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DNA GYRASE INHIBITORY ACTIVITY OF ELLAGIC ACID DERIVATIVES

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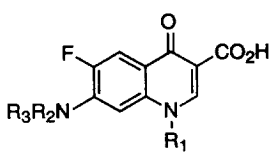
Abstract: Ellagic acid was found to inhibit *E. coli* DNA gyrase supercoiling with approximately the same potency as nalidixic acid. Tricyclic analogs of ellagic acid, which vary in the number and position of the hydroxy groups as well as their replacement with halogens, have been synthesized. The biological activity of these analogs is discussed.

DNA gyrase, a member of the topoisomerase class of enzymes, is an essential bacterial enzyme that catalyzes the introduction of negative superhelical turns into closed-circuit double-stranded DNA.[3] The gyrase enzyme has been shown to be an A_2B_2 tetramer. The 4-quinolone antibacterials **1** target the Gyr A subunit of the DNA gyrase holoenzyme, inhibiting supercoiling by repressing the religation sequence-specific 4-base pair staggered cuts on the DNA duplex.[4,5] Such inhibition can be observed and quantitated by performing the in vitro DNA gyrase supercoiling inhibition assay.[6]



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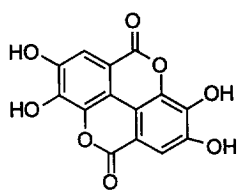

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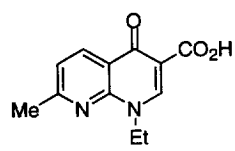

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The β-keto acid moiety of the quinolones **1** has been postulated to be essential for binding to the DNA.DNA gyrase complex. Though the quinolones are potent broad spectrum antibacterials, several suffer from central nervous system (CNS) side effects in humans as well as articular cartilage damage side effects in juvenile animals. It is believed that the β-keto acid functionality is also responsible for many of the unwanted side effects.[4]

In our search for novel inhibitors of DNA gyrase, ellagic acid (**2**) was discovered to inhibit the supercoiling of the DNA gyrase with a potency (IC_{50} = 47 μg/mL) approximately equal to that of nalidixic acid (**3**) (IC_{50} = 52 μg/mL).[7] We have since tried to delineate the phamacophore for the DNA gyrase activity of ellagic acid (**2**) and report herein some of our findings on tricyclic analogs **8**, which vary the number and the position of hydroxyl groups, as well as their replacement with halogens.

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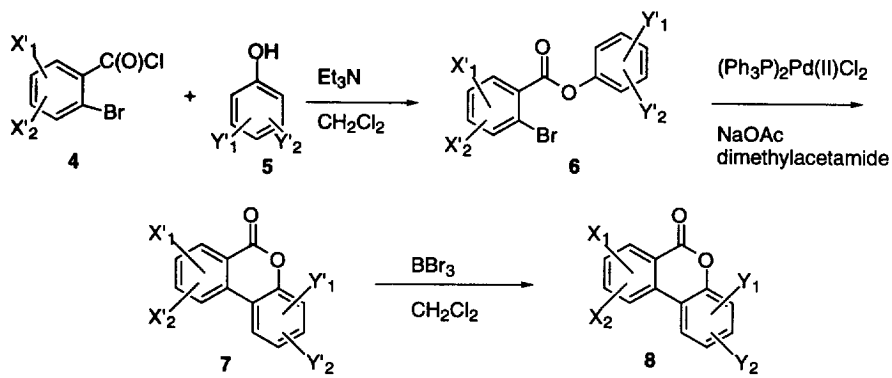
Scheme 1.



The general synthesis for the dibenzo[b,d]pyran-6-one series **8** of tricyclic analogs of ellagic acid (**2**) is outlined above (Scheme 1).[8] The appropriately substituted acid chloride **4** is condensed with phenol derivative **5** to produce the ester **6**. The biaryl coupling of ester **6** affords the methoxylated lactone **7** in modest yields (usually 20–30%). Demethylation of the methyl ethers proceeds smoothly with boron tribromide in methylene chloride to afford the target lactones **8**.

The direct tricyclic analog **8a** of ellagic acid (**2**) is approximately three times less potent than the parent compound (Table 1). Interestingly, the regioisomeric tetraphenol **8b** is more active than **8a**, but still twofold less potent than ellagic acid (**2**). Next, the number of hydroxyl groups on the tricyclic framework was investigated. Complete removal, **8c**, as well as removal of either the 8,9-dihydroxy groups, **8d**, or the 3,4-dihydroxy groups, **8e**, resulted in compounds devoid of any activity. It should also be mentioned that all of the intermediate methoxy derivatives were also inactive thus indicating that the phenol is important for activity.

Replacement of the hydroxyl groups with either chlorine or fluorine was investigated. Incorporation of a fluorine at C-9, **8g**, results in an increase in activity relative to the unsubstituted case. Compound **8j**, with a 3,4-difluoro substitution pattern, is 3.5 times more potent than the tetraphenol analog **8a** and the most potent tricyclic analog found with an IC_{50} = 40 μg/mL. The analogous 3,4-dichloro compound **8i** is significantly less active than **8j**.

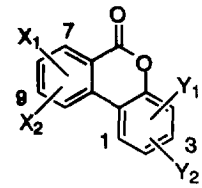


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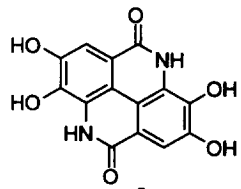
Table 1. DNA Gyrase Supercoiling Inhibition[6] by Dibenzo[*b*,*d*]pyran-6-ones (**8**)

Compd 8	Y_1	Y_2	X_1	X_2	IC_{50} (μg/mL)[1]
a	3-OH	4-OH	8-OH	9-OH	130
b	2-OH	3-OH	8-OH	9-OH	80
c	H	H	H	H	>500
d	3-OH	4-OH	H	H	>500
e	H	H	8-OH	9-OH	>500
f	3-OH	4-OH	8-OH	H	80
g	3-OH	4-OH	H	9-F	110
h	2-OH	3-OH	H	9-F	370
i	3-Cl	4-Cl	8-OH	9-OH	280
j	3-F	4-F	8-OH	9-OH	40
k	1-Me	3,4-OH	H	9-F	>500
l	1-Me	3,4-OH	8-OH	9-OH	140
Ellagic acid (**2**)	--	--	--	--	47
Nalidixic acid (**3**)	--	--	--	--	52
Ofloxacin	--	--	--	--	1.8

[1] IC_{50} values are averages of several runs

Since removal of one of the lactone bridges of ellagic acid (**2**) decreases the activity, one can postulate that the fourth ring of ellagic acid may serve to "flatten" the biphenyl portion of the molecule and therefore the molecule may better interact with the DNA enzyme complex. Therefore, compounds which have a less planar conformation should be less active. Addition of a group at the C-1 postion would cause the molecule to be less planar due to steric interactions between the C-1 methyl group and the C-10 hydrogen. To test this hypothesis, **8k** was synthesized and found to be considerably less active than the analogous desmethyl compound **8g**, as anticipated. Further evidence for this hypothesis is that the tetracyclic aza analog of ellagic acid **9** exhibits an IC_{50} = 24 μg/mL, approximately twofold more active than ellagic acid thus indicating that planarity is crucial to good potency.[9] In addition, coworkers have shown for the aza flavones **10** and **11** that increasing the planarity of the molecule via a carbonyl bridge greatly improves the potency of the compounds as DNA gyrase inhibitors (IC_{50} = 81 μg/mL and 22 μg/mL, respectively).[10]



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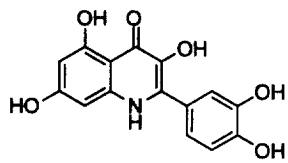

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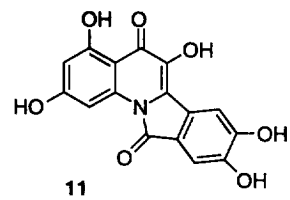

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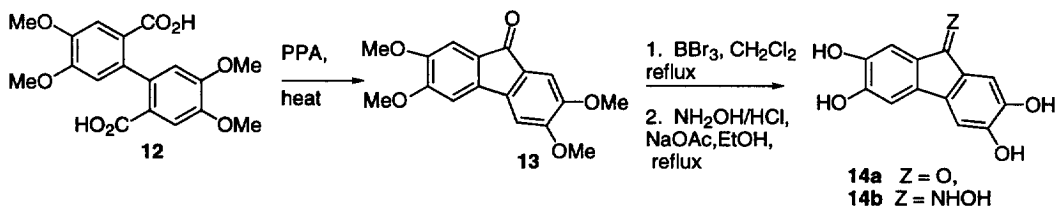
Consequently we designed several rigid fluorenone analogs **14** where the two phenyl rings are coplanar. These compounds were synthesized in several steps from biaryl derivative **12**.[9] Friedel–Crafts acylation of **12** in hot polyphosphoric acid afforded tricyclic ketone **13**. Demethylation of the ethers with boron tribromide gave **14a**, which was then converted into oxime **14b**.

Scheme 2.



12 13

14a Z = O,
14b Z = NHOH

Fluorenone **14a** inhibited the supercoiling of DNA gyrase with an IC_{50} = 13 μg/mL, which is approximately fourfold more potent than ellagic acid (**2**). The ketone in **14a** was replaced by an oxime which resulted in an additional sixfold increase in potency (IC_{50} = 2 μg/mL) of fluorenone **14b** relative to fluorenone **14a**. Encouraged by these results, we are continuing to explore this series of DNA gyrase inhibitors and our progress will be reported in due course.

References and Notes

1. Author to whom correspondence should be addressed.
2. Present address- Bristol Myers Squibb Pharmaceutical Research Institute, Wallingford, CT.
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