Synthesis of hydrazides of heterocyclic amines and their antimicrobial and spasmodolytic activity

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Abstract
Un unsolvable issue of a significant number increase of drug multi resistant strains of microorganisms including Mycobacterium tuberculosis force researchers for continuous design novel pharmaceuticals. The purpose of the study is the establishment of the correlation between the structure of novel heterocyclic hydrazide derivatives and their biological activity. Several hydrazide derivatives of N-piperidinyl and N-morpholinyl and propionic acids and N-piperidinyl acetic and their derivatives were synthesized via condensation of corresponding esters with hydrazine hydrate. The structure of synthesized compounds were confirmed by the use of FTIR, H1NMR, Mass-spectroscopy and element analysis. Investigation of synthesized substances using PASS software was carried out to predict probability of pharmacological activity in silico. The antibacterial, antifungal and spasmodolytic activity as well as acute toxicity of obtained compounds were evaluated in vivo. 2-(N-piperidinyl)acetic acid hydrazide and 2-methyl-3-N-piperidinyl)propanacid hydrazide revealed antibacterial and spasmodolytic activities comparable to the model drugs (drotaverin) in vitro study. Synthesized compounds in in vivo experiment showed significantly low acute toxicity (LD50 520–5750 mg/kg) compared to commercially available drugs (streptomicine, ciprofloxacinum and drotaverin LD50 100–215 mg/kg). The structure-activity relationship was established that the increasing of the length of the linker between heterocyclic amine and hydrazide group results in a decrease of antimicrobial activity against studied strains (Escherichia coli, Salmonella typhymurium, Salmonella choleraesuis, Staphylococcus aureus).

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1. Introduction

Past several decades illustrated a growing interest to synthesis of novel precursors of drugs and modification of commercially available drug due to unsolvable issue with an increasing number of drug multi resistant strains of microorganisms including Mycobacterium Tuberculosis (Gegia, Winters et al. 2017, Vilchèze and Jacobs Jr 2019, Zhang, Jiang et al. 2019) and advances in medicine related to discovery of new diseases. Moreover, a synthesis of novel active pharmaceutical substances have a great interest for development of fundamental organic chemistry and drug discovery the establishment of a relation between 3-D structure of isomers and their biological activity Moreover, the rising interest in fundamental relationship between 3-D structure of organic compounds and their biological activity facilitates the need for the synthesis of new active pharmaceutical substances. (Domagala 1994, Dudek, Arodz et al. 2006, Koçyigit-Kaymakçıoglu, Oruç-Emre et al. 2009, Roveda, Clavette et al. 2009, Popiołek 2017). The interest of the synthesis of novel heterocyclic amines and particularly to hydrazide derivatives of piperidine and morpholine is attributed to their wide biological activity. The particular interest is in the synthesis of piperidine and morpholine derivatives due to their wide spectrum of biological activity (Eswaran, Adhikari et al. 2009, Pillai, Rajeswari et al. 2014, Jachak, Ramesh et al. 2015, RK, BEGUM et al. 2018, Tikhov and Kuznetsov 2020). A recent review paper illustrated comprehensive overview of chemical modifications (Dieckmann cyclization, Mannich reaction/amidation, Diels-Alder reaction, δ-amino-β-ketoester condensation etc.) including enolate-carbodiimide rearrangement of piperidine and

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piperidine-2,4-dion derivatives. For these structures various biological activities were found as selective inhibitors of the enzymes dihydroorotase and thiodihydroorotase, that is promising forthethe development of anti-cancer drugs and antimicrobial substances (Tikhov and Kuznetsov 2020). Quantitative structure–activity relationship (QSAR), Structure–Activity Relationship (SAR) and other modern methods provide opportunity to predict potential biological activity prior testing it in vitro and in vivo (Alamine, Anselm et al. 2004, Dudek, Arodz et al. 2006, Roveda, Clavette et al. 2009, El-Shamy, Abdel-Mohsen et al. 2015, Mathew, Suresh et al. 2015, Sader, Castanheira et al. 2017, RK, Belgum et al. 2018). A number of highly active and at the same time quite toxic compounds were found among hydrazide derivatives of pyridine and other aromatic compounds (Sievers and Herrier 1975, Alamine, Anselm et al. 2004, Karaman, Oruç-Emre et al. 2016), therefore the search of less toxic and sufficiently active compounds remains a relevant problem of pharmaceutical chemistry (Ross 1958). For example, carbonic acid hydrazides (isonicotinic acid hydrazide) cause clinically apparent acute liver injury, that increase from 0.5% to 1% and is fatal in the range of 0.05–0.1% to the recipients (Shah, Santucci et al. 1995). Isoxazid and their derivatives were discovered and comprehensively studied in the middle of 20th century (Middlebrook 1954; Rollas and Kückgüzél, 2007). A great interest to carbonic acid hydrazides and thiodyrazides is related to simplicity of their synthesis and purification process as well as its high chemical reactivity (Alamine, Anselm et al. 2004, Mathew, Suresh et al. 2015).

Products of hydrazides condensation with aldehydes - hydrazones revealed a wide range of antimicrobial, antiviral, antitumor, hypolipidemic, hypotensive, diuretic activities and activity against M. Tuberculosis at a relatively low toxicity (Sievers and Herrier 1975, Alamine, Anselm et al. 2004, Narang, Narasimhan et al. 2012, Pillai, Rajaswari et al. 2014, Karaman, Oruç-Emre et al. 2016). Derivatives bearing a R-CONHR functionality at the 6-position of naphthyridine exhibited analgesic and/or anti-inflammatory activities (Di Braccio, Grossi et al. 2014). The reports describe antimicrobial, antioxidant, antimicrobial and antioxidant activity of various morpholine derivatives being attributed to Schiff bases and β-lactam functionalities. (Cebec, Bayrak et al. 2019) It was reported that glycine hydrazides derivatives possess high spasmolytic activity, transient hypotension and might have effects on the central nervous system and revealed low toxicity with LD50 more than 1000 mg/kg (Rips, Derappe et al. 1965). A detailed methods of synthesis of thiosemicarbazides of N-piperidinylacetic acid was described previously as well as and strategies of its cyclisation to bis-heterocyclic compounds (Misra, Dwivedi et al. 1978, Dyusebaeva and Kalugin 2015). In this work the synthesis of various hydrazides containing heterocyclic amine is described and results of antimicrobial, antifungal and spasmodic activity as well as acute toxicity is discussed.

2. Materials and methods

2.1. Chemistry

Hydrazine hydrate (50%) and 3,4-Dihydroxybenzaldehyde (97%); CD3OD (99.9%), DMSO d6 (99.9%); drugs of comparison: Amphotericin B solution (250 µg/mL in deionized water, 0.1 µm filtered, BioReagent), Miconazole (2)-[2-(2,4-Dichlorobenzyl)-2-(2,4-dichlorophenyl)-ethyl]-1H-imidazole, streptomycin (99.5%), ciprofloxacinum (1-Cyclopropyl-6-fluoro-4-oxo-7-(piperaizin-1-yl)-1,4-dihydro-quinoline-3-carboxylic acid) (99%); droxatervin (1-{[(3,4-diehtoxyphenyl)-methylene]-6,7-dioxythio-1,2,3,4-tetrahydroisoquinoline (99%), TLC Silufol UV-254 were obtained from Aldrich Chemical Co. KBr (99.5%), ethanol (95%), hexane (95%), benzene (95%), dichloromethane (99%), hydrochloride) (95%) and iodine (98%) were obtained from LabChemProm, which was then converted to absolute ethanol using a distillation with calcium oxide (technical grade). Prior to use all reagent were purified via distillation under reduced pressure. The control of purity of synthesized derivatives was controlled using TLC Silufol UV-254 (treatment with a vapour of iodine) (Tolstikova, Tolstikov et al. 1988).

FTIR spectra of obtained compounds were recorded using Spectord 75 IR spectrophotometer (in a thin layer, in tablets with KBr) with a resolution 2 cm⁻¹.

1H NMR spectroscopy (Bruker 300 NMR spectrometer, USA Bruker, and Bruker DRX 500 with a working frequency of 300, 500 MHz). Internal standard for 1H-NMR was hexamethyldisilane, deuterated solvents CD3OD, DMSO d6 was used for dissolution of compounds.

Agilent 6890 N 5973 N GC–MS (Palo Alto, CA, USA) with electronic ion sources was used for characterisation of novel compounds. The analysis was performed on a Agilent6890N Gas chromatograph equipped with Agilent 5973 N Mass selective detector. Capillary GC analysis was carried out on a HP–5MS (30 m x 0.25 mm ID, 0.25 µm) capillary column (5% diphenyl, 95% dimethylpolysiloxane, J&W Scientific, Folsom, CA, USA) with helium as mobile phase. Quadrupole analyzer temperatures were maintained at 280, 230, 180 °C. A solvent delay of 3 min was chosen. In the full-scan mode, electron ionization (EI) mass spectra in the range of 50–500 (m/z) were recorded.

2.2. Synthesis of aminoheterocyclic acids hydrazides (I-V)

Synthesis of ethyl(methyl) esters of α- β- aminocarboxylic acid (I-IV) were conducted according to previously described methods (Berillo and Sh.S. 2008, Berillo 2010, Dyusebaeva and Kalugin 2015). Briefly, piperidine of morpholine dissolved in absolute ethanol was added dropwise to the solution of methyacrylate or methylmethacrylate, which was taken with a 10% excess compared to heterocyclic amine under stirring at room temperature. The reaction mixture was incubated at elevated temperature overnight and the color change from transparent to reddish appeared. The solvent and unreacted initial compounds were removed under reduced pressure. The lead products were purified via distillation under vacuum. A reaction mixture containing 0.1 mol of ethyl (methyl) esters of α- β- heterocyclic amino functionality (morpholinyl or piperidinyl) acetic or propan acid, 6 g (0.12 mol) absolute hydrazine hydrate (95%) reflux in ethanol for 2 h at 75–80 °C. The kinetic of product formation was monitored using TLC. Compounds DB (III-V) were purified using a column chromatography with silica gel eluent of absolute ethanol or methanol. After evaporation of solvent under vacuum a crystal products were obtained.

The compound MAI was synthesised according to the report, [Alamine, A., et al. 2004] piperidine was treated with ethyl ester of bro-moacetic acid in presence of potassium carbonate. Then, the product ethyl ester of 2-(N-piperidyl) acetic acid was reacted with the hydrazine hydrate. Analogously 2-(4-hydroxylimino)-2,5-dimethyl piperidin-1-yl was alkylated by ethyl ester of bro-moacetic acid in acetone medium in the presence of dry potassium carbonate. Obtained ester of 2-(4-hydroxylimino)-2,5-dimethyl piperidin-1-yl acetic acid was reacted with hydrazide hydrate giving the substance MAI.

2-(N-piperidyl) acetic acid hydrazide (MAI) C176H126N3O Mr 157.21 g/mol, white crystals, yield 95%; m.p. 55–56 °C. FTIR, ν, cm⁻¹: 1245 (C=O–C), 1735 (C=O), 3422–3254 (–NH–NH2). 1H NMR (DMSO d6), δ ppm 1.3 (s, 2H, –CH2–), 1.5 (s, 4H, –CH2–), 2.1 (s, 4H, 2–CH2–), 2.4 (d, 2H, –NCH3–), 3.8 (d, 2H, –NH–), 8.1 (s, 1H, –NH–) (Bruker 300 NMR spectrometer, USA Bruker with a working frequency of 300 MHz). Elemental analysis of C176H126N3O,
2.3. In silico study

Prediction of Activity Spectra for Substances (PASS) predicts 4000 types of biological activity with the average prediction accuracy of about 95%. (Stasevych, Zvarych et al. 2017) http://www.way2drug.com/passonline/ Physicochemical properties, LogP and Lipinski constants of compounds (I-VI) calculated using Advanced Chemistry Development (ACD/Labs) Software V11.0 are provided in supplementary information.

2.4. Biological assays

Antimicrobial and spasmylic activity as well as acute toxicity of 2-(4-dihydroxyimino)-2,5-dimethyl piperidyl) acetic acid hydrazide (II); 2-methyl-3-(N-piperidyl)propionic acid hydrazide (III); 2-methyl-3-(N-morpholyl)propionic acid hydrazide (IV) were studied at the laboratory of Scientific and Production Association «MedBioPharm» (Almaty, Kazakhstan).

Antibacterial activity of compounds (I-IV) was evaluated against gram-positive (S. aureus) and gram-negative (E. coli and S. choleraeuis, S. Typhymurium) bacterial strains. The growth of microorganisms was determined on the meat and peptone broth and expressed through a turbidity standard procedure calibrated from 0 to 4. The score 4 is attributed to (control) the turbidity of the meat and peptone solution with bacterial growth (over 1 million microbial cells/mL of broth), score 3 corresponds to the range of concentrations from 1,000 up to 1 million cells/mL, score 2 is related to the range – from 50 to 1000 cells/mL, score 1 contains up to 50 microbial cells/mL, Score 0 - a clear solution indicating strong bacteriostatic activity. Streptomycin was used as a reference drug (control) in comparable concentration (Dyusebaeva, Elibaeva et al. 2017). Antimicrobial activity of 3-(N-morpholyl)propionic acid hydrazide (V) and 3,4-dihydroxybenzyliden hydrazide 2-methyl-3-(N-piperidinyl)propionic acid (VI) were investigated at the laboratory of microbiology at Department of Biotechnology Al-Farabi Kazakh National University (Almaty, Kazakhstan). The antibacterial activity of compounds V and VI was tested against strains of E. coli 251, S. Typhymurium, S. choleraeuis, S. aureus on meat and peptone broth by serial dilution. Incubation of tubes containing medium with and without precursor of drug was carried out at 37°C. The results of bacteriostatic activity were considered after 24, 48, 72 h, respectively. A comparable concentration of antibiotic drug ciprofloxacin was used as a control according to the standard protocol (Gein, Odegova et al. 2015). The compound III at concentration of 0.2 mg/mL was tested against antifungal activity, against C. albicans, A. flavus, M. canis, F. solani, C. glabrata. The incubation of plates was performed for 7 days at 27°C (Mittendorf, Kunisch et al. 2003).

The spasmylic activity was estimated using a model of intestine of white Outbred laboratory rats according a recognized method in vitro (Kulkarni, Patil et al. 2004). Briefly, the experiment was performed using male rats of the same age and weight. A segment of the intestine with a length of about 3 cm was used, incubated at room temperature for 6 h. The kinetics of product formation were monitored using TLC. After the initial compounds disappear from TLC the solvent was evaporated under vacuum at 50 °C, the obtained crude product was mixed with silica gel and purified using column chromatography CHCl3–hexane. Melting point 170–172 °C, Rf 0.303 (EtOH 96%), brown crystals, yield 46%. FTIR, ν cm⁻¹: 1669 (>C = O), 1115 (C–O–C), 3420–3250 (–NH–NH–). 1H NMR (DMSO d₆), δ ppm: 1.54 (t, 2H, –CH2–), 3.65 (m, 4H, 2(–OCH2–)) 4.15 (t, 2H, –CH2–), 2.62–2.44 (2H, –NHCH2–). Mass-spectrum main pattern, m/z (I): 187 (3.33%), 172 (0.66), 156 (0.66), 144 (0.66), 128 (1), 112 (0.66), 101 (7), 100 (0.00), 98 (7.3), 87 (2.5), 85 (8.8), 83 (2), 81 (2). (Figure S2).
The acute toxicity LD₅₀ of active pharmaceutical substances (I-IV) was studied by intraperitoneal administration using white outbred mice of both sexes weighing 17–23 g (n = 3) according to previously published method (Hooser, Beasley et al. 1989). The preclinical animal studies were carried out at the organisation “MedBioPharm” (Almaty, Kazakhstan). All animal studies were performed according the general guidelines of working with animals “Rules of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes” and in accordance with the requirements for the study of new pharmacological substances (Council 2010, Council 2011).

3. Results and discussion

3.1. Synthesis

Piperidine and morpholine containing α- & β-amino propionionic acids hydrazides were synthesized.

Using a well-known method of ester group condensation with hydrazine hydrate. The esters of α- & β aminoacids were obtained via nucleophile addition reaction of piperidine or morpholine to the double bond of acrylic(methacrylic) acid esters (Berillo 2010, Dyusebaeva and Kalugin 2015). The reflux of the ester of α- or β-aminoacids with hydrazine hydrate for several hours leads to corresponding hydrazides (Schema 1).

The structures of synthesized compounds are presented on the Fig. 1. Comprehensive physicochemical characterization is shown in experimental part and supplementary information. The FTIR spectrum of pharmaceutical active substance 2- (N-piperidinyl) acetoxyhydrazide(MAI) contains absorption bands of stretching vibrations of the NH2 group in the region of 3310–3260 cm⁻¹, the NH group in the region of 3180 cm⁻¹, and the carbonyl group at 1690 cm⁻¹. In the 1H NMR spectrum of the BDIII, the protons of the piperidine ring resonate at the field of 1.58 ppm (dd, 6H, –CH23,4,5) and 2.34 ppm (dd, 4H, –CH22,6). Protons at carbon atoms C2,6 are shifted to the region of downfield due to the influence of the nitrogen atom and appear at 2.29–2.32 ppm. Signals at 3.65 and 3.74 ppm attributed to the protons C3,5 of the methylene groups of the morpholine fragment of the molecule BDIV deshielded, due to the influence of the electronegative oxygen atom. The 3 protons of the methyl group (–CH–CH3) appears as a doublet signal at 1.14 ppm, and one proton (–CH–CH3) registered as a quadruplet signal at 2.74–2.78 ppm. The methyl protons of the N–CH2 resonance as doublet signal in the field of 2.44–2.60 ppm in the substance BDIV. Due to the fact that the 1H NMR spectrum was recorded in a protonic solvent D2O, the protons of the NH2 and NH groups are not identified in the spectrum, as these protons are attributed to easily exchangeable.

3.2. Pharmacological activity

The main scope of the current research is focused on the establishment of the correlation between the structure of novel heterocycle derivatives hydrazides and their biological activity (spasmolytic, antimicrobial, antifungal). Estimation of the acute toxicity is also an important parameter taken into account during novel pharmaceutical development. Results of acute toxicity, spasmolytic and antimicrobial activity of novel derivatives (MAI; 2-(4-hydroxylimino)-2,5-dimethyl piperidin-1-yl)acetic acid hydrazide (MAII); 2-methyl-3-(N-piperidyl)propionic acid hydrazide (BDIII) and BDIV) is presented in Table 1. Biological activity and the toxicity of compounds were compared to activity of model well known drugs ciprofloxacinum (antimicrobial activity), drotaverin containing piperidine fragment as spasmolytic substance. The toxicity of heterocyclic derivatives of hydrazides (MAI, MAII, BDIII, BDIV) revealed significantly lesser acute toxicity compared to model drugs (Table 1). In order to carry out the real correlation of toxicity to quantity of the active pharmaceutical substance the LD50 was represented in mmol/kg of mouse. In average synthesized drugs exhibited more than hundred times less toxicity in comparison with the model drugs (Streptomycin, Ciprofloxacin, Dotraverine) (Table 1). Obtained compounds have different pKa values (S4) and therefore pH of solutions in pure water were 11–9 and adjusted with hydrochloric acid to physiological values. It is known that the inhibitory activity of some substances is pH dependent due to existence of a mixture of tautomericforms and one has different pharmacological activity to the other (Tikhov and Kuznetsov, 2020). Lipinski parameters helps to estimate druglike-ness or evaluate if a derivative has a certain pharmacological or biological activity, as well as prognoses chemical and physical properties that would likely make it an orally active drug (Lipinski, Lombardo et al. 1997). Therefore, we provided entirely essential parameters prediction of biological activity such as polar surface area, solubility, pKa, pH of the unbuffered solution and LogP obtained using Advanced Chemistry Development (ACD/Labs) Software (supplementary information). Calculated Lipinski’s parameters for the synthesized compounds are in a correct range of values (no more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen – hydrogen bonds); no more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms) a molecular weight less than 500 g/mol) and therefore we can expect biological activity (Lipinski, Lombardo et al. 1997).
3.3. Structure activity relationships

In vivo toxicity was decreased with the substitution of a piperidinyl group to a morpholinyl one (Table 1). This transition also diminished the antimicrobial activity, which is in line with the previously reported data. It was established that the substitution of piperidinyl moiety to morpholinyl functionality resulted in the decrease of the toxicity in vivo. The transition from the piperidinyl to morpholinyl group led to diminish of antimicrobial efficiency, (Table 1) that correlates with previous studies containing morpholinyl cycle (Sensi, Maggi et al. 1964).

Partition coefficient LogP is an important parameter taken into account during drug design, allowing to predict some biological activity as well as adsorption and distribution of the drug within the body (Ben Arfa, Combes et al. 2006). The compound MAII and ciprofloxacinum have similar LogP and polar surface area, however MAII did not reveal significant antimicrobial activity. Compounds MAI, MAII, BDIII, BDIV, BDV and 3-(N-morpholyl)propionic acid (BDVI) were tested on Gramm positive and negative strains (Escherichia coli 251, S. choleræaus, S. Typhymurium, S. aureus F-21). It was found that most active compounds are MAI, MAII, BDIII having a harmacophore piperidyl functionality and hydrazide group (–NH-NH₂) in the structure. 2-methyl-3-(N-piperidyl) propionic acid hydrazide BDIII possesses a moderate antimicrobial activity compared to the model drug streptomycin, but it is significantly less toxic and therefore it is possible to continue the investigation the antimicrobial activity at higher doses. These data correlate well with SAR data obtained using PASS software. Thus, antituberculosic, antimycobacterial and antiviral (Picornavirus) probability of biological activity (Pa) for MAI were 0.541, 0.539 and 0.431, respectively. The computer modelling has revealed high probability for antituberculosic (Pa 0.433) antimycobacterial (Pa 0.424) and antiviral (Picornavirus) (Pa 0.398) activity for the substance BDIII. Nevertheless, the derivative MAII has Pa coefficient of 0.373 for antibacterial activity the software did not predict antituberculosic or antimycobacterial activity. Pharmaceutical active substance BDIV is very perspective for further investigation for antituberculosic (Pa 0.418) antimycobacterial (Pa 0.41) and antiviral (Picornavirus) (Pa 0.41) activities. The morpholine containing derivative IV did not reveal inhibiting activity against bacterial strains E. coli, S. Typhymurium, S. choleræaus and S. aureus. 2-(N-piperidinyl)-propionic acid hydrazide MAI revealed comparable level of antimicrobial activity of model drug ciprofloxacin. Previously, several 1,2,5-trimethylpiperidin-4-ol derivatives the precursor of MAII

Table 1

| Pharmaceutical active substance | LogP | LD₅₀, mmol/kg | LD₅₀, mg/kg | Antimicrobial activity |
|--------------------------------|------|---------------|------------|------------------------|
| MAI                            | −0.716 | 3.67 | 580 | 0 S.choleraesuis 1 E. coli 0 S. aureus |
| MAII                           | −1.043 | 2.40 | 520 | 3 S.choleraesuis 4 E. coli 4 S. aureus |
| BDIII                          | −0.254 | 4.31 | greater than 800 | 2 S.choleraesuis 2 E. coli 2 S. aureus |
| BDIV*                          | −1.834 | 30.73 | 5755 > 21 | 4 S.choleraesuis 4 E. coli 4 S. aureus |
| BDV                            | −2.118 | − | − | 4 S.choleraesuis 4 E. coli 4 S. aureus |
| Streptomycin                   | −6.4 | 3.676 E-4 | 213.8 | 0 S.choleraesuis 0 E. coli 0 S. aureus |
| Ciprofloxacin                  | −1.1 | 2.966E-4 | 98.3 | 0 S.choleraesuis 0 E. coli 0 S. aureus |
| Dotraverine                    | − | 37.7 E-3 | 15 | − S.choleraesuis − E. coli − S. aureus |
| Negative control               | − | − | 0 | − S.choleraesuis − E. coli − S. aureus |

*LD₅₀ with intraperitoneal administration to rats is equaled to 331 ± 11.5 mg/kg. 1.91 ± 0.066 mmol/kg.

Fig. 1. Structures of active pharmaceutical substances piperidine and morpholine derivatives.

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Table 1

Acute toxicity and antimicrobial activity of novel compounds (MAI; MAII; BDIII and BDIV).

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*LD₅₀ with intraperitoneal administration to rats is equaled to 331 ± 11.5 mg/kg. 1.91 ± 0.066 mmol/kg.
were investigated for antibacterial activity of against 16 strains illustrating effective bacteriostatic effect at concentration of 50 mg/mL (Dyusebaeva, Elibavea et al. 2017). 3-(N-morpholyl) propionic acid hydrazide BDV at concentrations of 0.5, 1.0, 2.0 and 4.0 mg/mL did not reveal the antimicrobial activity against E. coli 251 and S. aureus F-21 strains, respectively, which may be related to too hydrophobic structure (LogP = 2.118). 3,4- dihydroxybenzilidenhydrazide 2-methyl-3-(N-piperidinyl)propionic acid DBVI possesses antimicrobial activity comparable to ciprofloxacin and the initial hydrazide DBIII. Thus, the introduction of aromatic ring into the molecule did not increase nor decrease antimicrobial activity significantly compared to initial hydrazide BDIII, however LogP changed significantly from ~0.25 to + 1.4 and polar surface area from 58 to 100A2 (Supplementary). The derivative BDVI at the concentration of 2 mg/mL illustrated bacteriostatic activity against S. aureus F-21 (Table S2), however it did not inhibit the growth of E.coli 251. The minimal inhibition concentration level is comparable with other similar piperidine derivatives (Issayeva, Datkhayev et al. 2019). Among most widely used anti tubercular drugs is isonicotinic acid hydrazide (Isoniazide) and its derivatives (Kocyigit-Kaymakoglu, Oruc-Emre et al. 2009, Sterling, Moro et al. 2015, Vilchèze and Jacobs Jr 2019, Zhang, Jiang et al. 2019). Therefore, following study will be devoted to testing of these derivatives: (2-(N-piperidinyl)acetic acid hydrazide and 3,4-dihydroxybenziliden hydrazide 2-methyl-3-(N-piperidinyl)propiolic acid BDVI against M.Tuberculosis. Spasmolytic activity of compounds MAI, MAII, BDII and BDIV were tested in vitro (Table 2). Active pharmaceutical substances MAL and DBIII revealed spasmolytic activity comparable to the activity of model drug Dotraverin. Taking into account that LD50 mmol/kg of novel substances more than 100 times less than model drug Dotraverin, however LogP changed significantly from ~0.25 to + 1.4 and polar surface area from 58 to 100A2 (Supplementary). The derivative BDVI at the concentration of 2 mg/mL illustrated bacteriostatic activity against S. aureus F-21 (Table S2), however it did not inhibit the growth of E.coli 251. The minimal inhibition concentration level is comparable with other similar piperidine derivatives (Issayeva, Datkhayev et al. 2019). Among most widely used anti tubercular drugs is isonicotinic acid hydrazide (Isoniazide) and its derivatives (Kocyigit-Kaymakoglu, Oruc-Emre et al. 2009, Sterling, Moro et al. 2015, Vilchèze and Jacobs Jr 2019, Zhang, Jiang et al. 2019). Therefore, following study will be devoted to testing of these derivatives: (2-(N-piperidinyl)acetic acid hydrazide and 3,4-dihydroxybenziliden hydrazide 2-methyl-3-(N-piperidinyl)propiolic acid BDVI against M.Tuberculosis. Spasmolytic activity of compounds MAI, MAII, BDIII and BDIV were tested in vitro (Table 2). Active pharmaceutical substances MAL and DBIII revealed spasmolytic activity comparable to the activity of model drug Dotraverin. Taking into account that LD50 mmol/kg of novel substances more than 100 times less than model drugs illustrated in Table 1 these derivatives can be recommended for medical use at least in veterinary and recommended for comprehensive nonclinical studies. In vivo testing of the compounds MAL, MAII, BDIII and BDIV showed a spasmylytic activity comparable to a model drug – Dotraverin. LD50 mmol/kg 100 times less than the model drug is suitable for veterinary and medical uses. The derivative BDIV illustrated spasmylytic activity under acetylcholine induced spasm, however 2-methyl3-(N-morpholyl) propionic acid hydrazide was less active compared to the drug of comparison. The substitution of piperidine ring to morpholine moiety lead to a decreased of spasmolytic activity (Table 2). The incorporation of the spacer > CH(CH3) between heterocycle and hydrazide group did not affect the activity. Based on obtained biological screening, it was found that piperidine containing derivatives showed significant spasmylytic activity. The predicted activity level is represented in PASS software by the list of activities with the probabilities “to be active” (Pa) and “to be inactive” (Pi) generated for each biological activity. The obtained data is in excellent agreement with PASS software prediction data illustrating that substances MAI and BDIII should possess spasmylytic, urinary and spasmylytic activity with probabilities (Pa) of 0.426 ±0.34 and 0.400 ±0.516, respectively. The transformation of hydrazide group to hydrazone does not decrease the probability of spasmylytic activity and the pharmaceutically active substance BDIV has a coefficient of 0.511. Piperidine derivative MAI (Pa 0.498) BDIII (Pa 0.537) and DBIV (Pa 0.433) possess high probability of the Cytochromes P450 activator gene CYP2E1 inducing activity and therefore it would be interesting to check it in vivo studies in further study. Piperidine derivative MAI (Pa 0.498) BDIII (Pa 0.537) and DBIV (Pa 0.433) possess a high probability of inducing the expression of the CYP2E1 gene, which is a Cytochrome P450 activator. This lays an interest in the further studies to check that activity. Comparative analysis of predicted pharmacological activity of piperidine and morpholine derivatives revealed in Table 3. There is a report that secondary and tertiary amines were conveniently synthesized by alkylation amines of pyrrolidine, piperidine,morpholine, furfurylaine etc. with the applicable alkyl bromides, such as isoamyl bromide, 2-bromo-6-methyl- heptane, arid 2-bromo-6-methylene-5-ene and none of them revealed superior spasmylytic activity to either atropine or papaverine (Cocolas, Avakian et al. 1965).

### Table 2

| Pharmaceutical active substance | Change in intestinal length after drug administration | Acetylcholine spasm (1 mg Acetylcholine / 1 ml medium) | Histamine spasm activity (1 mg Histamine / 1 ml medium) |
|---------------------------------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
| MAI                             | 0                                              | 0                                              | 0                                              |
| MAL                             | 0                                              | Increased by 4 mm                              | Increased by 4 mm                              |
| BDIII                           | 0                                              | 0                                              | Increased by 2 mm                              |
| BDIV                            | 0                                              | Diminished by 3 mm                             | Diminished by 3 mm                             |
| Dotraverin                      | 0                                              | 0                                              | 0                                              |
| Acetylcholine                   | –                                              | Diminished by 4 mm                             | –                                              |
| Histamine                       | –                                              | Increased by 4 mm                              | –                                              |

### Table 3

| Pharmaceutical active substance | Pa     | Pi     | Pharmacological activity                          |
|---------------------------------|--------|--------|--------------------------------------------------|
| MAI C7H15N3O                   | 0.310  | 0.053  | Muscle relaxant                                  |
| BDIII C9H20N4O2                | 0.405  | 0.029  | Muscle relaxant                                  |
| BDIV C8H17N3O2                 | 0.345  | 0.013  | Central nervous system active muscle relaxant    |
| BDIII C9H20N4O2                | 0.372  | 0.038  | Skeletal muscle relaxant                         |
| BDIV C8H17N2O2                 | 0.357  | 0.040  | Muscle relaxant                                  |
| BDIV C8H17N3O2                 | 0.393  | 0.008  | Central nervous system active muscle relaxant    |
| MAI C7H15N3O                   | 0.684  | 0.031  | Antisiechemic cerebral                           |
| MAII C9H20N4O2                 | 0.804  | 0.014  | Antisiechemic cerebral                           |
| BDII C8H17N2O2                 | 0.716  | 0.026  | Antisiechemic cerebral                           |
| BDIV C8H17N4O2                 | 0.730  | 0.024  | Antisiechemic cerebral                           |
| MAI C7H15N3O                   | 0.655  | 0.013  | Anticonvulsant                                   |
| MAL C8H19N3O                   | 0.521  | 0.031  | Anticonvulsant                                   |
| BDII C9H20N4O2                 | 0.461  | 0.045  | Anticonvulsant                                   |
| BDIV C8H17N3O2                 | 0.501  | 0.035  | Anticonvulsant                                   |
| MAI C8H19N3O                   | 0.536  | 0.074  | Kidney function stimulant                        |
| BDII C9H20N4O2                 | 0.431  | 0.145  | Kidney function stimulant                        |
| MAI C7H15N3O                   | 0.570  | 0.046  | Antidysergenic                                   |
| MAL C9H19N3O                   | 0.425  | 0.090  | Antidysergenic                                   |
| BDII C9H20N4O2                 | 0.469  | 0.045  | Respiratory analeptic                            |
| BDIV C8H17N3O2                 | 0.499  | 0.038  | Respiratory analeptic                            |

### 4. Conclusion

It was illustrated that heterocyclic compounds 2-(N-piperidinyl)acetic acid hydrazide and 2-methyl-3-(N-piperidinyl) propionic acid hydrazide have high antimicrobial and spasmylytic activity concurrently with low toxicity. It was established that obtained hydrazides containing morpholinyl moiety did not possess antimicrobial and spasmylytic activity at tested range of concentrations. Nevertheless, synthesized heterocyclic derivatives have low acute toxicity and therefore perspective for study other
type of activities. 3,4-dihydroxybenzylidenazlocycla 2- methyl-3-(N-piperidinyl)propanoic acid may be used as a prodrug for delivery of active compound BDI intermediate via hydrolysis under acidic conditions. 3,4-dihydroxybenzyldiazylyzocycla 2-methyl-3-(N-piperidinyl)propanoic acid can be perspective for study its antioxidant and antianic activity due to presence of phenolic hydroxyl groups. It was observed that the introduction of methylene groups between heterocycle and hydrazide group led to significant decrease of antimicrobial activity. 2-methyl-3-(N-piperidinyl)propanoic acid hydrazide did not show antifungal activity against C. albicans, A. flavus, M. canis, F. solani, C. glabrata. The following study will be devoted to comprehensive study of the mechanism of analgesic and spasmylocytic activity of these heterocyclic hydrazides.

CRediT authorship contribution statement

Dmitry A. Berillo: Data curation, Conceptualization, Visualization, Moldyra A. Dyusebaeva: Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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