Bull. Chem. Soc. Ethiop. **2023**, 37(5), 1209-1220.
DOI: https://dx.doi.org/10.4314/bcse.v37i5.13

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

DESIGN AND SYNTHESIS OF INDOLE-BENZIMIDAZOLE HYBRID MOLECULES AND EVALUATION OF THEIR *IN-VITRO* CYTOTOXIC ACTIVITIES

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(Received January 25, 2023; Revised April 14, 2023; Accepted April 19, 2023)

ABSTRACT. Cancer is one of the most deadly diseases worldwide, challenging the world for effective treatment of the diseases, to tackle this problem a vast amount of therapeutic candidates are being investigated. Indole-benzimidazole structures have recently gained considerable attention, because compounds containing these structure exhibit a very good anticancer property. Two series of novel indole-benzimidazole hybrids molecules *viz.,* 2-(5-substituted-1*H*-indol-3-yl)-5-substituted-1*H*-benzo[*d*]imidazole **5(a-f)** and 2-(5-substituted-1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)-5-substituted-1*H*-benzo[*d*]imidazole **6(a-f)** were synthesized and characterized by spectroscopic techniques. The twelve target molecules have been investigated for their *in-vitro* cytotoxic activity against human ovarian carcinoma cells **(SKOV-3)**, human prostate cancer cells **(PC-3)**, human cervical cancer cells **(HeLa)** and human acute monocytic leukemia cells **(THP-1)** using MTT assay. Compound; 2-(5-bromo-1H-indol-3-yl)-5-methyl-1H-benzo[d]imidazole **(5e)** was interesting with IC_{50} (μM) values of 23.69 (SKOV-3), 73.05 (PC-3), 64.66 (HeLa) and 39.08 (THP-1), respectively.

KEY WORDS: Indole, Benzimidazole, Hybrid molecules, Anticancer activity

INTRODUCTION

Nitrogen-heterocycles play a vital role in medicinal chemistry and they have been intensively used as scaffolds for drug development [1]. Among nitrogen heterocyclic compounds indole nucleus is continuously drawing the interest of many researchers for the development of newer drug moiety [2]. Owing to its wide range of activities, a large number of indole derivatives have been synthesized and screened for their anticancer [3-14], antitumor [15-18], anti-inflammatory [19], antibacterial [20], antifungal [21], anti-malarial [22] and anticonvulsant activities [23]. In particular, N-1 and C-3-substituted indole derivatives have been found to play an important role in many biologically active compounds especially with anti-inflammatory [24, 25], anticancer [26-28], antineociceptive [29, 30], antipsychotic activity [31].

Benzimidazoles are a class of aromatic, bicyclic, heterocyclic chemical compounds that share the basic structure of 6-membered benzene united to 5-membered imidazole. These are the important class of heterocyclic scaffolds which have diverse biological activities and clinical applications, compounds carrying benzimidazole nucleus have been reported to have antiviral [32-34], anticancer [35], antibacterial [36], antimalaria [37], antifungal [38], antihelminthic [39], antiprotozoal (*Entamoeba histolytica* and *Trichomonas vaginalis*) and anticancer activities [40].

Moreover the presence of extra hetrocyclic ring to the position 3 of indole ring represents an important class of marine alkaloids, most of these marine alkaloids exhibit significant biological activities [41]. For example, marine alkaloids containing hetrocyclic ring at 3-position of indole is presented in Figure 1. Aplicyanins were isolated from the ascidian *Aplidium cyaneum* and meridianins were isolated from the tunicate *Aplidium meridianum*, the former contain a 3-(2-amino-1,4,5,6-tetrahydropyrimidin-4-yl)-5-bromoindole nucleus while the later contain 3-

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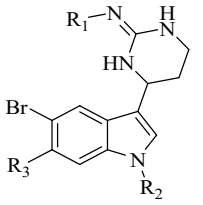
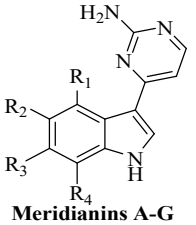
(pyrimid-4-yl) brominated and/or hydroxylated indole nucleus. These compounds are found to be cytotoxic to the human tumor cell lines MDA-MB-231 (breast adenocarcinoma), A549 (lung carcinoma), HT-29 (colorectal carcinoma) and cytotoxicity towards murine tumor cell lines and potent inhibitors of several protein kinases, respectively [42-44]



Aplicyanins A-F

A: $R_1 = R_2 = R_3 = H$
B: R_1= Ac, $R_2 = R_3 = H$
C: $R_1 = R_3 = H$, R_2 = OMe
D: R_1 = Ac, R_2 = OMe, $R_3 = H$
E: $R_1 = H$, R_2 = OMe, R_3= Br
F: R_1 = Ac, R_2 = OMe, R_3 =B r



Meridianins A-G

A: R_1 = OH, $R_2 = R_3 = R_4 = H$
B: R_1 = OH, $R_2 = R_4 = H$, R_3 = Br
C: $R_1 = R_3 = R_4 = H$, R_3 = Br
D: $R_1 = R_2 = R_4 = H$, R_3 = Br
E: R_1 = OH, $R_2 = R_3$ = H, R_4 = Br
F: $R_1 = R_4 = H$, $R_2 = R_3$ = Br
G: $R_1 = R_2 = R_3 = R_4 = H$

Figure 1. Structure of Aplicyanins A-F and Meridianins A-G.

Inspired by a wide range of biological activities and the favorable drug-like properties of indoles and benzimidazole, we envisioned that the combination of these two bioactive components especially benzimidazole ring at C-3 position of indole would afford compounds with a potential anticancer activity, therefore we designed and synthesized compounds containing both moieties in a single molecular frame and screened for their anticancer activities against the human ovarian carcinoma cell (SKOV-3), human prostate cancer cell, (PC-3), human cervical cancer cell (HeLa) and human acute monocytic leukemia cell lines (THP-1).

EXPERIMENTAL

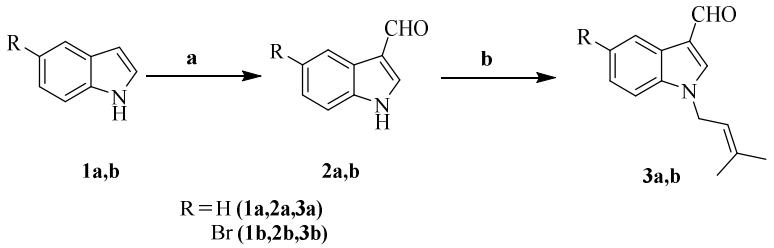
Materials and methods

All the chemicals used were of synthetic grade procured from Sigma Aldrich. Completion of the reactions was monitored by analytical thin layer chromatography (TLC) using E-Merck 0.25 mm silica gel plates using ethyl acetate/hexane as solvent system, visualization was accomplished with UV light (256 nm) and iodine chamber. All the synthesized compounds were purified by column chromatography (silica gel 100-200 mesh) using a mixture of hexane and ethyl acetate. Melting points were measured in open capillary tubes and were uncorrected. All the ^{1}H and ^{13}C spectra were recorded in $CDCl_3$ or DMSO-d_6 solvent (400 MHz for ^{1}H and 100 MHz for ^{13}C) relative to TMS internal standard. Infrared (IR) spectra were recorded using FT-IR Bruker Alpha spectrometer. The electron ionization mass spectra were recorded on Agilent 1100.

Chemistry

Synthesis of novel indole-benzimidazole hybrids viz., 2-(5-substituted-1H-indol-3-yl)-5-substituted-1H-benzo[d]imidazole **5(a-f)** and 2-(5-substituted-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-5-substituted-1H-benzo[d]imidazole **6(a-f)** have been accomplished by adopting known synthetic routes. Formylation of indole/ substituted indole using $POCl_3$ in dry DMF gave 1H-indole-3-carboxaldehyde (**2a,b**), reaction of (**2a,b**), with 3,3-dimethylallyl bromide in the

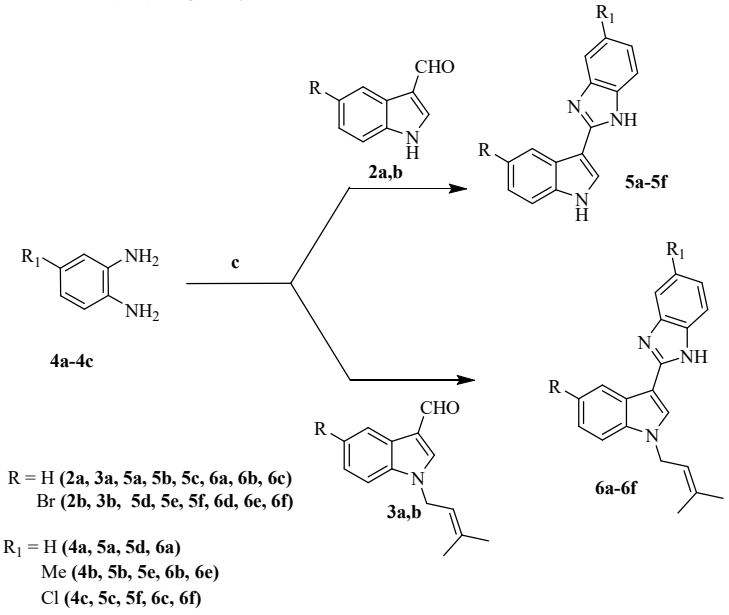
presence NaH gave substituted 1-(3-methylbut-2-enyl)-1H-indole-3-carboxaldehyde (**3a,b**) in good yield (Scheme 1).



Reagent and conditions: a) $POCl_3$, dry DMF; 0 °C; $NaOH_{(aqua)}$, 40 °C; b) 3,3-dimethylallyl bromide, NaH, dry DMF, 0 °C

Scheme 1. Synthesis of 5-substituted-1H-indole-3-carbaldehyde (**2a**,**b**) and 5-substituted-1-(3-methylbut-2-enyl)-1H-indole-3-carbaldehyde (**3a**,**b**).

The target molecules were achieved by the reaction of substituted/unsubstituted phenylene diamine (**4)** in the presence of *p*-toluene sulfonic acid (PTSA) in anhydrous DMF under refluxing conditions with 5-substituted-1H-indole-3-carboxaldehyde (**2a,b**) yields 2-(5-substituted-1H-indol-3-yl)-5-substituted-1H-benzo[d]imidazole **5(a-f)**. Compound **4** under the same reaction conditions reacts with 5-substituted-1-(3-methylbut-2-enyl)-1H-indole-3-carboxaldehyde (**3a,b**) to give 2-(5-substituted-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-5-substituted-1H-benzo[d] imidazole **6(a-f)** in good yield (Scheme 2).



Reagents and conditions: c) *p*-TSA, DMF, reflux 100 °C

Scheme 2. Synthesis of 2-(5-substituted-1*H*-indol-3-yl)-5-substituted-1*H*-benzo[*d*]imidazole **5(a-f)** and 2-(5-substituted-1-(3-methylbut-2-enyl)-1*H*-indol-3-yl)-5-substituted-1*H*-benzo[*d*]imidazole **6(a-f)**.

Synthesis and characterization of the intermediates and target compounds

*General procedure for the synthesis of 5-substituted 1H-indole-3-carboxaldehyde **(2a,b)***

To a solution of substituted indoles **(1a,b)** (42.6 mmol) in dry DMF (187.4 mmol) in an ice-salt bath $POCl_3$ (47.1 mmol) was subsequently added with stirring over a period of 30 min. After completion of addition, the temperature rose to 40 °C and stirred the syrup for 1.5 h at that temperature. At the end of the reaction (as indicated by TLC) 25 g crushed ice was added to the reaction mixture. The obtained solution was transferred into 250 mL RB flask, NaOH (470 mmol) dissolved in 50 mL water was added with constant stirring and the resultant suspension was heated rapidly to the boiling point and allowed to cool to room temperature, after which it was placed in refrigerator overnight. The precipitate was filtered off, washed thrice with 100 mL water, yielding 5-substituted 1*H*-indole-3-carbaldehyde **(2a,b)**.

*[1]H-Indole-3-carboxaldehyde **(2a)***. Yield: 92%, brownish yellow solid, Mp: 196-198 °C; IR (KBr, cm^{-1}): 3442 (N-H), 1632 (C=N), 1229; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.14 (t, J = 7.2 Hz, 1H), 7.22 (t, J = 7.2 Hz, 1H), 7.34 (d, J = 8 Hz, 1H), 7.52 (s, 1H), 7.62 (d, J = 8 Hz, 1H), 8.12 (s, 1H), 9.52 (s, 1H); ^{13}C NMR (DMSO-d_6, 22.4 MHz): δ 111.4, 118.0, 119.4, 120.5, 122.4, 127.7, 131.82, 137.2, 182.7; ESI-MS: *m/z* 146.20 $[M+H]^+$; anal. calcd. for C_9H_7NO: C, 74.47, H, 4.86, N, 9.65%, found: C, 74.44, H, 4.91, N, 9.64%.

*5-Bromo-1H-indole-3-carboxaldehyde **(2b)***. Yield: 90%, cream colored solid, Mp: 192 °C; IR (KBr, cm^{-1}): 3312 (N-H), 1643 (C=N), 1229; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.34 (d, J = 8.8 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 8.25 (s, 1H), 8.32 (s, 1H), 9.94 (s, 1H); ^{13}C NMR (DMSO-d_6, 22.4 MHz): δ 113.0, 114.8, 117.3, 123.1, 125.6, 135.2, 136.7, 144.4, 183.9. ESI-MS: *m/z* 245.95 $[M+Na]^+$; anal. calcd. for C_9H_6BrNO: C, 48.25, H, 2.70, N, 6.25%, found: C, 48.22, H, 2.76, N, 6.24%.

*General procedure for the synthesis of substituted 1-(3-methylbut-2-enyl)-1H-indole-3-carboxaldehyde (**3a,b**)*

To a solution of substituted 1*H*-indole-3-carbaldehydes **(2a,b)** (2.20 mmol) in dry DMF (5 mL) was added NaH (2.64 mmol, 60% oil dispersion) and the resulting mixture was stirred for 10 min in an ice bath. 3,3-dimethylallyl bromide (2.20 mmol) was added and the resulting mixture stirred for 15 min at 0 °C. The mixture was diluted with EtOAc (20 mL), washed five times with distilled water (50 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the solvent was removed under reduced pressure to get crude residue which was purified by column chromatography (silica gel 100-200 mesh) using 8:2 (hexane : EtOAc) as eluents affording **3a,b**.

*1-(3-Methylbut-2-enyl)-1H-indole-3-carboxaldehyde **(3a)***. Yield: 87%, brownish yellow crystals, Mp: 79-81 °C; IR (KBr, cm^{-1}): 1640 (C=N) 1224; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.85 (s, 3H), 1.86 (s, 3H), 4.74 (d, J = 7.2 Hz, 2H), 5.44 (t, J = 7.2 Hz, 1H), 7.28-7.41 (m, 4H) 7.76 (s, 1H), 9.99 (s, 1H); ^{13}C NMR ($CDCl_3$, 22.4 MHz): δ 17.9, 25.4, 44.6, 110.0, 117.7, 117.9, 121.8, 122.7, 123.6, 125.5, 137.3, 137.5, 138.7, 184.3; ESI-MS: *m/z* 214.20 $[M+H]^+$; anal. calcd. for $C_{14}H_{15}NO$: C, 78.84, H, 7.09, N, 6.57%, found: C, 78.80, H, 7.16, N, 6.55%.

*5-Bromo-1-(3-methylbut-2-enyl)-1H-indole-3-carboxaldehyde **(3b)***. Yield: 89%, pale pink solid, Mp: 95-98 °C; IR (KBr, cm^{-1}): 1654(C=N), 1162; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.85 (s, 3H), 1.86 (s, 3H), 4.71 (d, J =7.2 Hz, 2H), 5.42 (t, J =7.2 Hz, 1H), 7.25 (d, J = 9.6 Hz, 1H), 7.43 (dd, J = 2.0, 8.8 Hz, 1H), 7.73 (s, 1H), 8.47 (d, J = 2.0 Hz, 1H), 9.96 (s, 1H); ^{13}C NMR ($CDCl_3$, 22.4 MHz): δ 12.7, 20.1, 39.4, 106.0, 110.8, 111.7, 119.0, 121.1, 121.4, 130.3, 132.6, 132.8, 133.8,

178.6; ESI-MS: *m/z* 314.01 $[M+Na]^+$; anal. calcd. for $C_{14}H_{14}BrNO$: C, 57.55, H, 4.83, N, 4.79%, found: C, 57.53, H, 4.90, N, 4.77%.

General procedure for the synthesis of 2-(5-substituted-1H-indol-3-yl)-5-substituted-1H-benzo[d]imidazole ***(5a-f)***

5-Substituted-1*H*-indole-3-carbaldehyde (1.5 mmol) **(2a,b)** and *o*-phenylenediamine (1.5 mmol) **(4a-c)** were thoroughly dissolved in dry DMF (10 mL), 40 mol % of PTSA (103.2 mg, 0.6 mmol) was added and the solution was refluxed at 100 °C for appropriate time. After completion of the reaction (monitored by TLC), the solution was allowed to room temperature and extracted with ethyl acetate; the combined organic layer was dried over anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure, the crude product was purified by column chromatography using silica gel (100-200 mesh), hexane : ethyl acetate (7:3) as eluents and recrystallized from ethanol to afford the target compounds **(5a-f)**.

2-(1H-Indol-3-yl)-1H-benzo[d]imidazole ***(5a)***. Yield: 74%; Mp: 106-108 °C; IR (KBr cm^{-1}): 3424 (N-H), 3048, (C-H aromatic) 1613(C=N); ^{1}H NMR (DMSO-d_6, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.67(s, 1H, NH), 8.50-8.16 (m, 2H, Ar-H), 7.56-7.48 (m, 2H, Ar-H),7.22-7.13 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.1, 138.2, 137.8, 136.6, 130.3, 129.2, 123.2, 122.3, 121.2, 120.3, 118.5, 114.3, 110.2; 106.4; anal. calc. (%) for $C_{15}H_{11}N_3$: C, 77.23; H, 4.75; N, 18.01; found: C, 77.16; H, 4.78; N, 17.98.

2-(1H-Indol-3-yl)-5-methyl-1H-benzo[d]imidazole ***(5b)***. Yield: 72%; Mp: 210-212 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.81 (s, 1H, NH), 8.46 (d, J = 8.4, 1H, Ar-H), 8.24 (d, J = 2.4, 1H, Ar-H) 7.54-7.47 (m, 2H Ar-H), 7.38 (s, 1H, H-2′), 7.26-7.20 (m, 2H ArH), 7.05 (d, J = 7.6 1H, Ar-H), 1.92 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 171.9, 151.8, 148.4, 138.2, 136.5, 131.1, 127.2, 127.1, 124.7, 121.0, 119.3, 112.1, 105.0, 21.2; anal. calc. (%) for $C_{16}H_{13}N_3$: C, 77.71; H, 5.30; N, 16.99; found: C, 77.63; H, 5.33; N, 16.97.

5-Chloro-2-(1H-indol-3-yl)-1H-benzo[d]imidazole ***(5c)***. Yield: 70 %; Mp: 94-96 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.60 (s, 1H, benzimidazole NH), 11.67 (s, 1H, indole NH), 8.49 (d, J = 6.8 Hz, 1H, Ar-H), 8.24 (d, J = 2.8 Hz, 1H, Ar-H) 7.96 (s, H-2′), 7.58-7.50 (m, 3H Ar-H), 7.25-7.14 (m, 3H, Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 162.3, 162.2, 150.9, 136.5, 126.5, 125.3, 125.0, 122.3, 121.3, 121.1, 120.4, 112.0, 111.8, 106.1; anal. calc. (%) for $C_{15}H_{10}ClN_3$: C, 67.30; H, 3.77; Cl, 13.24; N, 15.70; found: C, 67.16; H, 3.79; N, 15.68.

2-(5-Bromo-1H-indol-3-yl)-1H-benzo[d]imidazole ***(5d)***. Yield: 68 %; Mp: 146-148 °C; IR (KBr cm^{-1}) 3407 (N-H), 3079, (C-H aromatic), 1652(C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.53 (s, 1H benzimidazole NH), 11.84 (s, 1H indole NH), 8.73 (d, J = 2 Hz, 1H, Ar-H), 8.21 (d, J = 2.8 Hz, 1H, Ar-H) 7.96-7.51 (m, 2H, Ar-H), 7.49 (s,1H, H-2′), 7.37-7.15 (m, 3H Ar-H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 160.1, 147.2, 136.4, 130.3, 130.1, 128.2, 126.4, 124.3, 123.1, 122.8, 121.8, 120.6, 112.9, 110.7, 105.5, anal. calc. (%) for $C_{15}H_{10}BrN_3$: C, 57.75; H, 3.23; Br, 25.60; N, 13.46; found: C, 57.62; H, 3.27; N, 13.44.

2-(5-Bromo-1H-indol-3-yl)-5-methyl-1H-benzo[d]imidazole ***(5e)***. Yield: 70 %; Mp: 110-112 °C; IR (KBr cm^{-1}): 3424 (N-H), 3228(N-H), 1655(C=N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.81 (s, 1H, NH), 8.66 (d, J = 1.6 Hz, 1H, Ar-H), 8.17 (d, J = 2.4 Hz, 1H, Ar-H) 7.47 (d, J = 8.8 Hz, 1H Ar-H), 7.43 (s, 1H, H-2′), 7.34 -7.31 (m, 2H Ar-H), 6.97 (d, J = 8 Hz, 1H, Ar-H), 2.41(s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 162.3, 148.4, 135.2, 130.5, 130.5, 127.3, 126.8, 124.7, 123.4, 123.3, 122.7, 120.8, 113.5, 110.9, 106.1, 21.2; anal. calc. (%) for $C_{16}H_{12}BrN_3$: C, 58.91; H, 3.71; Br, 24.50; N, 12.88; found: C, 58.01; H, 3.72; Br, 24.50; N, 12.86.

2-(5-Bromo-1H-indol-3-yl)-5-chlorol-1H-benzo[d]imidazole ***(5f)***. Yield: 66 %; Mp: 134-136 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 12.69 (s, 1H benzimidazole NH), 11.89 (s, 1H indole NH), 8.68 (d, *J* = 2 Hz, 1H, Ar-H), 8.22 (d, *J* = 2.8 Hz, 1H, Ar-H), 7.96 (s, 1H, H-2′), 7.67-7.16 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.2, 150.3, 142.3, 135.2, 127.8, 126.8, 124.8, 123.3, 121.3, 114.0, 113.1, 105.8; anal. calc. (%) for $C_{15}H_9BrClN_3$: C, 51.98; H, 2.62; Br, 23.05; Cl, 10.23; N, 12.12; found: C, 51.89; H, 2.61; N, 12.08.

General procedure for the synthesis of 2-(5-substituted-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-5-substituted-1H-benzo[d]imidazole ***(6a-f)***

Substituted 1-(3-methylbut-2-enyl)-1*H*-indole-3-*carboxaldehyde* (1 mmol) (**3a,b)** and *o*-phenylenediamine (1 mmol) **(4a-c)** were dissolved in dry DMF (10 mL), 40 mol % of PTSA (68.2 mg, 0.4 mmol) was added and the solution was refluxed at 100 °C for appropriate time. After completion of the reaction (monitored by TLC), the solution was cooled to room temperature and extracted with ethyl acetate; the combined organic layer was dried over Anhydrous Na_2SO_4, the solvent was evaporated under reduced pressure, the crude product was purified by column chromatography using silica gel (100-200 mesh) and hexane : ethyl acetate (7:3) as eluents and recrystallized from ethanol to afford target compounds **(6a-f)**.

2-(1-(3-Methylbut-2-enyl)-1H-indol-3-yl)-1H- benzo[d]imidazole ***(6a)***. Yield: 79%; Mp: 142-144 °C; IR (KBr cm^{-1}): 3443 (N-H), 3095, (C-H aromatic), 2966 (C-H, aliphatic), 1625(C=N); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.24 (d, *J* = 7.61, 1H, Ar-H), 7.89 (s, 1H, H-2′), 7.58-7.56 (m, 2H Ar-H), 7.31-7.15 (m, 5H Ar-H), 5.19 (t, *J* = 6.8 Hz, 1H), 4.48 (d, *J* = 6.8 Hz, 2H), 1.65 (s, 3H), 1.62 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.1, 138.2, 137.8, 136.6, 129.3, 129.2, 125.5, 122.3, 121.2, 121.2, 120.3, 120.3, 118.5, 114.3, 110.2, 110.2, 105.2, 44.3, 25.5, 17.9; MS m/z: 302 [M + H]$^+$; anal. calc. (%) for $C_{20}H_{19}N_3$: C, 79.70; H, 6.35; N, 13.94; found: C, 79.12; H, 6.33; N, 13.92.

5-Methyl-2-(1-(3-methylbut-2-enyl)-1H-indol-3-yl)-1H- benzo[d]imidazole ***(6b)***. Yield: 77.2%; Mp: 138-140 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.35 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.72 (s, 1H, H-2′), 7.51-7.04 (m, 6H Ar-H), 5.26 (t, *J* = 6.8 Hz, 1H), 4.56 (d, *J* = 7.2 Hz, 2 H), 2.46 (s, 3H), 1.72 (s, 3H), 1.69 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.2, 138.3, 135.2, 132.0, 129.0, 128.9, 128.90, 127.2, 123.0, 122.9, 118.2, 118.2, 114.5, 105.9, 44.4, 25.6, 21.6, 18.0; MS m/z: 302.21 [M + H]$^+$; anal. calc. (%) for $C_{21}H_{21}N_3$: C, 79.97; H, 6.71; N, 13.32; found: C, 79.88; H, 6.72; N, 13.29.

5-Chloro-2-(1-(3-methylbut-2-enyl)-1H-indol-3-yl)-1H- benzo[d]imidazole ***(6c)***. Yield: 62%; Mp: 204-206 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20 (d, *J* = 7.6, 1H, Ar-H), 7.80 (s, 1H, H-2′), 7.50-7.12 (m, 6H Ar-H), 5.26 (t, *J* = 6.8 Hz, 1H), 4.49 (d, *J* = 7.2 Hz, 2 H), 1.70 (s, 3H), 1.66 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.5, 138.0, 136.7, 128.8, 128.7, 127.5, 125.6, 122.5, 121.4, 121.3, 120.2, 120.2, 118.4, 110.3, 105.4, 44.3, 25.5, 17.9; MS m/z: 336.25 (M + H)$^+$; anal. calc. (%) for $C_{20}H_{18}ClN_3$: C, 71.53; H, 5.40; Cl, 10.56; N, 12.51; found: C, 70.89; H, 5.41; N, 12.48.

2-(5-Bromo-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-1H-benzo[d]imidazole ***(6d)***. Yield: 78%; Mp: 238-240 °C; IR (KBr cm^{-1}) 3423 (N-H), 3084, (C-H aromatic) 2924 (C-H aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.37 (s, 1H, Ar-H), 7.55 (s, 1H, H-2′), 7.27-7.06 (m, 6H, Ar-H), 5.17(t, *J* = Hz, 1H), 4.53 (d, *J* = 5.2Hz, 2 H), 1.69(s, 3H), 1.65(s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.5, 138.4, 129.1, 125.9, 122.3, 118.2, 114.7, 111.6, 44.5, 30.9, 25.63, 18.0; MS m/z: 382.1/380.1 (M$^+$ + H) (for ^{81}Br/^{79}Br, 100%, 99%); anal. calc. (%) for $C_{20}H_{18}BrN_3$: C, 63.17; H, 4.77; Br, 21.01; N, 11.05; found: C, 62.88; H, 4.76; N, 11.02.

2-(5-Bromo-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-5-methyl-1H-benzo[d]imidazole ***(6e)***. Yield: 72%; Mp: 240-242 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.35 (d, *J* = 1.6 Hz, 1H, Ar-H), 7.72 (s,1H, H-2′), 7.51-7.04 (m, 5H, Ar-H), 5.27 (t, *J* = 7.2 Hz, 1H), 4.57 (d, *J* = 7.2 Hz, 2 H), 2.46 (s, 3H), 1.72 (s, 3H), 1.69 (s, 3H); ^{13}CNMR ($CDCl_3$, 100 MHz): δ 148.2, 138.3, 135.2, 132.0, 129.0, 128.9, 128.9, 127.2, 123.0, 122.9, 118.2, 118.2, 114.5, 101.9, 44.4, 25.6, 21.6, 18.0; anal. calc. (%) for $C_{21}H_{20}BrN_3$: C, 63.97; H, 5.11; Br, 20.26; N, 10.66; found: C, 63.12; H, 5.12; N, 10.63.

2-(5-Bromo-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-5-chloro-1H-benzo[d]imidazole ***(6f)***. Yield: 73%; Mp: 250-252 °C; IR (KBr cm^{-1}) 3424 (N-H), 3096, (C-H aromatic) 2969 (C-H aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.35 (s, 1H, Ar-H), 7.72 (s, 1H, H-2′), 7.50-7.03 (m, 5H, Ar-H), 5.26 (t, *J* = 6.8 Hz, 1H), 4.56 (d, *J* = 7.2 Hz, 2 H), 1.72 (s, 3H), 1.69 (s, 3H); ^{13}C NMR (DMSO-d6), 100 MHz): δ 148.2, 138.3, 135.2, 132.0, 129.0, 12.8, 127.2, 118.2, 114.5, 105.8, 44.4, 25.6, 18.0; anal. calc. (%) for $C_{20}H_{17}BrClN_3$: C, 57.92; H, 4.13; Br, 19.27; Cl, 8.55; N, 10.13; found: C, 57.78; H, 4.11; N, 10.09.

RESULTS AND DISCUSSION

In-vitro cytotoxic activity

The anti-proliferative/cytotoxic activities of indole-benzimidazole hybrids molecules **5(a-f)** and **6(a-f)** were evaluated against four different types of human cancer cell lines, viz., human ovarian carcinoma cells (SKOV-3), human prostate cancer cells (PC-3), human cervical cancer cells (HeLa) and human acute monocytic leukemia cells (THP-1) using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the method of Mossman [43] and the IC_{50} values determined are summarized in Table 1. The cytotoxic investigations were carried out at IICT, Hyderabad in association with Dr. J.V. Rao, Scientist, Biology Division.

Table 1. *In-vitro* cytotoxicity of target molecules **(5a-f)** and **(6a-f)** against SKOV-3, Hela, PC-3 and THP-1 human cancer cells by MTT assay was expressed in both μg/mL and μM.

Entry	Compounds	Cytotoxicity IC_{50} μg/mL (μM).			
		SKOV-3	PC-3	Hela	THP-1
1	**5a**	23.45±0.91 (100.64)	29.11±1.75 (124.91)	39.21±3.26 (168.28)	37.01±0.31 (158.84)
2	**5b**	17.92±1.02 (72.46)	47.93±1.62 (193.82)	40.40±2.11 (163.37)	26.66±1.94 (107.80
3	**5c**	26.69±2.83 (99.70)	47.19±3.08 (176.27)	41.44±1.89 (154.81)	23.23±3.07 (86.77)
4	**5d**	40.31±2.61 (129.13)	28.85±2.85 (92.42)	33.32±1.32 (106.74)	28.25±2.36 (90.49)
5	**5e**	7.73±1.96 (23.69)	23.83±1.72 (73.05)	21.09±1.65 (64.66)	12.75±0.39 (39.08)
6	**5f**	29.86±1.52 (86.14)	27.67±3.89 (79.86)	32.46±2.67 (93.64)	51.01±0.24 (147.16)
7	**6a**	N.A (N.A)	40.29±1.07 (133.68)	N.A (N.A)	38.94±0.52 (129.20)
8	**6b**	N.A (N.A)	39.01±0.25 (123.65)	89.34±2.18 (283.19)	42.59±1.56 (135.00)
9	**6c**	93.74±3.58 (279.12)	78.28±1.54 (233.09)	N.A (N.A)	75.03±1.93 (223.41)
10	**6d**	56.02±1.59 (147.31)	62.57±0.53 (164.53)	N.A (N.A)	92.31±0.26 (242.74)

11	**6e**	N.A (N.A)	73.63±0.21 (186.73)	N.A (N.A)	84.27±0.13 (213.71)
12	**6f**	60.49±1.62 (145.85)	91.22±1.21 (219.95)	N.A (N.A)	67.43±2.64 (162.58)
13	**5FU***	0.49±0.06 (3.82)	1.46±0.14 (11.25)	1.28±0.07 (9.84)	0.54±0.08 (4.15)

*5-Fluorouracil (standard drug molecule) was employed as positive control.

Exponentially growing cells were treated with different concentrations of **5(a-f)** and **6(a-f)** compounds for 24 h and cell growth inhibition was analyzed through MTT assay.

IC_{50} is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor and were calculated using the respective regression analysis. The values represent the mean ± SE of three individual observations.

From the results obtained (Table 1), it is clear that majority of the indole-benzimidazole hybrids **(5a-f)** and **(6a-f)** reduce the cancer cell viability significantly with IC_{50} values ranging from 25 μM to 150 μM. MTT assay reveals that compounds **5b**, **5c**, **5d**, **5e** and **5f** are active with an IC_{50} values less than 100 μM. Compound **5e** was the lead against all the tested cell lines with an IC_{50} values ranging between 23.69-73.05 μM. The IC_{50} values of **5e** against SKOV-3, PC-3, HeLa and THP-1 were 23.69, 73.05, 64.66 and 39.08 μM, respectively. Compound **6c** was found to be weak against SKOV-3 (279.12 μM) and PC-3 (233.09 μM). Concerning Hela, compound **6b** (283.19 μM) and for THP-1 cell lines compound **6d** (242.74 μM) were the weak candidates. 25% (3 out of 12 compounds) and 41% (5 out of 12 compounds) were inactive against SKOV-3 and Hela, respectively. From the results of Table 1 the series of 2-(5-substituted-1*H*-indol-3-yl)-5-substituted-1*H*- benzo[d]imidazole **5(a-f)** were found to be more active than the series 2-(5-substituted-1-(3-methylbut-2-enyl)-1H-indol-3-yl)-5-substituted-1H-benzo[d]imidazole **6(a-f)**.

Cell lines and cell culture

The human ovarian carcinoma cells (SKOV-3), human prostate cancer cell (PC-3), human cervical cancer cells (HeLa) and human acute monocytic leukemia cells (THP-1) were obtained from the National Centre for Cellular Sciences (NCCS), Pune, India. The cells were cultured in their respective media, *i.e.*, SKOV-3 cells in Dulbecco's Modified Eagle Medium (DMEM); HeLa cells in Eagle's Minimum Essential Medium (EMEM) and remaining two cell lines PC-3 and THP-1 in Roswell Park Memorial Institute media (RPMI-1640), supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1 mM $NaHCO_3$, 2 mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin. The cells were cultured in a 5% CO_2 incubator, kept at 37 °C in a humidified atmosphere.

Test concentrations

Initially, stock solutions of each test substances were prepared in 100% dimethyl sulfoxide (DMSO, Sigma Chemical Co., St. Louis, MO) with a final concentration of 8 mg/mL. Exactly 25 μL of stock was diluted to 1 mL in culture medium to obtain experimental stock concentration of 200 μg/mL. This solution was further serially diluted with media and exactly 100 μL of each diluent was added to 100 μL of cell suspension (total assay volume of 200 μL) to obtain the concentrations of 10 to 100 μg/mL, and incubated in 5% CO_2, at 37 °C for 24 h. Bioassays were repeated three times and their mean values were used to determine the IC_{50} values using the respective regression analysis.

Cytotoxicity

Cytotoxicity was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, according to the method of Mossman [45]. Briefly, the cells (2 x 104) were seeded in each well containing 0.1 mL of medium in 96 well plates. After overnight incubation at 37 °C in 5% CO_2, the cells were treated with 100 µL of different test dilutions with five replicates each. The test concentrations were maintained ranging from10 to100 µg/mL, which is equivalent 10 to 100 ppm. The cell viability was assessed after 24 h, by adding 10 µL of MTT (5 mg/mL) per well. The plates were incubated at 37 °C for additional three hours. The medium was discarded and the formazan blue, which formed in the cells, was dissolved with 100 µL of DMSO. The rate of color formation was measured at 570 nm in a spectrophotometer (Spectra MAX Plus; Molecular Devices; supported by SOFTmax PRO-5.4). The percent inhibition of cell viability was determined with reference to the control values (without test compound). The data were subjected to linear regression analysis and the regression lines were plotted for the best straight-line fit. The IC_{50} (inhibition of cell viability) concentrations were calculated using the respective regression equation.

Structure activity relationship studies (SARS)

The SAR study reveals that, hydrophobic substituent (Br) and electron releasing group (methyl) at position 5 of indole and benzimidazole rings respectively enhances the cytotoxic activity. Brominated analogues were active with respect to indole motif and the order of activity with respect to substituents on 5^{th} position of benzimidazole was methyl > chloro > hydrogen.

CONCLUSION

In conclusion, we have successfully synthesized 12 indole-benzimidazole hybrids molecules. These hybrids were screened for their *in-vitro* anticancer activity. Among the indole-benzimidazole hybrids evaluated for cytotoxic activity, **5e** exhibited better inhibition concentration against all the screened cell lines. Compounds without prenyl group exhibited better activity than prenylated compounds.

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