

α -Glucosidase Inhibitors with a 4,5,6,7-Tetrachlorophthalimide Skeleton Pendanted with a Cycloalkyl or Dicarba-*closo*-dodecaborane Group

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Previous studies of α -glucosidase inhibitors derived from thalidomide revealed that 4,5,6,7-tetrachloro-*N*-alkylphthalimide derivatives are superior lead compounds. Structure–activity relationship studies indicated that a hydrophobic group at the N(2) position is mandatory for potent activity. Accordingly, we have designed and synthesized some 4,5,6,7-tetrachloro-*N*-cycloalkylphthalimide and 4,5,6,7-tetrachloro-*N*-dicarba-*closo*-dodecaborane derivatives. The prepared compounds exhibited potent α -glucosidase-inhibitory activity. Among them, 4,5,6,7-tetrachloro-*N*-cycloheptylphthalimide (**9**) showed the most potent activity, being approximately 30 times more active than the classical inhibitor, 1-deoxynojirimycin (**1**).

Key words α -glucosidase; enzyme inhibitor; phthalimide; tetrachlorophthalimide; structure–activity relationship

α -Glucosidase (EC 3.2.1.20) catalyzes the final step in the digestion of carbohydrates. Inhibitors of the enzyme may retard the uptake of dietary carbohydrates and suppress postprandial hyperglycemia, and may therefore have considerable potential for the treatment of various diseases, including diabetes, certain forms of hyperlipoproteinemia, and obesity.^{1–4} α -Glucosidase inhibitors also have potential as anti-viral agents controlling viral infectivity through interference with the normal biosynthesis of N-linked oligosaccharides by glycosylation of viral coat/envelope glycoproteins,^{5–7} and are being investigated for the treatment of both cancer and acquired immunodeficiency syndrome (AIDS).^{8–10} Recently, effectiveness of α -glucosidase inhibitors in the treatment of B- and C-type viral hepatitis has been reported.^{11,12}

Several low-molecular-weight α -glucosidase inhibitors have been identified as a result of screening of natural products. Among them, 1-deoxynojirimycin (**1**, Fig. 1) is a well established inhibitor,¹³ and various mechanistic and structural development studies have been reported.^{14–18} We have been engaged in structural development and activity expansion studies of thalidomide (**2**, Fig. 1),^{19,20} and we recently reported that some 4,5,6,7-tetrachloro-*N*-alkylphthalimide derivatives derived from thalidomide (**2**) possess potent α -glucosidase-inhibitory activity.^{20–22} Structure–activity relationship studies on 4,5,6,7-tetrachloro-*N*-alkylphthalimide derivatives indicated that the hydrophobic group at the N(2) position is a critical factor for potent activity.^{21,22} These findings led us to design several 4,5,6,7-tetrachlorophthalimide derivatives pendanted with a secondary alkyl group (**5**, **6**), a cycloalkyl group (**7**–**10**), or a dicarba-*closo*-dodecaborane (carborane) (**11**) as the hydrophobic group at the N(2) position (Fig. 2). The carborane skeleton is stabilized by 26 delocalized skeletal electrons, and exhibits unusual properties, such as high boron content, remarkable thermal and chemical stability, a hydrophobic surface, and spherical geometry.²³ The carboranes moiety has been utilized in medicinal chemistry for boron neutron capture therapy (BNCT),²³ and has been established to be a superior hydrophobic pharmacophore.^{24–27}

In this paper, we describe preparation of *N*-cycloalkyl- and



Fig. 1. Structures of 1-Deoxynojirimycin (**1**) and Thalidomide (**2**)

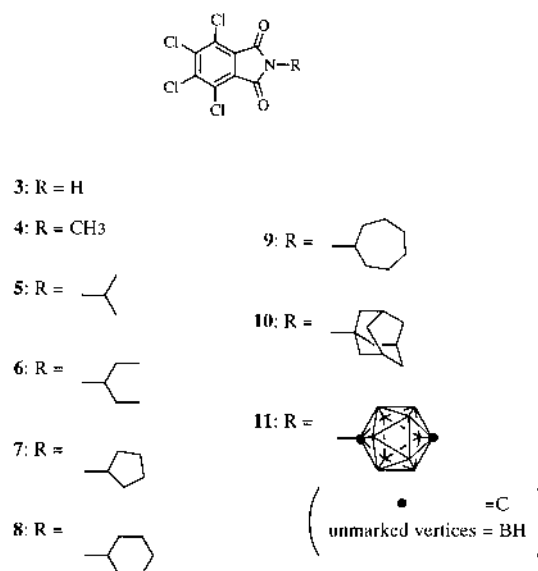


Fig. 2. Structures of 4,5,6,7-Tetrachlorophthalimide Derivatives (**3**–**11**)

N-carborane-substituted 4,5,6,7-tetrachlorophthalimide derivatives and evaluation of their α -glucosidase-inhibitory activity.

Results and Discussion

4,5,6,7-Tetrachlorophthalimide derivatives were prepared by condensation of tetrachlorophthalic anhydride with appropriate amines by usual organic synthetic methods in good yields.^{21,22,24–27} The structures were confirmed by spectroscopic data (NMR, Mass) and gave appropriate analytical

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Table 1. α -Glucosidase-Inhibitory Activity of 4,5,6,7-Tetrachlorophthalimide Derivatives

Compound	IC ₅₀ (μ M)	Compound	IC ₅₀ (μ M)
1	30.7	7	6.4
3	49.3	8	3.7
4	22.1	9	1.0
5	10.3	10	1.7
6	24.0	11	1.5

values (see Experimental section).

The α -glucosidase-inhibitory activity of the compounds (**1**, **3**—**11**) was assayed by the reported method as described in the Experimental Section (*vide infra*).^{21,22,28–30} A classical α -glucosidase inhibitor, 1-deoxynojirimycin (**1**), was adopted as a positive control. All of the tested compounds showed dose-dependent inhibitory activity, and the IC₅₀ values (μ M) are presented in Table 1. Though the IC₅₀ values of the test compounds showed some variation from experiment to experiment, they were basically reproducible. The experiments were performed in triplicate and repeated at least three times, and the mean values are presented.

All of the compounds described in this article were derived from 4,5,6,7-tetrachlorophthalimide (**3**: IC₅₀=49.3 μ M), which is a less active inhibitor than 1-deoxynojirimycin (**1**: IC₅₀=30.7 μ M.) Introduction of a methyl group at the N(2) position (**4**: IC₅₀=22.1 μ M) resulted in a slightly more active compound than 1-deoxynojirimycin (**1**). We prepared three other types of derivatives, *i.e.*, compounds pendanted with a secondary alkyl group (**5**, **6**), a cycloalkyl group (**7**—**10**), and a carborane group (**11**). All of these compounds (**5**—**11**) showed more potent activity than 1-deoxynojirimycin (**1**), reconfirming that a hydrophobic group at the N(2) position is necessary for potent activity.

Though introduction of an isopropyl group (**5**: IC₅₀=10.3 μ M) potently enhanced the activity of the non-substituted (**3**) or methylated (**4**) derivative, exchange of the isopropyl group of **5** for a larger group, an isopentanyl group (**6**: IC₅₀=24.0 μ M), diminished the activity of **5**. Compound **6** showed almost the same activity as the methylated derivative (**4**). However, the cyclization of the C₅-unit to afford the cyclopentanyl derivative (**7**: IC₅₀=6.4 μ M) resulted in a far more active compound than 1-deoxynojirimycin (**1**) or compounds **4**—**6**. These results suggest that the shape of the N(2)-substituent is critically recognized by α -glucosidase. It appears that isopropyl group represents the minimum size of the N(2)-hydrophobic group necessary for potent activity, *i.e.*, for high-affinity fitting/binding of the compound to the binding site of α -glucosidase.²¹ In addition, the far more potent activity of **7** than **5** or **6** suggests that a compact hydrophobic structure with a certain bulkiness is important for the activity. Therefore, we designed conformationally restricted ring-expanded derivatives (**8**, **9**), and the compounds with a spherical hydrophobic substituent, *i.e.*, adamantane- and carborane-pendant derivatives (**10**, **11**, respectively).

In the series of prepared compounds with a monocyclic alkyl substituent (**7**—**9**), a compound with a larger ring showed more potent activity; *i.e.*, the activity increased in the order of **7** (IC₅₀=6.4 μ M) < **8** (IC₅₀=3.7 μ M) < **9** (IC₅₀=1.0 μ M). The two compounds with a spherical substituent, **10** and **11**, showed comparable activity (IC₅₀=1.7 and 1.5 μ M, re-

spectively), though they are slightly less potent than **9**. The similar effects of adamantyl and carborane groups are in agreement with our previous finding²⁴ and may reflect the similar size of the two skeletons.

The cycloheptanyl derivative (**9**) is the most potent inhibitor among the prepared compounds, being approximately 30 times more potent than 1-deoxynojirimycin (**1**). Our studies imply usefulness of the 4,5,6,7-tetrachlorophthalimide skeleton as a superior pharmacophore for α -glucosidase inhibitors. Active compounds presented in this paper should be superior lead compounds for medicaments for the treatment of various diseases, including diabetes, obesity, AIDS, and B- and C-type viral hepatitis. Application of these α -glucosidase inhibitors as hypoglycemic agents and anti-viral agents, as well as mechanistic studies of the enzyme inhibition, are scheduled.

Experimental

Assay of α -Glucosidase Inhibition Assessment of the α -glucosidase-inhibitory activity of the compounds (**1**, **3**—**11**) was performed by the reported method.^{21,22,28,29} Briefly, α -glucosidase (*Saccharomyces* sp., Wako Fine Chemicals Co. Ltd., final concentration: 25 mU/ml) was incubated with *para*-nitrophenylglucopyranoside (p-NPG, final concentration: 80 μ M) in the presence or absence of various concentrations of test compound in 10 mM phosphate buffer (pH 7.28) containing 1% v/v dimethyl sulfoxide (DMSO) at 37 °C for exactly 10 min. After the incubation, an equal volume of stop solution (0.5 M Na₂CO₃) was added, and the amount of released *para*-nitrophenol was measured in terms of the absorbance at 400 nm. The assay was performed in triplicate with five different concentrations around the IC₅₀ values which were roughly estimated in the first round of experiments, and the mean values were taken. The IC₅₀ values calculated from the dose–response curves thus obtained in these experiments are presented in the text.

Chemicals All the compounds (**4**—**11**) were prepared by simple short-step procedure, though the yields were not high. Compound **4** was prepared by means of the Gabriel synthesis. In brief, compound **3** (commercially available) in ethanol was treated with aqueous KOH, and the resulting precipitate was collected. The precipitate was added to iodomethane dissolved in *N,N*-dimethylformamide (DMF). The mixture was stirred at room temperature overnight, then filtered, and CH₃COOEt and water were added to the filtrate. The organic phase was separated, washed with brine, dried over MgSO₄, and evaporated to dryness. The residue was recrystallized from a mixture of CH₃COOEt and *n*-hexane to give **4** in the yield of 34% (not optimized). Compounds **5**—**10** were prepared by condensation of 4,5,6,7-tetrachlorophthalic anhydride with appropriate amines in dry pyridine. Typically, to a mixture of 4,5,6,7-tetrachlorophthalic anhydride (1.0—2.0 mmol) and amine (1 eq) was added dry pyridine (3—6 ml). The mixture was heated to reflux for 1.5—3 h with periodic checking by means of thin layer chromatography. Then CH₃COOEt was added and the solution was evaporated. The residue was separated by silica gel column chromatography. The yields were 30 to 50% (not optimized). Compound **11** was prepared by a similar method, except for the use of dry toluene as the condensation solvent, from 4,5,6,7-tetrachlorophthalic anhydride and *para*-carboramine in the yield of 16% (not optimized).

4,5,6,7-Tetrachloro-*N*-methylphthalimide (**4**): mp 190—192 °C. ¹H-NMR (500 MHz, CDCl₃) δ : 3.20 (3H). FAB-MS *m/z*: 300 (M+)⁺. Anal. Calcd for C₉H₃Cl₄NO₂: C, 36.16; H, 1.01; N, 4.69. Found: C, 36.17; H, 1.28; N, 4.80.

4,5,6,7-Tetrachloro-*N*-isopropylphthalimide (**5**): mp 205—206 °C. ¹H-NMR (500 MHz, CDCl₃) δ : 1.49 (6H), 4.55 (1H). FAB-MS *m/z*: 326 (M+)⁺. Anal. Calcd for C₁₁H₇Cl₄NO₂: C, 40.40; H, 2.16; N, 4.28. Found: C, 40.23; H, 2.27; N, 4.13.

4,5,6,7-Tetrachloro-*N*-isopentylphthalimide (**6**): mp 104—106 °C. ¹H-NMR (500 MHz, CDCl₃) δ : 0.87 (6H), 1.80 (2H), 2.04 (2H), 4.55 (1H). FAB-MS *m/z*: 354 (M+)⁺. Anal. Calcd for C₁₃H₁₁Cl₄NO₂: C, 43.98; H, 3.12; N, 3.95. Found: C, 43.85; H, 3.12; N, 3.81.

4,5,6,7-Tetrachloro-*N*-cyclopentylphthalimide (**7**): mp 196—198 °C. ¹H-NMR (500 MHz, CDCl₃) δ : 1.65 (2H), 1.96 (4H), 2.07 (2H), 4.64 (1H). FAB-MS *m/z*: 352 (M+)⁺. Anal. Calcd for C₁₃H₉Cl₄NO₂: C, 44.23; H, 2.57; N, 3.97. Found: C, 44.16; H, 2.65; N, 4.05.

4,5,6,7-Tetrachloro-*N*-cyclohexylphthalimide (**8**): mp 270—271 °C. ¹H-

NMR (500 MHz, CDCl₃) δ : 1.27 (1H), 1.37 (2H), 1.72 (3H), 1.88 (2H), 2.18 (2H), 4.12 (1H). FAB-MS m/z : 366 (M+1)⁺. *Anal.* Calcd for C₁₄H₁₁Cl₄NO₂: C, 45.81; H, 3.02; N, 3.82. Found: C, 45.78; H, 3.12; N, 4.08.

4,5,6,7-Tetrachloro-*N*-cycloheptylphthalimide (**9**): mp 239–240 °C. ¹H-NMR (500 MHz, CDCl₃) δ : 1.51 (3H), 1.64 (3H), 1.82 (4H), 2.24 (2H), 4.28 (1H). FAB-MS m/z : 380 (M+H)⁺. *Anal.* Calcd for C₁₅H₁₃Cl₄NO₂: C, 47.28; H, 3.44; N, 3.68. Found: C, 47.18; H, 3.51; N, 3.88.

4,5,6,7-Tetrachloro-*N*-adamantylphthalimide (**10**): mp 206–210 °C. FAB-MS m/z : 418 (M+H)⁺. *Anal.* Calcd for C₁₈H₁₅Cl₄NO₂: C, 51.58; H, 3.61; N, 3.34. Found: C, 51.57; H, 3.87; N, 3.07.

4,5,6,7-Tetrachloro-*N*-dicarba-closo-dodecaboranylphthalimide (**11**): mp >300 °C. HR-MS for C₁₀H₁₁B₁₀Cl₄NO₂: Calcd; 427.0517. Found; 427.0518.

Acknowledgements The work described in this paper was partially supported by funds for the Promotion of Fundamental Studies in Health Science from the Organization for Pharmaceutical Safety Research, and Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan.

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