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Potential Anticancer Agents: Design, Synthesis of New Pyrido[1,2-a] benzimidazoles and Related Derivatives Linked to Alkylating Fragments

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Abstract

The incentive of the present work has been primarily directed towards the design and synthesis of some novel pyrido[1,2-a]benzimidazoles with specific functionalities believed to have alkylation ability. This combination of pharmacological agents may enable synergistic anticancer effect. Nine compounds **5b, 13a, 13d, 13e, 14b, 14c, 15, 16,** and **17** were selected by the National Cancer Institute (NCI), Bethseda, Maryland, USA to be evaluated for their *in vitro* antitumor activity. All the selected compounds were tested initially at a single dose (10 μM) in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. Majority of the test compounds exhibited moderate cytotoxic activity. The highest activity in all the investigated cancer cells was displayed by **14c** against melanoma SK-MEL-5 cell line. This may be due to the impact of the lipophilic trifluoromethyl substitution on the biological activity profile.

Keywords: Design; Synthesis; Substituted pyridine; 2-pyridone; Antitumor activity

Introduction

Cancer is a devastating affliction, the frequency of which is progressively increasing all over the world. Its occurrence is escalating rapidly and is a major cause in health complications [1]. The treatment approach dictates that the treatment of cancer is directed toward eradication of all cancer cells and this is attained by frenziedly discovery of new candidates of anticancer activity [2].

Previously, we have utilized pyrido[1,2-a]benzimidazole (PBI) as a privileged scaffold for the design of many PBI derivatives of potential cytotoxic activity [3-8]. In fact, this ring system is characterized by the presence of pyridine or 2-pyridone units which constitute a subject of great interest due to their extensive presence in the skeletal backbone of many biologically active compounds. They possess a wide variety of biological activities such as antiulcer [9], antidiabetic [9], anti-inflammatory [10], anticoagulant [11], antiviral [12], antibacterial [13], antifungal [14] and anticancer activities [15]. Pyridine moiety is one of the building units of some tyrosine-kinase inhibitors; imatinib is used in the treatment of multiple cancers; whereas, sorafenib is used in the treatment of advanced renal and hepatocellular carcinoma [16]. The 2-pyridone unit is an integral part of some cytotoxic agents such as roquinimex which investigated as adjuvant therapy after bone marrow transplantation in chronic myelogenous leukemia [17] and diazaquinomycin A which demonstrates *in vitro* cytotoxicity against some tumor cell lines [18].

Among the investigated PBI series,NSC649900 [3], NSC682011 [4] and NSC699944 [5] (Figure 1) were identified by the NCI as promising candidates for further testing in an *in vivo* anticancer hollow fiber assay because of their good cytotoxic activity and subpanel disease selectivity especially against leukemic cell in the *in vitro* screen. In fact, the PBI backbone of these compounds demonstrates structural complementarity with the isosteric β-carboline and pyrido[2,3-a] indolizine (PI) which constitute the key scaffolds of many cytotoxic agents such as the β-carboline alkaloid harmine which is identified as a useful inhibitor of tumor development [19] and the antitumor antibiotic camptothecin [20].

Biochemical data suggests that camptothecin act as DNA topoisomerase I inhibitor. It possesses a novel mechanism of action involving the inhibition of DNA relaxation by DNA topoisomerase I, and more specifically the stabilization of a covalent binary complex formed between topoisomerase I and DNA [20]. In addition, it is proposed that the planar nature of camptothecin allows its intercalation between DNA base pairs at the site of single-strand cleavage [21]. For this reason, it may be worthy to study the possible interactions of NSC649900, NSC682011 and NSC699944 with topisomerase-I as a target enzyme because of the evident structural complementarity between PBI scaffold of these agents and pyrido[2,3-a]indolizine (PI) backbone of camptothecin (Figure 2). Docking results revealed that NSC649900, NSC682011 and NSC699944 displayed arene-arene interactions with one or more amino acid residues similar to camptothecin.NSC649900 showed arene-arene interactions with DAC113 and TGPB11 residues, in addition to hydrogen bonding with ArgD364; whereas, NSC682011 displayed arene-arene interactions with TGPB11and ArgD364

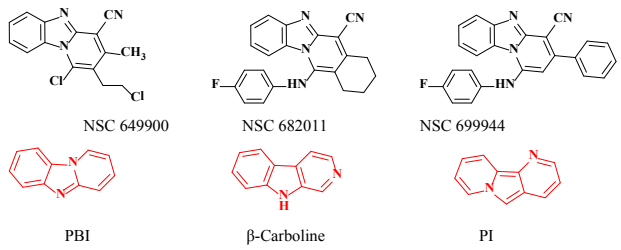

Figure 1: Structural complementarity between PBI, β-carboline and PI.

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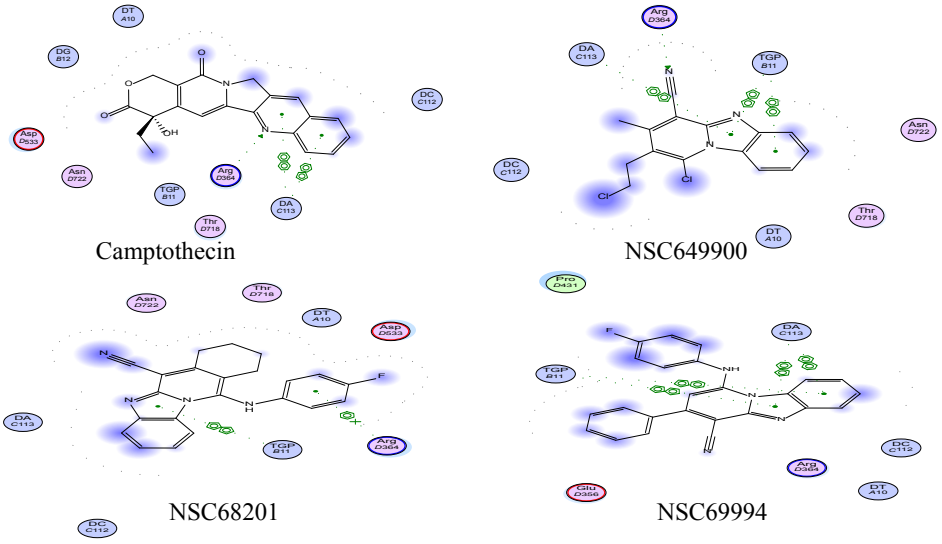


Figure 2: Binding mode of Camptothecin, NSC649900, NSC682011 and NSC699944 into the active sites of topo I-DNA complex (Crystal structure; PDB ID: 1T8I, was obtained from the Protein Data Bank). Docking studies was performed using (MOE 2008.10).

amino acid residues. In addition, NSC699944 revealed arene-arene interactions with TGPB11 and DAC113 amino acid residues. The result indicates that the planar PBI scaffold contribute to binding to the main active sites similar to camptothecin and it is possible that these PBIs may intercalate between DNA base pairs of topo I- DNA complex through arene-arene interactions (Figure 2). Inspired by these findings and in a continuation of our efforts to discover and explore new heterocyclic compounds of promising anticancer activities two series of PBIs comprising pyridine and 2-pyridone nuclei (Figure 3) were designed to be synthesized and evaluated for their *in vitro* anticancer activity. Both series are designed with specific functionalities such as 2-hydoxyalkyl, 2-chloroalkyl, piperidino- and morpholino alkyl moieties at postion-2 through an aminomethylene spacer (Figure 3, Scheme 1) or at position-1(Figure 3, Scheme 2). Other analogues comprising aliphatic amino and aryl amino moieties are proposed.

Alkylating fragments such as 2-chloroethylamino and N,N-bis(2-choroethyl)amino and selected substituents such as 2-hydoxyethylamino and morpholinoalkyl are incorporated in the PBI scaffold of the proposed compounds because of their importance in the backbone structure of some antineoplastic drugs; namely, Mitoxantrone [22], Lomustine [23], Bendamustine [24] and Gefitinib [25] .

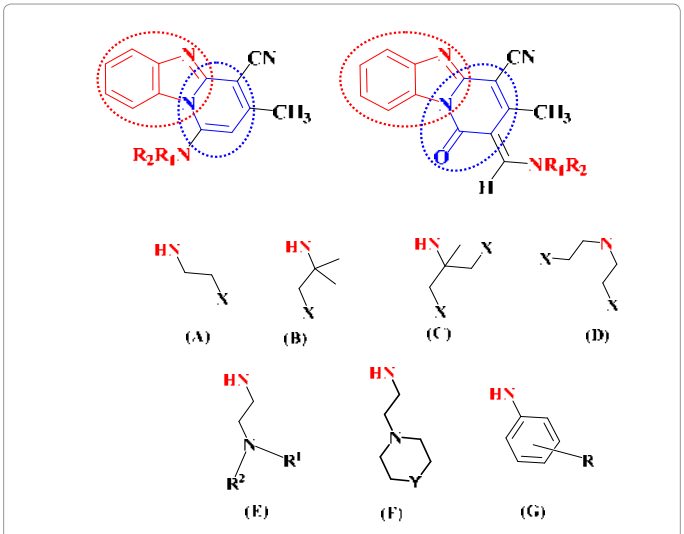


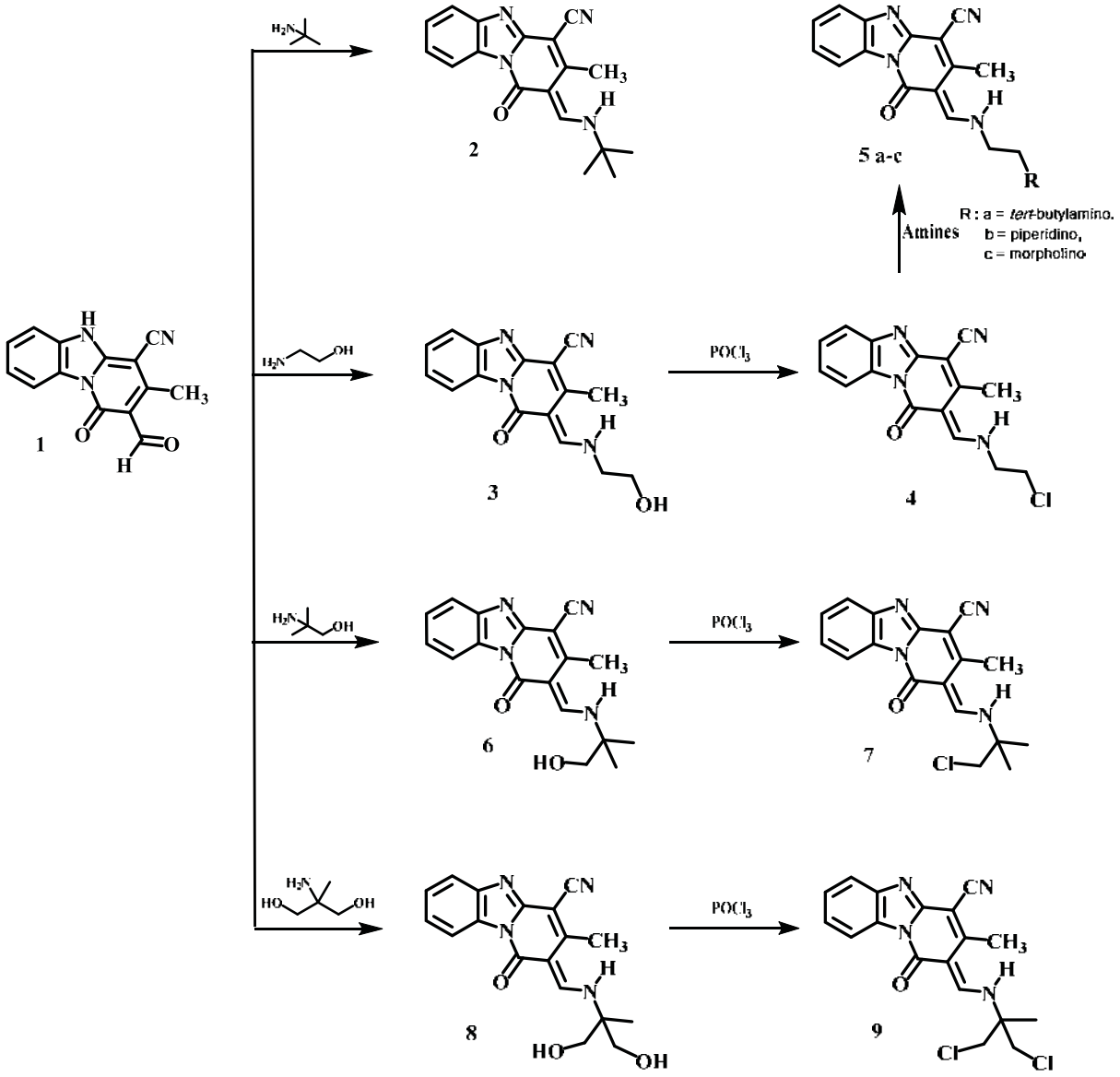
Figure 3: A display of the proposed PBIs and the adopted alkylating fragments and other selected substituents.

Experimental

Chemistry

All reagents and solvents were purchased from commercial suppliers and were purified and dried when necessary by standard techniques. Melting points were determined in open glass capillaries using Stuart capillary melting point apparatus (Stuart Scientific Stone, Staffordshire, UK) and are uncorrected. IR spectra were recorded, for potassium bromide discs, ύ (cm^{-1}), on Perkin Elmer 1430 spectrophotometer. ^{1}H-NMR spectra were determined either on a Bruker Avance spectrometer (400 MHz) at the microanalytical unit, Faculty of Science, Cairo University, or on Jeol (125 MHz) at the microanalytical unit, Faculty of Science, Alexandria University, using DMSO-d_6 as a solvent and TMS as internal standard. The chemical shifts are given in δ ppm values (s, singlet; d, doublet; t, triplet and m, multiplet). ^{13}C-NMR spectra were determined on Jeol (125 MHz), Faculty of Science, Alexandria University, using TMS as internal standard. Mass spectra were run on a Finnigan mass spectrometer model SSQ/7000 (70 eV), Faculty of Science, Cairo University. Microanalyses were performed at the microanalytical unit, Faculty of Science, Cairo University. The results of the microanalyses were within ± 0.4% of the calculated values. Follow-up of the reactions and checking the homogeneity of the compounds were made by ascending TLC run on silica gel G (Merck 60) coated glass plates. The spots were visualized by exposure to iodine vapor or UV lamp at λ 254 nm for few seconds.

Pyridobenzimidazole-4-carbonitrile was prepared according to a reported procedure in a good yield through cyclocondensation of 1H-benzimidazol-2-yl-acetonitrile with ethyl acetoacetate in presence of ammonium acetate [7]. Formylation of pyridobenzimidazole-4-

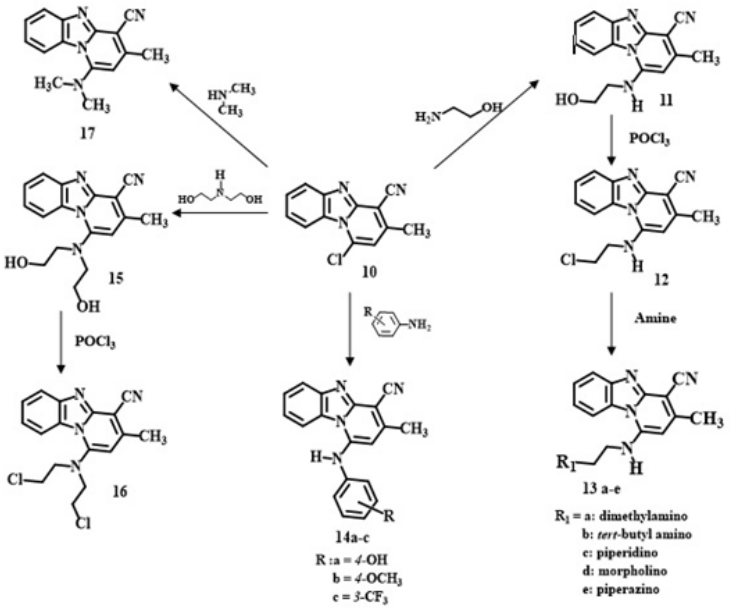


Scheme 1: Synthesis of pyridobenzimidazole derivatives 2-9.

carbonitrile was achieved by applying Vilsmeier Haack reaction by the addition of phosphorous oxychloride to a well stirred suspension of pyridobenzimidazole-4-carbonitrile to afford the aldehyde **(1)** [7], while 1-Chloro-3-methylpyridobenzimidazole-4-carbonitrile (**10**) was prepared by refluxing pyridobenzimidazole-4-carbonitrile in excess phosphorous oxychloride according to a reported procedure [7].

2-(*tert*-Butylamino)methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (2): A mixture of 2-formyl-3-methyl-1-oxo-1,5-dihydropyridobenzimidazole-4-carbonitrile **(1)** (2 mmol, 0.50 g) and 2-methylpropan-2-amine (3 mmol, 0.22 g) in dimethylformamide (10 ml) was stirred at room temperature for 20 h**.** The reaction mixture was then diluted with ice cold water. The obtained product was filtered, washed with water, dried and crystallized from dimethylformamide/ethanol. Yield 69.63%, M.P.>300°C; IR (KBr, cm^{-1}): 3231 (NH), 3107, 3055, 3024, 2968, 2930, 2873 (C-H), 2218 (C≡N), 1655 (C=O), 1614 (C=N), 1561 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.50 (s, 9H, 3 CH_3), 2.67 (s, 3H, CH_3 at C_3), 7.34 (t, J=7.64 Hz, 1H, pyridobenzimidazole C_7-H), 7.44 (t, J=7.64 Hz, 1H, pyridobenzimidazole C_8-H), 7.74 (d, J=7.98 Hz, 1H, pyridobenzimidazole C_6-H), 8.35 (d, J=14.65 Hz, 1H, methine H), 8.41 (d, J=7.97 Hz, 1H, pyridobenzimidazole C_9-H), 11.60 (d, J=14.23 Hz 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{18}H_{18}N_4O$ (306.37): C, 70.57; H, 5.92; N, 18.29. Found: C, 70.74; H, 5.97; N, 18.47.

2-[(2-Hydroxyethyl)amino]methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (3): A mixture of 2-formyl-3-methyl-1-oxo-1,5-dihydropyridobenzimidazole-4-carbonitrile **(1)** (2 mmol, 0.50 g) and 2-aminoethan-1-ol (3 mmol, 0.18 g) in dimethylformamide (10 ml) was stirred at 60-80°C for 4-6 h .The reaction mixture was then diluted with ice cold water. The obtained product was filtered, washed with water, dried and crystallized from



Scheme 2: Synthesis of pyridobenzimidazole derivatives 11-17.

dimethylformamide/ethanol. Yield 50.97%, M.P. 282°C; IR (KBr, cm^{-1}): 3463 (OH), 3250 (NH), 2227 (C≡N), 1649 (C=O), 1615 (C=N), 1562, 1448 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.59 (s, 3H, CH_3), 3.69 (t, *J*=7.72 Hz, 4H, 2 CH_2), 5.07 (t, J=4.53 Hz, 1H, OH, D_2O exchangeable), 7.33 (t, J=7.64 Hz, 1H, pyridobenzimidazole C_7-H), 7.44 (t, J=7.64 Hz, 1H, pyridobenzimidazole C_8-H), 7.73 (d, J=8.02 Hz, 1H, pyridobenzimidazole C_6-H), 8.40 (d, J=8.02 Hz, 1H, pyridobenzimidazole C_9-H), 8.46 (d, J=14.59 Hz, 1H, methine H), 11.16-11.33 (m, 1H, NH , D_2O exchangeable). ^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 17.98 (CH_3), 53.12 (NCH_2), 60.14 (OCH_2), 87.10 (pyridobenzimidazole C_4), 98.38 (CN), 115.46 (pyridobenzimidazole C_9), 116.83 (pyridobenzimidazole C_2), 119.03, 122.91, 125.63 (pyridobenzimidazole $C_{6,7,8}$), 130.81 (pyridobenzimidazole C_{9a}), 144.22 (pyridobenzimidazole C_{5a}), 148.72 (pyridobenzimidazole C_3), 153.84(pyridobenzimidazole C_{4a}), 161.56 (methine CH), 161.63 (pyridobenzimidazole C_1). Anal. Calcd. for $C_{16}H_{14}N_4O_2$ (294.31): C, 65.30; H, 4.79; N, 19.04 . Found: C, 65.43; H, 4.85; N, 19.18.

2-[(2-Chloroethyl)amino]methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (4): A suspension **3** (2 mmol, 0.59 g) in phosphorous oxychloride (6 ml) was heated under reflux for 3 h while stirring. The reaction mixture was allowed to cool to room temperature and then poured onto crushed ice. The mixture was neutralized with Na_2CO_3 and the obtained product was filtered, washed with water, dried and crystallized from DMF/ethanol. Yield 89.53%, M.P.>300°C; IR (KBr, cm^{-1}): 3231 (NH), 3091, 3018, 2962, 2935, 2870 (C-H), 2214 (C≡N), 1655 (C=O), 1610 (C=N), 1556 (C=C), 770 (C-Cl). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.59 (s, 3H, CH_3), 3.91-4.06 (m, 4H, 2 CH_2), 7.34 (t, J=7.56 Hz, 1H, pyridobenzimidazole C_7-H), 7.44 (t, J=7.56 Hz, 1H, pyridobenzimidazole C_8-H), 7.73 (d, J=7.85 Hz, 1H, pyridobenzimidazole C_6-H), 8.38 (d, J=7.83 Hz, 1H, pyridobenzimidazole C_9-H), 8.52 (d, J=14.32 Hz, 1H, methine H), 11.11-11.35 (m, 1H, NH, D_2O exchangeable).^{13}C-NMR (125 MHz, DMSO-d_6, δ ppm): 18.01 (CH_3), 44.15 (NCH_2), 52.02 (CH_2Cl), 87.75 (pyridobenzimidazole C_4), 98.65 (CN), 115.46(pyridobenzimidazole C_9), 116.74 (pyridobenzimidazole C_2), 118.93(pyridobenzimidazole C_6), 122.93, 125.48 (pyridobenzimidazole $C_{7,8}$), 130.79(pyridobenzimidazole C_{9a}), 144.18 (pyridobenzimidazole C_{5a}), 148.51(pyridobenzimidazole C_{4a}), 153.78 (pyridobenzimidazole C_3), 161.53(pyridobenzimidazole C_1), 161.76 (methine CH). Anal. Calcd. for $C_{16}H_{13}ClN_4O$ (312.76): C, 61.44; H, 4.19; N, 17.91. Found: C, 61.59; H, 4.24; N, 18.07.

3-Methyl-1-oxo-2-{[2-(substituted)ethyl]amino}methylene-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (5a-c): A suspension of 2-[(2-chloroethyl)amino]methylene-3-methyl-1-oxo-1,2-dihydropyridobenzimidazole-4-carbonitrile **(4)** (2 mmol, 0.63 g) and the corresponding amine (6 mmol) in a mixture of absolute ethanol (15 ml) and dimethylformamide (6 ml) was stirred at room temperature for 10 h in case of compound **(5a)** and was refluxed for 10 h in case of both compounds **(5b, c).** Crushed ice was added to the reaction mixture and the obtained product was filtered, washed with water, dried and crystallized from the proper solvent.

2-{[2-(*tert*-Butylamino)ethyl]amino}methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a] benzimidazole-4-carbonitrile (5a): Yield 51.43%, M.P.>300°C; crystallization solvent: dioxane/ethanol; IR (KBr, cm^{-1}): 3419 (NH), 3058, 2969, 2935 (C-H), 2218 (C≡N), 1655 (C=O), 1615 (C=N), 1560 (C=C).^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.50 (s, 9H, 3 CH_3), 2.50 (under DMSO, 4H, 2 NCH_2), 2.68 (s, 3H, CH_3 at C_3), 7.34 (t, J=7.37 Hz, 1H, pyridobenzimidazole C_7-H), 7.45 (t , J=7.37 Hz, 1H, pyridobenzimidazole C_8-H), 7.74 (d, J=7.73 Hz, 1H, pyridobenzimidazole C_6-H), 8.36 (d, J=14.26 Hz, 1H, methine H), 8.42 (d, J=7.88 Hz, 1H, pyridobenzimidazole C_9-H), 11.61 (m, J=13.82 Hz, 1H, enamine NH, D_2O exchangeable). Anal. Calcd. for $C_{20}H_{23}N_5O$ (349.44): C, 68.74; H, 6.63; N, 20.04. Found: C, 68.98; H, 6.69; N, 20.21.

3-Methyl-1-oxo-2-{[2-(piperidin-1-yl)ethyl]amino}methylene-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (5b): Yield 42.88%, M.P. 175°C;crystallization solvent: Dichloro-methane/ethyl acetate; IR (KBr, cm^{-1}): 3420, 3246 (NH), 3050, 2919, 2848, 2802 (C-H), 2213 (C≡N), 1659 (C=O), 1612 (C=N), 1552 (C=C). ^{1}H-NMR (400

MHz, DMSO-d_6) δ (ppm): 1.40-1.53 (m, 6H, piperidine $C_{3,4,5}$-H_2), 2.44-2.50 (m, 6H, CH_2N and piperidine $C_{2,6}$-H_2), 2.60 (s, 3H, CH_3), 3.71 (m, 2H, CH_2-N), 7.33 (t, J=7.64 Hz, 1H, pyridobenzimidazole C_7-H), 7.43 (t, J=7.64 Hz, 1H, pyridobenzimidazole C_8-H), 7.72 (d, J=7.93 Hz, 1H, pyridobenzimidazole C_6-H), 8.39 (d, J=8.01 Hz, 1H, pyridobenzimidazole C_9-H), 8.46 (d, J=14.32 Hz, 1H, methine H), 11.18 (m, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{21}H_{23}N_5O$ (361.45): C, 69.78; H, 6.41; N, 19.38. Found: C, 69.93; H, 6.52; N, 19.62.

3-Methyl-2-{[2-(morpholin-4-yl)ethyl]amino}methylene-1-oxo-1,2-dihydropyrido[1,2-a] benzimidazole-4-carbonitrile (5c): Yield 39.73%, M.P. 240°C;crystallization solvent: ethanol; IR (KBr, cm^{-1}): 3415, 3227 (NH), 3050, 2920, 2866, 2804 (C-H), 2214 (C≡N), 1665 (C=O), 1612 (C=N), 1559 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.42-2.52 (m, 5H, CH_3 and N-CH_2), 2.54-2.66 (m, 4H, morpholine $C_{3,5}$-H_2), 3.55-3.62 (m, 4H, morpholine $C_{2,6}$-H_2), 3.68-3.78 (m, 2H, CH_2-N), 7.33 (t, J=7.65 Hz, 1H, pyridobenzimidazole C_7-H), 7.43 (t, J=7.65 Hz, 1H, pyridobenzimidazole C_8-H), 7.72 (d, J=8.09 Hz, 1H, pyridobenzimidazole C_6-H), 8.39 (d, J=8.10 Hz, 1H, pyridobenzimidazole C_9-H), 8.47 (d, J=14.42 Hz, 1H, methine H), 11.20 (m, J=13.10 Hz, 1H, NH , D_2O exchangeable). Anal. Calcd. for $C_{20}H_{21}N_5O_2$ (363.42): C, 66.10; H, 5.82; N, 19.27. Found: C, 66.37; H, 5.87; N, 19.43.

2-[(1-Hydroxy-2-methylprop-2-yl)amino]methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (6): A mixtureof2-formyl-3-methyl-1-oxo-1,5-dihydropyridobenzimidazole-4-carbonitrile **(1)** (2 mmol, 0.50 g) and 2-amino-2-methylpropan-1-ol (3 mmol, 0.27 g) in dimethylformamide (10 ml) was stirred at 60-80°C for 4-6 h .The reaction mixture was then diluted with ice cold water. The obtained product was filtered, washed with water, dried and crystallized from dimethylformamide/ethanol. Yield 83.75%, M.P.>300°C; IR (KBr, cm^{-1}): 3456 (OH), 3280 (NH), 3055, 2931, 2860 (C-H), 2218 (C≡N), 1656 (C=O), 1615 (C=N), 1561, 1446 (C=C).^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.42 (s, 6H, 2 CH_3), 2.64 (s, 3H, CH_3 at C_3), 3.50 (d, J=5.16 Hz, 2H, CH_2-O), 5.53 (t, J=5.26 Hz, 1H, OH , D_2O exchangeable), 7.33 (t, J=7.67 Hz, 1H, pyridobenzimidazole C_7-H), 7.4 (t, J=7.67 Hz, 1H, pyridobenzimidazole C_8-H), 7.72 (d, J=8.03 Hz, 1H, pyridobenzimidazole C_6-H), 8.31 (d, J=14.71 Hz, 1H, methine H), 8.39 (d, J=7.48 Hz, 1H, pyridobenzimidazole C_9-H), 11.68 (d, J=14.20 Hz, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{18}H_{18}N_4O_2$ (322.37): C, 67.07; H, 5.63; N, 17.38. Found: C, 67.21; H, 5.72; N, 17.52.

2-[(1-Chloro-2-methylprop-2-yl)amino]methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a] benzimidazole-4-carbonitrile (7): A suspension **6** (2 mmol, 0.64 g) in phosphorous oxychloride (6 ml) was heated under reflux for 3 h while stirring. The reaction mixture was allowed to cool to room temperature and then poured onto crushed ice. The mixture was neutralized with Na_2CO_3 and the obtained product was filtered, washed with water, dried and crystallized from dioxane/ethanol. Yield 58.68%, M.P. 276°C; IR (KBr, cm^{-1}): 3231 (NH), 3054, 3022, 2977, 2929 (C-H), 2220 (C≡N), 1655 (C=O), 1611 (C=N), 1561, 1436 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.57 (s, 6H, 2 CH_3), 2.66 (s, 3H, CH_3 at C_3), 4.02 (s, 2H, CH_2-Cl), 7.34 (t, J=7.74 Hz, 1H pyridobenzimidazole C_7-H), 7.45 (t, J=7.74 Hz, 1H, pyridobenzimidazole C_8-H), 7.74 (d, J=7.81 Hz, 1H, pyridobenzimidazole C_6-H), 8.32 (d, J=14.29 Hz, 1H, methine H), 8.40 (d, J=7.94 Hz, 1H, pyridobenzimidazole C_9-H), 11.60 (d, J=14.43 Hz, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{18}H_{17}ClN_4O$ (340.81): C, 63.44; H, 5.03; N, 16.44 . Found: C, 63.59; H, 5.11; N, 16.72.

2-[(1,3-Dihydroxy-2-methylprop-2-yl)amino]methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (8): A mixture of 2-formyl-3-methyl-1-oxo-1,5-dihydropyridobenzimidazole-4-carbonitrile **(1)** (2 mmol, 0.50 g) and 2-amino-2-methylpropane-1,3-diol (3 mmol, 0.315 g) in dimethylformamide (10 ml) was stirred at 60-80°C for 4-6 h The reaction mixture was then diluted with ice cold water. The obtained product was filtered, washed with water, dried and crystallized from dimethylformamide/ethanol. Yield 63.54%, M.P.>300°C; IR (KBr, cm^{-1}): 3472, 3361 (OH), 3160 (NH), 2942, 2865 (C-H), 2212 (C≡N), 1661 (C=O), 1610 (C=N), 1556, 1446 (C=C).^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.36 (s, 3H, CH_3), 2.63 (s, 3H, CH_3 at C_3), 3.51 , 3.55 (2d, J_1=5.44 Hz, J_2=5.21 Hz, each 1H, CH_2), 3.62 , 3.66 (2d, J_1=4.91 Hz, J_2=5.16 Hz, each 1H, CH_2), 5.41 (t, J=5.08 Hz, 2H, 2 OH, D_2O exchangeable), 7.33 (t, J=7.67 Hz, 1H, pyridobenzimidazole C_7-H), 7.44 (t, J=7.67 Hz, 1H, pyridobenzimidazole C_8- H), 7.73 (d, J=8.04 Hz, 1H, pyridobenzimidazole C_6-H), 8.32 (d, J=14.77 Hz, 1H, methine H), 8.40 (d, J=8.48 Hz, 1H, pyridobenzimidazole C_9-H), 11.70 (d, J=14.64 Hz, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{18}H_{18}N_4O_3$ (338.37): C, 63.89; H, 5.36; N, 16.56. Found: C, 64.06; H, 5.40; N, 16.73.

2-[(1,3-Dichloro-2-methylprop-2-yl)amino]methylene-3-methyl-1-oxo-1,2-dihydropyrido[1,2-a]benzimidazole-4-carbonitrile (9): A suspension **8** (2mmol, 0.68 g) in phosphorous oxychloride (6 ml) was heated under reflux for 3 h while stirring. The reaction mixture was allowed to cool to room temperature and then poured onto crushed ice. The mixture was neutralized with Na_2CO_3 and the obtained product was filtered, washed with water, dried and crystallized from absolute ethanol. Yield 15.99%, M.P.>300°C; IR (KBr, cm^{-1}): 3365 (NH), 3232, 2965 (C-H), 2218 (C≡N), 1653 (C=O), 1608 (C=N), 1559, 1444 (C=C).^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.66 (s, 3H, CH_3), 2.65 (s, 3H, CH_3 at C_3), 4.10, 4.15 (2s, each 2H, 2 CH_2-Cl), 7.34 (t, J=7.67 Hz, 1H, pyridobenzimidazole C_7-H), 7.43 (t, J=7.67 Hz, 1H,pyridobenzimidazole C_8-H), 7.73 (d, J=8.04 Hz, 1H, pyridobenzimidazole C_6-H), 8.35 (d, J=14.18 Hz, 1H, methine H), 8.40 (d, J=8.48 Hz, 1H, pyridobenzimidazole C_9-H), 11.63 (d, J=12.96 Hz, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{18}H_{16}Cl_2N_4O$ (375.25): C, 57.61; H, 4.30; N, 14.93. Found: C, 57.84; H, 4.36; N, 15.18.

1-(2-Hydroxyethyl)amino-3-methylpyrido[1,2-a] benzimidazole-4-carbonitrile (11): A mixture of the chloro derivative **(10)** (4 mmol, 0.97 g) and ethanolamine, (12 mmoles, 0.73 g) in dioxane (20 ml) was heated at 60-80°C for 10 h. The reaction mixture was then poured into ice cold water. The product was filtered, washed with water, dried and crystallized from DMF/ ethanol. Yield 93.46%, M.P. 286°C; IR (KBr, cm^{-1}): 3423 (OH), 3256 (NH), 2923, 2872(C-H), 2212 (C≡N), 1630 (C=N), 1590, 1563 (C=C), 1084 (CH_2-OH). 1H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.51 (s, 3H, CH_3), 3.55 (q, 2H, J=5.74 Hz, N-CH_2), 3.76 (q, 2H, J=5.73 Hz, O-CH_2), 5.01 (t, J=5.57, 1H, OH, D2O exchangeable), 6.18 (s, 1H, pyridobenzimidazole C_2-H), 7.24 (t, J=5.43 Hz, 1H, NH, D_2O exchangeable), 7.31 (t, J=7.73 Hz, 1H, pyridobenzimidazole C_7-H), 7.51 (t, J=7.73 Hz, 1H, pyridobenzimidazole C_8-H), 7.77 (d, J=8.14 Hz, 1H, pyridobenzimidazole C_6-H), 8.37 (d, J=8.37 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. for $C_{15}H_{14}N_4O$ (266.30): C, 67.65; H, 5.30; N, 21.04. Found: C, 67.89; H, 5.39; N, 21.25.

1-(2-Chloroethyl)amino-3-methylpyrido[1,2-a]benzimidazole-4-carbonitrile (12): A suspension of 1-(2-hydroxyethyl)amino-3-methylpyrido[1,2-a]benzimidazole-4-carbonitrile **(11)** (5 mmol, 1.3 g) in phosphorous oxychloride (5 ml) was heated under reflux while stirring for 3 h. The reaction mixture was allowed to cool to room temperature, then poured onto crushed ice and neutralized with Na_2CO_3. The product was filtered, washed with water and crystallized from dioxane. Yield 17.56%, M.P. 244°C; IR (KBr, cm^{-1}): 3413 (NH), 3104, 3069, 3023, 2966, 2921 (C-H), 2211 (C≡N), 1630 (C=N), 1600,

1552, 1489, 1462 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.54 (s, 3H, CH_3), 3.85 (q, J=5.67 Hz, 2H, N-CH_2), 3.98 (t, J=6.14 Hz, 2H, CH_2-Cl), 6.30 (s, 1H, pyridobenzimidazole C_2-H), 7.35 (t, J=7.81 Hz, 1H, pyridobenzimidazole C_7-H), 7.49-7.58 (m, 2H, pyridobenzimidazole C_8-H and NH, D_2O exchangeable), 7.79 (d, J=8.16 Hz, 1H, pyridobenzimidazole C_6-H), 8.42 (d, J=8.42 Hz, 1H, pyridobenzimidazole C_9-H). ^{13}C-NMR (125MHz, DMSO-d_6, δ ppm): 21.11 (CH_3), 42.95 (NCH_2), 44.84 (CH_2Cl), 85.28 (pyridobenzimidazole C_4), 91.84 (pyridobenzimidazole C_9), 115.18 (pyridobenzimidazole C_2), 119.06 (CN), 118.62, 120.47, 126.11 (pyridobenzimidazole $C_{6,7,8}$), 128.25 (pyridobenzimidazole C_{9a}), 145.36 (pyridobenzimidazole C_{4a}), 148.52 (pyridobenzimidazole C_{5a}), 149.19 (pyridobenzimidazole C3), 151.60 (pyridobenzimidazole C1). Anal. Calcd. for $C_{15}H_{13}ClN_4$ (284.75): C, 63.27; H, 4.60; N, 19.68. Found: C, 63.49; H, 4.66; N, 19.85.

3-Methyl-1-[2-(substituted)ethyl]aminopyrido[1,2-a] benzimidazole-4-carbonitrile (13a-e): A mixture of 1-(2-chloroethyl) amino-3-methylpyridobenzimidazole-4-carbonitrile **(12)** (2 mmol, 0.57 g) and dimethylamine, *tert*-butylamine, piperidine, morpholine or piperazine (6 mmol) respectively, in 10:8 mixture of dioxane/ dimethylformamide (18 ml) was stirred at room temperature for 24 hrs in case of compounds **(13a,b)** or at 60-80°C for 12 hrs in case of compounds **(13c-e).** Crushed ice was added to the reaction mixture and the separated product was filtered, washed with water and crystallized from the proper solvent.

1-[2-(Dimethylamino)ethyl]amino-3-methylpyrido[1,2-a] benzimidazole-4-carbonitrile (13a): Yield 37.29%, M.P.>258°C; crystallization solvent: DMF/ethanol. IR (KBr, cm^{-1}): 3382 (NH), 3036, 3010, 2978, 2950, 2918, 2861, 2816 (C-H), 2205 (C≡N), 1632 (C=N), 1598, 1559, 1462, 1431 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.30 (s, 6H, 2 CH_3), 2.53 (s, 3H, CH_3 at C_3), 2.69 (t, J=6.29 Hz, 2H, N-CH_2), 3.52 (t, J=6.25 Hz, CH_2-NH), 6.15 (s, 1H, pyridobenzimidazole C_2-H), 7.28 (s, 1H, NH, D_2O exchangeable), 7.34 (t, J=7.80 Hz, 1H, pyridobenzimidazole C_7-H), 7.53 (t, J=7.80 Hz, 1H, pyridobenzimidazole C_8-H), 7.79 (d, J=8.32 Hz, 1H, pyridobenzimidazole C_6-H), 8.23 (d, J=8.37 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. for $C_{17}H_{19}N_5$(293.37): C, 69.60; H, 6.53; N, 23.87. Found: C, 69.87; H, 6.59; N, 24.12.

1-[2-(*tert*-Butylamino)ethyl]amino-3-methylpyrido[1,2-a] benzimidazole-4-carbonitrile (13b): Yield 78.13%, M.P.>286°C; crystallization solvent: ethanol. IR (KBr, cm^{-1}): 3536 (NH), 3302, 3113, 2966 (C-H), 2210 (C≡N), 1631 (C=N), 1595 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.21 (s, 9H, 3 CH_3), 2.54 (s, 3H, CH_3 at C_3), 2.90-3.11 (m, 2H, N-CH_2), 3.49-3.73 (m, 2H, CH_2-NH), 6.25 (s, 1H, pyridobenzimidazole C_2-H), 7.32 (t, J=7.47 Hz, 1H, pyridobenzimidazole C_7-H), 7.53 (t, J=7.47 Hz, 1H, pyridobenzimidazole C_8-H), 7.79 (d, J=8.05 Hz, 1H, pyridobenzimidazole C_6-H), 8.45 (d, J=8.18 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. for $C_{19}H_{23}N_5$ (321.43): C, 71.00; H, 7.21; N, 21.79. Found: C, 71.24; H, 7.28; N, 21.88.

3-Methyl-1-[2-(piperidin-1-yl)ethyl]aminopyrido[1,2-a] benzimidazole-4-carbonitrile (13c): Yield 89.55%, M.P.>264°C; crystallization solvent: dioxane/ ethanol. IR (KBr, cm^{-1}): 3360 (NH), 3041, 3012, 2975, 2931, 2853, 2829 (C-H), 2205 (C≡N), 1633 (C=N), 1598, 1549, 1463 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 1.39-1.51 (m, 2H, piperidine C_4-H_2), 1.51-1.64 (m, 4H, piperidine $C_{3,5}$-H_2), 2.50 (under DMSO, 7H, CH_3 and piperidine $C_{2,6}$-H_2), 2.72 (t, J=6.31 Hz, 2H, ethyl N-CH_2), 3.51 (t, J=6.07 Hz, 2H, NCH_2), 6.10 (s, 1H, pyridobenzimidazole C_2-H), 7.28-7.39 (m, 2H, pyridobenzimidazole C_7-H and NH, D_2O exchangeable), 7.53 (t, 1H, J=7.69 Hz, pyridobenzimidazole C_8-H), 7.8 (d, J=8.00 Hz, 1H, pyridobenzimidazole C_6-H), 8.26 (d, J=8.25 Hz, 1H,pyridobenzimidazole C_9-H).EI-Mass spectrum m/z (relative abundance%) 334.25 (0.91); 333.25 (3.25); 235.14 (1.64); 206.10 (1.03); 194.09 (0.87); 102.06 (0.79); 99.16 (6.71); 98.11 (100.00); 96.10 (1.72); 83.12 (0.85); 70.09(3.82); 55.07(2.03). Anal. Calcd. for $C_{20}H_{23}N_5$ (333.44): C, 72.04; H, 6.95; N, 21.00. Found: C, 72.18; H, 7.02; N, 21.17.

3-Methyl-1-[2-(morpholin-4-yl)ethyl]aminopyrido[1,2-a] benzimidazole-4-carbonitrile (13d):

Yield 47.76%, M.P.>258°C; crystallization solvent: dioxane. IR (KBr, cm^{-1}): 3377 (NH), 3070, 2957, 2834, 2803 (C-H), 2201 (C≡N), 1629 (C=N), 1596, 1558, 1458 (C=C), 1265, 1028 (C-O-C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.51 (s, 3H, CH_3), 2.55 (t, J=4.44 Hz, 4H, morpholine $C_{3,5}$-H_2), 2.76 (t, J=6.30 Hz, 2H, N-CH_2), 3.54 (q, J=6.2 Hz, 2H, CH_2-NH), 3.64 (t, J=4.49 Hz, 4H, morpholine $C_{2,6}$-H_2), 6.13 (s, 1H, pyridobenzimidazole C_2-H), 7.27 (t, J=4.06 Hz, 1H, NH , D_2O exchangeable), 7.36 (t, J=7.66 Hz, 1H, pyridobenzimidazole C_7-H), 7.53 (t, J=7.66 Hz, 1H, pyridobenzimidazole C_8-H), 7.8 (d, J=8.02 Hz, 1H, pyridobenzimidazole C_6-H), 8.28 (d, J=8.34 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. for $C_{19}H_{21}N_5O$ (335.41): C, 68.04; H, 6.31; N, 20.88. Found: C, 68.31; H, 6.34; N, 21.06.

3-Methyl-1-[2-(piperazin-1-yl)ethyl]aminopyrido[1,2-a] benzimidazole-4-carbonitrile (13e):

Yield 74.63%, M.P.>276°C; crystallization solvent: DMF/ H_2O. IR (KBr, cm^{-1}): 3387, 3206 (NH), 3073, 3049, 2945, 2890, 2814 (C-H), 2200 (C≡N), 1630 (C=N), 1596, 1561, 1458 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.52 (s, 3H, CH_3), 2.57-2.68 (m, 4H, piperazine $C_{2,6}$-H_2), 2.77 (t, J=6.22 Hz, 2H, N-CH_2), 2.93 (t, J=4.66 Hz, 4H, piperazine $C_{3,5}$-H_2), 3.53 (t, J=6.23 Hz, 2H, CH_2-NH), 6.13 (s, 1H, pyridobenzimidazole C_2-H), 7.34 (t, *J*=7.72 Hz, 1H, pyridobenzimidazole C_7-H), 7.35 (t, *J*=7.72 Hz, 1H, pyridobenzimidazole C_8-H), 7.8 (d, J=8.05 Hz, 1H, pyridobenzimidazole C_6-H), 8.3 (d, J=8.29 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. for $C_{19}H_{22}N_6$ (334.43): C, 68.24; H, 6.63; N, 25.13. Found: C, 68.42; H, 6.68; N, 25.37

3-Methyl-1-(4-substituted phenylamino)pyrido[1,2-a] benzimidazole-4-carbonitrile (14a-c): A mixture of the chloro derivative **(10)** (4 mmol, 0.97 g) and the proper arylamines, (12 mmoles) in dioxane (20 ml) was heated at 60-80°C for 13-38 h. The reaction mixture was then poured into ice cold water. The product was filtered, washed with water, dried and crystallized from DMF/ethanol.

1-(4-Hydroxyphenyl)amino-3-methylpyrido[1,2-a] benzimidazole-4-carbonitrile (14a): Yield 71.58%, M.P.>300°C; IR (KBr, cm^{-1}): 3368 (OH), 3100 (NH), 3006, 2929, 2875 (C-H), 2209 (C≡N), 1626 (C=N), 1592, 1544, 1512, 1461 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.42 (s, 3H, CH_3), 5.91 (s, 1H, pyridobenzimidazole C2-H), 6.88 (d, J=7.98 Hz, 2H 4-hydroxyphenyl $C_{2,6}$-H), 7.23 (d, J=7.98 Hz, 2H, 4-hydroxyphenyl $C_{3,5}$-H), 7.32 (t, J=7.70 Hz, 1H, pyridobenzimidazole C_7-H), 7.53 (t, J=7.70 Hz, 1H, pyridobenzimidazole C_8-H), 7.80 (d, J=7.84 Hz, 1H, pyridobenzimidazole C_6-H), 8.49 (d, J=8.00 Hz, 1H, pyridobenzimidazole C_9-H), 9.09 , 9.57 (2s, each 1H, NH and OH, D_2O exchangeable). Anal. Calcd. for $C_{19}H_{14}N_4O$ (314.35): C, 72.60; H, 4.49; N, 17.82. Found: C, 72.78; H, 4.58; N, 18.01.

1-(4-Methoxyphenyl)amino-3-methylpyrido[1,2-a] benzimidazole-4-carbonitrile (14b): Yield 95.20%, M.P.>300°C; IR (KBr, cm^{-1}): 3367 (NH), 3073, 3052, 3010, 2950, 2909, 2830 (C-H), 2205 (C≡N), 1629 (C=N), 1593, 1546, 1512, 1473 (C=C), 1263, 1029 (C-O-C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.42 (s, 3H, CH_3), 3.80 (s, 3H, O-CH_3), 5.98 (s, 1H, pyridobenzimidazole

C_2- H), 7.04 (d, J=8.5 Hz, 2H, methoxyphenyl $C_{2,6}$-H), 7.29-7.36 (m, 3H, pyridobenzimidazole C_7-H and methoxyphenyl $C_{3,5}$-H), 7.54 (t, J=7.64 Hz, 1H of pyridobenzimidazole C_8-H), 7.81 (d, J=6.75 Hz, 1H, pyridobenzimidazole C_6-H), 8.48 (d, J=7.435 Hz, 1H, pyridobenzimidazole C_9-H), 9.2 (s, 1H, NH, D_2O exchangeable). ^{13}C-NMR (125 MHz, DMSO-d6, δ ppm): 21.02 (CH_3), 55.73 (OCH_3), 87.15 (pyridobenzimidazole C_4), 95.32 (pyridobenzimidazole C_9), 115.36 (methoxyphenyl $C_{2,6}$), 116.24 (pyridobenzimidazole C_2), 116.97 (CN), 118.77, 120.56, 126.11 (pyridobenzimidazole $C_{6,7,8}$), 126.27 (methoxyphenyl $C_{3,5}$), 128.80 (pyridobenzimidazole C_{9a}), 131.96 (methoxyphenyl C_1), 145.15 (pyridobenzimidazole C_{4a}), 148.92 (pyridobenzimidazole C_{5a}), 149.16 (methoxyphenyl C_4), 151.15 (pyridobenzimidazole C_3), 157.62 (pyridobenzimidazole C_1). Anal. Calcd. for $C_{20}H_{16}N_4O$ (328.38): C, 73.15; H, 4.91; N, 17.06. Found: C, 73.24; H, 4.98; N, 17.14.

3-Methyl-1-[3-(trifluoromethyl)phenylamino]pyrido[1,2-a] benzimidazole-4-carbonitrile (14c): Yield 68.21%, M.P. 299°C; IR (KBr, cm^{-1}): 3469 (NH), 2211 (C≡N), 1636 (C=N), 1548, 1454 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.4 (s, 3H, CH_3), 6.31 (s, 1H, pyridobenzimidazole C_2-H), 7.28-7.85 (m, 7H, pyrido-benzimidazole $C_{6,7,8}$-H and trifluoromethylphenyl $C_{2,4,5,6}$-H), 8.51 (d, J=8.00 Hz, 1H, pyrido-benzimidazole C_9-H), 9.69 (s, 1H, NH, D_2O exchangeable). Anal. Calcd. for $C_{20}H_{13}F_3N_4$ (366.35): C, 65.57; H, 3.58; N, 15.29. Found: C, 65.72; H, 3.56; N, 15.43.

1-Dimethylamino-3-methylpyrido[1,2-a]benzimidazole-4-carbonitrile (15): A mixture of the chloro derivative **(10)** (4 mmol, 0.97 g) and dimethylamine, (12 mmoles, g) in dioxane (20 ml) was stirred at room temperature for 31 h. The reaction mixture was then poured into ice cold water. The product was filtered, washed with water, dried and crystallized from dioxane. Yield 94.88%, M.P.>257°C; IR (KBr, cm^{-1}): 3044, 3009, 2952, 2882, 2850 (C-H), 2218 (C≡N), 1626 (C=N), 1593, 1511, 1481, 1442 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.60 (s, 3H, CH_3 at C_3), 2.93 (s, 6H, 2 CH_3), 6.59 (s, 1H, pyridobenzimidazole C_2-H), 7.37 (t, J=7.72 Hz, 1H, pyridobenzimidazole C_7-H), 7.54 (t, J=7.72 Hz, 1H, pyridobenzimidazole C_8-H), 7.83 (d, J=8.56 Hz, 1H, pyridobenzimidazole C_6-H), 8.14 (d, J=8.33 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. for $C_{15}H_{14}N_4$ (250.31): C, 71.98; H, 5.64; N, 22.38 . Found: C, 72.15; H, 5.73; N, 22.51.

1-Bis(2-hydroxyethyl)amino-3-methylpyrido[1,2-a] benzimidazole-4-carbonitrile (16): A mixture of the chloro derivative **(10)** (4 mmol, 0.97 g) and diethanolamine (12 mmoles) in dioxane (20 ml) was heated at 60-80°C for 27. The reaction mixture was then poured into ice cold water. The product was filtered, washed with water, dried and crystallized from DMF/H_2O. Yield 82.26%, M.P. 257°C; IR (KBr, cm^{-1}): 3381 (OH), 2976, 2927, 2867 (C-H), 2214 (C≡N), 1624 (C=N), 1591, 1512, 1477, 1432 (C=C). ^{1}H-NMR (400 MHz,DMSO-d_6) δ (ppm): 2.58 (s, 3H, CH_3), 3.36-3.69 (m, 8H, 2 N-CH_2 and 2 CH_2-O), 4.56 (t, J=4.40 Hz, 2H, 2 OH, D_2O exchangeable), 6.69 (s, 1H, pyridobenzimidazole C_2-H), 7.34 (t, J=7.69 Hz, 1H, pyridobenzimidazole C_7-H), 7.52 (t, J=7.69 Hz, 1H, pyridobenzimidazole C_8-H), 7.81 (d, J=8.06 Hz, 1H, pyridobenzimidazole C_6-H), 8.20 (d, J=8.38 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. for $C_{17}H_{18}N_4O_2$ (310.36): C, 65.79; H, 5.85; N, 18.05. Found: C, 66.01; H, 5.94; N, 18.31.

1-Bis(2-chloroethyl)amino-3-methylpyrido[1,2-a] benzimidazole-4-carbonitrile (17): A suspension of 1-bis(2-hydroxyethyl)amino-3-methylpyrido[1,2-a]benzimidazole-4-carbonitrile **(16)** (5 mmol) in phosphorous oxychloride (5 ml) was heated under reflux while stirring for 3 h. The reaction mixture was allowed to cool to room temperature, then poured onto crushed ice and neutralized with Na_2CO_3. The product was filtered, washed with water and crystallized from dioxane/ ethanol. Yield 89.93%, M.P. 237°C; IR (KBr, cm^{-1}): 3055, 3020, 2961, 2920, 2874 (C-H), 2214 (C≡N), 1624 (C=N), 1593, 1511, 1442 (C=C). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.60 (s, 3H, CH_3), 3.63-3.96 (m, 8H, 2 NCH_2 and 2 CH_2-Cl), 6.88 (s, 1H, pyridobenzimidazole C_2-H), 7.38 (t, *J*=7.72 Hz, 1H, pyridobenzimidazole C_7-H), 7.55 (t, *J*=7.72 Hz, 1H, pyridobenzimidazole C_8-H), 7.85 (d, J=8.20 Hz, 1H, pyridobenzimidazole C_6-H), 8.23 (d, J=8.83 Hz, 1H, pyridobenzimidazole C_9-H). Anal. Calcd. For $C_{17}H_{16}C_{l2}N_4$ (347.24): C, 58.80; H, 4.64; N, 16.14. Found: C, 58.97; H, 4.61; N, 16.32.

Antitumor activity

Nine compounds **5b, 13a, 13d ,13e,14b, 14c, 15, 16,** and **17** were selected by the National Cancer Institute (NCI), Bethseda, Maryland, USA to be evaluated for their *in vitro* antitumor activity. Effective one-dose assay has been added to the NCI 60 Cell screen in order to increase compound throughput and reduce data turnaround time to suppliers while maintaining efficient identification of active compounds. All the selected compounds were tested initially at a single dose (10 μM) in the full NCI 60 cell panel including leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cell lines. The results are presented in Table 1 as growth (G%) and only compounds which satisfy pre-determined threshold inhibition criteria would progress to the five-dose screen [26-29].

Results and Discussion

Chemistry

The synthetic procedures implemented to obtain the newly synthesized compounds are demonstrated in Schemes 1 and 2.

In Scheme 1, the enamine tautomers of Schiff bases **2, 3, 6** and **8** were synthesized by reacting the aldehyde **1** [7] with the proper amine. ^{1}HNMR spectrum of compound **2** displayed a singlet at 1.50 ppm due to *tert*-butyl protons and a doublet at 8.35 ppm integrated for one proton attributed to the methine proton. It also revealed one D_2O exchangeable doublet at 11.60 ppm characteristic for NH proton. Meanwhile, the ^{1}HNMR spectrum of compound **3** revealed one triplet at 3.69 ppm attributed to the four protons of the ethanolamine side chain and a D_2O exchangeable triplet at 5.07 ppm due to OH proton. One doublet at 8.46 ppm integrated for one proton representing the methine proton. Also its ^{13}CNMR spectrum showed peaks at around 53 and 60 ppm corresponding to NCH_2 and OCH_2 moieties of the ethanolamine side chain. ^{1}HNMR spectrum of compound **6** revealed a singlet at 1.42 ppm due to the six protons of the two CH_3 groups of the side chain and a doublet at 3.50 ppm corresponding to the CH_2-O protons. In addition, a D_2O exchangeable triplet at 5.53 ppm due to OH proton was observed. The spectrum also showed one doublet at 8.31 ppm attributed to the methine proton and a doublet at 11.68 ppm characteristic for the NH proton. Additionally, ^{1}HNMR spectrum of compound **8** revealed a singlet at 1.36 ppm due to the CH_3 protons of the side chain and a D_2O exchangeable triplet at 5.41 ppm due to two OH protons. Two doublets at 8.32 and 11.70 ppm, each integrated for one proton attributed to the methine and the NH protons, respectively were shown.

Chlorination of aliphatic OH group was achieved by heating the starting material **3, 6** and **8** in excess phosphorous oxychloride to give the corresponding chlorinated compounds **4, 7** and **9**. The ^{1}HNMR spectrum of compound **4** lacked the D_2O exchangeable triplet of the OH proton and revealed a doublet at 8.52 ppm attributed to the

Comp No.	Leukemia		Non-Small Cell Lung Cancer			Colon Cancer		CNS Cancer			Melanoma			Ovarian Cancer	Renal Cancer			PC	Breast Cancer			
	HL- 60 (TB)	RPMI- 8226	A549/ ATCC	HOP- 62	NCI- H522	HCT- 116	HT29	SNB- 19	SNB- 75	U251	LOX IMVI	SK- MEL- 5	UACC -62	SK- OV-3	786- 0	CAKI- 1	UO- 31	PC- 3	MCF 7	MDA- MB- 231/ ACTCC	BT- 549	T-47D
5b	90.61	96.51	97.75	90.76	93.55	95.44	98.19	97.25	83.72	95.67	92.41	97.03	92.28	98.36	106.13	89.03	79.55	88.18	86.44	93.83	91.19	86.26
13a	91.62	92.00	96.43	87.18	97.86	97.20	101.65	90.08	94.77	96.58	89.05	100.64	81.60	97.87	98.30	89.91	80.97	93.28	98.55	84.44	90.84	88.55
13d	97.26	92.87	83.42	93.43	72.54	90.60	89.72	115.47	73.27	95.36	99.56	93.57	110.15	88.23	92.90	85.61	82.36	82.44	88.74	104.11	87.01	68.31
13e	91.60	95.78	87.59	84.71	85.52	75.01	84.65	100.01	83.41	90.71	99.29	95.30	94.46	82.51	86.64	93.00	85.67	88.12	86.84	94.11	83.52	89.04
14b	43.63	93.34	72.60	79.96	82.00	77.64	98.55	98.11	95.65	85.60	94.41	103.67	86.58	90.41	105.31	100.31	88.24	77.45	90.16	93.42	87.45	108.08
14c	31.80	22.82	43.73	-2.32	20.69	39.61	13.19	40.70	59.41	11.62	59.64	4.24	11.90	46.41	58.95	38.74	28.32	21.80	36.31	54.18	19.39	8.68
15	101.30	103.96	97.24	96.90	97.98	101.40	109.59	95.59	90.68	98.10	102.37	105.95	90.63	97.48	94.27	99.21	93.44	102.40	98.18	93.53	99.25	90.71
16	85.53	85.36	94.77	90.03	83.88	22.15	86.43	92.78	79.85	98.81	84.85	92.56	76.98	101.95	83.66	90.37	81.23	88.87	77.13	81.11	97.73	82.20
17	113.20	95.29	66.70	80.34	69.49	75.33	83.41	111.75	82.92	90.68	101.06	95.37	95.25	88.29	80.56	82.60	77.15	81.89	92.84	107.73	77.67	65.75

*<20%, Significant; 20-<40%, Remarkable; 40-<60%, Considerable; 60-<90%, Weak

Table 1: The growth percentage (G %) in single-dose assay for the selected compounds*.

methine proton. The spectrum also showed a multiplet at around 11.2 ppm characteristic for the NH proton. Furthermore, the ^{13}CNMR spectrum displayed peaks at 44.15 and 52.02 ppm attributed to NCH_2 and CH_2Cl moieties of the side chain, respectively. Nucleophilic displacement of aliphatic chlorine atom by aliphatic amines was achieved by reacting a suspension of **4** and the proper amine in a mixture of absolute ethanol and dimethylformamide to furnish compounds **5a- c**. their IR spectrum showed absorption bands between 3227-3420 cm^{-1} corresponding to the NH groups. ^{1}HNMR spectrum of compound **5a** revealed a singlet at 1.50 ppm representing the nine protons of the *tert*-butyl group and one doublet at 8.36 ppm corresponding to the methine proton. A mulitplet at 11.61 ppm attributed to the enamine NH proton was also displayed whereas, ^{1}HNMR spectrum of compound **5b** revealed two multiplets at 1.40-1.53 and 2.44-2.50 ppm corresponding to the piperidine protons. The spectrum also showed one doublet at 8.46 ppm integrated for one proton attributed to the methine proton. A mulitplet at 11.18 ppm attributed to the enamine NH proton was also displayed. Furthermore, the ^{1}HNMR spectrum of compound **5c** showed two multiplets due to the morpholine protons, one doublet at 8.47 ppm attributed to the methine proton and a mulitplet at 11.20 ppm attributed to the enamine NH proton. ^{1}HNMR spectrum of the chlorinated compound **7** also lacked the D_2O exchangeable triplet of the OH proton and revealed one doublet at 8.32 ppm characteristic for the methine proton. A D_2O exchangeable doublet at 11.60 ppm attributed to the NH was also displayed. Moreover, ^{1}HNMR spectrum of compound **9** lacked the D_2O exchangeable triplet of the two OH protons and revealed one doublet at 8.35 ppm integrated for one proton characteristic for the methine proton. A doublet at 11.63 ppm attributed to the NH proton was also displayed.

In Scheme 2, the following amines **11, 14a-c** and **16** were prepared by reacting the chloro derivative **10** [7] with ethanolamine, substituted aryl amines and diethanolamine in dioxane at 60-80°C. ^{1}HNMR spectrum of compound **11** revealed two quartets at 3.55 and 3.76 ppm representing the four protons of ethanolamine moiety. The presence of both the OH proton and the NH proton was confirmed by the presence of two D_2O exchangeable triplets at 5.01 and 7.24 ppm, respectively. While ^{1}HNMR spectrum of compound **14b** showed a singlet at 3.80 ppm representing the OCH_3 protons and a doublet and a multiplet at 7.04 and around 7.3 ppm, respectively, indicating the presence of the 4-methoxyphenyl group. Also the ^{13}CNMR spectrum displayed signals at around 55.73, 115.36 and 126.27 ppm attributed to OCH_3 carbon and methoxyphenyl $C_{2,6}$ and $C_{3,5}$ respectively. In addition, peaks at 131.96 and 149.16 ppm corresponding to methoxyphenyl C_1 and C_4 were observed. ^{1}HNMR spectrum of compound **16** revealed multiplet at 3.36-3.69 ppm representing the eight protons of the diethanolamine group. The presence of both OH protons was confirmed by the presence of a D_2O exchangeable triplet at 4.56ppm. The hydroxyl precursors **11** and **16** were chlorinated by heating in excess phosphorous oxychloride to produce compounds **12** and **17**. Their IR spectra lacked the absorption bands attributed to the OH groups. In addition; their ^{1}HNMR spectra revealed disappearance of the D_2O exchangeable OH proton signal confirming its replacement by the chloro group.^{1}HNMR spectrum of compound **12** revealed a quartet at 3.85 ppm and a triplet at 3.98 ppm representing the four protons of the chloroethyl group. Its ^{13}CNMR spectrum showed peaks at 42.95 and 44.84 ppm corresponding to NCH_2 and CH_2Cl, respectively. Scheme 2 also describes the reaction of chloro derivative **12** with dimethylamine, *tert*-butylamine, piperidine, morpholine and piperazine to give the corresponding amines **13 a-e**, respectively. Their IR spectra showed absorption bands in the range between 3206 and 3536 cm^{-1} corresponding to the NH groups. ^{1}HNMR spectrum of compound **13a** revealed a singlet at 2.30 ppm integrated for six protons indicating the presence of two CH_3 groups. The reaction of 1-Chloro-3-methylpyridobenzimidazole-4-carbonitrile **10** [7] with dimethylamine in dioxane at room temperature led to compounds **15**; its ^{1}HNMR spectrum revealed a singlet at 2.93 ppm representing six protons of the two CH_3 groups.

In vitro antitumor activity

Nine compounds **5b, 13a, 13d, 13e, 14b, 14c, 15, 16,** and **17** were selected by NCI and tested initially at a single dose (10 μM) in the full NCI 60 cell line panel. The results are recorded as percentage growth (G%); for example, a value of (100%) means no growth inhibition. A value of (20%) would mean (80%) growth inhibition.

Results obtained for **5b** revealed that it possesses a weak activity against CNS SNB-75 (83.72%); renal cancer CAKI-1 (89.03%) and UO-31(79.55%); prostate cancer PC-3 (88.18%) and breast cancer MCF7 (86.44%) and T-47D (86.26%) cell lines.

The PBIs with substituted aminoethlyl moiety at position-1 **(13a, 13d and 13e)** showed weak activity as indicated from (G%) for some cell lines; for compound**13a**: non-small cell lung cancer HOP-62 (87.18%); melanoma SK-MEL-5 (89.05%) and UACC-62 (81.60%); renal cancer CAKI-1 (89.91%) and UO-31 (80.97%) and breast cancer MDA-MB-231/ATCC (84.44%) and T-47D (88.55%) cell lines. The (G%) for PBI **(13d)**: non-small cell lung cancer A549/80CC (83.42%) and NCI-H522 (72.54%); renal cancer CAKI-1 (85.61%) and UO-31 (82.36%); and breast cancer MCF7 (88.74%), BT-549 (87.01%)

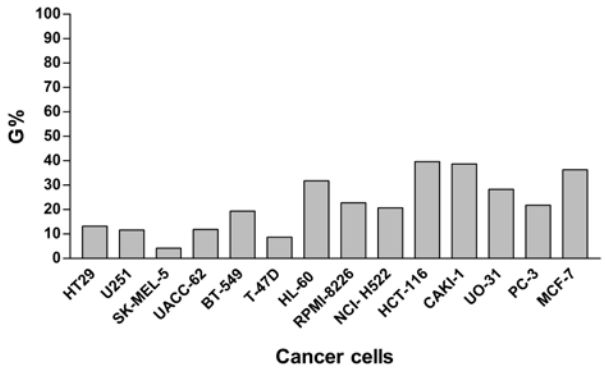


Figure 4: *In vitro* antitumor activity of compound **14c** against different cancer cells.

and T-47D (68.31%) cell lines. The (G%) for related analogue **(13e)**: non-small cell lung cancer A549/80CC (87.59%), HOP-62 (84.71%) and NCI-H522 (85.52%); colon cancer HCT-116 (75.01%) and HT29 (84.65%); renal cancer 786-0 (86.64%) and UO-31 (85.67%); and breast cancer MCF7 (86.84%), BT-549 (83.52%) and T-47D (89.04%) cell lines.

The PBIs with aryl substituents **(14b,c)** showed different cytotoxic profiles. Compound **14b** demonstrates a weak activity against non-small lung cancer A549/ATCC (72.60%), HOP-62 (79.96%) and NCI-H5-2 (82.00%); melanoma UACC-62 (86.58%); renal cancer UO-31 (88.24%); and breast cancer BT-549 (87.45%) cell lines. However, it displayed considerable activity against leukemia HL-60(TB) (43.63%) cell line. On the other hand significant activity was recorded for the PBI analogue **(14c)** against colon cancer HT-29 (13.19%); CNS cancer U251 (11.62%); melanoma SK-MEL-5 (4.24%) and UACC-62 (11.90%) and breast cancer BT- 549 (19.39%) and T-47D (8.68%) cell lines. In addition, it showed a remarkable activity against leukemia HL-60(TB) (31.80%) and RPMI-8226 (22.82%); non-small cell lung cancer NCI-H522 (20.69%); colon cancer HCT-116 (39.61%); renal cancer CAKI-1 (38.74%) and UO-31 (28.32%); prostate cancer PC-3 (21.80%) and breast cancer MCF 7 (36.31%) cell lines. Furthermore compound **(14c)** showed a considerable activity against non-small cell lung cancer A549/ATCC (43.73%); CNS cancer SNB-19 (40.70%) and SNB-75 (59.41%); melanoma LOX IMVI (59.64%); ovarian cancer SK-OV-3 (46.41%); renal cancer 786-0 (58.95%) and breast cancer MDA-MB-231/ATCC (54.18%) cell lines (Figure 4).

The PBIs with dimethylamino substituent **(15)** showed weak cytotoxic activity against some cell lines as indicated from the recorded (G%): non-small cell lung cancer A549/ATCC (66.70%), HOP-62 (80.34%) and NCI-H522 (69.49%); renal cancer 786-0 (80.56%), CAKI-1 (82.60%) and UO-31 (77.15%) and breast 80 cancer BT-549 (77.67%) and T-47D (65.75%) cell lines. The PBI (**16)** was completely inactive; whereas, its chloro analogue (**17)** displayed remarkable activity against colon cancer HCT-116(22.15%) cell line. It also showed weak inhibitory effect against leukemia HL-60 (TB) (85.53%) and RPMI-226 (85.36%); non-small cell lung cancer NCI-H522 (83.88%); colon cancer HT29 (86.43%); melanoma LOXIMVI (84.85%) and UACC-62 (76.98%); renal cancer 786-0 (83.66%) and UO-31 (81.23%); prostate cancer PC-3 (88.87%) and breast cancer MCF-7 (77.13%), MDA-MB-231/ATCC (81.11%) and T-47D (82.20%) cell lines.

Structure Activity Relationship (SAR)

Compound **5b** revealed a weak inhibitory effect against many cell lines from some types of cancer. This would indicate that the presence of 2-pyridone scaffold did not result in a significant improvement on the activity. Structural activity correlation revealed that the PBIs which lack the 2-pyridone unit but have instead a pyridine moiety showed variable cytotoxic activity. For instance, substitution at position-1 with N,N-dimethylaminoethylamino **(13a)**, morpholinoethylamino **(13d)** and piperazinylethylamino **(13e)** did not let significant activity. On the other hand, the PBI which carry 4-methoxyphenylamino moiety **(14b)** exhibited weak inhibitory effects against several cell lines from non-small cell lung cancer, melanoma, renal and breast cancer and it demonstrated remarkable cytotoxic activity against one cell line from leukemia. It is worthy to mention that the highest anticancer activity was recorded for compounds **14c**. Results revealed that replacement of the 4-methoxy **(14b)** with 3-trifluoromethyl group **(14c)** resulted in broad spectrum and variable degree of activity against many of the tested cell lines. In fact, this finding would indicate the impact of the lipophilic 3-trifluoromethylphenylamino substituents on the activity.

The presence of bis(2-hydroxyethyl)amino group **(16)** did not show any significant impact on the activity; whereas, the PBI **(17)** which carry an alkylating fragment, bis(2-choloroethyl)amino, displayed a remarkable inhibitory effect against colon cancer HCT-116 cell line and weak inhibitory effects against many cell lines from leukemia, melanoma, non-small lung cancer and colon, renal, prostate and breast cancer.

Conclusion

In conclusion, two series of benzimidazoles comprising pyridine and 2-pyridone nuclei together with various functionalities believed to have alkylation ability were synthesized. These series were designed as an example of a new molecular hybrids having anticancer activity. The anticancer activity results revealed that among the tested compounds, compound **(14c)** was found to possess promising anticancer activity and the most significant inhibition as revealed from the growth percentage (G%)was found against melanoma SK-MEL-5 (4.24%) and UACC-62 (11.90%), CNS cancer U251 (11.62%), colon cancer HT-29 (13.19%); and breast cancer BT- 549 (19.39%) and T-47D (8.68%) cell lines. In fact, this finding together with the remarkable antineoplastic activity reported to the related PBI analogues (NSC682011 and NSC699944, Figure 1) would indicate the impact of the lipophilic 3-tripfluoromethylphenylamino and 4-fluorophenylamino substituents on the activity. Although, none of the screened compounds satisfied the threshold inhibition criteria to pass for evaluation in the full panel five-dose *in vitro* antitumor screen, The PBI **14c** can be considered starting structure that merit further optimization in order to design more active lead compounds for further studies.

Declaration of Interest

The authors declared no conflicts of interest. Only the authors are responsible for the content and writing of the paper.

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