Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 4130–4135
Bioorganic & Medicinal Chemistry Letters

Identification of potent type I MetAPs inhibitors by simple bioisosteric replacement. Part 2: SAR studies of 5-heteroalkyl substituted TCAT 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*

Received 17 March 2005; revised 27 May 2005; accepted 2 June 2005
Available online 6 July 2005

Abstract—Systematic SAR studies on the thiazole ring 5-substituent of TCAT derivatives revealed that the introduction of a β-alkoxy or an amino group enhanced the inhibitory activity significantly. The present compounds are representative of specific Co(II)-MetAP1 inhibitors. Before the physiologically relevant metal ions for MetAPs are established, these small molecular compounds could be used as tools for detailed biological studies.

The methionine aminopeptidases (MetAPs) are a novel class of dinuclear metalloprotease responsible for removal of the initiator N-terminal methionine residue of nascent proteins.[1] It is widely found in prokaryotic and eukaryotic cells and exists in two forms: type I (MetAP1) and type II (MetAP2).[2] The removal of methionine represents a critical step in the maturation of proteins for proper function, targeting, and eventual degradation.[3–6] MetAPs present good targets for new antibiotic drug discovery because of their important physiological functions.[7–9] Moreover, MetAPs have been shown, biochemically and structurally, to be the molecular target of the antiangiogenesis agent fumagillin and its derivatives.[10] And inhibitors of MetAPs offer hope as new treatments for bacterial and fungal infections and cancers.[11]

In the preceding paper, we obtained a new series of potent MetAP1 inhibitors through simple bioisosteric replacement from the PCAT series of compounds.[12] Preliminary systematic SAR studies of these TCAT series compounds demonstrated that the introduction of β-methoxy to the 5-alkyl-substituted compounds improved the inhibitory activity against the enzymes dramatically. These series of β-methoxy-containing compounds interested us particularly, because of their prominent potency against *Ec*MetAP1, as well as the structural feature of β-methoxy-containing alkyl substituents at the 5-position, which stimulated us to investigate the effect of the heteroalkyl group at the 5-position on the inhibition of MetAP1s. In this study, we report the synthesis and evaluation of a series of heteroalkyl-containing TCAT derivatives (Fig. 1).

Initially, we synthesized a series of β-alkoxy-containing compounds from the corresponding α,β-unsaturated aldehyde using a similar method with the syntheses of

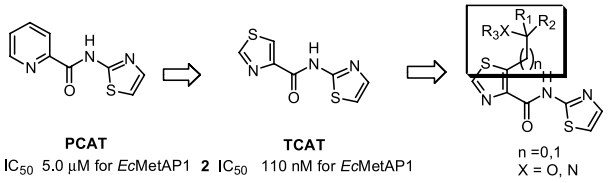


Figure 1.

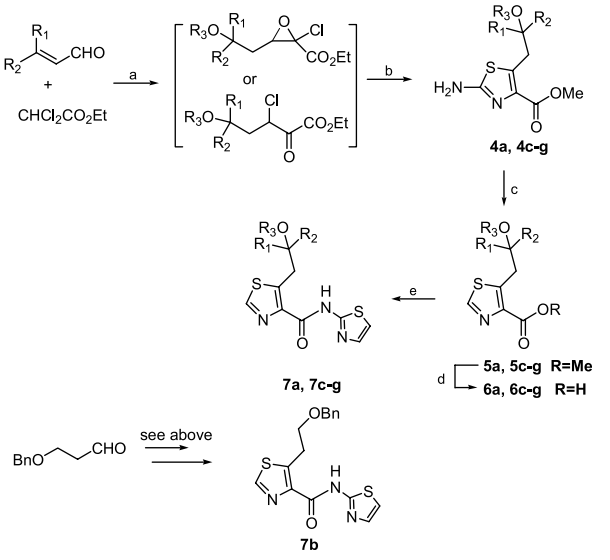
Keywords: Type I MetAPs inhibitors; Bioisosteric replacement.
* Corresponding authors. Tel.: +86 21 50800954; fax: +86 21 50800721 (F.-J.N.); tel.: +86 21 50801313x132; fax: +86 21 50800721 (J.L.); e-mail addresses: fjnan@mail.shcnc.ac.cn; jli@mail.shcnc.ac.cn

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.06.005

3a and **3b**. As shown in Scheme 1, the Michael addition reaction took place in the first Darzens reaction of ethyl dichloroacetate and the α,β-unsaturated aldehyde with NaOMe or NaOEt in Et_2O, and the resulted intermediates reacted with thiourea to form 2-aminothiazole-4-carboxylate **4a**, **4c**–**g**.[13] Replacement of the 2-amino-group with hydrogen and further basic hydrolysis gave **6a**, **6c**–**g**, followed by condensation with 2-aminothiazole in the presence of EDC in DMF afforded **7a**, **7c**–**g**. Similarly compound **7b** was synthesized from 3-benzyloxypropionaldehyde.

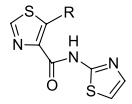
As shown in Table 1, all 7-alkoxy derivatives **7a**–**g** showed good inhibition of *Ec*MetAP1 with IC_{50} values less than 100 nM except **7a**. In particular, the ethoxy derivative **7e** exhibited potent inhibition activity for *Ec*MetAP1 (IC_{50} = 28 nM) and *Sc*MetAP1 (IC_{50} = 150 nM). Although the introduction of methoxy (**7a**) or benzyloxy (**7b**) to the β-position had little effect on the activity against the enzymes, the effect of adding a methyl group to the end of **7a** was striking (**3a** vs **7a**). However, an additional methyl group (**7c**) decreased the *Sc*MetAP1 activity and improved the *Ec*MetAP1 selectivity. Both straight chain and branched chain alkyl derivatives (**7d** and **3b**, respectively) showed good activity against two enzymes. In addition, changing the methoxy to ethoxy increased the activity (**7e** vs **3b**). The results may suggest that besides the importance of the oxygen of the alkoxy, these alkyl groups are reaching out to fill a hydrophobic pocket on the active site of the enzymes. This hydrophobic pocket was able to accommodate medium-sized alkyl groups such as isobutyl and cyclohexyl (**7f** and **g**, respectively).

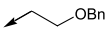
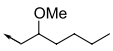
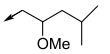
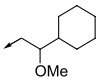
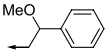
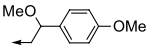
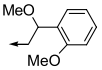
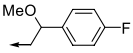
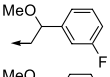
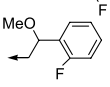
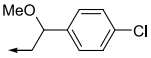
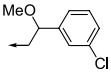
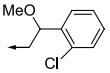
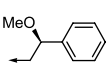
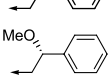
On the basis of the above analyses, we became interested in the syntheses of analogues containing β-methoxy and aryl groups at the 5-position of TCAT. However, attempts to prepare analogous compounds by the condensation of ethyl dichloroacetate with cinnamaldehyde



Scheme 1. Reagents: (a) NaOMe or NaOEt, 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.

Table 1. Inhibition of *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
2	—	0.11 ± 0.02	2.26 ± 0.38
3a		0.074 ± 0.008	0.88 ± 0.08
3b		0.089 ± 0.006	0.50 ± 0.05
7a		0.15 ± 0.02	6.43 ± 0.57
7b		0.09 ± 0.02	4.25 ± 1.47
7c		0.069 ± 0.005	3.21 ± 0.47
7d		0.10 ± 0.01	0.45 ± 0.05
7e		0.028 ± 0.001	0.15 ± 0.02
7f		0.09 ± 0.01	0.50 ± 0.07
7g		0.054 ± 0.010	0.40 ± 0.01
7h		0.043 ± 0.002	0.44 ± 0.06
7i		0.023 ± 0.002	0.31 ± 0.07
7j		0.035 ± 0.006	0.57 ± 0.08
7k		0.057 ± 0.003	0.39 ± 0.08
7l		0.037 ± 0.008	0.34 ± 0.05
7m		0.034 ± 0.002	0.27 ± 0.01
7n		0.033 ± 0.002	0.57 ± 0.13
7o		0.09 ± 0	0.52 ± 0.08
7p		0.17 ± 0.01	0.23 ± 0.05
7q		0.097 ± 0.020	7.53 ± 1.22
7r		0.061 ± 0.008	0.75 ± 0.04
7h(R)		0.036 ± 0.004	0.45 ± 0.02
7h(S)		0.066 ± 0.004	0.45 ± 0.03

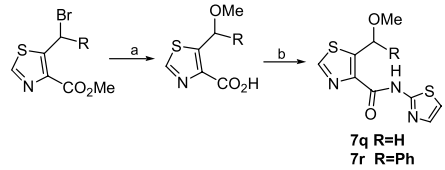
[a] Assays were performed as previously described.[12a]
[b] See Ref. 12a.

were unsuccessful, and an unexpected mixture of products was obtained. Therefore, β-methoxy aldehyde was first prepared, as shown in Scheme 2. Treatment of the corresponding aldehyde with allylmagnesium bromide gave homoallylic alcohol **8h**–**p** in high yields. This product was protected as the methyl ether **9h**–**p**, which was then converted into the corresponding aldehyde **10h**–**p** by ozonolysis in MeOH–CH_2Cl_2 solution followed by reductive treatment with Me_2S. The resulting β-methoxy aldehydes were further converted to the corresponding compounds **7h**–**p** by using the same procedure shown in Scheme 1.

As expected, all these nine compounds (**7h**–**p**) showed good inhibitory activity against both *Ec*MetAP1 and *Sc*MetAP1. The effects of substitution on the phenyl group derived are summarized in Table 1. In general, substituents on the phenyl ring are well tolerated, regardless of their electronic properties, and a number of very potent derivatives were obtained with IC_{50} values less than 50 nM against *Ec*MetAP1. Except **7p**, the Cl- and F-substituted derivatives (**7k**–**m**, **7n**–**o**) showed good inhibitory activity against *Ec*MetAP1. Although not so obvious, all these compounds showed good inhibition activity for *Sc*MetAP1 ($IC_{50} < 0.57$ μM).

We also synthesized two typical α-methoxy-containing derivatives **7q** and **r**. Both could be prepared by the corresponding brominated ester with replacement by methoxide anion, hydrolysis, and condensation with 2-aminothiazole (Scheme 3). As shown in Table 1, **7q** showed similar activity against the enzymes to **7a**, and **7r** showed weaker activity than **7h**, which showed that the β-methoxy derivatives may fit better in the pockets of the enzymes.

To understand further the subtle stereochemical requirement of protein to small molecular inhibitors, because its X-ray structure is currently unavailable, we synthesized the enantiomers of **7h**, as shown in Scheme 4. The optical isomers of 3-methoxy-3-phenylpropionaldehyde **16** were prepared from cinnamyl alcohol in six steps. Catalytic asymmetric epoxidation of cinnamyl alcohol using L-(+)-diethyl tartrate (DET) gave (−)-(2*S*,3*S*)-2,3-epoxycinnamyl alcohol **11R**.[14] Regioselective reduction of **11R** with Red-Al in DME gave (*R*)-3-phenyl-1,3-dihydroxypropane **12R** in high yield.[15] Treatment of the crude diol **12R** with 1.1 equiv TBDMSCl led to monosilicified compound **13R**. This



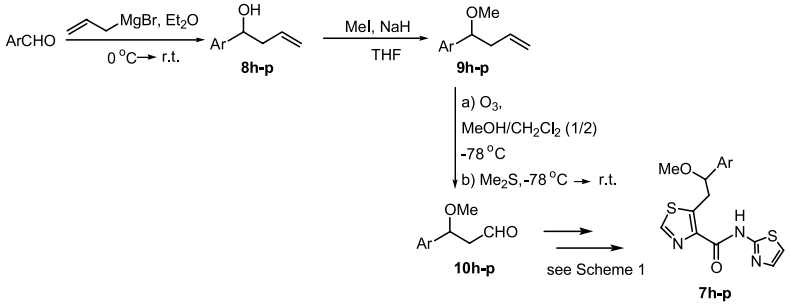
Scheme 3. Reagents: (a) LiOH, MeOH–H_2O; (b) 2-aminothiazole, DCC, HOBt, DMF.

product was protected as methyl ether **14R** and then desilicated by TBAF in THF giving the alcohol **15R**. Swern oxidation of **15R** gave (*R*)-3-methoxy-3-phenylpropionaldehyde **16R**, which could be converted into **7h**(**R**) using the method described in Scheme1. Compound **7h**(**S**) was obtained similarly, except employing D-(−)-diethyl tartrate as the chiral ligand.

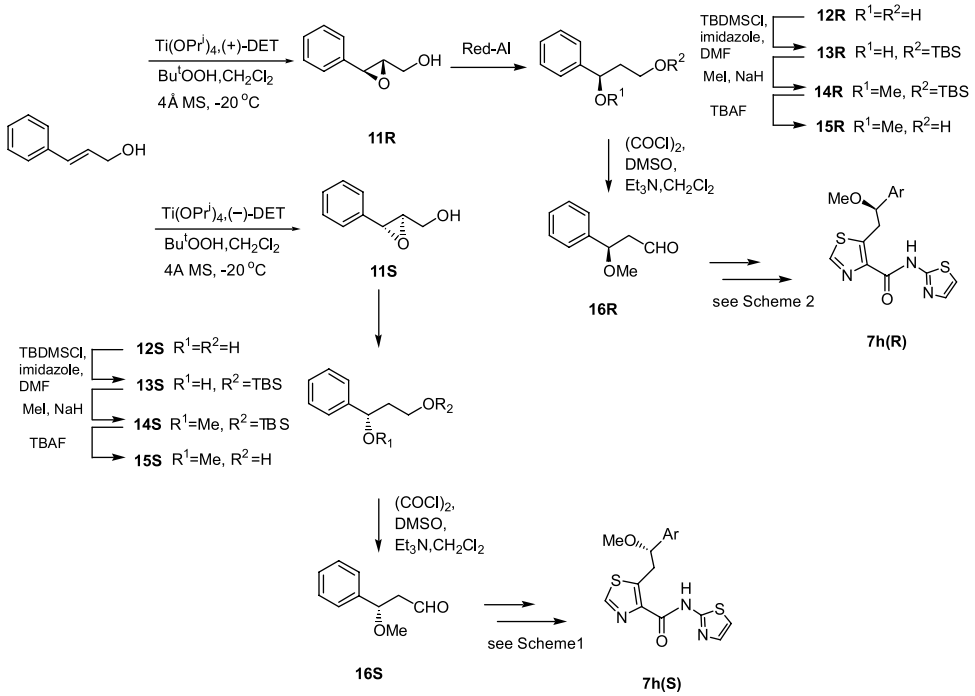
The results are shown in Table 1, from which we can see that there is a nearly 2-fold activity difference between the two stereoisomers of **7h**(**R**) and **7h**(**S**) against *Ec*MetAP1, although they showed similar activity against *Sc*MetAP1. These differences may reflect the subtle differences in the active sites of *Ec*MetAP1 and *Sc*MetAP1.

To understand the subtle differences between the enzymes and obtain some more potent and selective inhibitors, we considered other hydrogen-bonding derivatives. Starting from compound **8a**, a hydrogen-bonding acceptor alcohol, we synthesized a series of its derivatives, such as the ester, sulfonamide and the results are shown in Table 2. Compounds **8b**–**h** were obtained by acylation of **8a**, which was obtained by debenzylation of **7b** with the corresponding carboxylic chlorides under basic conditions (Scheme 5).

Except for compounds **8c** and **8f**, most of these compounds showed moderate or weak activity against *Sc*MetAP1, and were selective *Ec*MetAP1 inhibitors. Although **8a** showed weak activity against the enzymes, its ester derivatives showed increased *Ec*MetAP1 activity with increasing bulk of the end substituents (**8a**–**e**), especially the aryl containing group (**8d** and **8e**). However, insertion of an ether and a methylene spacer (**8f**) or increasing the bulk of the phenyl ring (**8g**) reduced the *Ec*MetAP1 activity. In addition, sulfonylation of the alcohol oxygen (**8h**) dramatically reduced the *Ec*MetAP1 activity.

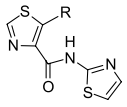


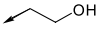
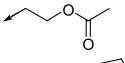
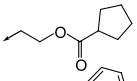
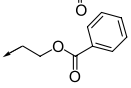
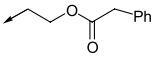
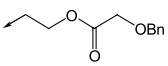
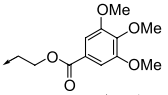
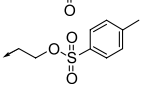
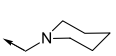
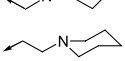
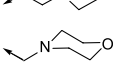
Scheme 2. The syntheses of compounds **7h**–**p**.



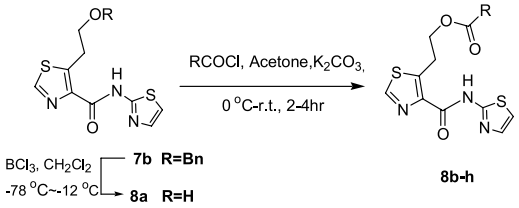
Scheme 4. The syntheses of enantiomers of **7h.**

Table 2. Inhibition of *Ec*MetAP1 and *Sc*MetAP1[a]



Compound	R	IC$_{50}$ (μM)	
		*Ec*MetAP1	*Sc*MetAP1
2	—	0.11 ± 0.02	2.26 ± 0.38
8a		0.47 ± 0.10	10.39 ± 4.78
8b		0.31 ± 0.06	17.92 ± 3.31
8c		0.14 ± 0.01	0.80 ± 0.05
8d		0.041 ± 0.011	3.08 ± 0.43
8e		0.033 ± 0.011	6.04 ± 0.51
8f		0.13 ± 0.01	0.55 ± 0.08
8g		0.16 ± 0.01	1.56 ± 0.43
8h		1.53 ± 0.11	3.72 ± 0.26
9a		0.025 ± 0.001	0.35 ± 0.007
9b		0.010 ± 0.001	0.075 ± 0.007
9c		0.045 ± 0.005	0.41 ± 0.04
9d		0.055 ± 0.003	1.76 ± 0.28

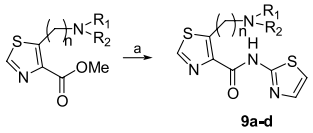
[a] Assays were performed as previously described.[12a]



Scheme 5. The syntheses of **8a**–**h**.

Finally, we synthesized several amino-containing derivatives. As shown in Scheme 6, compounds **9a**–**d** were prepared by condensation of the anion of a 2-aminothiazole with the corresponding 5-substituted-thiazole-4-carboxylic acid methyl ester in THF.

As shown in Table 2, all these four compounds **9a**–**d** showed good inhibition of *Ec*MetAP1 with IC$_{50}$ values less than 60 nM. The introduction of pyrrolidinyl (**9a**) or piperidinyl (**9b**) to the α-position of 5-methyl of TCAT dramatically increased the activity, especially for compound **9b**, which is the best inhibitor described in the literature to date with IC$_{50}$ values of 10 nM for *Ec*MetAP1 and 75 nM for *Sc*MetAP1. However, insertion of an additional methylene spacer (**9c**) or oxygen atom to the piperidine (**9d**) decreased the activity. This may be due to the appropriate size and shape of the



Scheme 6. Reagents and conditions: (a) NaH, 2-aminothiazole; THF, reflux.

pockets in which MetAP1 accommodates its inhibitor. Therefore, we did not attempt to prepare analogues containing substituents of increased chain length.

Of the above TCAT inhibitors, we tested several typical compounds for antibacterial activity against *Staphylococcus aureus*, Enteropathogenic *Escherichia coli*, and *Pseudomonas aeruginosa*, and the activity is poor (MIC > 16 μg/mL, data not shown). We also tested the compounds more active in inhibiting *Sc*MetAP1 for antifungal activity. Most of the inhibitors tested showed no antifungal activity against *Candida albicans*, *Aspergillus niger*, *Trichophyton rubrum*, *Saccharomyces cerevisiae*, *Epidermophyton floccosum*, and *Microsporum canis* (MIC > 128 μg/mL, data not shown). Although we have obtained very potent inhibitors of *S. cerevisiae* MetAP1 through rational structural modification, it seemed that there was little relationship between the in vitro inhibitory potencies for the Co(II)–*Sc*MetAP1 and in vivo efficacy in antifungal activity. One possibility is that the poor antifungal activity may be derived from poor penetration of the fungus wall. However, in the case of these in vitro Co(II)–*Sc*MetAP1 inhibitors, the lack of antifungal activity might also be related to the metal ion present in physiological situations. The physiological metal ions for MetAPs have not been established and are controversial at the moment. MetAPs of bacteria and yeast have been categorized as cobalt-dependent metalloenzymes, based on the observations that the purified enzymes show highest activity in the presence of cobalt as compared with that of other divalent metal ions.[2] A more recent study showed that Zn(II) was a superior cofactor to Co(II) for yeast MetAP1 because Co(II) did not stimulate yeast MetAP1 activity in the presence of physiological concentrations of reduced glutathione.[16] Another study demonstrated that the physiologically relevant metal ion for *E. coli* MetAP1 was probably Fe(II), on the basis of whole cell metal analyses.[17] Most recently, D'souza et al. showed the kinetic and structural characterization of manganese-loaded MetAPs from *E. coli* and the hyperthermophilic archaeon *Pyrococcus furiosis*, and implicated manganese as a metal cofactor for MetAPs.[18] Identification of physiological metal cofactors for MetAPs is critical for discovery of small molecule therapeutic inhibitors because their potency may vary for different metal ions. In a study on yeast MetAP1 that argues for Zn(II) as the cofactor, manganese is also shown to induce enzyme activity both in the absence and in the presence of glutathione. Manganese is shown to be concentrated in bacteria overexpressing *E. coli* MetAP1 by a factor of 2.2, similar to the increase of iron, and it also induced *E. coli* MetAP1 enzyme activity.[2] More recently, Ye et al. reported the discovery and characterization of two groups of potent and highly metallo form-selective inhibitors of the Co(II)-form, and of the Mn(II)-form, and an X-ray structure of a di-Mn(II)-form of *E. coli* MetAP complexed with the Mn(II)-form selective inhibitor was also obtained.[19] These results partially supported the contention that Mn(II) ions could be physiological metal cofactors for MetAPs. However, the relevant details of physiological metal cofactors for MetAPs will still depend on the exact match of in vitro data and their in vivo antibacterial activity.

Although the in vitro potency could not match the in vivo activity, the present compounds are representative of specific Co(II)–MetAP1 inhibitors. Before the physiologically relevant metal ions for MetAPs are established, these small molecular compounds could be used as tools for detailed biological studies.

In summary, we obtained a new series of potent MetAP1 inhibitors through simple bioisosteric replacement from the PCAT series of compounds. The detailed SAR showed that these TCAT series of compounds showed different activity and selectivity compared with the corresponding PCAT compounds. These differences may reflect subtle differences in the active sites of *Ec*MetAP1 and *Sc*MetAP1. Further efforts in modifying these and other lead structures with the aim of improving potency as well as specificity in vitro, and efficacy in vivo, are in progress.

Acknowledgments

This work was supported by the National Natural Science Foundation of China Grants 30271528 (F.-J.N.), the Qi Ming Xing Foundation of Shanghai Ministry of Science and Technology Grant 02QB14013 (F.-J.N.).

References and notes

1. Bradshaw, R. A.; Brickey, W. W.; Walker, K. W. *Trends Biochem. Sci.* **1998**, *23*, 263.
2. Arfin, S. M.; Kendall, R. L.; Hall, L.; Weaver, L. H.; Stewart, A. E.; Matthews, B. W.; Bradshaw, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7714.
3. Lowther, W. T.; Matthews, B. W. *Chem. Rev.* **2002**, *102*, 4581.
4. Prchal, J. T.; Cashman, D. P.; Kan, Y. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 24.
5. Boissel, J. P.; Kasper, T. J.; Shah, S. C.; Malone, J. I.; Bunn, H. F. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 8448.
6. Tso, J. Y.; Hermodson, M. A.; Zalkin, H. *J. Biol. Chem.* **1982**, *257*, 3532.
7. Chang, S. Y.; McGary, E. C.; Chang, S. *J. Bacteriol.* **1989**, *171*, 4071.
8. Miller, C. G.; Kukral, A. M.; Miller, J. L.; Movva, N. R. *J. Bacteriol.* **1989**, *171*, 5215.
9. Li, X.; Chang, Y. H. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12357.
10. (a) Griffith, E. C.; Su, Z.; Niwayama, S.; Ramsay, C. A.; Chang, Y. H.; Liu, O. J. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15183; (b) Sin, N.; Meng, L.; Wang, M. Q.; Wen, J. J.; Bornmann, W. G.; Crews, C. M. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 6099.
11. Vaughan, M. D.; Sampson, P. B.; Honek, J. F. *Curr. Med. Chem.* **2002**, *9*, 385.
12. (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.

13. Barton, A.; Breukelman, S. P.; Kaye, P. T.; Denis Meakins, G.; Morgan, D. J. *J. Chem. Soc. Perkin. Trans. 1* **1982**, 159.
14. Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.
15. Gao, Y.; Sharpless, K. B. *J. Org. Chem.* **1988**, *53*, 4081.
16. Walker, K. W.; Bradshaw, R. A. *Protein Sci.* **1998**, *7*, 2684.
17. D'souza, V. M.; Holz, R. C. *Biochemistry* **1999**, *38*, 11079.
18. D'souza, V. M.; Swierczek, S. I.; Cosper, N. J.; Meng, L.; Ruebush, S.; Copik, A. J.; Scott, R. A.; Holz, R. C. *Biochemistry* **2002**, *41*, 13096.
19. Ye, Q. Z.; Xie, S. X.; Huang, M.; Huang, W. J.; Lu, J. P.; Ma, Z. Q. *J. Am. Chem. Soc.* **2004**, *126*, 13940.