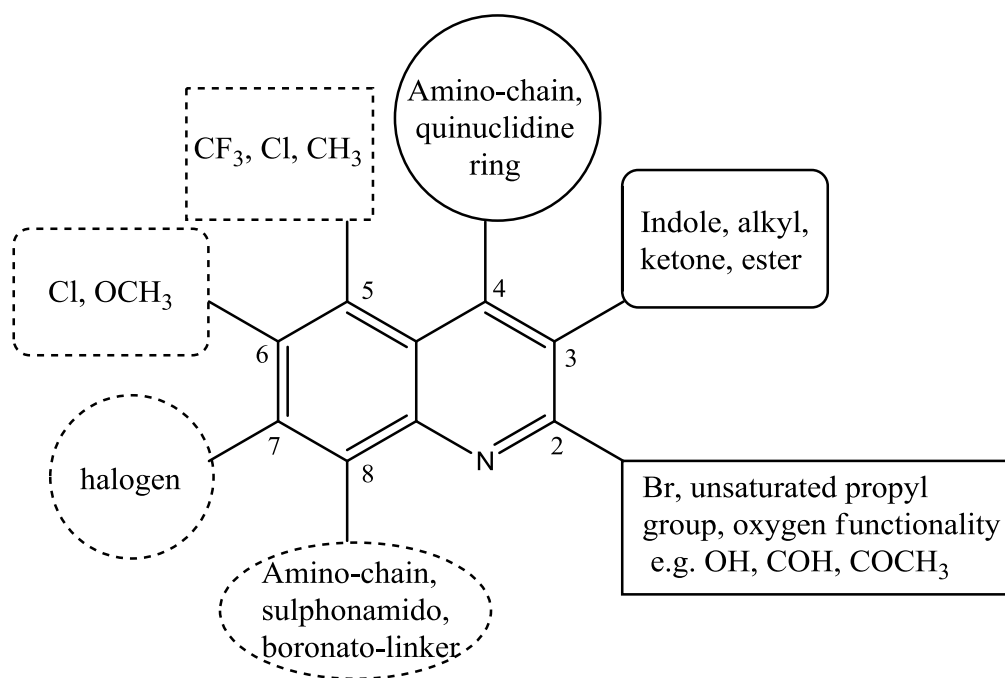
Fig. (**4**) General SAR of favourable substituents at each position quinoline nucleus.

This meta-analysis indicates that substitution at any position of the quinolone ring can potentially lead to improved leishmanicidal activity and improved activity is often associated with multiple substitutions. Simple mono-substituted quinolines show a variety of activities when positioned around the ring. The 2-substituted analogs, for example, exhibit greater potency if a three- to five-carbon alkyl chain is employed and activity increases if (a) C1-C2 of the chain is saturated (**1** (IC_{50} = 50 µg/mL), **3** (IC_{50} = 12 µM)); (b) substitution includes (i) an allylic alcohol **5**; (ii) an α,β-unsaturated aldehyde **8;** (iii) an α,β-unsaturated ester **9**; (iv) an allylic bromine **11** and **12**; or (v) an alkyne **14** (IC_{50} = 2µM). However, activity decreases for substitution of larger groups at the 2 position (2-CH=CHCH$_2$CH$_2$CH$_2$, IC_{50} = >30 µM, 2-C$_6$H$_5$, IC_{50} = >100µM).

There are relatively few studies of quinolines with single substitutents at the 3- to 7-positions. Mono-substitution at the 3-position yields compounds with limited *in vitro* activity or compounds that are highly cytotoxic. The same is true for 5- and 6-monosubstitution. The paucity of studies involving mono-substituted 3-, 5- and 6-quinolines is in part due to the increased difficulty of preparing these compounds. However, multiple substituents including di-, tri- and poly-substituted, methoxy, halogen and /or methyl functionalities at the 5-, 6-, and / or 7-positions can yield

potent quinolines such as sitamaquine and chloroquine. Conversely long chain substituents at any of these positions decreases activity dramatically. A combination of substitution at the 3-position with a 4-substituted indole, alkyl, ketone or ester can yield potent leishmanicidal compounds [16,32].

Moderate activity is seen for 2,4- and 2,6-substitution with either a 4-carboxylic acid or 6-methoxy, halogen or methyl groups combined with a 2-aryl substituent. Even more active are 4- and 8- mono-substitution involving certain alkylamines, complex ring systems, or aryl substitutions tethered via sulfonamide or boronic acid linkers. Bisquinoline derivatives (2,8- and 5,8-) exhibit potent activity when an alkyl chain (n = 1, 4, 5) is used to connect the two quinoline moieties (IC_{50} = 2.0-3.4 µM). Chloroquine-like (4,7-substituted) derivatives demonstrate similar patterns in activity, with alkylamine substituents (IC_{50} = 2.3-14 µM), piperazine (IC_{50} = 32-70 µM) and quinuclidine rings (IC_{50} = 1.9-3.8 µM). 4-Substituted quinine analogues are active at low to moderate concentrations (IC_{50} = 10-50 µM).

Primaquine and other 6,8-substituted derivatives exhibit modest activity, increasing 10-fold when a quinuclidine is added to the end of the 8-substituted, six-carbon chain. Extending this amino chain to eight carbons in combination with a 4-methyl (EC_{50} = 5.09 µM) or 5-chloro substituent (MEC = 5-10 µg/mL) is also effective. Other structural variations in the 8-amino side chain have either a detrimental or beneficial effect on activities, e.g. replacing the diethylamino (G-index = 474) with a dipropylamine increases activity (G-index = 333), as does, shortening the tether from hexyl to pentyl (G-index = 178) or lengthening from hexyl to heptyl (G-index = 96.7). However, introducing a substituted piperazine or other heterocycle to an eight-carbon amino chain reduces potency.

Both, 8-sulphonamido and 8-boronato quinolines can be highly leishmanicidal compounds. The 8-arylsulphonamido substituents were less active, eg benzenesulphonamido (IC_{50} = 0.45-13.96 µM), pyridinesulphonamido (IC_{50} = 2.25 µM), naphthalenesulphonamido (IC_{50} = 3.53 µM) and thiophenesulphonamido (IC_{50} = 0.53 µM). Increased activity was seen in all of the boronate derivatives, regardless of other quinoline ring substitution (IC_{50} = 0.02-1.1 µM).

7. Preclinical and Clinical Development.

Although aspects of the mechanism of action, metabolism and toxicity of selected quinolines have been described, in general these areas of preclinical development are not well understood. This is partly due to the patchy approach to these studies. A quinoline, active against another target, and for which drug approval has been obtained, is then tested in leishmaniasis studies, but the mechanism of action is not generally pursued – it is either effective or it is not. Imiquimod, for example, has been approved for external warts in adults [69] and is a successful leishmanicide, but its mechanism of action is not fully understood. Saquinavir, an HIV-protease inhibitor has also proved ineffective against VL [70]. Progression of quinolines through preclinical studies is inconsistent with most interest on chloroquine, Imiquimod, mefloquine, primaquine, sitamaquine and a few, selected substituted quinolines. A précis of the current knowledge in this area is presented below.

It is important to note that preclinical studies have involved a variety of animal models. In mice tests, for example, mice (such as random-bred albino mice (TFW line) [71] or Balb/c mice [25]) are infected subcutaneously on their back and median lesions scores determined. Other animals (mongrel and parasite-free beagle dogs [72], opossums [73] and rats [74]) have also been used for screening with quite different responses between species. The most widely used and reliable model is the *L. donovani*-hamster model. In this system, the suppressive effect of a compound on the parasite densities in the hamster's liver is compared to that of a reference compound, usually meglumine antimoniate.

The anti-leishmanial efficacies of medicinal plant extracts [15] **1**, **2**, **3**, **4**, 2-phenylquinoline and 2-(3,4-methylenedioxyphenyl ethyl) quinoline, were evaluated in BALB/c mice infected with *L. amazonensis* or *L. venezuelensis* [15,16,36,75] *or L. donovani* [76]. Subcutaneous treatment of *L. donovani*-infected BALB/c mice infected with **2** for 10 days at 0-54 mmol/kg per day resulted in 86-6% parasite suppression in the liver [76]. Oral administration of 0-54 mmol/kg of 2-n-propylquinoline once daily for 5 or 10 days to *L. donovani*-infected mice suppressed parasite burdens in the liver by 87-8 and 99-9%, respectively [76]. In a study with *L. amazonensis* or *L. venezuelensis* infected BALB/c mice, three-carbon chain quinolines (**1** and **2**) were more potent than *N*-methylglucamine antimonate against *L.*

amazonensis, and other 2-substituted quinoline alkaloids such as [2-(3,4 methylenedioxy phenylethyl) quinoline, 2-(3,4-dimethoxyphenylethyl)quinoline, and **3**, were as effective as the reference drug [15]. In another study, also with BALB/c mice infected with *L. amazonensis* or *L. venezuelensis*, five injections of chimanine B at intervals of 4 days reduced the lesion weight by 74% and the parasite loads in the lesion by 90% compared with the values for the group of untreated mice [16]. Other 2-substituted quinolines, **1** administered by the oral and intralesional routes, 2-phenylquinoline administered by the oral route, **4** administered by intralesional injection, and two total alkaloidal extracts of *G. longiflora* administered by the oral route, had intermediate effects [16]. The variable leishmanicidal activities could not be explained because the modes of action of these compounds are unknown. However, the active 2-substituted quinolines, **1** or **3**, were reported to have good absorption and to be metabolized at low levels, resulting in the choice of **3** as the lead compound [16].

Subsequent to the above studies on 2-substituted quinolines, the development of a high-performance liquid chromatographic method for the specific determination of **1**, in plasma and liver homogenates of mice was reported [77]. This method was an aid to preclinical studies and was used to determine the pharmacokinetic profile of **1** following oral administration to mice [77]. More recently, the *in vivo* efficacy of 2-substituted quinolines on *L. amazonensis* CL in BALB/c mice and on *L. infantum* and *L. donovani* visceral infection in BALB/c mice established differential efficacy [17]. 2-Substituted quinolines (where the 2 substituent was $-C\equiv CH$, $-CH=CHC(O)H$, $-CH=CHCH_2OH$, or $-CH=CHCH_2OCH_3$) were significantly more effective than meglumine antimoniate in reducing the parasite burden in both the liver and spleen. Quinoline **1** and **5** at 12.5 mg/kg significantly reduced the parasite burdens in the liver by ~ 60-70%. Quinoline **5** was the most promising compound against both CL and VL. These compounds displayed double anti-leishmanial and anti-viral activities, which suggested they could be potentially used as a treatment for co-infection of *Leishmania*-HIV [17].

Preclinical studies have included a range of di-, tri- and tetra-substituted quinoline derivatives that could be considered to be derivatives of the frontline, potential anti-leishmanial lead compounds primaquine and sitamaquine. However, there are a few

notable exceptions such as an indolylquinoline [33], Imiquimod [78], chloroquine [79] and mefloquine [62], which will be discussed first. An indolylquinoline derivative (2-(2''-dichloroacetamidobenzyl)-3-(3'-indolylquinoline)) has been reported to reduce the parasite burdens in the spleen and liver by >93% in *L. donovani*-infected hamsters. Combination therapy of the indolylquinoline derivative with sodium antimony gluconate cured established VL in hamsters, with no residual living parasites in the livers and spleens of infected animals [33]. Chloroquine did not inhibit the multiplication of *L. donovani* in hamster livers when administered at 100 mg/kg/day for 12 days [79], or *L. major* in TFW mice when administered at 90 mg/kg/day for 5 days [62]. These results stand in contrast to the activity observed *in vitro* (Section 3). In the latter study, mefloquine displayed moderate activity levels against *L. major* in TFW mice when administered at 70 mg/kg/day for 5 days [62]. However, in a non-blinded, therapeutic trial conducted in Columbia, 1.25-1.5 grams of mefloquine given as a single oral dose or a dose of 250 mg a day for 5-6 days was not efficacious in the treatment of New world CL[80], whereas a trial in Ecuador gave a cure to 68% of patients (11 patients) in four weeks or less [81,82]. Issues such as patient compliance, early vomiting, superinfection of lesions, a potential difference in the bioavailability of the drug due to methods of manufacture, and classification of lesions at a point in time (lesion might initially enlarge before healing) complicate the direct comparison of the two mefloquine trials.

Imiquimod

Topical treatment with 5% Imiquimod cream (compared with a placebo cream) of *L. major*-infected BALB/c mice significantly reduced the severity of the lesions on the footpads of the mice, with less swelling due to infection, fewer parasites, and inhibition of surface ulceration. However, as Imiquimod is unlikely to penetrate as far as the inner regions of the foot, infection was expected to spread further if treatment were stopped in this animal model [78]. Recently, clinical trials using Imiquimod, topical treatment three times a week with Imiquimod cream (5%) on patients (from Damascus) led to regression of lesions of CL after 4 weeks. However after this time clinical deterioration occurred. It was concluded that topical application of Imiquimod alone was ineffective in treating Old World CL [83]. A further two Imiquimod trials, gave more promising outcomes. In the first trial, topical treatment of the drug (over 20 days) in combination with ongoing antimonial therapy led to 57% of lesions and 50%

of patients (12 patients in total) being clinically 'cured' at day 20, with 90% patients being cured at 6 months [69,84]. In the second trial (which was a double-blind randomized trial), treatment of forty patients (in Peru) with clinical resistance to antimony, received megluamine antimoniate (20 mg/kg/day intramuscularly (IM) or intravenously (IV)) and were randomized to receive topical Imiquimod (5%). The Imiquimod group achieved a 72% cure at 3 months versus 35% of the vehicle cream group (control group). Residual scarring was less prominent when Imiquimod was used [85].

Primaquine

Interest in use of primaquine as an anti-leishmanical agent traces back almost forty years. Although quite toxic, primaquine, administered in conjunction with either quinine or chloroquine, is used to treat the *P. vivax* and *P. ovale* malaria [86], and is used if no other alternatives are effective [87]. In an initial clinical trial (Ethiopia, 1970), primaquine displayed minor efficacy (1 patient of 7 patients was cured) against diffuse CL [88]. However, an observation that the 2-benzyloxy derivative of primaquine was less toxic [89,90], suggested the possibility of a more effective and less toxic agent may be found and stimulated work on derivatives of primaquine. This included work on the 2-methoxy or 2-ethyl derivatives of primaquine, with activity against *L. donovani* in hamsters [60]; a 8-piperazin-1'-ylhexyl amino derivative (**83**) (moxipraquine) which was effective against *L. major*, *L. m. mexicana* and *L. brasiliensis* but displayed significant foetal toxicity [91]; 4-methyl-5-fluoro derivatives (**84**) of primaquine, which displayed a ten-fold increase against *L. donovani* in golden hamsters, as compared to primaquine [92]; 4-amino-2-methoxyacridine derivatives (**85-87**) of primaquine, which had poor activity against *L. donovani* in hamsters [57]; a survey of 8-aminoquinolines including several 4-methylquinolines (also known as lepidines, e.g. **88-90**), with low activity against *L. major* or *L. amazonesis* in random bred albino mice [62]; and 8-amino-6-methoxy lepidine derivatives (e.g. **91-93**), active against *L. donovani* in hamsters [93]. It was demonstrated in the latter study that the presence of a 4-methyl group on primaquine (**94**) analogues produced a marked enhancement in anti-leishmanial effectiveness, and that a 6-methoxy lepidine (**95**) bearing a 8-(6-diethylaminohexyl amino) group was also effective [93]. The quinoline derivative that possessed a 4-methyl-6-methoxy-8-(6-diethylaminohexyl amino) group was to become known as sitamaquine [93].

MeO— ... 2-OMe quinoline structure

83

84

MeO— acridine structure with R

85 $NH_2CH(CH_3)CH_2CH_2CH_2NH_2$,
86 $NH_2CH_2CH_2CH_2CH_2CH_2N(C_2H_5)_2$,
87 $NH_2CH_2CH_2CH_2CH(CH_3)NH_2$

88

89

90

91

92

93

94

95
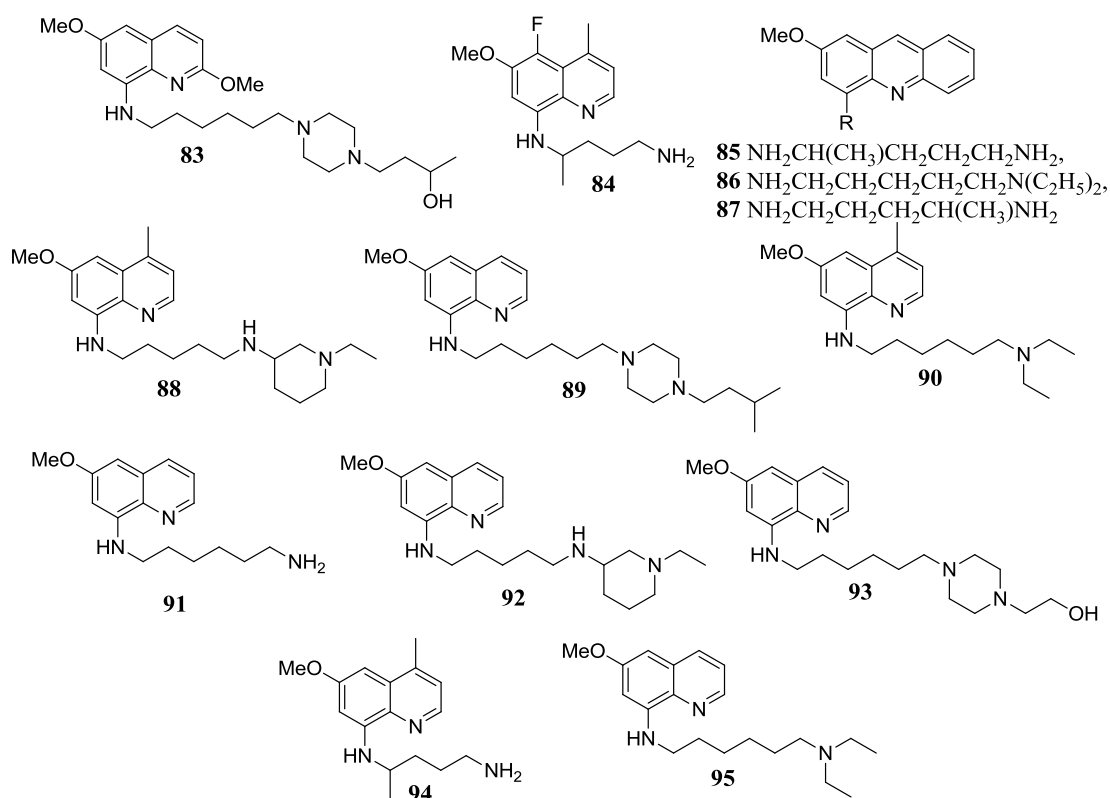
Studies during the last 20 years into the improved delivery of primaquine have included use of liposome carriers [94,95], peptide grafted liposomes [96], a self-gelling primaquine-gum arabic conjugate [97], nanoparticles (typically 150 to 200 nm in size) [96,98-100] and biodegradable microspheres (1-20 μM in size) [101]. Aspects such as polymer-drug interactions, in vitro drug release and physiochemical stability and toxicity were considered in some of these studies [97,99,101,102]. These delivery systems were aimed at increased effectiveness, prolonged duration of action and / or reduced toxicity of the drug. The effectiveness of the primaquine-loaded nanoparticles was 3.3 times as high as the free drug [96], whereas microspheres were 100 times more efficient than the free drug, primaquine [101].

Sitamaquine

Sitamaquine (formerly WR-6026) is an orally active 8-aminoquinoline in development for treatment of VL and has been reviewed elsewhere [103]. Early studies identified sitamaquine as more active against *L. major in vivo* (random-bred albino mice, TFW line) than in tissue culture [62]; active in mice infected with *L. donovani* [104] and active in mongrel and parasite-free beagle dogs infected with *L.*

d. khartoum [69]. Sitamaquine also displayed parasite suppression in the liver and spleen of opposums infected with *L. donovani* [85]. These results stimulated the synthesis and evaluation of a variety of 8-amino side chain analogues against *L. donovani* in hamsters as well as other ring substituted quinoline analogues [55,105-107]. Although several analogues had meglumine antimoniate indexes in excess of 300 [55], none was superior to sitamaquine. Topical formulations of sitamaquine (anhydrous gel emulsions) did not affect lesion progression or reduce parasite burden in BALB/c mice infected with *L. major* [109]. Encapsulated within liposomes prior to injection into hamsters was 700 to 1800 times more effective than the control antimonial drug (meglumine antimoniate). In addition, the duration of action was prolonged, and the drug toxicity decreased [110,111]. Similarly, N-(2-hydroxypropyl)methacrylamide (HPMA) co-polymer − NPC1161 sitamaquine conjugate containing lysosomally degradable side chains, was shown to be more effective than the free drug in BALB/c mice infected with *L. donovani*, indicating the potential for targeted delivery [112].

While the mode of action of 8-aminoquinolines and sitamaquine is not yet understood. It has been suggested that 8-aminoquinolines interfere with mitochondrial respiration, possibly through interactions with ubiquinones [62]. The major metabolites of sitamaquine have been identified as the desmethyl and 4-CH_2OH derivatives in rats and hamsters [113], with 5-hydroxylation and side chain oxidation being important steps in the metabolic pathway [114]. The 4-CH_2OH derivative is the major urinary metabolite in humans, however only 14% of the drug could be accounted for [114]. Analysis of the physiochemical interactions of sitamaquine were studied using phospholipids to represent a parasite membrane, the first barrier to be crossed by a drug. This study indicated that initial electrostatic interaction between positively charged sitamaquine and the negative headgroups of the anionic phospholipids was required for subsequent hydrophobic interactions between the aromatic ring of sitamaquine and the phospholipid alkyl chains which led to drug insertion into the monolayer [53].

A major concern with the clinical development of 8-aminoquinolines, such as primaquine, is that such compounds can cause oxidant-induced hemolytic anemia with methemoglobinemia (a higher than normal level of methemoglobin, metHb, in

the blood, resulting in tissue hypoxia). A subchronic study of sitamaquine in Sprague-Dawley rats (receiving up to 12 mg/kg/day for 13 weeks) and Beagle dogs (receiving up to 3 mg/kg/day for 13 weeks) indicated that only the highest doses caused hemolytic anemia and methemoglobinemia, along with assocated hepatoxicity. The toxic effects of sitamaquine were essentially resolved by the end of the recovery period in both species [74].

Sitamaquine has been in development for 15 years and has attracted interest in India and the Drugs for Neglected Diseases Group [115-117]. It was initially developed at the Walter Reed Army Institute of Research for treatment of leishmaniasis, with GlaxoSmithKline helping with the final development and FDA submission. After a pilot study in India [118], several small phase I/II clinical trials have been run. In Brazil 67% of patients (total patients = 95) treated with sitamaquine (2 mg/kg/day for 28 days) were cured of *L. Chagasi*[119]. In Kenya, 83% of patients (total patients = 46) were cured of VL when treated with sitamaquine (1.7 mg/kg/day for 28 days) [51]. Similarly, in India 81% of patients (total patients = 31) were cured of VL when treated with sitamaquine (1.5 mg/kg/day for 28 days). Sitamaquine was generally well tolerated by the patients in these studies, however side effects such as renal adverse events [101] were observed in a few subjects and this appeared to be treatment-related. Interpretation of these data is complicated because VL itself is associated with renal impairment. Sitamaquine has also been used in cross disease studies where a single report of the drug used in a HIV-positive patient with VL indicated that the patient responded to the use of sitamaquine [120].

7. Concluding Remarks

A new treatment for VL is urgently needed. Current medicines suffer from impractical treatment regimens, emerging drug resistance, or are simply unaffordable. Quinolines are a promising class of candidates in this respect and certain examples are potentially effective and affordable chemotherapies for this devastating disease. Unfortunately, the molecular targets of these drugs are not known and rational drug design is further hampered by the wide variety of disease models and methods of reporting leishmanicidal efficacy. In particular, a standardized *in vivo* model would

allow quantitative comparisons of drug potency. Nevertheless, the above metastudy provides qualitatively trends useful for further investigation.

Conflict of Interest and Patient Consent

DJY is grateful to the Australian Academy of Science for financial assistance to travel to the laboratory of Professor Margaret Phillips, University of Texas, Southwestern Medical Center.

Abbreviations
CL = cutaneous leishmaniasis

MCL = mucocutaneous leishmaniasis

VL = visceral leishmaniasis

HAART = highly active anti-retrovial therapy

IC_{50} = Concentration of the drug to achieve a 50% of inhibition of disease

IC_{90} = Concentration of the drug to achieve a 90% of inhibition of disease

EC_{50} = Concentration of the drug that produces 50% of the maximum possible result

EC_{90} = Concentration of the drug that produces 90% of the maximum possible result

MIC = Minimum Inhibitory Concentration

MEC = minimum effective concentration

G-index = G= SD_{90} for meglumine antimoniate / SD_{90} for test compound

HPMA = *N*-(2-hydroxypropyl)methacrylamide

GI = growth inhibition

IP = Intraperoteneal (10µg/ml, 48hrs)

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