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Synthesis, crystal structure determination and antiproliferative activity of novel 2-amino-4-aryl-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles

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ABSTRACT

This manuscript describes the synthesis of novel 2-amino-4-aryl-4,10-dihydro-[1,3,5]triazino[1,2-*a*]benzimidazoles as hydrochloride salts **4a-n** and **5b** which were prepared in the reaction of cyclocondensation between 2-guanidinobenzimidazole and versatile heteroaromatic aldehydes. Structures of all prepared compounds have been studied by using ^{1}H and ^{13}C NMR, IR and UV/Vis spectroscopy.

The crystal and molecular structure of **4f** was determined by X-ray diffraction on single crystals. The molecule of 2-amino-4-(4′-methylphenyl)-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole hydrochloride **4f** ($C_{16}H_{16}N_5^+ \cdot Cl^-$) exists in the solid state in one of the possible tautomeric forms, being protonated at the one of the nitrogen atoms of the 1,4-dihydrotriazine ring. The molecule is highly delocalized within the 4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole moiety with the highest deviation from the plane for the methine carbon atom and the protonated nitrogen atom of the 1,4-dihydrotriazine ring. The cations are joined *via* N–H···N hydrogen bonds into $R_2^2(8)$ centrosymmetric dimers. Cation dimers are further connected with Cl^- ions *via* N–H···Cl and C–H···Cl hydrogen bonds into 2D chains spreading along the *b* axis. The obtained single-crystal X-ray structure determination unequivocally confirms tautomeric form of the compound present in the solid-state and can represent tantative pattern for other prepared compounds.

All prepared compounds were tested on their antiproliferative activity *in vitro* on several human cancer cell lines. Compound **4m** was the most active one ($IC_{50} \approx 20$ μM), while compounds **4d**, **4f**, **4k**, **4l** **4m** showed moderate, but non-selective, antiproliferative activity with IC_{50} 25–60 μM.

1. Introduction

Benzimidazoles represent the key building block for a variety of numerous medical and biochemical agents possessing different chemical and pharmacological features. A large number of benzimidazoles derivatives play also crucial roles in the functions of a number of biologically important molecules. In addition, benzimidazole derivatives posses diverse biological properties like anticancer [1–3], antiviral [4,5], antibacterial [6,7], antifungal [8], antihistaminic [9] or anticonvulsant activity [10]. We have recently reported the synthesis and antiproliferative activity of versatile heterocyclic benzimidazole derivatives as well as fused benzoannulated benzimidazole derivatives related to benzimidazo[1,2-*a*]quinolines [11–13]. Thus, our previously published results reveal that amidino substituted 2-styrylbenzimidazoles and their cyclic analogs benzimidazo[1,2-*a*]quinolines showed significant antiproliferative effects and pronounced selectivity toward tumor cells in regard to normal cells. We also showed that some positively charged analogs of benzimidazo[1,2-*a*]quinolines and diaza-cyclopenta[*c*]fluorenes intercalate into ds DNA or RNA, which might account for their accentuated antiproliferative effect.

On the other hand, guanidine functionalities are found in a large number of synthetic and naturally occurring compounds such as sponges, algae or different microorganisms [14]. Their synthesis has attracted much attention from organic and medicinal chemists and many synthetic methods were developed [15]. Guanidine group can be found either as a terminal group of substituents or in the form of five- or six-membered heterocycles. Their derivatives posses the diversity of the chemical, physicochemical and biological properties [16–19]. Guanidines are also efficient ligands for the synthesis of various organometallic catalysts with a various metal ions [20,21]. Triazino[1,2-*a*]benzimidazole derivatives which posses a guanidine group as a part of six-membered ring, have a wide range of biological effects including antibacterial and antifungal activity and dihydrofolate reductase (DHFR) inhibitory activities [22,23].

Above mentioned considerations prompted us to explore a new series of different substituted heteroaromatic 2-amino-

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4-aryl-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles as hydrochloride salts. The hydrochloride salts were prepared to improve their solubility in water. Full details about the synthesis, single crystal X-ray structure determination and evaluation of antiproliferative activity on the panel of several human tumor cell lines *in vitro* are reported herein.

2. Experimental section

2.1. Synthesis

2.1.1. Instrumental methods of detection

All chemicals were purchased from commercial suppliers. Melting points were recorded on SMP11 Bibby apparatus. The ^{1}H 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. NMR spectra were measured in DMSO-d_6 solutions using TMS as an internal standard. IR spectra were recorded on a Bruker Vertex 70 spectrophotometer with diamond crystal. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates. Elemental analysis for carbon, hydrogen and nitrogen were performed on a Perkin–Elmer 2400 elemental analyzer. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value. All compounds were routinely checked by thin layer chromatography (TLC) using precoated *Merck* silica gel 60F-254 plates and the spots were detected under UV light (254 and 366 nm).

2.1.2. General method for preparation of compounds ***4a-n***

Solution of equimolar amounts of 2-guanidinobenzimidazole **1**, corresponding heteroaromatic aldehydes **2a-n** and few drops of piperidine in absolute ethanol, were refluxed for 2–4 h. After reaction mixture was cooled to the room temperature, the crude product was filtered off and recrystallized from ethanol to obtain powder products **3a-n**. A stirred suspension of compounds **3a-n** in absolute ethanol (15 ml) was cooled in an ice-salt bath and saturated with $HCl_{(g)}$. After 24 h of stirring small amount of diethylether was added, resulting product was filtered off and washed with diethylether to obtain hydrochlorides **4a-n**.

*2.1.2.1. 2-Amino-4-phenyl-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride **4a**.* Compound **4a** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and benzaldehyde **2a** (0.18 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 2 h and recrystallization from ethanol to yield 0.31 g (70%) of white powder **3a** which was suspended in absolute ethanol (15 ml) and saturated with $HCl_{(g)}$ to gave 0.32 g (90%) of white powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3420, 3310, 3209, 2980, 1675, 1635; ^{1}H NMR (600 MHz, DMSO-d_6): 13.30 (s, 1H, NH), 9.35 (s, 1H, $H_{arom.}$), 8.03 (brs, 1H, NH^+), 7.52 (brs, 2H, $H_{arom.}$), 7.48–7.44 (m, 3H, $H_{arom.}$), 7.38 (d, 1H, J = 7.85 Hz, $H_{arom.}$), 7.23 (t, 1H, J = 7.68 Hz, $H_{arom.}$), 7.12 (1H, J = 7.80 Hz, $H_{arom.}$), 6.99 (s, 1H, $H_{arom.}$), 6.92 (d, 1H, J = 7.98 Hz, $H_{arom.}$); ^{13}C NMR (150 MHz, DMSO-d_6): 157.2 (s), 151.2 (s), 138.4 (d), 130.6 (d), 129.8 (d, 2C), 128.2 (s), 127.3 (d, 2C), 126.4 (s), 124.4 (d), 123.4 (d), 112.2 (d), 110.9 (d), 66.3 (d); Anal. Calcd. for $C_{15}H_{14}ClN_5$: C, 60.10; H, 4.71; N, 23.36. Found: C, 59.96; H, 5.00; N, 23.60.

*2.1.2.2. 2-Amino-4-(4′-fluorophenyl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride **4b**.* Compound **4b** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 4-fluorobenzaldehyde **2b** (0.21 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.30 g (63%) of white powder **3b** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.28 g (83%) of white powder; mp = 212–213 °C; IR (KBr) υ/cm^{-1}: 3467, 3298, 3220, 2964, 1659, 1625; ^{1}H NMR (600 MHz, DMSO-d_6): 13.31 (s, 1H, NH), 9.36 (s, 1H, $H_{arom.}$), 7.95 (d, 2H, J = 8.13 Hz, $H_{arom.}$), 7.72 (d, 2H, J = 8.19 Hz, $H_{arom.}$), 7.39 (d, 1H, J = 7.83 Hz, $H_{arom.}$), 7.26 (t, 1H, J = 7.64 Hz, $H_{arom.}$), 7.16 (d, 1H, J = 7.92 Hz, $H_{arom.}$), 7.12 (s, 1H, $H_{arom.}$), 6.99 (d, 1H, J = 7.89 Hz, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): 157.0 (s), 151.1 (s), 143.2 (s), 133.8 (d, 2C), 129.8 (s), 128.3 (d, 2C), 128.0 (s), 124.6 (d), 123.6 (d), 118.6 (s), 113.3 (s), 112.3 (d), 110.8 (d), 65.4 (d); Anal. Calcd. for $C_{15}H_{13}ClFN_5$: C, 56.70; H, 4.12; N, 22.04. Found: C, 56.49; H, 4.08; N, 21.88.

*2.1.2.3. 2-Amino-4-(4′-cyanophenyl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride **4c**.* Compound **4c** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 4-cyanobenzaldehyde **2c** (0.22 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 4 h and recrystallization from ethanol to yield 0.31 g (64%) of white powder **3c** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.30 g (86%) of white powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3398, 3304, 3199, 2984, 2225, 1690, 1624; ^{1}H NMR (600 MHz, DMSO-d_6): 13.29 (s, 1H, NH), 9.33 (s, 1H, $H_{arom.}$), 8.01 (brs, 1H, NH^+), 7.61 (d, 2H, J = 8.58 Hz, $H_{arom.}$), 7.38 (t, 1H, J = 7.78 Hz, $H_{arom.}$), 7.31 (d, 2H, J = 8.52 Hz, $H_{arom.}$), 7.13 (t, 2H, J = 7.68 Hz, $H_{arom.}$), 7.00 (s, 1H, $H_{arom.}$), 6.90 (d, 1H, J = 7.98 Hz, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): 164.9 (s), 161.8 (s), 157.1 (s), 151.1 (s), 134.8 (s), 134.1 (s), 129.8 (s), 129.7 (d), 129.6 (d), 128.1 (s), 124.4 (d), 123.5 (d), 116.9 (d), 116.5 (d), 112.6 (d), 110.9 (d), 65.6 (d); Anal. Calcd. for $C_{16}H_{13}ClN_6$: C, 59.17; H, 4.03; N, 25.88. Found: C, 59.05; H, 4.12; N, 26.02.

*2.1.2.4. 2-Amino-4-(4′-N,N-dimethylaminophenyl)-4,10-dihydro [1,3,5]-triazino[1,2-a]benzimidazole hydrochloride **4d**.* Compound **4d** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 4-*N,N*-dimethylaminobenzaldehyde **2d** (0.26 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 2 h and recrystallization from ethanol to yield 0.33 g (63%) of yellow powder **3d** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.32 g (86%) of orange powder; mp = 263–264 °C; IR (KBr) υ/cm^{-1}: 3409, 3312, 3199, 2944, 1689, 1641; ^{1}H NMR (300 MHz, DMSO-d_6): 13.30 (s, 1H, NH), 9.33 (s, 1H, $H_{arom.}$), 8.00 (brs, 1H, NH^+), 7.42 (d, 2H, J = 8.46 Hz, $H_{arom.}$), 7.36 (d, 1H, J = 7.92 Hz, $H_{arom.}$), 7.22 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 7.11 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 7.02 (brs, 2H, $H_{arom.}$), 6.89 (d, 2H, J = 8.42 Hz, $H_{arom.}$), 5.42 (brs, 3H, NH_3^+), 2.93 (s, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 156.8 (s), 150.7 (s), 129.3 (s), 128.0 (d, 2C), 127.8 (s), 126.4 (s), 124.8 (d, 2C), 123.8 (d), 122.9 (d), 121.4 (s), 111.6 (d), 110.6 (d), 65.7 (d), 41.6 (q, 2C); Anal. Calcd. for $C_{17}H_{19}ClN_6$: C, 59.56; H, 5.59; N, 24.51. Found: C, 59.82; H, 5.70; N, 24.61.

*2.1.2.5. 2-Amino-4-(N-methylpyrol-2-yl)-4,10-dihydro[1,3,5]triazino [1,2-a]benzimidazole hydrochloride **4e**.* Compound **4e** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and *N*-methyl-pyrole-2-carboxaldehyde **2e** (0.19 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.22 g (50%) of white powder **3e** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.20 g (80%) of yellow powder; mp = 289–290 °C; IR (KBr) υ/cm^{-1}: 3395, 3291, 3203, 2963, 1666, 1629; ^{1}H NMR (600 MHz, DMSO-d_6): 13.26 (s, 1H, NH), 9.30 (s, 1H, $H_{arom.}$), 8.04 (brs, 1H, NH^+), 7.39 (d, 1H, J = 7.86 Hz, $H_{arom.}$), 7.25 (t, 1H, J = 7.65 Hz, $H_{arom.}$), 7.13 (t, 1H, J = 7.68 Hz, $H_{arom.}$), 7.04 (s, 1H, $H_{arom.}$), 6.91 (d, 2H, J = 7.98 Hz, $H_{arom.}$), 6.26 (t, 2H, J = 3.24 Hz, H_{Py}), 6.04 (t, 2H, J = 3.24 Hz, H_{Py}), 3.56 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): 157.6 (s), 151.6 (s), 129.8 (s),

128.5 (s), 126.7 (d), 126.1 (s), 125.9 (s), 124.4 (d), 123.5 (d), 112.2 (d), 111.7 (d), 110.8 (d), 107.4 (d), 60.8 (d), 34.5 (q); Anal. Calcd. for $C_{14}H_{15}ClN_6$: C, 55.54; H, 4.99; N, 27.76. Found: C, 55.72; H, 5.12; N, 27.62.

*2.1.2.6. 2-Amino-4-(4′-methylphenyl)-4,10-dihydro[1,3,5]triazino [1,2-a]benzimidazole hydrochloride **4f**.* Compound **4f** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 4-methylbenzaldehyde **2f** (0.20 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.34 g (72%) of white powder **3f** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.26 g (68%) of white powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3412, 3324, 3221, 3004, 1698, 1638; ^1H NMR (600 MHz, DMSO-d_6): 13.18 (s, 1H, NH), 9.17 (s, 1H, $H_{arom.}$), 7.94 (brs, 1H, NH^+), 7.38 (d, 2H, J = 8.01 Hz, $H_{arom.}$), 7.34 (d, 1H, J = 7.98 Hz, $H_{arom.}$), 7.24 (d, 2H, J = 7.92 Hz, $H_{arom.}$), 7.21 (d, 1H, J = 7.78 Hz, $H_{arom.}$), 7.18 (d, 1H, J = 7.78 Hz, $H_{arom.}$), 7.10 (t, 1H, J = 7.72 Hz, $H_{arom.}$), 6.89 (s, 1H, $H_{arom.}$), 6.88 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 2.26 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): 157.2 (s), 151.2 (s), 140.3 (s), 135.5 (s), 130.2 (d, 2C), 129.8 (s), 128.2 (s), 127.3 (d, 2C), 124.4 (d), 123.4 (d), 112.2 (d), 111.0 (d), 66.2 (d), 21.3 (q); Anal. Calcd. for $C_{16}H_{16}ClN_5$: C, 61.24; H, 5.14; N, 22.32. Found: C, 61.36; H, 5.28; N, 22.55.

*2.1.2.7. 2-Amino-4-(furan-2-yl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride **4g**.* Compound **4g** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and furane-2-carboxaldehyde **2g** (0.16 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 4 h and recrystallization from ethanol to yield 0.24 g (57%) of light yellow powder **3g** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.25 g (92%) of white powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3411, 3323, 3198, 2956, 1684, 1642; ^1H NMR (300 MHz, DMSO-d_6):): 13.21 (s, 1H, NH), 9.27 (s, 1H, $H_{arom.}$), 8.06 (brs, 1H, NH^+), 7.67 (d, 1H, J = 1.80 Hz, $H_{arom.}$), 7.35 (d, 1H, J = 7.36 Hz, $H_{arom.}$), 7.30–7.18 (m, 2H, $H_{arom.}$), 7.17 (s, 1H, $H_{arom.}$), 6.82 (d, 1H, J = 3.15 Hz, $H_{arom.}$), 6.48 (dd, 1H, J_1 = 3.05 Hz, J_2 = 1.78 Hz, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): 157.6 (s), 150.9 (s), 149.5 (s), 145.4 (d), 129.6 (s), 127.9 (s), 126.8 (s), 124.6 (d), 123.6 (d), 112.2 (d), 111.3 (d), 110.7 (d), 59.4 (d); Anal. Calcd. for $C_{13}H_{12}ClN_5O$: C, 53.89; H, 4.17; N, 24.17. Found: C, 54.16; H, 4.25; N, 24.32.

*2.1.2.8. 2-Amino-4-(imidazole-2-yl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole dihydrochloride **4h**.* Compound **4h** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and imidazole-2-carboxaldehyde **2h** (0.16 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.17 g (40%) of white powder **3h** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.18 g (80%) of yellow powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3413, 3356, 3245, 3029, 1698, 1621; ^1H NMR (600 MHz, DMSO-d_6): 13.20 (s, 1H, NH), 9.31 (s, 1H, $H_{arom.}$), 8.64 (brs, 1H, $H_{arom.}$), 8.18 (brs, 1H, NH^+), 7.90 (s, 1H, $H_{arom.}$), 7.36 (d, 1H, J = 7.72 Hz, $H_{arom.}$), 7.23 (t, 1H, J = 7.28 Hz, $H_{arom.}$), 7.14 (d, 1H, J = 7.36 Hz, $H_{arom.}$), 7.13 (s, 1H, $H_{arom.}$), 6.95 (d, 1H, J = 7.65 Hz, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): 157.0 (s), 150.9 (s), 147.7 (d), 130.6 (s), 130.5 (s), 129.8 (s), 128.1 (s), 124.5 (d), 123.5 (d), 120.1 (d), 112.1 (d), 110.5 (d), 59.1 (d); Anal. Calcd. for $C_{12}H_{12}ClN_7$: C, 49.75; H, 4.17; N, 33.84. Found: C, 49.98; H, 4.32; N, 33.60.

*2.1.2.9. 2-Amino-4-(pyridine-4-yl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride **4i**.* Compound **4i** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and pyridine-2-carboxaldehyde **2i** (0.16 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.25 g (56%) of white powder **3i** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.23 g (82%) of white powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3398, 3314, 3202, 2986, 1664, 1650; ^1H NMR (300 MHz, DMSO-d_6): 13.42 (s, 1H, NH), 9.64 (d, 1H, J = 1.44 Hz, $H_{arom.}$), 8.77 (d, 2H, J = 5.97 Hz, H_{Py}), 8.17 (brs, 1H, NH^+), 7.71 (d, 2H, J = 5.94 Hz, H_{Py}), 7.39 (d, 1H, J = 7.80 Hz, $H_{arom.}$), 7.26 (t, 1H, J = 7.24 Hz, $H_{arom.}$), 7.19–7.09 (m, 2H, $H_{arom.}$), 7.08 (s, 1H, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): 157.0 (s), 152.8 (s), 151.0 (s), 145.8 (d, 2C), 129.8 (s), 127.9 (s), 124.8 (d), 124.3 (d, 2C), 123.7 (d), 112.5 (d), 110.9 (d), 64.3 (d); Anal. Calcd. for $C_{14}H_{13}ClN_6$: C, 55.91; H, 4.36; N, 27.94. Found: C, 55.72; H, 4.25; N, 27.71.

*2.1.2.10. 2-Amino-4-(thiophene-2-yl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride **4j**.* Compound **4j** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and thiophene-2-carboxaldehyde **2j** (0.19 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.42 g (93%) of white powder **3j** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.43 g (90%) of white powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3432, 3318, 3226, 2988, 1681, 1642; ^1H NMR (300 MHz, DMSO-d_6): 13.24 (s, 1H, NH), 9.45 (s, 1H, $H_{arom.}$), 8.11 (brs, 1H, NH^+), 7.61 (d, 1H, J = 4.95 Hz, H_{thioph}), 7.45 (d, 1H, J = 8.15 Hz, $H_{arom.}$), 7.38 (d, 1H, J = 5.20 Hz, H_{thioph}), 7.35 (d, 1H, J = 8.10 Hz, $H_{arom.}$), 7.26–7.16 (m, 2H, $H_{arom.}$), 7.05 (t, 1H, J = 4.86 Hz, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): 157.1 (s, 2C), 150.7 (s), 141.8 (s), 129.7 (s), 129.1 (d), 128.8 (d), 127.9 (s), 127.6 (d), 124.6 (d), 123.6 (d), 112.3 (d), 111.1 (d), 61.9 (d); Anal. Calcd. for $C_{13}H_{12}ClN_5S$: C, 51.06; H, 3.96; N, 22.90. Found: C, 50.89; H, 4.17; N, 22.70.

*2.1.2.11. 2-Amino-4-(4′-chlorophenyl)-4,10-dihydro[1,3,5]triazino [1,2-a]benzimidazole hydrochloride **4k**.* Compound **4k** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 4-chlorobenzaldehyde **2k** (0.24 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.35 g (70%) of white powder **3k** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.36 g (91%) of white powder; mp >290 °C; IR (KBr) υ/cm^{-1}: 3433, 3298, 3199, 2976, 1685, 1649; ^1H NMR (600 MHz, DMSO-d_6): 13.26 (s, 1H, NH), 9.30 (s, 1H, $H_{arom.}$), 7.70 (brs, 1H, NH^+), 7.52 (s, 4H, $H_{arom.}$), 7.38 (d, 1H, J = 7.83 Hz, $H_{arom.}$), 7.25 (t, 1H, J = 7.38 Hz, $H_{arom.}$), 7.14 (t, 2H, J = 7.62 Hz, $H_{arom.}$), 7.01 (s, 1H, $H_{arom.}$), 6.94 (d, 1H, J = 7.86 Hz, $H_{arom.}$); ^{13}C NMR (75 MHz, DMSO-d_6): 157.1 (s), 157.0 (s), 137.4 (s), 135.2 (s), 129.8 (d, 2C), 129.6 (s), 129.3 (d, 2C), 128.1 (s), 124.5 (d), 123.5 (d), 112.3 (d), 110.9 (d), 65.6 (s); Anal. Calcd. for $C_{15}H_{13}Cl_2N_5$: C, 53.91; H, 3.92; N, 20.96. Found: C, 54.10; H, 4.11; N, 20.69.

*2.1.2.12. 2-Amino-4-(2′-chloro-5′-nitrophenyl)-4,10-dihydro[1,3,5] triazino[1,2-a]benzimidazole hydrochloride **4l**.* Compound **4l** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 2-chloro-5-nitrobenzaldehyde **2l** (0.32 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.34 g (59%) of yellow powder **3l** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.30 g (80%) of yellow powder; mp = 256–258 °C; IR (KBr) υ/cm^{-1}: 3432, 3299, 3205, 2969, 1655, 1639; ^1H NMR (600 MHz, DMSO-d_6): 13.29 (s, 1H, NH), 8.83 (brs, 1H, NH^+), 8.40 (s, 1H, $H_{arom.}$), 8.29 (dd, 1H, J_1 = 8.70 Hz, J_2 = 2.70 Hz, $H_{arom.}$), 7.85 (d, 1H, J = 8.76 Hz, $H_{arom.}$), 7.34 (d, 1H, J = 7.92 Hz, $H_{arom.}$), 7.20 (brs, 1H, $H_{arom.}$), 7.13 (t, 1H, J = 7.62 Hz, $H_{arom.}$), 6.97 (t, 1H, J = 7.74 Hz, $H_{arom.}$), 6.72 (d, 1H, J = 7.98 Hz, $H_{arom.}$); ^{13}C NMR (150 MHz, DMSO-d_6): 156.3 (s), 152.0 (s), 147.2

(s), 139.1 (s), 136.5 (s), 133.0 (d), 128.9 (s), 126.8 (d), 126.1 (d), 123.7 (d), 122.4 (d), 113.7 (d), 109.9 (d), 65.4 (d); Anal. Calcd. for $C_{15}H_{12}Cl_2N_6O_2$: C, 47.51; H, 3.19; N, 22.16. Found: C, 47.70; H, 3.32; N, 22.25.

*2.1.2.13. 2-Amino-4-(4′-N,N-diethylamino-2′-hydroxyphenyl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride **4m**.* Compound **4m** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 4-*N,N*-diethylamino-2-hydroxybenzaldehyde **2m** (0.33 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 4 h and recrystallization from ethanol to yield 0.32 g (54%) of light yellow powder **3m** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.31 g (88%) of white powder; mp = 209–210 °C; IR (KBr) υ/cm^{-1}: 3395, 3312, 3215, 2965, 1663, 1631; ^1H NMR (600 MHz, DMSO-d_6): 13.05 (s, 1H, NH), 8.92 (s, 1H, $H_{arom.}$), 7.70 (brs, 1H, NH^+), 7.34 (d, 2H, J = 7.74 Hz, $H_{arom.}$), 7.21 (t, 1H, J = 7.56 Hz, $H_{arom.}$), 7.12 (t, 2H, J = 7.62 Hz, $H_{arom.}$), 6.98 (s, 1H, $H_{arom.}$), 6.91 (d, 1H, J = 8.14 Hz, $H_{arom.}$), 6.62 (brs, 1H, $H_{arom.}$), 3.36 (m, 4H, CH_3), 1.01 (t, 6H, J = 6.81 Hz, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): 157.3 (s, 2C), 151.3 (s), 143.0 (s), 129.6 (s), 128.3 (s), 126.0 (s), 124.1 (d), 123.3 (d), 123.2 (d), 119.4 (d), 117.6 (d), 114.6 (d), 111.9 (d), 110.5 (d), 64.3 (d), 48.9 (t), 47.7 (t), 42.7 (q), 41.6 (q); Anal. Calcd. for $C_{19}H_{23}ClN_6O$: C, 58.99; H, 5.99; N, 21.72. Found: C, 59.20; H, 6.13; N, 21.55.

*2.1.2.14. 2-Amino-4-[2′-(4″-N,N-dimethylaminophenyl)ethylene]-4,10-dihydro [1,3,5]triazino[1,2-a]benzimidazole dihydrochloride **4n**.* Compound **4n** was prepared from 2-guanidinobenzimidazole **1** (0.30 g, 1.70 mmol) and 4-*N,N*-diethylamino-2-hydroxybenzaldehyde **2n** (0.26 g, 1.70 mmol) in absolute ethanol (10 ml) and piperidine (0.15 ml) after refluxing for 3 h and recrystallization from ethanol to yield 0.45 g (80%) of orange powder **3n** which was suspended in absolute ethanol (10 ml) and saturated with $HCl_{(g)}$ to gave 0.47 g (87%) of light pink powder; mp = 227–229 °C; IR (KBr) υ/cm^{-1}: 3431, 3318, 3224, 2999, 1684, 1645; ^1H NMR (300 MHz, DMSO-d_6): 13.17 (s, 1H, NH), 9.15 (s, 1H, $H_{arom.}$), 7.98 (brs, 1H, NH^+), 7.48 (d, 2H, J = 8.25 Hz, $H_{arom.}$), 7.37 (d, 2H, J = 8.28 Hz, $H_{arom.}$), 7.30–7.21 (m, 2H, $H_{arom.}$), 7.04 (d, 1H, J = 15.51 Hz, $H_{arom.}$), 7.00 (s, 1H, $H_{arom.}$), 6.54 (d, 1H, J = 8.40 Hz, $H_{arom.}$), 6.33 (dd, 1H, J_1 = 15.48 Hz, J_2 = 8.30 Hz, $H_{arom.}$), 4.10 (brs, 2H, NH^+), 2.97 (s, 6H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): 157.3 (s, 2C), 150.8 (s), 147.1 (s), 139.2 (s), 134.9 (d, 2C), 129.8 (s), 129.0 (d, 2C), 128.3 (s), 124.3 (s), 124.2 (d), 123.5 (d), 112.1 (d), 111.1 (d), 66.0 (d), 43.5 (q, 2C); Anal. Calcd. for $C_{21}H_{25}ClN_6$: C, 63.55; H, 6.35; N, 21.17. Found: C, 63.80; H, 6.44; N, 21.30.

*2.1.3. 2Preparation of 2-amino-4-(2′-chloro-5′-aminophenyl)-3,4-dihydro[1,3,5]triazino [1,2-a]benzimidazole **5a***

Compound **4l** (0.25 g, 7.30 mmol) and solution of $SnCl_2x2H_2O$ (1.48 g, 6.57 mmol) in H_2O (2 ml) and concentrated HCl (2 ml) were refluxed for 30 min. After cooling, the reaction mixture was evaporated under vacuum and dissolved in water (50 ml). The resulting solution was treated with 20% NaOH to pH 14. The resulting product was filtered off and washed with water to gave 0.15 g (66%) of dark yellow powder; mp = 223–224 °C; IR (KBr) υ/cm^{-1}: 3456, 3322, 3268, 3110, 2977, 1658, 1625; ^1H NMR (600 MHz, DMSO-d_6): 7.88 (s, 1H, $H_{arom.}$), 7.25 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 7.12 (d, 1H, J = 8.28 Hz, $H_{arom.}$), 6.94 (t, 1H, J = 8.22 Hz, $H_{arom.}$), 6.88 (s, 1H, $H_{arom.}$), 6.80 (t, 1H, J = 7.56 Hz, $H_{arom.}$), 6.61 (d, 1H, J = 7.68 Hz, $H_{arom.}$), 6.55 (dd, 1H, J_1 = 8.60 Hz, J_2 = 2.08 Hz, $H_{arom.}$), 6.31 (d, 1H, J = 1.98 Hz, $H_{arom.}$), 6.26 (brs, 2H, NH_2), 5.34 (brs, 2H, NH_2); ^{13}C NMR (75 MHz, DMSO-d_6): 153.8 (s), 152.9 (s), 148.8 (s), 145.2 (s), 143.3 (s), 135.9 (s), 131.2 (s), 130.1 (d), 122.5 (d), 120.7 (d), 118.2 (s), 116.0 (d), 115.2 (d), 112.2 (d), 109.4 (d), 63.9 (d); Anal. Calcd. for $C_{15}H_{13}ClN_6$: C, 57.60; H, 4.19; N, 26.87. Found: C, 57.81; H, 4.26; N, 26.70.

*2.1.4. Preparation of 2-amino-4-(2′-chloro-5′-aminophenyl)-4,10-dihydro[1,3,5]triazino [1,2-a]benzimidazole hydrochloride **5b***

A stirred suspension of compound **5a** (0.05 g, 0.16 mmol) in absolute ethanol (5 ml) was cooled in an ice-salt bath and saturated with $HCl_{(g)}$. After 24 h of stirring small amount of diethylether was added, resulting product was filtered off and washed with diethylether to gave 0.45 g (80%) of yellow powder; mp 264–265 °C; IR (KBr) υ/cm^{-1}: 3452, 3328, 3253, 3119, 2989, 1652, 1644; ^1H NMR (600 MHz, DMSO-d_6): 13.30 (s, 1H, NH), 9.33 (s, 1H, $H_{arom.}$), 8.00 (brs, 1H, NH^+), 7.40 (d, 1H, J = 7.92 Hz, $H_{arom.}$), 7.31–7.24 (m, 2H, $H_{arom.}$), 7.14 (d, 1H, J = 7.68 Hz, $H_{arom.}$), 7.08 (s, 1H, $H_{arom.}$), 6.91 (brs, 1H, $H_{arom.}$), 6.85 (d, 1H, J = 7.60 Hz, $H_{arom.}$), 6.75 (d, 1H, J = 8.10 Hz, $H_{arom.}$), 4.00 (brs, 3H, NH_3^+); ^{13}C NMR (150 MHz, DMSO-d_6): 155.5 (s), 154.0 (s), 149.1 (s), 145.0 (s), 144.1 (s), 137.2 (s), 131.7 (s), 130.6 (d), 121.3 (d), 119.4 (d), 117.1 (s), 116.8 (d), 116.5 (d), 112.8 (d), 108.1 (d), 64.1 (d); Anal. Calcd. for $C_{15}H_{14}Cl_2N_6$: C, 51.59; H, 4.04; N, 24.07. Found: C, 51.80; H, 4.22; N, 24.30.

2.2. Single-crystal X-ray diffraction experiment

Selected crystallographic and refinement data for structure **4f** obtained by the single-crystal X-ray diffraction experiment are reported in Table 1.

Data collection has been performed by applying the CrysAlis Software system, Version 1.171.33.55 [24]. The Lorentz-polariza-

Table 1
General and crystal data and summary of intensity data collection and structure refinement for compound **4f**.

	4f
Formula	$C_{16}H_{16}ClN_5$
M_r	313.79
Crystal system, colour and habit	Triclinic, colourless, prism
Space group	*P*-1
Crystal dimensions (mm^3)	0.49 × 0.37 × 0.29
Unit cell parameters	
a (Å)	8.6725(4)
b (Å)	9.0395(3)
c (Å)	10.9905(6)
α (°)	88.008(4)
β (°)	72.587(5)
γ (°)	80.615(4)
V (Å^3)	811.02(7)
Z	2
D_c (g cm^{-3})	1.285
μ (mm^{-1})	0.239
$F(000)$	328
θ range for data collection (°)[a]	3–30
h, k, l range	−12 to 11 −12 to 12 −15 to 15
Scan type	ω
No. measured reflections	8462
No. independent reflections ($R_{int.}$)	4724 (0.017)
No. refined parameters/restraints	212/0
No. observed reflections, $I \geqslant 2\sigma(I)$	3060
g_1, g_2 in w	0.0689, 0
R, wR [$I \geqslant 2\sigma(I)$]	0.0431, 0.1133
R, wR [all data]	0.0705, 0.1211
Goodness of fit on F^2, S	0.979
Min. and max. electron density (e Å^{-3})	−0.19, 0.25
Maximum Δ/σ	0.001

[a] The data collection were collected by ω-scans on an Oxford Xcalibur diffractometer equipped with 4-circle kappa geometry and CCD Sapphire 3 detector and graphite-monochromated MoKα radiation (λ = 0.71073 Å) at ambient temperature (296 K).

tion effect was corrected and the intensity data reduced by the CrysAlis RED application of the CrysAlis Software system, Version 1.171.33.55 [24]. The diffraction data have been scaled for absorption effects by the multi-scanning method. Structure was solved by direct methods and refined on F^2 by weighted full-matrix least-squares. Programs SHELXS97 and SHELXL97 integrated in the WinGX v. 1.70.01 [25] software system were used to solve and refine structure. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms belonging to Csp^2 and Csp^3 (C3 and C16) carbon atoms were placed in geometrically idealized positions [Csp^2–H 0.93 Å with $U_{iso}(H) = 1.2\ U_{eq}(C)$; Csp^3–H 0.96 Å with $U_{iso}(H) = 1.5\ U_{eq}(C)$] and they were constrained to ride on their parent atoms by using the appropriate SHELXL97 HFIX instructions. The positions of hydrogen atoms belonging to nitrogen N1, N4 and N5 atoms were determined from difference Fourier syntheses and their coordinates were refined freely, while isotropic displacement parameters were refined with $U_{iso}(H) = 1.2\ U_{eq}(N)$. The molecular geometry calculations and graphics were done using ORTEP-3 [26,27] integrated in the WinGX software system, PLATON [28] programme and Mercury [29].

2.3. Antiproliferative evaluation assay

The experiments were carried out on five human cell lines, which are derived from four cancer types. The following cell lines were used: HCT 116 (colon carcinoma), H 460 (lung carcinoma) and MCF-7 (breast carcinoma). MCF-7, HCT 116 and H 460 cells were cultured as monolayers and maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C. The growth inhibition activity was assessed as described previously. The panel cell lines were inoculated onto a series of standard 96-well microtiter plates on day 0, at 1×10^4–3×10^4 cells/ml, depending on the doubling times of a specific cell line. Test agents were then added in ten-fold dilutions (10^{-8}–10^{-4} M) and incubated for further 72 h. Working dilutions were freshly prepared on the day of testing. After 72 h of incubation the cell growth rate was evaluated by performing the MTT assay, which detects dehydrogenAse activity in viable cells. The absorbance (A) was measured on a microplate reader at 570 nm. The absorbance is directly proportional to the number of living, metabolically active cells. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

$$\text{If } (\text{mean } A\text{test} - \text{mean } A_{\text{tzero}}) \geqslant 0, \quad \text{then}$$
$$\text{PG} = 100 \times (\text{mean}A_{\text{test}} - \text{mean } A_{\text{tzero}})/(\text{mean } A_{\text{ctrl}} - \text{mean } A_{\text{tzero}}).$$

$$\text{If } (\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}}) < 0, \quad \text{then PG} = 100 \times (\text{mean } A_{\text{test}} - \text{mean } A_{\text{tzero}})/A_{\text{tzero}},$$

where the mean A_{tzero} is the average of optical density measurements before exposure of cells to the test compound, the mean A_{test} is the average of optical density measurements after the desired period of time and the mean A_{ctrl} is the average of optical density measurements after the desired period of time with no exposure of cells to the test compound. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from concentration–response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (*i.e.* 50%). If however, for all of the tested concentrations produce PGs exceeding the respective reference level of effect (*e.g.* PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each test was performed in quadruplicate in at least two individual experiments.

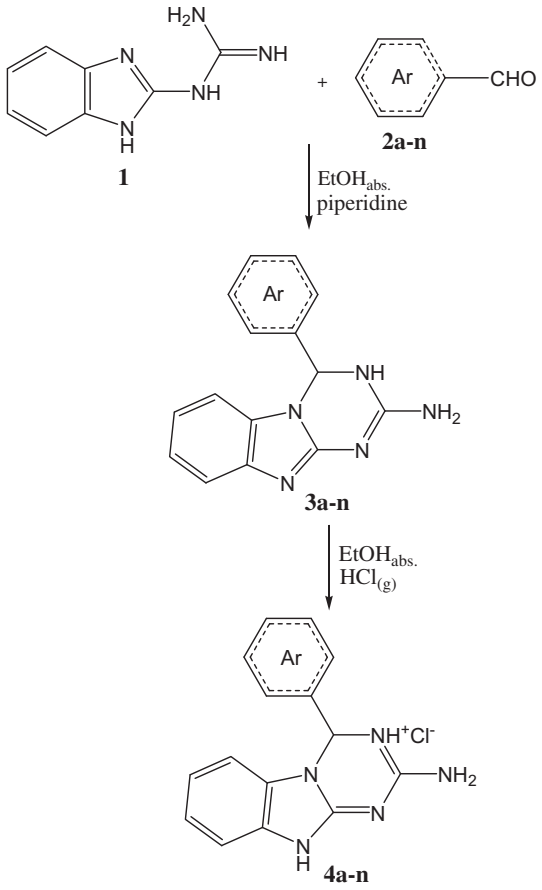
3. Results and discussion

3.1. General aspects of preparation

All newly prepared 2-amino-4-aryl-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles as hydrochloride salts were synthesized according to the procedure shown in Scheme 1 and Table 2, by the conventional methods of organic synthesis for the preparation of similar heterocyclic compounds [23].

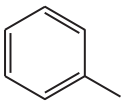
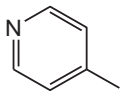
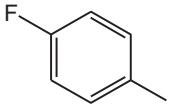
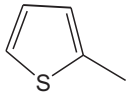
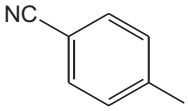
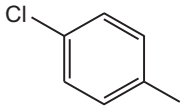
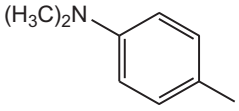
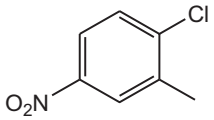
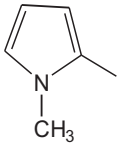
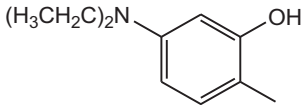
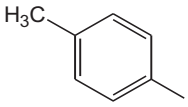
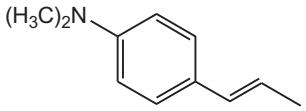
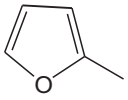
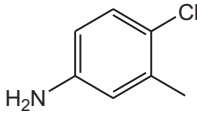
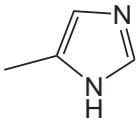
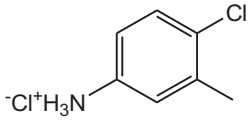
In the cyclocondensation reaction between the 2-guanidinobenzimidazole **1** and versatile heteroaromatic benzaldehydeys **2a-n** in absolute ethanol by using piperidine as a catalysts, 2-amino-4-aryl-3,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles were prepared. Hydrochloride salts of 2-amino-4-aryl-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles **4a-n** were prepared in ethanolic solution with HCl_{gas} in order to ensure better solubility. Amino substituted derivative **5a** was prepared according to the experimental procedure shown in Scheme 2, from nitro substituted derivative **3l** by reduction with $SnCl_2{\cdot}2H_2O$ in MeOH and concentrated HCl.

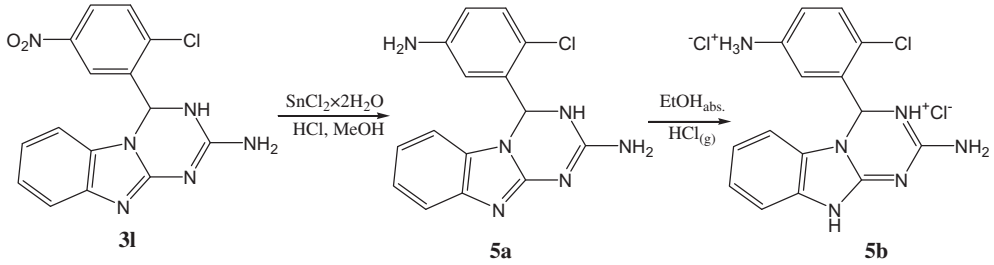
All structures of novel prepared compounds **4a-n** and **5b** were determined by the NMR analysis based on the analysis of H–H coupling constants as well as chemical shifts. The formation of the *s*-triazine ring was confirmed by the NMR spectral data. Since prepared compounds could exist in several tautomeric forms, according to the ^1H NMR data, 3,4-dihydro[1,3,5]triazino[1,2-



Scheme 1. Reaction scheme for preparation of 2-amino-4-aryl-4,10 dihydro[1,3,5]triazino[1,2-*a*]benzimidazole hydrochlorides **4a-n**.

Table 2
Prepared compounds **3a–3n**, **4a–4n** and **5a–5b**.

Comp.	Ar	Comp.	Ar
3a, **4a**		**3i**, **4i**	
3b, **4b**		**3j**, **4j**	
3c, **4c**		**3k**, **4k**	
3d, **4d**		**3l**, **4l**	
3e, **4e**		**3m**, **4m**	
3f, **4f**		**3n**, **4n**	
3g, **4g**		**5a**	
3h, **4h**		**5b**	



3l **5a** **5b**

Scheme 2. Reaction scheme for preparation of 2-amino-4-aryl-4,10 dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles hydrochlorides **5a-b**.

a]benzimidazole structures were confirmed. These spectra showed the presence of NH signal around 8 ppm in ^{1}H NMR as well as singlet of triazine ring at 6.89–7.20 ppm in ^{1}H NMR as it can be viewed from the ^{1}H NMR spectra of compound **3d** (Fig. 1a) and at 59.1–66.3 ppm in ^{13}C NMR spectra.

It is well known that compounds **4a-n** could obtain in three tautomeric forms as 3,4-dihydro, 4,10-dihydo and 1,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles. By the protonation of 3,4-dihydro[1,3,5]-triazino[1,2-*a*]benzimidazoles **4a-n** with HCl_{gas} 4,10-dihydro forms obtained. ^{1}H NMR spectra have shown broadening effect of the NH signal as well as presence of NH singlet of benzimidazole nuclei at 13.18–13.40 ppm as it can be viewed from the ^{1}H NMR spectra of compound **4c** (Fig. 1b). Some of specific carbon and proton chemical shifts of compounds **4a-n** and **5b** are given in Table 3.

3.2. Crystal structure description

ORTEP drawing of molecular structure for compound **4f** is depicted in Fig. 2, while crystal structure is shown in Fig. 3.

The selected molecular geometry is listed in Table 4 and hydrogen bonding geometry in Table 5, respectively.

The structure of compound **4f** is isostructural with 2-amino-4-phenyl-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole hydrochloride [30]. The molecule of 2-amino-4-(4′-methylphenyl)-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole hydrochloride, **4f**,

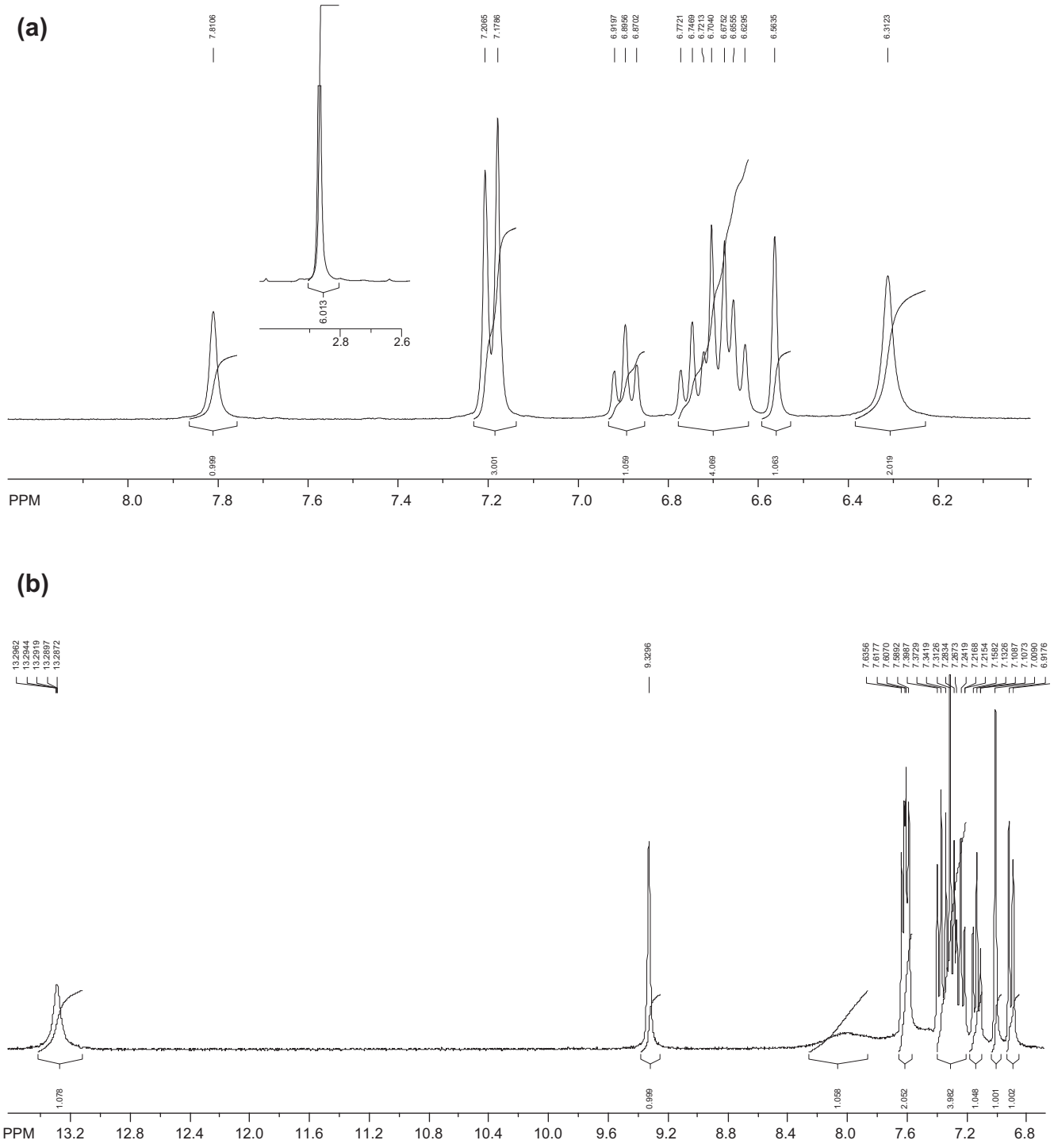


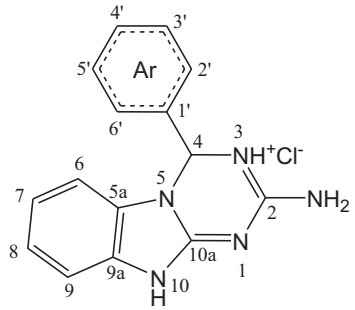
Fig. 1. ^{1}H NMR spectra of compounds **3d** (a) and **4c** (b).

$C_{16}H_{16}N_5^+ \cdot Cl^-$, exists in the solid state in one of the possible tautomeric forms being protonated at the N5 atom of the 1,4-dihydrotriazine ring (the value of the C3–N5 bond distance of 1.457(2) Å indicates dominantly σ bond character). The molecule is not planar containing the sp^3 methine C3 atom. Benzimidazole ring is fused with the dihydrotriazine one containing the C3 atom in a relatively planar moiety (the atoms N5 and C3 deviate 0.123(1) Å and 0.123(1) Å, respectively, from the dihydrotriazine ring best calculated plane). The phenyl and dihydrotriazine rings are inclined mutually by 86.65(8)°. The cations are mutually link *via* N–H···N hydrogen bond between the amino N4 atom acting as proton donor and the dihydrotriazine N3 atom acting as proton acceptor (Table 5) into $R_2^2(8)$ centrosymmetric dimers. Dimers further connected with Cl^- anions *via* N–H···Cl and C–H···Cl hydrogen bonds including dihydrotriazine and imidazole NH proton groups as well as the remaining amino NH group (Table 5). The Cl^- anion acts as a quadruple proton acceptor.

3.3. UV/Vis spectroscopy

In order to study the spectroscopic properties of prepared hydrochloride salts of 2-amino-4-aryl-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles **4a-n** and **5b**, UV/Vis absorption spectra were undertaken in ethanol. The UV/Vis spectra of all were

Table 3
Characteristic NMR data of compounds **4a–4n** and **5b**.



Comp.	^{1}H NMR (δ/ppm)			^{13}C NMR (δ/ppm)	
	NH$^+$-3	NH-10	H-4	C-4	C-10a
4a	8.03	13.30	6.99	66.3	157.2
4b	–	13.31	6.99	65.4	157.0
4c	8.01	13.29	7.00	65.6	161.8
4d	8.00	13.30	7.02	65.7	156.8
4e	8.04	13.26	7.04	60.8	157.6
4f	7.94	13.18	6.89	66.2	157.2
4g	8.06	13.21	7.17	59.4	157.6
4h	8.18	13.20	7.13	59.1	157.0
4i	8.17	13.42	7.08	64.3	157.0
4j	8.11	13.24	7.22	61.9	157.1
4k	7.70	13.26	7.01	65.6	157.1
4l	8.83	13.29	7.20	65.4	156.3
4m	7.70	13.05	6.98	64.3	157.3
4n	7.98	13.17	7.00	66.0	157.3
5b	8.00	13.30	6.91	64.1	155.5

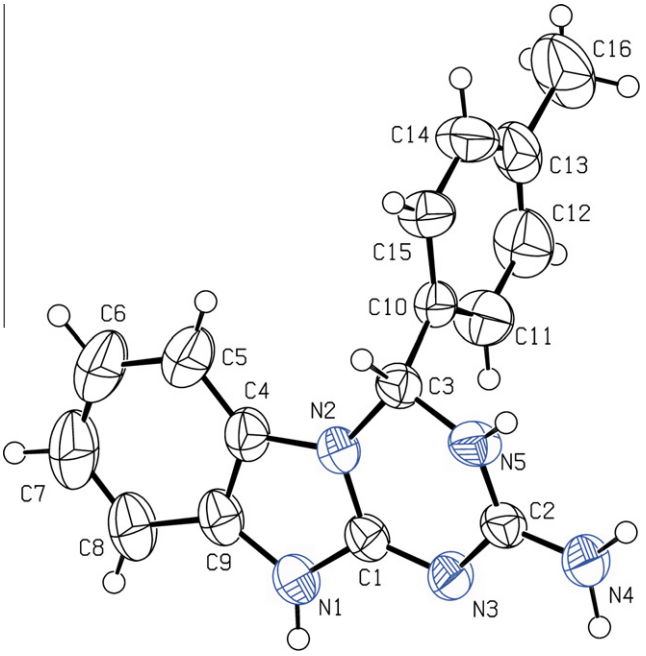


Fig. 2. ORTEP drawing of the molecular structure of 2-amino-4-(4′-methylphenyl)-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole hydrochloride, **4f**, with the atomic numbering scheme. The thermal ellipsoids are drawn at the 50% probability level at 296 K. The Cl^- anion is omitted.

recorded at the same concentration of 2×10^{-5} mol dm^{-3}. Spectra can be visualized in Fig. SM1 (Supplementary material) and all the results are summarized in Table 6.

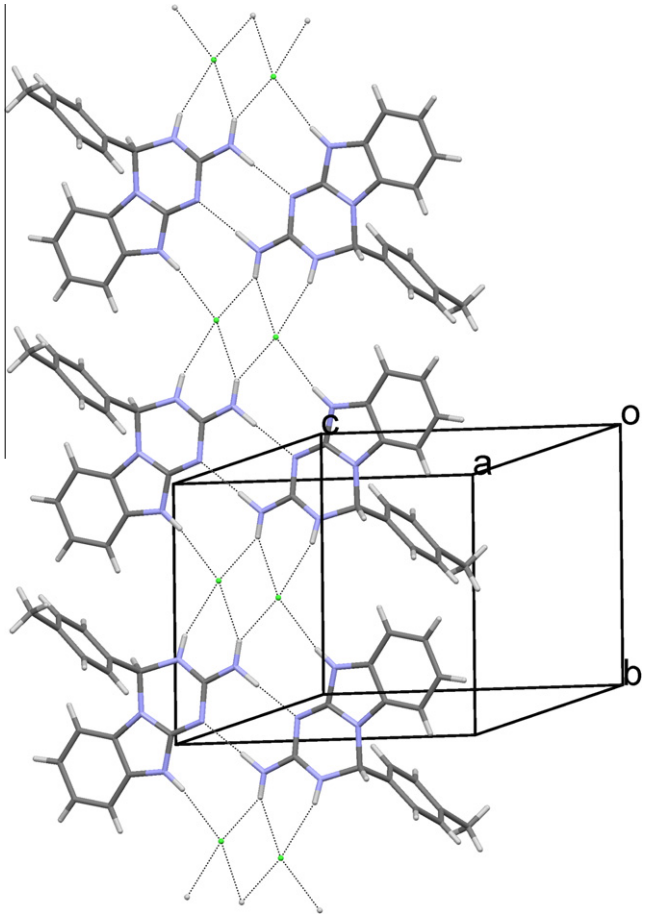


Fig. 3. Crystal packing of 2-amino-4-(4′-methylphenyl)-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole hydrochloride, **4f** showing assembling of molecules *via* N–H···Cl, N–H···N and C–H···Cl hydrogen bonds.

Table 4
Selected interatomic distances (Å) and valence and torsion angles (°) for the compound **4f**.

	4f
	Selected bond distances
C2–N4	1.320(2)
C2–N5	1.332(2)
C1–N3	1.321(2)
C2–N3	1.351(2)
C3–N5	1.457(2)
C3–N2	1.459(2)
C1–N2	1.350(2)
C1–N1	1.346(2)
	Bond angles
N1–C1–N2	108.0(1)
N3–C2–N5	122.8(1)
N5–C3–N2	105.7(1)

As it might be observed from obtained spectra (Fig. SM1a) most of aromatic derivatives exhibit one characteristic strong absorption maxima with one main absorption band at ~300 nm. 4-*N*,*N*-dimethylamino **4d**, 2-chloro-5-nitro **4l**, 4-*N*,*N*-diethylamino-2-hydroxy **4m** and 2-chloro-5-amino **5b** substituted compounds showed two absorption bands of similar intensity at 301, 300, 302 and 303 nm and at 266, 263, 268 and 252 nm respectively. 4-*N*,*N*-(dimethylaminophenyl)ethylene substituted compound **4n** showed hyperchromic shift of absorption maxima at 304 nm in

Table 5
Hydrogen bond geometry (Å, °) for compound **4f**.

D–H···A	D–H	H···A	D···A	∠D–H···A	Symmetry code
4f					
C3–H3···Cl1	0.98	2.575	3.517(1)	161	–
N1–H1N1···Cl1	0.86(2)	2.258(17)	3.109(1)	168(2)	$-x, -y, -z+2$
N5–H1N5···Cl1	0.83(2)	2.335(18)	3.130(1)	162(2)	$-x, -y+1, -z+2$
N4–H1N4···Cl1	0.86(2)	2.720(21)	3.379(2)	134(2)	$x+1, +y, +z$
N4–H2N4···N3	1.00(2)	1.976(19)	2.971(2)	174(2)	$-x+1, -y, -z+2$

Table 6
Electronic absorption data of **4a–n** and **5b** in ethanol ($c = 2.0 \times 10^{-5}$ mol dm^{-3}).

Comp.	λ_{max} (nm)	$\varepsilon \times 10^3$ (dm^3 mol^{-1} cm^{-1})	Comp.	λ_{max} (nm)	$\varepsilon \times 10^3$ (dm^3 mol^{-1} cm^{-1})
4a	302	20.7	**4i**	302	17.95
	260	9.8		259	11.1
4b	301	18.93	**4j**	303	18.65
	261	13.4		260	9.85
4c	303	22.75	**4k**	303	21.35
	261	11.1		260	11.45
4d	301	21.8	**4l**	300	19.3
	266	23.7		263	19.35
4e	301	25.95	**4m**	302	21.45
	270	11.7		268	18.8
	261	12.35			
4f	302	21.1	**4n**	332	13.45
	270	8.25		304	27.25
	260	9.45		260	8.1
4g	301	18.3	**5b**	303	21.3
	270	8.6		252	21.4
	261	8.85			

Table 7
GI_{50} values (in μM).

Compound ID	Cell lines		
	HCT 116	MCF-7	H460
GI_{50}[a] (μM)			
4a	⩾100	⩾100	>100
4b	⩾100	>100	>100
4c	⩾100	>100	>100
4d	65 ± 36	51 ± 28	72 ± 27
4e	>100	>100	>100
4f	52 ± 20	38 ± 19	43 ± 4
4g	>100	>100	>100
4h	>100	>100	>100
4i	>100	>100	>100
4j	>100	⩾100	>100
4k	34 ± 14	52 ± 45	31 ± 0.05
4l	52 ± 39	60 ± 40	49 ± 0.5
4m	17 ± 4	22 ± 7	18 ± 3
4n	24 ± 19	23 ± 19	35 ± 15
5a	⩾100	⩾100	>100
5b	⩾100	>100	>100

[a] GI_{50}; the concentration that causes 50% growth inhibition.

comparison to all other compounds as well as appearance of one absorption band at 332 nm. 4-fluoro substituted compound **4b** showed hypochromic shift of absorption maxima at 301 nm. UV/Vis spectra of heteroaromatic derivatives **4e**, **4g**, **4h**, **4i** and **4j** (Fig. SM1b) showed that all compounds exhibit one main strong absorption band at ~300 nm and one lower absorption band at ~260 nm, while compounds **4e** and **4g** showed two lower absorption bands at 270 and 261 nm. 2-*N*-methylpyrolyl **4e** and 5-imidazolyl **4h** substituted derivatives showed hyperchromic shift of absorption maxima at 301 and 300 nm.

3.4. Antiproliferative evaluation

All prepared 10,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles **4a-n** and **5b** were tested for their antiproliferative activities against human malignant tumor cell lines in the Laboratory of Experimental Therapy, Division of Molecular Medicine, Ruđer Bošković Institute, Croatia. The following cell lines were used: H460 (colon carcinoma), MCF-7 (breast carcinoma) and HCT 116 (colon carcinoma). Most of the tested compounds did not showed antiproliferative activity while some of compounds showed moderate activity (Table 7). The most active compound is 2-hydroxy-4-*N,N*-diethylamino substituted derivative **4m** ($IC_{50} \approx 20$ μM), which show cytotoxicity at the maximal tested concentration (100 μM). 4-*N,N*-dimethylamino **4d**, 4-methyl **4f**, 4-chloro **4k**, 2-chloro-5-nitro **4l** and 4-*N,N*-dimethyl-aminophenyl)ethylene **4m** substituted derivatives showed also moderate antiproliferative activity with IC_{50} 25–60 μM. Also, there is no significant selectivity of the above mentioned compounds between the cell lines. Introduction of heteroaromatic rings caused only decrease of antiproliferative activity.

4. Conclusions

In this work we have presented the synthesis of novel 2-amino-4-aryl-4,10-dihydro-[1,3,5]triazino[1,2-*a*]benzimidazoles, crystal structure determination of compound **4f** and antiproliferative activity *in vitro* of all prepared derivatives. Novel 2-amino-4-aryl-4,10-dihydro-[1,3,5]triazino[1,2-*a*]benzimidazoles **4a-n** and **5b** as hydrochloride salts were prepared from 2-amino-4-aryl-3,4-dihydro-[1,3,5]triazino[1,2-*a*]benzimidazoles **3a-n** and **5a** in ethanolic solution saturated with HCl_{gas}. Compounds **3a-n** and **5a** were prepared in cyclocondensation reaction between 2-guanidinobenzimidazole and versatile heteroaromatic compounds.

The molecular and crystal structure of compound 2-amino-4-(4′-methylphenyl)-4,10-dihydro[1,3,5]triazino[1,2-a]benzimidazole hydrochloride, **4f**, reveals that one of the nitrogen atoms of 4,10-dihydro[1,3,5]triazine ring is protonated causing that the Csp^3 methine atom and protonated Nsp^3 nitrogen atom deviate most from planarity. The cations are joined *via* N–H···N hydrogen bonds into $R_2^2(8)$ centrosymmetric dimers. Cation dimers are further connected with Cl^- ions into 2D chains spreading along the *b* axis. Single-crystal X-ray structure determination has been performed for compound 2-amino-4-(4′-methylphenyl)-4,10-dihydro[1,3,5]triazino[1,2-*a*]benzimidazole hydrochloride, **4f**, in order to establish correlation between the solid-state molecular structure with IR spectroscopic data in the context of tautomeric variability of this class of compounds and further with possible interpretation of biological activity. Moreover, the obtained structure unequivocally confirms tautomeric form of the compound present in the solid-state and can represent tantative pattern for other prepared compounds.

All prepared 10,4-dihydro[1,3,5]triazino[1,2-*a*]benzimidazoles **4a-n** and **5b** were tested for their antiproliferative activities and showed in general no to modest activity. 2-Hydroxy-4-*N,N*-diethylamino substituted derivative **4m** was the most active compound ($IC_{50} \approx 20$ μM), while compounds **4d**, **4f**, **4k**, **4l 4m** showed also moderate antiproliferative activity with IC_{50} 25–60 μM with no significant selectivity between the tumor cell lines.

Acknowledgments

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(Ruđer Bošković Institute, Zagreb, Croatia) for antiproliferation assays on a panel of human tumor cell lines.

Appendix A. Supplementary material

CCDC number 43927 for compound **4f** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 (0)1223 336033; email: deposit@ccdc.cam.ac.uk]. Structure factors table is available from the authors. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2011.10.054.

References

[1] V. Martínez, V. Burgos, J. Alvarez-Builla, G. Fernández, A. Domingo, R. Garcia-Nieto, F. Gago, I. Manzanares, C. Cuevas, J.J. Vaquero, J. Med. Chem. 47 (2004) 1136.
[2] J. Pastor, G. Siro, J.L. García-Navío, J.J. Vaquero, J. Alvarez-Builla, F. Gago, B. Pascual-Teresa, M. Pastor, M.M. Rrodrigo, J. Org. Chem. 62 (1997) 5476.
[3] M. Hranjec, K. Starčević, I. Piantanida, M. Kralj, M. Marjanović, M. Hasani, G. Westman, G. Karminski-Zamola, Eur. J. Med. Chem. 43 (2008) 2877.
[4] K. Starčević, M. Kralj, K. Ester, I. Sabol, M. Grce, K. Pavelić, G. Karminski-Zamola, Bioorg. Med. Chem. 15 (2007) 4419.
[5] L. Yun-Fei, W. Gui-Feng, L. Yu, H. Wei-Gang, W. Tang, C.L. Feng, L.P. Shi, Y.D. Ren, J.P. Zuo, W. Lu, Eur. J. Med. Chem. 42 (2007) 1358.
[6] Z. Ates-Alagoz, S. Yildiz, E. Buyukbingol, Chemotherapy 53 (2007) 110.
[7] S. Yadav, P. Kumar, E. De Clercq, J. Balzarini, C. Pannecouque, S.K. Dewan, B. Narasimhan, Eur. J. Med. Chem. 45 (2010) 5985.
[8] H.M. Grogan, Pest. Manag. Sci. 62 (2006) 153.
[9] J. Velík, V. Baliharová, J. Fink-Gremmels, S. Bull, J. Lamka, L. Skálová, Res. Vet. Sci. 76 (2004) 95.
[10] I. Kucukguzel, S.G. Kucukguzel, S. Rollas, G.O. Sanis, O. Ozdemir, I. Bayrak, T. Altug, J. Stables, Il Farmaco 59 (2004) 839.
[11] M. Hranjec, M. Kralj, I. Piantanida, M. Sedić, L. Šuman, K. Pavelić, G. Karminski-Zamola, J. Med. Chem. 50 (2007) 5696.
[12] M. Hranjec, I. Piantanida, M. Kralj, L. Šuman, K. Pavelić, G. Karminski-Zamola, J. Med. Chem. 51 (2008) 4899.
[13] M. Hranjec, G. Pavlović, M. Marjanović, M. Kralj, G. Karminski-Zamola, Eur. J. Med. Chem. 45 (2010) 2405.
[14] A.N. Pearce, E.W. Chia, M.V. Berridge, E.W. Maas, M.J. Page, J.L. Harper, V.L. Webb, B.R. Copp, Tetrahedron 64 (2008) 5748.
[15] (a) M.A. Poss, E. Iwanowicz, J.A. Reid, J. Lin, Z. Gu, Tetrahedron Lett. 33 (1992) 5933;
(b) M. Cortes-Salva, B.L. Nguyen, C. Javier, K.R. Pennypacker, J.C. Antilla, Org. Lett. 12 (2010) 1316.
[16] R.K. Arafa, M.A. Ismail, M. Munde, W.D. Wilson, T. Wenzler, R. Brun, D.W. Boykin, Eur. J. Med. Chem. 43 (2008) 2901.
[17] S. Lee, T. Kim, B.H. Lee, S. Yoo, K. Leeb, K.Y. Yi, Bioorg. Med. Chem. Lett. 17 (2007) 1291.
[18] J.U. Peters, T. Lubbers, A. Alanine, S. Kolczewski, F. Blasco, L. Steward, Bioorg. Med. Chem. Lett. 18 (2008) 262.
[19] W. Zeghida, J. Debray, C. Michel, A. Milet, P. Dumy, M. Demeunynck, Bioorg. Med. Chem. Lett. 18 (2008) 4779.
[20] H. Ube, D. Uraguchi, M. Terada, J. Organometall. Chem. 692 (2007) 545.
[21] J. Maaranen, O.S. Andell, T. Vanne, I. Mutikainen, J. Organometall. Chem. 691 (2006) 240.
[22] G. Gulyas, T. Emri, A. Simon, Z. Gyorgydeak, Folia Microbiol. 47 (2002) 29.
[23] A.V. Dolzhenko, W.-K. Chui, A.V. Dolzhenko, L.-W. Chan, J. Fluor. Chem. 126 (2005) 759.
[24] Oxford Diffraction Ltd., *Xcalibur CCD system*, *CrysAlis Software system* Versions 1.171.33.55. Abingdon, Oxfordshire, England, 2008.
[25] G.M. Sheldrick, Acta Crystallogr. A 64 (2008) 112.
[26] L.J. Farrugia, J. Appl. Cryst. 32 (1999) 837.
[27] L.J. Farrugia, J. Appl. Cryst. 30 (1997) 565.
[28] L. Spek, J. Appl. Cryst. 36 (2003) 7.
[29] C.F. Macrae, I.J. Bruno, J.A. Chisholm, P.R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek, P.A. Wood, J. Appl. Cryst. 41 (2008) 466.
[30] S.K. Mohamed, M.A.A. El-Remaily, A.M. Soliman, H. Abdel-Ghany, S.W. Ng, Acta Cryst E 67 (2011) 1154.