Synthesis of new fluorescent compounds from 5-nitro-1H-indazole

Vahid Pakjoo, Mina Roshani, Mehdi Pordel,* and Toktam Hoseini

Department of Chemistry, Mashhad Branch, Islamic Azad University, Mashhad, Iran
E-mail: mehdipordel58@yahoo.com

DOI: http://dx.doi.org/10.3998/ark.5550190.0013.917

Abstract
Nucleophilic substitution of hydrogen followed by intramolecular electrophilic aromatic substitution in nitro dervitavies of indazole has been used as a key step in the one pot synthesis of new fluorescent heterocyclic compounds 3H-pyrazolo[4,3-a]acridin-11-carbonitriles.

Keywords: Nucleophilic substitution of hydrogen, 5-nitro-1H-indazole, heterocyclization, fluorescence, emission and absorption spectra

Introduction
Fluorescence is used as an analytical tool to determine the concentrations of various species, either neutral or ionic. When the analyte is fluorescent, direct determination is possible; otherwise, a variety of indirect methods using derivatization, formation of a fluorescent complex or fluorescence quenching have been developed. Fluorescence sensing is the method of choice for the detection of analytes with a very high sensitivity, and often has an outstanding selectivity thanks to specially designed fluorescent molecular sensors.1-3 Fluorescence is also a powerful tool for investigating the structure and dynamics of matter or living systems at a molecular or supramolecular level. Polymers, solutions of surfactants, solid surfaces, biological membranes, proteins, nucleic acids and living cells are well-known examples of systems in which estimates of local parameters such as polarity, fluidity, order, molecular mobility and electrical potential is possible by means of fluorescent molecules playing the role of probes.4-7 The latter can be intrinsic or introduced on purpose. The high sensitivity of fluorimetric methods in conjunction with the specificity of the response of probes to their microenvironment contribute towards the success of this approach.8-10 Fluorescent heterocyclic compounds are of interest as functional materials in many disciplines such as emitters for electroluminescence devices,11 molecular probes for biochemical research,12 in traditional textile and polymer fields,13 whitening agents14 and photo conducting materials.15
We have previously described a process for the relatively feasible production of some new fluorescent compounds\textsuperscript{16,17} by reacting the imidazo[1,2-a]pyridine with arylacetonitriles via nucleophilic substitution of hydrogen\textsuperscript{18-21} Now we describe here the syntheses of new fluorescent heterocyclic compounds from nitro derivatives of indazole by this method and evaluation of their spectroscopic properties.

**Results and Discussion**

The new 3-alkyl-3\textit{H}-pyrazolo[4,3-\textit{a}]acridin-11-carbonitrile 3\textit{a}-\textit{d} were synthesized via the nucleophilic substitution of hydrogen of 1-alkyl-1\textit{H}-indazole 1\textit{a, b} with arylacetonitriles 2\textit{a, b} in basic MeOH solution and then intramolecular electrophilic aromatic substitution in moderate yields\textsuperscript{16,17-22} (Scheme 1). When R' is an electron withdrawing group such as NO\textsubscript{2} group, the yield of the reaction is very low, since the corresponding conjugated base is a weak nucleophile. A proposed mechanism to explain the formation of compounds 3\textit{a}-\textit{d} is shown in Scheme 2.\textsuperscript{16,17}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme1.png}
\caption{Scheme 1}
\end{figure}

The structural assignments of compounds 3\textit{a}-\textit{d} were based on the analytical and spectral data. For example, in the \textsuperscript{1}H NMR spectrum of 3\textit{a}, there are the signals at \(\delta\ 4.07\) and 4.28 ppm assignable to protons of methoxy and methyl group and the doublet of doublet signal at \(\delta\ 7.53\) ppm (\(J\ 9.2\) Hz and \(J'\ 2.4\) Hz), the doublet signals at \(\delta\ 7.61\) (d, \(J\ 2.4\) Hz), \(\delta\ 7.90\) (d, \(J\ 9.6\) Hz), \(\delta\ 8.07\) (d, \(J\ 9.6\) Hz), \(\delta\ 8.35\) (d, \(J\ 9.2\) Hz) ppm and singlet signal at \(\delta\ 9.15\) ppm attributed to six protons of aromatic rings. Moreover, the FT-IR spectrum of 3\textit{a} in KBr showed the absorption band at 2240 cm\textsuperscript{-1} corresponding to cyanide group. All this evidence plus the \textsuperscript{13}C NMR spectrum, molecular ion peak at \textit{m/z} 288 and microanalytical data strongly support the tetracyclic structure of compound 3\textit{a}. 

©ARKAT-USA, Inc
Scheme 2

The fluorescence absorption and emission spectra of compounds 3a-d were recorded at the concentration of $10^{-5}$ and $10^{-6}$ M in chloroform. Figure 1 and Figure 2 show the visible absorption and emission spectra of compounds 3a-d. The $\lambda_{\text{abs}}$, values of extinction coefficient ($\varepsilon$), $\lambda_{\text{ex}}$, $\lambda_{\text{em}}$ and fluorescence quantum yield ($\Phi_F$) data are presented in Table 1. Values of extinction coefficient ($\varepsilon$) were determined as the slope of the plot of absorbance vs concentration. The fluorescence quantum yields ($\Phi_F$) of compounds 3a-d were determined via comparison methods, using fluorescein as a standard sample in 0.1 M NaOH and MeOH solution. Also the fluorescence absorption and emission spectra of compound 3a were measured in different solvents (Figure 3 and Figure 4). As it is demonstrated in these figures, the fluorescence absorption and emission spectra of 3a in polar solvents exhibit solvatochromic red shift with the increasing solvent polarity. Solvent effects shift the emission to lower energy owing to stabilization of the excited state by the polar solvent molecules (Table 2). This type of behavior is observed for most of the dyes. For example, in Table 2, one can see that in the absorption spectrum for 3a, $\lambda_{\text{abs}}$ shifts from 369 to 397 nm, and in the emission spectrum, $\lambda_{\text{em}}$ shifts from 447 to 456 nm as the solvent is changed from n-hexane to methanol.
**Figure 1.** Visible absorption spectra of compounds 3a-d in dilute (1 × 10^{-5} M) chloroform solution.

**Figure 2.** Emission spectra of compounds 3a-d in dilute (1 × 10^{-6} M) chloroform solution.
Table 1. Photophysical data for absorption (abs) and emission (em) of 3a-d in chloroform

<table>
<thead>
<tr>
<th>Dye</th>
<th>3a</th>
<th>3b</th>
<th>3c</th>
<th>3d</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\lambda_{\text{abs}} \text{ (nm)})(^a)</td>
<td>380</td>
<td>380</td>
<td>395</td>
<td>395</td>
</tr>
<tr>
<td>(\varepsilon \times 10^{-4} \text{ (M}^{-1} \text{ cm}^{-1})) (^b)</td>
<td>3.8</td>
<td>3.8</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>(\lambda_{\text{ex}} \text{ (nm)})(^c)</td>
<td>375</td>
<td>375</td>
<td>375</td>
<td>375</td>
</tr>
<tr>
<td>(\lambda_{\text{em}} \text{ (nm)})(^d)</td>
<td>454</td>
<td>454</td>
<td>438</td>
<td>438</td>
</tr>
<tr>
<td>(\Phi_{\text{F}})(^e)</td>
<td>0.45</td>
<td>0.53</td>
<td>0.51</td>
<td>0.59</td>
</tr>
</tbody>
</table>

\(^a\) Wavelengths of maximum absorbance.
\(^b\) Extinction coefficient.
\(^c\) Wavelengths of fluorescence excitation.
\(^d\) Wavelengths of fluorescence emission.
\(^e\) Fluorescence quantum yield.

Figure 3. Visible absorption spectra of compound 3a in different solvents (1 \(\times\) 10\(^{-5}\) M).
**Figure 4.** Emission spectra of compound 3a in different solvents (1 × 10⁻⁶ M).

**Table 2.** Spectroscopic data for 3a at 298K in dependence of the solvent

<table>
<thead>
<tr>
<th>Solvent</th>
<th>λ_{abs} (nm)</th>
<th>λ_{flu} (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>369</td>
<td>447</td>
</tr>
<tr>
<td>Chloroform</td>
<td>375</td>
<td>450</td>
</tr>
<tr>
<td>DMF</td>
<td>380</td>
<td>460</td>
</tr>
<tr>
<td>DMSO</td>
<td>385</td>
<td>456</td>
</tr>
<tr>
<td>Ethanol</td>
<td>395</td>
<td>460</td>
</tr>
<tr>
<td>Methanol</td>
<td>397</td>
<td>456</td>
</tr>
</tbody>
</table>

**Conclusions**

We have presented a new, facile, efficient and useful protocol for the synthesis of new derivatives of pyrazolo[4,3-a]acridin which have fluorescent properties and research into their possible applications is in progress. For example, these compounds can be used as new molecular probes for biochemical research with hydrolyzing the cyanide group to corresponded carboxylic acid and linking the latter compounds to biologically important molecules such as carbohydrates, lipids, proteins and nucleic Acids.

**Experimental Section**

**General.** Melting points were recorded on an Electrothermal type-9100 melting-point apparatus. The IR spectra were obtained on a Tensor27 spectrometer and only noteworthy absorptions are
listed. The $^{13}$C NMR (100 MHz) and the $^1$H NMR (400 MHz) spectra were recorded on a Bruker Avance DRX-400 Fouriertransformer spectrometer. Chemical shifts are reported in ppm downfield from TMS as internal standard; coupling constant $J$ are given in Hz. The mass spectra were recorded on a Varian Mat, CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyzer. Absorption spectra were recorded on Varian 50-bio UV-Visible spectrophotometer. Fluorescence spectra were recorded using Varian Cary Eclipse spectrofluorophotometer. UV–vis and fluorescence scans were recorded from 350 to 700 nm. All measurements were carried out at room temperature. Compounds 1a, b $^{24}$ were obtained according to the published methods. Other reagents were commercially available.

**General procedure for the synthesis of 3a-d from 1a,b and 2a,b.** Compounds 1a,b (10 mmol) and 2a,b (12 mmol) were added with stirring to a solution of KOH (20 g, 357 mmol) in methanol (50 mL). The mixture was stirred in rt for 24 h. After concentration at reduced pressure, the precipitate was collected by filtration, washed with water, following with EtOH, and then air dried to give crude 3a-d.

**8-Methoxy-3-methyl-3H-pyrazolo[4,3-a]acridin-11-carbonitrile (3a).** Compound 3a was obtained as pale yellow needles (EtOH), yield (72%), mp 325-327 °C. $^1$H NMR (CDCl$_3$) δ 4.07 (s, 3H), 4.28 (s, 3H), 7.53 (dd, $J$ 9.2 Hz, $J'$ 2.4 Hz, 1H), 7.61 (d, $J$ 2.4 Hz, 1H), 7.90 (d, $J$ 9.6 Hz, 1H), 8.07 (d, $J$ 9.6 Hz, 1H), 8.35 (d, $J$ 9.2 Hz, 1H), 9.15 (s, 1H) ppm. $^{13}$C NMR (CDCl$_3$): δ 36.26, 55.85, 106.62, 116.80, 117.22, 120.59, 122.16, 124.22, 125.63, 129.28, 129.75, 129.89, 134.51, 137.76, 147.50, 149.94, 161.21 ppm; IR (KBr disk): ν 2240 cm$^{-1}$ (CN). MS (m/z) 288 (M$^+$). Anal. Calcd for C$_{17}$H$_{12}$N$_4$O (288.3): C, 70.82; H, 4.20; N, 19.43. Found: C, 70.45; H, 4.12; N, 19.29.

**3-Ethyl-8-methoxy-3H-pyrazolo[4,3-a]acridin-11-carbonitrile (3b).** Compound 3b was obtained as pale yellow needles (EtOH), yield (69%), mp 310-312 °C. $^1$H NMR (CDCl$_3$) δ 1.65 (t, $J$ 7.2 Hz, 3H), 4.06 (s, 3H), 4.61 (q, $J$ 7.2 Hz, 2H), 7.51 (dd, $J$ 9.2 Hz, $J'$ 2.0 Hz, 1H), 7.59 (d, $J$ 2.0 Hz, 1H), 7.90 (d, $J$ 9.6 Hz, 1H), 8.05 (d, $J$ 9.6 Hz, 1H), 8.33 (d, $J$ 9.2 Hz, 1H), 9.15 (s, 1H) ppm. $^{13}$C NMR (CDCl$_3$): δ 15.50, 44.49, 55.83, 106.61, 110.40, 116.04, 116.76, 117.20, 120.69, 122.10, 124.14, 125.61, 129.11, 134.55, 136.83, 147.54, 147.85, 161.16 ppm. IR (KBr disk): ν 2240 cm$^{-1}$ (CN). MS (m/z) 302 (M$^+$). Anal. Calcd for C$_{18}$H$_{14}$N$_4$O (302.3): C, 71.51. H, 4.67; N, 18.53. Found: C, 71.41; H, 4.60; N, 18.71.

**3,8-Dimethyl-3H-pyrazolo[4,3-a]acridin-11-carbonitrile (3c).** Compound 3c was obtained as pale yellow needles (EtOH), yield (71%), mp 261-264 °C. $^1$H NMR (CDCl$_3$) δ 2.70 (s, 3H), 4.27 (s, 3H), 7.69 (dd, $J$ 8.4 Hz, $J'$ 1.2 Hz, 1H), 7.88 (d, $J$ 9.6 Hz, 1H), 8.08 (d, $J$ 9.6 Hz, 1H), 8.11 (d, $J$ 1.2 Hz, 1H), 8.34 (d, $J$ 8.4 Hz, 1H), 9.15 (s, 1H) ppm. $^{13}$C NMR (CDCl$_3$): δ 22.02, 36.26, 110.10, 115.84, 116.69, 117.28, 121.79, 124.18, 124.39, 128.84, 129.77, 132.09, 134.84, 137.98, 140.69, 146.43, 147.35 ppm. IR (KBr disk): ν 2240 cm$^{-1}$ (CN). MS (m/z) 272 (M$^+$). Anal. Calcd for C$_{17}$H$_{12}$N$_4$ (272.3): C, 74.98; H, 4.44; N, 20.57. Found: C, 74.59; H, 4.37; N, 20.32.

**3-Ethyl-8-methyl-3H-pyrazolo[4,3-a]acridin-11-carbonitrile (3d).** Compound 3d was obtained as pale yellow needles (EtOH), yield (67%), mp 255-256 °C. $^1$H NMR (CDCl$_3$) δ 1.66 (t, $J$ 7.2 Hz, 3H), 2.70 (s, 3H), 4.62 (q, $J$ 7.2 Hz, 2H), 7.69 (dd, $J$ 8.8 Hz, $J'$ 1.2 Hz, 1H), 7.90 (d,
$J$ 9.6 Hz, 1H), 8.09 (d, $J$ 9.6 Hz, 1H), 8.12 (d, $J$ 1.2 Hz, 1H), 8.35 (d, $J$ 8.8 Hz, 1H), 9.18 (s, 1H) ppm. $^{13}$C NMR (CDCl$_3$): $\delta$ 15.50, 22.01, 44.50, 109.97, 115.84, 116.64, 117.25, 121.88, 124.15, 124.33, 128.81, 129.60, 132.01, 134.86, 137.04, 140.60, 146.33, 146.37 ppm. IR (KBr disk): $\nu$ 2240 cm$^{-1}$ (CN). MS (m/z) 286 (M$^*$$^+$). Anal. Calcd for C$_{18}$H$_{14}$N$_4$ (286.3): C, 75.51; H, 4.93; N, 19.57. Found: C, 75.91; H, 5.08; N, 19.71.

References