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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

1-(7-Chloroquinolin-4-yl)-2-[(1H-pyrrol-2-yl)methylene] hydrazine: a potent compound against cancer

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Abstract Heteroaromatic derivatives (**3a–f**) have been synthesized and evaluated for their activity against four cancer cell lines. Among the studied compounds, 1-(7-Chloroquinolin-4-yl)-2-[(1H-pyrrol-2-yl)methylene]hydrazine (**3e**) exhibited an excellent cytotoxic activity against the referred lines, and especially on melanoma cells (MDAMB-435). In this case, compound **3e** is four times more active than the standard substance Doxorubicin. Together with other results from our group, 7-chloro-4-quinolinylhydrazones derived from chloroquine could be considered a relevant finding toward the rational design of new leads for antitumor compounds.

Keywords Antitumor activity · Cancer · Chloroquine · Drugs · Hydrazones · Quinoline

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Introduction

In the field of drug discovery, the quinoline nucleus, found in many synthetic and natural products, is an important class of heterocyclic compound, since it presents a wide range of pharmacological activities, such as antiviral (Font *et al.*, 1997), antibacterial (Kaminsky and Meltzer, 1968), antifungal (Musiol *et al.*, 2006), antiobesity (Warshakoon *et al.*, 2006), and anti-inflammatory (Sloboda *et al.*, 1991) and antimalarial (Foley and Tilley, 1998) properties. Such a variety is well illustrated by the availability of a large number of drugs containing this heterocyclic class.

Quinoline derivatives are particularly relevant in antimalarial drug research. Quinine, an alkaloid which was originally isolated from the bark of the cinchona tree in Peru, was the first effective treatment for malaria (Foley and Tilley, 1998). This substance played the role of starting point to design new antimalarial drugs, such as, for instance, Chloroquine (Fig. 1, CQ), that is reputed as the drug of choice for malaria, due to its effectiveness, low toxicity, and reduced costs. In view of these characteristics, the therapeutic activity of CQ in other diseases, such as lupus erythematosus, rheumatoid arthritis and amoebic hepatitis (Augustijns *et al.*, 1992), HIV-1/AIDS and chikungunya fever (Savarino *et al.*, 2006), has been widely studied.

Furthermore, as CQ was recently found to present activities against different types of human cancers (Chuandong *et al.*, 2006; Kim *et al.*, 2010), the drug emerged as a potential anticancer agent. Thanks to these properties, CQ could be considered a lead compound to design new antitumor drugs. Based on this hypothesis, our research group has recently studied two series of mono and polysubstituted 7-chloro-4-quinolinylhydrazone derivatives designed by molecular hybridization, which have shown promising cytotoxic activities (Fig. 1).

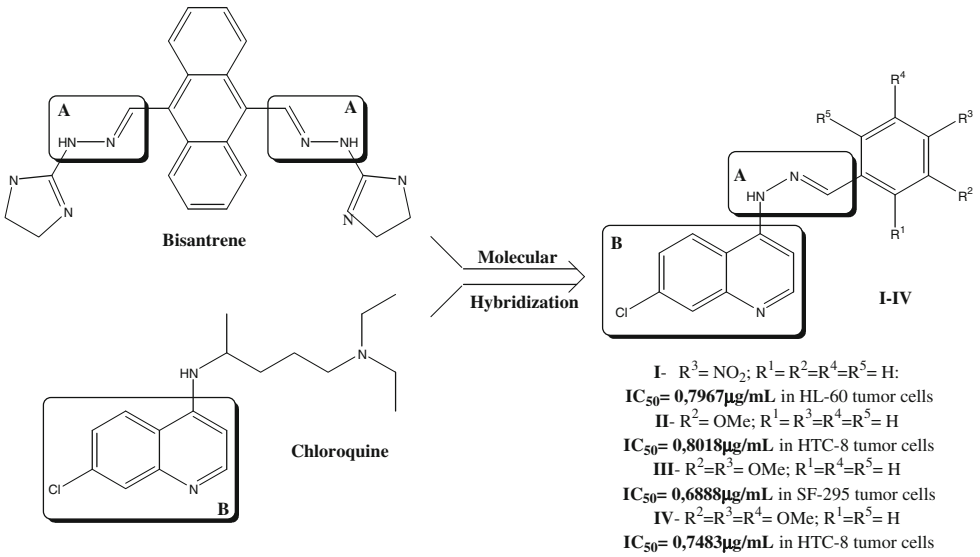


Fig. 1 7-chloro-4-quinolinylhydrazones derivatives designed by molecular hybridization between hydrazone scaffold (*A*) existing in bisantrene and 7-chloro-quinoline nucleus (*B*) found in CQ

Therefore, in the present study, we decided to synthesize and evaluate a series of heteroaromatic 7-chloro-4-quinolinylhydrazones against cancer cell lines. Hence, four heterocyclic nuclei were selected (furan, thiophene, pyrrole, and imidazole) based on isosteric replacements: (i) substitution of oxygen atom from the furane ring (**3b**) by sulfur (**3d**) or nitrogen (**3e**); (ii) substitution of –CH=by –N=in pyrrole ring (**3e**) to provide an imidazole ring (**3f**). Besides, a nitro group has been introduced on furan (**3a**) and thiophene (**3c**) rings, in an attempt to analyze the influence of this group on the biological activity of said series, since nitro compounds have been recently studied as radio sensitizers in the antitumor therapy (Krause *et al.*, 2005).

Materials and methods

Chemistry

Melting points were determined on a Buchi apparatus and are uncorrected. Infrared spectra were recorded in a Thermo Nicolet Nexus 670 spectrometer, as potassium bromide pellets and frequencies are expressed in cm^{-1}. Mass spectra (ESI assay in solution of ammonium chloride) were recorded in Micromass ZQ Waters mass spectrometer. NMR spectra were recorded in a Bruker Avance 500 spectrometer operating at 500.00 MHz (^1H), in deuterated dimethylsulfoxide. Chemical shifts are reported in ppm (δ) relative to tetramethylsilane and *J*-coupling in Hertz (Hz). Proton spectra were typically obtained at room temperature. For TLC plates, coated with silica gel, were run in chloroform/methanol (9:1) mixture and spots were developed in ultraviolet and solution of ninhidrine (0.2% (w/v) in ethanol).

General procedures for synthesis of 7-chloro-4-quinolinylhydrazones derivatives (**3a–f**)

The 7-chloro-4-quinolinylhydrazones derivatives (**3a–f**) were obtained by the reaction between 7-chloro-4-hydrazinoquinoline (1.03 mmols) and the appropriate heteroaromatic aldehyde (**2a–f**) (1.24 mmols) in ethanol (5 ml). After stirring for 12–24 h at room temperature, the resulting mixture was concentrated under reduced pressure. Then, the residue was filtered under vacuum and purified by washing with cold Et_2O (3 × 10 ml), leading to the pure derivatives (**3a–f**) as solids in 78–92% yields.

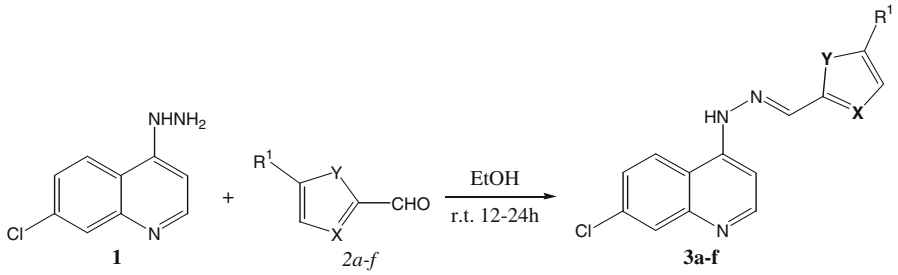
*1-(7-Chloroquinolin-4-yl)-2-[(5-nitro-furan-2-yl)methylene]hydrazine (**3a**)*

Yield: 85%; Mp: 238–240°C; ^1H NMR [500 MHz (FIDRES ± 0.15 Hz), DMSO-d_6] δ: 11.70 (1H, br, NH), 8.35 (2H, m, H_2 and H_5), 8.30 (1H; s; N=C–H), 7.81 (2H, d, $J = 3{,}7$ Hz, $H_{7'}$ and H_6), 7.55 (1H, br, H_8); 7.22–7.26 (2H, m, H_3 *e* $H_{8'}$). IRλ_{max} (cm^{-1}; KBr pellets): 3,158 (N–H); 1,571 (C=N). MS/ESI: *m/z* $[M - H]^{+\cdot}$: 315. Anal. Calcd for $C_{14}H_9ClN_4O_3$: C 53.09, H 2.86, N 17.69. Found: C 52.93, H 2.78, N 17.74.

*1-(7-Chloroquinolin-4-yl)-2-[(5-nitro-thiophen-2-yl)methylene]hydrazine (**3c**)*

Yield: 92%; Mp: 189–190°C; ^1H NMR [500 MHz (FIDRES ± 0.15 Hz), DMSO-d_6] δ: 11.91 (1H, br, NH), 8.55 (2H, m, H_2 and H_5), 8.22 (1H; s; N=C–H), 8.08 (1H, d, $J = 4.4$ Hz, $H_{7'}$); 7.53 (1H, d, $J = 1.9$ Hz, H_8); 7.48–7.51 (2H, m, $H_{8'}$ *e* H_6); 6.98 (1H, d, $J = 7.4$ Hz, H_3). IRλ_{Max} (cm^{-1}; KBr pellets): 3,182 (NH); 1,585 (C=N). MS/ESI:

Scheme 1 Synthesis of heteroaromatic 7-chloro-4-quinolinylhydrazone derivatives **3a–f**



m/z $[M + H]^{+\cdot}$: 333. Anal. Calcd for $C_{14}H_9ClN_4O_2S$: C 50.53, H 2.73, N 16.84. Found: C 50.36, H 2.68, N 16.91.

Biological assays

Cytotoxicity against cancer cell lines

Compounds **3a–f** (0.009–5 μg/ml) were tested for their cytotoxic activity against three four cancer cell lines: SF-295 (glioblastoma), HCT-8 (colon), MDA-MB-435 (melanoma), HL60 (leukaemia) (National Cancer Institute, Bethesda, MD). All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C with 5% CO_2. Each compound was dissolved with DMSO, until reaching a concentration of 1 mg/ml. The final concentration of DMSO in the culture medium was kept constant, below 0.1% (v/v). Compounds **3a–f** were incubated with the cells for 72 h. The negative control received the same amount of DMSO (0.001% in the highest concentration). The cell viability was determined by reduction of the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann (Ahmed *et al.*, 1994).

Cell membrane disruption

The test was performed in 96-well plates using a 2% mouse erythrocyte suspension in 0.85% NaCl containing 10 mM $CaCl_2$ (Sharma and Sharma 2001). The compounds **3a–f** were diluted as mentioned above, and tested at 250 μg/ml. After incubation at room temperature for 30 min, centrifugation, and removal of the supernatant, the released hemoglobin was measured by spectrophotometry at 540 nm. DMSO was used as a negative control, and Triton X-100 (1%) as positive control. After incubation at room temperature for 1 h, centrifugation, and removal of the supernatant, the released hemoglobin was measured by spectrophotometry at 540 nm. (EC_{50} is the calculated effective concentration that induced lysis on 50% that of the Triton X-100).

Results and discussion

All the 7-chloro-4-quinolinylhydrazones derivatives **3a–f** were synthesized by our research group (Scheme 1). Compounds **3a–f** were obtained through reaction between 7-Chloro-4-hydrazinoquinoline **1** and the appropriate aldehydes **2a–f**, as described in "Materials and Methods" section (Table 1). In general, ^{1}H NMR spectra showed the imine proton (N=C–H) as a singlet at 8.81–8.29 ppm. Furthermore, IR spectra presented N–H and N=C stretching vibrations at 3,197–3,247 and 1,612–1,576 cm^{-1}, respectively.

All synthesized compounds **3a–f** were tested in vitro against three cancer cells at 25 μg/ml, by using MTT assay (Table 2) (Ahmed *et al.*, 1994), and then, according to their growth inhibition (GI) percentage in, at least, one cell line, they were classified as active (100% GI), moderately active (75% < GI < 100%), or inactive (GI < 50%).

Among all tested compounds, only the 1-(7-Chloroquinolin-4-yl)-2-[(1H-pyrrol-2-yl)methylene]hydrazine (**3e**) was found to be active against all cancer cell lines, with a GI higher than 90%. Furthermore, derivative **3f** was moderately active on MDAMB-435 cells, while all other compounds were inactive against all cancer cell lines at 25 μg/ml.

In view of the above data, the compound **3e**, that presented a GI above 90%, was selected for in vitro anticancer activities evaluation against four human cancer cell lines, by using MTT assay (Table 3) (Ahmed *et al.*, 1994). The concentrations that induce 50% inhibition of cell

Table 1 Yields and melting points of 7-chloro-4-quinolinylhydrazones derivatives **3a–f**

Entry	Y	X	R^1	Yield (%)	Mp (°C)
3a	O	CH	NO_2	79	238–240[b]
3b	O	CH	H	83	210–212[a]
3c	S	CH	NO_2	85	189–190[b]
3d	S	CH	H	78	231–232[a]
3e	NH	CH	H	92	219–220[a]
3f	NH	N	H	83	274–275[a]

[a] Fattorusso *et al.* (2008)

[b] Ferreira *et al.* (2010)

Table 2 Growth inhibition percentage (GI%) for three tumor cell lines, measured by MTT assay

Entry	SF-295 GI%	SD (%)	MDAMB-435 GI%	SD (%)	HCT-8 GI%	SD (%)
3a	7.53	2.26	38.02	2.35	24.10	4.65
3b	61.92	0.51	68.47	0.27	53.25	2.16
3c	33.43	2.29	42.07	3.08	48.95	3.15
3d	1.03	3.27	21.58	1.49	21.09	5.67
3e	**100.00**	**0.12**	**100.00**	**0.51**	**100.00**	**0.05**
3f	30.17	4.31	82.59	0.59	33.95	5.37

Bold values indicate the best results

Table 3 Cytotoxic activity of 7-chloro-4-quinolinylhydrazones derivatives **3e** on tumor cell lines

Entry	IC_{50} (μg/ml)			
	MDAMB-435	SF-295	HCT-8	HL-60
3e	**0.1168**	0.5271	0.4410	0.09521
	0.1042–0.131	0.3973–0.699	0.3327–0.584	0.0845–0.107
Doxorubicin	0.48	0.23	0.01	0.02
	0.34–0.66	0.19–0.25	0.01–0.02	0.01–0.02

Bold values indicate the best results

Data are presented as IC_{50} values, with 95% confidence intervals obtained by nonlinear regression for all cell lines [leukemia (HL-60), melanoma (MDAMB-435), nervous system (SF-295), colon (HCT-8)] from three independent experiments. Doxorubicin (Dox) was used as positive control. The trials were performed in triplicate. *nd* Not determined. IC_{50} = concentrations that induce 50% inhibition of cell growth in μg/ml

growth (IC_{50}) in μg/ml are presented in Table 3. This compound was not able to disrupt the cell membrane integrity of erythrocytes in mouse model (data not shown) (Sharma and Sharma, 2001).

These results showed that compound **3e** exhibited an excellent cytotoxic activity against the four cancer cell lines, mainly on melanoma cells (MDAMB-435). In this case, compound **3e** is four times more active than the standard substance Doxorubicin, which could be considered a relevant finding toward the rational design of new leads for antitumor compounds.

In general, when compared to the monosubstituted and polysubstituted 7-chloro-4-quinolinylhydrazones previously evaluated by our research group (Montenegro *et al.*, 2011), the heteroaromatic derivatives **3a-f** showed worst antitumor activities than these other two series, except for **3e**. Furthermore, in a previous study (Montenegro *et al.*, 2011), the presence of nitro group was important to trigger the biological activity of monosubstituted quinolinylhydrazones (IC_{50} ranging from 0.7967 to 1.200 μg/ml). This behavior was already expected, since nitro compounds have been recently studied as radio sensitizers in antitumor therapy, due to the bioreduction capacity of the nitro group, which releases intermediates in the redox process (Krause *et al.*, 2005). However, in contrast with these findings, the presence of nitro group in heteroaromatic series did not lead to improved anticancer activities (please refer to **3a** vs. **3b,** and **3c** vs. **3d,** as shown in Table 3).

Conclusion

Six 7-chloro-4-quinolinylhydrazones were synthesized and evaluated against four different types of cancer cell lines. Among them, derivative 1-(7-Chloroquinolin-4-yl)-2-[(1H-pyrrol-2-yl)methylene]hydrazine (**3e**) could be considered a relevant finding toward the rational design of new leads for antitumor compounds, since it is four times more active than the standard substance Doxorubicin on melanoma cells (MDAMB-435). Further studies for the purpose of obtaining more information about structure–activity relationship, as well as researches to elucidate the molecular mechanisms of cytotoxicity presented by these compounds are still in progress.

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