EVALUATION OF ANTITUMOR ACTIVITY OF SOME 4-AMINOPIPERIDINE DERIVATIVES — LOW MOLECULAR WEIGHT HSP70 INHIBITORS — ON TRANSPLANTABLE MOUSE TUMORS

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Low molecular weight compounds targeting chaperone proteins Hsp90 and Hsp70 have opened up a new avenue in the therapy of neoplasms. In 2020, we tested 3 Hsp70 inhibitors from the class of 4-aminopiperidine derivatives for their antitumor activity on *in vivo* models. The list of the tested compounds included N-(2-chlorobenzyl)-N-ethyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (compound 1), 4-((methyl(1-(2-(methylthio)pyrimidin-4-yl) piperidin-4-yl)amino)methyl) benzonitrile (compound 2) and N-(2,6- dichlorobenzyl)-1-(1-(2-(ethylthio)pyrimidin-4-yl)piperidin-4-yl)-N-methylmethaneamine (compound 3). The aim of this study was to compare the efficacy of 4-aminopiperidine derivatives *in vivo* using the models of transplantable murine L1210 lymphocytic leukemia and B16 melanoma. Compounds 2 and 3 used in combination with cyclophosphamide exhibited high cytotoxic activity ($p = 0.05$) against L1210 leukemia (an 80-82% increase in survival time) and B16 melanoma (98-99.7% tumor growth delay). For L1210 lymphocytic leukemia, compounds 2 and 3 used in combination with cyclophosphamide fell into the low (+) therapeutic potential category. For B16 melanoma, compounds 1, 2 and 3 used in combination with cyclophosphamide fell into either low (+) or moderate (++) therapeutic potential categories. On the whole, the tested doses of the compounds used in combination with cyclophosphamide hold promise for the therapy of L1210 leukemia and B16 melanoma in mouse models. Our findings confirm the potential of low molecular weight Hsp70 inhibitors for combination chemotherapy against cancer.

Keywords: heat shock proteins, Hsp70 inhibitors, transplantable tumor, L1210 leukemia, B16 melanoma

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Author contribution: Aldobaev VN planned the experiment, summarized its results and wrote this manuscript; Mikhina LV carried out the experiment in animal models; Present MA synthesized the tested compounds.

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ОЦЕНКА ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ РЯДА ПРОИЗВОДНЫХ 4-АМИНОПИПЕРИДИНА, НИЗКОМОЛЕКУЛЯРНЫХ ИНГИБИТОРОВ HSP70, НА ПЕРЕВИВАЕМЫХ ОПУХОЛЯХ МЫШЕЙ

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Применение низкомолекулярных агентов, мишенью которых являются молекулярные шапероны Hsp90 и Hsp70, стало основой для целого направления в терапии новообразований. В 2020 г. была проведена сравнительная оценка противоопухолевой активности на моделях *in vivo* трех производных 4-аминопиперидина ингибиторов Hsp70: N-(2-хлоробензил)-N-этил-1-(2-(метилтио)пиримидин-4-ил)пиперидин-4-амина (№ 1); 4-((метил(1-(2-(метилтио)пиримидин-4-ил)пиперидин-4-ил)амино)метил) бензонитрила (№ 2); N-(2,6-дихлорбензил)-1-(1-(2-(этилтио)пиримидин-4-ил)пиперидин-4-ил)-N-метилметанамина (№ 3). Целью работы было провести сравнительные испытания эффективности производных 4-аминопиперидина *in vivo* на перевиваемых опухолях мышей. Противоопухолевую активность исследуемых веществ изучали на моделях лимфоидной лейкемии L1210 и меланомы B16. Субстанции № 2 и 3 продемонстрировали высокую статистически значимую ($p = 0,05$) активность в случае комбинированной терапии с циклофосфамидом для лейкоза L1210 (увеличение продолжительности жизни — 80–82%) и для меланомы B16 (торможение роста опухоли — 98–99,7%). В случае L1210 вещества № 2 и 3 в комбинации с цитостатиком попали в низшую категорию перспективности «+» для модельных лейкозов животных. В случае B16 вещества № 1–3 в комбинации с цитостатиком попадали либо в низшую категорию перспективности «+», либо в категорию «++» для модельных солидных опухолей животных. Испытанные дозировки субстанций продемонстрировали обещающие результаты лечения в комбинации с циклофосфамидом на перевиваемых опухолях лимфоидной лейкемии L1210 и меланомы B16 мышей. Полученные эффекты подтверждают перспективность применения низкомолекулярных ингибиторов Hsp70 в составе комбинированной химиотерапии в онкологии.

Ключевые слова: белки теплового шока, ингибиторы Hsp70, перевиваемая опухоль, лейкемия L1210, меланома B16

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Low molecular weight compounds targeting molecular chaperones like Hsp90 and Hsp70 have opened up a new avenue in the therapy of neoplasms. Heat shock proteins Hsp90 and Hsp70 are overexpressed in many tumors, which explains selective accumulation of Hsp90 inhibitors in tumor tissue [1]. Inhibited expression and/or reduced functional activity of heat shock proteins result in the accumulation of damaged, partially denatured, functionally altered proteins in the cell. It is hypothesized that Hsp90 and Hsp70 might enhance the anticancer effect of cytotoxic drugs and help to overcome drug resistance when used in combination with chemotherapy agents. Some recent publications [2–4] discuss the synthesis of Hsp70 inhibitors, the small molecules designed by means of molecular docking. An article [4] describes the synthesis of 67 candidate Hsp70 inhibitors from the class of 4-aminopiperidine derivatives [4], whose activity was tested on cell cultures in vitro. The article provides information on the kinetic rate constants for each compound measured by surface plasmon resonance and evaluates the inhibitory effect of the synthesized compounds on Hsp70 ATPase activity. Another publication [5] describes an alternative technique for the synthesis of some of the 4-aminopiperidine derivatives from [4], including 1-(2-alkylthiopyrimidin-4-yl)piperidin-4-N-alkyl,N-hetaryl/aryl amines. The proposed technique allowed us to obtain larger combinatorial libraries for further screening tests on cell cultures and the subsequent optimization of candidate cytotoxic drugs.

In 2018–2019, our team synthesized a collection of 4-aminopiperidine derivatives, which partially overlapped with the collection described in [4] and performed an *in vitro* screening test of their cytotoxic activity on cell cultures. Three 4-aminopiperidine derivatives were selected for further in vivo tests on animal tumor models. The antitumor activity of the synthesized compounds was studied on transplantable mouse lymphocytic leukemia (L1210) and solid melanoma tumors (B16). Cyclophosphamide was used as a positive control following recommendations in [8] and as a treatment against induced cancers.

METHODS

Laboratory animals

The tumors were maintained in female C_{57}Bl/6 and DBA/2 mice. The specific activity of the synthesized compounds was assessed *in vivo* on inoculated female hybrid BDF1 mice (C_{57}Bl/6 × DBA/2). At the beginning of the study, the mice weighted 20 to 30 g. The animals were purchased from the Research Center for Biomedical Technology, FMBA (the branch of Stolbovaya breeding nursery, Moscow region). The animals were housed in a conventional room in the absence of other animal species.

The mice were kept in 26 × 17 × 12 cm^3 polycarbonate cages (Tecniplast; Italy) at 6 animals per cage. The cages were fitted with stainless-steel wire lids and in-built feed hoppers, a stainless-steel divider to separate the food zone, and label holders. The cages were arranged on stainless steel racks (Tecniplast; Italy).

Wood chips were used as a bedding (Laboratorkorm; Russia); the bedding was 5–10 mm thick.

The animals were allowed ad libitum access to a standard chow diet (Kombikorm PK-120 for laboratory rats, mice and hamsters by Laboratorkorm; Russia). The food was supplied via a food hopper.

The animals also had ad libitum access to filtered tap water supplied in standard drinking bottles with steel caps and sipper tubes. Water quality satisfied Sanitary Regulations 2.1.4.1074-01 (updated on April 2, 2018). Filtering was necessary to avoid water contamination that could have affected the results of the study.

The mice were housed under artificial 12 : 12 light-dark conditions in a controlled environment at 20–26 °C and 30–70% air humidity. The temperature and humidity in the room were maintained by an automated climate control system. The air exchange rate was 15 air changes per hour. Before the experiment, the animals were quarantined for 14 days.

Maintenance of transplantable tumors

The antitumor activity of the synthesized compounds was studied *in vivo* using the transplantable murine lymphocytic leukemia (L1210) and solid B16 melanoma models.

Cancer cell lines were provided by Blokhin Cancer Research Center (Russia), where they had been cryopreserved in 1 ml ampoules and stored in liquid nitrogen. The cells were shipped in liquid nitrogen. Protocols used to recover the cryopreserved cells were the same for both cancer cell lines. Briefly, the ampoules were retrieved from liquid nitrogen and left in an incubator at 37 °C for 30 min. After that, 0.5 ml of the cell suspension was administered to each animal. Lymphocytic leukemia L1210 was maintained in DBA/2 mice, B16 melanoma was maintained in C_{57}Bl/6 mice. The neoplasms were maintained by inoculation. To maintain L1210 lymphocytic leukemia, intact mice were inoculated intraperitoneally with 0.3 ml of L1210 ascitic fluid derived from hosts on days 5 or 6 and diluted with normal saline 1 : 60. To keep B16 melanoma viable, intact mice were subcutaneously inoculated with 0.5 ml of B16 melanoma preparation derived from hosts on day 16–20 (1 g of the tumor was homogenized in 10 ml of normal saline).

Treatment

Lymphocytic leukemia and solid melanoma cells were transplanted to female BDF1 mice (C_{57}Bl/6 × DBA/2) using the same protocol as for tumor maintenance. For inoculations, we used cells that had undergone at least 2 passages in mice after thawing. Therapy against L1210 lymphocytic leukemia was initiated 24 h after inoculation; therapy against B16 melanoma was initiated 48 h after inoculation [6]. As part of the experiment, we determined effective cyclophosphamide doses and regimens against the induced murine cancers. The choice of cyclophosphamide as a positive control for the L1210 model was dictated by the results of our previous study [7]. With the melanoma model, the choice of cyclophosphamide was based on our practical experience. Mice inoculated with L1210 cells received IM injections of 50 mg/kg cyclophosphamide twice, 24 h and 72 h after inoculation. This treatment regimen allowed us to prolong survival by an average of 31–43%, as compared with the negative control group (NC). For mice inoculated with L1210 lymphocytic leukemia cells, survival times ranged from 2 to 3 weeks.

Mice inoculated with B16 melanoma cells received 3 IM injections of 80 mg/kg cyclophosphamide on days 2, 5 and 9 after inoculation. This regimen resulted in 62–100% tumor growth delay during the observation period (1 month) and prolonged survival by 10–23%, as compared with the NC group. For mice inoculated with B16 melanoma cells, survival times ranged from 4 to 5 weeks.

For the experiment, 4-aminopiperidine derivatives were formulated as water-soluble hydrochlorides. Mice with lymphocytic leukemia received daily injections of

Table 1. Primary tests of antitumor activity in the murine L1210 model

Group	Number of animals in the group	Treatment	Dosage, mg/kg	Total number of injections
1	6	No treatment (NC)	–	–
2	6	Cyclophosphamide (PC)	50	2
3	6	Compound 1 + Cyclophosphamide	200 50	7 2
4	6	Compound 1	200	7
5	6	Compound 2 + Cyclophosphamide	150 50	7 2
6	6	Compound 2	150	7
7	6	Compound 3 + Cyclophosphamide	250 50	7
8	6	Compound 3	250	7

Note: NC — negative control; PC — positive control.

4-aminopiperidine derivatives for 7 days; the first injection was administered the day after inoculation. Mice with B16 melanoma received daily intraperitoneal injections of the synthesized compounds for 10 days; the first injection was administered 48 h after inoculation. The formulations were prepared in a laminar flow cabinet using a ready-to-use sterile normal saline solution.

Statistical analysis

The efficacy of treatment was evaluated relative to the outcomes in the NC group (inoculated mice, no treatment received). We compared the increase in the survival time, tumor growth delay and a related T/C parameter [6]. The increase in survival time and tumor growth delay were calculated from the average tumor volume at a specific point in time after inoculation (for B16 melanoma) and the survival time within the experiment (for both cancers). Differences between the studied parameters were measured using the approach of a function or several random variables [7]. Mean squared deviations (MSD) were calculated for the tumor volume at a specific point in time after inoculation (for B16 melanoma) and survival time within the observation period (both cancers). Based on mean values, MSDs and sample sizes (the number of animals in the groups), mathematical expectations (ME), 95% CI for tumor growth delay (TGD) and survival time increase (STI) were calculated using a code written in *Mathematica 9* [7].

RESULTS

Efficacy of synthesized 4-aminopiperidine derivatives in L1210 lymphocytic leukemia model

Prior to evaluating the efficacy of N-(2-chlorobenzyl)-N-ethyl-1-(2-(methylthio)pyrimidin-4-yl)piperidin-4-amine (compound 1), 4-((methyl(1-(2-(methylthio)pyrimidin-4-yl) piperidin-4-yl)amino)methyl) benzonitrile (compound 2) and N-(2,6-dichlorobenzyl)-1-(1-(2-(ethylthio)pyrimidin-4-yl)piperidin-4-yl)-N-methylmethaneamine (compound 3), we conducted a series of preliminary experiments to determine their maximum tolerated dose (MTD, single intraperitoneal injection) for BDF1 (C_{57}Bl/6 × DBA/2) hybrid mice. After the injection, the animals were closely monitored and their weight was measured daily for 7 days. Based on clinical observations and weight dynamics, MTDs for compounds 1, 2 and 3 were 250 mg/kg, 200 mg/kg and 300 mg/kg, respectively. At these doses, the tested compounds increased the heart rate, induced rapid breathing and provoked clonic or tonic seizures in most experimental animals. These symptoms resolved within 10–15 min after the

Table 2. Antitumor activity of 3 synthesized compounds administered intraperitoneally to mice with transplantable L1210

Group	Treatment	STI, %		T/C	
		I*	II	I	II
1	No treatment (NC)	–		–	
2	CPA** (PC)	31	43	131	143
		37 ***		137	
3	Compound 1 + CPA	60	71	160	171
		65,5		166	
4	Compound 1	10	17	110	117
		13,5		114	
5	Compound 2 + CPA	63	80	163	180
		71,5		172	
6	Compound 2	5	11	105	111
		8,0		108	
7	Compound 3 + CPA	72	82	172	182
		77,0		177	
8	Compound 3	0	5	100	105
		2,5		103	

Note: * — experiment number; ** — cyclophosphamide; *** — mean value.

Table 3. Mathematical expectations (ME) for STI and their 95% CI

Group	Treatment	I* ME for STI, % [CI] %	II ME for STI, % [CI] %
1	No treatment (NC)	–	–
2	CPA ** (PC)	32 [6; 66]	43 [29; 60]
3	Compound 1 + CPA	62 [23; 110]	71 [48; 98]
4	Compound 1	11 [–17; 46]	17 [–4; 40]
5	Compound 2 + CPA	64 [34; 104]	80 [62; 100]
6	Compound 2	6 [–21; 40]	11 [–7; 30]
7	Compound 3 + CPA	74 [37;121]	82 [61; 106]
8	Compound 3	5 [–25; 41]	11 [–16; 40]

Note: * — experiment number; ** — cyclophosphamide.

injection. During the first 2–3 days after the injection, half of the animals lost 2-8% of their weight. By the end of the observation period, all animals had gained weight.

Considering the obtained MTDs and the guidelines on primary antitumor activity testing [8], we selected the following dosing regimen for the animals inoculated with L1210 cells: compound 1 — 200 mg/kg, compound 2 — 150 mg/kg, compound 3 — 250 mg/kg. The compounds were administered intraperitoneally, daily, for 7 days. The first injection was administered 24 h after inoculation. The animals were divided into 8 groups (6 animals per group except for the NC group, which consisted of 8 animals). The NC group did not receive any treatment. The positive control (PC) group was treated with cyclophosphamide (Table 1).

The results generated by a series of 2 experiments conducted on the L1210 model are provided in Table 2. Ranges and mean values for STI and T/C were used as repeatability indicators.

As seen from Table 2, a relatively high increase in the survival time was achieved only when the tested doses of compounds 1, 2 and 3 were used in combination with cyclophosphamide.

For L1210 lymphocytic leukemia, compounds 2 and 3 used in combination with the cytotoxic drug fall into the low therapeutic potential category (designated as + ; T/C ≥ 175%) [6].

A potentiating effect was demonstrated by compounds 1, 2 and 3 used in combination with cyclophosphamide.

Table 3 shows mathematical expectations for STI and their 95% CI in the L1210 model.

Considering CI shown in Table 3, it can be concluded that differences in STI were significant ($p = 0.05$) between groups 2 and 5 and between groups 2 and 7 in experiment II.

Efficacy of synthesized 4-aminopiperidine derivatives in B16 melanoma model

Based on the results of antitumor activity tests conducted on the L1210 leukemia model, we selected the following dosing regimen for the B16 melanoma model: compound 1 — 200 mg/kg, compound 2 — 150 mg/kg, compound 3 — 250 mg/kg. The compounds were administered intraperitoneally, daily, over the course of 10 days. The first injection was administered 48 h after inoculation. The animals were divided into 8 groups (6 animals per group except for the NC group, which consisted of 8 animals). The NC group did not receive any treatment. The PC group was treated with cyclophosphamide (Table 4).

The results generated by a series of 2 experiments conducted on the B16 melanoma model are provided in Table 5. Ranges (STI and TGD) and mean values (TGD) were used as repeatability indicators.

As seen from Table 5, relatively high TGD levels were achieved only when the tested doses of compounds 1, 2 and 3 were used in combination with cyclophosphamide.

For solid B16 melanoma, compounds 1, 2 and 3 used in combination with the cytotoxic drug fall into the low therapeutic potential category (designated as +; TGD < 51–80%) or the

Table 4. Primary antitumor activity tests conducted on the B16 melanoma model

Group	Number of animals in the group	Treatment	Dose, mg/kg	Total number of injections
1	8	No treatment (NC)	–	–
2	6	Cyclophosphamide (PC)	80	3
3	6	Compound 1 + Cyclophosphamide	200 80	10 3
4	6	Compound 1	200	10
5	6	Compound 2 + Cyclophosphamide	150 80	10 3
6	6	Compound 2	150	10
7	6	Compound 3 + Cyclophosphamide	250 80	10 3
8	6	Compound 3	250	10

Table 5. Antitumor activity of 3 synthesized compounds administered intraperitoneally to mice with transplantable B16 melanoma measured in a series of 2 experiments

Group	Treatment	TGD, %								STI, %	
		Day 13		Day 21		Day 28		Day 33			
		I*	II	I	II	I	II	I	II	I	II
1	No treatment (NC)	–		–		–		–		–	
2	CPA* (PC)	77	87	62	83	62	78	31	65	18	23
		82***		72.5		70		48			
3	Compound 1 + CPA	71	93	67	86	52	76	40	66	–	
		82		76.5		64		53			
4	Compound 1	–		–		2	11	5	12	–	
		–		–		8.5		8.5			
5	Compound 2 + CPA	89	100	75	98	69	82	46	55	24	30
		94.5		86.5		75.5		50.5			
6	Compound 2	–		–		17	22	8	17	–	
		–		–		19.5		12.5			
7	Compound 3 + CPA	82	100	67	94	56	86	47	89	–	
		91		80.5		71		68			
8	Compound 3	13	21	–		15	28	12	18	–	
		17		–		21.5		15			

Note: * — experiment number; ** — cyclophosphamide; *** — mean value.

Table 6. Mathematical expectations (ME) for TGD, STI and their 95% CIs

Group	Treatment	ME for TGD, % [CI] %					ME for STI, % [CI] %
		Day 13		Day 21	Day 28	Day 33	
1	No treatment (NC)	–		–	–	–	–
2	CPA* (PC)	I*	82 [23; 99]	57 [–51; 115]	59 [16; 83]	24 [–51; 70]	22 [–24; 86]
		II	85 [55; 95]	80 [47; 94]	76 [50; 91]	63 [29; 82]	29 [–23; 112]
3	Compound 1 + CPA	I	78 [–41; 148]	62 [–14; 92]	49 [–12; 83]	35 [–46; 88]	–
		II	92 [75; 96]	84 [53; 99]	72 [38; 98]	64 [–23; 89]	–
4	Compound 1	I	–	–	–5 [–157; 96]	–3 [–122; 72]	–
		II	–	–	–3 [–97; 58]	5 [–82; 54]	–
5	Compound 2 + CPA	I	92 [68; 95]	71 [6; 104]	67 [16; 102]	40 [–19; 77]	28 [–13; 89]
		II	99 [95; 104]	98 [94; 99]	79 [57; 95]	51 [–3; 88]	19 [–10; 115]
6	Compound 2	I	–	–	11 [–108; 87]	0 [–110; 68]	–
		II	–	–	9 [–75; 65]	10 [–77; 63]	–
7	Compound 3 + CPA	I	86 [43; 96]	62 [–20;99]	55 [4; 84]	42 [–17; 77]	–
		II	99,7 [95; 104]	93 [82;97]	84 [67; 96]	88 [78; 94]	–
8	Compound 3	I	32 [–189; 95]	–	9 [–113; 86]	4 [–98; 66]	–
		II	7 [–183; 83]	–	16 [–72; 78]	11 [–66; 54]	–

Note: * — experiment number; ** — cyclophosphamide.

moderate therapeutic potential category (designated as ++; TGD < 81–90%) on days 13, 21 and 28 after inoculation [6].

In combination with cyclophosphamide, compounds 2 and 3 demonstrated the potentiating effect with respect to TGD on day 13; compounds 1, 2 and 3, on day 21; compound 3, on day 33; the additive effect was demonstrated by compound 1 on day 33, compound 2 on days 28 and 33 and compound 3 on day 28.

A significant increase in the average survival time was observed only in the groups undergoing therapy with cyclophosphamide or cyclophosphamide + compound 2.

This, along with the comparable efficacy of compounds 1 and 3 demonstrated on the B16 melanoma model (Table 4) and the lymphocytic leukemia model (Table 2), allowed us to single out compound 2 as the most promising candidate for further dose optimization and development of effective treatment regimens.

Mathematical expectations for TGD, STI and their 95% CIs are shown in Table 6.

Considering CI shown in Table 6, it can be concluded that differences in TGD were significant ($p = 0.05$) between groups 2 and 5 and between groups 2 and 7 on day 13; between groups 2 and 5 on day 21 in experiment II. In all other cases of combination therapy, TGD trended towards significance.

Based on the increase in TGD and survival time achieved by applying the combination regimen vs monotherapy with cyclophosphamide (see Tables 3 and 6), compound 2 was singled out as the most promising candidate for further research.

DISCUSSION

Hsp70 inhibitors are traditionally classified by their mechanism of action and structure. As a rule, Hsp70 inhibitors bind to the nucleotide-binding domain and block the interaction of other factors with the nucleotide-binding and substrate-binding Hsp70 domains [9–11]; these agents also inhibit Hsp70 ATPase activity (the mechanisms are not specified) [12–15], selectively suppress GRP78 [16–18], interact with the EEVD Hsp70 domain [19], disrupt the interaction between Hsp70 and BAG3 [20–22], etc. At the same time, Hsp inhibitors can be grouped into the following classes by their chemical structure: ATP analogues (Ver-155008) [9, 23], dihydropyridines (MAL3-101, DMT3132, NSC 630668-R/1) [14, 15, 24], flavonoids (epigallocatechin-3-gallate, quercetin) [25, 26], imidazoles (Apoptozole, Az-TPP-O3) [27, 28], phenylethylsulfonamides (Pifithrin-μ) [29, 30], rhodocyanines and their derivatives (YM-1, MKT-077, JG-98) [21, 31, 32], methylene blue [33] and some other compounds.

The compounds described in this article belong to the class of Hsp70 APTase activity inhibitors, but due to their chemical structure they have opened up a new avenue in the study of non-specific low molecular weight Hsp70 inhibitors. This study is a logical continuation of the study [4], which modeled chemical structures showing affinity for the ATP binding site of the Hsp70 molecule, described conditions for the synthesis of 4-aminopiperidine derivatives (potential Hsp70 inhibitors), analyzed their kinetic rate constants by means of surface plasmon resonance and demonstrated the inhibition of Hsp70 ATPase activity using a colorimetric test. In addition, the authors of the study screened the original collection of the synthesized compounds for their activity against 16 cancer cell lines and 2 human fibroblast cell lines. The most toxic compounds demonstrated LC_{50} in the range from 0.7 to 2.0 μM. In the acute toxicity test, one of the compounds orally administered to model mice was found to have LD_{50} of 870 mg/kg.

Summing up, the aim of the study was achieved: we successfully evaluated the Hsp70-inhibiting potential of 4-aminopiperidine derivatives using murine models of transplantable L1210 lymphocytic leukemia and solid B16 melanoma.

CONCLUSION

As expected, our preliminary experiments showed that high doses of the synthesized 4-aminopiperidine derivatives used in combination with cyclophosphamide hold promise as chemotherapeutic drugs.

It was shown that therapy with compounds 2 and 3 resulted in significant differences in treatment efficacy ($p = 0.05$) between the groups that received combination therapy and monotherapy with cyclophosphamide. Specifically, combination therapy resulted in longer survival times in the groups with transplantable L1210 leukemia and in significant tumor growth delay on days 13 and 21 after inoculation in the groups with B16 melanoma.

Based on the obtained data, the most active compound 4-((methyl(1-(2-(methylthio)pyrimidin-4-yl) piperidin-4-yl)amino) methyl) benzonitrile formulated as a hydrochloride was selected for further optimization of dosing regimens and administration routes.

The strength of the sytotoxic effect observed in this study confirms the promise of low molecular weight Hsp inhibitors for combination therapy of cancer.

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