A FAMILY OF CYCLOPHELLITOL ANALOGS: SYNTHESIS AND EVALUATION

Sir:

Cyclophellitol (1) was isolated from culture filtrates of mushroom, *Phellinus* sp.\(^1\), and found to be a highly specific and effective irreversible inactivator of \(\beta\)-glucosidases\(^2,3\). It is generally believed that the flattened half-chair conformation of the glycosyl intermediate is important for transition state binding by the enzyme\(^4,5\). The ground-state conformation of cyclophellitol (1) resembles the flattened half-chair conformation. Therefore, it is anticipated that the cyclophellitol analogs would have a variety of glycosidase-inhibitory activities. Recently, we have synthesized \(\beta,\beta\)-epi-cyclophellitol (2)\(^6,7\), the \(\alpha\)-manno type analog 3\(^8\), and the aziridine analog 4\(^9\) (7-azabicyclo[4.1.0]heptane derivative), together with cyclophellitol (1) itself\(^7,9\). In a limited inhibitory activity study\(^6~9\), it was shown that the glycoside-cleaving enzymes recognized the configurations of these compounds. It is noteworthy that the aziridine analog 4 showed a high inhibitory activity against almond \(\beta\)-glucosidase of IC\(_{50}\) 0.22 \(\mu\)g/ml\(^9\). To better understand the structure-inhibition relationship, we synthesized another aziridine analog 5, the thiirane analogs 6 and 7, the \(N\)-alkyl aziridine analogs 8~10, and the \(N\)-acyl aziridine analog 11. A preliminary glucosidase inhibitory activity study was also performed.

The synthesis of 5 began with natural cyclophellitol (1) according to the same procedures used for the synthesis of 4\(^9\). Cyclophellitol (1) was benzylated with BnBr and NaH in DMF at 25°C for 0.5 hour to give the tetra-O-benzyl derivative 12 in 90% yield. Treatment of 12 with NaN\(_3\) in DMF (110°C, 12 hours) afforded 13 and 14 in 27% and 41% yield, respectively: 13: \([\alpha]_D^{25} +15^\circ\) (c 0.34, CHCl\(_3\)); \(^1\)H NMR (270 MHz, CDCl\(_3\)) \(\delta\) 1.90 (1H, m, 5-H\(^*\)), 4.02 (1H, s, OH); Anal Calcd for C\(_{32}\)H\(_{34}\)N\(_2\)O\(_5\): C 72.52, H 6.43, N 7.25. Found: C 72.92, H 6.97, N 6.86. 14: \([\alpha]_D^{25} -2.4^\circ\) (c 0.76, CHCl\(_3\)); \(^1\)H NMR (270 MHz, CDCl\(_3\)) \(\delta\) 1.48 (1H, dddd, \(J_{5,8} = J_{5,6} = 10.8\) Hz, \(J_{5,8} = J_{5,6} = 2.0\) Hz, 5-H\(^*\)), 2.51 (1H, d, \(J = 2.0\) Hz, OH), 3.49 (1H, ddd, \(J_{1,2} = J_{1,6} = 9.8\) Hz, 1-H), 3.70 (1H, ddd, d, 6-H; Anal Calcd for C\(_{32}\)H\(_{34}\)N\(_2\)O\(_5\): C 72.52, H 6.43, N 7.25. Found: C 72.59, H 6.31, N 7.03. The \(^1\)H NMR spectrum of 14 clearly indicated \(J_{1,2} = J_{1,6} = 9.8\) Hz, \(J_{5,6} = 10.8\) Hz) that the C-1 hydroxyl group and the C-6 azide group of 14 are oriented equatorially. Since the one-step procedure (Ph\(_3\)P, toluene, 110°C, 0.5 hour)\(^9\) to obtain the aziridine 15 from a mixture of 13 and 14 did not succeed, a three-step procedure was necessary for this transformation: i) MsCl, pyridine, 25°C, 12 hours, ii) Ph\(_3\)P, THF, 25°C, 0.5 hour, then H\(_2\)O, 25°C, 12 hours, iii) NaOMe, MeOH, 25°C, 1.5 hours, 40% overall yield. Finally, de-O-benzylation of 15 (Li, liq NH\(_3\), ether, -78°C, 1 hour) afforded the aziridine analog 5 in 60% yield: \([\alpha]_D^{25} +28^\circ\) (c 0.12, H\(_2\)O); \(^1\)H NMR (270 MHz, D\(_2\)O, DOH = 4.80) \(\delta\) 1.94 (1H, m, 5-H), 2.41 (1H,
The thiirane analogs 6 and 7 were prepared as follows. 1,6-epi-Cyclophellitol (2) was protected as its tetra-O-methoxybenzyl ether 16 in 60% yield by treatment with 4-methoxybenzyl (MPM) chloride and NaH in DMF at 25°C for 20 hours. Thiirane formation was realized by treatment of 16 with Ph3P=S and trifluoroacetic acid in benzene at 60°C for 48 hours to give 17 in 52% yield: [α]D +73° (c 0.18, CHCl3); 1H NMR (270MHz, CDCl3) δ 3.17 (1H, d, J1,6=6.2Hz, J1,2=0Hz, 1-H), 3.57 (1H, dd, J5,6=4.0Hz, 6-H); Anal Calcd for C39H44O8S: C 69.62, H 6.59. Found: C 69.55, H 6.45. It was assumed by the proposed reaction mechanism that the Cl- and the C6-configurations were inverted under these conditions. Finally, de-0-methoxybenzylation of 17 (DDQ, CH2Cl2-MeOH-H2O, 25°C, 12 hours) afforded the thiirane analog 6 in 65% yield: [α]D5 +110° (c 0.16, MeOH); 1H NMR (270MHz, CD3OD) δ 3.31 (1H, dd, J1,6=6.0Hz, J1,2=0Hz, 1-H), 3.49 (1H, dd, J5,6=8.4Hz, 6-H), 3.69 (1H, dd, J5,8=10.8Hz, 8-H), 3.89 (1H, dd, J5,8=10.8Hz, 8'-H). In an analogous fashion, cyclophellitol (1) was transformed to 7 via 18 in 20% overall yield: 18: 1H NMR (270MHz, CDCl3) δ 3.08 (1H, dd, J1,6=6.8Hz, J5,6=2.0Hz, 6-H), 3.33 (1H, dd, J1,2=3.8Hz, 1-H), 3.77, 3.78, 3.80, 3.82 (each 3H, each s, 4 × OMe). 7: [α]D5 +128° (c 0.12, MeOH); 1H NMR (270 MHz, CD3OD) δ 1.62 (1H, d, J1,6=6.4Hz, J1,2=0Hz, 1-H), 1.89 (1H, m, 5-H), 1.99 (1H, dd, J5,6=3.2Hz, 6-H), 2.33 (3H, s, NMe), 2.97 (1H, dd, J3,4=J5,6=10.0Hz, 4-H), 3.08 (1H, dd, J5,6=8.0Hz, 3-H), 3.57 (1H, d, 2-H), 3.62 (1H, dd, J1,6=6.6Hz, J1,2=0Hz, 1-H), 3.15-3.25 (2H, m, 3- and 4-H), 3.52 (1H, dd, J5,6=4.0Hz, 6-H), 3.56 (1H, dd, J5,6=10.4Hz, 6-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H). In an analogous fashion, cyclophellitol (1) was transformed to 7 via 18 in 20% overall yield: 18: 1H NMR (270MHz, CDCl3) δ 3.08 (1H, dd, J1,6=6.8Hz, J5,6=2.0Hz, 1-H), 3.77, 3.78, 3.80, 3.82 (each 3H, each s, 4 × OMe). 7: [α]D5 +128° (c 0.12, MeOH); 1H NMR (270 MHz, CD3OD) δ 1.62 (1H, d, J1,6=6.4Hz, J1,2=0Hz, 1-H), 1.89 (1H, m, 5-H), 1.99 (1H, dd, J5,6=3.2Hz, 6-H), 2.33 (3H, s, NMe), 2.97 (1H, dd, J3,4=J5,6=10.0Hz, 4-H), 3.08 (1H, dd, J5,6=8.0Hz, 3-H), 3.57 (1H, d, 2-H), 3.62 (1H, dd, J1,6=6.6Hz, J1,2=0Hz, 1-H), 3.15-3.25 (2H, m, 3- and 4-H), 3.52 (1H, dd, J5,6=4.0Hz, 6-H), 3.56 (1H, dd, J5,6=10.4Hz, 6-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H). In an analogous fashion, cyclophellitol (1) was transformed to 7 via 18 in 20% overall yield: 18: 1H NMR (270MHz, CDCl3) δ 3.08 (1H, dd, J1,6=6.8Hz, J5,6=2.0Hz, 1-H), 3.77, 3.78, 3.80, 3.82 (each 3H, each s, 4 × OMe). 7: [α]D5 +128° (c 0.12, MeOH); 1H NMR (270 MHz, CD3OD) δ 1.62 (1H, d, J1,6=6.4Hz, J1,2=0Hz, 1-H), 1.89 (1H, m, 5-H), 1.99 (1H, dd, J5,6=3.2Hz, 6-H), 2.33 (3H, s, NMe), 2.97 (1H, dd, J3,4=J5,6=10.0Hz, 4-H), 3.08 (1H, dd, J5,6=8.0Hz, 3-H), 3.57 (1H, d, 2-H), 3.62 (1H, dd, J1,6=6.6Hz, J1,2=0Hz, 1-H), 3.15-3.25 (2H, m, 3- and 4-H), 3.52 (1H, dd, J5,6=4.0Hz, 6-H), 3.56 (1H, dd, J5,6=10.4Hz, 6-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H). In an analogous fashion, cyclophellitol (1) was transformed to 7 via 18 in 20% overall yield: 18: 1H NMR (270MHz, CDCl3) δ 3.08 (1H, dd, J1,6=6.8Hz, J5,6=2.0Hz, 1-H), 3.77, 3.78, 3.80, 3.82 (each 3H, each s, 4 × OMe). 7: [α]D5 +128° (c 0.12, MeOH); 1H NMR (270 MHz, CD3OD) δ 1.62 (1H, d, J1,6=6.4Hz, J1,2=0Hz, 1-H), 1.89 (1H, m, 5-H), 1.99 (1H, dd, J5,6=3.2Hz, 6-H), 2.33 (3H, s, NMe), 2.97 (1H, dd, J3,4=J5,6=10.0Hz, 4-H), 3.08 (1H, dd, J5,6=8.0Hz, 3-H), 3.57 (1H, d, 2-H), 3.62 (1H, dd, J1,6=6.6Hz, J1,2=0Hz, 1-H), 3.15-3.25 (2H, m, 3- and 4-H), 3.52 (1H, dd, J5,6=4.0Hz, 6-H), 3.56 (1H, dd, J5,6=10.4Hz, 6-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H), 3.98 (1H, dd, J5,8=9.0Hz, 8-H).
Table 1. Glucosidase inhibitory activities of 5~11. [IC50 (µg/ml) (1% at 100 µg/ml in parentheses)].

<table>
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<tr>
<th>Enzyme tested</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
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<tr>
<td>β-Glucosidase</td>
<td>32</td>
<td>(84)</td>
<td>(2)a</td>
<td>(8)</td>
<td>(82)</td>
<td>(18)</td>
<td>(98)</td>
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<td>(almond)</td>
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<tr>
<td>α-Glucosidase</td>
<td>(12)</td>
<td>(44)</td>
<td>(55)</td>
<td>(46)</td>
<td>(38)</td>
<td>(20)</td>
<td>(66)</td>
</tr>
<tr>
<td>(baker yeast)</td>
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a 30 µg/ml.
b 10 µg/ml.

2-H), 3.61 (1H, dd, \(J_{\text{gem}} = 10.0\) Hz, 8-H), 3.99 (1H, dd, \(J_{S_5, 8} = 4.0\) Hz, 8'-H), 10: \([\alpha]_D^{20} + 65^\circ\) (c 0.33, MeOH); \(^1\)H NMR (270 MHz, CD3OD) \(\delta 0.94\) (3H, t, \(J = 7.0\) Hz, Me), 1.36, 1.56 (each 2H, each m, CH2CH2), 1.64 (1H, d, \(J_{1,6} = 6.4\) Hz, \(J_{1,2} = 0\) Hz, 1-H), 1.88 (1H, m, 5-H), 1.98 (1H, dd, \(J_{5,6} = 3.2\) Hz, 6-H), 2.16, 2.34 (each 1H, each m, NCH2), 3.02 (1H, dd, \(J_{3,4} = J_{4,5} = 10.0\) Hz, 4-H), 3.10 (1H, dd, \(J_{5,6} = 8.0\) Hz, 3-H), 3.58 (1H, d, 2-H), 3.62 (1H, dd, \(J_{\text{gem}} = 10.0\) Hz, 8-H), 3.99 (1H, dd, \(J_{S_5, 8} = 4.6\) Hz, 8'-H). The N-butyryl analog 11 was directly prepared from 4 by treatment with butyryl chloride and triethylamine in MeOH at 25°C for 15 minutes in 65% yield: \([\alpha]_D^{20} + 56^\circ\) (c 0.26, MeOH); \(^1\)H NMR (270 MHz, D2O, DOH = 4.80) \(\delta 0.91\) (3H, t, \(J = 7.0\) Hz, Me), 1.62 (2H, sextet, \(J = 7.0\) Hz, CH2), 2.08 (1H, m, 5-H), 2.50 (2H, t, \(J = 7.0\) Hz, COCH2), 2.86 (1H, d, \(J_{5,6} = 6.4\) Hz, \(J_{1,2} = 0\) Hz, 1-H), 3.17 (1H, dd, \(J_{3,4} = J_{4,5} = 10.4\) Hz, 4-H), 3.21 (1H, dd, \(J_{S_5, 8} = 10.4\) Hz, 8-H), 3.34 (1H, dd, \(J_{3,4} = J_{4,5} = 8.4\) Hz, 3-H), 3.76 (1H, dd, \(J_{\text{gem}} = 11.0\) Hz, \(J_{S_5, 8} = 8.4\) Hz, 8-H), 3.81 (1H, d, 2-H), 4.04 (1H, dd, \(J_{S_5, 8} = 4.4\) Hz, 8'-H).

The glycosidase inhibitory activities of 5~11 were generally assayed according to the method reported by SAUL et al.\(^{12}\) and are shown in Table 1. The previous evaluation\(^6~9\) of 1, 2, 3 and 4 revealed that the glycoside-cleaving enzymes recognized the configurations of these compounds including the epoxide and the aziridine configurations. On the contrary, the new aziridine analog 5 showed inhibitory activity only against almond \(\beta\)-glucosidase with an IC50 of 32 µg/ml (indeed, 5 was a weak inhibitor of baker yeast \(\alpha\)-glucosidase, \textit{Escherichia coli} \(\beta\)-galactosidase, and jack bean \(\alpha\)-mannosidase, data not shown). These findings reflect that the inhibition mechanisms of the epoxide and the aziridine analogs are different. Neither the thirane analog 6 nor 7 showed significant activities. Various \(N\)-alkyl derivatives of 1-deoxy-1-nojirimycin were shown to have different inhibition properties, especially anti-HIV activity\(^{13}\). Among 8, 9 and 10, the \(N\)-butyl aziridine analog 10 is a better almond \(\beta\)-glucosidase inhibitor (IC50 1.3 µg/ml) than the \(N\)-methyl and \(N\)-ethyl derivatives. Furthermore, the \(N\)-butyryl analog 11 showed inhibitory activity against almond \(\beta\)-glucosidase of IC50 0.3 µg/ml. These results suggest that the \(N\)-substituent may play a key role for inhibition. Further study along this line is now in progress.

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