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ON THE BINDING SITE OF QUINOLONE ANTIBACTERIALS. AN ATTEMPT TO PROBE THE SHEN MODEL

Stephen Hanessian,* Raffaele Saladino, and Jose Cid Nunez

Department of Chemistry, Université de Montréal
C.P. 6128, Centre-Ville, Montréal, P.Q., CANADA.,H3C 3J7

Abstract: Quinolone-nucleic acid base hybrids were synthesized in an effort to probe a mechanistic model and a proposed mode of antibacterial action where stacked pairs of quinolones interact with DNA through H-bonding.

The quinolones represent a class of potent antibacterial agents that have acquired widespread importance clinically.[1] In spite of their comparatively simple structures *vis-a-vis* other more complex antibiotics,[2] their mode of action at the molecular level remains a subject of debate and some controversy. There is a general consensus that the functional target of the quinolones is the bacterial enzyme DNA gyrase, which is required for the initiation and propagation of DNA synthesis.[3] Since DNA is involved, it is also agreed that the mode of action of quinolones comprises a three component complex, the molecular nature of which is currently of great interest.[4] In 1989, Shen, Pernet, Mitscher and their coworkers[5] proposed a cooperative quinolone-DNA binding model in which DNA-gyrase is intimately involved in a drug-enzyme recognition process. Through extensive SAR and the synthesis of

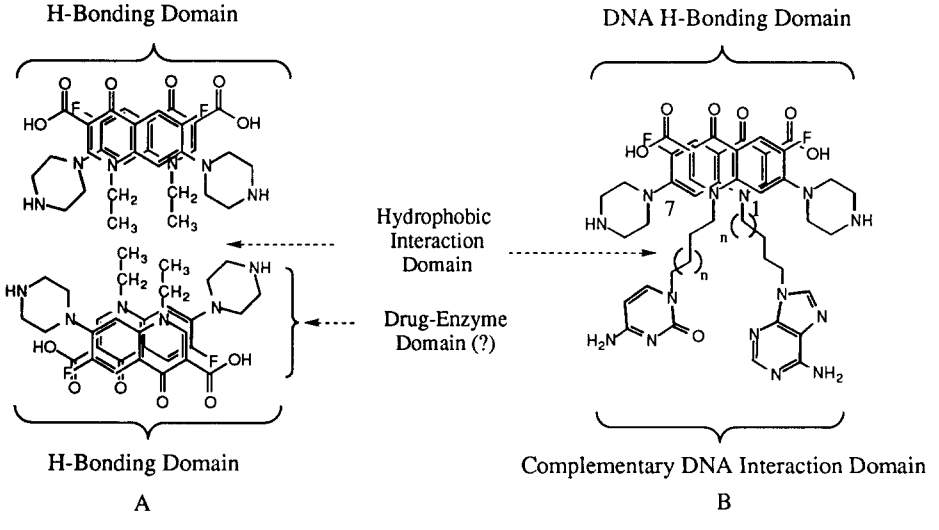


Figure 1. A. Shen model of quinolone-DNA gyrase-DNA interaction. B. Quinolone-nucleic acid base hybrid interactions with DNA gyrase and DNA.

novel compounds, Shen and coworkers were able to propose functional domains of quinolone antibiotics which are depicted in Figure 1A. Central to their theme is an interaction between antiparallel stacked dimers of the drug with single stranded DNA through H-bonding. Hydrophobic contact between two pairs of stacked quinolones and a gyrase interaction domain that comprises the basic C-7 appendage, completes the assembly and the cooperative recognition of the three-component complex. Observation of biological activity with tethered homodimeric quinolones seem to provide relevant experimental evidence for the model.[5] More recently, Palumbo and coworkers[6] have suggested a different model for the mode of DNA binding of the quinolones. They advance the notion that the drug acts by intercalating between nucleotide base pairs of a single stranded DNA. Stabilization of such drug-DNA interaction can be achieved by forming Mg^{+2} bridges between the aromatic carbonyl-carboxylate system and the phosphate esters of the DNA. Hurley and coworkers[7] have further elaborated on the Palumbo model, and other proposals are also available.[8] Shen has also suggested that the effect of Mg^{+2} is catalytic in helping the entry of the quinolones into their binding site. While the role of the enzyme is not as clearly defined as in the cooperative Shen model[5] in these proposals, there is agreement that a single stranded DNA is involved. The Palumbo model[6] differs from the Shen model by proposing stacking and Mg^{+2} bridges to account for molecular events leading to antigyrase action, rather than cooperative H-bonding, drug-drug interaction, and drug-enzyme recognition as proposed by Shen.[5] Cedergren and coworkers[9] have recently reported an SAR and QSAR study including molecular recognition to provide a rational explanation for the mode of action of quinolones. They conclude that the intercalation and Mg^{+2} ion-dependent model is consistent with data provided by modeling.

In an effort to probe the Shen model,[5] we reasoned that the synthesis of quinolones in which the N-1 alkyl substituent was replaced by a three or four carbon chain terminating with the four representative purine and pyrimidine bases would provide complementary binding interactions with the corresponding base pairs in the DNA.[10] A pictorial representation of such quinolone-nucleic acid base hybrids is shown in Figure 1B utilizing A-C as examples. A more concise schematic is shown in Figure 2 where the presumed quinolone carbonyl H-bonded interaction with an A-C pair on the DNA is further complemented by an "extended" A-C/T-G pairing from the drug to another segment of DNA.

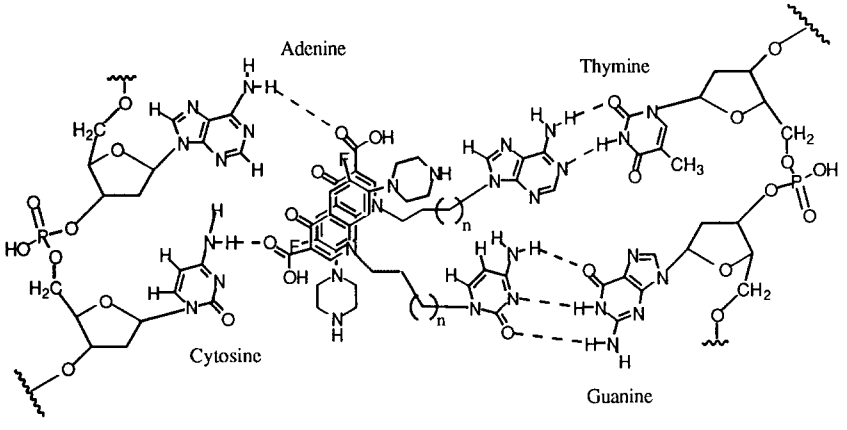
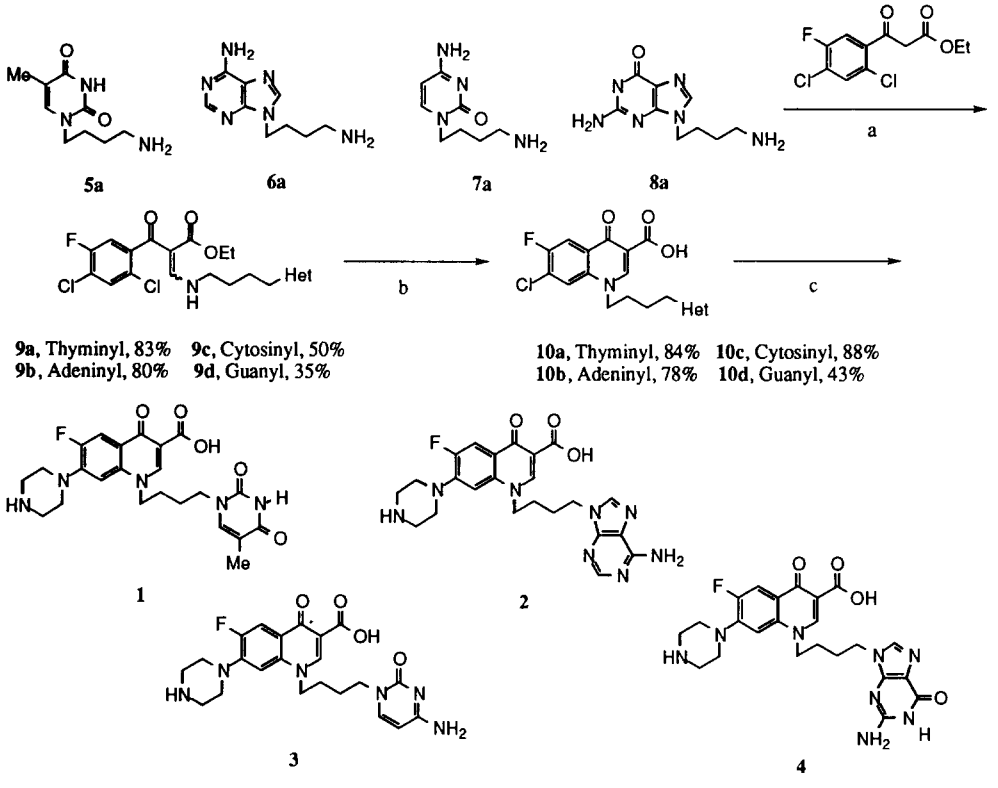


Figure 2. Hypothetical interactions of proposed quinolone-adenine/cytosine hybrids with complementary bases in DNA.

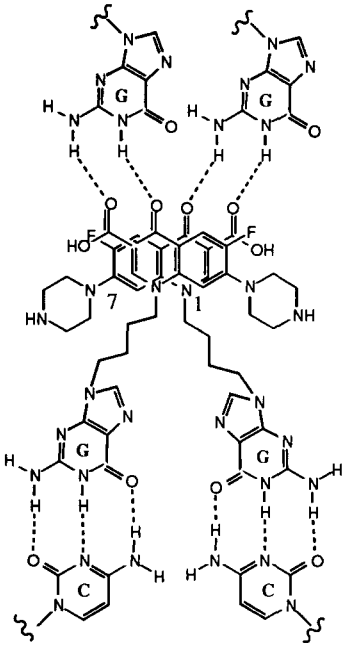
Although we had no quantitative measure that such quinolone-nucleic acid base hybrids would indeed mimic the drug-drug associated model, we were encouraged by the results of binding specificities of quinolones to a single as compared to double stranded DNA.[11] It has been demonstrated that the binding of norfloxacin to poly(dG), poly(dA), poly(dT), and poly(dC) decreases in that order, with a distinct preference for the poly(dG). Binding to double stranded poly(dA)-poly(dT) was virtually undetectable. These and other results have led Shen and coworkers[3-5] to suggest that quinolones exhibit preferential binding to unpaired guanine bases in a single stranded DNA region, possibly through hydrogen bonds involving the 4-keto/3-carboxyl group. We therefore proceeded with the synthesis and biological evaluation of several quinolone-base hybrids, based on the predictions of the Shen model. The synthesis of four quinolone-purine/pyrimidine hybrid molecules **1-4** is shown in Scheme 1.

Scheme 1

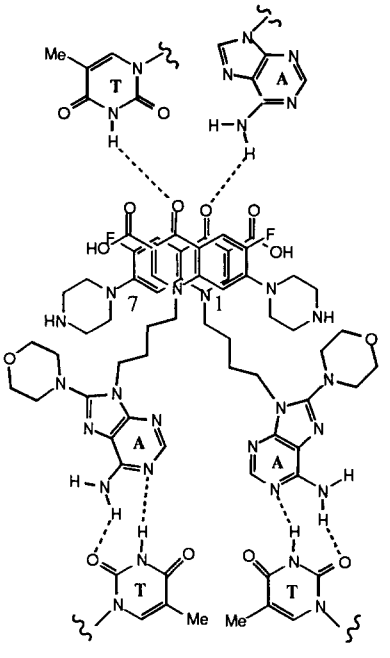


(a) i: Ethyl-2,4-dichloro-5-fluoro benzoylacetate (1.2 equiv.), $CH(OC_2H_5)_3$, Ac_2O, 130 °C, 3 h. ii: add **5a, 6a, 7a, 8a** CH_2Cl_2/EtOH (1:1 v/v), 25 °C, 12 h.; (b) i: NaH (1.5 equiv.), toluene, reflux, 12 h.; ii: NaOH, THF/H_2O, 80 °C, 16 h. (c) Piperazine, 1-methyl-pyrrolidone, 80 °C, 12-24 h., for **1, 2, 3, 4**; 76%, 90%, 75%, 54% respectively.

The required 4-amino-1-substituted bases were prepared by reacting 1-bromo-4-chlorobutane with the respective bases , followed by displacement of the chloro group with sodium azide (NaN_3, Bu_4NBr, 25 °C, MeCN reflux), then hydrogenation with Ra-Ni. The 4-aminobutyl heterocycles **5a-8a** were then condensed with the readily available[12] aromatic precursor (Scheme 1) to yield the enamino derivatives **9a-d** in good to excellent yields. Solubility problems intervened to provide a more modest yield for the guanine analog **9d**. Cyclization to the quinolones **10a-d** were carried out in a routine manner, and subsequent introduction of the piperazine unit at C-7 led to the intended hybrid analogs **1-4**, obtained as amorphous solids. Although these were sparingly soluble in common organic solvents, they were all adequately characterized by NMR and other analytical techniques.[13]



A. Complementary DNA Interaction Domain of compound **4**.



B. Complementary DNA and Gyrase interaction domain of compound **5**.

Figure 3. A. Proposed interaction of the quinolone-guanine hybrid **4** with a GGCC nucleotide sequence.
B. Proposed interaction of a quinolone-8-morpholinoadenine hybrid with a TTAT nucleotide sequence.

Gyrase-dependent DNA cleavage assays by the standard agarose gel electrophoresis method[14] at 50 μg/mL or less of compounds **1-3** in 5% DMSO as solvent, exhibited no drug-stimulated DNA cleavage. In an effort to probe the influence of the nature of the C-7 substituent, we prepared the N-methylpiperazine, morpholino, and pyrrolidino analogs related to the quinolone-thymine hybrid **1**. Also, the adenine analog **2** was prepared with a three-carbon linker chain. Again, biological activity was disappointingly absent, although the C-7 morpholino derivative of **1** gave values approaching Nalidixic acid (70 μg/mL vs 58 μg/mL respectively). The base-pairing design in compounds **1-3** was inspired in part from the fact that the nucleotide sequence in *Micrococcus Luteus*

DNA is TTAT,[15] hence the attachment of T and A units in hybrids **1** and **2**. However, the cleavage site on pBR322 DNA (a derivative of ColE1 DNA) has the nucleotide sequence CGCC between the staggered cuts with *E. coli* DNA gyrase.[16] We therefore prepared the quinolone-guanine hybrid **4** in anticipation of a complementary interaction as shown in Figure 3A. Again, no gyrase-dependent DNA cleavage was observed in the appropriate test system involving these preparations with compound **4**. We reasoned that the lack of a second enzyme-binding domain in the inactive analogs **1-4** was in part the reason for their inactivity. Accordingly, we prepared the 8-morpholinoadeninyl hybrid molecule **5**[17] depicted in Figure 3B. In such a derivative the stacked quinolone and adeninyl portions would still satisfy H-bonded interactions with the DNA, with the added feature that the piperazino and morpholino groups might effectively bind to the enzyme as in the original proposal especially if they are properly orientated in space. Unfortunately, this "rationally designed" hybrid also lacked activity in the gyrase-dependent DNA cleavage assays.

In conclusion, we have attempted to probe the Shen model[5] for the interaction of quinolone antibacterials with DNA and DNA gyrase from bacterial sources. The hybrid molecules, consisting of a quinolone linked to purine and pyrimidine bases through three and four methylene bridges were designed and predicated upon the presumed existence of H-bonding and enzyme binding domains as in the proposed three-component model.[5] The absence of enhancement of gyrase-dependent DNA cleavage in these analogs may be due to unfavorable three-dimensional features, and the deployment of critical functionality that deviate substantially from the elusive yet prevailing situation with the quinolones themselves. Thus, while the results of this study do not support the Shen model,[5] we cannot exclude its validity at this time. Clearly, additional studies are needed to shed some light on the fascinating roles that the quinolones play at the molecular level in conjunction with DNA gyrase and DNA itself. We are currently reevaluating our options for the design and synthesis of other quinolone prototypes in order to gain a better understanding of the relevance of the currently proposed models[5-7] with regard to their antibacterial mode of action.

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17. Prepared from 8-bromoadenine via: (a) morpholine, 140 °C, 4 h, 75%; (b) NaH, THF, then 4-bromo-1-chlorobutane, 25 °C, 2d; (c) NaN_3, Me_4NBr, NaI, MeCN, reflux 24h, 50-50% (2 steps); (d) Pd/C, H_2, EtOH, 90%; (e) condensation according to Scheme 1.

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