Notes

ISOLATION OF GLUTATHIONE-*S*-TRANSFERASE INHIBITORS

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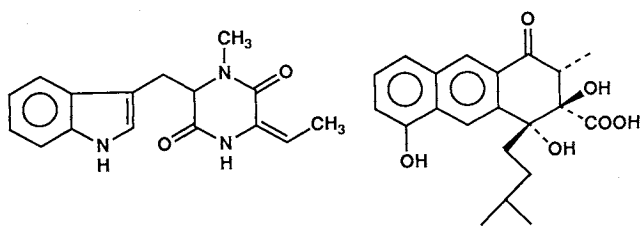
Glutathione *S*-transferase isozyme family is one of the most important drug-detoxifying enzymes in the liver. On the other hand, reports have suggested that increased glutathione *S*-transferase activity may create resistance against a few anticancer drugs, for example, alkylating agents[1~4], cisplatin[5~7] and doxorubicin[8]. Inhibitors of this enzyme, ethacrynic acid and piriprost are already known and their potential therapeutic utility in combination with alkylating agents has been suggested by TEW and his colleagues[9]. Our group has been screening for novel inhibitors of glutathione *S*-transferase from microbial origin and we recently isolated tryptophandehydrobutyrine diketopiperazine[10] (TDD) and rishirilide B[11] (Fig. 1). Although the structures of these compounds are already known, it seems to be worth while to report their enzymological and biological properties because of their potential usefulness. We report here the inhibitory effect of these compounds on glutathione-*S*-transferase activity, the kinetic data and the potentiation effect of these compounds on chlorambucil.

Glutathione *S*-transferase was prepared from doxorubicin-resistant P388 cells. Exponentially growing P388/ADR cells were homogenized using a teflon-glass homogenizer in double distilled water. The homogenized cells were centrifuged at $105,000 \times g$ for 1 hour at 4°C and the supernatant was used for the enzyme assay. The enzyme activity was measured according to the method of HABIG *et al.*[12]. 1-Chloro-2,4-dinitrobenzene (CDNB) was used as the second substrate. After screening of microbial culture filtrates, we isolated TDD and rishirilide B. For isolation of TDD, *Streptomyces* sp. MI513-bF5 was cultured in media containing galactose 2%, dextrin 2%, soy peptone 1%, corn steep liquor 0.5%, $(NH_4)_2SO_4$ 0.2% and $CaCO_3$ 0.2% (pH 7.4) for 4 days at 28°C on a reciprocal shaker. The active principle was extracted from broth filtrate (13 liters) with BuOAc and successively purified by Sephadex LH-20 column chromatography (MeOH) and preparative TLC ($CHCl_3$-MeOH = 10 : 1).

UV, FAB-MS, ^1H and ^{13}C NMR data revealed that it was identical to TDD. It had already been recognized as a metabolite of *Streptomyces spectabilis*[10]. For isolation of rishirilide B, *Streptomyces* sp. SA-3093 was cultured in media containing glycerol 2%, Bacto-soytone 1% and $CaCO_3$ 0.4% with a quarter strength artificial seawater (Jamarin S) for 4 days at 28°C on a reciprocal shaker. The active principle was extracted from broth filtrate (8.6 liters) with BuOAc at pH 3.0 and successively purified by silica gel column chromatography ($CHCl_3$-MeOH = 5 : 1), Sephadex LH-20 column chromatography (MeOH) and preparative TLC (BuOH-MeOH-H_2O = 4 : 1 : 2).

UV, FAB-MS, ^1H and ^{13}C NMR data revealed that it was identical to rishirilide B. It had already been recognized as the inhibitor of α_2-macroglobulin[11].

Fig. 1. Structures of TDD and rishirilide B.



TDD Rishirilide B

The inhibitory activity of various inhibitors against glutathione *S*-transferase are shown in Table 1. Inhibition of TDD against glutathione S-transferase is competitive with glutathione (Fig. 2-a $Ki=6.0\times10^{-6}$ M) and competitive with CDNB (Fig. 2-b, $Ki=1.6\times10^{-5}$ M), too.

Table 1. Inhibitory effects of various inhibitors on glutathione *S*-transferase.

Inhibitor	IC_{50} (μM)
Ethacrynic acid	16.0
TDD	26.5
Rishirilide B	26.9
TA-3037A[15]	8.9

Inhibition of rishirilide B against glutathione *S*-transferase is competitive with glutathione (Fig. 3-a, $Ki=9.4\times10^{-6}$ M) and competitive with CDNB (Fig. 3-b, $Ki=1.9\times10^{-5}$ M), too.

Cytotoxic activity was assayed using Walker chlorambucil-resistant and -sensitive cells. Walker cells were cultured in DULBECCO's modified EAGLE's medium (DMEM) supplemented with 10% foetal bovine serum (Gibco) with or without an inhibitor. After 72 hours, the viable cells were counted by MTT method[13]. For potentiation tests, practically non-cytotoxic concentration of TDD and rishirilide B were used. TDD and rishirilide B showed little

Fig. 2. Lineweaver-Burk plot of inhibition of glutathione-*S*-transferase by TDD.
(a): ○ I=0, ● I=1, ▲ I=3, ■ I=6 μg/ml, (b): ○ I=0, ● I=1, ▲ I=5, ■ I=8 μg/ml.

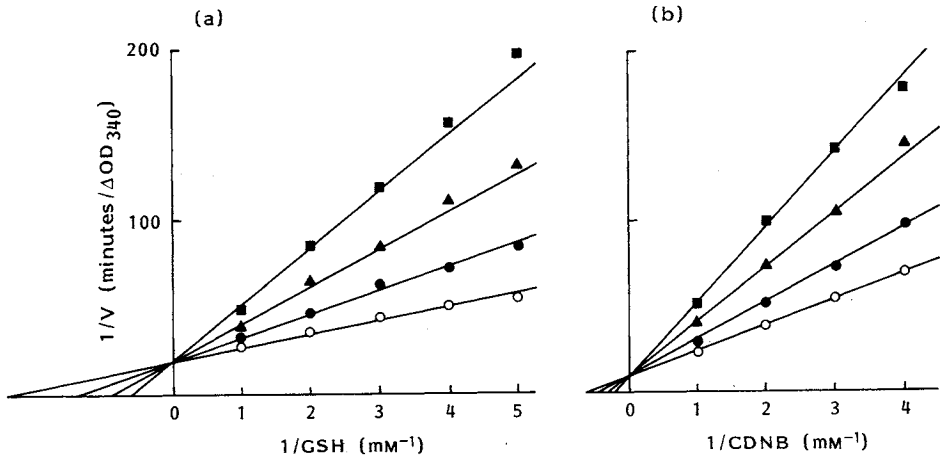
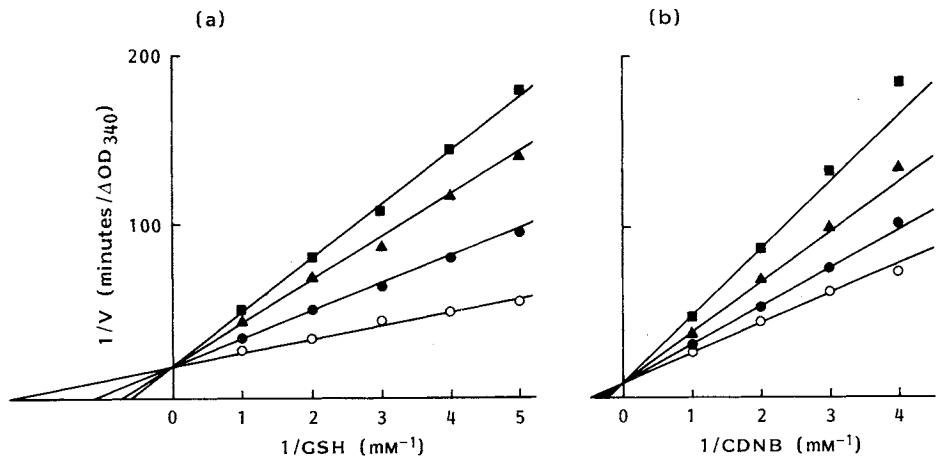


Fig. 3. Lineweaver-Burk plot of inhibition of glutathione-*S*-transferase by rishirilide B.
(a): ○ I=0, ● I=5, ▲ I=8, ■ I=10 μg/ml, (b): ○ I=0, ● I=2, ▲ I=5, ■ I=8 μg/ml.



potentiation effect on chlorambucil.

KAMEI *et al.* reported piperafizines A and B, potentiators of cytotoxicity of vincristine[14]. It is interesting that both piperafizines and TDD are structurally classified as diketopiperazines.

Potentiation tests with other resistant cells are now under study.

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