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SYNTHESIS, SPECTRAL CHARACTERIZATION, AND *IN-VITRO* ANTIOXIDANT ACTIVITY SCREENING OF SOME NOVEL 2-HYDROXY QUINOLINE DERIVATIVES

Pradeep Kumar M. R. [*1] and Hunashal R. D. [2]

Department of Pharmaceutical Chemistry [1], KLE College of Pharmacy, A Constituent Unit of KLE Academy of Higher Education and Research [KAHER], Hubli - 580031, Karnataka, India.
Department of Pharmacology [2], Karnataka Institute of Medical Science, Hubli - 580031, Karnataka, India.

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Correspondence to Author:
Dr. Pradeep Kumar M. R.

Assistant Professor,
Department of Pharmaceutical Chemistry, KLE College of Pharmacy, Hubli - 580031, Karnataka, India.

E-mail: pradeepmrpk@yahoo.co.in

ABSTRACT: In an attempt of synthesizing some novel and potent antioxidant activity having compounds, here some novel quinoline derivatives are reported. Initially using Vilsemeir-Hack reagent method 7-methyl or 8-methyl substituted 2-cholro-3-formylquinolines (Ia, b) were prepared. Further 7-methyl or 8-methyl substituted 2-hydroxy quinoline-3-carbaldehyde (IIa, b) were synthesized by the reaction of compound (I) on microwave irradiation with 4M HCl, which on further treatment with different substituted hydrazides yielded the novel Schiff bases of quinoline III (a-f). Purity of the synthesized compounds was determined by TLC and melting point. The structure of all newly synthesized compounds was confirmed by spectral studies such as IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. All the synthesized compounds were screened for *in-vitro* antioxidant activity by free radical scavenging activity by DPPH assays method and ferric ion reduction method using ascorbic acid as the standard drug. Screening results showed that all compounds are exhibiting moderate to good activity. Especially compounds III b, III d, and III f showed significant antioxidant activity.

INTRODUCTION: Oxidation processes are essential for the energy management of all living organisms and are therefore maintained under strict control by several cellular mechanisms [1]. Free radicals are molecules, ions, or atoms with unpaired electrons in their outermost shell of electrons [2]. These are constantly formed in human body, can become toxic when generated in excess or during the deficiency of naturally occurring antioxidant defenses. High levels of free radicals can cause damage to biomolecules such as lipids, proteins, enzymes and DNA in cells and tissues. This may result in many diseases such as cancer, diabetes, cardiovascular, autoimmune diseases, neurodegenerative disorders and other diseases through the violent reactivity of the free radicals [3-5]. Antioxidants are important compounds that reduce or neutralize the free radicals, thus protecting the cells from oxidative injury [6]. Therefore, considerable research has been directed towards the development and identification of new antioxidants to prevent radical-induced damage.

Quinoline ring system is a ubiquitous pharmacophore and an essential structural fragment of a large number of natural and synthetic compounds possessing versatile pharmacological activities like antidiabetic [7], anti-HIV [8], antioxidant [9], anti-inflammatory [10], antifungal [11], antimicrobial [12,13], antihypertensive [14], analgesic [15], antiparasitic [16], antitubercular [17, 18, 19], antiviral [20], anticonvulsant [21,

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[22], antimalarial [23], anticancer [24, 25, 26,] *etc*. These results encouraged us to synthesize some novel quinoline derivatives for antioxidant activity.

In continuation of our research work on quinolines to explore their potent therapeutic properties [27, 28, 29], here we have reported the synthesis, spectral characterization, and *in-vitro* antioxidant screening of some novel quinoline derivatives.

In this research work, initially, compounds I (a, b) were synthesized by the microwave irradiation of 7 or 8-substituted-2-cholro-3-formylquinolines with 4M HCl. IR spectra of these compounds showed characteristic peaks at around 3180 cm^{-1} (OH), 1680 cm^{-1} (C=O), 1600 cm^{-1} (C=N), Further a novel series of quinoline derivatives III (a-f) were synthesized by the reaction between compounds II (a, b) and different hydrazides. IR spectra of these compounds showed characteristic peaks at around 3400 cm^{-1} (OH), 3100 cm^{-1} (NH), 1660 cm^{-1} (C=O), 1600 cm^{-1} (C=N). ^{1}H NMR spectra of these compounds showed characteristic peaks at around δ 2.1, 8.32 and 12.24 ppm due to protons of CH_3, CH=N, and OH respectively. ^{13}C NMR spectra of these compounds showed characteristic peaks at around δ 22, 135, and 160 ppm due to carbon of CH_3, CH=N, and C=O respectively.

MATERIALS AND METHODS:

General Considerations: The various chemicals used in the synthesis of the titled compounds were purchased from Sigma-Aldrich Pvt. Ltd, Spectrochem Pvt. Ltd, S.D. Fine Chem Pvt. Ltd. Absolute ethanol and DMF utilized were purified according to the literature.

Melting points of all synthesized compounds were determined by open capillary method and are uncorrected. FTIR spectra were recorded on Bruker Alpha-T by using KBr pellets. The $^{1}HNMR$ were recorded on Bruker Avance II NMR 400 MHz instruments using DMSO as solvent and TMS as internal standard; chemical shifts are expressed as δ values (ppm). Elemental analysis was carried out for the synthesized compounds. All the physicochemical data are given in **Table 1**.

Antioxidant Activity Screening:

Free Radical Scavenging Activity by DPPH Assays Method: [30] DPPH (1, 1-diphenyl-2-picryl-hydrazil) is stable free radical. Methanol solution of DPPH is used to evaluate the antioxidant activity of several synthetic compounds. Antioxidant on interaction with DPPH, both transfer electron or hydrogen atom to DPPH, thus neutralizing its free radical character and convert it to1, 1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging activity of the drug. The change in absorbance produced at 517 nm has been used as a measure of its antioxidant activity. For this method 10, 20, 30, 40, 50 µg/ml concentrations of ligands and ascorbic acid were prepared. From this stock solution, 1ml has been pipette out, and 5ml methanol solution of DPPH was added, shaken well, and the mixture was incubated at 37 °C for 30 min absorbance of all samples measured against blank 517 nm. The absorbance of the DPPH reagent alone was taken as control. The % radical scavenging activity can be calculated following formula:

% free radical Scavenging activity = Absorbance of control - Absorbance of sample × 100 / Absorbance of control

All the synthesized compounds were subjected to anti-oxidant activity screening using this method, and results are shown in **Table 2**.

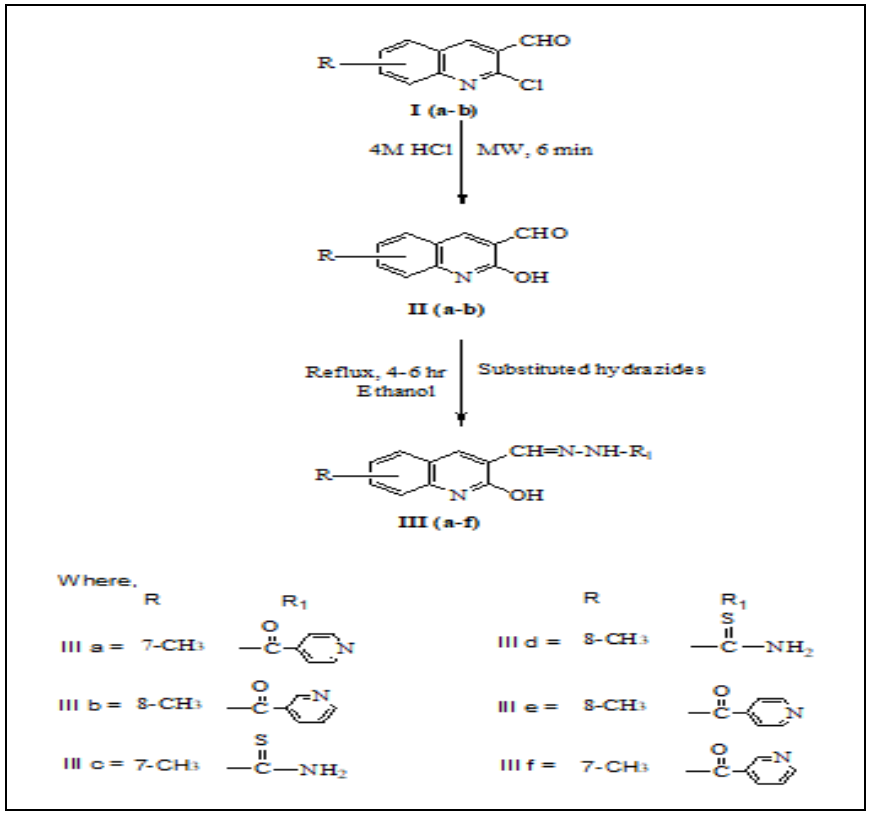
Ferric Ion Reduction Method: [31] The reaction mixture containing 1, 10-o-phenanthroline (0.5 mL), ferric chloride 1mL (0.02 mM) and test compound (solution of different concentration of synthesized compounds and ascorbic acid as standard drug) in a final volume of 5 mL with methanol was incubated for 15-20 min at ambient temperature and absorbance was measured at 510 nm. In another set, sodium dithionate (0.3 mM) was added instead of the compounds, and absorbance was taken as equivalent to 100% reduction of all the ferric ions present.

Anti-oxidant activity by ferric ion reduction method can be calculated by the following formula:

$$\% \text{ Activity} = [At / As] \times 100$$

Where, As = absorbance by standard drug solution at 510 nm; At = absorbance by the sample solution at 510 nm.

All the synthesized compounds were subjected for anti-oxidant activity screening using this method and results are shown in **Table 3**.



SCHEME

RESULTS:

General Procedure for the Synthesis of 7/ 8-methyl-2-hydroxyquinoline-3-carbaldehyde II (a, b): A mixture of 7 or 8-methyl-2-chloro-3-formylquinolines (0.01 mol) and 4M HCl solution (30 ml) was subjected for Microwave irradiation (120 W) for 6 min. Yellow solid was precipitated on cooling. This was poured into a beaker containing crushed ice (80 g), filtered, dried, and recrystallised from glacial acetic acid.

II a: IR (KBr) cm^{-1}: 3190.12 (OH), 1601.03 (C=O), 1622.14 (C=N). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.13 (s, 3H, CH_3), 7.21 (d, 1H, C_6-H of quinoline), 7.42 (d, 1H, C_8-H of quinoline), 7.90 (d, 1H, C_5-H of quinoline), 8.38 (s, 1H, C_4-H of quinoline), 10.26 (s, 1H, CHO), 12.63 (s, 1H, OH). Mass (m/z): 187.19

II b: IR (KBr) cm^{-1}: 3189.11 (OH), 1684.76 (C=O), 1621.17 (C=N). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.1 (s, 3H, CH_3), 7.28 (d, 1H, C_6-H of quinoline), 7.51 (d, 1H, C_8-H of quinoline), 7.89 (d, 1H, C_5-H of quinoline), 8.32 (s, 1H, C_4-H of quinoline), 10.43 (s, 1H, CHO), 12.25 (s, 1H, OH).

Mass (m/z): 187.19.

General procedure for the synthesis of compounds III (a-f): A mixture of compound II (a, b) (0.005 mol) in 25 ml of absolute ethanol, substituted hydrazides (0.005 mol), and a catalytic amount of glacial acetic acid was added. The mixture was refluxed for 6 h. A solid compound was obtained on cooling the reaction mixture. It was filtered, dried, and recrystallized from ethanol and DMF mixture to get the final compounds III (a-f).

III a: IR (KBr) cm^{-1}: 3406.16 (OH), 3159.24 (NH), 1649.61 (C=O), 1604.17 (C=N). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.18 (s, 3H, CH_3), 7.35 - 8.38 (m, 9H, C_4, C_5, C_6, C_8-H of quinoline, CH=N & C_2, C_3, C_5, C_6-H of pyridine), 12.09 (s, 1H, NH), 12.31 (s, 1H, OH). Mass (m/z): 306.32

III b: IR (KBr) cm^{-1}: 3406.37 (OH), 3159.33 (NH), 1644.21 (C=O), 1615.18 (C=N), ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 2.19 (s, 3H, CH_3), 7.34 (s, 2H, C_3 & C_4-H of pyridine), 7.48 (s, 1H, C_8-H of quinoline), 7.77 (s, 2H, C_2 & C_5-H of pyridine), 7.86 (s, 1H, C_6-H of quinoline), 8.13 (s, 1H, C_5-H of quinoline), 8.34 (s, 1H, CH=N), 8.55 (s, 1H, C_4-H of quinoline), 11.68 (s, 1H, NH), 12.26 (s, 1H, OH). ^{13}C NMR (400 MHz, DMSO-d_6, δ ppm):

22.08 (CH_3), 161.98 (quinoline- C_2), 160.16 (C=O), 151.30 (quinoline C_9), 145.26 (CH=N), 141.75 (pyridine- C_4), 136.43 (quinoline- C_4), 131.62 (quinoline- C_7), 127.58 (pyridine- C_3 and -C_5), 126.03 (quinoline- C_5), 122.18 (pyridine- C_2 and -C_6), 121.39 (quinoline- C_8), 120.55 (quinoline- C_6), 118.32 (quinoline- C_{10}), 115.36 (quinoline- C_3). Mass (m/z): 306.11

III c: IR (KBr) cm^{-1}: 3410.17 (OH), 3106.34 (NH), 1681.02 (C=O), 1613.05 (C=N), ^{1}H NMR (400 MHz, DMSO-*d_6*, δ ppm): 2.13 (s, 3H, CH_3), 7.35 (d, 1H, C_8-H of quinoline), 7.41 (d, 1H, C_6-H of quinoline), 7.56 (s, 1H, C_5-H of quinoline), 8.35(s, 1H, C_4-H of quinoline), 8.59(s, 2H, NH_2), 8.76(s, 1H, CH=N), 11.24 (s, 1H, NH), 12.31 (s, 1H, OH). Mass (m/z): 260.07

III d: IR (KBr) cm^{-1}: 3414.22 (OH), 3136.17 (NH), 1658.38 (C=O), 1605.94 (C=N), ^{1}H NMR (400 MHz, DMSO-*d_6*, δ ppm): 2.12 (s, 3H, CH_3), 7.36 (d, 1H, C_8-H of quinoline), 7.47 (d, 1H, C_6-H of quinoline), 7.58 (s, 1H, C_5-H of quinoline), 8.29 (s, 1H, C_4-H of quinoline), 8.62 (s, 2H, NH_2), 8.79 (s, 1H, CH=N), 11.52 (s, 1H, NH), 12.19 (s, 1H, OH).Mass (m/z): 260.31

III e: IR (KBr) cm^{-1}: 3412.44 (OH), 3109.38 (NH), 1643.25 (C=O), 1611.36 (C=N), ^{1}H NMR (400 MHz, DMSO-*d_6*, δ ppm): 2.1 (s, 3H, CH_3), 7.25 (s, 2H, C_3 & C_4-H of pyridine), 7.38 (s, 1H, C_8-H of quinoline), 7.64 (s, 2H, C_2 & C_5-H of pyridine), 7.80(s, 1H, C_6-H of quinoline), 7.97 (s, 1H, C_5-H of quinoline), 8.58 (s, 1H, CH=N), 8.85 (s, 1H, C_4-H of quinoline), 11.18 (s, 1H, NH), 12.24 (s, 1H, OH). Mass (m/z): 306.28

III f: IR (KBr) cm^{-1}: 3419.66 (OH), 3143.20 (NH), 1642.53 (C=O), 1621.08 (C=N), ^{1}H NMR (400 MHz, DMSO-*d_6*, δ ppm): 2.14 (s, 3H, CH_3), 7.45-8.78 (m, 9H, C_4, C_5, C_6, C_8-H of quinoline, CH=N & C_2, C_3, C_5, C_6-H of pyridine), 11.25 (s, 1H, NH), 12.42 (s, 1H, OH).^{13}C NMR (400 MHz, DMSO-*d_6*, δ ppm): 22.13 (CH_3), 162.39 (quinoline- C_2), 161.43 (C=O), 150.98 (quinoline C_9), 141.88 (CH=N), 139.62 (pyridine- C_4), 137.04 (quinoline- C_4), 131.28 (quinoline- C_7), 128.06 (pyridine- C_3 and -C_5), 122.02 (quinoline- C_5), 122.13 (pyridine- C_2 and -C_6), 121.88 (quinoline- C_8), 120.52 (quinoline- C_6), 118.34 (quinoline- C_{10}), 115.96 (quinoline- C_3). Mass (m/z): 306.14

TABLE 1: PHYSICO-CHEMICAL DATA OF THE SYNTHESIZED COMPOUNDS III (a-f)

Compound	Molecular formula	M.P °C	%Yield	Solubility
III a	$C_{17}H_{14}N_4O_2$	256-258	64	DMSO
III b	$C_{17}H_{14}N_4O_2$	236-238	58	DMSO
III c	$C_{17}H_{14}O_3S$	228-230	70	DMSO
III d	$C_{17}H_{14}N_4OS$	270-272	66	DMSO
III e	$C_{17}H_{14}N_5O_4$	262-264	59	DMSO
III f	$C_{17}H_{14}N_5O_4$	244-246	62	DMSO

TABLE 2: *IN-VITRO* ANTIOXIDANT ACTIVITY SCREENING RESULTS OF NEWLY SYNTHESIZED COMPOUNDS III (a-f) BY DPPH RADICAL SCAVENGING ASSAY

S. no.	Compound	Mean abs ± S.E.M	% inhibition
1	III a	0.5230±0.0012	56.20
2	III b	0.5120±0.0014	59.10
3	III c	0.5530±0.0008	52.60
4	III d	0.5996±0.0009	58.80
5	III e	0.5328±0.0010	52.10
6	III f	0.5410±0.0006	57.30
7	Ascorbic acid	0.5260±0.05	68.50

TABLE 3: *IN-VITRO* ANTIOXIDANT ACTIVITY SCREENING RESULTS OF NEWLY SYNTHESIZED COMPOUNDS BY FERRIC REDUCING ACTIVITY

S. no.	Compound	Ferric reducing activity (100µg/mL) %
1	III a	0.38
2	III b	0.46
3	III c	0.34
4	III d	0.44
5	III e	0.40
6	III f	0.42
7	Ascorbic acid	0.0865

DISCUSSION: A novel series of 2-hydroxy quinoline derivatives III (a-f) have been synthesized by reacting 7 or 8-substituted -2-hydroxy-3-formylquinolines I (a, b) with substituted hydrazides. The purity of the synthesized compounds was confirmed by TLC. The structure of all the newly synthesized compounds was confirmed by physicochemical and spectral data.

All the compounds were screened for their *in–vitro* antioxidant activities using free radical scavenging activity by DPPH assays method and ferric ion reduction method using ascorbic acid as the standard drug. Compounds III b, III d, and III f showed significant antioxidant activity.

CONCLUSION: In series III (a-f) it is evidently observed that the antioxidant activity of the compounds has been greatly influenced by the presence of methyl as a substituent on the quinoline nucleus. Quinoline moiety and its Schiff bases do contribute for the observed activity but the presence of methyl at $7^{th}/8^{th}$ position of quinoline contributed to its significant antioxidant activity. The preliminary antioxidant activity screening result of these compounds depicted them as potential antioxidant leads endowed with moderate to excellent activity. Further enhancement in the activity can be achieved by slight modifications in the ring substituent.

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CONFLICTS OF INTEREST: No conflict of interest.

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