Synthesis, characterisation, optical properties and theoretical calculations of a new fluorescent heterocyclic system: 3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridine

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Four new fluorescent dyes derived from the 3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridine system were synthesised and fully characterised by ^{1}H NMR, ^{13}C NMR, mass and analytical data. These new fluorophores were prepared from the reaction of 1-alkyl-5-nitro-1*H*-indazoles with 1-naphthylacetonitrile *via* nucleophilic substitution of hydrogen, in high yields. The optical properties of the dyes were also investigated and the results revealed that, in some cases, they have higher quantum yields compared with well-known fluorescent dyes such as fluorescein. Solvent effects on the fluorescence characteristics of the four compounds indicated that the emission wavelength is red-shifted with increasing solvent polarity. Furthermore, density functional theory calculations using the B3LYP hybrid functional and the 6-311++G(d,p) basis set provided the optimised geometries and relevant frontier orbitals of the compounds. Calculated electronic absorption spectra were also obtained using the time-dependent density functional theory method.

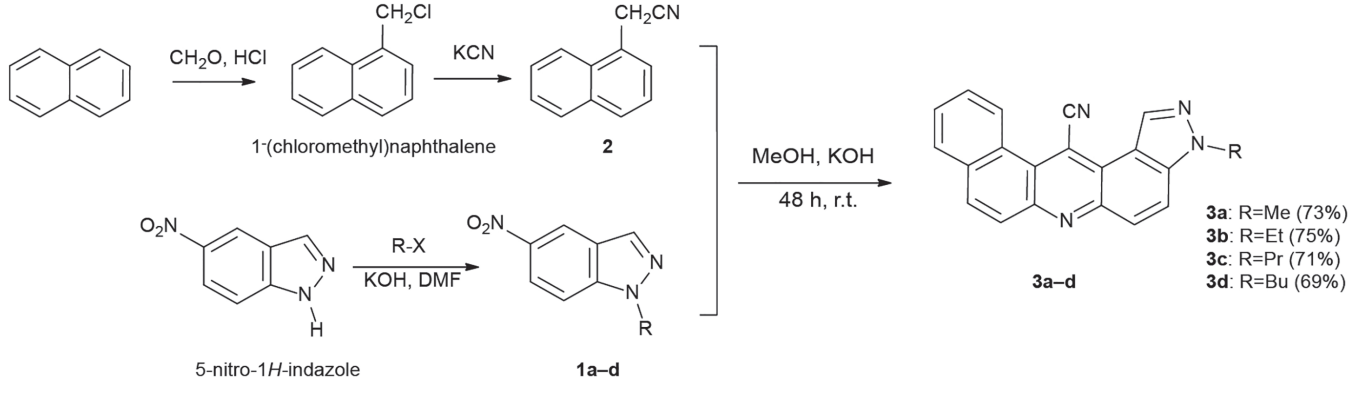
Keywords: 5-nitro-1*H*-indazoles, 3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridine, chromophore, emission and absorption spectra, density functional theory

The synthesis of fluorescent heterocyclic compounds is particularly important because of their increasing use in emitters for electroluminescence devices,[1] molecular probes for biochemical research,[2,3] traditional textile and polymer fields,[4] fluorescent whitening agents[5] and photo-conducting materials.[6] They also find applications in the search for new biologically active compounds and diagnostic methods.[7] Among the various fluorescent heterocyclic compounds, acridines are one of the oldest classes of heterocyclic compounds extensively used as dyes and pigments since the 19th century[8,9] and have been commercialised for more than a century. In particular, some of them have been found to be efficient fluorescent chemosensors for the recognition of transition metal ions, such as Hg^{2+},[10] and emitters for luminescence studies.[11] Also, acridine orange (3,6-dimethylaminoacridine) is a nucleic acid-selective metachromatic stain valuable for cell cycle determination.[12] The acridine nucleus, condensed with additional heterocyclic rings and in particular with the incorporation of five- and six-membered heterocyclic rings into the acridine chromophore, strongly favours passive cellular drug uptake, rendering the efflux by multidrug resistance transporters inefficient.[13] In contrast, pyrazole derivatives have recently received considerable attention due to their optical properties. For example, pyrazoles with suitable functionalisation can be used as dyes,[14] couplers for photographic materials,[15] herbicides,[16] and luminescent and fluorescent substances.[17] The combination of the pyrazole nucleus with the acridine moiety may enhance these properties.

We recently focused on the synthesis of new fluorescent heterocyclic compounds[18–22] and the development of their application.[23] Prompted by the abovementioned optical properties of acridine and pyrazole heterocycles, we herein report a synthesis of a new pentacyclic heterocyclic system, the 3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridines, with the purpose of studying their spectral and fluorescence properties. In addition, density functional theory (DFT) calculations of the fluorophores using the B3LYP hybrid functional and the 6-311++G(d,p) basis set were performed to gain a deeper insight into the fluorescence properties of the compounds. Calculated electronic absorption spectra were also obtained using the time-dependent density functional theory (TD-DFT) method.

Results and discussion

Compounds **3a–d** derived from the new heterocyclic system of 3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridines were synthesised from the reaction of 1-alkyl-5-nitro-1*H*-indazoles **1a–d** with 1-naphthylacetonitrile **2** in basic MeOH solution *via* the nucleophilic substitution of hydrogen[24] in high yields (Scheme 1). The precursors 1-alkyl-5-nitro-1*H*-indazoles **1a–d** were obtained from the alkylation of 5-nitro-1*H*-indazole with various alkyl halides in DMF and KOH according to a literature method.[25] The other precursor, 1-naphthylacetonitrile **2**, was prepared in two steps. Initial reaction of naphthalene with paraformaldehyde and HCl in HOAc led to the formation of 1-chloromethylnaphthalene.[26] Subsequent treatment of 1-chloromethylnaphthalene with KCN in alcohol and water produced 1-naphthylacetonitrile (**2**) in good yield.[27]



Scheme 1 Synthesis of compounds **3a–d** derived from the new heterocyclic system.

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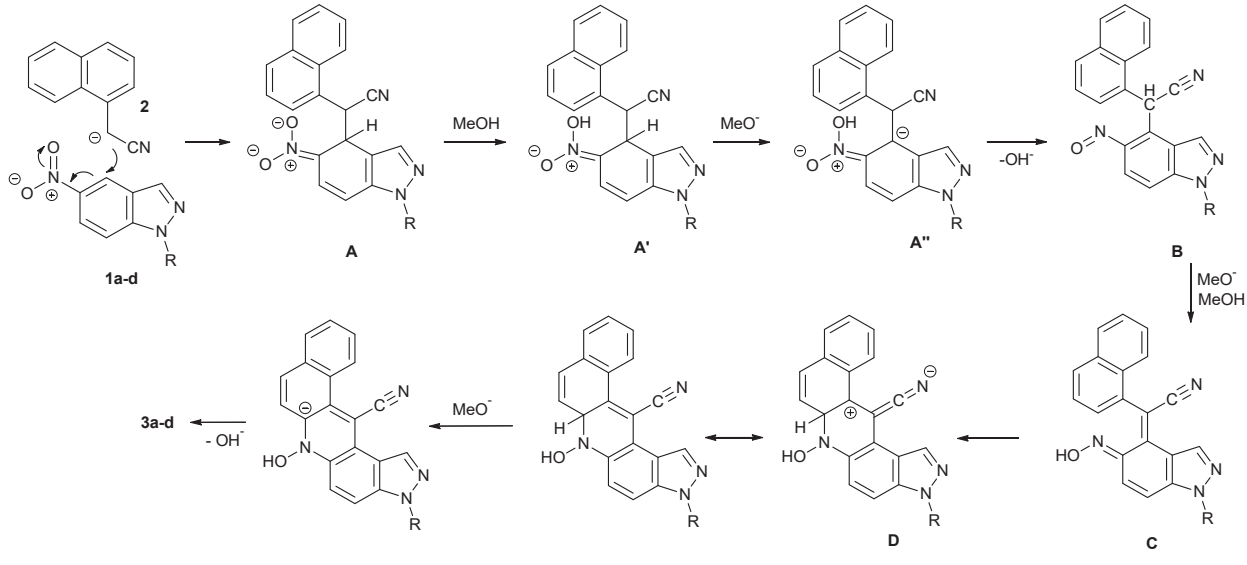
Recently, we performed DFT calculations using the B3LYP hybrid functional and the 6-311++G(d,p) basis set to gain the most reasonable mechanism for the formation of 3*H*-imidazo[4,5-*a*]acridines.[28] The results obtained revealed that intramolecular electrophilic aromatic substitution is the most likely mechanism for cyclisation of the intermediate in these reactions. According to these results,[28] the most plausible reaction mechanism to explain the formation of fluorophores **3a–d** involves the attack of the anion derived from **2** on compounds **1a–d** and the generation of intermediates **A** and **A′** followed by dehydration of **A″** and the formation of intermediate **B** in basic media, as depicted in Scheme 2. Finally, intramolecular electrophilic aromatic substitution of **C** to **D** led to the formation of the compounds **3a–d**.

The structures of products **3a–d** were confirmed by NMR techniques, IR spectroscopy, and mass spectral and microanalytical data. The spectral details of the compounds are given in the 'Experimental' section. For example, in the ^{1}H NMR spectrum of compound **3b** in $CDCl_3$, there is a multiplet signal at δ = 7.80–8.01, two doublet signals at δ = 8.11 and 9.96, and a singlet signal at δ = 9.40 attributed to the nine protons from the aromatic rings. Also, there are 21 signals in the ^{13}C NMR spectrum of compound **3b**. In addition, in the FTIR spectrum of **3b**, the CN absorption peak was observed at 2221 cm^{-1}. All this evidence, in conjunction with a molecular ion peak at *m*/*z* 322 (M^+), supports a pentacyclic structure for compound **3b**.

Photophysical properties of compounds **3a–d**

Based on our previous experiences that acridine moieties exhibit very interesting fluorescence properties,[18,19,21] compounds **3a–d** were characterised by UV-Vis and fluorescence spectroscopy in the wavelength range of 200–1000 nm. Figure 1 shows the visible absorption and emission spectra of compounds **3a–d** and numerical spectral data are presented in Table 1. Extinction coefficient (ε) values were calculated as the slope of the plot of absorbance *versus* concentration. As can be seen from Table 1, the extinction coefficient (ε) of compound **3a** (R = Me) and fluorescence intensity of compound **3d** (R = Bu) were the highest values. The fluorescence quantum yields of compounds **3a–d** were determined *via* comparison methods, using fluorescein as a standard sample in 0.1 M NaOH and MeOH solution.[29] The value of the fluorescein emission quantum yield is 0.79, and the



Scheme 2 The most plausible reaction mechanism for the formation of compounds **3a–d**.

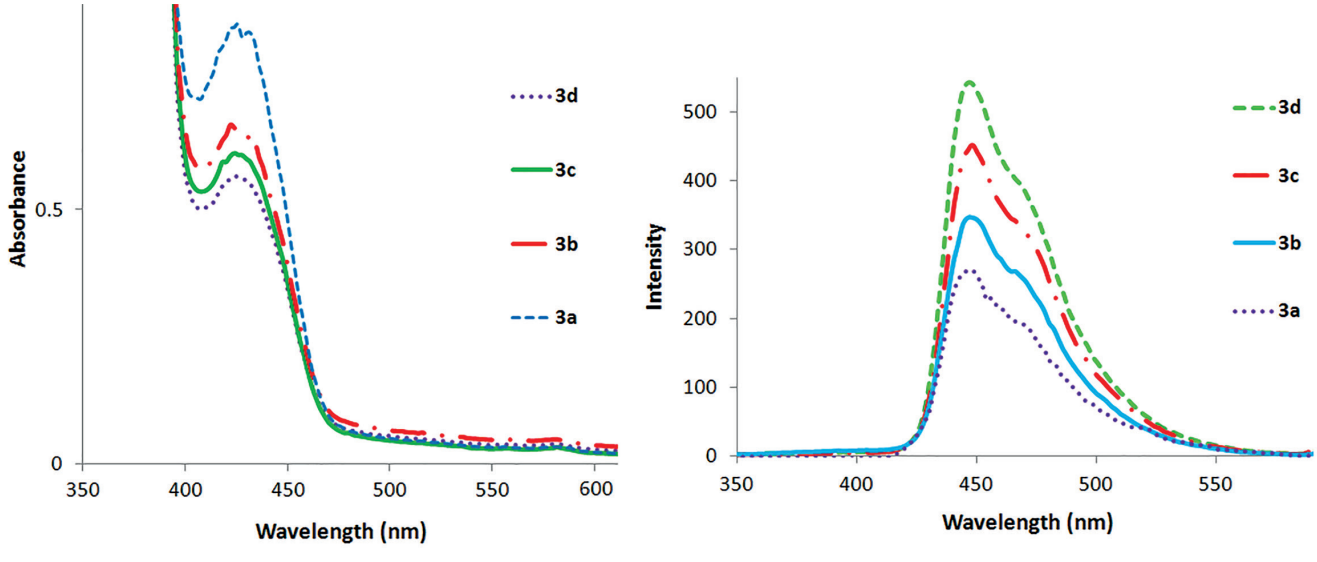


Fig. 1 UV-Vis absorption spectra (left) (2 × 10^{-5} mol L^{-2}) and fluorescence emission spectra (right) (1 × 10^{-6} mol L^{-2}) of compounds **3a–d** in chloroform.

Table 1 Spectroscopic data for compounds **3a–d** at 298 K

Dye	3a	3b	3c	3d
Wavelength of maximum absorbance, λ_{abs} (nm)	420	420	420	420
Extinction coefficient, ε (×10^{-3} (mol L^{-1})$^{-1}$ cm^{-1})	47.5	32.5	30.0	27.0
Wavelength of fluorescence excitation, λ_{ex} (nm)	420	420	420	420
Wavelength of fluorescence emission, λ_{flu} (nm)	445	445	450	450
Fluorescence quantum yield, Φ_F^e	0.67	0.75	0.80	0.81

Table 2 Spectroscopic data for compounds **3a–d** at 298 K in different solvents

Solvent	λ_{abs} (nm)	λ_{flu} (nm)
MeOH	430	460
Chloroform	420	450
THF	423	445
DCM	420	450
CCl_4	417	435
n-Hexane	410	430

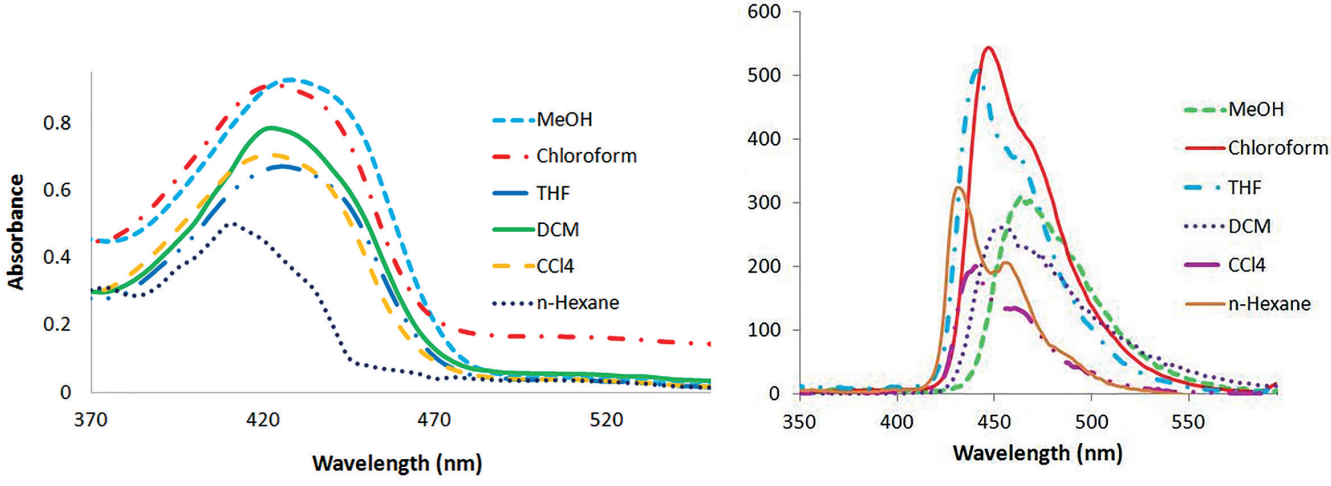


Fig. 2 UV-Vis absorption spectra (left) (2 × 10^{-5} mol L^{-2}) and fluorescence emission spectra (right) (1 × 10^{-6} mol L^{-2}) of compound **3d** in different solvents with excitation at λ_{abs}.

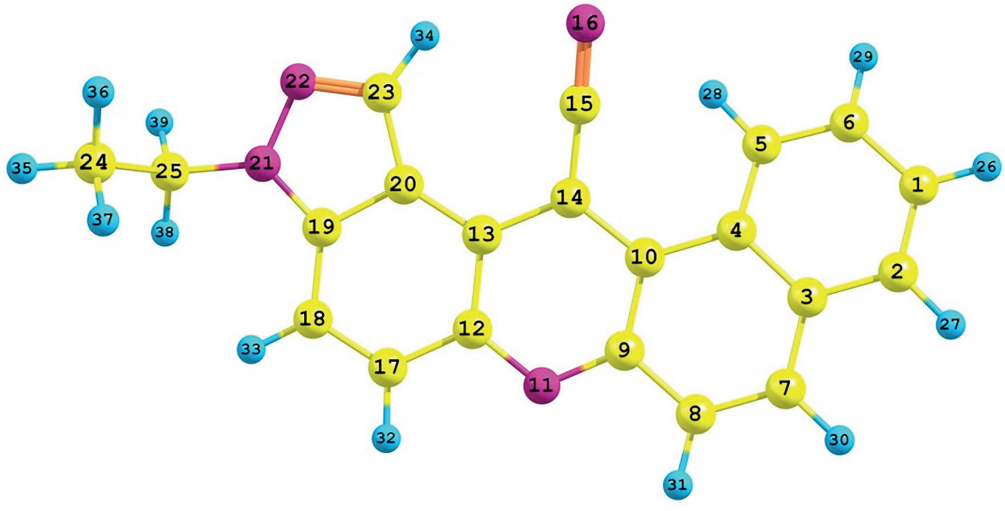


Fig. 3 Optimised geometry of compound **3b**.

obtained emission quantum yields of compounds **3a–d** were around 0.67–0.81.

Fluorescent chromophores are usually known to have planar and rigid π-conjugated systems, and many fluorescent chromophores are based on rigid ring systems. The fluorescence emission of the new compounds **3a–d** can be explained in terms of the extended conjugation pathway. Some charge-separated mesomeric structures of **3a–d** are shown in Scheme S1 in the ESI.

The solvatochromic properties of compound **3d** were also studied in different solvents, such as *n*-hexane, dichloromethane (DCM), tetrahydrofuran (THF), CCl_4 and MeOH (Fig. 2). As can be seen in this figure, the absorption and emission spectra of compound **3d** undergo a red-shift with increasing solvent polarity. Increasing the solvent polarity stabilises the excited state of the molecule relative to the ground state, causing the experimentally observed red-shift of the absorption and emission spectra (Table 2). For example, in the absorption and emission spectra of compound **3d**, the wavelength of maximum absorbance (λ_{abs}) and wavelength of fluorescence emission (λ_{flu}) shift from 410 to 430 nm and 430 to 460 nm, respectively, as the solvent changes from *n*-hexane to MeOH.

DFT calculations

As depicted in Fig. 3, the pentacyclic ring and cyano group are essentially rigid and planar in the optimised geometry of compound **3b**. Moreover, the C=C bond lengths (1.35–1.45 Å) of the aromatic rings are in the expected range (Table S1 in the ESI).[30]

The energy difference between the HOMO and LUMO frontier orbitals is one of the important characteristics of

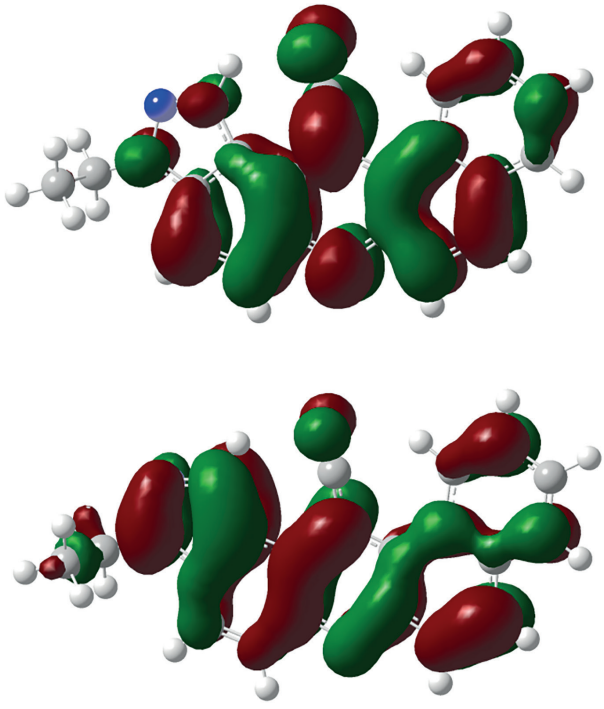


Fig. 4 The HOMO and LUMO maps of compound **3b**.

molecules, and has a determining role in, for example, electronic properties, electronic spectra and photochemical reactions. As can be seen in Fig. 4, the frontier molecular orbitals of **3b** are mainly composed of *p* atomic orbitals in the HOMO and LUMO maps of dye **3b**, so the electronic transitions in the spectra above correspond to π–π* electronic transitions. The energy separation between the HOMO and LUMO is 3.09 eV (401.2 nm). As can be seen, the three-dimensional maps are mostly localised on the centre of the ring system.

The TD-DFT electronic spectra calculations for **3b** demonstrate that there is an electronic transition band: a relatively sharp peak at 433.6 nm (oscillator strength: 0.4208) that can be linked to ð–ð* transitions (donor endocyclic nitrogen (N3) to the acceptor CN group). This electronic transition band is comparable to the experimental value of 420 nm. The calculated electronic absorption spectral data of compound **3b** are provided in Table S2 the ESI.

Experimental

Absorption and fluorescence spectra were recorded on a Varian 50-bio UV-Vis spectrophotometer and a Varian Cary Eclipse spectrofluorophotometer. UV-Vis and fluorescence scans were recorded from 200 to 1000 nm. All measurements were carried out at r.t. The IR (as KBr discs) spectra were obtained on a Tensor 27 spectrophotometer and only noteworthy absorptions are listed. The ^{1}H NMR and ^{13}C NMR spectra (300 and 75 MHz, respectively) were recorded on a Bruker Avance DRX-300 FT spectrometer in $CDCl_3$. Chemical shifts are reported in ppm downfield from TMS as an internal standard. Electron impact ionisation mass spectra were recorded on a Varian Mat CH-7 instrument at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyser. Melting points were measured on an Electrothermal type 9100 melting point apparatus.

Compounds **1a**–**d**[25] and **2**[26,27] were synthesised according to the literature procedures. Other reagents and solvents were purchased from Merck. All solvents were dried according to standard procedures.

Computational methods

All of the calculations were performed using the DFT method with the B3LYP functional[31] as implemented in the Gaussian 03 program package[32] and the 6-311++G(d,p) basis set.

First, the geometry of the compound **3b** was fully optimised in the EtOAc solution. Here, one self-consistent reaction field method, the sophisticated polarised continuum model (PCM),[33] was used for investigation of the solvent effects. The PCM calculations were performed in EtOAc solution and the zero-point corrections were considered to obtain energies. Based on the optimised geometries and using TD-DFT[34–36] methods, the electronic spectrum of compound **3b** was predicted.

Synthesis of compounds **3a**–**d** *from* **1a**–**d** *and* **2***; general procedure*

1-alkyl-5-nitro-1*H*-indazoles **1a**–**d** (10 mmol) and 1-naphthylacetonitrile (**2**) (13 mmol) were added with stirring to a solution of KOH (20 g, 357 mmol) in methanol (40 mL). The mixture was stirred at r.t. for 48 h. After concentration at reduced pressure, the precipitate was collected by filtration, washed with water, followed by cold EtOH and acetone, and then air dried to give the crude products **3a**–**d**. Further purification was achieved by crystallisation from a suitable solvent such as EtOH or acetone.

*3-Methyl-3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridine-13-carbonitrile* (**3a**): Shiny yellow needles (acetone); m.p. 320–322 °C; yield 73%; IR (KBr) (ν_{max} cm^{-1}): 2223 (CN); ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.17 (3H, s, NCH_3), 7.78–7.99 (7H, m, ArH), 8.09 (1H, d, *J* = 9.3 Hz, ArH), 9.42 (1H, s, ArH), 9.98 (1H, d, *J* = 7.5 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 36.2, 107.3, 115.3, 116.4, 120.5, 123.2, 124.1, 126.0, 127.5, 128.1, 128.3, 129.0, 129.3, 129.3, 132.5, 133.4, 135.5, 137.4, 145.7, 147.6; MS (*m*/*z*) 308 [M]$^{+}$ found: C, 78.05; H, 3.94; N, 17.90; calcd for $C_{20}H_{12}N_4$ (308.3): C, 77.91; H, 3.92; N, 18.17%.

*3-Ethyl-3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridine-13-carbonitrile* (**3b**): Shiny yellow needles (EtOH); m.p. 288–289 °C; yield 66%; IR (KBr) (ν_{max} cm^{-1}): 2221 (CN); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.68 (3H, t, *J* = 7.2 Hz, $NCH_2C\underline{H}_3$), 4.62 (2H, q, *J* = 7.2 Hz, $NC\underline{H}_2CH_3$), 7.80–8.01 (7H, m, ArH), 8.09 (1H, d, *J* = 9.3 Hz, ArH), 9.42 (1H, s, ArH), 9.98 (1H, d, *J* = 7.5 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.5, 44.5, 107.7, 116.0, 116.1, 120.8, 123.3, 124.1, 126.0, 127.7, 128.0, 128.4, 129.1, 129.2, 129.3, 132.3, 133.0, 135.3, 137.5, 145.7, 146.8. MS (*m*/*z*) 322 [M]$^{+}$ found: C, 78.29; H, 4.40; N, 17.21; calcd for $C_{21}H_{14}N_4$ (322.4): C, 78.24; H, 4.38; N, 17.38%.

*3-Propyl-3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridine-13-carbonitrile* (**3c**): Shiny yellow needles (MeOH); m.p. 268–270 °C; yield 67%; IR (KBr) (ν_{max} cm^{-1}): 2225 (CN); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05 (3H, t, *J* = 7.2 Hz, $NCH_2CH_2C\underline{H}_3$), 2.07–2.14 (2H, m, $NCH_2C\underline{H}_2CH_3$), 4.54 (2H, t, *J* = 7.2 Hz, $NC\underline{H}_2CH_2CH_3$), 7.81–8.04 (7H, m, ArH), 8.13 (1H, d, *J* = 9.3 Hz, ArH), 9.44 (1H, s, ArH), 9.98 (1H, d, *J* = 7.5 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 12.1, 24.9, 49.3, 107.5, 116.3, 116.1, 120.7, 123.1, 124.5, 125.8, 127.4, 127.7, 128.5, 129.2, 129.2, 129.5, 132.6, 133.1, 135.3, 137.6, 145.6, 146.7. MS (*m*/*z*) 336 [M]$^{+}$ found: C, 78.79; H, 4.83; N, 16.49; calcd for $C_{22}H_{16}N_4$ (336.4): C, 78.55; H, 4.79; N, 16.66%.

*3-Butyl-3*H*-benzo[*a*]pyrazolo[3,4-*j*]acridine-13-carbonitrile* (**3d**): Shiny yellow needles (MeOH); m.p. 260–262 °C; yield 70%; IR (KBr) (ν_{max} cm^{-1}): 2224 (CN); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.01 (6H, d, *J* = 6.9 Hz, $NC\underline{H}_2CH_2CH_2CH_3$), 1.41–1.45 (1H, m, $NCH_2C\underline{H}_2CH_2CH_3$), 2.02–2.05 (1H, m, $NCH_2CH_2C\underline{H}_2CH_3$), 4.57 (2H, d, *J* = 7.2 Hz, $NCH_2CH_2CH_2C\underline{H}_3$), 7.81–8.03 (7H, m, ArH), 8.12 (1H, d, *J* = 9.3 Hz, ArH), 9.43 (1H, s, ArH), 9.98 (1H, d, *J* = 7.5 Hz, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.3, 22.3, 32.8, 46.8, 107.2, 116.1, 116.2, 120.5, 123.0, 124.3, 125.7, 127.4, 127.5, 128.1, 129.3, 129.0, 129.6, 132.1, 133.3, 135.6, 137.8, 145.4, 146.9. MS (*m*/*z*) 350 [M]$^{+}$ found: C, 78.98; H, 5.20; N, 15.79; calcd for $C_{23}H_{18}N_4$ (350.4): C, 78.83; H, 5.18; N, 15.99%.

Acknowledgement

We express our sincere gratitude to the Research Office, Mashhad Branch, Islamic Azad University, Mashhad-Iran, for financial support of this work.

Electronic Supplementary Information

Charge-separated mesomeric structures for compounds **3a**–**d**, and the structural parameters for compound **3b** and its calculated electronic absorption spectra are available through stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data

Received 19 January 2017; accepted 25 May 2017
Paper 1704550
https://doi.org/10.3184/174751917X14967701766914
Published online: 16 June 2017

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