CHAPTER-I

1.1. Introduction:
Natural, synthetic and semisynthetic heterocyclic compounds play an important role in drug discovery and chemical biology. The heterocycles are mainly of the classes of alkaloids, flavones, isoflavones, chromones, coumarins, chromenes. Synthetic compounds of these classes show different biological activity. More than 50% of the drugs used in the modern medicine are either derived from natural or synthetic heterocyclic system. A brief account of the biologically active oxygen heterocycles which are used as drugs or in various stages of development as drugs are reviewed in Chapter I.

1.2. Bioactive coumarins, chromans, chromones and triterpenoids:
Warfarin (1) and related 4-hydroxycoumarins decrease blood coagulation by inhibiting vitamin K epoxide reductase, an enzyme that recycles oxidized vitamin K$_1$ to its reduced form after it has participated in the carboxylation of several blood coagulation proteins, mainly prothrombin and factor VII. The aminocoumarin antibiotics novobiocin (2) and clorobiocin (3) are very potent inhibitors of DNA gyrase and are produced by different Streptomyces strains. Dicoumarol (4) is an anticoagulant that functions as a vitamin K antagonist. It is also used in biochemical experiments as an inhibitor of reductases.

Chromakalim (5) is a potassium channel opening vasodilator used to treat cardiac problems. Ormeloxifene (centochroman) (6) is used in India as a birth control pill (contraceptive agent). Flavone 8-acetic acid also known as mitoflaxone (7) is an adjuvant anti-cancer drug. 7, 4-Dihydroxy isoflav-3-ene (8) is under clinical trials as an anticancer drug. Flavopyridol (alvocidib) (9) is under clinical development for chronic lymphocytic
leukemia. Disodium cromoglycate (10) is an anti allergic drug used to treat asthma. Flavoxate (11) is used to treat urinary bladder spasms, eupatilin (12) is used to treat acid-related disorders and demiflin (13) is a marketed drug used as coronary vasodilator. Silybin (14) is a hepatoprotective agent used in the treatment of jaundice.

Betulinic acid (15) is a naturally occurring pentacyclic triterpenoid which has antiretroviral, antimalarial and anti-inflammatory properties as well as a more recently discovered potential as an anticancer agent by inhibition of topoisomerase. It is found in the bark of several species of plants principally the white birch (Betula pubescens). Bevirimat (16) [3-O-(3’,3’-dimethylsuccinyl)betulinic acid] was found to exhibit remarkable anti-HIV-1 activity against primary and drug resistant HIV-1 isolates.
1.3. OBJECTIVES AND SCOPE OF THE PRESENT STUDY:
Since many substituted coumarins have a diverse range of biological activities, the present work is planned so as to synthesize new heterocycles pendent on the coumarin ring at 4,6,7,8 positions and fused to coumarin ring at 7,8 positions by applying the various synthetic strategies.

The results of the present study are discussed in 6 chapters

1) Biological activity of oxygen heterocyclic systems and triterpene betulinic acid is reviewed in Chapter-I.

2) i) Chapter II (Section A) describes the regioselective synthesis of ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates via NCS mediated intermolecular 1,3-dipolar cycloaddition of coumarin nitrile oxides.

ii) Chapter II (Section B) describes the synthesis of 8,9-substituted-3a,4-dihydropyran[2',3':5,6]chromeno[4,3-c]isoxazol-10(3H)-ones via CAN mediated intramolecular 1,3-dipolar cycloaddition of O-allyl oximes.

iii) Chapter II (Section C) describes the synthesis of 8,9-substituted-pyrano [2',3':5,6]chromeno[4,3-c]isoxazol-10(4H)-ones via CAN mediated intramolecular 1,3 dipolar cycloaddition of O-propargyl oximes.

3) Chapter III describes the synthesis of 4/6/7-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-coumarins by using O-alkylated substituted hydroxy coumarins and amide oximes.
4) Chapter IV describes the synthesis of O-alkyl hydroxamic acids, N-(benzyloxy)-2-(4-H/Methyl-coumarin-4/6/7-yloxy)-acetamides by amide coupling of coumarin-4,6,7-yloxyacetic acids and O-(substituted benzyl)-hydroxylamines.

5) Chapter V describes the synthesis of C-28 modified 1,2,4-oxadiazole esters and amides of betulinic acid via esterification and amide coupling strategies.

6) Chapter VI, Section A describes the biological screening of molecules in the Eli Lilly-Open Innovation Drug Discovery Programme, Section B deals with the evaluation of the antibacterial and antifungal activity of synthetic coumarin derivatives and Section C describes the cytotoxicity of semisynthetic analogues of betulinic acid.

CHAPTER-II:
This chapter describes the generation and reactions of coumarin nitrile oxides using intermolecular and intramolecular cycloaddition strategies.

Section A describes the the regioselective synthesis of ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates (25 a-e). Isoxazolines and isoxazoles with various functionalization patterns are among the most interesting bioactive compounds. The target heterocycles have been frequently prepared using the 1,3-dipolar cycloaddition of nitrile oxides to alkenes as the key step an approach which allowed us to achieve novel derivatives with different pharmacological activities.

2.1. Regioselective synthesis of ethyl-3-[7-benzyloxy-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates (25 a-e):
Isoxazoline derivatives have been reported to possess antifungal, antibacterial, anticonvulsant, anti-inflammatory, anti-viral, analgesic, antitumor, chemotherapeutic activity. Penicillin derivatives containing isoxazole ring were found to be antibacterial agents. In the present study novel substituted isoxazoliny coumarins (25 a-e) were developed using NCS mediated intermolecular 1,3-dipolar cycloadditions of 7-benzyloxy-4-methyl-coumarin aldehyde chlorooxime (22) with various (E)-ethyl-3-aryl-prop-2-enoates (24 a-e).
2.2. Synthesis of 7-benzyloxy-4-methyl-coumarin aldehyde chlorooxime (22):
Duff formylation of 7-Hydroxy-4-methyl-coumarin (17) in the presence of hexamine and acetic acid at 90-95 °C gave 4-methyl-7-hydroxy-8-formyl-coumarin (18). 4-Methyl-7-hydroxy-8-formyl-coumarin (18) was protected in the form of benzyl derivative (20) with benzylbromide (19) in the presence of K$_2$CO$_3$ in DMF at 80-90 °C. The reaction of 7-benzyloxy-4-methyl-coumarin aldehyde (20) with NH$_2$OH.HCl in the presence of NaOAc at RT gave oxime (21) which on chlorination with NCS in chloroform at 0 °C gave 7-benzyloxy-4-methyl-coumarin aldehyde chlorooxime (22) and 7-benzyloxy-3-chloro-4-methyl-coumarin aldehyde chlorooxime (22a) as a minor product.

Scheme 1: Synthesis of 7-benzyloxy-4-methyl-coumarin aldehyde chlorooxime (22):

2.3. Synthesis of (E)-ethyl-3-aryl-prop-2-enoates (24 a-e):
(E)-Ethyl-3-aryl-prop-2-enoates (24 a-e) were synthesized from the esterification of substituted cinnamic acids (23 a-e) in the presence of ethanol and catalytic amount of Con.H$_2$SO$_4$.

Scheme 2: Synthesis of (E)-ethyl-3-aryl-prop-2-enoates (24 a-e):

\[
\begin{align*}
23 \text{ a-e} & \xrightarrow{\text{Con H$_2$SO$_4$, EtOH}} 24 \text{ a-e} \\
a) R = H & \quad b) R = 3-CF$_3$ \quad c) R = 4-CF$_3$ \quad d) R = 3,5\text{ Difluoro} \quad e) R = \text{Indoly}l
\end{align*}
\]
2.4. Synthesis of ethyl-3-[7-benzylxylo-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates (25 a-e):

7-Benzylxylo-4-methyl-coumarin aldehyde chlorooxime (22) on reaction with (E)-ethyl-3-phenylprop-2-enoate (24a) in the presence of Et$_3$N in chloroform at RT for 24 h gave regioselectively ethyl-3-[7-benzylxylo-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (25a). Under similar reaction conditions 7-benzylxylo-3-chloro-4-methyl-coumarin aldehyde chlorooxime (22a) gave ethyl-3-[7-benzylxylo-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (26a). 25a and 26a are characterized by $^1$H-NMR, NOESY, $^{13}$C-NMR and MS.

Scheme 3: Synthesis of ethyl-3-[7-benzylxylo-4-methyl-2-oxo-2H-8-chromenyl]-5-aryl-4,5-dihydro-4-isoxazole carboxylates (25 a-e):

In the $^1$H NMR of ethyl-3-[7-benzylxylo-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (25a) shows the H-4'' of the newly formed isoxazoline appeared at $\delta$ 4.68 (d, J=8.8Hz) and H-5'' at $\delta$ 6.11 (d, J=9.2Hz). Ethyl ester protons at
\[ \delta \ 0.95 \ (t, \ -\text{COOCH}_2\text{CH}_3) \ \text{and} \ \delta \ 3.95 \ (qq, \ -\text{COOCH}_2\text{CH}_3) \ \text{phenyl protons attached to isoxazoline ring at} \ \delta \ 7.43-7.46 \ (m, \ H-2'' \ & H-6''). \ \text{Coumarin H-3, H-5 and H-6 appeared at} \ 6.17 \ (s), \ 7.6 \ (d, \ J=8.8Hz) \ \text{and} \ 6.96 \ (d, \ J=8.8Hz). \ \text{4-CH}_3 \ \text{and benzyloxy protons at} \ 2.72 \ (s), \ 7.33-7.39 \ (m).

\text{In the} \ ^{13}\text{C NMR of} \ 25a \ \text{isoxazoline carbons appeared at} \ \delta \ 159.8 \ (C-3''), \ 61.7 \ (C-4''), \ 85.6 \ (C-5''), \ \text{ethyl ester carbons at} \ 63.0 \ (-\text{COOCH}_2\text{CH}_3), \ 13.7 \ (-\text{COOCH}_2\text{CH}_3) \ \text{and phenyl ring carbons attached to the isoxazoline ring at} \ 139.1 \ (C-1''), \ 126.9 \ (C-2'' \ & C-6''), \ 128.8 \ (C-3'' \ & C-5''), \ 126.6 \ (C-4''). \ \text{The coumarin moiety carbons appeared as follows} \ \delta \ 159.5 \ (C-2), \ 108.5 \ (C-3), \ 147.1 \ (C-4), \ 112.6 \ (C-4a), \ 128.6 \ (C-5), \ 114.1 \ (C-6), \ 153.0 \ (C-7), \ 106.8 \ (C-8), \ 152.0 \ (C-8a), \ 71.1 \ (7-OCH_2), \ 135.4 \ (C-1'), \ 128.7 \ (C-2' \ & C-6'), \ 128.3 \ (C-3' \ & C-5'), \ 127.2 \ (C-4').

\text{In the DIPMS of} \ 25a \ \text{the quasi-molecular ion peak was observed at} \ m/z \ 484 \ (M+1).

\text{In the} \ ^1\text{H NMR of ethyl-3-[7-benzyloxy-3-chloro-4-methyl-2-oxo-2H-8-chromenyl]-5-phenyl-4,5-dihydro-4-isoxazole carboxylate (26a) the protons of the newly formed isoxazoline} \ H-4'' \ \text{appeared at} \ \delta \ 4.62 \ (d, \ J=8.8Hz) \ \text{and} \ H-5'' \ \text{at} \ \delta \ 6.08 \ (d, \ J=8.8Hz). \ \text{Ethyl ester protons at} \ \delta \ 0.95 \ (t, \ -\text{COOCH}_2\text{CH}_3) \ \text{and} \ \delta \ 3.95 \ (qq, \ -\text{COOCH}_2\text{CH}_3) \ \text{and the phenyl protons attached to isoxazoline ring at} \ \delta \ 7.40-7.41 \ (m, \ H-2'' \ & H-6''). \ \text{Coumarin ring H-5 and H-6 appeared at} \ 7.6 \ (d, \ J=8.9Hz) \ \text{and} \ 6.96 \ (d, \ J=9.2Hz). \ \text{4-CH}_3 \ \text{and benzyloxy protons at} \ 2.54 \ (s), \ 7.31-7.34 \ (m).

\text{In the} \ ^{13}\text{C NMR of} \ 26a \ \text{the isoxazoline carbons appeared} \ \delta \ 159.4 \ (C-3''), \ 61.8 \ (C-4''), \ 85.7 \ (C-5''), \ \text{ethyl ester carbons at} \ 63.0 \ (-\text{COOCH}_2\text{CH}_3), \ 13.7 \ (-\text{COOCH}_2\text{CH}_3) \ \text{and phenyl ring carbons attached to the isoxazoline ring at} \ 139.0 \ (C-1''), \ 126.5 \ (C-2'' \ & C-6''), \ 128.8 \ (C-3'' \ & C-5''), \ 126.5 \ (C-4''). \ \text{The coumarin moiety carbons appeared at} \ \delta \ 156.1 \ (C-2), \ 107.0 \ (C-3), \ 146.7 \ (C-4), \ 113.9 \ (C-4a), \ 128.4 \ (C-5), \ 113.9 \ (C-6), \ 150.8 \ (C-7), \ 106.8 \ (C-8), \ 147.4 \ (C-8a), \ 71.2 \ (7-OCH_2), \ 128.8 \ (C-1'), \ 128.7 \ (C-2' \ & C-6'), \ 127.3 \ (C-3' \ & C-5'), \ 127.2 \ (C-4').

\text{In the DIPMS of} \ 26a \ \text{the quasi-molecular ion peak was observed at} \ m/z \ 518 \ (M+1).
Section B describes the synthesis of 8,9-substituted-3a,4-dihydropyrano[2′,3′:5,6]chromeno[4,3-c]isoxazol-10(3H)-ones (31 a-e)

2.5. Synthesis of 8,9-substituted-3a,4-dihydropyrano[2′,3′:5,6]chromeno[4,3-c]isoxazol-10(3H)-ones (31 a-e):
Isoxazolines have been described as antimicrobial, protein tyrosine phosphatase IB (PTPIB) inhibitors and anti-inflammatory agents. In view of the several biological activities of isoxazolines, we planned to synthesize fused isoxazolines (31 a-e) at 7,8 positions of coumarin ring.

7-Allyloxy-3,4-substituted-coumarin-8-carbaldehydes (29 a-e) were obtained from the allylation of 3,4-disubstituted-7-hydroxy-8-formyl coumarin (28 a-e) with allyl bromide in the presence of KF/DMF at 60-65 °C for 2-3 h in 60-75% yields. These on reaction with NH$_2$OH.HCl in the presence of NaOAc in methanol at RT to give 7-allyloxy-3,4-substituied-coumarin-8-aldehydeoximes (30 a-e) which on CAN mediated intramolecular 1,3-dipolar cycloaddition gave 8,9-substituted-3a,4-dihydropyrano[2′,3′:5,6]chromeno[4,3-c]isoxazol-10(3H)-ones (31 a-e).

Scheme 4: Synthesis of 8,9-substituted-3a,4-dihydropyrano[2′,3′:5,6]chromeno[4,3-c]isoxazol-10(3H)-ones (31 a-e):

a) $R_1 = H, R_2 = CH_3$   b) $R_1 = H, R_2 = H$   c) $R_1 = H, R_2 = Ph$   d) $R_1 = Br, R_2 = Ph$   e) $R_1 = Cl, R_2 = Ph$
In the $^1$H NMR of 8,9-methyl-3a,4-dihydropyano[2',3':5,6]chromeno[4,3-c]isoxazol-10(3H)-one (31a) the protons of the newly formed fused isoxazoline ring protons appeared at $\delta$ 3.92-4.22 (m, H-3a & 3-CH$_2$), 4.73-4.81 (m, 4-CH$_2$), coumarin protons appeared at 2.43 (s, 8-CH$_3$), 6.23 (s, H-9), 6.92 (d, J=8.8Hz, H-7), 7.55 (d, J=8.8Hz, H-6).

In the $^{13}$C NMR of 31a fused isoxazoline ring carbons appeared at $\delta$ 69.5 (C-3), 45.8 (C-3a), 69.7 (C-4), 158.1 (C-11c), other carbons appeared at 159.9 (10C=O), 152.0 (C-5a), 51.6 (C-8), 148.9 (C-11a), 127.2 (C-7), 114.0 (C-11b), 113.6 (C-6), 112.7 (C-9), 102.7 (C-7a), 18.9 (8-CH$_3$).

In the DIPMS of 31a the quasi-molecular ion peak was observed at m/z 280 (M+Na).

Section C describes synthesis of 8,9-substituted-pyrao[2',3':5,6]chromeno[4,3-c]isoxazol-10(4H)-ones (34 a-e)

Recent reports have described isoxazoles as lysophosphatidic acid (LPA) antagonists, inhibitors of human rhinovirus 2 replication and they also exhibit antitubulin, insect antifeedant activities. In view of the several biological activities of isoxazoles, we planned to synthesize fused isoxazoles (34 a-e) at 7,8 positions of coumarin ring.

7-Propargyloxy-3,4-substituted-coumarin-8-carbaldehydes (32 a-e) were obtained from the O-propargylation of 3,4-substituted-7-hydroxy-8-formyl coumarins (28 a-e) with propargyl bromide in the presence of KF/DMF at 60-65 °C for 2-3 h in 60-75% yields. These are on reaction with NH$_2$OH.HCl in the presence of NaOAc in methanol at RT gave 7-propargyloxy-3,4-substitued-coumarin-aldehydeoximes (33 a-e) which on CAN mediated intramolecular 1,3-dipolar cycloaddition gave 8,9-substituted-3a,4-dihydropyano[2',3':5,6]chromeno[4,3-c]isoxazol-10(3H)-ones (34 a-e).

In the $^1$H NMR of 8-methylpyrano[2',3':5,6]chromeno[4,3-c]isoxazol-10(4H)-one (34a) the proton of the newly formed fused isoxazole ring appeared at $\delta$ 8.31 (s, H-3), 5.35 (s, 4-CH$_2$) and coumarin protons at $\delta$ 2.43 (s, 8-CH$_3$), 6.24 (s, H-9), 6.96 (d, J=8.8Hz, H-7), 7.56 (d, J=8.8Hz, H-6).

In the $^{13}$C NMR of 34a the fused isoxazole ring carbons appeared at $\delta$ 157.7 (C-11c), 150.9 (C-3), 103.2 (C-3a), 61.9 (C-4), coumarin carbons appeared at 159.9 (10-C=O), 152.3 (C-5a), 150.8 (C-8), 150.5 (C-11a), 127.3 (C-7), 114.7 (C-11b), 114.2 (C-7a), 112.8 (C-9), 110.5 (C-6), 18.8 (8-CH$_3$)

In the DIPMS of 34a the quasi-molecular ion peak was observed at m/z 278 (M+Na).
CHAPTER-III:

Synthesis of 4/6/7-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-4-methyl-coumarins (43-47), (50-55), (58-63):

1,2,4-Oxadiazoles have been described as bioisosteres of amide and esters. Furthermore, they have been reported to have agonist activity for cortical muscarinic receptors, 5-HT1D (5-hydroxytryptamine), 5-HT3 and sphingosine-1-phosphate-1 (S1P1) receptors. They also display activity as anti-inflammatory and anti-tumor agents, anti-diabetic, anti-asthmatic and growth hormone secretagogues. They exhibit signal transduction, monoamine oxidase, cell adhesion and tryptase inhibitor properties. Furthermore, they show activity against several breast and colorectal cancer cell lines, human neutrophil elastase and human DNA topoisomerases. In view of the several biological activities of 1,2,4-oxadiazoles, we plan to synthesize new 4/6/7-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-coumarins (43-47), (50-55), (58-63). 4,6,7 substituted coumarins (35, 48 and 56) were prepared from Pechmann condensation, these are alkylated with ethylbromoacetate to give esters (36, 49 and 57), which are reacted with amide oximes (37-42) in the presence of K$_2$CO$_3$ to afford 4/6/7-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-coumarins (43-47), (50-55), (58-63).

3.1. Synthesis of 4-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-coumarins (43-47):

4-Hydroxy coumarin (35) on alkylation with ethylbromoacetate in the presence of K$_2$CO$_3$ in DMF gave ethyl-2-(coumarin-4-yloxy)-acetate (36) which on further reaction with different amide oximes (37-42) gave 4-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-coumarins (43-47).
Scheme 6: Synthesis of 4-[(3-ary-1,2,4-oxadiazol-5-yl)methoxy]-coumarins (43-47):

37, 43) Ar = Benzyl
38, 44) Ar = Phenyl
39, 45) Ar = o-Methoxy phenyl
40, 46) Ar = o-Benzylxy phenyl
41, 47) Ar =

In the $^1$H NMR of 4-[(3-Benzyl-1,2,4-oxadiazol-5-yl)methoxy]-coumarin (43) the 4-OCH$_2$ appeared at $\delta$ 5.38 (s), 3’-CH$_2$ at 4.14 (s) and other protons appeared at 5.77 (s, H-3), 7.29-7.33 (m, H-6 & H-8), 7.33-7.35 (m, H-2” to H-6”), 7.57-7.61 (m, H-7), 7.85 (dd, J=1.6Hz, J=1.6Hz, H-5).

In the $^{13}$C NMR of 43 the newly formed oxadiazole ring carbons appeared at $\delta$ 170.0 (C-3’), 164.3 (C-5’). Other carbons appeared at 162.0 (2-C=O), 153.2 (C-8a), 134.8 (C-1”), 132.9 (C-2”), 129.0 (C-6”), 128.9 (C-3”), 128.8 (C-5”), 128.7 (C-7), 127.4 (C-4”), 124.2 (C-6), 123.2 (C-5), 116.8 (C-4a), 116.7 (C-8), 91.6 (C-3), 61.2 (4-OCH$_2$), 32.2 (3’-CH$_2$).

In the DIPMS of 43 the quasi-molecular ion peak was observed at $m/z$ 357 (M+Na).

3.2. Synthesis of 6-[(3-ary-1,2,4-oxadiazol-5-yl)methoxy]-4-methyl-coumarins (50-55):
6-[(3-Aryl-1,2,4-oxadiazol-5-yl)methoxy]-4-methyl-coumarins (50-55) were synthesized from the reaction of ethyl-2-(4-methyl-coumarin-6-yl oxy)-acetate (49) with amide oximes (37-42) in the presence of K$_2$CO$_3$, toluene at reflux temperature for 48 h.
Scheme 7: Synthesis of 6-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-4-methyl-coumarins (50-55):

In the $^1$H NMR of 6-[(3-Benzyl-1,2,4-oxadiazol-5-yl)methoxy]-4-methyl-coumarin (50) 6-OCH$_2$ protons appeared at $\delta$ 5.29 (s), 3'-CH$_2$ protons at 4.11 (s) other protons appeared at 6.33 (s, H-3), 7.13 (dd, J=3.2Hz, J=2.8Hz, H-7 & H-8), 7.27 (s, H-5), 7.31 (m, H-2" to H-6").

In the $^{13}$C NMR of 50 the newly formed oxadiazole ring carbons appeared at $\delta$ 174.3 (C-3'), 169.7 (C-5'). Other carbons appeared at 160.6 (2-C=O), 153.7 (C-6), 151.7 (C-4), 148.8 (C-8a), 135.0 (C-1''), 128.9 (C-2''), 128.8 (C-6''), 127.3 (C-4''), 120.6 (C-8), 119.3 (C-4a), 118.8 (C-3''), 118.2 (C-7), 118.0 (C-5''), 115.8 (C-3), 109.7 (C-5), 61.7 (-OCH$_2$), 32.2 (3'-CH$_2$), 18.6 (4-CH$_3$).

In the DIPMS of 50 the quasi-molecular ion peak was observed at $m/z$ 348 (M+Na).

3.3. Synthesis of 7-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-4-methyl-coumarins (58-63):
7-[(3-Aryl-1,2,4-oxadiazol-5-yl)methoxy]-4-phenyl-coumarins (58-63) were synthesized from the reaction of ethyl-2-(4-phenyl-coumarin-7-yloxy)-acetate (57) with amide oximes (37-42) in the presence of K$_2$CO$_3$, toluene at reflux temperature for 24 h.
Scheme 8: Synthesis of 7-[(3-aryl-1,2,4-oxadiazol-5-yl)methoxy]-4-methyl-coumarins (58-63):

In the $^1$H NMR of 7-[(3-Benzyl-1,2,4-oxadiazol-5-yl)methoxy]-4-phenyl-coumarin (58) the 7-OCH$_2$, 3'-CH$_2$ appeared at $\delta$ 5.33 (s), 4.11 (s) and other protons at 6.26 (s, H-3), 6.85 (dd, J=1.4Hz, J=0.4Hz, H-8), 6.96 (d, J=8.6Hz, H-6), 7.31-7.33 (m, Ar-H), 7.41-7.44 (m, Ar-H), 7.51 (d, J=2Hz, H-5).

In the $^{13}$C NMR of 58 the newly formed oxadiazole ring carbons appeared at 173.8 (C-3'), 169.8 (C-5') and other carbons at 167.9 (2-C=O), 160.9 (C-7), 160.7 (C-4), 160.2 (C-8a), 155.7 (C-1''), 155.5 (C-1''), 135.2 (C-2''), 134.9 (C-6''), 129.7 (C-2'''), 129.0 (C-3'''), 128.9 (C-5'''), 128.8 (C-6'''), 128.4 (C-3''), 127.3 (C-4'''), 113.8 (C-5), 112.8 (C-4''), 112.5 (C-3), 112.4 (C-4a), 102.3 (C-6), 101.9 (C-8a), 61.1 (7-OCH$_2$), 32.2 (3'-CH$_2$).

In the DIPMS of 58 the quasi-molecular ion peak was observed at $m/z$ 410.6 (M+Na).

**CHAPTER-IV:**

Synthesis of N-benzyloxy-2-(4-H/methyl-coumarin-4/6/7-yloxy)-acetamides (91-96), (98-103), (105-110):

Recent reports suggest that N-benzyloxy benzamide and its analogs with the O-benzylhydroxylamine moiety (-CONHOCH$_2$-Ar) possess antibacterial, herbicidal and enzyme inhibiting activities. The pharmacophore (-CONHOCH$_2$-) was generally
considered to be the bioisosteric analog of \((-\text{CONHCH}_2\text{CH}_2-)\) in drug design. Considering the potential antifungal activity of the coumarin derivatives and the fungicidal activity contribution of the O-benzylhydroxylamine group to the N-(benzyloxy)benzamide derivatives, a series of new O-alkyl hydroxamic acid analogues were designed, synthesized and tested against various fungi \textit{in vitro}.

Objective of the present work is to develop O-alkyl hydroxamic acid analogues of coumarin 4,6,7-yloxyacetic acids. O-alkylated esters (64-66) on base hydrolysis gave substituted coumarin 4,6,7-yloxyacetic acids (67-69). Substituted benzylhalides (71-76) reacted with N-hydroxy phthalimide (70) and subsequent reaction with hydrazine hydrate to give o-substituted benzyl-hydroxylamines (78-83). Coumarin-4,6,7-yloxyacetic acids (67-69) coupled with O-substituted benzyl-hydroxylamines (78-83) via acid chloride method to achieve a series of N-benzyloxy-2-(4-H/Methyl-coumarin-4/6/7-yloxy)-acetamides (91-96), (98-103), (105-110).

\textbf{4.1. Synthesis of coumarin-4,6,7-yloxyacetic acids (67-69):}

Coumarin-4,6,7-yloxyacetic acids (67-69) were synthesized from the base hydrolysis of esters (64-66) with sodium hydroxide in ethylalcohol at RT.

\textit{Scheme 9: Synthesis of coumarin-4,6,7-yloxyacetic acids (67-69):}

\[
\begin{array}{c}
\text{64-66} \quad \xrightarrow{\text{NaOH, Ethanol}} \quad \text{67-69} \\
R_1 \quad R_2 \quad R_3 \quad \text{CO} \\
\end{array}
\]

64) R\_1 = \text{OCH}_2\text{COOEt}, R\_2 = R\_3 = \text{H} \quad \Rightarrow \quad 67) R\_1 = \text{OCH}_2\text{COOH}, R\_2 = R\_3 = \text{H}  \\
65) R\_2 = \text{OCH}_2\text{COOEt}, R\_1 = \text{CH}_3, R\_3 = \text{H} \quad \Rightarrow \quad 68) R\_2 = \text{OCH}_2\text{COOH}, R\_1 = \text{CH}_3, R\_3 = \text{H}  \\
66) R\_3 = \text{OCH}_2\text{COOEt}, R\_1 = \text{CH}_3, R\_2 = \text{H} \quad \Rightarrow \quad 69) R\_3 = \text{OCH}_2\text{COOH}, R\_1 = \text{CH}_3, R\_2 = \text{H}

2-(4-Methyl-coumarin-7-yloxy)-acetic acid (69) is characterized from its spectral data. In its \(^1\text{H NMR} \) spectrum (CDCl\(_3\), 400 MHz) the 4-\text{CH}_3, 7-\text{OCH}_2 \) appeared at \( \delta 2.39 \) (s), 4.84 (s) and coumarin ring protons at 6.22 (s, H-3), 6.96 (s, H-8), 6.96 (d, J=8Hz, H-6), 7.68 (d, J=8.8Hz, H-5).
4.2. Synthesis of O-(substituted benzyl)-hydroxylamines (78-83):

N-Hydroxy phthalimide (70) was alkylated with different substituted benzylhalides (71-76) in the presence of K₂CO₃ and DMF at RT. These are reacted with hydrazine hydrate in ethanol at reflux temperature to give O-(substituted benzyl)-hydroxylamines (78-83).

Scheme 10: Synthesis of O-(substituted benzyl)-hydroxylamines (78-83):

O-(4-Fluorobenzyl)-hydroxylamine (78) is characterized from its spectral data. In its 
¹H NMR (CDCl₃, 400 MHz), the -NH₂ & -CH₂ appeared at δ 5.4 (bs), 4.6 (s) and aromatic protons at 7.1 (d, J=8Hz, H-3 & H-5), 7.3 (d, J=7.5Hz, H-2 & H-6)

4.3. Synthesis of N-(benzyloxy)-2-(4-H/methyl-coumarin-4/6/7-yloxy)-acetamides:

4.3.1 Synthesis of N-(benzyloxy)-2-(coumarin-4-yloxy)-acetamides (84-89):

N-Benzzyloxy-2-(coumarin-4-yloxy)-acetamides (84-89) were synthesized from the amide coupling of 2-(coumarin-4-yloxy)-acetic acid (67) with O-substituted benzylhydroxylamines (78-83) via acid chloride method at RT. These were purified by acid-base workup and characterized from their spectra.
Scheme 11: Synthesis of N-(benzyloxy)-2-(coumarin-4-yloxy)-acetamides (84-89):

In the $^1$H NMR of N-(4-fluorobenzyloxy)-2-(coumarin-4-yloxy)-acetamide (84) the newly formed amide proton appeared at 11.59 (s, O=C-NH); the 4-OCH$_2$ & 1'-OCH$_2$ at 4.79 (s), 4.85 (s) and other protons at 5.86 (s, H-3), 7.19-7.24 (m, H-3' & H-5), 7.38-7.5 (m, H-2', H-3' & H-6, H-8), 7.67-7.69 (m, H-7), 7.96 (d, J=9.2Hz, H-5).

In the $^{13}$C NMR of 84 the newly formed amide carbon appeared at 163.5 (O=C-NH) and other carbons at 164.7 (C-4), 161.8 (2-C=O), 153.1 (C-8a), 133.3 (C-1'), 132.3 (C-3' & C-5'), 131.7 (C-7), 124.8 (C-4'), 123.9 (C-2' & C-6'), 116.8 (C-6), 115.7 (C-5), 115.3 (C-4a), 115.3 (C-8), 91.7 (C-3), 76.7 (1'-CH$_2$), 66.8 (4-OCH$_2$).

In the DIPMS of 84 showed the quasi-molecular ion peak at m/z 344 (M+1).

4.3.2 Synthesis of N-(benzyloxy)-2-(4-methyl-coumarin-6-yloxy)-acetamides (90-95):
N-(Benzyloxy)-2-(4-methyl-coumarin-6-yloxy)-acetamides (90-95) were synthesized from the amide coupling of 2-(4-methyl-coumarin-6-yloxy)-acetic acid (68) with O-substituted benzylhydroxylamines (78-83) via acid chloride method at RT. These were purified by acid-base workup and characterized from their spectra.
Scheme 12: Synthesis of N-(benzyloxy)-2-(4-methyl-coumarin-6-yloxy)-acetamides (90-95):

![Scheme Image]

In the $^1$H NMR of N-(4-fluorobenzyloxy)-2-(4-methyl-coumarin-6-yloxy)-acetamide (90), the newly formed amide proton appeared at 9.15 (s, O=C-NH) and the 4-CH$_3$, 6-OCH$_2$ & 1'-OCH$_2$ at 2.38 (s), 4.61 (s) & 4.95 (s). Other protons appeared at 6.3 (s, H-3), 7.01-7.06 (m, H-5 & H-7, H-3' & H-5'), 7.24 (d, J=1.6Hz, H-2'), 7.37 (m, H-8 & H-6').

In the $^{13}$C NMR of 90 the newly formed amide carbon appeared at 164.9 (O=C-NH), other carbons at 161.3 (C-4'), 160.2 (2-C=O), 154.3 (C-6), 153.2 (C-4), 148.1 (C-8a), 132.4 (C-7), 131.5 (C-2' & C-6'), 125.0 (8), 120.0 (C-4a), 117.9 (C-3' & C-5'), 115.6 (C-7), 114.3 (C-3), 109.9 (C-5), 91.7 (1'-CH$_2$), 76.7 (6-OCH$_2$), 66.8 (4-CH$_3$).

In the DIPMS of 90 showed the quasi-molecular ion peak at m/z 358.4 (M+1).

4.3.3 Synthesis of N-(benzyloxy)-2-(4-methyl-coumarin-7-yloxy)-acetamides (96-101):
N-(Benzyloxy)-2-(4-methyl-coumarin-7-yloxy)-acetamides (96-101) were synthesized from the amide coupling of 2-(4-methyl-coumarin-7-yloxy)-acetic acid (69) with O-substituted benzylhydroxylamines (78-83) via acid chloride method at RT. These were purified by acid-base workup and characterized from their spectra.
Scheme 13: Synthesis of N-(benzyloxy)-2-(4-methyl-coumarin-7-yloxy)-acetamides (96-101):

In the $^1$H NMR of N-(4-fluorobenzyloxy)-2-(4-methyl-coumarin-7-yloxy)-acetamide (96), the newly formed amide proton appeared at 9.13 (s, O=\text{C-NH}) and the 4-CH$_3$, 7-OCH$_2$ & 1'-OCH$_2$ at 2.4 (s), 4.62 (s) & 4.95 (s). Other protons appeared at 6.17 (s, H-3), 6.81 (s, H-8), 6.83 (d, J=2.4Hz, H-6), 7.01-7.05 (m, H-3' & H-5'), 7.37-7.4 (m, H-2' & H-6'), 7.5 (d, J=8.8Hz, H-5).

In the $^{13}$C NMR of 96 the newly formed amide carbon appeared at 164.6 (O=\text{C-NH}) and the other carbons at 161.3 (C-4'), 161.0 (C-7), 160.4 (2-C=O), 154.9 (C-8a), 153.7 (C-4), 131.6 (C-1'), 131.5 (C-2' & C-6'), 126.9 (C-5), 115.6 (C-3' & C-5'), 114.4 (C-3), 112.8 (C-4a), 111.9 (C-6), 102.0 (C-8), 91.7 (1'-CH$_2$), 76.7 (7-OCH$_2$), 66.8 (4-CH$_3$).

In the DIPMS of 96 showed the quasi-molecular ion peak at m/z 358 (M+1).

CHAPTER-V:

Betulinic acid (104) (3β-hydroxy-lup-20(29)-en-28-oic acid) is a naturally occurring pentacyclic lupine-type triterpenoid which exhibits a variety of biological and medicinal properties such as inhibition of human immunodeficiency virus (HIV), anti-bacterial, anti-malarial, anti-inflammatory, anthelmintic, anti-HSV-1 and anti-cancer activities. The molecule offers several hot spots (e.g. C-3, C-20 and C-28) to provide enough space for chemical modification.
SECTION A: Synthesis of C-28 modified 1,2,4-oxadiazole esters of betulinic acid (105-112):

1,2,4 oxadiazole esters of betulinic acid (105-112) were synthesized from the reaction of betulinic acid (104) with 5-(bromomethyl)-3-aryl-1,2,4-oxadiazoles (103 a-h) in the presence of K$_2$CO$_3$ in DMF.

5.1. Synthesis of 5-(bromomethyl)-3-aryl-1,2,4-oxadiazoles (103 a-h):

5-(Bromomethyl)-3-aryl-1,2,4-oxadiazoles (103 a-h) were synthesized from the reaction of different substituted amide oximes (102 a-h) with bromoacetyl bromide and immediate cyclisation reaction in toluene at reflux temperature.

*Scheme 14: Synthesis of 5-(bromomethyl)-3-aryl-1,2,4-oxadiazoles (103 a-h):*

![Scheme 14](image)

- a) Ar = 4-Methyl phenyl
- b) Ar = 4-Methoxy phenyl
- c) Ar = Phenyl
- d) Ar = 2-Methoxy phenyl
- e) Ar = 2-Benzyl
- f) Ar = 2-Benzoyloxy phenyl
- g) Ar = Phenyl
- h) Ar = Benzyl

In $^1$H NMR of 5-(bromomethyl)-3-p-tolyl-1,2,4-oxadiazole (103a), the 4'-CH$_3$, 5-CH$_2$ appeared at δ 2.41 (s), 4.54 (s) and aromatic protons at 7.28 (d, J=7.8Hz, H-2' & H-6'), 7.97 (d, J=8.4Hz, H-3' & H-5').

5.2. Synthesis of C-28 modified 1,2,4-oxadiazole esters of betulinic acid (105-112):

1,2,4-oxadiazole esters of betulinic acid (105-112) were synthesized from the reaction of betulinic acid (104) with 5-(bromomethyl)-3-aryl-1,2,4-oxadiazoles (103 a-h) in the presence of K$_2$CO$_3$/DMF.
Scheme 15: Synthesis of C-28 modified 1,2,4-oxadiazole esters of betulinic acid (105-112):

103a, 105) Ar = 4-Methyl phenyl
103b, 106) Ar = 4-Methoxy phenyl
103c, 107) Ar = 4-Cyano pyridyl
103d, 108) Ar = 2-Methoxy phenyl
103e, 109) Ar = 2-Benzyl ether phenyl
103f, 110) Ar = 3-Cyano pyridyl
103g, 111) Ar = Phenyl
103h, 112) Ar = Benzyl

In the $^1$H NMR of 105, the 5'-CH$_2$, 4''-CH$_3$ appeared at δ 5.31-5.39 (m), 2.42 (s), and aromatic protons at 7.27 (d, J=8Hz, H-2'' & H-6''), 7.94 (d, J=8Hz, H-3'' & H-5''). Betulinic acid protons appeared at δ 0.74, 0.77, 0.85 (all s, each 3H, 24-CH$_3$, 25-CH$_3$, 26-CH$_3$), 0.97 (s, 23-CH$_3$, 27-CH$_3$), 1.68 (s, 30-CH$_3$), 0.69-2.28 (all m, remaining protons), 3.01 (m, H-19), 3.17 (dd, J=9.1Hz, 4.3Hz, H-3), 4.58 (d, J=6.4Hz, H-29α), 4.73 (d, J=1.6Hz, H-29β).

In the $^{13}$C NMR 105 the newly formed ester carbonyl and oxadiazole carbons appeared at 175.0 (-COOCH$_3$), 174.3 (C-3''), 168.4 (C-5'') and olefin carbons at 150.2 (C-20), 109.7 (C-29). Aromatic carbons appeared at 141.7 (C-4''), 129.5 (C-3'' & C-5''), 127.4 (C-2'' & C-6''), 123.5 (C-1''). Betulinic acid carbons appeared at 78.9 (C-3), 64.2 (5'-CH$_2$), 56.82 (C-17), 56.0 (C-5), 55.3 (C-9), 50.5 (C-18), 49.5 (C-19), 46.7 (C-14), 42.4 (C-8), 40.7 (C-4), 38.8 (C-13), 38.7 (C-10), 38.1 (C-1), 37.1 (C-13), 36.8 (C-22), 34.3 (C-7), 31.9 (C-16), 30.4 (C-21), 29.6 (C-15), 29.5 (C-2), 29.0 (C-12), 27.9 (C-23), 27.4 (C-24), 27.2 (C-11), 25.4 (C-30), 21.5 (4''-CH$_3$), 20.8 (C-6), 19.3 (C-26), 18.2 (C-25), 16.1 (C-27).

In the DIPMS of 105 showed the quasi-molecular ion peak at m/z 630 (M+1).
SECTION B: Synthesis of C-28 modified 1,2,4-oxadiazole amides of betulinic acid (118-124):

1,2,4-oxadiazole amides of betulinic acid (118-124) were synthesized from the amide coupling reaction of 3-O-acetyl betulinic acid (113) with (3-aryl-1,2,4-oxadiazol-5-yl)methanamines (117 a-g).

5.3. Synthesis of 3-O-acetyl betulinic acid (113):

Betulinic acid (104) is treated with acetic anhydride in the presence of pyridine at RT for overnight to yield 3-O-acetyl betulinic acid (113).

Scheme 16: Synthesis of 3-O-acetyl betulinic acid (113):

In $^1$H NMR of 3-O-acetyl betulinic acid (113), acetyl protons appeared at δ 2.21 (s, -OCOCH$_3$) and betulinic acid protons appeared at 0.84, 0.85, 0.86, 0.94, 0.98, 1.7 (each 3H, s, 6×CH$_3$), 3.01-3.05 (m), 4.48 (dd, J=5.5Hz, 10.5Hz), 4.62 and 4.75 (each 1H, br s).

5.4. Synthesis of (3-aryl-1,2,4-oxadiazol-5-yl)-methanamines (117 a-g):

Substituted amide oximes (114 a-g) were synthesized from the reaction of different nitriles with NH$_2$OH.HCl in the presence of Et$_3$N in ethanol. 5-(Chloromethyl)-3-aryl-1,2,4-oxadiazoles (115 a-g) were synthesized from the reaction of different substituted amide oximes (114 a-g) with chloroacetyl chloride and immediate cyclisation reaction in toluene. (3-Aryl-1,2,4-oxadiazol-5-yl)-methanamines (117 a-g) were synthesized from the alkylation of potassium phthalimide (116) with 5-(chloromethyl)-3-aryl-1,2,4-oxadiazoles (115 a-g) in DMF followed by the reaction with NH$_2$.NH$_2$.H$_2$O in ethylalcohol.
Scheme 17: Synthesis of (3-aryl-1,2,4-oxadiazol-5-yl)-methanamines (117 a-g):

a) Ar = 4-Methyl phenyl  
b) Ar = 4-Methoxy phenyl  
c) Ar = 4-Fluoro phenyl  
d) Ar = 2-Methoxy phenyl  
e) Ar = 2-Chloro phenyl  
f) Ar = Phenyl  
g) Ar = Benzyl

In $^1$H NMR of (3-p-Tolyl-1,2,4-oxadiazole-5-yl)-methanamine (117a) 4'-CH$_3$, 5-CH$_2$ protons appeared at δ 2.4 (s), 4.13 (s) and aromatic protons at 7.27 (d, J=6.8Hz, H-2' & H-6'), 7.95 (d, J=8.4Hz, H-3' & H-5').

5.5 Synthesis of C-28 modified 1,2,4-oxadiazole amides of betulinic acid (118-124):

1,2,4-oxadiazole amides of betulinic acid (118-124) were synthesized from the amide coupling reaction of 3-O-acetyl betulinic acid (113) with (3-aryl-1,2,4-oxadiazol-5-yl)-methanamine (117 a-g) via acid chloride method.

Scheme 18: Synthesis of C-28 modified 1,2,4-oxadiazole amides of betulinic acid (118-124):

117a, 118) Ar = 4-Methyl phenyl  
117b, 119) Ar = 4-Methoxy phenyl  
117c, 120) Ar = 4-Fluoro phenyl  
117d, 121) Ar = 2-Methoxy phenyl  
117e, 122) Ar = 2-Chloro phenyl  
117f, 123) Ar = Phenyl  
117g, 124) Ar = Benzyl
In the $^1$H NMR of 118 newly formed -CONH proton appeared at 6.33 (t); H-3, 5' CH$_2$ and olefin protons appeared at 4.52 (t), 4.69 (dd, J=5.6Hz), 4.76 (dd, J=5.6Hz), 4.61 (dd, J=0.4Hz, H-29α), 4.75 (d, J=1.6Hz, H-29β), aromatic protons at 7.28 (d, J=8Hz, H-3'' & H-5''), 7.94 (d, J=8Hz, H-2'' & H-6''). Betulinic acid protons appeared at δ 0.8, 0.83, 0.85, 0.86, 0.98, 1.7, 2.05 (all s, each 3H, 24-CH$_3$, 25-CH$_3$, 26-CH$_3$, 23-CH$_3$, 27-CH$_3$, 30-CH$_3$, -COOCH$_3$), 0.69-2.28 (all m, remaining protons), 2.43 (s, 4''-CH$_3$), 3.08-3.11 (m, H-19).

In the $^{13}$C NMR of 118 the newly formed amide and oxadiazole carbons appeared at δ 176.6 (-CONH), 171.0 (C-3'), 168.3 (C-5'), olefin carbons and -COOCH$_3$ at 150.6 (C-20), 109.5 (C-29), 176.5 (3-COOCH$_3$). Aromatic carbons appeared at 141.6 (C-4''), 129.5 (C-3'' & C-5''), 127.4 (C-2'' & C-6''), 123.7(C-1''). Betulinic acid carbons appeared at δ 14.5 (C-27), 16.0 (C-25), 16.1 (C-26), 16.4 (C-6), 18.1 (4''-CH$_3$), 19.4 (-COOCH$_3$), 20.8 (C-30), 21.3 (C-11), 21.5 (C-23), 23.6 (C-24), 25.5 (C-2), 27.9 (C-12), 29.4 (C-15), 30.7 (C-21), 33.6 (C-16), 34.2 (C-22), 35.7 (C-7), 37.1 (C-4), 37.6 (C-10), 37.7 (C-13), 38.1 (C-1), 38.3 (C-8), 40.7 (C-14), 42.5 (5'-CH$_2$), 46.6 (C-19), 50.1 (C-18), 50.5 (C-17), 55.4 (C-9), 55.9 (C-5), 81.1 (C-3).

In the DIPMS of 118 showed the quasi-molecular ion peak at m/z 692.6 (M+Na).

**CHAPTER-VI:**

**Section-A:**

**6.0. Eli Lilly-Open Innovation Drug Discovery Programme:**

As part of the collaborative drug discovery programme between Osmania University and Eli Lilly, sixty eight analogues were submitted for Eli Lilly-Open Innovation Drug Discovery programme. Out of which forty analogues were accepted for biological screening after cheminformatics filter studies. Compounds are screened for anti cancer, CGRP Receptor Antagonists, G protein coupled receptors, HK2 Inhibitors, Apelin inhibitors, GLP 1 receptor antagonists, GPR 119 Receptor Agonists, EZH2 inhibitors, Tuberculosis activity. Some of the compounds showed moderate activity. Most of the molecules are in various stages of *in vitro* screening for further studies. Screening results of the molecules in nine different therapeutic areas are mentioned in Chapter VI.
Section-B:
6.1. Evaluation of antibacterial, antifungal and cytotoxicity of heterocycle substituted comarins and O-alkyl hydroxamic acid analogues of coumarin:
Natural, synthetic heterocyclic compounds and semi synthetic analogues are reported to have anticancer, anti-inflammatory, antileukemic, antiviral, antibacterial, antifungal and MAO & HDAC inhibitors. Therefore it is considered to screen representative compounds obtained in the present study for their antibacterial, antifungal and cytotoxicity.

6.2. Antibacterial activity and antifungal activity:
Among the synthetic heterocycles obtained, fifty three compounds were tested for antibacterial activity by using ampicillin as standard. Among the compounds tested, coumarin derivatives 25c, 26d, 31b, 34b, 34d, 44, 45, 46, 88, 97, 101 showed moderate inhibition towards Escheria Coli. 25d, 26c, 31a, 34d, 46, 50, 53, 61, 85, 88, 89, 94, 101 showed inhibition towards against Staphylococcus aureus.

Among the fifty three compounds tested for antifungal activity by using clotrimazole as reference, among the compounds tested, coumarin derivatives 25d, 26a, 31c, 34c, 46, 53, 55, 61, 63, 87, 88, 95, 101 showed moderate inhibition towards Aspergillus niger 25d, 26c, 31c, 34c, 45, 46, 53, 54, 61, 62, 87, 88, 94, 95, 100 showed inhibition towards against Rhizoctonia solani.

Section-C:
6.3. Cytotoxicity of semisynthetic analogues of betulinic acid:
Out of 15 oxadiazole derivatives of betulinic acid, 8 analogues were screened for their cytotoxicity by MTT assay method using betulinic acid (104) as internal standard tested for their cytotoxicity, four compounds 105, 107, 120, 121 displayed better and low cytotoxic activities against the tested cells than betulinic acid.