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# Synthesis and antiproliferative activity in vitro of 2-aminobenzimidazole derivatives

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**Abstract**

A novel series of Schiff bases **1–11**, the derivatives of 2-aminobenzimidazole and substituted aromatic aldehydes, has been synthesised. Compounds **1–11** reduced by $NaBH_4$ formed 2-benzylaminobenzimidazoles **12–21**. 2-(*o*-Bromobenzylamino)benzimidazole (**15**) acylated by cinnamoyl chloride gave 2-(*o*-bromobenzylamino)-1-cinnamoylbenzimidazole (**22**). Long heating of **15** and **19** with *p*-nitrocinnamoyl or cinnamoyl chloride led to the formation of pyrimido[1,2-a]benzimidazol-4-ones **23** and **24**. The structures of **1–24** were identified by the results of elemental analysis and their IR, $^1H$ NMR and MS spectra. Among the compounds **1–24** evaluated for their antiproliferative activity in vitro, **16, 19, 20** and **22** exhibited cytotoxic activity against the cells of human cancer cell lines, namely SW707 (rectal), HCV29T (bladder), A549 (lung) and T47D (breast cancer).



*Keywords:* 2-Aminobenzimidazole derivatives; Schiff bases; 2-Benzylidene-; 2-Benzylaminobenzimidazoles; 2-Benzylamino-1-cinnamoylbenzimidazole; Pyrimido[1,2-a]benzimidazol-4-ones; Antiproliferative activity in vitro

## 1. Introduction

There are ca. 30 derivatives of 2-aminobenzimidazole registered in the world as drugs, which exhibit diverse pharmacological activities, e.g. antiparasitic, antifungal, antiviral and antiallergic [1]. The structures, syntheses and pharmacological properties of these compounds have been presented in a recently published review [2]. 2-Aminobenzimidazole derivatives active against herpes simplex virus (HSV), human cyclomegalovirus (HCMV) and HIV were also described and patented [3–7]. A group of complex compounds of 2-aminobenzimidazole derivatives with some metals, such as cobalt, zinc and copper, showed also antifungal and antibacterial activity [2,8,9], while complexes with ruthenium [10] were cytotoxic in vitro against SKW-8 cells (human T-lymphoma). The derivatives of pyrimido[1,2-a]benzimidazoles recently described [11] show slight antibiotic and antidysrhythmic properties. The synthesis of substituted and tricyclic derivatives of 2-aminobenzimidazole, exhibiting high immunotropic activity, has been presented in our previous papers [12,13]. The aim of this work was to synthesise Schiff bases, the derivatives of 2-aminobenzimidazole and substituted aromatic aldehydes, then—after their reduction and acylation—to examine them for their antiproliferative activity in vitro against the cells of various human tumour cell lines. Available data confirm that Schiff bases exhibit antineoplastic activity [14], while their reactions with active methylene compounds give pyrimido[1,2-a]benzimidazole derivatives [14–16].

## 2. Results and discussion

### 2.1. Chemistry

Schiff bases **1–11** have been obtained in the reactions of 2-aminobenzimidazole with aromatic aldehydes:

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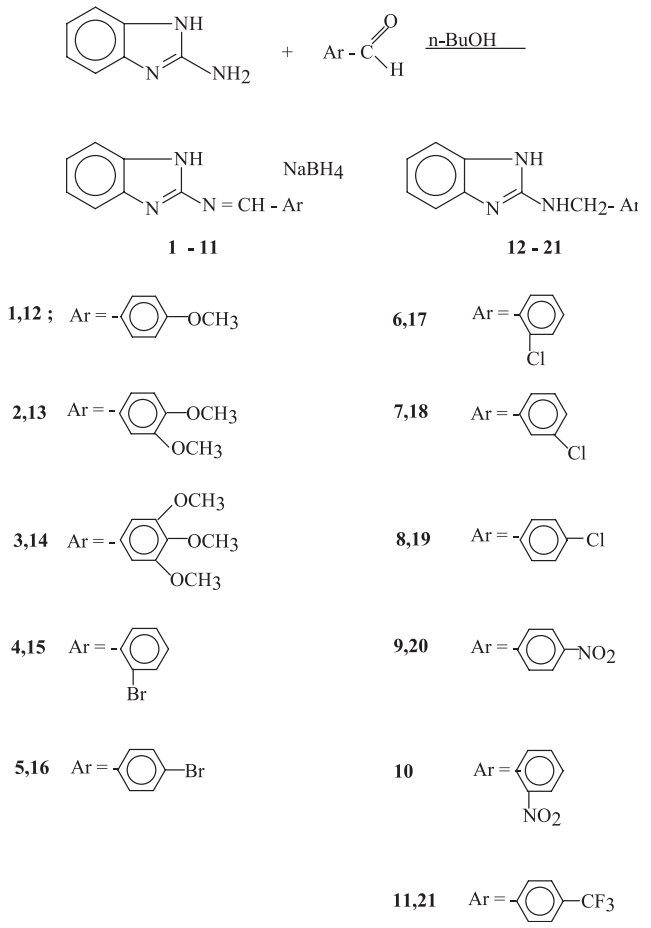



Scheme 1

4-methoxy- [17], 3,4-dimethoxy-, 3,4,5-trimethoxy-, 2-bromo-, 4-bromo-, 3-chloro-, 4-chloro- [17], 4-nitro- [17], 2-nitro- and 4-trifluoromethylbenzaldehyde (Scheme 1). The reactions were carried out in boiling toluene, then in dioxane in significant excess of aldehyde [18]. The best method appeared to be heating of 2-aminobenzimidazole in the mixture of absolute ethanol/benzene (5:1) in the presence of catalytic amounts of glacial acetic acid. The shortest time of reactions and the highest yields were observed in the reactions of 2-aminobenzimidazole with *p*-bromo-, *p*-nitro- and *o*-chlorobenzaldehyde. Schiff bases **1–11** 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 their MS, IR and $^1$H NMR spectra.

IR spectra of compounds **1–11** contain, among other absorption bands, those in the range of $\nu$ = 1630–1645 cm$^{-1}$ characteristic for the chain groups C=N. The presence of CH=N protons was confirmed by $^1$H NMR spectra of all aldimines in which one-proton singlets at δ ∼ 9.35–9.87 ppm are observed. One-proton singlets at δ ∼ 12.57–12.85 ppm were assigned to the imidazole group NH. The signals corresponding to aromatic protons were observed in the range of δ 7.10–8.45 ppm.

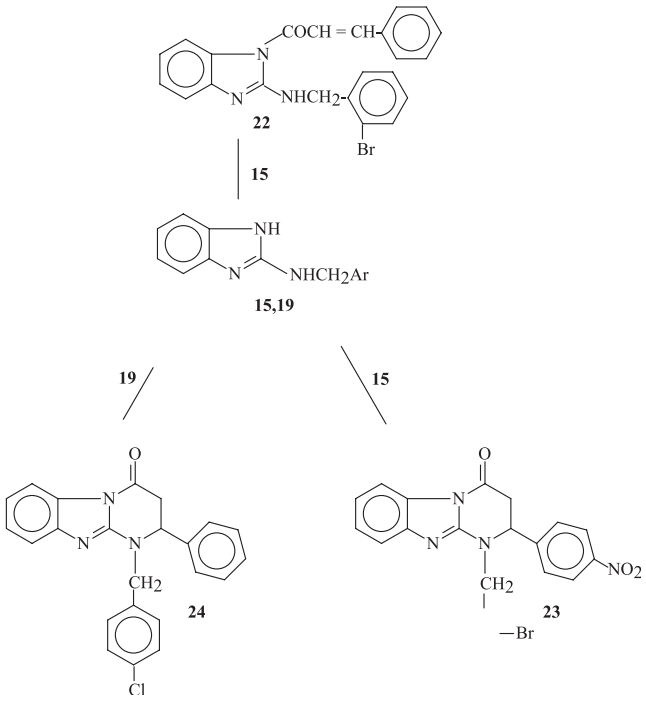
MS spectra of aldimines **1–11** show the presence of their molecular ions. In the case of 2-(4-bromobenzylideneamino)benzimidazole (**5**) isotopic ions (M-1), *m/z* (%): 298 (100) and 300 (98) were recorded. Elimination of proton from the molecules **1, 2** and **9–11** or fragmentation followed by elimination of Br, Cl from molecular ions of **4** and **6** resulted in the formation of corresponding base ions. Molecular ion of **3, 7** and **8** shows the highest intensity in the spectrum.

Benzimidazole fragment, *m/z* (%) = 118, was found in MS spectra of aldimines **1–11**. This ion undergoes typical fragmentation and elimination described earlier [12].

Aldimines have been subjected to reduction using $NaBH_4$ in ethanol replaced later by boiling isopropanol, which has increased the rate of reduction. Moreover, less amount of $NaBH_4$ could be used. The extent of the reduction has been monitored by TLC and the decolouring of yellow reaction mixture. The structures of 2-benzylaminobenzimidazole derivatives **12–21** were confirmed by the results of elemental analysis and their IR, MS and $^1$H NMR spectra. Particularly, the latter show some characteristic features, e.g. the absence of one-proton singlets at δ ∼ 9.50 ppm (CH=N) observed in the spectra of imines **1–11**, whereas, two-proton doublets at δ ∼ 4.50 ppm, $J$ = ∼4.50 Hz ascribed to methylene protons are present. Protons of amine groups NH–$CH_2$ were recorded as triplets among aromatic protons, e.g. in the case of compound **21** at δ = 7.23 ppm. Broad one-proton signal at δ = 10.89 ppm was assigned to proton of the imidazole group NH. Proton signals of both amine groups disappear in the presence of $D_2O$. MS spectra of derivatives **12** and **13** contain base ions—substituted benzyl ions. Base ions of **15** and **17–19** resulted from the elimination of Br and Cl from molecular ions. In the case of **16** and **21** molecular ions are the most intensive ones.

In the next stage of our work, we decided to modify chemically molecules of **15** and **19** (Scheme 2), which exhibited antiproliferative activity in vitro (Table 1). These compounds have been subjected to reaction with cinnamoyl and *p*-nitrocinnamoyl chlorides under different experimental conditions.

Generally, the presence of cinnamoyl substituents in various heterocyclic systems leads to their high biological activity. Some derivatives of 1,5-benzodiazepines synthesised in our laboratory were shown to be psychotropic [19], antiproliferative in vitro [20] and immunotropic [21]. The derivatives of 3(4H)-chinazolinone [22] and 2-aminobenzimidazole [12,13] display high immunotropic activity along with lack of toxicity. It is known from the literature, that the derivatives of cinnamic acid, isolated from various plants, are selective 5-lipoxygenase enzymes (5-LO) inhibitors and active as stimulators of platelet aggregation and prostaglandin inhibitors. The cinnamic acid and its derivatives exhibited antimicrobial, anti-inflammatory, anticancer and analgesic activities [23–30].




Scheme 2

The compound **15**, acylated by using cinnamoyl chloride in boiling THF, formed single product. As could be expected, there are two possible acylation paths: one at the position 1 or 2 and second at the positions 1 and 2. Elemental analysis and MS spectra have confirmed the addition of one cinnamoyl group to the molecule of 15. $^1$H NMR spectrum of **22** reveals the presence of vinylene (CH=CH) protons and no signals, which could be assigned to the imidazole NH groups. The signals of methylene protons were recorded as doublets, at δ = 4.71 ppm, $J$ = 4.51 Hz, whereas those of the NH–$CH_2$ groups were found among aromatic protons. These findings indicate that an acylation has occurred at position 1, which is more favourable than at position 2 due to the steric hindrance of the substituents. MS spectrum recorded for **22** reveals the presence of two isotopic ions of nearly the same intensity (∼18): $m/z$ (%) = 433 and 431, related to the presence of Br atom in the molecule. Two main fragmentation routes were observed for **22**: the first step occurs by the releasing of cinnamoyl ion $m/z$ (%) = 131 (100) and subsequent formation of isotopic ions $m/z$ (%) = 303 (13) and $m/z$ (%) = 301 (14). After Br elimination ion $m/z$ (%) = 222 (36) was observed and its subsequent fragmentation to benzimidazole ion $m/z$ (%) = 118. Base ion $m/z$ (%) = 131 (100) undergoes the fragmentation in the way described earlier for 2-cinnamoylaminobenzimidazole [12,13].

Table 1
Antiproliferative activity of the compounds **1–24** against the cells of human rectal and bladder cancer cell lines: SW707 and HCV29T

| Compound | Cell line/$ID_{50}$ (μg/ml) ± S.D. | |
|---|---|---|
| | SW707 | HCV29T |
| **1** | 46.0 ± 1.1 | 44.9 ± 1.1 |
| **2** | 43.2 ± 2.2 | 29.8 ± 1.5 |
| **3** | 15.7 ± 1.3 | 16.3 ± 2.0 |
| **4** | 7.9 ± 1.2 | 10.6 ± 1.6 |
| **5** | 10.0 ± 1.5 | 9.6 ± 1.3 |
| **6** | 16.2 ± 2.3 | 8.1 ± 1.8 |
| **7** | 13.4 ± 1.9 | 19.3 ± 1.4 |
| **8** | Negative | Negative |
| **9** | 23.2 ± 1.8 | 23.5 ± 1.1 |
| **10** | Negative | Negative |
| **11** | Negative | 58.0 ± 1.0 |
| **12** | 38.6 ± 1.0 | 24.5 ± 1.2 |
| **13** | Negative | Negative |
| **14** | Negative | Negative |
| **15** | 23.3 ± 1.3 | 8.7 ± 1.6 |
| **16** | 6.1 ± 1.4 | 3.6 ± 1.3 |
| **17** | 20.5 ± 1.4 | 6.0 ± 1.7 |
| **18** | 31.9 ± 1.1 | 14.1 ± 1.2 |
| **19** | 7.4 ± 1.3 | 5.5 ± 1.2 |
| **20** | 3.5 ± 1.1 | 0.4 ± 1.7 |
| **21** | 34.3 ± 1.1 | 33.0 ± 1.3 |
| **22** | 4.0 ± 1.0 | 2.9 ± 1.0 |
| **23** | Negative | Negative |
| **24** | Negative | Negative |
| Cisplatin control | 4.9 ± 1.5 | 0.7 ± 1.5 |

Long heating of derivative **14** together with *p*-nitrocinnamoyl chloride and derivative **19** with cinnamoyl chloride in boiling THF led to the formation of pyrimido[1,2-a]benzimidazol-4-ones **23** and **24** (Scheme 2). Their structures were confirmed by the results of elemental analysis and MS, IR and $^1$H NMR spectra. The absence of vinylene (CH=CH) protons signals in the $^1$H NMR spectra confirms three-cyclic structure of compounds **23** and **24**. The $^1$H NMR spectrum of **24** contains three one-proton doublets with different coupling constants. Two of them, at 3.13 and 3.71 ppm, can be ascribed to diastereotypic methylene protons, which split each other. In addition to this interaction, methylene protons are split by methine proton at δ = 6.01 ppm. Methylene proton at δ = 3.71 ppm is additionally deshielded by the benzene ring at position 2. The signals of methylene protons at position 1 were recorded as two-proton singlet at δ = 5.21 ppm. There are 13 aromatic protons within the range of δ = 6.97–7.53 ppm.

The MS spectrum of **24** contains two peaks corresponding to isotopic ions $m/z$ (%) = 389 (35) and 387 (100) due to the presence of Cl in parent molecule. Further analysis of this spectrum shows that there are several fragmentation paths, none of which leads to the elimination of substituents or Cl. The first step involves degradation of pyrimidine ring via elimination of CO and subsequent formation of two isotopic ions, $m/z$ (%) = 361 (**4**) and 359 (11). Elimination of [Cl]$^{\cdot}$ from both ions gives ion $m/z$ (%) = 324 (3). Further fragmentation involves elimination of ions $[C_6H_5]^+$, $m/z$ (%) = 77 (7) and $[C_6H_6]^+$, $m/z$ (%) = 78 (5) leading to the formation of ion $m/z$ (%) = 247 (8) from which benzylaminobenzimidazole fragment, $m/z$ (%) = 222 (8), is formed after $C_2H_2$ elimination. Another fragmentation route of **24** was also observed, viz. degradation of pyrimidine ring through the abstraction of $COCH_2CHPh$ group. This results in the formation of two

Table 2
Antiproliferative activity of the compounds **16, 19, 20** and **22** against the cells of human lung and breast cancer cell lines: A549 and T47D

| Compound | Cell line/$ID_{50}$ (μg/ml) ± S.D. | |
|---|---|---|
| | A549 | T47D |
| **16** | 10.9 ± 1.9 | 9.3 ± 1.5 |
| **19** | 9.5 ± 1.4 | 5.4 ± 1.3 |
| **20** | 3.4 ± 1.1 | 3.3 ± 1.1 |
| **22** | 3.8 ± 1.0 | 3.4 ± 1.4 |
| Cisplatin control | 3.3 ± 1.4 | 2.1 ± 1.8 |

ions, *m/z* (%) = 257 (13) and 255 (34), followed by releasing of $[Cl]^{\cdot}$ and formation of two fragments, *m/z* (%) = 220 (6) and 219 (9). Their fragmentations have been described previously [12,13]. The presence of ions *m/z* (%) = 285 (6), 284 (3) and 283 (18) were also observed in the MS spectrum of **24** formed after elimination of ions $[C_8H_7]^+$ and $[C_8H_8]^+$ from parent molecule. Their further fragmentation involves elimination of Cl radicals giving isotopic ions, *m/z* (%) = 248 (5), 247 (8) and 246 (3). The next steps occur: first as elimination of benzyl ion *m/z* (%) = 90 (12) followed by CO abstraction and formation of ion *m/z* (%) = 130 (7.89), and second as releasing of HCN and formation of ion *m/z* (%) = 103 (12). Degradation of latter ion has been described earlier [12].

### 2.2. Biological activity

#### 2.2.1. Antiproliferative assay in vitro

The results of cytotoxic activity in vitro were expressed as an $ID_{50}$—the dose of compound (in μg/ml), that inhibits proliferation rate of the tumour cells by 50% as compared to control untreated cells.

The compounds **1–24** evaluated for antiproliferatve activity have different chemical structures: derivatives **1–11** are aldimines, **12–21**—2-benzylaminobenzimidazoles, **22** is 1-cinnamoyl-2-benzyl while **23** and **24** are pyrimido[1,2-a]benzimidazoles. Their cytotoxic activity against the cells of two human cancer cell lines is presented in Table 1.

2-(*p*-Chlorobenzylideneamino)- (**8**), 2-(*o*-nitrobenzylideneamino)- (**10**), 2-(3,4-dimethoxybenzylamino)- (**13**), 2-(3,4,5-trimethoxybenzylamino)benzimidazole (**14**) and **23, 24** did not reveal cytotoxic activity ($ID_{50}$ > 100 μg/ml) against the cells of human cancer lines applied.

The selected compounds approaching the cytotoxic activity criterion ($ID_{50}$ ≤ 4 μg/ml) against SW707 and HCV29T cells, namely: 2-(*p*-bromobenzylamino)- (**16**), 2-(*p*-chlorobenzylamino)- (**19**), 2-(*p*-nitrobenzylamino)benzimidazole (**20**) and 1-cinnamoyl-2-(*o*-bromobenzimidazole (**22**) were tested also against the cells of A549 and T47D human lung and breast cancer cell lines (Table 2). The obtained results indicate on these compounds as good candidates for further studies in vitro against a broad panel of human and murine tumour cell lines, with an aim to select the most active compounds for further preclinical in vivo studies.

#### 2.2.2. Structure–activity relationship

Majority of Schiff bases studied **1–11**, represented by general formula (HetN=CHAr) exhibited antiproliferative activity in vitro at rather high doses (Table 1). Since all these compounds contain imine bond N=CH in their structure, it seems that their potential antiproliferative activity is decreased or even abolished (**8, 10** and **11**) by the presence of substituents in the phenyl ring. Among the compounds **1–3** substituted by one, two or three O–$CH_3$ groups, 2-(2,4,5-trimethoxybenzylideneamino)benzimidazole (**3**) is the most active. Brominated derivatives, **4** and **5**, containing Br at the *ortho* or *para* positions, were found to be slightly more active than chloroderivatives, **6** and **7**. Schiff bases containing $NO_2$ group exhibited weak activity in the case of *p*-substituted derivative **9**, but did not in the case of *o*- and *p*-$CF_3$ group-substituted compounds **10** and **11**.

Reduction of imine bond N=CH in Schiff base gave products **12–21**, which were usually more active than their precursors. Comparison of benzylamino derivatives with –$OCH_3$ substituents shows that only compound **12** (*p*-$OCH_3$) is active. In the group of brominated derivatives compound **16** containing Br at *para* position is more active than its *ortho* analogue **15**. Among chlorinated compounds **17–19**, *para* derivative **19** appeared to be the most active, although its analogue, Schiff base **8** was inactive. Compound **20** (*p*-$NO_2$) was found to be the most active in comparison with all other products. Introduction of cinnamoyl substituent, COCH=CHPh, at the position 1 of **15** gave one of the most active compounds, 1-cinnamoyl -2-(*o*-bromobenzylamino)-benzimidazole (**22**). The presence of vinylene bond (CH=CH) in 22 leads to significant antiproliferative activity in vitro as compared to tricyclic derivatives **23** and **24**, which were not active in biological tests. It should be noted that compounds, **16, 19 20** and **22**, approached the international criterion of antiproliferative activity in vitro against all human cancer cell lines applied in our studies (Tables 1 and 2).

## 3. Experimental

### 3.1. Chemistry

Melting points (uncorrected) were measured with a Boethius melting point apparatus. Analyses were performed on a Perkin Elmer 2400 analyser and satisfactory results within ±0.4% calculated values were obtained for the new compounds. IR spectra (in KBr) were recorded with an IR 75 spectrophotometer, $^1H$ NMR spectra on a Bruker AVANCE DRX 300 using DMSO-$d_6$ as an internal standard. Mass spectra were determined on a GCMS-LK 82091 spectrometer at the ionisation energy 15 or 70 eV. The course of reaction and the purity of products were checked by TLC (Kiesselgel G. Merck) in diethyl ether/ethanol = 5:1 as eluent.

*3.1.1. Reactions of 2-aminobenzimidazole with selected aromatic aldehydes: 4-methoxy-, 3,4-dimethoxy-, 3,4,5 trimethoxy-, 2-bromo-, 4-bromo-. 2-chloro-, 3-chloro-, 4-chloro-, 4-nitro-, 2-nitro- and 4-trifluoromethylbenzoate (**1–11**)*

General method: To a mixture of 2-aminobenzimidazole (1.33 g, 0.01 mol) dissolved in anhydrous ethanol (50 ml) and benzene (10 ml) an appropriate aldehyde (0.012 mol) and a few drops of glacial acetic acid were added. The reactants were heated for 8–24 h at boiling temperature. At the end of the reaction the solvents were partially evaporated under reduced pressure and the residue left for crystallisation. The precipitate was collected by filtration, washed with ether, dried and recrystallised from appropriate solvents.

*2-(p-Methoxybenzylideneamino)benzimidazole* (**1**)

Yield 1.53 g (61%), yellow crystals from ethanol, m.p. 220–222 °C. IR (KBr): ν $cm^{-1}$ = 3300 (*sec* NH), 2840 (Ar–$OCH_3$), 1630 (N=C), 1560 (*sec* NH), 1420 ($OCH_3$), 1250 (–C–O–C). $^1$H NMR (DMSO): δ = 3.96 (s, 3H), 7.03 (d, *J* = 8.7 Hz, 2H), 7.15 (m, 5H), 7.48 (s, 1H), 9.37 (s, 1H), 12.57 (s, 1H). Anal.: $C_{15}H_{13}N_3O$ (251.29 ). MS (70 eV): *m/z* (%) = 252 (4), 251 (41), 250 (100), 253 (3), 207 (9), 118 (13), 91 (7).

*2-(3,4-Dimethoxybenzylideneamino)benzimidazole* (**2**)

Yield 1.79 g (64%), yellow crystals from acetonitrile, m.p. 254–255 °C. IR (KBr): ν $cm^{-1}$ = 3300 (*sec* NH), 3105 ($OCH_3$), 2900 (CH), 1620 (N=CH), 1600 (C=N), 1580 (*sec* NH), 1430 ($OCH_3$), 1275 (750 aromat.). $^1$H NMR (DMSO): δ = 3.86 (s, 6H), 7.15 (m, 2H), 6.84 (m, 3H), 7.12 (m, 1H), 7.52 (m, 3H), 9.35 (s, 1H), 12.62 (s, 1H). Anal.: $C_{16}H_{15}N_3O_2$ (281.31). MS (70 eV): *m/z* (%) = 282 (7), 281 (65), 280 (100), 236 (5), 133 (50), 118 (17), 105 (11), 95 (10), 85 (12), 77 (6), 57 (33), 43 (32).

*2-(3,4,5-Trimethoxybenzylideneamino)benzimidazole* (**3**)

Yield 0.45 g (15%), yellow crystals from acetonitrile, m.p. 115–120 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 2900 (CH), 2850 (–$OCH_3$), 1635 (C=N), 1690, 1570 (C=N), 1430 (–$OCH_3$), 1340 (CH), 1325 (NH), 1220 (C–O–C), 980, 830, 730 (CH aromat.). $^1$H NMR (DMSO): δ = 3.77 (s, 3H), 3.87 (s, 6H), 7.19 (m, 1H), 7.43 (m, 5H), 9.87 (s, 1H), 12.68 (s, 1H). Anal.: $C_{17}H_{17}N_3O_3$ (311.34). MS (70 eV): *m/z* (%) = 312 (35), 311 (100), 297 (5), 296 (25), 280 (30), 269 (12), 266 (10), 265 (7), 235 (15), 210 (55), 140 (12), 110 (56), 118 (16), 91 (17), 65 (8), 64 (16), 63 (15).

*2-(o-Bromobenzylideneamino)benzimidazole* (**4**)

Yield 1.98 g (63%), yellow precipitate from ethanol, m.p. 216–217 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 3000, 2800 (CH), 1640 (C=N), 1690, 1600 (*sec* NH), 1500 (C=N), 1340 (CH), 1340 (=NH), 1280 (NH), 980, 830, 760 (CH aromat.). $^1$H NMR (DMSO): δ = 7.21 (m, 2H), 7.53 (m, 4H), 7.81 (m, 1H), 8.26 (m, 1H), 9.73 (s, 1H), 12.85 (s, 1H). Anal.: $C_{14}H_{10}N_3Br$ (300.16); MS (70 eV): *m/z* (%) = 302 (2), 301(18), 300 (18), 222 (2), 221(16), 220 (100), 155 (13), 149 (25), 110 (25), 91 (16), 90 (13), 65 (11), 57 (20), 55 (18).

*2-(p-Bromobenzylideneamino)benzimidazole* (**5**)

Yield 2.0 g (66%), yellow precipitate from ethanol, m.p. 256–257 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 3000, 2810 (CH), 1635 (C=N), 1600 (*sec* NH), 1510 (C=N), 1340 (=CH), 830, 760 (CH aromat.). $^1$H NMR (DMSO): δ = 7.01 (m, 2H), 7.45 (m, 2H), 7.64 (d, *J* = 8 Hz, 2H), 8.01 (d, *J* = 8 Hz, 2H), 9.61 (s, 1H), 12.67 (s, 1H). Anal.: $C_{14}H_{10}N_3Br$ (300.16). MS (70 eV): *m/z* (%) = 300 (98), 298 (100), 220 (90), 219 (17), 218 (11), 118 (31), 91 (21), 90 (20), 76 (10), 75 (8), 39 (14).

*2-(o-Chlorobenzylideneamino)benzimidazole* (**6**)

Yield 1.80 g (70%), yellow precipitate from ethanol, m.p. 212–217 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 2880 (CH), 1640 (C=N), 1690, 1560 (*sec* NH), 1420 (–$CH_2$–), 1330, 1300 (=NH), 1280 (NH), 820, 740 (CH aromat.). $^1$H NMR (DMSO): δ = 7.20 (m, 2H), 7.57 (m, 4H), 7.82 (m, 1H), 8.26 (m, 1H), 9.72 (s, 1H), 12.81 (s, 1H). Anal.: $C_{14}H_{10}N_3Cl$ (255.71). MS (70 eV): *m/z* (%) = 255 (10), 220 (100), 193 (5), 118 (15), 91 (8), 90 (11), 76 (4), 65 (7), 63 (8), 39 (7).

*2-(m-Chlorobenzylideneamino)benzimidazole* (**7**)

Yield 1.50 g (59%), yellow precipitate from ethanol, m.p. 200–202 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 2880 (CH), 1640 (C=N), 1560 (*sec* NH), 1420 (–CH–), 1300 (=NH), 810, 740 (CH aromat.). $^1$H NMR (DMSO): δ = 7.07 (m, 2H), 7.49 (m, 2H), 7.87 (d, *J* = 8.02 Hz, 2H), 8.07 (m, 2H), 9.68 (s, 1H), 12.77 (s, 1H). Anal.: $C_{14}H_{10}N_3Cl$ (255.71). MS (70 eV) *m/z* (%) = 257 (48), 255 (100), 220 (30), 193 (7), 118 (18), 91 (26), 90 (12), 65 (33), 39 (20).

*2-(p-Chlorobenzylideneamino)benzimidazole* (**8**)

Yield 1.53 g (60%) yellow precipitate crystallised from ethanol, m.p. 179–181 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 2880 (CH), 1640 (C=N), 1560 (*sec* NH), 1500 (C=N), 1420 (–CH–), 1300 (=NH), 820, 740 (CH aromat). $^1$H NMR (DMSO): δ = 7.01 (m, 2H), 7.45 (m, 2H), 7.65 (d, *J* = 8.15 Hz, 2H), 8.02 (d, *J* = 8.15 Hz, 2H), 9.62 (s, 1H), 12.67 (s, 1H). Anal.: $C_{14}H_{10}N_3Cl$ (255.71). MS (70 eV): *m/z* (%) = 257 (48), 255 (100), 220 (28), 193 (5), 118 (15), 91 (25), 90 (11), 76 (4), 65 (71), 65 (31), 39 (17).

*2-(p-Nitrobenzylideneamino)benzimidazole* (**9**)

Yield 1.95 g (73%), yellow precipitate from ethanol, m.p. 266–268 °C. IR (KBr): ν $cm^{-1}$ = 3380 (*sec* NH), 2880 (CH), 1640 (C=N), 1565 (*sec* NH, C=N), 1520, 1350 ($NO_2$), 830 (CH). $^1$H NMR (DMSO): δ = 7.19 (m, 2H), 7.56 (m, 2H), 8.22 (d, 2H, *J* = 9 Hz), 8.30 (d, 2H, *J* = 9 Hz), 9.57 (s, 1H), 12.73 (s, 1H). Anal.: $C_{14}H_{10}N_4O_2$ (266.26). MS (70 eV): *m/z* (%) = 266 (71), 265 (100), 220 (12), 193 (15), 118 (29), 91 (12), 90 (15), 76 (7), 65 (6), 64 (8), 63 (14), 39 (9).

*2-(o-Nitrobenzylideneamino)benzimidazole* (**10**)

Yield 0.70 g (26%), yellow precipitate from ethanol, m.p. 297–299 °C. IR (KBr): ν $cm^{-1}$ = 3390 (*sec* NH), 2890 (CH), 1645 (C=N), 1560 (*sec* NH), 1530, 1355 ($NO_2$), 760 (CH aromat.). $^1$H NMR (DMSO): δ = 7.22 (m, 2H), 7.60 (m, 4H), 7.93 (m, 1H), 8.43 (m, 1H), 9.75 (s, 1H), 12.85 (s, 1H). Anal.: $C_{14}H_{10}N_4O_2$ (266.26). MS (70 eV) *m/z* (%) = 266 (72), 265 (100), 219 (27), 193 (18), 118 (35), 91 (15) 76 (16), 65 (16), 39 (12).

*2-(p-Trifluoromethylbenzylideneamino)benzimidazole* (**11**)

Yield 1.95 g (67%) yellow precipitate crystallised from ethanol, m.p. 238–240 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 3000 (CH aromat.), 2840 (CH), 1635 (C=N), 1580 (C=N ring), 870 (CH aromat. 1,4-disubstituted), 750 (CH aromat. 1,2-disubstituted). $^1$H NMR (DMSO): δ = 7.21 (m, 2H), 7.54 (m, 2H), 7.93 (d, *J* = 8.04 Hz, 2H), 8.27 (d, *J* = 7.99 Hz, 2H), 9.56 (s, 1H), 12.83 (br, 1H). Anal.: $C_{15}H_{10}N_3F_3$ (289.26). MS (70 eV): *m/z* (%) = 289.1 (47.36), 288.1 (100.00), 262.1 (6.52), 261.1 (4.84), 220.1 (2.36), 118.1 (6.83), 91.1 (3.18), 90.1 (3.39), 65.1 (0.98), 64.1 (1.21), 39.1 (0.44).

### *3.1.2. Reduction of Schiff bases* ***1–11*** *with* $NaBH_4$, ***12–21***

One portion of $NaBH_4$ (0.01 mol) was added to boiling mixture of 0.01 mol of appropriate compound in 250 ml of isopropanol during heating for 3–4 h (TLC). Then the alcohol was refluxed under reduced pressure and 200 ml of water was added to cold and dry residue. Undissolved residue was filtered, washed with water (ca. 1500 ml) to neutral reaction. After drying precipitates were crystallised.

*2-(p-Methoxybenzylamino)benzimidazole* (**12**)

Yield 1.27 g (50%), white precipitate crystallised from acetonitrile, m.p. 207–208 °C. IR (KBr): ν $cm^{-1}$ = 3300 (*sec* NH), (CH), 2820 (–$OCH_3$), 1625 (NH, C=N), 1500 (C=N), 1335 (*sec* NH, CH), 1050 (NH), 810, 730 (CH aromat.). $^1$H NMR (DMSO): δ = 3.70 (s, 3H), 4.42 (d, *J* = 4.42 Hz, 2H), 6.85 (m, 4H), 7.05 (t, 1H), 7.17 (m, 2H), 7.29 (d, *J* = 8.58 Hz, 2H), 10.87 (br, 1H). Anal.: $C_{15}H_{15}N_3O$ (253.29). MS (70 eV): *m/z* (%) = 254 (6), 253 (40), 122 (10), 121 (100), 119 (3), 105 (3), 91 (7), 90 (3), 78 (9), 77 (9), 65 (3), 51 (3), 41 (4), 39 (3).

*2-(3,4-Dimethoxybenzylamino)benzimidazole* (**13**)

Yield 0.75 g (27%), white precipitate crystallised from acetonitrile, m.p. 234.5–236 °C. IR (KBr): ν $cm^{-1}$ = 3280 (NH), 3020 (CH), 2810 (–$OCH_3$), 1625 (*sec* NH), 1500 (C=N), 1400 (–$OCH_3$), 1255 (C–O–C), 1220, 880, 730 (CH). $^1$H NMR (DMSO): δ = 3.72(s, 6H), 4.41(d, *J* = 4.48 Hz, 2H), 6.84(m, 4H), 6.98(t, 1H), 7.01(s, 1H), 7.11(t, 2H), 10.79 (br, 1H). Anal.: $C_{16}H_{17}N_3O_2$ (283.33). MS (70 eV): *m/z* (%) = 284 (4), 283 (24), 152 (8), 151 (100), 133 (4), 121 (5), 107 (5), 106 (5), 105 (5), 91 (4), 90 (4), 78 (4), 77 (3), 51 (31), 39 (3).

*2-(3,4,5-Trimethoxybenzylamino)benzimidazole* (**14**)

Yield 0.56 g (18%), white solid from ethanol, m.p. 107–111 °C. IR (KBr) ν $cm^{-1}$ = 3380 (*sec* NH), 2860, 2845 (CH), 1625 (*sec* NH), 1500 (C=N), 1440 (–$OCH_3$), 1225 (C–O–C), 810, 760 (CH aromat.). $^1$H NMR (DMSO): δ = 3.72 (s, 3H), 3.85 (s, 6H), 4.42 (d, *J* = 4.60 Hz, 2H), 6.83 (m, 4H), 6.88 (t, 1H), 6.97 (s, 1H), 7.15 (m, 1H), 10.83 (br, 1H). Anal.: $C_{17}H_{19}N_3O_3$ (313.36). MS (70 eV) *m/z* (%) = 315 (12), 313 (100), 298 (5), 282 (25), 267 (8), 212 (8), 142 (18), 118 (20), 91 (25), 65 (10), 39 (8).

*2-(o-Bromobenzylamino)benzimidazole* (**15**)

Yield: 2.15 g (70%), white solid crystals from ethanol, m.p. 213–215 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 3040 (CH), 2920 (–$CH_2$–), 1650 (C=N), 1600 (*sec* NH), 1530 (C=N), 1480, 1450 ($CH_2$), 1280 (NH), 960, 760 (aromat.). $^1$H NMR (DMSO): δ = 4.55 (d, *J* = 4.52 Hz, 2H), 6.87 (s, 2H), 7.16 (m, 4H), 7.36 (m, 2H), 7.63 (d, *J* = 7.80 Hz, 1H), 10.91 (s, 1H). Anal.: $C_{14}H_{12}N_3Br$ (302.17). MS (70 eV): *m/z* (%) = 304 (3), 303 (17), 302 (5), 301 (18), 223 (13), 222 (100), 220 (11), 171 (11), 105 (9), 91 (8), 90 (15).

*2-(p-Bromobenzylamino)benzimidazole* (**16**)

Yield 1.83 g (61%), white crystal from ethanol, m.p. 195–197 °C. IR (KBr): ν $cm^{-1}$ = 3330 (NH), 3030 (–CH–), 2920 (–$CH_2$–), 1650 (C=N), 1620, 1330 (NH), 860, 760 (CH aromat.). $^1$H NMR (DMSO): δ = 4.45 (d, *J* = 4.31 Hz, 2H), 7.00 (br, 1H), 7.12 (m, 2H), 7.35 (d, *J* = 8.60 Hz, 2H), 7.60 (m, 2H), 8.03 (d, *J* = 8.60 Hz, 2H), 11.0 (s, 1H). Anal.: $C_{14}H_{12}N_3Br$ (302.17). MS (70 eV): *m/z* (%) = 304 (12), 303 (74), 302 (38), 301 (100), 223 (5), 222 (8), 221 (6), 220 (9), 146 (13), 132 (26), 105 (19), 91 (13), 90 (47), 89 (18), 63 (11), 39 (12).

*2-(o-Chlorobenzylamino)benzimidazole* (**17**)

Yield 2.01 g (78%), white crystals from ethanol, m.p. 212–214 °C. IR (KBr): ν $cm^{-1}$ = 3320 (*sec* NH), 1620 (C=N), 1600, 1580 (*sec* NH), 1450 ($CH_2$), 1350, 1260 (NH), 830, 730 (CH aromat.), $^1$H NMR (DMSO): δ = 4.55 (d, *J* = 4.52 Hz, 2H), 6.95 (m, 2H), 7.12 (m, 3H), 7.19 (td, 1H), 7.32 (td, 1H), 7.40 (m, 1H), 7.61 (dd, 1H), 10.88 (s, br, 1H). Anal.: $C_{14}H_{12}N_3Cl$ (257.72). MS (70 eV): *m/z* (%) = 258 (6), 257 (43), 223 (30), 222 (100), 220 (25), 171 (36), 132 (32), 91 (21), 90 (79), 89 (32), 78 (13), 77 (13), 76 (6), (10), 64 (12), 63 (23), 39 (15).

*2-(m-Chlorobenzylamino)benzimidazole* (**18**)

Yield 0.98 g (38%), white solid from ethanol, m.p. 170–172 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 3040 (CH), 2920 (–$CH_2$–), 1650 (C=N), 1600 (*sec* NH), 1530 (C=N), 1480, 1450 ($CH_2$), 1280 (NH), 900, 760 (CH aromat), $^1$H NMR (DMSO): δ = 4.40 (d, *J* = 4.49 Hz, 2H), 6.74 (s, 1H), 6.89 (s, 1H), 7.18 (m, 4H), 7.48 (m, 3H), 10.87 (s, br, 1H). Anal.: $C_{14}H_{12}N_3Cl$ (257,12). MS (70 eV): *m/z* (%) = 259 (14), 258 (7), 257 (45), 223 (28), 222 (100), 171 (35), 132 (30), 90 (80), 77 (18), 65 (8), 63 (21), 39 (12).

*2-(p-Chlorobenzyloamino)benzimidazol* (**19**)

Yield 2.00 g (78%) white solid crystallised from ethanol, m.p. 166–168 °C. IR (KBr): ν $cm^{-1}$3400 (NH, *sec.* ring), 3080 (C–H aromat), 2830 (–$CH_2$–), 1630 (C=N), 1590 (C–N ring) 1460 (–$CH_2$), 820, 745 (CH aromat.). $^1$H NMR (DMSO): δ = 4.49 (d, *J* = 4.82 Hz, 2H), 6.12 (s-br, 1H), 6.75 (m, 2H), 7.13 (m, 3H), 7.45 (m, 3H), 10.87 (s-br, 1H). Anal.: $C_{14}H_{12}N_3Cl$ (257.12). MS (70 eV): *m/z* (%) = 258 (7), 257 (44), 223 (30), 222 (100), 171 (37), 132 (30), 90 (79), 77 (14), 65 (10), 64 (5), 63 (24), 39 (16).

*2-(p-Nitrobenzylamino)benzimidazole* (**20**)

Yield 0.72 g (27%), bricky solid from DMF, m.p. 252–254 °C. IR (KBr): ν $cm^{-1}$ = 3400 (*sec* NH), 3010, 2860 (CH), 1625 (*sec* NH), 1500 (C=N), 1535, 1360 ($NO_2$), 760 (CH aromat.). $^1$H NMR (DMSO): δ = 4.50 (d, *J* = 4.20 Hz, 2H), 6.78 (s, 2H), 7.12 (m, 4H), 7.32 (m, 2H), 7.69 (m, 1H), 10.78 (s, 1H). Anal.: $C_{14}H_{12}N_4O_2$ (268.29). MS (70 eV) *m/z* (%) = 268 (11), 267 (100), 222 (17), 171 (27), 132 (15), 90 (18), 77 (8), 65 (12), 39 (7).

*2-(p-Trifluoromethylbenzylamino)benzimidazole* (**21**)

Yield 2.01 g (69%) white crystal from ethanol, m.p. 222–223 °C. IR (KBr): ν $cm^{-1}$ = 3470, 3440 (*sec*. NH), 3050 (CH aromat.), 2920 (–$CH_2$–), 1630 (*sec* NH), 1580 (C=N ring), 1465 (–$CH_2$–), 845, 745 (CH aromat.). $^1$H NMR (DMSO): δ: = 4.59 (d, *J* = 4.15 Hz, 2H), 6.85 (t, 2H), 7.11 (d, *J* = 8.79 Hz, 2H), 7.23 (t, 1H), 7.57 (d, *J* = 7.40 Hz, 2H), 7.68 (d, *J* = 8.37 Hz, 2H), 10.89 (s, 1H). $^1$H NMR (DMSO + $D_2O$): δ: = 4.56 (s), 6.88 (m), 7.11 (m), 7.55 (d, *J* = 8.20 Hz), 7.62 (d, *J* = 8.28 Hz). Anal.: $C_{15}H_{12}N_3F_3$ (291.28). MS (70 eV): *m/z* (%) = 292.1 (16), 291.1 (100), 290.1 (39), 263.1 (3), 174.1 (5), 159.1 (12), 146.1 (7), 132.1 (26), 118.1 (14), 105.1 (11), 90.1 (6), 65.1 (1).

### *3.1.3. Reactions of compounds **15** and **19** with α-, β-unsaturated chlorides, cinnamoyl chloride,* p-*nitrocinnamoyl chloride*

General procedure: To the suspension of **15** or **19** (0.01 mol) in anhydrous THF (50 ml) cinnamoyl chloride (0.01 mol) or *p*-nitrocinnamoyl chloride was added. The mixture was heated and stirred for 5 h (**22**), or 40 h (**23** and **24**). At the end of the reaction precipitate was filtered, washed with ether, dried and neutralized with 5% solution of $NaHCO_3$.

*2-(o-Bromobenzylamino)-1-cinnamoylbenzimidazole* (**22**)

Yield 1.7 g (40%), yellow precipitate from ethanol, m.p. 238–240 °C. IR (KBr): ν $cm^{-1}$ = 3310 (–NH–$CH_2$–), 3040 (CH=CH–, *trans*), 2920($CH_2$), 1675 (–CH=CH– *trans* or NCO), 1630 (C=N ring), 1580 (*sec* NH), 1460 ($CH_2$), 1345 (*sec* NH), 1300, 990 (–CH=CH– *trans*), 750 (C–Br), 700 (CH aromat). $^1$H NMR (DMSO): δ (ppm) = 4.71 (d, *J* = 4.51 Hz, 2H), 7.16 (t, 1H), 7.33 (m, 4H), 7.50 (m, 6H), 7.65 (d, *J* = 7.74 Hz, 1H), 7.87 (t, 2H), 7.95 (d, *J* = 15.42 Hz, 1H), 8.18 (t, 1H). Anal.: $C_{23}H_{18}N_3OBr$ (432.32). MS (70 eV): *m/z* (%) = 433 (18), 431 (18), 352 (8), 303 (13), 301 (14), (16), 222 (36), 220 (13), 132 (8), 131 (100), 118 (13), 103 (54), 91 (16), 77 (26), 76 (3).

*1-(o-Bromobenzyl)-2-(p-nitrophenyl)-1,2,3,4-tetrahydropyrimido[1,2-a]-benzimidazol-4-one* (**23**)

Yield 3.09 g (64%) yellow precipitate crystallised from ethanol, m.p. 176–178 °C. IR (KBr): ν $cm^{-1}$ = 3040 (CH), 2940 (–$CH_2$–), 1625 (C=O), 1580, 1520 (pyrimid. ring), 1455 (–$CH_2$–), 1345 ($NO_2$), 1020 (CH pyrimid.), 850 (1,4-disubstituted benzene, CH), 750 (CH, 1,2-disubstituted benzene). $^1$H NMR (DMSO): δ = 3.16 (dd, *J* = 16.92 Hz, *J* = 3.53 Hz, 1H), 3.80 (dd, *J* = 16.92 Hz, *J* = 2.62 Hz, 1H), 5.19 (s, 2H), 6.04 (m, 1H), 7.08 (m, 2H), 7.19 (m, 2H), 7.25 (m, 2H), 7.39 (m, 2H), 7.51 (d, *J* = 8.73 Hz, 1H), 7.63 (m, 1H), 7.85 (d, *J* = 7.83 Hz, 1H), 8.21 (d, *J* = 8.73 Hz, 1H). Anal.: $C_{23}H_{17}N_4O_3Br$ (477.32). MS (70 eV): *m/z* (%) = 479.0 (6), 478.0 (25), 477.1 (7), 476.0 (25), 397.1 (100), 355.1 (4), 302.0 (4), 300 (6), 222.1 (10), 220.1 (14), 171.0 (24), 169.0 (26), 90.1 (7), 77.1 (1).

*1-(p-Chlorobenzyl)-2-phenyl-1,2,3,4-tetrahydropyrimido[1,2-a]benzimidazol-4-one* (**24**)

Yield 1.98 g (52%) white precipitate crystallised from ethanol, m.p 186–187 °C. IR (KBr): ν $cm^{-1}$ = 3020 (CH), 2920 (–$CH_2$–), 1630 (C=O amid *tert*.), 1610, 1535 (pyrimid. ring), 1450 (–$CH_2$–), 1010 (CH pyrimid.), 850 (CH, 1,4-disubstituted benzene), 750 (CH, 1,2-disubstituted benzene). $^1$H NMR (DMSO): δ = 3.13 (dd, *J* = 16.12 Hz, *J* = 3.14 Hz, 1H), 3.71 (dd, *J* = 16.37 Hz, *J* = 2.34 Hz, 1H), 5.21 (s, 2H), 6.01 (dd, *J* = 6.68 Hz, *J* = 3.02 Hz, 1H), 6.97 (m, 2H), 7.11 (m, 3H), 7.28 (m, 3H), 7.35 (m, 4H), 7.53 (m, 1H). Anal.: $C_{23}H_{18}N_3OCl$ (387.87). MS (70 eV): *m/z* (%) = 390.1 (8), 389 (35), 388.1 (29), 387.1 (100), 361 (4), 324 (3), 285 (6), 284 (3), 283 (18), 257 (13), 256.0 (42), 255.0 (35), 254.1 (93), 248 (5), 247 (8), 246 (3), 222 (8), 220 (6), 219 (9), 131.1 (8), 127.0 (24), 126.0 (6), 125.0 (76). 104.1 (17), 103.1 (12), 91.1 (4), 90.0 (12), 78.1 (5), 77.1 (7), 65.1 (2).

### *3.2. Antiproliferative assay in vitro*

#### *3.2.1. Compounds*

The compounds **1–24** were examined in in vitro screening assay. Test solutions of the compounds (1 mg/ml) were prepared ex tempore by dissolving the substance in 100 μl of DMSO completed with 900 μl of tissue culture medium. Afterwards, the compounds were diluted in culture medium (described below) to reach the final concentrations of 100, 10, 1 and 0.1 μg/ml. The solvent (DMSO) in the highest concentration used in test did not reveal any cytotoxic activity (data not shown). Cisplatin was applied as a test referential agent.

#### *3.2.2. Cells*

The following established in vitro human cancer cell lines were applied: SW707 (rectal adenocarcinoma), A549 (non-

small cell lung carcinoma) and T47D (breast cancer). All lines were obtained from the American Type Culture Collection (Rockville, Maryland, USA) and are maintained in the Cell Culture Collection of the Institute of Immunology and Experimental Therapy, Wroclaw, Poland. Human uroepithelial cell line HCV29T established in Fibiger Institute, Copenhagen, Denmark, was obtained from Dr. J. Kieler in 1982 and maintained at the Institute of Immunology and Experimental Therapy, Wroclaw, Poland.

Twenty-four hours before addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, USA) at a density of $10^4$ cells per well. The cells were cultured in the opti-MEM medium supplemented with 2 mM glutamine (Gibco, Warsaw, Poland), streptomycin (50 μg/ml), penicillin (50 U/ml) (both antibiotics from Polfa, Tarchomin, Poland) and 5% feotal calf serum (Gibco, Grand Island, USA). The cell cultures were maintained at 37 °C in humid atmosphere saturated with 5% $CO_2$.

### *3.2.3. Sulphorhodamine B assay*

The details of this technique were described by Skehan et al. [31]. The cytotoxicity assay was performed after 72 h exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/ml) of the tested agents. The cells attached to the plastic were fixed by gently layering cold 50% trichloroacetic acid (TCA, Aldrich-Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4 °C for 1 h and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma, Germany) dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 min. Unbound dye was removed by rinsing (4×) with 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCh, Gliwice, Poland) for determination of optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland). Each compound in given concentration was tested in triplicates in each experiment repeated three to five times.

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