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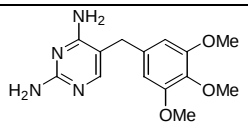
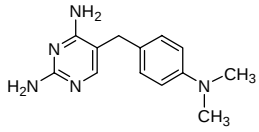
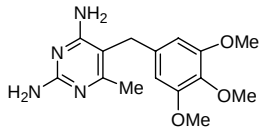
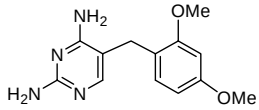
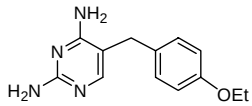
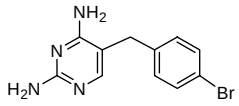
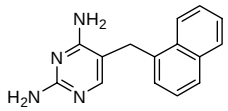
Immobilization of Malarial (*Plasmidium falciparum*) Dihydrofolate Reductase for the Selection of Tight-Binding Inhibitors from Combinatorial Library

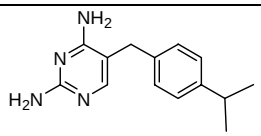
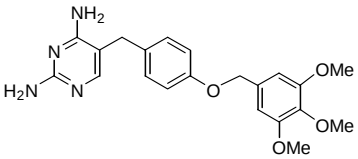
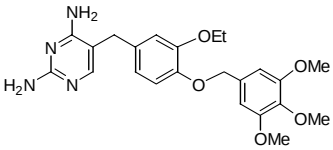
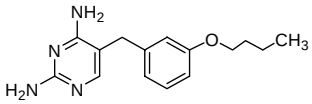
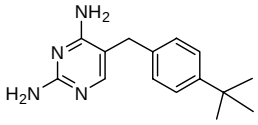
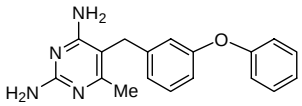
S2 **Table S-1** Spectroscopic data of trimethprim derivatives

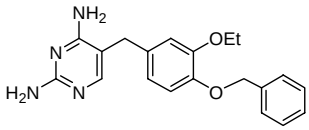
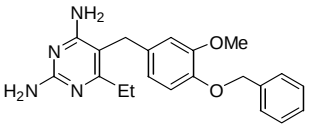
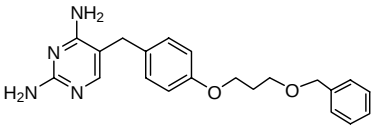
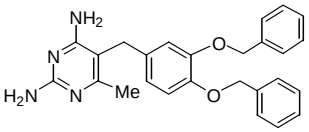
S6 **Figure S-1** HPLC spectrum of trimethoprim derivatives in control

S7 **Figure S-2** Lineweaver-Burk plots of the Pf DHFR-C59RS108N by Tmp(A) and T9 (B)

Table S-1 Spectroscopic data of Tmp derivatives

Cpd	structure	^{1}H NMR
Tmp		3.52 (2H, s, CH_2), 3.61 (3H, s, OCH_3), 3.71 (6H, s, 2xOCH_3), 5.70 (2H, bs, NH_2), 6.10 (2H, bs, NH_2), 6.54 (2H, s, ArH) and 7.51 (1H, s, H-pyrimidine)
T1		2.83 (6H, s, 2xCH_3), 3.47 (2H, s, CH_2), 5.66 (2H, bs, NH_2), 6.01 (2H, bs, NH_2), 6.65 (2H, d, *J* = 8.6 Hz, ArH), 7.03 (2H, d, *J* = 8.6 Hz, ArH) and 7.43 (1H, s, H-pyrimidine)
T2		2.07 (3H, s, CH_3), 3.61 (3H, s, OCH_3), 3.66 (2H, s, CH_2), 3.69 (6H, s, 2xOCH_3), 5.63 (2H, bs, NH_2), 5.96 (2H, bs, NH_2) and 6.44 (2H, s, ArH)
T3		3.43 (2H, s, CH_2), 3.73 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 5.65 (2H, bs, NH_2), 6.02 (2H, bs, NH_2), 6.45 (1H, dd, *J* = 8.20, 2.28 Hz, ArH), 6.55 (1H, d, *J* = 2.22 Hz, ArH), 6.96 (1H, d, *J* = 8.35 Hz, ArH) and 7.32 (1H, s, H-pyrimidine)
T4		1.30 (3H, t, *J* = 6.97 Hz, CH_2*CH_3*), 3.53 (2H, s, CH_2), 3.97 (2H, q, *J* = 6.97, O*CH_2*CH_3), 5.69 (2H, bs, NH_2), 6.06 (2H, bs, NH_2), 6.82 (2H, d, *J* = 8.6 Hz, ArH), 7.11 (2H, d, *J* = 8.6 Hz, ArH) and 7.46 (1H, s, H-pyrimidine)
T5		3.59 (2H, s, CH_2), 5.75 (2H, bs, NH_2), 6.13 (2H, bs, NH_2), 7.18 (2H, d, *J* = 8.6 Hz, ArH), 7.46 (2H, d, *J* = 8.4 Hz, ArH) and 7.51 (1H, s, H-pyrimidine)
T6		4.05 (2H, s, CH_2), 5.73 (2H, bs, NH_2), 6.28 (2H, bs, NH_2), 7.14 (1H, s, H-pyrimidine) and 7.24 -7.98 (7H, m, ArH)

T7		1.17 (6H, d, *J* = 6.9 Hz, 2xCH_3), 2.83 (1H, hept, *J* = 6.9 Hz, CH), 3.56 (2H, s, CH_2), 5.71 (2H, bs, NH_2), 6.08 (2H, bs, NH_2), 7.13 (4H, s, ArH) and 7.49 (1H, s, H-pyrimidine)
T8		3.53 (2H, s, CH_2Ar), 3.65 (3H, s, OCH_3), 3.77 (6H, s, 2xOCH_3), 4.94 (2H, s, OCH_2Ar), 5.69 (2H, bs, NH_2), 6.06 (2H, bs, NH_2), 6.76 (2H, s, ArH), 6.93 (2H, d, *J* = 8.6 Hz, ArH), 7.14 (2H, d, *J* = 8.6 Hz, ArH) and 7.46 (1H, s, H-pyrimidine)
T9		1.32 (3H, t, *J* = 6.9Hz, CH_2*CH_3*), 3.52 (2H, s, CH_2), 3.65 (3H, s, OCH_3), 3.77 (6H, s, 2xOCH_3), 4.00 (2H, q, *J* = 6.9Hz, O*CH_2*CH_3), 4.96 (2H, s, OCH_2), 5.68 (2H, bs, NH_2), 6.05 (2H, bs, NH_2), 6.70 (1H, dd, *J* = 1.7, 8.2 Hz, ArH), 6.76 (2H, s, ArH), 6.88 (1H, d, *J* = 1.8 Hz, ArH), 6.94 (1H, d, *J* = 8.2 Hz, ArH) and 7.47 (1H, s, H-pyrimidine)
T10		0.92 (3H, t, *J* = 7.43 Hz, CH_3), 1.41 (2H, hex, *J* = 7.44 Hz, CH_2), 1.67 (2H, pent, *J* = 6.57 Hz, CH_2), 3.55 (2H, s, CH_2), 3.91 (2H, t, *J* = 6.5 Hz, CH_2), 5.70 (2H, bs, NH_2), 6.07 (2H, bs, NH_2), 6.71-6.78 (3H, m, ArH), 7.15 (1H, t, *J* = 7.85 Hz, ArH) and 7.49 (1H, s, H-pyrimidine)
T11		1.25 (9H, s, 3xCH_3), 3.56 (2H, s, CH_2), 5.70 (2H, bs, NH_2), 6.07 (2H, bs, NH_2), 7.13 (2H, d, *J* = 8.24 Hz, ArH), 7.29 (2H, d, *J* = 8.26 Hz, ArH) and 7.49 (1H, s, H-pyrimidine)
T12		2.01 (3H, s, CH_3Ar), 3.74 (2H, s, CH_2Ar), 5.66 (2H, bs, NH_2), 6.04 (2H, bs, NH_2), 6.77 (1H, d, *J* = 7.4 Hz, ArH), 6.82 (1H, s, ArH), 6.90 (1H, d, *J* = 7.66 Hz, ArH), 6.98 (2H,

		d, J = 7.85 Hz, ArH), 7.13 (1H, t, J = 7.36 Hz, ArH), 7.27 (1H, t, J = 7.85 Hz, ArH) and 7.38 (2H, t, J = 7.7 Hz, ArH)
T13		1.31 (3H, t, J = 6.9 Hz, CH_2CH_3), 3.51 (2H, s, CH_2), 4.00 (2H, q, J = 6.9 Hz, OCH_2CH_3), 5.05 (2H, s, CH_2), 5.68 (2H, bs, NH_2), 6.05 (2H, bs, NH_2), 6.68 (1H, dd, J = 1.7, 8.2 Hz, ArH), 6.88 (1H, d, J = 1.7 Hz, ArH), 6.92 (1H, d, J = 8.2 Hz, ArH), 7.30-7.44 (5H, m, ArH) and 7.47 (1H, s, H-pyrimidine)
T14		1.01 (3H, t, J = 7.5 Hz, CH_2CH_3), 2.36 (2H, q, J = 7.5 Hz, CH_2CH_3), 3.67 (2H, s, CH_2Ar), 3.72 (3H, s, OCH_3), 5.01 (2H, s, OCH_2Ar), 5.62 (2H, bs, NH_2), 5.91 (2H, bs, NH_2), 6.54 (1H, dd, J = 1.6, 8.26 Hz, ArH), 6.83 (1H, d, J = 1.6 Hz, ArH), 6.91 (1H, d, J = 8.26 Hz, ArH) and 7.30-7.43 (5H, m, ArH)
T15		1.96 (2H, quint, J = 6.26 Hz, $CH_2CH_2CH_2$), 3.52 (2H, s, CH_2Ar), 3.57 (2H, t, J = 6.28 Hz, OCH_2CH_2), 3.99 (2H, t, J = 6.27 Hz, $ArOCH_2CH_2$), 4.47 (2H, s, OCH_2Ar), 5.68 (2H, bs, NH_2), 6.05 (2H, bs, NH_2), 6.82 (2H, d, J = 8.48 Hz, ArH), 7.11 (2H, d, J = 8.48 Hz, ArH), 7.26-7.32 (5H, m, ArH) and 7.46(1H, s, H-pyrimidine)
T16		1.99 (3H, s, CH_3Ar), 3.62 (2H, s, CH_2Ar), 5.05 (4H, s, 2xOCH_2Ar), 5.63 (2H, bs, NH_2), 5.95 (2H, bs, NH_2), 6.59 (1H, dd, J = 1.2, 8.1 Hz, ArH), 6.89 (1H, d, J = 1.3 Hz, ArH), 6.93 (1H, d, J = 8.2 Hz, ArH) and 7.28-7.43 (10H, m, ArH)

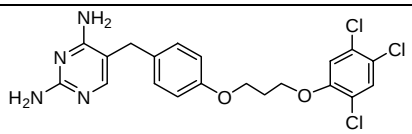
T17		2.16 (2H, quint, *J* = 6.2 Hz, $CH_2\mathit{CH_2}CH_2$), 3.52 (2H, s, CH_2Ar), 4.09 (2H, t, *J* = 6.2 Hz, $O\mathit{CH_2}CH_2$), 4.25 (2H, t, *J* = 6.1 Hz, $O\mathit{CH_2}CH_2$), 5.67 (2H, bs, NH_2), 6.04 (2H, bs, NH_2), 6.85 (2H, d, *J* = 8.5 Hz, ArH), 7.10 (2H, d, *J* = 8.5 Hz, ArH), 7.45 (1H, s, ArH), 7.49 (1H, s, ArH) and 7.78 (1H, s, H-pyrimidine)

Figure S-1 HPLC spectrum of trimethoprim derivatives in control

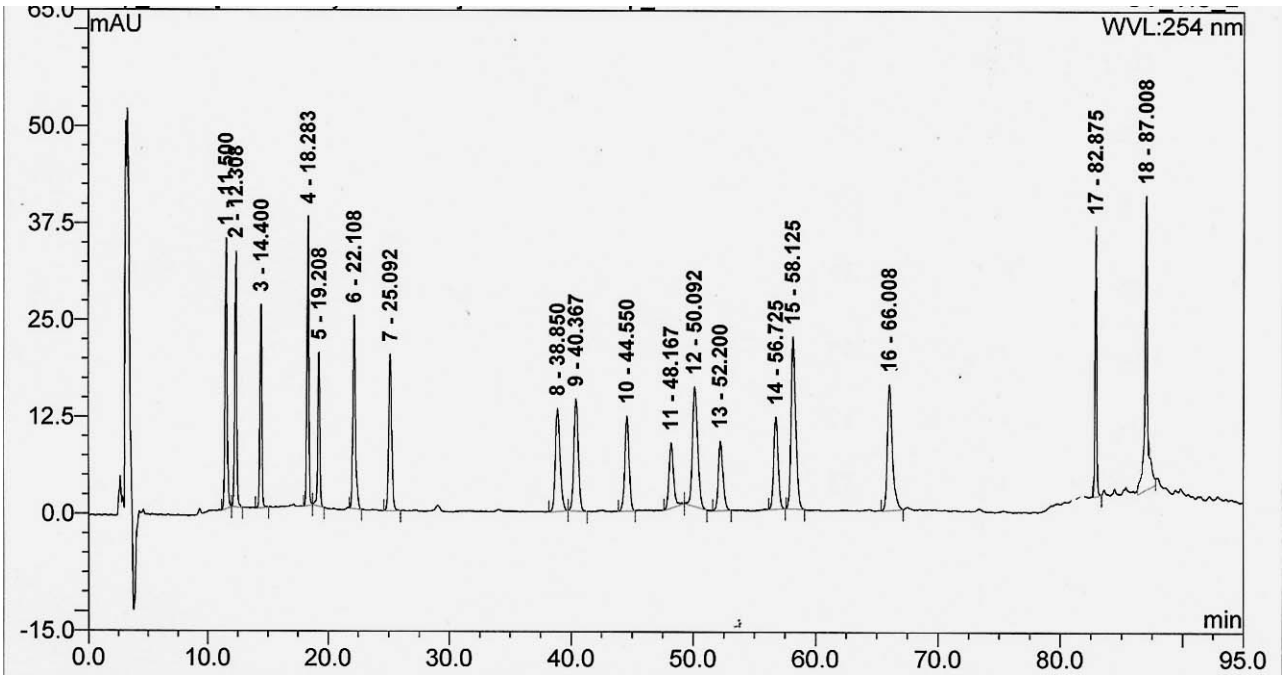
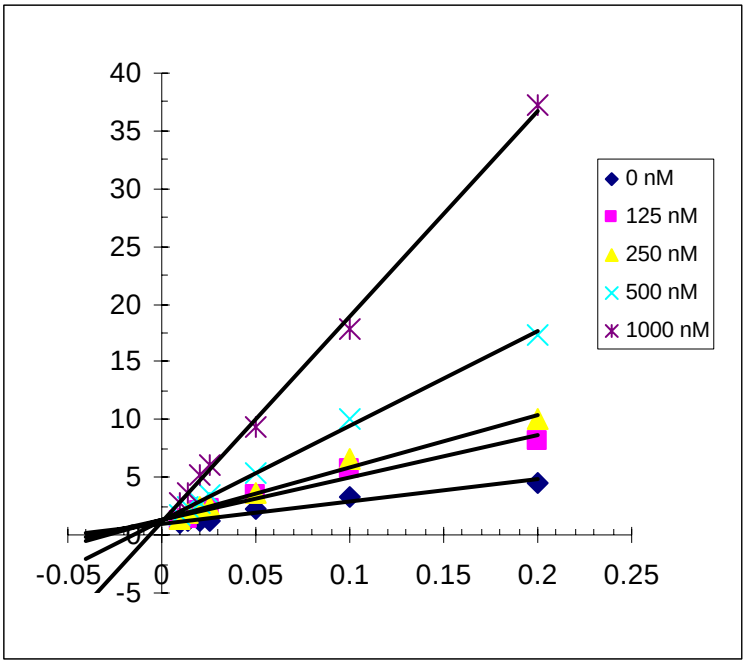
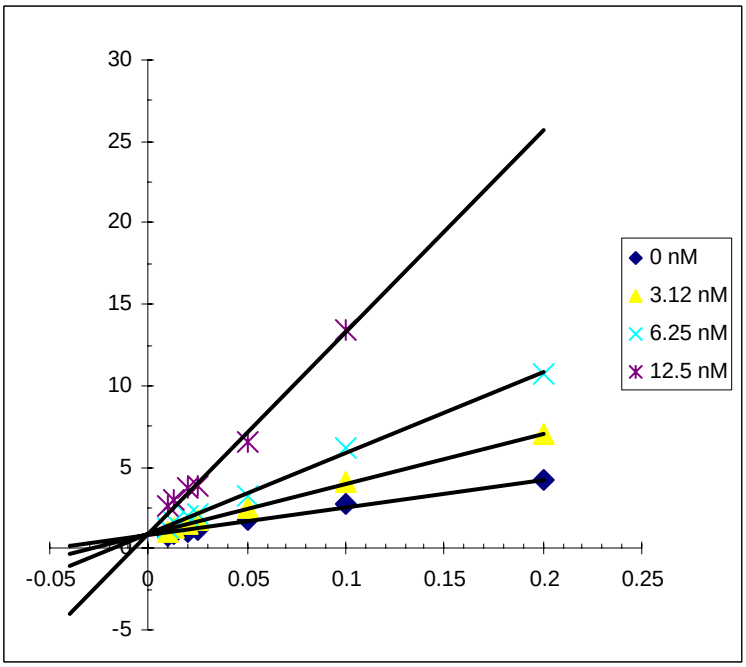


Figure S-2 Lineweaver-Burk plots of the Pf DHFR-C59RS108N by Tmp(A) and T9 (B)



A



B