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Synthesis and antitumor evaluation of some new substituted amidino-benzimidazolyl-furyl-phenyl-acrylates and naphtho[2,1-*b*]furan-carboxylates

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Abstract

The multistep synthesis of a series of substituted amidino-benzimidazolyl-furyl-phenyl-acrylic acid's esters and substituted amidino-benzimidazolyl-naphtho[2,1-*b*]furan-carboxylic acid's esters is described starting from corresponding 3-(2-furyl)-2-phenyl-acrylic acids. The new compounds were tested on the cytostatic activities against malignant cell lines: pancreatic carcinoma (MiaPaCa2), breast carcinoma (MCF7), cervical carcinoma (HeLa), laryngeal carcinoma (Hep2), colon carcinoma (HT 29), melanoma (HBL), and human fibroblasts cell line (WI38). All compounds inhibited the proliferation of tumor cell lines. Inhibitory effect of examined compounds depended on concentration, but without significant difference among the type of tumor cells. The compounds **2** and **5** exerted very low inhibitory effect on the growth of human fibroblasts. Unsubstituted derivative **8** has not inhibited any tested cell lines.

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Keywords: Pancreatic carcinoma; Laryngeal carcinoma; Breast carcinoma; Melanoma; Amidinobenzimidazolyl-furans

1. Introduction

Amidino-substituted aromatic and heteroaromatic compounds are widely investigated on their different biological activity in the recent time [1–12] including some phenyl substituted monoamidines which are synthesized and their anti HIV activity examined [13]. On the other side, there is no much literature evidence about the antitumor activity of amidines. A few papers describe the synthesis of the natural oligopeptide antiviral antibiotics *netropsyne* and *distamycine* and their synthetic derivatives. They have attracted considerable attention on the part of synthetic chemists and pharmacists because some representatives exhibit anticancer activity [14]. The effect of the synthetic derivative of *distamycine* on molecular interaction between DNA and transcription factor Sp 1 were studied [15], while a new derivatives of *distamycine* bearing one or more pyrazole rings are synthesized and examined in vitro and in vivo activities against L1210 leukaemia [16]. DNA-binding properties and cytotoxic activity of novel aromatic amidines in cultured human skin fibroblasts were examined recently [17]. Antitumor activity of a series of cyclic amidines of benzodiazepine series was investigated too [18].

2. Results and discussion

In this paper we describe the preparation of six new substituted amidino-benzimidazolyl heterocyclic compounds from furan series which could serve as potential anticancer agent. The preparation of the target compounds is outlined in the scheme. Amidino compounds were prepared in the several steps from methyl (*Z*)-3-(5-formyl-2-furyl)-2-phenyl-acrylic acrylate, **1** [19] with

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earlier prepared 3,4-diamino-substituted-benzamidines in the Pinner reaction [20,21]. In this way were prepared methyl ester of 3-[5-(*N*-isopropylamidino-benzamidazolyl)-2-furyl]-2-phenyl-acrylic acid **2**, 3-[5-(*N*-2-imidazolinyl-benzimidazolyl-2-furyl]-2-phenyl-acrylic acid **3** and 3-[5-(*N*-morpholinyl-amidino-benzimidazolyl)-2-furyl]-2-phenyl-acrylic acid **4** as hydrochloride salts. The prepared compounds **2**, **3** and **4** were photochemically dehydrogenated into methyl esters of 8-[5-(*N*-isopropylamidino-benzamidazolyl)]naphtho[2,1-*b*]-furan-5-carboxylic acid **5**, 8-[5-(*N*-2-imidazolinyl-benzimidazolyl)]naphtho[2,1-*b*]furan-5-carboxylic acid **6** and 8-[5-(*N*-morpholinyl-amidino-benzimidazolyl)]naphtho[2,1-*b*]furan-5-carboxylic acid **7** as hydrochloride salts. Compound **4** photolitically rearranged into methyl ester of 8-(2-benzimidazolyl)naphtho[2,1-*b*]furan-5-carboxylic acid **8** by the prolonged irradiation in abs. ethanol (Scheme 1).

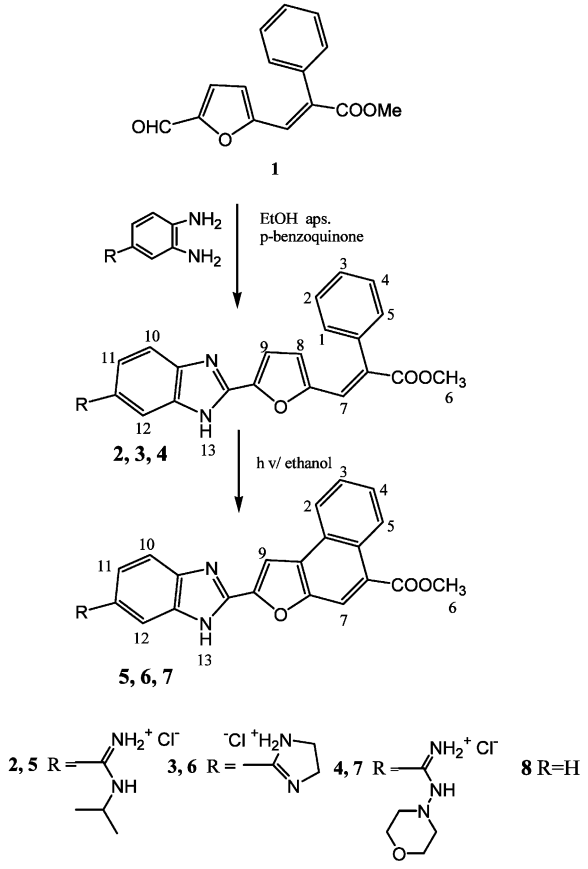
Structure identification of the synthetic products were determined by ^{1}H NMR, ^{13}C NMR spectroscopy and elemental analysis.

2.1. Biological activity

All new prepared compounds **2**, **3**, **4**, **5**, **6**, **7** and **8** were tested on their antitumor activity, using different cell lines HeLa – cervical carcinoma, Hep2 – laryngeal carcinoma, HT29 – colon carcinoma, MiaPaCa2 – pancreatic carcinoma, HBL – melanoma, MCF7 – breast cancer and normal human fibroblasts – WI38. The inhibitory effect varied between the compounds, and depended on the concentration (as illustrated in Fig. 1).

All results are summarised in Table 1.

They are expressed as IC_{50}, a concentration at which compound inhibit the cell proliferation for 50%. Very strong inhibition was achieved on Hep2 ($IC_{50} = 1.66$–39.8 μM), MiaPaCa2 ($IC_{50} = 2.45$–46.7 μM), MCF7 ($IC_{50} = 2.63$–10 μM) and HBL ($IC_{50} = 1.78$–39.8 μM) cell lines. On the HeLa cells very good inhibition was achieved with compound **2**, **3**, **4**, **6** and **7** ($IC_{50} = 3.16$–5.07 μM) and slight inhibition with compound **5** ($IC_{50} =$ 199 μM). Similar observation was on HT29 cell line. A compound **4** inhibited very strong HT29 cells ($IC_{50} =$ 1.38 μM), but other compounds exerted a wicker effect ($IC_{50} = 42.7$–1580 μM). The best inhibition was achieved with compound **4**, but it inhibits also the growth of normal fibroblasts at the same extend ($IC_{50} =$ 5.5 μM). Very strong inhibition of all examined tumor cell lines was achieved with compound **2**, and without influence on proliferation of normal fibroblasts (WI38). To compare an inhibitory effect among the compounds, we can conclude that the basic structure of compounds **3**, **4**, **6** and **7** and substituents (**R**) did not influence on their inhibitory effect. But in compounds **2** and **5** the substituent (**R**) has an influence on the inhibitory effect. Unsubstituted derivative **8** has not inhibited any tested cell lines. Thus, the inhibitory effect moderated with substituent (**R**) and the concentration of substance, rather than with type of tumor cell line.



Scheme 1.

3. Experimental

3.1. General

Melting points were determined on a Kofler block apparatus and are uncorrected. IR spectra were determined with a Nicolet Magna 760 infrared spectrophotometer in KBr pellets. ^{1}H NMR and ^{13}C NMR spectral data were determined using Brucker Avance DPX 300 MHz NMR or Varian-Gemini 300 MHz spectrometers with tetramethylsilane as an internal standard. Elemental analyses were carried out in the Microanalitical laboratory at the 'Rugjer Boskovic' Institute.

3.2. Methyl E-3-(5-formyl-2-furyl)-2-phenylacrylate (*1*)

Corresponding methyl-*E*-3-(2-furyl)-2-phenylacrylate was formylated by Vilsmeier formilation.

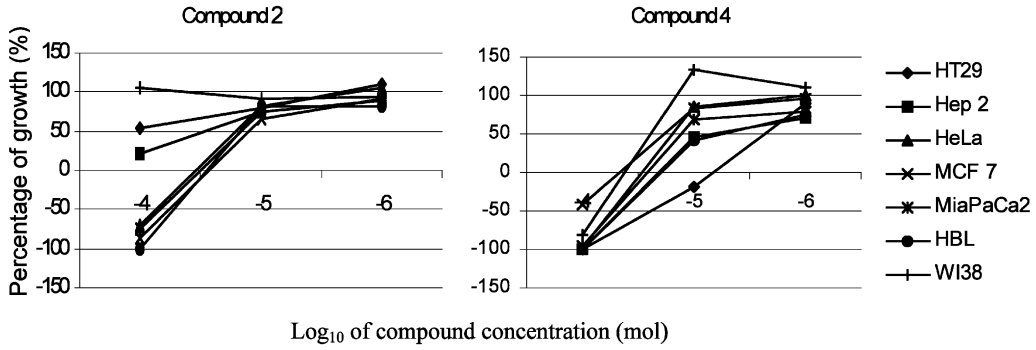


Fig. 1. The inhibitory effect of compound **2** and **4** on the growth of tumor cell lines (HT29, Hep2, HeLa, MCF7, MiaPaCa2, HBL) and normal human fibroblasts (WI38).

Phosphorus oxychloride (22.8 ml, 250 mmol) was added dropwise with cooling to DMF (20 ml, 250 mmol). The reaction mixture was stirred for half-an-hour by cooling on ice, the apparatus being protected with a calcium chloride tube. A solution of methyl-*E*-3-(2-furyl)-2-phenylacrylate (19 g, 86 mmol) in DMF (10 ml) was added dropwise to the mixture. After the addition was completed, the mixture was stirred for 1 h at room temperature, then heated at 90 °C for 3 h, cooled and poured onto crushed ice and made weakly alkaline with sodium carbonate solution, and left overnight on ice. The solid was filtered off, washed with water and recrystallized from methanol. Orange crystals 12.2 g (83.5%) are obtained, m.p. 103–105 °C (literature [19] m.p. 103–105 °C.

3.3. Methyl-E-3-[5-(5-N-isopropylamidino-2-benzimidazolyl)-2-furyl]-2-phenyl-acrylate hydrochloride (2)

Compound **2** was prepared using the method described earlier [20]. A mixture of **1** (1.01 g, 4.0 mmol), 3,4-diamino-*N*-isopropylbenzamidine [21] (0.90 g, 4.0 mmol) and *p*-benzoquinone (0.43 g, 4.0 mmol) in abs. EtOH (25 ml) was stirred at reflux for 4 h (under nitrogen). The reaction mixture was cooled to room temperature, and diethylether was added and the resulting brown solid was filtered off. The crude product was suspended in abs. ethanol and cooled to 0–5 °C. Into the suspension was introduced HCl gas until the suspension was saturated and the content was stirred over night on the room temperature. Dry ether was added and the precipitated yellow–green powder was filtered off and washed with dry ether. It was repeated few times until the powder was analytically pure. It was obtained 0.95 g (41%) slightly green powder. m.p. 235–237 °C. IR (cm^{-1}; KBr): 3300, 1675, 1590. ^{1}H NMR (δ ppm) (DMSO-d_6): 9.60 (s, 1H, $NH_{amid.}$), 9.57 (s, 1H, $NH_{amid.}$), 9.49 (bs, 1H, $NH_{amid.}$), 8.01 (s, 1H, $NH_{benzim.}$, H-13), 7.77 (d, 1H, $H_{arom.}$, H-10, $J = 8.64$ Hz), 7.73 (s, 1H, $H_{arom.}$, H-12), 7.61 (d, 1H, $H_{arom.}$, H-11, $J = 8.84$ Hz), 7.60 (s, 1H, $H_{ethen.}$, H-7), 7.54–7.46 (m, 3H, $H_{arom.}$, H-2, H-3, H-4), 7.38 (d, 1H, $H_{fur.}$, H-9, $J = 3.83$ Hz), 7.31 (d, 2H, $H_{arom.}$, H-1,5, $J = 7.76$ Hz), 5.71 (d,1H, $H_{fur.}$, H-8, $J = 3.78$ Hz), 4.07 (m, 1H, $CH_{i\text{-}pr}$), 3.90 (s, 3H, H-6, OCH_3), 1.34 (d, 6H, $2CH_{3i\text{-}Pr.}$, $J = 6.21$ Hz). ^{13}C NMR (δ ppm) (DMSO-d_6): 166.9, 162.5, 152.9, 144.6, 144.4, 135.5, 132.9, 129.6, 129.3, 129.1, 127.1, 124.7, 124.2, 116.8, 116.6, 116.2, 115.3, 53.1, 45.7, 21.9. *Anal*. Calc. for $C_{25}H_{27}N_4O_3Cl_3 \cdot 3H_2O$: C 50.71, H 5.62, N 9.43; Found: C 50.34, H 5.68, N 9.04.3%.

Table 1
Inhibitory effect of compounds on tumor cell lines and normal fibroblasts

Comp.	IC_{50} (μM)						
	HT29	Hep2	HeLa	MCF7	MiaPaCa2	HBL	WI38
2	75.8	12.6	3.24	2.63	3.09	2.57	> 1 000 000
3	42.7	5.01	5.62	5.12	35.5	41.7	56
4	1.38	1.66	3.16	4.37	2.45	1.78	5.5
5	1580	39.8	199	6.3	21.4	6.3	> 1 000 000
6	398	6.02	4.26	10	3.72	7.58	63
7	42.7	4.17	5.01	4.89	46.7	39.8	60.2
8	> 1 000 000	> 1 000 000	> 1 000 000	> 1 000 000	> 1 000 000	> 1 000 000	> 1 000 000

3.4. Methyl E-3-{5-[5-N-(2-imidazolinyl)-benzimidazolyl-2-furyl]}-2-phenylacrylate hydrochloride (3)

Compound **3** was prepared using the method described for the preparation of **2** from **1** (0.72 g, 2.8 mmol), 4-[*N*-(2-imidazolinyl)]-1,2-phenylenediamine [21] (0.50 g, 2.8 mmol) and *p*-benzoquinone (0.3 0 g, 2.8 mmol) in abs. ethanol (25 ml). It was obtained 0.80 g, (54%) olive green powder m.p. 243–246 °C. IR (cm^{-1}; KBr): 3350, 1700, 1610. ^1H NMR (δ ppm) (DMSO-d_6): 10.76 (s, 2H, $NH_{amid.}$), 8.38 (s, 1H, $NH_{benzimid.}$, H-13), 7.91 (d, 1H, $H_{arom.}$, H-10, $J = 8.64$ Hz), 7.78 (d, 1H, $H_{arom.}$, H-11, $J = 8.64$ Hz), 7.70 (s, 1H, $H_{arom.}$, H-12), 7.50 (s, 1H, $H_{ethen.}$, H-7), 7.47–7.44 (m, 3H, $H_{arom.}$, H-2, H-3, H-4), 7.36 (d, 1H, $H_{fur.}$, H-9, $J =$ 3.66 Hz), 7.30 (d, 1H, $H_{arom.}$, H-5, $J = 7.80$ Hz), 7.29 (d, 1H, $H_{arom.}$, H-1, $J = 7.64$ Hz), 5.68 (d, 1H, $H_{fur.}$, H-8, $J = 3.66$ Hz), 3.99 (bs, 4H, $CH_{2\ imid.}$), 3.41 (s, 3H, H-6, OCH_3). ^{13}C NMR (δ ppm) (DMSO-d_6): 166.3, 165.1, 151.9, 145.5, 145.0, 135.1, 131.8, 128.9, 128.7, 128.4, 126.6, 122.9, 115.9, 115.6, 114.9, 52.7, 44.2 (2C). *Anal*. Calc. for $C_{24}H_{22}N_4O_3Cl_2 \cdot 2H_2O$: C 55.34, H 5.03, N 10.76; Found: C 55.73, H 4.81, N 10.75%.

3.5. Methyl E-3-{5-[5-N-(N-morpholinylamidino)-benzimidazolyl-2-furyl]}-2-phenylacrylate hydrochloride (4)

Compound **4** was prepared using the method described for the preparation of **2** from **1** (0.54 g, 2.1 mmol), 4-(*N*-morpholynilylamidino)-1,2-phenylenediamine [21] (0.50 g, 2.1 mmol) and *p*-benzoquinone (0.23 g, 2.1 mmol) in abs. ethanol (25 ml). It was obtained 0.57 g, (54%) slightly green powder mp. 221–224 °C. IR (cm^{-1}; KBr): 3300, 1720, 1650, 1620. ^1H NMR (δ ppm) (DMSO-d_6): 11.17 (s, 1H, $H_{amid.}$), 9.81 (bs, 1H, $NH_{amid.}$), 9.11 (s, 1H, $NH_{amid.}$), 8.10 (s, 1H, $H_{benzimid.}$, H-13), 7.80 (d, 1H, $H_{arom.}$, H-10, $J = 8.45$ Hz), 7.73 (s, 1H, $H_{arom.}$, H-12), 7.65 (d, 1H, $H_{arom.}$, H-11, $J = 8.48$ Hz), 7.53 (s, $1H_{ethen.}$, H-7), 7.52–7.49 (m, 3H, $H_{arom.}$, H-2, H-3, H-4), 7.36 (d, 1H, $H_{fur.}$, H-9, $J = 3.8$ Hz), 7.32 (d, 2H, $H_{arom.}$, H-1, H-5, $J = 7.81$ Hz), 5.71 (d, 1H, $H_{fur.}$, H-7, $J = 3.6$ Hz), 3.96 (s, 3H, H-6, OCH_3), 3.77 (t, 4H, $2CH_{2\ morph.}$, $J = 7.20$ Hz), 2.94 (bs, 4H, $2CH_{2\ morph.}$). ^{13}C NMR (δ ppm) (DMSO-d_6): 170.1, 166.3, 162.0, 152.2, 145.4, 144.5, 136.8, 132.1, 130.6, 130.1, 128.9, 128.4, 123.6, 120.5, 117.0, 116.4, 115.7, 115.6, 66.1, 53.8 (2C), 52.8 (2C). *Anal*. Calc. for $C_{26}H_{28}N_5O_4Cl_3 \cdot 2H_2O$: C 50.61, H 5.24, N 11.35; Found: C 50.31, H 5.62, N 11.16%.

3.6. Methyl 2-[(5-N-isopropylamidino)benzimidazolyl]naphtho[2,1-b]furan-5-carboxylate hydrochloride (5)

A saturated solution of compound **2** (0.90 g, 2.0 mmol) in abs. ethanol (140 ml) was irradiated by pyrex filtered light with UV light of 400 W high pressure mercury arch lamp for 15 h at room temperature. Air was bubbled through the solution. Irradiated solution was concentrated under reduced pressure to 1/3 of the volume. Diethylether was added to the solution and the precipitated powder was filtered off and recrystallized from ethanol. It was obtained 0.37 g (35%) yellow powder m.p. 255–257 °C. IR (cm^{-1}; KBr): 3300, 1640. ^1H NMR (δ ppm) (DMSO-d_6): 9.62 (bs, 2H, $NH_{amid.}$), 9.52 (s, 1H, $NH_{amid.}$), 9.07 (s, 1H, $NH_{benzimid.}$, H-13), 8.88 (d, 1H, $H_{arom.}$, H-10, $J = 8.67$ Hz), 8.63 (s, 1H, $H_{arom.}$, H-12), 8.50 (d, 1H, $H_{arom.}$, H-11, $J = 8.30$ Hz), 8.47 (d, 1H, $H_{arom.}$, H-5, $J = 7.60$ Hz), 8.11 (s, 1H, $H_{naphthofur.}$, H-7), 7.87–7.70 (m, 3H, $H_{arom.}$, H-2, H-3, H-4), 7.63 (bs, 1H, $H_{fur.}$, H-9), 4.46 (m, 1H, $CH_{i\text{-}pr}$), 4.07 (s, 3H, H-6, OCH_3), 1.24 (d, 6H, $2CH_{3\ i\text{-}pr.}$, $J = 6.21$ Hz). ^{13}C NMR (δ ppm) (DMSO-d_6): 167.5, 162.7, 158.9, 151.2, 148.7, 145.6, 143.8, 141.7, 128.3, 128.1, 127.5, 127.0, 126.3, 124.9, 124.3, 123.7, 117.3, 116.3, 115.7, 108.4, 53.1, 45.6, 21.9. *Anal*. Calc. for $C_{25}H_{24}N_4O_3Cl_2 \cdot 2H_2O$: C 56.12, H 5.27, N 10.47; Found: C 56.30, H 5.16, N 10.72%.

3.7. Methyl 2-{[(5-N-(2-imidazolinyl)]benzimidazolyl}naphtho[2,1-b]furan-5-carboxylate hydrochloride (6)

Compound **6** was prepared on the way described for **5** by irradiation of saturated solution of **3** (0.50 g, 1.1 mmol) in ethanol: water (15 ml: 10 ml) during 35 h. It was obtained 0.075 g (16%) yellow–green powder, m.p. > 300 °C. IR (cm^{-1}; KBr): 3350, 1690. ^1H NMR (δ ppm) (DMSO-d_6): 10.67 (bs, 2H, $NH_{amid.}$), 8.87 (d, 1H, $H_{arom.}$, H-10, $J = 8.98$ Hz), 8.59 (s, 1H, $NH_{benzimid}$, H-13), 8.53 (d, 1H, $H_{arom.}$, H-11, $J = 8.54$ Hz), 8.46 (s, 1H, $H_{arom.}$, H-12), 8.44 (d, 1H, $H_{arom.}$, H-5, $J = 7.38$ Hz), 7.90 (bs, 1H, $H_{arom.}$, H-2), 7.84 (s, 1H, H-7), 7.80-7.70 (m, 2H, $H_{arom.}$, H-3, H-4), 7.65 (s, 1H, $H_{furan.}$, H-9), 4.25–4.21 (bs, 4H, $2CH_{2\ imid.}$), 4.16 (s, 3H, H-6, OCH_3). ^{13}C NMR (δ ppm) (DMSO-d_6): 166.8, 165.0, 150.6, 148.1, 146.5, 142.2, 127.6, 127.5, 127.4, 127.3, 126.8, 126.3, 125.2, 124.3, 123.0, 116.1, 115.6, 115.4, 107.8, 107.3, 52.5, 44.2 (2C). *Anal*. Calc. for $C_{24}H_{20}N_4O_3Cl \cdot 3H_2O$: C 53.67, H 4.88, N 10.43; Found: C 53.66, H 4.87, N 10.68%.

3.8. Methyl 2-{5-[5-N-(N-morpholinylamidino)]benzimidazolyl}naphtho[2,1-b]furan-5-carboxylate hydrochloride (7)

Compound **7** was prepared on the way described for **5** by irradiation of saturated solution of **4** (0.75 g, 1.5 mmol) in abs. ethanol (150 ml) during 10 h. It was obtained 0.21 g (23%) yellow powder, mp. 270–272 °C. IR (cm^{-1}; KBr): 3300, 1650. ^1H NMR (δ ppm) (DMSO-d_6): 11.32 (bs, 1H, $NH_{amid.}$), 9.85 (bs, 1H, $NH_{amid.}$), 9.20 (s, 1H, $NH_{amid.}$), 8.23 (bs, 1H, $NH_{benzimid.}$, H-13), 7.90 (d, 1H, Harom., H-10, $J = 8.40$ Hz), 7.85 (s, 1H, $H_{arom.}$, H-12), 7.77 (d, 1H, $H_{arom.}$, H-11, $J = 8.70$ Hz), 7.70 (s, 1H, H-7), 7.68 (d, 1H, $H_{arom.}$, H-5, $J = 7.80$ Hz), 7.48-7.37 (m, 3H, $H_{arom.}$, H-2, H-3, H-4), 7.28 (bs, 1H, $H_{fur.}$, H-9), 3.76 (bs, 4H, $2CH_2$ morf.), 3.72 (s, 3H, H-6, OCH_3), 2.96 (bs, 4H, $2CH_2$ morph.). ^{13}C NMR (δ ppm) (DMSO-d_6): 162.20, 162.12, 161.57, 150.66, 148.27, 145.41, 129.03, 128.49, 127.72, 127.61, 127.54, 127.50, 125.68, 125.62, 125.14, 124.41, 123.03, 122.75, 120.31, 116.17, 115.78, 115.05, 65.51 (2C), 62.02 (2C), *Anal*. Calc. for $C_{26}H_{26}N_5O_4Cl_3 \cdot 3H_2O$: C 49.37, H 5.10, N 11.07; Found: C 49.38, H 5.31, N 11.21%.

3.9. Methyl 2-(benzimidazolyl)naphtho[2,1-b]furan-5-carboxylate (8)

When the saturated solution of compound **4** (0.25 g, 0.5 mmol) in abs. ethanol (35 ml) was prolonged irradiated (during 15 h and in the quantity of the 1/3 of the experiment 3.8) by pyrex filtered light with UV light of 400 W high pressure mercury arch, only photolitic product **8** (after recrystallization in ethanol) was isolated in the yield of 0.015 g (14%). Yellow powder m.p. 230–232 °C. IR (cm^{-1}; KBr): 3310, 1650. ^1H NMR (δ ppm) (DMSO-d_6):, 8.87 (d, 1H, $H_{arom.}$, H-10, $J = 8.87$ Hz), 8.55 (s, 1H, $NH_{benzimid.}$, H-13), 8.54 (d, 1H, $H_{arom.}$, H-11, $J = 8.50$ Hz), 8.45 (s, 1H, $H_{arom.}$, H-12), 8.22 (s, 1H, H-7), 7.83(d, 1H, $H_{arom.}$, H-5, $J = 7.60$ Hz), 7.81 (s, 1H, $H_{fur.}$, H-9), 7.76(d, 1H, $H_{arom.}$, H-2, $J = 7.70$ Hz), 7.72(dd, 1H, $H_{arom.}$, H-4, $J_1 = 8.40$ Hz, $J_2 = 8.38$ Hz), 7.66(dd, 1H, $H_{arom.}$, H-3, $J_1 = 8.36$ Hz, $J_2 = 8.40$ Hz), 4.44 (s, 3H, H-6, OCH_3). ^{13}C NMR (δ ppm) (DMSO-d_6): 167.5, 151.2, 148.6, 146.0, 128.2, 128.1, 128.0 (2C), 127.4, 126.9, 126.2, 125.0, 121.7, 120.3, 116.5, 116.2, 108.2, 105.3, 53.1. *Anal*. Calc. for $C_{21}H_{14}N_2O_3$: C 70.06.37, H 4.48, N 7.78; Found: C 70.35, H 4.24, N 8.03%.

3.10. Biological assays

Biological activity of the compounds **2**, **3**, **4**, **5**, **6**,**7** and **8** was measured by their influence on growth of cell cultures in vitro. The following human carcinoma cell lines were used: HeLa (cervical carcinoma), Hep2 (laryngeal carcinoma), HT29 (colon carcinoma), MiaPaCa2 (pancreatic carcinoma), HBL (melanoma), MCF7 (breast cancer) and normal human fibroblasts WI38. The cells were treated with different concentration of compounds (10^{-4}, 10^{-5}, 10^{-6} M) during 72 h. All cell lines were grown in DMEM medium (supplemented with 10% heat inactivated fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin) at 37 °C in a humidified atmosphere with 5% CO_2. For the purpose of the experiment, the cells were plated in 96-microwell flat bottom plates at a concentration of 2×10^4 cells/ml (all tumor cell lines) and 3×10^4 cells/ml (WI38). The next day (24 h later) compounds were added to the cells at different concentrations (10^{-4}, 10^{-5} and 10^{-6} M). Compounds were dissolved in DMSO at a concentration of 10^{-1} M and diluted with DMEM medium into working concentrations. The concentration of DMSO was less than 0.1% and at that concentration it does not affect the growth. Control cells (without any compound) were growing under the same conditions. Cell viability was measured at day 0 and 72 h after addition of compounds, using MTT assay, which detects dehydrogenase activity in viable cell [22]. For this purpose the medium was discarded and MTT was added to each well at a concentration of 20 μg/40 μl. After 4 h of incubation at 37 °C, the precipitates were dissolved in 160 μl of DMSO. The absorbance was measured on an ELISA reader at 570 nm, and the percentage of growth was calculated. Each number was the mean of three individual experiments done in quadruplicate. The results are expressed as IC_{50}, what represent a concentration for 50% inhibitory effect.

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