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Microwave Assisted Synthesis, Physico-chemical Properties and Antioxidant Activity of α,β-Unsaturated Benzimidazole Derivatives Incorporated with Baritone Moiety

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A series of (2E)-1-(H-benzimidazol-2-yl)-3-substituted phenyl 2-propen-1-one linked with barbitone (**5a-g**) are synthesized by both conventional method and microwave assisted method. The benzimidazole chalcones (**4a-g**) were prepared from the condensation of 2-acetyl benzimidazole (**3a**) with different aromatic aldehydes. These chalcones on reaction with barbituric acid in presence of acetic acid medium gave the α,β-unsaturated benzimidazole derivatives. The structures of the all the final compounds were established on the basis of IR, ^{1}H NMR, mass spectra and elemental analysis. The druglikeness and physicochemical properties of the derivatives were determined by actelion, molsoft, molinspiration and ACD ChemDraw Ultra 11.0 software. The final products possess a favourable drug likeness and drug score. All the final synthesized compounds were screened for their antioxidant properties like free radical scavenging by DPPH method. Among the synthesized compounds (**5f**), (**5c**) and (**5d**) were exhibited a good antioxidant activity and all the other derivatives showed a moderate activity.

Key Words: 2-Acetyl benzimidazole, Barbituric acid, Chalcones, Antioxidant activity.

INTRODUCTION

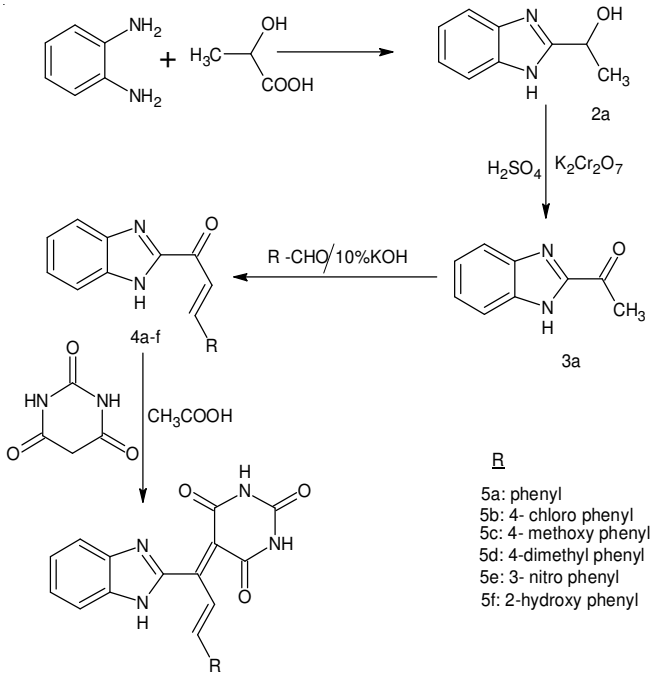
Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules and which are generally very reactive. There is an increasing evidence of the implication of free radicals and reactive oxygen species in a variety of diseases and pathophysiological events including cancer, inflammations and myocardial infraction[1,2]. Benzimidazoles are regarded as a promising class of biologically active agents. The benzimidazole nucleus exhibit a wide range of biological profile such as, antimicrobial[3-6], antitubercular[7,8], anticancer[9-13], angiotensin II receptor antagonists[14]. The benzimidazole also known to exhibit significant activity against several viruses such as human cytomegalovirus, HIV[15], herpes[16], RNA[17] and influenza[18].

Barbituric acid is hydroxy pyrimidine nucleus extensively used in the class of hypnotics[19-22]. The nucleus has highly reactive acidic hydrogen atoms on the carbon atom α to both carbonyl groups. The ability of these hydrogen atoms to react with a carbonyl compound is already reported[23]. Inspired from these findings, our research was focused on the connection between α, β unsaturated ketone of benzimidazole derivatives with barbituric acid. To our best of knowledge, no screening of the free radical scavenging effect of the synthesized barbitone derivatives has yet been undertaken. The two pharmacophores are linked by means of carbon-carbon double bond. Some experimental protocols have been made for this purpose and excellent results have been obtained. The synthesis of 5-[(2E)-1-(1*H*-benzimidazol-2-yl)-3-substituted phenylprop-2-en-1-ylidene]pyzrimidine-2,4,6-(1*H*, 3*H*, 5*H*)-trione (**5a-g**) described in this study are outlined in **Scheme-I**. *Ortho*-phenyelenediamine reacts with lactic acid gave 2(α-hydroxyethyl)benzimidazole (**2a**), which on subjected with oxidation in presence of potassium dichromate produced 2- acetyl benzimidazole (**3a**). The chalcones (**4a-g**) were prepared by reacting 2-acetyl benzimidazole with appropriate aldehydes in the presence of a base by Claisen-Schmidt condensation[24]. The condensation of benzimidazole chalcones (**4a-g**) with barbituric acid in acetic acid gave (**5a-g**)[25,26]. The acidic hydrogen present in the pyrimidine nucleus of barbituric acid undergoes interamolecular acid catalyzed dehydration resulting an activated C=C bond in the final derivatives. The structures of the all the final compounds were established on the basis of spectral analysis.

EXPERIMENTAL

Melting points were determined by using melting point apparatus MP-DS TID 2000 V and the values were uncorrected. Reactions were monitored by thin layer chromatography (TLC)

on pre coated silica gel G plates using iodine vapour as visualizing agent. IR spectra were recorded on JASCO FT/IR-140 spectrophotometer by using KBr pellets technique. PMR spectra were recorded using BRUCKER FT-NMR-500 MHz spectrophotometer by using DMSO as solvent and TMS as internal standard. The chemical shift was expressed in δ ppm. Mass spectra were recorded on a JEOL GCmate mass spectrometer.



Scheme-I: Synthetic route of the titled compounds

***In silico* screening:** *In silico* screening is an effective tool for the determination of the druglikeness of a molecule. Parameters related to druglikeness of the derivatives were established on the basis of Lipinski's Rule of 5[27]. The drug likeness and physicochemical properties of the derivatives were determined by actelion, molsoft and molinspiration and ACD ChemDraw Ultra 11.0 software. Blood-brain barrier prediction of the set of compounds was calculated by DEC-1 model[28]. The results were indicated in Table-1. It has been noted that the better brain penetration is predicted for compounds have high calculated log P and low TPSA. Polar surface area being an indication of compound's capacity to form hydrogen bonds. It is noted that the incorporation of a barbitone moiety in the benzimidazole chalcone gave favourble positive druglikeness score determined by both Actelion and Molsoft. Druglikeness is a measure of similarity of a proposed drug compound to general population of drugs in use[29]. The predicted log BB decreases in the final derivatives due to the more polar group participation. The presence of NO_2 in the aromatic nucleus increases the TPSA and decreases the predicted log. The physico-chemical properties of the newly synthesized candidates were shown in Table-1.

Synthesis of 2-(α-hydroxyethyl) benzimidazole (2a): *Ortho*-phenylenediamine (0.25 mol) was mixed with lactic acid (0.35 mol) in a round bottom flask and refluxed for 3 h. The reaction mixture was cooled added with 10 % NaOH until bacisity to litmus paper. The crude pink colured product obtained was thoroughly washed with water and dissolved in 400 mL of boiling water. To this add 2 g of decolourising carbon was added and heated for 15 min. The mixture was filtered rapidly at the pump through a preheated buchner funnel. The product obtained was further filtered and washed with (25 X3) mL cold water and dried at 100 ºC. The yield was found to be 78 %.

Synthesis of 2-acetyl benzimidazole (3a): To a solution of 2-(α-hydroxy) ethyl benzimidazole **2a** (0.01 mol) in dil. H_2SO_4 (5 %, 40 mL) was drop wise added the solution of $K_2Cr_2O_7$ (0.15 mol) and H_2SO_4 (25 %, 80 mL) with constant stirring at room temperature over a period of 20 min. Further the reaction mixture was stirred at room temperature for 2 h. After completion of the reaction (monitored by TLC), the reaction mixture was neutralized with aqueous NH_3 solution (1:1) and resultant orange solid was filtered, washed with water and dried, recrystallized from ethyl acetate.

General Synthesis of (2*E*)-1-(1*H*-benzimidazol-2-yl)-3-phenylprop-2-en-1-one (4a-h): 2-Acetyl benzimidazole (0.01 mol) and appropriately substituted aromatic aldehydes (0.012 mol) were mixed in ethanol (20 mL) containing 10 % aq. KOH (8 mL) and magnetically stirred the solution constantly at room temperature for 10 h. The whole mixture was transferred in to 100 mL ice cold water and acidified with dil.

TABLE-1
PHYSICOCHEMICAL PROPERTIES OF THE SYNTHESIZED DERIVATIVES

Compound	ACD logP[a]	C logP[b]	mi logP[c]	TPSA[d]	log BB[e]	Drug likeness	
						Molsoft	Actelion
4a	3.27	3.04	3.40	45.75	-0.07	0.39	-2.47
4b	4.05	3.65	4.08	45.75	0.017	0.63	1.82
4c	3.32	2.93	3.46	54.98	-0.11	0.29	1.09
4d	3.88	3.03	3.51	48.99	-0.12	-0.49	-3.19
4e	2.85	3.04	3.34	91.57	-0.15	-0.86	0.07
4f	3.01	2.74	3.16	65.98	-0.42	0.06	0.99
4g	3.82	3.61	3.92	45.75	0.01	-0.38	-4.07
5a	3.41	1.88	2.31	111.47	-1.22	1.02	0.82
5b	4.08	2.49	2.99	111.48	-1.13	1.15	0.60
5c	3.46	1.77	2.36	120.71	-1.37	0.63	0.81
5d	3.51	1.88	2.41	114.71	-1.27	0.79	0.20
5e	3.34	1.89	2.24	157.302	-1.90	0.01	0.22
5f	3.21	1.58	2.07	131.71	-1.57	0.47	0.84

[a]Calculated by ACD ChemDraw Ultra 11.0 software; [b]Calculated by Actelion; [c & d] Calculated by Molinspiration; [e]Calculated by DEC-I model [log BB = -0.0148 (miTPSA) + 0.152 (C log P) + 0.139]

HCl. The solid formed was washed, filtered and dried, recrystallized from ethanol yield a product of (**4a-f**).

Synthesis of (5a-f): To a solution of (**4a-g**) (0.01 mol) suspended in 7 mL acetic acid, barbituric acid (0.01 mol) was added with constant stirring. The reaction mixture was then refluxed for 7 h with occasional stirring. The resultant contents were poured in to in to crushed ice. The crude product was filtered and recrystallized from methanol.

Microwave assisted synthesis: Barbituric acid (0.01 mol) was added to a mixture of chalcone (**4a-g**) (0.01 mol) in acetic acid. The reaction mixture was irradiated for 2-3 min at 60 % microwave power with 30 sec interval using a domestic microwave oven. The reaction progress was monitored by TLC. The resultant contents were poured in to in to crushed ice with constant stirring. The isolated product was recrystallized from methanol.

5-[(2*E*)-1-(1*H*-benzimidazol-2-yl)-3-phenylprop-2-en-1-ylidene]pyrimidine-2,4,6-(1*H*, 3*H*, 5*H*)-trione (5a): Brown solid, yield 77 %, m.p. 255-258 ºC; IR(KBr) :3230 (NHstr), 1693 (C=O), 1587 (C=N), ^{1}H NMR (DMSO d_6 + $CDCl_3$) in δ ppm: 9.1 (1H, s, NH benzimidazole) 8.0-8.4 (2H, s, pyrimidine NH) 6.48.1 (11H, m, 9 ArH, 2CH=CH). MS: *m/z* = 359.21 (M^++1). Calcd/Anal. (%) [C67.03/67.12, H3.94/3.81, N15.63/15.59].

5[(2*E*)-1-(1H-benzimidazol-2-yl)-3-(4 chlorophenyl)-prop-2-en-1-ylidene]pyrimidine2,4,6(1*H*,3*H*,5*H*)-trione (5b): Yellow solid, yield 77 %, m.p. 265-267 ºC; IR (KBr): 3238 (NHstr), 1696 (C=O), 1584 (C=N), ^{1}H NMR (DMSO d_6 + $CDCl_3$) in δ ppm: 8.9 (1H, s, NH benzimidazole) 8.4-8.6 (2H, s, pyrimidine NH), 6.4-8.3 (10 H, m, 8ArH, 2CH=CH). MS: *m/z* = 394.1 (M+1). Calcd/Anal. (%) [C 61.16/61.23, H 3.34/3.32, N 14.26/14.28].

5-[(2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-ylidene]pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (5c): Pale green solid, yield 67 %, m.p. 225-2228ºC; IR (KBr): 3239 (N-H str), 1681 (C=O), 1576 (C=N), ^{1}H NMR (DMSO d_6 + $CDCl_3$) in δ ppm: 8.9 (1H, s, NH benzimidazole) 8.4-8.6 (2H, s, pyrimidine NH), 6.4-8.3 (10H, m, 8 ArH, 2CH=CH). 3.3 (3H, s, OCH_3) MS: m/z = 390.12 (M+1). Calcd./Anal. (%) [C61.16/61.23, H 3.34/3.32, N 14.26/14.28].

5-{(2*E*)-1-(1*H* benzimidazol-2-yl)-3-[4(dimethylamino)-phenyl]prop-2-en-1-ylidene}pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (5d): Brown solid, yield 76 %, m.p.-210-213 ºC; IR (KBr) 3227 (N-H str). 1678 (C=O), 1569 (C=N), ^{1}H NMR (DMSO d_6 + $CDCl_3$) in δ ppm: 8.9 (1H, s, NH benzimidazole), 8.5-8.7 (2H, s, pyrimidine NH), 6.3-8.4 (10 H, m, 8Ar H, 2CH=CH). 3.3 (6H, s, $N(CH_3)_2$. MS: 402.56 (M+1). Calcd./Anal. (%) [C 65.83/65.91, H 4.77/4.72, N 17.45/17.53].

5-[(2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(3-nitrophenyl)-prop-2-en-1-ylidene]pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (5e): Pale brown solid, yield 66 %, m.p.-239-240 ºC; IR (KBr) 3227 (N-H str). 1678 (C=O), 1569 (C=N) 1310 (Ar-NO_2), ^{1}H NMR (DMSO-d_6 + $CDCl_3$) in δ ppm: 8.5 (1H, s, NH benzimidazole), 8.2-8.4 (2H, s, pyrimidine NH), 6.4-8.4 (10H, m, 8ArH, 2CH=CH). MS: 405.6 (M+2). Calcd./Anal. (%) [C 59.56/59.71, H 3.25/3.29, N 17.36/17.43].

5-[(2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(2-hydroxyphenyl)prop-2-en-1-ylidene]pyrimidine-2,4,6-(1*H*,3*H*,5*H*)-trione (5f): Yellow solid, yield 61 %, m.p. 200-203 ºC; IR (KBr) 3379 (Ar-OH), 3231 (NH str). 1680 (C=O), 1562 (C=N) ^{1}H NMR (DMSO d_6 + $CDCl_3$) in δ ppm: 9.1 (1H, s, NH benzimidazole), 8.4-8.8 (2H, s, pyrimidine NH), 6.4-8.5 (10H, m, 8ArH, 2 CH=CH), 5.62 (1H, s, ArOH) MS: 375.64 (M+1). Calcd./Anal. (%) [C 61.17/61.25, H 3.77/3.79, N 14.97/14.86].

Antioxidant activity by DPPH method: The scavenging activity of the synthesized candidates (**5a-f**) on DPPH radical was determined according to the methods of Shimada *et al.*[30]. Different concentration of the test compounds in 1.5 mL of methanol were added to a 1.5 mL (0.2 mM) solution of DPPH radical in methanol. The above solutions were allowed to react at room temperature for 0.5 h. After 0.5 h the absorbance values were measured at 517 nm and converted to percentage of scavenging activity, which was calculated by using the following formula. The IC_{50} and percentage inhibition of the newly synthesized candidates were compared with standard ascorbic acid and the values were shown in Table-2.

$$\text{Percentage of scavenging activity} = \left[\frac{(Ab + As) - Am}{Ab}\right] \times 100$$

where, [Ab: absorbance of 1.5 mL DPPH + 1.5 mL methanol, Am: Absorbance of 1.5 mL DPPH + 1.5 mL drug solution, As: Absorbance of 1.5 drug + 1.5 mL methanol solution].

RESULTS AND DISCUSSION

The highly reactive acidic hydrogen present in the barbituric acid has a tendency to react with ketone group in the, α,β-unsaturated carbonyl system in presence of an acetic acid medium. The structures of the final products were confirmed on the basis of spectral studies. All the newly synthesized compounds were characterized by IR, ^{1}H NMR and mass spectroscopic data. IR spectrum of the 5-[(2*E*)-1-(1*H*-benzimidazol-2-yl)-3-(4-chlorophenyl)prop-2-en-1-ylidene]pyrimidine-2,4,6-(1*H*,3*H*,5H)-trione (**5b**) showed a strong absorption band at 3238 cm^{-1} corresponding to benzimidazole NH. The absorption at 2910 and 2878 cm^{-1} corresponds to pyrimidine NH/NH. A sharp absorption at 1696 cm^{-1} corresponds to carbonyl stretching and 1232 cm^{-1} due to aryl chloride. The ^{1}H NMR spectra showed the singlet peak at δ 8.4 and 8.6 were assigned to pyrimidine NH/NH. The singlet peak at δ 9.1 corresponding to benzimidazole NH. The doublet for *vicinal* protons along with the aromatic multiplet between δ 6.4-8.2 ppm. Mass spectrum of compound **5b** revealed the molecular ion peak [M+2] at *m/z* 394 corresponding to the molecular mass of the compound.

Free radical scavenging properties of the synthesized derivatives were evaluated by decrease in the absorption of the stable DPPH radical at 517 nm. This bleaching of DPPH absorption occurs when the odd electron of the radical is paired[30]. All the newly synthesized barbitone derivatives incorporated with a α,β unsaturated benzimidazole derivatives were showed a good DPPH free radical scavenging activity. In this study we proposed that decrease in the DPPH absorption due to the two NH group in the pyrimidine moiety, which can donate hydrogen atom and exist in radical form and the electron conjugation effect in the structure stabilizes the radical so that it does not involved in a destructive biochemical reaction. The highest activity is shown in the participation of electron donating group in the aromatic nucleus of the final products.

TABLE-2
DETERMINATION OF ANTIOXIDANT ACTIVITY OF BARBITONE MOIETIES BY DPPH ASSAY

Compound code	Absorbance at 517 nm						
	Absorbance	0.1 mM	0.2 mM	0.5 mM	0.7 mM	1 mM	$IC_{50\ (mM)}$
5a	Am	0.8735	0.7532	0.6743	0.5432	0.4219	
	As	0.0056	0.0037	0.0023	0.0011	0.0007	
	% of activity	8	20.73	28.75	42.52	55.34	0.84
5b	Am	0.8652	0.6928	0.5832	0.5128	0.4156	
	As	0.0042	0.0031	0.0019	0.0011	0.0008	
	% of activity	8.71	26.87	38.36	45.74	56.01	0.81
5c	Am	0.7435	0.6739	0.4312	0.3678	0.2932	
	As	0.0032	0.0024	.0019	0.0011	0.0007	
	% of activity	21.50	28.80	54.38	61.11	68.98	0.42
5d	Am	0.7568	0.6854	0.4976	0.3952	0.3129	
	As	0.0054	0.0032	0.0021	0.0016	0.0011	
	% of activity	20.33	27.67	47.46	58.27	66.94	0.46
5e	Am	0.8679	0.7431	0.6552	0.5327	0.4019	
	As	0.0065	0.0042	0.0024	0.0012	0.0004	
	% of activity	8.67	21.65	30.78	43.64	57.43	0.75
5f	Am	0.6976	0.5682	0.4367	0.2865	.2182	
	As	0.0054	0.0039	0.0028	0.0017	0.0011	
	% of activity	26.60	40.17	54.00	69.80	76.98	0.37
Ascorbic acid	Absorbance	0.5692	0.4821	0.2431	0.1191	0.0431	
	% of activity	39.65	48.89	74.22	87.37	95.43	0.21

The IC_{50} and percentage inhibition of the newly synthesized products were compared with standard ascorbic acid and the values were shown in Table-2.

Conclusion

In conclusion, we have synthesized some novel benzimidazole products incorporated with barbitone moiety and evaluated for these compounds for their antioxidant activity by DPPH assay. All the derivatives showed a favourable range of activity. The products like **5f**, **5c** and **5d** were concluded as the most potent derivatives among them. This study focused a relation between scavenging and blood brain barrier prediction of DEC-I model. It has been concluded than an increase in this value will not favours for the DPPH scavenging activity in the titled derivatives except in **5e**. Introduction of electron donating group in the *ortho* and *para* position gets less C log P value and increases the percentage inhibition. Therefore our research will provide a great impact on the medicinal chemist for the further research in the benzimidazole connecting barbituric acid derivatives possessing potent antioxidant and anticancer activities.

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