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Research paper

Design, synthesis, and anti-proliferative activity of 1-(4-methoxyphenyl)-12-hydroxymethyl-*p*-carborane derivatives

Asako Kaise, Kiminori Ohta*, Yasuyuki Endo

Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai, 981-8558, Japan

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ABSTRACT

1-(4-Methoxyphenyl)-12-hydroxymethyl-*p*-carborane (**2a**), which is a precursor to the previously developed potent carborane-containing ER agonist BE120, exhibited weak cell growth inhibitory activity against four human cancer cell lines (MCF-7, MDA-MB-453, LNCaP, and PC-3). The biological evaluation of a series of derivatives of **2a** revealed that an increased number of methoxy groups on the benzene ring of **2a** enhanced the cell growth inhibitory activity. Trimethoxyphenyl derivative **2g** afforded the most potent cell growth inhibitory activity (mean GI_{50} value: 5.8 μM) in a panel screening using 39 human cancer cell lines. Moreover, **2g** induced for MDA-MB-453 breast cancer cell lines an arrest of the cell cycle in the G2/M phase and apoptosis mediated by caspase-3/7.

1. Introduction

Dicarba-*closo*-dodecaboranes (henceforth: carboranes) contain an icosahedral C_2B_{10} skeleton and exhibit a spherical geometry, a highly hydrophobic surface, as well as high levels of resistance toward a wide range of reagents and thermal stability (Fig. 1) [1]. Areas concerned with applied and materials chemistry have recently seen a surge of scientific papers focusing on carborane-containing compounds [2]. In medicinal chemistry, for example, carboranes have been used in the context of boron neutron capture therapy (BNCT) to incorporate high numbers of boron atoms into tumor cells [3]. Nearly two decades ago, our group started to work on the hypothesis that the exceptional hydrophobic character and the spherical geometry of the carboranes might render them effective hydrophobic pharmacophores [4–7]. Subsequently, we demonstrated that the carborane cage may serve as a novel hydrophobic pharmacophore for drug development, and we also designed and synthesized various carborane-containing nuclear receptor ligands [4,5,8–11]. Several carborane-containing compounds were used as pharmacological tools and some of their biological effects were examined *in vivo* [12–15]. The first successful example, 1-(4-hydroxyphenyl)-12-hydroxymethyl-*p*-carborane (BE120, **1**), showed a more potent binding affinity to the estrogen receptor α (ERα) and thus a more potent estrogenic activity than estradiol, which is one of the endogenous female estrogen hormones (Fig. 1) [4]. The evaluation of the physicochemical properties of carborane isomers, using various carboranyl phenols in a quantitative structure-activity relationship (QSAR) study as ER ligands, revealed that these carborane isomers may fulfill various roles for different targets [16,17]. Currently, drug design approaches that are based on the carborane cage as a hydrophobic pharmacophore, e.g. structure-based drug design (SBDD) or ligand-based drug design (LBDD), are established via the development of various carborane-containing ligands, and inhibitors for receptors or enzymes [18,19].

Preliminary results suggested that 1-(4-methoxyphenyl)-12-hydroxymethyl-*p*-carborane (**2a**), the precursor for **1**, exhibits weak cell growth inhibitory activity against the human breast cancer cell line MCF-7, which expresses ERα and proliferates in an estrogen-dependent manner (Fig. 1 and Table 1). Surprisingly, the extremely potent cell proliferation-inducing activity of **1** against the MCF-7 cell line was reversed into a cell growth inhibitory activity by substitution of the phenyl-attached hydroxyl group in **1** with a methoxy group in **2a**. A further investigation into the biological activity of **2a** revealed cell growth inhibitory activity toward four human cancer cell lines (Table 1).

The diametrally opposed activity between **1** and **2a** regarding the cell growth regulatory activity for MCF-7 prompted us to develop novel carborane-containing anti-cancer drugs. Cell growth inhibitory mechanisms and target proteins for **2a** still remain

* Corresponding author.
E-mail address: k-ohta@tohoku-mpu.ac.jp (K. Ohta).

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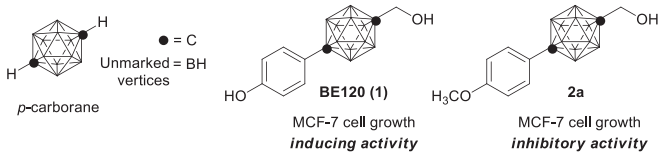


Fig. 1. Chemical structures of *p*-carborane, BE120 (**1**), and **2a**.

Table 1
GI_{50} values for **2a**–**2g** against four human cancer cell lines.

Compound	GI_{50} (μM)[a]			
	Breast		Prostate	
	MCF-7	MDA-MB-453	LNCaP	PC-3
2a	36.5 ± 3.2	21.4 ± 4.0	15.3 ± 4.1	41.5 ± 1.7
2b	9.1 ± 2.9	13.8 ± 1.8	26.1 ± 5.3	33.5 ± 3.5
2c	40.3 ± 2.5	19.1 ± 1.0	30.6 ± 3.1	46.8 ± 6.1
2d	4.7 ± 0.8	5.1 ± 0.1	6.3 ± 1.0	42.3 ± 5.7
2e	6.1 ± 0.8	5.8 ± 0.2	19.5 ± 6.2	18.1 ± 3.4
2f	4.5 ± 0.3	4.8 ± 0.3	4.4 ± 0.5	10.7 ± 1.1
2g	8.3 ± 1.9	5.0 ± 0.5	3.2 ± 0.4	6.4 ± 1.6

[a] GI_{50} values represent the average of at the least three individual measurements ($n = 3$).

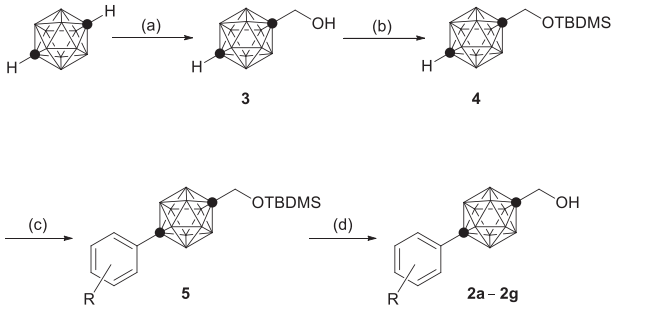
unknown, and their identification is as challenging as it is exciting. Elucidating the impact carborane cages may exert on the biological activity of e.g. **2a** might also lead to the discovery of new functions and/or properties of these carborane cages.

After identifying these attractive research targets, we focused on increasing the cell growth inhibitory activity of **2a** and the associated mechanisms; for that purpose we designed methoxyphenyl-*p*-carborane derivatives **2a**–**2g** and examined their potential as novel anti-cancer drug candidates (Fig. 2). In this paper, we report structure-activity relationship (SAR) studies of **2a**–**2g**, which vary in number and position(s) of the methoxy group(s) attached on the benzene ring, with respect to a panel of 39 cancer cell lines. Moreover, we deliver a cell-based mechanistic analysis, and the inhibitory activity toward the polymerization of tubulin.

2. Results and discussion

2.1. Chemistry

The synthesis of methoxyphenyl-*p*-carborane derivatives **2a**–**2g** is summarized in Scheme 1. Key intermediate **4** was prepared in 65% overall yield from *p*-carborane by a hydroxymethylation with paraformaldehyde, followed by a protection of the hydroxyl group with *tert*-butyldimethylsilyl chloride (TBDMSCl) [20]. Subsequently, **4** was treated with *n*-BuLi and transmetalated *in situ* with

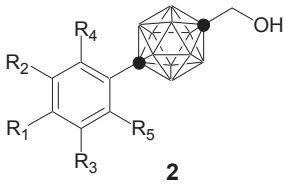


Scheme 1. Reagents and conditions: (a) (i) *n*-BuLi, THF, 0 °C; (ii) $(CHO)_n$, 0 °C; (b) TBDMSCl, imidazole, CH_2Cl_2, rt; (c) (i) *n*-BuLi, DME, 0 °C; (ii) CuCl, rt; (iii) pyridine, Ar-I, 100 °C; (d) TBAF, THF, rt.

CuCl. The obtained *p*-carboranyl copper salt was treated with various iodomethoxy benzene reagents to afford the corresponding coupling products (**5**) [21], which could not be separated from the iodomethoxy benzene starting materials by column chromatography on silica gel. Thus, these mixtures were treated with tetra-*n*-butyl ammonium fluoride (TBAF) to afford target compounds **2a**–**2g** in 40–67% yield over two steps.

2.2. Cell growth inhibitory activity of methoxyphenyl-*p*-carborane derivatives **2a**–**2g** against four human breast and prostate cancer cell lines

Subsequently, *p*-carboranes **2a**–**2g** were evaluated with respect to their cell growth inhibitory activity against the human breast cancer cell lines MCF-7 and MDA-MB-453, as well as against the human prostate cancer cell lines LNCaP and PC-3 [22]. The concentration values that caused 50% cell growth inhibition (GI_{50}) are summarized in Table 1. While MCF-7 cells showed an ER-dependent cell growth, MDA-MB-453 cells showed an ER-independent cell growth, as the latter do not express ERs. Regardless of ER expression, lead compound **2a** showed cell growth inhibitory activity for both breast cancer cell lines. These results suggest that the cell growth inhibitory activity is unrelated to the direct activation of ER. For **2a**, GI_{50} values of a few dozen μM were observed for these four cancer cell lines. Other monomethoxyphenyl derivatives, such as **2b** and **2c**, exhibited similar GI_{50} values regardless of the position of the methoxy group. Compound **2b** showed the most potent GI_{50} value (9.1 μM) against the MCF-7 cell line. Relative to monomethoxy derivatives **2a**–**2c**, dimethoxy derivatives **2d** and **2e** inhibited the cell growth of the four cancer cell lines more potently, and more than half of the estimated GI_{50} values were found in the range 1–10 μM. Trimethoxy derivatives **2f** and **2g** showed GI_{50} values of several μM for all



Compound	R_1	R_2	R_3	R_4	R_5
2a	OCH_3	H	H	H	H
2b	H	OCH_3	H	H	H
2c	H	H	H	OCH_3	H
2d	OCH_3	H	H	OCH_3	H
2e	H	H	H	OCH_3	OCH_3
2f	OCH_3	OCH_3	H	OCH_3	H
2g	OCH_3	OCH_3	OCH_3	H	H

Fig. 2. Chemical structures of **2a**–**2g**.