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Synthesis, photophysical property study of novel fluorescent 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-*d*][1,3]oxazole derivatives and their antimicrobial activity

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Abstract. A series of 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-*d*][1,3]oxazole derivatives have been synthesized from intermediate 1-amino-3-(1,3-benzoxazol-2-yl)naphthalen-2-ol. This intermediate was obtained by coupling 3-(1,3-benzoxazol-2-yl)naphthalen-2-ol with 4-sulphobenzenediazonium chloride followed by reduction with sodium dithionate in water at pH 8–9. 3-(1,3-benzoxazol-2-yl)naphthalen-2-ol was synthesized from 3-hydroxynaphthalene-2-carboxylic acid and 2-amino phenol in the presence of PCl_3 in chlorobenzene at 130–135°C. All these compounds were characterized by FT-IR, ^{1}H NMR, mass spectral and elemental analysis. The synthesized compounds are fluorescent which absorbs in the range of 296 to 332 nm while emits in the ranges of 368 to 404 nm with excellent quantum yield. All compounds were evaluated for *in vitro* antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* strains and *in vitro* antifungal activity against *Candida albicans* and *Aspergillus niger* strains by using serial dilution method.

Keywords. Heterocyclic synthesis; benzoxazole; naphthoxazole; fluorescence; photophysical properties; antibacterial activity; antifungal activity.

1. Introduction

Benzoxazoles and naphthoxazoles are important class of heterocyclic compounds because of their wide spectrum of biological and photochromatic activities. 2-Substituted naphthoxazole is a major subunit occurring in a number of biologically active compounds[1] and natural products.[2] They find extensive use as fluorescent probes[3] and as intermediates for dyes.[4] Ortho-substituted naphthoxazole derivatives show promising inhibitory activity for protein tyrosine phosphatase-1B (PTB-1B) and *in vivo* antidiabetic activity in SLM, STZ-S and db/db mice models.[5–7]

A number of benzoxazole and naphthoxazole derivatives possessing antifungal,[8] antiinflammatory,[9] antitumour[10] and antiHIV[11] activities have been reported. Some of the benzoxazole derivatives exhibit diverse chemotherapeutic activity including anticancer agent[12] NSC-693638 and antibacterial agents;[13] some examples are UK-1, MUK-1 and DMUK-1 (figure 1).

The excellent photophysical properties like broad spectral windows, high molar absorptivity values and reasonably good fluorescence quantum efficiency have made them to be of use as fluorescent probe and sensing materials.[14] In material chemistry, benzoxazoles find applications as laser dyes and photochromatic agents.[15] Some of the phenylaminonaphtho[1,2-*d*]oxazol-2-yl-type compounds were reported as a sensor for water in organic solvents by photo-induced electron transfer (PET).[16]

There are reports available describing the synthesis and biological activity of naphtho[1,2-*d*][1,3]oxazole[5] and benzoxazole[17] separately. However, there are no reports available describing synthesis and biological activity of benzoxazole derivatives incorporated with naphthoxazole heterocycles in a single moiety. Therefore, as a part of ongoing research work on synthesis of biologically important fused heterocycles,[18] we report here the synthesis of novel 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-*d*][1,3]oxazole derivatives and their antimicrobial activity study.

The synthesized derivatives are the structural analogues of *bis*(benzoxazole) natural product UK-1 (scheme 1), a secondary metabolite with interesting biological activity. This UK-1 was also found to be

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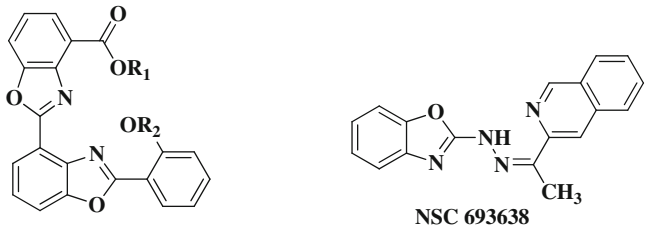


UK-1 (R_1 = Me, R_2 = H)

MUK-1 (R_1 = Me, R_2 = Me)

DMUK-1 (R_1 = H, R_2 = H)

Figure 1. Various bioactive molecules containing benzoxazole.

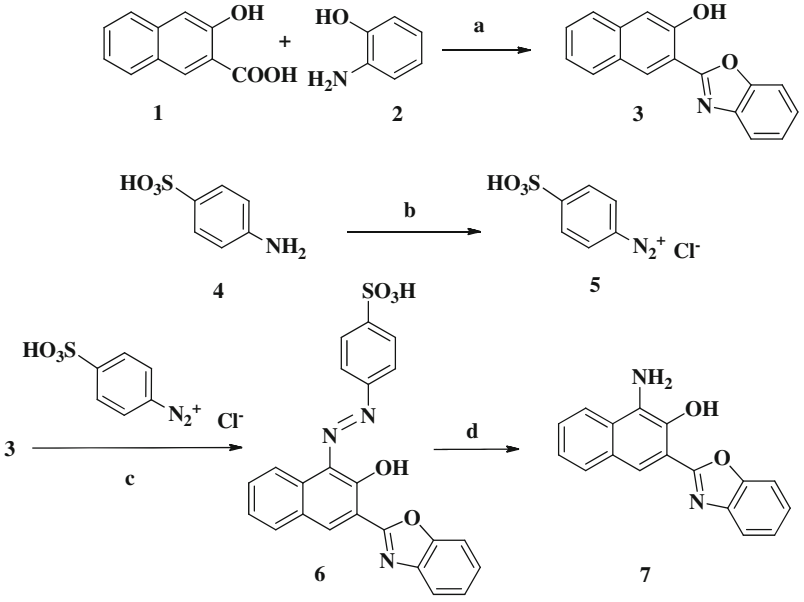
fluorescent, on excitation at 335 nm, it emits at 530 nm. It binds to double strand DNA 10 times more tightly in the presence of Mg^{2+} than in its absence. The decrease in emission intensity is the detection tool for its binding with DNA strand. It is also used as inhibitor of human topoisomerase II.[19] It shows wide spectrum of potential anticancer activity against leukemia, lymphoma, and certain solid tumour derived cell lines.[20] The benzoxazole derivatives are used as fluorescent probes[21,22] and sensors for the detection of different metal ions.[23,24] In this study, one of the 1,3-benzoxazole ring of UK-1 is replaced by naphtho[1,2-*d*][1,3]oxazole ring and studied for their biological activities.

2. Experimental

All commercial reagents were used as received without purification and all solvents were reagent grade. The reaction was monitored by TLC using 0.25 mm E-Merck silica gel 60 F254 precoated plates, which were visualized with UV light. Melting points were measured on standard melting point apparatus from Sunder Industrial Product, Mumbai and were uncorrected. The FT-IR spectra were recorded on a Perkin Elmer 257 spectrometer using KBr discs. ^{1}H NMR spectra were recorded on a Varian Cary Eclipse Australia, MR-300 MHz, USA instrument using TMS as an internal standard. Mass spectra were recorded on Finnigan Mass spectrometer. The absorption spectra of the compounds were recorded on a Spectronic Genesys 2 UV-Visible spectrophotometer; UV-Visible emission spectra were recorded on JASCO – FP 1520. Simultaneous DSC-TGA measurements were performed on simultaneous DSC-TGA Waters (India) Pvt. Ltd.

2.1 *Experimental procedure for the synthesis of 3-(1,3-benzoxazol-2-yl)naphthalen-2-ol (**3**)*

A mixture of 2-aminophenol **2** (1.09 g, 0.01 mol) and 3-hydroxynaphthalene-2-carboxylic acid **1** (1.88 g, 0.01 mol) was refluxed (133–135°C) in chlorobenzene



Scheme 1. Synthesis of 1-amino-3-(1,3 benzoxazol-2-yl)naphthalene-2-ol (**7**). Reagents and conditions: (a) PCl_3, Chlorobenzene, Reflux (133–135°C), 4 h, 80%; (b) $NaNO_2$, HCl, 0–5°C, 45 min, 98%; (c) Methanol: Water, pH 8–9, 0–5°C (80: 20) 3 h, 90%; (d) $Na_2S_2O_4$, NaOH, H_2O, 90°C, 1.5 h, 66%.

(10 mL) in the presence of PCl_3 (2 g, 1.3 mL, 0.01 mol) for 4 h. After completion of reaction, the solid product was precipitated out and was filtered to get crude product **3** (yield 80%) which was further recrystalized form ethanol.

2.2 *Experimental procedure for the synthesis of 4-{[3-(1,3-benzoxazol-2-yl)-2-hydroxynaphthalen-1-yl]diazenyl}benzenesulphonic acid (**6**)*

Sulphanilic acid **4** (2.07 g, 0.012 mol) was dissolved in water (10 mL) containing sodium carbonate of (0.62 g) at 50–55°C. A solution $NaNO_2$ (0.84 g, 0.012 mol) in water (10 mL) was added to sulphanilic acid solution in water and then this mixture was added slowly to the conc. HCl (4 mL) at 0–5°C for 30 min and further stirred at this temperature for 1.5 h to get 4-sulphobenzenediazonium chloride salt **5**. This was further coupled with the 3-(1,3-benzoxazol-2-yl)naphthalene-2-ol **3** (2.61 g, 0.010 mol) at 0–5°C at pH 8–9 for 3 h and then at room temperature for 1 h in methanol:water mixture (80:20). Then reaction mixture was neutralized with acetic acid (9–10 mL) at pH 7, methanol was removed under reduced pressure and then filtered out to afford 4-{[3-(1,3-benzoxazol-2-yl)-2-hydroxynaphthalen-1-yl]diazenyl}benzenesulphonic acid **6** (yield 90%).

2.3 *Experimental procedure for the synthesis of 1-amino-3-(1,3-benzoxazol-2-yl)naphthalen-2-ol (**7**)*

The azo compound **6** (4.45 g, 0.010 mol) was heated in water containing NaOH (3.51 g, 0.088) at 50°C and sodium dithionate (3.78 g, 0.025 mol) was added at 90°C in portions over period of 1 h. The mixture was heated at 90°C at pH 8–9 until the colour of azo get disappeared (1.5 h). The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mass was neutralized up to pH 7, filtered and washed well with water 2–3 times and dried well to afford crude 1-amino-3-(1,3-benzoxazol-2-yl)naphthalen-2-ol **7** in 66% yield. Crude product was recrystalized in ethyl alcohol.

2.4 *General experimental procedure for the synthesis of 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-d][1,3]oxazole derivatives from intermediate **7** and substituted aromatic acids (**8–11**)*

1-Amino-3-(1,3-benzoxazol-2-yl) naphthalen-2-ol **7** (0.010 mol), corresponding substituted aromatic acid (0.010 mol) and PCl_3 (0.015 mol) were refluxed in chlorobenzene (130–133°C) (6 mL) up to the completion of reaction (16–18 h) and was confirmed by TLC. After cooling the reaction mass, solid product was filtered out and recrystalized from chloroform to afford corresponding compounds **8–11** in 57–65% yield.

2.5 *General experimental procedure for the synthesis of 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-d][1,3]oxazole derivatives from intermediate **7** and substituted aromatic aldehydes (**8–11**)*

1-Amino-3-(1,3-benzoxazol-2-yl) naphthalen-2-ol **7** (0.010 mol) and corresponding aromatic substituted aldehydes (0.010 mol) were heated in dimethylsulphoxide (6 mL) at 158–160°C up to the completion of reaction (10–12 h), confirmed by TLC. After cooling the reaction mass, solid product was filtered out and recrystalized form chloroform to afford corresponding compounds **8–11** in 62–72% yield.

2.6 *Experimental procedure for the synthesis of 4-(1,3-benzoxazol-2-yl)-2-methylnaphtho [1,2-d] [1,3] oxazole (**12**)*

1-Amino-3-(1,3-benzoxazol-2-yl)naphthalen-2-ol (1.38 g, 0.005 mol) **7** was refluxed in the presence of acetic anhydride (5 mL) up to completion of reaction (30 min) which was confirmed by TLC. Reaction mass was cooled and slowly poured on crushed ice with efficient stirring. Solid product was filtered out and recrystalized form ethyl alcohol to afford compound **12** in 73% yields.

2.7 *Spectral data of compounds (**7**–**12**)*

2.7a *1-Amino-3-(1,3-benzoxazol-2-yl)naphthalen-2-ol (**7**)*: **Mp:** 206–208°C. FT-IR (KBr): 3510, 3452, 3357 (-NH_2, -OH), 3028 (Aromatic -CH Stretching), 1615, 1585, 1477, (C=C, C=N ring stretching), 1222, 1132 (C–O stretching), 750 (Aromatic -CH out of plane bending) cm^{-1}. ^{1}**H NMR:** (DMSO-*d*6)δ = 6.98 (d, J = 7.9 Hz, 1H, Ar-H), 7.30–7.43 (dd, J = 7.8, 8.2, 1.8 Hz, 5H, Ar-H), 7.58 (d, J = 8.2, 1.9 Hz, 1H, Ar-H), 7.93 (d, J = 8.5, 1.6 Hz, 1H, Ar-H), 8.83 (s, 1H, Ar-H), 9.96 (s, 2H, -NH_2), 10.12 (s, 1H, -OH) ppm. **MS (*m/z*):** 277.1 (M+1, 98%), 276.1 (M^+, 26%), 275.1 (22%), 250.1 (89%) 248.2 (65%), 232.2 (23%) 124 (24%). Anal. Calcd. for $C_{17}H_{12}N_2O_2$: C, 73.90; H, 4.38; N, 10.14. Found: C, 73.87; H, 4.48; N, 10.03.

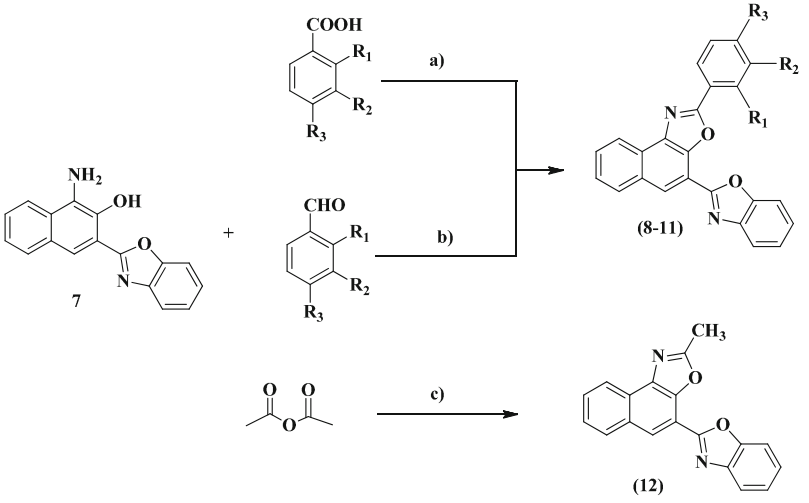
2.7b *4-(1,3-Benzoxazol-2-yl)-2-phenylnaphtho[1,2-d][1,3]oxazole (**8**)*: **Mp:** 210–212°C. **FT-IR (KBr):** 3040 (Aromatic -CH streching), 1615, 1576 (C=C,

C=N ring stretching), 1217, 1126 (C–O stretching), 740, 695 (Aromatic -CH out of plane bending vibration) cm^{-1}. ^{1}**H NMR:** ($CDCl_3$) $\delta = 7.43–7.46$ (m, $J =$ 8.2, 2.1 Hz, 2H, Ar–H), 7.59–7.66 (m, $J = 8.4$, 1.8 Hz, 4H, Ar–H), 7.73–7.84, (m, $J = 8.7$, 7.3, 0.2 Hz, 2H, Ar–H), 7.94 (dd, $J = 7.2$, 2.1 Hz, 1H, Ar–H), 8.12 (d, $J = 8.4$ Hz, 1H, Ar–H), 8.48 (dd, $J = 8.1$, 1.8, 3 Hz, 2H, Ar–H), 8.64 (d, $J = 8.1$ Hz, 1H, Ar–H), 8.75 (s, 1H, Ar–H) ppm. **MS (*m/z*):** 363.3 (M+1, 100%), 277.3 (54%), 251.6 (48%), 233.1 (37%). Anal. Calcd. for $C_{24}H_{14}N_2O_2$: C, 79.55; H, 3.89; N, 7.73. Found: C, 79.51; H, 3.92, N, 7.70.

2.7c *4-(1,3-Benzoxazol-2-yl)-2-(4-nitrophenyl) naphtho[1,2-d][1,3]oxazole (**9**)*: **Mp:** >300°C. **FT-IR (KBr):** 3042 (Aromatic -CH streching), 1624, 1575 (C=C, C=N ring stretching), 1538, 1330 (-NO_2) 1210, 1126 (C–O stretching), 835, 740 (Aromatic -CH out of plane bending vibration) cm^{-1}. ^{1}**H NMR:** ($CDCl_3$)$\delta =$ 7.43–7.49 (m, $J = 9.9$, 9.3 Hz, 2H, Ar–H), 7.64–7.82 (m, $J = 7.8$, 7.9 Hz, 3H, Ar-H), 7.94 (t, $J = 9.3$, 1.5 Hz, 1H, Ar-H), 8.11 (d, $J = 8.1$, 1.8 Hz, 1H, Ar-H), 8.43–8.46 (d, $J = 9$ Hz, 2H, Ar-H), 8.59–8.65 (t, $J =$ 9.0, 7.2 Hz, 3H, Ar-H), 8.79 (s, 1H, Ar-H), ppm. **MS (*m/z*):** 408.2 (M+1, 99%), 407.3 (M^+, 21%), 378.1 (23%), 362.2 (90%), 350.2 (12%), 301.1 (19%). Anal. Calcd. for $C_{24}H_{13}N_3O_4$: C, 70.76; H, 3.22; N, 10.31; Found: C, 70.71; H, 3.26; N, 10.39.

2.7d *4-(1,3-Benzoxazol-2-yl)-2-(3-phenoxyophenyl) naphtho[1,2-d][1,3]oxazole (**10**)*: **Mp:** 160–162°C. **FT-IR (KBr):** 3065 (Aromatic -CH streching), 1628, 1565 (C=C, C=N ring stretching), 1214, 1134 (C–O stretching), 780, 695 (Aromatic -CH out of plane bending vibration) cm^{-1}. ^{1}**H NMR:** (DMSO-*d*6)$\delta =$ 7.10–7.26 (m, $J = 8.4$, 8.6, 2.2 Hz, 4H, Ar-H), 7.41–7.47 (m, $J = 8.9$, 7.1, 1.5 Hz, 4H, Ar-H), 7.52–7.78 (m, $J = 8.5$, 7.2, 1.8 Hz, 4H, Ar-H), 7.90 (m, $J = 2.1$, 0.9 Hz, 1H, Ar-H), 8.08–8.12 (d, $J = 8.1$, 1.8 Hz, 2H, Ar-H), 8.22 (d, $J = 6.9$, 0.9 Hz, 1H, Ar-H), 8.60 (d, $J = 8.4$ Hz, 1H, Ar-H), 8.74 (s, 1H, Ar-H) ppm. **MS (*m/z*):** 455.3 (M+1, 99%), 454.1 (M^+, 57%), 362 (47%), 250 (38%), 232 (41%). Anal. Calcd. for $C_{30}H_{18}N_2O_3$: C, 79.28; H, 3.99; N, 6.16. Found: C, 79.35; H, 3.89; N, 6.11.

2.7e *4-[4-(1,3-Benzoxazol-2-yl)naphtho [1,2-d][1,3] oxazol-2-yl]phenol (**11**)*: **Mp:** 272–275°C. **FT-IR (KBr):** 3365, 3352 (O–H), 3045 (-CH streching), 1626, 1575 (C=C, C=N ring stretching), 1215, 1122 (C–O stretching), 845, 730 (Aromatic -CH out of plane bending vibration) cm^{-1}. ^{1}**H NMR:** (DMSO-*d*6)$\delta =$ 6.90 (t, $J = 7.8$, 1.4 Hz, 1H, Ar-H), 7.00 (d, $J = 7.9$ Hz, 1H, Ar-H), 7.10–7.18 (t, $J = 10.8$, 7.8 Hz, 2H, Ar–H), 7.38–7.48, (m, $J = 7.2$, 1.8 Hz, 2H, Ar–H), 7.58 (d, 7.4 Hz, 1H, Ar–H), 7.68 (m, $J = 8.2$, 1.8 Hz, 1H, Ar–H), 7.90–8.02 (dd, $J = 9.2$, 1.2 Hz, 2H, Ar–H), 8.36 (dd, $J = 8.1$, 1.5 Hz, 1H, Ar–H), 8.72 (d, $J = 8.2$ Hz, 1H, Ar–H), 8.84 (s, 1H, Ar–H), 10.48 (s 1H, Ar-OH) ppm. **^{1}H NMR (D_2O Exchange of Comp 11):** (DMSO-*d*6) $\delta = 4.81$ (s, 1H, H-OD), 6.90 (t, $J = 7.8$, 1.4 Hz, 1H, Ar–H), 7.00 (d, $J = 7.9$ Hz, 1H, Ar–H), 7.10–7.18 (t, $J = 10.8$, 7.8 Hz, 2H, Ar–H), 7.38–7.48 (m, $J = 7.2$, 1.8 Hz, 2H, Ar–H), 7.58 (d, $J = 7.4$ Hz, 1H, Ar-H), 7.68 (m, $J = 8.2$, 1.8 Hz, 1H, Ar-H), 7.90–8.02 (dd, $J = 9.2$, 1.2 Hz, 2H, Ar-H), 8.36 (dd, $J = 8.1$, 1.5 Hz, 1H, Ar-H), 8.72 (d,



Scheme 2. Synthesis of 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-d][1,3]oxazole derivatives (**8–12**). Reaction conditions: (a) Chlorobenzene, PCl_3 130–133°C, 16–18 h (57–65%); (b) DMSO, 158–160°C, 10–12 h (62–72%); (c) 100–104°C, 1–1.5 h (73%).

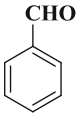
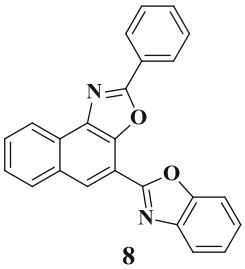
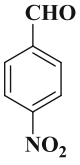
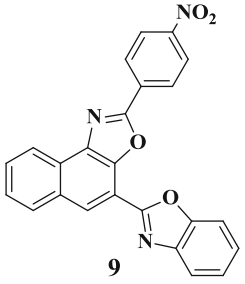
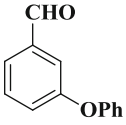
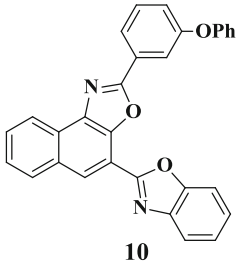
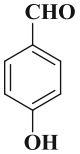
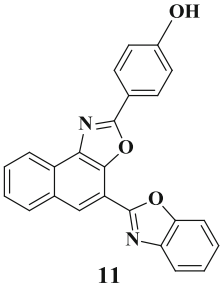
J = 8.2 Hz, 1H, Ar–H), 8.84 (s, 1H, Ar–H), ppm. **MS (*m/z*):** 379.6 (M+1, 91%), 378 (M^+, 51%), 362.2 (58%), 350.2 (52%), 277.2 (16%), 261.1 (27%), 250.6 (76%), 232.2 (37%). Anal. Calcd. for $C_{24}H_{14}N_2O_3$: C, 76.18; H, 3.73; N, 7.40; Found: C, 76.69; H, 3.82; N, 7.56.

2.7f *4-(1,3-Benzoxazol-2-yl)-2-methylnaphtho[1,2-d][1,3]oxazole (**12**)*: **Mp:** 190–192°C. **FT-IR (KBr):** 3055 (Aromatic -CH streching), 1616, 1555 (C=C, C=N ring stretching), 1205, 1110 (C-O stretching), 740 (Aromatic C-H out of plane bending vibration) cm^{-1}. ^{1}**H NMR**: (DMSO-*d*6)δ = 2.64 (s, 3H, -CH_3), 7.35–7.43 (m, J = 7.8, 6.9, 1.4 Hz, 2H, Ar-H), 7.52–7.78 (m, J = 8.8, 7.6, 2.2 Hz, 5H, Ar-H), 8.12 (d, J = 8.1 Hz, 1H), 8.96 (s, 1H, Ar-H) ppm. **MS (*m/z*):** 301.7 (M+1, 100%), 300.2 (M^+, 51%), 260.1 (16%), 250.2 (89%), 232.2 (32%). Anal. Calcd. for $C_{19}H_{12}N_2O_2$: C, 75.99; H, 4.03; N, 9.33; Found: C, 75.89; H, 4.12; N, 9.26.

3. Results and discussion

The synthetic scheme for the preparation of 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-*d*][1,3]oxazole

Table 1. Synthesis of 4-(1,3-benzoxazol-2-yl)naphtho[1,2-*d*][1,3]oxazole derivatives from intermediate **7** and substituted aromatic aldehydes[a].

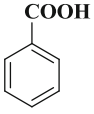
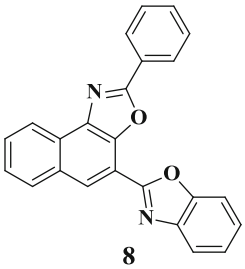
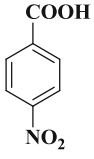
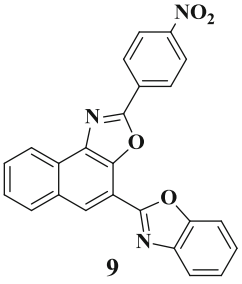
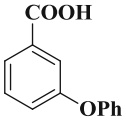
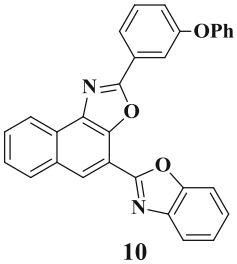
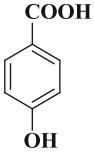
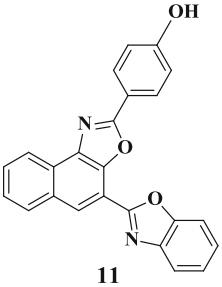
Sr. No.	Substituted aromatic aldehydes	Product	Time (h)	Yield[b] (%)
1		 **8**	10	68
2		 **9**	12	72
3		 **10**	11	69
4		 **11**	10.5	62

[a]Solvent: DMSO, Temp: 158–160°C, Time: 10–12 h.
[b]Isolated yield

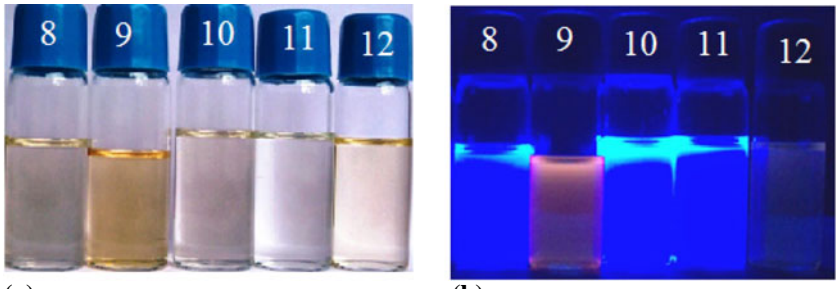
derivatives is shown in schemes 1 and 2. 3-(1,3-Benzoxazol-2-yl) naphthalen-2-ol **3** was prepared by reported procedure from 3-hydroxynaphthalene-2-carboxylic acid **1** and 2-aminophenol **2** in the presence of PCl_3 in chlorobenzene at 130–135°C.[25] Sulphanilic acid was diazotized to get 4-sulphobenzenediazonium chloride **5**, which was further coupled with **3** to get azo compound **6**. This azo compound **6** was reduced by using sodium dithionate at pH 8–9 to get 1-amino-3-(1,3-benzoxazol-2-yl)naphthalene-2-ol **7**[26] (scheme 1) which was confirmed by FT-IR, ^{1}H-NMR, mass spectral analysis and its M+1 peak was found to be at 277.1.

The series of 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho [1,2-*d*][1,3]oxazole derivatives were synthesized by three different routes from intermediate 1-amino-3-(1,3-benzoxazol-2-yl) naphthalen-2-ol **7** as shown in scheme 2. Compound **7** on treatment with different aromatic acids in the presence of phosphorus trichloride (PCl_3) in chlorobenzene at reflux temperature (130–133°C) for 15–18 h or with different aromatic aldehydes in dimethylsulphoxide at 158–160°C for 10–12 h gives 4-(1,3-benzoxazol-2-yl)naphtho[1,2-*d*][1,3] oxazole derivatives **8–11**. Substituted aromatic aldehyde gives product with good yield in short reaction

Table 2. Synthesis of 4-(1,3-benzoxazol-2-yl)naphtho[1,2-*d*][1,3]oxazole derivatives from intermediate **7** and substituted aromatic carboxylic acids[c].

Sr. No.	Substituted aromatic acids	Product	Time (h)	Yield[d] (%)
1		 **8**	18	62
2		 **9**	16 65	
3		 **10**	16.5	63
4		 **11**	16	57

[c]Solvent: Chlorobenzene, Reagent: PCl_3 Temp: 130–133°C, Time: 16–18 h.
[d]Isolated yield



(a) **(b)**

Figure 2. Day light and UV light photographs of the molecules in DMF. (**a**) Day light photograph and (**b**) UV-light photograph.

time (table 1) as compared to substituted aromatic acids (table 2).

Treatment of **7** with acetic anhydride at reflux temperature furnishes 4-(1,3-benzoxazol-2-yl)-2-methylnaphtho[1,2-*d*][1,3]oxazole **12**. All synthesized compounds were confirmed by FT-IR, ^{1}H NMR and mass spectral analysis. FT-IR spectra of compound **9** shows peak at 1538 and 1330 cm^{-1} clearly indicates the presence of –NO_2 group in the structure. Compound **11** shows distinct peak for –OH in FTIR at 3352–3365 cm^{-1} and phenolic –OH signal at 10.48 δ ppm in ^{1}H-NMR and was confirmed by D_2O exchange. The phenolic –OH peaks were disappeared in D_2O exchange with additional peak at 4.80–4.82 δ ppm. Mass spectral data for compounds **9** and **12** show M+1 peak at 408.2 and 301.1, respectively which are in well agreement with their molecular weight.

3.1 *Biological activity*

All compounds were evaluated for *in vitro* antibacterial activities against *E. coli* and *S. aureus* strains and *in vitro* antifungal activity against *C. albicans* and *A. niger* strains by using serial dilution method.

3.2 *General*

Incubator at 37°C; pipettes of various sizes (Gilson); sterile tips, 100, 200, 500, and 1000 μL; sterile normal saline; sterile isosensitest agar (Southern Group Laboratory, SGL); antibiotic solutions (Sigma–Aldrich); sterile solution of 10% (v/v) DMSO in water (Sigma–Aldrich) were used. Isosensitest medium was used throughout the assay, as it is pH buffered. Although NCCLS recommends the use of Mueller Hinton medium for susceptibility testing,[27] the isosensitest medium had comparable results for most of the tested bacterial strains.[28]

3.3 *Preparation of the plates*

Plates were prepared under aseptic conditions. A sterile 96 well plate was labelled. A volume of 100 μL of test material in 10% (v/v) DMSO (usually a stock concentration of 4 mg/ml) was pipetted into the first row of the plate. To all other wells 50 μL of nutrient broth was added. Serial dilutions were performed using a multichannel pipette. Tips were discarded after use such that each well had 50 μL of the test material in serially descending concentrations. To each well, 10 μL of resazurin indicator solution was added. Using a pipette 30 μL of 3.3 × strength isosensitized broth added to each well to ensure that the final volume was single strength of the nutrient broth. Finally, 10 μL of bacterial suspension (5 × 10^6 cfu/ mL) was added to each well to achieve a concentration of 5 × 10^5 cfu/ mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a broad-spectrum antibiotic as positive control, a column with all solutions with the exception of the test compound, and a column with all solutions with the exception of the bacterial solution, adding 10 μL of nutrient broth instead. The plates were

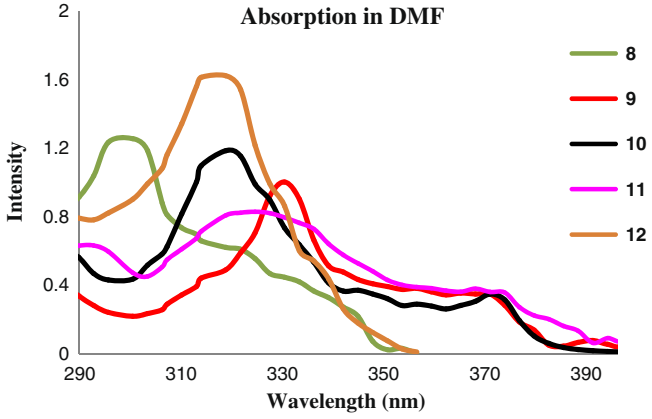


Figure 3. Absorption of compounds **8–12** in DMF.

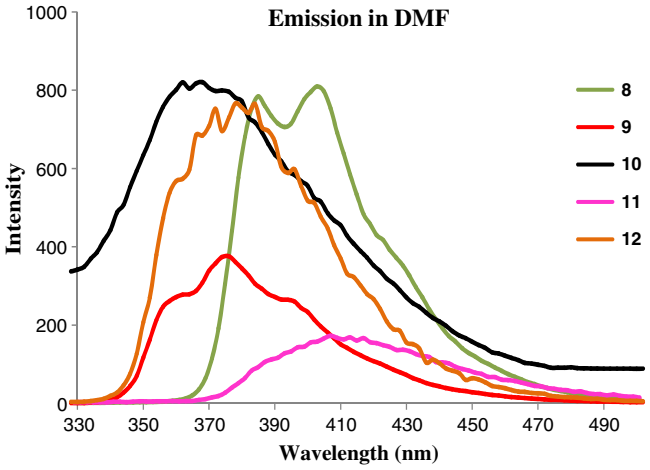


Figure 4. Emission of compounds **8–12** in DMF.

prepared in triplicate, and placed in an incubator set at 37°C for 18–24 h. The colour change was assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial or fungal strain.[29]

3.4 *Photophysical properties*

All synthesized compounds are fluorescent (figure 2b) and were studied for their photophysical properties. Their absorption and emission properties were recorded in DMF. All these compounds absorb from 296 to 332 nm (figure 3) and emit from 368 to 404 nm (figure 4) with good Stokes shift ranges from 39 to 108 nm. Out of these compounds, compound **8** shows good Stokes shift with 108 nm. As compared to compound **9**, compound **11** shows larger Stokes shift, this property attributed due to electron donating ability of –OH in compound **11** for easy flow of electron from 4-OH phenyl ring to benzoxazole ring via fused naphthoxazole ring system and electron withdrawing ability of $–NO_2$ in compound **9** (table 3)

Table 3. Absorption and emission at 1×10^{-6} M concentration of compounds **8–12** in DMF.

Compounds	Absorption λ_{max}(nm)	Emission λ_{max}(nm)	Stokes shift (nm)
8	296	404	108
9	332	371	39
10	320	368	48
11	326	404	78
12	320	384	64

Absorption λ_{max} and emission λ_{max} were measured in nm.
Samples were prepared in DMF.
Analyses were carried out at room temperature

Table 4. Quantum yield of compounds **8–12** in DMF.

Compound No	Quantum yield
8	0.206
9	0.129
10	0.308
11	0.047
12	0.274

Absorption λ_{max} and emission λ_{max} were measured in nm.
Samples were prepared in DMF.
Analyses were carried out at room temperature

An effective compound for the biological application should have good fluorescent intensity, high quantum yield and high photostability. Quantum yield of all compounds were recorded by using tinopal as a reference standard. Absorption and emission characteristics of standard as well as unknown samples were measured at different concentration of unknown samples and standard at (2, 4, 6, 8 and 10 ppm level). Absorbance intensity values were plotted against emission intensity values. A linear plot was obtained. Gradients were calculated for each unknown compound and for standard. All the measurements were done by keeping the parameters such as solvent and slit width constant. Relative quantum yield of all synthesized compounds **8–12** were calculated by using the formula 1.[30]

Formula 1: Relative fluorescence quantum yield

$$\Phi_X = \Phi_{ST}\left(\mathrm{Grad}_X/\mathrm{Grad}_{ST}\right)\left(\eta_X^2/\eta_{ST}^2\right),$$

where

Φ_X: Quantum yield of unknown sample
Φ_{ST}: Quantum yield of standard used
Grad_X: Gradient of unknown sample
Grad_{ST}: Gradient of standard used

Table 5. Thermal gravimetric analysis (TGA) of compound **8–12**.

Compound	TGA
8	222 (97.50%)
9	223 (98.41%)
10	236 (98.34%)
11	216 (99.67%)
12	255 (98.12%)

TGA was measured in °C

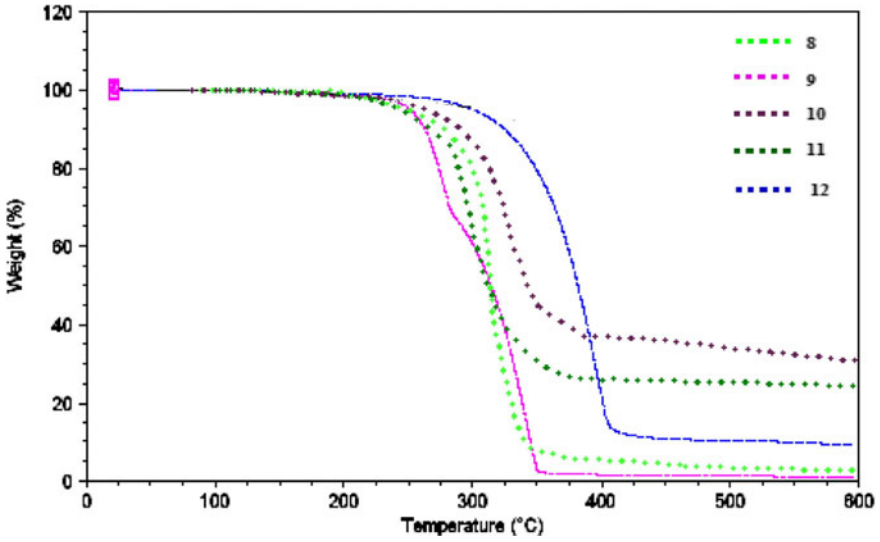


Figure 5. TGA curves of compound **8–12**.

η_X^2: Refractive index of solvent for standard sample

η_{ST}^2: Refractive index of solvent for sample.

The fluorescence quantum yields of all compounds were recorded in DMF at room temperature as shown in table 4. Quantum yields of all compounds are found to be good and ranges from 0.047 to 0.308 (table 4).

3.5 *Thermal stability*

To investigate the thermal stability of synthesized compounds **8–12** the thermal stability studies have been carried out using thermo gravimetric analysis (TGA) technique. The thermal gravimetric analysis has been carried out in the temperature range of 50–600°C under nitrogen atmosphere. TGA result in table indicates that the naphthoxazole ring skeleton is 98% stable up to 216°C (compound **11**). Above this temperature all compounds start to decompose and show the major loss in weight. The compound **12** shows the highest thermal stability up to 255°C (98.12%). The comparison of T_d (decomposition temperature) showed that the thermal stability of the compound **8–12** decreases in the order **12** > **10** > **9** > **8** > **11** as shown in table 5. TGA analysis curve of the compounds **8–12** is as shown in figure 5.

3.6 *Antimicrobial activity*

The novel compounds **8–14** were evaluated for their *in vitro* antibacterial activity against *E. coli* and *S. aureus* strains and *in vitro* antifungal activity against *C. albicans* and *A. niger* strains by using serial dilution method. The minimum inhibitory concentration (MIC) was determined for the compounds. The MIC (μg/mL) values recorded in table 6 indicate that most of the tested compounds showed variable inhibitory growth effects against tested bacterial and fungal strains. Antimicrobial data were compared with standard drug Streptomycin and Fluconazole.

The MIC values from table 6 reveals that compound **10**, **11** and **12** showed good to moderate activity against *E. coli* and *S. aureus*. Compound **8** shows activity against *S. aureus* while compound **9** showed good activity against *E. coli*.

As compared to the antibacterial activity all synthesized compounds (**8–12**) showed good antifungal activity against antifungal strains *C. albicans* and *A. niger*. Results mentioned in table 6 showed that compounds

Table 6. Antibacterial and antifungal activities of newly synthesized compounds indicated by MIC (μg/mL) using the modified resazurin assay.

Compounds	Bacterial strain		Fungal strain	
	E. coli	*S. aureus*	*C. albicans*	*A. niger*
8	625	156.2	156.2	78
9	156.2	625	78	78
10	156.2	156.2	312.5	312.5
11	156.2	156.2	78	78
12	156.2	156.2	156.2	156.2
Streptomycin	125	125	–	–
Fluconazole	–	–	125	125

Antimicrobial activities were expressed in MIC.
MIC: Minimal inhibitory concentration values.
Bacterial strain: *E. coli*; *S. aureus*.
Fungal Strain: *C. albicans*; *A. niger*.
Solvent used: DMSO (Dimethyl sulphoxide).
Standard: Bacterial strain: Streptomycin- 125 μg/mL, Fungal strains: Fluconazole- 125 μg/mL.

8, **9**, **11** and **12** showed good inhibition of growth in case of *C. albicans* as well as *A. niger* while compound **10** showed moderate activity against both antifungal strain. Electron donating and electron withdrawing groups present on phenyl ring does not affect the growth inhibitory activity against tested bacterial and fungal strains.

In general, most of the tested compounds revealed better activity against the antibacterial strain (*E. coli*, *S. aureus*) and antifungal strain (*C. albicans*, *A. niger*). Novel compounds are reactive against fungal strain as compared to bacterial strain tested over microorganisms.

4. Conclusion

We have designed and synthesized a series of novel 4-(1,3-benzoxazol-2-yl)-2-phenylnaphtho[1,2-*d*]1,3]oxazole derivatives. The photophysical property study shows that all are fluorescent and absorbs from 296 to 332 nm while emits in the ranges of 368 to 404 nm with excellent quantum yield. These novel compounds were evaluated for *in vitro* antibacterial activity against *E. coli* and *S. aureus* and antifungal activity against *C. albicans* and *A. niger* using serial dilution technique. The synthesized compounds show good antifungal activity than that of antibacterial. All synthesized compounds were confirmed by FT-IR, ^{1}H NMR and mass spectral analysis. We believe that the insights gained in this study would be useful for the development of potential drug candidates derived from naphtho [1,2-*d*] [1,3] oxazole derivatives in the development of novel antiinfective agents.

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References

1. (a) Devinder K, Jacob M R, Reynolds M B and Kerwin S M 2002 *Biorg. Med. Chem.* **10** 3997; (b) Sato Y, Yamada M, Yoshida S, Soneda T, Ishikawa M, Nizato T, Suzuki K and Konno F 1998 *J. Med. Chem.* **41** 3015; (c) Katz L 1953 *J. Am. Chem. Soc.* **75** 712
2. (a) Rodriguez A D, Ramirez C, Rodriguez I I and Gonzalez E 1999 *Org. Lett.* **1** 527; (b) Kobayashi J, Madono T and Shigemori H 1995 *Tetrahedron Lett.* **36** 5589
3. Tanaka K, Kumagai T, Aoki H, Deguchi M and Iwata S 2001 *J. Org. Chem.* **66** 7328
4. Abbady M A 1966 *Preparation and application of some new naphthoxazole dyes*. M. Sc. Thesis, Assiut University Assiut, Egypt
5. Kumar A, Ahmad P, Maurya R A, Singh A B and Srivastava A K 2009 *Eur. J. Med. Chem.* **44** 109
6. Malamas M S, Sredy J, Katz Moxham A, Xu W X, Mcdevitt R, Adedayo F O, Sawiciki D R, Seetaller L, Sullivan L and Taylor J R 2000 *J. Med. Chem.* **43** 1293
7. Malamas M S, Sredy J, Gunavan I, Mihan B, Sawichi D R, Seestaller L, Sullivan D and Flam B R 2000 *J. Med. Chem.* **43** 995
8. Ertan T, Yildiz I, Tekiner-Gulbas B, Bolelli K, Temiz-Arpaci O, Yalcin I, Aki E, Ozkan S and Kaynak F 2009 *Eur. J. Med. Chem.* **44** 501
9. Dunwell D W and Delme 1977 *Eur. J. Med. Chem.* **20** 797
10. White A W, Curtin N J, Eastman B W, Golding B T, Hostomsky Z, Kyle S, Li J, Maegley K A, Skalitzky D J, Webber S E, Yu X H and Griffin R 2004 *J. Bioorg. Med. Chem. Lett.* **14** 2433
11. Novelli F, Tasso B, Sparatore F and Sparatore A 1997 *Farmaco* **52** 499
12. (a) McKee M L and Kerwin S M 2008 *Bioorg. Med. Chem.* **16** 1775; (b) Oksuzoglu E, Temiz-Arpaci O, Tekiner-Gulbas B, Eroglu H, Sen G, Alper S, Yildiz I, Diril N, Aki-Sener E and Yalcin I 2008 *Med. Chem. Res.* **16** 1
13. Sun L Q, Chen J, Bruce M, Deskus J A, Epperson J R, Takaki K, Johnson V G, Iben L, Mahle C D, Ryan E and Xu C 2004 *Bioorg Med. Chem. Lett.* **14** 3799
14. (a) Costa S P G, Oliveira E, Lodeiro C and Raposo M M 2007 *Sensors* **7** 2096; (b) Shamsipur M, Poursaberi T, Karami A R, Hosseini M, Momeni A, Alizadeh N, Yousefi M and Ganjali M R 2004 *Anal. Chim. Acta.* **501** 55; (c) Zang X B, Peng J, He C L, Shen G L and Yu R Q 2006 *Anal. Chim. Acta.* **567** 189
15. (a) Heathcock C H 1991 In: *Comprehensive organic synthesis* B M Trost, and I Flemming (eds) New York: Pergamon Press **Vol 2**; (b) Varga A, Aki Sxener E, Yalcin I, Temiz Arpaci O, Tekiner Gulbasx B, Cherepnev G and Molnar J 2005 *In Vivo* **19** 1087
16. Yousuke O, Haruka E and Katsuhira Y 2009 *Dyes Pigments* **82** 58
17. Ooyama Y, Nonami K, Watanabe S and Yoshida K 2008 *Dyes Pigments* **77** 315
18. (a) Padalkar V S, Borse B N, Gupta V D, Phatangare K R, Patil V S and Sekar N 2011 *J. Het. Chem.* (Article Accepted, Manuscript ID: JHET-11–0566.R1); (b) Padalkar V S, Borse B N, Gupta V D, Phatangare K R, Patil V S, Umape P G and Sekar N 2011 *Arab. J. Chem* (Article Accepted, doi:10.1016/j.arabjc.2011.12.006)
19. Reynolds M b, DeLuca M R and Kerwin S M 1999 *Bioorg. Chem.* **27** 326
20. Devinder K, Melissa R J, Michael B R and Sean M K 2002 *Bioorg. Med. Chem.* **10** 3997
21. Xu Y and Pang Y 2010 *Chem. Commun.* **46** 4070
22. Kim T I, Kang H J, Han G, Chung S J and Kim Y 2009 *Chem. Commun.* **45** 5895
23. Chen W, Wright B D and Pang Y 2012 *Chem. Commun.* **48** 3824

24. Yan L, Ye Z, Peng C and Zhang S 2012 *Tetrahedron* **68** 2725
25. Matsui, Kuroda 1967 *Kogyo Kagaku Zasshi* **70** 2325
26. Rangnekar D W and Rajadhyaksha D D 1986 *Dyes Pigments* **7** 365
27. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. 2000 *National Committee for Clinical Laboratory Standards*, PA: Wayne
28. Koeth L M, King A, Knight May J, Miller Phillips I and Poupard J A 2000 *J. Antimicrob. Chemother.* **46** 369
29. Sarker S D, Nahar L and Kumarasamy Y 2007 *Methods* **42** 321
30. Padalkar V S, Tathe A B, Gupta V D, Patil V S, Phatangare K R and Sekar N 2012 *J. Fluoresc.* **22** 311