



Investigation of the activity of new derivatives of 1,3-diazinone-4 and their acyclic precursors with respect to bacteria of the genus *Proteus*

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Abstract

Introduction: The present paper provides a study of the activity of the new 1,3-diazinon-4 derivatives and their acyclic precursors under the laboratory cipher PYaTd1, PYaTs2, PYaTs3 and PYaTs4 against microorganisms of the genus *Proteus*, which is of high importance at the moment as the growing resistance of the *Proteus* to previously highly active antibiotics dictates the need to search for effective antimicrobial agents that meet modern safety requirements.

Materials and Methods: The study of the activity of the compounds was carried out on collection and freshly isolated strains from patients with different pathologies. The strains were identified using the BIOMIC V3 apparatus (Giles Scientific, USA) to verify genus and species identity. The strains used in the study were previously examined for susceptibility to antibacterial drugs by the Disc Method to assess the presence or absence of resistance. The activity of the new compounds was studied by the serial dilution method.

Results: The results of the study showed that the compounds PYaTd1, PYaTs2, PYaTs3 and PYaTs4 show a different activity against bacteria of the genus *Proteus*. The substance PYaTs2 is ineffective. With respect to strains *P. mirabilis* and *P. rettgeri*, the minimum inhibitory concentration of the compounds PYaTs3, PYaTs4 and PYaTd1 ranges from 4 µg/ml to 16 µg/ml.

Conclusion: Thus, by the average aggregate indices, regardless of the species and strain of bacteria, the most effective compound is PYaTd1, the MIC₅₀ of which is within 10 µg/ml, which proves it to be promising and makes further development worthwhile.

Keywords

compounds PYaTd1, PYaTs2, PYaTs3, PYaTs4, *P. mirabilis*, *P. rettgeri*, *P. vulgaris*, activity, MIC.

Introduction

Urinary tract infections are amongst the most common bacterial infections in humans caused by bacteria of the genus *Proteus* (*P. vulgaris*, *P. mirabilis*, *P. rettgeri*) (Pellegrino et

al. 2013, Budnik 2015, Liu et al. 2015). In 70% of cases, there are protei in cases of urolithiasis (Schaffer et al. 2016). *P. vulgaris* along with *Escherichia* and *Pseudomonas aeruginosa*, staphylococci and streptococci, as well as with anaerobic clostridia, often complicates the course of puru-

lent and anaerobic infections. *P. mirabilis* often insulates wounds, forming biofilms, which protects the pathogen from the effects of antimicrobial agents and makes treatment difficult (Leblebicioglu and Esen 2003, Schaffer et al. 2016). *P. mirabilis* is known as an etiological agent of the onset of nosocomial pneumonia (Palagin et al. 2012). There is an increased interest towards the role of *Proteus* in the etiology of rheumatoid arthritis (RA) and towards the development of chemotherapeutic drugs which are active against protei that can reduce the risk of and/or worsening RA development (Ushakova et al. 2001, Disaanayake et al. 2014).

Recent studies have shown that the problems of treating *Proteus* infections are linked to a growing number of strains resistant to antibiotics. In various countries of the world, studies of the resistance of protei to antimicrobial agents prove the proliferation of *Proteus* – producers of extended-spectrum β -lactamases (Cremet et al. 2011, Schmie-mann et al. 2012, Corvec et al. 2013, Chen et al. 2015).

Despite intensive activities to contain and control antibiotic resistance, the problem remains extremely urgent. In this regard, WHO has published a list of “priority pathogens”, which includes enterobacteria (Budnik 2015).

The growing resistance of the pathogen to the previously highly active antibiotics makes it a high priority to search for effective antimicrobial agents that meet modern safety requirements.

Currently, there are opportunities to significantly increase the effectiveness of research on the synthesis of new pharmacologically active substances by using modern computer technologies that allow molecular design of target structures with a predictable effect (Filimonov and Porojkov 2006, WHO 2017).

New derivatives of 1,3-diazinon-4 and their acyclic precursors were created and synthesised by the staff of the Department of Organic Chemistry of Pyatigorsk Pharmaceutical Institute (Kodonidi 2010, Oganesyana et al. 2013). A preliminary analysis of the potential biological properties of these compounds was carried out using the PASS programme, which made it possible to conclude that the occurrence of various types of biological activity was likely. According to the computer forecast, they should have antibacterial actions. The primary screening of fifteen new derivatives of diazinon showed that they exhibited activity against protei. Some promising leaders were chosen for further development (Luzhnova et al. 2017).

Goal

To conduct a study of the activity of the new 1,3-diazinon-4 derivatives and their non-cyclic precursors coded in the laboratory as PYaTd1, PYaTs2, PYaTs3 and PYaTs4 for microorganisms of the genus *Proteus*.

Materials and methods of research

Investigation of the activity of the compounds PYaTd1, PYaTs2, PYaTs3 and PYaTs4 with reference to *Proteus* was carried out on the collection strains: *P. mirabilis* NIIL

tya1, NIIL *tya2* (collection “NIIL”, Astrakhan), *P. mirabilis* VK 294, VK 299 (State-funded Hospital JSC “SM Kirov City Clinical Hospital No. 3”, Astrakhan); isolates - *P. rettgeri* SES 11/01, SES 11/02 (isolated from the intestines of the patients - Sanitary and Epidemiological Station, Astrakhan); *P. mirabilis* VK 190, VK 194 (isolated from trophic ulcers), *P. vulgaris* VK 01, VK 02 (isolated from the intestine) - State-funded Hospital JSC “SM Kirov City Clinical Hospital No. 3”, Astrakhan.

All the strains used in the work were identified by means of the BIOMIC V3 software system (Giles Scientific, USA) to determine their belonging to the genus and species by using the bio Merieux APL kit, which includes strips containing individual biochemical markers, to identify protei.

The activity of the compounds was studied by the serial dilution method (Navashin and Fomin 1974). In these studies, the concentration of the substances under study in the series of serial dilutions was decreased in a geometric progression by a factor of 2: 128 $\mu\text{g/ml}$, 64 $\mu\text{g/ml}$, 32 $\mu\text{g/ml}$, 16 $\mu\text{g/ml}$, 8 $\mu\text{g/ml}$, 4 $\mu\text{g/ml}$, 2 $\mu\text{g/ml}$, 1 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$ and 0.25 $\mu\text{g/ml}$. Control was the tubes containing meat-peptone broth (MPB) without adding the compound and microorganisms (control of the medium sterility), with adding microorganisms without adding the substances (positive control), inoculations with a solvent (DMSO in equivalent volumes) and serial dilution series of the reference substances: sulphodimethoxin and streptocid for PYaTs2, PYaTs3 and PYaTs4 containing a fragment of sulphanilamide and dapsone for PYaTd1 containing a dapsone fragment.

The quantity of the compound (drug) of 4 mg was dissolved in 0.5 ml of dimexide, then 4.5 ml of physiological solution was added thereto to prepare a working solution from which, by means of dilution, a number of tubes with preset concentrations were formed.

To prepare a suspension of bacteria, a one-day culture was used. The bacterial suspension (0.05 ml) of a certain density (0.5 McFarland standard) was added to each tube with MPB BCH of a series of dilutions of the tested substances, including positive control. This culture was incubated for 24 hours at a temperature of +37 °C. At the end of this period, the presence of microorganisms growth was visually assessed in each of the tubes; then the contents of the tubes were centrifuged at 1500 rpm for 10 minutes. The supernatant was removed. The deposit was washed twice with physiologic saline solution. From each tube, 0.02 ml of the deposit was plated on Ploskirev's medium. The culture was incubated for 24 hours at a temperature of +37 °C; then the grown colonies were counted, using the BIOMIC V3 microbiology system (Giles Scientific, USA). Based on the results obtained, the minimum inhibitory concentrations of the compounds – MIC₅₀ and MIC₉₀₋₁₀₀ were calculated (LibUSSR.RU 2014, Semina et al. 2004).

The strains used in this work were previously tested for susceptibility to antibacterial drugs by the Disc Test to determine the presence or absence of resistance (Semina et al. 2004). A set of indicator discs DI-PLS-50-01 (CJSC Research Centre for Pharmacotherapy, St. Petersburg) was used. The results were read using the BIOMIC V3.

The results were statistically processed using computer software BIOSTAT 2009 (Analist Soft Inc., USA). The variation series was checked for normality by the Kolmogorov-Smirnov criterion. The index of statistical significance was determined by the Student's t-test. Statistically significant were the variations at $p \leq 0.05-0.01$.

Results and discussion

The strains of *Proteus* used in the work displayed a wide range of antibiotic susceptibility: from high sensitivity to antibacterial drugs to multidrug resistance (Table 1).

Analysis of the cultured collection strains *P. mirabilis* showed that the compounds codified as PYaTs3 and PYaTs4 suppress their growth within the concentration range of 128-4 $\mu\text{g/ml}$, with a concentration of 8-4 $\mu\text{g/ml}$ being the MIC_{50} and 128 $\mu\text{g/ml}$ corresponding to the MIC_{85} - MIC_{100} , i.e. close to bactericidal. These strains also displayed sensitivity for the PYaTs2 compound in, but within, the concentration ranges of 128-16 $\mu\text{g/ml}$: the MPC_{50} corresponded in most cases to 32 $\mu\text{g/ml}$ and, with 128 $\mu\text{g/ml}$, the growth rate decreased by 65-6% at the most.

The reference drugs for this group of compounds were less effective: the MIC_{50} of sulphadimethoxin was usually 32 $\mu\text{g/ml}$ and its concentration of 128 $\mu\text{g/ml}$ inhibited the growth of 55% of the population at most; MIC_{50} of streptocid was somewhat lower (Table 2) and the effect when exposed to 128 $\mu\text{g/ml}$ was similar to that of sulphadimethoxin.

The compound PYaTd1 suppressed the growth of strains in the same ranges as PYaTs3 and PYaTs4. In comparison with dapsone, it was somewhat less active.

The analysis of cultured freshly isolated strains of *P. mirabilis* showed that the PYaTs3 and PYaTs4 compounds had an MIC_{50} of 8 $\mu\text{g/ml}$ and, at a concentration of 128 $\mu\text{g/ml}$, the growth of 65% of the population at most was inhibited. The compound PYaTd1 also retained its activity within the concentration range of 4-8 $\mu\text{g/ml}$ (MIC_{50}) and the concentration of 128 $\mu\text{g/ml}$ corresponded to IPC65-70 (Table 3).

The effect of the reference drugs sulphanilamides was twice as weak as PYaTs3 and PYaTs4 (Table 3). High activity was shown by dapsone.

The analysis of the cultured strains of *P. rettgeri* isolated from patients showed that the activity of the compounds towards this type of protei is somewhat lower than that of *P. mirabilis*: the range of MIC_{50} in the PYaTs3 and PYaTs4 compounds was 8-16 $\mu\text{g/ml}$, the concentration of 128 $\mu\text{g/ml}$ inhibited the growth of 65-75% of the population and the PYaTs2 functioned much more weakly (Table 4). The activity of the substance PYaTd1 towards this species remained at the same level. Sulphadimethoxin was ineffective. The activity of streptocid was higher: its MIC_{50} was 1-2 $\mu\text{g/ml}$, the maximum level of growth inhibition (128 $\mu\text{g/ml}$) not exceeding 65%. It was dapsone that actively inhibited the growth of the pathogen: at high concentrations, its effect was bactericidal and the MIC_{50} was 1 $\mu\text{g/ml}$ (Table 4).

The results of the cultured *P. vulgaris* can be seen in Table 5, which shows that, for the given type of bacteria, the use of the tested compounds and reference drugs is less effective. In all the compounds of "s" group, MIC_{50} is 64 $\mu\text{g/ml}$ and application of a dose of 128 $\mu\text{g/ml}$ inhibits no more than 60-65% of the population of strains. The MIC_{50} range of PYaTd1 shifts to higher concentrations (Table 5) and, at the concentration of 128 $\mu\text{g/ml}$,

Table 1. Sensitivity of strains of the genus *Proteus* to antibiotics

Antibiotics	<i>P. mirabilis</i>						<i>P. rettgeri</i>		<i>P. vulgaris</i>	
	<i>NIIL ty1</i>	<i>NIIL ty2</i>	<i>BK 294</i>	<i>BK 299</i>	<i>BK 190</i>	<i>BK 194</i>	<i>SES 11/01</i>	<i>SES 11/02</i>	<i>BK 01</i>	<i>BK 02</i>
Ampicillin	R	R	S	R	S	S	S	S	R	R
Amoxicillin-clavulanate	I	I	S	I	S	S	S	S	R	R
Ciprofloxacin	R	R	S	I	S	S	S	S	R	R
Gentamicin	S	S	S	S	S	S	S	S	R	R
Levofloxacin	I	I	S	S	S	S	S	S	R	R
Ceftazidime	I	I	S	S	S	S	S	S	S	R
Cefixime	S	S	S	S	S	S	S	S	S	S
Cefotaxime	R	I	S	S	S	S	S	S	S	S
Cefepime	I	I	S	S	S	S	S	S	S	I
Ampicillin-sulbactam	I	I	S	I	S	S	S	S	R	S
Ticarcillin-clavulanate	I	I	S	S	S	S	S	S	S	S
Imipenem	S	S	S	I	S	S	S	S	S	S
Ceftriaxone	R	R	S	I	S	I	S	S	S	S
Amikacin	R	I	S	I	S	I	I	I	S	S
Norfloxacin	I	I	S	S	S	I	S	S	S	S

Note: R – resistant; I – moderately resistant; S – sensitive.

Table 2. Activity of the compounds towards collection strains *P. mirabilis* ($\mu\text{g/ml}$)

Compound	<i>P. mirabilis</i> NIL tya1		<i>P. mirabilis</i> NIL tya2	
	MIC ₅₀	MIC ₉₀₋₁₀₀	MIC ₅₀	MIC ₉₀₋₁₀₀
PYATs2	8	–	16	–
PYATs3	4	128	8	–
PYATs4	4	128	4	–
PYATd1	4	128	4	–
Sulphadimethoxin	16	–	32	–
Streptocide	8	–	16	–
Dapson	1	128	2	128
	<i>P. mirabilis</i> BK 294		<i>P. mirabilis</i> BK 299	
PYATs2	16	–	16	–
PYATs3	8	–	8	–
PYATs4	8	–	4	–
PYATd1	8	–	4	–
Sulphadimethoxin	32	–	32	–
Streptocide	16	–	16	–
Dapson	2	128	4	128

Table 3. Activity of compounds towards *Proteus mirabilis* strains ($\mu\text{g/ml}$) isolated from patients

Compound	<i>P. mirabilis</i> BK194		<i>P. mirabilis</i> BK190	
	MIC ₅₀	MIC ₉₀₋₁₀₀	MIC ₅₀	MIC ₉₀₋₁₀₀
PYATs2	32	–	64	–
PYATs3	8	–	8	–
PYATs4	8	–	8	–
PYATd1	4	–	8	–
Sulphadimethoxin	64	–	128	–
Streptocide	16	–	16	–
Dapson	1	100	1	100

Table 4. Activity of compounds towards *Proteus rettgeri* Strains ($\mu\text{g / ml}$) isolated from patients

Compound	<i>P. rettgeri</i> SES 11/01		<i>P. rettgeri</i> SES 11/02	
	MIC ₅₀	MIC ₉₀₋₁₀₀	MIC ₅₀	MIC ₉₀₋₁₀₀
PYATs2	64	–	32	–
PYATs3	8	–	8	–
PYATs4	16	–	8	–
PYATd1	8	–	4	–
Sulphadimethoxin	128	–	64	–
Streptocide	1	–	2	–
Dapson	1	128	1	128

Table 5. Activity of compounds towards *Proteus vulgaris* strains ($\mu\text{g/ml}$) isolated from patients

Compound	<i>P. vulgaris</i> BK 01		<i>P. vulgaris</i> BK 02	
	MIC ₅₀	MIC ₉₀₋₁₀₀	MIC ₅₀	MIC ₉₀₋₁₀₀
PYATs2	64	–	64	–
PYATs3	64	–	64	–
PYATs4	64	–	64	–
PYATd1	32	–	16	–
Sulphadimethoxin	128	–	128	–
Streptocide	128	–	128	–
Dapson	32	128	8	128

Table 6. Average statistical values of the activity of compounds towards strains of the genus *Proteus*

Compound	MIC ₅₀	
	M±m, mkg/ml	Me±m, mkg/ml
PYATs2	37.6±7.5*#	32.0±2.3*#
PYATs3	18.8±7.5**###	8.0±3.0**###
PYATs4	18.8±7.6**###	8.0±3.0**###
PYATd1	9.2±2.8	6.0±1.0^
Sulphadimethoxin	75.2±15.1	64.0±6.0
Streptocide	34.7±15.6	16.0±6.2
Dapson	5.3±3.0	1.5±1.2

Note: * – $p \leq 0.05$, ** – $p \leq 0.01$ relating to sulphadimethoxin; # – $p \leq 0.05$, ## – $p \leq 0.01$ relating to streptocide; ^ – $p \leq 0.05$ relating to dapson.

65-75% inhibition of the growth of the microorganism population can be seen.

The reference drugs sulphadimethoxin and streptocide were ineffective: at a concentration of 128 µg/ml, they inhibited the growth of no more than 50-65% of the strain. The activity of dapson towards these strains was also reduced, but it acted efficiently in the zone of comparatively lower concentrations (Table 5).

Mean values of the activity of the compounds are shown in Table 6. It follows from the table that the PYATs3 and PYATs4 compounds containing a fragment of sulphonamides are much more active than the reference drugs. PYATs2 was also more active than the reference drugs, but it was inferior to PYATs3 and PYATs4, since its MIC₅₀ was twice as high.

The PYATd1 compound, by activity, outperformed the compounds containing a fragment of sulphonamide: its MIC₅₀ was 2-4 times less. The ability of PYATd1 to suppress the growth of strains of the genus *Proteus* was somewhat inferior to that of the reference drug dapson.

The difference in the value of their MIC₅₀ was not statistically significant, but the dapson median value was statistically significantly lower.

Conclusion

Thus, the tested compounds display different activities towards bacteria of the genus *Proteus*. The PYATs2 substance is ineffective. With respect to strains of *P. mirabilis* and *P. rettgeri*, the minimum inhibitory concentration of the compounds PYATs3, PYATs4 and PYATd1 ranges from 4 µg/ml to 16 µg/ml, which falls within an allowable range of concentrations required for antibacterial drugs. With respect to strains of *P. vulgaris*, the activity of the compounds is much lower. The most effective compound, according to the mean values regardless of the species and strain of bacteria, is the compound PYATd1, having an MIC₅₀ within 10 µg/ml, which proves it to be promising and makes further development worthwhile.

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