Med Chem Res (2014) 23:2690–2697
DOI 10.1007/s00044-013-0856-1

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Design, synthesis, and PASS-assisted evaluation of novel 2-substituted benzimidazole derivatives as potent anthelmintics

Kamalpriya Garg · Yogita Bansal · Gulshan Bansal · R. K. Goel

Received: 29 March 2013 / Accepted: 17 October 2013 / Published online: 31 October 2013

Abstract A series of novel 2-substituted benzimidazole analogs has been designed and synthesized by connecting the benzimidazole nucleus with variedly substituted chalcone moieties through an amino linker. The designed analogs were predicted for their biological activity profile through the computer software PASS. The compounds were predicted to have potent anthelmintic activity. These were synthesized and the activity of each compound was evaluated experimentally at the concentrations of 0.1, 0.2, and 0.5 % in terms of mortality time and paralysis time for the helminthes. The experimentally observed activity was found to comply with the PASS predicted activity. All compounds showed dose-dependent activities. The compounds with an electron releasing group at the *para* position on phenyl ring in the chalcone moiety (**8** and **9**) were the most active in comparison to those bearing electron withdrawing groups. The corresponding *ortho* analogs (**4** and **5**) also revealed good paralytic and lethal activities. The higher activities of **8** and **9** may be attributed to the favorable electronic interactions of the electron releasing groups present at *para* position of the phenyl ring. Comparative analysis of the Lipinski's parameters and the activities of the compounds revealed all the compounds to comply with the Lipinski's rule of five. Further an optimum hydrophilicity and total polar surface area in the range of 65–80 of the molecule are required for the potent activity, but Molar refractance is not found to have any significant role in determining the anthelmintic activity.

K. Garg · Y. Bansal (✉) · G. Bansal · R. K. Goel
Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147 002, India
e-mail: yogitabansalp@rediffmail.com

Keywords Benzimidazole · Chalcones · Lipinski · Pharmacophore · Helminthes · PASS

Introduction

Benzimidazoles nucleus has received worldwide attention for the development of new molecules of therapeutic interest due to its presence in numerous bioactive molecules. Many drugs derived from benzimidazole belonging to different categories of pharmacological activities include omeprazole, enviradine, candesartan, bendamustine, astemizole, albendazole, mebendazole, thiabendazole, and fenbendazole (Bansal and Silakari, 2012). An elaborate survey of the literature has revealed the importance of 2-position of benzimidazole for a majority of the therapeutic activities and a substituted amino group is proved to be the most versatile substituent at this position (Navrocka, 1996; Mor *et al*., 2004; Caroti *et al*., 1989; Akul *et al*., 2011; Nawrocka *et al*., 2004). Chalcones (1,3-diaryl-2-propen-1-ones) are open chain flavonoids, distributed widely in fruits, vegetables, spices, tea, and soy-based foodstuff. These also possess many useful pharmacological activities such as anti-inflammatory, antimicrobial, antifungal, antioxidant, anthelmintic, antitumor, and anticancer (Settimo *et al*., 1991; Zdzislawa, 2007; Di Carlo *et al*., 1999). These activities are attributed to the α,β-unsaturated keto group owing to its propensity to be attacked by the nucleophilic groups present in proteins. Coupling of benzimidazole and chalcone moieties has produced compounds (Fig. 1) exhibiting antitumor, anti-depressant, antimicrobial activities (Dimmock *et al*., 2005; Mishra *et al*., 2001; Shaharyar *et al*., 2010; Babu and Selvakumar, 2012; Mathew *et al*., 2012). Hence, based upon the chemotherapeutic importance of 2-aminobenzimidazoles and

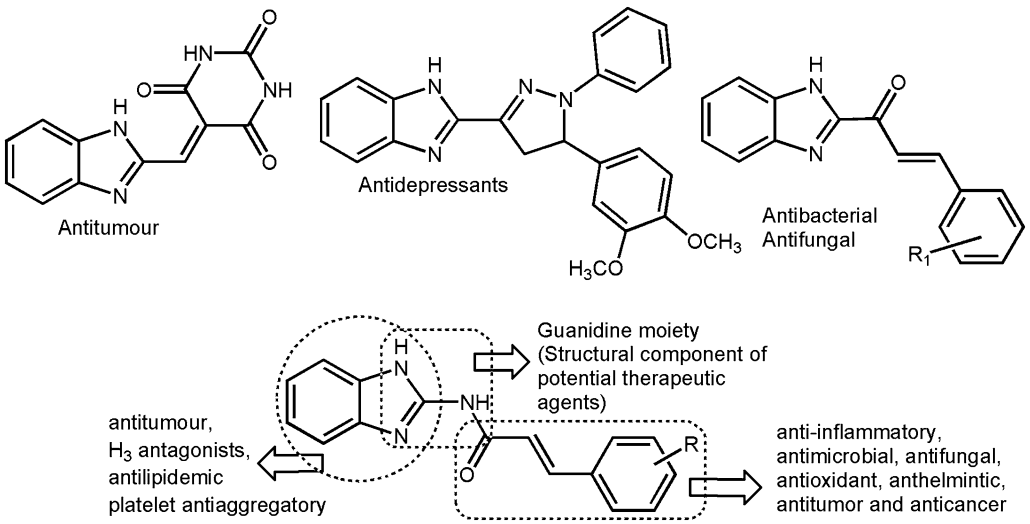

Fig. 1 Benzimidazole–chalcones as important therapeutic agents

α,β-unsaturated keto group of chalcone, novel compounds have been designed by coupling the two vital moieties through an amino linker in the present study (Fig. 1). The three components of the designed compounds, i.e., benzimidazole nucleus, guanidine, and chalcone moiety are responsible for varied activities and hence these are expected to exhibit wide spectra of pharmacological activities. The compounds were synthesized and predicted for their pharmacological activities through a computer-aided program, prediction of activity spectrum of substances (PASS). The most probable activity predicted for the synthesized compounds was evaluated experimentally.

Results and discussion

Chemistry

A series of benzimidazole–chalcone conjugates was designed and synthesized based on the importance of 2-aminobenzimidazole and chalcones toward varied biological activities. The target compounds were synthesized through a three step process (Fig. 2). In the first step, 2-aminobenzimidazole (**1**) was prepared from *o*-phenylenediamine and cyanogen bromide as reported by Leonard *et al*. (1947). In the second step, the **1** was acetylated using acetic anhydride by stirring at 5–10 °C in chloroform to form 2-acetamidobenzimidazole (2). Finally, **2** was coupled with variedly substituted benzaldehydes to produce the target compounds (**3–11**) through conventional refluxing as well as microwave-assisted method (Wang *et al*., 2007). Structures of the target compounds were confirmed through IR, ^{1}H NMR, ^{13}C NMR, and high resolution mass spectral (HRMS) data. In general, the structure was ascertained by appearance of Amide-I bands of medium intensity in the range of 1,670–1,700 cm^{-1} in IR spectra and confirmed by accurate mass of quasimolecular ion ($M+H^+$) in the +ESI-TOF mass spectrum of each compound. Finally, elementary composition of each compound was confirmed through calculation of molecular formula corresponding to mass of its $(M+H)^+$ ion using elemental composition calculator. The calculated molecular formula of $(M+H)^+$ ion of each compound was found to be same as the actual one.

Predicted biological activities of benzimidazole derivatives by PASS

PASS predicts the pharmacological effects and different side effects of a molecule based on the analysis of structure–activity relationships for the training set of database currently including more than 46,000 drugs, drug candidates, and lead compounds having biological activities determined experimentally. Two probabilities are calculated for each activity, i.e., probability of compound being active (P_a) and probability of compound being inactive (P_i). Being probabilities, the P_a and P_i values vary from 0.000 to 1.000 and in general, $P_a + P_i < 1$, since these probabilities are calculated independently. The PASS predictions can be interpreted and used in a flexible manner: (i) only activities with $P_a > P_i$ are considered as possible for a particular compound; (ii) If $P_a > 0.7$, the chance to find the activity experimentally is high; (iii) If $0.5 < P_a < 0.7$, the chance to find the activity experimentally is less, but the compound is probably not so similar to known pharmaceutical agents; and (iv) If $P_a < 0.5$, the chance to find the activity experimentally is even less, but the chance to find a structurally new

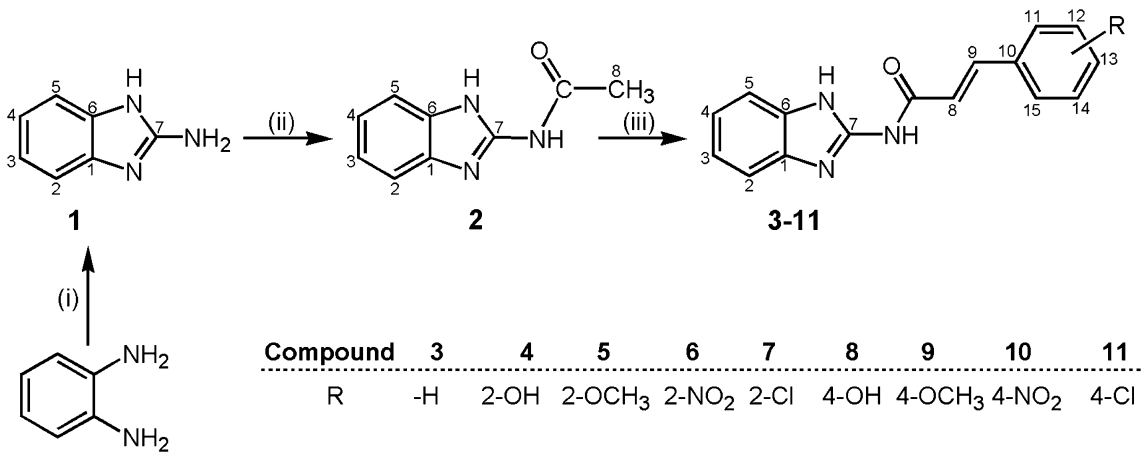

Fig. 2 Synthetic scheme for 2-substituted benzimidazole derivatives. (*i*) CNBr, methanol, stirring, 24 h, NH_4OH; (*ii*) acetic anhydride, $CHCl_3$, stirring, 5–10 °C; (*iii*) ArCHO, KOH, microwave (2 min, 170 W)/conventional (reflux, 5 h)

Table 1 PASS predicted activity spectrum of the target compounds

Compound	Anthelmintic activity		Antineoplastic activity		Leukotriene antagonist		Cytokinine modulator	
	P_a	P_i	P_a	P_i	P_a	P_i	P_a	P_i
3	0.818	0.003	0.792	0.015	0.692	0.04	0.591	0.049
4	0.833	0.023	0.809	0.011	0.726	0.029	0.556	0.063
5	0.836	0.003	0.679	0.042	0.746	0.025	–	–
6	0.698	0.005	0.703	0.031	–	–	–	–
7	–	–	0.531	0.029	–	–	–	–
8	0.850	0.003	0.640	0.050	0.754	0.022	–	–
9	0.836	0.003	0.679	0.042	0.75	0.021	0.636	0.032
10	0.776	0.018	0.587	0.074	–	–	–	–
11	–	–	0.686	0.021	–	–	–	–

compound, i.e., novel chemical entity (NCE) increases (Poroikov *et al*., 2000; Marawaha *et al*., 2007). Recently, an internet version of PASS has been made available at the PASS developer's web site. The accuracy of prediction is reported to be as high as 85 %. The predicted activity spectrum of the synthesized compounds (Table 1) revealed that most of the compounds possess high probability of showing anthelmintic and antineoplastic activities. The compounds bearing no or electron releasing groups (hydroxyl and methoxy) on the chalconic phenyl ring were predicted to have higher anthelmintic activity than the compounds bearing electron withdrawing groups (chloro and nitro).

Anthelmintic activity

The earthworms are used to study the anthelmintic activity because of their resemblance, both anatomically and physiologically, with the intestinal roundworm parasites in human beings. All test compounds exhibited concentration-dependent paralytic as well as lethal activities and the maximum anthelmintic activity was observed at 0.5 % of the test compounds (Table 2; Figs. 3, 4). The activity was in concordance with their PASS predicted scores that support the applicability of the PASS in reliable prediction of the activity spectrum of the compounds. The **8** and **9** (*p*-hydroxyl and *p*-methoxy derivatives) were the most active compounds from the series and slightly less active than albendazole at all concentrations. The corresponding *ortho* analogs (**4** and **5**) also revealed good paralytic and cidal activities. On the contrary, the chloro and nitro analogs in the series (**6**, **7**, **10**, and **11**) were found to be the least active of all even at 0.5 % concentration. It revealed that the higher activities of **8** and **9** may be attributed to the favorable electronic interactions of the electron releasing groups present at *para* position of the phenyl ring. Comparative analysis of the anthelmintics activities of the compounds and the Lipinski's parameters for a bioactive drug (Tables 2, 3, respectively) has revealed that all calculated parameters comply with the Lipinski's rule of five

Table 2 Anthelmintic activity of the compounds at concentrations of 0.1, 0.2, and 0.5 % w/v

Compound	Time of paralysis (min)			Time of death (min)		
	0.1	0.2	0.5	0.1	0.2	0.5
Albz.	8.8 ± 0.3	7.6 ± 0.2	6.1 ± 0.3	12.7 ± 0.3	9.8 ± 0.4	7.0 ± 0.3
3	17.3 ± 0.1	14.2 ± 0.1	11.3 ± 0.1	18.9 ± 0.1	15.9 ± 0.2	13.2 ± 0.1
4	14.1 ± 0.2	10.8 ± 0.2	8.9 ± 0.2	16.3 ± 0.1	12.9 ± 0.3	10.1 ± 0.1
5	13.8 ± 0.1	11.8 ± 0.1	9.5 ± 0.1	14.6 ± 0.1	12.4 ± 0.1	10.3 ± 0.1
6	14.4 ± 0.1	12.3 ± 0.1	10.3 ± 0.1	16.3 ± 0.1	14.4 ± 0.1	11.2 ± 0.1
7	13.7 ± 0.2	12.8 ± 0.1	12.3 ± 0.1	16.4 ± 0.1	14.9 ± 0.2	14.3 ± 0.1
8	11.3 ± 0.1	10.7 ± 0.1	8.5 ± 0.1	12.8 ± 0.2	11.9 ± 0.2	10.2 ± 0.1
9	10.5 ± 0.1	9.2 ± 0.1	8.7 ± 0.1	12.0 ± 0.2	11.7 ± 0.2	10.7 ± 0.1
10	13.1 ± 0.2	12.2 ± 0.2	11.7 ± 0.2	15.5 ± 0.2	14.6 ± 0.2	14.3 ± 0.1
11	14.3 ± 0.1	11.1 ± 0.1	10.5 ± 0.1	16.1 ± 0.2	13.3 ± 0.1	12.3 ± 0.1
Control	>25	>25	>25	>25	>25	>25

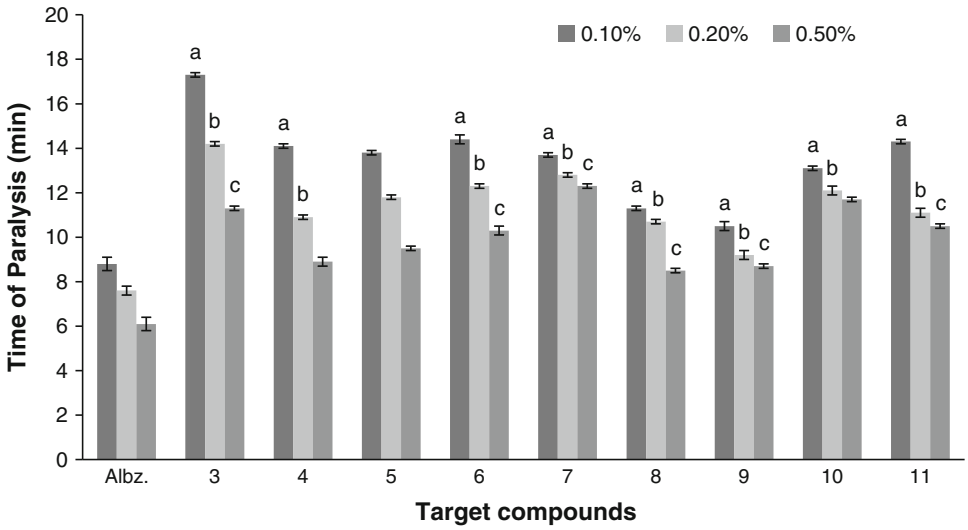

Fig. 3 Time of paralysis of compounds **3**–**11**. Each value is expressed as mean ± SD ($n = 6$). Statistical significant difference was obtained at $p < 0.05$, *a*, *b*, and *c* as compared to Albendazole (Albz.) at the respective concentration

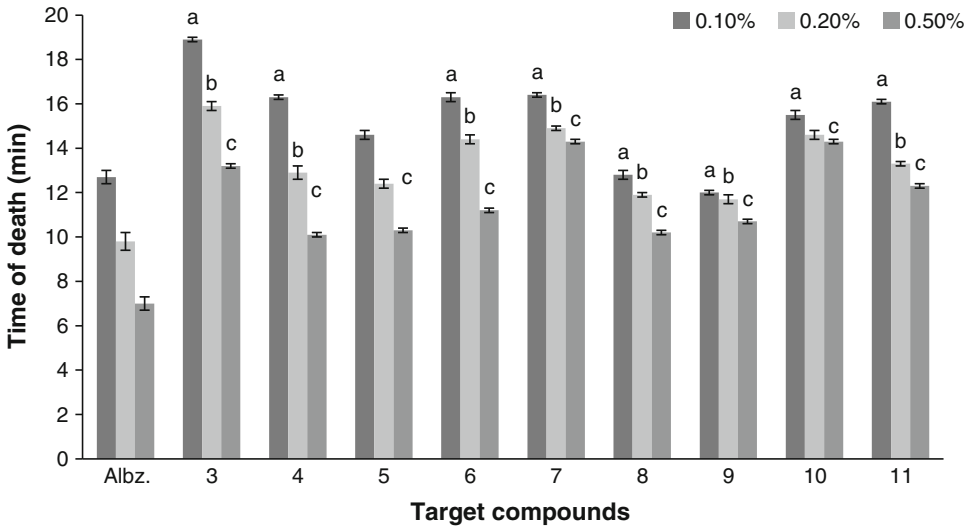

Fig. 4 Time of death of compounds **3**–**11**. Each value is expressed as mean ± SD ($n = 6$). Statistical significant difference was obtained at $p < 0.05$, *a*, *b*, and *c* as compared to Albendazole (Albz.) at the respective concentration

Table 3 TPSA, molar refractance, and calculated Lipinski's parameters for the test compounds

Compound	TPSA[a]	MR[b]	Log P[c]	M_W^d	nON[e]	nOHNH[f]	$n$violations[g]	$n$rotb[h]
3	57.78	77.94	3.28	263.30	4	2	0	3
4	78.01	79.63	3.04	279.30	5	3	0	3
5	67.02	84.40	3.11	293.33	5	2	0	4
6	103.60	ND[i]	3.01	308.30	7	2	0	4
7	57.78	82.75	3.73	297.75	4	2	0	3
8	78.01	79.63	2.80	279.30	5	3	0	3
9	67.02	84.40	3.34	293.33	5	2	0	4
10	103.60	ND[i]	3.24	308.30	7	2	0	4
11	57.78	82.75	3.96	297.75	4	2	0	3

[a] Total polar surface area (hydrophilicity)

[b] Molar refractance (cm^3/mol)

[c] Calculated lipophilicity

[d] Molecular weight

[e] Number of hydrogen bond acceptor

[f] Number of hydrogen bond donors

[g] Number of violations from Lipinski's rule of five

[h] Number of rotatable bonds

[i] Not determined/calculated

(Lipinski *et al*., 2001), i.e., a molecular weight of 500 or less, a log P not more than 5, number of hydrogen bond donor sites not exceeding five, and number of hydrogen bond acceptor sites not exceeding ten. However, a wide variability in the total polar surface area (TPSA), molar refractance (MR, a measure of steric factor), and log P values of the compounds have indicated that though an optimum hydrophilicity and a TPSA in the range of 65–80 of the molecule may be required for the potent activity but MR does not play any significant role in determining the anthelmintic activity.

Conclusion

The ABC series of benzimidazole derivatives bearing variedly substituted chalcone moieties have been synthesized and evaluated through PASS software for predicting the activity spectrum of the compound. All compounds were predicted to have potent anthelmintic activity which was found to comply with the activity observed experimentally. The SAR designed from the in silico as well as experimental study reveals that the electron releasing groups increases the anthelmintic activity. On the contrary, an electron withdrawing group at both *ortho* as well as *para* position of the phenyl ring incurs lesser activity. Further, the activity is higher for the analog bearing the group at *para* position on phenyl ring in the chalcone moiety. All compounds have been found to comply with the Lipinski's rule of five for a bioactive drug.

Materials and methods

The reactions requiring anhydrous conditions were conducted in flame-dried apparatus. Melting points were recorded on electronic melting point apparatus (Perfit, India). The microwave-assisted reactions were carried out in a microwave oven (IFB23SCI, 850 W). IR spectra were recorded on IR Spectrophotometer (Perkin Elmer Fx) as KBr disks. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance II NMR spectrometer (400 MHz), using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. In ^{1}H NMR, chemical shifts were reported in δ values with number of protons, multiplicities (s—singlet, m—multiplet, dd—double doublet, br—broad), and coupling constants (J) in Hertz (Hz). The mass spectra were recorded on Bruker Daltonics microTOF instrument using electrospray ionization in positive and/or negative polarity mode. Each compound was purified by crystallization and purity of all synthesized compounds was ascertained by TLC using pre-coated Silica gel G plates (Merck, Mumbai, India) using chloroform:methanol (96:4) as developing solvent system. The dried developed plates were visualized in UV chamber at both short and long wavelengths as well as by exposure to iodine vapors in a tightly closed chamber. Earth worms (*Pheretima posthuma*) of nearly equal size (6 ± 1 cm, in expanded form) were procured from the vermicompost (Jagatpur, Chandigarh, India) and were selected randomly for the present study. The species of earthworms was authenticated from the Department of Zoology, Punjabi University, Patiala (India).

Chemistry

2-Aminobenzimidazole (1)

A mixture of 3.2 g (30 mM) of cyanogen bromide and 2.1 g (20 mM) of *o*-phenylenediamine in aqueous methanol was stirred at room temperature for 24 h. Thereafter, the solution was heated on water bath to remove the methanol. The solution was cooled and basified with ammonia. The crude precipitates obtained were recrystallized from ethanol–water. Yield 88 %; m.p. 234 °C; IR: 3500–3400 (N–H str.), 3116 (C–H, Ar, str.), 1636 (C=N str.), 1483 (N–H scissoring), 1451 (C=C str.), 1239 (C–N str.), 846 (N–H wagging), 719 (Ar–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.2 (dd, 2H, $J = 5.8$, 3 Hz, ArH), 6.9 (dd, 2H, $J = 5.8$, 3 Hz, ArH), 5.4 (br s, 2H, NH_2), 4.8 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 117.2 (C-2, C-5), 123.9 (C-3, C-4), 139.1 (C-1, C-6), 142.5 (C-7). HRMS (+ESI): *m/z* 134.0729 ($C_7H_8N_3^+$).

2-Acetylamino benzimidazole (2)

The **1** (1.33 g, 0.01 M) was dissolved in 15 mL of chloroform and 50 mL of pyridine. Acetic anhydride (1.02 g, 0.01 M) was added dropwise in the reaction mixture with constant stirring over 4 h at 5–10 °C. The solvent was recovered on rotary vacuum evaporator, the contents were dried and recrystallised from ethanol to give pure compound. Yield 52 %; m.p. 280–282 °C; IR: 3500–3400 (N–H str.), 3052 (C–H, Ar, str.), 2920 (C–H, Ali, str.), 1685 (C=O str.), 1473 (N–H scissoring), 1458 (C=C str.), 1316 (C–H bend), 1234 (C–N str.); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0 (s, 1H, NH), 7.5–7.4 (m, 2H, ArH), 7.3–7.1 (m, 2H, ArH), 5.1 (br s, 1H, NH), 2.5-2.1 (s, 3H, CH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 19.4 (CH_3) 117.1 (C-2, C-5), 124.2 (C-3, C-4), 138.6 (C-1, C-6), 143.1 (C-7), 170.2 (C=O). HRMS (+ESI): *m/z* 176.0838 ($C_9H_{10}N_3O^+$).

Target compounds (3–11)

Conventional method (Method A) The **2** (1.75 g, 0.01 M) was dissolved in ethanol (30 mL) and aqueous solution of KOH (2 %, 5 mL). The aromatic aldehyde (0.01 M) was added and the reaction mixture was refluxed for 5 h. The solvent was recovered on rotary vacuum evaporator, the contents were poured onto crushed ice and acidified with dilute HCl. The solid separated was filtered and recrystallised from ethanol to obtain the pure product.

Microwave irradiation method (Method B) The mixture of **2** (1.75 g, 0.01 M) and aromatic aldehyde (0.01 M) in aqueous solution of KOH (2 %, 5 mL) was irradiated in microwave for 2 min at 20 % power output. The reaction mixture were poured into crushed ice and acidified with dilute HCl. The solid separated was filtered and recrystallised from ethanol to obtain the pure product.

*2-[3-Phenylacrylamido]-benzimidazole (**3**)* Yield: 62 % (Method A), 56 % (Method B); m.p. 118–120 °C; IR: 3500–3400 (N–H str.), 3048 (C–H, Ar, str.), 2880 (C–H, Ali, str.), 1685 (C=O str.), 1642, 1453 (C⋯C str.), 922 (Ar C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.9–7.8 (br s, 1H, NH), 7.5–7.4 (m, 2H, ArH), 7.3–7.1 (m, 8H, ArH, CH=CH), 6.9–6.8 (m, 1H, CH=CH), 5.0 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 117.4 (C-2, C-5), 121.2 (C-8), 124.3 (C-3, C-4), 127.6 (C-11, C-15), 129.3 (C-13), 130.2 (C-12, C-14), 136.1 (C-10), 138.5 (C-1, C-6), 142.4 (C-7), 143.5 (C-9), 166.2 (C=O). HRMS (+ESI): *m/z* 264.1151 ($C_{16}H_{14}N_3O^+$).

*2-[3-(2-Hydroxyphenyl)acrylamido]-benzimidazole (**4**)* Yield: 69 % (Method A), 65 % (Method B); m.p. 290–294 °C; IR: 3619 (O–H str), 3500–3400 (N–H str.), 3052 (C–H, Ar, str.), 2883 (C–H, Ali, str.), 1680 (C=O str.), 1631, 1450 (C⋯C str.), 901 (C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.9–7.8 (br s, 1H, NH), 7.8–7.5 (m, 3H, ArH, CH=CH), 7.4–7.3 (m, 3H, ArH), 7.2–6.9 (m, 2H, ArH), 6.6–6.5 (m, 3H, ArH, CH=CH), 5.0 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 117.2 (C-2, C-5), 120.5 (C-8), 122.3 (C-14), 123.6 (C-10), 124.1 (C-3, C-4), 128.7 (C-15), 130.7 (C-13), 138.7 (C-1, C-6), 156.7 (C-11), 142.8 (C-7, C-9), 165.8 (C=O). HRMS (+ESI): *m/z* 280.1198 ($C_{16}H_{14}N_3O_2^+$).

*2-[3-(2-Methoxyphenyl)acrylamido]-benzimidazole (**5**)* Yield: 60 % (Method A), 67 % (Method B); m.p. 129 °C; IR: 3500–3400 (N–H str.), 3053 (C–H, Ar, str.), 2872 (C–H, Ali, str.), 1685 (C=O str.), 1422 (C=C str.), 1218 (C–O str.), 927 (Ar–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.9–7.8 (br s, 1H, NH), 7.7–7.6 (m, 2H, ArH), 7.5 (m, 1H, CH=CH), 7.3–7.1 (m, 5H, ArH, CH=CH), 6.9-6.7 (m, 2H, ArH), 5.0 (br s, 1H, NH), 3.6 (s, 3H, OCH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 57.1 (OCH_3), 115.7 (C-12), 116.9 (C-2, C-5), 121.1 (C-9), 122.3 (C-10, C-14), 124.2 (C-3, C-4), 128.7 (C-15), 129.6 (C-13), 138.9 (C-1, C-6), 142.2 (C-7, C-9), 161.6 (C-11), 165.2 (C=O). HRMS (+ESI): *m/z* 294.1261 ($C_{17}H_{16}N_3O_2^+$).

*2-[3-(2-Nitrophenyl)acrylamido]-benzimidazole (**6**)* Yield: 82 % (Method A), 78 % (Method B); m.p. 122–125 °C; IR: 3500–3400 (N–H str.), 3078 (C–H, Ar, str.), 2875 (C–H, Ali, str.), 1685 (C=O str.), 1516,1341 (O⋯N⋯O str.), 1646,1429 (C⋯C str.), 924 (C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0–7.8 (m, 4H, NH, ArH, CH=CH), 7.7–7.6 (m, 2H, ArH), 7.5–7.4 (m, 5H, ArH), 5.0 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 117.3 (C-2, C-5), 120.7 (C-8), 121.4 (C-10), 124.2 (C-3, C-4), 125.4 (C-12), 128.6 (C-15), 129.9 (C-13), 136.3 (C-14), 139.1 (C-1, C-6), 142.9 (C-7, C-9), 148.2 (C-11), 166.2 (C=O). HRMS (+ESI): *m/z* 309.1003 ($C_{16}H_{13}N_4O_3^+$).

2-[3-(2-Chlorophenyl)acrylamido]-benzimidazole ***(7)*** Yield: 65 % (Method A), 72 % (Method B); m.p. 111–115 °C; IR: 3500–3400 (N–H str.), 3054 (C–H, Ar, str.), 2886 (C–H, Ali, str.), 1682 (C=O str.), 1652, 1425 (C⋯C str.), 930 (C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0–7.9 (br s, 1H, NH), 7.7–7.6 (m, 3H, ArH, CH=CH), 7.3–7.1 (m, 7H, ArH, CH=CH), 5.0 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 116.8 (C-2, C-5), 120.9 (C-8), 124.1(C-3, C-4), 127.6 (C-14), 128.5 (C-15), 130.1 (C-12, C-13), 132.8 (C-11), 136.7 (C-10), 138.5(C-1, C-6), 143.2 (C-7, C-9), 165.3 (C=O). HRMS (+ESI): *m/z* 298.0759 ($C_{16}H_{13}N_3OCl^+$).

2-[3-(4-Hydroxyphenyl)acrylamido]-benzimidazole ***(8)*** Yield: 75 % (Method A), 68 % (Method B); m.p. 256–260 °C; IR: 3610 (Free O–H str.), 3500–3400 (N–H str.), 3035 (C–H, Ar, str.), 2887 (C–H, Ali, str.), 1680 (C=O str.), 1636,1421 (C⋯C str.), 923 (C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0–7.9 (br s, 1H, NH), 7.7-7.6 (m, 3H, ArH, CH=CH), 7.3–7.1 (m, 4H, ArH), 6.9–6.8 (m, ArH, CH=CH), 5.0 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 116.9 (C-2, C-5, C-12, C-14), 121.3 (C-8), 123.8 (C-3, C-4), 128.4 (C-10, C-11, C-15), 138.7 (C-1, C-6), 143.5 (C-7, C-9), 157.9 (C-13), 165.4 (C=O). HRMS (+ESI): *m/z* 280.1195 ($C_{16}H_{14}N_3O_2^+$).

2-[3-(4-Methoxyphenyl)acrylamido]-benzimidazole ***(9)*** Yield: 67 % (Method A), 60 % (Method B); m.p. 196–200 °C; IR: 3500–3400 (N–H str.), 3047 (C–H, Ar, str.), 2870 (C–H, Ali, str.), 1682 (C=O str.), 1643, 1420 (C⋯C str.), 927 (C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.9–7.8 (br s, 1H, NH), 7.7–7.6 (m, 2H, ArH), 7.5 (m, 1H, CH=CH), 7.3–7.1 (m, 4H, ArH), 6.9–6.7 (m, 3H, ArH, CH=CH), 5.0 (br s, 1H, NH), 3.41 (m, 3H, OCH_3). ^{13}C NMR (400 MHz, $CDCl_3$): δ 57.4 (OCH_3), 115.2 (C-12, C-14), 117.4 (C-2, C-5), 121.4 (C-8), 124.3 (C-3, C-4), 128.3 (C-10, C-11, C-15), 138.8 (C-1, C-6), 142.9 (C-7, C-9), 162.7 (C-13), 166.1 (C=O). HRMS (+ESI): *m/z* 294.1256 ($C_{17}H_{16}N_3O_2^+$).

2-[3-(4-Nitrophenyl)acrylamido]-benzimidazole ***(10)*** Yield: 73 % (Method A), 85 % (Method B); m.p. 226–230 °C; IR: 3500–3400 (N–H str.), 3048 (C–H, Ar, str.), 2874 (C–H, Ali, str.), 1680 (C=O str.), 1523,1336 (O⋯N⋯O str.), 1640, 1449 (C⋯C str.), 927 (C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0–7.8 (m, 5H, NH, ArH), 7.7–7.4 (m, 4H, ArH, CH=CH), 7.4–7.3 (m, 2H, ArH), 5.0 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 116.9 (C-2, C-5), 120.7 (C-8), 124.5 (C-3, C-4), 126.4 (C-12, C-14), 128.2 (C-11, C-15), 139.2 (C-1, C-6), 141.9 (C-10), 142.8 (C-7, C-9), 149.3 (C-13), 165.3 (C=O). HRMS (+ESI): *m/z* 309.0997 ($C_{16}H_{13}N_4O_3^+$).

2-[3-(4-Chlorophenyl)acrylamido]-benzimidazole ***(11)*** Yield: 76 % (Method A), 70 % (Method B); m.p. 276–280 °C; IR: 3500–3400 (N–H str.), 3050 (C–H, Ar, str.), 2873 (C–H, Ali, str.), 1680 (C=O str.), 1652,1423 (C⋯C str.), 936 (C–H bending); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0–7.9 (br s, 1H, NH), 7.7–7.6 (m, 3H, ArH, CH=CH), 7.3–7.1 (m, 6H, ArH), 6.9–6.8 (m, 1H, CH=CH), 5.0 (br s, 1H, NH). ^{13}C NMR (400 MHz, $CDCl_3$): δ 116.9 (C-2, C-5), 121.3 (C-8), 124.3 (C-3, C-4), 128.8 (C-11, C-15), 130.2 (C-12, C-14), 134.5 (C-10, C-13), 138.7 (C-1, C-6), 142.3 (C-7, C-9), 165.1 (C=O). HRMS (+ESI): *m/z* 298.0756 ($C_{16}H_{13}N_3OCl^+$).

PASS prediction of the target compounds

Biological activities of the target compounds were predicted by a computer software PASS. The activities predicted for each target compound (**3**–**11**) having $P_a > 0.5$ are listed in Table 1. The Lipinski's parameters were calculated (Table 3) through Molinspiration software (Lipinski *et al*., 2001).

Anthelmintic activity

The anthelmintic activity of each compound was evaluated on Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and cleaned by washing with normal saline. The activity was evaluated using the method described in various reports (Ajaiyeoba *et al*., 2001; Dash *et al*., 2002; Shivkumar and Kumar, 2003; Kosalge and Fursule, 2009). Briefly, the worms were divided into different groups containing six earthworms in each group and washed with the normal saline. All the target compounds were evaluated at three concentrations (0.1, 0.2, and 0.5 % w/v) taking albendazole as standard drug. The helminthes were placed in solutions of each compound at each concentration individually in Petri plates. The time taken for worms to become motionless was noted as paralysis time, whereas the time taken for death of the worm was noted as lethal time. To ascertain death, each worm was frequently subjected to external stimuli that stimulate and induce movement in earth worms, if alive. The time taken for complete paralysis and death was recorded. The mean paralysis time and mean lethal time were calculated for each compound at each concentration.

Statistical analysis

Results are expressed as mean ± SD. The statistical significance of the observed data was determined by one-way analysis of variance (ANOVA) followed by Tukey's test for the anthelmintic activity of the synthesized compounds and the results were reported to be statistically significant at $p < 0.05$.

Acknowledgments The authors are thankful to Punjabi University, Patiala for providing the infrastructure and financial assistance, to All India Council for Technical Education (AICTE), New Delhi for

fellowship to one of the authors (Kamalpriya Garg), to Crystal Pharmaceuticals (Ambala, India) for albendazole as a generous gift sample, and to molsinpiration.com for free access to the software for calculation of the Lipinski's parameters. Authors acknowledge the free PASS software license provided by Dr. V.V. Poroikov and IBMC, Russian Academy of Medical Sciences, Moscow.

References

Ajaiyeoba EO, Onocha PA, Olarenwaju OT (2001) In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extract. Pharm Biol. 39:217–220

Akul G, Srinivas B, Vidyasagar M, Kandikonda S (2011) Synthesis of 3-(1*H*-benzimidazol-2-yl-amino)-2-phenyl-1,3-thiazolidin-4-one as potential CNS depressant. Int J Pharm Tech Res. 3:360–364

Babu IS, Selvakumar S (2012) Synthesis, characterization, antibacterial and antifungal activities of some potent 2-substituted benzimidazolyl chalcones. Int J Biol Pharm Res. 3:275–283

Bansal Y, Silakari O (2012) Therapeutic journey of benzimidazole: a review. Bioorg Med Chem 20(21):6208–6236

Caroti P, Ceccotti C, Settimo FD, Primofiore G, Franzone JG, Reboani MC, Cravanzola C (1989) Synthesis, antilipidemic and platelet antiaggregatory activity of 2-aminobenzimidazole amide derivatives. Farmaco 44:227–255

Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB (2002) Evaluation of *Evolvulus alsinoides* Linn. for anthelmintic and antimicrobial activities. J Nat Rem. 2:182–185

Di Carlo G, Mascolo N, Izzo AA, Capasso F (1999) Flavanoids: old and new aspects of a class of natural therapeutic drugs. Life Sci 65(4):337–353

Dimmock JR, Elias DW, Beazely MA, Kandepu NM (2005) Bioactivities of chalcones. Curr Med Chem 12:481–499

Kosalge SB, Fursule RA (2009) Investigation of in vitro anthelmintic activity of *Thespesia lampas* (Cav.). Asian J Pharm Clin Res. 2:69–71

Leonard NL, Daviy CY, Karl BM (1947) Sulfonate salts of substituted benzimidazoles. J Med Chem 69:2459–2461

Lipinski CA, Lombardo F, Dominy BW, Feeney P (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. J Adv Drug Deliv Rev. 46:3–26. Calculation of molecular properties and bioactivity score was done by software available at website http://www.molinspiration.com/

Marawaha A, Goel RK, Mahajan MP (2007) PASS-predicted design, synthesis and biological evaluation of cyclic nitrones as nootropics. Bioorg Med Chem Lett 17:5251–5255

Mathew B, Suresh J, Anbazhagan S (2012) Development of novel (1*H*) benzimidazole bearing pyrimidine-trione based MAO-A inhibitors: synthesis, docking studies and antidepressant activity. J Saudi Chem Soc. doi:10.1016/j.jscs.2012.09.015

Mishra L, Sinha R, Itokawa H, Kenneth FB, Tachibana Y, Nakanishi Y, Kilgore N, Lee KH (2001) Anti-HIV and cytotoxic activities of Ru(II)/Ru(III) polypyridyl complexes containing 2,6-(2′-Benzimidazolyl)-pyridine/chalcone as co-ligand. Biorg Med Chem. 9:1607–1617

Mor M, Bordi F, Silva C, Rivara S, Zuliani V, Vacondio F, Rivara M, Barocelli E, Bertoni S, Ballabeni V, Magnanini F, Impicciatore M, Plazzi PV (2004) Synthesis, biological activity, QSAR and QSPR study of 2-aminobenzimidazole derivatives as potent H_3-antagonists. Bioorg Med Chem 12:663–674

Navrocka W (1996) Synthesis and pharmacological properties of new 2-aminobenzimidazole derivatives. Boll Chim Farm 135:18–23

Nawrocka W, Sztuba B, Kowalska MW, Liszkiewicz H, Wietrzyk J, Nasulewicz A, Pełczyńska M, Opolski A (2004) Synthesis and antiproliferative activity in vitro of 2-aminobenzimidazole derivatives. Farmaco 59:83–91

Poroikov VV, Filimonov DA, Borodina VY, Lagunin AA, Kos A (2000) Robustness of biological activity spectra predicting by computer program PASS for non congeneric sets of chemical compounds. J Chem Inf Comput Sci 40:1349–1355

Settimo AD, Marini AM, Primofiore G, Subissi A (1991) Synthesis and antihypertensive activity of some 2-aminobenzimidazole and indole derivatives. Farmaco 46:357–367

Shaharyar M, Abdullah MM, Bakht MA, Majeed J (2010) Pyrazoline bearing benzimidazoles: search for anticancer agents. Eur J Med Chem 45:114–119

Shivkumar YM, Kumar VL (2003) Anthelmintic activity of latex of *Calotropis procera*. Pharma Biol. 41:263–265

Wang R, Lu X, Yu X, Shi L, Sun Y (2007) Acid-catalyzed solvent-free synthesis of 2-arylbenzimidazoles under microwave irradiation. J Mol Catal A. 266:198–201

Zdzislawa N (2007) A review of anti-infective anti-inflammatory chalcones. Eur J Med Chem 42:125–137