



SYNTHESIS OF SCHIFF'S BASES OF DIHYDROPYRIMIDINONES WITH SULPHAMETHOXAZOLE BY MICROWAVE IRRADIATION TECHNIQUE AND THEIR EVALUATION AS ANTIBACTERIAL AGENTS

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ABSTRACT

The combination of Sulphamethoxazole and Trimethoprim (Cotrimaxazole) is effective against various bacterial infections including pneumonia. But this suffers from the drawbacks in its efficiency to eradicate the infections. In the present study, we developed Schiff's bases of dihydropyrimidinones (DHPMs) with sulphamethoxazole by using microwave irradiation technique. DHPMs contains basic pyrimidine nucleus and mimics the structure of trimethoprim. By combining these sulphomethoxazole and DHPMs, a single moiety is expected to inhibit both the enzymes Dihydropteroate synthase and Dihydrofolate reductase sequentially. A simple multicomponent one pot method was developed for synthesis of DHPMs by combining substituted aromatic aldehydes, substituted aromatic ketones with urea in the presence of $AlCl_3$ as catalyst by microwave irradiation technique. The compounds were obtained in good yield. Then these were combined with sulphamethoxazole to form their schiff's bases. 10 Schiff's bases were prepared and were evaluated for their antibacterial activity against various gram positive and gram-negative bacteria. The compounds IId and III were found to have good antibacterial activity compared to standard cotrimaxazole.

KEY WORDS

Schiff's bases, Sulphamethoxazole, Dihydropyrimidinones, Microwave irradiation technique, Antibacterial activity.

1. Introduction:

Sulphamethoxazole, a sulfonamide is well known for its effects against bacterial infections such as urinary tract infections, bronchitis and prostatitis¹. The blockbuster combination of Sulphomethoxazole with Trimethoprim (Cotrimaxazole) is the choice of treatment for pneumonia caused by *Pneumocystis carinii*, but this combination suffers in terms of its efficiency to eradicate pneumonia like infections².

In the present study, we aimed to develop a single moiety by combining the structural features of both sulphamethoxazole and trimethoprim. Trimethoprim is a pyrimidine derivative³, hence we designed dihydropyrimidinones (DHPMs) which resembles the structure of trimethoprim and are allowed to combine with sulphamethoxazole by forming Schiff's bases.

DHPMs were prepared by a multicomponent one pot synthesis (Biginelli reaction)⁴ by using microwave irradiation technique in the presence of anhydrous $AlCl_3$ as catalyst and acetonitrile as solvent. Then these DHPMs were combined with sulphamethoxazole to form their Schiff's bases by microwave heating. Microwaves produces rapid intense heating, with good atom economy and all the drawbacks of original Biginelli reaction to synthesize DHPMs could overcome by using this technique. By less solvents consumption and energy conservation, it meets the principles of green chemistry⁵⁻⁹.

Schiff's bases are the products formed when primary amines react with carbonyl compounds such as aldehydes and ketones by the loss of a water molecule¹⁰. Here the carbonyl group of DHPMs reacts

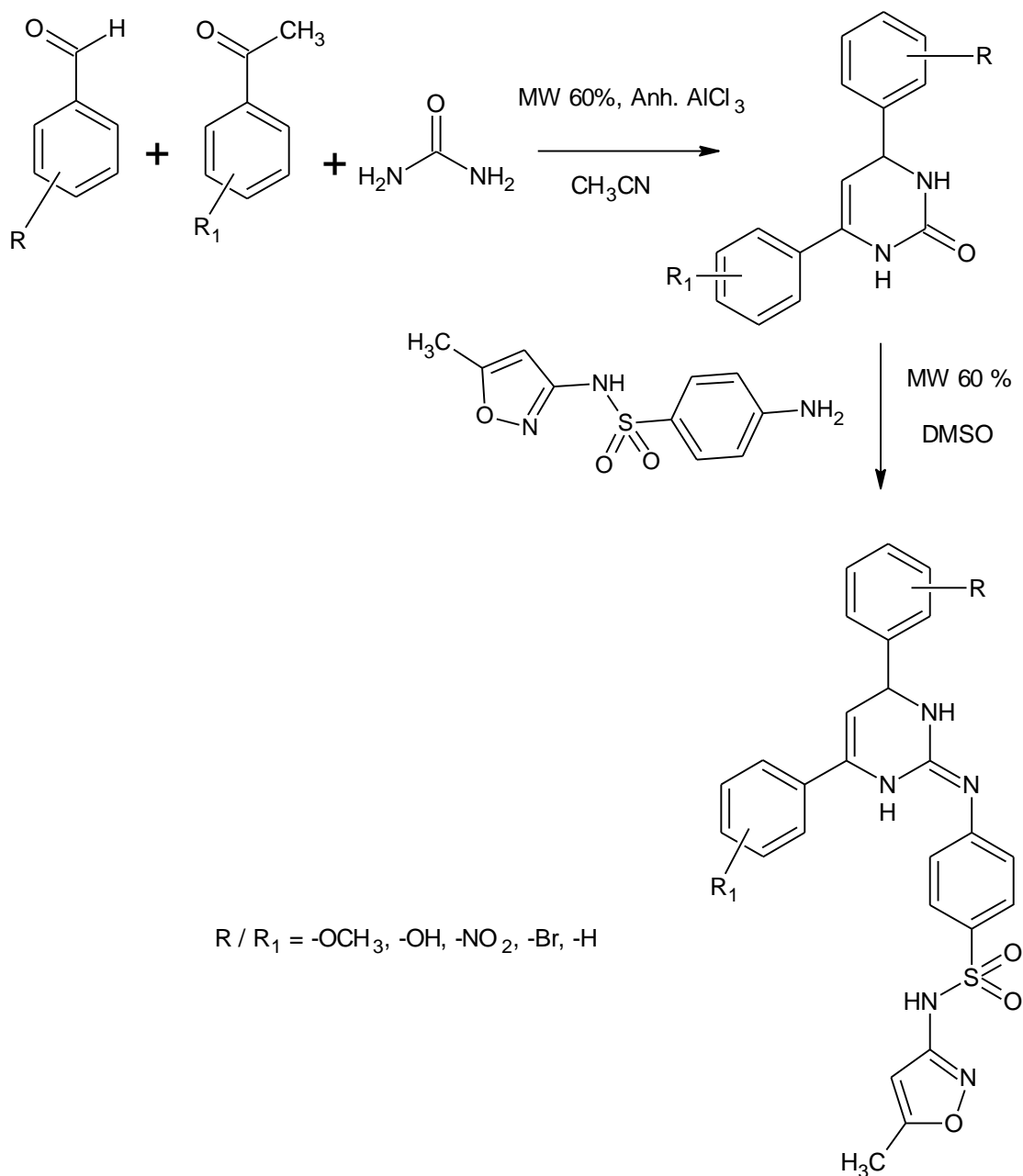
with primary amine moiety of sulphamethoxazole and forms Schiff's bases¹¹.

2. Experimental Methods:

2.1. Chemistry:

All the chemicals were obtained from SD fine chemicals Ltd and the solvents were of laboratory grade. Each

reaction was monitored by TLC by using appropriate solvent system, which was selected by trial and error method. Precoated TLC plates (Silicagel GF₂₅₄) were obtained from E. Merck. All the synthesized compounds were purified by recrystallisation. Melting points were noted on open capillary and they are uncorrected.



Schiff's bases of DHPMs with Sulphamethoxazole

Figure 1: Scheme: Synthesis of Schiff's bases of Dihydropyrimidinones with Sulphamethoxazole

General procedure for the synthesis of DHPMs:

Step1: A mixture of 0.01moles of urea, 0.01moles of substituted aromatic aldehydes and substituted aromatic ketones, acetonitrile and anhydrous AlCl_3 catalyst were taken and mixed thoroughly in a beaker and this reaction mixture was irradiated in microwave oven at watts (of 46.66). The reaction was monitored by TLC. After the completion of reaction, the mixture was poured in 50ml of ice cold water with continuous stirring. Then the mixture was neutralized with NaOH using litmus paper. The product formed was filtered and then dried^{12,13}.

General procedure for synthesis of schiff's bases of 3,4-Dihydropyrimidine-2-one with sulphamethoxazole:

Step2: A solution of sulfamethoxazole (0.001 mol) in absolute ethanol (30 ml) was slowly added to a solution of DHPM (0.001 mol) in absolute ethanol (20 ml). The reaction mixture was stirred for about 2 hrs. Then ethanol was allowed to evaporate, and the residue was dissolved in suitable quantity of DMSO. This mixture was irradiated in a microwave oven for about 2-3 min. After cooling, a precipitate was formed which was collected by filtration, then washed with cold ethanol, and recrystallized from ethanol¹⁴.

2.2. Antibacterial activity:

Microorganisms: The test organisms included the gram-positive bacteria *Bacillus cereus* (ATCC 11778), *Staphylococcus aureus* (ATCC 25923) and gram-negative bacteria, *Escherichia coli* (ATCC 25922), *Proteus vulgaris*

(ATCC 17440). All the bacterial strains were obtained from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Method: Antibacterial activities of Ila - Iij were evaluated using well diffusion method on Mueller-Hinton agar (MHA). The inhibition zones were reported in millimeter (mm). MHA agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter=6mm) were filled with 50 μl (Concentration of 100 $\mu\text{g/ml}$) of the test samples and incubated at 37°C for 24 hours. After the incubation period, the diameter of the growth inhibition zones was measured. Cotrimaxazole was used as the standard^{15,16}.

3. Results and discussion:

The synthesis of Schiff's bases with different specific DHPMs in DMSO as a solvent under microwave irradiation resulted in 10 new Schiff's bases. All the compounds were characterized by different physicochemical techniques like melting point, FTIR spectroscopy, Mass and NMR (¹H) and evaluated for their antibacterial activity.

3.1. Physical and spectroscopic properties of the prepared Schiff's bases:

The physical properties of Schiff's bases of sulfamethoxazole including melting point, yield and R_f are tabulated in Table 1.

Table 1: Physical data of the synthesized compounds

Compound Code	R	R ₁	Reaction time	% yield	M.p (°C)	R _f *
Ila	4-OCH ₃	4-Br	3min. 20 s	88.75	242-245	0.67
Ilb	4-OH	4-Br	3min. 40s	86.05	218-220	0.73
Ilc	2-OH	4-NO ₂	2min. 15s	95.03	230-232	0.63
Ild	-H	3-NO ₂	1min. 55s	89.2	264-266	0.56
Ile	2-OH	-H	3min. 15s	82.2	210-212	0.52
Ilf	-H	4-NO ₂	2min. 25s	88.3	248-250	0.71
Ilg	4-OCH ₃	3-NO ₂	3min. 31s	98.06	236-238	0.83
Ilh	4-OH	3-OH	1min. 25s	91.6	198-200	0.48
Ili	4-OH	3-NO ₂	3min. 35s	84.5	254-256	0.59
Ilj	4-OCH ₃	3-OH	2min. 45s	78.3	260-262	0.77

*Solvent system for R_f: n-hexane : ethyl acetate: methanol in ratio of 1:1:0.4.

The structure of the prepared Schiff's bases was confirmed by infrared spectroscopy. The FTIR spectra of sulfamethoxazole and its prepared compounds, showed that the band of NH₂ was found in sulfamethoxazole in

the location 3,298 cm⁻¹ and then disappeared. After that, the band of NH appeared in the prepared Schiff's bases with different shifting from 3,250 to 3,287 cm⁻¹. The band of C=N for imine stretching vibration was also

not found in sulfamethoxazole, and it appeared in the prepared Schiff's bases with shifting from 1,603 to 1,650 cm^{-1} . Also, the band of C=N for ring stretching vibration shifted from 1,620 cm^{-1} in sulfamethoxazole to 1,615 to 1,630 cm^{-1} in the prepared compounds.

The $^1\text{H-NMR}$ spectrum of compound IIa showed the following characteristic chemical shifts (DMSO as a solvent): the singlet signal at $\delta = 2.21$ ppm suggested the attribution of the protons of the CH_3 group, the singlet signal at $\delta = 6.0$ ppm suggested the attribution of the proton of CH of the isoxazole ring, the multiplet signal at $\delta = 6.7$ to 7.7 ppm suggested the attribution of the protons of three aromatic benzene rings, the doublet signal at $\delta = 6.1$ ppm suggested the attribution of the proton of the $-\text{C} = \text{CH}$ group, the singlet signal at $\delta = 9.3$ ppm suggested the presence of sulphonamide -NH, a doublet at $\delta = 9.8$ ppm and a singlet at 10.7 ppm suggested the attribution of the proton of the NH groups, and the singlet signal at $\delta = 3.5$ ppm suggested the attribution of the 3 protons of $-\text{OCH}_3$ and a doublet at 5.5 ppm suggested the presence of $-\text{CH}$ of dihydropyrimidine ring.

Characterization of the Synthesized compounds:

6-(4-bromophenyl)-4-(4-methoxyphenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIa):

Mol. For: $\text{C}_{27}\text{H}_{24}\text{BrN}_5\text{O}_4\text{S}$; Mol.Wt:594; Solubility : Chloroform; IR (KBr, Cm^{-1}): 3042, 1536, 3250, 1650, 1616; $^1\text{H NMR}$ Spectrum (DMSO, δ ppm): 2.21(s, 3H, $-\text{CH}_3$), 6.0(s, 1H, $-\text{CH}=\text{}$), 9.3(s, 1H, -NH, sulphonamide), 6.7-7.7(m, 12Ar-H), 10.7(s, 1H, -NH), 9.8(d, 1H, -NH), 3.5(s, 3H, OCH_3), 6.1(d, 1H, $-\text{C}=\text{C-H}$), 5.5(d, 1H, $-\text{CH}$). Mass m/z: 594.

6-(4-bromophenyl)-4-(4-hydroxyphenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIb):

Mol. For: $\text{C}_{26}\text{H}_{22}\text{BrN}_5\text{O}_4\text{S}$; Mol.Wt:580; Solubility : Chloroform; IR (KBr, Cm^{-1}): 3048, 1590, 3276, 1633, 1620; $^1\text{H NMR}$ Spectrum (DMSO, δ ppm): 2.3(s, 3H, $-\text{CH}_3$), 5.8(s, 1H, $-\text{CH}=\text{}$), 8.6(s, 1H, -NH, sulphonamide), 6.8-7.9(m, 12Ar-H), 9.5(s, 1H, -NH), 10.2(s, 1H, -NH), 10.8(s, 1H, OH), 6.2(d, 1H, $-\text{C}=\text{C-H}$), 5.1(d, 1H, $-\text{CH}$). Mass m/z: 580.

6-(4-nitrophenyl)-4-(2-hydroxyphenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIc):

Mol. For: $\text{C}_{26}\text{H}_{22}\text{N}_6\text{O}_6\text{S}$; Mol.Wt:546; Solubility : Chloroform; IR (KBr, Cm^{-1}): 3053, 1582, 3263, 1603, 1622; $^1\text{H NMR}$ Spectrum (DMSO, δ ppm): 2.6(s, 3H, $-\text{CH}_3$), 5.4(s, 1H, $-\text{CH}=\text{}$), 9.4(s, 1H, -NH, sulphonamide), 6.6-8.1(m, 12Ar-H), 10.3(s, 1H, -NH), 9.9(s, 1H, -NH), 10.9(s, 1H, -OH), 5.8 (d, 1H, $-\text{C}=\text{C-H}$), 5.0(d, 1H, $-\text{CH}$). Mass m/z: 546.

6-(3-nitrophenyl)-4-(4-phenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIId):

Mol. For: $\text{C}_{26}\text{H}_{22}\text{N}_6\text{O}_5\text{S}$; Mol.Wt:530; Solubility : Chloroform; IR (KBr, Cm^{-1}): 3058, 1546, 3282, 1648, 1630; $^1\text{H NMR}$ Spectrum (DMSO, δ ppm): 2.4(s, 3H, $-\text{CH}_3$), 5.6(s, 1H, $-\text{CH}=\text{}$), 9.4(s, 1H, -NH, sulphonamide), 6.4-7.6(m, 13Ar-H), 10.7(s, 1H, -NH), 8.8(s, 1H, -NH), 5.9 (d, 1H, $-\text{C}=\text{CH}$), 4.8(d, 1H, $-\text{CH}$). Mass m/z: 530.

6-(4-phenyl)-4-(2-hydroxyphenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIe):

Mol. For: $\text{C}_{26}\text{H}_{25}\text{N}_5\text{O}_4\text{S}$; Mol.Wt:501; Solubility : Chloroform; IR (KBr, Cm^{-1}): 3038, 1568, 3296, 1642, 1618; $^1\text{H NMR}$ Spectrum (DMSO, δ ppm): 1.6(s, 3H, $-\text{CH}_3$), 6.1(s, 1H, $-\text{CH}=\text{}$), 9.4(s, 1H, -NH, sulphonamide), 6.5-7.8(m, 13Ar-H), 10.4(s, 1H, -NH), 9.8(s, 1H, -NH), 10.6(s, 1H, -OH), 2.6(s, 3H, OCH_3), 5.7(d, 1H, $-\text{C}=\text{C-H}$), 5.2(d, 1H, $-\text{CH}$). Mass m/z: 502.

6-(4-Nitrophenyl)-4-(4-phenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIIf):

Mol. For: $\text{C}_{26}\text{H}_{22}\text{N}_6\text{O}_5\text{S}$; Mol.Wt:530; Solubility : Chloroform; IR (KBr, Cm^{-1}): 3062, 1554, 3248, 1636, 1615; $^1\text{H NMR}$ Spectrum (DMSO, δ ppm): 2.1(s, 3H, $-\text{CH}_3$), 5.9(s, 1H, $-\text{CH}=\text{}$), 8.4(s, 1H, -NH, sulphonamide), 6.6-7.8(m, 13Ar-H), 10.64(s, 1H, -NH), 9.6(s, 1H, -NH), 3.1(s, 3H, OCH_3), 5.8(d, 1H, $-\text{C}=\text{C-H}$), 4.1(d, 1H, $-\text{CH}$). Mass m/z: 530.

6-(3-nitrophenyl)-4-(4-methoxyphenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIg):

Mol. For: $\text{C}_{27}\text{H}_{24}\text{N}_6\text{O}_6\text{S}$; Mol.Wt:560; Solubility : Chloroform; IR (KBr, Cm^{-1}): 3062, 1554, 3268, 1650, 1610; $^1\text{H NMR}$ Spectrum (DMSO, δ ppm): 1.9 (s, 3H, $-\text{CH}_3$), 5.6(s, 1H, $-\text{CH}=\text{}$), 9.8(s, 1H, -NH, sulphonamide), 6.4-8.0(m, 12Ar-H), 10.5(s, 1H, -NH), 10.9(s, 1H, -NH), 3.1(s, 3H, OCH_3), 6.0(d, 1H, $-\text{C}=\text{C-H}$), 5.2(d, 1H, $-\text{CH}$). Mass m/z: 560.

6-(3-hydroxy phenyl)-4-(4-hydroxy phenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIh):

Mol. For: $C_{26}H_{23}N_5O_5S$; Mol.Wt:518; Solubility : Chloroform; IR (KBr, cm^{-1}): 3054, 1586, 3272, 1646, 1612 ; 1H NMR Spectrum (DMSO, δ ppm): 1.7 (s, 3H,-CH₃), 6.2(s,1H,-CH=), 8.6(s, 1H, -NH,sulphonamide), 6.7-7.9(m,12Ar-H), 10.1(s,1H,-NH), 9.5(s,1H, -NH), 11.2(s,1H, -OH), 11.8(s,1H, - OH), 6.0(d,1H, -C=C-H), 5.6(d, 1H, -CH). Mass m/z: 518.

6-(3-nitro phenyl)-4-(4-hydroxy phenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIi):

Mol. For: $C_{26}H_{22}N_6O_6S$; Mol.Wt:546; Solubility : Chloroform; IR (KBr, cm^{-1}): 3046, 1574, 3258, 1630, 1618 ; 1H NMR Spectrum (DMSO, δ ppm): 1.8 (s, 3H,-CH₃), 6.2(s,1H,-CH=), 9.2(s, 1H, -NH,sulphonamide), 6.5-7.7(m,12Ar-H), 10.2(s,1H,-NH), 9.6(s,1H, -NH), 11.2(s,1H, - OH), 5.7 (d,1H, -C=C-H), 5.2(d, 1H, -CH). Mass m/z: 546.

6-(3-hydroxy phenyl)-4-(4-methoxy phenyl)-3,4-dihydropyrimidine -2-imine- benzene sulfonamide -N-(5-methyl)-3-isoxazole(IIj):

Mol. For: $C_{27}H_{25}N_5O_5S$; Mol.Wt:531; Solubility : Chloroform; IR (KBr, cm^{-1}): 3066, 1542, 3270, 1634, 1621; 1H NMR Spectrum (DMSO, δ ppm): 2.4(s, 3H,-CH₃), 5.7(s,1H,-CH=), 8.3(s, 1H, -NH,sulphonamide), 6.6-7.8(m,12Ar-H), 10.2(s,1H,-NH), 9.6(s,1H, -NH), 10.8(s,1H, - OH), 3.1(s,3H, OCH₃), 6.0(d,1H, -C=C-H), 5.3(d, 1H, -CH). Mass m/z: 531.

3.2. Antibacterial activity:

Among the synthesized Schiff's bases of sulphomethoxazole with DHPMs the compounds, all the compounds showed antibacterial effect against cultures of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Proteus vulgaris*, the compounds IId and IIi showed promising antibacterial activity, and the compounds IIa, IIh, IIj found to contain good activity compared to the standard Cotrimaxazole. The zone of inhibition of IId is 3mm greater than standard against *Bacillus cereus*, 6mm greater than standard against *Staphylococcus aureus*, 5mm greater than *Escherichia coli* and 2mm greater than standard against *Proteus vulgaris*. The details of zone of inhibition were posted in table 2.

Table 2: Zone of Inhibition (mm) of the synthesized compounds:

S.No	Compound Code	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Proteus vulgaris</i>
1	IIa	20	21	18	21
2	IIb	13	11	12	15
3	IIc	11	14	16	10
4	IId	24	26	28	25
5	IIe	11	16	15	12
6	IIf	18	20	21	19
7	IIg	10	13	17	15
8	IIh	19	18	21	20
9	IIi	23	21	19	22
10	IIj	18	19	21	21
11	Standard	21	20	23	23

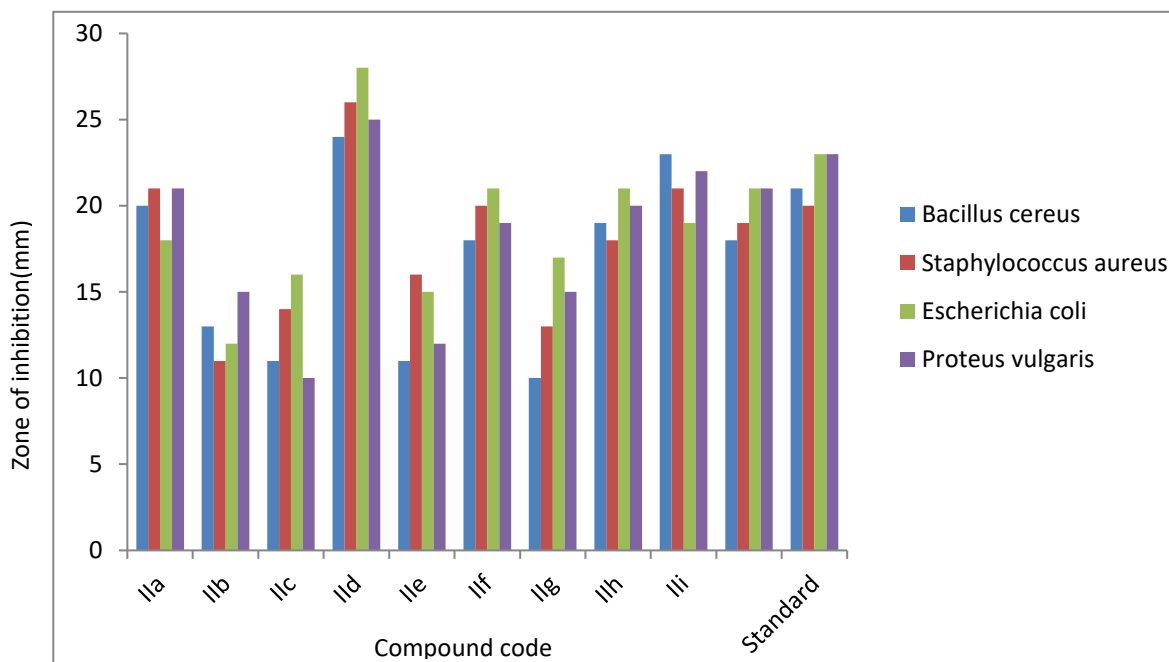


Figure 2: Comparison of zone of inhibition against various bacterial cultures

4. Conclusion:

We have developed a simple method for the preparation of dihydropyrimidinones by following a three component one pot method by using microwave irradiation technique. The yield of the compounds was greatly improved from biginelli reaction. Then Schiff's bases of these DHPMs were prepared by reaction with antibiotic sulphamethoxazole by employing microwave heating. 10 compounds were synthesized and evaluated for their antibacterial actions against various gram positive and gram-negative bacteria. The compound IId exhibited excellent antibacterial effect against all cultures compared to standard cotrimaxazole. This extreme antibacterial effect may be attributed to the combination of dihydropyrimidine ring with sulphomethoxazole. It appears that the combination of these two moieties is causing sequential blockade of folate synthesis in bacteria, by inhibiting dihydropteroate synthase and dihydrofolate reductase and might exhibiting synergistic effect greater than the sum of individual effects of sulphamethoxazole and trimethoprim.

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