Research article

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Received: 26 May 2011, Revised: 30 July 2011, Accepted: 1 September 2011 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/bio.1364

Effects of homogeneous media, binary mixtures and microheterogeneous media on the fluorescence and fluorescence probe properties of some benzo[b][1,8] naphthyridiens with HSA and BSA

Deepak P. Shelar, Ramhari V. Rote, Sandeep R. Patil and Madhukar N. Jachak*

ABSTRACT: A rapid and efficient method for the synthesis of various poly-substituted benzo[b][1,8]naphthyridines in high yield has been developed via the Friedländer condensation of 2-aminoquinoline-3-carbaldehyde 1 with various alicyclic ketones in a base catalyst (aq. potassium hydroxide). A series of benzo[b][1,8]naphthyridines branched with various side-chains and substituents were prepared with the aim of being investigated as a fluorescent agents. Electronic absorption and fluorescence properties of some representative benzonaphthyridines (3d, 5b and 21f) in homogeneous organic solvents, dioxane–water binary mixtures and in the microheterogeneous media (sodium dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB) and Triton-X100 micelles) have been examined. A linear correlation between solvent polarity and fluorescence properties was observed. Further, the interaction of these benzonaphthyridines (3d, 5b and 21f) with human serum albumin (HSA) and bovine serum albumin (BSA) in phosphate buffer have been examined by UV-vis absorption and fluorescence spectroscopy. The fluorescence intensity of 3d, 5b and 21f increases with the increasing HSA and BSA concentration. These benzonaphthyridines also quench the 345 nm fluorescence of BSA in phosphate buffer (λ_{ex} 280 nm). These compounds have potential for use as neutral and hydrophobic fluorescence probes for examining the microenvironments in proteins, polymers, micelles, etc. Copyright © 2011 John Wiley & Sons, Ltd.

The following supporting information may be found in the online version of this article.

Keywords: benzo[b][1,8]naphthyridines; microheterogeneous media; human serum albumin (HSA); bovine serum albumin (BSA); quenching

Introduction

Every protein has a unique function in metabolism. Proteins are important for the structural and functional organization of cells and various cell organelles. Albumins are major proteins in blood plasma and functions as a transport protein for many compounds, such as fatty acids, hormones, bilirubin and drugs. Many drugs and other bioactive small molecules can bind reversibly to albumins, whereby the latter serves as a carrier. Furthermore, albumins are the principal biomacromolecules that are involved in the maintenance of colloid-blood pressure and are implicated in the facilitated transfer of many ligands across organ–circulatory interfaces such as in the liver, intestine, kidney and brain (1).

Serum albumin is the most abundant transport protein of all the proteins in blood plasma, accounting for approximately 60% of the total serum protein content, it has many physiological and pharmacological functions and have been extensively studied (2–6). Serum albumin is involved in binding and carriage of an array of biologically important compounds (exogenous ligands), such as fatty acids, bilirubin, bile salts and lecithin, and this binding mechanism was examined using absorption, fluorescence and circular dichroism spectroscopy. Based on such studies, information on the binding process of many exogenous ligands has been reported at the molecular level (7–11). However, major interest in albumins is a result of their strong drug-binding capacity; it served as model protein for a large variety of biochemical and biophysical studies and a large volume of literature is available. Numerous experiments are performed for characterizing the binding capacity and sites of albumins (12,13). Structural aspects and properties of this transport protein are well explored. The primary structure is made up of 580 amino acid residues and secondary structure is constituted of 67% of helix of six turns and 17 disulphide bridges. The tertiary structure is composed of three domains I, II and III; each domain is constituted by two subdomains, named as IA, IB, IIA, IIB, IIIA and IIIB (2,4,5). The principal function of

* Correspondence to: M. N. Jachak, Organic Chemistry Research Centre, Department of Chemistry, KTHM College, Gangapur Road, Nashik 422002, MS, India. E-mail: mnjachak@hotmail.com

Organic Chemistry Research Centre, Department of Chemistry, KTHM College, Gangapur Road, Nashik, 422002, MS, India

serum albumin is to transport a wide variety of fatty acids and metabolites (4) via the main binding regions located in subdomains IIA (site 1) and IIIA (site 2) (4,14). Bovine and human serum albumins (BSA and HSA) display approximately 80% sequence homology and a repeating pattern of disulphides, which are strictly conserved.

Human and bovine serum albumins (HSA and BSA) are probably the most-studied serum albumin proteins. The protein HSA has the ability to bind several ligands, including small aromatic and heterocyclic carboxylic acids, such as non-steroidal antiflammatory drugs (7). Bovine serum albumin (BSA) has been one of the most extensively studied proteins of this group. Various methods are reported to determine bovine serum albumin, such as resonance light scattering techniques (15–17), chemiluminescence (18), the Bradford assay method (19), circular dichroism, fluorescence spectroscopy (20), Fourier transform infrared spectroscopy (21), ultraviolet absorption spectroscopy, differential scanning calorimetry (22), etc. Because proteins bear tryptophan, tyrosine and phenylalanine residues, which have intrinsic fluorescence, it is seen that fluorescence spectroscopy could serve as an effective and cheaper method for such studies (23,24).

Fluorescence probe techniques represent the most important area of fluorescence spectroscopy to study biologically important systems and are rapidly gaining popularity in the study of biological microheterogeneous systems, due to their non-invasive nature and the large amount of information gleaned thereby (25,26). Probes that show massive spectral changes on being bound to such systems are being designed and developed, and their spectral responses are then exploited to gain information about the structure and dynamics of the system under study. Probes such as ANS (27–31) show polarity-dependent spectral characteristics and are being widely used as fluorescent labels for studying proteins, vesicles, micelles, mixed micelles and similar biological or biomimetic micro-heterogeneous systems. Fluorescence probe techniques are one of the most powerful methodologies, yielding structural and dynamical information concerning the fluorophore environment (8,25,32–37). Fluorescence spectroscopy and probe molecules that exhibit solvatochromic fluorescence have been particularly useful for this purpose (8,31,38–41).

We have recently reported the synthesis of fluorescent benzo[b][1,8]naphthyridine-3-carbonitrile using 2-aminoquinoline-3-carbaldehyde (*o*-aminoaldehyde) **1** (42) and also reported that certain banzonaphthyridines are capable of solvatochromic fluorescence emission and these are used as a fluorescence probe for studying the microenvironment of organized assemblies, such as bovine serum albumin (BSA) (43). Herein, we report a useful and simple approach toward the synthesis of various benzo[b][1,8]naphthyridines via Friedländer condensation of **1** with amides, benzylcyanide and various cyclic ketones in different reactions and conditions are discussed. Some representative benzonaphthyridines (**3d**, **5b** and **21f**) are investigated for their absorption and fluorescence properties in homogeneous media of organic solvents, 1,4-dioxane–water binary mixtures and in the microheterogeneous media of sodium dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB) and Triton-X100 micelles. Moreover, to examine the usefulness of these benzonaphthyridines as a fluorescence probes for proteins, it has been covalently attached to human serum albumin (HSA) and bovine serum albumin (BSA).

Experimental

General

Human serum albumin (HSA) and bovine serum albumin (BSA) were purchased from Hi-Media Laboratories Pvt. Ltd (Mumbai, India). Surfactants [cetyl trimethyl ammonium bromide (CTAB), sodium dodecyl sulphate (SDS) and Triton-X100 for micelle preparation] and quinine sulphate [for determination of Φ_f] were purchsaed from Hi-Media Laboratories and Research-Lab Fine Chem Industries (Mumbai, India), respectively. All other chemicals, reagents and solvents [such as acetonitrile, dimethylformamide (DMF), dioxane, ethanol, ethyl acetate, *n*-hexane, pet-ether methanol and tetrahydrofuran (THF)], used in spectroscopic and other studies, were obtained from LOBA Chemie. Pvt. Ltd (Mumbai, India), Spectrochem (Mumbai, India) and E. Merck (India). Deionized, double-distilled water (Millipore) was used for preparing the micelle solutions. All AR-grade organic solvents were dried and freshly distilled prior to use. The UV-grade solvents were used for spectral studies. Melting points were determined on a Gallenkamp melting-point apparatus, Mod. MFB595, in open capillary tubes and were uncorrected. Fourier transform infrared (FTIR) spectra in KBr disk were measured on a Shimadzu FTIR-408 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian XL-300 MHz spectrometer, using tetramethylsilane (TMS) as the internal standard, and the solvents were deuterio-chloroform ($CDCl_3$) and deuterio-dimethylsulphoxide (DMSO-d_6). Chemical shifts were reported in ppm from an internal tetramethylsilane standard and were given in δ-units. Mass spectra were recorded on a Shimadzu GC–MS QP 2010A mass spectrometer with an ionization potential of 70 eV. Elemental analyses were performed on a Hosli CH-analyzer and within $\pm$ 0.3 of the theoretical percentage. For the microwave irradiation, a conventional (unmodified) domestic BPL (BMO 700T) microwave oven was used. The absorption spectra were measured using a Shimadzu UV-1601 UV-VIS spectrophotometer. The fluorescence spectra were recorded on a RF-5301 PC spectrofluorophotometer by exciting the samples at their absorption maximum ($\lambda_{abs.\ max.}$). Compounds for UV and fluorescence measurements were dissolved in DMF, UV and fluorescence scan were recorded in the range 200–600 nm. The Φ_f relative to quinine sulphate in 1.0×10^{-3} mol/L H_2SO_4 ($\Phi_f = 0.57$) was measured at room temperature by a standard literature procedure (65–67). Both samples and standard were excited at the same excitation wavelength and the optical density (OD) of the standard and the sample were adjusted to be nearly equal. For all electronic spectroscopic studies (such as absorption, fluorescence excitation and emission) 1.0×10^{-3} mol/L solutions of the compounds were used. All reactions were monitored by thin-layer chromatography, carried out on 0.2 mm silica gel 60 F_{254} (Merck) plates, using UV light (250 and 400 nm) and fluorescence light (400 and 600 nm) for detection. For fluorescence quenching studies, the required amount of HSA and BSA solution (in phosphate buffer) and the required amount of benzo[b][1,8] naphthyridines solution in dimethyl sulphoxide (DMSO) (1.0×10^{-4} mol/L) were taken in a 5 mL volumetric flask. Since the benzo[b][1,8]naphthyridines were sparingly soluble in water, their stock solutions were prepared in DMSO, which was used as a solvent for interaction studies of serum albumins (37).

Synthesis

Synthesis of pyrimido[4,5-b]quinoline (3a–d)

Method1. A solution of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and formamide (**2a**; 0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 3 h. Completion of the reaction was monitored by thin-layer chromatography (TLC). The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with pet-ether, dried and recrystallized from ethanol.

Method 2. The compound **1a-b** (0.001 mmol) and the corresponding formamide (**2a**; 0.001 mol) was heated at 175–180 °C for 1*H* (TLC check). On cooling, the molten mass was stirred in ethanol (2 mL) for 15 min. The solid obtained on cooling was collected by filtration, washed with cold ethanol and crystallized from ethanol.

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Method 3. A solution of **1a-b** (0.001 mol) and the corresponding formamide (**2a**; 0.001 mol) was heated in diphenyl ether at about 190–210 °C for 2.5 h (monitored by TLC check). On cooling, the molten mass was stirred in ethanol (2 mL) for 15 min. The solid obtained on cooling was collected by filtration, washed with cold ethanol and recrystallized from ethanol.

Method 4. A solution of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and formamide (**2a**; 0.001 mol) in aqueous HCl solution (10 mL, 2%) was refluxed for 6 h. Completion of the reaction was monitored by TLC. The mixture was then cooled at room temperature. The separated solid product was collected by suction filtration, washed with *n*-hexane, dried and recrystallized from ethanol.

Method 5. A solution of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and corresponding amide (**2a**; 0.001 mol) in the catalytic amount of $SnCl_2 2H_2O$ reaction mass was refluxed for 15–140 min. The reaction was monitored with the help of TLC. After completion of the reaction (TLC check), the reaction mass was poured over crushed ice. The reaction mass was stirred and neutralized with aq. $NaHCO_3$ solution, extracted with ethyl acetate (25 mL) and concentrated over a rotary evaporator. The solid was collected by suction filtration and recrystallized from cold ethanol.

Method 6. A mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and formamide (**2a**; 0.001 mol) and conc. HCl (2–3 drops, AR grade) was taken in a borosil round-bottomed flask attached to a condenser and irradiated in an unmodified domestic microwave oven at 70 W for 1.5–12 min (monitored by TLC). The resultant mixture was cooled to room temperature; cold water was added, the mixture was neutralized to pH 7 with aq. $NaHCO_3$ solution (10% v/v). The separated solid was filtered by suction, washed with cold water, dried and recrystallized using ethanol.

Pyrimido[4,5-b]quinoline (***3a***). Yield: 0.155 g (85%), recrystallized from ethanol to afford a faint yellow prisms; m.p. 164–167 °C. IR (KBr): 3012 m, 2968 m, 1618 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 7.34 (dd, 1*H*, *J* = 8.2 and 8.7 Hz, Ar-H), 7.79 (dd, 1*H*, *J* = 8.7 and 8.1 Hz, Ar-H), 8.09 (d, 1*H*, *J* = 8.2 Hz, Ar-H), 8.36 (d, 1*H*, *J* = 8.1 Hz, Ar-H), 8.69 (s, 1*H*, Ar-H), 8.78 (s, 1*H*, Ar-H), 9.19 (s, 1*H*, Ar-H). Anal. calcd. for $C_{11H7}N_3$ (181.19): C, 72.92; H, 3.86; N, 23.20%; found, C, 72.94; H, 3.83; N, 23.19%.

7-Methoxypyrimido[4,5-b]quinoline (***3b***). Yield: 0.172 g (81%), recrystallized from ethanol to afford faint yellow needles; m.p. 175–178 °C. IR (KBr): 3023 m, 2960 m, 1620 s, 1031 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 4.02 (s, 3H, OCH_3), 7.43 (d, 1*H*, *J* = 9.0 Hz, Ar-H), 7.51 (s, 1*H*, Ar-H), 8.24 (d, 1*H*, *J* = 9.0 Hz, Ar-H), 8.68 (s, 1*H*, Ar-H), 9.21 (s, 1*H*, Ar-H), 9.44 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 59.2, 112.7, 125.4, 127.5, 130.6, 131.7, 136.7, 143.7, 148.7, 157.1, 158.9, 160.1. Anal. calcd. for $C_{12}H_9N_3O$ (211.22): C, 68.24; H, 4.26; N, 19.90%; found, C, 68.25; H, 4.23; N, 19.93%.

2-Phenylpyrimido[4,5-b]quinoline (***3c***). Yield: 0.217 g (84%), recrystallized from ethanol to afford a yellow solid; m.p. 194–196 °C. IR (KBr): 3010 m, 2958 m, 1628 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 7.12–7.40 (m, 5H, Ar-H), 7.48 (dd, 1*H*, *J* = 8.3 and 8.5 Hz, Ar-H), 7.84 (dd, 1*H*, *J* = 8.5 and 7.9 Hz, Ar-H), 8.12 (d, 1*H*, *J* = 8.3 Hz, Ar-H), 8.29 (d, 1*H*, *J* = 7.9 Hz, Ar-H), 8.58 (s, 1*H*, Ar-H), 9.06 (s, 1*H*, Ar-H). Anal. calcd. for $C_{17}H_{11}N_3$ (257.29): C, 79.37; H, 4.28; N, 16.34%; found, C, 79.34; H, 4.32; N, 16.35%.

7-Methoxy-2-phenylpyrimido[4,5-b]quinoline (***3d***). Yield: 0.236 g (82%), recrystallized from ethanol to afford a yellow solid; m.p. 201–204 °C. IR (KBr): 3007 m, 2960 m, 1616 m, 1099 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 3.98 (s, 3H, OCH_3), 7.12–7.29 (m, 5H, Ar-H), 7.39 (d, 1*H*, *J* = 9.2 Hz, Ar-H), 7.56 (s, 1*H*, Ar-H), 8.20 (d, 1*H*, *J* = 9.2 Hz, Ar-H), 8.51 (s, 1*H*, Ar-H), 9.04 (s, 1*H*, Ar-H). ^{03}C-NMR (75 MHz $CDCl_3$) δ: 58.9, 111.9, 122.5, 124.7, 127.5 (2 × Cs), 128.2, 129.4 (2 × Cs), 129.9, 131.9, 133.1, 136.7, 144.9, 151.2, 157.7, 158.2, 163.1. Anal. calcd. for $C_{18}H_{13}N_3O$ (287.32): C, 75.26; H, 4.52; N, 14.63%; found, C, 75.25; H, 4.50; N, 14.65%.

Synthesis of 3-Phenylbenzo[b][1,8]naphthyridin-2-amine (***5a-b***). The mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and benzylcyanide (**4**; 0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 6 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with pet-ether, dried and recrystallized from ethanol.

3-Phenylbenzo[b][1,8]naphthyridin-2-amine (***5a***). Yield: 0.222 g (81%), recrystallized from ethanol to afford faint yellow prisms; m.p. 218–221 °C. IR (KBr): 3321 m, 3259 m, 3014 m, 2977 m, 2968 w, 1624 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 6.68 (bs, 2H, NH_2), 7.21–7.33 (m, 5H, Ar-H), 7.49 (dd, 1*H*, *J* = 8.0 and 8.4 Hz, Ar-H), 7.61 (dd, 1*H*, *J* = 8.4 and 8.5 Hz, Ar-H), 8.13 (d, 1*H*, *J* = 8.0 Hz, Ar-H), 8.29 (d, 1*H*, *J* = 8.5 Hz, Ar-H), 8.63 (s, 1*H*, Ar-H), 8.94 (s, 1*H*, Ar-H). Anal. calcd. for $C_{18}H_{13}N_3$ (271.32): C, 79.70; H, 4.79; N, 15.49%; found, C, 79.74; H, 4.80; N, 15.45%.

7-Methoxy-3-phenylbenzo[b][1,8]naphthyridin-2-amine (***5b***). Yield: 0.254 g (84%), recrystallized from ethanol to afford yellow needles; m.p. 227–229 °C. IR (KBr): 3349 m, 3278 m, 3029 m, 2951 m, 2904 m, 1617 m, 1022 w cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 4.09 (s, 3H, OCH_3), 6.89 (bs, 2H, NH_2), 7.08–7.21 (m, 5H, Ar-H), 7.40 (d, 1*H*, *J* = 8.8 Hz, Ar-H), 7.69 (s, 1*H*, Ar-H), 8.14 (d, 1*H*, *J* = 8.8 Hz, Ar-H), 8.51 (s, 1*H*, Ar-H), 8.84 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 59.2, 112.4, 120.3, 122.9, 124.1, 126.9 (2 × Cs), 128.5, 129.6 (2 × Cs), 130.1, 131.7, 137.2, 137.9, 138.4, 143.6, 156.5, 157.8, 158.3. Anal. calcd. for $C_{19}H_{15}N_3O$ (301.34): C, 75.74; H, 4.98; N, 13.95%; found, C, 75.76; H, 4.98; N, 13.99%.

Synthesis of 2,3-dihydro-1H-benzo[b]cyclopenta[g][1,8]naphthyridine (***7a-d***). The mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and cyclopentanone **6a** (0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 5 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with pet-ether, dried and recrystallized from ethanol.

2,3-Dihydro-1H-benzo[b]cyclopenta[g][1,8]naphthyridine (***7a***). Yield: 0.178 g (80%), recrystallized from ethanol to afford a faint yellow solid; m.p. 171–173 °C. IR (KBr): 3012 m, 2968 m, 2912 w, 1610 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.95 (m, 2H, CH_2), 2.49 (t, 2H, *J* = 7.4 Hz, CH_2), 3.09 (t, 2H, *J* = 6.8 Hz, CH_2), 7.34 (dd, 1*H*, *J* = 7.9 and 8.3 Hz, Ar-H), 7.74 (dd, 1*H*, *J* = 8.3 and 8.4 Hz, Ar-H), 8.09 (d, 1*H*, *J* = 7.9 Hz, Ar-H), 8.36 (d, 1*H*, *J* = 8.4 Hz, Ar-H), 8.78 (s, 1*H*, Ar-H), 9.19 (s, 1*H*, Ar-H). Anal. calcd. for $C_{15}H_{12}N_2$ (220.27): C, 81.81; H, 5.45; N, 12.72%; found, C, 81.80; H, 5.48; N, 12.70%.

8-Methoxy-2,3-dihydro-1H-benzo[b]cyclopenta[g][1,8]naphthyridine (***7b***). Yield: 0.211 g (84%), recrystallized from ethanol to afford faint yellow needles; m.p. 184–186 °C. IR (KBr): 3019 m, 2972 m, 2929 m, 1619 s, 1026 s cm^{-1}.^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.88 (m, 2H, CH_2), 2.37 (t, 2H, *J* = 7.1 Hz, CH_2), 3.14 (t, 2H, *J* = 6.9 Hz, CH_2), 4.02 (s, 3H, OCH_3), 7.43 (d, 1*H*, *J* = 7.7 Hz, Ar-H), 7.51 (s, 1*H*, Ar-H), 8.24 (d, 1*H*, *J* = 7.7 Hz, Ar-H), 9.21 (s, 1*H*, Ar-H), 9.44 (s, 1*H*, Ar-H). MS (70 eV) *m/z* (%): 250 [M^+] (83), 206 (37), 125 (19), 77 (22), 44 (85), 32 (47). Anal. calcd. for $C_{16}H_{14}N_2O$ (250.29): C, 76.80; H, 5.60; N, 11.20%; found, C, 76.82; H, 5.62; N, 11.22%.

1,2,3,4-Tetrahydrodibenzo[b,g][1,8]naphthyridine (***7c***). Yield: 0.202 g (86%), recrystallized from ethanol to afford a yellow solid; m.p. 206–209 °C. IR (KBr): 2994 m, 2971 m, 2917 m, 1622 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.67–1.74 (m, 4H, CH_2), 2.60 (t, 2H, *J* = 6.8 Hz, CH_2), 2.94 (t, 2H, *J* = 6.9 Hz, CH_2), 7.27 (dd, 1*H*, *J* = 8.2 and 8.6 Hz, Ar-H), 7.61 (dd, 1*H*, *J* = 8.6 and 8.1 Hz, Ar-H), 7.97 (d, 1*H*, *J* = 8.2 Hz, Ar-H), 8.31 (d, 1*H*, *J* = 8.1 Hz, Ar-H), 8.68 (s, 1*H*, Ar-H), 9.17 (s, 1*H*, Ar-H). Anal. calcd. for $C_{16}H_{14}N_2$ (234.30): C, 82.05; H, 5.98; N, 11.96%; found, C, 82.03; H, 6.00; N, 11.98%.

9-Methoxy-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine (***7d***). Yield: 0.231 g (87%), recrystallized from ethanol to afford yellow needles; m.p. 231–234 °C. IR (KBr): 3006 m, 2968 m, 1618 s, 1011 s cm^{-1}.^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.55–1.68 (m, 4H, CH_2), 2.74 (t, 2H, *J* = 7.1 Hz, CH_2), 3.01 (t, 2H, *J* = 6.5 Hz, CH_2), 4.02 (s, 3H, OCH_3), 7.38 (d, 1*H*, *J* = 8.8 Hz, Ar-H), 7.58 (s, 1*H*, Ar-H), 8.17 (d, 1*H*, *J* = 8.8 Hz, Ar-H), 9.02 (s, 1*H*, Ar-H), 9.31 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 21.9, 22.2, 32.5, 34.7, 58.1, 112.9, 123.4, 125.1, 129.1, 131.2, 133.5, 135.1, 137.8, 144.7, 156.2, 157.9, 159.9. MS (70 eV) *m/z* (%): 264 [M^+] (72), 220 (42), 132 (29), 110 (33), 44 (77), 32 (68). Anal. calcd. for $C_{17}H_{16}N_2O$ (264.32): C, 77.27; H, 6.06; N, 10.60%; found, C, 77.29; H, 6.09; N, 10.58%.

*Synthesis of 4-methyl-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine (**9a-b**).* A mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and 2-methylcyclohexanone (**8**) (0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 6 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with cold methanol, dried and recrystallized from ethanol.

*4-Methyl-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine (**9a**).* Yield: 0.204 g (82%), recrystallized from ethanol to afford yellow prisms; m.p. 211–213 °C. IR (KBr): 3015 m, 2968 m, 2912 m, 1618 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.52 (d, 3H, J = 5.5 Hz, CH_3), 1.89 (m, 2H, CH_2), 2.04 (m, 2H, CH_2), 2.49 (t, 2H, J = 7.4 Hz, CH_2), 3.26 (m, 1*H*, CH), 7.16 (dd, 1*H*, J = 7.9 and 8.3 Hz, Ar-H), 7.77 (dd, 1*H*, J = 8.3 and 8.5 Hz, Ar-H), 8.14 (d, 1*H*, J = 7.9 Hz, Ar-H), 8.27 (d, 1*H*, J = 8.5 Hz, Ar-H), 8.82 (s, 1*H*, Ar-H), 9.07 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 19.1, 20.5, 28.7, 29.9, 31.2, 119.9, 126.1, 127.8, 128.5, 129.5, 131.1, 133.9, 135.3, 138.2, 149.5, 158.1, 161.5. Anal. calcd. for $C_{17}H_{16}N_2$ (248.32): C, 82.25; H, 6.45; N, 11.29%; found, C, 82.27; H, 6.46; N, 11.30%.

*9-Methoxy-4-methyl-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine (**9b**).* Yield: 0.239 g (85%), recrystallized from ethanol to afford a yellow solid; m.p. 171–173 °C. IR (KBr): 3021 m, 2959 m, 2926 m, 1622 s, 1029 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.47 (d, 3H, J = 5.8 Hz, CH_3), 1.84 (m, 2H, CH_2), 2.12 (m, 2H, CH_2), 2.45 (t, 2H, J = 6.7 Hz, CH_2), 3.12 (m, 1*H*, CH), 3.98 (s, 3H, OCH_3), 7.33 (d, 1*H*, J = 8.3 Hz, Ar-H), 7.44 (s, 1*H*, Ar-H), 7.97 (d, 1*H*, J = 8.3 Hz, Ar-H), 9.32 (s, 1*H*, Ar-H), 9.49 (s, 1*H*, Ar-H). MS (70 eV) *m/z* (%): 278 [M^+] (100), 263 (92), 249 (47), 206 (22), 131 (12), 109 (22). Anal. calcd. for $C_{18}H_{18}N_2O$ (278.35): C, 77.69; H, 6.47; N, 10.07%; found, C, 77.70; H, 6.48; N, 10.09%.

*Synthesis of 3,4-dihydrodibenzo[b,g][1,8]naphthyridin-1(2H)-one (**11a-d**).* A mixture of **1a-b** (0.001 mol) and dimedone (**10a-b**; 0.001 mol) was heated at 180–185 °C for 15 min. On cooling, the molten mass was stirred in methanol (10 mL) for 15 min. The separated solid was collected by suction filtration and recrystallized from ethanol.

*3,4-Dihydrodibenzo[b,g][1,8]naphthyridin-1(2H)-one (**11a**).* Yield: 0.199 g (80%), recrystallized from ethanol to afford a faint yellow solid; m.p. 239–241 °C. IR (KBr): 3071 m, 2977 m, 2931 w, 1705 s, 1604 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.88 (m, 2H, CH_2), 2.56 (t, 2H, J = 6.5 Hz, CH_2), 2.94 (t, 2H, J = 6.7 Hz, CH_2), 7.21 (dd, 1*H*, J = 8.2 and 8.6 Hz, Ar-H), 7.55 (dd, 1*H*, J = 8.6 and 8.1 Hz, Ar-H), 8.11 (d, 1*H*, J = 8.2 Hz, Ar-H), 8.47 (d, 1*H*, J = 8.1 Hz, Ar-H), 8.68 (s, 1*H*, Ar-H), 9.19 (s, 1*H*, Ar-H). Anal. calcd. for $C_{16}H_{12}N_2O$ (248.28): C, 77.41; H, 4.83; N, 11.29%; found, C, 77.40; H, 4.80; N, 11.31%.

*9-Methoxy-3,4-dihydrodibenzo[b,g][1,8]naphthyridin-1(2H)-one (**11b**).* Yield: 0.227 g (81%), recrystallized from ethanol to afford yellow needles; m.p. 184–186 °C. IR (KBr): 3009 m, 2970 m, 2918 m, 1698 s, 1615 s, 1019 s cm^{-1}.^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.94 (m, 2H, CH_2), 2.47 (t, 2H, J = 7.2 Hz, CH_2), 2.83 (t, 2H, J = 6.6 Hz, CH_2), 4.05 (s, 3H, OCH_3), 7.37 (d, 1*H*, J = 9.0 Hz, Ar-H), 7.55 (s, 1*H*, Ar-H), 8.20 (d, 1*H*, J = 9.0 Hz, Ar-H), 9.27 (s, 1*H*, Ar-H), 9.38 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 21.8, 31.7, 39.7, 58.3, 113.2, 123.3, 124.9, 128.9, 130.5, 134.5, 136.5, 137.2, 144.7, 156.7, 158.1, 166.5, 197.2. MS (70 eV) *m/z* (%): 279 [M + H]$^+$ (100), 236 (22), 202 (48), 174 (37), 131 (38), 77 (57), 51(49). Anal. calcd. for $C_{17}H_{14}N_2O_2$ (278.30): C, 73.38; H, 5.03; N, 10.07%; found, C, 73.40; H, 5.05; N, 10.08%.

*3,3-Dimethyl-3,4-dihydrodibenzo[b,g][1,8]naphthyridin-1(2H)-one (**11c**).* Yield: 0.229 g (82%), recrystallized from ethanol to afford a faint green solid; m.p. 201–203 °C. IR (KBr): 3033 m, 2960 m, 2914 m, 1696 s, 1626 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.19 (s, 6H, CH_3), 2.44 (s, 2H, CH_2), 2.89 (s, 2H, CH_2), 7.39 (dd, 1*H*, J = 7.9 and 8.5 Hz, Ar-H), 7.70 (dd, 1*H*, J = 8.5 and 8.1 Hz, Ar-H), 8.09 (d, 1*H*, J = 7.9 Hz, Ar-H), 8.30 (d, 1*H*, J = 8.1 Hz, Ar-H), 8.71 (s, 1*H*, Ar-H), 9.10 (s, 1*H*, Ar-H). MS (70 eV) *m/z* (%): 277 [M + H]$^+$ (100), 248 (22), 201 (28), 183 (27), 152 (22), 77 (52), 51 (41), 31 (63). Anal. calcd. for $C_{18}H_{16}N_2O$ (276.33): C, 78.26; H, 5.79; N, 10.14%; found, C, 78.29; H, 5.80; N, 10.15%.

*9-Methoxy-3,3-dimethyl-3,4-dihydrodibenzo[b,g][1,8]naphthyridin-1(2H)-one (**11d**).* Yield: 0.256 g (83%), recrystallized from ethanol to afford green needles; m.p. 229–231 °C. IR (KBr): 3018 m, 2966 m, 2913 m, 1703 s, 1604 s, 1010 s cm^{-1}.^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.25 (s, 6H, CH_3), 2.39 (s, 2H, CH_2), 2.94 (s, 2H, CH_2), 4.09 (s, 3H, OCH_3), 7.36 (d, 1*H*, J = 8.8 Hz, Ar-H), 7.51 (s, 1*H*, Ar-H), 8.24 (d, 1*H*, J = 8.8 Hz, Ar-H), 9.28 (s, 1*H*, Ar-H), 9.46 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 23.4, 25.7, 30.6, 44.8, 50.7, 58.7, 113.4, 123.8, 125.3, 131.2, 132.7, 134.1, 136.9, 138.3, 144.1, 156.6, 158.7, 166.2, 197.7. MS (70 eV) *m/z* (%): 307 [M + H]$^+$ (100), 278(29), 250(22), 179(18), 55(12), 41(26). Anal. calcd. for $C_{19}H_{18}N_2O_2$ (306.36): C, 74.50; H, 5.88; N, 9.15%; found, C, 74.52; H, 5.90; N, 9.14%.

*Synthesis of 7-(3,4-dichlorophenyl)-7,12-dihydrobenzo[b]naphtho[2,3-g][1,8] naphthyridine (**13a-b**).* The mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and cetraline (**12**; 0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 3 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with pet-ether, dried and recrystallized from ethanol.

*5-(3,4-Dichlorophenyl)-5,6-dihydrobenzo[b]naphtho[2,1-g][1,8]naphthyridine (**13a**).* Yield: 0.358 g (84%), recrystallized from ethanol to afford a yellow solid; m.p. 284–286 °C. IR (KBr): 2991 m, 2959 m, 1620 s cm^{-1}. ^{1}H-NMR (300 MHz DMSO-d_6) δ: 3.84 (d, 2H, J =5.8 Hz, CH_2), 5.09 (t, 1*H*, J = 5.8 Hz, CH_2), 6.89–7.09 (m, 4H, Ar-H), 7.15 (dd, 1*H*, J = 8.6 and 3.2 Hz, Ar-H), 7.24 (d, 1*H*, J = 3.2 Hz, Ar-H), 7.39 (dd, 1*H*, J = 8.0 and 8.5 Hz, Ar-H), 7.49 (d, 1*H*, J = 8.6 Hz, Ar-H), 7.70 (dd, 1*H*, J = 8.5 and 8.1 Hz, Ar-H), 8.14 (d, 1*H*, J = 8.0 Hz, Ar-H), 8.44 (d, 1*H*, J = 8.1 Hz, Ar-H), 8.81 (s, 1*H*, Ar-H), 9.08 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz DMSO-d_6) δ: 38.3, 43.5, 120.2, 121.9, 122.5, 123.1, 124.9, 125.1, 125.8, 127.2, 128.1, 128.7, 129.2, 129.8, 131.4, 132.9, 133.9, 135.6, 136.4, 137.1, 137.9, 141.4, 144.9, 148.3, 159.3, 162.7. Anal. calcd. for $C_{26}H_{16}Cl_2N_2$ (427.33): C, 73.23; H, 3.75; N, 6.57%; found, C, 73.20; H, 3.74; N, 6.55%.

*5-(3,4-Dichlorophenyl)-10-methoxy-5,6-dihydrobenzo[b]naphtho[2,1-g][1,8] naphthyridine (**13b**).* Yield: 0.391 g (85%), recrystallized from ethanol to afford a yellow solid; m.p. 291–293 °C. IR (KBr): 3011 m, 2975 m, 2934 m, 1609 s, 1029 s cm^{-1}. ^{1}H-NMR (300 MHz DMSO-d_6) δ: 3.77 (d, 2H, J = 5.9 Hz, CH_2), 4.04 (s, 3H, OCH_3), 4.88 (t, 1*H*, J = 5.9 Hz, CH_2), 6.94–7.13 (m, 4H, Ar-H), 7.19 (dd, 1*H*, J = 8.5 and 2.9 Hz, Ar-H), 7.29 (d, 1*H*, J = 2.9 Hz, Ar-H), 7.37 (d, 1*H*, J = 8.5 Hz, Ar-H), 7.47 (d, 1*H*, J = 8.9 Hz, Ar-H), 7.62 (s, 1*H*, Ar-H), 8.24 (d, 1*H*, J = 8.9 Hz, Ar-H), 9.21 (s, 1*H*, Ar-H), 9.31 (s, 1*H*, Ar-H). MS (70 eV) *m/z* (%): 456 [M^+] (86), 458 [M + 2] (59), 460 [M + 4] (9), 441 (29), 268 (31), 171 (47), 193 (40), 44 (84), 32 (49). Anal. calcd. for $C_{27}H_{18}Cl_2N_2O$ (457.35): C, 71.05; H, 3.94; N, 6.14%; found, C, 71.07; H, 3.91; N, 6.16%.

*Synthesis of 3-methoxy-5,6-dihydrobenzo[b]naphtho[2,1-g] [1,8]naphthyridine (**15a–b**).* The mixture of 2-aminoquinoline-3-carbaldehyde (**1a–b**; 0.001 mol) and 6-methoxy-tetralone (**14**; 0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 2.5 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with *n*-hexane, dried and recrystallized from ethanol.

*3-Methoxy-5,6-dihydrobenzo[b]naphtho[2,1-g][1,8]naphthyridine (**15a**).* Yield: 0.260 g (83%), recrystallized from ethanol to afford a faint yellow solid; m.p. 275–277 °C. IR (KBr): 2986 m, 2969 m, 2911 m, 1624 s, 1029 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 2.26 (d, 2H, J = 4.8 Hz, CH_2), 2.39 (d, 2H, J = 4.8 Hz, CH_2), 3.94 (s, 3H, OCH_3), 6.89 (dd, 1*H*, J = 7.7 and 3.4 Hz, Ar-H), 7.08 (d, 1*H*, J = 3.4 Hz, Ar-H), 7.16 (d, 1*H*, J = 7.7 Hz, Ar-H), 7.34 (dd, 1*H*, J = 7.9 and 8.7 Hz, Ar-H), 7.73 (dd, 1*H*, J = 8.7 and 8.0 Hz, Ar-H), 8.18 (d, 1*H*, J = 7.9 Hz, Ar-H), 8.31 (d, 1*H*, J = 8.0 Hz, Ar-H), 8.85 (s, 1*H*, Ar-H), 9.14 (s, 1*H*, Ar-H). Anal. calcd. for $C_{21H16}N_2O$ (312.37): C, 80.76; H, 5.12; N, 8.97%; found, C, 80.75; H, 5.12; N, 8.99%.

*3,10-Dimethoxy-5,6-dihydrobenzo[b]naphtho[2,1-g][1,8]naphthyridine (**15b**).* Yield: 0.285 g (83%), recrystallized from ethanol to afford faint green solid; m.p. 297–299 °C. IR (KBr): 3017 m, 2979 m, 2928 m, 1609 s, 1043 s cm^{-1}. ^{1}H-NMR (300 MHz DMSO-d_6) δ: 2.35 (d, 2H, J = 5.3 Hz, CH_2), 2.51 (d, 2H,

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

J = 5.3 Hz, CH_2), 3.90 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.72 (dd, 1*H*, J = 8.2 and 3.1 Hz, Ar-H), 7.06 (d, 1*H*, J = 3.1 Hz, Ar-H), 7.18 (d, 1*H*, J = 8.2 Hz, Ar-H), 7.33 (d, 1*H*, J = 8.5 Hz, Ar-H), 7.42 (s, 1*H*, Ar-H), 8.31 (d, 1*H*, J = 8.5 Hz, Ar-H), 8.68 (s, 1*H*, Ar-H), 9.21 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz DMSO-d_6) δ: 29.1, 34.7, 58.8, 59.7, 114.3, 117.2, 118.1, 124.5, 125.3, 126.1, 128.3, 129.8, 131.7, 133.8, 135.2, 137.1, 138.2, 144.1, 156.2, 158.3, 159.1, 162.5. Anal. calcd. for $C_{22}H_{18}N_2O_2$ (342.39): C, 77.19; H, 5.26; N, 8.18%; found, C, 77.20; H, 5.25; N, 8.21%.

Synthesis of 5′,5′-Dimethyl-3,4-dihydro-1H-spiro[dibenzo[b,g][1,8]naphthyridine-2,2′-[1,3]dioxane] (***17a-b***). The mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and 3,3-dimethyl-1,5-dioxaspiro[5,5]undecan-9-one (**16**; 0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 6 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with pet-ether, dried and recrystallized from ethanol.

5′,5′-Dimethyl-3,4-dihydro-1H-spiro[dibenzo[b,g][1,8]naphthyridine-2,2′-[1,3] dioxane] (***17a***). Yield: 0.271 g (81%), recrystallized from ethanol to afford yellow needles; m.p. 231–233 °C. IR (KBr): 3022 m, 2974 m, 1613 s, 1033 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.21 (s, 6H, $(CH_3)_2$), 2.35 (t, 2H, J = 4.5 Hz, CH_2), 2.67 (s, 2H, CH_2), 3.02 (t, 2H, J = 4.5 Hz, CH_2), 4.31 (s, 4H, CH_2), 7.44 (dd, 1*H*, J = 8.3 and 8.8 Hz, Ar-H), 7.81 (dd, 1*H*, J = 8.8 and 8.0 Hz, Ar-H), 8.12 (d, 1*H*, J = 8.3 Hz, Ar-H), 8.30 (d, 1*H*, J = 8.0 Hz, Ar-H), 8.88 (s, 1*H*, Ar-H), 9.14 (s, 1*H*, Ar-H). Anal. calcd. for $C_{21H22}N_2O_2$ (334.41): C, 75.44; H, 6.58; N, 8.38%; found, C, 75.49; H, 6.59; N, 8.42%.

9-Methoxy-5′,5′-dimethyl-3,4-dihydro-1H-spiro[dibenzo[b,g][1,8]naphthyridine-2,2′-[1,3]dioxane] (***17b***). Yield: 0.298 g (81%), recrystallized from ethanol to afford a yellow solid; m.p. 214–216 °C. IR (KBr): 3017 m, 2970 m, 1615 s, 1041 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.17 (s, 6H, $(CH_3)_2$), 2.42 (t, 2H, J = 5.3 Hz, CH_2), 2.71 (s, 2H, CH_2), 2.94 (t, 2H, J = 5.3 Hz, CH_2), 4.07 (s, 3H, OCH_3), 4.38 (s, 4H, CH_2), 7.29 (d, 1*H*, J = 8.7 Hz, Ar-H), 7.49 (s, 1*H*, Ar-H), 8.26 (d, 1*H*, J = 8.7 Hz, Ar-H), 8.73 (s, 1*H*, Ar-H), 9.17 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 18.4, 19.2, 21.3, 28.4, 30.5, 41.5, 58.9, 71.7 (2 × Cs), 88.8, 114.1, 123.1, 125.7, 129.8, 131.7, 133.4, 136.0, 138.3, 145.3, 158.2, 159.5, 161.5. Anal. calcd. for $C_{22}H_{24}N_2O_3$ (364.44): C, 72.52; H, 6.59; N, 7.69%; found, C, 72.55; H, 6.60; N, 7.67%.

Synthesis of ethyl-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine-4-carboxylate (***19a-b***). The mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and ethyl-2-oxocyclohexane-carboxylate (**18**; 0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 2.5 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with pet-ether, dried and recrystallized from ethanol.

Ethyl-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine-4-carboxylate (***19a***). Yield: 0.244 g (79%), recrystallized from ethanol to afford a faint green solid; m.p. 117–119 °C. IR (KBr): 3009 m, 2961 w, 2914 m, 1732 s, 1623 s, 1024 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.30 (t, 3H, J = 6.2 Hz, CH_3), 1.78 (m, 2H, CH_2), 2.25 (m, 2H, CH_2), 2.61 (t, 2H, J = 5.9 Hz, CH_2), 3.93 (t, 1*H*, J = 5.3 Hz, CH), 4.09 (q, 2H, J = 6.2 Hz, CH_2), 7.51 (dd, 1*H*, J = 8.2 and 8.7 Hz, Ar-H), 7.67 (dd, 1*H*, J = 8.7 and 8.1 Hz, Ar-H), 8.15 (d, 1*H*, J = 8.2 Hz, Ar-H), 8.30 (d, 1*H*, J = 8.1 Hz, Ar-H), 8.77 (s, 1*H*, Ar-H), 9.23 (s, 1*H*, Ar-H). Anal. calcd. for $C_{19}H_{18}N_2O_2$ (306.36): C, 74.50; H, 5.88; N, 9.15%; found, C, 74.52; H, 5.94; N, 9.22%.

Ethyl-9-methoxy-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine-4-carboxylate (***19b***). Yield: 0.274 g (81%), recrystallized from ethanol to afford a green solid; m.p. 81–83 °C. IR (KBr): 3026 m, 2976 m, 2942 m, 1738 s, 1620 s, 1041 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.31 (t, 3H, J = 5.9 Hz, CH_3), 1.66 (m, 2H, CH_2), 2.21 (m, 2H, CH_2), 2.59 (t, 2H, J = 6.5 Hz, CH_2), 3.79 (t, 1*H*, J = 6.0 Hz, CH), 4.02 (s, 3H, OCH_3), 4.11 (q, 2H, J = 5.9 Hz, CH_2), 7.48 (d, 1*H*, J = 8.2 Hz, Ar-H), 7.59 (s, 1*H*, Ar-H), 8.28 (d, 1*H*, J = 8.2 Hz, Ar-H), 8.92 (s, 1*H*, Ar-H), 9.33 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 12.2, 22.7, 28.3, 35.3, 49.3, 58.2, 62.3, 116.3, 123.3, 124.3, 129.3, 131.3, 133.7, 135.2, 138.3, 145.3, 158.3, 159.4, 163.7, 177.3. Anal. calcd. for $C_{20}H_{20}N_2O_3$ (336.38): C, 71.42; H, 5.95; N, 8.33%; found, C, 71.40; H, 5.95; N, 8.30%.

Synthesis of 2-methyl-1,2,3,4-tetrahydroquinolino[2,3-b][1,6]naphthyridine (***21a-f***). The mixture of 2-aminoquinoline-3-carbaldehyde (**1a-b**; 0.001 mol) and piperidone (**20a-c**; 0.001 mol) in ethanolic potassium hydroxide solution (10 mL, 2%) was refluxed for 3 h. Completion of the reaction was monitored by TLC. The mixture was then cooled to room temperature; the separated solid product was collected by suction filtration, washed with pet-ether, dried and recrystallized from ethanol.

2-Methyl-1,2,3,4-tetrahydroquinolino[2,3-b][1,6]naphthyridine (***21a***). Yield: 0.207 g (83%), recrystallized from ethanol to afford a yellow solid; m.p. 256–258 °C. IR (KBr): 3007 m, 2974 m, 2942 m, 1616 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 2.42 (s, 3H, CH_3), 2.95 (t, 2H, J = 6.5 Hz, CH_2), 3.12 (t, 2H, J = 6.5 Hz, CH_2), 3.89 (s, 2H, CH_2), 7.38 (dd, 1*H*, J = 8.1 and 8.5 Hz, Ar-H), 7.67 (dd, 1*H*, J = 8.5 and 8.6 Hz, Ar-H), 8.16 (d, 1*H*, J = 8 .1 Hz, Ar-H), 8.27 (d, 1*H*, J = 8.6 Hz, Ar-H), 8.67 (s, 1*H*, Ar-H), 9.12 (s, 1*H*, Ar-H). Anal. calcd. for $C_{16}H_{15}N_3$ (249.31): C, 77.10; H, 6.02; N, 16.86%; found, C, 77.12; H, 6.04; N, 16.88%.

9-Methoxy-2-methyl-1,2,3,4-tetrahydroquinolino[2,3-b][1,6]naphthyridine (***21b***). Yield: 0.236 g (84%), recrystallized from ethanol to afford a faint brown solid; m.p. 267–269 °C. IR (KBr): 2994 m, 2945 m, 2911 m, 1618 s, 1029 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 2.38 (s, 3H, CH_3), 2.70 (t, 2H, J = 5.8 Hz, CH_2), 3.14 (t, 2H, J = 5.8 Hz, CH_2), 4.03 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 7.37 (d, 1*H*, J = 8.8 Hz, Ar-H), 7.56 (s, 1*H*, Ar-H), 8.17 (d, 1*H*, J = 8.8 Hz, Ar-H), 8.77 (s, 1*H*, Ar-H), 9.17 (s, 1*H*, Ar-H). MS (70 eV) *m/z* (%): 278 $[M\text{-}H]^+$ (100), 236 (33), 202 (37), 174 (28), 131 (42), 117 (22), 77 (12), 44 (19). Anal. calcd. for $C_{17}H_{17}N_3O$ (279.34): C, 73.11; H, 6.09; N, 15.05%; found, C, 73.12; H, 6.11; N, 15.07%.

1-(3,4-Dihydroquinolino[2,3-b][1,6]naphthyridin-2(1H)-yl)ethanone (***21c***). Yield: 0.237 g (85%), recrystallized from ethanol to afford a faint brown solid; m.p. 214–217 °C. IR (KBr): 3019 m, 2966 w, 2928 m, 1679 s, 1610 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 2.13 (s, 3H, CH_3), 3.22 (t, 2H, J = 6.6 Hz, CH_2), 3.49 (t, 2H, J = 6.6 Hz, CH_2), 4.73 (s, 2H, CH_2), 7.42 (dd, 1*H*, J = 8.4 and 8.5 Hz, Ar-H), 7.68 (dd, 1*H*, J = 8.5 and 8.3 Hz, Ar-H), 8.11 (d, 1*H*, J = 8.4 Hz, Ar-H), 8.39 (d, 1*H*, J = 8.3 Hz, Ar-H), 8.82 (s, 1*H*, Ar-H), 9.08 (s, 1*H*, Ar-H). ^{13}C-NMR (75 MHz $CDCl_3$) δ: 23.5, 35.2, 41.3, 44.2, 121.7, 126.2, 126.9, 127.6, 129.1, 129.9, 130.5, 133.7, 138.1, 148.1, 154.1, 157.2, 173.3. Anal. calcd. for $C_{17}H_{15}N_3O$ (277.32): C, 73.64; H, 5.41; N, 15.16%; found, C, 73.61; H, 5.40; N, 15.16%.

1-(9-Methoxy-3,4-dihydroquinolino[2,3-b][1,6]naphthyridin-2(1H)-yl)ethanone (***21d***). Yield: 0.269 g (87%), recrystallized from ethanol to afford a yellow solid; m.p. 244–247 °C. IR (KBr): 3012 m, 2959 m, 2936 w, 1683 s, 1607 s, 1024 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 2.09 (s, 3H, CH_3), 2.78 (t, 2H, J = 6.4 Hz, CH_2), 3.19 (t, 2H, J = 6.4 Hz, CH_2), 4.05 (s, 3H, OCH_3), 4.81 (s, 2H, CH_2), 7.36 (d, 1*H*, J = 7.9 Hz, Ar-H), 7.67 (s, 1*H*, Ar-H), 8.19 (d, 1*H*, J = 7.9 Hz, Ar-H), 8.87 (s, 1*H*, Ar-H), 9.23 (s, 1*H*, Ar-H). ^{13} C-NMR (75 MHz $CDCl_3$) δ: 24.1, 36.1, 40.9, 45.1, 59.1, 114.5, 123.1, 125.4, 128.5, 129.9, 131.5, 133.7, 138.6, 146.3, 157.3, 159.2, 161.9, 174.8. MS (70 eV) *m/z* (%): 307 $[M^+]$ (94), 277 (79), 264 (88), 249 (47), 221 (25), 141 (34), 113 (59), 98 (31), 57 (39), 43 (92). Anal. calcd. for $C_{18}H_{17}N_3O_2$ (307.35): C, 70.35; H, 5.53; N, 13.68%; found, C, 70.37; H, 5.50; N, 13.65%.

tert-butyl-3,4-dihydroquinolino[2,3-b][1,6]naphthyridine-2(1H)-carboxylate (***21e***). Yield: 0.279 g (83%), recrystallized from ethanol to afford a faint purple solid; m.p. 267–269 °C. IR (KBr): 3007 m, 2987 m, 2938 m, 1732 s, 1617 s, 1019 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.59 (s, 9H, CH_3), 3.26 (t, 2H, J = 6.7 Hz, CH_2), 3.37 (t, 2H, J = 6.7 Hz, CH_2), 4.34 (s, 2H, CH_2), 7.32 (dd, 1*H*, J = 8.2 and 8.5 Hz, Ar-H), 7.67 (dd, 1*H*, J = 8.5 and 8.1 Hz, Ar-H), 8.17 (d, 1*H*, J = 8.2 Hz, Ar-H), 8.30 (d, 1*H*, J = 8.1 Hz, Ar-H), 8.88 (s, 1*H*, Ar-H), 9.24 (s, 1*H*, Ar-H). Anal. calcd. for $C_{20}H_{21}N_3O_2$ (335.40): C, 71.64; H, 6.26; N, 12.53%; found, C, 71.65; H, 6.26; N, 12.53%.

tert-butyl-9-methoxy-3,4-dihydroquinolino[2,3-b][1,6]naphthyridine-2(1H)-carboxylate (***21f***). Yield: 0.301 g (82%), recrystallized from ethanol to afford a faint green solid; m.p. 234–237 °C. IR (KBr): 3019 m, 2954 m, 1736 s, 1612 s, 1028 s cm^{-1}. ^{1}H-NMR (300 MHz $CDCl_3$) δ: 1.48 (s, 9H, CH_3), 3.27 (t, 2H, J = 5.9 Hz, CH_2), 3.42 (t, 2H, J = 5.9 Hz, CH_2), 4.07 (s, 3H, OCH_3), 4.42 (s, 2H, CH_2), 7.47 (d, 1*H*, J = 8.4 Hz, Ar-H), 7.63 (s, 1*H*, Ar-H), 8.37 (d, 1*H*, J = 8.4 Hz, Ar-H), 8.94 (s, 1*H*, Ar-H), 9.24 (s, 1*H*, Ar-H). ^{13} C-NMR (75 MHz $CDCl_3$) δ: 25.7 (3 × Cs), 31.7, 42.1, 45.3, 59.1, 82.7, 115.3, 123.2, 125.7,

128.7, 129.9, 132.7, 135.1, 137.9, 145.2, 158.8, 159.1, 159.9, 161.2. MS (70 eV) *m/z* (%): 365 [M^+] (76), 308 (48), 277 (37), 264 (59), 236 (19), 221 (32), 153 (38), 77 (26), 57 (94), 44 (57), 41 (84). Anal. calcd. for $C_{21}H_{23}N_3O_3$ (365.43): C, 69.04; H, 6.30; N, 11.50%; found, C, 69.04; H, 6.32; N, 11.52%.

Results and discussion

Annelation reaction with heterocyclic aminoaldehydes provides synthetic entry into heterocyclic systems fused to a pyridine or pyrimidine (44–47) nucleus by condensation reaction. *o*-Aminoaldehyde has fascinating potential, remarkable versatility and utility for the annelation of heterocyclic ring structures to synthesize various heterocycles (48–53). Known 2-aminoquinoline-3-carbaldehyde (*o*-aminoaldehyde) **1** has been synthesized by a novel method reported in our previous communication (42).

Friedländer synthesis was originally described by Friedländer in 1882 and has been accepted to be one of the most convenient routes to this scaffold (54). The reaction advances by the condensation of α-amino carbonyl compound (N–C–C–C unit) with a derivative having α-methylene group with respect to the carbonyl (C–C unit), in the presence of a suitable catalyst. During recent years there has been a rapid increase in the number of publications describing the successful accomplishment of the Friedländer reaction. This was achieved by making changes in the catalyst or reaction medium or physical parameters, although the starting substrates were conceptually similar in these endeavours. From the literature, we observe that Friedländer condensation is generally carried out either with base catalysis (sodium or potassium hydroxide, sodium alkoxide, piperidine) in water or alcoholic solvents, or by heating a mixture of the components in the absence of solvent, or heating in high-boiling solvent; also, Friedländer condensation is carried out with acid catalysis or with Lewis acid or in under microwave irradiation.

Hence, to determinate suitable reaction conditions for Friedländer condensation, we performed cyclocondensation of **1a** or **1b** with formamide **2a** in various reaction conditions, such as: (i) in ethanolic KOH under reflux; (ii) neat heat about 175–180 °C without solvent; (iii) reflux in high-boiling solvent, i.e. in diphenyl ether for 2–3 h; (iv) in aq. HCl as strong acid at 90 °C for 6 h; (v) heat with $SnCl_2 2H_2O$ (Lewis acid); (vi) use of HCl as a catalyst for a similar reaction under microwave irradiation for benzonaphthyridine synthesis (unlike the conventional heating, which takes about 6 h for completion of the reaction, microwave speeds up the reaction and completes it within 1.5–12 min). From the above studies, we observed that the base-catalysed reaction, i.e. milder reaction conditions, gave good yield and, more importantly, lack of side-products as compared to other reaction conditions. Thus, cyclocondensation of **1a** or **1b** with amide **2(a-b)** achieved in ethanolic KOH to furnished pyrimido[4,5-b]quinoline **3(a-d)** in 81–85% yield. On the other hand, a mild base, such as piperidine, was sufficiently strong for condensation of *o*-aminoaldehyde **1** with benzyl cyanide. Hence, cyclocondensation of **1a** or **1b** with benzyl cyanide **4** in ethanolic piperidine gave 3-phenylbenzo[b][1,8]naphthyridin-2-amine **5(a-b)** in 81–84% yield (Scheme 1).

Reactions of *o*-aminoaldehydes with cyclic ketones are especially valuable for the construction of polycondensed heterocyclic systems. Herein, we extend Friedländer condensation using different cyclic ketones having a reactive methylene group to generate libraries of new heterocycles. And mild reaction conditions are employed in the Friedländer condensation to permit the transformation of functional groups from the starting ketone into the annelated heterocyclic ring. Thus, base-catalysed condensation (in ethanolic KOH) of **1** with cyclopentanone **6a** gave the strained heterocycle 2,3-dihydro-1*H*-benzo[b]cyclopenta[g][1,8] naphthyridine **7(a-b)** in 80–84% yield, also available via acid catalysed condensation (in aq. HCl) of the components although in much lower yield, i.e. 40–45%, hence we performed further cyclocondensation in the base-catalysed condensation. Therefore, cyclocondensation of 2-aminoquinoline-3-carbaldehyde **1a** or **1b** with 2-methylcyclohexanone **8** in ethanolic KOH furnished into 4-methyl-1,2,3,4-tetrahydrodibenzo[b,g][1,8]naphthyridine **9 (a-b)** in 82–85% yield. However, reaction of 1 with dimedone **10(a-b)** was unsuccessful in ethanolic KOH due to its more enolic character; hence this condensation is achieved by heating without solvent at 180–185 °C to afford 3,3-dimethyl-3,4-dihydrodibenzo[b,g][1,8]naphthyridin-1(2H)-one **11(a-d)** in good yield (Scheme 2).

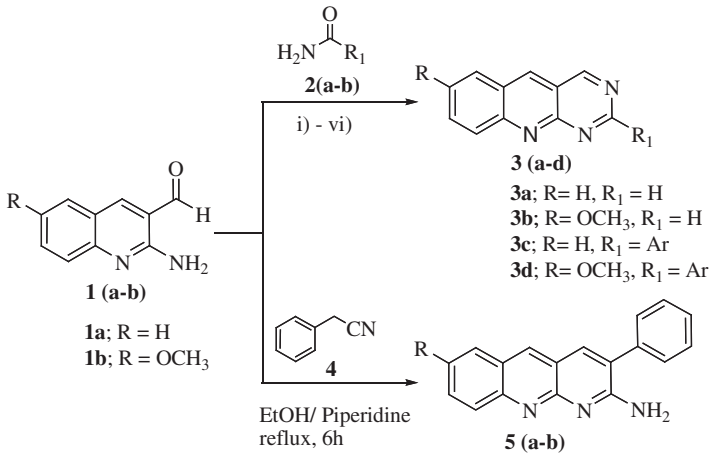
Numerous six-membered ring ketones have been converted into fused system via their annelation with *o*-aminoaldehyde. From the literature, we observe that a large number of substituted piperidones (55,56) and pyrrolidones have been condensed with *o*-aminobenzaldehyde in the base-catalysed reaction; thus, herein, we utilized the piperidones for generating the various heterocycles, by Friedländer condensation with 2-aminoquinoline-3-carbaldehyde (*o*-aminoaldehyde) **1**.

Because of useful applicability of base-catalysed condensation, we performed further condensations in ethanolic KOH. Thus, condensation of **1a** or **1b** with cetralene **12** and 6-methoxy-tetralone **14** in ethanolic KOH under reflux gave 5-(3,4-dichloro-phenyl)-5,6-dihydrobenzo[b]naphtho[2,1-g][1,8]naphthyridine **13(a-b)** and 3-methoxy-5,6-dihydrobenzo[b]naphtho[2,1-g][1,8]naphthyridine **15(a-b)**, respectively, in good yield. Analogue cyclocondensation of 3,3-dimethyl-1,5-dioxaspiro[5,5]undecan-9-one **16**, ethyl-2-oxocyclohexane-carboxylate **18** and substituted piperidone **20(a-c)** under similar reaction conditions yielded 5′,5′-dimethyl-3,4-dihydro-1*H*-spiro[dibenzo[b,g]-[1,8]naphthyridine-2,2′-[1,3]dioxane] **17(a-b)**, ethyl-1,2,3,4,-tetrahydrodibenzo[b,g][1,8]-naphthyridine-4-carboxylate **19(a-b)**, and 2-methyl-1,2,3,4-tetrahydroquinolino[2, 3-b][1,6]- naphthyridine **21(a-f)**, respectively, in good yield. All synthesized compounds were characterized by spectroscopic and analytical methods and all spectra is provided in Supporting information section. For example, the IR of **21d** shows stretching frequency at 1683 cm^{-1} for C = O. The ^{1}H-NMR spectrum of **21d** in $CDCl_3$ shows three singlets at 2.09, 4.05 and 4.81 ppm corresponding to protons of methyl, methoxy and methylene groups, respectively. Two triplets at 2.78 and 3.19 ppm with $J = 6.4$ Hz observed for the $–CH_2–CH_2–$ group. All aromatic protons show expected chemical shifts and splitting patterns which resemble the structure proposed. The mass spectrum of **21d** reveals a molecular ion peak *m/z* at 307. The ^{13}C-NMR spectrums of this compound are in agreement with the structure proposed (Scheme 3). The above study revealed that base-catalysed condensation is a more elegant method than other reaction conditions reported in literature. Synthesized benzonaphthyridines were further utilized for study of their photophysical properties.

Photophysical properties

Absorption and fluorescence studies in homogeneous media (organic solvents) and microheterogeneous media (micelles) and dioxane–water system. A variety of environmental factors affect fluorescence emission, including interactions

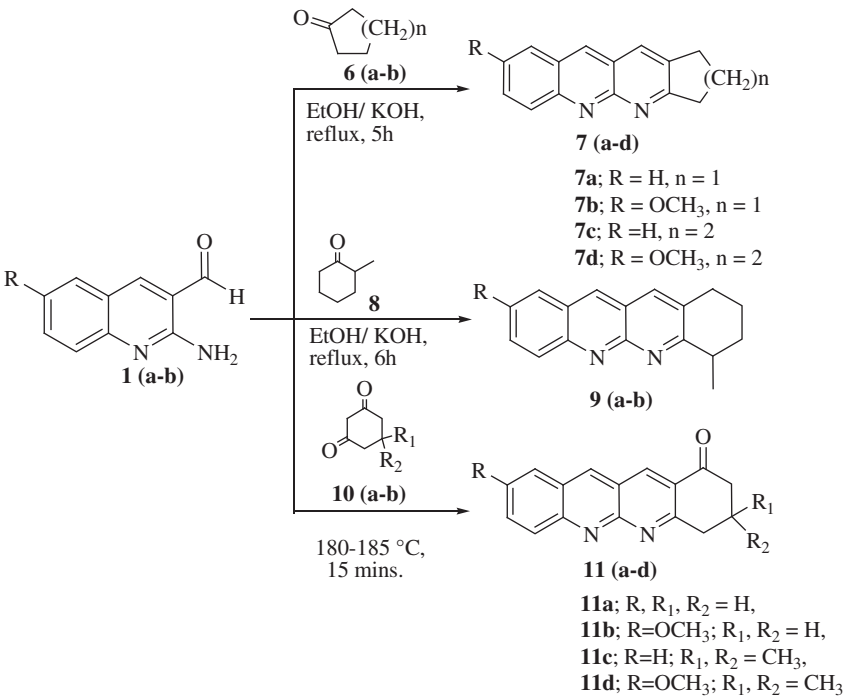
LUMINESCENCE
The Journal of Biological and Chemical Luminescence



i) EtOH/ KOH, reflux, 3h ii) 175-180 °C, neat heat, 1h iii) Diphenyl ether, reflux, 2.5h

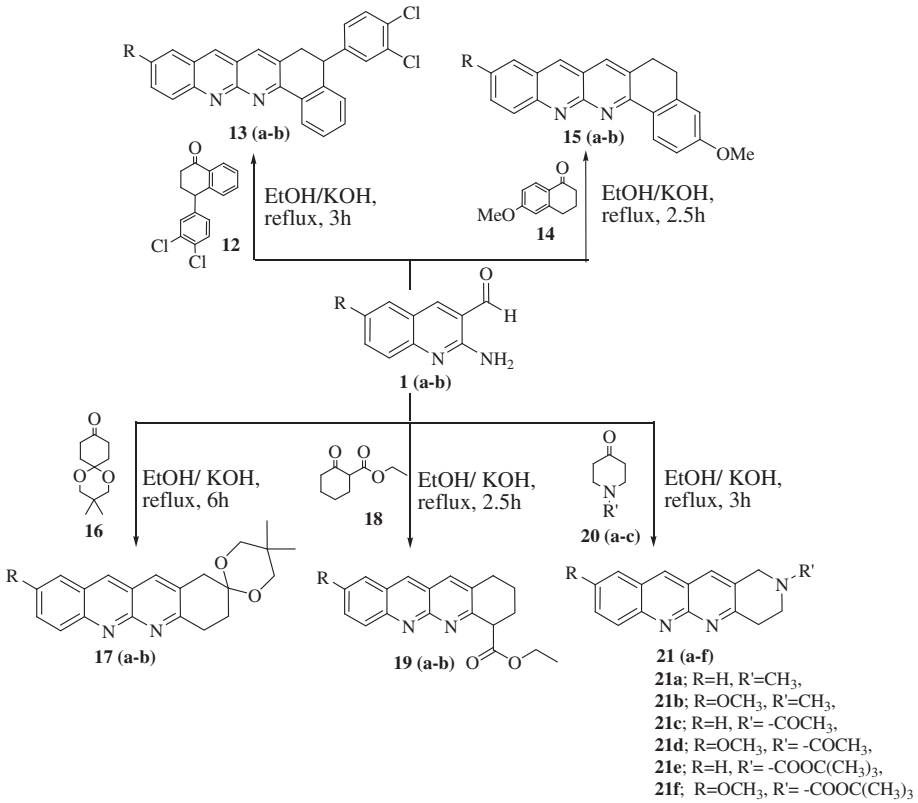
iv) HCl (1.0 eq.) H_2O, 90 °C, 6h v) $SnCl_2$. $2H_2O$, Neat heat, 15-140 min. vi) HCl Cat., MW, 1.5-12min.

Scheme 1. Synthesis of benzo[b][1,8]naphthyridines by condensation of *o*-aminoaldehyde with amides and benzylcyanide.



Scheme 2. Synthesis of benzo[b][1,8]naphthyridines by condensation of *o*-aminoaldehyde with alicyclic ketones.

between the fluorophore and surrounding solvent molecules (dictated by solvent polarity), other dissolved inorganic and organic compounds, temperature, pH and the localized concentration of the fluorescent species. The effect of these parameters varies widely from one fluorophore to another, but the absorption and emission spectra, as well as quantum yields, can be heavily influenced by environmental variables. In fact, the high degree of sensitivity in fluorescence is primarily due to interactions that occur in the local environment during the excited state lifetime. Thus, in this paper, we mainly give emphasis on to the study of effect of solvent on absorption and fluorescence emission, because solvents play an important role in physical and chemical processes. Solvent effects are related to the nature and the extent of the solute–solvent interactions developed in the solvation shell of the solutes (57). Organic mixed solvents are widely used as the mobile phase in liquid chromatography and capillary electrophoresis as a reaction medium. Solvent mixtures have improved physical properties, such as solvation power, density, viscosity and refractive index, compared with their neat solvents (58). When the solute is dissolved in a solvent, the solvent exerts a definite influence on the solute. This influence depends on the nature of the solvent. This influence reflects changes in the absorption and fluorescence spectrum (59) and this phenomenon is known as solvatochromism. Solvatochromism is used to describe the pounced change in position that sometimes occurs in the intensity of an absorption band, accompanying a change in the polarity of the medium. The preferential solvation phenomenon that is the selective



Scheme 3. Synthesis of benzo[b][1,8]naphthyridines by condensation of *o*-aminoaldehyde with alicyclic ketones.

enrichment of a certain solvent component in the solvation shell of a given solute is of paramount importance in the interpretation of many physiochemical parameters measured in the mixtures (60). Hydrogen bonding plays an important role in the study of preferential solvation and has been widely investigated, because it is present in large variety of chemical, biochemical and pharmacological events (61).

Prompted by this literature survey, in this study we investigated the electronic absorption and fluorescence properties of some representative benzonaphthyridines (**3d**, **5b** and **21f**) in the homogeneous media of organic solvents with different solvent polarities, dioxane–water binary mixtures and in the microheterogeneous media of SDS, CTAB and Triton-X100. UV-vis absorption and fluorescence spectral data of the benzonaphthyridines **3d**, **5b** and **21f** in organic solvents (homogeneous media) of different polarities are given in Table 1. Representative fluorescence spectra in different organic solvents are shown in Fig. 1. A moderate red shift in the absorption λ_{abs} max of these compounds is observed, whereas the fluorescence maximum (λ_f max) of these compounds is greatly affected by the solvent polarity, where a much red-shifted fluorescence is observed on increasing the solvent polarity from *n*-hexane to acetonitrile (Fig. 1). We found that the fluorescence intensity of these compounds in non-polar *n*-hexane is low as compared to polar aprotic and polar protic solvents (Fig. 1).

As compared to the homogeneous media of organic solvents, the micellar environment does not cause a significant change in the absorption spectra of these benzonaphthyridines (Table 2). Only a very moderate red shift in the absorption λ_{abs} maxima of compounds **3d** and **21f** is observed. The absorption and fluorescence spectral data of benzonaphthyridines **3d**, **5b** and **21f** in various compositions of 1,4-dioxane–water are summarized in Table 3 and graphically represented in Figs 2 and 3 for **5b** and **21f**, respectively. From Table 3 we observe that, in 1,4-dioxane–water mixtures the absorption maximum (λ_{abs} max) tends to decrease as the water content is increased. These absorption spectral features can be due to ground state hydrogen bond interactions and aggregate formation, particularly in water, in which benzonaphthyridines are not freely soluble. On the other hand, fluorescence maximum (λ_f max) is highly red-shifted as the water content in the solvent mixture is increased; also it was observed that enhancement in quantum yield (Φ_f) is linear with the increase in the percentage of water in the solvent mixtures. This observation is similar to those from studies in organic solvents of different polarity.

In contrast to a rather moderate solvent polarity effect on the absorption spectra, significant solvent polarity effects are seen in the fluorescence behaviour of these benzonaphthyiridines. In organic solvents and dioxane–water systems, as the polarity (relative permittivity, ε) of the medium is increased, the λ_f max undergoes a red shift.

In micellar media, benzonaphthyridines **3d** shows a fluorescence peak at around 505–508 nm, similar to its fluorescence in polar solvents such as methanol and acetonitrile. The fluorescence spectrum of compounds **21f** in micelles is significantly red-shifted as compared to that in non-polar *n*-hexane. However, the fluorescence maxima (λ_f max) in micelles are similar to those in polar solvents such as DMF, MeCN and MeOH. As compared to in polar solvents, the fluorescence maximum (λ_f max) of **21f** is blue-shifted in Triton-X100 micelles (Table 2, Fig. 4). The fluorescence intensity of these studied compounds in CTAB micelles is high compared to the SDS and Triton-X100 micelles. In general, as compared to the fluorescence of benzopanaphthyridines **3d**, **5b** and **21f** in non-polar *n*-hexane, in

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

Table 1. UV-vis absorption (λ_{abs} max) and fluorescence (λ_f max) spectral data of benzonaphthyridines 3 d, 5b and 21f in homogeneous media (organic solvents)

Compound	Solvent	Maximal wavelength (nm)		Stokes' shift (cm^{-1})	Φ_f (± 0.002)
		Absorption	Emission		
3d	*n*-Hexane	377	460	3712	0.252
	Dioxane	381	475	4452	0.317
	THF	385	486	5712	0.343
	DMF	394	512	6109	0.427
	MeCN	389	507	7223	0.419
	MeOH	388	505	7118	0.412
5b	*n*-Hexane	382	472	4672	0.322
	Dioxane	387	487	5125	0.359
	THF	390	492	6029	0.363
	DMF	398	525	7548	0.438
	MeCN	393	514	8230	0.429
	MeOH	392	512	8470	0.423
21f	*n*-Hexane	360	448	3724	0.222
	Dioxane	367	465	4210	0.256
	THF	371	473	4968	0.316
	DMF	378	495	5670	0.366
	MeCN	375	484	5951	0.339
	MeOH	374	482	6170	0.335

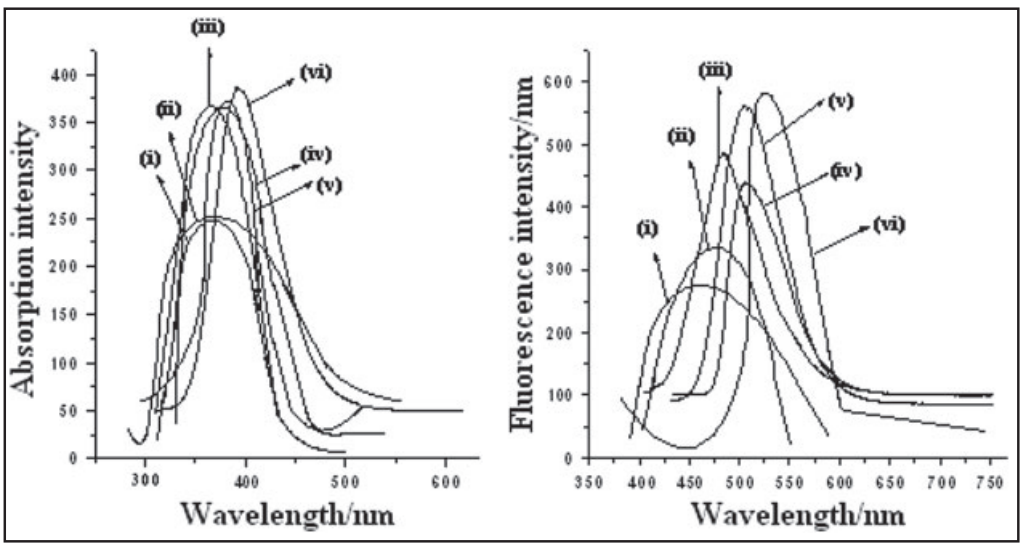


Figure 1. UV-vis absorption (λ_{abs} max) and fluorescence (λ_f max) spectra of **5b** in: (i) *n*-hexane; (ii) dioxane; (iii) THF; (iv) methanol; (v) MeCN; and (vi) DMF.

micellar media the fluorescence of these benzonaphthyridines is red-shifted; the maximum red shift is observed for **21f**. A comparison of the λ_f max of benzopanthyridines **3d**, **5b** and **21f** in polar organic solvents such as methanol with those in micelles reveals that the benzonaphthyridines experience relatively polar environment in the micelles.

The quantum yield (Φ_f) of benzonaphthyridines (**3d**, **5b** and **21f**) in organic solvents is greatly dependent on solvent polarity. The fluorescence efficiency of benzonaphthyridines **5b** and **21f** is generally decreased in polar solvents. The quantum yield (Φ_f) of these benzonaphthyridines (**3d**, **5b** and **21f**) is relatively higher in Triton-X100 as compared to either SDS or CTAB. The fluorescence efficiency is lowest in SDS micelles for all three benzonaphthyridines. Since we have already shown that there is an increase in quantum yield (Φ_f) with increase in solvent polarity of the medium, the increase in Φ_f in micelles is attributable to the greater polarity of the medium.

The Stokes' shift for all three benzonaphthyridines in polar homogeneous media of organic solvents such as DMF, acetonitrile and methanol is greater than that in micellar media. Among the three micelles studied, the smallest Stokes' shift is observed in neutral Triton-X100. Thus, the ionic micelles of SDS and CTAB affect the fluorescence spectra most. This can be due to interactions between the micellar charges and the polar benzonaphthyridine fluorophore. Thus, while benzonaphthyridine **21f** shows significant micelle-charge dependent fluorescence

Table 2. UV-vis absorption (λ_{abs} max) and fluorescence (λ_f max) spectral data of benzonaphthyridines 3d, 5b and 21f in microheterogeneous media (micelles)

Compound	Media	Maximal wavelength (nm)		Stokes' shift (cm^{-1})	Φ_f (± 0.002)
		Absorption	Emission		
3d	CTAB	397	508	5992	0.419
	SDS	397	508	5992	0.212
	Triton-X-100	397	505	5746	0.437
5b	CTAB	395	519	7322	0.391
	SDS	392	504	6177	0.242
	Triton-X-100	395	511	6171	0.437
21f	CTAB	386	489	4839	0.378
	SDS	381	490	5340	0.312
	Triton-X-100	386	469	4187	0.412

Table 3. UV-vis absorption (λ_{abs} max) and fluorescence (λ_f max) spectral data of 3d, 5b and 21f in 1,4-dioxane–water mixtures

Compound	Water in 1,4-dioxane (%)	λ_{abs} max (nm)	λ_f max (nm)	Stokes' shift (cm^{-1})	Φ_f (± 0.002)
3d	0	381	475	4452	0.310
	10	378	485	5789	0.297
	20	375	488	5610	0.287
	30	373	488	5610	0.287
	40	370	491	5475	0.289
	50	368	493	5475	0.290
	60	366	493	5701	0.290
5b	0	387	487	5125	0.359
	10	385	503	5852	0.317
	20	384	503	5892	0.310
	30	380	503	5891	0.310
	40	376	504	5908	0.312
	50	374	504	6102	0.312
	60	371	504	6227	0.316
21f	0	367	465	4210	0.256
	10	364	469	4109	0.247
	20	360	483	5127	0.230
	30	359	498	6451	0.212
	40	355	505	6869	0.192
	50	353	508	7223	0.187
	60	349	519	7610	0.172

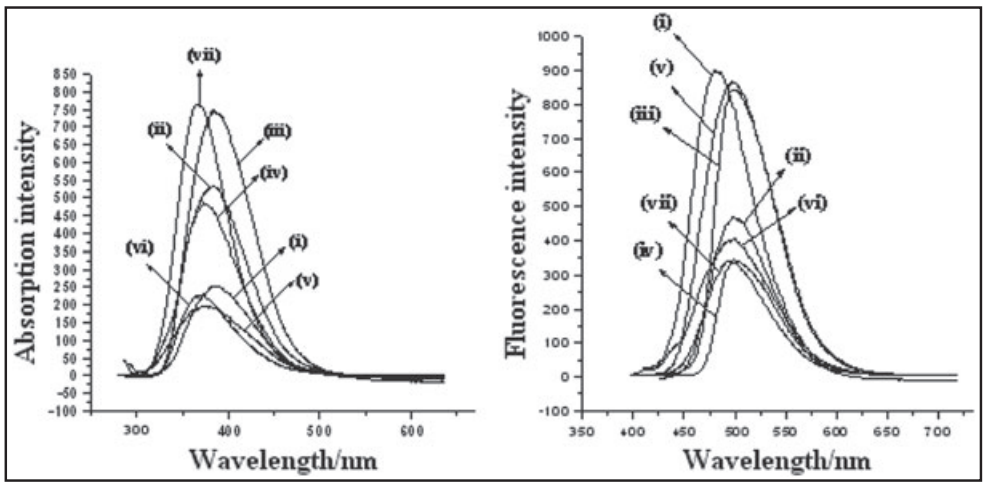


Figure 2. UV-vis absorption and fluorescence emission (λ_f max) spectra of **5b** in different concentrations of water: (i) 0%; (ii) 10%; (iii) 20%; (iv) 30%; (v) 40%; (vi) 50%; and (vii) 60%.

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

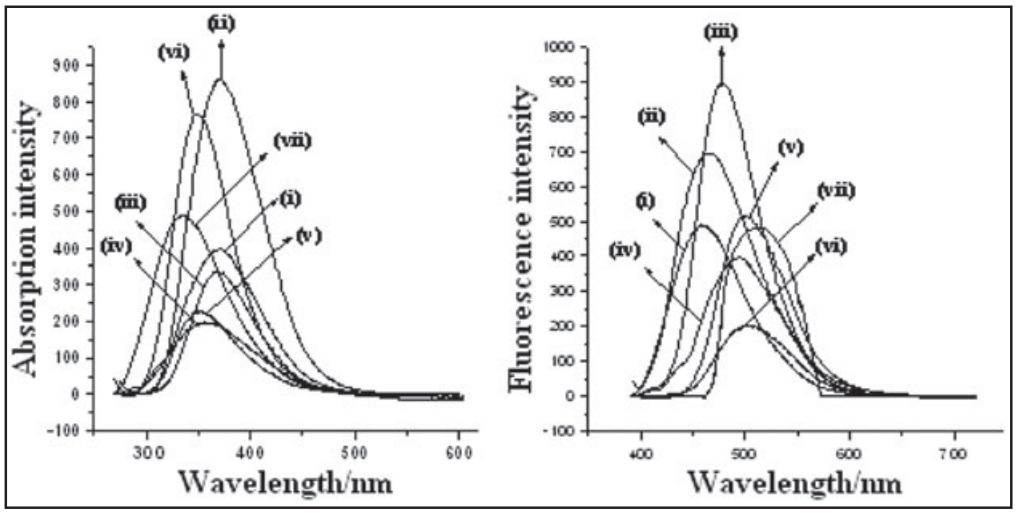


Figure 3. UV-vis absorption and fluorescence emission (λ_f max) spectra of **21f** in different concentration of water: (i) 0%; (ii) 10%; (iii) 20%; (iv) 30%; (v) 40%; (vi) 50%; and (vii) 60%.

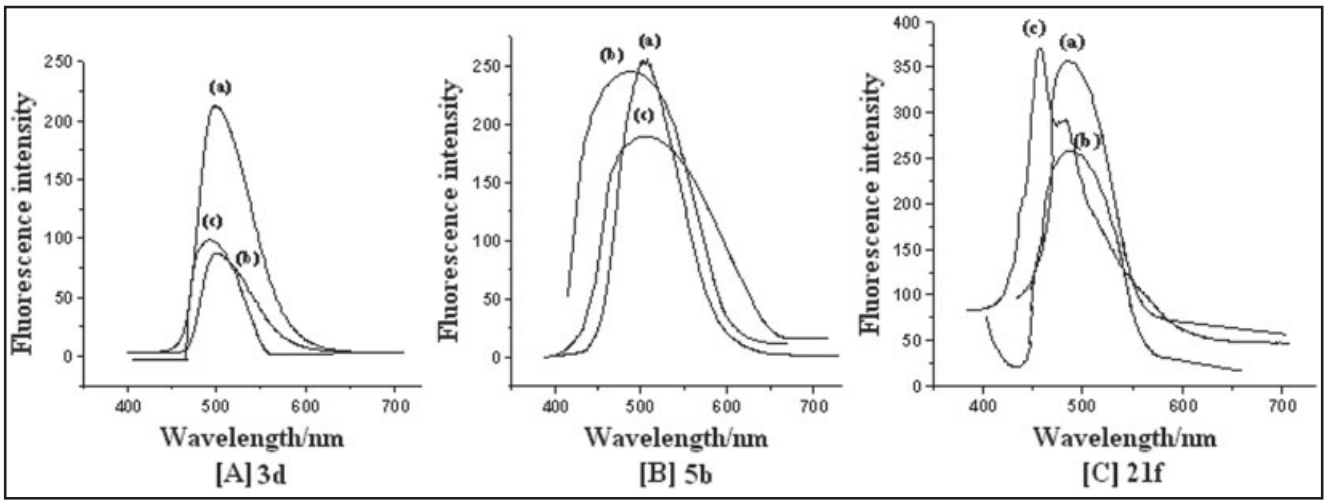


Figure 4. Fluorescence spectra of all three benzonaphthyridines (**3d**, **5b** and **21f**) in micelles of: (a) CTAB; (b) SDS; and (c) Triton-X100.

peak shifts, **3d** and **5b** do not show such marked changes in their fluorescence λ_f max. Further, UV-vis and fluorescence spectra of compounds **3(a-d)**, **5(a-b)**, **7(a-d)**, **9(a-b)**, **11(a-d)**, **13(a-b)**, **15(a-b)**, **17(a-b)**, **19(a-b)** and **21(a-f)** are taken in DMF as solvent. Fluorescence quantum yields of all the synthesized compounds were determined by a standard literature procedure using quinine sulphate as a reference standard (62,63) and are given in Table 4.

Electronic absorption and fluorescence properties of human serum albumin (HSA) and bovine serum albumin (BSA) with compounds 3d, 5b and 21f in phosphate buffer

HSA and BSA are homologous proteins composed of single polypeptide chains with 583 and 585 amino acids, respectively, with a similar sequence and a similar conformation (64). From the spectroscopic point of view, one of the main differences between the two proteins is that in HSA there is only one tryptophan residue, which is located at position 214 (Trp-214), buried in a hydrophobic pocket at sub-domain IIA, whereas in BSA there are two tryptophan amino acid residues (Trp-134 and Trp-212). This additional tryptophan residue in BSA is located at position 134, buried in a hydrophobic pocket and it has been proposed to lie near the surface of the albumin molecule in the second helix of the first domain (2,4,5). The tryptophan residue plays an important role as a chromophore and a fluorophore in optical studies of proteins.

Recently, we bound some representative benzonaphthyridines with BSA and studied the effect of protein–probe interaction on electronic absorption and fluorescence emission (43). In continuation of our efforts to study the effect of protein–probe (benzonaphthyridine) interaction (in phosphate buffer) on fluorescence emission, herein we study the binding effect of synthesized benzonaphthyridines (**3d**, **5b**, **21f**) with human serum albumin (HSA) and bovine serum albumin (BSA) on absorption and fluorescence properties. In order to examine the interaction of **3d**, **5b** and **21f** with HSA and BSA, the fluorescence titrations with HSA and BSA were carried out in phosphate buffer of pH 7.4. The absorption and fluorescence spectral data for **3d**, **5b** and **21f** are collected in Tables 5 and 6 and fluorescence emission (λ_f max) is graphically represented in Figs 5 and 6 with HSA and BSA, respectively. From Tables 5 and 6, we observed

Table 4. UV-vis absorption (λ_{abs} max) and fluorescence (λ_f max) spectral data of 3(a–d), 5(a–b), 7(a–d), 9(a–b), 11(a–d), 13(a–b), 15 (a–b), 17(a–b), 19(a–b) and 21(a–f) in DMF as the solvent (ca. 10^{-3}) at room temperature

Compound	λ_{abs} max (nm)	λ_f max (nm)	ϕ_F (± 0.002)	Compound	λ_{abs} max (nm)	λ_f max (nm)	ϕ_F (± 0.002)
3a	335	451	0.256	11d	407	500	0.403
3b	361	467	0.294	13a	398	472	0.351
3c	365	461	0.284	13b	418	487	0.372
3d	394	512	0.427	15a	370	479	0.337
5a	365	474	0.315	15b	407	507	0.392
5b	398	525	0.438	17a	366	466	0.310
7a	360	472	0.294	17b	370	471	0.329
7b	365	481	0.327	19a	397	489	0.387
7c	362	477	0.301	19b	402	521	0.419
7d	369	488	0.345	21a	361	482	0.367
9a	365	472	0.284	21b	368	494	0.388
9b	368	481	0.378	21c	334	449	0.290
11a	368	478	0.311	21d	338	458	0.312
11b	373	498	0.397	21e	360	445	0.281
11c	370	489	0.350	21f	378	495	0.366

Table 5. Absorption maximum (λ_{abs} max), fluorescence emission (λ_f max) and fluorescence quantum yield (Φ_f) of benzonaphthyridines (3d, 5b and 21f) in phosphate buffer, pH 7.4, and HSA (5.0×10^{-6} mol/L)

Compound	λ_{abs} max (nm)		λ_f max (nm)		ϕ_F (± 0.002)	
	Buffer	HSA	Buffer	HSA	Buffer	HSA
3d	407	403	512	529	0.421	0.433
5b	390	387	488	494	0.382	0.405
21f	401	399	502	517	0.409	0.425

Table 6. Absorption maximum (λ_{abs} max), fluorescence emission (λ_f max) and fluorescence quantum yield (Φ_f) of benzonaphthyridines (3d, 5b and 21f) in phosphate buffer, pH 7.4, and BSA (5.0×10^{-6} mol/L)

Compound	λ_{abs} max (nm)		λ_f max (nm)		ϕ_F (± 0.002)	
	Buffer	BSA	Buffer	BSA	Buffer	BSA
3d	415	409	509	535	0.419	0.437
5b	427	406	521	551	0.428	0.448
21f	374	375	502	479	0.407	0.413

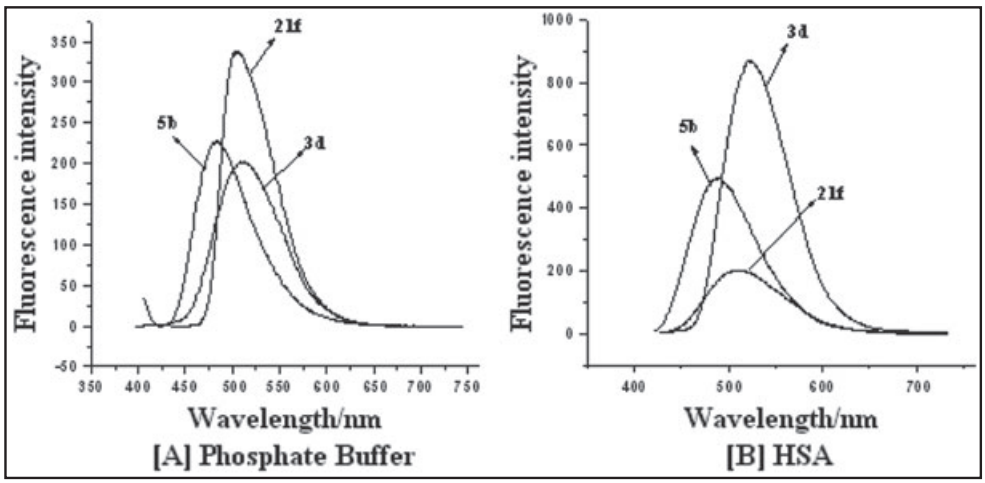


Figure 5. Comparative fluorescence emission (λ_f max) spectra of **3d**, **5b** and **21f**: (A) phosphate buffer; (B) in HSA.

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

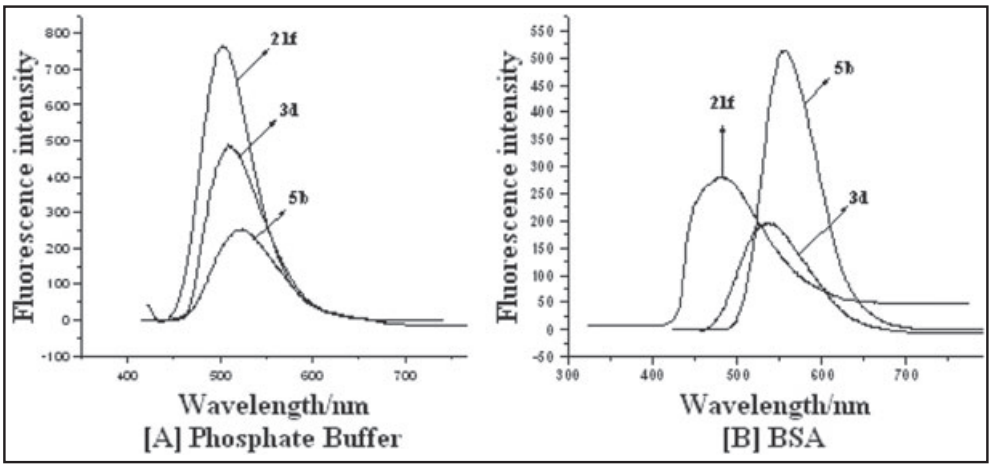


Figure 6. Comparative fluorescence emission (λ_f max) spectra of **3d**, **5b** and **21f**: (A) phosphate buffer; (B) in BSA.

that binding with BSA and HSA in the solutions of benzonaphthyridines in phosphate buffer results in an enhancement of the fluorescence emission (red-shift) and quantum yield (Φ_f) for all compounds, and blue shift in the λ_f max is observed for **5b** with HSA and **21f** with BSA. The blue shift in λ_f max suggests that the polarities of the protein environments in which the benzonaphthyridines are located are less than the polarity of the bulk aqueous phase, since similar blue shifts are observed in less polar organic solvents. This proves the binding of the probes to a hydrophobic site of the protein. Further, we examined the effect of increasing HSA and BSA concentration on fluorescence emission and noted that a gradual increase in the concentration of HSA and BSA in the solution of benzonaphthyridines (**3d**, **5b** and **21f**) in phosphate buffer results in an enhancement of the fluorescence intensity and quantum yield (Φ_f) for all three benzonaphthyridines. Representative fluorescence spectra in different concentration of HSA and BSA for **5b** and **21f** are shown in Figs 7 and 8, respectively.

Fluorescence quenching study with BSA

The interaction of benzonaphthyridines (**3d**, **5b** and **21f**) with BSA was also monitored by studying the quenching of BSA fluorescence with the increasing concentration of benzonaphthyridines. Thus, the fluorescence spectra of BSA in the presence of different concentrations of **3d**, **5b** and **21f** are recorded in the range 300–500 nm by exciting the protein at 280 nm. The representative fluorescence emission spectra are given in Fig. 9. The fluorescence intensity of BSA decreased regularly with the increasing concentration of the probes, which indicates that the probes are binding to the protein. Upon addition of increasing amounts of compounds **3d**, **5b** and **21f**, the fluorescence intensity of BSA at 345 nm decreased, along with the appearance of new peaks at 505, 512 and 497 nm, respectively.

Conclusions

In conclusion, the formation of linear tetracyclic and pentacyclic benzonaphthyridines from dicyclic *o*-aminoaldehyde **1** represents a remarkable efficient heteroannelation reaction. All these synthesized compounds are included in the library of fluorescent heterocyclic compounds. Synthesized benzonaphthyridines were studied for their photophysical properties and certain benzonaphthyridines (**3d**, **5b** and **21f**) were studied for UV-vis absorption (λ_{abs} max) and fluorescence emission (λ_f max) in homogeneous media of organic solvents, 1,4-dioxane–water binary mixtures and in the micellar environment. From these studies, we reveal that, these benzonaphthyridines (**3d**, **5b** and **21f**) exhibit solvatochromic fluorescence emission that may be

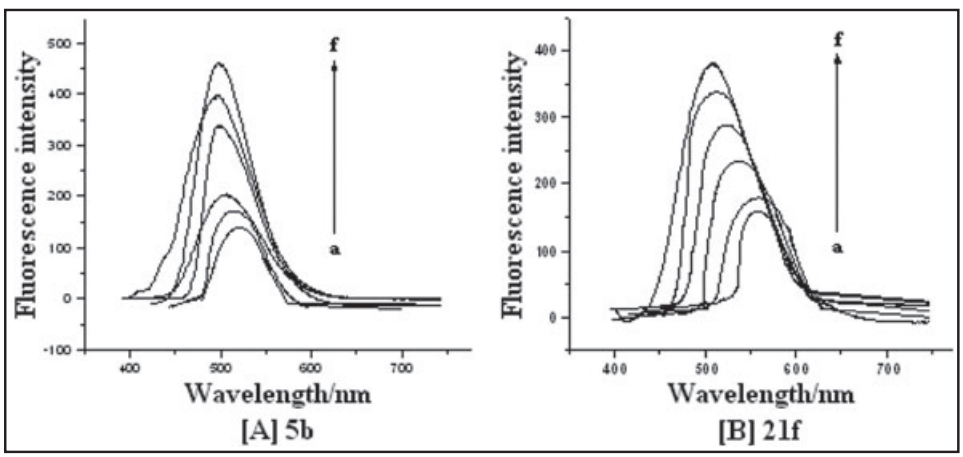


Figure 7. Fluorescence emission (λ_f max) spectra of **5b** and **21f** with increasing HSA concentration (A) for **5b**; curves a–f correspond to [HSA] 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 ($\times 10^{-6}$ mol/L), respectively. (B) For **21f**; curves a–f correspond to [HSA] 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 ($\times 10^{-6}$ mol/L), respectively.

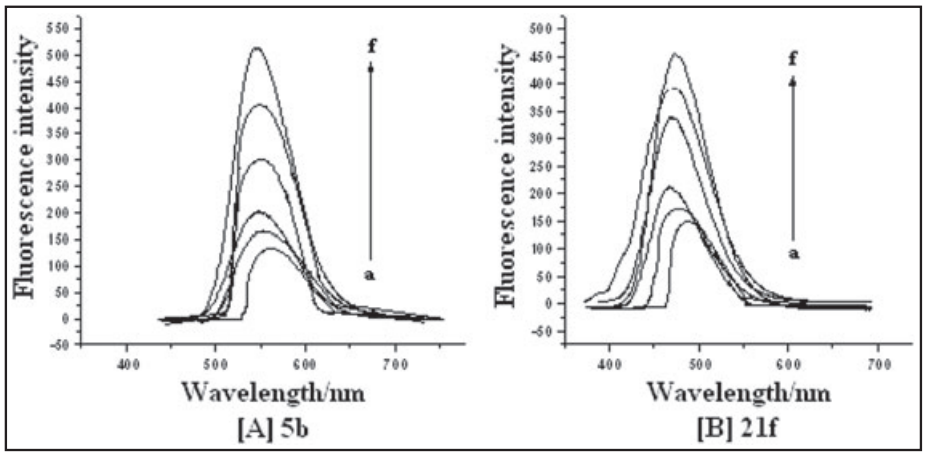


Figure 8. Fluorescence emission (λ_f max) spectra of **5b** and **21f** with increasing BSA concentration: (A) for **5b**; curves a–f correspond to [BSA] 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 ($\times 10^{-6}$ mol/L), respectively; (B) for **21f**; curves a–f correspond to [BSA] 0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 ($\times 10^{-6}$ mol/L), respectively.

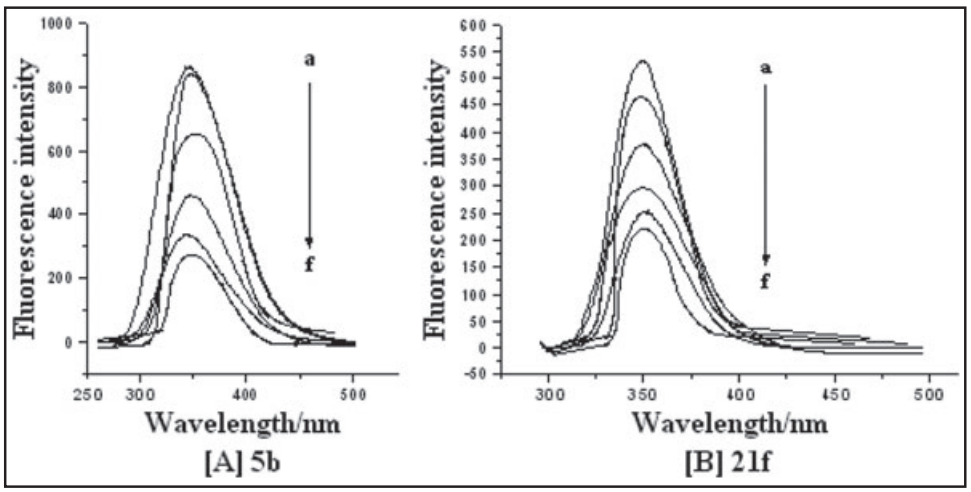


Figure 9. Fluorescence emission (λ_f max) spectra of BSA (1.0×10^{-6} mol/L) in the presence of different concentrations of **5b** and **21f**: (A) for **5b**, curves a–f correspond to 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 ($\times 10^{-6}$ mol/L); (B) for **21f**, curves a–f correspond to 0.0, 0.2, 0.4, 0.6, 1.2 and 1.6 ($\times 10^{-6}$ mol/L).

due to a polar, conformationally relaxed, intramolecular charge-transfer excited state. The absorption maximum (λ_{abs} max) of these compounds undergoes a moderate red shift with increasing solvent polarity; on the other hand, the fluorescence maximum (λ_f max) becomes highly red-shifted with increasing solvent polarity. However, these benzonaphthyridines (**3d**, **5b** and **21f**) exhibit micellar charge-dependent changes in their fluorescence intensity in the micellar environment. These fluorescence properties of benzonaphthyridines can be utilized to investigate the microenvironment of ionic micelles and related organized assemblies.

Further, fluorescence probe properties of these benzonaphthyridines (**3d**, **5b** and **21f**) with HSA and BSA were investigated using UV-vis absorption and fluorescence spectroscopy. Upon binding with HSA and BSA, these compounds exhibit enhanced fluorescence intensity and quantum yield (Φ_f), except for **5b** with HSA and **21f** with BSA, which show blue-shifted fluorescence intensity. In general, fluorescence intensity enhancement is linear with the increase in HSA and BSA concentration. The results indicate that these benzonaphthyridines can be used for studying the polarities of protein cavities. This study provides new directions for the design of neutral and hydrophobic fluorescence probes. In general, these compounds have good potential for use as fluorescence probes to study the microenvironments of biomolecules, polymers, organized assemblies, etc. Quantum yields (Φ_f) of all synthesized compounds are calculated.

SUPPORTING INFORMATION

The supporting information i.e. 1H and 13C NMR, mass spectra may be found in the online version of this article.

Acknowledgements

The authors would like to thank the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for financial support for this research project. They thank Professor D. D. Dhavale, Department of Chemistry, University of Pune, India, for his valuable cooperation in the measurement of ^{1}H-NMR, ^{13}C-NMR, mass and elemental analysis. We thank Dr V. B. Gaikwad, Principal, KRT Arts, BH Commerce and AM Science College, Nashik-02, MS, India, for valuable cooperation in the measurement of IR, UV and fluorescence.

LUMINESCENCE
The Journal of Biological and Chemical Luminescence

References

1. Pardridge WM. Plasma protein-mediated transport of steroid and thyroid hormones. Am J Physiol 1987;252:157–64.
2. Peters T Jr. Serum albumin. Adv Protein Chem 1985;37:161–245.
3. Brown JR. In: Rosenoer VM, Oratz M, Rothschild MA (eds). *Albumin structure, function and uses*. Pergamon: Oxford, 1977; 27–51.
4. Peters T Jr. *All about albumin*. Academic Press: San Diego, CA, 1996.
5. Min HX, Carter DC. Atomic structure and chemistry of human serum albumin. Nature 1992;358:209–15.
6. Helms MK, Paterson CE, Bhagavan NV, Jameson DM. Time-resolved fluorescent studies on site-directed mutants of human serum albumin. FEBS Lett 1997;408:67–70.
7. Sudlow G, Birkett DJ, Wade DN. Further characterization of specific drug binding sites on human serum albumin. Mol Pharmacol 1977;12:1052.
8. Moreno F, Cortijo M, Gonzalez-Jimenez J. The fluorescent probe prodan characterizes the warfarin binding site on human serum albumin. Photochem Photobiol 1999;69:8.
9. Kumar CV, Buranaprapuk A. Tuning the selectivity of protein photocleavage:spectroscopic and photochemical studies. J Am Chem Soc 1999;121:4262.
10. Reed RG. Nucleus-dependent regulation of tyrosine aminotransferase degradation in hepatoma tissue culture cells. J Biol Chem 1977;252:7483.
11. Jacobsen J, Brodersen R. Location of long chain fatty acid-binding sites of bovine serum albumin by affinity labeling. J Biol Chem 1983;258:6319.
12. Kessler MA, Wolfbeis OS. Laser-induced fluorometric determination of albumin using longwave absorbing molecular probes. Anal Biochem 1992;200(2):254–9.
13. Stamler JS, Singel DL, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. Science 1992;258:1898–902.
14. Ghuman J, Zunszain PA, Petitpas I, Bhattacharya AA, Otagiri M, Curry S. Structural basis of the drug-binding specificity of human serum albumin. J Mol Biol 2005;353(1):38–52.
15. Ma CQ, Li KA, Tong SY. Microdetermination of proteins by Rayleigh light scattering technique with acid green 25. Anal Chim Acta 1997;338(3):255–60.
16. Yin YB, Wang YN, Ma JB. Aggregation of two carboxylic derivatives of porphyrin and their affinity to bovine serum albumin. Spectrochim Acta A Mol Biomol Spectrosc 2006;64(4):1032–8.
17. Huang CZ, Lu W, Li YF, Huang YM. On the factors affecting the enhanced resonance light scattering signals of the interactions between proteins and multiply negatively charged chromophores using water blue as an example. Anal Chim Acta 2006;556 (2):469–75.
18. Yakovleva J, Davidsson R, Bengtsson M, Laurell T, Emnéus J. Microfluidic enzyme immunosensors with immobilised protein A and G using chemiluminescence detection. Biosens Bioelectron 2003;19:21–34.
19. Zor T, Selinger Z. Linearization of the Bradford protein assay increases its sensitivity:theoretical and experimental studies. Anal Biochem 1996;236:302–8.
20. Fan HH, Kashi RS, Middaugh CR. Conformational lability of two molecular chaperones Hsc70 and gp96:Effects of pH and temperature. Arch Biochem Biophys 2006;447:34–45.
21. Meersman F, Smeller L, Heremans K. A comparative FTIR study of cold-, pressure- and heat-induced unfolding and aggregation of myoglobin. Biophys J 2002;82:2635–44.
22. Fitzsimons SM, Mulvihill DM, Morris ER. Denaturation and aggregation processes in thermal gelation of whey proteins resolved by differential scanning calorimetry. Food Hydrocoll 2007;21:638–44.
23. Wang YP, Wei YL, Dong C. Study on the interaction of 3,3-bis(4-hydroxy-1-naphthyl)-phthalide with bovine serum albumin by fluorescence spectroscopy. J Photochem Photobiol A Chem 2006;177:6–11.
24. Wang F, Yang JH, Wu X, Sun CX, Liu SF, Guo CY, Jia Z. The interaction mechanism and fluorescence enhancement in morin–Al^{3+}–sodium dodecyl benzene sulfonate–protein system. Chem Phys Lett 2005;409:14–22.
25. Mallick A, Halder B, Chattopadhyay N. Spectroscopic investigation on the interaction of ICT probe 3-acetyl-4-oxo-6,7-dihydro-12*H*-indolo-[2,3-a]quinolizine with serum albumin. J Phys Chem B 2005;109:14683–90.
26. Grabowski Z, Rotkiewicz K, Rettig W. Structural changes accompanying intramolecular electron transfer: focus on twisted intramolecular charge-transfer states and structures. Chem Rev 2003;103:3899–4032.
27. Poklar N, Lah J, Salobir M, Maek P, Vesnaver G. pH and temperature-induced molten globule-like denatured states of equinatoxin II: a study by UV-Melting, DSC, far- and near-UV CD spectroscopy and ANS fluorescence. Biochemistry 1997;36:14345–52.
28. Haskard CA, Li-Chan ECY. Hydrophobicity of bovine serum albumin and ovalbumin determined using uncharged (PRODAN) and anionic (ANS) fluorescent probes. J Agric Food Chem 1998;46:2671–7.
29. Cardamone M, Puri NK. Spectrofluorimetric assessment of the surface hydrophobicity of proteins. Biochem J 1992;282:589–93.
30. Matulis D, Baumann CG, Bloomfield VA, Lovrien RE. 1-Anilino-8-naphthalene sulphonate as a protein conformational tightening agent. Biopolymers 1999;49:451–8.
31. Daniel E, Weber G. Cooperative effects in binding by bovine serum albumin. I. The binding of 1-anilino-8-naphthalenesulfonate. Fluorimetric titrations. Biochemistry 1966;5:1893–900.
32. Banerjee A, Basu K, Sengupta PK. Interaction of 7-hydroxyflavone with human serum albumin: a spectroscopic study. J Photochem Photobiol B Chem 2008;90:33.
33. Davis DM, Birch DJS. Extrinsic fluorescence probe study of human serum albumin using Nile red. J Fluoresc 1996;6:23.
34. Wu FY, Ji ZJ, Wu YM, Wan XF. Interaction of ICT receptor with serum albumins in aqueous buffer. Chem Phys Lett 2006;424:387.
35. Singh RB, Mahanta S, Guchhait N. Study of interaction of proton transfer probe 1-hydroxy 2-naphthaldehyde with serum albumins: spectroscopic study. J Photochem Photobiol B Biol 2008;91:1.
36. Kaldas MI, Walle UK, van der Woude H, McMillan JM, Walle T. Covalent binding of the flavonoid quercetin to human serum albumin. J Agric Food Chem 2005;53:4194.
37. Papadopoulou A, Green RJ, Frazier RA. Interaction of flavonoids with bovine serum albumin: a fluorescence quenching study. J Agric Food Chem 2005;53:158–63.
38. Turner DC, Brand L. Quantitative estimation of protein binding site polarity. Fluorescence of *N*-arylaminonaphthalenesulfonates. Biochemistry 1968;7(10):3381–90.
39. Sytnik A, Gormin D, Kasha M. Interplay between excited-state intramolecular proton transfer and charge transfer in flavonols and their use as protein-binding-site fluorescence probes. Proc Natl Acad Sci USA 1994;91(25):11968–72.
40. Tsuchida T, Zheng G, Pandey RK, Potter WR, Bellnier DA, Henderson BW, Kato H, Dougherty TJ. Correlation between site II-specific human serum albumin (HSA) binding affinity and murine *in vivo* photosensitizing efficacy of some photofrin components. Photochem Photobiol 1997;66(2):224–8.
41. Rettig W, Lapouyade R. Fluorescence probes based on TICT states and other adiabatic photoreactions. In: Lakowicz JR (ed). *Topics in fluorescence spectroscopy*, Vol. 4. Probe design and chemical sensing. Plenum Press: New York, 1994.
42. Shelar DP, Birari DR, Rote RV, Patil SR, Toche RB, Jachak MN. Novel synthesis of 2-amino-3-quinoline-carbaldehyde, benzo[b][1,8] naphthyridines and study of their fluorescence behavior. J Phys Org Chem 2011;24:203–11.
43. Shelar DP, Patil SR, Rote RV, Toche RB, Jachak MN. Synthesis and fluorescence investigation of differently substituted benzo[b][1,8] naphthyridines: interaction with different solvents and bovine serum albumin (BSA). J Fluoresc 2011;21(3):1033–47.
44. Hawes EM, Wibberley DG. 1,8-Naphthyridines. J Chem Soc 1966;3: 315–21.
45. Majewicz TG, Caluwe P. Chemistry of *o*-aminoaldehydes. Reactions of 2-aminonicotinaldehyde and cyclohexanediones. J Org Chem 1975;40(23):3407–10.
46. Hawes EM, Wibberley DG. 1,8-Naphthyridines. Part II. Preparation and some reactions of 2-substituted derivatives. J Chem Soc C 1967;16:1564–8.
47. Hawes EM, Gorecki DKJ, Gedir RG. 2,3-Disubstituted 1,8-naphthyridines as potential diuretic agents. 2. 5,7-Dimethyl derivatives. J Med Chem 1977;20(6):838–41.
48. Clemo GR, Felton DGI. The influence of structure on the ultraviolet absorption spectra of heterocyclic systems. Part II. Nitrogen analogues of the monobenzofluorenes. J Chem Soc 1952; 1658–67.

49. Fehnel EA. Friedländer syntheses with *o*-aminoaryl ketones. I. Acid-catalyzed condensations of *o*-aminobenzophenone with ketones. J Org Chem 1966;31(9):2899–902.
50. Markgraf JH, Katt RJ, Scott WL, Shefrin RN. Strained heterocyclic systems. IV. 1,2-Dihydrocyclobuta[b]quinoline and derivatives. J Org Chem 1969;34(12):4131–3.
51. Liao TK, Nyberg WH, Cheng CC. Total synthesis of camptothecin. I. Synthesis of ethyl 8-(α-chlorobutyryloxymethyl)-7,9-dioxo-7,8,9,11-tetrahydroindolizino[1,2-a]quinoline-8-carboxylate and related tetracyclic compounds. J Heterocycl Chem 1971;8:373–7.
52. Tang CSF, Morrow CJ, Rapoport H. Total synthesis of D,L-camptothecin. J Am Chem Soc 1975;97:159.
53. Settimo ADa, Primofiore G, Livi O, Ferrarini PL, Spinelli S. Synthesis of some 3-substituted quino[3,2-c][1,8]naphtyridines. A new heterocyclic ring system. J Heterocycl Chem 1979;16:169.
54. Friedlander P. Friedlander synthesis. Ber Dtsch Chem Ges 1882;15:2572–5.
55. Kempter G, Hirschberg S. Heterocycles from amino ketones. V. The friedlander synthesis with nitrogen-, oxygen-, or sulfur-containing five- and six-membered ring ketones. Chem Ber 1965;98:419.
56. Shamma M, Novak L. Preparation of some tricyclic analogs of camptothecin. Collect Czech Chem Commun 1970;35:3280.
57. Mancini PM, del Perez AC, Vottero LR. Nonspecific solute–solvent interactions in binary solvent mixtures containing an aprotic hydrogen-bond acceptor and a hydrogen-bond donor:dipolarity/polarizability and refractive index. J Sol Chem 2001;30(8):695–707.
58. Reichardt C. Solvents and solvent effects: an introduction. Org Process Res Dev 2007;11(1):105–103.
59. Sasirekha V, Vanelle P, Terme T, Meenakshi C, Umadevi M, Ramakrishnan V. Solvatochromism, preferential solvation of 2, 3-bis(chloromethyl)-1,4-anthraquinone in binary mixtures and the molecular recognition towards *p-tert*-butyl-calix[4]arene. J Fluoresc 2007;17(5):528–39.
60. Hagazi MF, Al-Mudhaf HF, Abu-Shady AI. Preferential solvation of acetic acid in binary mixtures of carbon tetrachloride–cyclohexane and carbon tetrachloride–1,2-dichloroethane. J Sol Chem 2007;36 (4):467–78.
61. Bevilaqua T, da Silva DC, Machado VG. Preferential solvation of Brooker's merocyanine in binary solvent mixtures composed of formamides and hydroxylic solvents. Spectrochim Acta A Mol Biomol Spectrosc 2004;60(4):951–8.
62. Lakowicz JR. *Principles of fluorescence spectroscopy*, 2nd edn. Springer: New York, USA, 1999.
63. Fletecher AN. Quinine sulfate as a fluorescence quantum yield standard. Photochem Photobiol 1969;9(5):439–44.
64. Carter DC, Ho JX. Structure of serum albumin. Adv Protein Chem 1994;45:153.
65. Eastman JW. Quantitative spectrofluorimetry-the fluorescence quantum yield of quinine sulfate. Photochem Photobiol 1967;6 (1):55–72.
66. Adams MJ, Highfield JG, Kirkbright GF. Determination of absolute fluorescence quantum efficiency of quinine bisulfate in aqueous medium by optoacoustic spectrometry. Anal Chem 1977;49 (12):1850–2.
67. Meech SR, Phillips D. Photophysics of some common fluorescence standards. J Photochem 1983;23:193–217.