Investigations on the 4-Quinolone-3-carboxylic Acid Motif. 1. Synthesis and Structure−Activity Relationship of a Class of Human Immunodeficiency Virus type 1 Integrase Inhibitors†

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A set of 4-quinolone-3-carboxylic acids bearing different substituents on the condensed benzene ring was designed and synthesized as potential HIV-1 integrase inhibitors structurally related to elvitegravir. Some of the new compounds proved to be able to inhibit the strand transfer step of the virus integration process in the micromolar range. Docking studies and quantum mechanics calculations were used to rationalize these data.

Introduction

The HIV-1 integrase enzyme (IN)[a], which is essential for the replication of the virus and has no cellular counterparts, is an intriguing target for the development of anti-AIDS drugs.[1] Recently, Sato and co-workers[2] reported that the 4-quinolone-3-carboxylic acid antibiotics can be used as an alternative scaffold to diketo acids (DKA) in order to identify new IN inhibitors. These novel quinolone IN inhibitors, such as **1** (Chart 1), were structurally optimized into the highly potent compound **2** (GS-9137, elvitegravir), exhibiting potent inhibitory activity against IN-catalyzed DNA strand transfer ($IC_{50} = 7.2$ nM) and antiviral activity in vitro ($EC_{50} = 0.9$ nM).[2] Despite the high interest generated by these compounds,[3,4] only scattered examples of quinolones used as the supporting scaffold for the keto acid motif are described in the literature as IN inhibitors,[5] resulting in a total lack of structure−activity relationships information for this class of inhibitors. On the basis of these considerations as well as on our interest in the development of new anti-IN agents,[6] we planned to explore the effect of chemical modifications on the 4-quinolone-3-carboxylic acid scaffold, with the aim of gaining new insight in the fundamental structural requirements for anti-IN activity. Keeping in mind the pharmacophoric groups of **1** and **2** and the synthetic accessibility of the new molecules, the basic scaffold was decorated at N-1 and C-6 to provide the final compounds **3** and **4**, according to the following considerations: (i) Hydroxyalkyl chains of variable length were introduced at N-1 to study the effect of the spacer between the heterocyclic nitrogen and the OH group; moreover, chains with a terminal alkoxy moiety or basic nitrogen were used to evaluate the importance of a free OH and the influence of a protonatable group on the anti-IN activity of the molecule, respectively. (ii) In most of the compounds, the benzyl group of **1** and **2** was replaced with potential bioisosteric moieties with the aim of exploring novel structural motifs and establishing structure−activity relationships. (iii) In none of the new compounds a methoxy group at C-7 was introduced because this substituent, although important for pharmacokinetic and in vivo activity profiles, does not affect in vitro binding affinity, as demonstrated by the comparable IC_{50} values of **2** and its 7-demethoxy derivative (7.2 nM vs 8.1 nM, respectively).[2] Finally, quinone derivatives of general structure **5** were also synthesized where a second carbonyl group at C-5 might act as an additional chelating moiety, leading to an improvement of the inhibitory potency against integrase.

Results and Discussion

Chemistry. Easily accessible anilines **6a**−**d** (Scheme 1) were condensed with diethyl ethoxymethylenemalonate (EMME) to give the intermediate enaminoesters that were directly subjected to cyclization by heating in diphenyl ether to yield **7a**−**d**. These compounds were then alkylated using an appropriate alkyl halide to provide derivatives **8a**−**j** in low to good yield (19−85%). After hydrolysis of **8a**−**j** in 10% aq NaOH, the corresponding quinolone-3-carboxylic acids **3a**−**j** were obtained in yields ranging from 17% to 100%.

With this first set of compounds in our hands, different modifications at the C-6 position of the quinolone scaffold were made, keeping the 2-hydroxyethyl chain at N-1 fixed. The 6-bromo derivative **9** (Scheme 2), in turn prepared by alkylation of ethyl 6-bromo-4-oxo-1,4-dihydroquinoline-3-carboxylate, was subjected to different metal-catalyzed coupling reactions under high density microwave irradiation[7] to give, after basic hydrolysis of the intermediate products, the quinolone-3-carboxylic acids **3k**−**n** and **4a**,**b**. Compound **3o** was prepared in 28% overall yield by hydrolysis of the corresponding ester, which was in turn synthesized by a Buchwald−Hartwig amination of ethyl 6-bromo-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate in order to assess the actual relevance for biological activity of the hydroxyalkyl side chain at N-1 by direct comparison between **3o** and **3n**.

Quinone derivative **5a**, **5b**, and **5c** were obtained in satisfactory yield of 30−44% by reacting 1-ethyl-4,5,8-trioxo-1,4,5,8-tetrahydroquinolin-3-carboxylic acid (**10**)[8] with 3-chloro-2-

† Dedicated with respect and gratitude to Professor Marino Artico on the occasion of his retirement.

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[a] Abbreviations: IN, integrase; 3′-P, 3′-processing; ST, strand transfer; DKA, β-diketoacid; EMME, diethyl ethoxymethylenemalonate; BINAP, 2,2′-bis(diphenylphosphino)-1′,1-binaphthyl; Herrmann's palladacycle, *trans*-di(μ-acetato)bis[*o*-tolylphosphino)benzyl]dipalladium(II); Xantphos, 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene; MW, microwave; CPE, cytophatic effect; Tnp, transposase; vdW, van der Waals.

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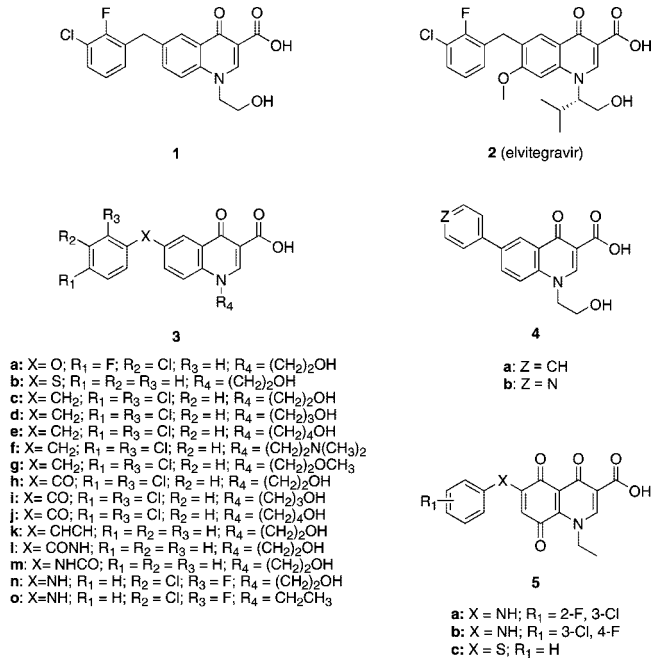
Chart 1. Structure of Elvitegravir and New 4-Quinolones Inhibiting HIV-1 Integrase

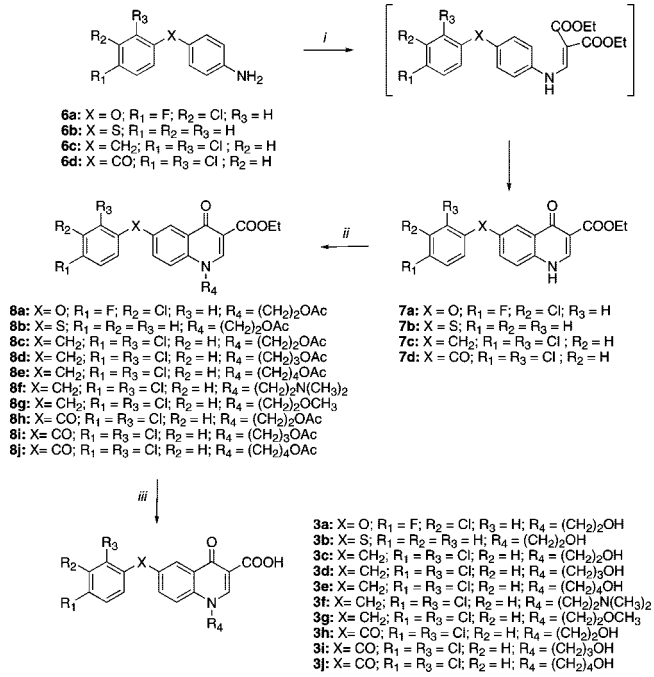


a: X= O; R_1 = F; R_2 = Cl; R_3 = H; R_4 = $(CH_2)_2OH$
b: X= S; R_1 = R_2 = R_3 = H; R_4 = $(CH_2)_2OH$
c: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OH$
d: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_3OH$
e: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_4OH$
f: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2N(CH_3)_2$
g: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OCH_3$
h: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OH$
i: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_3OH$
j: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_4OH$
k: X= CHCH; R_1 = R_2 = R_3 = H; R_4 = $(CH_2)_2OH$
l: X= CONH; R_1 = R_2 = R_3 = H; R_4 = $(CH_2)_2OH$
m: X= NHCO; R_1 = R_2 = R_3 = H; R_4 = $(CH_2)_2OH$
n: X=NH; R_1 = H; R_2 = Cl; R_3 = F; R_4 = $(CH_2)_2OH$
o: X=NH; R_1 = H; R_2 = Cl; R_3 = F; R_4 = CH_2CH_3

a: Z = CH
b: Z = N

a: X = NH; R_1 = 2-F, 3-Cl
b: X = NH; R_1 = 3-Cl, 4-F
c: X = S; R_1 = H

Scheme 1. Synthesis of 6-Substituted 4-Quinolone-3-carboxylic Acids **3a**−**j**[a]



6a: X = O; R_1 = F; R_2 = Cl; R_3 = H
6b: X = S; R_1 = R_2 = R_3 = H
6c: X = CH_2; R_1 = R_3 = Cl ; R_2 = H
6d: X = CO; R_1 = R_3 = Cl ; R_2 = H

7a: X = O; R_1 = F; R_2 = Cl; R_3 = H
7b: X = S; R_1 = R_2 = R_3 = H
7c: X = CH_2; R_1 = R_3 = Cl ; R_2 = H
7d: X = CO; R_1 = R_3 = Cl ; R_2 = H

8a: X= O; R_1 = F; R_2 = Cl; R_3 = H; R_4 = $(CH_2)_2OAc$
8b: X= S; R_1 = R_2 = R_3 = H; R_4 = $(CH_2)_2OAc$
8c: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OAc$
8d: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_3OAc$
8e: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_4OAc$
8f: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2N(CH_3)_2$
8g: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OCH_3$
8h: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OAc$
8i: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_3OAc$
8j: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_4OAc$

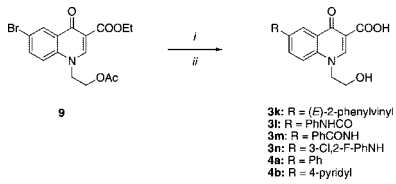
3a: X= O; R_1 = F; R_2 = Cl; R_3 = H; R_4 = $(CH_2)_2OH$
3b: X= S; R_1 = R_2 = R_3 = H; R_4 = $(CH_2)_2OH$
3c: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OH$
3d: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_3OH$
3e: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_4OH$
3f: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2N(CH_3)_2$
3g: X= CH_2; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OCH_3$
3h: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_2OH$
3i: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_3OH$
3j: X= CO; R_1 = R_3 = Cl; R_2 = H; R_4 = $(CH_2)_4OH$

[a] Reagents and conditions: (*i*) (1) EMME, 120 °C, 1 h; (2) Ph_2O, reflux, 2 h. (*ii*) R_4Y, K_2CO_3, DMF, 90 °C, 12 h. (*iii*) 10% aq NaOH, reflux, 2 h.

fluoroaniline, 3-chloro-4-fluoroaniline, and thiophenol, respectively, according to a known procedure (see Supporting Information).[8]

Inhibition of HIV-1 IN Activity. Compounds **3c**−**e**, structurally related to **2** and characterized by a hydroxyalkyl chain of variable length at N-1 and a 2,4-dichlorobenzyl moiety at C-6, showed anti-IN activity in the low micromolar range, being at least 20 times less potent than the reference compound **2** in the strand transfer (ST) assay[1] (Table 1). The introduction of a methoxyethyl or a dimethylaminoethyl chain at N-1 resulted in **3g** and **3f**, respectively, for which a further significant decrease in activity was observed. This result highlights the fundamental role played by the free terminal hydroxy group on the N-1 chain. The replacement of the benzyl group of **3c**−**e** with a bioisosteric benzoyl group resulted in **3h**, **3i**, and **3j**, respectively, which, independently by the length of the chain at N-1, proved to be completely inactive in the ST assay (IC_{50} > 100 μM). When the 2,4-dichlorobenzyl moiety of **3c** was replaced with a 3-chloro-4-fluorophenoxy group (**3a**), a loss of activity in the ST assay was observed as well; the inhibitory potency was partially restored with a thiophenyl group even if unsubstituted (**3b**, IC_{50} = 18.5 μM). Removal of the spacer between the two aromatic rings gave **4a** and **4b**, characterized by a phenyl or a pyridyl group directly linked to C-6, which proved to be completely inactive in inhibiting the enzyme. Similarly, by increasing the distance between the two aromatics by the use of rigid systems such as double bond (**3k**),

Scheme 2. Synthesis of 6-Substituted 4-Quinolone-3-carboxylic Acids **3k**−**n** and **4a**,**b** by Metal-Catalyzed, Microwave-Assisted Reactions[a]



[a] Reagents and conditions: (*i*) (for **3k**, **4a**, **4b**) appropriate arylboronic acid, $Pd(PPh_3)_2Cl_2$, 2 M Na_2CO_3, EtOH/DME, MW, 120 °C, 30 min; (for **3l**) aniline, $Mo(CO)_6$, Herrmann's palladacycle, $[(t\text{-}Bu)_3PH]BF_4$, 2 M Na_2CO_3, H_2O, MW, 150 °C, 15 min; (for **3m**) benzamide, $Pd(dba)_2$, Xantphos, Cs_2CO_3, NMP, dioxane, MW, 140 °C, 1 h; (for **3n**) 3-chloro-2-fluoroaniline, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, MW, 150 °C, 15 min. (*ii*) 10% aq NaOH, reflux, 2 h.

amide (**3l**), or inverse amide (**3m**), a loss of activity was observed, thus confirming the importance of a methylene or sulfur spacer.

Most of the tested compounds displayed in the overall assay[9] IC_{50} values lower than those found in the ST test, thus raising the question whether their activity might be more likely due to the inhibition of DNA binding or 3′-processing[1] (3′-P) steps. Accordingly, some selected compounds were subjected to biological evaluation in the 3′-P assay in order to assess their actual mechanism of IN inhibition. However, none of the compounds proved to be more active in this test than in either the overall or the ST assays. At the moment, no explanation can be given for this result except that the three reactions (DNA binding, 3′-P, ST) occur in a coupled mode in the overall integration assay. Finally, the results obtained for the class of quinone derivatives (**10**, **5a**−**c**), all characterized by the presence of an ethyl chain at N-1, deserve some attention. Starting from the unsubstituted compound **10** (IC_{50} = 58.7 μM), the introduction of a substituted phenylamino (**5b**, IC_{50} = 2.1 μM) or phenylthio moiety (**5c**, IC_{50} = 1.6 μM) at C-6 gave compounds with IC_{50} values in the low micromolar range. This positive effect on the inhibitory activity could be ascribed to the introduction on the molecule of an additional chelating moiety. Moreover, **10** and **5b**,**c** exhibited IC_{50} values of the same order of magnitude in both the ST and overall assays, thus suggesting a completely selective mechanism of action for this class of compounds.

In Vitro HIV-1 Assay and Drug Susceptibility Assay. The antiviral activity of the new compounds on the HIV-induced cytopathic effect (CPE) in human lymphocyte MT-4 cell culture was determined by the MT-4/MTT-assay. All compounds proved to be inactive in inhibiting HIV-1 replication at subcytotoxic concentration (Table 1). It should be noted that **2** proved to be the most cytotoxic compound under the test conditions. However, due to its high antiviral potency (EC_{50} = 0.00037 μM), it elicited a very favorable selectivity index (3108).

Docking Studies. It has previously been reported that the quinolone integrase inhibitor **2** selectively inhibits the ST step via a mechanism similar to that of the DKA.[2] Accordingly, the activity of quinolones is likely due to sequestration of divalent metal ions within IN active site in complex with viral DNA.[10]

Although the crystallographic structure of IN in complex with viral DNA has not yet been resolved, it has been reported that Tn5 transposase (Tnp), which belongs to the superfamily of polynucleotidyl transferases as IN, can be considered as an excellent surrogate model for studying the mechanism of action of ST inhibitors.[11,12] In this context, docking calculations on our compounds together with **2** were performed on Tn5 Tnp−DNA complex by following the computational protocol described by Barreca et al.[12] In its best docking pose (conformation with the highest fitness score and belonging to the most populated cluster), compound **2** (Figure 1A) presented the carboxylate group, hydrogen-bonded to H329, chelating the metal ion between E326 and D97 and the β-ketone oxygen together with the fluorine atom coordinating the other metal ion. Moreover, the hydroxy group of the N-1 chain made two H-bonding interactions with S100, while the C-7 methoxy group did not seem to be involved in any interaction. Finally, the 2-F, 3-Cl substituted benzyl group was perpendicular to the quinolone ring, establishing favorable vdW interactions with E190 and D188. Interestingly, the binding mode proposed for compound **2** is in perfect agreement with its mutation profile (see validation of docking studies in Supporting Information) as well as with the pharmacophoric model for quinolones recently reported.[3]

To facilitate the analysis of the biological data, quinolones **3a**−**o**, **4a**,**b**, **5a**−**c**, and **10** were divided into two main groups on the basis of their IC_{50} value in the ST assay: compounds with IC_{50} < 100 μM were classified as active, while those endowed with an IC_{50} > 100 μM were considered inactive. The active compounds **3b**−**f** displayed a binding mode similar to that of **2**, showing the same important interactions with the exception of that involving the fluorine atom on the benzyl moiety, which, in our compounds, is replaced by a chlorine or a hydrogen atom. The lower activity of **3f** and **3g** with respect to **3c** could be explained by the lack of the hydrogen bond between the side chain OH and S100. On the other hand, the inactive compounds (**3a**, **3k**−**o**, and **4a**,**b**), because of their enhanced conformational rigidity, were not able to assume a conformation similar to that of the active compounds (Figure 1B).

It is worth noting that compounds **3h**−**j**, with a carbonyl substituent at C-6, showed a peculiar behavior, binding the enzyme in a similar way as the active compounds although showing no activity in the ST assay. To explain this finding, theoretical quantum mechanics calculations were performed on these molecules with the aim to measure the partial charge on each atom. These calculations suggest for **3 h**−**j** an electron-withdrawing effect on the chelating system due to the C-6 carbonyl substituent, which results in a poor interaction of the molecules with the divalent metal ions of the active site. As an example, results obtained for **3c** and **3h** are comparatively shown in Figure 2S of Supporting Information.

With respect to the quinone series (compounds **5a**−**c** and **10**), docking results were unable to completely explain their activity profile. In agreement with biological results, **5c** showed a similar orientation to that described above for the active compounds. Conversely, a different docking mode was observed for compounds **5a**,**b** and **10** (Figure 1C). In fact, these compounds presented the carboxylate group, hydrogen-bonded to K164, chelating the metal ion between D188 and D97 and the two carbonyl oxygens coordinating the ion between D97 and E326. Moreover, for compounds **5a** and **5b**, the anilino moiety and the quinone ring established favorable interaction with W125. This type of binding mode was not able to explain the inactivity of **5a** with respect to **5b** because both of these two compounds are expected to interact in the same way with the enzyme. Further studies are ongoing to clarify the structure−activity relationship for the quinone series.

Conclusions

The results of this first attempt aimed at rationalizing the influence on the anti-IN activity of the different decorating elements introduced on the 4-quinolone-3-carboxylic acid scaffold allowed us to highlight new aspects of structure−activity relationships for

Table 1. Inhibition of HIV-1 Integrase Enzymatic Activity, Replication of HIV-1 (III_B), and Cytotoxicity in MT-4 cells

	IC_{50} [a] (μM)				
compd	overall	3′-P	ST	EC_{50} [b] (μM)	CC_{50} [c] (μM)
3a	86.9 ± 24.4	>100	>100	>9.9	9.9
3b	4.8 ± 2.6		18.5 ± 3.7	>22.7	22.7
3c	0.07 ± 0.03		0.2 ± 0.0	>5.3	5.3
3d	0.9 ± 0.4		1.3 ± 0.1	>3.6	3.6
3e	0.05 ± 0.0	16.6 ± 4.8	0.6 ± 0.3	>4.2	4.2
3f	10.8 ± 5.4		24.1 ± 5.9	>5.2	5.2
3g	5.6 ± 2.6		16.5 ± 0.7	>4.2	4.2
3h	18.7 ± 11.7	>100	>100	>21.1	21.1
3i	73.9 ± 49.0	>100	>100	>17.5	17.5
3j	54.9 ± 33.9	>100	>100	>17	17
3k	71.9 ± 13.6		>100	>22.6	22.6
3l	>100		>100	>224	224
3m	>100		>100	>220.5	220.5
3n	>100		>100	>27.4	27.4
3o	38.0 ± 0.83	>100	>100	>9.8	9.8
4a	>100		>100	>44.9	44.9
4b	77.6 ± 19.0	>100	>100	>138.1	138.1
5a	9.7 ± 1.6	>100	>100	>2.4	2.4
5b	1.0 ± 0.1		2.1 ± 0.5	>2.2	2.2
5c	4.6 ± 0.1		1.6 ± 0.3	>30.0	30.0
10	43.7 ± 18.2		58.7 ± 5.7	>42.8	42.8
1			0.0242 ± 0.0116[d]	0.0763 ± 0.0037[d]	>15[d]
2	0.002 ± 0.001	0.05 ± 0.01	0.007 ± 0.0007	0.00037 ± 0.00004	1.15

[a] Concentration required to inhibit by 50% the in vitro integrase activities. [b] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells. [c] Cytotoxic concentration to reduce MT-4 cell viability by 50%. [d] Taken from ref 2.

IN inhibiting quinolones. In particular, the substitution of the benzyl group with aryl, styryl, aroylamino, and aniline groups results in inactive compounds that are not able to adopt the bioactive conformation; conversely, the phenylthio group is able to mimic quite efficiently the benzyl group. On the other hand, the substitution of the benzyl group with the electron-withdrawing benzoyl moiety gives compounds that can be accommodated in the same orientation as the active molecules within the binding site but show a reduced chelating ability. Finally, the replacement of the benzene ring of the quinolone with a quinone moiety gives compounds that, with the exception of **5c**, adopt a different orientation in the binding site and likely act through a pure ST inhibition mechanism.

Experimental Section

Synthesis of 6-Substituted Quinolones 7a−d: General Procedure. A mixture of the appropriate aniline **6a−d** (1 mmol) and diethyl ethoxymethylenemalonate (200 μL, 1 mmol) was heated at 120 °C for 1 h. The crystalline solid obtained after cooling at room temperature was dissolved in diphenyl ether (3 mL), and the solution was refluxed for 2 h. After cooling to room temperature, diethyl ether (4 mL) was added and the precipitated solid was filtered and recrystallized from DMF.

Synthesis of 1,6-Disubstituted Quinolones 8a−j and 9: General Procedure. A mixture of the appropriate quinolone derivative **7a−d** (1 mmol) or ethyl 6-bromo-4-oxo-1,4-dihydroquinoline-3-carboxylate (296 mg, 1 mmol), the appropriate alkyl halide (2.8 mmol), and solid K_2CO_3 (386 mg, 2.8 mmol) in dry DMF (1 mL) was heated under N_2 atmosphere at 90 °C for 12 h and then poured into an ice−water mixture. The precipitated *N*-alkylquinolone was collected by filtration and recrystallized or extracted into DCM and purified by flash chromatography on silica gel eluting with DCM/MeOH (98:2).

Synthesis of 4-Quinolone-3-carboxylic Acids 3a−j: General Procedure. A suspension of the appropriate ester (1 mmol) in 10% aq. NaOH (10 mL) was refluxed for 2 h. After cooling at room temperature, the reaction mixture was acidified using conc. HCl. The resulting precipitate was filtered and washed with water to give the corresponding 4-quinolone-3-carboxylic acid.

Synthesis of Quinolones 3k and 4a,b by Suzuki Coupling: General Procedure. A 5 mL process vial was charged with **9** (95.5 mg, 0.25 mmol), the appropriate boronic acid (1.25 mmol for phenylboronic acid and 4-pyridineboronic acid, 0.75 mmol for *trans*-2-phenylvinylboronic acid), $Pd(PPh_3)_2Cl_2$ (8.8 mg, 0.0125 mmol), 2 M Na_2CO_3 (0.2 mL, 0.4 mmol, for **4a**; 0.38 mL, 0.75 mmol, for **4b**; 0.25 mL, 0.5 mmol, for **3k**), 0.6 mL of EtOH, and 2.4 mL of DME. The vessel was sealed under air and exposed to microwave heating for 30 min at 120 °C (for **4a**,**b**) or 20 min. at 130 °C (for **3k**). The reaction mixture was thereafter cooled down to room temperature, diluted with DCM, filtered through a short plug of neutral aluminum oxide, and purified by flash chromatography on silica gel (99:1 to 95:5 DCM/MeOH). The ester so obtained was hydrolyzed with 10% NaOH as described for **3a−j**.

1-(2-Hydroxyethyl)-4-oxo-6-(phenylcarbamoyl)-1,4-dihydroquinoline-3-carboxylic Acid (3l). A 5 mL microwave vial was charged with **9** (149.0 mg, 0.39 mmol), aniline (0.071 mL, 0.78 mmol), molybdenum hexacarbonyl (103.6 mg, 0.39 mmol), 2 M Na_2CO_3 (0.59 mL, 1.2 mmol), *trans*-di(μ-acetato)bis[*o*-tolylphosphino)benzyl]dipalladium(II) (Herrmann's palladacycle) (18.3 mg, 0.019 mmol), [(*t*-Bu)$_3$PH]BF_4 (22.8 mg, 0.078 mmol), and water (1.1 mL). Subsequent to the addition of reagents and solvents, the vial was capped under air with a Teflon septum. The mixture was thereafter irradiated with microwaves to 150 °C for 15 min. After cooling, the crude mixture was filtered through a plug of celite and the mixture of partially hydrolyzed compounds so obtained was treated with 10% NaOH (4 mL) under reflux for 2 h. After cooling at room temperature, the reaction mixture was acidified to pH 3 using conc. HCl. The resulting precipitate was filtered and washed with water to give **3l** (27.5 mg, 20% overall yield).

6-Benzamido-1-(2-hydroxyethyl)-4-oxo-1,4-dihydroquinoline-3-carboxylic Acid (3m). A 5 mL microwave vial was charged with **9** (105.0 mg, 0.27 mmol), benzamide (99.8 mg, 0.82 mmol), Cs_2CO_3 (358.2 mg, 1.1 mmol), $Pd(dba)_2$ (9.5 mg, 0.016 mmol), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (Xantphos) (19.0 mg, 0.033 mmol), and 10% NMP in dioxane (3.6 mL). Subsequent to the addition of reagents and solvents, the vial was capped under air with a Teflon septum. The mixture was irradiated with microwaves at 140 °C for 1 h. After cooling, the crude mixture was filtered through a plug of celite and hydrolyzed with 10% NaOH as described for **3l**.

Synthesis of Quinolones 3n and 3o by Buchwald−Hartwig Reaction: General Procedure. A 5 mL microwave vial was charged with **9** (149.0 mg, 0.39 mmol) or ethyl 6-bromo-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (126.0 mg, 0.39 mmol), 3-chloro-2-fluoroaniline (142.0 mg, 0.97 mmol), Cs_2CO_3 (381.0 mg,

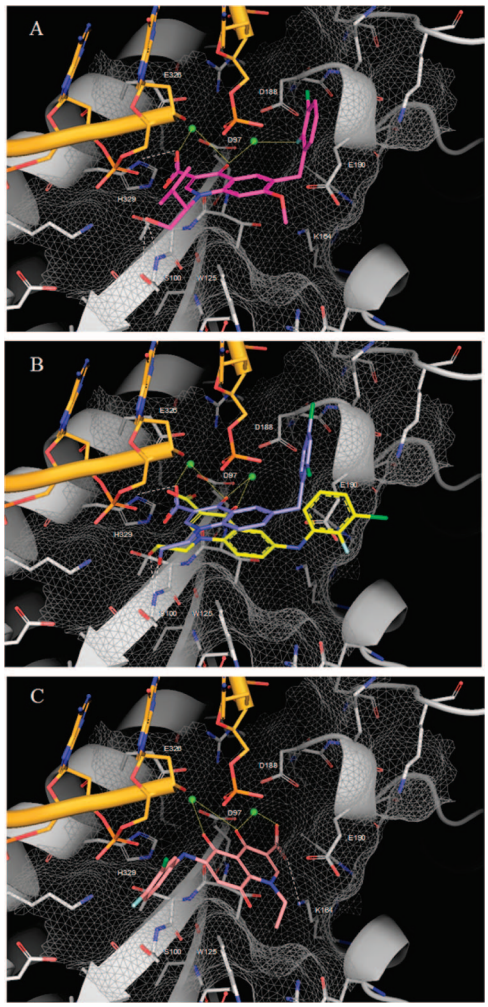


Figure 1. Binding mode of the studied compounds within the Tnp Tn5 binding site (PDB code 1MUS). (A) Binding mode of compound **2** (magenta). (B) Binding mode of compound **3n** (yellow) superimposed to **3c** (blue). Interestingly, the anilino moiety of **3n** is not able to correctly overlay the benzyl group of **3c**. (C) Binding mode of compound **5b** (pink). Metal ions are presented as green balls. Tn5 Tnp/ligand interactions are shown as dashed lines (metal coordination in yellow, hydrogen bonds in white).

1.17 mmol), $Pd(OAc)_2$ (4.3 mg, 0.0195 mmol), BINAP (24.3 mg, 0.039 mmol), and toluene (4.15 mL). After the addition of reagents and solvents, the vial was capped with a Teflon septum. The reaction mixture was irradiated with microwaves at 150 °C for 15 min. The crude mixture was filtered through a plug of celite and then purified by flash chromatography on silica gel (hexane:AcOEt gradient 40:60 to 20:80 for the precursor of **3n**, and DCM:MeOH 98:2 to 94:6 for the precursor of **3o**). The product so obtained was hydrolyzed as described above to give the title compound.

Integrase Assays. The enzymatic integration reactions were carried out as described previously with minor modifications.[9]

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Supporting Information Available: Detailed biological and molecular modeling protocols; analytical and spectral data for compounds **3a**−**o**, **4a**,**b**, **5a**−**c**, **7a**−**d**, **8a**−**j**, **9.** This material is available free of charge via the Internet at http://pubs.acs.org.

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