Symmetrical Bis-Quinolinium Compounds: New Human Choline Kinase Inhibitors with Antiproliferative Activity against the HT-29 Cell Line

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Studies have been aimed at the establishment of structure−activity relationships that define choline kinase inhibitory and antiproliferative activities of 40 bisquinolinium compounds. These derivatives have electron-releasing groups at position 4 of the quinolinium ring. It is found that the enzymatic inhibition is closely related to the size of the linker, the 3,3′-biphenyl moiety being the most suitable. On the other hand, the antiproliferative activity against the HT-29 cancer cell line is less influenced by the linker type and by substituent R_4. The corresponding QSAR equation was obtained for the whole set of compounds for the antiproliferative activity, the electronic parameter σ_R of R_4, the molar refractivity of R_8, and the lipophilic parameters clog *P* and π_{linker}. The most potent antiproliferative agent so far described is **40** for which an $IC_{50} = 0.45$ μM was predicted by the QSAR equation, while its experimental value is $IC_{50} = 0.20$ μM.

Introduction

Choline kinase (ChoK) is the first enzyme in the Kennedy pathway for the synthesis of phosphatidylcholine (PC), and it phosphorylates choline to phosphorylcholine (*P*Cho) using adenosine 5′-triphosphate (ATP) as phosphate donor.[1] *ras* genes are the most extensively studied oncogenes in human cancer to date. Ras proteins play a pivotal role in cellular signal transduction and help to regulate cellular proliferation and terminal differentiation.[2] The family of *ras* oncogenes has been implicated in up to 30% of all human tumors and in some of them up to 90%.[3] Overexpression of several oncogenes induces increased levels of ChoK and the intracellular levels of *P*Cho.[4] Additional evidence gives support for a role of ChoK in the generation of human tumors. Studies using nuclear magnetic resonance (NMR) techniques have demonstrated elevated levels of *P*Cho in human tumoral tissues with respect to normal ones, including breast, colon, lung, and prostate tumors among others.[5] It is generally accepted that *ras* is the most widely studied oncogene in human carcinogenesis, and inhibition of ChoK has been demonstrated to be a novel efficient antitumor strategy in oncogene-transformed cells.[6] These primary observations were later extrapolated in vivo in the nude mice system.[7]

Research on ChoK inhibitors has correlated the inhibitory effect on proliferation by symmetrical bis-quaternary compounds with the ability to inhibit the production of *P*Cho in whole cells.[6c] When the 1,2-ethylene-*p*-(bisbenzyldimethyl-diyl) moiety was used as a linker between the two 4-substituted pyridinium cationic heads (**1**, Figure 1),[8] the structures were screened for their activity inhibiting isolated ChoK (under ex vivo conditions). The 4-NR_2 group made a substantial contribution and it was suggested[8] that the role of the 4-NR_2 group was electronic, via the delocalization of the positive charge. The importance of frontier orbital energies (LUMO) of model compounds has been emphasized and interpreted.[9] We have very recently published a review on ChoK inhibitors.[10] It has been reported that increased activity of ChoK was observed in a variety of human breast carcinomas.[11] It has recently been reported that ChoK dysregulation is a frequent event found in a variety of human tumors such as lung, colorectal, and prostate tumors.[12] All these encouraging results are the basis of the development of a novel strategy in cancer treatment.

Trispyridinium compounds (**3**, Figure 1) are more potent than the bispyridinium ones (**2**, Figure 1) as inhibitors of human ChoK.[13] Nevertheless, the trispyridinium structures are less active than the bispyridinium ones as antiproliferative agents because the latter show better lipophilicity to cross the cytosolic membranes. On the other hand, cyclizing open structures or creating an additional ring system in a given structure represents one of the useful methods in the search for biologically active conformations. The result is a more constrained molecule with an imposed conformation. To begin with, we designed and synthesized the most simple model of macrocyclic compounds that have only one benzene ring as a linker (**4**−**7**).[14] These compounds are dissimilar to each other in the substitution pattern shown by the upper (first prefix) and lower (second prefix) benzene rings (Figure 1). We have recently studied the conformational dynamics of cyclophane **4**[15] and the inhibitory activities against human ChoK of a set of 25 bispyridinium compounds with electron-releasing groups at position 4 and found that the 3,3′-biphenyl linker is the most suitable.[16] We have

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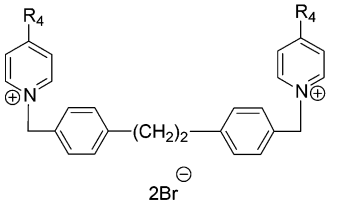

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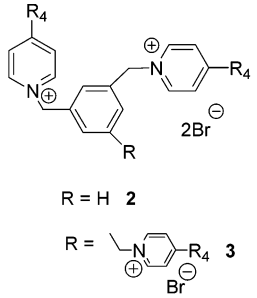
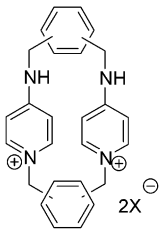
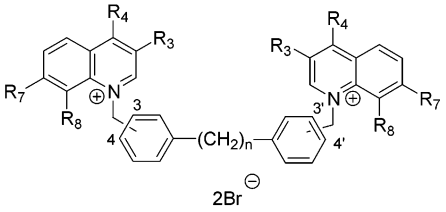


R_4: NMe_2, pyrrolidino, piperidino, several *N*-methylanilino groups.



Compound	Isomer
4	*p,p*
5	*m,m*
6	*p,m*
7	*m,p*

X = Br for all compounds and moreover PF_6 for **4**



R_3 = H, Me
R_4 = cyclic tertiary and acyclic amino groups
R_7 = H, Cl, NH_2
R_8 = H, Me
n = 0, 2
isomers: 3,3' and 4,4' when n = 0
4,4' when n = 2

8-48

Figure 1.

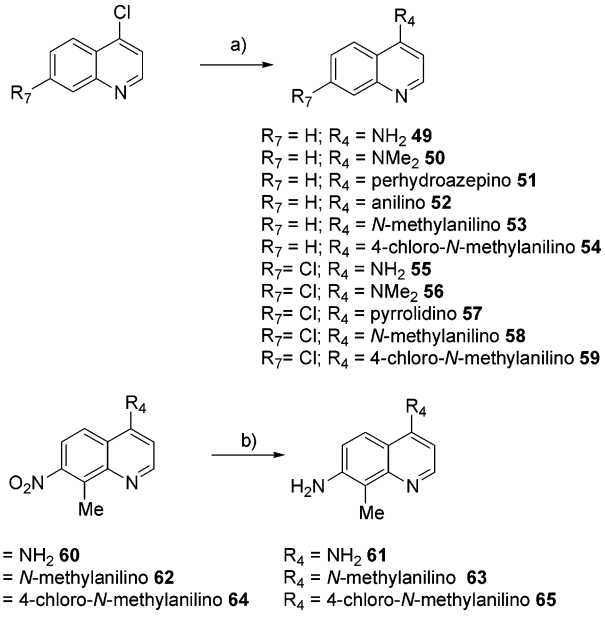
proposed a model for the inhibition of ChoK by using cyclophanes **4**−**7**.[17]

With the aim of obtaining new structure−activity relationships and studying in depth the structural parameters that define the ChoK inhibitory and antiproliferative activities, the synthesis of a new set of compounds is proposed based on changing the pyridinium for quinolinium moieties of the biscationic acyclic compounds.

The aim of this paper specifically focuses on the two following aspects: (1) the effect to be expected on the ex vivo human ChoK inhibitory activity by a variation in the linker that connects the quinolinium cations having electron-releasing groups at their position 4, with other different groups at positions 3, 7, and 8 of the heterocycle (compounds **8**−**48**, Figure 1); (2) the influence of the factors that control the antiproliferative activities of such compounds.

Chemistry. Forty end compounds, included in three series (according to their cationic heads), of which each is divided in three subseries (according to their linker), have been synthesized (Tables 1−3). They are biscationic compounds that consist of a linker and two cationic heads which are 4-substituted quinolinium rings with tertiary cyclic and acyclic amino groups. Figure 2 and Scheme 1 (compound **68**) show the quinoline structures that are to be quaternized.

The synthesis of 7-unsubstituted-4-aminoquinolines such as 4-aminoquinoline (**49**),[18] 4-(dimethylamino)-quinoline (**50**),[19] and 4-*N*-(methylanilino)quinoline (**53**)[20] have been reported. Although 4-anilinoquinoline (**52**)[21]



R_7 = H; R_4 = NH_2 **49**
R_7 = H; R_4 = NMe_2 **50**
R_7 = H; R_4 = perhydroazepino **51**
R_7 = H; R_4 = anilino **52**
R_7 = H; R_4 = *N*-methylanilino **53**
R_7 = H; R_4 = 4-chloro-*N*-methylanilino **54**
R_7= Cl; R_4 = NH_2 **55**
R_7= Cl; R_4 = NMe_2 **56**
R_7= Cl; R_4 = pyrrolidino **57**
R_7= Cl; R_4 = *N*-methylanilino **58**
R_7= Cl; R_4 = 4-chloro-*N*-methylanilino **59**

R_4 = NH_2 **60**
R_4 = *N*-methylanilino **62**
R_4 = 4-chloro-*N*-methylanilino **64**

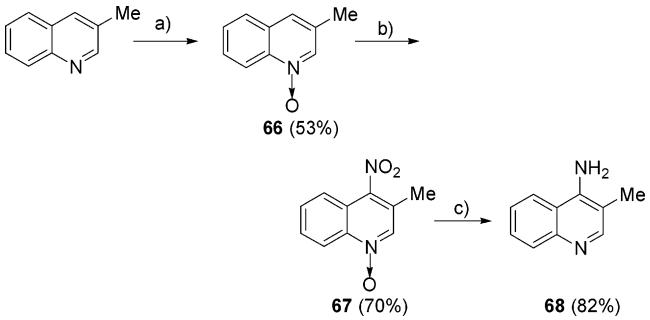
R_4 = NH_2 **61**
R_4 = *N*-methylanilino **63**
R_4 = 4-chloro-*N*-methylanilino **65**

Figure 2. Structures of quinolines **49**−**65**. Reagents: (a) R_4H, heating; (b) $SnCl_2$, glacial acetic acid.

was first reported by Backeberg, we decided to prepare it by heating at reflux a mixture of 4-chloroquinoline and aniline in glacial acetic acid (88%). When this procedure was applied for the reaction between 4-chloroquinoline and 4-chloro-*N*-methylaniline, the new compound 4-(4-chloro-*N*-methylanilino)quinoline (**54**) was obtained (97%).

7-Chloro-4-aminoquinoline (**55**)[22] was prepared according to a known procedure. Although 7-chloro-4-

Scheme 1[a]



[a] Reagents and conditions: (a) Peracetic acid, glacial acetic acid, reflux, 3 h. (b) HNO_3/H_2SO_4, 100 °C, 3 h. (c) H_2, Pd/C, MeOH, 3 h.

(dimethylamino)quinoline (**56**)[23] was first reported by Gupton et al., we decided to apply the same methodology used for the synthesis of **50**, but changing 4-chloroquinoline for 4,7-dichloroquinoline. The target molecule was obtained with a 40% yield, while in the original paper[23] compound **56** was obtained with a 24% yield. Although the synthesis of 7-chloro-4-*N*-(methylanilino)-quinoline (**58**)[24] was known, a different route from the one published was used. The procedure used for the synthesis of **52** was applied for the synthesis of **57** between 4,7-dichloroquinoline and pyrrolidine (50% yield), 4,7-dichloroquinoline and *N*-methylaniline (to yield **58·HBr** with a 75% yield, after adding a solution of hydrogen bromide in glacial acetic acid), and 4,7-dichloroquinoline and 4-chloro-*N*-methylaniline (to produce **59** with a 59% yield). Before the quaternization reaction, **58·HBr** was converted to the free base by treating it with a sodium hydroxyde aqueous solution and subsequent extraction with diethyl ether.

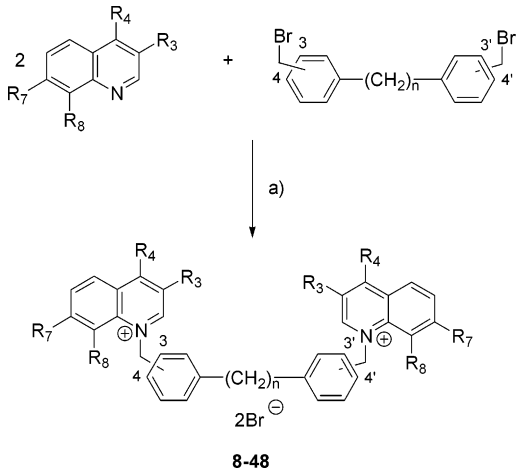
For the quinolines belonging to the third series, 4-chloro-7-nitro-8-methylquinoline[25] was treated with ammonia gas in phenol to produce **60** (with a 65% yield), with *N*-methylaniline in glacial acetic acid at reflux to give **62** (with a 60% yield), and with 4-chloro-*N*-methylaniline in glacial acetic acid at reflux to provide **64** (with a 50% yield). Reduction of nitro compounds **60**, **62**, and **64** with $SnCl_2$ in glacial acetic acid rendered **61** (75%), **63** (75%), and **65** (70%), respectively.

Finally, the starting material for the synthesis of 4-amino-3-methylquinoline (**68**) was 3-methylquinoline (Scheme 1). The sequence of reactions was the following: (a) oxidation with peracetic acid in glacial acetic acid under reflux to yield **66** (53%); (b) nitration at position 4 of the heterocyclic structure to yield **67** (70%); and (c) reduction of both the nitro and the *N*-oxide groups to produce **68** (82%).

Three different types of linkers have been used. The 3,3′-bis(bromomethyl)biphenyl linker was obtained through a radical benzylic halogenation of the commercially available 3,3′-dimethylbiphenyl with *N*-bromosuccinimide (NBS) and benzoyl peroxide as initiator.[26] The syntheses of 4,4′-bis(bromomethyl)biphenyl[27,28] and 4,4′-bis(bromomethyl)bibenzyl[29] were carried out by reaction between biphenyl (or bibenzyl), formaldehyde (or its polymers), and hydrogen bromide in the presence of *o*-phosphoric acid.

The synthesis of the acyclic final compounds was carried out by heating the corresponding bromide and heterocyclic derivatives (in a molar ratio 1:2) using butanone as solvent (Scheme 2). The reaction was

Scheme 2[a]



[a] Reagents and conditions: (a) Butanone, sealed tube, 100 °C.

carried out in a sealed tube and at a temperature of 100 °C. Compounds **8**, **15**, and **22** have been previously described by one of us.[30]

Biological Testing. Compounds **8**−**48** were tested in an ex vivo system using human ChoK as a target. This assay allowed us to evaluate the affinity of the compounds for ChoK, without considering the possible passage through biological membranes. The effects on cell proliferation by the ChoK inhibitors in *ras*-transformed cells were next investigated on the HT-29 cell line (in vitro assay for 6 days). This cell line was established from a colon adenocarcinoma, one of the most frequent solid human cancers that is resistant to chemotherapy,[31] making these cells appropriate for the search of new antitumor drugs. IC_{50} values were obtained from nonlinear least-squares fit of the Hill equation to the data. The activity in the in vitro assay reflected the pharmacodynamic properties of the compounds rather than their affinity for the enzyme.

Results and Discussion

Structure−Activity Relationships (SAR). Biological results of compounds **8**−**48** are shown in Tables 1, 2, and 3, corresponding to series **A**, **B** and **C**, as a function of group located at position 7 of the quinolinium ring. Each series has been divided into three subseries depending on the linker nature.

7-Unsubstituted Bisquinolium Derivatives (series A). Biological results of compounds **8**−**27** (series **A**) are shown in Table 1. From the biological results obtained in the subfamily with the 3,3′-biphenyl linker, it can be deduced that a good correlation generally exists between ChoK inhibition and antiproliferative activity. Compound **11** shows an excellent IC_{50} against ChoK inhibition, and it suggests that the introduction of cycloalkylamino groups favors the inhibitory activity. Moreover, its antiproliferative activity is equivalent to the ChoK inhibition. When the substituent R_4 is an anilino group, it is observed that the three derivatives **12**, **13**, and **14** are potent ChoK inhibitors, the *N*-methylanilino group being better than anilino and 4-chloro-*N*-methylanilino. This might indicate that the *N*-methylanilino group is the most appropriate substituent in this type of biscationic structures. If this series is analyzed as a group it is observed that the

Table 1. IC_{50} ChoK and HT-29 Values for the Bisquinolinium Compounds (series **A**)

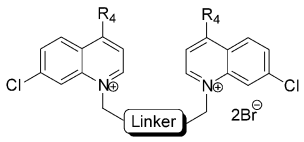
Comp.	Linker	R_3	R_4	IC_{50} ChoK (μM)	IC_{50} HT-29 (μM)
9		H	amino	1.20	1.90
10		Me	amino	11.9	4.40
11		H	dimethylamino	4.40	1.60
12		H	perhydroazepino	0.50	0.50
13		H	anilino	1.30	1.60
14		H	*N*-methylanilino	0.40	0.80
15		H	4CNMA[a]	2.10	1.50
16		H	amino	81.1	2.20
17		Me	amino	> 200	3.30
18		H	dimethylamino	39.7	1.70
19		H	perhydroazepino	2.20	0.50
20		H	anilino	17.8	0.70
21		H	*N*-methylanilino	3.00	0.60
22		H	4CNMA[a]	2.00	1.20
23		H	amino	80.0	2.00
24		H	dimethylamino	10.2	0.50
25	$(CH_2)_2$	H	perhydroazepino	0.60	0.30
26		H	anilino	2.30	0.30
27		H	*N*-methylanilino	1.40	0.70
28		H	4CNMA[a]	4.80	0.70

[a] 4CNMA = 4-chloro-*N*-methylanilino.

linker type has an influence on the ChoK inhibition, the 3,3′-biphenyl being the most suitable one. The IC_{50} values against ChoK of 4,4'-bibenzyl derivatives are slightly poorer, and a clear decrease of such an activity is observed with the 4,4'-biphenyl spacer. This fact reaffirms previous results related to the influence of the linker on the ChoK activity.[16] Thus, the bibenzyl spacer allows the compounds to adopt the appropriate conformation on binding to the enzyme, due to the higher flexibility caused by the ethylene bridge between the two phenyl rings. On the other hand, the antiproliferative capacity against the human colon cell line HT-29 is less influenced by both the linker spacer and the R_4 substituent, the obtained values being similar for the three subseries. This fact leads us to think that these structures could act, apart from on ChoK, at another level in the cellular signaling triggered by the activation of *ras* oncogene. It can be deduced that substituents R_4 that give rise to higher ChoK inhibitory and antiproliferative activities are cycloalkylamino and phenylamino groups.

On the other hand, if each subfamily is analyzed individually, on comparing the bis(4-aminoquinolinium) derivatives (**8** and **15**) with the analogous derivatives with a methyl group at position 3 (**9** and **16**, respectively), both the ChoK inhibitory and the antiproliferative activities decrease (Table 1). This may be due to a steric hindrance caused by the methyl group that might

Table 2. IC_{50} ChoK and HT-29 Values for the Bisquinolinium Compounds (series **B**)



Comp.	Linker	R_4	IC_{50} ChoK (μM)	IC_{50} HT-29 (μM)
29		amino	20.6	1.90
30		dimethylamino	9.60	0.70
31		pyrrolidino	1.20	0.40
32		*N*-methylanilino	3.10	1.00
33		4CNMA[a]	5.70	1.90
34		amino	63.3	3.20
35		dimethylamino	20.6	0.80
36		pyrrolidino	19.8	2.40
37		*N*-methylanilino	11.4	0.50
38		4CNMA[a]	11.4	1.20
39		amino	> 200	1.90
40		dimethylamino	9.00	0.27
41	$(CH_2)_2$	pyrrolidino	1.00	0.20
42		*N*-methylanilino	3.50	0.50
43		4CNMA[a]	5.70	0.80

[a] 4CNMA = 4-chloro-*N*-methylanilino.

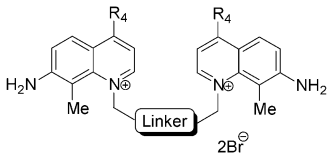
interfere with the interaction between the compound and the enzyme.

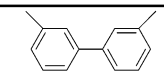
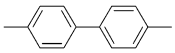
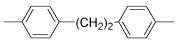
7-Chloro-Substituted Bisquinolium Derivatives (series B). Table 2 shows the biological results for compounds **28**−**42** (series **B**). The synthesis of series **B** of this bis(7-chloroquinolinium) compounds was carried out to analyze the influence that an electron-withdrawing group has on the ChoK inhibitory activity. If the human ChoK inhibitory activities of series **B** is compared with the same values for the 7-unsubstituted series (series **A**), it is deduced that such an inhibition is influenced by the chlorine atom. Thus, the inhibitory activity always decreases regardless of the linker nature. Nevertheless, the antiproliferative activity is barely affected by the introduction of the chlorine atom.

The bis(7-chloroquinolinium) compounds with the 3,3′-biphenyl spacer are more active than compounds with the 4,4′-biphenyl spacer, as happened with the bis(7-unsubstituted quinolinium) salts. It must be pointed out that cycloalkylamino or arylamino substituents are markedly better enzymatic inhibitors than their amino or dimethylamino derivatives. Within series **B** the most active antiproliferative agent against HT-29 so far described, **40**, is found with an IC_{50} in the in vitro assay = 0.20 μM.

Influence of the Introduction of an Amino Group at Position 7 of the Quinolinium Ring (series C). The biological results of compounds **43**−**48** (series **C**) are shown in Table 3. In the following phase of this study, the biscationic structures with an amino group at position 7 of the quinolinium ring were assayed. Moreover, in such compounds a methyl group was introduced at position 8 to simplify the synthesis. The

Table 3. IC_{50} ChoK and HT-29 Values for the Bisquinolinium Compounds (series **C**)



Comp.	Linker	R_4	IC_{50} ChoK (μM)	IC_{50} HT-29 (μM)
44		*N*-methylanilino	56.8	3.80
45		4CNMA[a]	147	33.0
46		*N*-methylanilino	96.1	25.2
47		4CNMA[a]	46.1	19.4
48		*N*-methylanilino	133	7.00
49		4CNMA[a]	57.5	19.5

[a] 4CNMA = 4-chloro-*N*-methylanilino.

ChoK inhibitory activity of these bis(7-amino-8-methylquinolinium) compounds (series **C**) was clearly lower than the corresponding series **A** and **B**. **46** and **48** were the only compounds, whose substituent at position 4 is the 4-chloro-*N*-methylanilino group, that showed notable IC_{50} ex vivo values (although such activities were not outstanding compared with the values obtained for the previously mentioned bisquinolinium derivatives). Interestingly, despite showing a marginal ChoK inhibitory activity, compounds of series **C** showed antiproliferative activities against HT-29, which reinforced the above-mentioned hypothesis that these types of structures could act in another point of the transduction process (it must be remembered that the mechanism by which *P*Cho transmits the mitogenic signal to the nucleus is still unknown). The starting hypothesis supposed that the introduction of an electron-releasing group at position 7 should increase the ChoK inhibitory activity in these biscationic type structures. On the contrary, the enzymatic inhibitory potency was drastically diminished. It is probable that the methyl group at position 8, introduced to simplify the synthetic route, caused a steric hindrance in its interaction with the enzyme that would explain the decrease of ChoK activity. Therefore, it was not possible to assess the influence of the amino group at position 7 of the quinolinium ring with the results obtained. The future synthesis and biological tests of their analogous compounds without the methyl group in such a position will confirm this hypothesis.

QSAR of the Antiproliferative Activity against the HT-29 Cell Line. We have tried to correlate ChoK inhibitory activity for the whole set of compounds (series **A**, **B**, and **C**) with the electronic and lipophilic parameters, but all the attempts turned out to be fruitless. In general it can be deduced (see Tables 1, 2, and 3) that the activity against the HT-29 cell line is greater than the corresponding activity against ChoK, with which the symmetrical bisquaternized salts could act on another point of the pathway triggered by *ras* activation. Table 4 (see Supporting Information) shows all the ChoK inhibitors arranged according to decreasing antiproliferative activity, where $p(IC_{50})_{HT-29} = -\log(IC_{50})_{HT-29}$, bearing in mind that the higher the value of $p(IC_{50})_{HT-29}$ the more potent is the compound, together with the descriptors necessary for the establishment of the corresponding QSAR equation. The octanol−water partition coefficient, used in its logarithmic form (log *P*), is the most widely accepted measure of lipophilicity. Reproducibility and accuracy of experimental log *P* determinations are not exact for extremely lipophilic and/or hydrophilic compounds such as the bisquinolinium structures **8**−**48**. Fragmental methods make it possible to create data banks and to perform log *P* calculations by computer.

The clog *P* values of the bis-salts were calculated by using the Ghose−Crippen modified atomic contribution system[32] (ATOMIC5 option) of the PALLAS 2.0 program.[33] π_{spacer} is the substituent constant for the linker calculated by using the Ghose−Crippen modified atomic contribution system[32] (ATOMIC5 option) of the PALLAS 2.0 program.[33] One of the most important chemicophysical properties used in QSAR studies is the molar refractivity (MR). It has been shown to be related to lipophilicity, molar volume, and steric bulk.[34] Most often the MR values are scaled by a factor of 0.1 to achieve reasonable values of the regression coefficients of the resulting QSAR equations. The significance of MR in QSAR equations of some ligand−enzyme interactions has being interpreted with the help of 3D structures. These investigations showed that substituents modeled by MR bind in polar areas, while substituents modeled by π bind in a hydrophobic space.[35,36] Correspondingly, a positive sign of MR in a QSAR equation can be explained by binding the substituents to a polar surface, while a negative sign or a nonlinear relationship indicates a limited area or steric hindrance at this binding site.[34] The Hammett-type σ_R values for the amino, dimethylamino, and anilino groups were taken from the Hansch and Leo tables,[37] while we have previously published[38] such values for the pyrrolidino, perhydroazepino, and *N*-methylanilino groups. The same methodology[38] was used to estimate the unknown σ_R value for the 4-chloro-*N*-methylanilino group by using the ^{13}C chemical shifts of the previously reported compounds 1,1′-(biphenyl-3,3′-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)pyridinium] dibromide and 1,1′-(biphenyl-4,4′-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)pyridinium] dibromide.[16]

When the volume effects (MR_8) (the subscript refers to the position of the substituent), calculated global lipophilicity (clog *P*), the substituent constant of the linker, and the electronic parameters (σ_{R4}) of the R_4 substituent were taken into account for the antiproliferative activity, eq 1 was obtained:

$$p(IC_{50})_{HT-29} = -2.66 - 0.03\ (\pm 0.00)\ MR_8^2 + 0.10\ (\pm 0.02)\ \text{clog}\ P + 1.05\ (\pm 0.31)\ \pi_{linker} - 3.73\ (\pm 0.71)\ \sigma_R \quad (1)$$

$$n = 40,\ r = 0.920,\ s = 0.223,\ F_{4,35} = 47.856,\ \alpha < 0.001$$

where *n* is the number of compounds, *r* is the correlation coefficient, and *s* is the standard deviation between estimated and actual antiproliferative values. The Fisher test is highly significant here ($\alpha < 0.001$). The number in parentheses accounts for the standard error of the regression coefficients.

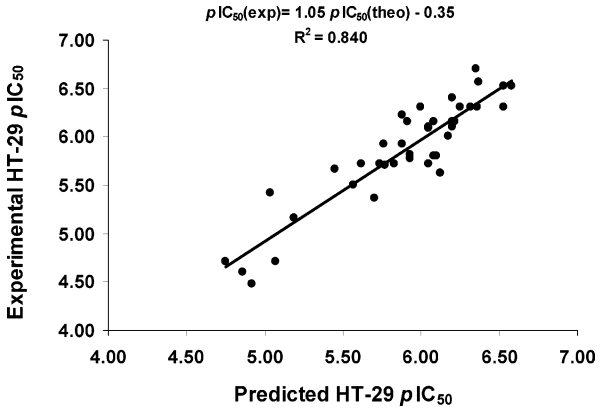


Figure 3. Graph between experimental and predicted antiproliferative activities for the test set.

In deriving eq 1, **16** was not included because this compound is not a ChoK inhibitor (see Table 1). Equation 1 gives a good cross-validated r^2_{CV} value (q^2) of 0.837. A most significant aspect of this study is that every data point was included in the formulation of eq 1. Such an outcome is rarely found and merits a special consideration. We find this to be quite unusual since one usually finds some outliers in QSAR works which must be omitted to obtain a high correlation.

From eq 1 the following aspects must be highlighted: (i) the coefficient of MR_8 is a squared negative term, and hence the presence of the methyl group at position 8 is detrimental in relation to the antiproliferative activity; accordingly, it is advisable that a hydrogen atom be in this position because this atom has a smaller value for MR; (ii) there is no electronic contribution of the group located at position 7 of the quinolinium ring. This is most remarkable in the case of the 7-NH_2 group, which is located at the same relative position (although in the other aromatic ring) as the 4-substituent in relation to the N^+ atom, and its presence should have facilitated even more the delocalization of the positive charge of the endocyclic N atom. A plausible explanation is that the 8-methyl group exerts a steric hindrance making it impossible for the 7-NH_2 group to adopt a coplanar disposition in relation to the aromatic ring. This is necessary for the electronic delocalization to take place. Therefore, 7−substituent contributes only to the global lipophilicity of the molecules; (iii) finally, lipophilicity contributes in two aspects to the antiproliferative activity: on one hand, a global contribution (clog *P*) and on the other, a contribution at a specific site on the molecules (π_{linker}). Both descriptors are orthogonal, and therefore the participation of both is justified in the QSAR equation. The relative contribution of π_{linker} in eq 1 is higher than that of log *P,* and it can be hypothesized that favorable hydrophobic interactions between the enzyme and the linker would modulate the coupling inhibitor-ChoK. Although, according to eq 1 the increase in the global lipophilicity and in the lipophilicity of the linker would augment the antiproliferative activity, the solubility was the reason for limiting the spacers to the 3,3′-, 4,4′-biphenyl, and 4,4′-bibenzyl moieties.

When the experimental $p(IC_{50})_{HT-29}$ values are correlated with the theoretical ones (see Table 4) calculated by eq 1, eq 2 is obtained corresponding to the straight line represented in Figure 3:

$$p(IC_{50})_{HT-29\ exptl} = -0.35 + 1.05\ (\pm 0.08)\ p(IC_{50})_{HT-29\ theoret} \quad (2)$$

$$n = 40,\ r = 0.916,\ s = 0.220,\ F_{1,38} = 198.854,\ \alpha < 0.001$$

Conclusions

From the biological assays obtained under ex vivo conditions on human ChoK from all the final compounds, it can be deduced that an increase of lipophilicity generated by the introduction of two quinolinium rings as cationic heads (in relation to the two pyridinium rings[16]) improves the antiproliferative activity. On the other hand, the ChoK inhibition is influenced by the introduction of a chlorine atom at position 7 of the quinolinium rings. Thus, the inhibitory activity against the enzyme always decreases regardless of the nature of the linker. Nevertheless, the antiproliferative activity is scarcely affected by the introduction of a chlorine atom, which is logical because many other factors have an influence on the HT-29 activity. The bis(7-aminoquinolinium) compounds show antiproliferative activities despite showing a marginal ChoK inhibitory activity. This strongly supports the hypothesis that these types of structures might act either in another point of the signaling pathway triggered after the activation of the *ras* oncogene or in another pathway involved in the cellular proliferation. Finally, the ChoK inhibition is influenced by the type of arylalkyl spacer of compounds. Thus, the best spacer is the 3,3′-biphenyl. Moreover, the positive influence of the introduction of electron-releasing substituents at position 4 in the quinolinium ring is confirmed, and the introduction of a methyl group at position 3 of the quinolinium ring gives rise to a decrease in the ChoK inhibitory activity.

Experimental Section

(a) Chemistry. For general procedures see ref 14. All compounds were dried at 40 °C and 0.1 mmHg for 24 h, but many which appear to be solvates held tenaciously to water. **49**,[18] **50**,[19] **53**,[20] **55**,[22] 4-chloro-7-nitro-8-methylquinoline,[25] 3,3′-Bis(bromomethyl)biphenyl,[26] 4,4′-bis(bromomethyl)biphenyl,[27,28] and 4,4′-bis(bromomethyl)bibenzyl[29] were synthesized according to literature procedures. In the NMR data, the abbreviation pst means a pseutotriplet.

General Experimental Procedure for the Preparation of Bisquinolinium Compounds. A solution of the linker [bis(bromomethyl) compound][26−29] and the corresponding (4-substituted)quinoline (in a 1/2 molar ratio) was heated at 100 °C in a sealed tube for a period of time that went from 15 and 192 h. After filtration and thorough washing with butanone, ethyl acetate, and diethyl ether, the solid was purified by recrystallization from EtOH or MeOH after adding diethyl ether to turbidity.

1,1′-(Biphenyl-3,3′-diylmethylene)bis(4-amino-3-methylquinolinium) dibromide (9): Yield: 77%. Mp 291−292 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.23 (bs, 2H); 8.93 (s, 2H); 8.60 (d, J = 8.4, 2H); 8.46 (bs, 2H); 8.04 (d, J = 8.4, 2H); 7.89 (pst, J = 7.1, 2H); 7.74 (s, 2H); 7.68 (pst, J = 7.5, 2H); 7.56 (d, J = 7.7, 2H); 7.41 (t, J = 7.7, 2H); 7.12 (d, J = 7.7, 2H); 5.88 (s, 4H); 2.31 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{34}H_{32}N_4Br_2$ (M − HBr − Br)$^+$ 495.2549; found 495.2549. Anal. ($C_{34}H_{32}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis(4-dimethylaminoquinolinium) dibromide (10): Yield: 60%. Mp 278−279 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (d, J = 7.6, 2H); 8.41 (d, J = 8.6, 2H); 8.10 (d, J = 8.7, 2H); 7.91 (pst, J = 8.1, 2H); 7.80 (s, 2H); 7.63 (pst, J = 8.1, 2H); 7.58 (d, J = 7.7, 2H); 7.43

(t, J = 7.7, 2H); 7.17 (d, J = 7.7, 2H); 7.12 (d, J = 7.6, 2H); 5.93 (s, 4H); 3.49 (s, 12H). HR LSIMS (*m/z*) calcd for $C_{36}H_{36}N_4Br_2$ $(M - Br)^+$ 603.2123; found 603.2123. Anal. ($C_{36}H_{36}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(perhydroazepino)quinolinium] dibromide (11): Yield: 84%. Mp 293−294 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (d, J = 7.7, 2H); 8.34 (d, J = 8.2, 2H); 8.05 (d, J = 8.5, 2H); 7.89 (pst, J = 7.8, 2H); 7.76 (s, 2H); 7.61 (pst, J = 7.5, 2H); 7.57 (d, J = 7.7, 2H); 7.43 (t, J = 7.7, 2H); 7.15 (m, 4H); 5.90 (s, 4H); 4.00 (t, 8H); 1.94 (bs, 8H); 1.58 (bs, 8H). HR LSIMS (*m/z*) calcd for $C_{44}H_{48}N_4Br_2$ $(M - Br)^+$ 711.3062; found 711.3062. Anal. ($C_{44}H_{48}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis(4-anilinoquinolinium) dibromide (12): Yield: 65%. Mp 316−318 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 2H); 8.95 (d, J = 7.4, 2H); 8.84 (d, J = 8.5, 2H); 8.20 (d, J = 8.9, 2H); 8.02 (pst, J = 7.8, 2H); 7.82 (pst, J = 7.7, 2H); 7.76 (s, 2H); 7.62-7.53 (m, 8H); 7.48−7.41 (m, 6H); 7.21 (d, J = 7.7, 2H); 6.87 (d, J = 7.4, 2H); 5.99 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{44}H_{36}N_4Br_2$ $(M - HBr - Br)^+$ 619.2862; found 619.2861. Anal. ($C_{44}H_{36}N_4Br_2$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(*N*-methylanilino)quinolinium] dibromide (13): Yield: 64%. Mp 274−275 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.25 (d, J = 7.4, 2H); 8.17 (d, J = 8.9, 2H); 7.85 (s, 2H); 7.81 (pst, J = 8.0, 2H); 7.63 (d, J = 8.3, 2H); 7.52−7.39 (m, 16H); 7.32 (pst, J = 7.7, 2H); 7.23 (d, J = 7.4, 2H); 6.09 (s, 4H); 3.76 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{40}N_4Br_2$ $(M - Br)^+$ 727.2436; found 727.2436. Anal. ($C_{46}H_{40}N_4Br_2 \cdot 3H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)quinolinium] dibromide (14): Yield: 45%. Mp 217−218 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.24 (d, J = 7.4, 2H); 8.18 (d, J = 8.9, 2H); 7.84 (s, 2H); 7.63 (d, J = 7.5, 2H); 7.56−7.43 (m, 18H); 7.23 (d, J = 7.4, 2H); 6.08 (s, 4H); 3.74(s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{38}N_4Cl_2Br_2$ $(M - Br)^+$ 795.1657; found 795.1656. Anal. ($C_{46}H_{38}N_4Cl_2Br_2 \cdot 3H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis(4-amino-3-methylquinolinium) dibromide (16): Yield: 64%. Mp 299−301 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.31 (bs, 2H); 8.93 (s, 2H); 8.56 (d, J = 8.5, 2H); 8.47 (bs, 2H); 8.00 (d, J = 8.2, 2H); 7.90 (m, 8H); 7.89 (pst, J = 8.2, 2H); 7.68 (pst, J = 8.5, 2H); 5.87 (s, 4H); 2.25 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{34}H_{32}N_4Br_2$ $(M - HBr - Br)^+$ 495.2549; found 495.2549. Anal. ($C_{34}H_{32}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(dimethylamino)quinolinium] dibromide (17): Yield: 61%. Mp 224−225 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.86 (d, J = 7.6, 2H); 8.41 (d, J = 8.6, 2H); 8.03 (d, J = 8.9, 2H); 7.90 (pst, J = 8.1, 2H); 7.63 (pst, J = 8.1, 2H); 7.61 (d, J = 8.3, 4H); 7.34 (d, J = 8.3, 4H); 7.12 (d, J = 7.6, 2H); 5.90 (s, 4H); 3.48 (s, 12H). HR LSIMS (*m/z*) calcd for $C_{36}H_{36}N_4Br_2$ $(M - Br)^+$ 603.2123; found 603.2123. Anal. ($C_{36}H_{36}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(perhydroazepino)quinolinium] dibromide (18): Yield: 82%. Mp 273−274 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.78 (d, J = 7.7, 2H); 8.34 (d, J = 7.9, 2H); 8.00 (d, J = 8.5, 2H); 7.88 (pst, J = 7.8, 2H); 7.62 (d, J = 8.4, 4H); 7.60 (pst, J = 7.5, 2H); 7.33 (d, J = 8.4, 4H); 7.14 (d, J = 7.7, 2H); 5.87 (s, 4H); 4.01 (t, 8H); 1.94 (bs, 8H); 1.59 (bs, 8H). HR LSIMS (*m/z*) calcd for $C_{44}H_{48}N_4Br_2$ $(M - Br)^+$ 711.3062; found 711.3060. Anal. ($C_{44}H_{48}N_4Br_2 \cdot 0.5H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(anilino)quinolinium] dibromide (19): Yield: 66%. Mp 326−327 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.10 (s, 2H); 8.88 (d, J = 7.4, 2H); 8.83 (d, J = 8.5, 2H); 8.14 (d, J = 8.9, 2H); 8.02 (pst, J = 7.8, 2H); 7.82 (pst, J = 7.7, 2H); 7.62 (d, J = 8.1, 4H); 7.61 (t, J = 7.8, 4H); 7.53 (d, J = 7.8, 4H); 7.47 (t, J = 7.3, 4H); 7.35 (d, J = 8.1, 4H); 6.90 (d, J = 7.4, 2H); 5.97 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{44}H_{36}N_4Br_2$ $(M - HBr - Br)^+$ 619.2862; found 619.2862. Anal. ($C_{44}H_{36}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(*N*-methylanilino)quinolinium] dibromide (20): Yield: 58%. Mp 276−278 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.15 (d, J = 7.4, 2H); 8.09 (d, J = 8.9, 2H); 7.80 (pst, J = 8.3, 2H); 7.66 (d, J = 8.3, 2H); 7.52−7.38 (m, 20H); 7.32 (t, J = 7.6, 2H); 6.03 (s, 4H); 3.75 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{40}N_4Br_2$ $(M - HBr - Br)^+$ 647.3175; found 647.3177. Anal. ($C_{46}H_{40}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[4-(4-chloro-*N*-methylanilino)quinolinium] dibromide (21): Yield: 30%. Mp 255−257 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.19 (d, J = 7.4, 2H); 8.12 (d, J = 8.9, 2H); 7.83 (pst, J = 7.5, 2H); 7.66 (d, J = 8.2, 2H); 7.55 (d, J = 8.8, 4H); 7.44 (d, J = 8.9, 4H); 7.56−7.39 (m, 12H); 6.05 (s, 4H); 3.73 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{38}N_4Cl_2Br_2$ $(M - Br)^+$ 795.1657; found 795.1658. Anal. ($C_{46}H_{38}N_4Cl_2Br_2 \cdot 2H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(dimethylamino)quinolinium] dibromide (23): Yield: 93%. Mp 255−257 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.82 (d, J = 7.6, 2H); 8.40 (d, J = 8.6, 2H); 7.99 (d, J = 8.7, 2H); 7.88 (pst, J = 8.1, 2H); 7.62 (pst, J = 7.9, 2H); 7.20 (d, J = 8.3, 4H); 7.17 (d, J = 8.3, 4H); 7.10 (d, J = 7.6, 2H); 5.81 (s, 4H); 3.48 (s, 12H); 2.76 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{38}H_{40}N_4Br_2$ $(M - Br)^+$ 631.2436; found 631.2437. Anal. ($C_{38}H_{40}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(perhydroazepino)quinolinium] dibromide (24): Yield: 76%. Mp 292−293 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, J = 7.7, 2H); 8.33 (d, J = 8.2, 2H); 7.96 (d, J = 8.5, 2H); 7.87 (pst, J = 7.8, 2H); 7.60 (pst, J = 7.5, 2H); 7.20 (d, J = 8.5, 4H); 7.17 (d, J = 8.5, 4H); 7.13 (d, J = 7.7, 2H); 5.79 (s, 4H); 3.99 (t, 8H); 2.77 (s, 4H); 1.94 (bs, 8H); 1.58 (bs, 8H). HR LSIMS (*m/z*) calcd for $C_{46}H_{52}N_4Br_2$ $(M - Br)^+$ 739.3375; found 739.3375. Anal. ($C_{46}H_{52}N_4Br_2 \cdot 1H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(anilino)quinolinium] dibromide (25): Yield: 77%. Mp 284−286 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 2H); 8.85 (d, J = 7.4, 2H); 8.83 (d, J = 8.5, 2H); 8.01 (d, J = 8.9, 2H); 8.00 (pst, J = 7.8, 2H); 7.82 (pst, J = 7.7, 2H); 7.60 (t, J = 7.6, 4H); 7.53 (d, J = 7.6, 4H); 7.46 (t, J = 7.6, 4H); 7.19 (s, 8H); 6.88 (d, J = 7.4, 2H); 5.88 (s, 4H); 2.77 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{46}H_{40}N_4Br_2$ $(M - HBr - Br)^+$ 647.3174; found 647.3176. Anal. ($C_{46}H_{40}N_4Br_2 \cdot 1.9H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(*N*-methylanilino)quinolinium] dibromide (26): Yield: 64%. Mp 279−280 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.14 (d, J = 7.4, 2H); 8.06 (d, J = 8.9, 2H); 7.79 (pst, J = 8.3, 2H); 7.52−7.39 (m, 14H); 7.31 (t, J = 7.6, 2H); 7.24 (s, 8H); 5.96 (s, 4H); 3.74 (s, 6H); 2.80 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{48}H_{44}N_4Br_2$ $(M - Br)^+$ 755.2749; found 755.2749. Anal. ($C_{48}H_{44}N_4Br_2 \cdot H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[4-(4-chloro-*N*-methylanilino)quinolinium] dibromide (27): Yield: 20%. Mp 212−214 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.19 (d, J = 7.4, 2H); 8.10 (d, J = 8.9, 2H); 7.82 (pst, J = 7.5, 2H); 7.54 (d, J = 8.8, 4H); 7.44 (d, J = 8.9, 4H); 7.52−7.39 (m, 6H); 7.24 (s, 8H); 5.98 (s, 4H); 3.73 (s, 6H); 2.80 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{48}H_{42}N_4Cl_2Br_2$ $(M - Br)^+$ 823.1970; found 823.1972. Anal. ($C_{48}H_{42}N_4Cl_2Br_2 \cdot H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis(4-amino-7-chloroquinolinium) dibromide (28): Yield: 91%. Mp 277−278 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 and 9.31 (bs , 4H); 8.76 (d, J = 7.3, 2H); 8.54 (d, J = 9.0, 2H); 8.17 (d, J = 1.5, 2H); 7.81 (dd, J = 9.0, 1.5, 2H); 7.68 (s, 2H); 7.59 (d, J = 7.7, 2H); 7.45 (t, J = 7.7, 2H); 7.18 (d, J = 7.7, 2H); 6.93 (d, J = 7.3, 2H); 5.89 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{32}H_{26}N_4Br_2Cl_2$ $(M - HBr - Br)^+$ 535.1456; found 535.1457. Anal. ($C_{32}H_{26}N_4Br_2Cl_2 \cdot 0.2H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[7-chloro-4-(dimethylamino)quinolinium] dibromide (29): Yield: 45%. Mp 285−286 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.85 (d, J = 7.7, 2H); 8.42 (d, J = 9.2, 2H); 8.18 (d, J = 2.0, 2H); 7.78 (s, 2H); 7.65 (dd, J = 9.2, 2.0, 2H); 7.59 (d, J = 7.7, 2H); 7.46 (t, J = 7.7, 2H); 7.18 (d, J = 7.7, 2H); 7.13 (d, J = 7.7, 2H); 5.93 (s, 4H); 3.49 (s, 12H). HR LSIMS (*m/z*) calcd for $C_{36}H_{34}N_4Br_2Cl_2$ $(M - HBr - Br)^+$ 591.2082; found 591.2081. Anal. ($C_{36}H_{34}N_4Br_2Cl_2 \cdot 0.7H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[7-chloro-4-(pyrrolidino)quinolinium] dibromide (30): Yield: 48%. Mp 299−301 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83 (d, J = 7.7, 2H); 8.58 (d, J = 9.3, 2H); 8.16 (d, J = 1.8, 2H); 7.78 (s, 2H); 7.65 (dd, J = 9.3 and 1.8, 2H); 7.60 (d, J = 7.7, 2H); 7.46 (t, J = 7.7, 2H); 7.18 (d, J = 7.7, 4H); 6.95 (d, J = 7.7, 2H); 5.93 (s, 4H); 4.16−3.77 (bd, 8H); 2.04 (bs, 8H). HR LSIMS (*m/z*) calcd for $C_{40}H_{38}N_4Br_2Cl_2$ (M − Br)$^+$ 723.1657; found 723.1657. Anal. ($C_{40}H_{38}N_4Br_2Cl_2\cdot H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[7-chloro-4-(*N*-methylanilino)quinolinium] dibromide (31): Yield: 40%. Mp 275−277 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.15 (d, J = 7.4, 2H); 8.09 (s, 2H); 7.80 (d, J = 8.3, 2H); 7.73 (s, 2H); 7.66 (d, J = 8.3, 2H); 7.52−7.32 (m, 16H); 7.24 (d, J = 7.7, 2H); 6.03 (s, 4H); 3.75 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{38}N_4Br_2Cl_2$ (M − Br)$^+$ 795.1657; found 795.1657. Anal. ($C_{46}H_{38}N_4Br_2Cl_2\cdot 3.9H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[7-chloro-4-(4-chloro-*N*-methylanilino)quinolinium] dibromide (32): Yield: 45%. Mp 220−221 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.19 (d, J = 7.5, 2H); 8.29 (d, J = 1.7, 2H); 7.85 (s, 2H); 7.64 (d, J = 7.2, 2H); 7.57−7.45 (m, 16H); 7.25 (d, J = 7.7, 2H); 6.08 (s, 4H); 3.73 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{36}N_4Cl_4Br_2$ (M − HBr − Br)$^+$ 783.1616; found 783.1616. Anal. ($C_{46}H_{36}N_4Cl_4Br_2\cdot 1.5H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis(4-amino-7-chloroquinolinium) dibromide (33): Yield: 70%. Mp 273−274 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.38 and 9.31 (bs , 4H); 8.73 (d, J = 7.3, 2H); 8.54 (d, J = 9.0, 2H); 8.14 (d, J = 1.8, 2H); 7.80 (dd, J = 9.0, 1.8, 2H); 7.64 (d, J = 8.3, 4H); 7.32 (d, J = 8.3, 4H); 6.92 (d, J = 7.3, 2H); 5.87 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{32}H_{26}N_4Br_2Cl_2$ (M − Br)$^+$ 615.0718; found 615.0718. Anal. ($C_{32}H_{26}N_4Br_2Cl_2\cdot 0.7H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[7-chloro-4-(dimethylamino)quinolinium] dibromide (34): Yield: 45%. Mp 225−226 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (d, J = 7.7, 2H); 8.42 (d, J = 9.2, 2H); 8.13 (d, J = 2.0, 2H); 7.69−7.62 (m, 6H); 7.34 (d, J = 8.3, 4H); 7.13 (d, J = 7.7, 2H); 5.90 (s, 4H); 3.49 (s, 12H). HR LSIMS (*m/z*) calcd for $C_{36}H_{34}N_4Br_2Cl_2$ (M − HBr − Br)$^+$ 591.2082; found 591.2082. Anal. ($C_{36}H_{34}N_4Br_2Cl_2\cdot 1.2H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[7-chloro-4-(pyrrolidino)quinolinium] dibromide (35): Yield: 47%. Mp 288−289 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, J = 7.7, 2H); 8.58 (d, J = 9.3, 2H); 8.10 (s, 2H); 7.65 (m, 6H); 7.33 (d, J = 8.2, 4H); 6.95 (d, J = 7.7, 2H); 5.89 (s, 4H); 4.16−3.77 (m, 8H); 2.04 (bs, 8H). HR LSIMS (*m/z*) calcd for $C_{40}H_{38}N_4Br_2Cl_2$ (M − HBr − Br)$^+$ 643.2395; found 643.2396. Anal. ($C_{40}H_{38}N_4Br_2Cl_2\cdot 1.5H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[7-chloro-4-(*N*-methylanilino)quinolinium] dibromide (36): Yield: 34%. Mp 284−286 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (d, J = 7.5, 2H); 8.21 (s, 2H); 7.70 (d, J = 8.3, 2H); 7.50 (t, J = 8.3 and 6.9, 2H); 7.46−7.40 (m, 20H); 6.06 (s, 4H); 3.75 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{38}N_4Br_2Cl_2$ (M − Br)$^+$ 795.1657; found 795.1657. Anal. ($C_{46}H_{38}N_4Br_2Cl_2\cdot 1.5H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[7-chloro-4-(4-chloro-*N*-methylanilino)-quinolinium] dibromide (37): Yield: 48%. Mp 276−277 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.14 (d, J = 7.4, 2H); 8.23 (d, J = 1.6, 2H); 7.73 (d, J = 8.3, 2H); 7.69 (d, J = 8.4, 4H); 7.56 (d, J = 8.8, 4H); 7.46 (d, J = 8.9, 4H); 7.50−7.46 (m, 6H); 7.41 (d, J = 8.4, 4H); 6.04 (s, 4H); 3.73(s, 6H). HR LSIMS (*m/z*) calcd for $C_{46}H_{36}N_4Cl_4Br_2$ (M − HBr − Br)$^+$ 783.1616; found 783.1614. Anal. ($C_{46}H_{36}N_4Cl_4Br_2$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis(4-amino-7-chloroquinolinium) dibromide (38): Yield: 47%. Mp 285−290 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.42 and 9.32 (bs , 4H); 8.72 (d, J = 7.3, 2H); 8.55 (d, J = 9.0, 2H); 8.07 (s, 2H); 7.76 (dd, J = 9.0, J = 1.1, 2H); 7.17 (d, J = 8.2, 4H); 7.14 (d, J = 8.2, 4H); 6.94 (d, J = 7.3, 2H); 5.79 (s, 4H); 2.77 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{34}H_{30}N_4Br_2Cl_2$ (M − HBr − Br)$^+$ 563.1769; found 563.1769. Anal. ($C_{34}H_{30}N_4Br_2Cl_2\cdot 1.5H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[7-chloro-4-(dimethylamino)quinolinium] dibromide (39): Yield: 38%. Mp 252−253 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (d, J = 7.7, 2H); 8.41 (d, J = 9.2, 2H); 8.07 (d, J = 2.0, 2H); 7.65 (dd, J = 9.0, J = 2.0, 2H); 7.20 (d, J = 8.3, 4H); 7.17 (d, J = 8.3, 4H); 7.11 (d, J = 7.7, 2H); 5.81 (s, 4H); 3.48 (s, 12H); 2.79 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{38}H_{38}N_4Br_2Cl_2$ (M − HBr − Br)$^+$ 619.2395; found 619.2393. Anal. ($C_{38}H_{38}N_4Br_2Cl_2\cdot 0.8H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[7-chloro-4-(pyrrolidino)quinolinium] dibromide (40): Yield: 63%. Mp 285−286 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (d, J = 7.7, 2H); 8.57 (d, J = 9.2, 2H); 8.03 (d, J =2.0, 2H); 7.64 (dd, J = 9.2, J = 2.0, 2H); 7.20 (d, J = 8.2, 4H); 7.15 (d, J = 8.2, 4H); 6.93 (d, J = 7.7, 2H); 5.78 (s, 4H); 4.16−3.76 (m, 8H); 2.78 (s, 4H); 2.04 (bs, 8H). HR LSIMS (*m/z*) calcd for $C_{42}H_{42}N_4Br_2Cl_2$ (M − HBr − Br)$^+$ 671.2708; found 671.2709. Anal. ($C_{42}H_{42}N_4Br_2Cl_2\cdot 0.5H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[7-chloro-4-(*N*-methylanilino)quinolinium] dibromide (41): Yield: 43%. Mp 240−242 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (d, J = 7.4, 2H); 8.14 (s, 2H); 7.51−7.38 (m, 16H); 7.23 (s, 8H); 5.95 (s, 4H); 3.73 (s, 6H); 2.81 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{48}H_{42}N_4Br_2Cl_2$ (M − Br)$^+$ 823.1970; found 823.1968. Anal. ($C_{48}H_{42}N_4Br_2Cl_2\cdot 4H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[7-chloro-4-(4-chloro-*N*-methylanilino)quinolinium] dibromide (42): Yield: 48%. Mp 256−257 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.11 (d, J = 7.4, 2H); 8.18 (d, J = 1.5, 2H); 7.55 (d, J = 8.8, 4H); 7.46 (d, J = 8.8, 4H); 7.56−7.44 (m, 6H); 7.24 (s, 8H); 5.97 (s, 4H); 3.72 (s, 6H); 2.82 (s, 4H). HR LSIMS (*m/z*) calcd for $C_{48}H_{40}N_4Cl_4Br_2$ (M − HBr − Br)$^+$ 811.1927; found 811.1926. Anal. ($C_{48}H_{40}N_4Cl_4Br_2\cdot 2H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[7-amino-8-methyl-4-(*N*-methylanilino)-quinolinium] dibromide (43): Yield: 25%. Mp 327−329 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.43 (d, J = 7.0, 2H); 7.57 (s, 2H); 7.46−7.32 (m, 12H); 7.29 (d, J = 7.8, 4H); 7.23 (d, J = 7.8, 2H); 7.09 (d, J = 9.7, 2H); 6.98 (d, J = 7.0, 2H); 6.74 (d, J = 9.7, 2H); 4.72 (s, 2H); 4.70 (s, 2H); 3.59 (s, 6H); 2.40 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{48}H_{46}N_6Br_2$ (M − HBr − Br)$^+$ 705.3706; found 705.3706. Anal. ($C_{48}H_{46}N_6Br_2\cdot 2.1H_2O$) C, H, N.

1,1′-(Biphenyl-3,3′-diylmethylene)bis[7-amino-4-(4-chloro-*N*-methylanilino)-8-methylquinolinium] dibromide (44): Yield: 55%. Mp 314−315 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.47 (d, J = 6.9, 2H); 7.58 (s, 2H); 7.40 (m, 6H); 7.39 (d, J = 8.7, 2H); 7.34 (t, J = 7.7, 2H); 7.27 (d, J = 7.7, 2H); 7.23 (d, J = 8.7, 2H); 7.13 (d, J = 9.7, 2H); 7.03 (d, J = 6.9, 2H); 6.82 (d, J = 9.7, 2H); 4.58 (s, 2H); 4.56 (s, 2H); 3.56 (s, 6H); 2.41 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{48}H_{44}N_6Cl_2Br_2$ (M − HBr − Br)$^+$ 773.2926; found 773.2916. Anal. ($C_{48}H_{44}N_6Cl_2Br_2\cdot H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[7-amino-8-methyl-4-(*N*-methylanilino)quinolinium] dibromide (45): Yield: 45%. Mp 311−313 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (bs, 2H); 7.47 (d, J = 8.2, 4H); 7.41−7.29 (m, 14H); 7.21 (d, J = 8.2, 4H); 7.07 (d, J = 9.4, 2H); 6.94 (d, J = 6.9, 2H); 6.64 (d, J = 9.4, 2H); 4.68 (s, 2H); 4.66 (s, 2H); 3.55 (s, 6H); 2.43 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{48}H_{46}N_6Br_2$ (M − HBr − Br)$^+$ 705.3706; found 705.3706. Anal. ($C_{48}H_{46}N_6Br_2\cdot 2.1H_2O$) C, H, N.

1,1′-(Biphenyl-4,4′-diylmethylene)bis[7-amino-4-(4-chloro-*N*-methylanilino)-8-methylquinolinium] dibromide (46): Yield: 42%. Mp 315−317 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.46 (d, J = 6.9, 2H); 7.51 (d, J = 8.1, 4H); 7.41 (m, 8H); 7.34 (d, J = 8.1, 4H); 7.23 (d, J = 8.7, 4H); 7.12 (d, J = 9.7, 2H); 7.02 (d, J = 6.9, 2H); 6.80 (d, J = 9.7, 2H); 4.56 (s, 2H); 4.54 (s, 2H); 3.56 (s, 6H); 2.41 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{48}H_{44}N_6Cl_2Br_2$ (M − HBr − Br)$^+$ 773.2926; found 773.2926. Anal. ($C_{48}H_{44}N_6Cl_2Br_2\cdot H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[7-amino-8-methyl-4-(*N*-methylanilino)quinolinium] dibromide (47): Yield: 37%. Mp 288−290 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.41 (d, J = 7.0, 2H); 7.44 (t, J = 7.8, 4H); 7.41

(m, 8H); 7.39 (t, J = 7.8, 2H); 7.26 (m, 8H); 7.18− 6.97 (m, 4H); 6.70 (d, J = 9.4, 2H); 4.62 (s, 2H); 4.59 (s, 2H); 3.61 (s, 6H); 2.79 (s, 4H); 2.27 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{50}H_{50}N_6Br_2$ (M − HBr − Br)$^+$ 733.4019; found 733.4019. Anal. ($C_{50}H_{50}N_6Br_2 \cdot 3.5H_2O$) C, H, N.

1,1′-[Ethylenebis(benzene-1,4-diylmethylene)]bis[7-amino-4-(4-chloro-*N*-methylanilino)-8-methylquinolinium] dibromide (48): Yield: 36%. Mp 299−300 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.45 (d, J = 7.0, 2H); 7.41 (d, J = 8.7, 4H); 7.23 (m, 8H); 7.17 (d, J = 8.2, 4H); 7.10 (d, J = 8,2, 4H); 7.09 (d, J = 9.8, 2H); 7.02 (d, J = 7.0, 2H); 6.75 (d, J = 9.7, 2H); 4.48 (s, 2H); 4.45 (s, 2H); 3.56 (s, 6H); 2.78 (s, 4H); 2.38 (s, 6H). HR LSIMS (*m/z*) calcd for $C_{50}H_{48}N_6Cl_2Br_2$ (M − HBr − Br)$^+$ 801.3240; found 801.3238. Anal. ($C_{50}H_{48}N_6Cl_2Br_2 \cdot H_2O$) C, H, N.

(b) Pharmacology. ChoK Inhibition and Cell Proliferation Assays. The ex vivo human ChoK inhibition and antiproliferative assays against HT-29 cells were followed in accordance with the protocols previously reported.[39,40] The results are recorded in Tables 1−3.

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Supporting Information Available: Table 4 and full experimental procedures and characterization data for quinolines **51**, **52**, **54**, **56**−**59**, and **62**−**68**. This material is available free of charge via Internet at http://pubs.acs.org.

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