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Novel α-Glucosidase Inhibitors with a Tetrachlorophthalimide Skeleton

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Abstract—Novel α-glucosidase inhibitors with a tetrachlorophthalimide skeleton were prepared and their structure–activity relationships were analyzed. Among them, *N*-phenyl-4,5,6,7-tetrachlorophthalimide (CP0P: **2**) and *N*-(4-phenylbutyl)-4,5,6,7-tetrachlorophthalimide (CP4P: **6**) showed very potent inhibitory activity, being more potent than 1-deoxynojirimycin (dNM: **1**). Mechanistic studies revealed that CP0P (**2**) and CP4P (**6**) inhibit α-glucosidase non-competitively and competitively, respectively.

α-Glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the uptake dietary carbohydrates and suppress postprandial hyperglycemia, and could be useful to treat diabetic and/or obese patients.[1–3] α-Glucosidase inhibitors are also known to possess potential as broad-spectrum anti-viral agents.[4] For example, 1-deoxynojirimycin (dNM: **1**) exhibits potential anti-human immunodeficiency virus (HIV) activity.[5] This activity is thought to arise because of its ability to inhibit trimming glucosidases involved in the biosynthesis of the *N*-linked oligosaccharides on the envelope glycoprotein.[6] Consequently, many efforts have been made to isolate α-glucosidase inhibitors from natural sources, in addition to structural development studies of dNM (**1**).[7–10]

We have engaged in structural development studies of thalidomide (*N*(α)-phthalimidoglutarimide), which has pleiotropic activities including anti-HIV activity and hypoglycemic activity.[11,12] Previously, these activities were thought to arise from its inhibitory effect on tumor necrosis factor (TNF)-α production.[13,14] Therefore, we focused on isolating the TNF-α production-regulating activity, and have synthesized various TNF-α production-regulators with a phthalimide skeleton.[14–17] Recent studies revealed that hypoglycemic activity of our phthalimide analogues derived from thalidomide does not correlate with their TNF-α production-regulating activity, suggesting that the hypoglycemic activity is elicited through a mechanism other than TNF-α production-regulation. This led us to suppose that α-glucosidase might be a candidate target molecule of our phthalimide analogues, and screening of α-glucosidase-inhibiting activity was performed. The screening suggested that tetrachlorophthalimide derivatives possess potent α-glucosidase inhibiting activity.

In this paper, we describe the α-glucosidase-inhibiting activity of a series of tetrachlorophthalimide derivatives and we discuss their structure–activity relationship and inhibitory mechanism.

Tetrachlorophthalimide analogues (**2**–**8**; Table 1) and other phthalimide derivatives (**9**–**18**; Table 1) were prepared by condensation of tetrachlorophthalic anhydride or substituted (or unsubstituted) phthalic anhydride with appropriate amines by the usual synthetic method (4- and 5-amino derivatives (**15** and **16**) were obtained by hydrogenation of the corresponding nitro derivatives (**13** and **14**, respectively)).[15–18] Structures of the compounds were confirmed by spectroscopic data (NMR, Mass) and appropriate analytical values were obtained for all the compounds. The inhibitory effect of the compounds on α-glucosidase activity was assessed by the usual method.[19–21] Briefly, α-glucosidase (Wako Pure Chemical Industries, Ltd., 25 mU/mL) was incubated with various concentrations of test compounds (**1**–**18**) in 10 mM phosphate buffer (pH 7.2) at 37 °C for 10 min. The substrate (*p*-nitrophenyl-α-D-glucopyranoside) was added at the final concentration of 0.16 mM and the mixture was incubated at 37 °C for 30 min. It was

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basified by adding 0.5 M Na_2CO_3, and the amount of released *p*-nitrophenol (Abs. 400 nm) was measured. All the compounds inhibited α-glucosidase dose-dependently, and their IC_{50} values are shown in Table 1.

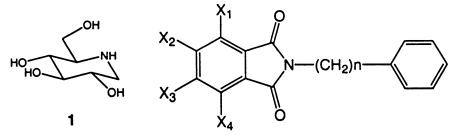
Binding of each test compound to α-glucosidase was analyzed by the surface plasmon resonance (SPR) method using an SPR670 instrument (Nippon Laser & Electronics Lab.) according to the protocol recommended by the supplier. Briefly, the sensor chip (Au film on a glass plate) was treated with 10 μM 4,4-dithiobutyric acid, and the carboxyl group was activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS). Then α-glucosidase was covalently fixed to the sensor chip. Phosphate-buffered saline (PBS(−), pH 7.4) was passed over the α-glucosidase monolayer at 15 μL/min. Various concentrations of test compounds were injected and the signal changes were monitored. Kinetic constants were obtained by analysis of the sensorgram curves using kinetic evaluation software, and the data are shown in Table 1 as association constants (K_a values).

As shown in Table 1, all of the tetrachlorophthalimide derivatives (**2**–**8**) showed more potent α-glucosidase-inhibiting activity (IC_{50} of 2.0–10.9 μM) than dNM (**1**, IC_{50} = 47.6 μM). Tetrachlorophthalimide structure seems to be necessary for potent activity, because the corresponding unsubstituted phthalimide derivatives (**9**–**12**) were inactive (IC_{50} = >500 μM).

Though introduction of a nitro group as an electron-withdrawing group at position 4 or 5 of the phthalimide moiety of inactive P0P (**9**) (4NP0P (**13**) and 5NP0P (**14**)) resulted in appearance of α-glucosidase-inhibiting activity, the activity was weak (IC_{50} values of 23.7–25.9 μM), though stronger than that of dNM (**1**). The introduction of an electron-donating amino or hydroxyl group at position 4 or 5 of the phthalimide moiety (**15**–**18**) had no effect on the activity (Table 1).

Comparison of the α-glucosidase-inhibiting activity (IC_{50} values; Table 1) of the methylene-spacered tetrachlorophthalimide derivatives (**3**–**8**: n = 1–6; Table 1) showed a clear tendency for potency to be dependent on the length of the methylene spacer (number n, Fig. 1; the potency increased in the order of **3 < 4 < 5 < 6**, and decreased in the order of **6 > 7 > 8**), and the maximum potency was found for CP4P (**6**: n = 4). The α-glucosidase-inhibiting activity seems to be elicited by direct binding of tetrachlorophthalimide derivatives (**3**–**8**), because the association constants (K_a values, Table 1) are in approximately the same order as the IC_{50} values. However, some deviation was observed in the correlation between IC_{50} and K_a values, i.e., CP4P (**6**) showed the lowest IC_{50} value, while CP3P (**5**: n = 3, Table 1)

Table 1. α-Glucosidase-inhibiting activity and binding activity of 1-deoxy-nojirimycin (dNM: **1**), tetrachlorophthalimide derivatives (**2**–**8**), and other phthalimide derivatives (**9**–**18**)



Compound		X_1	X_2	X_3	X_4	n	mp (°C)	IC_{50}[a] (μM)	K_a[a] (× 10^5 M^{-1})
dNM	(**1**)	—	—	—	—	—	—	47.6	2.1
CP0P	(**2**)	Cl	Cl	Cl	Cl	0	258–260	2.6	1.4
CP1P	(**3**)	Cl	Cl	Cl	Cl	1	202–205	10.9	0.15
CP2P	(**4**)	Cl	Cl	Cl	Cl	2	177–178	6.0	0.25
CP3P	(**5**)	Cl	Cl	Cl	Cl	3	163	4.5	0.33
CP4P	(**6**)	Cl	Cl	Cl	Cl	4	118–119	2.0	0.22
CP5P	(**7**)	Cl	Cl	Cl	Cl	5	125–126	3.5	0.15
CP6P	(**8**)	Cl	Cl	CI	Cl	6	115–117	7.0	n.d.[b]
POP	(**9**)	H	H	H	H	0	209–211	>500	n.d.
P1P	(**10**)	H	H	H	H	1	115	>500	n.d.
P2P	(**11**)	H	H	H	H	2	129	>500	n.d.
P3P	(**12**)	H	H	H	H	3	48	>500	n.d.
4NP0P	(**13**)	NO_2	H	H	H	0	160–162	25.9	n.d.
5NP0P	(**14**)	H	NO_2	H	H	0	192–193	23.7	n.d.
4AP0P	(**15**)	NH_2	H	H	H	0	180	>500	n.d.
5AP0P	(**16**)	H	NH_2	H	H	0	207–208	>500	n.d.
4HP0P	(**17**)	OH	H	H	H	0	165–167	>500	n.d.
5HP0P	(**18**)	H	OH	H	H	0	248–250	>500	n.d.

[a]IC_{50} and K_a values were determined as described in the text.
[b]n.d., not determined.

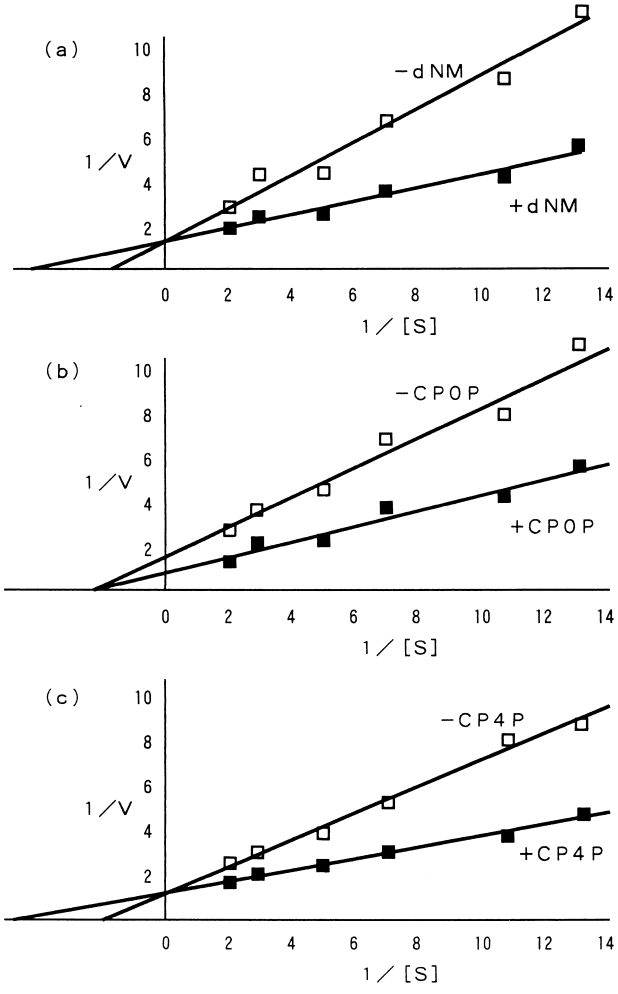


Figure 1. Lineweaver–Burk plot analysis of (a) dNM (**1**), (b) CP0P (**2**), and (c) CP4P (**6**): □ in the absence of inhibitors; ■ in the presence of inhibitors. Vertical scale: 1/V (× 10^5 M^{-1} min); horizontal scale: 1/[S] (× 10^3 M^{-1}).

showed the highest K_a value (Table 1). Though the reasons for this are unclear at this stage, possible explanations are: (i) difference of binding site (vide infra), and (ii) difference of hydrophobicity (vide infra).

The compound without a methylene spacer, CP0P (**2**: $n=0$; Table 1), showed exceptional character in both α-glucosidase-inhibiting activity and binding to the enzyme. Its potent α-glucosidase-inhibiting activity deviates from the general trend of the activity of methylene-spacered tetrachlorophthalimide derivatives (**3**–**8**), i.e. CP0P (**2**) possesses potent inhibitory activity which is comparable to that of CP4P (**6**). Moreover, CP0P (**2**) possesses exceptionally high affinity toward α-glucosidase, which is one order of magnitude higher than that of CP4P (**6**).

These results suggest that the mechanism of α-glucosidase inhibition elicited by CP0P (**2**) is different from that elicited by CP4P (**6**). In fact, Lineweaver–Burk plot analysis (Fig. 1) indicated that CP0P (**2**) inhibits the enzyme non-competitively (the inhibition curves cross on the horizontal axis) and CP4P (**6**) inhibits it competitively (the inhibition curves cross on the vertical axis). As reported by other researchers,[22,23] dNM (**1**) was a competitive inhibitor in the same assay system.

CP0P (**2**) is also exceptional in its selectivity. We assessed the inhibitory effect of our compounds on β-glucosidase activity by the method same with that applied to the β-glucosidase inhibition assay (vide supra), except β-glucosidase (Sweet Almond) and *p*-nitrophenyl-β-D-glucopyranoside were used as the enzyme and the substrate, respectively. Compounds **3**–**18** were all inactive (IC_{50} values were >500 μM), suggesting these compounds are selective to α-glucosidase. On the other hand, only CP0P (**2**) showed moderate inhibitory activity toward α-glucosidase with the IC_{50} value of 17.9 μM, though the efficacy was lower than that toward α-glucosidase ($IC_{50}=2.6$ μM).

Our results suggest that CP4P (**6**) (and possibly other methylene-spacered tetrachlorophthalimide derivatives (**3**–**5**, **7** and **8**)) inhibits α-glucosidase by binding to its catalytic site in a mutually competitive manner with the cognate substrate, which implies that the tetrachlorophthaloyl group could be a sugar mimic. The structure–activity relationships of the methylene-spacered analogues indicated that optimum number of methylene units for fitting to the binding site is $(CH_2)_4$. The presence of a hydrophobic pocket close to the catalytic (substrate-binding) domain of α-glucosidase has been proposed on the basis of the potent activity of *N*-butyl-1-deoxynojirimycin,[22–24] and the phenyl group of CP4P (**6**) might interact with this putative pocket. Recently reported structural development studies of dNM based on the NMR-studies suggested the existence of the local aromatic environment in the enzyme's active site.[10] In the studies, the *N*-benzyl derivative of dNM was reported to be inactive possibly due to steric factors, and the less bulky *N*-glycyl derivative of dNM, whose carbonyl group can interact with the local aromatic environment via π–π stacking, was more potent α-glucosidase inhibitor than dNM.[10] This structure–activity relationship of the derivatives of dNM cannot be simply applied to our tetrachlorophthalimide analogues at this stage.

Though CP0P (**2**) showed the highest affinity for the enzyme among the prepared compounds, its binding site appear to be different from the substrate binding domain. Studies to identify the binding site are under way.

In conclusion, we have prepared novel and potent α-glucosidase inhibitors with a tetrachlorophthalimide skeleton and with different inhibitory mechanisms. The compounds should be useful as lead compounds for medicaments to treat diabetic and/or obese patients, as well as for anti-viral agents.

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References and Notes

1. Yu, C.; Lee, A. M.; Bassler, B. L.; Roseman, S. *J. Biol. Chem.* **1991**, *266*, 24260.
2. Legler, G. *Adv. Carbohydrate Chem. Biochem.* **1990**, *48*, 319.
3. Yoshikawa, M.; Shimada, H.; Nishida, N.; Li, Y.; Toguchida, I.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 113.
4. Mehta, A.; Zitzmann, N.; Rudd, P. M.; Block, T. M.; Dwek, R. A. *FEBS Lett.* **1998**, *430*, 17.
5. Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 9229.
6. de Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedema, F.; Ploegh, H. L. *Nature* **1987**, *380*, 74
7. Watanabe, J.; Kawabata, J.; Kurihara, H.; Niki, R. *Biosci. Biotech. Biochem.* **1997**, *61*, 177.
8. Shiozaki, M.; Yoshiike, R.; Ando, O.; Ubukata, O.; Haruyama, H. *Tetrahedron* **1998**, *54*, 15167.
9. van den Broek, L. A. G. M.; den Nieuwenhof, M. W. P. K.; Butters, T. D.; van Boeckel, C. A. A. *J. Pharm. Pharmacol.* **1996**, *48*, 172.
10. Hines, J. V.; Chang, H.; Gerdeman, M. S.; Warn, D. E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1225.
11. Makonkawkeyoon, S.; Limson-Pombre, R. N. R.; Moreira, A. L.; Shauf, V.; Kaplan, G. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5974.
12. Randall, T. J. *Amer. Med. Assoc.* **1990**, *263*, 1467.
13. Sampio, E. P.; Sarno, E. N.; Gaillily, R.; Chon, Z. A.; Kaplan, G. J. *Exp. Med.* **1991**, *173*, 699.
14. Hashimoto, Y. *Curr. Med. Chem.* **1998**, *5*, 163.
15. Miyachi, H.; Azuma, A.; Ogasawara, A.; Uchimura, E.; Watanabe, N.; Kobayashi, Y.; Kato, F.; Kato, M.; Hashimoto, Y. *J. Med. Chem.* **1997**, *40*, 156.
16. Miyachi, H.; Ogasawara, A.; Azuma, A.; Hashimoto, Y. *Bioorg. Med. Chem.* **1997**, *5*, 2095.
17. Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S. *Chem. Pharm. Bull.* **1996**, *44*, 156.
18. Sasaki, K.; Shibata, Y.; Hashimoto, Y.; Iwasaki, S. *Biol. Pharm. Bull.* **1995**, *18*, 1228.
19. Walker, J. M.; Winder, J. S.; Kellam, J. *Appl. Biochem. Biotech.* **1993**, *38*, 141.

20. Kang, M. S.; Zwolshen, J. H.; Harry, B. S.; Sunkara, P. *Anal. Biochem*. **1989**, *181*, 109.
21. Yoshikawa, K.; Yamamoto, K.; Okada, S. *Biosci. Biotech. Biochem*. **1994**, *58*, 1392.
22. Karlsson, G. B.; Butters, T.; Dwek, R. A.; Platt, F. M. *J. Biol. Chem*. **1993**, *268*, 570.
23. Herre, E.; Genghof, D. S.; Sternlicht, H.; Brewer, C. F. *Biochemistry* **1977**, *16*, 1780.
24. Asano, N.; Nishida, M.; Kato, A.; Kizu, H.; Matsui, K.; Shimada, Y.; Itoh, T.; Baba, M.; Watson, A. A.; Nash, R. J.; Lilley, P. M. Q.; Watkin, D. J.; Fleet, G. W. J. *J. Med. Chem*. **1998**, *41*, 2565.