4.1. INTRODUCTION

Urea is a functional moiety that is frequently found in the natural products and often displays a broad range of biological activities. In meticulous, substituted urea derivatives have attracted attention due to their wide range of applications as agricultural pesticides and anticonvulsants. Unsymmetrical substituted urea derivatives at amino groups have also been shown to have a potent HIV-1 protease inhibitor activity. Hetrazane (I) and thiocarlide (II) are well known urea and thiourea scaffold drugs used in the treatment for filarial and tuberculosis respectively.

One very interesting and promising class of heterocycles is piperazine and its derivatives. Piperazine derivatives have been extensively investigated by the organic chemists due to their close association with various types of biological activities. Moreover, they have wide clinical applications in the therapy of functional diseases and exhibit insecticidal, antidiabetic, antimicrobial, acetyl cholinesterase inhibitors, antimalarial, dopamine transporter, MC4 receptor and HIV-protease inhibitors.

Urea derivatives have been used for the treatment of a wide range of solid tumours. Urea based prodrugs have been reported as candidates for melanocyte-directed enzyme prodrug therapy (MDEPT), in which they release the drug upon exposure to tyrosinase. Some urea related derivatives also have been reported as protein tyrosine kinases (PTKs) inhibitors, and have become an important class of potential anticancer drugs. Urea and thiourea derivatives have also been used for brain cancer treatment and as potent inhibitors of human DNA topoisomerase II. Moreover, other properties were attributed to urea derivatives such as HIV-1 protease and cholesterol acyltransferase (ACAT) inhibitory activities. They are also promising therapeutic agents for hypercholesteremia and atherosclerosis. Urea/thiourea
derivatives display a wide range of biological activities including antibacterial, antifungal, antitubercular, antithyroid, antihelmintic, rodenticidal, insecticidal, herbicidal, and plant growth regulator properties.20-25

Mitsunori Kono et al.26 have designed and synthesized a series of piperazine ureas (III-V) and evaluated for their potential orally available fatty acid amide hydrolase (FAAH) inhibitors that are therapeutically effective against pain.

\[
\text{(III)} \quad \text{(IV)} \quad \text{(V)}
\]

Where, \(X = \) S

\[Z = \]

\[X1 = N, \text{CH} & R = H, 2-F, 3-F, 2,4-F, \text{etc.}\]

S Perveen et al.27 reported urea and thiourea derivatives (IX & X) from the reaction of 1-phenyl piperazine (VI) with various aliphatic/ aromatic isocyanates (VII) and isothiocyanates (VIII) respectively in 1,4-dioxane for 1-1.5 h and tested for their antidepressant activity in mice.

\[
\text{(VI)} + \text{(VII)} \xrightarrow{1,4-\text{Dioxane} \ 0 \degree C, 1-1.5 \ h} \text{(IX)}
\]

\[
\text{(VI)} + \text{(VIII)} \xrightarrow{1,4-\text{Dioxane} \ 0 \degree C, 1-1.5 \ h} \text{(X)}
\]

A Sunil Kumar Reddy et al.28 reported the synthesis and antimicrobial activity of urea and thiourea derivatives of oxazolidinones (XIII) by the reaction of oxazolidinone basic moiety (XI) in the presence of trifluoroacetic acid (TFA),
thioanisole, TEA in DCM followed by various substituted isocyanates and isothiocyanates respectively in the presence of TEA in DCM.

![Chemical structure](image)

P K Priyanka et al.\textsuperscript{29} reported the green synthesis of symmetrical N, N'-disubstituted thiourea derivatives (XV) by the reaction of available aromatic primary amine (XIV) with CS\textsubscript{2} and water in sun light for 7 h.

\[
\begin{align*}
2 \text{NH}_2 &+ \text{CS}_2 + \text{H}_2\text{O} &\xrightarrow{\text{sun light}} \text{NH}_2\text{S}\text{NH} \\
\text{(XIV)} & & \text{(XV)}
\end{align*}
\]

A series of aryl substituted urea and thiourea derivatives (XVII-XIX) were reported\textsuperscript{30} from aminopyridine and aminodiazine, 2-chlor-6-methyl(is(thio)cyanates (XVI) in THF at reflux condition and screened for their potential anticonvulsant activity.
Literature study revealed that the importance of urea and thiourea derivatives having versatile biological applications. The above importance of the research work lies in the possibility that the next generation urea and thiourea derivatives might be more efficacious as antimicrobial and anticancer agents. Based on these facts, we synthesized biologically active piperazine doped urea and thiourea derivatives of cyclopentyl 3-(2-methoxy-4-(o-tolylsulfonylcarbamoyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (zafir-lukast intermediate) and evaluated for their antimicrobial activity.

4.2. PRESENT WORK

4.2.1. SYNTHESIS

Synthesis of tert-butyl 4-(4-((5-(cyclopentyl oxycarbonylamino)-1-methyl-1H-indol-3-yl)methyl)-3-methoxybenzoyl)piperazine-1-carboxylate (3) by the reaction of cyclopentyl 3-(2-methoxy-4-(o-tolylsulfonylcarbamoyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (1) (ZAK intermediate) with Boc piperazine (2) in the presence of N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC.HCl), 1-hydroxybenzotriazole (HOBt), triethylamine (TEA) in dimethylformamide (DMF) at room temperature for 3 h followed by simple work up procedure. Consequently, the intermediate 3 was treated with 2N HCl in acetone at room temperature for 2 h to afford compound 4. Finally, compound 4 was reacted with various substituted phenyl isocynates and isothiocyanates in the presence of TEA in tetrahydrofuran (THF) at room temperature for 3 h to obtain final compounds 5a-e & 6a-e in good yields (Scheme 4.1). The newly synthesized compounds were confirmed by characterizing IR, $^1$H NMR, $^{13}$C NMR and mass spectral data.
Scheme 4.1. Synthesis of novel zafirlukast urea and thiourea derivatives (5a-e & 6a-e)

4.2.2. BIOLOGICAL ACTIVITY

4.2.2.1. Antibacterial activity

All the synthesized compounds 5a-e & 6a-e were evaluated for their antimicrobial activity against two gram +ve bacterial strains, *Streptococcus* and *Lactobacillus* and two gram -ve bacterial strains, *Vibrio cholerae* and *Escherichia coli* at 200 µg/mL concentration using the disc diffusion method and Tetracycline was used as a standard drug. The obtained results are presented in Table 4.2. Experimental procedure and the results were discussed in the experimental and results and discussion sections respectively.
4.2.2.2. Antifungal activity

All the newly synthesized compounds were evaluated for their antifungal activity against *Aspergillus niger*, *Aspergillus fumigates* and *Clostridium tetani* fungal strains using the disc diffusion method at 200 μg/mL concentration. Nystatin was used as a standard drug. Detailed experimental procedure and the results were discussed in the experimental and results and discussion sections respectively.

4.3. EXPERIMENTAL SECTION

4.3.1. MATERIALS

All the chemicals are procured from Sigma Aldrich, India and are used without further purification. The solvents were purchased from Merck, India and were purified by distillation process. The progress of the reaction was monitored by thin layer chromatography (TLC) using UV light, iodine and ninhydrin for visualization of components. Column chromatography was performed with silica gel (Merck 100-200 mesh). Melting points were taken in open capillaries by Guna digital melting point apparatus and are uncorrected. FT-IR spectra were recorded using Bruker FT-IR Spectrometer on ALPHA interferometer (ECO-ATR) and bands are reported in cm⁻¹. ¹H NMR and ¹³C NMR spectra were recorded at 400 MHz and 100 MHz using Bruker AV 400 spectrometer in DMSO-d6 solvent with respect to tetramethylsilane (TMS) as internal standard. LC-MS spectra were recorded on an Acquity Ultra Performance Liquid Chromatography instrument coupled with an API 3000 mass spectrometer operating in electrospray positive ionization mode.

4.3.2. SYNTHESIS

**Synthesis of tert-butyl 4-(4-((5-(cyclopentyloxycarbonylamino)-1-methyl-1H-indol-3-yl)methyl)-3-methoxybenzoyl)piperazine-1-carboxylate (3)**

To a solution of ZAK intermediate (1) (2.0 g, 1.0 eq.) and Boc piperazine (0.93 g, 1.05 eq.) in DMF at room temperature, EDC·HCl (1.18 g, 1.3 eq.), HOBt (0.83 g, 1.3 eq.) and TEA (16.20 mmol) were added successively. The reaction mixture was stirred at room temperature for 3 h. The progress of the reaction was monitored by thin layer chromatography (TLC). After completion of the reaction, the reaction mixture was poured into 100 mL of cold water and stirred at room temperature for 15 min. Filtered the solid formed, washed with 20 ml of n-hexane and then dried to get compound 3 as pale brownish color solid with 92% yield.
Synthesis of cyclopentyl 3-(2-methoxy-4-(piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (4)

A mixture of compound 3 (4.4 g, 1.0 eq.) and 2N HCl or 35 % HCl (1.5 eq.) in acetone was stirred at room temperature for 1 h. After completion of the reaction, it was basified with saturated NaHCO₃ solution. The reaction mixture was extracted with ethyl acetate (4 × 15 mL) and the combined organic layer was dried over with anhydrous Na₂SO₄ and the solvent was evaporated by using rota-evaporator to obtain the crude product. The crude product was purified by column chromatography using 100-200 silica gel mesh eluting with 50-60% ethyl acetate and n-hexane to obtain compound 4 as a pale brown solid with 90 % yield.

General procedure for the synthesis of title compounds (5a-e & 6a-e)

Compound 4 (0.20g, 1.0 eq.) was taken in tetrahydrofuran (THF) and cooled 0-5 °C in ice bath and then TEA (1.5 eq.) was added to the cold reaction solution and stirred for 10 min and then various substituted phenyl isocyanates and phenyl isothiocyanates (1.0 eq.) were added. The reaction mixture was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC. After completion of reaction, the solvent was removed under reduced pressure and residue was taken in water (30 mL) and extracted with ethyl acetate (4 × 15 mL). The combined organic layer was dried and concentrated to get crude compound. The crude compound was purified by column chromatography using ethyl acetate:hexane (6:4) as eluent to afford the title compounds 5a-e & 6a-e in excellent yields.

4.3.3. BIOLOGICAL ACTIVITY

4.3.3.1.Antibacterial activity

The antibacterial activity of the newly synthesized compounds (5a-e & 6a-e) was screened against Streplococcus, Lactobacillus, Vibrio cholerae, and Escherichia coli using the agar disc diffusion method.³¹,³² The nutrient agar medium in each petri plate was homogeneously spread with a bacterial strains and incubated at 25±2 °C for 24 h. All the test compounds were dissolved in DMSO. Sterile discs of Whatmann No.1 filter paper of about 6 mm diameter were impregnated on the surface of the medium. 200 µg/mL concentrations of the test compounds were prepared and applied on the discs and incubated for 48-72 h at 37 °C. The zone inhibition around the disc was calculated edge to edge zone of the confluent growth corresponds to the sharpest edge of the zone and was measured in millimeters. All the tests were repeated three times.
and average value was taken as the final result. Tetracycline was used as a standard drug and inhibition zones of the test compounds were compared with the standard and control.

4.3.3.2. Antifungal Activity

The antifungal activity of synthesized compounds (5a-e & 6a-e) were evaluated against *Aspergillus niger*, *Aspergillus fumigates* and *Clostridium tetani* using the agar disc diffusion method. The fungal strains were maintained on Potato Dextrose Agar (PDA) medium (Hi-Media). A loopful of culture from the slant was inoculated into the Potato Dextrose broth and incubated at 37 °C for 48-72 h. 0.1 mL of the culture was spread on the potato dextrose agar plate and a sterile glass spreader was used for even distribution of the inoculum. All the tested compounds were dissolved in sterile discs of Whatmann No.1 filter paper of about 6 mm diameter were impregnated on the surface of the media. Blank test showed that DMSO does not affect the test organisms. 200 µg/mL concentrations of the test compounds were prepared and applied on the discs and incubated for 48-72 h at 37 °C. The zone inhibition around the disc was calculated edge to edge zone of the confluent growth corresponds to the sharpest edge of the zone and was measured in millimeters. All tests were repeated three times and average data taken as final result. Nystatin was used as a standard drug and the inhibition zones of the test compounds were compared with controls.

4.4. RESULTS AND DISCUSSION

In the first step, synthesis of tert-butyl 4-(4-((5-(cyclopentyloxy carbonylamino)-1-methyl-1H-indol-3-yl)methyl)-3-methoxybenzoyl)piperazine-1-carboxylate (3) was achieved by the reaction of ZAK intermediate (1) with Boc piperazine (2) in the presence of EDC.HCl, HOBt and TEA in DMF at room temperature for 2.5-3 h. The reaction was carried out with acid amine coupling reagents, (1H-Benzotriazol-1-yloxy)(dimethylamino)-N,N-dimethylmethaniminium hexafluorophosphate (HBTU) and 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) in the presence of bases N,N-diisopropylethylamine (DIPEA) and 4-dimethylaminopyridine (DMAP). The resulted compound was obtained around 55-65 % yields with incompletion of starting materials. Using EDC.HCl, HOBt in the presence of TEA at room temperature for 2.5 h reaction occurs smoothly and form an intermediate amide compound 3 in excellent yield (90 %) in a short reaction
time. This reaction condition has good advantages like simple condition, easy workup process with excellent yield of resultant compound. In the next step, synthesis of cyclopentyl 3-(2-methoxy-4-(piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (4) was achieved by using 2N HCl solution in acetone at room temperature for 1 h. At first, we tried this reaction with trifluoroacetic acid (TFA) and resulted incompletion of starting material 3. In the final step, synthesis of title compounds 5a-e & 6a-e was achieved smoothly by reacting of compound 4 with various substituted phenyl isocyanates and phenyl isothiocyanates in the presence of TEA in THF at room temperature for 3 h.

4.4.1. SPECTRAL CHARACTERIZATION FOR TITLE COMPOUNDS

The synthesized title compounds 5a-e & 6a-e were characterized using IR, $^1$H NMR, $^{13}$C NMR and mass spectral data. The detailed spectral characterization data have discussed in the following sections.

**FT-IR Spectra**

The IR spectra were recorded for all the synthesized compounds 5a-e & 6a-e and the data are coincided to the respective functional groups. IR spectra showed characteristic absorption bands for all the synthesized compounds at 3313–3291 cm$^{-1}$ for -NH stretching, 2954–2923 cm$^{-1}$ for –CH stretching (aliphatic), 1799–1693 cm$^{-1}$ for C=O stretching (carbamate) and 1627–1606 cm$^{-1}$ for -N-CO stretching (amide) and 1229-1220 cm$^{-1}$ for C=S stretching vibrations. The spectra of compounds, cyclopentyl 3-(4-(4-(4-chlorophenylcarbamoyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (5b) and cyclopentyl 3-(2-methoxy-4-(4-(p-tolylcarbamothioyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (6e) are given in Figure 4.1 & 4.2 respectively.

**NMR Spectra**

The $^1$H NMR spectra were recorded for the title compounds 5a-e & 6a-e and the spectra confirmed their respective structures. The $^1$H NMR spectra of all the compounds, showed a singlet in the region δ 9.65-8.42 corresponded to NH, all the aromatic protons were shown in the region δ 7.67-6.81 as multiplet, cyclopentane CH protons were shown in the region δ 5.08-5.06 as broad singlet, δ 3.99-3.93 as singlet for CH$_2$, δ 3.70-3.31 as multiplet for piperazine CH$_2$ and δ 1.92-1.86 as multiplet corresponding to cyclopentane CH$_2$. The $^1$H NMR spectra of compound cyclopentyl 3-
(4-(4-(4-chlorophenylcarbamoyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (5b) and cyclopentyl 3-(2-methoxy-4-(4-(p-tolylcarbamothioyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (6e) are given in Figure 4.3 & 4.4 respectively as models. Standard abbreviations for multiplicity are used as follows: ‘s’ for singlet, ‘d’ for doublet, ‘dd’ for doublet of doublet, ‘t’ for triplet and ‘m’ for multiplet.

$^{13}$C NMR chemical shift values of all the synthesized compounds observed in the region $\delta$ 32.8-32.1 for N-CH$_3$, 56.0-55.2 for O-CH$_3$, 154.1-152.6 for C=O (carbamate), 159.6-156.5 for Ar-C=O, 169.1-166.0 for C=O (amide) and 182.8-181.8 for C=S (thiourea). All other $^{13}$C NMR signals corresponded to their respective carbons of the structures. $^{13}$C NMR spectral data of all the synthesized compounds have provided in experimental section. $^{13}$C NMR spectra of compounds, cyclopentyl 3-(4-(4-chlorophenylcarbamoyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (5b) and cyclopentyl 3-(2-methoxy-4-(4-(p-tolylcarbamothioyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (6e) are given in Figure 4.5 & 4.6 respectively as models of the series of compounds.

**Mass Spectra**

The mass spectra were recorded for all the synthesized compounds 5a-e & 6a-e and the spectra confirmed their respective structures by showing their respective molecular ion peaks in positive mode. The molecular ion for compound 5b appeared at $m/z$ 644.5 (M+H)$^+$ and the fragment ion appeared at $m/z$ 532.2 due to the loss of C$_6$H$_9$O$_2$ radical ion from molecular ion. The molecular ion for compound 6e appeared at $m/z$ 640.6 (M+H)$^+$. The fragment ion appeared at $m/z$ 528.5 due to the loss of C$_6$H$_9$O$_2$ radical ion from molecular ion. The mass spectra of the compounds, cyclopentyl 3-(4-(4-chlorophenylcarbamoyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (5b) and cyclopentyl 3-(2-methoxy-4-(4-(p-tolylcarbamothioyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (6e) are given as models in Figure 4.7 & 4.8 respectively. The mass data for all the compounds are given along with other spectral data below in experimental section.
Mass fragmentations pattern of compound 5b

Mass fragmentations pattern of compound 6e

Cyclopentyl 3-(4-(4-(4-bromophenylcarbamoyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (5a)

Molecular formula: C$_{35}$H$_{38}$BrN$_{5}$O$_{5}$
Molecular weight: 687.20
Physical state: Pale brown solid
Yield: 92 %
Melting point: 120-122 °C
IR (KBr) : ν 3295 (NH, str), 2927 (Alip -CH, str), 1700 (C=O, carbamate), 1625 (N-C=O, amide) cm$^{-1}$
$^1$H NMR (400 MHz, DMSO-$d_6$) : δ 9.21 (s, 1H, -NH), 8.71 (s, 1H, -NH), 7.65 (s, 1H, Ar-H), 7.45-7.02 (m, 9H, Ar-H), 6.88 (d, $J = 8.0$ Hz, 1H, N-CH,
indole), 5.06 (bs, 1H, CH, cyclopentane), 3.93 (s, 2H, CH2), 3.89 (s, 3H, OCH3), 3.70 (s, 3H, N-CH3), 3.48-3.31 (m, 8H, CH2, piperazine), 1.84-1.57 (m, 8H, CH2, cyclopentane) ppm.

$^{13}$C NMR (100.6 MHz, DMSO-$d_6$) : δ 169.1 (C7), 156.5 (C2), 154.6 (C12), 153.6 (C31), 138.3 (C14), 134.1 (C4), 133.2 (C29), 131.1 (C16,18,26), 129.6 (C6), 128.3 (C28), 127.2 (C1, C22), 121.2 (C15,17,19), 118.7 (C5,25), 111.4 (C3,21), 109.4 (C24,27), 76.1 (C32), 55.5 (C2'), 45.6 (C8,9,10,11), 32.3 (C20,23,33,36), 24.3 (C34,35) ppm.

LC-MS : $m/z$ 710.2 [M+Na]$^+$ (10 %), 688.2 [M+H]$^+$ (90 %), 578.3 [(M-C$_6$H$_9$O)+Br]$^+$ (93 %)

Cyclopentyl 3-(4-(4-(4-chlorophenylcarbamoyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (5b)

Molecular formula: C$_{35}$H$_{38}$ClN$_5$O$_5$

Molecular weight: 643.26

Physical state: Pale brown solid

Yield: 92 %

Melting point: 151-153 °C

IR (KBr) : ν 3313 (-N-H, str), 2926 (Aliph C-H, str), 1721 (carbamate, C=O, str), 1625 (amide, N-C=O, str) cm$^{-1}$

$^1$H NMR (400 MHz, DMSO-$d_6$) : δ 9.21 (s, 1H, -NH), 8.71 (s, 1H, -NH), 7.66 (s, 1H, Ar-H), 7.50 (d, J = 8.0 Hz, 2H, Ar-H), 7.29-7.26 (m, 3H, Ar-H), 7.16 (d, J = 8.0 Hz, 1H, Ar-H), 7.08-7.02 (m, 3H, Ar-H), 6.89 (d, J = 8.0 Hz, 1H, N-CH, indole), 5.07 (bs, 1H, CH, cyclopentane), 3.94 (s, 2H, CH$_2$), 3.89 (s, 3H, OCH$_3$), 3.70 (s, 3H, N-CH$_3$), 3.49-3.27 (m, 8H, CH$_2$, piperazine), 1.86-1.57 (m, 8H, CH$_2$, cyclopentane) ppm.

$^{13}$C NMR (100 MHz, DMSO-$d_6$) : δ 169.1 (C7), 156.5 (C2), 154.7 (C12), 153.6 (C31), 139.4 (C14), 134.5 (C4), 133.2 (C17,29), 131.0 (C26), 129.1 (C6), 128.3 (C16,18), 127.3 (C28), 125.3 (C1,22), 120.9 (C15,19), 118.7 (C5,25), 111.4 (C3,21), 109.4 (C24,27), 76.2 (C32), 55.5 (C2'), 43.7 (C8,9,10,11), 32.3 (C20,23,33,36), 24.4 (C34,35) ppm.

LC-MS : $m/z$ 666.3 [M+Na]$^+$ (5 %), 644.5 [M+H]$^+$ (98 %), 532.2 [(M-C$_6$H$_9$O)+Cl]$^+$ (100 %)

73
Cyclopentyl 3-(4-(4-(4-fluorophenylcarbamoyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (5c)

Molecular formula: C_{35}H_{38}FN_{5}O_{5}
Molecular weight: 627.71
Physical state: Pale orange solid
Yield: 90 %
Melting point: 132-134 °C

IR (KBr) : ν 3274 (-N-H, str), 2923 (Aliph C-H, str), 1696 (carbamate, C=O, str), 1614 (amide, N-C=O, str) cm⁻¹

¹H NMR (400 MHz, DMSO-d₆) : δ 9.21 (s, 1H, -NH), 8.62 (s, 1H, -NH), 7.66 (s, 1H, Ar-H), 7.47-7.43 (m, 2H, Ar-H), 7.28 (d, J = 8.0 Hz, 1H, Ar-H), 7.15 (d, J = 8.0 Hz, 1H, Ar-H), 7.09-7.02 (m, 5H, Ar-H), 6.89 (d, J = 8.0 Hz, 1H, N-CH, indole), 5.07 (bs, 1H, CH, cyclopentane), 3.94 (s, 2H, CH₂), 3.89 (s, 3H, OCH₃), 3.70 (s, 3H, N-CH₃), 3.49-3.32 (m, 8H, CH₂, piperazine), 1.92-1.57 (m, 8H, CH₂, cyclopentane) ppm.

¹³C NMR (100 MHz, DMSO-d₆) : δ 169.0 (C₇), 157.4 (C₁₇), 156.2 (C₂), 155.3 (C₁₂), 153.6 (C₃₁), 135.6 (C₄,₁₄), 133.1 (C₂₉), 133.0 (C₂₆), 131.2 (C₆), 129.4 (C₂₈), 127.1 (C₂₂), 126.8 (C₁), 121.0 (C₂₅), 118.4 (5,1₅,1₉), 114.3 (C₁₆,₁₈), 111.1 (C₃,₂₁), 108.9 (C₂₄,₂₇), 76.1 (C₃₂), 55.2 (C₂), 43.8 (C₈,₉,₁₀,₁₁), 32.1 (C₂₀,₂₃,₃₃,₃₆), 24.3 (C₃₄,₃₅) ppm.

LC-MS : m/z 628.3 [M+H]^+ (95 %), 629.3 [M+2H]^+ (30 %), 630.3 [M+3H]^+ (15 %)

Cyclopentyl 3-(2-methoxy-4-(4-(4-methoxyphenylcarbamoyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (5d)

Molecular formula: C_{3₆}H₄₁N₅O₆
Molecular weight: 639.31
Physical state: Pale brown solid
Yield: 91 %
Melting point: 136-138 °C

IR (KBr) : ν 3307 (NH, str), 2927 (Aliph -CH, str), 1701(C=O, carbamate), 1618 (N-C=O, amide) cm⁻¹
1H NMR (400 MHz, DMSO-d6) : δ 9.21 (s, 1H, -NH), 8.42 (s, 1H, -NH), 7.65 (s, 1H, Ar-H), 7.35-6.81 (m, 10H, Ar-H), 5.07 (bs, 1H, CH, cyclopentane), 3.94 (s, 2H, CH₂), 3.89 (s, 3H, OCH₃), 3.71 (s, 3H, N-CH₃), 3.70 (s, 3H, OCH₃), 3.47-3.32 (m, 8H, CH₂, piperazine), 1.86-1.57 (m, 8H, CH₂, cyclopentane) ppm.

13C NMR (100 MHz, DMSO-d6) : δ 169.1 (C₇), 155.2 (C₁₇), 154.5 (C₂), 154.3 (C₁₂), 153.6 (C₃₁), 134.6 (C₄), 133.2 (C₂₉), 132.9 (C₁₄), 131.0 (C₂₆), 129.1 (C₆), 128.3 (C₂₈), 127.3 (C₂₂), 121.5 (C₁), 119.9 (C₁₅), 118.7 (C₁₉,₂₅), 113.9 (C₁₆,₁₈), 111.4 (C₃,₂₁), 109.4 (C₂₄,₂₇), 76.6 (C₃₂), 55.5 (C₂, C₁₇), 43.6 (C₈,₉,₁₀,₁₁), 32.3 (C₂₀,₂₃,₃₃,₃₆), 24.4 (C₃₄,₃₅) ppm.

LC-MS : m/z 640.6 [M+H]⁺ (100 %), 641.7 [M+2H]⁺ (50 %), 642.6 [M+3H]⁺ (10 %)

Cyclopentyl 3-(2-methoxy-4-(4-(p-tolylcarbamoyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (5e)

Molecular formula: C₃₆H₄₁N₅O₅
Molecular weight: 623.74
Physical state: Pale brown solid
Yield: 93 %
Melting point: 158-160 °C
IR (KBr) : ν 3300 (NH, str), 2924 (Alip -CH, str), 1693 (C=O, carbamate), 1627 (N-C=O, amide) cm⁻¹

1H NMR (400 MHz, DMSO-d6) : δ 9.21 (s, 1H, -NH), 8.49 (s, 1H, -NH), 7.65 (s, 1H, Ar-H), 7.33-7.02 (m, 9H, Ar-H), 6.89 (d, J = 8.0 Hz, 1H, N-CH, indole), 5.07 (bs, 1H, CH, cyclopentane), 3.94 (s, 2H, CH₂), 3.89 (s, 3H, OCH₃), 3.70 (s, 3H, OCH₃), 3.48 (s, 3H, N-CH₃), 3.21-3.18 (m, 8H, CH₂, piperazine), 1.86-1.57 (m, 8H, CH₂, cyclopentane) ppm.

13C NMR (100 MHz, DMSO-d6) : δ 169.4 (C₇), 156.7 (C₂), 154.2 (C₁₂), 152.6 (C₃₁), 137.2 (C₁₄,₁₇), 133.6 (C₄), 132.4 (C₂₉), 130.4 (C₂₆), 129.1 (C₆,₁₆,₁₈), 128.3 (C₂₈), 126.4 (C₁₂₂), 119.8 (C₁₅,₁₉), 118.7 (C₃₅,₂₅), 111.3 (C₃,₂₁), 109.4 (C₂₄,₂₇), 76.5 (C₃₂), 55.5 (C₂'), 44.7 (C₈,₉,₁₀,₁₁), 32.3 (C₂₀,₂₃,₃₃,₃₆), 23.2 (C₃₄,₃₅) 20.28 (C₁₇) ppm.
LC-MS: 
\( m/z \) 646.4 [M+Na]\(^+\) (5 %), 624.3 [M+H]\(^+\) (65 %), 512.3 [M-C\(_6\)H\(_9\)O\(_2\)]\(^+\) (60 %)

**Cyclopentyl 3-(4-(4-(4-chlorophenylcarbamothioyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (6a)**

Molecular formula: C\(_{35}\)H\(_{38}\)ClN\(_5\)O\(_4\)S
Molecular weight: 660.23
Physical state: Pale brown solid
Yield: 91 %
Melting point: 131-133 °C

IR (KBr): 
\( v \) 3295 (NH, str), 2940 (Alip -CH, str), 1799 (C=O, carbamate), 1614 (N-C=O, amide), 1225 (C=S) cm\(^{-1}\)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)):
\( \delta \) 9.43 (s, 1H, -NH), 9.20 (s, 1H, -NH), 7.65 (s, 1H, Ar-H), 7.53-7.03 (m, 9H, Ar-H), 6.91 (d, \( J = 8 \) Hz, 1H, N-CH, indole), 5.06 (bs, 1H, CH, cyclopentane), 3.94 (s, 2H, CH\(_2\)), 3.89 (s, 3H, OCH\(_3\)), 3.70 (s, 3H, N-CH\(_3\)), 3.31-3.25 (m, 8H, CH\(_2\), piperazine), 1.85-1.56 (m, 8H, CH\(_2\), cyclopentane) ppm.

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)):
\( \delta \) 182.0 (C\(_{12}\)), 169.7 (C\(_7\)), 157.0 (C\(_2\)), 154.1 (C\(_{31}\)), 140.4 (C\(_{4,14}\)), 134.8 (C\(_{17}\)), 133.7 (C\(_{29}\)), 131.5 (C\(_{15,19,26}\)), 129.6 (C\(_6\)), 128.8 (C\(_{16,18}\)), 128.3 (C\(_{28}\)), 127.7 (C\(_1\)), 127.2 (C\(_{22}\)), 119.3 (C\(_{5,25}\)), 111.8 (C\(_{3,21}\)), 109.9 (C\(_{24,27}\)), 76.6 (C\(_{32}\)), 55.0 (C\(_2\)), 46.0 (C\(_{8,9,10,11}\)), 32.8 (C\(_{20,23,33,36}\)), 24.9 (C\(_{34,35}\)) ppm.

MS (ESI): 
\( m/z \) 659.4 [M-H]\(^+\) (25 %)

**Cyclopentyl 3-(4-(4-(2,4-dichlorophenylcarbamothioyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (6b)**

Molecular formula: C\(_{35}\)H\(_{37}\)Cl\(_2\)N\(_5\)O\(_4\)S
Molecular weight: 693.60
Physical state: Pale brown solid
Yield: 91 %
Melting point: 121-123 °C

IR (KBr): 
\( v \) 3274 (NH, str), 2947 (Alip -CH, str), 1699 (C=O, carbamate), 1617 (N-C=O, amide), 1220 (C=S) cm\(^{-1}\)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)):
\( \delta \) 9.32 (s, 1H, -NH), 9.20 (s, 1H, -NH), 7.65 (s, 2H, Ar-H),
MHz, DMSO-d6) 7.43-7.04 (m, 7H, Ar-H), 6.92 (d, J = 8 Hz, 1H, N-CH, indole), 5.07 (bs, 1H, CH, cyclopentane), 3.99 (s, 2H, CH$_2$), 3.94 (s, 3H, OCH$_3$), 3.90 (s, 3H, N-CH$_3$), 3.70-3.31 (m, 8H, CH$_2$, piperazine), 1.86-1.57 (m, 8H, CH$_2$, cyclopentane) ppm.

$^{13}$C NMR (100 MHz, DMSO-d6): δ 182.4 (C$_{12}$), 169.7 (C$_{7}$), 157.0 (C$_{2}$), 154.1 (C$_{31}$), 137.9 (C$_{4,14}$), 134.8 (C$_{15,29}$), 133.7 (C$_{19}$), 132.6 (C$_{17,26}$), 131.7 (C$_{16}$), 129.7 (C$_{6}$), 129.3 (C$_{28}$), 128.8 (C$_{1}$), 127.8 (C$_{22}$), 119.4 (C$_{18}$), 114.7 (C$_{5,25}$), 111.2 (C$_{3,21}$), 109.9 (C$_{24,27}$), 76.6 (C$_{32}$), 56.0 (C$_{35}$), 46.2 (C$_{8,9,10,11}$), 32.8 (C$_{20,23,33,36}$), 24.9 (C$_{34,35}$) ppm.

LC-MS: m/z 694.2 [M+H]$^+$ (82 %), 582.2 [M-C$_6$H$_9$O]$^+$ (65 %)

Cyclopentyl 3-(4-(4-(2,4-difluorophenylcarbamothioyl)piperazine-1-carbonyl)-2-methoxybenzyl)-1-methyl-1H-indol-5-ylcarbamate (6c)

Molecular formula: C$_{35}$H$_{37}$F$_2$N$_5$O$_4$S
Molecular weight: 661.76

Physical state: Pale brown solid

Yield: 93 %

Melting point: 124-126 ºC

IR (KBr): ν 3274 (NH, str), 2954 (Alip -CH, str), 1701 (C=O, carbamate), 1612 (N-C=O, amide), 1229 (C=S) cm$^{-1}$

$^1$H NMR (400 MHz, DMSO-d6): δ 9.20 (s, 1H, -NH), 9.08 (s, 1H, -NH), 7.65 (s, 1H, Ar-H), 7.49-7.04 (m, 8H, Ar-H), 6.91 (d, J = 8 Hz, 1H, N-CH, indole), 5.07 (s, 1H, CH, cyclopentane), 3.99 (s, 2H, CH$_2$), 3.94 (s, 3H, OCH$_3$), 3.90 (s, 3H, N-CH$_3$), 3.70-3.31 (m, 8H, CH$_2$, piperazine), 1.86-1.57 (m, 8H, CH$_2$, cyclopentane) ppm.

$^{13}$C NMR (100 MHz, DMSO-d6): δ 182.8 (C$_{12}$), 169.7 (C$_{7,15}$), 160.3 (C$_{17}$), 157.9 (C$_{2}$), 154.1 (C$_{31}$), 134.8 (C$_{4}$), 133.7 (C$_{29}$), 131.5 (C$_{26}$), 129.6 (C$_{6,19}$), 128.8 (C$_{27}$), 127.8 (C$_{1,22}$), 119.4 (C$_{25}$), 118.7 (C$_{5}$), 114.9 (C$_{14}$), 112.2 (C$_{18}$), 111.9 (C$_{3,21}$), 109.9 (C$_{24,27}$), 108.7 (C$_{16}$), 76.6 (C$_{32}$), 56.0 (C$_{35}$), 46.1 (C$_{8,9,10,11}$), 32.8 (C$_{20,23,33,36}$), 24.5 (C$_{34,35}$) ppm.

LC-MS: m/z 662.4 [M+H]$^+$ (83 %), 550.4 [M-C$_6$H$_9$O]$^+$ (75 %)
Cyclopentyl 3-(2-methoxy-4-(4-(3-(trifluoromethyl)phenylcarbamothioyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (6d)

Molecular formula: C₃₆H₃₈F₃N₅O₄S
Molecular weight: 693.78
Physical state: Pale brown solid
Yield: 95%
Melting point: 126-128 °C

IR (KBr) : \( \nu \) 3291 (NH, str), 2949 (Alip-CH, str), 1700 (C=O, carbamate), 1613 (N-C=O, amide), 1224 (C=S) cm\(^{-1}\)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) : \( \delta \) 9.65 (s, 1H, -NH), 9.20 (s, 1H, -NH), 7.67-7.03 (m, 10H, Ar-H), 6.92 (d, \( J = 8 \) Hz, 1H, N-CH, indole), 5.06 (s, 1H, CH, cyclopentane), 3.99 (s, 2H, CH\(_2\)), 3.94 (s, 3H, OCH\(_3\)), 3.70 (s, 3H, N-CH\(_3\)), 3.57-3.31 (m, 8H, CH\(_2\), piperazine), 1.85-1.56 (m, 8H, CH\(_2\), cyclopentane) ppm.

\(^13\)C NMR (100 MHz, DMSO-\(d_6\)) : \( \delta \) 181.8 (C\(_{12}\)), 169.7 (C\(_7\)), 157.0 (C\(_2\)), 154.1 (C\(_{31}\)), 142.2 (C\(_{14}\)), 134.8 (C\(_4\)), 133.7 (C\(_{29}\)), 131.5 (C\(_{16,26}\)), 130.0 (C\(_{15}\)), 129.5 (C\(_{6,19}\)), 129.3 (C\(_{18}\)), 128.6 (C\(_{28}\)), 127.7 (C\(_{22}\)), 125.9 (C\(_1\)), 123.2 (C\(_{16'}\)), 121.5 (C\(_{17}\)), 119.4 (C\(_{5,25}\)), 111.8 (C\(_{3,21}\)), 109.9 (C\(_{27}\)), 108.7 (C\(_{24}\)), 76.6 (C\(_{32}\)), 56.0 (C\(_2'\)), 46.0 (C\(_{8,9,10,11}\)), 32.8 (C\(_{20,23,30,36}\)), 23.7 (C\(_{34,35}\)) ppm.

LC-MS : \( m/z \) 694.1 [M+H\(^+\)]\(^{78}\%)\), 582.2 [M-C\(_6\)\(_H_9\)O\(^+\)]\(^{76}\%\)

Cyclopentyl 3-(2-methoxy-4-(4-(p-tolylcarbamothioyl)piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate (6e)

Molecular formula: C\(_{36}\)H\(_{41}\)N\(_5\)O\(_4\)S
Molecular weight 639.81
Physical state: pale brown solid
Yield: 93%
Melting point: 138-140 °C

IR (KBr) : \( \nu \) 3305 (NH, str), 2924 (Alip-CH, str), 1699 (C=O, carbamate), 1606 (N-C=O, amide), 1226 (C=S) cm\(^{-1}\)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) : \( \delta \) 9.29 (s, 1H, -NH), 9.21 (s, 1H, -NH), 7.65 (s, 1H, Ar-H), 7.28 (d, \( J = 8.0 \) Hz, 1H, Ar-H), 7.17-7.04 (m, 8H, Ar-H), 6.91 (d, \( J = 8.0 \) Hz, 1H, N-CH, indole), 5.08 (s, 1H, CH,
cyclopentane), 3.94 (s, 2H, CH₂), 3.89 (s, 3H, OCH₃), 3.70 (s, 3H, N-CH₃), 3.52-3.23 (m, 8H, CH₂, piperazine), 2.51 (d, J = 4.0 Hz, 3H, CH₃), 1.86-1.56 (m, 8H, CH₂, cyclopentane) ppm.

\(^{13}\text{C NMR (100 MHz, DMSO-}d_6\text{)}:\) δ 182.2 (C₁₂), 169.7 (C₇), 157.0 (C₂), 154.1 (C₃₁), 138.7 (C₁₇), 135.1 (C₁₄), 134.9 (C₄), 133.7 (C₂₀), 131.4 (C₂₀), 130.6 (C₆), 129.7 (C₁₆,₁₈), 128.9 (C₂₈), 127.7 (C₁₅,₁₉,₂₂), 125.9 (C₁), 122.0 (C₂₅), 119.3 (C₃), 111.8 (C₃,₂₁), 109.9 (C₂₇), 108.9 (C₂₄), 76.6 (C₃₁), 56.0 (C₂), 46.2 (C₈,₉,₁₀,₁₁), 32.8 (C₂₀,₂₃,₃₀,₃₆), 24.9 (C₃₄,₃₅), 20.9 (C₁₇) ppm.

LC-MS : \(m/z\) 640.6 [M+H]⁺ (100 %), 528.5 [M-C₆H₉O.]⁺ (15 %)

### 4.4.2. BIOLOGICAL ACTIVITY

**Antibacterial activity**

All the synthesized compounds were screened for their *in vitro* antibacterial activity against *Streptococcus*, *Lactobacillus*, *Vibrio cholera* and *Escherichia coli* bacterial strains using the disc diffusion method at 200 µg/mL concentration. Tetracycline was used as a standard drug. Results revealed that compounds 6a and 6d exhibited good antibacterial activity against tested strains. The results are tabulated in **Table 4.1**.

**Table 4.1** Antibacterial activity of novel zafirlukast urea and thiourea derivatives (5a-e & 6a-e)

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Streptococcus</th>
<th>Lactobacillus</th>
<th>Vibrio cholera</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5b</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5c</td>
<td>0.6</td>
<td>0.6</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>5d</td>
<td>0.8</td>
<td>0.6</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>5e</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6a</td>
<td>1.0</td>
<td>1.2</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>6b</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>6c</td>
<td>0.5</td>
<td>0.5</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>6d</td>
<td>1.0</td>
<td>1.1</td>
<td>1.0</td>
<td>1.1</td>
</tr>
<tr>
<td>6e</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1.8</td>
<td>1.8</td>
<td>1.7</td>
<td>1.6</td>
</tr>
</tbody>
</table>

NA : No Activity
Antifungal activity

All the synthesized compounds were screened for their in vitro antifungal activity against *Aspergillus niger*, *Aspergillus fumigates* and *Clostridium tetani* using the disc diffusion method at 200 µg/mL concentration. Nystatin was used as standard drug. The results revealed that no antifungal activity shown against tested strains.

4.5. CONCLUSION

Synthesis of cyclopentyl 3-(2-methoxy-4-(piperazine-1-carbonyl)benzyl)-1-methyl-1H-indol-5-ylcarbamate urea and thiourea derivatives by zafirlukast (ZAK) intermediate 1 with Boc piperazine 2 followed by treatment with 2N HCl and various substituted phenyl isocyantes and phenyl isothiocyanates in simple procedure to afford titled compounds 5a-e & 6a-e respectively. All the synthesized compounds were characterized by IR, 1H NMR, 13C NMR and mass spectral analysis. The synthesized compounds were evaluated for their antibacterial and antifungal activities. Compounds 6a and 6d shown good antibacterial activity.

4.6. DIRECTIONS FOR FUTURE RESEARCH

As part of extension work to the present work, synthesis of N-oxides for all the titled compounds may be done to study and compare the biological and pharmacological applications.
REFERENCES