# Inhibition of UDP-glucuronosyltransferase by 5'-O-amino Acid and Oligopeptide Derivatives of Uridine: Structure-Activity Relationships

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The inhibitory effect of a series of 5'-O-amino acid and oligopeptide derivatives of uridine on rat liver UDP-glucuronosyltransferase (UGT) activities was investigated using two assay systems. A quantitative structure-activity relationship (QSAR) study was performed. The compounds include a lipophilic residue linked to the nucleoside by a variable spacer. Moreover, half of the derivatives have two spacers linked to the uridine moiety. Compound **1**, a serine derivative of isopropylideneuridine, was found to be the most potent inhibitor of both 4-nitrophenol (4-NP) and phenolphthalein (PPh) glucuronidation, with an $I_{50}$ of 0.45 mM and 0.22 mM, respectively. Kinetic studies with this substance revealed a mixed type of inhibition towards 4-NP and UDP-glucuronic acid, with apparent Ki values of 150 μM and 120 μM, respectively. The dipeptide derivatives **11**-**14** exhibited a low activity against 4-NP conjugation. However, a marked suppression of PPh glucuronidation was found with compounds **11** and **13**. Generally, compounds with two spacers are more inhibitory against the UGT activities studied. The QSAR analysis outlined the significance of the spacers with a minimum length of 5 atoms and lipophilic residues linked to them for the inhibitory effect of the compounds. The most significant contribution to this effect is given by the six-atom spacer for both, 4-NP and PPh substrates. 4-NP converting UGT isoforms seem to respond more specifically to the inhibitors: a five-atom for the first and a six-atom for the second spacer enhance binding to both 4-NP and PPh conjugating isoenzymes, while a long second spacer contributes to inhibitor binding to UGT isoforms only converting PPh.

## Introduction

UDP-glucuronosyltransferase (UGT, EC 2.4.1.17) is a family of membrane-bound isoenzymes that play an important role in the biotransformation and detoxification of a large variety of xenobiotics and endogenous compounds (Bock *et al.,* 1986; Tephly and Burchell, 1990; Clarke and Burchell, 1994; Bock and Lilienblum, 1994). The enzymes catalyze the transfer of glucuronic acid from UDP-glucuronic acid (UDPGA) to the respective aglycones containing hydroxyl-, amino-, carboxyl- or sulfhydryl-groups, forming water-soluble β-(D)-glucuronides. Various drugs are extensively converted to inactive glucuronides in this way and subsequently excreted from the organism (Miners and Mackenzie, 1991). Thus, the inhibition of UGT could increase the plasma level and therapeutic efficacy of a number of drugs. Simultaneously, the development of selective inhibitors of UGT could also be an useful approach in studying the active sites of different UGT isoforms.

Several classes of UGT inhibitors have been recently developed, including alkanoic and arylalkanoic acids and related derivatives (Fournel *et al.,* 1986; Fournel-Gigleux *et al.,* 1989; Noort *et al.,* 1990; Said *et al.,* 1992). We found some potent and specific inhibitors of UGT among a wide range of pyrimidine derivatives, considered as analogs of uracil or uridine moiety of UDPGA (Naydenova *et al.,* 1995). According to the current presentations, the UDP part or the uridine moiety is thought to provide most of the free binding energy of the ligand-enzyme complex (Hochman and Zakim, 1983). Thus, the synthesis of new inhibitors is directed to structures with full analogy to either UDP part (Noort *et al.,* 1990) or uridine moiety

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(Paul *et al.*, 1993). Linkage of lipophilic aryl- or arylalkyl-residues to UDP led to powerful selective UGT inhibitors, considered as possible transition-state analogs (Noort *et al.*, 1990; Said *et al.*, 1992). These compounds were designed to take advantage of both, the high affinity of UGT for UDP part of UDPGA and the specific structural requirements for an acceptor substrate (aglycone). It was suggested that the both phosphate groups might be required for correct binding (Noort *et al.*, 1990). However, structurally related compounds, in which the linker between uridine and aglycone was replaced by a diphosphate-like five-atom spacer (-OCONHSO$_2$O-), have been reported to be strong inhibitors of diverse UGT isoforms and some of them to behave as possible transition-state analogs (Paul *et al.*, 1993; Radominska *et al.*, 1994; Battaglia *et al.*, 1995). This offers the possibility, by varying this spacer, to design novel active-site directed inhibitors, based on the presumed transition- state for the glucuronidation reaction.

In an attempt to develop such UGT inhibitors, various protected amino acids and some dipeptides were linked to 5'-O-position of isopropylideneuridine, thus introducing different spacers between the nucleoside and the lipophilic residue. Moreover, two spacers linked to the uridine moiety could be suggested for the half of the derivatives. As these spacers contain a number of single bonds allowing rotation, one can presume that they could behave in a similar way in relation to the active-site binding of the nucleoside and aglycone residues. In this work the inhibitory effect of these compounds on UGT in rat liver microsomes is presented, using 4-nitrophenol (4-NP) and phenolphthalein (PPh) as substrates. Glucuronidation of these aglycones is associated with different UGT isoforms, present in the rat liver (Wishart, 1978). To estimate the contribution of the different spacers and acceptor substrates to the active-site binding, a quantitative structure-activity relationship (QSAR) study was performed to outline the structural features of importance for the inhibitory potency of these compounds and possibly further to direct the rational design of new UGT inhibitors.

## Materials and Methods

### *Materials*

Derivatives **1–8** were synthesized as described in detail (Alargov *et al.*, 1997). The synthesis of compounds 5'-O-(N-*tert.*butyloxycarbonyl-L-seryl)-2',3'-O-isopropylideneuridine, 5'-O-(N-benzyloxycarbonyl-O-benzyl-D,L-threonyl)-2',3'-O-isopropylideneuridine (**9**), 5'-O-(O-benzyl-D,L-threonyl)-2',3'-O-isopropylideneuridine (**10**) and dipeptide derivatives 5'-O-(N-*tert.*butyloxycarbonyl-O-benzyl-L-seryl-L-valyl)-2',3'-O-isopropylideneuridine (**11**), 5'-O-(O-benzyl-L-seryl-L-valyl)-uridine (**12**), 5'-O-(N-*tert.*butyloxycarbonyl-L-valyl-O-benzyl-L-seryl)-2',3'-O-isopropylideneuridine (**13**), 5'-O-(L-valyl-O-benzyl-L-seryl)-uridine (**14**) is comprehensively described in a concomitant paper. Briefly, compound **11** was synthesized by the DCC/HOBt method from N-*tert.*butyloxycarbonyl-O-benzyl-L-serine and 5'-O-L-valyl-2',3'-O-isopropylideneuridine in 95% yield after gel chromatography. In a similar way, compound **13** was obtained from N-*tert.*butyl-oxycarbonyl-L-valine and 5'-O-(O-benzyl-L-seryl)-2',3'-O-isopropylideneuridine in 93% yield. Treatment of **11** and **13** with HCl/EtOAc at room temperature for 30 min led to removal of both, Boc- and 2',3'-O-isopropylidene groups giving compounds **12** and **14** in 94% and 91% yields, respectively. The new derivatives were TLC pure and were characterized by MS, $^1$H NMR and elemental analysis. All other chemicals were purchased from Sigma.

### *Preparation of microsomes*

Liver microsomes from male Wistar rats were prepared by differential centrifugation (10000× *g* for 10 min and 105000× *g* for 60 min). The microsomal pellets were suspended in 0.1 M Tris [Tris(hydroxymethyl)aminomethane]/HCl buffer (pH 7.4) containing 0.25 M sucrose and stored at −70 °C until used (Matsui and Watanabe, 1982). The microsomal protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard.

### *Enzyme assay*

UGT activities towards 4-nitrophenol (4-NP) or phenolphthalein (PPh) were assayed as described previously (Naydenova *et al.*, 1995). Glucuronida-

tion was measured on microsomes activated by the nonionic detergent Lubrol-17A. The optimal mass ratio of detergent to protein was 0.25. The mixture of microsomes and detergent was preincubated at 4 °C for 30 min. The transferase activity was measured colorimetrically at 405 nm for 4-NP or at 550 nm for PPh. The specific activity was expressed as nmoles of glucuronide formed per min per mg protein. All derivatives tested were colorless and were added at a final concentration of 1 mM, dissolved in DMSO. The final concentration of DMSO in the incubation mixture was 2%. The control specific activity of UGT towards 4-NP in the presence of DMSO was 17.23±2.16 nmol/min×mg. For glucuronidation of PPh this value was 2.87±0.4 nmol/min×mg.

The apparent Ki values were determined from graphical Dixon's plots of 1/V versus [I] varying the inhibitor concentrations between 0.2 and 0.8 mM.

*QSAR analysis*

The Fujita-Ban variant of the Free-Wilson analysis was used as a basic method in the QSAR study (Seydel and Schaper, 1979). It is a multivariate technique for analyzing QSARs by dividing the chemical structures into structural parts and features: substructures, substituents, residues, etc. The biological activity, A, is expressed as a sum of activity contributions: $A = \Sigma_j\Sigma_k C_{jk}X_{jk} + A_0$, where $X_{jk}$ has a value of one if the feature k is present in part j and zero otherwise, $C_{jk}$ is the corresponding regression coefficient, and $A_0$ is activity of a reference structure. Multiple linear regression (MLR) was applied for a structural feature selection. The statistical evaluation of the QSARs was done on the basis of the coefficient of multiple determination $R^2$, regression coefficient B and variable significance p.

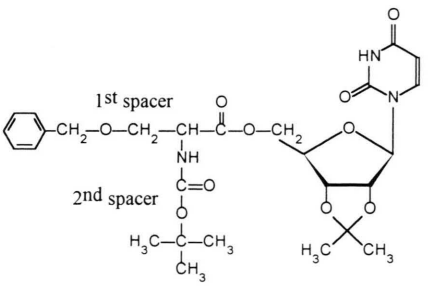
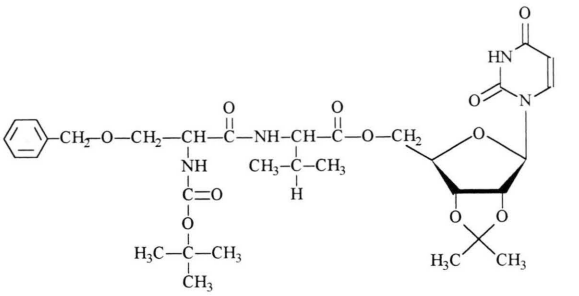
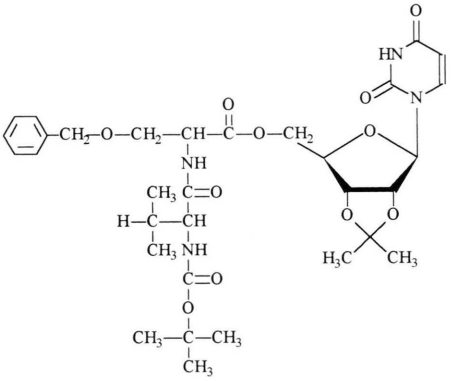



Fig. 1. Chemical structure of Boc-L-Ser(Bzl)-iUrd (compound **1**).
Boc, *tert.* butyloxycarbonyl; Bzl, benzyl; iUrd, isopropylideneuridine.



**11**



**13**

Fig. 2. Structure of dipeptide uridinyl derivatives Boc-L-Ser(Bzl)-L-Val-iUrd (**11**) and Boc-L-Val-L-Ser(Bzl)-iUrd (**13**).
Boc, *tert.* butyloxycarbonyl; Bzl, benzyl; iUrd, isopropylideneuridine.

## Results

*Structure of the inhibitors*

The compounds investigated in the present study contain a hydrophilic uridine moiety connected to lipophilic residues by varying spacers (amino acid residues). The most common is the five-atom ω-oxypropionate (-$OCH_2CH(NH)$-COO-) spacer (see Fig. 1), which links benzyl (**1**, **2**, **9**, **10**, **13**, **14**) or *tert.*butyl (**7**, **8**) moieties to uridine (Urd) or isopropylideneuridine (iUrd). In compounds **3**, **4**, **5** and **6** this spacer is shortened by omitting the ω-oxygen. Besides this first spacer, the compounds **1**, **3**, **5**, **7** and **9** could be suggested to contain also a second six-atom bridge

(-OCONHCHCOO-) linking *tert.*butyl (**1**, **3**, **5**) or benzyl (**7**, **9**) moieties to the nucleoside. In dipeptide derivatives **11** and **12** the ω-oxypropionate spacer is linked to Urd (iUrd) via a valyl residue, forming an eight-atom serylvalyl first spacer (-$OCH_2$CH(NH)CONHCHCOO-). In compounds **11** and **13**, a second nine-atom spacer (-OCONHCHCONHCHCOO-) could be suggested (Fig. 2).

*Inhibitory effect on the UGT activities*

In order to determine the inhibitory potency of these compounds they were tested on the glucuronidation of 4-NP and PPh by microsomal UGTs. The results expressed as a percent inhibition of the control enzyme activities are presented in Table I.

Table I. Inhibitory effect of 5'-O-amino acid and oligopeptide derivatives of uridine on UGT activities. 4-NP or PPh-glucuronidation was assayed in a standard medium containing an activated microsomal fraction (802 μg/ml protein), 0.1 M Tris/Cl (pH 7.4), 40 μM EDTA, 10 mM $MgCl_2$, 2 mM UDPGA and 500 μM 4-NP or 120 μM PPh, in the presence of 1 mM uridinyl analogs, dissolved in DMSO. The glucuronidation reaction was started by addition of UDPGA and was carried out at 37 °C for 10 min. The UGT activity was measured at 405 nm for 4-NP or at 550 nm for PPh, and expressed as nmoles of glucuronide formed per min per mg protein. The results are expressed as percent inhibition of enzyme activity measured in the absence of the analogs.
Mean values of at least three experiments and the standard deviations are given.

| | Compound | Inhibition (%) | |
|---|---|---|---|
| | (1 mM concentration) | 4-NP-UGT | PPh-UGT |
| **1** | Boc-L-Ser(Bzl)-iUrd | 75 ± 9 | 77 ± 7 |
| **2** | H-L-Ser(Bzl)-Urd | 15 ± 1 | 18 ± 3 |
| **3** | Boc-L-Leu-iUrd | 49 ± 2 | 55 ± 2 |
| **4** | H-L-Leu-Urd | 26 ± 4 | 0 |
| **5** | Boc-L-Val-iUrd | 52 ± 4 | 50 ± 3 |
| **6** | H-L-Val-Urd | 17 ± 3 | 10 ± 2 |
| **7** | Z-L-Thr(Bu$^t$)-iUrd | 47 ± 8 | 0 |
| **8** | H-L-Thr(Bu$^t$)-iUrd | 22 ± 3 | 11 ± 2 |
| **9** | Z-D,L-Thr(Bzl)-iUrd | 25 ± 4 | 0 |
| **10** | H-D,L-Thr(Bzl)-iUrd | 30 ± 5 | 36 ± 3 |
| **11** | Boc-L-Ser(Bzl)-L-Val-iUrd | 34 ± 6 | 70 ± 2 |
| **12** | H-L-Ser(Bzl)-L-Val-Urd | 22 ± 3 | 25 ± 2 |
| **13** | Boc-L-Val-L-Ser(Bzl)-iUrd | 25 ± 1 | 65 ± 7 |
| **14** | H-L-Val-L-Ser(Bzl)-Urd | 21 ± 2 | 25 ± 4 |

4-NP-UGT, 4-nitrophenol UDP-glucuronosyltransferase activity.
PPh-UGT, phenolphthalein UDP-glucuronosyltransferase activity.
Boc, *tert.* butyloxycarbonyl; Bzl, benzyl; iUrd, isopropylideneuridine; Urd, uridine; Bu$^t$, *tert.* butyl; Z, benzyloxycarbonyl.

Compound **1**, the serine derivative of iUrd (Fig. 1), was found to be the most potent inhibitor of both, 4-NP and PPh glucuronidation, with $I_{50}$ values of 0.45 mM and 0.22 mM, respectively. Leucine and valine derivatives **3** and **5** were also active, exerting at 1 mM concentration a similar decrease in 4-NP or PPh conjugation (49–55%). These data have been published in a preliminary note (Naydenova *et al.*, 1996).

The compounds containing the respective unprotected L-amino acids, **2**, **4** and **6**, were low active towards the UGT activities studied. Among the threonine derivatives, compound **7** showed a selective inhibition potency against 4-NP glucuronidation (47%). Derivatives **8**, **9** and **10** were less inhibitory towards 4-NP conjugation, and only compound **10** showed some activity against PPh convertion. Dipeptide derivatives **11–14** exerted as a whole a low inhibitory effect on the 4-NP-UGT activity. However, a significant decrease in PPh glucuronidation was registered with compounds **11** and **13** (70% and 65%, respectively).

Kinetic studies were carried out with the most potent inhibitor, compound **1**. Its interactions with the microsomal UGT isoforms, converting 4-NP, were characterized by varying both the concentration of 4-NP (0.1 to 0.8 mM) and UDPGA (0.25 to 8 mM), using inhibitor concentrations between 0.2 and 0.8 mM. A mixed type of inhibition towards 4-NP and UDPGA was found (Fig. 3 a, b), and apparent Ki values of 150 μM and 120 μM, respectively, were determined by Dixon's plots of the data (1/V versus [I]). These results suggest that compound **1** competes, at least in part, with both substrates for their binding sites in the molecules of 4-NP converting UGT isoenzymes.

*QSAR study*

In the QSAR analysis the same mechanism of action for all compounds is assumed. This demand is a prerequisite for each QSAR study although its fulfilment is not known, especially in the case of complex enzyme-inhibitor interactions, where closely related analogs may bind in very different manners. Several ways of structural data organization (data structuring) were experimented. The uridine moiety was considered as a conservative part of the structure independently of its form (iUrd or Urd) as no definitive information about its influ-

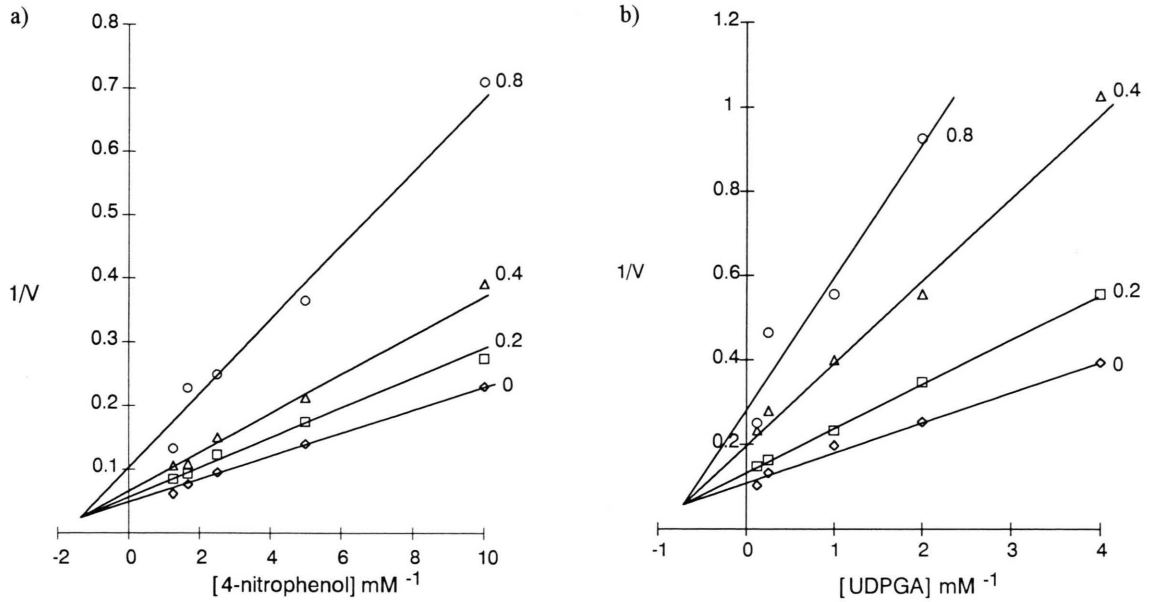



Fig. 3. Lineweaver-Burk plots for the inhibition of 4-NP glucuronidation in rat liver microsomes by Boc-L-Ser(Bzl)-iUrd (compound **1**). The 4-NP- glucuronidation was assayed as in Table I at: (a) a constant UDPGA concentration (2 mM), varying the 4-NP concentration from 0.1 to 0.8 mM; (b) a constant concentration of 4-NP (0.5 mM), varying the UDPGA concentration from 0.25 to 8 mM. Inhibitor concentrations (mM) are indicated on the right side of the plot.
Boc, *tert.* butyloxycarbonyl; Bzl, benzyl; iUrd, isopropylideneuridine; 4-NP, 4-nitrophenol; PPh, phenolphthalein.

ence on the inhibitory effect was available in advance. As well, the results of other authors do not allow any particular conclusion to be drawn on the higher inhibitory potency of the isopropylideneuridine versus uridine form (Battaglia *et al.*, 1995). In the study a percent inhibition effect was used although not recommendable for QSARs due to the nonlinear characteristics of dose-response curves. Nevertheless, the presented QSAR analysis aims some quantitative estimation to be done as to which structural features might be responsible for the biological activity of interest presuming equal possibilities for active-site binding of nucleoside and aglycone residues by more than one spacer.

The structures were considered as consisting of two main variable parts: related to first (1st) and second (2nd) spacers (Table II, see Fig. 1).

In the first structuring the length of the spacers (here and further every spacer is considered to start from the 5'-O- atom of the uridine moiety) was experimented as indicator variables: short (less than 5 atoms), five-atom and long (more than 5 atoms) for the 1st spacer; no (no 2nd spacer presented), six-atom and long (more than 6 atoms) for the 2nd spacer. The structure with a short 1st spacer and no 2nd spacer was chosen to be a reference. No significant structural features were identified for PPh and the six-atom 2nd spacer only was estimated as contributing significantly to the inhibitory effect for 4-NP with a moderate fitting quality ($R^2$=0.614, $p<0.05$). Taking into account the possible rotational hindrance around the single bonds of the 3rd carbon atom (where the two spacers split) by the methyl-group substituent on the next carbon atom (compounds **7** and **9**), an additional structural feature was experimented for these inhibitors. Once again the six-atom 2nd spacer was estimated as significant for 4-NP ($R^2$= 0.813, $p<0.008$). For PPh the five-atom and a long 1st spacer and the six-atom and a long 2nd spacer were estimated as significant ($R^2$=0.955, $p<0.00004$). For both substrates the presence of methyl-group next to the 3rd spacer atom was also significant with a negative contribution to the inhibitory effect. These results directed the final structuring to be detailed on the five-atom 1st spacer and the six-atom 2nd.

Table II. Indicator variables used in the QSAR study of the UGT inhibitors.

| | 1st spacer[a)] | | | | 2nd spacer[b)] | | | |
|---|---|---|---|---|---|---|---|---|
| **Compound** | **short** | **five-Bu$^t$** | **five-Bzl** | **long 1st** | **no** | **six-Bu$^t$** | **six-Bzl** | **long 2nd** |
| **1** | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 2 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| **3** | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **4** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| **5** | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **6** | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| **7** | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| **8** | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| **9** | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| **10** | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| **11** | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| **12** | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 |
| **13** | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| **14** | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |

**a) 1st spacer**

short: —$O^1$—$C^2$(O)—$C^3$H—$C^4H_2$—$CH(CH_3)_2$ and —$O^1$—$C^2$(O)—$C^3$H—$CH(CH_3)_2$
|

five-Bu$^t$: —$O^1$—$C^2$(O)—$C^3$H—$C^4H_2$—$O^5$—$C(CH_3)_3$
|

five-Bzl: —$O^1$—$C^2$(O)—$C^3$H—$C^4H_2$—$O^5$—$CH_2(C_6H_5)$
|

long 1st: —$O^1$—$C^2$(O)—$C^3$H—$N^4$H—$C^5$(O)—$C^6$H—$C^7H_2$—$O^8$—$CH_2(C_6H_5)$
|

**b) 2nd spacer**

six-Bu$^t$: —$O^1$—$C^2$(O)—$C^3$H—$N^4$H—$C^5$(O)—$O^6$—$C(CH_3)_3$
|

six-Bzl: —$O^1$—$C^2$(O)—$C^3$H—$N^4$H—$C^5$(O)—$O^6$—$CH_2(C_6H_5)$

long 2nd: —$O^1$—$C^2$(O)—$C^3$H—$N^4$H—$C^5$(O)—$C^6$H—$N^7$H—$C^8$(O)—$O^9$—$C(CH_3)_3$
| |

Bu$^t$, *tert.* butyl; Bzl, benzyl.

The indicator variables used for structural features designation in the final QSAR study are presented in Table II: 1st spacer (*short*, consisting of less than 5 atoms; *five-Bu$^t$*, five-atom spacer with *tert.*butyl residue; *five-Bzl*, five-atom spacer with benzyl residue; *long 1st*, eight-atom or dipeptide spacer with benzyl residue); 2nd spacer (*no*, no spacer presented; *six-Bu$^t$*, six-atom spacer with *tert.*butyl residue; *six-Bzl*, six-atom spacer with benzyl residue; *long 2nd*, nine-atom or dipeptide spacer with *tert.*butyl residue). As compounds **3** and **5**, and **4** and **6** are presented by the same structural features and possess very close inhibitory effects, the final MLR run was performed without compounds **5** and **6**. Again, the structure with a short 1st and no 2nd spacer was chosen as a reference (compound **4**).

The final MLR results are presented in Table III a, b and Fig. 4 a, b for 4-NP and PPh substrates, respectively. In case of 4-NP, the six-atom 2nd spacer with *tert.*butyl residue is significant (Table III a). In case of PPh, the five-atom and a long 1st spacer with benzyl residue and the six-atom and a long 2nd spacer with butyl and benzyl residues are significant (Table III b). Suggesting a positive influence of the lipophilicity of the aglycone analog residue for the enzyme inhibition (Noort *et al.*, 1990) one could expect that more lipophilic the residue more potent the inhibitor. However, taking into account that the lipophilicities of the benzyl and *tert.*butyl substituents are very close (Rekker and Mannhold, 1992), neither of them was considered as an individual indicator variable. Both the substituents were estimated to

Table III. Regression summary results of the QSAR study of the UGT inhibitors: N, number of compounds; B, regression coefficient; SE, standard error of estimate; t, Student's test; p-level, statistical significance; $R^2$, coefficient of multiple determination; Bu$^t$, *tert.* butyl; Bzl, benzyl; 4-NP, 4-nitrophenol; PPh, phenolphthalein.

a) 4-NP substrate: N=12; $R^2$=0.785; p<0.00338; SE= 9.7669

| Variable | B | SE | t(9) | p-level |
|---|---|---|---|---|
| intercept | 21.37500 | 4.229203 | 5.054143 | 0.001473 |
| **six_Bu$^t$** | 40.62500 | 8.098308 | 5.016480 | 0.001537 |
| six_Bzl | 10.75000 | 8.458407 | 1.270925 | 0.244359 |
| long_2nd | 8.12500 | 8.098308 | 1.003296 | 0.349131 |
| five_Bu$^t$ | 7.75000 | 8.458407 | 0.916248 | 0.390010 |

b) PPh substrate: N=12; $R^2$=0.972; p<0.00096; SE=6.8944

| Variable | B | SE | t(5) | p-level |
|---|---|---|---|---|
| intercept | 0.8333 | 5.745369 | 0.14504 | 0.890341 |
| **six_Bu$^t$** | 53.3333 | 6.080327 | 8.77146 | 0.000319 |
| **long_2nd** | 40.6667 | 6.080327 | 6.68824 | 0.001130 |
| **six_Bzl** | −20.0000 | 6.080327 | −3.28930 | 0.021730 |
| **five_Bzl** | 23.6667 | 6.080327 | 3.89233 | 0.011498 |
| **long_1st** | 24.3333 | 7.961016 | 3.05656 | 0.028212 |
| five_Bu$^t$ | 14.6667 | 7.961016 | 1.84231 | 0.124776 |

* Significant structural features are given in bold.

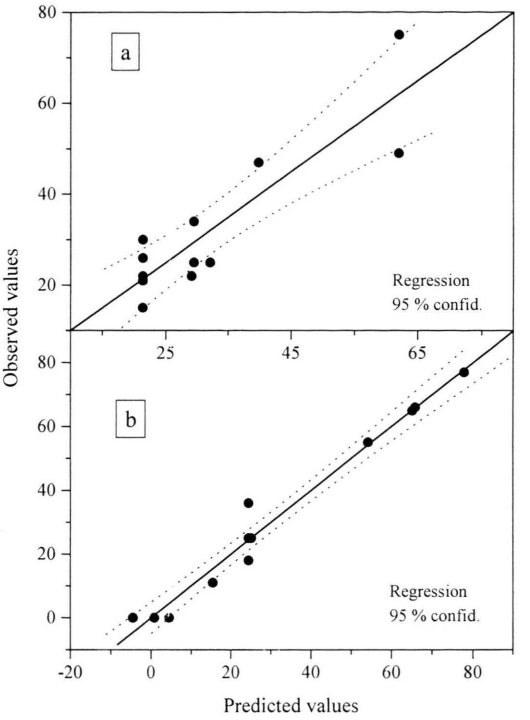



Fig. 4. Plots of predicted versus observed inhibition effects of the UGT inhibitors:
a) 4-NP substrate b) PPh substrate
4-NP, 4-nitrophenol; PPh, phenolphthalein.

be significant when linked to the relevant spacers. The fitting quality of the models could be considered as very good according to $R^2$ values (0.785 and 0.972 for 4-NP and PPh, respectively, Table III) and the plots of predicted versus observed inhibitory effects (Fig. 4).

## Discussion

In the past years, several active-site directed UGT inhibitors have been designed, and some of them shown to behave as analogs of the presumed transition- state of the glucuronidation reaction, catalyzed by UGT. They combined structural features of the both substrates: the hydrophilic uridine moiety and the lipophilic acceptor residue, connected by diphosphate or diphosphate-like five-atom spacers.

In this study, novel uridinyl analogs, modified at 5'-O-position by protected and unprotected amino acids, were tested as inhibitors of diverse rat liver UGTs and their structure-activity relationships analyzed by a relevant QSAR approach.

In general, the QSAR analysis outlined the significance of the spacers with length of minimum five atoms and lipophilic residues linked to them for the UGT inhibitory effect of the compounds studied. This finding is in agreement with the existing understanding for a five-atom spacer as in the UDP part (Noort *et al.*, 1990; Battaglia *et al.*, 1995). However, according to the variable significance, the most significant contribution to the inhibitory effect is given to the six-atom spacer (starting and ending by oxygen atoms and containing rotatable bonds) for both, 4-NP and PPh substrates.

One can presume that both the spacers could play a role with a stronger influence of the six-atom 2nd spacer. Generally, the compounds suggested to carry two spacers linked to Urd (iUrd) are more inhibitory against enzyme activities studied than those with one spacer only. In our preliminary publication we have assumed that both, the lipophilic benzyl moiety and the five-atom ω-oxypropionate spacer (compound **1**) contribute to the high inhibitory potential (Naydenova *et al.*, 1996). However, in compound **1** a second six-atom spacer could be considered, linking

iUrd and a lipophilic *tert.*butyl moiety. The abolishment of this spacer by removing of the N-protecting *tert.*butyloxycarbonyl residue abolished the inhibitory activity (compound **2**). The same holds true for derivatives **4** and **6**, which are unprotected forms of **3** and **5** (Table I). Obviously, the six-atom spacer linking *tert.*butyl to uridine residue contributes significantly to the inhibitory potency of the compounds.

The dipeptide derivatives containing a long (eight-atom) first (**11**, **12**) and/or a long (nine-atom) second (**11**, **13**) spacers are much less active against 4-NP glucuronidation as compared with compound **1**. However, the nine-atom second spacer in compounds **11** and **13** is favourable for their binding to the molecules of PPh conjugating UGT isoenzymes. Additionally, the *tert.*butyl residue in these compounds also seems to facilitate this binding.

To check the role of the first spacer on the inhibitory effect, a new compound, 5'-O-(N-*tert.*butyloxycarbonyl-L-seryl)-2',3'-O-isopropylideneuridine, similar to the most potent inhibitor was synthesized with no aglycone residue linked to the first spacer (see Fig. 1). The lack of such residue particularly eliminated the possible contribution of the first spacer in the inhibitor-enzyme interaction. Indeed, the new compound proved to be half less potent towards 4-NP glucuronidation and one third less potent towards PPh conversion (32% and 46% inhibition, respectively) than compound **1**. These results confirm again the possible role of both spacers as well the stronger influence of the six-atom spacer on the UGT inhibition activity of the derivatives studied

In conclusion, 4-NP converting UGT isoforms respond more specifically to the inhibitors: a five-atom first and a six-atom second spacers enhance binding to both, 4-NP and PPh conjugating isoenzymes, while a long second spacer contributes to inhibitor binding only to UGT isoforms converting PPh.

Thus, on the basis of the presented study, one can expect that new and potent UGT inhibitors could be designed not only by varying the acceptor substrate analogs but also allowing alternative spacers between the nucleoside and the aglycone moieties possibly to be involved in the enzyme-inhibitor interaction.

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