Biotechnological Journal of Environmental Microorganisms (BJEM)

Biotechnological Journal of Environmental Microorganisms(BJEM) 2(6) 2023 295-300

6-Chloro-3-nitro-4-hydroxyquinolin-2(*1H*)-one as an efficient antibacterial agent

Enayatollah Moradi Rufchahi[1*]

[1]Department of Chemistry, Faculty of Basic Sciences, Lahijan branch, Islamic Azad University, Lahijan, Iran

Received: 20 July 2023/ Revised: 17 August 2023/ Accepted: 22 August 2023

Abstract

In this study, 4-chloroaniline was first reacted with diethyl malonate to yield the corresponding malonodianilide. Then, this compound was cyclized to 6-chloro-4-hydroxyquinolin-2(*1H*)-one in melted polyphosphoric acid at 140-150°C. 6-Chloro-3-nitro-4-hydroxyquinolin-2(*1H*)-one was successfully synthesized from 6-chloro-4-hydroxyquinolin-2(*1H*)-one through a traditional nitration process. After workup and purification process, this compound was characterized using Fourier transform infrared (FT-IR) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopic techniques. The antibacterial activities of the nitration product dissolved in DMSO were then evaluated using the well diffusion method against Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), Proteus vulgaris (ATCC 49132), and Listeria monocytogenes (ATCC 1298) bacterial strains.

Key words: 4-hydroxy quinolin-2(*1H*)-one, Nitration, Spectroscopy, Antibacterial activities

*Corresponding Author: E-mail: em.Rufchahi@iau.ac.ir, moradierufchahi@gmail.com

Biotechnological Journal of Environmental Microorganisms (BJEM)

Biotechnological Journal of Environmental Microorganisms(BJEM) 2(6) 2023 295-300

Introduction

Over the years, interest in quinolin-2-one derivatives has been growing due to their potential biological and chemical benefits [O'Donnell et al.,2010; Abdou, 2017]. Quinoline-2-one is an important structural component of several synthetic and natural compounds with notable medicinal properties [Bisacch, 2015; Michael, 2003]. Many papers have reported the synthesis and properties of quinoline-2-ones and a large number of their derivatives [Aly et al., 2020]. For example, antimicrobial tests were conducted on a novel series of symmetrically substituted 3,3-dibenzyl-4-hydroxy-3,4-dihydro-*1H*-quinolin-2-ones. The results showed that the minimum inhibitory concentration (MIC) values of these active heterocycles were even slightly higher than those exhibited by levofloxacin, employed as a comparator medicinal reference [Ferretti et al., 2014].

A series of 4-hydroxy-3-nitro-2-quinolones were synthesized, and their biological activity was discussed and compared with their related analogs [Dolle et al., 1995; Audisio et al., 2011; Cai et al., 1996; Shukla et al., 2011; Oeveren et al.,2007]. The ability of these antiallergic agents to prevent the rat's homocytotropic antibody-antigen-induced passive cutaneous anaphylactic reaction has been used to measure their antiallergic activity [Buckle et al., 1975].

Similarly, it has been demonstrated that 4-hydroxy-3-nitro-2(*1H*)-quinolone (compound 1, Scheme 1), a nitrated derivative of 4-hydroxyquinolin-2(*1H*)-one, exhibits anticancer action against cancer cells (Figure 1). It can be applied to the treatment of lung cancer, breast cancer, and colon cancer, among other cancer types. 4-Hydroxy-3-nitro-2(*1H*)-quinolone is an epidermal growth factor receptor (EGFR) antagonist and blocks epidermal growth factor signaling through its inhibition of EGFR tyrosine kinase activity [https://www.biosynth.com].

Inspired by the aforementioned encouraging results, we first prepared 6-chloro-4-hydroxyquinolin-2(*1H*)-one (**16**) and characterized its structure. This compound was then reacted with concentrated nitric acid to obtain the corresponding 3-nitro derivative (**17**) (Figure 2). Additionally, the antibacterial activities of the compound dissolved in DMSO were evaluated using the well diffusion method against the bacterial strains Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus vulgaris ATCC 49132, and Listeria monocytogenes ATCC 1298.

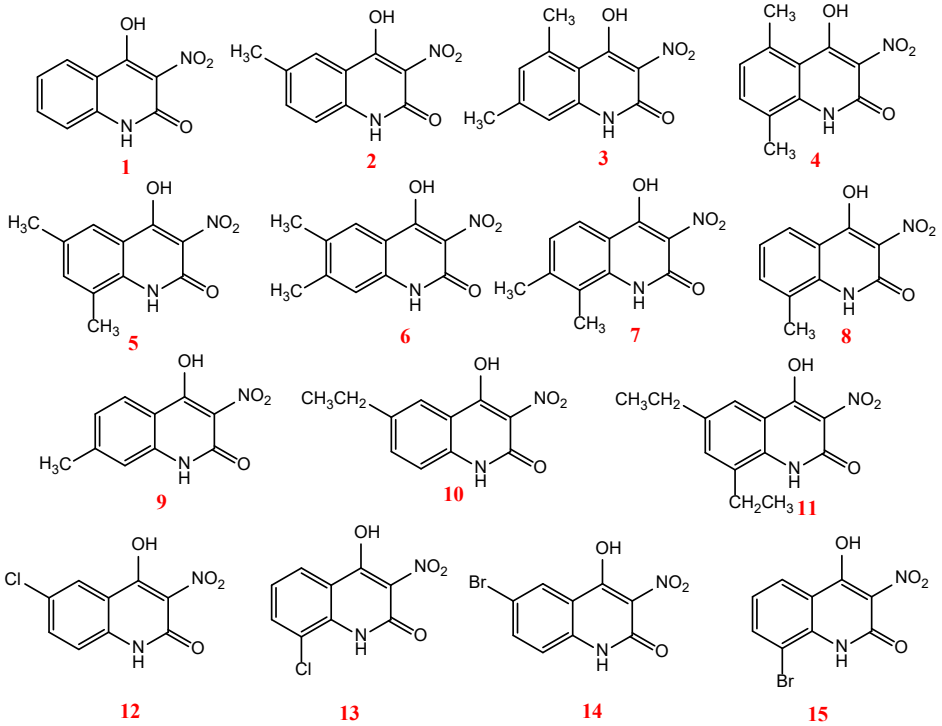

Figure 1. Some biologically active 3-nitro derivatives of 4-hydroxyquinolin-2(*1H*)-ones [Cai et al., 1996].

Materials and methods

All chemicals and solvents were obtained from commercial suppliers, namely Aldrich–Sigma and Merck chemical companies. The FT-IR spectra of the samples were obtained using a Perkin Elmer FT-IR Spectrophotometer, employing pressed KBr discs. NMR spectra were acquired using a Brucker Avance spectrometer in DMSO-d_6 with TMS serving as an internal standard. Melting points were determined using a Barnstead Electrothermal 9100 melting point apparatus in open capillary tubes, and the values were left uncorrected.

Preparation of N, N-Di-(4-chlorophenyl) malonamide (16)

A mixture of 4-chloroaniline (100 mmol, 12.8 g) and dimethylmalonate (50 mmol, 5.7 ml) was refluxed for 4 hours in an oil bath. After cooling, the mixture was treated with diethyl ether, filtered by suction, and recrystallized from ethanol. The yield was 95%, resulting in a white solid with a melting point of 218-220 °C (reported as 217 °C [Ziegler et al., 1965]).

Synthesis of 6-chloro-4-hydroxyquinoline-2-(*1H*)-one (17)

N, N′-Di-(4-chlorophenyl) malonamide (0.576 g, 2 mmol) was dissolved in 3.5 ml of methane sulfonic acid containing 10% phosphorus pentoxide and heated in an oil bath at 150°C for 90 minutes. The resulting dark viscous solution was allowed to cool, followed by adding water. The precipitated compound was filtered, washed with water, and air-dried. The crude product was then dissolved in 20 ml of 0.1 mol·L^{-1} sodium hydroxide solution, and any undissolved material was removed by filtration. The filtrate was neutralized with concentrated hydrochloric acid, and the resulting solid was recrystallized from DMF, yielding 6-chloro-4-hydroxyquinolin-2-(*1H*)-one as a white powder.

6-Chloro- 4-hydroxyquinoline -2-(*1H*)-one: white solid; Yield 42%; m.p. >350°C (350°C Ref. [Moradi-e-Rufchahi, 2010]); FT-IR(KBr) ν (cm-1) 3420(OH), 3100(NH), 3025(=C-H), 1658(C=O); ^1H NMR (400MHz, DMSO-d6) δ ppm 12.01(NH, br), 7.78(*1H*, d, J=2.4 Hz), 7.60(*1H*, dd, J= 8.8, 2.4Hz), 7.26(*1H*, d, J= 8.8 Hz), 5.99(*1H*, s).

Synthesis of 6-chloro-3-nitro-4-hydroxyquinoline-2-(*1H*)-one (**18**)

A suspension of 6-Chloro- 4-hydroxyquinolin-2-(*1H*)-one (**17**) (2.0 mmol, 0.39 g) in glacial acetic acid (6 mL) was heated for 10-15 minutes in an oil bath at 70 °C and then treated with nitrating agent prepared from concentrated nitric acid (0.2 mL) and concentrated sulphuric acid (0.25 mL) to start the exothermic reaction. The starting material dissolved and the solution was stirred for an additional 10 min at this temperature. The resulting solution was then poured into ice water (100 mL) and the precipitate was filtered and recrystallized from glacial acetic acid to afford 6-chloro-3-nitro-4-hydroxyquinolin-2-(*1H*)-one (**18**) as a deep orange powder; Yield 51%; m.p. >350°C; FTIR (KBr): 3371 (OH), 3181 (NH), 3078 (=C–H), 1657 (C=O), 1597 (C=C),1597 (N=O), 1499 (N=O); ^1H NMR (400 MHz, DMSO-d_6), δ(ppm): 11.12 (*1H*, OH), 7.89 (*1H*, d, J = 2.0 Hz), 7.51(*1H*, dd, J=2, 8.4 Hz), 7.19 (*1H*, d, J=8.4 Hz).

Results and Discussions

Synthesis and characterization

6-chloro-4-Hydroxyquinolin-2(*1H*)-one (**17**) was prepared following the reported procedure [Moradi Rufchahi and Ghanadzadeh Gilani, 2012]. In this method, N, N′-di(4-chlorophenyl) malonamide (**16**) was synthesized by reacting 4-chloroaniline with diethyl malonate under reflux conditions. This obtained di-anilide was then cyclized to 6-chloro-4-Hydroxyquinolin-2(*1H*)-one in reaction with polyphosphoric acid under thermal conditions at 140-150 °C. The ^1H NMR data were used to distinguish the structure of this compound from the starting materials in which, the signal at 5.99 ppm (=CH) confirmed the formation of the desired quinolone ring. The preparation of the 6-chloro-4-Hydroxyquinolin-2(*1H*)-one and its nitration is illustrated in Scheme 2. Compound (**17**) was nitrated with concentrated nitric acid in glacial acetic acid at 80 °C for 10 min to afford 6-chloro-3-nitro-4-hydroxyquinolin-2-(*1H*)-one (**18**) in a good yield. The purity of the compounds was confirmed by thin-layer chromatography (TLC) using diethyl

Biotechnological Journal of Environmental Microorganisms (BJEM)

Biotechnological Journal of Environmental Microorganisms(BJEM) 2(6) 2023 295-300

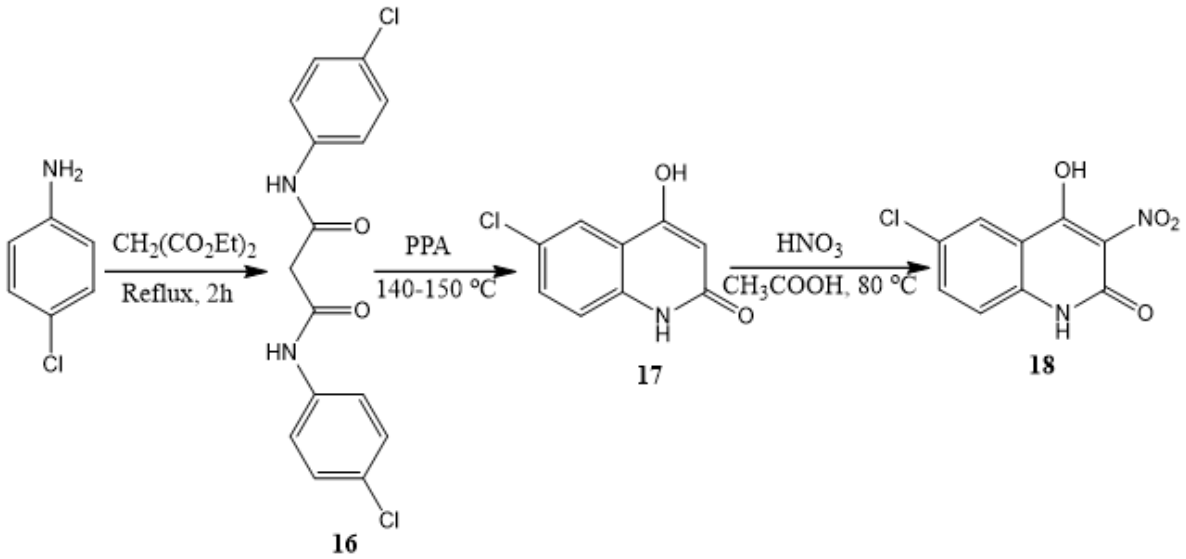

Figure 2. Synthetic route to 6-chloro-3-nitro-4-hdroxyquinolin-2(*1H*)-one.

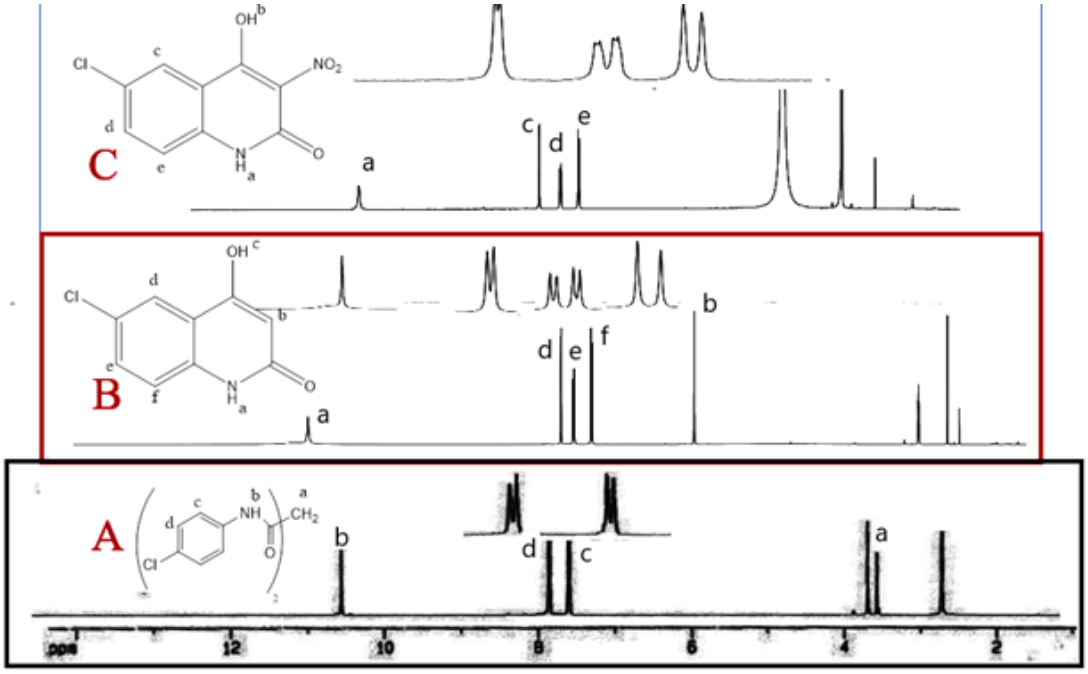

Figure 3. ^{1}H NMR spectra of the compounds **16** (A), **17** (B), and **18** (C) in DMSO-d6 at ambient temperature.

ether/ethyl acetate (20:80) as the mobile phase, along with melting point determination. All compounds exhibited a single TLC spot and sharp melting points.

Compound (**18**) possesses a nitro group; therefore, the stretching vibration of N=O linkage shows two bands at 1592 and 1334 cm–1 in its IR spectra. The signal assigned to the olefinic proton was not observed in the *1H* NMR spectrum of this compound.

Figure 3 shows *1H* NMR spectra of compounds (**16**), (**17**), and (**18**) in one sheet. As obvious in the figure, the step-by-step transformation of each starting material to the corresponding product can be assigned through the changes in the spectra.

3.2. Evaluation of Antibacterial Activity

The synthesized 6-chloro-3-nitro-4-hdroxyquinolin-2(*1H*)-one derivative (**18**) was tested for its antibacterial activities by using the well diffusion method on Mueller-Hinton agar (MHA).

Table 1. Minimal inhibitory concentrations (MIC, g/mL) of the prepared compound against the tested microorganisms.

Microorganism	concentration of the compound (mg/ml)			
	2	4	6	8
Staphylococcus aureus	-*	13mm	21mm	37mm
Escherichia coli	-	-	-	17mm
Proteus vulgaris	-	7mm	10mm	12mm
Listeria monocytogenes	-	-	8mm	15mm

* It does not show activity.

Table 2. Evaluation of the antimicrobial activity of compound 18 against studied bacterial strains by using the well diffusion method.

Microorganism	Diameter of inhibition zone* (mm)	
	6-chloro-3-nitro-4-hydroxyquinolin-2-(1*H*)-one **18**	Gentamycin**
Staphylococcus aureus	35	38
Escherichia coli	16	18
Proteus vulgaris	15	18
Listeria monocytogenes	20	31

The MIC values for some of the synthesized product was tested against the mentioned bacterial and are listed in Table 1. Briefly, the bacterial strains were suspended with a turbidity of 0.5 McFarland (equal to 1.5×108 colony-forming units (CFU)/ml) and cultured on MHA under aseptic conditions. Wells with 6mm in diameter were filled with 2 mg/ml, 4 mg/ml, 6 mg/ml and 8 mg/ml of dye solution and incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zone was measured in mm (Table 1).

The compound was dissolved in DMSO and Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Proteus vulgaris ATCC 49132 and Listeria monocytogenes 1298 ATCC were used as references for the antibacterial assay and the inhibition zones were reported in millimeter (mm) after 24 hours. The results are depicted in Table 2.

According to the activity index, the well diffusion results show that the compounds (18) has reasonable antimicrobial activity against the tested microbes, which was confirmed by an inhibition zone (see Figure 4). In conclusion, the aim of the present study was to synthesize and investigate the antimicrobial activities of a new quinolone compound in the hope of discovering new lead structure that serve as potent antimicrobial agents compared to the standard drug Gentamycin.

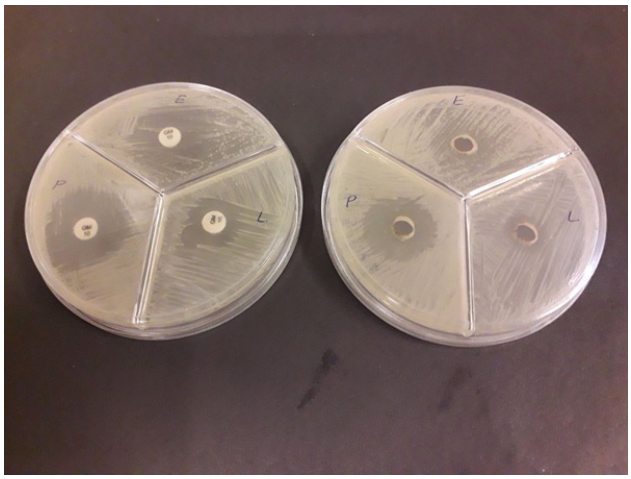

Figure 4. Zone of inhibition for (**18**) (E: Escherichia coli, P: Proteus vulgaris, L: Listeria monocytogenes) by well diffusion method.

Conclusion

Conclusively, in the presented research work, 6-chloro-4-hydroxyquinoli-2(*1H*)-one was used as an enol type coupling component for the synthesis and characterization of 6-chloro-3-nitro-4-hdroxyquinolin-2(*1H*)-one.

Additionally, the evaluation of the antibacte-

rial activities of the compound showed that this nitro product has good antibacterial activity, emphasizing the potential of this compound (**18**) for its dual efficacy against both Gram-positive and Gram-negative bacteria. Further exploration into the mechanisms and selectivity of this compound could provide valuable insights for its applications in combating bacterial infections.

Acknowledgment

The author is grateful to the Islamic Azad University of Lahijan authorities for providing the necessary facilities to carry out the present work.

References

Abdou M M (2017) Chemistry of 4-Hydroxy-2(*1H*)-quinolone. Part 1: Synthesis and reactions Arab J Chem, 10, S3324–S3337.

Aly A, El-Sheref E M, Mourad A F E, Bakheet M E M, Bräse S (2020) 4-Hydroxy-2-quinolones: syntheses, reactions and fused heterocycles. Mol Divers, 24, 477-524.

Audisio D, Messaoudi D S, Peyrat J F, Brion J D, Alami M, Cegielkowski L, Methy-Gonnot D, Radanyi C, Renoir J M (2011) Discovery and Biological Activity of 6BrCaQ as an Inhibitor of the Hsp90 Protein Folding Machinery. Chem Med Chem 6, 804-815.

Bisacch G S (2015) Origins of the quinolone class of antibacterials: an expanded "discovery story". J Med Chem, 58, 4874–4882.

Buckle D R, Cantello B C C, Smith H, Spicer B A (1975) 4-Hydroxy-3-nitro-2-quinolones and related compounds as inhibitors of allergic reactions. J Med Chem 18, 726–732.

Cai S X, Zhou Z L, Huang J C, Whittemore E R, Egbuwoku Z O, Egbuwoku J E, Hawkinson J E, Woodward R M, Weber E, Keana J F W (1996) Structure−Activity Relationships of 4-Hydroxy-3-nitroquinolin-2(*1H*)-ones as Novel Antagonists at the Glycine Site of N-Methyl-d-aspartate Receptors. J Med Chem 39, 4682-4686.

Dolle V, Fan E, Nguyen C H, Aubertin A M, Kirn A, Andreola M L, Jamieson G, Litvak LT, Bisagni E (1995) A New Series of Pyridinone Derivatives as Potent Non-Nucleoside Human Immunodeficiency Virus Type 1 Specific Reverse Transcriptase Inhibitors. J Med Chem 38, 4679-4686.

Ferretti M D, Neto A T, Morel A F, Kaufman T S, Larghi E L (2014) Synthesis of symmetrically substituted 3,3-dibenzyl-4-hydroxy-3,4-dihydro-*1H*-quinolin-2-ones, as novel quinoline derivatives with antibacterial activity. Eur J Med Chem, 81, 253-266.

Michael J P (2003) Quinoline, quinazoline and acridone alkaloids. Nat Prod Rep, 20, 476-493.

Moradi Rufchahi EO, Ghanadzadeh Gilani A (2012) Synthesis, characterization and spectroscopic properties of some new azo disperse dyes derived from 4- hydroxybenzo [h]quinolin-2-(*1H*)-one as a new synthesized enol type coupling component, Dyes Pigm 95, 632-638.

Moradi-e-Rufchahi, EO (2010) Synthesis of 6-chloro and 6-fluoro-4-hydroxyl-2-quinolone and their azo disperse dyes. Chin. Chem. Lett. 21, 542-546.

O'Donnell F, Smyth T J P, Ramachandran V N, Smyth W F (2010) A study of the antimicrobial activity of selected synthetic and naturally occurring quinolones. Int J Antimicrob Agents, 35, 30-38.

Oeveren A, Motamedi M, Martinborough E, Zhao S, Shen Y, West S, Chang W, Kallel A, Marschke K B, Lopez F J, Negro-Vilar A, Zhi L (2007) Novel selective androgen receptor modulators: SAR studies on 6-bisalkylamino-2-quinolinones. Bioorg Med Chem Lett 17, 1527-1531.

Shukla N M, Malladi S S, Mutz C A, Balakrishna R, David S A (2010) Structure−Activity Relationships in Human Toll-Like Receptor 7-Active Imidazoquinoline Analogues. J Med Chem 53, 4450-4465.

Ziegler E, Wolf R, Kappe J (1965) Eine einfache synthese des 4- hydroxycarboxytyrils und seiner derivate. Monatsch 96, 418.