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ORIGINAL ARTICLE

Antioxidative and antiproliferative activities of novel pyrido[1,2-*a*]benzimidazoles

Martina Tireli[1] · Kristina Starčević[2] · Tamara Martinović[3] · Sandra Kraljević Pavelić[3] · Grace Karminski-Zamola[4] · Marijana Hranjec[4]

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Abstract A series of pyrido[1,2-*a*]benzimidazoles has been designed, and novel examples are synthesized and evaluated for their potential antiproliferative activity against four human tumour cell lines—cervical (HeLa), colorectal (SW620), breast (MCF-7) and hepatocellular carcinoma (HepG2). In addition, their antioxidative potency has been evaluated by in vitro spectrophotometric assays. Preliminary structure–activity relationships among the synthesized compounds are discussed. Evaluation of their antioxidative capacity has shown that two compounds (**25** and **26**) possess promising reducing characteristics and free radical scavenging activity. Selective antiproliferative effect in the single-digit micromolar range was observed for compound **25** on MCF-7 (IC_{50} = 6 μM) and HeLa (IC_{50} = 8 μM) cell lines, comparable to the standards 5-fluorouracil and cisplatin. The combination of the radical scavenging activity and antiproliferative activity of compound **25** positions this compound as a potential lead candidate for further optimization.

Keywords Pyrido[1,2-*a*]benzimidazoles · Amides · Antiproliferative activity · Antioxidative activity · Cyclization

Introduction

Azine azoles, including pyrido-, pyrimido-, and triazinobenzimidazole heterocycles, have attracted considerable attention in recent years because of their promising biological activities and their use as important building blocks in natural and synthetic bioactive compounds [1–3]. The benzimidazole nucleus itself has been widely incorporated as one of the most important heterocyclic pharmacophores in various biomedical compounds, thus possessing a wide spectrum of biological activities. Furthermore, benzimidazole derivatives as a structural isostere of nucleotides could easily interact with the biomacromolecules of a living system and significantly impact their biological activities and functions. In addition to their well-known anticancer [4], antibacterial [5], antiviral [6] and others activities, in the last decade, the antioxidative potency of versatile benzimidazole derivatives has also been studied [7]. The implication of free radicals in the cell signalling pathway of many chronic diseases, such as cancer, inflammation, neurodegenerative syndrome or diabetes is very important. In general, the rate of reactive oxygen species (ROS) production is increased in most diseases and could be regulated by a proper application of antioxidants [8]. Antioxidants are considered as compounds that can prevent oxidation of easily oxidizable substrates and prevent excess of ROS. Therefore, the antioxidative evaluation of natural

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✉ Marijana Hranjec
mhranjec@fkit.hr

[1] Laboratory for synthesis and processes of self-assembling of organic molecules, Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

[2] Department of Animal Husbandry, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, 10000 Zagreb, Croatia

[3] Department of Biotechnology, Centre for high-throughput technologies, University of Rijeka, Radmile Matejčić 2, 51000 Rijeka, Croatia

[4] Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, P. O. Box 177, 10000 Zagreb, Croatia

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and synthetic compounds is extremely important in terms of prevention of diseases mentioned above.

Pyrido[1,2-*a*]benzimidazoles are attractive compounds due to their wide range of significant biological activities including anticancer, antineoplastic [9,10], antiviral [11], anxiolytic [12,13], antimalarial [14,15] and antimicrobial activities [16] and potential pharmaceutical applications. Pyrido[1,2-*a*]benzimidazoles with substitution on the N5-nitrogen have been found to possess high affinity for the benzodiazepine (BZD) site on the GABA-A receptor [17,18]. The most promising of these compounds was an ethoxymethyl analogue which has activity in the nanomolar concentration range for the BZD site on the GABA-A receptor and has been advanced to human clinical trials [15]. Moreover, Badaway et al. have published the synthesis and antineoplastic activity of several substituted pyrido[1,2-*a*]benzimidazole derivatives where some analogues exhibited moderate in vitro antineoplastic activity against most of the leukaemia and *Artina salina larvae* cell lines [19,20]. From previous studies, it was concluded that the biological activity is strongly dependent on the pyrido[1,2-*a*]benzimidazole tricyclic core as well as the position and type of the side chain substituents.

Recently, our attention was focused on fused, benzannulated benzimidazole derivatives with a highly conjugated planar tetracyclic chromophore bearing different side chain substituents that could be important for additional interactions with possible biological targets or could improve their spectroscopic properties, namely fluorescence intensity, due to their applications in fluorescent microscopy as potential fluorescent probes for the detection of biomacromolecules in living systems [21,22]. Additional biological activity studies of the above-mentioned class of compounds including spectroscopic characterization of interactions with DNA, topoisomerase I-mediated DNA relaxation, DNase I footprinting and fluorescent microscopy experiments confirmed their promising anticancer potential [23].

Based on previous knowledge, and considering the promising anticancer potential of fused benzimidazole derivatives, herein we present the design and synthesis of novel amino and amido-substituted pyrido[1,2-*a*]benzimidazoles via a key catalytic amidation reaction and their antiproliferative and antioxidative biological profiles.

Results and discussion

Chemistry

All novel pyrido[1,2-*a*]benzimidazoles **9**–**26** were prepared according to the experimental procedure shown in Scheme 1 starting from 4-substituted-1,2-phenylenediamines. The main precursors for the synthesis of pyrido[1,2-*a*]benzimidazoles, substituted 2-cyanomethylbenzimidazoles **5**–**8**, were prepared through a cyclocondensation reaction between 4-substituted-1,2-phenylenediamines **1**–**4** and 2-cyanoacetamide at high temperature.

The synthesis of cyano-substituted pyrido[1,2-*a*]benzimidazoles was accomplished by heating an ethanolic solution of 2-cyanomethylbenzimidazoles **5**–**8** with perchloric acid at reflux producing cyclic derivatives **9**–**12** in good yields. Within the acidic hydrolysis, the corresponding carboxylic acids **13**–**16** were obtained as main precursors for the synthesis of amido-substituted pyrido[1,2-*a*]benzimidazoles. Activation of **13**–**16** with 1,1′-carbodiimidazole in DMF followed by the addition of an amine afforded desired amido-substituted derivatives. In light of the poor solubility of these compounds, these compounds were dissolved in ethanol and converted to their hydrochloride salts **17**–**24** by bubbling excess HCl(g) through the ethanolic solution.

8-Amino-substituted pyrido[1,2-*a*]benzimidazole **25** was synthesized by reducing the 8-nitro precursor **10** which was isolated from a regioisomer mixture *via* column chromatography. All substituted pyrido[1,2-*a*]benzimidazoles **17**–**24** were prepared as a mixture of two regioisomers. The structures of all pyrido[1,2-*a*]benzimidazoles **17**–**24** were confirmed by ^{1}H and ^{13}C NMR spectroscopy, and mass spectrometry.

NMR analysis was based on the values of H-H coupling constants and chemical shifts in the ^{1}H and ^{13}C NMR spectra.

Antiproliferative activity

Antiproliferative activities of the pyrido[1,2-*a*]benzimidazole derivatives **17**–**26** were assessed against four human tumour cell lines: cervical carcinoma (HeLa), colorectal metastatic adenocarcinoma (SW620), breast metastatic epithelial adenocarcinoma (MCF-7), hepatocellular carcinoma (HepG2) cell lines, and against normal diploid human fibroblasts (BJ). Cisplatin and 5-fluorouracil were used as positive controls and the results for the most active compounds **19**, **23**–**26** are summarized in Table 1.

Compounds **19**, **25** and **26** exhibited antiproliferative activity against three cancer cell lines (MCF7, SW620 and HeLa), with IC_{50} values of 6–43 μM and no activity towards HepG2 cells. The pyrido[1,2-*a*]benzimidazole **23**, bearing a nitro group at position 3 and an *N*,*N*-dimethylamino-1-propyl carboxamide moiety at position 7(8), exhibited moderate cytotoxicity towards two cell lines: SW620 and HeLa (IC_{50} = 28 and 36 μM, respectively). The most potent and selective growth inhibitory activity observed in the series was with compound **25** against MCF-7 and HeLa cancer cell lines (IC_{50} = 6 μM), with a comparable inhibitory value to 5-fluorouracil and cisplatin.

All other analogues **17**, **18**, **20**–**22** and **24** did not show any effect on tested tumour cell lines (IC_{50} > 100 μM).

Scheme 1 Synthesis of pyrido[1,2-*a*]benzimidazoles

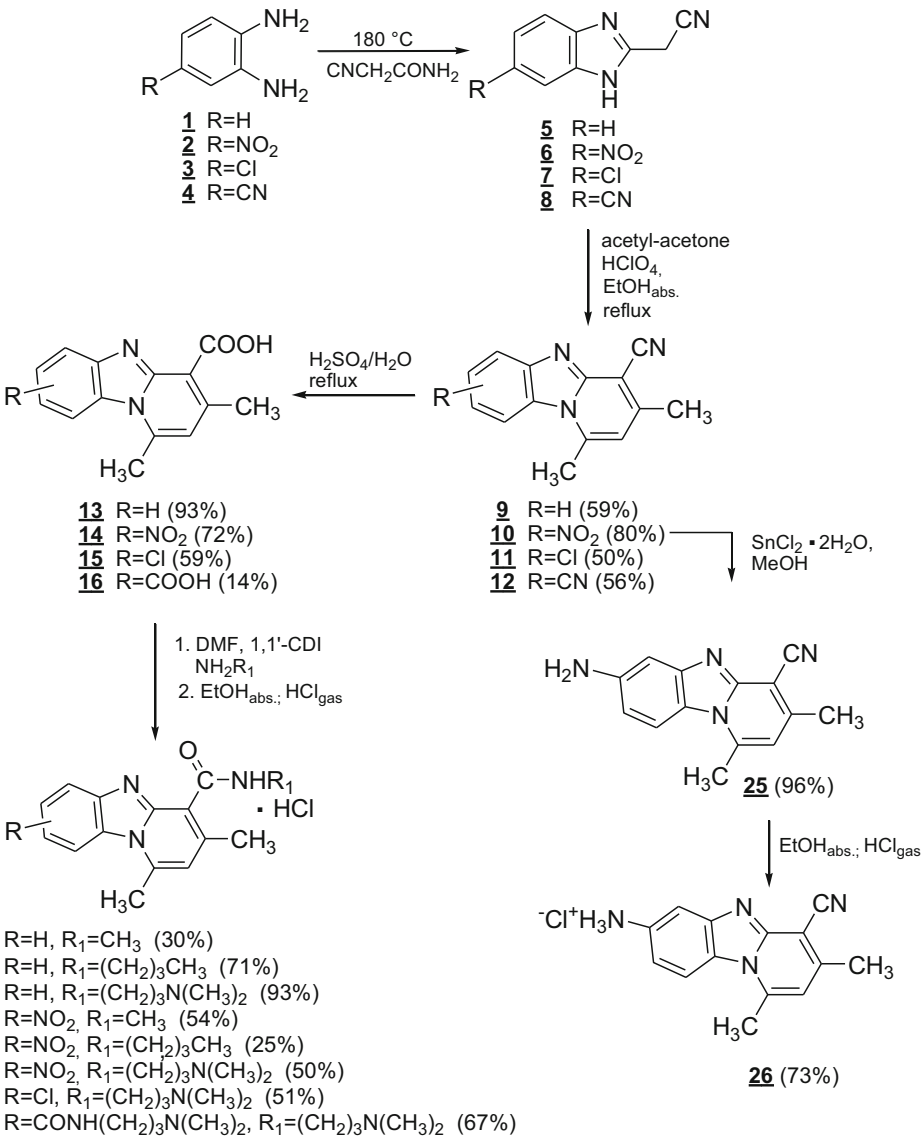


Table 1 In vitro anticancer activity ($^{a}IC_{50}$) of selected pyrido[1,2-*a*]benzimidazole derivatives

Comp.	IC_{50}^{a} (μM)				
	MCF-7	HepG2	SW620	HeLa	BJ
19	43	>100	35	34	26
23	>100	>100	28	36	22
25	6	>100	27	8	35
26	41	>100	24	34	26
Cisplatin	12.61	2.41	4.01	0.35	0.22
5-FU	42.0	>100	3.4	10.3	18.5

[a] IC_{50} values are the concentrations that cause 50 % inhibition of cancer cell growth (μM)

We also investigated the effect of these compounds on normal fibroblasts (BJ) proliferation and found they exhibited IC_{50} values in the range of 21–100 μM which are comparable to the two standards used in this study. By exploring the variation in selectivity of the compounds towards the four tumour cell lines used in our work, it was revealed that variations of substituents at positions 3 and 7(8) of the pyrido[1,2-*a*]benzimidazole derivatives led to different cytotoxic activities. Structure–activity relationships (SAR) demonstrated that compounds with hydrogen or nitro substituent at position 7(8), and a methyl or butyl carboxamide group at position 3 of the pyrido[1,2-*a*]benzimidazole skeleton did not exhibit any activity against the tumour cell lines. Placing a *N*,*N*-dimethylamino-1-propyl carboxamide group at position 3 of unsubstituted or 7(8)-chloro-substituted derivative exerted moderate activity towards three tumour cell lines (MCF-7, SW620 and HeLa). However, the presence of a nitro group at the 7(8)-position was detrimental for antiproliferative activity. The best observed antiproliferative activity was for compound **25** with an amino and

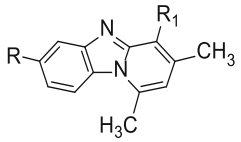
cyano group at positions 7(8) and 3, respectively. Interestingly, the hydrochloride salt of **25** (**26**) showed a decrease in antiproliferative activity and selectivity compared to the salt-free precursor **25**. This could be probably addressed to the pKa of the aniline NH_2 group which is known to be 9.2. Therefore, we could assume that at physiological pH of the biological assay medium, the aniline NH_2 group of compound **26** is still protonated. In addition, the presence of two electron-donating methyl groups and one electron-accepting cyano group probably does not have a significant influence on the pKa value of the aniline NH_2 group. The presence of a positive charge in compound **26** is unfavourable to enter the cell membrane which results in a weaker antiproliferative activity.

Nevertheless, the observed SAR among the evaluated compounds suggests that an increase of antiproliferative activity is due to the placement of an electron-withdrawing group at the 3-position and an electron-donating substituent at 7(8)-position of the pyrido[1,2-*a*]benzimidazole scaffold. Hence, the targeted compounds could efficiently enter the cell and act as radical scavengers. Overall, the most potent and selective growth inhibitory activity was observed in the micromolar range against the MCF-7 and HeLa cancer cell lines, with compound **25** being most potent ($IC_{50} = 6\,\mu M$), comparable to the standards, 5-fluorouracil and cisplatin.

It is accepted that adequate lipophilicity is necessary for compounds to penetrate and cross the cell membrane. LogP is defined as 1-octanol/water partition coefficient and is indicative for lipophilicity or hydrophilicity of novel compounds. The calculated LogP values, also known as clogP values, were obtained using ChemDraw Ultra 10.0 [24]. The clogP values for the neutral form of the prepared compounds are presented in Table 2. The cLogP values of compounds **17**–**26** increased with the length of the alkyl groups on position R1 resulting in reduced or no antiproliferative activity for compounds **17**, **18**, **20** and **21**. Increasing further the length of the alkyl chain by *N*-dimethylamino group led to a decrease in cLogP values and enhancement of antiproliferative activity. From the results, it is clear that despite of increasing the clogP of the compounds, the enhancement of their antimicrobial activities is not consistent indicating there is a poor correlation between the clogP values and antiproliferative activity of these compounds.

Thus, this suggests that the observed antiproliferative activity could be explained by the fact that protonated species were unfavourable for being delivered through the cell membrane.

Table 2 cLogP values of compounds **17**–**26**



Comp		clogP[a]
17	R=H, R_1=$CONHCH_3$ × HCl	2.88
18	R=H, R_1=$CONH(CH_2)_3CH_3$ × HCl	4.47
19	R=H, R_1=$CONH(CH_2)_3N(CH_3)_2$ × HCl	3.57
20	R=NO_2, R_1=$CONHCH_3$ × HCl	2.74
21	R=NO_2, R_1=$CONH(CH_2)_3CH_3$ × HCl	4.33
22	R=NO_2, R_1=$CONH(CH_2)_3N(CH_3)_2$ × HCl	3.43
23	R=Cl, R_1=$CONH(CH_2)_3N(CH_3)_2$ × HCl	4.28
24	R=$CONH(CH_2)_3N(CH_3)_2$, R_1=$CONH(CH_2)_3N(CH_3)_2$	3.47
25	R=NH_2, R_1=CN	3.07
26	R=NH_2x HCl, R_1=CN	3.07

[a] Calculated as neutral species

The radical scavenging capacity of final compounds was measured against DPPH and ABTS stable radicals as well as ferric reducing ability/antioxidant power (FRAP) ability to reduce ferric to ferrous ion. The radical scavenging method using DPPH stable radical indicates the ability of tested species to donate proton/electron. In particular, this is a measure of reducing ability in iron-free medium [26,27]. The DPPH final concentration of the test compounds was 100 μM and results were read after 30 min (Table 3). The results obtained in this study were expressed as IC_{50} values with the exception of **19**, **20**, **21, 23** and **25** which did not react under the experimental condition. Compounds **17**, **18** and **22** showed poor reducing ability expressed as a very high concentration of IC_{50} (410 and 450 μM, respectively). The best activity was observed for compound **24** (100 μM) in comparison to BHT (IC_{50} 25 ± 4.2 μM). Regarding the substitution pattern of pyrido[1,2-*a*]benzimidazole scaffold, the best activity was observed for compound bearing *N*,*N*-dimethylamino-1-propyl carboxamide group at positions 3 and 7(8). The different alkyl substitutions of carboxamide at 3-position of heterocycle scaffold exhibited poor reducing ability (high IC_{50}). The expansion of alkyl chain from 1 to 4 carbon atoms of carboxamide at 3-position in combination with EWG substituent at 7(8)-position showed no activity at all.

Compound **25** bearing a nitrile group at the 3-position and an amino group at 7(8)-position of the pyrido[1,2-*a*]benzimidazole scaffold did not show any reduction in activity under the experimental condition, while its hydrochloride salt **26** exhibited limited activity ($IC_{50} = 262\,\mu M$), about 10× higher than BHT. The radical scavenging activity of all other compounds was very week in comparison to BHT.

Antioxidative activity

Antioxidative activity could be estimated by different in vitro and in vivo biological assays. In vitro assays rely on spectrophotometric measurements [25].

Table 3 Antioxidant activity of compounds **13**–**21** and **27** by DPPH, FRAP and ABTS methods

Comp	DPPH IC_{50} μM	FRAP $mmolFe^{2+}/mmol_C$	ABTS $mmolTEAC/mmol_C$
17	>300	8.54 ± 0.34	41.36 ± 4.75
18	>300	8.64 ± 0.95	55.01 ± 15.54
19	No	48.15 ± 1.43	52.60 ± 2.73
20	No	78.48 ± 6.91	19.52 ± 3.62
21	No	57.79 ± 14.28	23.02 ± 2.99
22	>300	5.45 ± 0.06	92.97 ± 1.44
23	No	21.64 ± 1.17	177.59 ± 1.47
24	100 ± 40	23.69 ± 1.25	176.40 ± 5.82
25	No	4407.21 ± 93.46	754.09 ± 0.71
26	261 ± 30	4838.34 ± 105.81	680.88 ± 1.69
BHT	25 ± 4.2	2089.34 ± 55.98	679.15 ± 37.48

no no activity under the experimental condition

The reducing power of tested compounds refers to their antioxidant activity. The ferric reducing ability of plasma (FRAP) assay [28,29] was used to determine the reducing power of novel compounds and the results are presented in Table 3. The FRAP values showed that compounds **25** and **26** (4407.21 ± 93.46 and 4838.34 ± 105.81 $mmolFe^{2+}/mmol_C$, respectively) have notable radical scavenging activity, while the rest of the compounds showed very low activity (8–78 $mmolFe^{2+}/mmol_C$). It should to be emphasized that compounds **25** and **26** showed the highest level of scavenging in comparison to BHT.

Conclusions

In this manuscript, we present the synthesis, antiproliferative and antioxidative activities of novel amido-substituted pyrido[1,2-*a*]benzimidazoles with different types and lengths of amide side chains placed on the tricyclic skeleton. The most pronounced antiproliferative effect in the micromolar range among all newly synthesized derivatives was observed for compound **25** on MCF-7 (IC_{50} = 6μM) and HeLa (IC_{50} = 8μM) cell lines. Additionally, *N*,*N*-dimethylamino-1-propyl carboxamide group in the unsubstituted or 7(8)-chloro-substituted derivative slightly enhanced the antiproliferative activity unlike the nitro group that decreased antiproliferative activity. On the other hand, protonated amino derivative **26** showed decrease in the antiproliferative activity in comparison to **25** probably due to reduced cell membrane penetration.

A biological evaluation concerning antioxidant activity of novel pyrido[1,2-*a*]benzimidazoles indicates that the compounds **25** and **26** have significant reducing power and promising radical scavenging activity in the FRAP and ABTS assays with comparable activity to the reference compound BHT. All these results position pyrido[1,2-*a*]benzimidazole **25** as an attractive lead compound for further optimization and development against cancer.

Materials and methods

Synthesis

General methods

All chemicals and solvents were purchased from commercial suppliers Aldrich and Acros. Chemicals were used without further purification, while solvents were distilled from appropriate drying agents. Melting points were determined by using SMP11 Bibby and Büchi 535 apparatus and were uncorrected. The ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or Varian Gemini 600 at 300, 600 and 150 and 75 MHz, respectively, in DMSO-d_6 solutions. Chemical shifts in ppm (δ) are in reference to TMS (s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet). TLC was carried out on Merck silica gel 60F-254 glass plates. Elemental analysis obtained on a Perkin-Elmer 2400 elemental analyser and the obtained analytical results are within 0.4 % of the theoretical value.

*General method for preparation of amido-substituted pyrido[1,2-a]benzimidazoles hydrochloride salts **17–24***

A suspension containing a pyrido[1,2-*a*]benzimidazolecarboxylic acid (**13**–**16**) in anhydrous DMF (2–7 mL) was stirred for several minutes at room temperature and the 1,1′-carbonyldiimidazole was added. After stirring for 1.5 h at room temperature, an amine was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was evaporated to reach a volume of 1–2 mL and the precipitate was filtered off and washed with small amount

of water (2 mL). The product was dried and suspended in absolute ethanol (5 mL), saturated with the gaseous HCl and stirred for 24 h at room temperature. After the filtration, the product was washed with small amount of diethylether (10 mL) to obtain hydrochloride salt.

N-methyl-1,3-dimethylpyrido[1,2-a]benzimidazole-4-carboxamide hydrochloride ***17***

From **13** (0.40 g, 1.60 mmol), 1,1′-carbonyldiimidazole (0.28 g, 1.70 mmol) and a 30 % solution of methylamine in water (0.17 mL, 1.60 mmol) were added in DMF (4 mL) to obtain 0.061 g (15 %) of crude amide (grey powder) which in turn afforded desired hydrochloride salt (0.19 g, 30 %) as grey powdered; mp 232–235 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.98 (d, 1H, J = 8.8 Hz), 8.70 (bs, 1H), 8.53 (t, 2H, J = 8.4 Hz), 7.95 (d, 1H, J = 8.0 Hz), 7.85 (t, 2H, J = 8.4 Hz), 7.70 (d, 1H, J = 8.0 Hz), 7.41 (s, 1H), 3.18 (s, 3H, CH_3), 2.93 (d, 3H, J = 4.6 Hz, CH_3), 2.57 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 162.4, 149.6, 148.7, 146.4, 135.1, 128.7, 122.3, 121.8, 118.7, 117.9, 117.5, 96.1, 23.1, 21.9; UV (EtOH) λ_{max}: 339, 250, 243; IR (diamond) (ν/cm^{-1}): 3394, 3206, 3039, 2573, 1713, 1662, 1646, 1540; Anal. ($C_{15}H_{16}ClN_3O$) Calc. C 62.18, H 5.57, N 14.50 %; Found C 62.04, H 5.69, N 14.73 %.

N-butyl-1,3-dimethylpyrido[1,2-a]benzimidazole-4-carboxamide hydrochloride ***18***

From **13** (0.40 g, 1.60 mmol), 1,1′-carbonyldiimidazole (0.28 g (1.70 mmol) and a 30 % solution of *n*-buthylamine in water (0.16 mL, 1.60 mmol) were added in DMF (4 mL) to obtain 0.035 g (7 %) of crude amide (grey powder) which in turn afforded desired hydrochloride salt (0.024 g, 71 %) as grey powdered; mp 239–243 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ8.97(t, 1H, J = 4.8 Hz), 8.80 (bs, 1H), 8.51 (d, 1H, J = 8.5 Hz), 7.93 (d, 1H, J = 8.4 Hz), 7.78 (t, 1H, J = 7.8 Hz), 7.61 (t, 1H, J = 8.0 Hz), 7.37 (s, 1H), 3.42 (q, 2H), 3.15 (s, 3H), 2.57 (s, 3H), 1.57 (m, 2H), 1.39 (m, 2H), 0.94 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.8, 147.6, 143.9, 140.8, 139.7, 129.3, 125.2, 120.5, 119.0, 118.5, 115.4, 114.8, 56.3, 44.6, 36.8, 264, 20.4, 20.1; UV (EtOH) λ_{max}: 343, 250, 244; IR (diamond) (ν/cm^{-1}): 3492, 3197, 2941, 2606, 1526; Anal. ($C_{18}H_{22}ClN_3O$) Calc. C 65.15, H 6.68, N 12.66 %; Found C 65.02, H 6.80, N 12.87 %.

N-(3-N,N-dimethylamino-1-propyl)-1,3-dimethylpyrido[1,2-a]benzimidazole-4-carboxamide hydrochloride ***19***

From **13** (0.50 g, 2.10 mmol), 1,1′-carbonyldiimidazole (0.37 g, 2.25 mmol) and 3-*N*,*N*-dimethylamino-1-propylamine (0.29 mL, 2.30 mmol) were added in DMF (7 mL) to obtain 0.262 g (40 %) of crude amide (grey powder) which in turn afforded desired hydrochloride salt (0.275 g, 93 %) as grey powdered; mp 275–279 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.20 (bs, 1H), 9.16 (t, 1H, J = 5.8 Hz), 8.50 (d, 1H, J = 8.4 Hz), 7.95 (d, 1H, J = 8.3 Hz), 7.77 (t, 1H, J = 8.8 Hz), 7.61 (t, 1H, J = 8.85 Hz), 7.35 (s, 1H), 3.17 (q, 2H, J = 6.1 Hz), 3.05–2.98 (m, 4H), 2.78 (s, 6H), 2.22 (s, 3H), 1.98 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 163.1, 147.8, 143.0, 142.6, 141.9, 128.8, 128.2, 124.1, 119.5, 118.4, 117.7, 114.8, 54.9, 42.2 (2C), 37.2, 24.3, 21.1, 20.2; UV (EtOH) λ_{max}: 350, 250, 245; IR (diamond) (ν/cm^{-1}): 3567, 3356, 2700, 1670, 1639; Anal. ($C_{19}H_{25}ClN_4O$) Calc. C 63.24, H 6.98, N 15.53 %; Found C 62.98, H 6.80, N 15.38 %.

N-methyl-1,3-dimethyl-7(8)-nitropyrido[1,2-a]benzimidazole-4-carboxamide hydrochloride ***20***

From **14** (0.40 g,1.40 mmol), 1,1′-carbonyldiimidazole (0.28 g,1.70 mmol) and a 30 % solution of methylamine in water (0.17 mL,1.60 mmol) were added in DMF (2.5 mL) to obtain 0.29 g (69 %) of crude amide (grey powder) which in turn afforded desired hydrochloride salt (0.155 g, 54 %) as light yellow powdered; mp 265–267 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 90.20 (bs, 1H), 9.16 (bs, 1H), 9.05 (bs, 1H), 8.92 (bs, 1H), 8.85 (s, 1H), 8.76 (s, 1H), 8.46 (d, 1H, J = 8.4 Hz), 8.38 (d, 1H, J = 8.5 Hz), 8.14 (d, 1H, J = 8.5 Hz), 7.94 (d, 1H, J = 8.7 Hz), 6.88 (s, 1H), 6.87 (s, 1H), 3.19 (s, 3H), 2.99 (d, 3H, J = 4.6 Hz), 2.54 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 164.5, 164.4, 159.7, 159.6, 150.9, 150.1, 149.4, 149.1, 144.2, 144.0, 140.9, 140.6, 140.3, 139.5, 132.2, 128.9, 121.1, 120.8, 118.9, 117.1, 116.8, 115.3, 114.5, 113.00, 44.5, 44.1, 31.8, 30.9, 21.1, 20.2; UV (EtOH) λ_{max}: 385, 293, 276, 237; IR (diamond) (ν/cm^{-1}): 3220, 3054, 2918, 1662, 1504; Anal. ($C_{15}H_{15}ClN_4O_3$) Calc. C 63.24, H 6.98, N 15.53 %; Found C 62.98, H 6.80, N 15.38 %.

N-butyl-1,3-dimethyl-7(8)-nitropyrido[1,2-a]benzimidazole-4-carboxamide hydrochloride ***21***

From **14** (0.40 g,1.40 mmol), 1,1′-carbonyldiimidazole (0.28 g, 1.70 mmol) and a 30 % solution of *n*-buthylamine in water (0.17 mL, 1.60 mmol) were added in DMF (2.5 mL) to obtain 0.153 g (32 %) of crude amide (grey powder) which in turn afforded desired hydrochloride salt (0.045 g, 25 %) as light yellow powdered; mp 214–217 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ **14a**: 9.30 (bs, 1H), 9.24 (bs, 1H), 9.03 (t, 1H, J = 2.3 Hz), 9.02 (t, 1H, J = 2.4 Hz), 8.89 (bs, 1H), 8.58 (d, 1H, J = 2.2 Hz), 8.44 (d, 1H, J = 9.2 Hz), 8.35 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.1 Hz), 8.11 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.3 Hz), 7.88 (d, 1H, J = 9.1 Hz), 6.93 (s, 1H), 6.83 (s, 1H), 3.41–3.34 (m, 2H), 3.33–3.30 (m, 2H), 3.10 (s, 3H), 3.04 (s, 3H), 2.63 (s, 3H), 2.54 (s, 3H), 1.63–1.56 (m, 2H), 1.53–1.48 (m, 2H), 1.49–1.42 (m, 2H), 1.41–1.32 (m, 2H), 0.97 (t, 3H, J = 7.3 Hz), 0.96 (t, 3H, J = 7.3 Hz); ^{13}C NMR

(DMSO-d_6, 150 MHz) δ 164.3, 164.0, 159.9, 159.4, 151.4, 151.1, 149.8, 149.6, 145.0, 144.8, 141.2, 140.9, 140.7, 139.9, 131.0, 129.9, 122.0, 121.7, 118.6, 118.1, 117.0, 116.5, 114.6, 113.1, 36.4, 35.7, 32.7, 31.9, 31.6, 30.8, 28.5, 28.2, 24.4, 24.2, 21.4, 20.8; UV (EtOH) λ_{max}: 390, 296, 238; IR (diamond) (ν/cm^{-1}): 3197, 2964, 1653, 1616; Anal. ($C_{18}H_{21}ClN_4O_3$) Calc. C 57.37, H 5.62, N 14.87 %; Found C 57.55, H 5.80, N 15.05 %.

N-(3-N,N-dimethylamino-1-propyl)-1,3-dimethyl-7(8)–nitropyrido[1,2-a]benzimidazole-4-carboxamide hydrochloride ***22***

From **14** (0.80 g, 0.70 mmol), 1,1′-carbonyldiimidazole (0.15 g, 0.77 mmol) and 3-*N*,*N*-dimethylamino-1-propylamine (0.10 ml, 0.77 mmol) were added in DMF (3.6 mL) to obtain 0.020 g (20 %) of crude amide (yellow powder) which in turn afforded desired hydrochloride salt (0.011 g, 50 %) as yellow powdered; mp 209–211 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.46 (bs, 1H), 9.40 (bs, 1H), 9.14 (t, 1H, J = 2.2 Hz), 9.08 (t, 1H, J = 2.3 Hz), 8.40 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.2 Hz), 8.34 (dd, 1H, J_1 = 9.1 Hz, J_2 = 2.2 Hz), 7.91 (d, 1H, J = 9.0 Hz), 7.83 (d, 1H, J = 9.0 Hz), 7.62 (s, 1H), 7.57 (s, 1H), 6.99 (s, 1H), 6.93 (s, 1H), 3.39 (s, 6H), 3.34 (s, 6H), 3.30 (s, 3H), 3.22 (s, 3H), 2.92 (q, 2H, J = 6.1 Hz), 2.88 (q, 2H, J = 6.0 Hz), 2.73–2.66 (m, 4H), 2.66 (s, 3H), 2.60 (s, 3H), 1.24–1.19 (m, 4H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 166.2, 166.1, 162.4, 162.0, 153.3, 153.0, 150.7, 150.4, 147.3, 146.8, 143.0, 142.6, 141.6, 140.6, 133.0, 132.4, 125.2, 124.7, 119.8, 119.1, 118.3, 117.8, 114.9, 113.9, 41.8 (2C), 37.6, 36.7, 33.8, 33.2, 28.9, 28.3, 27.9, 27.5, 24.2, 23.8 (q); UV (EtOH) λ_{max}: 389, 292, 237; IR (diamond) (ν/cm^{-1}): 3209, 2988, 1664, 1646; Anal. ($C_{19}H_{24}ClN_5O_3$) Calc. C 56.22, H 5.96, N 17.25 %; Found C 56.50, H 5.82, N 17.09 %.

N-(3-N,N-dimethylamino-1-propyl)-7(8)-chloro-1,3-dimethylpyrido[1,2-a]benzimidazole-4-carboxamide hydrochloride ***23***

From **15** (0.40 g, 0.72 mmol), 1,1′-carbonyldiimidazole (0.14 g, 0.72 mmol) and 3-*N*,*N*-dimethylamino-1-propylamine (0.16 mL, 0.98 mmol) were added in DMF (7.5 mL) to obtain 0.20 g (80 %) of yellow oil which in turn afforded desired hydrochloride salt (0.11 g, 51 %) as yellow powdered; mp 239–241 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.91 (bs, 2H), 9.25 (t, 1H, J = 2.4 Hz), 9.12 (t, 1H, J = 2.3 Hz), 8.54 (s, 1H), 8.47 (s, 1H), 8.38 (d, 1H, J = 8.3 Hz), 8.30 (d, 1H, J = 8.4 Hz), 7.90 (d, 1H, J = 8.4 Hz), 7.71 (d, 1H, J = 8.4 Hz), 7.10 (s, 1H),7.06 (bs, 1H), 3.68 (s, 6H), 3.64 (s, 6H), 2.64 (s, 6H), 3.50–3.43 (t, 4H, J = 6.9 Hz), 3.40–3.22 (m, 4H), 2.83 (s, 3H), 2.81 (s, 3H), 2.54 (s, 6H), 2.03–1.97 (m, 4H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 165.6, 165.1, 162.1, 161.5, 153.8, 153.3, 150.5, 150.0, 147.1, 146.5, 143.4, 143.0, 142.1, 141.9, 133.2, 132.9, 125.0, 124.6, 119.6, 119.3, 118.1, 117.7, 114.3, 113.4, 41.7 (2C, q), 38.0, 37.7, 33.5, 33.1, 29.1, 28.7, 27.6, 27.2, 24.1, 23.7 (q); UV (EtOH) λ_{max}: 352, 303, 281, 273, 254, 247, 219; IR (diamond) (ν/cm^{-1}): 3214, 2989, 1656, 1649; Anal. ($C_{19}H_{24}Cl_2N_4O$) Calc. C 57.73, H 6.12, N 14.17 %; Found C 57.90, H 5.98, N 14.05 %.

4,7(8)-N,N-di(3-N,N-dimethylamino-1-propyl)-1,3-dimethylpyrido[1,2-a]benzimidazole-4-carboxamide dihydrochloride ***24***

From **16** (0.05 g, 0.17 mmol), 1,1′-carbonyldiimidazole (0.07 g, 0.37 mmol) and 3-*N*,*N*-dimethylamino-1-propylamine (0.15 mL, 0.74 mmol) to obtain 0.09 g of yellow oil which in turn afforded desired hydrochloride salt (0.07 g, 67 %) as yellow oil; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.85 (bs, 2H), 9.80 (bs, 2H), 9.31 (bs, 2H), 9.10 (d, 1H, J = 9.0 Hz), 9.00 (d, 1H, J = 8.9 Hz), 8.16 (bs, 4H), 7.80 (s, 1H), 7.78 (s, 1H), 7.68 (d, 1H, J = 8.8 Hz), 7.62 (d, 1H, J = 8.8 Hz), 3.42 (bs, 24H), 3.19 (s, 3H), 3.15 (s, 3H), 2.90 (q, 8H, J = 6.4 Hz), 2.79–2.74 (m, 8H), 2.74 (s, 3H), 2.70 (s, 3H), 2.16–1.97 (m, 8H); UV (EtOH) λ_{max}: 345, 258, 219, 216, 210; IR (diamond) (ν/cm^{-1}): 3202, 2995, 1666, 1660; Anal. ($C_{25}H_{38}Cl_2N_6O_2$) Calc. C 57.14, H 7.29, N 15.99 %; Found C 56.98, H 7.44, N 15.70 %.

8-Amino-1,3-dimethylpyrido[1,2-a]benzimidazole-4-carbonitrile **25**

From **10** (0.20 g, 0.75 mmol) and solution of $SnCl_2 \times 2H_2O$ (1.40 g, 0.00585 mmol) in MeOH (2.2 mL) and concentrated HCl (2.2 mL) after stirring at refluxed for 0.5 h. After cooling, the reaction mixture was evaporated under vacuum and dissolved in water (25 mL). The resulting solution was treated with 20 % NaOH to pH = 14. Resulting product was filtered off and washed with water (10 mL) to obtain 0.17 g (96 %) of crude product (yellow powder); mp 285–286 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 7.48 (d, 1H, J = 1.9 Hz), 7.42 (d, 1H, J = 1.8 Hz), 7.00 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.9 Hz), 6.92 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.9 Hz), 6.70 (s, 1H), 6.66 (s, 1H), 5.25 (s, 2H), 5.20 (s, 2H), 2.95 (s, 3H), 2.90 (s, 3H), 2.55 (s, 3H) 2.53 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 146.9, 146.93, 144.9, 144.7, 144.4, 144.3, 143.6, 143.4, 136.5, 136.3, 130.7, 130.5, 123.4, 123.1, 119.6, 119.2, 115.7, 115.6, 115.4, 115.2, 111.9, 111.7, 98.0, 97.8, 20.6, 20.4, 20.3, 20.0 (q); UV (EtOH) λ_{max}: 420, 312, 271, 219; Anal. ($C_{14}H_{12}N_4$) Calc. C 71.17, H 5.12, N 23.71 %; Found C 71.40, H 5.02, N 23.90 %.

8-Amino-1,3-dimethylpyrido[1,2-a]benzimidazole-4-carbonitrile hydrochloride **26**

A suspension of **25** (0.05 g, 0.0021 mmol) in absolute ethanol (15 mL) was saturated with $HCl_{(g)}$. After 24 hours

of stirring, small amount of diethylether (5 mL) was added, and the resulting product was filtered off and washed with diethylether (10 mL) to obtain 0.06 g (73 %) of crude product (yellow powder); mp > 250 °C; ^{1}H NMR (DMSO-d_6, 600 MHz) δ 10.42 (bs, 3H), 10.40 (bs, 3H), 8.47 (d, 1H, J = 1.7 Hz), 8.45 (d, 1H, J = 1.6 Hz), 8.04 (d, 1H, J = 8.8 Hz), 8.00 (d, 1H, J=8.7 Hz), 7.59 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.9 Hz), 7.57 (dd, 1H, J_1 = 8.7 Hz, J_2 = 1.9 Hz), 7.03 (s, 1H), 7.00 (s, 1H), 3.07 (s, 3H), 3.05 (s, 3H), 2.66 (s, 3H), 2.64 (s, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz) δ 150.7, 150.4, 148.0, 147.6, 144.6, 144.5, 142.1, 141.9, 140.1, 139.9, 129.0, 128.7, 121.5, 121.1, 119.3, 119.0, 114.7, 114.6, 114.0, 113.7, 110.9, 110.6, 96.4, 96.2, 21.0, 20.6, 20.5, 20.4 (q); UV (EtOH) λ_{max}: 407, 271, 218; Anal. ($C_{14}H_{13}ClN_4$) Calc. C 61.65, H 4.80, N 20.54 %; Found C 61.75, H 4.64, N 20.70 %.

Antiproliferative activity

Cell culturing

The experiments were carried out on four human cell lines HeLa (cervical carcinoma), SW620 (colorectal adenocarcinoma, metastatic), MCF-7 (breast epithelial adenocarcinoma, metastatic), HepG2 (hepatocellular carcinoma); and BJ (normal diploid human fibroblasts) which were cultured as monolayers. The cell lines were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 % foetal 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.

Proliferation assays

The panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 5000 cells per well according to the doubling times of specific cell line. Test compounds and control compounds (cisplatin and 5-fluorouracile, 5–FU) were then added in five 10-fold dilutions (0.01–100 μM) followed by a 72-h incubation. The experiments were implemented according to the standard previously published experimental procedure [30].

Antioxidative activity

Each in vitro experiment was performed at least in triplicate, the results were averaged and the standard deviation of absorbance was less than 10 % of the mean, and the results are presented in Table 3.

Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH)

The determination of reducing activity was measured according to the previously reported procedure with some modification [25,26]. Briefly, to solution of DPPH (final concentration 100 μM) in absolute ethanol was added equal volume of tested compounds of various concentrations dissolved in DMSO. The assay was carried out in a 96-well microtitre plate. Ethanol and DMSO were used as control solution. Sample blank was also performed. After 30 min in dark at room temperature, the absorbance was recorded at 517 nm on microplate reader μQuant (Biotec Inc.). All measures were done in triplicate and the results presented in Table 3 were averaged. The percentage scavenging (PS) of test samples at each concentration was calculated using the following formula:

$$[(\text{Acontrol} - \text{Acompound})/\text{Acontrol}] \times 100$$

The IC_{50} values for each compound were calculated from dose–response curves using linear regression analysis by using GraphPad Prism 6 program.

Determination of ferric reducing/antioxidant power (FRAP assay)

The FRAP method was conducted according to the previously reported procedure [28] with minor modifications for assay on a 96-well microplate. The antioxidant capacity of the tested compounds was estimated as their power to reduce the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex, which is simple, fast and reproducible. A solution of 10 mM TPTZ and 20 mM ferric chloride was diluted in 300 mM sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. The tested compound solution (20 μL) was added to the 96-well microplate followed by working FRAP solution (280 μL). The mixture was shaken and incubated 30 min at 37 °C in the dark. Final concentration of tested compounds was (0.01 mM). The absorbance at 593 nm was recorded using microplate reader μQuant (Biotec Inc.). For FRAP assay, ferrous sulphate ($FeSO_4 \times 7H_2O$) was used to develop a 20–2000 μmol/L standard curve.

All results were then expressed as Fe^{2+} equivalents (Fe^{2+} μmol). All tests were done in triplicate and the results were averaged and presented in Table 2.

Free radical scavenging ability by the use of a stable ABTS radical cation (2,20 azinobis-(3-ethylbenzthiazoline-6-sulphonic acid)

The total antioxidant activity (TEAC) method [29–31] was modified and adjusted for microtiter plate reader. For the standard TEAC assay, $ABTS^{\cdot+}$ was prepared by mixing an ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture was allowed to stand for 12–16 h at room temperature in the dark until reaching a stable oxidative state. On the day of analysis, the $ABTS^{\cdot+}$ solution was diluted

with PBS (pH 7.4) to an absorbance of 0.700 ± 0.01 at 734 nm. The radical was stable in this form for more than two days when stored in the dark at room temperature. Standards and solutions of tested compounds (10 μL) were mixed with working $ABTS^{\cdot+}$ radical cation solution (200 μL) to each well of the microplate, shake and incubate at room temperature for 5 min. The decrease of absorbance at 734 nm was recorded by μQuant (Biotec Inc.). Aqueous phosphate buffer solution (without $ABTS^{\cdot+}$ solution) and Trolox (0.2–1.25 mmol/L) were used as a control and main calibrating standard, respectively. Results were expressed as average of three independent measures as Trolox equivalents (mmol TEAC/$mmol_C$) and presented in Table 2.

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References

1. Frolov AN (2004) Photochemic synthesis of pyrimido[1,2-*a*]benzimidazole derivatives. Russ J Gen Chem 74:1949–1951. doi:10.1007/s11176-005-0125-1
2. Monika G, Sarbjot S, Chander M (2014) Benzimidazole: an emerging scaffold for analgesic and anti-inflammatory agents. Eur J Med Chem 76:494–505. doi:10.1016/j.ejmech.2014.01.030
3. Perin N, Nhili R, Ester K, Laine W, Karminski-Zamola G, Kralj M, David-Cordonnier MH, Hranjec M (2014) Synthesis, antiproliferative activity and DNA binding properties of novel 5-amino-benzimidazo[1,2-*a*]quinoline-6-carbonitriles. Eur J Med Chem 80:218–227. doi:10.1016/j.ejmech.2014.04.049
4. Hranjec M, Kralj M, Piantanida I, Sedić M, Šuman L, Pavelić K, Karminski-Zamola G (2007) Novel cyano- and amidino-substituted derivatives of styryl-2-benzimidazoles and benzimidazo[1,2-*a*]quinolines. Synthesis, photochemical synthesis, DNA binding and antitumor evaluation, Part 3. J Med Chem 50:5696–5711. doi:10.1021/jm070876h
5. Ates-Alagoz Z, Yildiz S, Buyukbingol E (2007) Antimicrobial activities of some tetrahydronaphthalene-benzimidazole derivatives. Chemotherapy 53:110–113. doi:10.1159/000100011
6. Starčević K, Kralj M, Ester K, Sabol I, Grce M, Pavelić K, Karminski-Zamola G (2007) Synthesis, antiviral and antitumor activity of 2-substituted-5-amidino-benzimidazoles. Bioorg Med Chem 15:4419–4426. doi:10.1016/j.bmc.2007.04.032
7. Kathrotiya HG, Patel MP (2012) Microwave-assisted synthesis of 3′-indolyl substituted 4H-chromenes catalyzed by DMAP and their antimicrobial activity. Med Chem Res 21:3406–3416. doi:10.1007/s00044-011-9861-4
8. Goshev I, Mavrova A, Mihaylova B, Wesselinova D (2013) Antioxidant activity of some benzimidazole derivatives to definite tumor cell lines. J Cancer Res Ther 1:87–91. doi:10.14312/2052-4994.2013-13
9. Demirayak S, Kayagil I, Yurttas L (2011) Microwave supported synthesis of some novel 1,3-Diarylpyrazino[1,2-*a*]benzimidazole derivatives and investigation of their anticancer activities. Eur J Med Chem 46:411–416. doi:10.1016/j.ejmech.2010.11.007
10. Refaat HM (2012) Synthesis of potential anticancer derivatives of pyrido[1,2 *a*]benzimidazoles. Med Chem Res 21:1253–1260. doi:10.1007/s00044-011-9636-y
11. Badawey E, Kappe T (1995) Benzimidazole condensed ring system. IX. Potential antineoplastics. New synthesis of some pyrido[1,2-*a*]benzimidazoles and related derivative. Eur J Med Chem 30:327–332. doi:10.1016/0223-5234(96)88241-9
12. Maryanoff BE, Nortey SO, McNall JJ, Sanfilippo PJ, McComsey DF, Dubinsky B, Shank RP, Reitz AB (1999) Potential anxiolytic agents. 3. Novel A-ring modified pyrido[1,2-*a*]benzimidazoles. Bioorg Med Chem Lett 9:1547–1552. doi:10.1016/S0960-894X(99)00240-1
13. Kotovskaya SK, Baskakova ZM, Charushin VN, Chupakhin ON, Belanov EF, Bormotov NI, Balakhnin SM, Serova OA (2005) Synthesis and antiviral activity of fluorinated pyrido[1,2-*a*]benzimidazoles. Pharm Chem J 39:574–578. doi:10.1007/s11094-006-0023-9
14. Reitz AB, Gauthier DA, Ho W, Maryanoff BE (2000) Tautomerism and physical properties of pyrido[1,2-*a*]benzimidazole (PBI) GABA-A receptor ligands. Tetrahedron 56:8809–8812. doi:10.1016/S0040-4020(00)00874-7
15. Jordan AD, Vaidya AH, Rosenthal DI, Dubinsky B, Kordik CP, Sanfilippo PJ, Wu WN, Reitza AB (2002) Potential anxiolytic agents. Part 4: novel orally-active N5-substituted pyrido[1,2-a]benzimidazoles with high GABA-A receptor affinity. Bioorg Med Chem Lett 12:2381–2386. doi:10.1016/S0960-894X(02)00463-8
16. Ndakala AJ, Gessner RK, Gitari PW et al (2011) Antimalarial pyrido[12-a]benzimidazoles. J Med Chem 54:4581–4589. doi:10.1021/jm200227r
17. Maryanoff BE, Ho W, McComsey DF, Reitz AB, Grous PP, Nortey SO, Shank RP, Dubinsky B, Taylor RJ Jr, Gardocki JF (1995) Potential anxiolytic agents. Pyrido[1,2-a]benzimidazoles: a new structural class of ligands for the benzodiazepine binding site on GABA-A receptors. J Med Chem 38:16–20. doi:10.1021/jm00001a005
18. Sangani CB, Jardosh HH, Patel MP, Patel RG (2013) Microwave-assisted synthesis of pyrido[1,2-*a*]benzimidazole derivatives of *β*-aryloxyquinoline and their antimicrobial and antituberculosis activities. Med Chem Res 22:3035–3047. doi:10.1007/s00044-012-0322-5
19. Badawey ES, Kappe T (1995) Benzimidazole condensed ring system. IX. Potential antineoplastics. New synthesis of some pyrido[1,2-*α*]benzimidazoles and related derivative. Eur J Med Chem 30:327–332. doi:10.1016/0223-5234(96)88241-9
20. Badawey EA, Kappe T (1999) Benzimidazole condensed ring systems. XI. Synthesis of some substituted cycloalkyl pyrido[1,2-*a*]benzimidazoles with anticipated antineoplastic activity. Eur J Med Chem 34:663–667. doi:10.1016/S0223-5234(00)80036-7
21. Hranjec M, Pavlović G, Marjanović M, Kralj M, Karminski-Zamola G (2010) Benzimidazole derivatives related to 2,3-acrylonitriles, benzimidazo[1,2-*a*]quinolines and fluorenes: synthesis, antitumor evaluation in vitro and crystal structure determination. Eur J Med Chem 45:2405–2417. doi:10.1016/j.ejmech.2010.02.022
22. Hranjec M, Kralj M, Piantanida I, Sedic M, Suman L, Pavelic K, Karminski-Zamola G (2007) Novel cyano- and amidino-substituted derivatives of styryl-2-benzimidazoles and benzimidazo[1,2-a]quinolines. Synthesis, photochemical synthesis, DNA binding and antitumor evaluation, Part 3. J Med Chem 50:5696–5711. doi:10.1021/jm070876h
23. Perin N, Martin-Kleiner I, Nhili R, Laine W, David-Cordonnier MH, Vugrek O, Karminski-Zamola G, Kralj M, Hranjec M (2013) Biological activity and DNA binding studies of 2-substituted benzimidazo[1,2-*a*]quinolines bearing different amino side chains. Med Chem Comm 4:1537–1550. doi:10.1039/C3MD00193H

24. http://www.cambridgesoft.com
25. Kulišić T, Radonić A, Katalinić V, Miloš M (2004) Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem 85:633–640. doi:10.1016/j.foodchem.2003.07.024
26. Doulou I, Kontogiorgis C, Koumbis AE, Evgenidou E, Hadjipavlou-Litina D, Fylaktakidou KC (2014) Synthesis of stable aromatic and heteroaromatic sulfonyl-amidoximes and evaluation of their antioxidant and lipid peroxidation activity. Eur J Med Chem 80:145–153. doi:10.1016/j.ejmech.2014.04.040
27. Aksu K, Topal F, Gulcin I, Tümer F, Göksu S (2015) Acetylcholinesterase inhibitory and antioxidant activities of novel symmetric sulfamides derived from phenethylamines. Arch Pharm 348:446–455. doi:10.1002/ardp.201500035
28. Benzie IFF, Strain JJ (1996) The reducing ability of plasma as a measure of 'antioxidant' power—the FRAP assay. Anal Biochem 239:70–76. doi:10.1006/abio.1996.0292
29. Khan I, Ali S, Hameeda S, Ramaa NH, Hussainb MT, Wadood A, Uddin R, Ul-Haq Z, Khan A, Ali S, Choudhary MI (2010) Synthesis, antioxidant activities and urease inhibition of some new 1,2,4-triazole and 1,3,4-thiadiazole derivatives. Eur J Med Chem 45:5200–5207. doi:10.1016/j.ejmech.2010.08.034
30. Hranjec M, Lučić B, Ratkaj I, Kraljević Pavelić S, Piantanida I, Pavelić K, Karminski-Zamola G (2011) Novel imidazo[4,5-b]pyridine and triaza-benzo[c]fluorene derivatives: synthesis, antiproliferative activity and DNA binding studies. Eur J Med Chem 46:2748–2758. doi:10.1016/j.ejmech.2011.03.062
31. Gülçin I, Huyut Z, Elmastas M, Aboul-Enein HY (2014) Radical scavenging and antioxidant activity of tannic acid. Arab J Chem 3:43–53. doi:10.1016/j.arabjc.2009.12.008