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New thiosemicarbazones based on quinoline scaffold as anticancer iron chelators

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Abstract

Novel quinoline derivatives was designed as anticancer iron chelators. Structurally they combine active moieties of known quinoline and thiosemicarbazone bioeffectors. For the synthetic part of study we applied microvawe assisted techniques MAOS. Resulted compounds exhibited interesting anticancer activities against HCT116 cancer cells.

Keywords: Thiosemicarbazones, quinoline, anticancer, microwave.

Introduction

The quinoline scaffold is present in many classes of biologically-active compounds [1]. A series of compounds derived from 8-hydroxyquinoline and styrylquinoline derivatives were recently synthesized as potential HIV-1 integrase inhibitors [2,3]. These compounds show a significant similarity to some novel antifungal agents, namely homoallylamines, and therefore possess potential antifungal activity [4]. Our previous study dealing with 8-hydroxyquinoline and styrylquinoline derivatives showed that they could also possess a strong antifungal activity [5-7]. According to the results reported recently, some new hydroxyquinoline derivatives also possess

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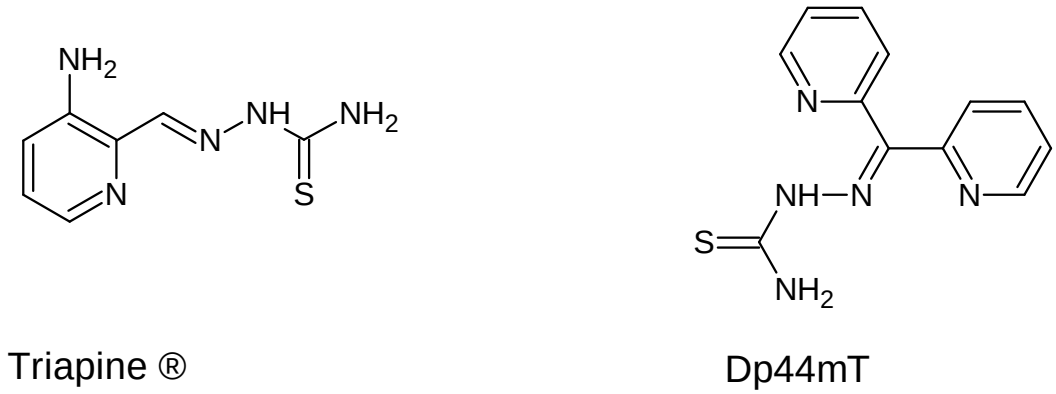
interesting herbicidal activities [6,8-13]. Some investigated compounds also showed antineoplastic activity [14]. This encouraged us to exploit the usability of quinoline scaffold in anticancer iron chelators. We aimed to functionalization of simple quinoline moiety with thiosemicarbaides, key part of another important bioeffectors and highly potential iron chelators: thiosemicarbazones.

This class of chelators was one of the first groups of ligands to be characterised for potent anti-tumour activity [15-18]. Particular structure of these class of compounds i.e. vicinity of sulfur and nitrogen atoms makes them valuable chelators for iron and other similar metal cations as Cu^{2+}, Co^{2+}, Mn^{2+}, Zn^{2+} [16, 19-22].



Triapine ® Dp44mT

Fig.1. Thiosemicarbazones with high anticancer potency.

There are two possible way of exhibition of anticancer potency through blocking of Ribonucleotide reductase (RR) [15-18] or by specific red-ox activity [23]. Especially the redox activity of Fe-complexes should be emphasized. Reduction of complex of Fe^{3+}-thiosemicarbazone lead to generation of reactive oxygen species (ROS) whose may be responsible also for inhibition of RR as they are able to quench the tyrosyl radical of the R2 subunit of RR [24]. This was proven by Shiao et al. who shown that Fe-Triapine complex is better inhibitor of RR than chelator alone [25]. Nevertheless many potential mechanisms of action of thiosemicarbazones should be highlighted as triggers for the designing of new drugs.

Results and discussion

In our approach we combined two moieties of known potency to design new anticancer agents **I** and **II** (Fig. 2.). For synthetic part we have applied our experience in microwave assisted chemistry carrying most of the steps in this efficient and green technique [26]. Microwave heating was especially useful in synthesis of structures **Ia** and **Ib**, where facilitated 3 out of 4 steps (with preparation of polyphosphoric acid).

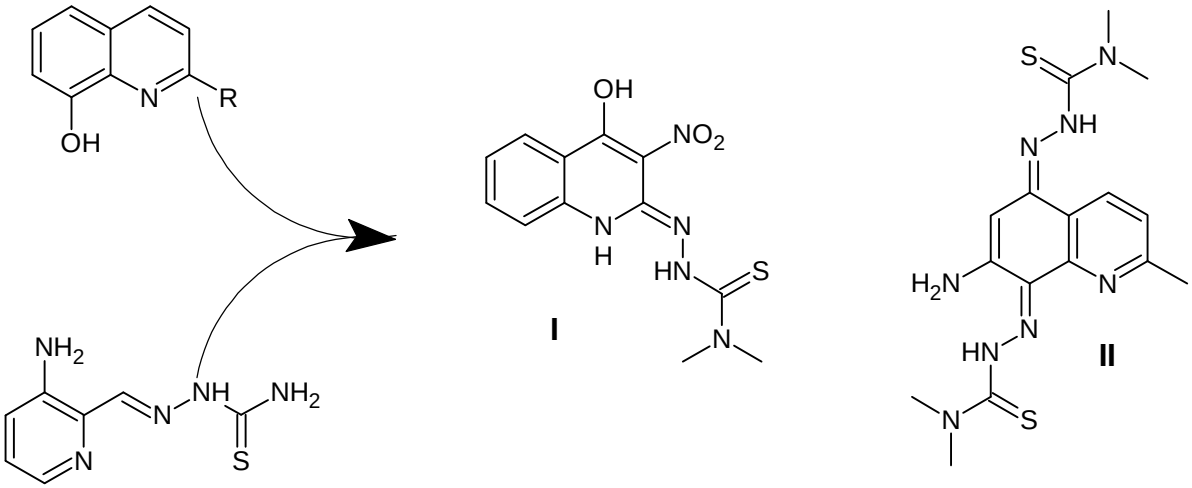


Fig.2. Quinoline as a scaffold for new iron chelators.

Yields of main product was good (above 80%) and the purity satisfying even without column chromatography.

Discovery microwave reactor from CEM was used for all synthesis. Structure of all new compounds was confirmed with NMR, MS and IR techniques. For purification we used Sepacore preparative chromatography from Buchi.

The anti-proliferative activity of the synthesized compounds were assessed by the MTT assay against the HCT116 (human colon carcinoma) cell line by the MTS assay. Cells obtained from the ATCC were grown as monolayer cultures in 75 cm2 flasks (Nunc) in Dulbecco's modified Eagle's medium supplemented with 12% foetal bovine serum (Gibco-BRL), 100 μg/mL of gentamycin, 100 μg/mL of streptomycin and 100 IU/mL of crystalline penicillin (Polfa). Cells were cultured under standard conditions at 37°C, in a humidified atmosphere at 5% CO_2.

Twenty four hours before addition of the tested compounds, the cells were seeded in 96-well plates. The assay was performed following a 72 h incubation with varying concentrations of the tested agents. The results were calculated as IC_{50} values. Each individual compound was tested in triplicate in a single experiment, with each experiment being repeated 3-7 times. After a 72 h incubation with tested compounds, 10 μL of MTT solution (MTT: stock solution: 5 mg/mL) was added to each well and incubated for 2 h at 37°C. After this incubation, 100 μL of the lysis mixture was added to each well. The optical densities of the samples were analyzed after an incubation of 24 h at 570 nm.

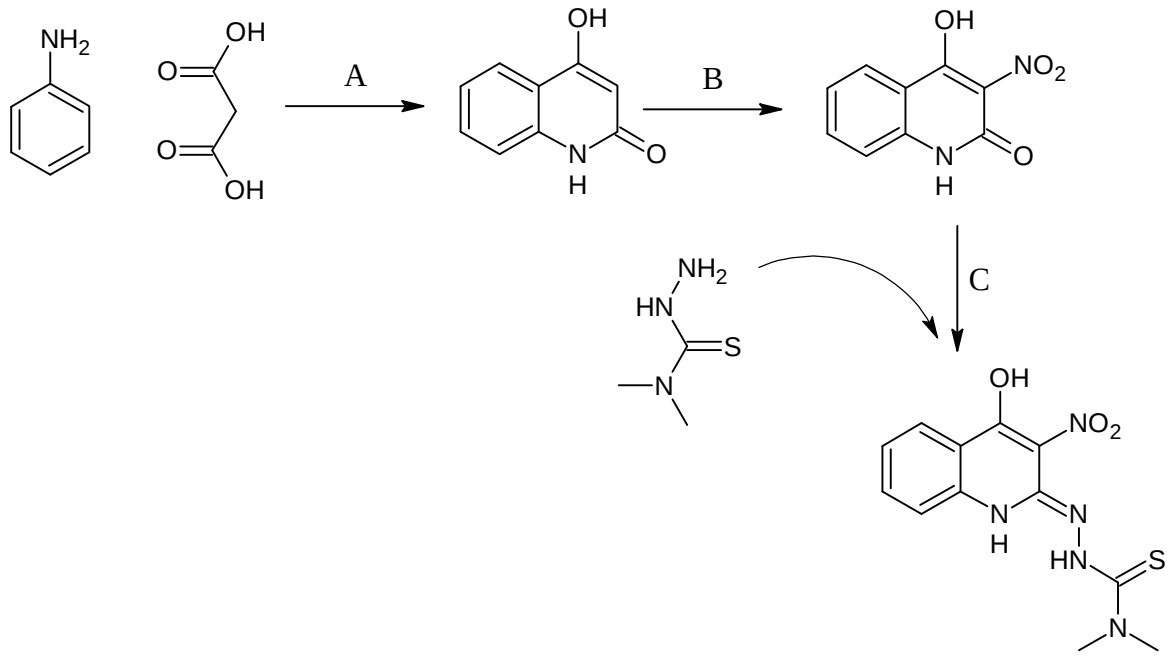


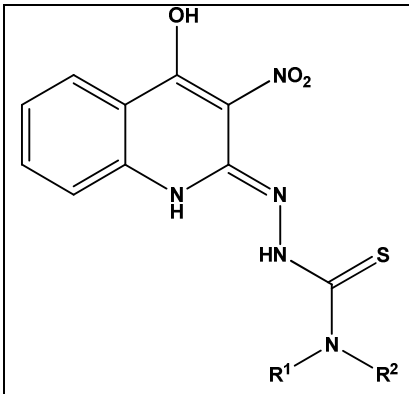
Fig.3. Synthesis of quinoline-ylidene I bearing thiosemicarbazone moiety. A- μWave/ polyphosphoric acid; B-nitration; C- μWave/EtOH.

4-Hydroxyquinolin-2(1H)-one Preparation of PPA: P_2O_5 (287.9 g) was added to 85% phosphoric acid (200 g, 118.4 mL) under stirring and microwave heating. The mixture was then heated for 15 min. Aniline (7 mL, 5 mmol) and malonic acid (5.2 g, 5 mmol) were thoroughly mixed with 20 g PPA and heated under stirring in microwave reactor at 400 W during 2×20 min with 5 min interval. The temperature reached 210 °C. Then the mixture was poured into crushed ice and the beige solid was filtered and purified by extraction with EtOH and a white crystalline compound was obtained. Yield 35%; Mp 340 °C; HPLC purity 97.12%; UV (nm), λ_{max}/log ε: 231.3/3.51; IR (cm^{-1}): 3618, 1180 (OH), 3043 (=CH-), 1670 (lactam), 1650 (C=O), 1593 (Ph), 1522 (NH).

4-Hydroxy-3-nitroquinolin-2(1H)-one. The product was obtained according to the described nitration procedure as a yellow crystalline compound. Yield 71%; Mp 252-255 °C; HPLC purity 99.72%; UV (nm), λ_{max}/log ε: 336.8/3.57; IR (cm^{-1}): 3620, 1181 (OH), 1712 (C=O), 1682 (lactam), 1622 ($C=C_{cycle}$), 1595 (Ph), 1547 (NO_2), 1525 (NH).

General procedure for synthesis Ia and Ib

The appropriate quinoline derivative was dissolved in ethanol, then thiosemicarbazide was added and microwave tube was closed using special capper and heated for 8 minutes in 82 °C. Pressure and temperature was controlled. The crude product was purified by simple crystallization using ethanol and diethyl ether.

	Where: **Ia**= $R^1 = CH_3$ $R^2=CH_3$ **MiLogP = 2.41** **AlogP = 1.43** **IC_{50}= 24 µM** **Ib**= R^1=H; $R^2=CH_2CH_3$

Ia ^{1}H-NMR (400 MHz, DMSO): 10,90 (bs, 1H); 7,95 (d, *J*=7,2 Hz, 1H); 7,46 (t, *J*= 7,6 Hz); 7,17 (d, *J*= 8 Hz, 1H); 7,09 (t, *J*= 7,6 Hz, 1H), 3.19 (s, 6H).

Ib ^{1}H-NMR (400 MHz, DMSO): 11,2 (bs, 1H); 7,98 (d, *J*=7,6 Hz, 1H); 7,40 (t, *J*= 7,2 Hz); 7,17 (d, *J*= 8,4 Hz, 1H); 7,02 (t, *J*= 7,6 Hz, 1H), 3.65 (q, 2H, *J*=7,4 Hz); 1,19 (t, 3H, *J*= 7,2 Hz)

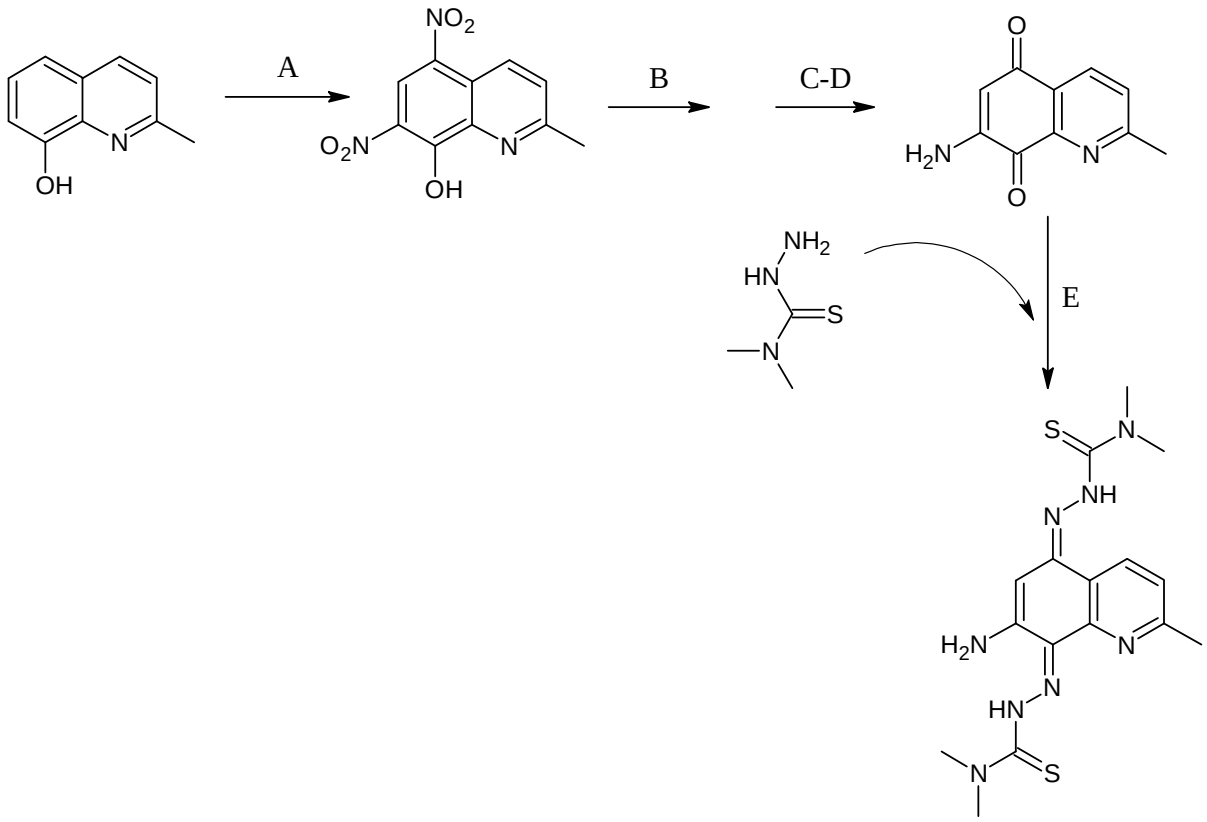
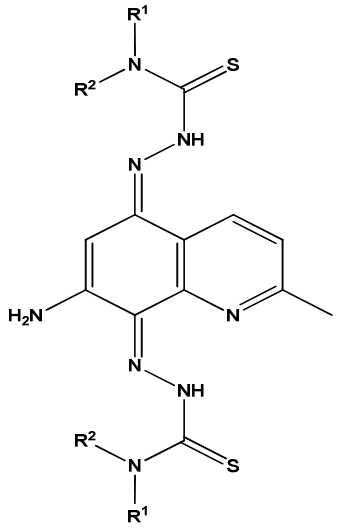


Fig.4. Synthesis of quinoline-diylidene II bearing thiosemicarbazone moiety. A-nitration; B-Reduction/acylation; D-oxidation; E- µWave/EtOH.

	Where: **IIa**= R^1=CH_3; R^2=CH_3 **MiLogP = 2.05** **ALogP = 1.18** **IC_{50}= 19 µM** **IIb**= R^1=H; R^2=CH_2CH_3

IIa ^{1}H-NMR (400 MHz, DMSO): 15,89 (bs, 1H); 8,29 (d, *J*= 8,0 Hz, 1H); 7,54 (d, *J*= 8,0 Hz, 1H); 5,74 (s, 1H); 3,18 (s, 12H).

IIb ^{1}H-NMR (400 MHz, DMSO): 15,02 (bs, 1H); 8,35 (d, *J*= 8,6 Hz, 1H); 7,63 (d, *J*= 8,0 Hz, 1H); 5,84 (s, 1H); 3,53 (bs, q, 4H,); 1,32 (t, 6H, *J*=6,8 Hz).

Further studies on structure optimization as well as elucidation of mechanism of action will be performed. Synthetic procedure presented here is time and cost efficient and allows obtaining relatively wide group of compounds for biological tests. Additional assays as redox potential measurement of Fe-complexes and prolonged cytotoxicity as clonogenity are currently running. We hope these studies will open new promising field of research.

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