Potent, Selective Inhibitors of Fibroblast Growth Factor Receptor Define Fibroblast Growth Factor Dependence in Preclinical Cancer Models

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Abstract

We describe here the identification and characterization of 2 novel inhibitors of the fibroblast growth factor receptor (FGFR) family of receptor tyrosine kinases. The compounds exhibit selective inhibition of FGFR over the closely related VEGFR2 receptor in cell lines and in vivo. The pharmacologic profile of these inhibitors was defined using a panel of human tumor cell lines characterized for specific mutations, amplifications, or translocations known to activate one of the four FGFR receptor isoforms. This pharmacology defines a profile for inhibitors that are likely to be of use in clinical settings in disease types where FGFR is shown to play an important role. Mol Cancer Ther; 10(9); 1542–52. ©2011 AACR.

Introduction

The fibroblast growth factor (FGF) family and their 4 receptor tyrosine kinases, FGFR1/2/3/4, mediate numerous physiologic processes including cell migration, proliferation, survival, and differentiation. Given the importance of FGF/FGFR, it is unsurprising that aberrant FGFR signaling is found in many tumor types including multiple myeloma, gastric, endometrial, prostate, and breast (1, 2). Gain-of-function mutations in FGFRs are the most common kinase abnormality in cancer with activation occurring via a range of mechanisms such as point mutation, amplification, chromosomal translocation, and aberrant splicing (3). For example, the t(4;14)(p16;q32) chromosomal translocation found in 15% of multiple myeloma patients often results in overexpression of FGFR3 (4–6). The overexpressed FGFR3 is usually wild type and although somatic mutations are occasion-

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with fragment-derived hits, we used a structure-based design (25–27) to optimize lead molecules to potent FGFR inhibitors with selectivity against VEGFR2, which shares 57% sequence identity with the kinase domain of FGFR1–3 and 54% with that of FGFR4. Further optimization of pharmacokinetic properties resulted in a series of imidazopyridine leads, the properties of which are described here. The profile in preclinical models presented here describes a paradigm for the response of cellular systems with a defined genetic background to a specific FGFR inhibitor.

Materials and Methods

Compounds 1 and 2 are 1-[(4-fluorophenyl)imidazo[1,2-a]pyridin-3-yl]phenyl]-3-(2,2,2-trifluoroethyl)urea and 1-[(5-methyl-[1,3,4]oxadiazol-2-yl)imidazo[1,2-a]pyridin-3-yl]phenyl]-3-(2,2,2-trifluoroethyl)urea, respectively. These and additional compounds used were synthesized by Astex Therapeutics Ltd. or Ortho Biotech (28).

Cell lines

KG-1, MFE-296, and RT112/84 cell lines were from the European Collection of Animal Cell Cultures. Snu-1, Snu-16, Kato-III, Hec-1A, and AN3-CA cell lines were obtained from the American Type Culture Collection. RPMI-8226 and wild-type Ba/F3 cell lines were obtained from German Collection of Microorganisms and Cell Cultures. The KMS-11 cell line was obtained from the Japanese Collection of Research Biorepositories. The Jim-1 cell line was obtained under license from Cancer Research Technology. Stably transfected Ba/F3 cell lines expressing TEL-FGFR1/3/4 were generated from fusion expression constructs of the TEL oligomerization domain linked to the 5' end of FGFR kinase domains in pcDNA3.1 (Invitrogen) and electroporation into wild-type Ba/F3 cells. Selection of stable cell lines was conducted with Geneticin (Invitrogen) in the absence of mouse interleukin 3. Human umbilical vein endothelial cells (HUVEC) were obtained from Clonetics and grown in EGM2 medium (Clonetics). Antibodies were from Cell Signaling Technology, except total FRS2-α from Santa Cruz Biotechnology.

Cloning, expression, and purification of kinase domains for FGFR1 and VEGFR2

A construct spanning residues 455 to 763 of the wild-type human FGFR1 with L455V, C486A, and C582S mutations was expressed as a His-tagged protein in Sf9 insect cells.

Cell pellets were lysed by sonication in a buffer containing 25 mmol/L Tris-HCl at pH 8.0, 250 mmol/L sodium chloride, 10% glycerol, 10 mmol/L imidazole, and 5 mmol/L β-mercaptoethanol. After centrifugation, the supernatant was incubated with Ni-NTA fast flow resin and eluted with an imidazole gradient. The eluate was incubated overnight with TEV protease to remove the His-tag and purified on a Ni column. Ion exchange and size exclusion chromatography were used to further purify the protein.

A construct encompassing residues 805 to 1,171, but omitting 50 residues of the kinase insert domain, was made for the kinase domain of VEGFR2. This construct was expressed and purified as described (29).

Crystallization, diffraction data collection, structure determination, and refinement

Crystals were obtained by the hanging drop method. FGFR1 crystals were obtained by seeding into a solution containing 10 to 15 mg/mL of protein, 100 mmol/L Bis-Tris buffer at pH 6.5, 300 mmol/L ammonium sulfate, 12% to 16% PEG10000, and 5% ethylene glycol.

VEGFR2 crystals were obtained from drops containing 7 mg/mL of protein mixed with 100 mmol/L HEPES, pH 7.2, 2.0 to 2.2 mol/L ammonium sulfate, and 4% mPEG550.

Complexes of FGFR1 with compounds 1, 2, and 4 were prepared by soaking the crystals in saturated solutions in 10% dimethyl sulfoxide (DMSO) and well solution for 3, 3, and 2 days, respectively. The complex of VEGFR2 with compound 4 was prepared by soaking the compound into the crystal for 3 hours in 10% DMSO and well solution.

All data for FGFR were collected using a Jupiter CCD detector mounted on an R200 rotating anode x-ray generator. Data were processed and scaled using d*trek. X-ray data for VEGFR2 were collected at the ESRF on ID23.1 and processed and scaled using MOSFLM. All structures were solved using molecular replacement and difference Fourier methods and refined using REFMAC (CCCP4, United Kingdom).

In vitro kinase activities

Inhibition of kinase activity in vitro was conducted as described in Supplementary Data.

Proliferation assays

Cells were seeded into 96-well plates at 5 × 10³ cells per well before addition of compound in 0.1% DMSO for 72 hours. A solution of 10% v/v Alamar Blue (Biosource International) was added following compound incubation and cells incubated for a further 6 hours. Plate fluorescence was read at λex = 535 nm and λem = 590 nm.

Phospho-FGFR3 and phospho-VEGFR2 ELISA

Phospho-FGFR3 was measured in KMS-11 cell lysates using a DuoSetIC ELISA (R&D Systems). KMS-11 cells were plated out in serum-free medium (2 × 10⁶ cells per well) and treated with compounds for 30 minutes before lysing in 125 μL of TG lysis buffer [20 mmol/L Tris, pH 7.6, 0.14 mol/L NaCl, 1% (v/v) Triton X-100, 10% (v/v) glycerol, 0.05 mol/L NaF, and 1 mmol/L Na3VO4 + protease inhibitor tablet from Roche (Mini Complete; used at 1 tablet/10 mL)] for 30 minutes at 4°C. Lysates (100 μL) were assayed according to the manufacturer’s protocol.
Phospho-VEGFR2 was measured using a DuoSet IC ELISA (R&D Systems). HUVECs (5 × 10⁴ cells per well) were plated out in EGM2 medium (including 1% serum) and left to recover overnight. The cells were switched to serum-free medium and left for further 16 hours and then treated with compounds for 30 minutes and stimulated with recombinant human VEGF165 (R&D Systems) at 100 ng/mL for 5 minutes at 37°C before lysing in 125 µL of TG lysis buffer for 30 minutes at 4°C. Lysates (100 µL) were assayed according to the manufacturer’s protocol.

Western blotting

Cell or xenograft lysates were prepared in TG lysis buffer (see above), cleared by centrifugation, and normalized to total protein by BCA assay (Thermo Scientific). Equal quantities of total protein were denatured and run on SDS-PAGE gels (Invitrogen) and transferred to nitrocellulose blots (Invitrogen). Bound primary antibodies were detected using IR-labeled secondary antibodies (Li-Cor) and an Odyssey imager (Li-Cor).

Xenograft models

All animal studies were conducted according to the relevant national regulatory guidelines and individual experiments approved by the appropriate institutional animal welfare committee. Cells from tissue culture were implanted subcutaneously in the right flank of 8- to 10-week-old BALB/c Foxn1nu/nu mice (Charles River) or BALB/c Irsd athymic nude-Foxn1nu (Harlan) at 5 × 10⁶ cells per animal in 50% Matrigel basement media (BD Biosciences) and 50% (v/v) RPMI 1640 media (Invitrogen). Treatment commenced when tumors were palpable (approximately 5 mm × 5 mm, 10–14 days postimplantation). Groups of tumor-bearing animals (n = 8) received dosing vehicle (control) by oral gavage or FGFR3 inhibitor as indicated in the schedule in a dosing volume of 10 mL/kg. Tumor volume was calculated by caliper (Mitutoyo) measurements using the equation \( a^2 \times b/2 \), where \( a \) is the smallest measurement and \( b \) the largest. Data are presented as mean relative tumor volume, where the tumor volume on the initial day of treatment (day 0) is assigned a relative tumor volume value of 1.

A complete regression was defined as a decrease in tumor volume to an undetectable size, less than 3 mm in any dimension. Tolerability was estimated by monitoring body weight loss, clinical signs, and survival. Statistical significance between control and treatment was determined by using ANOVA with Dunnnett’s post test for 3 or more groups or Student’s t test for 2 groups.

Pharmacodynamic studies

Subcutaneous xenograft tumors were removed from nude mice at the indicated times following a single oral administration of FGFR inhibitor. Tumor samples were ground to a fine powder under liquid nitrogen and protein extracted by addition of 1 mL triton lysis buffer. Western blots were conducted as outlined in Materials and Methods.

Results

Structure-based design of compounds 1 and 2, inhibitors of FGFR, and basis for VEGFR2 selectivity

Fragment screening against FGFR was conducted using a combination of nuclear magnetic resonance spectroscopy, thermal denaturation, and x-ray crystallography, resulting in the identification of greater than 30 x-ray structures of fragments in FGFR1. One attractive starting point for medicinal chemistry was the imidazopyridine fragment (compound 3). Because of the low molecular weight of this fragment hit, it had a relatively low potency with an IC₅₀ of 120 µmol/L versus FGFR3. However, when potency was normalized with respect to size, an encouraging value of 0.38 kcal per heavy atom was obtained as a measure of the fragment’s ligand efficiency (30). The FGFR1 crystal structure shows that the imidazopyridine binds in the ATP site of the kinase forming a single hydrogen bond to the backbone NH of Ala564 (see Fig. 1B). The binding site suggests that the agent acts as an inhibitor by competing for binding with ATP. The experimentally determined binding mode suggested how potency might be readily improved. First, replacement of the chlorine with substituted aromatics could be used to form hydrogen bonds with the side chain of Asp641 and in addition, access to this part of the enzyme might be used to drive selectivity through interactions with Ala640, which in VEGFR2 is substituted by the larger amino acid, cystine. Second, the crystal structure indicated that the ester group at position 6 of the imidazopyridine template was probably not useful for affinity, whereas elaboration at position 7 might facilitate good surface complementarity between the ligand and a region of the protein where affinity increases are often observed in kinases.

This structure-based drug design approach led to identification of the selective and potent FGFR inhibitor compound 1 that has an FGFR3 potency of 3 nmol/L and a selectivity of about 30-fold over VEGFR2 (Fig. 1A and C) with reduced lipophilicity relative to compound 1. Both compounds were potent inhibitors of all 4 FGFR isoforms with greater selectivity over VEGFR2, platelet-derived growth factor receptor (PDGFR) β, and the epidermal growth factor receptor (EGFR) family than TKI-258 or brivanib (Table 1). Compound 1 in particular retained activity against VEGFR1 and VEGFR3 and Flt-3, although a screen conducted against a larger panel of kinases indicated that only MKNK and RIPK2 were sensitive to the compounds at 100 nmol/L and below (Supplementary Table S1). More details on the fragment screening, the structure-based drug design approach, and the associated structure activity relationships will be reported elsewhere in due course.

Crystal structures of compounds 1 and 2 bound to FGFR1 were obtained (Fig. 1C). Both compounds showed a positioning of the imidazopyridine template similar to
compound 3 and the formation of the hydrogen bond with the N–H of Ala564 on the hinge of the kinase. As expected, the aromatic group at position 7 of the imidazo[1,2-a]pyridine showed good surface complementarity with the protein. From the structure activity relationships of related compounds (data not shown), it is apparent that

Table 1. *In vitro* assays were conducted for the indicated kinases

<table>
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<tr>
<th>Kinase</th>
<th>Compound 1 (IC_{50}, nmol/L)</th>
<th>Compound 2 (IC_{50}, nmol/L)</th>
<th>Brivanib (IC_{50}, nmol/L)</th>
<th>TKI-258 (IC_{50}, nmol/L)</th>
<th>ZD6474 (IC_{50}, nmol/L)</th>
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NOTE: IC_{50} was calculated using Prism software and expressed as mean of at least 2 independent experiments conducted in duplicate. Abbreviation: ND, not determined.
the urea group is important for both potency and selectivity of the compounds. The 2 N–Hs of the urea formed a dual hydrogen bond with the carboxylate of Asp641, whereas the carbonyl of the urea formed a water-mediated hydrogen bond with side chain of Arg627. To our knowledge, this positioning for Arg627 has not been seen before in FGFR1 crystal structures and it appeared to be induced by the water-mediated interaction with the urea.

Figure 2. Inhibition of proliferation and survival in FGFR-dependent cell lines. Antiproliferative activity of compounds 1 and 2 were assessed in a panel of human tumor cell lines as described in Materials and Methods. Cell lines known not to be dependent upon FGFR signaling were included (*). Experiments were conducted in triplicate, and data presented are composed of the mean of at least 2 independent experiments and values expressed as the fold change from the mean log IC50.
The lack of potency of compounds 1 and 2 against VEGFR2 and their low solubility in the crystallography buffer system precluded us from obtaining a structure of either in complex with VEGFR2. However, we did succeed in obtaining a VEGFR2 crystal structure for the relatively potent and selective compound 4 (Fig. 1A) from the same series (FGFR3 IC₅₀ = 12 nmol/L; VEGFR2 IC₅₀ = 220 nmol/L). Figure 1D shows the experimentally determined binding mode of compound 4 in VEGFR2 superimposed on the experimental binding mode of the same compound in FGFR1. The 2 binding modes for this ligand are very similar and selectivity in the series must be driven by subtle energetic differences. One possible explanation for the selectivity is induced movement of Arg627 in the FGFR1 structure that does not occur with the corresponding residue (Arg1032) in VEGFR2. This lack of movement of Arg1032 is also seen in other VEGFR2 structures from this series, suggesting a higher energetic penalty associated with this protein movement in VEGFR2. However, it should be noted that our FGFR1 protein structures are obtained with nonphosphorylated protein, whereas the VEGFR2 crystals are obtained from a mixture of mono- and diphosphorylated protein, so differences in conformational mobility associated with different phosphorylation states cannot be discounted. Another possible driver for selectivity is that in VEGFR2, the sulfur of Cys1045 (equivalent to Ala640 in FGFR1) is approximately 3.4 Å away from the N–H and 3.5 Å away from the carbon of the urea group and that these close contacts are slightly unfavorable in VEGFR2.

Compounds 1 and 2 inhibit proliferation and survival of a panel of FGFR-dependent cell lines

Compounds 1 and 2 were assayed for antiproliferative activity against a panel of 14 cell lines, comprising Ba/F3 cells engineered to express constitutively active forms of FGFR1, 3, and 4 and lines representative of a number of diseases in which FGFR signaling is known to be upregulated. In addition, 3 control cell lines were included [Snu-1, a gastric line harboring mutant ras; HEC-1A, a wild-type FGFR2 endometrial line; and RPMI-8226, a multiple myeloma line without the t(4;14) translocation].

Figure 3. Compounds 1 and 2 exhibit selectivity for FGFR3 over VEGFR2 in cells. Serum-starved HUVECs (VEGFR2) or KMS-11 cells (FGFR3) were incubated with the indicated compounds for 30 minutes. HUVECs were stimulated by the addition of 100 ng/mL VEGF165 for 5 minutes. An ELISA was used to monitor either pVEGFR2 or pFGFR3. Curves are representative of at least 3 individual experiments in each case.
Finally, wild-type Ba/F3 cells that are dependent upon cytokine signaling for survival were included. Both compounds 1 and 2 were potent inhibitors of FGFR-dependent cell survival with mean IC$_{50}$ values in FGFR-dependent cells of 320 and 670 nmol/L and FGFR-independent cells of 3,500 and >6,500 nmol/L, respectively. Examples of cell lines activated by each of the FGFR isoforms were included in the panel, and cell proliferation was inhibited in all of these consistent with the pan-FGFR activity of the compounds (Table 1). Figure 2 is a graphical representation of the data with log IC$_{50}$ values expressed as a fold change from the mean value. Brivanib alaninate, an example of a broad-spectrum tyrosine kinase inhibitor, was significantly less potent in the panel overall and exhibited minimal selectivity for FGFR-dependent versus independent lines with a mean IC$_{50}$ of 2,500 and 6,500 nmol/L, respectively. These data clearly illustrate the selectivity of compounds 1 and 2 in FGFR-dependent systems compared with those cell lines transformed by other mechanisms.

**Compounds 1 and 2 selectively inhibit FGFR in cells**

An ELISA was used to monitor levels of phospho-FGFR3 in KMS-11 multiple myeloma cells and phospho-VEGFR2 in HUVECs following incubation with a concentration range of compound 1, compound 2, the mixed VEGFR2/FGFR inhibitors TKI-258 and brivanib, or the VEGFR inhibitor ZD6474. Both compounds 1 and 2 inhibited phospho-FGFR at concentrations around 10-fold lower than those required to inhibit phospho-VEGFR2 (Fig. 3). This is consistent with the selectivity of these compounds *in vitro* (Table 1). The less selective compounds TKI-258, brivanib, and ZD6474 were more potent against VEGFR2 in HUVECs (28, 27, and 190 nmol/L) than against FGFR in KMS-11 (300, 200, and >10,000 nmol/L; Fig. 3).

Inhibition of FGFR2 signaling in cells was confirmed in the FGFR2-amplified gastric cancer cell line Snu-16 (Fig. 4). Snu-1 gastric lines were included as a control line, as they express only low levels of FGFR2 and harbor a Ras mutation. Both compounds 1 and 2 inhibited phosphorylation of the FGFR2 receptor in Snu-16 cells at concentrations above 100 nmol/L. Inhibition of downstream signaling in the mitogen-activated protein kinase (MAPK) and AKT pathways was also observed at the levels of extracellular signal–regulated kinase (ERK), AKT, and S6. Incubation of Snu-1 cells with the same concentrations had no inhibitory effect on these signaling pathways consistent with the lower antiproliferative activity of the compounds in this cell line (Fig. 2). Inhibition of phospho-FGFR3 and down-
stream signaling was observed in KMS-11 cells at concentrations below 100 nmol/L (Fig. 4C). Inhibition of FGFR1 signaling in KG-1 cells, FGFR2 in AN3-CA and MFE-296, and FGFR3 in 97/7, RT-4, and RT112/84 bladder cell lines was also confirmed by the same methods (Supplementary Fig. S1).

Pharmacokinetic characterization of compounds 1 and 2

Supplementary Figure S2 shows the pharmacokinetic profiles of compounds 1 and 2 following oral dosing in the mouse. Compound 1 exhibits high oral bioavailability in the mouse (79%) and excellent dose linearity with respect to C\(_{\text{max}}\) (19.5 and 28.6 μg/mL) and area under curve (AUC; 179 and 287 h μg/mL for 50 and 100 mg/kg, respectively). Compound 2 exhibits similarly favorable kinetics with an oral bioavailability of 100% and dose linearity (C\(_{\text{max}}\) values of 16, 33, and 49 μg/mL and AUC values of 65, 130, and 330 h μg/mL for 12.5, 25, and 50 mg/kg, respectively).

Stability in liver microsomes suggested low clearance for both compounds (Supplementary Table S2) and this was confirmed in vivo. Metabolic clearance was 2.3 and 5.1 mL/min/kg, respectively, for compound 1 in mouse and rat and 5.2 and 1.1 mL/min/kg for compound 2 following intravenous dosing. Low turnover in human microsomes suggested that therapeutic exposures should be achievable in human subjects.

Pharmacodynamic studies in human tumor xenografts

Inhibition of FGFR and downstream signaling was investigated in human tumor xenograft models (Fig. 5). A single dose of either compound 1 or 2 at 50 mg/kg orally completely ablated phospho-FGFR3 in the Ba/F3-TEL/FGFR3 model (Fig. 5A). This inhibition was shown to be dose dependent for compound 1 (Supplementary Fig. S3). In Snu-16 (Fig. 5B) and KMS-11 (Fig. 5C) xenografts, phospho-FGFR2 and phospho-FGFR3 were inhibited for more than 8 hours following a single dose of compound 1. Inhibition of downstream MAPK and AKT signaling and an increase in the levels of cleaved PARP, a marker of apoptosis, were observed at 8 hours in the Snu-16. Figure 5D shows RPMI-8226 tumors removed from animals 4 hours after treatment with the indicated doses of compound 1. In this FGFR-independent model, no effect was observed on either MAPK or PARP.

Efficacy in human tumor xenografts

The antitumor efficacy of both compounds 1 and 2 was investigated in FGFR3-dependent multiple myeloma xenografts (Fig. 6A and C). For this purpose, we used KMS-11 cells that express high levels of a mutant FGFR3 (Fig. 4C) and Jim-1 cells that express physiologic levels of wild-type FGFR3 (Supplementary Fig. S3). Compounds were administered orally, once daily at doses that tolerability studies suggested were below the maximum tolerated doses. Administration of 50 mg/kg compound 1 for up to 21 days resulted in minimal body weight loss and no observations of gross toxicity were made. For compound 2, no adverse toxicities were observed at doses up to 100 mg/kg once daily when dosed for up to 26 days. Compound 1 caused tumor growth inhibition at 12.5 mg/kg orally in the Jim-1 model with regression of tumor volume observed at both 25 and 50 mg/kg. At 50 mg/kg, all animals showed tumor shrinkage by day 5, which persisted to day 10. By day 16, 50% of these tumors were still smaller than on day 1. Compound 2 was less effective in the Jim-1 model at 50 mg/kg consistent with the lower potency of this compound. The higher dose of

Figure 5. Inhibition of FGFR signaling in xenograft tumors. Mice bearing Ba/F3-TEL/FGFR3 (A), Snu-16 (B), or KMS-11 (C) xenografts were treated with a single dose of compound 1 or 2, at 50 mg/kg, as indicated. Triplicate tumor samples were removed at subsequent times and processed to determine levels of phospho-signaling by immunoblotting. Nude mice bearing RPMI-8226 xenografts (D) had compound 1 administered daily for 21 days at the indicated doses. Tumors were removed at 4 hours following the final dose.
100 mg/kg, however, caused an equivalent growth inhibition in the KMS-11 model to that caused by 50 mg/kg compound 1. Both compounds were also efficacious in the endometrial xenograft model AN3-CA. Compound 1 was not efficacious even at the highest dose (50 mg/kg) tested in the FGFR-independent RPMI-8226 model (Fig. 6B), a dose and schedule that resulted in complete growth inhibition in the FGFR-positive Jim-1 model. Compound 2 was administered orally to mice bearing FGFR-dependent (KMS-11, Jim-1, AN3-CA); C or FGFR-independent xenografts (MDA-MB-231); D once daily for the indicated doses and time periods. Groups were taken off study at the end of the study period or when tumors reached greater than 1,000 mm³.

Figure 6. Compounds 1 and 2 are efficacious in FGFR-dependent tumor xenograft models. Compound 1 was administered orally to mice bearing FGFR-dependent (KMS-11, Jim-1, AN3-CA; A) or FGFR-independent xenografts (RPMI-8226; B) once daily at the indicated doses and time periods. For the Jim-1 and RPMI-8226 studies, TKI-258 was included as a positive control. Compound 2 was administered orally to mice bearing FGFR-dependent (KMS-11, Jim-1, AN3-CA; C) or FGFR-independent xenografts (MDA-MB-231); D once daily for the indicated doses and time periods. Groups were taken off study at the end of the study period or when tumors reached greater than 1,000 mm³.

Discussion

Here, we describe the characterization of 2 fragment-derived, specific inhibitors of the FGFR family of kinases. Compounds 1 and 2 are potent inhibitors of all 4 isoforms of the FGFR family with no appreciable activity in a panel of related receptor tyrosine kinases including EGFR and PDGFRβ. The compounds were around 20-fold selective against the highly homologous VEGFR2 kinase. They were selective both in cell-based assays and in vivo where this apparently modest level of selectivity in vitro translated to a highly specific tumor inhibitory effect in FGFR-dependent xenografts with no activity in xenografts not dependent upon FGFR signaling. These data more than any other confirm that the compounds are acting in an FGFR-specific manner when exerting their effects in these model systems.
This is the first detailed pharmacologic characterization of compounds that exhibit selectivity for FGFR family members over VEGFR2. With the growing interest in targeting FGFR, broad studies such as those presented here will help define the pharmacologic profile of a specific FGFR inhibitor across a relevant panel of preclinical systems. Several studies in defined tumor types have been conducted with PD173074 (12, 31, 32), but they predominantly use engineered cell lines or those representing a single disease type. A lack of small molecule and biological inhibitors specific for FGFR and a lack of well-characterized reagents have, to date, prevented similar studies.

There are several indications within oncology in which targeting the FGFR pathway specifically may be of benefit. These include tumor types with specific activating mutations in FGFR (5–7, 31), those with amplification of one of the receptor family members (2) or more broadly as an antiangiogenic therapy following VEGFR therapy in tumors in which FGF-2 has been upregulated (24). Several molecules are or have been investigated with the aim of potentially exploiting their FGFR activity. PD173074 showed selectivity for the FGFR receptor in vitro and a number of reports described its activity in individual FGFR-dependent systems (12, 14), but it did not proceed into clinical development. Compounds explored clinically include brivanib alaninate (BMS-582664; ref 29, 33) in hepatocellular carcinoma (NCT00355238), endometrial (NCT00888173), and colorectal cancer (NCT00207051) and TKI-258 (Novartis) in multiple myeloma (NCT01058434), renal cell carcinoma (NCT00715182), urethelial cell carcinoma (NCT00709426), and breast cancer (NCT 00958971). Isomof-specific antibody therapies are also in preclinical development. Two recent publications describe the potent activity of FGFR2-IIIb–specific (34) and FGFR2-specific (35) antibodies in preclinical models of gastric cancer, harboring FGFR2 amplification. This further supports the important role of FGFR2 in this indication.

Data presented herein show that broad spectrum compounds have in vivo activity against both FGFR-dependent and -independent models, most likely driven by their antiangiogenic activity via VEGFR2 inhibition. Thus, it is difficult to show FGFR-specific effects attributable to these compounds either preclinically or in clinical studies. One possible limitation of these inhibitors in FGFR-driven disease is the appearance of intervening toxicities due to inhibition of additional kinases, which prevents maximal inhibition of FGFR in relevant tumor settings.

Compounds 1 and 2 described here exhibit very distinct activity profiles in FGFR-dependent and -independent in vitro and in vivo systems. As such, the compounds offer an exciting and innovative approach to targeting cancers dependent upon FGFR signaling.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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