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Identification of a novel series of potent RON receptor tyrosine kinase inhibitors

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ABSTRACT

A novel series of *N*-(3-fluoro-4-(2-substituted-thieno[3,2-*b*]pyridin-7-yloxy)phenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides targeting RON receptor tyrosine kinase was designed and synthesized. SAR study of the series allowed us to identify compounds possessing either inhibitory activity of RON kinase enzyme in the low nanomolar range with low residual activity against the closely related c-Met or potent dual inhibitory activity against RON and c-Met, – with no significant activity against VEGFR2 in both cases.

Receptor tyrosine kinases are implicated in tumor development and progression and are validated targets for the development of therapeutics in oncology.[1] Deregulation of RON (recepteur d'origine nantais) has been described in numerous types of cancers including colorectal, breast, lung, pancreas, prostate and bladder and occurs mainly through wild type receptor overexpression or expression of variants harboring different deletions within the extracellular domain, leading to constitutive receptor activation.[2] Importantly, increased RON activation in tumor tissues correlated with histological grading and tumor staging, and was a predictor of poorer patient prognosis.[3] RON is mainly expressed on epithelial cells but also on macrophages, and is activated following ligation by macrophage stimulating protein (MSP). The molecular mechanisms through which RON contributes to tumorigenesis are beginning to unfold and involve multiple biological functions including proliferation, migration, invasion and the induction of angiogenic/chemotactic factors in endothelial cells.

Recently, ImClone Systems (now a division of Eli Lilly & Co.) developed IMC-41A10, a human IgG1 monoclonal antibody that binds with high affinity to human RON RTK and blocks MSP ligand binding.[4] IMC-41A10 inhibited RON phosphorylation and signaling following activation of cancer cell lines with MSP, and suppressed biological functions such as MSP-dependent cell migration. Importantly, IMC-41A10 inhibited tumor growth by 50–60% in several human xenograft tumor models including colon, lung and pancreatic carcinoma models, thus further validating RON as a valuable oncology target. Small molecule inhibitors of RON have been described as well. These chemical entities inhibit both Ron and the closely related c-Met kinase.[5] c-Met is found to be activated in a large number of different cancers and small molecule inhibitors targeting Met/Ron are presently under clinical evaluation in patients with solid tumors.[6] For example, a potent small-molecule dual inhibitor of c-Met/RON was disclosed by Amgen.[7] This quinoline based compound having the 1-(2-hydroxy-2-methylpropyl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-carboxamide head group (Fig. 1) inhibits both Met and RON enzymes in the low nanomolar range and demonstrates anti-tumor activity in a colorectal xenograft model in mice when dosed po at 100 mg/kg once daily. Furthermore, Bristol-Myers Squib described BMS-777607 as a new pyridine based selective and orally efficacious inhibitor of the Met/RON kinase superfamily having a new cyclic head group that has advanced into phase I clinical trials.[8] To the

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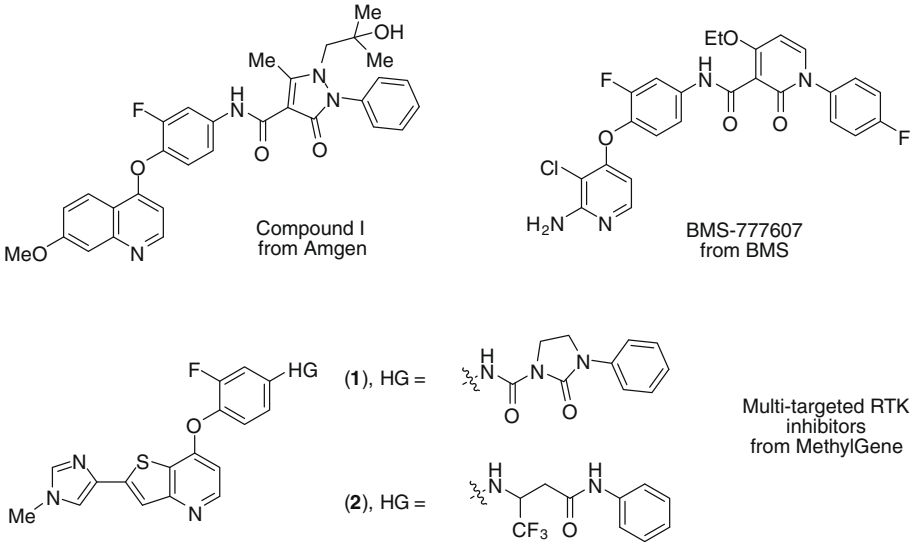
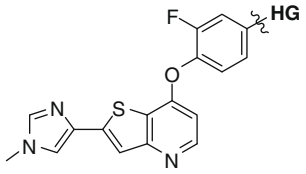


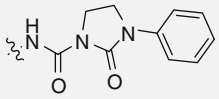
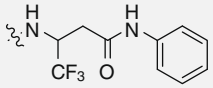
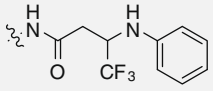
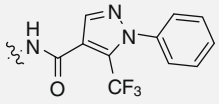
Figure 1. c-Met and/or VEGFR-2 inhibitors **1** and **2** as well as the Amgen and BMS c-Met/Ron inhibitors.

best of our knowledge, small molecules that specifically target RON (but not c-Met) have not been described yet. The high sequence similarity between the kinase catalytic domains of Met and RON makes the design of such inhibitors challenging.[6i]

We have previously reported on the two novel thieno[3,2-*b*]pyridine based series of either dual c-Met/VEGFR2 inhibitors with a five-membered cyclic urea head group[9] or selective VEGFR family inhibitors with an acyclic amide isostere head group,[10] exemplified by structures **1** and **2**, respectively (Fig. 1). These compounds were evaluated for their inhibitory activity against the RON kinase in a cell-based assay we developed, where TPR-RON was stably expressed in a cell line and is constitutively active.[11] Compound **1** showed a submicromolar activity against TPR-RON while compound **2** was a micromolar inhibitor of this enzyme (Table 1). However compound **3**—the regioisomer of compound **2**—which was identified as a weak inhibitor of both c-Met and VEGFR2,[12] surprisingly showed activity against TPR-RON comparable to that of **1**. Thus, we hypothesized that combining the structural features of the head groups of compounds **1** and **3,** that is, rigidifying the 4,4,4-trifluoro-3-(phenylamino)butanamide moiety into the 1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide fragment as illustrated by compound **4**, may affect the kinase selectivity profile of the resultant chemical entities, and perhaps yield a molecule with an even greater selectivity towards RON. Indeed, when compound **4** was synthesized and evaluated it showed a different kinase selectivity profile compared to compounds **1** and **3**: it demonstrated a potent RON kinase inhibition in a Ron-specific cell-based assay, reduced activity against c-Met and lack of activity against VEGFR2 in in vitro kinase assays. As a consequence, we initiated the SAR study around compound **4** as a prototype of selective RON inhibitor.

Table 1
Thieno[3,2-*b*]pyridine based kinase inhibitors **1**–**4** with different head groups



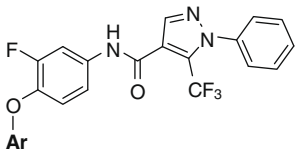
Compd	Head groups (HG)	TPR-RON ELISA[a] IC_{50} (μM)	c-Met[b] IC_{50} (μM)	VEGFR2[b] IC_{50} (μM)
1		0.17	0.02	0.01
2		1.03	0.12	0.01
3		0.41	0.70	0.21
4		0.05	0.15	>10

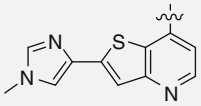
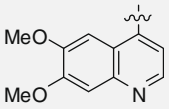
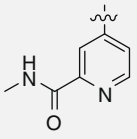
[a] Cell-based assay.
[b] Enzymatic assays.

First, we exercised a limited scaffold hopping—thieno[3,2-*b*]pyridine, quinoline or pyridine—by keeping unchanged the new cyclic head group and the central aromatic ring (Table 2). These scaffolds are thought to bind to the ATP catalytic site in the hinge region of the target kinase enzymes. Not surprisingly, the quinoline-based 1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide **5** was equipotent to compound **4** and showed a similar kinase profile. However, the *N*-methylpicolinamide-based analogue **6** was completely inactive against both TPR-RON and c-Met enzymes. With the ultimate goal to improve both the enzymatic and cellular activities of the newly discovered 1-aryl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides against RON we could explore either analogues of **4** or congeners of **5**. We chose to focus on the thieno[3,2-*b*]pyridine series of compounds—analogues of **4** since we had in place chemistry leading to the substituted thienopyridine derivatives.[9] A typical synthesis of the target molecules is illustrated in Scheme 1 in which the aniline derivatives **A**[9,10] react with 1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid (commercially available from Maybridge) in the presence of HATU reagent and DIPEA to afford compounds **B**.[13]

Next, we investigated the substitution pattern of the pyrazole ring, which is thought to occupy the hydrophobic back pocket of the kinase catalytic domain of the target enzymes (Table 3). Replacement of the bulky and hydrophobic trifluoromethyl group by a small alkyl substituent (compounds **7** and **8**) decreased the activity against TPR-RON and slightly increased the activity against c-Met and VEGFR2. The 'unsubstituted' compound **9** demonstrated a further decrease of activity against TPR-RON showing the same

Table 2
1-Phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide kinase inhibitors based on different scaffolds



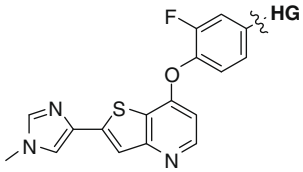
Compd	Ar	TPR-RON ELISA[a] IC_{50} (μM)	c-Met[b] IC_{50} (μM)	VEGFR2[b] IC_{50} (μM)
4		0.05	0.15	>10
5		0.08	0.20	>2
6		>10	>15	>20

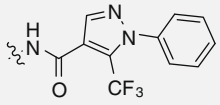
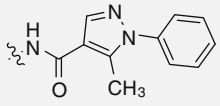
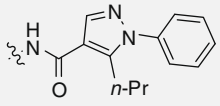
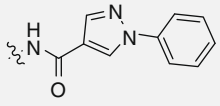
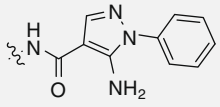
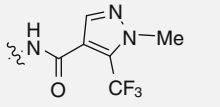
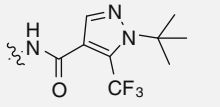
[a] Cell-based assay.
[b] Enzymatic assays.

activity against c-Met and VEGFR2. Replacement of the trifluoromethyl group by an amino group, a potential hydrogen bond donor (compound **10**) abolished the activity against TPR-RON, slightly decreased the activity against c-Met and maintained measurable activity against VEGFR2. The replacement of the terminal phenyl ring by a methyl group (compound **11**) reduced the inhibition of TPR-RON, slightly improving the activity against c-Met, and to a great extent restored the activity against VEGFR2. Finally, introduction of the bulky *tert*-butyl group instead of the methyl restored the activity against RON and maintained the inactivity against the VEGFR2 enzyme (compound **12**).

Thus, a bulky and an electronegative trifluoromethyl group at the 5-position of the pyrazole system as well as an aromatic phenyl ring at the position 1 of the same ring seem to be essential for the potent inhibition of RON enzyme and the desired decreased inhibition against both c-Met and VEGFR2. The presence of these two substituents in compound **4** may allow the head group to adopt a specific conformation and interact favorably with the hydrophobic back pocket of the RON kinase active site, possibly via a hydrogen bond network and/or hydrophobic contacts of the trifluoromethyl group and a π–π stacking and/or hydrophobic interactions of the phenyl group (e.g., compound **4** vs compounds **9** and **11**). At the same time, the presence of these two substituents on the head group may preclude compound **4** from binding properly

Table 3
Head group variations (compounds **4** and **7**–**12**)

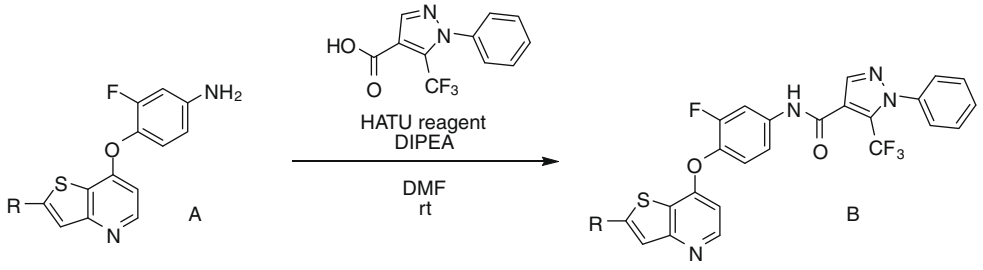


Compd	Head groups (HG)	TPR-RON ELISA[a] IC_{50} (μM)	c-Met[b] IC_{50} (μM)	VEGFR2[b] IC_{50} (μM)
4		0.05	0.15	>10
7		0.15	0.07	2.0
8		0.21	0.07	1.9
9		0.59	0.08	1.4
10		>1	0.18	0.8
11		>1	0.06	0.7
12		0.20	0.07	3.0

[a] Cell-based assay.
[b] Enzymatic assays.

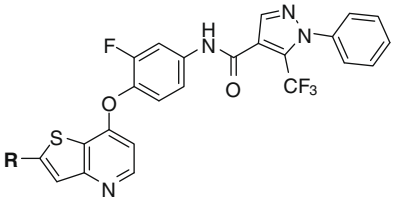
to the kinase active site of VEGFR2 and—although to a lesser extent—to that of c-Met as well.

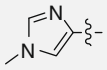
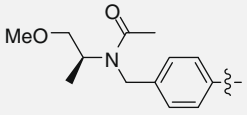
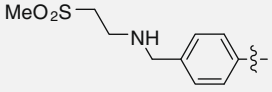
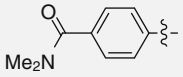
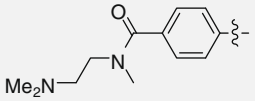
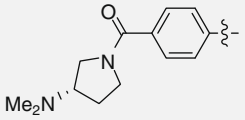
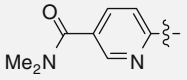
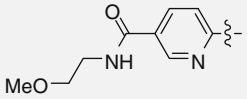
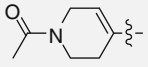
We also explored the nature of the substituent R—presumed to extend into the solvent exposed area and potentially capable of modulating physicochemical and pharmacokinetic properties of the inhibitors—in *N*-(3-fluoro-4-(thieno[3,2-*b*]pyridin-7-yloxy) phenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides (compounds **13–21,** Table 4). Compound **13**—an isomer of **4** (both bearing a rather small *N*-methylimidazole substituent)—maintained relatively weak inhibitory activity against c-Met (150 nM), a micromolar activity against VEGFR2 enzyme but importantly, showed



Scheme 1. General synthesis of *N*-(3-fluoro-4-(2-substituted-thieno[3,2-*b*]pyridin-7-yloxy)phenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides.

Table 4
Variation of substituents in *N*-(3-fluoro-4-(2-substituted-thieno[3,2-*b*]pyridin-7-yloxy)phenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides series



Compd	R	TPR-RON ELISA[a] IC_{50} (μM)	c-Met[b] IC_{50} (μM)	VEGFR2[b] IC_{50} (μM)
4		0.05	0.15	>10
13		0.06	0.15	1.4
14		0.06	0.03	2.5
15		0.08	0.06	1.1
16		0.03	0.05	1.4
17		0.09	0.03	0.7
18		0.10	0.04	1.0
19		0.03	0.04	2.5
20		0.04	0.06	5.3
21		0.09	0.05	1.4

[a] Cell-based assay.
[b] Enzymatic assays.

nanomolar activity in the TPR-RON assay. However to our surprise, compounds **14**–**21**, bearing bulkier substituents R of different shape and/or electronic properties, while maintaining good activities against RON (30–100 nM), were equally active against c-Met in the range of 30–90 nM and generally much less active against VEGFR2. Thus, compounds **4**, **13–21** showed good selectivity for the RON and c-Met enzymes with compounds **4** and **13** being the most RON-selective.

Compound **4** was profiled and tested at 0.1 μM against 23 kinases using Millipore's Kinaseprofiler™ assay services. In addition to targeting RON receptor tyrosine kinase (>95% of inhibition), it was also active against Flt3 (96% of inhibition) and moderately active against TrkA (81% of inhibition) but importantly, did not show significant inhibitory activity against a representative panel of human kinases (<40% of inhibition): ALK, Bmx, CHK1, cKit, c-Raf, EphB4, FAK, GSK3β, Haspin, IKKβ, JAK2, JAK3, LIMK1, MEK1, PDK1, PI3-β, Pim-1, PKBα, Ret and Tie2.

Compound **4** was evaluated for its ability to inhibit c-Met and RON in multiple cell-based assays (Table 5). Consistent with its sub-micromolar inhibitory activity against the c-Met enzyme (0.15 μM), compound **4** displayed weak inhibition of c-Met phosphorylation in the MKN-45 gastric carcinoma cell line which over-expresses c-Met, as well as c-Met-dependent cell migration and scattering mediated by HGF, in two other cancer cell lines, a non-small-lung carcinoma cell line A549 and a prostate carcinoma cell line DU145. As predicted, these results demonstrate that compound **4** is a weak inhibitor of c-Met when compared to compound **1**. However, when compound **4** was evaluated for it ability to inhibit RON phosphorylation, it demonstrated potent inhibition of RON phosphorylation in several models. RON has recently been described to play an important role in prostate tumorigenesis: knockdown of RON in a prostate carcinoma cell line, PC3, that overexpresses RON, resulted in reduced tumor growth.[14] Therefore, we determined in this model whether compound **4** inhibited the phosphorylation of endogenously expressed full length RON. Compound **4** potently inhibited RON phosphorylation in these cancer cells with an IC_{50} of 30–40 nM (Fig. 2).[15] This is consistent with the low-nanomolar IC_{50} values for compound **4** obtained in our RON kinase cell-based assay (50 nM). Furthermore, the inhibition of RON phosphorylation was also analyzed in another cancer cell model, the HCT116 colon carcinoma, where RON is expressed and phosphorylated in the absence of stimulation with exogenously added RON ligand (MSP).[16] In these cells, compound **4** significantly inhibited RON phosphorylation in a dose-dependent manner (Fig. 3).[15] Interestingly, although the proliferation of these cells may not be driven by RON exclusively, compound **4** potently inhibited cellular proliferation in this model, as detected in a MTT assay (Table 5).

Compound **4** was further evaluated for its pharmacokinetic properties in the rat (Table 6). Compound **4** showed a 2.7 h half-life, a reasonably low rate of clearance and good oral bioavailability.

Table 5
Effect of compounds **1** and **4** on c-Met- mediated cellular endpoints and on human cancer cell proliferation

Compd	c-Met-driven cell-based assay			Tumor MTT assay
	c-Met in MKN-45 IC_{50} (μM)	A549 Wound Healing inh. IC_{50} (μM)	DU145 Scattering inh. IC_{50} (μM)	HCT116 IC_{50} (μM)
1	0.07	0.08	0.08	n.a.
4	0.5	0.4	2	0.08

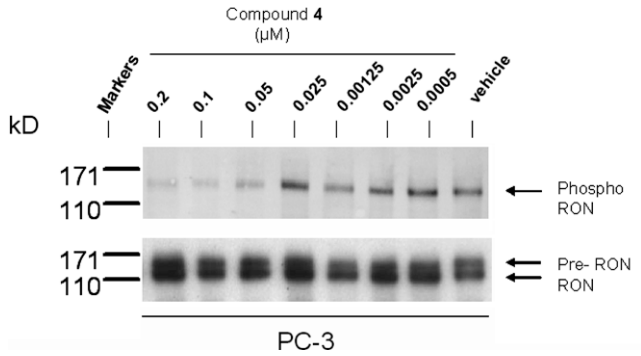


Figure 2. Effect of compound **4** against endogenous RON enzyme in the PC-3 prostate cancer cells.

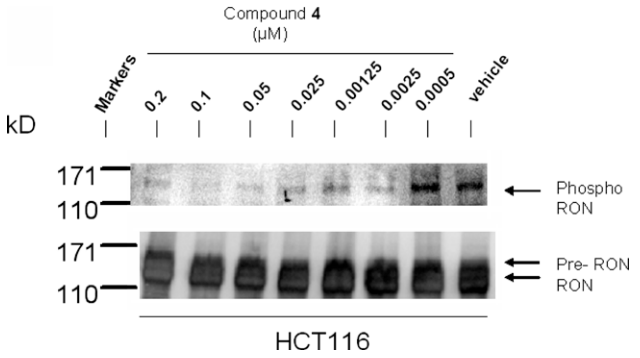


Figure 3. Effect of compound **4** against endogenous RON enzyme in the HCT116 lung cancer cells.

Table 6
Rat pharmacokinetic profile of compound **4**

PK properties[a]	Compd **4**
$T_{1/2}$ (h), iv	2.7
Cl (L/h/kg)	0.63
Vss (L/kg)	2.4
T_{max} (h), po	6.0
C_{max} (μM/(mg/kg)), po	0.25
AUC_{0_6} (μM h/(mg/kg)), po	0.84
% *F*	>30

[a] iv dose: 2.5 mg/kg, po dose: 5.0 mg/kg.

In conclusion, a novel series of *N*-(3-fluoro-4-(2-substituted-thieno[3,2-*b*]pyridin-7-yloxy)phenyl)-1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamides was designed and synthesized. The most attractive compounds of the series—**4** and **13**—are selective for RON kinase enzyme with only residual activity against c-Met and no significant inhibitory activity against VEGFR2. These novel chemical entities represent a valuable starting point in design and synthesis of RON selective inhibitors both as a tool to further validate RON as a biological target and for the development of potential anti-cancer therapeutics.

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