# Elucidating features that drive the design of selective antifolates using crystal structures of human dihydrofolate reductase

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Contents:

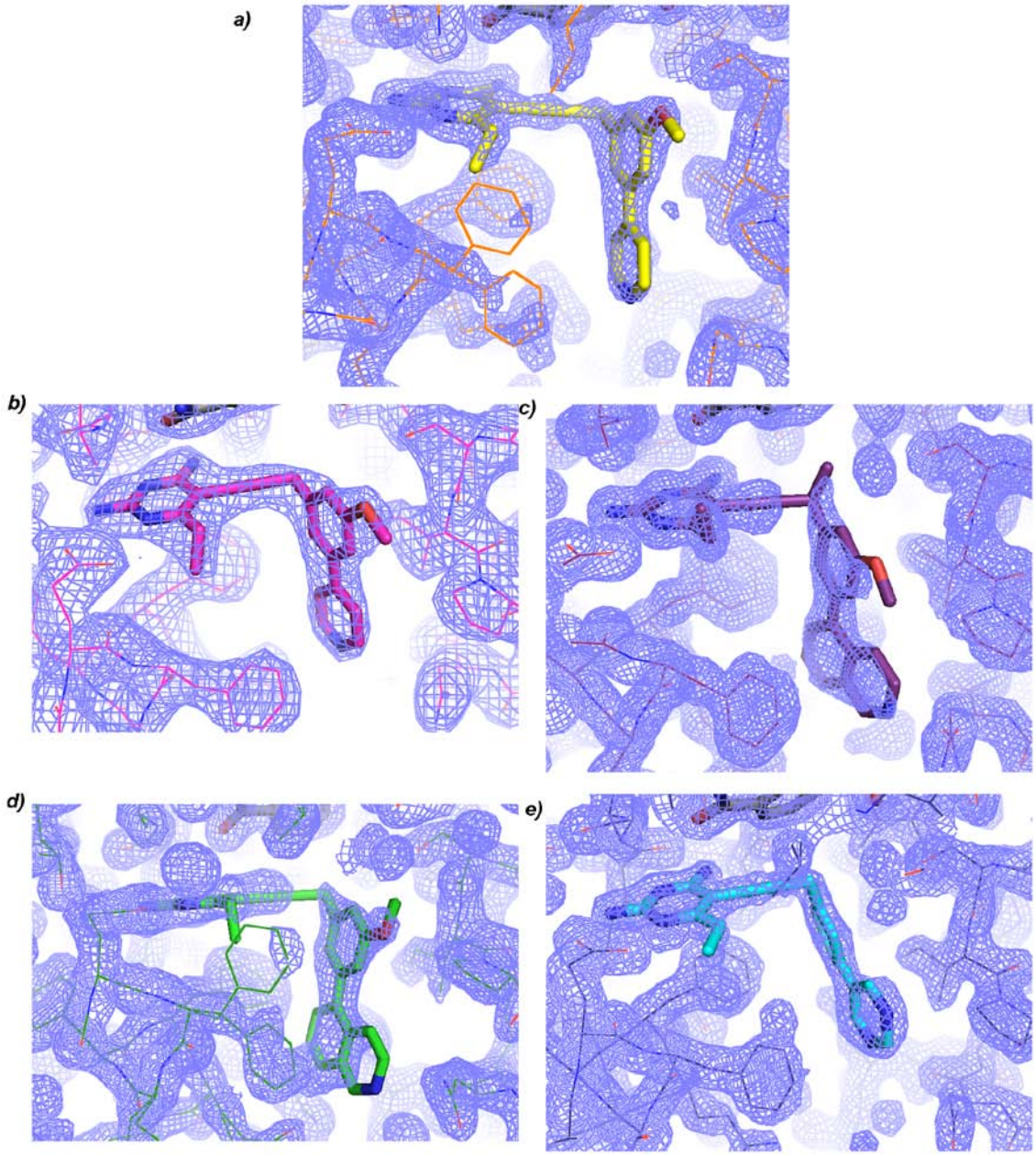


**Figure S1.** Electron density (2Fo-Fc) for ligands in the active site of human DHFR. a) UCP1006, b) UCP1015, c) UCP1019, d) UCP1017, e) UCP1025. Density is contoured at 1.5 σ for UCP1006, UCP1015 and UCP1025; density is contoured at 1.0 σ for UCP1017 and UCP1019.

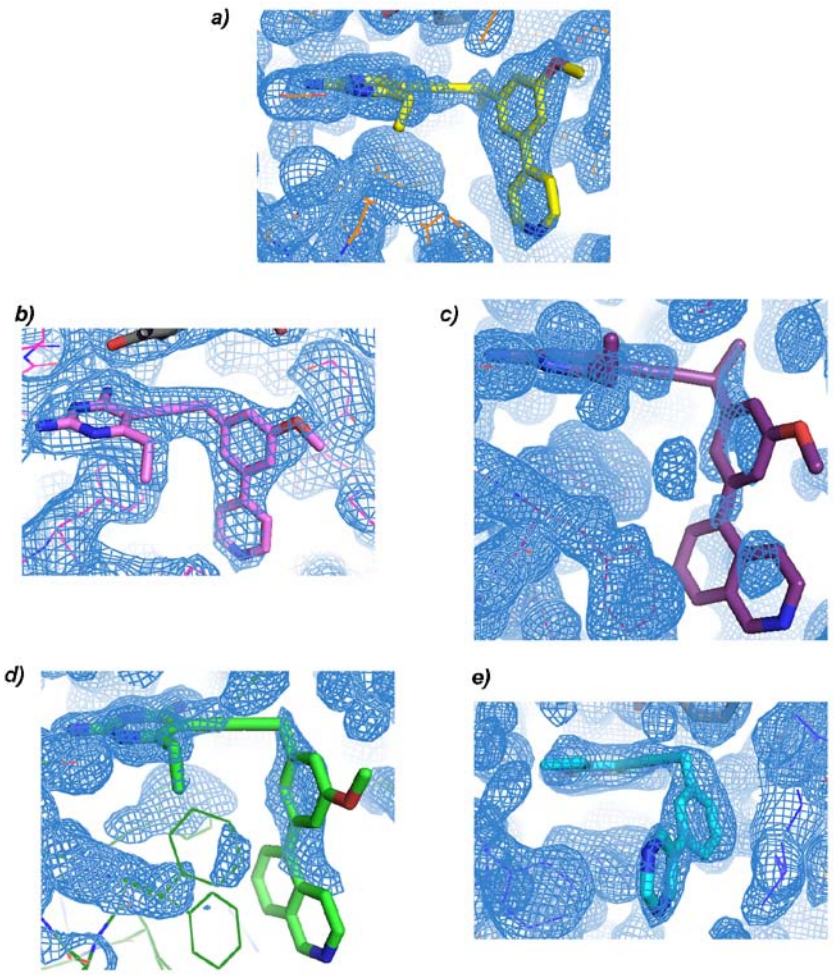


**Figure S1, cont.** Electron density visualized using simulated annealing omit maps for ligands in the active site of human DHFR. a) UCP1006, b) UCP1015, c) UCP1019, d) UCP1017, e) UCP1025.

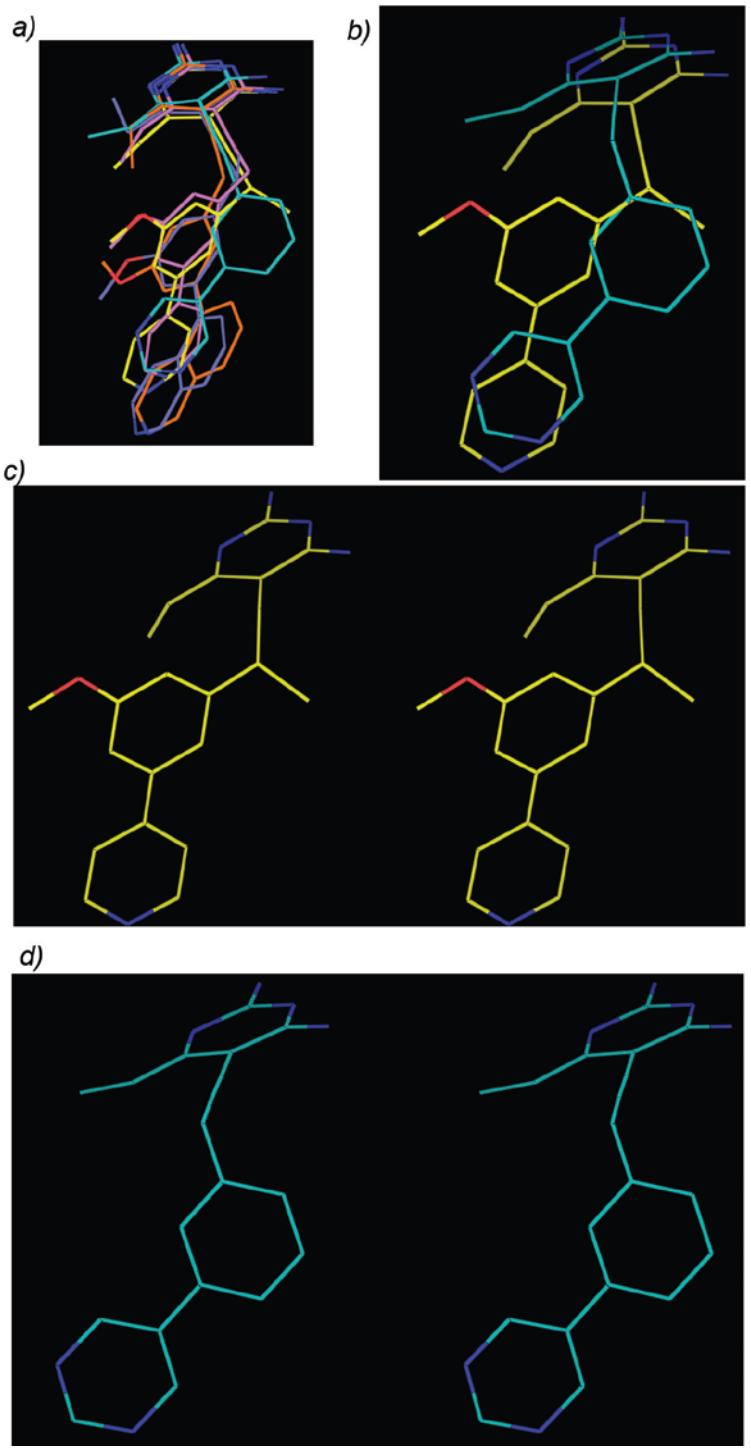


**Figure S2.** Ligand conformations as bound to human DHFR. All of the inhibitors with the exception of UCP1025 adopt approximately the same dihedral angle around the propargylic carbon. UCP1025 adopts a different angle, forcing the biaryl system "backward" in this orientation. a) all inhibitors superposed (yellow: UCP1006, blue: UCP1025, orange: UCP1017, purple: UCP1019, pink: UCP1015) b) UCP1006 superimposed with only UCP1025 for clarity c) stereoview of UCP1006, d) stereoview of UCP1025

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h  1   MVGSLNCIVAVSQ-NMGIGKNGDLPWPPLRNEFRYFQRMTTTSSVEGKQNL
Sa 0     MTLSILVAHDL-QRVIGFENQLPWH-LPNDLKHVKKLSTG-------HT
Cg 1  MSKPVVGIVAALLPPEMGIGFQGNLPW-RLAKEMKYFREVTTLTNDNSKQNV

h  51  VIMGKKTWFSIPEKNRPLKGRINLVLSRELKEPP---QGAHFLSRSLDDALK
Sa 40  LVMGRKTFESIG---KPLPNRRNVVLTSDTSFNV---EGVDVIH-SIEDIYQ
Cg 52  VIMGRKTWESIPQKFRPLPKRINVVVSRSFDGELRKVEDGIYHSNSLRNCLT

h  100 LTEQ-PELAN-KVDMVWIVGGSSVYKEAMNHPGHLKLFVTRIMQ-----DF
Sa  86 LPG-----------HVFIFGGQTLFEEMIDKVD--DMYITVIEG-----KF
Cg 104 ALQSS-LANENKIERIYIIGGGEIYRQSMDL--ADHWLITKIMPLPETTIP

h  145 ESDTFFPE--IDLEKYKLLP-EYPG----------------------VLSD
Sa 119 RGDTFFPP--YTFEDWEVAS-SVEG----------------------KLD-
Cg 152 QMDTFLQKQELEQ-RFYDNSDKLVDFLPSSIQLEGRLTSQEWNGELVKGLP

h  172 VQEEKGIKYKFEBYEKND
Sa 144 --EKNTIPHTFLHLIRKK
Cg 202 -VQEKGYQFYFTLYTKK
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**Figure S3.** Structural alignment of human, *S. aureus* and *C. glabrata* DHFR

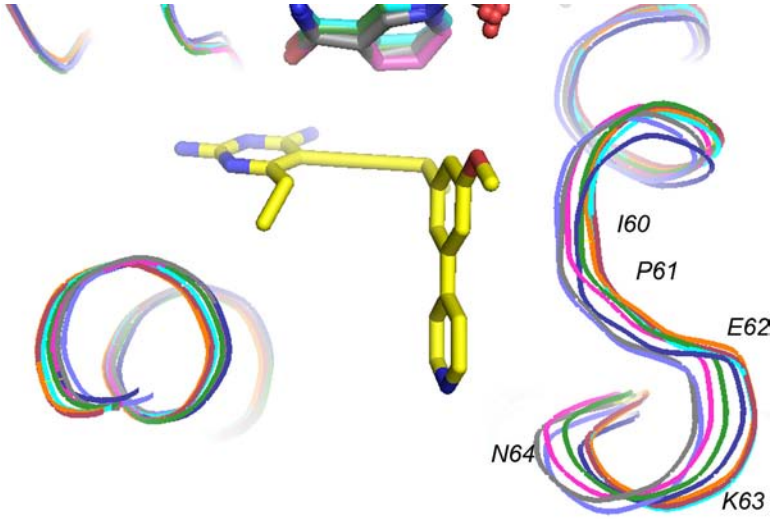


**Figure S4.** Superpositions of loop region in a survey of crystal structures of human DHFR bound to a variety of ligands.

| PDB ID | Color | Distance I60 | Distance E62 | Distance K63 | Distance N64 |
|---|---|---|---|---|---|
| **4KAK** | Orange | | | | |
| **1KMV** | Periwinkle | 1.0 | 1.7 | 2.1 | 1.5 |
| **1S3W** | Green | 0.5 | 1.1 | 1.5 | 1.2 |
| **3NZD** | Magenta | 0.8 | 1.3 | 1.9 | 1.6 |
| **2C2S** | Light Blue | 0.2 | 0.4 | 0.4 | 0.1 |
| **2W3A** | Raspberry | 0.2 | 0.3 | 0.3 | 0.2 |
| **1OHJ** | Dark Blue | 0.9 | 0.8 | 0.9 | 0.4 |
| **4DDR** | Light Gray | 1.0 | 1.8 | 2.6 | 1.8 |