Anti-proliferative Activities of Some Bivalent Symmetrical 5-Substituted Hydantoin Derivatives towards Human Brain Glioma U251 Cells (U251) and Human Carcinoma Cells (KB3-1)

Makoto Furutachi, a Kaori Ota, a Fumiko Fujisaki, a Ryuji Ikeda, b Naoki Yoshikawa, b Tsubasa Yokota, b Yasuo Takeda, c Kazumi Yokomizo, d Jian-Rong Zhou, d Nobuhiro Kashige, a Fumio Miake, a and Kunihiro Sumoto* a

a Faculty of Pharmaceutical Sciences, Fukuoka University; 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan; b Department of Pharmacy, University of Miyazaki Hospital; 5200 Kihara, Kiyotake, Miyazaki 889–1692, Japan; c Kagoshima University Hospital; 8–35–1 Sakuragaoka, Kagoshima 890–8520, Japan; and d Faculty of Pharmaceutical Sciences, Sojo University; 4–22–1 Ikeda, Nishi-ku, Kumamoto 860–0082, Japan.

Received June 10, 2019; accepted August 2, 2019

Novel bivalent twin-drug type hydantoin derivatives were evaluated in vitro using a human brain glioma cell line (U251) and a human carcinoma cell line (KB3-1). Among the 5-substituted hydantoin derivatives (1a–b and 2a–d) examined in this study, bivalent symmetrical 5-substituted hydantoin derivative 1b showed the highest anti-proliferative activity towards both U251 and KB3-1 cells. The values of anti-proliferative activity (IC₅₀) of this hydantoin derivative against the two cell lines (U251 and KB3-1) were 0.46 and 5.21 µM, respectively. The anti-proliferative activity of all of the compounds except for compounds 2a and 2d against U251 cells was higher than that of cisplatin. Bivalent symmetrical compound 1b had a biphenylmethane linker in the molecule. All of the tested bivalent hydantoin derivatives showed higher activity against U251 cells than against KB3-1 cells. For twin-drug type hydantoin derivatives 2a–d, which have a linear methylene linker in the molecules, it was found that methylene linker length in these molecules have an effect on the anti-proliferative activity against U251 and KB3-1 cells.

Key words hydantoin; symmetrical bivalent molecule; anti-proliferative activity; U251 cell; KB3-1 cell; cytotoxic activity

INTRODUCTION

Many natural and synthetic bioactive symmetrical bivalent molecules have been studied for the development of new agents to treat various infectious diseases or for the development of new valuable ligands including anticancer agents to treat many types of diseases. 1–5) A bivalent (multivalent) ligand is expected to show enhanced affinity or biological potential compared to that of the corresponding monovalent ligand. Therefore, many scientists studying bioactive molecules have an interest in the development of such twin-drug type bivalent (multivalent) molecules. 6–9)

From the viewpoint of molecular geometry, many host receptors that consist of homo-oligometric units often constitute symmetric macromolecule architectures such as C₂-s or C₂-symmetrical geometric systems. 10) As a useful study for such molecular recognition, a structural investigation of multivalent carbohydrate-protein interactions using synthetic biomolecules has been reported. 11) We have also been interested in molecules that interfere with carbohydrate recognition stages by directing a controlled biological response in order to find new bioactive leads. 12–21)

In connection with the above projects, we have recently reported some symmetrical 5-substituted hydantoin derivatives and the results of biological evaluation of the synthesized symmetrical twin-drug type 5-substituted hydantoin derivatives. 8–11) Among previously targeted bivalent twin-drug type 5-substituted hydantoin derivatives, we found that a few derivatives showed a considerable level of biological activity and also affinity to a few sulfated glycosaminoglycans such as heparan sulfate and dermatan sulfate. 10,11)

The sulfated glycosaminoglycans have been shown to be deeply involved in many stages as regulators for the growth of cancer cells. 22) These bivalent hydantoin-related derivatives are considered to be potential leads in the search for preferred anticancer activity, and we selected a few bivalent twin-drug type hydantoin derivatives for evaluation of their anti-proliferative activities.

Here we report the results of evaluation of the anti-proliferative activities of some of our newly designed bivalent twin-drug type bivalent symmetrical 5-substituted hydantoin derivatives and related compounds using a human brain glioma cell line (U251) and a human carcinoma cell line (KB3-1).

MATERIALS AND METHODS

Preparation of Twin-Drug Type Symmetrical Derivatives (1a–b and 2a–d) Twin-drug type symmetrical compounds (1a–b and 2a–d) were prepared according to the reported procedure. The physical and spectroscopic data for these compounds have already been reported. 11,12) From a stereochemical viewpoint, those products 1 and 2 can be considered to be a mixture of three twin-drug type molecules, i.e., two C₂-symmetrical molecules that have the same absolute configuration (R,R or S,S) regarding the 5-position carbons of two chiral hydantoin rings in the molecules and a C₅-symmetrical meso compound having different absolute configurations (R,S).

We distinguished the presence of three predominant stereoisomers...
in the free base of product 1a by an HPLC method. We used stereoisomeric mixtures for biological evaluation [anti-proliferative activity (IC50)]. Since anticancer activities against two human cancer cell lines were confirmed in this study, we hope to clarify the isolation of the three isomers and anticancer evaluation of compound 1 in our future studies.

**Cell Culture** Two human cancer cell lines (U251 and KB3-1) were used. The cells were incubated at 37°C in an atmosphere of 5% CO2 and 95% air.

**Anticancer Assay** Anticancer activities of the synthesized twin-drug type hydantoin derivatives against the two cancer cell lines (U251 and KB3-1) were evaluated by the reported procedure. Cell proliferation in vitro was estimated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay. Cell proliferation in vitro was assessed by the MTT colorimetric assay in 96-well plates. Cells (5 × 10^4) were inoculated into each well. After overnight incubation (37°C in 5% CO2), target compounds (1a–b and 2a–d) were added to the culture and incubated for 5 d. Thereafter, 50 µL of MTT (1 mg/mL) was added to each well and the plates were incubated for an additional 4 h. After aspiration of the culture medium, the resulting formazan was dissolved with 100 µL of dimethyl sulfoxide (DMSO). The plates were read at 570 nm using a micro-plate reader. The 50% tumor growth inhibitory ratios (IC50 values) were estimated. The determined IC50 values are summarized in Table 1 and they were used for structure–activity relationship discussion of the target hydantoin derivatives.

**RESULTS AND DISCUSSION**

The structures of the tested bivalent symmetrical 5-substituted hydantoin-related derivatives are shown in Fig. 1. The anti-proliferative activities (IC50 values) against a human brain glioma cell line (U251) and a human carcinoma cell line (KB3-1) are summarized in Table 1. As shown in Table 1, all of the tested twin-drug type derivatives showed significant anti-proliferative activities against U251 and KB3-1 cells.

Among the tested compounds, symmetrical bivalent 5-substituted hydantoin derivatives 1a and 1b showed high activities against both cancer cell lines (IC50 = 0.46–9.85 µM, respectively). The anti-proliferative activities (IC50 values) of compounds 1a and 1b against U251 cells were higher than the anti-proliferative activity of cisplatin (IC50 = 3.06 µM). These active bivalent hydantoin derivatives had a bifurfenylmethane linker in the molecule. Compounds 1a and 1b also showed approx. 6–9-times higher selectivity of anti-proliferative activity against U251 cancer cells than values against KB3-1 cells. The reason for the higher activities of compound 1b than those of compound 1a against both U251 and KB3-1 cancer cells may be that either the factor of basicity or stereoelectronic effect of the 5-substituted amino moiety or both factors are involved in the expression of anticancer activity (see Table 1).

Regarding anti-proliferative activities against U251 cancer cells, among the tested symmetrical bivalent 5-substituted hydantoin derivatives 2a–d, derivative 2c showed the highest anti-proliferative activity (IC50 = 1.05 µM) and had a linker methylene chain length (n) of 8 methylene groups. On the other hand, regarding anti-proliferative activities against KB3-1 cells, 5-substituted hydantoin 2d having the longest linker length (n = 12) showed the highest anti-proliferative activity (IC50 = 7.12 µM) and the activity tended to increase as the linker methylene chain became longer [from n = 4 (2a) to n = 12 (2d)] (see Table 1). The results obtained indicate that additional detailed experiments on the effects of longer methylene linkers on anti-proliferative activities against KB3-1...
cancer cells may be needed. A possible reason for the structural difference in the potentials of anti-proliferative activity against U251 and KB3-1 cells is that the three-dimensional supramolecular interaction of these bivalent hydantoin molecules with active site macromolecules on the cell surfaces contributes to the potential of anti-proliferative activity. An indicative review regarding the challenge to define structural determinants and the relevance of targets for optimal activity has recently been published.29 In our previous paper,23 we reported that bivalent free phenylboronic acid derivatives having a methylene chain linker showed higher activity against KB3-1 cancer cells than against U251 cells. It is noteworthy that reverse results showing that bivalent 5-substituted hydantoin derivatives (1 and 2) have higher activity against U251 cancer cells than against KB3-1 cells were obtained in this study. The finding of selectivity of twin-drug type 5-substituted hydantoin derivatives 2 against species of cancer cells is also interesting, and we are considering addressing this issue in the future.

The results obtained in this study and a previous study on bivalent symmetrical phenylboronic acid derivatives31 may be explained by the fact that target bivalent molecules have a property of affinity to sulfated glycosaminoglycans such as heparan sulfate or 1,2-diol functionality contained in sugar chains. The results of these studies have also published a few new anticancer active leads. Further molecular modifications, together with a comparison with single-drug type compounds, and studies on the modes of action for high levels of anticancer activities of compounds will need to be undertaken. The results of bioassays for the development of newly targeted bivalent (or oligovalent) symmetrical molecules using a few human cancer cell lines will be reported separately.

Conflict of Interest The authors declare no conflict of interest.

REFERENCES AND NOTES

1) Duran AM, Meiler J. Inverted topologies in membrane proteins: a mini-review. Comput. Struct. Biotechnol. J., 8, e201308004 (2013).
2) Oshovsky GV, Reinholdt DN, Verbouw W. Supramolecular chemistry in water. Angew. Chem. Int. Ed., 46, 2366–2393 (2007).
3) Gibson SE, Castaldi MP. R, C, symmetry: molecular design inspired by nature. Angew. Chem. Int. Ed., 45, 4718–4720 (2006).
4) Béroué G. Natural and synthetic biologically active dimeric molecules: anticancer agents, anti-HIV agents, steroid derivatives and opioid antagonists. Curr. Med. Chem., 13, 131–154 (2006).
5) Micewicz ED, Luong HT, Jung C-L, Waring AJ, McBride WH, Ruchala P. Novel dimeric Smae analogs as prospective anticancer agents. Bioorg. Med. Chem. Lett., 24, 1452–1457 (2014).
6) Wermuth CG. The Practice of Medicinal Chemistry, 3rd ed., Academic Press, San Diego (2008) and related references cited therein.
7) Wittmann V. Structural investigation of multivalent carbohydrate-protein interactions using synthetic biomolecules. Curr. Opin. Chem. Biol., 17, 982–989 (2013).
8) Furutachi M, Fujisaki F, Tsuru R, Ejima A, Takeda Y, Ohata T, Ito M, Nakamura M, Aki H, Kashige N, Miake F, Sumoto K. Synthesis and antibacterial evaluation of some new 5-substituted hydantoin derivatives and novel twin-drug type derivatives. Heterocycles, 92, 1111–1120 (2016).
9) Fujisaki F, Furutachi M, Fujisara R, Okabe M, Aki H, Kashige N, Miake F, Sumoto K. Preparation and antibacterial evaluation of some symmetrical twin-drug type bivalent molecules. Heterocycles, 91, 1668–1677 (2015).
10) Fujisaki F, Fujisara R, Okabe M, Naito A, Fukami E, Aki H, Kashige N, Miake F, Sumoto K. Reaction of 5-methylenehydantoins and their chemical modification to twin-drug type symmetrical molecules. Heterocycles, 89, 2745–2759 (2014).
11) Fujisaki F, Aki H, Naito A, Fukami E, Kashige N, Miake F, Sumoto K. Synthesis of new 5-substituted hydantoins and symmetrical twin-drug type hydantoin derivatives. Chem. Pharm. Bull., 62, 429–438 (2014).
12) Fujisaki F, Toyofuku K, Egami M, Ishida S, Nakamoto N, Kashige N, Miake F, Sumoto K. Antibacterial activity of some 5-diallylamino-nomethylhydantoin derivatives and related compounds. Chem. Pharm. Bull., 61, 1090–1093 (2013).
13) Mibu N, Aki H, Ikeda H, Saito A, Uchida W, Yokomizo K, Zhou J-R, Miyata T, Sumoto K. Carbohydrate recognition of symmetrical tripod receptor type tris(2-aminopyridyl)methane derivatives. J. Therm. Anal. Calorim., 113, 1015–1018 (2013).
14) Mibu N, Ohata T, Sano M, Zhou J-R, Yokomizo K, Aki H, Sumoto K. Carbohydrate recognition of C3-symmetrical tripod receptor type 2,4,6-trisubstituted 1,3,5-triazine derivatives with antiviral activities. J. Therm. Anal. Calorim., 135, 2807–2811 (2019).
15) Furutachi M, Ejima A, Tsuru R, Goto S, Takeda Y, Ako K, Fuchigami S, Fujii S, Okumura A, Tozuka A, Yokomizo K, Zhou J-R, Inao H, Ono Y, Kashige N, Miake F, Sumoto K. Novel twin-drug type C3-symmetrical phenylboronic acid pinacol esters. Heterocycles, 95, 517–524 (2017).
16) Furutachi M, Ejima A, Tsuru R, Goto S, Takeda Y, Ako K, Fujii S, Okumura A, Tozuka A, Yokomizo K, Zhou J-R, Inao H, Ono Y, Kashige N, Miake F, Sumoto K. Novel trivalent C3-symmetrical phenylboronic acid pinacol esters. Org. Prep. Proced. Int., 49, 287–292 (2017).
17) Furutachi M, Fuchigami S, Goto S, Takeda Y, Ako K, Sumoto K. Novel trivalent C3-symmetrical phenylboronic acid pinacol esters and their biological activities. Heterocycles, 96, 144–151 (2018).
18) Furutachi M, Matsumoto A, Tamagnagata T, Sugita A, Kurokawa M, Yokomizo K, Zhou J-R, Kashige N, Miake F, Sumoto K. Novel trivalent C3-symmetrical phenylboronic acid pinacol esters and their biological evaluation. Heterocycles, 98, 148–158 (2017).
19) Fujisaki F, Yokomizo K, Sano M, Kagauchi Y, Morimoto K, Shimo mura S, Sato R, Hiraga N, Matsunaga A, Zhou J-R, Ohata T, Aki H, Sumoto K. Preparation and antiviral activity of some new C4- and C6-symmetrical tri-substituted triazine derivatives having benzylamino substituents. Chem. Pharm. Bull., 66, 830–838 (2018).
20) Mibu N, Yokomizo K, Yamada K, Matsuyma J, Tomonaga S, Sakai I, Sato R, Kawano Y, Matsumoto, Fujita Y, Inoue Y, Iida M, Hashiguchi K, Zhou J-R, Furutachi M, Sumoto K. Preparation of some novel trisubstituted 1,3,5-triazines and hybrid linker mode 1,3,5-triazine derivatives and their biological evaluation. Heterocycles, 98, 489–508 (2019).
21) Furutachi M, Gondo T, Ikedad H, Yosikawa N, Yokota T, Takeda Y, Yokomizo K, Zhou J-R, Kashige N, Miake F, Sumoto K. Anti proliferative activities towards human brain glioma U251 cells and human carcinoma cells (KB-3-1) of some twin-drug type bivalent C3-symmetrical phenylboronic acid derivatives. Biol. Pharm. Bull., 42, 833–836 (2019).
22) Vitale D, Kakama SK, Greve B, Jang B, Oh E-S, Alaniz L, Gottle
M. Proteoglycans and glycosaminoglycans as regulators of cancer stem cell function and therapeutic resistance. FEBS J., 286, 2870–2882 (2019).

25) Ikeda R, Vermeulen LC, Lau E, Jiang Z, Sachidanandam K, Yamada K, Kolesar JM. Isolation and characterization of gemcitabine-resistant human non-small cell lung cancer A549 cells. Int. J. Oncol., 38, 513–519 (2011).

26) The Japanese Society of Chemotherapy (1989), Chemotherapy, 38, 102–105 (1990).

27) The Japanese Society of Chemotherapy (1992), Chemotherapy, 41, 184–189 (1993).

28) Schinazi RF, Peters J, Williams C, Chance D, Nahmias AJ. Effect of combinations of acyclovir with vidarabine or its 5’-monophosphate on herpes simplex viruses in cell culture and in mice. Antimicrob. Agents Chemother., 22, 499–507 (1982).

29) Lanzi C, Cassinelli G. Heparan sulfate mimetics in cancer therapy: the challenge to define structural determinants and the relevance of targets for optimal activity. Molecules, 23, 2915 (2018).