Synthesis antimicrobial and antioxidant activities of some new 3-indolyl pyrazolo[2,3-c]pyran and its derivatives

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Some new 6-amino-4-(2′,5′-disubstituted 1H-indol-3′-yl)-3-methyl-N′-isonicotinyl-1,4-dihydropyran[2,3-c]pyrazoles have been synthesized and characterized by spectroscopic techniques (1H NMR, IR and MS) and elemental analysis. These compounds exhibit good antimicrobial and antioxidant activities on screening.

Keywords: Indole, isoniazid, pyrano[2,3-c]pyrazole, antimicrobial, antioxidant

The present studies are a part of the continuing interest on the synthesis of new indole congeners possessing diverse biological activities viz., anti-inflammatory, antimicrobial, antidiabetic, anti-HIV, anticancer, COX-II inhibitors, lyphoxygenase activity, antioxidant and antituberculosis. Further, pyrazoles have played a crucial part in the development of heterocyclic chemistry and useful as synthetic organic synthesis. Many pyrazole analogues possess broad spectrum of pharmacological properties, such as, analgesic, antipyretic, antidepressant and antirheumatic, and are also well known for their pronounced anti-inflammatory activity as well as potent antidiabetic agents. Also, isoniazid has been used in combination with ethambutol, rifampicin, streptomycin and pyrazinamide to treat tuberculosis. Literature survey also reveals that substituted pyranol[2,3-c]pyrazoles have been synthesized in the attempt to find new physiologically active compounds with applications as drugs, pesticides and several derivatives of practical significance.

If indole nucleus is coupled to other biologically active heterocycles, the resulting system is expected to show an increased spectrum of biological activities. Moreover, no report has been cited earlier on 2,5-disubstituted indoles containing isoniazid and pyrazol[2,3-c]pyrazole moieties for any kind of biological studies. In view of above observations, title compounds were synthesized in order to obtain novel heterocyclic systems containing four biodynamic heterocyclic moieties viz., indole, isoniazid, pyrazole and pyran, and evaluated for their antimicrobial and antioxidant activities.

Results and Discussion

The general synthetic pathway is discussed in Schemes I and II. The required intermediate 2-isonicotinoyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one 1 was prepared by reaction of isoniazid with ethyl acetoacetate in absolute ethanol. The other synthetic intermediates 2-[2′,5′-disubstituted 1H-indol-3′-yl]-methylene]malanonitrile 3 and ethyl 3-[2′,5′-disubstituted 1H-indol-3′-yl]-2-cyanoacrylate 4 were synthesized by reacting 2,5-disubstituted indole-3-carboxaldehydes 2 with malanonitrile and ethylcyanoacetate in alcohol containing catalytic amount of piperidine under reflux temperature, respectively. Compound 3a in its IR spectrum exhibited absorption band at 3270 cm\(^{-1}\) due to indole NH and a sharp peak at 2208 cm\(^{-1}\) due to CN functions. In the \(^1\)H NMR spectrum of 3a, the downfield singlet appeared at \(δ\) 13.10 which corresponds to indole NH, another singlet observed at \(δ\) 8.10 was attributed to methine proton and the multiplet of eight aromatic protons resonated between \(δ\) 7.30-8.00. Mass spectrum of 3a exhibited isotopic molecular ion peak at \(m/z\) 303 and 305. The spectral data supports the formation of 3a from 2a. Compound 4a in its IR spectrum showed absorption peaks at 3250, 2300 and 1670 cm\(^{-1}\) corresponding to indole NH, CN and C=O functions, respectively. In its \(^1\)H NMR spectrum, signals due to various protons resonated at \(δ\) 12.9 (s, 1H, NH), 8.2 (s, 1H, =CH), 7.3-7.6 (m, 8H, ArH), 4.2 (q, 2H, CH\(_2\)) and 1.2 (t, 3H, CH\(_3\)). The mass spectrum of 4a exhibited isotopic molecular ion peak at \(m/z\) 350 and 352. The spectral data supports the formation of 4a from 2a (Scheme I).

When compound 1 (Ref. 30) was subjected to cyclocondensation with compound 3 or 4 in alcohol...
containing catalytic amount (99%) of triethylamine, it yielded 6-amino-5-cyano-4-(2',5'-disubstituted-1H-indol-3'-yl)-3-methyl-N'-isonicotinoyl-1,4-dihydropyrano[2,3-c]pyrazole 5a and ethyl 6-amino-4-(2',5'-disubstituted-1H-indol-3'-yl)-3-methyl-N'-isonicotinoyl-1,4-dihydropyrano[2,3-c]pyrazol-5-carboxylate 6a, respectively (Scheme II).

The formation of compounds 5a and 6a were confirmed by IR, ¹H NMR and mass spectral data. In the IR spectrum of compounds 5 and 6, the absorption frequency due to pyrazolyl carbonyl function at 1685 cm⁻¹ was missing, whereas, the new absorption band due to amino group was observed at 3322-3431 cm⁻¹. The ¹H NMR spectrum of compound 5a exhibited a singlet at δ 12.8 which was assigned to indole NH, a multiplet between δ 7.10-8.30 accounted for twelve aromatic protons and two protons of amino group. The singlet due to methyl protons was noticed at δ 2.30 and the appearance of new singlet at δ 5.10 due to pyran proton confirmed the formation of 5a. The mass spectrum of 5a exhibited the isotopic molecular ion peak at m/z 506 and 508. In the ¹H NMR of 6a, signals due to various protons resonated at δ 12.80 (s, 1H, indole NH), 7.00-8.70 (m, 14H, ArH and NH₂), 4.70 (s, 1H, pyran H), 4.20 (q, 2H, CH₂), 2.40 (s, 3H, CH₃) and 1.80 (t, 3H, CH₃). Its mass spectrum exhibited molecular ion peaks at m/z 553 and 555, which confirms the formation of 6a.

**Biological activities**

**Antimicrobial activity:** Antimicrobial activity results of the test compounds revealed that compound 3a (Table I) exhibited maximum zone of inhibition against all the three bacteria *S. aureus*, *P. aeruginosa* and *K. pneumonia*. On the other hand, compounds 4a and 4b exhibited maximum zone of inhibition against *S. aureus* and *P. aeruginosa*. Compounds 4d, 5b and 6d exhibited maximum zone of inhibition against *S.*
Antifungal screening of test compounds (Table I) indicated that compound 3a exhibited maximum zone of inhibition against all three fungi A. niger, A. terrus and A. oryzae. However, compounds 3b, 5b and 6b showed the maximum zone of inhibition against A. oryzae and A. niger. Compounds 3c, 3d, 4a, 4b, 4e and 6c exhibited maximum zone of inhibition against A. oryzae. Compounds 4c and 6a were found to exhibit maximum zone of inhibition against A. niger.

2-[(2′-Phenyl-5′-chloro-1H-indol-3′-yl)methylene]-malononitrile 3a was found to be active against all the six microorganisms. The activity of this compound may be attributed to the presence of highly polar and electronegative groups viz., chloro and cyanoacrylate and/or malononitrile present at 5- and 3-position of the indole nucleus, respectively. In addition to this, the 2-phenyl indole nucleus itself is an electron rich moiety. These groups may be responsible for inhibiting the growth of microorganisms. More or less the same is true for the rest of the active compounds.

### Table I — Antimicrobial activity of compounds 3-6

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Std. I: Gentamycin; Std. II: Fluconazole

Antioxidant activity

Radical Scavenging Activity Assay (RSA): All the synthesized compounds were tested for their radical scavenging activity (RSA) using stable free radical DPPH. The results were compared with the standards 2-tert-butyl-4-methoxyphenol (butylated hydroxy anisole, BHA) and 2-(1,1-dimethylethyl)-1,4-benzenediol (2-tert-butyl hydroquinone, TBHQ). Compounds 3b (IC<sub>50</sub> 15.59 µg/mL), 3c (IC<sub>50</sub> 16.16 µg/mL) and 3d (IC<sub>50</sub> 15.78 µg/mL) exhibited 63-65% radical scavenging activity at a concentration of 40 µg/mL. Compound 3d (IC<sub>50</sub> 15.59 µg/mL) exhibited 69 and 68% scavenging activity at a concentration 60 and 80 µg/mL, respectively. Compounds 6a (IC<sub>50</sub> 15.95 µg/mL), 6d (IC<sub>50</sub> 12.59 µg/mL) and 6e (IC<sub>50</sub> 13.19 µg/mL) exhibited 76.65, 76.1 and 70.2% radical scavenging activity, respectively at a concentration of 80 µg/mL. Compounds 6d and 6e exhibited 76.1 and 74.5% radical scavenging activity at 60 µg/mL, respectively. Compound 6d exhibited highest radical...
scavenging activity 80.1% and 79.6% at a concentration of 20 and 40 µg/mL, respectively. Whereas, compounds 6b (IC₅₀ 14.93 µg/mL) and 6e (IC₅₀ 13.19 µg/mL) exhibited scavenging activity ranging from 66.57-76.1% at a concentration 20 and 40 µg/mL, respectively. The results were compared to that of BHA (IC₅₀ 11.21 µg/mL) and TBHQ (IC₅₀ 11.81 µg/mL). The RSA of compounds 3 and 6 may be due to the presence of malanonitrile function in 3 and pyrano[2,3-c] pyrazole and isonicotyl groups in 6 at position-3 of indole nucleus. These groups may help to stabilize the free radical formed after transfer of an electron or hydrogen to the stable DPPH free radical as shown in Scheme III.

**Experimental Section**

All the reagents were obtained commercially and used after further purification. Melting points were determined by an open capillary method and are uncorrected. The IR (KBr) spectra were recorded with a Perkin-Elmer spectrum one FT-IR spectrometer. The ¹H NMR (DMSO-d₆) spectra were recorded with a Bruker NMR (400 MHz) and the chemical shifts were expressed in ppm (δ scale) with TMS as internal standard. Mass spectra were recorded with a JEOL-AcuTOF JMS-T100LC mass spectrometer. Elemental analysis was carried out using Flash EA1112 series elemental analyzer.

**General procedure for the synthesis of 2-isonicotinyl-5-methyl-2,4-dihydro-3H-pyrazol-3-one, 1**

This compound was prepared from isoniazid and ethylacetoacetate according to the following literature procedure³⁰.

**General procedure for synthesis of 2,5-disubstituted indole-3-carboxaldehyde, 2a-e**

These compounds were prepared by following literature procedure³¹.
General procedure for the synthesis of 3a-e and 4a-e

These two precursors 3a, 3e, 4a and 4e were synthesized according to literature procedure. A mixture of 2,5-disubstituted indole-3-carboxaldehyde (0.0056 mol), malonitrile (or ethylcyanoacetate) (0.0056 mol) in dry ethanol (18 mL) and few drops of piperidine, was refluxed for 1 hr. It was then cooled to RT and poured into ice-cold water. The separated solid was filtered, washed with water, dried and purified by recrystallization from ethanol.

Physical constants and spectral data of the following synthesized compounds are in good agreement with the reported values.

2-[(2-Phenyl-5-chloro-1H-indol-3′-yl)methylene]malonitrile, 3a

73% yield. Pale yellow crystals (ethanol). m.p. 230-31°C. IR (KBr): 3272 (NH), 2208 (CN), 1661 cm⁻¹ (CO); ¹H NMR (DMSO-d₆): δ 12.8 (s, 1H, NH), 8.2 (s, 1H, =CH), 7.1-8.1 (m, 7H, ArH), 1.35 (s, 3H, CH₃); MS: m/z 317, 319 (M⁺, M⁺2). Anal. Calcd for C₁₉H₁₅N₂Cl: C, 71.93; H, 3.79; N, 13.27%.

Ethyl 3-(5′-chloro-2′-(phenyl-1H-indol-3′-yl)-2-cyanoacrylate, 4a

Ethyl 3-(2′-phenyl-1H-indol-3′-yl)-2-cyanoacrylate, 4e

2-[(2-p-Toly)-5-chloro-1H-indol-3′-yl)methylene]malonitrile, 3b

76% yield. Yellow solid (ethanol). m.p. 239-41°C. IR (KBr): 3245 (NH), 2290 (CN), 1673 cm⁻¹ (CO); ¹H NMR (DMSO-d₆): δ 12.8 (s, 1H, NH), 8.0 (s, 1H, =CH), 7.4-7.9 (m, 8H, ArH), 1.3 (t, 3H, CH₂), 1.4 (s, 3H, CH₃); MS: m/z 350, 352 (M⁺, M⁺2). Anal. Calcd for C₁₉H₁₈N₂Cl: C, 76.85; H, 4.20; N, 8.00%. Found: C, 76.85; H, 4.30; N, 8.01%.

Ethyl 3-(2′-(p-chlorophenyl)-1H-indol-3′-yl)-2-cyanoacrylate, 4c

68% yield. Yellow solid (ethanol). m.p. 237-38°C. IR (KBr): 3245 (NH), 2290 (CN), 1673 cm⁻¹ (CO); ¹H NMR (DMSO-d₆): δ 12.8 (s, 1H, NH), 8.2 (s, 1H, =CH), 7.4-7.9 (m, 8H, ArH), 4.3 (q, 2H, CH₂), 1.3 (t, 3H, CH₃); MS: m/z 350, 352 (M⁺, M⁺2). Anal. Calcd for C₂₁H₁₅N₂O₂Cl: C, 76.57; H, 4.29; N, 8.00%. Found: C, 68.50; H, 4.30; N, 8.01%

General procedure for the synthesis of compounds 5a-e and 6a-e

A solution of compound 1 (0.001 mol) and compound 3 or 4 (0.001 mol) in ethanol (30 mL) containing triethylamine (0.5 mL) was heated under reflux for 5 hr. The reaction mixture was cooled to RT and poured over crushed ice containing a few drops of HCl. The solid formed was collected by filtration, washed with water, dried and purified by recrystallization from ethanol.

6-Amino-5-cyano-4-(5′-chloro-2′-phenyl-1H-indol-3′-yl)-3-methyl-N′-isonicotinyl-1,4-dihydropyrazino[2,3-c]pyrazole, 5a

69% yield. Yellow needles. m.p. 256-57°C. IR (KBr): 3322 (NH₂), 3270 (NH), 2208 (CN), 1661 (CO), 1608 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆): δ 12.8 (s, 1H, NH), 7.1-8.3 (m, 12H, ArH and NH₂), 5.1 (s, 1H, pyran H), 2.3 (s, 3H, CH₃); MS: m/z 506, 508. 
Ethyl 6-amino-4-(5′-chloro-2′-phenyl-1H-indol-3′-yl)-3-methyl-N′-isonicotinyl-1,4-dihydropyran-2,3-c]pyrazol-5-carboxylate, 6a

60% yield. Yellow needles. m.p. 271-72°C. IR (KBr): 3431 (NH), 3295 (NH), 1670 (CO), 1660 (CO), 1608 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆): δ 12.8 (s, 1H, NH), 7.0-8.7 (m, 12H, ArH and NH₂), 4.7 (s, 1H, pyran H), 4.2 (q, 2H, CH₂), 2.4 (s, 3H, CH₃), 1.8 (t, 3H, CH₃); MS: m/z 553, 555 (M⁺, M⁺+2). Anal. Calcld for C₃₀H₂₈N₅O₄Cl: C, 65.10; H, 4.34; N, 12.66. Found: C, 65.15; H, 4.31; N, 12.62%.

Ethyl 6-amino-4-(5′-chloro-2′-(p-tolyl)-1H-indol-3′-yl)-3-methyl-N′-isonicotinyl-1,4-dihydropyran-2,3-c]pyrazol-5-carboxylate, 6b

62% yield. Yellow needles. m.p. 275-76°C. IR (KBr): 3434 (NH), 3290 (NH), 1671 (CO), 1662 (CO), 1608 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆): δ 12.9 (s, 1H, NH), 7.1-8.8 (m, 12H, ArH and NH₂), 4.7 (s, 1H, pyran H), 4.3 (q, 2H, CH₂), 2.5 (s, 3H, CH₃), 2.0 (s, 3H, CH₃), 1.8 (t, 3H, CH₃); MS: m/z 576, 569 (M⁺+2). Anal. Calcld for C₃₀H₂₈N₅O₄Cl: C, 65.61; H, 4.59; N, 12.35. Found: C, 65.63; H, 4.62; N, 12.37%.

Ethyl 6-amino-4-(2′-(p-chlorophenyl)-1H-indol-3′-yl)-3-methyl-N′-isonicotinyl-1,4-dihydropyran-2,3-c]pyrazol-5-carboxylate, 6c

64% yield. Yellow crystals. m.p. 279-80°C. IR (KBr): 3430 (NH), 3285 (NH), 1676 (CO), 1656 cm⁻¹ (C=N), ¹H NMR (DMSO-d₆): δ 12.7 (s, 1H, NH), 7.2-8.7 (m, 12H, ArH and NH₂), 4.6 (s, 1H, pyran H), 4.2 (q, 2H, CH₂), 2.5 (s, 3H, CH₃), 1.9 (t, 3H, CH₃); MS: m/z 553, 555 (M⁺+2). Anal. Calcld for C₂₉H₂₄N₅O₄Cl: C, 65.10; H, 4.34; N, 12.66. Found: C, 65.12; H, 4.32; N, 12.70%.

Ethyl 6-amino-4-(2′-(p-tolyl)-1H-indol-3′-yl)-3-methyl-N′-isonicotinyl-1,4-dihydropyran-2,3-c]pyrazol-5-carboxylate, 6d

59% yield. Yellow solid. m.p. 280-82°C. IR (KBr): 3425 (NH), 3283 (NH), 1673 (CO), 1656 (CO), 1605 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆): δ 12.8 (s, 1H, NH), 7.1-8.6 (m, 12H, ArH and NH₂), 4.5 (s, 1H, pyran H), 4.3 (q, 2H, CH₂), 2.6 (s, 3H, CH₃), 2.1 (s, 3H, CH₃), 1.8 (t, 3H, CH₃); MS: m/z 533 (M⁺). Anal. Calcld for C₃₅H₂₇N₅O₄: C, 69.80; H, 5.07; N, 13.13. Found: C, 69.83; H, 5.09; N, 13.10%.
Ethyl 6-amino-4-(2′-phenyl-1H-indol-3′-yl)-3-methyl-4H-isonicotinyl-1,4-dihydro pyrano[2,3-c]-pyrazol-5-carboxylate, 6e

61% yield. Yellow solid. m.p. 283-85°C. IR (KBr): 3420 (NH₂), 3280 (NH), 1679 (CO), 1655 (CO), 1604 cm⁻¹ (C=N); ¹H NMR (DMSO-d₆): δ 12.9 (s, 1H, NH), 7.1-8.6 (m, 13H, ArH and NH₂), 4.6 (s, 1H, pyran H), 4.4 (q, 2H, CH₂), 2.6 (s, 3H, CH₃), 1.8 (t, 3H, CH₃); MS: m/z 519 (M⁺).

Analytical Calcd for C₃₀H₂₅N₅O₄: C, 69.36; H, 4.82; N, 13.49. Found: C, 69.40; H, 4.85; N, 13.51%.

Antimicrobial activity

All the synthesized compounds (3-6) were evaluated for their antibacterial activity against S. aureus, P. aeruginosa and K. pneumonia, while antifungal activity was evaluated against A. niger, A. terrus and A. oryzae by cup plate method at a concentration of 1mg/mL following literature procedure. The zone of inhibition (in mm) was compared with the standard gentamycin and fluconazole for antibacterial and antifungal activities, respectively. The results are tabulated in Table I.

Antioxidant activity: 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) radical scavenging activity (RSA)

The free radical scavenging activity of 3-6 was carried out in the presence of the stable free radical DPPH following Hatano’s method using 2-tert-butyl-4-methoxyphenol (butylated hydroxy anisole, BHA) and 2-(1,1-dimethylthyl)-1,4-benzenediol (2-tert-butyl hydroquinone, TBHQ) as standards. The radical scavenging activity (RSA) for methanolic solutions of compounds 3-6 at concentrations 25, 50, 75 and 100 µg/mL containing freshly prepared DPPH solution (0.004% w/v) was carried out and compared with those of standards BHA and TBHQ. All the test analyses were performed on three replicates and the results are averaged. The results in percentage are expressed as the ratio of absorption decrease of DPPH in the presence of test compounds and absorption of DPPH in the absence of test compounds at 517 nm using ELICO SL 171 Mini Spec spectrophotometer. The percentage scavenging activity of the DPPH free radical was measured using the following equation:

\[
\text{% DPPH radical scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100
\]

The results are shown in Figures 1 and 2.

Conclusion

The present work describes antimicrobial assay and free radical scavenging activity of the synthesized compounds. Compound 3a exhibited maximum zone of inhibition against all the six organisms compared to other compounds. On the other hand, good radical scavenging activity was exhibited by 6d (80.1%) at a concentration of 80 µg/mL. However, none of the compound exhibited antimicrobial and radical scavenging activity better than the standards.

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