Chloroquine analogues as leads against *Pneumocystis* lung pathogens

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Running Title: Chloroquine analogues as anti-*Pneumocystis* leads

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Abstract

The impact of *Pneumocystis* pneumonia (PcP) on morbidity and mortality remains substantial for immunocompromised individuals, including those afflicted by HIV infection, organ transplantation, cancer, auto-immune diseases, or subject to chemotherapy or corticosteroid-based therapies. Previous work from our group has shown that repurposing antimalarial compounds for PcP holds promise for treatment of this opportunistic infection. Following our previous discovery of chloroquine analogues with dual-stage antimalarial action both *in vitro* and *in vivo*, we now report the potent action of these compounds on *Pneumocystis carinii, in vitro*. Identification of chloroquine analogues as anti-PcP leads is an unprecedented finding.

Introduction

Pneumocystis jirovecii pneumonia (PcP) is a potentially fatal opportunistic infection for immunocompromised individuals, such as those infected with HIV, or receiving immunosuppressive therapies for organ transplantation, cancer or autoimmune diseases. (1-3) Despite fatality rates due to PcP in HIV-infected people have significantly dropped due to highly active antiretroviral therapies (HAART), PcP remains a major cause of death or of chronic lung dysfunction amongst people affected by diseases as diverse as lymphoma,(4) rheumatoid arthritis,(5) lupus erythematosus,(6) or renal dysfunction due to IgA nephropathy.(7) Moreover, atypical radiological presentation of PcP sometimes occur, leading to delayed diagnosis and intervention, with frequently discouraging end results.(1,7) Though considered as an innocuous infection in immunocompetent people, *P. jirovecii* has been suggested as a morbidity factor in sudden infant death syndrome, given its high incidence in healthy infants.(8)

P. jirovecii, formerly known *P. carinii* f. sp. *hominis*, is the causative agent of PcP or pneumocystosis in humans, and was originally identified as the pathogen responsible for the epidemics of interstitial plasma cell pneumonitis that infected premature infants in Europe during World War II.(2,9) *Pneumocystis* spp. are unicellular eukaryotic organisms now included in the Fungi kingdom, due to their high genetic homology with other fungi.(2,10) These fungi are thought to be host-specific and infect a wide variety of mammals.(11) *P. jirovecii* infects humans, while *P. carinii* and *P. murina* infect rats and mice, respectively. Pre-clinical drug screening is conducted using the latter two species, as a culture system to properly propagate *P. jirovecii in vitro* was developed only recently, and still requires validation.(12)

Pneumocystis spp. have several features that are atypical amongst fungi, such as the lack of efficacy of common anti-fungal agents like amphotericin B and the ketoconazoles, likely due to the lack of ergosterol as the major sterol in their membranes.(13) Initially, these microorganisms were thought to be protozoans, as they were susceptible to antiprotozoals like primaquine, trimethoprim, sulfa drugs, pentamidine, or atovaquone, among others; these are still present in anti-PcP therapies, where the recommended first-line regimen for PcP prophylaxis and treatment is the well-known trimethoprim/sulfamethoxazole (TMP-SMX) combination, used for more than 50 years as a wide-spectrum antibiotic, and also employed against malaria.(13,14)

Some reports documenting *P. jirovecii* gene mutations in the target of the sulfamethoxazole component, dihydropteroate synthase, (15,16) may indicate resistance of this pathogen to TMP-SMX, supporting earlier reports.(17-19) Alternative options for salvage therapy include intravenous pentamidine, whose adverse effects are usually worse than those of TMP-SMX, or primaquine/clindamycin combinations.(20) Clinical use of the latter still lacks

approval in several countries, partly because primaquine (PQ, **1**) is hemotoxic, in particular for people with 6-glucose-phosphate dehydrogenase (6GPD).(21,22)

As hemotoxicity and low oral bioavailiability are major downsides of PQ's use in the clinical practice, we have been working on its chemical modification over the past decade, in an attempt to improve its action on malaria,(23-28) leishmaniasis,(29,30) and pneumocystosis,(24,25) with overall promising results. These successes provided the rationale to extend such "chemical recycling" efforts to other antimalarials, as chloroquine (CQ, **2** in Fig. 1),(31,32) and mepacrine (MPR, **3** in Figure 1).(32) This effort delivered *N*-acyl chloroquine analogues (**2a-j** in Figure 1) as relevant antiparasitic leads, active against liver and blood-stage *Plasmodia*,(31,32) as well as *Leishmania* parasites,(28) while being generally non-cytotoxic.(31,32) Moreover, one of such leads, **2c**, displayed remarkable *in vivo* performance in immunocompetent mice, when encapsulated in immunoPEGliposomes specifically targeted at *Plasmodium*-infected erythrocytes (PiRBC).(33)

In view of the above, we have now investigated the anti-*Pneumocystis* activity of *N*-acyl chloroquine derivatives **2a-j** (Fig. 1) using a standardized *in vitro* assay with *P. carinii*.(34) The study included *N*-acyl primaquine and *N*-acyl mepacrine analogues, **1a,b** and **3a**, respectively, as well as all relevant parent antimalarials, for comparison (see Figure 1). The effects of replacing the covalent amide bond between the antimalarial pharmacophore and the cinnamoyl group in PQ derivatives **1a,b** and CQ derivatives **2a,d**, by an ionic ammonium carboxylate bond, respectively producing PQ-derived ionic liquids **1'a,b**,(26) and CQ-derived ionic liquids **2'a,d** (Figure 1), were also assessed.

[insert Figure 1 here]

Antimicrobial Agents and Chemotherapy

Results

Chemical synthesis. The test compounds were prepared with a minimum 95% purity as described elsewhere and presented structural data consistent with previous reports.(27,31,32) Novel ionic liquids **2'a,d**, were successfully synthesized and presented adequate purity and spectral data, as given in detail under Materials&Methods.

Anti-*Pneumocystis* activity and cytotoxicity assays. All the test compounds (including parent drugs **1-3**) were first screened for their anti-*Pneumocystis* activity at 100 μg/mL (not shown); remarkably, with exception of PQ derivatives **1a,b** and **1'b**, and of CQ derivative **2h**, all compounds reduced *P. carinii* viability (ATP content) by at least 94% after 24 h, and reached nearly 100% reduction after 72 h. Such potent anti-*Pneumocystis* activity of most compounds was confirmed by subsequent determination of their respective IC_{50} values at 72 h, as shown in Table 1, which includes the compounds' toxicity for two mammalian cell lines (equally expressed as IC_{50} at 72 h), namely, A549 human lung adenocarcinoma and L2 rat pulmonary epithelial cells.

[insert Table 1 here]

Interestingly, despite the fact that, in most cases, activity differences are not markedly significant within experimental error, the following trends for structure-activity relationships (SAR) were apparent:

(i) the anti-*Pneumocystis* activity of PQ (**1**) is markedly decreased upon *N*-acylation of the drug's primary amine with a cinnamoyl group, as in **1a,b**; in turn, when the amide bond

between PQ and the cinnamic acid building blocks is replaced by a non-covalent ionic bond, as in **1'a,b**, the activity of the parent drug is restored;

(ii) in contrast with the previous situation, the moderate anti-*Pneumocystis* activity of CQ (**2**) is preserved or even improved in the respective *N*-acyl analogues, except when a flexible alkyl spacer between the 4-aminoquinoline core and the *N*-acyl group is absent, as in **2h**; moreover, replacement of the amide bond as in **2a,d** by its ammonium carboxylate ionic counterpart, as in **2'a,d**, does not affect activity expressively;

(iii) within the subset of CQ analogues **2a-g**, compounds **2b** (R = Me) and **2d** (R = OMe) are markedly more active than the others, with **2b** being non-toxic to both mammalian cell lines tested; IC_{50} values for this subset suggest that:

(a) the ideal length spacer between the 4-aminoquinoline core and the cinnamoyl group is butyl (n=4), as activity slightly drops when this length is either decreased (**2b** *vs.* **2f**) or increased (**2d** vs. **2g**); notably, this was equally observed regarding anti-malarial activity;(31)

(b) there seems to be no obvious correlation between compounds' lipophilicity (clogP values, not shown) and anti-*Pneumocystis* activity, whereas former observations regarding the antimalarial activity of these compounds indicated that more lipophilic ones seemed preferred;(31,35)

(c) the influence of the stereoelectronic properties of the *p*-substituent R on the anti-*Pneumocystis* activity of compounds **2a-e** was also surveyed, by searching possible correlations between IC_{50} values and classical descriptors, namely, Hammett constant σ_{para}, inductive sigma constant σ_I, Charton's sterical constant σ_v, Taft sterical parameter E_s, hydropho-

bicity parameter π, and molar refractivity, MR;(36) interestingly, as shown in Figure 2, a linear correlation (r^2 = 0.96) seems to exist with the Hammett's constant σ_{para}, where the positive slope of the linear fit suggests that electrodonating R groups favor anti-*Pneumocystis* activity;

(iv) replacement of the aryl group (**2a**) by a non-aromatic cyclohexyl (**2i**) or propyl (**2j**) seems to slightly decrease activity, while improving selectivity as compared to the rest of the **2a-g** series, except when it comes to the best overall performer (activity plus selectivity), **2b**. MPR (**3**) and its *N*-acyl analogue **3a** were included in this study only for comparison, as MPR was earlier reported as having low *in vivo* efficacy against *Pneumocystis*;(37) indeed, despite its marked activity, MPR was too toxic, whereas **3a** was not, but still did not outshine the activity and safety performance of its CQ counterparts **2b,d**.

[insert Figure 2 here]

Finally, all covalent compounds herein reported were run through the following publicly available filters for Pan Assay Interference Compounds (PAINS),(38) namely, ZINC (http://zinc15.docking.org/patterns/home),(39) PAINS Remover (http://www.cbligand.org/PAINS/),(40) and Aggregator Advisor (http://advisor.docking.org),(41) for identification of potential PAINS and aggregators. With the exception of compounds **3** and **3a**, whose aminoacridine moiety triggered a PAINS alert,(39,40) and **2h**, whose structural similarity to reported aggregators has been identified,(39,41) all compounds passed all filters used. In the case of organic salts **1'a**, **1'b**, **2'a** and **2'd**, only ZINC was apparently able to conveniently process their ionic structures, returning no PAINS or aggregator alerts.

Discussion

Globally, IC_{50} values against *P. carinii* (Table 1) confirm that, with exception of **1a,b** (slightly active) and of **2h** (inactive), all test compounds range from moderately to markedly active, particularly in the cases of CQ analogues **2b,d** and of MPR (**3**) and its analogue **3a**. Moreover, the vast majority of the compounds displayed only mild or no cytotoxic activity against both mammalian cell lines used, being the only exceptions compounds **2a**, **2c**, **2e**, and **3**, which displayed IC_{50} values below 10 μg/mL against A549 human lung carcinoma cells. Remarkably, one of the most active compounds against *P. carini*, **2b**, was also the most selective.

Altogether, the above observations show that, in contrast to PQ, whose well-known anti-*Pneumocystis* activity is practically lost upon acylation of the drug's aliphatic amine, *N*-acyl chloroquine analogues, especially those embedding a cinnamoyl group, are quite promising anti-PcP leads. Interestingly, this is not the first time that *N*-cinnamoyl chloroquine analogues like **2a-g** perform surprisingly well against pathogens, e.g. *P. carinii* as herein described, or specific pathogen developmental stages, e.g. liver-stage *Plasmodia* as previously reported by us,(31) for which PQ, not CQ, was one of the gold standards.

The significant activity of the test compounds against *Pneumocystis carinii* may be different against *P. jirovecii* or *P. murina*, given the biological divergence amongst these species. Still, the predictive value of *in vitro* methods herein applied has been previously validated *in vivo* on the mouse model of *Pneumocystis* pneumonia.(42-44)

In conclusion, *N*-cinnamoyl chloroquine analogues like **2a-g** are a notable family of anti-infective compounds, in particular because they have been revealing remarkable activity on pathogens against which CQ itself, or other related compounds, have not been currently

considered as useful. In the particular case of PcP, these compounds hold great promise, and best performers **2a,b** will be taken forward for *in vivo* assays. To the best of our knowledge, unveiling CQ analogues as potent anti-PcP leads is unprecedented.

Ongoing work is addressing evaluation of the potential activity of these CQ analogues against another concerning class of lung pathogens, *Mycobacteria*,(45) as CQ itself has been reported as potentially useful in the fight against these bacteria.(46) Results will be timely reported.

Materials&Methods

Chemical Synthesis. *Chemicals and Instrumentation.* Primaquine and chloroquine biphosphates, as well as mepacrine hydrochloride, were from Sigma-Aldrich. Cinnamic acids were all from Acros Organics. The coupling agent 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate (TBTU) was from Bachem, and all other chemicals were from Sigma-Aldrich. All solvents were of analytical grade, and purchased to VWR International. NMR analyses were carried out on a Brucker Avance III 400 MHz spectrometer, and samples were prepared in either deuterated chloroform ($CDCl_3$) or deuterated dimetylsulfoxide ($(CD_3)_2SO$) with tetramethylsilane (TMS) as an internal reference. Chemical shifts are reported downfield in parts per million (ppm) and multiplicity of proton signals is indicated as s (singlet), d (doublet), dd (double doublet), t (triplet), m (unresolved multiplet). High resolution mass spectra (HRMS) were obtained on an LTQ Orbitrap XL / LTQ Tune Plus 2.5.5 spectrometer, and processed with the 2.1.0 Xcalibur software (Thermo Scientific). Compounds purity was based on peak areas obtained through HPLC analyses that were run using the following conditions: 0−100% of B in A (A = H_2O with 0.05% of

trifluoroacetic acid; B = acetonitrile) in 30 min with a flow rate of 1 mL/min on a Merck-Hitachi Lachrom Elite instrument equipped with a diode array detector (DAD) and thermostated (Peltier effect) autosampler, using a Purospher STAR RP-18e column (150 mm × 4.0 mm; particle size, 5 µm).

*Synthesis of primaquine derivatives **1a,b** and **1'a,b**.* *N*-cinnamoyl-primaquine derivatives **1a,b** were prepared by previously described methods, and their analytical and structural data were consistent with those formerly reported.(27) Similarly, the synthesis of the ionic counterparts of **1a** and **1b**, respectively, ionic liquids **1'a** and **1'b**, was carried out as reported earlier, and compounds' structural and analytical data concurred with those hitherto published.(28)

*Synthesis of N-acyl-chloroquine (**2a-j**) and of N-acyl-mepacrine (**3a**) analogues.* The chemical synthesis of compounds **2a-j** and **3a** followed similar and previously reported procedures, yielding the expected compounds whose structural and analytical data agreed with those earlier described.(31,32,35,36,47)

*Synthesis of the new chloroquine/cinnamic acid-derived ionic liquids **2'a,d**.* Organic salts **2'a,d**, representative of the ionic counterparts of compounds **2a-g**, were prepared as follows. Chloroquine was first obtained as a free base, by addition of triethylamine (0.25 mL) to a suspension of chloroquine biphosphate (0.1298 g; 0.285 mol) in dichloromethane (4 mL). The mixture was stirred for 30 minutes in ice and in the dark, after which the organic layer was washed with water (3×10 mL), dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. Free chloroquine base, thus quantitatively obtained as a pale yellow oil, was next dissolved in methanol, and titrated with an equimolar amount of the relevant cinnamic acid, upon dropwise addition of a methanolic solution of the acid.

The neutralization reaction proceeded in the dark at room temperature for 30 minutes, after which methanol was removed by evaporation at reduced pressure and target organic salts **2'a,d** obtained as chromatographically homogeneous colorless viscous liquids, whose spectral data were in agreement with the target structures, as follows.

2-((7-chloroquinolin-4-yl)amino)-*N,N*-diethylpropan-1-aminium cinnamate (2'a)

Colorless oil (51 mg, 81%) δ_H (400 MHz, DMSO-d6) 8.37 (s, 1H), 8.35 (d, 1H, J=4.4 Hz), 7.76 (d, 1H, J=2.2Hz), 7.66 (m, 2H), 7.55 (d, 1H, J=16.0 Hz), 7.42 (m, 5H), 6.90 (d, 1H, J=7.9 Hz), 6.52 (m, 2H), 2.42 (m, 6H), 1.69 (m, 1H), 1.50 (m, 4H),1.23 (d, 4H, J=6.3 Hz), 0.91 (t, 6H, J=7.1 Hz); δ_C (100 MHz, DMSO-d6) 167.8, 151.8, 149.5, 149.2, 143.2, 134.4, 133.3, 130.0, 128.8, 128.0, 127.4, 124.3, 123.8, 120.1, 117.5, 98.8, 51.9, 47.5, 46.1, 33.3, 23.0, 19.8, 11.2

(EI^+) $^m/_z$ calculated for $C_{18}H_{27}ClN_3^+$: 320.19, found 320.33; (EI^-) $^m/_z$ calculated for $C_9H_7O_2^-$ 147.05, found 147.33

2-((7-chloroquinolin-4-yl)amino)-*N,N*-diethylpropan-1-aminium p-methoxycinnamate (2'd)

Colorless oil (52 mg, 87%) δ_H (400 MHz, DMSO-d6) 8.37 (s, 1H), 8.34 (d, 1H, J=4.4Hz), 7.76 (d, 1H, J=2.2Hz), 7.62 (d, 2H, J=8.8 Hz), 7.51 (d, 1H, J=15.9 Hz), 7.42 (dd, 1H, J=9.0 Hz, J= 2.2 Hz), 6.96 (d, 2H, J=8.8Hz), 6.89 (d, 1H, J= 8.0 Hz), 6.50 (d, 1H, J= 5.6 Hz), 6.36 (d, 1H, J= 15.9 Hz), 3.79 (s, 3H), 2.40 (m, 6H) 1.69 (m, 1H), 1.50 (m, 4H), 1.22 (m, 4H), 0.91 (t, 6H, J=7.1 Hz); δ_C (100 MHz, DMSO-d6) 167.9, 160.8, 151.9, 149.5, 149.3, 143.4, 133.3, 129.8, 127.4, 126.9, 124.3, 123.7, 117.5, 116.9, 114.3, 98.8, 55.3, 52.0, 47.5, 46.1, 33.3, 23.3, 19.8, 11.5

253 (EI^+) $^m/_z$ calculated for $C_{18}H_{27}ClN_3^+$: 320.19, found 320.35; (EI^-) $^m/_z$ calculated for $C_{10}H_9O_3^-$

254 177.06, found 177.05

255

256 ***In vitro* assays.** *Compound preparation*. Test compounds were stored at -20 ºC without ex-

257 posure to light, until use. Immediately prior to the first screen, compounds were solubilized

258 in 100% DMSO at a concentration of 20 mg/mL. For testing, the compounds were diluted

259 directly into culture medium at dilutions of 100, 10, 1, and 0.1 µg/mL. Culture medium

260 consisted of RPMI-1640 containing 20% horse serum, 1% MEM vitamin solution, 1%

261 MEM NEAA, 200 units/mL penicillin and 0.2 mg/mL streptomycin, and 50 µg/mL vanco-

262 mycin. Negative controls were medium alone, and 10 µg/mL ampicillin. Positive control

263 was 1 µg/mL pentamidine isethionate. Medium with the vehicle alone (DMSO) was tested

264 at the highest dilution to identify any associated toxicity.

265 *P. carinii ATP assay. P. carinii* viability was assessed through a non-specific ATP assay pre-

266 viously validated for this purpose (42-44,48,49). Briefly, cryopreserved and characterized *P.*

267 *carinii* isolated from rat lung tissue was distributed into triplicate wells of 48-well plates

268 with a final volume of 500 µL and a final concentration of 5×10^7 nuclei/ml *Pj* and 5×10^6

269 *Pm*. Controls and compound dilutions were added and incubated at 36 ºC, 5% CO_2. At 24,

270 48, and 72 hours, 10% of the well volume was removed and the ATP content was measured

271 using Perkin Elmer ATP-liteM luciferin-luciferase assay. The bioluminescence generated by

272 the ATP content of the samples was measured with a BMG POLARstar OPTIMA spectro-

273 photometer and expressed as Relative Light Units (RLUs). A sample of each group was

274 examined microscopically on the final assay day to rule out the presence of bacteria or oth-

275 er fungi. A quench control assay to determine compound interference in the lucifer-

in/luciferase revealed quenching with six compounds at the highest (100 µg/mL) concentration. No quench was observed at 50 µg/mL. Test concentrations of these compounds were lowered accordingly.

L2 and A549 Toxicity assay. For mammalian cell toxicity testing, A549 cells were cultured in DMEM medium and L2 cells with F12 medium. Both were supplemented with 10% fetal calf sera, 1× MEM vitamins, and 1X NEAA. Cultured cells were plated at 2×10^5/mL and grown to confluent monolayers. Media was removed and replaced with fresh media containing controls and test compound in appropriate dilutions. Assays of three time points (24, 48, 72 h) with triplicate wells were sampled. Media was aspirated from the wells. Adherent cells were stained with MTT reagent (thiazolyl blue) and incubated for one hour. The MTT reagent was aspirated from the cells and the MTT solvent added to the stained cells and rocked at room temperature until the crystals were dissolved. The plate was read in absorbance mode on a BMG POLARstar OPTIMA spectrophotometer.

Calculations. For the *P. carinii* ATP assay, background bioluminescence was subtracted and triplicate well readings were averaged. Using EXCEL software, % reduction in ATP for all groups was calculated (vehicle control - experimental/vehicle control ×100); results of triplicate assays were averaged and standard deviation determined. For the cell toxicity assay, triplicate well readings were averaged; for each day's readings, % reduction in absorbance for all groups was calculated: media control - experimental/media control ×100. In both assays, the 50% inhibitory concentration (IC_{50}) was calculated using InSTAT linear regression program.

Abbreviations

299 6GPD, 6-glucose-phosphate dehydrogenase; ATP, adenosine triphosphate; CQ, chloroquine;
300 DAD, diode array detector; DMEM, Dulbecco's modified Eagle medium; DMSO, dime-
301 thylsulfoxide; PcP, *Pneumocystis carinii* pneumonia; HIV, human immunodeficiency virus;
302 HAART, highly active antiretroviral therapy; HPLC, high performance liquid chromatog-
303 raphy; HRMS, high resolution mass spectrometry; IC_{50}, 50% inhibitory concentration;
304 MEM, minimum essential medium; MPR, mepacrine; MTT, 3-(4,5-dimethylthiazol-2-yl)-
305 2,5-diphenyltetrazolium bromide; NEAA, non-essential amino acid solution; NMR, nuclear
306 magnetic resonance; PEG, polyethyleneglycol; PiRBC, *Plasmodium*-infected erythrocytes;
307 PQ, primaquine; SD, standard deviation; SI, selectivity index; TBTU, 2-(1*H*-Benzotriazole-
308 1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; TMP-SMX, trime-
309 thoprim/sulfamethoxazole; TMS, tetramethylsilane; RPMI, Roswell Park Memorial Insti-
310 tute medium.
311
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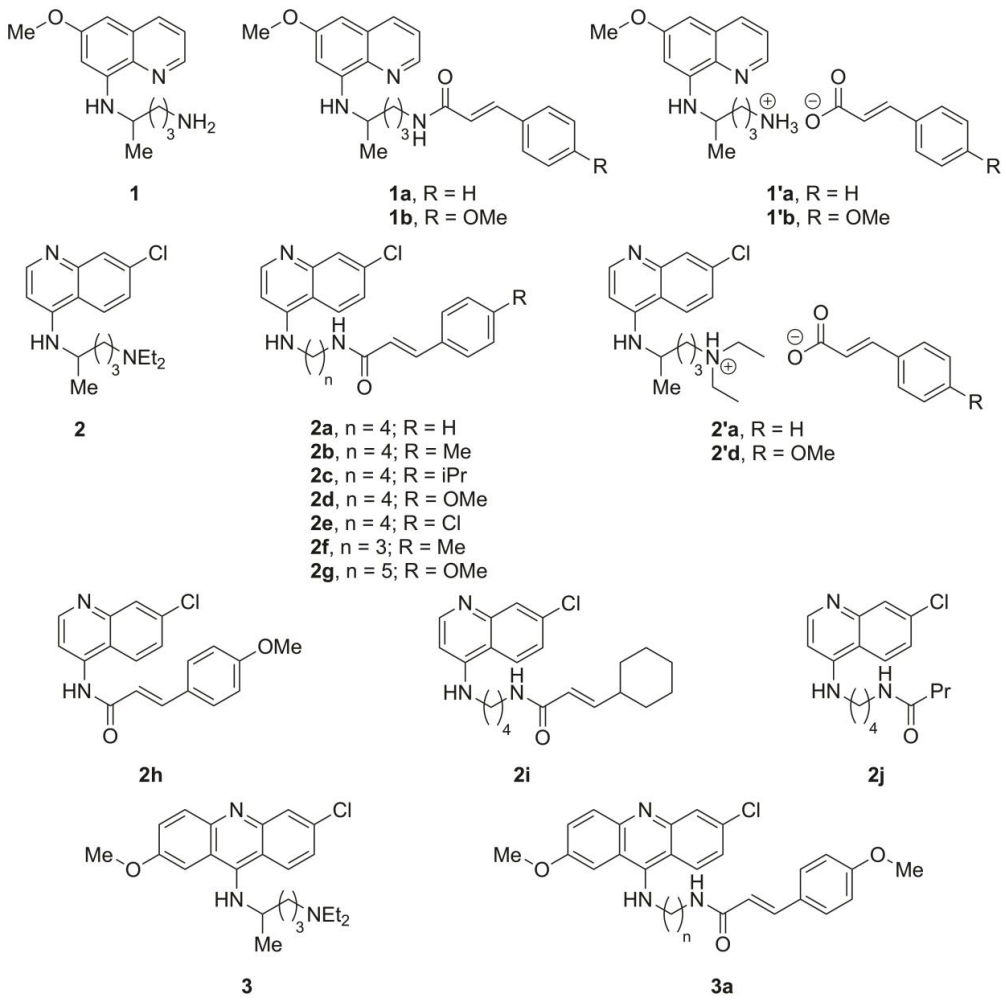

Figure 1. Classical antimalarial drugs and respective analogues, herein under scrutiny as potential anti-*Pneumocystis* agents.

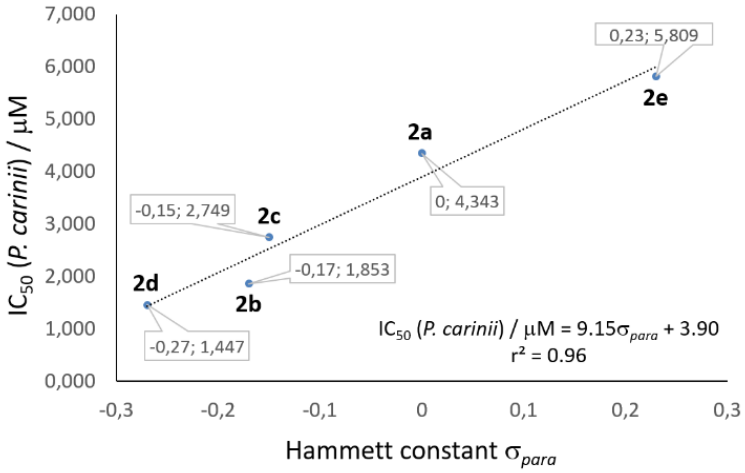

Figure 2. Correlation between *in vitro* anti-*Pneumocystis* activity (IC_{50} at 72 h / µM) of compounds **2a-e** and Hammett constants σ_{para} for the aryl substituent group R.

Antimicrobial Agents and Chemotherapy

Table 1. Minimal inhibitory concentrations at 50% (IC_{50}) of test compounds, at 72 h, against *P.carinii*, A549 human lung adenocarcinoma cells, and L2 rat pulmonary epithelial cells. IC_{50} values both in µM and in µg/mL units (the latter, within parenthesis).

Cpd	IC_{50} ± SD / µM (IC_{50} ± SD / µg/mL)			SI1[c]	SI2[d]
	P. carinii[a]	A549 cells[b]	L2 cells[b]		
1	4.92 ± 0.85 (1.28 ± 0.221)	110.04 ± 49.93 (28.5 ± 13.0)	>200 (>100)	22.4	>41
1a	43.58 ± 3.14 (16.97 ± 1.22)	>200 (>100)	>200 (>100)	>5	>5
1b	35.42 ± 7.52 (14.86± 3.16)	>200 (>100)	>200 (>100)	>6	>6
1'a	5.17 ± 4.46 (2.11 ± 1.82)	120.49 ± 43.56 (49.1 ± 17.8)	>200 (>100)	23.3	>39
1'b	5.09 ± 1.11 (2.23 ± 0.486)	100.15 ± 41.53 (43 ± 18.2)	>200 (>100)	19.7	>39
2	3.13 ± 2.14 (1.00± 0.685)	61.55 ± 6.38 (19.7 ± 2.04)	>200 (>100)	19.7	>64
2a	4.34 ± 1.34 (1.65 ± 0.509)	19.01 ± 14.29 (7.22 ± 5.43)	82.84 ± 7.27 (31.5 ± 2.76)	4.38	4.36
2b	1.85 ± 2.75 (0.730 ± 1.08)	>200 (>100)	>200 (>100)	>100	>100
2c	2.75 ± 2.30 (1.16 ± 0.971)	12.94 ± 6.99 (5.46 ± 2.95)	75.01 ± 16.35 (31.6 ± 6.90)	4.71	5.80
2d	1.45 ± 0.06 (0.593 ± 0.023)	80.02 ± 46.11 (32.8 ± 18.9)	128.59 ± 72.75 (52.7 ± 29.8)	55.3	1.61
2e	5.81 ± 2.19 (2.41 ± 0.906)	17.79 ± 12.41 (7.37 ± 5.14)	48.13 ± 23.48 (19.9 ± 9.73)	3.06	2.71
2f	4.09 ± 3.21 (1.55 ± 1.22)	41.04 ± 14.85 (15.6 ± 5.64)	139.62 ± 17.98 (53.0 ± 6.83)	10.0	3.40
2g	2.61 ± 0.80 (1.10 ± 0.340)	51.68 ± 30.19 (21.9 ± 12.8)	79.26 ± 50.24 (33.6 ± 21.3)	19.8	1.53
2h	>200 (>100)	-	-	-	-
2i	7.00 ± 1.06 (2.70 ± 0.411)	>200 (>100)	>200 (>100)	>29	>29
2j	8.45 ± 2.58 (2.79 ± 0.850)	166.60 ± 53.27 (55.0 ± 17.6)	>200 (>100)	19.7	>24
2'a	4.99 ± 1.64 (2.34 ± 0.769)	65.74 ± 7.84 (30.8 ± 3.67)	>200 (>100)	13.2	>40
2'd	4.60 ± 2.39	59.23 ± 22.19	>200	12.9	>43

Antimicrobial Agents and Chemotherapy

	(2.29 ± 1.19)	(29.5 ± 11.0)	(>100)		
3	0.90 ± 0.21 (0.358 ± 0.083)	17.58 ± 1.50 (7.03 ± 0.600)	124.19 ± 11.45 (49.7 ± 4.58)	19.6	7.07
3a	2.27 ± 0.50 (1.11 ± 0.245)	72.04 ± 60.20 (35.3 ± 29.5)	>200 (>100)	31.7	>88

[(a)] Activity scale: high, <0.010 µg/mL; very marked, 0.011 to 0.099 µg/mL; marked, 0.10 to 0.99 µg/mL; moderate, 1.0 to 9.99 µg/mL; slight, 10.0 to 49.9 µg/mL; none, ≥ 50 µg/mL.(24,25) [(b)] Cytotoxicity scale: high, < 1 µg/mL; moderate, 1.1 to 10 µg/mL; mild, 10.1 to 99.9 µg/mL; none, ≥ 100 µg/mL.(24,25) SI = selectivity index, where [(c)]SI1 = IC_{50}(A549) / IC_{50}(*P. carinii*), and [(d)] SI2 = IC_{50} (L2) / IC_{50} (*P. carinii)*.