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SYNTHESIS, CONFORMATION, CRYSTAL STRUCTURES AND DNA CLEAVAGE ABILITIES OF TETRACYCLIC ANALOGS OF QUINOCARCIN

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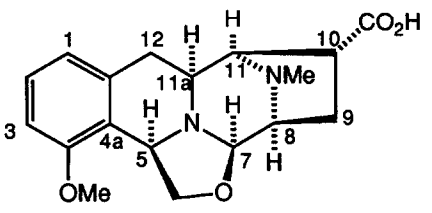
Abstract. Two totally synthetic, racemic analogs of quinocarcin have been designed and their crystal structures determined. Both substances effect the modest cleavage of plasmid DNA. Alteration of the conformation of the reactive oxazolidine fused to the piperazine ring by selecting the stereochemistry at C-11a through synthesis drastically attenuates the relative ability of these substances to cleave DNA.

Introduction

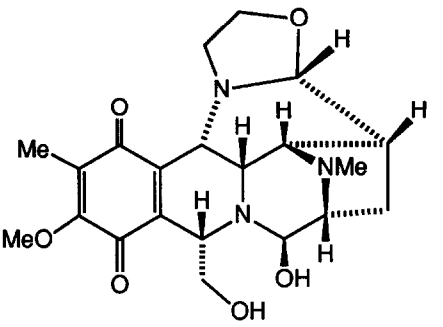
Quinocarcin (**1**) is a natural secondary metabolite produced by *Streptomyces melanovinaceus* and is the simplest member of the naphthyridinomycin (**3**)/saframycin (**4**) class of anti-tumor agents.[1,2] Quinocarcin has been shown[1a,3] to display weak antimicrobial activity against several Gram-positive microbes but is inactive toward Gram-negative bacteria. As its citrate salt, quinocarcin (named quinocarmycin citrate or KW2152) displays promising anti-tumor activity[3] against several lines of solid mammalian carcinomas including St-4 gastric carcinoma; Co-3 human colon carcinoma; MX-1 human mammary carcinoma; M5076 sarcoma; B16 melanoma and P388 leukemia. This substance is currently under evaluation in human clinical trials by the Kyowa Hakko Kogyo Co., Japan.

Our interest in this substance stems from a report by Tomita, et. al.[4] that recorded the remarkable observation that **1** cleaves plasmid DNA in an O_2-dependent fashion that was reported: 1) to not require metal ions (Fe^{2+} or Cu^{2+}); 2) to be stimulated by dithiothreitol; 3) to be inhibited by oxygen free radical scavengers such as methanol, *tert*-butanol, α-tocopherol and; 4) to be inhibited by superoxide dismutase (SOD) and catalase. Quinocarcin blocks RNA synthesis in preference to DNA and protein synthesis in P388 leukemia cells.[3d] On the other hand, in *Bacillus subtilis*, quinocarcin inhibited [^{3}H] thymidine incorporation suggesting inhibition of DNA polymerase; therefore, DNA synthesis is thought[4] to be preferentially inhibited in *Bacillus subtilis*. It has also been reasonably proposed[3d,4,5] that quinocarcin alkylates DNA in the minor groove[5] through the ring-opened form of the oxazolidine (imminium **5**); similar DNA alkylation has been invoked for **3** and **4**. Indirect support[3d,4] for the involvement of the oxazolidine ring in the above context comes from the lack of biological activity displayed by quinocarcinol (**2**) which is coproduced with **1** by *Streptomyces melanovinaceus*. Quinocarcinol also does not cleave plasmid DNA[4] which forces the conclusion that the oxazolidine moiety is also responsible for the oxidative degradation of DNA by a unique mechanism.

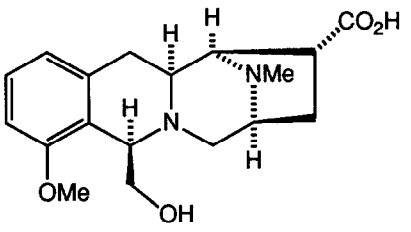
While it is not yet clear whether the anti-tumor properties of quinocarmycin citrate are a manifestation of only one mode of action (i.e., DNA alkylation) or both (DNA alkylation and oxidative DNA cleavage), we were intrigued by the oxidative cleavage observations of the Kyowa-Hakko group[4] since **1** does not contain any readily recognizable functionality that would be associated with the capacity for oxidative DNA cleavage,[6] such as metal



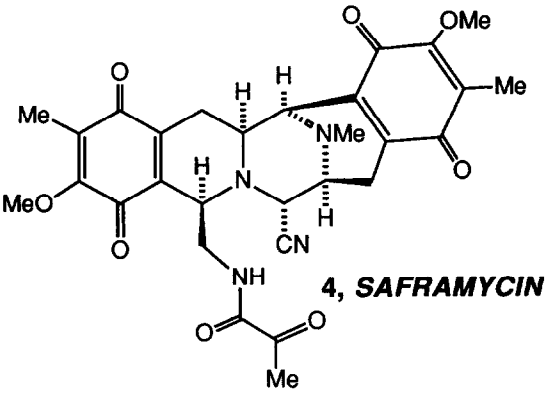
1, *QUINOCARCIN, DC-52*



3, *NAPHTHYRIDINOMYCIN*



2, *QUINOCARCINOL, DC-52d*

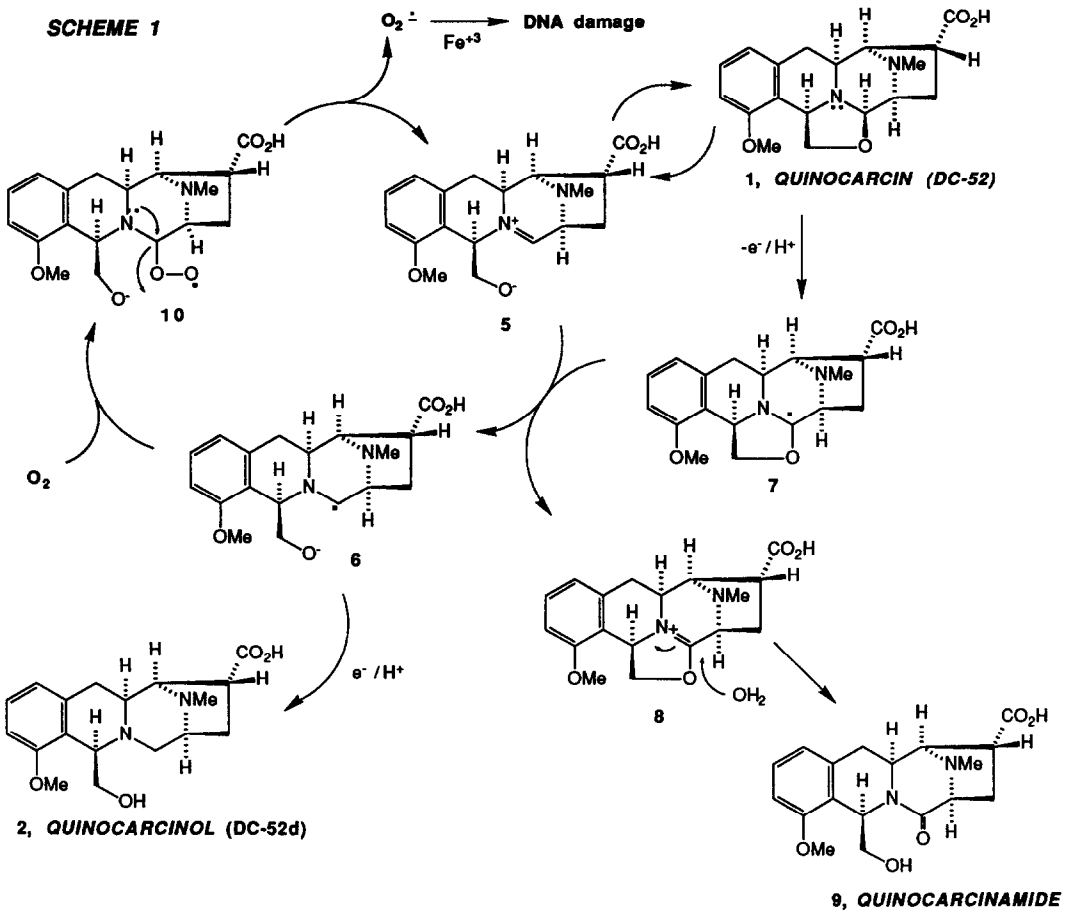


4, *SAFRAMYCIN A*

chelation sites, quinones, and ene-diynes amongst others. Most likely, the efficacy of this drug is a delicate and intimate combination of multiple effects that are brought to bear on its macromolecular targets. We have recently found[7] that quinocarcin undergoes a redox self-disproportionation reaction that we have invoked is coupled to the capacity of this substance to effect the production of superoxide in the presence of molecular oxygen and results, at least in part, to Fenton-mediated lesions in DNA; a mechanism for this process is reviewed[7] in Scheme 1. At the heart of this process, the oxazolidine ring is functioning as its own reductant which ultimately results in the reduction of oxygen and the cleavage of DNA. Such a process would also presumably be relevant to possible oxidative damage to RNA *in vitro* and *in vivo*. In the present study, we wished to examine the intrinsic capacity of simpler oxazolidine-containing analogs to effect the DNA cleavage reaction. Most significantly, we wished to experimentally determine whether pre-designed and synthetically[8] incorporated stereoelectronic control elements into simpler analogs could attenuate the capacity of this ring system to oxidatively damage DNA relative to **1**.

Design Criteria

Remers[5] has conducted molecular mechanics calculations on quinocarcin by docking the drug in the minor groove. From this study, it was concluded that the absolute configuration of quinocarcin is most likely that depicted in Scheme 1. The calculations suggested that the lowest energy conformer of **1** orients the piperazine ring in a chair-like conformation which therefore places the oxazolidine nitrogen lone pair in an antiperiplanar



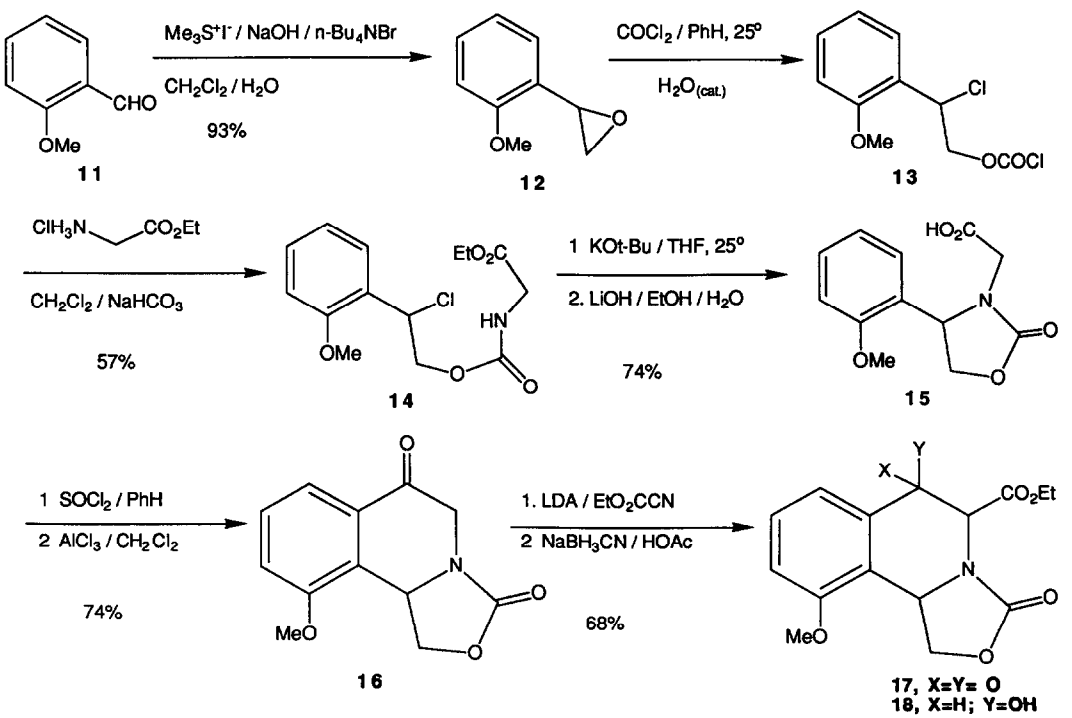
orientation to the oxazolidine methine (Figure 1, *anti*-**1**). Ring opening of the oxazolidine to the imminium species (see **5**, Scheme 1) requires nitrogen pyrimidal inversion to a higher energy twist boat conformer (Figure 1, *syn*-**1**) that was calculated to lie ~10 kcal mol^{-1} above the other conformer. In this situation, the oxazolidine nitrogen lone pair is *syn*- to the methine and antiperiplanar to the C–O bond. It was postulated[5] that the imminium species should be a good alkylator for N-2 of guanine in the minor groove of the sequence $d(ATGCAT)_2$. Based on the similarity to **3** and **4**,[2k] this is a reasonable expectation. In the present study, we wished to ask a different question regarding the conformational significance of the oxazolidine moiety. As shown in Scheme 1, the initial step in the electron-transfer between the oxazolidine and the imminium species involves 1-electron loss from the oxazolidine nitrogen with loss of the oxazolidine methine as a proton producing the reduction and oxidation radicals **6** and **7**, respectively. It is reasonable to expect that the *trans*, antiperiplanar arrangement of the oxazolidine methine and nitrogen lone pair in the lower energy conformer predicted by calculation, should also be the most favorable geometry for concomitant electron and proton loss in the redox self-disproportionation. We hoped to test this idea by synthesizing two simple analogs of quinocarcin that would each mirror one of the two conformational states of the natural product depicted in Figure 1. Thus, analog **23a** which has all three methines

oriented *syn* (same as **1**) would be expected to have the same relative conformation as *anti*-**1** with respect to the oxazolidine and piperazine rings. Analog **23b**, on the other hand, which has inverted stereochemistry at C-11a (quinocarcin numbering) is predicted to exist in a conformation that mirrors *syn*-**1**. Both of these predictions were based on examination of Dreiding stereomodels and molecular mechanics calculations.[9] From this stereoelectronic analysis, **23a** should be much faster at effecting oxidative DNA cleavage relative to **23b**. The synthesis, structures and relative DNA cleavage ability of these materials is presented below.

Results

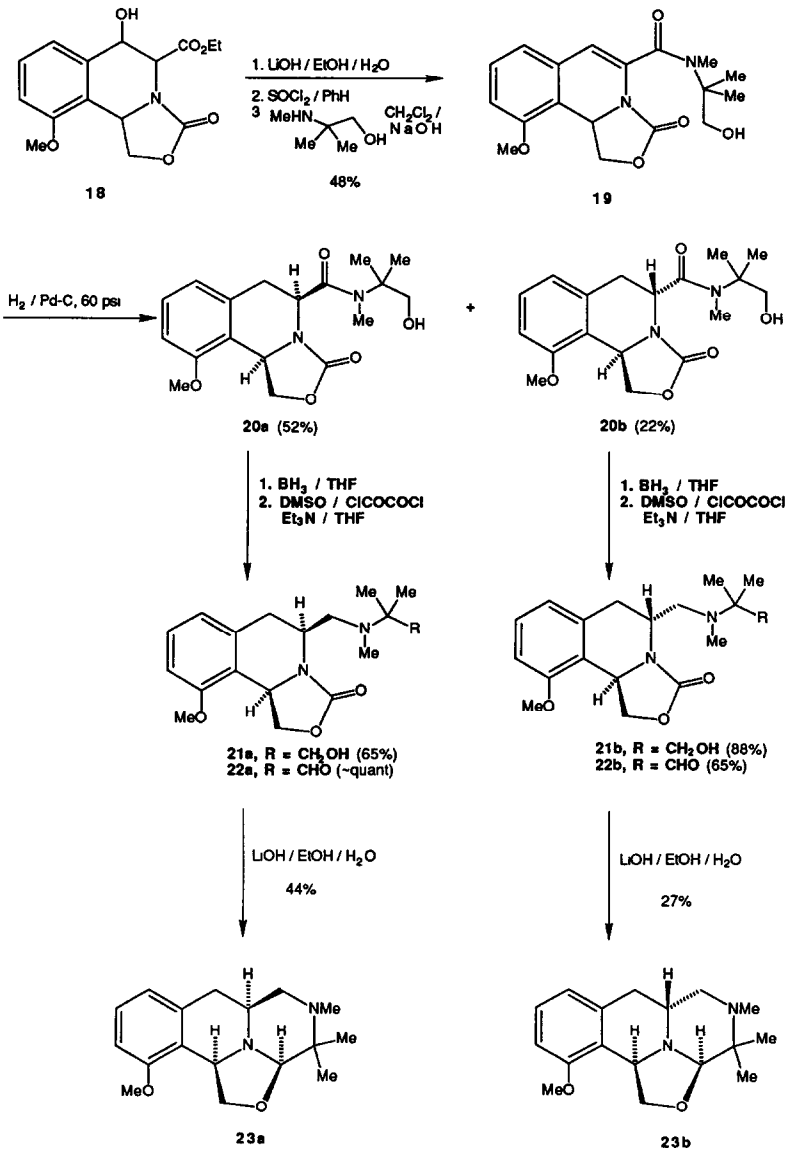
The preparation of the key isoquinoline **18** is detailed in Scheme 2 and was made by a modification of a known procedure.[10] *Ortho*-anisaldehyde (**11**) was treated with trimethylsulfonium iodide under phase-transfer conditions to afford the epoxide **12** in high yield. The epoxide was regioselectively opened with phosgene as a solution in benzene containing a catalytic amount of water to afford the chloroformate **13**. Without purification, **13** was subjected to acylation under Schotten-Baumann conditions to provide the urethane **14** in 57% overall yield from **12**. Treatment of **14** with potassium t-butoxide in THF at room temperature effected cyclization to the corresponding oxazolidinone which was saponified to the acid[10] **15** (74%, two steps). Acid chloride formation and intramolecular Friedel-Crafts acylation provided the isoquinolone **16** in 74% yield from **15**. The procedure described herein is an optimized preparation (from **15**) based on our previously reported[10] synthesis of **16**. The overall sequence from **11** is considerably more efficient and is amenable to multi-gram scale (10 gm scale is described in the experimental section).

SCHEME 2



C-Homologation of **16** proved to be troublesome and required extensive examination of various electrophilic species and reaction conditions due to the propensity of the ketone enolate to undergo O-acylation. Eventually, it was found that the lithium enolate condensed smoothly with ethyl cyanoformate to provide the β-ketoester **17**. Reduction of the ketone with sodium cyanoborohydride gave a single diastereomer (**18**) of unknown relative stereochemistry. This substance served as the key substrate from which various stereoselective and non-stereoselective routes to the target oxazolidine analogs (**23**) were examined. We found two parallel,

SCHEME 3



stereoselective routes[11] to **23a** and **23b** from **18**, wherein the relative stereochemistry at C-11a and the oxazolidine methine could be controlled. More recently, we have found it to be more efficient in terms of man-hours, to employ a common, non-stereoselective route that furnishes both stereochemical series and requiring only a simple chromatographic separation as described in Scheme 3.

Saponification of **18** furnished a crude carboxylic acid which was directly treated with thionyl chloride in benzene. The crude product was directly subjected to Schotten-Baumann acylation with 2,2,3-trimethyl ethanolamine to give the unsaturated amide **19** in 48% overall yield from **18**. This substance was then hydrogenated to give 52% of the *syn*-isomer **20a** and 22% of the *anti*-isomer **20b**. These materials were separated by silica gel chromatography and carried on separately to the final oxazolidines.

For **20a**, borane reduction furnished the tertiary amine **21a** in 65% yield without loss of stereochemical integrity. Swern oxidation to the aldehyde **22a** proceeded in essentially quantitative yield. The crucial oxazolidine-forming step proved somewhat capricious, but could be achieved in 44% yield by refluxing **22a** in basic ethanol for two days. Silica gel purification and crystallization furnished a single oxazolidine diastereomer for which a single crystal x-ray analysis[12] has been secured (Figure 2).

Similarly, **20b** was converted into **23b** in modest overall yield. The nicely crystalline **23b** also proved amenable to x-ray crystallographic analysis[12] as shown in Figure 2.

As is evident from the crystal structures, **23a** and **23b** differ with respect to the orientation of the oxazolidine nitrogen lone pair relative to the oxazolidine methine in the crystal. Substance **23a** positions the nitrogen lone pair *trans*-antiperiplanar to the oxazolidine methine; whereas **23b** positions the nitrogen lone pair *trans*-antiperiplanar to the oxazolidine C–O bond. These conformations are exactly those predicted from examination of Dreiding molecular models and molecular mechanics calculations.[9] The inversion of stereochemistry at C-11a (quinocarcin numbering) in **23b** from the all *syn*-situation in **23a** induces sufficient ring strain to favor nitrogen pyramidal inversion and results in a geometry similar to that evident in the crystal structure. These results confirm our predictions alluded to above that **23a** should mirror the ground state conformation (*anti*-**1**, Figure 1) of the oxazolidine for quinocarcin that was postulated in Remers computational study.[5] Similarly, **23b** can be thought of as mirroring the higher energy conformer of quinocarcin (*syn*-**1**, Figure 1) that Remers postulated as being the reactive conformation that precedes oxazolidine ring-opening to the imminium species (see **5**, Scheme 1) which should (quite reasonably) alkylate DNA.

Reactions with DNA

Compounds **23a** and **23b** as the free bases were virtually insoluble in aqueous buffers and were used as either water-soluble citrate or hydrochloride salts. Reaction of these materials with supercoiled plasmid DNA (pBR 322) were examined at various concentrations and conditions; the results are collected in Table 1 and Figure 3. Reactions were conducted in pH 8, 20 mmol phosphate buffer at 37°C for 2 hours and were analyzed by 0.8% agarose gel electrophoresis. The DNA bands were visualized by staining with ethidium bromide after running the gel and were quantitated by scanning densitometry. The supercoiled plasmid (form I, ccc DNA, fastest band) when nicked is first converted to open circular plasmid (form II, cc DNA, slowest band) and after extensive scission, linear DNA (form III, intermediate band) was observed. Both analogs required fairly high concentrations relative to **1** to produce observable damage to the DNA. As predicted, compound **23b** is significantly inferior to **23a** in effecting DNA cleavage and required dithiothreitol (DTT) even at concentrations as high as 5 mmol (entries 2 and 3, Table 1, lanes 2 and 3, Figure 3; compare entry 22). Since **23a** displayed

Figure 1

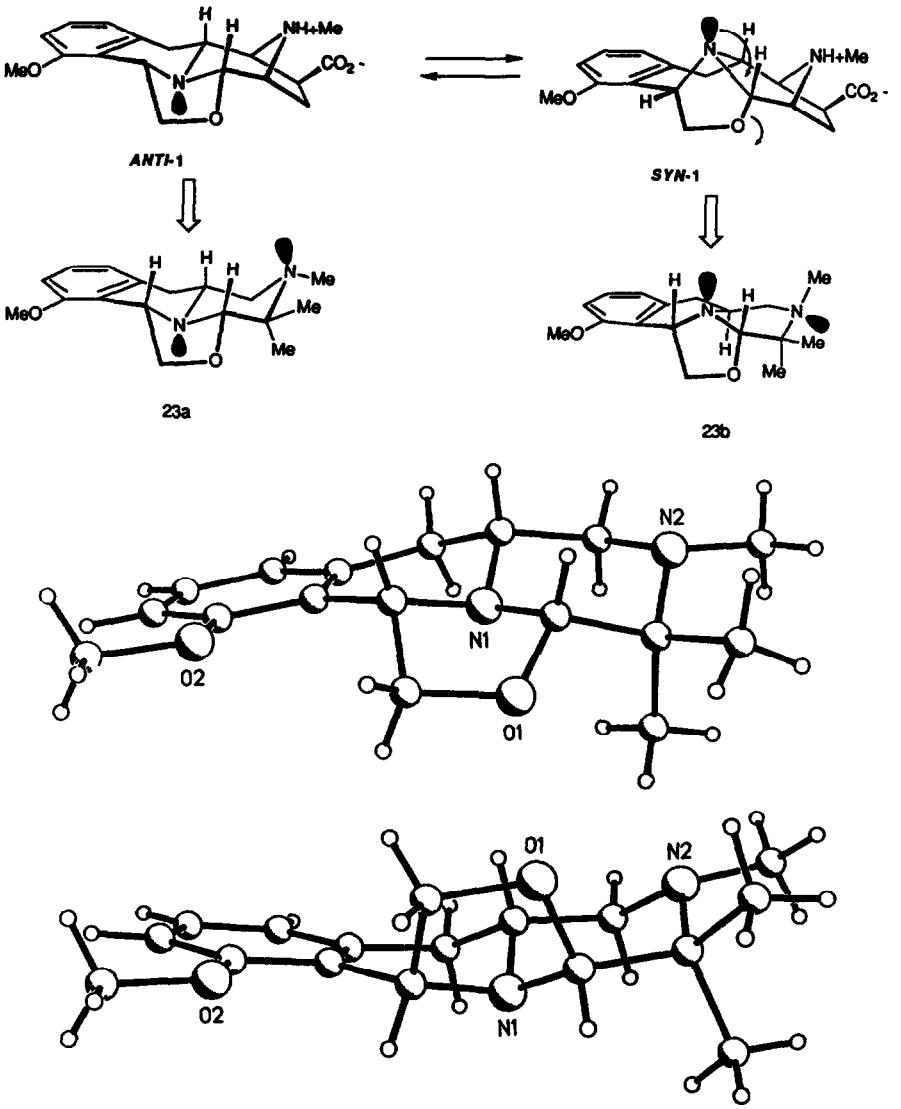


Figure 2. X-Ray Molecular Structures of 23a and 23b. Spheres are of fixed arbitrary radius.

superior DNA cleavage relative to **23b**, the reactivity of this material was examined in more detail and compared to that of **1**. As with quinocarcin, the DNA cleavage by **23a** is enhanced by the addition of DTT (compare entries 7, 16 and 17, Table 1; lanes 7, 16, and 17, Figure 3). It is significant to note that DTT by itself is capable of modest DNA cleavage (entry 17, Table 1, lane 17, Figure 3) *via* Fenton-mediated production of hydroxyl radical; this reaction is a manifestation of superoxide production during thiol autoxidation.[13] The DNA cleavage observed by **23a** in the presence of DTT is at least an order of magnitude greater than DTT alone; the DNA cleavage is thus clearly not due to DTT alone (compare lanes 16 and 17, Figure 3). Superoxide dismutase and catalyse both inhibit

Figure 3. 0.8% Agarose gel electrophoresis of plasmid pBR322 DNA cleavage experiments. Lanes 1-21 correspond to entries 1-21 from Table I. See Table I for visualization details.

the cleavage; SOD being the more potent inhibitor (entries 10 and 11, Table 1; lanes 10 and 11, Figure 3). This is consistent with superoxide production and subsequent Fenton-mediated DNA cleavage.[14] Hydrogen peroxide strongly stimulates the DNA cleavage by **23a** (compare entries 7 and 20, Table 1; lanes 7 and 20, Figure 3). Quinocarcin is significantly better than either **23a** or **23b** at effecting DNA cleavage and gives comparable DNA scission at 1 mmol without external reductants that **23a** gives at 5 mmol with hydrogen peroxide (compare entries 9 and 20, Table 1; lanes 9 and 20, Figure 3).

Quinocarcin has been shown[4,7] to produce superoxide (see Scheme 1) and therefore, Fenton-mediated production of hydroxyl radical with adventitious iron must be invoked for the DNA cleavage event by these molecules. However, hydrogen peroxide alone at 0.1 mmol causes virtually no significant DNA damage (entry 21, Table 1). Since hydrogen peroxide is reduced by Fe(II) in the Fenton reaction, producing hydroxyl radical, the oxazolidine can be functioning indirectly in cycling adventitious Fe(III) to Fe(II) *via* superoxide production or may directly effect the redox cycling of the metal. This point has not yet been addressed. Attempts to sequester adventitious iron and uncouple[15] the presumed Haber-Weiss/Fenton reaction was performed by the addition of the potent Fe(III) chelator desferal (log k_f = 30.7). Addition of desferal to the reaction of **23a** with DNA showed very little inhibition at 0.1 mmol (entry 12, Table 1; lane 12, Figure 3) and partial protection at 10 mmol (entry 14, Table 1; lane 14, Figure 3). However, since the citrate salt of these materials proved to be less effective than the hydrochloride salts in effecting DNA cleavage, we suspect that both citrate and desferal are functioning as competitive CH substrates for the reactive oxidant with DNA (present in very low concentration relative to citrate or desferal) rather than as efficient metal sequestering agents. Additional experimental evidence for direct metal mediation in the DNA cleavage event is not yet available. Ascorbate, a powerful oxygen reductant, is very effective at mediating DNA cleavage which is completely inhibited by catalase (entries 23 and 24, Table 1). The incomplete inhibition of DNA cleavage by **23a** with either catalase of SOD (entries 10 and 11, Table 1) and the incomplete protection afforded by desferal suggests that the mechanism of DNA cleavage by this heterocycle may involve other pathways that are distinct from most recognized DNA oxidants. These possibilities are currently being pursued.

However, indirect experimental evidence points to a significant difference in the capacity of **23a** and **23b** to produce superoxide, paralleling their DNA cleavage abilities. Reduction of nitroblue tetrazolium (NBT)[18] by the HCl salts of **23a** and **23b** were determined at pH 8.0 (20 mM phosphate buffer) containing 1% Triton X-100 at 25°C. For **23a** (1.0 mM) ΔOD_{500nm}/minute = 0.0003 and for **23b** (1.0 mM) ΔOD_{500nm}/minute ≈ 0.0000. For reference[4] 1.0 mM quinocarcin has a ΔOD_{500nm}/minute = 0.002 in the absence of any external reductant

Table I

Entry	Substrate[a]	Concentration [mmol]	% DNA Form I	% DNA Form II	% DNA Form III	S	DNA Cleavage Yield[b] ppm
1	DNA Control		75	25	---	0.29[c]	---
2	**23b** citrate	1.0	77	23	---	-0.03	0.0
3	**23b** citrate	5.0	69	31	---	0.08	0.08
4	**23a** citrate	1.0	63	37	---	0.17	0.85
5	**23a** citrate	5.0	46	54	---	0.49	0.51
6	**23a** hydrochloride	1.0	51	49	---	0.38	2.0
7	**23a** hydrochloride	5.0	19	81	---	1.37	1.4
8	quinocarcin (**1**)	0.1	62	38	---	0.19	9.9
9	quinocarcin (**1**)	1.0	---	84	16	9.5	49
10	**23a** hydrochloride + catalase (10 *ug*/mL)	5.0	39	61	---	0.65	0.68
11	**23a** hydrochloride + SOD (10 *ug*/mL)	5.0	60	40	---	0.22	0.23
12	**23a** hydrochloride + desferal	5.0 0.1	27	73	---	1 0	1.0
13	**23a** hydrochloride + desferal	5.0 1.0	37	63	---	0.70	0.73
14	**23a** hydrochloride + desferal	5.0 10	59	41	---	0.24	0.25
15	DNA Control		81	19	---	0.21[c]	---
16	**23a** hydrochloride + DTT	5.0 0.1	10	87	3	3.8	3.9
17	DTT	0.1	61	39	---	0.28	---
18	DTT + catalase (10 *ug*/mL)	0.1	78	22	---	0.04	---
19	**23a** hydrochloride + DTT + catalase (10 *ug*/mL)	5.0 0.1	32	68	---	0.93	0.97
20	**23a** hydrochloride + H_2O	5.0 0.1	3.5	89	7.5	6.0	6.2
21	H_2O_2	0.1	74	26	---	0.08	---
22	**23b** citrate + DTT	5.0 0.1	50	50	---	0.45	0.47
23	ascorbic acid	0.1	15	79	6	5.4	280
24	ascorbic acid + catalase (10 *ug*/mL)	0.1	80	20	---	0.02	1.0

a. Reaction mixtures were 20 mmol in pH 8 phosphate buffer and contained 0.15 *ug* of pBR 322 plasmid DNA. b. The cleavage yield is expressed by the term S[ccc DNA]/[substrate] and describes the number of single hits per cleavage substrate molecule and allows for a comparison of the relative efficiency of DNA cleavage. c. The S value for the DNA control represents the amount of oc (form II) DNA present in the starting plasmid DNA solution and was subtracted from the S values calculated for the individual cleavage reactions. Measurements of the relative intensity of DNA bands were obtained by scanning densitometry of black and white (Polaroid instant) photographs of the gels (0.8% agarose) visualized by ethidium bromide staining and UV illumination. The mean number of single strang scissions (S)[16] per supercoiled DNA substrate was calculated using the Poisson distribution. When only forms I (ccc or covalently closed circular supercoiled) and forms II (oc or open circular) are present, the equation simplifies to $S = -\ln f_I$, where f_I is the fraction of form I molecules. In those cases where form III (linear) DNA was present, S was calculated from $f_I + f_{II} = [1 - S(2h + 1)/(2L)]^{S/2}$ where h is the distance between hits on opposite strands to produce a linear molecule (16 base pairs)[17] and L is the total number of base pairs in pBR 322 (4362 base pairs). The film used to photograph the gels is assumed to have a linear response to the range of DNA quantities used.[16] Supercoiled DNA is restricted with respect to its ability to bind ethidium bromide and the densitometry values obtained for form I were multiplied by 1.22 as described by Dervan.[16]

(such as DTT). Finally, these substances cleave both double-stranded and single-stranded DNA in a non-sequence specific manner with essentially equal efficacy[7] which argues against the significance of any relative difference in the capacity of these compounds to dock to DNA as a mechanistic determinant relevant to DNA cleavage.

This work demonstrates that simple oxazolidine-containing isoquinolines based on the quinocarcin structure are intrinsically capable of cleaving plasmid DNA and that stereoelectronic elements can markedly attenuate the capacity of these systems to damage nucleic acids. Efforts to attach DNA-binding domains to the synthetic analog nucleus and evaluation of the relative ability of these materials to alkylate DNA as well as their biological activities is under study.

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EXPERIMENTAL

DNA Nicking Experimentals

DNA nicking reaction mixtures were made up by addition at 0° of appropriate amounts of reagent stock solutions to a stock solution of pBR 322 DNA plasmid (Boehringer-Mannheim Biochemical Co.) containing 0.15 μg DNA per reaction (20 μM base pair concentration). The total volumes of the reaction mixtures were brought up to 10 μl with distilled and deionized water when necessary and the reaction mixtures were incubated at 37° for 2 hours in tightly capped plastic tubes. Stock solutions for DNA including experiments were prepared using distilled, deionized water and commercially available reagents: DTT - Sigma; sodium phosphate monobasic - EM Science; sodium phosphate dibasic, 30% hydrogen peroxide - Malinckrodt; superoxide dismutase, beef liver catalase (suspension in water) - Boehringer Mannheim Biochemical. Desferal was the generous gift from Ciba-Geigy Co. From quinocarcin citrate which was a generous gift from Kyowa Hakko Kogyo Co., Japan, free quinocarcin was obtained by passing it through HP-20 ion exchange resin (Mitsubishi Corp.) at 4°. Citric acid was eluted with water and subsequently free quinocarcin was eluted in methanol/water - 3/1 fraction. Free quinocarcin was further purified by HPLC on C-18 Resolve Column (Waters) using 5% methanol/5% acetonitrile in 6.0mM, pH 6.8 potassium phosphate buffer. To remove the phosphate buffer from lyophilized quinocarcin fraction it was passed through HP-20 column in the same manner as described above.

The degree of DNA nicking was monitored by horizontal gel electrophoresis on 0.8% agarose gel onto which the whole volumes of the reaction mixtures were loaded after prior addition of 3 μl of loading buffer (0.25% bromophenol blue, 40% sucrose). The electrophoreses were run for 2 h at 55V and the gels were submerged for 15 min in ethidium bromide solution. The electrophoresis gels were immediately visualized on a UV transilluminator and photographed using black and white instant films (Polaroid T667). The measurements of the relative intensities of DNA bands were performed on the photographs using the Dell System 325 computer and Technology Resources Inc. image processing software. The average number of nicks per DNA molecule S was calculated according to the method described by Dervan.[16]

Synthesis of Quinocarcin Analogs

(2-Methoxyphenyl)oxirane **12**. A nonhomogenous mixture of o-anisaldehyde (20.0 g, 0.147 mol, 1.0 eq.), trimethylsulfonium iodide (37.0 g, 0.177 mol, 1.2 eq.), tetra n-butylammonium iodide (0.52 g, 0.0014 mol, 0.01 eq.), CH_2Cl_2 (500 mL) and aqueous NaOH (50%, 330 mL) was vigorously stirred at room temperature for 5 days. After dilution with water the organic layer was separated, washed with water, dried over Na_2SO_4, concentrated in vacuum and the residue was Kugelrohr distilled to yield the pure product in form of colorless liquid (20.4 g, 92.5%).

12. ^{1}H NMR (270 MHz) ($CDCl_3$) TMS: 2.69 (1 H, q, J = 2.4 Hz), 3.12 (1 H, q, J = 4.6 Hz), 3.85 (3 H, s), 4.20 (1 H, t, J = 2.7 Hz), 6.91 (2 H, m), 7.15 (1 H, q, J = 1.6 Hz), 7.25 (1 H, m). IR (NaCl, neat):

3051, 3002, 2941, 2838, 1689, 1602, 1496, 1466, 1439, 1391, 1287, 1256, 1103, 1048, 1027, 989, 880, 755 cm^{-1}.

Glycine ethyl ester N-carbamate **14**. A solution of epoxide **12** (0.69 g, 4.60 mmol, 1.0 eq.) and phosgene (0.85 g, 8.58 mmol, 1.86 eq.) in benzene (10 mL) was kept in sealed flask for 48 h. The solvent was removed under reduced pressure (prior to this with larger scale runs the reaction mixture has to be purged with the flux of dry nitrogen and the excessive phosgene should be deactivated by passing through aqueous solution of alkali). The oily residue of crude chloroformate **13** was redissolved in CH_2Cl_2 (15 mL), and a solution of saturated $NaHCO_3$ was added (15 mL), followed by a solution of glycine ethyl ester hydrochloride (0.64 g, 4.6 mmol, 1.0 eq.) in small volume of water. After 10 min of vigorous stirring at room temperature the organic layer was separated, washed with water, dried over $MgSO_4$ and concentrated to yield crude product as yellow oil from which pure **14** was isolated by radial chromatography (CH_2Cl_2/CH_3OH - 10/1) 0.83 g (57%). Analytical sample was obtained by recrystallization from isopropyl alcohol, mp = 61-63°.

13. ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 3.85 (3 H, s); 4.55 (1 H, dd, J = 11.3 Hz, J = 5.0 Hz); 4.67 (1 H, dd, J = 11.3 Hz, J = 7.9 Hz); 5.62 (1 H, dd, J = 7.9 Hz, J = 5.0 Hz); 6.90 (1 H, d, J = 8.3 Hz); 6.98-7.03 (1 H, m); 7.30-7.36 (1 H, m); 7.48 (1 H, dd, J = 7.6 Hz, J = 1.6 Hz); IR (NaCl, neat): 1779, 1492, 1252, 1145, 755 cm^{-1}.

14. ^1H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.28 (1 H, t, J = 7.2 Hz); 3.85 (3 H, s); 3.95 (2 H, d, J = 5.5 Hz); 4.21 (2 H, q, J = 7.2 Hz); 4.44-4.48 (2 H, m); 5.32 (1 H, br s); 5.58 (1 H, t, J = 6.3 Hz); 6.88 (1 H, d, J = 8.4 Hz); 6.98 (1 H, t, J = 7.2 Hz); 7.30 (1 H, m); 7.48 (1 H, dd, J = 7.7 Hz, J = 1.6 Hz). IR (NaCl, neat): 3357, 1729, 1533, 1495, 1252, 1201, 1052, 1026, 757 cm^{-1}. Anal. Calcd for $C_{14}H_{18}ClNO_5$: C, 53.25; H, 5.74; N, 4.43. Found: C, 53.40; H, 5.81; N, 4.39.

Cyclic urethane **15**. To a solution of **14** (15.7 g, 50.0 mmol, 1.0 eq.) in THF (150 mL) cooled to 0°C a solution of potassium t-butoxide (6.13 g, 55.0 mmol, 1.1 eq.) in THF (75 mL) was added slowly with stirring. After 0.5 h the reaction mixture was diluted with water, slightly acidified with dilute HCl and extracted with CH_2Cl_2. The organic extract was dried over Na_2SO_4, filtered and concentrated in vacuum to yield crude **11**[5] as brownish oil (14.1 g, quant.). Ethanol (150 mL) was added followed by LiOH monohydrate (2.8 g, 66.7 mmol, 1.3 eq.) in water (60 mL) at 0°C. After 0.5 h the reaction mixture was concentrated under reduced pressure to a half volume at room temperature, diluted with water, acidified and extracted with ethyl acetate. The extract was dried over Na_2SO_4, filtered and concentrated. From the oily residue pure **12**[5] was obtained by crystallization from ethyl acetate (9.3 g, 74%).

Isoquinolone **16**. **14** was obtained from acid **12** as previously described.[5] Substituting CH_2Cl_2 for tetrachloroethane as a solvent for Friedel-Crafts cyclization improved the yield to 74% (on 40.0 mmol scale).

β-Ketoester **17**. To a stirred solution of **16** (120 mg, 0.515 mmol, 1.0 eq.) in 10% HMPA/THF (8.8 mL) cooled to -78°C was added $(TMS)_2NLi$ in THF (0.60 mL, 1.0 M, 0.60 mmol, 1.15 eq.). The resulting solution was stirred at -78°C for 45 min when cyanoethylformate (51 mg, 0.515 mmol, 1.0 eq.) was added in one portion. The reaction was stirred at -78°C for 2 h and quenched with saturated aqueous NH_4Cl (0.60 mL, 1.0 M, 1.5 mL), diluted with CH_2Cl_2 (50 mL) and washed with water and brine. The organic layer was dried over $MgSO_4$, filtered, evaporated and the residue was chromatographed on silica gel (hexane/ethyl acetate - 3/2) yielding **17** as an amorphous solid (126 mg, 80%).

17. ^1H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 1.24 (3 H, t, J = 8.2 Hz), 3.87 (3 H, s); 4.11-4.25 (3 H, m); 5.05 (1 H, t, J = 8.5 Hz); 5.32 (1 H, s); 5.57 (1 H, t, J = 8.5 Hz), 7.16 (1 H, d, J = 7.7 Hz), 7.43 (1 H, t, J = 8.0 Hz); 7.71 (1 H, d, J = 7.8 Hz); IR (NaCl, CH_2Cl_2): 1765, 1750, 1700, 1250 cm^{-1}; mass spectrum, $CI(NH_3)$, m/e = 305 (100), 233 (12).

β-Hydroxyester **18**. To a solution of ketoester **17** (267 mg, 0.87 mmol, 1.0 eq.) $NaBH_3CN$ (89 mg, 1.50 mmol, 1.72 eq.) was added at room temperature and the reaction mixture was stirred for 4 h. After diluting with CH_2Cl_2 the organic layer was washed with water, Na_2CO_3 solution, water and dried over Na_2SO_4. Evaporation of the solvent yielded yellowish oily product which crystallized on standing (226 mg, 84%). Analytical sample was obtained by recrystallization from ethyl acetate/hexane mp = 119-121°.

18. ^1H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.23 (3 H, t, J = 7.0); 3.83 (3 H, s); 4.03 (1 H, t, J = 8.8 Hz); 4.92-5.18 (4 H, m); 6.81-6.84 (1 H, m); 7.26-7.37 (2 H, m). IR (KBr pellet): 3520, 1753, 1717, 1413, 1221. Anal. Calcd for $C_{15}H_{17}NO_6$: C, 58.62; H, 5.57; N, 4.56. Found: C, 58.72; H, 5.60; N, 4.44.

Hydroxyamide **19**. To a solution of crude β-hydroxyester **18** (148 mg, 0.48 mmol, 1.0 eq.) in ethanol (5.0 mL) aqueous LiOH (2.0 M, 0.36 mL, 0.72 mmol, 1.5 eq.) was added at room temperature. After 0.5 h at room temperature the reaction mixture was diluted with water and washed with CH_2Cl_2. The aqueous layer was acidified with HCl (2.0 M, 0.4 mL) and extracted with CH_2Cl_2. Drying of the organic extract over Na_2SO_4 and

evaporation of the solvent yielded slightly yellowish solid (105 mg) which was refluxed in benzene (2.0 mL) with thionyl chloride (134 mg, 1.13 mmol, 3.0 eq.) for 1.5 h. The reaction mixture was concentrated in vacuum, the oily residue was redissolved in dry CH_2Cl_2 and excess of 2,2,3-trimethylethanolamine (118 mg, 1.13 mmol, 3.0 eq., obtained from 2,2-dimethylethanoloamine by treatment with methylchloroformate and subsequent reduction of the methylurethane with excess of lithium aluminum hydride) in CH_2Cl_2 (2.0 mL) was added with ice-water cooling. After 20 min at room temperature the reaction mixture was washed with water and the hydroxyamide **19** (63 mg, 48%) was isolated by radial chromatography on silica gel (ethyl acetate/hexane - 2/1) as crystalline colorless solid. Analytical sample was obtained by recrystallization from methanol mp = 188-190°.

19. ^{1}H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.43 (3 H, s); 1.46 (3 H, s); 3.06 (3 H, s); 3.83 (3 H, s); 3.80-3.92 (2 H, m); 4.56 (1 H, dd, J = 10.8, J = 9.3 Hz); 5.09 (1 H, dd, J = 8.8 Hz, J = 8.1 Hz); 5.39 (1 H, dd, J = 10.9 Hz, J = 8.2 Hz); 6.03 (1 H, br s); 6.77-6.85 (2 H, m); 7.23-7.30 (1 H, m). IR (NaCl, neat): 3500, 1755, 1633, 1575 cm^{-1}. Anal. Calcd for $C_{18}H_{22}N_2O_5$: C, 62.41; H, 6.40; N, 8.09. Found: C, 62.21; H, 6.44; N, 7.89.

Hydroxyamides **20A,B**. A solution of unsaturated amide **19** (252 mg, 0.728 mmol) in ethanol (100 mL) was hydrogenated under 60 psi H_2 at room temperature with 5% palladium on charcoal catalyst (240 mg) for 12 h. The mixture of diastereoisomeric products was separated by PTLC chromatography on silica gel (CH_2Cl_2/THF - 20/1) to yield amides **20A** (131 mg, 52%) and **20B** (55 mg, 22%) as colorless oils. Analytical samples were obtained by recrystallization. **20A** (ethyl acetate) mp= 197.5-198.5°; **20B** (ethyl acetate/hexane) mp = 131-132°.

20A. ^{1}H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.39 (3 H, s); 1.42 (3 H, s); 2.88 (1 H, dd, J = 16.9 Hz, J = 3.5 Hz); 3.06 (3 H, s); 3.20 (1 H, br s); 3.41 (1 H, dd, J = 16.3 Hz, J = 11.6 Hz); 3.83 (3 H, s); 4.12 (1 H, dd, J = 11.3 Hz, J = 3.6 Hz); 4.23 (1 H, dd, J = 9.0 Hz, J = 6.3 Hz); 4.63 (1 H, dd, J = 11.4 Hz, J = 1.2 Hz); 4.85 (1 H, t, J = 8.9 Hz); 5.14-5.20 (1 H, m); 6.75-6.82 (2 H, m); 7.20-7.27 (1 H, m). IR (NaCl, neat): 3474, 1731, 1645, 1583 cm^{-1}. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.92; H, 6.75; N, 7.73.

20B. ^{1}H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 1.22 (3 H, s); 1.23 (1 H, s); 3.02 (3 H, s); 2.98-3.12 (2 H, m); 3.59 (2 H, br s); 3.78 (3 H, s); 3.87-3.94 (1 H, m); 4.09 (1 H, t, J = 8.7 Hz); 4.90 (1 H, t, J = 8.8 Hz); 5.11 (1 H, dd, J = 7.5 Hz, J = 4.6 Hz); 5.18 (1 H, t, J = 8.6 Hz); 6.68 (1 H, d, J = 8.2 Hz); 6.75 (1 H, d, J = 8.2 Hz); 7.16 (1 H, t, J = 7.9 Hz). IR (NaCl, neat): 3459, 1745, 1648, 1591, 1084, 731 cm^{-1}. Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04. Found: C, 61.99; H, 6.86; N, 7.82.

Hydroxyamines **21A,B**. To a suspension of **20A** (129 mg, 0.37 mmol, 1.0 eq.) in dry THF (10 mL) solution of borane in THF (1.0 M, 1.85 mL, 5.0 eq.) was added at room temperature under N_2. After 5 h at room temperature 1.0 M aqueous $(NH_4)_2CO_3$ was added and stirring was continued for another 5 h. The reaction mixture was concentrated and partitioned between water and methylene chloride. Separation on silica gel by radial chromatography (CH_2Cl_2/MeOH - 10/1) yielded starting amide (13 mg, 10%) and amine **21A** (80 mg, 65%) as colorless oil.

21A. ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.03 (3 H, s); 1.07 (3 H, s); 2.31 (3 H, s); 2.70 (1 H, br s); 2.76-2.85 (2 H, m); 3.06 (1 H, dd, J = 15.8 Hz, J = 8.8 Hz); 3.16 (1 H, dd, J = 13.3 Hz, J = 5.3 Hz); 3.30 (1 H, 1/2 ABq, J = 10.8 Hz); 3.45 (1 H, 1/2 ABq, J = 10.8 Hz); 3.58-3.67 (1 H, m); 3.82 (3 H, s); 4.31 (1 H, dd, J = 9.0 Hz, J = 6.6 Hz); 4.76 (1 H, t, J = 8.7 Hz); 5.00 (1 H, t, J = 7.2 Hz); 6.78 (1 H, d, J = 8.1 Hz); 6.82 (1 H, d, J = 7.6 Hz); 7.23 (1 H, t, J = 8.1 Hz). IR (NaCl, neat): 3457, 1747, 1586, 1070 cm^{-1}.

By analogous procedure **21B** was obtained from **20B** in 88% yield as colorless oil.

21B. ^{1}H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 0.89 (3 H, s); 0.92 (3 H, s); 2.24-2,34 (1 H, m); 2.29 (3 H, s); 2.53 (1 H, dd, J = 13.1 Hz, J = 8.8 Hz); 2.71 (1 H, d, J = 12.9 Hz); 3.05-3.25 (3 H, m); 3.80 (3 H, s); 4.10 (1 H, t, J = 7.9 Hz); 4.35-4.43 (1 H, m); 4.90 (1 H, t, J = 8.1 Hz); 4.96-5.02 (1 H, m); 6.71-6.75 (2 H, m); 7.18 (1 H, t, J = 8.0 Hz). IR (NaCl, neat): 3455, 1751, 1587, 1077 cm^{-1}; mass spectrum m/e = 335 (M^+ +1), 333, 303, 263.

Aldehydes **22A,B**. To a solution of DMSO (7 mg, 0.09 mmol, 3.0 eq.) in dry methylene chloride (0.2 mL) at -78° oxalyl chloride (5.7 mg, 0.045 mmol, 1.5 eq.) was added and after 15 min at -78° addition of hydroxyamine **21A** (11 mg, 0.03 mmol, 1.0 eq.) in methylene chloride (0.2 mL) followed. After 1.5 h at -78°C triethylamine (30 mg, 0.30 mmol, 1.0 eq.) was added and stirring was continued for 30 min. The reaction mixture was concentrated under reduced pressure, diluted with methylene chloride and washed with water. The organic layer was dried over Na_2SO_4 and concentrated to furnish pure aldehyde **22A** (11 mg, quant.) as colorless oil.

22A. ^{1}H NMR (300 MHz) ($CDCl_3$) δ TMS: 1.09 (3 H, s); 1.12 (3 H, s); 2.30 (3 H, s); 2.70 (1 H, dd, J = 13.3 Hz, J = 9.2 Hz); 2.88 (1 H, dd, J = 15.7 Hz, J = 4.1 Hz); 3.02 (1 H, dd, J = 13.2 Hz, J = 4.5 Hz);

3.09 (1 H, dd, J = 15.8 Hz, J = 8.1 Hz); 3.62-3.68 (1 H, m); 3.82 (3 H, s); 4.30 (1 H, dd, J = 8.9 Hz, J = 7.2 Hz); 4.77 (1 H, dd, J = 9.0 Hz, J = 8.1 Hz); 4.98 (1 H, t, J = 7.4 Hz); 6.78 (1 H, d, J = 8.3 Hz); 6.82 (1 H, d, J = 7.7 Hz); 7.24 (1 H, t, J = 7.7 Hz); 9.47 (1 H, s). IR (NaCl, neat): 1730, 1586, 1470, 1070 cm^{-1}

By analogous procedure aldehyde **22B** was obtained from **21B** in 65% yield as colorless oil.

22B. ^1H NMR (270 MHz) ($CDCl_3$) δ $CHCl_3$: 0.93 (3 H, s); 0.99 (3 H, s); 2.23-2.42 (5 H, m); 2.88 (1 H, d, J = 16.3 Hz); 3.06 (1 H, dd, J = 16.2 Hz, J = 5.8 Hz); 3.80 (3 H, s); 3.98-4.07 (1 H, m); 4.37-4.45 (1 H, m); 4.86-4.94 (1 H, m); 6.71-6.75 (2 H, m); 7.20 (1 H, t, J = 7.9 Hz); 9.25 (1 H, s). IR (NaCl, neat): 1756, 1587, 1472, 1258, 1078 cm^{-1}.

Quinocarcin analogs **23A,B**. To a solution of crude **22A** (11.0 mg, 0.03 mmol) in ethanol (2 mL) aqueous LiOH solution (2.0 M, 0.2 mL) was added and the mixture was refluxed under N_2 for 48 h. The reaction mixture was diluted with methylene chloride and washed with brine. The organic layer was dried over Na_2SO_4, concentrated and the oily residue was separated by silica gel PTLC (CH_2Cl_2/MeOH - 10/1) to yield starting aldehyde **22A** (2.2 mg, 20%), oxazolidine **23A** (4.2 mg, 44%) and alcohol **21A** (1.0 mg, 10%) as colorless oils. Recrystallization from pentane produced crystalline **23A** mp = 111-113° which was used for X-ray structure determination.

23A. ^1H NMR (300 MHz) ($CDCl_3$) δ TMS: 0.95 (3 H, s); 1.23 (3 H, s); 2.32 (3 H, s); 2.39 (1 H, dd, J = 11.5 Hz, J = 9.5 Hz); 2.73-2.98 (4 H, m); 3.52-3.67 (3 H, m); 3.77 (3 H, s); 4.55 (1 H, t, J = 6.2 Hz); 6.67 (1 H, d, J = 8.2 Hz); 6.77 (1 H, d, J = 7.6 Hz); 7.14 (1 H, t, J = 8.1 Hz). IR (NaCl, neat): 1581, 1470, 1260, 1087, 1018, 779 cm^{-1}.

By analogous procedure **23B** was obtained from **22B** as colorless oil with 27% yield. Recrystallization from ethyl acetate/hexane produced crystalline product mp = 159-160° which was used for X-ray structure determination.

23B. ^1H NMR (270 MHz) ($CDCl_3$) δ TMS: 1.16 (3 H, s); 1.33 (3 H, s); 2.34 (3 H, s); 2.52-2.75 (4 H, m); 2.95-3.05 (1 H, m); 3.62 (1 H, d, J = 7.3 Hz); 3.77 (3 H, s); 4.16 (2 H, t, J = 7.4 Hz); 4.42 (1 H, t, J = 7.7 Hz); 6.71 (1 H, d, J = 7.5 Hz); 6.74 (1 H, d, J = 7.6 Hz); 7.18 (1 H, t, J = 7.9 Hz). IR (NaCl, neat): 1588, 1473, 1260, 1018, 786, 744 cm^{-1}.

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