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A NEW SERIES OF CYCLIC AMINO ACIDS AS INHIBITORS OF S-ADENOSYL L-METHIONINE SYNTHETASE

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Abstract : Optically active 3-amino-3-(tetrahydrofuranyl) carboxylic acid, 3-amino-3-(tetrahydrothienyl) carboxylic acid and their corresponding six membered ring analogues have been synthesised and examined as potential inhibitors of the enzyme S-adenosylmethionine (AdoMet) synthetase. The kinetic behaviour of these compounds was studied using recombinant rat liver AdoMet synthetase (α-isoform) fractionated from *E. coli* transformed with the plasmid pSSRL-T7N. All the compounds tested were competitive inhibitors of the enzyme with respect to L-methionine.

KEYWORDS: Amino acids derivatives; Enzyme inhibitors; Kinetics.

S-Adenosylmethionine synthetase (EC. 2.5.1.6, ATP: L-methionine-S-adenosyltransferase) catalyses the reaction of ATP and L-methionine to yield S-adenosylmethionine (AdoMet), pyrophosphate and orthophosphate[1]. AdoMet is utilised by methyltransferases for the methylation of RNA, DNA, histones, proteins, polysaccharides, steroids and numerous other metabolites[2]. S-Adenosylhomocysteine (AdoHcy) is the by-product of these AdoMet-dependent methyltransferases. The cellular concentration of S-adenosylmethionine, and hence the level of methylation activity, is controlled primarily by S-adenosylmethionine synthetase and by S-adenosylhomocysteine hydrolase that degrades adenosylhomocysteine, a potent product inhibitor of most S-adenosylmethionine utilizing enzymes, to homocysteine and adenosine[3].

The rational design of methylation inhibitors based on the inhibition of each of these enzymes involved in AdoMet metabolism has attracted the attention of medicinal chemists in the search for antitumor[4] and antiviral agents[5]. Recent reports concerning combination drugs studies performed with AdoMet synthetase and AdoHcy hydrolase inhibitors demonstrated their synergetic effects in altering nucleic acid methylation resulting in an improvement of the antiproliferative or antiviral potency of each of the drugs studied[6]. Given this new interest in S-adenosylmethionine synthetase inhibitors, we initiated a search for new series of inhibitors of AdoMet synthetase. Among the large variety of L-methionine (Met) analogues identified as inhibitors of the enzyme[7], L-cis-AMB and L-cis-AMTB (Fig. 1) are of particular interest[4b,8],although their availability is limited by a rather difficult chemical access[9a,b].

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The inhibitory potency of these two constrained Met analogues results from their ability to mimic the conformation of L-methionine at the active site of the enzyme[9b]. From these results we considered that easily accessible heterocyclic amino acids **I-IV** (Figure 1), which have close similarity in size and molecular feature with L-cis-AMB and L-cis-AMTB, might be good candidates for AdoMet synthetase inhibition.

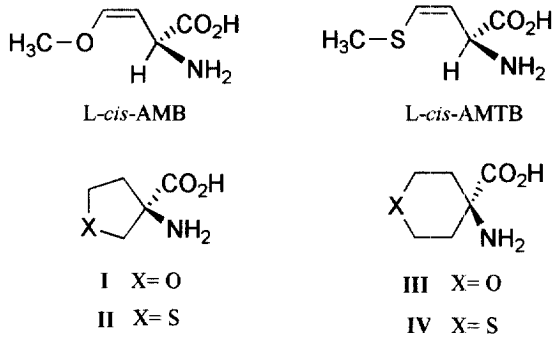
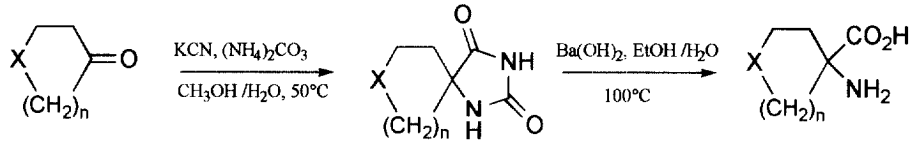


Figure 1

Compounds **I-IV** are readily prepared through a Bucherer reaction[10] starting with commercially available carbonyl precursors (Scheme 1).

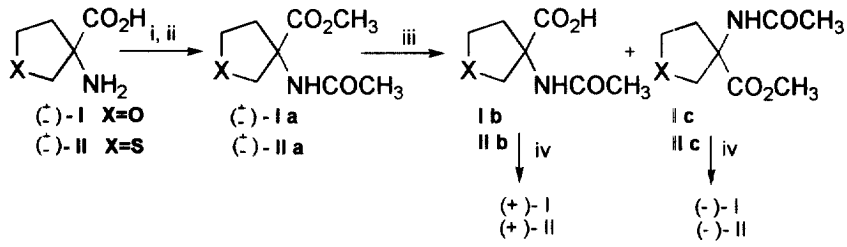


Scheme 1

Thus, when submitted to classical Bucherer conditions, tetrahydrofuran-3-one **1**(accessible through PCC oxidation of commercially available tetrahydrofuran-3-ol), tetrahydrothiophen-3-one **2**, tetrahydropyran-4-one **3** and tetrahydrothiopyran-4-one **4** provided spirohydantoins **5-8** respectively, in excellent yields (≥ 90%). Hydrolysis of hydantoins **5-8** was accomplished by treatment with barium hydroxide at 100°C for 24 hours to give cyclic amino acids (**cAAs**) **I-IV** in good yields (≥ 80%). These amino acids have been purified on HP 20 SS hydrophobic resin and fully characterised[11].

Alcalase from *Bacillus licheniforms* (the major component is subtilisin A) is a useful enzyme that selectively catalyses the hydrolysis of D,L-amino acid esters to provide L-aminoacid and D-aminoacid ester with

high optical purity[12]. We tentatively used this enzyme for the resolution of methyl ester corresponding to racemic cyclic amino acids **I** and **II** reported herein. Best results were obtained using their N-acetyl derivatives **Ia** and **IIa** as illustrated in Scheme 2.



i: CH_2N_2, MeOH; ii$(CH_3CO)_2O$, MeOH; iii:Alcalase (1,25 U/mmole of **Ia** or **IIa**), H_2O, pH 7; iv : NaOH 2.5 N, 80°, 8 h.

Scheme 2

The course of the enzymatic hydrolysis was followed at constant pH (7.0) by means of a pH-stat. (0.1 N NaOH as titrant) and the reaction stopped at 50% conversion (3-4 h). Unchanged esters were extracted with ethyl acetate (75% based on a single enantiomer). The aqueous solution was neutralised before concentration. Residual aqueous solution was desalted by column chromatography (HP 20 SS hydrophobic resin). The fractions containing the hydrolysed products were eluted with H_2O/MeOH (80/20) and lyophilised (70% yield). The enantiomeric excesses of the recovered esters : **Ic**, $[\alpha]_D^{25°} = -22°$ (c 0.1, $CHCl_3$), 84% ee and **IIc**, $[\alpha]_D^{25°} = -27°$ (c 0.1, $CHCl_3$), 92% ee, were determined by NMR[13]. Purified **Ib-c** and **IIb-c** were hydrolysed with NaOH 2.5 N at 80° for 8 h to afford the corresponding free amino acids : (+)-**I**, $[\alpha]_D^{25°}$ +7° (c 0.3, H_2O) and (+)-**II**, $[\alpha]_D^{25°}$ +10° (c 0.3, H_2O).

For the first time, the utility of alcalase as a biocatalyst in the enantioselective hydrolysis of cyclic amino acid esters has been demonstrated.

The inhibitory activities of **I-IV** against the tetramer (α-isoform) of recombinant rat liver AdoMet synthetase[14] were examined. The enzyme was fractioned from the cell free extract of *Escherichia coli* BL 21 (BE3) strain transformed with the plasmid pSSRT-7N, by a procedure reported by Alvarez et al[14]. The purified enzyme used in our experiments has a specific activity of 2 U/mg (Km for Met = 70μM). S-Adenosylmethionine synthetase activity was measured as described by Sullivan and Hoffman[15] using (^{14}C-*methyl*)-L-methionine. Kinetic analysis of the inhibition exerted by **I** is reported (Figure 2). The double reciprocal plots are indicative of competitive inhibition. Similar experiments were carried out with the other derivatives including racemic and both enantiomers of **I** and **II.** These also exhibit the same type of competitive inhibition pattern. The Ki values obtained are listed in Table 1.

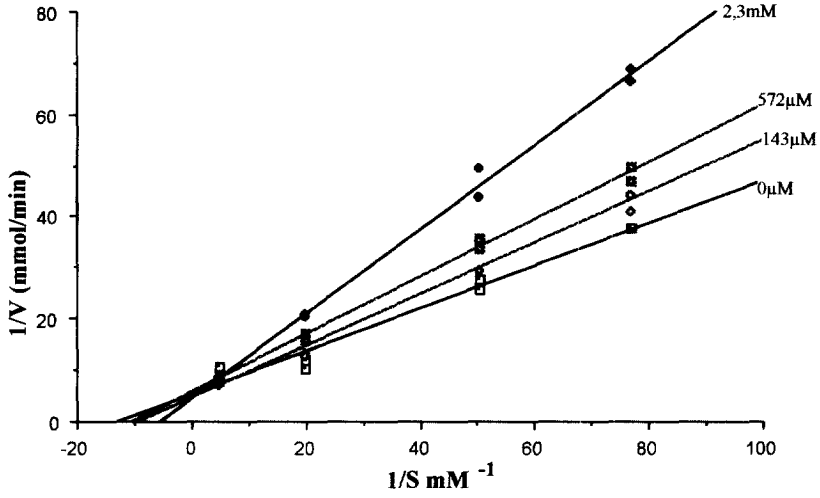


Figure 2:Lineweaver-Burk plots of AdoMet synthetase inhibition by **I**. Purified enzyme preparation (30 µU) was incubated at 37°C in 240 mM Hepes (pH 7.5), 700 mM KCl, 36 mM $MgSO_4$, 9,6 mM DTT, 5 mM ATP. The concentration of ($^{14}CH_3$) methionine was varied from 30 to 200 mM, in absence or presence of varied concentrations of inhibitor as indicated. Double reciprocal plots of the data were computer-generated. Linear regression analyses were used to determine the x-axis intercept values for calculation of the kinetic constants.

Table 1. Kinetic constants of **cAA I - IV** for rat liver recombinantAdoMet synthetase (α-isoform).

cAA	(+)-I*	(+)-II*	III	IV
K_i (mM)	0.75 ± 0.01	1.04 ± 0.05	1.0 ± 0.05	3 ± 0.1

*(-)-I and (-)-II are devoided of significant activity.

The four cAAs synthesised exhibited K_i values in the mM range. From these results it appeared that enlargement of the ring size tend to reduce inhibitory activity.The best result was obtained with the five membered ring enantiomer (+)-**I**. Among the large number of cyclic amino acids screened on different isoforms of rat liver AdoMet synthetase[7a,c] the inhibitory potency of (+)-**I** is comparable with that of cycloleucine (K_i 516 ± 33µM)[8] which emerged from these early studies, however its affinity to the target enzyme is still 15 times inferior to that of L-cis-AMB (K_i 56 ± 16 µM, for the same isozyme of rat liver)[8]. These preliminary results obtained with (+)-**I** and **III** cAAs are promising. It is therefore of interest to synthesise the corresponding C-3 unsaturated analogs, the flattened structures of which could provide both geometric and electronic mimics of the most active L-cis-AMB inhibitor. The synthesis of these interesting compounds is currently in progress.

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References

1. Cantoni, G.L. *J. Biol. Chem.*, **1953**, *204*, 403.
2. *Biochemistry of S-adenosylmethionine and related compounds*; Usdin, E. ; Borchardt, R.T. ; Creveling,. C.R., Ed., Macmillan & Co : London, **1982**.
3. Cantoni, G.L ; Chiang, P.K. In *Natural Sulfur compounds: novel biochemical and structural aspecst*, Cavallini, D., Gauld, G.E., Zappia, V., Ed., Plenum Publishing Corp : New York, **1980**,pp 67-80.
4. (a) Porter, C.W. ; Sufrin, J.R. *Anticancer Res.* **1986**, *6*, 525. (b) Sufrin, J.R. ; Lombardini, J.B. ; Alks, V. *Biochem. Biophys. Acta*, **1992**. *1202*, 87. (c) Peeg, A.E.. *Cancer Res.*, **1988**, *48*, 759.
5. (a) Liu, S. ; Wolfe, M.S. ; Borchardt, R.T. *Antiviral Res.*, **1992**, *19*, 247. (b) Yuan, C.S. ; Liu, S. ; Wnuk, S.F. ; Robins, M.J. ; Borchardt, R.T. *Nucleosides & Nucleotides* **1995**, *14*, 439.
6. Kramer, D.L. ; Porter, C.W. ; Borchardt, R.T. ; Sufrin, J.R. *Cancer. Res.* 1990, *50*, 3828. (b) Ishii, H. ; Hasobe, M. ; McKee, J.G. ; Ault-Riche, D.B. ; M., Borchardt, R.T., *Antiviral Chem. & Chemother.* **1993**, *4*, 127.
7. a) Lombardini, J.B. ; Coulter, A.W. ; Talalay, P. *Mol. Pharmacol.* **1970**, *4*, 481. (b) Coulter, A.W. ; Lombardini, J.B. ; Talalay, P. *Mol. Pharmacol.* **1974**, *10*, 29. (c) Coulter, A.W. ; Lombardini, J.B. ; Talalay, P. *Mol. Pharmacol.* **1974**, *10*, 319. (d) Sufrin, J.R. ; Coulter, A.W. ; Talalay, P. *Mol. Pharmacol.* **1979**, *15*, 661. (e) Sufrin, J.R. In *Transmethylation* ; Usdine, I. ; Borchardt, R.T., Creveling,. C.R., Ed. , Elsevier North Holland, **1979**, p 27.
8. Sufrin, J.R. ; Lombardini, J.B. ; Keith, D.D. *Biochem. Biophys. Res. Com.* **1982**, *106*, 25.
9. (a) Alks, V. ; Sufrin, J.R. *Tet. Lett.* **1990**, *37*, 5257. (b) Alks, V. ; Keith, D.D. ; Sufrin, J.R. *Synthesis* **1992**, 623.
10. a) Bucherer, H.T. ; Steiner, W. *J. Prakt Chem.* **1934**, *140*, 291. (b) Bucherer, H.T. ; Liev, V.A. *J. Prakt Chem.* **1934**, *141*, 5.
11. **I** - Mp : >220°C; MS (DCI/ NH_3) : 132 $(MH)^+$; ^1H NMR (250 MHz, D_2O), δ ppm, J Hz : 2.21 (1H, ddd, J 7.0 , 7.0, 14, H-4a) ; 2.60 (1H, ddd, J 6.8 , 8.0, 14 , H-4b) ; 4.05 (4H, m, H-2 , H-5); ^{13}C NMR (D_2O), δ ppm: 39 .0 (C-4) ; 69.0 (C-3) ; 70.6 (C-5) ; 77.8 (C-2) ; 177,3 (CO_2H).

    II - Mp : > 220°C; MS (EI) : 148 $(M)^+$; NMR ^1H (250 MHz, D_2O) δ ppm, J Hz: 2.45 (1H, ddd, J 3.4 , 7.3 , 14.1 , H-4a) ; 2.64 (1H, ddd, J 8.4 , 9.2 , 14.1 , H-4b) ; 3.05 (1H, ddd, J 7.3 , 9.2 , 11.4 , H-5a) ;

3.12 (1H, d, J 12.6 , H-2a) ; 3.25 (1H, ddd, J 3.4 , 8.4 , 11.4 , H-5b) ; 3.48 (1H, d, J 12.6 , H-2b); ^{13}C MNR (D_2O), δ ppm: 29.2 (C-4) ; 39.3 , 39.6 (C-2, C-5) ; 70.0 (C-3) ; 174.7 (CO_2H).

III - Mp : >220°C; MS (DCI/ Methane) : 146 $(MH)^+$; ^1H NMR (250 MHz, D_2O), δ ppm, J Hz: 1.50 (2H, ddd, J 4.25 , 7.0 , 13.7 , H-3a, H-5a) ; 1.86 (2H, ddd, J 4.3 , 7 .1 , 13.7 , H-3b , H-5b) ; 3.47 (2H, ddd, J 4.25 , 7.1 , 12.2 , H-2a , H-6a) , 3.58 (2H, ddd, J 4.3 , 7.0 , 12.2 , H-2b, H-6b) ; ^{13}C NMR (D2O), δ ppm: 32,0 (C-3 , C-5) ; 58.2 (C-4) ; 63.6 (C-2 , C-6) ; 175,8 (CO_2H).

IV - Mp: >220°C; MS (DCI/ Methane) : 162 $(MH)^+$; ^1H NMR (250 MHz, D_2O), δ ppm, J Hz: 2.10 (2H, m, H-3a , H-5a) ; 2.40 (2H, m, H-3b , H-5b) ; 2.90 (4H, H-2 , H-6); ^{13}C NMR (D_2O), δ ppm: 22.9 (C-2, C-6); 33.0 (C-3 , C-5) ; 60.1 (C-4) ; 176.8 (CO_2H).

12. Chen, S.T. ; Wang, K.T. ; Wong, G.H. *J. Chem. Commun.* **1986**, 1514.
13. The enantiomeric excess (ee) was determined by using $Eu(hfc)_3$ as a chiral shift reagent (^1H NMR, integration of the separated ester *O-CH₃* signals of **Ic** and **IIc**). The ee for the hydrolysed products **Ib** and **IIb** were also determined by the same procedure after their conversion to their corresponding methyl ester derivatives by treatement with CH_2N_2. The absolute configuration of hydrolysed product was not determined. Since it is too hasardous to assume the stereospecificity of Alcalase towards natural AAs is the same that for α,α-substituted AAs like **Ib** or **IIc**, (+) and (-) enantiomers obtained were tested on the enzyme, only (+) enantiomers have significant inhibitory property.
14. Alvarez, L. ; Mingorance, J. ; Pajares, M.A. ; Mato, J.M. *Biochem. J.* **1994**, *301*, 557.
15. Sullivan, D.M. ; Hoffman, J.L. *Biochemistry* **1983**, *22*, 1636.