SUPPORTING INFORMATION

Structural Studies of Pterin-Based Inhibitors of Dihydropteroate Synthase

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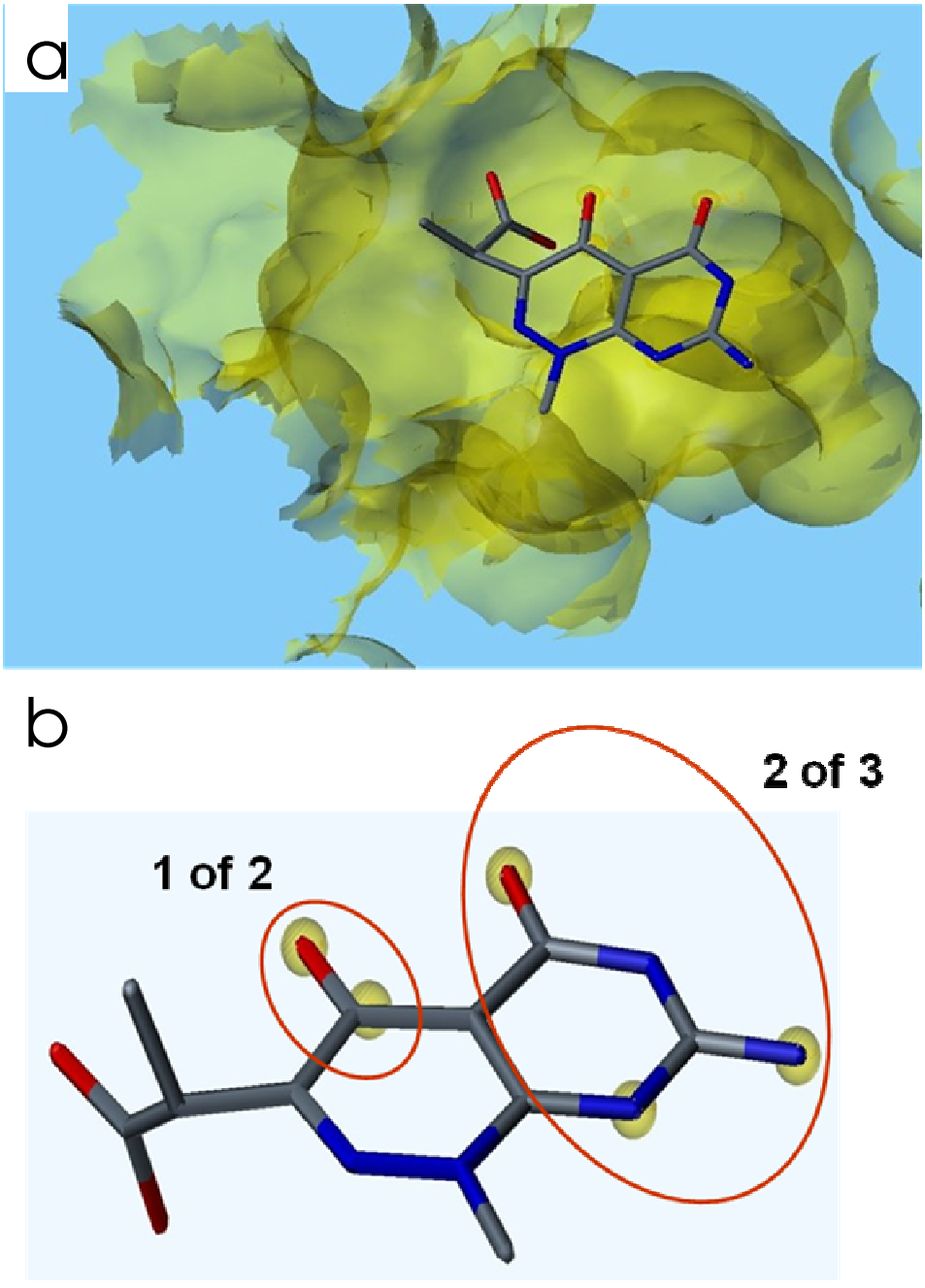


Figure S1 UNITY pharmacophore filters applied to DHPS. a) Surface volume constraint. b) Donor and Acceptor Constraints.

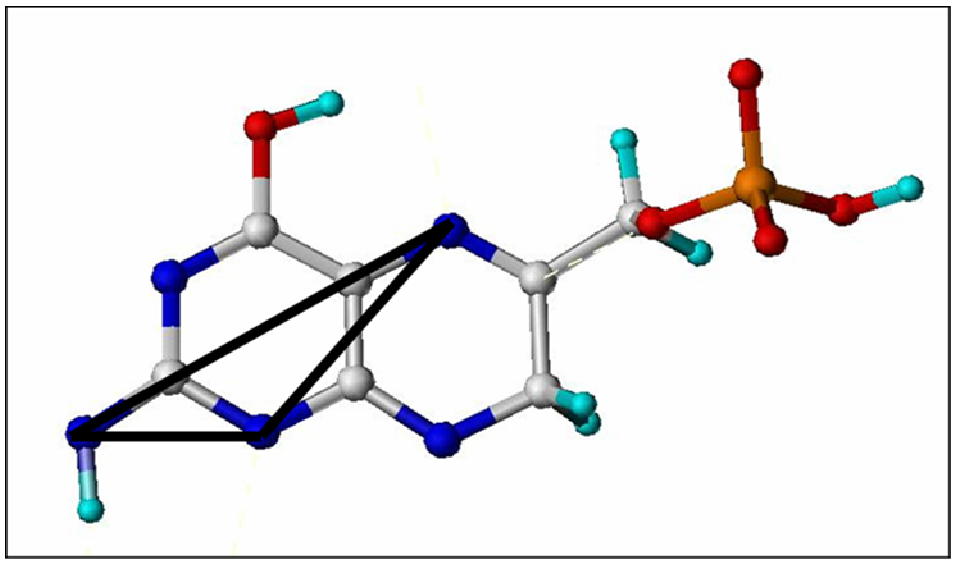


Figure S2 Unity query #1, Maybridge database.

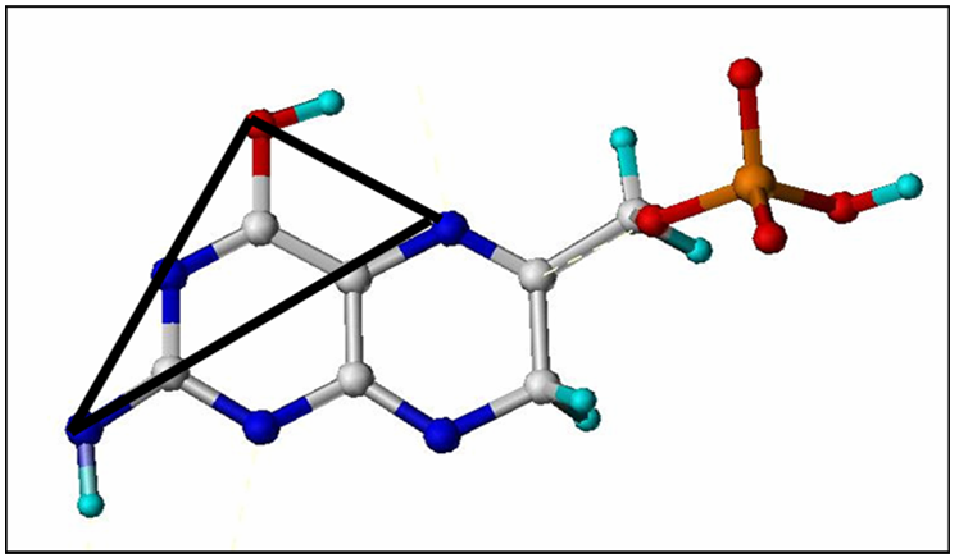
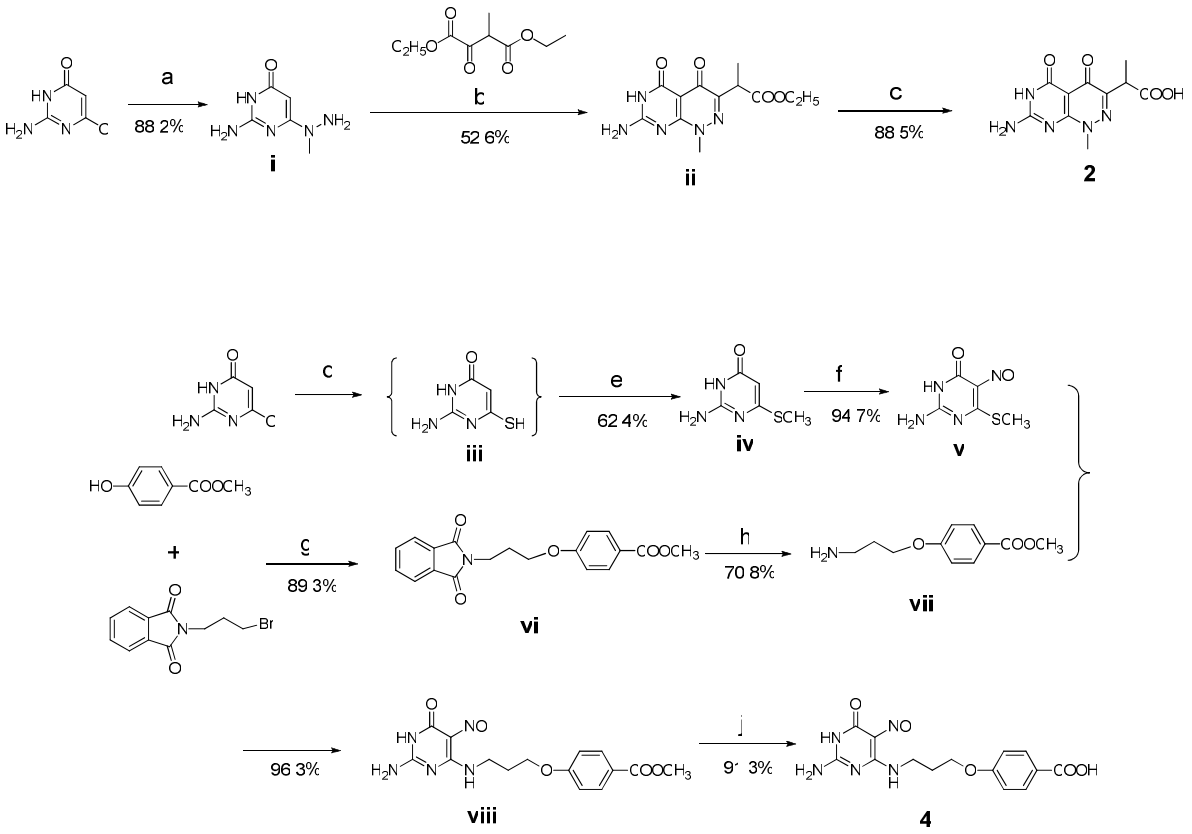


Figure S3 Unity query #2, NCI database.

Scheme S1



Conc tions anc reagents a CH_3NHNH_2 H_2O ref ux 3h b Methy oxa acet c Acc Dethy Ester H_2O ref ux 3h c (1) 5%NaOH THF RT overn ght (2) 1N HC c NaHS $(CH_2OH)_2$ 135°C 6h e CH_3 1NNaOH 50%EtOH RT 3h f $NaNO_2$ H_2O 40°C 2NAcOH 2h g NaH DMSO RT 70h h 55%NH_2NH_2 MeOH ref ux 4h MeOH ref ux 20h j (1) 0 5NNaOH RT overn ght (2) 1N HC

Scheme S1 Synthesis scheme utilized in the synthesis of the known DHPS inhibitors 2 &4.

Synthesis Methods

General Methods: The reactions were monitored by thin layer chromatography (TLC) on pre-coated Merck 60 F_{254} silica gel plates and visualized by UV detection. All ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 or Varian INOVA-500 spectrometers. Chemical shifts are reported in ppm (δ) relative to residual solvent peak or internal standard (tetramethylsilane) and coupling constants (J) are reported in hertz (Hz). Mass spectra were recorded on a Bruker Esquire LC/MS using ESI.

The synthesis schemes for compounds **2** & **4** is described in Scheme S1, the experimental methods and analytical data follows:

*2-amino-6-(1-methylhydrazinyl)pyrimidin-4(3H)-one (**i**)*

To a mixture of 2-Amino-4-chloro-6-hydroxypyrimidine (3.50g, 24.14mmol) in water (180ml) was added methylhydrazine (5.40g, 117.14mmol) and refluxed for 3h. The reaction solution was cooled to room temperature and filtered to give crystal **i,** 3.30g, yield 88.2%. ^{1}HNMR (DMSO-d_6, 300MHz) δ 9.70 (s, 1H), 6.20 (s, 2H), 4.95 (s, 1H), 4.45 (ss, 2H), 3.10 (s, 3H). ^{13}CNMR (DMSO-d_6, 75MHz) δ 165.86, 163.10, 154.21, 92, 75.84. MS (ESI): 178.2 (M^++ Na).

*Ethyl 2- (7-amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido (4,5-c) pyridazin-3-yl) propanoate (**ii**)*

To a mixture of **i** (3.00g, 19.35mmol) in water (150ml) was added Methyloxalacetic Acid Diethyl Ester (7.80g, 38.61mmol). The reaction mixture was refluxed for 3h, then cooled to 70^{o}C and filtered to give white solid 6 2.98g, yield 52.6%. ^{1}HNMR (DMSO-d_6, 300MHz) δ 10.9 (s, 1H), 4.05 (q, 2H, J=Hz), 3.78 (q, 1H, J=7.5Hz), 3.75 (s, 3H), 1.32 (3H, d, J=7.0Hz), 1.15 (3H, t, J=7.5Hz). MS (ESI): 316.3 (M^++Na).

*2-(7-amino-1-methyl-4,5-dioxo-1,4,5,6-tetrahydropyrimido(4,5-c)pyridazin-3-yl)propanoic acid (**2**)*

To a solution of **ii** (0.3g, 1.02mmol) in THF(10ml) was added 5% NaOH solution (7ml) at RT and stirred overnight, followed by solvent evaporation. The resulting solution was acidified with 1N HCl to pH 4~5 and filtered to give white solid **2** 239mg, yield 88.5%. ^{1}HNMR (DMSO-d_6, 500MHz) δ 10.96 (s, 1H), 3.82 (q, 1H, J=7.0Hz), 3.78 (s, 3H), 1.33 (d, 3H, J=7.0Hz). ^{13}CNMR (75MHz, DMSO-d_6) δ 173.68, 166.78, 159.42, 157.44, 155.38, 154.42, 100.68, 40.88, 40.00, 14.17. MS (ESI): 288 (M^++Na)

*2-amino-6-(methylthio)pyrimidin-4(3H)-one (**iv**)*

To a solution of 2-Amino-4-chloro-6-hydroxypyrimidine (3.0g, 21mmol) in ethane-1,2-diol (12ml) was added NaHS (4.8g, 86mmol), and slowly heated to 135°C and stirred for 6h. After cooling to RT, 30ml of EtOH and 15ml of acetic acid were added, and stirred overnight at RT. The reaction mixture was filtered, washed with EtOH to give yellow solid **iii** 3.1g which was directly used in the next step reaction without further purification. To a solution of above product (21mmol) in 1N NaOH (18ml) and 50% EtOH (60ml) was added CH_3I (3.1g, 22mmol) and stirred for 3h at RT, filtered, the solid was washed with water to give yellow solid **iv** 2.03g, yield 62.4%. ^{1}HNMR (DMSO-d_6, 500MHz) δ 10.56 (1H, s), 6.58 (2H, s), 5.36 (1H, s), 2.32 (3H, s). MS (ESI) m/z: 158 (M^++1).

*2-amino-6-(methylthio)-5-nitrosopyrimidin-4(3H)-one (**v**)*

A mixture of **iv** (1.59g, 10.13mmol) and $NaNO_2$ (1.55g, 22.46mmol) in water (30ml) was stirred at 40°C to allow for disolution. Then to this mixture was added 2N AcOH (15ml) slowly maintaining the temperature at 40°C. The reaction mixture was then kept stirring for 2h at 40°C, cooled to RT, filtered, washed with water to give blue solid **v** 1.78g, yield 94.7%. ^{1}H NMR (DMSO-d_6, 300MHz) δ 11.61 (1H, s), 8.71 (1H, s), 7.38 (1H, s), 2.26 (3H, s). MS (ESI) m/z: 185 (M^-).

*Methyl 4-(3-(1,3-dioxoisoindolin-2-yl)propoxy)benzoate (**vi**)*

400mg (90%, 66.67mmol) of NaH was slowly added to DMSO (20ml). To this basic reaction solution was added a solution of Methyl 4-hydroxybenzoate (2.28g, 15mmol) in DMSO (15ml) and stirred for 3h at RT. Then a solution of N-(3-Bromopropyl)phthalimide (4.0g, 15mmol) in DMSO(13ml) was added dropwise at RT. The reaction mixture was kept stirring for 70h, quenched the reaction solution with water, filtered, washed with water

to give white solid **vi**, 4.54g, yield 89.3%. ^{1}HNMR ($CDCl_3$, 300MHz) δ 7.94 (2H,d, J=9.0Hz), 7.84 (2H,dd, J1=5.0Hz, J2= 3.0Hz)), 7.72 (2H, dd, J1=5.0Hz, J2=3.0Hz), 6.80 (2H, d, J=9.0Hz), 4.09 (2H, t, J=6.5Hz), 3.92 (2H, t, J=6.5Hz), 3.90 (s, 3H), 2.21 (2H, m).

*Methyl 4-(3-aminopropoxy)benzoate (**vii**)*

To a solution of **vi** (340mg, 1.0mmol) in MeOH (10ml) was added 55% hydrazine (116mg) and refluxed for 4h, then extracted with EtOAc and washed with 1N NaOH, water, dried over Na_2SO_4 and evaporated to give oil **vii** 148mg, yield 70.8%. ^{1}HNMR ($CDCl_3$, 500MHz) δ 7.98 (2H, d, J=8.5Hz), 6.91 (2H, d, 9.0Hz), 4.11 (2H, t, J=6.5Hz), 3.88 (3H, s), 2.922 (2H, t, 7.0Hz), 1.95 (2H, m), 1.28 (br, 2H). MS (ESI) m/z: 210 (M^++H).

*Methyl 4-(3-(2-amino-5-nitroso-6-oxo-1,6-dihydropyrimidin-4-ylamino)propoxy)benzoate (**viii**)*

To a solution of **vii** (140mg, 0.67mmol) in MeOH (12ml) was added **10** (125mg, 0.67mmol) and refluxed for 20h, cooled to RT, and filtered, washed with MeOH to give tan powder solid **15** 224mg, yield 96.3%. ^{1}H NMR (DMSO-d_6, 500MHz) δ 12.60 (1H, s), 10.82 (1H, s), 8.21 (1H, s), 7.91 (2H, d, J=9.0Hz), 7.11 (2H, d, J=9.0Hz), 6.91 (1H, s), 4.11 (2H, t, J=6.0Hz), 3.71 (s, 3H), 3.58 (2H, m) 2.02 (2H, m), MS (ESI) m/z: 346 (M^-).

*4-(3-(2-amino-5-nitroso-6-oxo-1,6-dihydropyrimidin-4-ylamino)propoxy)benzoic acid(**4**)*

170mg (0.49mmol) of **vii** was disolved in 0.5N NaOH solution (4ml) and stirred overnight at RT, then acidified by 1N HCl and filtered, washed with water to give tan powder solid **16** 152mg, yield 91.3%. ^{1}HNMR (MDSO-d_6, 500MHz) 12.60 (1H, t, J=5.5Hz), 10.83 (1H, s), 8.24 (1H, s), 7.89 (2H, d, J=8.5Hz), 7.08 (2H, d, J=9.0Hz), 6.91 (1H, s), 4.09(2H, t, J=6.0Hz), 3.58 (2H, m), 2.02 (2H, m). MS (ESI) m/z: 334(M^++H).

Table S1 Statistics of data collection.

Parameter	**Compound 2**	**Compound 3**	**Compound 4**	**Compound 5**	**Compound 6**	**Compound 7**
Space group	$P6_222$	$P6_222$	$P6_222$	$P6_222$	$P6_222$	$P6_222$
Unit cell dimensions (Å) a,b,c	97.5, 97.5, 263.8	98.8, 98.8, 263.9	96.8, 96.8, 263.6	97.5, 97.5, 264.0	97.9, 97.9, 262.9	97.5, 97.5, 261.6
Resolution range(Å)[a]	50.0-2.32(2.4-2.32)	50.0-2.3(2.38-2.3)	50.0-2.1(2.18-2.1)	80.3-2.4(2.53-2.4)	84.8-2.5(2.64-2.5)	50.0-2.4(2.49-2.4)
R_{sym}[ab]	0.057(0.206)	0.070(0.208)	0.094(0.347)	0.065(0.280)	0.091(0.281)	0.087(0.171)
I/□[a]	40.0(4.7)	35.9(9.6)	29.7(2.0)	23.0(6.3)	19.6(4.6)	33.9(11.5)
Completeness(%)[a]	92.2(57.6)	89.0(52.0)	96.5(89.6)	100.0(100.0)	98.7(92.3)	90.2(54.1)
Redundancy[a]	8.0(5.9)	12.3(9.4)	13.5(6.4)	10.9(11.1)	11.4(7.7)	11.1(8.7)
Unique reflectionos	30,565	30,569	41,961	30,034	26,335	27,556

Parameter	**Compound 10**	**Compound 11**	**Compound 16**	**Compound 17**	**Compound 18**	**Compound 19**
Space group	$P6_222$	$P6_222$	$P6_222$	$P6_222$	$P6_222$	$P6_222$
Unit cell dimensions (Å) a,b,c	97.7, 97.7, 263.6	98.1, 98.1, 263.9	99.3, 99.3, 264.4	99.2, 99.2, 262.1	98.3, 98.3, 263.1	99.3, 99.3, 263.1
Resolution range(Å)[a]	50.0-2.4(2.49-2.4)	50.0-2.0(2.07-2.0)	50.0-2.2(2.28-2.2)	50.0-2.3(2.38-2.3)	50.0-2.4(2.49-2.4)	50.0-2.6(2.69-2.6)
R_{sym}[ab]	0.101(0.309)	0.102(0.346)	0.099(0.350)	0.113(0.326)	0.049(0.132)	0.066(0.160)
I/□[a]	19.0(1.6)	32.3(3.1)	29.4(3.6)	26.5(4.6)	47.5(10.8)	38.2(7.1)
Completeness(%)[a]	88.5(58.2)	94.2(60.8)	97.7(91.7)	89.8(56.2)	91.5(57.9)	93.9(62.0)
Redundancy[a]	7.6(2.9)	11.5(6.7)	12.0(7.7)	10.5(6.0)	11.9(8.4)	11.9(7.2)
Unique reflectionos	26,492	48,615	39,203	30,988	27,831	22,826

[a] Values in parentheses refer to the highest resolution shell.

[b] $R_{sym} = \sum | (I - \langle I \rangle) | / \sum (I)$, where I is the observed intensity.

TableS 2 Statistics of refinement.

Parameter		**Compound 2**	**Compound 3**	**Compound 4**	**Compound 5**	**Compound 6**	**Compound 7**
Resolution range(Å)		50.0-2.32	50.0-2.4	50.0-2.2	50.0-2.5	50.0-2.5	50-2.4
No. of reflection		28,987	27,239	35,180	25,244	24,997	25,876
Completeness(%)		92.2	93.2	97.3	99.9	100.0	91.5
R_{work} / R_{free}[c]		0.205/0.264	0.221 / 0.268	0.217 / 0.279	0.224 / 0.269	0.236 / 0.278	0.225 / 0.280
No. of atoms	Protein	4,050	4,165	4,176	4,073	4,138	4,169
	Non-protein	199	134	303	147	156	164
Mean B overall (Å^2)		61	67	53	69	47	59
Rmsd from ideal values							
Bond lengths(Å)		0.022	0.019	0.021	0.022	0.007	0.012
Bond angles(°)		1.872	1.862	1.909	2.116	1.071	1.302
Ramanchandran plot							
Most favored region(%)		91.4	92.3	91.5	90.7	90.9	92.5
Additional allowed region(%)		8.4	7.5	8.5	9.1	8.6	7.5
Generously allowed region(%)		0.2	0.2	0.0	0.2	0.4	0.0
Disallowed region(%)		0.0	0.0	0.0	0.0	0.0	0.0

Table S2, Cont. Statistics of Refinement

Parameter		Compound 10	Compound 11	Compound 16	Compound 17	Compound 18	Compound 19
Resolution range(Å)		50.0-2.6	50.0-2.0	50.0-2.2	50.0-2.3	50.0-2.4	50.0-2.7
No. of reflection		24,151	46,100	37,178	29.375	25,193	20,296
Completeness(%)		94.8	94.2	97.8	89.8	87.3	97.3
R_{work}/R_{free}[c]		0.246 / 0.298	0.204 / 0.238	0.205 / 0.247	0.203 / 0.265	0.208 / 0.268	0.271 / 0.311
No. of atoms	Protein	4,022	4,137	4,100	4,102	4,090	3,996
	Non-protein	110	329	257	233	135	73
Mean B overall (Å^2)		60	40	54	65	64	50
Rmsd from ideal values							
Bond lengths(Å)		0.025	0.013	0.020	0.022	0.023	0.022
Bond angles(°)		2.148	1.310	1.741	1.970	1.993	2.127
Ramanchandran plot							
Most favored region(%)		86.3	93.8	92.9	92.0	90.0	83.1
Additional allowed region(%)		13.4	6.2	6.9	8.0	10.0	16.3
Generously allowed region(%)		0.2	0.0	0.0	0.0	0.0	0.7
Disallowed region(%)		0.0	0.0	0.2	0.0	0.0	0.0

[c] R_{free} is the R value obtained for a test set of reflections consisting of randomly selected 5% subset of the data set excluded from refinement.