

Antibacterial studies of dihydropyrimidinones and pyrimidinethiones

Abstract

Some new dihydro pyrimidinone and pyrimidinethione compounds have been synthesized and their antibacterial activities have been studied in dimethyl formamide and dimethyl sulphoxide against some Gram positive and Gram negative bacteria. For this, Agar well diffusion method is used. It is observed that inhibition depends on solvent, bacterial strain, nature and position of substitution in a compound. When the same substitution is attached to different moieties its effect on bacterial strains changes in different solvents.

Keywords: dihydro pyrimidinone, pyrimidinethione, dimethyl formamide

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Introduction

The extensive use of antibiotics resulted in the multiple drug resistant pathogens. This highlights the need for the development of new classes of antimicrobial agents and alteration of known drugs in such a way that would allow them to retain their resistance to the pathogen. So, chemists and pharmacists are always in search of new drugs molecules.

Heterocyclic compounds containing nitrogen are promising structure moiety for drug design. Dihydropyrimidines are one of the important heterocyclic compounds, which are of interest due to its efficiency towards various pharmacological uses. These are large group of structurally diverse compounds which are known to possess some characteristic properties like manifestations of novel structures, thermal stability, relevant biological properties, high synthesis flexibility and medicinal utility.¹⁻¹² Further, some of dihydropyrimidines are reported to overcome multidrug resistance efficiently.¹³

Thus, in the present paper, some new dihydropyrimidinone and pyrimidinethione compounds (shown in Figure 1) have been synthesized and their antibacterial activities have been studied in dimethyl formamide and dimethyl sulphoxide against some Gram positive and Gram negative bacteria.

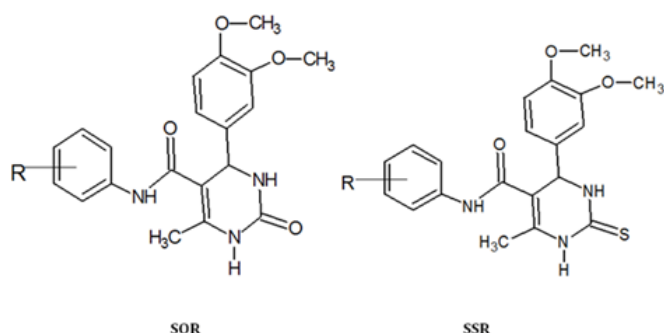


Figure 1 General structure of dihydropyrimidinone (SOR) and pyrimidinethione (SSR) compounds.

Materials and methods

Synthesis

Synthesis of dihydropyrimidinone derivatives: (i) Synthesis of 3, 4-dimethoxybenzaldehyde: An aqueous solution of vanillin was refluxed for half an hour with stirring at 100°C. To this solution, few drops of NaOH and dimethyl sulphate were added slowly and the reaction mixture was again refluxed with stirring for about 4 hours. In this reaction mixture, diethyl ether was added and the solvent was evaporated to get the crude product 3,4-dimethoxybenzaldehyde. (ii) Synthesis of 3-oxa-N-phenylbutanamide: To an equimolar mixture of substituted aniline and ethyl acetoacetate in toluene, few drops of NaOH solution were added and the solution was refluxed for 12 hours. The excess of toluene was distilled out and the reaction mixture was taken in hexane and stirred. The product was isolated and was dissolved in NaOH solution. This solution was then neutralized with dilute hydrochloric acid and product was separated. (iii) Synthesis of 4-(3,4-dimethoxyphenyl)-6-methyl-2-oxa-N-phenyl-1, 2, 3, 4-tetrahydropyrimidin-5-carboxamide: A methanolic solution of 3, 4-dimethoxy aldehyde, substituted diketones and urea was refluxed for 12 hours using conc. HCl as catalyst. After the completion of reaction, product was isolated and recrystallized from ethanol. Similarly other compounds were synthesized.

Synthesis of pyrimidinethione derivatives: (i) Synthesis of 3, 4-dimethoxybenzaldehyde: As above. (ii) Synthesis of 3-oxa-N-phenylbutanamide: As above. (iii) Synthesis of 4-(3,4-dimethoxyphenyl)-6-methyl-N-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidin-5-carboxamide: A methanolic solution of 3,4-dimethoxy aldehyde, 3-oxo-N-phenylbutanamide and thiourea was refluxed for 12 hours using conc. HCl as catalyst. After the completion of reaction, product was isolated and recrystallized from ethanol. Similarly other compounds were synthesized.

The physical parameters along with substitution groups of synthesized compounds are listed in Table 1.

Antimicrobial activity

The antibacterial activities of all the synthesized compounds were

studied in dimethyl sulphoxide (DMSO) and dimethylformamide (DMF) using Agar well diffusion method. The solvent DMSO and DMF were also purified before use by standard method.¹⁴

Preparation of the test compound: The synthesized compounds were dissolved in DMF and DMSO at a concentration of 1mg/μl.

Table 1 Physical data and substitution groups of synthesized compounds

Compound code	R	M.Wt. (g)	M.F.	RfValue	M.P.°C	Yield %
RVG series						
SOR-1	4-OCH ₃	397	C ₂₁ H ₂₃ N ₃ O ₅	0.61	168	53
SOR-2	4-CH ₃	381	C ₂₁ H ₂₃ N ₃ O ₄	0.54	152	57
SOR-3	4-Cl	401	C ₂₀ H ₂₀ N ₃ O ₄ Cl	0.49	179	46
SOR-4	2-CH ₃	381	C ₂₁ H ₂₃ N ₃ O ₄	0.47	182	62
SOR-5	3-OCH ₃	397	C ₂₁ H ₂₃ N ₃ O ₅	0.54	164	45
SOR-6	4-F	385	C ₂₀ H ₂₀ N ₃ O ₄ F	0.71	160	59
SOR-7	2,5-di Cl	436	C ₂₁ H ₁₉ N ₃ O ₄ Cl ₂	0.37	171	72
SOR-8	3-Cl	401	C ₂₁ H ₂₀ N ₃ O ₄ Cl	0.62	198	62
SOR-9	3,4- di Cl	436	C ₂₁ H ₁₉ N ₃ O ₄ Cl ₂	0.66	169	77
SOR-10	3-Cl, 4-F	419	C ₂₁ H ₁₉ N ₃ O ₄ ClF	0.52	145	78
SRG series						
SSR-1	4-OCH ₃	413	C ₂₁ H ₂₃ N ₃ O ₄ S	0.49	211	63
SSR-2	4-CH ₃	397	C ₂₁ H ₂₃ N ₃ O ₃ S	0.53	194	56
SSR-3	4-Cl	417	C ₂₀ H ₂₀ N ₃ O ₃ ClS	0.46	224	66
SSR-4	2- CH ₃	397	C ₂₁ H ₂₃ N ₃ O ₃ S	0.49	232	61
SSR-5	3-OCH ₃	413	C ₂₁ H ₂₃ N ₃ O ₄ S	0.62	215	75
SSR-6	4-F	401	C ₂₀ H ₂₀ N ₃ O ₃ FS	0.7	248	59
SSR-7	2,5-di Cl	452	C ₂₁ H ₁₉ N ₃ O ₃ Cl ₂ S	0.59	251	70
SSR-8	3-Cl	417	C ₂₁ H ₂₀ N ₃ O ₃ ClS	0.82	219	65
SSR-9	3,4- di Cl	452	C ₂₁ H ₁₉ N ₃ O ₃ Cl ₂ S	0.63	263	67
SSR-10	3-Cl, 4-F	435	C ₂₁ H ₁₉ N ₃ O ₃ ClFS	0.7	201	72

Test microorganisms: The synthesized compounds were tested for its antibacterial activity against Gram positive bacteria viz. *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Bacillus megaterium* ATCC 9885 and Gram negative bacteria viz. *Salmonella typhimurium* ATCC 23564, *Proteus mirabilis* NCIM 2241 and *Klebsiella pneumoniae* NCIM 2719. Microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India. These microorganisms were maintained at 4°C using nutrient agar slants.

Agar well diffusion method: The antimicrobial study of synthesized compounds were done by agar well diffusion method. The bacterial strains were activated by inoculating in 25ml of N-broth. This mixture was incubated for 24h in an incubator at 37°C. 0.2ml of the activated strain was incubated in Mueller Hinton Agar. The nutrient medium Mueller Hinton agar was kept at 45°C and was then poured in Petri dishes. These dishes are then left without disturbance so that the medium should be solidified. Using a sterile cork borer, 0.85cm well was made in the Petri dishes and in each well, 0.1ml of test solution was filled. These dishes were then incubated for 24h at 37°C. For each bacterial strain, three experiments were done for each compound and mean value was used as zone of inhibition for each sample. The

inhibition was also measured for pure DMSO or DMF for each strain, which was considered as control. The zone of inhibition for each compound in each strain is given after subtraction of zone of control.

Results and discussion

For all the synthesized compounds, physical parameters are given in Table 1 along with substitution group.

Antibacterial activity

1, 4-dihydropyrimidinones: Figure 2 shows the zone of inhibition against the Gram positive bacteria in DMSO. It is observed in DMSO that against *S. aureus*, SOR-7 exhibited maximum inhibition and SOR-8 showed minimum inhibition. SOR-1, SOR-2, SOR-4, SOR-6, SOR-9 and SOR-10 showed no inhibition at all. For *B. cereus*, again, SOR-7 showed maximum inhibition whereas SOR-9 and SOR-10 exhibited minimum inhibition. SOR-4 and SOR-6 showed no inhibition at all. Against *B. megaterium*, only SOR-7 exhibited maximum inhibition whereas SOR-10 showed minimum inhibition. SOR-4 had no inhibition. Thus, in DMSO, SOR-7 is most effective compound in the studied bacteria.

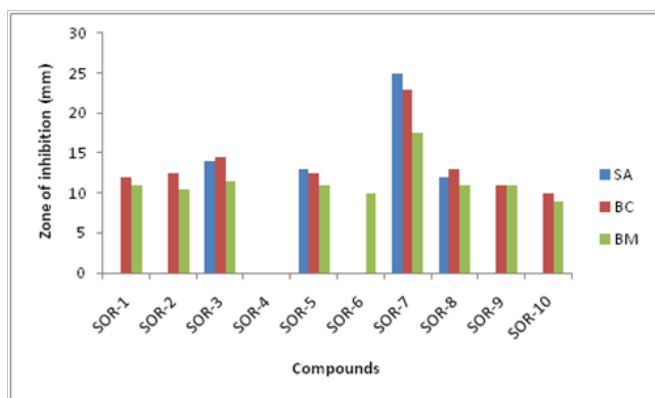


Figure 2 Zone of inhibition of SOR compounds against Gram positive bacteria in DMSO.

In DMF, zone of inhibition against Gram positive bacteria is given in Figure 3. It is observed that again SOR-7 showed maximum inhibition for all the three bacterial strains. For *S. aureus* and *B. cereus*, SOR-4 and SOR-6 shows no inhibition at all whereas for *B. megaterium*, all the compounds show inhibition.

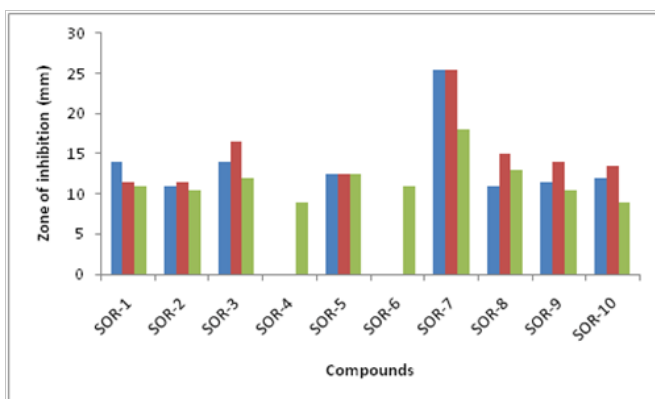


Figure 3 Zone of inhibition of SOR compounds against Gram positive bacteria in DMF.

Thus, for both DMSO and DMF, *B. megaterium* is the most susceptible bacteria and SOR-4 and SOR-6 show no inhibition against *S. aureus* and *B. cereus*. The inhibition depends on solvent, strain and structure of the compound. SOR-4 and SOR-6 contain 2-methyl and 4-fluoro group respectively. Thus, these substitutions are not effective against *S. aureus* and *B. cereus*. On the other hand, the presence of 2,5-dichloro groups (as in SOR-7) increases the inhibition in the studied solvents for these bacteria. Other substitutions have an intermediate effect.

Figure 4 shows the zone of inhibition against Gram negative bacteria in DMSO. It is observed again the inhibition is maximum for SOR-7 against all the three bacterial strains. For *S. typhimurium*, only SOR-5 and SOR-7 showed inhibition and other compounds had no inhibition at all. For *K. pneumonia*, SOR-1, SOR-4, SOR-5 and SOR-9 did not effect. Against *P. mirabilis*, SOR-4 and SOR-6 showed no inhibition. Thus, for Gram negative bacteria also, maximum inhibition is observed by SOR-7 containing 2, 5-dichloro substitution. Whereas, SOR-4 and SOR-6 containing 2-methyl and 4-fluoro substitutions in aromatic ring had no effect on these bacteria. Thus, in DMSO, SOR-7 is the most effective compound for these bacteria and *P. mirabilis* is the most susceptible bacteria.

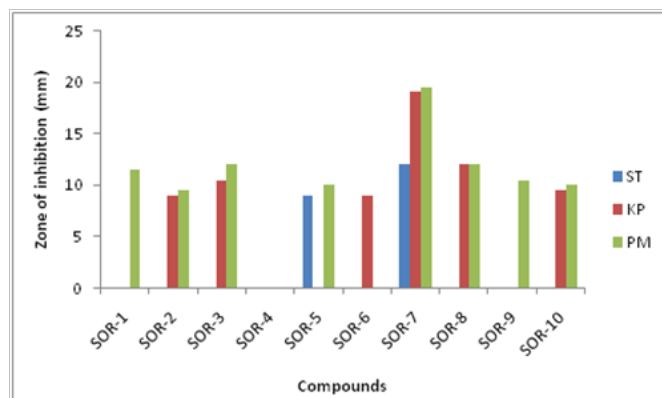


Figure 4 Zone of inhibition of SOR compounds against Gram negative bacteria in DMSO.

Figure 5 shows the zone of inhibition against Gram negative bacteria in DMF. Against all the three bacteria, again SOR-7 exhibited maximum inhibition. For *S. typhimurium*, inhibition is quite less in comparison to other two Gram negative bacteria; *K. pneumonia* and *P. mirabilis*. For this strain, inhibition is more or less same for all the compounds except SOR-9 which had no effect on this bacterium. The comparison of inhibition of SOR-9 (containing 3, 4-dichloro substitution) with that of SOR-7 (containing 2, 5-dichloro substitution) suggests that position of substitution also plays an important role in inhibition. For *K. pneumonia*, all the compounds show inhibition and maximum inhibition is observed for SOR-7 containing 2, 5-dichloro substitution. Against *P. mirabilis*, SOR-10 exhibited minimum inhibition. SOR-1, SOR-2, SOR-4, SOR-5 and SOR-6 showed no inhibition at all. Thus, in DMF *K. pneumonia* is the most susceptible bacteria and compound with chloro substitution at 2nd and 5th position is most effective. So, overall, in SOR series, DMF is good solvent and nature and position of substituent play important role in inhibition.

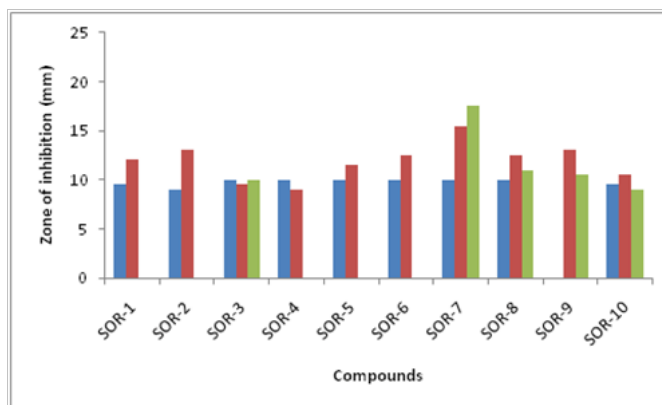


Figure 5 Zone of inhibition of SOR compounds against Gram negative bacteria in DMF.

Dihydropyrimidinethiones: Figure 6 shows the zone of inhibition against the Gram positive bacteria in DMSO for these synthesized compounds. It is observed that against *S. aureus*, SSR-1, SSR-2 and SSR-7 are not effective whereas SSR-6 exhibited maximum inhibition. For *B. cereus*, all the compounds showed inhibition and maximum is observed by SSR-5. Against *B. megaterium*, only SSR-4 had no inhibition and SSR-5 and SSR-6 exhibited maximum inhibition. So, overall, SSR-6 containing 4-fluoro group as substitution is effective in DMSO for these three Gram positive bacteria and *B. cereus* is the most susceptible bacteria.

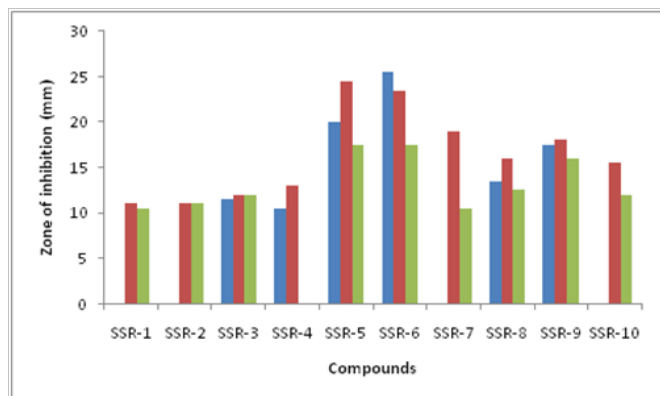


Figure 6 Zone of inhibition of SSR compounds against Gram positive bacteria in DMSO.

Figure 7 shows the zone of inhibition against the Gram positive bacteria in DMF where all the compounds exhibited inhibition against studied bacterial strains. However, again SSR-6 containing 4-fluoro substitution showed maximum inhibition for all the three bacterial strains.

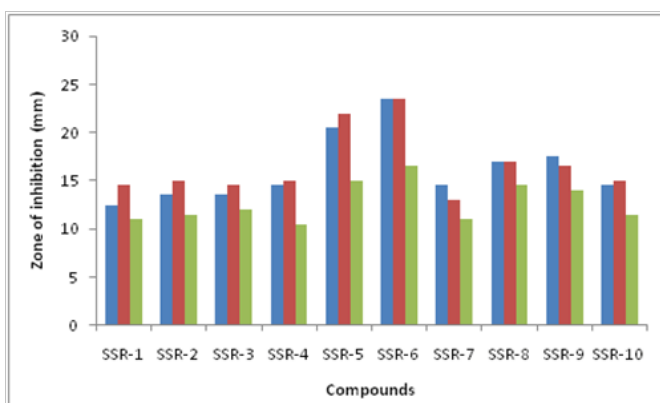


Figure 7 Zone of inhibition of SSR compounds against Gram positive bacteria in DMF.

Comparison of inhibition in DMSO and DMF shows that inhibition is greater in DMSO than in DMF

Figure 8 shows the zone of inhibition against Gram negative bacteria in DMSO. It is observed that for *S. typhimurium*, only SSR-5 showed inhibition and other compounds had no inhibition at all. Thus, 3-methoxy is effective in DMSO. Against *K. pneumonia*, SSR-10 had no effect whereas and SSR-6 exhibited maximum inhibition. Against *P. mirabilis*, all the compounds exhibited inhibition and SSR-5 and SSR-6 showed maximum inhibition. Thus, the presence of 3-methoxy (as in SSR-5) and 4-fluoro (as in SSR-6) are equally effective. Overall, 3-methoxy and 4-fluoro substitutions are most effective and *S. typhimurium* is the most resistant bacteria.

Figure 9 shows the zone of inhibition against Gram negative bacteria in DMF. In DMF, against all the three bacteria, SSR-5 exhibited maximum inhibition. For *S. typhimurium*, SSR-8 showed no inhibition. So, 3-chloro substitution is not effective. For *K. pneumonia*, SSR-1 and SSR-2 showed no inhibition whereas against *P. mirabilis*, inhibition is not shown by SSR-1 and SSR-4.

Thus, in DMF, overall, *S. typhimurium* is most susceptible bacteria and SOR-6 exhibited maximum inhibition in both the solvents. Thus,

for these compounds, solvent and substitutions both play an important role in inhibition. The 4-fluoro substitution in SSR-6 is most effective in both DMSO and DMF against Gram positive bacteria. Against Gram negative bacteria also, DMF is found to be good solvent.

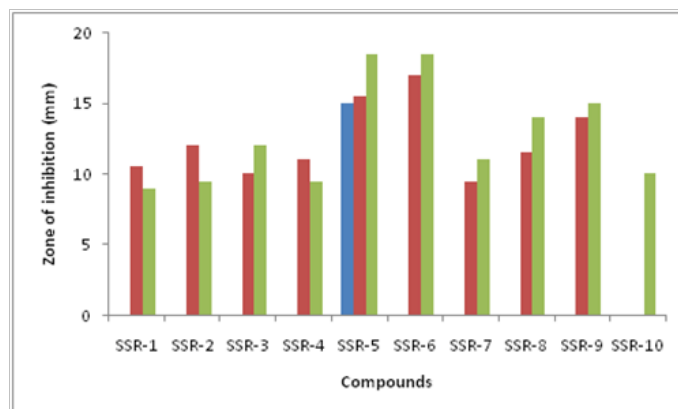


Figure 8 Zone of inhibition of SSR compounds against Gram negative bacteria in DMSO.

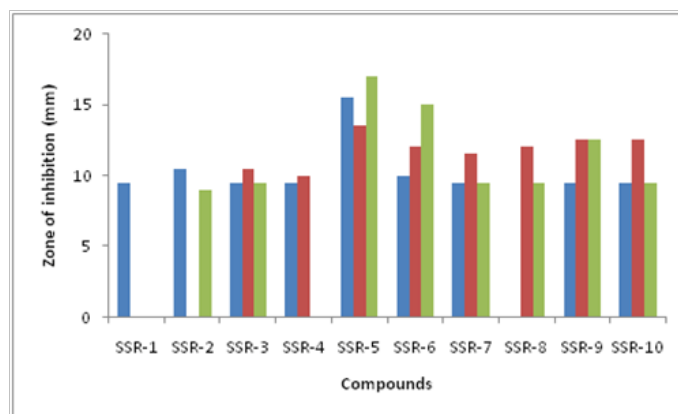


Figure 9 Zone of inhibition of SSR compounds against Gram negative bacteria in DMF.

Conclusion

Comparison of structures of compounds of SOR and SSR series shows that there is slight change in moiety but substitutions are same. When the inhibition of these compounds is compared then a substitution which causes increase in inhibition in one series was not much effective in other series. This suggests that moiety also play an important role in inhibition.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

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