Synthesis and cytotoxicity of natural (-)-quinocarcin and its related compounds

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Abstract: The title synthesis was accomplished by featuring highly diastereoselective reduction of the 1,3-disubstituted isoquinoline 10 to control stereochemistries at the C5 and C11a positions in 1a simultaneously in a single step. In vitro cytotoxicity assay of the synthesized quinocarcin congeners including their enantiomers disclosed novel aspects of structure-activity relationships and explored the unnatural 10-decarboxyquinocarcin derivatives 1b, 3b, and 4b which are more highly cytotoxic than the corresponding natural 10-carboxy compounds 1a, 3a, and 4a.

(-)-Quinocarcin [(-)-1a] isolated from the culture broth of Streptomyces melanovinaceus in 1983, exhibits prominent antitumor activity against various types of mammalian solid tumors (ref.1 and 2). Quinocarcinol (2a), the pharmacologically inactive dihydroderivative of 1a, was also isolated from the same culture broth (ref.1 and 2). While 1a is fairly unstable, cyanation of 1a with sodium cyanide can produce the more stable hydrogen cyanide adduct, DX-52-1 (3a), which still retains significant antitumor activity (ref. 2). It is also reported that treatment of 3a with silver nitrate cleanly regenerates 1a (ref. 2).

The stereostructure of 1a except its absolute configuration was revealed by a combination of X-ray diffraction and spectral studies to have a novel 8,11-iminoazepino[1,2-b]isoquinoline skeleton with six asymmetric carbons (ref. 2). Although the absolute configuration of 1a had been suggested based on the computer simulation of binding of 1a to DNA (ref. 3), Garner completed the first asymmetric synthesis of (-)-1a in 1992, leading to confirmation of its absolute configuration (ref. 4).

1. Synthesis of (-)-1a, (-)-1b, (+)-3a, (+)-3b, (+)-4a, (+)-4b, and Their Enantiomers (ref. 7 and 8)

At the time when our project was started, the absolute configuration of natural (-)-1a had not been established. Accordingly, prior to embarking on the total synthesis of (-)-1a, the preparations of the ABE ring systems 5-7, the ABC ring systems 8 and 9, and their enantiomers were first carried out to establish an efficient and reliable synthetic method for constructing asymmetric centers at the C5 and C11a positions of (-)-1a with the definite absolute configurations (ref. 9 and 10). On the basis of the results accumulated
in the successful syntheses of 5-9 and their enantiomers, a novel synthetic strategy for (-)-1a was designed as shown in Scheme 1, which features highly diastereoselective reduction of the 1,3-disubstituted isoquinoline 10a accessible from the bromobenzene 11, the D-threose 12, and the 2-formylpyrrolidine 13a. In addition to 1a, its 10-decarboxy derivative 1b was selected as a target molecule since 1b was anticipated to be synthesized more easily than 1a and antitumor activity of unnatural 1b was very intriguing. Preparations of 11 and 12 were readily achieved from commercially available 2-amino-3-nitrotoluene and tartaric acid, respectively, according to the reported procedures.

As shown in Scheme 2, optically pure 13a,b required for the synthesis of (-)-1a and (-)-1b, were prepared starting with commercially available (S)-glutamic acid (14) and/or (S)-pyroglutamic acid (17). Thus, N-p-methoxybenzylation of 14 followed by sequential five step operations produced the 2-pyrrolidinone 15b. Additional reaction of 15b with Bredereck reagent followed by acidic hydrolysis, reduction, and O-protection afforded 15a. Reduction of 15a,b and subsequent aminal formation provided the 2-methoxypyrrolidines 16a,b. The same compound 16b was prepared more effectively from 17 by sequential five step operations. Treatment of 16a,b with trimethylsilyl cyanide in the presence of boron difluoride etherate gave epimeric mixtures of the amino nitriles 18a,b and 19a,b. The desired 2,5-cis isomers 19a,b separated by column chromatography on silica gel were reduced to 13a,b. On the other hands, the undesired 2,5-trans isomers 18a,b were also derived to 13a,b by sequential operations including base catalyzed epimerization of the aldehydes 20a,b.

**Scheme 2**

With highly functionalized 13a,b in hand, we next focused our attention on the synthesis of (-)-1a and (-)-1b. As shown in Scheme 3, lithiation of 11 followed by coupling reaction with 12 gave the ketone 21 having a chiral auxiliary after Collins oxidation of the initially formed secondary alcohol. Exchange of the O-protected group provided the methoxymethyl (MOM) ether 23 via the alcohol 22. Deprotonation of 23 followed by the reaction with 13a,b and Jones oxidation produced the diketones 24a,b, which were further treated with aqueous ammonia to furnish the key intermediates 10a,b. Crucial reduction of 10a,b with sodium cyanoborohydride under acidic conditions proceeded in a highly diastereoselective manner at 0°C, giving rise to the 1,3-cis-disubstituted tetrahydroisoquinolines 25a,b as sole products in excellent yields. The observed high stereoselectivity can be accounted for by the sequential two step asymmetric inductions which proceeded under chelation and stereoelectronic controls, respectively. The reduction products 25a,b were elaborated to the aldehydes 28a,b by sequential seven
Synthesis and cytotoxicity of natural (-)-quinocarcin

Scheme 3

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\begin{align*}
\text{a) } & \text{BuLi; } \text{b) Collins oxid. } 97\% \text{(2 steps)}
\text{c) } & \text{H}_2, \text{Pd(OH)}_2\text{-C-d) MOMCl, } \text{P}^\text{2-En, } 90\% \text{(2 steps)}
\text{e) } & \text{LDA, TMEDA; } 51\% \text{(for } 13a)\text{, } 75\% \text{(for } 13b)
\text{f) } & \text{Jones oxid. } 42\% \text{(for } 24a)\text{, } 66\% \text{(for } 24b)
\text{g) } & \text{NH}_2\text{OH, } 72\% \text{(for } 10a)\text{, } 72\% \text{(for } 10b)
\text{h) } & \text{H}_2, \text{Pd-C}\text{-d) MOMCl, } \text{Pr,EtN, Wk (2 steps)}
\text{i) } & \text{FeCl}_3\text{-SiCl}_4 \text{(for } 13a), 12\% \text{HCl (for } 24b)
\text{j) } & \text{Jones oxid., } 4\% \text{(for } 32a)\text{, } 68\% \text{(for } 24b)
\text{k) } & \text{NH}_2\text{OH, } 67\% \text{(for } 25a)\text{, } 7\% \text{(for } 25b)
\text{l) } & \text{TrocCl, Py, } 85\% \text{(for } 26a)\text{, } 93\% \text{(for } 26b)
\text{m) } & \text{NH}_2\text{OH, } 68\% \text{(for } 27a)\text{, } 6\% \text{(for } 27b)
\text{n) } & \text{FeCl}_3\text{-SiCl}_4 \text{(for } 28a)\text{, } 74\% \text{(for } 28b)
\text{o) } & \text{Zn, AcOH \text{TMSCN, ZnCl}_2, } 39\% \text{(for } 31a)(2 \text{ steps), } 56\% \text{(for } 31b)(2 \text{ steps)}
\text{p) } & \text{TFA, } 82\% \text{(for } 32a)\text{, } 91\% \text{(for } 32b)
\text{q) } & \text{NaBH}_4, 72\% \text{HCl} \text{(for } 31b)(2 \text{ steps), } 56\% \text{(for } 31b)(2 \text{ steps)}
\text{r) } & \text{FeCl}_3\text{-SiCl}_4 \text{(for } 35a), 12\% \text{HCl (for } 26b)
\text{s) } & \text{NalO, I) NaBH}_4, 7\% \text{(for } 27a)(3 \text{ steps), } 68\% \text{(for } 27b)(3 \text{ steps)}
\text{t) } & \text{Jones oxid. } 79\% \text{u) HCHO, NaBH}_4, 72\% \text{v) } 1\text{M NaOH, } 83\% \text{(for } 3b)\text{, } 81\% \text{(for } 3a)
\text{w) } & \text{AgNO}_3, 81\% \text{(for } -1a)\text{, } 83\% \text{(for } -1b)
\end{align*}
\]

step operations. Removal of the trichloroethoxycarbonyl (Troc) group in 28a,b afforded the amino aldehydes 29a,b which directly cyclized to the unstable hemiaminals 30a,b. These were immediately derived to the more stable amino nitriles 31a,b. Removal of the tert-butoxycarbonyl (Boc) group in 31b followed by reductive N-methylation yielded the 10-decarboxylated acetate (+)-4b via amine 32b. On the other hands, simultaneous removals of both the Boc and MOM groups in 31a followed by N-methylation and Jones oxidation gave the 10-carboxylated acetate (+)-4a. Saponification of (+)-4a and (+)-4b provided (+)-3a and (+)-3b, which were further treated with silver nitrate to furnish (-)-1a and (-)-1b, respectively. In addition to these products, their enantiomers were similarly prepared starting with L- tartaric acid and (R)-glutamic acid and/or (R)-pyroglutamic acid as starting materials. Ready access of enantiomeric pairs of 1a,b, 3a,b, and 4a,b obviously disclosed effectiveness of our explored synthetic scheme.

It was found that the unnatural 10-decarboxyquinocarcin derivatives (-)-1b,(+)-3b, and (+)-4b are more highly cytotoxic than the corresponding natural 10-carboxy derivatives (-)-la, (+)-3a, and (+)-4a (vide infra). Since a large quantity of (-)-1a is available from the fermentation broth of Streptomyces sp., these highly cytotoxic congeners were also prepared from naturally occurring (-)-1a by featuring the Barton’s radical decarboxylation (ref. 11). Taking into account the potent cytotoxicity of 10-decarboxylated derivatives (-)-1b, (+)-3b, and (+)-4b, various quinocarcin congeners 33-45 bearing methoxycarbonyl, hydroxymethyl, acetoxymethyl, benzoyloxymethyl, formyl, or fluoromethyl group at their 10-positions were synthesized starting with (-)-1a by featuring efficient reduction of (+)-4a. From congener 35, the compound 43 corresponding to (-)-1a was also prepared (ref. 11).

2. In Vitro Cytotoxicity of (-)-1a and Its Related Compounds (ref. 11)

In vitro cytotoxicity assay against P388 murine leukemia was carried out by employing various structural types of quinocarcin congeners prepared in the course of our synthetic studies. IC50 values (µg/ml) collected are shown in Table 1. These results clearly disclosed that 10-decarboxyquinocarcin (1b) and its 7-cyano congeners 3b and 4b were 10^1-3 times more cytotoxic than the corresponding 10-
carboxy compounds 1a, 3a, and 4a. It is also noteworthy that 1a, 3a, 4a, 1b, 3b, 4b, and 32b bearing natural configurations were found to be 10^1-4 times more cytotoxic than the corresponding enantiomers possessing unnatural absolute configurations. Cytotoxicity of N13-Boc derivative 31b and N13-H derivative 32b was obviously inferior to that of the corresponding N13-Me derivative 4b. The ABE ring systems 5-7, the ABC ring systems 8 and 9, and their enantiomers showed very weak cytotoxicity. Almost all of the 10-substituted quinocarcin congeners 33-45 derived from 1a exhibit superior cytotoxicity to 1a.

Table 1. *In Vitro* Cytotoxicity of (-)-Quinocarcin and Its Related Compounds against P388 Murine Leukemia 

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (µg/mL)</th>
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<tbody>
<tr>
<td>1a</td>
<td>3.3 x 10^2 (3.2) b</td>
</tr>
<tr>
<td>3a</td>
<td>3.6 x 10^2 (5.1) b</td>
</tr>
<tr>
<td>4a</td>
<td>1.0 x 10^-1 (&gt;100) b</td>
</tr>
<tr>
<td>1b</td>
<td>3.9 x 10^-3 (34) b</td>
</tr>
<tr>
<td>3b</td>
<td>8.2 x 10^-6 (&gt;3.3) b</td>
</tr>
<tr>
<td>4b</td>
<td>2.0 x 10^-6 (&gt;3.6) b</td>
</tr>
<tr>
<td>5</td>
<td>4.5 (4.5) b</td>
</tr>
</tbody>
</table>

a) Concentration required for 50% inhibition of the cell growth after incubation for 96 h at 37°C (initial cell density: 1 x 10^4 cells/mL). b) Value in parenthesis shows the cytotoxicity of enantiomer.

Summarizing the results of *in vitro* cytotoxicity assay, it appeared evident that (i) all the carbon framework (the ABCDE ring system or the ABCD ring system bearing the C7-cyano group) with natural absolute configuration is indispensable for significant cytotoxicity, wherein the natural configuration would provide a key structural feature for molecular recognition by DNA, (ii) the N13-methyl group plays an important role for prominent cytotoxicity, and (iii) the C10-carboxyl group is not always necessary for potent activity. The 10-decarboxy congeners (-)-1b, (+)-3b, (+)-4b, 37, and 45 showing promising *in vitro* cytotoxicity were subjected to *in vivo* antitumor activity assay, whose detailed results will be reported in due course.

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References:


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