 doi:10.20944/preprints202208.0110.v1

Article

Cytoprotective activity of newly synthesized 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1*H*)-ones derivatives

Shynggys Sergazy*[1,2], Zarina Shulgau[1], Aigerim Zhulikeyeva[1], Yerlan Ramankulov[1], Irina V. Palamarchuk[3] and Ivan V. Kulakov[1,3]

[1] RSE "National Center for Biotechnology", 13/5 Kurgalzhynskoe road, Nur-Sultan, Kazakhstan; shulgau@biocenter.kz
[2] National Laboratory Astana, Nazarbayev University, Nur-Sultan, Kazakhstan; shynggys.sergazy@gmail.com
[3] Institute of Chemistry, Tyumen State University, 15a Perekopskaya St., Tyumen 625003, Russia; i.v.kulakov@utmn.ru
* Correspondence: shynggys.sergazy@gmail.com; Tel.: +7 702 1999758

Abstract: Currently, studies are being conducted on the possible role of the cytoprotective effect of biologically active substances in conditions of cerebral hypoxia or cardiomyopathies. At the same time, oxidative stress is considered as one of the important mechanisms of cellular cytotoxicity and a target for the action of cytoprotectors. The aim of this study is to search for derivatives of 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1H)-ones.

The probability of cytoprotective action was assessed by two tests by measuring cell viability (with neutral red dye and MTT test). It was found that some derivatives of 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1H)-ones under the conditions of our experiment have a pronounced cytoprotective activity, providing better cell survival *in vitro*, including the MTT test and conditions of blood hyperviscosity.

To correlate the obtained results in vitro, molecular docking of the synthesized derivatives was also carried out. The standard drug omeprazole (co-crystallized with the enzyme) was used as a standard. It was shown that all synthesized derivatives of 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1H)-ones had higher affinity for the selected protein than the standard gastro-cytoprotector omeprazole.

The studied derivatives of 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1H)-ones also fully satisfy Lipinski's rule of thumb of five (RO5), which increases their chances for possible use as orally active drugs with a good ability to absorption and moderate lipophilicity.

Thus, the results obtained make it possible to evaluate derivatives of 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1H)-ones as having a relatively high cytoprotective potential.

Keywords: 3-aminopyridin-2(1*H*)-one derivatives; 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1*H*)-ones; antiradical activity; cytoprotective activity

1. Introduction

The search for new highly effective antioxidant and cytoprotective compounds is an urgent task for medical chemistry, since many diseases, such as diabetes mellitus, hepatitis of various etiologies, strokes, atherosclerosis and many others, occur against a background of pronounced oxidative stress and are accompanied by mass cell death.

Previously we have reported the method for the preparation of 4-aryl(hetaryl)-substituted 3-aminopyridin-2(1*H*)-ones based on the intramolecular cyclization of N-(3-oxoalkenyl)amides. Almost all of the obtained 3-aminopyridin-2(1*H*)-ones possess a high antiradical activity [1]. In addition, 3-aminopyridin-2(1*H*)-one derivatives are of interest as potential biologically active

 doi:10.20944/preprints202208.0110.v1

compounds [2,3]. For example, Amrinone is a pyridine phosphodiesterase 3 inhibitor [4]. Some 3-aminopyridin-2(1*H*)-one derivatives show antiviral activity, including against the AIDS virus [5,6]. The presence of an "embedded" amino acid fragment makes them attractive building blocks for the synthesis of novel derivatives with promising biological applications [7–9].

We have previously shown that the reaction of 3-amino-6-methyl-4-phenylpyridin-2(1H)-one with aromatic aldehydes afforded the corresponding Schiff bases, the reduction of which with sodium borohydride led to the formation of 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1*H*)-ones (Scheme 1.) [10]. Among a series of novel compounds, 3-(arylmethyl)-6-methyl-4-phenylpyridin-2(1*H*)-one derivatives showed antiradical activity against DPPH and ABTS radicals.

In addition, an initial assessment of these synthesized compounds in vivo for tranquilizing (anxiolytic) activity by «light-dark box test» and antidepressant activity by Porsolt's «behavioral despair test». Several derivatives of 3-(arylmethylamino)-6-methyl-4-phenylpyridin-2(1*H*)-one with higher potential neurotropic activity than that of the comparator drugs (Mexidol and Amitriptyline) were detected [11].

Undoubtedly, an important problem of pharmacology is the search for universal protection mechanisms that allow protecting the cell from death in order to preserve its structural integrity and functional activity. It should be taken into account that one of the most common mechanisms of cell damage is the formation of reactive oxygen species that induce free radical lipid peroxidation of membranes with the development of oxidative stress [12,13]. In this regard, the detection of antiradical properties in newly synthesized substances predetermines the search for manifestations of cytoprotective action. Derivatives of 3-arylmethyl-aminopyridone in preliminary experiments demonstrated some potential for antioxidant/antiradical activity, and it is logical that the present attempt to clarify the presence of an antiradical effect in combination with a cytoprotective effect is natural.

2. Results

2.1. Antiradical activity tests

The following tables and figures contain the results of a study of the antiradical activity of eight 3- (arylmethyl)-6-methyl-4-phenylpyridin-2(1*H*)-one compounds taken into the study. The final value is presented as IC50 (50% reduction in DPPH radical activity). Ascorbic acid was used as a standard for antiradical activity against DPPH (DPPH).

Below, as an example, the results of a single determination of optical density and a single calculation of IC50 (DPPH) for ascorbic acid are given. In total, three similar determinations were carried out.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

Table 1. Optical density values of a 100 μM DPPH radical solution after 10-minute incubation with ascorbic acid at final concentrations in the reaction mixture of 50, 25, 20, 15, 10, 5, and 2.5 μM

Final concentration of ascorbic acid in the reaction mixture, μM	Absorbance
50	0,106
25	0,586
20	0,674
10	0,857
5	0,946
Control (DPPH solution without test sample)	1,038

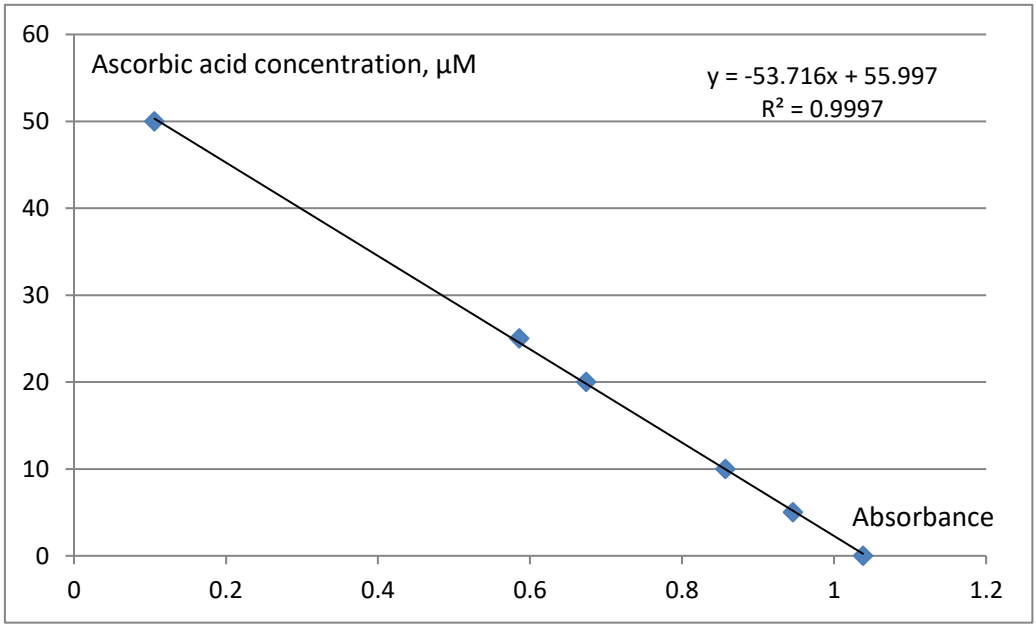


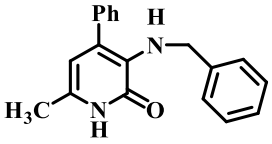
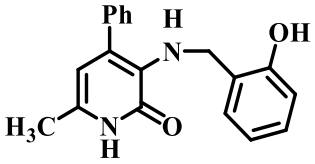
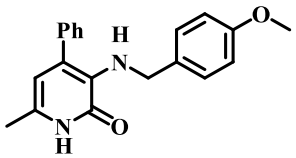
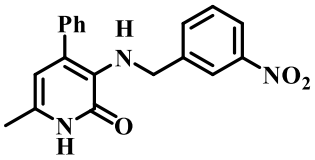
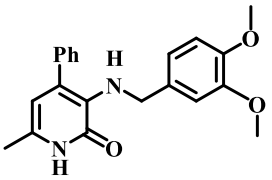
Figure 1. Calibration curve for calculating the IC50(DPPH) of ascorbic acid.

Using the constructed calibration curve, we determined the concentration of ascorbic acid capable of reducing the optical density of a 100 μM solution of the DPPH radical by 50%. For ascorbic acid, IC50(DPPH) was found to be 28.1 μM.

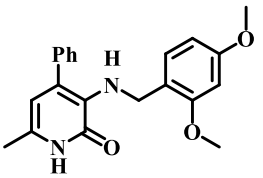
For the studied compounds, the results were obtained in the form of fixed optical density indicators and calculated IC50(DPPH) levels in each case according to three variants of experiments. The results are presented in table 2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

Table 2. Antiradical activity of the studied substances in relation to the DPPH radical (n=3)

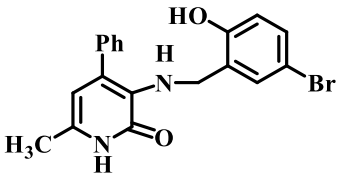
Sample	IC50, μM.
Standard - ascorbic acid	28,1
3a  Chemical Formula: $C_{19}H_{18}N_2O$ Molecular Weight: 290,37	28,5
3b  Chemical Formula: $C_{19}H_{18}N_2O_2$ Molecular Weight: 306,36	25,9
3c  Chemical Formula: $C_{20}H_{20}N_2O_2$ Molecular Weight: 320,39	26,7
3d  Chemical Formula: $C_{19}H_{17}N_3O_3$ Molecular Weight: 335,36	24,6
3e  Chemical Formula: $C_{21}H_{22}N_2O_3$ Molecular Weight: 350,42	27,0
3f	33,4

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1



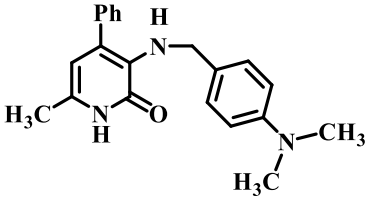
Chemical Formula: $C_{21}H_{22}N_2O_3$
Molecular Weight: 350,42

3g 47,5



Chemical Formula: $C_{19}H_{17}BrN_2O_2$
Molecular Weight: 385,2610

3h 19,5



Chemical Formula: $C_{21}H_{23}N_3O$
Molecular Weight: 333,44

As can be seen from the presented data, the ability to "extinguish" DPPH is inherent in all eight compounds under study, approximately to the same extent with a small scatter in quantitative characteristics. The antiradical activity of the studied compounds is comparable to that of ascorbic acid.

In a similar mode, the study of the antiradical activity of the compounds with respect to the ABTS•+ radical was carried out. As a standard of antiradical activity, vitamin E (Trolox) is present in the form of trolox equivalent of antioxidant capacity – TEAC (Table 3).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

Table 3. Antiradical activity of the studied substances in relation to ABTS•+ radical (n=3)

Sample	TEAC	Note
Standard - ascorbic acid	1,01 ± 0,06	Antiradical activity is 101% of the activity of Trolox
3a	0,52 ± 0,09	Antiradical activity is 52% of the activity of Trolox
3b	1,09 ± 0,02	Antiradical activity is 109% of the activity of Trolox
3c	-	precipitated during the experiment
3d	0,92 ± 0,17	Antiradical activity is 92% of the activity of Trolox
3e	0,61 ± 0,08	Antiradical activity is 61% of the activity of Trolox
3f	-	precipitated during the experiment
3g	-	precipitated during the experiment
3h	0,55 ± 0,12	Antiradical activity is 55% of the activity of Trolox

Evaluating the antiradical effect of the presented samples in relation to the ABTS•+ radical, it can be seen that the studied compounds have a moderate potential for antiradical activity, approaching in terms of the severity of the quantitative assessment the indicators inherent in vitamin E (Trolox).

The results presented in tables 2 and 3 prove the presence of an antiradical potential in six derivatives of 3-arylmethyl-aminopyridone comparable to the antiradical activity of known standards - ascorbic acid and vitamin E.

2.2. Cytoprotective activity tests

In accordance with the aim of the study, the next step after establishing the antiradical effect of the investigated compounds of 3-arylmethyl-aminopyridone was to study the probable cytoprotective effect. The results of measuring the level of viability of transplanted MCF-7 cells during incubation with the studied compounds 3-arylmethyl-aminopyridone are presented in table 4.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

Table 4. The level of viability of MCF-7 cells under *in vitro* conditions under the influence of the studied samples in the MTT test

Sample	Absorbance λ=570 nm	Absorbance λ=690 nm	Δ(Abs570-Abs690)	Percentage of living cells, %
3a	0,210	0,169	0,041	25
3b	0,528	0,404	0,124	74
3c	0,740	0,711	0,029	17
3d	3,697	1,565	2,132	1269
3e	0,240	0,147	0,093	56
3f	0,116	0,077	0,039	23
3g	0,475	0,423	0,052	31
3h	1,930	1,617	0,313	186
Control	0,363	0,194	0,168	100

The table 4 shows the indicators of cell viability after incubation with 3-arylmethyl-aminopyridone compounds as a percentage relative to the cell line MCF-7 taken as 100% viability in the control (cells without the addition of test compounds).

As can be seen, when cells are incubated with **3e**, cell viability is approximately halved. But the incubation of MCF-7 cells with **3h** and **3d** gives the opposite result: these compounds apparently prevent cell damage during the 24-hour incubation period and after a day their viability significantly exceeds the viability of cells in the control.

It can be considered proven that samples **3h** and **3d** have a pronounced cytoprotective activity under the conditions of our experiment, contributing to a better survival of the MCF-7 cell line.

For the compound with the most pronounced ability to protect cells under conditions of cultivation of the MCF-7 monolayer during the day, namely the compound **3d**, additional studies were performed to confirm the level of viability of MCF-7 cells in the test with neutral red. This dye is known to selectively stain only intact cells with fully functionally preserved membranes.

The results of the study of changes in the viability of MCF-7 cells under in vitro conditions under the influence of the studied samples in the test with neutral red are presented in Table 5. The table shows the indicators of cell viability as a percentage relative to the MCF-7 cell line taken as 100% viability in the control (cells without addition of test compound)

Table 5. Test results for MCF-7 cells (vitamin stained with neutral red) after 24 incubations with **3d**

Sample	Absorbance λ=570 nm	Absorbance λ=690 nm	Δ(Abs570-Abs690)	Percentage of living cells, %
3d	0,800	0,628	0,172	904
Control	0,097	0,078	0,019	100

As can be seen, the results of the interaction of cells pre-incubated with the **3d** compound with the neutral red dye, which is able to selectively stain the cell only with a functionally and structurally preserved cell membrane, indicate that the cell viability after contact with the **3d** compound is significantly increases.

In experiments on the study of hemorheological activity of samples (table 6), it was found that incubation of blood for 60 minutes at a temperature of 43.0 °C leads to a

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

significant increase in blood viscosity at various spindle speeds from 2 to 60 rpm, which indicates the formation of blood hyperviscosity.

Table 6. Effect of studied samples and pentoxifylline on blood viscosity (mPa*s) at different spindle speeds in an in vitro blood hyperviscosity model

Researched indicator	Blood viscosity (mPa*s) at various spindle speeds, revolutions per minute							
	2	**4**	**6**	**8**	**12**	**20**	**40**	**60**
3a								
Initial viscosity, n=2	5,87±0,11	4,38±0,01	3,62±0,06	3,32±0,02	2,76±0,02	2,33±0,03	2,14±0,03	2,07±0,06
Blood viscosity after incubation, n=4	8,50±0,19 p1=0,0009	6,31±0,04 p1=0,000004	5,71±0,17 p1=0,0013	4,95±0,16 p1=0,0025	4,73±0,11 p1=0,0003	3,79±0,20 p1=0,0077	3,32±0,11 p1=0,0022	3,19±0,05 p1=0,0002
Blood viscosity after incubation with 3a, n=4	7,08±0,02 p1=0,0001 p2=0,0004	5,34±0,03 p1=0,00004 p2=0,000001	4,56±0,16 p1=0,0186 p2=0,0028	4,24±0,10 p1=0,0039 p2=0,0092	3,73±0,20 p1=0,0337 p2=0,0049	3,33±0,03 p1=0,00003 p2=0,0597	3,13±0,02 p1=0,00002 p2=0,1509	3,07±0,02 p1=0,0001 p2=0,0783
3b								
Initial viscosity, n=2	3,29±0,03	3,07±0,04	2,50±0,02	2,36±0,02	2,28±0,02	2,18±0,01	2,11±0,01	2,06±0,04
Blood viscosity after incubation, n=4	9,40±0,07 p1=0,000001	7,31±0,04 p1=0,0000004	5,43±0,06 p1=0,00001	4,45±0,05 p1=0,00001	4,20±0,04 p1=0,000004	3,34±0,09 p1=0,0010	3,25±0,07 p1=0,0005	3,16±0,04 p1=0,0001
Blood viscosity after incubation with 3b, n=4	7,13±0,08 p1=0,00001 p2=0,000001	5,52±0,30 p1=0,0052 p2=0,0010	4,44±0,09 p1=0,0001 p2=0,0001	4,11±0,03 p1=0,000004 p2=0,0009	3,62±0,15 p1=0,0042 p2=0,0104	3,21±0,04 p1=0,0001 p2=0,2239	3,12±0,03 p1=0,00003 p2=0,1609	3,08±0,03 p1=0,00003 p2=0,1576
3d								
Initial viscosity, n=2	3,55±0,55	3,43±0,60	2,92±0,36	2,80±0,35	2,74±0,36	2,27±0,09	2,19±0,06	2,16±0,05
Blood viscosity after incubation, n=4	9,61±0,26 p1=0,0003	6,67±0,30 p1=0,0052	5,07±0,04 p1=0,0007	4,29±0,01 p1=0,0023	3,82±0,15 p1=0,0263	3,51±0,06 p1=0,0003	3,36±0,05 p1=0,0001	3,23±0,02 p1=0,00003
Blood viscosity after incubation with 3d, n=4	7,62±0,23 p1=0,0010 p2=0,0012	5,37±0,13 p1=0,0096 p2=0,0075	4,29±0,12 p1=0,0082 p2=0,0008	3,83±0,14 p1=0,0264 p2=0,0162	3,54±0,03 p1=0,0243 p2=0,1159	3,32±0,05 p1=0,0003 p2=0,0580	3,23±0,03 p1=0,00005 p2=0,0771	3,14±0,02 p1=0,00003 p2=0,0349
3e								
Initial viscosity, n=2	3,29±0,03	3,07±0,04	2,50±0,02	2,36±0,02	2,28±0,02	2,18±0,01	2,11±0,01	2,06±0,04
Blood viscosity after incubation, n=4	9,40±0,07 p1=0,000001	7,31±0,04 p1=0,0000004	5,43±0,06 p1=0,00001	4,45±0,05 p1=0,00001	4,20±0,04 p1=0,000004	3,34±0,09 p1=0,0010	3,25±0,07 p1=0,0005	3,16±0,04 p1=0,0001
Blood viscosity after incubation with 3e, n=4	7,13±0,08 p1=0,00001 p2=0,000001	5,52±0,30 p1=0,0052 p2=0,0010	4,44±0,09 p1=0,0001 p2=0,0001	4,11±0,03 p1=0,000004 p2=0,0009	3,62±0,15 p1=0,0042 p2=0,0104	3,21±0,04 p1=0,0001 p2=0,2239	3,12±0,03 p1=0,00003 p2=0,1609	3,08±0,03 p1=0,00003 p2=0,1576
3f								
Initial viscosity, n=2	3,42±0,31	3,27±0,25	2,88±0,24	2,58±0,05	2,37±0,05	2,20±0,07	2,14±0,07	3,42±0,31
Blood viscosity after incubation, n=4	7,20±0,04 p1=0,00005	6,16±0,22 p1=0,0013	5,12±0,03 p1=0,0001	4,93±0,04 p1=0,00001	4,33±0,02 p1=0,000002	3,38±0,06 p1=0,0003	3,26±0,08 p1=0,0011	7,20±0,04 p1=0,00005
Blood viscosity after	6,41±0,02	5,21±0,04	4,72±0,09	4,47±0,06	4,11±0,04	3,25±0,03	3,13±0,03	6,41±0,02

incubation with 3f, n=4	p1=0,0001 p2=0,0000004	p1=0,0003 p2=0,0049	p1=0,0007 p2=0,0048	p1=0,00005 p2=0,0009	p1=0,00002 p2=0,0050	p1=0,0001 p2=0,0835	p1=0,0001 p2=0,1991	p1=0,0001 p2=0,0000004
3g								
Initial viscosity, n=2	5,71±0,88	4,14±0,23	3,62±0,01	3,19±0,12	2,74±0,34	2,23±0,05	2,14±0,03	2,03±0,005
Blood viscosity after incubation, n=4	9,50±0,02 p1=0,0022	6,67±0,09 p1=0,0002	5,58±0,22 p1=0,0041	4,82±0,32 p1=0,0295	3,99±0,06 p1=0,0051	3,40±0,14 p1=0,0055	3,03±0,06 p1=0,0005	2,90±0,07 p1=0,0014
Blood viscosity after incubation with 3g, n=4	7,37±0,10 p1=0,0414 p2=0,000001	5,28±0,17 p1=0,0176 p2=0,0003	5,11±0,14 p1=0,0020 p2=0,1200	4,28±0,10 p1=0,0031 p2=0,1657	3,62±0,10 p1=0,0263 p2=0,0204	3,17±0,06 p1=0,0006 p2=0,1777	2,93±0,05 p1=0,0006 p2=0,2655	2,62±0,11 p1=0,0259 p2=0,0876
3h								
Initial viscosity, n=2	5,02±0,34	3,87±0,04	3,39±0,17	3,09±0,25	2,78±0,34	2,37±0,36	2,15±0,22	2,02±0,22
Blood viscosity after incubation, n=4	8,67±0,29 p1=0,0016	6,13±0,28 p1=0,0055	5,18±0,45 p1=0,0595	4,26±0,02 p1=0,0017	3,77±0,10 p1=0,0190	3,65±0,09 p1=0,0079	3,13±0,01 p1=0,0018	2,95±0,07 p1=0,0051
Blood viscosity after incubation with 3h, n=4	6,37±0,14 p1=0,0104 p2=0,0004	4,89±0,17 p1=0,0179 p2=0,0088	4,34±0,32 p1=0,1267 p2=0,1826	3,94±0,13 p1=0,0254 p2=0,0438	3,47±0,14 p1=0,0795 p2=0,1318	3,33±0,15 p1=0,0365 p2=0,1162	2,98±0,04 p1=0,0045 p2=0,0121	2,57±0,14 p1=0,0912 p2=0,0485
Pentoxifylline								
Initial viscosity, n=6	5,94±0,59	4,90±0,43	4,10±0,38	3,87±0,34	3,40±0,29	2,69±0,26	2,32±0,12	2,21±0,12
Blood viscosity after incubation, n=12	7,53±0,45 p1=0,0519	6,36±0,40 p1=0,0364	5,79±0,44 p1=0,0250	5,19±0,31 p1=0,0184	4,37±0,13 p1=0,0026	3,56±0,15 p1=0,0065	2,76±0,09 p1=0,0098	2,53±0,07 p1=0,0218
Blood viscosity after incubation with pentoxifylline, n=12	7,03±0,43 p1=0,1584 p2=0,4306	5,81±0,30 p1=0,1009 p2=0,2800	5,00±0,21 p1=0,0357 p2=0,1205	4,56±0,16 p1=0,0532 p2=0,0855	4,05±0,10 p1=0,0171 p2=0,0631	3,24±0,14 p1=0,0563 p2=0,1353	2,56±0,08 p1=0,0960 p2=0,0999	2,39±0,07 p1=0,1887 p2=0,1590

Note:

n is the number of samples in a group; p is the significance level;

p1<0.05 - statistically significant differences compared to baseline;

p2<0.05 - statistically significant differences compared to the corresponding values in control samples

2.3. Molecular doking and drug-likeness properties evaluation

Currently, modern cytoprotectors are successfully used in the treatment of cerebral hypoxia of various etiologies, as well as in acute and chronic cardiovascular pathology [14-16]. It is known that oxidative stress is considered as one of the important mechanisms of cell cytotoxicity. In medical practice, there is a sufficient arsenal of effective cytoprotectors from the group of pharmacological agents with different mechanisms of action. All of them protect cells from cytotoxic effects of various etiologies [17-20]. For example, a cell dies as a result of exposure to highly reactive oxygen radicals that destroy all types of macromolecules (RNA, DNA, proteins, lipids). At the same time, antioxidants that neutralize harmful oxygen radicals have a cytoprotective effect on cells [21, 22].

In this regard, to evaluate a possible biological target and confirm the previously obtained results of the cytoprotective activity of compounds 3a-h, we used the method of molecular docking. The oxidoreductase enzyme receptor (PDB identifier: 4KEW) [23],

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

which is directly involved in the catalytic processes of biological oxidation, was chosen as the target protein.

Three-dimensional (3D) structures were obtained from the RCSB Protein Data Bank [24], while the ligand molecules were plotted using ChemBio3D Ultra 14.0. The protein structure was prepared for docking by removing a water molecule, a native ligand and adding polar hydrogen atoms, and converted to pdbqt format using the AutoDock MGL software package [25]. The docking process was carried out using AutoDock Vina [26]. For the oxidoreductase enzyme receptor (PDB: 4KEW) [23], active site grid coordinates (X = 14.1084, Y = 19.9038, and Z = 9.73737; size 26 × 20 × 20 Å) were used. The interaction of ligands at binding sites was interpreted using the Discovery Studio Visualizer [27].

The docking results showed that for the studied structures, the free energies (kcal/mol) of complexes with the selected receptors were higher than the free energy of the complex of this protein with the corresponding native ligand (comparison drug Omeprazole).

Table 7. Docking score values of compound **3a-h** and reference drug (**Omeprazole**) for target protein 4KWE, kcal/mol.

Ligand \ **Receptor**	**4KWE**
Omeprazole (co-crystallized)	-8.2
3a	-8.1
3b	-9.2
3c	-8.6
3d	-9.6
3e	-8.9
3f	-8.9
3g	-8.9
3h	-8.8

For compounds 3a–h, the number of intermolecular hydrogen bonds, the binding energies of the stable ligand–receptor complex (4KWE), and the number of nearest amino acid residues were determined (Table 8).

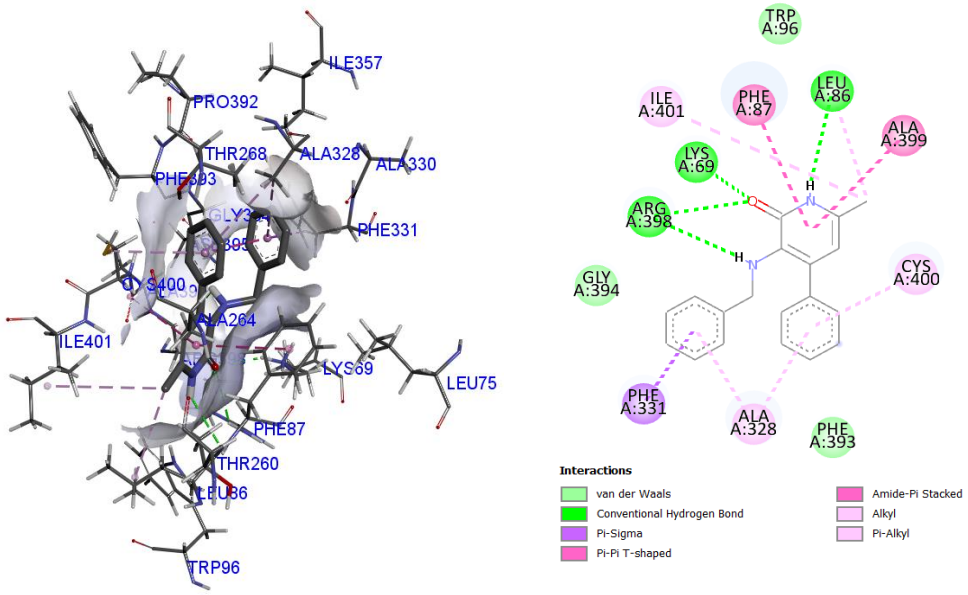
Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

Table 8. Interactions of basic amino acids and H-bonds.

Compound	Receptor	H-bond	Residual Amino acid Interactions	
			Pi-Alkyl, Pi-Sigma, Alkyl, Amide-Pi Stacked, Pi-Pi T-shaped, Pi-Sulfur, Unfavorable Donor-Donor Pi-Sulfur/Pi-Pi Stacked/ Pi-Pi T-shaped/Halogen/ Unfavorable Donor-Donor	Van-der Walls interactions
3a	4KWE	ARG398, LYS69, LEU86	PHE331, ALA328, CYS400, ALA399, LEU86, PHE87, ILE401	PHE393, GLY394, TRP96, THR260
3b		LYS69, ARG398	LEU86, ILE401, CYS400, ALA264, PHE261,PHE87, ALA328	THR268, GLY402, THR260, ARG398, TRP96
3c		LYS69, ARG398, PRO392	PHE393, CYS400, ALA264, PHE87, ILE401, LEU86, TRP96, ALA328	LEU322, THR260, GLY402, HIS100, ALA399
3d		ILE401, ARG398, TRP96, LYS69	LEU86, ALA264, PHE87, ALA328, PHE331, ILE357, CYS400	GLY402, GLY394, PHE393, ASN395
3e		ARG398, LYS69, ALA330	ALA328, PHE87, LEU437, ALA74, ALA330, LEU75, PHE331, LYS69, CYS400	GLY394, PHE393, LEU181, PRO329, VAL78, LEU188, MET354, SER72, SER332, ASN395
3f		ARG398, LYS69, PRO392, THR268	ALA328, PHE393, CYS400, ALA264, TRP96, LEU86, ILE401	ALA399, PHE261, PHE87, THR327, THR260, GLY402, LEU322, HIS100
3g		ARG398, LYS69, ALA399,	CYS400, ALA264, PHE261, ILE401, HIS100, LEU86, TRP96,	PHE87, ASN395, GLY402, THR260, THR268, GLY265, PHE393
3h		ARG398, LYS69, PRO392	ALA328, PHE393, ALA264, CYS400, PHE261, ILE401, TRP96, LEU86, HIS100	GLY402, GLY265, THR260, PHE87, ALA399

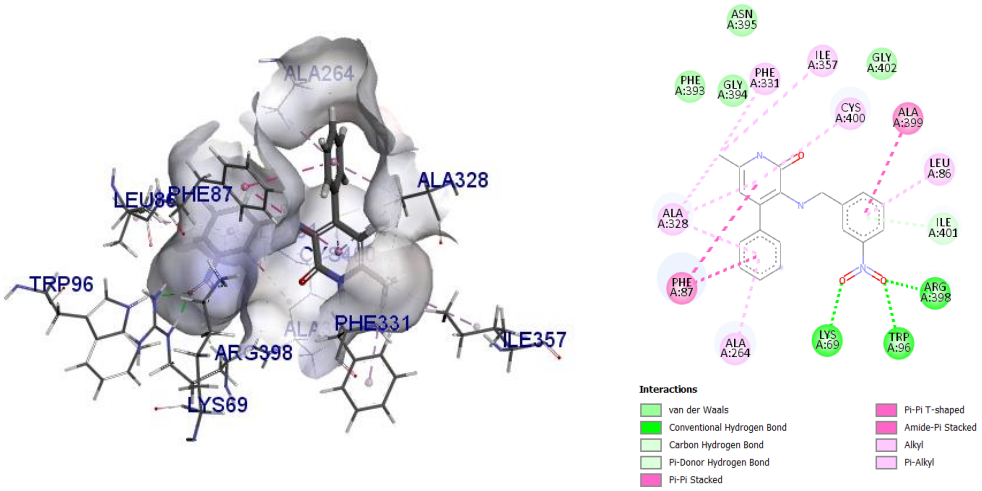
Analysis of the interactions between the 4KWE protein complex and ligands 3a-h showed that all the studied derivatives formed fairly strong complexes with the target receptor protein (Figure 2-5).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1



3D docking model 2D docking model

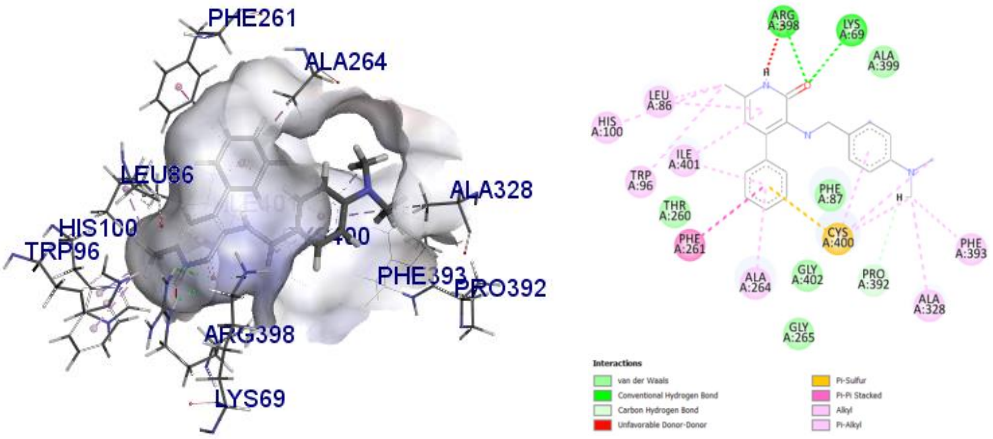
Figure 2. Complex between **3a** and oxidoreductase enzyme receptor (4KWE).



3D docking model 2D docking model

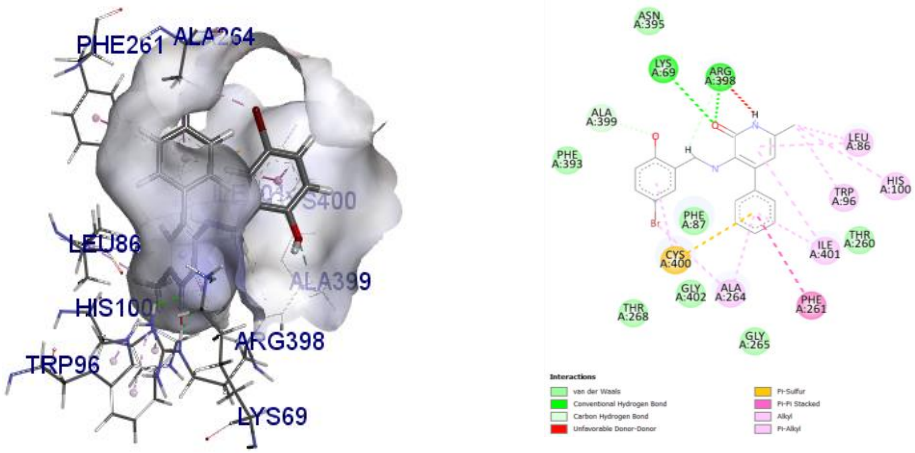
Figure 3. Complex between **3d** and oxidoreductase enzyme receptor (4KWE).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1



3D docking model 2D docking model

Figure 4. Complex between **3h** and oxidoreductase enzyme receptor (4KWE).



3D docking model 2D docking model

Figure 5. Complex between 3g and oxidoreductase enzyme receptor (4KWE).

Thus, the results of computer docking testify to their potential cytoprotective activity. In this case, the possible presence of both acceptor and donor substituents in the methylaryl fragment increases the affinity for the selected receptor protein.

2.4. Compound screening using Lipinski's rule of five

To model the bioavailability of new physiologically active substances for their possible use as an orally active drug, one of the ways to identify compounds with good absorption capacity and moderate lipophilicity is to screen compounds using Lipinski's five rule of thumb rule (RO5). The rule states that good absorption or penetration of a drug is possible if the chemical structure of the drug does not violate more than one of the following criteria/rules:

1) molecular weight less than or equal to 500;
2) LogP (octanol-water partition coefficient) must be less than or equal to 5;
3) Hydrogen bond donors must be less than or equal to 5;
4) There should not be more than 10 hydrogen bond acceptors [28].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

The Molinspiration online tool [29] was used to assess the selected compounds 3a-h with potential cytoprotective activity as the likelihood of their use in medical practice. The results obtained are shown by us in Table 9.

Table 9. Calculated values of compounds 3a-h according to Lipinski's five rule

Ligand	Molecular Weight	miLogP	nHBA	nHBD	nViolation
3a	290,36	3,73	3	2	0
3b	306,36	3,67	4	3	0
3c	320,39	3,79	4	2	0
3d	335,36	3,67	6	2	0
3e	350,41	3,38	5	2	0
3f	350,41	3,77	5	2	0
3g	385,25	4,46	4	3	0
3h	333,43	3,83	4	2	0

The results of the computer screening indicate that all the studied compounds 3a-h did not violate any of the RO5 rules, indicating their possible use as drugs with antiradical and cytoprotective activity.

3. Discussion

The results of the MTT test and the neutral red test can be considered evidence of the presence of cytoprotective activity in 3-arylmethylaminopyridone compounds, in the compound codenamed **3d**. As was determined in the same series of experiments, the investigated compounds of 3-arylmethyl-aminopyridone, including **3d**, have a pronounced potential for antiradical activity in the standard DPPH and ABTS•+ tests. The data obtained allow us to suggest a relationship between the presence of an antiradical potential and manifestations of a cytoprotective effect. The data obtained under the conditions of our experiment support the hypothesis of the possible protection of cellular and intracellular membranes of eukaryotic cells from the damaging effects of free radicals by substances that bind and neutralize free radicals [30]. One can especially confidently judge the relationship between antiradical and cytoprotective actions under conditions of cellular aging [31].

An absolute limitation of this study is the lack of comparison of antiradical and cytoprotective effects *in vivo* using adequate models. This is what is expected to be done in the future.

Thus, for 3-arylmethyl-aminopyridone compounds, an antiradical effect on free radicals DPPH and ABTS•+ has been proven, and the results of in vitro studies on cell culture have been obtained, suggesting the possibility of a cytoprotective effect. Both results obtained under the conditions of this experiment deserve, in our opinion, detailed consideration in the forthcoming extended experiments.

4. Materials and Methods

4.1. Synthesis of compounds

IR spectra were registered on a FT-IR spectrometer FT-801 in KBr pellets. ^{1}H and ^{13}C NMR spectra were acquired on a Jeol JNM-ECA 400 spectrometer (400 and 100 MHz, respectively) in $CDCl_3$ or DMSO-d_6, using TMS or residual signals of the deuterated solvent as internal standard. Signals of protons and ^{13}C atoms were assigned with the aid of APT (Attached Proton Test). The reaction course and purity of the products were

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

checked by thin-layer chromatography on Sorbfil UV-254 plates. Melting points were determined on a Kofler bench. Elemental analysis was performed with a Carlo Erba 1106 CHN analyzer. Aldehydes were commercially available and used as received.

3-Amino-6-methyl-4-phenylpyridine-2(1*H*)-one **1** was synthesized according to procedure [1].

In order to create a library of 3-arylmethyl-aminopyridone derivatives and then evaluate them for possible antiradical and cytoprotective activity, we performed the synthesis of compounds **3a–h** based on 3-amino-6-methyl-4-phenylpyridine-2(1*H*)-one **1**. 3-(Arylmethylamino)-pyridones **3a–g** were obtained in good yields by reacting pyridine-2-one **1** with a series of aromatic aldehydes followed by reduction of imines **2a–g** with sodium borohydride in 2-propanol at 25–35 °C (Scheme 1), their physico-chemical and spectral characteristics are given in the literature [10, 11].

Synthesis of compounds **2a-g** and **3a-g**

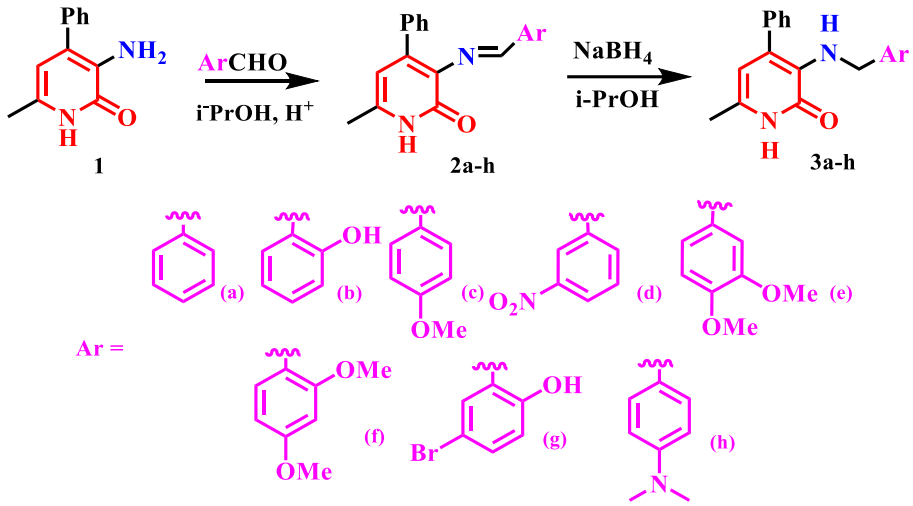


Figure 6. Synthesis of 3-(arylmethylamino)pyridone derivatives **3a–h.**

Preparation of the compounds

(*E*)-3-((4-Dimethylamino)benzylidene)amino)-6-methyl-4-phenylpyridin-2(1*H*)-one (**2h**). The mixture of 3-amino-pyridin-2(1*H*)-one **1** (200 mg, 1 mmol), 654 mg 4-(dimethylamino)benzaldehyde (43 mmol) and catalytic amount of formic acid in 30 ml 2-propanol was refluxed for 8 h. After cooling the reaction mixture, precipitated imine **2h** was filtered off and washed with hexane.Yield: 310 мг (93%), yellow crystals, m.p.: 258-261 °C (2-propanol). IR (KBr): ν, cm^{-1}: 3748, 2894, 1614, 1633. ^{1}H NMR (400 МГц, $CDCl_3$, δ м.д.): 2.36 (s, 3H, CH_3); 3.01 (s, 6H, $N(CH_3)_2$); 6.20 (s, 1H, H-5); 6.66 (d, 2H, J = 7.3, H-3',5' Ar); 7.33-7.36 (m, 3H, H-3,4,5 Ph); 7.51 (d, 2H, J = 7.3, H-2,6 Ph); 7.63 (d, 2H, J = 7.3, H-3',5' Ar); 9.20 (s, 1H, N=CH); 12.55 (br. s, 1H, NH). ^{13}C NMR (100 МГц, $CDCl_3$, δ м.д.): 18.8 (CH_3); 40.2 ($N(\underline{C}H_3)_2$) 108.5 (C-5); 111.4 (C-3',5' Ar); 125.7; 127.5 (C-3,5 Ph); 127.7 (C-4 Ph); 130.1 (C-2',6' Ar); 130.2 (C-2,6 Ph); 133.3 (C-1' Ar); 138.2; 139.1; 143.9; 152.2; 161.3 (C-2); 162.9 (N=$\underline{C}$H). Anal. Calcd for $C_{21}H_{21}N_3O$: C, 76.11; H, 6.39; N, 12.68. Found: C, 75.74; H, 6.81; N, 12.28.

3-((4-Dimethylaminobenzyl)amino)-6-methyl-4-phenylpyridin-2(1*H*)-one (3h). To a suspension of imine **2h** (1.23 g, 3.7 mmol) in 2-PrOH (50 ml) were added water (3 ml) and sodium borohydride (1.119 g, 30 mmol) with stirring at a temperature of 25-35 °C; the reaction mixture was stirred for 10 h. Then the reaction mixture was poured into a beaker with ice-cold water (150 ml). The aqueous layer was extracted with chloroform (3×25 ml), the organic layer was dried over Na_2SO_4, the solvent was removed by distillation, and the residue was triturated with hexane. The crude product was recrystallized from a 1:2 mixture of 2-PrOH and hexane. Yield: 250 mg (75%), yellow crystals, m.p.: 165-168 °C (2-propanol-hexane). IR (KBr, cm^{-1}): 2922, 1643, 1518. ^{1}H NMR (400 MHz,

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 4 August 2022 doi:10.20944/preprints202208.0110.v1

$CDCl_3$) δ: 2.27 (s, 3H, CH_3); 2.89 (s, 6H, $N(CH_3)_2$); 3.70 (s, 2H, N-$C\underline{H}_2$); 4.97 (br. s, 1H, N-H); 5.93 (s, 1H, H-5); 6.60 (d, 2H, *J* = 9.2, H-3',5' Ar); 6.95 (d, 2H, *J* = 8.5 , H-3',5' Ar); 7.32 (t, 1H, *J* = 7.3, H-4 Ph); 7.41 (т, *J* = 7.3, 1H, H-4 Ph) 7.49 (*д*, 2H, *J* = 7.3, H-2,6 Ph); 12.83 (уш.с, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$, δ м.д.): 18.3 (CH_3); 40.7 ($N(\underline{C}H_3)_2$); 50.0 (CH_2-Ar); 109.4 (C-5); 112.5 (C-3',5' Ar); 127.4 (C-4 Ph); 128.2; 128.2 (C-2',6' Ar); 128.3 (C-2,6 Ph); 128.5 (C-3,5 Ph); 131.0; 131.9; 133.4; 139.5; 149.6 (C-4' Ar); 161.6 (C-2). Anal. Calcd for $C_{21}H_{23}N_3O$: C, 75.65; H, 6.95; N, 12.60; Found: C, 75.22; H, 5.65; N, 9.94.

4.2. DPPH and ABTS Radical Scavenging Assay

The antiradical action of the compounds **3h**, **3e**, **3d** was routinely studied in relation to the radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH or DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) [32].

To assess the antiradical activity of the studied samples (**3h**, **3e**, **3d**), the standard DPPH test "Sigma Aldrich" was used in accordance with the manufacturer's instructions. To select substances with pronounced antiradical activity, 2 ml of a 100 μM ethanol solution of DPPH were mixed with 20 μl of the test samples dissolved in methanol at a concentration of 5 mM. Thus, the final concentration of the test substance in the reaction mixture was 50 μM. Ten minutes after adding the test compound solution to the DPPH radical solution, the decrease in absorbance at 515 nm was measured. For substances capable of reducing optical density by more than 50%, a test was performed for interaction with DPPH radical at final concentrations of the studied substances of 50, 25, 20, 15, 10, 5, and 2.5 μM. After that, the concentration of the test substance capable of reducing the optical density by 50% was determined - IC50(DPPH). As a reference drug, a substance with known antiradical properties, ascorbic acid, was used.

The antiradical activity of the presented samples was studied against the radical cation 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) using the Antioxidant Assay Kit (Sigma Aldrich). The principle of the method is the formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes ABTS with the formation of a cation radical - ABTS•+. ABTS•+ is a stable radical that can exist in aqueous solutions for quite a long time, however, the introduction of various antiradical agents into the solution leads to their interaction with ABTS•+ and rapid consumption ("quenching") of the latter. The consumption of ABTS•+ is accompanied by characteristic spectral changes, which make it possible to record the reaction rate. The ability to dose the initial concentration of radicals in the system and control the rate of their "quenching" led to the widespread use of ABTS•+ to standardize the antiradical activity of various compounds [33]. We compared the rate of "quenching" of ABTS•+ by the test substances and the standard, which was used as a semi-synthetic water-soluble analogue of vitamin E, which has the commercial name "Trolox". The use of "Trolox" allows you to evaluate the effectiveness of anti-radical action through the so-called "Trolox equivalent of antioxidant efficiency" - TEAC (Trolox Equivalent Antioxidant Capacity). The TEAC values indicate how much Trolox in mmol/l (mM) "extinguishes" ABTS•+ with the same efficiency as 1 mM of the analyte. The analyzed samples (**3h**, **3e**) were dissolved in DMSO to a concentration of 10 mM. Then twice 10-fold dissolved in 1×Assay Buffer to a final concentration of 0.1 mm. All test substances were tested at a concentration of 0.1 mM.

4.3. Cell Viability Assays

The cytoprotective properties of the presented samples (**3h**, **3e**, **3d**) were evaluated in the MTT test on the breast cancer cell line MCF-7. 1 mg of the test sample was dissolved in 1 ml of DMSO. 10 μl of the dissolved substance was added to 100 μl of the nutrient medium with MCF-7 cells. Cells in nutrient medium without the addition of test compounds served as controls. After cells were incubated for 24 hours with the test objects, viability was determined in the MTT test using the In Vitro Toxicology Assay Kit, MTT based (Sigma Aldrich) according to the manufacturer's instructions.

 doi:10.20944/preprints202208.0110.v1

In Vitro Toxicology Assay Kit Neutral Red Based (Sigma Aldrich) was used on the MCF-7 cell line according to manufacturer's manual to access compounds with pronounced cytoprotective activity.

4.4 Study of hemorheological effects on the model of blood hyperviscosity in vitro

Hyperviscosity syndrome (HBIS) was reproduced in vitro by blood incubation at 43.0°C for 60 minutes. Blood viscosity was measured on a Brookfield DV2T rotational viscometer at various spindle speeds (from 2 to 60 rpm).

Studies of the hemorheological activity of 6 samples were carried out on 12 Wistar female rats, 12 weeks old, weighing 220–240 g. After blood sampling, the initial blood viscosity was determined in laboratory animals, and then blood samples were incubated with the test substances at a temperature of 43.0 °C for 60 min and then measured the parameters under study. The blood was incubated with the test objects dissolved in DMSO, the final concentration of the compounds was 10-5 g/ml of blood. Blood samples to which DMSO solvent was added in an equivolume amount served as controls. As a reference drug, a substance with known hemorheological properties, pentoxifylline, was used at a concentration of 10-5 g/ml of blood.Studies of the hemorheological activity of 6 samples were carried out on 12 Wistar female rats, 12 weeks old, weighing 220–240 g. After blood sampling, the initial blood viscosity was determined in laboratory animals, and then blood samples were incubated with the test substances at a temperature of 43.0 °C for 60 min and then measured the parameters under study. The blood was incubated with the test objects dissolved in DMSO, the final concentration of the compounds was 10-5 g/ml of blood. Blood samples to which DMSO solvent was added in an equivolume amount served as controls. As a reference drug, a substance with known hemorheological properties, pentoxifylline, was used at a concentration of 10-5 g/ml of blood. [34, 35]. Blood incubation for 1 hour under these conditions was accompanied by the formation of blood hyperviscosity [36]. The initial blood viscosity from each animal was measured once, the blood viscosity after incubation was measured in two samples from each animal, both in control and experimental samples.

4.5 Ethical considerations

All research work with laboratory animals was performed in accordance with generally accepted ethical standards for the treatment of animals, based on standard operating procedures that comply with the rules adopted by the European Convention for the Protection of Vertebrate Animals used for Research and other Scientific Purposes (Strasbourg, 1986). The study protocol of the project "Search for means of pharmacological correction of increased blood viscosity syndrome associated with endocrine pathology" was approved on August 7, 2020 (Protocol No. 3) by the Local Ethics Commission of the Republican State Enterprise "National Center for Biotechnology" (IRB00013497 National Center of Biotechnology IRB #1).

4.6. Data Evaulation

Statistical processing of the results was carried out using the Excel program. The results obtained are presented as "mean ± standard error of the mean".

Author Contributions: Conceptualization, S.S., Z.S., Y.R. and I.V.K.; Methodology, Z.S., A.Z. and I.P.; validation, S.S., Y.R. and I.V.K.; investigation, Z.S. and A.Z.; writing—original draft preparation, S.S.; writing—review and editing, Y.R.; visualization, I.V.K.; supervision, Y.R.; funding acquisition, Z.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan (grant "Search for means of pharmacological correction of increased blood viscosity syndrome associated with endocrine pathology," Grant no. AP09058121).

Institutional Review Board Statement: IRB00013497 National Center of Biotechnology IRB #1

Informed Consent Statement: Not applicable

Data Availability Statement: Not applicable

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Sample Availability: Samples of the compounds are available from the authors.

References

1. Kulakov, I.V.; Matsukevich, M.V.; Shulgau, Z.T.; Sergazy, S.; Seilkhanov, T.M.; Puzari, A.; Fisyuk, A.S. Synthesis and antiradical activity of 4-aryl(Hetaryl)-substituted 3-aminopyridin-2(1*H*)-ones. *Chem. Heterocycl. Compd.* **2015**, *51*, doi:10.1007/s10593-016-1809-7.
2. Kusakabe, K.I.; Tada, Y.; Iso, Y.; Sakagami, M.; Morioka, Y.; Chomei, N.; Shinonome, S.; Kawamoto, K.; Takenaka, H.; Yasui, K.; et al. Design, synthesis, and binding mode prediction of 2-pyridone-based selective CB2 receptor agonists. *Bioorganic Med. Chem.* **2013**, doi:10.1016/j.bmc.2013.01.006.
3. Zhang, Y.M.; Fan, X.; Chakaravarty, D.; Xiang, B.; Scannevin, R.H.; Huang, Z.; Ma, J.; Burke, S.L.; Karnachi, P.; Rhodes, K.J.; et al. 1-Hydroxy-2-pyridinone-based MMP inhibitors: Synthesis and biological evaluation for the treatment of ischemic stroke. *Bioorganic Med. Chem. Lett.* **2008**, doi:10.1016/j.bmcl.2007.10.045.
4. Ward, A.; Brogden, R.N.; Heel, R.C.; Speight, T.M.; Avery, G.S. Amrinone: A Preliminary Review of its Pharmacological Properties and Therapeutic Use. *Drugs* **1983**, doi:10.2165/00003495-198326060-00002.
5. Hoffman, J.M.; Wai, J.S.; Thomas, C.M.; Levin, R.B.; O'Brien, J.A.; Goldman, M.E. Synthesis and Evaluation of 2-Pyridinone Derivatives as HIV-1 Specific Reverse Transcriptase Inhibitors. 1. Phthalimidoalkyl and -alkylamino Analogs. *J. Med. Chem.* **1992**, doi:10.1021/jm00099a006.
6. Saari, W.S.; Wai, J.S.; Fisher, T.E.; Thomas, C.M.; Hoffman, J.M.; Rooney, C.S.; Smith, A.M.; Jones, J.H.; Bamberger, D.L.; Goldman, M.E.; et al. Synthesis and Evaluation of 2-Pyridinone Derivatives as HIV-1-Specific Reverse Transcriptase Inhibitors. 2. Analogues of 3-Aminopyridin-2(1*H*)-one. *J. Med. Chem.* **1992**, doi:10.1021/jm00099a007.
7. Verissimo, E.; Berry, N.; Gibbons, P.; Cristiano, M.L.S.; Rosenthal, P.J.; Gut, J.; Ward, S.A.; O'Neill, P.M. Design and synthesis of novel 2-pyridone peptidomimetic falcipain 2/3 inhibitors. *Bioorganic Med. Chem. Lett.* **2008**, doi:10.1016/j.bmcl.2008.05.068.
8. Zhu, S.; Hudson, T.H.; Kyle, D.E.; Lin, A.J. Synthesis and in vitro studies of novel pyrimidinyl peptidomimetics as potential antimalarial therapeutic agents. *J. Med. Chem.* **2002**, doi:10.1021/jm020104f.
9. Kulakov, I. V.; Nikitina, O.S.; Fisyuk, A.S.; Goncharov, D.S.; Shul'Gau, Z.T.; Gulyaev, A.E. Synthesis and intramolecular cyclization of N-acyl- and N-allyl-N'-(2-oxo-1,2-dihydro-pyridin-3-yl)thiourea. *Chem. Heterocycl. Compd.* **2014**, doi:10.1007/s10593-014-1519-y.
10. Kulakov, I. V.; Palamarchuk, I. V.; Shulgau, Z.T.; Seilkhanov, T.M.; Gatilov, Y. V.; Fisyuk, A.S. Synthesis, structure and biological activity 3-(arylmethyl)aminopyridine-2(1*H*)-ones and 1*H*-pyrido[2,3-b][1,4]oxazin-2(3*H*)-ones. *J. Mol. Struct.* **2018**, doi:10.1016/j.molstruc.2018.04.036.
11. Palamarchuk, I. V.; Shulgau, Z.T.; Kharitonova, M.A.; Kulakov, I. V. Synthesis and

neurotropic activity of new 3-(arylmethyl)aminopyridine-2(1*H*)-one. *Chem. Pap.* **2021**, doi:10.1007/s11696-021-01696-7.

12. Pizzino, G.; Irrera, N.; Cucinotta, M.; Pallio, G.; Mannino, F.; Arcoraci, V.; Squadrito, F.; Altavilla, D.; Bitto, A. Oxidative Stress: Harms and Benefits for Human Health. *Oxid. Med. Cell. Longev.* **2017**, doi:10.1155/2017/8416763.
13. Parcheta, M.; Świsłocka, R.; Orzechowska, S.; Akimowicz, M.; Choińska, R.; Lewandowski, W. Recent developments in effective antioxidants: The structure and antioxidant properties. *Materials (Basel).* **2021,** doi:10.3390/ma14081984.
14. Hill, M.D.; Goyal, M.; Menon, B.K; Nogueira, R.G.; McTaggart, R.A.; Demchuk, A.M.; et.al. Efficacy and safety of nerinetide for the treatment of acute ischaemic stroke (ESCAPE-NA1): a multicentre, double-blind, randomised controlled trial. *Lancet,* **2020,** doi: 10.1016/S0140-6736(20)30258-0.
15. Martinel Lamas, D.J.; Nicoud, M.B.; HA Sterle, H.A.; Cremaschi, G.A.; Medina, V.A. Histamine: a potential cytoprotective agent to improve cancer therapy? *Cell Death and Disease,* **2015,** doi:10.1038/cddis.2015.378.
16. Labiche, L.A.; Grotta, J.C. Clinical Trials For Cytoprotection In Stroke. *NeuroRX,* **2004,** doi:10.1602/neurorx.1.1.46
17. Zhao, B. Natural antioxidants protect neurons in Alzheimer's disease and Parkinson's disease. *Neurochem. Res.* **2009,** doi: 10.1007/s11064-008-9900-9.
18. Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Med. Cell. Longev.* **2009,** doi: 10.4161/oxim.2.5.9498.
19. Mehta, J.; Rayalam, S.; Wang, X. Cytoprotective Effects of Natural Compounds against Oxidative Stress. *Antioxidants*, **2018,** doi:10.3390/antiox7100147.
20. Dinkova-Kostova, A.T.; Talalay, P. Direct and indirect antioxidant properties of inducers of cytoprotective proteins. *Mol. Nutr. Food Res.* **2008,** doi: 10.1002/mnfr.200700195.
21 Clark, I.A.; Cowden, W.B.; Hunt N.H. Free radical-induced pathology. *Med Res Rev.* **1985**, doi: 10.1002/med.2610050303.
22. Sarangarajan, R.; Meera, S.; Rukkumani, R.; Sankar, P.; Anuradha, G. Antioxidants: Friend or foe? *Asian Pac J Trop Med.* **2017**, doi: 10.1016/j.apjtm.2017.10.017.
23. Lorthiois, E.; Roache, J.; Barnes-Seeman, D.; Altmann, E.; Hassiepen, U.; Turner, G.; et al. Structure-Based Design and Preclinical Characterization of Selective and Orally Bioavailable Factor XIa Inhibitors: Demonstrating the Power of an Integrated S1 Protease Family Approach. *J Med Chem.* **2020,** doi: 10.1126/science.1239248.
24. https://www.rcsb.org/
25. Morris, G.M.; Huey, R.; Lindstrom, W.; Sanner, M.F.; Belew, R.K.; Goodsell, D.S.; Olson, A.J.; Autodock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *J. Comp. Chem.,* **2009,** doi:10.1002/jcc.21256.
26. Trott, O.; Olson, A.J.; AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.,* **2010,** doi: 10.1002/jcc.21334.
27. BIOVIA, Dassault Systemes,v20. 1. 0. 19,295, BIOVIA Discovery Studio, Dassault Systemes: San Diego, CA, USA, **2020.**
28. Lipinski, C.A.; Lead- and drug-like compounds: the rule-of-five revolution. *Drug Discov. Today Technol.,* **2004,** doi: 10.1016/j.ddtec.2004.11.007.

29. https://molinspiration.com/
30. Egea, J.; Fabregat, I.; Frapart, Y.M.; Ghezzi, P.; Görlach, A.; Kietzmann, T.; Kubaichuk, K.; Knaus, U.G.; Lopez, M.G.; Olaso-Gonzalez, G.; et al. European contribution to the study of ROS: A summary of the findings and prospects for the future from the COST action BM1203 (EU-ROS). *Redox Biol.* **2017**, doi: 10.1016/j.redox.2017.05.007.
31. Vatner, S.F.; Zhang, J.; Oydanich, M.; Berkman, T.; Naftalovich, R.; Vatner, D.E. Healthful aging mediated by inhibition of oxidative stress. *Ageing Res. Rev.* **2020,** doi: 10.1016/j.arr.2020.101194.
32. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm Wiss Technol.* **1995**, doi: 10.1016/S0023-6438(95)80008-5.
33. Miller, N.J.; Rice-Evans, C.; Davies, M.J.; Gopinathan, V.; Milner, A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin. Sci.* **1993**, doi:10.1042/cs0840407.
34. Plotnikov M.B.; Shamanaev A.Y.; Aliev O.I.; Sidekhmenova A.V.; Anishchenko A.M.; Arkhipov A.M. Pentoxifylline treatment enhances antihypertensive activity of captopril through hemorheological improvement in spontaneously hypertensive rats during development of arterial hypertension. *J Am Soc Hypertens.* **2017,** doi: 10.1016/j.jash.2017.09.007.
35. Słoczyńska K.; Kózka M.; Pękala E.; Marchewka A.; Marona H. In vitro effect of pentoxifylline and lisofylline on deformability and aggregation of red blood cells from healthy subjects and patients with chronic venous disease. *Acta Biochim Pol.* **2013**, doi: 10.18388/ABP.2013_1962.
36. Plotnikov, M.B.; Koltunov, A.A.; Aliyev, O.I. Method of selection of medicinal substances affecting the rheological properties of blood in vitro // Experimental and clinical pharmacology. - **1996**. - No. 6. - pp. 57-58.