Synthesis and evaluation of the 7,8-substituted-deaza-dGTP/dGMP derivatives as hMTH1 inhibitors and conferring their triphosphates cell membrane permeability

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論　文　名	Synthesis and evaluation of the 7,8-substituted-deaza-dGTP/dGMP derivatives as hMTH1 inhibitors and conferring their triphosphates cell membrane permeability (hMTH1 阻害剤としての 7,8-置換-デアザ-dGTP / dGMP 誘導体の合成と評価およびそれらのトリリン酸の細胞膜透過性の付与)
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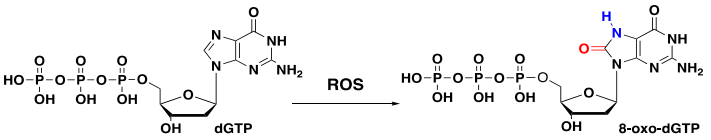
論　文　審　査　の　結　果　の　要　旨

Cellular DNA is continuously damaged by reactive oxygen species (ROS). 8-Oxo-2'-deoxyguanosine (8-oxo-dG) is a representative nucleoside damage that is formed by oxidation of the 8 position of 2'-deoxyguanosine. 8-Oxo-dG triphosphates (8-oxo-dGTP) is formed from dGTP with ROS (Scheme 1), and is incorporated into DNA for both 2'-deoxycytidine (dC) and 2'-deoxyadenosine (dA) as the template at the stage of replication (Fig 1). The misincorporation of 8-oxo-dG into DNA for the dA template cause the transversion mutation, therefore, effective repair systems remove 8-oxo-dG from the cells. hMTH1 is an essential repair enzyme that hydrolyzes 8-oxo-dGTP to 8-oxo-dGMP to prevent its misincorporation into DNA. The cancer cells generate much amount of 8-oxo-dGTP than normal cells by excessive ROS, overexpressed hMTH1 hydrolyzes it to prevent cell death. It has been reported that hMTH1 activity is essential for cancer cell survival and the selective inhibitors for hMTH1 activity is possible as anticancer agents to suppress cancer cells growth by incorporating 8-oxo-dGTP into the DNA. Recently, we reported that 8-iodo-7-deaza-dGTP as an 8-oxo-dGTP mimicry efficiently inhibited the hMTH1 hydrolysis activity. In this study, Hui Shi designed and synthesized the novel 7,8-substituted 7-deaza-dGTP/dGMP derivatives. Moreover, she evaluated these nucleoside analogues to inhibition of hMTH1 activity and conferring their triphosphates cell membrane permeability.



Scheme 1. Oxidation of dGTP by ROS

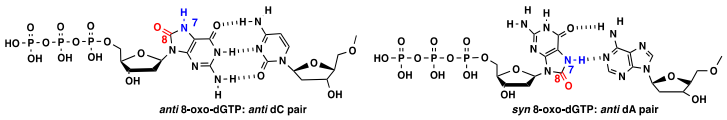
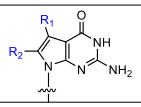


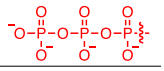
Fig 1. Base pair of 8-oxo-dGTP with dC and dA

According to the result of the X-ray crystal structural analysis of the complex between hMTH1 and 8-iodo-7-deaza-dGTP, Hui Shi designed the 7- or 8-substituted 7-deaza-dGTP derivatives. In addition, they have different polarizability and dipole moment between 7- or 8-substituted 7-deaza-dG and 8-oxoG by the computational method. She expected that modification of nucleobase induced the change of electronic environment of the nucleobase moiety, then the binding affinity and π-π interaction with the enzyme are further enhanced. In order to reveal the triphosphate of 7- or 8-substituted 7-deaza-dG, she also designed and tested the corresponding monophosphate compounds.

Firstly, Hui Shi synthesized these triphosphate and monophosphate derivatives and evaluated the

Table 1. The IC_{50} values of hMTH1 inhibitors





Entry	R_1	R_2	Diol IC_{50} (μM)	Monophosphate IC_{50} (μM)	Triphosphate IC_{50} (μM)
1	H	Ph	≥ 1000	-	-
2	H	TMS(ethynyl)	67.1 ± 4.3	-	-
3	H	CF_3	64.3 ± 12.6	0.76 ± 0.12	0.51 ± 0.06
4	H	Ethynyl	27.8 ± 6.6	-	-
5	Cl	Cl	22.5 ± 3.1	0.13 ± 0.02	0.16 ± 0.02
6	Br	Br	10.7 ± 3.2	0.26 ± 0.03	0.11 ± 0.03
7	I	I	17.0 ± 7.2	0.13 ± 0.02	0.13 ± 0.04
8	H	H	47.9 ± 7.9	10.4 ± 0.12	1.57 ± 0.12[1)]
9	H	Cl	-	0.58 ± 0.08	0.86 ± 0.16[1)]
10	H	I	44.8 ± 7.4[1)]	0.84 ± 0.19[1)]	0.42 ± 0.06[1)]

Conditions: 20mM Tris-HCl PH 7.5, 4mM $MgCl_2$, 40mM NaCl, 80 μg/mL BSA, 8mM DTT, 10% glycerol; 50 μM 8-oxo-dGTP, 5 nM hMTH1;various concentrations of of 8-Ph-7deazadG, 8-TMS(ethynyl)-7-deazadG, 8-ethynyl-7-deazadG, 8-CF_3-7-deazadG, 7,8-dihalogenated-7- deazadG, in 1% DMSO, 8-Cl-7-deazadGMP, 8-I-7-deazadGMP, 8-CF_3-7-deazadGMP, 7,8-dihalogenated-7-deazadGMP, 8-I-7-deazadGTP, 8-CF_3-7-deazadGTP and 7,8-di- halogenated-7-deazadGTP.
1) Yin Y. Sasaki S. Taniguchi Y. ChemBioChem.

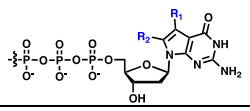
inhibitory effect of these derivatives. These triphosphate derivatives could not be hydrolyzed by hMTH1.

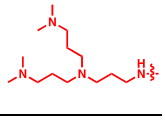
The IC_{50} values were shown in Table 1. She found that 7,8-dihalogenated triphosphate and monophosphate derivatives (**Entry 5-7**) have stronger inhibitory activity than the 8-iodo-7-deazadGTP (**Entry 8**). Additionally, the phosphate at the 5′ position is very important for inhibiting hMTH1 by comparing the IC_{50} values of diol derivatives and triphosphate, monophosphate derivatives. According to steady-state kinetic parameters, these 7- or 8-substituted 7-deaza-dGTP and 7-deaza-dGMP exhibited competitive inhibition.

However, since these triphosphate derivatives have various negative charges of the triphosphate moiety, and are very hydrophilic, they cannot penetrate the hydrophobic cell membrane composed of phospholipids into the target cells to inhibit hMTH1. Recently, it has been reported that cationic groups attached to ATP analogues promote cell permeability and a cell-permeable ATP analogue ATP–polyamine–biotin (APB) was developed. Therefore, she designed the two kinds of γ-aminomodified-7,8-dihalogenated-7-deazadGTP derivatives to expect to promote membrane permeability.

According to the parallel artificial membrane permeability assay, the log P_e values of γ-aminomodified-dGTP and -7,8-dihalogenated-7-deazadGTP were higher than that of unmodified dGTP and 7,8-dihalogenated-7-deazadGTP, which showed that the amine modification at the γ position can improve the membrane permeability (Table 2).

Table 2. The log P_e (cm/s) values of dGTP and 7,8-dihalogenated-7-deazadGTP and γ-modified-dGTP and 7,8-dihalogenated -7-deazadGTP



Compounds	log P_e (cm/s)	log P_e (cm/s)	log P_e (cm/s)
dGTP	-7.23 ± 0.10	-6.69 ± 0.12	-6.31 ± 0.25
$R_1 = R_2 =$ Cl	-7.11 ± 0.15	-6.62 ± 0.11	-6.56 ± 0.04
$R_1 = R_2 =$ Br	-6.93 ± 0.04	-6.48 ± 0.03	-6.45 ± 0.29
$R_1 = R_2 =$ I	-6.81 ± 0.08	-6.58 ± 0.23	-6.52 ± 0.04

condition: PBS (PH 7.4), dGTP, 7,8-dihalogenated-7-deazadGTP, γ-aminomodified-dGTP and γ-aminomodified-7,8-dihalogenated-7-deazadGTP in PBS solution (500 μM).

In this study, Hui Shi designed and successfully synthesized various 7- and 8-position modified 7-deazadG triphosphate and monophosphate derivatives. She found that 7,8-dihalogenated 7-deaza-dGTP/dGMP derivatives cannot be hydrolyzed by hMTH1 but strongly inhibit hMTH1 activity. Additionally, the phosphate at the 5′ position is very important for inhibiting hMTH1 by comparing the IC_{50} values of diol derivatives and triphosphate, monophosphate derivatives. The log Pe values of 7,8-dihalogenated-7-deazadGTP and γ-aminomodified-7,8-dihalogenated-7-deazadGTP showed that the amine modification at the γ position can improve cell membrane permeability.

Therefore, this study reveals new possibilities for new functions of nucleic acid derivatives, and we admit that this paper is worth the doctoral degree of (Medicinal Sciences).