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The effect of some 1,4-dihydropyridine and 1,4-dihydroindeno[1,2-b]pyridine derivatives on glutathione *S*-transferase activity *in vitro*

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Abstract—The 1,4-dihydropyridine (1,4-DHP) and 1,4-dihydroindeno[1,2-b]pyridine (1,4-DHIP) derivatives were investigated as glutathione *S*-transferase (GT) inhibitors. The obtained results indicate that some of 1,4-DHP's containing lipophylic and bulky substituents have inhibitory effects on GT *in vitro*. The derivatives of 1,4-DHIP are more pronounced inhibitors.

Glutathione *S*-transferase (GT*, EC 2.5.1.18) has been noted as a major detoxificant that catalyses many electrophilic xenobiotic conjugations with glutathione (GSH) [1]. The electrophilic carcinogens, mutagens and some cytostatics can be substrates of GT. Recently it has been postulated that GT isoenzymes may play an important role in the intrinsic and the acquired resistance of cells to carcinogens and to anticancer drugs. Thus the inhibition of GT would be potentially beneficial during cancer chemotherapy. The diuretic drug ethacrynic acid and the prostaglandin I_1 analog piriprost are inhibitors of GT and enhance the cytotoxicity of chloroambucil in Walker 256 rat breast carcinoma drug resistant cell lines [2]. The possible ways by which the activity of GT may be modulated for cancer chemotherapy have been summarized [3]. Many structurally diverse chemical compounds are effective inhibitors of GT. Among them are plant phenols [4], quinones [5], GSH peptide analogs [6] and indomethacine [7].

1,4-Dihydropyridine (1,4-DHP) as well as 1,4-dihydroindeno[1,2-b]pyridine (1,4-DHIP) derivatives have a wide spectrum of physiological activities such as cardiovascular [8], antitumor [9] and radioprotective [10] actions. During our studies of 1,4-DHP and 1,4-DHIP derivatives we have investigated their influence on GT activity *in vitro*.

Materials and Methods

Chemicals. The derivatives of 1,4-DHP were synthesized in the Latvian Institute of Organic Synthesis. The identity and purity of the compounds were confirmed by proton magnetic resonance spectrometry (Bruker WH-DS spectrometer, 90 MHz) and by mass spectrometry (AEI MS 905 spectrometer, 70 eV). The purity of the synthesized compounds was tested by HPLC. GT (from bovine liver, protein content 56%), the Sigma Chemical Co. (St. Louis, MO, U.S.A.); GSH (Reanal, Hungary); 1-chloro-2,4-dinitrobenzene (CDNB), Fluka AG (Buchs, Switzerland).

Assay of GT activity. The reaction was performed in a 10 mm rectangular spectrophotometric cuvette. To 1.75 mL of buffer (pH 6.5, 0.1 M potassium phosphate, 1.0 mM EDTA) 0.05 mL of the GT solution in the same buffer was added. The concentration of GT solution was 0.1 mg GT per mL. Depending on their solubilities the derivatives of 1,4-DHP and 1,4-DHIP were dissolved, either in ethanol or buffer. 1,4-DHP and 1,4-DHIP (0.02 mL) solution was added (concentration of compounds in the reaction mixture in the cuvette was 5×10^{-5} M, if not otherwise indicated). This reaction mixture was incubated for 20 min at 25°. The GT activity was determined according to the previously described enzymatic reaction [11]. In our experiment the reaction was started by the addition of 0.08 mL 25 mM CDNB in 95% ethanol (concentration of CDNB in cuvette: 1 mM) and 0.1 mL of 50 mM GSH in buffer (the concentration of GSH in cuvette: 2.5 mM). The concentration of ethanol in the cuvette did not exceed 5% (v/v). The blank cuvette contained all reagents except the enzyme. The reaction was monitored spectophotometrically ("Hitachi 557", Japan) at 340 nm. The control experiments were carried out in the absence of 1,4-DHP or 1,4-DHIP. The results were expressed as % of GT activity against the control.

Results and Discussion

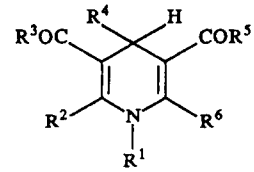
The values of GT activities in the reaction mixtures in the presence of 1,4-DHP or 1,4-DHIP derivatives are summarized in Tables 1 and 2, respectively.

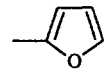
Our obtained results from Table 1 indicate that derivatives with hydrophobic bulky substituents in positions 3 and 5 show the more pronounced inhibition on GT. Compound 3, containing a menthyl substituent in positions 3 and 5, is one of the most active inhibitors. The menthol itself does not show inhibition (GT activity 99.4%). Also in the case of menthylester of acetoacetic acid we observed only a weak inhibition effect (85.3% GT activity). These observations suggest that bulky lipophilic substituents themselves do not ensure inhibition activity of the compounds. The inhibition effect appears when the menthyl oxycarbonyl substituent is attached to the 1,4-DHP system. From the determinated data we conclude that the dihydropyridine ring system is not crucial for inhibition of GT activity. Thus compound 17, which is the corresponding pyridine derivative of compound 3, at the same concentration as compound 3 manifests very similar inhibition activity to GT. Although it is known [12] that the furyl substituent enhances the GT activity, in the case of 1,4-DHP the introduction of furyl substituent (compound 10) does not influence GT activity. The calcium antagonists, nifedipine (compound 14) and riodipine (compound 16), show slight inhibition activities. The electron donor or electron acceptor properties of substituents in position 4 seem to be of no consequence on the inhibition activity. Substitution in position 1 slightly enhances the inhibition for example, comparing compounds 7 and 12. The inhibition effect of 1,4-DHP is dose dependent, IC_{50} for compound 3 is 2.5×10^{-5} M.

Among 1,4-DHIP derivatives (Table 2) the influence of substituents differs. Thus, if compound 15 from the 1,4-DHP series possesses only moderate inhibition activity, the corresponding derivative from the 1,4-DHIP series (compound 21) has strong inhibition activity. In general, derivatives of 1,4-DHIP have a more pronounced inhibition activity on GT than the 1,4-DHP derivatives. The presence of the sulfur as a part of substituents in 1,4-DHP and 1,4-DHIP derivatives also enhances the inhibition activity on GT (compounds 2 and 15 in Table 1 and compounds 19, 21 and 22 in Table 2).

* Abbreviations: GT, glutathione *S*-transferase; GSH, glutathione; 1,4-DHP, 1,4-dihydropyridine; 1,4-DHIP, 1,4-dihydroindeno[1,2-b]pyridine; CDNB, 1-chloro-2,4-dinitrobenzene.

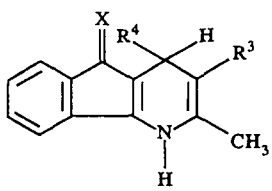
Table 1. Chemical structure of 1,4-DHP and its influence on activity of GT



Nr	R^1	R^2=R^6	R^3=R^5	R^4	GT activity (%)
—	—	—	—	—	100.0
1	H	CH_3	OC_2H_5	H	98.6 ± 5.4
2	H	CH_3	SC_2H_5	H	89.3 ± 3.6
3	H	CH_3	O-menthyl	H	21.0 ± 2.7
4	H	CH_3	O-bornyl	H	20.5 ± 2.3
5	H	CH_3	O-i-bornyl	H	25.8 ± 3.0
6	H	CH_3	OC_2H_5	COONa	99.4 ± 5.4
7	H	CH_3	OC_2H_5	C_6H_5	94.0 ± 5.5
8	H	CH_3	OC_2H_5	C_6H_4OH-4	102.9 ± 2.7
9	H	CH_3	OC_2H_5	$C_6H_4N(CH_3)_2$-4	96.5 ± 2.6
10	H	CH_3	OC_2H_5		100.0 ± 3.9
11	H	CH_3	OC_2H_5	5-norbornen-2-yl	76.3 ± 3.5
12	C_6H_5	H	OC_2H_5	C_6H_5	85.1 ± 4.2
13	H	CH_3	OCH_2COONa	H	95.8 ± 5.0
14	H	CH_3	OCH_3	$C_6H_4NO_2$-2	91.2 ± 4.4
15	H	CH_3	SC_2H_5	$C_6H_4NO_2$-2	69.8 ± 2.1
16	H	CH_3	OCH_3	$C_6H_4OCHF_2$-2	97.6 ± 3.2
17	2,6-dimethyl-3,5-bis(menthyloxycarbonyl)pyridine				38.3 ± 2.4

The results are means of at least three experiments ± SD.

Table 2. Chemical structure of 1,4-DHIP and its influence on activity of GT



Nr	X	R^3	R^4	GT activity (%)
18	O	$COOC_2H_5$	C_6H_5	90.0 ± 5.2
19	O	$CSOC_2H_5$	C_6H_5	17.0 ± 3.4
20	O	COOmenthyl	$C_6H_4NO_2$-2	46.0 ± 4.2
21	O	$COSC_2H_5$	$C_6H_4NO_2$-2	24.7 ± 1.5
22	O	$COSC_2H_5$	$C_6H_4NO_2$-3	45.4 ± 2.0
23	S	$COOC_2H_5$	C_6H_5	51.8 ± 3.4

The results are means of at least three experiments ± SD.

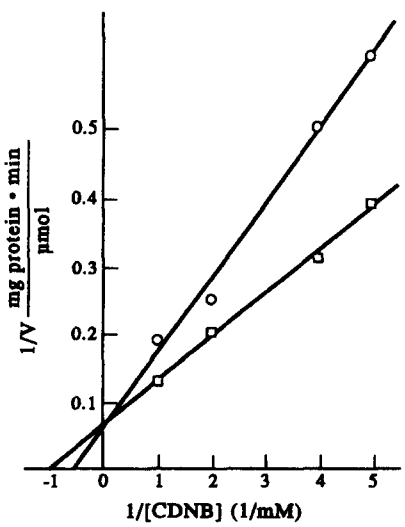


Fig. 1. Inhibition of GT in the presence of compound 3 (○). Control (□).

To understand better the manner of inhibition, GT activity was measured in the presence of compound 3 with varying concentrations of CDNB. Figure 1 shows that the inhibition can be competitive with respect to CDNB. Further investigations are necessary for the complete explanation of the manner of inhibition.

Our results show that 1,4-DHP derivatives with lipophilic (hydrophobic) bulky substituents (bornyl, isobornyl, menthyl) in the positions 3 and 5 as well as the related condensed systems possess inhibition activity on GT *in vitro*. Further studies are needed to determine if this inhibition ability of 1,4-DHP or 1,4-DHIP plays a role in the modulation of GT activity *in vivo*.

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