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Enhanced *Pneumocystis carinii* Activity of New Primaquine Analogues

Thomas E. Goodwin,[a,*] Carole J. Boylan,[b] William L. Current,[b] John C. Byrd,[a] Clint B. Edwards,[a] Danny A. Fuller,[c] Jennifer L. Green,[a] Christine D. Larocca,[a] Kevin D. Raney,[a] Ashley S. Ross[a] and W. Andrew Tucker[a]

[a]*Department of Chemistry, Hendrix College, 1600 Washington Avenue, Conway, AR 72032, USA*
[b]*Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA*
[c]*Parkview Arts/Science Magnet High School, 2501 John Barrow Road, Little Rock, AR 72204, USA*

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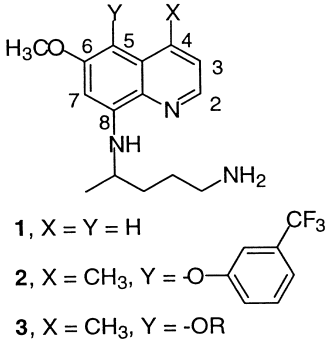
Abstract—New analogues of the venerable antimalarial drug primaquine have been synthesized and bioassayed in vivo against *Pneumocystis carinii*, a life-threatening infection common among immunosuppressed patients. Two of these new compounds are significantly more active than primaquine itself, and provide new information for future drug design and development in this area.

Introduction

Primaquine (**1**), an 8-aminoquinoline antimalarial,[1] in combination with clindamycin, an antibacterial agent, was shown in model assays to be effective in vitro and in vivo against *Pneumocystis carinii* pneumonia (PCP), a leading cause of morbidity and mortality in immunosuppressed patients.[2] This treatment was subsequently used successfully in human AIDS patients.[3]

In the necessary and continual search for new and more active drugs in this area, primaquine analogues with improved therapeutic ratios versus malaria[4] have also exhibited improved efficacy against PCP.[5] Compound **2**,[6]



for example, is much more effective than primaquine against malaria, and in both an in vitro culture model of *P. carinii* proliferation and a rat model of PCP.[5c] Thus, drug design can take advantage of apparent synchrony between structure–activity relationships (SARs) for two devastating and quite different human diseases, one protozoal in origin (malaria), and one fungal (PCP). The rationale for a new foray along these lines is presented below.

Following the general SAR guidelines of Pick,[4b] Chen and co-workers prepared a homologous series of primaquine analogues (**3**, R = *n*-C_3–C_{12}) and tested them against malaria as blood schizonticides in mice, and as tissue schizonticides in monkeys.[7] The C_5 and C_6 compounds were said be "remarkably effective blood and tissue schizonticides", and one of these (**3**, R = *n*-C_6) was later shown to be quite active against *P. carinii* in a rat model.[5a]

Synthesis

Based on the leads described above, a homologous series of 5-(4-alkoxyphenoxy)-4-methyl primaquine analogues was deemed worthy of testing against PCP and malaria. Initially, three homologues (**8**, **9**, and **10**) were synthesized from 5-chloro-6-methoxy-4-methyl-8-nitroquinoline (**5**) starting from 1,2-dimethoxybenzene (**4**).[6,8,9] Following substitution by the appropriate 4-alkoxyphenol

*Corresponding author. E-mail: goodwin@hendrix.edu

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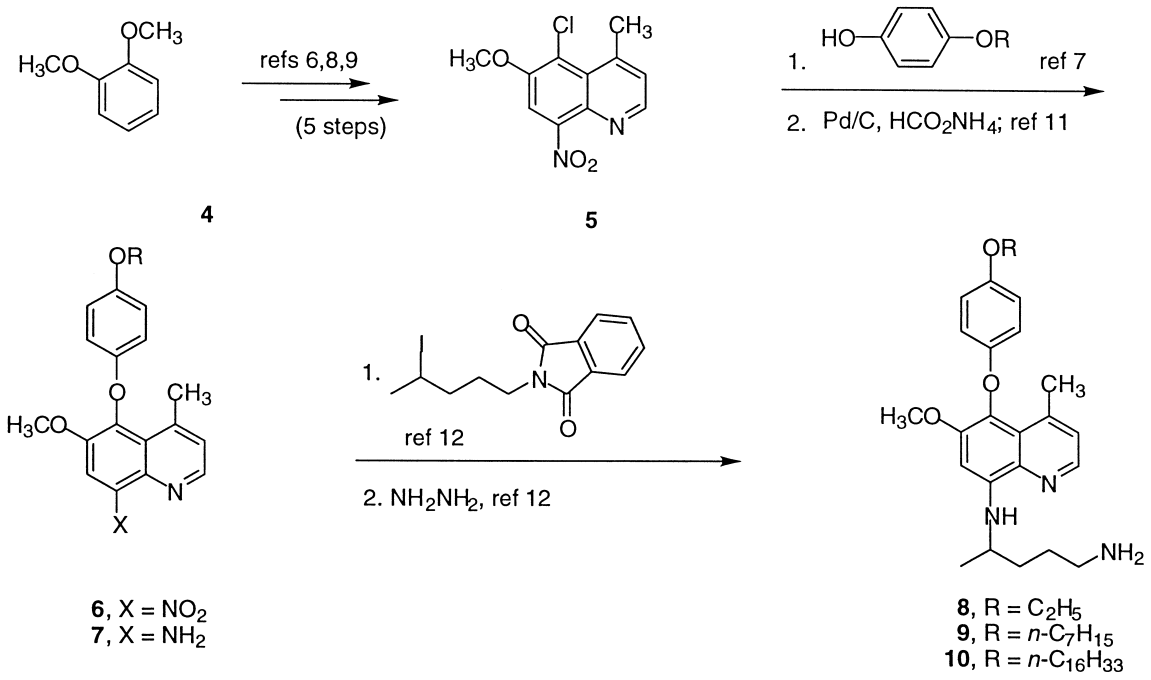

Scheme 1.

to provide compounds **6**,[7,10] the nitro group was reduced by palladium-catalyzed transfer hydrogenation using ammonium formate to give amine **7**,[11] followed by attachment of the primaquine sidechain[12] to provide the desired products (Scheme 1). Fumarate salts were prepared for stability and bioassay purposes.[13,14]

PCP Bioassay

Materials and methods

Primaquine analogues were tested in the immunosuppressed rat model described previously.[15] Five female Lewis rats (Harlan Sprague–Dawley, Inc., Indianapolis, IN) were assigned to each of the following treatment groups: *P. carinii*-infected, nontreated rats (infected controls), *P. carinii*-infected rats given the positive control drugs, 0.2 mg/mL of trimethoprim and 1 mg/mL of sulfamethoxazole (TMP/SMX), and *P. carinii*-infected animals receiving primaquine analogues. Three weeks after intratracheal inoculation with *P. carinii*, immunosuppressed rats with heavy infection (approximately 10^7 *P. carinii* organisms/g lung) were used to screen drugs for therapeutic activity. Experimental compounds were administered by intraperitoneal injection or oral gavage one to two times daily. Infected controls received vehicle only, and positive drug controls received TMP/SMX ad libitum in the drinking water. After 2 weeks of therapy, animals were euthanized and the lungs were removed and homogenized. Methenamine silver-stained lung homogenates were examined microscopically and infection scores, a logarithmic representation of the actual number of *P. carinii* cysts, were determined. Efficacy was determined by comparing the *P. carinii* cyst burden in lungs of treated rats to that of infected controls and positive control compounds.

Results

Primaquine analogues **8** and **9** were very effective against established *P. carinii* infections in the rat model when administered intraperitoneally or orally. Compound **8** was the more active, and when given at 2 mg/kg intraperitoneally once or twice daily, or orally once daily, it was more effective than the positive control drug, TMP/SMX (Table 1 and Fig. 1). A dose–response relationship was demonstrated when the fumarate of compound **8** was administered intraperitoneally once daily at doses from 0.25 to 2 mg/kg (Fig. 1). When this

Table 1. Efficacy of primaquine analogues in a rat model of *P. carinii*

Compound[a]	Dose	Route of administration	Infection score[b] Mean ± SEM
8	2 mg/kg	Intraperitoneal	0
9	2 mg/kg	Intraperitoneal	0.71 ± 0.38
10	2 mg/kg	Intraperitoneal	4.50 ± 0.35
9	2 mg/kg	Oral	1.25 ± 0.39
TMP–SMX[c]	Approx. 2–10 mg/day	Drinking water	1.31 ± 0.42
Infected control[d]	Vehicle	Intraperitoneal	4.90 ± 0.21
Primaquine[e]	2 mg/kg	Subcutaneous	4.30

[a]Primaquine analogues were tested as their fumarate salts.
[b]Infection scores are a logarithmic representation based upon blinded microscopic evaluation (400×) of numbers of cysts quantified in lung homogenates: 0, no cysts found in 30 microscopic fields; 1, 1–5 cysts per 10 fields; 2, approximately 1 cyst per field; 3, 2–10 cysts per field; 4, >10 but < 100 cysts per field; 5, >100 but < 1000 cysts per field.
[c]Trimethoprim (0.2 mg/mL)–sulfamethoxazole (1 mg/mL), positive treatment control. Mean ± SEM for three studies.
[d]Vehicle-treated (10% DMSO in water), *P. carinii*-infected controls. Mean ± SEM for three studies.
[e]For comparison; infection scores were based upon microscopic evaluation (1000×) of numbers of cysts and trophozoites in 50 microscopic fields. Primaquine was tested as its phosphate. SEM were not reported. Untreated infected controls in these studies had scores of 4.3–4.6 ± 0.2 SEM. Primaquine data are from Ref. 5c.

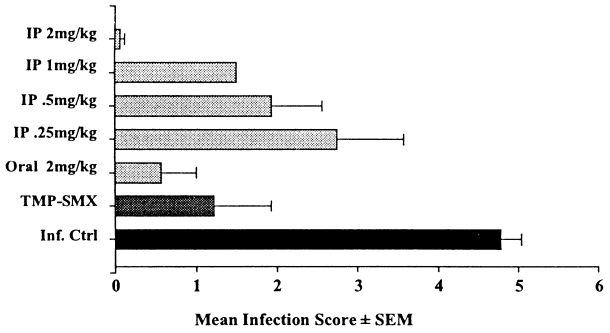

Figure 1. In vivo dose response of primaquine analogue **8** in an immunosuppressed rat model of PCP.[15] The experimental compound was administered ip or oral once daily for approximately 14 days to immunosuppressed rats with heavy *P. carinii* infections. See footnote b, Table 1, for an explanation of infection scores.

salt form was given intraperitoneally at 2 mg/kg twice daily, no cysts were found in lung homogenates (Table 1). The fumarate of the more hydrophobic compound **10** was not active against *P. carinii* when given intraperitoneally at 2 mg/kg twice daily (Table 1). Based on studies in a comparable animal model,[5c] primaquine alone, when given at 2 mg/kg, appeared similar to compound **10** with no efficacy observed against rat PCP (Table 1).

Conclusion

After a review of the literature describing bioassays of primaquine and primaquine analogues against malaria and *P. carinii*, a short homologous series of new primaquine analogues was designed, prepared, and tested against PCP in vivo. Two of these compounds (**8** and **9**) proved to be significantly more efficacious than primaquine, thus providing additional information for the design of new drugs in this area.[5c]

Acknowledgements

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9. The reported[8] quinoline ring-forming conditions for compound **5** were improved by omitting arsenic acid from the reaction mixture. With the aid of a Soxhlet extractor using 1:1 ether:petroleum ether, the desired product was obtained from the initially-formed black solid in yields comparable to those in the literature.
10. 4-Hexadecyloxyphenol was prepared by minor modifications of a published procedure: Neubert, M. E.; Laskos, S. J., Jr.; Maurer, L. J.; Carlino, L. T.; Ferrato, J. P. *Mol. Cryst. Liq. Cryst*. **1978**, *44*, 197. 4-Ethoxyphenol and 4-heptyloxyphenol are commercially available.
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13. Representative data are given below for primaquine analogue **10**. All stable new compounds gave a satisfactory elemental analysis or high resolution mass spectrum. IR and NMR spectra were consistent with the assigned structures. Experimental details and data for all new compounds discussed herein will be furnished upon request. **8-(4-Amino-1-methylbutyl)amino-5-(4-*n*-hexadecyloxyphenoxy)-6-methoxy-4-methylquinoline (10, R = *n*-$C_{16}H_{33}$).** ^{1}H NMR ($CDCl_3$) δ 8.38 (1H, d, J = 4.1 Hz), 7.04 (1H, d, J = 4.1 Hz), 6.77 (2H, m, AA′), 6.69 (2H, m, BB′), 6.47 (1H, s), 3.87 (2H, t, J = 6.3 Hz), 3.84 (3H, s), 3.68 (2H, m), 2.64 (3H, s), 2.77 (2H, br), 1.80–1.60 (6H, m), 1.40–1.20 (28H, m), 1.35 (3H, d, J = 6.3 Hz), 0.88 (3H, t, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$) δ 154.0, 153.8, 151.5, 144.7, 143.5, 143.3, 133.2, 127.2, 125.1, 124.8, 115.5 (2C, overlapping resonances), 94.2, 68.7, 57.1, 48.3, 34.3, 31.8 (29.6, 29.5, 29.4, 29.3, 29.2 (11C, overlapping resonances)), 26.0, 22.9, 22.6, 20.6, 13.9 (two resonances were obscured). The free base of this compound was quite unstable in air and $CDCl_3$; it was quickly converted to its fumarate salt (heat with 1 equiv of fumaric acid in 2-propanol) for storage, bioassay and further characterization: mp 137–138 °C; FABHRMS (free base): calcd for $^{12}C_{38}{}^{1}H_{59}{}^{14}N_3{}^{16}O_3$: 605.455643. Found: 605.455231.
14. The activities of compounds **8**, **9**, and **10** were compared to that of chloroquine, a 4-aminoquinoline antimalarial, when measured against cultured intraerythrocytic asexual forms of the human malaria parasite *Plasmodium falciparum*, clones Dd2 (chloroquine resistant) and HB3 (chloroquine sensitive). A published protocol was followed which measures incorporation of hypoxanthine into nucleic acid, a good measure of parasite activity.[16] The fumarate salts of primaquine analogues **8** and **9** were dissolved in DMSO, while compound **10**

was taken up in a 1:1 mixture of 90% aqueous ethanol and DMSO. Compound **10** was not active up to a concentration of 5 μM. IC_{50} data for chloroquine (CQ) and compounds **8** and **9** are as follows: CQ, IC_{50} for HB3 = 20 nM, IC_{50} for Dd2 = 200 nM; **8**, IC_{50} for HB3 (CQ sens) = 2000 nM, IC_{50} for Dd2 (CQ resist) = 1800 nM; **9**, IC_{50} for HB3 = 900 nM; IC_{50} for Dd2 = 1500 nM.

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