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Research Article

Biological Evaluation of Pyrrolo [3, 2-d] pyrimidine Derivatives as Antibacterial Agents against Pathogenic Bacteria

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Abstract

Background: Pyrimidine molecules' biological and chemotherapeutic importance in the medicinal world has been overlooked in many reports. We have previously synthesized new series of pyrrolo [3,2-d]pyrimidine derivatives (4a-4f) and here, we evaluate the antibacterial activity of these derivatives against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Salmonella bacteria.*

Materials and Methods: The agar well diffusion and agar dilution methods were used for determining inhibition zone diameter and minimum inhibitory concentration during preliminary evaluation of antimicrobial activity against gram-positive and gram-negative bacteria. Statistical analysis using Microsoft Excel 2010 was based on three independent experiments and the results were expressed as mean.

Results: Some of the synthesized compounds exhibited antibacterial activity against the tested *bacteria*.

Conclusion: Our findings indicate the antibacterial potential of the six novel synthetic pyrrolo[3,2-d]pyrimidine compounds.

Keywords: antibacterial activity, pyrimidine, antimicrobial activity

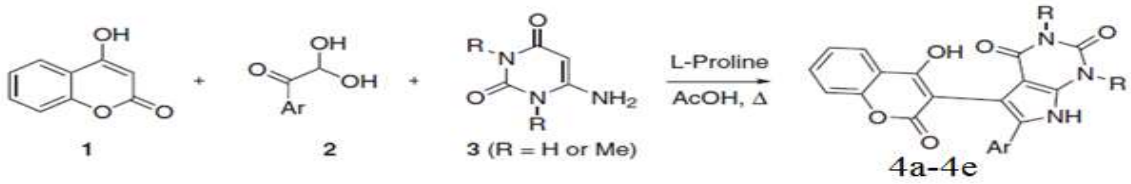
Introduction

Among various classes of heterocyclic compounds containing nitrogen, pyrimidines have attracted considerable attention as potential bioactive molecules. Pyrimidine derivatives are known as new cytostatic drugs which are used as basic structure of marketed drugs like anticancer (Gemcitabin, Flourouracil and Floxuridin) [1,2], antihyperlipidemic (Aronixil) [3], antipsychotic (Buspirone and and Risperidone) [4], antihistaminic (Thonzylamine) [5], antiviral (Etravirine) [6-8], antipsoriatic (Enazardem) [9], antilipidemic drugs (Rosuvastatin) [10]. Other drugs in which pyrimidine plays a key role include antivirals and antibiotics. Etravirine and Iclaprim are respectively antiviral and antibiotic drugs, which have a pyrimidine ring in their structure [11-13]. All of the studies on syntheses of these compounds are aimed at the condensation of the pyrimidine ring with other aromatic or heterocyclic rings to found pharmaceutical molecules. This information clearly reveals the important role of these derivatives as new pharmaceuticals active compounds and therefore the advancement of efficient methods to synthesize these biological active compounds is very important. Because pathogenic bacteria are constantly resisting current antibiotics, we always need to discover and identify alternative drugs to overcome this problem. In our previous study we synthesized pyrrolo [2,3-d]pyrimidine derivatives by one-pot and three-component reaction [14]. As synthesis and evaluation of antimicrobial activity is an important part of our research program herein, we have evaluated antibacterial and activity of these derivatives against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Salmonella bacteria.*

Materials and Methods

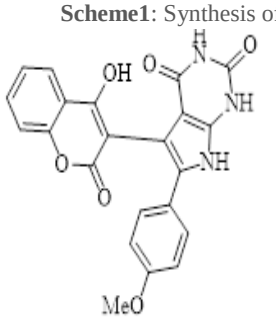
General procedure for synthesis of pyrrolo[3,2-*d*]pyrimidine derivatives

To synthesize pyrrolo[3,2-d]pyrimidine derivatives, stirred solution of 4-hydroxycoumarin, arylglyoxal hydrate , 6-aminouracil or 1,3-dimethyl-6-aminouracil and L-proline in acetic acid (5 mL) were heated under reflux for 4 h. The mixture was left to cool in room temperature and the resulting precipitate was filtered, washed with cold EtOH and dried to give products **4a-4g** as orange needles in 73-86% yields [14, 15].



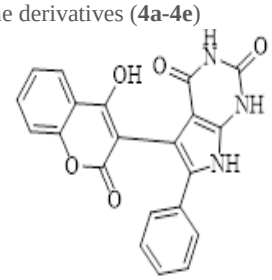
Scheme1: Synthesis of pyrrolo [3,2-d]pyrimidine derivatives (**4a-4e**)

4a)



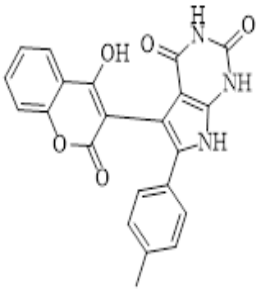
5-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-6-(4-methoxyphenyl)
-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione

4b)



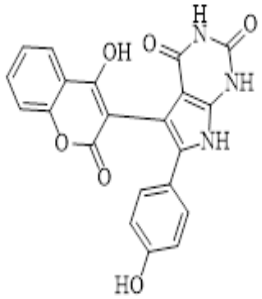
5-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-6-phenyl-
1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione

4c)



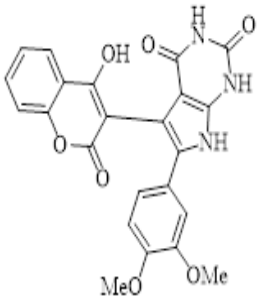
5-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-6-(*p*-tolyl)-
1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione

4d)



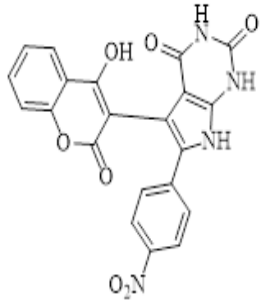
5-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-6-(4-hydroxyphenyl)
-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione

4e)



5-(3,4-dimethoxyphenyl)-5-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)
-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione

4f)



5-(4-hydroxy-2-oxo-2*H*-chromen-3-yl)-6-(4-nitrophenyl)
-1,7-dihydro-2*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*)-dione

Fig 1. *Chemical properties of pyrrolo[3,2-d]pyrimidine derivatives (4a-4e)*

The in vitro antimicrobial study of the pyrrolo[3,2-d]pyrimidine derivatives was evaluated against *Staphylococcus aureus* ATCC **25923**, *Pseudomonas aeruginosa* ATCC **27853**, *Escherichia coli* ATCC **25922** *and Salmonella* ATCC 1730 bacteria, by Well agar diffusion and Agar dilution methods. All microorganisms were obtained from the culture collection of Urmia University of Medical Sciences.The stock solution

was prepared for pyrimidine derivatives (4a-4e) and reference drugs (Ceftizoxime and Ciprofloxacin) in 99.5% Dimethyl Sulfoxide (DMSO) to get a concentration of 1000 µg/mL. This stock solution was used for Agar well diffusion method [16, 17]. Briefly 80 µL of pyrimidine derivatives (in 1000 µg/ml concentration), were moved into the plates which tested bacteria, aseptically swabbed on their surface and wells were created by a sterile borer. The plates were incubated at 35°C for 24h. The experiment was carried out twice and the zone of inhibition was recorded. Agar dilution method was used in the second step of antibacterial tests. In this step MIC (Minimum Inhibitory Concentration) values that were defined as lowest concentration of compound for inhibiting the growth of tested bacteria were determined. Experiments were performed with five different concentrations of each pyrimidine derivative (1000 µg/mL, 500 µg/mL, 250 µg/mL, and 125 µg/mL, 62.5 µg/mL µg/mL) to prepare doubling serial dilutions of compounds in medium up to the fifth concentration. Negative (DMSO) and positive Control (Ceftizoxime and Ciprofloxacin) plates were prepared at the same way [18, 19]. Statistical analysis using Microsoft Excel 2010 was based on three independent experiments and the results were expressed as mean.

Result

Antimicrobial evaluations were determined by Agar diffusion and Agar dilution methods for each bacterial strain and were shown in (Table 1). The antibacterial nature was categorized following the Rota et al. scale, which reports weak activity with a Inhibition Zone ≤ 12 mm, moderate activity with a Inhibition Zone ranging between >12 and <20 mm, and strong potential with a Inhibition Zone ≥ 20 mm [20,21]. Based on the inhibition zone diameter measured for the stock solution, all tested compounds exhibited weak antibacterial activity against Gram-negative and Gram-positive bacteria. The antibacterial activity of all synthesized compounds was weaker than ciprofloxacin and ceftizoxim. As shown in table 1, all synthesized compounds exhibited antibacterial activity against tested bacteria with inhibition zone diameters ranging from 12 to 13 mm and the MICs of the synthesized compounds were lower than the MIC obtained from reference drugs. The MBC of the pyrimidine derivatives was similar or two-fold higher than the MIC values.

Compounds	***Staphylococcs uaureus***	***Escherichi coli***	***Pseudomons aeruginosa***	***Salmonella***
4a	1000(≥12)	1000(≥12)	1000(≥12)	1000(≥12)
4b	1000(≥12)	500(13)	1000(≥12)	1000(≥12)
4c	1000(≥12)	1000(≥12)	1000(≥12)	1000(≥12)
4d	1000(≥12)	1000(≥12)	1000(≥12)	1000(≥12)
4e	1000(≥12)	500(13)	500(13)	1000(≥12)
ceftizoxim	125(25)	100(16)	250(14)	100(14)
ciprofloxacin	0.07(40)	15(45)	1.5(42)	35(36)

Table1. *Minimum inhibitory concentrations (µg/mL) and inhibition zone diameter (1mg/ml) of the pyrrolo [3,2-d]pyrimidine derivatives against the tested bacteria*

Discussion

Previous reports indicated that the 4,4′-(1,4-phenylene)bis(pyrimidin-2-amine) derivatives against Gram positive and Gram negative bacterial strains have shown significant antibacterial activity as compared to Cefadroxil (standard drug)[22]. Mohamed *et al* found that the Thieno [2,3-d]pyrimidin-4(3H)-one derivatives have good antibacterial activity[23]. Moreover, Verma et al. reported pyridopyrimidine carboxylate derivatives showed moderate antibacterial activity [24]. Interestingly (5-bromo-2-chloro-pyrimidin-4-ylsulfanyl)-4-methoxy-phenylamine derivativs showed a good antibacterial activity against *Bacillus subtilis,Staphylococcus aureus, Xanthomonas campestris and Escherichiacoli* [25].Our pyrimidin series (4a–4f), utilizing Methoxyphenyl (4a), Methylphenyl (4b), Dimethoxyphenyl (4c), Phenyl (4d), Hydroxyphenyl (4e) and Nitrophenyl (4f) substitutions (Figure 1) did not lead to significant antibacterial activity. The growth of *Escherichi coli* was inhibited by the compounds 4b and 4e at the range of 250-500 µg/ mL. In addition, compound 4e was active against *Pseudomons aeruginosa*. All of the compounds showed poor activity compared with ciprofloxacin and ceftizoxim. The lowest activity of compounds was observed against *Staphylococcs uaureus* and *Salmonella.* The MBC of compounds was the same or three fold higher than the corresponding MIC results.In order to improve and increase the antimicrobial nature of the synthesized derivatives, it is necessary to make fundamental changes in the structure and side substitutions of the synthesized derivatives.As mentioned before, the proposed one-step method for the synthesis of compounds, can provide an efficient and impressive method for other pharmaceutical fields with time efficiency and less energy input. Continuation of study is required to evaluate the synergistic effect of synthesized compounds, and assess their safety and efficacy. But the proposed one-step method can provide an efficient and impressive method for other pharmaceutical fields with time efficiency and less energy input.

Conclusion

In this study, the antibacterial activity of a new series of pyrrolo[3,2-d]pyrimidine derivatives (**4a**-**4e**)was tested against four pathogenic bacteria. The test results showed that the compounds 4a-4f exhibited weak antibacterial activity.

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Ethical Considerations

No manipulation or something similar were carried out on any animals.

Compliance with ethical guidelines

This article does not contain any studies involving animals or human participants performed by any of the authors.

Funding

The authors received no specific funding for this work."

Conflict of interest

We declare that we have no conflict of interest and the ethical principles for this research have been respected.

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