# Supporting Information

## Chemo-Immunotherapeutic Anti-Malarials Targeting Isoprenoid Biosynthesis

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**Table of Contents:**



| Contents | Pages |
|---|---|
| **Table S1** | **S2** |
| **Experimental Section** | **S3** |
| **References** | **S11** |

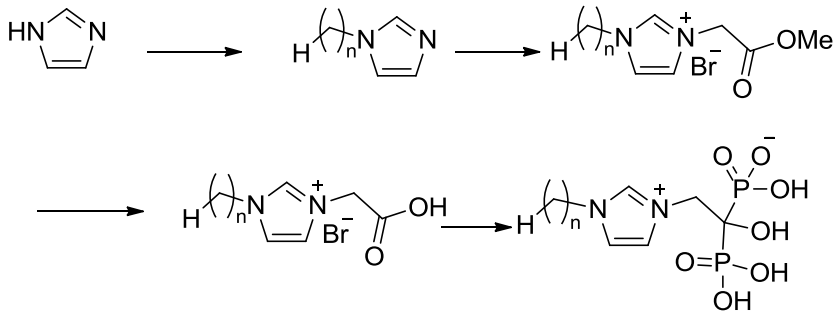
**Table S1.** Data collection and refinement statistics

| Crystal | HsFPPS/**5** |
|---|---|
| (PDB ID) | (4GA3) |
| | Co-crystallization |
| Data collection | |
| Radiation source | LS-CAT 21-ID-G |
| Wavelength (Å) | 0.97857 |
| Space group | $P4_12_12$ |
| *a* (Å) | 112.29 |
| *b* (Å) | 112.29 |
| *c* (Å) | 68.55 |
| Resolution (Å) | 50.0-2.40 (2.44-2.40) |
| No. of reflections | 17314 (882) |
| Completeness (%) | 97.4 (99.7) |
| Redundancy | 16.1 (16.4) |
| $R_{merge}$ (%) | 8.3 (63.5) |
| I/σ(I) | 53.9 (7.52) |
| Refinement | |
| Resolution (Å) | 40.5-2.40 (2.44-2.40) |
| No. of reflections | 16394 (987) |
| $R_{work}$ (%) | 20.4 (26.0) |
| $R_{free}$ (%) | 28.2 (35.1) |
| Geometry deviations | |
| Bond lengths (Å) | 0.016 |
| Bond angles (°) | 1.937 |
| Mean B-values (Å$^2$) / number of non-H atoms | |
| All refined atoms | 43.5/2909 |
| Compound atoms | 30.2/20 |
| $PO_4$ ions | 54.4/10 |
| Mg ions | 20.4/3 |
| Water molecules | 39.0/70 |
| Ramachandran plot (%) | |
| Most favored | 93.9 |
| Additionally allowed | 6.1 |
| Generously allowed | 0 |
| Disallowed | 0 |

## Experimental Section

**Synthetic Aspects. 1** and **17** were available from previous work.[1] The synthesis of **2-16** and **18-32** are described below.

**General procedure for the synthesis of lipophilic zoledronate analogs (2-16):**



A mixture of imidazole (100 mmol), alkyl bromide (100 mmol) and $K_2CO_3$ (200 mmol) in acetone (200 mL) was refluxed overnight. Upon filtration and removal of solvent, the residue was subjected to flash chromatography with ethyl acetate as eluant to give product with > 90 % yield. *N*-alkylimidazole was stirred with methyl bromoacetate in ethyl acetate at room temperature to give the imidazolium salt which was hydrolyzed under reflux in HCl (6 M) to give the carboxylic acid in quantitative yield. A mixture of the carboxylic acid (3 mmol), $H_3PO_3$ (15 mmol), and toluene (8 mL) was heated to 80 °C with stirring. After all solids melted, $POCl_3$ (15 mmol) was added slowly and the reaction mixture vigorously stirred at 80 °C for 5 h. The mixture was cooled, toluene decanted and 6 M HCl (3 mL) added to the residue. The resulting solution was refluxed for 1 h, then most of the solvent was removed *in vacuo*. *i*-PrOH (25 mL) was added to precipitate a 1-hydroxymethylene bisphosphonate as a white powder, which was filtered, washed with 2-propanol (5 x 5 mL), dried, and further purified by recrystallization in $H_2O$/*i*-PrOH. In some cases, the bisphosphonate was neutralized with NaOH and crystallized as its sodium salt, from $H_2O$/*i*-PrOH.

**1-Hydroxy-2-(*N*-methyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (2).**
$^1$H NMR (400 MHz, $D_2O$) $\delta$ : 8.50 (s, 1H), 7.28 (s, 1H), 7.13 (s, 1H), 4.44 (t, *J* = 7.2 Hz, 2H), 3.66 (s, 3H). $^{31}$P NMR (162 MHz, $D_2O$) $\delta$ : 15.09. Anal. Calcd. for $C_6H_{12}N_2O_7P_2 \cdot 0.5H_2O$ C, 24.42; H, 4.44; N, 9.49. Found, C, 24.36; H, 4.18; N, 9.18.

**1-Hydroxy-2-(*N*-ethyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (3).**
$^1$H NMR (400 MHz, $D_2O$) $\delta$ : 8.60 (s, 1H), 7.32 (s, 1H), 7.25 (s, 1H), 4.48 (t, *J* = 7.2 Hz, 2H), 4.04 (q, *J* = 7.2 Hz, 2H), 1.31 (t, *J* = 6.8 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) $\delta$ : 15.15. Anal. Calcd. for $C_7H_{14}N_2O_7P_2$: C, 28.01; H, 4.70; N, 9.33. Found: C, 28.32; H, 4.57; N, 8.96.

**1-Hydroxy-2-(*N*-propyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (4).**

$^1$H NMR (400 MHz, $D_2O$) δ : 8.61 (s, 1H), 7.34 (s, 1H), 7.25 (s, 1H), 4.48 (t, *J* = 7.2 Hz, 2H), 3.99 (m, 2H), 1.70 (q, *J* = 6.8 Hz, 2H), 0.73 (t, *J* = 6.8 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ : 15.14. Anal. Calcd. for $C_8H_{16}N_2O_7P_2$: C, 30.58; H, 5.13; N, 8.92. Found: C, 30.33; H, 5.07; N, 8.64.

**1-Hydroxy-2-(*N*-butyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (5).**
$^1$H NMR (400 MHz, $D_2O$) δ : 8.55 (s, 1H), 7.29 (s, 1H), 7.19 (s, 1H), 4.43 (t, *J* = 9.6 Hz, 2H), 3.97 (t, *J* = 7.2 Hz, 2H), 1.66 (m, 2H), 1.07 (m, 4H), 0.64 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ : 15.07.
Anal. Calcd. for $C_{10}H_{20}N_2O_7P_2$: C, 35.10; H, 5.89; N, 8.19. Found: C, 35.01; H, 5.67; N, 8.24.

**1-Hydroxy-2-(*N*-pentyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (6).**
$^1$H NMR (400 MHz, $D_2O$) δ : 8.55 (s, 1H), 7.29 (s, 1H), 7.19 (s, 1H), 4.43 (t, *J* = 9.6 Hz, 2H), 3.97 (t, J = 7.2 Hz, 2H), 1.66 (m, 2H), 1.07 (m, 4H), 0.64 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ : 15.07. Anal. Calcd. for $C_{10}H_{20}N_2O_7P_2$: C, 35.10; H, 5.89; N, 8.19. Found: C, 35.01; H, 5.67; N, 8.24.

**1-Hydroxy-2-(*N*-hexyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (7).**
$^1$H NMR (400 MHz, $D_2O$) δ : 8.55 (s, 1H), 7.29 (s, 1H), 7.20 (s, 1H), 4.43 (t, *J* = 10.0 Hz, 2H), 3.97 (t, *J* = 7.2 Hz, 2H), 1.64 (m, 2H), 1.07 (m, 6H), 0.64 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ : 15.20
Anal. Calcd. for $C_{11}H_{22}N_2O_7P_2$: C, 37.09; H, 6.22; N, 7.86. Found: C, 36.79; H, 6.59; N, 7.48.

**1-Hydroxy-2-(*N*-heptyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (8).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.57 (s, 1H), 7.31 (s, 1H), 7.21 (s, 1H), 4.45 (t, *J* = 10.0 Hz, 2H), 3.99 (t, *J* = 7.2 Hz, 2H), 1.67 (m, 2H), 1.07 (m, 8H), 0.64 (t, J = 6.8 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.07. Anal. Calcd. for $C_{12}H_{24}N_2O_7P_2$: C, 38.92; H, 6.53; N, 7.57. Found: C, 38.63; H, 6.50; N, 7.47.

**1-Hydroxy-2-(*N*-octyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (9).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.54 (s, 1H), 7.30 (s, 1H), 7.18 (s, 1H), 4.42 (t, *J* = 10.0 Hz, 2H), 3.98 (t, *J* = 6.4 Hz, 2H), 1.67 (m, 2H), 1.07 (m, 10H), 0.64 (t, *J* = 6.8 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 14.85.
Anal. Calcd. for $C_{13}H_{26}N_2O_7P_2 \cdot 0.7H_2O$ C, 39.34; H, 6.96; N, 7.06. Found: C, 39.04; H, 6.68; N, 7.00.

**1-Hydroxy-2-(*N*-nonyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (10).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.56 (s, 1H), 7.03 (s, 1H), 7.20 (s, 1H), 4.44 (t, *J* = 9.6 Hz, 2H), 3.98 (t, *J* = 7.2 Hz, 2H), 1.66 (m, 2H), 1.05 (m, 12H), 0.64 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.04
Anal. Calcd. for $C_{14}H_{28}N_2O_7P_2 \cdot 0.25H_2O$: C, 41.74; H, 7.13; N, 6.95. Found: C, 41.65; H, 6.99; N, 6.83.

**1-Hydroxy-2-(*N*-decyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (11).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.55 (s, 1H), 7.30 (s, 1H), 7.20 (s, 1H), 4.46 (t, *J* = 9.6 Hz, 2H), 3.98 (t, *J* = 7.2 Hz, 2H), 1.66 (m, 2H), 1.05 (m, 14H), 0.64 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.10
Anal. Calcd. for $C_{15}H_{30}N_2O_7P_2$: C, 43.69; H, 7.33; N, 6.79. Found: C, 43.69; H, 7.47; N, 6.58.

**1-Hydroxy-2-(*N*-undecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (12).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.54 (s, 1H), 7.30 (s, 1H), 7.19 (s, 1H), 4.43 (t, *J* = 9.6 Hz, 2H), 3.98 (t, *J* = 7.2 Hz, 2H), 1.66 (m, 2H), 1.05 (m, 16H), 0.64 (t, J = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 14.81
Anal. Calcd. for $C_{16}H_{32}N_2O_7P_2 \cdot 0.33H_2O$: C, 44.44; H, 7.61; N, 6.48. Found: C, 44.35; H, 7.62; N, 6.40.

**1-Hydroxy-2-(*N*-dodecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (13).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.65 (s, 1H), 7.39 (s, 1H), 7.14 (s, 1H), 4.42 (t, *J* = 9.6 Hz, 2H), 3.98 (t, *J* = 7.2 Hz, 2H), 1.65 (m, 2H), 1.05 (m, 18H), 0.64 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.18.

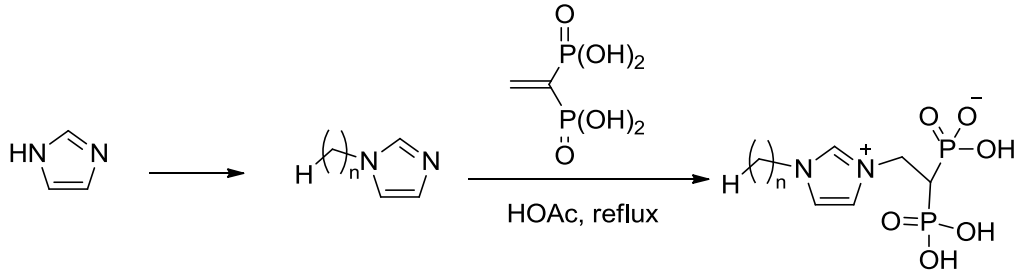
Anal. Calcd. for $C_{17}H_{34}N_2O_7P_2 \cdot H_2O$: C, 44.54; H, 7.92; N, 6.11. Found: C, 44.72; H, 7.83; N, 6.36.

**1-Hydroxy-2-(*N*-tridecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (14).**
$^1$H NMR (400 MHz, DMSO-d6) δ: 9.01 (s, 1H), 7.59 (s, 2H), 4.48 (s, 2H), 4.02 (m, 2H), 1.69 (m, 2H), 1.19 (m, 20H), 0.80 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.27. Anal. Calcd. for $C_{18}H_{36}N_2O_7P_2 \cdot H_2O$: C, 45.76; H, 8.11; N, 5.93. Found: C, 5.65; H, 7.82; N, 5.69.

**1-Hydroxy-2-(*N*-tetradecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (15).**
$^1$H NMR (400 MHz, DMSO-d6) δ: 9.01 (s, 1H), 7.59 (s, 2H), 4.48 (s, 2H), 4.02 (m, 2H), 1.69 (m, 2H), 1.19 (m, 22H), 0.80 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, DMSO-d6) δ: 15.17. Anal. Calcd. for $C_{19}H_{38}N_2O_7P_2 \cdot 0.2H_2O$: C, 48.34; H, 8.20; N, 5.93. Found: C, 48.07; H, 8.13; N, 5.88.

**1-Hydroxy-2-(*N*-pentadecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (16).**
$^1$H NMR (400 MHz, DMSO-d6) δ: 9.01 (s, 1H), 7.59 (s, 2H), 4.48 (s, 2H), 4.02 (m, 2H), 1.69 (m, 2H), 1.19 (m, 24H), 0.80 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, DMSO-d6) δ: 15.20. Anal. Calcd. for $C_{20}H_{38}N_2Na_2O_7P_2 \cdot 0.1H_2O$: C, 45.47; H, 7.29; N, 5.30. Found: C, 5.12; H, 7.68; N, 5.33.

**General procedure for the synthesis of lipophilic deoxy-zoledronate analogs (18-32)**




To a solution of ethene-1,1-diyldiphosphonic acid (1 mmol) in HOAc (5 mL) was added an *N*-alkylimidazole (1 mmol), and the solution refluxed overnight with stirring. Upon removal of solvent, recrystallization was carried out from $H_2O$/*i*-PrOH (1/10) to give the products as white powders.

**2-(*N*-methyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (18).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.56 (s, 1H), 7.34 (s, 1H), 7.18 (s, 1H), 4.41 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.67 (s, 3H), 2.54 (tt, *J* = 6.4, 21.2 Hz, 1H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 16.05. Anal. Calcd. for $C_6H_{12}N_2O_6P_2 \cdot 0.1H_2O$: C, 26.68; H, 4.48; N, 10.37; Found: C, 26.94; H, 4.30; N, 10.32.

**2-(*N*-ethyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (19).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.68 (s, 1H), 7.40 (s, 1H), 7.31 (s, 1H), 4.47 (dt, *J* = 7.2, 13.2 Hz, 2H), 4.05 (t, *J* = 7.2 Hz, 2H), 2.58 (tt, *J* = 7.2, 21.6 Hz, 1H), 1.31 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 16.19. Anal. Calcd. for $C_7H_{14}N_2O_6P_2$: C, 29.59; H, 4.97; N, 9.86; Found: C, 29.71; H, 4.76; N, 9.90.

**2-(*N*-propyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (20).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.65 (s, 1H), 7.38 (s, 1H), 7.27 (s, 1H), 4.45 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.96 (t, *J* = 6.8 Hz, 2H), 2.58 (tt, *J* = 7.2, 21.6 Hz, 1H), 1.68 (m, 2H), 0.70 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 16.20. Anal. Calcd. for $C_8H_{16}N_2O_6P_2 \cdot 0.4H_2O$: C, 31.46; H, 5.55; N, 9.17. Found: C, 31.25; H, 5.33; N, 8.84.

**2-(*N*-butyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (21).**

$^1$H NMR (400 MHz, $D_2O$) δ: 8.60 (s, 1H), 7.35 (s, 1H), 7.28 (s, 1H), 4.36 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.97 (t, *J* = 7.2 Hz, 2H), 2.10 (tt, *J* = 6.8, 20.4 Hz, 1H), 1.64 (m, 2H), 1.12 (m, 2H), 0.70 (t, *J* = 7.6 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.36. Anal. Calcd. for $C_9H_{18}N_2O_6P_2$: C, 34.62; H, 5.81; N, 8.97. Found: C, 34.42; H, 5.92; N, 9.05;

**2-(*N*-pentyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (22).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.66 (s, 1H), 7.39 (s, 1H), 7.21 (s, 1H), 4.48 (dt, *J* = 6.8, 12.8 Hz, 2H), 4.00 (t, *J* = 7.2 Hz, 2H), 2.59 (tt, *J* = 6.4, 20.0 Hz, 1H), 1.68 (m, 2H), 1.12 (m, 4H), 0.68 (t, *J* = 7.2 Hz, 3H).
$^{31}$P NMR (162 MHz, $D_2O$) δ: 16.23. Anal. Calcd. for $C_{10}H_{20}N_2O_6P_2 \cdot 0.8H_2O$: C, 35.26; H, 6.39; N, 8.22. Found: C, 34.93; H, 6.00; N, 8.36.

**2-(*N*-hexyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (23).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.60 (s, 1H), 7.36 (s, 1H), 7.21 (s, 1H), 4.36 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.97 (t, *J* = 7.2 Hz, 2H), 2.26 (tt, *J* = 6.8, 20.4 Hz, 1H), 1.66 (m, 2H), 1.10 (m, 6H), 0.66 (t, *J* = 4.8 Hz, 3H).
$^{31}$P NMR (162 MHz, $D_2O$) δ: 15.34. Anal. Calcd. for $C_{11}H_{22}N_2O_6P_2 \cdot 0.1$*i*-PrOH: C, 37.11; H, 6.26; N, 7.87. Found: C, 7.17; H, 6.38; N, 7.88.

**2-(*N*-heptyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (24).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.61 (s, 1H), 7.35 (s, 1H), 7.21 (s, 1H), 4.38 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.96 (t, *J* = 7.2 Hz, 2H), 2.30 (tt, *J* = 6.8, 20.4 Hz, 1H), 1.66 (m, 2H), 1.08 (m, 8H), 0.66 (t, *J* = 4.8 Hz, 3H).
$^{31}$P NMR (162 MHz, $D_2O$) δ: 15.33. Anal. Calcd. for $C_{12}H_{24}N_2O_6P_2$: C, 40.68; H, 6.83; N, 7.91; Found: C, 40.41; H, 6.78; N, 7.59.

**2-(*N*-octyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (25).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.61 (s, 1H), 7.36 (s, 1H), 7.23 (s, 1H), 4.43 (dt, *J* = 7.2, 13.6 Hz, 2H), 3.96 (t, *J* = 7.2 Hz, 2H), 2.45 (tt, *J* = 7.6, 21.2 Hz, 1H), 1.66 (m, 2H), 1.06 (m, 10H), 0.63 (t, *J* = 6.8 Hz, 3H).
$^{31}$P NMR (162 MHz, $D_2O$) δ: 15.75. Anal. Calcd. for $C_{13}H_{26}N_2O_6P_2 \cdot 0.7H_2O$: C, 40.99; H, 7.25; N, 7.35. Found: C, 40.92; H, 7.07; N, 7.19.

**2-(*N*-nonyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (26).**
$^1$H NMR (500 MHz, $D_2O$) δ: 8.63 (s, 1H), 7.40 (s, 1H), 7.24 (s, 1H), 4.40 (dt, *J* = 7.5, 13.0 Hz, 2H), 4.00 (t, *J* = 6.0 Hz, 2H), 2.21 (tt, *J* = 7.5, 20.0 Hz, 1H), 1.71 (m, 2H), 1.10 (m, 12H), 0.69 (t, *J* = 7.5 Hz, 3H).
$^{31}$P NMR (202 MHz, $D_2O$) δ: 15.75. Anal. Calcd. for $C_{14}H_{27}N_2NaO_6P_2$: C, 41.59; H, 6.79; N, 6.93. Found: C, 41.91; H, 6.85; N, 6.66.

**2-(*N*-decyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (27).**
$^1$H NMR (500 MHz, $D_2O$) δ: 8.65 (s, 1H), 7.40 (s, 1H), 7.28 (s, 1H), 4.45 (dt, *J* = 7.0, 13.5 Hz, 2H), 4.01 (t, *J* = 7.5 Hz, 2H), 2.45 (tt, *J* = 7.5, 21.5 Hz, 1H), 1.70 (m, 2H), 1.13 (m, 14H), 0.68 (t, *J* = 7.5 Hz, 3H). $^{31}$P NMR (202 MHz, $D_2O$) δ: 15.54; Anal. Calcd. for $C_{15}H_{30}N_2O_6P_2$: C, 45.45; H, 7.63; N, 7.07. Found: C, 45.40; H, 7.56; N, 7.00.

**2-(*N*-undecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (28).**
$^1$H NMR (400 MHz, $D_2O$) δ: 8.61 (s, 1H), 7.38 (s, 1H), 7.22 (s, 1H), 4.39 (dt, *J* = 7.2, 13.6 Hz, 2H), 3.98 (t, *J* = 7.2 Hz, 2H), 2.23 (tt, *J* = 6.8, 20.4 Hz, 1H), 1.68 (m, 2H), 1.07 (m, 16H), 0.68 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.17; Anal. Calcd. for $C_{16}H_{32}N_2O_6P_2$: C, 43.54; H, 7.31; N, 6.35. Found: C, 43.67; H, 7.29; N, 7.43.

**2-(*N*-dodecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (29).**
$^{1}$H NMR (400 MHz, $D_2O$) δ: 8.68 (s, 1H), 7.42 (s, 1H), 7.22 (s, 1H), 4.42 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.43 (t, *J* = 7.2 Hz, 2H), 2.42 (tt, *J* = 7.2, 21.6 Hz, 1H), 1.65 (m, 2H), 1.04 (m, 18H), 0.68 (t, *J* = 7.2 Hz, 3H).
$^{31}$P NMR (162 MHz, $D_2O$) δ: 15.33. Anal. Calcd. for $C_{17}H_{34}N_2O_6P_2 \cdot 1.55H_2O$: C, 39.93; H, 6.72; N, 5.48. Found: C, 40.30; H, 7.11; N, 5.45.

**2-(*N*-tridecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (30).**
$^{1}$H NMR (400 MHz, $D_2O$) δ: 8.61 (s, 1H), 7.38 (s, 1H), 7.22 (s, 1H), 4.37 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.98 (t, *J* = 7.2 Hz, 2H), 2.19 (tt, *J* = 7.2, 21.6 Hz, 1H), 1.67 (m, 2H), 1.07 (m, 20H), 0.66 (t, *J* = 7.2 Hz, 3H).
$^{31}$P NMR (162 MHz, $D_2O$) δ: 15.40. Anal. Calcd. for $C_{18}H_{36}N_2O_6P_2 \cdot H_2O$: C, 47.36; H, 8.39; N, 6.14. Found: C, 47.4; H, 8.11; N, 6.05.

**2-(*N*-tetradecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (31).**
$^{1}$H NMR (400 MHz, $D_2O$) δ: 8.75 (s, 1H), 7.48 (s, 1H), 7.21 (s, 1H), 4.44 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.99 (t, *J* = 7.2 Hz, 2H), 2.38 (tt, *J* = 7.2, 21.6 Hz, 1H), 1.66 (m, 2H), 1.07 (m, 22H), 0.66 (t, *J* = 7.2 Hz, 3H). $^{31}$P NMR (162 MHz, $D_2O$) δ: 15.40. Anal. Calcd. for $C_{19}H_{38}N_2O_6P_2 \cdot 0.5H_2O$: C, 49.45; H, 8.52; N, 6.07. Found: C, 49.33; H, 8.63; N, 6.02.

**2-(*N*-pentadecyl-imidazolium-1-yl)ethylidene-1,1-bisphosphonic acid (32).**
$^{1}$H NMR (400 MHz, $D_2O$) δ: 8.68 (s, 1H), 7.45 (s, 1H), 7.16 (s, 1H), 4.40 (dt, *J* = 7.2, 13.2 Hz, 2H), 3.97 (t, *J* = 7.2 Hz, 2H), 2.16 (tt, *J* = 7.2, 21.6 Hz, 1H), 1.66 (m, 2H), 1.07 (m, 24H), 0.67 (t, *J* = 7.2 Hz, 3H).
$^{31}$P NMR (162 MHz, $D_2O$) δ: 15.40. Anal. Calcd. for $C_{20}H_{40}N_2O_6P_2 \cdot 0.5H_2O$: C, 50.52; H, 8.69; N, 5.89. Found: C, 50.30; H, 8.72; N, 5.96.

**Human FPPS expression.** Truncated human FPPS (6-353) was cloned into NdeI/BamHI restriction sites of pET28a vector by using forward primer (5'-CTTCATATGAATTCAGATGTTTATGCCCAAGAAAAGCAGGATTTCG-3') and reverse primer (5'-CTTGGATCCTCACTTTCTCCGCTTGTAGATTTTGCGCG-3'). pET28a-$(His)_6$HsFPPS_6~353 was transformed into BL21(DE3) Tuner cells to ensure proper distribution of inducers across the cells. Transformed cells were spread onto LB plates with 50 μg/mL kanamycin and were incubated for 16 hours. A single colony that carried pET28a-$(His)_6$HsFPPS 6-353 was inoculated in 100 mL LB broth with 50 μg/mL kanamycin and incubated at 37 °C overnight. 10 mL of inoculated cells were added into 1L LB broth with 50 μg/mL kanamycin and incubated until the $OD_{600}$ reached 0.6~0.8. Cells were induced by 1 mM IPTG and incubated at 24°C for at least 16 hours, then centrifuged, and the pellets frozen at -80 °C. The cell pellets were then thawed in wash buffer (10 mM HEPES, pH 7.5, 500 mM

NaCl and 35 mM imidazole) with addition of Benzonase (EMD Millipore) and EDTA-free protease cocktail (Roche). Thawed cells were sonicated (10 sec active, 20 sec rest, for 10 min) then centrifuged at 23,000 rpm for 30 min. The supernatant was loaded on a Ni-NTA column and eluted with 0~100 % elution buffer (10 mM HEPES, 500 mM NaCl and 500 mM imidazole). Fractions were subjected to SDS-PAGE and only pure $(His)_6$HsFPPS was collected. The protein was digested with thrombin and dialyzed against dialysis buffer (10 mM HEPES, pH 7.4 and 150 mM NaCl) at 4 °C for 30 hours to remove the N-terminal His-tag. HsFPPS was further purified by using S200 gel filtration chromatography with storage buffer (10 mM Tris, pH 7.4 and 25 mM NaCl). The pure HsFPPS fraction was concentrated to 37 mg/mL, then quickly frozen in liquid nitrogen and stored at -80°C.

**Human FPPS inhibition assays.** Human FPPS inhibition assays were carried out using 96 well plates with 200 μL reaction mixture in each well. The condensation of geranyl diphosphate (100 μM final) and isopentenyl diphosphate (100 μM final) was monitored at room temperature by using a continuous spectrophotometric assay for phosphate-releasing enzymes.[2] The reaction buffer contained 50 mM Tris-HCl (pH 7.4), 1 mM $MgCl_2$, and 0.01 % Triton X100. The compounds investigated were pre-incubated with enzyme for 30 min at room temperature. The $IC_{50}$ values were obtained from fitting dose-response curve using Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, www.graphpad.com).

***P. vivax* GGPPS expression.** A clone encoding *P. vivax* GGPPS (PlasmoDB gene ID: Pv092040) with an N-terminally $His_6$-tagged fusion protein and a tobacco etch virus protease site was expressed in *Escherichia coli* BL21-codon Plus (DE3) RIL (Stratagene) at 15 °C in baffled flasks. Cells were lysed by sonication in the presence of Benzonase Nuclease (Novagen) and a protease inhibitor cocktail (Sigma), and the protein purified chromatographically by using a Ni-nitrilotriacetate resin. EDTA was added immediately to the elution fraction to 1 mM, and 5 mM DTT added after 15 min. The eluted

protein was then concentrated and loaded onto a Sephadex S-200 gel filtration column, and fractions containing PvGGPPS collected.

***P. vivax* GGPPS inhibition assays.** The *P. vivax* GGPPS inhibition assays were carried out by using 96-well plates with 200 μL of reaction mixture in each well. The condensation of geranyl diphosphate (100 μM) with isopentenyl diphosphate (100 μM) was monitored at room temperature by using a continuous spectrophotometric assay for phosphate-releasing enzymes[2] in a reaction mixture containing 50 mM Tris-HCl (pH 7.4), 1 mM $MgCl_2$, and 0.01% Triton X100. The inhibitors were pre-incubated with the enzyme for 30 min, at room temperature. The $IC_{50}$ values were obtained from fitting the dose-response curve using Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, www.graphpad.com).

**γδ T cell activation assays.** Vγ2Vδ2T cell activation was assessed by TNF-α release as described previously.[3] Briefly, the $CD4^+$ Vγ2Vδ2T cell clone, JN.23, was stimulated with bisphosphonates in the presence of the antigen presenting cell line, CP.EBV (an EBV transformed human B cell line). For TNF-α release, supernatants were harvested 16 h later and assayed for TNF-α levels by sandwich ELISA (R&D Systems). Concentrations required to achieve 50% of the observed maximal T cell response ($EC_{50}$) were obtained by using the Prism 4.0 program (Graphpad Software, La Jolla, CA, www.graphpad.com), using a sigmoidal dose-response function. Curve fitting minima for each experiment were determined using the Global Fitting technique, as implemented in Prism 4.0. Curve fitting maxima were optimized for each individual compound without the use of any constraints.

**Crystallization, data collection and refinement of the HsFPPS/5 complex.** Co-crystallization of human FPPS was carried out as follows. 34 mg/mL HsFPPS was mixed with 1 mM **5** and 2 mM $MgCl_2$. The mixture was then incubated at 4 °C overnight. The mixture was centrifuged and any precipitate discarded. The protein solution was then mixed with mother liquor (1.2 M Na/K phosphate, pH 5.2 and

25% glycerol) in a ratio of 1:1. Hanging drops were incubated at 18 °C. Large, hexagon-like crystals appeared in ~ 1-3 days and grew to maximum size in one week. The crystals were mounted, then frozen in liquid nitrogen. Diffraction data was collected at the Life Science Collaborative Access Team (LS-CAT) at the Argonne National Laboratory (Argonne, IL). Data was processed by using HKL2000[4] and refined by using Refmac[5,6] and Coot[7]. Refined statistics are shown in Table S1. Graphics were created by using PyMOL[8].

***P. falciparum* growth inhibition assays.** *P. falciparum* growth inhibition assays were carried out as described in our previous work.[9] A *P. falciparum* culture was adjusted to 2% hematocrit, 0.5% parasitemia, then dispensed by a WellMate (Thermo) into 384 well plates (Greiner) containing the compounds (final volume 50 μL) and incubated for 72 h. Chloroquine, artemisinin, and DMSO were used within the assay plates to serve as controls. After 3 d, a parasite lactate dehydrogenase (pLDH) assay was used to assess compound efficacy. At the end of 72 h, the plates were frozen overnight at −20 °C. After thawing, the plates were shaken for 45 s at 1,700 rpm in a Mix Mate (Eppendorf) and 5 μL of the lysate transferred into the corresponding well of another plate containing 30 μL of Malstat Reagent[10] and incubated for 2 h. The absorbance (650 nm) was read using a Spectramax M5 (Molecular Devices). $IC_{50}$ values were obtained from fitting the dose-response curve using Prism 4.0 (GraphPad Software, Inc., La Jolla, CA, www.graphpad.com).

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