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Synthesis and Antioxidant Studies of Schiff Bases of 2-Pyrazole Substituted Quinoline Derivatives.

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ABSTRACT

In the present work synthesis of new Schiff bases of 2-pyrazole substituted quinoline derivatives has been reported. New Schiff bases of 2-(3,5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, **4(a-f)** were synthesized by straightforward condensation with heterocyclic amines, **3(a, b)**. Recrystallization of the final compounds was achieved in good yields avoiding the tedious column purification methods. The synthesized compounds were successfully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR and mass analysis. We further explored the antioxidant efficacy of synthesized compounds using DPPH and H_2O_2 assays. The results obtained from antioxidant efficacy showed that compounds **4c** and **4f** are having good antioxidant activity showing IC_{50} values of 1.96, 1.97 for DPPH radical scavenging efficacy and 1.88, 1.90 respectively for H_2O_2 scavenging efficacy which was comparable to the standard ascorbic acid.

Keywords: Quinoline, Pyrazole, Schiff base, Antioxidant.

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INTRODUCTION

Nitrogen containing heterocycles, particularly the functionalized quinolines are crucial structural units and have exhibited considerable attention owing to their pronounced biological activities such as antioxidant [1], antibacterial [2], anti-inflammatory [3], anti-HIV [4] and anti-cancer activities [5]. Functionalized quinolines are prevalently found in many biologically active molecules and the wide range of physical and chemical properties of these derivatives have led to their successful use in pharmaceuticals [6] and material chemistry [7]. Consequently, numerous reports have been described for developing novel functionalized quinoline derivatives which are of paramount importance in the organic and medicinal community [8]. Pyrazole, on the other hand has attracted considerable attention for their vital role in synthetic organic chemistry and are extensively found in biologically active compounds as anti-cancer [9], anti-tubercular [10], anti-diabetic [11], anti-bacterial [12] and anti-fungal agents [13].

Schiff bases, the condensation products of aldehydes or ketones with amines are a prominent class of biologically active drug molecules due to their wide range of pharmacological activities [14]. They have some interesting applications in clinical, biological, analytical and industrial fields [15]. Due to their coordination capability these are considered to be one of the most important ligands in modern coordination chemistry [16]. Schiff base derivatives have been reported to exhibit biological activities like anti-bacterial [17], anti-fungal [18], antitumor [19], anti-viral [20], DNA-binding properties [21]. So, many researchers have been synthesizing these compounds as potential drug candidates with low toxicity and maximal effects [22].

Free radicals play a vital role in the pathogenicity of various dreadful diseases necessitating for developing a therapeutic pathway to identify and synthesize new effective antioxidants which prevent radical-induced damage. Experimental and clinical reports make it evident that oxidative stress-induced by reactive oxygen species generated during normal metabolic processes results in various diseases like cancer, inflammation, atherosclerosis and aging [23, 24]. Overproduction of free radicals and reactive oxygen species (ROS) such as superoxide radical anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (HO) are derived either exogenously or endogenously through different processes like pollution, radiation and metabolic reactions happening in the human body [25, 26].

Hence, keeping in view of the above mentioned facts and in continuation of our research on functionalized quinolines [27, 28], the current study was carried out to synthesize structurally distinct new Schiff bases on quinoline ring system having pyrazole moiety substituted at the 2nd position in a simple, yet effective yielding method and then explore their antioxidant potential by DPPH and H_2O_2 scavenging assay methods.

MATERIAL AND METHODS

The materials were purchased from Sigma-Aldrich and Merck and were utilized without any additional purification. Column chromatography was performed using silica–gel (100-200 mesh size) purchased from Merck and all the reactions were observed by thin layer chromatography (TLC) on aluminum sheets pre-coated with silica gel 60F254 purchased from Merck. Melting points were recorded on an Elchem digital melting point apparatus in open capillaries and are uncorrected. The ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance-400 MHz instrument at room temperature in $CDCl_3$ with TMS as an internal standard. Mass spectra were obtained using LCMS or GC-MS spectrometry.

Experimental

Synthesis of 2-(3,5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, 2(a-c)

Compounds, **1(a-c)** were prepared according to the method by our research group [29]. To a stirred solution of 2-(3, 5-disubstituted-1*H*-pyrazol-yl)-3-(1,3-dioxolan-2yl)quinolines, **1(a-c)** (1 mmol) in acetone as solvent, catalytic amount of PTSA (0.2 mmol) was added and refluxed for 1 h. Completion of the reaction was monitored by TLC. Excess solvent was evaporated and then water was added to the left over crude, extracted with DCM, washed with saturated $NaHCO_3$ (2×15 ml), dried over anhydrous sodium sulphate and concentrated under reduced pressure to afford products which were pure enough to proceed further without any further purification.

Synthesis of Schiff bases of 2-(3,5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, 4(a-f)

Equimolar mixture of 2-(3,5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, **2(a-c)** and heterocyclic amines, **3(a, b)** were taken in ethanol and catalytic amount of glacial acetic acid(1-2 drops) was added and refluxed for 4 h. On completion of the reaction, excess solvent was evaporated using rotary evaporator to yield the solids which were later slurred and recrystallized with ethanol to afford the target compounds, **4(a-f)** in good yields.

(*E*)-*N*-((2-(3,5-dimethyl-1*H*-pyrazol-1-yl)quinolin-3-yl)methylene)pyridin-4-amine, 4a

89% yield. M.p.: >300 °C IR (KBr pellets, cm^{-1}) υ: 2899, 2222, 1621, 1567. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 2.14 (3H, s, CH_3), 2.53 (3H, s, CH_3), 6.08 (s, 1H, CH), 7.41-7.44 (2H, t, *J* = 8.4 Hz, CH), 7.68-7.73 (2H, t, *J* = 8 Hz, CH), 7.97-7.99 (2H, d, *J* = 8 Hz, CH), 8.40-8.42 (2H, d, *J* = 8.4 Hz, CH), 8.61 (1H, s, Imine CH), 8.82 (1H, s, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 12.3, 14.1, 107.0, 110.2, 115.3 x 2, 124.4, 125.6, 126.8, 128.3 x 2, 130.2, 135.2, 144.5, 148.0 x 2, 149.4, 151.9, 159.8, 161.1 (C=N). Mol. formula: $C_{20}H_{17}N_5$ requires 327.15; LCMS *m/z* found to be 328.18[M+1].

(*E*)-*N*-((2-(3-methyl-5-phenyl-1*H*-pyrazol-1-yl)quinolin-3-yl)methylene)pyridin-4-amine, 4b

85% yield. M.p.: 292-294 °C IR (KBr pellets, cm^{-1}) υ: 2921, 2221, 1626, 1541. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 2.35 (3H, s, CH_3), 6.10 (s, 1H, CH), 7.08-7.11 (2H, d, *J* = 8.4 Hz, CH), 7.41-7.46 (3H, m, CH), 7.51-7.56 (2H, t, *J* = 9 Hz, CH), 7.68-7.73 (2H, t, *J* = 8.4 Hz, CH), 7.97-7.99 (2H, d, *J* = 8 Hz, CH), 8.41-8.43 (2H, d, *J* = 8.4 Hz, CH), 8.65 (1H, s, Imine CH), 8.84 (1H, s, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 12.3, 107.1, 110.3, 116.2 x 2, 124.5, 125.8, 127.1 x 2, 128.5, 131.2 x 2, 135.6, 143.2 x 2, 147.2, 149.7, 152.2, 158.4, 162.2 (C=N). Mol. formula: $C_{25}H_{19}N_5$ requires 389.16; LCMS *m/z* found to be 390.23[M+1].

(*E*)-*N*-((2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-7-methylquinolin-3-yl)methylene)pyridin-4-amine, 4c

86% yield. M.p.: >300 °C IR (KBr pellets, cm^{-1}) υ: 3053, 2887, 2224, 1606, 1563. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 2.20 (3H, s, CH_3), 2.38 (3H, s, CH_3), 2.53 (3H, s, CH_3), 6.08 (s, 1H, CH), 7.41-7.46 (3H, m, CH), 7.64 (1H, s, CH), 7.97-7.99 (1H, d, *J* = 8.4 Hz, CH), 8.40-8.43 (2H, d, *J* = 8.8 Hz, CH), 8.61 (1H, s, Imine CH), 8.82 (1H, s, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 12.3, 14.2, 21.4, 107.2, 110.4, 115.5 x 2, 123.9, 125.9, 126.3, 129.4, 130.4, 134.6 x 2, 144.5, 148.2 x 2, 149.7, 152.6, 159.4, 161.6 (C=N). Mol. formula: $C_{21}H_{19}N_5$ requires 341.16; GC-MS *m/z* found to be 341.76(M^+).

(*E*)-*N*-((2-(3,5-dimethyl-1*H*-pyrazol-1-yl)quinolin-3-yl)methylene)thiazol-2-amine, 4d

94% yield. M.p.: 283-285 °C IR (KBr pellets, cm^{-1}) υ: 2919, 2221, 1614, 1597, 1082. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 2.16 (3H, s, CH_3), 2.52 (3H, s, CH_3), 6.08 (s, 1H, CH), 7.68-7.75 (3H, m, CH), 7.97-8.02 (3H, m, CH), 8.41 (1H, s, Imine CH), 8.82 (1H, s, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 12.4, 14.2, 109.8, 116.5, 124.3, 125.8, 126.3, 128.9 x 2, 131.3, 135.3, 141.1, 148.0 x 2, 151.6, 159.9, 161.6 (C=N), 170.2. Mol. formula: $C_{18}H_{15}N_5S$ requires 333.1; LCMS *m/z* found to be 334.16[M+1].

(*E*)-*N*-((2-(3-methyl-5-phenyl-1*H*-pyrazol-1-yl)quinolin-3-yl)methylene)thiazol-2-amine, 4e

86% yield. M.p.: >300 °C IR (KBr pellets, cm^{-1}) υ: 2923, 2232, 1632, 1587, 1086. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 2.35 (3H, s, CH_3), 6.12 (s, 1H, CH), 7.08-7.12 (2H, d, *J* = 7.6 Hz, CH), 7.41-7.45 (1H, m, CH), 7.51-7.56 (2H, t, *J* = 7.6 Hz, CH), 7.67-7.73 (3H, m, CH), 7.96-8.01 (3H, m, CH), 8.45 (1H, s, Imine CH), 8.84 (1H, s, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 12.3, 108.4, 116.7 x 2, 124.1, 125.4, 126.3 x 2, 127.5, 128.9 x 2, 129.4, 130.2 x 2, 135.1 x 2, 141.6, 147.7, 151.0 x 2, 158.3, 162.3 (C=N), 170.7. Mol. formula: $C_{23}H_{17}N_5S$ requires 395.12; LCMS *m/z* found to be 396.21[M+1].

(*E*)-*N*-((2-(3,5-dimethyl-1*H*-pyrazol-1-yl)-7-methylquinolin-3-yl)methylene)thiazol-2-amine, 4f

92% yield. M.p.: >300 °C IR (KBr pellets, cm^{-1}) υ: 2931, 2239, 1628, 1591, 1081. ^{1}H NMR ($CDCl_3$, ppm, 400 MHz) δ: 2.25 (3H, s, CH_3), 2.37 (3H, s, CH_3), 2.56 (3H, s, CH_3), 6.08 (s, 1H, CH), 7.53-7.58 (2H, m, CH), 7.64 (1H, s, CH), 7.97-7.99 (2H, m, CH), 8.42 (1H, s, Imine CH), 8.82 (1H, s, CH). ^{13}C NMR ($CDCl_3$, ppm, 100 MHz) δ: 12.4, 14.3,

21.5, 108.2, 116.7, 124.4, 125.7, 126.5, 128.4 x 2, 130.9, 135.6, 142.1, 148.0 x 2, 151.7, 159.8, 161.4 (C=N), 170.4. Mol. formula: $C_{19}H_{17}N_5S$ requires 347.12; GC-MS *m/z* found to be 347.58(M^+).

Antioxidant activity

Oxidative stress occurs when the production of free radicals in the body goes beyond the protective defenses, which initiates early stages of cancer and heart disease. The free radicals are also suspected in the development of arthritis, Alzheimer's disease, arthritis, cataracts, diabetes, kidney disease and age related blindness. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit cell death and other oxidation reactions. In present study, Schiff bases of 2-(3,5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, **4(a-f)** derivatives were evaluated for their free radical scavenging activity using the DPPH radical assay. Reduction of DPPH radicals can be observed by decrease in absorbance at 517 nm. Different Schiff base derivatives of pyrazole substituted quinolines reduced DPPH and peroxide radicals significantly and the activity of the derivatives was compared with commercial antioxidant Ascorbic acid.

DPPH radical scavenging assay

Free radical scavenging is one of the familiar mechanisms through which antioxidants inhibits oxidation. In the present study, the radical scavenging ability of all the synthesized compounds was determined spectrophotometrically using the stable DPPH radical method. DPPH assay is a quite simple and standard method for evaluating antioxidant activity. One of the best advantages of this assay is the commercial availability of DPPH radical, so there is no need of it to be generated in advance like in other assays. In the present study DPPH radical scavenging efficacy were carried out as per the reported method [30]. Compound solutions, **4(a-f)** of various concentrations were prepared by dissolving an appropriate amount of each compound in ethanol. The solution of DPPH in ethanol was prepared just before UV measurements. 3 ml of sample and 1 ml of DPPH solutions were mixed and kept in the dark for 30 min at room temperature and then the decrease in absorption was measured. For the control absorption blank sample containing the same amount of ethanol and DPPH solution was measured. BHT was used as standard. The experiment was carried out in triplicate. Radical scavenging activity was calculated by following formula:

$$I\%\ (percentage\ inhibition) = \frac{[AB - AA]}{AB} \times 100$$

Where AB = absorption of blank sample
AA = absorption of sample

H_2O_2 scavenging assay

The hydrogen peroxide scavenging assay was carried out as per the protocol [31]. A Solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS) [pH 7.4]. Various concentrations of the sample **4(a-f)** were added to 2 ml of hydrogen peroxide solution in PBS. Ascorbic acid was taken as standard. After 10 min, the absorbance was measured at 230 nm. For control absorption blank sample containing same amount of methanol and H_2O_2 solution was measured. Radical scavenging activity was calculated by formula used in the above method.

RESULTS AND DISCUSSION

Chemistry

In the current study, the reaction sequences involved in synthesis of the desired products are depicted in the following **Scheme 1**. Thus, the starting materials, 2-(3,5-disubstituted-1*H*-pyrazol-yl)-3-(1,3-dioxolan-2yl)quinolines, **1(a-c)** were synthesized by the cyclo- condensation reaction of 2-hydrazino-3-(1,3-dioxolan-2-yl)quinolines with substituted 1, 3-diketones according to the reported method by our research group [29].

Initially, the compounds, **1(a-c)** were subjected to deprotection in the presence of catalytic amount of PTSA and acetone as solvent under reflux condition to afford the 2-(3,5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, **2(a-c)** in excellent yields within 1 h which were carried on to next step without any additional purification. The carbaldehyde group of intermediates, **2(a-c)** is an attractive functionality to discover many new possibilities and are valuable synthons for the synthesis of fused quinoline systems. Our interest was to synthesize substituted quinoline Schiff base derivatives, **4(a–f)** which was achieved through straight forward condensation of heterocyclic amines, **3(a, b)** with various substrates, **2(a-c)** by refluxing in ethanolic solution in the presence of catalytic amount of glacial acetic acid. Most of the reactions got completed within 4 h and the final Schiff bases of 2-(3, 5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, **4(a-f)** were recrystallized in ethanol in excellent yields with 84-94% yields avoiding the tedious column purification techniques. The structures of all the compounds, **4(a-f)** were confirmed by the FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectra.

The mass spectrum of **4d** demonstrated a molecular ion peak at *m*/*z* 334.21 $[M + H]^{+}$, which indicates the condensation of 2-aminothiazole, **3b** to the 2-(3,5-dimethyl-1H-pyrazol-1-yl)quinoline-3-carbaldehyde, **1a**. The appearance of three methyl protons at δ 2.32 ppm is attributable to the $-CH_3$ group at 7th position of the quinoline system. The three methyl protons appeared as singlet each at δ 2.16 ppm, δ 2.52 ppm and a peak at δ 6.08 ppm attributable to the two methyl groups and CH in the pyrazole moiety. Similarly, the existence of proton at δ 8.61 ppm corresponds to imine proton and the peaks observed at δ 7.68, 7.97 and δ 8.82 ppm are attributable to the aromatic protons in ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of compound **4d** demonstrated carbon peaks at δ 12.43 and δ 14.28 which were attributable to the two methyl carbons and peaks from δ 107.63 to δ 161.64 ppm are related to the aromatic carbons.

Antioxidant activity

In DPPH assay, the scavenging efficacy of antioxidants towards the stable radical DPPH is considered as a measure and a strong absorption band can be seen at 517 nm in the visible region whereas the underlying principle in hydrogen peroxide scavenging assay is that there is a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. The scavenging ability of all the compounds, **4(a-f)** were tested in three different varied concentrations **10**, **50** and **100 µg/ml** and compared with the standard reference compound Ascorbic acid. The results showed that most of the synthesized compounds were showing moderate to good antioxidant efficacy.

Out of all the synthesized compounds **4c** and **4f** are having good antioxidant efficacy showing IC_{50} values of 1.96, 1.97 for DPPH radical scavenging efficacy and 1.88, 1.90 respectively for H_2O_2 scavenging efficacy which was comparable to that of standard ascorbic acid (**Table 1 & Table 2**). As per structural and chemical features it is quite obvious that structural variations bring about changes in bioactivity. In addition to this it also alters the biological properties of the molecules in regular trend. Presence of more number of inductively electron donating ($-CH_3$) groups in compound **4f** significantly enhances its antioxidant activity mainly due to its ability to form intra and intermolecular hydrogen bonding and also thiazole ring presence in the compound has further enhanced the scavenging efficacy. The introduction of phenyl ring in case of **4b** and **4e** did not show significant effect in its antioxidant efficacy.

CONCLUSION

In conclusion, we have reported an adaptable synthesis related to Schiff bases of 2-(3,5-disubstituted-1*H*-pyrazol-1-yl)quinoline-3-carbaldehydes, **4(a-f)** which were isolated in good to excellent yields with deprotection and condensation of heterocyclic amines as key steps. All the analogues were tested for their antioxidant ability by DPPH method and H_2O_2 scavenging method. The results obtained from antioxidant studies have revealed that compounds **4c** and **4f** demonstrated good efficacy.

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