

SUPPORTING INFORMATION: Sequence alignment of class B1 GPCRs, binding sites of CRF1 antagonists in stereo representation and table showing correlation between protein-ligand interaction energies and the experimental binding affinities of three chemical series of CRF1 antagonists.

Secretin family receptors alignment

ADCYAP1R1	-WSEFPF-----HYFDACG-FDEYESETGDQ-----DYYYLSVKALYTVGYST	
VIPR2	-WSETFP-----DFVDACG-YSDPEDESKIT-----FYILVKAIYTLGYSV	
VIPR1	-WTHLEPG-----PYPIACG-LDDKAASLDEQOT-----MFYGSVKTGYTIGYGL	
GHRHR	-WSEFPF-----PYPVACP--VPLELLAEEES-----YFSTVKIIYTVGHSI	
SCTR	-WSETFP-----RPNLACG--VNVNDS SNEKRHS-----YLLKLKVMYTVGYSS	
GIPR	-QWGLW-----RDHTQCENPEKNEAFLDQR-----LILERLQVMYTVGYSL	
GLP1R	-LWLQKDNS--SLPWRDLSECEESKRGRSSPEE-----QLFLYIIYTVGYAL	
GLP2R	-TWQTIENA--TDIWQDDSECSENHSFKQNVDRYA-----LLSTLQLMYTVGYSF	
PTHR1	-SWELVPGH--NRTWANYSECVKFLTNETRERE-----VFDRLGMIYTVGYSV	
PTHR2	-TWDFMHS--NKTWANYSDCLRFLQPDISIGKQE-----FFERLYVMYTVGYSI	
CALCR	VWFKHPEN--NRTWSNYTMCNAFTPEKLNAY-----VLYYLAIVGHSL	
CALCRL	NWFRHPAS--NRTWTNYTQC NVNTHEKVKTALN-----LFYLTIIIGHGL	
CRHR1	SWAAR-----VNYSECQEILNE-EKSKSV-----HYHVAVIINYLGHCI	TM1
CRHR2	TWASK-----INYSQCEPILDDKQRKYDL-----HYRIALVVNYLGHCV	

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ADCYAP1R1	SLVTLTTAMVILCRFRKLHCTR-----NFIHMNLFVSFMLRAISVFIKDWILYAE----	
VIPR2	SLMSLATGSIILCLFRKLHCTR-----NYIHLNLFSLFILRAISVLKDDVLYSS----	
VIPR1	SLATLLVATAILSLFRKLHCTR-----NYIHMHLFISFILRAAAVFIKDLALFDS----	
GHRHR	SIVALFVAITILVALRRLHCPR-----NYVHTQLFTTFILKAGRVFLKDAALFHS----	
SCTR	SLVMLLVALGILCAFRRHLHCTR-----NYIHMHLFVSFILRALS NFIKDAVLFSS----	
GIPR	SLATLLLALLILSLFRRLHCTR-----NYIHINLFTSFMLRAAAILSRDRLLPRPG---	
GLP1R	SFSALVIASAILLGFRRHLHCTR-----NYIHLNLFASFILRALS VFIKDAALKWY---	
GLP2R	SLISLFLAL TLLLFLRKLHCTR-----NYIHMNLFASFILRTLAVLVKDVVFYNSY---	
PTHR1	SLASLTVAVLILAYFRRLHCTR-----NYIHMHLFSLFMLRAVSI FVKDAVLYSGATLD	
PTHR2	SFGSLAVAILIIGYFRRLHCTR-----NYIHMHLFVSFMLRATSIFVKDRVVHAHIGVK	
CALCR	SIFTLVISLGI FVFRSLGCQR-----VTLHKNMFLTYILNSMII IHLVEVVPNGELV	
CALCRL	SIASLLISLGIFFYFKSLSCQR-----ITLHKNLFFSFVCSNVVTI IHLTAVANNQALV	
CRHR1	SLVALLVAFVFLRLRSIRCLR-----NI IHWNLISAFILR--NATWFVVQLTMSPEVH	TM2
CRHR2	SVAALVA AFLFLALRSIRCLR-----NVIHWNLITTFILR--NVMWFLLQL--VDHEVH	

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Secretin family receptors alignment

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ADCYAP1R1 -----QDSNHC---FISTVECK-----AVM
VIPR2      -----SGTLHCPDQPSSWVGCK-----LSL
VIPR1      -----GESDQCS---EGSVGCK-----AAM
GHRHR      -----DDTDHCS---FSTVLCK-----VSV
SCTR       -----DDVTYCD---PHRAGCK-----LVM
GIPR       -----PYLGDQALA---LWNQALAACR-----TAQ
GLP1R      -----STAAQQHQWDGLLSYQDSLSCR-----LVF
GLP2R      -----SKRPDNEGWMSYLSEMSTSCR-----SVQ
PTHR1      EAERLTEEELRAIAQAPPPATAAAGYAGCR-----VAV
PTHR2      ELES LIMQDD--PQNSIEATSVDKSQYIGCK-----IAV
CALCR      R-----RDPV SCK-----ILH
CALCRL     A-----TNPV SCK-----VSQ
CRHR1     Q-----SNV G WCR-----LVT
CRHR2     E-----SNEV WCR-----CIT
  
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TM3

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ADCYAP1R1 VFFHYCVVSNYFWLFI EGLYLFTLLVETFFPERRY-----FYWYTIIGWGTPTVCV
VIPR2     VFLQYCI MANFFWLLVEGLYLHTLLV-AMLP PRC-----FLAYLLIGWGLPTVCI
VIPR1     VFFQYCVMANFFWLLVEGLYLHTLLAVSFFSERKY-----FWGYILIGWGV PSTFT
GHRHR     AASHFATMTNFSWLLAEAVYLNCLLASTSPSSRA-----FWWLVLAGWGLPV LFT
SCTR      VLFQYCI MANYSWLLVEGLYLHTLLAISFFSERKY-----LQGFVAFGWGSPAIFV
GIPR      IVTQYCVGAN YTWLLVEGVYLHSLLVLVGGSEEGH-----FRY YLLGWGAPALFV
GLP1R     LLMQYCVAA NYWLLVEGVYLYTLLAFSVFSEQWI-----FRLYV SIGWGV PLLFV
GLP2R     VLLHYFVGANYLWLLVEGLYLHTLLEPTVLPERRL-----WPRYLLLGWAFPV L FV
PTHR1     TFFLYFLATNYYWILVEGLYLHSLIFMAFFSEKKY-----LWGF TVFGWGLPAV FV
PTHR2     VMFIYFLATNYYWILVEGLYLHNLIFVAFFSDTKY-----LWGFILIGWGFPAAFV
CALCR     FFHQYMMACNYFWMLCEGIYLHTLIVVAVFTEKQR-----LRWY YLLGWGFPLVPT
CALCRL    FIHL YLMGCNYFWMLCEGIYLHTLIVVAVFAEKQH-----LMWY YFLGWGFPLIPA
CRHR1     AAYNYFHV TN FFWMFGE GCYLHTAIVLTYSTDRLR-----KWMFICIGWGV PFP I I
CRHR2     TIFNYFVV TN FFWMFVE GCYLHTAIVMTYSTERLR-----KCLFLFIGWCIPFP I I
  
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Residues in red are conserved residues which contact NBI 27914

TM4

Secretin family receptors alignment

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ADCYAP1R1 TVWATL-----RLYFDDTGCWDMNDSTAL-----WWVIKGPVVG
VIPR2      GAWTAA-----RLYLEDTGCWDTNDHSVP-----WWVIRIPILIS
VIPR1      MVWTIA-----RIHFEDYGCWDTINSSL-----WWIIKGPILTS
GHRHR     GTWVSC-----KLAFEDIACWDLDDTSPY-----WWIIKGPIVLS
SCTR      ALWAIA-----RHFLEDVGCWDINANASI-----WWIIRGPIVLS
GIPR      IPWVIV-----RYLYENTQCWERNEVKAI-----WWIIRTPILMT
GLP1R     VPWGIV-----KYLYEDEGCWTRNSNMNY-----WLIIRLPILFG
GLP2R     VPWGFA-----RAHLENTGCWTTNGNKKI-----WWIIRGPMMLC
PTHR1     AVWVSV-----RATLANTGCWDLSSGNK-----KWI IQVPILAS
PTHR2     AAWAVA-----RATLADARCWELSAGDI-----KWIYQAPILAA
CALCR     TIHAIT-----RAVYFNDNCWLSVETHL-----LYIIHGPMVMAA
CALCRL    CIHAIA-----RSLYNDNCWISSDTHL-----LYIIHGPICAA
CRHR1     VAWAIG-----KLYYDNEKCFWFGKRPGVYT-----DYIYQGPMILV
CRHR2     VAWAIG-----KLYYENEQCWFGKEPGDLV-----DYIYQGPILV

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TM5

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ADCYAP1R1 IMVNFVLFIGIIVILVQKLQSPDMGGNESSIYLRLAR-----
VIPR2      IIVNFVLFISIIIRILLQKLTSPDVGGNDQSQYKRLAK-----
VIPR1      ILVNFILFICIIIRILLQKLRPPDIRKSDSSPYSRLAR-----
GHRHR     VGVNFGLFLNIIRILVRKLEPAQGSLSHTQSQYWRLSK-----
SCTR      ILINFILFINILRILMRKLRTQETRGNEVSHYKRLAR-----
GIPR      ILINFLIFIRILGILLSKLRTRQMRCRDYR--LRLAR-----
GLP1R     IGVNFLIFVRVICIVVSKLKANLMCKTDIK--CRLAK-----
GLP2R     VTVNFFIFLKILKLLISKLKAHQMCFRDYK--YRLAK-----
PTHR1     IVLNFILFINIVRVLATKLRRETNAGRCDTRQYRKLLK-----
PTHR2     IGLNFILFLNTVRVLATKIWETNAVGHDRKQYRKLA-----
CALCR     LVVNFFLLNIVRVLVTKMRET--HEAESHMYLKAVK-----
CALCRL    LLVNLFLLNIVRVLITKLVKVT--HQAESNLYMKAVR-----
CRHR1     LLINFIFLFNIVRILMTKLRAS--TTSETIQYRKAVK-----
CRHR2     LLINFVFLFNIVRILMTKLRAS--TTSETIQYRKAVK-----

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Residues in red are conserved residues which contact NBI 27914

TM6

Secretin family receptors alignment

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ADCYAP1R1  -----STLLLIPLFGIHYTVFAFSPENVSK-----RERLVF
VIPR2      -----STLLLIPLFGVHYMVFAVFPISISS-----KYQILF
VIPR1      -----STLLLIPLFGVHYIMFAFFPDNFKP-----EVKMFV
GHRHR      -----STLFLIPLFGIHYIIFNFLPDNAGL-----GIRLPL
SCTR       -----STLLLIPLFGIHYIVFAFSPEDAM-----EIQLFF
GIPR       -----STLTLVPLLGVHEVVFAPVTEEQARGALR-----FAKLGf
GLP1R      -----STLTLIPLLGTHEVIFAFVMDEHARGTLR-----FIKLfT
GLP2R      -----STLVLIPLLGVHEILFSFITDDQVEGFAK-----LIRLFI
PTHR1      -----STLVLMPLFGVHYIVFMATPYTEVSGTLW-----QVQMHy
PTHR2      -----STLVLVLVFGVHYIVFVCLP-HSFTGLGW-----EIRMHC
CALCR      -----ATMILVPLLGIQFVVPWRPSNKMLG-----KIYDYV
CALCRL     -----ATLILVPLLGIEFVLIPWRPEGKIAE-----EVYDYI
CRHR1      -----ATLVLLPLLGITYMLFFVNPGEDEVSR-----VVFfYf
CRHR2      -----ATLVLLPLLGITYMLFFVNPGEDDLSQ-----IMfYf

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TM7

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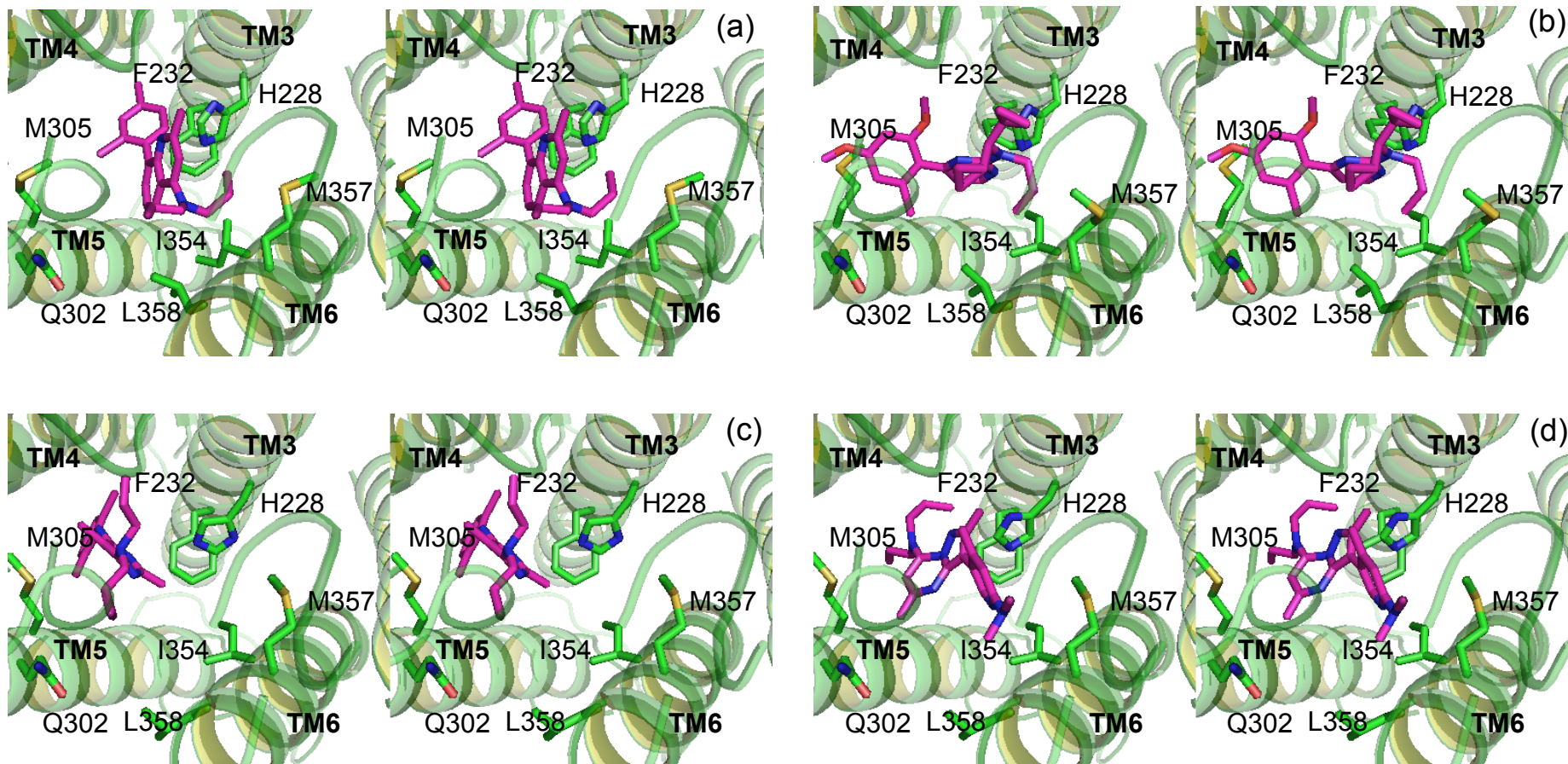
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ADCYAP1R1  ELGLGSFQGFVVAVLYCFLNGEVQAEIKR--KWRSWKVNRYFAVDFKHRHPSLASSGVNG
VIPR2      ELCLGSFQGLVVAVLYCFLNSEVQCELKR--KWRSRCPTPSASRDYRVCGSSFSRNGSEG
VIPR1      ELVVGSFQGFVVAIlyCFLNGEVQAE LR--KWRRWHLQGV LGWNPKYRHPSGGSNGATC
GHRHR      ELGLGSFQGFIVAIlyCFLNQEV RTEISR--KWHGHDPPELLPAWRTRAKWTTPSR SAAKV
SCTR       ELALGSFQGLVVAVLYCFLNGEVQLEVQK--KWQWHLREFPLHPVASFSNSTKASHLEQ
GIPR       EIFLSSFQGFVSVLYCFINKEVQSEIRR--GWHHCRLRRSLGEEQRQLPERAFRALPSG
GLP1R      ELSFTSFQGLMVAIlyCFVNNEVQLEFRK--SWERWRL-EHLHI-QRD---SSMKPLKCP
GLP2R      QLTLS SFHGFLVALQYGFANGEVKAELRK--YWVRFLLARHSGCRACVLG-KDFRFLGKC
PTHR1      EMLFNSFQGFVVAI IYCFNNGEVQAEIKK--SWSRWTLALDFKRKARSGSSSYSGPMVS
PTHR2      ELFFNSFQGFVSI IYCYCNGEVQAEVKK--MWSRWNLSDWKRTPPCGSRRCGS-VLTT
CALCR      MHSLIHFQGFVATIYCFNNEVQTTVKR--QWAQFKIQWNQRWGRRPSNRSARAAAAAA
CALCRL     MHILMHFQGLLVSTIFCFFNNGEVQAILRR--NWNQYKIQFGNSFSNSEALRSASyTVSTI
CRHR1      NSFLESFQGFVSVFVlyCFLNSEVRSAIRK--RWHRWQDKHSIRARVARAMSIPTSPTRVS
CRHR2      NSFLOSFQGFVSVFYCFNNGEVRS AVRK--RWHRWQDHHS LRVP MARAMSIPTSPTRIS

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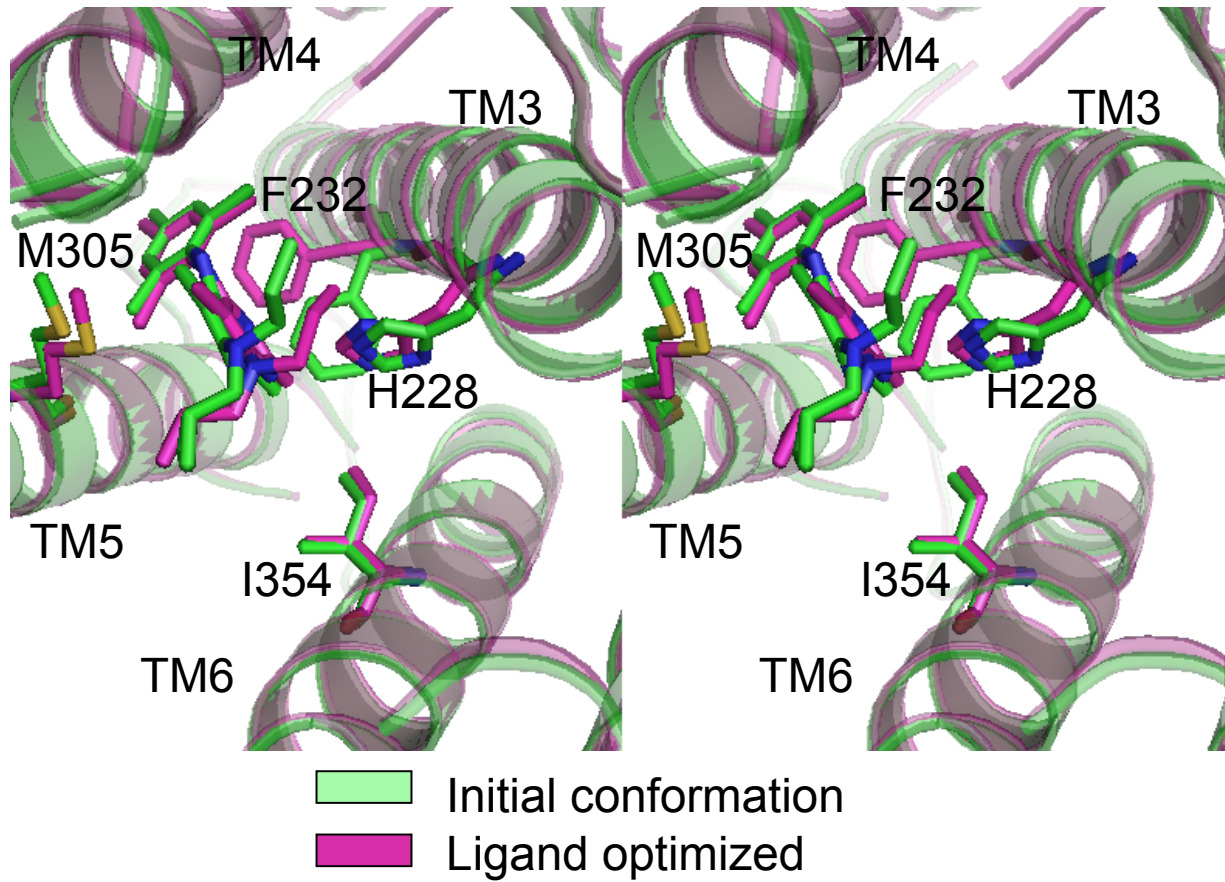
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Fig. S1



Predicted binding sites of selected compounds in *hCRF*₁-R. Residues within 5 Å of the ligand are shown in each figure in stereo representation. (a) binding site for compound **1**; (b) compound **4**; (c) NBI-27914; (d) NBI-30775.

Fig. S2



Comparison of the apo receptor structure of *hCRF*₁-R to the NBI-27914 stabilized *hCRF*₁-R structure from LITiCon shown in stereo representation.

Table S1.

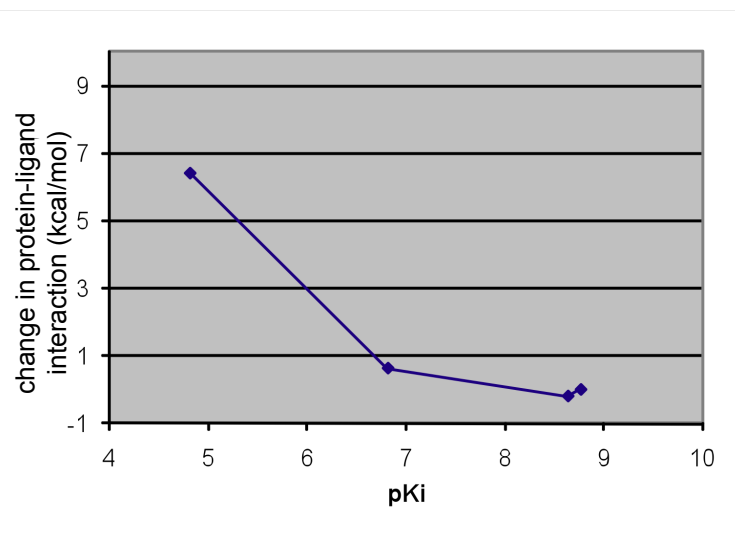
4 series

compound	Change in protein-ligand interaction (Kcal/mol)	Experimental binding affinity (pKi)
1	0	9.05
2	1.47	7.49
3	4.37	6.38

7 series

compound	Change in protein-ligand interaction (Kcal/mol)	Experimental binding affinity (pKi)
4	0	7.96
5	1.47	6.07
6	6.69	inactive

compound	Change in protein-ligand interaction (Kcal/mol)	Experimental binding affinity pKi
NBI27914	0	8.77
66221	-0.2	8.64
66225	0.6	6.82
66226	6.4	4.82



Comparison of the protein-ligand interaction energies and the experimental binding affinities of three chemical series of CRF1 antagonists. The best binder in each series is marked in pink and the weakest binder is marked in blue. Also the change in binding energy vs experimental binding affinity for the NBI-27914 series is shown graphically.