

HETEROCYCLES, Vol. 102, No. 12, 2021, pp. 2372 - 2378. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 1st September, 2021, Accepted, 29th September, 2021, Published online, 8th October, 2021
DOI: 10.3987/COM-21-14547

INVERTED POSITIONING OF DNMT1 INHIBITOR IN THE ACTIVE SITE OF DNMT1 CAUSED BY HYDROPHOBICITY/HYDROPHILICITY OF THE TERMINAL STRUCTURE

Toshifumi Tojo,^{a*} Yuhei Kubo,^a Takeshi Kondo,^{a,b} and Makoto Yuasa^{a,b}

^a Department of Pure and Applied Chemistry, Faculty of Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan

^b Research Institute for Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan

Corresponding author; E-mail: tojo-t@rs.tus.ac.jp

Abstract – DNA (cytosine-5)-methyltransferase 1 (DNMT1) is one of the enzymes that regulate DNA modification. It has been demonstrated that overexpression of DNMT1 is associated with the development of cancer, making DNMT1 an attractive molecular target for cancer therapy. Focused on the terminal structures of existing DNMT1 inhibitors, we designed and screened test compounds that possessed another functional group. Binding simulations identified compounds with a trifluoromethylphenyl group to insert in an inverted position against DNMT1 compared to existing DNMT1 inhibitors. These results suggest that the binding form against DNMT1 may depend on the hydrophobicity/hydrophilicity of the inhibitor's terminal structure.

Recently, 'epigenetics', which is the study of modifications to gene expression rather than to the DNA sequence itself, has become an attractive avenue for cancer therapy.¹ Epigenetics involves the methylation of DNA and acetylation of histones, and is important in various processes such as embryogenesis, cell differentiation, other fundamental life processes.¹ DNA (cytosine-5)-methyltransferase (DNMT), which is one of the enzymes that regulate DNA modification, catalyses the methylation at the 5-carbon position of cytosine's pyrimidine ring.² DNMT1, which is part of the DNMT family, selectively recognizes hemimethylated DNA and catalyses the methylation of specific CpG structures in DNA. In recent studies, overexpression of DNMT1 was shown to result in the developments of lung cancer, oesophageal cancer, gastric cancer and colorectal cancer,³⁻⁶ making DNMT1 an attractive target molecule for cancer therapy. Many DNMT1 inhibitors have since been reported, one of which is SGI-1027, which shows growth

inhibitory activity against human colon cancer cells (HCT116) and human breast cancer cells (MDA-MB-231) (Figure 1).^{7,8} The inhibitory effect of SGI-1027 functions through its competitive interaction with the DNMT1 pocket, which interacts with *S*-adenosylmethionine (SAM).⁸

It has been reported that the quinoline ring of SGI-1027 interacts with the hydrophobic pocket of DNMT1, which associates with SAM,⁸ and contains residues of hydrophobic amino acids (e.g. leucine, phenylalanine).^{9,10} It was suggested that the pyrimidine ring of SGI-1027 interacts with the pocket that associates with DNA bases.⁸ The correlation between DNMT1 inhibitory activity and the inhibitor structure where the functional group of pyrimidine ring was replaced is limited to the amino group, while another functional group (carboxy group, trifluoromethyl group), which can construct hydrogen bond with a residue of amino acid has not been investigated because of the difficulty of introducing of the functional group against the pyrimidine ring.

Herein, we focused on the structure of the pyrimidine ring in DNMT1, and elucidated the correlation between the inhibitory activity of DNMT1 and its structure. In this study, we used a benzoic acid group and trifluoromethylphenyl group instead of pyrimidine ring. We then efficiently synthesized test compounds using a Cu-catalysed coupling reaction.

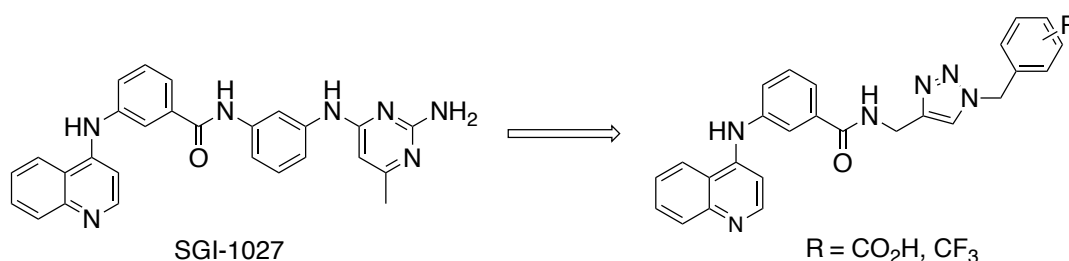
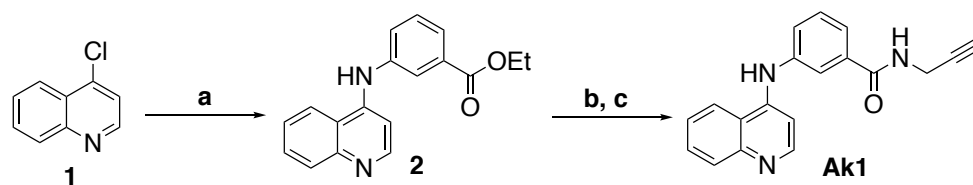


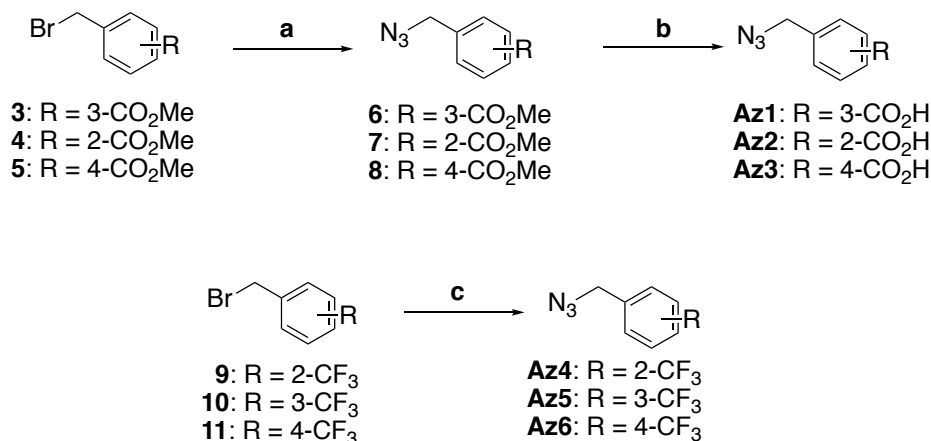
Figure 1. Structures of represent DNMT1 inhibitor (SGI-1027) and test compounds

Compounds used in this study are shown in Figure 1. First, compound **2** was obtained by reacting ethyl 3-aminobenzoate and 4-chloroquinoline under acidic conditions.⁸ **Ak1** was synthesized by a condensation reaction with propargylamine. As shown in Scheme 2, **Az1-3**, which contain a carboxy group, were synthesized by azidation and hydrolysis.^{13,14} Compounds **9-11** were treated with sodium azide to obtain **Az4-6**. The Cu-catalysed coupling reaction between **Ak1** and **Az1-6** provided compounds **A-F** (Scheme 3).¹⁵ All test compounds were purified by HPLC to show >95% purity. In this study, DNMT1 activity assays were performed using a commercially available DNMT1 assay kit and DNMT1 recombinant enzyme. The test compounds (final concentration: 1 μ M) were incubated with DNMT1 for 1 h at 37 °C. Results from these inhibitory activity assays are shown in Figure 2.

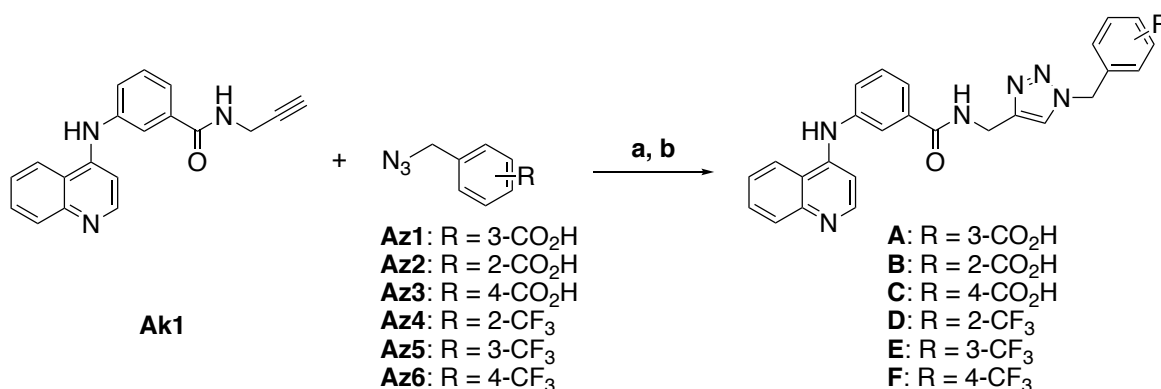
Test compounds with a trifluoromethylphenyl group (compounds **D-F**) demonstrated higher inhibitory activity than the benzoic acid group (compounds **A-C**). In addition, compound **F** showed stronger DNMT1 inhibitory activity than SGI-1027 (compound **F**: 40% inhibition, SGI-1027: 28% inhibition).



Scheme 1. Synthesis of Ak1^a (^aReagents and conditions: (a) ethyl 3-aminobenzoate, concHCl, EtOH, 90 °C, 2 h, 91%; (b) 2M KOH, EtOH, rt, overnight; (c) Propargylamine, EDC·HCl, HOBt, DMF, rt, overnight, 50-64% over 2 steps)



Scheme 2. Synthesis of Az1-6^a (^aReagents and conditions: (a) NaN₃, DMF, 80 °C, overnight, (b) 2 M LiOH, 1,4-dioxane/H₂O, rt, overnight, 87-92% over 2 steps, (c) NaN₃, DMSO, rt, overnight, 53-88%)



Scheme 3. Synthesis of test compounds A-F^a (^aReagents and conditions: (a) CuSO₄, sodium ascorbate, DMSO/H₂O, 60 °C, 64-67% (compound A), (b) CuSO₄, sodium ascorbate, DMF/H₂O, 60 °C, 16-22.5 h, 53-87% (compounds B-F))

Next, we focused on the correlation between the position of the functional group and its inhibitory activity. In the benzoic acid group, compound **A** (3-carbon) presented with the greatest inhibitory activity, followed in order by compound **C** (4-carbon) and compound **B** (2-carbon) (compound **A**: 26% inhibition, compound **B**: 12% inhibition, compound **C**: 17% inhibition). In addition, to investigate concentration dependence, we tested the inhibitory activity of compound **A** and compound **A** showed 42% inhibition at 100 μM. On the other hand, in the trifluoromethylphenyl group, compound **F** (4-carbon) presented with the greatest inhibitory activity, followed in order by compound **D** (2-carbon) and compound **E** (3-carbon)

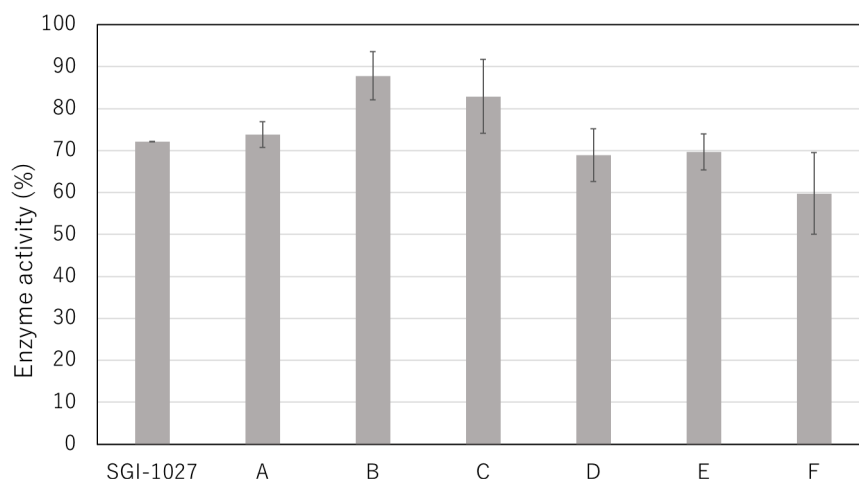
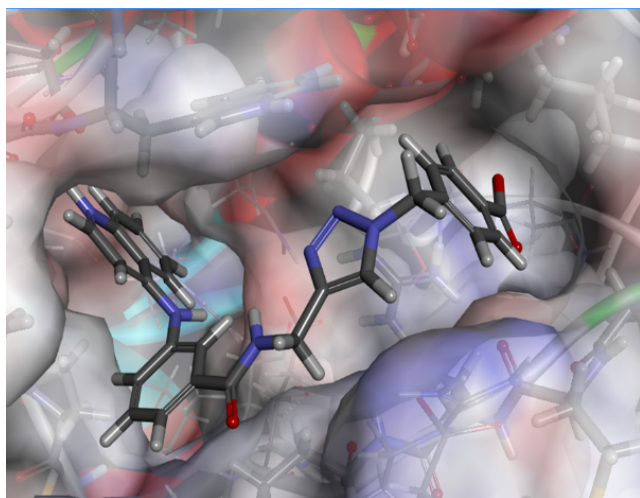


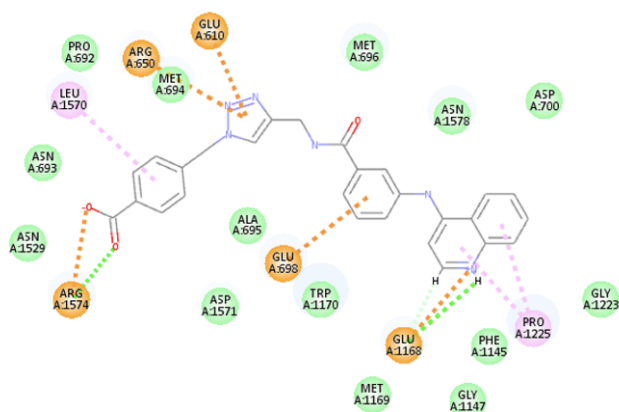
Figure 2. *In vitro* DNMT1 inhibitory activity of test compounds A-F and SGI-1027. The % enzyme activity in the presence of 1 μ M of test compounds A-F or SGI-1027.

(compound **D**: 31% inhibition, compound **E**: 30% inhibition, compound **F**: 40% inhibition). In addition, to investigate concentration dependence, we tested the inhibitory activity of compound **F** and compound **F** showed 61% inhibition at 100 μ M. Altogether, these suggest that the strength of DNMT1 inhibitory activity depends on the kind of functional group at specific positions. In this study, we showed that compounds with a benzoic acid group or trifluoromethylphenyl group show great potential as a DNMT1 inhibitor. Interestingly, inhibitor assays revealed a difference in the strength of DNMT1 inhibitory activity that depended on the position of the functional group. To investigate the mechanism behind this observation, we performed binding simulations of compounds **C** and **F** with DNMT1 using the Discovery Studio software. As shown in Figures 3 (A) and (B), the quinoline ring was positioned in the pocket of DNMT1, which interacts with SAM, while the benzoic acid group associated with DNMT1 in the pocket, which interacts with the base of DNA. This result suggested that compound **C** associates with DNMT1 in the same mechanism as that of SGI-1027.⁸ On the other hand, the quinoline ring of compound **F** coordinated with DNMT1 within its pocket, with its trifluoromethylphenyl group aiding in the positioning (Figures 3 (C) and (D)). Interestingly, this result revealed that compound **F** interacts with DNMT1 in an inverted position compared to SGI-1027 or compound **C** (compound **C**: the triazole ring coordinates with Met 694, the phenyl ring coordinates with Glu 698, and the quinoline ring coordinates with Pro 1225; compound **F**: *N-H* group of the quinoline ring coordinates with Met 694, the triazole ring coordinates with Glu 698, and trifluoromethylphenyl group coordinates with Pro1225). For SGI-1027, it has been reported that the quinoline ring coordinates with the pocket of DNMT1, which interacts with SAM, while its pyrimidine ring coordinates with the pocket, which interacts with the base of DNA.⁸ Compared with the pocket of DNMT1, which interacts with SAM, studies have shown that there are many residues of hydrophilic amino acids (i.e. arginine, glutamic acid) in the pocket, which interacts with the base of DNA.¹⁷

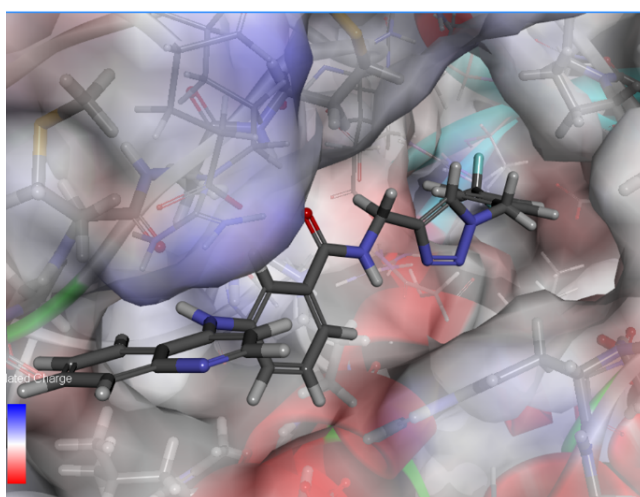
(A) compound C (benzoic acid group)



(B) compound C (benzoic acid group)



(C) compound F (trifluoromethylphenyl group)



(D) compound F (trifluoromethylphenyl group)

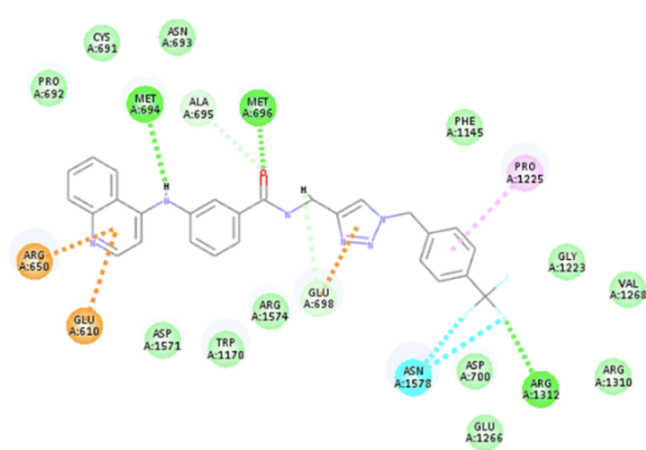


Figure 3. (A), (C) View of the conformation of compounds **C** and **F** docked in the DNMT1 catalytic core. (B), (D) View of schematic diagram of compounds **C** and **F** docked in the DNMT1 catalytic core. These compounds were docked into a model based on the crystal structure of DNMT1 (PDB code: 3SWR) using Discovery Studio software.

Our binding simulations between compound **C** and DNMT1 revealed that a hydrogen bond and electrostatic interaction were formed between the carboxy group of compound **C** and Arg 1574 of DNMT1. On the other hand, the affinity between the trifluoromethylphenyl group of compound **F** and the hydrophilic pocket of DNMT1 may be lower than that of compound **C**. In addition, the quinoline ring may show some affinity for both the hydrophilic and hydrophobic pockets of DNMT1. Therefore, we have identified the aforementioned factors to potentially drive the inverted binding between compound **F** and DNMT1. A study has shown that a bisquinoline derivative, which has a quinoline ring instead of a pyrimidine ring, shows the same level of inhibitory activity against DNMT1 as SGI-1027.⁸ These results suggest that a quinoline ring may coordinate with both pockets of DNMT1, and compound **F** may interact with DNMT1 in an inverted position relative to SGI-1027.

Next, we performed binding simulations between DNMT1 and compounds **D** and **E**, which have different positioning of the trifluoromethyl group from compound **F**. Interestingly, compounds **D** and **E** also showed similar inverted binding as with compound **F** (SI). These results suggested that the trifluoromethylphenyl group may coordinate with the DNMT1 pocket regardless of its position, and the binding form against DNMT1 may depend on the hydrophobicity of the DNMT1 inhibitor terminal structure.

In conclusion, we elucidated that a benzoic acid or trifluoromethylphenyl group show the inhibitor activity against DNMT1 instead of pyrimidine ring. Interestingly, our results also suggest that the binding form against DNMT1 may invert depending on the hydrophobicity/hydrophilicity of the DNMT1 inhibitor terminal structure. We expect future studies to further investigate the correlation between DNMT1 inhibitory activity and the structure of the inhibitor as it is straightforward to introduce a functional group into a phenyl group. We conclude that it is important that the inverted position of a DNMT1 inhibitor be considered when designing DNMT1 inhibitors.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Shin Aoki for the technical support.

REFERENCES

1. S. Sharma, T. K. Kelly, and P. A. Jones, *Carcinogenesis*, 2010, **31**, 27.
2. R. Z. Jurkowska, T. P. Jurkowski, and A. Jeltsch, *ChemBioChem, A.*, 2011, **12**, 206.
3. F. Chik and M. Szyf, *Carcinogenesis*, 2011, **32**, 224.
4. J. F. Linnekamp, R. Butter, R. Spijker, J. P. Medema, and H. W. M. van Laarhoven, *Cancer Treat. Rev.*, 2017, **54**, 10.
5. A. Erdmann, L. Halby, J. Fahy, and P. B. Arimondo, *J. Med. Chem.*, 2015, **58**, 2569.
6. L. Halby, Y. Menon, E. Rilova, D. Pechalrieu, V. Masson, C. Faux, M. A. Bouhleb, M. H. David-Cordonnier, N. Novosad, Y. Aussagues, A. Samson, L. Lacroix, F. Ausseil, L. Fleury, D. Guianvarc'h, C. Ferroud, and P. B. Arimondo, *J. Med. Chem.*, 2017, **60**, 4665.
7. J. Datta, K. Ghoshal, W. A. Denny, S. A. Gamage, D. G. Brooke, P. Phiasivongsa, S. Redkar, and S. T. Jacob, *Cancer Res.*, 2009, **69**, 4277.
8. S. Valene, Y. Liu, M. Schnkenburger, C. Zwergel, S. Cosconti, C. Gros, M. Tardugno, D. Labella, C. Florean, S. Minden, H. Hasimoto, Y. Chang, X. Zhang, G. Kirsch, E. Novellino, P. B. Arimondo, E. Miele, E. Ferretti, A. Gulino, M. Diederich, X. Cheng, and A. Mai, *J. Med. Chem.*, 2014, **57**, 701.
9. C. Zwergel, M. Schnkenburger, F. Sarno, C. Battistelli, M. C. Manara, G. Stazi, R. Mazzone, R. Fioravanti, C. Gros, F. Ausseil, C. Florean, A. Nebbioso, R. Strippoli, T. Ushijima, K. Scotlandi, M.

- Tripodi, P. B. Arimondo, L. Altucci, M. Diederich, A. Mai, and S. Valente, *Clin. Epigenet.*, 2019, **11**, 68.
10. E. Rilova, A. Erdmann, C. Gros, V. Masson, Y. Aussagues, V. Poughon-Cassabois, A. Rajavelu, A. Jeltsch, Y. Menon, N. Novosad, J. M. Gregoire, S. Vispé, P. Schambel, F. Ausseil, F. Sautel, P. B. Arimondo, and F. Cantagrel, *ChemMedChem*, 2014, **9**, 590.
 11. J. Yoo, S. Choi, and J. L. Medina-Franco, *PLoS One*, 2013, **8**, e62152.
 12. J. P. Wolfe and S. L. Buchwald, *J. Org. Chem.*, 2000, **65**, 1144.
 13. J. M. Smith, F. Vitali, S. A. Archer, and R. Fasan, *Angew. Chem. Int. Ed.*, 2011, **50**, 5075.
 14. B. Peschke, M. Zundel, S. Bak, T. R. Clausen, N. Blume, A. Pedersen, F. Zaragoza, and K. Madsen, *Bioorg. Med. Chem.*, 2007, **15**, 4382.
 15. J. J. Yang, W. W. Yu, L. L. Hu, W. J. Liu, X. H. Lin, W. Wang, Q. Zhang, P. L. Wang, S. W. Tang, X. Wang, M. Liu, W. Lu, and H. K. Zhang, *J. Med. Chem.*, 2020, **63**, 569.
 16. M. Esteller, *Oncogene*, 2002, **21**, 5427.
 17. Z. M. Zhang, S. Liu, K. Lin, Y. Luo, J. J. Perry, Y. Wang, and J. Song, *J. Mol. Biol.*, 2015, **427**, 2520.