

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

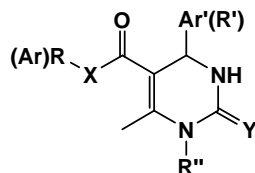
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MATERIALS AND METHODS

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 ₆ H ₄	NH	O	2-ClC ₆ H ₄	H
C ₂	4-BrC ₆ H ₄	NH	O	C ₆ H ₅	H
C ₃	4-FC ₆ H ₄	NH	O	C ₆ H ₅	H
C ₄	C ₆ H ₁₁	NH	O	C ₆ H ₅	H
C ₅	2-ClC ₆ H ₄	NH	O	2-ClC ₆ H ₄	H
C ₆	2-ClC ₆ H ₄	NH	O	4-ClC ₆ H ₄	H
C ₇	2-ClC ₆ H ₄	NH	O	3-BrC ₆ H ₄	H
C ₈	2-ClC ₆ H ₄	NH	O	4-BrC ₆ H ₄	H
C ₉	2-ClC ₆ H ₄	NH	O	4-CH ₃ C ₆ H ₄	H
C ₁₀	2-ClC ₆ H ₄	NH	O	3-NO ₂ C ₆ H ₄	H
C ₁₁	2-ClC ₆ H ₄	NH	O	4-NO ₂ C ₆ H ₄	H
C ₁₂	2-ClC ₆ H ₄	NH	O	3-CH ₃ OC ₆ H ₄	H
C ₁₃	2-ClC ₆ H ₄	NH	O	4-CH ₃ OC ₆ H ₄	H
C ₁₄	2-ClC ₆ H ₄	NH	O	C ₆ H ₅ CHCH ₃	H
C ₁₅	C ₆ H ₅	NH	O	C ₆ H ₅	H
C ₁₆	4-CH ₃ OC ₆ H ₄	NH	O	C ₆ H ₅	H
C ₁₇	2-ClC ₆ H ₄	NH	O	4-FC ₆ H ₄	H
C ₁₈	2-ClC ₆ H ₄	NH	S	4-BrC ₆ H ₄	H
C ₁₉	2-ClC ₆ H ₄	NH	S	C ₆ H ₅	H
C ₂₀	C ₆ H ₅ CH ₂	NH	S	C ₆ H ₅	H
C ₂₁	C ₆ H ₅ CH ₂	NH	S	2-ClC ₆ H ₄	H
C ₂₂	C ₆ H ₅ CH ₂	NH	S	4-CH ₃ C ₆ H ₄	H
C ₂₃	C ₂ H ₅	O	O	C ₆ H ₅	H
C ₂₄	C ₂ H ₅	O	O	C ₆ H ₅	CH ₃
C ₂₅	C ₂ H ₅	O	O	4-FC ₆ H ₄	H
C ₂₆	C ₂ H ₅	O	O	4-FC ₆ H ₄	CH ₃
C ₂₇	CH ₃	O	O	4-FC ₆ H ₄	H
C ₂₈	CH ₃	O	O	4-FC ₆ H ₄	CH ₃
C ₂₉	CH ₃	O	O	<i>n</i> -C ₃ H ₇	H
C ₃₀	C ₂ H ₅	O	S	C ₆ H ₅	CH ₃
C ₃₁	CH ₃	O	S	C ₆ H ₅	CH ₃
C ₃₂	C ₂ H ₅	O	O	4-BrC ₆ H ₄	H
C ₃₃	CH ₃	O	O	4-BrC ₆ H ₄	H
C ₃₄	C ₂ H ₅	O	S	4-BrC ₆ H ₄	H
C ₃₅	CH ₃	O	S	4-BrC ₆ H ₄	H
C ₃₆	C ₂ H ₅	O	O	4-BrC ₆ H 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 lipophilic 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-isopropoxycarbonyl-6-methyl-3,4-dihydropyrimidine-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 lipophilic 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	
	<i>E. coli</i>	<i>S. enteritidis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>	<i>B. subtilis</i>	<i>A. niger</i>	<i>C. albicans</i>
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.

ACKNOWLEDGMENT

This project was funded by the School of Nutrition and Food Sciences, Isfahan University of Medical Sciences.

REFERENCES

1. Kappe CO. Biologically active dihydropyrimidones of the Biginelli-type – A literature survey. *Eur J Med Chem.* 2000;35:1043-1052.
2. Klein E, DeBonis S, Thiede B, Skoufias DA, Kozielski F, Lebeau L. New chemical tools for investigating human mitotic kinesin Eg5. *Bioorg Med Chem.* 2007;15:6474-6488.
3. Hurst EW, Hull R. Two new synthetic substances active against viruses of the psittacosis-lymphogranuloma-trachoma group. *J Med Pharm Chem.* 1961;3:215-229.
4. Zabihollahi R, Vahabpour R, Hartoonian C, Sedaghati B, Sadat SM, Soleymani M, *et al.* Evaluation of the *in vitro* antiretroviral potential of some Biginelli-type pyrimidines. *ActaVirologica.* 2012;56:11-18.
5. Stefani HA, Oliveira CB, Almeida RB, Pereira CMP, Braga RC, Cell R, *et al.* Dihydropyrimidin-(2H)-ones obtained by ultrasound irradiation: A new class of potential antioxidant agents. *Eur J Med Chem.* 2006;41:513-518.
6. Mansouri M, Movahedian, Rostami M, Fassihi A. Synthesis and antioxidant evaluation of 4-(furan-2-yl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate esters. *Res Pharm Sci.* 2012;4:257-264.
7. Chitra S, Devanathan, Pandiarajan DK. Synthesis and *in vitro* microbiological evaluation of novel 4-aryl-5-isopropoxycarbonyl-6-methyl-3,4-dihydropyrimidinones. *Eur J Med Chem.* 2010;45:367-371.
8. Kumar R, Malik S, Chandra R. Synthesis and antimicrobial activity of 4-[5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl]-dihydropyrimidines and 4-[5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl]-3,4-dihydropyrimidin-2-ones. *Ind J Chem.* 2009;48:718-724.
9. Singh OM, Singh SJ, Devi MB, Devi LN, Singh NI, Lee SG. Synthesis and *in vitro* evaluation of the antifungal activities of dihydropyrimidinones. *Bioorg Med Chem Lett.* 2008;18:6462-6467.
10. Sedaghati B, Fassihi A, Arbabi S, Ranjbar M, Memarian HR, Saghaie L, *et al.* Synthesis and antimicrobial activity of novel derivatives of Biginellipyrimidines. *Med Chem Res.* In Press. DOI 10.1007/s00044-011-9931-7
11. Memarian HR, Ranjbar M. Synthesis of Biginelli compounds using cobalt hydrogen sulfate. *J Chin Chem Soc.* 2011;58:522-527.
12. Soleymani M, Memarian HR. Synthesis of 3,4-dihydropyrimidin-2(1H)-one-5-carboxamides. *Z Naturforsch.* 2010;65:485-492.
13. Kirilmis C, Ahmedzade M, Servi S, Koca M, Kizirgil A, Kazaz C. Synthesis and antimicrobial activity of some novel derivatives of benzofuran: Part 2. The synthesis and antimicrobial activity of some novel 1-(1-benzofuran-2-yl)-2-mesitylethanone derivatives. *Eur J Med Chem.* 2008;43:300-308.