Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 5 pp. 951–963, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

SYNTHESIS AND *IN VITRO* ANTIPROLIFERATIVE ACTIVITY OF NOVEL 2-ARYLIDENEAMINOBENZIMIDAZOLE DERIVATIVES

ANNA NOWICKA[1]*, HANNA LISZKIEWICZ[1], WANDA P. NAWROCKA[1]
JOANNA WIETRZYK[2] and JOANNA SADOWSKA[2]

[1]Wroclaw Medical University, Department of Drug Technology, Borowska 211A, 50-556 Wrocław, Poland
[2]Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Science, "NeoLek" Laboratory of Experimental Anticancer Therapy, Wrocław, Poland

Abstract A new class of Mannich bases **9-26**, derivatives of 2-amino-1H-benzimidazole, were obtained in the condensation of Schiff bases **1-4** or 2-benzylaminobenzimidazoles **5-8** with selected secondary amines: morpholine, piperidine, N-methylpiperazine, N-phenylpiperazine, 1-(2-pyridyl)piperazine, 1(2-methoxyphenyl)piperazine, 1-(2-pyrimidinyl)piperazine and formaldehyde in ethanol. The pyrimido[1,2-a]benzimidazole derivatives **27-29** have been synthesized in the reactions of Schiff base **2** with selected compounds containing active methylene group: acetylacetone, benzoylacetone and malononitrile. The structures **1-29** were confirmed by the results of elementary analysis and their IR, ^{1}H- and ^{13}C-NMR spectra. The products **1-29** are of interest for biological studies and can be substrates for further synthesis. All compounds were screened against the cells of MV4-11 human leukemia and then the most active of them **5**, **7**, **9-16**, **24-26**, **28**, **29** were tested towards human T47D breast and A549 lung cancer cells as well as normal mouse fibroblasts (BALB/3T3). The most active compound against the cancer cell lines was 4-amino-3-cyano-2-(4-hydroxyphenylene)-1,2-dihydropyrimido[1,2-a]benzimidazole (**29**) (IC_{50} 0.23 ± 0.05 μg/mL against MV4-11 cells) showing in parallel very low cytotoxicity towards mouse fibroblasts. Cisplatin was the control drug.

Keywords: 2-arylideneaminobenzimidazole, 2-benzylaminobenzimidazole, Mannich bases, pyrimido[1,2-a]benzimidazole, antiproliferative activity *in vitro*

The Mannich reaction is very important for the synthesis of biologically active compounds (1). Many studies have shown that Mannich bases possess potent biological activities: analgesic (2), antimalarial (3), anticonvulsant (4, 5), antipsychotic (6) or antimicrobial (7-10). The structure of the Mannich bases possess currently used drugs (11) with diverse pharmacological activity e.g., tramadol - an opioid pain reliever, procyclidine - used for the treatment of drug-induced parkinsonism, molindon - neuroleptic, falicain - used for local anesthesia in laryngology and rolitetracycline - a tetracycline antibiotic (Fig. 1).

The problem of treatment of neoplastic diseases forces to search for new compounds, also Mannich bases. The most recent medicinal chemistry publications (12-23) have reported that the Mannich bases, derivatives of the various heterocycles, show in preliminary tests antiproliferative activity *in vitro* against human tumor cell lines. In a few publications (12, 13), the authors revealed the possible mechanism of their antitumor effects by molecular docking studies.

In our recently published work (14) Mannich bases, derivatives of 2-thioxo-imidazo[4,5-*b*]pyridine, exhibited good antiproliferative activity *in vitro* against cancer cell lines: breast (MCF-7), lung (A549) and leukemia (MV4-11). The most active and in parallel selective towards cancer cells was 1-benzyl-6-bromo-3-morpholinemethyl-2-thioxoimidazo[4,5-b]pyridine. Compounds derived from 4-piperazinylquinoline and isatin (15) exhibited promising anticancer activity *in vitro* against human breast cancer cell lines (MDA-MB468, MCF-7). The Mannich bases, derivatives of 3-aminomethyl-2-aryloimidazo[2,1-b]benzothiazole were active against two breast cancer cell lines (MCF-7, HeLa) and liver cancer cell line (HepG2). The most active, among all obtained compounds, was 3-pyrrolidineamino-2-phenyl-6-fluoroimidazo[2,1-b]benzotiazole (16).

There is a new approach in the drug discovery that combines two or more pharmacophores with

* Corresponding author: e-mail: anna.nowicka@umed.wroc.pl

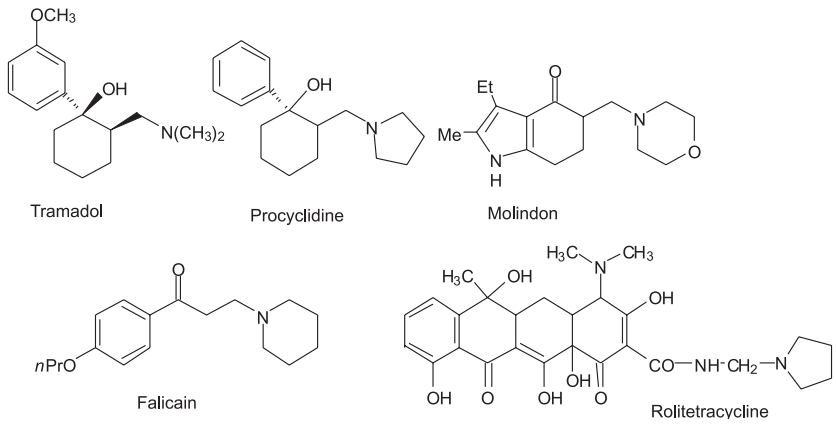


Figure 1. Selected drugs – Mannich bases

anticancer activity into a single molecule. Schiff and Schiff-Mannich bases are also common pharmacophores in the design and development of anticancer agents. Schiff bases also demonstrate interesting pharmacological activities including antidiabetic (24), antioxidant (25), antimicrobial (26-28), antitumor and anticancer (29) activities.

Significant activity against breast cancer line (MCF-7) exhibited Mannich-Schiff base derivatives of isatin-benzmidazole and isatin-thiazoline (17). Higher activities possess isatin-benzimidazole derivatives. They contained in their structures substituents e.g., morpholinmethyl, piperidinmethyl, N-phenylpiperazinemethyl, N-(2-methoxyphenyl)piperazinemethyl or N-methylpiperazinemethyl. The cytotoxic effects *in vitro* against liver cancer cell line (HepG2) demonstrated Schiff and Mannich bases, derivatives of 5-substituted-4-amino-3-thioxo-1,2,4-triazole (18).

Another way to search for biologically active compounds, including the anticancer activity, is to modify the chemical structure of known drugs of various pharmacological activity or natural compounds. This chemical modification could change the profile of action of new synthesized compounds. An interesting example was the use of ofloxacin – antibacterial drug, as a substrate in Schiff and then Mannich condensations with N-methylpiperazine and formaldehyde. New products which possess anticancer activity *in vitro*, have been obtained (19). *Via* the Mannich condensation, anticancer prodrugs of anthracycline antibiotics: doxorubicin and daunorubicin, which contain in their structure polyethylene glycols (PEGs), have been synthesized. PEGs are potential drug carriers for improving the therapeutic index of anticancer agents. Obtained prodrugs, at the same dose, showed comparable cytotoxicity as anthracyclines, but have prolonged time of action (20). 6α7β-Dihydroxyvoucapan-17β-oic acid (21), isolated from the fruit of the Brazilian plant *Pterodon polygalaeflorus*, possesses anti-inflammatory and analgesic activity. The aminomethylation of this diterpenoid led to six compounds, which showed antiproliferative activity *in vitro* against nine human cell lines (UACC-62, MCF-7, NCI-ADR/RES, 786-0, NCI-H460, PC-3, OVCAR-036, HT-29, K562).

Synthesis of new 2-aminobenzimidazole derivatives, possessing anticancer activity, is now one of the most important directions of research conducted on this group of compounds. 2-Aminobenzimidazole-based compounds demonstrated high cytotoxic activity against human cancer cell lines: lung (A549), breast (MCF-7) and leukemia (HL-60) (30). The main goal of this paper was to synthesise Schiff bases, in reaction of 2-aminobenzimidazole with selected aromatic aldehydes and its chemical modification: by reduction of obtained imines, Mannich condensation or/and reaction with selected compounds containing active methylene group. All the synthesised compounds were examined for their antiproliferative activity *in vitro* against human cancer cell lines: leukemia (MV4-11), breast (T47D) and lung (A549) and also mouse fibroblast cell line (BALB/3T3).

EXPERIMENTAL

Chemistry

Melting points were measured with a Boetius melting point apparatus. The new products were analyzed using a Perkin Elmer 2400 analyzer. IR spectra (in KBr) were recorded with an IR 75 spec-

trophotometer, ^{1}H- and ^{13}C NMR spectra – on a Bruker AVANCE DRX 300 MHz apparatus using DMSO-d_6 as an internal standard. The course of reaction and the purity of products were checked by TLC (Kieselgel G, Merck) in diethyl ether : ethanol 5 : 1, v/v as eluent.

The synthesis of 2-aminobenzimidazole (31) and Schiff base **4** (32) have been presented in our previous articles.

General procedure for the preparation of compounds 1-4

Reaction of 2-aminobenzimidazole with selected aromatic aldehydes: salicylic, 4-hydroxy-, 3-hydroxy- and 2-chlorobenzaldehyde. To a solution of 2-aminobenzimidazole (0.01 mol) in ethanol (30 mL) appropriate aromatic aldehydes were added and catalyst Triflate was used. The solution was refluxed ca. 8-10 h (TLC control). After cooling, the precipitate was filtered, washed with diethyl ether, dried and crystallized from appropriate solvent.

2–(Salicylideneamino)benzimidazole (1)

Obtained as a yellow precipitate, yield 1.80 g (76%); crystallized from ethanol; m.p. 222-223°C; IR (KBr, cm^{-1}): 3650 (OH), 3420 (NH), 3080, 1600 (ring), 1520 (C=C, C=N), 1260, 1230 (OH), 760 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 7.01 (m, 2H, Ar-H), 7.20 (m, 2H, Ar-H), 7.48 (m, 3H, Ar-H), 7.86 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.67 (s, 1H, -CH=N-), 12.13 (s, 1H, -OH), 12.73 (s, 1H, -NH, imidazole); Analysis: calcd. for $C_{14}H_{11}N_3O$ (237.26): C 70.87, H 4.67, N 17.71%; found: C 70.47, H 4.63, N 17.70%.

2-(4-Hydroxybenzylideneamino)benzimidazole (2)

Obtained as a yellow precipitate, yield 1.88 g (79%); crystallized from ethanol; m.p. 267-269°C; IR (KBr, cm^{-1}): 3360 (NH), 3080 (CH arom.), 1635 (CH=N, ring), 1500, 1520 (C=C), 1260 (C-O-H), 840 (CH), 730 (CH); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 6.95 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.15 (m, 2H, Ar-H), 7.47 (m, 2H, Ar-H), 7.92 (d, 2H, *J* = 8.7 Hz, Ar-H), 9.31 (s, 1H, -CH=N-), 10.44 (s, 1H, -OH), 12.51 (s, 1H, -NH-, imidazole). Analysis: calcd. for $C_{14}H_{11}N_3O$ (237.26): C 70.87, H 4.67, N 17.71%; found: C 70.53, H 4.84, N 17.16%.

2-(3-Hydroxybenzylideneamino)benzimidazole (3)

Obtained as a yellow precipitate, yield 1.82 g (77%); crystallized from toluene; m.p. 221-223°C; IR (KBr, cm^{-1}): 3380 (NH), 3070 (-CH-), 1680 (CH=N ring), 1230 (C-O-H), 890, 780 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 7.04 (m, 1H, Ar-H); 7.18 (m, 2H, Ar-H); 7.38 and 7.49 (t, 5H, *J* = 7.8 Hz + m, Ar-H); 9.37 (s, 1H, -CH=N-); 9.87 (s, 1H, -OH); 12.67 (s, 1H, -NH-, imidazole). Analysis: calcd. for $C_{14}H_{11}N_3O$ (237.26): C 70.87, H 4.67, N 17.71%; found: C 70.82, H 4.90, N 17.59%.

General procedure for the preparation of compounds 5-8

Reduction of azamethine bond in Schiff bases. To a solution of Schiff bases **1-5** (0.01 mol) in isopropanol (60 mL) $NaBH_4$ (0.01 mol) was added. The solution was refluxed for ca. 6 h. The solvent was evaporated under reduced pressure, 200 mL of cold water and ice was added to cold and dry residue. The precipitate formed was filtered, washed with water to neutral reaction. After drying, precipitates were crystallized from appropriate solvent.

2-(Salicylamino)benzimidazole (5)

Obtained as a white precipitate, yield 1.82 g (76%); crystallized from ethanol; m.p. 225-226°C; IR (KBr, cm^{-1}): 3420 (NH), 2920 (-CH_2-), 1600 (-CH-, arom.), 1280 (C-OH), 760 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 4.39 (d, 2H, *J* = 5.30 Hz, -*CH_2*-NH-), 6.83 (m, 2H, Ar-H), 6.92 (m, 2H, Ar-H), 7.17 (m, 4H, Ar-H), 7.30 (br, 1H, -CH_2-*NH*-), 9.70 (s, 1H, -OH), 11.67 (s, 1H, -NH-, imidazole). Analysis: calcd. for $C_{14}H_{13}N_3O$ (239.27): C 70.28, H 5.48, N 17.56%; found: C 70.17, H 5.47, N 17.53%.

2-(4-Hydroxybenzylamino)benzimidazole (6)

Obtained as a white precipitate, yield 1.82 g (76%); crystallized from ethanol; m.p. 225-226°C; IR (KBr, cm^{-1}): 3420 (NH), 3200 (OH), 2920 (CH), 2850 (-CH_2-), 1600 (C=C), 1280 (C-OH), 1175, 820, 760; ^{1}H NMR (300 MHz, DMSO, δ, ppm): 4.45 (d, 2H, *J* = 5.4Hz, -*CH_2*-NH-), 7.10 (br, 1H, -CH_2-*NH*-), 7.12 (m, 2H, Ar-H), 7.35 (d, 2H, *J* = 8.6 Hz, Ar-H), 7.60 (m, 2H, Ar-H), 8.03 (d, 2H, *J* = 8.6 Hz, Ar-H), 9.30 (s, 1H, -OH); 11.0 (s, 1H, -NH-, imidazole). Analysis: calcd. for $C_{14}H_{13}N_3O$ (239.27): C 70.28, H 5.48, N 17.56%; found: C 70.16, H 5.75, N 17.23%.

2-(3-Hydroxybenzylamino)-1H-benzimidazole (7)

Obtained as a white precipitate, yield 1.76 g (74%); crystallized from ethanol; m.p. 200-202°C; IR (KBr, cm^{-1}): 3410 (NH), 3080 (CH arom.), 2920 (-CH_2-), 1280 (-C-O-H), 880, 730 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 4.43 (d, 2H, *J* = 5.5 Hz, -*CH_2*-NH-); 6.61 + 6.80 +7.08 (m, 9H, -CH_2-*NH*- and Ar-H); 9.39 (s, 1H, -OH), 11.76 (s, 1H, -

NH-, imidazole). Analysis: calcd. for $C_{14}H_{10}ClN_3$ (255.7): C 70.28; H 5.48; N 17.56%; found: C 70.44; H 5.62; N 17.55%.

2-(2-Chlorobenzylamino)-1H-benzimidazole (8)

Obtained as a white precipitate, yield 2.42 g (94%); crystallized from dioxane; m.p. 104-106°C; IR (KBr, cm^{-1}): 3080 (CH arom.); 3300 (NH); 2845 (-CH_2-); 1600 (NH); 1575 (ring); 770 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 4.59 (d, 2H, *J* = 5,4 Hz, -*CH*$_2$-NH-); 6.87 (m, 2H, Ar-H); 7.12 (m, 3H, -CH_2-*NH*- and Ar-H); 7.27 (m, 2H, Ar-H); 7.44 (m, 2H, Ar-H); 10.87 (s, 1H, -NH-, imidazole). Analysis: calcd. for $C_{14}H_{13}N_3O$ (239.5): C 65.25, H 4.88, N 16.30%; found: C 65.06, H 5.30, N 15.96 %.

General procedure for the preparation of 9-26

The Mannich reaction. To a solution of appropriate Schiff bases **1-4** (0.01 mol) or 2-arylaminobenzimidazoles **5-8** (0.01 mol) in ethanol (25 mL) selected secondary amines: morpholine, piperidine, N-methylpiperazine, N-phenylpiperazine, 1-(2-pyridyl)piperazine, 1(2-methoxyphenyl)piperazine, 1-(2-pyrimidinyl)piperazine (0.01 mol) and 37% formaldehyde (0.03 mol) were added. The mixture was stirred at room temperature for ca. 4-6 h (TLC control). The precipitate formed was filtered, washed with diethyl ether, dried and crystallized form appropriate solvent.

1-[(Piperidin-1-yl)methyl]-2-(salicylideneamino) benzimidazole (9)

Obtained as a yellow precipitate, yield 2.10 g (63%); crystallized from ethanol; m.p. 149-151°C; IR (KBr, cm^{-1}): 3030 (CH, arom.), 2920 (-CH_2-), 1600 (ring), 1580 (C=N), 1180 (C-O-H), 760 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 1.45 (m, 6H, -CH_2-CH_2-CH_2-, piperidine), 2.49 (m, 4H, -CH_2-N-CH_2-, piperidine), 5.11 (s, 2H, -CH_2-), 6.99 (m, 2H, -Ar-H), 7.23 (m, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 7.64 (m, 2H, Ar-H), 7.97 (d, 1H, *J* = 6.6 Hz, Ar-H), 9.65 (s, 1H, -CH=N-), 9.72 (s, 1H, -OH). ^{13}C NMR (300 MHz, DMSO, δ, ppm): 165.57, 160.35, 154.31, 140.99, 135.90, 134.91, 131.28, 122.33, 122.24, 120.03, 119.75, 118.59, 116.82, 111.32, 64.46, 51.25 (2C), 25.33 (2C), 23.39; Analysis: calcd. for $C_{20}H_{22}N_4O$ (334.41): C 71.83, H 6.63, N 16.75%; found: C 71.64, H 6.54, N 16.40%.

1-[(Morpholin-4-yl)methyl]-2-(salicylideneamino)benzimidazole (10)

Obtained as a yellow precipitate, yield 2.28 g (68%); crystallized from ethanol; m.p. 153-154°C; IR (KBr, cm^{-1}): 2920 (-CH_2-), 1600 (ring), 1580 (C=N), 1180 (C-O-H), 760 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.56 (m, 4H, -CH_2-N-CH_2-, morpholine), 3.53 (m, 4H, -CH_2-O-CH_2-, morpholine), 5.13 (s, 2H, -CH_2-), 7.02 (m, 2H, Ar-H), 7.24 (m, 2H, Ar-H), 7.49 (m, 1H, Ar-H), 7.65 (m, 2H, Ar-H), 7.98 (m, 1H, Ar-H), 9.65 (s, 1H, CH=N-), 9.74 (s, 1H, -OH). ^{13}C NMR (300 MHz, DMSO, δ, ppm): 164.51, 160.38, 154.29, 141.03, 135.75, 134.92, 131.21, 122.43, 122.32, 120.08, 119.76, 118.67, 116.80, 111.17, 66.06 (2C), 63.73, 50.46 (2C); Analysis: calcd. for $C_{14}H_{13}N_3O$ (239.5): C 67.84, H 5.99, N 16.66%; found: C 67.61, H 5.67, N 16.54%.

1-[(4-Methyl-piperazin-1-yl)methyl]-2-(salicylideneamino)benzimidazole (11)

Obtained as a yellow solid, yield 1.88 g (51%); crystallized from DMF; m.p. 157-161°C; IR (KBr, cm^{-1}): 2950 (CH_3), 2920 (-CH_2-), 1600 (ring), 1540 (C=N), 1180 (C-O-H), 760 (CH, arom.). ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.13 (s, 3H, -CH_3), 2.42 (m, 4H, -CH_2-N-CH_2-, piperazine), 2.80 (m, 4H, -CH_2-N-CH_2-, piperazine), 5.13 (s, 2H, -CH_2-), 7.03 (d, 2H, *J* = 7.8 Hz, Ar-H), 7.24 (m, 2H, Ar-H), 7.58 (m, 3H, Ar-H), 7.97 (d, 1H, *J* = 7.2 Hz, Ar-H), 9.41 (s, 1H, -CH=N-), 9.73 (s, 1H, -OH). ^{13}C NMR (300 MHz, DMSO, δ, ppm): 164.65, 160.33, 154.32, 141.00, 135.90, 134.90, 131.27, 122.32, 122.23, 120.03, 118.60, 116.81, 116.73, 111.33, 64.46, 55.34 (2C), 51.25 (2C), 43.39; Analysis: calcd. for $C_{20}H_{23}N_5O$ (349.43): C 68.74, H 6.63, N 20.04%; found: C 68.02, H 6.72, N 20.01%.

1-(Piperidin-1-yl)-2-(4-hydroxybenzylideneamino)benzimidazole (12)

Obtained as a yellow precipitate, yield 0.841 g (25%); crystallized from ethanol; m.p. 187-190°C; IR (KBr, cm^{-1}): 2940 (-CH_2-), 1600 (ring), 1450 (C=N), 1140 (C-O-H), 840 (CH), 760 (CH); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 1.49 (m, 6H, -CH_2-CH_2-CH_2-, piperidine), 2.55 (m, 4H, -CH_2-N-CH_2-, piperidine), 5.19 (s, 2H, -CH_2-), 6.96 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.18 (m, 2H, Ar-H), 7.60 (m, 2H, Ar-H), 7.92 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.97 (d, 1H, *J* = 8.4Hz, Ar-H), 9.34 (s, 1H, -CH=N-), 9.72 (s, 1H, -OH). ^{13}C NMR (300 MHz, DMSO, δ, ppm): 158.24, 154.64, 152.82, 141,50, 137.23, 136.58, 128.27, 126.48, 121.63 (2C), 118.25, 115.97 (2C), 111.19, 64.08), 51.75 (2C), 25.46 (2C), 23.41; Analysis: calcd. for $C_{20}H_{22}N_4O_1$ (334.41): C 70.94, H 5.54, N 17.03%; found: C 71.02, H 5.62, N 16.93%.

1-(Morpholin-4-yl-methyl)-2(-4-hydroxybenzylideneamino)benzimidazole (13)

Obtained as a yellow precipitate, yield 1.59 g (47%); crystallized from dioxane; m.p. 200-204°C; IR (KBr, cm^{-1}): 2940 (-CH_2-), 1600 (ring), 1450 (C=N ring), 1360 (NH), 1140 (C-O-H), 840, 760 (CH); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.58 (m, 4H, CH_2-N-CH_2-, morpholine), 3.55 (m, 4H, -CH_2-O-CH_2-, morpholine), 5.21 (s, 2H, -CH_2-), 6.95 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.23 (m, 2H, Ar-H), 7.61 (m, 2H, Ar-H), 7.98 (d, 2H, *J* = 8.4 Hz, Ar-H), 9.36 (s, 1H, -CH=N-), 9.46 (s, 1H, -OH); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 164.59, 160.27, 155.79, 141.21, 138.54, 136.77, 131.97 (2C), 122.11, 121.77, 118.34, 116.62, 116.06, 111.08, 66.17 (2C), 63.32, 50.42 (2C). Analysis: calcd. for $C_{19}H_{20}N_4O_2$ (336.39): C 70.43, H 5.62, N 16.97%; found: C 70.71, H 5.23, N 16.63%.

1-[(Piperidin-1-yl)methylo]-2-(3-hydroxybenzylideneamino)benzimidazole (14)

Obtained as a yellow precipitate, yield 1.60 g (48%), crystallized from toluene; m.p. 161-163°C; IR (KBr, cm^{-1}): 3080 (CH arom.), 2940 (-CH_2-), 1610 (arom., ring), 1480 (C=N ring), 1280 (C-O-H), 900, 760 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 1.24 (m, 2H, -CH_2-*CH_2*-CH_2-, piperidine); 1.46 (m, 4H, -*CH_2*-CH_2-*CH_2*-, piperidine); 2.56 (m, 4H, -*CH_2*-*N*-*CH_2*-, piperidine); 5.22 (s, 2H, Ar-H); 7.04 (m, 1H, Ar-H); 7.22 (m, 1H, Ar-H); 7.39 (t, 2H, *J* = 7.8 Hz, Ar-H); 7.60 (m, 4H, Ar-H); 9.40 (s, 1H, -OH); 9.86 (s, 1H, -CH=N-); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 160.25, 157.8, 155.15, 141.14, 136.99, 130.15, 128.84), 127.58, 122.21, 122.08, 120.32, 118.60, 114.77, 111.43, 64.28, 51.20 (2C), 25.47 (2C), 23.39. Analysis: calcd. for $C_{20}H_{22}N_4O$ (334.4): C 71.83, H 6.63, N 16.75%; found: C 72.23, H 6.77, N 16.79%.

1-[(Morpholin-1-yl)methyl]-2-(3-hydroxybenzylideneamino)benzimidazole (15)

Obtained as a yellow precipitate, yield 2.05 g (61%); crystallized from toluene; m.p. 163-165°C; IR (KBr, cm^{-1}): 3080 (CH), 2920 (-CH_2-), 1610 (arom., ring), 1480 (C=N ring), 1280 (C-O-H), 900, 780 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.59 (t, 4H, *J* = 4.2 Hz, -CH_2-N-CH_2-, morpholine); 3.54 (t, 4H, *J* = 4.2 Hz, -CH_2-O-CH_2-, morpholine); 5.23 (s, 2H, -CH_2-); 7.04 (m, 1H, Ar-H); 7.24 (m, 2H, Ar-H); 7.40 (m, 1H, Ar-H); 7.54 (m, 2H, Ar-H); 7.62 (m, 1H, Ar-H); 7.95 (m, 1H, Ar-H); 9.41 (s, 1H, -OH); 9.84 (s, 1H, -CH=N-). ^{13}C NMR (300 MHz, DMSO, δ, ppm): 159.85, 158.78, 155.07, 141.11, 136.50, 135.93, 130.15, 122.37, 122.21, 121.65, 120.37, 118.68, 114.93, 111.31, 66.02 (2C), 63.47, 50.41 (2C); Analysis: calcd. for $C_{19}H_{20}N_4O$ (336,4): C 67.84, H 5.99, N 16.66%; found: C 69.45, H 6.00, N 16.20%.

1-[(4-Phenylpiperazin-1-yl)methyl]-2-(3-hydroxybenzylideneamino)benzimidazole (16)

Obtained as a yellow precipitate, yield 2.84 g (64%); crystallized from toluene; m.p. 149-150°C; IR (KBr, cm^{-1}): 3010 (CH arom.), 2940 (-CH_2-), 1610 (arom., ring), 1480 (C=N ring), 1280 (C-O-H), 800, 770 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.74 (t, 4H, -CH_2-N-CH_2-, piperazine); 3.09 (t, 4H, -CH_2-N-CH_2-, piperazine); 5.31 (s, 2H, -CH_2-); 6.70 (t, 1H, *J* = 7.2 Hz, Ar-H); 6.84 (d, 1H, *J* = 7.8 Hz, Ar-H); 7.13 (m, 6H, Ar-H); 7.36 (t, 1H, *J* = 7.8 Hz, Ar-H); 7.56 (m, 2H, Ar-H); 7.73 (m, 2H, Ar-H), 9.41 (s, 1H, -OH); 9.61 (s, 1H, -CH=N-). Analysis: calcd. for $C_{25}H_{25}N_5O$ (411.50): C 72.97, H 6.12, N 17.02%; found: C 72.82, H 6.28, N 17.27%.

1-[((4-Pyridin-2-yl)piperazin-1-yl)methyl]-2-(3-hydroxybenzylideneamino)benzimidazole (17)

Obtained as a yellow precipitate, yield 1.45 g (35%); crystallized from toluene; m.p. 199-200°C; IR (KBr, cm^{-1}): 3010 (CH arom.), 2920 (-CH_2-), 1610 (arom.), 1480 (C=N ring), 1260 (C-O-H), 890, 780 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.49 (m,4H, -CH_2-N-CH_2-, piperazine); 3.26 (m, 4H, -CH_2-N-CH_2-, piperazine); 5.29 (s, 2H, -CH_2-); 6.61 (m, 1H, Ar-H); 7.38 (m, 8H, Ar-H); 8.28 (m, 3H, Ar-H); 9.38 (s, 1H, -OH); 9.85 (s, 1H, -CH=N-); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 160.84, 157.83, 155.43, 155.09, 149.51, 141.10, 136.51, 135.81, 135.17, 127.5, 122.40, 122.21, 121.66, 121.32, 120.39, 119.9, 118.68, 114.85, 109.99, 63.42, 49.85 (2C), 43.18 (2C); Analysis: calcd. for $C_{24}H_{22}N_6O$ (412,49): C 69.88, H 5.86, N 20.37%; found: C 69.64, H 5.62, N 20.57%.

1-[(4-(2-Methoxyphenyl)piperazin-1-yl)methyl]-2-(3-hydroxybenzylideneamino)-benzimidazole (18)

Obtained as a yellow precipitate, yield 2.39 g (54%); crystallized from toluene; m.p. 147-149°C; IR (KBr, cm^{-1}): 3040 (CH arom.), 2940 (-CH_2-), 2840 (Ar-OCH_3), 1610 (arom., ring), 1480 (C=N), 1280 (C-O-H), 910, 770 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.74 (m, 4H, -CH_2-N-CH_2-, piperazine), 2.98 (m, 4H, -CH_2-N-CH_2-, piperazine), 3.65 (s, 3H, -OCH_3); 5.31 (s, 2H, -CH_2-); 6.84 (m, 4H, Ar-H); 7.04 (m, 1H, Ar-H); 7.24 (m, 2H, Ar-H); 7.39 (m, 1H, Ar-H); 7.52 (m, 3H, Ar-H); 7.71 (m, 1H, Ar-H); 9.41 (s, 1H, -OH); 9.85 (s, 1H, -CH=N-). Analysis: calcd. for $C_{26}H_{27}N_5O_2$ (441.53): C 70.73, H 6.16, N 15.86%; found: C 70.65, H 6.19, N 15.83%.

1-[(Piperidin-1-yl)methyl]-2-(2-chlorobenzylideneamino)benzimidazole (19)

Obtained as a yellow precipitate, yield 2.65 g (75%); crystallized from toluene; m.p. 123-125°C; IR (KBr, cm^{-1}): 3050 (CH) 2940, 2870 (-CH_2-); 1600 (ring); 1480 (C=N); 750 (-CH_2-); 770 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 1.45 (m, 6H, -CH_2-CH_2-CH_2-, piperidine); 2.45 (m, 4H, -CH_2-N-CH_2-, piperidine); 5.26 (s, 2H, -CH_2-); 7.23 (m, 2H, Ar-H); 7.59 (m, 1H, Ar-H); 7.68 (m, 4H, Ar-H); 8.74 (d, 1H, *J* = 7.5 Hz, Ar-H); 9.81 (s, 1H, -CH=N-); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 160.29, 154.62, 141.04, 136.21, 136.09, 134.26, 131.87, 130.39, 128.72, 127.98, 127.86, 122.48, 118.95, 111.64, 64.31, 51.13 (2C), 25.45 (2C), 23.36; Analysis: calcd. for $C_{20}H_{21}N_4Cl$ (352.9): C 68.08, H 6.00, N 15.88%; found: C 68.44, H 6.07, N 15.91%.

1-[(4-Phenylpiperazin-1-yl)methyl]-2-(2-chlorobenzylideneamino)-1H-benzimidazole (20)

Obtained as a yellow precipitate, yield 2.45 g (56%); crystallized from toluene; m.p. 174-175°C; IR (KBr, cm^{-1}): 3075 (CH arom.); 2925 (-CH_2-); 1590 (ring); 1500 (C=N); 750 (CH arom.). ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.75 (m, 4H, CH_2-N-CH_2-, piperazine); 3.10 (m, 4H, -CH_2-N-CH_2-, piperazine); 5.38 (s, 2H, -*CH_2*-); 6.72 (d, 1H, *J* = 7.24 Hz, Ar-H); 6.85 (d, 2H, *J* = 8.1 Hz, Ar-H); 7.14 and 7.27 (m, 4H, Ar-H); 7.63 (m, 4H, Ar-H); 8.32 (d, 1H, *J* = 7.5 Hz, Ar-H); 9.84 (s, 1H, -CH=N-); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 160.55, 154.69, 150.84, 141.02, 136.27, 135.90, 134.39, 131.85, 130.42, 128.96, 128.94 (2C), 128.75, 128.05, 122.64, 119.03, 118.84, 115.53 (2C), 111.62, 63.29, 49.87 (2C), 48.42 (2C); Analysis: calcd. for $C_{25}H_{24}N_5Cl$ (429.9): C 69.84, H 5.63, N 16.29%; found: C 70.15, H 5.78, N 16.22%.

1-[(4-(Pyridin-2-yl)piperazin-1-yl)methyl]-2-(2-chlorobenzylideneamino)-1H-benzimidazole (21)

Obtained as a yellow precipitate, yield 2.48 g (57%); crystallized from toluene; m.p. 168-169°C; IR (KBr, cm^{-1}): 3010 (CH); 2950 (-CH_2-); 1600 (ring); 1480 (C=N); 775, 760 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.78 (t, 4H, -CH_2-N-CH_2-, piperazine); 3.54 (t, 4H, CH_2-N-CH_2-, piperazine); 5.28 (s, 2H, -CH_2-); 6.56 (m, 2H, Ar-H); 7.28 (m, 2H, Ar-H); 7.40 (m, 2H, Ar-H); 7.50 (m, 3H, Ar-H); 7.77 (m, 1H, Ar-H); 8.18 (m, 1H, Ar-H); 8.40 (d, 1H, *J* = 7.5 Hz, Ar-H); 9.94 (s, 1H, -CH=N-); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 161.62, 159.14, 154.89, 149.15, 141.73, 137.60, 137.41, 135.94, 135.47, 132.72, 130.45, 128.79, 127.14, 122.99, 122.88, 119.68, 113.28, 110.67, 106.98, 64.22, 50.46 (2C), 45.07 (2C); Analysis: calcd. for $C_{24}H_{23}N_6Cl$ (430.9): C 66.89, H 5.38, N 19.50%; found: C 67.02, H 5.44, N 19.80%.

1-[(4-(2-Methoxyphenylene)piperazin-1-yl)methyl]-2-(2-chlorobenzylideneamino)-1H-benzimidazole (22)

Obtained as a yellow precipitate, yield 3.22 g (69%); crystallized from toluene; m.p. 159-161°C; IR (KBr, cm^{-1}): 3080 (CH arom.); 2940 (-CH_2-); 2850 (-OCH_3); 1600 (ring); 1450 (-CH_2-); 750 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.35 (m, 4H, -CH_2-N-CH_2-, piperazine); 2.91 (m, 4H, -CH_2-N-CH_2-, piperazine); 3.66 (s, 3H, -OCH_3); 5.37 (s, 2H, -CH_2-); 6.87 (m, 4H, Ar-H); 7.27 (d, 2H, *J* = 7.5 Hz, Ar-H); 7.64 (m, 4H, Ar-H); 7.69 (m, 1H, Ar-H); 8.42 (d, 1H, *J* = 7.5 Hz, Ar-H); 9.85 (s, 1H, -CH=N-); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 160.52, 154.62, 151.92, 141.04, 141.02, 136.30, 136.01, 134.39, 131.86, 130.41, 128.90 (2C), 128.04, 122.63, 122.48, 120.67, 119.02, 118.00, 111.66 (2C), 65.16, 50.12, 50.05 (2C), 40.30, 39.80; Analysis: calcd. for $C_{26}H_{26}N_5ClO$ (459.9): C 67.89, H 5.70, N 15.23%; found: C 67.59, H 5.58, N 14.99%.

1-[(4-(Pyrimidin-2-yl)piperazin-1-yl)metylo]-2-(2-chlorobenzylideneamino)benzimidazole (23)

Obtained as a yellow precipitate, yield 2.49 g (57%); crystallized from toluene; m.p. 164-170°C; IR (KBr, cm^{-1}): 3060 (CH arom.); 2950 (-CH_2-); 1600 (ring); 1550 (C=N); 760 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.64 (m, 4H, -CH_2-N-CH_2-, piperazine); 2.72 (m, 4H, -CH_2-N-CH_2-, piperazine); 5.34 (s, 2H, -CH_2-); 6.54 (m, 1H, Ar-H); 7.26 (m, 2H, Ar-H); 7.65 (m, 5H, Ar-H); 8.33 (m, 3H, Ar-H); 9.81 (s, 1H, -CH=N-); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 160.90, 160.66, 157.81, 154.56, 141.03, 136.28, 135.89, 134.35, 131.86, 130.38, 128.92, 128.01, 122.59 (2C), 119.02, 117.55, 109.98, 63.47, 49.77 (2C), 43.16 (2C); Analysis: calcd. for $C_{23}H_{22}N_7Cl$ (431.9): C 63.96, H 5.13, N 22.70%; found: C 63.61, H 4.90, N 22.04%.

1-[(Piperidin-1-yl)methyl]-2-(salicylamino)benzimidazole (24)

Obtained as a white precipitate, yield 2.08 g (62%); crystallized from ethanol; m.p. 159-161°C; IR (KBr, cm^{-1}): 3320 (NH), 3050 (CH, arom.), 2940, 2860 (-CH_2-), 1600 (ring), 1200 (C-O-H), 760 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 1.53 (m, 6H, -CH_2-CH_2-CH_2-, piperidine), 2.78 (m, 4H,

-CH_2-N-CH_2-, piperidine), 4.45 (d, 2H, *J* = 5.7 Hz, -CH_2-NH-), 5.13 (s, 2H, -CH_2-), 6.77 (m, 2H, Ar-H), 6.97 (m, 2H, Ar-*H*), 7.11 (H, Ar-H), 7.27 (m, 4H, -NH- and Ar-H), 9.72 (s, 1H, -OH); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 156.20, 147.66, 140.52, 138.65, 135.01, 128.93, 126.50, 124.35, 121.50, 120.76, 117.15, 116.90, 108.57, 63.84, 50.76 (2C), 42.04, 25.30 (2C), 23.40; Analysis: calcd. for $C_{20}H_{24}N_4O$ (336.43): C 71.40, H 7.19, N 16.65%; found: C 71.29, H 7.11, N 15.97%.

1-[(Morpholin-4-yl)methyl]-2-(salicylamino)benzimidazole (25)

Obtained as a white precipitate, yield 1.89 g (56%); crystallized from dioxane; m.p. 169-170°C; IR (KBr, cm^{-1}): 3350 (NH), 2920, 2860 (-CH_2-), 1600 (ring), 1580 (C=N), 1200 (C-O-H), 760 (CH, arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.87 (m, 4H, -CH_2-N-CH_2-, morpholine), 3.66 (m, 4H, -CH_2-O-CH_2-, morpholine), 4.47 (d, 2H, *J* = 6.0 Hz, -*CH_2*-NH-), 5.18 (s, 2H, -CH_2-), 6.78 (m, 2H, Ar-H), 6.97 (m, 2H, Ar-H), 7.10 (m, 1H, Ar-H), 7.29 (m, 4H, -NH- and Ar-H), 9.70 (s, 1H, -OH); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 155.34, 148.47, 140.59, 135.02, 134.25, 128.96, 126.40, 121.56, 120.91, 119.13, 117.15, 116.87, 108.53, 66.00 (2C), 63.14, 50.23 (2C), 42.58; Analysis: calcd. for $C_{19}H_{22}N_4O_2$ (338.40): C 67.44, H 6.55, N 16.56%; found: C 67.22, H 6.66, N 16,46%.

1-(Morpholin-1-yl)-methyl)-2-(4-hydroxybenzylamino)benzimidazole (26)

Obtained as a white precipitate, yield 0.95 g (28%); crystallized from dioxane; m.p. 164-165°C; IR (KBr, cm^{-1}): 3380 (NH), 2870 (-CH_2-), 1600 (ring), 1580 (NH), 1450 (C=N), 1140 (C-O-H), 820, 760 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.67 (m, 4H, -CH_2-N-CH_2-, morpholine); 3.54 (m, 4H, -CH_2-O-CH_2-, morpholine), 4.45 (d, 2H, *J* = 5.7 Hz, -*CH_2*-NH-), 5.29 (s, 2H, -CH_2-), 6.70 (d, 2H, *J* = 8.7 Hz, Ar-H), 6.92 (m, 3H, Ar-H), 7.21 (m, 4H, -NH- and Ar-H), 9.26 (s, 1H, -OH); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 156.48, 145.3, 141.52, 135.37, 131.25, 128.52, 128.36, 120.40, 120.37, 118.36, 114.95, 114.86, 108.08, 66.32, 66.14, 62.83, 51.57, 50.20, 45.29; Analysis: calcd. for $C_{19}H_{22}N_4O_2$ (338.40): C 67.84, H 5.99, N 16.66%; found: C 68.09, H 6.19, N 16.40%.

General procedure for the preparation of compounds 27-29

Reaction of 2-(4-hydroxybenzyl)aminobenzimidazole (**2**) with selected compound containing active methylene group: acetylacetone, benzoylacetone and malononitrile. To a solution of Schiff base **2** (0.01 mol) in ethanol (30 mL) containing triethylamine (0.3 mL) selected compounds containing active methylene group were added. The solution was refluxed for ca 8-10 h (TLC control). After cooling, the precipitate was filtered, washed with diethyl ether, dried and crystallized form appropriate solvent.

3-Acetyl-2-(4-hydroxyphenylene)-4-methyl-1,2-dihydropyrimido[1,2-a]benzimidazole (27)

Obtained as a white precipitate, yield 1.62 g (51%); crystallized from ethanol; m.p. 298-302°C; IR (KBr, cm^{-1}): 3230 (NH), 2950, 2860 (CH_3), 1600 (ring), 1500 (NH), 1340 (CH), 1230 (C-O-H), 1200, 860, 740 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 2.20 (s, 3H, -CH_3), 3.40 (s, 3H, -OCH_3), 6.50 (s, 1H, -NH-*CH*-), 6.64 (d, 2H, *J* = 8.7 Hz, Ar-H), 7.01 (m, 2H, Ar-H), 7.23 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.33 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.41 (d, 1H, *J* = 8.4 Hz, Ar-H), 9.42 (s, 1H, -OH), 10.70 (s, 1H, -NH-, pyrimidine); ^{13}C NMR (300 MHz, DMSO, δ, ppm): 194.42, 158.93, 156.22, 142.31, 145.45, 132.03, 131.60, 128.64, 128.49 (2C), 121.61, 121,28, 119.97, 116.72, 115.13 (2C), 110.06, 30.43, 19.58; Analysis: calcd. for $C_{19}H_{17}N_3O_2$ (319.36): C 71.46, H 5.37, N 16.66%; found: C 71.43, H 5.42, N 16.27%.

3-Benzoyl-2-(4-hydroxyphenylene)-4-methyl-1,2-dihydropyrimido[1,2-a]benzimidazole (28)

Obtaind as a white precipitate, yield 1.57 g (41%); crystallized from ethanol; m.p. 291-294°C; IR (KBr, cm^{-1}): 3240 (NH), 2840 (CH_3), 1665 (C=O), 1230 (C-O-H), 835, 740 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 1.82 (s, 3H, -CH_3), 6.49 (s, 1H, -NH-*CH*-), 6.61 (d, 2H, *J* = 8.4 Hz, Ar-H), 6.90 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.04 (t, 1H, *J* = 7.2 Hz, Ar-H), 7.10 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.35 (d, 3H, *J* = 7.8 Hz, Ar-H), 7.48 (m, 5H, Ar-H), 9.38 (s, 1H, -OH), 10.62 (s, 1H, -NH-, pyrimidine); Analysis: calcd. for $C_{24}H_{19}N_3O_2$ (381.43): C 70.72, H 5.28, N 17.10%; found: C 70.45, H 5.16, N 17.67%.

4-Amino-3-cyano-2-(4-hydroxyphenylene)-1,2-dihydropyrimido[1,2-a]benzimidazole (29)

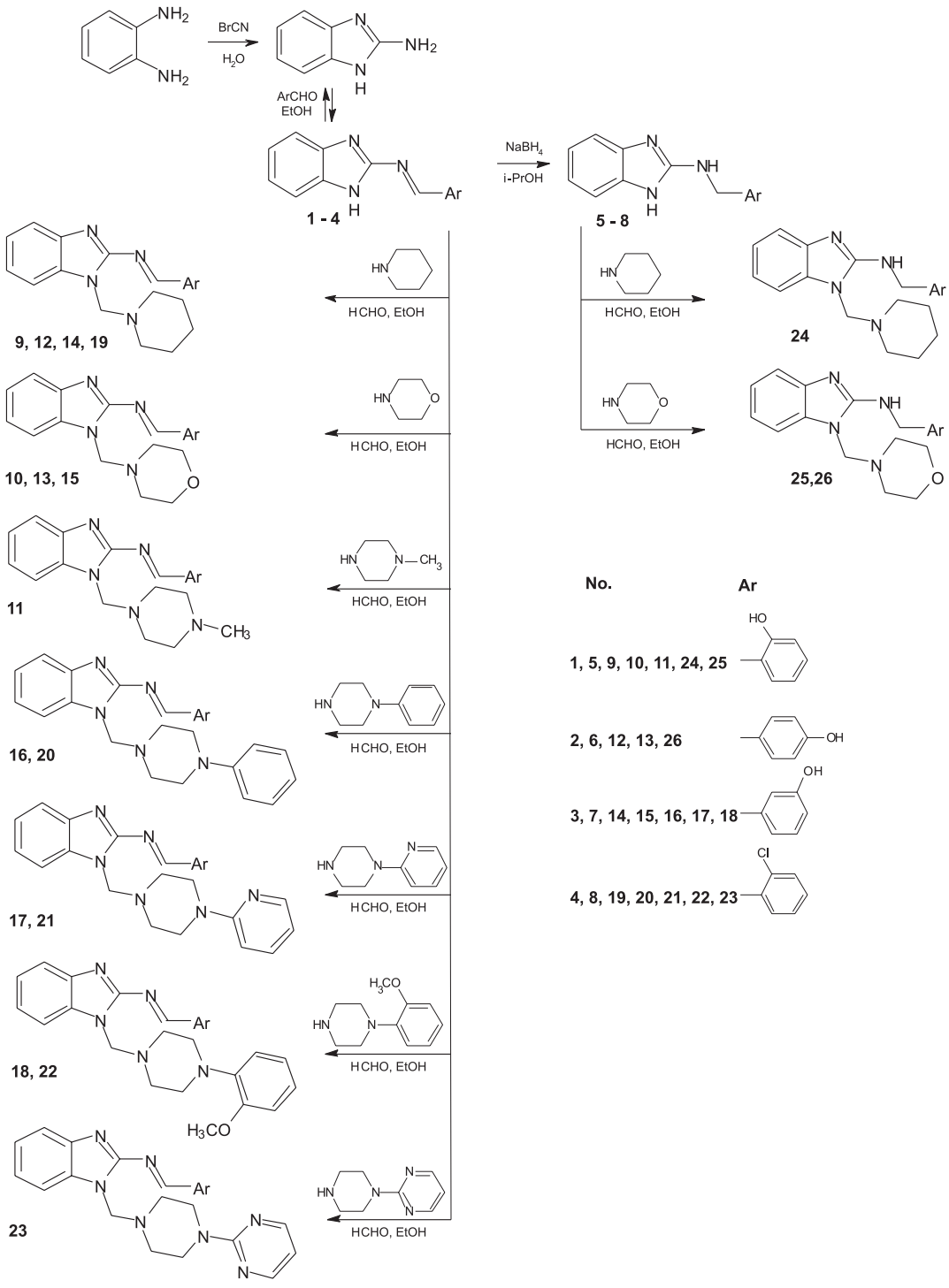
Obtained as a white precipitate, yield 1.62 g (54%); crystallized from butanol; m.p. 223-225°C; (KBr, cm^{-1}): 3500, 3400 (NH_2), 3350 (NH), 2900 (CH), 2200 (CN), 1260 (C-O-H), 830, 760 (CH arom.); ^{1}H NMR (300 MHz, DMSO, δ, ppm): 5.09 (s, 1H, -CH-), 6.73 (m, 4H, -NH_2 and Ar-H), 6.98 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.10 (m, 3H, Ar-H); 7.21 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.63 (d, 1H, *J* = 7.8 Hz, Ar-

H), 8.46 (s, 1H, -NH-, pyrimidine), 9.46 (s, 1H, -OH); Analysis: calcd. for $C_{17}H_{13}N_5O_1$ (303.32): C 67.51, H 5.67, N 22.21%; found: C 67.02, H 5.32, N 22.43%.

Biology

In vitro antiproliferative assay

Antiproliferative tests were performed on human cancer cell lines: A549 (lung), T47D



Scheme 1. Synthesis of Mannich bases

(breast), leukemia MV4-11 and mouse embryonic fibroblast BALB/3T3 according to standard procedure (33). All cell lines were obtained from American Type Culture Collection (Rockville, Maryland, USA) and have been maintained in culture or frozen in thaw Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences (IIET, PAS, Wrocław, Poland). The A549 and T47D cells were cultured in a mixture of Opti-MEM and RPMI 1640 medium (1 : 1, both from Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 5% fetal bovine serum. The culture of T47D cells were supplemented with 0.8 mg/L of insulin (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). MV4-11 cells were cultured in RPMI 1640 medium (Gibco, Scotland, UK) with 2 mM L-glutamine, adjusted to contain 1.5 g/L sodium bicarbonate and 1.0 mM sodium pyruvate, 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Mouse fibroblasts BALB/3T3 were maintained in Dulbecco medium (DMEM, Gibco, Scotland, UK) supplemented with 2 mM L-glutamine and 10% fetal bovine serum (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). All culture media were supplemented with 100 units/mL penicillin, and 100 μg/mL streptomycin (Polfa Tarchomin S.A., Warszawa, Poland). Cell lines were grown at 37°C with 5% CO_2 humidified atmosphere. The anti-proliferative effect of the tested compound was examined after 72 h exposure of the cultured cells to varying concentrations of the test compound (total plate incubation time: 96 h), using the sulforhodamine B (SRB) assay for adherent cells (A549, BALB/3T3 and MCF-7) and MTT assay for leukemia cells (MV4-11) (33). The results were shown as an IC_{50} value (inhibitory concentration 50% - a concentration in μg/mL of tested agent which inhibits proliferation of 50% of cancer cells population). Each compound was tested at every concentration in triplicate in a single experiment, which was repeated 3 times. The activity of tested compound was compared to the activity of cisplatin, used as a reference agent.

RESULTS AND DISCUSSION

Chemistry

Schiff bases **1-4** have been obtained in the reactions of 2-aminobenzimidazole with selected aromatic aldehydes: salicylic-, 4-hydroxy, 3-hydroxy, 2-chloro- (32) benzaldehyde (Scheme 1). The reactions were carried out in boiling ethanol with the presence of catalytic amounts of Triflate. Schiff bases **1-4** formed crystals of different shades of yellow due to the presence of chromophoric groups (CH=N) in their molecules. The product structures have been confirmed by elemental analysis and IR, ^1H and ^{13}C NMR spectra.

IR spectra of Schiff bases contain, among other absorption bands, those in the range of ν = 1635-1680 cm^{-1} characteristic for the chain groups C=N. The presence of CH=N proton was confirmed by ^1H NMR spectra of all imines in which one-proton singlets at δ = 9.31-9.67 ppm were observed. One-proton singlets at δ = 12.51-12.73 ppm were assigned to the imidazole group NH. For compounds **1-3** one-proton singlets at δ = 9.87-12.13 ppm characteristic for -OH group were observed. The signals corresponding to aromatic protons were observed in the range of δ = 7.01-7.92 ppm.

Azomethine bond (–N=CH-) in imines **1-4** have been subjected to selective reduction using $NaBH_4$ in boiling *i*-propanol. The extent of the hydrogenation has been monitored by TLC and decoloring of yellow solution. In ^1H NMR spectra of 2-benzylaminobenzimidazoles **5-8** the absence of one-proton singlets at δ = 9.31-9.67 ppm were observed, whereas, two-proton doublets at $\delta \sim 4.50$ ppm ($J \sim 5.50$ Hz) ascribed to NH-C**H**$_2$ protons are present. Broad one-proton signal at $\delta \sim 7.10$ ppm was ascribed to N**H**-CH_2 protons. Other signals of protons are observed in the similar places like in Schiff bases.

In the next stage of our work, Schiff bases **1-4** and 2-benzylaminobenzimidazole derivatives **5-8** were used as substrates for the Mannich condensation with, selected, pharmacophore, secondary amines: morpholine, piperidine, N-methylpiperazine, N-phenylpiperazine, 1-(2-pyridyl)piperazine, 1(2-methoxyphenyl)piperazine, 1-(2-pyrimidinyl) piperazine and formaldehyde. For the synthesis we have chosen amines that were used in the Mannich condensations and gave active antiproliferative compounds (12-23). The reactions were carried out in ethanol at room temperature. Under these reaction conditions, only the compounds presented in Scheme 1 have been obtained.

In ^1H NMR spectra of compounds **9-23** two-proton singlets at $\delta \sim 5.25$ ppm characteristic for -CH_2- group were observed, instead of one-proton singlet of NH imidazole. In case of 2-benzylaminobenzimidazoles **5-8** there are two possible aminomethylation paths: one in the position 1 or 2 or second at 1 and 2 positions. The elementary analyses confirm one aminomethylene group in obtained compounds **24-26**. In ^1H NMR spectra the absence of signal characteristic for NH imidazole was observed. Two-proton singlets at $\delta \sim 5.15$ ppm from -CH_2- group were assigned.

Another way of chemical modification of Schiff base **2** were reactions with selected compounds containing active methylene group; 1,3-diketones: acetyl-, benzoylacetone, or malononitrile in boiling ethanol with catalyctic amounts of triethylamine (Scheme 2). Tricyclic 2-(4-hydroxybenzylidene)-aminobenzimidazole (**2**) derivatives were obtained. In the first step of the reaction an unstable adducts **A** and **B** were formed. Elementary analysis confirmed that elimination of one molecule of water form adducts **A** lead to pyrimido[1,2-a]benzimidazole derivatives **27**, **28**. Cyclization of unstable adduct **B** gave 4-amino-3-cyano-2-(4-hydroxyphenylene)-1,2-dihydropyrimido[1,2-*a*]benzimidazole (**29**).

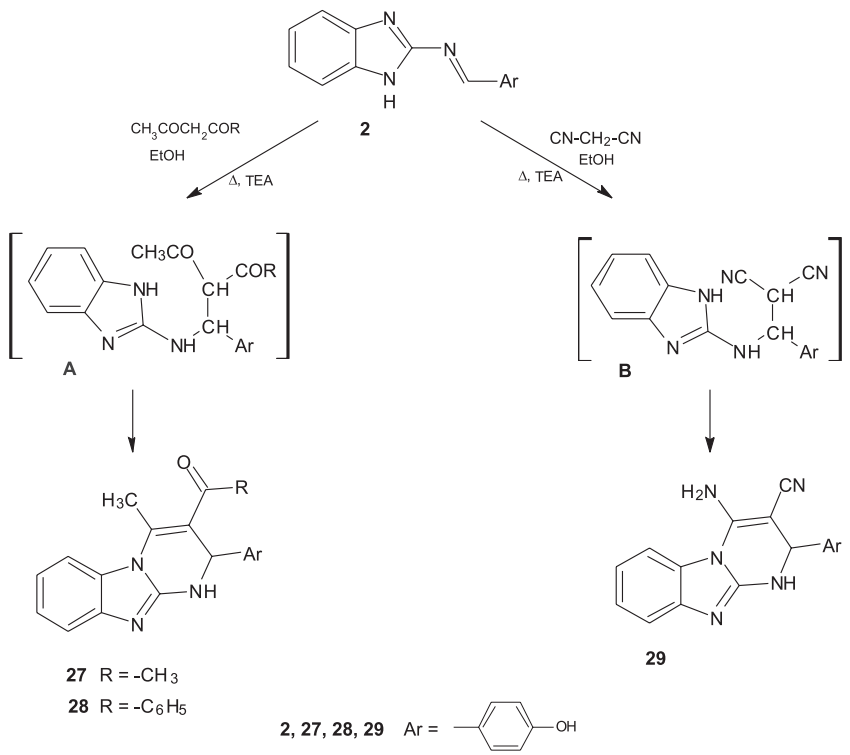
In the ^{1}H NMR spectra of 3-acetyl- (**27**), 3-benzoyl-2-(4-hydroxyphenylene)-4-methyl-1,2-dihydropyrimido[1,2-*a*]benzimidazole (**28**) and 4-amino-3-cyano-2-(4-hydroxyphenylene)-1,2-dihydropyrimido[1,2-a]benzimidazole (**29**) are displayed two one proton singlet signals at $\delta \sim 6.50$ ppm and $\delta \sim 10.65$ ppm or at $\delta = 5.09$ ppm and $\delta = 8.46$ ppm which were assigned to the -C**H**-N**H**- protons, respectively. Mutual splitting of signals was not recorded because of fast protons exchange from N**H** group.

Number of signals for the aromatic protons in the ^{1}H NMR spectra of all compounds is in good agreement with their structures.

Twenty seven **1-3**, **5-29** new compounds of various chemical structures were obtained from the syntheses described here. These derivatives may also be used as starting materials for further syntheses. All synthesized compounds were screened for their antiproliferative activity *in vitro* against the cells of 4 human tumor cell lines. The obtained compounds seem to be suitable candidates for further chemical modifications and might be of interest as pharmacologically active compounds.

Biological activity

To screen the antiproliferative activity of the compounds, the cell lines of various origin: leukemia, breast, lung cancer have been chosen. Selected types of cancer are the examples of diseases frequently afflicted people worldwide. Moreover, we have performed the comparative evaluation of cytotoxicity on mouse fibroblast cell line BALB/3T3. This cell line is recommended by various agencies introducing alternative methods for testing toxicity of compounds. For example, these



Scheme 2. Reaction of 2-(4-hydroxybenzylideneamino)benzimidazole with compounds containing active methylene group

cells were used in some methods aiming to estimate starting doses for oral acute systemic toxicity of compounds under the European Center of Validation of Alternative Methods (ECVAM) guidelines (OECD guidance document (GD) 129 published in 2010).

The synthesized compounds were screened for their antiproliferative activity using cells of MV4-11 human biphenotypic B myelomonocytic leukemia (Table 1). In comparison to cisplatin, the activity of tested compounds was lower, however we selected some compounds with IC_{50} value ranged between 0.23-4.25 μg/mL for further studies on the cells of breast and lung cancer, as well as on normal mouse fibroblasts to assess their selectivity towards cancer cells.

The antiproliferative activity *in vitro* of selected compounds **5**, **7**, **9-16**, **24-26**, **28**, **29** was significantly lower against lung and breast cancer cells, comparing to the results obtained against MV4-11 cells. However, one of them showed interesting profile of activity. Namely, the activity of compound **29** towards mouse fibroblasts was significantly lower than towards cancer cells, suggesting low toxicity (Table 2). Particularly, the most active on all cancer cells compound **29**, was inactive against normal fibroblasts.

The results revealed that all screened compounds demonstrated good to promising antiproliferative activity *in vitro* against MV4-11 human leukemia cell line. Among Schiff bases **1-4** the highest anticancer activity shows 2–(2-chlorobenzylideneamino)-1*H*-benzimidazole (**4**), containing in his structure 2-chlorophenylene substituent. Selective reduction of azomethine bond in Schiff bases **1-4** provides 2-benzylaminobenzimidazoles **5-8**, which were more active than the substrates. The presence of chlorine group in aromatic ring increases the anticancer activity, whereas the hydroxyl group decreases it. The most active compound was 2-(2-chlorobenzylamino)-1*H*-benzimidazole (**8**). All obtained Mannich bases **9-26** showed good antiproliferative activity *in vitro*. The promising activity possesses 2-salicylideneamino-1*H*-benzimidazoles **9-11** and 3-hydroxyamino-1*H*-benzimidazole **14**, substituted in 1 position with: piperidine, morpholine and 4-methylpiperazine. Comparable activity was shown by 1-[(piperidin-1-yl)methyl]- (**24**), 1-[(morpholin-4-yl)methyl]-2-(salicylamino)benzimidazole (**25**) and 1-(morpholin-1-yl)-methyl)-2-(4-hydroxybenzylamino)benzimidazole (**26**).

Among series of tricyclic 2-(4-hydroxyphenylene)pyrimido[1,2-*a*]benzimidazole derivatives the highest antiproliferative activity possesses compound **29**, substituted in position 3 and 4 with cyano and amino group, respectively. Replacement of cyano group in 3 position with benzoyl and amino group in position 4 with methyl decreased 10 times the antiproliferative activity. 3-Acetyl-2-(4-hydroxyphenylene)-4-methyl-1,2-dihydropyrimido[1,2-

Table 1. The antiproliferative activity of compounds against the cells of MV4-11 human leukemia cell line.

Compound	IC_{50} [mg/mL] mean ± SD	Compound	IC_{50} [mg/mL] mean ± SD
1	14.47 ± 0.96	**16**	3.40 ± 0.14
2	28.10 ± 2.77	**17**	4.80 ± 1.37
3	18.64 ± 2.23	**18**	4.34 ± 0.55
4	4.33 ± 4.87	**19**	3.31 ± 0.1
5	2.41 ± 0.58	**20**	6.67 ± 3.87
6	5.52 ± 0.11	**21**	4.72 ± 0.74
7	3.83 ± 0.55	**22**	5.88 ± 2.13
8	1.88 ± 1.42	**23**	4.75 ± 2.66
9	2.32 ± 0.11	**24**	2.48 ± 0.60
10	2.56 ± 0.53	**25**	3.07 ± 0.61
11	2.13 ± 0.28	**26**	2.41 ± 0.18
12	4.20 ± 1.78	**27**	n.a.
13	4.25 ± 1.2	**28**	2.65 ± 0.47
14	2.84 ± 0.31	**29**	0.23 ± 0.05
15	3.08 ± 0.24	Cisplatin	0.04 ± 0.01

n.a. = not active in the range of concentrations used

Table 2. The antiproliferative activity of selected compounds against human breast (T47D) and lung (A549) cancer and normal mouse fibroblasts (BALB/3T3) cell lines.

Compound	Cel line/ IC_{50} mg/mL		
	T47D	A549	BALB/3T3
5	9.55 ± 1.26	24.9 ± 1.71	38.10 ± 2.31
7	23.19 ± 1.97	36.62 ± 1.88	n.a.
9	24.55 ± 1.48	37.10 ± 0.96	49.27 ± 3.82
10	18.52 ± 3.79	36.59 ± 0.25	44.05 ± 3.26
11	20.92 ± 4.55	35.16 ± 1.98	38.01 ± 1.53
12	38.12 ± 3.51	36.16 ± 0.66	43.91 ± 1.67
13	38.59 ± 1.73	36.45 ± 2.71	36.68 ± 1.59
14	39.75 ± 0.81	34.94 ± 2.91	41.61 ± 2.28
15	33.11 ± 2.29	34.67 ± 2.39	38.42 ± 2.03
16	35.33 ± 3.29	39.61 ± 0.90	44.49 ± 1.63
24	15.83 ± 1.00	33.31 ± 1.18	40.23 ± 2.88
25	17.83 ± 0.44	31.60 ± 1.00	45.29 ± 2.76
26	24.95 ± 1.70	35.29 ± 2.12	38.59 ± 2.61
28	n.a.	n.a.	n.a.
29	6.69 ± 0.24	5.72 ± 0.81	n.a.
cisplatin	2.78 ± 0.55	2.40 ± 0.64	3.04 ± 0.83

n.a. = not active in the range of concentrations used

a]benzimidazole (**27**) was inactive in the range of concentrations used.

It is difficult, on the basis of obtained results, to make a broader SAR discussion.

CONCLUSIONS

Twenty seven new compounds **1-3**, **5-29** of various chemical structure: Schiff bases, 2-benzylaminobenzimidazole, Mannich bases and pyrimido[1,2-*a*]benzimidazole derivatives were obtained by the syntheses described here. Their structures were confirmed by elemental analysis and IR, ^{1}H and ^{13}C NMR spectra. All synthesized compounds **1-29** were screened for their antiproliferative activity *in vitro* on MV4-11 human leukaemia cell line. The results revealed that all screened compounds demonstrated good to promising antiproliferative activity against human leukemia cell line. The most active compounds were then tested towards human breast T47D and lung A549 cancer cell lines and normal mouse fibroblasts.

The most active compound against the cells of cancer cell lines was 4-amino-3-cyano-2-(4-hydroxyphenylene)-1,2-dihydropyrimido[1,2-*a*]benzimidazole (**29**) (IC_{50} 0.23 ± 0.05 μg/mL against MV4-11 cells) showing in parallel very low cytotoxicity towards mouse fibroblasts. Cisplatin was used as reference drug (IC_{50} 0.04 ± 0.01 μg/mL).

REFERENCES

1. Tramontini M., Angiolini L.: Tetrahedron 46, 1791 (1990).
2. Jesudason E.P., Sridhar S.K., Padma Malar E.J., Shanmugapandiyan P., Inayathullah M. et al.: Eur. J. Med. Chem. 44, 2307 (2009).
3. Singh B., Chetia D., Puri S.K., Srivastava K., Prakash A.: Med. Chem. Res. 20, 1523 (2011).
4. Obniska J., Rzepka S., Kamiński K.: Bioorg. Med. Chem. 20, 4872 (2012).
5. Kamiński K., Obniska J., Chlebek I., Liana P., Pękala E.: Eur. J. Med. Chem. 66, 12 (2013).
6. Linz S., Müller J., Hübner H., Gmeiner P., Troschütz R.: Bioorg. Med. Chem. 17, 4448 (2009)
7. Plech T., Wujec M., Majewska M., Kosikowska U., Malm A.: Med. Chem. Res. 22, 2531 (2013).
8. Koparir M., Orek C., Parlak A.E., Söylemez A., Koparir P. et al.: Eur. J. Med. Chem. 63, 340 (2013).

9. Emami S., Ghafouri E., Faramarzi M.A., Samadi N., Irannejad H., Foroumadi A.: Eur. J. Med. Chem. 68, 185 (2013).
10. Nowicka A., Liszkiewicz H., Nawrocka W.P.: Wiad. Chem. 68, 161 (2014)
11. The Merck Index, 14th edn., Whitehouse Station, USA 2006.
12. Chaudhary A., Sharma P.P., Bhardwaj G., Jain V., Bharatam P.V. et al.: Med. Chem. Res. 22, 5654 (2013).
13. Shahzad S.A., Yar M., Bajda M., Jadoon B., Khan Z.A. et al.: Bioorg. Med. Chem., 22, 1008 (2014).
14. Nowicka A., Liszkiewicz H., Nawrocka W.P., Wietrzyk J., Zubiak A., Kołodziejczyk W.: Acta Pol. Pharm. Drug Res. 72, 101 (2015).
15. Solomon V.R., Hu C., Lee H.: Bioorg. Med. Chem. 18, 1563 (2010).
16. Kumbhare R.M., Kumar K.V., Ramaiah M.J., Dadmal T., Pushpavalli S.N.C.V.L. et al.: Eur. J. Med. Chem. 46, 4258 (2011).
17. Taher A.T., Khalil N.A., Ahmed E.M.: Arch. Pharm. Res. 34, 1615 (2011).
18. Sunil D., Isloor A.M., Shetty P., Chandrakantha B., Satyamoorthy K.: Med. Chem. Res. 20, 1024 (2011).
19. Hu G., Wang G., Duan N., Wen X., Cao T. et al.: Acta Pharm. Sinica B, 2, 312 (2012).
20. Zhao Y.J., Wei W., Su Z.G., Ma G.H.: Int. J. Pharm. 379, 90 (2009).
21. Euzebio F.P.G., dos Santos F.J.L., Pilo-Veloso D., Alcantara A.F.C., Ruiz A.L.T.G. et al.: Bioorg. Med. Chem. 18, 8172 (2010).
22. Shaw A.Y., Chang C.Y., Hsu M.Y., Lu P.J., Yang C.N. et al.: Eur. J. Med. Chem. 45, 2860 (2010).
23. Savariz F.C., Foglio M.A., Goes Ruiz A.L., da Costa W.F., de Magalhães Silva M. et al.: Bioorg. Med. Chem. 22, 6867 (2014).
24. Wang H., Yan J., Song X., Fa L., Xu J. et al.: J. Med. Chem. 20, 2119 (2012).
25. Bayoumi W.A., Elsayed M.A.: Med. Chem. Res. 21, 1633 (2012).
26. Anand P., Patil V.M., Sharma V.K., Khosa R.L., Masand N.: Int. J. Drug Discov. 60, 851 (2012).
27. Przybylski P., Huczyński A., Pyta K., Brzeziński B., Bartl F.: Curr. Org. Chem. 13, 124 (2009).
28. da Silva C.M., da Silva D.L., Modolo L.V., Alves R.B., de Resende M.A. et al.: J. Adv. Res. 2, 1 (2011).
29. Sondhi S.M., Arya S., Rani R., Kumar N., Roy P.: Med. Chem. Res. 21, 3620 (2012).
30. Nowicka A., Liszkiewicz H., Nawrocka W.P., Wietrzyk J., Kempińska K., Dryś A.: Cent. Eur. J. Chem. 12, 1047 (2014).
31. Leonard N.J., Curtin D.Y., Beck K.M.: J. Am. Chem. Soc. 69, 2459 (1947).
32. Nawrocka W.P., Sztuba B., Kowalska M., Liszkiewicz H., Wietrzyk J. et al.: Farmaco 59, 83 (2004).
33. Wietrzyk J., Chodynski M., Fitak H., Wojdat E., Kutner A., Opolski A.: Anti-cancer Drugs 18, 447 (2007).

Received: 24. 10. 2014