Design, synthesis and molecular modeling of novel pyrido[2,3-*d*]pyrimidine analogs as antifolates: Application of Buchwald-Hartwig aminations of heterocycles

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Supporting Information

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Table S1. High-Resolution Mass Spectra (HRMS)

Compd	Ionization method	Formula	Calcd mass	Found mass
4	EI	$C_{13}H_{12}N_6$	252.1123	252.1125
5	EI	$C_{14}H_{14}N_6O$	282.1229	282.1228
6	EI	$C_{14}H_{14}N_6O$	282.1229	282.1225
7	ESI	$C_{15}H_{17}N_6O_2$ $(M+H)^+$	313.1413	313.1403
8	EI	$C_{16}H_{18}N_6O_3$	342.1440	342.1445
9	ESI	$C_{14}H_{15}N_6$ $(M+H)^+$	267.1358	267.1372
10	ESI	$C_{16}H_{19}N_6$ $(M+H)^+$	295.1671	295.1656
11	EI	$C_{17}H_{14}N_6$	302.1279	302.1285
12	EI	$C_{13}H_{11}N_7O_2$	297.0974	297.0966
13	ESI	$C_{17}H_{15}N_6$ $(M+H)^+$	303.1358	303.1352
14	EI	$C_{13}H_{10}N_6F_2$	288.0935	288.0931
15	ESI	$C_{13}H_{10}N_6F_3$ $(M+H)^+$	307.0919	307.0927
16	ESI	$C_{14}H_{15}N_6$ $(M+H)^+$	267.1358	267.1375
17	EI	$C_{15}H_{16}N_6O$	294.1468	294.1452
18	ESI	$C_{15}H_{17}N_6O$ $(M+H)^+$	297.1464	297.1469
19	ESI	$C_{16}H_{19}N_6O_2$ $(M+H)^+$	327.1569	327.1557
20	ESI	$C_{17}H_{21}N_6O_3$ $(M+H)^+$	357.1675	357.1680
21	ESI	$C_{15}H_{17}N_6$ $(M+H)^+$	281.1515	281.1520
22	ESI	$C_{17}H_{21}N_6$ $(M+H)^+$	309.1828	309.1807
23	ESI	$C_{14}H_{14}N_7O_2$ $(M+H)^+$	312.1209	312.1237
24	ESI	$C_{18}H_{17}N_6$ $(M+H)^+$	317.1515	317.1542
25	ESI	$C_{14}H_{13}N_6F_2$ $(M+H)^+$	303.1170	303.1154
26	ESI	$C_{14}H_{12}N_6F_3$ $(M+H)^+$	321.1076	321.1068
31p	EI	$C_9H_{13}NO_2$	167.0946	167.0947
31s	EI	$C_{10}H_{15}N$	149.1204	149.1220
31v	EI	$C_7H_7NF_2$	143.0546	143.0545
31w	EI	$C_7H_6NF_3$	161.0452	161.0444
32a	EI	$C_{23}H_{28}N_6O_2$	420.2273	420.2268
32b	ESI	$C_{24}H_{31}N_6O_3$ $[M+H]^+$	451.2458	451.2456

32c	ESI	$C_{24}H_{31}N_6O_3$ $[M+H]^+$	451.2458	451.2455
32d	ESI	$C_{25}H_{33}N_6O_4$ $[M+H]^+$	481.2563	481.2564
32e	EI	$C_{26}H_{34}N_6O_5$	510.2590	510.2580
32f	ESI	$C_{24}H_{30}N_6O_2Na$ $[M+Na]^+$	457.2328	457.2300
32g	ESI	$C_{26}H_{35}N_6O_2$ $[M+H]^+$	463.2821	463.2796
32h	EI	$C_{27}H_{30}N_6O_5$	470.2430	470.2422
32i	ESI	$C_{23}H_{28}N_7O_4$ $[M+H]^+$	466.2203	466.2179
32j	EI	$C_{23}H_{28}N_7O_4$	470.2430	470.2423
32m	ESI	$C_{24}H_{30}N_6O_2Na$ $[M+Na]^+$	457.2328	457.2314
32n	ESI	$C_{24}H_{30}N_6O_2Na$ $[M+H]^+$	465.2614	465.2578
32p	ESI	$C_{26}H_{35}N_6O_4$ $[M+H]^+$	495.2720	495.2749
32q	ESI	$C_{27}H_{36}N_6O_5$ $[M+Na]^+$	547.2645	547.2615
32s	ESI	$C_{27}H_{37}N_6O_2$ $[M+H]^+$	477.2978	477.2979
32t	ESI	$C_{24}H_{29}N_7O_4Na$ $[M+Na]^+$	502.2179	502.2181
32u	EI	$C_{28}H_{32}N_6O_2$	484.2586	484.2594
32v	ESI	$C_{24}H_{28}N_6O_2F_2Na$ $[M+Na]^+$	493.2140	493.2178
32w	ESI	$C_{24}H_{28}N_6O_2F_3$ $[M+H]^+$	489.2226	489.2234

Table S2. Elemental analysis:

Compd	**Formula**	**Calcd %**				**Found%**			
		C	H	N	F	C	H	N	F
4	$C_{13}H_{12}N_6 \cdot 0.11\ H_2O$	61.41	4.84	33.05		61.46	5.03	32.99	
5	$C_{14}H_{14}N_6O \cdot 0.38\ H_2O$	58.13	5.15	29.05		57.96	5.26	29.42	
6	$C_{14}H_{14}N_6O \cdot 0.57\ H_2O$	57.48	5.21	28.73		57.37	5.41	28.98	
7	$C_{15}H_{16}N_6O_2 \cdot 0.20\ H_2O$	57.02	5.23	26.60		57.13	5.20	26.35	
8	$C_{16}H_{18}N_6O_3 \cdot 0.37\ H_2O$	55.07	5.41	24.08		55.05	5.36	24.12	
9	$C_{14}H_{14}N_6$	63.14	5.30	31.56		63.19	5.20	31.47	
10	$C_{16}H_{18}N_6 \cdot 0.11\ H_2O$	64.84	6.20	28.35		64.77	6.29	28.53	
11	$C_{17}H_{14}N_6$	67.53	4.67	27.80		67.49	4.78	27.46	
12	$C_{13}H_{11}N_7O_2$	52.52	3.73	32.98		52.81	3.93	32.83	
13	$C_{17}H_{14}N_6 \cdot 0.38\ H_2O$	66.02	4.81	27.17		65.88	4.97	27.55	
14	$C_{13}H_{10}N_6F_2 \cdot 0.55\ H_2O$	52.35	3.75	28.18	12.74	52.11	3.91	28.55	12.99
15	$C_{13}H_9N_6F_3 \cdot 0.44\ H_2O$	49.71	3.17	26.76	18.14	49.47	3.19	26.97	18.48
16	$C_{14}H_{14}N_6 \cdot 0.25\ H_2O$	62.11	5.39	31.04		62.05	5.51	31.18	
17	$C_{15}H_{16}N_6O \cdot 0.29\ H_2O$	59.73	5.54	27.86		59.63	5.79	28.13	
18	$C_{15}H_{16}N_6O \cdot 0.36\ H_2O$	59.48	5.57	27.74		59.40	5.83	27.96	
19	$C_{16}H_{18}N_6O_2$	58.88	5.56	25.75		58.91	5.49	25.78	
20	$C_{17}H_{20}N_6O_3 \cdot 0.16\ H_2O$	56.83	5.70	23.39		56.89	5.77	23.25	
21	$C_{15}H_{16}N_6$	64.27	5.75	29.98		63.89	5.86	29.58	
22	$C_{17}H_{20}N_6$	66.21	6.54	27.25		65.91	6.46	27.01	
23	$C_{14}H_{13}N_7O_2 \cdot 0.39\ H_2O$	52.81	4.36	30.79		52.77	4.56	30.91	
24	$C_{18}H_{16}N_6$	68.34	5.10	26.56		68.56	5.03	26.35	
25	$C_{14}H_{12}N_6F_2$	55.63	4.00	27.80	12.57	55.81	3.91	27.68	12.47
26	$C_{14}H_{11}N_6F_3 \cdot 0.15\ H_2O$	52.07	3.52	26.03	17.65	52.05	3.49	26.11	17.58

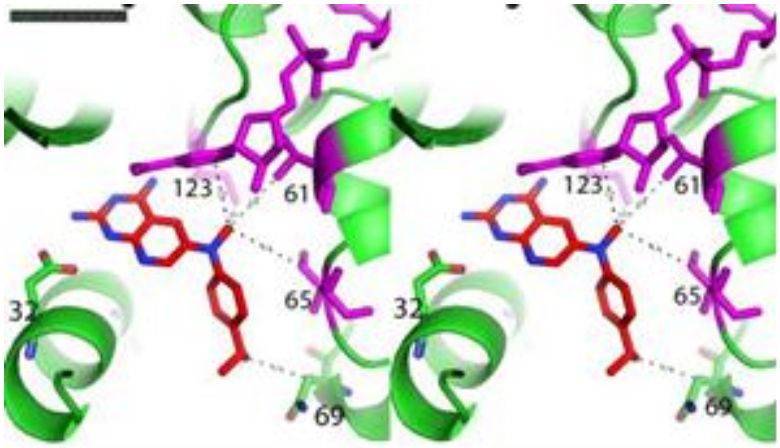

Figure S1. Stereoview of the active site of F69N mutant of *pc*DHFR (green) as a ternary complex with NADPH (violet) and **22** (red). Those residues that contact the N-methyl and 4'-isopropyl are illustrated: Glu32, Ile123, Thr61, NADPH, Ile65 and Asn69 (violet).

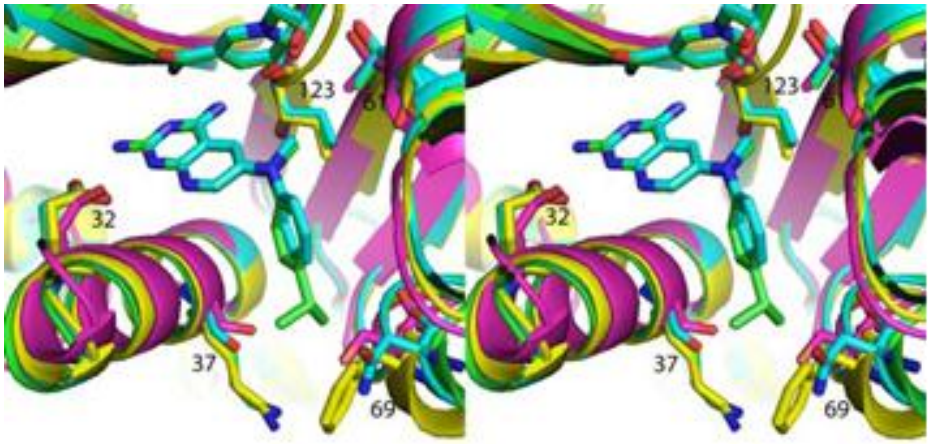

Figure S2. Stereoview of the active site comparing the structures of K37S/F69N double mutant of pcDHFR as a ternary complex with **16** (cyan), with **22** (green), with wild type pcDHFR (yellow) and with a homology model of pjDHFR (vc) (violet). The residues Glu/Asp32, Lys/Ser37, Asn/Ser/Phe69, Thr61 and Ile123 are labeled.

Table S3. Alignment of human (h), *Pneumocystis carinii* (pc) and *Pneumocystis jirovecii* (pj DHFR.

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 hDHFR       --MVGSLNCIVAVSQNMGIGKNGDLPWPPLRNEFRYFQRMTT---TSSVEGKQNLVIMGK
pcDHFR       MNQQKSLTLIVALTTSYGIGRSNSLPWK-LKKEISYFKRVTSFVPTFDSFESMNVVLMGR
pcDHFR       MDWQKSLTLIVALTLSRGIGLKNDLPWK-LKSDMMFFSRVTSGLLVTRSTGQMNVVLMGR

hDHFR       KTWFSIPEKNRPLKGRINLVLSRELKEPPQGAHFLSRSLDDALKLTEQ---PELANKVDM
pcDHFR      KTWESIPLQFRPLKGRINVVITRNESLDLGNGIHSAKSLDHALELLYRTYGSESSVQINR
pjDHFR      KTWESLPAHSRPLKNRINVVISRQEVLDLGGGAYHARSLDDALALLSQIYDSTSKIQLNR

hDHFR       VWIVGGSSVYKEAMNHPGHLKLFVTRIMQDFESDTFFPEIDLEKYKLLPEYPGVLSDVQ-
pcDHFR      IFVIGGAQLYKAAMDHPKLDRIMATIIYKDIHCDVFFPLKFRDKEWSSVWKKEKHSDLES
pjDHFR      VFVIGGGELYKAAMEHSRLNRIIATVIHNEVDCDVFFPIDFRSSQSCLPWRKQDHSVLEA

hDHFR       -----------EEKGIKYKFEVYEKND
pcDHFR      WVGTKVPHGKINEDGFDYEFEMWTRDL
pjDHFR      WVGSKVPQGKINENGFIYEFEMWIRDI
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