Supporting Information

Origins of the Specificity of Inhibitor P218 towards Wild-Type and Mutant *PfDHFR* : A Molecular Dynamics Analysis

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I. Binding mode analysis of WR99210 in the active-site of wild-type and quadruple-mutant PfDHFR.

Plasmodium falciparum DHFR has developed resistance to all known antifolate drugs such as pyrimethamine and cycloguanil. Mutation at position 108 from Ser to Asn is reported to be the main cause of resistance developed by *Pf*DHFR. Mutated residue Asn108 cause steric clash with the antifolate drugs (pyrimethamine and cycloguanil), hence these drugs are not able to bind efficiently in the active-sites of mutant *Pf*DHFR. WR99210 is a lead molecule known to bind efficiently to both wild-type and multiple-resistant *Pf*DHFR enzyme. It binds in a similar fashion to both wild-type and quadruple-mutant *Pf*DHFR (Asn51Ile, Cys59Arg, Ser108Asn and Ile164Leu). WR99210 is designed such that it has a flexible linker chain by which it avoids any steric clash with Asn108 in the mutant *Pf*DHFR. Binding pose analysis shows that it interacts with Ile14, Asp54 and Ile164 to form H-bond interaction and orients in a way such that it avoids any steric hindrance due to Ser to Asn mutation at position 108 in *Pf*DHFR (Fig. S1).

Figure S1: WR99210 in the active sites of wild-type (A) and quadruple-mutant *Pf*DHFR (B). The active-site residues that form H-bonds with WR99210 are shown. Ser108 and Asn108 are mentioned to depict that WR99210 binding is not affected by Ser108Asn mutation.

II. The energy minimized and bioactive conformations of P218

For the purpose of molecular docking exercise P218 structure was built using sketch module of SYBYL7.1 and subjected to 1000 cycle minimization with standard Tripos force field and 0.005 kcal/mol energy gradient convergence criterion using Powell's method. The 3D

structure of P218 obtained from the above mentioned exercise adopts a linear structure such that the hydrophobic regions C_6H_4 and C_2H_5 exist in the opposite directions, whereas, the bioactive conformation of P218 (PDB code: 4DP3) adopts a bent shape bringing the C_2H_5 and C_6H_4 groups into close proximity such that hydrophobic features bind in the hydrophobic cavity of *Pf*DHFR.

Figure S2: The energy minimized and the bioactive conformation of P218.

There are total of 10 rotatable bonds which contribute to the various possible conformations of P218. A molecular mechanics based systematic conformational search followed by energy minimization resulted in total 257 conformations. The relative energies of all these conformations with respect to bioactive conformation are given in Table S1. Out of these the most stable conformation adopts an almost U-shaped arrangement, similar to the bioactive conformation. The energy difference between these two is estimated to be about 8.5 kcal/mol. Further, quantum chemical analysis has been carried out on three P218 conformations (bioactive conformation, most stable P218 conformation and linear conformation) using B3LYP/6-311+G(d) method. The relative energy for most stable conformation and linear conformation as compared to the bioactive conformation is estimated to be -3.15 and 3.03 kcal/mol. The most stable P218 conformation adopts a boat-shaped conformation of macrocycle (P218) and intramolecular hydrogen bonding, whereas, the bioactive conformation adopts a chair-shaped conformation of macrocycle and intramolecular hydrogen bond. These intramolecular hydrogen bonds may not be favoured under solvent

conditions. Hence, it can be envisaged that P218 undergoes conformational change in the active-site of *Pf*DHFR to adopt its bioactive conformation and bind stably to the enzyme.

Table S1: Relative energies and corresponding torsion angles for P218 conformations, as obtained using molecular mechanics method.

Conf.	Relative energy	Angle1	Angle2	Angle3	Angle4	Angle5	Angle6	Angle7	Angle8	Angle9	Angle10
1 ^a	0.00	285.48	167.16	99.93	12.17	203.94	26.17	170.02	177.76	359.35	91.31
2	45.50	289.65	166.61	218.59	14.27	206.15	140.78	170.01	57.15	359.51	329.82
3	64.75	49.65	46.61	218.59	254.27	326.15	20.78	50.01	177.15	359.51	329.82
4	-2.09	49.65	166.61	218.59	254.27	206.15	20.78	170.01	57.15	359.51	89.82
5	27.50	49.65	166.61	218.59	254.27	326.15	260.78	50.01	57.15	239.51	89.82
6	35.68	289.65	46.61	218.59	134.27	86.15	20.78	290.01	177.15	119.51	209.82
7	65.30	289.65	166.61	218.59	134.27	326.15	20.78	50.01	297.15	119.51	329.82
8	70.57	289.65	166.61	338.59	254.27	326.15	20.78	50.01	57.15	359.51	329.82
9	53.08	289.65	46.61	218.59	254.27	86.15	20.78	290.01	297.15	239.51	329.82
10	51.43	289.65	166.61	98.59	14.27	206.15	140.78	170.01	177.15	359.51	329.82
11	39.61	289.65	166.61	218.59	134.27	206.15	140.78	170.01	297.15	119.51	329.82
12	42.31	49.65	46.61	218.59	134.27	86.15	260.78	290.01	297.15	239.51	89.82
13	36.89	49.65	166.61	98.59	254.27	326.15	20.78	50.01	57.15	119.51	89.82
14	77.59	169.65	166.61	338.59	254.27	206.15	140.78	170.01	57.15	239.51	209.82
15	32.85	49.65	166.61	218.59	134.27	326.15	20.78	170.01	177.15	239.51	209.82
16	4.94	49.65	166.61	218.59	254.27	86.15	20.78	290.01	297.15	239.51	329.82
17	56.89	289.65	286.61	218.59	14.27	86.15	20.78	50.01	57.15	119.51	89.82
18	69.66	169.65	166.61	338.59	254.27	326.15	260.78	170.01	57.15	239.51	209.82
19	28.97	289.65	286.61	98.59	134.27	206.15	20.78	170.01	57.15	119.51	89.82
20	52.30	289.65	166.61	218.59	254.27	326.15	140.78	170.01	177.15	119.51	329.82
21	65.40	169.65	286.61	218.59	134.27	206.15	260.78	50.01	57.15	239.51	209.82
22	47.44	169.65	166.61	338.59	134.27	206.15	20.78	290.01	57.15	359.51	89.82
23	69.01	289.65	286.61	98.59	134.27	326.15	260.78	50.01	297.15	239.51	89.82
24	72.38	49.65	46.61	218.59	134.27	86.15	140.78	170.01	57.15	119.51	89.82
25	81.76	289.65	166.61	338.59	134.27	86.15	140.78	170.01	177.15	239.51	329.82

26	63.69	49.65	166.61	218.59	134.27	326.15	140.78	290.01	177.15	119.51	209.82
27	83.20	289.65	166.61	218.59	134.27	86.15	20.78	170.01	57.15	119.51	329.82
28	77.92	49.65	166.61	98.59	134.27	326.15	140.78	290.01	57.15	359.51	89.82
29	40.71	49.65	46.61	98.59	254.27	206.15	260.78	50.01	57.15	359.51	329.82
30	69.12	289.65	166.61	98.59	14.27	86.15	20.78	170.01	297.15	119.51	329.82
31	-6.72	289.65	166.61	218.59	254.27	206.15	20.78	290.01	297.15	119.51	89.82
32	70.13	49.65	166.61	338.59	254.27	206.15	260.78	170.01	177.15	239.51	329.82
33	65.11	169.65	166.61	338.59	254.27	86.15	260.78	170.01	297.15	239.51	209.82
34	20.91	169.65	166.61	338.59	134.27	206.15	20.78	170.01	177.15	119.51	89.82
35	67.06	289.65	286.61	218.59	254.27	326.15	260.78	170.01	57.15	239.51	209.82
36	53.83	49.65	46.61	98.59	134.27	326.15	20.78	170.01	177.15	239.51	329.82
37	51.17	49.65	166.61	338.59	254.27	326.15	20.78	290.01	177.15	359.51	209.82
38	71.99	49.65	46.61	338.59	134.27	86.15	20.78	170.01	57.15	239.51	329.82
39	76.49	169.65	166.61	218.59	134.27	326.15	140.78	170.01	57.15	239.51	89.82
40	59.14	289.65	286.61	98.59	14.27	206.15	140.78	290.01	57.15	239.51	209.82
41	69.84	49.65	286.61	218.59	134.27	206.15	20.78	290.01	297.15	239.51	209.82
42	36.37	289.65	166.61	218.59	254.27	326.15	140.78	290.01	297.15	239.51	209.82
43	-6.02	289.65	166.61	218.59	134.27	206.15	20.78	290.01	177.15	359.51	89.82
44	30.71	49.65	46.61	98.59	134.27	206.15	260.78	170.01	297.15	359.51	209.82
45	16.35	289.65	166.61	338.59	254.27	206.15	20.78	170.01	297.15	239.51	209.82
46	39.33	169.65	166.61	98.59	254.27	206.15	20.78	170.01	177.15	239.51	209.82
47	78.81	169.65	166.61	218.59	254.27	206.15	260.78	290.01	177.15	119.51	209.82
48	37.62	289.65	166.61	98.59	254.27	326.15	20.78	290.01	297.15	239.51	89.82
49	59.48	169.65	166.61	338.59	254.27	326.15	260.78	50.01	57.15	239.51	209.82
50	15.22	49.65	166.61	218.59	14.27	206.15	20.78	170.01	57.15	359.51	329.82
51	18.23	49.65	166.61	98.59	14.27	206.15	260.78	290.01	177.15	359.51	89.82
52	53.64	49.65	166.61	338.59	254.27	326.15	20.78	50.01	57.15	359.51	209.82
53	26.69	289.65	166.61	218.59	134.27	206.15	260.78	170.01	177.15	119.51	89.82
54	45.43	49.65	46.61	218.59	134.27	206.15	260.78	170.01	297.15	359.51	329.82
55	79.46	169.65	166.61	218.59	14.27	86.15	140.78	170.01	297.15	119.51	89.82
56	70.25	49.65	46.61	218.59	254.27	326.15	20.78	170.01	57.15	359.51	329.82
57	56.11	169.65	166.61	218.59	14.27	206.15	260.78	170.01	57.15	239.51	89.82

58	36.21	169.65	166.61	98.59	134.27	206.15	260.78	170.01	297.15	359.51	89.82
59	39.81	169.65	166.61	218.59	134.27	206.15	20.78	290.01	177.15	119.51	209.82
60	49.15	169.65	166.61	218.59	14.27	86.15	20.78	290.01	297.15	359.51	89.82
61	61.86	169.65	286.61	218.59	134.27	86.15	260.78	290.01	297.15	119.51	209.82
62	40.82	289.65	166.61	218.59	14.27	206.15	140.78	170.01	177.15	359.51	329.82
63	48.29	289.65	166.61	98.59	134.27	326.15	20.78	290.01	57.15	359.51	89.82
64	21.93	49.65	46.61	218.59	134.27	206.15	20.78	170.01	297.15	239.51	329.82
65	8.10	49.65	166.61	98.59	254.27	206.15	260.78	170.01	177.15	239.51	89.82
66	31.93	289.65	166.61	338.59	134.27	206.15	140.78	170.01	297.15	359.51	209.82
67	43.59	169.65	166.61	218.59	254.27	206.15	20.78	50.01	177.15	359.51	209.82
68	74.32	289.65	286.61	218.59	134.27	326.15	140.78	170.01	297.15	359.51	209.82
69	34.17	49.65	166.61	218.59	254.27	326.15	140.78	290.01	177.15	119.51	209.82
70	-8.25	49.65	46.61	218.59	254.27	86.15	20.78	290.01	177.15	359.51	89.82
71	45.57	289.65	166.61	218.59	134.27	86.15	20.78	50.01	297.15	359.51	89.82
72	37.34	289.65	286.61	98.59	134.27	86.15	20.78	170.01	57.15	119.51	209.82
73	64.41	289.65	166.61	338.59	254.27	326.15	20.78	50.01	297.15	119.51	209.82
74	52.67	169.65	286.61	338.59	254.27	206.15	140.78	170.01	297.15	359.51	209.82
75	26.07	289.65	166.61	218.59	134.27	206.15	140.78	170.01	297.15	359.51	209.82
76	27.09	49.65	46.61	218.59	254.27	206.15	260.78	50.01	57.15	119.51	89.82
77	10.88	49.65	166.61	218.59	134.27	206.15	140.78	290.01	177.15	359.51	209.82
78	75.84	169.65	166.61	218.59	134.27	326.15	140.78	290.01	57.15	119.51	89.82
79	36.12	49.65	46.61	98.59	134.27	206.15	260.78	50.01	177.15	239.51	209.82
80	35.32	289.65	286.61	218.59	254.27	206.15	140.78	170.01	177.15	359.51	89.82
81	42.22	289.65	46.61	98.59	14.27	206.15	20.78	290.01	177.15	119.51	89.82
82	61.18	289.65	286.61	218.59	14.27	86.15	260.78	290.01	177.15	359.51	209.82
83	61.44	169.65	286.61	218.59	254.27	326.15	140.78	290.01	297.15	119.51	209.82
84	17.28	289.65	166.61	338.59	254.27	206.15	20.78	50.01	177.15	359.51	209.82
85	64.18	289.65	46.61	98.59	134.27	86.15	260.78	170.01	57.15	239.51	89.82
86	19.53	169.65	166.61	98.59	134.27	86.15	20.78	50.01	177.15	239.51	89.82
87	15.77	49.65	166.61	338.59	134.27	86.15	20.78	50.01	57.15	239.51	209.82
88	17.28	289.65	286.61	218.59	254.27	206.15	20.78	170.01	57.15	359.51	209.82
89	11.26	49.65	166.61	98.59	254.27	86.15	20.78	170.01	57.15	239.51	329.82

90	23.51	169.65	166.61	98.59	254.27	206.15	20.78	290.01	57.15	239.51	89.82
91	53.90	49.65	286.61	218.59	254.27	86.15	20.78	290.01	297.15	119.51	89.82
92	21.05	49.65	46.61	98.59	14.27	206.15	140.78	290.01	177.15	359.51	89.82
93	66.05	49.65	166.61	218.59	254.27	86.15	20.78	170.01	57.15	119.51	209.82
94	41.54	289.65	166.61	338.59	254.27	86.15	260.78	50.01	297.15	119.51	89.82
95	-8.08	49.65	166.61	218.59	254.27	206.15	20.78	50.01	177.15	359.51	89.82
96	5.59	49.65	166.61	218.59	134.27	206.15	20.78	290.01	297.15	119.51	329.82
97	34.12	169.65	166.61	98.59	254.27	86.15	20.78	290.01	177.15	239.51	209.82
98	35.00	169.65	166.61	98.59	254.27	86.15	20.78	290.01	177.15	359.51	209.82
99	51.31	49.65	46.61	98.59	134.27	86.15	260.78	50.01	297.15	239.51	209.82
100	70.64	289.65	46.61	218.59	14.27	206.15	140.78	170.01	297.15	119.51	89.82
101	41.50	289.65	46.61	98.59	14.27	86.15	20.78	290.01	177.15	239.51	89.82
102	83.18	289.65	46.61	218.59	254.27	86.15	20.78	50.01	297.15	359.51	89.82
103	46.95	169.65	166.61	218.59	14.27	206.15	260.78	290.01	297.15	119.51	89.82
104	20.35	49.65	46.61	218.59	14.27	206.15	140.78	290.01	297.15	239.51	209.82
105	6.99	289.65	166.61	218.59	14.27	86.15	20.78	170.01	297.15	359.51	209.82
106	35.72	49.65	46.61	218.59	254.27	206.15	260.78	290.01	297.15	359.51	209.82
107	38.00	49.65	46.61	98.59	14.27	86.15	20.78	50.01	177.15	239.51	329.82
108	55.59	49.65	166.61	218.59	14.27	86.15	260.78	290.01	177.15	359.51	329.82
109	56.99	49.65	166.61	338.59	134.27	206.15	140.78	290.01	57.15	359.51	89.82
110	34.93	49.65	46.61	218.59	254.27	206.15	260.78	170.01	297.15	359.51	329.82
111 ^b	-8.52	49.65	166.61	98.59	254.27	206.15	20.78	170.01	177.15	239.51	89.82
112	64.47	49.65	166.61	338.59	134.27	86.15	20.78	50.01	177.15	239.51	329.82
113	43.32	49.65	166.61	98.59	134.27	86.15	260.78	290.01	177.15	239.51	329.82
114	33.40	49.65	166.61	218.59	14.27	206.15	140.78	290.01	177.15	119.51	329.82
115	89.31	49.65	46.61	98.59	14.27	86.15	140.78	170.01	177.15	359.51	329.82
116	53.40	289.65	46.61	98.59	254.27	206.15	20.78	170.01	297.15	119.51	89.82
117	79.67	49.65	46.61	218.59	134.27	326.15	260.78	50.01	297.15	119.51	209.82
118	55.71	49.65	46.61	218.59	254.27	86.15	260.78	50.01	297.15	239.51	329.82
119	30.80	49.65	46.61	218.59	134.27	86.15	260.78	170.01	297.15	359.51	209.82
120	5.15	49.65	166.61	338.59	134.27	86.15	20.78	50.01	57.15	239.51	89.82
121	43.97	169.65	166.61	338.59	134.27	206.15	20.78	170.01	297.15	119.51	209.82

122	48.84	289.65	166.61	218.59	254.27	326.15	20.78	50.01	57.15	239.51	209.82
123	90.62	169.65	166.61	98.59	254.27	86.15	260.78	290.01	177.15	119.51	209.82
124	53.32	289.65	286.61	218.59	14.27	206.15	260.78	170.01	57.15	119.51	209.82
125	45.00	289.65	166.61	98.59	134.27	326.15	20.78	170.01	297.15	239.51	209.82
126	39.50	49.65	166.61	218.59	254.27	86.15	260.78	50.01	297.15	239.51	209.82
127	72.55	289.65	46.61	218.59	254.27	86.15	260.78	170.01	177.15	119.51	329.82
128	60.11	289.65	166.61	338.59	14.27	86.15	20.78	290.01	297.15	239.51	209.82
129	9.25	289.65	286.61	218.59	254.27	206.15	20.78	290.01	177.15	119.51	209.82
130	27.36	289.65	166.61	98.59	14.27	206.15	140.78	170.01	57.15	239.51	209.82
131	41.92	289.65	46.61	218.59	14.27	206.15	20.78	170.01	297.15	239.51	89.82
132	70.50	289.65	46.61	98.59	134.27	326.15	260.78	170.01	297.15	239.51	209.82
133	53.12	49.65	166.61	338.59	254.27	326.15	20.78	50.01	57.15	119.51	209.82
134	62.91	49.65	46.61	98.59	254.27	326.15	140.78	290.01	297.15	119.51	209.82
135	70.61	289.65	166.61	338.59	134.27	86.15	260.78	50.01	297.15	119.51	209.82
136	35.98	289.65	166.61	98.59	134.27	326.15	260.78	50.01	57.15	119.51	209.82
137	35.02	49.65	166.61	218.59	134.27	326.15	260.78	170.01	297.15	119.51	209.82
138	66.53	169.65	166.61	338.59	254.27	206.15	260.78	170.01	57.15	119.51	89.82
139	26.12	289.65	166.61	338.59	254.27	206.15	260.78	170.01	57.15	359.51	89.82
140	-8.17	49.65	166.61	218.59	254.27	206.15	20.78	50.01	57.15	119.51	89.82
141	47.65	49.65	166.61	98.59	14.27	86.15	260.78	290.01	177.15	119.51	209.82
142	82.86	169.65	166.61	98.59	254.27	326.15	140.78	290.01	177.15	119.51	89.82
143	77.09	289.65	166.61	338.59	134.27	86.15	140.78	290.01	297.15	359.51	209.82
144	40.00	169.65	286.61	218.59	254.27	86.15	20.78	290.01	177.15	359.51	209.82
145	10.68	289.65	166.61	218.59	134.27	206.15	20.78	290.01	57.15	119.51	209.82
146	49.01	49.65	46.61	218.59	134.27	206.15	260.78	170.01	297.15	239.51	329.82
147	84.81	49.65	286.61	338.59	254.27	86.15	20.78	290.01	177.15	119.51	329.82
148	31.13	289.65	166.61	98.59	134.27	206.15	140.78	290.01	177.15	359.51	329.82
149	38.00	49.65	46.61	218.59	14.27	206.15	140.78	170.01	297.15	359.51	329.82
150	5.88	289.65	166.61	218.59	134.27	86.15	20.78	170.01	177.15	119.51	329.82
151	77.06	49.65	286.61	338.59	134.27	206.15	20.78	50.01	177.15	119.51	89.82
152	90.62	49.65	46.61	98.59	134.27	206.15	20.78	50.01	297.15	239.51	89.82
153	79.20	49.65	286.61	218.59	254.27	206.15	260.78	170.01	57.15	359.51	89.82

154	22.81	289.65	166.61	218.59	254.27	86.15	260.78	170.01	177.15	239.51	329.82
155	33.32	289.65	286.61	218.59	134.27	206.15	20.78	50.01	297.15	119.51	209.82
156	74.37	49.65	286.61	218.59	14.27	206.15	20.78	290.01	297.15	359.51	329.82
157	88.48	49.65	286.61	218.59	14.27	206.15	260.78	170.01	297.15	239.51	209.82
158	12.71	289.65	286.61	218.59	134.27	206.15	20.78	50.01	177.15	239.51	209.82
159	77.41	289.65	46.61	218.59	14.27	206.15	20.78	170.01	57.15	119.51	209.82
160	42.95	169.65	286.61	338.59	254.27	86.15	260.78	50.01	177.15	119.51	209.82
161	6.78	289.65	166.61	98.59	134.27	86.15	20.78	50.01	177.15	119.51	329.82
162	85.41	169.65	166.61	98.59	134.27	206.15	20.78	50.01	297.15	359.51	89.82
163	82.43	289.65	166.61	98.59	134.27	326.15	140.78	170.01	297.15	239.51	329.82
164	5.64	289.65	166.61	218.59	134.27	86.15	20.78	170.01	177.15	239.51	329.82
165	31.28	289.65	286.61	98.59	14.27	86.15	260.78	50.01	57.15	359.51	209.82
166	64.87	289.65	166.61	218.59	134.27	326.15	260.78	50.01	297.15	119.51	329.82
167	77.57	289.65	46.61	218.59	14.27	206.15	260.78	290.01	177.15	119.51	89.82
168	59.59	289.65	166.61	338.59	134.27	86.15	140.78	170.01	297.15	119.51	89.82
169	42.40	289.65	166.61	338.59	134.27	326.15	260.78	170.01	177.15	119.51	329.82
170	86.82	49.65	286.61	338.59	254.27	86.15	20.78	170.01	177.15	239.51	329.82
171	0.24	49.65	166.61	338.59	134.27	86.15	20.78	290.01	177.15	359.51	89.82
172	19.46	49.65	166.61	98.59	254.27	206.15	260.78	290.01	177.15	359.51	89.82
173	73.43	289.65	46.61	218.59	254.27	86.15	260.78	50.01	57.15	119.51	329.82
174	-7.33	49.65	166.61	98.59	134.27	206.15	20.78	50.01	57.15	119.51	89.82
175	81.91	49.65	286.61	218.59	14.27	206.15	260.78	170.01	57.15	119.51	89.82
176	33.14	49.65	166.61	98.59	134.27	326.15	20.78	290.01	297.15	119.51	209.82
177	56.33	289.65	46.61	218.59	14.27	86.15	20.78	290.01	57.15	239.51	329.82
178	24.21	49.65	46.61	98.59	14.27	86.15	20.78	50.01	177.15	119.51	89.82
179	53.98	289.65	286.61	218.59	134.27	326.15	140.78	290.01	177.15	359.51	89.82
180	31.53	289.65	166.61	98.59	134.27	326.15	20.78	290.01	177.15	239.51	89.82
181	23.65	49.65	166.61	98.59	134.27	206.15	260.78	170.01	57.15	239.51	209.82
182	-3.31	289.65	166.61	218.59	134.27	206.15	20.78	170.01	297.15	119.51	89.82
183	29.15	289.65	166.61	98.59	14.27	86.15	260.78	170.01	177.15	119.51	209.82
184	78.87	289.65	286.61	218.59	134.27	86.15	140.78	170.01	297.15	239.51	209.82
185	31.07	49.65	46.61	98.59	14.27	206.15	140.78	170.01	297.15	359.51	89.82

186	32.56	49.65	166.61	218.59	14.27	86.15	260.78	50.01	177.15	359.51	329.82
187	17.60	49.65	46.61	98.59	14.27	206.15	20.78	170.01	297.15	359.51	329.82
188	64.69	169.65	166.61	338.59	134.27	326.15	260.78	170.01	57.15	359.51	89.82
189	15.55	49.65	46.61	98.59	14.27	206.15	20.78	290.01	177.15	239.51	329.82
190	36.42	49.65	46.61	98.59	14.27	206.15	140.78	170.01	297.15	119.51	329.82
191	37.81	289.65	166.61	218.59	254.27	326.15	260.78	50.01	57.15	119.51	209.82
192	23.45	49.65	166.61	218.59	254.27	86.15	260.78	50.01	57.15	359.51	329.82
193	70.00	169.65	166.61	338.59	134.27	206.15	140.78	290.01	57.15	119.51	209.82
194	70.62	289.65	46.61	98.59	134.27	206.15	140.78	170.01	177.15	119.51	89.82
195	30.18	49.65	46.61	218.59	134.27	86.15	260.78	170.01	177.15	119.51	209.82
196	30.73	49.65	166.61	218.59	254.27	206.15	140.78	170.01	57.15	239.51	329.82
197	25.19	49.65	166.61	98.59	14.27	86.15	260.78	170.01	177.15	359.51	209.82
198	26.86	49.65	46.61	218.59	254.27	206.15	140.78	290.01	177.15	119.51	89.82
199	91.43	49.65	286.61	338.59	254.27	206.15	20.78	170.01	57.15	119.51	89.82
200	60.92	49.65	166.61	98.59	134.27	326.15	20.78	50.01	297.15	119.51	329.82
201	41.24	49.65	166.61	98.59	254.27	326.15	260.78	50.01	57.15	239.51	209.82
202	31.01	289.65	46.61	218.59	134.27	86.15	20.78	170.01	177.15	359.51	89.82
203	41.33	289.65	166.61	98.59	14.27	86.15	140.78	170.01	297.15	239.51	209.82
204	55.48	289.65	286.61	218.59	134.27	326.15	140.78	170.01	177.15	119.51	209.82
205	52.49	49.65	166.61	338.59	254.27	326.15	20.78	290.01	57.15	239.51	209.82
206	81.40	49.65	286.61	338.59	134.27	206.15	20.78	290.01	177.15	239.51	209.82
207	18.07	49.65	46.61	218.59	254.27	206.15	20.78	50.01	57.15	239.51	329.82
208	41.06	169.65	286.61	218.59	254.27	86.15	20.78	170.01	297.15	119.51	209.82
209	53.91	289.65	166.61	218.59	254.27	326.15	20.78	50.01	297.15	119.51	89.82
210	7.50	289.65	286.61	218.59	134.27	86.15	20.78	170.01	297.15	239.51	89.82
211	22.48	289.65	166.61	218.59	254.27	86.15	260.78	50.01	57.15	239.51	209.82
212	45.09	169.65	286.61	218.59	134.27	206.15	20.78	170.01	177.15	359.51	209.82
213	2.87	289.65	166.61	338.59	254.27	206.15	20.78	170.01	177.15	119.51	89.82
214	75.00	49.65	286.61	218.59	254.27	206.15	260.78	170.01	297.15	359.51	89.82
215	47.73	49.65	46.61	98.59	134.27	326.15	260.78	50.01	57.15	119.51	329.82
216	80.76	49.65	286.61	218.59	254.27	206.15	260.78	170.01	57.15	119.51	89.82
217	18.73	49.65	166.61	338.59	134.27	86.15	260.78	50.01	177.15	239.51	89.82

218	4.77	49.65	166.61	98.59	254.27	206.15	20.78	290.01	177.15	359.51	329.82
219	20.45	49.65	46.61	218.59	134.27	206.15	20.78	50.01	177.15	359.51	329.82
220	24.76	49.65	166.61	98.59	134.27	86.15	260.78	170.01	57.15	359.51	209.82
221	77.89	289.65	286.61	98.59	254.27	206.15	20.78	170.01	57.15	359.51	209.82
222	48.52	49.65	46.61	98.59	134.27	86.15	260.78	290.01	177.15	119.51	209.82
223	20.84	49.65	46.61	98.59	14.27	206.15	140.78	290.01	177.15	359.51	209.82
224	28.33	49.65	166.61	218.59	134.27	206.15	140.78	170.01	297.15	119.51	329.82
225	83.53	49.65	166.61	338.59	254.27	86.15	260.78	50.01	57.15	239.51	89.82
226	25.48	289.65	166.61	338.59	254.27	206.15	140.78	170.01	297.15	119.51	89.82
227	73.10	289.65	166.61	218.59	134.27	206.15	260.78	290.01	297.15	239.51	89.82
228	33.71	289.65	166.61	338.59	14.27	206.15	140.78	290.01	177.15	359.51	89.82
229	5.09	289.65	166.61	98.59	134.27	86.15	20.78	170.01	177.15	239.51	329.82
230	87.55	169.65	166.61	218.59	254.27	326.15	260.78	50.01	177.15	119.51	209.82
231	1.72	49.65	166.61	338.59	134.27	86.15	20.78	170.01	297.15	359.51	89.82
232	60.07	49.65	46.61	218.59	134.27	86.15	140.78	170.01	297.15	359.51	89.82
233	82.87	169.65	166.61	218.59	134.27	206.15	260.78	290.01	177.15	239.51	89.82
234	51.95	289.65	286.61	218.59	254.27	326.15	260.78	170.01	177.15	359.51	209.82
235	28.77	289.65	166.61	98.59	14.27	206.15	140.78	290.01	177.15	359.51	209.82
236	64.94	289.65	166.61	218.59	254.27	326.15	140.78	290.01	57.15	119.51	329.82
237	43.82	49.65	46.61	98.59	134.27	326.15	20.78	290.01	177.15	239.51	89.82
238	40.76	49.65	166.61	338.59	254.27	326.15	260.78	170.01	57.15	359.51	209.82
239	11.70	289.65	286.61	218.59	254.27	86.15	20.78	50.01	57.15	239.51	209.82
240	22.61	49.65	46.61	98.59	134.27	206.15	20.78	170.01	57.15	239.51	329.82
241	49.93	49.65	46.61	218.59	254.27	326.15	140.78	170.01	57.15	359.51	209.82
242	37.33	169.65	166.61	98.59	14.27	206.15	20.78	290.01	297.15	119.51	209.82
243	22.54	49.65	46.61	98.59	254.27	206.15	20.78	170.01	57.15	359.51	209.82
244	31.00	289.65	166.61	98.59	134.27	206.15	140.78	290.01	297.15	359.51	329.82
245	26.51	49.65	166.61	218.59	14.27	86.15	20.78	290.01	57.15	359.51	329.82
246	40.61	289.65	166.61	218.59	134.27	326.15	260.78	170.01	177.15	359.51	329.82
247	46.21	169.65	166.61	218.59	134.27	86.15	260.78	170.01	297.15	119.51	89.82
248	89.87	169.65	286.61	338.59	134.27	326.15	20.78	290.01	57.15	239.51	209.82
249	54.00	49.65	46.61	98.59	134.27	326.15	20.78	170.01	177.15	119.51	329.82

250	65.70	169.65	166.61	338.59	134.27	206.15	260.78	170.01	297.15	239.51	209.82
251	57.04	289.65	166.61	98.59	134.27	326.15	140.78	170.01	177.15	239.51	89.82
252	36.08	169.65	166.61	98.59	134.27	86.15	20.78	290.01	297.15	359.51	209.82
253	88.86	169.65	166.61	338.59	254.27	206.15	20.78	50.01	297.15	359.51	89.82
254	22.26	169.65	166.61	98.59	134.27	206.15	20.78	170.01	297.15	119.51	89.82
255	19.96	289.65	166.61	338.59	254.27	86.15	20.78	290.01	177.15	359.51	89.82
256	51.15	169.65	166.61	218.59	134.27	326.15	260.78	50.01	177.15	239.51	89.82
257	66.26	49.65	46.61	338.59	254.27	206.15	20.78	170.01	297.15	239.51	89.82

^abioactive conformation; ^bmost stable conformation

Figure S3: The B3LYP/6-311+G(d) optimized structures for P218 conformations and their relative energies compared to bioactive conformation (after minimization).

III. Root Mean Square Deviation (RMSD) of redocked ligands with the co-crystallized conformation of ligands.

To validate the molecular docking protocol, it is a common practice to redock the already reported ligands in the active site of X-ray crystal structures. The X-ray crystal structure of human DHFR (*h*DHFR; PDB: 4DDR) and quadruple-mutant *Pf*DHFR (qm*Pf*DHFR; PDB: 4DP3) have P218 as bound ligand; wild-type *Pf*DHFR (wt*Pf*DHFR; PDB: 4DPD) have dihydrofolate (DHF) as bound ligand and double-mutant *Pf*DHFR complexes (dm1*Pf*DHFR; PDB: 1J3J and dm2*Pf*DHFR; PDB: 3UM5) have pyrimethamine as bound ligand. These bound ligands were redocked in the active-sites of respective enzymes and the RMSD value of the docked pose and co-crystallized conformation of bound ligand was calculated. The RMSD values were found to be in an acceptable range (Fig. S4) and all the ligands in their

best docked pose showed the same molecular recognition interactions as shown in crystal structures.

Figure S4: Superimposed structures of co-crystallized ligand (green) and docked conformation of ligands (pink) A) P218 in the active site of *h*DHFR (RMSD = 0.3124 Å) B) P218 in the active site of *qmPf*DHFR (RMSD = 0.2914 Å) C) DHF in the active site of *wtPf*DHFR (RMSD = 0.7435 Å) D) Pyrimethamine in the active site of *dm1Pf*DHFR (RMSD = 0.2013 Å) E) Pyrimethamine in the active site of *dm2Pf*DHFR (RMSD = 0.2754 Å).

IV. Energy graphs of all the five complexes during the molecular dynamics simulation run.

Molecular Dynamics (MD) simulation was carried out for 35 ns for all the five complexes (i) *h*DHFR-P218 (ii) *wtPf*DHFR-P218 (iii) *qmPf*DHFR-P218 (iv) *dm1Pf*DHFR-P218 and (v) *dm2Pf*DHFR-P218. Initially kinetic energy, potential energy and total energy (kinetic + potential energy) of the complex during the entire simulation run of 35 ns were studied to check the quality of simulation run. These energy parameters were plotted from the obtained MD output files. Probing these properties allowed us to check that nothing strange happened during the simulation run. Any abrupt and unexpected spikes in the energy plots indicates a problem with the simulation protocol, bad starting structure; too long time step, inappropriate parameters etc. In the present work, the energy graph for kinetic energy for all the five complexes remained constant for the entire simulation run implying that the kinetic energy for the complexes was stable and also the temperature thermostat, which acts on the kinetic

energy, was working correctly (Fig. S5). The potential energy, and consequently the total energy since total energy = potential energy + kinetic energy, also remained constant with no wild oscillations, indicating that complex attained stability during the simulation run (Fig. S5).

Figure S5: Kinetic energy, potential energy and total energy graphs for A) *hDHFR* B) *wtPfDHFR* C) *qmPfDHFR* D) *dm1PfDHFR* and E) *dm2PfDHFR* during the production run of 35ns. The kinetic energy of the system is positive while its potential energy and total energy is negative.

V. Interaction of P218 with Arg59 in qmPfDHFR and dm1PfDHFR.

Quadruple-mutant *Pf*DHFR (qm*Pf*DHFR) and double-mutant *Pf*DHFR (dm1*Pf*DHFR) have Cys59Arg mutation. From the molecular docking and dynamics studies on P218 binding to these two enzymes it was noted that the mutations do not hamper P218 binding mode in the active-sites of mutant enzymes. The ‘per-residue’ decomposition energies noted for this residue (Arg59) in qm*Pf*DHFR and dm1*Pf*DHFR was -10.57 and -3.33 kcal/mol, respectively. Hence, it can be envisaged that mutated residue Arg59 strongly favors P218 binding in qm*Pf*DHFR, as compared to P218 binding to dm1*Pf*DHFR. Structural investigation revealed that Arg59 in qm*Pf*DHFR and dm1*Pf*DHFR are oriented differently. For simplicity, the lowest energy structure from the entire simulation run of 35 ns for both the complexes is shown in Fig. S6. From the figure it is clear that Arg59 in qm*Pf*DHFR adopts an orientation such that it can easily form H-bond with P218 (Fig. S6A), while in dm1*Pf*DHFR, Arg59 is oriented such that it cannot form stable H-bond during the simulation (Fig. S6B).

Figure S6: The lowest energy structures of qm*Pf*DHFR-P218 complex (A) and dm1*Pf*DHFR-P218 complex (B) to show Arg59 orientation in both the complexes.

VI. Hydrogen bond analysis of all the five complexes.

Hydrogen bond analysis was carried out for last 5 ns simulation run to identify the crucial active-site amino acids that form H-bonds with the bound ligand P218. The analysis showed that Ile14, Asp54, Arg59, Arg122 and Ile164 in *Pf*DHFR and Ile7 and Glu30 in *h*DHFR

formed H-bond interaction with the ligand P218 (Fig. 7, main article). The detailed H-bond distances for these amino acids in the active-sites of all the five enzymes are given in Fig. S7. From the distance graphs it can be envisaged that in all the five complexes two stable hydrogen bonds were formed between N7 amino group and Asp54 (*Pf*DHFR)/Glu30 (*h*DHFR); and N1 of P218 and Asp54 (*Pf*DHFR)/Glu30 (*h*DHFR). The distance variation with Asp54/Glu30, follow H-bond criteria (distance cut-off of 3.2 Å), while there exists slight high fluctuation in H-bond distance between N1 of ligand and Glu30 of *h*DHFR (Fig. S7A-B). P218 shows interaction with Arg122 while it does not form the same interaction with Arg70 of *h*DHFR (structural homolog of Arg122 in *Pf*DHFR). All the *Pf*DHFR complexes form two stable hydrogen bonds with Arg122 (Fig. S7C-D). All the four *Pf*DHFR-P218 complexes also involve H-bond interaction between N8 amino group and main chain of Ile14, and similar kind of H-bond interaction also exists between P218 and main chain of Ile7 in *h*DHFR (Fig. S7E). The H-bond distance analysis shows that hydrogen bond between P218 and Ile14 of qm*Pf*DHFR is not stable as compared to all other complexes, as it has high distance fluctuations. Ile164 forms a stable hydrogen bond between N8 amino group of P218 in the wt*Pf*DHFR, dm1*Pf*DHFR and dm2*Pf*DHFR, while, mutated residue Leu164 in qm*Pf*DHFR does not form stable hydrogen bond with P218 as shown in (Fig. S7F). This reveals that mutation at position 164 from Ile to Leu in qm*Pf*DHFR does not favor P218 binding as shown by the longer distance (> 3.2 Å) in the graph. Mutation at position 59 from Cys to Arg in qm*Pf*DHFR and dm1*Pf*DHFR has different effects on P218 binding, where Arg59 favors P218 binding in qm*Pf*DHFR as compared to dm1*Pf*DHFR. This can be seen from the H-bond distance graph of Arg59 and P218 in qm*Pf*DHFR which is within the H-bond range for the last 5 ns simulation run while the same distance between Arg59 and P218 in dm1*Pf*DHFR is high revealing decreased interaction in the double-mutant form (Fig S7G). P218 binding to human DHFR is not favored by Arg70, whereas, it was noticed that in the

dynamical conditions P218 tends to stabilize itself by interacting with Asn64, but this bond was not stable for the entire simulation run as shown in (Fig. S7H). Overall, it was noticed that on an average the number of hydrogen bonds formed in human DHFR was three-four and five-six hydrogen bonds in *Pf*DHFR complexes (Fig. S8).

Figure S7: Hydrogen bond distances between P218 and active-site residues of *h*DHFR, wt*Pf*DHFR, qm*Pf*DHFR, dm1*Pf*DHFR and dm2*Pf*DHFR, formed during last 5ns of simulation run. Angle and distance cut off for hydrogen bonding was set to 120° (i.e. 120° - 180°) and 3.2 Å.

Figure S8: Number of hydrogen bonds formed by P218 over the last 5ns simulation with *h*DHFR, *wtPj*/DHFR, *qmPj*/DHFR, *dm1Pj*/DHFR and *dm2Pj*/DHFR. Analysis was carried out on the last 5 ns (2500 frames) of simulation run. Angle and distance cut off for hydrogen bonding was set to 120° (i.e. 120° - 180°) and 3.2 Å.