Supporting Information for:

Fragment-based design of symmetrical bis-benzimidazoles as selective inhibitors of the trimethoprim-resistant, Type II R67 dihydrofolate reductase

Dominic Bastien, Maximilian C. C. J. C. Ebert, Delphine Forge, Jacynthe Toulouse, Natalia Kadnikova, Florent Perron, Annie Mayence, Tien L. Huang, Jean Jacques Vanden Eynde and Joelle N. Pelletier

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Table S1 : List of the compounds screened against R67 DHFR

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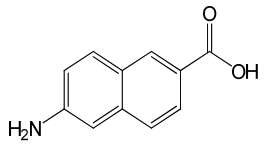
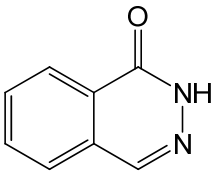
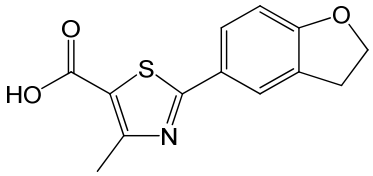
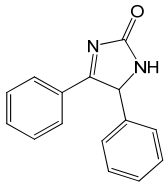
Structure				
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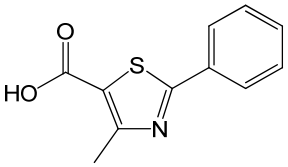
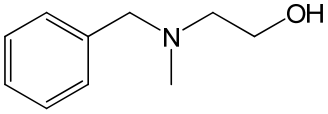
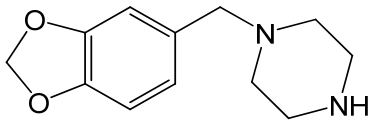
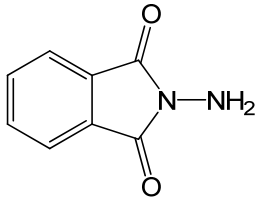
Structure				
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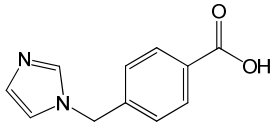
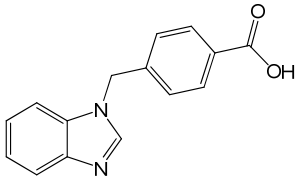
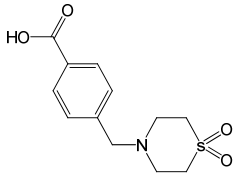
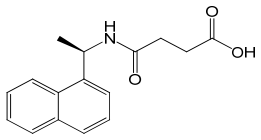
Structure				
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Source	Maybridge	Maybridge	Not available	Aldrich
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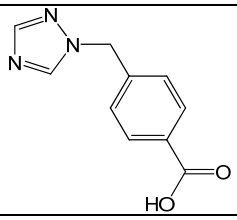
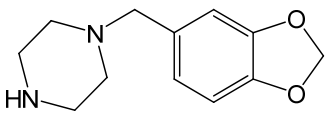
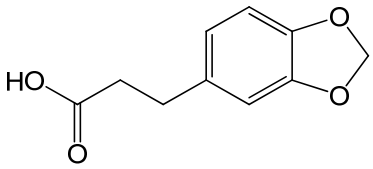
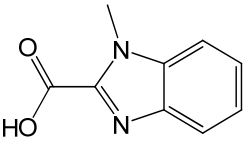
Structure				
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Source	Maybridge	Aldrich	Maybridge	Maybridge

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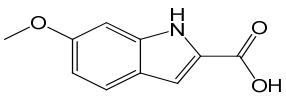
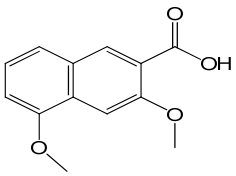
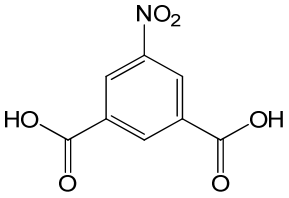
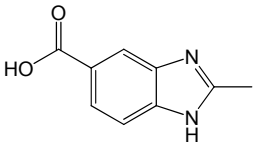
Structure				
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Source	Aldrich	Aldrich	Aldrich	Maybridge

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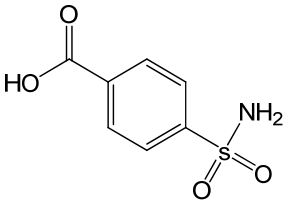
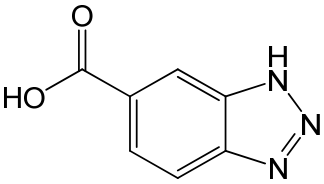
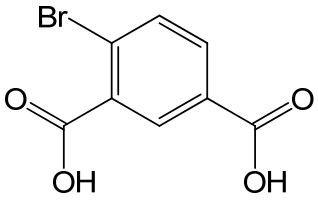
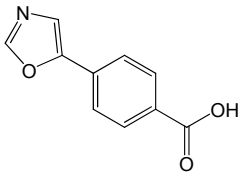
Structure				
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Source	Maybridge	Maybridge	Not available	Maybridge

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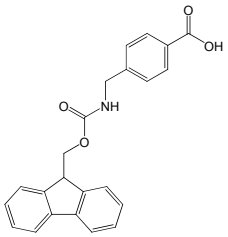
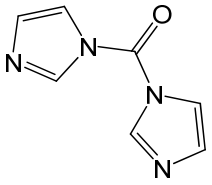
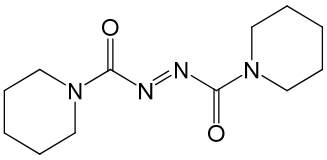
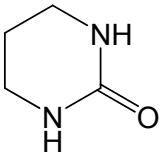
Structure				
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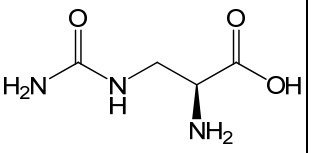
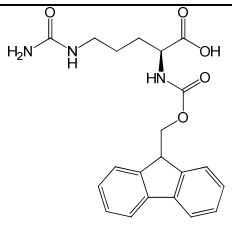
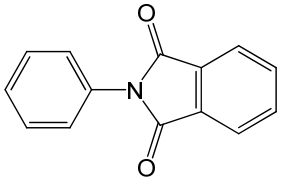
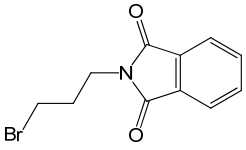
Structure				
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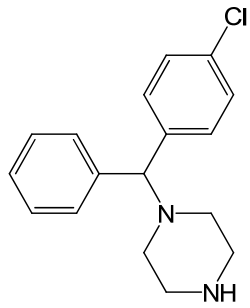
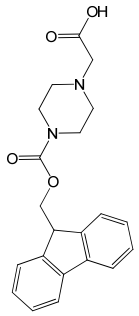
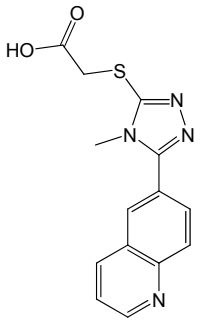
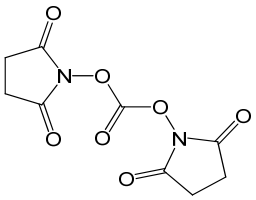
Structure				
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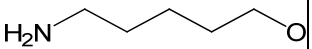
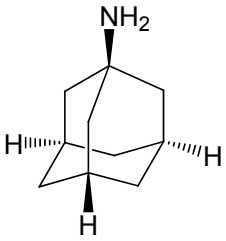
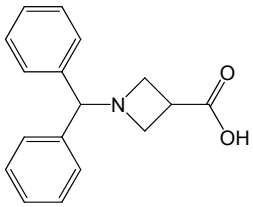
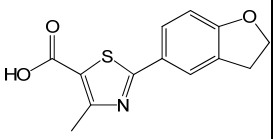
Structure				
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Source	Aldrich	Aldrich	Maybridge	Maybridge

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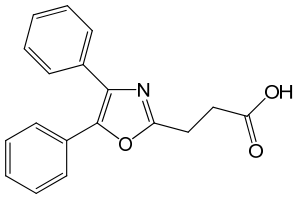
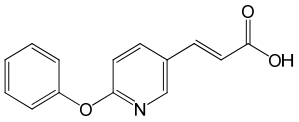
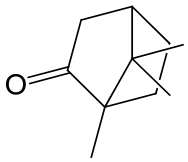
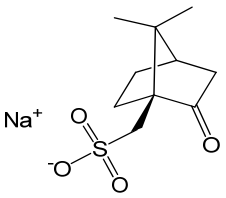
Structure				
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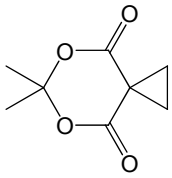
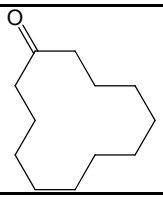
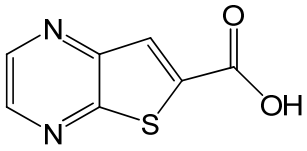
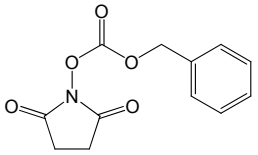
Structure				
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Source	Aldrich	Aldrich	Maybridge	Aldrich

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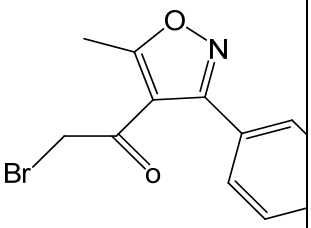
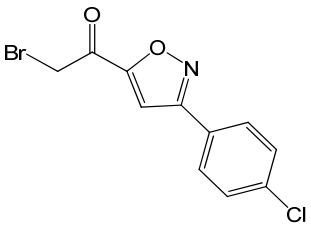
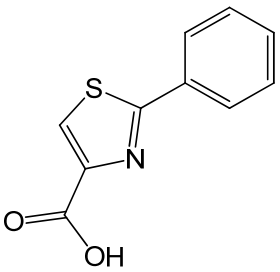
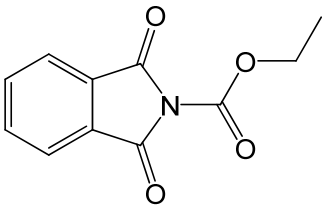
Structure				
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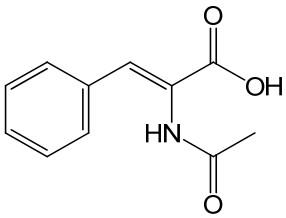
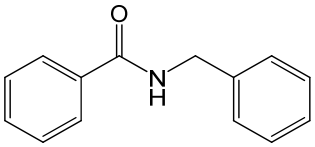
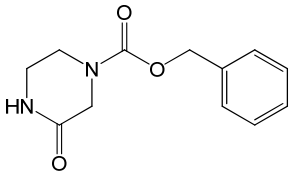
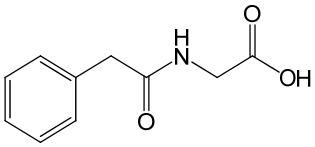
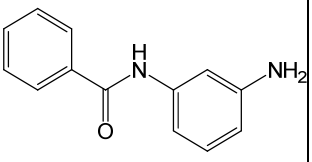
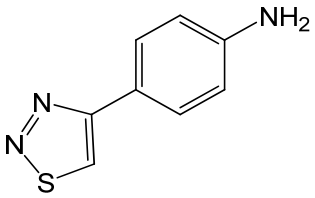
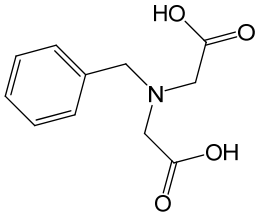
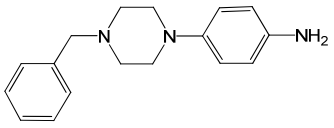
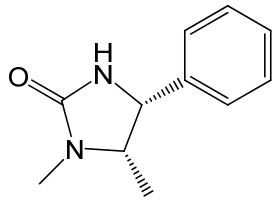
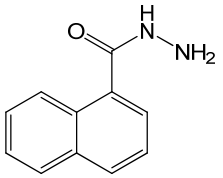
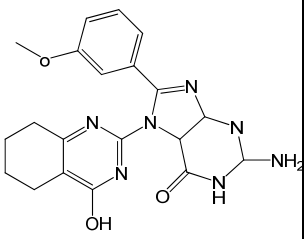
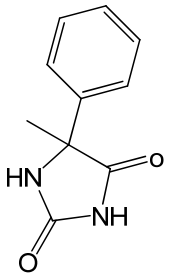
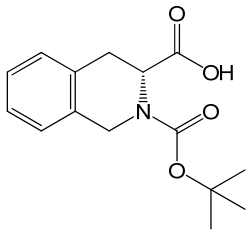
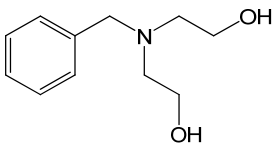
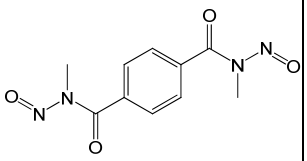
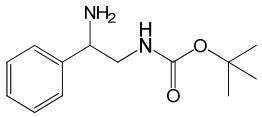
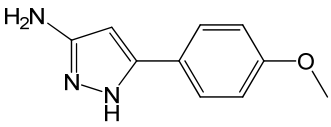
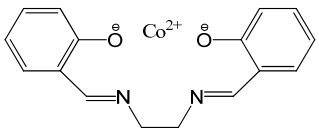
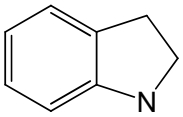
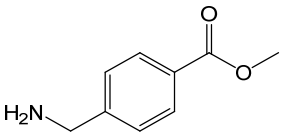
Source	Maybridge	Not available	Maybridge	Lancaster

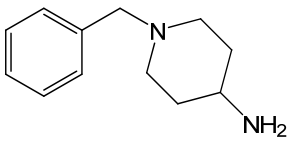
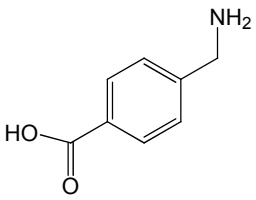
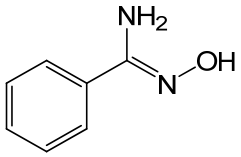
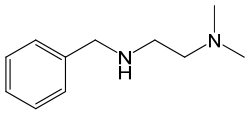
#	53	54	55	56
Structure				
CAS	5469-45-4	1485-70-7	78818-15-2	500-98-1
Source	Aldrich	Aldrich	Maybridge	TCI

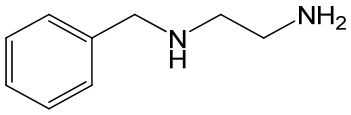
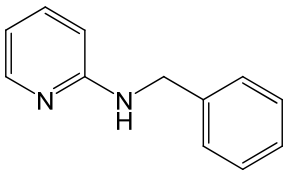
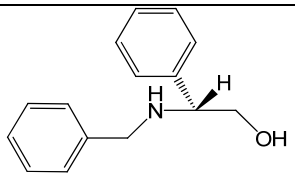
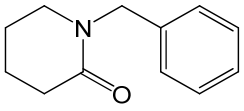
#	57	58	59	60
Structure				
CAS	16091-26-2	Not available	3987-53-9	16154-69-1
Source	Trans World	Not available	Aldrich	Bionet

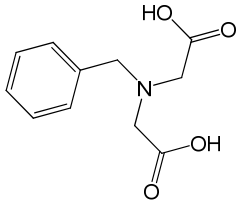
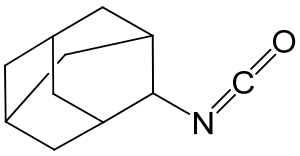
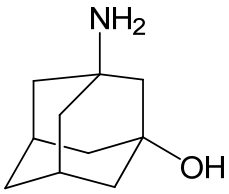
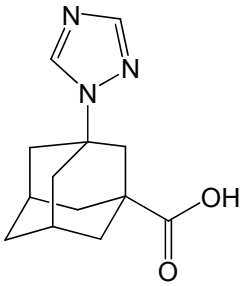
#	61	62	63	64
Structure				
CAS	92841-65-1	43038-45-5	Not available	6843-49-8
Source	Fluka	Lancaster	Not available	Acros

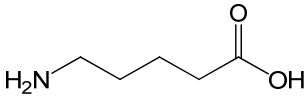
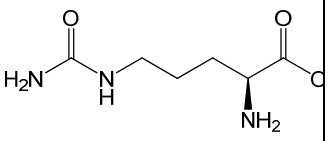
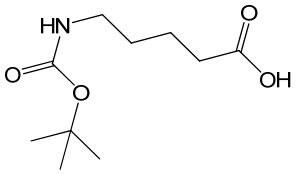
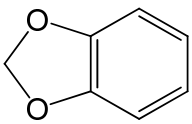
#	65	66	67	68
Structure				
CAS	115962-35-1	101-32-6	133-55-1	167298-44-4
Source	Pep Tech	Aldrich	Aldrich	Infarmatik

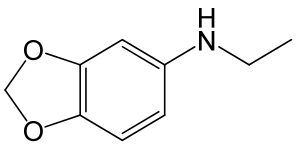
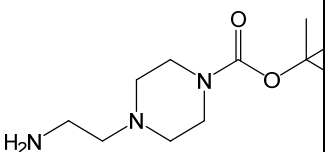
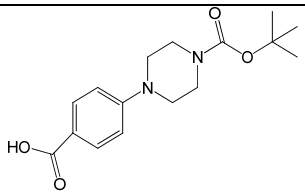
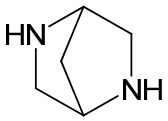
#	69	70	71	72
Structure				
CAS	19541-95-8	14167-18-1	496-15-1	6232-11-7
Source	Aldrich	Aldrich	Aldrich	Aldrich

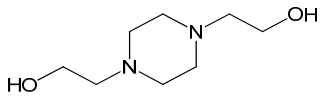
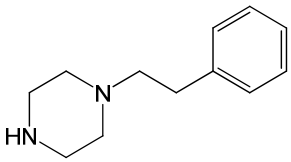
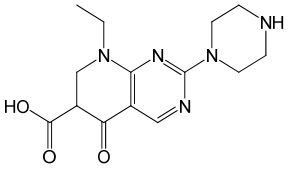
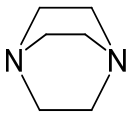
#	73	74	75	76
Structure				
CAS	50541-93-0	56-91-7	613-92-3	103-55-9
Source	Aldrich	Aldrich	Maybridge	Lancaster

#	77	78	79	80
Structure				
CAS	4152-09-4	6935-27-9	14231-57-3	4783-65-7
Source	TCI	Lancaster	Aldrich	Aldrich

#	81	82	83	84
Structure				
CAS	3987-53-9	4411-25-0	702-82-9	Not available
Source	Aldrich	Aldrich	Aldrich	Matrix

#	85	86	87	88
CAS				
CAS	660-88-8	372-75-8	27219-07-4	274-09-9
Source	Aldrich	Aldrich	Aldrich	Aldrich

#	89	90	91	92
Structure				
CAS	32953-14-3	192130-34-0	162046-66-4	132747-20-7
Source	TCI	Chembasics	Jw-Pharmlab	Alfa

#	93	94	95	96
Structure				
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Source	Aldrich	Not available	Aldrich	Aldrich
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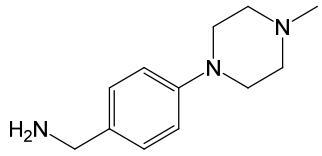
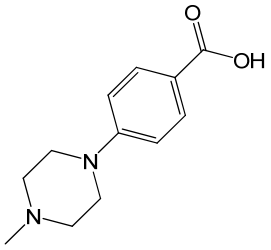
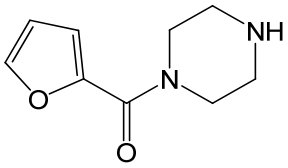
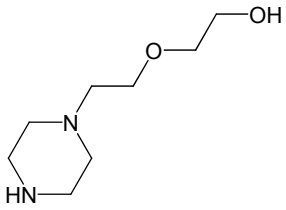
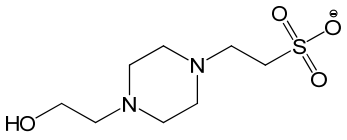
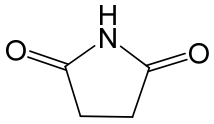
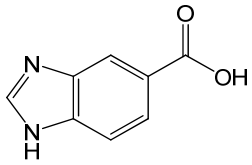
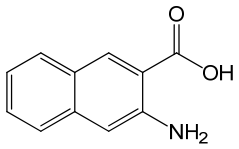
Structure				
CAS	216144-45-5	86620-62-4	40172-95-0	13349-82-1
Source	Maybridge	Maybridge	Acros	Aldrich
#	101	102	103	104
Structure				
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Source	Fluka	Eastman	Aldrich	Aldrich
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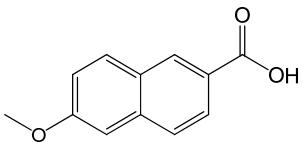
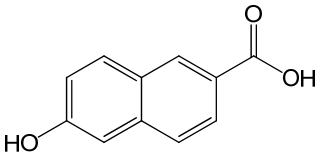
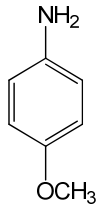
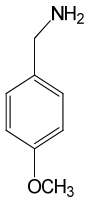
Structure				
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Source	Aldrich	Aldrich	Aldrich	Aldrich

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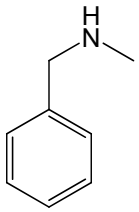
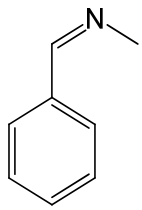
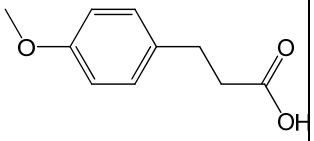
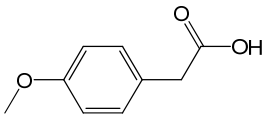
Structure				
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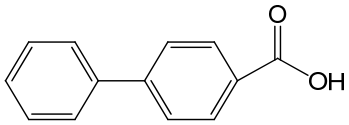
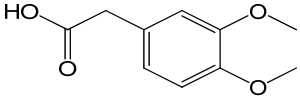
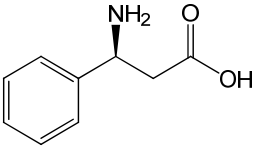
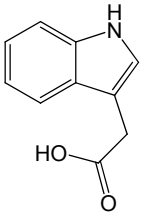
Structure				
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Source	Accurate Chemicals & Scientific	Aldrich	CSPS	Not available

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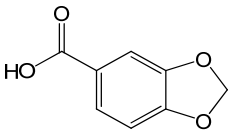
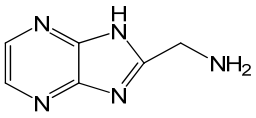
Structure		
CAS	94-53-1	5993-91-9
Source	Aldrich	Aldrich

Characterization of compounds 8, 8a and 9:

2,2'-[1,5-pentanediylbis(4-oxyphenylene)]-1H-benzimidazole-5-carboxylic acid; (8)

IR(KBr, cm-1): 3691-2362, 1680, 1609, 1505,1256.

1H NMR (DMSO-d6, ppm): 1,6 (m, 2H, J = 6Hz, CH_2-CH_2-CH_2-O), 1.8 (m, 4H, J = 6Hz, CH_2-CH_2-O), 4.1 (t, 4H,J= 6Hz, CH_2-CH_2-CH_2-O), 7.2 (d, 4H, J= 8Hz, C_6H_4:H5' and H3'), 7.6 (d, 2H, J= 8Hz, benz: H7), 7.8 (d, 2H, J= 8Hz, benz: H6), 8.2 (m, 6H, C_6H_4:H6', H2' and benz: H4), 13 (s, 2H, COOH).

Yield: 97%

4,4'-[1,5-pentanediyl-(oxy)]-bisbenzoic acid(8a)

IR(KBr, cm-1): 1683,1605, 1254,1170,771.

1H NMR (DMSO-d6, ppm): 1.5 (m, 2H, J = 6Hz, O-CH_2-CH_2-CH_2), 1.8 (m, 4H, J = 6Hz,O-CH_2-CH_2-CH_2),4 (t, 4H, J = 6Hz, O-CH_2-CH_2-CH_2),7 (d, 4H, J = 8 Hz, C_6H_4: H3, H5), 7.9 (d, 4H,J = 8Hz, C_6H_4: H2,H6), 12.6 (s, 2H, COOH).

Yield: 72%

2'-(4,4'-(2-hydroxypropane-1,3-diyl)bis(oxy)bis(4,1-phenylene))bis(1H-benzimidazole-5-carboxylic)acid (9)

IR(KBr, cm-1): 3400, 2950, 1685, 1603.

1H NMR (DMSO-d6, ppm): 4.24 (m, J = 6Hz, 5H, -CH_2-CH(OH)-CH_2), 5.51 (sl, 1H, -OH), 7.22 (d, 4H, J = 8.7 Hz, C_6H_4: H3', H5'), 7.66 (d, 2H, benz: H6), 7.86 (d, 2H, benz: H7), 8.18 (m, 6H, C_6H_4: H2', H6' and benz: H4), 13.02 (s, 2H, COOH).

Yield: 94%

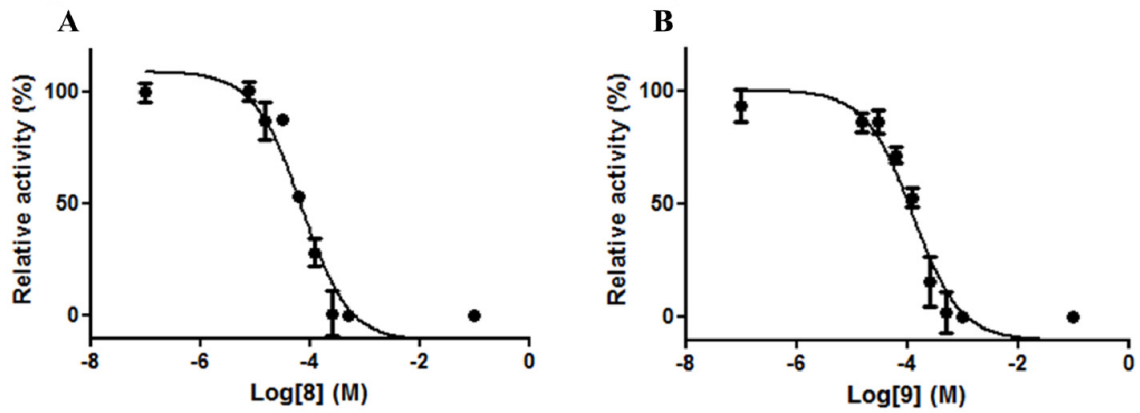


Figure S1: **IC_{50} plot for compounds 8 and 9**. The activity of R67 DHFR was measured in presence of (A) compound **8** or (B) compound **9**, and was corrected according to the control without inhibitor. The data were fit to the 'one-site binding' equation. Values are given as the mean ± standard deviation for triplicate results.

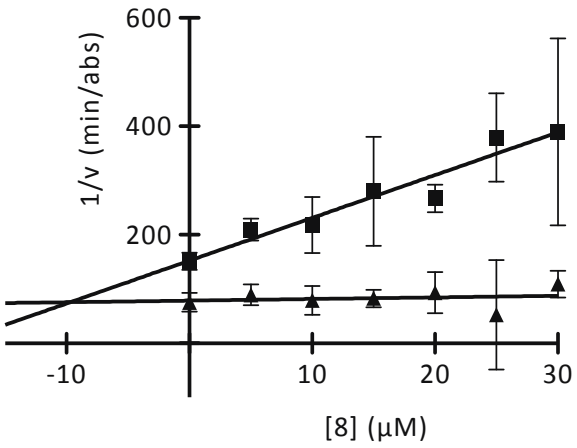


Figure S2. Dixon plot for determination of type of inhibition of R67 DHFR by 8, relative to the DHF substrate. The reciprocal rates of DHFR activity were plotted as a function of inhibitor concentration. NADPH co-factor was held constant at 50 μM. DHF substrate was held at either 25 μM (~ K_M^{DHF}, ■) or 164 μM (~ 7 × K_M^{DHF}, ▲). Values are given as the mean ± standard deviation for triplicate results. The intercept of the two slopes gave K_i (**8**) = 9.7 μM.

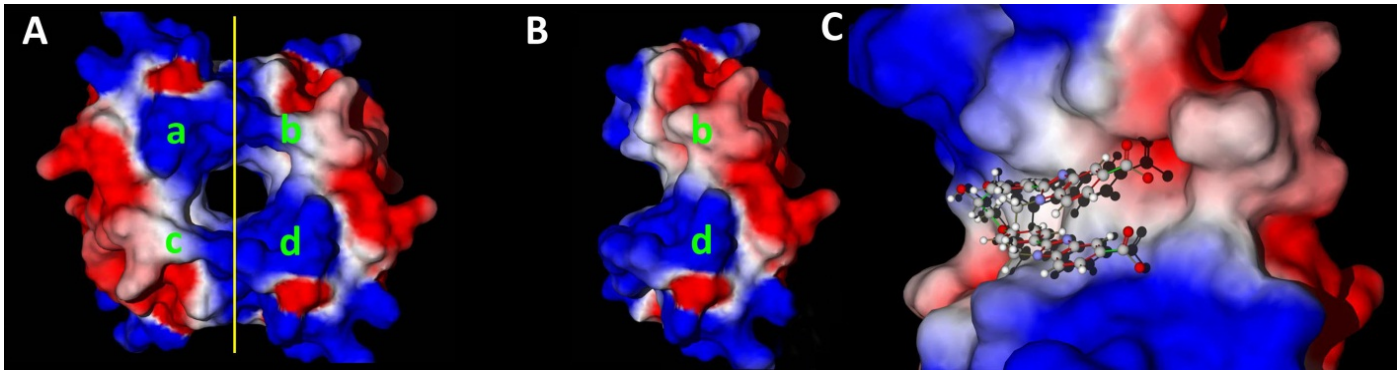


Figure S3: Docking of compound 9 on R67 DHFR using AutodockVina. (A) R67 DHFR is shown as an electrostatics surface with the basic, acidic and hydrophobic regions shown in blue, red and white, respectively, and monomers labelled a-d. (B) Two monomers are shown, where the tetramer was split as indicated in panel A. (C) Conformation of **9** shown in ball and sticks representation in the active site of the DHFR R67. Hydrogen, carbon, nitrogen and oxygen are in white, grey, blue and red, respectively.