Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 433–437

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Synthesis of Mimics of *S*-Adenosyl-L-Homocysteine as Potential Inhibitors of Erythromycin Methyltransferases

Stephen Hanessian* and Paulo W. M. Sgarbi

Department of Chemistry, Université de Montréal, PO Box 6128, Station Centre-ville, Montréal, QC, Canada H3C 3J7

Received 23 November 1999; accepted 22 December 1999

Abstract—A series of indanotriazine C-ribosides were prepared as SAH mimics, and tested for their ability to inhibit erythromycin resistance methylases Erm AM and Erm C′. A carbocyclic analogue derived from quinic acid was also synthesized and tested.

Erythromycin resistance methylase (Erm) is an enzyme involved in the base-specific *N*-methylation of bacterial 23S ribosomal RNA, utilizing *S*-adenosylmethionine as the methylating agent.[1] Since this modification of the r-RNA takes place near or at the binding sites of macrolide antibiotics, their interaction with the ribosome is rendered ineffective with consequent loss of antibacterial activity. This phenomenon of resistance imparted by methyl transferases such as Erm AM and Erm C′ extends to the macrolide–lincosamine–streptogramin type B (MLS) group of antibiotics.[2,3] The three-dimensional (3-D) structure of Erm AM has been determined by NMR spectroscopy.[4] The crystal structure of Erm C complexed with *S*-adenosylmethionine, reveals binding interactions in the catalytic domain.[5,6]

In view of the importance of these enzymes, efforts have been made to develop inhibitors. Indeed such inhibitors of Erm C′ can sensitize bacteria that are resistant to MLS in vitro and in vivo. In this regard it is of interest to point out that *S*-adenosyl-L-homocysteine (SAH, **1**, Fig. 1) is a naturally occurring inhibitor of methyl transferases (K_i∼40 μM).

Fesik and co-workers[7] have screened a library of small heterocyclic molecules against Erm AM using SAR by NMR. Some of the lead compounds such as **2** and **3** (Fig. 1), have shown binding to Erm AM similar to SAH, as judged by their K_i values and chemical shift changes for the amide NH groups in NMR spectra of drug–enzyme complexes. The triazine core compound **2** and the pyrimidine analogue **3** showed K_i values of 8 and 10 μM, respectively. Fesik and co-workers[7] have postulated specific interaction domains with the enzyme Erm C′ based on a 3.0 Å X-ray crystal structure of the complex. The indane moiety was viewed as filling a hydrophobic crevice composed of the side chains of I185, L86, I106, D84 and D109. H-Bonding of the amino triazine unit was proposed as another interaction with Erm AM and Erm C′. The piperidine and aniline side-chains in **2** and **3** respectively were found to only partially fill the space occupied by the ribose ring in SAH, while the amino acid portion of the latter had no counterpart in the lead inhibitors.

These findings prompted us to consider the design and synthesis of mimics of SAH that would encompass some of the beneficial features of the Abbott compounds, while adding complementary functionality.

We report herein the synthesis of two prototypical SAH mimics of generalized structures **4** and **5** (Fig. 1). We envisaged hybrid structures that contain a common indanotriazine scaffold as an adenine surrogate capable of interacting with a hydrophobic side-chain and of H-bonding. The diol groups in the anhydro sugar unit in **4** and the cyclohexane ring in **5** were intended to mimic the ribose ring in SAH. Functional diversity would be provided by an ether or thioether type side-chain that would correspond to the anilino appendage in **3** and the amino acid unit in SAH, respectively.

Considering that diversification was envisaged at the hydroxymethyl group corresponding to the ribose unit

*Corresponding author. Tel.: +1-514-343-6738; fax: +1-514-343-5728; e-mail: hanessia@ere.umontreal.ca

0960-894X/00/$ - see front matter
PII: S0960-894X(00)00021-4

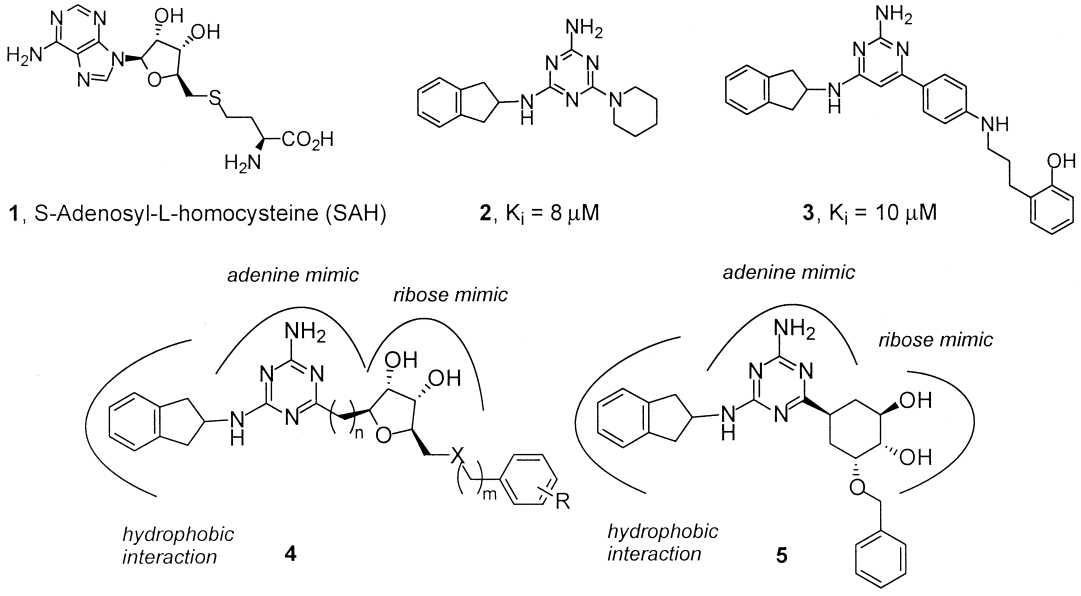
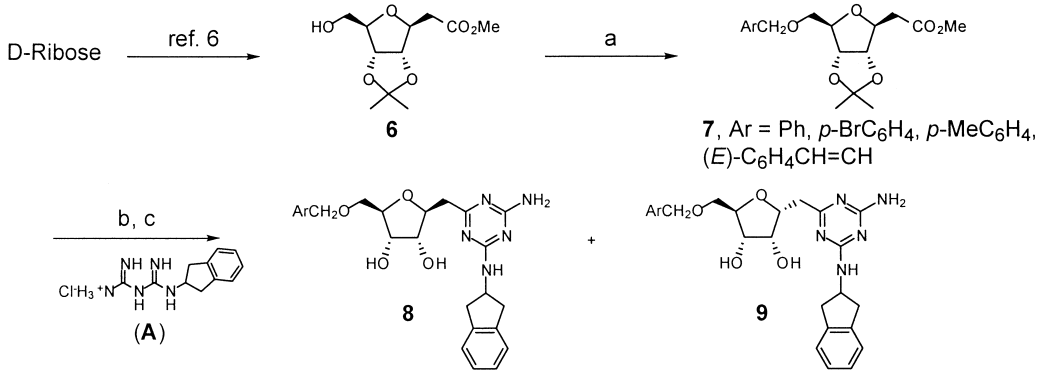


Figure 1.

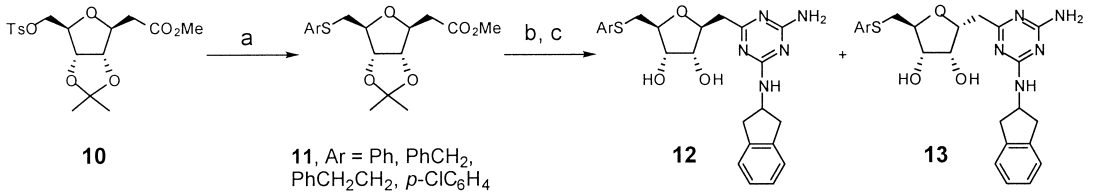
in SAH, the logical strategy was to prepare a core anhydrosugar structure containing the indanotriazene unit, then to conduct appropriate modifications at the intended primary site (Scheme 1). This strategy, simple as it appeared to be, had to be abandoned when it was found that selective alkylation of the primary hydroxyl group in the presence of the aminotriazine unit was not possible. Temporary protection of the heterocycle as the bis-*N*-Boc derivative was not entirely satisfactory, due to the sensitivity of the urethane group to cleavage under basic conditions, and the tendency for anomerization via a base-catalyzed β-elimination and reclosure. In view of the exploratory nature of the project, we proceeded to reverse the order of substitution of the anhydro sugar core (Scheme 1).

Etherification of the readily available hydroxyester **6**,[8,9] led to a series of aralkyl ethers expressed as **7** in excellent yields. Introduction of the indanotriazine was done under basic conditions[10,11] as shown in Scheme 1. Unfortunately, this led to a mixture of anomers because of the acidity of the methylene group in the resulting triazine derivative and an inevitable β-elimination and reclosure. Separation of the isomers and deprotection of the acetal led to the intended prototypes **8** and **9** in each case. Individual compounds as well as anomeric mixtures were tested for inhibition of Erm AM and Erm C′ (vide infra). A series of thioethers represented by **12** and **13** were also prepared by the same protocol (Scheme 2). In order to avoid the isomerization issue, we also prepared the extended-chain analogue **18** shown in Scheme 3.

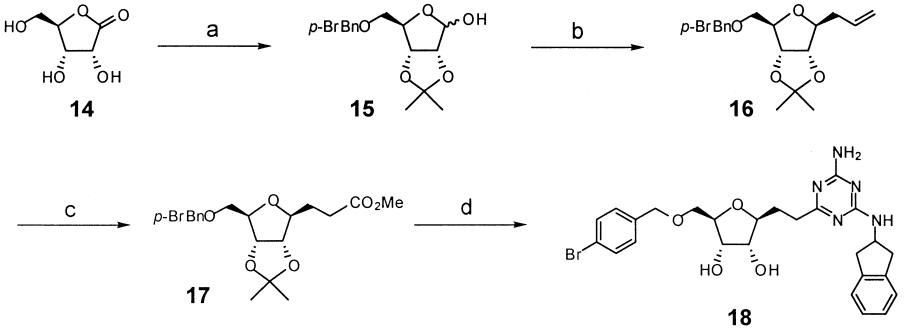
Finally, we used the readily available quinic acid **19**[12] as a template to arrive at the intended carbocyclic SAH mimic **5** as shown in Scheme 4. Deoxygenation[13] of **20**



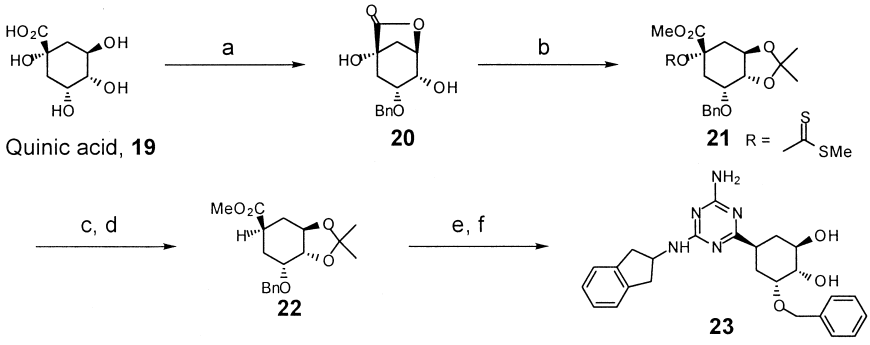
Scheme 1. Conditions: (a) $ArCH_2Br$, NaH, Bu_4NI, DMF, rt, 12 h (85–90%); (b) indanyl-guanidine **A**, NaOMe, MePH, 80 °C; (c) THF/H_2O 9:1, *p*-TsOH, 40 °C (54–78%).



Scheme 2. Conditions: (a) ArSH, NaH, DMF, rt, 6 h (75–88%); (b) indanyl-guanidine **A** (see Scheme 1), NaOMe, MeOH, 80 °C; (c) THF/H_2O 9:1, *p*-TsOH, 40 °C (56–87%).



Scheme 3. Conditions: (a) i. acetone, H_2SO_4, rt, 12 h (85%), ii. NaH, DMF, 0 °C, 30 min; then 4-bromobenzyl bromide, 0 °C to rt, 2 h (90%), iii. DIBAL-H, $PhCH_3$, −78 °C, 12 h (91%); (b) i. phenyl disulfide, PBu_3, $PhCH_3$ (95%), ii. 5.0 eq allyltributyltin, 2.0 eq Bu_3SnOTf, $PhCH_3$, 110 °C, 12 h (82%); (c) i. $BH_3{\cdot}Me_2S$, THF, 0 °C; ii. EtOH, NaOH, H_2O_2 (80% over 2 steps), iii. Jones reagent, acetone, 0 °C, iv. CH_2N_2, ether (95% over 2 steps); (d) indanyl-guanidine **A** (see Scheme 1), NaOMe, MeOH, 80 °C; f. THF/H_2O 9:1, *p*-TsOH, 40 °C (80% over 2 steps).



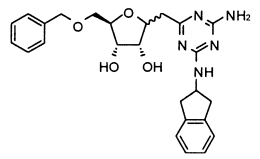
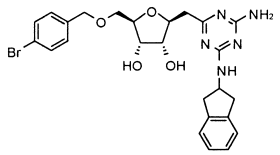
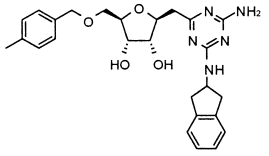
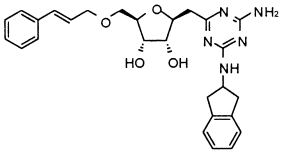
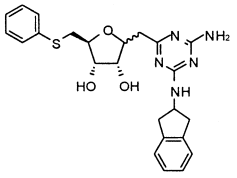
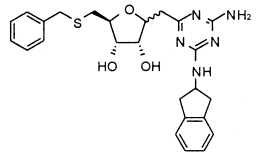
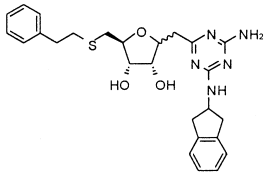
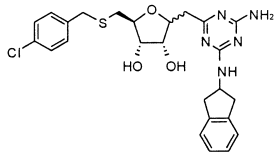
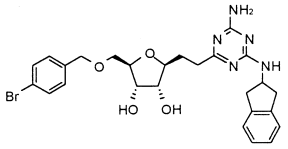
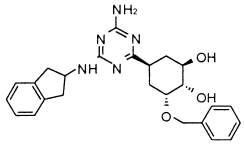
Scheme 4. Conditions: (a) i. *p*-TsOH, PhH, ii. Bu_2SnO, iii. BnBr (75% over 3 steps); (b) i. K_2CO_3, MeOH (100%), ii. 2,2-DMP, CSA, THF, rt (96%); (c) i. NaH, THF, ii. CS_2, iii. MeI (94%); (d) Bu_3SnH, AlBN, $PhCH_3$ (75%); (e) indanyl-guanidine **A** (see Scheme 1), NaOMe, MeOH, 80 °C; f. THF/H_2O 9:1, *p*-TsOH, 40 °C (83% over 2 steps).

via its xanthate ester **21** led to the differentially protected cyclohexane carboxylic acid derivative **22**, which was transformed into **23**.

Table 1 shows the results of the inhibition studies on Erm AM and Erm C′.[14] Regrettably, only two compounds (entries 2 and 8), showed weak but promising activity at K_i values ranging from 40 to 80 μM. It is of interest that even within this limited set of potential inhibitors, some selectivity was observed based on subtle functional changes. A *p*-substituted halogen or a chain extended aromatic ether group appears to be well tolerated (compare entries 1, 3 and 6 with 2, 4 and 8). A methylene bridge between the anhydro sugar and the indanotriazine seems better than an ethylene bridge (entries 2 and 9). Finally, it appears that the anomeric configuration is not playing a crucial role in the interaction with Erm C′ and Erm AM (entries 2 and 8).

These preliminary observations could be useful in designing more effective inhibitors of these important enzymes in the future.

Table 1.

Entry		Erm AM est. K_i (μM)	Erm C′ est. K_i (μM)
1		>100 (16.9%@100)	> (5.5%@100)
2		~75	~40
3		>100 (15%@100)	>100 (0%@100)
4		~90	~60
5		>100 (3.4%@100)	>100 (28%@100)
6		>100 (10%@100)	>100 (10%@100)
7		>100 (18%@100)	50–100 (39.9%@100)
8		~55	~80
9		~50 (55%@100)	50–100 (37.5%@100)
10		>100 (9.9%@100)	>100 (17.7%@100)

Acknowledgement

We thank Abbott Laboratoires for generous financial assistance and for biological testing.

References and Notes

1. Denoya, C.; Dubnam, D. *J. Biol. Chem.* **1989**, *264*, 2615.
2. For a perspective, see Chu, D. T. W.; Katz, L. *J. Med. Chem.* **1996**, 39, 3853.
3. Eady, E. A.; Ross, J. I.; Cove, J. H. *Antimicrob. Chemother.* **1990**, *26*, 461.
4. Yu, L.; Petros, A. M.; Schnuchel, A.; Zhong, P.; Severin, J. M.; Walter, K.; Holzman, T. F.; Fesik, S. W. *Nature Struct. Biol.* **1997**, *4*, 483.
5. Bussière, D. E.; Muchmore, S. W.; Dealwis, C. G.; Schluckebier, G.; Nienaber, V. L.; Edalji, R. P.; Walter, K. A.; Ladrov, U. S.; Holzman, T. F.; Abad-Zapatero, C. *Biochemistry* **1998**, *37*, 7103.
6. Schluckebier, G.; Zhong, P.; Steward, K. D.; Kavanaugh, T. J.; Abad-Zapatero, C. *J. Mol. Biol.* **1999**, *289*, 277.
7. Hajduk, P. J.; Dinges, J.; Schkeryantz, J. M.; Janowick, D.; Kaminski, M.; Tufano, M.; Augeri, D. J.; Petros, A.; Nienaber, V. L.; Zhong, P.; Hammond, R.; Coen, M.; Bentel, B.; Katz, L.; Fesik, S. W. *J. Med. Chem.* **1999**, *42*, 3852.
8. Ohrui, H.; Jones, G. H.; Moffatt, J. G.; Maddox, M. L.; Christensen, A. T.; Byran, S. K. *J. Am. Chem. Soc.* **1975**, *97*, 4602.
9. Hanessian, S.; Ogawa, T.; Guindon, Y. *Carbohydr. Res.* **1974**, *38*, C12.
10. For preparation of indanyl-guanidine, see Shapiro, S. L.; Parrino, V. A.; Geiger, K.; Kobrin, S.; Freedman, L. *J. Am. Chem. Soc.* **1957**, *77*, 5064.
11. For cyclization into a triazine, see Modest, E. J. In *Heterocyclic Compounds*; Elderfield, R. C., Ed.; John-Wiley & Sons, New York, 1961; Vol. 7, pp 627.
12. For a review on the utilization of quinic acid as a chiron in synthesis, see Barco, A.; Benetti, S.; DeRisi, C.; Marchetti, P.; Pollini, E. P.; Zanirato, V. *Tetrahedron: Asymmetry* **1997**, *8*, 3515.
13. Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574
14. For details for the inhibition assay, see ref 7.