Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Design, synthesis and RON receptor tyrosine kinase inhibitory activity of new head groups analogs of LCRF-0004

Franck Raeppel, Stéphane L. Raeppel *, Eric Therrien †

*Laboratoires ChemRF Inc./ChemRF Laboratories Inc., 3194 rue Claude-Jodoin, Montréal, QC H1Y 3M2, Canada*

ARTICLE INFO

*Article history:*
Received 19 June 2015
Revised 21 July 2015
Accepted 23 July 2015
Available online xxxx

*Keywords:*
RON
c-Met
RTK inhibitors
Head group
Molecular docking
RON homology model
Oncology

ABSTRACT

New heteroarylcarboxamide head groups substituted with two aromatic rings analogs of thieno[3,2-*b*]pyridine-based kinase inhibitor **LCRF-0004** were designed and synthesized. Potent inhibitors of RON tyrosine kinase with various level of selectivity for c-Met RTK 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 cells. Activation of RON RTK by MSP triggers downstream signaling pathways (β-catenin, PI3K/Akt, MAPK, NF-κB and STAT3) which mediate a number of biological events including macrophage activity and tissue repair, and epithelial cell behavior (cell growth, motility, and epithelial to mesenchymal transition). 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 interest as promising therapeutic targets for cancer therapy. Importantly, overexpression of RON in tumor tissues correlates with increased metastasis and poor prognosis in human 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 we have shown in a recent study that it was possible to replace the pyrazole head group of **LCRF-0004**,[7] known to be a potent and selective RON RTK inhibitor, by different five-membered heterocycles such as pyrrole, imidazole and triazole (Fig. 1, structure **A**)[8] without loss of RON enzyme inhibitory activity. In a second study, we have disclosed that it was also possible to replace 1-phenyl-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxamide head group of **LCRF-0004** by a fused bicyclic lactam head group (Fig. 1, structure **B**)[9] as mimetic of its potential bioactive conformation.

In our first study,[8] our design was guided by molecular docking study using an X-ray crystal structure of c-Met kinase domain (PDB: 3U6I)[10] which shares a high sequence homology with RON. The docking of **LCRF-0004** using the Fitted program[11] revealed a class II extended conformation with the head group deeply engaged in the hydrophobic back pocket of the enzyme. A probable intramolecular hydrogen bond between a fluorine atom from the trifluoromethyl group and the N*H*-acidic carboxamide seems to rigidify the head group in the desired bioactive conformation (Fig. 2). By scrutinizing carefully this predicted binding mode we deemed it possible to replace the trifluoromethyl substituent from the pyrazole head group by something bulkier and potentially with a modulated electrostatic environment. In order to validate this

* Corresponding author at present address: SagitheR Consulting, 2620 rue de l'Étrier, Saint-Lazare, QC J7T 3L8, Canada.
*E-mail address:* stephane.raeppel@gmail.com (S.L. Raeppel).
† Present address: Molecular Forecaster Inc., 969 rue Marc-Aurèle Fortin, Laval, QC H7L 6H9, Canada.

http://dx.doi.org/10.1016/j.bmcl.2015.07.080
0960-894X/

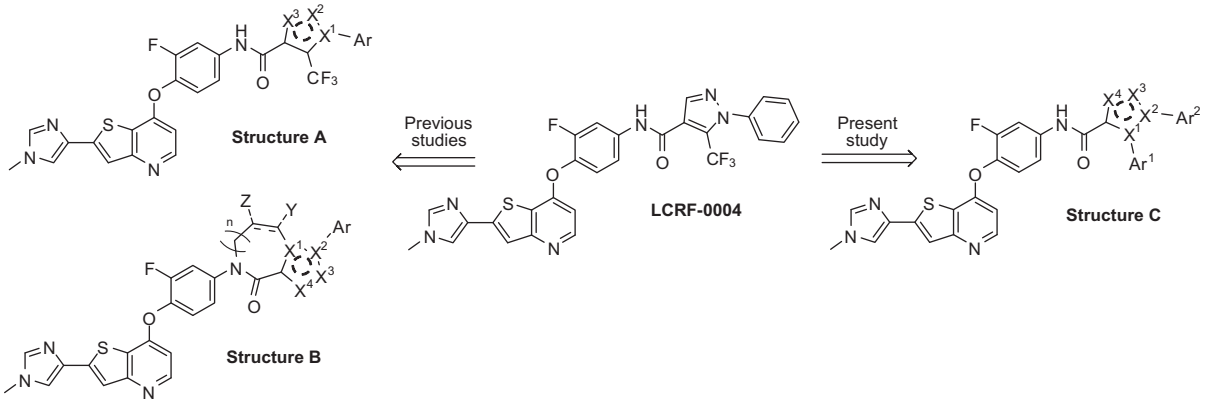



**Figure 1.** Structure of **LCRF-0004** and generic structures for new analogs (**A**, **B** and **C**).

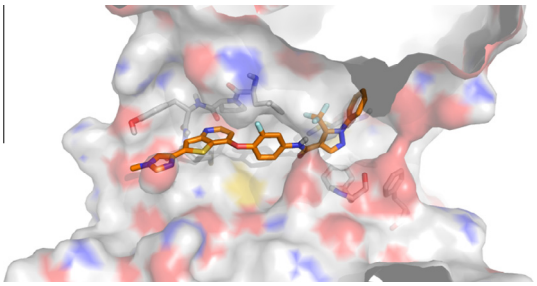
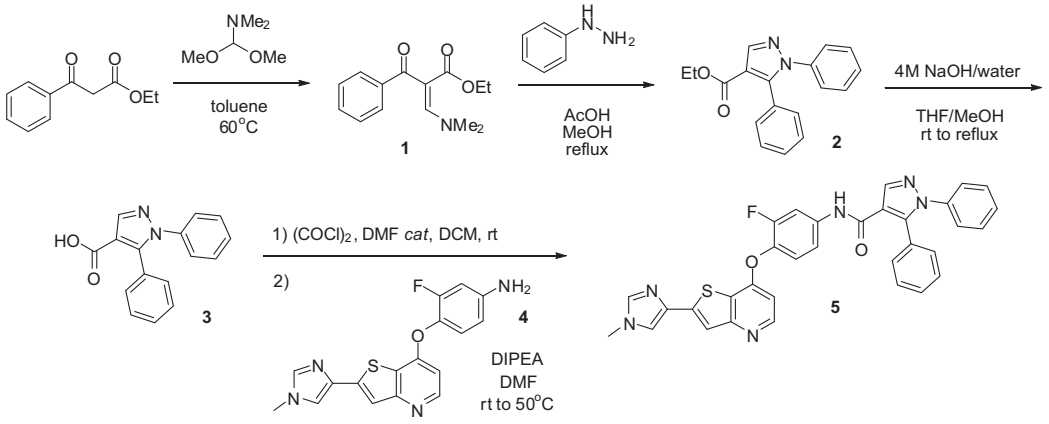


**Figure 2.** Docking pose of **LCRF-0004** in c-Met kinase domain.

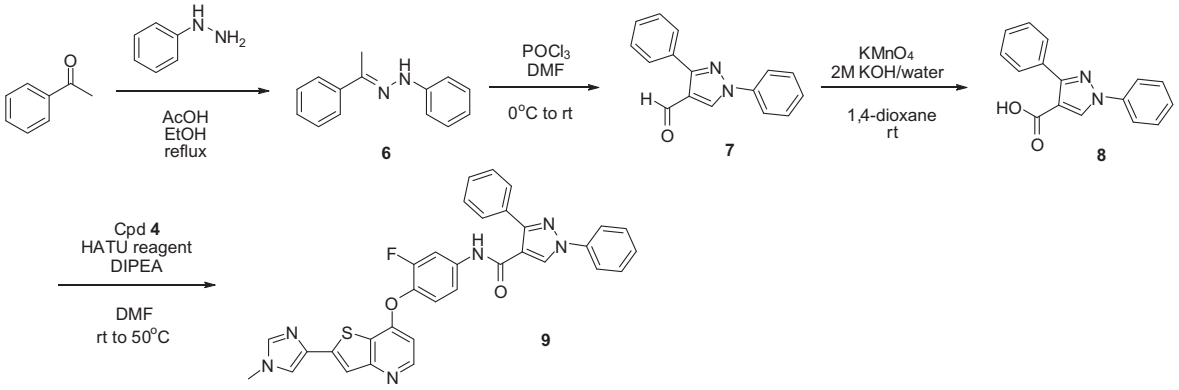
hypothesis, we describe herein our efforts in the design and synthesis of small molecules with new heteroarylcarboxamide head groups substituted with two aromatic rings (Fig. 1, structure **C**) as analogs of **LCRF-0004**. These new compounds demonstrated high potency for the inhibition of RON tyrosine kinase with various levels of selectivity for c-Met RTK.

The synthesis of new analogs is described as follows: compound **5** was prepared in ten steps from commercially available starting materials (Scheme 1). 1,5-Diphenyl-1*H*-pyrazole-4-carboxylic acid **3** was first prepared in three steps from ethyl 3-oxo-3-phenylpropanoate by an enamine formation, followed by a condensation with phenyl hydrazine and a saponification reaction. After acid chloride formation of intermediate **3** and amide coupling with known aniline **4**,[12] the desired final compound **5** was obtained with a 1,5-diphenyl-1*H*-pyrazole-4-carboxamide head group. Compound **9** was obtained in four steps from acetophenone and intermediate **4** (Scheme 2). Thus, condensation of phenyl hydrazine with acetophenone to produce hydrazone **6** followed by a Vilsmeier–Haack reaction gave rise to 1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **7**.[13] Oxidation of the formyl substituent of compound **7** by potassium permanganate under aqueous alkaline conditions[14] provided carboxylic acid **8**. Amide coupling between intermediates **4** and **8** afforded compound **9** having a 1,3-diphenyl-1*H*-pyrazole-4-carboxamide head group. Compound **13** was obtained in four steps from ethyl 3-oxo-3-phenylpropanoate and intermediate **4** (Scheme 3). Ethyl 3-oxo-3-phenylpropanoate was first transformed into the activated tosyloxy derivative **10** by the action of Koser's reagent (HITB), then treated with thiobenzamide, under Hantzsch conditions, to afford thiazole **11**[15] and finally saponified to afford 2,4-diphenylthiazole-5-carboxylic acid **12**. Amide coupling between intermediates **4** and **12** provided compound **13** having a 2,4-diphenylthiazole-5-carboxamide head group. Compound **19** was obtained in six steps from 2-bromo-1-phenylethanone and intermediate **4** (Scheme 4). 2-Bromo-1-phenylethanone was first alkylated by potassium 2-cyano-1-ethoxyethenolate formed in situ, cyclized under acidic conditions to form the pyrrole ring and submitted to a palladium-catalyzed dehalogenation reaction under hydrogen atmosphere to provide ethyl 5-phenyl-1*H*-pyrrole-3-carboxylate **16**.[16] After copper-catalyzed N-arylation[17] of pyrrole **16** and a saponification reaction, 1,5-diphenyl-1*H*-pyrrole-3-carboxylic acid **18** was obtained. Amide coupling between intermediates **4** and **18** provided the final compound **19** having a 1,5-diphenyl-1*H*-pyrrole-3-carboxamide head group.

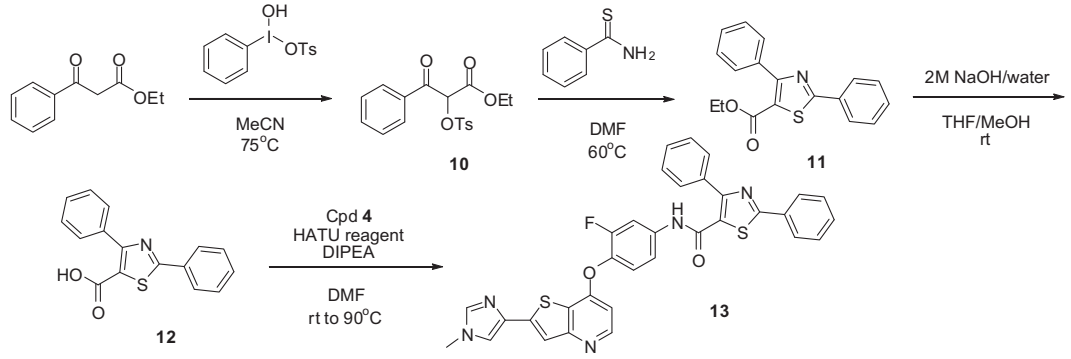
Compounds having a 1,5-bis-aryl-1*H*-pyrazole-4-carboxamide head group were prepared using the following general synthesis[18] (Scheme 5). Methyl or ethyl aryl ester was reacted with lithium



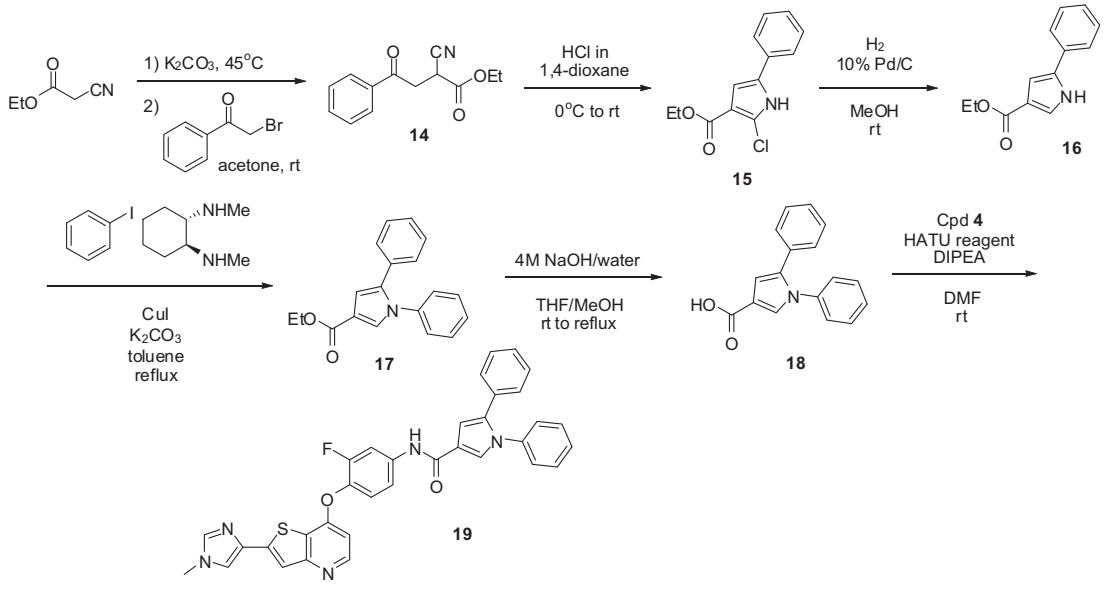

**Scheme 1.** Synthesis of compound **5**.




**Scheme 2.** Synthesis of compound **9**.




**Scheme 3.** Synthesis of compound **13**.

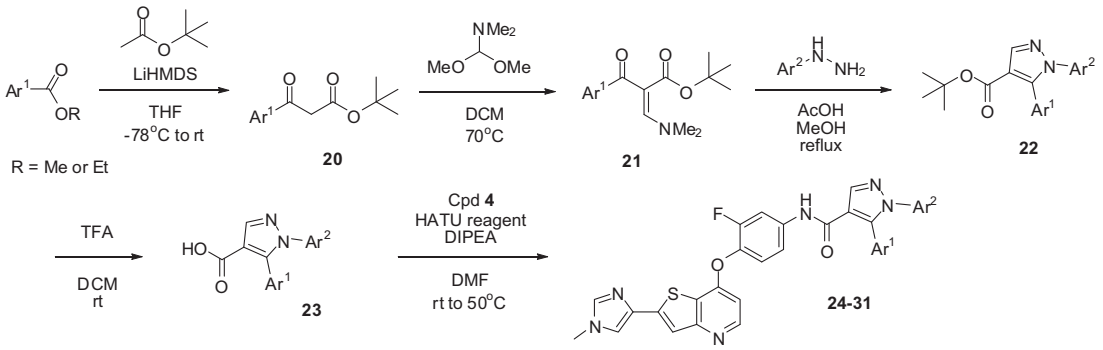



**Scheme 4.** Synthesis of compound **19**.

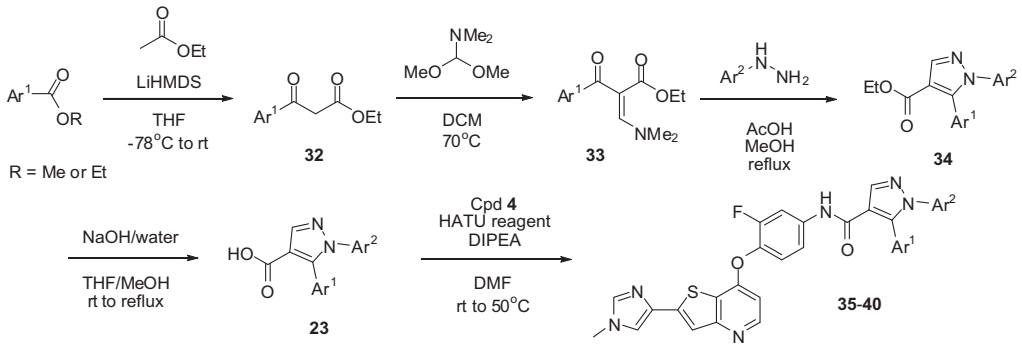
1-*tert*-butoxyethenolate prepared in situ, followed by the enamine formation and a condensation reaction with phenyl hydrazine. After acidic *tert*-butyl ester hydrolysis and amide coupling with aniline **4**, final compounds **24**–**31** were isolated in pure form after flash chromatography purification under normal and/or reverse phases.

An alternative synthesis is described below which was used for the preparation of new analogs featuring an acid sensitive 1, 5-bis-aryl-1*H*-pyrazole-4-carboxylic acid **23** such as $Ar^1$ = 2-thienyl, 3-thienyl, 2-furyl, 5-methylisoxazol-3-yl, 4-nitrophenyl, and 4-acetamidophenyl, and $Ar^2$ = phenyl (Scheme 6). Methyl or ethyl aryl ester was reacted with lithium 1-ethoxyethenolate prepared in situ, followed by the enamine formation and a condensation reaction with phenyl hydrazine. After saponification and amide coupling with aniline **4**, final compounds **35**–**40** were isolated in pure form after flash chromatography purification under normal and/or reverse phases.

In this present study, our design was guided by molecular docking studies using an homology model of RON kinase domain built from the X-ray crystal structure of c-Met kinase domain (PDB: 3U6I)[10] (Fig. 3). The docking using the Fitted program[11] of




**Scheme 5.** General synthesis of compounds **24**–**31**.




**Scheme 6.** Alternative synthesis of compound **35**–**40**.

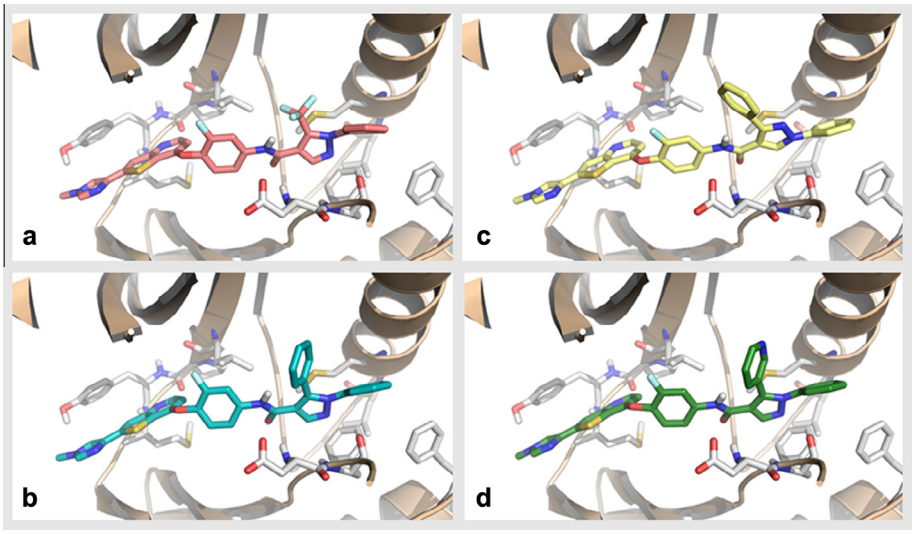
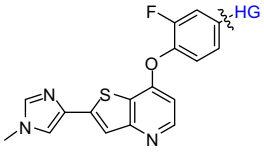


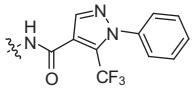
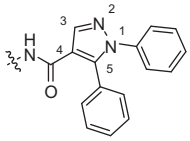
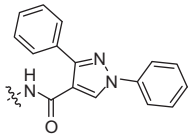
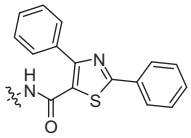
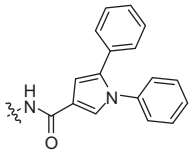

**Figure 3.** Docking poses in RON kinase domain homology model. (a) **LCRF-0004**; (b) compound **5**; (c) compound **9**; (d) compound **28**.

**LCRF-0004** in the model of the active site of RON showed a predicted docking score of −24.6 versus −23.1 for compound **5**. Predicted docking scores for compounds **9** and **28** were −22.4 and −22.6, respectively.

As previously disclosed, the presence of both trifluoromethyl and phenyl substituents on the pyrazole head group of **LCRF-0004** is essential for the inhibition of RON activity.[7] In the present study, we decided to explore the replacement of the bulky and electro-negative trifluoromethyl moiety by a phenyl ring and to study its influence when transposed around the heteroaryl head group as well. At first, to verify our hypothesis compound **5** was prepared as a prototype in this new chemical series (Table 1). Although twice less active against the inhibition of RON tyrosine kinase when compared to **LCRF-0004**, this compound turned out to be more active for the inhibition of phospho-c-Met in MKN-45 gastric cancer cells (c-Met RTK is present in full-length and is over-expressed). On the other hand, moving the phenyl substituent on position 3 instead of position 5 on the pyrazole ring of compound **5**, such as in compound **9**, starts to be detrimental for RON inhibition. Likewise, replacing the pyrazole by a thiazole ring, such as in compound **13** or moving the phenyl substituent on position 2, such as in pyrrole **19**, reinforces this trend, probably due to steric hindrances. Thus, the three substituents on the heteroaryl head group shall be contiguous in order to respect the 5-atom linker characteristic[19] and based on this exploratory work we decided to further investigate close analogs of compound **5**.

**Table 1**
Enzymatic and cellular inhibitory activities of **LCRF-0004** and bis-aryl compounds



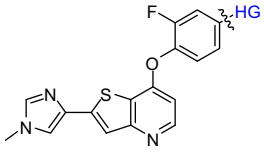

| Compds | Head group (HG) | RON (h) kinase $IC_{50}$ (μM) | Phospho-c-Met in MKN-45 $IC_{50}$ (μM) |
|---|---|---|---|
| **LCRF-0004** |  | 0.012 | 6.87 |
| **5** |  | 0.023 | 0.74 |
| **9** |  | 0.032 | 2.07* |
| **13** |  | 0.045 | 2.26* |
| **19** |  | 0.101 | 4.84* |

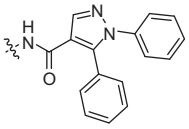
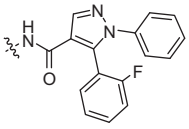
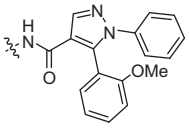
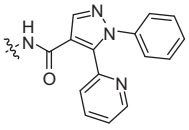
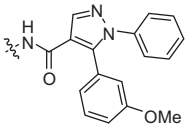
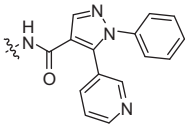
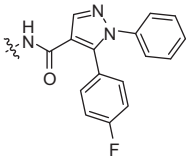
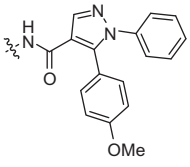
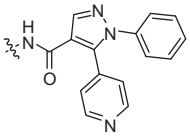
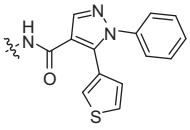
* 'Plateau' was observed.

As shown in Table 2, substituents in *ortho* or *meta* positions on the phenyl ring or heteroaryls ($Ar^1$) are well accepted and have only a slight impact on the inhibition of RON tyrosine kinase. Indeed, compound **28** having a 3-pyridinyl ring as a replacement of the trifluoromethyl substituent is equipotent to **LCRF-0004**. Moreover, compound **28** is a highly potent inhibitor of c-Met (h) kinase domain with an $IC_{50}$ = 0.010 μM. This result was translated into the inhibition of phospho-c-Met in MKN-45 cell-based assay with an $IC_{50}$ = 0.23 μM. Likewise, compound **27** showed a high potency against c-Met (h) kinase domain with an $IC_{50}$ = 0.007 μM. In addition, compounds **27** and **28** exhibit good inhibitory activity against the phosphorylation of RON in HT29 colon cancer cells (preliminary data). Although, compound **29** ($Ar^1$ = 4-fluorophenyl) and compound **36** ($Ar^1$ = 3-thienyl, as bio-isostere of phenyl ring) proved to be quite potent in our phospho-c-Met cell-based assay, compounds **30** and **31** turned out to be inactive. This result could be explained by a possible clash between the *para*-methoxy substituent of **30** and an electrostatic repulsion between 4-pyridinyl of **31** in the active site of c-Met kinase domain, respectively.

In conclusion, we have designed and synthesized new potent inhibitors of RON based on **LCRF-0004** by replacing its trifluoromethyl substituent with an aromatic ring. Furthermore, this new search allowed us to study the effect of various heteroarylcarboxamide head groups[20] substituted with two aromatic rings on the RON inhibitory activity and selectivity over c-Met. Thus, potent dual RON/c-Met inhibitors were discovered, and these new chemical agents may be preferable over specific agents, especially when both RTKs are co- and overexpressed in cancer patients. Future works will optimize these new head groups and explore the other parts of **LCRF-0004** as well, which should allow us to further modulate and optimize the in vitro and in vivo activities and DMPK

**Table 2**
Enzymatic and cellular inhibitory activities of new bis-aryl compounds




| Compds | Head group (HG) | RON (h) kinase $IC_{50}$ (μM) | Phospho-c-Met in MKN-45 $IC_{50}$ (μM) |
|---|---|---|---|
| **5** |  | 0.023 | 0.74 |
| **24** |  | 0.025 | 1.09 |
| **25** |  | n.a. | 0.35 |
| **26** |  | 0.023 | 1.23 |
| **27** |  | 0.027 | 0.30 |
| **28** |  | 0.014 | 0.23 |
| **29** |  | n.a. | 0.19 |
| **30** |  | n.a. | >10 |
| **31** |  | n.a. | >10 |
| **36** |  | n.a. | 0.34 |

n.a. (data not available).

profile of our inhibitors. More studies from our academic collaborators using **LCRF-0004** as a pharmacological tool for the drug target validation of RON will be also disclosed soon.[21]

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16. Compound **16** was prepared according to Arikawa, Y.; Nishida, H.; Kurasawa, O.; Hasuoka, A.; Hirase, K.; Inatomi, N.; Hori, Y.; Matsukawa, J.; Imanishi, A.; Kondo, M.; Tarui, N.; Hamada, T.; Takagi, T.; Takeuchi, T.; Kajino, M. *J. Med. Chem.* **2012**, *55*, 4446.
17. Reaction conditions performed according to Antilla, J. C.; Baskin, J. M.; Barder, T. E.; Buchwald, S. L. *J. Org. Chem.* **2004**, *69*, 5578.
18. Carboxylic acid **23** were prepared according to US 2005/0080087 A1.
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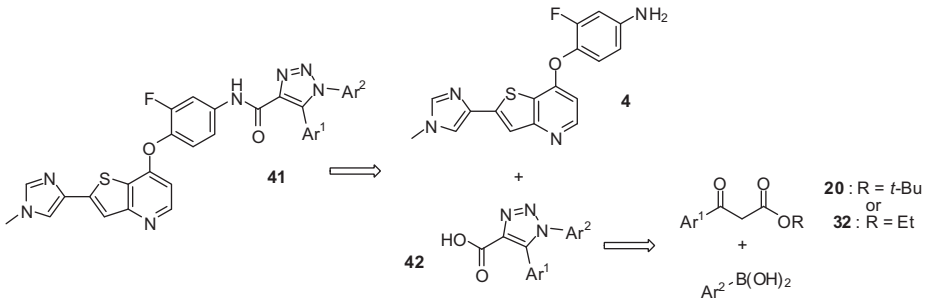
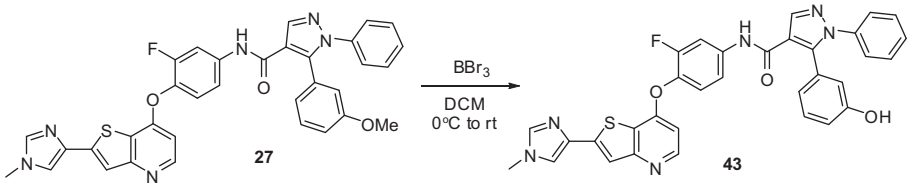
20. During this study we have also synthesized a new series of compounds (**41**) with a bis-aryl triazole head group via the retrosynthetic scheme described below. Carboxylic acids **42** were prepared in two or three steps after β-keto ester synthesis (intermediates **20** or **32**, if not commercially available), triazole ring formation (reaction conditions performed according to Zhang, J.; Jin, G.; Xiao, S.; Wu, J.; Cao, S. *Tetrahedron* **2013**, *69*, 2352) and ester hydrolysis (acidic or basic conditions).




During this study, we have also prepared compounds of generic structure C (Fig. 1) in which $Ar^1$ = hydroxyarene (e.g., phenol, pyridone, hydroxypyridine, etc.) and $Ar^2$ = phenyl by a demethylation reaction of the corresponding methoxy substituent using $BBr_3$ reagent. See one example illustrated below:

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