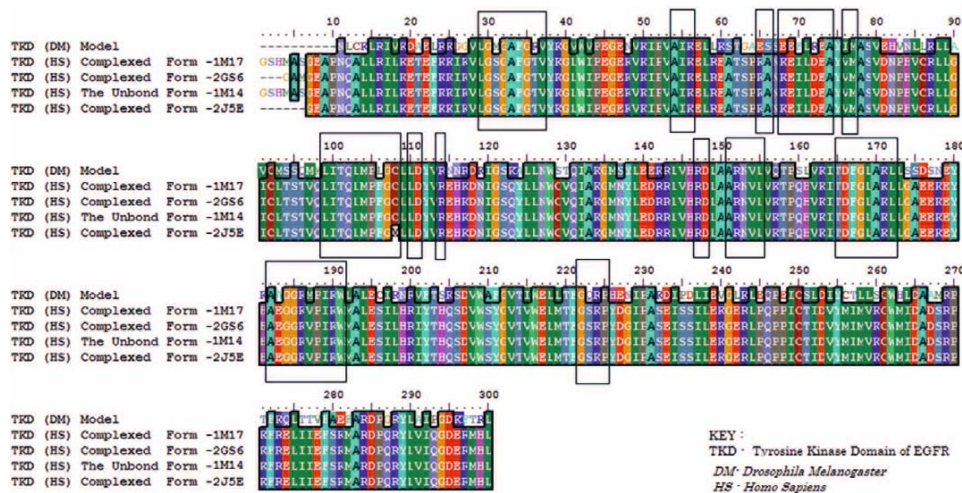


Drosophila-based *in vivo* assay for the validation of inhibitors of the epidermal growth factor receptor/Ras pathway

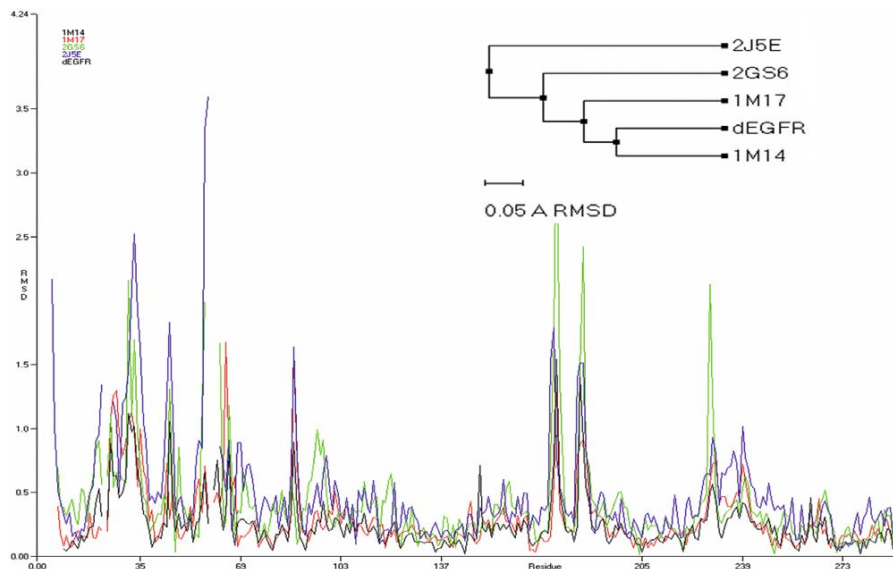
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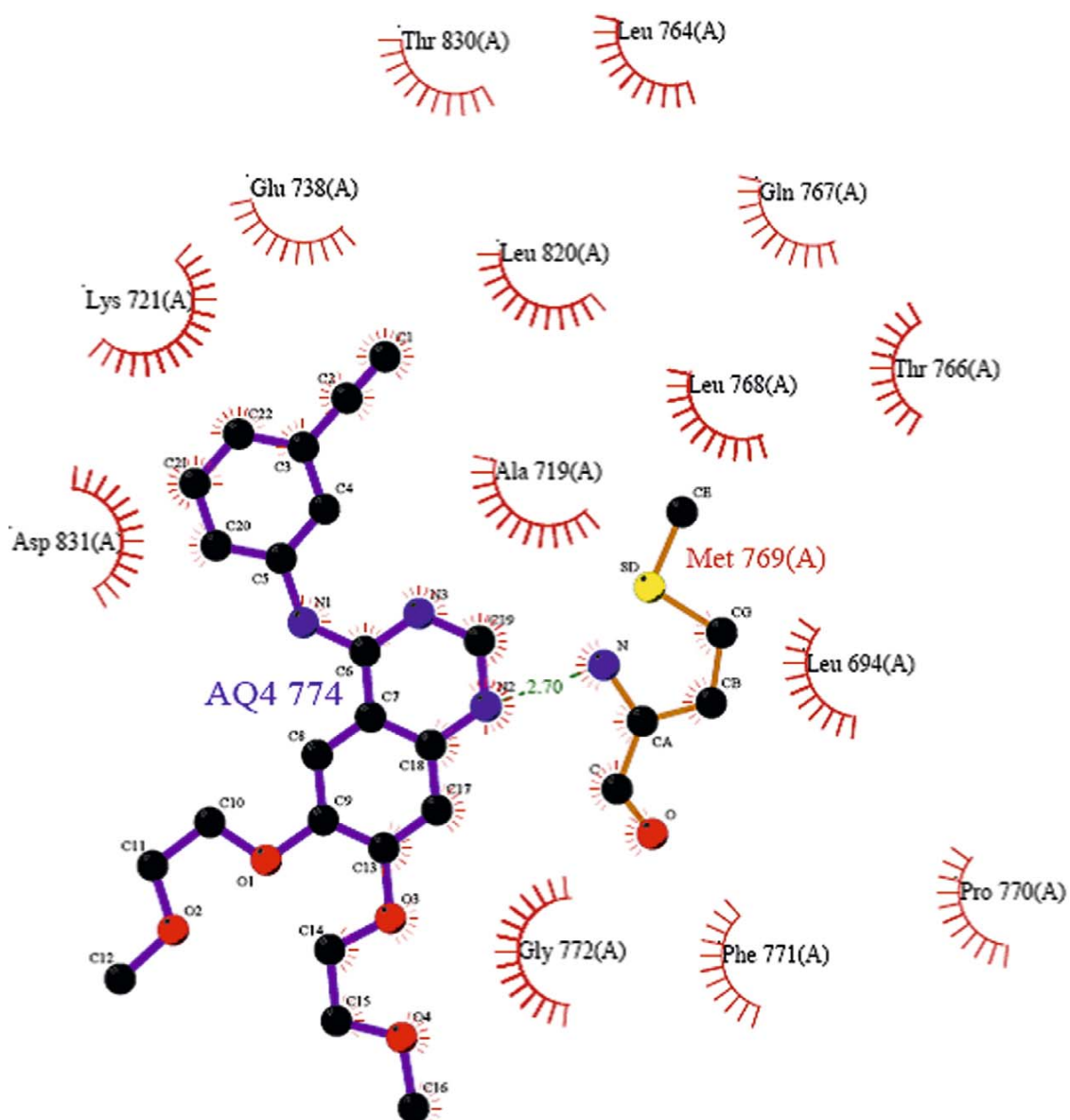
Supplementary Figures and Tables



Supplementary figure 1. Protein sequence alignment of the EGFR TK domain with mammalian crystal structures. The *Drosophila* TK sequence is followed by four human sequences which are crystallized TK domain structures obtained from PDB and are in complex with different drugs except for 1M14 (the list is elaborated in table 1). The top bordered line in the alignment window shows the sequence identity among the species and the alignment region encapsulated in the boxes is the active site of the TK domain.



Supplementary figure 2. Superimposed structure studies on the TK domain of *Drosophila* and *Homo sapiens* EGFR sequence. (A) RMSD plot derived as a result of superimposition studies of the four crystal structures (given in table 1) and the modelled *Drosophila* EGFR-TK (dEGFR) domain (B) The summarized relational tree was calculated and plotted from the structural alignment studies.



Supplementary figure 3. LIGPLOT of interactions involving 1M17 obtained from PDBsum. The green dotted line between the nitrogen of the quinazoline moiety and the nitrogen of Met 769 is the hydrogen bond interaction and the red dotted line depicts the hydrophobic interactions.

Supplementary table 1. Total emergence, phenotypes and lethality of EGFR pathway components overexpressed with *ey-Gal4* and loss- and gain-of-function mutants treated with gefitinib. Different concentrations of gefitinib were mixed with fly food and the parental flies were released. Adult flies that emerged were scored separately for their eye and wing phenotypes. The values indicated in the table are an average of 4 experimental vials. Standard error bars are indicated in the graph as per the values documented in the table. Standard error was seen in figure 4.

Concentration of gefitinib	Total progeny	Pupal lethality	Adult emergence	Adult eye phenotype	Adult wing phenotype	Adult flies with no phenotype
CS 0 ppm	64.25±2.95	0	64.25±2.95	0	0	64.25±2.95
UAS-Lambdatop++ 0 ppm	28±1.68	0	28±1.68	28±1.68	0	0
Ras 'e' B++ 0 ppm	27.75±3.64	0	27.75±3.64	0	0	27.75±3.64
UAS-argos++ 0 ppm	41.25±2.81	0	41.25±2.81	0	0	41.25±2.81
CS 15 ppm	58.25±5.88	0	58.25±5.88	1.75±1.44	5.5±1.19	51±5.96
UAS-Lambdatop++ 15 ppm	31.25±0.63	0	31.25±0.63	9±7.43	0	22.25±7.55
Ras 'e' B++ 15 ppm	25.5±2.63	0	25.5±2.63	24.75±3.12	0	0.75±0.75
UAS-argos++ 15 ppm	29.5±1.76	0	29.5±1.76	3.75±2.25	0	25.75±3.99
CS 25 ppm	61±3.70	0	61±3.70	7.25±0.25	4±1.78	49.75±4.97
UAS-Lambdatop++ 25 ppm	32.5±2.60	0	32.5±2.60	0	0	32.5±2.60
Ras 'e' B++ 25 ppm	10±0.41	0	10±0.41	10±0	0	0
UAS-argos++ 25 ppm	24.25±2.17	0	24.25±2.17	11±1.29	0	13.25±1.38

Supplementary table 2. Total emergence, phenotypes and lethality of the EGFR pathway components overexpressed with *ey-Gal4* and loss- and gain-of-function mutants treated with erlotinib. Different concentrations of erlotinib were mixed with the fly food and the parental flies were released. Adult flies that emerged were scored separately for their eye and wing phenotypes. The values indicated in the table are an average of 4 experimental vials. Standard error bars are indicated in the graph as per the values documented in the table. Standard error was seen in figure 4.

Concentration of erlotinib	Total progeny	Pupal lethality	Adult emergence	Adult eye phenotype	Adult wing phenotype	Adult flies with no phenotype
CS 0ppm	59.5±5.38	0	59.5±5.38	0	0	59.5±5.38
UAS-Lambdatop++ 0 ppm	26.25±2.50	0	26.25±2.50	26.25±2.50	0	0
Ras 'e' B++ 0 ppm	31±2.46	0	31±2.46	0	0	31±2.46
UAS-argos++ 0 ppm	43±1.91	0	43±1.91	0	0	43±1.91
CS 150 ppm	40.5±2.72	0	40.5±2.72	0	0	40.5±2.72
UAS-Lambdatop++ 150 ppm	26±2.48	0	26±2.48	3.75±1.65	0	22.25±3.97
Ras 'e' B++ 150 ppm	33.25±2.10	0	33.25±2.10	12.25±1.93	0	21±3.34
UAS-argos++ 150 ppm	17.25±1.48	0	17.25±1.49	13.25±1.55	0	4±0.58
CS 200 ppm	40±3.94	0	40±3.94	5.25±2.39	0	34.75±3.62
UAS-Lambdatop++ 200 ppm	23.75±4.37	0	23.75±4.37	0.75±0.48	0	23±4.80
Ras 'e' B++ 200 ppm	21.25±8.53	0	21.25±8.53	20.5±8.87	0	0.75±0.48
UAS-argos++ 200 ppm	14±1.68	0	14±1.68	11.5±1.66	0	2.5±0.65