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ANTIMYCOBACTERIAL PROPERTIES OF 5H-[1,3,4]THIADIAZOLO[2,3B]QUINAZOLIN-5-ONE DERIVATIVES

PROPRIETĂȚILE ANTIMICOBACTERIENE ALE DERIVATILOR DE 5H-[1,3,4]THIADIAZOLO[2,3B] QUINAZOLIN-5-ONĂ

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Rezumat. Triptantrinul este un inhibitor cunoscut al Mycobacterium tuberculosis purtător de enoyl-acyl protein-reductază (InhA). La modificarea structurii triptantrinului au fost obținuți analogi de 5H- [1,3,4] tiadiazolo [2,3b] chinazolin-5-onă cu proprietăți antimicobacteriene diverse potență. Ca rezultat al experimentelor dedicate studiului proprietăților antimicobacteriene ale derivaților 5H- [1,3,4] tiadiazolo [2,3b] chinazolin-5-onă, sa constatat că trei derivați ai acesteia au activitate antituberculoasă semnificativă. Compusul 2-Mercapto-5H- [1,3,4] tiadiazolo [2,3-b] chinazolin-5-ona în 4 experimente din 5, în concentrația de 200 μg/ml (concentrația minimă inhibitoare (MIC)) a prezentat activitatea de inhibare superioară împotriva creșterii micobacteriene (până la 100%). Compusul 2-Mercapto-5H- [1,3,4] tiadiazolo [2,3-b] chinazolin-5-one sulfat în 3 experimente din 5, în concentrația 200 μg/mL (MIC) a prezentat activitate de inhibare superioară împotriva creșterii micobacteriene (până la 100%). Compusul complex al 2-Mercapto-5H- [1,3,4] tiadiazolo [2,3-b] chinazolin-5-onei cu β-ciclodextrină în 4 experimente din 5, în concentrația 200 μg/mL (MIC) a prezentat activitate de inhibare superioară împotriva creșterii micobacteriene (până la 100%). Acești trei derivați ai 5H- [1,3,4] tiadiazolo [2,3b] chinazolin-5-onei au prezentat activitate de inhibare superioară împotriva creșterii micobacteriene la aceeași concentrație ca și medicamentul antituberculos de primă linie rifampicina în condiții experimentale - 200 μg/mL, aceasta fiind o caracteristică pozitivă pentru studiul ulterior al derivaților 5H-[1,3,4] tiadiazolo [2,3b] chinazolin-5-onă cu perspectiva dezvoltării unui nou medicament antituberculos.
Cuvinte cheie: derivați de 5H-[1,3,4]tiadiazolo[2,3b]chinazolin-5-onă, activitate antimicobacteriană, concentrație minimă inhibitorie.

Abstract. Tryptanthrin is a known inhibitor of Mycobacterium tuberculosis enoyl-acyl carrier protein reductase (InhA) and modifications in its structure gave a group of 5H-[1,3,4]thiadiazolo[2,3b] quinazolin-5-one analogues with antimycobacterial various potency. As a result of experiments devoted to the study of the antimycobacterial properties of derivatives of 5H-[1,3,4] thiadiazolo[2,3b] quinazolin-5-one, it was found that three derivatives of it have antituberculosis activity. The compound 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b] quinazolin-5-one in 4 experiments out of 5 in the concentration 200 μg/mL (minimum inhibitory concentration (MIC)) exhibited the superior inhibition activity against mycobacterial growth (up to 100%). The compound 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b]quinazolin-5-one sulfate in 3 experiments out of 5 in the concentration 200 μg/mL (MIC) exhibited the superior inhibition activity against mycobacterial growth (up to 100%). The compound complex of 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b] quinazolin-5-one with ß-cyclodextrin in 4 experiments out of 5 in the concentration 200 μg/mL (MIC) exhibited the superior inhibition activity against mycobacterial growth (up to 100%). Three derivatives of 5H-[1,3,4]thiadiazolo[2,3b]quinazolin-5-one exhibited the superior inhibition activity against mycobacterial growth at the same concentration as the first-line antituberculosis drug rifampicin in experimental conditions – 200 μg/mL, which is a positive characteristic for further study of derivatives of 5H-[1,3,4] thiadiazolo[2,3b] quinazolin-5-one with the perspective of developing a new antituberculosis medicine.
Keywords: derivatives of 5H-[1,3,4] thiadiazolo[2,3b] quinazolin-5-one, antimycobacterial activity, minimum inhibitory concentration.

Introduction

According to the estimates of the World Health Organization 8.9 - 11.0 million people worldwide fell ill with tuberculosis in 2019. Between 2015 and 2019, the number of deaths from tuberculosis decreased by 14%. However, multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) pose major threats to national TB control programs. In 2019 3.3% of new MDR-TB cases and 18% of MDR-TB cases were detected among patients previously treated for tuberculosis. There were an estimated 400 000 – 535 000 incident cases of rifampicin resistant tuberculosis, 78% had MDR-TB [1]. Thus, with an increase in the incidence rates, prevalence and absolute number of patients with MDR of mycobacterium tuberculosis, the need for new compounds with anti-tuberculosis activity increases.

Derivatives of 5H- [1,3,4] thiadiazolo[2,3b] quinazolin-5-one are trypthantrin (1, indolo[2,1-b]quinazoline-6,12-dione) analogues. Tryptanthrin is a known inhibitor of Mycobacterium tuberculosis enoyl-acyl carrier protein reductase (InhA) and modifications in its structure gave a group of 5H-[1,3,4]thiadiazolo[2,3b]quinazolin-5-one analogues with antimycobacterial various potency. Tryptanthrin is a naturally occurring compound from the class of tryptophan-derived alkaloids produced by different plants and fungi [2]. It has been established that tryptantrin and its derivatives have antimycobacterial activity with different activity in vitro and in vivo [3].

The purpose – study of antimycobacterial properties of derivatives of 5H-[1,3,4]thiadiazolo[2,3b]quinazolin-5-one

Materials and Methods

The study of the antimycobacterial properties of derivatives of 5H-[1,3,4]thiadiazolo[2,3b]quinazolin-5-one was carried out on the strain *Mycobacterium terrae 15755*. This strain is non-pathogenic and is recommended for use as a model for determining anti-tuberculosis activity [4]. Antimycobacterial properties were evaluated based on the minimum inhibitory concentration (MIC, μg/mL), which are shown in the table. To study the antimycobacterial properties against *Mycobacterium terrae 15755*, the method of dilutions in solid nutrient medium in Petri dishes was used. For this, the initial solution of the compound in dimethyl sulfoxide (concentration 2000 μg/mL) was added to Middlebrook 7H9 broth with glycerol to obtain the required concentrations (200; 100; 50; 25; 12.5 and 6.25 μg/mL). Then, a culture of mycobacteria was inoculated into all analyzed solutions. For blank controls, two samples were used. In order to control the effect of the solvent, dimethyl sulfoxide was added to the first sample in the same amount as in the samples with the maximum concentration of the analyte – 200 μg/mL. The second sample did not contain any additives (culture growth control). All samples were kept in a thermostat at 37°C for three weeks. To assess the antimicrobial properties, the MIC (μg/mL) was determined, which corresponds to the concentration of the analyte at which the growth of mycobacteria in the Petri dish was not observed. In parallel experiments, rifampicin, isoniazid, and ethambutol, which have a mycobactericidal effect and are used to treat tuberculosis, were used as reference standards [5].

Results and discussions: The results of determining the antimycobacterial properties of derivatives of 5H-[1,3,4]thiadiazolo[2,3b]quinazolin-5-one are shown in the table, which shows the observed growth of Mycobacterium terrae 15755 at various concentrations of the studied compounds – 6.25 μg / mL, 12.5 μg / mL, 25.0 μg / mL, 50 μg / mL, 100 μg / mL and 200 μg / mL (table 1).

The compound 2-Mercapto-5H-[1,3,4] thiadiazolo[2,

Table 1. Antimycobacterial properties of derivatives of 5H-[1,3,4]thiadiazolo[2,3b]quinazolin-5-one

Chemical name of the compound	6,25 μg/mL	12,5 μg/mL	25,0 μg/mL	50,0 μg/mL	100,0 μg/mL	200,0 μg/mL
2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b]quinazolin-5-one	+ + + +	+ + + +	+ + +	+ + +	+	-
	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ + +
	+ + + +	+ + + +	+ + +	+ + +	+ +	-
	+ + + +	+ + + +	+ + +	+ + +	+ + +	-
	+ + + +	+ + + +	+ + +	+ + +	+ +	-
sulfate 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b]quinazolin-5-one	+ + + +	+ + + +	+ + + +	+ + +	+	-
	+ + + +	+ + + +	+ + + +	+ + +	+	-
	+ + + +	+ + + +	+ + +	+ + +	+ + +	+
	+ + + +	+ + + +	+ + +	+ + +	+ + +	-
	+ + + +	+ + + +	+ + +	+ + +	+ + +	+
complex of 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b]quinazolin-5-one with ß-cyclodextrin	+ + + +	+ + + +	+ + + +	+ + +	+	-
	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +	+ +
	+ + + +	+ + + +	+ + + +	+ + +	+ +	-
	+ + + +	+ + + +	+ + + +	+ + +	+ +	-
	+ + + +	+ + + +	+ + + +	+ + +	+ + +	-
rifampicin	+ + +	+ + +	+ +	+ +	+	-
	+ + + +	+ + + +	+ + +	+ +	+	-
isoniazid	+ +	+ +	+	-	-	-
	+ + +	+ +	+	+	-	-
ethambutol	+ +	+ +	-	-	-	-
	+ +	+ +	+	-	-	-

+ + + + bountiful growth; + + + strong growth; + + weak growth, + slight growth; - lack of growth

3-b]quinazolin-5-one in 4 experiments out of 5 in the concentration 200 μg/mL (minimum inhibitory concentration (MIC)) exhibited the superior inhibition activity against mycobacterial growth (up to 100%).

The compound 2-Mercapto-5H-[1,3,4] thiadiazolo[2, 3-b]quinazolin-5-one sulfate in 3 experiments out of 5 in the concentration 200 μg/mL (MIC) exhibited the superior inhibition activity against mycobacterial growth (up to 100%).

The compound complex of 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b] quinazolin-5-one with ß-cyclodextrin in 4 experiments out of 5 in the concentration 200 μg/mL (MIC) exhibited the superior inhibition activity against mycobacterial growth (up to 100%).

Rifampicin, which is currently used for the treatment of tuberculosis and is a first-line drug, in the concentration 200 μg/mL exhibited the superior inhibition activity against mycobacterial growth (up to 100%).

Isoniazid, which is currently used for the treatment of tuberculosis and is a first-line drug, exhibited the superior inhibition activity against mycobacterial growth (up to 100%) in concentrations 50 μg/mL and 100 μg/mL.

Ethambutol, which is currently used for the treatment of tuberculosis and is a first-line drug, exhibited the superior inhibition activity against mycobacterial growth (up to 100%) in the concentrations 25 μg/mL and 50 μg/mL.

Thus, the antimycobacterial properties of three derivatives of 5H-[1,3,4]thiadiazolo[2,3b]quinazolin-5-one, such as 2-Mercapto-5H-[1,3,4]thiadiazolo[2,3-b]quinazolin-5-one, 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b] quinazolin-5-one sulfate and complex of 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b]quinazolin-5-one with ß-cyclodextrin was found to be the same as rifampicin, which is currently used to treat tuberculosis and is a first-line drug, which is a positive characteristic for further study of derivatives of 5H-[1,3,4] thiadiazolo[2,3b]quinazolin-5-one.

Conclusions

As a result of experiments devoted to the study of the antimycobacterial properties of derivatives of 5H-[1,3,4] thiadiazolo[2,3b] quinazolin-5-one, it was found that three studied derivatives of 5H-[1,3,4] thiadiazolo[2,3b] quinazolin-5-one have anti-tuberculosis activity. The compound 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b] quinazolin-5-one in 4 experiments out of 5 in the concentration 200 μg/mL (MIC) exhibited the superior inhibition activity against mycobacterial growth (up to 100%). The compound 2-Mercapto-5H-[1,3,4] thiadiazolo [2,3-b] quinazolin-5-one sulfate in 3 experiments out of 5 in the concentration 200 μg/mL (MIC) exhibited the superior inhibition activity against mycobacterial growth (up to 100%). The compound complex of 2-Mercapto-5H-[1,3,4] thiadiazolo[2,3-b] quinazolin-5-one with ß-cyclodextrin in 4 experiments out of 5 in the concentration 200 μg/mL (MIC) exhibited the superior inhibition activity against mycobacterial growth (up to 100%). Three studied derivatives of 5H-[1,3,4] thiadiazolo[2,3b] quinazolin-5-one exhibited the superior inhibition activity against mycobacterial growth at the same concentration as the first-line anti-tuberculosis drug rifampicin in experimental conditions - 200 μg/mL, which is a positive characteristic for further study of derivatives of 5H-[1,3,4]thiadiazolo[2,3b] quinazolin-5-one with the perspective of developing a new antituberculosis medicine.

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