Bioorganic & Medicinal Chemistry Letters 20 (2010) 1124–1127

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis, inhibitory activity, and binding mode study of novel DNA methyltransferase 1 inhibitors

Takayoshi Suzuki *, Rikako Tanaka, Shohei Hamada, Hidehiko Nakagawa, Naoki Miyata *

Graduate School of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabe-dori, Mizuho-ku, Nagoya, Aichi 467-8603, Japan

ARTICLE INFO

Article history:
Received 5 November 2009
Revised 30 November 2009
Accepted 3 December 2009
Available online 21 December 2009

Keywords:
DNA methyltransferase
Inhibitor
Non-nucleoside

ABSTRACT

To identify novel non-nucleoside DNA methyltransferase (DNMT) inhibitors, we designed and synthesized a series of maleimide derivatives. Among this series, compounds **5**–**8** were found to be more potent DNMT1 inhibitors than RG108, a DNMT1 inhibitor reported previously by Siedlecki et al. The binding mode analysis of compound **5** is also reported.

© 2009 Elsevier Ltd. All rights reserved.

DNA methylation at the 5-position of cytosine in CpG dinucleotides is a pivotal mechanism for the epigenetic regulation of gene expression. Hypermethylation occurs in the CpG-rich sequence, the so-called CpG islands, where core promoters and transcription initiation sites are located. The hypermethylation in the CpG islands leads to the silencing of genes.[1–3] CpG island-specific hypermethylation is a characteristic common to cancer cells. This causes the silencing of tumor suppressor genes, such as *p16*^{INK4a} and *human mutL homologue 1*, which are involved in the tumorigenic process, including DNA repair, cell cycle regulation, and apoptosis.[4–7] Therefore, DNA methylation inhibitors are regarded as potential anticancer agents.[8]

DNA methylation is catalyzed by DNA methyltransferases (DNMTs) using *S*-adenosyl-L-methionine (SAM) as the methyl donor (Fig. 1). At present, four mammalian DNMTs, namely, DNMT1,[9] DNMT2,[10] DNMT3A, and DNMT3B,[11] are known. Among these, DNMT1 is regarded as the major contributor to DNMT activity and a maintenance methyltransferase in human cells because it shows preference for hemi-methylated DNA substances that are methylated in one strand and unmethylated in the other. The primary function of DNMT1 may be the copying of methylation patterns from the parent DNA strand to the newly replicated daughter strand during the DNA replication process.[12]

The catalytic mechanism for the methylation of cytosine-5 has been studied extensively.[13–15] As depicted in Figure 2, a thiol of the cysteine residue in the active site of DNMTs serves as a nucleophile that attacks the 6-position of cytosine to generate a covalent

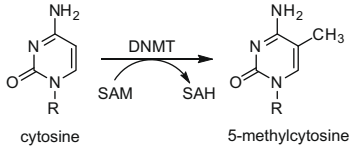


Figure 1. DNA methylation at the 5-position of cytosine catalyzed by DNMT. SAH: *S*-adenosyl-L-homocysteine.

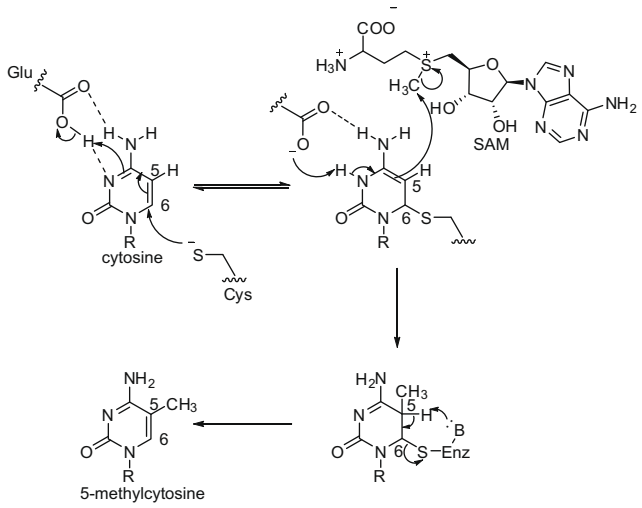


Figure 2. Proposed catalytic mechanism for the methylation of cytosine by DNMT.

* Corresponding authors. Tel./fax: +81 52 836 3407 (N.M.).
E-mail addresses: suzuki@phar.nagoya-cu.ac.jp (T. Suzuki), miyata-n@phar.nagoya-cu.ac.jp (N. Miyata).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.016

DNA-protein intermediate that possesses nucleophilic properties at the 5-position. This reactive intermediate accepts a methyl group from SAM to form the 5-methyl covalent adduct and *S*-adenosyl-L-homocysteine (SAH). Following the methyl transfer, the proton at the 5-position is abstracted by a basic residue in the active site of the enzyme that is removed from the 6-position by β-elimination to generate the methylated cytosine and the free enzyme.

To date, several DNMT inhibitors have been developed.[16–20] Nucleoside analogues, such as 5-azacytidine **1**, 5-fluoro-2′-deoxycytidine **2**, and zebularine **3** (Fig. 3), are DNMT inhibitors that show antiproliferative activity against cancer cells.[16,21–23] However, many nucleoside analogues have problems, such as severe toxicity, which are probably associated with their incorporation into DNA.[24] We therefore started a search for non-nucleoside DNMT inhibitors from the point of view of drug discovery and the discovery of a tool for biological research. In this letter, we report the design, synthesis, inhibitory activity, and binding mode study of novel non-nucleoside DNMT inhibitors.

In designing novel non-nucleoside DNMT inhibitors, we focused on the structure of RG108 **4** (Fig. 3), a DNMT1 inhibitor reported previously by Siedlecki et al.[25] From a computational study, it has been predicted that the carboxylate anion and the carbonyl of the phthalimide form hydrogen bonds with Arg 1310 and Arg 1308, respectively, and the phthalimide group lies next to Cys 1226 in the active site of DNMT1 (Fig. 4a). Based on the structure of the RG108/DNMT1 complex, we designed maleimide derivatives **5**–**13** (Fig. 5). Succinimide **14** (Fig. 5) was also designed as a reference compound. These analogues were expected to undergo conjugate addition from a thiol of Cys 1226 in the active site of DNMT1, which would lead to DNMT1 inhibition (Fig. 4b).

A series of compounds modeled after RG108 were synthesized, as shown in Schemes 1 and 2. Compounds **5**–**13** were synthesized from corresponding maleic anhydrides **15a**–**d** and amines **16a**–**d** in only one step via the route shown in Scheme 1. Heating maleic anhydrides **15a**–**d** and amines **16a**–**d** in refluxing AcOH afforded desired compounds **5**–**13**. The hydrogenation of maleimide **5** using Pd/C produced succinimide **14** (Scheme 2).

The compounds synthesized in this study were tested in an in vitro assay using human DNMT1.[26] The results are summarized in Figure 6. In this enzyme assay, RG108 **4** showed 34% inhibition of DNMT1 activity at the concentration of 1000 μM. Compounds **5**–**8**, in which the phthalimide of RG108 **4** is replaced with various maleimides, exerted more potent DNMT1 inhibitory activity than RG108 **4**. Among these, compound **5** was the most potent DNMT1 inhibitor, displaying over 60% inhibition even at 10 μM. Compounds **9** and **10**, in which the indole ring of **5** is replaced with a phenyl ring and a hydrogen, respectively, resulted in less potent inhibition, suggesting the importance of the indole ring. In addition, compounds **11**–**13** that had no carboxylic acid moiety tended to show less potent inhibitory activity than corresponding carboxylic acids **5**–**7**. These results suggest that the interaction between the carboxylate anion and Arg 1310 is responsible for the DNMT1 inhibitory activity. Compound **14**, in which the maleimide of **5** is replaced with a succinimide, was completely inactive. The reason for the loss of activity is unclear, although it seems reasonable to assume that it is because compound **14** does not have an α,β-unsaturated keto structure and cannot undergo addition from a thiol of Cys 1226 in the active site of DNMT1.

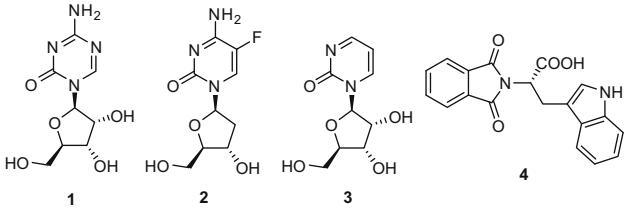


Figure 3. Reported DNMT inhibitors.

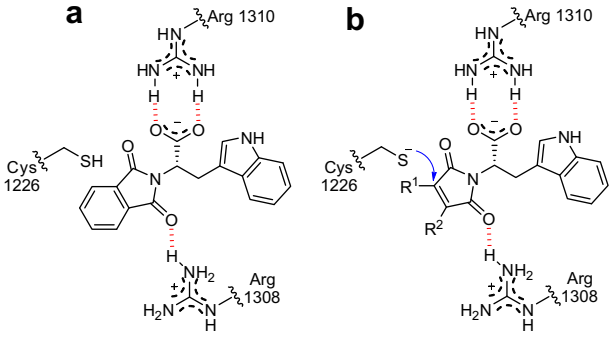
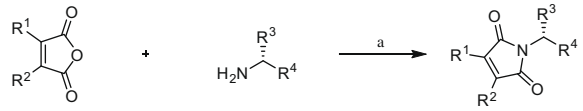


Figure 4. Simulated binding mode of RG108 **4** in the active site of DNMT1 (a), and model for the binding of maleimide derivatives (b).

5-**13** **14**

5: $R^1 = R^2 = H$, $R^3 = COOH$, R^4 = 3-indolyl
6: $R^1 = H$, $R^2 = CH_3$, $R^3 = COOH$, R^4 = 3-indolyl
7: $R^1 = H$, $R^2 = Ph$, $R^3 = COOH$, R^4 = 3-indolyl
8: $R^1 = R^2 = CH_3$, $R^3 = COOH$, R^4 = 3-indolyl
9: $R^1 = R^2 = H$, $R^3 = COOH$, $R^4 = Ph$
10: $R^1 = R^2 = H$, $R^3 = COOH$, $R^4 = H$
11: $R^1 = R^2 = R^3 = H$, R^4 = 3-indolyl
12: $R^1 = H$, $R^2 = CH_3$, $R^3 = H$, R^4 = 3-indolyl
13: $R^1 = H$, $R^2 = Ph$, $R^3 = H$, R^4 = 3-indolyl

Figure 5. Structures of compounds **5**–**14**.

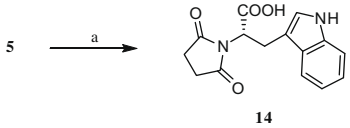


15a: $R^1 = R^2 = H$
15b: $R^1 = H$, $R^2 = CH_3$
15c: $R^1 = H$, $R^2 = Ph$
15d: $R^1 = R^2 = CH_3$

16a: $R^3 = COOH$, R^4 = 3-indolyl
16b: $R^3 = COOH$, $R^4 = Ph$
16c: $R^3 = COOH$, $R^4 = H$
16d: $R^3 = H$, R^4 = 3-indolyl

5-**13**

Scheme 1. (a) AcOH, reflux, 5–68%.



14

Scheme 2. (a) Pd/C, H_2, MeOH, rt, 78%.

To study the binding mode of compound **5** in the active site, we calculated the lowest energy conformation of **5** when it has been docked into the model based on the crystal structure of M.*Hha* I,[27] a DNA methyltransferase from *Haemophilus haemolyticus*, using software packages Glide 3.5 and Macromodel 8.1.[28] An inspection of the complex shows that the indole ring of compound **5** is located in the hydrophobic region formed by the benzene ring of Tyr 254 and the methylene chains of Gly 88 and Gly 255 (Fig. 7). In addition, it is suggested that the carboxylate anion of **5** forms two

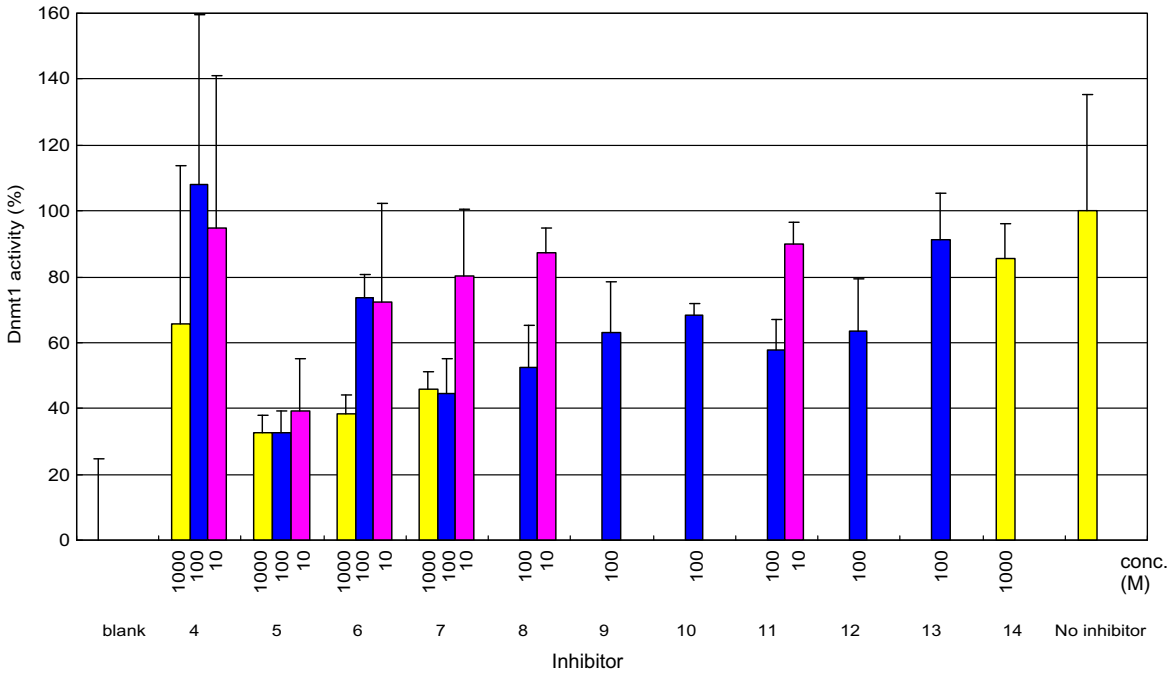


Figure 6. DNMT1 inhibitory activity of compounds **4**–**14**.

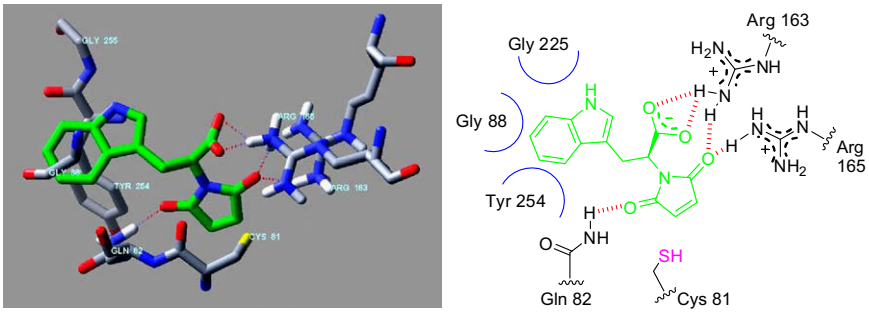


Figure 7. View of the conformation of compound **5** (green) docked in the active site of M.*Hha* I (left) and its schematic representation (right).

hydrogen bonds with Arg 163. It is also shown that one carbonyl of the maleimide forms two hydrogen bonds with Arg 163 and Arg 165, and the other carbonyl forms a hydrogen bond with Gln 82. Further, the maleimide moiety of **5** lies next to Cys 81, where the conjugate addition of a thiol to the maleimide can occur.

In summary, we have identified novel non-nucleoside lead structures from which more potent DNMT inhibitors can be developed. The findings of this study should pave the way for the development of new anticancer drugs. Detailed studies of compound **5** and its analogues are under way.

Acknowledgments

This work was supported in part by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, and a Grant-in Aid for Research in Nagoya City University.

References and notes

1. Ballestar, E.; Esteller, M. *Carcinogenesis* **2002**, *23*, 1103.
2. Herman, J. G.; Baylin, S. B. *N. Eng. J. Med.* **2004**, *350*, 947.
3. Karpf, A. R.; Jones, D. A. *Oncogene* **2002**, *21*, 5496.
4. Li, E.; Beard, C.; Jaenisch, R. *Nature* **1993**, *366*, 362.
5. Li, E.; Bestor, T. H.; Jaenisch, R. *Cell* **1992**, *69*, 915.
6. Okano, M.; Bell, D. W.; Haber, D. A.; Li, E. *Cell* **1999**, *99*, 247.
7. Esteller, M. *Oncogene* **2002**, *21*, 5427.
8. Yu, N.; Wang, M. *Curr. Med. Chem.* **2008**, *15*, 1350.
9. Bestor, T.; Laudano, A.; Mattaliano, R.; Ingram, V. *J. Mol. Biol.* **1988**, *203*, 971.
10. Yoder, J. A.; Bestor, T. H. *Hum. Mol. Genet.* **1998**, *7*, 279.
11. Okano, M.; Xie, S.; Li, E. *Nat. Genet.* **1998**, *19*, 219.
12. Pradhan, S.; Bacolla, A.; Wells, R. D.; Roberts, R. J. *J. Biol. Chem.* **1999**, *274*, 33002.
13. Wu, J. C.; Santi, D. V. *J. Biol. Chem.* **1987**, *262*, 4778.
14. Chen, L.; MacMillan, A. M.; Chang, W.; Ezaz-Nikpay, K.; Lane, W. S.; Verdine, G. L. *Biochemistry* **1991**, *30*, 11018.
15. O'Gara, M.; Klimasauskas, S.; Roberts, R. J.; Cheng, X. *J. Mol. Biol.* **1996**, *261*, 634.
16. Suzuki, T.; Miyata, N. *Curr. Med. Chem.* **2006**, *13*, 935.
17. Kumar, R.; Srivastava, R.; Singh, R. K.; Surolia, A.; Rao, D. N. *Bioorg. Med. Chem.* **2008**, *16*, 2276.
18. Castellano, S.; Kuck, D.; Sala, M.; Novellino, E.; Lyko, F.; Sbardella, G. *J. Med. Chem.* **2008**, *51*, 2321.
19. Isakovic, L.; Saavedra, O. M.; Llewellyn, D. B.; Claridge, S.; Zhan, L.; Bernstein, N.; Vaisburg, A.; Elowe, N.; Petschner, A. J.; Rahil, J.; Beaulieu, N.; Gauthier, F.; MacLeod, A. R.; Delorme, D.; Besterman, J. M.; Wahhab, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2742.
20. Saavedra, O. M.; Isakovic, L.; Llewellyn, D. B.; Zhan, L.; Bernstein, N.; Claridge, S.; Raeppel, F.; Vaisburg, A.; Elowe, N.; Petschner, A. J.; Rahil, J.; Beaulieu, N.; MacLeod, A. R.; Delorme, D.; Besterman, J. M.; Wahhab, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2747.
21. Lubbert, M. *Curr. Top. Microbiol. Immunol.* **2000**, *249*, 135.
22. Christman, J. K. *Oncogene* **2002**, *21*, 5483.
23. Egger, G.; Liang, G.; Aparicio, A.; Jones, P. A. *Nature* **2004**, *429*, 457.

24. Brueckner, B.; Kuck, D.; Lyko, F. *Cancer J.* **2007**, *13*, 17.
25. Siedlecki, P.; Boy, R. G.; Musch, T.; Brueckner, B.; Suhai, S.; Lyko, F.; Zielenkiewicz, P. *J. Med. Chem.* **2006**, *49*, 678.
26. For the in vitro methylation assay, EpiQuik™ DNA Methyltransferase Activity/Inhibitor Assay Kit (Epigentek Group Inc., Brooklyn, NY, USA) and human DNA (cytosine-5) methyltransferase (DNMT1) (New England Biolabs, Inc., Bevery, MA, USA) were used. Test samples were dissolved in DMSO and the solution was diluted by adding 10 parts of PBS. To the DHMT assay buffer in 0.5 mL tubes were added 1.6 mM *S*-adenosyl-L-methionine (AdoMet) and inhibitor solution. Then, DNMT1 was added to the tubes. The reaction mixture was mixed, transferred to the substrate DNA-coated strip wells, and incubated at 37 °C for 60 min. The reaction solution was discarded, methylation DNA capture antibody was added to the strip wells, and the mixture was incubated at room temperature for 60 min. After the capture antibody was aspirated, the detection antibody was added to the strip wells and incubation was carried out at room temperature for 30 min. Then, the antibody was removed, the developing solution was added to the strip wells, and incubation was carried out at room temperature for 10 min. Thereafter, the stop solution was added to the strip wells. The resultant mixture was transferred to a 96-well plate and absorbance at 450 nm was measured.
27. Zhou, L.; Cheng, X.; Connolly, B. A.; Dickman, M. J.; Hurd, P. J.; Hornby, D. P. *J. Mol. Biol.* **2002**, *321*, 591.
28. The X-ray structure of M.*Hha* I (PDB code 1M0E) was used as the target structure for docking. Protein preparation, receptor grid generation, and ligand docking were performed using the software Glide 3.5. Compound **5** was docked into the DNA binding site of M.*Hha* I. The standard precision mode of Glide was used to determine favorable binding poses, which allowed the ligand conformation to be flexibly explored while holding the protein as a rigid structure during docking. Then, the predicted complex structure was fully energy-minimized with both the protein and the ligand allowed to move using Macromodel 8.1 software. The conformation of compound **5** in the DNA binding site was minimized by MM calculation based on the OPLS-AA force field with the following parameter set: solvent: water; method: LBFGS; max # of iterations: 10,000; converge: gradient; convergence threshold: 0.05.