Bioorganic & Medicinal Chemistry Letters 22 (2012) 138–143

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of potent and selective matrix metalloprotease 12 inhibitors for the potential treatment of chronic obstructive pulmonary disease (COPD)

Yuchuan Wu [a,*], Jianchang Li [a], Junjun Wu [a], Paul Morgan [a], Xin Xu [a], Fabio Rancati [b], Stefania Vallese [b], Luca Raveglia [b], Rajeev Hotchandani [a], Nathan Fuller [a], Joel Bard [a], Kristina Cunningham [a], Susan Fish [a], Rustem Krykbaev [a], Steve Tam [a], Samuel J. Goldman [a], Cara Williams [a], Tarek S. Mansour [a], Eddine Saiah [a], Joseph Sypek [a], Wei Li [a]

[a] Pfizer Global Research & Development, 200 CambridgePark Drive, Cambridge, MA 02140, USA
[b] NiKem Research S.r.L., Via Zambeletti 25, 20021 Baranzate, MI, Italy

ARTICLE INFO

Article history:
Received 25 September 2011
Revised 9 November 2011
Accepted 14 November 2011
Available online 20 November 2011

Keywords:
MMP-12
COPD
SAR
Dibenzofuran

ABSTRACT

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease associated with irreversible progressive airflow limitation. Matrix metalloproteinase-12 (MMP-12) has been characterized to be one of the major proteolytic enzymes to induce airway remodeling, destruction of elastin and the aberrant remodeling of damaged alveoli in COPD and asthma. The goal of this project is to develop and identify an orally potent and selective small molecule inhibitor of MMP-12 for treatment of COPD and asthma. Syntheses and structure–activity relationship (SAR) studies of a series of dibenzofuran (DBF) sulfonamides as MMP-12 inhibitors are described. Potent inhibitors of MMP-12 with excellent selectivity against other MMPs were identified. Compound **26** (MMP118), which exhibits excellent oral efficacy in the MMP-12 induced ear-swelling inflammation and lung inflammation mouse models, had been successfully advanced into Development Track status.

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease which is characterized by a progressive airflow limitation and associated with an abnormal inflammatory response of the lungs to noxious particles or gases, mainly caused by cigarette smoke.[1,2] This disease affects ~16 million people in the US alone[3] and ranks among the top five leading causes of death worldwide, and it is estimated that it will be in the mortality top three by 2020.[4]

The matrix metalloproteinases (MMPs) are a protein family of zinc dependent endopeptidases which can degrade a variety of matrix components in both normal physiological states and in abnormal pathological process. They are mainly involved in extracellular cleavage and play important roles in diverse biological and especially pathological process such as inflammation, fibrosis and cancer.[5] Increased MMP expression in COPD development has been demonstrated in animal studies and preliminary studies in humans. However, the poor outcomes observed in preclinical studies using broad spectrum inhibitors of MMPs[6] suggest the need for MMP inhibitors with better selectivity profile. Recently, there is increasing experimental evidence indicating that some lung diseases such as COPD, asthma and lung cancers are associated with MMP-12 mediated pathologic degradation.[7] MMP-12 is mainly produced by macrophage and involved in acute and chronic pulmonary inflammatory disease associated with intense airway remodeling. As a consequence our efforts were focused on the identification of potent and highly selective inhibitors of MMP-12.

We have recently disclosed the identification of orally efficacious MMP-12 inhibitors MMP408 and MMP145 for the potential treatment of COPD and asthma.[8,9] MMP408 in particular (compound **1**, Fig. 1) has shown good potency against hMMP-12 (IC_{50} = 2.0 nM), and excellent selectivity (>150- to ~1000-fold) over other MMPs. This compound also demonstrated oral efficacy in an hMMP-12 induced lung inflammation mouse model with a good dose response relationship.[7]

As aryl carbamate **1** moved forward in development, trace amount of its toxic aniline metabolite **2** (Fig. 1) was identified in vivo. It raised major concerns over the further progression of candidate **1** down the development pathway. As a result, we set out to make structural changes to our lead compounds that would allow us to maintain the desirable pharmacologic profile of potency and MMP-selectivity while reducing the risk of metabolic degradation to provide aniline **2**.

Several chemistry plans were proposed to address this metabolic problem. The first strategy explored toward this goal involved the synthesis of a series of compounds wherein the secondary aryl carbamate was replaced with N-linked heterocycles. The general synthetic route for this series commenced with nitration of dibenzofuran **3** using fuming HNO_3 in TFA, followed by hydrogenation in

* Corresponding author.
E-mail address: charliewu09@yahoo.com (Y. Wu).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.11.046

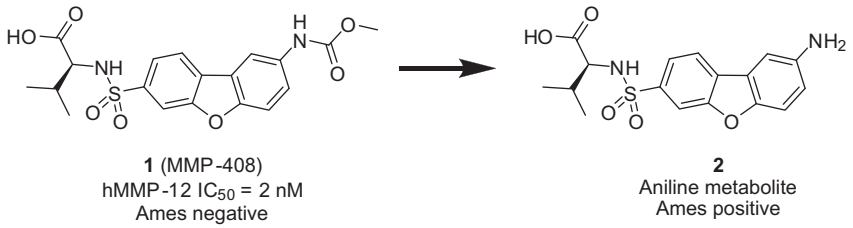
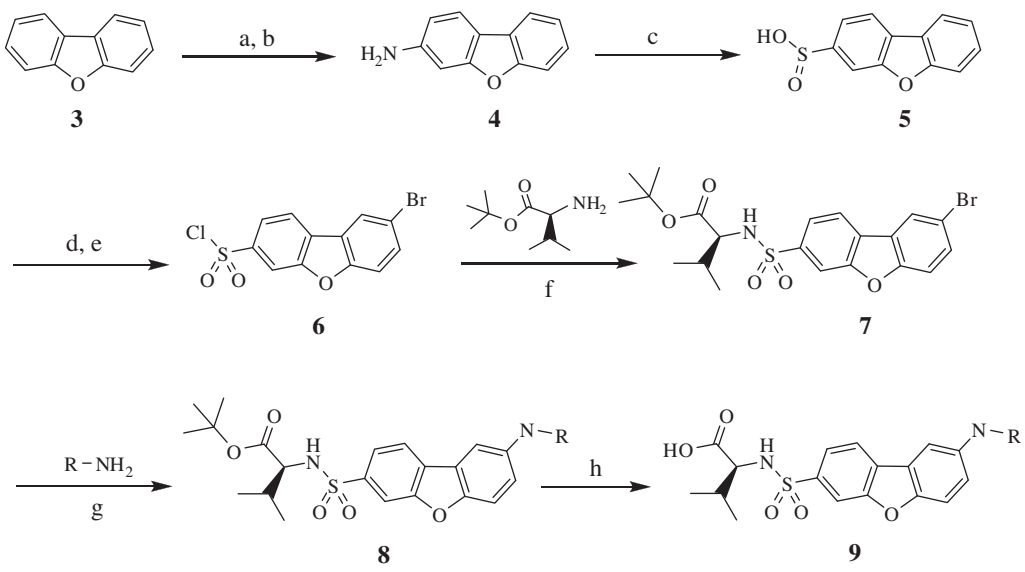
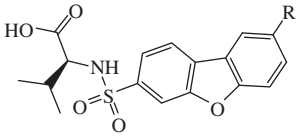


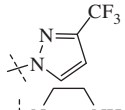
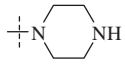
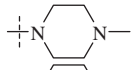
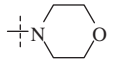
Figure 1. Metabolic pathway of compound **1**.

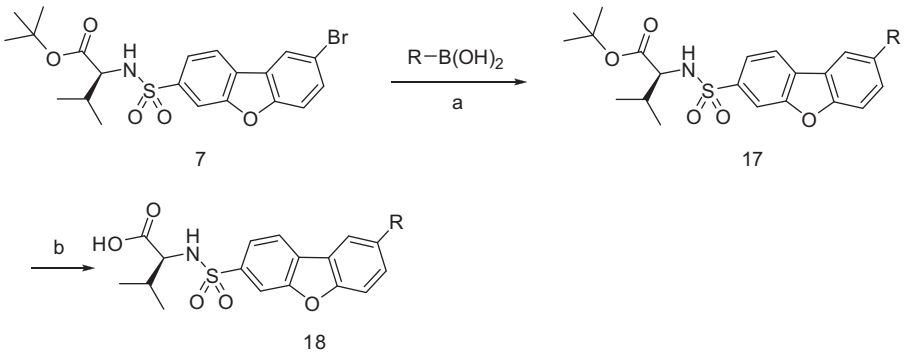


Scheme 1. Reagents and conditions: (a) HNO_3 (fuming), TFA, rt, 2 h, 70%. (b) H_2, Pd/C, MeOH, 100%. (c) (i) AcOH, HCl. $NaNO_2$, (ii) $CuCl_2$, AcOH, SO_2, 0 °C to rt, 20 h, 60%. (d) Cl_2, $AcOH/H_2O$, 0 °C to rt, 8 h, 80%. (e) Br_2, AcOH, 76 °C, 6 h, 90%. (f) *i*-Pr_2NEt, CH_2Cl_2, rt, 18 h, 95%. (g) $Pd(DBA)_2$, P(*O*-tolyl)$_3$, NaOBu, toluene, 105 °C, 18 h, 20–60%. (h) TFA, CH_2Cl_2, rt, 6 h, 70–95%.

Table 1
IC_{50} SAR summary of N-linked dibenzofuran analogs

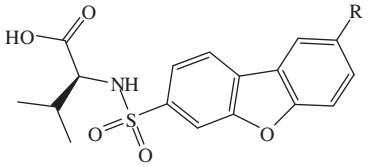


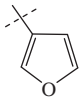
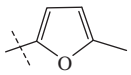
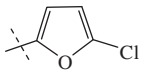
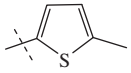
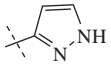
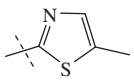
Entry	R	hMMP-12 IC_{50} (nM)	Selectivity (fold)	
			MMP-13/MMP-12	MMP-8/MMP-12
10		2.0	88	19
11		13	34	4
12		15	37	15
13		0.7	349	44
14		23	34	2
15		18	38	0.5
16		2.2	154	3



Scheme 2. Reagents and conditions: (a) $Pd(PPh_3)_4$, K_2CO_3, dioxane, microwave, 120 °C, 15 min, 60–95%. (b) TFA, CH_2Cl_2, rt, 6 h, 70–95%.

Table 2
IC_{50} SAR summary of C-linker dibenzofuran analogs



Entry	R	hMMP-12 IC_{50} (nM)	Selectivity (fold)	
			MMP-13/MMP-12	MMP-8/MMP-12
19		0.4	900	33
20		0.1	3270	300
21		0.1	1440	70
22		0.2	1690	100
23		0.2	1200	125
24		<1.0	>1130	>2500
25		0.4	525	143
26		1.0	1980	154
27		1.2	460	158

the presence of Pd/C to provide aniline **4** in quantitative yield (Scheme 1). Conversion to sulfinic acid **5** from aniline **4** took placed in two steps in 60% yield.[10] Further oxidation to the sulfonyl chloride,[11] and subsequent bromination provided aryl bromide **6**. Treatment with L-valine *tert*-butyl ester furnished the sulfonamide, and subsequent Buchwald–Hartwig[12] cross-coupling of aryl halide **7** followed by hydrolysis under acidic conditions provided the desired target compounds **9**.

The IC_{50} data of these N-heterocyclic analogs measured against human MMP-12 are shown in Table 1. In general, these analogs are potent MMP-12 inhibitors, with compound **13** reaching sub-nanomolar potency. While moderate selectivity over MMP-13 was observed (30- to 150-fold), the major shortcoming for this series of analogs is the lack of MMP-8 selectivity, a major concern based on the recent literature.[13] In addition to the poor selectivity over MMP-8, the pharmacokinetic (PK) data of N-linked heterocycle

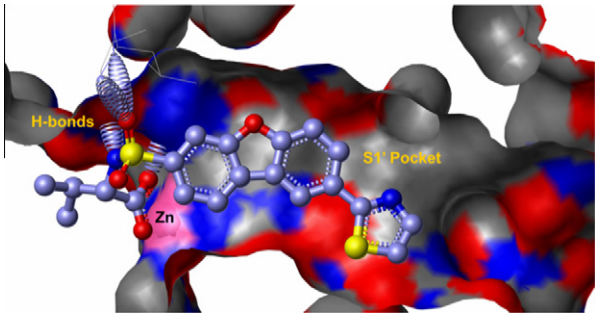


Figure 2. X-ray co-structure of compound **26**.

analogs (not shown here) were only moderate (bioavailability range of 14%–27%), the poor bioavailability may be related to their high clearances of those N-linked compounds. Although these analogs reduce the risk of metabolic degradation to the corresponding aniline, the team decided to further explore an alternative approach targeting C-linked analogs.

Preparation of the C-linker analogs was straightforward and the synthetic scheme is shown below (Scheme 2).

Suzuki–Miyaura coupling of compound **7** (from Scheme 1) with aryl bromides under microwave irradiation afforded a variety of C-linked compounds **17**,[14] which were treated with TFA to convert the *tert*-butyl ester into the desired carboxylic acids **18** in high yields.

A variety of C-linked analogs have been synthesized and the IC_{50} values of selected compounds are summarized in Table 2 below.

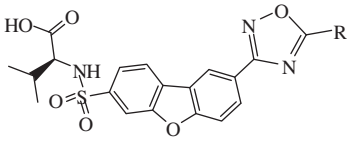
Based on the data listed in Table 2, significant improvements on potency against MMP-12 and selectivity over MMP-8 and MMP-13 have been achieved. For example, the potency of compounds **19**–**25** reached the sub-nanomolar range. As for the selectivity over MMP-13, it had been achieved greater than 1000-fold as indicated by compounds **21**, **22**, **23** and **24**. The most remarkable improvement is the selectivity over MMP-8. For the first time in our lab, selectivity over MMP-8 with more than a 100-fold is obtained (compounds **20**, **24**, **26**, and **27**).

In addition, we were able to obtain the X-ray co-crystal structure of compound **26** with MMP-12, which provides valuable information for understanding its interaction with the enzyme. As illustrated in Figure 2, the tricyclic dibenzofuran fully occupies the S1′ pocket, while the carboxylic acid coordinates with the zinc atom. The sulfone oxygen interacts with the backbone through hydrogen bonding. The specificity loops of MMPs have great similarities in size and shape, with MMP-12 having relatively small and predominantly hydrophobic pocket among those enzymes. By fine tuning the structure of the cyclic hetero-atom ring in the deep S1′ pocket, we were able to improve the potency and selectivity.

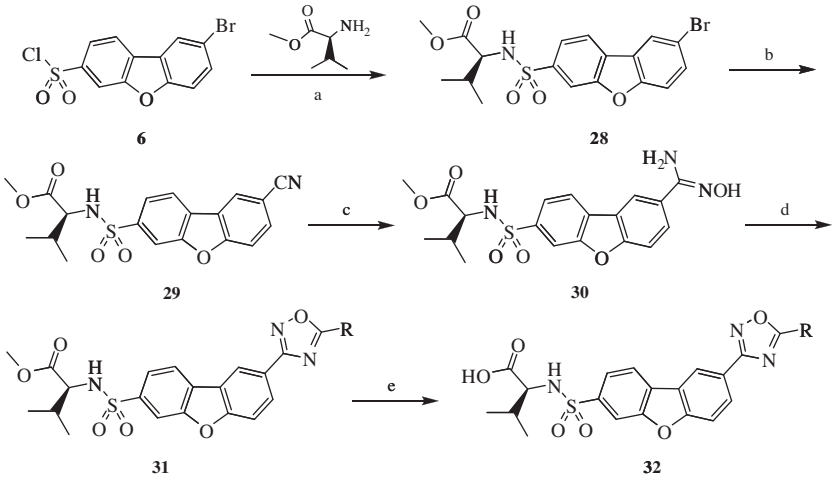
With the guidance of X-ray co-crystal structure and also based on the previous modeling, our strategy was to design and incorporate a functional group to optimize the occupancy of the S1′ pocket and hence maximize the hydrophobic interactions to gain the selectivity. It seems that small five-membered heterocyclic rings fit well in the S1′ pocket. One of our investigations was focused on oxadiazole heterocyclic ring substitutes on the DBF scaffold. The exemplary synthetic route is outlined below (Scheme 3).

The synthesis started with the intermediate **6**, which reacts with L-valine methyl ester to form sulfonamide **28** in 92%. Cyanation of the aryl bromide **28** with zinc cyanide catalyzed by $Pd(PPh_3)_4$ under microwave irradiation afforded the cyanide **29** in good yield. The compound **29** was treated with hydroxyamine in the presence of a base to produce the desired hydroxyamidine

Table 3
SAR summary of oxadiazole analogs



Entry	R	hMMP-12 IC_{50} (nM)	Selectivity (fold) MMP-8/MMP-12
33	Me	8.6	0.2
34	Et	2.9	1.4
35	*i*-Pr	4.5	19
36	*t*-Bu	17	11
37	*i*-Bu	5.4	21
38	CH_2OCH_3	1.9	10
39	CF_3	2.2	45
40	Cyclopropyl	1.6	56
41	Cyclobutyl	2.3	44
42	Cyclohexyl	7.2	92
43	Ph	4.0	60
44		0.3	100



Scheme 3. Reagents and conditions: (a) *i*-Pr_2NEt, CH_2Cl_2, rt, 18 h, 92%. (b) CuCN, $Pd(PPh_3)_4$, NMP, microwave, 120 °C, 1 h, 80%. (c) NH_2OH, TEA, rt, 6 h, 95%. (d) (i) RCOCl, DCM, 0 °C, 2 h, (ii) DMSO, 90 °C, 1 h, 75–88%. (e) LiOH, THF, water, 24 h, 60–95%.

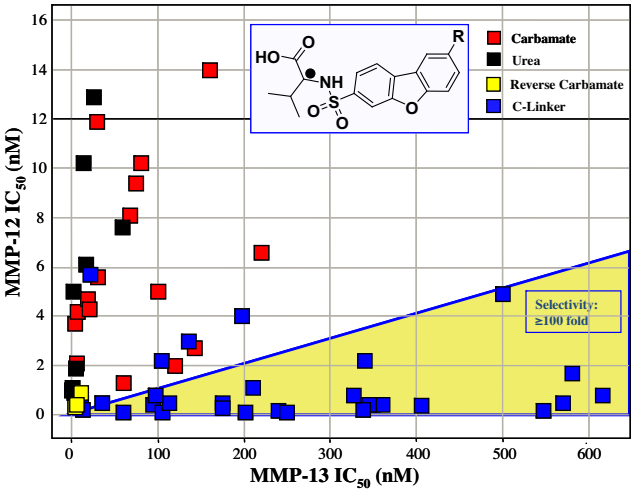


Figure 3. Comparison of N-linked compounds versus C-linked compounds.

30.[15] Formation of the oxadiazole compound **31** from **30** was carried out in DMSO at 90 °C. Saponification of **31** (LiOH in aqueous THF) afforded the final product **32** in good yield.

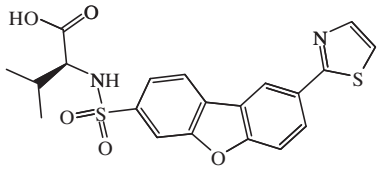
The IC_{50} values of the oxadiazole analogs are summarized in Table 3 below. These analogs are very potent MMP-12 inhibitors in general. It appears that there is a limited tolerance for the size of the R group. The enzyme can tolerate a small aliphatic chain (compounds **34**, **37**, **38**) or rings (**40**, **41**, and **42**), but, not the bulky *t*-butyl (**36** vs **37**) group. It is also notable that the oxygen-containing R groups give better potency (**38** and **44**). Selectivity over MMP-8 is moderate with only compound **44** reaching 100-fold.

To compare the selectivity between the C-linker and the N-linker compounds, the IC_{50} values of MMP-12 were plotted against those values for MMP-13 (Fig. 3 below). It is clear that the selectivity over MMP-13 for the C-linker analogs (blue rectangles) is greater than for both the carbamate (red rectangles) and the urea (black rectangles) counterparts. It is probably due to the better hydrophobic interaction of C-linked compounds.

Based on the above data and the physical properties, compound **26** was chosen as the lead for further in vitro profiling and PK studies (Table 4 below). As shown, the crystalline form of compound **26** has good aqueous solubility and exhibits negative results for the Ames and hERG tests. It has excellent selectivity over other human MMPs, which is a remarkable improvement over the N-linker series.[8,9] For example, the selectivity over MMP-13 for compound **26** has been improved to about 2000-fold comparing with only 60-fold of MMP408. Compound **26** was also profiled for cross-species MMP-12 activities. As shown at the Table 4, this compound has nearly equal potency for sheep MMP-12, while retaining a moderate potency profile against the MMP-12 from other species. It also has improved PK profile over the N-linker DBF analogs, especially the lower clearance (5 mL/min/kg) and higher bioavailability (F = 63%).

Thus, compound **26** was further evaluated orally in two animal models: the mouse ear-swelling model and the lung inflammation model. To evaluate the compound in vivo initially, a MMP-12 dependent pulmonary inflammatory response was induced in

Table 4
Selectivity and PK profile of compound **26**



Compound 26
M.W.: 430
tPSA: 129
cLogP: 4.31

Solubility: 0.55mg/ml (pH=7.4)
Ames Test: Negative
hERG Test: Negative

Selectivity profile (IC_{50} in nM)						
MMP-12	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-13
1.0	501	2530	57,700	154	1970	1980

MMP-12 activities over species (IC_{50} in nM)					
Human	Mouse	Rabbit	Dog	Monkey	Sheep
1.0	85	57	70	244	5.4

PK properties							
Dose iv (mg/kg)	Vdss (L/kg)	CL (mL/min/kg)	Dose PO (mg/kg)	$T_{1/2}$ (h)	Cmax (ng/mL)	AUC (h ng/mL)	F %
2	0.8	5	10	2.9	5550	20,317	63

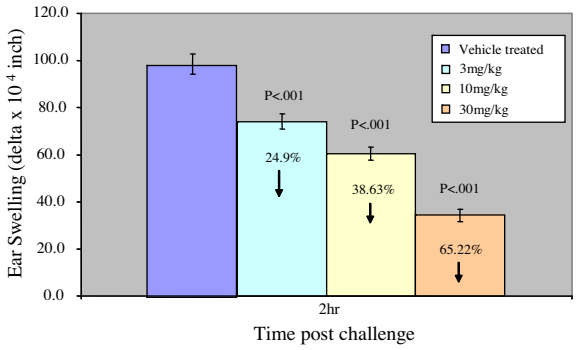


Figure 4. Effect on rhMMP-12-induced mouse ear swelling model (3, 10 and 30 mg/kg, PO, BID) of compound **26**.

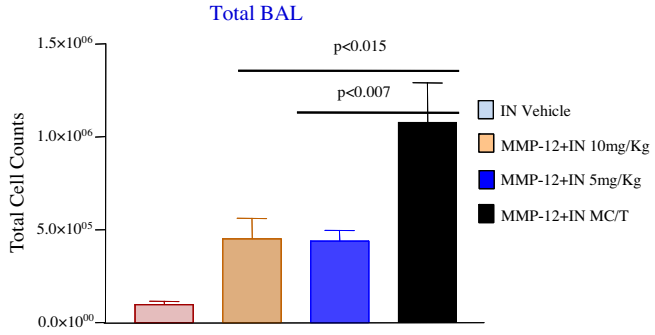


Figure 5. Effect on rhMMP-12 induced lung inflammation (5 and 10 mg/kg, PO (BID)) of compound **26**.

C57BL/6 mice by a single intradermal injection of recombinant human MMP-12 (rhMMP-12) in the ear. Compound **26** was administered orally (PO, BID) 12 h and 2 h prior to rhMMP-12 challenge. Swelling was measured as an increase in ear thickness 2 h post rhMMP-12 challenge. To evaluate the compound in the lung, an MMP-12 dependent pulmonary response was induced in C57BL/6 mice by intranasal administration of rhMMP-12 for three consecutive days. Compound **26** was administered (PO, BID) 2 h prior to, and 12 h after each of the three rhMMP-12 challenges. Lung inflammation was assessed 24 h after the final rhMMP-12 challenge. The detailed procedures for the in vivo pharmacology tests have been described previously.[8,9]

The mouse ear-swelling model is shown in Figure 4 below. Compound **26** has showed significant reduction in ear-swelling model at all the three doses tested (3, 10, and 30 mg/kg, p <0.001) when comparing to the vehicle control. The results of mouse ear-swelling model of compound **26** are comparable with that of compound MMP408.

Figure 5 below illustrates the efficacy of compound **26** against the rhMMP-12 induced mouse lung inflammation model. It showed significant reduction in total BAL inflammation (>50% inhibition, p <0.007) compared to the control (the black bar—MMP-12 with the vehicle). Further study demonstrated that the minimum efficacious dose for compound **26** was 5 mg/kg (PO, BID) in this lung inflammation model.

In general, the lung inflammation model may be more stringent than the ear-swelling model in that it required a higher dose of compound **26** to reduce the response. The time course for the rhMMP-12 induced lung inflammation being affected was over a 24–72 h time period, rather than a more acute 2 h time period evaluated in the ear-swelling. In addition to induction of endogenous mouse MMP-12, other induced mediators are likely affecting the resulting inflammatory response as well. In other studies we demonstrated that the no effect dose for compound **26** in the lung inflammation model was 3-mg/kg PO, BID in contrast to the ear-swelling model where an effect was seen with this dose. We believe there was a good correlation with efficacy and exposure with the compound in this model.

In summary, we have successfully identified a series of potent and selective MMP-12 inhibitors, which have the DBF core with a variety of C-linked heterocyclic rings as attachments. These new analogs exhibit improved MMP-12 potency and selectivity against other human MMPs over the N-linker analogs reported previously. Among these new analogs, compound **26** was selective for further in vitro and in vivo profiling. In addition to an overall improvement, this compound showed good oral efficacy in both the mouse ear-swelling model and lung inflammation model at the desired dose regimen. Compound **26** was further advanced to the Development Track status.

Acknowledgments

The authors are grateful to Dr. Nelson Huang and Ms. Ning Pan for LC-MS measurements, Drs. Walter Massefski and Vasilios Marathias for the contribution of NMR analysis, Drs. John Kubera and Franklin Schlerman for the lung inflammation experiments and Drs. Iain McFadyen and Diane Joseph-McCarthy for molecular modeling help.

References and notes

1. Celli, B. R.; MacNee, W. *Eur. Respir. J.* **2004**, *23*, 932.
2. Pauwel, R. A.; Rabe, K. F. *Lancet* **2004**, *364*, 613.
3. Jemal, A.; Ward, E.; Hao, Y.; Thun, M. *JAMA* **2005**, *294*, 1255.
4. Buist, A. S. *Proc. Am. Thorac. Soc.* **2008**, *5*, 796.
5. Vanlaere, I.; Libert, C. *Clin. Microbiol. Rev.* **2009**, *22*, 224.
6. Coussens, L. M.; Fingleton, B.; Matrisian, L. M. *Science* **2002**, *296*, 2387.
7. Mukhopadhyay, S.; Sypek, J.; Li, W.; Tavendale, R.; Page, K.; Fleming, M.; Brady, J.; O'Toole, M.; Macgregor, D. F.; Goldman, S.; Tam, S.; Abraham, W.; Williams, C.; Miller, D. K.; Palmer, C. N. *J. Allergy Clin. Immunol.* **2010**, *126*, 70.
8. Li, W.; Li, J.; Wu, Y.; Wu, J.; Hotchandani, R.; Cunningham, K.; McFadyen, I.; Bard, J.; Morgan, P.; Schlerman, F.; Xu, X.; Tam, S.; Goldman, S.; Williams, C.; Sypek, J.; Mansour, T. *J. Med. Chem.* **2009**, *52*, 1799.
9. Li, W.; Li, J.; Wu, Y.; Rancati, F.; Vallese, S.; Raveglia, L.; Wu, J.; Hotchandani, R.; Fuller, N.; Cunningham, K.; Morgan, P.; Fish, S.; Krykbaev, R.; Xu, X.; Tam, S.; Goldman, S.; Abraham, W.; Williams, C.; Sypek, J.; Mansour, T. *J. Med. Chem.* **2009**, *52*, 5408.
10. (a) Todd, H. R.; Shriner, R. L. *J. Am. Chem. Soc.* **1934**, *56*, 1382; (b) Sidduri, A.; Tilley, J. W.; Lou, J. P.; Chen, L.; Kaplan, G.; Mennona, F.; Campbell, R.; Guthrie, R.; Huang, T.; Rowan, K.; Schwinge, V.; Renzetti, L. M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2479.
11. Muramoto, Y.; Asakura, H.; Suzuki, H.; Kinki *Daigaku Kogakubu Kenkyu Hokoku* **1991**, *25*, 73.
12. (a) Zim, D.; Buchwald, S. L. *Org. Lett.* **2003**, *5*, 2413; (b) Vo, G. D.; Hartwig, J. F. *J. Am. Chem. Soc.* **2009**, *131*, 11049.
13. Palavalli, L. H.; Prickett, T. D.; Wunderlich, J. R.; Wei, X.; Burrell, A. S.; Porter-Gill, P.; Davis, S.; Wang, C.; Julia C Cronin, J. C.; Agrawal, N. S.; Lin, J. C.; Westbroek, W.; Hoogstraten-Miller, S.; Molinolo, A. A.; Fetsch, P.; Filie, A. C.; O'Connell, M. P.; Banister, C. E.; Howard, J. D.; Buckhaults, P.; Weeraratna, A. T.; Brody, L. C.; Steven A Rosenberg, S. A.; Samuels, Y. *Nat. Genet.* **2009**, *41*, 518.
14. (a) Molander, G. A.; Bernardi, C. R. *J. Org. Chem.* **2002**, *67*, 8424; (b) Nguyen, H. N.; Huang, X.; Buchwald, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 11818.
15. Baillie, C.; Zhang, L.; Xiao, J. *J. Org. Chem.* **2004**, *69*, 7779.