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Synthesis, Spectral Characterization and In-Vitro Screening of Some Novel Tetrahydroquinoline Derivatives for Their Antitubercular, Antioxidant Activities

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Abstract: In an attempt to synthesize some novel, potent antitubercular and antioxidant agents here we have reported the synthesis of a novel series of tetrahydroquinoline derivatives (III a-g). In this the titled compounds were synthesized by the reaction between substituted aromatic primary amines, *N*-vinylpyrrolidin-2-one and 4-nitro phthalic acid in acetonitrile. Structure of the synthesized compounds were confirmed on the basis of their physico-chemical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and Mass). All the synthesized compounds were screened for their antitubercular and antioxidant activities using the Microplate Alamar Blue Assay (MABA) method and nitric oxide scavenging, DPPH method respectively. For antitubercular and antioxidant activities screening isoniazid and ascorbic acid were used as the standard drugs respectively. Among the synthesized compounds III a, III d and III e have shown significant anti-tubercular activity and compounds III a, III b and III e have shown significant antioxidant activity.
Keywords: Tetrahydroquinoline, antitubercular, antioxidant, MABA and DPPH.

INTRODUCTION

Tuberculosis (TB) is one of the oldest and most prevalent diseases in history [1, 2]. According to World Health Organization (WHO) report, TB has spread to every corner of the world. It is estimated that between 2002 and 2020, approximately 1000 million people will be newly infected, over 150 million people will develop diseases and 36 million will die due to TB if proper control measures are not established [3].

The directly observed treatment, short course (DOTS) strategy, constitutes the cornerstone of the current protocol for control of TB. However, the three key drugs, isoniazid, pyrazinamide and rifampicin, used in the regimen are potentially hepatotoxic and may lead to drug associated hepatitis. Despite the undoubted success of DOTS strategy, the emergence of multi drug resistant (MDR) and extensive drug resistant (XDR) strains, intermittently isolated from patient's sputum, darken the future. Furthermore, one of the main cases for the prevalence of TB is synergy with HIV epidemic, where 31% of new TB cases are attributable for HIV co-infection [4].

Many compounds are in clinical trials and it is surprising that with this serious background, there have been no new potent drugs registered to treat TB in the last four decades. This reveals the inherent difficulties in discovery and clinical testing of new agents and also the resistance developed by the causative agent.

Another field of challenge to medicinal chemist is to develop some novel, potent and less side effect having antioxidant agents. It has been identified that antioxidants, which exhibit DPPH radical scavenging activity are increasingly receiving attention because of their interesting pharmacological activities like anticancer, antiinflammatory [5].

Tetrahydroquinoline derivatives have been an important class of heterocyclic compounds that exhibit biological activities. Imino Diels-Alder reaction is a well established route for the synthesis of nitrogen containing six membered heterocycles. Lewis acids [6] and protic acids [7] have been found to catalyze imino Diels-Alder reaction with electron-rich dienophiles. Literature study reveals that Diels-Alder reactions are executed by Lewis acids and also by using urea nitrate. Recently, photochemically catalyzed Diels–Alder reaction of arylimines with *N*-vinylpyrrolidin-2-one has also been reported [8]. In this research work we have reported the efficacy of 4-nitro phthalic acid as catalyst in imino Diels-Alder reaction.

Tetrahydroquinoline exhibit versatile pharmacological actions like antifungal [9], anticancer

[10], antitubercular [11], antibacterial [12], analgesic and antiinflammatory [13] etc. In continuation of our research work on tetrahydroquinoline, we made use of 4-nitro phthalic acid in synthesis of 2-methyl-4-amino-tetrahydroquinoline derivatives (IIIa-g) and screening them for antitubercular activity. Here all the compounds (IIIa-g) were obtained as *cis*-diastereoisomers. Their structural elucidations were based on ^{1}H NMR spectral data of the column-purified products. The relative *Trans* orientations of H-2, H-3, and H-4 were established from the large vicinal coupling constants between H-4 and H-3 (J = 11.9) and H-2 and H-3 (J = 12.3). From these results, we proposed the possible following mechanism to account for this reaction. An aromatic amine first reacts with *N*-vinyl pyrrolidin-2-one (NVP) to afford *N*-vinyl aniline, and the second step proceeds via the imino Diels-Alder reaction between *N*-acetylidene phenylamine and another molecule of NVP**.** In conclusion, a very remarkable and a facile synthesis of 1-(2-methyl-1,2,3,4-tetrahydroquinolin-4-yl)pyrrolidin-2-ones via 4-nitro phthalic acid-catalyzed hetero cyclization addition reaction between aryl amines and *N*-vinyl pyrrolidin-2-one has been established and screened for their antitubercular property.

Pharmacological activity

Antitubercular activity

MIC values were determined for the newly synthesised compounds against *M. tuberculosis* strain H_{37}Rv using the Microplate Alamar Blue assay (MABA) method [14], using isoniazid as the standard drug. The 96 wells plate received 100 µl of Middlebrook 7H9 broth and serial dilution of compounds were made directly on the plate with drug concentrations of 0.2, 0.4, 0.8, 1.6, 3.125, 6.25, 12.5, 25, 50 and 100 µg/ml. Plates were covered and sealed with parafilm and incubated at 37ºC for 5 days. Then, 25 µl of freshly prepared 1:1 mixture of almar blue reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The MIC was defined as the lowest drug concentration, which prevented color change from blue to pink. Compounds showed antitubercular activity between MIC of >100- 6.25 µg/ml.

Compounds III a, III d and III e showed significant antitubercular activity. Table 2 reveals antitubercular activity (MIC) data for all the synthesized compounds.

Antioxidant activity

The synthesized compounds were screened for their antioxidant property by nitric oxide [15] and DPPH [16] methods.

Assay for Nitric Oxide (NO) scavenging activity

Sodium nitroprusside (5 mM) in phosphate buffer pH 7.4 was incubated with 100 mM concentration of test compounds dissolved in a suitable solvent (dioxane/methanol) and tubes were incubated at 25^{0} C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5 ml of incubation solution was taken and diluted with 0.5 ml of Griess reagent (1% Sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was observed at 546 nm. The experiment was repeated in triplicate.

DPPH (1,1-diphenyl-2-picrylhydrazyl) reduction method

The nitrogen centered stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm, which is purple in color. This property makes it suitable for spectrophotometer studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts it into 1,1-diphenyl-2- picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. The solutions of test compounds (100 mM) were added to DPPH (100 mM) in dioxane/ethanol. The tubes were kept at an ambient temperature for 20 min and the absorbance was measured at 517 nm.

The inhibition percentage was calculated by using the following formula-

% inhibition = [(A Control - A Sample)/ A Control] x 100.

Where, A Control is the absorbance of the L-ascorbic acid. A Sample is the absorbance of different compounds.

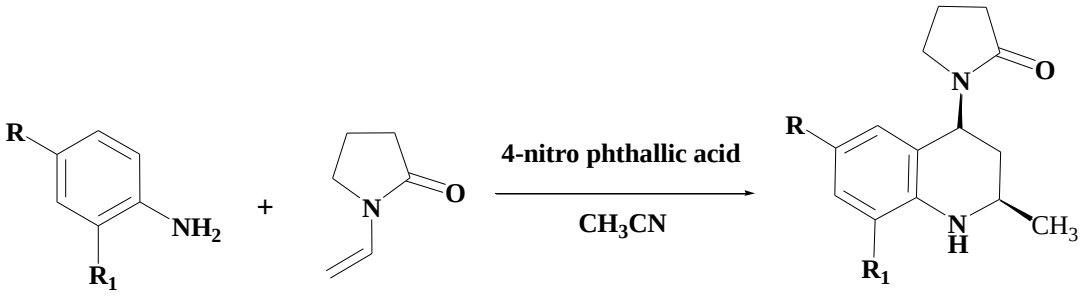
Compounds III a, III b and III e showed significant antioxidant activity. Table 3 shows antioxidant activity data for all the synthesized compounds.

EXPERIMENTAL SECTION

General considerations

The various chemicals used in the synthesis of the titled compounds were purchased from, sigma-aldrich pvt ltd, spectrochem pvt ltd and s.d. fine chem pvt ltd. Melting point of all synthesized compounds was determined by open capillary method and is 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 $cdcl_3$ / dmso as solvent and tms as internal standard, chemical shifts are expressed as δ values (ppm).

Scheme



(I) (II) (III a-g)

Where,

III a = R= Br and R_1 = H, III b = R= Cl and R_1 = H, III c = R= H and R_1 = H, III d = R= F and R_1 = H

III e = R= H and R_1 = Cl, III f = R = CH_3 and R_1 = H, III g = R= OCH_3 and R_1 = H,

Table-1: Physico-chemical data of the synthesized compounds (IIIa-g)

Compound	Molecular formula	M.P ^{0}C	%Yield	Elemental Analysis Found (Calcd) %		
				C	H	N
III a	$C_{14}H_{17}Br\ N_2O$	158-160	88	54.38 (54.36)	5.54 (5.52)	9.06 (9.04)
III b	$C_{14}H_{17}ClN_2O$	150-152	92	63.51 (63.50)	6.47 (6.46)	10.58 (10.56)
III c	$C_{14}H_{17}N_2O$	70-72	95	73.01 (73.00)	7.88 (7.86)	12.16 (12.14)
III d	$C_{14}H_{17}F\ N_2O$	138-140	90	67.72 (67.70)	6.90 (6.88)	11.28 (11.26)
III e	$C_{14}H_{17}ClN_2O$	150-152	92	63.51 (63.50)	6.47 (6.46)	10.58 (10.56)
III f	$C_{15}H_{20}\ N_2O$	96-98	90	73.74 (73.72)	8.21 (8.20)	11.47 (11.46)
III g	$C_{15}H_{20}\ N_2O_2$	90-92	88	69.20 (69.18)	7.74 (7.72)	10.76 (10.74)

Table-2: Antitubercular activity screening results of newly synthesized compounds III (a-g)

Compound	MIC values (µg/ ml) *M. tuberculosis* $H_{37}Rv$
III a	50
III b	>100
III c	100
III d	25
III e	50
III f	100
III g	>100
Isoniazid	0.25

Table 3: Antioxidant activity results of the synthesized compounds III (a-g)

Compound	Nitric oxide scavenging (%)	DPPH scavenging (%)
III a	74.95	71.80
III b	69.02	67.53
III c	60.85	59.02
III d	61.14	65.33
III e	72.48	70.81
III f	65.76	70.06
III g	58.62	56.27
Ascorbic acid	97.70	95.52

General procedure for the synthesis of 2-methyl-4-amino-tetrahydroquinolines (IIIa-g)

The mixture of primary aromatic amines (5 mmol), *N*-vinyl pyrrolidin-2-one (12 mmol), and 4-nitro phthalic acid (2.5 mmol) in acetonitrile (5 ml) was stirred at 50^0 C for an optimum time. After completion of reaction as indicated by TLC, the reaction mixture was quenched with saturated aqueous sodium bicarbonate solution (20 ml) and then extracted with three fractions of ethyl acetate (each 15 ml). The combined organic layer was dried over anhydrous sodium sulphate and concentrated. The crude product was further purified by column chromatography using ethyl acetate and petroleum ether (3:7) as mobile phase and iodine vapors as visualizing agent.

III a: IR (KBr, cm^{-1}): 3312 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 1.13 (d, 3H, CH_3), 2.03-2.11 (m, 6H, C_3-quinoline, C_3 & C_4-pyrrolidine), 2.77 (t, 1H, C_2-quinoline), 3.38 (t, 2H, C_5- pyrrolidine), 4.05 (d, 1H, NH), 4.82 (t, 1H, C_4-quinoline), 6.28 (d, 1H, C_8-quinoline), 7.07-7.13 (d, 2H, C_5 & C_7-quinoline); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm); 17.1, 21.7, 37.2, 38.3, 44.0, 47.6, 51.1, 111.8, 114.5, 125.9, 130.1, 133, 142.5, 173.3; MS: *m/z* = 309 (M+1).

III b: IR (KBr, cm^{-1}): 3395 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.23 (d, 3H, CH_3), 2.00-2.13 (m, 6H, C_3-quinoline, C_3 & C_4-pyrrolidine), 2.79 (t, 1H, C_2-quinoline), 3.40 (t, 2H, C_5-pyrrolidine), 4.00 (d, 1H, NH), 4.87 (t, 1H, C_4-quinoline), 6.32 (d, 1H, C_8-quinoline), 6.87-6.93 (d, 2H, C_5 & C_7-quinoline); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 18.2, 22.0, 31.3, 33.5, 42.2, 47.0, 47.8, 116.0, 120.8, 122.7, 126.3, 129.0, 144.0, 175.8; MS: m/z = 265 (M+1).

III c: IR (KBr, cm^{-1}): 3348 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 1.15 (d, 3H, CH_3), 2.02-2.11 (m, 6H, C_3-quinoline, C_3 & C_4-pyrrolidine), 2.82 (t, 1H, C_2-quinoline), 3.45 (t, 2H, C_5-pyrrolidine), 4.05 (d, 1H, NH), 4.88 (t, 1H, C_4-quinoline), 6.28 (d, 1H, C_8-quinoline), 6.84-6.91 (d, 2H, C_5 & C_7-quinoline), 6.53 (d, 1H, C_6-quinoline). MS: m/z = 231 (M+1).

III d:IR (KBr, cm^{-1}): 3341 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 1.26 (d, 3H, CH_3), 2.07-2.16 (m, 6H, C_3-quinoline, C_3 & C_4-pyrrolidine), 2.72 (t, 1H, C_2-quinoline), 3.36 (t, 2H, C_5-pyrrolidine), 4.00 (d, 1H, NH), 4.76 (t, 1H, C_4-quinoline), 6.36 (d, 1H, C_8-quinoline), 6.59-6.63 (d, 2H, C_5 & C_7-quinoline); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm); 17.6, 21.6, 30.6, 33.2, 41.6, 46.3, 47.4, 116.0, 120.8, 122.7, 126.3, 129.0, 142.7, 174.7; MS: m/z = 249 (M+1).

III e: IR (KBr, cm^{-1}): 3395 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.37 (d, 3H, CH_3), 2.08-2.33 (m, 6H, C_3-quinoline, C_3 & C_4-pyrrolidine), 2.59 (t, 1H, C_2-quinoline), 3.19 (t, 2H, C_5-pyrrolidine), 4.11 (d, 1H, NH), 4.78 (t, 1H, C_4-quinoline), 6.41 (d, 1H, C_8-quinoline), 6.90-7.09 (d, 2H, C_5 & C_7-quinoline); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 19.3, 22.4, 31.7, 33.8, 42.1, 47.3, 48.5, 116.2, 120.8, 122.9, 126.4, 129.2, 144.3, 175.2; MS: m/z = 265 (M+1).

III f: IR (KBr, cm^{-1}): 3316 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 1.27 (d, 3H, CH_3), 2.00-2.13 (m, 6H, C_3-quinoline, C_3 & C_4-pyrrolidine), 2.35 (s, 3H, CH_3), 2.73 (t, 1H, C_2-quinoline), 3.34 (t, 2H, C_5-pyrrolidine), 4.12 (d, 1H, NH), 4.81 (t, 1H, C_4- quinoline), 6.26 (d, 1H, C_8-quinoline), 6.66-6.73 (d, 2H, C_5 & C_7-quinoline). MS: m/z = 245 (M+1).

III g: IR (KBr, cm^{-1}): 3319 (NH); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm); 1.33 (d, 3H, CH_3), 2.10-2.23 (m, 6H, C_3-quinoline, C_3 & C_4-pyrrolidine), 2.59 (t, 1H, C_2-quinoline), 3.43 (t, 2H, C_5-pyrrolidine), 3.73 (s, 3H, CH_3), 4.04 (d, 1H, NH), 4.87 (t, 1H, C_4- quinoline), 6.22 (d, 1H, C_8-quinoline), 6.37-6.43 (d, 2H, C_5 & C_7-quinoline); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm); 17.8, 22.0, 31.8, 33.4, 42.2, 46.7, 48.8, 56.7, 112.6, 115.2, 115.7, 124.3, 139.1, 152.7, 175.6; MS: m/z = 261 (M+1).

CONCLUSION

In this research work we accomplished the synthesis of some novel series of tetrahydroquinoline derivatives. The *In-vitro* antitubercular and antioxidant activity screening result of these compounds depicted them as potential antitubercular and 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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