Heterocyclic chemistry has gained immense importance pharmacologically and continues to be a major contributing arena in organic chemistry. Amongst different heterocyclic compounds oxazoline, thiazoline, imidazoline, pyrimidine, benzoxazole, benzothiazole and benzimidazole derivatives constitute the core structures of numerous natural products and biologically active synthetic compounds. In this perspective the present work entitled “SYNTHESIS, CHARACTERIZATION AND BIOASSAY OF A NEW CLASS OF MONO AND BIS HETEROCYCLES” has been taken up.

INTRODUCTION

A brief introduction on the importance of five and six membered heterocycles and the methods of syntheses pertaining to oxazolines, thiazolines, imidazolines, pyrimidines, benzoxazoles, benzothiazoles and benzimidazoles were described.

PRESENT WORK

The widespread applications of heteroarenes as scaffold in medicinal chemistry and in materials science have driven the development of a new class of heterocyclic cores. In fact the design, modification and optimization of reaction conditions, characterization of the structures of new molecules is a worthwhile contribution in the field of heterocyclic chemistry. In this perspective, the author did considerable work on the development of bis and tris heterocycles from simple substrates. The results were presented in four sections.
SECTION-I

Synthesis and antimicrobial activity of 2-(((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)methyl)benzoxazole, 2-(((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)methyl)benzothiazole, 2-(((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)methyl)-1H-benzimidazole, 2-(((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)methyl)benzoxazole, 2-(((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)methyl)benzothiazole, 2-(((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)methyl)-1H-benzimidazole, 2-(((4',5'-dihydro-1'H-imidazol-2'-yl)methylsulfonyl)methyl)benzoxazole, 2-(((4',5'-dihydro-1'H-imidazol-2'-yl)methylsulfonyl)methyl)benzothiazole and 2-(((4',5'-dihydro-1'H-imidazol-2'-yl)methylsulfonyl)methyl)-1H-benzimidazole.

The synthetic intermediates 2-(chloromethyl)benzoxazole (1) and 2-(chloromethyl)benzothiazole (2) were prepared by the irradiation of 2-aminophenol / 2-aminothiophenol and chloroacetyl chloride in acetic acid for 10 min at a power of 500 W. However, 2-(chloromethyl)-1H-benzimidazole (3) was obtained by treating o-phenylenediamine with chloroacetic acid in the presence of 5N HCl (Scheme I.1).
The reaction of compounds 1, 2 and 3 with thioglycolic acid in the presence of sodium hydroxide in methanol resulted in 2-((benzoxazol-2-yl)methylthio)acetic acid (4), 2-((benzothiazol-2-yl)methylthio)acetic acid (5) and 2-((1H-benzimidazol-2-yl)methylthio)acetic acid (6). Oxidation of latter compounds with hydrogen peroxide furnished 2-((benzoxazol-2-yl)methylsulfonyl)acetic acid (7), 2-((benzothiazol-2-yl)methylsulfonyl)acetic acid (8) and 2-((1H-benzimidazol-2-yl)methylsulfonyl)acetic acid (9). Esterification of acid functionality in the compounds 7, 8 and 9 with methanol and conc. H$_2$SO$_4$ produced methyl 2-((benzoxazol-2-yl)methylsulfonyl)acetate (10), methyl 2-((benzothiazol-2-yl)methylsulfonyl)acetate (11) and methyl 2-((1H-benzimidazol-2-yl)methylsulfonyl)acetate (12) (Scheme I.2).
The cyclocondensation of compounds 10, 11 and 12 with 2-aminoethanol and \textit{n}-butyllithium complexed with a suspension of 5-10\% molar equivalent of anhydrous samarium(III) chloride in toluene afforded 2-(((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)methyl)benzoxazole (13), 2-(((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)methyl)benzothiazole (14) and 2-(((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)methyl)-1\textit{H}-benzimidazole (15). On the other hand, the reaction of compounds 10, 11 and 12 with 2-aminoethanethiol in the presence of \textit{n}-butyllithium and anhydrous samarium(III) chloride gave 2-(((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)methyl)benzoxazole (16), 2-(((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)methyl)benzothiazole (17) and 2-(((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)methyl)-1\textit{H}-benzimidazole (18). Adopting similar methodology, 2-(((4',5'-dihydro-1'\textit{H}-imidazol-2'-yl)methylsulfonyl)methyl)benzoxazole (19), 2-(((4',5'-dihydro-1'\textit{H}-imidazol-2'-yl)methylsulfonyl)methyl)benzothiazole (20) and 2-(((4',5'-dihydro-1'\textit{H}-imidazol-2'-yl)methylsulfonyl)methyl)-1\textit{H}-benzimidazole (21).
were prepared from the compounds 10, 11, 12 and 1,2-ethanediamine in the presence of n-butyllithium and samarium(III) chloride (Scheme I.3). The spectral and analytical tools were employed to establish the structures of the new compounds.

\[
\text{i) } n\text{-BuLi / Sm(III)Cl / HOCH}_2\text{CHNH}_2 / \text{Toluene}
\]
\[
\text{ii) } n\text{-BuLi / Sm(III)Cl / HSCH}_2\text{CHNH}_2 / \text{Toluene}
\]
\[
\text{iii) } n\text{-BuLi / Sm(III)Cl / NH}_2\text{CH}_2\text{CHNH}_2 / \text{Toluene}
\]

**SCHEME I.3**

**Antimicrobial activity**

The lead compounds 13-21 were tested for antimicrobial activity at two concentrations, 50 and 100 µg/ml. For antibacterial studies, the microorganisms employed were *Staphylococcus aureus, Bacillus subtilis* (Gram-positive) and *Pseudomonas aeruginosa, Klebsiella pneumoniae* (Gram-negative). For antifungal, *Aspergillus niger* and *Penicillium chrysogenum* were used as microorganisms. Chloramphenicol and Ketoconazole were employed as standard drugs for antibacterial and antifungal studies, respectively. All the tested compounds showed higher antibacterial activity towards Gram-negative bacteria than Gram-positive bacteria. The
thiazolinyl and imidazolinyl benzimidazoles (18 & 21) displayed excellent activity than the other tested compounds. However, oxazolinyl benzimidazole (15), thiazolinyl benzothiazole (17), imidazolinyl benzoxazole (19) and imidazolinyl benzothiazole (20) exhibited moderate to good activity. On the other hand, oxazolinyl benzoxazole (13), oxazolinyl benzothiazole (14) and thiazolinyl benzoxazole (16) displayed least activity.

The compounds 13-21 inhibited the spore germination against tested fungi. All the compounds displayed slightly higher antifungal activity towards *P. chrysogenum* than *A. niger*. The imidazolinyl benzimidazole (21) exhibited relatively good activity against *P. chrysogenum*. However, the compounds 13, 14, 15 and 16 showed least activity.

**SECTION-II**

*Synthesis and antimicrobial activity of 2-((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)benzoxazole, 2-((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)benzothiazole, 2-((4',5'-dihydrooxazol-2'-yl)methylsulfonyl)-1H-benzimidazole, 2-((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)benzoxazole, 2-((4',5'-dihydrothiazol-2'-yl)methylsulfonyl)benzothiazole, 2-((4',5'-dihydro-1'H-imidazol-2'-yl)methylsulfonyl)benzoxazole and 2-((4',5'-dihydro-1'H-imidazol-2'-yl)methylsulfonyl)-1H-benzimidazole.*

The reactive intermediates benzoxazole-2-thiol (22), benzothiazole-2-thiol (23) and 1H-benzimidazole-2-thiol (24) were prepared by the reaction of 2-aminophenol / 2-aminothiophenol / *o*-phenylenediamine with carbon disulfide in the presence of potassium hydroxide in ethanol (Scheme II.1).
The reaction of compounds 22, 23 and 24 with chloroacetic acid in the presence of sodium hydroxide in methanol resulted in 2-(benzoxazol-2-ylthio)acetic acid (25), 2-(benzothiazol-2-ylthio)acetic acid (26) and 2-(1H-benzimidazol-2-ylthio)acetic acid (27). Oxidation of latter compounds with hydrogen peroxide afforded 2-(benzoxazol-2-ylsulfonyl)acetic acid (28), 2-(benzothiazol-2-ylsulfonyl)acetic acid (29) and 2-(1H-benzimidazol-2-ylsulfonyl)acetic acid (30). The compounds 28, 29 and 30 on esterification with methanol in the presence of conc. H$_2$SO$_4$ produced methyl 2-(benzoxazol-2-ylsulfonyl)acetate (31), methyl 2-(benzothiazol-2-ylsulfonyl)acetate (32) and methyl 2-(1H-benzimidazol-2-ylsulfonyl)acetate (33) (Scheme II.2).
The reaction of compounds 31, 32 and 33 with 2-aminoethanol and n-butyllithium complexed with a suspension of 5-10% molar equivalent of anhydrous samarium(III) chloride in toluene gave 2-((4′,5′-dihydrooxazol-2′-yl)methylsulfonyl)benzoxazole (34), 2-((4′,5′-dihydrooxazol-2′-yl)methylsulfonyl)benzothiazole (35) and 2-((4′,5′-dihydrooxazol-2′-yl)methylsulfonyl)-1H-benzimidazole (36). Similar reaction of compounds 31, 32 and 33 with 2-aminoethanethiol furnished 2-((4′,5′-dihydrothiazol-2′-yl)methylsulfonyl)benzoxazole (37), 2-((4′,5′-dihydrothiazol-2′-yl)methylsulfonyl)benzothiazole (38) and 2-((4′,5′-dihydrothiazol-2′-yl)methylsulfonyl)-1H-benzimidazole (39). Analogously, 2-((4′,5′-dihydro-1′H-imidazol-2′-yl)methylsulfonyl)benzoxazole (40), 2-((4′,5′-dihydro-1′H-imidazol-2′-yl)methylsulfonyl)benzothiazole (41) and 2-((4′,5′-dihydro-1′H-imidazol-2′-yl)methylsulfonyl)-1H-benzimidazole (42) were prepared from the compounds 31, 32, 33 and 1,2-ethanediame in the presence of n-butyllithium and
samarium(III) chloride (Scheme II.3). The structures of the new compounds were confirmed by IR, NMR, mass and elemental analyses.

![Scheme II.3](image)

**Antimicrobial activity**

The bis heterocycles 34-42 were screened for their antimicrobial activity at two different concentrations, 50 and 100 µg/ml. Bacterial strains *Staphylococcus aureus*, *Bacillus subtilis*, (Gram-positive bacteria), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram-negative bacteria) and fungi *Aspergillus niger*, *Penicillium chrysogenum* were used to test the antimicrobial activity. Simultaneously the standard antibiotics Chloramphenicol for antibacterial activity Ketoconazole for antifungal activity were tested against the pathogens. The results indicated that Gram-negative bacteria were more susceptible towards the tested compounds than Gram-positive ones. The
benzimidazole in combination with thiazoline (39) and imidazoline (42) exhibited greater activity towards *P. aeruginosa*. Besides, benzothiazole in combination with thiazoline (38) and imidazoline (41) showed moderate to good activity. However, the compounds 34, 35, 36 and 37 displayed least activity against the tested bacteria.

All the compounds inhibited the spore germination against tested fungi. The compounds showed slightly higher antifungal activity towards *P. chrysogenum* than *A. niger*. The compound 42 displayed excellent activity particularly against *P. chrysogenum*. On the other hand, the compounds 34, 35, 36 and 37 exhibited least activity.

**SECTION-III**

*Synthesis, antimicrobial and cytotoxic activities of 2-(2-((benzoxazol-2-yl)-methylthio)-6-methylpyrimidin-4-ylthio)benzoxazole, 2-(2-((benzothiazol-2-yl)methylthio)-6-methylpyrimidin-4-ylthio)benzothiazole, 2-(2-((1H-benzimidazol-2-yl)methylthio)-6-methylpyrimidin-4-ylthio)-1H-benzimidazole, 2-((4-((benzoxazol-2-yl)methylthio)-6-methylpyrimidin-2-ylthio)methyl)benzoxazole, 2-((4-((benzothiazol-2-yl)methylthio)-6-methylpyrimidin-2-ylthio)methyl)benzothiazole, 2-((4-((1H-benzimidazol-2-yl)methylthio)-6-methylpyrimidin-2-ylthio)methyl)-1H-benzimidazole, N-(2-((benzoxazol-2-yl)methylthio)-6-methylpyrimidin-4-yl)benzoxazol-2-amine, N-(2-((benzothiazol-2-yl)methylthio)-6-methylpyrimidin-4-yl)benzothiazol-2-amine and N-(2-((1H-benzimidazol-2-yl)methylthio)-6-methylpyrimidin-4-yl)-1H-benzimidazol-2-amine.*

The synthon 4-chloro-6-methylpyrimidine-2-thiol (43) was prepared as follows. The reaction of ethyl acetoacetate with thiourea in the presence of NaOMe produced 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one. This on treatment with POCl₃ resulted in compound 43 (Scheme III.1).
The reactive intermediates benzoxazol-2-ylmethanethiol (44), benzothiazol-2-ylmethanethiol (45) and 1H-benzimidazol-2-ylmethanethiol (46) were obtained by the reaction of 2-aminophenol, 2-aminothiophenol and o-phenylenediamine with thioglycolic acid in the presence of 5N HCl. The benzoxazol-2-amine (47) and 1H-benzimidazol-2-amine (49) were prepared by the treatment of o-aminophenol or o-phenylenediamine with cyanogen bromide. However, benzothiazol-2-amine (48) was obtained from aniline and ammonium thiocyanate in the presence of bromine in acetic acid (Scheme III.2).

The reaction between compounds 1 and 43 in the presence of triethylamine in tetrahydrofuran resulted in 2-((4-chloro-6-methylpyrimidin-2-ylthio)methyl)benzoxazole (50). Similarly, 2-((4-chloro-6-methylpyrimidin-2-ylthio)methyl)benzothiazole (51) and 2-((4-chloro-6-methylpyrimidin-2-ylthio)methyl)-1H-benzimidazole (52) were prepared by treating the compounds 2 and 3 with 43 (Scheme III.3).
The tris heterocyclic compounds linked by methanesulfanyi and sulfanyl moieties were prepared as follows. Treatment of compound 22 with 50 in the presence of a catalytic amount of methanesulfonic acid produced 2-(2-((benzoxazol-2-yl)methylthio)-6-methylpyrimidin-4-ylthio)benzoxazole (53). Adopting similar procedure, 2-(2-((benzothiazol-2-yl)methylthio)-6-methylpyrimidin-4-ylthio)benzothiazole (54) and 2-(2-((1H-benzimidazol-2-yl)methylthio)-6-methylpyrimidin-4-ylthio)-1H-benzimidazol (55) were prepared by performing the reaction of 23 with 51 and 24 with 52 (Scheme III.4).

In a much similar way, 2-((4-((benzoxazol-2-yl)methylthio)-6-methylpyrimidin-2-ylthio)methyl)benzoxazole (56) was obtained by treating 44 with 50 in the presence of a catalytic amount of methanesulfonic acid. Likewise, 2-((4-((benzothiazol-2-yl)methyl-
thio)-6-methylpyrimidin-2-ylthio)methyl)benzothiazole (57) and 2-((4-((1H-benzimidazol-2-yl)methylthio)-6-methylpyrimidin-2-ylthio)methyl)-1H-benzimidazole (58) were prepared by the reaction of 45 with 51 and 46 with 52 (Scheme III.5).

On the other hand, tris heterocycles linked by methanesulfanyl and amino groups-
\[N-(2-((benzoxazol-2-yl)methylthio)-6-methylpyrimidin-4-yl)benzoxazol-2-amine \quad (59),\]
\[N-(2-((benzothiazol-2-yl)methylthio)-6-methylpyrimidin-4-yl)benzothiazol-2-amine \quad (60)\]
and \(N-(2-((1H-benzimidazol-2-yl)methylthio)-6-methylpyrimidin-4-yl)-1H-benzimidazol-2-amine \quad (61)\) were prepared by the reaction of compounds 47, 48 and 49 with 50, 51 and 52, respectively. The structures of all the compounds were established by IR, \(^1\)H NMR, \(^{13}\)C NMR, mass and elemental analyses (Scheme III.6).
**Antimicrobial activity**

The compounds 50-61 were screened for antimicrobial activity at three different concentrations 25, 50 and 100 µg/ml. The *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria), *Aspergillus niger*, *Penicillium chrysogenum* (fungi) were employed to test the antimicrobial activity. Ciprofloxacin, Ketoconazole were the standard drugs used in antibacterial and antifungal studies. The results of antibacterial activity revealed that all the tested compounds exhibited more activity towards Gram-positive bacteria than Gram-negative bacteria. The tris heterocyclic compounds exhibited greater activity than the bis heterocyclic systems. The 4-chloropyrimidinylsulfanyl methyl benzoxazole (50), benzothiazole (51) and benzimidazole (52) displayed least activity. Replacement of chloro substituent by heterocyclic moiety enhanced the activity. Further, the amino linked heterocycles 59, 60 and 61 showed slightly higher activity than those having sulfanyl group 53, 54 and 55. Amongst tris heterocyclic compounds, pyrimidinyl bis methylthio benzoxazole (56), benzothiazole (57) and benzimidazole (58) displayed greater activity. This may be due to more flexibility of these compounds. In fact, compound 58 showed activity higher than the standard Ciprofloxacin at all tested concentrations towards *S. aureus*.

All the tested compounds inhibited the spore germination against tested fungi. In general most of the compounds showed slightly higher antifungal activity towards *P. chrysogenum* than *A. niger*. The compound 58 displayed excellent activity particularly against *P. chrysogenum* equivalent to the standard drug Ketoconazole at 100 µg/ml.

**Cytotoxicity activity**

The compounds 50-61 were subjected to MTT assay to determine growth inhibitory/cytotoxic capability. The compounds 54, 60 and the reference MPK-09 showed cytotoxic activity on A549 cells with IC$_{50}$ values 70 µM, 10.5 µM and 2.85 µM, respectively. However, the remaining compounds do not display any cytotoxicity when used up to 200 µM concentration. The cytotoxic activity observed with compounds 54
and 60 was concentration dependent. Compound 60 at concentrations 12.5-200 µM showed lowest viability, while viability more than 50% was observed when this compound was used at a concentration below 12.50 µM on A549 cells. This suggests that compound 60 as a noticeable lead molecule for cytotoxic activity against tumor cells.

**SECTION-IV**

*Synthesis and antimicrobial activity of N-(1,3-benzoxazol-2-yl)-N'-{4-(1,3-benzoxazol-2-ylamino)-6-methyl-2-pyrimidinyl}sulfamide, N-(1,3-benzothiazol-2-yl)-N'-{4-(1,3-benzothiazol-2-ylamino)-6-methyl-2-pyrimidinyl}sulfamide, N-(1H-benzimidazol-2-yl)-N'-{4-(1H-benzimidazol-2-ylamino)-6-methyl-2-pyrimidinyl}sulfamide, N-(1,3-benzoxazol-2-ylmethyl)-N'-4-[(1,3-benzoxazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide, N-(1,3-benzothiazol-2-ylmethyl)-N'-4-[(1,3-benzothiazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide, N-(1H-benzimidazol-2-ylmethyl)-N'-4-[(1H-benzimidazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide.*

The synthetic intermediates (benzoxazol-2-yl)methanamine (62), (benzothiazol-2-yl)methanamine (63) and (1H-benzimidazol-2-yl)methanamine (64) were prepared by the cyclocondensation of 2-aminophenol, 2-aminophenol and o-phenylenediamine with aminoacetic acid in the presence of 5N HCl (Scheme IV.1).

![Scheme IV.1](image-url)
The reaction of compounds 47, 48 and 49 with 4-chloro-6-methylpyrimidin-2-amine (65) in ethanol in the presence of methanesulfonic acid resulted in \(N^4\)-(1,3-benzoxazol-2-yl)-6-methyl-2,4-pyrimidinediamine (66), \(N^4\)-(1,3-benzothiazol-2-yl)-6-methyl-2,4-pyrimidinediamine (67) and \(N^4\)-(1\(H\)-benzimidazol-2-yl)-6-methyl-2,4-pyrimidinediamine (68). In order to develop tris heterocycles linked by a variety of pharmacophoric units, the compounds 66, 67 and 68 were treated with sulfonyl chloride to get sulfamoyl chloride derivatives- (4-(benzoxazol-2-ylamino)-6-methylpyrimidin-2-yl)sulfamoyl chloride (69), (4-(benzothiazol-2-ylamino)-6-methylpyrimidin-2-yl)sulfamoyl chloride (70) and (4-((1\(H\)-benzimidazol-2-yl)amino)-6-methylpyrimidin-2-yl)sulfamoyl chloride (71). The treatment of compounds 47 with 69, 48 with 70 and 49 with 71 in the presence of methanesulfonic acid afforded \(N\)-(1,3-benzoxazol-2-yl)-\(N^\prime\)-[4-(1,3-benzoxazol-2-ylamino)-6-methyl-2-pyrimidinyl]sulfamide (72), \(N\)-(1,3-benzothiazol-2-yl)-\(N^\prime\)-[4-(1,3-benzothiazol-2-ylamino)-6-methyl-2-pyrimidinyl]sulfamide (73) and \(N\)-(1\(H\)-benzimidazol-2-yl)-\(N^\prime\)-[4-(1\(H\)-benzimidazol-2-ylamino)-6-methyl-2-pyrimidinyl]-sulfamide (74) (Scheme IV.2).

The tris heterocycles 72, 73 and 74 were also prepared by another route. The reaction of compound 65 with sulfonyl chloride produced (4-chloro-6-methylpyrimidin-2-yl)sulfamoyl chloride (75). The latter compound on treatment with two moles of 47, 48 and 49 in the presence of methanesulfonic acid gave the desired tris heterocycles 72, 73 and 74 (Scheme IV.2).
On the other hand, the reaction of compounds 62, 63 and 64 with 65 in the presence of methanesulfonic acid in ethanol furnished $N^4$-(1,3-benzoxazol-2-ylmethyl)-6-methyl-2,4-pyrimidinediamine (76), $N^4$-(1,3-benzothiazol-2-ylmethyl)-6-methyl-2,4-pyrimidinediamine (77) and $N^4$-(1$H$-benzimidazol-2-ylmethyl)-6-methyl-2,4-pyrimidine-diamine (78), respectively. Functionalization of amino group in the compounds 76, 77
and 78 with sulfuryl chloride provided (4-((benzoxazol-2-ylmethyl)amino)-6-methylpyrimidin-2-yl)sulfamoyl chloride (79), (4-((benzothiazol-2-ylmethyl)amino)-6-methylpyrimidin-2-yl)sulfamoyl chloride (80) and (4-(((1H-benzimidazol-2-yl)methyl)amino)-6-methylpyrimidin-2-yl)sulfamoyl chloride (81). The treatment of compounds 62 with 79, 63 with 80 and 64 with 81 in the presence of methanesulfonic acid resulted in N-(1,3-benzoxazol-2-ylmethyl)-N′-4-[(1,3-benzoxazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide (82), N-(1,3-benzothiazol-2-ylmethyl)-N′-4-[(1,3-benzothiazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide (83) and N-(1H-benzimidazol-2-ylmethyl)-N′-4-[(1H-benzimidazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide (84) (Method A; Scheme IV.3). The latter compounds were also prepared as follows. The reaction of compound 65 with sulfuryl chloride produced (4-chloro-6-methylpyrimidin-2-yl)sulfamoyl chloride (75). The treatment of 75 with two moles of 62, 63 and 64 in the presence of methanesulfonic acid afforded 82, 83 and 84 (Method B; Scheme IV.3). The structures of all the new compounds were confirmed by spectral parameters and elemental analyses.

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<th>75</th>
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**SCHEME IV.3**

**Antimicrobial activity**

The compounds 66-84 were tested for antimicrobial activity at three different concentrations 25, 50, and 100 µg/ml. The Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*) and fungi (*Aspergillus niger, Penicillium chrysogenum*) were used to test the antimicrobial activity. The standard drugs used for bacterial and fungal studies are Ciprofloxacin and Ketoconazole. The results of antibacterial activity revealed that all the tested compounds
showed more activity towards Gram-positive bacteria than Gram-negative bacteria. The tris heterocyclic compounds 72-74 and 82-84 displayed higher activity than the corresponding bis heterocyclic systems 66-71 and 76-81. Amongst the latter compounds those having N-chlorosulfonyl group (69-71 & 79-81) exhibited slightly higher activity than the compounds with amino group (66-68 & 76-78). Introduction of one heterocyclic ring by the replacement of chlorine in 69-71 and 79-81 increases the activity. The compounds $N$-(1,3-benzothiazol-2-yl)-$N'$-[4-(1,3-benzothiazol-2-ylamino)-6-methyl-2-pyrimidinyl]sulfamide (73), $N$-(1H-benzimidazol-2-yl)-$N'$-[4-(1H-benzimidazol-2-ylamino)-6-methyl-2-pyrimidinyl]sulfamide (74), $N$-(1,3-benzothiazol-2-ylmethyl)-$N'$-4-[(1,3-benzothiazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide (83) and $N$-(1H-benzimidazol-2-ylmethyl)-$N'$-4-[(1H-benzimidazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide (84) exhibited excellent antibacterial activity against S. aureus greater than the standard drug, Ciprofloxacin at all tested concentrations. On the other hand, $N$-(1,3-benzoxazol-2-yl)-$N'$-[4-(1,3-benzoxazol-2-ylamino)-6-methyl-2-pyrimidinyl]sulfamide (72) and $N$-(1,3-benzoxazol-2-ylmethyl)-$N'$-4-[(1,3-benzoxazol-2-ylmethyl)amino]-6-methyl-2-pyrimidinylsulfamide (82) displayed least activity. Further it was noticed that (4-chloro-6-methylpyrimidin-2-yl)sulfonyl chloride (75) exhibited no activity.

All the compounds inhibited the spore germination of the tested fungi except compound 75. All the compounds displayed slightly higher antifungal activity towards P. chrysogenum than A. niger. The compounds 72, 82 and 84 showed excellent activity against P. chrysogenum greater than the standard drug Ketoconazole.