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Synthesis and cytotoxic activity of a novel dihydroisoquinoline-derive hydroxamic acid

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ABSTRACT

Naturally occurring hydroxamic acid derivatives are biosynthesized by microorganisms (siderophores) and plants (benzoxazinoids). Recent developments in drug related research have highlighted the promising biological and pharmacological properties that the hydroxamic acid function may offer for the enhancement of therapeutic applications. This study reports on the full synthesis of a new dihydroisoquinoline hydroxamic acid (2-Hydroxy-3,3-dimethyl-7-nitro-3,4-dihydroisoquinolin-1(2H)-one). It also describes its cytotoxicity with regard to the human hepatocarcinoma cell line Hep3B.

Keywords

Nitrone; hydroxamic acid; cytotoxicity; Hep3B cells.

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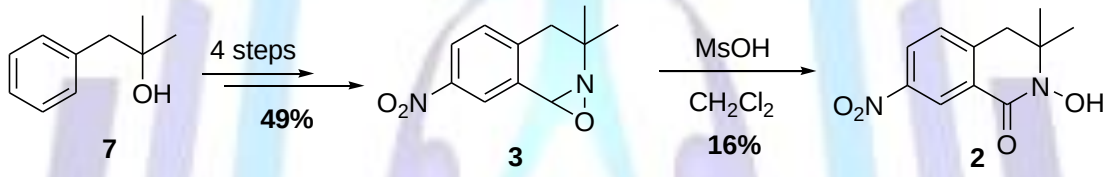
INTRODUCTION

Hydroxamic acids have attracted an increasing interest during the last few decades due to their wide range of biological activities and low toxicities. These properties have generally been attributed to their ability to chelate metals via the presence of two oxygen atoms [1-3] which confer them antibacterial, antifungal, anti-inflammatory, anti-asthmatic, and anticancer activities [4, 5].

Several synthetic methods have been reported, with varying degrees of success, for the preparation of hydroxamic acids. These methods include the amidation of esters with hydroxylamine [6, 7], nucleophilic displacement of carboxylates linked to an oxime resin [8, 9], amidation of carboxylic acids with hydroxylamine using various coupling reagents [10-15], amidation of N-acyloxazolidinones with hydroxylamines using samarium triflate [16], reaction of esters with O-benzylhydroxylamine [17,18], and the Angelini–Rimini reaction on a solid support [19]. Most of these methods have, however, used stringent basic conditions, intricate experimental procedures, high reaction temperatures, and complex purification methods, which limit their utility for large scale synthesis.

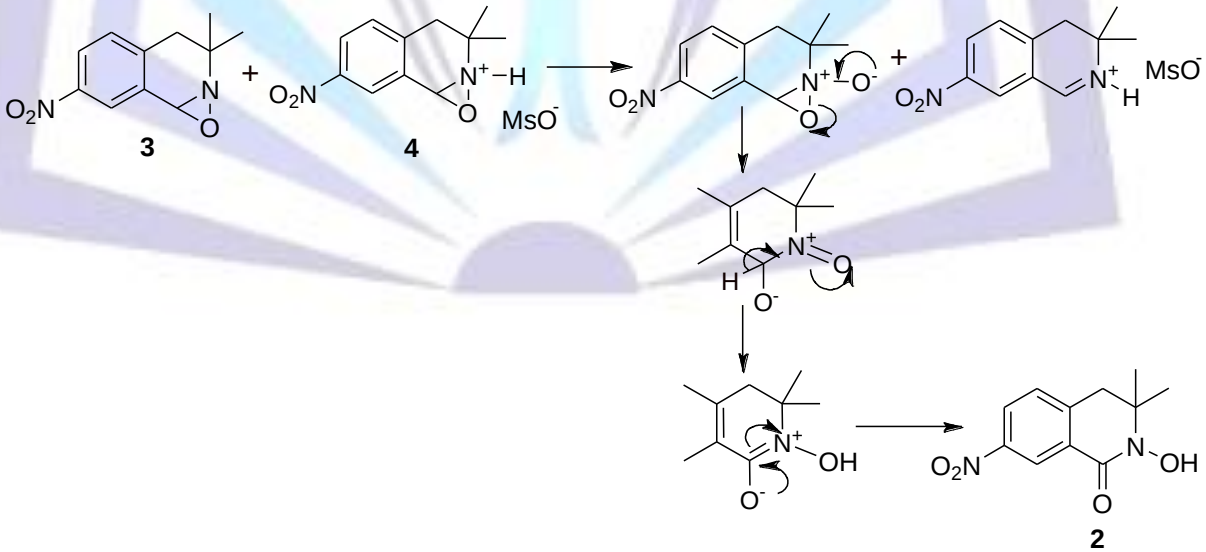
In our previous works, we studied the synthesis of the hydroxamic acid **2 (**2-Hydroxy-3,3-dimethyl-7-nitro-3,4-dihydroisoquinolin-1(2H)-one) by two different methods [20,21].

The first method consisted in the attainment of compound **2** starting from the commercially available tertiary alcohol **7** through 5 steps and with an overall yield of 7.8 %. In the last step, compound **2** was obtained by the acid-catalysed reaction of oxaziridine **3**, with a yield of 16 % [20] (scheme 1).



Scheme 1. Synthesis of the hydroxamic acid **2** from Oxaziridines **3** under the action of methanesulfonic acid.

Hydroxamic acid **2** was formed following the oxidation of oxaziridine **3** by N-protonated oxaziridine **4** as illustrated in the mechanism presented in scheme 2.



Scheme 2. Mechanistic hypothesis of hydroxamic acid **2** formation.

The second method consisted in the attainment of hydroxamic acid **2** from the same tertiary alcohol **7** through 4 steps and with an overall yield of 23.2 %. In the last step, compound **2** was obtained directly from the peracid oxidation of imine **5** [21] (scheme 3) with a yield of 40 %.

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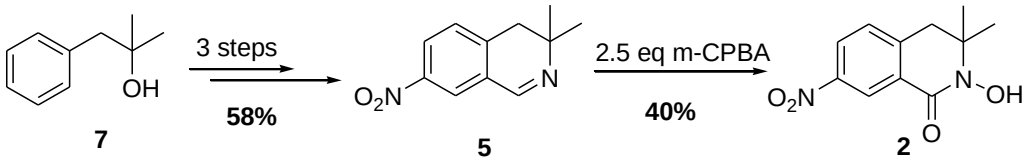
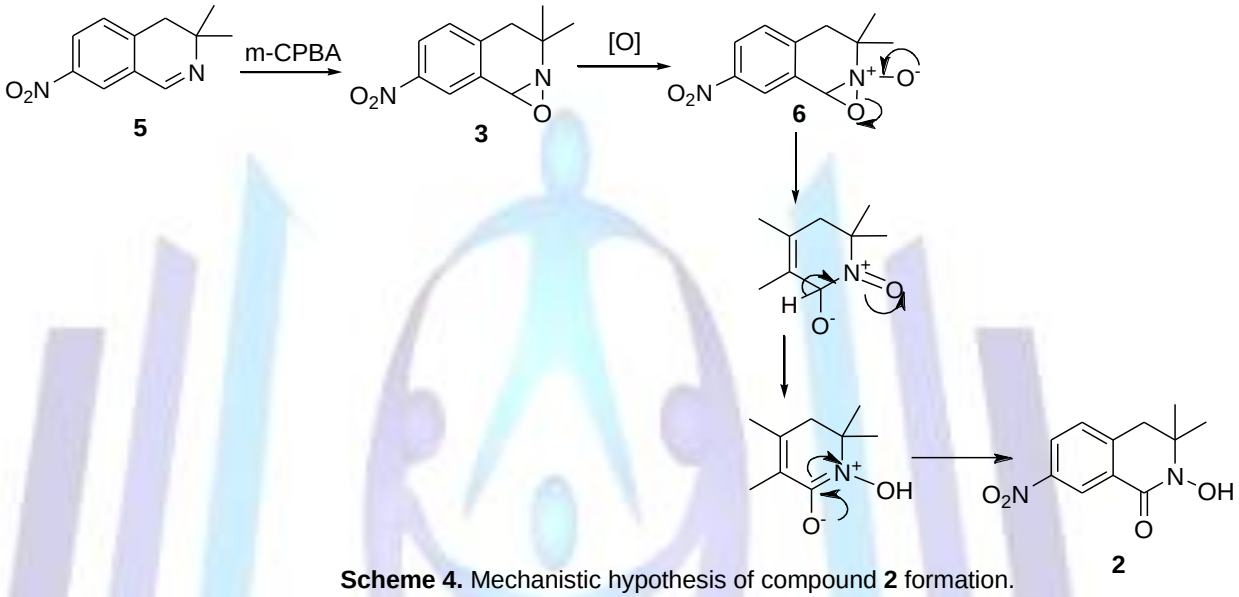


Scheme 3. Synthesis of the hydroxamic acid **2** from imine **5** under the action of m-chloroperbenzoic acid.

The oxidation of imine **5** by m-chloroperbenzoic acid gave oxaziridine **3**, which was sensitive to over-oxidation and, by rearrangement, yielded into hydroxamic acid **2** (scheme 4).



Scheme 4. Mechanistic hypothesis of compound **2** formation.

In this study, we investigate the feasibility and potential gain effects of a third method for the optimization of the total synthesis of hydroxamic acid **2**. We also explore the cytotoxic property of the cyclic hydroxamic acid **2** on the human hepatocarcinoma cell line Hep3B, currently studied in our laboratory.

RESULTS AND DISCUSSION

Total synthesis of hydroxamic acid 2 through nitrone 1

Compound **2** was obtained from alcohol **7** through Nitrone **1**. The latter was synthesized from the commercial tertiary alcohol **7** according to scheme 6. In step a, formamide **8** was formed by the Ritter reaction [22] and further cyclized into imine **9** [23,24]. The nitration of the dihydroisoquinoline **9** under soft conditions [25,26] selectively led to the nitro derivative **5**. The oxidation of **5** by dimethyldioxirane (DMD), however, rapidly led to nitrone **1** with a yield of 85% (scheme 5).

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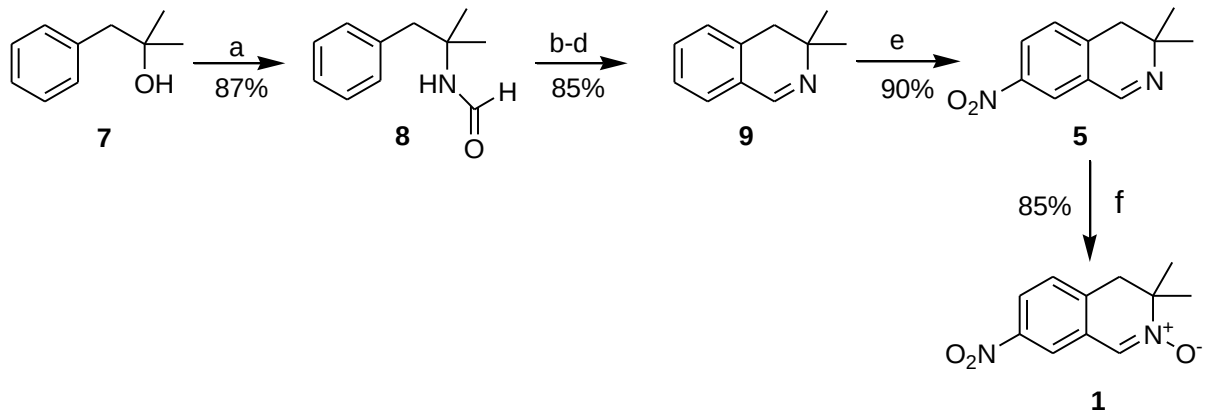
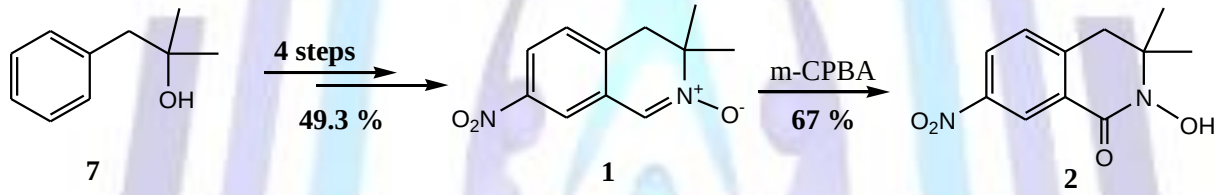


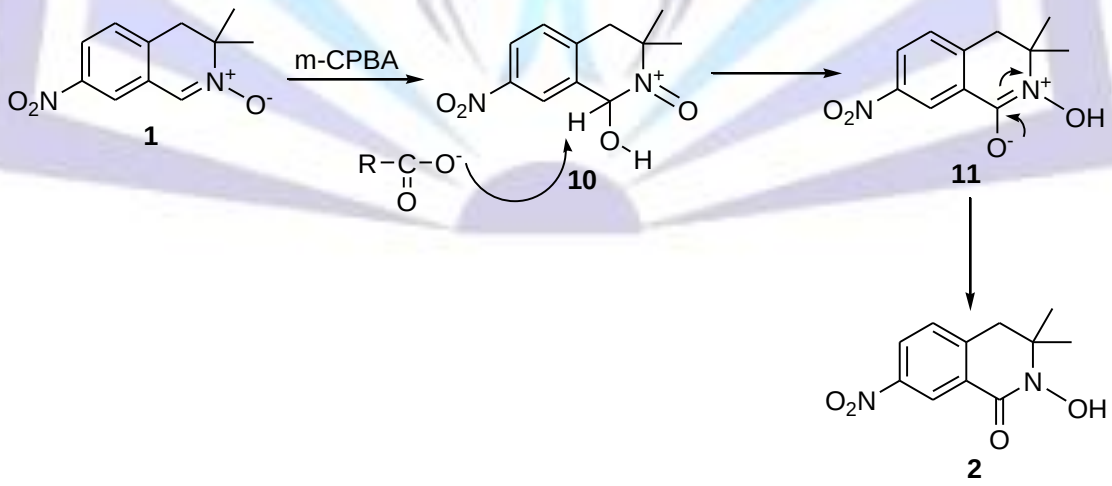
Scheme 5. Reagents and conditions: (a) KCN, AcOH-H_2SO_4,rt; (b) chlorure d'oxalyle; CH_2Cl_2; (c) $FeCl_3$; (d) MeOH, H_2SO_4; (e) KNO_3-H_2SO_4, rt 2h, 60°C 4h; (f) DMD (1,1eq), Acetone, rt.

Hydroxamic acid **2** was then obtained by the peracid oxidation of nitrone **1** with 1.1 equivalent of m-CPBA at room temperature in dichloromethane (Scheme 6). The m-CPBA disappeared after 5 h, leading to compound **2** with a yield of 67% (and with an overall yield of 33% from compound **7)**.



Scheme 6. Synthesis of the hydroxamic acid **2** from niitrone **1** under the action of m-chloroperbenzoic acid.

We hypothesize that the oxidation mechanism of nitrone **1** occurred on compound **10**, which, by deprotonation and tautomerization, gave compound **11** (scheme 7). The further rearrangement of **11** yielded into the expected compound **2**.



Scheme 7. Mechanistic hypothesis of compound **2** formation.

Cytotoxic activity of hydroxamic acid 2 on Hep3B cells

The anti-proliferative effect of compound **2** on the chemoresistant cell line Hep3B was assessed using the ATPLite™ assay. The results showed that this compound exhibited a cytotoxic but moderate activity on this cell line with an IC_{50} of 56.9 µM.

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CONCLUSION

We have prepared hydroxamic acid **2** starting the commercially available tertiary alcohol **7** through a five-step procedure with a better overall yield of 33 %. To the authors' knowledge, this study is the first to report on a hydroxamic acid **2** belonging to the dihydroisoquinoline family that exhibit a cytotoxic activity.

EXPERIMENTAL

Chemistry

Solvents were purified by standard methods. Melting points (mp) were determined under a microscope using a Leitz Wetzlair device and were uncorrected. Mass spectra (MS) were obtained by electronic impact (EI) (70 eV) on an AEI MS-50 spectrometer and in high resolution (HR) on a Kratos MS-80 spectrometer. NMR spectra were recorded at 250 or 300MHz for ^{1}H and 62.5 or 75MHz for ^{13}C. Chemical shifts (δ) are given in parts per million relative to tetramethylsilane (TMS), and coupling constants (*J*) are expressed in hertz (Hz). All reactions were monitored by TLC using commercial silica-gel plates, and visualization was accomplished by ultraviolet (UV) light or stained with a Draggendorf reagent. The presence of oxidizing species in the reaction mixtures was determined by potassium the iodide test. Formamide **8** [22] and imine **9** [23, 24] were synthesized by a known procedure. Imine **5** was prepared as described in a previous work by the authors [20].

Typical Dimethyldioxirane (DMD) oxidation procedure for imine

Dimethyldioxirane (DMD) was prepared as an acetone solution according to well established procedures in the literature [27]. In brief, the dimethyldioxirane product was obtained after distillation as an acetone solution (250-275 ml) using acetone (320 ml, 4.35 mol), water (440 ml), sodium hydrogen carbonate (240 g), and potassium peroxymonosulphate (500 g, 0.813 mol). The solution was analysed by iodometric titration and found to be consistently within the range 0.077-0.085M.

Preparation of nitrone 1

Imine **5** (0.1 g) in dichloromethane solution and pre-dried dimethyldioxirane (10% excess) were stirred together [CH_2Cl_2-Me_2CO (2:1)] at ice-bath temperature for 2 h. The removal of the solvent under reduced pressure gave nitrone **1**, which was purified by preparative TLC using chloroform-hexane (1:1) as an eluant and analysed by ^{1}H NMR spectroscopy. The product was isolated with a yield of 85%.

Mp: 152–154 °C. ^{1}H NMR ($CDCl_3$, 300MHz, δ): 1.47 s (6H, $2CH_3$), 3.19 s (2H, CH_2 H-4), 7.4 d (1H arom. H-5, *J* = 8.2 Hz), 7.79 s (1H, H-1), 7.97 d (1H arom. H-8, *J* = 2 Hz), 8.10 dd (1H, H-6, *J* = 2, *J* = 8.2 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz, δ): 24.82 [$C(CH_3)_2$], 41.73 (CH_2 C-4), 67.49 (CMe_2 C-3), 119.10, 123.46 and 128.65 (CH arom.), 129.73, 131.17, 136.59 (C arom.) and 148 (CH C-1). MS (EI): 221 [$(M+H)^{+\cdot}$., base peak]. Anal. calcd. For $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72; O, 21.79. Found: C, 60.07; H, 5.76; N, 12.59; O, 21.65%.

Preparation of hydroxamic acid 2

A solution of nitrone **1** (3 mmol) in CH_2Cl_2 (100ml) was stirred at room temperature while a solution of m-chloroperbenzoic acid 86% (3.3 mmol) in CH_2Cl_2 (50 ml) was added slowly. After 5h, the mixture was washed with saturated aqueous sodium bicarbonate (3 x 150ml), dried, and evaporated. The product was separated on silica gel chromatography using di-ethyl-ether as eluent, with a yield of 67%.

Mp: 175–176 °C. ^{1}H NMR (CD_3SOCD_3, 300MHz, δ): 1.26 s (6H, $2CH_3$), 3.23 s (2H, CH_2 H-4), 7.58 d (1H arom. H-5, *J* = 8Hz), 8.33 dd (1H, H-6, *J* = 2.4, *J* = 8Hz), 8.55 d (1H arom. H-8, *J* = 2.4Hz); ^{13}C NMR (CD_3SOCD_3 75MHz, δ): 25.24 [$C(CH_3)_2$], 41.80 (CH_2 C-4), 60.66 (CMe_2 C-3), 122.02, 126.87 and 130.41 (CH arom.), 129.91, 144.05 and 147.21(C arom.), 160.08 [-C(O) C-1]. MS (IE): 236 ($M^{+\cdot}$), 221 [$(M - 15)^{+\cdot}$, base peak]; MS (HR): found mass: 236.0844 mass calculated for $C_{11}H_{12}N_2O_4$: 236.0875.

Biology

The human cancer cell line Hep3B (HB-8064™, ATCC) was cultivated as previously described elswhere [28] with a doubling time of 20h (37°C, 5% CO_2). Hep3B cells were seeded in 96-well sterile plates and, after 18 h, were further incubated for 48 h with compound **2** at various concentrations (ranging from 0 to 200 µM) in 0.1% of DMSO. The proliferation of treated Hep3B cells was compared to control cells, which were treated only with 0.1% of DMSO, and measured by an ATP-based luminescence assay (ATPlite™kit, Perkin Elmer, France) and a Trilux reader (Perkin Elmer). The half maximal inhibitory concentration (IC_{50}) values were determined based on 3 independent experiments using the GraphPad Prism software (GraphPad Software Inc., USA).

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