Bis-8-hydroxyquinoline and Bis-8-hydroxyquinaldine *N*-Substituted Amines: A Single Methyl Group Structural Difference between the Two Heterocycles, Which Modulates the Antiproliferative Effects

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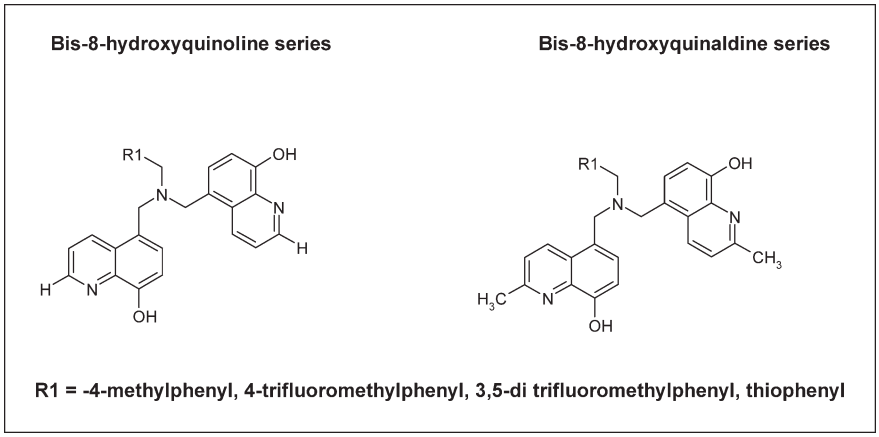

The synthesis of a series of bis-8-hydroxyquinoline- and bis-8-hydroxyquinaldine-substituted *N*-benzyl or thiophenyl amines and their corresponding bis-8-hydroxyquinoline is reported. *In vitro* growth inhibitory effects of both series have been evaluated. It has been observed that analogs from the bis-8-hydroxyquinoline series exert nanomolar range activity, whereas the antiproliferative activity of the corresponding analogs from the bis-8-hydroxyquinaldine series was found to be drastically lower. Molecular docking and chemical–physical properties account for these observed growth inhibitory differences between the two series of analogs, which differ only by the presence of a methyl group at the 2 position of the heterocyclic ring.

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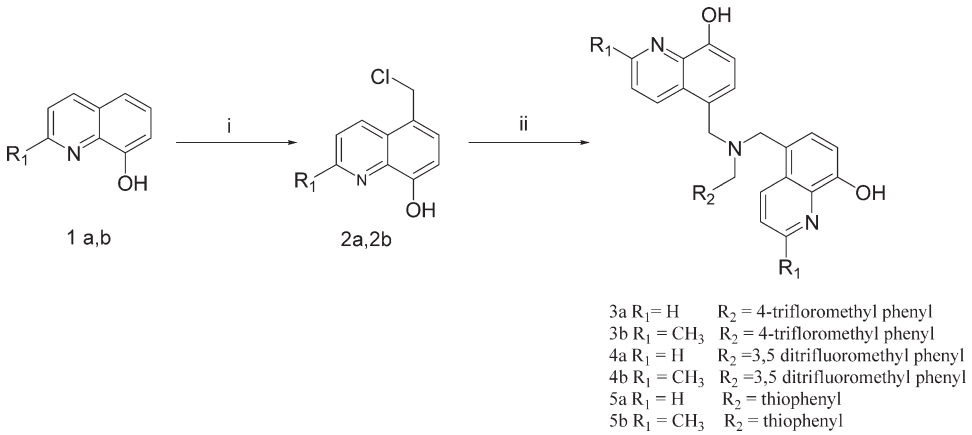
INTRODUCTION

Hydroxyquinoline is a privileged structural moiety observed in many biologically active natural products; it is used as the source for many drugs diversely prescribed among a wide range of pathologies, including neurodegenerative [1], parasitic amoebic dysentery [2], and herpes viral diseases [3]. More specifically, 8-hydroxyquinoline (8-HQ) moiety has been mostly used for its capacity to strongly chelate metal ions, particularly Cu^{++} and Zn^{++} [4].

We have reported preliminary results on the antitumor activity of two analogs JLK1486 and JLK1472, which belong to the family of bis-8-hydroxyquinoline-substituted benzylamines [5]. From this work, we discover that a single methyl subtituent at the position 2 of the quinoline ring lead to what is called quinaldine series drastically that diminish the antiproliferative effect of the resulting compounds. For this purpose, we synthesized a new series of bis-8-hydroxyquinaldine *N*-substituted analogs, and we studied their anticancer activities in comparison with that of the corresponding bis-8-

Scheme 1. Reagents and conditions: (i) HCHO, 37% HCl in H_2O, HCl (gas), r.t., overnight, 80%; (ii) corresponding primary amine K_2CO_3, CH_3CN, r.t., overnight.



hydroxyquinoline *N*-substituted series. Molecular docking techniques and physicochemical studies were carried out to account for the observed differences in antitumor activity between the two families of 8-HQ and 8-hydroxyquinaldine (8-HQD) analogs.

CHEMISTRY

Starting from 8-HQ (**1a**) or 8-HQD (**1b**), commercially available compounds, the corresponding 5-chloromethyl derivatives (**2a** and **2b**) were obtained in good yields by direct reaction with 37% formaldehyde in strong acidic conditions [6,7]. The solid compounds were directly collected by simple filtration and used without purification. The next step consisted in an addition on **2a** and **2b** of an excess of selected *N*-substituted primary amines that preferentially led to the formation of the desired bis-8-hydroxyquinoline or bis-8-hydroxyquinaldine benzyl or thiophenyl amines (Scheme 1). Under these experimental conditions, the desired bis-8-hydroxyquinoline and quinaldine derivatives (**3a**, **3b**, **4a**, **4b**, **5a**, **5b**) were obtained with moderate yields (40–50%), whereas mono-8-hydroxyquinoline or mono-8-hydroxyquinaldine by-products were only present as traces. The desired compounds were purified by column chromatography and fully identified by conventional spectral and centesimal analysis.

RESULTS AND DISCUSSION

Compounds from the bis-8-hydroxyquinoline and bis-8-hydroxyquinaldine series were evaluated for their antiproliferative effect on a panel of 15 cancer cell lines [8–11].

The data in Table 1 clearly show that bis-8-hydroxyquinoline derivatives display higher *in vitro* antitumor activity than bis-8-hydroxyquinaldine derivatives. Indeed, in contrast to these bis-8-hydroxyquinoline derivatives for some of which antiproliferative effect could be observed in two digit n*M* range, the corresponding bis-8-hydroxyquinaldine analogs (**3b**, **4b**, **5b**) displayed antitumor effects higher than 5 μ*M*, and thus, revealing themselves 100–1000-fold weaker antiproliferative compounds when compared with 8-HQ derivatives. In addition, it must also be highlighted that the large variations observed in terms of antitumor activity for various bis-8-hydroxyquinoline derivative series on a given cancer cell line, say for example the BxPC3 pancreas cancer and the VM-47 melanoma cell lines.

These data prompted us to envisage preliminary structure–activity relationship analysis. Compounds (**2a**) and (**2b**), which are mono-8-hydroxyquinoline analogs and which include a chloromethyl moiety at the position 4 of the 8-HQ or 8-HQD nucleus, are found totally inactive in comparison with the *N*-substituted benzyl or thiophenylamine derivatives, a feature that reveals that the presence of a *N*-substituted amine moiety is essential for anticancer activity and also confirm that bis-8-hydroxyquinoline analogs are more potent antiproliferative compounds than the corresponding mono-8-hydroxyquinoline analog [5]. These observations led us, therefore, to try to understand why bis-8-hydroxyquinoline and bis-8-hydroxyquinaldine derivatives display such marked differences in terms of antiproliferative effect. Such observation that bis-8-hydroxyquinoline analogs are more potent bioactive molecules than their corresponding mono-8-hyroxy analog has been reported in the case of neurodegenerative diseases [12]. We have, thus, considered the possible electronic effects induced by the electron pair of the amine group in the 8-HQ or quinaldine system in terms of chemical reactivity, and the possible physical–chemical properties differences between the two scaffolds mainly in terms of nucleophilicity,

Table 1

Determination of the IC_{50} (n*M*) *in vitro* growth inhibitory in 5 carcinoma, 5 glioma, and 5 melanoma.

	Carcinoma					Glioma					Melanoma				
N°	A549	BxPC3	LoVo	MCF7	PC3	HS683	T98G	U373	U138	GL19	VM-21	VM-48	VM-47	SKMEL-28	B16F10
3a	8	4299	10	34	66	10	99	44	49	1241	81	1584	97	>10 μ*M*	92
4a	9	79	10	38	71	181	72	96	46	2143	47	98	146	>10 μ*M*	76
5a	27	89	40	35	44	35	88	85	44	3451	79	1757	1726	4826	86
3b	>10 μ*M*	nd	5565	nd	9605	>10 μ*M*	nd	7209	nd	>10 μ*M*	nd	nd	nd	nd	nd
4b	9713	nd	7954	nd	8787	>10 μ*M*	nd	8695	nd	>10 μ*M*	nd	nd	nd	nd	nd
5b	>10 μ*M*	nd	>10 μ*M*	nd	>10 μ*M*	>10 μ*M*	nd	>10 μ*M*	nd	>10 μ*M*	nd	nd	nd	nd	nd

Compound *in vitro* antiproliferative effect has been performed in 5 carcinoma, 5 glioma, and 5 melanoma cell lines. The cancer cells have been cultured in the presence of the drugs for 3 days. The IC_{50} values were determined by means of the MTT colorimetric assay as detailed previously [6,8]. The values reported in this table are means obtained on six distinct values. The standard errors are not reported for the sake of clarity, because they are <5% as compared with the mean values.

IC_{50} are expressed in n*M* unless in μ*M* when specified in the table.

Cell lines: A549, human alveolar epithelial; BxPC3, pancreatic cancer; LoVo, colon cancer; MCF7, breast cancer; PC3, humanprozstate cancer; HS683, human glioma; T98G, human glioblastoma; U373, human glioblastoma-astrocytoma epithelial; U318, human glioma; GL19, glioblastoma multiform; VM-21 and VM-47 are mutant melanoma; SKMEL-28 and B16F10 are human melanoma.

nd: not determined.

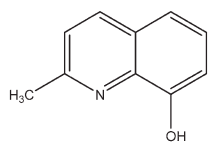
basicity, and hydrophobicity. There is a conceptual relationship between nucleophilicity and basicity. Both properties are involved in the formation of a new bond by donation of an electron pair to an electrophilic species. Basicity in the Brønsted sense involves formation of a bond to hydrogen, while generally nucleophilicity refers to the effect of a Lewis base on the rate of nucleophilic substitution reaction. The relative nucleophilicities may differ from reaction to reaction, and several parameters have significant influence on nucleophilicities. From literature reports [13], it can be seen that the presence of a methyl group in 8-HQD makes the nitrogen donor more basic in comparison with 8-HQ (Fig. 1).

Considering the possible biological mode of action of the compounds under study in which the proton of the hydroxyl group in the ground state could be involved in their antitumor mechanism(s) of action, the observed differences in pKa values (Fig. 1) could account for the observed differences in antiproliferative effects. Differences in the pKA values between the two systems could induce variations in the electronic driving force from the exocyclic nitrogen lone pair electron to the hydroxy group through the aromatic ring. Electronic effects induced by the presence of the methyl substituent at the 2 position of the quinoline ring influence the acidity of the OH proton and consequently, the conjugation between the nitrogen atom lone pair electron from the benzylamine moiety and the phenol group through the aromatic system.

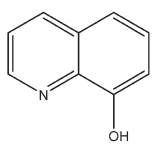
Next, we considered the differences in terms of hydrophobicity between the two scaffolds as a possible parameter which could influence cell permeation and consequently, the observed anticancer activities. The calculated clog*P* values determined for analogs **3a** (quinoline series) and **3b** (quinaldine series) were, respectively, 6.16 and 7.64 (calculated from ACD Labs/Log*P* dB 3.5 and ChemSketch 3.5). As expected, 8-HQD derivatives were slightly more hydrophobic than their corresponding analogs from the bis-8-hydroxyquinoline series, but these rather small differences in hydrophobicity cannot account alone for the observed drastic differences in antiproliferative effect.

As steric effects of substituted quinolines on lithium coordination geometry have been reported [14], we next examined the possible influence of steric hindrance induced by the methyl group at position 2 of the quinoline heterocycle. For this purpose, compounds **3a** and **3b** were selected as representative compounds for minimal energy conformational search. Minimal energy conformations for both analogs were generated and superimposed after a Monte Carlo Conformational Search (Macromodel version 6.5 was used for molecular mechanics calculations) [15].

After calculations, both lowest energy conformations for **3a** (132.8 kcal/mol) and **3b** (134.5 kcal/mol) were



8-hydroxy quinaldine scaffold



8 –Hydroxy-quinoline scaffold

Figure 1. pKa values: 8-hydroxyquinoline [pK_{A1} = 5.13 (NH_4+/NH_2), pK_{A2} = 9.89 (OH/O^-)] and 8-hydroxyquinaldine [pK_{A1} = 5.67 (NH_4+/NH_2), pK_{A2} = 9.97 (OH/O^-)].

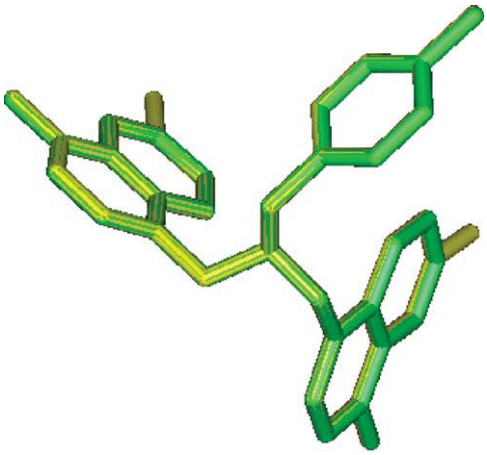


Figure 2. Superimposition of compounds **3a** (green) and **3b** (yellow)—best conformations obtained through a Monte Carlo conformational search. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

identical. They exhibit an energetic difference inferior to 2 kcal/mol between the two analogs, which could not account for the drastic drop in biological activity of the methylated compounds. Further, superimposition of the successively obtained conformations within a 3 kcal/mol span (from the lowest representative conformation) between these analogs revealed no differences between both analogs, which displayed similar poses with a similar energy difference in favor of the quinoline scaffold (Fig. 2).

From these results, steric effects induced by the presence of a methyl group at the position 2 appear to be too weak to entirely abolish the anticancer activity of all the 8-HQD analogs irrespective of the cancer cell lines (carcinoma, glioma, or melanoma) used in the assay.

CONCLUSIONS

In conclusion, a series of bis-8-hydroxyquinolines *N*-benzyl or thiophenyl amines and their corresponding bis-8-hydroxyquinaldine derivatives have been prepared. We found that compounds that belong to the bis-8-hydroxyquinoline series have an *in vitro* potent antiproliferative effect on a large panel of cancer cell lines at lower nanomolar IC_{50} values, while their corresponding 8-HQD counterparts display only weak (in the μ*M* range), if any, antiproliferative effect. Molecular docking studies and physico-chemical properties suggest that the presence of a methyl group in position 2 vicinal to the endocyclic nitrogen atom induces electronic factors (pKa values) that greatly influence the driving electronic force all along the aromatic system, including the exocyclic nitrogen. In contrast, Monte Carlo studies reveal only a small energy difference with less than 2 kcal/mol having been observed between the two series differing in the 2-methyl group only. The steric effect of a methyl group at the 2 position of the heterocycle and the difference in clog*P* values appeared to be too weak to account for the observed drastic antiproliferative differences between both series of analogs. Investigations on the mechanism of action and on the possible biological targets of those are underway.

EXPERIMENTAL

Compounds **2a** and **3a** have been already reported [5]. Starting quinaldin-8-ol intermediate **2b** has been synthesized as follows:

5-(Chloromethyl)-2-methylquinolin-8-ol hydrochloride (2b). A mixture of 7.3 g (0.045 mol) of quinaldin-8-ol (Aldrich), 8 mL of concentrated hydrochloric acid, and 8 mL (0.05 mol) of 37% formaldehyde was treated with hydrogen chloride gas for 90 min. The yellow solid was collected on a filter and dried to give 8.5 g of compound (70% yield) mp 280°C ^{1}NMR (250 MHz, DMSO): 9.14 (d, 1H, J = 8.75 Hz), 8.05 (d, 1H, J = 8.75 Hz), 7.83 (d, 1H, J = 8 Hz), 7.56 (d, 1H, J = 8 Hz), 5.233 (s, 2H), 2.5 (s, 3H). Anal. Calcd. for $C_{11}H_{11}NOCl_2$: C, 54.09; H, 4.51; N, 5.73. Found: C, 54.19; H, 4.45; N, 5.81.

General procedure for the reaction of 5-chloromethyl 8-hydroxyquinaldine (2a) and (2b) with primary amines for the synthesis of analogs 4a, 5a, 3b, 4b, and 5b. All of these reactions were carried out under a nitrogen atmosphere. To a solution of 5-chloromethyl quinolin-8-ol dihydrochloride (**2a)** or 5-chloromethylquinaldin-8-ol dihydrochloride (**2b)** (1.3 mmol) at 50°C in ethyl acetate (10 mL) was added appropriate primary amines (3.91 mmol). Stirring is maintained overnight. Then, the solution is cooled down to 0°C and filtrated; the filter cake is washed with cold ethyl acetate (5 mL). The filtrate is concentrated *in vaccuo*, diluted in diethyl ether (5 mL), and centrifuged. The ethereal phase is removed, and the obtained solid is washed two more time by centrifugation with diethyl ether at 0°C to give the desired products.

5,5′-(4-(Trifluoromethyl)benzylazanediyl)bis(methylene)bis(2-methylquinolin-8-ol) (3b). This compound was obtained as colorless solid. (42%), mp = 205°C ^{1}NMR (250 MHz, $CDCl_3$): 7.64 (d, 2H, J = 7.75 Hz), 7.41–7.39 (m, 2H), 7.22–7.06 (m, 4H), 6.95–6.8 (m, 4H), 3.61 (s, 4H), 3.44 (s, 2H), 2.61 (s, 6H). MS, *m/z* ($C_{30}H_{26}F_3N_3O_2$): calcd. 518, $[M + H]^+$; found 518. Anal. Calcd. for $C_{30}H_{26}F_3N_3O_2$: C, 69.50; H, 5.02; N, 8.11. Found: C, 69.37; H, 5.04; N, 8.14.

5,5″-(3,5-Bis(trifluorométhyl)benzylazanediyl)bis(methylene)diquinoline-8ol (4a). This compound was obtained as a yellow solid. (50%), mp = 193°C ^{1}NMR (250 MHz, $CDCl_3$): 9.8 (m, 2H), 6.7–8.05 (m, 13H aromatic) 3.6–3.8 (m 6H, CH_2). MS, *m/z* ($C_{29}H_{21}F_6N_3O_2$): calcd. MW 558; found 558. Anal. Calcd. for $C_{29}H_{21}F_6N_3O_2$: C, 62.48; H, 3.80; N, 7.54. Found: C, 62.10; H, 3.89; N, 7.33.

5,5′-(3,5-Bis(trifluorométhyl)benzylazanediyl)bis(methylene)diquinaldine-8-ol (4b). This compound was obtained as brown solid. (38%), mp = 207°C H^{1}NMR (250 MHz, $CDCl_3$): 8.76–8.74 (m, 2H), 7.96–7.92 (m, 2H), 7.64 (s, 1H), 7.43–7.38 (m, 4H), 724, (m, 5H) 7.19–7.13 (m, 4H), 7.08–7.05 (m, 2H), 3.89 (br s, 4H), 3.61 (s, 2H). 2,8 (3H). MS, *m/z* ($C_{31}H_{25}F_6N_3O_2$): calcd. 586, $[M + H]^+$; found 587. Anal. Calcd. for

$C_{31}H_{25}F_6N_3O_2$: C, 63.59; H, 4.30; N, 7.18. Found: C, 64.12; H, 4.26; N, 7.11.

5,5′-(Thiophen-2-ylmethylazanediyl)bis(methylene)diquinolin-8-ol (5a). This compound was obtained as a brown solid. (48%), mp = 167 (decomp) ^{1}NMR (250 MHz, MeOD): 8.79 (m, 2H), 8.13 (m, 2H), 7.43 (m, 3H), 7.23 (m, 2H), 7.01 (m, 4H), 3.86 (br s, 4H), 3.76 (s, 2H). MS, *m*/*z* ($C_{25}H_{21}N_3O_2S$): calcd. 428.1, $[M + H]^+$; found 428.1. Anal. Calcd. for $C_{25}H_{21}N_3O_2S$: C, 72.24; H, 9.83; N, 4.95. Found: C, 71.98; H, 9.86; N, 4.93.

5,5′-(Thiophen-2-ylmethylazanediyl)bis(methylene)diquinaldine-8-ol (5b). This compound was obtained as a gray solid. (36%), mp = 205°C (decomp) ^{1}NMR (250 MHz, MeOD): 9.83 (m, 2H), 6.8–8.05 (m, 8H), 6.74–7.06 (m, 3H thiophenyl ring), 3.5–3.6 (m 6H), 2.55 (s, 6H). MS, *m*/*z* ($C_{27}H_{25}N_3O_2S$): calcd. 455, $[M+H]^+$; found 55. Anal. Calcd. for $C_{27}H_{25}N_3O_2S$: C, 71.21; H, 5.50; N, 9.23. Found: C, 71.35; H, 5.38; N, 9.19.

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