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Original article

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

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ABSTRACT

The present article describes the synthesis of dihydrobenzo[4,5]imidazo[1,2-*a*]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-*a*]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*, *Aspergillus flavus*, *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-*a*]pyrimidin-4-one (**4g**) (R = *i*-Pr, R_1 = 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.

1. Introduction

Dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidin-4-one is a class of fused tricyclic system having three nitrogen atoms. The design concept of dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones has arised 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.

6-Butylfuro[2,3-*d*]pyrimidine derivatives [6] showed the highest cytostatic activity against Malignant leukemia and T-lymphocyte cells. 5-Benzylidine barbiturate derivatives [7] inhibited the growth of mushroom tyrosinase and Gram-positive bacteria *Staphylococcus aureus*. Pyrimidine bases [8] exerted pronounced antiproliferative activity against the HeLa and MiaPaCa-2 cell lines. 1-Adamantyl thiopyrimidines [9] displayed the significant cytotoxic activity particularly against H69AR cell line. Pyrimidine analogs of indane-1,3-diones [10] showed significant reduction in ulcerogenic activity when compared to standard drug *Indomethacin*.

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.

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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 straight forward method for the synthesis of dihydrobenzo[4,5]imidazo [1,2-*a*]pyrimidin-4-one derivatives. On the otherhand, 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 carraggeenin-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. Benzothiazolyl 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 and in our effort to discover novel antimicrobial and anticancer agents, the aim of our work is synthesis of coumarin substituted dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones and to evaluate them for their therapeutic importance.

2. Chemistry

The synthesis of compounds (**2a**–**h**) (R: a; *i*-Pr, b; 4-$CF_3C_6H_4$, c; 3-FC_6H_4, d; CF_3, e; C_6H_5, f; 4-FC_6H_4, g; 3-ClC_6H_4, h; 4-$CH_3OC_6H_4$) 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 the condensation of β-ketoesters (**1a**–**h**) with 2-aminobenzimidazole under microwave irradiation. The best conditions to obtain these compounds were achieved at 130 °C using DMF as a reaction media. This method gave the higher yield (74–94%) and required a shorter reaction time (3 min). The compounds (**2d**–**h**) have already been reported by the thermal method (Table 1) in the literature [11].

4-Bromomethyl coumarins [17] (**3a**–**f**) were synthesized by Pechmann cyclization of phenols with 4-bromoethylacetoacetate [18] using conc. H_2SO_4 as a cyclizing agent. The synthesis of coumarin substituted dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones (Scheme 2) were carried out by reaction of 4-bromomethyl coumarins (**3a**–**f**) (R_1 = a; 6-OMe, b; 6-F, c; 6-CH_3, d; 6,8-dimethyl, e; 6-Cl, f; 6-Br) with dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones (**2a**, **b**) (R = a; *i*-Pr, b; 4-$CF_3C_6H_4$) in the presence of anhydrous K_2CO_3 in dry acetone at room temperature for 24 h. Removal of solvents under reduced pressure afforded the title compounds (**4a**–**h**) as solids which were purified by routine methods. The numbering of the skeleton is shown in Fig. 1.

Table 1
Synthesis of reported compounds (**2d**–**h**) under microwave irradiation.

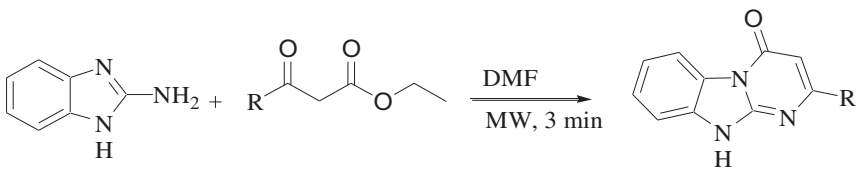
Compounds	Thermal (reported)			Microwave		
	Time	Temperature	Yield (%)	Time	Temperature	Yield (%)
2d	60 min	140 °C	55	3 min	130 °C	74
2e	60 min	140 °C	75	3 min	130 °C	87
2f	60 min	140 °C	87	3 min	130 °C	91
2g	60 min	140 °C	80	3 min	130 °C	90
2h	60 min	140 °C	87	3 min	130 °C	94

Synthesis of reported compounds (**2d**–**h**) under microwave irradiation.

2.1. Result and discussion

In the IR spectrum of the compound 2-isopropyl-10*H*-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 C_3–H proton. A multiplet was observed at δ 2.79 due to methine proton of isopropyl group. The methyl protons of isopropyl group and C_7–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 C_8–H proton. The multiplet was observed in the region between δ 7.42–7.50 due to the presence of C_9–H and C_{10}–H protons. The N–H proton was resonated as a singlet at δ 12.88 which was further confirmed by its D_2O exchange. The mass spectrum (ESI-MS) of the compound (**2a**) showed a [M + 1] peak at 228. The ^{13}C NMR spectral data of all the compounds is given in Experimental section.

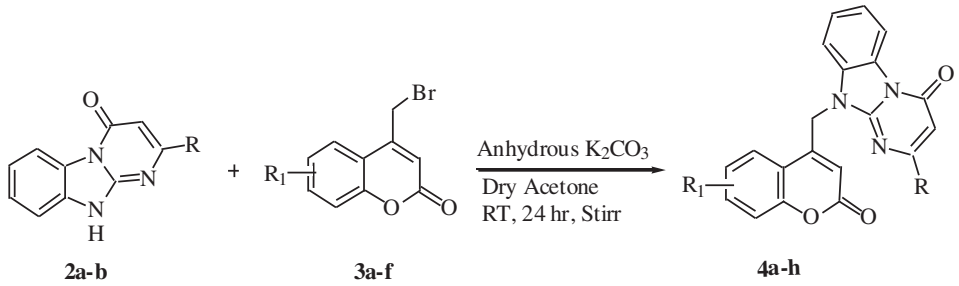
The IR spectrum of the compound 10-(6-methoxy-2-oxo-2*H*-chromen-4-ylmethyl)-2-trifluoromethyl-10*H*-benzo[4,5]imidazo [1,2-*a*]pyrimidin-4-one (**4a**) (R = CF_3 & R_1 = 6-OCH_3) displayed the benzimidazopyrimidine carbonyl stretching frequency at 1685 cm^{-1}, where as the lactone carbonyl stretching frequency appeared at 1712 cm^{-1}. The ^1H NMR spectrum of the compound (**4a**) showed a singlet at δ 3.50, 5.82, 6.02 and 6.26 due to 6-OCH_3, N–CH_2, C_3–H of coumarin and C_3–H of benzimidazopyrimidine protons respectively. The aromatic protons resonated as a multiplet in the range of δ 7.45–8.32. The mass spectrum (ESI-MS) of the compound (**4a**) displayed a [M + 1] peak at 442. The ^{13}C NMR spectral data of all the compounds is given in Experimental section.



1a-h **2a-h**

R; a; *i*-Pr, b; 4-$CF_3C_6H_4$, c; 3-FC_6H_4, d; CF_3, e; C_6H_5, f; 4-FC_6H_4, g; 3-ClC_6H_4, h; 4-$OCH_3C_6H_4$

Scheme 1.



2a-b **3a-f** **4a-h**

Compounds	4a	4b	4c	4d	4e	4f	4g	4h
R	CF_3	CF_3	CF_3	CF_3	*i*-Pr	*i*-Pr	*i*-Pr	*i*-Pr
R_1	6-OMe	6-F	6-CH_3	6,8-dimethyl	6-F	6-OMe	6-Cl	6-Br

Scheme 2.

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 = CF_3, R_1 = 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, R_1 = 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-FC_6H_4) and (**2g**) (R = 4-ClC_6H_4) were found to be highly active against *A. fumigatus* and *A. flavus* with MIC of 0.2 μg/mL. The compounds (**2b**) (R = 4-$CF_3C_6H_4$), (**2d**) (R = CF_3), (**2e**) (R = C_6H_5), (**2g**) (R = 3-ClC_6H_4), (**2h**) (R = 4-$CH_3OC_6H_4$) 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-$CF_3C_6H_4$), (**2c**) (R = 3-FC_6H_4), (**2e**) (R = C_6H_5), (**4b**) (R = CF_3, R_1 = 6-F), (**4c**) (R = CF_3, R_1 = 6-CH_3), (**4d**) (R = CF_3, R_1 = 6,8-dimethyl) and (**4h**) (R = *i*-Pr, R_1 = 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, 10^6 cells/mL), ice cold phosphate buffer saline (1 mL, p^H = 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 CO_2 incubator at 37 °C with continuous flow of 5% CO_2 for 3 h.

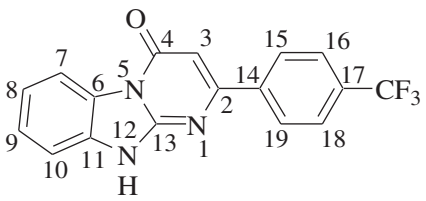


Fig. 1. Numbering of the compound (**2b**).

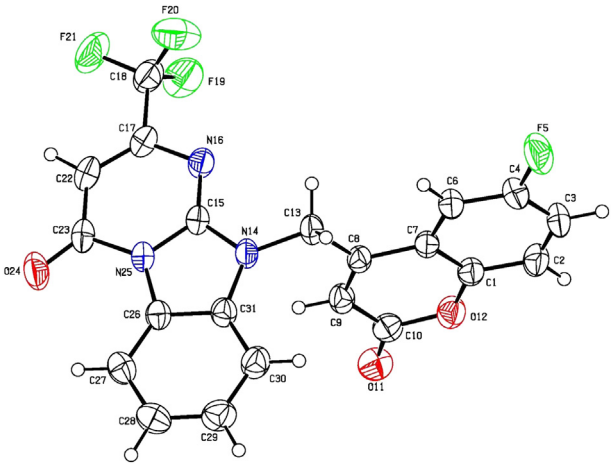


Fig. 2. ORTEP diagram with the displacement at 50% probability level (**4b**).

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

Compounds	Gram-positive			Gram-negative		
	S. aureus	*E. faecalis*	*S. mutans*	*E. coli*	*K. penumoniae*	*P. aeruginosa*
2a	100	0.2	25	12.5	100	12.5
2b	>100	0.2	>100	>100	>100	>100
2c	100	0.2	12.5	100	100	25
2d	100	0.2	50	100	100	100
2e	100	0.2	100	100	100	100
2f	100	0.2	12.5	100	100	100
2g	100	0.2	25	100	100	100
2h	100	0.2	100	100	100	100
4a	100	0.4	12.5	100	100	>100
4b	100	0.8	12.5	100	100	100
4c	100	0.4	12.5	100	100	>100
4d	100	0.4	0.2	100	100	100
4e	100	0.4	0.8	100	100	100
4f	100	0.4	3.16	100	100	>100
4g	100	0.4	12.5	100	100	100
4h	>100	0.8	>100	100	>100	>100
Ciprofloxacin	2	2	2	1	1	2

Then, previous mixture (0.2 mL), ice cold phosphate buffer saline (0.3 mL, $p^H = 7.4$) 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-$CF_3C_6H_4$), (**2d**) (R = CF_3), (**2fb**) (R = 4-FC_6H_4), (**2g**) (R = 4-ClC_6H_4), (**4b**) (R = CF_3, R_1 = 6-F), (**4d**) (R = CF_3, R_1 = 6,8-dimethyl), (**4e**) (R = *i*-Pr, R_1 = 6-F) and (**4g**) (R = *i*-Pr, R_1 = 6-Cl) were found to be highly active (>70%) against DAL cell at the concentration of 100 μg/mL. The rest of the compounds were found to be moderately active (>40%) against DAL cell at the concentration of 100 μg/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}H NMR spectra were recorded on Bruker 300 MHz spectrometer. ^{13}C NMR, H–H Cosy, HSQC and ^{19}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 δ 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-aminobenzimadzole (0.5 g, 3.75 mmol) and β-ketoesters (**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

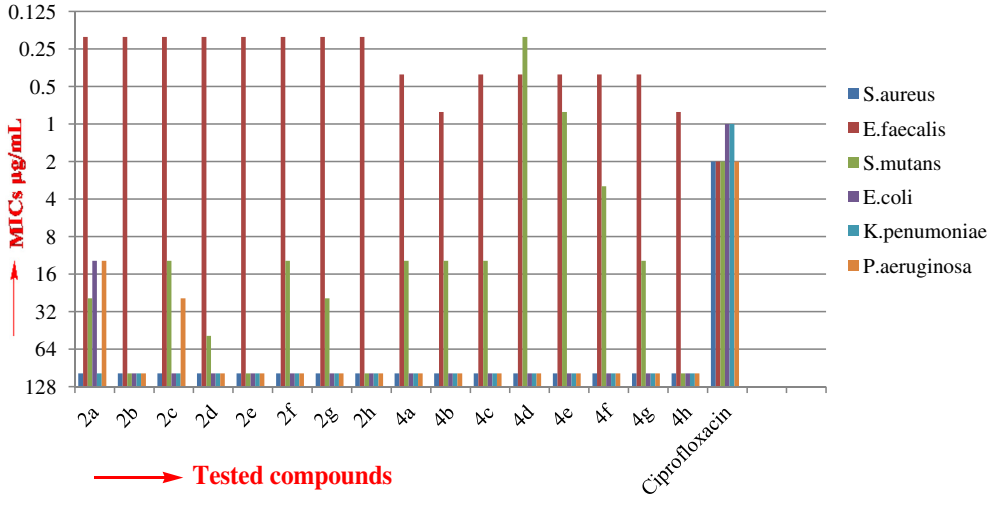


Fig. 3. Antibacterial activity of compounds (**2a**–**h**) and (**4a**–**h**).

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

Compounds	*C. albicans*	*A. niger*	*A. fumigatus*	*A. flavus*	*F. oxysporum*	*P. chrysogenum*
2a	3.12	25	1.6	1.6	0.4	0.2
2b	6.25	100	1.6	1.6	0.2	0.2
2c	1.6	12.5	0.8	0.8	0.4	0.2
2d	1.6	6.25	0.8	0.8	0.2	0.4
2e	1.6	6.25	0.8	0.8	0.2	0.2
2f	1.6	1.6	0.2	0.2	0.4	0.4
2g	1.6	25	0.2	0.2	0.2	0.4
2h	6.25	25	1.6	0.8	0.2	0.4
4a	50	25	6.25	3.12	0.2	0.4
4b	50	25	1.6	1.6	0.2	0.2
4c	50	12.5	25	12.5	0.2	0.2
4d	50	3.12	1.6	3.12	0.2	0.2
4e	25	1.6	0.8	1.6	0.2	0.4
4f	25	50	0.8	0.8	0.2	0.8
4g	50	12.5	0.8	1.6	0.2	0.4
4h	50	25	0.8	0.8	0.2	0.2
Fluconazole	16	8	8	8	8	8

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); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.22 (d, 6H, 2-CH_3 of *i*-Pr, J = 6.9 Hz), 2.79 (m, 1H, CH of *i*-Pr), 5.87 (s, 1H, C_3–H), 7.27 (t, 1H, C_8–H, J = 7.8 Hz), 7.42–7.50 (m, 2H, C_9–H & C_{10}–H), 8.38 (d, 1H, C_7–H, J = 8.1 Hz), 12.88 (s, 1H, N–H, D_2O exchangeable) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 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 + 1] 228; Anal. $C_{13}H_{13}N_3O$. Calcd for: C, 68.70; H, 5.77; N, 18.49. Found: C, 68.60; H, 5.71; N, 18.37.

*3.1.1.2. 2-(4-Trifluoromethyl-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); ^{1}H NMR (300 MHz, DMSO-d_6): δ 6.82 (s, 1H, C_3–H), 7.34–7.39 (m, 1H, C_{10}–H), 7.51–7.53 (d, 2H, C_8–H & C_9–H, J = 3.9 Hz), 8.05 (d, 2H, C_{15}–H & C_{19}–H, J = 6.0 Hz), 8.48 (d, 1H, C_7–H, J = 8.1 Hz), 8.73 (d, 2H, C_{16}–H & C_{18}–H, J = 3.6 Hz), 13.14 (s, 1H, N–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): 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, $^2J_{CF}$ = 32 Hz), 140.99, 148.14, 149.54, 158.93, 166.44 ppm; ESI-MS: m/z [M + 1] 330; Anal. $C_{17}H_{10}F_3N_3O$. Calcd for: C, 62.01; H, 3.06; N, 12.76. Found: 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); ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.65 (s, 1H, C_3–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; ^{13}C NMR (100 MHz, DMSO-d_6): δ 97.62, 110.97, 113.42, (d, $^2J_{CF}$ = 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 + 1] 280; Anal. $C_{16}H_{10}FN_3O$. Calcd for: C, 68.81; H, 3.61; N, 15.05. Found: C, 68.70; H, 3.55; N, 14.97.

*3.1.2. Synthesis of 10-(2-oxo-2H-chromen-4-ylmethyl-10H-benzo[4,5]imidazo[1,2-a])pyrimidin-4-ones (**4a**–**h**)*

A mixture of 10*H*-benzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones (**2a**, **b**) (2.20 mmol) and anhydrous K_2CO_3 (0.6 g, 4.4 mmol) was stirred in 30 mL of dry acetone for 20 min. 4-Bromomethylcoumarins (**3a**–**f**) (2.20 mmol) was added and

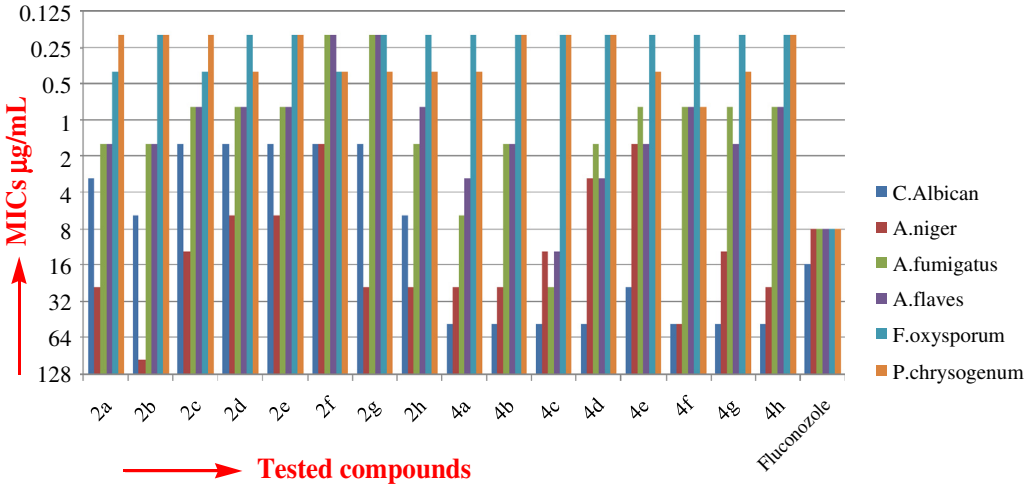


Fig. 4. Antifungal activity of compounds (**2a**–**h**) and (**4a**–**h**).

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

Type of cancer cell (1×10^5)	Concentration of compounds (μg/ml)	Number of compounds	Number of cells		% of dead cells
			Live	Dead	
Dalton's Ascitic Lymphoma	100	**2a**	43	57	57
	100	**2b**	21	79	79
	100	**2c**	46	54	54
	100	**2d**	20	80	80
	100	**2e**	42	58	58
	100	**2f**	17	83	83
	100	**2g**	29	71	71
	100	**2h**	36	64	64
	100	**4a**	42	58	58
	100	**4b**	21	79	79
	100	**4c**	31	69	69
	100	**4d**	22	78	78
	100	**4e**	19	81	81
	100	**4f**	38	62	62
	100	**4g**	12	88	88
	100	**4h**	53	47	47
5-Flourouracil	100	–	12	88	88

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-a]pyrimidin-4-one (**4a**).* Colorless solid, Yield: 93%. Mp: 228–230 °C, IR (KBr, cm^{-1}): 1685 cm^{-1} (C=O), 1712 cm^{-1} (lactone C=O); ^1H NMR (300 MHz, DMSO-d_6): δ 3.50 (s, 3H, OCH_3), 5.82 (s, 2H, N–CH_2), 6.02 (s, 1H, C_3–H of coumarin), 6.26 (s, 1H, C_3–H of benzimidazopyrimidine), 7.45–8.32 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.64, 55.86, 100.84, 107.75, 100.52, 112.32, 116.02, 117.56, 117.70, 119.87, 123.36, 125.36, 126.84, 130.74, 147.37, 148.48, 149.41 (q, $^2J_{CF}$ = 64 Hz), 150.05, 155.64, 158.96, 159.57 ppm; ESI-MS: *m/z* [M + 1] 442; Anal. $C_{22}H_{14}F_3N_3O_4$. Calcd for: C, 59.87; H, 3.20; N, 9.52. Found: C, 59.68; H, 3.06; N, 9.30.

*3.1.2.2. 10-(6-Fluoro-2-oxo-2H-chromen-4-ylmethyl)-2-trifluoromethyl-10H-benzo[4,5]imidazo [1,2-a]pyrimidin-4-one (**4b**).* Colorless solid, Yield: 90%. Mp: 240–245 °C, IR (KBr, cm^{-1}): 1673 cm^{-1} (C=O), 1738 cm^{-1} (lactone C=O); ^1H NMR (300 MHz, DMSO-d_6): δ 5.86 (s, 2H, N–CH_2), 6.28 (s, 1H, C_3–H of coumarin), 6.64 (s, 1H, C_3–H of benzimidazopyrimidine), 7.47–8.58 (m, 7H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 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, $^2J_{CF}$ = 34 Hz), 156.91, 158.96, 159.23, 159.30 ppm; ESI-MS: *m/z* [M + 1] 430; Anal. $C_{21}H_{11}F_4N_3O_3$. Calcd for: C, 58.75; H, 2.58; N, 9.79. Found: C, 58.63; H, 2.47; N, 9.56.

*3.1.2.3. 10-(6-Methyl-2-oxo-2H-chromen-4-ylmethyl)-2-trifluoromethyl-10H-benzo[4,5]Imidazo [1,2-a]pyrimidin-4-one (**4c**).* Colorless solid, Yield: 92%. Mp: 211–213 °C, IR (KBr, cm^{-1}): 1665 cm^{-1} (C=O), 1734 cm^{-1} (lactone C=O); ^1H NMR (300 MHz, DMSO-d_6): δ 2.45 (s, 3H, CH_3), 5.86 (s, 2H, N–CH_2), 6.15 (s, 1H, C_3–H of coumarin), 6.64 (s, 1H, C_3–H of benzimidazopyrimidine), 7.37–8.58 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.31, 42.63, 100.81, 110.23, 111.70, 115.73, 116.63, 119.74, 121.15, 122.32, 123.34, 125.17, 125.32, 125.56, 127.16, 130.81, 133.18, 134.38, 148.81, 150.04 (q, $^2J_{CF}$ = 34 Hz), 158.93 ppm; ESI-MS: *m/z* [M + 1] 426; Anal. $C_{23}H_{16}F_3N_3O_3$. Calcd for: C, 62.12; H, 3.32; N, 9.88. Found: C, 62.01; H, 3.21; N, 9.74.

*3.1.2.4. 10-(6,8-Dimethyl-2-oxo-2H-chromen-4-ylmethyl)-2-trifluoromethyl-10H-benzo[4,5] imidazo[1,2-a]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); ^1H NMR (300 MHz, DMSO-d_6): δ 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, C_3–H of coumarin), 6.64 (s, 1H, C_3–H of benzimidazopyrimidine), 7.43–8.58 (m, 6H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 15.16, 20.30, 42.62, 100.08, 110.52, 111.69, 115.98, 116.62, 122.46, 122.31, 123.33, 125.16, 125.31, 126.86, 130.80, 133.17, 134.37, 148.79, 149.35, 149.46, 150.03 (q, $^2J_{CF}$ = 35 Hz), 158.92, 159.53 ppm; ESI-MS: *m/z* [M + 1] 440; Anal. $C_{23}H_{16}F_3N_3O_3$. Calcd For: C, 62.87; H, 3.67; N, 9.56. Found: C, 62.67; H, 3.58; N, 9.40.

*3.1.2.5. 10-(6-Fluoro-2-oxo-2H-chromen-4-ylmethyl)-2-isopropyl-10H-benzo[4,5]imidazo[1,2-a] 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); ^1H NMR (300 MHz, DMSO-d_6): δ 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, C_3–H of coumarin), 6.24 (s, 1H, C_3–H of benzimidazopyrimidine), 7.45–8.50 (m, 7H, Ar–H) ppm; ^{13}C NMR

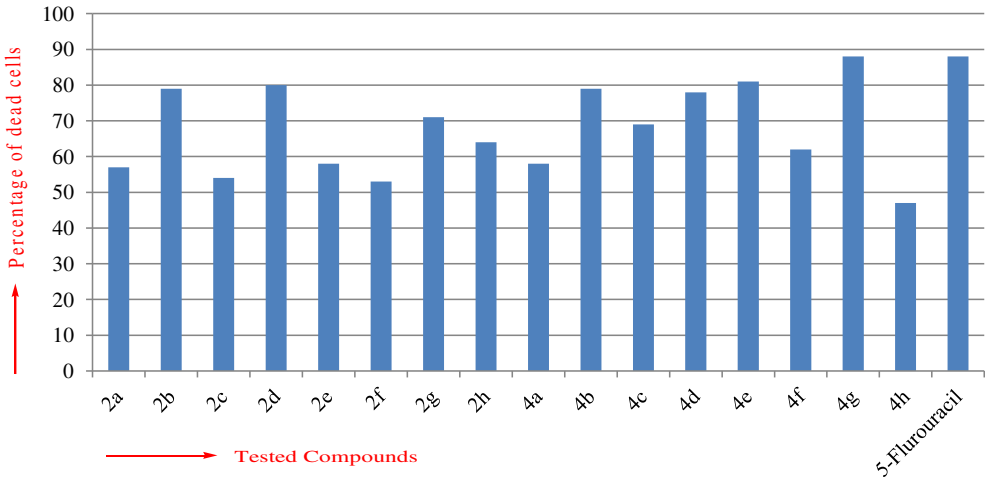


Fig. 5. *In vitro* cell cytotoxicity of (**2a**–**h**) and (**4a**–**h**).

(100 MHz, DMSO-d_6): δ 21.77, 33.95, 42.98, 96.88, 101.97, 110.54, 112.88, 116.92, 117.76, 121.47, 122.76, 123.85, 125.91, 128.52, 131.27, 132.69, 142.60, 144.96, 145.10, 152.93 (d, $^2J_{CF}$ = 66 Hz), 153.66, 161.23 ppm; LC-MS: m/z [M + 1] 404; Anal. $C_{23}H_{18}FN_3O_3$. Calcd for: C, 68.48; H, 4.50; N, 10.42. Found: C, 68.34; H, 4.40; N, 10.29.

*3.1.2.6. 10-(6-Methoxy-2-oxo-2H-chromen-4-ylmethyl)-2-isopropyl-10H-benzo[4,5]imidazo[1,2-a] pyrimidin-4-one (**4f**).* Colorless solid, Yield: 94%. Mp: 210–213 °C, IR (KBr, cm^{-1}): 1670 cm^{-1} (C=O), 1740 cm^{-1} (lactone C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.20 (d, 6H, 2-CH_3 of *i*-Pr, J = 6.0 Hz), 2.80 (m, 1H, CH of *i*-Pr), 3.52 (s, 3H, OCH_3), 5.82 (s, 2H, N–CH_2), 6.01 (s, 1H, C_3–H of coumarin), 6.10 (s, 1H, C_3–H of benzimidazopyrimidine), 7.25–8.52 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 23.90, 32.27, 42.37, 55.85, 101.49, 110.01, 112.38, 114.46, 115.06, 115.67, 116.20, 117.42, 117.73, 119.86, 122.49, 122.60, 126.01, 130.59, 147.39, 149.04, 155.65, 159.52, 163.87 ppm; ESI-MS: m/z [M + 1] 416; Anal. $C_{24}H_{21}N_3O_4$. Calcd for: C, 69.39; H, 5.10; N, 10.11. Found: C, 69.17; H, 4.86; N, 10.01.

*3.1.2.7. 10-(6-Chloro-2-oxo-2H-chromen-4-ylmethyl)-2-isopropyl-10H-benzo[4,5]imidazo[1,2-a] pyrimidin-4-one (**4g**).* Colorless solid, Yield: 89%. Mp: 237–239 °C, IR (KBr, cm^{-1}): 1669 cm^{-1} (C=O), 1732 cm^{-1} (lactone C=O): ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.20 (d, 6H, 2CH_3 of *i*-Pr, J = 6.0 Hz), 2.78 (m, 1H, CH of *i*-Pr), 5.82 (s, 2H, N–CH_2), 6.03 (s, 1H, C_3–H of coumarin), 6.26 (s, 1H, C_3–H of benzimidazopyrimidine), 7.39–8.51 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 20.34, 33.33, 42.53, 100.81, 110.56, 111.98, 115.99, 116.39, 116.87, 123.35, 124.80, 125.34, 126.87, 130.83, 133.21, 133.85, 142.81, 148.61, 151.15, 153.36, 158.96 ppm; ESI-MS: m/z [M + 2] 421; Anal. $C_{23}H_{18}ClN_3O_3$. Calcd for: C, 65.79; H, 4.32; N, 10.01. Found: C, 65.65; H, 4.16; N, 9.86.

*3.1.2.8. 10-(6-Bromo-2-oxo-2H-chromen-4-ylmethyl)-2-isopropyl-10H-benzo[4,5]imidazo[1,2-a] pyrimidin-4-one (**4h**).* Colorless solid, Yield: 95%. Mp: 213–215 °C, IR (KBr, cm^{-1}): 1672 cm^{-1} (C=O), 1731 cm^{-1} (lactone C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.24 (d, 6H, 2CH_3 of *i*-Pr, J = 6.0 Hz), 2.73 (m, 1H, CH of *i*-Pr), 5.80 (s, 2H, N–CH_2), 6.03 (s, 1H, C_3–H of coumarin), 6.34 (s, 1H, C_3–H of benzimidazopyrimidine), 7.32–8.55 (m, 7H, Ar–H) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ 21.64, 34.87, 40.13, 98.44, 113.60, 115.08, 116.21, 116.85, 118.83, 119.08, 121.30, 125.44, 126.79, 127.73, 134.80, 135.10, 148.22, 150.07, 152.53, 159.04, 159.20 ppm; ESI-MS: m/z [M + 2] 466; Anal. $C_{23}H_{18}BrN_3O_3$. Calcd for: C, 59.50; H, 3.91; N, 9.05. Found: C, 59.38; H, 3.79; N, 8.91.

4. Conclusion

In conclusions, we have developed a simple method for the synthesis of dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidin-4-ones under microwave irradiation giving excellent yields of the products (74–94%) in shorter reaction time (3 min). These molecules further reacted with various substituted 4-bromomethylcoumarins to yield a new series of coumarin substituted dihydrobenzo[4,5]imidazo [1,2-*a*]pyrimidin-4-ones. The *in vitro* antimicrobial screening revealed that all the tested compounds possessed better antifungal properties than antibacterial properties. The coumarin substituted dihydrobenzo[4,5]imidazo[1,2-*a*]pyrimidin-4-one (**4g**) (R = *i*-Pr, R_1 = 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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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.

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