Microwave assisted synthesis of dihydrobenzo[4,5]imidazo[1,2-α]pyrimidin-4-ones; synthesis, in vitro antimicrobial and anticancer activities of novel coumarin substituted dihydrobenzo[4,5]imidazo [1,2-α]pyrimidin-4-ones

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ARTICLE INFO

Article history:
Received 19 February 2013
Received in revised form 17 July 2013
Accepted 22 July 2013
Available online 15 August 2013

Keywords:
4-Bromomethylcoumarins
Coumarins
Benzimidazole
Pyrimidine

ABSTRACT

The present article describes the synthesis of dihydrobenzo[4,5]imidazo[1,2-α]pyrimidin-4-one (2a–h) under microwave irradiation. The product was obtained in excellent yield (74–94%) in a shorter reaction time (2 min). These molecules (2a, b) further reacted with various substituted 4-bromomethylcoumarins (3a–f) to yield a new series of coumarin substituted dihydrobenzo[4,5]imidazo[1,2-α]pyrimidin-4-ones (4a–h). The structure of all the synthesized compounds were confirmed by spectral studies and screened for their in vitro antibacterial activity against three Gram-positive bacteria viz., Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans and three Gram-negative bacteria viz., Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and antifungal activity against Candida albicans, Aspergillus niger, Aspergillus fumigatus, Fusarium oxysporum, Penicillium chrysogenum and anticancer activity against Dalton’s Ascitic Lymphoma (DAL) cell line.

In general, all the compounds possessed better antifungal properties than antibacterial properties. The coumarin substituted dihydrobenzo[4,5]imidazo[1,2-α]pyrimidin-4-one (4g) (R = i-Pr, R1 = 6-Cl) was found to be the most potent cytotoxic compound (88%) against Dalton’s Ascitic Lymphoma cell line at the concentration of 100 μg/mL.

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1. Introduction

Dihydrobenzo[4,5]imidazo[1,2-α]pyrimidin-4-one is a class of fused tricyclic system having three nitrogen atoms. The design concept of dihydrobenzo[4,5]imidazo[1,2-α]pyrimidin-4-ones has arisen from the broad spectrum and the wide range of biological activities of the benzimidazole and pyrimidine.

Benzimidazole derivatives [1] exhibited high cytotoxicity against HepG-2 cells and good EGFR inhibitory activity. 2-Substituted-5-amino-benzimidazoles [2] possessed significant cytotoxicity against breast cancer cell line MCF-7. 1,2,5-Trisubstituted benzimidazoles [3] and benzimidazolo pyrimidine conjugates [4] were found to be antitumor agents against Melanoma cell lines. QSAR analyses of 2-aminobenzimidazole derivatives [5] were studied on the relation between acute toxicity and the octanol/water partition coefficient.


The fusion of benzimidazole and pyrimidine pharmacophores in a single molecular frame work and the study of subsequent influence on the biological activities are of current interest.
The known synthetic method of dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-4-one derivatives [11] had many demerits such as long reaction time, drastic condition, tedious experimental procedure and low yield. Hence, there is a need for a simple and straightforward method for the synthesis of dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-4-one derivatives. On the other hand, to date, neither the synthesis nor the biological evaluation of dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-4-ones has been reported in the literature. For all these reasons, we have done laboratory work on the synthesis of fused dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-4-ones.

Coumarins are known to be biologically versatile compounds possessing several biological properties. Coumarin Mannich bases [12] inhibited carrageenin-induced hind paw edema and found to possess protective properties against adjuvant-induced arthritis in rats. 4-Amino-3-(2-methylbenzyl)coumarin derivatives [13] exhibited potent estrogenic activity on the estrogen receptor positive (ER+) human MCF-7 breast cancer cell line. 4-Hydroxy coumarin derivatives [14] showed pronounced prolongation of prothrombin time with anticoagulant values similar to that of warfarin. Benzo[b]thiazolyl coumarin acetamide derivatives [15] exhibited strong in vitro anti-HIV effect against the wild-type HIV-1 cell line. The in vitro antioxidant activities of 4-schiff bases-7-benzyloxy coumarin derivatives [16] revealed that DPPH and ABTS+ radical scavenging activities were better than that of the commercial antioxidant BHT.

Based on the survey of recent literature studies on benzimidazoles, pyrimidines and coumarins in our effort to discover novel antimicrobial and anticancer agents, the aim of our work is the synthesis of reported compounds (2a-h) and to evaluate them for their therapeutic importance.

2. Chemistry

The synthesis of compounds (2a–h) (R: a; i-Pr; b; 4-CF3C6H4; c; 3-FC6H4; d; CF3; e; C6H5; f; 4-FC6H4; g; 3-ClC6H4; h; 4-OCH3C6H4) was accomplished by synthetic sequence shown in Scheme 1. The preparation of dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-4-ones was carried out by routine methods. The IR spectrum of the compound 10-(6-methoxy-2-oxo-2H-chromen-4-ylmethyl)-2-trifluoromethyl-10H-benzoz[4,5]imidazo[1,2-a]pyrimidin-4-one (2a) (R = i-Pr) showed a carbonyl stretching frequency at 1664 cm⁻¹, where as the N-H stretching frequency showed a strong absorption band at 3230 cm⁻¹. The 1H NMR spectrum of compound (2a) exhibited a singlet in the region δ 5.87 due to presence of C3-H proton. A multiplet was observed at δ 2.79 due to methine proton of isopropyl group. The methyl protons of isopropyl group and C7-H proton were found to be a doublet at δ 1.22 (J = 9 Hz) and 8.38 (J = 9 Hz) respectively. A triplet was appeared at δ 7.27 (J = 6 Hz) due to the presence of C6-H proton. The multiplet was observed in the region between δ 7.42–7.50 due to the presence of C9–H and C10–H protons. The N-H proton was resonated as a singlet at δ 12.88 which was further confirmed by its D2O exchange. The mass spectrum (ESI-MS) of the compound (2a) showed a [M + 1] peak at 228. The 13C NMR spectral data of all the compounds is given in Experimental section.

In the IR spectrum of the compound 2-isopropyl-10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-one (2a) (R = i-Pr), the carbonyl stretching frequency was observed at 1664 cm⁻¹, where as the N–H stretching frequency showed a strong absorption band at 3230 cm⁻¹. The 1H NMR spectrum of compound (2a) exhibited a singlet in the region δ 5.87 due to presence of C3-H proton. A multiplet was observed at δ 2.79 due to methine proton of isopropyl group. The methyl protons of isopropyl group and C7-H proton were found to be a doublet at δ 1.22 (J = 9 Hz) and 8.38 (J = 9 Hz) respectively. A triplet was appeared at δ 7.27 (J = 6 Hz) due to the presence of C6-H proton. The multiplet was observed in the region between δ 7.42–7.50 due to the presence of C9–H and C10–H protons. The N–H proton was resonated as a singlet at δ 12.88 which was further confirmed by its D2O exchange. The mass spectrum (ESI-MS) of the compound (2a) showed a [M + 1] peak at 228. The 13C NMR spectral data of all the compounds is given in Experimental section.

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The IR spectrum of the compound 2-isopropyl-10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-one (2a) (R = i-Pr) showed a carbonyl stretching frequency at 1664 cm⁻¹, where as the lactone carbonyl stretching frequency appeared at 1712 cm⁻¹. The 1H NMR spectrum of compound (2a) exhibited a singlet in the region δ 12.88 which was further confirmed by its D2O exchange. The mass spectrum (ESI-MS) of the compound (2a) showed a [M + 1] peak at 228. The 13C NMR spectral data of all the compounds is given in Experimental section.
The molecular structure of the compound (4b) is also established by single crystal analysis [19] as shown in Fig. 2.

### 2.2. Antimicrobial activity

All the newly synthesized compounds (2a–h) and (4a–h) were screened for their antibacterial and antifungal activity at different concentrations of 100, 50, 25, 12.5, 6.25, 3.125, 1.6, 0.8, 0.4 and 0.2 μg/mL by the broth micro dilution method. The minimum inhibitory concentrations (MIC) were determined by serial dilution method [20].

Antibacterial activity was carried out against three Gram-positive bacteria viz., Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans and three Gram-negative bacteria viz., Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa. Ciprofloxacin was used as a standard. Antifungal activity was carried out against six fungi viz., Candida albicans, Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Fusarium oxysporum and Penicillium chrysogenum. Fluconazole was used as a standard.

The investigation of antibacterial data (Table 2) showed that most of the tested compounds exhibited good bacterial inhibition. The compounds (2a–h) were found to be highly active against *E. faecalis* with MIC of 0.2 μg/mL. The compound (4d) (R = CF3, R1 = 6,8-dimethyl) was found to be highly active against *S. mutans* with MIC of 0.2 μg/mL. The compound (4e) (R = i-Pr, R1 = 6-F) was found to be most active against *S. mutans* with MIC of 0.8 μg/mL (Fig. 3). It is interesting to note that all the tested compounds were found to be most potent against *E. faecalis* when compared to standard drug Ciprofloxacin. The rest of the compounds were found to be inactive.

The investigation of antifungal data (Table 3) showed that most of the tested compounds exhibited good fungal inhibition. The compounds (2f) (R = 4-FC6H4) and (2g) (R = 4-ClC6H4) were found to be highly active against *A. fumigatus* and *A. flavus* with MIC of 0.2 μg/mL. The compounds (2b) (R = 4-CF3C6H4), (2d) (R = CF3), (2e) (R = C6H5), (2g) (R = 4-ClC6H4), (2h) (R = 4-CH3OOC6H4) and (4a–h) were found to be highly active against *F. oxysporum* with MIC of 0.2 μg/mL. The compounds (2a) (R = i-Pr), (2b) (R = 4-CF3C6H4), (2c) (R = 3-FC6H4), (2e) (R = C6H5), (4b) (R = CF3, R1 = 6-F), (4c) (R = CF3, R1 = 6-CH3), (4d) (R = CF3, R1 = 6,8-dimethyl) and (4h) (R = i-Pr, R1 = 6-Br) were found to be highly active against *P. chrysogenum* with MIC of 0.2 μg/mL (Fig. 4). It is interesting to note that all the tested compounds were found to be most potent against *A. fumigatus, F. oxysporum* and *P. chrysogenum* when compared to standard drug Fluconazole.

### 2.3. In vitro cell cytotoxicity

The newly synthesized compounds (2a–h) and (4a–h) were determined in vitro cell cytotoxicity by using trypan blue dye exclusion assay method [21]. In this test, only the dead cells took up the dye due to lack of intact membranes. Dalton’s Ascitic Lymphoma (DAL) cells (0.2 mL, 10⁶ cells/mL), ice cold phosphate buffer saline (1 mL, pH = 7.4) and one of the compounds (2a–h) and (4a–h) (0.2 mL) were taken in an Eppendorf tube. They were incubated in CO₂ incubator at 37 °C with continuous flow of 5% CO₂ for 3 h.
Then, previous mixture (0.2 mL), ice cold phosphate buffer saline (0.3 mL, pH = 7.4) and trypan blue solution (0.5 mL, 0.4% in normal saline) were taken in an Eppendorf tube and kept for 5–15 min at room temperature. The percentage of dead cells was calculated with the following formula using Neubauer chamber.

\[
\text{% Dead cell} = \frac{\text{Number of dead cells}}{\text{Sum of dead cells and living cells}} \times 100
\]

The investigation of in vitro cell cytotoxicity (Table 4) revealed that most of the tested compounds exhibited good activity. The compounds \((2b) (R = 4-\text{CF}_3\text{C}_6\text{H}_4), (2d) (R = \text{CF}_3), (2f) (R = 4-\text{CF}_3\text{C}_6\text{H}_4), (2g) (R = 4-\text{ClC}_6\text{H}_4), (4b) (R = \text{CF}_3, R_1 = 6-\text{F}), (4d) (R = \text{CF}_3, R_1 = 6,8-\text{dimethyl}), (4e) (R = \text{i-Pr}, R_1 = 6-\text{F})\) and \((4g) (R = \text{i-Pr}, R_1 = 6-\text{Cl})\) were found to be highly active (>70%) against DAL cell at the concentration of 100 \(\mu\text{g/mL}\). The rest of the compounds were found to be moderately active (>40%) against DAL cell at the concentration of 100 \(\mu\text{g/mL}\) (Fig. 5).

3. Experimental section

The melting points were determined by open capillary method using electric melting point apparatus and are uncorrected. The IR spectra (KBr disc) were recorded on a Shimadzu-8400S FT-IR Spectrophotometer. \(^1\text{H}\) NMR spectra were recorded on Bruker 300 MHz spectrometer, \(^{13}\text{C}\) NMR, \(^1\text{H}\) H COSY, HSQC and \(^{19}\text{F}\) NMR spectra were recorded on Bruker 400 MHz spectrometer by using DMSO-\(d_6\) as a solvent and TMS as an internal standard. The chemical shifts are expressed in \(\delta\) ppm. The mass spectra were recorded using Agilent-Single Quartz ESI-MS and Agilent-Single Quartz LC-MS. The purity of the compounds was checked by TLC. Milestone laboratory’s microwave reactor was used to carry out the microwave reactions. The elemental analyses were carried out using Elemental Vario Micro Cube CHN Rapid Analyzer. All the compounds gave satisfactory elemental analysis.

3.1. General procedure for the preparation of compounds

3.1.1. Synthesis of 10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-ones (2a–h)

An equimolar mixture of 2-aminobenzimidazole (0.5 g, 3.75 mmol) and \(\beta\)-ketesters (1a–h) (3.75 mmol) in DMF (10 mL) was added to a microwave tube equipped with a magnetic stir bar. The microwave tube was fitted with a reflux condenser and irradiated in a microwave reactor at a temperature of 130 °C for 3 min.
at a maximum power of 320 W. Then, the reaction mixture was poured on to crushed ice. The solid was filtered and washed with 100 mL of cold water. The crude product was dried and recrystallized from 1:3 ethyl acetate and chloroform.

### 3.1.1.1. 2-Isopropyl-10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-one (2a).

Colorless solid, Yield: 93%. Mp: 189–191 °C, IR (KBr, cm⁻¹): 3230 cm⁻¹ (N–H), 1664 cm⁻¹ (C=O); ¹H NMR (300 MHz, DMSO-d₆): δ 1.22 (d, 6H, 2-CH₃ of i-Pr, J = 6.9 Hz), 2.79 (m, 1H, CH of i-Pr), 5.87 (t, 1H, C₆–H, J = 7.8 Hz), 7.47–7.50 (m, 2H, C₉–H & C₁₀–H), 8.38 (d, 1H, C₇–H, J = 8.1 Hz), 12.88 (s, 1H, N–H, D₂O exchangeable) ppm; ¹³C NMR (100 MHz, DMSO-d₆): δ 21.65, 34.87, 98.42, 113.59, 115.08, 121.28, 125.92, 126.81, 135.16, 148.25, 158.95, 159.22 ppm; ESI-MS: m/z [M + H]+ 228; Anal. C₁₃H₁₃N₃O. Calcd for: C, 69.60; H, 5.71; N, 24.87, 28.42, 31.97, 35.52, 39.07, 42.62, 46.17, 49.72, 53.27 ppm.

### 3.1.1.2. 2-(4-Fluoromethyl-phenyl)-10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-one (2b).

Colorless solid, Yield: 87%. Mp: 233–235 °C, IR (KBr, cm⁻¹): 3236 cm⁻¹ (N–H), 1687 cm⁻¹ (C=O); ¹H NMR (300 MHz, DMSO-d₆): δ 6.82 (s, 1H, C₁–H), 7.34–7.39 (m, 1H, C₆–H), 7.51–7.53 (d, 2H, C₆–H & C₇–H, J = 3.9 Hz), 8.05 (d, 2H, C₁₀–H & C₁₁–H, J = 6.0 Hz), 8.48 (d, 1H, C₉–H, J = 8.1 Hz), 8.73 (d, 2H, C₁₆–H & C₁₈–H, J = 3.6 Hz), 13.14 (s, 1H, N–H) ppm; ¹³C NMR (100 MHz, DMSO-d₆): δ 98.18, 111.02, 115.69, 117.46, 120.56, 121.93, 125.45, 126.30, 126.92, 127.71, 129.12, 129.89 (q, JCF = 32 Hz), 140.99, 148.14, 149.54, 158.93, 166.44 ppm; ESI-MS: m/z [M + H]+ 330; Anal. C₁₄H₁₀F₄N₅O. Calcd for: C, 61.92; H, 2.91; N, 12.60.

### 3.1.1.3. 2-(3-Fluoro-phenyl)-10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-one (2c).

Colorless solid, Yield: 94%. Mp: 213–215 °C, IR (KBr, cm⁻¹): 3237 cm⁻¹ (N–H), 1681 cm⁻¹ (C=O); ¹H NMR (300 MHz, DMSO-d₆): δ 6.65 (s, 1H, C₁–H), 7.31–7.38 (m, 3H, Ar–H), 7.49 (d, 2H, J = 3.9 Hz), 8.17–8.21 (m, 2H, Ar–H), 8.46 (d, 1H, J = 8.1 Hz, Ar–H), 13.11 (s, 1H, N–H) ppm; ¹³C NMR (100 MHz, DMSO-d₆): δ 97.62, 110.97, 113.42, (d, JCF = 23 Hz), 115.67, 116.81, 121.89, 123.0, 125.69, 126.25, 130.47, 130.58, 139.56, 139.64, 149.46, 159.06, 159.71, 161.20, 163.61 ppm; ESI-MS: m/z [M + H]+ 280; Anal. C₁₄H₁₀F₃N₅O. Calcd for: C, 68.81; H, 3.61; N, 15.05. Found: C, 68.70; H, 3.55; N, 14.97.


A mixture of 10H-benzo[4,5]imidazo[1,2-a]pyrimidin-4-ones (2a, b) (2.20 mmol) and anhydrous K₂CO₃ (0.6 g, 4.4 mmol) was stirred in 30 mL of dry acetonitrile for 20 min. 4-Bromomethylcoumarins (3a–f) (2.20 mmol) was added and

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**Table 3** Results of antifungal activities of compounds (2a–h) and (4a–h) MICs (μg/mL).

<table>
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<tr>
<th>Compounds</th>
<th>C. albicans</th>
<th>A. niger</th>
<th>A. fumigatus</th>
<th>A. flavus</th>
<th>F. oxysporum</th>
<th>P. chrysogenum</th>
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<td>1.6</td>
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<tr>
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<tr>
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<tr>
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**Fig. 4.** Antifungal activity of compounds (2a–h) and (4a–h).
stirring was continued for 24 h. The reaction mixture was concentrated to one fourth volume and poured on to crushed ice. The solid separated was filtered and washed with 5% HCl (10 mL). Then, it was washed with 50 mL of cold water. The crude product was dried and recrystallized from ethanol.

3.1.2.1. 10-(6-Methoxy-2-oxo-2H-chromen-4-ylmethyl)-2-trifluoromethyl-10H-benzo[4,5]imidazo[1,2-α]pyrimidin-4-one (4a). Colorless solid, Yield: 93%. Mp: 228–230 °C, IR (KBr, cm\(^{-1}\)): 1670 cm\(^{-1}\) (C=O), 1738 cm\(^{-1}\) (lactone C=O); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 5.86 (s, 2H, N–CH\(_2\)), 6.28 (s, 1H, C3–H of coumarin), 6.64 (s, 1H, C3–H of benzimidazopyrimidine), 7.47–8.58 (m, 7H, Ar–H); \(^13\)C NMR (100 MHz, DMSO-d\(_6\)): \(\delta\) 42.44, 100.88, 110.54, 111.44, 113.12, 116.01, 118.16, 118.46, 118.55, 119.49, 119.74, 123.38, 125.39, 126.83, 130.71, 148.15, 149.44, 150.03 (q, \(\delta\)JCF = 34 Hz), 156.91, 158.96, 159.23, 159.30 ppm; ESI-MS: m/z [M + 1] 442; Anal. C\(_{22}\)H\(_{14}\)F\(_3\)N\(_3\)O\(_4\). Calcd for: C, 59.87; H, 3.20; N, 9.74. Found: C, 59.63; H, 2.47; N, 9.56.

3.1.2.4. 10-(6,8-Dimethyl-2-oxo-2H-chromen-4-ylmethyl)-2-trifluoromethyl-10H-benzo[4,5]imidazo[1,2-α]pyrimidin-4-one (4d). Colorless solid, Yield: 91%. Mp: 216–218 °C, IR (KBr, cm\(^{-1}\)): 1670 cm\(^{-1}\) (C=O), 1730 cm\(^{-1}\) (lactone C=O); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 2.36 (s, 3H, 6-CH\(_3\)), 2.41 (s, 3H, 8-CH\(_3\)), 5.84 (s, 2H, N–CH\(_2\)), 6.13 (s, 1H, C3–H of coumarin), 6.64 (s, 1H, C3–H of benzimidazopyrimidine), 7.43–8.58 (m, 6H, Ar–H) ppm; \(^13\)C NMR (100 MHz, DMSO-d\(_6\)): \(\delta\) 15.16, 20.30, 42.62, 100.08, 110.52, 111.69, 115.98, 116.62, 122.46, 122.31, 123.33, 125.16, 125.36, 126.10, 130.81, 131.18, 134.38, 148.81, 150.04 (q, \(\delta\)JCF = 34 Hz), 158.93 ppm; ESI-MS: m/z [M + 1] 426; Anal. C\(_{23}\)H\(_{16}\)F\(_3\)N\(_3\)O\(_2\). Calcd For: C, 62.12; H, 3.32; N, 9.88. Found: C, 62.01; H, 3.21; N, 9.74.

3.1.2.5. 10-(6-Fluoro-2-oxo-2H-chromen-4-ylmethyl)-2-trifluoromethyl-10H-benzo[4,5]imidazo[1,2-α]pyrimidin-4-one (4e). Colorless solid, Yield: 88%. Mp: 230–232 °C, IR (KBr, cm\(^{-1}\)): 1664 cm\(^{-1}\) (C=O), 1736 cm\(^{-1}\) (lactone C=O); \(^1\)H NMR (300 MHz, DMSO-d\(_6\)): \(\delta\) 1.20 (d, 6H, 2CH\(_3\) of i-Pr, \(J\) = 6.0 Hz), 2.80 (m, 1H, CH of i-Pr), 5.82 (s, 2H, N–CH\(_2\)), 6.03 (s, 1H, C3–H of coumarin), 6.24 (s, 1H, C3–H of benzimidazopyrimidine), 7.45–8.50 (m, 7H, Ar–H) ppm; \(^13\)C NMR

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**Table 4**

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<th>Number of % of dead cells</th>
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<td></td>
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<td></td>
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<td>2c</td>
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</table>

In vitro cytotoxicity of compounds (2a–h) and (4a–h).

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**Fig. 5.** In vitro cell cytotoxicity of (2a–h) and (4a–h).
properties than antibacterial properties. The coumarin substituted dihydrobenzo[4,5]imidazo[1,2-a]pyrimidin-4-one (4g) (R = i-Pr, R1 = 6-Cl) was found to be the most potent cytotoxic compound (88%) against Dalton’s Ascitic Lymphoma cell line at the concentration of 100 µg/mL.

Acknowledgments

We are grateful to the University Grant Commission, New Delhi, India for the financial support [F.NO.37-76/2009 (SR)]. We are thankful to Prof. Y.S. Bhat, Bangalore Institute of Technology, Bangalore, for providing Microwave Reactor facility and for his encouragement. We are also thankful to Indian Institute of Science, Bangalore for the spectral analysis.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.07.015.

References

[20] CDC 910400 (4b) contains the supplementary crystallographic data for this paper. This data can be obtained free of charge at www.ccdc.cam.ac.uk or email: deposit@ccdc.cam.ac.uk.