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Identification of potent type I MetAP inhibitors by simple bioisosteric replacement. Part 1: Synthesis and preliminary SAR studies of thiazole-4-carboxylic acid thiazol-2-ylamide derivatives

Yong-Mei Cui,[a] Qing-Qing Huang,[a] Jie Xu,[a] Ling-Ling Chen,[a] Jing-Ya Li,[a] Qi-Zhuang Ye,[a,b] Jia Li[a,*] and Fa-Jun Nan[a,*]

[a] *Chinese National Center for Drug Screening, Shanghai Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Graduate School of the Chinese Academy of Sciences, Chinese Academy of Sciences, 189 Guoshoujing Road, Zhangjiang Hi-Tech Park, Shanghai, 201203, PR China*

[b] *High-Throughput Screening Laboratory, University of Kansas, Lawrence, KS 66047, USA*

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Abstract—A series of thiazole-4-carboxylic acid thiazol-2-ylamide (TCAT, **4**) derivatives were designed and synthesized according to simple bioisosteric replacement from previously reported pyridine-2-carboxylic acid thiazol-2-ylamide (PCAT) MetAP inhibitors. The preliminary SAR studies demonstrated that these TCAT series of compounds showed different activity and selectivity compared with those of the corresponding PCAT compounds. These findings provide useful information for the design and discovery of more potent inhibitors of type I MetAPs.

The methionine aminopeptidases (MetAPs) represent a unique class of proteinases that are responsible for removing the N-terminal methionine residue from nascent polypeptide chains.[1] There are two major classes of MetAPs (type I and type II), each of which can be subdivided into two subclasses, those with N-terminal extensions and those without it. Eubacteria (for example, *Escherichia coli*, *Bacillus subtilis*, and *Salmonella typhimurium*) have only type I MetAPs, while archaea (for example, *Methanobacterium thermoautotrophicum*, *Sulfolobus solfataricus*, and *Pyrococcus furiosis*) have only type II MetAPs. Eukaryotic cells contain both type I and type II MetAPs.[2] Although the enterohemorrhagic *E. coli* strain O157:H7 has more than 20% of its 5416 genes different from the *E. coli* laboratory strain K-12,[3] it has exactly the same MetAP sequence. Deletion of the MetAP gene from *E. coli*[4] or *S. typhimurium*[5] proved to be lethal, demonstrating the essential role of MetAP enzymes in bacteria. Deletion of either type I or type II MetAP from the yeast *Saccharomyces cerevisiae* produced a slow-growth phenotype, and removal of both MetAPs made the yeast nonviable.[6] Our previous work has also indicated that the selective *Sc*MetAP1 inhibitor significantly inhibited *Sc*MetAP1 deletion strain growth, but had no effect on *Sc*MetAP2 deletion strain growth.[7] Therefore, MetAPs are potential targets for developing antibacterial and antifungal drugs, and the inhibitors against MetAPs offer new hope for treatment of bacterial and fungal infections.[8]

Various inhibitors have been identified for MetAPs. Natural product, fumagillin, and its derivatives[9] are potent inhibitors of type II MetAP in humans, and covalently modified the enzyme. Until recently, only weak inhibitors of type I MetAPs, such as fumagillin,[10] phosphorus-based Met analogs,[11] and bestatin-derived peptide inhibitors, have been identified.[12] Various 1,2,4-triazole-based derivatives have recently been reported to represent a new class of more potent inhibitors for *Staphylococcus aureus* MetAP-I (*Sa*MetAP-I).[13]

In our previous work,[14] starting with compound **1**, with an IC_{50} of 5.0 μM for *Ec*MetAP1, which was identified

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[*] Corresponding authors. Tel.: +86 21 50801313, ext. 132; fax: +86 21 50800721 (J.L.); tel.: +86 21 50800954; fax: +86 21 50800721 (F.-J.N.); e-mail addresses: jli@mail.shcnc.ac.cn; fjnan@mail.shcnc.ac.cn

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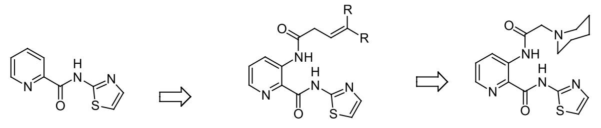
as a hit in a random screening of a diverse molecule library of 4500 compounds for *Ec*MetAP1 inhibitors, we have subsequently carried out successful structural modifications and structure–activity relationship (SAR) analyses and obtained potent inhibitors (for example, for **2a**, IC_{50} = 130 nM for *Ec*MetAP1; for **2b**, IC_{50} = 140 nM for *Sc*MetAP1; for **3**, IC_{50} = 53 nM for *Ec*MetAP1). They represent small-molecule MetAP inhibitors with novel structures different from those of alkylating fumagillin derivatives and peptidic bestatin-based MetAP inhibitors.



1 IC_{50} 5.0 μM for *Ec*MetAP1

2a R = H, IC_{50} 130 nM for *Ec*MetAP1
2b R = Me, IC_{50} 140 nM for *Sc*MetAP1

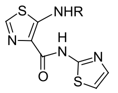
3 R = H, IC_{50} 53 nM for *Ec*MetAP1

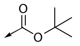
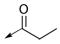
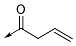
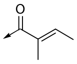
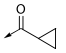
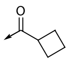
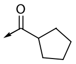
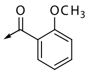
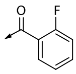
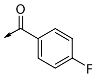
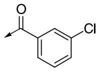
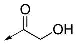
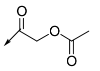
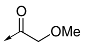
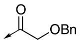
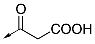
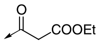
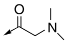
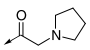
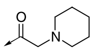
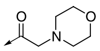
To find new MetAP inhibitors with improved potency and selectivity, good specificity in vitro, and good efficacy in vivo, we set out to explore the SAR of the above inhibitors.

Our previous work revealed that the pyridine-2-carbonyl unit is very important in pyridine-2-carboxylic acid thiazol-2-ylamide (PCAT) inhibitors. It was regarded as the key chelator for the cobalt ion of the enzyme. The 1,3-thiazole ring is a classical isostere of pyridine, and therefore, a logical first step was to design new analogs in which the pyridine ring in **1** was replaced by a thiazole moiety, which gave the thiazole-4-carboxylic acid thiazol-2-ylamide (TCAT) **4** with greatly enhanced in vitro potency toward *Ec*MetAP1 (Table 1). The potency toward *Ec*MetAP1 increased about 50-fold compared with the lead compound **1**, with an IC_{50} value of 110 nM. The introduction of the 3-*N*-acyl group greatly enhanced the activity against *Ec*MetAP1 and *Sc*MetAP1 compared with that of PCAT itself in the PCAT series of compounds, by analogy with which two series of new compounds **A** and **B** were designed, bearing a 5-*N*-acyl group or an alkyl group, respectively (Fig. 1).

Initially, a series of 5-*N*-acyl derivatives **5a–l** were synthesized (Table 1). These molecules shared the substructural diversity of the 5-position of the thiazole ring of TCAT as that of fatty acylamino (without substituent or with short saturated or unsaturated side chain, compounds **5a–h**) and aromatic acylamino (compounds **5i–l**). Compounds **5c–l** were readily available from common intermediate **5a** and various carboxylic acids or chlorides, as shown in Scheme 1. Ethyl 5-aminothiazole-4-carboxylate **8** was prepared according to the literature.[15] Treatment of **8** with Boc_2O, followed by base hydrolysis, afforded **10**. Condensation of compound **10** with 2-aminothiazole in the presence of DCC in DMF, followed by amine deprotection and acylation with various carboxylic acids or chlorides under neutral or basic conditions, afforded 5-*N*-acyl-substituted compounds **5c–l**.

Table 1. Inhibitory activity of TCAT derivatives on *Ec*MetAP1 and *Sc*MetAP1[a]



Compound	R	IC_{50} (μM)	
		*Ec*MetAP1	*Sc*MetAP1
1[b]	—	5.0 ± 0.8	7.0 ± 0.1
4	—	0.11 ± 0.02	2.26 ± 0.38
5a	H	1.97 ± 0.37	13.21 ± 2.19
5b		0.06 ± 0.01	4.62 ± 0.36
5c		0.081 ± 0.011	0.22 ± 0.01
5d		0.054 ± 0.014	2.63 ± 0.61
5e		0.044 ± 0.014	0.37 ± 0.05
5f		0.042 ± 0.007	6.38 ± 1.05
5g		0.040 ± 0.009	0.25 ± 0.03
5h		0.024 ± 0.002	0.13 ± 0.02
5i		0.19 ± 0.01	3.32 ± 0.34
5j		0.076 ± 0.003	0.99 ± 0.02
5k		0.11 ± 0.02	0.48 ± 0.05
5l		0.27 ± 0.04	1.83 ± 0.24
5m		0.19 ± 0.01	1.81 ± 0.40
5n		0.076 ± 0.011	2.65 ± 0.36
5o		0.092 ± 0.016	1.53 ± 0.21
5p		0.029 ± 0.003	1.12 ± 0.24
5q		0.088 ± 0.013	4.91 ± 0.65
5r		0.066 ± 0.002	0.67 ± 0.09
5s		0.30 ± 0.02	10.50 ± 0.74
5t		0.16 ± 0.02	0.86 ± 0.07
5u		0.14 ± 0.02-	1.45 ± 0.16
5v		0.11 ± 0.01	4.71 ± 0.63

[a] Assays were performed, as previously described.[14a]

[b] See Ref. 14a.

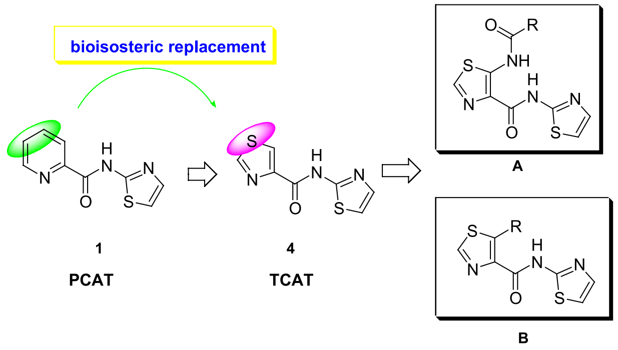
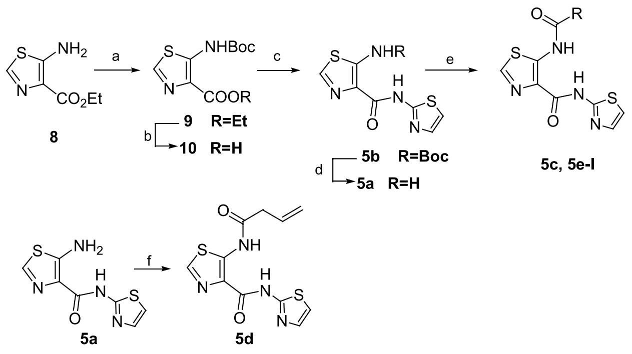


Figure 1.



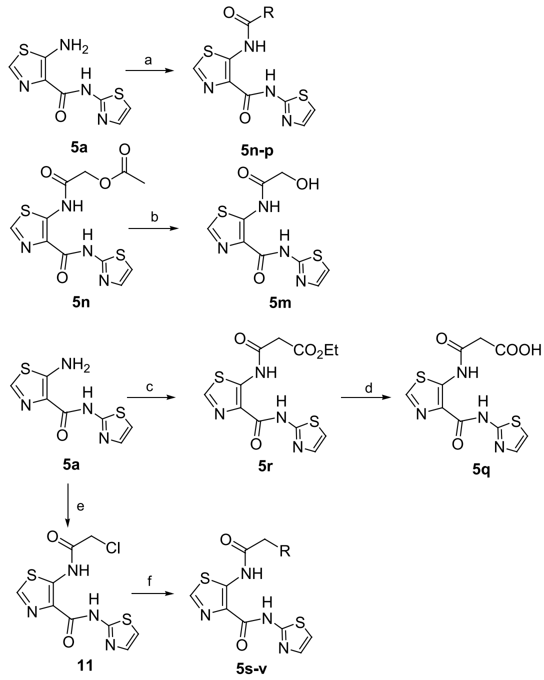
Scheme 1. Reagents and conditions: (a) (i) Boc_2O, DMAP, CH_3CN, (ii) Bu^{t}OH, reflux, 95%; (b) LiOH, MeOH–H_2O, 100%; (c) 2-aminothiazole, DCC, HOBt, DMAP, DMF, 81%; (d) TFA, CH_2Cl_2, 100%; (e) appropriate acyl chloride, Py, AcOEt; (f) 2-butenic acid, pivaloyl chloride, CH_2Cl_2, Py.

Simple replacement of a pyridine ring of the original inhibitor with a 4-thiazolyl unit usually gave compounds that were more active against the enzymes than the corresponding PCAT inhibitors, the results of which are shown in Table 1. Most of the 12 compounds tested showed excellent inhibition of *Ec*MetAP1 (IC_{50} values in the range of tens to hundreds of nanomolar). Saturated or unsaturated alkyl acylamino derivatives (**5c–e**) showed excellent activity against *Ec*MetAP1, with IC_{50} values less than 100 nM. Derivatives with three-, four-, and five-membered rings (**5f–h**) also retained good activity to *Ec*MetAP1, indicating that the small-sized hydrophobic group in this position was involved in the interaction of the inhibitor with *Ec*MetAP1. On the other hand, the four compounds with aromatic groups directly connected to the carbonyl carbon (**5i–l**) showed less significant activity against the enzymes, except for the F-containing derivatives. Inhibition of *Sc*MetAP1 was not as outstanding as that of *Ec*MetAP1, but the results were satisfactory, except for **5d** and **5f**, which showed that similar cyclopropyl and allyl groups inhibited the interaction with *Sc*MetAP1.

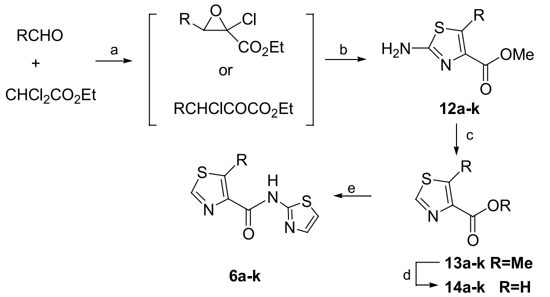
It has been pointed out that the hydrogen bond formed between the enzyme and 3-position substituents of PCAT contributed to the binding affinity. Similar to PCAT series of compounds, we synthesized compounds **5m–v** for the purpose of forming extra hydrogen bonds between MetAP1 and inhibitors. The preparation of these compounds is summarized in Scheme 2. Direct amidation of **5a** produced **5n–p** and**11**. Compound **5m** was obtained by the base hydrolysis of **5n**. Compound **5a** reacted with malonic acid diethyl ester to give compound **5r**, the base hydrolysis of which afforded compound **5q**. Compounds **5s–v** were prepared via aminolysis of **11** by the corresponding amines.

As shown in Table 1, all of the 10 compounds showed good inhibition of *Ec*MetAP1, with IC_{50} values less than 0.3 μM. The hydroxy derivative **5m** was apparently less active than the other derivatives (**5n–p**) against *Ec*MetAP1, indicating that the free hydroxy group is unfavorable on the side chain of these compounds. However, all these four derivatives (**5m–p**) showed moderate activity against *Sc*MetAP1. In addition, although similar in inhibiting *Ec*MetAP1, the carboxyl-substituted derivative **5q** was apparently less active than **5r** against *Sc*MetAP1, indicating that a free carboxyl on the side chain of TCAT is unfavorable in inhibiting *Sc*MetAP1 and may reflect subtle differences in the active sites of *Ec*MetAP1 and *Sc*MetAP1. Surprisingly, the amino-derived compounds (**5s–v**) showed less activity against the enzymes than the corresponding PCAT derivatives, which were the best inhibitors of PCAT against MetAP1. This may suggest that simple replacement of an ethylenic group of a pyridine ring by its isosteric S atom significantly affects the interaction of the inhibitor with the enzymes.

From the above results, we can see that simple replacement of the pyridine ring of original PCAT inhibitors



Scheme 2. Reagents and conditions: (a) appropriate acyl chloride, Py, AcOEt; (b) LiOH, dioxane–H_2O; (c) diethyl malonate, heat; (d) LiOH, MeOH–H_2O; (e) chloroacetyl chloride, Py, AcOEt; (f) appropriate amine, dioxane.



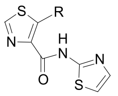
Scheme 3. Reagents and conditions: (a) NaOMe, Et_2O; (b) NH_2CSNH_2, MeOH, reflux; (c) $NaNO_2$, H_3PO_2; (d) LiOH, MeOH–H_2O; (e) 2-aminothiazole, DCC, HOBt, DMF.

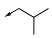
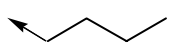
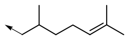
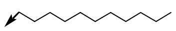
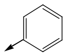
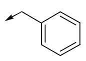
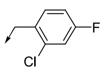
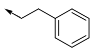
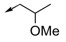
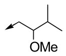
gave a new type of potent MetAP1 inhibitor, with IC_{50} values between tens to hundreds of nanomolar against *Ec*MetAP1. Systematic SAR studies of introducing 5-*N*-acyl substituents demonstrated that these TCAT series of compounds showed different activity and selectivity compared with those of the corresponding PCAT compounds. This may suggest that simple replacement of an ethylenic group of a pyridine ring by its isosteric S atom significantly affects the interaction of the inhibitor with the enzymes. Therefore, to obtain more potent MetAP1 inhibitors, we synthesized several derivatives of the original TCAT compound **4** with simple alkyl modifications at the 5-position.

As shown in Scheme 3, the syntheses of 5-alkyl-substituted compounds **6a–k** could be effected by the condensation of 5-alkyl-substituted-thiazole-4-carboxylic acid, which was obtained by several transformations from the corresponding aldehyde and ethyl dichloroacetate,[16] with 2-aminothiazole in the presence of DCC or EDC in DMF.

Derivatives of the original TCAT compound **4** with simple alkyl modifications at the 5-position are given in Table 2. Small electron-donating substituents, such as methyl, ethyl, isopropyl, isobutyl, and *n*-pentyl, had little effect on the activity against *Ec*MetAP1 (compounds **6a–e**), with IC_{50} values between 110 and 220 nM. In addition, unsaturation had no significant effect on the activity of these alkyl derivatives against *Ec*MetAP1 (**6f**). However, these compounds demonstrated a different activity trend toward *Sc*MetAP1; the short straight-chain alkyl derivatives **6a** and **6b** showed decreased activity against *Sc*MetAP1, and the derivative with an unsaturated side chain (**6f**) showed a similar activity to **4**. Although the branched side-chain derivative **6c** showed weak activity against *Sc*MetAP1, insertion of a methylene spacer between the thiazole and the isopropyl groups significantly improved the activity (**6d** vs. **6c**). Although not so apparent, this trend also persisted between these two compounds against *Ec*MetAP1. In addition, moderate chain length alkyl derivative **6e** showed good activity against *Sc*MetAP1, with an IC_{50} value less than 1.0 μM. This may be attributed to appropriate space and shape of the pockets with which *Sc*MetAP1 accommodates its inhibitor. Considering the substrate specificity of MetAPs, too long or a too large substituent chain may decrease the binding affinity, as is the case with compound **6g**, almost with no inhibitory activity against *Ec*MetAP1 or *Sc*MetAP1. At the same time, we synthesized several aryl-group-containing derivatives (**6h–k**). From the results, we can see that substitution by a phenyl ring (**6h**) or benzyl group (**6i**) showed lower activity than **4** against both *Ec*MetAP1 and *Sc*MetAP1. However, introduction of an electron-withdrawing 2-chloro-4-fluoro group to the phenyl group of the benzyl substituent (**6j**) or an additional insertion of a methylene spacer (**6k**) dramatically increased the activity. From the above results, we deduce that substituents at the 5-position with appropriate size and spatial arrangement may contribute to activity against the enzymes. The SAR analyses may reflect subtle differences in the active site of *Ec*MetAP1 and *Sc*MetAP1, and the difference in the mode of interaction between the inhibitors and the enzymes.

Table 2. Inhibition of EcMetAP1 and ScMetAP1[a]



Compound	R	IC_{50} (μM)	
		*Ec*MetAP1	*Sc*MetAP1
1[b]	—	5.0 ± 0.8	7.0 ± 0.1
4	—	0.11 ± 0.02	2.26 ± 0.38
6a		0.18 ± 0.03	9.82 ± 0.43
6b		0.18 ± 0.03	7.64 ± 0.99
6c		0.22 ± 0.02	7.20 ± 0.54
6d		0.10 ± 0.02	0.99 ± 0.08
6e		0.16 ± 0.02	0.98 ± 0.10
6f		0.17 ± 0.00	2.11 ± 0.22
6g		76.50 ± 11.70	> 100
6h		0.21 ± 0.02	3.41 ± 0.44
6i		0.17 ± 0.03	3.80 ± 0.74
6j	 F Cl	0.082 ± 0.009	0.90 ± 0.11
6k		0.065 ± 0.006	0.66 ± 0.10
7a	 OMe	0.074 ± 0.008	0.88 ± 0.08
7b	 OMe	0.089 ± 0.006	0.50 ± 0.05

[a] Assays were performed, as previously described.[14a]

[b] See Ref. 14a.

During our process of synthesizing the above 5-alkyl-substituted compounds, we first intended to introduce an unsaturated alkyl group by using an α,β-unsaturated aldehyde, such as crotonaldehyde; however, we finally obtained the β-methoxy product **7a**, as the Michael addition reaction took place in the first Darzens reaction of ethyl dichloroacetate and the α,β-unsaturated aldehyde with NaOMe in Et_2O.[16] Using the same method, we obtained **7b** from the corresponding α,β-unsaturated aldehyde, 4-methyl-2-pentenyl. The inhibitory activities of these two compounds to *Ec*MetAP1 and *Sc*MetAP1 are shown in Table 2. Both showed good inhibition of *Ec*MetAP1 with IC_{50} values less than 100 nM. Although not so outstanding as those for *Ec*MetAP1, the inhibition activities against *Sc*MetAP1 are satisfying, compared with the simple 5-alkyl-substituted compounds (**6a–c**, **6g–i**). This strongly indicates that the introduction of β-methoxy dramatically improved activity against the enzymes.

In conclusion, we obtained a new series of potent MetAP1 inhibitors through simple bioisosteric replacement from the PCAT series of compounds. Preliminary systematic SAR studies of introducing 5-*N*-acyl substituents demonstrated that these TCAT series of compounds showed different activity and selectivity compared with those of the corresponding PCAT compounds. The introduction of β-methoxy unexpectedly during the syntheses of 5-alkyl-substituted compounds improved the inhibitory activity against the enzymes dramatically. These findings provide a new starting point for the design and discovery of more potent MetAP1 inhibitors.

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References and notes

1. (a) Arfin, S. M.; Kendall, R. L.; Hall, L.; Weaver, L. H.; Stewart, A. E.; Matthews, B. W.; Bradshaw, R. A. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7714; (b) Lowther, W. T.; Matthews, B. W. *Biochim. Biophys. Acta* **2000**, *1477*, 157.
2. Bradshaw, R. A.; Brickey, W. W.; Walker, K. W. *Trends Biochem. Sci.* **1998**, *23*, 263.
3. Perna, N. T.; Plunkett, G.; Burlandm, V.; Mau, B.; Glasner, J. D.; Rose, D. J.; Mayhew, G. F.; Evans, P. S.; Gregor, J.; Kirkpatrick, H. A.; Posfai, G.; Hackett, J.; Klink, S.; Boutin, A.; Shao, Y.; Miller, L.; Grotbeck, E. J.; Davis, N. W.; Lim, A.; Dimalanta, E. T.; Potamousis, K. D.; Apodaca, J.; Anantharaman, T. S.; Lin, J.; Yen, G.; Schwartz, D. C.; Welch, R. A.; Blattner, F. R. *Nature* **2001**, *409*, 529.
4. Chang, S. Y.; McGary, E. C.; Chang, S. *J. Bacteriol.* **1989**, *171*, 4071.
5. Miller, C. G.; Kukral, A. M.; Miller, J. L.; Movva, N. R. *J. Bacteriol.* **1989**, *171*, 5215.
6. Li, X.; Chang, Y. H. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 12357.
7. Chen, L. L.; Li, J.; Li, J. Y.; Luo, Q. L.; Mao, W. F.; Nan, F. J.; Ye, Q. Z. *Acta. Pharmacol. Sin.* **2004**, *25*, 907.
8. Vaughan, M. D.; Sampson, P. B.; Honek, J. F. *Curr. Med. Chem.* **2002**, *9*, 385.
9. (a) Griffith, E. C.; Su, Z.; Niwayama, S.; Ramsay, C. A.; Chang, Y. H.; Liu, O. J. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 15183; (b) Sin, N.; Meng, L.; Wang, M. Q.; Wen, J. J.; Bornmann, W. G.; Crews, C. M. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 6099.
10. Lowther, W. T.; McMillen, D. A.; Orville, A. M.; Matthews, B. W. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 12153.
11. Lowther, W. T.; Zhang, Y.; Sampson, P. B.; Honek, J. F.; Matthews, B. W. *Biochemistry* **1999**, *38*, 14810.
12. Keding, S. J.; Dales, N. A.; Lim, S.; Beaulieu, D.; Rich, D. H. *Synth. Commun.* **1998**, *28*, 4463.
13. Oefner, C.; Douangamath, A.; D'Arcy, A.; Hafeli, S.; Mareque, D.; Mac Sweeney, A.; Padilla, J.; Pierau, S.; Schulz, H.; Thormann, M.; Wadman, S.; Dale, G. E. *J. Mol. Biol.* **2003**, *332*, 13.
14. (a) Luo, Q. L.; Li, J. Y.; Liu, Z. Y.; Chen, L. L.; Li, J.; Qian, Z.; Shen, Q.; Li, Y.; Livingston, G. H.; Ye, Q. Z.; Nan, F. J. *J. Med. Chem.* **2003**, *46*, 2631; (b) Luo, Q. L.; Li, J. Y.; Liu, Z. Y.; Chen, L. L.; Li, J.; Ye, Q. Z.; Nan, F. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 635; (c) Luo, Q. L.; Li, J. Y.; Chen, L. L.; Li, J.; Ye, Q. Z.; Nan, F. J. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 639.
15. Sen, A. K.; Mukhopadhyay, A. K. *Ind. J. Chem. B* **1981**, *20*, 275.
16. Barton, A.; Breukelman, S. P.; Kaye, P. T.; Denis Meakins, G.; Morgan, D. J. *J. Chem. Soc. Perkin I.* **1982**, 159.