Benzimidazole quinoline derivatives — An effective green fluorescent dye for bacterial imaging

Mahalingam Malathi, Palathurai Subramaniam Mohan, Raymond J. Butcher, and Chidambaram Kulandaisamy Venil

Abstract: A one-pot synthesis of benzimidazoles by condensing naphthyl or quinoline aldehyde with benzene-1,2-diamine has been reported. IR, ^{1}H and ^{13}C NMR, mass spectral, and CHN analyses were used to elucidate the structures of the products. The molecular structural correlation in the optical properties of the quinoline and naphthalene benzimidazoles was explored. The fluorescence quantum yield (ϕ_f) and time-resolved fluorescent lifetime of the quinoline benzimidazoles derivatives were estimated. The influence of solvent polarity and pH on the optical property of quinoline derivatives was illustrated. To explore the bioanalytical applicability, the thermal stability by TG–DTA analysis and the cytogenetic analysis of 3-(1*H*-benzoimidazol-2-yl)-2-chloro-8-methyl-quinoline (**1b**) compound were carried out. The fluorescent staining ability of **1b** was analyzed and also compared with the normal Gram staining in the bacterium.

Key words: benzimidazole quinolines, optical property, fluorescence quantum yield, cytogenetic analysis, fluorescence staining.

Résumé : On a réalisé une synthèse monotope de benzimidazoles par condensation de naphtyl- et de quinoléinealdéhydes avec de la benzène-1,2-diamine. On a fait appel aux analyses CHN et aux spectroscopies IR, RMN ^{1}H et ^{13}C et de masse pour élucider la structure des produits. On a exploré la corrélation de la structure moléculaire dans les propriétés optiques des benzimidazolylquinoléines et des benzimidazolylnaphtalènes. On a évalué le rendement quantique de fluorescence (ϕ_f) et le temps de vie fluorescent résolu en fonction du temps des dérivés benzimidazolylquinoléines. On a aussi illustré l'influence de la polarité du solvant et du pH sur les propriétés optiques de ces dérivés de la quinoléine. Afin de pouvoir évaluer les possibilités de l'utiliser dans le domaine des analyses biologiques, on a aussi effectué une analyse cytogénétique et on a évalué la stabilité thermique de la 3-(1*H*-benzimidazol-2-yl)-2-chloro-8-méthylquinoléine (**1b**) par analyse thermogravimétrique (TG) et analyse thermique différentielle (ATD). On a analysé la capacité du composé 1b à agir comme colorant fluorescent et on a comparé ses propriétés à celles de la coloration Gram normale dans les bactéries.

Mots-clés : benzimidazolylquinoléines, propriété optique, rendement quantique de fluorescence, analyse cytogénétique, coloration de fluorescence.

[Traduit par la Rédaction]

Introduction

Rapid advances in optical technologies have recently led to significant progress in medical diagnostics, drug discovery, and (bio)chemical analysis, in particular for methods that use fluorescence detection in microscopy, for microarrays or single-molecule tracking.[1] These developments have fueled the search for novel fluorophore (stains, labels, and indicators)[2] with high brightness that can be excited and which could emit well within the visible or near-infrared (NIR) region of the spectrum.[3] This is a part of continual shift away from the use of radioactive tracers in the bioanalytical study. Particularly, it eliminates the dangers of handling carcinogenic dyes, radioactive materials, and the cost of their proper disposal such as crystal violet, ethidium bromide, and ^{32}P in DNA staining analysis.

Accordingly, much research has been done to develop one-photon fluorescent (OPF) probes because they are useful tools for know how the functions in biological systems. One of the major drawbacks of such probes is that the excitation wavelengths are in the range of 350–560 nm, which may cause damage to the substrates specifically in the living

Received 23 March 2009. Accepted 27 August 2009. Published on the NRC Research Press Web site at canjchem.nrc.ca on 6 November 2009.

Abbreviations: OPF: one-photon fluorescent. TPF: two-photon fluorescent. FTIR: fourier transform infrared. UV–vis: ultraviolet–visible. NMR: nuclear magnetic resonance. XRD: X-ray diffraction. TG–DTA: thermogravimetric and differential thermal analysis. EtOAc: ethyl acetate. EtOH: ethanol.

M. Malathi and P.S. Mohan.[1] Department of Chemistry, Bharathiar University, Coimbatore 641 046, India.
R.J. Butcher. Department of Chemistry, Howard University, WA, USA.
C.K. Venil. Department of Environmental Science, Division of Microbiology, Bharathiar University, Coimbatore 641 046, India.

[1]Corresponding author (e-mail: ps_mohan_in@yahoo.com).

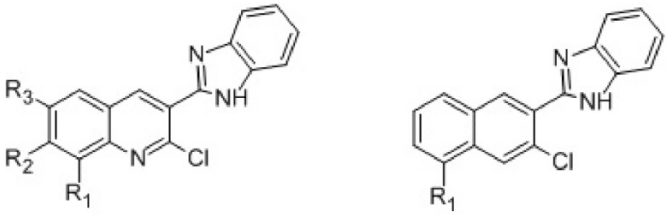
cells.[5] A fluorescent probe having higher excitation wavelength[6] overcomes these shortcomings in two ways: (*i*) using lower energy photon can cause less damage to the cells or tissues and (*ii*) lower energy photon has longer wavelength that can penetrate deep inside the tissue. Many applications of such materials have become reality, some of which include optical power limiting,[7] two-photon (TP) upconversion lasing,[8] TP fluorescence excitation microscopy,[9] three-dimensional optical-data storage,[10] and photodynamic therapy.[11] An important addition to such applications would be the development of TP probes for bioanalytical applications. As a result, the design and synthesis of fluorescent probes with higher excitation wavelength have attained the central position of the current research.[12]

A detailed search of structural correlation in the fluorescent probes reveals that the benzimidazole derivatives could act as an effective fluorescent probe.[13] In addition to the biological activity,[14] many of benzimidazole derivatives are studied as luminescent singling sensors for anions.[15] Recently, Zang et al.[16] have reported a Y-shaped TP-active material based on the imidazole core. Consecutively, Iyer et al.[17] have reported that the benzimidazole ring in a heteroaromatic system could exhibit efficient fluorescence-light-emitting property. All the reports reveal that the benzimidazole and its derivatives have different fluorescence efficiencies, depending on the molecular structure. Consequently, the molecular-structure-based designing is one of the convenient tools for the innovation of the new efficient fluorescent probes.

In this connection, quinoline system gains importance due to its recent revolution in optical chemistry.[18] Basically, quinoline derivatives have been synthesized and widely utilized as drugs for antimalarial, anticancer, and also as DNA intercalators.[19] In contrary, there has been a paucity of imperative papers account that the quinoline derivatives could be used as selective fluorescence chemosensors for biologically important metal ions, as fluorescent dopent in OLEDs and electrochemiluminescence,[20] and so forth. This kindles interest to study the optical property of the benzimidazole-substituted quinoline derivatives. Consecutively, the synthesis of benzimidazole naphthalene derivatives also intended to study the role of quinoline system in the optical property of the synthesized quinoline benzimidazole derivatives.[4]

On par with our objective, we propose a conventional synthetic methodology for the synthesis of quinoline and naphthalene benzimidazole derivatives (BIQ, BIN) in high yield (see Scheme 1 for structures). The optical properties of the synthesized compounds were analyzed by using absorption and emission spectral studies. Based on the fluorescence property, the applicability of the compounds in the bioanalytical studies is focused.

Scheme 1. Quinoline and naphthalene benzimidazoles.



1 a R_1, R_2, and $R_3 = H$
1 b $R_1 = CH_3$, R_2, and $R_3 = H$
1 c R_1, $R_3 = H$, $R_2 = CH_3$
1 d R_1, $R_2 = H$, $R_3 = CH_3$

2 a $R_1 = H$
2 b $R_1 = CH_3$

Experimental section

Materials and physical measurements

All reagents and chemicals were purchased from Ranchem chemicals. All the solvents were HPLC-grade. Thin-layer chromatography (TLC) was performed using glass plates coated with silica gel-G containing 13% calcium sulphate as binder. Melting points were determined using a Raga melting point apparatus and are uncorrected. The starting quinoline carbaldehyde compounds were synthesized according to the reported procedure.[21] The naphthalene carbaldehyde starting material was purchased from Sigma-Aldrich chemicals and used without further purification. FTIR spectra (4000–400 cm^{-1}) of compounds were recorded as KBr pellets with a Nicolet Avatar Model FTIR spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 (400 MHz) spectrometer in $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Trace DSQ mass spectrograph. Microanalyses (C, H, and N) were performed on a Vario EL-III Elementar elemental analyzer. UV–vis absorption spectra (200–800 nm) were recorded using a Shimadzu UV/2501PC spectrophotometer. The fluorescence spectra were recorded using JASCO FP-6600 spectrofluorometer (Xenon lamp). The fluorescence microscopy analysis was done by using Nikon fluorescence microscopes.

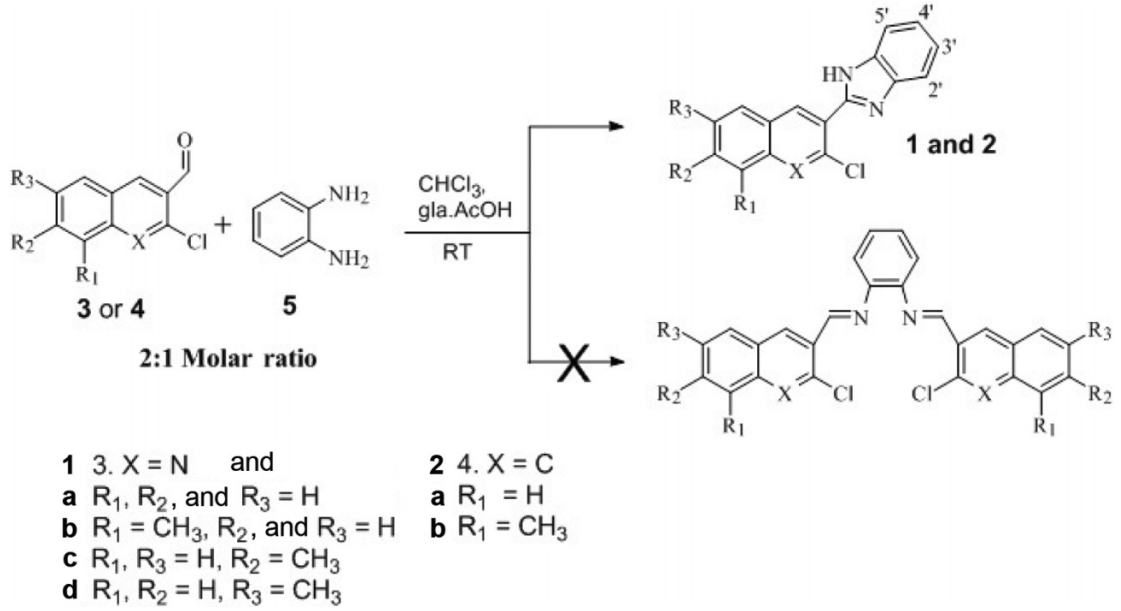
Synthesis of benzimidazole compounds

Scheme 2 outlines the general procedure for the synthesis of quinoline and naphthalene benzimidazole derivatives. The aldehyde compound (**3** or **4**) in chloroform was stirred with two drops of glacial acetic acid. To this, benzene-1,2-diamine (**5**) was added, and the stirring was continued for about 15 min. The formation of product was confirmed by TLC. Excess $CHCl_3$ was evaporated, and the resulting crude was washed with water. The dried crude was adsorbed for column chromatography using petroleum ether and EtOAc mixture as eluent. The benzimidazole product was isolated in petroleum ether/EtOAc (9:1) medium. The obtained product was characterized by IR, ^1H and ^{13}C NMR, mass, and elemental analyses.

Synthesis of 3-(1H-benzoimidazol-2-yl)-2-chloro-quinoline (1a)

The compound was prepared as described above by the reaction of 2-chloro-quinoline-3-carbaldehyde (10 mmol, 1.73 g) and benzene-1,2-diamine (5 mmol, 0.541 g). Off-white solid (yield: 1.25 g, 89.33%); mp 202 °C. IR (KBr): 3424, 1607, 1405, 1034, 741. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.27 to 7.38 (m, 2H, C6–quinoline–Q–H, C3′–benzo–BE–H), 7.60–7.64 (m, 2H, C2′ and C5′ of BE–H), 7.79–7.83 (m, 2H, C7–Q–H and C4′–BE–H), 7.92–7.94 (d, 1H, J = 8 Hz, C5–Q–H), 8.03–8.05 (d, 1H, J = 8 Hz, C8–Q–H), 9.26 (s, 1H, C4–Q–H), 10.59 (bs, NH–imidazole–IMD–H). ^{13}C NMR δ: 120.22, 122.66, 123.84, 126.31, 127.10, 127.45, 127.81, 130.59, 131.30, 131.80, 136.21, 140.83, 141.11,

Scheme 2. Preparation of quinoline and naphthalene benzimidazole derivatives.



146.68, 147.03, 147.38. EI-MS: m/z = [M$^+$] 279, [M + 2]$^+$ 281. Anal. calcd. for $C_{16}H_{10}N_3Cl$: C, 68.70; H, 3.60; N, 15.02. Found: C, 68.55; H, 3.48; N, 14.78.

*Synthesis of 3-(1*H*-benzoimidazol-2-yl)-2-chloro-8-methyl-quinoline (1b)*

The compound was prepared as described above by the reaction of 2-chloro-8-methyl-quinoline-3-carbaldehyde (9 mmol, 1.85 g) and benzene-1,2-diamine (4.5 mmol, 0.4866 g). Off-white solid (yield: 1.4 g, 89.82%); mp 215 °C. IR (KBr): 3432, 1604, 1330, 1024, 760. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.78 (s, 3H, C8–Q–CH_3–H), 7.34–7.38 (m, 2H, C3′ and C4′ BE–H), 7.48–7.52 (t, 1H, C6–Q–H, J = 8 Hz), 7.62–7.64 (d, 1H, C7–Q–H, J = 8 Hz), 7.74–7.76 (d, 3H, C5–Q–H, C2′ and C5′ BE–H, J = 8 Hz), 9.22 (s, 1H, C4–Q–H), 10.40 (bs, NH–IMD–H). ^{13}C NMR δ: 22.05, 120.31, 122.26, 123.42, 126.27, 126.99, 127.52, 127.82, 130.59, 131.34, 131.81, 136.22, 140.84, 141.11, 146.69, 147.12, 147.38. EI-MS: m/z = [M$^+$] 293, [M + 2]$^+$ 295. Anal. calcd. for $C_{17}H_{12}N_3Cl$: C, 69.51; H, 4.12; N, 14.31. Found: C, 69.43; H, 3.98; N, 14.18.

*Synthesis of 3-(1*H*-benzoimidazol-2-yl)-2-chloro-7-methyl-quinoline (1c)*

The compound was prepared as described above by the reaction of 2-chloro-7-methyl-quinoline-3-carbaldehyde (9 mmol, 1.85 g) and benzene-1,2-diamine (4.5 mmol, 0.4866 g). Off-white solid (yield: 1.305 g, 88.80%); mp 220 °C. IR (KBr): 3435, 1606, 1370, 1044, 752. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.57 (s, 3H, C7–Q–CH_3–H), 7.33 to 7.38 (m, 3H, C6–Q–H, C3′ and C4′–BE–H), 7.48–7.45 (dd, 1H, J_1 = 8 Hz, J_2 = 4 Hz, C5–Q–H), 7.83–7.86 (m, 3H, C8–Q–H, C2′ and C5′–BE–H), 9.25 (s, 1H, C4–Q–H), 10.42 (bs, NH–IMD–H). ^{13}C NMR δ: 22.03, 120.25, 121.92, 123.39, 126.32, 124.82, 127.15, 127.78, 130.13, 131.32, 131.81, 136.21, 140.82, 142.75, 145.80, 147.39, 147.66. EI-MS: m/z = [M$^+$] 293, [M + 2]$^+$ 295. Anal. calcd. for $C_{17}H_{12}N_3Cl$: C, 69.51; H, 4.12; N, 14.31. Found: C, 69.42; H, 3.97; N, 14.12.

*Synthesis of 3-(1*H*-benzoimidazol-2-yl)-2-chloro-6-methyl-quinoline (1d)*

The compound was prepared as described above by the reaction of 2-chloro-6-methyl-quinoline-3-carbaldehyde (9 mmol, 1.85 g) and benzene-1,2-diamine (4.5 mmol, 0.4866 g). Off-white solid (yield: 1.305 g, 88.80%); mp 220 °C. IR (KBr): 3443, 1605, 1334, 1024, 762. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.64 (s, 3H, C6–Q–CH_3–H), 7.34–7.38 (m, 2H, C3′ and C4′–BH–H), 7.48–7.52 (m, 2H, C5 and C7–Q–H), 7.76–7.81 (d, 3H, C8–Q–H, C2′ and C5′–BH–H, J = 8 Hz), 9.25 (s, 1H, C4–Q–H), 10.42 (bs, NH–IMD–H). ^{13}C NMR δ: 22.03, 120.25, 122.67, 123.02, 126.32, 127.11, 127.45, 127.81, 130.59, 131.32, 131.81, 136.21, 140.83, 141.11, 146.69, 147.03, 147.40. EI-MS: m/z = [M$^+$] 293, [M + 2]$^+$ 295. Anal. calcd. for $C_{17}H_{12}N_3Cl$: C, 69.51; H, 4.12; N, 14.31. Found: C, 69.32; H, 3.89; N, 14.14.

*Synthesis of 2-(3-chloro-naphthalen-2-yl)-1*H*-benzoimidazole (2a)*

The compound was prepared as described above by the reaction of 3-chloro-naphthalene-2-carbaldehyde (9 mmol, 1.72 g) and benzene-1,2-diamine (4.5 mmol, 0.4866 g). Yellowish orange solid (yield: 1.125 g, 89.70%); mp 210 °C. IR (KBr): 3470, 1614, 1321, 744. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.25–7.34 (m, 4H, C6 and C7–naphthalene–NA, C3′ and C4′–BH–H), 7.65–7.67 (d, 2H, C5 and C8–NA, J = 8 Hz), 7.69–7.75 (m, 3H, C4–NA, C2′ and C5′–BE–H), 7.84 (s, 1H, C1–NA–H), 9.5 (bs, NH–IMD–H). ^{13}C NMR δ: 115.4, 115.5, 122.9, 123.0, 126.1, 126.9, 127.0, 127.1, 127.8, 128.0, 130.1, 132.2, 134.3, 134.7, 137.9, 138.0, 141.5. EI-MS: m/z = [M$^+$] 278, [M + 2]$^+$ 280. Anal. calcd. for $C_{17}H_{11}N_2Cl$: C, 73.26; H, 3.98; N, 10.05. Found: C, 73.15; H, 3.79; N, 9.85.

*Synthesis of 2-(3-chloro-5-methyl-naphthalen-2-yl)-1*H*-benzoimidazole (2b)*

The compound was prepared as described above by the

reaction of 3-chloro-5-methyl-naphthalene-2-carbaldehyde (9 mmol, 1.84 g) and benzene-1,2-diamine (4.5 mmol, 0.4866 g). Yellowish orange solid (yield: 1.132 g, 85.65%); mp 225 °C. IR (KBr): 3456, 1607, 1332, 756. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.61 (s, 3H, C6–Q–CH_3–H), 7.20–7.29 (m, 4H, C6 and C7–NA–H, C3′ and C4′–BE–H), 7.59–7.63 (d, 2H, C5 and C8–NA–H, J = 8 Hz), 7.65–7.72 (m, 3H, C4–NA–H, C2′ and C5′–BE–H), 7.82 (s, 1H, C1–NA–H), 9.2 (bs, 1H, NH–IMD–H). ^{13}C NMR δ: 20.63, 114.2, 114.5, 121.9, 122.0, 125.1, 125.9, 127.2, 127.5, 127.9, 128.0, 130.2, 132.2, 134.2, 134.5, 137.5, 138.2, 140.5. EI-MS: m/z = [M$^+$] 292, [M + 2]$^+$ 294. Anal. calcd. for $C_{17}H_{11}N_2Cl$: C, 73.85; H, 4.48; N, 9.57. Found: C, 73.65; H, 4.34; N, 9.47.

Optical measurements

For the absorption analysis, stock solution was prepared by dissolving 10 mg of BIQ derivatives in 100 mL ethanol. The solution was diluted to 10^{-5} mol/L concentration for the absorption and 10^{-7} mol/L for the emission analysis. But in the case of solvent polarity study of **1b**, the compounds in the specified solvent medium were only used for both absorption and emission analysis. The fluorescence intensity vs. pH titration was carried out using 10% aqueous alcoholic solution of the BIQ-**1b** compound. 0.1 mol/L HCL and 0.1 mol/L NaOH were used to adjust the pH of the medium.

Fluorescence decays were recorded using TCSPC method using the following setup. A diode-pumped millena CW laser (Spectra Physics) 532 nm was used to pump the Ti:sapphire rod in Tsunami picosecond mode locked laser system (Spectra Physics). The 750 (80 MHz) was taken from the Ti:sapphire laser and passed through pulse picker (Spectra Physics, 3980 2S) to generate 4 MHz pulses. The seond harmonic output (375 nm) was generated by a flexible harmonic generator (Spectra Physics, GWU 23PS). The vertically polarized 375 nm laser was used to excite the sample. The fluorescence emission at magic angle (54.7°) was dispersed in a monochromator (f/3 aperture), counted by a MCP PMT (Hamamatsu R 3809), and processed through CFD, TAC, and MCA. The instrument response function for this system is ~52 ps. The fluorescence decay was analyzed by using the software provided by IBH (DAS-6) and PTI global analysis software.[22]

Cytogenetic analysis

Cytogenetic analysis was performed for compound **1b** in human peripheral blood lymphocyte culture (PBLC). They were obtained from peripheral blood set-up following the method of Hungerford.[23] Two experiments were carried out using four smoking and non-smoking healthy male donors, aged 23, 24, and 28 years. The testing compound was dissolved in 1% methanol. Its four different concentrations of 0.02, 0.2, 2, and 20 μg mL^{-1} were added to the culture medium (0.1 mL solution of compound **1b** per 8 mL of the medium) after 48 h culture initiation. Duplicate cultures for each dose were maintained for the study of chromosomal aberrations. Then, cultures were incubated at 37 °C for a period of 24 (total 72 h) h. Methanol (1%) was applied to control I and II, respectively.

The cultures were shaken periodically up to three times a day. During the two hours before the culture harvest, the dividing cells were arrested in the metaphase by adding 0.05 mL of colchicine solution (w = 0.01%). The contents in the vial were centrifuged at 1200 rpm for 5 min at the end of the colchicine treatment. The supernatant was discarded, and 5 mL of pre-warmed hypotonic solution (0.075 mol L^{-1} KCl) was added to the cell button. The cells were incubated for 20 min, sedimented after centrifugation at 1200 rpm for 10 min, and then fixed in a freshly prepared fixative (methanol/glacial acetic acid, 3:1, v/v). Two or three changes of the fixative were applied. Slides were prepared by placing a drop of the cell suspension on a clean chilled slide and dried immediately at 40 °C for a few seconds. The slides were routinely stained in a 2% buffered solution of Giemsa.[23] Three-hundred-and-fifty well-banded metaphases were analyzed for each treatment under an oil immersion lens.

Staining analysis in the bacterial microbe

Potent strains of bacteria were isolated from the gut of sulfur butterfly (*Kricogonialyside*). The isolation and culturing experimental procedures of the bacteria were carried out using the reported methods.[24] The confirmed *Serratia marcescens* SB08 was used in the staining process. Initially, the bacterium was heat-fixed in the clean glass slide. Two or three drops of 10% ethanolic solution of **1b** (10^{-9} mol/L) was added to the heat-fixed bacterial smear and allowed to bind for 15–60 s. After that, the slide was rinsed with distilled water and air-dried. Then, the slide was examined under the fluorescence microscopy.

Results and discussion

Synthesis of benzimidazole derivatives

Recent exploration of pyrene- and benzimidazole-based compounds as long-wavelength fluorophores[25] raise our interest to synthesize quinoline and naphthalene derivatives containing benzimidazole unit and study their structural correlation in the optical property. To achieve our objective, the work is started by synthesizing benzimidazole compounds from the derivatives of quinoline and naphthalene aldehydes with 1,2-diaminobenzene (Scheme 2). Such quinoline benzimidazole derivative was first prepared by Bhanumathi et al.[26] in two-stage approach. Their synthetic method was low-yielding, time-consuming, and did not seem amenable for the synthesis of benzimidazole compounds as demanded by the need of their application. Additionally, Kidwai et al.[27] reported a bisquinoline formation in the condensation reaction of quinoline aldehyde with benzene-1,2-diamine. Their reaction conditions and medium are similar to the Bhanumathi et al. synthetic methodology. Recently, Chen reported a one-pot synthetic methodology for the synthesis of 2-benzimidazolyl quinoline by the direct condensation of diamine with quinoline acid.[28] In this sequence, the exploration of reaction conditions and temperature for the synthesis of benzimidazole derivatives from the diamine and aldehydes could be significant. It may give a valuable outlook for the synthesis of complex benzimidazole derivatives.

Initially, the reaction was carried out with the 1:1 molar ratio of reactants in chloroform with catalytic amount of glacial acetic acid. This reaction conditions resulted in the formation of a mixture of products. However, 2:1 molar ratio

Table 1. Yields, NH ^{1}H NMR, ν_{NH} IR, and absorption λ_{max} values of quinoline, naphthalene benzoimidazoles **1a**–**1d** and **2a**, **2b**.

Serial No.	Compound	Yield (%)	δ_H [a] (ppm)	IR $\nu_{(NH)}$ (cm^{-1})	λ_{max} in nm[b] ($\varepsilon \times 10^5$ M^{-1} cm^{-1})
1	**1a**	89.33	10.59	3424	346 (1.89)
2	**1b**	89.82	10.40	3432	354 (1.94)
3	**1c**	88.80	10.42	3435	351 (1.92)
4	**1d**	88.80	10.42	3443	352 (1.93)
5	**2a**	89.70	9.5	3470	317 (2.55)
6	**2b**	85.65	9.2	3456	332 (2.62)

[a]NH proton of the benzimidazole ring in the BIQ and BIN (400 MHz, $CDCl_3$).
[b]The 30 μmol/L ethanolic solution of BIQ and BIN compounds were used in the UV–vis absorption analysis.

of the reactants, i.e., aldehyde and diamine, respectively, produced a single product in the same reaction medium after 15 min. The purification of the products from the crude mixture was done by column chromatography using petroleum ether:ethyl acetate (9:1) mixture as eluent. The structure of the products was confirmed by IR, NMR, mass, and elemental analyses. The single-crystal structure of one of the quinoline benzimidazole derivatives (**1b**) was solved by X-ray crystallographic technique and was reported.[29] The characterization analysis supports the formation of benzimidazole derivatives using the 2:1 molar ratio of reactants. The same reaction was carried out smoothly in a beaker without changing the environmental condition, such as the high temperature, inert atmosphere, and expensive catalysts. It is believed that the presence of excess aldehydes might help to stop the backward reaction.

In the characterization analysis, a broad spectral correlation could be observed specifically in the benzimidazole N–H functional group of BIQ and BIN derivatives (Table 1). The IR spectrum of the BIQ and BIN derivatives shows ν_{NH} band in the region of 3420–3445 and 3455–3470 cm^{-1}, respectively. The lower ν_{NH} wavelength for BIQ compounds illustrates the existence of strong hydrogen bonding (Imida–N–H···Cl–Q) in their molecular structure. In addition, the ^{1}H NMR spectra of BIQ and BIN derivatives show a pronounced deshielding effects in the δ value of BIQ–N–H functional group (δ 10.40–10.59 and 8.52–8.55, respectively), which further confirms the existence of strong hydrogen bonding (Imida–N–H···Cl–Q) in the BIQ derivatives. It is concluded that the nitrogen lone-pair electrons in the quinoline ring could facilitate the existence of strong hydrogen bonding in the BIQ derivatives. This specific molecular structural conformation will play a vital role in the optical behavior of BIQ compounds compared with BIN compounds.

Absorption and emission spectral studies

The optical analyses of BIQ and BIN compounds were recorded in ethanol. Figure 1 shows the absorption spectra of compounds **1b** and **2b**. In general, UV–vis absorption spectra of BIQ and BIN ethanolic solutions exhibit peaks with λ_{max} in the range of 340 to 355 nm (Table 1). λ_{max} values of the respective compounds was used to excite the corresponding compound in the spectrofluorimetric analysis. The resulting fluorescence spectra of BIQ and BIN compounds prove the existence of fluorescence property only in BIQ compounds.

The absorption λ_{max} of **1b** is 354 nm in ethanol. This solution, on excitation in the range of λ_{max} 350–355 nm (single excitation), exhibits two fluorescence peaks around λ_{max} 432 and 851 nm (Fig. 2*a*). But the intensity of the first fluorescence peak is 10 times higher compared with the following peak (10:1). The same solution, on excitation in the range of λ_{max} 700–710 nm (double excitation), exhibits a fluorescence peak and a hump (Fig. 2*b*) around λ_{max} 433 and 849 nm, respectively. In this double excitation, the intensity ratio of the fluorescence peak and hump is also found to be 10:1. However, the fluorescence peak intensity in the single excitation (350–355 nm) is five times higher than the doubly excited (700–710 nm) fluorescence peak. This shows the sensitivity of the double excitation process. All the spectrofluorimetric experiments of **1b** were repeated in different solvents, i.e., in CH_2Cl_2, $CHCl_3$, DMF, DMSO, and EtOH. The combined spectral data expose the double-excitation property of **1b** in the above mentioned solvent. Additionally, it illustrates that the intensity of the fluorescent λ_{max} is higher in polar solvents than in non-polar solvents.

All the observed results propose that the BIQ derivatives should have intramolecular charge transfer (ICT) interaction in the solution form. ICT occurs between the quinoline and imidazole ring systems during electronic excitation. It restricts the internal rotation of imidazole unit within the molecule, which creates a self-organized supramolecular architecture. This molecular conformational arrangement of

Fig. 1. The UV–vis absorption spectra of the BIQ and BIN compounds in 30 μmol/L concentration: (*i*) absorption spectra of BIQ-**1b** with λ_{max} = 336 and 354; (*ii*) absorption spectra of BIN-**2b** with λ_{max} = 332 and 350 nm.

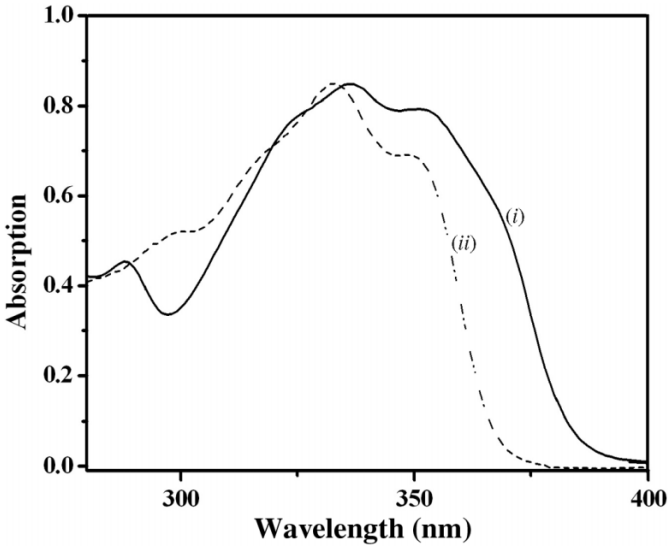
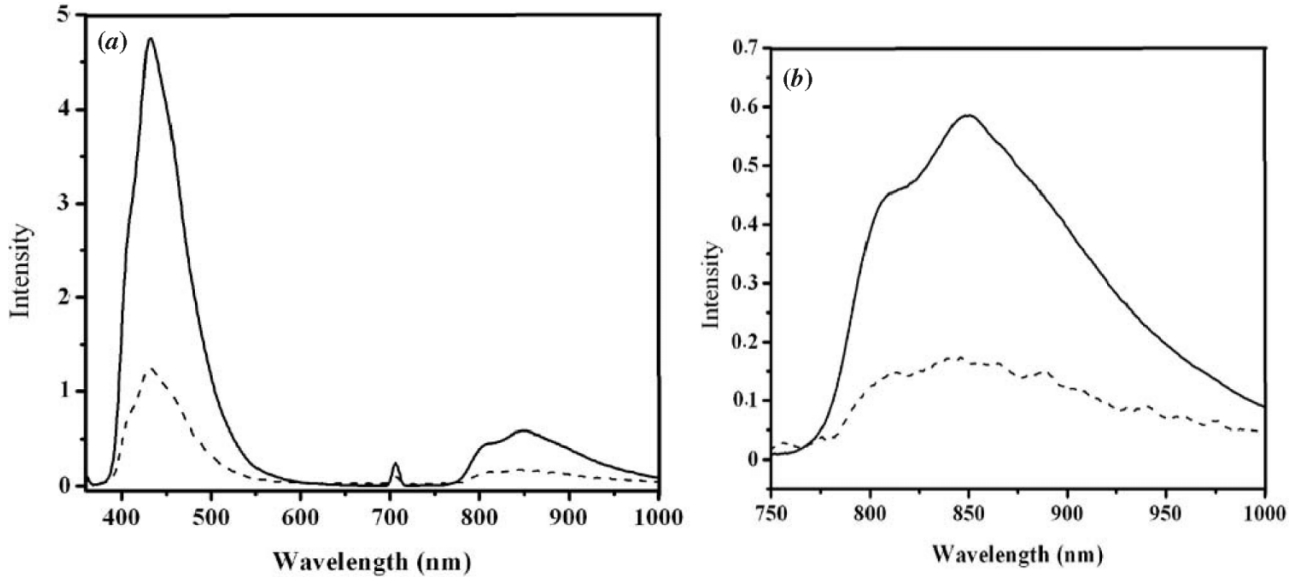


Fig. 2. Fluorescence spectra of ethanolic solution of BIQ-**1b** in 0.3 μmol/L concentration: (*a*) Solid line: emission spectra of **1b** in the excitation wavelength λ_{exc} 354 nm and the resulting emission λ_{emi} = 434 and 852 nm; dashed line: emission spectra of **1b** in the excitation wavelength λ_{exc} = 708 nm and the resulting emission λ_{emi} = 447 nm. (*b*) Expansion form of fluorescence spectra of **2a** from 750 to 1000 nm.



excited molecules can facilitate radiative decay during the electronic relaxation process, while in the case of naphthalene bearing imidazole moiety, the absence of ICT can sustain the internal rotation, thereby the electronic relaxation frequently takes place as non-radiative decay process.[30] Spectrofluorimetric analysis of other derivatives of BIQ (**1a**, **1c**, and **1d**) also exhibits the higher wavelength excitation and fluorescence emission property in solution.

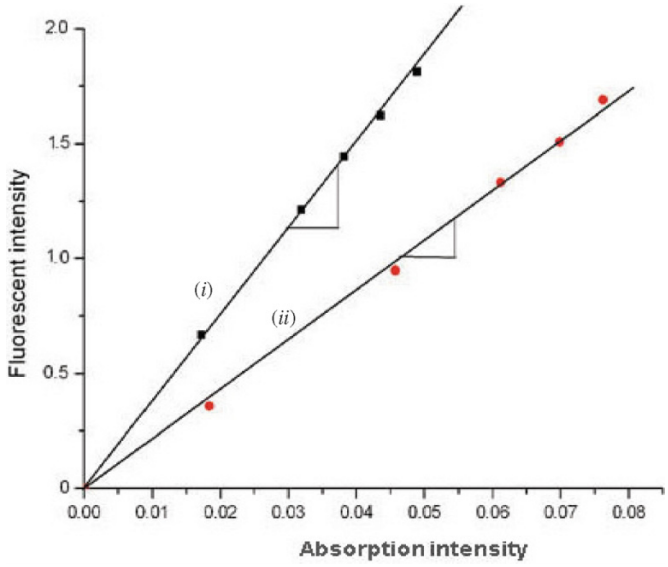
Fluorescence quantum-yield measurement

Fluorescence quantum yield (ϕ_f) of the BIQ derivatives (x) in ethanol was determined using 2-amino pyridine as a standard (std), since both compounds have excitation wavelength in the range of 340–450 nm. 0.1 mol/L H_2SO_4 solution was used in the preparation of 2-amino pyridine solution in different concentrations. For quantum-yield measurement, the absorption and fluorescence spectra of 2-amino pyridine solution were recorded in the following concentrations: 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1 μmol/L. The spectral experimental procedures were repeated in the ethanolic solution of BIQ derivatives in the same concentrations. The integrated fluorescence intensity vs. absorbance was plotted (Fig. 3), and the slope was calculated. The quantum yields of the benzimidazolyl quinoline compounds were calculated by applying the observed experimental data in the following equation:

$$[1] \qquad \phi_x = \phi_{std}\left(\frac{Grad_{std}}{Grad_x}\right)\left(\frac{\eta_x^2}{\eta_{std}^2}\right)$$

Here, ϕ_x and ϕ_{std} denote the fluorescence quantum yields of unknown (BIQ derivatives) and standard (2-amino pyridine) compounds, respectively. The "Grad" and η denote the gradient (slop) of the graph and refractive index of the respective solvent, respectively. The ϕ_{std} of the 2-amino pyridine in 0.1 mol/L H_2SO_4 solution is 0.6. The refractive index of the 0.1 mol/L H_2SO_4 and ethanol is 1.36 and 1.003, respectively. By doing the calculation, the fluorescence quantum yields (ϕ_f) of the BIQ derivatives are determined, and the values are tabulated (Table 2).

Fig. 3. Fluorescence quantum-yield measurement of **1b** (μmol/L concentration range). (*i*) Fluorescent vs. absorption intensities of 2-aminopyridine in 0.1 mol/L H_2SO_4 solution, λ_{exc} = 335 nm and λ_{emi} = 370 nm. (*ii*) Fluorescent vs. absorption intensities of BIQ-**1b** in ethanol, λ_{exc} = 354 nm and λ_{emi} = 434 nm.



Fluorescence lifetime measurement

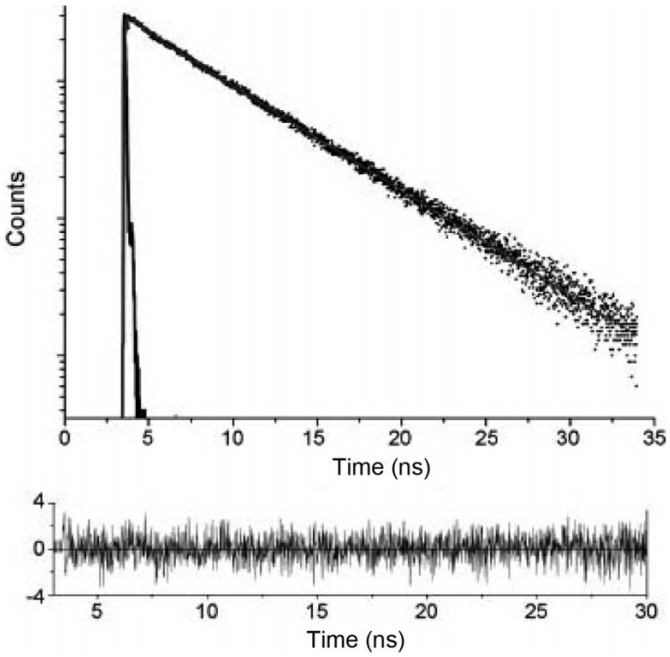
Time-dependent measurements of receptors have been performed using time-correlated single-photon counting (TCSPC) technique (Fig. 4). The fluorescecent lifetime of

Table 2. UV–vis, fluorescence spectra, quantum yield, and lifetime measurement data of compounds **1a–1d** in selected solvents.

	DMF			Ethanol			Water[b]				
Compounds[a]	$\lambda_{max\ ab}$	$\lambda_{max\ fl}$ [c]	$\lambda_{max\ fl}$ [d]	$\lambda_{max\ ab}$	$\lambda_{max\ fl}$ [c]	$\lambda_{max\ fl}$ [d]	$\lambda_{max\ ab}$	$\lambda_{max\ fl}$ [c]	$\lambda_{max\ fl}$ [d]	ϕ_f [e]	τ [e]
1a	317 332	415 805	410	322 346	424 824	430	348 365	432 861	432	0.501	2.12
1b	326 342	425 838	421	337 354	434 852	447	354 375	442 875	456	0.629	3.03
1c	322 339	424 839	423	331 351	433 851	445	350 372	441 874	455	0.617	3.09
1d	327 341	423 838	424	334 352	432 853	448	352 376	440 875	454	0.622	3.03

[a]30 and 0.3 μmol/L concentration of BIQ compounds were used to measure the absorption and emission spectra, respectively.
[b]10% ethanolic medium was used.
[c]Single-photon excitation.
[d]Double-photon excitation.
[e]The fluorescence quantum-yield measurements were done in ethanol using 2-aminopyridine as a standard.

Fig. 4. Fluorescence decay and residual curves profiles of BIQ-**1b** in ethanol, λ_{exc} = 354 nm and λ_{emi} = 432 nm. The solid lines represent the best fit to the data.



each BIQ derivative was measured in ethanol using the corresponding solution absorption λ_{max} wavelength as the excitation wavelength. The fluorescence lifetimes (τ_f) of the compounds are found to be in the range of 2–3 ns (Table 2).

Fluorescence efficiency

To optimize the fluorescence efficiency of BIQ derivatives, the influence of solvent polarity and pH of the medium on the optical property of **1b** was studied. In this fluorescence-optimizing spectrofluorimetric analysis, the single excitation of **1b** (428–435 nm) is mainly accounted.

Solvent-polarity study

The absorption and emission of **1b** were studied in different solvents with increasing polarity (toluene, CH_2Cl_2, $CHCl_3$, DMSO, DMF, EtOH, and 10% EtOH). Absorption spectra of the quinoline benzimidazole derivatives showed a gradual red shift with the increase in solvent polarity. This shift is much pronounced in the last three solvents in the following order: DMF < EtOH < H_2O (10% ethanol) (Fig. 5). Absorption λ_{max} values of **1b** in different solvents were used to record fluorescence spectra of the corresponding solutions. The recorded fluorescence spectrum shows red shift with the increase in solvent polarity. This illustrates the utility of this compound as a polar fluorescent probe (Fig. 6).[31] The experiment was repeated with double-excitation wavelength λ_{max}. The resulting spectra in the combined form were shown in Fig. 7. Absorbance and fluorescence wavelength λ_{max} of all other BIQ derivatives (**1a**, **1c**, and **1d**) in DMF, EtOH, and water (10% ethanol) were measured and tabulated (Table 2).

Figures 5, 6, and 7 point out that **1b** in the excited state is more polar than in the ground state. For the increase of the polarity of the solvent, the energy level which corresponds to a more polar molecular conformation will be more inten-

Fig. 5. UV–vis absorption spectra of BIQ-**1b** in (*i*) toluene, λ_{max} = 312 nm; (*ii*) DMF, λ_{max} = 326 and 342 nm; (*iii*) EtOH, λ_{max} = 330 and 354 nm; (*iv*) 10% aqueous ethanol, λ_{max} = 354 and 375 nm.

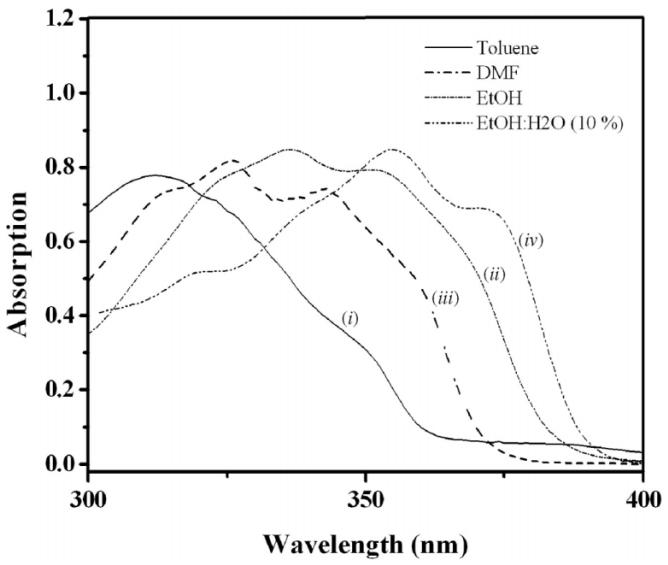
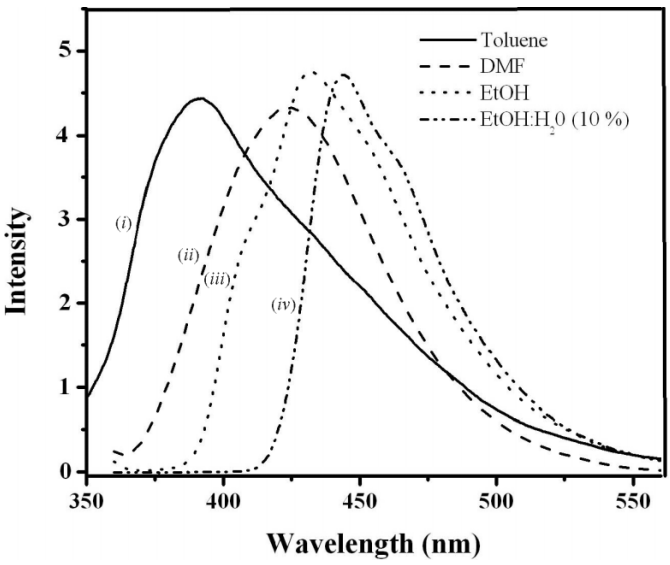


Fig. 6. Emission spectra of **1b** (single excitation) in (*i*) toluene, λ_{exc} = 312 nm and λ_{emi} = 370 nm; (*ii*) DMF, λ_{exc} = 342 and λ_{emi} = 425 nm; (*iii*) EtOH, λ_{exc} = 354 nm and λ_{emi} = 434 nm; (*iv*) 10% aqueous ethanol, λ_{exc} = 375 nm and λ_{emi} = 442 nm.



sively lowered.[32] Hence, a red shift of the wavelength is observed in the absorption and emission spectra of **1b** with the increase in solvent polarity.

In addition to the red shift of wavelength, Figs. 6 and 7 illustrate marked difference in the λ_{max} of the solution during double excitation compared with the single excitation. This further confirms the sensitivity of the double-excitation processes. This type of shifting also suggests that the excited geometrical conformation of **1b** may be suitable for the model of twisted intramolecular charge transfer (TICT).[32]

Fluorescence pH titration

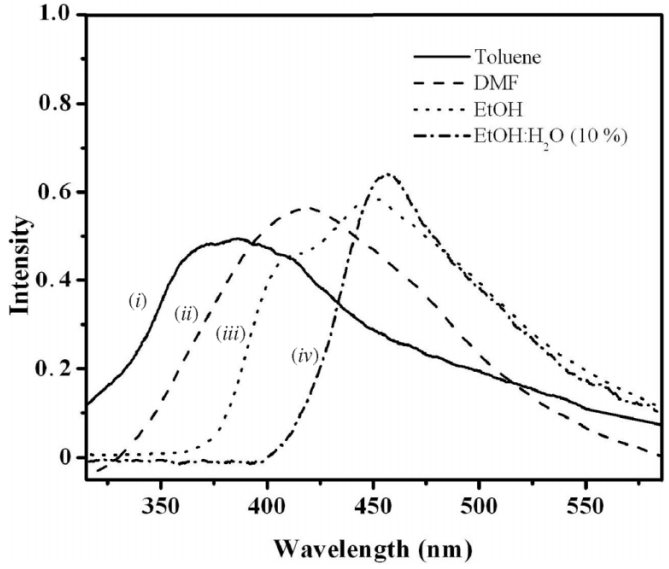
Quantitative pH titration was performed to find the fluorescent efficiencies of BIQ compounds in the wide range of pH. In this analysis, the intensity of first fluorescence peak during the single excitation of **1b** (λ_{exc} = 354 nm and λ_{emi} = 432 nm) was accounted. The fluorescence spectrum of the 3 μmol/L aqueous ethanol solution of **1b** was recorded in the pH range of 2 to 14, and a plot was drawn between the fluorescence intensity and pH. Figure 8 shows the fluorescent intensity vs. pH titration plot of **1b**. From the graph, it is observed that the fluorescent intensity is higher in the lower pH range and continuously decreases in higher pH range, owing to the deprotonation of the N atoms in the basic medium. However, it should be noted that the difference in the fluorescent intensity is very little when the pH is greater than 6.5. The fluorescent stability of **1b** in this pH range might be helpful to avoid the interference of possible pH change induced by biological stimulation.

Bioanalytical study of 1b

Cytogenetic analysis

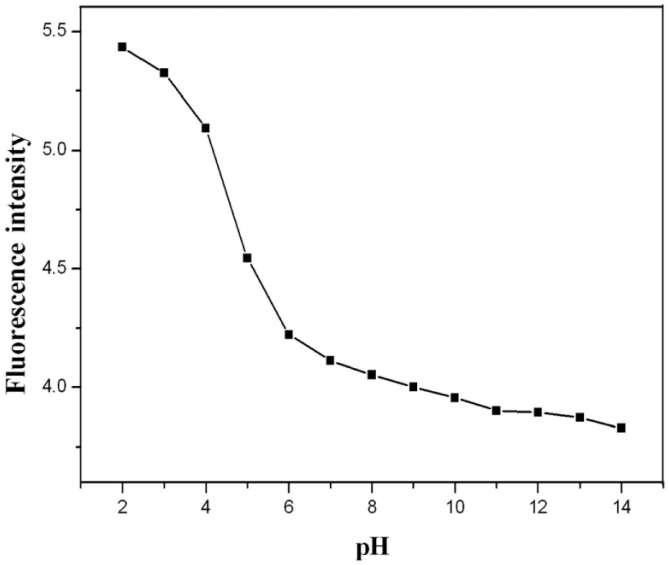
The observed results in the optical measurements support the suitability of BIQ derivatives as efficient polar fluorescent probes for bioanalytical study. But in the case of in vivo analysis, the cytotoxicity of the compound is the main factor which determines the suitability of the compound in cell-line analysis. With this necessity, the cytogenetic analysis was performed by using one of the BIQ compounds **1b** to scale up the utility of the derivatives in living-cells analysis. The preparation of the metaphase plates is detailed in the Experimental section.

Fig. 7. Emission spectra of **1b** (double excitation) in (*i*) toluene, λ_{exc} = 624 nm and λ_{emi} = 385 nm; (*ii*) DMF, λ_{exc} = 684 nm and λ_{emi} = 421 nm; (*iii*) EtOH, λ_{exc} = 708 nm and λ_{emi} = 447 nm; (*iv*) 10% aqueous ethanol, λ_{exc} = 750 nm and λ_{emi} = 456 nm.



All the prepared slides in well metaphase plates were screened for chromosomal aberrations (Table 3). The aberration includes endoreduplication, fragments, chromatid breaks, and chromatid gaps. The clastogenic properties of the compound were studied by investigating the effects of the compounds on human chromosomes through in vitro

Fig. 8. Fluorescence pH titration of BIQ-**1b** (3 μmol/L of 10% aqueous ethanolic solution) with λ_{exc} = 354 nm and λ_{emi} = 432 nm. The pH of the solution was adjusted by using 0.1 mol/L HCl and 0.1 mol/L NaOH to get acidic and basic pH, respectively.



analysis in the lymphocyte cultures. The data of the compound are pooled and shown in Table 3. It was noted that the increase in the chromosomal aberrations is dose-dependent. Chromosomal aberrations like chromatid gaps and chromatid breaks were also observed. Polyploid cells were absent in all examined slides. From Table 3, it is inferred that the compound did not show any aberration up to 10^{-4} mol/L concentration, which is essential for cell-lines in vitro studies.

Thermal analysis

BIQ compounds are remarkably stable towards air. It can be kept under air for a long time without any noticeable decomposition. Figure 9 shows the TG–DTA curves of **1b**. Compound **1b** exhibits two steps of decomposition in TG. The endothermic peak at 200 °C corresponds to the melting point of the compound. The first exothermic peak at 275 °C corresponds to the loss of chlorine. Then, a complete exothermic decomposition of the molecule occurs between 500 and 800 °C. During the decomposition, the masses of the compound, intermediate, and final product are those which best fit with observed mass loss in TG curve. The TG results are in good agreement with the DTA data. From this study, it is inferred that the compound is highly stable up to 250 °C.

Staining study

All the photophysical, cytotoxicity, and thermal analyses of BIQ derivatives support its applicability for bioanalytical studies. This creates an intention to carry out the staining analysis using BIQ compounds as staining dye. The appreciable fluorescence efficiency (high ϕ_f, water solubility, and thermal stability analysis) of **1b** makes it attractive for the staining analysis. For the initial staining analysis, we prefer small microorganism selectively bacteria because they are one of the primary microbes used in the drug discovery. Apart from the pathogenic activity of bacteria, they have significant applications in the industrial biotech processes, such as biocatalysis,[33] bioremediation[34], and so forth, in such a way that leads to the implementation of green chemistry in various industrial processes. Additionally, the enzyme-producing bacteria have promising medicinal activity.[35] In this regard, many enzyme-producing bacteria are analyzed in anticancer studies.[36]

In this margin, *S. marcescens* a Gram-negative bacterium produces L-asparaginase enzyme that has anti-proliferative and anti-tumor activities.[37] Accordingly, its identification, proliferation, and its structural analysis become significant in the bacterial research. Recently, Venil et al.[24] discussed the factors affecting the production of enzyme L-asparaginase from the bacterium *S. marcescens* SB and optimized an effective medium for its production. In this progress, the fluorescence imaging of the bacteria should have significant value. The surface morphological study will reveal many fundamental features of this organism and produce novel tools to study previously inaccessible problems. Additionally, this staining analysis can give a fundamental idea about the affinity of the BIQ compounds in the cell wall and also decides its applicability for further bioanalytical studies, such as optical imaging of living systems, detection of intracellular free metal ions, and so forth. With this intension preliminarily, we concentrate the staining analysis in the Gram-negative bacterium *S. marcescens* SB08.

Bacterial staining analysis

The fluorescent microscopic view shows that **1b** is an excellent green-fluorescence light-emitting material (Fig. 10*a*). The details of the slide preparation are explained in the Experimental section. In addition to the fluorescence staining, normal Gram staining of the bacterium were also done. The normal Gram-stained bacterial smear in the slide was viewed in the microscope (Fig. 10*b*), whereas the BIQ-stained one was viewed in the fluorescence microscope. The clean rod-shape image (Fig. 10*c*) of the bacterium confirms the binding of **1b** to the surface of the bacterium.

Conclusion

A specific molar ratio is implemented for the synthesis of BIQ and BIN compounds at room temperature. This short-reaction-time method offers high yield with reference to the reported procedures. All the synthesized compounds are characterized by various spectral and analytical techniques (IR, ^{1}H, ^{13}C NMR, mass, and CHN analyses).

The preliminary optical analysis confirms the fluorescence property of the BIQ derivatives and also reveals that such property is silent in the BIN derivatives. This optical behavior of BIQ derivatives can be attributed to the quinoline moiety facilitating a strong charge-transfer interaction with the imidazole group during the electronic relaxation. Due to that, its derivatives alone show intense absorption and fluorescence bands with ϕ_f approximately close to 1. In addition to the fluorescence property, the spectrofluorimetric analysis of BIQ derivatives exhibits a higher excitation wavelength (λ_{exc} = 708 nm) and emission property (λ = 434 and 850 nm) in different solvents, since it is also an essential parameter for the recognition of TP fluorescence probes. This low energy excitation and emission photophys-

Table 3. Cytogenetic analysis of the BIQ compound **1b**.

	Control		**1b** (24 h)[a]			
Mean number of aberrations	0 h	24 h	0.02 μg mL^{-1}	0.2 μg mL^{-1}	2 μg mL^{-1}	20 μg mL^{-1}
Average No. of metaphases (n = 8)	400	400	400	400	400	400
Endoreduplication	—	0.57±0.20	0.59±0.23	0.60±0.25	0.62±0.27	1.69±0.24
Fragments	—	0.32±0.35	0.34±0.30	0.37±0.22	0.45±0.28	1.49±0.52
Chromatid breaks	—	0.89±0.318	0.94±0.20	0.97±0.19	1.02±0.15	1.34±0.47
Chromatid gaps	—	0.02±0.17	0.05±0.10	0.07±0.10	0.09±0.14	0.69±0.29

[a]Chromosomal aberrations after 24 h incubation of 48 h initiated human peripheral blood lymphocyte culture with compound **1b**.

Fig. 9. TG–DTA of BIQ-**1b**: (*i*) TG of BIQ-**1b**; (*ii*) DTA of BIQ-**1b**.

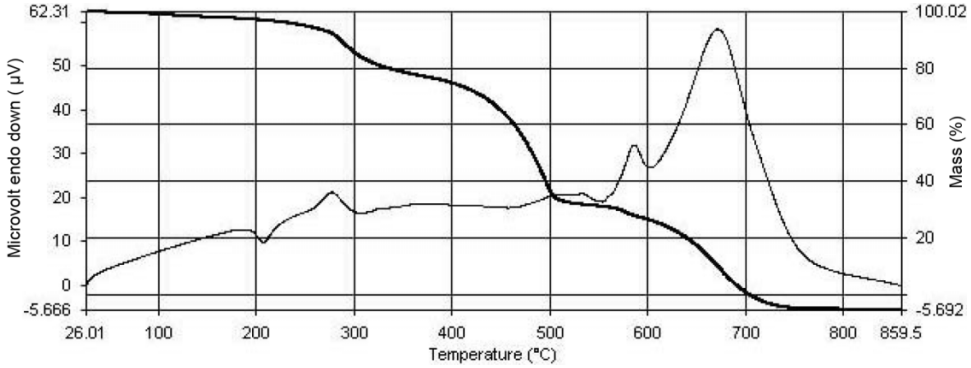
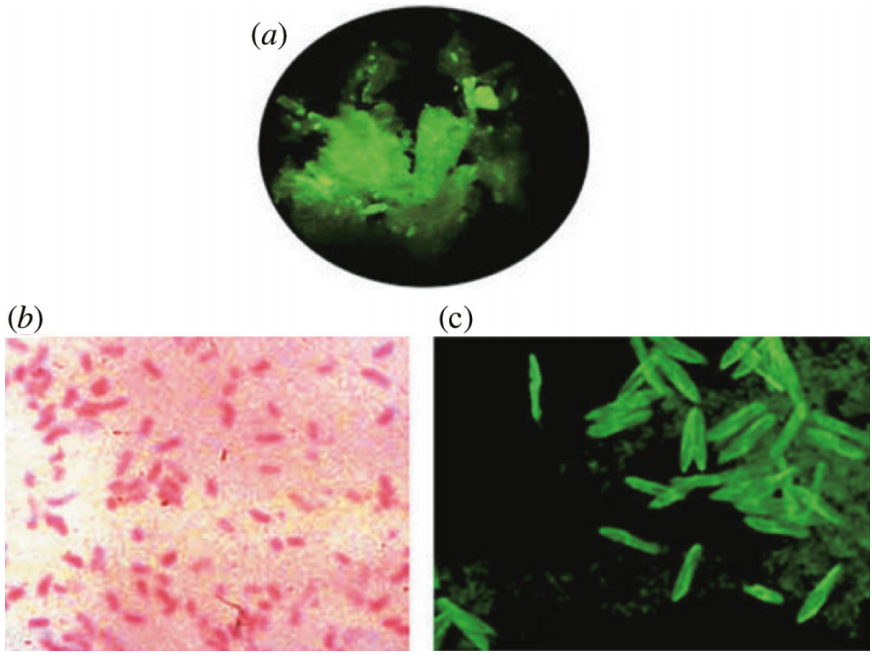


Fig. 10. Staining analysis of BIQ-**1b**: (*a*) fluorescent green-light-emitting view of BIQ-**1b**; (*b*) Gram-stained microscopic view of the *S. marcescens* SB; (*c*) BIQ-**1b**-stained fluorescence microscopic view of the *S. marcescens* SB.



ical property of BIQ compounds is significantly economic in the preliminary bioanalytical study of the living systems.

The fluorescence quantum yield (ϕ_f) and time-resolved fluorescent lifetime (T_f) of the BIQ derivatives were measured to evaluate its fluorescence efficiency. The optical analysis with increasing solvent polarity exposed the polar fluorescent probe nature of the BIQ derivatives. The fluorescence pH-titration measurement confirms the applicability of the BIQ derivatives in a wide range of pH.

In general, the combined optical spectral data of the BIQ and BIN derivatives conclude that the methyl derivatives have better photophysical properties in comparison with the unsubstituted derivatives. In addition to the optical property, a vast difference also exists in the IR and NMR spectral data of the substituted and unsubstituted benzimidazole derivatives. This is mainly due to the extension of conjugation (hyperconjugation)[38] facilitated by the methyl protons.

Based on the optical properties, one of the BIQ deriva-

tives (**1b**) was completely examined to evaluate its applicability in the bioanalytical studies. The TG–DTA analysis of **1b** exhibits that it is stable around the 250 °C and can withstand up to 500 °C with the loss of chlorine atom. The cytogenetic analysis exposed that up to the 10^{-4} mol/L concentration of **1b** can be used in living cells, which is expected to be sufficient for the in vitro analysis.

From the optimization, the bacterial staining applicability of **1b** was attempted. The **1b**-stained bacterial view in the fluorescence microscopy explores its staining ability. It reveals that BIQ derivatives could be good alternative for green beads in fluorescence-imaging studies. Solvent polarity and pH-sensitivity analyses illustrate the promising platform to design TP biomarkers from the BIQ derivatives. The low energy excitation and emission property of the BIQ derivatives in the solid form and its TP analysis are underway.

Supplementary data

Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 5304. For more information on obtaining material, refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.shtml.

Acknowledgement

One of the authors (MM) thanks the Council of Science and Industrial Research (CSIR), New Delhi, for the award of Senior Research Fellowship (SRF). Authors are thankful to the analytical instrumentation support from the NMR research center, IISc., Bangalore, India, and National Centre for Ultrafast Processes, University of Madras, Taramani Campus, India.

References

(1) (a) Cornish, P. V.; Ha, T. *ACS Chem. Biol.* **2007**, *2* (1), 53. doi:10.1021/cb600342a. PMID:17243783.; (b) Fricker, M.; Runions, J.; Moore, I. *Annu. Rev. Plant Biol.* **2006**, *57* (1), 79. doi:10.1146/annurev.arplant.57.032905.105239. PMID:16669756.; (c) Nagl, S.; Schaferling, M.; Wolfbeis, O. S. *Mikrochim. Acta* **2005**, *151*, 1.

(2) (a) Johnsson, N.; Johnsson, K. *ACS Chem. Biol.* **2007**, *2* (1), 31. doi:10.1021/cb6003977. PMID:17243781.; (b) Carol, P.; Sreejith, S.; Ajayaghosh, A. *Chem. Asian J.* **2007**, *2* (3), 338. doi:10.1002/asia.200600370. PMID:17441169.; (c) Wang, F.; Tan, W. B.; Zhang, Y.; Fan, X.; Wang, M. *Nanotechnology* **2006**, *17* (1), R1. doi:10.1088/0957-4484/17/1/R01.

(3) (a) Ghoroghchian, P. P.; Frail, P. R.; Susumu, K.; Park, T.-H.; Wu, S. P.; Uyeda, H. T.; Hammer, D. A.; Therien, M. J. *J. Am. Chem. Soc.* **2005**, *127* (44), 15388. doi:10.1021/ja055571b. PMID:16262400.; (b) Achilefu, S. *Technol. Cancer Res. Treat.* **2004**, *3* (4), 393. PMID:15270591.

(4) (a) Au, W.; Pathak, S.; Collie, C. J.; Hsu, T. C. *Mutat. Res.* **1978**, *58* (2-3), 269. doi:10.1016/0165-1218(78)90019-8. PMID:745616.; (b) Mohanty, K.; Naidu, J. T.; Meikap, B. C.; Biswas, M. N. *Ind. Eng. Chem. Res.* **2006**, *45* (14), 5165. doi:10.1021/ie060257r.; (c) Downing, T. W.; Garner, D. L.; Ericsson, S. A.; Redelman, D. *J. Histochem. Cytochem.* **1991**, *39* (4), 485. PMID:1900872.

(5) Haugland, R. P., Ed. *The Handbooks — A Guide to fluorescent Probes and Labeling Technologies,* 10th ed.; Molecular Probes: Eugene, OR, 2005.

(6) (a) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Anal. Chem.* **2004**, *327* (1), 82; (b) Woodroofe, C. C.; Won, A. C.; Lippard, S. J. *Inorg. Chem.* **2005**, *44* (9), 3112. doi:10.1021/ic048789s. PMID:15847416.

(7) (a) Fleitz, P. A.; Brant, M. C.; Sutherland, R. L.; Strohkendl, F. P.; Larson, J. R.; Dalton, L. R. *SPIE Proc.* **1998**, *91*, 3472; (b) He, G. S.; Bhawalkar, J. D.; Zhao, C. F.; Prasad, P. N. *Appl. Phys. Lett.* **1995**, *67* (17), 2433. doi:10.1063/1.114598.; (c) Ehrlich, J. E.; Wu, X. L.; Lee, I.-Y. S.; Hu, Z.-Y.; Röckel, H.; Marder, S. R.; Perry, J. W. *J. Opt. Lett.* **1997**, *22* (24), 1843. doi:10.1364/OL.22.001843.

(8) (a) Bhawalkar, J. D.; He, G. S.; Prasad, P. N. *Rep. Prog. Phys.* **1996**, *59* (9), 1041. doi:10.1088/0034-4885/59/9/001.; (b) He, G. S.; Zhao, C. F.; Bhawalkar, J. D.; Prasad, P. N. *Appl. Phys. Lett.* **1995**, *67* (25), 3703. doi:10.1063/1.115355.; (c) Zhao, C. F.; He, G. S.; Bhawalkar, J. D.; Park, C. K.; Prasad, P. N. *Chem. Mater.* **1995**, *7* (10), 1979. doi:10.1021/cm00058a032.

(9) (a) Denk, W.; Strickler, J. H.; Webb, W. W. *Science* **1990**, *248* (4951), 73. doi:10.1126/science.2321027. PMID:2321027.; (b) Mertz, J.; Xu, C.; Webb, W. W. *Opt. Lett.* **1995**, *20* (24), 2532. doi:10.1364/OL.20.002532.; (c) Denk, W.; Svoboda, K. *Neuron* **1997**, *18* (3), 351. doi:10.1016/S0896-6273(00)81237-4. PMID:9115730.; (d) Köhler, R. H.; Cao, J.; Zipfel, W. R.; Webb, W. W.; Hanson, M. R. *Science* **1997**, *276* (5321), 2039. doi:10.1126/science.276.5321.2039. PMID:9197266.; (e) Xu, C.; Zipfel, W. R.; Shear, J. B.; Williams, R. M.; Webb, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93* (20), 10763. doi:10.1073/pnas.93.20.10763. PMID:8855254.; (f) Denk, W. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91* (14), 6629. doi:10.1073/pnas.91.14.6629. PMID:7517555.

(10) (a) Parthenopoulos, D. A.; Rentzepis, P. M. *Science* **1989**, *245* (4920), 843. doi:10.1126/science.245.4920.843. PMID:17773360.; (b) Dvornikov, A. S.; Rentzepis, P. M. W. *Opt. Commun.* **1995**, *119* (3-4), 341. doi:10.1016/0030-4018(95)00338-9.; (c) Cumpston, B. H.; Ananthavel, S. P.; Barlow, S.; Dyer, D. L.; Ehrlich, J. E.; Erskine, L. L.; Heikal, A. A.; Kuebler, S. M.; Lee, I.-Y. S.; McCord-Maughon, D.; Qin, J.; Röckel, H.; Rumi, M.; Wu, X.-L.; Marder, S. R.; Perry, J. W. *Nature* **1999**, *398* (6722), 51. doi:10.1038/17989.

(11) Bhawalkar, J. D.; Kumar, N. D.; Zhao, C. F.; Prasad, P. N. *J. Clin. Laser Med. Surg.* **1997**, *15* (5), 201. PMID:9612170.

(12) (a) Gómez-Hens, A.; Aguilar-Caballos, M. P. *Trends Analyt. Chem.* **2004**, *23* (2), 127. doi:10.1016/S0165-9936(04)00305-X.; (b) Lord, S. J.; Lu, Z.; Wang, H.; Willets, K. A.; Schuck, P. J.; Lee, H.-L. D.; Nishimura, S. Y.; Twieg, R. J.; Moerner, W. E. *J. Phys. Chem. A* **2007**, *111* (37), 8934. doi:10.1021/jp0712598. PMID:17718454.; (c) Aslan, K.; Zhang, Y.; Geddes, C. D. *Anal. Chem.* **2009**, *81* (10), 3801. doi:10.1021/ac9001673. PMID:19354285.; (d) Cuppoletti, A.; Cho, Y.; Park, J.-S.; Strässler, C.; Kool, E. T. *Bioconjug. Chem.* **2005**, *16* (3), 528. doi:10.1021/bc0497766. PMID:15898718.

(13) (a) Jung, H. J.; Singh, N.; Jang, D. O. *Tetrahedron Lett.* **2008**, *49* (18), 2960. doi:10.1016/j.tetlet.2008.03.002.; (b) Joo, T. Y.; Singh, N.; Lee, G. W.; Jang, D. O. *Tetrahedron Lett.* **2007**, *48* (50), 8846. doi:10.1016/j.tetlet.2007.10.060.; (c) Krishnamoorthy, G.; Dogra, S. K. *J. Photochem. Photobiol. Chem.* **1999**, *123* (1–3), 109. doi:10.1016/S1010-6030(99)00030-1.

(14) (a) Reed, J. E.; Arnal, A. A.; Neidle, S.; Vilar, R. *J. Am. Chem. Soc.* **2006**, *128* (18), 5992. doi:10.1021/ja058509n.

PMID:16669641.; (b) Mandal, S. S.; Varshney, U.; Bhattacharya, S. *Bioconjug. Chem.* **1997**, *8* (6), 798. doi:10.1021/bc970121x. PMID:9404652.; (c) Chaudhuri, P.; Majumder, H. K.; Bhattacharya, S. *J. Med. Chem.* **2007**, *50* (10), 2536. doi:10.1021/jm0610604. PMID:17444624.; (d) Bhattacharya, S.; Chaudhuri, P. *Curr. Med. Chem.* **2008**, *15* (18), 1762. doi:10.2174/092986708785133013. PMID:18691037.

(15) (a) Tobey, S. L.; Anslyn, E. V. *Org. Lett.* **2003**, *5* (12), 2029. doi:10.1021/ol034427x. PMID:12790520.; (b) Kang, J.; Kim, H. S.; Jang, D. O. *Tetrahedron Lett.* **2005**, *46* (36), 6079. doi:10.1016/j.tetlet.2005.07.002.

(16) Zhang, M.; Li, M.; Zhao, Q.; Li, F.; Zhang, D.; Zhang, J.; Yi, T.; Huang, C. *Tetrahedron Lett.* **2007**, *48* (13), 2329. doi:10.1016/j.tetlet.2007.01.158.

(17) (a) Chetia, B.; Iyer, P. K. *Tetrahedron Lett.* **2008**, *49* (1), 94. doi:10.1016/j.tetlet.2007.11.011.; (b) Chetia, B.; Iyer, P. K. *Tetrahedron Lett.* **2007**, *48* (1), 47. doi:10.1016/j.tetlet.2006.11.022.; (c) Chetia, B.; Iyer, P. K. *Tetrahedron Lett.* **2006**, *47* (46), 8115. doi:10.1016/j.tetlet.2006.09.042.

(18) (a) Liu, Y.; Zhang, N.; Chen, Y.; Wang, L.-H. *Org. Lett.* **2007**, *9* (2), 315. doi:10.1021/ol062816w. PMID:17217293.; (b) Gross, E. M.; Anderson, J. D.; Slaterbeck, A. F.; Thayumanavan, S.; Barlow, S.; Zhang, Y.; Marder, S. R.; Hall, H. K.; Flore Nabor, M.; Wang, J.-F.; Mash, E. A.; Armstrong, N. R.; Wightman, R. M. *J. Am. Chem. Soc.* **2000**, *122* (20), 4972. doi:10.1021/ja0005993.

(19) Bonjean, K.; De Pauw-Gillet, M. C.; Defresne, M. P.; Colson, P.; Houssier, C.; Dassonneville, L.; Bailly, C.; Greimers, R.; Wright, C.; Quetin-Leclercq, J.; Tits, M.; Angenot, L. *Biochemistry* **1998**, *37* (15), 5136. doi:10.1021/bi972927q. PMID:9548744.

(20) (a) Bronson, R. T.; Montalti, M.; Prodi, L.; Zaccheroni, N.; Lamb, R. D.; Dalley, N. K.; Izatt, R. M.; Bradshaw, J. S.; Savage, P. B. *Tetrahedron* **2004**, *60* (49), 11139. doi:10.1016/j.tet.2004.08.062.; (b) Bronson, R. T.; Bradshaw, J. S.; Savage, P. B.; Fuangswasdi, S.; Lee, S. C.; Krakowiak, K. E.; Izatt, R. M. *J. Org. Chem.* **2001**, *66* (14), 4752. doi:10.1021/jo0017584. PMID:11442399.; (c) Kulkarni, P.; Tonzola, C. J.; Babel, A.; Jenekhe, S. A. *Chem. Mater.* **2004**, *16* (23), 4556. doi:10.1021/cm049473l.

(21) Ali, M. M.; Rajanna, K. C.; Prakash, P. K. S. *Synlett* **2001**, *2*, 251.

(22) Thiagarajan, V.; Ramamurthy, P. *J. Lumin.* **2007**, *126* (2), 886. doi:10.1016/j.jlumin.2007.01.003.

(23) Jude, A. L. C.; Sasikala, K.; Ashok Kumar, R.; Sudha, S.; Raichel, J. *Int. J. Hum. Genet.* **2002**, *2*, 95.

(24) (a) Venil, C. K.; Nanthakumar, K.; Karthikeyan, K.; Lakshmanaperumalsamy, P. *Iranian J. Biotechnol.* **2009**, *7* (1), 10; (b) Venil, C. K.; Lakshmanaperumalsamy, P. *Pak. J. Sci. Ind. Res.* **2008**, *51* (6), 333.

(25) (a) Penedo, J. C.; Pérez Lustres, J. L.; Lema, I. G.; Rodriguez, M. C. R.; Mosquera, M.; Rodríguez-Prieto, F. *J. Phys. Chem. A* **2004**, *108* (29), 6117. doi:10.1021/jp049056w.; (b) Dogra, S. K. *J. Lumin.* **2006**, *118* (1), 45. doi:10.1016/j.jlumin.2005.06.008.

(26) Bhanumathi, N.; Rao, K. R.; Sattur, P. B. *Heterocycles* **1986**, *24* (6), 1683. doi:10.3987/R-1986-06-1683.

(27) Kidwai, M.; Negi, N. *Monatsh. Chem.* **1997**, *128* (1), 85. doi:10.1007/BF00807642.

(28) Chen, T. R. *Mater. Lett.* **2005**, *59* (8-9), 1050. doi:10.1016/j.matlet.2004.12.002.

(29) Rominger, F.; Malathi, M.; Mohan, P. S.; Dondeti, T. R.; Hashmi, A. S. K. *Acta Crystallogr.* **2009**, *E65*, o400.

(30) Jlakowicz, J. R. *Principles of Fluorescence Spectroscopy,* 2nd ed.; Kluwer Academic/Plenum Publishers: New York, 2006.

(31) Kim, H. M.; Choo, H. J.; Jung, S. Y.; Ko, Y. G.; Park, W. H.; Jeon, S. J.; Kim, C. H.; Joo, T.; Cho, B. R. *ChemBioChem* **2007**, *8* (5), 553. doi:10.1002/cbic.200700003. PMID:17300111.

(32) Liu, Z. Q.; Fang, Q.; Yu, W. T.; Xue, G.; Cao, D. X.; Wang, D.; Xia, G. M.; Jiang, M. H. *Chin. Chem. Lett.* **2002**, *13* (10), 997.

(33) (a) Leresche, J. E.; Meyer, H.-P. *Org. Process Res. Dev.* **2006**, *10* (3), 572. doi:10.1021/op0600308.; (b) Kafarski, P.; Lejczak, B. *J. Mol. Catal., B Enzym.* **2004**, *29* (1-6), 99. doi:10.1016/j.molcatb.2003.12.013.

(34) (a) Chaerun, S. K.; Tazaki, K.; Asada, R.; Kogure, K. *Environ. Int.* **2004**, *30* (7), 911. doi:10.1016/j.envint.2004.02.007. PMID:15196839.; (b) Zahoor, A.; Rehman, A. *J. Environ. Sci. (China)* **2009**, *21* (6), 814. PMID:19803088.; (c) Frische, T.; Höper, H. *Chemosphere* **2003**, *50* (3), 415. doi:10.1016/S0045-6535(02)00603-3. PMID:12656263.

(35) (a) Azuma, T.; Watanabe, N.; Yagisawa, H.; Hirata, H.; Iwamura, M.; Kobayashi, Y. *Immunopharmacology* **2000**, *46* (1), 29. doi:10.1016/S0162-3109(99)00153-8. PMID:10665777.; (b) Bennett, J. W.; Bentley, R. *Adv. Appl. Microbiol.* **2000**, *47*, 1. doi:10.1016/S0065-2164(00)47000-0. PMID:12876793.

(36) (a) Ryan, R. M.; Green, J.; Lewis, C. E. *BioEssay* **2006**, *28* (1), 84. doi:10.1002/bies.20336.; (b) Senter, P. D.; Springer, C. J. *Adv. Drug Deliv. Rev.* **2001**, *53* (3), 247. doi:10.1016/S0169-409X(01)00206-X. PMID:11744170.

(37) (a) Deorukhkar, A. A.; Chander, R.; Ghosh, S. B.; Sainis, K. B. *Res. Microbiol.* **2007**, *158* (5), 399. doi:10.1016/j.resmic.2007.02.010. PMID:17467243.; (b) Grabley, S.; Thiericke, R. *Adv. Biochem. Eng. Biotechnol.* **1999**, *64*, 101. PMID:9933977.

(38) (a) Testa, A. C.; Wild, U. P. *J. Phys. Chem.* **1979**, *83* (23), 3044. doi:10.1021/j100486a023.; (b) Pan, J. H.; Chou, Y. M.; Chiu, H. L.; Wang, B. C. *Tamkang J. Sci. Eng.* **2005**, *8* (20), 175; (c) Srividya, N.; Ramamurthy, P.; Ramakrishnan, V. T. *Spectrochimica Acta Part A* **1998**, *54* (2), 245. doi:10.1016/S1386-1425(97)00211-4.