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Original article

Synthesis and antitumour activity of trimethylsilylpropyl substituted benzimidazoles

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Abstract – The quaternisation of *N*-substituted benzimidazoles by heating with various alkyl, allyl, propargyl and benzyl chlorides and bromides leads to the formation of benzimidazolinium salts. The interaction of *N*-monosubstituted benzimidazoles with various salts ($CuCl_2$, $ZnCl_2$, $CoCl_2$, $PdCl_2$ and $AgNO_3$) yielded stable solid complexes. Potential cytotoxic activity of synthesised benzimidazolinium salts and benzimidazole metal complexes was tested in vitro on four monolayer tumour cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma) and normal mouse fibroblast cells. A preliminary analysis of the structure–activity relationship for the benzimidazole derivatives clearly indicates that the character of substituents in the benzimidazole ring has strong influence on the cytotoxic activity. The insertion of the silicon atom into the *N*-alkyl chain increases the cytotoxic activity of benzimidazolinium salts significantly, which show a very significant potency in vitro against all studied tumour cell lines, being particularly active in experiments with B16 (mouse melanoma). TD_{50} for the most active compounds are in the range 0.001–0.008 μg ml^{-1}. Cytotoxicity of benzimidazole metal complexes (L_2MX_2) strongly depends on the metal nature. 1-(3-Trimethylsilylpropyl)benzimidazole in dose 1 mg kg^{-1} inhibits carcinoma S-180 tumour growth by 62% (on ICR mice). © 2001 Éditions scientifiques et médicales Elsevier SAS

benzimidazoles / benzimidazolinium salts / benzimidazole complexes / cytotoxic activity / antitumour activity

1. Introduction

The benzimidazole ring is an important pharmacophore in drug discovery [1]. Copper(II) and silver(I) complexes of 2-pyridyl-1H-benzimidazoles possess considerable activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flexneri* and *Candida albicans* [2]. Some *N*-(nitrophenylsulphonyl)benzimidazoles show good antiviral properties against RNA (Coxsackie B5 and Mumps) viruses [3]. A series of 1,2,4-trisubstituted benzimidazoles was evaluated in vitro as potent and selective neuropeptide Y Y1 receptor antagonists. Within this series the activity was enhanced for compounds with two appropriately positioned aminoalkyl groups (e.g. 4-piperidinoalkyl functionalities) at either N(1) or N(1) and C(4) positions of aromatic heterocycle [4–6]. *N*-Substituted benzimidazol-2-ones have low nanomolar affinity for the human neuropeptide Y Y5 receptor and behave as functional antagonists of the Y5 receptor [7]. To discover new immunosuppressive compounds, a variety of 5-alkoxy substituted 2-(4-pyridylmethylsulfinyl)benzimidazoles were synthesised and evaluated for their ability to inhibit protein tyrosine phosphatase activities. Enzymatic analysis with several phosphatases reveals that 5-isopropoxy-2-[(4-methylthiopyridyl-2)methylsulfinyl]benzimidazole shows high specific inhibition against CD45 [8]. 1-Methyl-2-phenylethynyl-3-(3-phenylpropyn-2-yl)-benzimidazolinium tetrafluoroborate and trifluoromethanesulfonate salts cause extensive cleavage of super-coiled DNA when incubated under mild conditions. Frank single-strand DNA cleavage is observed at concentrations as low as 1 μM [9]. Cytotoxicity of

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2-alkyl, 2-aryl, and 2-piperazinyl benzimidazole-4,7-dione derivatives were tested against three cancer cell lines (mouse lymphocytic leukaemia P388, and human gastric carcinoma SNU-1 and SNU-16). These compounds showed potent cytotoxicity against all three cell lines tested and especially SNU-16 was sensitive to them. 2-Aryl and 2-piperazinyl benzimidazole-4,7-dione derivatives were more potent than mitomycin C against P388 and SNU-16 [10]. Significant cytotoxic activity against several human tumour cell lines was displayed by 5,6-dichloro-2-(tetrahydropyran-2-yl)-benzimidazole [11], 6-aziridinylbenzimidazoles [12], pyrido[1,2-*a*]benzimidazoles [13], 3-substituted pyrrolo[1,2-*a*]benzimidazoles [14] and 1-(2,6-difluorophenyl)-1H,3H-thiazolo[3,4-*a*]benzimidazole [15].

Taking into account extensive DNA-cleaving ability of benzimidazolinium tetrafluoroborate and trifluoromethanesulfonate salts [9], it may be concluded that they have the potential to function as effective antitumour agents. These results prompted us to synthesise new silyl substituted benzimidazolinium salts to investigate their in vitro cytotoxicity, in vivo antitumour activity and the influence on cell morphology.

2. Chemistry

The general synthetic route chosen for preparation of substituted benzimidazolinium salts included two stages. Among the possible methods for synthesis of *N*-alkylsubstituted imidazoles phase-transfer catalysed (PTC) *N*-alkylation is one of the simplest and most convenient routes [16]. The alkylation of benzimidazole by 1-trimethylsilylprop-3-yl iodide, allyl bromide and heptyl bromide in a two-phase benzene–potassium hydroxide system in the presence of 18-crown-6 used as phase-transfer catalyst (the molar ratio of benzimidazole:alkylating agent:18-crown-6 is 1:1:0.03) affords the corresponding *N*-alkyl substituted benzimidazoles **1**–**3** in high yields. The following quaternisation of compounds **1** and **2** by heating with various alkyl, allyl, propargyl and benzyl chlorides and bromides leads to the formation of benzimidazolinium salts **4**–**25** (*figure 1*). In the case of quaternisation by various (chloromethyl)silanes (e.g. trimethyl(chloromethyl)silane, methyldi(2-furyl)-(chloromethyl)silane, methyldi(2-thienyl)(chloromethyl)silane), the reaction with *N*-trimethylsilylpropylbenzimidazole (**1**) proceeds with desilylation of trimethylsilyl group in the alkylating agent and formation of *N*-trimethylsilylpropyl-*N*′-methylbenzimidazolyl chloride (**4**) in good yield. The ^{1}H-NMR spectroscopic data obtained for compound **4** are similar to those of corresponding iodide **5** prepared by heating of benzimidazole **1** with methyl iodide.

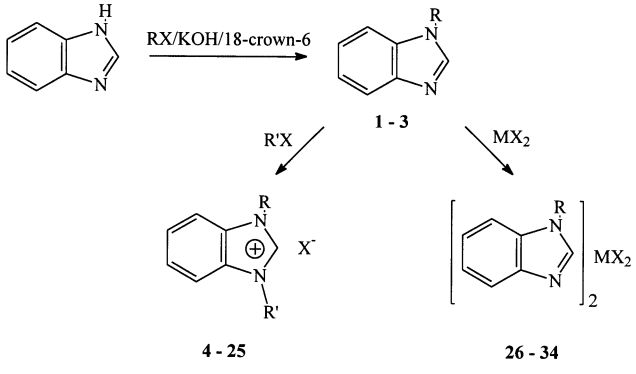


Fig. 1. Synthesis of benzimidazolinium salts **4**–**25** and benzimidazole metal complexes **26**–**34**.

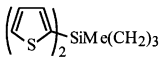
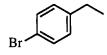
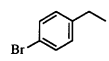
N-Monosubstituted benzimidazoles **1** and **3** were used as ligands for synthesis of complexes **26**–**34** by reaction with salts $CuCl_2$, $ZnCl_2$, $CoCl_2$, $PdCl_2$ and $AgNO_3$ (molar ratio of benzimidazole **1** or **3** with salt 2:1) in ethanol. All obtained complexes L_2MX_n are stable solids, poorly soluble in common organic solvents.

3. Results and discussion

3.1. In vitro cytotoxic activity

Potential cytotoxic activity of synthesised benzimidazolinium salts **6**–**25** and benzimidazole complexes **26**–**34** was tested in vitro on four monolayer tumour cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma) and normal mouse fibroblast cells. The experimental evaluations of cytotoxicity are presented in *tables I and II*. A preliminary analysis of the structure–activity relationship for the cytotoxic action clearly indicates the strong influence of the substituents in the benzimidazole heterocycle on toxic effects in vitro. Initial *N*-trimethylsilylpropylbenzimidazole (**1**) exhibits high cytotoxic activity ($TD_{50} = 0.55$–2.13 μg ml^{-1}) against all cell lines studied. The following quaternisation of this compound by various alkyl, allyl, propargyl and benzyl halides as a rule increases cytotoxic effect, which is especially expressed in the case of mouse

Table I. Cytotoxic activity of benzimidazolyl salts (TD_{50}, μg ml^{-1}).

N°	R	R'	X	HT - 1080		MG - 22A		B16		Neuro 2A		3T3
				TD_{50}[a]	NO, % CV[b]	TD_{50}[a]	NO, % CV[b]	TD_{50}[a]	NO, % CV[b]	TD_{50}[a]	NO, % CV[b]	TD_{50}[a]
	1	2	3	4	5	6	7	8	9	10	11	12
1	$Me_3Si(CH_2)_3$			1.2	-	2.13	-	0.6	12	0.55	12	>1
6	$Me_3Si(CH_2)_3$	C_2H_5	Br	0.2	250	0.45	150	0.001	40	0.5	13	0.5
7	$Me_3Si(CH_2)_3$	C_9H_{19}	Cl	0.07	133	0.03	150	0.008	44	0.6	200	0.25
8	$Me_3Si(CH_2)_3$	$Me_3Si(CH_2)_3$	Cl	0.25	150	0.5	250	0.001	50	0.55	23	0.35
9	$Me_3Si(CH_2)_3$	$(Me_3SiO)_3Si(CH_2)_3$	Cl	0.2	200	0.65	250	0.008	50	0.6	21	0.55
10	$Me_3Si(CH_2)_3$		Cl	0.55	54	0.75	21	0.25	75	3	300	0.2
11	$Me_3Si(CH_2)_3$	$CH_2{=}CHCH_2$	Br	0.35	150	0.35	200	0.002	30	0.8	8	0.5
12	$Me_3Si(CH_2)_3$	$HC{\equiv}CCH_2$	Br	0.4	150	0.35	75	0.02	3	2	6	0.6
13	$Me_3Si(CH_2)_3$	$Me_3SiC{\equiv}CCH_2$	Br	2.3	100	0.9	100	0.25	200	4.5	43	
14	$Me_3Si(CH_2)_3$	CH_2Ph	Br	1.4	200	1	25	0.005	29	0.85	9	0.8
15	$Me_3Si(CH_2)_3$		Br	0.15	200	0.35	200	0.003	40	0.85	11	0.6
16	$Me_3Si(CH_2)_3$		Br	0.45	100	0.3	180	0.14	150	0.85	150	
17	$CH_2{=}CHCH_2$	CH_3	I	98	6	*	3	*	5	62	31	-
18	$CH_2{=}CHCH_2$	C_2H_5	Br	66	19	87	11	*	20	-	-	-
19	$CH_2{=}CHCH_2$	C_4H_9	Cl	38	250	61	19	42	32	52	21	-
20	$CH_2{=}CHCH_2$	C_9H_{19}	Cl	0.65	125	0.4	175	0.4	300	0.75	250	4.7
21	$CH_2{=}CHCH_2$	$CH_2{=}CHCH_2$	Br	*	9	84	24	*	12	3.7	47	-
22	$CH_2{=}CHCH_2$	$HC{\equiv}CHCH_2$	Br	43	167	100	14	*	8	4	130	-
23	$CH_2{=}CHCH_2$	$Me_3SiC{\equiv}CHCH_2$	Br	14	300	82	12	70	14	-	-	-
24	$CH_2{=}CHCH_2$	CH_2Ph	Br	21	300	45	21	29	200	-	-	-
25	$CH_2{=}CHCH_2$		Br	4.2	200	32	83	5.9	300	1.8	550	>80

[a] Concentration (μg/mL) providing 50% cell killing effect [(CV+MTT)/2]
[b] NO Concentration (%) (CV: coloration)
* No cytotoxic effect was detected.
- Not tested.

melanoma B16. In the series of trimethylsilylpropylbenzimidazolinium salts **6**–**19** the TD_{50} values change from 0.25 μg ml^{-1} [$R' = (C_4H_3S)_2MeSi(CH_2)_3$ (**9**) and $Me_3SiC{\equiv}CCH_2$) (**13**)] to 0.001 μg ml^{-1} [$R' = Et$ (**6**) and $Me_3Si(CH_2)_3$ (**8**)] on B16 cell line. Methoxybenzyl derivative **15** exhibits the highest cytotoxicity on B16 among benzylbenzimidazolinium salts (**14**–**16**). For compounds with unsaturated substituent R′ (salts **11**–**13**) the activity increases in the order: $Me_3SiC{\equiv}CCH_2 < HC{\equiv}CCH_2 < CH_2{=}CHCH_2$ for all cell lines. The

Table II. Cytotoxic activity of benzimidazole metal complexes **26–34** (TD_{50}, μg ml^{-1}).

No.	R	MX_2	Colour	HT-1080		MG-22A		B16		Neuro 2A		3T3
				TD_{50} [a]	NO, %CV [b]	TD_{50} [a]	NO, %CV [b]	TD_{50} [a]	NO, %CV [b]	TD_{50} [a]	NO, %CV [b]	TD_{50} [a]
26	$Me_3Si(CH_2)_3$	$CuCl_2$	yellow	5	400	4.4	450	1.2	200	2	250	3.6
27	$Me_3Si(CH_2)_3$	$ZnCl_2$	white	0.6	250	0.75	250	0.4	300	4.2	300	4.1
28	$Me_3Si(CH_2)_3$	$CoCl_2$	blue	0.5	450	0.75	450	0.5	200	4.9	200	1.2
29	$Me_3Si(CH_2)_3$	$PdCl_2$	grey	2	500	0.8	500	0.45	200	3.9	200	4.1
30	$Me_3Si(CH_2)_3$	$AgNO_3$	white	2.9	250	3.6	250	1.5	350	5.4	250	2.2
31	C_7H_{15}	$CuCl_2$	yellow	1.5	450	2.5	300	1.8	333	0.25	1000	–
32	C_7H_{15}	$ZnCl_2$	white	35	300	21	300	–	–	–	–	–
33	C_7H_{15}	$CoCl_2$	blue	23	221	15	300	–	–	–	–	–
34	C_7H_{15}	$PdCl_2$	grey	52	400	33	158	–	–	–	–	–

[a] Concentration (μg ml^{-1}) providing 50% cell killing effect [(CV + MTT)/2].
[b] NO concentration (%) (CV: coloration).
–, Not tested.

influence of quaternisation is less effective on mouse hepatoma MG-22A ($TD_{50} = 1–0.03$ μg ml^{-1}) and human fibrosarcoma HT-1080 ($TD_{50} = 0.55–0.07$ μg ml^{-1}, with the exception of derivative **13**) being almost negligible on Neuro 2A cell line ($TD_{50} = 4.5–0.5$ μg ml^{-1}). *N*-Allylbenzimidazole **2** derivatives bearing methyl (**17**), ethyl (**18**), butyl (**19**), allyl (**21**), propargyl (**22**), trimethylsilylpropargyl (**23**), and benzyl (**24**) substituent show a slight cytotoxic effect (*table I*). The substitution of a short alkyl chain by a nonyl group (salt **20**) leads to a considerable increase of activity (0.4 μg ml^{-1} on mouse hepatoma MG-22A and mouse melanoma B16 cell lines). Comparison of the tumour growth inhibition for trimethylsilylpropyl **6–16** and allyl **17–25** benzimidazolinium salts confirms a significant influence of the long lipophilic trimethylsilylpropyl group or alkyl chain on cytotoxic action.

Inspection of the results presented in *table II* for di(*N*-trimethylsilylpropylbenziminazole) metal complexes **27–30** and its silicon free analogues **32–34** shows that the insertion of the silicon atom into the *N*-alkyl chain defines high level of cytotoxic activity. The only exception is di(*N*-trimethylsilylpropylbenzimidazole) copper dichloride **26** which is a weaker tumour growth inhibitor than the corresponding alkyl analogue **31**.

It has been found that the metal nature in the L_2MX_n complexes determine their in vitro activity. The inhibition ability of trimethylsilylpropyl substituted complexes **26–30** decreases in the following order: Zn>Co>Pd>Ag>Cu.

Under our experimental conditions several benzimidazolinium salts (**1**, **11–13**) exhibit remarkable NO-inhibitor properties, however, other active *N*-trimethylsilylpropylbenzimidazole derivatives are medium NO-inducers. In general, benzimidazolinium metal complexes **26–34** are more potent NO-inducers than the benzimidazolinium salts **1–25**, di(*N*-heptylbenzimidazole) copper dichloride **31** being the most active (1000% in the mouse neuroblastoma Neuro 2A).

3.2. Cell morphology

The influence of complexes **26–28** on tumour cell morphology was examined. *Figure 2* presents the morphologic structure of human fibrosarcoma HT-1080 cells at 30 °C (control). The copper complex of *N*-trimethylsilylpropylbenziminazole **26** induces the full necrosis of tumour cells (*figure 3*). In the case of zinc dichloride complex **27**, the cell size does not change after

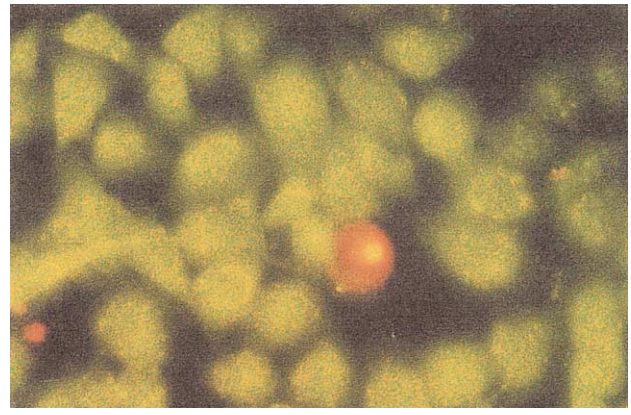


Fig. 2. View of human fibrosarcoma HT-1080 cell fenotype (30 °C, 24h).

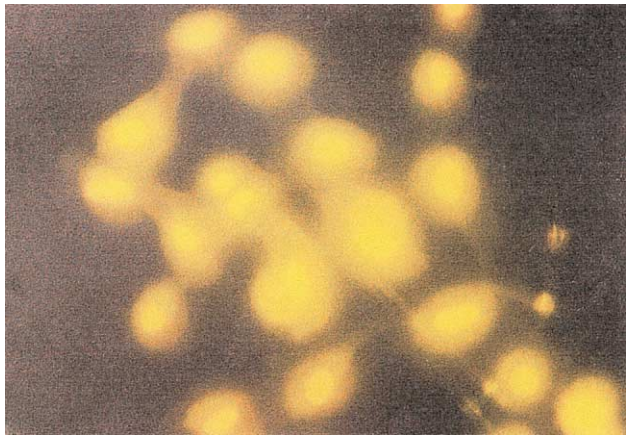


Fig. 3. View of HT-1080 cell fenotype with CU complex **26** (30 °C, 24 h).

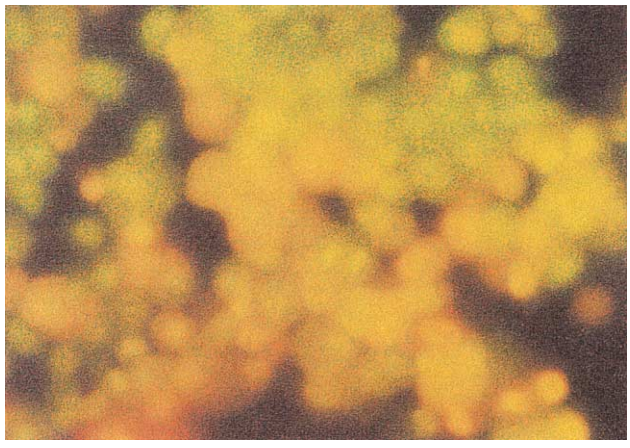


Fig. 4. View of HT-1080 cell fenotype with Zn complex **27** (30 °C, 24 h).

apoptosis (*figure 4*). Action of complexes **26** and **28** results in HT-1080 cells culture necrosis with cell nucleus fragmentation (*figure 5*).

3.3. In vivo antitumour activity

The antitumour activity of *N*-trimethylsilylpropylbenzimidazole (**1**) was tested against sarcoma S-180 in male ICR mice (18–20 g). In dose 1 mg kg^{-1} benzimidazole (**1**) inhibits the tumour growth by 62%. The higher concentration (3 mg kg^{-1}) decreases the tumour growth inhibition to 22% (*figure 6*).

4. Conclusions

N-Trimethylsilylpropylbenziminazole (**1**), and its benzimidazolinium salts and complexes show a very significant potency in vitro against different tumour cell lines, being particularly active in experiments with B16 (mouse melanoma) cell line (TD_{50} for the most active compounds are in the range 0.001–0.008 μg ml^{-1}). In vivo evaluation of benzimidazole (**1**) also confirms high antitumour activity against sarcoma S-180.

5. Experimental

^{1}H-NMR spectra were recorded on a Varian Mercury 200 spectrometer at 200.06 MHz at 303 K. The chemical shifts are given relative to TMS from solvent ($CDCl_3$) signal ($\delta_H = 7.25$). The melting points

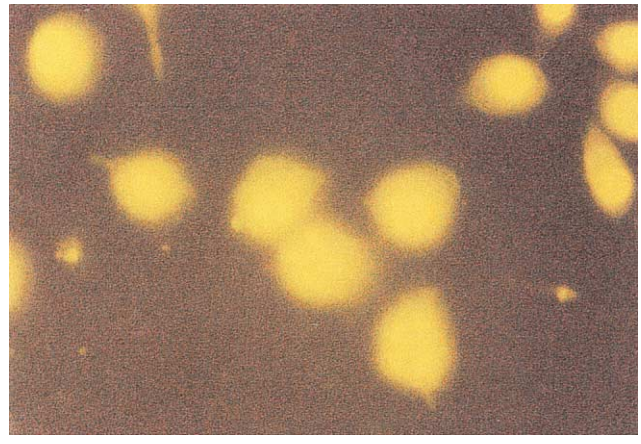


Fig. 5. View of HT-1080 cell fenotype with Co complex **28** (30 °C, 24 h).

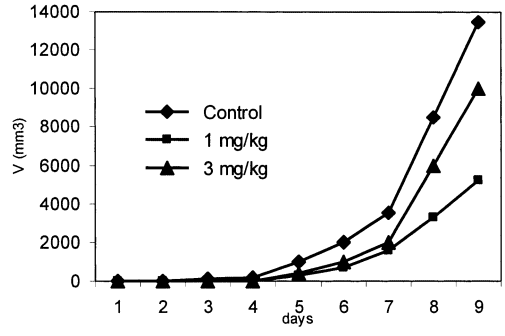


Fig. 6. Antitumour activity of 1-(3-trimethylsilylpropyl)-benzimidazole (**1**).

were determined on a 'Digital melting point analyser' (Fisher), and the results are given without correction.

5.1. Chemistry

5.1.1. General method for synthesis of N-substituted benzimidazoles **1**–**3**

The mixture of benzimidazole (0.02 mol), 1-trimethylsilylprop-3-yl iodide (allyl bromide or heptyl bromide) (0.02 mol), 18-crown-6 (0.6 mmol) and KOH (0.06 mol) in benzene (200 ml) was stirred for 6 h at reflux temperature (GLC control). After reaction completion the mixture was filtered. Benzene was evaporated and residue was dried in vacuum to give products as solids or oils.

5.1.1.1. N-(3-Trimethylsilylpropyl)benzimidazole (**1**)

Yield 83%, m.p. 63–64 °C. Impurities: <1% (HPLC on Symmetry C_{183} 3.9×150 mm. Mobile phase: 80% acetonitrile+20% H_2O. Detector UV 254 nm). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 9H, Me_3Si), 0.4–0.64 (m, 2H, CH_2Si), 1.65–2.04 (m, 2H, CH_2), 4.15 (t, 2H, $J = 7.6$ Hz, CH_2N), 7.22–7.38 (m, 3H, arom), 7.33–7.89 (m, 2H, arom). Anal ($C_{13}H_{20}N_2Si$) C, H, N.

5.1.1.2. N-Allylbenzimidazole (**2**)

Yield 80%. Impurities: <1% (HPLC on Symmetry C_{18}, 3.9×150 mm. Mobile phase: 80% acetonitrile+20% H_2O. Detector UV 254 nm). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 4.73 (dt, 2H, $J = 5.4$ Hz, $J = 1.4$ Hz, CH_2N), 5.09–5.26 (m, 2H, CH_2), 5.88–6.04 (m, 1H, CH), 7.24–7.36 (m, 4H, arom), 7.87 (s, 1H, CH). Anal ($C_{10}H_{10}N_2$) C, H, N.

5.1.1.3. N-Heptylbenzimidazole (**3**)

Yield 80%. Impurities: <1% (HPLC on Symmetry C_{18}, 3.9×150 mm. Mobile phase: 80% acetonitrile+20% H_2O. Detector UV 254 nm). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.85 (t, 3H, $J = 6.8$ Hz Me), 1.27 (m, 8H, $(CH_2)_4$), 1.85 (m, 2H, CH_2), 4.12 (t, 2H, $J = 7.0$ Hz, CH_2N), 7.24–7.29 (m, 3H, arom), 7.78–7.82 (m, 1H, arom), 7.88 (s, 1H, CH). Anal ($C_{14}H_{20}N_2$) C, H, N.

5.1.2. Preparation of benzimidazolinium salts

The mixture of *N*-substituted benzimidazole (0.02 mol) in toluene and alkylhalogenide (0.02 mol) was refluxed with stirring for about 4 h. Then it was allowed to stand at room temperature overnight to give a solid product. The precipitate was filtered, washed with toluene and recrystallised from ethanol.

5.1.2.1. N-Methyl-N′-(3-trimethylsilylpropyl)-benzimidazolyl chloride (**4**)

Yield 67%, m.p. 221 °C, TLC $R_f = 0.36$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 18H, Me_3Si), 0.45–0.65 (m, 2H, CH_2Si), 1.85–2.10 (m, 2H, CH_2), 4.44 (s, 3H, CH_3N), 4.67 (t, 6H, $J = 7.8$ Hz, CH_2N), 7.6–7.71 (4H, m, arom), 11.67 (s, 1H, CH). Anal. ($C_{22}H_{39}ClN_2Si$) C, H, N.

5.1.2.2. N-Methyl-N′-(3-trimethylsilylpropyl)-benzimidazolyl iodide (**5**)

Yield 87%, m.p. 98–99 °C, TLC $R_f = 0.38$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.07 (s, 18H, Me_3Si), 0.60–0.69 (m, 2H, CH_2Si), 2.07–2.12 (m, 2H, CH_2), 4.39 (s, 3H, CH_3N), 4.71 (t, 6H, $J = 7.8$ Hz, CH_2N), 7.77–7.81 (m, 4H, arom), 11.20 (s, 1H, CH). Anal ($C_{14}H_{23}IN_2Si$) C, H, N.

5.1.2.3. N-(3-Trimethylsilylpropyl)-N′-(ethyl)-benzimidazolyl bromide (**6**)

Yield 85%, m.p. 207–208 °C, TLC $R_f = 0.70$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.03 (s, 9H, Me_3Si), 0.48–0.7 (m, 2H, CH_2Si), 1.86–2.28 (m, 4H, CH_3+CH_2), 4.67 (t, 4H, $J = 7.6$ Hz, CH_2N+CH_2N), 7.67–7.76 (m, 4H, arom), 11.51 (s, 1H, CH). Anal ($C_{15}H_{25}BrN_2Si$) C, H, N.

5.1.2.4. N-Nonyl-N′-(3-trimethylsilylpropyl)-benzimidazolyl chloride (**7**)

Yield 80%, m.p. 164–165 °C, TLC $R_f = 0.43$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 18H, Me_3Si); 0.42–0.66 (m, 2H, CH_2Si); 0.90 (t, 3H, $J = 6.3$ Hz, CH_3); 1.20 (m, 14H, $(CH_2)_7$); 1.90–2.10 (m, 2H, CH_2); 4.67 (t, 6H, $J = 7.8$ Hz, CH_2N); 7.40 (m, 4H, arom); 11.68 (s, 1H, CH). Anal ($C_{22}H_{39}ClN_2Si$) C, H, N.

5.1.2.5. N,N′-Bis(3-trimethylsilylpropyl)benzimidazolyl chloride (**8**)

Yield 76%, m.p. >250 °C; TLC $R_f = 0.53$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 18H, Me_3Si), 0.4–0.64 (m, 4H, CH_2Si), 1.84–2.04 (m, 4H, CH_2), 4.62 (t, 4H, $J = 7.8$ Hz, CH_2N), 7.40 (m, 4H, arom), 11.62 (s, 1H, CH). Anal ($C_{19}H_{35}ClN_2Si_2$) C, H, N.

5.1.2.6. N-(3-Trimethylsilylpropyl)-N′-[3-tris(trimethylsiloxy)silylpropyl]benzimidazolyl chloride (**9**)

Yield 96%, m.p. 94–95 °C (from benzene). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 9H, Me_3Si), 0.03 (s, 27H, Me_3SiO), 0.51–0.61 (m, 4H, CH_2Si), 1.93–2.01 (m,

4H, CH_2), 4.10 (m, 4H, CH_2N), 7.54–7.70 (m, 4H, arom), 11.61 (s, 1H, CH). Anal ($C_{25}H_{53}ClN_2O_3Si_5$) C, H, N.

*5.1.2.7. N-(3-Trimethylsilylpropyl)-N′-[3-methyldi-(2-thienyl)silylpropyl]benzimidazolyl chloride (**10**)*

Yield 76%, m.p. 150–151 °C, TLC $R_f = 0.53$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 12H, Me_3Si, SiMe), 0.4–0.64 (m, 2H, CH_2Si), 1.15–1.20 (m, 2H, CH_2Si), 1.94–2.10 (m, 4H, CH_2), 4.66 (m, 4H, CH_2N), 7.20–7.29 (m, 4H, arom), 7.47–7.67 (m, 6H, arom), 11.67 (s, 1H, CH). Anal ($C_{25}H_{35}ClN_2S_2Si_2$) C, H, N.

*5.1.2.8. N-(3-Trimethylsilylpropyl)-N′-(allyl)-benzimidazolyl bromide (**11**)*

Yield 91%, m.p. 124–125 °C, TLC $R_f = 0.54$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.04 (s, 9H, Me_3Si), 0.47–0.64 (m, 2H, CH_2Si), 1.96–2.12 (m, 4H, CH_2, CH_2), 4.65 (dt, 2H, $J = 4$, $J = 7.6$Hz, CH_2N), 5.3–5.56 (m, 2H, CH_2N), 7.64–7.75 (m, 4H, arom), 11.47 (s, 1H, CH). Anal ($Cl_{16}H_{25}BrN_2Si$) C, H, N.

*5.1.2.9. N-(3-Trimethylsilylpropyl)-N′-(propargyl)-benzimidazolyl bromide (**12**)*

Yield 90%, m.p. 164–168 °C; TLC $R_f = 0.57$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.03 (s, 9H, Me_3Si), 0.46–0.72 (m, 2H, CH_2Si), 1.88–2.15 (m, 2H, CH_2), 2.64 (t, 2H, $J = 3$ Hz, CH_2), 4.61 (m, 2H, CH_2N); 5.71 (d, 1H, $J = 3$ Hz, CH), 7.55–7.97 (m, 4H, arom), 11.57 (s, 1H, CH). Anal ($C_{16}H_{23}BrN_2Si$) C, H, N.

*5.1.2.10. N-(3-Trimethylsilylpropyl)-N′-(trimethylsilylpropargyl)benzimidazolyl bromide (**13**)*

Yield 90%, m.p. 90–191 °C, TLC $R_f = 0.34$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 9H, Me_3Si), 0.14 (s, 9H, Me_3Si), 0.46–0.62 (m, 2H, CH_2Si), 1.88–2.15 (m, 2H, CH_2), 4.56 (m, 2H, CH_2N), 5.65 (s, 2H, CH_2N), 7.67–7.72 (m, 4H, arom), 11.54 (s, 1H, CH). Anal ($C_{19}H_{31}BrN_2Si_2$) C, H, N.

*5.1.2.11. N-(3-Trimethylsilylpropyl)-N′-benzylbenzimidazolyl bromide (**14**)*

Yield 85%, m.p. 128–130 °C, TLC $R_f = 0.38$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.04 (s, 9H, Me_3Si), 0.44–0.7 (m, 2H, CH_2Si), 1.90–2.06 (m, 2H, CH_2), 4.48 (t, 2H, $J = 7.6$ Hz, CH_2N), 5.93 (s, 2H, CH_2), 7.35–7.7 (m, 9H, arom), 11.71 (s, 1H, CH). Anal ($C_{20}H_{27}BrN_2Si$) C, H, N.

*5.1.2.12. N-(3-Trimethylsilylpropyl)-N′-(o-methoxybenzyl)benzimidazolyl bromide (**15**)*

Yield 85%, m.p. 175–176 °C, TLC $R_f = 0.46$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.04 (s, 9H, Me_3Si), 0.46–0.75 (m, 2H, CH_2Si), 1.95–2.26 (m, 2H, CH_2), 3.94 (s, 3H, MeO), 4.73 (t, 2H, $J = 7.6$ Hz, CH_2N), 5.80 (s, 2H, CH_2); 6.92–7.7 (m, 9H, arom); 11.42 (s, 1H, CH). Anal ($C_{21}H_{29}BrN_2OSi$) C, H, N.

*5.1.2.13. N-(3-Trimethylsilylpropyl)-N′-(p-bromobenzyl)benzimidazolyl bromide (**16**)*

Yield 75%, m.p. 154–155 °C, TLC $R_f = 0.44$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.02 (s, 9H, Me_3Si), 0.44–0.71 (m, 2H, CH_2Si), 1.90–2.28 (m, 2H, CH_2), 4.62 (q, 2H, $J = 6.4$ Hz, CH_2N), 5.98 (s, 2H, CH_2), 7.51–7.86 (m, 8H, arom), 11.53 (s, 1H, CH). Anal ($C_{16}H_{25}Br_2N_2Si$) C, H, N.

*5.1.2.14. N-Methyl-N′-allylbenzimidazolyl iodide (**17**)*

Yield 91%, m.p. 144–145 °C, TLC $R_f = 0.19$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 4.29 (s, 3H, CH_3), 5.27–5.30 (m, 2H, CH_2), 5.46–5.60 (m, 2H, CH_2), 6.10–6.19 (m, 1H, CH), 7.63–7.83 (m, 4H, arom), 10.75 (s, 1H, CH). Anal ($C_{11}H_{13}N_2I$) C, H, N.

*5.1.2.15. N-Ethyl-N′-allylbenzimidazolyl bromide (**18**)*

Yield 85%, m.p. 174–175 °C, TLC $R_f = 0.21$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.74 (t, 3H, $J = 7.4$ Hz, CH_3), 1.69 (t, 2H, $J = 7.4$ Hz, CH_2), 5.34–5.37 (m, 2H, CH_2), 5.43–5.56 (m, 2H, CH_2), 6.07–6.21 (m, 1H, CH), 7.63–7.83 (m, 4H, arom), 11.19 (s, 1H, CH). Anal ($C_{12}H_{15}N_2Br$) C, H, N.

*5.1.2.16. N-Butyl-N′-allylbenzimidazolyl chloride (**19**)*

Yield 85%, m.p. 95–97 °C, TLC $R_f = 0.21$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.98 (t, 3H, $J = 7.2$ Hz, CH_3), 1.40–1.47 (m, 2H, CH_2), 1.97–2.03 (m, 2H, CH_2), 4.59–4.68 (m, 2H, CH_2N), 5.33–5.38 (m, 2H, CH_2), 5.42–5.53 (m, 2H, CH_2), 6.04–6.13 (m, 1H, CH), 7.65–7.76 (m, 4H, arom), 11.61 (s, 1H, CH). Anal ($C_{14}H_{19}N_2Cl$) C, H, N.

*5.1.2.17. N-Nonyl-N′-allylbenzimidazolyl chloride (**20**)*

Yield 88%, m.p. 95–97 °C; TLC $R_f = 0.21$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.98 (t, 3H, $J = 6.4$ Hz, CH_3), 1.23–1.36 (m, 10H, CH_2), 2.05 (m, 2H, CH_2), 2.59 (m, 2H, CH_2), 4.58–4.64 (m, 2H, CH_2N), 5.36–5.39 (m, 2H, CH_2); 5.42–5.51 (2H, m, CH_2); 6.10–6.13 (1H, m, CH); 7.66–7.71 (4H, m, arom); 11.57 (1H, s, CH). Anal ($C_{19}H_{29}N_2Cl$) C, H, N.

*5.1.2.18. N,N′-Bis(allyl)benzimidazolyl bromide (**21**)*

Yield 78%, m.p. 95–97 °C, TLC $R_f = 0.21$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 5.28–5.31 (m, 4H, CH_2, CH_2), 5.41–5.51 (m, 4H, CH_2, CH_2), 6.00–6.19 (m, 2H, CH, CH), 7.56–7.74 (m, 4H, arom), 11.31 (s, 1H, CH). Anal ($C_{13}H_{15}N_2Br$) C, H, N.

*5.1.2.19. N-Allyl-N′-propargylbenzimidazolyl bromide (**22**)*

Yield 83%, m.p. 177–178 °C, TLC $R_f = 0.31$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 1.94 (d, 1H, $J = 1.4$ Hz, CH), 5.29–5.32 (m, 2H, CH_2), 5.48–5.86 (m, 2H, CH_2), 5.66–5.68 (m, 2H, CH_2), 6.03–6.21 (m, 1H, CH), 7.64–7.97 (m, 4H, arom), 11.45 (s, 1H, CH). Anal ($C_{13}H_{13}N_2Br$) C, H, N.

*5.1.2.20. N-Allyl-N′-(3-trimethylsilylpropargyl)-benzimidazolyl bromide (**23**)*

Yield 88%, m.p. 144–145 °C, TLC $R_f = 0.26$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.14 (s, 9H, Me_3Si), 5.29–5.32 (m, 2H, CH_2), 5.47–5.49 (m, 2H, CH_2), 5.57–5.61 (m, 2H, CH_2), 6.04–6.21 (m, 1H, CH), 7.64–7.95 (m, 4H, arom), 11.37 (s, 1H, CH). Anal ($C_{16}H_{21}N_2BrSi$) C, H, N.

*5.1.2.21. N-Allyl-N′-benzylbenzimidazolyl bromide (**24**)*

Yield 82%, m.p. 200–201 °C, TLC $R_f = 0.23$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 5.29–5.32 (m, 2H, CH_2), 5.45–5.49 (m, 2H, CH_2), 5.89 (s, 2H, CH_2), 6.04–6.20 (m, 1H, CH), 7.27–7.59 (m, 9H, arom), 11.66 (s, 1H, CH). Anal ($C_{17}H_{17}N_2Br$) C, H, N.

*5.1.2.22. N-Allyl-N′-(p-bromobenzyl)benzimidazolyl bromide (**25**)*

Yield 85%, m.p. 178–180 °C, TLC $R_f = 0.24$ (2-propanol). ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 5.26–5.29 (m, 2H, CH_2), 5.45–5.54 (m, 2H, CH_2), 5.94 (s, 2H, CH_2), 6.07–6.16 (m, 1H, CH), 7.47–7.72 (m, 8H, arom), 11.57 (s, 1H, CH). Anal ($C_{17}H_{16}N_2Br_2$) C, H, N.

5.1.3. Preparation of metal complexes in ethanol–water

A 5 ml aqueous solution of metal salts (0.02 mol) and 0.04 mol ligand in 10 ml of ethanol in a reaction tube was stirred vigorously. The mixture was refluxed by stirring for about 4 h. The mixture was then allowed to stand at room temperature overnight to give a solid product. This was then filtered, washed with water and ethanol, and dried in vacuo over anhydrous $CaCl_2$.

*5.1.3.1. Di[N-(3-trimethylsilylpropyl)benzimidazole] copper dichloride (**26**)*

Yield 81%, m.p. 243–245 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): −0.03 (s, 9H, Me_3Si), 0.26–0.48 (m, 2H, CH_2Si), 1.91–2.16 (m, 2H, CH_2), 4.43 (t, 2H, $J = 7.6$ Hz, CH_2N), 7.22–8.38 (m, 4H, arom), 9.78 (s, 1H, arom). Anal ($C_{26}H_{40}N_4Cl_2CuSi_2$) C, H, N.

*5.1.3.2. Di[N-(3-trimethylsilylpropyl)benzimidazole] zinc dichloride (**27**)*

Yield 85%, m.p. 154–156 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): −0.04 (s, 9H, Me_3Si), 0.31–0.48 (m, 2H, CH_2Si), 1.80–2.06 (m, 2H, CH_2), 4.30 (t, 2H, $J = 7.2$ Hz, CH_2N), 7.27–8.11 (m, 4H, arom), 8.53 (s, 1H, arom). Anal ($C_{26}H_{40}N_4Cl_2Si_2Zn$) C, H, N.

*5.1.3.3. Di[N-(3-trimethylsilylpropyl)benzimidazole] cobalt dichloride (**28**)*

Yield 78%, m.p. 140–143 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): −0.05 (9H, 5, Me_3Si); 0.31–0.48 (2H, m, CH_2Si); 1.64–1.98 (2H, m, CH_2); 5.62 (2H, t, $J = 7.2$ Hz, CH_2N); 7.27–8.04 (4H, m, arom); 7.76 (1H, s, arom). Anal ($C_{26}H_{40}N_4Cl_2CoSi_2$) C, H, N.

*5.1.3.4. Di[N-(3-trimethylsilylpropyl)benzimidazole] palladium dichloride (**29**)*

Yield 87%, m.p. 210–212 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): −0.03 (s, 9H, Me_3Si), 0.43–0.50 (m, 2H, CH_2Si), 1.81 (m, 2H, CH_2), 4.35 (t, 2H, $J = 7.2$ Hz, CH_2N), 7.38–8.37 (m, 4H, arom), 8.80 (s, 1H, arom). Anal ($C_{26}H_{40}N_4Cl_2Si_2Pd$) C, H, N.

*5.1.3.5. Di[N-(3-trimethylsilylpropyl)benzimidazole] silver nitrate (**30**)*

Yield 89%, m.p. 160–162 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): −0.02 (s, 9H, Me_3Si), 0.28–0.44 (m, 2H, CH_2Si), 1.90–2.12 (m, 2H, CH_2), 4.40 (t, 2H, $J = 7.6$ Hz, CH_2N), 7.12–8.38 (m, 4H, arom), 9.55 (s, 1H, arom). Anal ($C_{26}H_{40}N_5AgO_3Si_2$) C, H, N.

*5.1.3.6. Di[N-(heptyl)benzimidazole] copper dichloride (**31**)*

Yield 81%, m.p. 198–200 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.91 (t, 3H, $J = 6$ Hz, CH_3), 1.23 (m, 8H, $(CH_2)_4$), 1.81 (m, 2H, CH_2), 3.34 (m, 2H, CH_2N), 7.30–7.87 (m, 4H, arom), 8.22 (s, 1H, CH). Anal ($C_{28}H_{40}N_4Cl_2Cu$) C, H, N.

*5.1.3.7. Di[N-(heptyl)benzimidazole] zinc dichloride (**32**)*

Yield 80%, m.p. 134–136 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.82 (t, 3H, $J = 6$ Hz, CH_3), 1.20 (m,

8H, $(CH_2)_4$), 1.79 (m, 2H, CH_2), 4.36 (t, 2H, $J = 7.1$ Hz, CH_2N), 7.30–7.87 (m, 4H, arom), 8.64 (s, 1H, CH). Anal ($C_{28}H_{40}N_4Cl_2Zn$) C, H, N.

5.1.3.8. Di[N-(heptyl)benzimidazole] cobalt dichloride ***(33)***

Yield 90%, m.p. 120–121 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.89 (t, 3H, $J = 6$ Hz, CH_3), 1.31 (m, 8H, $(CH_2)_4$), 2.53 (m, 2H, CH_2), 3.57 (m, 2H, CH_2N), 7.30–7.87 (m, 4H, arom), 8.12 (s, 1H, CH). Anal ($C_{28}H_{40}N_4Cl_2Co$) C, H, N.

5.1.3.9. Di[N-(heptyl)benzimidazole] palladium dichloride ***(34)***

Yield 92%, m.p. 178–180 °C; ^{1}H-NMR ($CDCl_3$, TMS) δ (ppm): 0.83 (t, 3H, $J = 6.6$ Hz, CH_3), 1.22 (m, 8H, $(CH_2)_4$), 2.52 (m, 2H, CH_2), 4.37 (t, 2H, $J = 7.1$ Hz, CH_2N), 7.38–8.10 (m, 4H, arom), 8.74 (s, 1H, CH). Anal ($C_{28}H_{40}N_4Cl_2Pd$) C, H, N.

5.2. In vitro cytotoxicity assay

Monolayer tumour cell lines: MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neiroblastoma) and normal mouse fibroblast cells were cultivated with standard medium DMEM without an indicator ('Sigma') supplemented with 10% heat inactivated foetal bovine serum ('Sigma'). After the ampoule was defreezed the cells were used only from 1 to 4 passage. Cells in the range of $2–5\times10^4$ (cells ml^{-1}) (depending on line nature) were placed on 96-well plates immediately after compounds were inoculated to wells. The control cells without test compounds were cultivated on separate plates. The plates were cultivated for 72 h, 17 °C, 5% CO_2. A quantity of survived cells was determined using crystal violet (CV) or 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinium bromide (MTT). The quantity of live cells on control plates was taken in calculations for 100% [17, 18]. Concentration of NO was determined according to Gryess method (by NO_2 level in cultural medium). Sodium nitrite standard solution was used for the calibration curve [17].

5.3. In vivo activity assay

The compounds were tested in vivo against sarcoma S-180 cells. Sarcoma S-180 (5×10^6 cells) was inoculated s.c. to male ICR mice (6 weeks old, 18–20 g) on day 0. Drugs were administrated i.p., and the treatment was started 4 h after tumour transplantation. The number of mice used in each group was between six and ten. The efficacy of the treatment was estimated by the ellipsoid formula, and V of control group was taken in calculations for 100%.

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