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Synthesis and spectroscopic investigation of binding of novel Thiazolo [2, 3-a] isoquinoline analog with bovine serum albumin

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A condensation reaction between 1-cyclobutyl-3,4-dihydroisoquinoline and thioglycolic acid has been carried out using DCC yields 10b-cyclobutyl-5,6-dihydro-2H-thiazolo [2, 3-a] isoquinolin-3(10bH)-one [CBIQTGA]. The synthesized compound is characterised by using spectroscopic techniques. The interaction of CBIQTGA with bovine serum albumin has been studied using UV spectroscopy, spectrofluorimetry and circular dichroism techniques.

Keywords: DCC, Cyclisation, BSA, Fluorescence, Circular dichroism

Bovine serum albumin (BSA) is the most abundant and carrier protein which lies in blood plasma[1]. BSA consists of an amino acid chain of 537 residues. BSA gets divided into nine loops due to disulfide bonds into three structurally homologous domains. Intrinsic fluorescence is produced by tryptophan 134 which is located on the protein surface and tryptophan 212 lies in a hydrophobic binding pocket of protein[2-4]. Hence quenching of fluorescence is made useful in determining the binding affinities. Heterocyclic compounds are well known for their versatile biological activites[5]. Nitrogen and sulphur containing heterocycle are profoundly used as antimalarial[6] and antibacterial[7] agents, respectively.

Thiazolidinone[8] is a five member heterocycle found in various drugs and exhibits several biological activites. Thiazolidinones are known to exhibit anti HIV[9], anticancer[10], antimalarial[11], antitubercular[12] activites. Besides these they are also reported for their anticonvulsant[13], antihypnotic[14], antifungal[15], antibacterial[16] and anti-inflammatory[17] activites. Some of thiazolidinones are known to possess insecticidal activity[18]. Recently thiazolidinone derivatives were reported for their antiproliferative[19] activity. Moreover thiazolidinone-peptide hybrids were reported for their antidengue activity[20]. Thiazolidinone is the main functionality in known diabetic drugs including Rosiglitazone and Pioglitazone[21]. Rosiglitazone was investigated for their interaction with BSA[22]. Further the interaction of various thiazolo derivatives with BSA is also reported.[23-27] The study of interaction of proteins with small molecules is important in the field of pharmacokinetics and pharmacodynamics[28]. BSA has structural analogy to human serum albumin[29].

The present study deals with the synthesis of new thiazolo derivative with its characterization by using different spectroscopic methods[30]. The synthesized derivative 10b-cyclobutyl-5,6-dihydro-2H-thiazolo [2, 3-a] isoquinolin-3(10bH)-one [CBIQTGA] was further studied for its binding interaction with BSA using fluorescence spectroscopy, circular dichroism (CD) spectroscopy.[31]

Materials and Methods

Materials

All required chemicals and BSA was purchased from Sigma-Aldrich India and used without further purification[31]. BSA stock solution was prepared by dissolving BSA in Tris-HCl buffer with pH= 7.2. In all the experiments, double distilled water was used for dilution. In 5% ethanol, 10b-cyclobutyl-5,6-dihydro-2H-thiazolo[2,3-a] isoquinolin-3 (10bH) -one (CBIQTGA) stock solution (2.0×10^{-3} mol L^{-1}) was prepared. All required solvents were of analytical grade and purchased from Sigma Aldrich, India.

Synthesis of CBIQTGA

A mixture of 1-cyclobutyl-3,4-dihydroisoquinoline (0.1 mol), thioglycolic acid (0.3 mol) were stirred in tetrahydrofuran at 0-5 °C for 15 min and then N,N-dicyclohexylcarbodiimide (0.4 mol) was added[30]. The reaction mixture was stirred for additional 50 min at room temperature. The dicyclohexylurea was removed by filtration and filtrate was concentrated to dryness. The residue was taken in ethyl acetate and it was washed with 10% aq. citric acid, water and 10% aq. sodium hydrogen carbonate. Ethyl acetate extract was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue obtained was purified by column chromatography over silica gel (petroleum ether: chloroform 70:30) to obtain white solid. The reaction mechanism is shown in Scheme 1. The characterization spectra are given in Supplementary Data, Figs. S1-S4.

Characterisation: Yield: 71%; melting point: 91 °C; IR (cm^{-1}): 2971.41, 1667.28, 1411.92, 1330.27, 1293.09, 751.94; ^1H NMR ($CDCl_3$): 7.04-7.25 (m, 4H aromatic), 1.72-1.85 (m, 2H, aliphatic), 1.91-2.02 (m, 4H, aliphatic), 2.27-2.33 (m, 1H, aliphatic), 2.80-2.82 (d, 1H, aliphatic *J*=6 Hz), 3.00-3.12 (m, 1H, aliphatic), 3.23-3.25 (d, 1H, aliphatic *J*=6 Hz), 3.56-3.61 (dd, 1H, aliphatic *J*=15 Hz), 3.75-3.80 (dd, 1H, aliphatic *J*=15 Hz), 4.39-4.46 (m, 1H, aliphatic); ^{13}C NMR ($CDCl_3$): 16.75, 23.90, 25.42, 27.97, 34.26, 37.42, 46.44, 74.05, 125.66, 126.51, 127.48, 129.27, 131.42, 139.71, 169.24; Mass spectra: M^+ at m/z: 260.57.

Preparation of BSA and CBIQTGA solution

BSA solution (10 μM) was prepared based on a molecular weight of 66,000 Dalton in 0.1 M phosphate buffer[31]. Initially a stock solution (50 μM) of CBIQTGA was prepared and different concentrations (10, 20, 30, 40 and 50 μM) were prepared by serial dilution of the stock solution.

Characterisation techinques

Shimadzu RF-5301 PC spectrofluorophotometer was used to record the fluorescence. At 280 nm the excitation wavelength was set and emission was recorded in the range of 300-450 nm. Slit widths were kept at 5 nm each throughout the experiment.

At 293, 298, and 310 K temperatures with different concentrations of CBIQTGA solution in a 1 cm quartz cell, the fluorescence emission of BSA was recorded. An incubation time of 30 min was set for all fluorescence measurements for different temperatures[31-32]. Shimadzu UV-2401 PC spectrophotometer was used to record UV-visible spectra in the absence and presence of CBIQTGA at 298 K in the range of 250-310 nm. The quartz cuvette with 1 cm path length was used. The absorbance Tris-HCl buffer solution was recorded and subtracted. Jasco, J-815, CD spectrometer was used to record the circular dichroism measurements. CD spectrum measurements for BSA were recorded in the far UV region with scan speed of 10 nm/min, in presence and absence of CBIQTGA. The band width was fixed at 5 nm[31-33].

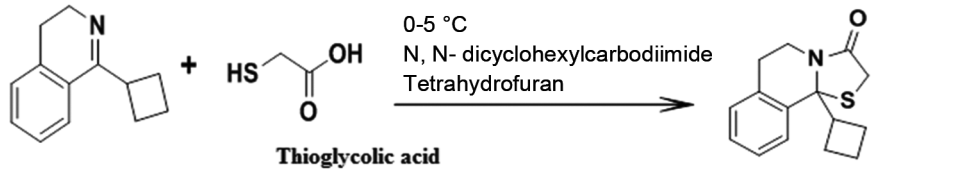
Results and Discussion

UV-visible spectra of BSA-CBIQTGA complex

UV-visible spectra of BSA in absence and presence of CBIQTGA show absorption peak at 280 nm, which increases with the increase in concentration of CBIQTGA as shown in Fig. 1. The shifting of absorption position to lower wavelength region may be due to formation of ground state complex BSA-CBIQTGA[31-32].

Fluorescence quenching of BSA in presence of CBIQTGA

The fluorescence of BSA is due to tryptophan (Trp) moiety which is very sensitive. The fluorescence spectra (Fig. 2a) were recorded for the free BSA and mixed with different concentrations of CBIQTGA to study the interaction between them. The spectra indicate that the BSA has a strong emission band observed between 345-380 nm when excited at 280 nm[31-32]. The intensity of this fluorescence band gradually decreases with increase in concentration of quencher i.e., CBIQTGA. This gradual decrease in the



Scheme 1 — Reaction mechanism for the synthesis of CBIQTGA

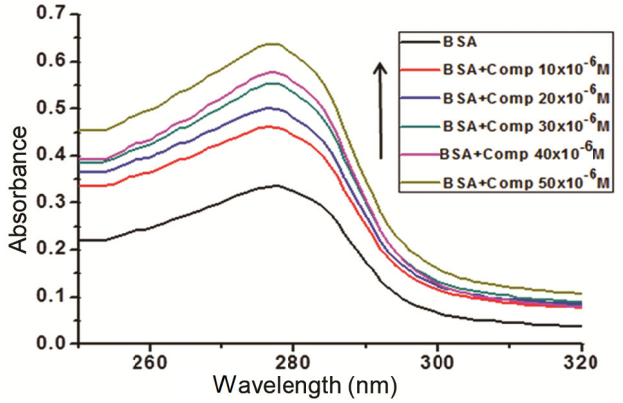


Fig. 1 — UV-visible spectra of BSA-CBIQTGA complex

fluorescence of BSA indicates the binding between CBIQTGA and BSA to form a complex.

The fluorescence quenching occurs either by dynamic or static mechanism, depending on the way of interaction between CBIQTGA and BSA[33-34]. The fluorescence quenching data at different temperatures as shown in Figs. 2b & 2c, were analyzed by the Stern-Volmer equation[35] as given below.

$$\frac{F0}{F} = 1 + K_{SV}\,Q = 1 + Kq_0Q$$

where, F_0 and F denote the steady-state fluorescence intensities in the absence and presence of CBIQTGA, respectively. K_{SV} is the Stern-Volmer quenching constant and [Q] is the concentration of CBIQTGA, K_q is the quenching rate constant of the biological macromolecule, τ_0 is the average lifetime of the molecule without any quencher. The Stern-Volmer plot of F_0/F vs. [Q] is presented in Fig. 3 and slope of this plot yields the Stern-Volmer constant (Table 1). The quenching constant decreases with the temperature thus indicating that fluorescence quenching occurs because of some specific binding between CBIQTGA and BSA by forming complexes which are destabilized at higher temperatures. The value of K_q was calculated by assuming fluorescence lifetime of the biopolymer of 10^{-8} s . The maximum scattering collision quenching constant of various quenchers with the biopolymer is 2 x 10^{10} L mol^{-1} s^{-1}. The value of quenching constant decreases with increasing temperature; this indicated that binding between CBIQTGA and BSA by complex formation which results in successful quenching. While in the present case the quenching constant K_q is of the order of 10^{11}. This indicates the presence of static quenching between CBIQTGA and BSA[34-35].

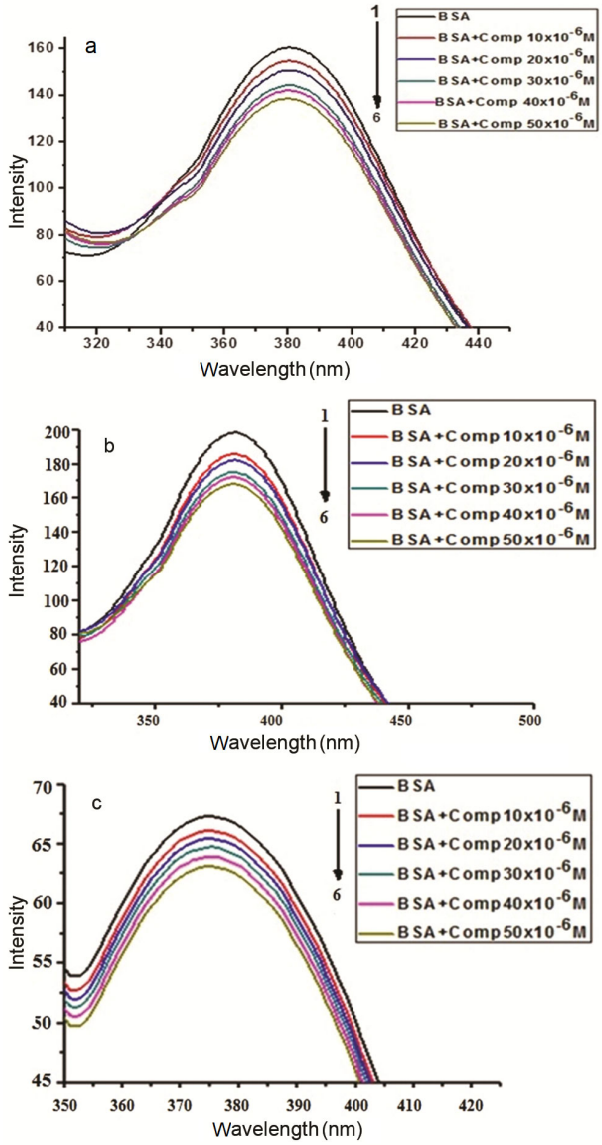


Fig. 2 — Fluorescence quenching of BSA in presence of CBIQTGA at (a) 298 K, (b) 293K and (c) 310 K

Binding constant and number of binding sites of BSA-CBIQTGA complex

The binding constant (K) and the number of binding sites (n) between the CBIQTGA and BSA were obtained from the equation as follows:

$$log\,\frac{F0 - F}{F} = \log K + n log[Q]$$

The plot of log $(F_0\text{-}F)/F$ vs. log [Q] is shown in Fig. 4 and the value of n and K are obtained from the slope and Y-axis intercept, respectively[34-35], and are summarized in Table 1. The value of number of binding sites between BSA and CBIQTGA is nearly

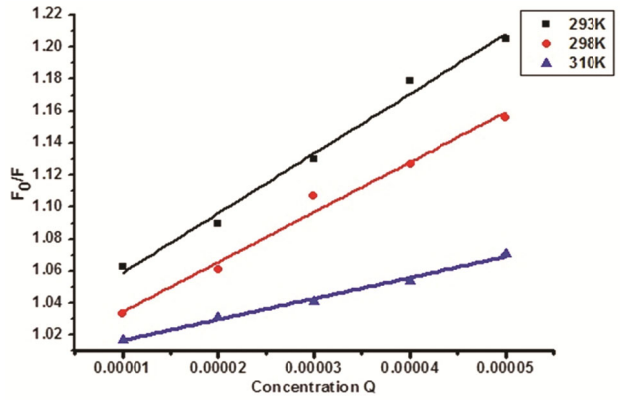


Fig. 3 — Stern-Volmer plots for the interaction of CBIQTGA with BSA at three different temperatures

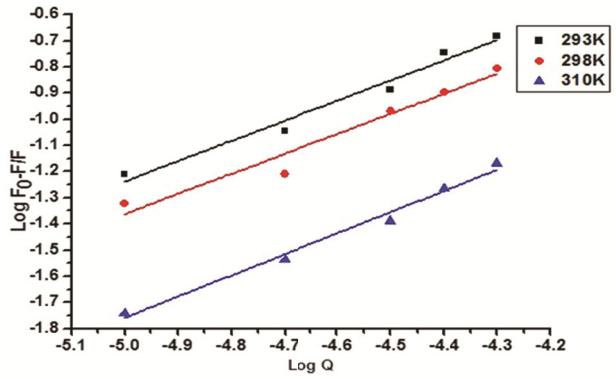


Fig. 4 — Double-logarithmic plot for the quenching of BSA with CBIQTGA

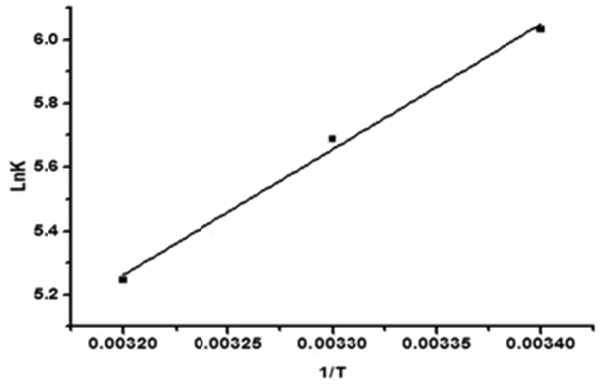


Fig. 5 — Plot of ln K vs. 1/T of the interaction between BSA and CBIQTGA at 293 K, 298 K and 310 K

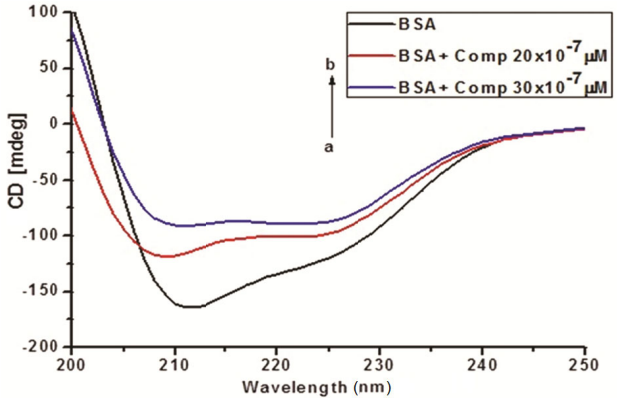


Fig. 6 — Circular dichroism spectra of BSA with CBIQTGA

Table 1 — Binding parameters of CBIQTGA with BSA at different temperatures

T (K)	Ksv (M^{-1})	Kq (M^{-1} S^{-1})	K (L M^{-1})	n
293	3.73×10^{3}	3.73×10^{11}	4.16×10^{2}	0.77
298	3.12×10^{3}	3.12×10^{11}	2.98×10^{2}	0.76
310	1.31×10^{3}	1.31×10^{11}	1.90×10^{2}	0.80

Table 2 — Thermodynamic Parameters of CBIQTGA with BSA at different temperatures

T (K)	ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})	ΔG^0 (kJ mol^{-1})
293			-14.693
298	-32.637	-60.675	-14.089
310			-13.523

1. Therefore the value of binding constant suggested that these compounds can easily form complex with the protein as well as released in desired target areas. This result suggests the formation of an unstable complex of BSA with CBIQTGA, which may partly decompose at higher temperatures[36]

Mode of binding

Usually van der Waals force, hydrogen bonding, hydrophobic interactions, hydrophilic interaction and electrostatic interactions are responsible for binding small molecules to macromolecule[37]. The enthalpy change ΔH^0 and entropy change ΔS^0 were obtained from van't Hoff's equation and change in free energy ΔG^0 can be obtained. The plot of ln*K* vs. 1/T is shown in Fig. 5 and the values of ΔH^0 and ΔS^0 were obtained from the slope and intercept of graph[38]. The negative value of ΔS^0 (-60.675 J mol^{-1} K^{-1}) as a proof for a hydrophilic interaction and negative value of ΔH^0 (-32.637 kJ mol^{-1}) indicate that hydrogen bonding and van der Waals forces are involved in the binding[39-40] (Table 2). The negative value of ΔG suggests that the binding between CBIQTGA and BSA occurred spontaneously.

Effect of CBIQTGA on the conformation of BSA

Main implication of circular dichroism, technique is its speed of analysis, sensitivity in analyzing the BSA-CBIQTGA interactions. The CD spectra of BSA with CBIQTGA are shown in Fig. 6, which shows two negative bands at 208 nm and 222 nm, respectively.

These bands are characteristics of α helical structure of BSA[39-40]. The band intensity of BSA decreases with the addition of CBIQTGA. CD spectra of BSA reveal that BSA retains its α-helix structure after binding to the CBIQTGA[40-41].

Conclusions

A new derivative CBIQTGA was synthesized by simple method with good yield. UV study reveals the formation of CBIQTGA–BSA complex. Fluorescence spectra conclude the static quenching mechanism for binding between CBIQTGA and BSA. Negative value of free energy indicates that CBIQTGA-BSA complex formation is spontaneous process and negative value of entropy indicates hydrophilic interaction between BSA and CBIQTGA. Circular dichroism spectra show the binding between CBIQTGA and BSA alters the conformation of BSA.

Supplementary Data

Supplementary data associated with this article are available in the electronic form at http://nopr.niscair.res.in/jinfo/ijca/IJCA_60A(08)1081-1085_SupplData.pdf.

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References

1 Majorek K A, Porebski P J, Dayal A, Zimmerman M D, Jablonska K, Stewart A J, Chruszcz M & Minor W, *Mol Immunol,* 52 (2012) 174.
2 Naik P N, Chimatadar S A & Nandibewoor S T, *J Photochem Photobiol B,* 100 (2010) 147.
3 He X M & Carter D C, *Nature,* 358 (1992) 209.
4 Jayabharathi J, Thanikachalam V & Perumal M V, *Spectrochimic Acta Part A,* 95 (2012) 622.
5 Al-Mulla A, *Der Pharma Chem*, 13 (2017) 141.
6 Chugh A, Kumar A, Verma A, Kumar S & Kumar P, *Med Chem Res,* 29 (2020) 1723.
7 Matada M N, Jathi K, Rangappa M M, Geoffry K, Kumar S R, Nagarajappa R B & Zahara F N, *J King Saud Univ Sci*, 32 (2020) 3313.
8 Verma A & Saraf S K, *Eur J Med.Chem*, 43 (2008) 897.
9 Barreca M L, Chimirri A, De Luca L, Monforte A-M, Monforte P, Rao A, Zappalà M, Balzarini J, De Clercq E, Pannecouque C & Witvrouw M, *Bioorg Med Chem Lett,* 11 (2001) 1793.
10 Bhaumik A, Upender K M, Khan K A & Srinivas Ch, *Int J Curr Res,* 6 (2014) 8508.
11 Shingade S G, Bari S B, Agarwal P & Srivastava K, *Indian J Chem,* 52B (2013) 1236.
12 Ahmed O, Salahuddin Md, Sharma P & Singhvi I, *Am J Pharm Tech Res,* 3(2013) 193.
13 Shingalapur R V, Hosamani K M, Keri R S & Hugar M H, *Eur J Med Chem,* 45 (2010)1753.
14 Ergenc N, Capan G, Gunay N S, Ozkirimli S, Gungor M, Ozbey S & Kendi E, *Archiv der Pharmazie,* 332 (1999) 343.
15 Singh B, Maheshwari A, Dak G, Sharma K & Talesara G L, *Indian J Pharm Sci*, 72 (2010) 607.
16 Patel V G, Goswami T K & Bhatt A R, *J Chem Pharma Res,* 6 (2014) 2760.
17 Ashour H M A, El-Ashmawy I M & Bayad A E, *Monatsh Chem,* 147 (2016) 605.
18 Tirlapur V K & Tadmalle T, *Der Pharmacia Sinica,* 2 (2011) 135.
19 Gududuru V, Hurh E, Dalton J T & Miller D D, *Bioorg Med Chem Lett*, 14 (2004) 5289.
20 Nitsche C, Schreier V N, Behnam M A M, Kumar A, Bartenschlager R & Klein C D, *J Med Chem*, 56 (2013) 8389.
21 Derosa G, D'Angelo A, Ragonesi P D, Ciccarelli L, Piccinni M N, Pricolo F, Salvadeo S A T, Montagna L, Gravina A, Ferrari I, Paniga S & Cicero A F G, *Internal Med J,* 37 (2007) 79.
22 Pawar S, Joshi R & Ottoor D, *J Lumin,* 197 (2018) 200.
23 Hou Z, Zhou N, He B, Yang Y & Yu X, *Spectrochim Acta Part A,* 79 (2011) 1931.
24 Yu X, Yang Y, Yao Q, Tao H, Lu S, Xie J, Zhou H & Yi P, , *Spectrochim Acta Part A,* 96 (2012) 690.
25 Yang Y, Yu X, Tong W, Lu S, Liu H, Yao Q & Zhou H, *J Solution Chem*, 42 (2013) 666.
26 Yu X, Ying Y, Lu S, Qing Y, Liu H, Li X & Yi P, *Spectrochim Acta Part A,* 83 (2011) 322.
27 Murthy M S, Pai P N, Sanjay R, Gopala K & Nargund L V G, *Asian J Chem,*17 (2005) 2841.
28 Jiao Q, Wang R, Jiang Y & Liu B, *Chem Cent J,* 12 (2018) 48.
29 Tian J N, Liu J Q, Tian X, Hu Z D & Chen X G, *J Mol Struct* 691 (2004) 197.
30 Srivastava T, Haq W & Katti S B, *Tetrahedron*, 58 (2002) 7619.
31 Bhogale A, Patel N, Sarpotdar P, Mariam J, Dongre P M, Miotello A & Kothari D C, *Colloids Surf B*, 102 (2013) 257.
32 J Jayabharathi, Thanikachalam V & Perumal M V, *J Lumin,* 132 (2012) 707.
33 Yu M, Ding Z, Jiang F, Ding X, Sun J, Chen S & Lv G, *Spectrochim Acta Part A,* 83 (2011) 453.
34 Mote U S & Kolekar G B, *Indian J Chem,* 55A (2016) 820.
35 Sarkar M, Paul S S & Mukherje K K, *J Lumin,* 142 (2013) 220.
36 Yu Z, Li D, Ji B & Chen J, *J Mol Struct,* 889 (2008) 422.
37 Ranade P B, Navale D N, Zote S W & Ramana M M V, *Indian J Chem,* 59A (2020) 569.
38 Kamat B P, Seetharamappa J & Melwanki M B, *Indian J Biochem Biophys,* 41 (2004) 173.
39 Ramana M M V, Betkar R, Nimkar A, Ranade P, Mundhe B & Pardeshi S, *Spectrochim Acta Part A,* 152 (2016) 165.
40 Tian Z, Zang F, Luo W, Zhao Z, Wang Y, Xu X & Wang C, *J Photochem Photobiol B,* 142 (2015) 103.
41 Yu J, Liu J-Y, Xiong W-M, Zhang X-Y & Zheng Y, *BMC Chemistry,* 13 (2019) 95.