Synthesis and Evaluation of 8-halogenated-7-deaza-2'-deoxy-guanosine as 8-oxo-2'-deoxy-guanosine analogues

尹, 貽貞

出版情報：九州大学, 2014, 博士（創薬科学）, 課程博士
バージョン：

Synthesis and Evaluation of 8-halogenated-7-deaza-2′-deoxy-guanosine as 8-oxo-2′-deoxy-guanosine analogues

Bioorganic and Synthetic Chemistry　　3PS12001P　　尹　貽貞

【Introduction】

8-Oxo-2′-deoxyguanosine (8-oxo-dG) is a representative nucleoside damage that is formed by oxidation of 2′-deoxyguanosine (dG) with reactive oxygen species (ROS), and its presence has been linked to aging, cancer, etc [1]. Unlike dG, 8-oxo-dG forms stable base pairs with both 2′-deoxycytidine (dC) and 2′-deoxyadenosine (dA). Based on the base-pairing properties of 8-oxo-dG, DNA polymerases incorporate 8-oxo-dGTP opposite dA and dATP opposite 8-oxo-dG, causing AT to CG and GC to TA transversion mutations. To suppress the genotoxicity of 8-oxo-dG and protect the genome integrity, hOGG1 can excise 8-oxo-dG from 8-oxo-dG:dC base pairs within duplex DNA. And hMYH provides the defense by removing dA opposite 8-oxo-dG. To prevent the incorporation of 8-oxo-dGTP into DNA, hMTH1 hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP that is further hydrolyzed by nucleotidase (Figure 1).

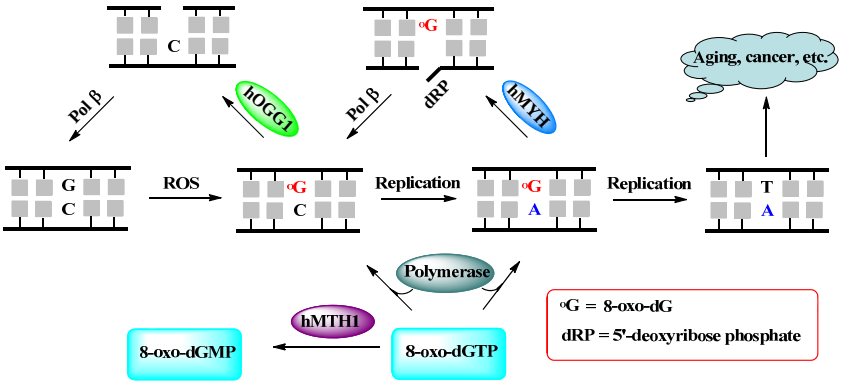


Figure 1. Mutation and repair of 8-oxo-dG

Recently, some DNA repair enzymes such as DNA polymerase β and hOGG1 have been regarded as antitumor targets. Especially, hMTH1 is responsible for removing of oxidized nucleotides and required for survival of cancer cells [2]. 8-Halogenated-7-deaza-dG derivatives were designed as 8-oxo-dG analogues to elucidate the contributions of N7-H and C8-oxygen to the base pairing, replication and repair of 8-oxo-dG. In this study, I have attempted to find out functional inhibitors of DNA repair enzymes among the 8-halogenated-7-deaza-dG derivatives (Figure 2).

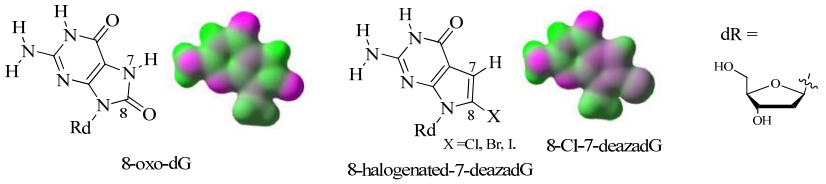
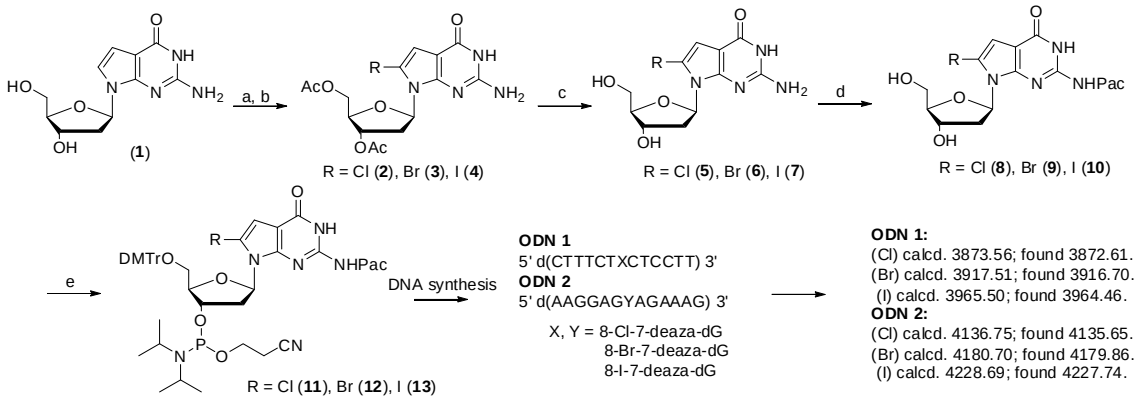


Figure 2. Structures of 8-oxo-dG and 8-halogenated-7-deazadG derivatives

【Experiments and Results】

1. Synthesis and base pairing properties of 8-halogenated-7-deaza-dG derivatives.

The syntheses of 8-halogenated-7-deaza-dG derivatives were achieved via the reaction between acetylated 7-deaza-dG and *N*-halogenated succinimides. These compounds were incorporated into the central part of 13-mer oligonucleotides (Scheme 1). The properties of these derivatives were investigated by computational, NMR and thermal denaturing studies. The significant upfield shift of the C-2′ signals and characteristic downfield shift of H-2′ signals indicated that 8-halogenated-7-deaza-dG derivatives prefer *syn*-conformation in DMSO solution similarly to 8-oxo-dG (Table 1). It was shown that the base pair of 8-halogenated-7-deaza-dG with dC was destabilized compared with dG, supporting their preference for *syn* conformation. Unlike 8-oxo-dG, 8-halogenated-7-deaza-dG did not form a stable base pair with dA, most likely due to the lack of N7-H hydrogen bonding with dA (Table 2 and Figure 3). Therefore, the newly-designed 8-halogenated-7-deaza-dG derivatives resemble 8-oxo-dG in shape and preference for *syn* conformation, but they do not form Hoogsteen base pair with the opposite dA.



Scheme 1. Reagents and conditions: (a) acetic anhydride, pyridine, 88%. (b) NRS (R = Cl, Br and I), DMF, 86%, 51% and 21%, respectively. (c) 7N ammonia in MeOH, 60-70%. (d) TMSCl, pyridine, followed by phenoxyacetic anhydride, pyridine, 70-80%. (e) (1) DMTrCl, pyridine. (2) 2-cyanoethyl-N,N'-diisopropyl-chlorophosphoroamidite, DIPEA, CH_2Cl_2, 39-61% over two steps.

Table 1. Selected chemical shift of corresponding diol compounds[a]

Nucleoside	C1'	C2'	H2'	C8
dG	82.5	39.5	2.49	135.2
8-oxodG	81.2	35.7	2.96	151.6
deazadG	82.2	39.2	2.30	116.7
Cl-deazadG (**5**)	83.0	37.2	2.99	115.0
Br-deazadG (**6**)	84.2	37.3	3.04	101.9
I-deazadG (**7**)	86.8	37.5	3.12	71.3

[a] ^{1}H and ^{13}C NMR shifts were recorded using 0.04 M corresponding diol compounds in DMSO-d6.

Table 2. Melting temperatures for the duplexes containing 8-halogenated-7-deaza-dG[a]

ODN 1: 5' d(CTTTCTXCTCCTT) 3'
ODN 2: 5' d(AAGGAGYAGAAAG) 3'

	X			
Y	dC	dA	dG	dT
dG	44.1	35.4	35.7	33.7
8-oxodG	42.3	39.2	33.4	32.2
deazadG	41.6	35.4	32.0	33.1
Cl-deazadG (**5**)	38.0	30.3	31.4	30.8
Br-deazadG (**6**)	37.0	30.1	31.0	30.5
I-deazadG (**7**)	35.3	29.4	31.4	29.3

[a] Conditions: 2 μM duplex, 100 mM NaCl and 10 mM sodium phosphate buffer, pH 7.0.

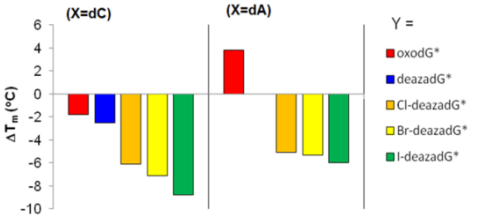


Figure 3. The bar graphs for the *ΔTm* values between the corresponding dG analogues and dG in 2 μM solutions of duplex DNA. (*ΔTm*(Z) = *Tm* (Z) - *Tm* (dG), Z represents modified dG).

2. Recognition and excision of 8-halogenated-7-deazadG in DNA duplex by 8-oxo-dG glycosidase.

I next tested the recognition and excision of 8-halogenated-7-deazadG derivatives in DNA duplex by Fpg and hOGG1. After incubation of the DNA duplex containing 8-oxo-dG analogues with Fpg and hOGG1 at 37°C, β-elimination and δ-elimination products are obtained which can be reflected on the gel (Figure 4). 8-Halogenated-7-deazadG derivatives, especially 8-Cl-7-deazadG, were good glycosidase substrates for Fpg. However, 8-halogenated-7-deazadG derivatives were slightly excised by hOGG1. Quartz crystal microbalance (QCM) provided the direct observation of the time courses of interaction between 8-halogenated-7-deazadG containing duplex and Fpg or hOGG1 (Figure 5 and Table 3). In the case of Fpg, the association rate constant (k_{on}) for dG or 7-deaza-dG was smaller than that for 8-oxo-dG and 8-halogenated-7-deazadG, suggesting that introducing C8-oxygen or C8-halogen help to the recognition by

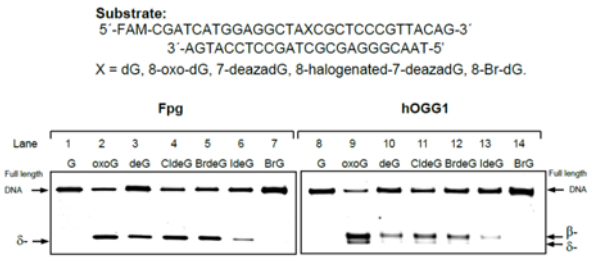


Figure 4. Glycosidase activity of 8-halogenated-7-deazadG in DNA with Fpg and hOGG1. β– and δ– indicate β emination and δ elimiantion prodcuts, respectively.

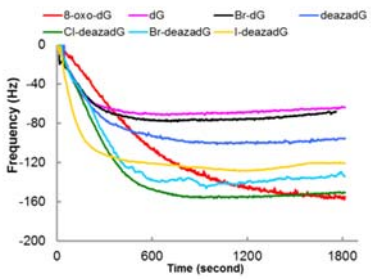


Figure 5. The time course interaction between hOGG1 and duplex DNA containing 8-oxo-dG analogues by QCM analysis.

Table 3. Kinetic parameters of duplex DNA containing 8-halogenated-7-deaza-dG derivatives for Fpg and hOGG1[a]

Duplex Sequence: 5'-CGATCATGGAGGCTAXCGCTCCCGTTACAG-3' / 3'-GCTAGTACCTCCGATCGCGAGGGCAATGTCTTTTT-Biotin-5'

		Fpg			hOGG1		
Entry	X =	k_{on} ($10^3 M^{-1}s^{-1}$)[b]	k_{off} ($10^{-3}s^{-1}$)[b]	K_d (nM)[c]	k_{on} ($10^3 M^{-1}s^{-1}$)[b]	k_{off} ($10^{-3}s^{-1}$)[b]	K_d (nM)[c]
1	8-oxo-dG	67.1 ± 24.8	0.78 ± 0.29	11.6 ± 2.8	70.1 ± 26.2	1.27 ± 0.47	18.1 ± 2.7
2	dG	25.6 ± 6.7	2.00 ± 0.52	78.2 ± 15	131.4 ± 26.6	3.44 ± 0.70	26.2 ± 7.0
3	Br-dG	50.9 ± 24.6	2.80 ± 1.35	55.0 ± 13	135.0 ± 47.7	3.56 ± 1.26	26.4 ± 3.6
4	deaza-dG	23.9 ± 12.0	0.54 ± 0.27	22.4 ± 5.7	97.6 ± 16.3	3.62 ± 0.60	37.1 ± 7.2
5	Cl-deaza-dG	40.6 ± 14.7	0.58 ± 0.21	14.3 ± 4.4	127.7 ± 54.2	2.44 ± 1.04	19.1 ± 4.9
6	Br-deaza-dG	42.2 ± 3.6	0.59 ± 0.05	14.0 ± 1.1	136.9 ± 46.0	2.77 ± 0.93	20.2 ± 4.6
7	I-deaza-dG	36.0 ± 13.6	1.17 ± 0.44	32.5 ± 3.1	181.9 ± 64.7	4.51 ± 1.60	24.8 ± 3.6

[a] Data are means (± standard deviation) of three or more independent experiments. [b] k_{on} and k_{off} were calculated from k_{obs} and *Kd*. [c] K_d (the dissociation constant) were obtained by stepwise injection.

Fpg. Interestingly, the dissociation rate constants (k_{off}) for 7-deaza-dG derivatives were similar to 8-oxo-dG, implying the importance of the presence of hydrogen at 7-position. In the case of hOGG1, 8-oxo-dG exhibited much lower k_{off} value than the other compounds, probably arising from the strong hydrogen bonding between 7-NH with Gly42 in the active site of hOGG1 (Figure 6). Although 8-Cl- and 8-Br-7-deazadG had lower k_{off} value than 8-oxo-dG, they exhibited higher k_{on} which resulted in the similar dissociate constant to 8-oxo-dG. Accordingly, it has been demonstrated that 8-halogenated-7-deaza-dG containing duplexes are competitive inhibitors for the glycosidase activity of hOGG1 to excise 8-oxo-dG in duplex DNA (Figure 7).

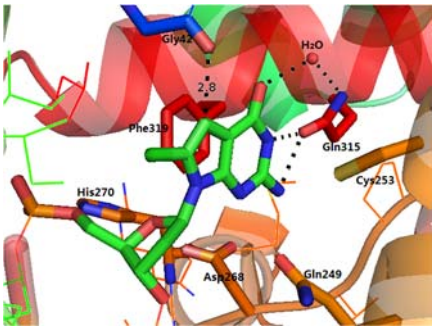


Figure 6. 8-Cl-7-deazadG in the active site of hOGG1.

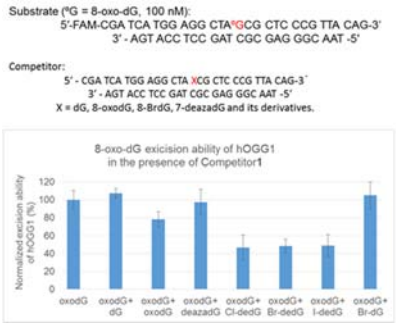
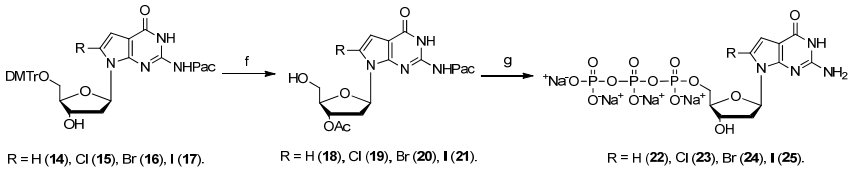


Figure 7. Bar graph of normalized excision ability of hOGG1 in the presence of corresponding 400 nM of competitor DNA (dG, oxodG, deazadG, Cl-deazadG, Br-deazadG, I-deazadG and Br-dG).

3. Synthesis and evaluation of 8-halogenated-7-deaza-dGTP.

I next synthesized 7-deaza-dGTP as well as 8-Cl-, 8-Br- and 8-I-7-deaza-dGTP using the conventional method (Scheme 2). Although 8-halogenated-7-deaza-dGTP derivatives were hardly hydrolyzed by hMTH1 (Figure 8A), they showed competitive inhibitory activity against 8-oxo-dGTP hydrolysis by hMTH1 (Figure 8B). Docking study (Autodock 4.2.6) and molecular dynamic simulation (NAMD 2.10) revealed that 8-I-7-deazadGTP may adopt *anti*-conformation and place itself in the almost same position as 8-oxo-dGTP. However, Trp117 in the active site of hMTH1 was shifted 2 Å as compared to its position in the complex of 8-oxo-dGMP and hMTH1, which might enhance the π-π interaction between Trp117 and 8-I-7-deazadGTP (Figure 8C). Furthermore, the IC_{50} values of 8-Cl-7-deazadGTP, 8-Br-7-deazadGTP and 8-I-7-deazadGTP were determined to be 0.857, 0.496 and 0.415 μM, respectively (Table 4). And their corresponding inhibition constants (K_i) were respectively calculated to be 116.8, 78.2 and 61.7 nM by Lineweaver-Burk plots (Table 5). The diol compound of 8-I-7-deazadG exhibited some inhibitory effect against hMTH1 but it is 100-fold weaker than 8-I-7-deazadGTP, implying the importance of the triphosphate group. Interestingly, 8-halogenated-7-deazadGTP exhibited much higher inhibitory activities against hMTH1 than SCH51344 and (S)-Crizotinib. Therefore, it is expected that 7-deazadGTP and 8-halogenated-7-deazadGTP would show antitumor activity by targeting hMTH1.



Scheme 2. (f) Acetic anhydride, pyridine, 61-74%; 3% trichloroacetic acid in CH_2Cl_2, 62-84%. (g) i: 2-chloro-4H-1,3,2-benzodioxaphosphorin-4-one, pyridine/1,4-dioxane (1:1); ii: $(HBu_3N^+)_2$ $H_2P_2O_7^{2-}$, Bu_3N, DMF; iii: I_2, pyridine/H_2O (98:2); iv: 28% ammonia solution, 27-43%.

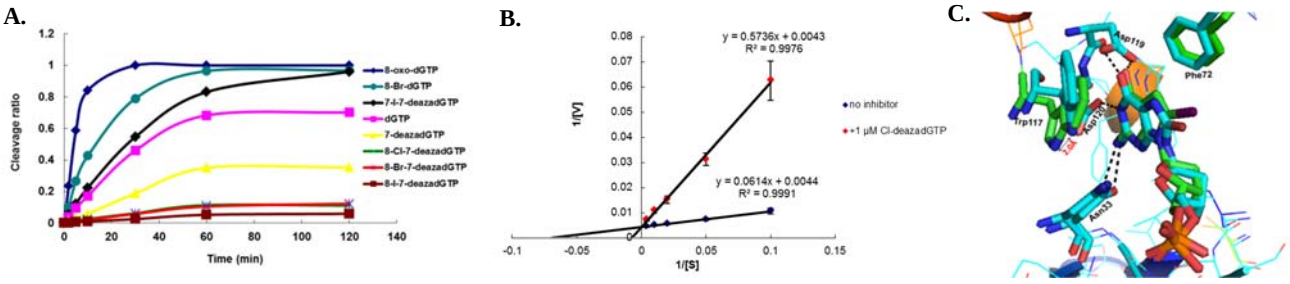


Figure 8. (A) Time course of hydrolysis of 7-deazadGTP and 8-halogenated-7-deaza-dGTP with hMTH1; (B) Lineweaver-Burk plots of 8-oxo-dGTP hydrolysis by hMTH1 in the absence of inhibitor or presence of 8-Cl-7-deazadGTP ; (C) Alignment of 8-I-deazadGMP after simulation (Autodock 4.2.6 and NAMD 2.10) with 8-oxo-dGMP crystal structure (PDB: 3ZR0).

Table 4. IC_{50} values of hMTH1 inhibitors[a]

	IC_{50} (μM)
dGTP	1997.8±337.4
7-deazadGTP	1.57±0.12
8-Cl-7-deazadGTP	0.857±0.155
8-Br-7-deazadGTP	0.496±0.021
8-I-7-deazadGTP	0.415±0.056
8-I-7-deazadG	44.81±7.44
8-Br-dGTP	167.49±40.83
7-I-7-deazadGTP	2.62±0.12
SCH51344	2.23±0.20
(S)-Crizotinib	1.00±0.13
TH287	0.0104±0.00101

[a]condition: 20 mM Tris-HCl PH 7.5, 4 mM $MgCl_2$, 40 mM NaCl, 80 μg/mL BSA, 8 mM DTT, 10% glycerol; 5 nM hMTH1; 50 μM 8-oxo-dGTP; various concentrations of dGTP, 7-deazadGTP, 8-halogenated-7-deazadGTP, 8-I-7-deazadG, 8-Br-dGTP, 7-I-7-deazadGTP, SCH51344, (S)-Crizotinib and TH287; 1% DMSO (for SCH51344, 8-I-7-deazadG, (S)-Crizotinib and TH287). Data are means (± standard deviation) of three or more independent experiments.

Table 5. Steady-state kinetic parameters and inhibition constants of 8-oxo-dGTP hydrolysis by hMTH1 in the absence or presence of inibitors[a]

Inhibitor	k_{cat} (S^{-1})	K_m (μM)	K_i (nM)
No inhibitor	18.94	13.95	
1 μM of 7-deazadGTP	17.73	79.45	213.0
1 μM of 8-Cl-7-deazadGTP	19.38	133.40	116.8
0.5 μM of 8-Br-7-deazadGTP	18.52	103.09	78.2
0.25 μM of 8-I-7-deazadGTP	17.01	70.43	61.7
3 μM of 7-I-7-deazadGTP	18.94	78.30	650.4
1 μM of SCH51344	14.12	65.78	—[b]
1 μM of (S)-Crizotinib	19.38	82.65	203.1
0.01 μM of TH287	17.01	63.33	2.83

[a]condition: 20 mM Tris-HCl PH 7.5, 4 mM $MgCl_2$, 40 mM NaCl, 80 μg/mL BSA, 8 mM DTT, 10% glycerol; 2 nM hMTH1; 10, 20, 50, 100, 250 μM 8-oxodGTP; in the absence of inhibitor or in the presence of inhibitor; 37°C; 1% DMSO (for SCH51344, S-Crizotinib, or TH287); incubated for 4 min (no inhibitor) or 10 min (with inhibitor). [b] K_i for SCH51344 was not calculated.

It was found that 8-halogenated-7-deazadGTP were only slightly incorporated into DNA to pair with dC and hardly incorporated to pair with dA by KF-exo⁻ and human polymerase β (Figure 9). Moreover, 8-halogenated-7-deazadG derivatives in duplex DNA were tested to be difficult to pair with dA during replication process. Therefore, 8-halogenated-7-deazadGTP derivatives are expected to have little side effects, further supporting their potentials as antitumor agents.

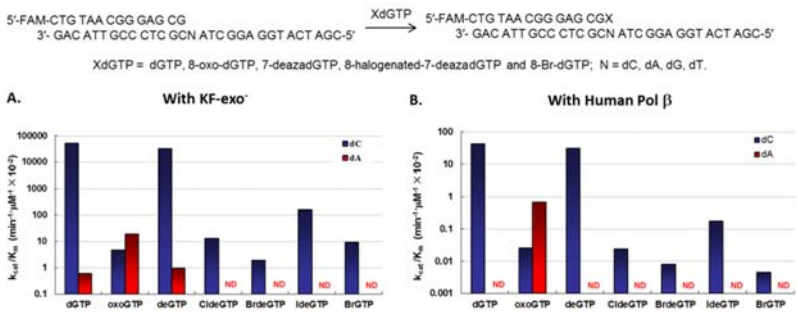


Figure 9. Incorporation of dGTP, 8-oxo-dGTP, 7-deazadGTP, 8-halogenated-7-deazadGTP and 8-Br-dGTP opposite dC, dA, dG and dT by KF-exo⁻ and Human polymerase β. A) Bar graph of incorporation efficiency (k_{cat}/K_m) with KF-exo⁻; B) Bar graph of incorporation efficiency (k_{cat}/K_m) with Human polymerase β.

【Conclusion】

8-Halogenated-7-deaza-dG derivatives were designed as 8-oxo-dG analogues, and their syntheses and incorporations into oligonucleotides were successful. 8-halogenated-7-deaza-dG derivatives resemble 8-oxo-dG in shape and preference for *syn*-conformation confirmed by the DFT calculations and NMR studies, but they do not form Hoogsteen base pair with the opposite dA based on the lower T_m values as compared to 8-oxo-dG. Interestingly, 8-halogenated-7-deaza-dG derivatives in duplex DNA, especially 8-Cl-7-deaza-dG, were good glycosidase substrates for Fpg and strong binders to hOGG1. Accordingly, 8-halogenated-7-deaza-dG derivatives in duplex DNA demonstrated competitive inhibition for the glycosidase activity of hOGG1 to excise 8-oxo-dG in duplex DNA. Furthermore, 8-halogenated-7-deazadGTP were successfully synthesized and demonstrated as strong inhibitors of hMTH1 at nanomolar concentrations. It is interesting that 8-halogenated-7-deazadGTP derivatives exhibited low mutagenic potential by DNA polymerases, implying that they might have little side effects. Thus, this study has clearly demonstrated that 8-halogenated-7-deazadG derivatives are potential to be used as probes and functional inhibitors of 8-oxo-dG repair enzymes.

【References】

1. Burrows, C. J.; Muller, J. G. *Chem. Rev.*, **1998**, *98*, 1109–1152.
2. Gad H.; Koolmeister T.; Jemth A.S.; Eshtad S.; Jacques S.A.; Ström C.E. et al. *Nature*, **2014**, *508*, 215-221; Huber K.V.; Salah E.; Radic B.; Gridling M.; Elkins J.M.; Stukalov A.; Jemth A.S. et al. *Nature*, **2014**, *508*, 222-227.

【Publications】

1. Yin, Y.; Taniguchi, Y.; Sasaki, S. Synthesis of 8-halogenated-7-deaza-2′-deoxyguanosine as an 8-oxo-2′-deoxyguanosine analogue and evaluation of its base pairing properties. *Tetrahedron*, **2014**, 70, 2040-2047.
2. Yin, Y.; Sasaki, S.; Taniguchi, Y. Recognition and excision properties of 8-Halogenated-7-deaza-2′-deoxyguanosine as 8-oxo-2′-deoxyguanosine analogue by Fpg and hOGG1. *Submitted*.