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Synthesis and inhibitory activity of new benzimidazole derivatives against Burkitt's lymphoma promotion

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Abstract—As a continuation to our previous work concerning antitumor benzimidazoles, we have synthesized series of new derivatives of 2-(1-benzyl-2-methyl-1*H*-benzimidazol-5-ylimino)-3-(substituted)-thiazolidin-4-one (**6a**–**e**), 3-(2-methyl-1*H*-benzimidazol-5-yl)-2-substituted-thiazolidin-4-one (**9a**–**f**) and we have studied their inhibitory activity against the Epstein–Barr Virus-early antigen (EBV-EA) activation introduced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Compound **6d** was found to be significantly active and compounds **5a** and **6e** were also active.
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1. Introduction

Benzimidazole derivatives are endowed with different types of biological activities especially antitumor activity.[1–3] Pyrrolo[1,2-*a*]benzimidazoles (PBIs) (**I, II,** and **III**) represent a new class of antitumor agents exhibiting cytotoxic activity against a variety of cancer cell lines.[4–9] Also other examples as the anticancer agents [Hoechst 33342], 2′-(4-ethoxyphenyl)-6-(5-methyl-1-piperazin-1-yl-1*H*-benzimidazol-2-yl)-1*H*-benzimidazole (**IV**)[10,11] and the bisbenzimidazole dye [Hoechst-33258] (**V**)[12,13] are inhibitors of DNA topoisomerase I. 5,6-Dichloro-2-pentafluoroethyl benzimidazole (**VI**) is an anticancer agent particularly against breast and prostatic cancer cell lines.[14] 1- or 5-Substituted 2-methyl benzimidazoles[15–17] (**VII**, **VIII**) were found by our laboratory group to have marked activity against breast cancer. Besides, the thiazolidinone ring is involved in many antitumor agents e.g. thiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide), NSC 286193 (**IX**),[18] and 5-alkylated thiazolidinones (**X**) which were found to have antiproliferative activity against human colon cancer cell lines.[19]

As a continuation to our previous work we had for aim in this manuscript to combine both nuclei, benzimidazole and thiazolidinone, in a new series of benzimidazoles having the thiazolidinone ring directly linked to the 5-position of the benzimidazole moiety or linked through a nitrogen atom to the 5-position. The inhibitory activity of the new compounds was studied against the Epstein–Barr virus-early antigen (EBV-EA) activation introduced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and a structure–activity relationship was deduced.

2. Results and discussion

2.1. Chemistry

2-Methyl-1*H*-benzimidazole (**1**) and 2-methyl-5(6)-nitro-1*H*-benzimidazole (**2**), previously described by our group,[15–17] were used as starting compounds to prepare the benzimidazole derivatives. Benzylation of compound **2** was performed by its reflux with benzyl chloride in acetone and potassium carbonate to yield 1-benzyl-2-methyl-5-nitro-1*H*-benzimidazole (**3**). Substitution at position 1 of the imino hydrogen eliminates the possibility for tautomerism and a definite assignment of the structure has to take place. The position of the nitro group in the benzene ring, whether it is in the 5 or in the 6 position, was determined by NOE ^{1}H NMR. To find out the position of attachment of the NO_2 group, NOE difference experiment has been performed for compound **3**. The received spectrum showed the disappearance of H4 and H6 with the recognition of the H7

Keywords: Benzimidazoles; Thiazolidinone; Schiff bases; EBV-EA induction.
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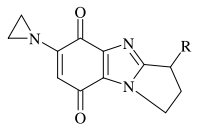

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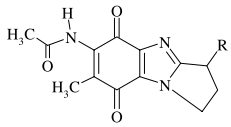

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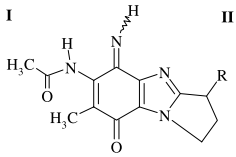
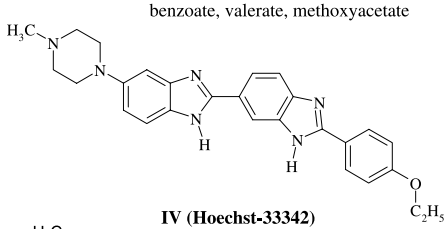


III

R=H, OH, OAc, carbamate, chloroacetate, propionate, benzoate, valerate, methoxyacetate



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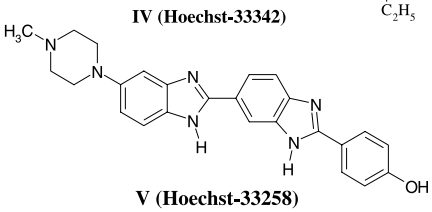

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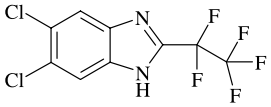

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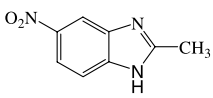

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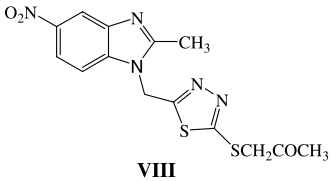

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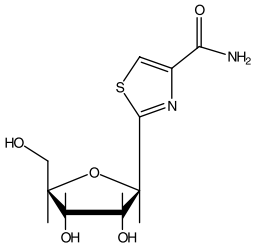

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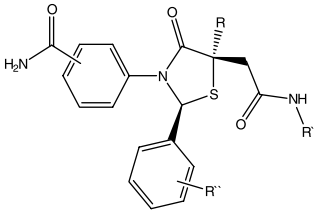


X

R = H , alkyl
R`= H, alkyl, aryl
R``= H, Cl , CH_3

resonance at δ (ppm) 7.61 together with the phenyl protons' multiplet at δ (ppm) 7.13, thus proving that the NO_2 group is attached at position 5 of the benzimidazole ring (Fig. 1).

Reduction of the nitro derivatives **3** was accomplished using concentrated hydrochloric acid and ferrous sulfate to produce 1-benzyl-2-methyl-1*H*-benzimidazol-5-amine monohydrochloride (**4**). Compound **4** reacted with methyl, ethyl, phenyl, benzoyl or *p*-methoxyphenyl isothiocyanate in ethanol to afford thiourea derivatives **5a**–**e**, respectively. Subsequent cyclization with ethyl bromoacetate in ethanol and anhydrous sodium acetate afforded the expected thiazolidin-4-ones **6a**–**e**, respectively. Reduction of compound **2**[20] using ferrous sulfate and concentrated hydrochloric acid yielded 5-amino-2-methyl-1*H*-benzimidazole monohydrochloride (**7**). Condensation of **7** with 5-methylfurfural, piperonal, indole-3-carbaldehyde, 2-thiophenecarboxaldehyde, 4-methylbenzaldehyde, and, or 1-naphthaldehyde in ethanol produced the Schiff bases **8a**–**f**, respectively. Treatment of the Schiff bases **8a**–**f** with thioglycolic acid yielded the thiazolidinone derivatives **9a**–**f**, respectively (Schemes 1 and 2).

3. Conclusions

Table 1 shows the inhibitory effect of the tested compounds against the EBV-EA induction. All assayed compounds demonstrated marked inhibitory activity at concentration 1000 mol ratio/TPA and fair low and no inhibition at concentration 500, 100, and 10 mol ratio/TPA, respectively. The most effective compound was found to be compound **6d** having the thiazololidinone ring linked to the 5 position of the benzimidazole nucleus through an imino nitrogen. Compounds **6d** and **6e** were found to be more active than the opened chain thiourea derivatives **5d** and **5e** having a benzyl or *p*-methoxyphenyl as substituents. For the other compounds, cyclization of the thiourea derivatives **5a**, **5b**, and **5c** having alkyl or phenyl groups as substituents to thiazolidinone derivatives **6a**, **6b**, and **6c** decreases the activity. Cyclization of the Schiff bases **8a**–**f** to the thiazolidinone derivatives **9a**–**f** attenuates the activity.

4. Experimental

Melting points were taken on a capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were measured in $CDCl_3$ and DMSO-d_6 on JEOL-300 Spectrometer with Me_4Si as an internal standard. Mass spectra were obtained with a Schimadzu GCS-QP 1000EX spectrometer at 70 eV. The IR spectra were recorded with a Philips Inflators Spectro-photometer Model PU9712 in KBr disks. Elemental analyses were performed at the Micro analytical Laboratory of the National Research Centre.

2-Methyl-1*H*-benzimidazole (**1**) was obtained by refluxing *o*-phenylene-diamine with acetic acid in 1:1 molar ratio in 4 N hydrochloric acid.[20] 5-Nitro-2-methyl-1*H*-benzimidazole (**2**) was prepared according to Bapat et al.'s method.[20]

4.1. 1-Benzyl-2-methyl-5-nitro-1*H*-benzimidazole (3)

To a solution of compound **2** (10 g, 56.5 mmol) and anhydrous potassium carbonate (7.8 g, 56.5 mmol) in acetone (30 ml), benzyl chloride (6.5 ml, 56.5 mmol)

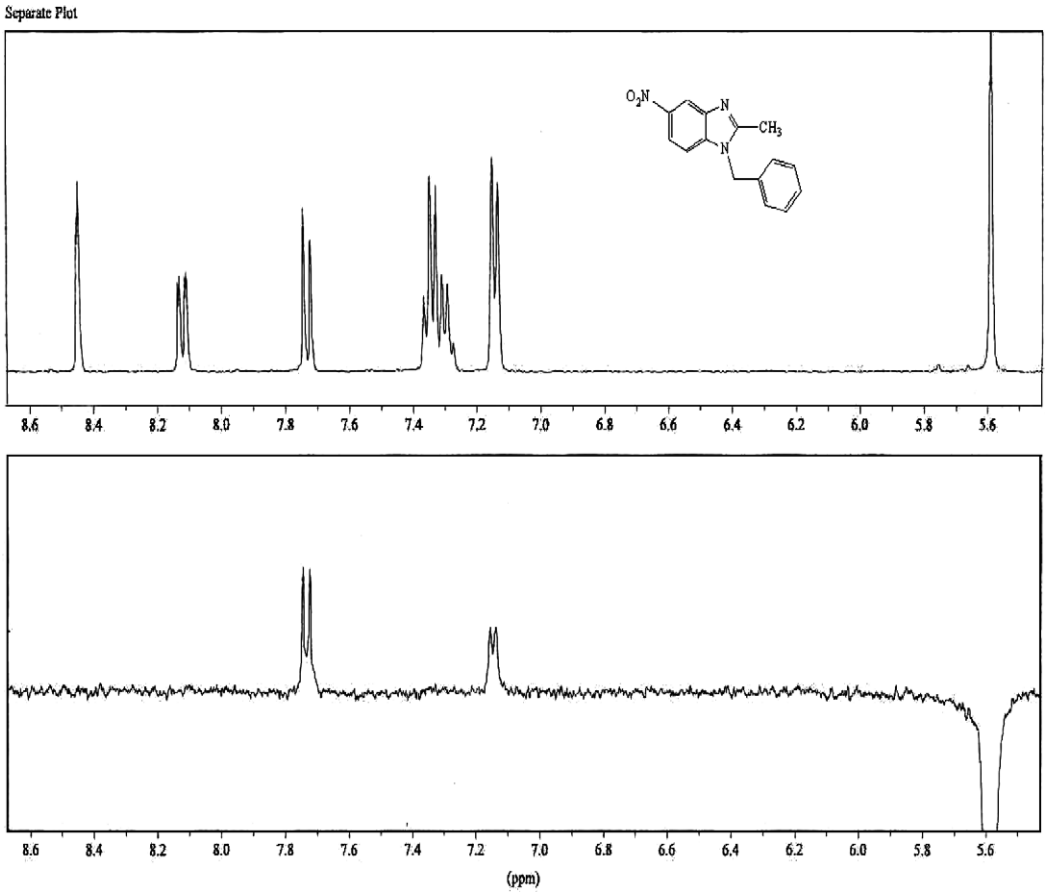


Figure 1. NOE spectrum of 1-benzyl-2-methyl-5-nitro-1*H*-benzimidazole (**3**).

was added dropwise. The mixture was stirred at room temperature for about 8 h. The mixture was then poured onto water and extracted with ethyl acetate, dried over sodium sulfate anhydrous, and concentrated under vacuum to give pure compound **3**, [yield: 89% (13.5 g)]; as yellow needles. Mp 152–154 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.59 (s, 3H, CH_3), 5.58 (s, 2H, CH_2), 7.13 (dd, $J_1 = 9$ Hz, $J_2 = 8.8$ H_Z, 2H, H′2 + H′6), 7.45 (m, 3H, H′3 + H′4 + H′5), 7.61 (d, $J = 9$ Hz, 1H, H7), 7.62 (dd, $J_{6\text{-}7} = 9$ Hz, $J_{6\text{-}4} = 8.8$ H_Z, 1H, H6), 8.01 (s, 1H, H4); ^{13}C NMR (DMSO-d_6) δ 15.01 (CH_3), 47.8 (CH_2), 111.9-129.9 (Ar.C), 157.5 (C=N); IR (cm^{-1}): 1618 (C=N), 1518, 1343 (NO_2); anal. calcd for $C_{15}H_{13}N_3O_2$ (267): C, 67.41; H, 4.86; N, 15.73. Found: C, 67.42; H, 4.85; N, 15.77.

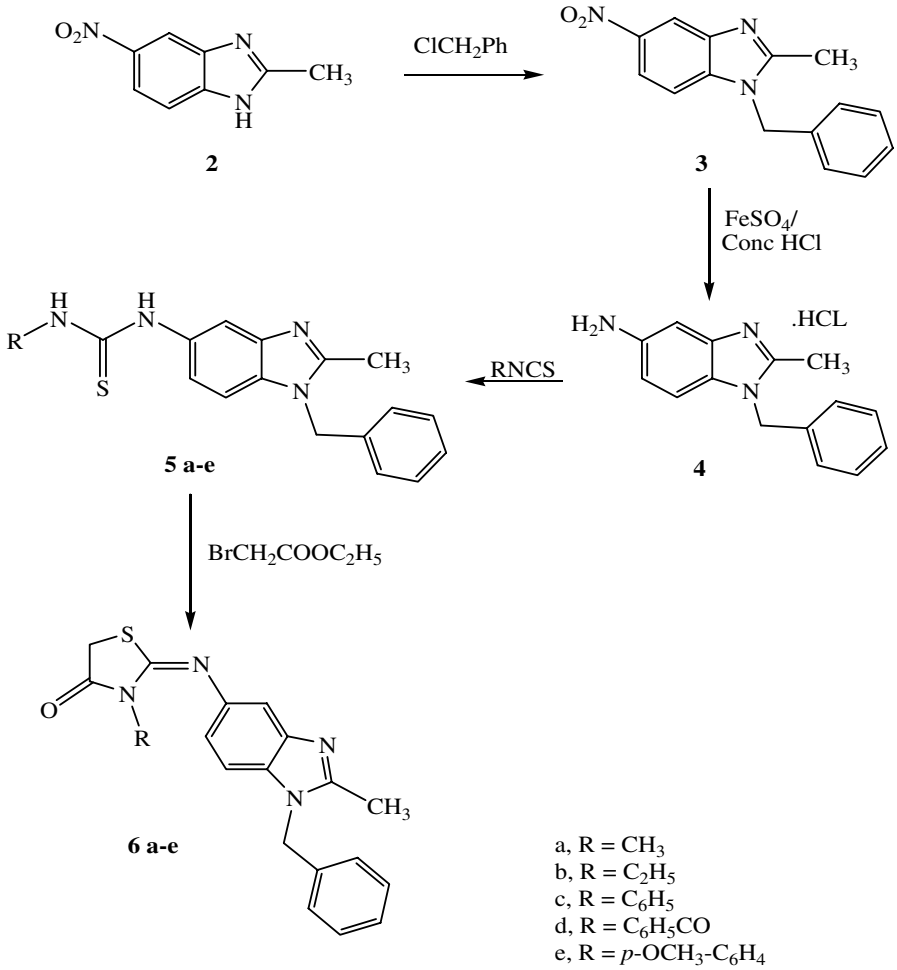
4.2. 1-Benzyl-2-methyl-1*H*-benzimidazol-5-amine monohydrochloride (4)

Compound **3** (3 g, 11.2 mmol) was dissolved in water (45 ml) containing concentrated hydrochloric acid (3.75 ml) to which an aqueous solution of ferrous sulfate (75 g) in water (225 ml) was added. To this solution was added excess of ammonia solution, the reaction mixture was heated to boiling for 15 min. The precipitate of ferric hydroxide was filtered off, the residue consisting of a mixture of monohydrochloride and ammonium salt was extracted with ethanol, and the ethanolic extract after treatment with charcoal was concentrated by distillation. The crude monohydrochloride of 2-methyl-5-aminobenzimidazole derivative obtained was purified by crystallization from ethanol/ether (5:1) mixture, [yield: 78% (2.4 g)]; as a brown powder. Mp <295 °C; ^{1}H NMR (300 MHz, DMSO:$CDCl_3$ 2:1) δ 2.80 (s, 3H, CH_3), 3.40 (s, br, 2H, NH_2), 5.60 (s, 2H, CH_2), 7.30 (d, 2H, $J = 9.1$ H_Z, H′2 + H′6),7.36 (m, 3H, H′3 + H′4+H′5), 7.39–7.76 (m, 2H, H7 + H6), 7.78 (s, 1H, H4); *MS*: *m/z* 237, 100% (M^+–HCl); 146 (71), 131(23), 116(51), 91(18), 76 (33). IR (cm^{-1}): 3415, 3220 (NH_2), 1610 (C=N); anal. calcd for $C_{15}H_{16}ClN_3$(273.5): C, 65.93; H, 5.86; Cl, 12.97; N, 15.35. Found: C, 65.94; H, 5.85; Cl, 12.91; N, 15.33.

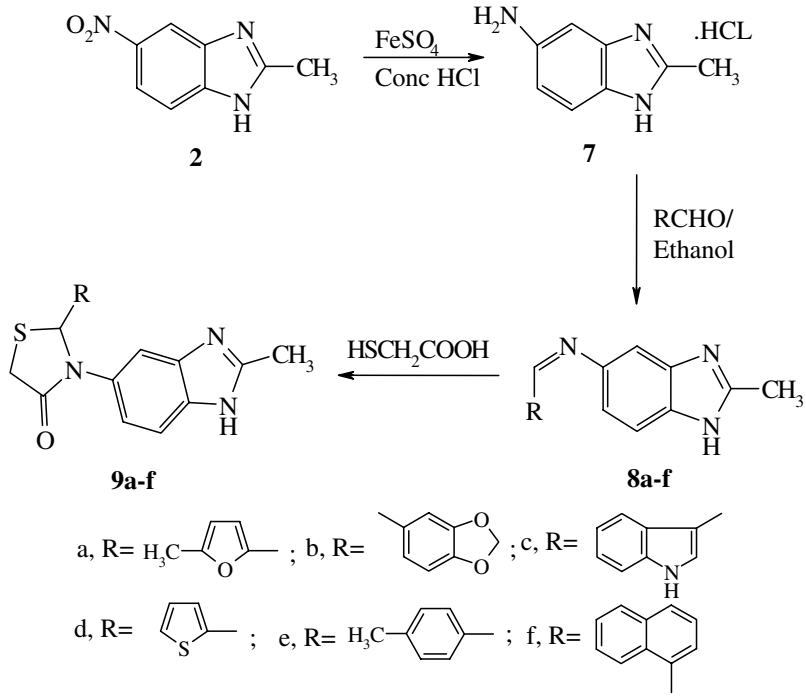
4.3. General procedure for the preparation of the compounds (5a–e)

Compound **4** (2 g, 6.3 mmol) was heated in (20 ml) ethanol until it dissolved. The proper isothiocyanate (methyl, ethyl, phenyl, benzoyl or *p*-methoxyphenyl isothiocyanate) (6.3 mmol) was added and the mixture was refluxed for 3 The crystalline product was recrystallized from appropriate solvent.

4.3.1. 1-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-yl)-3-methylthiourea (5a). Crystallized from ethanol, [yield: 66% (1.5 g)]; as a green powder. Mp 208–210 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.25 (s, 3H, CH_3), 2.55 (s, 3H, CH_3),3.60 (s, 1H, NH), 5.72 (s, 2H, CH_2), 7.47–7.65 (m, 5H, aromatic protons), 7.88 (d, 1H, H7), 8.15 (d, 1H, H6), 8.45 (s, 1H, H4); anal. calcd for



Scheme 1. Reaction of thiourea derivatives **5a-e** respectively ethyl bromoacetate to afford the Thiazolidin-4-ones **6a-e** respectively.



Scheme 2. Treatment of the Schiff bases **8a-f** with thioglycolic to yield the thiazolidinone derivatives **9a-f** respectively.

Table 1. Antitumor activity

Test materials	% EBV-EA positive cells[a]			
	Compound concentration[b] (mol ratio/32 pmol TPA)			
	1000	500	100	10
1	19.6 (60)[c]	63.6	88.3	100
2	18.4 (60)	62.5	88.0	100
3	18.3 (60)	62.4	87.4	100
4	18.5 (60)	62.2	86.3	100
5a	13.2 (60)	58.1	80.0	100
5b	14.0 (60)	59.6	81.2	100
5c	15.3 (60)	60.2	83.1	100
5d	17.8 (60)	60.6	82.0	100
5e	13.5 (60)	53.1	78.3	100
6a	20.7 (60)	61.2	83.4	100
6b	18.3 (60)	54.2	81.1	100
6c	19.4 (60)	61.0	82.5	100
6d	12.3 (60)	52.5	82.5	100
6e	13.2 (60)	53.0	78.0	100
7	20.3 (60)	64.3	88.9	100
8a	18.0 (60)	62.0	86.2	100
8b	15.1 (60)	60.2	85.4	100
8c	18.4 (60)	62.1	86.1	100
8d	22.0 (60)	65.5	89.1	100
8e	19.3 (60)	64.5	89.1	100
8f	20.6 (60)	65.8	89.6	100
9a	20.9 (60)	65.1	87.7	100
9b	21.6 (60)	65.0	88.4	100
9c	21.8 (60)	65.9	87.1	100
9d	22.1 (60)	66.5	88.9	100
9e	23.1 (60)	66.1	88.9	100
9f	17.1 (60)	61.0	85.1	100

[a] Positive control, 100%.
[b] Mol ratio/TPA (32 pmol = 20 ng/ml), 1000 mol ratio = 32 nmol; 500 mmol ratio = 16 nmol; 100 nmol ratio = 3.2 nmol; 10 nmol = .032 nmol.
[c] Values in parentheses are viability of Raji cells of the test compounds relative to the positive control (100%).

$C_{17}H_{18}N_4S$ (310): C, 65.80; H, 5.85; N, 18.06; S, 10.32. Found: C, 65.81; H, 5.89; N, 18.01; S, 10.29.

4.3.2. 1-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-yl)-3-ethylthiourea (5b). Crystallized from dichloromethane, [yield: 63% (1.5 g)]; as a yellow powder. Mp 179–181 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.28 (t, J = 6.9 Hz, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.62 (s, br, 2H, 2NH), 4.53 (q, J = 6.9 Hz, 2H, CH_2), 5.71 (s, 2H, CH_2), 7.47–7.64 (m, 5H, aromatic protons), 7.84–7.86 (m, 3H, H4 + H6 + H7); anal. calcd for $C_{18}H_{20}N_4S$ (324): C, 66.66; H, 6.17; N, 17.28; S, 9.87. Found: C, 66.67; H, 6.19; N, 17.26; S, 9.83.

4.3.3. 1-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-yl)-3-phenylthiourea (5c). Crystallized from methanol, [yield: 73% (2 g)]; as a brown powder. Mp 230–233 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, CH_3), 4.54 (s, 2H, 2NH), 5.60 (s, 2H, CH_2), 7.47–7.86 (m, 5H, aromatic protons + H7 benzimidazole), 7.85–7.98 (m, 5H, aromatic), 8.13 (d, J = 6.9 Hz, 1H, H6), 8.44 (s, 1H, H4); IR (cm^{-1}): 3365-3200 (NH groups), 1611 (C=N), 1338 (C=S); anal. calcd for $C_{22}H_{20}N_4S$ (372): C, 70.96; H, 5.37; N, 15.05; S, 8.60. Found: C, 70.87; H, 5.48; N, 15.19; S, 8.58.

4.3.4. 1-Benzoyl-3-(1-benzyl-2-methyl-1*H*-benzimidazol-5-yl)thiourea (5d). Crystallized from ethanol/diethyl ether (5:1), [yield: 76% (2.2 g)]; as a gray powder. Mp 192–194 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.30 (s, 3H, CH_3), 4.14 (s, 2H, 2NH), 5.20 (s, 2H, CH_2), 7.47-7.90 (m, 10H, aromatic protons + H7 benzimidazole), 7.91 (d, J = 6.9 Hz, 1H, H6), 8.01 (s, 1H, H4); IR (cm^{-1}): 3398, 3224 (NH groups), 1702 (C=O), 1622 (C=N), 1296 (C=S); anal. calcd for $C_{23}H_{20}N_4OS$ (400): C, 69.00; H, 5.00; N, 14.00; S, 8.09. Found: C, 69.03; H, 5.03; N, 13.94; S, 8.11.

4.3.5. 1-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-yl)-3-(4-methoxyphenyl)thiourea (5e). Crystallized from ethanol, [yield: 82% (2.4 g)]; as a brown powder. Mp 123–125 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.44 (s, 3H, CH_3), 3.64 (s, 3H, OCH_3), 4.28 (s, 2H, 2NH), 5.55 (s, 2H, CH_2), 7.51–7.93 (m, 9H, aromatic protons +H7 benzimidazole), 7.94 (d, J = 6.9 Hz, 1H, H6), 8.11 (s, 1H, H4);IR: 3269, 3223 (NH groups), 1596 (C=N), 1331 (C=S); anal. calcd for $C_{23}H_{22}N_4OS$ (402): C, 68.65; H, 5.47; N, 13.92; S, 7.96. Found: C, 68.64; H, 5.48; N, 13.88; S, 8.95.

4.4. General procedure for the preparation of the compounds (6a–e)

To a solution of compound **5** (2 g, 6.4 mmol) in absolute ethanol (20 ml), (0.8 ml, 6.4 mmol) ethyl bromoacetate and (2.08 g, 25.6 mmol) anhydrous sodium acetate were added. The reaction mixture was refluxed on a water bath for 2 h. cooled, diluted with water and allowed to

stand overnight; the precipitate was filtered and recrystallized from appropriate solvent.

4.4.1. 2-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-ylimino)-3-methyl-thiazolidin-4-one (6a). Crystallized from ethanol, [yield: 56% (1.1 g)]; as a green powder. Mp 221–223 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 3.35 (s, 2H, SCH_2CO), 4.51 (s, 2H, CH_2), 7.15–7.58 (m, 5H, aromatic protons), 7.90 (d, $J = 9$ Hz, 1H, H7), 8.16 (d, $J = 9$ Hz 1H, H6), 8.44 (s, 1H, H4); anal. calcd for $C_{19}H_{18}N_4OS$ (350): C, 65.14; H, 5.14; N, 16.00; S, 9.14. Found: C, 65.11; H, 5.13; N, 16.01; S, 9.10.

4.4.2. 2-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-ylimino)-3-ethyl- thiazolidin-4-one (6b). Crystallized from acetone, [yield: 77% (1.7 g)]; as a yellow powder. Mp 202–205 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.56 (t, 3H, CH_3), 2.84 (s, 3H, CH_3), 3.35 (s, 2H, SCH_2CO), 3.24 (q, 2H, CH_2), 4.51 (s, 2H, CH_2), 7.15–7.58 (m, 5H, aromatic protons), 7.90 (d, $J = 9$ Hz, 1H, H7), 8.16 (d, $J = 9$ Hz 1H, H6), 8.44 (s, 1H, H4); IR (cm^{-1}): 1706 (C=O), 1655, 1606 (C=N groups); anal. calcd for $C_{20}H_{20}N_4OS$ (364): C, 65.93; H, 5.54; N, 15.38; S, 8.79. Found: C, 65.90; H, 5.53; N, 15.41; S, 8.77.

4.4.3. 2-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-ylimino)-3-phenyl-thiazolidin-4-one (6c). Crystallized from dichloromethane/methanol (4:1), [yield: 72% (1.5 g)]; as a pale yellow powder. Mp 193–197 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.52 (s, 3H, CH_3), 3.35 (s, 2H, SCH_2CO), 5.50 (s, 2H, CH_2), 7.47–7.86 (m, 10H, aromatic protons + H7 benzimidazole), 8.04 (d, $J = 9$ Hz, 1H, H6), 8.45 (s, 1H, H4); IR (cm^{-1}): 1702 (C=O), 1611 (C=N); anal. calcd for $C_{24}H_{20}N_4OS$ (412): C, 69.90; H, 4.85; N, 13.59; S, 7.76. Found: C, 69.91; H, 4.83; N, 13.60; S, 7.80.

4.4.4. 3-Benzoyl-2-(1-benzyl-2-methyl-1*H*-benzimidazol-5-ylimino)thiazolidin-4-one (6d). Crystallized from dichloromethane/methanol (4:1), [yield: 81% (1.8 g)] ; as a gray powder. Mp 232–234 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.42 (s, 3H, CH_3), 3.35 (s, 2H, SCH_2CO), 5.50 (s, 2H, CH_2), 7.47–7.86 (m, 10H, aromatic protons + H7 benzimidazole), 8.04 (d, $J = 9$ Hz, 1H, H6), 8.45 (s, 1H, H4); IR (cm^{-1}): 1702, 1687 (C=O groups), 1615 (C=N). anal. calcd for $C_{25}H_{20}N_4O_2S$ (440): C, 68.18; H, 4.54; N, 12.72; S, 7.27. Found: C, 68.16; H, 4.51; N, 12.72; S, 7.29.

4.4.5. 2-(1-Benzyl-2-methyl-1*H*-benzimidazol-5-ylimino)-3-(4-methoxyphenyl)thiazoli-din-4-one (6e). Crystallized from ethanol, [yield: 77% (1.7 g)]; as a brown powder. Mp 147–149 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3H, CH_3), 3.35 (s, 2H, SCH_2CO), 3.75 (s, 3H, OCH_3), 5.50 (s, 2H, CH_2), 7.77–7.96 (m, 10H, aromatic protons + H7 benzimidazole), 8.04 (d, $J = 9$ Hz, 1H, H6), 8.15 (s, 1H, H4); IR (cm^{-1}): 1688.5 (C=O), 1580, 1589 (C=N groups) 1181.6 (O–C–O); anal. calcd for $C_{25}H_{22}N_4O_2S$ (442): C, 67.87; H, 5.97; N, 12.66; S, 7.23. Found: C, 67.85; H, 5.99; N, 12.65; S, 7.25.

4.5. 5-amino-2-methyl-1*H*-benzimidazole monohydrochloride (7)

Compound **7** was prepared according to Bapat et al.'s method[23]. IR (cm^{-1}): 3386, 3322 (NH_2), 3226.8 (NH), 1642(C=N); anal. calcd for $C_8H_{10}ClN_3$ (183): C, 52.31; H, 5.44; Cl, 19.34; N, 22.88. Found: C, 52.41; H, 5.35; Cl, 19.40; N, 22.67.

4.6. General procedure for the preparation of the compounds (8a–f)

A solution of the appropriate aromatic and heterocyclic aldehydes, namely, 5-methylfurfural, piperonal, indole-3-carbaldehyde, 2-thiophene carboxaldehyde, 4-methylbenzaldehyde and or 1-naphthaldehyde, (10 mmol) in ethanol (5 ml) was added dropwise to a well-stirred solution of compound **7** (10 mmol) in boiling ethanol (30 ml). The reaction mixture was refluxed for 5 h, then the solvent was removed under reduced pressure, the solid produced was filtered off, washed with dilute ethanol, and recrystallized from appropriate solvent.

4.6.1. 2-Methyl-*N*-[(5-methylfuran-2-yl)methylene]-1*H*-benzimidazol-5-amine (8a). Crystallized from ethanol, [yield: 77% (1.9 g)]; as a brown powder. Mp 250–252 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.71 (s, 3H, CH_3), 2.80 (s, 3H, CH_3), 5.91 (d, $J = 8.1$ Hz, 1H, H4′ furan), 6.20 (d, $J = 8.1$ Hz, 1H, H3′ furan), 7.61 (d, $J = 9.1$ Hz, 1H, H7), 7.84 (d, $J = 9.1$ Hz, 1H, H6), 8.10 (s, 1H, H4), 8.22 (s, 1H, CH=N); anal. calcd for $C_{14}H_{13}N_3O$ (239): C, 70.31; H, 5.38; N, 17.60. Found: C, 70.33; H, 5.39; N, 17.61.

4.6.2. *N*-[(Benzo(1,3-*d*)dioxol-6-yl)methylene]-2-methyl-1*H*-benzimidazol-5-amine (8b). Crystallized from ethanol, [yield: 71% (2 g)]; as a yellow powder. Mp 117–119 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.44 (s, 3H, CH_3), 6.10 (s, 2H, OCH_2O), 7.01 (d, $J = 7.70$ Hz, 1H, H7), 7.15 (d, $J = 7.70$ Hz, 1H, H6), 7.41 (m, 3H, aromatic protons), 7.52 (s, 1H, H4), 8.10 (s, 1H, CH=N).^{13}C NMR (DMSO-d_6) δ 14.85 (CH_3), 101.77 (O–CH2–O), 157.8 (C=N); IR (cm^{-1}): 3299 (NH), 1655, 1593 (C=N groups), 1114 (O–C–O); anal. calcd for $C_{16}H_{13}N_3O_3$ (279): C, 68.81; H, 4.65; N, 15.05. Found: C, 68.83; H, 4.61; N, 15.10.

4.6.3. *N*-[(1*H*-Indol-3-yl)methylene]-2-methyl-1*H*-benzimidazol-5-amine (8c). Crystallized from ethanol/diethyl ether (5:1), [yield: 67% (1.8 g)]; as a brown powder. Mp 173–175 °C; ^{1}H HMR (300 MHz, DMSO: CDCl3 2:1) δ 2.52 (s, 3H, CH_3), 6.82 (s, 1H, CH indole), 7.14–7.42 (m, 4H, H′5 + H′6 + H′7+H′8), 7.51 (s, 1H, CH), 7.71 (d, $J = 9.1$ Hz, 1H, H7), 7.84 (d, $J = 9.1$ Hz, 1H, H6), 8.10 (s, 1H, H4); IR (cm^{-1}): 3418, 3232 (NH groups), 1537, 1611 (C=N groups); anal. calcd for $C_{17}H_{14}N_4$ (274): C, 73.11; H, 5.10; N, 20.43. Found: C, 73.38; H, 5.09; N, 20.37.

4.6.4. 2-Methyl-*N*-[(thiophen-2-yl)methylene]-1*H*-benzimidazol-5-amine (8d). Crystallized from ethanol/diethyl ether (5:1), [Yield: 69% (1.7 g)]; as a pale yellow powder. Mp 230–232 °C; ^{1}H HMR (300 MHz, DMSO-

d_6) δ 2.39 (s, 3H, CH_3), 7.28–7.31 (m, 1H, H′4), 7.52 (d, J = 9.1Hz, 1H, H7), 7.62–7.80 (m, 4H, H6 + H′3 + H4 + CH=N), 7.91 (d, J = 9.1Hz, 1H, H′5); IR (cm^{-1}): 3403 (NH), 1611, 1598 (C=N groups); anal. calcd for $C_{13}H_{11}N_3S$ (241): C, 64.73; H, 4.56; N, 17.42; S, 13.27. Found: C, 64.75; H, 4.54; N, 17.46; S, 13.24.

4.6.5. (2-Methyl-1*H*-benzimidazol-5-yl)-(4-methylbenzylidene)-amine (8e). Crystallized from methanol, [yield: 76%, 1.9 g]; as a gray powder. Mp 192–194 °C; ^{1}H NMR (300 MHz, DMSO-d_6) 2.22 (s, 3H, CH_3), δ 2.42 (s, 3H, CH_3), 6.78 (d, J = 7.29 Hz, 2H, H′2 + H′6), 6.80 (d, J = 7.29 Hz, 2H, H′3 + H′5), 7.30 (d, J = 8.1 Hz, H7), 7.59 (d, J = 8.1 Hz, H6), 7.90 (s, 1H, H4), 7.93 (s, 1H, CH=N); anal. calcd for $C_{16}H_{15}N_3$(249): C, 77.08; H, 6.02; N, 16.86. Found: C, 77.05; H, 6.01; N, 16.89.

4.6.6. 2-Methyl-*N*-[(naphthalen-1-yl)methylene]-1*H*-benzimidazol-5-amine (8f). Crystallized from ethanol, [yield: 74%, 2.1 g]; as a brown powder. Mp 156–158 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.12 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 7.30–7.92 (m,10 H, aromatics protons), 7.93 (s, 1H, CH=N); IR (cm^{-1}): 3389 (NH), 1651, 1587 (C=N groups); anal. calcd for $C_{19}H_{15}N_3$(285): C, 80.10; H, 5.26; N, 14.73. Found: C, 80.19; H, 5.25; N, 14.71.

4.7. General procedure for the preparation of the compounds (9a–f)

A mixture of Schiff base **8a**–**f** (7.2 mmol) and thioglycolic acid (10 mmol) in dry toluene (50 ml) was refluxed for 5–8 h and the solvent was evaporated under reduced pressure. The residue was triturated with sodium bicarbonate solution, left overnight, filtered off, washed with water, dried, and recrystallized from appropriate solvent.

4.7.1. 3-(2-Methyl-1*H*-benzimidazol-5-yl)-2-(5-methylfuran-2-yl) thiazolidin-4-one (9a). Crystallized from methanol, [yield: 73% (1.9 g)]; as a brown solid. Mp 224–226 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.65 (s, 3H, CH_3), 2.85 (s, 3H, CH_3), 3.75 (s, 2H, CH_2), 4.24 (s, 1H, CH), 5.90 (d, J = 8.1 Hz, 1H, H4′ furan), 6.20 (d, J = 8.1 Hz, 1H, H3′ furan), 7.61 (d, J = 9.1 Hz, 1H, H7), 8.03 (d, J = 9.1 Hz, 1H, H6), 8.21 (s, 1H, H4); IR (cm^{-1}): 3329 (NH), 1681 (C=O), 1598 (C=N); anal. calcd for $C_{16}H_{15}N_3O_2S$ (313): C, 61.34; H, 4.79; N, 13.41; S, 10.22. Found: C, 61.32; H, 4.78; N, 13.39; S, 10.23.

4.7.2. 2-(Benzo(1,3-d)dioxol-6-yl)-3-(2-methyl-1*H*-benzimidazol-5-yl)thiazolidin-4-one (9b). Crystallized from ethanol, [yield: 70% (1.7 g)]; as a yellow powder. Mp 152–154 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.39 (s, 3H, CH_3), 3.18 (s, 2H, CH_2), 4.02 (s, 1H, CH–N), 6.08 (s, 2H, O–CH2–O), 6.75–6.83 (m, 3H, aromatic protons), 7.41 (d, J = 7.7 Hz, 1H, H7), 7.72 (d, J = 9.7 Hz, 1H, H6), 8.01 (s, 1H, H4); IR (cm^{-1}): 3214 (NH), 1670 (C=O), 1599 (C=N), 1111 (O–C–O); anal. calcd for $C_{19}H_{15}N_3O_3S$ (353): C, 61.18; H, 4.24; N, 11.89; S, 9.06. Found: C, 61.23; H, 4.15; N, 11.94; S, 9.12.

4.7.3. 2-(1*H*-Indol-3-yl)-3-(2-methyl-1*H*-benzimidazol-5-yl)thiazolidin-4-one (9c). Crystallized from ethanol/diethyl ether (5:2), [yield: 77% (1.9 g)]; as buff needles. Mp 175-177 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.34 (s, 3H, CH_3), 3.35 (s, 2H, CH_2), 4.01 (s, 1H, CH), 6.62 (s, 1H, CH), 7.23–7.34 (m, 5H, aromatic protons), 7.64-7.79 (m, 3H, H4+ H6+ H7); IR (cm^{-1}): 3398, 3217 (NH groups), 1680 (C=O), 1615 (C=N); anal. calcd for $C_{19}H_{16}N_3OS$ (334): C, 68.26; H, 4.79; N, 12.57; S, 9.58. Found: C, 68.24; H, 4.79; N, 12.55; S, 9.57.

4.7.4. 3-(2-Methyl-1*H*-benzimidazol-5-yl)-2-(thiophen-2-yl)thiazolidin-4-one (9d). Crystallized from ethanol, [yield: 80% (2.1 g)]; as pale yellow needles. Mp 194-196 °C; ^{1}H NMR (300 MH_Z, DMSO-d_6) δ 2.39 (2, 3H, CH_3), 3.32 (s, 2H, CH_2), 4.51 (s, 1H, CH), 7.25-7.38 (m, 1H, H′4), 7.52 (d, J = 9.1 Hz, 1H, H7), 7.63-7.78 (m, 3H, H′3 + H6 + H4), 7.91 (d, J = 9.1 Hz, 1H, H′5); IR (cm^{-1}): 3410 (NH), 1688 (C=O), 1629 (C=N); anal. calcd for $C_{15}H_{13}N_3OS_2$ (315): C, 57.14; H, 4.12; N, 13.33; S, 20.31. Found: C, 57.15; H, 4.11; N, 13.32; S, 20.28.

4.7.5. 3-(2-Methyl-1*H*-benzimidazol-5-yl)-2-*p*-tolylthiazolidin-4-one (9e). Crystallized from methanol, [yield: 84% (2.2 g)]; as a gray powder. Mp 267–269 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.27 (s, 3H, CH_3), 2.68 (s, 3H, CH_3), 3.22 (s, 2H, CH_2), 3.40 (s, 1H, CH-N), 6.78 (d, J =7.29 Hz, 2H, H′2 + H′6), 6.80 (d, J = 7.29 Hz, 2H, H′3 + H′5), 7.32 (d, J = 8.1 Hz, 1H, H7), 7.60 (d, J = 8.1 Hz, 1H, H6), 7.85 (s, 1H, H4); IR (cm^{-1}): 3343 (NH), 1689 (C=O), 1598 (C=N); anal. calcd for $C_{18}H_{17}N_3OS$ (323): C, 66.87; H, 5.26; N, 13.00; S, 9.90. Found: C, 66.88; H, 5.29; N, 12.91; S, 9.88.

4.7.6. 3-(2-Methyl-1*H*-benzimidazol-5-yl)-2-(naphthalen-1-yl)thiazolidin-4-one (9f). Crystallized from ethanol/diethyl ether (5:1), [yield: 74% (1.8 g)]; as brown prisms. Mp 211–213 °C; H NMR (300 MHz, DMSO-d_6) δ 2.56 (s, 3H, CH_3), 3.22 (s, 2H, CH_2), 3.40 (s, 1H, CH–N), 6.78 (m, 10 H, aromatic protons); IR (cm^{-1}): 3233 (NH), 1685 (C=O), 1619 (C=N); anal. calcd for $C_{21}H_{17}N_4OS$ (359): C, 70.19; H, 4.73; N, 11.69; S, 8.91. Found: C, 70.20; H, 4.72; N, 11.70; S, 8.89.

4.8. Antitumor promoting effect in vitro (Epstein–Barr virus activation test)

4.8.1. Materials and methods. The compounds **1, 2, 3, 4, 5a–c**, **6a–e**, **7, 8a–f**, and **9a–e** were tested for Epstein–Barr virus (EBV) Inhibitory Activity. The inhibition of EBV-EA activation was assayed using methods reported in the literature.[21a,b,22] The cells were incubated at 37 °C for 48 h in a medium containing butyric acid (4 nM), TPA (32 pM), and various amounts of test compounds. Smears were made from the cell suspensions and the EBV-EA inducing cells were stained using an indirect immunofluorescence technique. In each assay, at least

500 cells were counted, and the number of stained cells (positive cells) was recorded. Triplicate assays were performed for each data point. The EBV-EA inhibitory activity of the test compound was compared with that of the control experiment with butyric acid plus TPA. In control experiments, the EBV-EA inhibitory activities were generally around 40%, and these values were taken as a positive control. The viability of the cells was assayed by the Trypan-blue staining method. For the determination of cytotoxicity, the cell viability was required to be more than 60%.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.04.010.

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