Bioorganic & Medicinal Chemistry Letters 20 (2010) 3216–3218

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of a new potent bisamide FMS kinase inhibitor

Mohammed I. El-Gamal [a,b], Myung-Ho Jung [a], Chang-Hyun Oh [a,b,*]

[a] Biomaterials Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea
[b] Department of Biomolecular Science, University of Science and Technology, 113 Gwahangno, Yuseong-gu, Daejeon 305-333, Republic of Korea

ARTICLE INFO

Article history:
Received 16 January 2010
Revised 8 April 2010
Accepted 20 April 2010
Available online 24 April 2010

Keywords:
FMS
KDR
Tyrosine kinase
Kinase inhibitor
Antiinflammatory
Cancer
Selectivity
Bisamide

ABSTRACT

FMS is a type III receptor tyrosine kinase that binds to the macrophage or monocyte colony stimulating factor (M-CSF or CSF-1). Signal transduction through that binding results in survival, proliferation, and differentiation of monocyte/macrophage lineage. In this study, we report the discovery of a new potent inhibitor of FMS kinase. The synthesized pyrrolo[3,2-*c*]pyridine derivative (compound **1**) was initially tested at a single concentration of 1 μM against 47 different kinases. At this concentration, the% inhibitions of the enzymatic activities of FMS and KDR kinases were 90% and 71%, respectively, while the inhibition in activity was less than 58% for all of the other kinases. For compound **1**, the IC_{50} values against FMS and KDR were 96 and 1058 nM, respectively. So, compound **1** was found to be 11 times more selective for FMS kinase than KDR kinase. Compound **1** can be used as a promising lead for the development of new selective inhibitors of FMS kinase, which can be used as useful therapeutic tools for treatment of several inflammatory and cancer disorders.

The macrophage or monocyte colony stimulating factor (M-CSF or CSF-1) is the primary growth factor for the macrophage lineage.[1] It interacts with the cells through its specific trans-membrane receptor FMS (also known as CSF-1R)—a type III receptor tyrosine kinase.[1,2] Signal transduction through the CSF-1/FMS ligand–receptor complex results in survival, proliferation, and differentiation of cells of the monocyte/macrophage lineage.[2] Over-expression of CSF-1 and/or FMS has been implicated in a number of disease states such as the growth of metastasis of particular types of cancer,[3] in promoting osteoclast proliferation in bone osteolysis,[4] in inflammatory disorders such as rheumatoid arthritis,[5] atherosclerosis,[6] and Crohn's disease,[7] and in renal allograft rejection.[8] In addition, the expression of FMS in breast cancer has been linked to poor survivability and increased tumor size, where presumably the receptor is involved in local invasion and metastasis.[9–11]

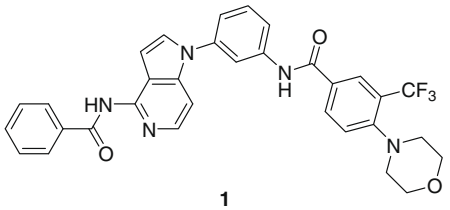
Tumor associated macrophages play a major role in the microtumor environment.[12,13]

The role of CSF-1-dependent macrophage proliferation has been evaluated by various animal studies.[14,15] CSF-1-deficient mice were reported to be resistant to collagen-induced arthritis (CIA). In a CIA model, CSF-1 was shown to increase the severity of the disease while a neutralizing anti-CSF-1 antibody had the opposite effect.

A monoclonal antibody to CSF-1 developed by Pfizer, PD-0360324, has recently entered phase 1 clinical trials for treatment of rheumatoid arthritis.[16]

Ki20227 is a urea type c-FMS tyrosine kinase inhibitor that has been reported to suppress osteoclast differentiation and osteolytic bone destruction in a bone metastasis model.[17] Imatinib mesylate, an arylamide derivative, has been recently reported to suppress bone metastases of breast cancer by inhibiting osteoclasts differentiation through blockade of c-FMS signals.[18] A number of reports have recently highlighted arylamides derivatives as potent FMS inhibitors.[19–23] Herein, we report the discovery of a potent FMS kinase inhibitor **1** with bisamide moieties (Fig. 1). The synthetic and screening protocols for this compound are illustrated in details. The kinase inhibitory activity of the synthesized compound was tested over 47 different kinases, and it showed selectivity for FMS kinase.

Synthesis of the target compound **1** was achieved according to the sequence illustrated in Scheme 1. 7-Hydroxy-1*H*-pyrrolo[2,3-*b*]pyridinium 3-chloroperoxybenzoate (**3**) was prepared by reacting 1*H*-pyrrolo[2,3-*b*]pyridine (**2**) with 3-chloroperoxybenzoic

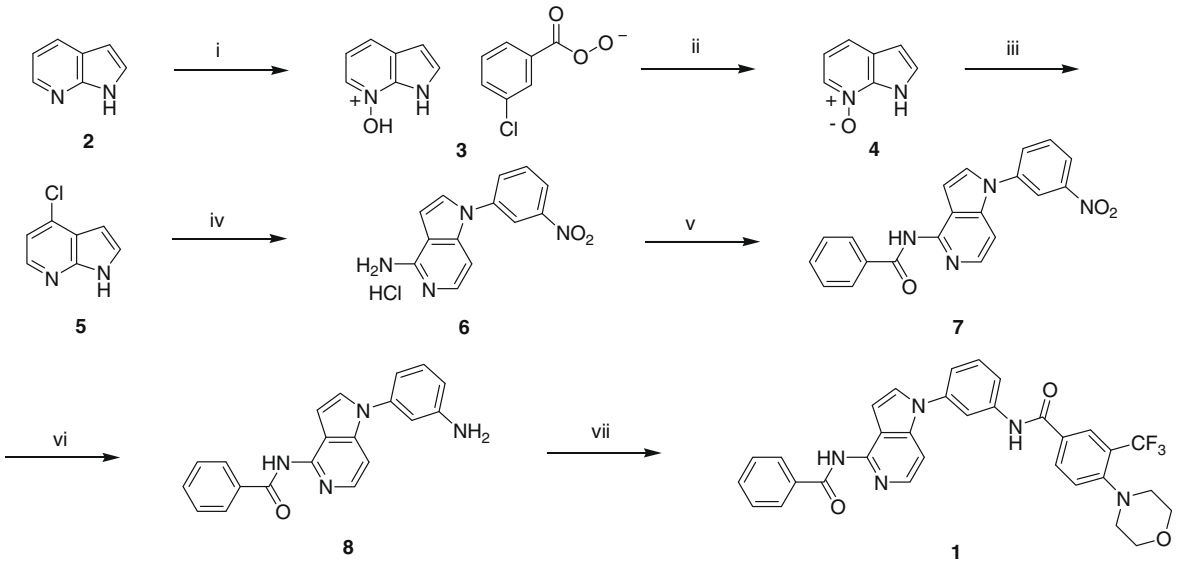


1

Figure 1. Structure of compound **1**.

* Corresponding author. Tel.: +82 2 958 5160; fax: +82 2 958 5189.
E-mail address: choh@kist.re.kr (C.-H. Oh).

0960-894X/$ - see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2010.04.088



Scheme 1. Reagents and conditions: (i) 3-chloroperoxybenzoic acid, DME/heptanes (1:2), 81%; (ii) K_2CO_3, H_2O, $CHCl_3$, 43%; (iii) CH_3SO_2Cl, DMF, 65 °C, 59%; (iv) *m*-nitroaniline, 180 °C, 10%; (v) benzoyl chloride, diisopropylamine, CH_3CN, 15%; (vi) Pd/C, H_2, THF, 32%; (vii) 3-(trifluoromethyl)-4-morpholinobenzoic acid, HOBt, EDCI, TEA, DMF, 80 °C, 16%.

acid.[24,25] Compound **3** was converted into the N-oxide compound **4** by reaction with potassium carbonate.[24,25] 4-Chloro-1*H*-pyrrolo[2,3-*b*]pyridine (**5**) was prepared by heating **4** with methanesulfonyl chloride in dry DMF. Compound **6** was prepared according to the method described by Girgis et al.[26] Heating **5** with *m*-nitroaniline led to nucleophilic displacement of the 4-chloro group by the *m*-nitroaniline amino group, followed by rearrangement of the resulting secondary amine to give the hydrochloride salt of 4-amino-1-(3-nitrophenyl)-1*H*-pyrrolo[3,2-*c*]pyridine (**6**), albeit in low yield. The amide derivative **7** was obtained by reaction of the amino group of **6** with benzoyl chloride in the presence of diisopropylamine as a base. Reduction of the nitro group of **7** using Pd–C/H_2 gave the corresponding amino compound **8**. The bisamide target compound **1** was obtained by condensation with 3-(trifluoromethyl)-4-morpholinobenzoic acid using HOBt/EDCI/TEA.

The screening results of the target compound **1** over 47 different kinases have revealed that the inhibitory activity of the compound was not exhibited over most of the tested kinases, while high inhibitory activity was selectively shown at FMS kinase only (Table 1).[27]

The compound was tested initially at a single dose concentration of 1 μM. At this concentration, 90% inhibition of the enzymatic activity of FMS kinase was observed. It also inhibited the enzymatic activity of KDR kinase by 71%. While the inhibition in activity was less than 58% in all of the other kinases, it was in the range of 30–57% in four kinases only (ABL1, FYN, LCK, and RET), and in the range of 20–30% in another four kinases only (Aurora A, IKKβ, PYK2, and SAPK2a).

Compound **1** was further tested over FMS and KDR kinases in order to determine its IC_{50}, where a 10-doses IC_{50} mode with threefold serial dilutions starting at 2 μM concentration was applied. Table 2 illustrates the IC_{50} values of compound **1** over FMS and KDR kinases. Compound **1** was found to be 11 times more selective for FMS kinase than KDR kinase.

The selectivity of compound **1** to FMS may be attributed to increased bulkness around the pyrrolo[3,2-*c*]pyridine moiety. Compound **1** includes two arylamide functionalities. This increase in bulkness is seemed to hinder the fitting of the compound to the binding sites of most of other kinases and to exclude it from their binding pockets. The difference in geometry between the binding

Table 1
Percentages of enzymatic inhibitions exerted by compound **1**[28] on 47 kinases

Kinase enzyme	% Inhibition[a–c]
ABL1	41
ALK	11
Aurora A	21
BTK	7
CaMKI	−1
CDK2/cyclinE	−12
CDK5/p25	8
CHK1	−8
c-Kit (D816V)	−2
c-SRC	10
DMPK	−18
EGFR (T790M)	1
EPHA1	9
FGFR1	6
FLT3 (D835Y)	16
FMS	90
FYN	44
GSK3β	4
HIPK2	9
IGF-1R	10
IKKβ	23
IR	2
JAK3	17
JNK1α1	−1
KDR	71
LCK	33
MAPK1	−9
MEK1	12
MET	−16
MLK1	−1
mTOR	−10
P70S6K	7
PAK2	2
PDK1	−1
PIM1	10
PKA	16
PKBα	18
PKCα	−5
PLK1	4
PYK2	23
RET	57
ROCK1	3
ROS/ROS1	19
RSK1	8
SAPK2a	25
SYK	6
TEC (activated)	14

[a] Test compound was used in a single dose concentration of 1 μM.
[b] 100% Activity refers to enzyme activity in negative control (DMSO).
[c] % Inhibition was calculated by subtracting% activity from 100.

Table 2
IC_{50} of compound **1** over FMS and KDR kinases

Kinase enzyme	IC_{50} (nM)
FMS	96
KDR	1058

pocket of FMS tyrosine kinase and other kinases may enable the selectivity of compound **1** for FMS. Moreover, the presence of two hydrogen bond donors and many hydrogen bond acceptors may enhance the selectivity of the compound for FMS kinase.

In conclusion, a new potent inhibitor for FMS kinase has been synthesized and can be used as a promising lead for the development of new selective inhibitors of FMS kinase, which can be used as useful therapeutic tools for treatment of several inflammatory and cancer disorders.

Acknowledgment

We are grateful to Korea Institute of Science and Technology (KIST) for financial support.

References and notes

1. Sherr, C. J. *Biochim. Biophys. Acta* **1988**, *948*, 225.
2. Woolford, J.; Rothwell, V.; Rohrschneider, L. *Mol. Cell. Biol.* **1985**, *5*, 3458.
3. Baiocchi, G.; Kavanagh, J. J.; Talpaz, M.; Wharton, J. T.; Gutterman, J. U.; Kurzrock, R. *Cancer* **1991**, *67*, 990.
4. El Hajj Dib, I.; Mélanie, G.; Valery, S.; Romuald, M.; Michel, B.; Sa, K. *Leuk. Res.* **2008**, *32*, 1279.
5. Ohno, H.; Uemura, Y.; Murooka, H.; Takanashi, H.; Tokieda, T.; Ohzeki, Y.; Kubo, K.; Serizawa, I. *Eur. J. Immunol.* **2008**, *38*, 283.
6. Inaba, T.; Gotoda, T.; Harada, K.; Shimada, M.; Ohsuga, J.; Ishibashi, S.; Yazaki, Y.; Yamada, N. *J. Clin. Invest.* **1995**, *95*, 1133.
7. Marshall, D.; Cameron, J.; Lightwood, D.; Lawson, A. D. G. *Inflamm. Bowel Dis.* **2007**, *13*, 219.
8. Wyburn, K. R.; Jose, M. D.; Wu, H.; Atkins, R. C.; Chadban, S. J. *Transplantation* **2005**, *80*, 1641.
9. Kluger, H. M.; Dollad-Filhart, M.; Rodov, S.; Kacinski, B. M.; Camp, R. L.; Rimm, D. L. *Clin. Cancer Res.* **2004**, *10*, 173.
10. Lin, E. Y.; Nguyen, A. V.; Russell, R. G.; Pollard, J. W. *J. Exp. Med.* **2001**, *193*, 727.
11. Yee, L. D.; Liu, L. *Anticancer Res.* **2000**, *20*, 4379.
12. Lewis, C. E.; Pollard, J. W. *Cancer Res.* **2006**, *66*, 605.
13. Sica, A.; Rubino, L.; Mancino, A.; Larghi, P.; Porta, C.; Rimoldi, M.; Solinas, G.; Locati, M.; Allavena, P.; Mantovani, A. *Expert Opin. Ther. Targets* **2007**, *11*, 1219.
14. Campbell, I. K.; Rich, M. J.; Bischof, R. J.; Hamilton, J. A. *J. Leukocyte Biol.* **2000**, *68*, 144.
15. Cook, A. D.; Braine, E. L.; Campbell, I. K.; Rich, M. J.; Hamilton, J. A. *Arthritis Res.* **2001**, *3*, 293.
16. Dose-escalating multiple dose study of PD-0360324 in combination with methotrexate in patients with rheumatoid arthritis. ClinicalTrials.gov Identifier NCT00550355.
17. Ohno, H.; Kubo, K.; Murooka, H.; Kobayashi, Y.; Nishitoba, T.; Shibuya, M.; Yoneda, T.; Isoe, T. *Mol. Cancer Ther.* **2006**, *5*, 2634.
18. Hiraga, T.; Nakamura, H. *Int. J. Cancer* **2009**, *124*, 215.
19. Patch, R. J.; Brandt, B. M.; Asgari, D.; Baindur, N.; Chadha, N. K.; Georgiadis, T.; Cheung, W. S.; Petrounia, I. P.; Chaikin, M. A.; Player, M. R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6070.
20. Illig, C. R.; Chen, J.; Wall, M. J.; Wilson, K. J.; Ballentine, S. K.; Rudolph, J. M.; DesJarlais, R. L.; Chen, Y.; Schubert, C.; Petrounia, I. P.; Crysler, C. S.; Molloy, C. J.; Chaikin, M. A.; Manthey, C. L.; Player, M. R.; Tomczuk, B. E.; Meegalla, S. K. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1642.
21. Irvine, K. M.; Burns, C. J.; Wilks, A. F.; Su, S.; Hume, D. A.; Sweet, M. J. *FASEB J.* **2006**, *20*, 1921.
22. Meegalla, S. K.; Wall, M. J.; Chen, J.; Wilson, K. J.; Ballentine, S. K.; DesJarlais, R. L.; Schubert, C.; Crysler, C. S.; Chen, Y.; Molloy, C. J.; Chaikin, M. A.; Manthey, C. L.; Player, M. R.; Tomczuk, B. E.; Illig, C. R. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3632.
23. Burns, C. J.; Harte, M. F.; Bu, X.; Fantino, E.; Giarrusso, M.; Joffe, M.; Kurek, M.; Legge, F. S.; Razzino, P.; Su, S.; Treutlein, H.; Wan, S. S.; Zeng, J.; Wilks, A. F. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1206.
24. Wang, X.; Zhi, B.; Baum, J.; Chen, Y.; Crockett, R.; Huang, L.; Eisenberg, S.; Ng, J.; Larsen, R.; Martinelli, M.; Reider, P. *J. Org. Chem.* **2006**, *71*, 4021.
25. Cheng, C.-C.; Chang, C.-P.; Yu, W.-S.; Hung, F.-T.; Liu, Y.-I.; Wu, G.-R.; Chou, P.-T. *J. Phys. Chem. A* **2003**, *107*, 1459.
26. Girgis, N. S.; Larson, S. B.; Robins, R. K.; Cottam, H. B. *J. Heterocycl. Chem.* **1989**, *26*, 317.
27. Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. *Nat. Biotechnol.* **2008**, *26*, 127.
28. *Preparation of compound* **1**: A mixture of compound **8** (17.0 mg, 0.05 mmol), 3-(trifluoromethyl)-4-morpholinobenzoic acid (28.5 mg, 0.1 mmol), HOBt (15.4 mg, 0.11 mmol), and EDCI (24.83 mg, 0.13 mmol) in dry DMF (1.0 mL) was cooled to 0 °C under nitrogen atmosphere. To the reaction mixture, TEA (0.02 mL, 0.01 mmol) was added at 0 °C. The mixture was then stirred at 80 °C for 12 h. The reaction mixture was cooled and then partitioned between water and ethyl acetate and the organic layer was separated. The aqueous layer was then extracted with ethyl acetate and the combined organic extracts were washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexanes–ethyl acetate 1:1 v/v) to yield compound **1** as a yellow crystalline solid (5.0 mg, 16%); mp 130–132 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.85 (br s, 1H), 10.65 (br s, 1H), 8.28–8.27 (m, 1H), 8.13–8.09 (m, 4H), 7.88–7.83 (m, 1H), 7.75 (d, J = 3.3 Hz, 2H), 7.67–7.54 (m, 6H), 7.41–7.37 (m, 1H), 6.69–6.67 (m, 1H), 3.75–3.72 (m, 4H), 2.97–2.89 (m, 4H); IR (KBr) 3418, 2919, 1607, 1496, 1323, 1121, 850, 709 cm^{-1}; MS m/z 586.2 $(M+H)^+$.