Potent and selective inhibitors of the Met [hepatocyte growth factor/scatter factor (HGF/SF) receptor] tyrosine kinase block HGF/SF-induced tumor cell growth and invasion

Xueyan Wang, Phuong Le, Congxin Liang, Julie Chan, David Kiewlich, Todd Miller, Dave Harris, Li Sun, Audie Rice, Stefan Vasile, Robert A. Blake, Anthony R. Howlett, Neela Patel, Gerald McMahon, and Kenneth E. Lipson

SUGEN, Inc., South San Francisco, CA

Abstract

The hepatocyte growth factor/scatter factor (HGF/SF) receptor, Met, mediates various cellular responses on activation with its ligand, including proliferation, survival, motility, invasion, and tubular morphogenesis. Met expression is frequently up-regulated in sarcomas and carcinomas. Experimental evidence suggests that Met activation correlates with poor clinical outcome and the likelihood of metastasis. Therefore, inhibitors of Met tyrosine kinase may be useful for the treatment of a wide variety of cancers that have spread from the primary site. We have discovered potent and selective pyrrole-indolinone Met kinase inhibitors and characterized them for their ability to inhibit HGF/SF-induced cellular responses *in vitro*. These compounds inhibit HGF/SF-induced receptor phosphorylation in a dose-dependent manner. They also inhibit the HGF/SF-induced motility and invasion of epithelial and carcinoma cells. Therefore, these compounds represent a class of prototype small molecules that selectively inhibit the Met kinase and could lead to identification of compounds with potential therapeutic utility in treatment of cancers. (Mol Cancer Ther. 2003;2:1085–1092)

Introduction

The receptor for hepatocyte growth factor (HGF), which is also known as scatter factor (SF), is c-Met. Normally, Met is predominantly expressed on epithelial and endothelial cells, while cells of mesenchymal origin generally express HGF, which tends to act in a paracrine manner. Activation of Met by HGF can induce a variety of cellular responses, including proliferation, survival, motility, invasion, and changes in morphology (see Refs. 1–3 for reviews).

Aberrant activation of Met can increase the tumorigenicity and metastatic potential of tumor cells (4, 5). Such activation can occur through several mechanisms, including overexpression of Met (with or without gene amplification), increased expression of HGF (through induction by tumor cells or by translocation to sites where endogenous levels are higher), formation of autocrine loops, or development of activating mutations.

Increased expression of Met or HGF has been reported in cancers of many organs, and shown to increase with tumor progression for several (see Ref. 3 for a review). Increased expression of HGF or Met has also been shown to correlate with shorter patient survival in several types of cancer (6–14). Therefore, it is logical to assume that inhibitors of Met could suppress tumor aggressiveness and increase survival.

In animal models, exposure of tumors to the HGF antagonists NK2 or NK4 suppressed tumor growth (15–19). Similarly, monoclonal antibodies that neutralize HGF or ribozymes that target HGF or Met expression exhibit antitumor activity in animal models (20–22). Two examples of small molecule inhibitors of Met have been reported in the literature. Geldanomycins are compounds related to anisamycin antibiotics that act as indirect inhibitors of Met signal transduction, by decreasing the cell surface expression of Met (23). More recently, the alkaloid kinase inhibitor K252a was reported to also inhibit Met kinase activity (24). In this paper, we report identification of synthetic indolinone inhibitors of Met kinase. We have characterized these inhibitors for their activity on Met as well as selectivity toward other kinases. The ability of these small molecules to inhibit HGF/Met-induced biological responses was also investigated.

Materials and Methods

Chemicals

SU11271 [(3*Z*)-5-(2,3-dihydro-1*H*-indol-1-ylsulfonyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1*H*-pyrrol-2-yl}methylene)-1,3-dihydro-2*H*-indol-2-one], *SU11274* [(3*Z*)-*N*-(3-chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1*H*-pyrrol-2-yl}methylene)-*N*-methyl-2-oxoindoline-5-sulfonamide], and *SU11606* [(3*Z*)-*N*-(3-chlorophenyl)-3-{[3,5-dimethyl-4-(3-morpholin-4-ylpropyl)-1*H*-pyrrol-2-yl]methylene}-*N*-methyl-2-oxoindoline-5-sulfonamide] were synthesized at SUGEN, Inc.

Cell Culture

All cell lines were obtained from American Type Culture Collection (ATCC, Rockville, MD), unless otherwise specified. The cell culture medium and supplements were purchased from Invitrogen (Carlsbad, CA) and fetal bovine

Received 6/20/03; revised 8/20/03; accepted 8/28/03.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Grant support: SUGEN, Inc., a subsidiary of Pharmacia Corporation, which was acquired by Pfizer in April 2003.

Requests for Reprints: Xueyan Wang, 3876 Bay Center Place, Hayward, CA 94545. Phone: (510) 293-8117. E-mail: xwang720@yahoo.com

serum (FBS) from Gemini Bio-products, Inc (Woodland, CA). Cells were cultured at 37°C under 5% CO_2 in basic medium with 10% FBS, 2 mM glutamine, and 1% antibiotic-antimycotic as recommended by ATCC.

Biochemical Kinase Assays

Met Kinase Assay. A chimeric protein was constructed containing the cytoplasmic domain of human c-Met fused to Glutathione *S*-transferase (GST) and expressed in SF9 cells. The c-Met kinase GST-fusion protein was used for an ELISA-based Met biochemical assay using the random copolymer poly(Glu:Tyr) (4:1) (P0275, Sigma, St. Louis, MO) immobilized on microtiter plates as a substrate. IC_{50} values were determined with various concentrations of the test compound in a buffer containing 5 μM ATP and 10 mM $MnCl_2$, 50 mM HEPES (pH 7.5), 25 mM NaCl, 0.01% BSA, and 0.1 mM Na orthovanadate. The kinase reaction was performed for 5 min at room temperature. The extent of substrate phosphorylation was measured using horseradish peroxidase-conjugated anti-pTyr antibodies (sc-7020HRP, Santa Cruz Biotechnology, Santa Cruz, CA).

Ron Kinase Assay. A chimeric protein was constructed consisting of the cytoplasmic domain of human Ron fused to *Escherichia coli* DNA gyrase B (GyrB), in a manner similar to that described for Kit by Krystal *et al.* (25). Chinese hamster ovary (CHO) cells expressing GyrB/Ron fusion protein were grown to confluence in a 15-cm plate in F-12 Ham's medium with 10% FBS and serum-starved with 0.1% BSA in DMEM for 16–24 h. Cells were lysed with HNTG buffer containing 50 mM HEPES, 150 mM NaCl, 10% glycerol, 0.5% Triton, 1 mM phenylmethylsulfonyl fluoride (PMSF), and a protease inhibitor cocktail (1.4 μM E-64, 10 μM bestatin, 1 μM leupeptin, 0.3 μM aprotinin, and 1 μM pepstatin). GyrB/Ron protein was isolated by immunoprecipitation with anti-Ron antibody (sc-322, Santa Cruz Biotechnology). Aliquots of immunocomplexes were incubated with 5 μM ATP in kinase buffer (25 mM HEPES, 100 mM NaCl, 5 mM $MgCl_2$, and 5 mM $MnCl_2$) in the presence or absence of test compound for 25 min at room temperature. After the reaction was stopped with EDTA, proteins were eluted with SDS sample buffer and resolved on an 8% polyacrylamide gel. Phosphorylation of Ron was detected by Western blotting with anti-pTyr antibody (sc-7020HRP, Santa Cruz Biotechnology) and IC_{50} values were estimated by visualizing the intensity of the protein band on film.

Other Kinase Assays. Recombinant human full-length or GST-cytoplasmic domain fusion proteins were utilized. IC_{50} values of compounds were determined by measuring phosphorylation of kinase peptide substrates or poly(Glu:Tyr) in the presence of ATP at a concentration of $2\times K_m$ and divalent cation ($MnCl_2$ or $MgCl_2$, 10–20 mM).

Met Cellular Kinase Assay

A549 cells were grown to confluence in F-12K medium with 10% FBS and serum-starved overnight. After treatment with test compound for 16–20 h, cells were stimulated with HGF/SF (50 ng/ml) for 10 min and then lysed with HNTG buffer plus 1 mM Na vanadate. Met or Gab1 proteins isolated by immunoprecipitation with anti-Met (sc-10, Santa Cruz Biotechnology) or anti-Gab1 (sc-9049, Santa Cruz Biotechnology) antibodies were fractionated by SDS-PAGE and transferred to a nitrocellulose membrane. The amount of phosphorylated Met or Gab1 was detected with anti-pTyr antibodies and the amount of target protein in each lane was visualized with anti-Met (sc-161, Santa Cruz Biotechnology) or anti-Gab1 antibodies (14-441, Upstate Cell Signaling Solutions, Waltham, MA).

Cell Proliferation Assay

BxPC3 cells were seeded in 96-well plates at 9000 cells/well in RPMI with 10% FBS. After incubation for 24 h, cells were washed with PBS and serum-starved for 48 h. Cells were then treated with HGF/SF along with different concentrations of compounds for 18 h at 37°C. After additional incubation with BrdUrd labeling reagent, cells were fixed and BrdUrd incorporation was assessed using anti-BrdUrd-peroxidase antibody conjugates followed by colorimetric determination. In the absence of inhibitors, HGF/SF induced a 2- to 3-fold increase in BrdUrd incorporation into BxPC3 cells. In parallel with the BrdUrd incorporation assay, the viability of cells exposed to different concentrations of the various compounds was evaluated by measuring the ability of live cells to metabolize resazurin (Tox-8 kit, Sigma).

Scatter Assay

Madin-Darby canine kidney (MDCK) cells were seeded at low density of 25 cells/well in a 96-well plate in MEM with 10% FBS and grown to small colonies of 10–15 cells. Cells were then stimulated with HGF/SF in the presence of various concentrations of the test compounds diluted in growth medium. After overnight incubation, colonies were fixed and stained with 0.2% crystal violet in 10% buffered formalin. Photographs were taken of individual colonies.

Three-Dimensional Invasion/Tubulogenesis Assay

Matrigel (Matrigel Basement Membrane Matrix, Becton Dickinson Labware, Franklin Lakes, NJ) was thawed on ice. Fifty microliters of Matrigel were added per well in a 96-well plate and incubated for 20 min at 37°C to induce gel formation. Rat intestinal epithelial 1 (RIE-1) cells were suspended at 2×10^4 cell/ml and mixed 1:1 with Matrigel on ice. 0.1 ml of the mixture was added per well. On the next day, 0.1 ml DMEM ± 80 ng/ml HGF (R&D 294-hg-005) was added on top of each Matrigel cell plug. Fresh medium ± HGF was added every 3 days. Photographs were taken after 6 days in culture.

Results

Identification of Pyrrole Indolinone as a Prototype of Potent and Selective Met Inhibitors

To identify potential Met kinase inhibitors, approximately 7500 compounds from an indolin-2-one library were screened in a high-throughput Met kinase assay for the ability to inhibit phosphorylation of poly(Glu:Tyr) (4:1). Although some weak inhibitors of Met kinase were identified, none demonstrated sub-micromolar potency.

To obtain some guidance on how the potency of these pyrrole indolinones might be enhanced, a homology model

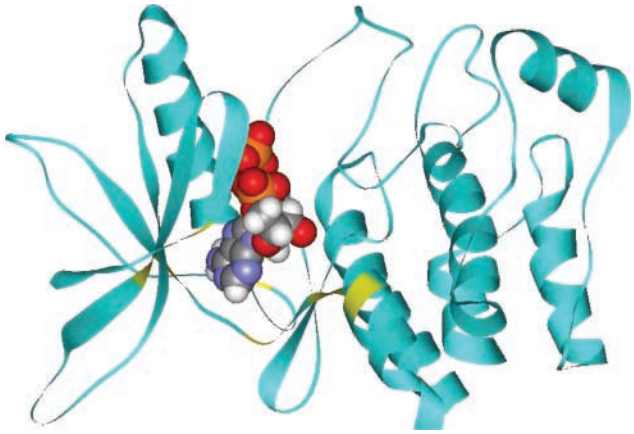

Figure 1. Met kinase homology model. A homology model of the Met kinase was developed based on the crystal structures of the FGFR1 kinase. ATP was docked into the active site based on the co-crystal of FGFR1 with ATP. Amino acids within 6 Å of ATP that differ between Met and FGFR1 are highlighted in *yellow*.

of Met kinase was developed, which was based on the crystal structures of the fibroblast growth factor receptor 1 (FGFR1) kinase (26). Fig. 1 shows a ribbon structure of this homology model, with ATP docked at its putative binding site. The position of amino acids that are within 6 Å of ATP, and which differ between Met and FGFR1, are highlighted in yellow (Fig. 1). One of these differences, Glu-1082 in Met, which corresponds to Lys-482 in FGFR1 (Fig. 2A), appeared to be a good candidate to try to exploit based on the charge difference and its position relative to the pyrrole substituents of compounds such as SU5402 which was co-crystallized with FGFR1 or SU6668, a vascular endothelial growth factor receptor 2 (VEGFR2) inhibitor. The fact that an acidic residue in Met had replaced a basic residue in FGFR1 suggested that amine-containing moieties on the pyrrole ring should be explored.

In addition to the differences in amino acids between Met and FGFR1 that were observed, homology modeling suggested that a similarity might also be exploited. The co-crystal structure of FGFR1 with SU5402 contained a water molecule near the 5-position of the indolinone ring. The nearest amino acid to this water molecule was Ala in both FGFR1 and Met (Fig. 2A). This similarity suggested that a sulfonamide substituent at the 5-position of the indolinone might be able to displace the water and the resulting hydrogen bonds might also enhance potency.

Using the ideas generated from homology modeling, a small combinatorial library was constructed with various amine substituents on the pyrrole and various C-5 sulfonamide substituents on the indolinone. Compounds with aromatic moieties on the sulfonamide demonstrated especially good potency, which led to synthesis of additional compounds to expand this class. Fig. 2B shows the chemical structures of several of the best compounds from this series. Fig. 2A shows the Met kinase domain homology model with SU11271 docked in the ATP binding site.

These compounds exhibited potent inhibitory activity toward Met kinase in an *in vitro* kinase assay (Table 1). The average IC_{50} values determined for these compounds are 0.04 μM for SU11271, 0.01 μM for SU11274, and 0.17 μM for SU11606. The selectivity of these compounds was determined by measuring their ability to inhibit several other kinases (Table 1). All three compounds showed only moderate inhibitory activity on Ron, the most closely related receptor tyrosine kinase with high sequence homology in the kinase domain. SU11271 inhibited FGFR1 and FLK-1 to a certain extent, whereas SU11606 exhibited quite potent activity toward FLK-1 with an IC_{50} value of 0.18 μM. Interestingly, SU11274, which combined the structure of SU11271 and SU11606 with 5-*N*-methyl-*N*-mCl-phenyl-sulfonamide on the oxindole core and the 4′-*N*-methypiperazine-amide substitution on the pyrrole, improved its overall selectivity profile (100-fold less potent on FLK-1 and

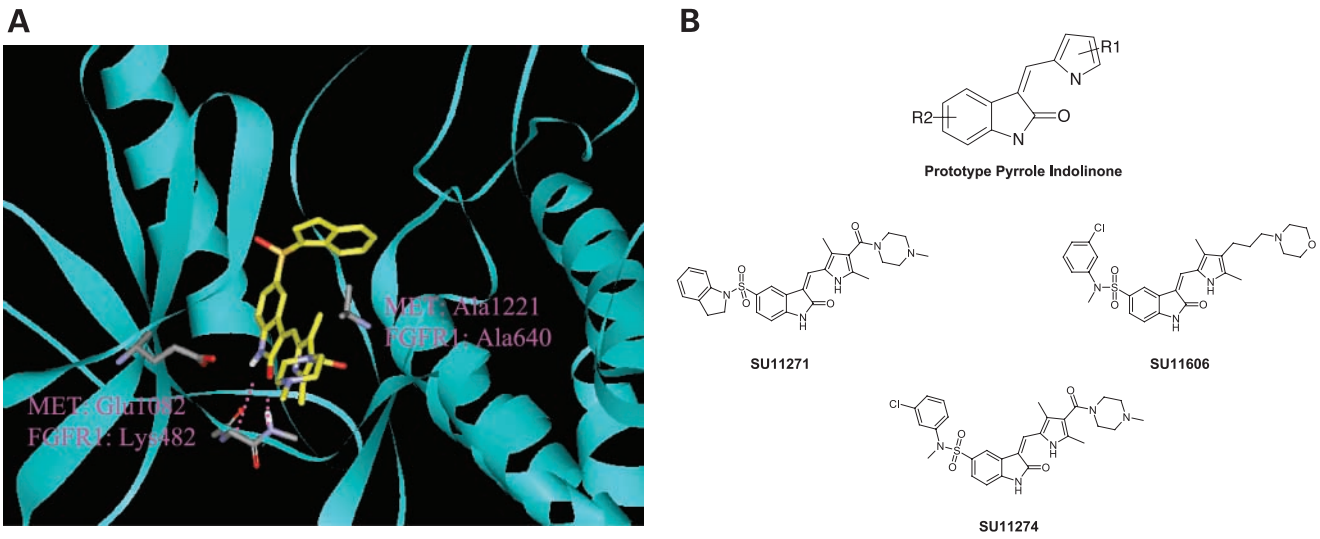

Figure 2. **A,** Met kinase homology model with SU11271 docked in ATP binding site. Amino acid residues that were used to design the compounds are indicated. **B,** core structure of pyrrole indoline-2-one and structures of three Met inhibitors, SU11271, SU11274, and SU11606.

800-fold less potent on FGFR1) while retaining similar potency toward Met. None of these compounds inhibited the enzymatic activities of two other receptor tyrosine kinases, platelet-derived growth factor receptor β (PDGFRβ) and epidermal growth factor receptor (EGFR), or of a serine/threonine kinase, cyclin-dependent kinase 2 (CDK2), at concentrations as high as 20 μM.

Met Inhibitors Inhibit Phosphorylation of Met and Signaling Molecules in Cells

To determine if SU11271, SU11274, and SU11606 can readily cross the plasma membrane and target the Met kinase within cells, A549 cells were exposed to different concentrations of the compounds. After stimulation with hHGF/SF, endogenous Met kinases were isolated with anti-hMet antibody and HGF-induced autophosphorylation of Met was detected by Western blotting using anti-pTyr antibodies. As shown in Fig. 3, unstimulated cells demonstrated low basal Met phosphorylation, which was significantly increased on stimulation with HGF/SF. Only the processed form of Met demonstrated HGF-induced phosphorylation on the p140 β subunit; the p170, unprocessed Met was insensitive to HGF stimulation. All three compounds inhibited HGF-induced Met phosphorylation in a dose-dependent manner, whereas total Met protein level was not significantly affected. Similar results were observed in other cell lines such as BxPC3 and B16F1. Consistent with the results from the biochemical assay, these compounds are much less potent at inhibiting Ron kinase in cells with estimated IC_{50} values of 16 μM for SU11274, 25 μM for SU11606, and >50 μM for SU11271 (data not shown).

Activation of Met kinase on binding of HGF/SF triggers a cascade of signaling events involving activation and phosphorylation of multiple kinases and signal transducers (27, 28). It has been reported that autophosphorylation of Met on its COOH-terminal domain provides a docking site for several Src homology 2 (SH2)-containing cellular proteins including Gab1, an Insulin Receptor Substrate (IRS)-like protein that is one of the major substrates for Met kinase (29, 30). Phosphorylation of Gab1 is important for the transforming activity of activated Met (31). To determine if inhibitors of Met kinase would affect this signaling process, Gab1 was isolated from cells that were treated with compounds and stimulated with HGF/SF. Phosphorylation of Gab1 was detected by Western blotting using anti-pTyr antibody. As expected, all three inhibitors, SU11271, SU11274, and SU011606, significantly reduced HGF-stimulated phosphorylation of Gab1 at a concentration of 10 μM (Fig. 4), suggesting that these inhibitors can block signal transduction from Met by inhibiting its kinase activity.

Met Inhibitors Reduce Cellular Activities in Response to HGF/SF

These Met kinase inhibitors were further evaluated for their ability to block cellular functions mediated by Met. HGF/SF stimulation induces many cellular functions, including cellular proliferation, motility, and invasion through extracellular matrix (1). Activation and phosphorylation of Met following binding of HGF is believed to be the initial step in HGF/Met signal transduction that leads to these cellular responses. Therefore, inhibitors of Met kinase would be expected to block Met-dependent cellular events. As shown in Fig. 5, SU11271, SU11274, and SU11606 inhibited HGF-induced BrdUrd incorporation into newly synthesized DNA in HGF/SF-induced BxPC3 cells, a human pancreatic carcinoma cell line, with IC_{50} values of 2.27, 2.02, and 0.89 μM, respectively. The inhibitory effect of these compounds on cell proliferation was not due to cytotoxicity because SU11271 and SU11274 did not cause cell death at concentrations as high as 50 μM (the highest concentration tested), whereas SU11606 started to show some cytotoxicity at 10 μM, a concentration well above the observed IC_{50} values in the BrdUrd incorporation assay (data not shown).

It has been well documented that HGF/SF induces movement of cells. In fact, one of the independent ways that scatter factor (HGF/SF) was originally discovered was as a fibroblast-derived factor that induced scattering of MDCK cells (32). As shown in Fig. 6, incubation of MDCK cells with HGF/SF induced a morphological change from epithelial colonies into scattered, individual cells. However, when cells were treated with inhibitors at increasing concentrations, the HGF-induced scattering process was significantly inhibited. SU011606 exhibited substantial inhibitory activity at a concentration as low as 0.3 μM, whereas SU011274 started to show such inhibitory effects at 1.25 μM and SU11271 at 2.5 μM. These compounds were also shown to inhibit the motility of NCI-H441 human lung carcinoma cells in a wound healing/scratch assay (data not shown).

HGF/Met signaling is very important in embryonic development for promoting branching morphogenesis (see review, Ref. 33). This involves coordinated regulation of a variety of processes such as motility and invasion that are critical for formation of organs. In cancer, HGF/Met signaling is thought to promote movement and invasion of tumor cells into extracellular matrix, which is associated with tumor metastasis. Fig. 7 demonstrates the effect of HGF on the growth of RIE-1 cell colonies suspended in Matrigel. In the absence of HGF, the colonies grow as compact spheres. HGF induced the formation of tubular structures and invasive growth in three dimensions. SU11271 inhibited the formation of tubular structures in a dose-dependent manner. At 5 and 10 μM, it also inhibited

Table 1. Effect of SU11271, SU11274, and SU11606 on enzymatic activity of Met and other kinases

Enzymatic Assays	SU11271 IC_{50} (μM)	SU11274 IC_{50} (μM)	SU11606 IC_{50} (μM)
Met	0.04	0.01	0.17
Ron	5.6	4.0	2.5
PDGFRβ	>20	>20	>20
FGFR1	0.5	8.5	1.8
EGFR	>100	>100	>100
FLK-1	1.28	1.25	0.18
CDK2	>20	>10	>20

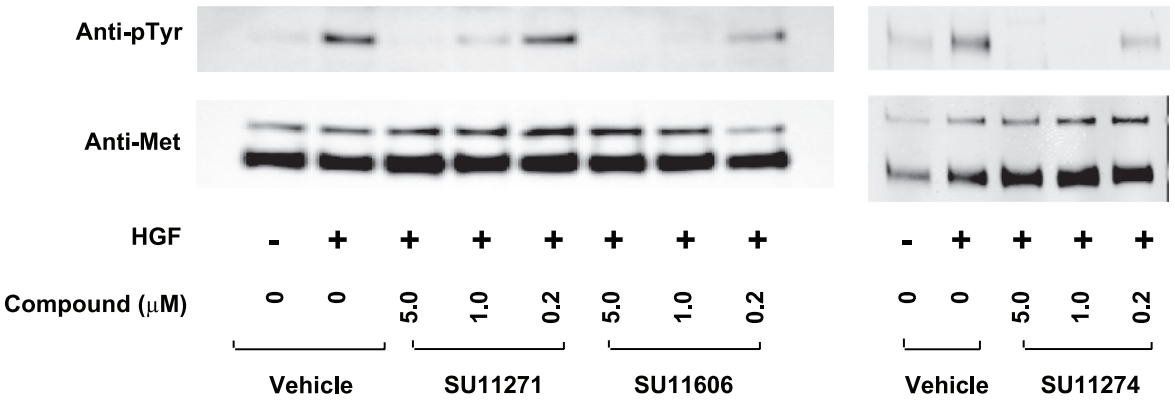

Figure 3. Effect of Met inhibitors on HGF-induced Met autophosphorylation in A549 cells. As described in ''Materials and Methods,'' A549 cells were exposed to the indicated concentrations of Met kinase inhibitors overnight and then stimulated with HGF/SF for 10 min. Lysates were made from the treated and untreated cells and Met was immunoprecipitated, fractionated by SDS-PAGE, and transferred to a nitrocellulose membrane. Phosphorylated Met was analyzed by Western blotting using anti-pTyr antibody, and the total amount of Met protein in each lane was visualized by stripping and reprobing the membrane with an anti-Met antibody.

the growth of RIE-1 colonies. SU11274 and SU11606 inhibited both processes at the lowest concentration tested, 1.25 μM. Similar observations were made with the human lung carcinoma cell line A549 (data not shown). All three compounds were also demonstrated to inhibit A549 and B16F1 cell invasion through Matrigel in a Boyden chamber invasion assay (data not shown).

Discussion

The bulk of clinical and experimental evidence has suggested that Met is a potential therapeutic target for development of inhibitors for treatment of cancers. Here we report identification and characterization of small molecule inhibitors that potently and, in the case of SU11274, selectively inhibit the enzymatic activity of Met kinase. These inhibitors, SU11271, SU11274, and SU11606, are derivatives of 3-pyrrole indoline-2-ones. With the guidance of a homology model of Met kinase developed from the FGFR1 kinase domain crystal structure, addition of a sulfonamide substituent at the 5-position of the indolinone and a basic group on the pyrrole ring led to significant inhibition of Met kinase activity. These compounds exhibit IC_{50} values for inhibition of Met kinase between 10 and ~200 nM. Different substituents on the sulfonamide and pyrrole amide resulted in changes in potency and selectivity of these compounds. The inhibitory activity of these compounds toward Ron, the tyrosine kinase that is most closely related to Met, is at least 10-fold less potent in the biochemical assay. As for other, more distantly related tyrosine and serine/threonine kinases, SU11274, which combines the structure of SU11271 and SU11606, exhibits the best overall selectivity. It is generally considered preferable to use selective inhibitors to avoid toxicities which could result from inhibition of additional targets. However, an inhibitor like SU11606 that inhibits both Met and receptors of VEGF (e.g., FLK-1/VEGFR2), may exhibit an additive anti-tumor effect by inhibiting multiple processes involved in Met-driven tumor growth and metastasis as well as VEGFR-driven angiogenesis.

These compounds were also assessed in a panel of cellular assays for their ability to inhibit Met kinase in cells. All of them inhibited HGF-induced autophosphorylation of Met in a dose-dependent manner in A549 cells, suggesting that they are able to penetrate plasma membrane and target the intracellular kinase domain of the receptor kinase. These inhibitors also significantly reduced phosphorylation of Gab1, an intracellular adaptor protein that binds to the COOH-terminal phosphorylated tyrosine residues of Met and is phosphorylated by the Met kinase. The kinase activity of Met and its interaction with Gab1 as well as several other signaling molecules have been shown to be essential for HGF-stimulated cellular responses (28, 29).

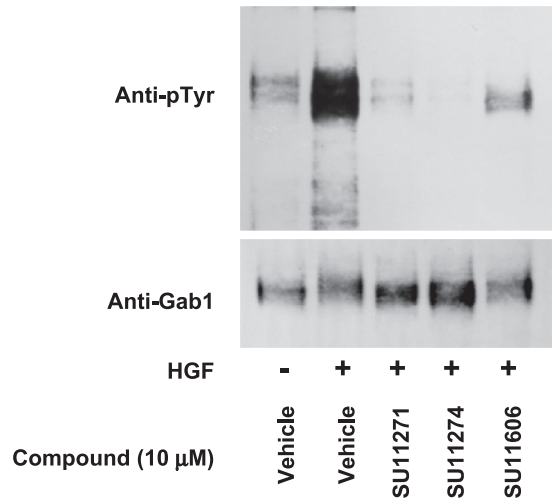

Figure 4. Effect of Met inhibitors on HGF-stimulated phosphorylation of Gab1 in A549 cells. A549 cells were treated with 10 μM Met inhibitors overnight and stimulated with HGF for 10 min. Cells were lysed and Gab1 was isolated by immunoprecipitation with anti-Gab1 antibody and then subjected to SDS-PAGE. Phosphorylated Gab1 was detected by Western blotting using anti-pTyr antibody and total Gab1 protein was visualized by reprobing with anti-Gab1 antibody.

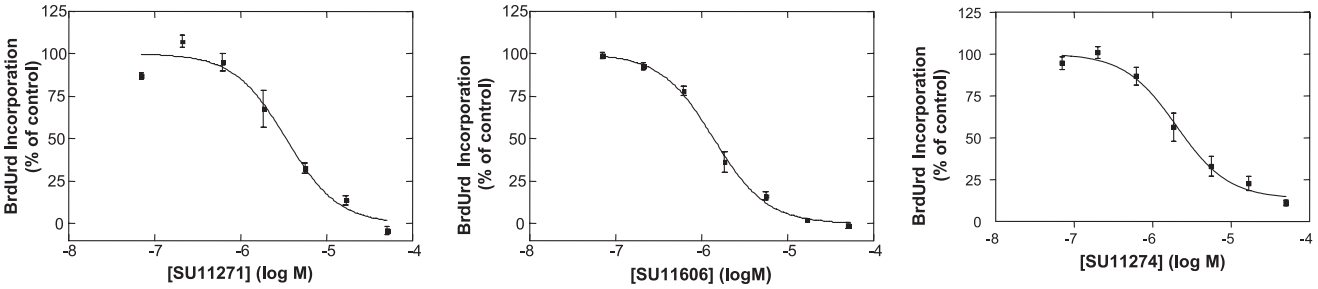

Figure 5. Treatment of Met kinase inhibitors blocks HGF-induced proliferation of BxPC3 cells. BxPC3 cells were serum-deprived and then stimulated with HGF/SF for 18 h in the presence of various concentrations of SU011271, SU11274, or SU011606. The stimulated cells were then exposed to BrdUrd for 1.5 h and fixed. The amount of incorporated BrdUrd was determined by ELISA. Representative data are shown. The average values from three experiments were used for calculating IC_{50}.

Consistent with these reports, we showed that the inhibitors of Met kinase significantly blocked a variety of HGF/Met-dependent cellular processes, including cell proliferation, motility, invasion, and morphogenesis. Interestingly, there appear to be some discrepancies between the relative potencies of these compounds toward Met in the biochemical and cellular assays. For example, SU11606 is less potent than SU11271 and SU11274 in the biochemical assay, but appears to be more potent in cells. The lipophilicity of SU11606 is slightly higher than that of the other two compounds (data not show), which may allow this compound to enter cells more readily and/or accumulate in cells to a greater extent yielding higher local concentration. It is also possible that the apparent potency of SU11606 results from the fact that it is a less selective inhibitor that may inhibit additional kinases involved in Met-dependent downstream responses. The cellular potencies of SU11271 and SU11274 appear to be less than their biochemical potencies and to vary between assays. Such behavior is common in kinase inhibitors and has previously been reported with commercially available inhibitors of PDGFR and EGFR (34).

In conclusion, we have generated a group of small molecules that show potent and selective inhibitory activity against Met kinase and demonstrate good cellular activity. Although the compounds described here lack pharmaceutical

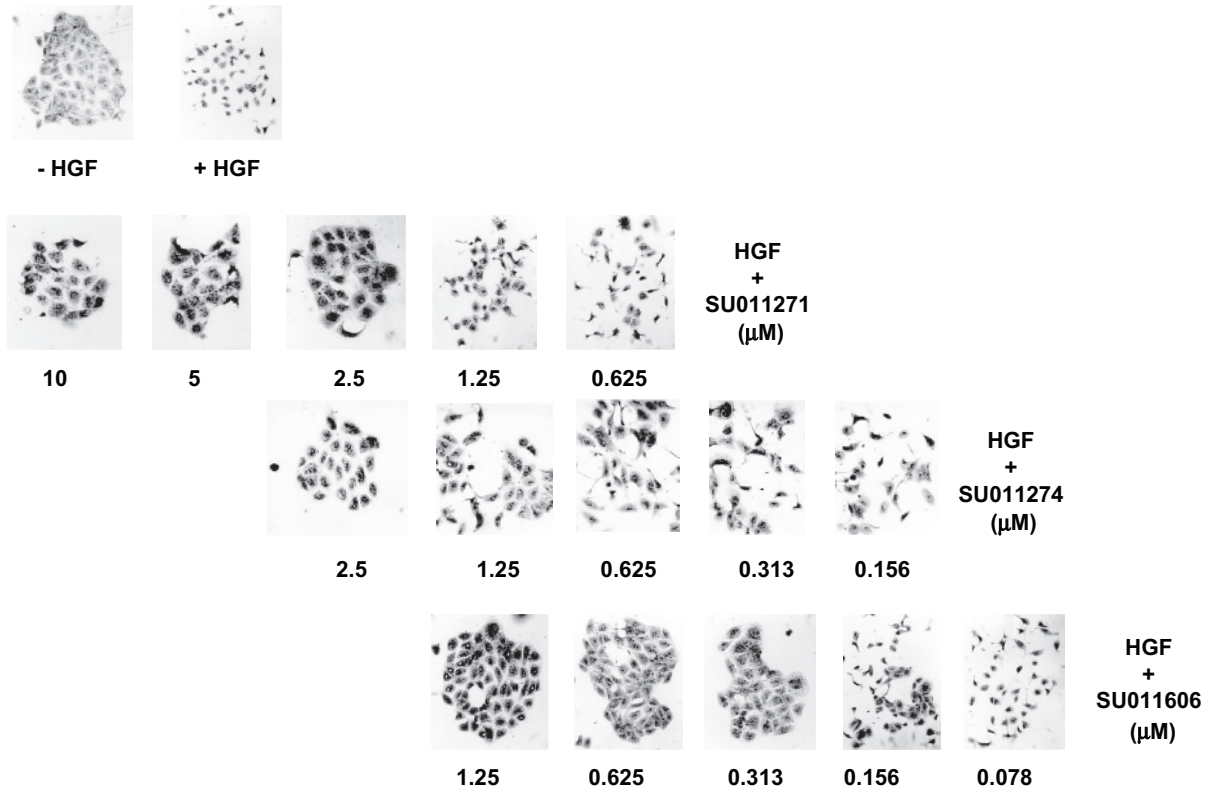

Figure 6. Met kinase inhibitors inhibit HGF-induced cell motility. Scatter assay: MDCK cells were stimulated with HGF/SF in the presence of various concentrations of the test compounds. After overnight incubation, photographs were taken of individual colonies.

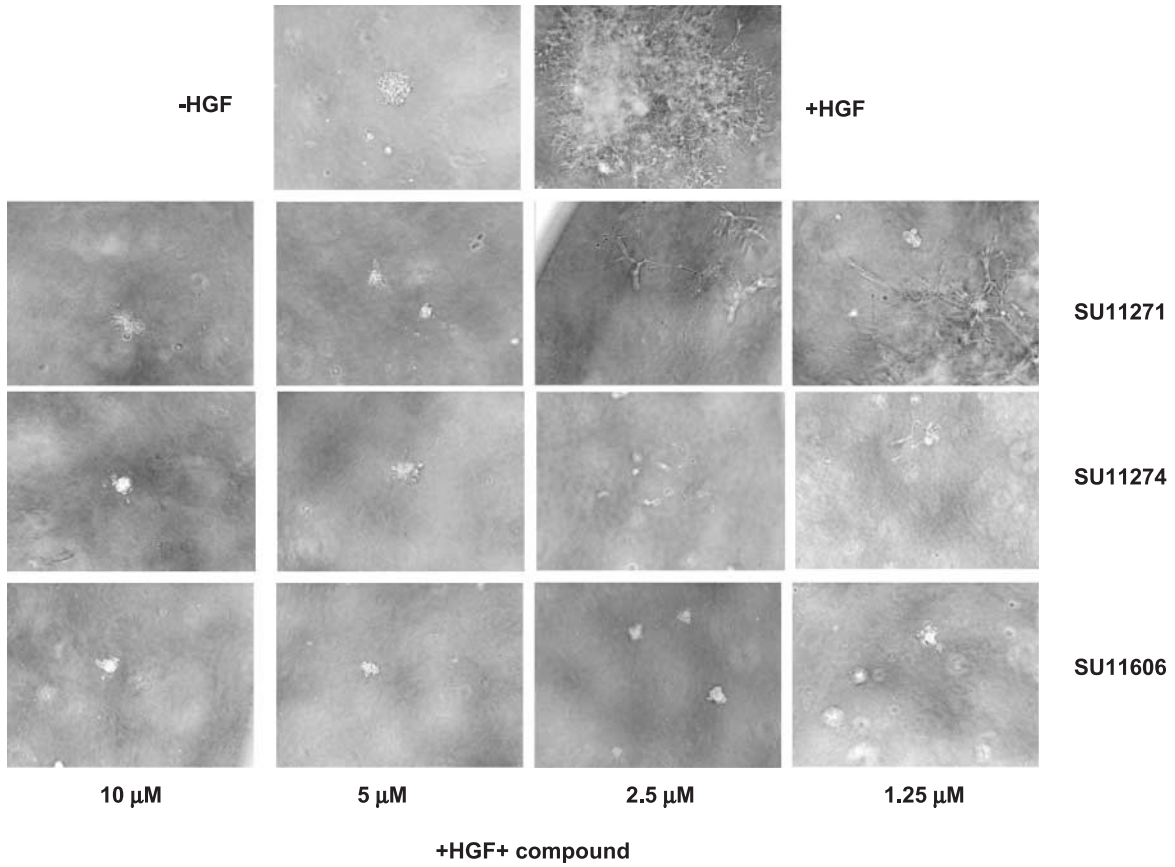

Figure 7. Met inhibitors block tubule formation of RIE-1 cells in Matrigel. RIE-1 cells were mixed with Matrigel and seeded in a 96-well plate. After incubation overnight, cells were fed with growth medium containing HGF and various concentrations of test compound. Tubular structures were observed by microscopy and photographs were taken on day 6.

properties (solubility, bioavailability, etc.) sufficient to achieve *in vivo* activity and necessary to be developed into therapeutics, observations in this report suggest that it will be feasible to generate specific Met kinase inhibitors with potential as a new treatment for cancer.

Acknowledgments

We thank Rachael Hawtin and Terence Hui for generating and preparing proteins used in the enzymatic assays. We thank Sheri Shirazian, Yong Zhou, Jeffrey Chen, James Rodda, Kim Lach, Danny Dam, Lany Ruslim, and Nghia Vu for technical support.

References

1. Haddad, R., Lipson, K. E., and Webb, C. P. Hepatocyte growth factor expression in human cancer and therapy with specific inhibitors. Anticancer Res., *21* (6B)*:* 4243 – 4252, 2001.

2. Maulik, G., Shrikhande, A., Kijima, T., Ma, P. C., Morrison, P. T., and Salgi, R. Role of the hepatocyte growth factor receptor, c-Met, in oncogenesis and potential for therapeutic inhibition. Cytokine Growth Factor Rev., *13:* 41 – 59, 2002.

3. Longati, P., Comoglio, P. M., and Bardelli, A. Receptor tyrosine kinases as therapeutic targets: the model of the Met oncogene. Curr. Drug Targets, *2:* 41 – 55, 2001.

4. Jeffers, M., Rong, S., and Vande Woude, G. F. Enhanced tumorigenicity and invasion-metastasis by hepatocyte growth factor/scatter factor-Met signaling in human cells concomitant with induction of the urokinase proteolysis network. Mol. Cell. Biol., *16:* 1115 – 1125, 1996.

5. Matsumoto, K., Date, K, Shimura, H., and Nakamura, T. Acquisition of invasive phenotype in gallbladder cancer cells via mutual interaction of stromal fibroblasts and cancer cells as mediated by hepatocyte growth factor. Jpn. J. Cancer Res., *87:* 702 – 710, 1996.

6. Yamashita, J., Ogawa, M., Yamashita, S., Nomura, K., Kuramoto, M., Saishoji, T., and Shin, S. Immunoreactive hepatocyte growth factor is a strong and independent predictor of recurrence and survival in human breast cancer. Cancer Res., *54:* 1630 – 1633, 1994.

7. Takada, N., Yano, Y., Matsuda, T., Otani, S., Osugi, H., Higashino, M. Kinoshita, H., and Fukushima, S. Expression of immunoreactive human hepatocyte growth factor in human esophageal squamous cell carcinomas. Cancer Lett., *97:* 145 – 148, 1995.

8. Nagy, J., Curry, G. W., Hillan, K. J., McKay, I. C., Mallon, E., Purushotham, A. D., and George, W. D. Hepatocyte growth factor/scatter factor expression and c-*met* in primary breast cancer. Surg. Oncol., *5:* 15 – 21, 1996.

9. Maemura, M., Iino, Y., Yokoe, T., Horiguchi, J., Takei, H., Koibuchi, Y., Horii, Y., Takeyoshi, I., Ohwada, S., and Morishita, Y. Serum concentration of hepatocyte growth factor in patients with metastatic breast cancer. Cancer Lett., *126:* 215 – 220, 1998.

10. Siegfried, J. M., Weissfeld, L. A., Luketich, J. D., Weyant, R. J., Gubish, C. T., and Landreneau, R. J. The clinical significance of hepatocyte growth factor for non-small cell lung cancer. Ann. Thorac. Surg., *66:* 1915 – 1918, 1998.

11. Seidel, C., Børset, M., Turesson, I., Abildgaard, N., Sundan, A., and Waage, A. Elevated serum concentrations of hepatocyte growth factor in patients with multiple myeloma. Blood, *91:* 806 – 812, 1998.

12. Ueki, T., Fujimoto, J., Suzuki, T., Yamamoto, H., and Okamoto, E. Expression of hepatocyte growth factor and its receptor, the c-*met* proto-oncogene, in hepatocellular carcinoma. Hepatology, *25:* 619 – 623, 1997.

13. Taniguchi, K., Yonemura, Y., Nojima, N., Hirono, Y., Fushida, S.,

Fujimura, T, Miwa, K., Endo, Y., Yamamoto, H., and Watanabe, H. The relation between the growth patterns of gastric carcinoma and the expression of hepatocyte growth factor receptor (c-*met*), autocrine motility factor receptor and the urokinase-type plasminogen activator receptor. Cancer, *82:* 2112 – 2122, 1998.

14. Wagatsuma, S., Konno, R., Sato, S., and Yajima, A. Tumor angiogenesis, hepatocyte growth factor, and c-*met* expression in endometrial carcinoma. Cancer, *82:* 520 – 530, 1998.

15. Guerin, C., Luddy, C., Abounader, R., Lal, B., and Laterra, J. Glioma inhibition by HGF/NK2, an antagonist of scatter factor/hepatocyte growth factor. Biochem. Biophys. Res. Commun., *273:* 287 – 293, 2000.

16. Date, K., Matsumoto, K., Kuba, K., Shimura, H., Tanaka, M., and Nakamura, T. Inhibition of tumor growth and invasion by a four-kringle antagonist (HGF/NK4) for hepatocyte growth factor. Oncogene, *17:* 3045 – 3054, 1998.

17. Kuba, K., Matsumoto, K., Date, K., Shimura, H., Tanaka, M., and Nakamura, T. HGF/NK4, a four-kringle antagonist of hepatocyte growth factor, is an angiogenesis inhibitor that suppresses tumor growth and metastasis in mice. Cancer Res., *60:* 6737 – 6743, 2000.

18. Tomioka, D., Maehara, N., Kuba, K., Mizumoto, K., Tanaka, M., Matsumoto, K., and Nakamura, T. Inhibition of growth, invasion, and metastasis of human pancreatic carcinoma cells by NK4 in an orthotopic model. Cancer Res., *61:* 7518 – 7524, 2001.

19. Maemondo, M., Narumi, K., Saijo, Y., Usui, K., Tahara, M., Tazawa, R., Hagiwara, K., Matsumoto, K., Nakamura, T., and Nukiwa, T. Targeting angiogenesis and HGF function using an adenoviral vector expressing the HGF antagonist NK4 for cancer therapy. Mol. Ther., *5:* 177 – 185, 2002.

20. Cao, B., Su, Y., Oskarsson, M., Zhao, P., Kort, E. J., Fisher, R. J., Wang, L-M., and Vande Woude, G. F. Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models. Proc. Natl. Acad. Sci. USA, *98:* 7443 – 7448, 2001.

21. Abounader, R., Ranganathan, S., Lal, B., Fielding, K., Book, A., Dietz, H. Burger, P., and Laterra, J. Reversion of human glioblastoma malignancy by U1 small nuclear RNA/ribozyme targeting of scatter factor/hepatocyte growth factor and c-*met* expression. J. Natl. Cancer Inst., *91:* 1548 – 1556, 1999.

22. Abounader, R., Lal, R., Luddy, C., Koe, G., Davidson, B., Rosen, E. M., and Laterra, J. *In vivo* targeting of SF/HGF and c-*met* expression via U1snRNA/ribozymes inhibits glioma growth and angiogenesis and promotes apoptosis. FASEB J., *16:* 108 – 110, 2002.

23. Webb, C. P., Hose, C. D., Koochekpour, S., Jeffers, M., Oskarsson, M., Sausville, E., Monks, A., and Vande Woude, G. F. The geldanamycins are potent inhibitors of the hepatocyte growth factor/scatter factor-met-urokinase plasminogen activator-plasmin proteolytic network. Cancer Res., *60:* 342 – 349, 2000.

24. Morotti, A., Mila, S., Accornero, P., Tagliabue, E., and Ponzetto, C. K252a inhibits the oncogenic properties of *met*, the HGF receptor. Oncogene, *21:* 4885 – 4893, 2002.

25. Krystal, G. W., Honsawek, S., Kiewlich, D., Liang, C., Vasile, S., Sun, L., McMahon, G., and Lipson, K. E. Indolinone tyrosine kinase inhibitors block Kit activation and growth of small cell lung cancer cells. Cancer Res., *61:* 3660 – 3668, 2001.

26. Mohammadi, M., McMahon, G., Sun, L., Tang, C., Hirth, P., Yeh, B. K., Hubbard, S. R., and Schlessinger, J. Structures of the tyrosine kinase domain of fibroblast growth factor receptor in complex with inhibitors. Science, *276:* 955 – 960, 1997.

27. Furge, K. A., Zhang, Y-W., and Vande Woude, G. F. Met receptor tyrosine kinase: enhanced signaling through adaptor proteins. Oncogene, *19:* 5582 – 5589, 2000.

28. Stuart, K. A., Riordan, S. M., Lidder, S., Crostella, L., Williams, R., and Skouteris, G. G. Hepatocyte growth factor/scatter factor-induced intracellular signaling. Int. J. Exp. Pathol., *81:* 17 – 30, 2000.

29. Weidner, K. M., Cesare, S. D., Sachs, M., Brinkmann, V., Behrens, J., and Birchmeier, W. Interaction between Gab1 and the c-Met receptor tyrosine kinase is responsible for epithelial morphogenesis. Nature, *384:* 173 – 176, 1996.

30. Nguyen, L., Holgado-Madruga, M., Maroun, C., Fixman, E. D., Kamikura, D., Fournier, T., Charest, A., Tremblay, M. L., Wong, A. J., and Park, M. Association of the multisubstrate docking protein Gab1 with the hepatocyte growth factor receptor requires functional Grb2 binding site involving tyrosine 1356. J. Biol. Chem., *272:* 20811 – 20819, 1997.

31. Bardelli, A., Longati, P., Gramaglia, D., Stella, M. C., and Comoglio, P. M. Gab1 coupling to the HGF/Met receptor multifunctional docking site requires binding of Grb2 and correlates with the transforming potential. Oncogene, *18:* 3103 – 3111, 1997.

32. Stoker, M., Gherardi, E., Perryman, M., and Gray, J. Scatter factor is a fibroblast-derived modulator of epithelial cell motility. Nature, *327:* 239 – 242, 1987.

33. Zhang, Y. W. and Vande Woude, G. F. HGF/SF-met signaling in the control of branching morphogenesis and invasion. J. Cell. Biochem., *88:* 408 – 417, 2003.

34. Lipson, K. E., Pang, L., Huber, J., Chen, H., Tsai, J-M., Hirt, P., Gazit, A., Levitzki, A., and McMahon, G. Inhibition of platelet-derived growth factor and epidermal growth factor receptor signaling events after treatment of cells with specific synthetic inhibitors of tyrosine kinase phopshorylation. J. Pharmacol. Exp. Ther., *285* (2)*:* 844 – 852, 1998.

Molecular Cancer Therapeutics

Potent and selective inhibitors of the Met [hepatocyte growth factor/scatter factor (HGF/SF) receptor] tyrosine kinase block HGF/SF-induced tumor cell growth and invasion

Xueyan Wang, Phuong Le, Congxin Liang, et al.

Mol Cancer Ther 2003;2:1085-1092.