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Structure-based drug design enables conversion of a DFG-in binding CSF-1R kinase inhibitor to a DFG-out binding mode

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ABSTRACT

The work described herein demonstrates the utility of structure-based drug design (SBDD) in shifting the binding mode of an HTS hit from a DFG-in to a DFG-out binding mode resulting in a class of novel potent CSF-1R kinase inhibitors suitable for lead development.

Colony-stimulating factor-1 receptor (CSF-1R or cFMS) is a receptor tyrosine kinase whose expression is restricted to macrophages, osteoclasts and trophoblasts and which is uniquely responsible for mediating the growth differentiation and survival effects of monocyte colony stimulating factor-1 (CSF-1).[1] CSF-1 signals through its receptor by means of ligand-induced dimerization and subsequent autophosphorylation. Macrophage proliferation, activation and survival are believed to be important for the progression of diseases such as rheumatoid arthritis (RA), osteoporosis, inflammatory bowel disease, and cancer. Thus, a reduction in the number of synovial macrophages through inhibition of CSF-1R signaling is expected to be therapeutic in RA, related inflammatory diseases, and cancer.[1,2]

There are a number of CSF-1R inhibitors with in vivo antiinflammatory efficacy reported in the literature, including **1**,[2a–c] **2**,[2d] **Ki20227**,[2e,f] and **GW2580**.[2g,h] As a part of our program targeting CSF-1R for RA, our internal kinase-targeted compound library was screened measuring the inhibition of autophosphorylation of CSF-1R in a cell-based assay.[3] Compounds **3a** and **3b** were identified as potent inhibitors of CSF-1R (IC_{50} = 40 nM and 57 nM, respectively). However, the high molecular weights (MW = 524–525) and poor ligand efficiencies (LE = 0.27–0.28) of these two hits required significant structural modifications to maximize their potential as leads. The work described herein demonstrates the utility of structure-based drug design (SBDD) in shifting the binding mode of this series from DFG-in to a DFG-out binding mode resulting in novel potent CSF-1R kinase inhibitor leads.

In our preliminary evaluation of this series, we were surprised to discover that compound **3a** was found to bind CSF-1R in a classical DFG-in binding mode (Fig. 2a).[4] Dramatic conformational differences in the juxtamembrane domain and activation loops are apparent when this DFG-in structure is compared with prior DFG-out CSF-1R structures, with some residues displaced by over 30 Å. Indeed, superposing the DFG-out and -in structures shows that elements of the juxtamembrane domain in the former (especially residues 550–554) mimic elements of the activation loop in the latter (residues 797–804). This is the first example of a DFG-in binding mode for a CSF-1R kinase inhibitor.[5,2i] It superposes well on the structure of the active conformation of cKit bound to ADP (RCSB code 1PKG).

Several known selective CSF-1R inhibitors bind the enzyme in a DFG-out mode. For example, **GW2580** is exquisitely selective for CSF-1R (Fig. 2b).[2g,h,5b] An important feature of this co-crystal structure is the bidentate hydrogen bonding network made by the oxygen atoms of the methoxy and benzyloxy groups of the 'DFG-out Tail' to the backbone amide NH of D796. We hypothesized that this is an important feature for locking in the DFG-out binding mode revealing access to the deep pocket.

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We desired a chemical series with a DFG-out binding mode, which would lock the CSF-1R kinase into an inactive confirmation.[6a,b] DFG-out inhibition is expected to increase the biochemical efficiency, defined as how effectively the binding of an inhibitor to the target results in blocking a physiological response.[6c,d] Furthermore, the likelihood for kinase selectivity is enhanced by a DFG-out binding mode as only a subset of kinases can achieve DFG-out conformations. We hypothesized that we could transform this aminopyrazine class of inhibitors into a class of DFG-out binders. An overlay of the crystal structures of **3a** and **GW2580** (Fig. 2c) illustrates the overlap of the hinge binding group aminopyrazine in **3a** and the corresponding diaminopyrimidine group in **GW2580**. Since the basic amine head group of **3a** reaches out to solvent, apparently contributing little to binding affinity, we hypothesized that the head group could be truncated to a simple aromatic group (Step 1 in Fig. 1). If this could be accomplished, then the DFG-out tail group found in **GW2580** could be incorporated (Step 2 in Fig. 1) giving hydrid compound **4** with a DFG-out binding mode, a hypothesis supported by molecular docking studies (Figs. 1 and 2d).

Compound **3a** and **3b**, while quite potent, have high molecular weights (MW) resulting in relatively poor ligand efficiencies (LE).[7] In order to transform the dichlorobenzyl ether group into the higher MW DFG-out tail group, which was anticipated to be necessary to bind into the DFG-out deep pocket, we initiated an effort to truncate the aryl amide head group. In addition to reducing MW, we desired to remove the basic amine as it puts this series at risk for binding the hERG channel.[8]

Compounds **8a–c** were prepared as shown in Scheme 1. Selective nucleophilic attack by benzylic alcohols or amines on dibromoaminopyrazine **5** results in bromopyrazine intermediate **6** which undergoes Suzuki coupling with aromatic boronic acids and esters such as pyrazole boronate **7** to give final products **8a–b**. Other aromatic and heteroaromatic boronic acids and esters were also prepared in a library format (data not shown).

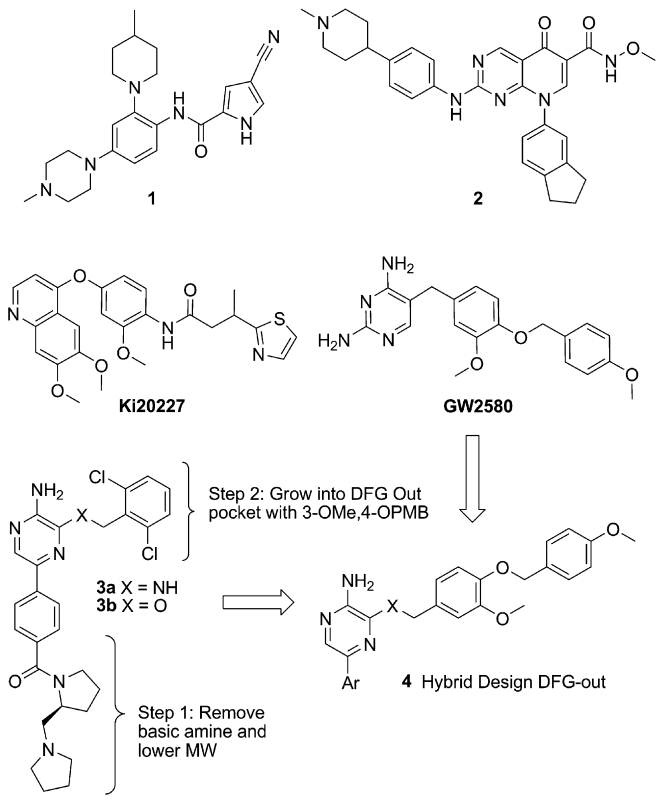


Figure 1. CSF-1R inhibitors. HTS hits and hybrid SBDD approach to designing a DFG-out kinase inhibitor.

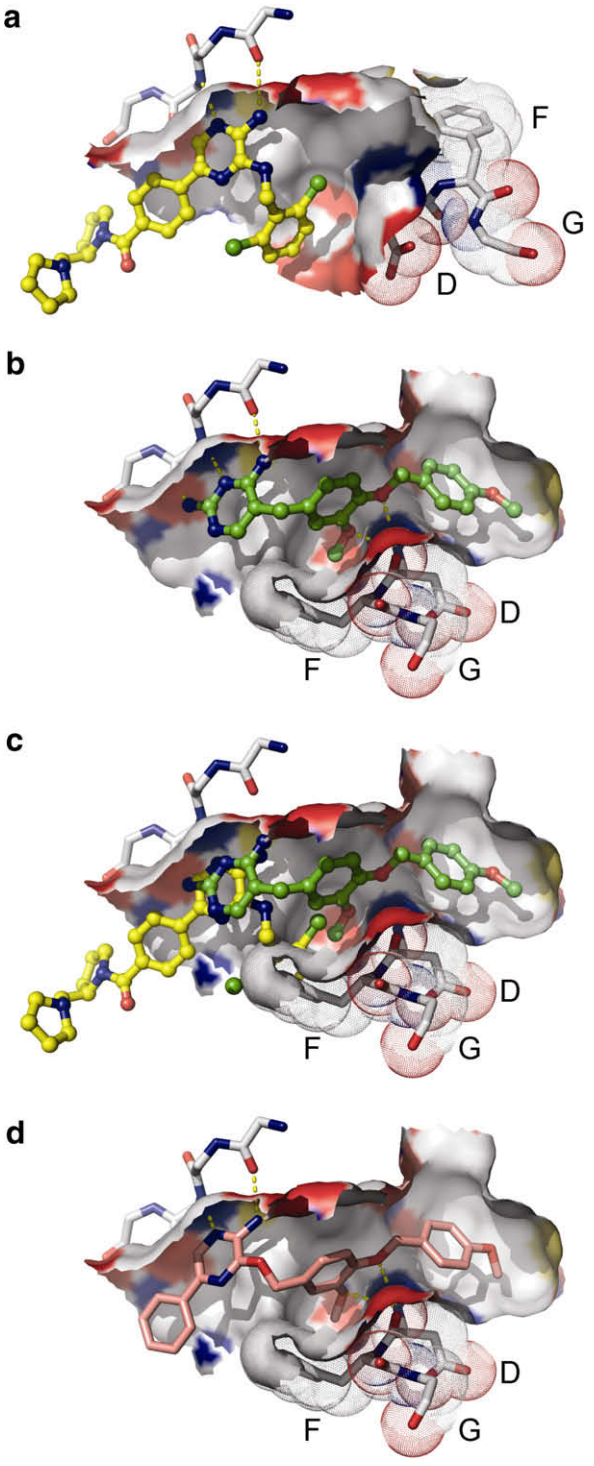
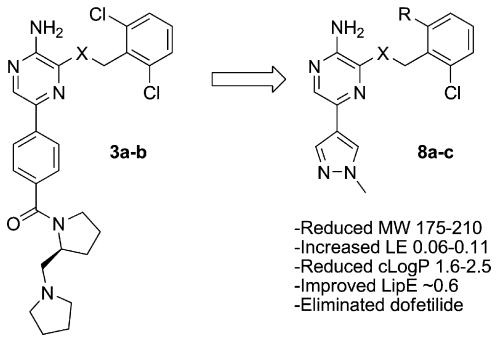


Figure 2. Binding surfaces are colored by electrostatic potential. (a) X-ray crystal structure of compound **3a** in CSF-1R in a DFG-in binding mode at 2.5 Å resolution (Ref. 4; RCSB code 3LCD). (b) X-ray crystal structure of **GW2580** in CSF-1R in a DFG-out binding mode (Ref. 5). (c) Overlay of compound **3a** (yellow) and **GW2580** (green) X-ray crystal structures in CSF-1R. (d) Molecular docking of hypothetical hybrid (pink) of compound **3a** and **GW2580** in the X-ray crystal structure of **GW2580**/CSF-1R.

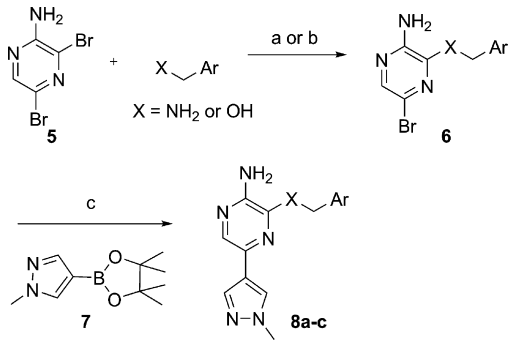
We were delighted to find that the entire aryl amide head group from compounds **3a–b** could be truncated to a simple methyl pyrazole as shown in compounds **8a–c** (Table 1). Although 9- and 30-fold less potent than the hits **3a–b**, compounds **8a–b** are significantly more ligand efficient (LE) than the screening hits

Table 1
Truncation of HTS hits



Compd	X	R	CSF-1R IC_{50}[a] (nM)	MW	LE	*c* Log *P*	LipE	Dof. % inh.[b]
3a	NH	—	40	524	0.28	5.5	1.9	78
3b	O	—	57	525	0.27	5.7	1.5	91
8a	NH	Cl	345	348	0.38	3.7	2.8	6
8b	O	Cl	1700	349	0.34	3.9	1.9	7
8c	O	H	1990	315	0.35	3.2	2.5	7

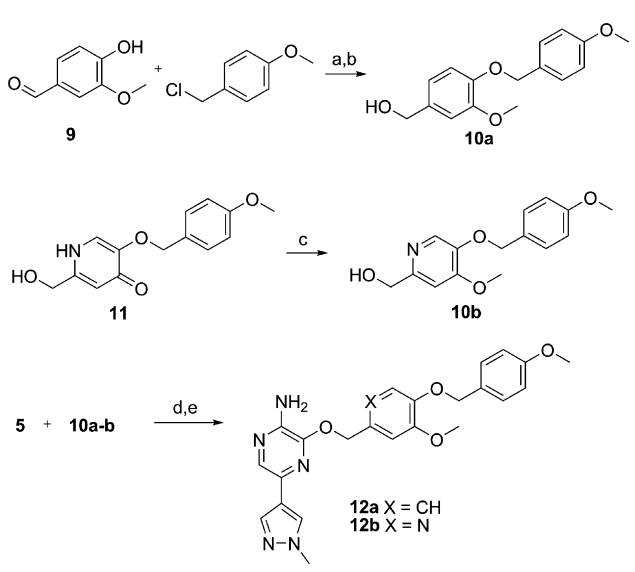
[a] Ref. 3.
[b] Dof. = dofetilide-Cy3B competitive binding assay, % inhibition at 10 μM of compound. See Ref. 8c.



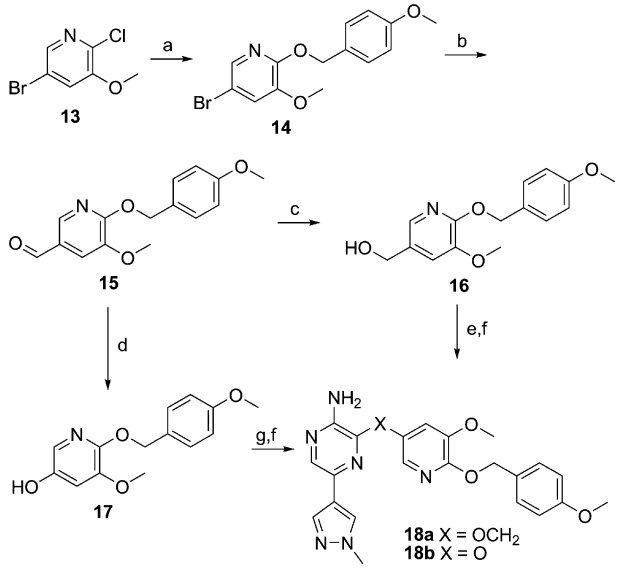
Scheme 1. Reagents and conditions: (a) X = NH_2; Na_2CO_3, NMP, 165 °C; (b) X = OH; KO*t*Bu, THF, 60 °C; (c) $Pd(PPh_3)_2Cl_2$, 2 M Na_2CO_3, DME, 80 °C.

due to their reduced MWs. Compound **8c** illustrates the second Cl atom was also unnecessary for CSF-1R potency. In terms of *c* Log *P* and lipophilic efficiency (LipE),[9] compounds **8a** and **8c** are an improvement of approximately one order of magnitude. Furthermore, the hERG potential of this series was significantly reduced as evidenced by the reduction in inhibition of the binding of fluorescently tagged dofetilide to the hERG channel.[8c] As a result, compound **8c** was then utilized as the core pharmacophore onto which the DFG-out tail group was incorporated.

The requisite extended benzyl alcohols **10a–b**, **16**, and **17** and final potential DFG-out binding hybrid inhibitors **12a–b** and **18a–b** were prepared as shown in Schemes 2 and 3. Benzaldehyde **9** was alkylated with *para*-methoxybenzyl chloride and then reduced with sodium borohydride to give benzyl alcohol **10a**. Pyridine derivative



Scheme 2. Reagents and conditions: (a) KOH, acetonitrile, reflux; (b) $NaBH_4$, MeOH, dioxane; (c) $TMSCHN_2$, MeOH, toluene; (d) KO*t*Bu, THF, 60 °C; (e) **7**, $Pd(PPh_3)_2Cl_2$, 2 M Na_2CO_3, DME, 80 °C.



Scheme 3. Reagents and conditions: (a) 4-methoxybenzyl alcohol, NaH, DMF; (b) (i) *n*-BuLi, THF, −60 °C; (ii) DMF; (c) $NaBH_4$, THF, MeOH. (d) (i) mCPBA, CH_2Cl_2; (ii) NaOMe, MeOH; (e) **5**, KO*t*Bu, THF, 60 °C; (f) **7**, $Pd(PPh_3)_2Cl_2$, 2 M Na_2CO_3, DME, 80 °C; (g) **5**, Cs_2CO_3, DMF, 70 °C.

10b was prepared by O-methylation of pyridone **11**[10] with trimethylsilyldiazomethane. Benzyl alcohols **10a**–**b** were then reacted with dibromoaminopyrazine **5**, followed by Suzuki coupling.

Pyridine derivatives **16** and **17** were prepared starting with 5-bromo-2-chloro-3-methoxypyridine (**13**) as shown in Scheme 3. Displacement of the chloride with 4-methoxybenzyl alcohol followed by lithium–halogen exchange and trapping with DMF gives aldehyde **15**. Aldehyde **15** was reduced with sodium borohydride to give alcohol **16**. Alcohol **16** was reacted with **5** followed by Suzuki coupling to give pyridine analog **18a**. Baeyer–Villiger rearrangement of aldehyde **15**, followed by hydrolysis in methanolic sodium methoxide gave phenol **17**. Nucleophilic displacement in the presence of cesium carbonate followed by Suzuki coupling gave analog **18b**.

Table 2 shows potency of compounds **12a**–**b** and **18a**–**b**. Incorporation of the **GW2580**-like DFG-out tail (**12a**) resulted in a dramatic 36-fold enhancement in CSF-1R potency over **8c**, presumably properly accessing the deep binding pocket revealed in a DFG-out binding mode. Similarly, pyridine variants of this methoxyphenyl-PMB tail were also quite potent (**12b** and **18a**). In contrast to **GW2580**, these analogs have an additional oxygen atom in the linker between the hinge binding aminopyrazine and the DFG-out tail group, yet still inhibit CSF-1R with a high degree of potency. Pyridine analog **18b** demonstrates that the OCH_2 linker can be shortened to a single oxygen atom, analogous to the 1-atom linker length of the **GW2580** scaffold, resulting in a slight improvement in CSF-1R potency.

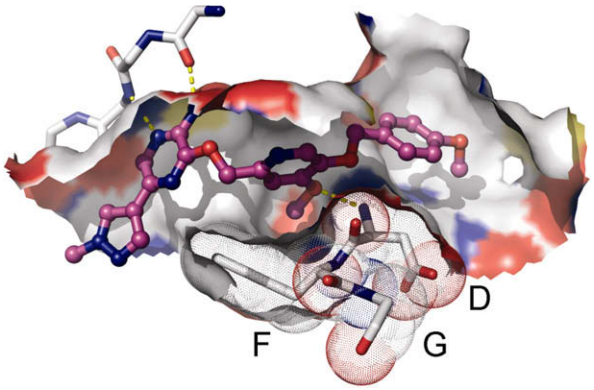


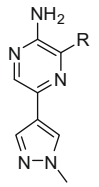
Figure 3. X-ray crystal structure of compound **12b** in CSF-1R at 3.4 Å resolution in a DFG-out binding mode (Ref. 4; RCSB code 3LCO).

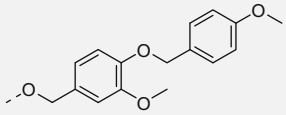
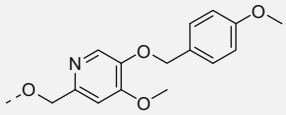
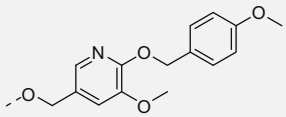
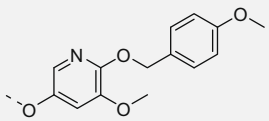
To ascertain whether compounds **12a**–**b** and **18a**–**b** were indeed binding in a DFG-out mode, compound **12b** was co-crystallized with CSF-1R. A relatively low resolution structure of **12b** with CSF-1R showed that **12b** was indeed binding in a DFG-out mode (Fig. 3).[4] Although hydrogen bonding interactions cannot be definitively determined given the resolution of the crystallographic data, the methoxy and benzyloxy oxygen atoms are near D796 in a manner similar to **GW2580**. A hydrogen bonding interaction with this residue is likely a key requirement for binding and accessing the DFG-out deep pocket.

By transforming a series of DFG-in CSF-1R inhibitors to DFG-out inhibitors, we have established a potent series more suitable for lead development. In contrast to **3a**–**b**, which were less attractive in terms of MW, LE, LipE, and hERG potential, **12a**–**b** and **18a**–**b** have significantly reduced MW, *c* Log *P*, improved LipE, and reduced hERG potential (Tables 1 and 2). For example, LipE's were improved 2–3.5 orders of magnitude and MW's were reduced into the 425–450 range. Furthermore, the potency and ligand/lipophilic efficiency enhancements were made in a more relevant cell-based assay system as opposed to a biochemical enzyme assay.

In summary, we have successfully utilized SBDD to transform a classical DFG-in binding mode HTS hit into a class of potent DFG-

Table 2
DFG-out binding leads



Compd		CSF-1R IC_{50}[a] (nM)	MW	LE	*c* Log *P*	LipE
12a		56	447	0.30	3.8	3.5
12b		143	448	0.28	3.1	3.7
18a		30	448	0.31	3.1	4.4
18b		12	434	0.34	2.9	5.0

[a] Ref. 3.

out binding CSF-1R inhibitors with potential to be developed into viable CSF-1R kinase inhibitor drug candidates.

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References and notes

1. Pixley, F. J.; Stanley, E. R. *Trends Cell Biol.* **2004**, *14*, 628.
2. (a) Patch, R. J.; Brandt, B. M.; Asgari, D.; Baindur, N.; Chadha, N. K.; Georgiadis, T.; Cheung, W. S.; Petrounia, I. P.; Donatelli, R. R.; Chaikin, M. A.; Player, M. R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6070; (b) Illig, C. R.; Chen, J.; Wall, M. J.; Wilson, K. J.; Ballentine, S. K.; Rudolph, M. J.; DesJarlais, R. L.; Chen, Y.; Schubert, C.; Petrounia, I.; Crysler, C. S.; Molloy, C. J.; Chaikin, M. A.; Manthey, C. L.; Player, M. R.; Tomczuk, B. E.; Meegalla, S. K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1642; (c) Meegalla, S. K.; Wall, M. J.; Chen, J.; Wilson, K. J.; Ballentine, S. K.; DesJarlais, R. L.; Schubert, C.; Crysler, C. S.; Chen, Y.; Molloy, C. J.; Chaikin, M. A.; Manthey, C. L.; Player, M. R.; Tomczuk, B. E.; Illig, C. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3632; (d) Huang, H.; Hutta, D. A.; Rinker, J. M.; Hu, H.; Parsons, W. H.; Schubert, C.; DesJarlais, R. L.; Crysler, C. S.; Chaikin, M. A.; Donatelli, R. R.; Chen, Y.; Cheng, D.; Zhou, Z.; Yurkow, E.; Manthey, C. L.; Player, M. R. *J. Med. Chem.* **2009**, *52*, 1081; (e) Uemura, Y.; Ohno, H.; Ohzeki, Y.; Takanashi, H.; Murooka, H.; Kubo, K.; Serizawa, I. *J. Neuroimmunol.* **2008**, *195*, 73; (f) Ohno, H.; Uemura, Y.; Murooka, H.; Takanashi, H.; Tokieda, T.; Ohzeki, Y.; Kubo, K.; Serizawa, I. *Eur. J. Immunol.* **2008**, *38*, 283; (g) Conway, J. G.; McDonald, B.; Parham, J.; Keith, B.; Rusnak, D. W.; Shaw, E.; Jansen, M.; Lin, P.; Payne, A.; Crosby, R. M.; Johnson, J. H.; Frick, L.; Lin, M.-H. J.; Depee, S.; Tadepalli, S.; Votta, B.; James, I.; Fuller, K.; Chambers, T. J.; Kull, F. C.; Chamberlain, S. D.; Hutchins, J. T. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16078; (h) Conway, J. G.; Pink, H.; Bergquist, M. L.; Han, B.; Depee, S.; Tadepalli, S.; Lin, P.; Crumrine, R. C.; Binz, J.; Clark, R. L.; Selph, J. L.; Stimpson, S. A.; Hutchins, J. T.; Chamberlain, S. D.; Brodie, T. A. *J. Pharmacol. Exp. Ther.* **2008**, *326*, 41; (i) Manthey, C. L.; Player, M. L. *Ann. Rep. Med. Chem.* **2009**, *44*, 211; (j) Manthey, C. L.; Johnson, D. L.; Illig, C. R.; Tuman, R. W.; Zhou, Z.; Baker, J. F.; Chaikin, M. A.; Donatelli, R. R.; Franks, C. F.; Zeng, L.; Crysler, C.; Chen, Y.; Yurkow, E. J.; Boczon, L.; Meegalla, S. K.; Wilson, K. J.; Wall, M. J.; Chen, J.; Ballentine, S. K.; Ott, H.; Baumann, C.; Lawrence, D.; Tomczuk, B. E.; Molloy, C. J. *Mol. Cancer Ther.* **2009**, *8*, 3151.
3. HEK293 Freestyle cells transiently transfected with human CSF-1R vector (Origene, SC116882) were suspended in DMEM supplemented with 0.1% fetal bovine serum, 1% L-glutamine, and 0.0405% bovine albumin (Sigma #A-8918) at 1.7×10^6. Thirty microlitre of the cell suspension were then dispensed into each well of plates containing 100 nL of compound in 100% DMSO. After 1 h of incubation at 37 °C, cells were lysed by adding 40 μL of 2× lysis buffer to each well and incubated for 4 min at room temperature. Forty microlitre of lysates were then transferred to a capture antibody coated and blocked ELISA plate. c-Fms/CSF-1R(C-20) antibody (Santa Cruz, cat.#: sc-692) was diluted to 1 μg/mL in Superblock (Pierce, cat #37545), and 50 μL was added to each well of goat anti-rabbit coated plates and incubated at room temperature for 60–90 min. Plates were washed three times with wash buffer (TBS + 0.1% Tween 20), and 40 μL of cell lysate was transferred to the ELISA capture plate and incubated for 2 h at room temperature. Plates were again washed three times with wash buffer, and 50 μL/well HRP-PY20 antibody (Santa Cruz, cat# SC508 Hrp) diluted to 1 μg/mL in Superblock was added to the plate and incubated for 30 min at room temperature. Plates were washed three times with wash buffer, and 50 μL/well of TMB solution (Bio-Rad, Cat# 172-1066) were added and plates were incubated at room temperature for 1–10 min (incubation time varies depending on color development). The color reaction was then stopped by addition of 50 μL/well of 0.1 N H_2SO_4 and plates were read on a spectrophotometer with 450 nm OD.
4. Compound **3a** bound to CSF-1R was crystallized following the protocol of Schubert et al. (Ref. 5a). CSF-1R protein for the co-crystal with **12b** was obtained following the protocol of Emerson et al. (Ref. 5b). 11 mg/mL protein in 20 mM HEPES pH 7.5, 300 mM NaCl, 5 mM DTT was incubated with 4 mM inhibitor at 4 °C overnight followed by the addition of 1 μg Arg-C purchased from Roche (per 50 μL sample) and further incubated at 22 °C for 24 h. The limited proteolysis was quenched with the addition of 0.5 μL of 5 mg/mL leupeptin. Hanging drop vapor diffusion experiments were set up by mixing 2.0 μL of protein solution with equal volumes of well solution (22.5–35% PEG 4000, 0.1 M Tris–HCL pH 8.5, 0.2 M $MgCl_2$). Crystallographic data were integrated and scaled using the HKL2000 software package (Ref. 11a), refinement was carried out using programs contained within the CCP4 suite (Ref. 11b), manual rebuilding was performed with COOT (Ref. 11c), ligands were docket with Afitt (OpenEye Scientific), and structural figures made using PyMol (http://www.pymol.org). CSF-1R bound to **3a** was refined to a crystallographic R value of 21.8% (R_{free} 26.5%) using all the data to 2.5 Å resolution and CSF-1R bound to **12b** was refined to a crystallographic R value of 24.7% (R_{free} 28.9%) using all the data to 3.4 Å resolution.
5. (a) Schubert, C.; Schalk-Hihi, C.; Struble, G. T.; Ma, H.-C.; Petrounia, I. P.; Brandt, B.; Deckman, I. C.; Patch, R. J.; Player, M. R.; Spurlino, J. C.; Springer, B. A. *J. Biol. Chem.* **2007**, *282*, 4094; (b) Emerson, H. K.; Musso, D. L.; Chamberlain, S. D.; Peckham, G. E. U.S. Patent 7,491,731, 2009.
6. (a) 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. Biol.* **2002**, *9*, 268; (b) Knight, Z. A.; Shokat, K. M. *Chem. Biol.* **2005**, *12*, 621; (c) Swinney, D. C. *Nat. Rev. Drug Disc.* **2004**, *3*, 801; (d) Swinney, D. C. *Curr. Top. Med. Chem.* **2006**, *6*, 461.
7. (a) Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430; (b) Reynolds, C. H.; Tounge, B. A.; Bembenek, S. D. *J. Med. Chem.* **2008**, *51*, 2432; (c) Tyrchan, C.; Blomberg, N.; Engkvist, O.; Kogej, T.; Muresan, S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6943.
8. (a) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. *J. Med. Chem.* **2006**, *17*, 5029; (b) Jalaie, M.; Holsworth, D. D. *Mini-Rev. Med. Chem.* **2005**, *5*, 1083; (c) Singleton, D. H.; Boyd, H.; Steidl-Nichols, J. V.; Deacon, M.; de Groot, M. J.; Price, D.; Nettleton, D. O.; Wallace, N. K.; Troutman, M. D.; Williams, C.; Boyd, J. G. *J. Med. Chem.* **2007**, *50*, 2931.
9. Ryckmans, T.; Edwards, M. P.; Horne, V. A.; Correia, A. M.; Owen, D. R.; Thompson, L. R.; Tran, I.; Tutt, M. F.; Young, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4406.
10. Miller, W. H.; Pendrak, I.; Seefeld, M. A. WO2006/002047, 2006.
11. (a) Otwinoski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307; (b) Collaborative Computational Project, Number 4. *Acta Crystallogr., Sect. D* **1994**, *50*, 760.; (c) Emsley, P.; Cowtan, K. *Acta Crystallogr.* **2004**, *D60*, 2126.