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FULL PAPER

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Isatin-3-acylhydrazones with Enhanced Lipophilicity: Synthesis, Antimicrobial Activity Evaluation and the Influence on Hemostasis System

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Water-soluble trialkylammonium isatin-3-hydrazone derivatives bearing phenolic substituent were easily synthesized with high yields. XRD studies confirmed the presence of these compounds as *trans*-(*Z*)-isomers in a crystal. It was shown that an increase in the lipophilicity of the cationic center leads to an increase in activity against Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*, including methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The MIC values of all compounds turned out to be 2–100 times higher than the MIC of norfloxacin against the MRSA strains in the absence of hemo- and cytotoxicity. Antiaggregation and anticoagulation properties were in vitro better than for acetylsalicylic acid and sodium heparin drugs. It has been shown by UV spectroscopy and fluorescence microscopy that the mechanism of antimicrobial action of new acylhydrazones is associated with their ability to destroy the bacterial cell membrane.

Keywords: quaternary ammonium salts, heterocycles, antimicrobial activity, cytotoxicity, isatin, hydrazones, hemostasis, lipophilicity.

Introduction

Currently, the World Health Organization names antibiotic resistance as one of the most serious threats to animal and human health. More than 700,000 people worldwide die each year from infections caused by resistant microbes. For other patients, the hospitalization time is lengthened. Leading researchers predict that in 30 years each year 10 million people will die from resistant microbes. At the same time, the economic damage is estimated at billions of dollars.[1–3] One of the ways to solve this acute problem is the search and development of new drugs that are effective against resistant pathogens. In this regard, one of the most promising classes of pharmacologically active compounds are quaternary ammonium compounds (QACs) - from small cationic molecules to complex polycyclic natural structures.[4–7]

In the search and development of new candidates for medicinal substances, not only their effectiveness and low toxicity are important, but also understanding the possibility of side effects. For example, due to the high risk of various cardiovascular diseases, the antiarthritic drug Vioxx (rofecoxib) was withdrawn from the market, the clinical studies of which did not include determining its effect on the hemostatic system[8,9] Thus, along with the determination of the

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target type of biological activity of new compounds, it seems appropriate to also study their antiaggregation and anticoagulant activities.

Being a member of the 'privileged structure' class, the isatin heterocyclic platform is widely used in pharmaceutical chemistry[10,11] Numerous studies have shown that derivatives of this heterocycle exhibit various types of biological activity, such as antiviral, antifungal, antibacterial, antiproliferative, antitumor, etc.[12–19] However, the usually low solubility of these compounds in biocompatible solvents does not allow more detailed studies of other types of their activity. In this regard, it is not unreasonable to believe that the combination of a planar and highly conjugated oxindole fragment and an ammonium center can lead to a strong change in the lipophilicity of such hybrid molecules and, consequently, to a change in antimicrobial efficiency. Moreover, it is known that the antimicrobial effect of ammonium compounds strongly depends on their lipophilicity.[20–23]

Several years ago, we first described the synthesis of a new type of water-soluble isatin acylhydrazones, containing a quaternized nitrogen atom, with low toxicity and selective activity against Gram-positive bacterial pathogens.[24–31] We showed that the most active derivatives of this series against *Staphylococcus aureus* (*S. aureus*) and *Bacillus cereus* (*B. cereus*) were hydrazones containing a sterically hindered phenolic fragment (*Figure 1*).

Results and Discussion

Continuing to develop this direction, we first began to study the platelet aggregations and blood coagulation activities of isatin-3-acylhydrazones and expanded the study of the effect of the ammonium center structure on the level of their antimicrobial activity. Thus, we decided to replace the structurally rigid cationic center with a more labile and lipophilic diethylmethyl one compared to the previously described structures of isatin acylhydrazones. In this work, we have evaluated the effect of a weak lipophilicity adjustment both on the level of antimicrobial activity and on some factors of hemostasis (platelet aggregations and blood coagulation).

For the synthesis of the target compounds, a simple synthetic approach was chosen, consisting in the condensation of benzylated isatins **1a**–**h** which were obtained by the procedure described earlier[27] with ammonium acetohydrazide **2** (*Scheme 1*). This interaction proceeds in ethanol at reflux temperature for 3 h to give desired compounds with 87–97% yields. Taking into account the influence of the nature of the substituent in the aromatic fragment on the degree of manifestation of the antimicrobial activity of the structures under study,[27] in this case, derivatives **3a**–**h** were obtained containing both electron-donor (methyl, methoxy groups) and electron-withdrawing substituents (halogen atoms, fused benzo fragment). All of the target compounds were obtained in pure form directly from the reaction vessel as yellow solids.

The structures of **3a**–**h** characterized by using their IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectral data. For compounds **3e** and **3g** the X-ray analysis has been also performed (crystal structure and supramolecular packaging of these molecules see in SI).

At the first step of biological activity evaluation of hydrazones **3a**–**h**, we assessed the solvent effect on the parameters of platelet aggregation and coagulograms. Coagulogram is a complex hematological study aimed at assessing the state of the hemostasis system (blood coagulation), or blood coagulation indicators. The hemostasis system includes blood cells (platelets) and specific substances (coagulation factors) dissolved in blood plasma and contained in platelets.

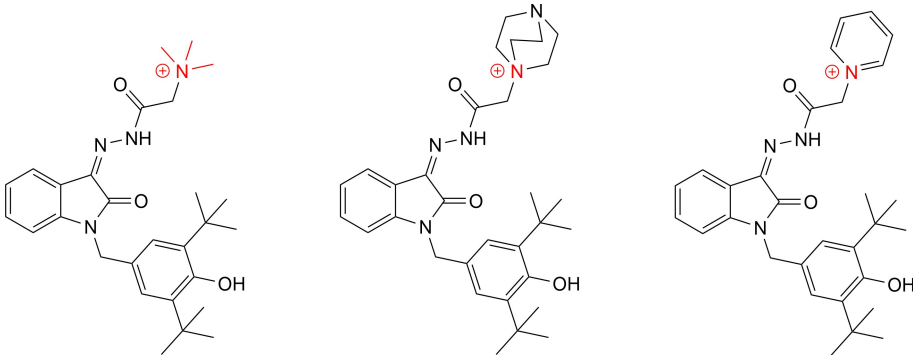
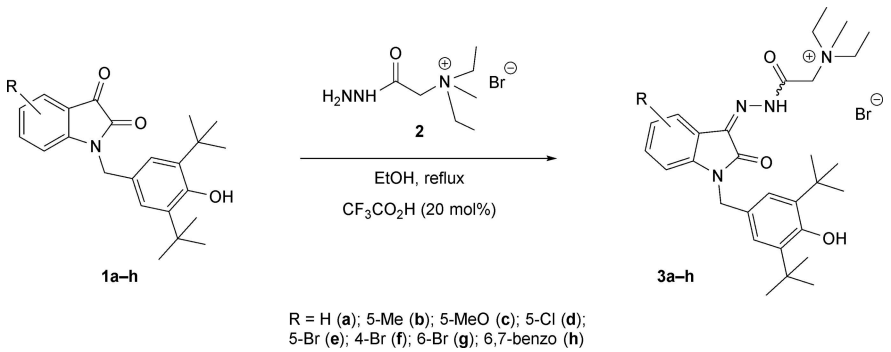


Figure 1. The previously described isatin-acylhydrazones with different types of rigidity and lipophilicity of ammonium cations.



Scheme 1. Synthetic pathway to methyl(diethyl)ammonium isatin-3-acylhydrazones.

Table 1. Anticoagulant and antiaggregation activity of compounds **3a**–**h**.

Compound	Platelets aggregation, % of control		APTT[$$], % of control
	ADP[$]	Collagen	
3a	−87.1 (82.3–89.1)**, ##	−79.4 (75.6–82.4)**, ##	+50.2 (47.8–63.2)**, †
3b	−11.7 (9.6–14.5)*	−10.4 (8.3–11.9)*	+10.4 (8.3–11.9)*, ††
3c	−21.9 (18.4–23.1)**, #	−18.6 (17.1–20.3)**, #	+18.6 (17.1–20.3)**
3d	−14.6 (13.7–16.8)*	−13.1 (11.6–15.2)**	+13.1 (11.6–15.2)*, †
3e	−14.2 (11.3–16.7)*	−12.3 (10.1–13.5)*	+12.3 (10.1–13.5)**, †
3f	−12.7 (11.3–15.2)*	−10.1 (9.1–12.3)*	+10.1 (9.1–12.3)**, ††
3g	−18.2 (17.4–20.1)**, #	−16.6 (14.8–19.5)**, #	+16.6 (14.8–19.5)**, †
3h	−21.3 (17.5–22.4)**, #	−16.7 (14.1–19.5)**	+16.7 (14.1–19.5)**, †
Acetylsalicylic acid	−13.7 (10.8–16.4)*	−14.7 (12.1–16.3)**	–
Heparin sodium	–	–	+20.3 (19.7–21.4)**

*$p \leq 0.05$, **$p \leq 0.001$ - compared to control; #$p \leq 0.05$, ##$p \leq 0.001$ - compared to acetylsalicylic acid; †$p \leq 0.05$, ††$p \leq 0.001$ - compared to Heparin sodium, [$] ADP – adenosine diphosphate, [$$] APTT - activated partial thromboplastin time.

DMSO did not produce any effect on the parameters of platelet aggregations and blood coagulation. The parameters of the anticoagulant and antiaggregation activity of the novel compounds are presented in *Table 1*.

The compounds demonstrated varying extent of the effect on the plasmatic component of hemostasis system that manifested only by a change in the parameter APTT of the intrinsic coagulation pathway. Compounds demonstrated anticoagulant activity $\geq$ 10% ($P < 0.05$). Regarding the impact on platelet aggregation the compounds demonstrated similar activity on both aggregation inductors. Compounds **3b**, **d**–**f** demonstrated antiaggregation activity *in vitro* at the level of acetylsalicylic acid. Compounds **3a**, **c**, **g**, **h** demonstrated antiaggregation activity exceeding indices of acetylsalicylic acid. The most promising active compound is **3a**. In screening concentrations *in vitro* this hydrazone is more active than acetylsalicylic acid (>80%) and heparin sodium (>59.2%) in terms of antiaggregation and anticoagulation activity, correspondingly.

The study of acylhydrazones **3b**–**h** showed high antimicrobial activity against *S. aureus* 209P (*Sa*), *B. cereus* 8035 (*Bc*), including methicillin-resistant strains of *S. aureus* MRSA 1 (resistant to fluoroquinolones) and MRSA 2 (resistant to both fluoroquinolones and b-lactams) (*Table 2*). Data for the compound **3a** are omitted since they were published earlier.[26]

The MBC/MIC ratios were calculated in order to determine bactericidal or bacteriostatic effect of the tested substances. As it can be seen from the obtained data, all the compounds (except for **3b** and **3c** containing electron donor groups) possess bactericidal properties (MBC/MIC $\leq$ 4) with respect to all *Sa* strains tested. Against *Bc* only **3b**, **c**, **e** are able to act bacteriostatically.

One of the important indicators of the effectiveness and safety of a new drug candidate is the level of its effect on red blood cells and normal human tissue cells. Following this requirement, we determined the

Table 2. Antimicrobial activity of compounds **3b**–**h***.

Compound	Minimum inhibitory concentration (MIC), μM			
	Sa	*Bc*	*MRSA-1*	*MRSA-2*
3b	3.2 ± 0.2	6.5 ± 0.5	13.0 ± 1.1	13.0 ± 1.2
3c	6.3 ± 0.5	12.6 ± 1.1	25.3 ± 1.9	25.3 ± 1.8
3d	12.6 ± 1.1	25.1 ± 1.9	50.0 ± 4.2	50.0 ± 4.4
3e	5.9 ± 0.4	5.9 ± 0.3	11.7 ± 9	23.4 ± 1.8
3f	5.9 ± 0.4	46.9 ± 3.7	23.4 ± 1.7	46.9 ± 3.8
3g	5.9 ± 0.4	5.9 ± 0.4	23.4 ± 1.7	23.4 ± 1.6
3h	6.1 ± 0.5	24.5 ± 1.7	24.5 ± 1.9	24.5 ± 1.8
Norfloxacin	7.5 ± 0.5	24.4 ± 2.1	391.4 ± 30	30.0 ± 2.6
	Minimum bactericidal concentration (MBC), μM			
3b	13.0 ± 10	104 ± 9.2	52 ± 4.6	13.0 ± 1.1
3c	51.0 ± 4.4	101 ± 9.2	25.3 ± 1.8	51.0 ± 4.6
3d	12.6 ± 1.2	50.0 ± 4.5	50.0 ± 4.4	50.0 ± 4.3
3e	5.9 ± 0.4	46.9 ± 3.9	23.4 ± 1.7	23.4 ± 1.8
3f	23.4 ± 1.7	93.8 ± 7.9	23.4 ± 1.8	46.9 ± 3.9
3g	5.9 ± 0.5	11.7 ± 1.1	23.4 ± 1.9	93.8 ± 8.6
3h	12.3 ± 1.1	24.5 ± 1.7	24.5 ± 1.9	24.5 ± 1.9
Norfloxacin	7.5 ± 0.6	24.4 ± 2.1	na	na

**Sa*, *Staphylococcus aureus*; *Bc*, *Bacillus cereus* MIC – minimal inhibitory concentration, MBC – minimal bactericidal concentration; n.a. – no activity. Average of three values measured; ± standard deviation (SD).

hemolytic activity and cytotoxicity of hydrazones **3a**–**h** (*Table 3*) in relation to liver cells (Chang liver cell lines). It can be seen that the values of these parameters are significantly higher than corresponding MICs and MBCs. The study showed that all the compounds obtained did not cause hemolysis in the MIC region. Among the obtained series of compounds, 5- and 6-halogenated acylhydrazones **3d**, **e**, **g** showed the highest hemotoxicity, although their HC_{50} values are 5–8 times better than Gramicidin S. The data obtained also indicate the absence of cytotoxicity against the Chang liver cell lines.

The selectivity of the compounds for microbial cells is an important criterion for assessing the cytotoxic effect. This indicator is characterized by the selectivity index (SI) value, which is calculated as the ratio between the HC_{50} value for erythrocytes (IC_{50} for eukaryotic cells) and the MIC value (MBC) for microbial cells. The results obtained indicate a high selectivity of the studied compounds to Gram-positive bacteria *S. aureus*, *B. cereus*, *MRSA-1* and *MRSA-2* at MIC(MBC) concentrations and low toxicity in relation to eukaryotic cells of human blood and liver (*Tables 4*, *5*) The highest potential in terms of hemotoxicity among the newly synthesized ammonium hydrazones is possessed by compounds **3f** and **3h** containing a bromine atom in position 4 and a fused [6,7]benzo fragment, respectively. Based on the calculated IC_{50}/MIC and IC_{50}/MBC values, the leaders in this series are halogen-containing hydrazones **3d**, **f**, **g**.

Table 3. Hemolytic activity and cytotoxicity of **3b**–**h**.

Compound	HC_{50} (μM)	IC_{50} (μM)
3b	144	217
3c	549	421
3d	73.8	2500
3e	57.5	204
3f	165	2345
3g	46.9	2345
3h	395	350
Gramicidin S	9.4 ± 0.8	–
Doxorubicin	–	3.0 ± 0.1

The membranotropic action of the studied ammonium salts was determined by the absorption of the crystal violet (CV) dye, since the increased penetrating ability of the CV dye implies a change in the permeability of the cytoplasmic membrane.[32] The data obtained showed that the most active membranotropic effect is manifested by 5-bromo derivative **3e**. At a concentration of 6 μM, corresponding to its MIC and MBC, the CV uptake was 55.4%. Significant changes in permeability caused by exposure to other compounds are observed at concentrations ≥ 12 μM (*Figure 2*). To visualize the effect of the bacterial cell membrane destruction, fluorescence microscopy was

Table 4. Hemolytic activity and selectivity index of **3b**–**h***.

Compound	*Sa*		*Ba*		MRSA-1		MRSA-2	
	HC_{50}/MIC	HC_{50}/MBC	HC_{50}/MIC	HC_{50}/MBC	HC_{50}/MIC	HC_{50}/MBC	HC_{50}/MIC	HC_{50}/MBC
3b	45	11	22.2	1.4	11	2.8	11	11
3c	87	11	43.6	5.4	21.7	21.7	21.7	11
3d	5.9	5.9	2.9	1.5	1.5	1.5	1.5	1.5
3e	9.7	9.7	9.7	1.2	4.9	2.5	2.5	2.5
3f	28.0	7	3.5	1.8	7	7	3.5	3.5
3g	7.9	7.9	7.9	4.0	2.0	2.0	2.0	0.5
3h	64.8	32	16.1	16.1	16.1	16.1	16.1	16.1

* – The experiments were repeated for three times.

Table 5. Cytotoxic activity and SI of **3b**–**h***.

Compound	*Sa*		*Ba*		MRSA-1		MRSA-2	
	IC_{50}/MIC	IC_{50}/MBC	IC_{50}/MIC	IC_{50}/MBC	IC_{50}/MIC	IC_{50}/MBC	IC_{50}/MIC	IC_{50}/MBC
3b	67.8	16.7	33.4	2.1	67.8	4.2	67.8	67.8
3c	66.8	8.3	33.4	4.2	16.6	16.6	16.6	4.1
3d	198	198	100	50	50	50	50	50
3e	34.6	34.6	34.6	4.3	17.4	8.7	8.7	8.7
3f	397	100	50	25	100	100	50	50
3g	397	397	397	200	100	100	100	25
3h	57.3	28.5	14.3	14.3	14.3	14.3	14.3	14.3

* – The experiments were repeated for three times.

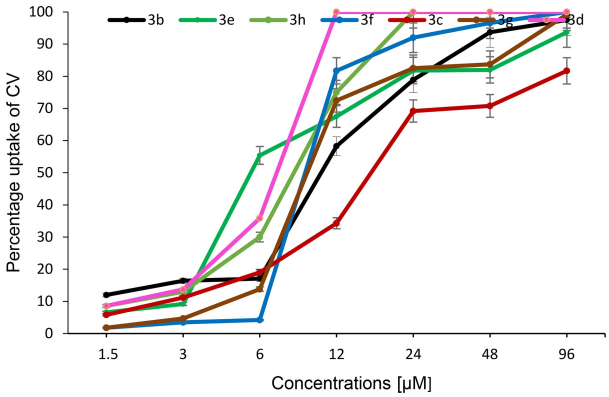


Figure 2. CV uptake analysis. Percentage of crystal violet in *Sa* supernatant after 30 min incubation with compounds **3b**–**h**. The optical density of the sample with the dye, in the absence of bacterial cells, was taken as 100 %.

used. The use of two dyes (green-fluorescent dye SYTO and red-fluorescent dye propidium iodide) makes it possible to assess the ratio of living and dead *S. aureus* cells after exposure to the tested compounds.[32] The results obtained showed that with an increase in the concentration of compounds, the number of dead cells (colored red) increases in comparison with the control (*Figure 3*). A quantitative assessment of the effects of tested compounds on *S. aureus* cells is presented in *Figure 4*.

The results obtained confirm the data of the experiment with CV. The effect of compounds **3b**–**h** was studied in the range of the corresponding MIC and MBC. It can be seen that the number of cells stained red (with damaged membranes) in all studied compounds significantly increases in MBC. Apparently, the processes of destabilization of the cytoplasmic membrane are just beginning when bacteria are cultured in the presence of the tested compounds in the MIC.

Enhanced lipophilicity of the compounds **3b**–**h** compared to previously reported trimethylammonium analogs indeed led to increase in activity level towards bacterial strains tested.[27] However, it appeared that another crucial factor is electron density change on isatin fragment provided by variation of substituents. Chemicalize was used for LogD(7.4) prediction, developed by ChemAxon.[33] As can be seen in the *Figure 5*, that represents these correlations, electron-donation leads to the highest activity in the range of LogD values between 1 and 2. Nonetheless, the most lipophilic derivatives are the ones with halogen and 6,7-benzo fragments still show activity below 15 μM, except for di-halogen derivative. This fact shows that

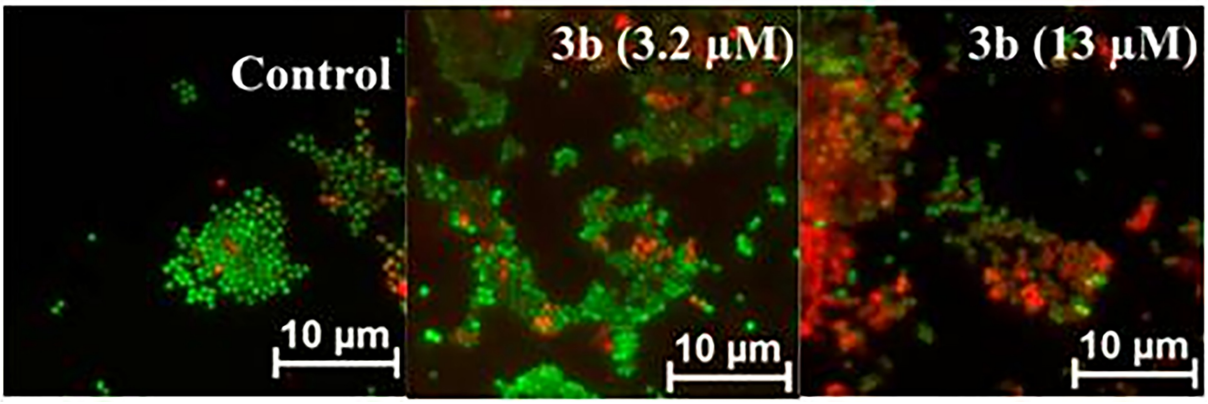


Figure 3. FM images of *Sa* bacteria stained by SYTO and propidium iodide in the presence of test compounds (on the example of **3b**).

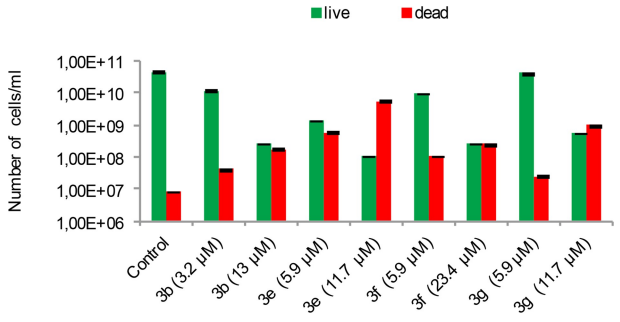


Figure 4. Quantitative assessment of the effect of test compounds on the permeability of the *S. aureus* cytoplasmic membrane. Control - intact *S. aureus* cells.

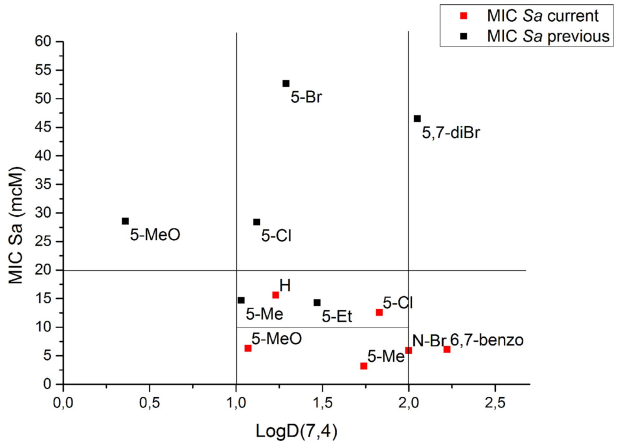


Figure 5. Summarized results from current and previous[27] works for *Sa* MIC correlation to LogD(7.4) and substituent on isatin fragment. Symbol N in N-Br is for 4/5/6-substituted derivatives.

both lipophilicity and increased electron density are of a high importance for antibacterial activity of the tested compounds. MIC values for *Bc* and MRSA strains follow almost the same pattern with slightly lower preference for substitution at position 4 for *Bc* and *MRSA-2*. Data for the chart can be found in SI.

Conclusions

In summary, a series of water-soluble methyl(diethyl)ammonium isatin-3-acylhydrazone analogs with different substituents in the aromatic ring have been synthesized and tested for their antimicrobial, anticoagulant and antiaggregation activities. Experimental data, coupled with theoretical calculations, proved the key role of the lipophilicity of ammonium isatin-3-acylhydrazones molecules and the electron density distribution on the oxindole cycle in the level of activity. Compounds **3e**, **g**, bearing bromine atoms in position 5 or 6, and naphtho-fused **3h** were found to be the most active against museum strains of *S. aureus* and *B. cereus*. In terms of MBC values, they are better or comparable to the activity of norfloxacin. With the exception of chloro derivative **3d**, all compounds were more active than norfloxacin against MRSA strains in the absence of hemo- and cytotoxicity in the MIC range. It was shown that the mechanism of the antimicrobial action of the new compounds is associated with a violation of the integrity of the bacterial cell membrane. The initial study of this series of compounds also showed good prospects in the search for new non-toxic anticoagulant drugs, superior in their activity to acetylsalicylic acid and heparin sodium.

Experimental Section

General

Commercially available starting materials and solvents were used without prior purification. Infrared spectra were measured with a Bruker Vector-22 instrument for the samples in KBr pellets. ^{1}H and ^{13}C-NMR spectra were recorded on a Bruker Avance-400 or Bruker Avance-600 Bruker spectrometers at 400, 600 and 100, 150 MHz, respectively. Chemical shifts were reported in ppm relative to residual signals of deuterated solvents. $CDCl_3$, D_2O, $(D_6)DMSO$ or $D_2O/(D_6)DMSO$ were used as the NMR solvents. ESI mass spectra were recorded on an AmazonX mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The measurements were carried out in the mode of registration of positive ions in the range of *m/z* from 100 to 2800. Elemental analysis was performed on a Euro Vector 2000 CHNS-3 instrument, halogen content was determined by pyrolysis in oxygen stream.

General Procedure for the Synthesis of Acylhydrazones **3a**–**h**

A mixture of substituted isatin **1a**–**h** (1 mmol), hydrazide **2** (1 mmol) and trifluoroacetic acid (20 mol%) in absolute ethanol (7 mL) was heated at reflux for 3 h. After cooling the solution to rt, solvent was rotary evaporated. The precipitate formed was washed with anhydrous diethyl ether, filtered off and dried in vacuum (12 mmHg) to give pure products **3a**–**h**. For the spectral data, please see the *Supporting Information*.

Antibacterial Activity Evaluation

The cultures used for testing included Gram-positive bacteria: *Staphylococcus aureus ATCC 209p*, *Bacillus cereus ATCC 8035*; Gram-negative bacteria: *Escherichia coli CDC F-50*, *Pseudomonas aeruginosa ATCC 9027*, and fungi: *Aspergillus niger BKMF-1119*, *Trichophyton mentagrophytes var. gypseum 1773*, and *Candida albicans 855–653*. The bacteriostatic and fungistatic properties were studied by a series of dilutions in liquid growth medium according to published procedure.[34] The bactericidal and fungicidal activities were studied by the well-established method.[35] Hemolytic action was determined using procedure which was described earlier.[36] To count living and dead cells, *Staphylococcus aureus 209 P* cell suspensions (control and test samples) were stained with a commercial LIVE/DEAD BacLight™ Bacterial Viability kit stain for 30 min in the dark. Next, the suspension was mixed with an equal volume of 0.5% low-melting agarose. The preparations were analyzed using a Nikon Eclipse Ci-S fluorescence microscope (Nikon, Japan). Cell counting in a given scan volume was carried out differentially according to red and green fluorescence.

Anticoagulant and Antiaggregation Activities Study

The in vitro experiments were performed using the blood of healthy male donors aged 18–24 years (total 54 donors). The study was approved by the Ethics Committee of Federal State Budgetary Educational Institution of Higher Education at the Bashkir State Medical University of the Ministry of Health of Russian Federation (No.2 dated 17.10.2012). Informed consent was obtained from all participants before blood sampling. The blood was collected from the cubital vein using the system of vacuum blood collection BD Vacutainer® (Becton, Dickinson and Company, USA). A 3.8% sodium citrate solution in 9:1 ratio was used as a venous blood stabilizer. The study of the effect on platelet aggregation was performed using the Born method[37] using the aggregometer «AT-02» (SPC Medtech, Russia). The assessment of antiplatelet activity of the studied compounds and reference preparations was started with the final concentration of 2×10^{-3} mol/L. Adenosine diphosphate (ADP; 20 μg/mL) and collagen (5 mg/mL) manufactured by Tehnologia-Standart Company, Russia, were used as inducers of aggregation. The study on the anticoagulant activity was performed by standard recognized clotting tests using the optical two-channel automatic analyzer of blood coagulation Solar CGL 2110 (CJSC SOLAR, Belarus). The following parameters were studied: activated partial thromboplastin time (APTT), prothrombin time (PT) and fibrinogen concentrations according to the Clauss method. The determination of anticoagulant activity of the studied compounds and reference preparation was performed in a concentration of 5×10^{-4} g/mL using the reagents manufactured by Tehnologia-Standart Company (Barnaul, Russia). The results of the study were processed using the statistical package Statistica 10.0 (StatSoft Inc, USA). The Shapiro-Wilk's test was used to check the normality of actual data distribution. The form of distribution of the data obtained differed from the normal one; therefore, for further analysis, the non-parametric methods were used. The data were presented as medians and 25 and 75 percentiles. Analysis of variance was conducted using Kruskal-Wallis test. A p value of 0.05 was considered statistically significant.

XRD Analysis

The X-Ray diffraction data for crystals of compounds **3e** and **3g** were collected on a Bruker AXS Smart Apex II and D8 Venture diffractometers, respectively in the ω and ϕ-scan modes using graphite monochromated MoK_α (λ 0.71073 Å) radiation. Structures was solved by direct methods and refined by the full matrix least-squares using SHELXTL program.[38] All non-hydrogen atoms were refined anisotropically. The positions of hydrogen atoms were located from the Fourier electron density synthesis and were included in the refinement in the isotropic riding model approximation. All figures were made using OLEX2.[39]

Supporting Information

Detailed biological evaluation methods and physicochemical experiments, copies of ^{1}H-NMR, ^{13}C-NMR and MS spectra can be found in the *Supporting Information*. *Supporting Information* for this article is available on the WWW under https://doi.org/10.1002/cbdv.202100496.

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Author Contribution Statement

Andrei V. Bogdanov - created the main idea of work. Sergey V. Bukharov, Andrei V. Bogdanov – synthesized starting materials and target compounds. Alexandra D. Voloshina, Anastasia S. Sapunova, Natalia V. Kulik – conducted biological tests. Natalia V. Terekhova – carried out molecular calculations. Alexey B. Dobrynin, Julia K. Voronina - conducted XRD experiments. Alexander V. Samorodov, Valentin N. Pavlov – evaluation of antiaggregation and anticoagulation activity. Vladimir F. Mironov - head of a research group.

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