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Original Article

Antimicrobial evaluation of some novel derivatives of 3,4-dihydropyrimidine-2(1*H*)-one

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Abstract

The antimicrobial activity of thirty six novel dihydropyrimidine derivatives was evaluated against common pathogenic bacteria. Significant antimicrobial activity (MIC=32, 64 µg/ml) was observed. *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria and *Staphylococcus aureus* as Gram-positive bacteria were determined to be the most susceptible pathogens in this study. The highest inhibitory activity was observed against Gram-negative microorganisms. The widest spectrum of antibacterial activity was exerted by **C6** and **C22**. Most of the compounds had remarkable antifungal activity (MIC=32 µg/ml).

Keywords: Antibacterial; Antifungal; 3,4-Dihydropyrimidine-2(1*H*)-one

INTRODUCTION

Despite the recent advances in medicine, antimicrobial chemotherapy still remains a significant problem in most under-developed and developed countries. Narrow spectrum of the activity of some antimicrobial drugs in the market, intolerable adverse effects and the inevitable emergence of resistant strains of pathologic microorganisms urge the replacement of these drugs with more acceptable compounds. Therefore, many different chemical entities are screened every day with the hope of identifying antimicrobial hits by pharmaceutical companies as well as academic institutions.

3, 4-dihydropyrimidine -2(1*H*)-one (DHPM) derivatives belong to an interesting class of heterocyclic compounds which has attracted considerable attention of medicinal chemists (1). DHPMs have been considered for a variety of biological activities such as antitumor (2), antiviral (3,4) and antioxidant (5,6) activities. There are also many reports of their antibacterial and antifungal properties. Many DHPMs have been examined for their antimicrobial effects in the recent years (7-10). They differ in the substituents on the C-4 and C-5 positions of the dihydropyridine ring, the two key positions on the structure of this type of pyrimidines.

In the present study, we decided to evaluate the antimicrobial activity of thirty six novel DHPM derivatives. Some common pathogenic microorganisms were used for the antimicrobial evaluations.The studied compounds possess (substituted) phenyl or benzyl ring at the C-4 position of the 3,4-dihydropyrimidine-2(1*H*)-one (thion) ring. They have also either an aryl carboxamide or an alkyl carboxylate ester at the C-5 position of this ring. We also evaluated the antimicrobial activity of another compound having an *n*-propyl group at the C-4 position. The aim of this study was to understand the effect of a non-aromatic moiety at the C-4 position on the antimicrobial activity of such pyrimidines. The role of substituents on the phenyl ring at the C-4 position and also the aryl carboxamide or alkyl carboxylate ester on the antimicrobial activity of this class of compounds were also observed through this study.

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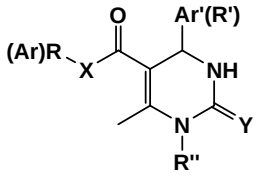
MATERIALS AND METHODS

Thirty six 3,4- dihydropyrimidine -2(1H)-one -5-carboxamides or 5-carboxylate esters were subjected to antimicrobial evaluation. The studied compounds were prepared in the Catalysis Division, Department of Chemistry, University of Isfahan, Isfahan, Iran. Synthesis of all compounds has been reported previously by this research group (11,12). General structure and structural details of the studied compounds are provided in Table 1.

Table 1. General structure and structural details of the compounds used in antimicrobial studies.



Compound	Ar(R)	X	Y	Ar′(R′)	R″
C_1	2-ClC_6H_4	NH	O	2-ClC_6H_4	H
C_2	4-BrC_6H_4	NH	O	C_6H_5	H
C_3	4-FC_6H_4	NH	O	C_6H_5	H
C_4	C_6H_{11}	NH	O	C_6H_5	H
C_5	2-ClC_6H_4	NH	O	2-ClC_6H_4	H
C_6	2-ClC_6H_4	NH	O	4-ClC_6H_4	H
C_7	2-ClC_6H_4	NH	O	3-BrC_6H_4	H
C_8	2-ClC_6H_4	NH	O	4-BrC_6H_4	H
C_9	2-ClC_6H_4	NH	O	4-$CH_3C_6H_4$	H
C_{10}	2-ClC_6H_4	NH	O	3-$NO_2C_6H_4$	H
C_{11}	2-ClC_6H_4	NH	O	4-$NO_2C_6H_4$	H
C_{12}	2-ClC_6H_4	NH	O	3-$CH_3OC_6H_4$	H
C_{13}	2-ClC6H4	NH	O	4-$CH_3OC_6H_4$	H
C_{14}	2-ClC6H4	NH	O	$C_6H_5CHCH_3$	H
C_{15}	C_6H_5	NH	O	C_6H_5	H
C_{16}	4-$CH_3OC_6H_4$	NH	O	C_6H_5	H
C_{17}	2-ClC_6H_4	NH	O	4-FC_6H_4	H
C_{18}	2-ClC_6H_4	NH	S	4-BrC_6H_4	H
C_{19}	2-ClC_6H_4	NH	S	C_6H_5	H
C_{20}	$C_6H_5CH_2$	NH	S	C_6H_5	H
C_{21}	$C_6H_5CH_2$	NH	S	2-ClC_6H_4	H
C_{22}	$C_6H_5CH_2$	NH	S	4-$CH_3C_6H_4$	H
C_{23}	C_2H_5	O	O	C_6H_5	H
C_{24}	C_2H_5	O	O	C_6H_5	CH_3
C_{25}	C_2H_5	O	O	4-FC_6H_4	H
C_{26}	C_2H_5	O	O	4-FC_6H_4	CH_3
C_{27}	CH_3	O	O	4-FC_6H_4	H
C_{28}	CH_3	O	O	4-FC_6H_4	CH_3
C_{29}	CH_3	O	O	*n*-C_3H_7	H
C_{30}	C_2H_5	O	S	C_6H_5	CH_3
C_{31}	CH_3	O	S	C_6H_5	CH_3
C_{32}	C_2H_5	O	O	4-BrC_6H_4	H
C_{33}	CH_3	O	O	4-BrC_6H_4	H
C_{34}	C_2H_5	O	S	4-BrC_6H_4	H
C_{35}	CH_3	O	S	4-BrC_6H_4	H
C_{36}	C_2H_5	O	O	4-BrC_6H_4	CH_3

Minimum inhibitory concentrations (MICs) were determined against three Gram-positive bacteria: *Staphylococcus aureus* PTCC 1337, *Listeria monocytogenes* RITCC 1293 4a serotype, and *Bacillus subtilis* PTCC 1023, and three Gram-negative bacteria: *Escherichia coli* PTCC 1330, *Pseudomonas aeruginosa* PTCC 1074, and *Salmonella enteritidis* RITCC 1624. The studied compounds were also screened for their antifungal activity against one yeast-like fungus: *Candida albicans* PTCC 5027 and one mold: *Aspergillus niger* PTCC5011. All microorganisms were obtained from Persian type culture collection. Muller-Hinton broth and Roswell Park Memorial Institute (RPMI) 1640 medium buffered with 3-(N-morpholino) propanesulfonic acid (MOPS) at pH 7.0 were used for growth of the bacteria and fungi, respectively (13). Microplate Alamar Blue® Assay (MABA) was used for antimicrobial assay. The inoculate of microorganism (10^6 CFU/ml) were prepared from overnight culture and suspensions were adjusted to 0.5 McFarland standard turbidity. The final size of inoculate was 1.5×10^4 CFU/ml for bacteria. The test compound dissolved in dimethyl sulfoxide (DMSO) was first diluted to the highest concentration to be tested and DMSO had no effect on the microorganisms in the concentrations studied. Serial two-fold dilutions were made in concentration range from 8 µg/ml to 512 µg/ml in sterile 96 well microplates. Then 20 µl of Alamar Blue® reagent was added to each well. Plates covered and sealed with parafilm and incubated for 24 h at 37°C. The MIC was defined as the lowest concentration, which prevented a color change from blue to pink. Ciprofloxacin was used as the standard antibacterial drug. The same method except some modifications was used for the antifungal studies. The final size of inoculate of microorganism was 1.5×10^5 CFU/ml for fungi. The incubation time was 48 h at 25°C. Ketoconazol was used as standard antifungal agent. All antimicrobial assay repeated at least three times.

RESULTS

The antibacterial and antifungal activity data are given in Table 2. Significant inhibitory activity, MIC=32, 64 µg/ml, was observed against the studied pathogenic bacteria. *Escherichia coli* and *Pseudomonas aeruginosa* as Gram-negative bacteria were the most susceptible microorganisms. *Staphylococcus aureus* was the most vulnerable Gram-positive bacteria in this study.

Most of the compounds had remarkable antifungal activity against the microorganisms employed in this study. Nine out thirty six studied compounds exerted a good antifungal activity (MIC=32 µg/ml) against both *Aspergillus niger* and *Candida albicans.* As can be seen in Table 2, *Candida albicans* was more susceptible than *Aspergillus niger* against the studied compounds.

DISCUSSION

The antibacterial data of present study (Table 2) was indicated that Gram-negative bacteria are more sensitive against most of the under investigation compounds. The higher inhibitory activity against Gram-negative microorganisms can be explained by the presence of lypophilic moieties in the dihydropyrimidine scaffold. *Bacillus subtilis* was the less prone bacteria against the studied pyrimidines. The widest spectrum of antibacterial activity was exerted by **C6** and **C22**. Phenyl ring, substituted with electron withdrawing or electron releasing substituents was the C-4 group in the most active compounds (MIC=32 µg/ml). The most potent antibacterial compounds in the present study belong to both C-5 aryl carboxamide and C-5 alkyl carboxylate ester containing ones compounds. Comparison of the results of **C23** and **C24**; **C25** and **C26**; and **C27** and **C28** reveals that methylation of the N-1 has no role on the antibacterial activity of the molecule. **C29** which contains an alkyl moiety at the C-4 position of the 3,4-dihydropyrimidine ring had a good antibacterial effect against *Staphylococcus aureus*.

Interestingly, nine compounds were indicated relatively high antifungal activity. They usually were compounds possessing a phenyl ring substituted with an electron withdrawing rather than an electron releasing group at the C-4 position. This is in

accordance with the results of other researches on the antifungal activity of DHPMs. Antimicrobial activity of some 4-aryl-5-isopropoxycarbony l-6- methyl -3,4- dihydro pyrimidine-2(1H) ones containing a *p*-substituted phenyl at C-4 position of the dihydropyrimidine ring has revealed that the most potent compounds were those with an electron withdrawing substituent on the C-4 phenyl group (7). **C29** had a good antifungal effect. The obtained results for the antimicrobial activity of this compound is not enough to claim that a substituted or unsubstituted phenyl ring is not necessary for the good antibacterial activity of this kind of pyrimidines. However, to the best of our knowledge there is not any other report of the antimicrobial activity of DHPMs containing any alkyl group at C-4.

CONCLUSION

Significant inhibitory activity (MIC=32, 64 µg/ml) was observed against the studied pathogenic bacteria. The best inhibitory activity was observed against Gram-negative microorganisms. This observation supposed to be related to the lypophilic character of the compounds. *Escherichia coli* and *Pseudomonas aeruginosa* among Gram-negative and *Staphylococcus aureus* in Gram-positive bacteria were determined to be the

Table 2. The In vitro antimicrobial activity of the synthesized pyrimidines against selected microorganisms.

Compound	Gram-negative bacteria			Gram-positive bacteria			Fungi	
	MIC (µg/ml)							
	E. coli	*S. enteritidis*	*P. aeruginosa*	*S. aureus*	*L. monocytogenes*	*B. subtilis*	*A. niger*	*C. albicans*
C_1	-	-	256	-	-	-	-	-
C_2	-	-	256	-	-	-	64	32
C_3	512	512	256	512	512	512	32	32
C_4	128	256	-	256	512	256	32	32
C_5	-	-	512	-	-	-	-	32
C_6	64	64	64	64	128	64	-	64
C_7	-	128	-	256	512	-	-	64
C_8	256	256	512	512	512	-	-	-
C_9	-	128	64	256	256	256	32	256
C_{10}	-	128	-	-	-	512	32	32
C_{11}	64	64	128	128	128	-	32	32
C_{12}	64	128	-	-	128	-	-	-
C_{13}	512	512	512	-	-	-	-	32
C_{14}	128	128	128	128	256	128	64	32
C_{15}	512	32	128	512	512	512	-	-
C_{16}	512	512	32	512	512	-	256	-
C_{17}	512	512	32	512	512	512	64	64
C_{18}	128	512	64	64	128	-	32	64
C_{19}	64	512	256	256	64	512	64	128
C_{20}	256	512	256	512	32	-	64	256
C_{21}	512	512	512	512	512	512	32	32
C_{22}	64	32	64	64	64	-	-	-
C_{23}	512	-	32	512	512	512	512	-
C_{24}	256	512	512	512	256	-	64	32
C_{25}	512	-	512	256	256	512	32	64
C_{26}	256	512	512	512	256	512	32	32
C_{27}	256	512	512	512	256	512	32	512
C_{28}	512	512	512	512	256	512	32	256
C_{29}	128	256	128	64	512	256	32	64
C_{30}	512	512	512	128	256	256	32	512
C_{31}	512	512	128	512	32	512	64	32
C_{32}	256	-	256	256	128	-	32	64
C_{33}	256	512	256	512	512	512	32	32
C_{34}	64	-	64	64	-	-	32	32
C_{35}	64	-	128	256	512	256	32	64
C_{36}	64	-	256	256	128	128	32	32
Ciprofloxacin					>32			
Ketoconazol					>32			

most susceptible pathogens in this study. The widest spectrum of antibacterial activity was exerted by **C6** and **C22**. Most of the compounds showed a remarkable antifungal activity (MIC=32 µg/ml). *Candida albicans* was more vulnerable than *Aspergillus niger* against the studied compounds. Structural modifications of the most active compounds determined in this work might provide good hit compounds for further studies as potential antimicrobial agents.

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