Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 8089–8103 **8089**
DOI: 10.1021/jm1009605

Design, Synthesis, and DNA-Binding of *N*-Alkyl(anilino)quinazoline Derivatives

Antonio Garofalo,[†,‡] Laurence Goossens,[†,‡] Brigitte Baldeyrou,[†,§,∥] Amélie Lemoine,[†,‡] Séverine Ravez,[†,‡] Perrine Six,[†,‡] Marie-Hélène David-Cordonnier,[†,§] Jean-Paul Bonte,[†,‡] Patrick Depreux,[†,‡] Amélie Lansiaux,[†,§,∥] and Jean-François Goossens*[,†,⊥]

[†]*Univ Lille Nord de France, F-59000 Lille, France,* [‡]*UDSL, ICPAL, EA 4481, F-59006 Lille, France,* [§]*INSERM U-837, IRCL, F-59045 Lille, France,* [∥]*Centre Oscar Lambret, IRCL, IMPRT, F-59045 Lille, France, and* [⊥]*UDSL, UFR de Pharmacie, EA 4481, F-59006 Lille, France*

Received July 28, 2010

New *N*-alkylanilinoquinazoline derivatives **5**, **12**, **20**, and **22** have been prepared from 4-chloro-6, 7-dimethoxyquinazoline **3**, 4-chloro-6,7-methylenedioxyquinazoline **19**, and commercially available anilines. Differents classes of compounds substituted by an aryloxygroup (**6a**−**c**, **16a**,**b**, and **17a**,**b**), (aminophenyl)ureas (**12a**,**b** and **13a**−**f**), anilines (**4a**−**m**, **20a**,**b**), *N*-alkyl(aniline) (**5a**−**m**, **21a**,**b**, **22a**,**d**), and *N*-aminoalkyl(aniline) (**22e**−**g**) have been synthesized. These molecules were evaluated for their cytotoxic activities and as potential DNA intercalating agents. We studied the strength and mode of binding to DNA of these molecules by DNA melting temperature measurements, fluorescence emission, and circular dichroism. The results of various spectral and gel electrophoresis techniques obtained with the different compounds, in particular compounds **5g** and **22f**, revealed significant DNA interaction. These experiments confirm that the *N*-aminoalkyl(anilino)-6,7-dimethoxyquinazoline nucleus is an efficient pharmacophore to trigger binding to DNA, via an intercalative binding process.

1. Introduction

Quinazoline-containing derivatives form an important class of synthetic products and represent an attractive scaffold for designing anticancer drugs. They have attracted interest over the past years because of their varied biological activity, notably as kinase inhibitors.[1−4] The 4-anilinoquinazoline scaffold has led to the development and the marketing of new series of antitumor agents such as gefitinib, erlotinib, and lapatinib.

In recent years several DNA-targeting agents consisting of a polycyclic planar moiety, such as quinoline, quinazoline, or indole linked to an alkyl or aniline, have been shown to be more active than kinase inhibitors.[5−9] These compounds interact with double stranded DNA and also present selective recognition of DNA sequences. They can be classified into two major categories, intercalating and minor groove binders.[10] Previously, our group has documented the DNA interaction capacity of the tyrosine protein kinase inhibitor **1a** (PD153035)[11] and its *N*-methyl analogue **1b** (EBE-A22)[12] (Figure 1).

The brominated anilinoquinazoline derivative **1a** also exhibits a very high affinity and selectivity for the epidermal growth factor receptor tyrosine kinase (EGFR TK[a]) and presents a remarkable cytotoxicity against several types of tumor cell lines.[12,13] In contrast, its *N*-methyl analogue **1b** retains good antiproliferative activities despite its total absence of EGFR TK inhibitory effect.[14] Many studies have been performed to define the strength and mode of DNA binding of both products, and the results have proved that **1b** binds to DNA as a typical intercalating agent. Therefore, this study demonstrated that the methylation of the anilino nitrogen of **1a** prevents binding to the EGFR TK but improves the interaction of the drug with DNA and confers a selectivity for GC-rich sequences. The addition of the *N*-methyl substituent on the anilinoquinazoline nucleus increased significantly the affinity of the ligand for specific sequences in DNA. The reduction of the conformational flexibility of this molecule maintains the anilinoquinazoline chromophore in a specific conformation favorable for the DNA intercalation and G−C base pairs recognition.[12]

The anilinoquinazoline skeleton targets DNA, and its N-methylated derivative represents a useful chemotype for the development of new DNA-targeted anticancer agent. These initial results prompted us to design new quinazoline derivatives and investigate their biological properties. Here, we report the synthesis, biological evaluation, and DNA binding of 55 new compounds. The interaction with DNA was studied and quantified by melting temperature, fluorescence, and circular dichroism experiments. The effects of the molecules on DNA topoisomerase I activities were evaluated. The cytotoxicity of the most active compounds was evaluated using three human tumor cell lines.

2. Chemistry

We have synthesized two series of new quinazolin compounds. These products form two groups distinguished by the

*To whom correspondence should be addressed. Phone: +33 (0)3 20 96 40 21. Fax: +33 (0)3 20 96 49 06. E-mail: jean-francois.goossens@univ−lille2.fr.

[a] Abbreviations: ATP, adenosine 5′-triphosphate; CD, circular dichroism; DM, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; EGFR TK, epidermal growth factor receptor tyrosine kinase; EtOAc, ethyl acetate; EtOH, ethanol; FC, flash chromatography; MeOH, methanol; rt, room temperature; sc, supercoiled DNA ; T_m, melting temperature; TLC, thick-layer chromatography.

Published on Web 10/29/2010

nature of the ether linker at the C-6 and C-7 positions of the quinazoline core: 6,7-dimethoxy− (**4**, **5**, **6**, **12**, **13**, **16**, **17**, **22**) and 6,7-methylenedioxyquinazoline (**20**, **21**) (Figure 2).

The synthesis of the 6,7-dimethoxyquinazoline derivatives is illustrated in Schemes 1−3. The key intermediate 4-chloro-6,7-dimethoxyquinazoline **3** was prepared according to described procedures[15] using methyl 2-amino-4,5-dimethoxybenzoate as a starting material. Compound **3** engaged in a nucleophilic substitution reaction in the presence of various commercial anilines in 2-propanol to obtain the final products **4a**−**m**. Compounds **5a**−**m** were synthesized by methylation reaction in the presence of iodomethane in *N*,*N*-dimethylformamide from **4a**−**m** with low yield (20−40%). Compound **3** on reaction with phenol derivatives by irradiation at 150 °C in DMSO gave compounds **6a**−**c** in good yields (Scheme 1).

As depicted in Scheme 2, compounds **8** and **9** were prepared differently: the intermediate **8** was synthesized using commercial *N*-methyl-4-nitroaniline, and compound **9** was prepared in a two-step sequence by nucleophilic substitution of **3**, giving anilinoquinazoline **7** that was finally N-methylated. Catalytic hydrogenation of the nitro group led to amino derivatives **10** and **11** (87−92% of yields) which were converted by condensation with commercial isocyanate in THF/H_2O mixture, in the presence of pyridine, to the corresponding ureas **12a**, **b** and **13a**−**f**.

Selective reaction of chloride derivative **3** with *m*- or *p*-aminophenol and tetra-*n*-butylammonium bromide in a mixture of methyl ethyl ketone and sodium hydroxide provided the intermediates **14**[15] and **15** which by condensation with the corresponding isocyanate in chloroform afforded the desired ureas **16a**,**b**[15] and **17a**,**b** in high yields and short time reactions (Scheme 3).

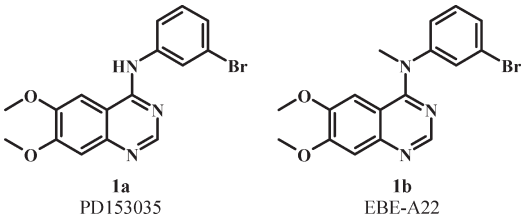


Figure 1. Structure of **1a** and its *N*-methyl analogue **1b**.

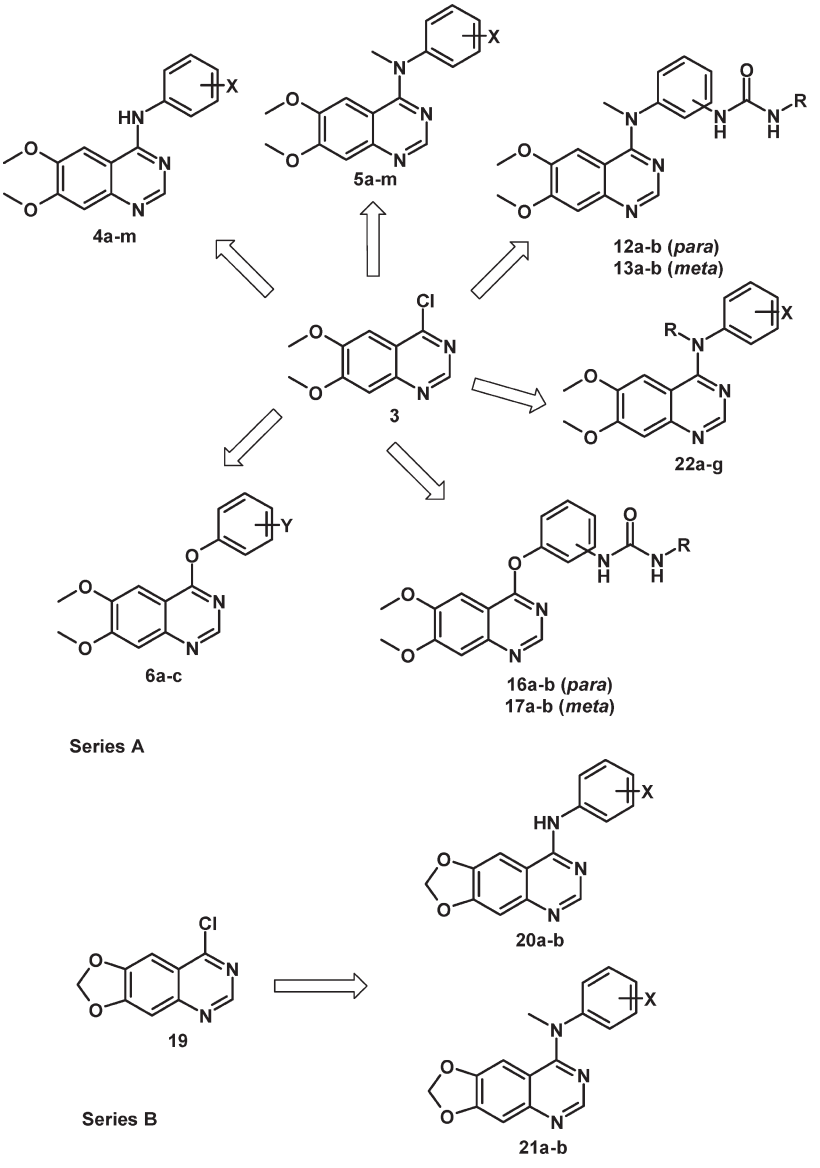
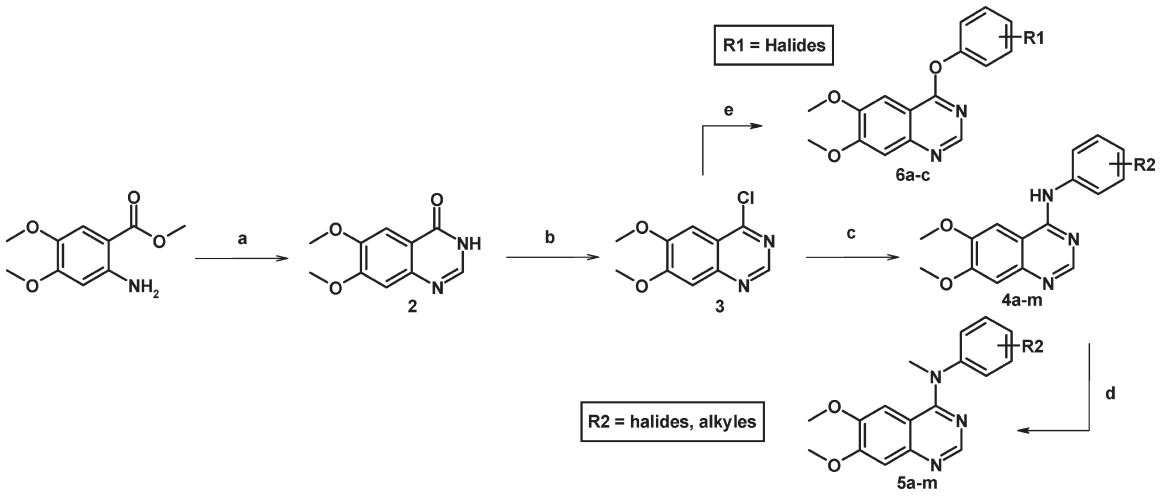


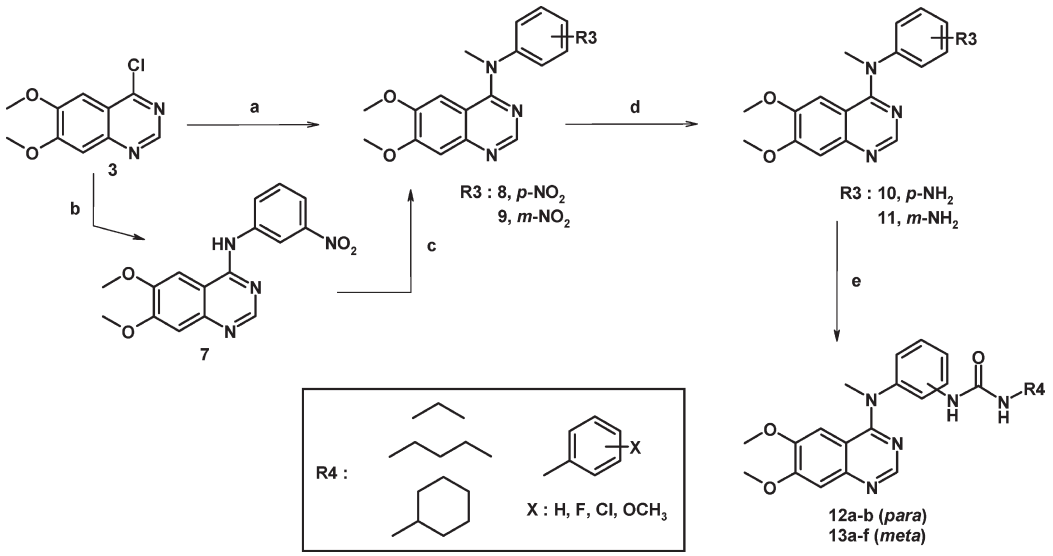
Figure 2. Series of compounds synthesized from 4-chloro-6,7-dimethoxyquinazoline **3** or 4-chloro-6,7-methylenedioxyquinazoline **19**.

Scheme 1[a]



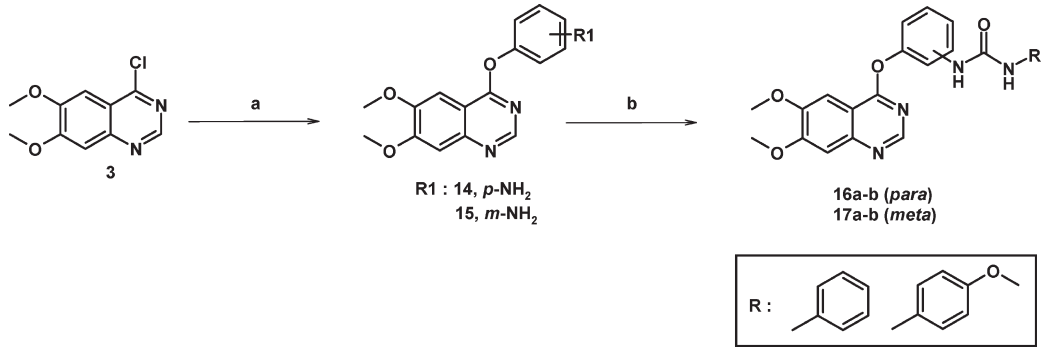
[a] Reagents and conditions: (a) NaOMe, $HCONH_2$, DMF/MeOH, 110 °C, 85%; (b) $POCl_3$, reflux, 92%; (c) aniline derivatives, $(CH_3)_2CHOH$, reflux, 80−95%; (d) CH_3I, NaH, DMF, room temperature, 25−40%; (e) phenol derivatives, NaH, DMSO, 150 °C, 100 W, microwave, 59−65%.

Scheme 2[a]



[a] Reagents and conditions: (a) *N*-methyl-4-nitroaniline, $(CH_3)_2CHOH$, reflux, 47%; (b) 3-nitroaniline, $(CH_3)_2CHOH$, reflux, 92%; (c) CH_3I, NaH, DMF, room temperature, 77%; (d) Raney Ni, H_2, MeOH, room temperature, 87% for **10** and 92% for **11**; (e) isocyanate derivatives, C_5H_5N, H_2O/THF, room temperature, 27−65%.

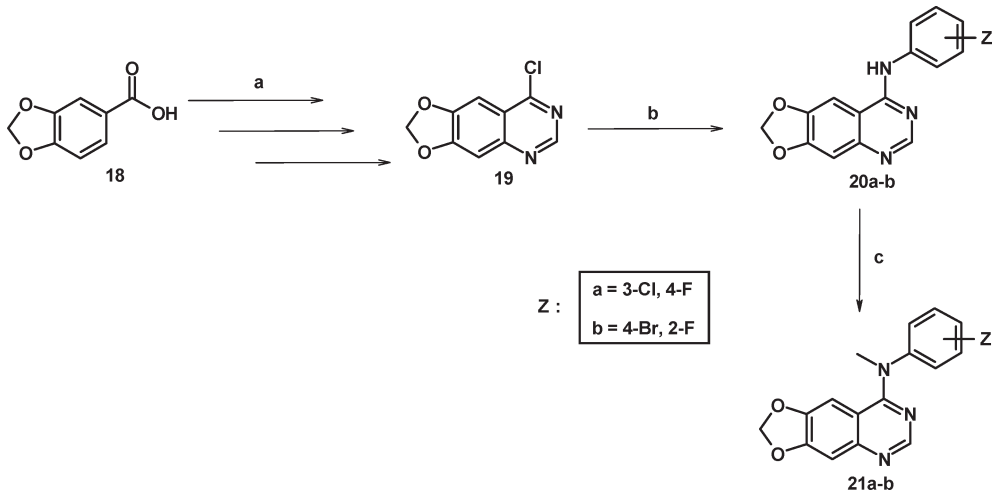
Scheme 3[a]



[a] Reagents and conditions: (a) *n*-$Bu_4N^+Br^-$, 2-butanone, 20% NaOH, reflux, 80% for **14** (with 4-nitrophenol) and 93% for **15** (with 3-nitrophenol); (b) isocyanate derivatives, NEt_3, $CHCl_3$, room temperature, 25−60%.

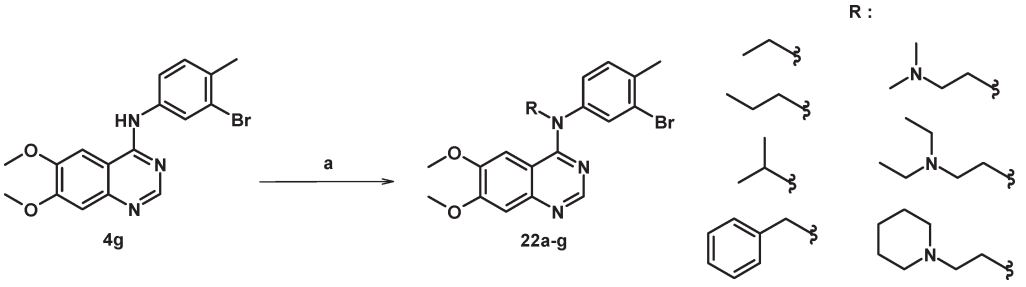
The synthesis of aniline derivatives **20a**,**b** and **21a**,**b** is outlined in Scheme 4. For the synthesis of 4-chloro-6,7-methylenedioxyquinazoline **19**, we employed a synthetic route according to described procedures[16] using piperonilic acid **18** as a starting

Scheme 4[a]



[a] Reagents and conditions: (a) (i) $SOCl_2$, MeOH, reflux, 70%; (ii) HNO_3, $SnCl_4$, CH_2Cl_2, −70 °C, 67%; (iii) Raney Ni, H_2, MeOH, room temperature, 70%; (iv) NaOMe, $HCONH_2$, DMF/MeOH, 110 °C, 84%; (v) $POCl_3$, reflux, 94%; (b) aniline derivatives, $(CH_3)_2CHOH$, reflux, 82−91%; (c) CH_3I, NaH, DMF, room temperature, 38−47%.

Scheme 5[a]



[a] Reagents and conditions: (a) R-I or R-Br or R-Cl, NaH, DMF, room temperature, 18−25%.

material. Obtention of the final 4-anilino-6,7-methylenedioxyquinazoline derivatives **20a**,**b** and **21a**,**b** was performed according to the same synthetic strategy: nucleophilic displacement of the chlorine atom with various arylamino groups in 2--propanol gave **20a**,**b**, and N-methylation reaction of these compounds provided **21a**,**b**.

From the bromide derivatives **4g**, a series of compounds was synthesized by modulation of the *N*-methyl group by alkyl- (**22a**−**d**) or cationic side chain (**22e**−**g**) to explore putative additional interaction site (Scheme 5).

3. Results and Discussion

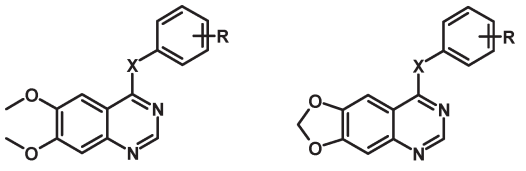
3.1. DNA-Binding Studies. A previous study performed with **1b** had demonstrated the occurrence of an intercalative binding process upon complex formation with DNA.[12] Similarly, we studied the capacity of the new quinazoline derivatives to bind to the DNA macromolecule. We engaged different methods to study the binding mode to DNA for the newly synthesized compounds, with the aim to characterize the DNA-binding process and measure the affinity of the compounds for DNA.

The ability of the drugs to protect calf thymus DNA (ctDNA) or the synthetic AT polynucleotide (poly $(dAdT)_2$) against thermal denaturation was used as an indicator of their capacity to bind DNA and to stabilize the DNA double strand. The variations of the temperature melting (T_m) values ($\Delta T_m = T_m^{\text{Drug-DNAcomplex}} - T_m^{\text{DNAalone}}$) are presented in Table 1 using a drug/DNA base pair ratio of 1.

ΔT_m values for **1b** are 8.1 and 12.2 °C for ctDNA and poly$(dAdT)_2$, respectively. These results are in agreement with our previous data. For the anilino (**4**b−**m**, **20a**,**b**) and aryloxy (**6a**−**c**, **16a**,**b**, **17a**,**b**) series, the low ΔT_m values (< 5 °C) indicate that these compounds have a weak DNA affinity. In the *N*-methyl analogue series (**5a**−**m**, **13a**−**f**, **21a**,**b**), the values range from 1.4 to 8.9 °C for ctDNA and from 3.3 to 15.0 °C for poly$(dAdT)_2$ (except for **12a**,**b**). This observation indicates that the substitution by a methyl group leads to compounds that stabilize DNA against heat denaturation. The 4-anilino-6,7-methylenedioxyquinazoline skeleton (series B), as in **21a**,**b**, is not adapted to stabilize the DNA duplex structure (ΔT_m < 5 °C) unlike the 6,7-dimethoxyquinazoline core (0 °C < ΔT_m < 15 °C) (series A).

Very interesting data were obtained with meta-position of halogens on the aniline ring. We demonstrated that this meta-position is more favorable than the para-position. Bisubstitution by halogens and methyl group (**5g**−**j**) was characterized by the best ΔT_m values especially for bromide (**5g**) and chloride (**5h**) with ΔT_m values for **5g** of 8.4 and 15 °C for ctDNA and poly$(dAdT)_2$, respectively (Table 1). A larger ΔT_m value was observed for **5g** compared to **1b**. Then the bisubstitution was considered as a positive element to reinforce DNA interaction. In addition, no significative differences of ΔT_m values were observed by replacement of bromine

Table 1. [a]



compd	series	X	R	ΔT_m (in °C) drug/DNA ratio in BPE buffer ctDNA	poly(dA-dT)$_2$
1a	A	NH	3-Br	0.2	0.4
1b	A	N−CH_3	3-Br	8.1	12.2
4a (AG14035)	A	NH	3-Cl	1.8	0.3
4b	A	NH	4-Br	3.9	0.2
4c	A	NH	3-F	0.2	0.4
4d	A	NH	3-CF_3	0.2	0.5
4e	A	NH	4-CF_3	0.1	0.5
4f	A	NH	3-Me	0.3	0.3
4g	A	NH	3-Br-4-Me	0.2	0.4
4h	A	NH	3-Cl-4-Me	0.3	0.4
4i	A	NH	3-F-4-Me	0.2	0.5
4j	A	NH	3-CF_3-4-Me	0.4	0.5
4k	A	NH	2,5-Br	0.4	0.5
4l	A	NH	3-Cl-4-F	0.2	0.4
4m	A	NH	4-Br-2-F	0.1	0.3
5a	A	N−CH_3	3-Cl	7.7 ± 0.6	12.4 ± 0.7
5b	A	N−CH_3	4-Br	6.0 ± 0.8	10.8 ± 0.6
5c	A	N−CH_3	3-F	7.3 ± 0.6	9.8 ± 0.8
5d	A	N−CH_3	3-CF_3	5.8 ± 0.5	9.5 ± 0.5
5e	A	N−CH_3	4-CF_3	6.5 ± 0.6	9.1 ± 0.6
5f	A	N−CH_3	3-Me	7.1 ± 0.7	9.7 ± 0.7
5g	A	N−CH_3	3-Br-4-Me	8.4 ± 0.6	15.0 ± 0.8
5h	A	N−CH_3	3-Cl-4-Me	8.9 ± 0.5	13.7 ± 0.6
5i	A	N−CH_3	3-F-4-Me	7.3 ± 0.5	11.3 ± 0.5
5j	A	N−CH_3	3-CF_3-4-Me	7.8 ± 0.6	11.0 ± 0.6
5k	A	N−CH_3	2,5-Br_2	1.6	3.3
5l	A	N−CH_3	3-Cl-4-F	6.1 ± 0.6	10.5 ± 0.7
5m	A	N−CH_3	4-Br-2-F	1.4	3.3
6a	A	O	3-Br	0.7	0.7
6b	A	O	3-Cl-4-F	1.2	0.5
6c	A	O	4-Br-2-F	0.8	0.5
12a	A	N−CH_3	*p*-NHCONH-(phenyl)	0.4	0.6
12b	A	N−CH_3	*p*-NHCONH-(butyl)	0.3	0.4
13a	A	N−CH_3	*m*-NHCONH-(phenyl)	6.0 ± 0.5	9.9 ± 0.8
13b	A	N−CH_3	*m*-NHCONH-(4-methoxyphenyl)	6.8 ± 0.5	11.0 ± 0.6
13c	A	N−CH_3	*m*-NHCONH-(3-chloro-4-fluorophenyl)	7.7 ± 0.6	10.5 ± 0.8
13d	A	N−CH_3	*m*-NHCONH-(ethyl)	5.2 ± 0.6	7.9 ± 0.6
13e	A	N−CH_3	*m*-NHCONH-(butyl)	5.7 ± 0.5	8.1 ± 0.7
13f	A	N−CH_3	*m*-NHCONH-(cyclohexyl)	5.7 ± 0.7	9.7 ± 0.6
16a	A	O	*p*-NHCONH-(phenyl)	0.4	0.5
16b	A	O	*p*-NHCONH-(4-methoxyphenyl)	0.3	0.5
17a	A	O	*m*-NHCONH-(phenyl)	0.4	0.5
17b	A	O	*m*-NHCONH-(4-methoxyphenyl)	0.2	0.3
20a	B	NH	3-Cl-4-F	0.3	0.4
20b	B	NH	4-Br-2-F	0.3	0.5
21a	B	N−CH_3	3-Cl-4-F	2.8	4.6
21b	B	N−CH_3	4-Br-2-F	3.6	4.2

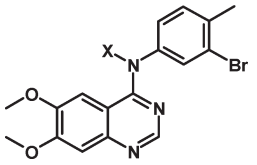
[a] Means values are calculated from at least two separate experiments with typical standard deviations of less than ±10%.

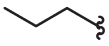
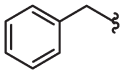
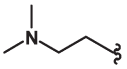
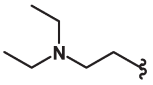
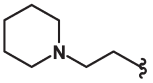
by chlorine (**5a**) on the aryl ring, whereas the introduction of F, CH_3, or CF_3 apparently reduces the DNA interaction capacity.

Introduction of a bulky substituent (**12a**,**b**, **13a**−**f**) decreases DNA interaction compared to halogen derivatives. In this series, the meta-position is also favorable when a urea group is introduced.

If we consider the compound **5g** in series A, its extent of interaction with DNA is similar to the reference compound as judged from the T_m measurements. To increase the DNA interaction, news compounds were synthesized bearing a *N*-alkyl or a cationic side chain. The addition of the *N*-alkyl substituent (ethyl, propyl, isopropyl) on the anilinoquinazoline

Table 2. [a]



Compd	X	ΔTm (in °C) drug/DNA ratio in BPE buffer	
		ctDNA	poly(dA-dT)
1b		**8.1 ± 0.5**	**12.2 ± 0.6**
5g		**8.4 ± 0.5**	**15 ± 0.7**
22a		**8.8 ± 0.5**	**13.4 ± 0.7**
22b		**7 ± 0.5**	**12.6 ± 0.8**
22c		**5.8 ± 0.5**	**12.4 ± 0.7**
22d		**6.5 ± 0.5**	**9.6 ± 0.8**
22e		**9.9 ± 0.6**	**20 ± 0.9**
22f		**14 ± 0.8**	**24.1 ± 0.8**
22g		**13.5 ± 0.7**	**27.7 ± 0.7**

[a] Means values are calculated from three separate experiments.

nucleus (compounds **22a**−**d**) did not improve the DNA interaction. However, the introduction of a cationic side chain (compounds **22e**−**g**) was found to strongly stabilize DNA (ΔT_m from 9.9 to 14 °C (ctDNA) and from 20 to 27.7 °C for poly(dAdT)$_2$) when compared with the *N*-methyl analogue **5g** (Table 2). This is most likely attributable to additional electrostatic interactions with DNA phosphodiesters groups.

The binding of four compounds, **1a**, **1b**, **5g**, and **22f** was studied by different methods to evaluate the spectroscopic changes of anilinoquinazoline chromophore (UV and CD experiments) or of DNA double helix (ΔT_m experiment). Figure 3 displays UV absorption measurements recorded upon ctDNA titration into a buffered aqueous solution of drugs. In both cases, the addition of DNA induces marked changes of the absorption spectra of **1a**, **1b**, **5g**, and **22f**. The binding to DNA remains more or less unchanged. In the medium-salt buffer, strong bathochromic and hypochromic shifts were observed with the three drugs with an absorption maximum red-shifted from 346 to 367 nm and a 2-fold decrease absorbance at 346 nm. During the titration with DNA, an isosbestic point at 362 nm was detected with the different compounds pointing to the existence of a single binding mode.

To confirm these results and to define more precisely the binding process, we deployed a spectroscopic method utilizing polarized light with circular dichroism measurements. Solutions of DNA in the presence of drugs **5g** and **22f** were analyzed by circular dichroism (CD) and compared with reference compounds (**1a** and **1b**). Circular dichroism measurements showed that a negative band is centered at 350−370 nm with **1b**, **5g**, and **22f** (Figure 4). Such a typical negative CD in the absorption band of the ligand is consistent with an intercalative DNA binding mode for these compounds. Under identical conditions with a low-salt medium (1 mM sodium cacodylate buffer, pH 7.0), the reduced dichroism of **1a** showed a 10 nm blue-shifted negative band. In addition, for **5g**, a decrease of the CD band at 330−340 nm is concomitant to an increase of the CD amplitude at 350−370 nm, with a relatively well resolved isodichroic crossover. This type of CD behavior may be assigned to excitonic coupling between adjacent intercalated molecules. This binding mode was observed with other intercalative agents.[17,18] At low binding ratios, a weak monomeric CD with the same shape as the absorption curve is observed, while at higher binding ratios, a strong exciton CD dominates because of interaction between pairs of molecules. For all tested compounds, a weak negative induced CD was observed, and this is consistent with an intercalative mode of binding to DNA, in particular for compound **5g**.

3.2. Fluorescence Measurements. Binding affinities for compounds **1b**, **5g**, and **22f** were quantified by means of intrinsic fluorescence of the compounds (Table 3). The fluorescence emission at 436 nm is weak when the drug is free in solution, but it is significantly enhanced when the drug is bound to DNA. The intrinsic fluorescence variation induced by DNA titration allows the determination of the apparent binding (K_D) constant using nonlinear least-squares analysis (GraphPad Prism software).

The data summarized in Table 3 attest that DNA ligand **5g** is a potent DNA binder, with an apparent binding constant of the same order as that of the reference **1b**. The binding affinity of **22f** is stronger compared to the K_D value calculated for **1b**. This molecule provides better stabilization of the DNA complex, as mentioned above using the T_m measurements. Compound **22f** displayed the highest ΔT_m value and the lowest K_D value, both indicating a marked duplex stabilization and a good affinity for DNA. These results led to the assumption that the introduction of cationic side chain triggers an additional mode of binding implicating the phosphate groups of DNA. In the case of **1a**, the changes of the fluorescence emission at 420 nm were very weak. Under such conditions, attempts to estimate the binding constant are generally doomed to failure.

3.3. DNA Sequence Selectivity. Sequence-specific DNA binding properties were determined by the DNase I footprinting methodology, using a radiolabeled 265-bp DNA restriction fragment as a substrate. A complete set of typical autoradiographs of the sequencing gels used to fractionate the products of partial digestion of DNA fragment complexed with the tested compounds is presented in Figure 5. These quinazoline derivatives strongly affected the cleavage of the DNA substrates by the nuclease. Visual inspections of the gels suffice to conclude that the synthesized compounds are sequence-selective binders, similar to **1b**.

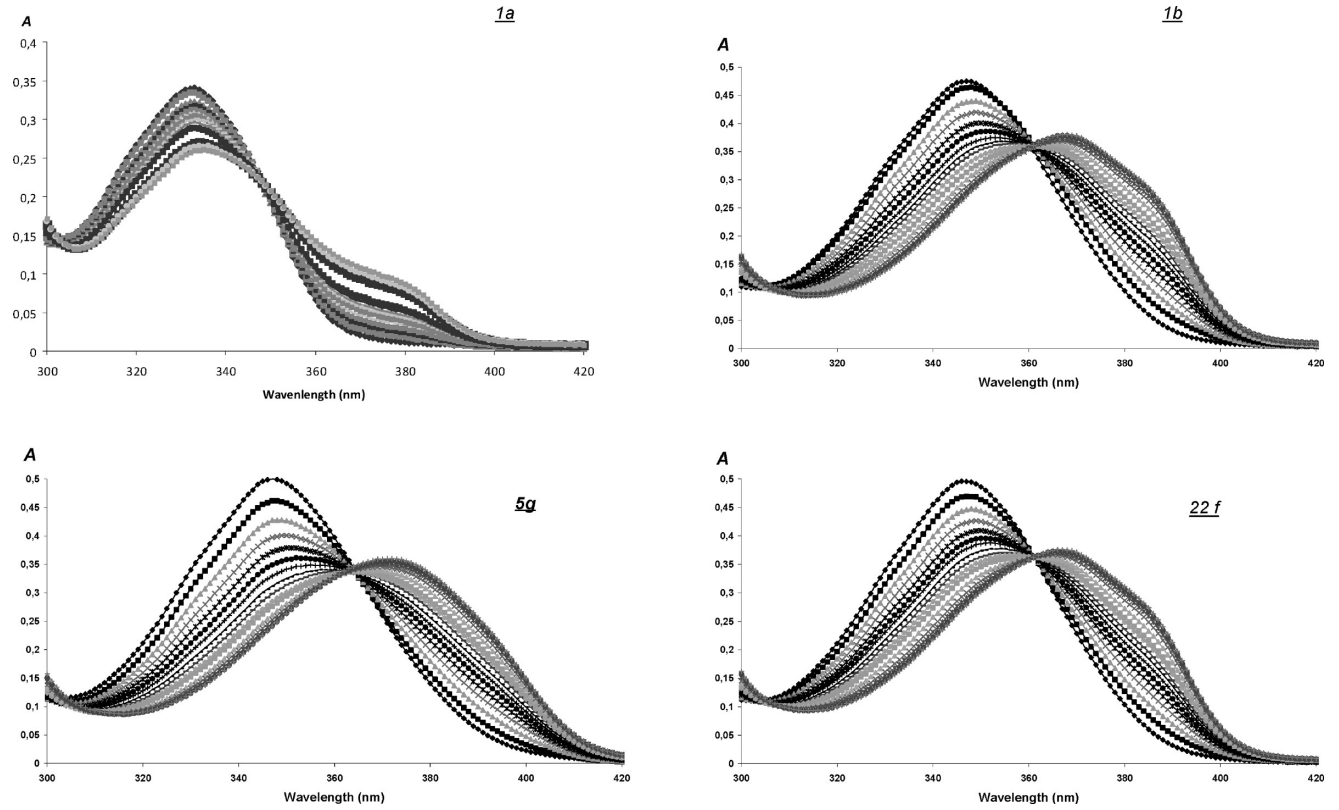


Figure 3. UV measurements. CT-DNA titrations of **1a**, **1b**, **5g**, and **22f**. The phosphate-DNA/drug ratio increased from 0 to 10 (top to bottom curves, $\lambda = 346$ nm).

A densiometry analysis of the autoradiograph obtained with the 265 bp fragments from plasmid pBS is presented in Figure 6. In the presence of drugs, several regions of attenuated DNA cleavage can be discerned around positions 43, 71, and 91. All footprintings coincide with the position of nucleotide sequences with a high GC content. These regions of drug-induced enhanced cleavage all correspond to AT-rich sequence, which is attributable to intercalation-induced perturbations of the double-helical structure of DNA. Plots show that compounds interact preferentially with sequences essentially composed of two or more consecutive G−C base pairs. Like most intercalating drugs, anilinoquinazoline derivatives strongly discriminate between runs of adenines or thymines.

3.4. Topoisomerase I Inhibition. A conventional DNA relaxation assay was used to access the effects of the compounds on the catalytic activity of human topoisomerase I. This enzyme introduces transient nicks in DNA at specific sites, leading to relaxation of the DNA helix. Incubation of the DNA−topoisomerase I mixture in the absence and in the presence of the test drugs results in different distribution of topoisomers which can be revealed by agarose gel electrophoresis.[19] Typical gels are presented in Figure 7. **1a** has practically no effect on the unwinding of DNA mediated by human topoisomerase I. In contrast, other products, in particular **5g**, induce a shift of the topoisomer distribution, indicating that these compounds affect the superhelical density of the plasmid. These topoisomerization assays thus reveal that these compounds unwind closed circular duplex DNA. These effects are typical of intercalating agents. These results suggest also that none of these molecules act as a poison for human topoisomerase. Cleavage experiments indicated no stabilization of the covalent complexes between DNA and topoisomerase I or II (data not shown).

3.5. In Vitro Antiproliferative Activity. The antiproliferative activities of the compounds were tested (Table 4) using three cancer cell lines: PC3 (hormono-independent prostate cancer), HT-29 (colon cancer), and MCF-7 (breast cancer).

Introduction of a methyl group on the aniline core (**5g**) induces a detectable antiproliferative effect more or less identical at **1b** (6.1 and 9.2 μM on HT-29 and MCF-7 cancers cells, respectively). The data indicate that molecules containing a cationic side chain exert an antiproliferative effect on the cell lines tested here, with IC_{50} values in the micromolar range. Compound **22f** emerges as the most cytotoxic agent in this series ($IC_{50} = 5.6$ μM in HT-29 cell line). **1b** has an inhibitory effect less pronounced than that of the aminoalkyl derivatives. The introduction of a cationic side chain tends to favor the antiproliferative activity.

4. Conclusion

Different conjugated structures were obtained by linking the planar quinazoline core with different aniline groups. Previous studies on DNA interaction of the tyrosine protein kinase inhibitor **1a** and its *N*-methyl analogue **1b** led to synthesis of new molecules which were evaluated as cytotoxic and DNA intercalating agents. It was shown that the methylation of the anilino nitrogen of quinazoline derivatives is essential to obtain an intercalating effect. This conformation must facilitate the interaction of the drug with DNA and confers sequence selectivity, compared to the corresponding anilino or aryloxy derivatives. The affinity of the N-methylated anilinoquinazoline derivatives for DNA is relatively weak compared to classical potent intercalators such ethidium

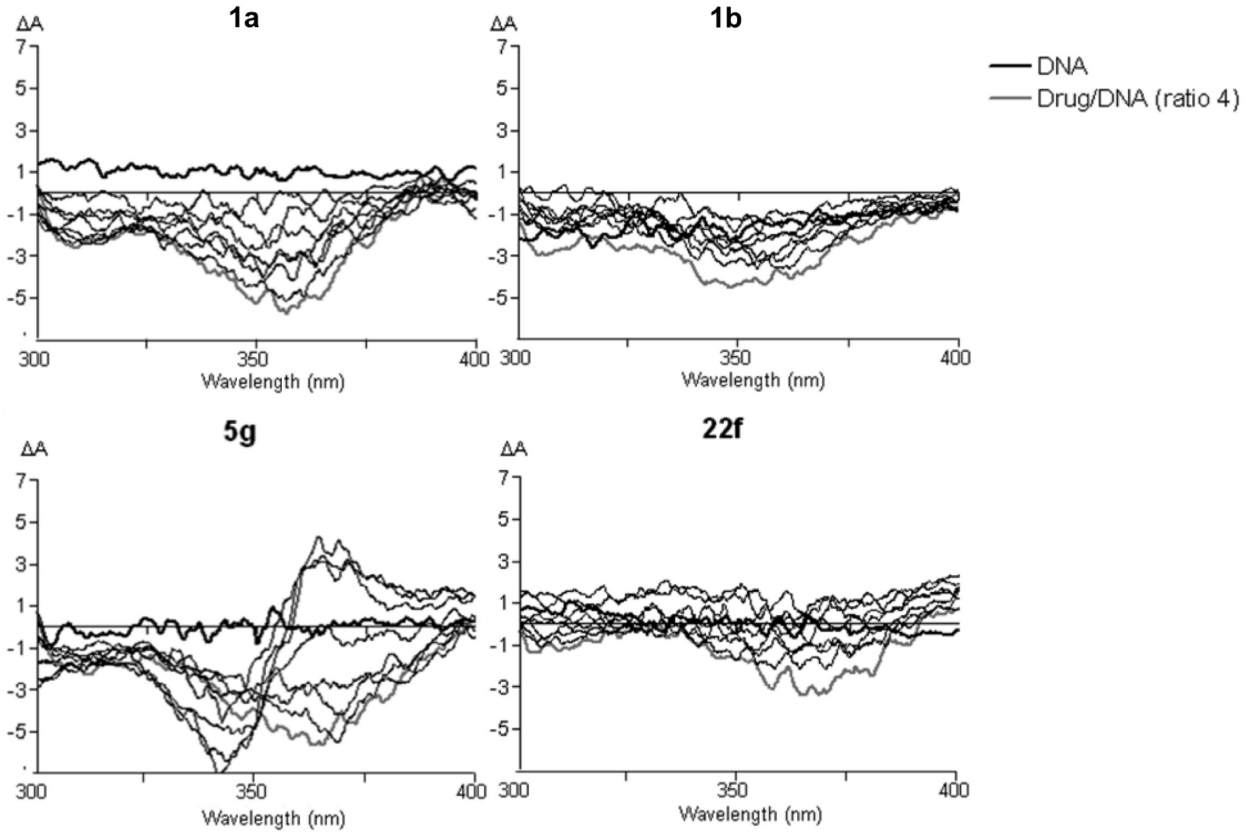


Figure 4. Circular dichroism measurements. CT-DNA titrations of **1a**, **1b**, **5g**, and **22f**. The DNA/drug ratio increased as follows from ratio 0 to 4.

bromide, but nevertheless, the DNA-binding studies confirm that the quinazoline core is a suitable carrier, allowing an effective DNA intercalative process. In this study, an additional cationic side chain was introduced on the anilino group substituting the quinazoline core. The designed molecules generally show an increased DNA interaction consistent with the formation of additional electrostatic contacts between the drug and the DNA receptor. Interestingly, a satisfactory correlation was observed between the extent of DNA interaction and the antiproliferative activity. Novel quinazoline molecules with a superior bioactivity profile compared to the reference compound **1b** have been described, and the objective is thus fully reached. Further development of these series should include another heterocycle as quinolein or acridine for more systematic structure−activity studies.

5. Experimental section

5.1. Chemistry. Melting points were determined with a Büchi 535 capillary melting point apparatus and are uncorrected. Kieselgel 60 F-254 commercial plates were used for analytical TLC as well as UV light and/or with iodine to follow the course of the reaction. Flash chromatography (FC) was performed with silica gel Kieselgel Si 60, 0.063−0.200 mm (Merck). The structure of each compound was confirmed by IR (Bruker VECTOR 22 instrument) and by ^{1}H NMR (300 MHz, Bruker AC300P spectrometer). Chemicals shifts (δ) are reported in ppm downfield from TMS. J values are in hertz, and the splitting patterns are designed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. APCI$^+$ (atmospheric pressure chemical ionization) mass spectra were obtained on an LC−MS system Thermo Electon Surveyor MSQ. Purity of the tested compounds was >95% using interpretation of a combination of LC−MS and NMR data.

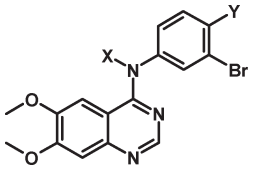
The synthesis of many compounds has been reported: **1b**[14] and **16a**,**b**.[15]

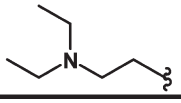
5.1.1. 4-Chloro-6,7-dimethoxyquinazoline (3).[15] To a solution of methyl-2-amino-4,5-dimethoxybenzoate (4.0 g, 19.0 mmol) in *N*,*N*-dimethylformamide (40 mL) and methanol (10 mL), formamide (76.0 mmol) and sodium methoxide (54.0 mmol) were added. The resulting mixture was refluxed for 16 h. After the reaction was quenched by water (100 mL), the mixture was neutralized by 1 M HCl solution. The precipitated was collected by filtration, washed with H_2O (30 mL) and Et_2O (30 mL), and dried in vacuo to give quinazolinone **2** as a white solid (82%) which used directly in the next step. A mixture of **2** (3.0 g, 15.0 mmol) and phosphorus oxychloride (30 mL) was refluxed for 2 h. After removal of the solvent, the residue was dissolved in ice−water (50 mL) and the mixture was neutralized by ammonium hydroxide. The precipitate was collected by filtration and dissolved in CH_2Cl_2 (100 mL). The organic layer was washed with a 1 M solution of K_2CO_3 (3 × 40 mL), brine (1 × 40 mL) and dried over $CaCl_2$, and the solvent was removed under reduced pressure. Spectroscopic data for compound **3** are in agreement with the data reported in the literature.[15]

5.1.2. General Procedure for Nucleophilic Substitution by Aniline (4a−m). 4-Chloro-6,7-dimethoxyquinazoline **3** (0.30 g, 1.34 mmol) was dissolved in refluxing 2-propanol (15 mL), and commercial aniline (1.60 mmol) was added dropwise. After 3 h, the precipitate was filtered off and washed with 2-propanol (10 mL) and Et_2O (10 mL). Spectroscopic data and melting point for compounds **4a** (AG14035),[20] **4b**,[21] **4c**,[21] **4d**,[20] **4f**,[22] and **4l**[23] are in agreement with those reported in the literature.

4-(4-Trifluoromethylanilino)-6,7-dimethoxyquinazoline Hydrochloride (4e). Crystallization from *N*,*N*-Dimethylformamide gave pure **4e** as a white solid (91%). Mp >250 °C. IR cm^{-1} 2467 (NH$^+$), 1320 (CF_3), 1076 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 11.72 (s, 1H, NH$^+$), 8.85 (s, 1H, ArH), 8.47 (s, 1H, ArH), 8.03 (d, J = 8.40 Hz, 2H, ArH), 7.82 (d, J = 8.40 Hz, 2H, ArH), 7.40 (s,

Table 3. [a]



Compd	X	Y	K_D (10^{-5} M)
1a	H	H	ND[b]
1b	-CH_3	H	21.4 ± 1.80
5g	-CH_3	-CH_3	32.62 ± 2.10
22f		-CH_3	4,02 ± 0.31

[a] The values are averages of three independent experiments. [b] ND: not determined. The weak fluorescence properties of **1a** could not allow us to determine an apparent binding constant.

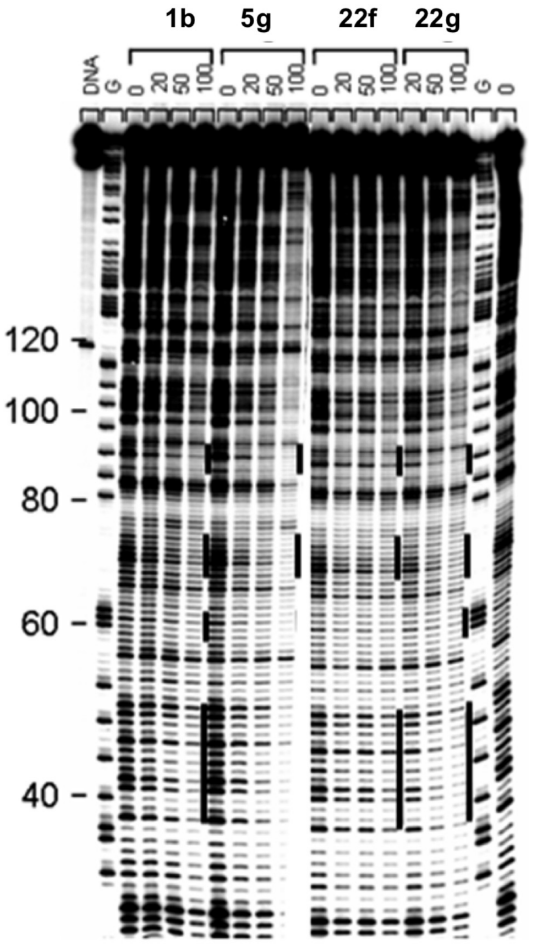


Figure 5. DNase I footprinting assay. Radiolabeled 265 bp DNA fragment was incubated alone (lane "DNA") or graded concentrations of the various compounds from 0 to 100 μM (as indicated at the top of each lane). The G track lanes labeled "G" were used as markers for guanines to locate the footprint areas and to establish the scale indicated from 40 to 120 bp. Vertical black lines localize the sites protected on the gel.

1H, ArH), 4.01 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{14}F_3N_3O_2$, *m/z*: 350 $(M + H)^+$.

4-(3-Bromo-4-methylanilino)-6,7-dimethoxyquinazoline Hydrochloride (4g). Crystallization from ethanol 95% gave pure **4g** as a white solid (95%). Mp >250 °C. IR cm^{-1} 2465 (NH^+), 1076 (C−O−C methoxy), 1056 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 11.42 (s, 1H, NH^+), 8.85 (s, 1H, ArH), 8.32 (s, 1H, ArH), 8.02 (s, 1H, ArH), 7.69 (d, J = 7.40 Hz, 1H, ArH), 7.42 (d, J = 7.40 Hz, 1H, ArH), 7.31 (s, 1H, ArH), 4.00 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 2.37 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{16}BrN_3O_2$, *m/z*: 374 [$(M + H)^+$ for ^{79}Br] and 376 [$(M + H)^+$ for ^{81}Br].

4-(3-Chloro-4-methylanilino)-6,7-dimethoxyquinazoline Hydrochloride (4h). Crystallization from *N*,*N*-dimethylformamide gave pure **4h** as a white solid (87%). Mp >250 °C. IR cm^{-1} 2466 (NH^+), 1088 (C−Cl), 1078 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 11.51 (s, 1H, NH^+), 8.82 (s, 1H, ArH), 8.38 (s, 1H, ArH), 7.88 (d, J = 2.10 Hz, 1H, ArH), 7.68 (dd, J = 2.10, 8.50 Hz, 1H, ArH), 7.45 (d, J = 8.50 Hz, 1H, ArH), 7.32 (s, 1H, ArH), 4.02 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{16}ClN_3O_2$, *m/z*: 330 [$(M + H)^+$ for ^{35}Cl] and 332 [$(M + H)^+$ for ^{37}Cl].

4-(3-Fluoro-4-methylanilino)-6,7-dimethoxyquinazoline Hydrochloride (4i). Crystallization from *N*,*N*-dimethylformamide gave pure **4i** as a white solid (95%). Mp >250 °C. IR cm^{-1} 2466 (NH^+), 1221 (C−F), 1076 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 11.51 (s, 1H, NH^+), 8.83 (s, 1H, ArH), 8.38 (s, 1H, ArH), 7.66 (dd, J = 2.10, 7.70 Hz, 1H, ArH), 7.51 (dd, J = 2.00, 8.30 Hz, 1H, ArH), 7.36 (m, 2H, ArH), 4.01 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 2.28 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{16}FN_3O_2$, *m/z*: 314 $(M + H)^+$.

4-(3-Trifluoromethyl-4-methylanilino)-6,7-dimethoxyquinazoline Hydrochloride (4j). Crystallization from *N*,*N*-dimethylformamide gave pure **4j** as a white solid (81%). Mp >250 °C. IR 2467 cm^{-1} (NH^+), 1324 (CF_3), 1079 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 11.51 (s, 1H, NH^+), 8.84 (s, 1H, ArH), 8.41 (s, 1H, ArH), 7.88 (d, J = 2.30 Hz, 1H, ArH), 7.45 (m, 2H, ArH), 7.34 (s, 1H, ArH), 4.01 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 2.34 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{18}H_{16}F_3N_3O_2$, *m/z*: 364 $(M + H)^+$.

4-(2,5-Dibromoanilino)-6,7-dimethoxyquinazoline Hydrochloride (4k). Crystallization from EtOH 95% gave pure **4k** as a white solid (95%). Mp >250 °C. IR cm^{-1} 2466 (NH^+), 1076 (C−O−C methoxy), 1059 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 11.71 (s, 1H, NH^+), 8.80 (s, 1H, ArH), 8.29 (s, 1H, ArH), 7.83 (d, J = 2.40 Hz, 1H, ArH), 7.79 (d, J = 8.60 Hz, 1H, ArH), 7.59 (dd, J = 2.40, 8.60 Hz, 1H, ArH), 7.39 (s, 1H, ArH), 4.02 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3). LC−MS ($APCI^+$), calcd for $C_{16}H_{13}Br_2N_3O_2$, *m/z*: 438 [$(M + H)^+$ for ^{79}Br] to 440 [$(M + H)^+$ for ^{79}Br and ^{81}Br] and 442 [$(M + H)^+$ for ^{81}Br].

4-(4-Bromo-2-fluoroanilino)-6,7-dimethoxyquinazoline Hydrochloride (4m). Crystallization from EtOH/cyclohexane gave pure **4m** as a white solid (87%). Mp >250 °C. IR cm^{-1} 2467 (NH^+), 1221 (C−F), 1076 (C−O−C methoxy), 1058 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 11.69 (s, 1H, NH^+), 8.82 (s, 1H, ArH), 8.31 (s, 1H, ArH), 7.79 (d, 1H, ArH), 7.60−7.50 (m, 2H, ArH), 7.41 (s, 1H, ArH), 4.02 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3). LC−MS ($APCI^+$), calcd for $C_{16}H_{13}BrFN_3O_2$, *m/z*: 378 [$(M + H)^+$ for ^{79}Br] and 380 [$(M + H)^+$ for ^{81}Br].

5.1.3. General Procedure for N-Alkylation (5a−m). A mixture of the N-substituted 6,7-dimethoxyquinazoline **4a−m** (0.60 mmol)

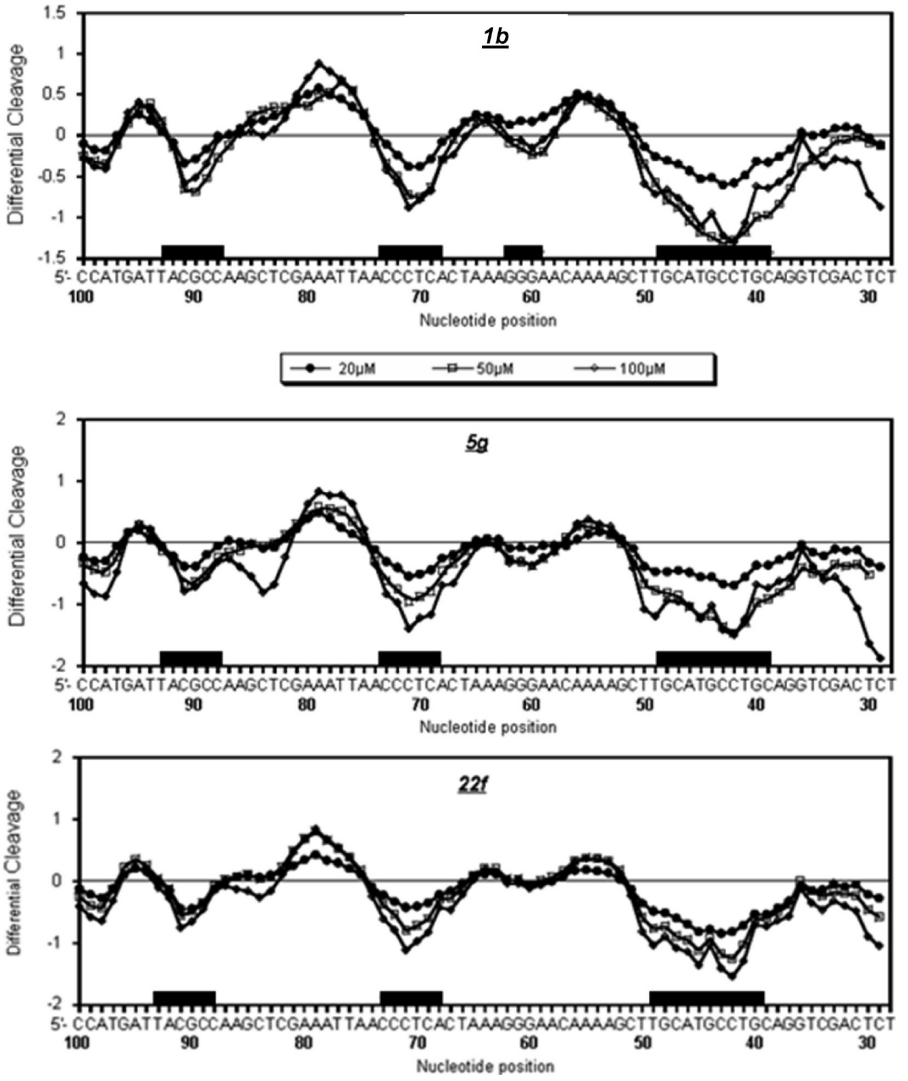


Figure 6. Densitometric analysis. Differential cleavage plots derived from the footprinting assay. Black boxes localize the sites protected on the gel.

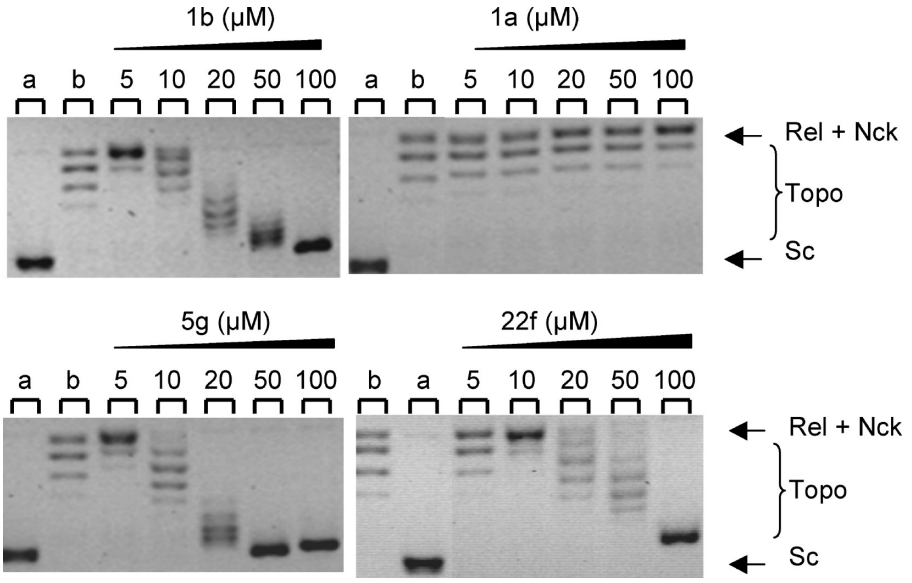
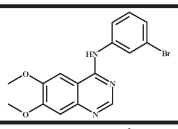
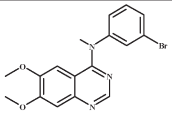
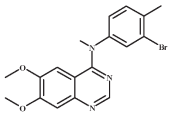
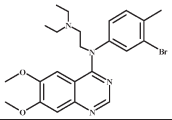


Figure 7. Topoisomerase I relaxation assay. Native supercoiled pUC19 (lane a) was incubated with 6 units of topoisomerase I in the absence (lane b) or in the presence of test compounds at the indicated concentration: Rel, relaxed; Nck, nicked; Topo, topoisomer products; Sc, supercoiled.

Table 4. [a]

Compd	Structure	Cell lines IC_{50} (μM)		
		PC3	HT-29	MCF-7
1a		> 10	10.1 ± 0.9	4.7 ± 0.4
1b		> 10	6.1 ± 0.5	9.2 ± 0.8
5g		9.5 ± 0.9	6.3 ± 0.6	9.9 ± 1.0
22f		> 10	5.6 ± 0.5	6.8 ± 0.7

[a] The cell growth rate was evaluated by performing the MTS assay (readout time: 72 h). Means values ± SD correspond to three independent experiments.

and sodium hydride (60% in oil) (1.20 mmol) in *N*,*N*-dimethylformamide (10 mL) was stirred 5 h at room temperature in a nitrogen atmosphere. Iodomethane (1.20 mmol) was added, and the mixture was stirred for 16 h. The reaction was quenched by water, and then the aqueous solution was extracted with EtOAc (3 × 40 mL), washed with a solution of $NaHCO_3$, and dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by FC (CH_2Cl_2/MeOH, 9:1) to give a white or yellow solid. Spectroscopic data and melting points for compounds **5a**, **5d**, **5f** are in agreement with those reported in the literature, and they were prepared by nucleophilic substitution using the commercial *N* -methylaniline. [24]

4-(*N*-Methyl-4-bromoanilino)-6,7-dimethoxyquinazoline (5b). Crystallization from toluene/petroleum ether gave pure **5b** as yellow solid (35%). Mp 205−207 °C. IR cm^{-1} 2957 (N−CH_3), 1076 (C−O−C methoxy), 1056 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 8.02 (s, 1H, ArH), 7.68 (s, 1H, ArH), 7.37 (d, J = 8.40 Hz, 2H, ArH), 7.02 (d, J = 8.40 Hz, 2H, ArH), 6.92 (s, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.67 (s, 3H, NCH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{16}BrN_3O_2$, m/z: 374 [(M + H)$^+$ for 79Br] and 376 [(M + H)$^+$ for ^{81}Br].

4-(*N*-Methyl-3-fluoroanilino)-6,7-dimethoxyquinazoline (5c). Crystallization from toluene/petroleum ether gave pure **5c** as yellow solid (29%). Mp 177−178 °C. IR cm^{-1} 2958 (N−CH_3), 1228 (C−F), 1077 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 7.92 (s, 1H, ArH), 3.63 (s, 3H, NCH_3), 7.65 (s, 1H, ArH), 7.20 (m, 1H, ArH), 6.91 (s, 1H, ArH), 6.80−6.65 (m, 3H, ArH), 3.97 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3). LC−MS ($APCI^+$), calcd for $C^{17}H^{16}FN^3O^2$, m/z: 314 (M + H)$^+$.

4-(*N*-Methyl-4-trifluoromethylanilino)-6,7-dimethoxyquinazoline (5e). Crystallization from toluene/cyclohexane gave pure **5e** as a white solid (31%). Mp 214−215 °C. IR cm^{-1} 2955 (N−CH_3), 1321 (CF_3), 1078 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 7.97 (s, 1H, ArH), 7.67 (s, 1H, ArH), 7.53 (d, J = 8.20 Hz, 2H, ArH), 7.11 (d, J = 8.20 Hz, 2H, ArH), 6.91 (s, 1H, ArH), 3.96 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.64 (s, 3H, NCH_3). LC−MS ($APCI^+$), calcd for $C_{18}H_{16}F_3N_3O_2$, m/z: 364 (M + H)$^+$.

4-(*N*-Methyl-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (5g). Crystallization from toluene/cyclohexane gave pure **5g** as a yellow solid (28%). Mp 228−229 °C. IR cm^{-1} 2956 (N−CH_3), 1076 (C−O−C methoxy), 1056 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 7.98 (s, 1H, ArH), 7.63 (s, 1H, ArH), 7.28 (d, J = 2.10 Hz, 1H, ArH), 7.17 (d, J = 8.60 Hz, 1H, ArH), 6.94 (dd, J = 2.10, 8.60 Hz, 1H, ArH), 6.91 (s, 1H, ArH), 3.96 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.61 (s, 3H, NCH_3), 2.30 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{18}H_{18}BrN_3O_2$, m/z: 388 [(M + H)$^+$ for ^{79}Br] and 390 [(M + H)$^+$ for ^{81}Br].

4-(*N*-Methyl-3-chloro-4-methylanilino)-6,7-dimethoxyquinazoline (5h). Crystallization from heptane/ethanol gave pure **5g** as a yellow solid (27%). Mp 195−196 °C. IR cm^{-1} 2955 (N−CH_3), 1088 (C−Cl), 1076 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.01 (s, 1H, ArH), 7.65 (s, 1H, ArH), 7.19 (d, J = 8.80 Hz, 1H, ArH), 7.13 (d, J = 2.10 Hz, 1H, ArH), 6.94 (s, 1H, ArH), 6.89 (dd, J = 2.10, 8.80 Hz, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.64 (s, 3H, NCH_3), 2.27 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{18}H_{18}ClN_3O_2$, m/z: 344 [(M + H)$^+$ for ^{35}Cl] and 346 [(M + H)$^+$ for ^{37}Cl].

4-(*N*-Methyl-3-fluoro-4-methylanilino)-6,7-dimethoxyquinazoline (5i). Crystallization from toluene/cyclohexane gave pure **5i** as a yellow solid (30%). Mp 193−194 °C. IR cm^{-1} 2956 (N−CH_3), 1224 (C−F), 1076 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.02 (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.11 (t, 1H, ArH), 6.92 (s, 1H, ArH), 6.84 (dd, J = 2.20, 7.90 Hz, 1H, ArH), 6.73 (d, J = 7.90 Hz, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.65 (s, 3H, NCH_3), 2.19 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{18}H_{18}FN_3O_2$, m/z: 328 (M + H)$^+$.

4-(*N*-Methyl-3-trifluoromethyl-4-methylanilino)-6,7-dimethoxyquinazoline (5j). Crystallization from H_2O/EtOH gave pure **5j** as yellow solid (28%). Mp 204−205 °C. IR cm^{-1} 2955 (N−CH_3), 1328 (CF_3), 1076 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 7.96 (s, 1H, ArH), 7.65 (s, 1H, ArH), 7.29 (d, J = 2.00 Hz, 1H, ArH), 7.26 (d, J = 8.40 Hz, 1H, ArH), 7.16 (dd, J = 2.00, 8.40 Hz, 1H, ArH), 6.91 (s, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.63 (s, 3H, NCH_3), 2.38 (s, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{19}H_{18}F_3N_3O_2$, m/z: 378 (M + H)$^+$.

4-(*N*-Methyl-2,5-dibromoanilino)-6,7-dimethoxyquinazoline (5k). Crystallization from toluene/EtOH gave pure **5k** as yellow solid (77%). Mp >250 °C. IR cm^{-1} 2958 (N−CH_3), 1078 (C−O−C methoxy), 1058 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 7.98 (s, 1H, ArH), 7.68 (s, 1H, ArH), 7.47 (d, J = 8.40 Hz, 1H, ArH), 7.20 (d, J = 2.30 Hz, 1H, ArH), 7.02 (dd, J = 2.30, 8.40 Hz, 1H, ArH), 6.91 (s, 1H, ArH), 3.96 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.65 (s, 3H, NCH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{16}Br_2N_3O_2$, m/z: 453 [(M + H)$^+$for ^{79}Br] to 455 [(M + H)$^+$ for ^{79}Br and ^{81}Br] and 457 [(M + H)$^+$ for ^{81}Br].

4-(*N*-Methyl-3-chloro-4-fluoroanilino)-6,7-dimethoxyquinazoline (5l). Crystallization from ethanol/cyclohexane gave pure **5l** as a yellow solid (39%). Mp >250 °C. IR cm^{-1} 2957 (N−CH_3), 1225 (C−F), 1086 (C−Cl), 1077 (C−O−C methoxy). ^{1}H NMR ($CDCl_3$) δ ppm 7.88 (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.22 (d, J = 7.90 Hz, 1H, ArH), 7.11 (m, 1H, ArH), 6.95 (dd, J = 2.20, 7.90 Hz, 1H, ArH), 6.58 (s, 1H, ArH), 4.01 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.61 (s, 3H, NCH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{15}ClFN_3O_2$, m/z: 348 [(M + H)$^+$ for ^{35}Cl] and 350 [(M + H)$^+$ for ^{37}Cl].

4-(*N*-Methyl-4-bromo-2-fluoroanilino)-6,7-dimethoxyquinazoline (5m). Crystallization from toluene/cyclohexane gave pure **5m** as yellow solid (29%). Mp >250 °C. IR cm^{-1} 2956 (N−CH_3), 1224 (C−F), 1078 (C−O−C methoxy), 1058 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 7.92 (s, 1H, ArH), 7.63 (s, 1H, ArH), 7.35 (dd, J = 2.00, 8.40 Hz, 1H, ArH), 7.23 (d, J = 8.40 Hz, 1H, ArH), 6.95 (m, 2H, ArH), 3.98 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.62 (s, 3H, NCH_3). LC−MS ($APCI^+$), calcd for $C_{17}H_{15}BrFN_3O_2$, m/z: 392 [(M + H)$^+$ for ^{79}Br] and 394 [(M + H)$^+$ for ^{81}Br].

5.1.4. General Procedure for Nucleophilic Substitution by the Commercial Phenol (6a−c). 4-Chloro-6,7-dimethoxyquinazoline **3** (0.20 g, 0.90 mmol) was added dropwise to a solution of the commercial phenol (1.07 mmol) and NaH (60% in oil) (1.60 mmol) in DMSO (4 mL). The mixture was placed into the cavity of a focused monomode microwave reactor and irradiated for 10 min at 150 °C (100 W). The reaction was quenched by water, and the precipitate was collected by filtration

and washed with water (20 mL). The residue was dissolved in EtOAc (50 mL), and the organic layer was washed with a 1 M solution of K_2CO_3, 1 M solution of HCl, and brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified by FC (CH_2Cl_2/EtOAc, 9:1) to give a white solid. Spectroscopic data and melting point for compound **6a** are in agreement with those reported in the literature.[25]

4-(3-Chloro-4-fluorophenoxy)-6,7-dimethoxyquinazoline (6b). Crystallization from heptane gave pure **6b** as a white solid (62%). Mp 204−205 °C. IR cm^{-1} 1223 (C−F), 1202 (C═C−O), 1086 (C−Cl), 1078 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.55 (s, 1H, ArH), 7.72 (dd, J = 2.20, 7.80 Hz, 1H, ArH), 7.55−7.50 (m, 2H, ArH), 7.45−7.35 (m, 2H, ArH), 4.02 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3). LC−MS (APCI$^+$), calcd for $C_{16}H_{12}ClFN_2O_3$, m/z: 335 [$(M + H)^+$ for ^{35}Cl] and [$(M + H)^+$ for ^{37}Cl].

4-(4-Bromo-2-fluorophenoxy)-6,7-dimethoxyquinazoline (6c). Crystallization from heptane gave pure **6b** as a white solid (59%). Mp 158−159 °C. IR cm^{-1} 1223 (C−F), 1202 (C═C−O), 1077 (C−O−C methoxy), 1058 (C−Br). ^{1}H NMR (DMSO-d_6) δ ppm 8.54 (s, 1H, ArH), 7.80 (dd, J = 2.10, 10.10 Hz, 1H, ArH), 7.51 (s, 1H, ArH), 7.55−7.45 (m, 2H, ArH), 7.37 (s, 1H, ArH), 4.02 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3). LC−MS (APCI$^+$), calcd for $C_{16}H_{12}BrFN_2O_3$, m/z: 379 [$(M + H)^+$ for ^{79}Br] and 381 [$(M + H)^+$ for ^{81}Br].

5.1.5. 4-(3-Nitroanilino)-6,7-dimethoxyquinazoline Hydrochloride (7).[15] Compound 7 was obtained by the same procedure used for **4a**−**m**. Starting from **3** (3.00 g, 13.35 mmol), a yellow solid was obtained by precipitation (92%). Spectroscopic data and melting point for this compound are in agreement with those reported in the literature.

5.1.6. 4-(*N*-Methyl-4-nitroanilino)-6,7-dimethoxyquinazoline Hydrochloride (8). Compound **8** was obtained by the same procedure used for **4a**−**m**. Starting from **3** (3.00 g, 13.35 mmol) and *N*-methyl-4-nitroaniline (2.64 g, 17.40 mmol), a yellow solid was obtained (47%). Mp 245−246 °C. IR cm^{-1} 2956 (N−CH_3), 1502 (NO_2), 1079 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 9.11 (s, 1H, ArH), 8.32 (d, J = 7.30 Hz, 2H, ArH), 7.70 (d, J = 7.30 Hz, 2H, ArH), 7.51 (s, 1H, ArH), 6.31 (s, 1H, ArH), 3.98 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.31 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{17}H_{16}N_4O_4$, m/z: 341 $(M + H)^+$.

5.1.7. 4-(*N*-Methyl-3-nitroanilino)-6,7-dimethoxyquinazoline (9). Compound **9** was obtained by the same procedure used for **5a**−**m**. Starting from **7** (2.00 g, 6.10 mmol), a yellow solid was synthesized which was recrystallized from CH_2Cl_2/petroleum ether (77%). Mp 237−238 °C. IR cm^{-1} 2957 (N−CH_3), 1502 (NO_2), 1079 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.01 (s, 1H, ArH), 7.86 (s, 1H, ArH), 7.75 (m, 1H, ArH), 7.65 (s, 1H, ArH), 7.55 (m, 2H, ArH), 6.91 (s, 1H, ArH), 3.97 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.65 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{17}H_{16}N_4O_4$, m/z: 341 $(M + H)^+$.

5.1.8. General Procedure for Catalytic Hydrogenation of Nitro Group (10, 11). Compound **8** or **9** (1.50 g, 4.40 mmol) was dissolved in methanol (50 mL), and Raney nickel (0.4 g) was added. Mixtures were stirred under hydrogen atmosphere at room temperature for 16 h. Reaction mixtures were passed through a plug of Celite, and solvents were evaporated under reduced pressure. Residues were stirred in CH_2Cl_2, filtered off, and washed with petroleum ether to give compounds **10** and **11** as yellow solids.

4-(*N*-Methyl-4-aminoanilino)-6,7-dimethoxyquinazoline Hydrocloride (10). Yield 87%. Mp 228−229 °C. IR cm^{-1} 3325 (NH_2), 2959 (N−CH_3), 1077 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.89 (s, 1H, ArH), 7.37 (s, 1H, ArH), 7.13 (d, J = 8.40 Hz, 2H, ArH), 6.72 (d, J = 8.40 Hz, 2H, ArH), 6.41 (s, 1H, ArH), 3.89 (s, 3H, OCH_3), 3.52 (s, 3H, OCH_3), 3.32 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{17}H_{18}N_4O_2$, m/z: 311 $(M + H)^+$.

4-(*N*-Methyl-3-aminoanilino)-6,7-dimethoxyquinazoline (11). Yield 92%. Mp 232−234 °C. IR cm^{-1} 3326 (NH_2), 2956 (N−CH_3), 1072 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.84 (s, 1H, ArH), 8.48 (s, 1H, ArH), 7.31 (s, 1H, ArH), 7.12 (m, 1H, ArH), 6.81 (s, 1H, ArH), 6.71 (d, J = 6.80 Hz, 1H, ArH), 6.52 (d, J = 7.60 Hz, 1H, ArH), 5.31 (s, 2H, NH_2), 4.03 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 3.95 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{17}H_{18}N_4O_2$, m/z: 311 $(M + H)^+$.

5.1.9. General Procedure for (*N*-Methylanilino)urea (12a,b). To a stirred solution of **10** (0.20 g, 0.63 mmol) and pyridine (0.16 g, 1.89 mmol) in 15 mL of a mixture H_2O/THF (5/5) were added isocyanate derivatives (1.26 mmol). After 16 h, the solvent was removed under reduce pressure and the oily residue was purified by FC ($CHCl_3$/MeOH, 8:2).

***N*-{4-[*N*-Methyl-6,7-dimethoxyquinazolin-4-ylamino]phenyl}-*N*′-phenylurea (12a).** Crystallization from cyclohexane/EtOH 95% gave pure **12a** as yellow solid (58%). Mp 110−111 °C. IR cm^{-1} 3212 (NH), 2958 (N−CH_3), 1696 (C═O), 1079 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.82 (s, 1H, NH), 8.71 (s, 1H, NH), 8.58 (s, 1H, ArH), 7.51 (d, J = 8.40 Hz, 2H, ArH), 7.45 (d, J = 8.20 Hz, 2H, ArH), 7.29 (m, 2H, ArH), 7.20 (d, J = 8.40 Hz, 2H, ArH), 7.15 (s, 1H, ArH), 6.96 (m, 1H, ArH), 6.40 (s, 1H, ArH), 3.85 (s, 3H, OCH_3), 3.52 (s, 3H, OCH_3), 3.22 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{24}H_{23}N_5O_3$, m/z: 430 $(M + H)^+$.

***N*-Butyl-*N*′-{4-[*N*-methyl-6,7-dimethoxyquinazolin-4-ylamino]-phenyl}urea (12b).** Crystallization from cyclohexane/EtOH 95% gave pure **12b** as yellow solid (46%). Mp 88 °C. IR cm^{-1} 3212 (NH), 2955 (N−CH_3), 1697 (C═O), 1075 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.61 (s, 1H, ArH), 8.55 (s, 1H, NH), 7.36 (d, J = 8.20 Hz, 2H, ArH), 7.15 (d, J = 8.20 Hz, 2H, ArH), 7.11 (s, 1H, ArH), 6.36 (s, 1H, ArH), 6.13 (s, 1H, NH), 3.88 (s, 3H, OCH_3), 3.51 (s, 3H, OCH_3), 3.21 (s, 3H, NCH_3), 3.05 (m, 2H, CH_2CH_3), 1.40−1.20 (m, 4H, CH_2CH_2), 0.98 (t, 3H, CH_2CH_3). LC−MS (APCI$^+$), calcd for $C_{22}H_{27}N_5O_3$, m/z: 410 $(M + H)^+$.

5.1.10. General Procedure for (*N*-Methylanilino)urea (13a−f). Compounds **13a**−**f** were obtained by the same procedure used for **12a**,**b**. Starting from **11** (0.20 g, 0.63 mmol) and isocyanate derivatives (1.26 mmol), the residues were purified by FC ($CHCl_3$/MeOH, 8:2).

***N*-{3-[*N*-Methyl-6,7-dimethoxyquinazolin-4-ylamino]phenyl}-*N*′-phenylurea (13a).** Crystallization from EtOH gave pure **13a** as yellow solid (50%). Mp 222−223 °C. IR cm^{-1} 3214 (NH), 2956 (N−CH_3), 1695 (C═O), 1079 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.63 (s, 1H, NH), 8.49 (s, 1H, NH), 7.88 (s, 1H, ArH), 7.76 (s, 1H, ArH), 7.50−7.25 (m, 4H, ArH), 7.20−6.90 (m, 5H, ArH), 6.53 (s, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.65 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{24}H_{23}N_5O_3$, m/z: 430 $(M + H)^+$.

***N*-{3-[*N*-Methyl-6,7-dimethoxyquinazolin-4-ylamino]phenyl}-*N*′-(4-methoxyphenyl)urea (13b).** Crystallization from EtOH gave pure **13b** as a white solid (27%). Mp 242−243 °C. IR cm^{-1} 3211 (NH), 2956 (N−CH_3), 1695 (C═O), 1078 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.91 (s, 1H, NH), 8.72 (s, 1H, NH), 8.53 (s, 1H, ArH), 7.50−7.35 (m, 4H, ArH), 7.20−6.90 (m, 5H, ArH), 6.42 (s, 1H, ArH), 3.98 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.62 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{25}H_{25}N_5O_3$, m/z: 444 $(M + H)^+$.

***N*-{3-[*N*-Methyl-6,7-dimethoxyquinazolin-4-ylamino]phenyl}-*N*′-(3-chloro-4-fluorophenyl)urea (13c).** Crystallization from EtOH 95% gave pure **13c** as yellow solid (65%). Mp 173−174 °C. IR cm^{-1} 3214 (NH), 2956 (N−CH_3), 1697 (C═O), 1223 (C−F), 1086 (C−Cl), 1078 (C−O−C methoxy). ^{1}H NMR (DMSO-d_6) δ ppm 8.87 (s, 1H, NH), 8.61 (s, 1H, NH), 7.91 (s, 1H, ArH), 7.79 (d, J = 4.40 Hz, 1H, ArH), 7.64 (s, 1H, ArH), 7.35−7.25 (m, 2H, ArH), 7.15−7.05 (m, 2H, ArH), 6.98 (d, J = 7.40 Hz, 1H, ArH), 6.87 (s, 1H, ArH), 6.58 (d, J = 7.40 Hz, 1H, ArH), 3.95 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.61 (s, 3H, NCH_3). LC−MS (APCI$^+$), calcd for $C_{24}H_{21}ClFN_5O_3$, m/z: 482 [$(M + H)^+$ for ^{35}Cl] and 484 [$(M + H)^+$ for ^{37}Cl].

***N*-Ethyl-*N*′-{3-[*N*-methyl-6,7-dimethoxyquinazolin-4-ylamino]-phenyl}urea (13d).** Crystallization from EtOH gave pure **13d** as yellow solid (37%). Mp 236−238 °C. IR cm^{-1} 3212 (NH), 2955 (N−CH_3), 1695 (C═O), 1079 (C−O−C methoxy). ^1H NMR (DMSO-d_6) δ ppm 8.95 (s, 1H, NH), 8.85 (s, 1H, ArH), 8.31 (s, 1H, ArH), 7.81 (s, 1H, ArH), 7.50−7.30 (m, 2H, ArH), 7.25−7.10 (m, 2H, ArH), 6.41 (s, 1H, NH), 4.06 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 3.97 (s, 3H, NCH_3), 3.10 (m, 2H, CH_2CH_3), 1.01 (m, 3H, CH_2CH_3). LC−MS ($APCI^+$), calcd for $C_{20}H_{23}N_5O_3$, *m*/*z*: 382 $(M + H)^+$.

***N*-Butyl-*N*′-{3-[*N*-methyl-6,7-dimethoxyquinazolin-4-ylamino]-phenyl}urea (13e).** Crystallization from cyclohexane/EtOH 95% gave pure **13e** as yellow solid (32%). Mp 180−181 °C. IR cm^{-1} 3213 (NH), 2955 (N−CH_3), 1696 (C═O), 1079 (C−O−C methoxy). ^1H NMR (DMSO-d_6) δ ppm 9.10 (s, 1H, NH), 8.88 (s, 1H, ArH), 8.35 (s, 1H, ArH), 7.79 (s, 1H, ArH), 7.40−7.15 (m, 4H, ArH), 6.51 (s, 1H, NH), 4.08 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 3.96 (s, 3H, NCH_3), 3.12 (m, 2H, CH_2CH_3), 1.40−1.25 (m, 4H, CH_2CH_2), 0.98 (m, 3H, CH_2CH_3). LC−MS ($APCI^+$), calcd for $C_{22}H_{27}N_5O_3$, *m*/*z*: 410 $(M + H)^+$.

***N*-Cyclohexyl-*N*′-{3-[*N*-methyl-6,7-dimethoxyquinazolin-4-ylamino]phenyl}urea (13f).** Crystallization from ethanol gave pure **13f** as yellow solid (42%). Mp 234−235 °C. IR cm^{-1} 3215 (NH), 2957 (N−CH_3), 1695 (C═O), 1077 (C−O−C methoxy). ^1H NMR (DMSO-d_6) δ ppm 8.68 (s, 1H, ArH), 8.49 (s, 1H, NH), 8.05 (s, 1H, ArH), 7.50 (s, 1H, ArH), 7.25−7.10 (m, 3H, ArH), 6.89 (s, 1H, ArH), 6.32 (s, 1H, NH), 4.03 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.88 (s, 3H, NCH_3), 1.70−1.10 (m, 11H, CH_2CH_2). LC−MS ($APCI^+$), calcd for $C_{24}H_{29}N_5O_3$, *m*/*z*: 436 $(M + H)^+$.

5.1.11. 3-[(6,7-Dimethoxy-4-quinazolinyl)oxy]aniline (15). A solution of **3** (2.00 g, 8.90 mmol), 3-aminophenol (1.17 g, 10.7 mmol) and tetra-*n*-butylammonium bromide (1.43 g, 4.45 mmol) in methylethylketone (20 mL) and 20% solution of NaOH (10 mL) was refluxed for 30 min. After dilution by $CHCl_3$ (100 mL) and H_2O (20 mL), the organic layer was washed with water, brine and dried over $MgSO_4$. The solvent was removed under reduced pressure and then the precipitated solid was collected by filtration and washed with MeOH (15 mL) to give **15** as a white solid (93%). Mp 203−204 °C. IR cm^{-1} 3384 (NH_2), 1205 (C═C−O), 1077 (C−O−C methoxy). ^1H NMR (DMSO-d_6) δ ppm 8.53 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.34 (s, 1H, ArH), 7.08 (m, 1H, ArH), 6.49 (m, 1H, ArH), 6.42 (m, 1H, ArH), 6.36 (m, 1H, ArH), 5.26 (s, 2H, NH_2), 4.02 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3). LC−MS ($APCI^+$), calcd for $C_{16}H_{15}N_3O_3$, *m*/*z*: 298 $(M + H)^+$.

5.1.12. General Procedure for Urea Derivatives (17a,b). To a stirred solution of **15** (0.20 g, 0.67 mmol) and NEt_3 (0.17 g, 1.68 mmol) in 10 mL of $CHCl_3$ were added phenyl isocyanate derivatives (0.80 mmol). After 5 h, the solvent was removed under reduced pressure and the oily residue was evaporated with EtOH (3 × 15 mL). The residue was filtered off, washed with EtOH (5 mL), and recrystallized.

***N*-{3-[6,7-Dimethoxyquinazolin-4-yloxy]phenyl}-*N*′-phenylurea (17a).** Crystallization from EtOH gave pure **17a** as a white solid (68%). Mp >250 °C. IR cm^{-1} 3212 (NH), 1696 (C═O), 1205 (C═C−O), 1077 (C−O−C methoxy). ^1H NMR (DMSO-d_6) δ ppm 8.88 (s, 1H, NH), 8.72 (s, 1H, NH), 8.55 (s, 1H, ArH), 7.65 (m, 2H, ArH), 7.60−7.45 (m, 4H, ArH), 7.30−7.20 (m, 3H, ArH), 7.00−6.90 (m, 2H, ArH), 3.99 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3). LC−MS ($APCI^+$), calcd for $C_{23}H_{20}N_4O_4$, *m*/*z*: 417 $(M + H)^+$.

***N*-{3-[6,7-Dimethoxyquinazolin-4-yloxy]phenyl}-*N*′-(4-methoxyphenyl)urea (17b).** Crystallization from EtOH gave pure **17b** as a white solid (70%). Mp 235 °C. IR cm^{-1} 3214 (NH), 1695 (C═O), 1208 (C═C−O), 1079 (C−O−C methoxy). ^1H NMR (DMSO-d_6) δ ppm 8.90 (s, 1H, NH), 8.75 (s, 1H, NH), 8.51 (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.40−7.30 (m, 3H, ArH), 7.09 (m, 1H, ArH), 6.85 (d, J = 8.80 Hz, 2H, ArH), 6.52 (m, 1H, ArH), 6.41 (m, 1H, ArH), 6.37 (m, 1H, ArH), 4.01 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3). LC−MS ($APCI^+$), calcd for $C_{24}H_{22}N_4O_5$, *m*/*z*: 447 $(M + H)^+$.

5.1.13. General Procedure for Nucleophilic Substitution by Aniline (20a,b). Compounds **20a**,**b** were obtained by the same procedure used for **4a**−**m**. Starting from **19** (0.20 g, 0.96 mmol) and the commercial aniline (1.15 mmol), the residue was filtered off, washed with 2-propanol (10 mL), Et_2O (10 mL), and recrystallized.

4-(3-Chloro-4-fluoroanilino)-6,7-methylenedioxyquinazoline Hydrochloride (20a). Crystallization from MeOH/petroleum ether gave pure **20a** as a white solid (82%). Mp >250 °C. IR cm^{-1} 2465 (NH^+), 1219 (C−F), 1052 (C−Cl), 925 (C−O−C). ^1H NMR (DMSO-d_6) δ ppm 11.51 (s, 1H, NH^+), 7.81 (s, 1H, ArH), 7.55 (s, 1H, ArH), 7.20−7.10 (m, 2H, ArH), 6.95 (dd, J = 2.20, 8.10 Hz, 1H, ArH), 6.61 (s, 1H, ArH), 6.41 (s, 2H, CH_2). LC−MS ($APCI^+$), calcd for $C_{15}H_9ClFN_3O_2$, *m*/*z*: 318 $[(M + H)^+$ for $^{35}Cl]$ and 320 $[(M + H)^+$ for $^{37}Cl]$.

4-(4-Bromo-2-fluoroanilino)-6,7-methylenedioxyquinazoline Hydrochloride (20b). Crystallization from EtOH gave pure **20b** as a yellow solid (91%). Mp >250 °C. IR cm^{-1} 2466 (NH^+), 1219 (C−F), 1056 (C−Br), 928 (C−O−C). ^1H NMR (DMSO-d_6) δ ppm 11.60 (s, 1H, NH^+), 8.81 (s, 1H, ArH), 8.39 (s, 1H, ArH), 7.77 (d, J = 9.30 Hz, 1H, ArH), 7.60−7.50 (m, 2H, ArH), 7.48 (s, 1H, ArH), 6.39 (s, 2H, CH_2). LC−MS ($APCI^+$), calcd for $C_{15}H_9BrFN_3O_2$, *m*/*z*: 362 $[(M + H)^+$ for $^{79}Br]$ and 364 $[(M + H)^+$ for $^{81}Br]$.

5.1.14. General Procedure for N-Alkylation (21a,b). Compounds **21a**,**b** were obtained by the same procedure used for **5a**−**m**. Starting from quinazoline derivatives **20a**,**b** (0.30 g, 0.94 mmol), the oily residue were purified by FC (CH_2Cl_2/MeOH, 9:1).

4-(*N*-Methyl-3-chloro-4-fluoroanilino)-6,7-methylenedioxyquinazoline (21a). Crystallization from toluene gave pure **21a** as a yellow solid (47%). Mp >250 °C. IR cm^{-1} 2955 (N−CH_3), 1219 (C−F), 1086 (C−Cl), 926 (C−O−C). ^1H NMR (DMSO-d_6) δ ppm 8.53 (s, 1H, ArH), 8.05 (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.50−7.35 (m, 3H, ArH), 6.32 (s, 2H, CH_2), 3.81 (s, 3H, NCH_3). LC−MS ($APCI^+$), calcd for $C_{16}H_{11}ClFN_3O_2$, *m*/*z*: 332 $[(M + H)^+$ for $^{35}Cl]$ and 334 $[(M + H)^+$ for $^{37}Cl]$.

4-(*N*-Methyl-4-bromo-2-fluoroanilino)-6,7-methylenedioxyquinazoline (21b). Crystallization from EtOH gave pure **21b** as yellow solid (38%). Mp 239−240 °C. IR cm^{-1} 2958 (N−CH_3), 1220 (C−F), 1056 (C−Br), 925 (C−O−C). ^1H NMR (DMSO-d_6) δ ppm 7.92 (s, 1H, ArH), 7.61 (s, 1H, ArH), 7.37 (m, 1H, ArH), 7.22 (m, 1H, ArH), 7.17 (s, 1H, ArH), 6.95 (m, 1H, ArH), 6.21 (s, 2H, CH_2), 3.60 (s, 3H, NCH_3). LC−MS ($APCI^+$), calcd for $C_{16}H_{11}BrFN_3O_2$, *m*/*z*: 376 $[(M + H)^+$ for $^{79}Br]$ and 378 $[(M + H)^+$ for $^{81}Br]$.

5.1.15. General Procedure for N-Alkylation (22a−g). Compounds **22a**−**g** were obtained by the same procedure used for **5a**−**m**. Starting from 4-(3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline hydrochloride (**4g**) (0.30 g, 0.73 mmol) and halogen derivatives (1.46 mmol), the oily residues were purified by FC (CH_2Cl_2/MeOH, 9:1).

4-(*N*-Ethyl-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (22a). Starting from iodoethane (116 μL, 1,46 mmol), compound **22a** was obtanied by crystallization from CH_2Cl_2/petrolum ether as a yellow solid (13%). Mp 160−161 °C. IR cm^{-1} 1078 (C−O−C methoxy), 1057 (C−Br). ^1H NMR (DMSO-d_6) δ ppm 8.03 (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.28 (d, J = 2.00 Hz, 1H, ArH), 7.18 (d, J = 8.60 Hz, 1H, ArH), 6.96 (dd, J = 2.00, 8.60 Hz, 1H, ArH), 6.92 (s, 1H, ArH), 4.18 (m, 2H, CH_2), 3.94 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.28 (s, 3H, CH_3), 1.31 (m, 3H, CH_3). LC−MS ($APCI^+$), calcd for $C_{19}H_{20}BrN_3O_2$, *m*/*z*: 402 $[(M + H)^+$ for $^{79}Br]$ and 404 $[(M + H)^+$ for $^{81}Br]$.

4-(*N*-Prop-1-yl-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (22b). Starting from 1-iodopropane (124 μL, 1,46 mmol),

compound **22b** was obtanied by crystallization from CH_2Cl_2/petrolum ether as a yellow solid (20%). Mp 143−144 °C. IR cm^{-1} 1078 (C−O−C methoxy), 1057 (C−Br). ^1H NMR (DMSO-d_6) δ ppm 8.01 (s, 1H, ArH), 7.67 (s, 1H, ArH), 7.35 (d, J = 2.00 Hz, 1H, ArH), 7.18 (d, J = 8.20 Hz, 1H, ArH), 6.96 (dd, J = 2.00, 8.20 Hz, 1H, ArH), 6.91 (s, 1H, ArH), 4.11 (m, 2H, CH_2), 3.95 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.28 (s, 3H, CH_3), 1.72 (m, 2H, CH_2), 0.91 (m, 3H, CH_3). LC−MS (APCI$^+$), calcd for $C_{20}H_{22}BrN_3O_2$, m/z: 416 [(M + H)$^+$ for ^{79}Br] and 418 [(M + H)$^+$ for ^{81}Br].

4-(*N*-Prop-2-yl-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (22c). Starting from 2-iodopropane (124 μL, 1,46 mmol), compound **22c** was obtanied by crystallization from CH_2Cl_2/petrolum ether as a yellow solid (17%). Mp 174−175 °C. IR cm^{-1} 1077 (C−O−C methoxy), 1058 (C−Br). ^1H NMR (DMSO-d_6) δ ppm 8.36 (s, 1H, ArH), 7.79 (s, 1H, ArH), 7.42 (d, J = 2.00 Hz, 1H, ArH), 7.28 (d, J = 8.40 Hz, 1H, ArH), 7.18 (s, 1H, ArH), 7.02 (dd, J = 2.00, 8.40 Hz, 1H, ArH), 4.98 (m, 1H, $CH(CH_3)_2$), 3.95 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 2.31 (s, 3H, CH_3), 1.49 (m, 6H, $CH(CH_3)_2$). LC−MS (APCI$^+$), calcd for $C_{20}H_{22}BrN_3O_2$, m/z: 416 [(M + H)$^+$ for ^{79}Br] and 418 [(M + H)$^+$ for ^{81}Br].

4-(*N*-Benzyl-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (22d). Starting from benzyl bromide (174 μL, 1,46 mmol), compound **22d** was obtanied by crystallization from heptane as a yellow solid (18%). Mp 138−140 °C. IR cm^{-1} 1078 (C−O−C methoxy), 1057 (C−Br). ^1H NMR (DMSO-d_6) δ ppm 8.28 (s, 1H, ArH), 7.63 (s, 1H, ArH), 7.40−7.25 (m, 6H, ArH), 7.20 (d, J = 8.20 Hz, 1H, ArH), 6.96 (dd, J = 2.00, 8.20 Hz, 1H, ArH), 6.81 (s, 1H, ArH), 5.38 (s, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3). LC−MS (APCI$^+$), calcd for $C_{24}H_{22}BrN_3O_2$, m/z: 464 [(M + H)$^+$ for ^{79}Br] and 466 [(M + H)$^+$ for ^{81}Br].

4-(*N*-(2-Dimethylaminoethyl)-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (22e). Starting from 2-dimethylaminoethyl chloride hydrochloride (210 mg, 1,46 mmol), compound **22e** was obtanied by crystallization from CH_2Cl_2/petrolum ether as a yellow solid (18%). Mp 107−109 °C. IR cm^{-1} 1078 (C−O−C methoxy), 1057 (C−Br). ^1H NMR (DMSO-d_6) δ ppm 8.04 (s, 1H, ArH), 7.72 (s, 1H, ArH), 7.37 (d, J = 2.10 Hz, 1H, ArH), 7.22 (d, J = 8.40 Hz, 1H, ArH), 7.01 (dd, J = 2.10, 8.40 Hz, 1H, ArH), 6.97 (s, 1H, ArH), 4.24 (m, 2H, CH_2), 3.95 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 2.57 (m, 2H, CH_2), 2.31 (s, 3H, CH_3), 2.19 (m, 6H, CH_3). LC−MS (APCI$^+$), calcd for $C_{21}H_{25}BrN_4O_2$, m/z: 445 [(M + H)$^+$ for ^{79}Br] and 447 [(M + H)$^+$ for ^{81}Br].

4-(*N*-(2-Diethylaminoethyl)-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (22f). Starting from 2-diethylaminoethyl chloride hydrochloride (251 mg, 1,46 mmol), compound **22f** was obtanied by crystallization from CH_2Cl_2/petrolum ether as a yellow solid (11%). Mp 115−116 °C. IR cm^{-1} 1078 (C−O−C methoxy), 1057 (C−Br). ^1H NMR (DMSO-d_6) δ ppm 7.92 (s, 1H, ArH), 7.68 (s, 1H, ArH), 7.30 (d, J = 2.10 Hz, 1H, ArH), 7.18 (d, J = 8.40 Hz, 1H, ArH), 7.01 (dd, J = 2.10, 8.40 Hz, 1H, ArH), 6.95 (s, 1H, ArH), 4.13 (m, 2H, CH_2), 3.95 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.63 (m, 2H, CH_2), 2.41 (m, 4H, CH_2CH_3), 2.29 (s, 3H, CH_3), 0.82 (m, 6H, CH_2CH_3). LC−MS (APCI$^+$), calcd for $C_{23}H_{29}BrN_4O_2$, m/z: 473 [(M + H)$^+$ for ^{79}Br] and 475 [(M + H)$^+$ for ^{81}Br].

4-(*N*-(2-Piperidin-1-ylethyl)-3-bromo-4-methylanilino)-6,7-dimethoxyquinazoline (22g). Starting from 2-chloroethylpiperidine hydrochloride (269 mg, 1,46 mmol), compound **22g** was obtained by crystallization from CH_2Cl_2/petrolum ether as a yellow solid (20%). Mp 139−140 °C. IR cm^{-1} 1078 (C−O−C methoxy), 1059 (C−Br). ^1H NMR (DMSO-d_6) δ ppm 7.94 (s, 1H, ArH), 7.68 (s, 1H, ArH), 7.30 (d, J = 2.10 Hz, 1H, ArH), 7.19 (d, J = 8.40 Hz, 1H, ArH), 6.98 (dd, J = 2.00, 8.40 Hz, 1H, ArH), 6.95 (s, 1H, ArH), 4.20 (m, 2H, CH_2), 3.95 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 2.55 (m, 2H, CH_2), 2.39 (m, 4H, CH_2CH_2), 2.29 (s, 3H, CH_3), 1.41 (m, 6H, CH_2CH_2). LC−MS (APCI$^+$), calcd for $C_{24}H_{29}BrN_4O_2$, m/z: 485 [(M + H)$^+$ for ^{79}Br] and 487 [(M + H)$^+$ for ^{81}Br].

5.2. DNA-Binding Methods. 5.2.1. Absorption Spectroscopy and Melting Experiments Studies. Absorption spectra and melting curves were determined using an Uvikon XL spectrophotometer coupled to a thermosystem. Titrations of the drug with DNA, covering a large range of DNA-phosphate/drug ratios (P/D), were performed by adding aliquots of a concentrated CT-DNA or poly(dA-dT)$_2$ solution to a drug solution at constant ligand concentration (20 μM). For each series of T_m measurements (λ = 260 nm), 10 samples were placed in a thermostatically controlled cell-holder, and the quartz cuvettes (10 mm path length) were Peltier heated. The measurements were performed in BPE buffer, pH 7.1 (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA) . The temperature inside the cuvette was measured with a platinum probe; it was increased over the range 20−100 °C with a heating rate of 1 °C/min. The "melting" temperature T_m, deduced from melting curve, was taken as the midpoint of the hyperchromic transition. DNA titrations were performed in the same medium, salt. To 1 mL of drug solution at 20 μM were added aliquots of a concentrated calf thymus DNA solution.

5.2.2. Fluorescence Titration Experiments. Fluorescence titration data were recorded at room temperature using SPEX fluorometer Fluorolog. Excitation was at 360 nm, and fluorescence emission was monitored over the range 380−520 nm. Samples used for titration experiments were prepared separately at a constant drug concentration of 10 μM and at DNA concentrations ranging from 0.01 to 1 mM. Fluorescence titration data were fitted directly to get apparent binding constants using a fitting function incorporated into Prism 3.0 defined by K_D = [ligand][DNA]/[ligand−DNA].

5.2.3. Circular Dichroism. CD spectra were recorded on a Jasco J-810 spectrometer. Solutions of drugs, nucleic acids, and their complexes (1 mL in a 1 mM sodium cacodylate buffer, pH 7.0) were scanned in 10 mm quartz cuvettes. Measurements were made by progressive dilution of drug−DNA complex at a high P/D (phosphate/drug) ratio with a pure ligand solution to yield the desired drug/DNA ratio. Four scans were accumulated and automatically averaged. Then 1 mL of drug solution at 50 μM was successively diluted with increased volumes of DNA at 5 mM.

5.2.4. DNase I Footprinting. Experiments were performed essentially as previously described.[26] Briefly, reactions were conducted in a total volume of 10 μL. Samples (3 μL) of the labeled DNA fragments were incubated with 5 μL of the buffered solution containing the ligand at the appropriate concentration. After 30 min of incubation at 37 °C to ensure equilibration of the binding reaction, the digestion was initiated by the addition of 2 μL of a DNase I solution whose concentration was adjusted to yield a final enzyme concentration of about 0.01 unit/mL in the reaction mixture. After 3 min, the reaction was stopped by freeze-drying. Samples were lyophilized and resuspended in 5 μL of an 80% formamide solution containing tracking dyes. The DNA samples were then heated at 90 °C for 4 min and chilled in ice for 4 min prior to electrophoresis.

5.2.5. DNA Topoisomerase I Relaxation Assay. Supercoiled pUC19 DNA (130 ng) was incubated with 4 units of recombinant human topoisomerase I (TopoGen Inc.) at 37 °C for 45 mn in relaxation buffer (50 mM Tris, pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA) in the presence of varying concentrations of the drug under study. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 μg/mL. DNA samples were then added to the electrophoresis drye mixture (3/l) and electrophoresed in a 1% agarose gel at room temperature for 2 h at 120 V. Gels were stained with ethidium bromide (1 μg/mL), washed, and photographed under UV light. Similar experiments were performed using ethidium-containing agarose gels.

5.3. Cell Culture and Cell Proliferation Assay. Human prostate cancer cells PC3, breast cancer cell line MCF7, and colon

cancer cell line HT29 were grown at 37 °C in a humidified atmosphere containing 5% CO_2, respectively, in RPMI-1640 medium (Sigma), MEM (Sigma), and DMEM (Gibco) supplemented with 10% fetal bovine serum, glutamine (2 mM), penicillin (100 IU/mL), and streptomycin (100 μg/mL).

In the cell proliferation assay, cells were plated in triplicate on 96-well plates (3.103 cells per well) and incubated for 72 h. The cell medium was changed to serum-free medium, and the cells were starved for 24 h for culture synchronization. Cells were then incubated in culture medium that contained various concentrations of tested compounds, each dissolved in less than 0.1% DMSO. After 72 h, cell growth was estimated by the colorimetric MTT test.

Acknowledgment. The authors are grateful to the institutions that support our laboratory: Université Lille Nord de France and the "Ligue Nationale contre le Cancer, Comité du Pas-de Calais", the PRIM, Pôle de Recherche Interdisciplinaire du Médicament (Région Nord-Pas-de-Calais, Ministère Délégué à la Recherche et aux Nouvelles Technologies, European Union (FEDER)).

References

(1) Traxler, P.; Bold, G.; Buchdunger, E.; Caravatti, G.; Furet, P.; Manley, P.; O'Reilly, T.; Wood, J.; Zimmermann, J. Tyrosine kinase inhibitors: from rational design to clinical trials. *Med. Res. Rev*. **2001**, *21*, 499–512.

(2) Denny, W. A. The 4-anilinoquinazoline class of inhibitors of erbB family of receptor tyrosine kinase. *Farmaco* **2001**, *56*, 51–56.

(3) Ranson, M.; Wardell, S. Gefitinib, a novel, orally administrated agent for the treatment of cancer. *J. Clin. Pharm. Ther*. **2004**, *29*, 95–103.

(4) Nelson, M. H.; Dolder, C. R. Lapatinib: a novel dual tyrosine kinase inhibitor with activity in solid tumors. *Ann. Pharmacother*. **2006**, *40*, 261–269.

(5) Gourdie, T. A.; Valu, K. K.; Gravatt, G. L.; Boritzki, T. J.; Baguley, B. C.; Wakelin, L. P. G.; Wilson, W. R.; Woodgate, P. D.; Denny, W. A. DNA-directed alkylating agents. 1. Structure−activity relationships for acridine-linked aniline mustards: consequences of varying the reactivity of the mustard. *J. Med. Chem*. **1990**, *33*, 117–1186.

(6) Prakash, A. S.; Denny, W. A.; Gourdie, T. A.; Valu, K. K.; Woodgate, P. D.; Wakelin, L. P. G. DNA-directed alkylating ligands as potential antitumor agents: sequence specificity of alkylation by intercalating aniline mustards. *Biochemistry* **1990**, *29*, 9799–9807.

(7) Ryckebusch, A.; Garcin, D.; Lansiaux, A.; Goossens, J. F.; Baldeyrou, B.; Houssin, R.; Bailly, C.; Hénichart, J. P. Synthesis, cytotoxicity, DNA interaction, and topoisomerase II inhibition properties of novel indeno[2,1-*c*]quinolin-7-one and indeno[1,2-*c*]isoquinolin-5,11-dione derivatives. *J. Med. Chem*. **2008**, *51*, 3617–3629.

(8) Chackal, S.; Facompré, M.; Houssin, R.; Goossens, J. F.; Pommery, N.; Hénichart, J. P.; Bailly, C. Highly cytotoxic benzo[*c*]pyrido[2,3,4-*k*,*l*]acridines. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 943–946.

(9) Anthony, S.; Agama, K. K.; Miao, Z. H.; Hollingshead, M.; Holbeck, S. L.; Wright, M. H.; Varticovski, L.; Nagarajan, M.; Morrell, A.; Cushman, M.; Pommier, Y. Bisindenoisoquinoline bis-1,3-{(5,6-dihydro-5,11-diketo-11*H*-indeno[1,2-*c*]isoquinoline)-6-propylamino}propane bis(trifluoroacetate) (NSC 727357), a DNA intercalator and topoisomerase inhibitor with antitumor activity. *Mol. Pharmacol*. **2006**, *70*, 1109–1120.

(10) Palchaudhuri, R.; Hergenrother, P. J. DNA as target for anticancer compounds: methods to determine the mode of binding and the mechanism of action. *Curr. Opin. Biotechnol*. **2007**, *18*, 497–503.

(11) Bridges, A. J.; Cody, D. R.; Zhou, H.; McMichael, A.; Fry, D. W. Enantioselective inhibition of the epidermal growth factor receptor tyrosine kinase by 4-(α-phenethylamino)quinazolines. *Bioorg. Med. Chem*. **1995**, *3*, 1651–1656.

(12) Goossens, J. F.; Bouey-Bencteux, E.; Houssin, R.; Hénichart, J. P.; Colson, P.; Houssier, C.; Laine, W.; Baldeyrou, B.; Bailly, C. DNA interaction of the tyrosine protein kinase inhibitor PD153035 and its *N*-methyl analogue. *Biochemistry* **2001**, *40*, 4663–4671.

(13) Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Hollis Showalter, H. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. Tyrosine kinase inhibitors. 8. An unusually steep structure−activity relationship for analogues of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor. *J. Med. Chem*. **1996**, *39*, 267–276.

(14) Bouey-Bencteux, E.; Loison, C.; Pommery, N.; Houssin, R.; Hénichart, J. P. Synthesis and antiproliferative properties of 4-aminoquinazoline derivatives as inhibitors of EGF receptor-associated tyrosine kinase activity. *Anti-Cancer Drug Des*. **1998**, *13*, 893–922.

(15) Furuta, T.; Sakai, T.; Senga, T.; Osawa, T.; Kubo, K.; Shimizu, T.; Suzuki, R.; Yoshino, T.; Endo, M.; Miwa, A. Identification of potent and selective inhibitors of PDGF receptor autophosphorylation. *J. Med. Chem*. **2006**, *49*, 2186–2192.

(16) Matsuno, K.; Ushiki, J.; Seishi, T.; Ichimura, M.; Giese, N. A.; Yu, J. C.; Takahashi, S.; Oda, S.; Nomoto, Y. Potent and selective inhibitors of platelet-derived growth factor receptor phosphorylation. 3. Replacement of quinazoline moiety and improvement of metabolic polymorphism of 4-[4-(*N*-substituted (thio) carbamoyl)-1-piperazinyl]-6,7-dimethoxyquinazoline derivatives. *J. Med. Chem*. **2003**, *46*, 4910–4925.

(17) Nordén, B.; Tjerneld, F. Structure of methylene blue-DNA complexes studied by linear and circular dichroism spectroscopy. *Biopolymers*. **1982**, *21*, 1713–1734.

(18) Facompré, M.; Carrasco, C.; Colson, P.; Houssier, C.; Chisholm, J. D.; Van Vranken, D. L.; Bailly, C. DNA binding and topoisomerase I poisoning activities of novel disaccharide indolocarbazoles. *Mol. Pharmacol*. **2002**, *62*, 1215–27.

(19) Bailly, C.; Waring, M. J. Comparison of different footprinting methodologies for detecting binding sites for a small ligand on DNA. *J. Biomol. Struct. Dyn*. **1995**, *12*, 869–898.

(20) VanBrocklin, H. F.; Lim, J. K.; Coffing, S. L.; Hom, D. L.; Negash, K.; Ono, M. Y.; Gilmore, J. L.; Bryant, I.; Riese, D. J. Anilinodialkoxyquinazolines: screening epidermal growth factor receptor tyrosine kinase inhibitors for potential tumor imaging probes. *J. Med. Chem*. **2005**, *48*, 7445–7456.

(21) Bridges, A. J.; Zhou, H.; Cody, D. R.; Rewcastle, G. W.; McMichael, A.; Showalter, H. D.; Fry, D. W.; Kraker, A. J.; Denny, W. A. Tyrosine kinase inhibitors. 8. An unusually steep structure−activity relationship for analogues of 4-(3-bromoanilino)-6,7-dimethoxyquinazoline (PD 153035), a potent inhibitor of the epidermal growth factor receptor. *J. Med. Chem*. **1996**, *39*, 267–276.

(22) Rocco, S. A.; Barbarini, E.; Rittner, R. *Synthesis* **2004**, *3*, 429–435.

(23) Gibson, K. H.; Grundy, W.; Godfrey, A.; Woodburn, J. R.; Ashton, S. E.; Curry, B. J.; Scarlett, L.; Barker, A. J.; Brown, D. S. Epidermal growth factor receptor tyrosine kinase: Structure−activity relationships and antitumour activity of novel quinazolines. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 2723–2728.

(24) Myers, M. R.; Setzer, N. N.; Spada, A. P.; Persons, P. E.; Ly, C. Q.; Maguire, M. P.; Zulli, A. L.; Cheney, D. L.; Zilberstein, A.; Johnson, S. E.; Franks, C. E.; Mitchell, K. J. The synthesis and SAR of new 4-(*N*-alkyl-*N*-phenyl)amino-6,7-dimethoxyquinazolines and 4-(*N*-alkyl-*N*-phenyl)aminopyrazolo[3,4-*d*]pyrimidines, inhibitors of CSF-1R tyrosine kinase activity. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 421–424.

(25) Kubo, K.; Shimizu, T.; Ohyama, S.; Murooka, H.; Nishitoba, T.; Kato, S.; Kobayashi, Y.; Yagi, M.; Isoe, T.; Nakamura, K.; Osawa, T.; Izawa, T. A novel series of 4-phenoxyquinolines: potent and highly selective inhibitors of PDGF receptor autophosphorylation. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 2935–2940.

(26) Bailly, C.; Kluza, J.; Martin, C.; Ellis, T.; Waring, M. J. DNase I footprinting of small molecule binding sites on DNA. *Methods Mol. Biol*. **2005**, *288*, 319–342.