Supporting Information for the manuscript entitled:

Dynamic Structure and Inhibition of a Malaria Drug Target: Geranylgeranyl Diphosphate Synthase

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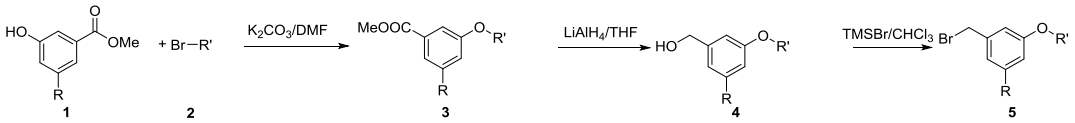
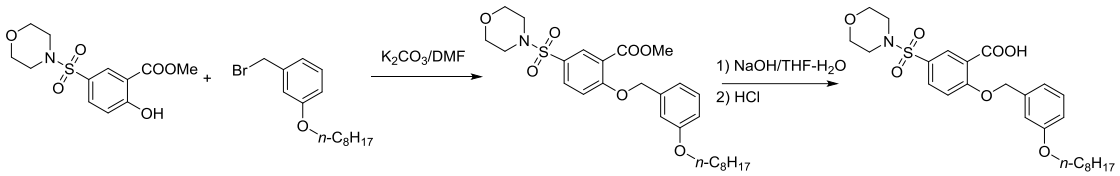
Scheme 1. Benzoic acids: General synthesis procedure for benzyl bromides.



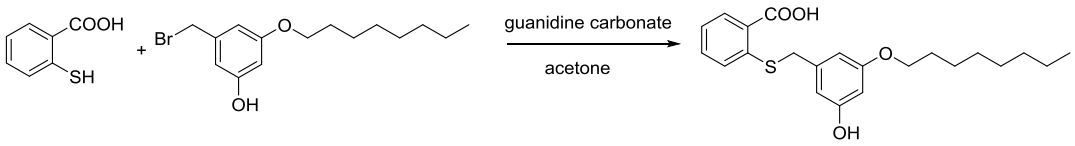
A mixture of methyl benzoate (**1**, 2 mmol), alkyl bromide (**2**, 2 mmol) and K_2CO_3 (0.28 g, 2 mmol) in DMF (5 mL) was stirred overnight. Ethyl acetate (20 mL) was added and the mixture washed with water (5 mL). The organic phase was dried and solvent removed under reduced pressure. The residue was then subjected to flash column chromatography to give **3** in almost quantitative yield. To a solution of $LiAlH_4$ (114 mg, 3 mmol) in anhydrous THF (10 mL) at 0 °C was slowly added **3** (1 mmol) and the mixture was then stirred at room temperature until **3** disappeared (~3 hrs). Workup with aqueous ammonia and then column chromatography afforded **4**. Treatment of **4** with 3 equivalents of TMSBr gave the title compound **5** after flash column chromatograpic separation.

Scheme 2. 5-morphinesulfamoyl-2-(3-(octyloxy)benzyloxy) benzoic acid (BPH-1158).



A mixture of methyl 2-hydroxy-5-(morpholinosulfonyl) benzoate (300 mg, 1 mmol), 1-(bromomethyl)-3-(octyloxy) benzene (300 mg, 1 mmol) and potassium carbonate (270 mg, mmol) was stirred in DMF (5 mL) overnight. Ethyl acetate (30 mL) was added and the mixture washed with water (5mL) and then concentrated under vacuum. After flash column chromatograpic separation (hexane/ethyl acetate, 5:1, v/v) the product was concentrated and then hydrolyzed using 1.2 equivalents of NaOH in THF-H_2O (1:1 v/v, 3 mL), followed by acidification with 1N HCl (3 mL) to afford the crude product. Further chromatograpic separation (hexane/ethyl acetate, 1:2, v/v) afforded the product as a white solid (BPH-1158, 350 mg, 70% yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.94 (d, J = 3 Hz, 1 H), 7.84 (dd, J = 3, 8.5 Hz, 1 H), 7.43 (d, J = 8.5 Hz, 1 H), 7.28 (t, J = 8.5 Hz, 1 H), 7.10 (s, 1 H), 7.02 (d, J = 8.5 Hz, 1 H), 6.86 (s, 1 H), 5,28 (s, 2 H), 3.95 (t, J = 6.5 Hz, 2 H), 3.62 (t, J = 4.5 Hz, 2 H), 2.84 (t, J = 4.5 Hz, 2 H), 1.69 (m, 2 H), 1.39 (m, 2 H), 1.26 (m, 8 H), 0.84 (t, J = 6.5 Hz, 3 H) HRMS (ESI): m/z $[M+H]^+$ calculated for $C_{26}H_{35}NO_7SNa^+$: 528.2032, found: 528.2029. Purity of the product determined by HPLC-MS (Phenomenex C6-Phenyl 110A. 100x2 mm, 210 nm, retention time = 7.9 min): 98.9%.

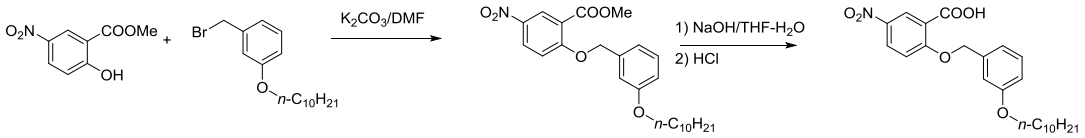
Scheme 3. 2-((3-hydroxy-5-(octyloxy)benzyl)sulfanyl)benzoic acid (BPH-1182).



A mixture of thiosalicyclic acid (150 mg, 1 mmol), guanidine carbonate (180 mg, 2 mmol) and 3-(bromomethyl)-5-(octyloxy) phenol (320 mg, 1 mmol) in acetone (5 mL) was stirred overnight at room temperature. The solid was collected and washed with 1N HCl (5 mL) and then water (5 mL). Crystallization

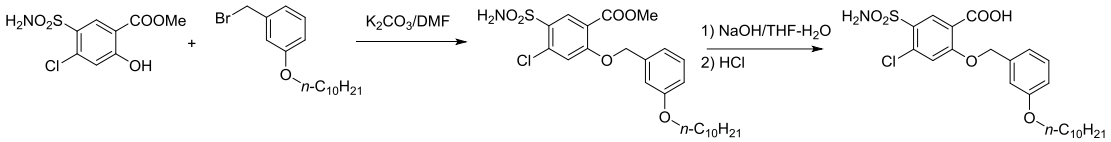
from acetone afforded the substituted thiosalicyclic acid as a solid (BPH-1182, 213 mg, 55 % yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 9.41 (s, 1 H), 7.87 (d, *J* = 10 Hz, 1 H), 7.48 (t, *J* = 7.5 Hz, 1 H), 7.42 (d, *J* = 10 Hz, 1 H), 7.18 (d, *J* = 7.5 Hz, 1 H), 6.42 (d, *J* = 5 Hz, 2 H), 6.19 (s, 1 H), 4.05 (s, 2 H), 3.85 (t, *J* = 6.5 Hz, 2 H), 1.65 (m, 2 H), 1.36 (m, 2 H), 1.24 (m, 8 H), 0.84 (t, *J* = 6.5 Hz, 3 H) ppm. HRMS (ESI): m/z $[M+H]^+$ calculated for $C_{22}H_{28}O_4NaS^+$: 411.1606, found: 411.1606. Purity of the product determined by HPLC-MS (Phenomenex C6-Phenyl 110A. 100x2 mm, 210 nm, retention time = 7.4 min): 98.5%.

Scheme 4. 2-(3-(decyloxy)benzyloxy)-5-nitrobenzoic acid (BPH-1186).



A mixture of methyl 2-hydroxy-5-nitrobenzoate (200 mg, 1 mmol), 1-(bromomethyl)-3-(decyloxy)benzene (320 mg, 1 mmol) and potassium carbonate (270 mg, mmol) was stirred in DMF (5 mL) overnight. Ethyl acetate (30 mL) was added and the mixture washed with water (5mL), dried, and then concentrated under vacuum. After flash column column separation (hexane/ethyl acetate, 5:1, v/v) and concentration the residue was hydrolyzed using 1.2 equivalents of NaOH in THF-H_2O (1:1 v/v, 3 mL), followed by acidification with 1N HCl (3 mL) to afford the crude product. Further chromatographic separation (hexane/ethyl acetate, 1:2, v/v) afforded the product as a white solid (BPH-1186, 300 mg, 72% yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 8.46 (d, *J* = 3 Hz, 1 H), 8.36 (dd, *J* = 3, 8.5 Hz, 1 H), 7.40 (d, *J* = 8.5 Hz, 1 H), 7.28 (t, *J* = 8.5 Hz, 1 H), 7.09 (s, 1 H), 7.01 (d, *J* = 8.5 Hz, 1 H), 6.86 (s, 1 H), 5,34 (s, 2 H), 3.94 (t, *J* = 6.5 Hz, 2 H), 1.68 (m, 2 H), 1.37 (m, 2 H), 1.24 (m, 12 H), 0.84 (t, *J* = 6.5 Hz, 3 H) ppm. HRMS (ESI): m/z $[M+H]^+$ calculated for $C_{24}H_{31}NO_6Na^+$: 452.2042, found: 452.2049. Purity of the product determined by HPLC-MS (Phenomenex C6-Phenyl 110A. 100x2 mm, 210 nm, retention time = 8.2 min): 99.2%.

Scheme 5. 4-chloro-2-((3-(decyloxy)-5-hydroxybenzyl)oxy)-5-sulfamoylbenzoic acid (BPH-1251).



A mixture of methyl 4-chloro-2-hydroxy-5-sulfamoylbenzoate (250 mg, 1 mmol), 1-(bromomethyl)-3-(decyloxy) benzene (320 mg, 1 mmol) and potassium carbonate (270 mg, mmol) was stirred in DMF (5 mL) overnight. Ethyl acetate (30 mL) was added and the mixture washed with water (5mL), dried, and then concentrated under vacuum. After flash column column separation (hexane/ethyl acetate, 5:1, v/v) and concentration the residue was hydrolyzed using 1.2 equivalents of NaOH in THF-H_2O (1:1 v/v, 3 mL), followed by acidification with 1N HCl (3 mL) to afford the crude product. Further chromatographic separation (hexane/ethyl acetate, 1:2, v/v) yielded the product as a white solid (BPH-1251, 330 mg, 65% yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ (ppm): 9.46 (s, 1 H), 8.27 (s, 1 H), 7.57 (s, 2 H), 7.44 (s, 1 H), 6.53 (s, 1 H), 6.41 (s, 1 H), 6.23 (s, 1 H), 5.20 (s, 2 H), 3.87 (t, *J* = 6.5 Hz, 2 H), 1.66 (m, 2 H), 1.36 (m, 2 H), 1.24 (m, 8 H), 0.84 (t, *J* = 6.5 Hz, 3 H) ppm. HRMS (ESI): m/z $[M+H]^+$ calculated for $C_{24}H_{32}O_7ClSN^+$: 513.1588, found: 513.1585. Purity of the product determined by HPLC-MS (Phenomenex C6-Phenyl 110A. 100x2 mm, 210 nm, retention time = 7.5 min): 99.5%.

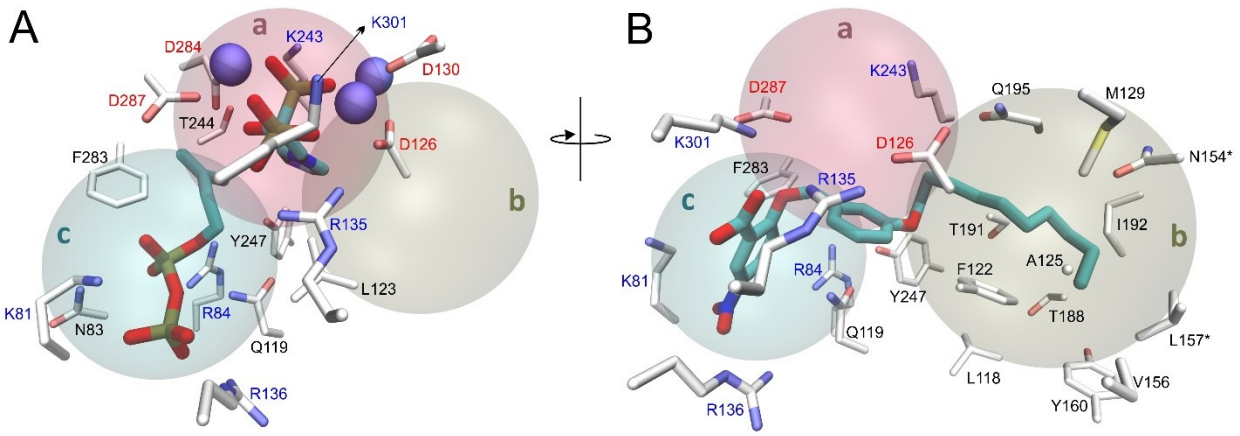
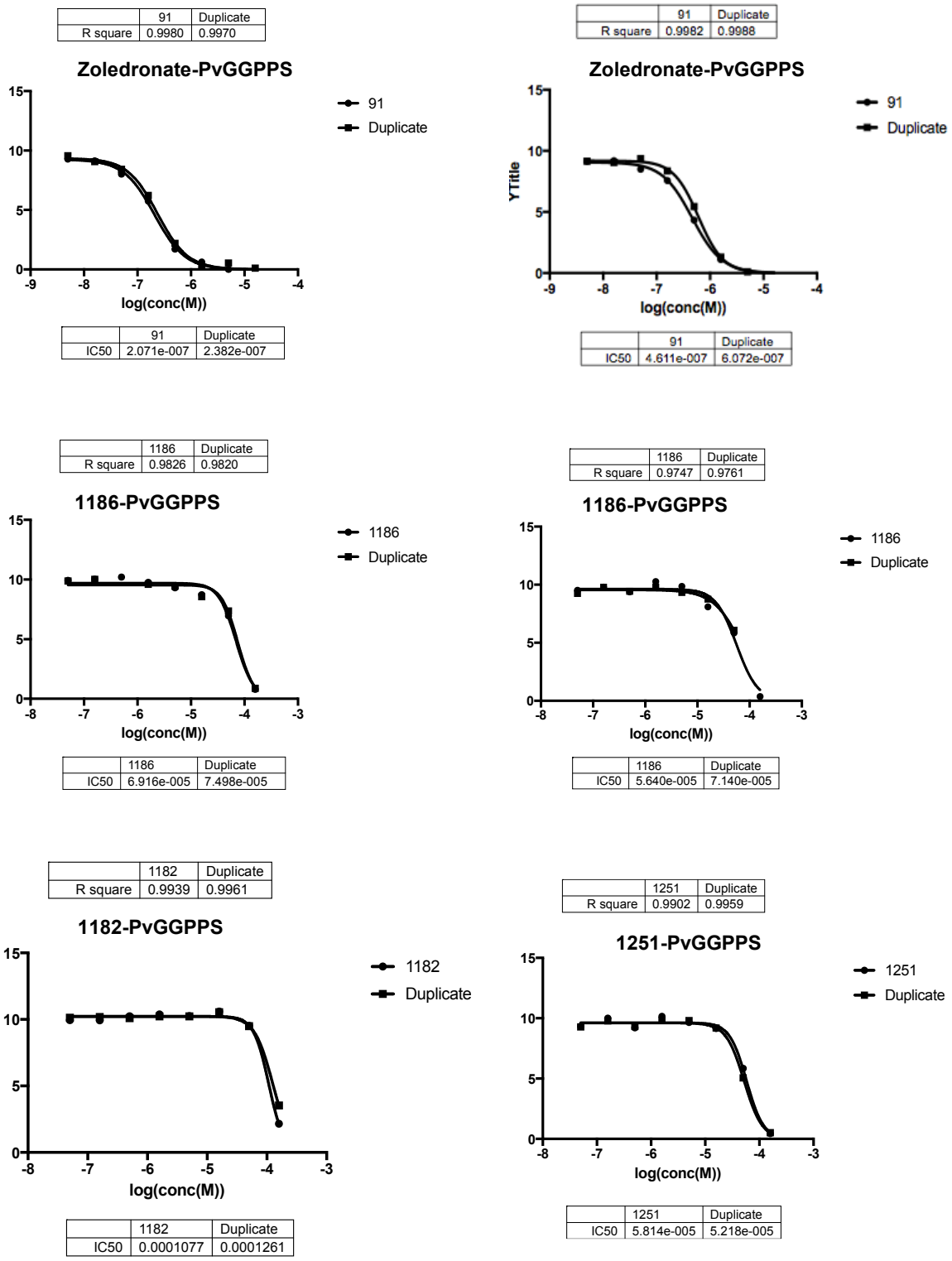


Figure S1. PvGGPPS pockets, as defined by side-chains located at less than 3.5 Å from the ligands. A) Residues forming pockets **a** and **c**, occupied by zoledronate and IPP (substrate-like binding mode). B) Residues forming pockets **b** and **c**, occupied by BPH1186 (product-like binding mode). Mg^{2+} ions in purple.



	91	Duplicate
R square	0.9980	0.9970

	91	Duplicate
IC50	2.071e-007	2.382e-007

	91	Duplicate
R square	0.9982	0.9988

	91	Duplicate
IC50	4.611e-007	6.072e-007

	1186	Duplicate
R square	0.9826	0.9820

	1186	Duplicate
IC50	6.916e-005	7.498e-005

	1186	Duplicate
R square	0.9747	0.9761

	1186	Duplicate
IC50	5.640e-005	7.140e-005

	1182	Duplicate
R square	0.9939	0.9961

	1182	Duplicate
IC50	0.0001077	0.0001261

	1251	Duplicate
R square	0.9902	0.9959

	1251	Duplicate
IC50	5.814e-005	5.218e-005

Figure S2. Representative dose-response curves for PvGGPPS inhibition by bisphosphonates.

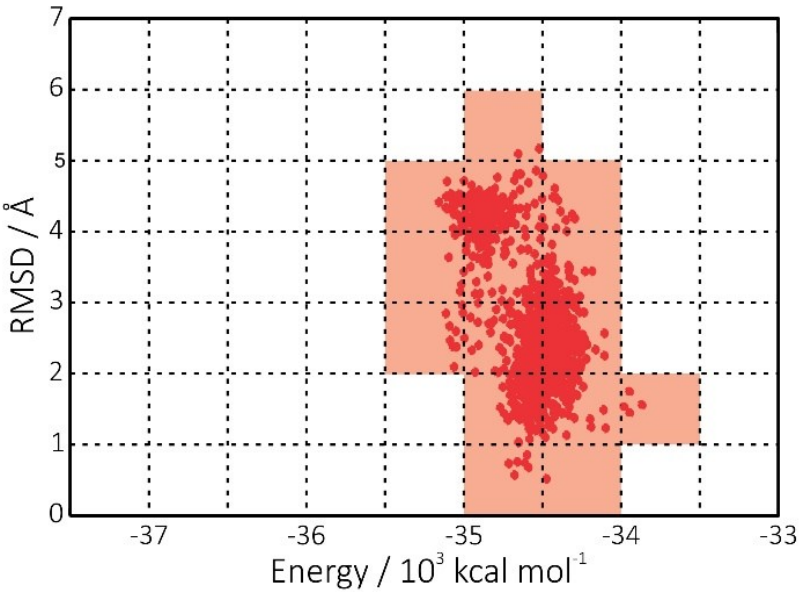


Figure S3. Estimating the conformational entropy associated with ligand mobility. The number of thermodynamically relevant microstates accessible to each ligand is obtained by dividing the colony space in a 2D grid and counting the number of bins that are occupied by at least one dot (pink bins). The distribution of dots corresponds to snapshots of the MD simulations.

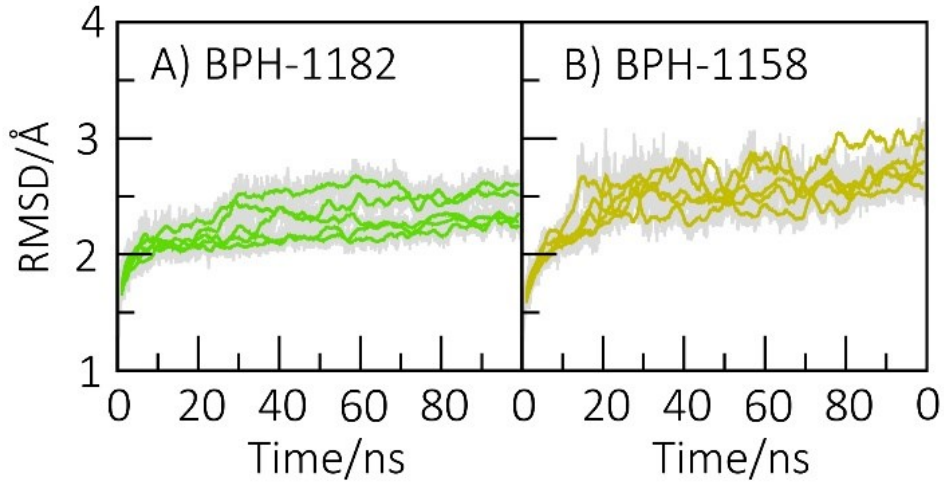


Figure S4. Mobility (RMSD) of GGPPS bound to BPH-1182 (A) and BPH-1158 (B).

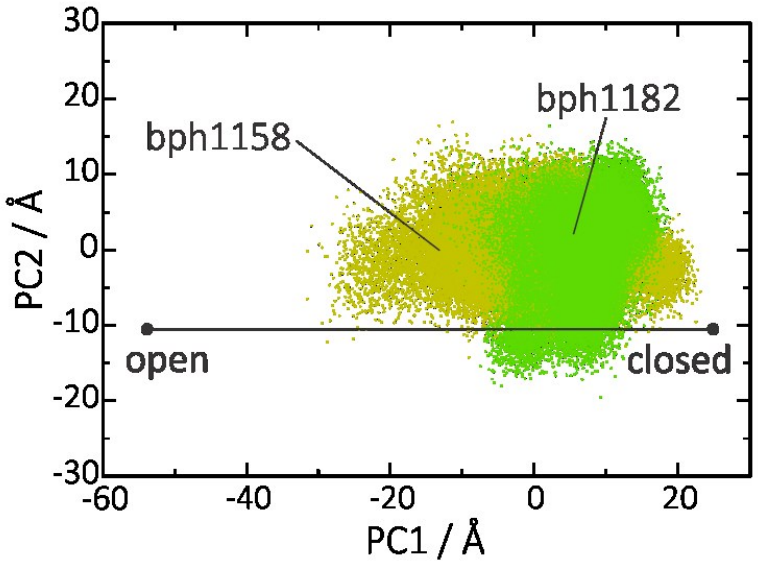


Figure S5. PCA analysis of benzoic acid inhibitors BPH-1182 (light green) and BPH-1158 (yellow). Open and closed conformations refer to the position of loop 9-10.

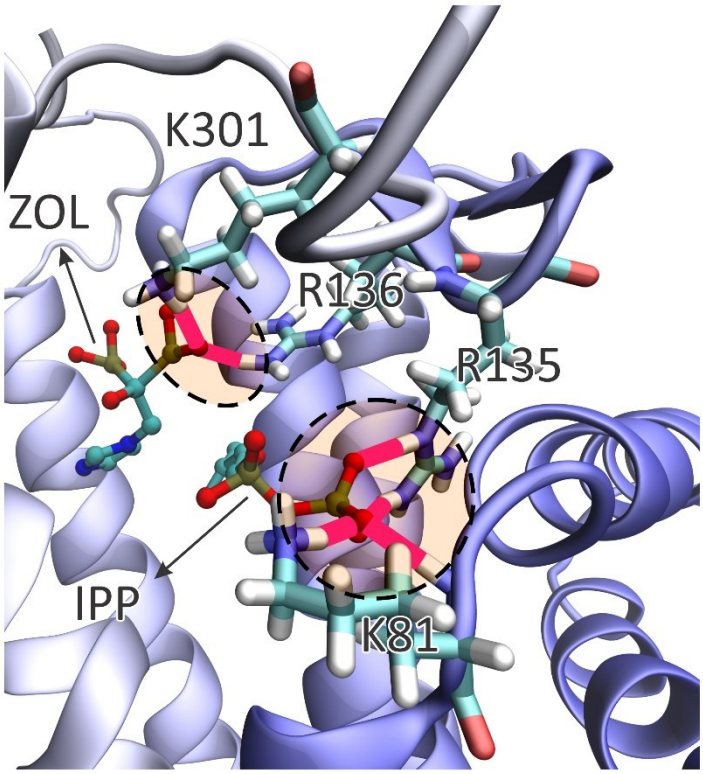


Figure S6. Hydrogen interactions (in pink) persistently formed in PvGGPPS complexed with Zoledronate (ZOL) and IPP.

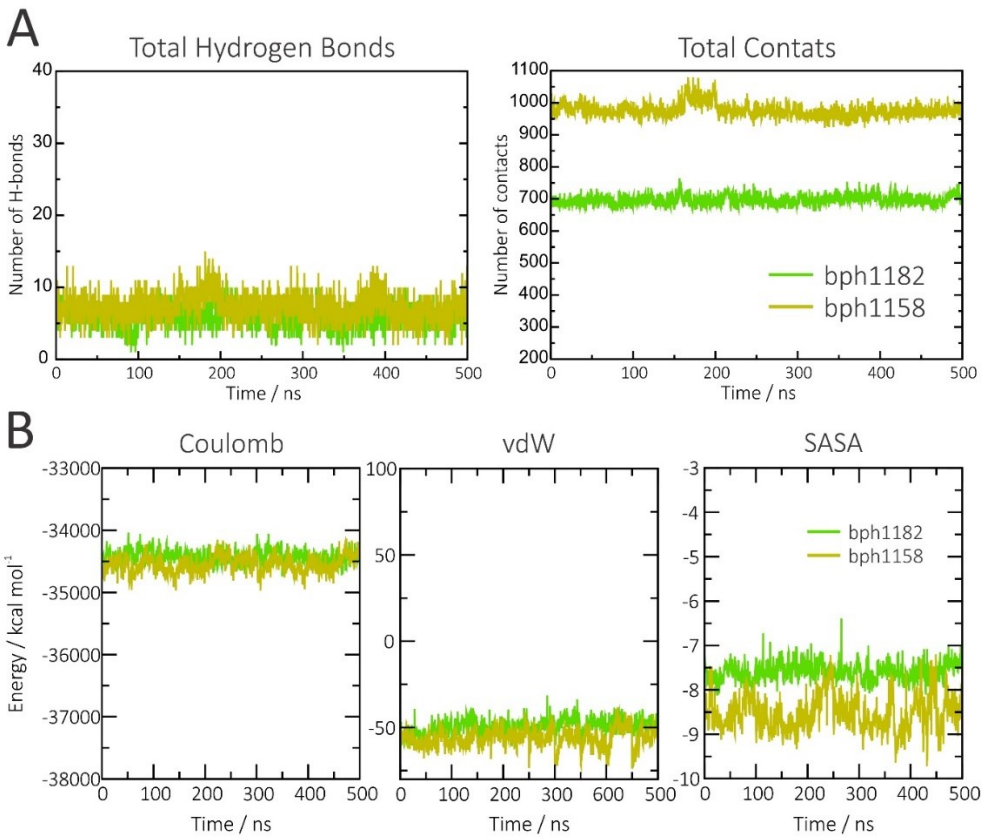


Figure S7. Interactions and energetics of benzoic acid inhibitors BPH-1182 (light green) and BPH-1158 (yellow). A, Total number of hydrogen bond interactions, and contacts, formed between benzoic inhibitors and GGPPS. A contact is formed whenever the inhibitor is less than 7Å from a protein atom. B, Coulombic, van der Waals and hydrophobic interaction energies (solvent-accessible surface area, SASA) computed for each ligand.

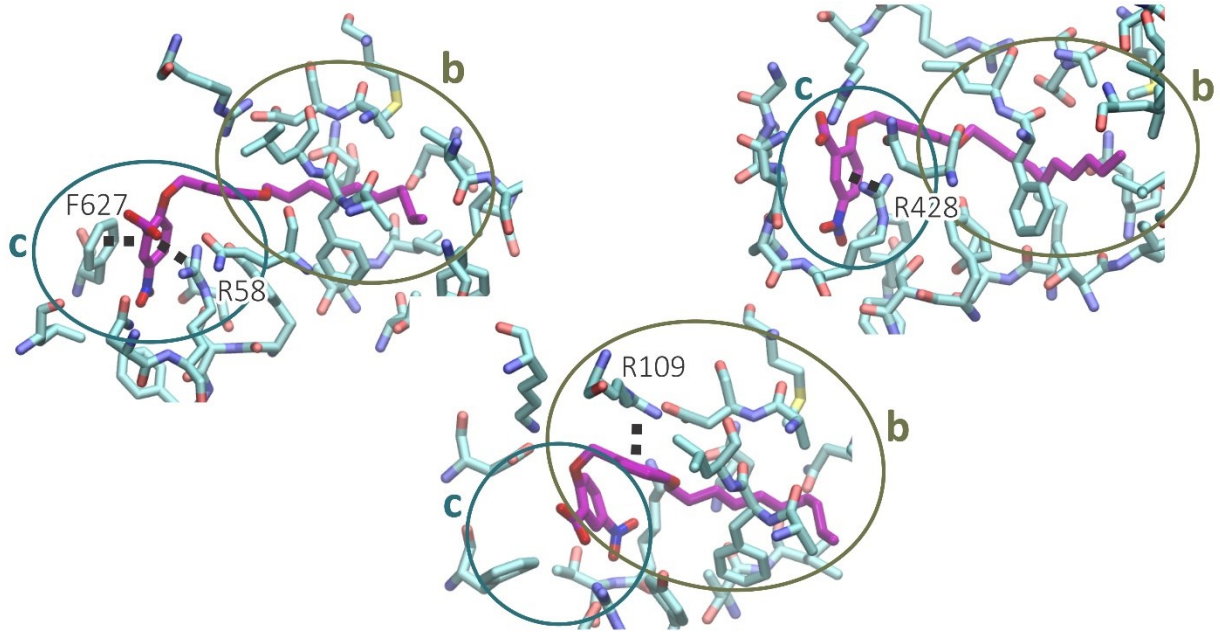


Figure S8. Examples of transient π-stacking interactions displayed by benzoic inhibitors during the MD simulations. Apart from hydrophobic interactions in the **b** pocket, benzoate and benzene rings of BPH-1186 form transient π-stacking interactions with aromatic and arginine side-chains from pocket **c**.

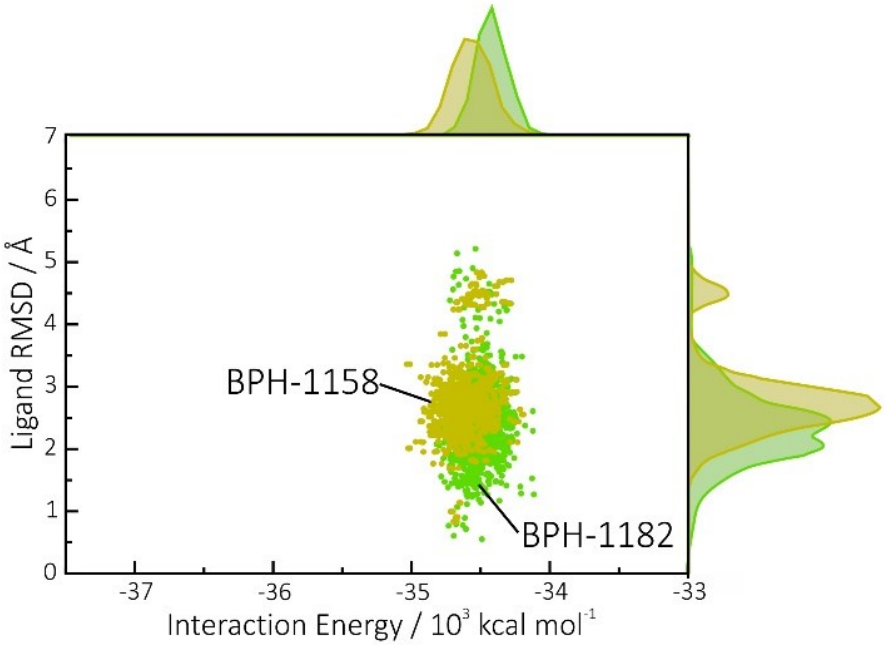


Figure S9. Conformational entropy of BPH-1182 (light green) and BPH-1158 (yellow). Projection of the simulations on the conformational colony space provides an estimate of the number of thermodynamically relevant microstates, related to entropic gain.

Table S1. Conformational entropy of ligands in the bound state.

Ligand	N	$ln(N)$	S_{conf} ($cal\ mol^{-1}\ K^{-1}$)
Zoledronate	27	3.30	6.55
BPH-703	21	3.04	6.03
GGPP	31	3.43	6.81
BPH-1186	50	3.91	7.76