ANTICANCER RESEARCH *38*: 4241-4246 (2018)
doi:10.21873/anticanres.12720

Structure-associated Functional Control of TX-1877 Series by Glyco-conjugation

KAZUTO OHKURA[1], YUKI KAWAGUCHI[2], YOHEI TATEMATSU[1], ATSUSHI TABATA[3], YOSHIHIRO UTO[3] and HITOSHI HORI[3]

[1]*Graduate School of Pharmaceutical Sciences, Suzuka University of Medical Science, Suzuka, Japan;*
[2]*Faculty of Pharmaceutical Sciences, Chiba Institute of Science, Choshi, Japan;*
[3]*Department of Bioengineering, Division of Bioscience and Bioindustry, Graduate School of Technology, Industrial and Social Sciences, Tokushima University, Tokushima, Japan*

Abstract. *Background/Aim: Sugar molecules are often used as a tool to structurally modify chemical compounds. The features of synthesized sugar-conjugated TX-1877 derivatives were herein examined. Materials and Methods: The molecular stabilities (reactivity) and hydrophobicities of sugar (e.g., monosaccharide and tetra-O-acetylated monosaccharide)-conjugated TXs were analyzed using a molecular simulation (e.g. molecular mechanics (MM) and molecular orbital (MO) analysis). Results: The hydrophobicities of TX-1877 derivatives were increased by tetra-O-acetylation, and TX-2244 exhibited the most potent radiosensitizing activity (enhancement ratio: ER=2.30). Conclusion: The conformations and hydrophobicities of chemical compounds may be controlled by adding monosaccharide- and tetra-O-acetyl-conjugated sugars to TX-1877.*

Sugar moieties have been effectively utilized in drug design to improve water solubility and for molecular recognition. Tumor tissues actively consume sugar, and the addition of a sugar moiety to designed drugs improves their uptake by them. The affinity of drugs to tumor tissue may be enhanced by the conjugation of sugar moieties. One of the strategies to potentially improve the affinity of synthesized compounds to tumor tissue is sugar conjugation to a pharmacophoric moiety. Sugar-hybrid molecules designed to exploit this strategy have been prepared by other groups (1, 2).

In the present study, the structural features of the TX-1877 glyco-conjugated series were analyzed. We previously designed and synthesized TX-1877 to be a more potent radiosensitizer than etanidazole and exhibit additional biological activities, such as anti-metastatic and immunopotentiative activity (3-6). TX-1877 was conjugated with several sugar moieties, such as β-glucose (β-Glc), β-galactose (β-Gal), α-mannose (α-Man), tetra-*O*-acetyl β-Glc (β-Glc(OAc)4), tetra-*O*-acetyl β-Gal (β-Gal$(OAc)_4$), tetra-*O*-acetyl α-Man (α-Man$(OAc)_4$), *N*-acetyl-β-galactosamine (β-GalNAc), and tri-*O*-acetyl β-GalNAc (β-GalNAc$(OAc)_3$), in order to examine the effects of sugar moieties on structure-associated functional control.

Correspondence to: Professor Kazuto Ohkura, Graduate School of Pharmaceutical Sciences, Suzuka University of Medical Science, 3500-3 Minamitamagaki-cho, Suzuka, Mie 513-8670, Japan. Tel: +81 593400611, Fax: +81 593681271, e-mail: kohkura@suzuka-u.ac.jp

Key Words: Molecular design, TX-1877, structure glyco-conjugation.

Materials and Methods

Molecular design and analysis. TX-1877 and sugar conjugated TX-1877 derivatives were designed and synthesized as previously described (4, 6). Conformation analysis of synthesized TX-1877 series were performed using CAChe-Conflex (Fujitsu Inc., Tokyo, Japan), and energy profiles were obtained as described previously (7, 8). Solvation free energy (stereo-hydrophobicity: dGW) was determined using Mopac (Fujitsu Inc., Tokyo, Japan) as previously described (9, 10).

In vitro radiosensitizing assay. *In vitro* radiosensitization was measured in EMT6/KU single cells under hypoxic conditions as previously described (11). Enhancement ratios (ERs) were determined from the ratio of radiation doses required to reduce the surviving fraction of EMT6/KU cells to 1%. Usually, each ER value of radiosensitizer was obtained from survival curves consisting of four or five points per curve and converted based on the ER value of etanidazole (ER=1.72) (12).

Results

Structure of glyco-conjugated TX-1877 series. Glyco-conjugated TX-1877 derivatives are shown in Figure 1. Monosaccharides were added to TX-1877, and TX-1877 derivatives (β-glucose: TX-2141, β-galactose: TX-2218, and α-mannose: TX-2217) were synthesized. The hydroxyl groups of the sugar moieties were acetylated and tetra-*O*-acetyl compounds (TX-2244, TX-2245, and TX-2246) were synthesized. Compounds in which an

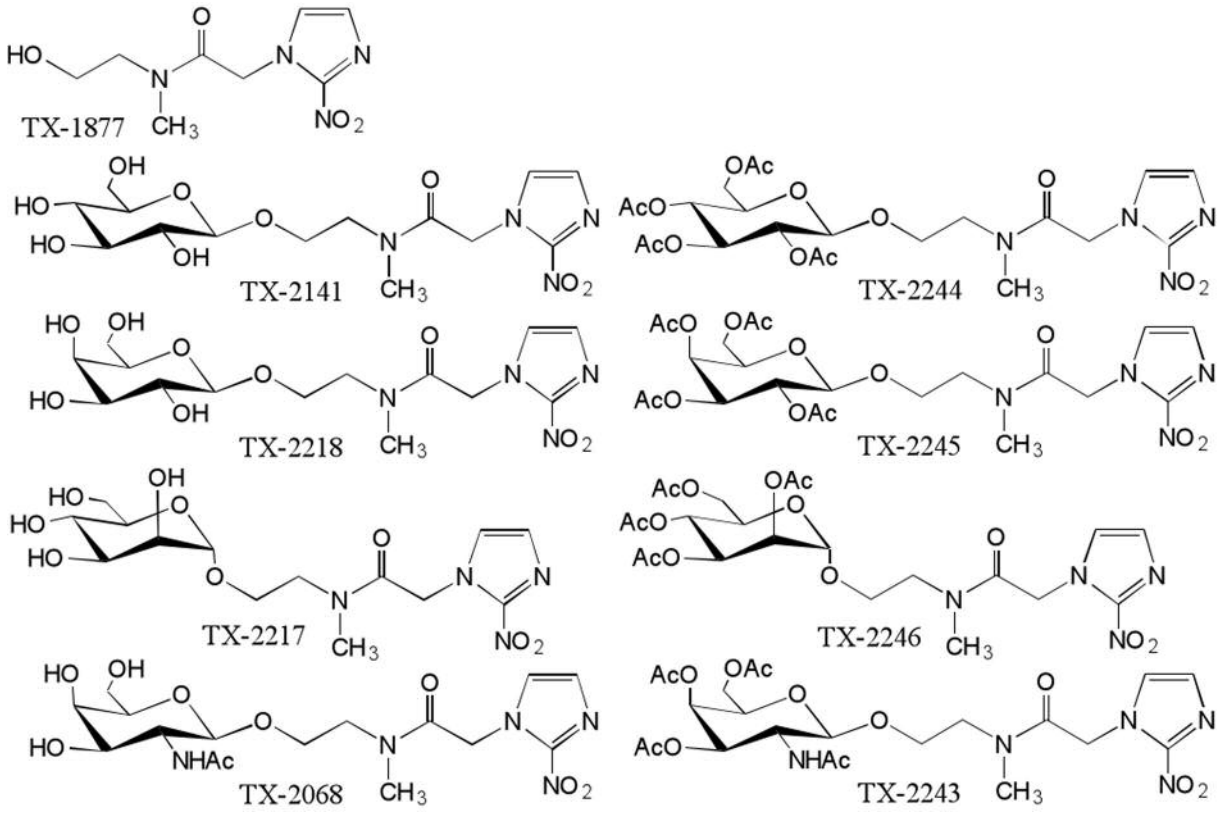

Figure 1. *Structure of sugar-conjugated TX-1877 and its derivatives.*

N-acetyl group (TX-2068) and *N*-,*O*-acetyl (TX-2243) had been introduced were synthesized from TX-2218 (11).

Energy profiles of glyco-conjugated series. TX-1877 generated 71 conformers (Table I), and their heat formation energies ranged between 12.881 and 18.520 kcal/mol (Figure 2A). The solvation-free energy (dGW: an index of stereo-hydrophobicity) of TX-1877 was approximately -160 kJ/mol (Figure 2E). β-glucose-conjugated TX-2141 had 402 conformers (27.712–43.694 kcal/mol), and their dGW values decreased from -170 kJ/mol to -238 kJ/mol (Figure 2B and 2F). TX-2218 and TX-2217 had 333 (24.177–45.751 kcal/mol) and 357 (26.307 – 42.406 kcal/mol) conformers, respectively (Figure 2C and 2D). The dGW values of TX-2218 (-246 – -166 kJ/mol, Figure 2G) and TX-2217 (-224 – -188 kJ/mol, Figure 2H) decreased during the conformational analysis. The molecular hydrophobicities of sugar-conjugated TX-1877 derivatives increased with increments of their heat formation energies.

More conformers were detected in the conformational analysis of tetra-*O*-acetyl-modified TX-1877 derivatives (TX-2244, -2245, and -2246) than in that of monosaccharide-conjugated derivatives (*e.g.*, TX-2141, -2218, and -2217). TX-2244 had 517 conformers (7.616-21.798 kcal/mol, Figure 3A), and dGW values decreased from -229 to -283 kJ/mol (Figure 3D). TX-2245 and -2246 had 659 (7.521-28.315 kcal/mol, Figure 3B) and 548 (6.082-20.343 kcal/mol, Figure 3C) conformers, respectively. Their dGW values decreased during

Table I. *Conformer and enhancement ratios (ER) of TX-1877 and its derivatives.*

Compound	Conformer No.	ER[a]
TX-1877	71	1.75
TX-2141	402	1.33
TX-2218	333	1.40
TX-2217	357	1.41
TX-2244	517	2.30
TX-2245	659	1.63
TX-2246	548	1.88
TX-2068	235	1.43
TX-2243	334	1.47

[a]Data from reference 11.

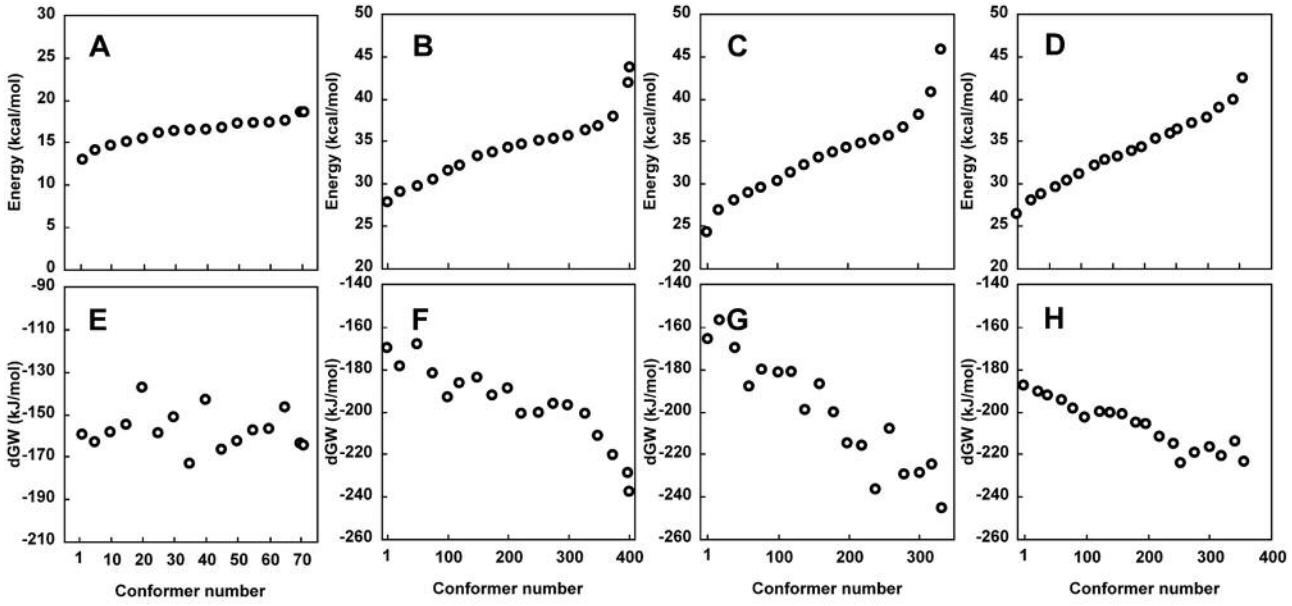

Figure 2. *Energy profile of conformation analysis of TX-1877 and monosaccharide conjugated derivatives. Heat formation energy profiles of TX-1877 (A), TX-2141 (B), TX-2218 (C), TX-2217 (D). Solvation free energy (stereo-hydrophobicity (dGW)) of TX-1877 (E), TX-2141 (F), TX-2218 (G), TX-2217 (H).*

the conformational analysis (TX-2245: from -245 to -281 kJ/mol (Figure 3E), TX-2246 from -229 to -264 kJ/mol (Figure 3F)). Heat formation energies decreased with the tetra-*O*-acetylation of sugar moieties, and molecular stability increased. The dGW values of *O*-acetylated TXs (*e.g.*, TX-2244, -2245, and -2246) decreased, while their hydrophobicities were increased by *O*-acetylation.

The *N*-acetylation of TX-2218 caused an increase in molecular stability, and heat formation energy decreased from 24.177 kcal/mol (Figure 2C, global minimum (GM) conformer of TX-2218) to 10.353 kcal/mol (Figure 4A, the GM conformer of TX-2068). The *N*-, *O*-acetylation of TX-2218 significantly increased molecular stability, while heat formation energy decreased (-4.344 kcal/mol in Figure 4B, the GM conformer of TX-2243).

The relationship between conformers and hydrophobicity was unaffected by the *N*-acetylation of TX-2218, and the dGW profile of TX-2068 (Figure 4C, -226 – -185 kJ/mol) was similar to that of TX-2218. Stereo-hydrophobicity was increased by the *N*-, *O*-acetylation of TX-2218, and a lower dGW value was obtained in the TX-2243 analysis (Figure 4D, -272 – -240 kJ/mol).

Radiosensitizing activity of TX-1877 series. The *in vitro* radiosensitizing activities of sugar-conjugated TX-1877 derivatives were measured at a dose of 1 mM in EMT6/KU cells under hypoxic conditions. ERs are shown in Table I, and the ER of TX-1877 was 1.75. Sugar conjugated TX-1877 derivatives (TX-2141: ER=1.33, TX-2218: ER=1.40, TX-2217: ER=1.41) did not exhibit any radiosensitizing activity; their activities were suppressed by sugar conjugation. In tetra-*O*-acetylated compounds, TX-2244 and TX-2246 exhibited radiosensitizing activities, with that of TX-2244 being significant (ER=2.30). *N*-acetyl (TX-2068: ER=1.43) and *N*-, *O*-acetyl (TX-2243: ER=1.47) compounds showed an ER decrease from 1.75 (TX-1877).

Discussion

TX derivatives were designed with a conjugated sugar moiety in anticipation of their precipitative incorporation into cancer cells (1, 2, 13-17). Heat formation energy (*i.e.* destabilization of the molecule) was greater in compounds with a conjugated monosaccharide than in the parent TX-1877 molecule. This result indicated that the reactivity of the monosaccharide-conjugated compound was greater than that of TX-1877. Moreover, the heat formation energies of tetra-*O*-acetylated compounds were lower than those of monosaccharide-conjugated compounds, and the molecule was stabilized by *O*-acetylation. In the conformation analysis of TX-1877, a correlation was not observed between stability and hydrophobicity, and its dGW value was approximately -160 kJ/mol. In monosaccharide- or tetra-*O*-acetyl-conjugated TX compounds, hydrophobicity increased with a decrease in stability. The conformations and the hydrophobicities of compounds may be controlled by the addition of a

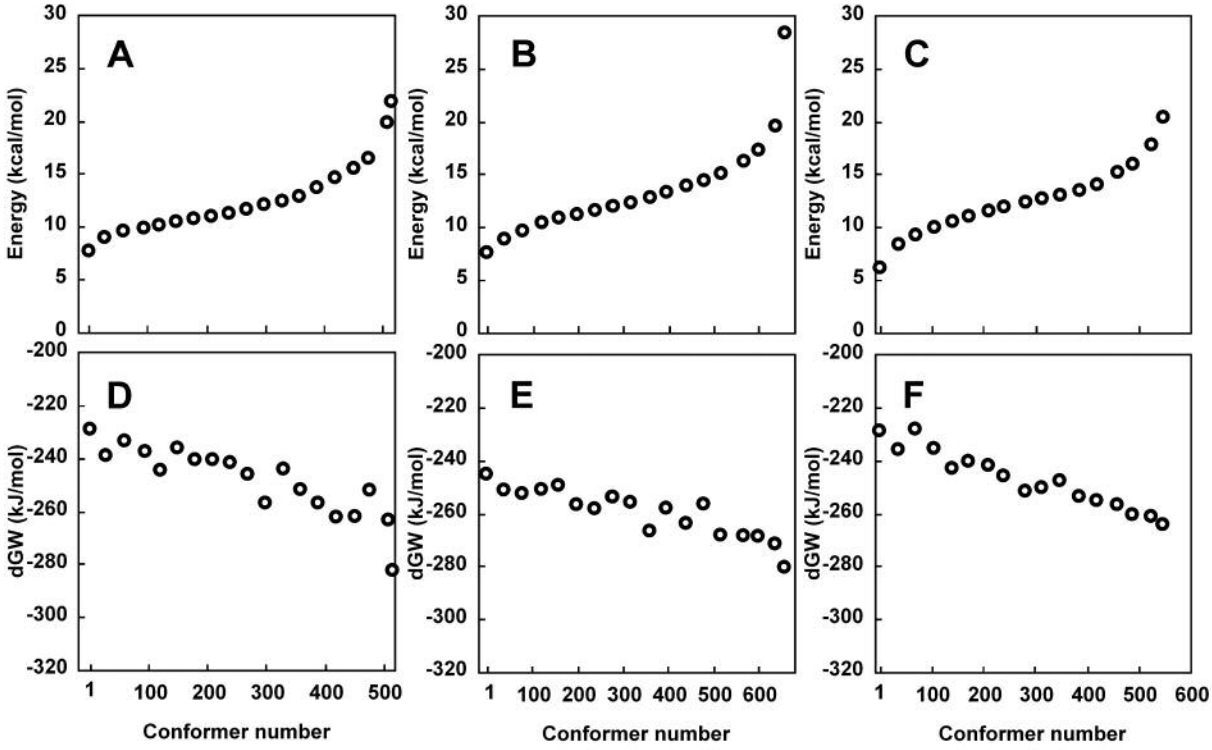

Figure 3. *Energy profile and stereo-hydrophobicity of tetra-O-acetylated TX derivatives. Heat formation energy of TX-2244 (A), TX-2245 (B), TX-2246 (C). Solvation free energy of TX-2244 (D), TX-2245 (E), TX-2246 (F).*

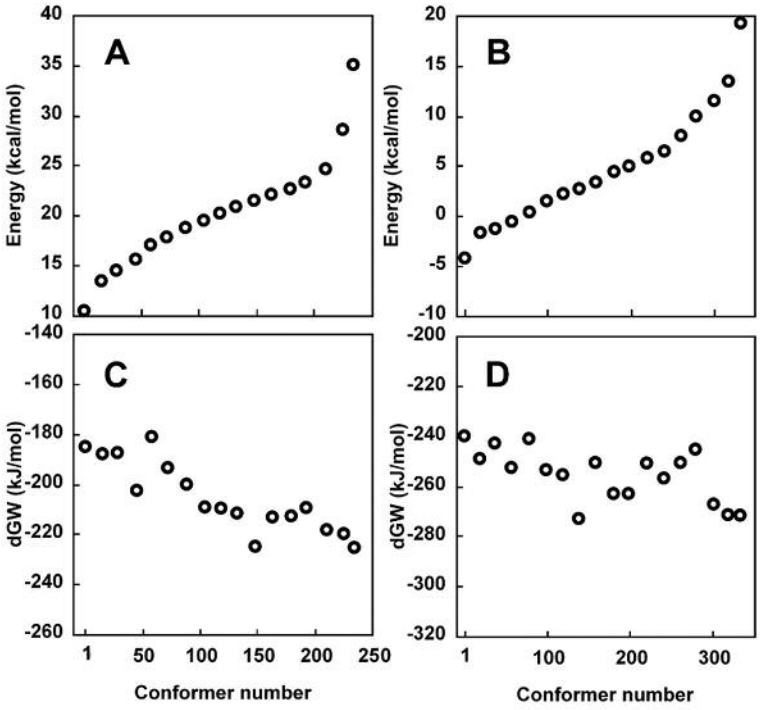

Figure 4. *Energy and hydrophobicity of N-acetylated, N-, O-acetylated derivatives. Energy of TX-2068 (A), TX-2243 (B). The dGW of TX-2068 (C), TX-2243 (D).*

monosaccharide- and tetra-*O*-acetyl-conjugated sugar to TX-1877. The tetra-*O*-acetylation of the β-glucose moiety of the parent TX-2141 molecule significantly improved radiosensitizing efficacy (TX-2244). Acetylation of the hydroxyl group in a glucose moiety appears to be advantageous for radiosensitization. Regarding TX-2244, the balance between molecular mobility (stability) and hydrophobicity was thought to work advantageously for radiosensitization.

ER was not increased by the *N*-acetylation of the β-galactose moiety (TX-2068). A strong ER-potentiating effect was not observed with the *N*-, *O*-acetylation of galactose (TX-2243). Based on these results, it can be speculated that the modification of glucose in the TX molecular design improves ER. Radiosensitization is now being investigated for TX-1877 derivatives with a conjugated disaccharide or polysaccharide moiety.

The hydrophobicities of tetra-*O*-acetylated compounds (*e.g.*, TX-2244, -2245, and -2246) were high, with that of TX-2244 (Figure 3D: -283 kJ/mol) being the highest. The hydrophobicities of monosaccharide-conjugated compounds (Figure 2F, 2G, 2H) were not higher than those of tetra-*O*-acetylated compound (Figure 3D, 3E, 3F). The interaction between *in vivo* water molecules and TX-1877 derivatives appears to control radiosensitizing activity (*e.g.*, ER) through hydrophobicity. In an electrostatic potential field (ESP) analysis of TX-1877 and -2244, positive and negative field developed with entire molecule evenly (data not shown). A detailed analysis of ESP field participation in radiosensitizing activity is in progress.

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Received April 11, 2018
Revised May 11, 2018
Accepted May 14, 2018