ISSN 1068-1620, Russian Journal of Bioorganic Chemistry, 2020, Vol. 46, No. 2, pp. 252–263. © Pleiades Publishing, Ltd., 2020.
Russian Text © The Author(s), 2020, published in Bioorganicheskaya Khimiya, 2020, Vol. 46, No. 2, pp. 207–219.

Effect of Derivatives of Hydroxamic Acids on Vasculogenic Mimicry

A. A. Vartanian[a], D. A. Khochenkov[a], Yu. A. Khochenkova[a], Yu. S. Machkova[a], D. S. Khachatryan[b], A. V. Kolotaev[b], A. N. Balaev[c], K. A. Ohmanovich[c], and V. N. Osipov[a, 1]

[a] *Blokhin National Medical Research Center of Oncology, Ministry of Health of the Russian Federation, Moscow, 115478 Russia*

[b] *Institute of Chemical Reagents and High Purity Chemical Substances, National Research Center Kurchatov Institute, Moscow, 123098 Russia*

[c] *JSC Farm-Sintez, Moscow, 121357 Russia*

Received September 25, 2019; revised November 8, 2019; accepted November 14, 2019

Abstract—Vasculogenic mimicry, the formation of vascular channels lined with tumor cells of a highly malignant phenotype, is currently considered as an additional system of blood supply of the tumor. Experimental studies in vivo have repeatedly demonstrated that vascular channels form in the areas of a tumor with a low density of blood vessels. It is supposed that the formation of a network of these channels inside the tumor maintains homeostasis and prevents early necrosis within it. In this work, bifunctional compounds based on a combination of quinazoline and hydroxamic acid in one molecule were examined for the ability to inhibit the migration of tumor cells and vasculogenic mimicry.

Keywords: hydroxamic acids, quinazoline derivatives, melanoma, breast cancer, kidney cancer, migration, vasculogenic mimicry

DOI: 10.1134/S106816202002017X

INTRODUCTION

The concept that VEGF-induced angiogenesis is a factor that limits tumor growth is currently generally accepted. According to the data of some clinical studies, antiangiogenic therapy significantly increases the total survival of oncological patients [1]. At the same time, evidence has accumulated indicating that most tumors barely respond to anti-VEGF therapy [2]. One reason for the survival of tumor cells during antiangiogenic therapy may be the heterogeneity of blood vessels. The formation of vessels in a tumor occurs against the background of uncontrolled mitogenic stimulation and the altered extracellular matrix. This leads to the replacement of vascular endothelium by tumor cells; sometimes, there can be no endothelial cells in vessels at all. The term vasculogenic mimicry (VM), the formation by tumor cells of vascular channels covered with the basal membrane was introduced late in 1999 [3]. The generation of vascular channels lined with tumor cells is a unique capacity of cells with a highly malignant phenotype. It was found that there is a high statistical correlation between the origination of VM in a tumor and the incidence of metastasizing [4]. A detailed examination of the role of some antiangiogenic drugs used in the clinic in the modulation of VM in vitro showed that these agents do not affect the formation of a vascular network by tumor cells [5]. At present, there is not a single physiological process in adult and children that is an analog of VM. The only example of VM in humans is the formation of vascular channels by cytotrophoblasts in the placenta during embryogenesis; therefore, VM can be considered as a tumor-specific process. This fact opens up new ways for blocking the tumor growth with the minimal impact on normal physiological processes.

A promising strategy for creating new pharmaceutical agents is currently the design and synthesis of hybrid compounds that consist of two or more different bioactive fragments and act through the activation/blocking of several targets. A combination of two active groups in one molecule can lead to a more pronounced therapeutic effect. Multipurpose hybrids also offer several advantages over a combined therapeutic approach. They are distinguished for better bioaccessibility, low toxicity, predictable pharmacokinetic and pharmacodynamic profiles, a simpler regime of application by a patient, a higher efficacy of treatment, and a lower cost of the therapy (see for the review [6]). Hydroxamic acids, which are capable of forming complexes with copper, zinc, magnesium, and calcium ions, block the activity of many metal-containing proteins. In addition, hydroxamic acid derivatives exhibit hypotensive [7], antimalarial [8], antituberculosis [9], and fungicidal activities [10]. On the other hand, the quinazoline cycle is present in the molecules of more than a hundred medicines. At present, various quinazoline and dihydroquinazoline derivatives are being used in clinical practice [11].

The modern methods of chemical synthesis make it possible to introduce a hydroxamic group into various natural and synthetic compounds, including the known

Abbreviations: HOBt, *N*-hydroxybenztriazole; NMM, *N*-methylmorpholine; TBTU, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; VEGF, vascular epithelial growth factor; FCS, fetal calf serum; VM, vasculogenic mimicry; CLS, capillary-like structure.

[1] Corresponding author: phone: +7 (916) 412-61-43; e-mail: ovn65@yandex.ru.

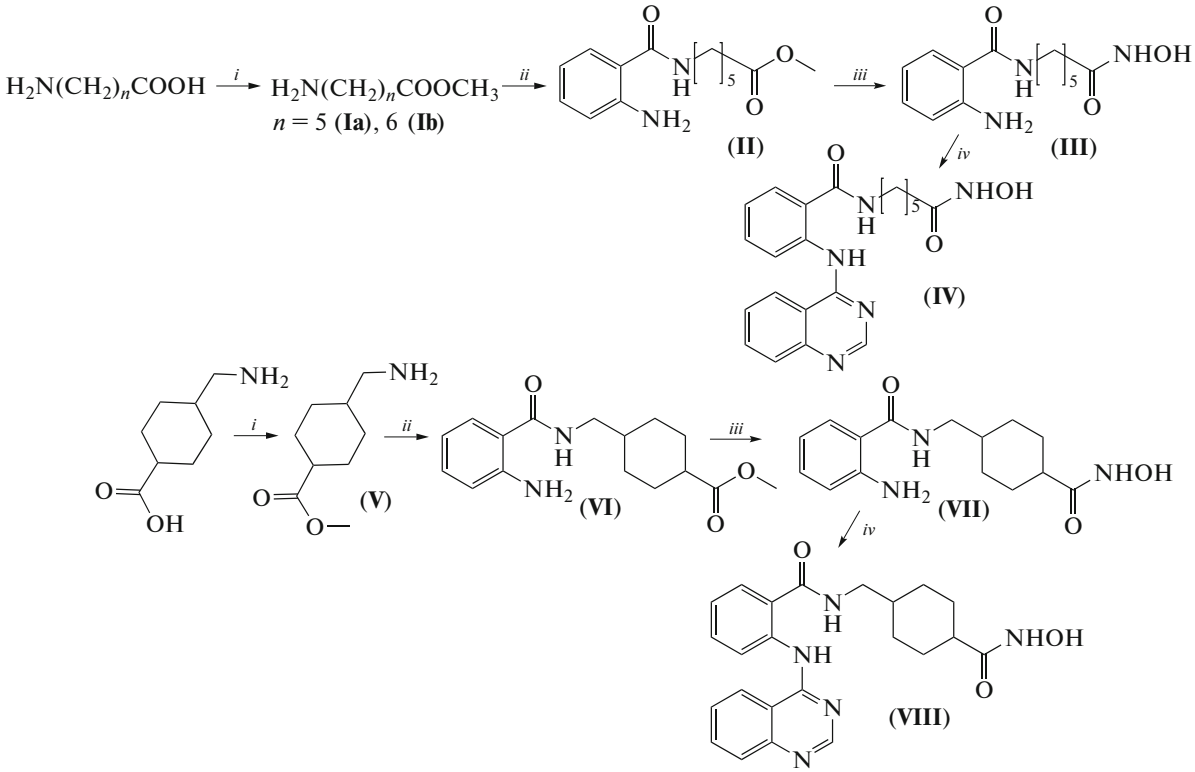
pharmaceuticals [12], increasing their therapeutic effect. We proposed that a combination of a quinazoline fragment and hydroxamic acid in one molecule would enable one to create promising new antitumor drugs. The goal of the present study was the screening of compounds combining the quinazoline cycle and a hydroxamic group in one molecule for the capacity to affect the migration of tumor cells and VM.

RESULTS AND DISCUSSION

Neoangiogenesis, the formation of microvessels on the basis of a network of preexisting vessels in the tissue, is a necessary condition for tumor growth [13]. A great number of microvessels in a tumor promote its rapid proliferation owing to the constant supply of nutrients and oxygen and the excretion of toxic products of metabolism. In recent years, more than 40 antiangiogenic agents have passed phase II and phase III clinical trials, and some of them in combination with chemotherapy have proved effective [1]. However, most tumors do not respond to antiangiogenic therapy [2]. The key factor may be the heterogeneity of tumor vessels: classical angiogenesis in a tumor goes parallel with the formation of mosaic vessels; in addition, the vessel cooption takes place in which the tumor grows along vessels already existing in the tissue [14]. Numerous recent investigations have shown that the presence of VM components in the tumor materials of patients correlates with the rapid progression of the tumor, an increase in the metastasizing rate, and, as a consequence, short-term survival of patients [3]. Interestingly, vascular channels lined with tumor cells are found in tumor areas with a low density of blood vessels. The results we have obtained in recent years enabled us to formulate the conception that antiangiogenic therapy activates the formation of vascular channels and transfers the tumor into a phase of more aggressive growth [15]. Taking into account that vascular channels are formed by tumor cells with a highly malignant phenotype (today we are unable to kill these cells), the search for a low-molecular-weight inhibitor of VM is becoming an increasingly urgent problem. The goal of our study was the synthesis of bifunctional compounds and their screening for the capacity to block VM.

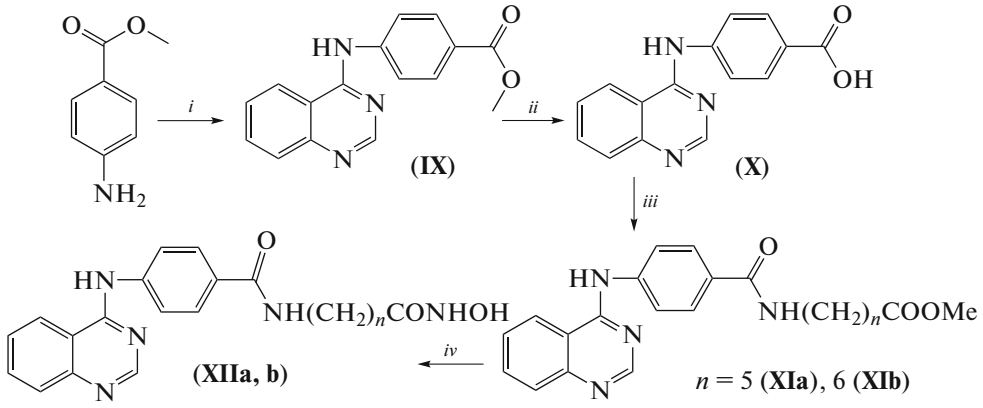
Synthesis

We proposed that bifunctional molecules that consist of two different bioactive fragments and act through the activation/blocking of several targets can effectively inhibit VM. We synthesized several groups of compounds containing a quinazoline cycle and a hydroxamic function. Compounds **(IV)** and **(VII)** were synthesized by a general method (Scheme 1). At the first stage, ethers were obtained from amino acids after which, by the reaction with isatoic anhydride, derivatives of anthranilic acid amides were synthesized. Then, the methyl esters of acids were converted into hydroxamates by the reaction with hydroxylamine. At the final stage, hydroxamic acids were condensed with 4-chloroquinazoline to form target compounds **(IV)** and **(VIII)**.



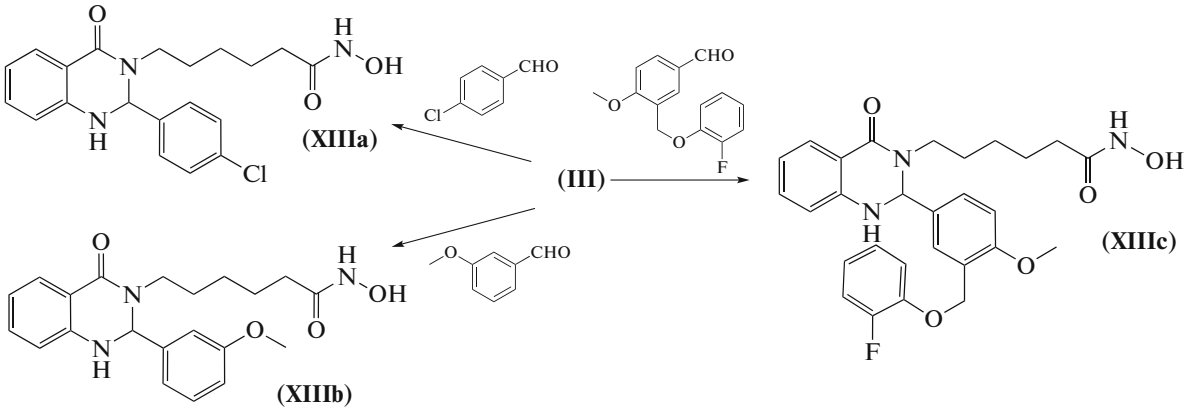
Scheme 1. Reagents and conditions: (*i*) $SOCl_2$/MeOH; (*ii*) isatoic anhydride/Na_2CO_3/DMF/H_2O; (*iii*) $HCl \cdot H_2NOH$/MeONa/MeOH; (*iv*) 4-chloroquinazoline/DMF/Δ.

For the synthesis of compounds **(XIIa)** and **(XIIb)**, methyl-4-aminobenzoate was first condensed with 4-chloroquinazoline, the ether was hydrolyzed, and aminoethers **(Ia)** and **(Ib)** were added; at the final stage, hydroxamic acids were obtained (Scheme 2).



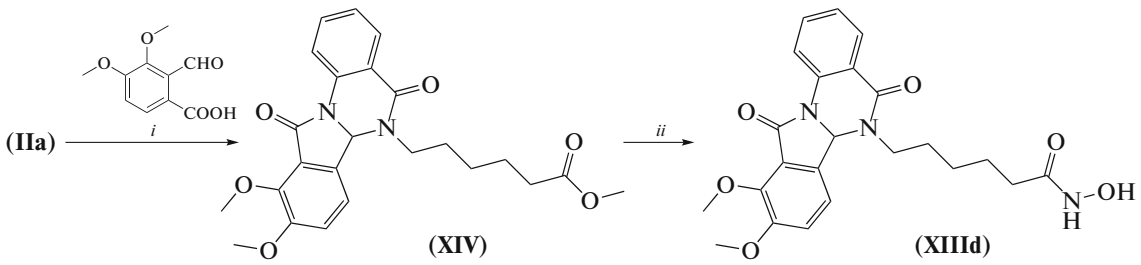
Scheme 2. Reagents and conditions: (*i*) 4-chloroquinazoline/DMF/Δ; (*ii*) LiOH/MeOH/H_2O; (*iii*) **(Ia)** or **(Ib)**/TBTU/HOBt/NMM/DMF; (*iv*) HCl · H_2NOH/MeONa/MeOH.

Compounds **(XIIIa)**–**(XIIIc)** were obtained by the condensation of **(III)** with different carbonyl compounds (Scheme 3). The reaction was carried out in boiling methanol or ethanol in the presence of catalytic amounts of *p*-toluenesulfonic acid (PTSA).



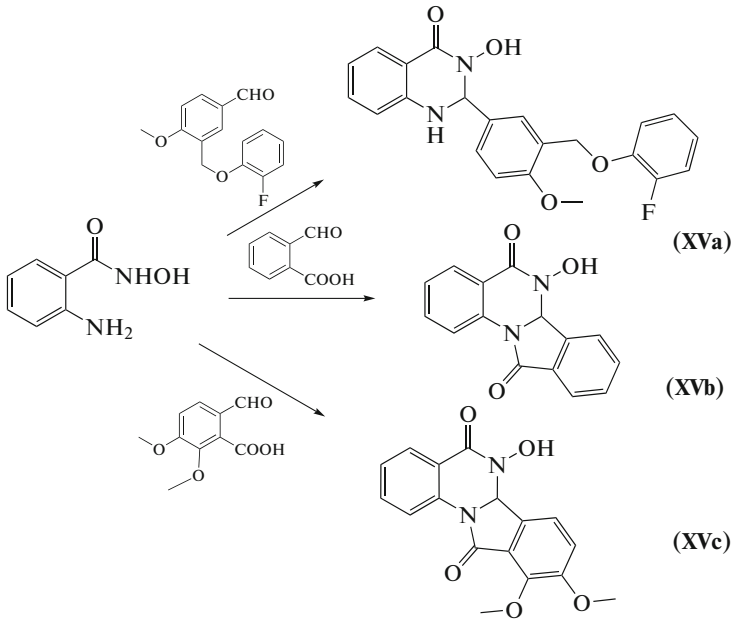
Scheme 3. Reagents and conditions: MeOH or EtOH/PTSA.

Under these conditions, the reaction of 4,5-dimethoxy-2-formylbenzoic acid with compound **(III)** leads to a complex mixture of products. The synthesis was conducted in two stages using compound **(II)** as an initial reagent; in this case, condensed tetracycle **(XIIId)** similar to those described in [16] formed (Scheme 4).



Scheme 4. Reagents and conditions: (*i*) PhCl/PTSA/Δ; (*ii*) HCl · H_2NOH/MeONa/MeOH.

Compounds **(XVa)**–**(XVc)** were obtained by the condensation of anthranilic acid hydroxyamide with different carbonyl compounds; as a result, the derivatives of cyclic hydroxamic acid were obtained (Scheme 5). With the use of 2-formylbenzoic acid derivatives, condensed tetracycles also formed.



Scheme 5. Reagents and conditions: MeOH or PhCl/PTSA/Δ.

Determination of Cytotoxic Activity

VM is differently represented in different tumor types. Thus, the blood supply through VM channels is more than 60% in the case of skin melanoma, 50% in the case of triple negative breast cancer, about 40% in the case of kidney cancer, more than 35% in the case of soft tissue sarcoma, more than 15% in the case of ovary cancer, and about 10% in the case of colon cancer [15]. Against the background of extremely imperfect blood circulation in a tumor, 10–15% of VM channels would hardly significantly change anything; however, in the amount of 40–60%, they clearly may be very important. Based on the above arguments, the cytotoxic activity of compounds was tested on melanoma, breast cancer, and kidney cancer cells.

Compounds **(IV)** and **(XIIIa)**–**(XIIId)** with the IC_{50} value less than 10 μM (Table 1) were chosen for further examination of functional activity.

Other tests (migration and formation of CLS) were performed at a noncytotoxic concentration of compounds, IC_{10} (90% live cells after 24 h). The use of this principle makes it possible to avoid false-positive results, when, e.g., the blocking of migration by 60% relative to the control is associated with the death of cells within 24 h.

Inhibition of Migration Capacity

In recent decades, the efforts of many oncologists were directed toward studying the mechanisms of initiation and progression of malignant tumors. With the passage of time, it became clear that the main threat of cancer is its spread throughout the body. Patients with malignant tumors die not from the original tumor but from metastases, which disorganize the functioning of tissues they damage. Metastasizing, a process of migration of tumor cells from the primary focus with subsequent formation of secondary tumor foci (metastases) is one of the major problems in the therapy of oncological diseases. The migration of tumor cells is currently considered as a key step of metastasizing. A search for preparations inhibiting the movement of tumor cells from the primary focus to adjacent or remote organs is of fundamental importance and can make possible the creation of new antitumor drugs increasing the efficacy of therapy.

The migration of tumor cells was assessed by the "wound-healing" method with some modifications. A cell monolayer was broken by scraping, and the width of the migration area was analyzed 24 h after the addition of compounds **(IV)** and **(XIIIa)**–**(XIIId)**. A monolayer of cells incubated in a culture medium containing 10% FCS served as a positive control. Cells growing in a serum-free medium served as a negative control, which determines the width of a "wound," a cell-free space on the surface of a well. It was shown

Table 1. Cytotoxic activity of compounds toward SN-12C kidney cancer, Mel Z metastatic human melanoma, and MCF-7 breast cancer cells in vitro

Number of compound	SN-12C		Mel Z		MCF-7	
	IC_{50}, μM	IC_{10}, μM	IC_{50}, μM	IC_{10}, μM	IC_{50}, μM	$IC_{10,}$ μM
(IV)	4.7 ± 1.5	2.1 ± 0.5	4.1 ± 0.8	0.9 ± 0.1	9.5 ± 1.58	2.8 ± 1.1
(VIII)	>50	>50	>50	>50	>50	>50
(XIIa)	>50	25.0 ± 3.2	>50	23.0 ± 4.8	>50	28.6 ± 2.7
(XIIb)	41.7 ± 11.1	6.2 ± 0.6	28.7 ± 11.2	6.6 ± 2.7	>50	>50
(XIIIa)	3.8 ± 1.2	1.7 ± 0.2	8.1 ± 0.3	1.2 ± 0.3	7.6 ± 2.8	0.7 ± 0.2
(XIIIb)	4.8 ± 0.8	3.1 ± 0.9	8.9 ± 1.3	>50	7.0 ± 0.1	0.7 ± 0.3
(XIIIc)	1.3 ± 0.6	0.7 ± 0.2	1.9 ± 0.3	0.9 ± 0.3	1.8 ± 0.5	0.7 ± 0.2
(XIIId)	5.7 ± 0.2	1.6 ± 0.3	5.7 ± 1.4	5.5 ± 1.2	24.4 ± 4.6	4.7 ± 1.8
(XVa)	>50	>50	>50	>50	>50	>50
(XVb)	>50	>50	>50	>50	>50	>50
(XVc)	>50	>50	>50	>50	>50	>50
Sunitinib	2.4 ± 0.3	1.0 ± 0.5	2.7 ± 0.4	1.2 ± 0.3	2.9 ± 0.3	1.3 ± 0.2

that compounds **(IV)**, **(XIIIa)**, **(XIIIb)**, and **(XIIId)** at nontoxic concentrations (IC_{10}) block the migration of cells of all three types of tumor cells into the region of the wound by 35 to 65% compared with the control. The target inhibitor VEGFR2 sunitinib (Selleckchem) at a nontoxic concentration of 1 μM, which inhibits migration activity of Mel Z cells by 22.3%, of MCF-7 cells by 28.9%, and of SN-12C cells by 38.5%, served as a reference preparation. Compound **(IV)** exhibited the highest inhibitory activity toward Mel Z melanoma and SN-12C kidney cancer cells: the migration activity was inhibited by 63.5 and 65.4%, respectively (Fig. 1). Compounds **(XIIIa)** and **(XIIId)** inhibited the migration of MCF-7 breast cancer cells by 64.9 and 69.3%, respectively. Compound **(XIIIc)** produced an insignificant inhibitory effect on the migration of melanoma and breast cancer cells (28–30%). With the use of compound **(XIIIc)**, the width of the migration area in the case of kidney cancer cells was comparable with that of the control sample (2.3%). The data on the effect of compounds **(IV)** and **(XIIIa)**–**(XIIId)** on the migration of tumor cells are presented in Table 2. The blocking of migration by compound **(IV)** was maximal in the case of SN-12C kidney cancer cells (65.4%) and Mel Z melanoma cells (63.5%). Compound **(XIIId)** appeared to be an efficient inhibitor of the migration of MCF-7 breast cancer cells (64.9%). Presumably, compounds **(IV)** and **(XIIId)** act on one of regulatory pathways underlying the rearrangement of the actin cytoskeleton of the cell. These results led us to the conclusion that it is advisable to create new analogs based on compound **(IV)** and, probably, compound **(XIIId)** as potential inhibitors of the migration of tumor cells.

Inhibition of Formation of CLS

The generation of vascular channels by tumor cells involves a number of successive events: migration of cells, recognition of homotypic cells, formation of cell–cell contacts, cell sprouting, and formation of homotypic structures similar to honey-like combs. The formation of CLS by tumor cells in a 3D culture serves as a test for VM in vitro [17]. In our studies, Matrigel, a lyophilized natural extracellular gel was used as a gel matrix. As noted above, compounds **(IV)** and **(XIIId)** produced the most pronounced inhibitory effect on cell migration. These compounds should also block CLS to one degree or another, since migration is the basic stage of VM. However, the other three compounds could also participate in the multistage VM process. Therefore, we examined the effect of all five compounds on the formation of CLS in the 3D culture. In the control on Matrigel, three types of selected tumor cells formed CLS. In melanoma cells growing in the presence of compounds **(IV)** and **(XIIIa)**, the formation of CLS was inhibited by 54.4 and 47.4%, respectively. Sunitinib inhibited the formation of a network of Mel Z cells; the total length of CLS was 64.3% of the control. On Matrigel, along with the formation of small clusters of cells, islets of the network characteristic of VM were seen. Compounds **(XIIIb)**–**(XIIId)** very little affected the ability of melanoma cells to participate in VM. Compound **(IV)** produced an unexpectedly strong effect on the formation of CLS by SN-12C kidney cancer cells; it inhibited the formation of CLS by 72.7% (Fig. 2). Compounds **(XIIIa), (XIIIb)**, and **(XIIId)** inhibited the formation of VLS by the same cells from 56.4 to 5.3%. Sunitinib induced the blocking of CLS from SN-12C cells by 47.2% of the control (Table 3). It should be noted that none of

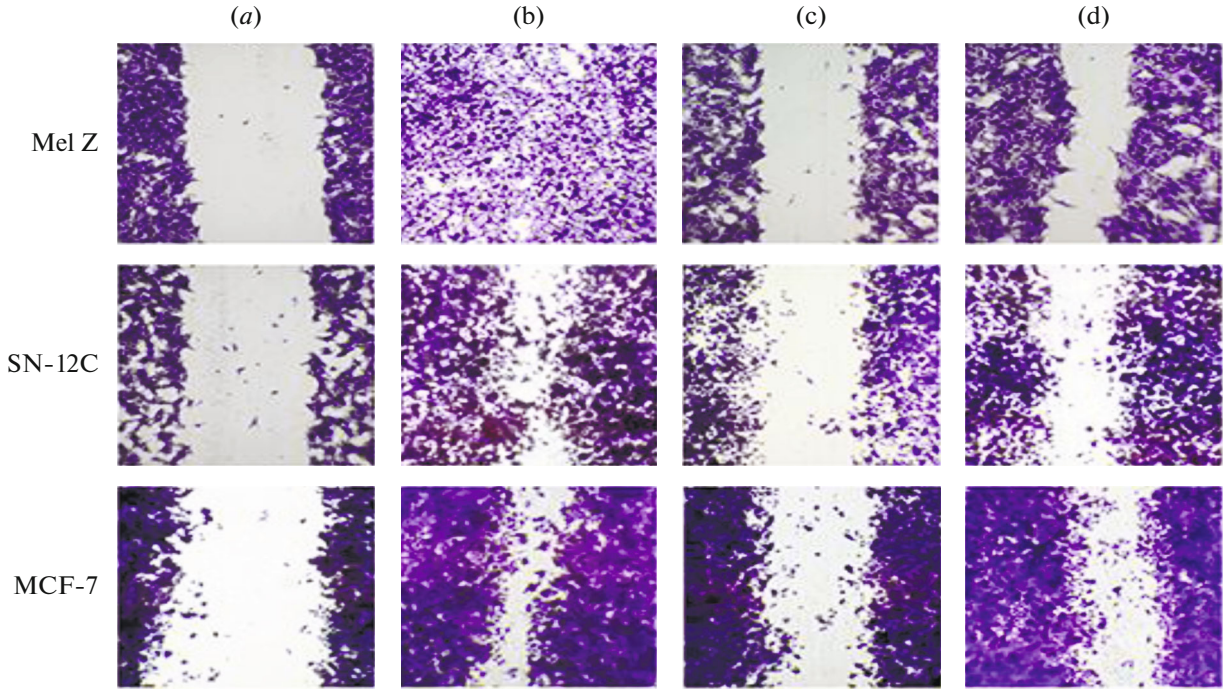


Fig. 1. Inhibition of the migration of melanoma Mel Z, kidney cancer SN-12C, and breast cancer MCF-7 cells (staining with trypan blue). (a) Negative control: wound width; (b) positive control: an increment in the cell monolayer growth in complete medium after 24 h; (c) an increment in the cell monolayer growth in complete medium in the presence of compound (**IV**) at IC_{10} after 24 h; (d) an increment in the cell monolayer growth in complete medium in the presence of sunitinib at IC_{10} after 24 h.

the five compounds affected the formation of CLS by breast cancer cells (data not shown).

Thus, new hydroxamic acid derivatives coupled with the quinazoline cycle were synthesized. Their effect on the migration of tumor cells and formation of CLS was examined. We showed using the wound-healing method that compound (**IV**) markedly blocks the migration of melanoma, breast cancer, and kidney cancer cells. In addition, in the presence of compound (**IV**) the capacity of kidney cancer cells for communication, which is necessary for the formation of CLS, significantly decreased. In metastatic melanoma cells, this effect was somewhat less pronounced. None of the five compounds had significant effect on the formation of CLS by breast cancer cells. The formation of VM is a complex biological process, which, along with the migration and homotypic recognition of cells, involves several signaling pathways. Presumably, compound (**IV**) is involved, in some or another way, in driver signaling pathways that control VM.

EXPERIMENTAL

All reagents and solvents were commercially available products and were used without additional purification. NMR spectra were recorded on a Bruker AVANCE III NanoBay Fourier NMR spectrometer (300 MHz for ^{1}H NMR and 76 MHz for ^{13}C NMR). Spectra were recorded in the deuterium stabilization

Table 2. Inhibition of migration activity of tumor cells by the action of hydroxamic acid derivatives at a concentration of IC_{10} in Mel Z metastatic human melanoma, SN-12C kidney cancer, and MCF-7 breast cancer cells

Compound OVFV	Inhibition of migration of tumor cells (% of cells without compounds)		
	SN-12C	Mel Z	MCF-7
(IV)	65.4	63.5	55.8
(XIIIa)	58.6	48.5	64.9
(XIIIb)	14.1	35.5	54.1
(XIIIc)	2.3	34.1	28.0
(XIIId)	45.7	62.0	69.4
Sunitinib	38.5	22.3	28.9

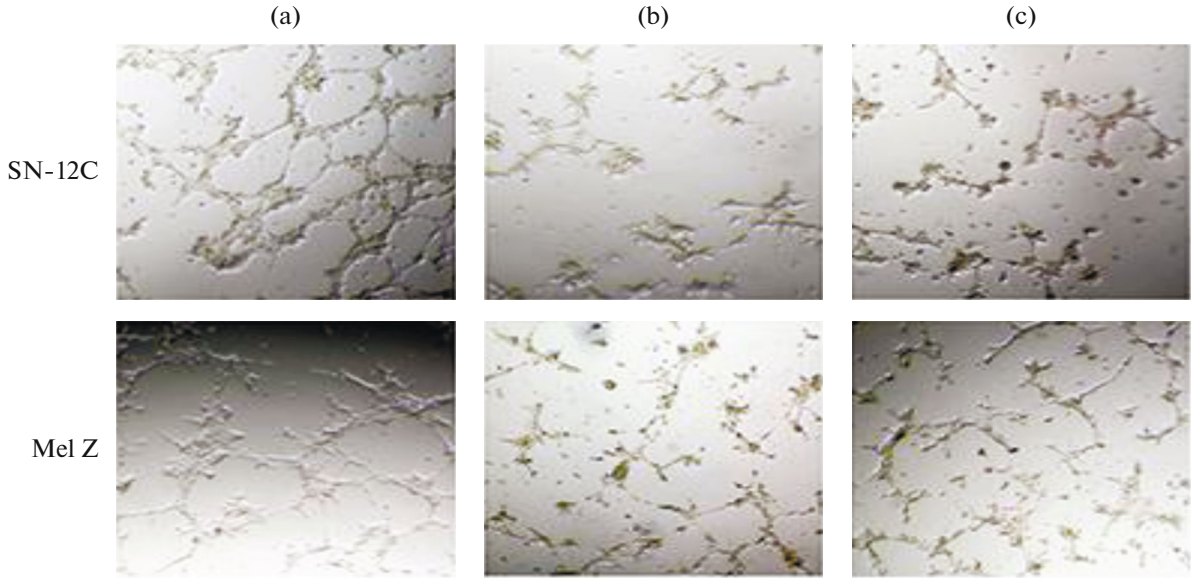


Fig. 2. Effect of compound (**IV**) on the formation of CLS on Matrigel by SN-12C kidney cancer cells and Mel Z melanoma cells. (a) Cells in complete medium RPMI-1640; (b) cells in complete medium RPMI-1640 in the presence of compound **(IV)** at IC_{10}, (c) cells in complete medium RPMI-1640 in the presence of sunitinib at IC_{10}.

mode; thermal stabilization at 25°C, with tetramethylsilane in DMSO-d_6 as an internal standard. Chemical shifts are given in ppm (δ), and the spin-spin coupling constants are in Hz. Electrospray ionization mass spectra (ESI-MS) were recorded on an Agilent LC/MS 1200 chromatographic system equipped with an Agilent Ion Trap 6310 mass detector. High-resolution mass spectra were measured in the electrospray ionization mode using an HPLC-MS analytical station Agilent Infinity 1260/Thermo Scientific Orbitrap Fusion Lumos. Melting points were determined in an open capillary on a Mettler Toledo MP 90 melting point analyzer.

Methyl (6-aminohexanoate) hydrochloride (Ia). Thionyl chloride (6.0 g, 50 mmol) was added dropwise at −5°C to methanol (50 mL). Then, 6-aminohexanoic acid (6.6 g, 25 mmol) was added, and the mixture was stirred for 3 h at room temperature. The solvent was removed in vacuum, methanol (20 mL) was added, and the solvent was removed once again. The product was obtained as white crystals. Yield 6.9 g (95.0%). ^{1}H NMR: 8.17 (s, 3H), 3.57 (s, 3H), 2.71 (t, *J* 7.4, 2H), 2.28 (t, *J* 7.4, 2H), 1.65–1.42 (m, 4H), 1.36–1.20 (m, 2H). ESI-MS, *m/z*: 146.1 $[M + H]^+$.

Methyl (7-aminoheptanoate) hydrochloride (Ib) was obtained from 7-aminoheptanoic acid in a similar way. Yield 54.2%; white crystals. ^{1}H NMR: 8.11 (br s, 3H), 3.57 (s, 3H), 2.77–2.65 (m, 2H), 2.29 (t, 2H, *J* 7.4), 1.61–1.44 (m, 4H), 1.36–1.18 (m, 4H). ESI-MS, *m/z* 160.1 $[M + H]^+$.

Methyl *trans*-4-aminomethylcyclohexan-1-carboxylate hydrochloride (V) was obtained in a similar way from *trans*-4-aminomethylcyclohexane carboxylic acid (tranexamic acid) as white crystals. Yield 93.4%. ^{1}H NMR: 8.14 (br s, 3H), 3.57 (s, 2H), 2.60 (d, 2H, *J* 6.9), 2.24 (tt, 1H, *J* 12.2, 3.5), 1.97–1.75 (m, 4H), 1.64–1.47 (m, 1H), 1.28 (qd, 2H, *J* 12.9, 3.2), 0.97 (qd, 2H, *J* 12.9, 3.3). ESI-MS, *m/z* 172.1 $[M + H]^+$.

Methyl 6-(2-aminobenzamido)hexanoate (II). Methyl ether of 6-aminohexanoic acid (15 g, 83 mmol) was dissolved in water (20 mL), and the pH of the solution was brought to 7–8 with a 10% sodium hydrocarbonate solution. The resulting solution was poured into a suspension of isatoic anhydride (10 g, 61 mmol) in dimethylformamide (DMF) (100 mL) in a flat-bottom flask of volume 250 mL, and the resulting mixture was stirred for 2 h at room temperature. The mixture was poured into water (200 mL), and the pH of the solution was brought to 3–4 with a 10% citric acid solution. After extraction with sulfuric ether (2 × 100 mL), the organic layer was washed with water (50 mL). The solvent was distilled off, and the residue was crystal-

Table 3. Formation of CLS by the action of hydroxamic acid derivatives

Compound OVFV	Length of CLS within the network (% of control)	
	SN-12C	Mel Z
(IV)	27.3	45.6
(XIIIa)	43.6	52.6
(XIIIb)	71.8	77.2
(XIIIc)	94.7	80.3
Sunitinib	47.2	64.3

lized from hexane (50 mL). Yield 15.0 g (92%); white crystals; mp 53–55°C. ^{1}H NMR: 8.16 (t, 1H, *J* 5.7), 7.46 (dd, 1H, *J* 7.9, 1.5), 7.15–7.08 (m, 1H), 6.68 (dd, 1H, *J* 8.3, 1.2), 6.36 (br s, 2H, NH_2), 3.58 (s, 3H), 3.26–3.13 (m, 2H), 2.30 (t, 2H, *J* 7.4), 1.64–1.42 (m, 4H), 1.39–1.22 (m, 2H). ESI-MS, *m/z* 265.1 $[M + H]^+$.

Methyl 4-((2-aminobenzamido)methyl)cyclohexan-1-carboxylate (VI) was obtained in a similar way from the methyl ester of tranexamic acid. Yield 14.3 g (89%); white crystals; mp 100–103°C. ^{1}H NMR: 8.17 (t, 1H, *J* 5.8), 7.47 (dd, 1H, *J* 8.0, 1.5), 7.15–7.08 (m, 1H), 6.67 (dd, 1H, *J* 8.2, 1.2), 6.54–6.46 (m, 1H), 6.36 (br s, 2H, NH_2), 3.58 (s, 3H), 3.06 (t, 2H, *J* 6.3), 2.33–2.17 (m, 1H), 1.90 (dd, 2H, *J* 13.4, 3.5), 1.77 (dd, 2H, *J* 13.2, 3.4), 1.59–1.40 (m, 1H), 1.28 (qd, 2H, *J* 13.0, 3.3), 0.96 (qd, 2H, *J* 12.9, 3.4). ESI-MS, *m/z* 292.2 $[M + H]^+$.

2-Amino-*N*-(6-(hydroxyamino)-6-oxohexyl)benzamide (III). A solution of hydroxylamine in methanol (30 mL) obtained from sodium (1.15 g, 0.05 mol) and hydroxylamine hydrochloride (2.10 g, 0.03 mol) was added to a solution of compound (**II**) (2.64 g, 0.01 mol) in methanol (30 mL). The reaction mixture was stirred for 6 h at room temperature. Methanol was distilled off in vacuum, water (20 mL) was added, and the solution was acidified to the pH 4 with 5% citric acid. The precipitate was filtered and washed with water (10 mL). Yield 2.20 g (83.3%); white crystals; mp 125–126°C. ^{1}H NMR: 10.28 (s, 1H, NH–O<u>H</u>), 8.60 (s, 1H, N<u>H</u>–OH) 8.11 (t, 1H, $CH_2N\underline{H}$, *J* 5.6), 7.44 (dd, 1H, Ar, *J* 8.0, 1.5), 7.14–7.08 (m, 1H, Ar), 6.67 (dd, 1H, Ar, *J* 8.2, 1.2), 6.55–6.45 (m, 1H, Ar), 6.30 (br s, 2H, NH_2), 3.19 (td, 2H, $NC\underline{H}_2$, *J* 7.5, 5.6), 1.95 (t, 2H, $COC\underline{H}_2$, *J* 7.5), 1.51 (m, 4H, $2C\underline{H}_2$), 1.28 (p, 2H, $C\underline{H}_2$, *J* 7.5). ESI-MS, *m/z* 266.2 $[M + H]^+$.

4-((2-Aminobenzamido)methyl)cyclohexan-1-hydroxyaminocarbamide (VII) was obtained from compound (**VI**) (164 mg, 1 mmol) in a similar way as compound (**III**). Yield 97.4%; white crystals. ^{1}H NMR: 10.33 (s, 1H, NH–O<u>H</u>), 8.62 (s, 1H, N<u>H</u>–OH), 8.16 (t, 1H, *J* 5.5), 7.46 (dd, 1H, Ar, *J* 7.9, 1.1), 7.12 (td, 1H, Ar, *J* 8.2, 1.4), 6.67 (dd, 1H, Ar, *J* 8.1, 1.3), 6.50 (td, 1H, Ar, *J* 8.0, 1.1), 6.34 (br s, 2H, NH_2), 3.05 (t, 2H, $C\underline{H}_2$, *J* 5.5), 2.31–2.14 (m, 1H), 1.88 (dd, 2H, $C\underline{H}_2$, *J* 13.2, 3.5), 1.75 (dd, 2H, $C\underline{H}_2$, *J* 13.2, 3.5), 1.57–1.41 (m, 1H), 1.27 (qd, 2H, $C\underline{H}_2$, *J* 13.0, 3.3), 0.95 (qd, 2H, $C\underline{H}_2$, *J* 12.9, 3.4). ESI-MS, *m/z* 290.3 $[M + H]^+$.

***N*-(6-(Hydroxyamino)-6-oxohexyl)-2-(quinazolin-4-ylamino)benzamide (IV)**. A solution of compound (**III**) (265 mg, 1 mmol) in DMF (5 mL) was added to a solution of 4-chloroquinazoline (164 mg, 1 mmol) in DMF (5 mL). The reaction mixture was stirred for 30 min at 40–50°C and cooled to room temperature. The sediment was filtered and washed with DMF (5 mL) and sulfuric ether (2 × 10 mL). Yield 384 mg (97.6%); light beige crystals; mp 180.8–187.5°C. ^{1}H NMR: 12.60 (s, 1H, N<u>H</u>–O<u>H</u>), 10.33 (s, 1H, N<u>H</u>–OH), 8.98 (d, 1H, NH, *J* 1.2), 8.90 (t, 1H, $CH_2N\underline{H}$, *J* 5.6), 8.72 (s, 1H, Ar), 8.66 (s, 1H, Ar), 8.17 (d, 1H, Ar, *J* 8.2), 7.95–7.81 (m, 3H, Ar), 7.75 (t, 1H, Ar, *J* 7.5), 7.60 (t, 1H, Ar, *J* 7.4), 7.19 (td, 1H, Ar, *J* 7.6, 1.2), 3.18 (td, 2H, *J* 6.9, 4.7), 1.94 (t, 2H, $COC\underline{H}_2$, *J* 7.3), 1.62–1.45 (m, 4H, $C\underline{H}_2$), 1.38–1.21 (m, 2H, $C\underline{H}_2$). ^{13}C NMR: 169.58, 169.24, 169.17, 157.23, 154.73, 149.78, 140.26, 140.11, 133.73, 132.38, 128.72, 128.55, 127.82, 122.73, 121.89, 121.34, 115.97, 32.65, 28.99, 26.54, 25.32. HRMS: *m/z* 394.1874 $[M]^+$. Calculated for $(C_{21}H_{23}N_5O_3)^+$: 394.1877.

***N*-((4-(Hydroxycarbamoyl)cyclohexyl)methyl)-2-(quinazolin-4-ylamino)-benzamide (VIII)** was obtained from 4-chloroquinazoline (164 mg, 1 mmol) and compound (**VII**) (291 mg, 1 mmol) in a similar way as compound (**IV**). Yield 97.4%; white crystals; mp 200.6–201.8°C. ^{1}H NMR: 12.48 (s, 1H, NH–O<u>H</u>), 10.32 (s, 1H, N<u>H</u>–OH), 8.94 (d, 1H, NH, *J* 1.2), 8.87 (t, 2H, $CH_2N\underline{H}$, *J* 5.0), 8.71 (s, 1H, Ar), 8.62 (s, 1H, Ar), 8.16 (d, 1H, Ar, *J* 7.8), 7.95–7.80 (m, 3H, Ar), 7.75 (t, 1H, Ar, *J* 7.2), 7.60 (t, 1H, Ar, *J* 7.8), 7.20 (t, 1H, Ar, *J* 7.6), 3.18 (t, 2H, $C\underline{H}_2$, *J* 6.3), 2.01–1.85 (m, 1H, CH), 1.79 (d, 2H, $C\underline{H}_2$, *J* 12.7), 1.64 (d, 2H, $C\underline{H}_2$, *J* 11.8), 1.34 (q, 2H, $C\underline{H}_2$, *J* 12.6), 0.95 (q, 2H, $C\underline{H}_2$, *J* 12.4). ^{13}C NMR: 172.23, 172.15, 168.89, 156.87, 156.78, 154.36, 149.54, 139.72, 139.56, 133.22, 131.90, 128.33, 128.26, 127.29, 122.31, 121.38, 115.52, 45.33, 45.21, 41.18, 36.77, 29.67, 28.62. HRMS: *m/z* 420.2030 $[M]^+$. Calculated for $(C_{23}H_{25}N_5O_3)^+$: 420.2034.

4-(Quinazolin-4-ylamino)benzoic acid (X). A solution of methyl 4-aminobenzoate (300 mg, 2 mmol) in DMF (5 mL) was added to a solution of 4-chloroquinazoline (230 mg, 2 mmol) in DMF (5 mL). The reaction mixture was stirred for 30 min at 40–50°C and cooled to room temperature. The precipitate was filtered and washed with DMF (5 mL) and sulfuric ether (2 × 10 mL). To the resulting compound (**IX**), a solution of NaOH (120 mg, 3 mmol) in a mixture of water (5 mL) and methanol (10 mL) was added. The mixture was refluxed for 2 h, water (10 mL) was added, and the solution was acidified with 5% citric acid to the pH 4. The sediment was filtered and washed with water (10 mL). Yield 416 mg (78.5%); white crystals; mp 296.1–299.2°C (with decomposition). ^{1}H NMR: 10.67 (s, 1H, OH), 8.85 (s, 1H, NH), 8.68 (d, 1H, Ar, *J* 8.3), 8.06–7.97 (m, 5H, Ar), 7.87 (d, 1H, Ar, *J* 7.2), 7.79 (t, 1H, Ar, *J* 7.7).

Methyl 6-((4-(quinazolin-4-ylamino)benzoyl)amino)-hexanoate (XIa). Compound (**X**) (530 mg, 2 mol) and thereafter methyl 6-amino hexanoate hydrochloride (362 mg, 2 mmol) were added to a solution of TBTU (642 mg, 2 mmol), hydroxybenztriazole hydrate (304 mg, 2 mmol), and *N*-methylmorpholine (606 mg, 6 mmol) in DMF (20 mL). The mixture was stirred for 5 h at room temperature, water (30 mL) was added,

and the extraction with ethyl acetate (2 × 25 mL) was carried out. The organic layer was washed with 5% citric acid (20 mL), water (10 mL), a 5% sodium bicarbonate solution (10 mL), and water (10 mL). The solvent was distilled off in vacuum. Yield 576 g (73.5%); beige crystals. ESI-MS, *m/z* 393.2 $[M + H]^+$. The product was used in further syntheses without additional purification.

Methyl 7-((4-(quinazolin-4-ylamino)benzoyl)amino)-heptanoate (XIb) was obtained in a similar way as compound **(XIa)** using methyl 7-aminoheptanoate hydrochloride (390 mg, 2 mmol). Yield 610 mg (75.1%). ESI-MS, *m/z* 407.3 $[M + H]^+$. The product was used in further syntheses without additional purification.

***N*-(6-(Hydroxyamino)-6-oxohexyl)-4-(quinazolin-4-ylamino)benzamide (XIIa)**. Hydroxylamine hydrochloride (208 mg, 3.00 mmol) was added to a solution of sodium methylate obtained by dissolving sodium (92 mg, 4.00 mmol) in methanol (10 mL) at 0°C, the mixture was stirred for 20 min at 0°C, and compound **(XIa)** (392 mg, 1 mmol) was added. The reaction mixture was stirred for 5 h at room temperature (TLC control), water (10 mL) was added, the mixture was acidified with 5% hydrochloric acid to the pH 4–5, and methanol was distilled off. The precipitate was filtered, washed with water, and air-dried. Yield 320 mg (81.2%); white crystals; mp 212.9°C. ^{1}H NMR: 10.34 (s, 1H, NH–O<u>H</u>), 9.94 (s, 1H, N<u>H</u>–OH), 8.67 (br s, 2H, NH + Ar), 8.59 (d, 1H, Ar, *J* 8.3), 8.37 (t, 1H, $CH_2N\underline{H}$, *J* 5.5), 8.01 (d, 2H, Ar, *J* 8.5), 7.93–7.79 (m, 4H, Ar), 7.67 (t, 1H, Ar, *J* 7.5), 3.30–3.19 (m, 1H, $C\underline{H}_2NH$), 1.96 (t, 2H, $COC\underline{H}_2$, *J* 7.3), 1.61–1.45 (m, 4H, CH_2), 1.38–1.20 (m, 2H, CH_2). HRMS: *m/z* 394.1800 $[M]^+$. Calculated for $(C_{21}H_{23}N_5O_3)^+$: 394.1877.

***N*-(7-(Hydroxyamino)-7-oxoheptyl)-4-(quinazolin-4-ylamino)benzamide (XIIb)** was obtained from compound **(XIb)** (406 mg, 1 mmol) in a similar way as compound **(XIIa)**. Yield 310 mg (76.1%); white crystals; mp 237.8–238.3°C. ^{1}H NMR: 10.34 (s, 1H, NHO<u>H</u>), 9.95 (s, 1H, N<u>H</u>OH), 8.66 (br s, 2H, NH + Ar), 8.60 (d, 1H, Ar, *J* 8.4), 8.37 (t, 1H, $CH_2N\underline{H}$, *J* 5.6), 8.01 (d, 2H, Ar, *J* 8.4), 7.93–7.79 (m, 4H, Ar), 7.67 (t, 1H, Ar), 3.30–3.18 (m, 2H, CH_2), 1.95 (m, 2H, CH_2, *J* 7.3), 1.60–1.41 (m, 4H, $2CH_2$), 1.39–1.24 (m, 4H, $2CH_2$). ^{13}C NMR: 169.19, 165.62, 157.49, 154.33, 149.74, 141.72, 133.21, 129.35, 127.88, 127.63, 126.47, 123.08, 121.00, 115.22, 32.25, 29.13, 28.39, 26.28, 25.12. HRMS: *m/z* 408.2030 $[M]^+$. Calculated for $(C_{22}H_{25}N_5O_3)^+$: 408.2034.

***N*-Hydroxy-6-(2-(4-chlorophenyl)-4-oxo-1,2-dihydroquinazolin-3(*4H*)-yl)hexamide (XIIIa)**. A solution of compound **(III)** (265 mg, 1 mmol) and 4-chlorobenzaldehyde (147 mg, 1.05 mmol) in ethanol (5 mL) was stirred for 6 h under boiling in an atmosphere of argon, and the solvent was distilled off in vacuum. The resulting solid residue was triturated with diethyl ether (5 mL) and air-dried. Yield 289 mg (74.8%); mp 111.4–112.3°C. ^{1}H NMR: 10.31 (s, 1H, N<u>H</u>–OH), 7.63 (d, 1H, Ar, *J* 7.4), 7.45–7.29 (m, 4H, Ar), 7.19 (t, 1H, Ar, *J* 7.6), 6.70–6.57 (m, 2H, Ar), 5.86 (s, 1H, N–C<u>H</u>–N), 3.96–3.79 (m, 1H, $NC\underline{H}_2$), 2.79–2.64 (m, 1H, $NC\underline{H}_2$), 1.91 (t, 2H, $COC\underline{H}_2$, *J* 7.3), 1.61–1.37 (m, 4H, $2CH_2$), 1.33–1.16 (m, 2H, $CH_2C\underline{H}_2$–CO). HRMS: *m/z* 388.1422 $[M]^+$. Calculated for $(C_{20}H_{22}ClN_3O_3)^+$: 388.1426.

***N*-Hydroxy-6-(2-(3-methoxyphenyl)-4-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)hexamide (XIIIb)**. A solution of compound **(III)** (265 mg, 1 mmol) and 4-chlorobenzaldehyde (147 mg, 1.05 mmol) in ethanol (5 mL) was stirred for 6 h under boiling in an atmosphere of argon, and the solvent was distilled off in vacuum. The resulting solid residue was triturated with diethyl ether (5 mL) and air-dried. Yield 299 mg (78.1%); mp 119.0–120.2°C. ^{1}H NMR: 10.31 (s, 1H, N<u>H</u>–OH), 7.66 (dd, 1H, Ar, *J* 7.9, 1.6), 7.28 (td, 1H, Ar, *J* 8.2, 1.8), 7.18 (td, 1H, Ar, *J* 7.6, 1.4), 7.13 (d, 1H, Ar, *J* 7.6), 7.05 (d, 1H, Ar, *J* 8.2), 6.86 (t 1H, Ar, *J* 7.5), 6.70–6.60 (m, 2H, Ar), 6.14 (s, 1H, N–C<u>H</u>–N), 3.96–3.82 (m, 1H, $NC\underline{H}_2$), 3.70 (s, 3H, $OC\underline{H}_3$), 2.80–2.63 (m, 1H, $NC\underline{H}_2$), 1.91 (t, 2H, CO–$\underline{CH}_2$, *J* 7.3), 1.63–1.40 (m, 4H, $2CH_2$), 1.37–1.17 (m, 2H, CH_2–$C\underline{H}_2$–CO). ^{13}C NMR: 169.09, 162.22, 159.31, 146.18, 142.77, 133.13, 129.64, 127.34, 118.14, 117.15, 115.03, 114.22, 113.25, 112.36, 69.81, 55.05, 44.29, 32.17, 27.22, 25.94, 24.85. HRMS: *m/z* 384.1918 $[M]^+$. Calculated for $(C_{21}H_{25}N_3O_4)^+$: 384.1922.

***N*-Hydroxy-6-(2-(4-methoxy)-3-(2-fluorophenoxy)-methylphenyl)-4-oxo-1,2-dihydroquinazolin-3(4*H*)-yl)-hexamide (XIIIc)**. A solution of compound **(III)** (159 mg, 0.60 mmol) and 4-methoxy-3-(2-fluorophenoxy)methylbenzaldehyde (164 mg, 0.63 mmol) in methanol (5 mL) was stirred for 6 h under boiling in an atmosphere of argon, and the solvent was distilled off in vacuum. The resulting solid residue was triturated with diethyl ether (5 mL) and air-dried. Yield 301 mg (98.8%); mp 102.8–104.7°C. ^{1}H NMR: 10.30 (s, 1H, N<u>H</u>–OH), 7.61 (dd, 1H, Ar, *J* 7.8, 1.6), 7.42 (d, 1H, Ar, *J* 2.3), 7.32–7.06 (m, 6H, Ar), 7.03 (d, 1H, Ar, *J* 8.6), 6.98–6.88 (m, 1H, Ar), 6.67–6.58 (m, 2H, Ar), 5.79 (s, 1H, N–C<u>H</u>–N), 5.06 (s, 2H, $OC\underline{H}_2$), 3.88–3.80 (m, 2H, $NC\underline{H}_2$), 3.79 (s, 3H, $OC\underline{H}_3$), 2.74–2.58 (m, 1H, $NC\underline{H}_2$), 1.90 (t, 2H, CO–$C\underline{H}_2$, *J* 7.3), 1.60–1.33 (m, 4H, $2C\underline{H}_2$), 1.25–1.11 (m, 2H, $2CH_2$). ^{13}C NMR: 169.11, 162.21, 157.04, 153.46, 150.24, 146.28, 146.23, 146.15, 133.06, 132.96, 127.41, 127.35, 124.78, 124.74, 124.23, 121.31, 121.22, 117.08, 116.15, 115.92, 115.42, 114.98, 114.19, 110.94, 69.64, 65.66, 55.68, 44.04, 32.19, 27.12, 25.97, 24.83. HRMS: *m/z* 508.2242 $[M]^+$. Calculated for $(C_{28}H_{30}FN_3O_5)^+$: 508.2246.

Methyl 6-(9,10-dimethoxy-5,11-dioxoindolo[2,1-*a*]quinazolin-6(5*H*,6*aH*,11*H*)-yl)hexanoate (XIV). A solution of compound (**IIa**) (264 mg, 1.0 mmol) and 2,3-dimethoxy-6-formylbenzoic acid (242 mg, 1.15 mmol) in chlorobenzene (5 mL) in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate was refluxed for 4 h and cooled to room temperature after which diethyl ether (10 mL) was added. The precipitate was filtered and air-dried. Yield 392 mg (89%); white crystals; mp 123.9–124.4°C. ^{1}H NMR: 8.04–7.90 (m, 2H, Ar), 7.75–7.64 (m, 1H, Ar), 7.60 (d, 1H, Ar, *J* 8.3), 7.46 (d, 1H, Ar, *J* 8.3), 7.36 (t, 1H, Ar, *J* 7.6), 6.40 (s, 1H, NC<u>H</u>N), 3.92 (s, 3H, OC<u>H</u>$_3$), 3.91 (s, 3H, OC<u>H</u>$_3$), 3.71–3.63 (m, 2H, NC<u>H</u>$_2$), 3.56 (s, 3H, CO_2C<u>H</u>$_3$), 2.22 (t, 2H, C<u>H</u>$_2$, *J* 7.3), 1.56–1.35 (m, 4H, 2C<u>H</u>$_2$), 1.34–1.07 (m, 2H, C<u>H</u>$_2$). ESI-MS, *m/z* 439.5 $[M + H]^+$.

6-(9,10-Dimethoxy-5,11-dioxoisoindolo[2,1-*a*]-quinazolin-6(5*H*,6*aH*,11*H*)-yl)-*N*-hydroxyhexanamide (XIIId). Hydroxylamine hydrochloride (139 mg, 2 mmol) was added to a solution of sodium methylate obtained by dissolving sodium (69 mg, 3 mmol) in methanol (7 mL) at 0°C. The mixture was stirred at 0°C for 20 min after which compound (**XIV**) (378 mg, 0.86 mmol was added. The reaction mixture was stirred for 3 h at room temperature (TLC control), water was added (5 ml), the solution was acidified with 5% hydrochloric acid to a pH value of 4–5, and methanol was evaporated. The precipitate was filtered, washed with water, and air-dried. Yield 200 mg (52.9%); white crystals; mp 136.1–141.8°C. ^{1}H NMR: 10.31 (s, 1H, N<u>H</u>OH), 8.65 (s, 1H, Ar), 7.97 (d, 1H, Ar, *J* 6.9), 7.94 (d, 1H, Ar, *J* 7.3), 7.68 (t, 1H, Ar, *J* 7.9), 7.59 (d, 1H, Ar, *J* 8.3), 7.47 (d, 1H, Ar, *J* 8.3), 7.36 (t, 1H, Ar, *J* 7.6), 6.40 (s, 1H, NC<u>H</u>N), 3.92 (s, 3H, OC<u>H</u>$_3$), 3.91 (s, 3H, OC<u>H</u>$_3$), 3.79–3.54 (m, 2H, NC<u>H</u>$_2$), 1.88 (t, 2H, COC<u>H</u>$_2$, *J* 7.3), 1.54–1.36 (m, 4H, 2C<u>H</u>$_2$), 1.34–1.10 (m, 2H, C<u>H</u>$_2$). ^{13}C NMR: 169.05, 162.71, 162.58, 153.83, 147.09, 136.51, 133.07, 130.75, 128.32, 124.99, 123.82, 121.64, 120.46, 120.24, 117.53, 68.85, 61.84, 56.44, 41.97, 32.13, 27.66, 25.80, 24.80. HRMS: *m/z* 440.1816 $[M]^+$. Calculated for $(C_{23}H_{25}N_3O_6)^+$: 440.1819.

3-Hydroxy-2-(3-(4-methoxy-(2-fluorophenoxy)-methyl)-phenyl)-2,3-dihydroquinazolin-4(1*H*)-one (XVa). A mixture of 2-aminobenzhydroxamic acid (0.30 g, 2 mmol) and 3-((2-fluorophenoxy)methyl)-4-methoxybenzaldehyde (0.52 g, 2 mmol) in methanol (7 mL) in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate was refluxed for 4 h in an atmosphere of argon, cooled to –15°C, and allowed to stand for 8 h. The precipitate was filtered, the filtrate was evaporated in vacuum, and the resulting residue was triturated with diethyl ether (10 mL) and air-dried to yield 0.62 g (79%) of the product as flesh-colored crystals; mp 209.7°C (with decomposition). ^{1}H NMR: 9.64 (s, 1H, NO<u>H</u>), 7.65 (dd, 1H, Ar, *J* 8.0, 1.6), 7.54 (d, 1H, N<u>H</u>, *J* 2.3), 7.41 (dd, 1H, Ar, *J* 8.5, 2.3), 7.36 (d, 1H, *J* 1.8), 7.28–7.15 (m, Ar, 4H), 7.15–7.03 (m, Ar, 2H), 7.01–6.88 (m, Ar, 1H), 6.76–6.63 (m, Ar, 2H), 5.87 (d, 1H, *J* 1.7), 5.07 (s, 2H, C<u>H</u>$_2$), 3.81 (s, 3H). ^{13}C NMR: 162.94, 157.35, 153.41, 150.18, 146.48, 146.35, 146.22, 134.59, 133.35, 131.74, 131.70, 129.50, 128.52, 128.39, 127.28, 127.19, 127.10, 124.85, 124.80, 121.31, 121.23, 121.15, 117.34, 114.06, 110.75, 74.60, 65.80, 55.74. HRMS: *m/z* 395.1402 $[M]^+$. Calculated for $(C_{22}H_{19}FN_2O_4)^+$: 395.1405.

6-Hydroxy-6,6*a*-dihydroisoindolo[2,1-*a*]quinazolin-5,11-dione (XVb). A solution of anthranilic acid hydroxyamide (0.45 g, 3.30 mmol) and 2-formylbenzoic acid (0.50 g, 3.30 mmol) in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate in chlorobenzene (13 mL) was refluxed for 7 h, and the reaction mixture was evaporated to dryness and triturated with diethyl ether (10 mL). The precipitate was filtered and air-dried to a constant weight. Yield 0.77 g (96%); white crystals; mp 198–199ºC. ^{1}H NMR: 9.41 (s, 1H, NO<u>H</u>), 8.08 (dd, 1H, Ar, *J* 8.2, 0.6), 7.97 (dd, 1H, Ar, *J* 7.8, 1.6), 7.89 (br d, 2H, Ar, *J* 8.1), 7.79 (td, 1H, Ar, *J* 7.4, 1.3), 7.74–7.64 (m, 2H, Ar), 7.35 (td, 1H, Ar, *J* 7.6, 1.1), 6.52 (s, 1H, NC<u>H</u>N). ^{13}C NMR: 165.26, 164.88, 139.55, 136.41, 133.68, 133.22, 131.27, 130.58, 128.22, 126.72, 125.24, 123.85, 120.00, 119.25, 72.72. HRMS: *m/z* 267.0764 $[M]^+$. Calculated for $(C_{15}H_{10}N_2O_3)^+$: 267.0769.

6-Hydroxy-9,10-dimethoxy-6,6*a*-dihydroisoindolo-[2,1-*a*]quinazolin-5,11-dione (XVc). A solution of anthranilic acid amide (0.45 g, 3.30 mmol) and 6-formyl-2,3-dimethoxybenzoic acid (0.80 g, 3.80 mmol) in the presence of a catalytic amount of *p*-toluenesulfonic acid monohydrate in chlorobenzene (15 mL) was refluxed for 7 h, and the reaction mixture was evaporated to dryness and triturated with diethyl ether (10 mL). The precipitate was filtered and air-dried to a constant weight. Yield 0.94 g (92%); white crystals; mp 240°C (with decomposition). ^{1}H NMR: 9.28 (s, 1H, NO<u>H</u>), 8.04 (d, 1H, Ar, *J* 7.8), 7.95 (dd, 1H, Ar, *J* 7.8, 1.6), 7.68 (td, 1H, Ar, *J* 7.8, 1.6), 7.52 (d, 1H, Ar, *J* 8.3), 7.44 (d, 1H, Ar, *J* 8.3), 7.33 (td, 1H, Ar, *J* 7.6, 1.1), 6.36 (s, 1H, NC<u>H</u>N), 3.91 (s, 3H, OC<u>H</u>$_3$), 3.88 (s, 3H, OC<u>H</u>$_3$). ^{13}C NMR: 164.76, 163.40, 153.81, 146.70, 136.47, 133.51, 131.79, 128.13, 125.16, 123.26, 122.27, 120.25, 119.39, 117.80, 71.65, 61.76, 56.50. HRMS: *m/z* 327.0903 $[M]^+$. Calculated for $(C_{17}H_{14}N_2O_5)^+$: 327.0979.

Cell Culture

Mel Z melanoma cells were used, which were derived as a cell line from metastatic nodes of a patient with skin melanoma, who underwent treatment at the Blokhin National Medical Research Center of Oncology, Ministry of Health of the Russian Federation [18]. MCF-7 breast cancer cells (ATCC® HTB-22™)

and SN-12C kidney cancer cells [19] were obtained from the bank of cell cultures at the Blokhin National Medical Research Center of Oncology, Ministry of Health of the Russian Federation.

Mel Z and SN-12C tumor cells were cultured on complete growth medium RPMI-1640 (Gibco) containing 10% FCS (HyClone), 2 mM glutamine, and 50 mg/mL of penicillin/streptomycin (PanEko, Russia) in Eppendorf cell culture flasks at 37°C in an atmosphere of 5% CO_2. MCF-7 cells were cultured on complete growth medium DMEM (Gibco) supplemented with 10% FCS (HyClone), 2 mM glutamine, and 50 mg/mL of penicillin/streptomycin (PanEko, Russia) in Eppendorf cell culture flasks at 37°C in an atmosphere of 5% CO_2. Cells were maintained in the logarithmic growth phase by the constant reseeding of the culture every two to three days.

MTT Test

The cytotoxic activity of compounds was estimated by the standard MTT test using the MTT reagent (3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide); the test is based on the ability of dehydrogenases of live metabolically active cells to reduce the MTT reagent to blue insoluble formazan crystals. For each compound, a graph of the dose-effect dependence was plotted, and the IC_{50} and IC_{10} values were determined using the program GraphPad Prizm 5.0 (GraphPad). The experimental error was no more than 5%.

MCF-7, Mel Z, and SN-12C tumor cells (5×10^4 cell/mL) were placed into the wells of a 96-well plate (Nunc) in complete growth medium RPMI-1640 or DMEM (for MCF-7) supplemented with 10% FCS (HyClone), 2 mM glutamine, and 50 mg/mL of penicillin/streptomycin (PanEko, Russia). After 24 h, compounds at concentrations of 10^{-4}–10^{-10} were added, and cells were incubated for 48 h after which the MTT solution (Sigma, Chemical Co, United States) at a final concentration of 0.5 mg/mL was added, 10 μL to each well. Cells were incubated for an additional 4 h and precipitated by the centrifugation of plates for 3 min at 1000 rpm on a Hettich 460R centrifuge (Hettich Lab Technology, Germany). Medium was taken, and DMSO (200 μL) was added to cells. Cells were resuspended and incubated for 10 min at 37°C after which the optical density of the formazan solution was immediately determined on a Multiskan FC microplate photometer (Thermo Scientific) at 570 nm using DMSO as a zero control.

Estimation of the Blocking of Migration Capacity of Cells by the Wound-Healing Method

Tumor cells (3×10^5 cell/mL) were placed into the wells of a 24-well plate (Nunc) in medium RPMI-1640 or DMEM containing 10% FCS (HyClone), 2 mM glutamine, and 50 mg/mL of penicillin/streptomycin (PanEko, Russia) and incubated until the formation of a monolayer. Then, the monolayer was broken by scraping a part of cells (by a 200 μL tip) in a straight line through the center. Cells were incubated for 24 h in complete growth medium RPMI-1640 or DMEM containing 10% FCS (HyClone), 2 mM glutamine, and each of the test compounds at noncytotoxic concentrations (IC_{10}). As a negative control, which determines the width of a "wound," cells cultured in serum-free medium RPMI-1640 or DMEM were used. Cells growing in complete medium RPMI-1640 or DMEM containing 10% FCS without test compounds served as a positive control, which determines a maximum growth of the monolayer in the wound area. Sunitinib at a noncytotoxic concentration of 1 μM (Selleckchem) served as a reference compound. After the termination of incubation, the cell monolayer on plates was washed twice with PBS and stained with trypan blue (PanEkon, Russia). The degree of the inhibition of migration activity was defined as the ratio (in percent) of the increment in the cell monolayer area by the action of a test compound to the increment in the growth of cells in the positive control. The width of the wound in the negative control was used as the basic value for the calculation of the wound width.

Capillary-Like Structure Formation in 3D Culture

Matrigel (Corning) was defrosted at 4°C, applied onto the wells of a 24-well plate (Nunc) (100 μL onto each) on ice, and allowed to stand under sterile conditions for matrix polymerization for 1 h at room temperature and for 30 min at 37°C in a CO_2 incubator. Mel Z and SN-12C cells at a concentration of 4×10^5 cell/mL in complete growth medium RPMI-1640 (Gibco) supplemented with 10% FCS were applied onto gel and incubated at 37°C for 12 h in the presence of test compounds at noncytotoxic concentrations (IC_{10}). Cells incubated in medium RPMI-1640 containing 10% FCS (Gibco) without test compounds served as a positive control. After the termination of incubation, the resulting network of CLS was photographed using a digital camera (Canon), and the length of CLS from tumor cells within the closed network was estimated using the program Image J v.1.73 (NIH, United States, freeware).

Statistical Analysis

All experiments were performed three times independently of one another. The data are given as the mean ± standard deviation. Differences were considered statistically significant if P values were less than 0.05. All statistical analyses were carried out using the software GraphPad Prism (GraphPad Software, La Jolla, San-Diego, California, United States).

FUNDING

The study was supported by the Ministry of Science and Higher Education of the Russian Federation (Agreement no. 075-11-2018-172 of 03.12.18). The unique identifier of the project is RFMEFI62418X0051.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants performed by any of the authors.

Conflict of Interests

The authors declare that there is no conflict of interest.

REFERENCES

1. Persy, I.S., Wick, J.Y., and Kaufman, B., *Nat. Rev. Clin. Oncol.*, 2009, vol. 6, pp. 569–577.
2. Azam, F., Mehta, S., and Harris, A.L., *Eur. J. Cancer*, 2010, vol. 46, pp. 1323–1332.
3. Maniotis, A., Folberg, R., Hess, A., Seftor, E.A., Gardner, L.M., Pe'er, J., Trent, J.M., Meltzer, P.S., and Hendrix, M.J., *Am. J. Pathol.*, 1999, vol. 55, pp. 739–752.
4. Zhang, S., Zhang, D., and Sun, B., *Cancer Lett.*, 2007, vol. 254, pp. 157–164.
5. Van der Schaft, W., Seftor, R.E., Seftor, E.A., Hess, A.R., Gruman, L.M., Kirschmann, D.A., Yokoyama, Y., Griffoen, A.W., and Hedrix, M.J., *J. Natl. Cancer Inst.*, 2004, vol. 96, no. 19, pp. 1473–1477.
6. Berube, G., *Expert Opin. Drug Discov.*, 2016, vol. 11, no. 3, pp. 281–305.
7. Yoon, S. and Eom, G.H., *Chonnam Med. J.*, 2016, vol. 52, no. 1, pp. 1–11.
8. Giannini, G., Battistuzzi, G., and Vignola, D., *Bioorg. Med. Chem. Lett.*, 2015, vol. 25, no. 3, pp. 459–461.
9. Rao, M., Valentini, D., Zumla, A., and Maeurer, M., *Int. J. Infect. Dis.*, 2018, vol. 69, pp. 78–84.
10. Muri, E.M., Nieto, M.J., Sindelar, R.D., and Williamson, J.S., *Curr. Med. Chem.*, 2002, vol. 9, pp. 1631–1653.
11. Ahmad, S. and Ahmad, I., *Med. Chem. Commun.*, 2017, vol. 8, pp. 871–885.
12. Asif, M., *Int. J. Med. Chem.*, 2014, article ID 395637.
13. Folkman, J., *Curr. Mol. Med.*, 2003, vol. 3, pp. 643–651.
14. Hillen, F. and Griffioen, A.W., *Cancer Metastasis Rev.*, 2007, vol. 26, pp. 489–502.
15. Vartanian, A., Golubeva, I., and Shprakh, Z., *Horizons Cancer Res.*, 2017, vol. 65, pp. 13–34.
16. Sashidhara, K.V., Palnati, G.R., Dodda, R.P., Avula, S.R., and Swami, P., *Synlett*, 2013, vol. 24, pp. 105–113.
17. Vailhe, B., Vittet, D., and Feige, J., *Lab. Invest.*, 2001, vol. 81, pp. 439–452.
18. Mikhailova, I.N., Lukashina, M.I., Baryshnikov, A.Yu., et al., *Vestn. Ross. Akad. Med. Nauk.*, 2005, vol. 7, pp. 37–40.
19. Naito, S., von Eschenbach, A.C., Giavazzi, R., and Fidler, I.J., *Cancer Res.*, 1986, vol. 46, pp. 4109–4115.

Translated by S. Sidorova