Design and synthesis of new N'-substituted-2-methylquinoline-3-carbohydrazides with antioxidant and antimicrobial activity

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Ten new N'-substituted-2-methylquinoline-3-carbohydrazide scaffolds have been synthesized, characterized by their physical and spectral data (IR, \(^1\)H NMR, and MS) and screened for \textit{in vitro} antimicrobial and antioxidant activities. Results clearly reveal that all the synthesized compounds possess \textit{in vitro} antioxidant activity at the tested dose as compared to the standard drug, ascorbic acid. From the results, it can be assumed that the presence of an electron donating group on the aromatic ring is an important requirement for the antioxidant activity of the synthesized compounds, 5a-j. The synthesized compounds have also been screened for antibacterial and antifungal activity against three different strains of Gram-positive (\textit{Bacillus subtilis}, \textit{S. pyogenes}, and \textit{Staphylococcus aureus}) and three strains of Gram-negative bacteria (\textit{Escherichia coli}, \textit{Enterobactor aerogens} and \textit{Klebsiella pneumoniae}) and two fungal strains (\textit{Candida albicans} and \textit{Fusarium oxysporium}). Some of the compounds are found to be active against all the tested organisms, but are equipotent and less active as compared to the standard drug streptomycin. Compounds 5b and 5j exhibit almost equipotent activity compared with the standard drug Itraconozol against fungal strain \textit{Fusarium oxysporium} and another compound 5f which is active against \textit{Candida albicans}.

\textbf{Keywords:} Quinoline, hydrazone, antibacterial, antifungal, antioxidant

Among the wide variety of heterocyclic compounds that have been explored for developing pharmaceutically important molecules, quinolines have played an important role in medicinal chemistry in last few decades and it is endowed with various activities, such as antibiotic\textsuperscript{1}, antituberculous\textsuperscript{2}, antimalarial\textsuperscript{3}, anti-inflammatory\textsuperscript{4}, anticancer\textsuperscript{5}, antihypertensive\textsuperscript{6}, tyrosine kinase inhibiting agents\textsuperscript{7} and anti-HIV\textsuperscript{8}. Hydrazones are active pharmocophores which possesses an azomethine-NH-N=C-proton constituting an important class of compounds for new drug development. They form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antibacterial, antifungal\textsuperscript{9} and antioxidant activities\textsuperscript{10}. Thus a program of synthesis of quinolinylhydrazones was envisaged to serve as a new scaffold for evaluation as antioxidant and antimicrobial agents (Scheme I). The search for new antimicrobial drugs is an area of active investigation with the goal of developing novel drugs in order to overcome the phenomenon of drug resistance. It is well-known that the quinoline nucleus and its derivatives play a vital role in the design of an important class of wide spectrum antibacterial agents. Structure-activity relationship (SAR) studies revealed that the antimicrobial activity in this class of compounds depends on the nature of the peripheral substituents and their spatial relationship within the quinoline nucleus.

\textbf{Results and Discussion}

The compounds having arylidene-hydrazide structure may exist as \textit{E/Z} geometrical isomers about –C=N double bond and \textit{cis-trans} amide conformers. According to the literature\textsuperscript{11}, the compounds containing imine bond are present in higher percentage in DMSO-\textit{d}_\textsubscript{6} solution in the form of geometrical \textit{E}-isomer about –C=N double bond. The \textit{Z}-isomer can be stabilized in less polar solvents by an intramolecular hydrogen bond. In the present study, the spectral data were obtained in DMSO-\textit{d}_\textsubscript{6} solution and no signal belonging to \textit{’Z’} isomer was observed. On the other hand, the \textit{cis-trans} conformers of \textit{’E’} isomer were present in the DMSO-\textit{d}_\textsubscript{6} solution. The structures of all the newly synthesized compounds were confirmed by IR, \(^1\)H NMR and LCMS studies. The IR spectrum of compound 3 revealed the presence of –ArH group due to the appearance of strong band at 3050 cm\textsuperscript{-1}, while that of –C=O of ester was observed at 1714 cm\textsuperscript{-1}. Further, in the \(^1\)H NMR spectra the signal derived from ester group (–OCH\textsubscript{2}CH\textsubscript{3}) was observed at \(\delta\) 4.4 (quartet) and 1.5 (triplet) integrating for two and three protons respectively. The LCMS showed its molecular ion peak at \textit{m/z} 216 (M+H), which is in accordance with its molecular formula C\textsubscript{13}H\textsubscript{13}NO\textsubscript{2}. The formation of
hydrazide 4 from ester 3 was evidenced by its IR, ¹H NMR and LCMS spectra. Its IR spectrum showed absorbance bands at 3259-3141 and 1620 cm⁻¹ indicating the presence of –NH₂ and –C=O group respectively, while its ¹H NMR spectrum showed disappearance of corresponding-OCH₂CH₃ peaks and appearance of peaks at δ 3.32 (-NH-NH₂) and 9.62 (-NH-NH₂) integrating for two protons and one proton respectively (D₂O exchangeable) clearly confirming the conversion of ester into hydrazide. The LCMS spectrum of compound 4 showed a molecular ion peak at m/z 290(M+H), which matches with its molecular formula C₁₈H₁₅N₃O. The yield and m.p. of compounds 5a-j are given in Table I. The structures of compounds 5a-j were interpreted by its IR, ¹H NMR and LCMS spectra. Its IR spectrum clearly reveals that the disappearance of peak due to –NH₂, ¹H NMR spectrum showed sharp singlet at δ 8.25 confirming that the formation of the (–N=C-) group. The LCMS spectrum of compound 5 showed a molecular ion peak at m/z 290(M+H), which matches with its molecular formula C₁₈H₁₅N₃O.

Antimicrobial evaluation
All the newly synthesized compounds 5a-j were evaluated for their in vitro antibacterial and antifungal activities against bacterial and fungal species by means of two-fold serial dilution¹². The in vitro antibacterial activity was performed against three Gram-positive bacterial strains such as Staphylococcus
aureus, Bacillus subtilis and Streptococcus pyogens and three Gram-negative strains including Klebsiella pneumonia, Enterococcus aerogens and Escherichia coli. Streptomycin was used as a reference drug. Further, the in vitro antifungal activity was performed against two fungal strains including Candida albicans and Fusarium oxysporium. The data generated from this study (Table II) showed that some of the target compounds exhibit good potency in inhibiting the growth of Gram-positive bacteria such as Staphylococcus aureus. The in vitro activity of compounds 5b, 5c and 5j against Gram-negative bacteria such as Klebsiella pneumonia and Escherichia coli are equipotent to the standard drug. The antifungal activity of compounds 5b, 5f and 5j against fungal strains such as Candida albicans and F. oxysporium are equivalent to or less potent than the standard drug Itraconazole.

Antioxidant activity

All synthesized compounds were evaluated for invitro antioxidant activity (Table III) using two different models. The radical scavenging ability of the compounds was tested against hydrogen peroxide. Also, measurement of the reducing ability using the Fe^{3+}–Fe^{2+} transformation was investigated.

Reducing power method

In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex was then monitored by measuring the formation of Perl’s Prussian blue at 700 nm. Increasing absorbance at 700 nm indicates an increase in reducing ability. It was found that the reducing power of all the synthesized compounds also increased with the increase in their concentrations. The best reducing power was presented by 5c containing 4-methoxy group (IC_{50} 54.51 ± 1.14µg/mL) and 5j (IC_{50} 53.79 ± 0.43 µg/mL) which exhibited satisfactory activity compared to reference compound Itraconazole. Reducing power seems to depend on electron donating group to increase the activity.

Hydrogen peroxide scavenging assay

The ability of the compounds to effectively scavenge hydrogen peroxide was determined according to the method of Ruch. The compounds were capable of scavenging hydrogen peroxide in a concentration dependent manner. The scavenging ability of all the compounds was found to be good compared to the

<table>
<thead>
<tr>
<th>Compd</th>
<th>Reducing power–IC_{50} (µg/mL) Mean ± S.D.</th>
<th>H_{2}O_{2} Scavenging ability–IC_{50} (µg/mL) Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>71.37 ± 1.55</td>
<td>205.23 ±0.32</td>
</tr>
<tr>
<td>5b</td>
<td>88.66 ± 0.78</td>
<td>166.47 ± 0.67</td>
</tr>
<tr>
<td>5c</td>
<td>54.51 ± 1.14</td>
<td>129.75 ±1.07</td>
</tr>
<tr>
<td>5d</td>
<td>93.08 ± 1.42</td>
<td>142.44 ± 1.47</td>
</tr>
<tr>
<td>5f</td>
<td>68.22 ± 0.32</td>
<td>142.73 ±0.97</td>
</tr>
<tr>
<td>5i</td>
<td>72.43 ± 0.43</td>
<td>155.50 ±1.15</td>
</tr>
<tr>
<td>5j</td>
<td>53.79 ± 0.97</td>
<td>127.83 ± 1.57</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>48.06 ± 0.54</td>
<td>123.67 ± 1.01</td>
</tr>
</tbody>
</table>
reference compound ascorbic acid (IC₅₀ 123.67 ± 1.01µg/mL). Among the tested compounds, the best scavenging activity was presented by 5c (IC₅₀ 129.75 ± 1.07 µg/mL) and 5j (IC₅₀ 127.83 ± 1.57 µg/mL). The presence of methoxy group on para position increases the hydrogen peroxide scavenging activity (Table III) compared to electron-withdrawing substituent at para position.

Experimental Section
All the chemicals used were of analytical grade obtained from S. D. Fine, Spectrochem and Aldrich chemicals. Completion of the reaction was monitored by thin layer chromatography (TLC) using E. Merck 0.25 mm silica gel plates. Visualization was accomplished with UV light (254 nm). All the solvents were distilled and dried using appropriate drying agents before use. Melting points were determined on Ana lab melting point apparatus and are uncorrected. All the ¹H NMR spectra were recorded on Avance 300 MHz and Innova 500 MHz instrument. The IR spectra were recorded on Shimadzu FT-IR spectrophotometer by using 1% KBr discs. The mass spectra were recorded on Agilent 1100 series instrument.

Procedure for the synthesis of ethyl 2-methylquinoline-3-carboxylate, 3
Iron powder (4 mmol) and 0.1M HCl (0.05 mmol) were sequentially added to a solution of an ortho-nitro benzaldehyde in methanol and the resulting mixture was stirred vigorously at 95°C (oil bath temperature) while progress of reaction was monitored by TLC. On completion of the reduction, the β-ketoester was added and the reaction mixture was refluxed. After completion of the reaction as indicated by TLC, the reaction mixture was cooled to RT and the mixture was filtered and washed with methanol to obtain pure product. TLC solvent system: Ethyl acetate: Hexane – 3:7. Yield 91%. m.p.182-84°C; IR (KBr): 3360 (NH), 1648 cm⁻¹ (C=O); ¹H NMR (300 MHz, DMSO-d₆): δ 11.69 (1H, s), 8.29 and 8.15 (1H, s, cis-trans conformer), 8.17-7.47 (1H, m), 7.88-7.61 (3H, m), 7.57-7.47 (1H, q), 7.42-7.32 (2H, m),7.24 (2H,d), 2.85 and 2.73 (3H, s, cis-trans conformer); LC-MS (ESI, m/z): 290(M+H).

Procedure for the synthesis of 2-methylquinoline-3-carbohydrazide, 4
Quinoline ester (1 eq) was dissolved in methanol (5 mL) and excess amount of hydrazine hydrate (99%, 3 eq) was added to the reaction mixture and refluxed for overnight. Completion of the reaction was monitored by TLC. The reaction mixture was cooled to RT; white crystals separated out from reaction mixture. These crystals were filtered and washed with methanol to obtain the pure compound. These crystals had enough purity to proceed for the next step. TLC solvent system: Ethyl acetate: Hexane – 3:7. Yield 89%, m.p. 221-23°C; IR (KBr): 3259-3141 (-NH-NH); 2928 (Ar-H), 1620 cm⁻¹ (–C=O); ¹H NMR (300 MHz, DMSO-d₆): δ 9.6 (1H); 8.18 (1H, s), 7.93 (1Ht), 7.7 (1H, t),8.73 (1H, d), 7.68 (1H, s),7.51 (1H, s), 1.8 (3H, s,CH₃); LC-MS (ESI, m/z): 202(M+H).

Procedure for the synthesis of 2-methyl-N’-[(E)-(4-methylphenyl)methylidene] quinoline-3-carbohydrazide, 5a-j
To a solution of quinolinehydrazide (1 mol) in methanol (5 mL), the corresponding arylaldehydes (1 mol) were added and the resulting mass refluxed for requisite time. Completion of reaction was monitored by TLC. The reaction mixture was cooled to RT. The solid thus obtained was filtered and washed with methanol to obtain pure product. TLC solvent system: Ethyl acetate: Hexane – 3:7. Yield 87%. m.p. 171-18°C; IR (KBr): 3050 (ArH),1714 cm⁻¹ (ester –C=O); ¹H NMR (300 MHz, DMSO-d₆): δ 8.7 (1H, s), 8.0 (1H, d), 7.8 (1H, d), 7.7 (1H, t), 7.5 (1H, t), 4.4 (2H, q, CH₂),3.0 (3H, s), 1.5 (3H, t,CH₃); LC-MS (ESI, m/z): 216 (M+H).
\(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 8.30 & 8.24 (1H, s, cis-trans conformer), 7.99 (1H, d), 7.93-7.64 (4H, m), 7.55 (1H, t), 7.29 (1H, d), 6.91 (1H, d), 6.74 (1H, d), 3.85 and 3.75 (3H, s, cis-trans conformer), 2.83 and 2.70 (3H, s, cis-trans conformer); LC–MS (ESI, \(m/z\)): 320(M+H), 342(M+Na).

\(N'\)-(E)-(3-Chlorophenyl) methylidene]-2-methylquinoline-3-carbohydrazide, 5g: Yield 92%. m.p. 200-02°C; IR (KBr): 3340(NH), 1664 cm\(^{-1}\) (C=O); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 11.85 and 11.87 (1H, s, cis-trans conformer), 8.27 and 8.24 (1H, s, cis-trans conformer), 7.99 (1H, t), 7.84 (1H, t), 7.73 (1H, q), 6.86 (1H, s), 6.51 (1H, s); LC–MS (ESI, \(m/z\)): 324(M+H).

\(N'\)-(E)-(4-Fluorophenyl) methylidene]-2-methylquinoline-3-carbohydrazide, 5i: Yield 91%. m.p. 210-12°C; IR (KBr): 3256(NH), 1681 cm\(^{-1}\) (C=O); \(^1\)H NMR (300 MHz, DMSO-\(d_6\)): \(\delta\) 11.71 and 11.63 (1H, s, cis-trans conformer), 8.27 and 8.24 (1H, s, cis-trans conformer), 7.99 (1H, t), 7.84 (1H, t), 7.73 (1H, q), 6.80 (1H, s), 6.51 (1H, s); LC–MS (ESI, \(m/z\)): 324(M+H).

Antimicrobial Activity

All the synthesized compounds 5 a-j were evaluated for their \textit{in vitro} antibacterial and antifungal activities using the two-fold serial dilution technique\(^{12}\). The analogs and standard drugs were further diluted in test medium to provide concentrations of 400, 200, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 µg/mL. Minimal inhibitory concentration (MIC) for each test compound was investigated against standard bacterial strains such as \textit{Staphylococcus aureus}, \textit{Bacillus subtilis} and \textit{Streptococcus pyogenes} (Gram-positive), \textit{Klebsiella pneumonia}, \textit{Enterococcus aerogens} and \textit{Escherichia coli} (gram negative); \textit{Candida albicans} and \textit{Fusarium oxysporum} (fungal strain). Itraconazole and Streptomycin were used as standard drugs. The MIC values for all the synthesized agents and reference drug are shown in Table II.

\textit{In vitro} antioxidant assays

\textit{In vitro} Antioxidant activity was performed by two methods.

Reducing power method

2 mL of each sample and standard solutions were spiked with 2.5 mL of 1% potassium ferricyanide solution. This mixture was maintained at 50°C in a water bath for 20 min. After cooling, 2.5 mL of 10% trichloroacetic acid was added (when precipitate formed, centrifuged at 3000 rpm for 10 min) 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 1 mL of 0.1% ferric chloride and kept for 10 min. Control was prepared in a similar manner excluding samples\(^{14}\). The absorbance of resulting solution was measured at 700 nm (Table III).

Hydrogen peroxide scavenging assay

The solution of hydrogen peroxide (100 mM) was prepared by the addition of various concentrations of compound (50–300 µg/mL) to hydrogen peroxide solution (2 mL) in phosphate buffered saline of pH 7.4. Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. For each concentration, a separate blank sample was used for background subtraction. For control sample, absorbance of hydrogen peroxide solution was taken at 230 nm\(^{15}\). The percentage inhibition activity (Table III) was calculated from the formula \([{(A_0 - A_1)} / A_0] \times 100\), where \(A_0\) is the absorbance of the control, and \(A_1\) is the absorbance of test/standard taken as ascorbic acid (50-300 µg/mL).

Conclusion

The ten new quinoline hydrazone compounds 5a-j were synthesized through multistep synthesis. The synthesized compounds were characterized by IR, \(^1\)H NMR and ESI-MS. \textit{In vitro} antibacterial, antifungal and antioxidant activities were examined using representative Gram-positive, Gram-negative, fungal strains and standard assays respectively. Out of the
ten derivatives, *para* substituted aryl derivatives (5b, 5c and 5j) exhibited very good potency in inhibiting the growth of *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Candida albicans* and *Fusarium oxysporium*. The antimicrobial activity of compounds 5b, 5c and 5j suggested that introduction of ring activating groups on *para* position of the aryl ring significantly improved the antimicrobial activity. Among the results obtained from the two methods for the evaluation of antioxidant activity of compounds 5c and 5j have shown the most promising antioxidant activity in reducing power assay and hydrogen peroxide scavenging assay. The activity is facilitated by the presence of electron donating groups on aryl ring.

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**References**