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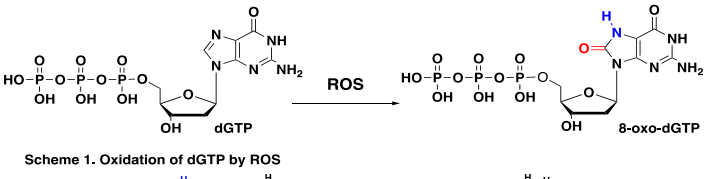
論文題名　：Synthesis and evaluation of the 7,8-substituted-deaza-dGTP/dGMP derivatives as hMTH1 inhibitors and conferring their triphosphates cell membrane permeability（hMTH1 阻害剤としての 7,8-置換-デアザ-dGTP/dGMP 誘導体の合成と評価およびそれらのトリリン酸の細胞膜透過性の付与）

区　　分　：甲

論　文　内　容　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, we designed and synthesized the novel 7,8-substituted 7-deaza-dGTP/dGMP derivatives. Moreover, we evaluated these nucleoside analogues to inhibit the hMTH1 activity.



Scheme 1. Oxidation of dGTP by ROS

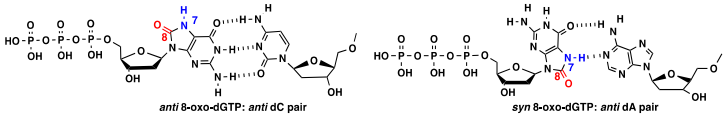
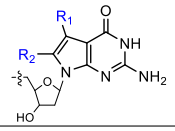
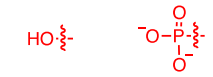


Fig 1. Base pair of 8-oxo-dGTP with dC and dA

According to the result of the X-ray crystal structural analysis of the complex between hMTH1 and 8-iodo-7-deaza-dGTP, we 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. We expect 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. Normally, this enzyme interacted with α-position of triphosphate. Therefore, in order to reveal the triphosphate of 7- or 8-substituted 7-deaza-dG, we also designed and tested the corresponding monophosphate compounds.

We synthesized these triphosphate and monophosphate derivatives and evaluated the inhibitory effect of these derivatives. These triphosphate derivatives could not be hydrolyzed by hMTH1. The IC_{50} values were





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	-	-
3	H	CF_3	64.3	0.76	0.51
4	H	Ethynyl	27.8	-	-
5	Cl	Cl	22.5	0.13	0.16
6	Br	Br	10.7	0.26	0.11
7	I	I	17.0	0.13	0.13
8*	H	I	44.8	0.84	0.42

Table 1. IC_{50} values of deazadG derivatives.
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-dichloro-7-deazadG, 7,8-dibromo-7-deazadG and 7,8-diiodo-7-deazadG in 1% DMSO, 8-iodo-7-deazadGMP, 8-CF_3-7-deazadGMP, 7,8-dichloro-7-deazadGMP , 7,8-dibromo-7-deazadGMP and 7,8-diiodo-7-deazadGMP, 8-iodo-7-deazadGTP, 8-CF_3-7-deazadGTP, 7,8-dichloro-7-deazadGTP , 7,8-dibromo-7-deazadGTP and 7,8-diiodo-7-deazadGTP.
*Yin Y. Sasaki S. Taniguchi Y. ChemBioChem.

shown in Table 1. We 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, we designed the two kinds of γ-polyamine-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 dGTP modified by two different amines are higher than that of dGTP at the same concentration, which showed that the amine modification at the γ position can improve the membrane permeability (Table 2). The membrane permeability of γ-modified-7,8-dihalogenated-7-deazadGTP derivatives are investigating by parallel artificial membrane permeability assay.

Table 2. The log P_e (cm/s) values of dGTP and γ-modified-dGTP

Entry	Compounds	Concentration (μM)	log P_e (cm/s)
1		10	-5.79 ± 0.38
2	dGTP	100	-6.36 ± 0.15
3		500	-7.23 ± 0.10
4	**32a**	10	-5.30 ± 0.27
5		100	-6.11 ± 0.25
6		500	-6.31 ± 0.25
7	**32b**	10	-5.25 ± 0.09
8		100	-6.13 ± 0.04
9		500	-6.69 ± 0.12

In this study, we designed and successfully synthesized various 7- and 8-position modified 7-deazadG triphosphate and monophosphate derivatives. We found that 7,8-dihalogenated 7-deaza-dGTP/dGMP derivatives strongly inhibit hMTH1 activity. The log Pe values of dGTP and γ-polyamine-dGTP showed that the amine modification at the γ position can improve cell membrane permeability. The membrane permeability of γ-modified-7,8-dihalogenated-7-deazadGTP derivatives are investigating.