Bioorganic & Medicinal Chemistry Letters, Vol.3, No.2, pp. 225-230, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00

DNA GYRASE INHIBITORY AND ANTIBACTERIAL ACTIVITY OF SOME FLAVONES(1)

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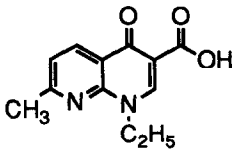
(*Received in USA* 12 *October* 1992)

Abstract: A series of flavones was studied for their DNA-gyrase inhibitory and antibacterial activities. This led to the identification of compounds with potent *Escherichia coli* DNA-gyrase inhibitory activity, and modest antimicrobial activity. The most active compound, ellagic acid has an IC_{50} = 3.3 μg/ml, which is comparable to some of the currently marketed 4-quinolone antibacterials.

The quinolones have become one of the most clinically useful classes of broad spectrum antibacterial drugs available today. Nalidixic acid (**1**), the first prototypic "quinolone" introduced into therapy, demonstrated activity against gram-negative bacteria, but lacked substantial gram-positive activity (2). Recently newer agents, for example ofloxacin (**2**), with a broader spectrum of activity have been discovered as a result of extensive variations of the ring system and substituents of (**1**) (3). In general the clinically useful members of this class of antibacterials invariably contain a β-keto acid moiety which has been postulated to be very crucial in their binding to the DNA•DNA-gyrase complex (4).



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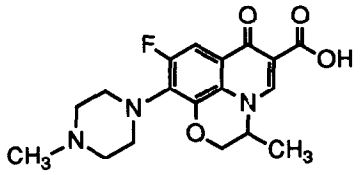

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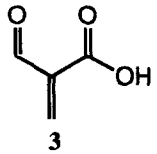

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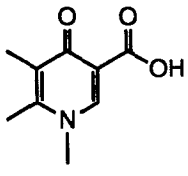

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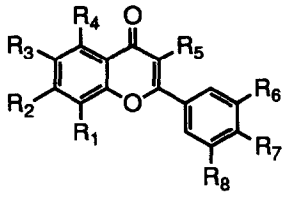

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It has therefore been our aim to identify novel inhibitors of DNA gyrase lacking the β-keto acid moiety as potential antibacterials. Based on the structures of known inhibitors of the enzyme, the pharmacophores (**3**) and (**4**) were generated and used for a MACCS structural search on our proprietary data base. The search identified the flavones (**5**) as potential inhibitors of the enzyme. Coupled with the fact that there are several reports in the literature which indicate that flavones and their derivatives exhibit antimicrobial

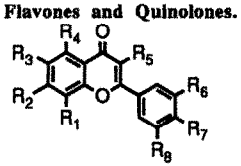
activities (5,6), we decided to study the DNA-gyrase inhibitory and antibacterial activities of a series of commercially available flavones (**5a-5n**) and ellagic acid (**6**)(Tables I & II). Since most of the compounds studied are hydrophilic and polyhydroxylated, with low c log P values and ionic at physiological pH, their penetration into the bacterial cell would be expected to be poor. Thus we observed that their antibacterial activity did not directly correlate with their in vitro DNA gyrase inhibition. Therefore, we studied their gram-negative antibacterial activity in a modified MIC assay by using polymyxin B nonapeptide (PMBN), which semi-permeabilizes gram-negative bacteria by forming selective membrane channels (7). We report our results herein.

All the compounds studied were tested in the DNA-gyrase supercoiling inhibition and the DNA-gyrase "cleavable complex" assay (8). The MIC's of the compounds against the six test organisms was determined in either Mueller Hinton Broth for gram-negatives, or Luria Broth for gram-positives in a microtiter well dilution series. Two-fold serial dilutions (range 0.015-500 μg/mL) of compound in broth were inoculated with adjusted suspensions of test organisms to approximately 5 x 10^5 CFU/mL. Microtiter plates were incubated overnight at 35°C. MIC was defined as the well concentration with no visible growth. PMBN (7) was used in conjunction with test compounds being screened at doses (at levels of PMBN exhibiting no antibacterial activity itself) lower than the MIC, and compared to PMBN-free controls.

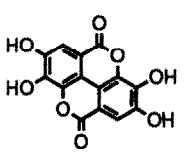
Results and Discussion

The inhibitory activities of the 15 compounds in the *E.coli* DNA-gyrase supercoiling assay and their ability to facilitate the "cleavable complex" are compared with some known 4-quinolone antibacterials (Table I). Eight of the 15 flavones tested inhibited the DNA-gyrase catalytic activity. The use of the DNA-gyrase supercoiling inhibition assay has been the classical approach in identifying and quantitating inhibition of DNA-gyrase by quinolones (9). This assay can detect both Gyr A and Gyr B subunit inhibitors, since the reaction exposes the holoenzyme to the potential inhibitor. Unfortunately, in a catalytic assay, reaction conditions such as pH, ionic strength, intercalation, chelation, and other non-specific effects can arise and be mistakenly read as specific inhibition. Since flavones are known to inhibit several enzymes by non-specific mechanisms (10,11), one could not conclude based on the results of the inhibition of the catalytic activity, that these flavones were bonafide inhibitors of the enzyme. A more specific variation on the supercoiling inhibition assay is the DNA-gyrase "cleavable complex" assay. In this assay, the endpoint is the detection and quantitation of linearized, cleaved fragments of the starting substrate, as compared to change in topomer position as with the supercoiling inhibition assay. A major difference in these assays is that the generation of the linearized fragment requires the catalytic activity of the holoenzyme to remain intact (i.e. free from non-specific inhibition) in order for the "cleavable complex" intermediate of DNA gyrase•DNA•drug to be formed, which in turn inhibits the resealing of the DNA. Any activity that prevents the binding or catalytic

Table I. Inhibition of DNA Gyrase Activity by Selected Flavones and Quinolones.



Cmpd.#	R1	R2	R3	R4	R5	R6	R7	R8	Supercoiling Inhibition IC_{50} (µg/mL)	Cleavable Complex CC_{50} (µg/mL)
5a	H	OH	H	OH	OH	OH	OH	H	47	45
5b	H	OH	H	OH	H	H	H	H	>500	NT
5c	H	OH	H	OH	OH	OH	H	OH	418	436
5d	H	OH	H	OH	OH	OH	OH	OH	225	195
5e	H	H	H	H	OH	H	H	H	>500	NT
5f	H	OH	H	OH	H	H	OH	H	67.6	63.2
5g	H	OH	H	OH	OH	H	H	H	>500	NT
5h	H	OH	H	H	OH	OH	OH	H	83.3	76.3
5i	H	OH	H	OH	H	OCH_3	H	H	>500	NT
5j	H	OH	H	OH	OH	H	OCH_3	H	>500	NT
5k	H	OH	OH	H	OH	H	OH	OH	55	46.9
5l	H	OH	H	OH	OH	OCH_3	OCH_3	OCH_3	>500	NT
5m	H	OCH_3	OH	OH	H	H	H	H	>500	NT
5n	OH	OH	H	H	H	H	H	H	89.2	95
6		ellagic acid							3.3	4.6



1	Nalidixic acid	52	41
2	Ofloxacin	1.75	2.1

NT = not tested if inactive in catalytic assay

Table II. MICs of Compounds 5a-5n, 6, and Quinolones against Gram-positive and Gram-negative (+/- PMBN) Bacteria.

		E.coli$_{ss}$		*S. typhimurium* 701		*P. maltophilia* 1057		*E. coli* KL-16		*S. epidermidis*	*S. aureus*
Compd #	**SCI# IC$_{50}$** (μg/ml)	**PMBN** (0 μg/ml)	**PMBN** (1μg/ml)	**PMBN** (0 μg/ml)	**PMBN** (1μg/ml)	**PMBN** (0 μg/ml)	**PMBN** (1μg/ml)	**PMBN** (0 μg/ml)	**PMBN** (1μg/ml)	strain OC2603	strain OC6538
5a	47	31.25	1.0	>125	31.25	>125	>125	>125	3.9	>125	>125
5b	>500	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
5c	418	125	3.9	>125	7.8	>125	>125	125	31.25	500	500
5d	225	62.5	3.9	>125	15.6	>125	125	>125	15.6	62.5	125
5e	>500	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
5f	67.6	>125	7.8	>125	15.6	>125	>125	>125	>125	125	>125
5g	>500	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
5h	83.3	62.5	3.9	>125	31.25	>125	>125	>125	15.6	>125	>125
5i	>500	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
5j	>500	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
5k	55	62.5	2.0	>125	15.6	>125	>125	>125	15.6	31.25	62.5
5l	>500	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
5m	>500	>125	>125	>125	>125	>125	>125	>125	>125	>125	>125
5n	89.2	125	7.8	>125	15.6	>125	125	>125	62.5	31.25	62.5
6	3.3	>125	2.0	>125	31.25	>125	>125	>125	7.8	>125	>125
1	52	0.5	0.125	3.9	2.0	62.5	15.6	2.0	0.5	250	500
2	1.75	<0.5	<0.5	0.125	0.0625	2.0	1.0	0.0625	<0.015	≤0.5	≤0.5

- SCI = Supercoiling Inhibition

$IC_{50}(CC_{50})$ = drug concentration to cause 50% inhibition of supercoiling or facilitate 50% of the maximum of the cleavable complex with the DNA gyrase cleavage assay (CC_{50}); IC_{50}'s determined by densitometric tracing of negatives of Polaroid 665 film of DNA fluoresced after EtBr staining;

activity of DNA-gyrase in a non-mechanism-based manner could prevent "cleavable complex" formation. Any gyrase inhibitor that specifically targets the Gyr A subunit in a mechanism-based manner such as the quinolones, would generate a "cleavable complex" and be reported as a bonafide active. However, any non-specific inhibition that would normally be detected as a "false positive" in the DNA-gyrase supercoiling inhibition assay (pH, temperature, chelation, intercalation, etc.) would not appear as active in the DNA cleavage assay. As seen in Table I, all of the flavones that inhibited the DNA gyrase catalytic activity also facilitated the cleavable complex. Thus the activity of all compounds with supercoiling inhibitory activities was shown to be against the Gyr A subunit of DNA gyrase by their ability to facilitate the "cleavable complex". The potencies of several active flavones at the enzyme level were in the range of the 4-quinolones in clinical practice, IC_{50} = 3.3 μg/mL for ellagic acid (**6**) is similar to IC_{50} = 1.75 μg/mL for ofloxacin (**2**); while (**5a**) (IC_{50} = 47 μg/mL), (**5f**) (IC_{50} = 67.6 μg/mL), and (**5k**) (IC_{50} = 55 μg/mL) are similar to that of the potency of nalidixic acid (**1**, IC_{50} = 52 μg/mL).

The antibacterial activities of the compounds are shown in Table II. Without PMBN, minimal antibacterial activity was observed only in the super-sensitive *E. coli* or the gram-positives. In general, antibacterial activity was observed only with the flavones with DNA-gyrase inhibitory activities, but not with the inactive flavones. This suggests the antibacterial activity of these flavones may be due in part to their inhibition of the DNA-gyrase. However the antibacterial activity observed did not parallel the potency at the enzyme level. In the presence of PMBN, some of the compounds exhibited antibacterial activity against some gram-negative organisms. The poor antibacterial activities could therefore be due to their inability to penetrate bacterial cell wall. It is worth mentioning that in the presence of PMBN, the MICs observed for some of the compounds were much lower than the IC_{50}s, suggesting that mechanisms other than the inhibition of the DNA gyrase may also play a role in the antibacterial activity.

In conclusion, as shown by the in vitro activities, some of these flavones studied are bonafide and potent inhibitors of the DNA gyrase enzyme. In general, the compounds show low or poor antibacterial activities due probably to their poor penetration into the bacterial cell wall as suggested by the PMBN studies. Current efforts are therefore trying to identify analogs which could overcome this barrier.

Acknowledgment. We thank Dr. Michael Corrado for many useful discussions and for his encouragement of this pursuit, and Ms. Heather Longwill with technical assistance with some of the MICs.

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