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Synthesis and antibacterial activity of amino acids modified with specifically substituted pyrrole heterocycle

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Since last century studying the properties of various heterocyclic compounds including pyrrole, as anti-inflammatory and anti-pain agents is explored strongly. It is interesting to note that a number of molecules containing in its structure pyrrole heterocycle are approved as drugs with diverse activities in medical practice.

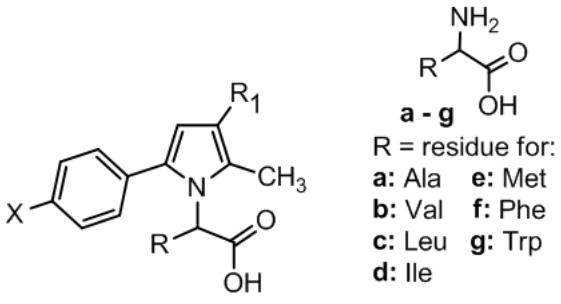
Design of a series of substituted with specifically modified pyrrole heterocycle at N-terminus amino acids was done. The aim compounds were synthesized in acid conditions using Paal-Knorr reaction between substituted pyrrole and natural amino acids. Further, after purification and characterization antibacterial activity against model Gram positive (*Bacillus cerreus 1085*), Gram negative (*Pseudomonas fluorescens*) microorganisms and fungi (*Candida lipolytica*) were studied by means of standard disk diffusion method. The highest activity against model strain Gram positive bacteria *(Bacillus cereus 1085)* show compounds Pyr-Ile (1d) and Pyr-β-Phe (1f). The best activity against model Gram negative microorganism (*Pseudomonas fluorescens 1442*) was revealed for compounds Pyr-Met (1e) and Pyr-β-Phe (1f). All tested compounds have not any activity against model strain fungi *Yarrowia lipolytica 3344*. Compound Pyr-β-Phe (1f) shows strong bacteriostatic effect against strain *Bacillus cereus 1085*.

Key words: pyrrole, Paal-Knor reaction, Bacillus cerreus 1085, Pseudomonas fluorescens, Candida lipolytica

INTRODUCTION

Since last century studying the properties of various heterocyclic compounds including pyrrole, as anti-inflammatory and anti-pain agents is explored strongly [1]. It is interesting to note that a number of molecules containing in its structure pyrrole heterocycle [2, 3] as well as unnatural amino acids are approved as drugs with diverse activities in medical practice [4-6]. Pyrrole derivatives are essential in finding new drugs with pharmacological activity as anti-inflammatory, cytotoxicity, in vitro cytotoxic activity against tumors [7], in the treatment of hyperlipidemia [8], etc. Pyrrole-containing heterocyclic derivatives show biological activity as COX-1/COX-2 inhibitors and cytotoxic activity against different human tumor models [9]. They also show antioxidant [10], anticonvulsant [11], HIV-inhibiting action [12]. Pyrrole heterocycle participate in the classic Non-steroidal anti-inflammatory drugs (NSAIDs): Tolmelin, Zomepirac and Klopirac, and in recent years their important biological activity is confirmed in many investigations in different directions [13-15].

Design of a series of substituted with specifically modified pyrrole heterocycle at N-terminus amino acids was done. The targeted products (fig. 1) were synthesized via Paal-Knorr cyclization by condensation of seven amino-acids (**a-g**), acting as primary amines and 1,4-dicarbonyl compounds. The later was prepared by C-alkylation of commercially available β-dicarbonyl compound with ω-bromoacetophenone and then used *in situ* [16].



where **1a-1g**: X = Cl, $R_1 = COOC_2H_5$.

Fig.1. Structure of pyrrolylamino acids tested for antibacterial activity

Herein we report the antibacterial activity against model Gram positive (*Bacillus cerreus 1085*), Gram negative (*Pseudomonas fluorescens*)

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microorganisms and fungi (*Candida lipolytica*) by means of standard disk diffusion method.

EXPERIMENTAL

Material and Methods

Pseudomonas fluorescens 1442, Bacillus cerreus 1085 and *Yarrowia lipolytica 3344*- microbial strains were supplied by the National Bank for Industrial Microorganisms and Cell Cultures (NBIMCC). Salts for nutrient medium were obtained from Merck (Germany). Glucose and bovine serum albumin (BSA) were obtained from Fluka (Switzerland). Agar and LB for nutrient microbial growth mediums were obtained from Sigma-Aldrich. Sterile disks impregnated with Gentamicin (10μg) and Fluconazole (25μg) were supplied by FOT (Bulgaria).

Cell cultures

Bacillus cerreus 1085, Pseudomonas fluorescens 1442 and *Yarrowia lipolytica 3344* were cultivated on solid agar nutrient medium containing meat extract, peptone, glucose, NaCl at 30°C for 24 h. After incubation process the bacterial colonies were picked up and suspended in liquid nutrient medium containing LB and 10% glucose for 24h in water bath shaker at 30 °C, pH 7.2-7.4. Further the cells were suspended in fresh liquid medium, containing LB and 10% glucose and the biomass was used for investigation of antimicrobial activity.

The yeast colonies were suspended in liquid nutrient medium containing yeast extract, malt extract, peptone and 10 % glucose. The biomass was cultivated for 24h in water bath shaker at 30^0C, pH 6.

Standard disk diffusion test was used for studding of antimicrobial activity of target compounds. Discs impregnated with Gentamicin (10μg) and fluconazole (25μg) were used as refers.

Standard DNS method was used for determination of glucose consumption by *Bacillus cereus 1085*.

RESULTS AND DISCUSSION

The activity of newly synthesized compounds are tested against model Gram negative microorganisms - *Pseudomonas fluorescens 1442*, Gram positive microorganisms - *Bacillus cerreus 1085*, and fungi - *Yarrowia lipolytica 3344.*

All impregnated sterile disks with different concentration of N –pyrrolyl amino acids (**1a-1g**), are incubated in petri dishes with biomass of strain *Bacillus cerreus 1085*. 24 hours later obtained inhibition zones are measured and all data are summarized in table 1. Sterile disks impregnated with 10μg Gentamicin (commercially available) were used as check samples.

Table 1. Inhibition zones in mm for tested compounds 1a-1g at concentrations 50mM and 25mM against *B.cereus*

Test compound	Zone of inhibition of *B. Cereus* at 50 mM concentration [mm]	Zone of inhibition of *B. Cereus* at 25mM concentration [mm]
1a	10	no effect
1b	13	10
1c	11	9
1d	16	15
1f	15	14
1g	8	8
Gentamicin	23	23

As it can be seen from the data in table 1, the highest activity reveals compounds Pyr-Ile (1d) and Pyr-β-Phe (1f) at both 50mM and 25mM concentrations. *Bacillus cereus1085* is resistant against compounds 1a, 1c and 1g at 50 mM and 25 mM concentrations. According to compound 1b *Bacillus cereus 1085* is moderately sensitive at 50 mM and microorganism is resistant at 25mM. Because of the good activity of compounds 1d and 1f they were also tested at 10 mM concentration. The obtained inhibition zones are 10mm which show that at this concentration strain *Bacillus cereus 1085* is resistant.

Table 2. Inhibition zones in mm for tested compounds1a-1g at concentrations 50mM and 25mM against *Pseudomonas fluorescens 1442*

Test compound	Zone of inhibition of *Ps. Fluorescens* at 50 mM [mm]	Zone of inhibition of *Ps.fluorescens* at 25 mM [mm]
1a	13	no effect
1b	10	8
1c	10	9
1d	15	11
1e	20	no effect
1f	23	12
1g	no effect	no effect
Gentamicin	23	23

The same methods were used for determination of antibacterial activity of newly synthesized compounds against Gram negative microorganism

Ps. fluorescens 144. All studies were done again at 25mM and 50mM concentrations.

Sterile disks impregnated with 10µg gentamicin (commercially available) were again used as check sample. Results are summarized at table 2.

Data in table 2 show that compounds 1b, 1c and 1g have no activity against strain *Pseudomonas fluorescens 1442*. Strain is moderately sensible to compounds 1a and 1d at 50mM concentration, but it is resistant at concentration 25mM.Compounds 1e and 1f have strong antibacterial activity at 50 mM concentration but strain is resistant at 25 mM

All compounds did not exhibit any antifungal activity against model strain *Yarrowia lipolytica 3344.*

Investigation of antimicrobial activity of Bacillus cereus 1085 in presence of compound Pyr-β-Phe

Monitoring of bacterial growth is an additional method for investigation of antibacterial Monitoring of bacterial growth is an additional method for investigation of antibacterial properties of compounds. Herein, we studied biomass growth of *Bacillus cereus 1085* in presence and absence of compound **1f**. For this purpose we used blank sample without compound and biomass in presence of 10 mM concentration of **1f**. The absorbance of the biomass were measured at 590 nm spectrophotometrically during 98h of incubation time (Fig.2)

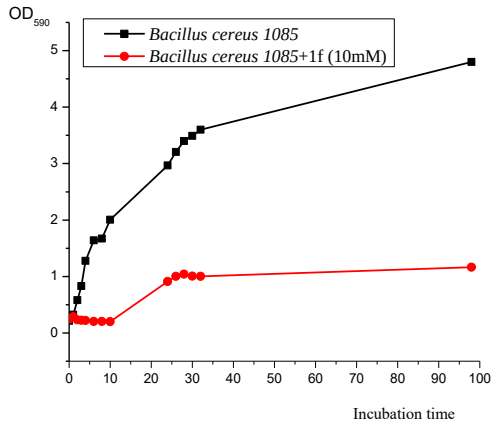

Fig. 2. Growth curves of *Bacillus cereus 1085* in presence and absence of 1f

From the figure is noticed that lag-phase of the cells without compound **1f** is shorter in comparison of those in presence of investigated compound. The cells are rapidly entered into exponential phase lasting up to 32 hours. After that the growth of cells is in steady state but don't enter in the stationary phase until the end of the incubation time. In terms of cultivation of test compound (1f) is observed a longer initial phase, approximately 10 hours of incubation time. The exponential phase is prolonged to 28h. After that is observed the stationary stage of the cells culture. It can be concluded from the obtained data that compound **1f** shows bacteriostatic effect to test microorganism *Bacillus cereus*.

In addition, the dynamic of glucose accumulation by *Bacillus cereus* cells in presence and absence of Pyr-β-Phe (1f) compound by using DNS method (Fig.3) were studied.

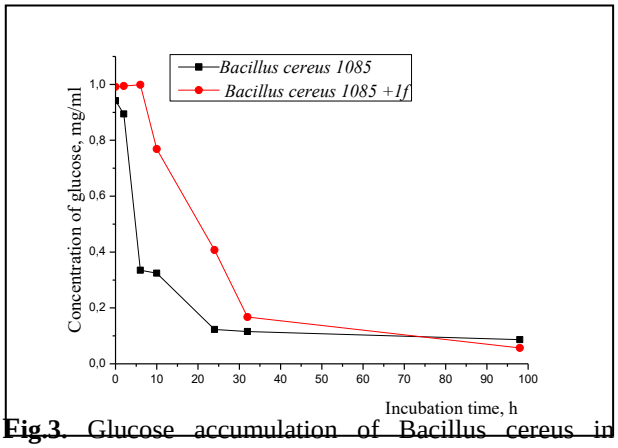

Fig.3. Glucose accumulation of Bacillus cereus in presence and absence of Pyr-β-Phe (1f)

The figure shows that the nutrient source accumulation by *Bacillus cereus* cells in the terms of cultivation without **1f** is proportional of the incubation time. In the presence of Pyr-β-Phe (1f) the assimilation of glucose begins after 10h of incubation time. The glucose is accumulated completely up to 35h of incubation time.

CONCLUSION

Antibacterial activity against model Gram positive (*Bacillus cerreus 1085*), Gram negative (*Pseudomonas fluorescens*) microorganisms and fungi (*Candida lipolytica*) of six newly synthesized pyrrolylamino acids were studied. The obtained results show that the highest activity against model strain Gram-positive bacteria *(Bacillus cereus 1085)* show compounds **Pyr-Ile (1d)** and **Pyr-β-Phe (1f)**. The best activity against model gram negative microorganism (*Pseudomonas fluorescens 1442*) was revealed for compounds **Pyr-Met (1e)** and **Pyr-β-Phe (1f)**. All tested compounds have no any activity against model strain fungi *Yarrowia lipolytica 3344.*

Bacteriostatic effect is observed onto growth of *Bacillus cereus* cells in the presence of **Pyr-β-Phe (1f).**

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СИНТЕЗ И АНТИБАКТЕРИАЛНА АКТИВНОСТ НА АМИНОКИСЕЛИНИ МОДИФИЦИРАНИ СЪС СПЕЦИФИЧНО ЗАМЕСТЕН ПИРОЛОВ ХЕТЕРОЦИКЪЛ

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(Резюме)

Свойствата на различни съединения, съдържащи пиролов хетероцикъл се изследват широко от началото на века като противовъзпалителни и противо-болкови агенти. Интересно е, също така, че редица молекули, съдържащи пиролов хетероцикъл са доказани лекарства с разнообразна активност в медицинската практика.

Беше направен дизайн на серия от аминокиселини, чиито N-край е включен в специфично заместени пиролови производни. Целевите съединения бяха синтезирани в кисела среда по реакцията на Паал-Кнор между заместен пирол и природни аминокиселини. След пречистване и охарактеризиране, съединенията бяха подложени на изследвания за антибактериална активност спрямо моделни Грам положителни (*Bacillus cerreus 1085*), Грам отрицателни (*Pseudomonas fluorescens*) микроорганизми и гъби (*Candida lipolytica*) чрез използване на стандартен дисково-дифузионен метод. Най-висока активност срещу моделния Грам положителен щам микроорганизми *(Bacillus cereus 1085)* показаха съединенията Pyr-Ile (1d) и Pyr-β-Phe (1f). Най-добра активност по отношение на моделните Грам отрицателни микроорганизми (*Pseudomonas fluorescens 1442*) беше установена за съединенията Pyr-Met (1e) и Pyr-β-Phe (1f). Всички тествани съединения не показаха активност спрямо моделният щам гъби *Yarrowia lipolytica 3344*. Съединението Pyr-β-Phe (1f) показва силен бактериостатичен ефект спрямо моделния щам *Bacillus cereus 1085*.