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SYNTHESIS OF GLUCURONIDES OF MULTIDRUG RESISTANCE REVERSING DRUG MS-209

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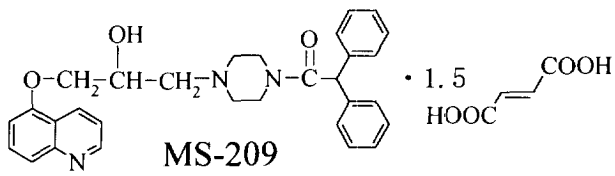
Abstract: We synthesized the glucuronides of MS-209 to identify the two main unknown metabolites in human urine. Reaction of MS-209 and glucuronyl trichloroacetimidate gave two β-isomers, which were each glucuronate of (*R*)- and (*S*)-MS-209. These spectrum data were identical with the metabolites.

Multidrug resistance (MDR) in cancer chemotherapy is a serious clinical problem. It is well known that P-170 glycoprotein (P-gp), which is expressed on the plasma membrane of drug-resistant tumor cells, actively effluxes antitumor agents from the cells. The resultant poor accumulation of these agents is the major cause of MDR,[1-5] and many drugs are known to reverse MDR.[6-10] However, the activity of these compounds is low and various side effects have been observed during clinical trials. Thus, it is necessary to develop more active and less toxic drugs, that are capable of reversing MDR of tumor cells.

We studied a number of quinoline derivatives and found MS-209.[11] It reverses MDR of tumor cells effectively and exhibits good pharmaceutical properties. MS-209 is currently in phase II clinical studies,



MS-209

but we found two unknown metabolites in human urine during phase I clinical studies. It is very important to determine these metabolites to the development of new drugs. Results of MS gave MH^+ = 658 respectively, so we assumed that these were glucuronides to a hydroxy group of MS-209. Therefore, we tried to synthesis the glucuronides of MS-209 and compare the results to the metabolites. However, some difficulties were expected since the hydroxy group of MS-209 is secondary and optically active. Therefore, reactivity was low and a four diastereo mixture was expected. Further, a basic nitrogen of MS-209 easily

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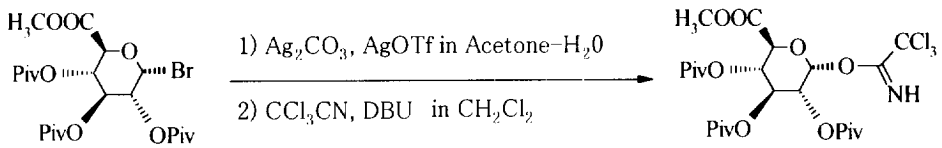
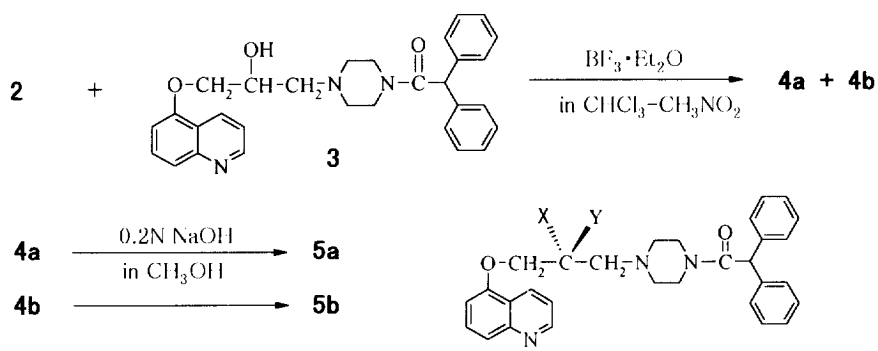
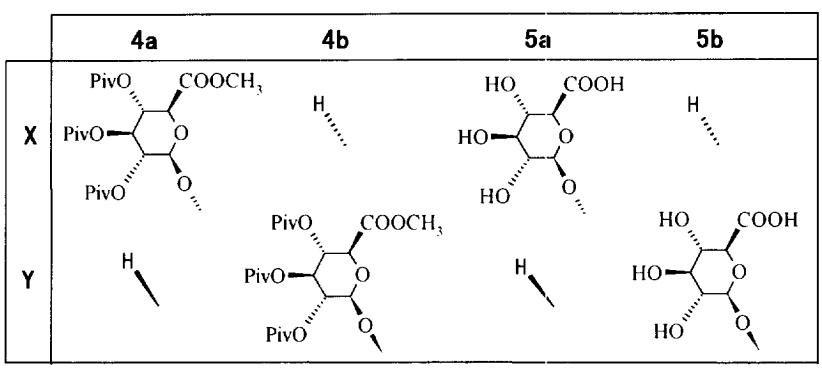
formed salts that were an acidic catalyst, such as Lewis acid, which was another problem. Indeed, reaction of commercially available methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-acetyl-β-D-glucuronate and MS-209 only gave *O*-acetylated MS-209 derivative. Then, we changed the acetyl groups to pivaloyl groups, leaving group to trichloroacetimidate, to prevent acylation of the hydroxy group and increase reactivity.



1 2

Methyl 1-bromo-1-deoxy-2,3,4-tri-*O*-pivaloyl-α-D-glucopyranuronate **1** was synthesized from D-glucuronolactone through three steps,[12] then converted to trichloroacetimidate **2** with a 55% yield.[13] To react **2** and a free form of MS-209 (**3**), an excess of $BF_3 \cdot Et_2O$ (2.5 eq.) was needed due to the formation of salt with basic nitrogen, and nitromethane was added to $CHCl_3$ to resolve precipitation of the salt.





Reaction products were easily separated with silica gel column, and these were β-glucuronide of (*R*)- and (*S*)- enantioisomers **4a** and **4b**.[14] It was interesting that the (*R*)-isomer was three times more reactive than the (*S*)-isomer on HPLC analysis. Conformation of the hydroxy group was determined using the (*R*)-isomer

of MS-209, which yielded only **4a** at 34 %. When **4a** and **4b** were deprotected under a basic condition, **5a**[15] and **5b**[16] were respectively yielded, and these HPLC and MS data agreed with those of the metabolites. Furthermore, these synthesized glucuronides **5a** and **5b** did not show MDR reversing activity *in vitro*.

Conclusions:

We found the two main metabolites of MS-209 in human urine and these spectrum data were identical with those of synthetic glucuronides of MS-209, which were β -isomers of (*R*)- and (*S*)-MS-209.

Reference and Notes:

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14. **Methyl 1-*O*-[(*R*)-3-{4-(2,2-diphenylacetyl)piperazin-1-yl}-1-(quinolin-5-yloxy)-2-propyl]-2,3,4-tri-*O*-pivaloyl-β-D-glucuronate 4a and Methyl 1-*O*-[(*S*)-3-{4-(2,2-diphenylacetyl)piperazin-1-yl}-1-(quinolin-5-yloxy)-2-propyl]-2,3,4-tri-*O*-pivaloyl-β-D-glucuronate 4b:** Free form of MS-209 (**3**) 1.04 g (2.15 mmol) and **2** 1.3 g (2.15 mmol) were dissolved in 16 ml of $CHCl_3$, then immersed in an ice bath. $BF_3 \cdot Et_2O$ 763 mg (5.4 mmol) was slowly added and stirred for 0.5 h. The salt was precipitated and CH_3NO_2 was added to resolve precipitation, then stirred for 4 h at room temperature. Pyridine (1 ml) and AcOEt were added, then the precipitation was filtered off. The filtrate was

evaporated and residue was purified by silica gel column (Hexane:AcOEt = 1:1) gave **4a** (13 %) and **4b** (2 %). **4a:** ^{1}H NMR δ ppm ($CDCl_3$) 1.10, 1.15 (sx2, 27H), 2.2 - 2.7 (m, 6H), 3.4 - 3.5 (m, 2H), 3.53 (s, 3H), 3.6 - 3.8 (m, 2H), 3.95 (d, 1H, *J* = 9.5 Hz), 4.2 - 4.3 (m, 3H), 4.92 (d, 1H, *J* = 8.1 Hz), 5.07 (t, 1H, *J* = 8.4 Hz), 5.18 (s, 1H), 5.20 (t, 1H, *J* = 9.5 Hz), 5.37 (t, 1H, *J* = 9 Hz), 6.84 (d, 1H, *J* = 7.3 Hz), 7.2 - 7.4 (m, 10H), 7.41 (dd, 1H, *J* = 4.4, 8.1 Hz), 7.58 (t, 1H, *J* = 8.1 Hz), 7.69 (d, 1H, *J* = 8.8 Hz), 8.54 (d, 1H, *J* = 7.3 Hz), 8.91 (dd, 1H, *J* = 2.2, 4.4 Hz). HPLC; Rt. 16.2 min. (1 ml/mim, YMC AM-312 ODS column, 10 mM KH_2PO_4-H_2O:CH_3CN=2:1). **4b:** ^{1}H NMR δ ppm ($CDCl_3$) 0.90, 1.10, 1.15 (sx3, 27H), 2.4-2.7 (m, 6H), 3.4 (br., 2H), 3.5 (br., 2H), 3.69 (s, 3H), 4.06 (d, 1H, *J* = 9.5 Hz), 4.15 (m, 1H), 4.2 - 4.3 (m, 2H), 4.95 (d, 1H, *J* = 8.1 Hz), 5.09 (t, 1H, *J* = 8.4 Hz), 5.19 (s, 1H), 5.26 (t, 1H, *J* = 9.5 Hz), 5.36 (t, 1H, *J* = 9.2 Hz), 6.80 (d, 1H, *J* = 7.3 Hz), 7.2 - 7.4 (m, 10H), 7.44 (dd, 1H, *J* = 4.4, 8.8 Hz), 7.58 (t, 1H, *J* = 8 Hz), 7.72 (d, 1H, *J* = 8.8 Hz), 8.48 (d, 1H, *J* = 7.3 Hz), 8.94 (dd, 1H, *J* = 1.5, 4.4 Hz). HPLC; Rt. 14.8 min. (1 ml/mim, YMC AM-312 ODS column, 10 mM KH_2PO_4-H_2O:CH_3CN=2:1).

15. **1-*O*-[(*R*)-3-{4-(2,2-diphenylacetyl)piperazin-1-yl}-1-(quinolin-5-yloxy)-2-propyl]-β-D-glucuronic acid sodium salt 5a: 4a** (176 mg, 0.19 mmol) was dissolved in MeOH (10 ml) and 0.2N NaOH (22 ml), then heated at 60℃ for 16 h. Reaction solution was washed with $CHCl_3$ three times and evaporated. Residue was purified by reverse phase column chromatography (H_2O/MeOH = 1/1) and crystallized with MeOH and Et_2O yielded title compound **5a** as a white solid 98 mg (78.5%). mp; > 240℃. ^{1}H NMR δ ppm (DMSO-d_6) 2.2 - 2.7 (m, 6H), 2.9 - 3.5 (m, 9H), 4.1 - 4.4 (m, 3H), 4.40 (d, 1H, *J* = 9.6 Hz), 4.8 (br., 1H), 5.1 (br., 1H), 5.52 (s, 1H), 7.1 - 7.4 (m, 11H), 7.5 - 7.7 (m, 3H), 8.69 (d, 1H, *J* = 8 Hz), 8.87 (d, 1H, *J* = 4 Hz). $[\alpha]_D$ -8.0° (c = 1.0, MeOH). EA for $C_{36}H_{38}N_3O_9Na \cdot 0.5H_2O$ (calc: C, 62.78; H, 5.51; N, 6.10. found: C, 62.91; H, 5.45; N, 6.06.). HPLC; Rt. 8.8 min. (1 ml/mim, YMC AM-312 ODS column, 10 mM KH_2PO_4-H_2O:CH_3CN=2:1). MS; MH^+ = 658.

16. **1-*O*-[(*S*)-3-{4-(2,2-diphenylacetyl)piperazin-1-yl}-1-(quinolin-5-yloxy)-2-propyl]-β-D-glucuronic acid sodium salt 5b:** Deprotection of **4b** with same manner as **4a** yielded **5b**. mp; 190 - 196℃. ^{1}H NMR δ ppm (DMSO-d_6) 2.2 - 2.7 (m, 6H), 2.9 - 3.6 (m, 9H), 4.2 - 4.3 (m, 3H), 4.44 (d, 1H, *J* = 7.7 Hz), 4.93 (br., 1H), 5.10 (br., 1H), 5.52 (s, 1H), 7.06 (d, 1H, *J* = 8 Hz), 7.2 - 7.3 (m, 10H), 7.50 (dd, 1H, *J* = 4, 8 Hz), 7.58 (d, 1H, *J* = 8.4 Hz), 7.65 (t, 1H, *J* = 8 Hz), 8.57 (d, 1H, *J* = 8.4 Hz), 8.88 (d, 1H, *J* = 4.4 Hz). $[\alpha]_D$ -13.0° (c = 0.43, MeOH). EA for $C_{36}H_{38}N_3O_9$ Na·$2H_2O$ (calc: C, 60.41; H, 5.91; N, 5.87. found: C, 60.50; H, 5.78; N, 5.88.). HPLC; Rt. 7.0 min. (1 ml/mim, YMC AM-312 ODS column, 10 mM KH_2PO_4-H_2O:CH_3CN=2:1). MS; MH^+ = 658.