Supporting Information for

Identification and structure-function analysis of sub-family selective G protein-coupled receptor kinase inhibitors

by

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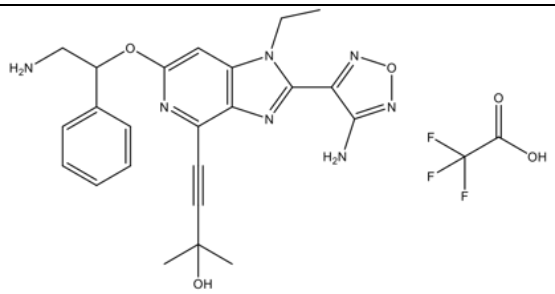
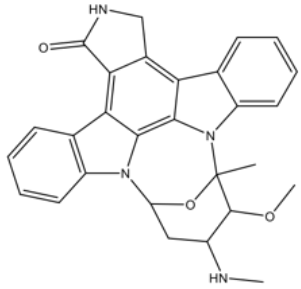
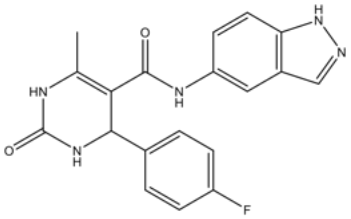
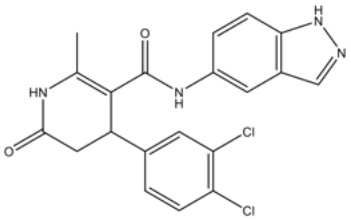
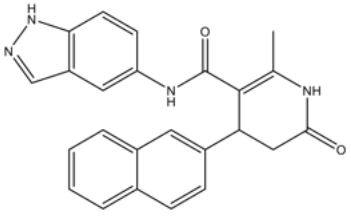
Supporting Results

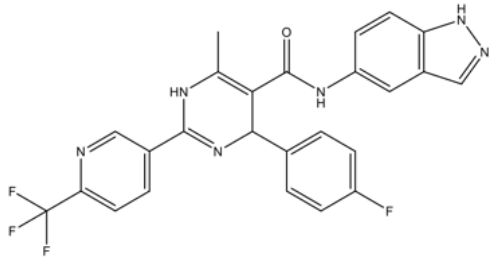
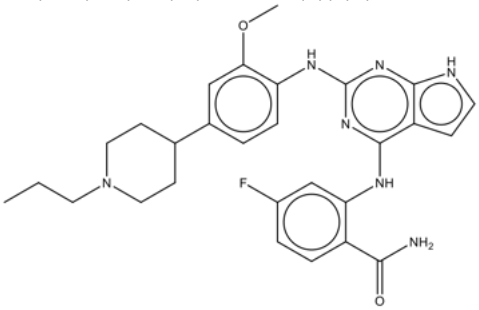
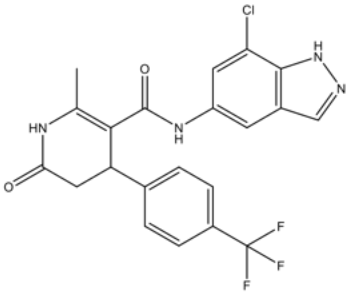
Structure-activity relationships. The GRK2-selective indazole compounds typified by GSK180736A all contain three ring systems (Figure 1a): an indazole ring that forms two hydrogen bonds with the kinase domain hinge, a dihydropyrimidine-like ring that is relatively solvent exposed, and a terminal fluorophenyl moiety that docks in a pocket formed between the P-loop and the side chain of active site Lys220. These rings thus occupy the so-called adenine, ribose, and polyphosphate subsites of the active site, respectively. The biochemical data for GSK466317A, GSK317354A, GSK270822A, and GSK299115A (Table 2) can thus be interpreted on the basis of the co-crystal structure of GSK180736A with GRK2. GSK466317A, 2 logs less potent than GSK180736A, contains a chloro substituent on the indazole benzene ring, a dihydropyridone instead of a dihydropyrimidine, and a trifluoromethyl instead of a fluoro substituent on the phenyl ring. Manual modeling predicts that the chloro group can be accommodated, but its dihydropyridone ring will not be able to form a hydrogen bond with the P-loop and the trifluoromethyl group is likely too bulky to fit in the polyphosphate subsite. GSK270822A, which exhibits about the same potency as GSK466317A, has a dihydropyridone ring and a bulky terminal naphthalene group that is also likely excluded from the polyphosphate subsite. GSK317354A, about 1 log less potent than GSK180736A, retains a dihydropyrimidine central ring but has a trifluoromethyl pyridine in place of the carbonyl oxygen of GSK180736A. Because this group is modeled to project out of the active site, its lower potency may result from steric clashes with nearby residues or less favorable solvent interactions. The addition of a second halogen to the terminal phenyl of GSK299115A can likely be accommodated, but, as in the case of GSK466317A, its pyridone ring cannot form a hydrogen bond with the P-loop. Thus loss of the P-loop hydrogen bond can be construed to cause ~10-

fold less potency of inhibition. Interestingly, these chemical differences do not as strongly affect potency for inhibition of GRK1 and GRK5, and in some cases even seem to improve potency (Table 2). This could be interpreted to mean that these compounds do not bind to these enzymes in an analogous manner.

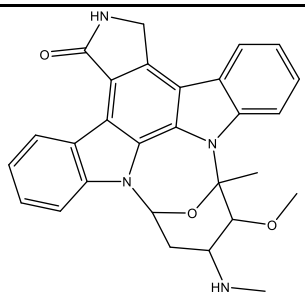
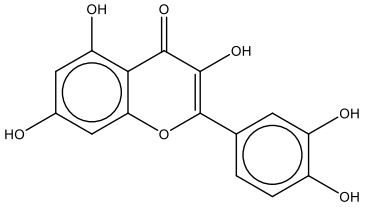
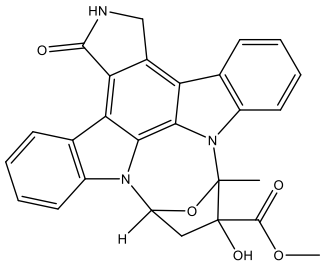
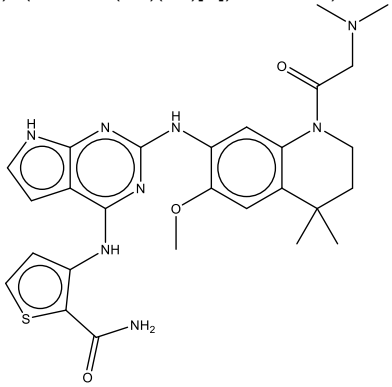
GSK2163632A also contains three ring systems, but they bind in a fundamentally different way than those of GSK180736A. Its central pyrrolopyrimidine ring system forms three hydrogen bonds with the hinge in the adenine subsite, and its thiophene carboxamide occupies the ribose subsite and interacts directly with the P-loop. The tetrahydroquinoline group extends along the hinge towards the large lobe where it is capped by residues in the AST loop. This last region is referred to as the "AST subsite" because it occupies a region of the active site that would normally contain residues from the AST in the active conformation of an AGC kinase. A similar region has been exploited to enhance the affinity of inhibitors of other kinases such as ERK5 [(1)]. Beneficial interactions with AST loop may thus prove to be critical for the development of inhibitors selective for GRK5. There are four related compounds in this study that provide the basis of a structure activity relationship: GSK2110263A, GSK2220400A, GSK1713088A, and GSK1326255A (Figure 1b). Absence of the gem-dimethyl substituent on the tetrahydroquinoline core of GSK2110263A decreases the potency for GRK1 inhibition, but does not affect potency against GRK5, confirming that the higher potency of GRK1 for GSK2163632A likely derives in part from the interactions with GRK1-specific residues in the AST. GSK2220400A lacks the gem-dimethyl group and has a methyl pyridine carboxamide in place of the thiophene carboxamide, which results in 100 fold lower potency against GRK1 but only a slight reduction in potency against GRK5. Thus it may be that the P-loop of GRK5 does not adopt the same "tucked under" conformation as seen in the GRK1 structure. Both GSK1713088A and GSK1326255A have a propyl piperidine moiety in place of the tetrahydroquinoline and a fluoro substituted pyridine carboxamide substituting for the thiophene carboxamide of GSK2163632A. These changes increase the IC_{50} against GRK1 by 2-3 logs, but do not affect GRK5 inhibition. Thus, GRK5 seems less sensitive to changes in the ribose and AST subsites than GRK1. GRK2, like GRK5, is relatively insensitive to these modifications, and some of them even decrease the IC_{50} (e.g. up to 1 log for GSK1326255A). Thus GRK2 may favor bulkier groups in its ribose subsite when a pyrrolopyrimidine is bound in the adenine subsite, although other explanations involving the other arm of the inhibitor are possible. Notably, PKA was not measurably inhibited by these compounds. This could reflect the unique sequence and the more ordered nature of the PKA AST region relative to those of GRKs [(2)], which could more efficiently compete for binding with this class of inhibitors.

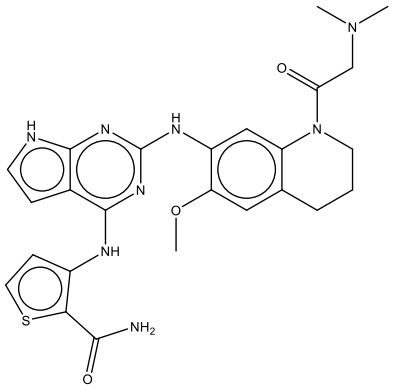
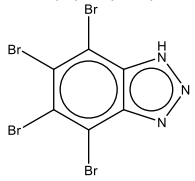
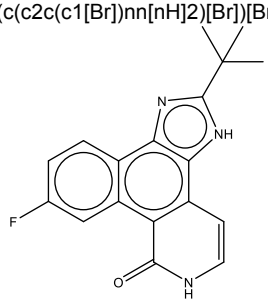
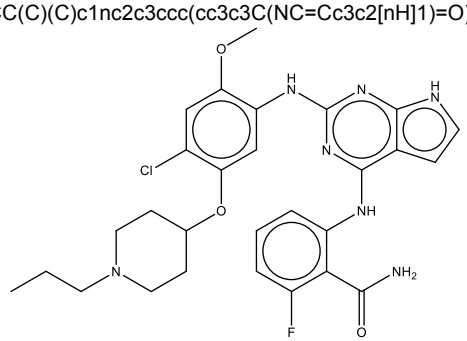
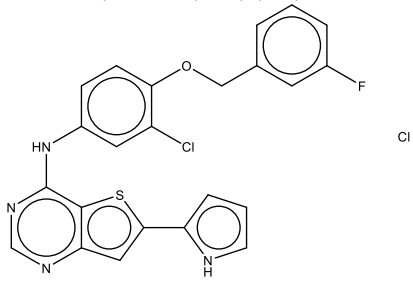
Supporting Table 1. DSF Screening Results for GRK2

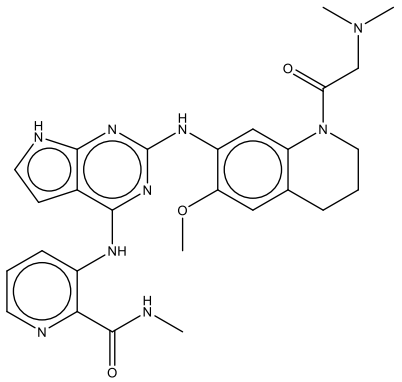
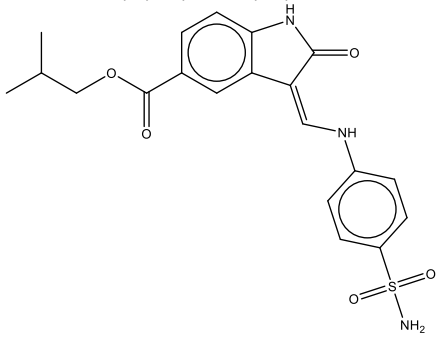
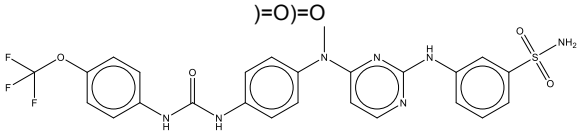
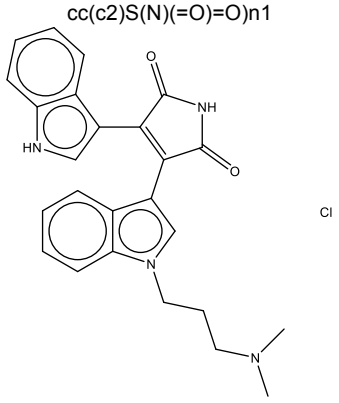
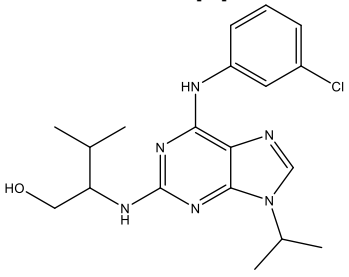
ΔT_m (°C)	Compound Description	Compound Supplier	Compound Supplier ID	Compound Structure and SMILES String
5.41	GSK PKI collection	GSK	GSK1007102B	 OC(=O)C(F)(F)F.CCN(C1C(=NON2)C=2N)C(=C(N=1)C1C#CC(C)(C)O)C=C(N=1)OC(CN)C(C=CC=C1)=C1
5.37	Staurosporine	AXXORA	S-9300	 C(C(C(O1)(C)N(C(=C(N(C12)C(=C1C=CC3)C=3)C13)C1=C(C=3C3=O)CN3)C(=CC=CC3)C1=3)OC)(C2)NC
5.11	GSK PKI collection	GSK	GSK180736A	 CC(=C(C1C(=CC=C(F)C2)C=2)C(=O)NC(C=CC(NN=C2)=C23)=C3)NC(N1)=O
4.83	GSK PKI collection	GSK	GSK299115A	 CC(=C(C1C(C=CC([Cl])=C2[Cl])=C2)C(=O)NC(C=CC(NN=C2)=C23)=C3)NC(C1)=O
3.82	GSK PKI collection	GSK	GSK270822A	 CC(=C(C1C(C=CC(=CC=CC2)C=23)=C3)C(=O)NC(C=CC(NN=C2)=C23)=C3)NC(C1)=O

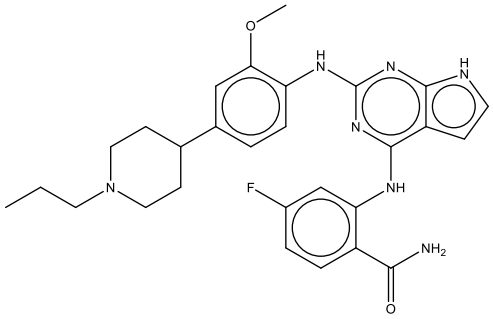
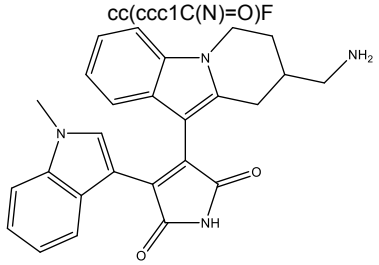
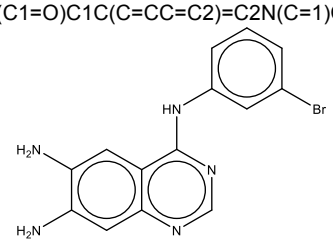
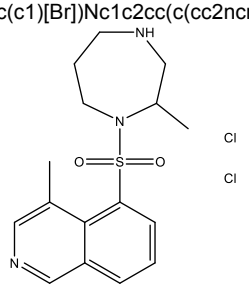
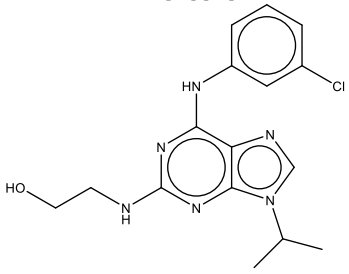
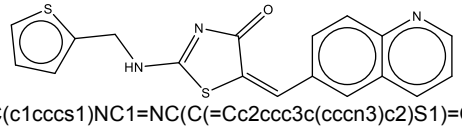
3.78	GSK PKI collection	GSK	GSK317354A		CC(=C(C1C(=CC=C(F)C2)C=2)C(=O)NC(C=CC(NN=C2)=C23)=C3)NC(=N1)C(=CC=C1C(F)(F)F)C=N1
3.29	GSK PKI collection	GSK	GSK1326255A		CCCN1CCC(CC1)c1ccc(c(c1)OC)Nc1nc(c2cc[nH]c2n1)Nc1cc(ccc1C(N)=O)F
2.94	GSK PKI collection	GSK	GSK466317A		CC(=C(C1C(=CC=C2C(F)(F)F)C=C2)C(=O)NC(C=C([Cl])C(NN=C2)=C23)=C3)NC(C1)=O

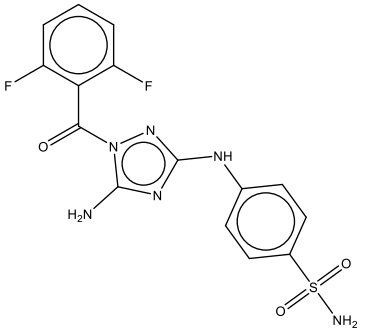
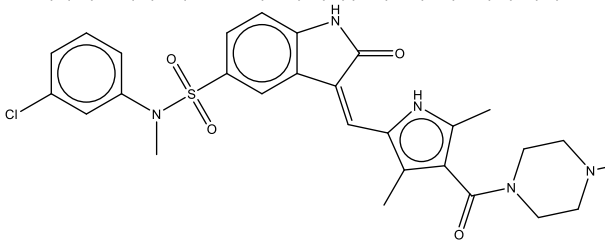
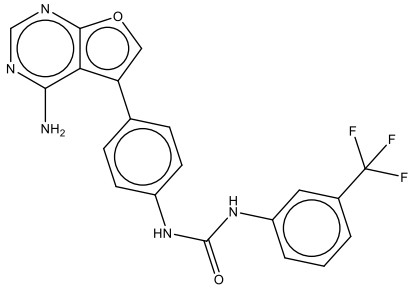
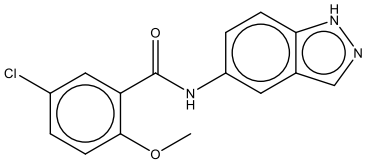
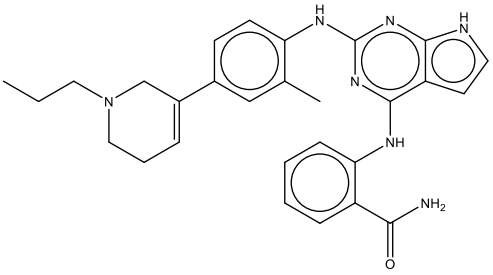
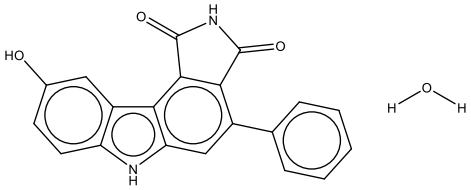
Supporting Table 2. DSF Screening Results for GRK5

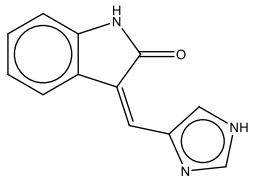
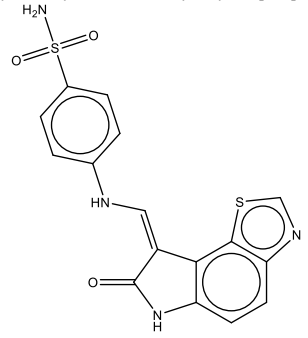
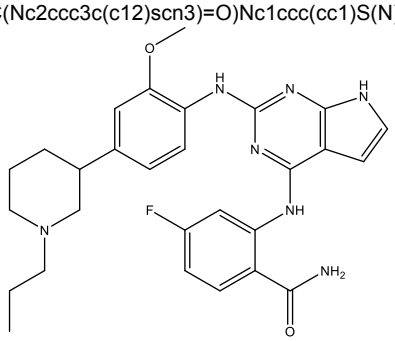
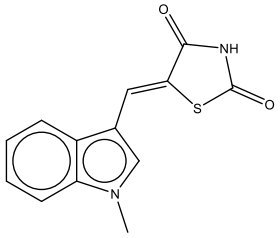
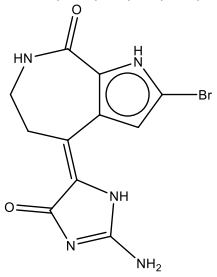
ΔT_m (°C)	Compound Description	Compound Supplier	Compound Supplier ID	Compound Structure and SMILES String
11.95	Staurosporine	AXXORA	S-9300	 C(C(C(O1)(C)N(C(=C(N(C12)C(=C1C=CC3)C=3)C13)C1=C(C=3C3=O)CN3)C(=CC=CC3)C1=3)OC)(C2)NC
10.59	Quercetin; 3,3',4',5,7-Pentahydroxyflavone	Calbiochem (EMD)	551600	 c1cc(c(cc1C1=C(C(c2c(cc(cc2O1)O)O)=O)O)O)O
9.89	K252a	Calbiochem (EMD)	420298	 C12C(C3N(C=1C=CC=C2)C1(C)C2(O)C(OC)=O)=C(C(=C(C=34)C(=C3N4C(O1)(C2)[H])C=CC=C3)C1=O)CN1
9.25	GSK PKI collection	GSK	GSK2163632A	 CC1(C)CCN(C(CN(C)C)=O)c2cc(c(cc12)OC)Nc1nc(c2cc[nH]c2n1)Nc1ccsc1C(N)=O

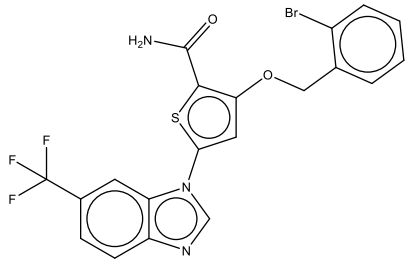
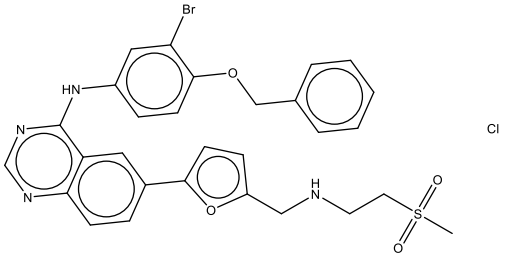
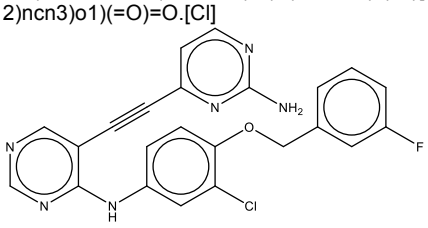
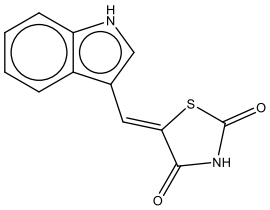
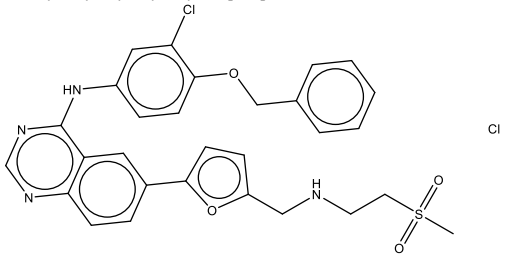
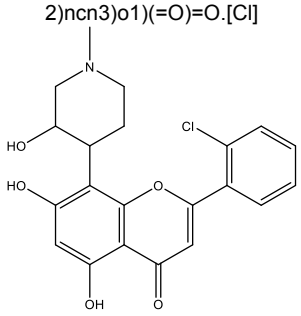
8.36	GSK PKI collection	GSK	GSK2110236A	 CN(C)CC(N1CCCc2cc(c(cc12)Nc1nc(c2cc[nH]c2n1)Nc1ccsc1C(N)=O)OC)=O
8.22	4,5,6,7-Tetrabromobenzo-triazole	Calbiochem (EMD)	218697	 c1(c(c(c2c(c1[Br])nn[nH]2)[Br])[Br])[Br]
8.13	JAK Inhibitor I; 2-(1,1-Dimethylethyl)-9-fluoro-3,6-dihydro-7H-benz[h]-imidaz[4,5-f]isoquinolin-7-one	Calbiochem (EMD)	420099	 CC(C)(C)c1nc2c3ccc(cc3c3C(NC=Cc3c2[nH]1)=O)F
7.37	GSK PKI collection	GSK	GSK1713088A	 CCCN1CCC(CC1)Oc1cc(c(cc1[Cl])OC)Nc1nc(c2cc[nH]c2n1)Nc1cccc(c1C(N)=O)F
6.93	GSK PKI collection	GSK	GW693881A	 C(c1cccc(c1)F)Oc1ccc(cc1[Cl])Nc1c2c(cc(c3ccc[nH]3)s2)ncn1.[Cl]

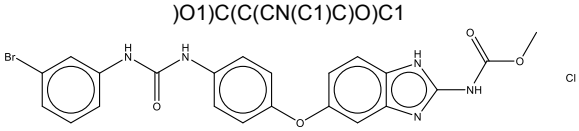
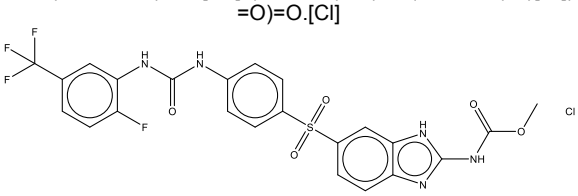
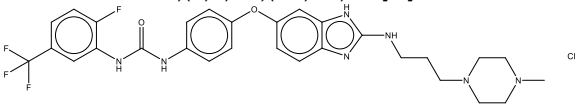
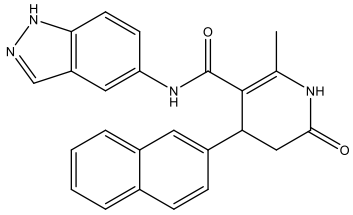
6.87	GSK PKI collection	GSK	GSK2220400A	 CNC(c1c(cccn1)Nc1c2cc[nH]c2nc(Nc2cc3c(CCCN3C(CN(C)C)=O)cc2OC)n1)=O
6.71	GSK PKI collection	GSK	GW416981X	 CC(C)COC(c1ccc2c(c1)C(=CNc1ccc(cc1)S(N)(=O)=O)C(N2)=O)=O
6.48	GSK PKI collection	GSK	GW806742X	 CN(c1ccc(cc1)NC(Nc1ccc(cc1)OC(F)(F)F)=O)c1ccnc(Nc2ccccc2)S(N)(=O)=O)n1
6.45	BIM I; Bisindolylmaleimide I; 2-[1-(3-Dimethylaminopropyl)-1H-indol-3-yl]-3-(1H-indol-3-yl)maleimide, HCl	Calbiochem (EMD)	203291	 CN(C)CCCn1cc(C2=C(C(NC2=O)=O)c2c[nH]c3ccccc23)c2ccccc12.[Cl]
6.44	Purvalanol A; 2-(1R-Isopropyl-2-hydroxyethylamino)-6-(3-chloroanilino)-9-isopropyl-purine	Calbiochem (EMD)	540500	 N(C(=C(C(=NC1NC(CO)C(C)C)N2C(C)C)N=C2)N=1)C(=CC(=CC1)[Cl])C=1

6.35	GSK PKI collection	GSK	GSK1326255A	 CCCN1CCC(CC1)c1ccc(c(c1)OC)Nc1nc(c2cc[nH]c2n1)Nc1cc(ccc1C(N)=O)F
6.19	BIM 10; Bisindolylmaleimide X, HCl	AXXORA	B-7204	 C(=C(C(C(C1=CC2)=CC=2)=C(N1C1)CC(C1)CN)C(N1)=O)(C1=O)C1C(C=CC=C2)=C2N(C=1)C
6.16	4-[(3-Bromophenyl)amino]-6,7-diaminoquinazoline	Calbiochem (EMD)	203697	 c1cc(cc(c1)[Br])Nc1c2cc(c(cc2ncn1)N)N
5.95	H-1152; (S)-(+)-2-Methyl-1-[(4-methyl-5-isoquinolinyl)sulfonyl]homopiperazine	Calbiochem (EMD)	555550	 N(CC(N1S(C(=C(C(=CC2)C3)C(=CN=3)C)C=2)(=O)=O)C)CCC1.[Cl].[Cl]
5.53	Compound 52; 2-(2-Hydroxyethylamino)-6-(3-chloroanilino)-9-isopropylpurine	Calbiochem (EMD)	234503	 CC(C)n1cnc2c(Nc3cccc(c3)[Cl])nc(NCCO)nc12
5.48	RO-3306	AXXORA	ALX-270-463	 C(c1cccs1)NC1=NC(C(=Cc2ccc3c(cccn3)c2)S1)=O

5.41	Aurora/Cdk Inhibitor	Calbiochem (EMD)	189406	 c1cc(c(C(n2c(N)nc(Nc3ccc(cc3)S(N)(=O)=O)n2)=O)c(c1)F)F
5.16	Met Inhibitor	Tocris	448101	 Cc1c(C(N2CCN(C)CC2)=O)c(C)[nH]c1C=C1C(Nc2ccc(cc12)S(N(C)c1cccc(c1)[Cl])(=O)=O)=O
4.96	GSK PKI collection	GSK	GW795493X	 c1cc(cc(c1)NC(Nc1ccc(cc1)c1coc2c1c(N)ncn2)=O)C(F)(F)F
4.92	9043251	ChemBridge	9043251	 COc1ccc(cc1C(Nc1ccc2c(c1)cn[nH]2)=O)[Cl]
4.92	GSK PKI collection	GSK	GSK994854A	 CCCN1CCC=C(C1)c1ccc(c(C)c1)Nc1nc(c2cc[nH]c2n1)Nc1ccccc1C(N)=O
4.89	Wee1/Chk1 inhibitor	Calbiochem (EMD)	681637	 [H]O[H].c1ccc(cc1)c1cc2c(c3C(NC(c13)=O)=O)c1cc(ccc1[nH]2)O

4.8	(Z,E)-3-(Imidazol-4-ylmethylene)indolin-2-one	Calbiochem (EMD)	175580	 C(=C1C(Nc2ccccc12)=O)c1c[nH]cn1
4.8	GSK PKI collection	GSK	GW589933X	 C(=C1C(Nc2ccc3c(c12)scn3)=O)Nc1ccc(cc1)S(N)(=O)=O
4.75	GSK PKI collection	GSK	GSK1173862A	 CCCN(CCCC1C(C=CC(NC(N=C(NC(=CC(F)=CC2)C=2C(N)=O)C(C=CN2)=C23)=N3)=C2OC)=C2)C1
4.52	STOCK4S-69416	InterBioScreen	STOCK4S-69416	 Cn1cc(C=C2C(NC(=O)S2)=O)c2ccccc12
4.5	10Z-Hymenialdisine; 4-(2-Amino-4-oxo-2-imidazolidin-5-ylidene)-2-bromo-4,5,6,7-tetrahydropyrrolo[2,3-c]azepin-8-one	Calbiochem (EMD)	400085	 C1CNC(c2c(cc([nH]2)[Br])C1=C1C(N=C(N)N1)=O)=O

4.43	GSK PKI collection	GSK	GW853606X	 C(c1ccccc1[Br])Oc1cc(n2cnc3ccc(cc23)C(F)(F)F)sc1C(N)=O
4.33	GSK PKI collection	GSK	GW580496A	 CS(CCNCc1ccc(c2ccc3c(c2)c(Nc2ccc(c(c2)[Br])OCc2ccccc2)ncn3)o1)(=O)=O.[Cl]
4.33	GSK PKI collection	GSK	GW799251X	 C(c1cccc(c1)F)Oc1ccc(cc1[Cl])Nc1c(C#Cc2ccnc(N)n2)cncn1
4.25	BAS 00253508	Asinex	BAS 00253508	 C(=C1C(NC(=O)S1)=O)c1c[nH]c2ccccc12
4.25	GSK PKI collection	GSK	GW574783B	 CS(CCNCc1ccc(c2ccc3c(c2)c(Nc2ccc(c(c2)[Cl])OCc2ccccc2)ncn3)o1)(=O)=O.[Cl]
4.13	Flavopiridol	AXXORA	ALX-430	 C(=C(C1)O)(C(=C(C=1O)C(C=C1C(=C(C=CC2)[Cl])C=2)=O

				)O1)C(C(CN(C1)C)O)C1
4.08	GSK PKI collection	GSK	GW694234A	 COC(Nc1nc2cc(ccc2[nH]1)Oc1ccc(cc1)NC(Nc1cccc(c1)[Br])=O)=O.[Cl]
4.07	GSK PKI collection	GSK	GW680908A	 COC(Nc1nc2ccc(cc2[nH]1)S(c1ccc(cc1)NC(Nc1cc(ccc1F)C(F)(F)F)=O)(=O)=O)=O.[Cl]
3.97	GSK PKI collection	GSK	GW700494A	 CN1CCN(CCCNc2nc3ccc(cc3[nH]2)Oc2ccc(cc2)NC(Nc2cc(ccc2F)C(F)(F)F)=O)CC1.[Cl]
3.92	GSK PKI collection	GSK	GSK270822A	 CC(=C(C1C(C=CC(=CC=CC2)C=23)=C3)C(=O)NC(C=CC(NN=C2)=C23)=C3)NC(C1)=O
3.89	GSK PKI Collection	GSK	GW300657X	C(c1ccncc1)NC(c1ccc2c(c1)C(C(N2)=O)=NNc1ccc(cc1)S(N)(=O)=O)=O
3.88	GSK PKI Collection	GSK	GW709042A	c1ccc(cc1)C(Nc1nc2cc(ccc2[nH]1)Oc1ccc(cc1)NC(Nc1cc(ccc1F)C(F)(F)F)=O)=O.[Cl]
3.82	GSK PKI Collection	GSK	GW796920X	'Cc1ccc(cc1c1ccc(cc1)C(NCC1CC1)=O)NC(CC1CC1)=O
3.72	GSK PKI Collection	GSK	GW290597X	C(CNC(c1ccc2c(c1)C(C(N2)=O)=NNc1ccc(cc1)S(N)(=O)=O)=O)Cn1ccnc1
3.6	GSK PKI Collection	GSK	GW284372X	C(c1ccccc1)Oc1ccc(cc1)Nc1c2cc(ccc2ncn1)c1ccco1
3.6	Cdk1/2 Inhibitor III	Calbiochem (EMD)	217714	c1cc(c(c(c1)F)NC(n1c(N)nc(Nc2ccc(cc2)S(N)(=O)=O)n1)=S)F
3.6	Syk Inhibitor	Calbiochem (EMD)	574711	Cn1cc(C=C2C(Nc3ccc(cc23)S(N)(=O)=O)=O)c2ccccc12
3.59	GSK PKI Collection	GSK	GW607049C	COC(Nc1nc2ccc(cc2[nH]1)Sc1ccc(cc1)NC(Nc1cc(ccc1F)C(F)(F)F)=O)=O.OS(O)(=O)=O
3.59	SU9516; 3-[1-(3H-Imidazol-4-yl)-meth-(Z)-ylidene]-5-methoxy-1,3-dihydro-indol-2-one	Calbiochem (EMD)	572650	COc1ccc2c(c1)C(=Cc1c[nH]cn1)C(N2)=O
3.58	GSK PKI Collection	GSK	GSK2219385A	Cc1cc(c(cc1N(C)C(CN(C)C)=O)Nc1nc(c2cc[nH]c2n1)Nc1ccc(c1C(N)=O)F)OC
3.55	GSK PKI Collection	GSK	GSK299115A	CC(=C(C1C(C=CC([Cl])=C2[Cl])=C2)C(=O)NC(C=CC(NN=C2)=C23)=C3)NC(C1)=O
3.54	GSK PKI Collection	GSK	GSK466314A	CC(=NN1)C2=C1C=CC(NC(=O)C(=C(C)NC(=O)C1)C1C(C=CC1C(F)(F)F)=CC=1)=C2
3.48	GSK PKI Collection	GSK	GSK466317A	CC(=C(C1C(=CC=C2C(F)(F)F)C=C2)C(=O)NC(C=C([Cl])C(NN=C2)=C23)=C3)NC(C1)=O
3.43	GSK PKI Collection	GSK	GSK1007102B	OC(=O)C(F)(F)F.CCN(C1C(=NON2)C=2N)C(=C(N=1)C1C#CC(C)(C)O)C=C(N=1)OC(CN)C(C=CC=C1)=C1
3.36	GSK PKI Collection	GSK	GW703087X	CC(Nc1cccc(C#Cc2cncnc2Nc2ccc(c(c2)[Cl])OCc2cccc(c2)F)c1)=O
3.29	GSK PKI Collection	GSK	GW616030X	CS(CCN(CC#N)Cc1ccc(c2ccc3c(c2)c(Nc2ccc(c(c2)[Cl])OCc2cccc(c2)F)ncn3)o1)(=O)=O
3.28	PDK/AKT inhibitor	Calbiochem (EMD)	521275	c1ccc2c(c1)C(c1c2nc2nnnn2n1)=O
3.27	GSK PKI Collection	GSK	GW633459A	C(CS(c1ccccc1)(=O)=O)NCc1ccc(c2ccc3c(c2)c(Nc2ccc(c(c

				2)[Cl])OCc2cccc(c2)F)ncn3)o1.[Cl]
3.25	GSK PKI Collection	GSK	GW701427A	COC(Nc1nc2cc(ccc2[nH]1)Oc1ccc(cc1)NC(Nc1cccc(c1)C(O)=O)=O)=O.[Cl]
3.25	GSK PKI Collection	GSK	GR105659X	C1Cc2cc(ccc2C1=C1C(Nc2ccccc12)=O)O
3.24	STO-609	Tocris	1551	c1ccc2c(c1)nc1c3ccc(C(O)=O)c4cccc(C(n12)=O)c34
3.19	GSK PKI Collection	GSK	GW770249X	c1cc(ccc1c1coc2c1c(N)ncn2)NC(Nc1cc(ccc1F)C(F)(F)F)=O
3.16	GSK PKI Collection	GSK	GW785804X	c1cc2c(ccc(c3c(c4ccc(cc4)F)nc(N)s3)n2)nc1
3.13	PKC beta inhibitor	Calbiochem (EMD)	539654	C(Cn1ccnc1)Cn1cc(C2=C(C(NC2=O)=O)Nc2ccccc2)c2cccc12
3.11	Oxindole I; 3-(1H-Pyrrol-2-ylmethylene)-1,3-dihydroindol-2-one	Calbiochem (EMD)	499600	C(=C1C(Nc2ccccc12)=O)c1ccc[nH]1
3.11	BIM 4; 8; bisindolylmaleimide IV	AXXORA	ALX-270-052-M001	c1ccc2c(c1)c(c[nH]2)C1=C(C(NC1=O)=O)c1c[nH]c2ccccc12
3.07	AOP 15805704	Asinex	AOP 15805704	C(=C(C(C=CC1F)=CC=1)N1)(C(C1=NC1)=CC=1)CN(CC(C1)O)CC1
3.05	GSK PKI Collection	GSK	GSK1220512A	CC(C)N1CCN(CC1)c1ccc(c(c1)OC)Nc1nc(c2cc[nH]c2n1)Nc1ccc(cc1C(N)=O)F
3.03	GSK PKI Collection	GSK	GW300660X	C(CNC(c1ccc2c(c1)C(C(N2)=O)=NNc1ccc(cc1)S(N)(=O)=O)=O)c1c[nH]cn1
2.98	3-(2-Chloro-3-indolylmethylene)-1,3-dihydroindol-2-one	Calbiochem (EMD)	217695	C(=C1C(Nc2ccccc12)=O)c1c2ccccc2[nH]c1[Cl]
2.98	PI3Kg inhib	Calbiochem (EMD)	528106	C(=C1C(NC(=O)S1)=O)c1ccc2c(c1)nccn2
2.97	GSK PKI Collection	GSK	GSK192082A	CCNC(=O)OC(CNC1C#CC(=CC(N=CN2)=C3C=2NC(C=CC(OCC(C=CC=C2F)=C2)=C2[Cl])=C2)S3)C1
2.96	GSK PKI Collection	GSK	GW831090X	c1cc(cc(c1)O)c1cc(Nc2ccc(cc2)S(N)(=O)=O)[nH]n1
2.93	GSK PKI Collection	GSK	GSK238063A	CN(C)C(=O)OC(CNC1C#CC(=CC(N=CN2)=C3C=2NC(C=CC(OCC(C=CC=C2F)=C2)=C2[Cl])=C2)S3)C1
2.9	Apigenin; 4',5,7-Trihydroxyflavone	Calbiochem (EMD)	178278	C1=C(c2ccc(cc2)O)Oc2cc(cc(c2C1=O)O)O
2.87	BAS 09687195	Asinex	BAS 09687195	C(=C1C(NC(=O)S1)=O)c1ccc(c2nc3ccccc3s2)o1
2.86	GSK PKI Collection	GSK	SB-736302	C1CC1n1c2ccncc2nc1c1c(N)non1
2.82	GSK PKI Collection	GSK	GW441756X	Cn1cc(C=C2C(Nc3cccnc23)=O)c2ccccc12
2.8	GSK PKI Collection	GSK	GW708336X	C1CC1Nc1nccc(c2cnn3c2ccc(n3)N2CCOCC2)n1
2.78	SB216763	AXXORA	10010246	Cn1cc(C2=C(C(NC2=O)=O)c2ccc(cc2[Cl])[Cl])c2ccccc12
2.68	GSK PKI Collection	GSK	GW759710A	c1cc(cc(c1)Nc1nccc(Nc2cccc(c2)F)n1)C(N)=O.[Cl]
2.67	PF 573228	Tocris	3239	CS(c1cccc(CNc2c(cnc(Nc3ccc4c(CCC(N4)=O)c3)n2)C(F)(F)F)c1)(=O)=O
2.66	GSK PKI Collection	GSK	GW439255X	Cc1cc(cc(C)c1C=Cc1cc(cnc1)C(=O)OC(C)(C)C)O
2.63	GSK PKI Collection	GSK	SB-476429-A	C(c1ccc(cc1)c1nc(c2ccc(c(c2)O)[Cl])c(c2ccncc2)[nH]1)N.[Cl]
2.58	GSK PKI Collection	GSK	GW566221A	CS(CCNCc1cc(c2ccc3c(c2)c(Nc2ccc(cc2)OCc2ccccc2)ncn3)oc1)(=O)=O.[Cl]
2.57	IKK-2 Inhibitor VI	Calbiochem (EMD)	401483	c1ccc(cc1)c1cc(C(N)=O)c(NC(N)=O)s1
2.51	GSK PKI Collection	GSK	GW659386A	COC(Nc1nc2cc(ccc2[nH]1)Oc1cccc(c1)NC(Nc1cc(ccc1F)C(F)(F)F)=O)=O.[Cl]
2.46	Genistein; 4',5,7-Trihydroxyisoflavone	Calbiochem (EMD)	345834	C1=C(C(c2c(cc(cc2O1)O)O)=O)c1ccc(cc1)O
2.43	Syk Inhibitor II	Calbiochem (EMD)	574712	C(CNc1ncc(C(N)=O)c(Nc2cccc(c2)C(F)(F)F)n1)N
2.42	IGF-1R Inhibitor II	Calbiochem (EMD)	407248	Cc1cc(c2ccccc2n1)NC(Nc1cc(ccc1OC)[Cl])=O
2.4	GSK PKI Collection	GSK	GW284408X	C(=C1C(Nc2ccccc12)=O)Nc1ccc2c(c1)NC(N2)=O
2.34	GSK PKI Collection	GSK	GW559768X	Cc1ccc(cc1Nc1ccnc2ccc(cc12)S(C)(=O)=O)O
2.32	GSK PKI Collection	GSK	GW683134A	c1cc(C(Nc2nc3cc(ccc3[nH]2)Oc2ccc(cc2)NC(Nc2cc(ccc2F)C(F)(F)F)=O)=O)oc1.[Cl]
2.29	GSK PKI Collection	GSK	GSK317354A	CC(=C(C1C(=CC=C(F)C2)C=2)C(=O)NC(C=CC(NN=C2)=C23)=C3)NC(=N1)C(=CC=C1C(F)(F)F)C=N1
2.27	GSK PKI Collection	GSK	GW781673X	C(c1ccc(cc1)[Cl])Nc1nccc(c2cnn3c2cccn3)n1
2.22	GSK PKI Collection	GSK	GW683768X	CCc1c(c2ccnc(NC3CC3)n2)c2cccnn2n1
2.22	5-Iodotubercidin; 4-	Calbiochem	407900	

	Amino-5-iodo-7-(b-D-ribofuranosyl)pyrrolo [2,3-d]pyrimidine	(EMD)		C(=NC=N1)(C(=C1N(C1)C(C(C(C2CO)O)O)O2)C=1I)N
2.21	TX-1918; 2-((3,5-dimethyl-4-hydroxyphenyl)-methylene)-4-cyclopentene-1,3-dione	Calbiochem (EMD)	655203	Cc1cc(C=C2C(C=CC2=O)=O)cc(C)c1O
2.19	JNK Inhibitor II; SAPK Inhibitor II; Anthra[1,9-cd]pyrazol-6(2H)-one	Calbiochem (EMD)	420119	c1ccc2c(c1)C(c1cccc3c1c2n[nH]3)=O
2.17	ART 16145605	Asinex	ART 16145605	CCN(CC)Cc1c2cccnc2[nH]c1c1ccncc1
2.14	GSK PKI Collection	GSK	GW622055X	CCS(c1ccc(c(c1)Nc1ncc(c2ccccc2[Cl])o1)OC)(=O)=O
2.12	AOP 16327220	Asinex	AOP 16327220	CCN(CCN(C)C)Cc1c2cccnc2[nH]c1c1ccc(cc1)F
2.11	GSK PKI Collection	GSK	GSK969786A	C(c1cccc(c1)F)Oc1ccc(cc1[Cl])Nc1c2cc(c3ccco3)sc2ncn1
2.08	GSK PKI Collection	GSK	GW396574X	CC(C)=Cc1cccc2c1C(C(N2)=O)=NNc1ccc(cc1)S(N)(=O)=O
2.08	BAS 00385194	Asinex	BAS 00385194	C(c1ccc(cc1[Cl])[Cl])Oc1cccc(C=C2C(NC(=O)S2)=O)c1
2.06	GSK PKI Collection	GSK	GSK326090A	CC(OC(C=C1N(C=N2)C3=C2C=CC(OCC(CCN(C)C2)C2)=C3)=C(S1)C(N)=O)C(=CC=CC1)C=1C(F)(F)F
2.03	GSK PKI Collection	GSK	SB-739452	C1CC1C(Nc1c2cc(c(c3ccco3)nc2[nH]n1)[Br])=O
2.01	Water			O

18 compounds that are not commercially available are omitted from the table.

Supporting References

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2. Boguth, C. A., Singh, P., Huang, C. C., and Tesmer, J. J. (2010) Molecular basis for activation of G protein-coupled receptor kinases, *EMBO J 29*, 3249-3259.