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Inhibition of protein kinase C by dequalinium analogues: Structure–activity studies on head group variations☆

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Abstract—New dequalinium analogues and related heteroaromatic systems were synthesized and evaluated for inhibition of protein kinase Cα. In vitro assays with recombinant human PKCα showed that the number of the aromatic ring head groups as well as their electron-richness, are critical factors that determine potency. The inhibitory strengths of the synthesized compounds are shown to correlate well with Mulliken charges on the head group ring nitrogen atoms making it possible to design likely candidate molecules having improved protein kinase Cα inhibitory activity.

1. Introduction

The role of protein kinase C (PKC) in signaling pathways that govern the proliferation, differentiation, and metastasis of cells makes this enzyme an attractive target for the design of chemotherapeutic agents.[1–10] Substantial effort has been directed over the years to inhibit PKC.[11–13] Among the most potent leads, bis-quinolinium compounds have received much attention.[14–19] An initial discovery[15] in 1990 that dequalinium diiodide **1**, a bis-quinolinium compound, inhibits PKC activity suggested that studies of structurally similar salts would be useful. Besides PKC inhibition, these compounds also exhibit an array of biological effects such as anti-microbial[16,20] and fungicidal activity,[21,22] potent anti-tumor activity,[23–25] potent and selective blockade of small conductance Ca^{2+}-activated K^+ channels,[26–32] and anti-malarial effects.[33]

We earlier reported that the PKC inhibitory action of bis-quaternary dequalinium salts is considerably greater than that of the corresponding mono-quaternary salts **A** and **B**.[16] For example **1** has an IC_{50} = 7 μM in contrast to that of **A** (IC_{50} = 117 μM) (Table 1). The significantly large difference led to the proposal of a "two point" binding model in which optimal activity occurs when the two head groups have coincident contact with two target sites in the catalytic domain of PKCα.[16] In addition, the inhibitory activity was shown to increase with increasing linker length.[16] The incorporation of a single *trans* double bond in the center of the C_{10}-linker yielded an inhibitory activity almost equivalent to that of the C_{10} saturated analogue, while the *cis* analogue was significantly weaker as an inhibitor. This finding suggested that when bound at its target site in PKCα, the dequalinium ion is positioned in a *trans*-oid geometry.[16]

This paper describes our continuing efforts to elucidate structure–activity relationships (SARs) of dequalinium and dequalinium-type analogues with respect to in vitro inhibition of PKC. In this study, we have focused on the effects of structural variations in the head groups while keeping the linkage constant, namely $[(CH_2)_{10}]$, with the aim of gaining insights into the rational design of new PKCα inhibitors.

2. Chemistry

Compounds **1**–**5** were prepared by reaction of the appropriate quinoline derivative with 1,10-diiododecane in 2-butanone or 4-methyl 2-pentanol (Scheme 1). Similar procedures were used for the synthesis of

Keywords: PKC Inhibition; Head group role; Substituent effects; Quinolinium analogues.
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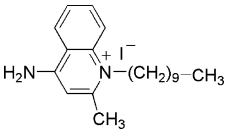
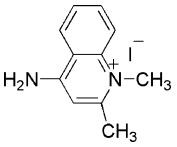
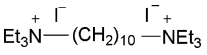
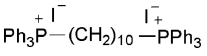
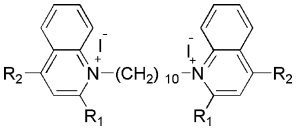
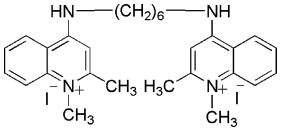
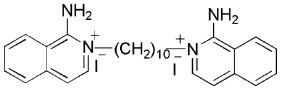
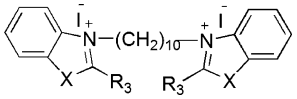
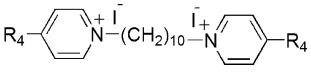
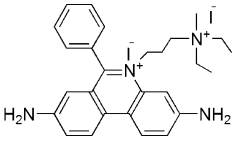
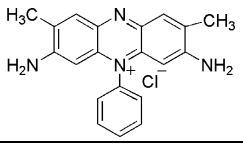
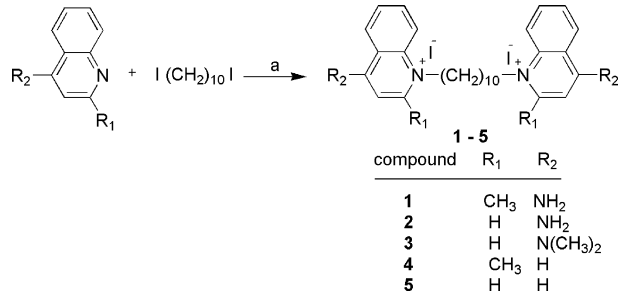
Table 1. IC_{50} values of compounds studied

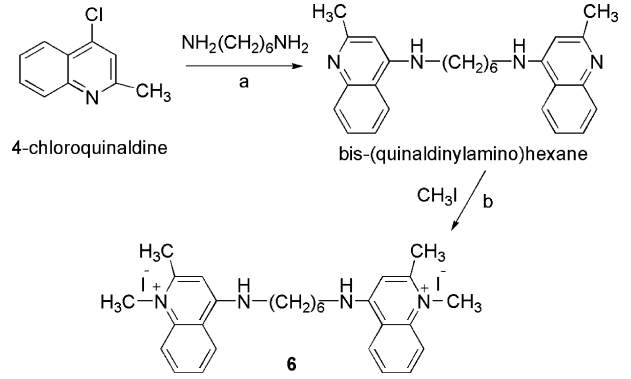
Compound	Structure	Substituents		IC_{50} (μM)
A[a]				117 ± 8
B[a]				3590 ± 510
C				>250
D				94 ± 16
		R_1	R_2	
1		CH_3	NH_2	7 ± 1
2		H	NH_2	30 ± 8
3		H	$N(CH_3)_2$	29 ± 12
4		CH_3	H	22 ± 2
5		H	H	72 ± 6
6				30 ± 11
7				30 ± 8
		X	R_3	
8		O	CH_3	36 ± 11
9		S	CH_3	17 ± 6
10		NCH_3	NH_2	14 ± 1
		R_4		
11		NH_2		112 ± 4
12		$N(CH_3)_2$		231 ± 26
13				16 ± 7
14				48 ± 4

The assay studies reported in above table were performed under reversible binding conditions as described in Section 6. IC_{50} represent the analogue concentrations needed for 50% inhibition, and are the average of two or more independent experiments, where PKC activity was analyzed in triplicate measurements.

[a] Previously reported.[16]



Scheme 1. Synthesis of dequalinium analogues. Reagents and conditions: (a) 2-butanone/4-methyl 2-pentanol, reflux, 48–72 h.



Scheme 2. Synthesis of exocyclic dequalinium analogue **6**. Reagents and conditions: (a) phenol, 180 °C; (b) acetone, reflux, 3d.

compounds **7**–**12**. Corresponding starting head groups for compounds **2** and **3** (4-aminoquinoline and 4-*N*,*N*-dimethylaminoquinoline) were synthesized by nucleophilic displacement of the chlorine atom from 4-chloroquinoline by ammonia and *N*,*N*-dimethylamine respectively, by following a modified literature procedure.[34] Scheme 2 shows the preparation of exocyclic dequalinium analogue **6** from 4-chloroquinaldine by reaction with 1,6 diaminohexane. This reaction produced bis(quinaldinylamino)hexane, which was then subjected to quaternization with methyl iodide to give **6**.

3. Biological activity

Recombinant human PKCα (95% pure) was used for testing the inhibitory action of dequalinium analogues **1**–**12**, and salts **13**, **14**, **C**, and **D** in vitro (Table 1). The total catalytic activity of PKCα was analyzed in triplicate at increasing concentrations of a selected dequalinium analogue. When compared to the level of activity measured in the absence of an analogue, the extent to which a given concentration of an analogue decreased the formation of phosphorylated peptide indicated the extent of PKCα inhibition. The error for these assays was typically less than 10%. IC_{50} values were calculated from dose–response curves as that concentration producing 50% inhibition. This analysis was carried out at least twice for each analogue and the IC_{50} values were averaged and are given in Table 1.

4. Results and discussion

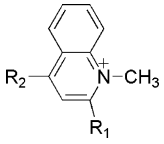
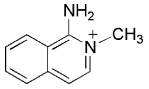
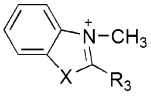
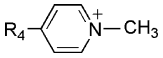
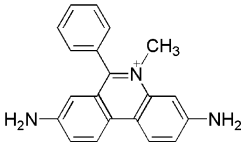
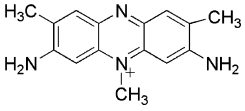
Most of the compounds analyzed in this study are structural analogues of the parent compound **1**. Compounds **A** and **B** are mono-quaternized quinoline derivatives and were included to provide reference benchmarks for PKC inhibition (Table 1).[16] For purposes of comparison, two aliphatic chain containing salts **C** and **D** lacking heteroaromatic head groups were also examined.

Our initial studies on the head groups were designed to explore the relative importance of the 2-methyl (R_1) and 4-amino (R_2) groups as contributors to inhibitory prowess in bis-quinolinium compounds. Dequalinium analogues **1**–**5** were synthesized in which these groups were replaced by hydrogen or other groups. The IC_{50} values of analogues **2** and **4** were both in the range 22–30 μM, indicating a weaker inhibition than that of **1**. For analogue **5**, in which both the 2-methyl and the 4-amino groups were replaced with H, a 10-fold lower potency than **1** was observed. These results suggest that both 2-methyl and 4-amino groups contribute to inhibitory activity.

The role of the 4-amino group was further investigated. In principle its contribution toward PKC inhibition could be related to its hydrogen bonding ability and/or to its ability to donate electron density into the aromatic head group via resonance. Analogue **3** was prepared to investigate these possibilities. This compound contains a 4-*N*,*N*-dimethylamino ($-NMe_2$) group rather than 4-amino. While the $-NMe_2$ group is approximately equivalent to $-NH_2$ group in terms of its ability to donate electron density into the aromatic ring, it cannot function as a hydrogen bond donor unlike the $-NH_2$ group. Revealingly (Table 1) the inhibitory activity of **3** was found to be essentially the same as that of **2**, implying that the electron-donating ability (by delocalization) of the 4-substituent (R_2) is more significant than its ability to function as a hydrogen bond donor. The 2-methyl group is an electron-donating group, albeit inferior to the 4-amino group. However, removal of the methyl group from parent compound **1** gives analogue **2**, that has almost the same IC_{50} as **4**, which is obtained when the 4-amino group is removed from **1**. This seems to reflect the closer proximity of methyl to the ring nitrogen. In summary, these results suggested to us that the degree of electron-richness in the heteroaromatic head group correlates with PKC inhibitory ability.

We therefore decided to seek a parameter that would quantitatively measure the electron-richness of the head groups represented in compounds **1**–**5**. We expected and found that the magnitude of the charge on N_1 (Mulliken charges) to be a suitable parameter.[26,28,29] The needed Mulliken charges were obtained from HF/6-31G MO calculations using Gaussian 03. To simplify the computations, the calculations were not performed on the whole bis-quinolinium compounds but rather on model compounds consisting of only one of the quinolinium head groups in which a simple methyl group was the quaternizing group in place of the C_{10} methylene linker present in the actual analogues (Table 2). Since there is

Table 2. Mulliken Charges on N_1 of the model compounds used for correlation in Figure 1

Compound[a]	Structure	Substituents		N_1 Charge
Ca	$Et_3N^+-CH_3$			−0.555
Da	$Ph_3P^+-CH_3$			1.135[b]
		R_1	R_2	
1a		CH_3	NH_2	−0. 790
2a		H	NH_2	−0. 743
3a		H	$N(CH_3)_2$	−0. 720
4a		CH_3	H	−0. 764
5a		H	H	−0. 683
7a				−0.723
		X	R_3	
8a		O	CH_3	−0.713
9a		S	CH_3	−0.633
10a		NCH_3	NH_2	−0.776
		R_4		
11a		NH_2		−0.624
12a		$N(CH_3)_2$		−0.624
13a				−0.771
14a				−0.814

HF/6-31G molecular orbital (MO) calculations were performed on model methylated quaternized derivatives as shown in this table using Gaussian 03.

[a] Model compound numbers correspond to the compound numbers of Table 1.

[b] Charge on P.

no electronic interaction between the two quinolinium head groups that are well separated by a C_{10} saturated linker, this is a valid approximation. Any errors introduced (due to different inductive and hyperconjugation effects) are expected to be common for all the compounds and the results should be comparable.[26–28,35] The calculated Mulliken charges on the ring nitrogen atoms for the model compounds are summarized in Table 2. A good correlation between IC_{50} values and the Mulliken charges on N_1 was obtained for compounds**1**, **2**, **4**, and **5** as shown in Figure 1. Here it can be seen that the analogues with lower IC_{50} values showed a larger negative Mulliken charge on N_1 and those with higher IC_{50} values showed a less negative charge on N_1.[36]

To determine whether this correlation could be used to predict the potency of novel analogues, we designed, synthesized, and studied a variety of electron-rich analogues (compounds **6**–**10**) in which the head groups consisted of rings other than quinoline, or in which the heteroaromatic rings are connected via the 4-amino groups rather than the ring nitrogen atoms. As expected, the calculated Mulliken charges for most of these newly designed model compounds were in agreement with a more negative charge density (Table 2). Preparation was carried out of two pyridine analogues having lower

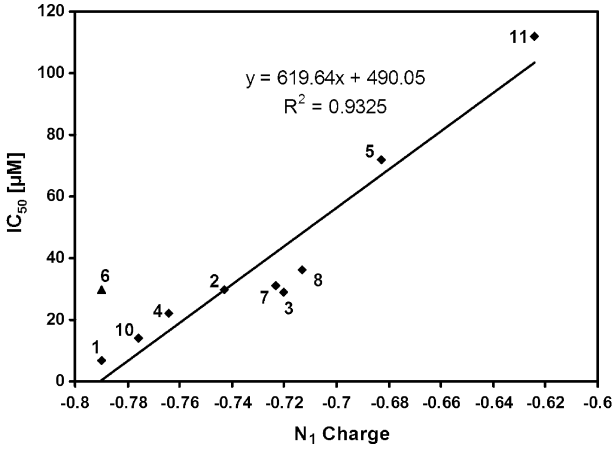


Figure 1. Plot of IC_{50} values of compounds studied (Table 1) vs Mulliken charges on N_1 of corresponding model compounds in Table 2. All compounds except **6** (exocyclic dequalinium analogue) were included into the calculation of R^2.

electron density (compounds **11** and **12**) that therefore were expected to produce weaker inhibition. In all cases, the IC_{50} values for all compounds exhibited good correlation with the magnitude of the N_1 Mulliken charge (Fig. 1). The weaker PKCα inhibitors produced with

pyridine head groups (compounds **11** and **12**) are consistent with the diminished electron-richness of these rings as compared to quinoline rings. In contrast, compounds **7**–**10** with isoquinoline, benzoxazole, benzthiazole, and benzimidazole head groups, respectively, have similar elevated PKC inhibition to those of quinoline derivatives **1**–**5**. Compound **6** deviates a little from the generally linear correlation of Figure 1, but this is hardly surprising as its structure is significantly different from those of analogues linked through their ring nitrogen atoms.

We had hoped to round out our studies by examining other dequalinium analogues of type **1**–**5** with a range of R_2 groups (electron-donating and electron-withdrawing) in the 4-position of the quinoline ring. However, attempts to prepare an analogue with $R_2 = –OCH_3$ were unsuccessful because reaction of 4-methoxyquinoline with 1,10 diiododecane results in the formation of 1,1′-(decane-1,10-diyl) bis(4-(1H)-quinolone) instead of the intended dequalinium analogue.[27] In addition, attempts failed to synthesize dequalinium analogues containing electron-withdrawing groups (such as $R_2 = –CO_2Et$ or –Cl), presumably because such groups cause the quinoline ring nitrogen atom to be so weakly nucleophilic toward alkylating agents that no evidence of reaction could be detected even after many weeks of reflux.[27,28] Therefore, all compounds successfully prepared in these studies possessed R_2 substituents that were either electron-donating or neutral.

Based on our finding of a correlation between electron density of the head groups and the corresponding PKCα inhibitory potency, it should be possible to make predictions about the PKC activity of other alkylated nitrogen heterocycles having no direct structural relationship to dequalinium salts. To test this correlation, we examined propidiumiodide, **13**, and safranine O, **14**,[37] which would be predicted to have appreciable PKC inhibitory activity due to the presence of both multiple electron-donating (NH_2 and CH_3) groups and several aromatic rings to augment electron-richness. Indeed, both these compounds were found to be much more potent as PKC inhibitors than previously investigated compounds such as **A** and **B** which also contain a single heteroaromatic head group. In fact, compound **13** proved to be almost as active as dequalinium diiodide itself (see Table 1). This finding suggests that preparation of an analogue based on **13** with two heteroaromatic head groups rather than one would be a worthwhile objective for future studies.

5. Conclusion

By SAR studies we demonstrated that there is a good correlation between the PKC potency and the N_1 charge for the compounds studied. The greater the electron density in the head group, the more negative is the N_1 charge and consequently the higher potency of PKCα inhibition. The influence of R_2 was found to be electronic via delocalization of positive charge thus making the head groups more electron-rich. In addition, varying the nature of the linkage of head groups is tolerated since two head groups linked by atoms other than the ring N atoms, as in **6**, do not appreciably alter inhibitory strength.

6. Experimental

6.1. Assay of inhibition of catalytic activity

Recombinant human PKCα (95% pure) (Pan Vera Corp., Madison, WI) was used for testing dequalinium analogues in vitro. Because of the purity and high basal activity of the enzyme preparation, it was possible to conduct assays in the absence of phosphatidylserine which is customarily added to assist in the activation of the enzyme.[16] It was excluded from these assays in order to avoid non-specific hydrophobic interactions that could pose a variable due to the different head groups represented in this collection of analogues.

To test the inhibitory potency of these compounds, the total catalytic activity of PKCα was analyzed in triplicate with increasing concentrations of a selected dequalinium analogue. PKCα activity was measured in a reaction volume of 0.12 mL consisting of 20 mM Tris, pH 7.4, 10 mM Mg^{2+}, 0.5 mM Ca^{2+}, 26.6 μM peptide substrate (RFARKGSLRQKNV), PKCα (28 ng per assay), 66 μM [γ-^{32}P]ATP, and 5 μL dequalinium analogue (or 5 μL DMSO as control) added to the specified concentration. Stock solutions for dequalinium analogues were prepared in DMSO and standardized spectrophotometrically on a Perkin-Elmer Lambda II spectrophotometer. The extinction coefficient for each compound is given in the Supplemental information.

Each phosphotransferase reaction was initiated by the addition of [γ-^{32}P]ATP to consecutive tubes in staggered 15-s intervals. After a 10 min reaction period, each tube was quenched in the same consecutive style by applying 100 μL of the reaction medium to a 2×2 cm square of phosphocellulose paper. The squares retained only the peptide following five wash steps with 1 L tap water, and the radioactive content of each square was analyzed by β-scintillation counting. When compared to the level of activity measured in the absence of an analogue, the extent to which a given concentration of an analogue decreased the formation of phosphorylated peptide indicated the extent of PKCα inhibition. The error for triplicate assays was typically less than 10%. IC_{50} values were calculated from dose–response curves as that concentration producing 50% inhibition. This analysis was carried out at least twice for each analogue and the IC_{50} values were averaged.

6.2. General experimental methods

^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz on a Bruker spectrometer, respectively. Chemical shifts were expressed in ppm relative to TMS (0.00 ppm), MeOD (3.31 ppm for ^{1}H and 49.01 ppm for ^{13}C), DMSO (2.50 ppm for ^{1}H and 39.51 ppm for ^{13}C) or $CDCl_3$ (7.26 ppm for ^{1}H and

77.22 ppm for ^{13}C). Elemental analyses were performed by Desert Analytics Laboratory, Arizona. Low and high resolution mass spectra were recorded by use of electrospray ionization at core facilities at Hunter College of the City University of New York and at University of Illinois Urbana-Champaign. Reaction solvents were distilled under N_2 as follows: hexane and Et_2O from sodium and benzophenone immediately before use, methanol from magnesium, CH_2Cl_2, and $CHCl_3$ from calcium hydride (CaH_2). 2 M ammonia in methanol was obtained from Aldrich in SureSeal™ bottles. TLC was carried out with Merck $60F_{254}$ (0.25 mm thick) sheets. All air- and water-sensitive reactions were performed under N_2 in oven- or flame-dried glassware.

6.2.1. 1,1′-(1,10-Decanediyl)bis[4-amino-2-methyl quinolinium] diiodide (1). 2.4 equivalents of 4-aminoquinaldine (0.5 g, 3.16 mmol) was refluxed with 1,10-diiododecane (0.518 g, 1.32 mmol) in 2-butanone for 48 h. The reaction was monitored by TLC using 2 M ammonia in MeOH/CH_2Cl_2 0.25:0.75 (R_f 0.45) and the presence of 1,10-diiododecane was observed by hexane. The precipitate obtained was filtered, washed thoroughly with 2-butanone, acetone, and anhyd Et_2O to yield 0.75 g (80%) of **1** as a pale yellow precipitate. ^1H NMR (DMSO) δ 1.31 (m, 10H), 1.72 (m, 6H), 2.74 (s, 6H), 4.47 (t, 4H, J = 7.9 Hz), 6.65 (s, 2H), 7.73 (t, 2H, J = 7.8 Hz), 8.03 (t, 2H, J = 7.8 Hz), 8.17 (d, 2H, J = 8.4 Hz), 8.45 (d, 2H, J = 8.4 Hz), 8.84 (br s, 4H); ^{13}C NMR (DMSO) δ 9.2, 21.6, 25.9, 29.8, 32.8, 47.9, 103.9, 116.6, 118.4, 124.3, 125.9, 134.4, 139.0, 155.0, 156.7; LRMS (M−2I/2) calcd for $C_{30}H_{40}N_4I_2$ *m/z* 228.2, found 228.2. Compounds **2**–**12** were synthesized following the procedure described above.

6.2.2. 4-Aminoquinoline. 4-Chloroquinoline (3 g, 0.018 mol) was heated to 180 °C with approximately 9 g of phenol. Ammonia dried over quicklime was passed through the solution for 3 h. The hydrochloride of the amine was separated and the excess of phenol was removed by steam-distillation.[34] The clear solution was concentrated by evaporation. The resulting solution was cooled, made alkaline with NaOH, and the crude obtained was recrystallized with MeOH to afford 2.26 g (87%) of 4-aminoquinoline as a pale yellow solid. ^1H NMR (DMSO) δ 6.55 (d, H, J = 4.8 Hz), 6.77 (br s, 2H), 7.38 (t, H, J = 7.6 Hz), 7.59 (t, H, J = 7.6 Hz), 7.76 (d, H, J = 8.0 Hz), 8.15 (d, H, J = 8.0 Hz), 8.32 (br s, H); ^{13}C NMR (DMSO) δ 102.3, 118.6, 122.3, 123.4, 128.6, 128.8, 148.8, 150.3, 151.4; LRMS (MH^+) calcd for $C_9H_8N_2$ *m/z* 145.1, found 145.1. 4-*N*,*N*-dimethylaminoquinoline and bis-(quinaldinylamino)hexane were synthesized following the procedure described above.

6.2.3. 4-*N*,*N*-Dimethylaminoquinoline. This compound was synthesized by nucleophilic displacement of chloride ion in 4-chloroquinoline with *N*,*N*-dimethylamine. The alkaline solution was extracted to CH_2Cl_2 and dried to afford 67% of 4-*N*,*N*-dimethylaminoquinoline as a brown liquid. ^1H NMR (DMSO) δ 2.97 (s, 6H), 6.85 (d, H, J = 4.8 Hz), 7.52 (dt, H, J = 1.2 Hz, J = 8.4 Hz), 7.69 (dt, H, J = 1.2 Hz, J = 8.4 Hz), 7.98 (d, H, J = 8.4 Hz), 8.08 (d, H, J = 8.4 Hz), 8.64 (d, H, J = 4.8 Hz); ^{13}C NMR (DMSO) δ 43.6, 107.3, 122.2, 124.2, 124.6, 128.8, 129.6, 149.5, 150.5, 156.8; LRMS (MH^+) calcd for $C_{11}H_{12}N_2$ *m/z* 173.1, found 173.1.

6.2.4. 1,1′-(1,10-Decanediyl)bis[4-aminoquinolinium] diiodide (2). The pale yellow precipitate isolated was 67%. ^1H NMR (DMSO) δ 1.17 (m, 12H), 1.74 (m, 4H), 4.5 (t, 4H, J = 7.1 Hz), 6.78 (d, 2H, J = 7.1 Hz), 7.73 (t, 2H, J = 7.7 Hz) 8.02 (t, 2H, J = 7.7 Hz), 8.15 (d, 2H, J = 8.6 Hz), 8.47 (d, 2H, J = 8.6 Hz), 8.52 (d, 2H, J = 7.1 Hz), 9.02 (br s, 4H); ^{13}C NMR (DMSO) δ 25.5, 28.2, 28.4, 28.5, 53.5, 101.7, 116.8, 118.0, 124.2, 126.1, 134.2, 137.7, 145.9, 157.5; Anal. Calcd for $C_{28}H_{36}N_4I_2$: C, 49.28; H, 5.32; N, 8.21; I, 37.19. Found: C, 49.15; H, 5.46; N, 8.09; I, 37.55. LRMS (M-2I)/2 calcd for $C_{28}H_{36}N_4I_2$ *m/z* 214.2, found 214.2.

6.2.5. 1,1′-(1,10-Decanediyl)bis[4-*N*,*N*,dimethylaminoquinolinium] diiodide (3). The pale yellow precipitate isolated was 73%. ^1H NMR (DMSO) δ 1.24 (m, 12H), 1.79 (m, 4H), 3.46 (s, 12H), 4.56 (t, 4H, J = 7.1 Hz), 7.01 (d, 2H, J = 7.5 Hz), 7.70 (t, 2H, J = 7.7 Hz), 8.01 (t, 2H, J = 7.7 Hz), 8.15 (d, 2H, J = 8.6 Hz), 8.47 (d, 2H, J = 8.6 Hz), 8.61 (d, 2H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 26.1, 28.4, 28.5, 28.9, 45.0, 55.0, 104.4, 117.9, 119.6, 125.7, 127.9, 134.0, 139.1, 146.3, 160.2; HRMS (M−I) calcd for $C_{32}H_{44}N_4I_2$ *m/z* 611.2611, found 611.2640.

6.2.6. 1,1′-(1,10-Decanediyl)bis[2-methylquinolinium] diiodide (4). The pale yellow precipitate isolated was 70%. ^1H NMR (DMSO) δ 1.35 (m, 8H), 1.57 (m, 4H), 1.90 (m, 4H), 3.12 (s, 6H), 4.92 (m, 4H), 8.00 (t, 2H, J = 7.6 Hz), 8.13 (d, 2H, J = 8.4 Hz), 8.24 (t, 2H, J = 7.6 Hz), 8.42 (d, 2H, J = 8.0 Hz), 8.59 (d, 2H, J = 8.0 Hz), 9.11 (d, 2H, J = 8.4 Hz); ^{13}C NMR (DMSO) δ 22.5, 25.9, 28.1, 28.6, 28.9, 51.4, 118.9, 125.6, 128.2, 129.0, 130.6, 135.3, 138.2, 145.7, 160.5; Anal. Calcd for $C_{30}H_{38}N_2I_2$: C, 52.95; H, 5.63; N, 4.12. Found: C, 52.87; H, 5.86; N, 3.89. HRMS (M−I) calcd for $C_{30}H_{38}N_2I_2$ *m/z* 553.2080, found 553.2100.

6.2.7. 1,1′-(1,10-Decanediyl)bis[quinolinium] diiodide (5). The pale yellow precipitate isolated was 73%. ^1H NMR (DMSO) δ 1.29 (m, 12H), 1.93 (m, 4H), 5.04 (t, 4H, J = 7.6 Hz), 8.06 (t, 2H, J = 7.6 Hz), 8.20 (t, 2H, J = 7.6 Hz) 8.30 (t, 2H, J = 8.1 Hz), 8.49 (d, 2H, J = 7.8 Hz), 8.65 (d, 2H, J = 8.6 Hz), 9.29 (d, 2H, J = 8.6 Hz), 9.54 (d, 2H, J = 5.8 Hz); ^{13}C NMR (DMSO) δ 25.8, 28.5, 28.8, 29.5, 57.3, 118.9, 122.1, 129.7, 129.9, 130.8, 135.6, 137.4, 147.4, 149.6; HRMS (M−I) calcd for $C_{28}H_{34}N_2I_2$ *m/z* 525.1767, found 525.1783.

6.2.8. 1,6-Bis[*N*-(1-methylquinolinium-2-methyl)amino]-hexane diiodide (6). 1,6-Diaminohexane (0.61 g, 5.25 mmol) was added dropwise to the reaction mixture containing 4-chloroquinaldine (2.06 g, 11.6 mmol) for 1 h. The crude product was recrystallized with ethanol to afford 1.46 g (70%) of bis-(quinaldinylamino)hexane as pale yellow crystals. ^1H NMR (DMSO) δ 1.45 (m, 4H), 1.70 (m, 4H), 2.98 (s, 6H), 3.52 (m, 4H), 7.3 (s, 2H), 7.90 (t, 2H, J = 7.6 Hz), 8.00 (t, 2H, J = 8.4 Hz),

8.48 (d, 2H, $J = 6.8$ Hz), 8.73 (d, 2H, $J = 8.4$ Hz), 9.56 (br s, 2H); ^{13}C NMR (DMSO) δ 26.0, 27.5, 42.7, 97.9, 116.7, 120.3, 123.5, 126.2, 133.0, 138.1, 142.3, 153.7, 155.1; HRMS (MH^+) calcd for $C_{26}H_{30}N_4$ *m/z* 399.2549, found 399.2568. Bis-(quinaldinylamino) hexane (0.246 g, 0.618 mmol) was refluxed with acetone (10 mL) and excess of MeI (0.25 g, 1.76 mmol) for 3d in order to methylate the ring nitrogen. The crude obtained was recrystallized with methanol to afford 0.34 g (80%) of **6** as a pale yellow precipitate. ^{1}H NMR (DMSO) δ 1.47 (m, 4H), 1.72 (m, 4H), 2.78 (s, 6H), 3.52 (m, 4H), 3.99 (s, 6H), 6.96 (s, 2H), 7.73 (t, 2H, $J = 7.6$ Hz), 8.01 (t, 2H, $J = 7.6$ Hz), 8.16 (d, 2H, $J = 8.4$ Hz), 8.51 (d, 2H, $J = 8.4$ Hz), 9.01 (br s, 2H); ^{13}C NMR (DMSO) δ 22.3, 26.1, 27.7, 36.4, 42.9, 100.2, 116.9, 118.5, 123.4, 126.0, 133.7, 139.4, 153.9, 156.5 Anal. Calcd for $C_{28}H_{36}N_4I_20.3HCl$: C, 48.49; H, 5.24; N, 8.08. Found: C, 48.20; H, 4.99; N, 7.95. HRMS (M−I) calcd for $C_{28}H_{36}N_4I_2$ *m/z* 555.1985 found 555.2000.

6.2.9. 1,1′-(1,10-Decanediyl)bis[1-amino isoquinolinium] diiodide (7). The pale yellow precipitate isolated was 79%. ^{1}H NMR (MeOD) δ 1.41 (m, 12H), 1.88 (m, 4H), 4.27 (t, 4H, $J = 7.7$ Hz), 7.25 (d, 2H, $J = 7.3$ Hz), 7.70 (d, 2H, $J = 7.7$ Hz), 7.79 (m, 2H), 7.94 (m, 4H), 8.48 (d, 2H, $J = 9.0$ Hz) (NH_2 signals were not observed due to exchange with the solvent); ^{13}C NMR (MeOD) δ 27.4, 28.7, 30.4, 30.5, 54.9, 113.9, 120.1, 126.1, 128.9, 130.5, 133.6, 135.7, 137.5, 155.2; HRMS (M−I) calcd for $C_{28}H_{36}N_4I_2$ *m/z* 555.1987, found 555.2007.

6.2.10. 1,1′-(1,10-Decanediyl)bis[2-methylbenzoxazolium] diiodide (8). The orange precipitate isolated was 80%. ^{1}H NMR (MeOD) δ 1.41 (m, 12H), 2.00 (m, 4H), 3.14 (s, 6H), 4.61 (t, 4H, $J = 7.8$ Hz), 7.80 (m, 4H), 8.00 (m, 2H), 8.08 (m, 2H); ^{13}C NMR (DMSO), δ 13.4, 27.2, 28.9, 29.7, 30.0, 48.1, 113.7, 115.2, 128.9, 129.9, 130.7, 149.2, 169.5; Anal. Calcd for $C_{26}H_{34}N_2O_2I_2$: C, 47.29; H, 5.19; N, 4.24; I, 38.43. Found: C, 47.05; H, 5.35; N 3.86; I, 38.65. LRMS (M−2I)/2 calcd for $C_{26}H_{34}N_2O_2I_2$ *m/z* 203.2, found 203.2.

6.2.11. 1,1′-(1,10-Decanediyl)bis[2-methylbenzothiazolium] diiodide (9). The purple precipitate isolated was 87%. ^{1}H NMR (DMSO) δ 1.28 (m, 8H), 1.31 (m, 4H), 1.84 (m, 4H), 3.22 (s, 6H), 4.71 (t, 4H, $J = 7.8$ Hz), 7.81 (m, 2H), 7.90 (m, 2H), 8.35 (d, 2H, $J = 8.2$ Hz), 8.46 (d, 2H, $J = 8.2$ Hz); ^{13}C NMR (DMSO) δ 16.9, 25.9, 27.8, 28.6, 28.7, 49.2, 116.9, 124.6, 128.1, 129.1, 129.4, 140.8, 177.0; Anal. Calcd for $C_{26}H_{34}N_2S_2I_2$: C, 45.09; H, 4.95; N 4.05; I, 36.65. Found: C, 45.09; H, 4.84; N 3.91; I, 36.34. HRMS (M−I) calcd for $C_{26}H_{34}N_2S_2I_2$ *m/z* 565.1242, found 565.1226.

6.2.12. 1,1′-(1,10-Decanediyl)bis[2-amino-1-methylbenzimidazolium] diiodide (10). The pale yellow precipitate isolated was 77%. ^{1}H NMR (MeOD) δ 1.30 (m, 12H), 1.78 (m, 4H), 3.71 (s, 6H), 4.17 (t, 4H, $J = 7.3$ Hz), 7.38 (m, 4H), 7.48 (m, 4H) (NH_2 signals were not observed due to exchange with the solvent); ^{13}C NMR (MeOD) δ 28.0, 29.5, 30.3, 30.8, 30.9, 44.8, 111.7, 111.9, 125.5, 125.6, 132.5, 132.2, 151.6; Anal. Calcd for $C_{26}H_{38}N_6I_2$: C, 45.36; H, 5.56; N 12.21; I, 36.87. Found: C, 45.50; H, 5.59; N 11.86; I, 36.40. HRMS (M−I) calcd for *m/z* $C_{26}H_{38}N_6I_2$ 561.2203, found 561.2216.

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Supplementary data

NMR spectral data for compounds **11**, **12**, **C**, and **D** and wavelengths (λ_{max}), and molar extinction coefficients (ε) of compounds studied. This material is available free of charge via the Internet at http://www.sciencedirect.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.07.067.

References and notes

1. Rotenberg, S. A.; Weinstein, I. B.. In *Biochemical and Molecular Aspects of Selected Cancers*; Pretlow, T. G., II, Pretlow, T. P., Eds.; Academic Press: Orlando, 1991; Vol. 1, pp 25–73.
2. Rotenberg, S. A.; Zhu, J.; Hansen, H.; Li, X.; Sun, X.; Michels, C. A.; Riedel, H. *J. Biochem.* **1998**, *124*, 756.
3. Krauss, R. S.; Housey, G. M.; Hsiao, W. L. W.; Johnson, M. K.; Rotenberg, S. A.; Borner, C. M. B.; Weinstein, I. B. *Prog. Clin. Biol. Res.* **1990**, *340*, 175.
4. Sun, X.; Rotenberg, S. A. *Cell Growth Differ.* **1999**, *10*, 343.
5. Sanz-Navares, E.; Fernandez, N.; Kazanietz, M. G.; Rotenberg, S. A. *Cell Growth Differ.* **2001**, *12*, 517.
6. Hanauske, A. R.; Sundell, K.; Lahn, M. *Curr. Pharm. Des.* **2004**, *10*, 1923.
7. Silva, D. *Curr. Cancer Drug Targets* **2004**, *4*, 327.
8. Chung, S. H.; Polgar, J.; Reed, G. L. *J. Biol. Chem.* **2000**, *275*, 25286.
9. House, C.; Kemp, B. E. *Science* **1987**, *238*, 1726.
10. Parker, P. J.; Coussens, L.; Totty, N.; Rhee, L.; Young, S.; Chen, E.; Stabel, S.; Waterfield, M. D.; Ullrich, A. *Science* **1986**, *233*, 853.
11. Rotenberg, S. A.; Calogeropoulou, T.; Jaworski, J. S.; Weinstein, I. B.; Rideout, D. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2490.
12. Rotenberg, S. A.; Huang, M. H.; Zhu, J.; Su, L.; Riedel, H. *Mol. Carcinog.* **1995**, *12*, 42.
13. Tammela, P.; Ekokoski, E.; Garcia-Horsman, A.; Talman, V.; Finel, M.; Tuominen, R.; Vuorela, P. *Drug Dev. Res.* **2004**, *63*, 76.
14. Rotenberg, S. A.; Baker, A. D. U.S. Patent 2002114769, 2002.
15. Rotenberg, S. A.; Smiley, S.; Ueffing, M.; Krauss, R. S.; Chen, L. B.; Weinstein, B. *Cancer Res.* **1990**, *50*, 677.
16. Qin, D.; Sullivan, R.; Berkowitz, W. F.; Bittman, R.; Rotenberg, S. A. *J. Med. Chem.* **2000**, *43*, 1413.
17. Rotenberg, S. A.; Sun, X.-g. *J. Biol. Chem.* **1998**, *273*, 2390.

18. Rotenberg, S. A.; Zhu, J.; Hansen, H.; Li, X.-d.; Sun, X.-g.; Michels, C. A.; Riedel, H. *J. Biochem.* **1998**, *124*, 756.
19. Sullivan, R. M.; Stone, M.; Marshall, J. F.; Uberall, F.; Rotenberg, S. A. *Mol. Pharmacol.* **2000**, *58*, 729.
20. Babbs, M.; Collier, H. O. J.; Austin, W. C.; Potter, M. D.; Taylor, E. P. *J. Pharm. Pharmacol.* **1956**, *8*, 110.
21. Toshio, A.; Shozo, K.; Sachiko, K. *Yakuzaigaku* **1966**, *26*, 131.
22. Toshio, A.; Shozo, K.; Sachiko, K. *Yakuzaigaku* **1966**, *26*, 189.
23. Weiss, M. J.; Wong, J. R.; Ha, C. S.; Bleday, R.; Salem, R. R.; Steele, G. D.; Chen, L. B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5444.
24. Zhuo, S.; Allison, W. S. *Biochem. Biophys. Res. Commun.* **1988**, *152*, 968.
25. Bodden, W. L.; Palayoor, S. T.; Hait, W. N. *Biochem. Biophys. Res. Commun.* **1986**, *135*, 574.
26. Galanakis, D.; Davis, C. A.; Herrero, B. D. R.; Ganellin, C. R.; Dunn, P. M.; Jenkinson, D. H. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 559.
27. Galanakis, D.; Davis, C. A.; Herrero, B. D. R.; Ganellin, C. R.; Dunn, P. M. *J. Med. Chem.* **1995**, *38*, 595.
28. Galanakis, D.; Calder, J. A. D.; Ganellin, C. R.; Owen, C. S.; Dunn, P. M. *J. Med. Chem.* **1995**, *38*, 3536.
29. Galanakis, D.; Davis, C. A.; Ganellin, C. R.; Dunn, P. M. *J. Med. Chem.* **1996**, *39*, 359.
30. Galanakis, D.; Ganellin, C. R.; Malik, S.; Dunn, P. M. *J. Med. Chem.* **1996**, *39*, 3592.
31. Cook, N. S. *Trends Pharmacol. Sci.* **1988**, *9*, 21.
32. Robertson, D. W.; Steinberg, M. I. *J. Med. Chem.* **1990**, *33*, 1529.
33. Makler, M. T. PCT Int. Appl. PIXXD2 WO 9004644 A1 19900503, 1990.
34. The procedure was a modification of that described by Backeberg, O. G.; Marais, J. L. C. *J. Chem. Soc. (London)* **1942**, 381.
35. Use of a methyl group to quaternize the head groups is a common practice in computational studies for this type of dimers.
36. A feature of MO computations of Mulliken charges on N_1 is that they come out negative even for N atoms with a formal plus charge. Electron-donating groups then make the Mulliken charges even more negative.
37. Compounds **13** and **14** were purchased from Aldrich.