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SYNTHESIS AND CYTOSTATIC EVALUATION OF PYRIDOPYRIMIDOBENZIMIDAZOLE DERIVATIVES

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Abstract – A set of novel 2,5-substituted benzimidazoles (**1**-**7**) and their cyclic derivatives (**8**-**11**) were synthesized and evaluated for their potential cytostatic effect on various tumor cell lines. The structures of the synthesized compounds were proved by means of IR, ^{1}H NMR and MS spectral data. Based on presented *in vitro* screening results we may conclude that cyclic compounds bearing nitro and amino substituents (**10** and **11**) showed the most pronounced growth inhibitory.

INTRODUCTION

Since, cancer still remains a major public health issue; there is a great medical need for new anticancer small molecule therapeutics.[1]

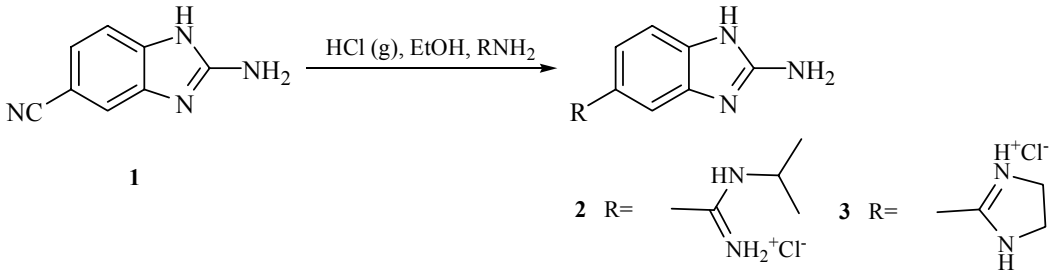
Most of planar polycyclic derivatives show an antiproliferative activity *in vitro* and some of them are important anticancer drugs.[2, 3] The biological activity of these compounds is related to their ability to form a complex with DNA, leading to cell death by inhibition of replicative enzymes and DNA repair systems or interfering with topoisomerases.[4]

Benzimidazole derivatives are structural isosters of naturally occurring nucleotides, which allows them to interact easily with the biopolymers of the living systems. Therefore, numerous biological activities and functions have been described: antihelmintic,[5] antifungal,[6] antiallergic, antimicrobial,[6, 8, 9] antiviral[10] and antineoplastic activity.[11]

In the search for new compounds with an antiproliferative activity, in this paper we report the synthesis of angular derivatives containing the benzimidazole nuclei as a part of their tetracyclic system. An evaluation of their antiproliferative activity was carried out using various human tumor cell lines and normal human fibroblasts as a control cell line. The chemical structures of all the new compounds were established by IR, ^{1}H NMR and MS spectral data for the compounds (**1**–**7**). The analysis of the ^{1}H NMR spectra showed that the compounds (**8**–**11**) present a mixture of two regioisomers—the 9- and 10-substituted derivatives in different ratio. The results are presented in Experimental Section.

RESULTS AND DISCUSSION

In continuation of our interest in the synthesis of novel polyfunctionalized heterocycles of biological importance, we report here the preparation of some new derivatives of 5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (**8**-**11**). Corresponding amidino substituted 2-aminobenzimidazoles (**2** and **3)** were prepared according to the **Scheme 1**.



Scheme 1 Synthesis of 2-amino-5-amidino substituted benzimidazoles

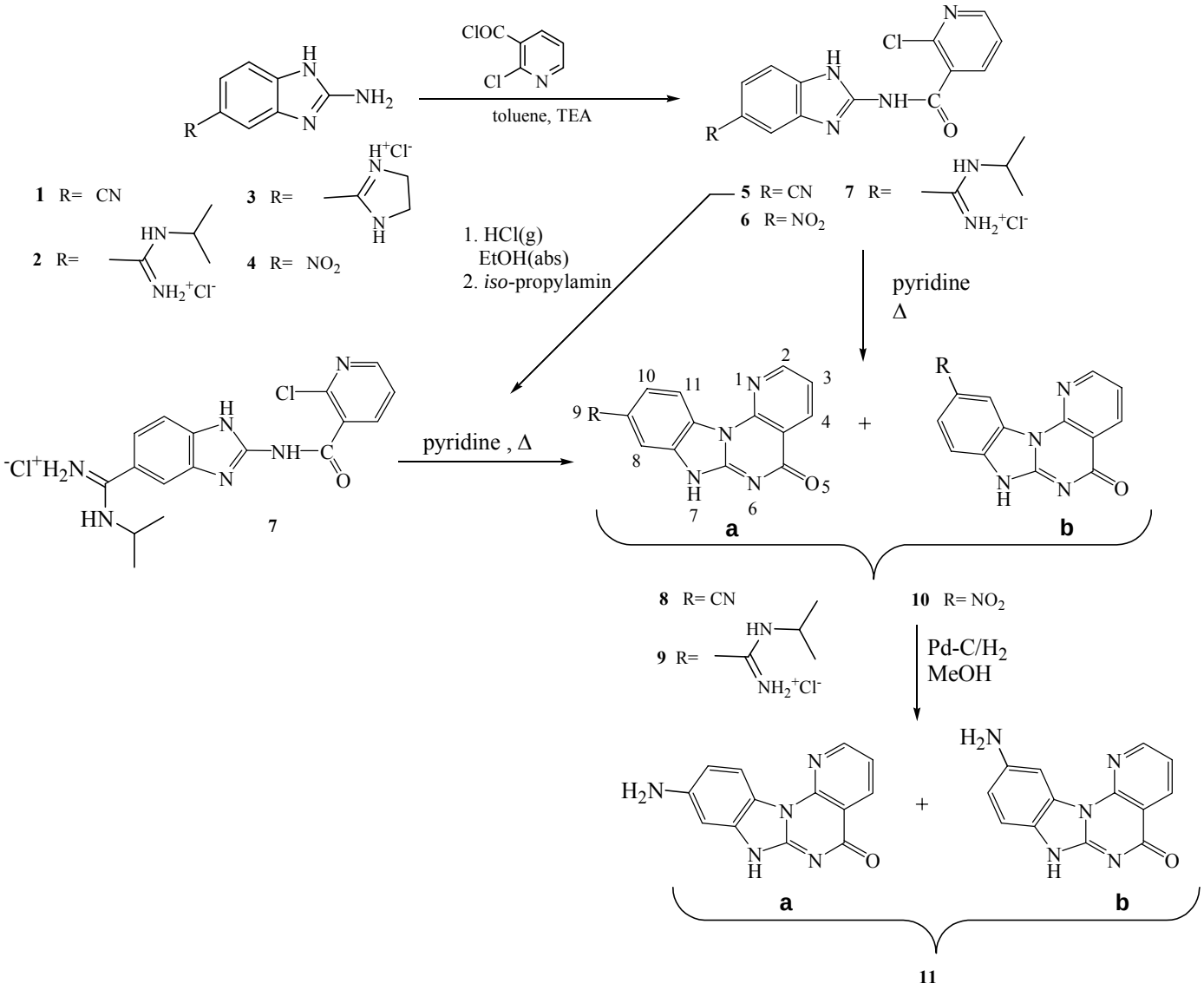
The 5-cyano-2-aminobenzimidazole (**1)** (**Scheme 1**) and 5-nitro-2-aminobenzimidazole (**4**) (**Scheme 2**), were conveniently obtained from corresponding 4-substituted-1,2-phenylendiamines using experimental conditions similar to those described in our previous paper.[12] Compound (**1)** was starting material for preparation of novel 5-amidino substituted 2-aminobenzimidazoles (**2**) and (**3)** in classical Pinner reaction.[13]

Above mentioned compounds (**1**, **2** and **3**) as well as (**4**) (**Scheme 1** and **Scheme 2**) represented the starting products employed for the preparation of the 9(10)-substituted-5,7-dihydro-5-oxopyrido[3',2': 5,6]pyrimido[1,2-*a*]benzimidazoles (**8**-**11**). The appropriate 6-substituted-2-aminobenzimidazoles (**1**-**4**) were allowed to react with 2-chloropyridine-3-carbonyl chloride in anhydrous toluene, in the presence of triethylamine, to obtain the amides (**5**, **6** and **7**) in good yields (respectively 40-60%) by published method.[14] It should be noted that the condensation of 5-(4,5-dihydro-1*H*-imidazol-2-yl)-1*H*-benzoimidazol-2-ylamine (**3**) and 2-chloronicotinoyl chloride did not yield the desired product because of low reactivity of imidazolinylamidino-substituted 2-aminobenzimidazole even thought we prolonged heating of reaction mixture. Amide (**7**) was also prepared by Pinner reaction but with rather small yield

~10%. Attempts to isolate imidazolinylamidino-substituted derivatives of compound (**5**), at the same way, was not successful because it polymerized during the isolation procedure owing to theirs instability and sensitivity to air moisture.

All prepared amides (**5**, **6** and **7**) were then cyclized to the desired heterocyclic derivatives (**8**, **9** and **10**) by reflux in pyridine for 48h in yields 30-50% (**Scheme 2**). Compound (**9**) could be prepared by converting 2-chloro-*N*-(5-cyano-1*H*-benzoimidazol-2-yl)-nicotinamide (**5**) in the Pinner reaction[13] into 2-chloro-*N*-[5-(*N*-isopropyl-carbamimidoyl)-1*H*-benzoimidazol-2-yl]-nicotinamide hydrochloride (**7**) which was than cyclized.

Amino derivative (**11**) of pyridopyrimidobenzimidazole was prepared by catalytic hydrogenation with Pd/C as catalyst in methanol (80%).



Scheme 2 Synthesis of novel pyridopyrimidobenzimidzoles

The prototropic properties of the benzimidazoles are well known. By the spectral assignment and multiplet analysis of ^{1}H NMR spectra, it was observed that the compounds (**8**-**11**) present a mixture of the 9 and

10-substituted derivatives in different ratio; compound (**8**) 1:1, compound (**9**) 1:1, compound (**10**) 1:3, compound (**11**) 1:6. The presence of the cyano group in the compound (**8**) in the 10 position leads to an upfield shift of the signal of H-8 proton 7.75 ppm, while the signal of H-8 proton in the 9-substituted isomer is shifted downfield 8.08 ppm (for the description of chemical shifts, see the numbering of the H-atoms in **Scheme 2**. If the signal of H-11 protons in both isomers is taken into consideration, it might be pointed out that the signal H-11 in the 10-cyano-sbstituted pyridopyrimidobenzimidazole (**8**) is shifted downfield 8.87 ppm, but signal of the H-11 proton in the 9-substituted isomer (**8**) is shifted upfield 7.71 ppm.

The amidino group exert on the signals of the H-8 proton in the both isomers, (**9**) similar influence, by the 7.77 ppm for the 10-amidino-substituted isomer and by 7.93 ppm for the 9-amidino isomer. The presence of amidino group in the 10 position leads to a downfield shift of the signal of the H-11 proton 8.84 ppm, while the signal of the H-11 in the 9-substituted isomer is shifted upfield H-11 7.62 ppm.

However, nitro group of the 9-substituted isomer (**10**) causes deshielding of the signal of the proton H-8 by 8.40 ppm, the nitro group at the C-10 position of the pyridopyrimidobenzimidazole (**10**) shift upfield the signal of the H-8 by 8.27 ppm, and the signals for the chemical shifts of H-11 are by 7.74 and 8.40 ppm.

Placing the amino group at the 10 position of the compound (**11**) causes upfield shift of the signals of the proton H-8 by 6.57 ppm, while the H-8 proton of the 9-substituted isomer shift downfield 7.91 ppm. The signal for the chemical shift of the H-11 proton is by 7.91 ppm for the 10-substituted isomer and by 6.65 ppm for the 9-amino-substituted isomer (**11**).

Furthermore, all ^{1}H chemical shifts of H-2, H-3 and H-4 were comparable for both isomers ($\Delta\delta$<1-0.5 ppm). In fact, perusal of chemical shift data showed that chemical shifts of H-2, H-3 and H-4 in the studied compounds change only marginally upon variation of the position of the different substituents.

Biological activity

Compounds (**1**-**11**) were evaluated for their cytostatic activity against several malignant tumor cell lines: cervical carcinoma (HeLa), pancreatic carcinoma (MiaPaCa-2), colon carcinoma (SW 620), breast carcinoma (MCF-7), lung carcinoma (H 460) and normal (diploid) human fibroblasts (WI 38, control cell line).

The tested compounds showed diverse antiproliferative effect on the presented panel cell lines (**Table 1**). The benzimidazoles (**1**, **2** and **3**) did not induce any significant inhibitory activity. The cytostatic activity is strongly increased (mostly to HeLa and MCF-7 cells) by the introducing the 2-chloro-nicotinamide at the position C-2 of benzimidazole ring (**5** and **7**). The cyclization of these molecules produced even stronger antiprolfierative effect (**10** and **11**). However, the nitro benzimidazole showed non-differential antiproliferative activity and cytotoxicity to normal cells, while the 9(10)-amino-5,7-dihydro-5-oksopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (**11**) showed the most pronounced selectivity to tumour cells, being less toxic to normal fibroblasts. Interestingly,

N-isopropylamidino-substituted compounds (**7**) and (**9**), either non-cyclic or cyclic (**7** and **9**, respectively) had very similar and modest cytostatic effect.

Table 1 Inhibitory effects of (**1**–**11**) on the growth of malignant tumor cell lines and normal human fibroblasts (WI 38)

Comp.	IC_{50} (μM)[a]					
	WI 38	**HeLa**	**MiaPaCa-2**	**SW 620**	**MCF-7**	**H 460**
1	>100	>100	>100	>100	>100	>100
2	>100	>100	>100	>100	97±2	>100
3	>100	>100	>100	>100	72±6	>100
5	74±15	33±24	72±28	83±14	30±23	62±11
7	>100	72±7	>100	>100	58±39	>100
8	>100	15±2	89±6	>100	65±2	86±10
9	>100	>100	≥100	>100	>100	>100
10	8±2	13±0,3	7±2	6,5±4,7	>100	39±12
11	≥100	7±6	30±8	8±4	19±12	43±0,4

[a]IC_{50}; the concentration that causes a 50% reduction of the cell growth

In conclusion, we have prepared a small set of 2,5-substituted benzimidazoles (**1-7**) and their cyclic derivatives (**8-11**). By the spectral assignment and multiplet analysis of ^{1}H NMR spectra, it was observed that the compounds (**8-11**) present a mixture of the 9 and 10-substituted derivates in different ratio; compound (**8**) 1:1, compound (**9**) 1:1, compound (**10**) 1:3, compound (**11**) 1:6.

Furthermore, all novel compounds (**1-11**) were tested on their antiproliferative activity. Based on presented *in vitro* screening results we may conclude that cyclic compounds bearing nitro and amino substituents (**10** and **11**) showed the most pronounced growth inhibitory effect and should be considered for further synthetic optimization.

EXPERIMENTAL

Chemistry

Melting points were obtained on an Original Kofler Mikroheitztisch apparatus (Reichert, Wien) and are uncorrected. IR spectra were recorded on a Nicolet Magna 760 spectrophotometer in KBr discs. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 and 600 at 300, 600 and 75 MHz, respectively. All NMR spectra were measured in DMSO-d_6 solutions using TMS as an internal standard. Elemental analysis for carbon, hydrogen and nitrogen were performed on a Perkin-Elmer 2400 elemental analyzer. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4 % of the theoretical value. MS spectra were recorded by using electrospray ionization technique (ESI) on the Micromass Platform LCZ single quadropole mass spectrometer. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates.

2-Amino-5(6)-cyanobenzimidazole (**1**)

3,4-diaminobenzonitrile (3.98g, 0.030mol) was added to a mixture of water (62.5mL), MeOH (62.5mL) and solution of cyanogen bromide (3.17g, 0.030mol) in MeCN (6mL) and left to stir overnight. The mixture was decolorized with charcoal and adjust to p*H*>9, with concd NH_4OH. White precipitate (**1)** was obtained 4.07g (86%); mp 212-215°C (Lit.,[12] mp 213-215°C).

2-Amino-*N*-isopropyl-1*H*-benzimidazole-5-carboxamidine hydrochloride (**2**)

A stirred suspension of (**1)** (0.5g, 3mmol) in anhydrous EtOH (5mL) was cooled in an ice-salt bath and was saturated with HCl gas. The flask was then tightly stoppered and the mixture was maintained at rt for 2 days, until nitrile band disappeared (monitored by IR analysis at 2200 cm^{-1}). The reaction mixture was purged with N_2 gas and diluted with Et_2O (50mL). The crude imidate was filtered off and was immediately suspended in anhydrous EtOH (5mL). The *N*-isopropylamine (0.94g, 1.6mmol) was added and the mixture was stirred for one day at rt. The reaction mixture was diluted with Et_2O (50mL) to give a precipitate. The precipitate was collected and recrystallized from EtOH-Et_2O to give white powder 0.55g (76%). mp 283-285°C; MS *m/z*: 218 (M+1); IR (cm^{-1}): 3359, 3166, 3074, 2973, 2358, 1675, 1612, 1562, 1442; ^{1}H NMR (DMSO-d_6) δ/ppm: 12.3 (brs, 1H, N<u>H</u>), 9.25 (s, 1H, N<u>H</u>), 9.15 (s,1H, N<u>H</u>), 8.82 (s, 1H, N<u>H</u>), 7.49 (s, 1H), 7.28 (d, 1H, *J*=8.30 Hz,), 7.23 (d, 1H, *J*=8.3 Hz), 6.65 (s, 2H, N<u>H</u>$_2$), 4.07 (m, 1H, C<u>H</u>$(CH_3)_2$), 1.26 (d, 6H, *J*=6.30 Hz, CH(C<u>H</u>$_3$)$_2$). *Anal.* Calcd for $C_{11}H_{15}N_5Cl$: C, 52.28; H, 5.98; N, 27.71. Found: C, 52.51; H, 6.01; N, 27.53.

5-(4,5-Dihydro-1*H*-imidazol-2-yl)-1*H*-benzoimidazol-2-ylamine (**3**)

A stirred suspension of **1** (1.0g, 6mmol) in anhydrous EtOH (15mL) was cooled in an ice-salt bath and was saturated with HCl gas. The flask was then tightly stoppered and the mixture was maintained at rt for 2 days, until nitrile band disappeared (monitored by IR analysis at 2200 cm^{-1}). The reaction mixture was purged with N_2 gas and diluted with Et_2O (50mL). The crude imidate was filtered off and was immediately suspended in anhydrous EtOH (10mL). The ethylenediamine (1.14g, 1.9mmol) was added and the mixture was refluxed for 4 h and than stirred for one day at rt. The reaction mixture was diluted with Et_2O (50mL) to give a precipitate. The precipitate was collected and recrystallized from EtOH-Et_2O to give white poweder 1.35g (90%). mp >300°C; IR (cm^{-1}): 3116, 2952, 1616, 1564, 1432, 1272; ^{1}H NMR (DMSO-d_6) σ/ppm: 8.4 (brs, 3H, N<u>H</u>), 7.77 (s, 1H), 7.58 (d, 1H, *J*=8.30 Hz), 7.25 (d, 1H, *J*=8.30 Hz), 6.72 (s, 2H, N<u>H</u>$_2$), 3.94 (s, 4H, C<u>H</u>$_2$); *Anal.* Calcd for $C_{10}H_{12}N_5Cl$: C, 50.53; H, 5.09; N, 29.54. Found: C, 50.51; H, 5.11; N, 29.54.

2-Amino-5-nitrobenzimidazole (**4**)

Compound (**4)** was prepared in a same manner as (**1**); 5-nitro-1,2-phenylendiamine (8.72g, 0.057mol) was added to a water (120mL), MeOH (120mL) and cyanogen bromide (6.04g, 0.057mol) in acetonitrile (11.3mL) afforded 0.120g (35.1%) of dark yellow powder; mp 190-192°C (mp[12]189-190°C).

General procedure for amide (**5**) *and* (**6**) *synthesis*

Reaction mixture of 2-chloro-nicotinoyl chloride, corresponding 2-aminobenzimidazole derivatives (**1**, **2** and **4**) and Et_3N in equimolar amounts in dry toluene was refluxed for 4-5h. After cooling, precipitate was filtered off, washed with water and recrystallized from MeOH.

2-Chloro-*N*-(5-cyano-1*H*-benzimidazol-2-yl)-nicotinamide (**5**)

Yield 0.75 g (39%) of white poweder. mp>300°C; MS *m/z*: 298 (M+1); UV (etanol, λ/nm (ε)): 307 (13569); IR (cm^{-1}): 3363, 2717, 2356, 2221, 1675, 1583, 1398, 1309; ^{1}H NMR (DMSO-d_6) σ/ppm: 12.70 (brs, 2H, NH), 8.57 (d, 1H, *J*=4.6 Hz,) 8.19 (d, 1H, *J*=6.1 Hz), 7.94 (s, 1H), 7.67-7.55- (m, 3H); ^{13}C NMR (DMSO-d_6) σ/ppm: 165.7, 151.7, 149.1, 146.9, 139.2 (2C), 131.9, 125.8, 123.7 (2C), 120.6, 103.7; *Anal.* Calcd for $C_{14}H_8N_5OCl$: C, 56.48; H, 2.71; N, 23.52. Found: C, 576.32; H, 2.80; N, 23.21.

2-Chloro-*N*-(5-nitro-1*H*-benzimidazol-2-yl)-nicotinamide (**6**)

Yield 0.921g (50.9%) of yellow poweder. mp 264-266°C; MS *m/z*: 317.8 (M+1); IR (cm^{-1}): 3366, 3050, 2917, 2846, 1677, 1641, 1574; ^{1}H NMR (DMSO-d_6) σ/ppm: 12.30 (brs, 2H, NH), 8.58 (d, 1H, *J*=4.86 Hz, *J*=1.68 Hz), 8.38 (s, 1H), 8.21 (d, 1H, *J*=7.44 Hz), 8.10 (d, 1H, *J*=8.58 Hz), 7.66 (d, 1H, *J*=8.34 Hz), 7.60 (dd, 1H, *J*=7.44 Hz, *J*=2.58 Hz). *Anal.* Calcd for $C_{13}H_8N_5O_3Cl$: C, 49.15; H, 2.54; N, 22.04. Found: C, 49.40; H, 2.35; N, 22.10.

2-Chloro-*N*-[5-(*N*-isopropyl)-1*H*-benzimidazol-2-yl]-nicotinamide hydrochloride (**7**)

Reaction mixture of 2-chloro-nicotinoyl chloride in dry toluene, corresponding 2-aminobenzimidazole derivative 2 in dry DMF and Et_3N in equimolar amounts was refluxed for 4-5h. After cooling, precipitate was filtered off, washed with water and recrystallized from MeOH. Yield 0.16 g (59%). mp >300°C; MS *m/z*: 357 (M+1); IR (cm^{-1}): 2927, 2813, 2727, 2615, 2526, 1672, 1558, 1510, 1394; ^{1}H NMR (DMSO-d_6) σ/ppm: 12.56 (brs, 2H, NH), 9.4 (brs, 3H, NH), 8.48 (d, 1H, *J*=7.60 Hz), 8.11 (d, 1H, *J*=7.50 Hz), 7.80 (s, 1H), 7.58 (d, 1H, *J*=8.30 Hz), 7.51-7.50 (m, 1H), 7.22 (d, 1H, *J*=8.30 Hz), 4.02 (m, 1H, CH(CH3)$_2$), 1.10 (d, 6H, *J*=6.50 Hz, CH(CH_3)$_2$); *Anal.* Calcd for $C_{17}H_{18}N_6OCl_2$: C, 51.92; H, 4.61; N, 21.37. Found: C, 52.01; H, 4.51; N, 21.12.

General procedure for synthesis of pyridopyrimidobenzimidazoles (**8-10**)

A suspension of the appropriate carboxamide (**5-7**) (1.0 mmol) in of pyridine (10 mL) was refluxed for 48 h. After cooling, the solution was filtered of and the residue obtained was washed with $NaHCO_3$, 10% aqueous solution and purified by recrystallized from EtOH to give compounds (**8-11**).

9(10)-Cyano-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (**8**)

Yield 0.065 g (27%). mp >300°C; MS *m/z*: 262 (M+1); UV (ethanol, λ/nm (ε)): 251 (26373), 321 (9523); IR (cm^{-1}): 3074, 2694, 2223, 1691, 1648, 1589, 1473, 1425, 1263; *Anal.* Calcd for $C_{14}H_7N_5O$: C, 64.37; H, 2.70; N, 26.81. Found: C, 64.23; H, 2.81; N, 26.92; ^{1}H NMR (DMSO-d_6) σ/ppm:

9-Cyano-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (8a)

12.56 (brs, 1H, NH), 8.92 (dd, 1H, *J*=4.83 Hz, *J*=1.53 Hz, H-2), 8.57 (d, 1H, *J*=7.68 Hz, H-4), 8.08 (s, 1H, H-8), 7.71 (d, 2H, *J*=8.48 Hz, H-10, H-11), 7.63 (d, 1H, *J*=7.75 Hz, H-3).

10-cyano-5,7-dihydro-5-oxopyrido[3',2':5,6]pirimido[1,2-*a*]benzimidazole (8b)

12.56 (brs, 1H, NH), 8.94 (dd, 1H, *J*=4.83 Hz, *J*=1.53 Hz, H-2), 8.87 (s, 1H, H-11), 8.68 (d, 1H, *J*=8.31 Hz, H-8), 8.58 (d, 1H, *J*=7.77 Hz, H-4), 7.75 (d, 1H, *J*=8.49 Hz, H-9), 7.61 (d, 1H, *J*=7.75 Hz, H-3).

9(10)-*N*-Isopropyl-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole-9-carboxamidine hydrochloride (9)

Yield 0.16 g (59%). mp >300°C; MS *m/z*: 321 (M+1); IR (cm^{-1}): 2927, 2813, 2727, 2615, 2526, 1672, 1558, 1510, 1394; *Anal.* Calcd for $C_{17}H_{17}N_6OCl$: C, 57.22; H, 4.80; N, 23.55. Found: C, 57.51; H, 4.91; N, 23.32; ^{1}H NMR (DMSO-d_6) σ/ppm:

9-*N*-Isopropyl-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole-9-carboxamidine hydrochloride (9a)

12.61 (brs, 1H, NH), 9.65 (brs, 2H, NH), 9.49 (brs, 1H, NH), 8.69 (dd, 1H, *J*=5.10 Hz, *J*=1.50 Hz, H-2), 8.60 (d, 1H, *J*=7.08 Hz, H-4), 7.93 (s, 1H, H-8), 7.65 (d, 1H, *J*=8.37 Hz, H-10), 7.62 (d, 1H, *J*=8.37 Hz, H-11), , 7.44 (dd, 1H, *J*=7.62 Hz, *J*=4.74 Hz, H-3), 4.09-4.08 (m, 1H, CH$(CH_3)_2$), 1.31 (d, 6H, *J*=6.01 Hz, CH(CH$_3$)$_2$).

10-*N*-Isopropyl-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole-9-carboxamidine hydrochloride (9b)

12.61 (brs, 1H, NH), 9.65 (brs, 2H, NH), 9.49 (brs, 1H, NH), 8.98 (dd, 1H, *J*=4.80 Hz, *J*=1.50 Hz, H-2), 8.84 (s, 1H, H-11), 8.63 (d, 1H, *J*=7.08 Hz, H-4), 8.49 (d, 1H, *J*=8.37 Hz, H-9), 7.77 (d, 1H, *J*=8.37 Hz, H-8), 7.71 (dd, 1H, *J*=7.56 Hz, *J*=4.02 Hz, H-3), 4.09-4.08 (m, 1H, CH$(CH_3)_2$), 1.31 (d, 6H, *J*=6.01 Hz, CH(CH$_3$)$_2$).

9(10)-Nitro-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (10)

Yield 0.921g (50.9%) yellow poweder. mp >300°C; IR (KBr)/(cm^{-1}): 3366, 3050, 2917, 1677, 1641, 1574; MS *m/z*: 317.8 (M+1); *Anal.* Calcd for $C_{13}H_7N_5O_3$: C, 55.52; H, 2.51; N, 24.90. Found: C, 55.34; H, 2.43; N, 24.56; ^{1}H NMR (DMSO-d_6) σ/ppm:

9-Nitro-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (10a)

12.61 (brs, 1H, NH), 8.94 (dd, 1H, *J*=4.80 Hz, *J*=1.50 Hz, H-2), 8.60 (d, 1H, *J*=7.77 Hz, H-4), 8.40 (s, 1H, H-8), 8.24 (d, 1H, *J*=8.64 Hz, H-10), 7.74 (d, 1H, *J*=8.85 Hz, H-11), 7.64 (dd, 1H, *J*=6.21 Hz , *J*=4.92 Hz, H-3).

10-Nitro-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (10b)

12.61 (brs, 1H, NH), 9.35 (s, 1H), 9.02 (dd, 1H, *J*=4.8 Hz, *J*=1.5 Hz, H-2), 8.73 (d, 1H, *J*=8.90 Hz, H-9), 8.60 (d, 1H, *J*=7.8 Hz, H-4), 8.40 (s, 1H, H-11) 8.27 (d, 1H, *J*=8.64 Hz, H-8), 7.66 (dd, 1H, *J*=4.89 Hz, *J*=6.42 Hz, H-3).

9(10)-Amino-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (**11**)

A solution of 9(10)-nitro-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (**10**) (0.250g, 0.89mmol) in mixture of MeOH (150mL) and DMSO (50mL) and 10% Pd-C (0.025g) was hydrogenated until the required quantity of H_2 was taken up. The solution was filtered through Celite to remove catalyst, and MeOH was removed under reduced pressure. The resulting oil was triturated with small amount of MeCN and resulted precipitate collected by filtration to afford 0.178g (79.8%). mp >300°C; IR (cm^{-1}): 3079, 2833, 2726, 1694, 1658, 1591; MS *m/z*: 251.9 (M+1); *Anal.* Calcd for $C_{13}H_9N_5O$: C, 62.15; H, 3.61; N, 27.87. Found: C, 62.35; H, 3.45; N, 27.63; ^{1}H NMR (DMSO-d_6) σ/ppm:

9-Amino-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (11a)

12.55 (brs, 1H, NH), 8.85 (dd, 1H, *J*=4.62 Hz, *J*=1.5 Hz, H-2), 8. 51 (d, 1H, *J*=7.89 Hz, H-4), 7.90 (s, 1H, H-8), 7.53 (dd, 1H, *J*=7.74, *J*=2.1 Hz, H-3), 7.23 (d, 1H, *J*=8.43 Hz, H-10), 6.65 (dd, 1H, *J*=8.43 Hz, *J*=1.98 Hz, H-11) 5.52 (brs, 2H, NH).

10-Amino-5,7-dihydro-5-oxopyrido[3',2':5,6]pyrimido[1,2-*a*]benzimidazole (11b)

12.55 (brs, 1H, NH), 8.85 (dd, 1H, *J*=4.62 Hz, *J*=1.5 Hz, H-2), 8.24 (d, 1H, *J*=7.90 Hz, H-4), 7.91 (s, 1H, H-11), 7.53 (dd, 1H, *J*=7.74 Hz, *J*=2.1 Hz, H-3), 7.23 (d, 1H, *J*=8.43 Hz, H-9), 6.57 (dd, 1H, *J*=8.58 Hz, *J*=1.71 Hz, H-8) 5.52 (brs, 2H, NH).

Antitumor activity assay

The HeLa (cervical carcinoma), MiaPaCa-2 (pancreatic carcinoma), SW 620 (colon carcinoma), MCF-7 (breast carcinoma), H460 (lung carcinoma), and WI 38 (normal fibroblasts) cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. The growth inhibition activity was assessed according to the slightly modified procedure performed at the National Cancer Institute, Developmental Therapeutics Program.[15]

The cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 1×10^4 to 3×10^4 cells/ml, depending on the doubling times of specific cell line. Test agents were then added in five, 10-fold dilutions (10^{-8} to 10^{-4} M) and incubated for a further 72 hours. Working dilutions were freshly prepared on the day of testing. After 72 hours of incubation the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenase activity in viable cells, as described previously.[14,16]

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