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Structure-based design of isoindoline-1,3-diones and 2,3-dihydrophthalazine-1,4-diones as novel B-Raf inhibitors

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ABSTRACT

Structure-guided design led to the discovery of novel chemical scaffolds for B-Raf inhibitors. Both type I and type II kinase inhibitors have been explored and lead compounds with good potency and excellent selectivity have been identified.

The RAS-RAF-MEK-ERK signal transduction pathway plays an important role in cell survival, growth, and proliferation.[1] Mutant B-Raf induces a constitutive activation of this pathway and is crucial for the development of certain types of tumors.[2] Therefore, inhibition of B-Raf offers a viable means for treating cancers.[3] In previous reports, we disclosed the development of a series of highly potent B-Raf inhibitors featuring a pyrazolopyrimidine core and a pyridyl hinge binder.[4,5] To further expand the chemical space of B-Raf inhibitors, we underwent a campaign to design novel scaffolds utilizing available structure information.

At the time we started our study both the X-ray structures of type I and II kinase inhibitors with B-Raf had been published[6] (Fig. 1). SB-590885[7,8] is a tri-substituted imidazole binding to the active conformation of B-Raf, while sorafenib[2,9] is a di-substituted urea interacting with the DFG-out inactive conformation of B-Raf. Both structures employ a nitrogen containing heterocycle as the hinge binder forming a hydrogen bond with hinge residue C532 which is also true for most of other B-Raf inhibitors.[10] We envisioned a different functional group as the hinge binder. As shown in a recent review,[11] amide groups are frequently seen in kinase inhibitors and it could form two hydrogen bonds with the hinge residue.[12] Our design started with the imidazole B-Raf inhibitors[8] where the pyridyl ring was replaced with an amide group and that was positioned toward C532 residue by the construction of a fused six-membered ring (Fig. 2). Dess–Martin oxidation of alcohol **4** provide aldehyde **5**, which underwent a Fischer indolization with 4-hydrazinylbenzoic acid followed by amidation and then demethylation to afford **3** (Scheme 1). The IC_{50} of compound **3** against B-Raf[13] was greater than 10 μM, although the modeling study suggested a favorable pose in the ATP binding pocket with the carbonyl group interacting with C532 of the active conformation model[14] (Fig. 3). We reasoned that there might be an energy penalty to fix the conformation of the amide group. A literature search revealed that imide groups can interact with the hinge residues when placed in appropriate positions.[15] Therefore, compound **11** with a tied imide group was proposed. The iodination of commercially available 5-aminoisoindoline-1,3-dione (**8**) followed by a intra-molecular Heck reaction gave tricycle compound **10**.

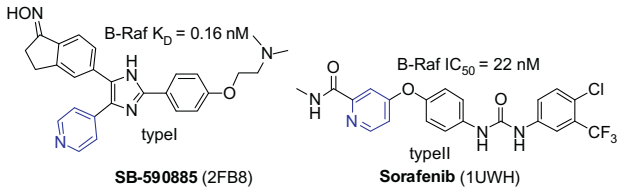

Figure 1. B-Raf inhibitors. Hinge binder highlighted as blue, PDB code shown in parentheses.

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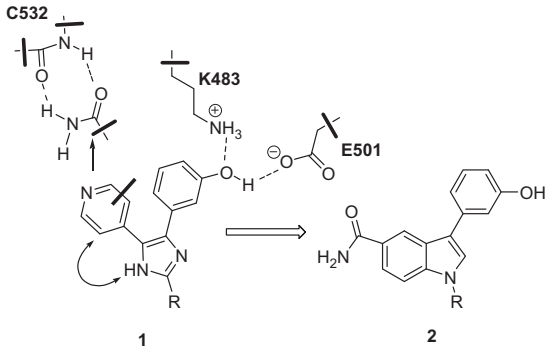
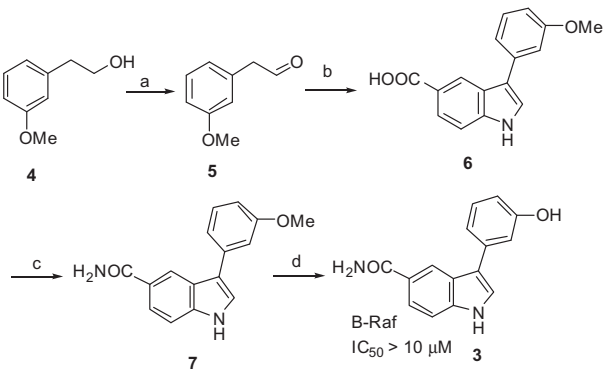


Figure 2. The design of B-Raf inhibitors.



Scheme 1. Reagents and conditions: (a) Dess–Martin periodinane, CH_2Cl_2, rt; (b) 4-hydrazinylbenzoic acid, HOAc, 130 °C, 45% two steps; (c). CDI, then NH_4OH, 50%; (d). BBr_3, CH_2Cl_2, rt, 50%.

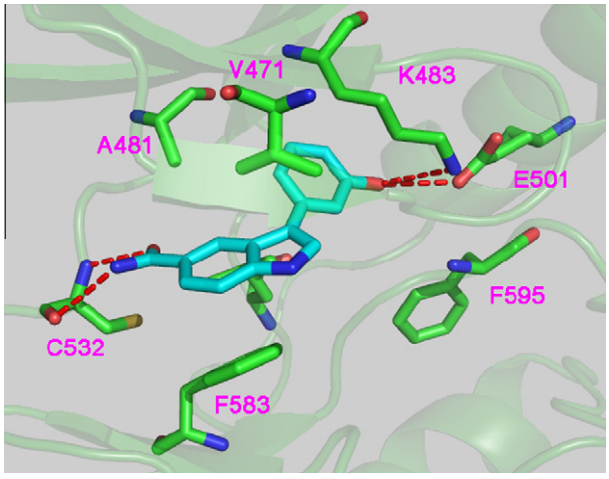
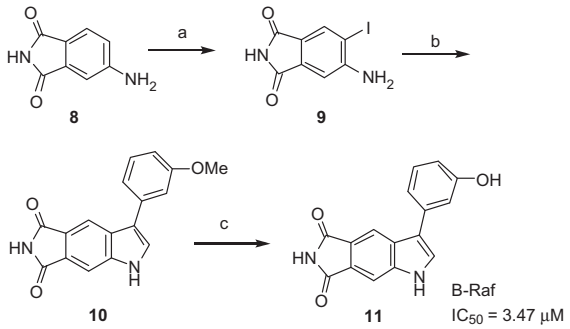


Figure 3. Model of compound **3a** with B-Raf.

Deprotection with boron tribromide afforded the desired product **11** (Scheme 2). To our delight, compound **11** was active against B-Raf with an IC_{50} of 3.47 μM.

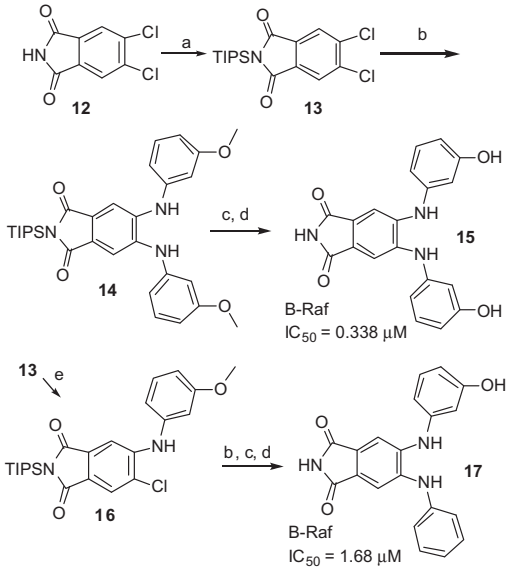
To further optimize this series, we wondered if the flat tricyclic core could be replaced with a more flexible system. To explore other favorable interactions and improve physical properties,[16] two bicyclic compounds **15** and **17** were designed and synthesized. The preparation of compound **15** started with 5,6-dichloroisoindoline-1,3-dione (**12**). The silyl protection of **12** followed by a



Scheme 2. Reagents and conditions: (a) 2 equiv NIS, DMF, 45 °C, 50%; (b) **5**, Pd, DABCO, 30%; (c) BBr_3, CH_2Cl_2, rt, 50%.

Buchwald coupling reaction[17] with two equivalents of 3-methoxyaniline afforded intermediate **14**. Subsequential deprotection with tetra-*n*-butylammonium fluoride and boron tribromide gave compound **15**. Compound **17** was synthesized in a similar route via selective stepwise Buchwald coupling reactions (Scheme 3). Both isoindoline-1,3-diones **15** and 2,3-dihydrophthalazine-1,4-dione **17** were more potent than tricyclic analog **11** supporting the hypothesis that a bicyclic scaffold affords a more potent B-Raf inhibitor. This data warranted optimization of the bicyclic scaffold. The model of **15** with B-Raf (Fig. 4) suggested there are favorable hydrogen bond interactions between the imide group and the hinge region. The isoindoline-1,3-dione core sits in a hydrophobic pocket surrounded by A481, V471, L514, and F583. One phenol group interacts with K483 and E501 while the other phenol group forms two hydrogen bonds with D594 and N581; this may explain fivefold increases in potency compared to analog **17**.

Our efforts for the further exploration of novel hinge binders led to the discovery 2,3-dihydrophthalazine-1,4-dione **18** and **19**, which were prepared by the reaction of compound **15** and **17** with hydrazine (Scheme 4). Interestingly, both compounds showed comparable activity to the isoindoline-1,3-dione analogs and a model of compound **19** with B-Raf suggests a binding mode very similar to that of **15** (Fig. 5). A substructure search of diacylhydrazide (CONHNHCO) motif against the RCSB protein data bank[18] did



Scheme 3. Reagents and conditions: (a) TIPSCl, Et_3N, rt, 74%; (b) $ArNH_2$, cat. XPhos, cat. $Pd_2(dba)_3$, K_3PO_4, toluene, reflux, 50–60%; (c) TBAF, rt; (d) BBr_3, CH_2Cl_2, rt, 40–60% two steps; (e) $Ar'NH_2$, cat. *t*-BuXPhos, cat. $Pd_2(dba)_3$, K_3PO_4, toluene, reflux, 37%.

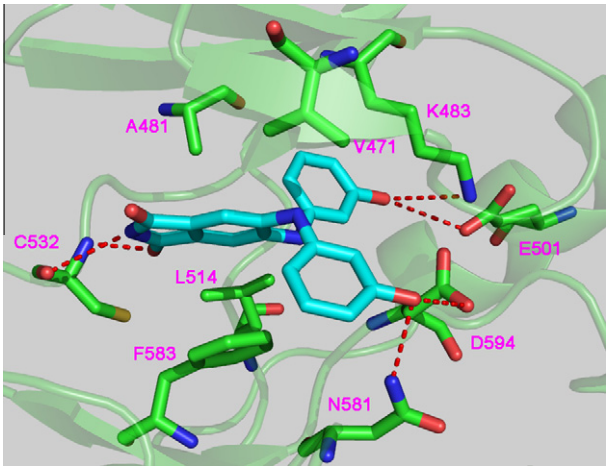
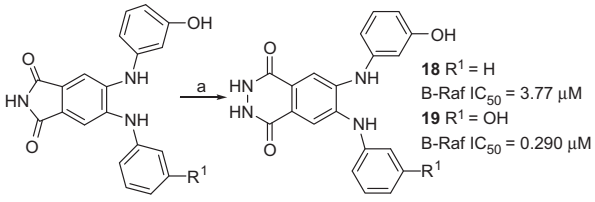


Figure 4. Model of compound **15** with B-Raf (active conformation).



Scheme 4. Reagents and condition: (a) N_2H_4, rt, DMF, 80%.

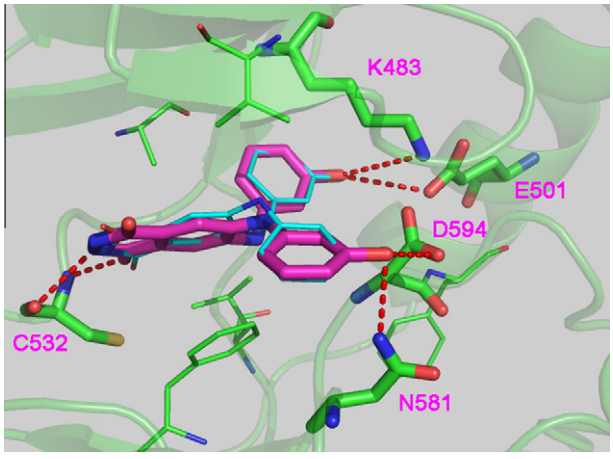


Figure 5. Model compound **19** with B-Raf (compound **15** shown as blue).

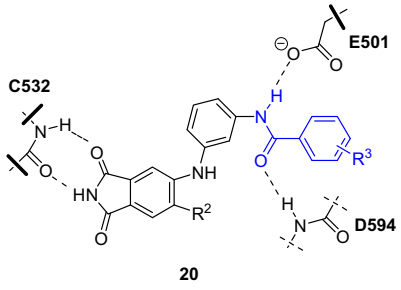


Figure 6. Design of type II B-Raf inhibitors.

not reveal any kinase X-ray crystal structures containing such a hinge binder suggesting it is an uncommon binding element.

After we identified potent type I B-Raf inhibitor leads containing novel scaffolds by using structure-based drug design strategies, we wondered if we could build a type II inhibitor using the same chemical scaffolds that might be beneficial for better selectivity and/or high biochemical efficiency.[19,20] It has been known[21] that type I kinase inhibitors can be converted to type II kinase inhibitors by the attachment of a lipophilic group at an appropriate position presumably filling the pocket created by the movement of DFG motif. Compound **20** was constructed by the replacement[22] of the phenol group with a benzamide group (Fig. 6). The amide group was designed to form two hydrogen bonds with E501 and D594, while the phenyl group could extend to the allosteric pocket in DFG-out conformation (Fig. 7).[23] The R^2 group was also changed

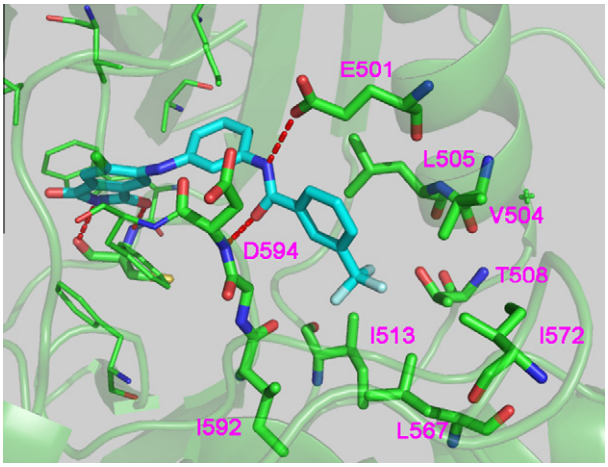
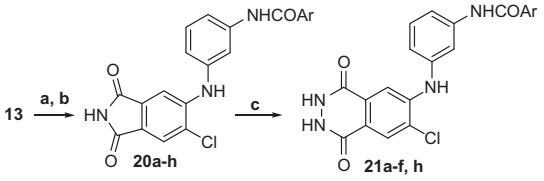
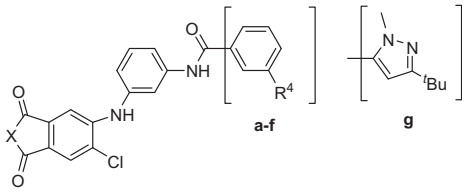


Figure 7. Model of compound **20d** with B-Raf (DFG-out conformation).



Scheme 5. Reagents and conditions: (a) ArCOCl, pyridine, rt; (b) TBAF, rt, 50–80% two steps; (c) N_2H_4 ~80%.

Table 1
Type II B-Raf inhibitors



X = NH	R^4	B-Raf IC_{50} (μM)	X = HNNH	R^4	B-Raf IC_{50} (μM)
20a	Cl	0.117	**21a**	Cl	0.035
20b	Br	0.035	**21b**	Br	0.019
20c	I	0.008	**21c**	I	0.008
20d	CF_3	0.010	**21d**	CF_3	0.017
20e	OCF_3	0.006	**21e**	OCF_3	0.018
20f	CN	0.297	**21f**	CN	0.296
20g	—	0.349	**21g**	—	0.293

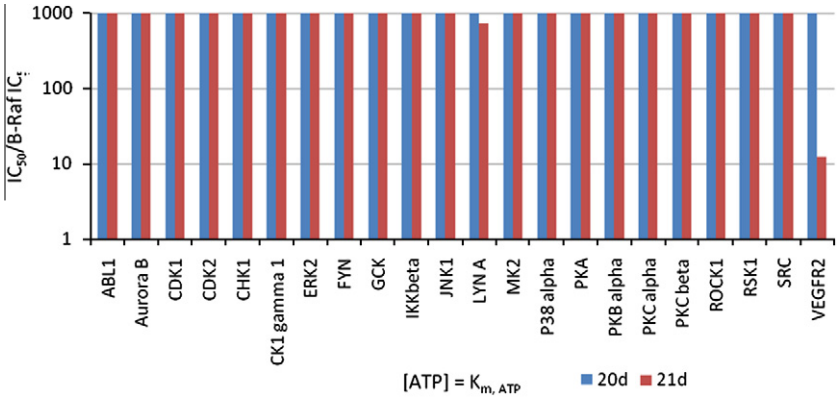


Figure 8. Selectivity of compounds **20d** and **21d**.

from a phenyl to a chlorine atom because the modeling study suggested that a large R^2 may have an unfavorable steric interaction with the transposed activation loop.[24] Isoindoline-1,3-diones **20a–g** were prepared from intermediate **13** by acylation and then deprotection (Scheme 5). Further reaction with hydrazine provided 2,3-dihydrophthalazine-1,4-diones **21a–g** (Scheme 5).

Both the isoindoline-1,3-dione analogs and 2,3-dihydrophthalazine-1,4-dione analogs exhibited good activity against B-Raf (Table 1). The potency increased from *meta*-chloro, bromo, to iodo analogs (**20a–c**/**21a–c**) indicated large *meta* lipophilic substituents on the phenyl ring were preferred to fill the hydrophobic pocket formed by seven non-polar amino acid residues (Fig. 7). The *meta*-trifluoromethylphenyl group has been documented[9,25] as a good hydrophobic group to fill the DFG-out pocket and analogs (**20d**, **21d**, **20e**, and **21e**) with such a group or *meta*-trifluoromethoxylphenyl indeed showed low nanomolar IC_{50}s. As we expected, compounds with a polar substitute (**20g**, **21g**) were less potent. Interestingly a *tert*-butyl pyrazole group, which was optimal for a p38 type II inhibitor,[26] did not give better potency.

The selectivity profile of compound **20d** and **21d** was assessed against a panel of 22 protein kinases (Fig. 8). Both of the compounds exhibited excellent selectivity against all of tested kinases including p38alpha, PKCbeta, and VEGFR2, which were inhibited by our previous pyrazolopyrimidine series[5] and the well known type II Raf inhibitor sorafenib.[9]

In summary, both type I and type II B-Raf inhibitor leads with novel chemical scaffolds and hinge binders have been developed with the aid of structure-based drug design technology. Compounds with low nanomolecular activity have been identified and they are suitable for further optimization.

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References and notes

1. Wellbrock, C.; Karasarides, M.; Marais, R. *Nat. Rev. Mol. Cell Biol.* **2004**, *5*, 875–885.
2. Wan, P. T. C.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R. *Cell* **2004**, *116*, 855–867.
3. Shepherd, C.; Puzanov, I.; Sosman, J. A. *Curr. Oncol. Rep.* **2010**, *12*, 146–152.
4. Wang, X.; Berger, D. M.; Salaski, E. J.; Torres, N.; Hu, Y.; Levin, J. I.; Powell, D.; Wojciechowicz, D.; Collins, K.; Frommer, E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6571–6574.
5. Wang, X.; Berger, D. M.; Salaski, E. J.; Torres, N.; Dutia, M.; Hanna, C.; Hu, Y.; Levin, J. I.; Powell, D.; Wojciechowicz, D.; Collins, K.; Frommer, E.; Lucas, J. *J. Med. Chem.* **2010**, *53*, 7874–7878.
6. The only known B-Raf crystal structures at that time were 1UWH, 1UWJ with sorafenib and 2FB8 with SB-590885. Updated to 8/2011, there are 18 X-ray crystal structures of B-Raf with small ligands in RCSB Protein Data Bank (1UWH, 1UWJ, 2FB8, 3C4C, 3C4D, 3D4Q, 3IDP, 3II5, 3OG7, 3PPJ, 3PPK, 3PRF, 3PRI, 3PSB, 3PSD, 3Q4C, 3Q96, 3SKC).
7. King, A. J.; Patrick, D. R.; Batorsky, R. S.; Ho, M. L.; Do, H. T.; Zhang, S. Y.; Kumar, R.; Rusnak, D. W.; Takle, A. K.; Wilson, D. M.; Hugger, E.; Wang, L.; Karreth, F.; Lougheed, J. C.; Lee, J.; Chau, D.; Stout, T. J.; May, E. W.; Rominger, C. M.; Schaber, M. D.; Luo, L.; Lakdawala, A. S.; Adams, J. L.; Contractor, R. G.; Smalley, K. S. M.; Herlyn, M.; Morrissey, M. M.; Tuveson, D. A.; Huang, P. S. *Cancer Res.* **2006**, *66*, 11100–11105.
8. Takle, A. K.; Brown, M. J. B.; Davies, S.; Dean, D. K.; Francis, G.; Gaiba, A.; Hird, A. W.; King, F. D.; Lovell, P. J.; Naylor, A.; Reith, A. D.; Steadman, J. G.; Wilson, D. M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 378–381.
9. Wilhelm, S. M.; Carter, C.; Tang, L.; Wilkie, D.; McNabola, A.; Rong, H.; Chen, C.; Zhang, X.; Vincent, P.; McHugh, M.; Cao, Y.; Shujath, J.; Gawlak, S.; Eveleigh, D.; Rowley, B.; Liu, L.; Adnane, L.; Lynch, M.; Auclair, D.; Taylor, I.; Gedrich, R.; Voznesensky, A.; Riedl, B.; Post, L. E.; Bollag, G.; Trail, P. A. *Cancer Res.* **2004**, *64*, 7099–7109.
10. Wong, K. K. *Recent Patents Anticancer Drug Discov.* **2009**, *4*, 28–35.
11. Ghose, A. K.; Herbertz, T.; Pippin, D. A.; Salvino, J. M.; Mallamo, J. P. *J. Med. Chem.* **2008**, *51*, 5149–5171.
12. Very recently a series of B-Raf inhibitors with an amide group as the hinged binder was published in this journal. Packard, G. K.; Papa, P.; Riggs, J. R.; Erdman, P.; Tehrani, L.; Robinson, D.; Harris, R.; Shevlin, G.; Perrin-Ninkovic, S.; Hilgraf, R.; McCarrick, M. A.; Tran, T.; Fleming, Y.; Bai, A.; Richardson, S.; Katz, J.; Tang, Y.; Leisten, J.; Moghaddam, M.; Cathers, B.; Zhu, D.; Sakata, S. *Bioorg. Med. Chem. Lett.* **2011**. doi:10.1016/j.bmcl.2011.03.006.
13. The B-Raf enzymatic assay protocol has been described in the following reference. Gopalsamy, A.; Ciszewski, G.; Hu, Y.; Lee, F.; Feldberg, L.; Frommer, E.; Kim, S.; Collins, K.; Wojciechowicz, D.; Mallon, R. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2735–2738.
14. A model built from 2FB8 (PDB) was used.
15. Zhao, B.; Bower, M. J.; McDevitt, P. J.; Zhao, H.; Davis, S. T.; Johanson, K. O.; Green, S. M.; Concha, N. O.; Zhou, B.-B. S. *J. Biol. Chem* **2002**, *277*, 46609–46615.
16. A recent analysis suggested that disruption of molecular planarity has a positive impact on the solubility. Ishikawa, M.; Hashimoto, Y. *J. Med. Chem.* **2011**, *54*, 1539–1554.
17. Hennessy, E. J.; Buchwald, S. L. *J. Org. Chem.* **2005**, *70*, 7371–7375.
18. RCSB Protein Data Bank website: www.pdb.org.
19. Backes, A.; Zech, B.; Felber, B.; Klebl, B.; Müller, G. *Expert Opin. Drug Discov.* **2008**, *3*, 1427–1449.
20. Alton, G. R.; Lunney, E. A. *Expert Opin. Drug Discov.* **2008**, *3*, 595–605.
21. Liu, Y.; Gray, N. S. *Nat. Chem. Biol.* **2006**, *2*, 358–364.
22. Wolin, R. L.; Bembenek, S. D.; Wei, J.; Crawford, S.; Lundeen, K.; Brunmark, A.; Karlsson, L.; Edwards, J. P.; Blevitt, J. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2825–2829.
23. A model built from 1UWH (PDB) was used.
24. To test this hypothesis a close analog to compound **20d** with a *meta*-methoxyphenyl replacing chlorine atom was prepared and it was eight fold less potent than **20d**.
25. Weisberg, E.; Manley, P. W.; Breitenstein, W.; Brüggen, J.; Cowan-Jacob, S. W.; Ray, A.; Huntly, B.; Fabbro, D.; Fendrich, G.; Hall-Meyers, E.; Kung, A. L.; Mestan, J.; Daley, G. Q.; Callahan, L.; Catley, L.; Cavazza, C.; Azam, M.; Mohammed, A.; Neuberg, D.; Wright, R. D.; Gilliland, D. G.; Griffin, J. D. *Cancer Cell* **2005**, *7*, 129–141.
26. Pargellis, C.; Tong, L.; Churchill, L.; Cirillo, P. F.; Gilmore, T.; Graham, A. G.; Grob, P. M.; Hickey, E. R.; Moss, N.; Pav, S.; Regan, J. *Nat. Struct. Mol. Biol.* **2002**, *9*, 268–272.