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# Discovery of a novel and potent series of thieno[3,2-*b*]pyridine-based inhibitors of c-Met and VEGFR2 tyrosine kinases

Stephen Claridge,[a,*] Franck Raeppel,[a] Marie-Claude Granger,[a] Naomy Bernstein,[a] Oscar Saavedra,[a] Lijie Zhan,[a] David Llewellyn,[a] Amal Wahhab,[a] Robert Deziel,[a] Jubrail Rahil,[c] Normand Beaulieu,[b] Hannah Nguyen,[b] Isabelle Dupont,[b] Annie Barsalou,[b] Carole Beaulieu,[b] Ian Chute,[b] Serge Gravel,[b] Marie-France Robert,[b] Sylvain Lefebvre,[b] Marja Dubay,[b] Roussen Pascal,[d] Jeff Gillespie,[d] Zhiyun Jin,[d] James Wang,[d] Jeffrey M. Besterman,[b] A. Robert MacLeod[b,†] and Arkadii Vaisburg[a]

[a]*Department of Medicinal Chemistry, MethylGene Inc., 7220 Frederick-Banting, Montréal, Que., Canada H4S 2A1*
[b]*Department of Cell Biology and Pharmacology, MethylGene Inc., 7220 Frederick-Banting, Montréal, Que., Canada H4S 2A1*
[c]*Department of Lead Discovery, MethylGene Inc., 7220 Frederick-Banting, Montréal, Que., Canada H4S 2A1*
[d]*Department of PK/Analytical Chemistry, MethylGene Inc., 7220 Frederick-Banting, Montréal, Que., Canada H4S 2A1*

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**Abstract**—A series of thieno[3,2-*b*]pyridine-based inhibitors of c-Met and VEGFR2 tyrosine kinases is described. The compounds demonstrated potency with $IC_{50}$ values in the low nanomolar range in vitro while the lead compound also showed in vivo activity against various human tumor xenograft models in mice. Further exploration of this class of compounds is underway.


c-Met (the receptor for hepatocyte growth factor/scatter factor, (HGF/SF)) and members of the vascular endothelial growth factor receptor (VEGFR) family[1] are among the attractive receptor tyrosine kinases (RTKs) pursued actively as potential targets for the development of cancer therapeutics. c-Met is primarily expressed on cells of epithelial and mesenchymal origin. Upon binding to its ligand, HGF, c-Met is activated, resulting in the phosphorylation of tyrosine residues within its kinase domain followed by phosphorylation of key residues in its unique multi-substrate-binding site. The phosphorylated residues provide sites for the recruitment of cellular adaptors and docking proteins leading to the activation of signaling pathways culminating in numerous biological responses, including cell migration and invasion, proliferation and survival, as well as morphogenesis and angiogenesis.[2]

While c-Met is involved in normal processes during development and wound healing, its deregulation is associated with tumorigenesis. The overexpression of c-Met or mutations within and outside of its kinase domain leading to its constitutive activation have been detected in cancer patients.[3] In addition, autocrine or paracrine activation of c-Met by its ligand, HGF, has also been described.[4] Together these mechanisms of c-Met activation have been associated with poor prognosis. In addition to its role in tumor cell survival and metastasis, c-Met is also implicated in angiogenesis and has been shown to cooperate synergistically with the vascular endothelial growth factor receptors (VEGFRs) including VEGFR1 (Flt1) and VEGFR2 (KDR). These receptors have also been implicated in the development and progression of various human cancers and, therefore, have been central in the development of anticancer therapies.[5] Molecules that potently inhibit both c-Met and VEGFR may have advantages over VEGFR-selective or c-Met-selective molecules since they can exploit several mechanisms of antitumor activity including anti-angiogenic activity from c-Met and VEGFR inhibition, potential synergistic anti-angiogenic activity from c-Met/VEGFR inhibition, and antitumor

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* Corresponding author. Tel.: +1 514 337 3333; fax: +1 514 337 0550; e-mail: claridges@methylgene.com
† Present address: Takeda San Diego, 10410 Science Center Drive, San Diego, CA 92121, USA.

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activity and anti-metastatic/anti-invasive activity from c-Met inhibition.[6] Thus, the combined inhibition of both c-Met and VEGFR signaling represents a promising approach to cancer treatment by directly targeting multiple pathways involved in tumor cell survival, as well as angiogenesis and metastasis.[7]

Figure 1 shows two small molecule inhibitors of VEGFR: Sunitinib (Sutent®) from Pfizer is approved for the treatment of both renal cell carcinomas (RCC) and imatinib mesylate-resistant gastrointestinal stromal tumor (GIST). Sorafenib (Nexavar ®) from Bayer/Onyx is approved for the treatment of advanced RCC and hepatocellular carcinoma (HCC).

The report of the c-Met/VEGFR2 inhibitor (**III**) discovered by Kirin Brewery in 2003[8] and shown in Figure 1 led to the genesis of the project with the initial goal to identify an alternative to the heavily utilized quinoline scaffold of **III**. Our preliminary investigations identified thieno[3,2-*b*]pyridine[9] as a good mimic of the quinoline moiety and this report will disclose their synthesis and the SAR of the 2-position substitution of these thienopyridine-based inhibitors, Scheme 1.

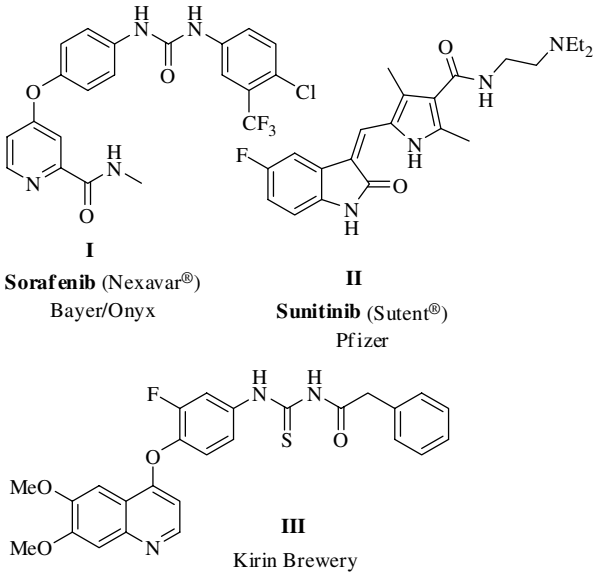
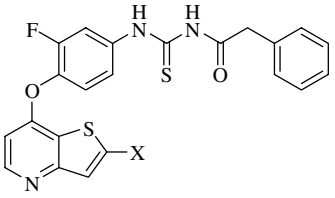



**Figure 1.** Examples of some known VEGFR and/or c-Met inhibitors.

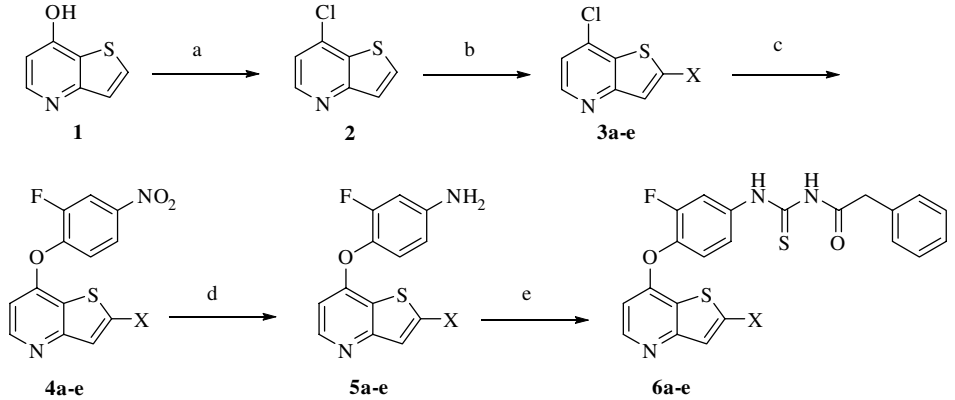
Thus, chlorination of **1** with $POCl_3$ afforded **2**[10] which upon selective deprotonation[11] with *n*-BuLi followed by quenching of the intermediate carbanion with various electrophiles afforded compounds **3a–e**. Heating **3a–e** in diphenyl ether at 180 °C with 2-fluoro-4-nitrophenol and potassium carbonate gave the desired nitrophenyl ethers **4a–e**, treatment of which with a mixture of $NaBH_4$ and $NiCl_2 \cdot 6H_2O$[12] gave anilines **5a–e**. Reaction of the aforementioned anilines with phenylacetyl isothiocyanate[13] afforded **6a–e**, Scheme 1. A detailed description of the syntheses and characterization of **6a–e** and subsequent compounds mentioned in this letter is provided.[14]

From Table 1, it may be seen that compounds **6d** and **6e** were the most potent against both enzymes when assayed in vitro. Although c-Met tolerated a range of 'simple' substituents in the 2-position of the thieno[3,2-*b*]pyridine ring system, VEGFR2 preferred a carbonyl. Thus, an additional set of molecules was made in efforts to obtain more potent inhibitors of *both* enzymes.

**Table 1.** In vitro[15] profile for compounds **III** and **6a–e**



| Compound | X | c-Met $IC_{50}$ (nM) | VEGFR2 $IC_{50}$ (nM) |
|---|---|---|---|
| **III** | — | 121 | 32 |
| **6a** | H | 116 | 1134 |
| **6b** | CH(OH)Me | 132 | 561 |
| **6c** | SMe | 130 | 346 |
| **6d** | –CO-(2-Furyl) | 130 | 127 |
| **6e** | $CO_2Me$ | 113 | 90 |




**Scheme 1.** Reagents and conditions: (a) $POCl_3$, reflux; (b) *n*-BuLi, −78 °C, THF then electrophile X; (c) 2-fluoro-4-nitrophenol, $Ph_2O$, $K_2CO_3$, 180 °C; (d) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, 0 °C, MeOH, water; (e) BnCONCS, THF, rt or EtOH/toluene, rt.

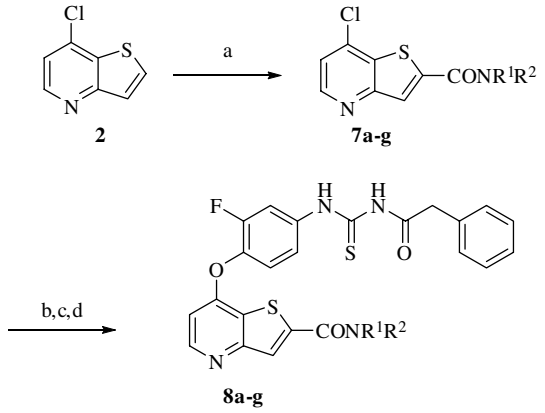
Hence, a series of amides was synthesized using the chemistry shown in Scheme 2 and their in vitro activities are shown in Table 2.

These data demonstrate that the amides are potent nanomolar inhibitors of both enzymes and that the amide functionality is well tolerated.

Unsubstituted (**8a**) or monosubstituted (**8b**) amides were reasonably potent against c-Met and slightly less so against VEGFR2. While dimethylamide **8c** showed a slight increase in potency against c-Met and a more pronounced increase in activity against VEGFR2, bulkier amides such as **8d**, **8e**, and **8g**, on the contrary, were less potent against both enzymes. Interestingly, compound **8f**, which was synthesized as a constrained analogue of compound **8d**, showed a 40-fold increase in activity against VEGFR2 suggesting that the active site of the VEGFR2 enzyme has a tolerance for only rigid and 'small' amides, such as **8c** and **8f**. The weak in vivo efficacy of the amides (data not shown), probably resulting from their poor pharmacokinetics, necessitated the search for an additional class of molecules in which the amide moiety was replaced with various heterocycles as amide isosteres. The chemistry used to achieve this is shown in Schemes 3 and 4.

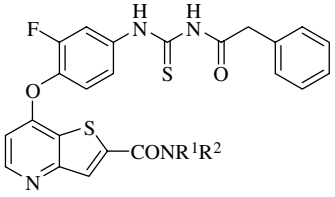
Table 3 shows the different 2-heteroaryl derivatives synthesized. The choice of the chemical method used was dependent on the availability of the respective halides or boronic acids. The chemistry in Scheme 4 was preferable over that shown in Scheme 3 due to the avoidance of the use of tin reagents.

Although all the compounds were active inhibitors, the imidazole substituted compounds were among the most potent of these analogues, when tested in in vitro kinase assays against the c-Met and VEGFR2 enzymes. Interestingly, in the TPR-Met assay, a cell-based assay which detected the phosphorylation of Y1230-34-35 in a fusion

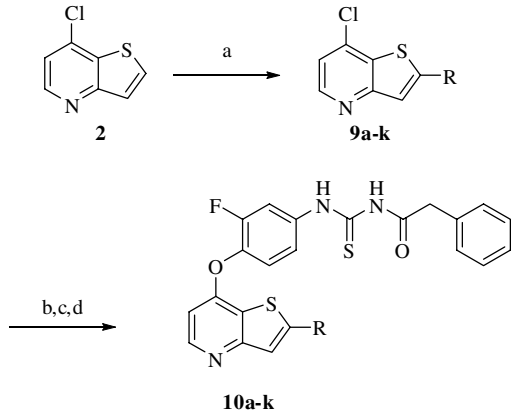



**Scheme 2.** Reagents and conditions: (a) i—*n*-BuLi, −78 °C, THF then $CO_2$ (g); ii—$(COCl)_2$, DCM, reflux; iii—$R^1R^2NH$, DCM, rt; (b) 2-fluoro-4-nitrophenol, $K_2CO_3$, 180 °C, $Ph_2O$; (c) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, 0 °C, MeOH, water; (d) BnCONCS, THF, rt or EtOH/toluene, rt.

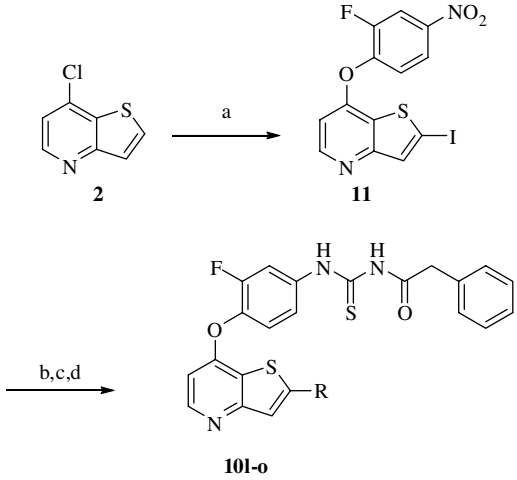
**Table 2.** In vitro[15] profile for compounds **8a–g**




| Compound | $R^1$ | $R^2$ | c-Met $IC_{50}$ (nM) | VEGFR2 $IC_{50}$ (nM) |
|---|---|---|---|---|
| **8a** | H | H | 65 | 129 |
| **8b** | H | Me | 80 | 133 |
| **8c** | Me | Me | 48 | 19 |
| **8d** | Et | Et | 114 | 386 |
| **8e** | *N*-Morpholine | | 125 | 289 |
| **8f** | *N*-Pyrrolidine | | 54 | 9 |
| **8g** | *N*-(4-Methylpiperazine) | | 193 | 344 |

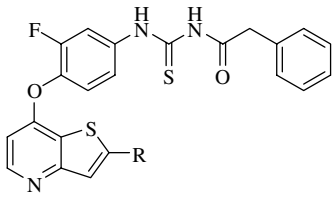



**Scheme 3.** Reagents and conditions: (a) i—*n*-BuLi, THF, −78 °C, then $Bu_3SnCl$; ii—heteroaryl bromide or iodide, $(PPh_3)_4Pd$, toluene, reflux, $N_2$; (b) 2-fluoro-4-nitrophenol, $K_2CO_3$, 180 °C, $Ph_2O$; (c) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, 0 °C, MeOH, water; (d) BnCONCS, THF, rt or EtOH/toluene, rt.




**Scheme 4.** Reagents and conditions: (a) i—*n*-BuLi, THF, −78 °C, iodine,[11]; ii—2-fluoro-4-nitrophenol, $K_2CO_3$, 180 °C, $Ph_2O$; (b) heteroaryl boronic acid, $(PPh_3)_4Pd$, DME, $NaHCO_3$, CsF, $H_2O$, reflux, $N_2$; (c) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, 0 °C, MeOH, water; (d) BnCONCS, THF, rt or EtOH/toluene, rt.

**Table 3.** In vitro[15] profile of compounds **10a**–**o**



| Compound | R | c-Met $IC_{50}$ (nM) | VEGFR2 $IC_{50}$ (nM) | $Y^{1230\text{-}34\text{-}35}$ TPR-Met $IC_{50}$ (nM) |
|---|---|---|---|---|
| **10a** | 1-Methyl-1H-imidazol-4-yl | 29 | 10 | 12 |
| **10b** | 1-Ethyl-1H-imidazol-4-yl | 52 | 11 | 22 |
| **10c** | 1-Methyl-1H-imidazol-2-yl | 51 | 10 | 2 |
| **10d** | 1-Ethyl-1H-imidazol-2-yl | 108 | 25 | 35 |
| **10e** | 1-Methyl-1H-pyrazol-4-yl | 24 | 25 | ND |
| **10f** | 1-Methyl-1H-1,2,4-triazol-5-yl | 69 | 28 | 20 |
| **10g** | Thiazol-2-yl | 65 | 17 | 28 |
| **10h** | Pyridin-2-y | 62 | 17 | 20 |
| **10i** | Pyridin-3-yl | 126 | 113 | 52 |
| **10j** | Pyrimidin-2-yl | 181 | 94 | 200 |
| **10k** | 1,3,4-Thiadiazol-2-yl | 60 | 145 | ND |
| **10l** | Thiophen-2-y | 70 | 32 | 300 |
| **10m** | 1-Methyl-1H-pyrrol-2-yl | 49 | 31 | 108 |
| **10n** | Furan-3-yl | 74 | 593 | ND |
| **10o** | Pyrimidin-5-yl | 63 | 175 | 5000 |

protein between c-Met kinase domain sequences and TPR, the pyrimidine-based compounds **10j** and **10o** were among the least active, possibly due to poor cellular penetration.

Compounds **10a** and **10c** were found to be potential lead candidates. However, the low stability found for **10c** in a human liver microsome test (data not shown) coupled with its poor solubility (1.7 μg/mL compared with 30 μg/mL for **10a**) led us to select **10a** for additional testing.

Table 4 shows a head to head comparison of the lead compound **10a** against Sutent® in various HGF- and VEGF-dependent cell-based assays. In HGF-driven cell migration and scattering assays, **10a** inhibited both responses efficiently, in contrast to Sutent®, which did not inhibit c-Met enzymatic activity. However, both inhibitors were comparable in VEGF-driven assays.

Given its good in vitro enzyme inhibition and cell-based profiles, the pharmacokinetic properties of **10a** were evaluated in vivo in rat and dog, Table 5.

Compound **10a** (>95% chromatographic purity) was administered to female Sprague–Dawley rats and male beagle dogs. DMSO was used in iv dosing to rats while a mixture of 5% DMSO, 1% Tween 80 in water and 0.1 N HCl in 40/60 PEG400/saline was used to dose rats po. For the dog studies, 0.04 N HCl in 25% HpbCD was used for iv dosing while a mixture of 5% DMSO, 1% Tween 80 in water and 0.1 N HCl in 40/60 PEG400/saline was used for po dosing to the dogs. Plasma samples were analyzed using an Agilent 1100 HPLC system coupled with an MDS Sciex API2000 triple quadrupole mass spectrometer. WinNonLin software was used to calculate the PK parameters.

**Table 5.** Pharmacokinetic profile for **10a** in two species

| Parameter | Rat[a] | Dog[b] |
|---|---|---|
| $t_{1/2}$, iv (h) | 1.2 | 5.8 |
| CL (L/(kg h)) | 0.33 | 1.1 |
| $V_{ss}$ (L/kg) | 0.25 | 1.5 |
| $T_{max}$, po (h) | 3.5 | 1.1 |
| $C_{max}$, po (μM/(mg/kg)) | 0.14 | 0.21 |
| AUC, po (μM h/(mg/kg)) | 0.74 | 0.74 |
| % *F* | 12 | 42 |

[a] Dose, iv 2.5 mg/kg (four animals used), po 5 mg/kg (nine animals used) and 25 mg/kg (three animals used).
[b] Dose, iv 0.8 mg/kg (two animals used), po 5 mg/kg (four animals used).

The results in Table 5 show that **10a** has a reasonable half-life, 1.2 h in rats and 5.8 h in dogs, and has an acceptable clearance, 0.33 L/(kg h) in rats and 1.1

**Table 4.** Comparison of **10a** with Sutent®

| | HGF-dependent | | VEGF-dependent | |
|---|---|---|---|---|
| | A549 cell migration $IC_{50}$ (μM) | DU145 cell scattering $IC_{50}$ (μM) | HUVEC ERK phosphorylation $IC_{50}$ (μM) | HUVEC proliferation $IC_{50}$ (μM) |
| Sutent | 2 | 10 | 0.03 | 0.025 |
| **10a** | 0.4 | 0.08 | 0.03 | 0.006 |

**Table 6.** The effect of oral dosage of **10a** on various human tumor models in vivo at 20 mg/kg once daily

| Tumor model | Experiment duration (days) | % Tumor growth inhibition |
|---|---|---|
| Colo205 (colorectal) | 16 | 41 |
| DU145 (prostate) | 11 | 57 |
| HCT116 (colorectal) | 14 | 41 |
| MNNGHOS (osteosarcoma) | 26 | 61 |
| MKN45 (gastric) | 13 | 94 |

L/(kg h) in dogs. The steady state volume of distribution was low in rats (0.25 L/kg) and reasonable in dogs (1.5 L/kg), while the oral bio-availability was determined to be 12% and 42% in rats and dogs, respectively.

As shown in Table 6, compound **10a** performed well in vivo against a panel of different human tumor types, particularly those that are driven by or overexpress c-Met (MNNGHOS and MKN45). Tumor growth inhibition at a dose of 20 mg/kg po once daily ranged from 41% to 94%. Compound **10a** was found to show spill-over inhibition of a number of kinases in addition to the intended c-Met/VEGFR2 activity.[16] Although disappointing as it was hoped that **10a** would be more selective, the inhibition of multiple kinases could help with the development of cancer treatments in which tumor growth due to up-regulation of alternative kinase signaling pathways, occurs. Furthermore, toxicity studies, using the MDS AdverseReactionEnzyme™ and Hit-Profiling™ Assay packages (MDS Pharma) showed that **10a** did not exhibit any binding to receptors, channels, or enzymes that could lead to potential toxicity liabilities. Compound **10a** (at 10 μM) did not inhibit CYP450 3A4, however, it showed some activity against CYP2C19, and 2DC (data not shown).

In conclusion, a series of novel c-Met/VEGFR2 tyrosine kinase inhibitors based upon the thieno[3,2-*b*]pyridine scaffold were designed and synthesized. These compounds exhibited excellent in vitro profiles and in particular, the most promising compound, **10a**, has significant antitumor activity in vivo. Additional SAR studies have been undertaken and these will be reported in due course.

## Acknowledgment

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15. In vitro kinase assays (c-Met and VEGFR-2/KDR): preparation of GST fusion proteins: recombinant baculo-

virus containing the catalytic domain of c-Met and of the VEGFR-2/KDR receptor fused to glutathione *S*-transferase (GST) fusion genes was used to infect high five (c-Met) or Sf9 (VEGFR-2/KDR) cells at a multiplicity of infection of 1 or 0.1, respectively. Cell lysates were prepared after ~72 h of infection in 1% Triton X-100, 2 μg of leupeptin/mL, and 2 μg of aprotinin/mL after ~72 h of infection in phosphate-buffered saline, and the fusion proteins were purified over glutathione agarose (Sigma) according to the manufacturer's instructions. Biochemical kinase assays for $IC_{50}$ determination and kinetic studies: Inhibition of c-Met and VEGFR2/KDR was measured in a DELFIA™ assay (Perkin-Elmer). The substrate poly($Glu_4$, Tyr) was immobilized onto black high-binding polystyrene 96-well plates (Nunc Maxisorp). The c-Met kinase reaction was conducted in 25 mM Hepes, pH 7.5, containing 20 mM NaCl, 10 mM $MgCl_2$, 5 mM β-mercaptoethanol, 0.1 mg/mL bovine serum albumin (BSA) and 20 μM vanadate, while the VEGFR-2/KDR reaction was conducted in 60 mM Hepes, pH 7.5, containing 3 mM $MgCl_2$, 3 mM $MnCl_2$, 1.2 mM β-mercaptoethanol, 0.1 mg/mL BSA and 3 μM vanadate. ATP concentrations in the assay were 10 μM for c-Met (5× the $K_m$) and 0.6 μM for VEGFR-2/KDR (2× the $K_m$). Enzyme concentration was 25 nM (c-Met) or 5 nM (VEGFR-2/KDR).The recombinant enzymes were preincubated with inhibitor and Mg-ATP on ice in polypropylene 96-well plates for 4 min, and then transferred to the substrate coated plates. The subsequent kinase reaction took place at 30 °C for 30 min. (c-Met) or 10 min. (VEGFR2/KDR). After incubation, the kinase reactions were quenched with EDTA and the plates were washed. Phosphorylated product was detected by incubation with Europium-labeled anti-phosphotyrosine MoAb. After washing the plates, bound MoAb was detected by time-resolved fluorescence in a Gemini SpectraMax reader (Molecular Devices). Inhibitors were tested at seven different concentrations each in triplicate. $IC_{50}$s were calculated in a four parameters equation curve plotting inhibition (%).

16. Compound **10a** at 100 nM concentration inhibited the following enzymes: VEGFR-1 (91%), VEGFR-3 (100%), Ron (80%), Tie-2 (81%), Flt-3 (96%), c-Kit (92%), Abl (91%), and TrkA (99%). Compound **10a** had <5% inhibitory activity against the following enzymes CHK1, EGFR, GSK3β, IGF-1R, IKK-β, JAK2, and JNKa1. Compound **10a** was evaluated using the Kinaseprofiler™ Kinase Selectivity Screening Service (radiometric protein kinase assays) by Millipore.