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Design and synthesis of close analogs of LCRF-0004, a potent and selective RON receptor tyrosine kinase inhibitor

Stéphane L. Raeppel *, Franck Raeppel, Eric Therrien

ChemRF Laboratories, 3194 rue Claude-Jodoin, Montréal, QC H1Y 3M2, Canada

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ABSTRACT

New carboxamide head group analogs of thieno[3,2-*b*]pyridine-based kinase inhibitor **LCRF-0004** were designed and synthesized. Potent and selective inhibitors of RON enzyme versus c-Met RTK were obtained.

The receptor tyrosine kinase RON (Récepteur d'Origine Nantais, also known as human MST1R, for macrophage stimulating 1 receptor) is the cell surface and transmembrane receptor with a cytoplasmic tyrosine kinase domain for its extracellular endogenous ligand MSP (for macrophage stimulating protein, also known as HGFL, for hepatocyte growth factor-like). RON belongs to the MET proto-oncogene family and shares significant structural and functional homology with c-Met RTK, and is normally expressed at low level in epithelial cells. The MSP/RON signaling pathway is involved in several important biological processes, including macrophage activity, wound healing, and epithelial cell behavior. Deregulation of RON has been described in numerous types of cancers[1] including colorectal,[2] breast,[3] lung,[4] pancreas,[5] prostate[6] 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.

Metastases in the body are the main cause of death for cancer patients. A recent study has further shown that MSP/RON axis facilitates tumor metastasis by suppressing host antitumor immunity.[7] The MSP/RON signaling pathway favors the conversion of micrometastatic lesions to overt metastases by suppressing antitumor immune responses. However, the loss of RON functions in the host promotes an effective antitumor $CD8^+$ T-cell (lymphocytes) response, hence inhibiting the outgrowth of metastatic cancer cells. Consequently, RON inhibitors may potentially prevent the outgrowth of micrometastases in cancer patients. A new study has reported an epigenetic reprogramming pathway that is required for breast cancer metastasis.[8] Concerted differential DNA methylation is initiated by the activation of the RON receptor tyrosine kinase by its ligand MSP. Analysis of human breast cancers revealed that this epigenetic program is significantly associated with poor clinical outcome. Furthermore, inhibition of RON kinase activity with a pharmacological agent blocks metastasis of patient-derived breast tumor grafts in vivo.

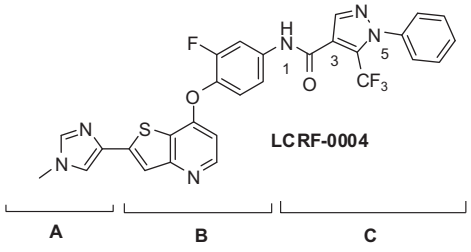

Figure 1. Structure of **LCRF-0004**.

As part of our internal research program focusing on the discovery of novel agents to fight and/or control the proliferation of metastatic cancers and metastasis, preliminary and new data were collected using **LCRF-0004** (Fig. 1) known to be a potent

* Corresponding author. Tel.: +1 514 244 1681.
E-mail address: stephane.raeppel@gmail.com (S.L. Raeppel).

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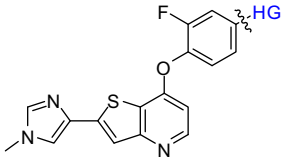
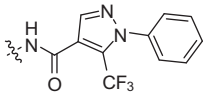
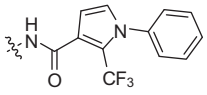
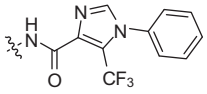
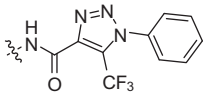
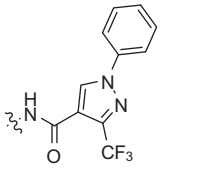
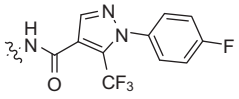
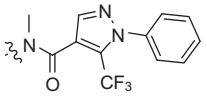
0960-894X/

Table 1
Enzymatic and cellular inhibitory activities of **LCRF-0004** and close analogs



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
6		0.010	n.a.
10		0.013	2.40*
13		0.017	0.67
16		0.095	3.77*
17		0.044	4.37*
18		0.580	n.a.

* 'Plateau' was observed; n.a. (data not available).

and selective inhibitor of RON receptor tyrosine kinase, reported by MethylGene.[9] Low nanomolar biological activity of **LCRF-0004** was determined (IC_{50} = 12 nM) in a RON kinase domain enzymatic assay (Table 1).[10] Furthermore, the selectivity of **LCRF-0004** for RON over c-Met was confirmed in c-Met- and RON-driven cellular assays. High micromolar inhibitory activities were observed on c-Met-phosphorylation in MKN-45 (gastric carcinoma, IC_{50} = 6.87 μM) cell line. On the other hand, **LCRF-0004** potently inhibited the RON-phosphorylation in the low nanomolar range in PC-3 (prostate cancer, IC_{50} = 30-40 nM)[9] and HT-29 (colon cancer, IC_{50} = 30 nM)[10] cancer cell lines where RON is overexpressed. These results demonstrate that this compound is selective for RON RTK versus c-Met RTK and penetrates into cancer cells. Moreover, **LCRF-0004** showed potent antiproliferative activities against HCT116 (colon carcinoma, IC_{50} = 0.08 μM), MDAMB-231 (breast adenocarcinoma, IC_{50} = 0.10 μM), and A549 (lung cancer, IC_{50} = 0.10 μM) cancer cell lines using MTT assays,[10] but we currently do not know by what exact mechanism takes place this biological effect. Through an academic collaboration, more advanced studies were conducted which further confirmed that targeting the kinase domain of the RON receptor with **LCRF-0004** is an effective interventional strategy in malignant pleural mesothelioma (MPM, H226 cancer cell line).[11] Asbestos exposure is the principal etiological agent of MPM cancer type.[12]

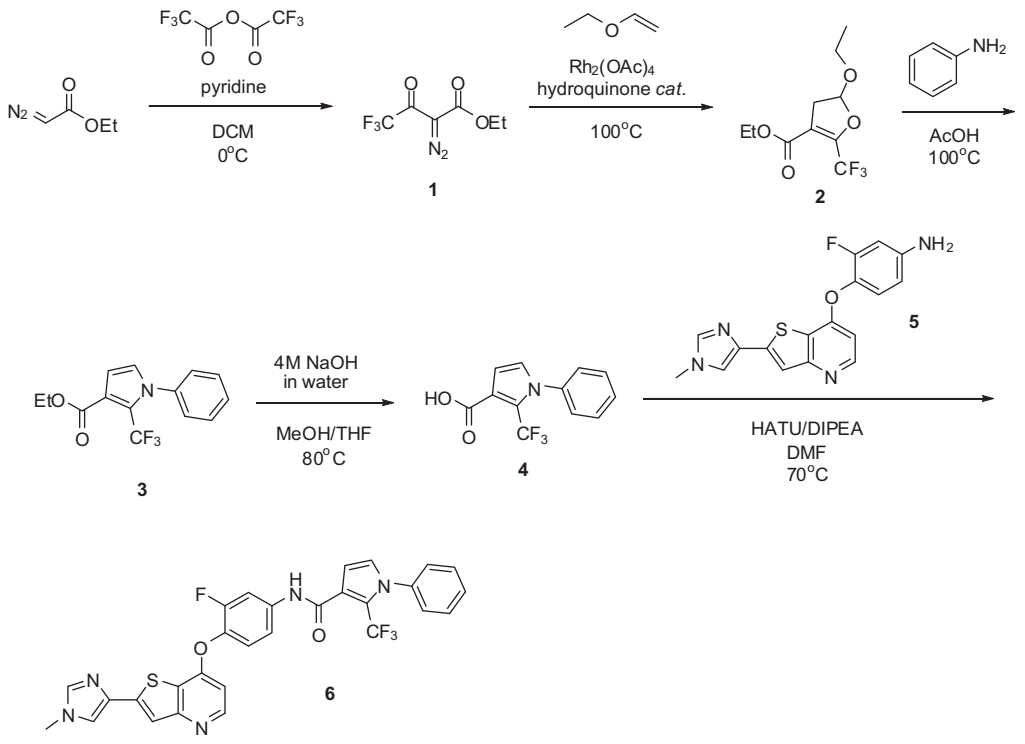
Deprived of structural information about the binding of **LCRF-0004** within the RON enzyme, we deemed important to synthesize and evaluate close analogs of **LCRF-0004** to gain insights about this inhibitor and establish structure activity relationships (SAR). As a first step, we decided to screen various head groups (Fig. 1, Part C), as this section of the molecule is believed to be the key motif for the observed selectivity over c-Met. Herein, we describe our efforts in the synthesis of small molecules, close analogs of **LCRF-0004**, as potent and selective inhibitors of RON tyrosine kinase versus c-Met RTK.

The synthesis of new analogs is described as follows: 1-phenyl-2-(trifluoromethyl)-1*H*-pyrrole-3-carboxylic acid **4** was prepared in 4 steps from ethyl 2-diazoacetate via ethyl 5-ethoxy-2-(trifluoromethyl)-4,5-dihydrofuran-3-carboxylate **2**.[13] Amide coupling between intermediates **4** and **5**[14] gave access to compound **6** with a pyrrole head group (Scheme 1). 1-Phenyl-5-(trifluoromethyl)-1*H*-imidazole-4-carboxylic acid **9** was prepared in 3 steps from aniline via ethyl 1-phenyl-5-(trifluoromethyl)-1*H*-imidazole-4-carboxylate **8**.[15] Amide coupling between intermediates **9** and **5** afforded compound **10** with an imidazole head group (Scheme 2). 1-Phenyl-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxylic acid **12** was prepared in 2 steps from phenylboronic acid via ethyl 1-phenyl-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxylate **11**.[16] Amide coupling between intermediates **12** and **5** provided compound **13** with a triazole head group[17] (Scheme 3).

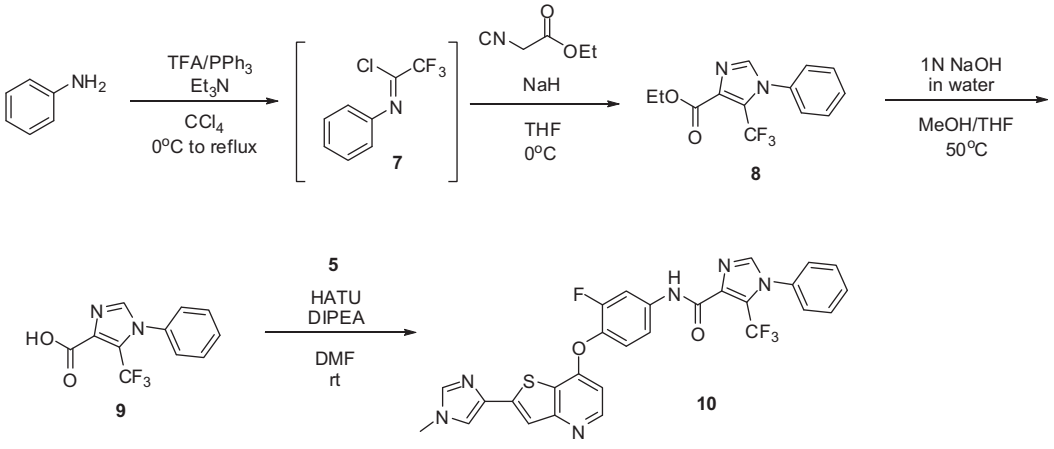
1-Phenyl-3-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid **15** was prepared in 2 steps from ethyl 5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylate via ethyl 1-phenyl-3-(trifluoromethyl)-1H-pyrazole-4-carboxylate **14**.[18] Amide coupling between intermediates **15** and **5** gave rise to compound **16** having a pyrazole isomer head group (Scheme 4). Amide coupling between 1-(4-fluorophenyl)-5-(trifluoromethyl)-1*H*-pyrazole-4-carboxylic acid and **5** afforded compound **17** with a *para*-substituted phenyl head group (Scheme 5). Amide methylation of **LCRF-0004** provided compound **18** with a tertiary amide linker connecting the head group to the central phenyl ring (Scheme 5).

Our design was guided by molecular docking studies using an X-ray crystal structure of c-Met kinase domain (PDB: 3U6I)[19] which shares a high sequence homology with RON. The docking of **LCRF-0004** using the Fitted program[20] revealed a class II extended conformation with the head group (Part C) deeply engaged in the hydrophobic back pocket of the enzyme interacting with the backbone N*H* of Asp1222. A possible intramolecular hydrogen bond between one fluorine atom from the trifluoromethyl group and the NH-acidic carboxamide of **LCRF-0004** may occur to form a 7-membered ring. The thieno[3,2-*b*]pyridine scaffold (Part B) interacts with the hinge binding domain (N*H* of Met1160) and 1-methyl-1*H*-imidazole substituent (Part A) is positioned in the solvent-exposed area (Fig. 2).

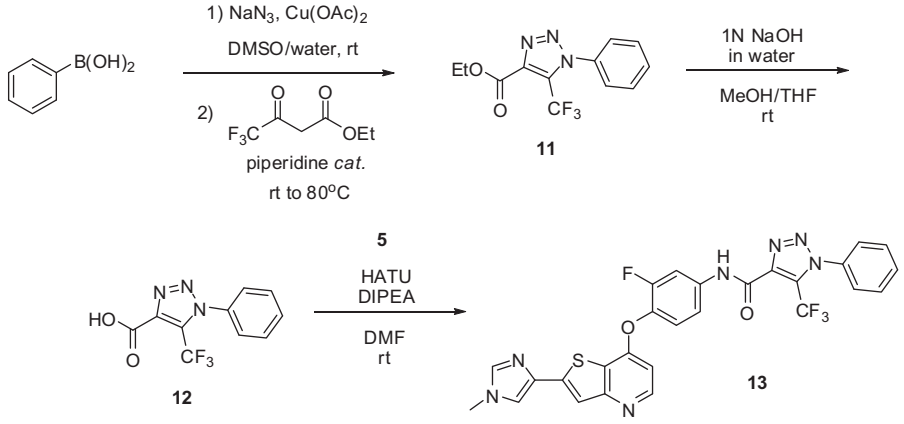
As previously disclosed, the presence of trifluoromethyl and phenyl substituents on the pyrazole head group of **LCRF-0004** is essential for the inhibition of RON activity.[9] In the present study, we demonstrated that it is possible to replace the pyrazole head group of **LCRF-0004** by other five-membered heterocycles such as pyrrole (**6**), imidazole (**10**), and triazole (**13**) without loss of activity for the RON enzyme inhibition. These new analogs are equipotent when compared to **LCRF-0004**, paving the way for more innovation (Table 1). On the other hand, some activity for c-Met starts to appear in MKN-45 cell-based assay in which c-Met RTK is overexpressed (e.g. compound **13** versus **LCRF-0004**). However, moving the phenyl substituent on the adjacent nitrogen of the pyrazole ring of **LCRF-0004** such as in compound **16** is detrimental for the RON inhibition. The three substituents on the pyrazole head group shall be contiguous in order to respect the 5-atom



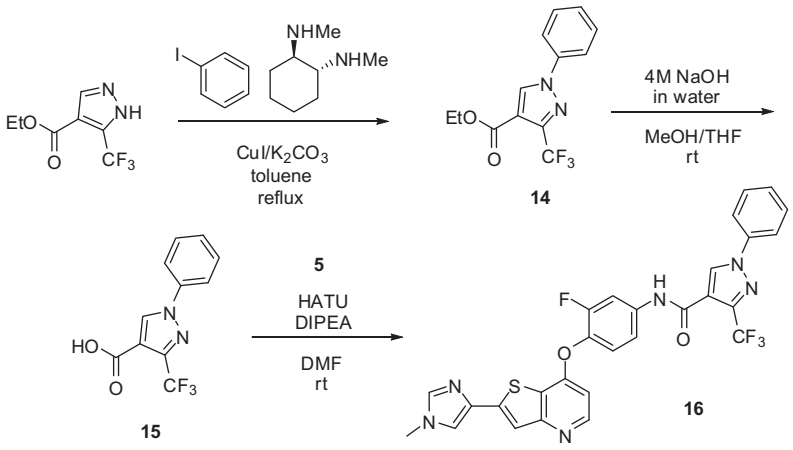
Scheme 1. Synthesis of compound **6**.



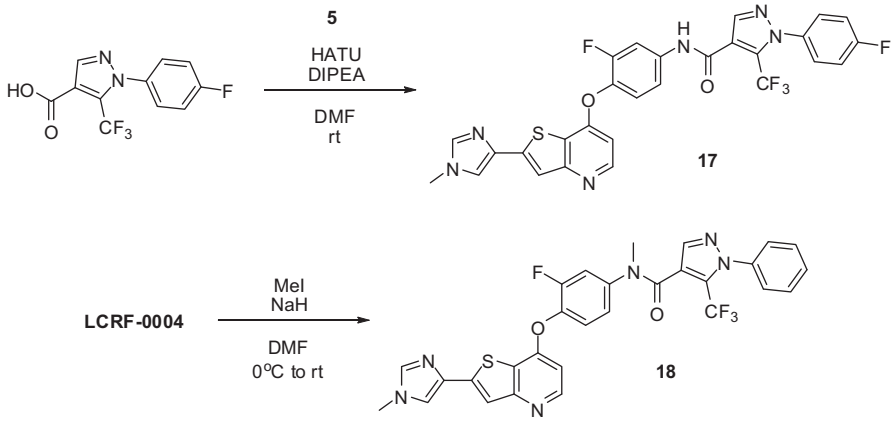
Scheme 2. Synthesis of compound **10**.



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



Scheme 4. Synthesis of compound **16**.



Scheme 5. Synthesis of compounds **17** and **18**.

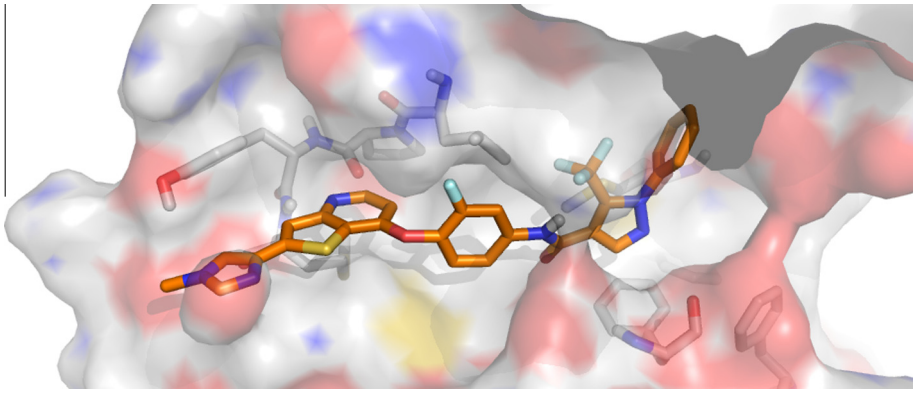


Figure 2. Docking pose of **LCRF-0004** in c-Met kinase domain.

linker characteristic[21] (Fig. 1). Likewise; introduction of a small substituent (fluorine) in *para* position of the phenyl ring such as in compound **17** has also a slight negative impact. The same trend was observed in a similar series with a different Part A. This effect was less pronounced with a substituent in *ortho* or *meta* positions.[10] Finally, introduction of a tertiary amide linker such as in compound **18** has a dramatic effect on the RON inhibition and this compound is 48-fold less active when compared to **LCRF-0004**. The presence at this position of a hydrogen bond donor (N*H* secondary amide bond) seems to be essential for the activity. Thus, compound **18** cannot adopt an 'ideal' bioactive conformation as **LCRF-0004** and/or very close analogs (e.g. compounds **6**, **10** and **13**) are able to do so, when bound in the catalytic kinase domain of RON.

In conclusion, we have designed and synthesized new potent inhibitors of RON based on **LCRF-0004**. Furthermore, this new search allowed us to study the effect of various head groups on the RON activity and selectivity over c-Met. Based on these results, we were able to design and synthesize two new series of potent RON inhibitors that will be disclosed in due course. Future work will explore parts A and B of **LCRF-0004** (Fig. 1) which should allow

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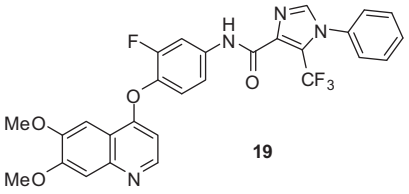
us to further modulate and optimize the in vitro and in vivo activities and DMPK profile of our inhibitors. More studies from our academic collaborators using **LCRF-0004** as a pharmacological tool for the drug target validation of RON will be also disclosed soon.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.04.056.

References and notes

1. (a) Yao, H.-P.; Zhou, Y.-Q.; Zhang, R.; Wang, M.-H. *Nat. Rev. Cancer* **2013**, *13*, 466; (b) Wang, M.-H.; Zhang, R.; Zhou, Y.-Q.; Yao, H.-P. *J. Biomed. Res.* **2013**, *27*, 1; (c) Wang, X.; Hankey, P. A. *Crit. Rev. Immunol.* **2013**, *33*, 549; (d) Benight, N. M.; Waltz, S. E. *Expert Opin. Ther. Targets* **2012**, *16*, 921; (e) Wagh, P. K.; Peace, B. E.; Waltz, S. E. *Adv. Cancer Res.* **2008**, 1; (f) Benvenuti, S.; Comoglio, P. M. *J. Cell Physiol.* **2007**, *213*, 316.
2. Tarang, S.; Wang, J. *Open Colorectal Cancer J.* **2012**, *5*, 15.
3. Kretschmann, K. L.; Eyob, H.; Buys, S. S.; Welm, A. L. *Curr. Drug Targets* **2010**, *11*, 1157.
4. (a) Kanteti, R.; Krishnaswamy, S.; Catenacci, D.; Tan, Y.-H. C.; El-Hashani, E.; Cervantes, G.; Husain, A. N.; Tretiakova, M.; Vokes, E. E.; Huet, H.; Salgia, R. *Gene. Chromosomes Cancer* **2012**, *51*, 841; (b) Han, W.-L.; Li, W.-D.; Hu, J.; Rusidanmu, A.; Chen, L.-F.; Shen, L.; Zheng, S.-S. *Chin. Med. J.* **2012**, *125*, 1110.
5. Kang, C. M.; Babicky, M. L.; Lowy, A. M. *Pancreas* **2014**, *43*, 183.
6. Gurusamy, D.; Gray, J. K.; Pathrose, P.; Kulkarni, R. M.; Finkleman, F. D.; Waltz, S. E. *Cancer Res.* **2013**, *73*, 1752.
7. Eyob, H.; Ekiz, H. A.; DeRose, Y. S.; Waltz, S. E.; Williams, M. A.; Welm, A. L. *Cancer Discov.* **2013**, 751.
8. Cunha, S.; Lin, Y.-C.; Goossen, E. A.; DeVette, C. I.; Albertella, M. R.; Thomson, S.; Mulvihill, M. J.; Welm, A. L. *Cell Rep.* **2014**, *6*, 141.
9. Raeppel, S.; Gaudette, F.; Mannion, M.; Claridge, S.; Saavedra, O.; Isakovic, L.; Déziel, R.; Beaulieu, N.; Beaulieu, C.; Dupont, I.; Nguyen, H.; Wang, J.; Macleod, A. R.; Maroun, C.; Besterman, J. M.; Vaisburg, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2745.
10. Unpublished results.
11. Baird, A. -M.; O'Byrne, K.; Easty, D.; Shiels, L.; Byrne, A.; Raeppel, S.; Soltermann, A.; Nonaka, D.; Fennell, D.; Mutti, L.; Pass, H.; Opitz, I.; Gray, S. 'The RON (MST1R)/MSP pathway is a potential therapeutic target in malignant pleural mesothelioma', 15th World Conference on Lung Cancer [P1.06-055 (Poster session)], 27–30 October 2013, Sydney, Australia.
12. Nowak, A. K. *Ann. Cardiothorac. Surg.* **2012**, *1*, 508.
13. Compound **2** was prepared according to Hoffmann, M. G.; Wenkert, E. *Tetrahedron* **1993**, *49*, 1057.
14. Compound **5** was prepared according to WO **2006**/010264 A1.
15. Compound **8** was prepared according to Huang, W. S.; Yuan, C. Y.; Wang, Z. Q. *J. Fluorine Chem.* **1995**, *74*, 279. During this study we have also synthesized compound **19**.



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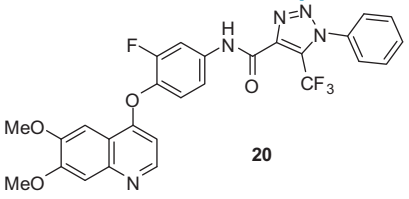
Compd **19**: RON (h) kinase: IC_{50} = 0.016 μM; Phospho-c-Met in MKN-45: IC_{50} = 1.06 μM.

16. Compound **11** was prepared according to Zhang, J.; Jin, G.; Xiao, S.; Wu, J.; Cao, S. *Tetrahedron* **2013**, *69*, 2352.
17. Compounds with 1-(substituted)-phenyl-5-(trifluoromethyl)-1*H*-1,2,3-triazole-4-carboxamide head group having a quinoline scaffold instead of thieno[3,2-*b*]pyridine was recently published as c-Met inhibitors by Zhou, S.; Liao, H.; Liu, M.; Feng, G.; Fu, B.; Li, R.; Cheng, M.; Zhao, Y.; Gong, P. *Bioorg. Med. Chem.* **2014**, *22*, 6438. During this study we have also synthesized compound **20** before the above-mentioned reference became public



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18. Compound **14** was prepared according to Antilla, J. C.; Baskin, J. M.; Barder, T. E.; Buchwald, S. L. *J. Org. Chem.* **2004**, *69*, 5578.
19. Liu, L.; Norman, M. H.; Lee, M.; Xi, N.; Siegmund, A.; Boezio, A. A.; Booker, S.; Choquette, D.; D'Angelo, N. D.; Germain, J.; Yang, K.; Yang, Y.; Zhang, Y.; Bellon, S. F.; Whittington, D. A.; Harmange, J.-C.; Dominguez, C.; Kim, T.-S.; Dussault, I. *J. Med. Chem.* **1868**, *2012*, 55.
20. (a) Corbeil, C. R.; Englebienne, P.; Moitessier, N. *J. Chem. Info. Model.* **2007**, *47*, 435; (b) Corbeil, C. R.; Moitessier, N. *J. Chem. Info. Model.* **2009**, *49*, 997.
21. (a) Tang, Q.; Zhao, Y.; Du, X.; Chong, L.; Gong, P.; Guo, C. *Eur. J. Med. Chem.* **2013**, *69*, 77; (b) Tang, Q.; Zhang, G.; Du, X.; Zhu, W.; Li, R.; Lin, H.; Li, P.; Cheng, M.; Gong, P.; Zhao, Y. *Bioorg. Med. Chem.* **2014**, *22*, 1236.