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Supporting Information

Lipophilic Bisphosphonates as Dual Farnesyl/Geranylgeranyl Diphosphate Synthase Inhibitors: An X-ray and NMR investigation

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Supplementary Tables

Table S1: pIC_{50} values for tumor cell growth inhibition and FPPS, GGPPS enzyme inhibition

	Experimental Values						Predicted Values [b]					
Compound [a]	FPPS pK_i (M)	GGPPS pK_i (M)	SlogP	SF-268 pIC_{50} (M)(exp)	MCF-7 pIC_{50} (M) (exp)	NCI-H460 pIC_{50} (M) (exp)	pIC_{50} (M) SF-268 pred	(residual)	pIC_{50} (M) MCF-7 pred	(residual)	pIC_{50} (M) NCI-H460 pred	(residual)
716	5.8	7.1	-0.4	7.9	6.4	8.3	6.4	(1.5)	5.6	(0.8)	6.7	(1.6)
715	7.3	8.1	-1.2	7.0	6.7	7.2	6.5	(0.5)	6.6	(0.1)	6.7	(0.5)
638	7.8	8.0	-1.1	6.7	6.8	6.8	6.4	(0.3)	6.3	(0.5)	6.5	(0.3)
722	8.9	7.2	-2.4	6.0	5.6	6.3	5.6	(0.4)	5.0	(0.6)	5.7	(0.6)
717	7.7	7.7	-1.8	6.2	6.3	6.2	6.2	(0.0)	5.4	(0.9)	6.3	(-0.1)
694	7.1	7.4	-0.1	5.9	4.9	6.0	6.6	(-0.7)	4.9	(0.0)	6.8	(-0.8)
604	8.9	7.3	-2.4	5.8	5.3	6.0	5.8	(0.0)	5.3	(0.0)	5.9	(0.1)
637	8.9	7.4	-2.5	5.9	6.6	5.7	5.5	(0.4)	5.6	(1.0)	5.6	(0.1)
688	8.6	7.5	-0.8	5.6	5.2	5.5	6.1	(-0.5)	5.0	(0.2)	6.3	(-0.8)
675	5.9	7.1	-0.2	5.2	5.2	5.3	4.9	(0.3)	5.6	(-0.4)	4.9	(0.4)
683	8.5	7.0	-2.4	4.9	4.7	4.8	5.0	(-0.1)	4.6	(0.1)	5.0	(-0.2)
261	8.9	5.5	-4.7	4.9	4.8	4.8	4.1	(0.8)	4.3	(0.5)	4.2	(0.6)
91	8.9	5.6	-5.5	4.8	4.7	4.8	3.8	(1.0)	4.6	(0.1)	3.8	(1.0)
678	7.9	5.7	-9.0	4.6	4.7	4.7	4.4	(0.2)	4.5	(0.2)	4.4	(0.3)
754	5.3	7.8	-0.9	4.6	4.6	4.7	5.2	(-0.6)	5.2	(-0.6)	5.2	(-0.5)
728	6.8	7.5	-1.5	4.9	4.5	4.6	5.3	(-0.4)	5.7	(-1.2)	5.4	(-0.8)
300	8.3	6.4	-3.8	4.5	4.4	4.5	4.8	(-0.3)	4.7	(-0.3)	4.9	(-0.4)
679	8.2	5.0	-5.0	4.5	4.4	4.3	4.8	(-0.3)	4.0	(0.4)	4.8	(-0.5)
472	8.6	6.6	-3.1	4.3	4.4	4.3	4.9	(-0.6)	4.8	(-0.4)	4.9	(-0.6)
474	8.4	5.8	-4.4	4.1	4.1	4.2	4.3	(-0.2)	4.5	(-0.4)	4.3	(-0.1)
278	8.6	5.4	-5.4	4.3	4.1	4.1	4.5	(-0.2)	4.1	(0.0)	4.6	(-0.5)
483	8.5	5.5	-3.9	4.0	4.0	4.1	3.8	(0.2)	4.3	(-0.3)	3.8	(0.3)
5	7.4	5.3	-7.0	3.5	3.9	4.0	4.1	(-0.6)	3.9	(0.0)	4.1	(-0.1)
685	8.3	5.7	-4.3	3.7	3.6	3.9	3.3	(0.4)	3.5	(0.1)	3.2	(0.7)
684	8.0	6.6	-3.6	3.7	3.6	3.8	4.2	(-0.5)	4.4	(-0.8)	4.2	(-0.4)
2	8.6	5.0	-5.0	3.9	3.8	3.7	3.8	(0.1)	4.1	(-0.3)	3.8	(-0.1)
24	8.3	5.6	-5.6	3.8	3.7	3.7	3.6	(0.2)	3.4	(0.3)	3.6	(0.1)
1	7.2	4.9	-6.7	3.5	3.3	3.5	4.1	(-0.6)	3.0	(0.3)	4.2	(-0.7)
727	8.2	5.6	-3.8	3.7	3.5	3.5	3.9	(-0.2)	3.8	(-0.3)	3.9	(-0.4)

[a] Chemical structures are shown in Figure S1 in the Supporting Information.
[b] Predicted values from leave-two-out cross validated models

This Table shows a compilation of enzyme (human FPPS, human GGPPS) inhibition data by a series of bisphosphonates together with computed SlogP values. The experimental $pIC_{50} = -\log_{10}IC_{50}[M]$ values are shown for all three cell lines. We also show the predicted (pred) pIC_{50} values from the combinatorial descriptor search and their residuals. The exp vs. pred R^2 value for MCF-7 is 0.78. For NCI-H460 the R^2 value was 0.77, for SF-268, the R^2 was 0.78. Full details are in Table S2-S4.

Table S2: Top ten "enzyme plus 2-descriptor" combinations with their coefficients and relative contribution for the MCF-7 tumor cell pIC_{50} predictions.

MCF7 pIC_{50} (cell) =	R^2	Relative Importance of pIC_{50}(enzyme)	Relative Importance of Descriptor B	Relative Importance of Descriptor C
25.59107 +0.74764 * GGPPS_pIC50 -9.24972 * BCUT_PEOE_3 -0.07518 * dipoleY	0.77530	1.000000	0.543553	0.451504
-0.37197 +0.53975 * GGPPS_pIC50 -0.09607 * E_tor +1.48546 * FCASA+	0.75706	0.573256	1.000000	0.990688
1.60321 +0.55960 * GGPPS_pIC50 +0.01527 * SMR_VSA5 -0.39560 * chi1_C	0.75231	0.450542	1.000000	0.829762
-6.36382 +0.98141 * GGPPS_pIC50 -7.96387 * GCUT_PEOE_1 +0.01284 * PEOE_VSA_POS	0.75219	1.000000	0.816069	0.567343
1.77550 +0.63325 * GGPPS_pIC50 -0.24596 * chi0_C +0.01308 * SMR_VSA5	0.74559	0.594899	0.864659	1.000000
28.48149 +0.69807 * GGPPS_pIC50 -9.18802 * BCUT_SMR_3 -0.04966 * dipoleY	0.74409	1.000000	0.471052	0.319424
-7.69477 +1.23574 * GGPPS_pIC50 -6.92773 * GCUT_PEOE_1 +5.94612 * PEOE_VSA_FPOS	0.74020	1.000000	0.563786	0.287592
-1.74865 +1.23574 * GGPPS_pIC50 -6.92773 * GCUT_PEOE_1 -5.94612 * PEOE_VSA_FNEG	0.74020	1.000000	0.563786	0.287592
-1.84405 +0.91410 * GGPPS_pIC50 +0.01280 * SMR_VSA5 -0.50160 * logP(o/w)	0.73981	0.800331	0.911887	1.000000
3.96512 +0.80121 * GGPPS_pIC50 +0.00015049 * pmi -0.20002 * KierA1	0.73936	0.867746	0.887623	1.000000

Table shows a compilation of the outputs of a combinatorial descriptor search that relates cell growth inhibition (pIC_{50} (cell)) with GGPPS inhibition plus two other descriptors (B, C).

$pIC_{50}(cell) = a \bullet pIC_{50}(enzyme) + b \bullet B + c \bullet C + d$

where a-d are linear regression coefficient and B, C are chosen from 230 descriptors in MOE and FPPS. The explanation of the meanings of each of the top-10 descriptor sets are given in the MOE manual and ref. 19 in the Text. GGPPS inhibition is the dominant descriptor in most cases, consistent with the high correlation between GGPPS inhibition and cell growth inhibition.

The following gives a summary of the R^2 values based on the combinatorial descriptor search using the top-ranked combination.

Target	Cell line	R^2, Enzyme vs. Cell	R^2, Training Set	R^2, Test Set[a]	Descriptors used
GGPPS	MCF-7	0.60	0.78	0.73	124

[a] Predicted values from leave-two-out cross validated models.

Table S3: Top ten "enzyme plus 2-descriptor" combinations with their coefficients and relative contribution for the NCI-H460 tumor cell pIC_{50} predictions.

NCI-H460 pIC_{50} (cell) =	R^2	Relative Importance of pIC_{50}(enzyme)	Relative Importance of Descriptor B	Relative Importance of Descriptor C
0.63207 +0.35568 * GGPPS_pIC50 -0.17238 * E_tor +2.17209 * FCASA+	0.76741	0.210523	1.000000	0.807295
-2.35689 -0.06808 * FPPS_pIC50 -0.21578 * E_tor +2.48428 * FCASA+	0.73463	0.030920	0.737612	1.000000
3.60781 +0.58855 * GGPPS_pIC50 -0.30001 * dipole +0.00033410 * pmiX	0.73343	0.345714	0.814374	1.000000
2.58980 +0.07950 * GGPPS_pIC50 -0.14206 * E_tor +0.00320 * CASA+	0.70560	0.057097	1.000000	0.691306
2.27359 +0.08322 * FPPS_pIC50 -0.15750 * E_tor +0.00355 * CASA+	0.70825	0.051781	0.691607	1.000000
4.80220 +0.64764 * GGPPS_pIC50 -0.22230 * KierA1 +0.00023492 * pmi	0.70361	0.506213	0.802098	1.000000
-0.38230 +0.63201 * GGPPS_pIC50 +8.92630 * FASA+ -0.11496 * E_tor	0.70163	0.560943	0.869247	1.000000
1.49330 +0.58087 * GGPPS_pIC50 +0.01907 * SMR_VSA5 -0.46209 * chi1_C	0.69768	0.374303	1.000000	0.775735
3.08296 +0.55624 * GGPPS_pIC50 +0.00026177 * pmi -0.23473 * dipole	0.69363	0.390173	1.000000	0.760871
-14.21073 +0.71487 * GGPPS_pIC50 +0.91522 * std_dim1 +6.50762 * a_ICM	0.69336	0.530967	1.000000	0.887648

Table shows a compilation of the outputs of a combinatorial descriptor search that relates cell growth inhibition (pIC_{50} (cell)) with GGPPS inhibition plus two other descriptors (B, C).

$pIC_{50}(cell) = a \bullet pIC_{50}(enzyme) + b \bullet B + c \bullet C + d$

where a-d are linear regression coefficient and B, C are chosen from 230 descriptors in MOE and FPPS. The explanation of the meanings of each of the top-10 descriptor sets are given in the MOE manual and ref. 19 in the Text. GGPPS inhibition is the dominant descriptor in most cases, consistent with the high correlation between GGPPS inhibition and cell growth inhibition.

The following gives a summary of the R^2 values based on the combinatorial descriptor search using the top-ranked combination.

Target	Cell line	R^2, Enzyme vs. Cell	R^2, Training Set	R^2, Test Set[a]	Descriptors used
GGPPS	NCI-H460	0.54	0.77	0.79	124

[a] Predicted values from leave-two-out cross validated models.

Table S4: Top ten "enzyme plus 2-descriptor" combinations with their coefficients and relative contribution for the SF-268 tumor cell pIC_{50} predictions.

SF268 pIC_{50} (cell) =	R^2	Relative Importance of pIC_{50}(enzyme)	Relative Importance of Descriptor B	Relative Importance of Descriptor C
0.68240 +0.37234 * GGPPS_pIC50 -0.15738 * E_tor +2.01914 * FCASA+	0.78095	0.241393	0.821984	1.000000
3.47134 +0.58562 * GGPPS_pIC50 -0.27717 * dipole +0.00030471 * pmiX	0.74359	0.377170	0.824941	1.000000
2.13378 -0.01874 * FPPS_pIC50 +2.38273 * FCASA+ -0.20582 * E_tor	0.73660	0.008925	1.000000	0.741719
1.90577 +0.13816 * FPPS_pIC50 -0.15338 * E_tor +0.00355 * CASA+	0.73530	0.088282	1.000000	0.709317
-0.34695 +0.62533 * GGPPS_pIC50 -0.10859 * E_tor +8.73213 * FASA+	0.73198	0.587560	1.000000	0.900202
1.65558 +0.53214 * GGPPS_pIC50 -0.43608 * chi1_C +0.01838 * SMR_VSA5	0.72967	0.355866	0.759749	1.000000
-13.73343 +0.67360 * GGPPS_pIC50 +6.36295 * a_ICM +0.89897 * std_dim1	0.72907	0.509358	0.883606	1.000000
1.83409 +0.18619 * FPPS_pIC50 -0.10393 * E_tor +0.04801 * E_vdw	0.72716	0.175579	1.000000	0.762609
2.52273 +0.10480 * GGPPS_pIC50 -0.13127 * E_tor +0.00303 * CASA+	0.72566	0.081452	1.000000	0.708243
4.66691 +0.64895 * GGPPS_pIC50 -0.21304 * KierA1 +0.00021779 * pmi	0.72203	0.547122	0.829133	1.000000

Table shows a compilation of the outputs of a combinatorial descriptor search that relates cell growth inhibition (pIC_{50} (cell)) with GGPPS inhibition plus two other descriptors (B, C).

$pIC_{50}(cell) = a \bullet pIC_{50}(enzyme) + b \bullet B + c \bullet C + d$

where a-d are linear regression coefficient and B, C are chosen from 230 descriptors in MOE and FPPS. The explanation of the meanings of each of the top-10 descriptor sets are given in the MOE manual and ref. 19 in the Text. GGPPS inhibition is the dominant descriptor in most cases, consistent with the high correlation between GGPPS inhibition and cell growth inhibition.

The following gives a summary of the R^2 values based on the combinatorial descriptor search using the top-ranked combination.

Target	Cell line	R^2, Enzyme vs. Cell	R^2, Training Set	R^2, Test Set[a]	Descriptors used
GGPPS	SF-268	0.56	0.78	0.66	124

[a] Predicted values from leave-two-out cross validated models.

Table S5: Data collection and refinement statistics for BPH-461[a] bound to human FPPS.

(PDB: 2OPM)

Crystals	461B[a]
Data collection	
Space group	$P4_12_12$
Unit cell dimension (Å)	
a = *b*, *c* (Å)	111.783, 66.525
X-ray source	BNL-X29[b]
Wavelength (Å)	1.1
Resolution (Å)	30-2.40 (2.49-2.40)
No. of reflection observed	204,362
Unique	16,818 (1,525)
Completeness (%)	98.6 (92.0)
R-merge	0.100 (0.412)
$I/\sigma I$	42.0
Multiplicity	12.2 (9.2)
Refinement statistics	
Resolution range (Å)	30.0–2.40
R-work/*R*-free (%)	23.35/26.93
RMSD	
Bond lengths	0.004
Bond angles	1.532
No. of atoms	
Protein	2,708
Ligand	18
PO_4^{3-}	10
Magnesium ion	3
Solvent (water)	130
B average (Å^2) of protein	51.20
B average (Å^2) of solvents	56.58
B average (Å^2) of ligands(Bisphosphonates,Mg^{2+} and PO_4^{3-})	68.93

[a]: BPH-461is 3-fluoro-1-(2-hydroxy-2,2-bisphosphonoethyl)-pyridinium
[b]: Brookhaven National Laboratory

BPH-461

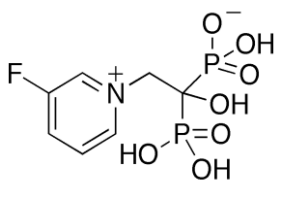


Table S6: Data collection and refinement statistics for BPH-461[a] bound to *T. brucei* FPPS (PDB: 3DYG).

Crystals	461B[a]
Data collection	
Space group	C2
Unit cell dimension (Å)	
a, b, c (Å)	135.565, 118.520, 63.186
β(°)	112.364
X-ray source	BNL-X8C[b]
Wavelength (Å)	1.1
Resolution (Å)	30-2.10 (2.18-2.10)
No. of reflection observed	406,549
Unique	53,536 (5,267)
Completeness (%)	99.1 (98.3)
R-merge	0.098 (0.530)
I/σI	28.0
Multiplicity	7.6 (7.6)
Refinement statistics	
Resolution range (Å)	30.0–2.10 (2.18-2.10)
R-work/*R*-free (%)	20.7/25.0 (25.8/33.0)
RMSD	
Bond lengths	0.008
Bond angles	1.24
No. of atoms	
Protein	5,745
Bisphosphonates	36
Magnesium ion	6
Solvent (water)	561
B average (Å^2) of protein	28.42
B average (Å^2) of solvents	27.60
B average (Å^2) of ligands(Bisphosphonates,Mg^{2+})	36.22

[a]: BPH-461is 3-fluoro-1-(2-hydroxy-2,2-bisphosphonoethyl)-pyridinium

[b]: Brookhaven National Laboratory

BPH-461

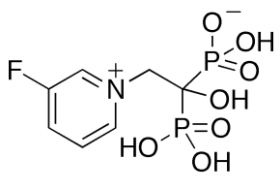
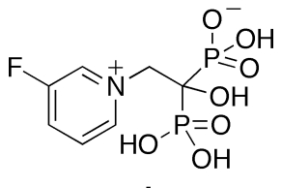


Table S7: Data collection and refinement statistics for BPH-461[a] and IPP bound to *T. brucei* FPPS. (PDB: 3DYF)

Crystals	461B[a]
Data collection	
Space group	C2
Unit cell dimension (Å)	
a,*b*,*c* (Å)	133.939, 117.898, 63.274
β(°)	112.364
X-ray source	BNL-X8C[b]
Wavelength (Å)	1.1
Resolution (Å)	30-2.65 (2.74-2.65)
No. of reflection observed	180,331
Unique	26,721 (2,681)
Completeness (%)	100.0 (100.0)
R-merge	0.135 (0.589)
$I/\sigma I$	52.7
Multiplicity	6.7 (6.5)
Refinement statistics	
Resolution range (Å)	30.0–2.65 (2.74-2.65)
R-work/*R*-free (%)	20.3/26.9 (28.7/34.0)
RMSD	
Bond lengths	0.006
Bond angles	1.10
No. of atoms	
Protein	5,733
Bisphosphonates	36
Magnesium ion	6
Solvent (water)	298
B average (Å^2) of protein	28.42
B average (Å^2) of solvents	27.60
B average (Å^2) of ligands(Bisphosphonates,Mg^{2+},IPP)	36.22

[a]: BPH-461is 3-fluoro-1-(2-hydroxy-2,2-bisphosphonoethyl)-pyridinium

[b]: Brookhaven National Laboratory



BPH-461

Table S8: Data collection and refinement statistics for BPH-721[a] bound to *T. brucei* FPPS. (PDB: 3DYH)

Crystals	721[a]
Data collection	
Space group	C2
Unit cell dimension (Å)	
a, *b*, *c* (Å)	133.915, 118.733, 63.036
β(°)	112.285
X-ray source	BNL-X29[b]
Wavelength (Å)	1.1
Resolution (Å)	30-1.95 (2.02-1.95)
No. of reflection observed	496,958
Unique	65,909(5,898)
Completeness (%)	98.8 (89.1)
R-merge	0.097 (0.509)
$I/\sigma I$	17.8
Multiplicity	7.5 (5.3)
Refinement statistics	
Resolution range (Å)	30.0–1.95 (2.02-1.95)
R-work/*R*-free (%)	23.9/28.1 (29.4/35.6)
RMSD	
Bond lengths	0.011
Bond angles	1.3
No. of atoms	
Protein	5,736
Bisphosphonates	42
Magnesium ion	6
Solvent (water)	349
B average (Å^2) of protein	45.80
B average (Å^2) of solvents	27.60
B average (Å^2) of ligands(Bisphosphonates,Mg^{2+})	30.60

[a]: BPH-721 is 2-(3-butoxypyridinium-1-yl)ethylidine-1,1-bisphosphonic acid

[b]: Brookhaven National Laboratory

BPH-721

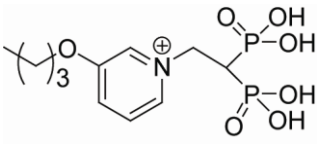


Table S9: Data collection and refinement statistics for BPH-722[a] bound to *T. brucei* FPPS (3EGT).

Crystals	722[a]
Data collection	
Space group	C2
Unit cell dimension (Å)	
a, *b*, *c* (Å)	133.227, 119.153, 62.957
β(°)	111.508
X-ray source	BNL-X29[b]
Wavelength (Å)	1.1
Resolution (Å)	30-2.00 (2.07-2.00)
No. of reflection observed	448,493
Unique	57,807(4,242)
Completeness (%)	94.0 (69.0)
R-merge	0.100 (0.518)
$I/\sigma I$	19.3
Multiplicity	7.8(5.1)
Refinement statistics	
Resolution range (Å)	30.0–2.00 (2.05-2.00)
R-work/*R*-free (%)	20.5/25.2 (29.0/33.0)
RMSD	
Bond lengths	0.011
Bond angles	1.3
No. of atoms	
Protein	5,736
Bisphosphonates	48
Magnesium ion	6
Solvent (water)	265
B average (Å^2) of protein	46.7
B average (Å^2) of solvents	47.79
B average (Å^2) of ligands(Bisphosphonates,Mg^{2+})	35.17

[a]: BPH-722 is 2-(3-heptyloxypyridinium-1-yl)ethylidine-1,1-bisphosphonic acid

[b]: Brookhaven National Laboratory

BPH-722

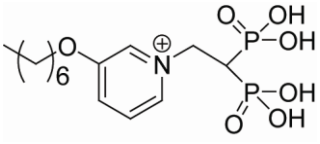


Table S10: Data collection and refinement statistics for BPH-714[a] bound to *T. brucei* FPPS (3EFQ).

Crystals	714[a]
Data collection	
Space group	C2
Unit cell dimension (Å)	
a, *b*, *c* (Å)	132.961, 119.157, 63.040
β(°)	111.383
X-ray source	BNL-X29[b]
Wavelength (Å)	1.1
Resolution (Å)	30-2.00 (2.07-2.00)
No. of reflection observed	465,810
Unique	58,529(4,662)
Completeness (%)	95.5 (75.8)
R-merge	0.099 (0.443)
$I/\sigma I$	17.8
Multiplicity	8.0(5.2)
Refinement statistics	
Resolution range (Å)	30.0–2.00 (2.05-2.00)
R-work/*R*-free (%)	21.1/24.8 (29.0/36.6)
RMSD	
Bond lengths	0.011
Bond angles	1.3
No. of atoms	
Protein	5,736
Bisphosphonates	50
Magnesium ion	6
Solvent (water)	256
B average (Å^2) of protein	43.15
B average (Å^2) of solvents	44.54
B average (Å^2) of ligands(Bisphosphonates,Mg^{2+})	33.84

[a]: BPH-714 is 2-(3-octyloxypyridinium-1-yl)ethylidine-1,1-bisphosphonic acid

[b]: Brookhaven National Laboratory

BPH-714

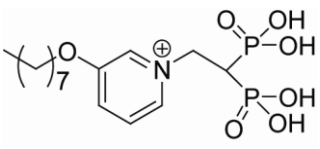


Table S11: Isothermal calorimetry results

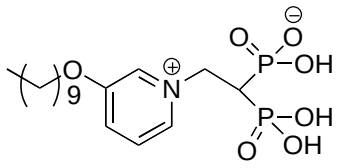
Cpd ID	ΔH (kcal/mol)	□ ΔS (cal/deg/mol)	ΔG (kcal/mol)
BPH-527	3.93	42.9	-8.9
BPH-536	4.01	43.1	-8.6
BPH-540	~0		
BPH-541	~0		
BPH-560	3.78	41.5	-8.5
BPH-571	2.49	36.5	-8.3
BPH-678	1.85	35.7	-8.7

Table S12: Data collection and refinement statistics for BPH-715[a] bound to *S. cerevisiae* GGPPS. (PDB: 2ZEU)

	BPH-715
Data collection	
Space group	$P2_12_12_1$
Unit cell dimension (Å)	
a, b, c (Å)	47.256 116.207 129.385
X-ray source	BL13B1[b]
Wavelength (Å)	1.0
Resolution (Å)	30-2.0 (2.07-2.0)
No. of reflection observed	376,311
Unique	48,744 (4,710)
Completeness (%)	99.4 (99.7)
R-merge	0.047 (0.383)
$I/\sigma I$	41.7
Multiplicity	7.7 (7.3)
Refinement statistics	
Resolution range (Å)	30-2.0
R-work/*R*-free (%)	18.92/23.61
RMSD	
Bond lengths	0.018
Bond angles	1.642
No. of atoms	
Protein	5025
Ligand	54
Magnesium ion	0
Solvent (water)	543
B average (Å^2) of protein	43.68
B average (Å^2) of solvents	58.32
B average (Å^2) of ligands (bisphosphonates)	68.65

[a]: BPH-715 is 2-(3-decyloxy-pyridinum-1-yl)-1,1-bisphosphonic acid

[b]: National Synchrotron Radiation Research Center (NSRRC, Hsinchu, Taiwan)



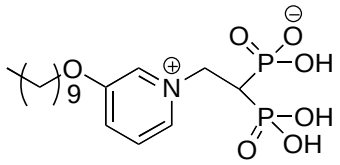
BPH-715

Table S13: Data collection and refinement statistics for BPH-715[a] bound to *S. cerevisiae* GGPPS. (PDB: 2ZEV)

	BPH-715 + IPP
Data collection	
Space group	$P2_12_12_1$
Unit cell dimension (Å)	
a, b, c (Å)	47.822 115.879 128.336
X-ray source	BL13B1[b]
Wavelength (Å)	1.0
Resolution (Å)	50-2.23 (2.31-2.23)
No. of reflection observed	207,561
Unique	33,587 (3,069)
Completeness (%)	93.3 (86.6)
R-merge	0.039 (0.452)
$I/\sigma I$	41.3
Multiplicity	6.2 (5.4)
Refinement statistics	
Resolution range (Å)	50.0–2.23
R-work/*R*-free (%)	19.99/26.55
RMSD	
Bond lengths	0.016
Bond angles	1.677
No. of atoms	
Protein	4,887
Ligand	68
Magnesium ion	4
Solvent (water)	452
B average (Å^2) of protein	46.42
B average (Å^2) of solvents	57.92
B average (Å^2) of ligands (bisphosphonates, Mg^{2+}, IPP)	69.25

[a]: BPH-715 is 2-(3-decyloxy-pyridinum-1-yl)-1,1-bisphosphonic acid

[b]: National Synchrotron Radiation Research Center (NSRRC, Hsinchu, Taiwan)



BPH-715

Supplementary Figures

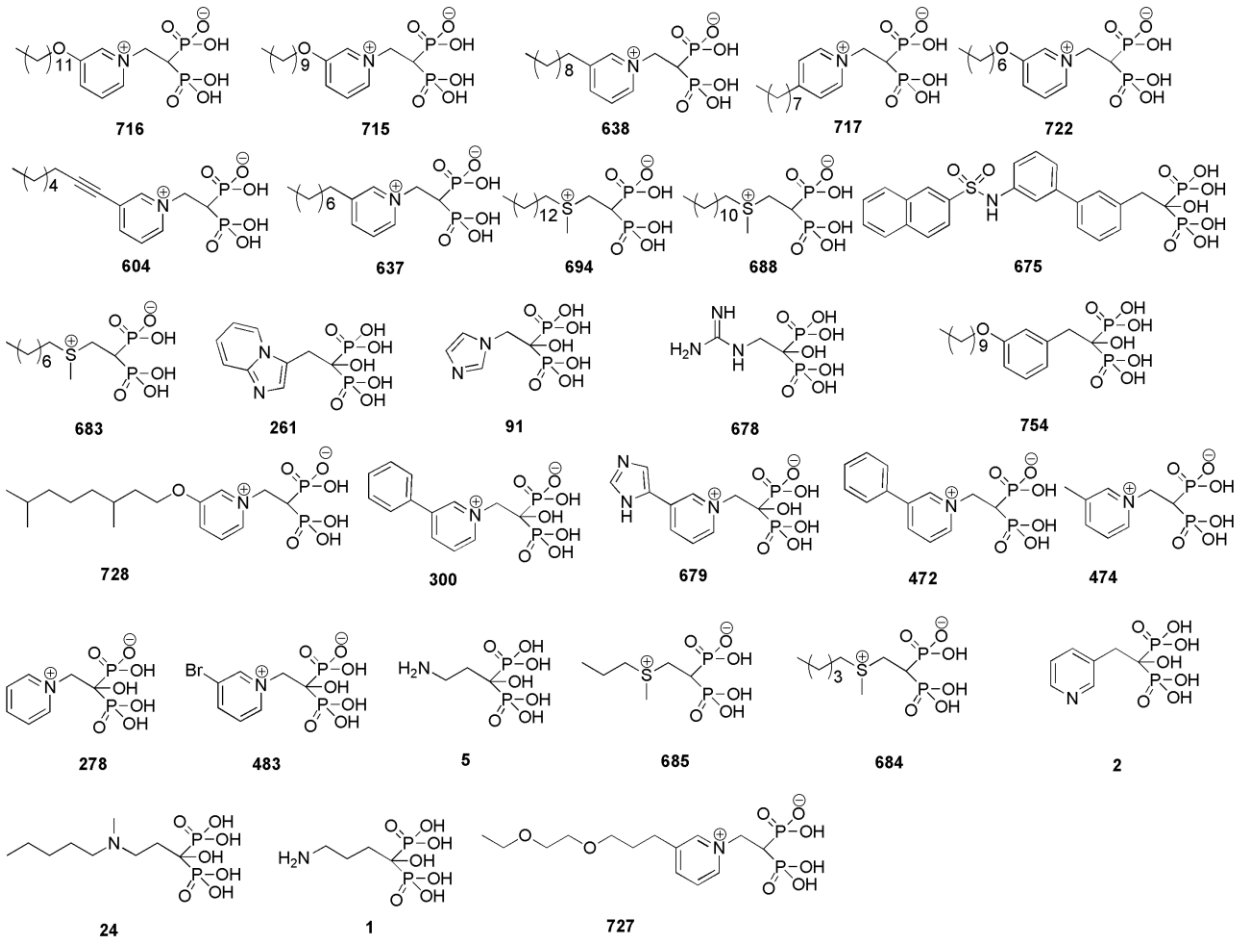


Figure S1: Structures of BPH-series of inhibitors investigated in cell growth inhibition, FPPS and GGPPS enzyme inhibition. Rank ordered in terms of decreasing potency in NCI-H460 cell growth inhibition (see Supporting Table S1).

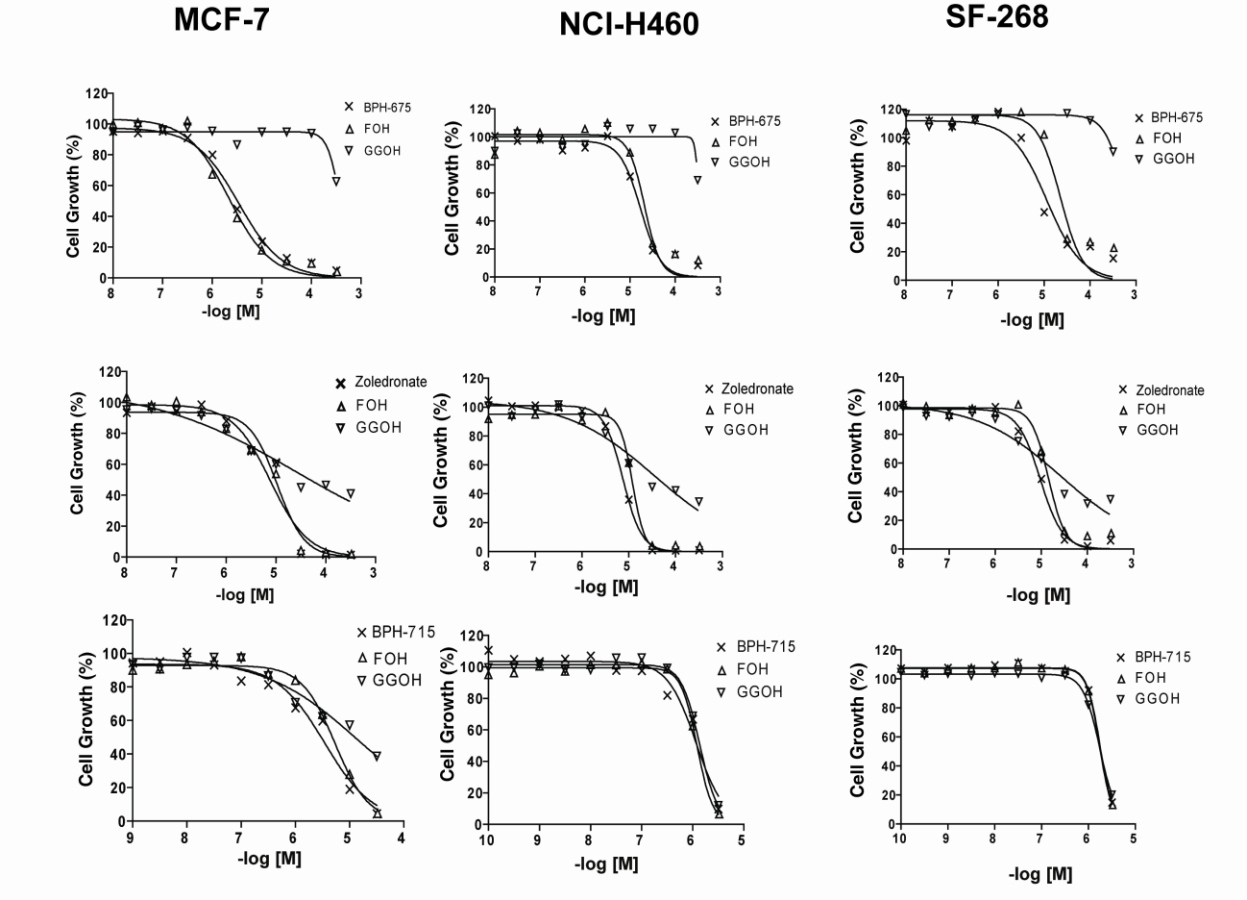


Figure S2: Rescue of cell growth inhibition for all three cell lines; BPH-675, zoledronate and BPH-715 inhibitors; 20 μM FOH (Δ) or GGOH ( ) concentrations.

Supporting Experimental Information

NMR sample preparation

T. brucei FPPS was expressed in BL21 (DE3) *E. coli* and purified as described previousy[1]. Microcrystalline samples for solid state NMR were prepared by serially adding bisphosphonate, IPP and Mg^{2+} in a ratio of 1:1:3. Typically, the starting FPPS protein concentration was between 10 to 20 mg/mL, and dilution during ligand addition was kept to a minimum. After incubating for 1-2 hrs, precipitation of crystalline protein[2] was induced by slowly adding first, β-mercaptoethanol (10 mM), then PEG-3350, to a maximum concentration of 20%. The solution was mixed gently and kept overnight until a white precipitate formed. The sample was transferred into a sealed 1 mL Eppendorf tube and then centrifuged at 3220g for 30 min and subsequently transferred into a 3.2 mm NMR rotor by centrifugation (3220g for 30 minutes). In some cases, precipitation was induced by adding Mg^{2+} to 2:2:9 (though this was rare). Typically, microcrystals precipitated out at ~ 10% PEG; at 20% or more PEG, a gel formed that was difficult to pellet. It is best to start with high protein concentration of at least 500 μM volume, and to use concentrated stock solutions of bisphosphonates, IPP, Mg^{2+}, so that the protein solution is not diluted.

1. Mao, J.; Gao, Y.G.; Odeh, S.; Robinson, H.; Montalvetti, A.; Docampo, R.; Oldfield, E. *Acta Crystallogr D Biol Crystallogr* **2004**, *60*, 1863-6.

2. Martin, R.W.; Zilm, K.M. *J. Mag. Res.* **2003**, *165*, 162-174.

Complete ref 5 from the article:

(5) Gnant, M.; Mlineritsch, B.; Schippinger, W.; Luschin-Ebengreuth, G.; Postlberger, S.; Menzel, C.; Jakesz, R.; Seifert, M.; Hubalek, M.; Bjelic-Radisic, V.; Samonigg, H.; Tausch, C.; Eidtmann, H.; Steger, G.; Kwasny, W.; Dubsky, P.; Fridrik, M.; Fitzal, F.; Stierer, M.; Rucklinger, E.; Greil, R.; Marth, C. *N. Engl. J. Med.* **2009**, *360*, 679-91.