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Design and synthesis of constrained analogs of LCRF-0004 as potent RON tyrosine kinase inhibitors

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This manuscript is dedicated to the memory of Dr. Daniel Delorme (1958–2014)

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ABSTRACT

New fused bicyclic lactam head groups as rigidified analogs of thieno[3,2-*b*]pyridine-based kinase inhibitor **LCRF-0004** were designed and synthesized. Depending on the functionalities and the size of these bicyclic head groups, potent inhibitors of RON tyrosine kinase with various level of selectivity against c-Met tyrosine kinase were obtained.

RON (Récepteur d'Origine Nantais, also known as human MST1R, for macrophage stimulating 1 receptor) is the transmembrane receptor tyrosine kinase (RTK) for its endogenous ligand MSP (for macrophage stimulating protein, also known as HGFL, for hepatocyte growth factor-like).[1] RON RTK belongs to the MET proto-oncogene family, one of the three protein families of the semaphorin superfamily. RON RTK shares significant structural and functional homology with c-Met RTK, and is normally expressed at low level in most epithelial tissues. The MSP/RON signaling axis is involved in several important biological processes, including macrophage activity, wound healing, and epithelial cell behavior. Aberrant activity of RON has been described in numerous types of cancers including colorectal,[2] breast,[3] lung,[4] pancreas[5] and prostate[6] and occurs mainly through wild type receptor overexpression or expression of isoform variants harboring different truncations within the extracellular domain, leading to enhanced and uncontrolled tyrosine kinase activity suggesting their potential importance as therapeutic targets.

Metastases in the body are the main cause of death for cancer patients. Two recent studies has further shown that MSP/RON axis facilitates tumor metastasis by suppressing host antitumor immunity[7] and by reprogramming an epigenetic pathway that is required for breast cancer metastasis.[8] Consequently, RON inhibitors may potentially prevent the outgrowth of micrometastases already seeded and/or the proliferation of metastases in cancer patients.

As part of our internal research program dedicated to the discovery of novel therapeutic agents to eradicate and/or control the proliferation of metastatic cancers and metastases, new data were collected using **LCRF-0004** (Fig. 1) known to be a potent and selective inhibitor of RON receptor tyrosine kinase, reported by MethylGene.[9] We have recently confirmed that **LCRF-0004** is a potent RON inhibitor (IC_{50} = 10 nM)[10] but a surprising result was also obtained regarding its c-Met inhibitory activity (Table 1). In the original publication,[9] it was showed that this

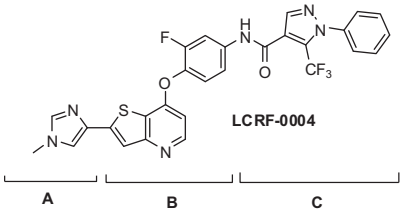

Figure 1. Structure of **LCRF-0004**.

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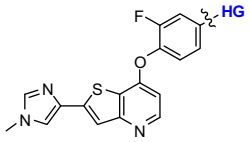
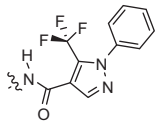
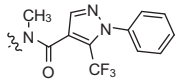
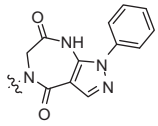
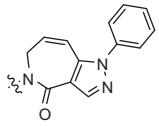
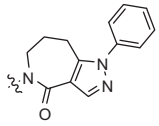
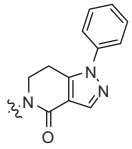
0960-894X/

Table 1
Enzymatic inhibitory activity of **LCRF-0004** and rigidified analogs



Compd	Head group (HG)	RON (h) kinase IC_{50} (μM)	c-Met (h) kinase IC_{50} (μM)
LCRF-0004		0.010	0.012
1		0.575	5.78
4		0.361	0.207
12		0.049	0.304
20		0.034	0.108
21		0.030	0.058

compound had an IC_{50} = 150 nM. In our assay, we obtained an IC_{50} = 12 nM, a factor of about 12-fold compared to the previous value. This factor may be due either to differences in the production of the enzyme (kinase domain) by cloning and/or the execution of the biochemical assay protocol, and can cause significant assay variability. Despite this divergence we have further confirmed that **LCRF-0004** was a less efficient ligand to inhibit the phosphorylation of c-Met (the protein is present in full-length and is overexpressed) in MKN-45 gastric cancer cells which are dependent on c-Met for survival with an IC_{50} = 6.87 μM. Furthermore, we confirmed that it was potent and selective for RON inhibition since it inhibited RON phosphorylation (the protein is present in full-length and is overexpressed) in HT29 colon cancer cells with an IC_{50} = 30 nM.[10]

Devoid of experimental structural information about the binding mode of **LCRF-0004** within the RON and/or c-Met kinase domains, we deemed important to synthesize and evaluate few constrained analogs of **LCRF-0004** to elucidate its bioactive conformation when bound in the active site of RON enzyme. Thus, we evaluated new bicyclic head groups (Fig. 1, Part C and Fig. 2), which are believed to be key motifs for the observed selectivity over c-Met. Herein, we describe our efforts in the design and synthesis of small molecules with a fused bicyclic head group as rigidified analogs of **LCRF-0004**. These rigidified analogs demonstrated high potency for the inhibition of RON tyrosine kinase with various levels of selectivity against c-Met tyrosine kinase.

To further support the predicted binding mode of **LCRF-0004** in the enzymatic site of RON, we synthesized compound **1**[10] in which we blocked the possibility of forming an intramolecular hydrogen bond, which potentially rigidifies the conformation by forming a seven-membered cyclic structure, as illustrated below in **LCRF-0004** (Fig. 2). Indeed the presence of the methyl amide probably should prevent the molecule to adopt a favorable bioactive conformation creating a 'clash' between the methyl and the trifluoromethyl substituents. The equilibrium is therefore shifted from the *syn*-**1** conformation to a more favorable *trans*-**1** from the energy point of view (Fig. 2). Consequently, the lowest energy conformation may not be favorable in the active site of the kinase domain, which should result in a decrease in the inhibitory potency of this molecule. Thus, when tested in enzymatic assays, compound **1** is 57 times less active in inhibiting RON and 481 times less active in inhibiting c-Met over **LCRF-0004** (Table 1). This result reinforces our hypothesis regarding the bioactive conformation of **LCRF-0004**.

The synthesis of new analogs is described as follows: Compound **4** was prepared in two steps from the known intermediate **2**[11] after acylation with chloroacetyl chloride followed by intramolecular

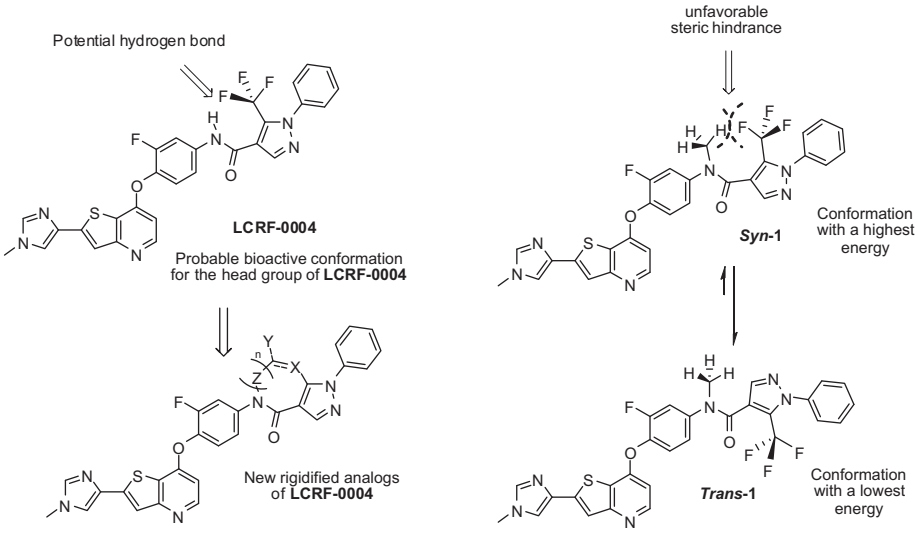


Figure 2. Possible head group conformations of **LCRF-0004** and compound **1**.