Synthesis, spectral characterizations and molecular docking studies of Montelukast Methyl Ketone, Lacosamide derivatives as promising antibacterial agents and antiviral activity towards New Castle Disease Virus (NDV) using Embryonated Chicken Eggs Assay (ECEA)

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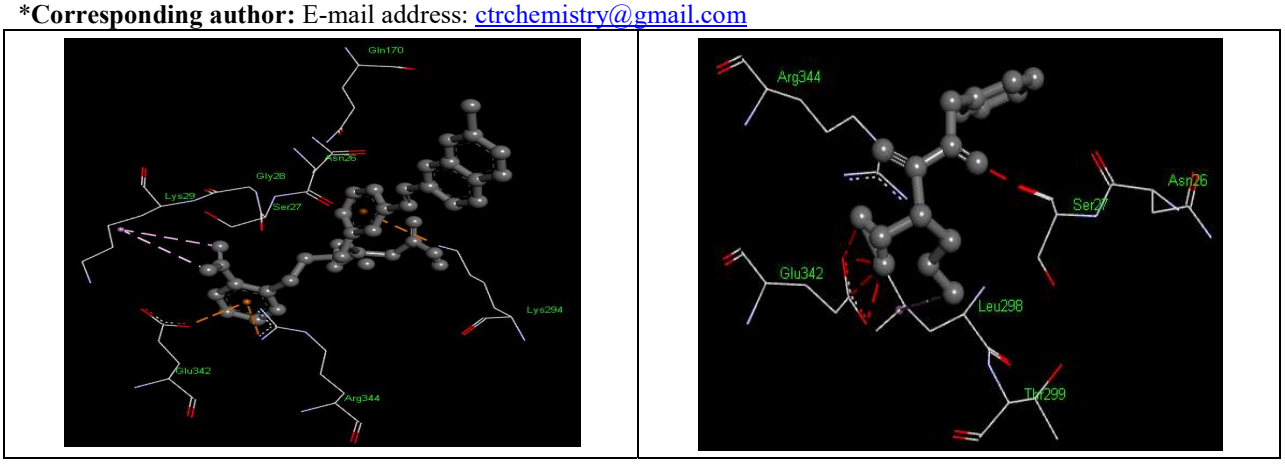

ABSTRACT:

In the present study, we report the synthesis of montelukast methyl ketone, lacosamide derivatives. The scalable procedure for the generation of montelukast (Singulair, drug for asthma) based on a novel and useful way of performing the key substitution reaction has been presented. The present approach is recognized from the prior solutions in the application of inexpensive and accessible materials, which ensures very simple and economic production method. Our suggested approach for the production of montelukast is capable to minimize an amount of impurities and lets the efficient production of montelukast and its scale-up. We retrospectively studied the efficacy and tolerability of lacosamide (LCM) in children with drug resistant epilepsy in a tertiary care centre in the Netherlands, from 2013 till 2019, with a follow-up of two years. 79 children, aged < 18 years, were included. Retention rate, effectiveness, reason for termination and side-effects were analyzed. Furthermore, prognostic variables for discontinuation as well as the incidence of side-effects were determined. The LCM retention rate and effectiveness of response were analyzed at three, twelve and twenty-four months. Lacosamide (LCM) is a new generation antiepileptic drug that affects the slow inactivation of voltage-gated sodium channels. We studied whether chronic LCM treatment prior to onset of absence seizures was able to prevent/reduce the development of absence seizures in GAERS rats, a well-validated animal model of absence epilepsy and epilepto genesis. Newcastle disease is highly infectious viral disease causing huge economic losses worldwide. These losses can be prevented by control of viral diseases. Medicinal plants have been traditionally used for treatment of different diseases since long. In this study the effect of from montelukast methyl ketone are investigated against Newcastle disease virus (NDV) by an *in-vivo* assay. Seven groups of nine-day-old embryo nated chicken eggs were inoculated with various treatments of different concentration. All the groups except uninoculated negative control group were inoculated with velogenic NDV strain; five groups received different concentrations. Daily observe the rate of embryo survival. Allantoic fluid from treated eggs was collected for hemagglutination test. Results showed that embryo survival rate was higher 300µg/ml treated antiviral activity. The synthesized heterocyclic compounds were evaluated for their *in vitro* antibacterial activity against pathogenic strains of both gram negative and gram positive bacteria. The **3a** derivative displayed superior antibacterial activity against *E. coli*, *Pseudomonas aeruginosa* in comparison to the reference drug. The molecular docking studies indicate that the montelukast methyl ketone **(3a)**, lacosamide derivative **(3b)** binds to the cell wall protein of *P. aeruginosa* and *E. coli by* formation of amino acid residues (GLU342, LEU398) of the bacterial cell wall protein. The **3b** derivatives were antiviral activity towards New Castle Disease Virus (NDV). All the products were thoroughly characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR, Mass spectral and CHN analysis. All the products were found to have good oral bioavailability.

KEYWORDS: Montelukast, THF, Antibacterial Activity, Antiviral Activity (New Castle Disease Virus (NDV)), Molecular docking studies and Spectral Characterization.

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1. INTRODUCTION:

Montelukast is a famous drug demonstrated for the chronic and prophylaxis therapy of asthma. It behaves as a selective antagonist of the leukotriene D4 receptor which causes the decrease of broncho constriction and eventuates in less inflammation. Montelukast is prescribed orally once daily which gives a profit in contrast with other drugs for pulmonary diseases such as asthma[1-4]. The sodium salt of montelukast characterized with formula is utilized for asthma therapy. The endeavors of merck frosst, causing to the finding and probable commercialization of Montelukast (at once $4.5 billion-a-year drug before the expiration of its patent in 2012), has frequently been indicated as a "case study in novel drug development and discovery"[5]. Montelukast is identified to be prone to multiple kinds of degradation. It is usually the instance of the following chemical transformation: dehydration at the *tert*-alcohol group, producing the corresponding olefin, dehalogenation reaction, photoisomerisation at the site of the double bond from geometry (*E*) to (*Z*), oxidation of the mercaptor group to the sulfoxide. The chemical impurities are mainly taken away by using crystallization in the phase of Montelukast acid or in the phase of montelukast salts with amines [6-15]. Lacos amide, one of the latest anti-epileptic drugs, is a functionalized amino acid with the chemical name (R) -2-acetyl-n-benzyl-3-methoxyacrylamide, which was widely used in adult and childhood epilepsy[16-21]. They function by selectively enhancing the slow inactivation of voltage-gated sodium channels without affecting fast inactivation; in this way, they reduce the excitability of pathological neurons without affecting physiological neuronal function[22]. Meanwhile, it appears to bind to collaps in response mediator protein 2 (CRMP-2) involved in neuro trophic signaling. This is hypothesized to produce a neuro protective effect preventing the formation of abnormal neuronal connections in the brain[23]. In addition, Ruffolo G et al[24] have recently found that lacos amide also acts on the GABA receptor; this mechanism is related to its ability to prevent the evolution of status epilepticus. Current anti-seizure treatments are effective in 70-80 % of children diagnosed with focal or generalized epilepsy; the remaining 20-30% still experience epileptic seizures[25]. Moreover, antiseizure medications (ASM) are known to induce major side effects, like dizziness, drowsiness, and mental slowing, leading to discontinuation of long-term treatments and reduced quality of life[26]. There is, therefore, a need for new ASM to treat drug resistant epilepsy. Lacosamide (LCM) has recently (2017) been approved for pediatric use[27,28]. By slowly enhancing the inactivation of voltage dependent sodium channels, it reduces neuronal over activity[29,30]. More recently we have reported the synthesis, spectral characterization, molecular docking, DFT studies and biological evaluation of N-(2-oxo-2-(phenylamino)ethyl)picolinamide derivatives as anti-inflammatory and antidiabetic activity[31]. More recently we have reported the synthesis, spectral characterization and biological evaluation of adamentanecarboxamides compounds[32]. Hence, in the present investigation, we report the synthesis, spectral characterizations and molecular docking studies of Montelukast Methyl Ketone, Lacosamide derivatives as promising antibacterial agents and antiviral activity towards New Castle Disease Virus (NDV) using Embryonated Chicken Eggs Assay (ECEA).

2. MATERIAL AND METHODS:

2.1. Experimental

All reagents are of analytical grade and were procured from sigma Aldrich. The glass substrates were soaked overnight in Jones reagent, washed with distilled water, cleaned ultrasonically first in acetone (3-4 minutes) followed by methanol and finally dried under vacuum oven at 100°C. ^{1}H-NMR spectra were recorded on 400MHz in DMSO-d_6 or $CDCl_3$ chemical shifts are given in δ value relative to the internal standard TMS. ^{13}C-NMR spectra were recorded on 100MHz in DMSO-d_6 or $CDCl_3$ and the chemical shifts are given in δ relative to the solvent. FT-IR of all the samples was recorded on a Nicolet Impact-400 spectrophotometer using KBr pellet technique. Mass spectra were recorded on a Varian VG 70-70H mass spectrometer. The identification of products was also made using GC-MS (Hewlett Packard, HP-5890) gas chromatography with a flame ionization detector (SB 30 packed column) equipped with a mass spectrometer, with helium as carrier gas at a flow rate of (1 ml/min). Analytical thin layer chromatography was performed on precoated sheets of silica gel of 0.25mm thickness containing PF 254 indicator (Merck, Darmstadt). Column

chromatography was performed with silica gel (60-120 mesh; SD Fine).

2.1.1. Typical Experimental Procedure For (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)propyl)thio)methyl)cyclopropyl)acetic acid

A mixture of (E)-methyl 2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(((1-(2-methoxy-2-oxoethyl)cyclopropyl)methyl)thio)propyl)benzoate **(2a)** (0.5g, 0.87mmol,) was dissolved in 5ml of diethyl ether at room temperature. The reaction mass cooled to 0^0C. The methyl magnesium bromide (1M in THF) (1.27ml, 1.3mmol) was added slowly drop wise at 0^0C and then reaction was warmed to room temperature, stirred at RT for 3hrs. The reaction was monitored by TLC using 30% ethyl acetate in hexane. The TLC shows that starting acid material was consumed and new non-polar spot was observed. The reaction mass was quenched with saturated solution of ammonium chloride (10ml) and washed with brine solution (5ml), extracted with ethyl acetate (3x30ml) and water (20ml) was added to the reaction mixture, stirred for 5 minutes and the reaction mixture was transferred to separating funnel. The organic layer was deried over sodium sulphate and concentrated the solvent was removed by using vacuum. The crude product was purified by column chromatography on silica gel (Merck, 60 - 120 mesh), ethyl acetate/ hexane, 30%) to give the product in 78% **(3a)** yield.

(E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)propyl)thio) methyl)cyclopropyl)acetic acid (3a)

FT- IR (KBr) V_{max} cm^{-1}: 3293, 2922, 1687, 1658, 1376, 1232, 1104, 760; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 11.1 (s. 1H, OH), 8.24 (d, 2H), 7.88 (d, 2H), 7.54 (d, 1H), 7.43 (d, 1H), 7.23-7.29 (m, 5H), 7.13-7.04 (m, 4H), 2.65 (d, 3H), 2.62 (s, 3H, -CH_3), 2.42 (s, 2H), 2.14 (s, 2H), 1.92 (t, 2H), 1.51 (s, 1H), 0.34-0.23 (m, 4H); **^{13}C NMR** (100MHz, $CDCl_3$) δ: 201.5, 177.5, 157.3, 147.3, 142.3, 138.7, 136.3, 135.4, 133.1, 130.1, 128.1, 127.4, 127.1, 126.4, 125.1, 118.1, 47.4, 36.4, 35.8, 32.8, 29.8, 28.5, 16.9, 9.9; **MS**: m/z: 570 (M^+); **Anal. Calcd. for** $C_{34}H_{33}ClNO_3S$: C, 71.50; H, 5.82; N, 2.45; **Found**: C, 70.57; H, 5.50; N, 2.34.

2.1.2. Typical Experimental Procedure For 2-amino-N-benzyl-3-methoxypropanamide

A mixture of 2-amino-N-benzyl-3-hydroxypropanamide (0.5g, 0.87mmol,) was dissolved in 10ml of DMF at RT, added potassium carbonate (0.533g, 3.86mmol) .The reaction mass cooled to 0^0C, methyl iodide (0.548g, 3.86mmol) was added slowly drop wise at 0^0C, and then reaction was heated to100^0C, stirred at RT for 10h. The reaction was monitored by TLC using 30% ethyl acetate in hexane. The TLC shows that starting material was consumed and new non-polar spot was observed. The reaction mass was quenched with ice cold water (10ml), extracted with ethyl acetate (3x30ml), the combined organic layer was washed with brine solution (5ml). The reaction mixture was stirred at room temperature. After complete conversion, as indicated by TLC, the solvent was removed by using vacuum. The organic layer was dried over sodium sulphate and concentrated at 48^0C by using vacuum. Ethyl acetate (30ml) and water (20ml) was added to the reaction mixture, stirred for 5 minutes and the reaction mixture was transferred to separating funnel. The organic layer was separated and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (Merck, 60 - 120 mesh), ethyl acetate/ hexane, 30%) to give the product in 75% **(3b)** yield.

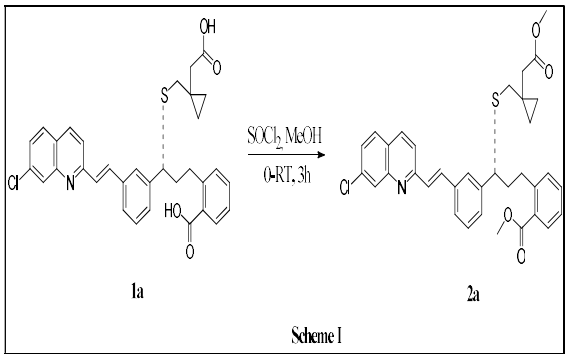
2-amino-N-benzyl-3-methoxypropanamide (3b)

FT- IR (KBr) V_{max} cm^{-1}: 3451, 2932, 1651, 1438, 1387, 1254, 1094, 843; **^{1}H NMR** ($CDCl_3$, 400 MHz) δ: 8.29 (s, 1H, NH), 7.34-7.23 (m, 5H), 5.11 (brs, 2H, NH), 4.40 (s, 2H), 3.73 (t, 1H), 3.31 (t, 2H), 3.25 (s, 3H, -CH_3); **^{13}C NMR** (100MHz, $CDCl_3$) δ: 172.5, 137.7, 128.4, 126.8, 126.4, 75.0, 58.4, 54.3, 43.1; **MS**: m/z: 208 (M^+); **Anal. Calcd. for** $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45; **Found**: C, 62.37; H, 7.59; N, 12.33.

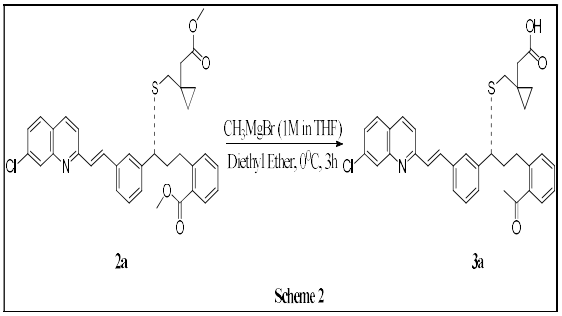
3. RESULTS AND DISCUSSION:

The target compound was synthesized a series of step given in scheme 1. Step one involves the (E)-2-(3-(((1-(carboxymethyl)cyclopropyl)methyl)thio)-3-(3-(2-(7-chloroquinolin-2 -yl)vinyl)phenyl)propyl)benzoic acid **(1a)** was dissolved in 5ml of methanol at room temperature. The reaction mass cooled to 0^0C. The thionyl chloride $SOCl_2$ was added slowly drop wise at 0^0C and then reaction was warmed to room temperature, stirred at RT for 3h (scheme - 1). The TLC shows that starting acid material was consumed and new non-polar spot was observed. The reaction mass was quenched with ice cold water (10ml), extracted with ethyl acetate (3x30ml), the combined organic layer was washed with washed with brine solution (5ml). The organic layer was deride over and concentrated. The precipitated product the corresponding (E)-methyl2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl) phenyl)-3-(((1-(2-methoxy-2-oxoethyl)cyclopropyl)methyl)thio)propyl) benzoate **(2a)** in good yields. The one-pot reaction of step two involves (E) methyl-2-(3-(3-(2-(7-

chloroquinolin-2-yl)vinyl)phenyl)-3-(((1-(2-methoxy-2-oxoethyl)zcyclopropyl)methyl)thio)propyl)benzoate **(2a)** was dissolved in 5ml of diethyl ether at room temperature. The reaction mass cooled to 0^0C. The methyl magnesium bromide (1M in THF) (1.27ml, 1.3mmol) was added slowly drop wise at 0^0C and then reaction was warmed to room temperature, stirred at RT for 3h. The TLC shows that starting material was consumed and new non-polar spot was observed. The reaction mass was quenched with saturated solution of ammonium chloride (10ml) and washed with brine solution (5ml), extracted with ethyl acetate (3x30ml), the combined organic layer was washed with brine solution (5ml). The organic layer was deried over sodium sulphate and concentrated. The corresponding product is (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)propyl)zthio)methyl) cyclopropyl) acetic acid **(3a)** were obtained in good yields. The reaction is illustrated in scheme 2.



Scheme 1: Synthesis of (E) methyl-2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(((1-(2-methoxy-2-oxoethyl)zcyclopropyl)methyl)thio)propyl)benzoate **(2a)**



Scheme 2: Synthesis of (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)propyl)zthio)methyl) cyclopropyl) acetic acid **(3a)**

3.1. Effect of temperature and solvent on (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloro quinolin-2yl)vinyl)phenyl)propyl)thio)methyl)cyclopropyl)acetic acid synthesis:

The reaction of (E)-methyl 2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(((1-(2-methoxy-2-oxoethyl)cyclopropyl)methyl)thio)propyl) benzoate **(2a)** was dissolved in 5ml of diethyl ether was chosen as a model reaction to study the effect of temperature and solvent. The results of the reaction are given in table 1. The reaction either did not progress or afforded lesser yields in more polar solvents such as ethanol, water, acetonitrile at room temperature or even at higher temperatures. However in the presence of less polar solvents such as toluene and benzene or diethyl ether the reaction afforded poor yields and took longer reaction times even at elevated temperatures. However highest yield of the (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloro quinolin-2yl)vinyl)phenyl)propyl)thio)methyl)cyclopropyl) acetic acid was obtained when the reaction was carried out under stirred at room temperature, over a period of 3.0 hours affording the corresponding (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl) propyl)thio)methyl)cyclopropyl)acetic acid **(3a)** in 78.0% yield. The results of the reaction are summarized in table 1.

Table 1 : Effect of temperature and solvent on (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)propyl)thio)methyl)cyclopropyl)acetic acid synthesis

S. No	Solvent	Temperature (^{0}C)	Time (h)	Yield[a] (%)
1	Toluene	25-27°C	8.0	55.0
2	Toluene	107-110°C	7.0	30.0
3	Benzene	78-80°C	8.0	28.0
4	CH_2Cl_2	25-27°C	7.0	No reaction
5	CH_3CN	80-82°C	8.0	30.0
6	H_2O	25-27°C	9.0	Incomplete
7	H_2O	98-100°C	8.0	40.0

8	C_2H_5OH	25-27°C	9.0	No reaction
9	C_2H_5OH	75-80°C	6.0	25.0
10	**Diethyl Ether**	**25-30°C**	**3.0**	**78.0**

3.2. The effect of substituent:

Inoder to prove the generality of our methodology, we extended the optimized reaction condition for synthesizing a series of (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)propyl)thio)methyl)cyclopropyl)acetic acid **(3a)** derivatives by reacting various other substituted (E) methyl-2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(((1-(2-methoxy-2-oxoethyl)zcyclopropyl)methyl)thio)propyl)benzoate **(2a)** was dissolved in 5ml of diethyl ether at room temperature. For all the studied examples, (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)propyl)thio)methyl)cyclopropyl)acetic acid **(3a)** were obtained in good yields. The results of the reaction are summarized in table 2 given below.

Table 2. Effect of substituents on (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)propyl)thio)methyl)cyclopropyl)acetic acid synthesis

S. No	(E)-methyl 2-(3-(3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl)-3-(((1-(2-methoxy-2-oxoethyl)cyclopropyl)methyl)thio)propyl)benzoate	Time [a] (h/mn)	Yield [b] (c)	Montelukast Methyl Ketone [c]
1		3.0	78	**(3a)**

[a] All the reactions were carried out according to typical experimental procedure.
[b] Isolated yield after Column purification.
[c] All the products were characterized by ^{1}HNMR, FT-IR and Mass spectral studies.

3.3. HPLC Chromatogram:

The HPLC Chromatogram were established based on (Xterra RP-18 reverse phase columns with acetonitrile/methanol/water as solvent, 0.02M ammonium acetate with UV detector and a flow rate of 1.0ml/min) and column chromatographic yields. The yields of the products were established after column chromatography.

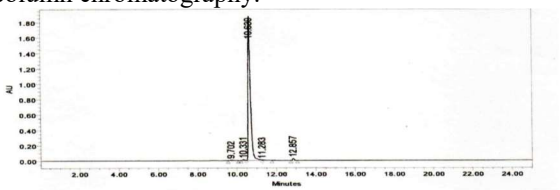
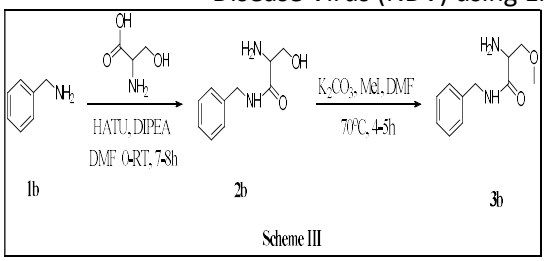


Fig.1 HPLC Chromatogram of (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl) phenyl)propyl)thio)methyl)cyclopropyl)acetic acid

S. No	RT	% Area
1	9.702	0.01
2	10.331	0.01
3	10.630	1.90
4	11.283	0.01
5	12.857	0.03
Sum		**98.04**

3.4. Synthesis of 2-amino-N-benzyl-3-methoxypropanamide:

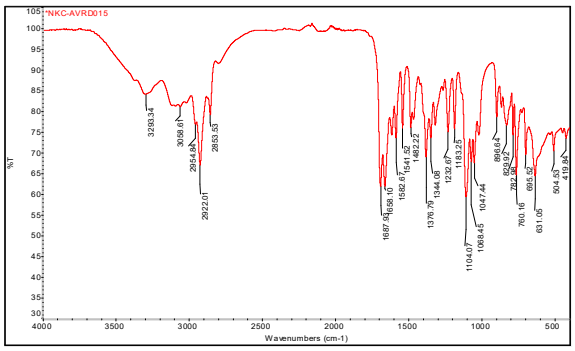
After synthesizing a series of 2-amino-N-benzyl-3-methoxypropanamide **(3b)** derivatives we focused our attention on extending the methodology for the synthesis of a series of 2-amino-N-benzyl-3-hydroxypropanamide **(2b)** was dissolved in 10ml of DMF at RT, added potassium carbonate. The reaction mass cooled to 0^0C, methyl iodide was added slowly drop wise at 0^0C and then reaction was stirred at 70^0C for 4-5h. The reaction was monitored by TLC using 30 % ethyl acetate in hexane. The TLC shows that starting material was consumed and new non-polar spot was observed. The reaction mass was quenched with ice cold water (10ml), extracted with ethyl acetate (3x30ml), the combined organic layer was washed with brine solution (5ml). The reaction mixture was stirred at room temperature. The reaction mixture was stirred at room temperature. After complete conversion, as indicated by TLC, the solvent was removed by using vacuum. The organic layer was dried over sodium sulphate and concentrated at 48^0C by using vacuum. Ethyl acetate (30ml) and water (20ml) was added to the reaction mixture, stirred for 5 minutes and the reaction mixture was transferred to separating funnel. The organic layer was separated and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (Merck, 60 - 120 mesh), ethyl acetate/ hexane, 30%) to give the product in 75% **(3b)** yield.



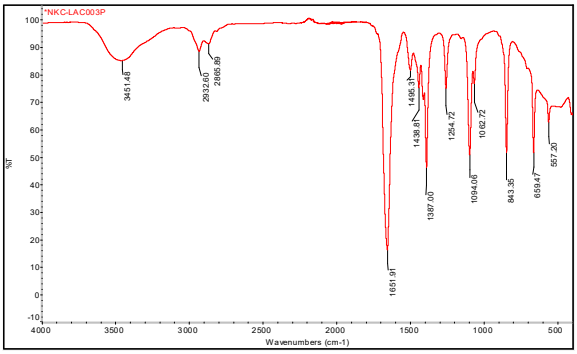
Scheme 3: Synthesis of 2-amino-N-benzyl-3-methoxypropanamide

3.5. FT-IR Spectral Studies:

The FT-IR spectroscopy studies were effectively used to identify the functional groups present in the synthesized (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl) phenyl)propyl)thio)methyl)cyclopropyl)acetic acid, 2-amino-N-benzyl-3-methoxypropan amide and 2-amino-N-benzyl-3-methoxypropanamide in the range of 400-4000cm^{-1}. The various bands obtained in FT-IR spectrum by using KBr pellet technique are shown in figure 2. The absorption bands at 3293.3cm^{-1} indicates the presence of intermolecular hydrogen bonds due to acid molecules in the lattice. The aromatic stretching vibrations are observed at 3058.6 cm^{-1}. The –C-H stretching vibrations are observed at 2954.8cm^{-1}. The carbonyl symmetric stretching vibrations are observed at 1687.9cm^{-1}. The aromatic stretching vibrations are observed at 2932.6 cm^{-1}. The –C-H stretching vibrations are observed at 2865.8 cm^{-1}. The carbonyl symmetric stretching vibrations are observed at 1651.9 cm^{-1}.



Compound – 1 (3a)



Compound – 2 (3b)

Figure 2 FI-IR spectra of the compounds 1, 2

4. BIOLOGICAL STUDIES:

4.1. *In vitro* evaluation antimicrobial assay:

Antimicrobial analysis of the test compounds were carried out by previously reported procedures[33-35] by using agar well diffusion method. All the compounds were prepared in acetic acid and DMSO solvent mixture. The test compounds were loaded into wells on the plates. The plates were incubated at 37^{0}C for 18-24h. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition (in mm) against the test microorganisms and the solvent mixture. Acetic acid and DMSO solvent mixture was used as control. Ciprofloxacin was used as reference drug. The tests were carried out in triplicates.

4.2. Antibacterial Studies:

All the synthesized compounds (**3a** and **3b**) were screened for antibacterial activity against pathogenic strains of gram negative and gram positive bacteria by using agar well diffusion method. Among the heterocyclic compounds (**3a** and **3b**) screened for *in vitro* antibacterial activity, the **3a** derivatives exhibited superior activity against pathogenic bacteria in comparison to the reference drug ciprofloxacin. The **3b** compounds exhibited relatively moderate bacterial activity. The **3a** derivatives exhibited superior antibacterial action against six strains of gram positive and gram negative bacteria with maximum zone of inhibition (11-28mm) against *E. coli, Kelbsiella pneumonia*, Pseudomonas *aeruginosa*, *Vibrio cholerae, Staphylococcus aureus Bacillus subtilis* and in comparison to ciprofloxacin antibiotic as shown in table 3.

Table.3 The *in vitro* antibacterial activity of synthesized compounds

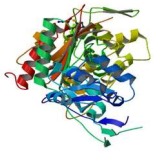
S. No	Number	Gram Positive		Gram Negative			
		Staphylococcus aureus*	Bacillus subtilis *	E. Coli*	Pseudomonas aeruginosa*	Kelbsiella pneumonia*	Vibrio cholerae *
1	**3a**	+++	++	+++	+++	+++	++
2	3b	++	++	+++	++	++	+
3	**Cipro-**	+++	++	++	+++	++	+++

	floxacin			+			

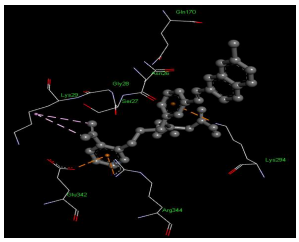
*Diametre of zone of inhibition in mm
(+++) = Highly active (20-30 mm); (++) = moderately active (15-20 mm); (+) = less active (5-15 mm)

4.3. Molecular Docking Studies:

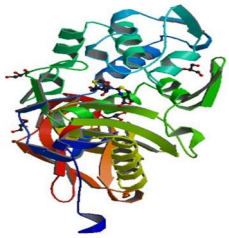
The molecular docking studies of the montelukast methyl ketone and lacosamide with cell wall protein of pseudomonas aeruginosa and E. coli was carried out with GOLD suite program to identify the binding modes of the compounds. GOLD suite program uses a genetic algorithm (GA) for protein-ligand docking with interactive docking set-up via Hermes. The crystal structure of penicillin-binding protein (PBP3) from Pseudomonas aeruginosa **(**PDB id: 3PBQ) and the crystal structure of penicillin-binding protein (PBP5) in E. coli ((PDB id: 3MZE) was obtained from the PDB sum protein data bank. The energy minimized structure of montelukast methyl ketone **(3a)** and lacosamide derivative **(3b)** was docked with the cell wall protein of *E. coli* and *P. aeruginosa* (Fig.4). The docking results indicate that the carbazolyl montelukast methyl ketone **(3a)** binds to the cell wall protein of *E. coli* docking score of 24.63 and lacosamide derivative **(3b)** binds to the cell wall protein of *P. aeruginosa* docking score of 84.3. The montelukast methyl ketone derivative **(3a)** forms the amino acid residue GLU342 of the cell wall protein of *P. aeruginosa*. The lacosamide derivative **(3b)** also forms the amino acid residue LEU398 of the cell wall protein of *E. coli.* Hence molecular docking studies establish the binding affinity of the montelukast methyl ketone derivatives for the cell wall protein of pathogenic bacteria and thereby inhibit the cell wall synthesis.



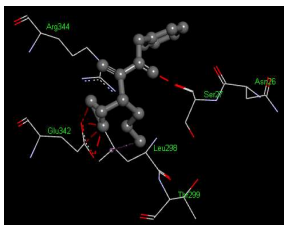
Crystal structure of PBP3 in Pseudomonas aeruginosa (PDB id: 3PBQ)



Molecular docking studies of 3a with PBP3 in Pseudomonas aeruginosa



Crystal structure of PBP5 in E. coli (PDB id: 3MZE)



Molecular docking studies of 3b with PBP5 in E. coli

Figure 4 Molecular docking studies of Montelukast Methyl Ketone and Lacosamide derivatives in penicillin binding protein

4.4. Determination of median embryo infectious dose (EID_{50}) of the virus:

The EID_{50} of the virus was determined using method of Young *et al*. (2002). 4.5ml each of PBS was dispensed into 4 sterile McCartney bottles, labeled 10^{-1} to 10^{-10}. 0.2ml of NDV viral stock was added to bottle1 labeled 10^{-1} and mixed thoroughly. 0.2ml of dilution in bottle 1 was transferred into bottle 2 and mixed thoroughly. The same dilution was done across the bottles until bottle 10 to make 10 fold serial dilutions so these diluents solutions were prepared as (10^{-1} to 10^{-10}). Nine- day- old embryonated chicken eggs, obtained from healthy flock of local poultry farm, were candled, labeled and swabbed with 70% ethanol. Five Eggs in each group were inoculated with 0.2ml of respective dilution. Eggs were candled after every 24 hours to confirm viability and also after 48 hours. Results were calculated appropriately. EID_{50} was calculated according to the method of Young *et al*. (2002).

4.5. Antiviral Activity:

The antiviral assay was performed by adopting the method described by Sally (2002) using NDV Lasota strain and non infectious 9-day-old embryonated chicken eggs. The eggs were grouped in a completely randomized pattern into seven groups. To prepare 10% w/v working stock solutions, 2g of aqueous, ethanolic and methanolic extracts were dissolved in 20ml dimethylsulphoxide (DMSO). From these working solutions, four different concentrations 300, 400, 500, 600µg/ml was prepared by serial dilution method. The different prepared inoculums were injected to the embryonated eggs according to the procedure of Chollom *et al*. (2012). A volume of 0.1ml of virus suspension and 0.9ml of different plant extract from the stock solution at different concentrations was mixed and kept for one hour at room temperature to react and then inoculated in eggs. The eggs were kept in incubator at 37^0C for 72 hrs. The eggs with virus suspension without

extract dole out as positive controls while uninoculated eggs dole out as negative controls.

The eggs were set in incubator for 24 hours with frequent candling after every 24 hours. Observations were made on the embryo movements, blood vessels and time of embryo death. After 72 hours the embryos were chilled at 4^0C and allantoic fluids from the treated eggs were collected for spot Hem agglutination test to detect the NDV in the eggs by following the procedure of Murakawa *et al*., 2003. The protocol of Young *et al*., 2002 was adopted to perform

Hem agglutination test to confirm the presence of NDV.

4.5.1. Antiviral activity in chick embryo viability using New Castle Disease Virus (NDV)

The table 4 illustrates the time of embryo deaths. Throughout the three days of experiment, the following groups did not show any embryo death: negative control (untreated eggs), aqueous and methanolic extract at 300µg/ml and ethanolic extract at 400µg/ml. The death of first embryo was observed in the positive control group (NDV alone) 24 hrs post inoculation; the mortality rate in this group at 48 hrs was 80% and at 72h all the embryos died. This was followed by the group aqueous extract at 600µg/ml. At the highest concentration 600µg/ml of different plant extracts, embryo deaths were recorded at 24h for aqueous extract and 48h for methanolic and ethanolic extract (table 4). Embryo survival rate and chicken embryo size to detect the NDV in the eggs. NDV embryo even though 10^{-1} suspension is expressed greater size embryo 6.56 cm compared to the rest of suspension and PBS control 3.88 cm. The alone virus control suspension showed greater size embryo 7.68cm, rest of the solvent and NDV control (5.87) therefore they have highest antiviral activity of rest of the solvent. In this study the effect of from montelukast methyl ketone are investigated against New castle disease virus (NDV) by an *in-vivo* assay. Seven groups of nine-day-old embryo nated chicken eggs were inoculated with various treatments of different concentration. All the groups except uninoculated negative control group were inoculated with velogenic NDV strain; five groups received different concentrations. Daily observe the rate of embryo survival. Allantoic fluid from treated eggs was collected for hem agglutination test.

Table. 4 The antiviral activity of Embryo deaths following inoculation of embryonated chicken eggs with New Castle Disease Virus (NDV) Synthesized compounds

Treatment	**Concentration (µg/ml)**	**Time of embryo death (h)**				**% Mortality**
		0	**24**	**48**	**72**	
Untreated egg	-	0	0	0	0	0%
NDV alone	-	0	2	2	-	80%
Aqueous extract + NDV(AE)	300	0	0	0	0	0%
	400	0	0	1	0	20%
	500	0	0	1	2	60%
	600	0	3	1	1	100%
Methanolic extract + NDV (ME)	300	0	0	0	0	0%
	400	0	0	0	0	0%
	500	0	0	1	2	60%
	600	0	0	1	1	40%
Ethanolic extract + NDV (EE)	300	0	0	1	2	60%
	400	0	0	0	2	40%
	500	0	0	2	0	40%
	600	0	0	2	2	80%

The antiviral activity was tested by the reduction of New Castle Disease Virus (NDV) assays.

(+) positive: reduction of viral NDV higher than 50% at both concentrations tested.

(+/−) positive/negative: reduction of viral NDV only achieved at 100 µg/ml.

(−) negative: without protection at 25 and at 100 µg/ml.

Yield (% w/w = g of extracts/100 g of dried and ground plant material).

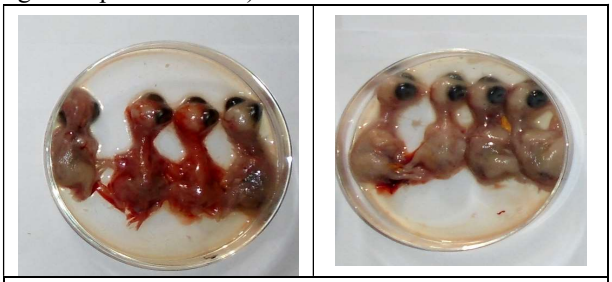


Figure 3 Embryo deaths following inoculation of embryonated chicken eggs with Newcastle disease virus (NDV) and incubation at different concentrations

5. CONCLUSION:

In the present study, we have developed a high yielding procedure for the divergent synthesis of (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)propyl) thio)methyl)cyclopropyl)acetic acid derivatives in good yields. This new method offers the advantages of methodological simplicity and offers well to moderate yields. The effect of temperature, substituents and mole ratio on the reaction was also

studied. All the products were thoroughly characterized by ^{1}H-NMR, ^{13}C-NMR, FT-IR, Mass spectral and CHN analysis. Among the synthesis of (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl) phenyl)propyl)thio)methyl)cyclopropyl)acetic acid **(3a)** derivatives exhibited superior antibacterial activity against seven strains of gram positive and gram negative bacteria. The (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)propyl)thio)methyl)cyclo propyl)acetic acid **(3a)** derivative displayed excellent antibacterial activity against *E. coli*, *Pseudomonas aeruginosa* and *E-coli* with the comparison to the reference drug. Hence based on ease of synthesis, potential antibacterial activity and antiviral activity it is evident that (E)-2-(1-(((3-(2-acetylphenyl)-1-(3-(2-(7-chloroquinolin-2yl)vinyl)phenyl)propyl)thio)methyl) cyclopropyl) acetic acid **(3a)** derivatives are a promising class antibacterial agents for clinical applications after necessary pharmacological studies. The molecular docking studies indicate that the montelukast methyl ketone **(3a)**, lacosamide derivative **(3b)** binds to the cell wall protein of *P. aeruginosa* and *E. coli by* formation of amino acid residues (GLU342, LEU398) of the bacterial cell wall protein. The alone virus control suspension showed greater size embryo 7.68cm, rest of the solvent and NDV control (5.87) therefore they have highest antiviral activity of rest of the solvent. In this study the effect of from montelukast methyl ketone are investigated against Newcastle disease virus (NDV) by an *in-vivo* assay. Seven groups of nine-day-old embryo nated chicken eggs were inoculated with various treatments of different concentration. All the groups except uninoculated negative control group were inoculated with velogenic NDV strain; five groups received different concentrations. Daily observe the rate of embryo survival. Allantoic fluid from treated eggs was collected for hem agglutination test.

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