Novel unit B cryptophycin analogues as payloads for targeted therapy

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Abstract

Cryptophycins are naturally occurring cytotoxins with great potential for chemotherapy. Since targeted therapy provides new perspectives for treatment of cancer, new potent analogues of cytotoxic agents containing functional groups for conjugation to homing devices are required. We describe the design, synthesis and biological evaluation of three new unit B cryptophycin analogues. The \(\text{O}\)-methyl group of the unit B D-tyrosine analogue was replaced by an \(\text{O}\)-(allyloxyethyl) moiety, an \(\text{O}\)-(hydroxyethyl) group, or an \(\text{O}\)-(((azidoethoxy)ethoxy)ethoxyethyl) substituent. While the former two maintain cytotoxicity in the subnanomolar range, the attachment of the triethylene glycol spacer with a terminal azide results in a complete loss of activity. Docking studies of the novel cryptophycin analogues to \(\beta\)-tubulin provided a rationale for the observed cytotoxicities.

Introduction

Cryptophycins are natural occurring cyclic depsipeptides that were first isolated from cyanobacteria Nostoc sp. ATCC 53789 in 1990 [1]. Cryptophycins target tubulin, in particular the peptide site of the vinca domain. They block microtubule formation, inhibiting their assembly and, hence, are antimitotic agents [2,3]. Their high cytotoxicity prompted manifold studies that were initially focussed on the total synthesis and structure–activity relationships [4-20]. This work resulted in the identification of cryptophycin-52, a highly biologically active analogue of cryptophycin-1 (Figure 1).

Eli Lilly took cryptophycin-52 into clinical trials. Although almost half of the patients obtained a benefit from the treatment, neurotoxic side effects forced the termination of the clinical trials [21-23]. In order to overcome the side effects of cryptophycin-52 and to better understand the fundamental structure for biological activity, numerous structure–activity relationship studies have been carried out [24-35]. However, like cryptophycin-52, the new analogues were not selective against cancer cells making them not better than its parent.

In recent years the targeted delivery of cytotoxic agents has emerged as a highly promising method to tackle selectivity issues [36-40]. Cryptophycin-52 and many analogues lack an addressable group to conjugate the toxin to a homing device. For this reason, new analogues containing functional groups...
Scheme 1: Synthesis of modified unit B (13 and 14). Reagents and conditions: (a) TsCl, DMAP, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, 4 h; (b) NaN<sub>3</sub>, DMF, 70 °C, overnight; (c) NaI, acetone, reflux, overnight; (d) TsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, rt, overnight; (e) NaI, acetone, reflux, overnight; (f) 6 or 9, K<sub>2</sub>CO<sub>3</sub>, DMF, 50 °C, overnight; (g) LiOH, H<sub>2</sub>O/MeOH/THF 1:1:1, rt, 2 h.
Scheme 2: Synthesis of cryptophycin analogues 22, 23 and 24. Reagents and conditions: (a) 4-DMAP, 2,4,6-trichlorobenzoyl chloride, Et$_3$N, THF, 0 °C, 3 h; (b) 1) piperidine, DMF, rt, 2 h; 2) 13 or 14, HOAt, EDC·HCl, Et$_3$N, CH$_2$Cl$_2$, 0 °C → rt, overnight; (c) 1) TFA/CH$_2$Cl$_2$/H$_2$O, rt, 2 h; 2) HATU, HOAt, DIPEA, DMF, rt, slow addition + 2 h; (d) 1) (CH$_3$O)$_3$CH, PPTS, CH$_2$Cl$_2$, rt, 2 h; 2) AcBr, CH$_2$Cl$_2$, rt, 4 h; 3) K$_2$CO$_3$, DME/ethylene glycol (2:1 v/v), rt, 5 min; (e) Pd(PPh$_3$)$_4$, phenylsilane, CH$_2$Cl$_2$, rt, 7 h.

2-allyloxyethanol (7) tosylations and nucleophilic displace-
ments by azide or iodide substitution provided 6 and 9 in good
yields. O-Alkylation of Boc-protected 3-chlorinated D-tyrosine
10 with 6 or 9 gave 11 and 12, again in satisfactory yields
(81–85%). Saponification of the ester moiety in 11 and 12 that
was formed concomitantly with the O-alkylation in the previous
reaction provided Boc-protected modified units B 13 and 14 in
76 and 90% yield, respectively.

The synthesis of units C–D and A succeeded as previously de-
scribed in the literature; unit A (15) and C–D (16) were
connected by Yamaguchi esterification to give 17 (Scheme 2)
[45]. Then, Fmoc was cleaved from the N-terminus of unit
C–D–A (17) using piperidine and the resulting crude amine was
coupled to the corresponding modified unit B (13 or 14),
affording the according linear cryptophycins 18 and 19 in
acceptable yields (51–59%). Compounds 18 and 19 were
treated with trifluoroacetic acid for simultaneous Boc and t-Bu
removal, which also cleaved the dioxolane ring. Subsequently,
macrolactamization was performed under pseudo-high-dilution
conditions to afford 20 and 21 as described previously [16].
Then the diol was transformed into the epoxide following a
three-step one-pot reaction as extensively used in the synthesis
of cryptophycin analogues [46]. Cryptophycin analogues 22 and
were obtained in good purity after column chromatography. The allyl ether in 23 was cleaved using Pd(PPh₃)₄ as Pd(0) source and phenylsilane as scavenger to obtain the cryptophycin analogue 24 in good purity.

### Biological evaluation

The biological activity of the modified unit B analogues was determined in a cell viability assay using the human cervix carcinoma cell line KB-3-1 (Table 1). The cryptophycin analogue 22 showed a dramatic loss of activity compared to cryptophycin-52 (2), while analogues 23 and 24 showed a reduced cytotoxicity although their IC₅₀ values are still in the low nanomolar range. The observed dramatic loss of activity of analogue 22 could be due to its poor internalization or the modification could alter the interaction with tubulin. In order to get an extensive knowledge of the novel analogues, we embarked in docking and modelling studies, herein reported, and internalization studies are ongoing in our research group.

### Docking and modelling of cryptophycin derivatives

There is no X-ray analysis of cryptophycin–tubulin complexes available to provide information on the binding site. Based on biochemical evidence, binding close to the vinca-alkaloid binding site of β-tubulin, the so called "peptide-site", has been proposed [2,52,53]. We performed a docking study to explain the different affinities of the newly synthesized derivatives. The parent compound 2 scored highest with respect to β-tubulin binding (Table 2). Three hydrogen bonds were detected to key residues in the peptide binding pocket of the vinca domain (Lys176, Val177 and Tyr210). Other than previously reported [52], the methoxy group of subunit B forms a hydrogen bond with Lys176 (Figure 2). Another binding mode of 2 with high binding affinity and hydrogen bond formation did not involve any interaction of subunit B, yet it was oriented towards the GDP binding site that might influence GTP hydrolysis.

### Table 1: Cytotoxicity of cryptophycin-52 and its unit B analogues.

<table>
<thead>
<tr>
<th>compd</th>
<th>unit B</th>
<th>IC₅₀ KB-3-1 (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>CH₂Ph(m-Cl,p-OMe)</td>
<td>0.015</td>
</tr>
<tr>
<td>22</td>
<td>CH₂Ph(m-Cl,p-(OCH₂CH₂)$_3$N₃)</td>
<td>195000</td>
</tr>
<tr>
<td>23</td>
<td>CH₂Ph(m-Cl,p-OCH₂CH₂OCH₂CHCH₂)</td>
<td>0.748</td>
</tr>
<tr>
<td>24</td>
<td>CH₂Ph(m-Cl,p-OCH₂CH₂OH)</td>
<td>0.184</td>
</tr>
</tbody>
</table>

Compound 22 with the triethylene glycol-based substituent prevents correct binding, the binding energy was decreased and mainly nonspecific interactions outside the binding pocket were observed (Figure 3). This was not the case for the other derivatives 23 and 24 (Figure 4).

### Table 2: Binding energies for the different cryptophycin analogues.

<table>
<thead>
<tr>
<th>compd</th>
<th>binding energy (kJ/mol)</th>
<th>max. binding energy (kJ/mol)</th>
<th>min. binding energy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>36.17</td>
<td>36.17</td>
<td>17.21</td>
</tr>
<tr>
<td>22</td>
<td>22.61</td>
<td>22.61</td>
<td>5.44</td>
</tr>
<tr>
<td>23</td>
<td>32.20</td>
<td>32.20</td>
<td>10.38</td>
</tr>
<tr>
<td>24</td>
<td>32.70</td>
<td>32.70</td>
<td>11.72</td>
</tr>
</tbody>
</table>

Besides hydrogen bond formation and binding affinity of inhibitors 2, 23 and 24, π-interactions and hydrophobic contacts with the binding pocket of the vinca domain were detected.
that would in turn increase the affinity of the inhibitor and its effect on the protein (Supporting Information File 1, Table S1).

Conclusion
In summary, three new cryptophycin analogues with a modified unit B have been designed and successfully synthesized. The novel analogues were less active than cryptophycin-52 in the KB-3-1 cell line. Analogue 22 showed a dramatic loss of activity whereas analogues 23 and 24 showed a reduced activity but were still very cytotoxic.

Supporting Information
Supporting Information File 1
Experimental part and analytical data.
[https://www.beilstein-journals.org/bjoc/content-supplementary/1860-5397-14-109-S1.pdf]

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References

Figure 4: Docking of 24 to β-tubulin. Surface and backbone of β-tubulin are shown in blue, GDP in yellow. H-bonding (yellow dots) were detected with Lys176 and Asp179 in magenta. The benzyl group and the epoxide of subunit A are directed towards the peptide binding pocket, while the hydroxyethyl group is positioned towards the GDP binding pocket forming an H-bond with Asp179.