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Inhibition of P-glycoprotein-mediated multidrug efflux by aminomethylene and ketomethylene analogs of reversins

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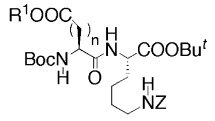
Abstract—Several aminomethylene analogs and a ketomethylene analog of reversins were synthesized in order to evaluate their ability to inhibit P-glycoprotein-mediated drug efflux in K562/R7 human leukemic cells overexpressing P-glycoprotein. These analogs retained good activity compared to cyclosporin A and the original reversins.

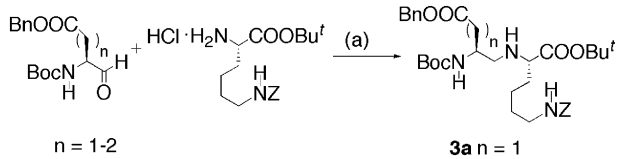
Multidrug resistance (MDR) to anticancer agents remains a major cause of treatment failure in cancer chemotherapy. MDR describes the cross-resistance of tumor cell lines to several structurally unrelated chemotherapeutic agents after exposure to a single cytotoxic drug. This phenomenon is often associated with overexpression of several proteins.[1] Among them P-glycoprotein (Pgp) is the most important one that belongs to the ABC superfamily of transporters which acts as a drug efflux pump.[2,3]

Numerous molecules have shown some activity on Pgp.[4] Among them short linear hydrophobic peptides were described as chemosensitizers.[5a] Seprõdi et al. showed that small hydrophobic peptide derivatives modulate Pgp-ATPase activity and inhibited the drug extrusion function of Pgp.[5b,c] These compounds are a family of di- and tripeptide derivatives sharing some common physico-chemical and structural features. Some of them are dimerized aminoacids from diacid derivatives. The enhanced affinity to Pgp of these chemosensitizers finally coined reversins is ascribed to the hydrophobic nature of the side chains protected with bulky aromatic or alkyl groups.[6] Among them reversin 121 **1a** showed the highest affinity and specificity for Pgp.



1a n = 1, R^1 = Bn, reversin 121
1b n = 2, R^1 = Dmp
1c n = 2, R^1 = *c*Hex

At 1–2 μM this reversin was more effective than cyclosporin A for blocking colchicine transport in isolated membranes and reconstituted systems. In order to improve proteolytic stability and bioavailability of reversins, we planned to synthesize aminomethylene and



n = 1-2

3a n = 1
3b n = 2

Scheme 1. Reagents and condition: (a) $NaBH_3CN$, MeOH, AcOH, rt, 1 h, 52% (*n* = 1) and 57% (*n* = 2).

Keywords: Multidrug resistance; P-glycoprotein; Reversin; Aminomethylene; Ketomethylene; Pseudopeptide; Chemosensitizer.
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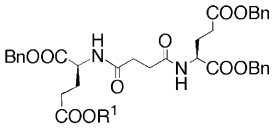
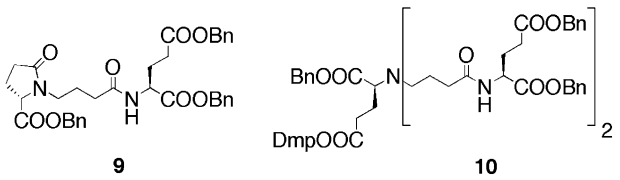
ketomethylene analogs of typical reversin representatives. We chose reversin 121 **1a** as our working model for dipeptide-type reversins and reversin 213 **2a** as a dimerized aminoacid containing succinyl unit. The reduced analogs **3a**–**b** were synthesized using the classical reductive amination strategy[7] starting from chiral pool-derived aminoaldehydes according to Scheme 1.[8]



2a R^1 = Bn, reversin 213
2b R^1 = *c*Hex

As far as reduced analogs of reversin 213 **2a** were concerned we started from 4-pentenoic acid which was activated as succinimidyl ester and then coupled to L-glutamic acid dibenzyl ester tosylate salt to obtain the amide **4** (Scheme 2). The corresponding aldehyde **6** was obtained by a two-step reaction with osmium tetroxide/4-methylmorpholine *N*-oxide[9] followed by oxidative cleavage of the diol **5** with sodium periodate. It is noteworthy that aldehyde **6** exists in equilibrium with the corresponding hemiaminals **6′** as previously described in similar reactions.[10a,b] Structure of the diastereoisomeric hemiaminals **6′** was assessed by ESIMS and HSQC and HMBC experiments.[10c] The last step consisted in obtaining the reduced analogs **8a**–**c** of reversin 213 **2a**. However in the standard reductive amination conditions, the reaction between diprotected L-glutamic acid **7b** and aldehyde **6** did not afford the corresponding secondary amine but instead the pyrrolidinone **9** in 38% yield (Fig. 1). This result is explained by nucleophilic attack of the generated secondary amine on the adjacent benzyl ester side chain as observed for similar derivatives.[11] So as to avoid cyclization, several protected derivatives of L-glutamic acid such as the 2,4-dimethyl-3-pentyl (Dmp)[12a] and cyclohexyl (*c*Hex)[12b] esters **7c** and **7d**[12c] known to prevent aspartimide formation were used to obtain, respectively, analogs **8b** and **8c**. Moreover by using an excess of aldehyde **6** a double addition product **10** was obtained in 47% yield in the case of reductive amination of L-glutamic acid derivative **7c** (Fig. 1).[13] Undescribed derivatives **1b** and **1c** along with compound **2b** were also synthesized by standard procedures for sake of comparison.

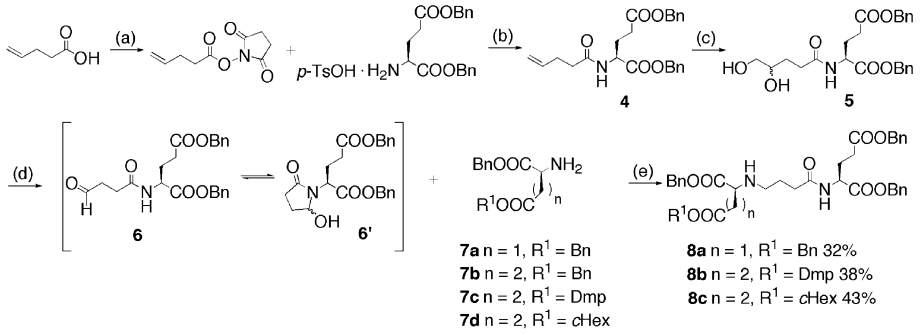


9 **10**

Figure 1. Structures of by-products **9** and **10** obtained respectively in the reductive amination of **7b** and **7c** with aldehyde **6**.

We thought it would be worthwhile to test the introduction of a spacer between the Asp and Lys residue of reversin 121 **1**. Thus, we chose to synthesize the protected Asp-ψ(CO–CH_2)Gly-Lys ketomethylene analog **16** as outlined in Scheme 3. The synthesis started from L-homoserine which was protected on the side chain as a *tert*-butyl dimethyl silyl ether followed by protection of the free α-amine as *tert*-butyloxycarbonyl derivative and finally transformed in Weinreb amide **11** in 40% overall yield over three steps. The alkene **12** was then obtained from Weinreb amide **11** by alkylation with butenyl magnesium bromide as described[14] in 55% yield. Transformation of the protected hydroxyl of **12** in benzyl ester **13** was carried out by oxidation of the deprotected hydroxyl with PDC and then esterification of the cesium salt in 53% overall yield as described.[15a,15b] Oxidation of alkene **13** with $RuCl_3{\cdot}xH_2O/NaIO_4$ afforded free acid **14** in 96% yield. The ketomethylene analog **16** was finally obtained by coupling the succinimidyl derivative **15** and diprotected L-lysine hydrochloride in 94% yield.

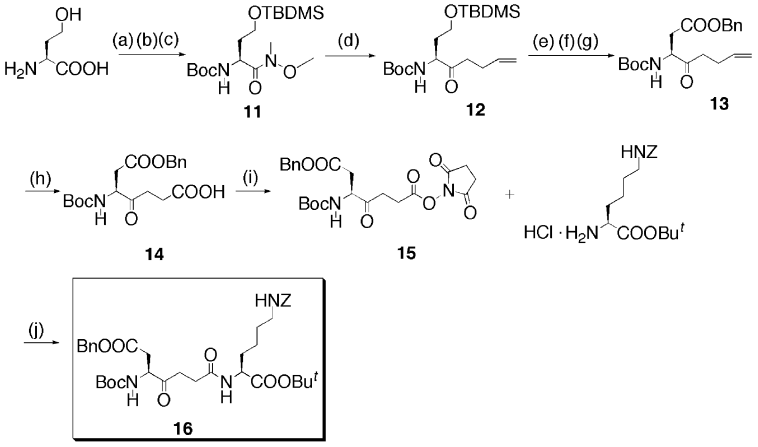
The efficiency of reversin analogs to inhibit Pgp-mediated daunorubicin efflux was investigated by monitoring the intracellular accumulation of this drug in K562/R7 human leukemic cells overexpressing Pgp in the presence of daunorubicin.[16a] Cyclosporin A was used as a positive control (Table 1). These analogs showed strong inhibitory activity comparable to that of reversin 121 **1a** except in the case of the tertiary amine **10** which caused an important decrease in activity. It was noteworthy that the replacement of the amide bond with



7a n = 1, R^1 = Bn
7b n = 2, R^1 = Bn
7c n = 2, R^1 = Dmp
7d n = 2, R^1 = *c*Hex

8a n = 1, R^1 = Bn 32%
8b n = 2, R^1 = Dmp 38%
8c n = 2, R^1 = *c*Hex 43%

Scheme 2. Reagents and conditions: (a) *N*-hydroxysuccinimide, DMAP, DCC, THF, 0 °C, 10 min then rt, 48 h, 94%; (b) DIEA, DMF, rt, 24 h, quant yield; (c) OsO_4 2.5% in *t*-BuOH, NMO, THF, H_2O, rt, 24 h, 86%; (d) $NaIO_4$, THF, H_2O, 3 h, quant yield; (e) $NaBH_3CN$, MeOH, AcOH, rt, 1–2 h.



Scheme 3. Reagents and conditions: (a) TBDMSCl, DBU, CH_3CN, 0 °C then rt, 24 h, 82%; (b) $(Boc)_2O$, Et_3N, acetone, H_2O, rt, 24 h, 80%; (c) *N*,*O*-dimethylhydroxylamine hydrochloride, TBTU, HOBt, DIEA, CH_2Cl_2, rt, 12 h, 61%; (d) butenyl magnesium bromide, THF, −78 °C then 3.5 h, rt, 30 min, 55%; (e) TBAF, THF, rt, 1 h, 87%; (f) PDC, DMF, rt, 3 h, 71%; (g) i—Cs_2CO_3, MeOH, H_2O then reduced pressure; ii—benzyl bromide, DMF, rt, 4.5 h, 86%; (h) $RuCl_3{\cdot}xH_2O$ $NaIO_4$, H_2O, CH_3CN, rt 1 h, 96%; (i) *N*-hydroxysuccinimide, DMAP, DCC, THF, 0 °C, 10 min then rt, 48 h, 49%; (j) DIEA, DMF, rt, 24 h, 94%.

Table 1. Mean inhibitory activity (%) of reversins and synthesized analogs on human leukemic cells K562/R7[16b]

Compound[a]	Mean inhibiting activity[b] %
1a Reversin 121	72.73 (±5.27)
1b	52.70 (±5.27)
1c	85.43 (±5.69)
2a Reversin 213	93.74 (±2.17)
2b	94.45 (±1.36)
3a	74.14 (±4.06)
3b	79.08 (±5.13)
8a	82.22 (±4.57)
8b	54.71 (±3.63)
8c	98.33 (±1.35)
10	3.35 (±3.44)
16	86.62 (±4.86)

[a] Compounds were tested at a 10 μM concentration.

[b] Cyclosporin A was used as positive control (mean inhibitory activity of 100%) at a final concentration of 2 μM. Standard deviation is given in parentheses.

an amino methylene isostere did not impair the capacity to inhibit Pgp-mediated drug efflux (**1a** vs **3a** and **2b** vs **8c**). Analogs with cyclohexyl ester presented an increased inhibitory activity when compared to 2,4-dimethyl-3-pentyl ester analogs (**1b** vs **1c** and **8b** vs **8c**). Moreover, exchanging benzyl ester by cyclohexyl ester was tolerated as shown in Table 1 by comparing activity for compounds **2a** and **2b**. Finally, the inhibitory activity of ketomethylene analog **16** was conserved indicating that inserting a succinyl moiety into reversin 121 **1a** was not detrimental to the Pgp-mediated drug efflux inhibitory activity.

We have designed aminomethylene and ketomethylene analogs with inhibitory activity of Pgp-mediated drug efflux comparable to that of reversins 121 **1a** and 213 **2a**. We envisage now the synthesis of ketomethylene analogs of reversin 121 and modified side chain analogs to confirm and refine our first results.

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(4H, m, 2 CH_2O Bn), 5.12 (4H, m, 2 CH_2O Bn), 4.77 (1H, dd, J = 4.5 and 9.8 Hz, C$H\alpha$), 4.64 (1H, t, J = 7.1 Hz, C$H\alpha$), 4.32 (1H, d, J = 4.5 Hz, exch OH), 3.41 (1H, d, J = 7.8 Hz, exch OH), 1.8–2.8 (16H, m, 2 –CH_2CH_2– and 2 –CH_2CH_2– Glu). ^{13}C NMR (75 MHz, $CDCl_3$, δ ppm): 176.3 (C=O), 175.8 (C=O), 173.7 (C=O), 173.1 (C=O), 172.8 (C=O), 171.0 (C=O), 136.0 (Cq Phe), 135.9 (Cq Phe), 135.5 (Cq Phe), 135.2 (Cq Phe), 129.1 (CH–Phe), 129.07 (CH–Phe), 129.02 (CH–Phe), 128.9 (CH–Phe), 128.8 (CH–Phe), 128.7 (CH–Phe), 128.6 (2 CH–Phe), 84.4 (*C*HOH), 82 (*C*HOH), 68.3 (*C*H_2O), 67.9 (*C*H_2O), 67.1 (*C*H_2O), 67.0 (*C*H_2O), 54.7 (*C*Hα), 54.3 (*C*Hα), 31.3 (*C*H_2), 30.0 (*C*H_2), 29.5 (*C*H_2), 29.3 (*C*H_2), 29.0 (*C*H_2), 28.8 (*C*H_2), 25.7 (*C*H_2), 24.6 (*C*H_2).

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