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SYNTHESIS, SPECTRAL STUDIES, INSILICO STUDIES, BRINE-SHRIMP CYTOTOXICITY, α- AMYLASE INHIBITION AND ANTI-UREASE ACTIVITIES OF MELOXICAM DERIVATIVES

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Abstract

The objective of this study was to deal with ester derivatives of meloxicam. In this connection, a series of meloxicam derivatives with carboxylic acids were synthesized and characterized using FTIR, HNMR and ^{13}CNMR. The in-vitro biological activities including brine shrimp cytotoxicity. α amylase inhibition and anti-urease activities were evaluated. The most active derived compounds M1, M2, M10 and M15 have shown 60%, 70%, 50% and 50% mortality rate respectively comparable to positive control. All derivatives were found to be non-toxic. Furthermore, the study suggests that synthesized meloxicam derivatives exhibit inhibitory potential against different biologically relevant enzyme targets.

Index terms: α-Amylase, Brine Shrimp Cytotoxicity, C^{13}NMR, FTIR, HNMR, Meloxicam, Urease

1. INTRODUCTION

Esters are organic compounds known due to their sweet taste and fruity smell. Chemically, ester functional group have one carbonyl carbon that is further attached to oxygen with one sigma bond (1), on the other side of oxygen, any alkyl or aryl moiety is attached. The general formula of esters are -COOR'. The R' may be alkyl or aryl and these groups may be same or different (2). If R and R' are linked together and form a cyclic ring, these compounds are known as lactone (3).

Esters are the condensation product of carboxylic acid and alcohol. These are classified on the basis of alcohol that condensed with carboxylic acid (4) The ester bond easily hydrolyzes by the enzyme group esterase (5). This property makes the ester functional group a good candidate of prodrug (6). Ester containing prodrugs are enalapril and aspirin (7). Aspirin is classified among Non-steroidal anti-inflammatory drug.

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Non-steroidal anti-inflammatory drugs (NSAIDs) are used all over the world for their analgesic, anti-inflammatory, and antipyretic effects (8). NSAIDs are, worldwide, the most commonly prescribed class of medications and account for approximately 5–10% of all medications prescribed each year (9). NSAIDs encompass a large class of drugs with extreme structural and functional diversity. These are mostly weak organic acids (comprising of an acidic moiety along with an aromatic functional group) (10). The general structure of a typical NSAID consists of an acidic moiety (carboxylic acid, enols) attached to a planar aromatic functional group. Salicylates were the first identified NSAIDs following extraction of salicylic acid from willow bark (11). These are carboxylic acid derivatives, i.e. propionic acid, acetic acid and salicylic acid derivatives (12).

All these drugs have potential to inhibit the prostaglandins and have anti-pyretic action as well. Prostaglandins protect the stomach from acid by its inhibitory potential otherwise ulcer related problem may arise (13). Meloxicam belongs to the class of mono-carboxylic acid amides and therapeutically used as NSAIDs (14). Meloxicam have potential to inhibit both COX-1 and COX-2 but it have more potential to inhibit the COX-2 (15). The structural features revealed that meloxicam have enolic group that is potential candidate for structural modification. Piroxicam belongs to the same chemical class and is structural analogue of meloxicam (16).

Several drugs modification of piroxicam have done and animal models revealed that the piroxicam derivatives have less ulcer like adverse effects. Esters derivative of piroxicam have potential pharmacological effect (17). To check the potential therapeutic effect of meloxicam derived compounds, brine shrimp toxicity studies were performed. Stomach related side effects are always associated with NSAIDs. NSAIDs are usually associated with gastric and duodenal ulcer (18).There is always a need of new drug molecules that have less side effects and better therapeutic effects. (19). The enolic group of meloxicam is responsible for the selective COX-2 inhibition activity (20). Piroxicam is lead molecule of enolic-carboxamide derivatives (21). Similarly, the enolic group of meloxicam have potential active site to develop the new pharmacophore.

Prodrugs of meloxicam have been synthesized and tested against urease inhibition test and brine shrimp toxicity test. The patient suffering with *H-pylori* infection have elevated level of urease enzyme produced by infectious organism (22). To reduce the pain related problems in ulcer patients, there is need to use such type of prodrugs that have less stomach irritation effect to reduce the urease activity in stomach. The aim of this study is to develop the new active derivative of meloxicam and to check its potential against the different biological enzymes like urease and alpha amylase.

In acute pancreatitis, the serum amylase level increased. The studies have proved the active role of NSAIDs in decreasing the level of serum amylase and lipase concentration in patients of pancreatitis. The patients suffering with peptic ulcer have elevated level of ureases. Meloxicam being COX-2 selective have less stomach related effect as compared to the non-selective COX-1 inhibitors. There is need to evaluate the effect of anti-urease activity of derived meloxicam esters.

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2. MATERIALS AND METHOD

Analytical grade (Merck/ sigma Aldrich) reagents were used. The solvents were purified by drying method. Gallen Kamp apparatus was used to check the melting point of synthesized meloxicam derivatives. FTIR spectrophotometer (Bruker-Tensor27) was recorded in the range of 400-4000 cm^{-1}.^{1}H NMR and ^{13}C NMR of synthesized derivatives were derived by Bruker- 400 spectrophotometer.

Synthesis of Meloxicam Derivatives (M1-M15)

M1-M15 derivatives of meloxicam were synthesized by reflux condensation of enolic group of parent molecule. Process was carried out in chloroform solution of suitable reagents containing carboxylic acid functional groups. Reaction mixture was refluxed for roughly 3hrs in the presence of a strong catalyst i.e. H_2SO_4. Excess volume of solvent was evaporated by using Rota-evaporator. Synthesized product was purified by recrystallization process by using chloroform. Melting points of synthesized derivatives were determined by GallenKemp melting point apparatus. Solubility of ester derivatives were analyzed in different organic solvents. The general structural scheme of M1-M9 derivatives is shown as in Figure 1 and their structures in Figure 3. Similarly, structural scheme of M10-M15 derivatives are shown in Figure 2 and their structures in Figure 3.

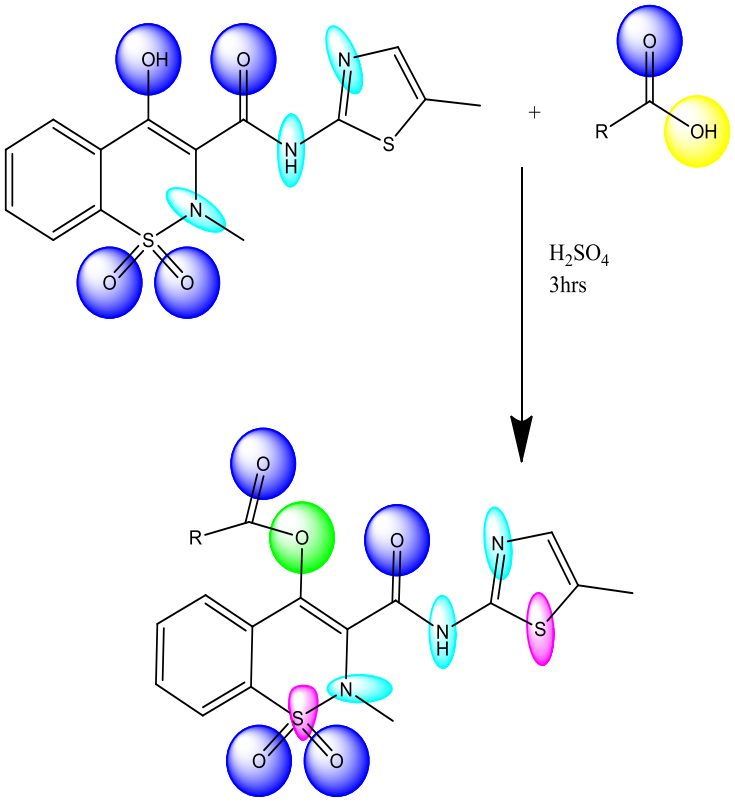

Figure 1: Synthesis of Meloxicam Derivative (M1-M9)

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Where,

Code	R
M1	$CH{=}CH\text{-}C_6H_5$
M2	$C_6H_2(OH)_3$
M3	$C_6H_4NH_2$
M4	C_6H_5
M5	C_6H_4OH
M6	$C_5H_{10}NH_2$
M7	C_4H_7NH
M8	$C_4H_8SNH_2$
M9	CH_2NH_2

Synthesis of M10-M15

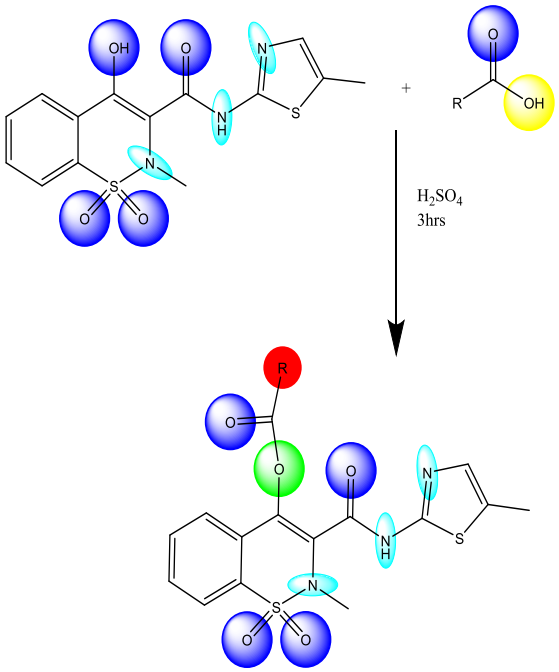

Figure 2: Synthesis of Meloxicam Derivatives (M10-M15)

Where R=,

Code	R=
M10	---------
M11	C_6H_4
M12	CH_2CH_2
M13	$CH_2CH_2CHNH_2$
M14	$CH(OH)CH(OH)$
M15	$CH_2CH(NH_2)$

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Structures of M1-M15

The structures of synthesized derivatives M1-15 are shown in Figure 3.

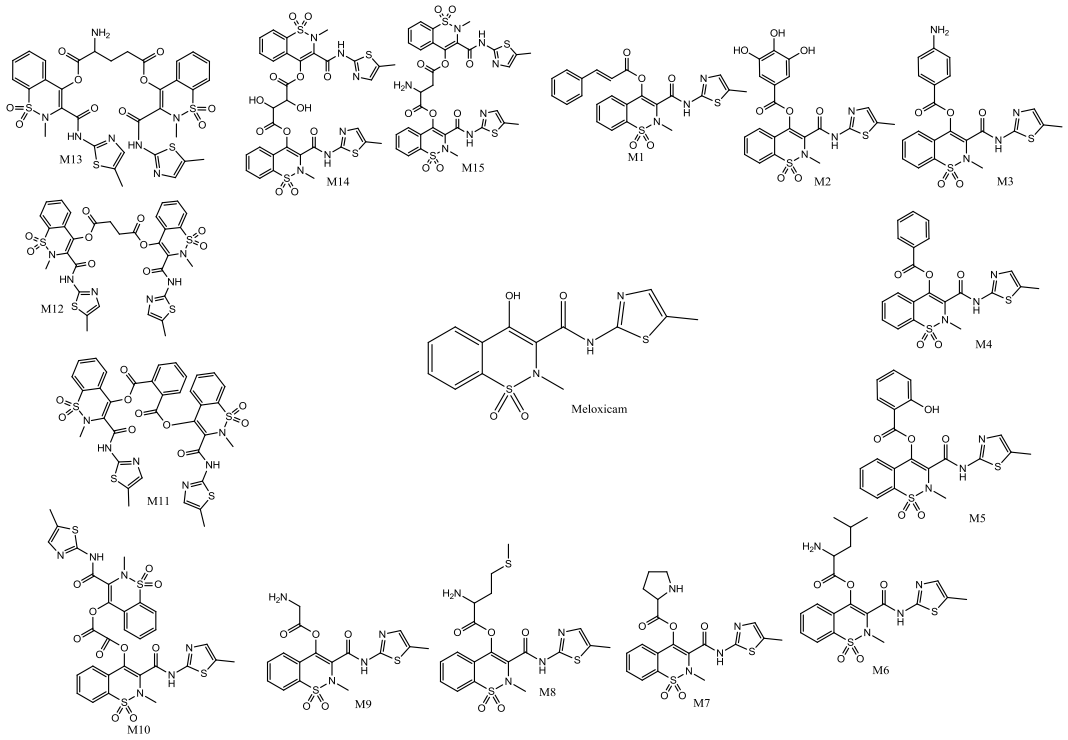

Figure 3: Structures of M1-15

Characterization of M1-M15

All the newly synthesized derivatives were checked for their yield, physical state, solubility and melting points. To check their solubility, derivatives were dissolved in different polar and non-polar solvents i.e., ethanol, methanol, chloroform, water, DMSO. Melting points were recorded using a Gallen kamp melting point apparatus and are uncorrected. Fourier transform infrared spectrum of samples was recorded using KBr pellet press method by Bruker TENSOR 27 FTIR spectrophotometer. ^{1}H and ^{13}C nuclear magnetic (NMR) analyses were performed on a spectrospin 400 MHz spectrometer (Bruker) equipped with a sample Xpress (from Bruker) auto sampler system, using deuterated solvents for the preparation of samples. The obtained spectra were analyzed using Topspin 7.1 software (Bruker). The chemical shifts were reported relative to Trimethylsilane (TMS) used as a standard (0.00 ppm). Signals were identified and described as singlet(s), doublet (d), triplet (t) (23).

Meloxicam, IUPAC Name: 4-hydroxy-2-methyl-N-(5-methylthiazol-2-yl)-2H-benzo[e][1,2]thiazine-3-carboxamide 1,1-dioxide, Chemical Formula: $C_{14}H_{13}N_3O_4S_2$, Mol. Weight: 351.40 gm/mol and M. P. 258 °C. Elemental analysis (calculated) for $C_{14}H_{13}N_3O_4S_2$: C, 47.85; H, 3.73; N, 11.96; S, 18.25 (found) C, 47.81; H, 3.80; N, 12.03;

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S, 18.21, FT-IR v (cm-1), 3400 (N-H), 1748 (C=O), 1652 (C=N), 1466 (CH=CH), 1134 (C−N), 1244 (C−O), ^{1}H NMR (DMSO, ppm) δ, 2.15s (3H, CH_3), 2.10s (3H, CH_3), 6.35 s, (1H, CH), 7.31-7.32 t, 7.44-7.45t, 7.55-7.56 d, 7.76-7.77d, (4H, CH), 8.75 s, (1H, NH), 9.17s, (1H, OH), ^{13}C NMR (DMSO, ppm) δ: 121.5(C1), 130.1 (C2), 130.5 (C3), 119.7 (C4), 132.1(C5), 109.6 (C6), 145.7 (C7), 126.4 (C8), 160.1 (C9), 154.3(C10), 119.6(C11), 133.5 (C12), 9.5(C13), 17.8(C14).

M1 IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl cinnamate. Yield (82%); white semi-solid. Molecular formula: $C_{23}H_{19}N_3O_5S_2$ and molecular weight: 481.54 gm/mol. Elemental analysis (calculated) for : $C_{23}H_{19}N_3O_5S_2$: C, 57.37; H, 3.98; N, 8.73; S, 13.32 (found) C, 57.41; H,4.05; N, 8.65; S, 13.28, FT-IR v (cm-1), 29841 (C−H), 1711 (C=O), 1636 (C=N), 1473 (CH=CH), 1175 (C−N), 1268 (C−O), ^{1}H NMR (DMSO, ppm) δ, 2.23s (3H, CH_3), 2.28s (3H, CH_3), 6.91 s, (1H, CH), 7.42-7.43 m, 7.46-7.47m, (4H, CH), 8.75 s, (1H, NH), 7.11-7.12 d, 5.31-5.32 d(2H, CH), 7.55-7.54d, 7.33-7.34 m(5H, CH), ^{13}C NMR (DMSO, ppm) δ: 120.3(C1), 130.4 (C2), 130.8 (C3), 121.7 (C4), 131.6(C5), 110.6 (C6), 144.0 (C7), 125.3 (C8), 159.1 (C9), 153.6 (C10), 119.6(C11), 132.5 (C12), 12.9(C13), 21.8(C14), 156.7(C15), 110.6(C16), 145.2(C17), 134.6(C18), 128.2 (C19,23), 127.9(C20,22), 127.5(C21) .

M2, IUPAC Name. 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl 3, 4, 5-trihydroxybenzoate. Yield (85%); Brown semi solid. Molecular formula: $C_{21}H_{17}N_3O_8S_2$ and molecular weight: 503.50gm/mol. Elemental analysis (calculated) for $C_{21}H_{17}N_3O_8S_2$: C, 50.09; H, 3.40; N, 8.35; S, 12.74 (found) C, 50.15; H, 3.35; N, 8.45; S, 12.70. FT-IR v (cm-1), 2981 (C−H), 1716 (C=O), 1683 (C=N) 1604, 1473 (CH=CH), 1152 (C−N), 1272 (C−O), 3366 (N−H), 3628, 3649 (O-H). ^{1}H NMR (DMSO, ppm) δ, 2.21s (3H, CH_3), 1.98s (3H, CH_3), 6.92 s, (1H, CH), 7.34-7.35t, 7.43-7.45 d, 7.46-7.48d, (4H, CH), 8.75 s, (1H, NH), 7.29s, (2H, CH), 5.30 s(3H, OH), ^{13}C NMR (DMSO, ppm) δ: 120.4 (C1), 129.8 (C2), 131.6 (C3), 120.3 (C4), 132.5(C5), 111.0 (C6), 146.7 (C7), 127.4 (C8), 162.1 (C9), 155.7 (C10), 121.6(C11), 134.5 (C12), 13.6 (C13), 19.8(C14), 153.7 (C15), 123.4 (C16), 108.7 (C17,21), 144.9 (C18,20), 140.7 (C19).

M3 IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl 4-aminobenzoate; Yield (80%); Yellow semi solid. Molecular formula: $C_{21}H_{18}N_4O_5S_2$ and molecular weight: 470.52 gm/mol. Elemental analysis (calculated) for $C_{21}H_{18}N_4O_5S_2$: C, 53.61; H, 3.86; N, 11.91; S, 13.63 (found) C, 53.55; H, 3.90; N, 11.85; S, 13.70, FT-IR v (cm-1), 3353, (N−H), , 1698 (C=O), 1683 (C=N), 1606, 1473 (CH=CH), 1153 (C−N), 1272 (C−O), ^{1}H NMR (DMSO, ppm) δ, 2.21s (3H, CH_3), 1.99s (3H, CH_3), 6.94 s, (1H, CH), 7.28-7.30 t, 7.33-7.36 d, 7.48-7.51d, (8H, CH), 7.95 s, 7.97 (3H, NH), ^{13}C NMR (DMSO, ppm) δ: 126.0 (C1), 128.1 (C2), 132.6 (C3), 120.3 (C4), 130.9 (C5), 129.1 (C6), 167.2 (C7), 128.4 (C8), 162.1 (C9), 172.0 (C10), 121.6(C11), 134.5 (C12), 13.9 (C13), 19.8 (C14), 153.7 (C15), 118.4 (C16), 129.1 (C17,21), 112.9 (C18,20), 148.7 (C19).

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M4 Yield IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl benzoate: Yield (90%); Semi solid. Molecular formula: $C_{21}H_{17}N_3O_5S_2$ and molecular weight: 455.51gm/mol. Elemental analysis (calculated) for $C_{21}H_{17}N_3O_5S_2$: C, 55.37; H, 3.76; N, 9.22; S, 14.08 (found) C, 55.28; H, 3.85; N, 9.15; S, 14.15

FT-IR v (cm-1), 3365 (N−H), 1698 (C=O), 1647 (C=N), 1473 (CH=CH), 1168 (C−N), 1273 (C−O), ^{1}H NMR (DMSO, ppm) δ, 2.01s (3H, CH_3), 1.97s (3H, CH_3), 6.92 s, (1H, CH), 7.32-7.35t, 7.44-7.45 d, 7.47-7.48d, (4H, CH), 8.75 s, (1H, NH), 8.14-8.15d, 7.87-7.88t, 7.65-7.66t, (5H, CH), ^{13}C NMR (DMSO, ppm) δ: 122.0 (C1), 129.6 (C2), 132.3 (C3), 121.3 (C4), 133.5(C5), 112.1 (C6), 147.6 (C7), 127.8 (C8), 160.9 (C9), 157.6 (C10), 123.7(C11), 134.2 (C12), 13.3 (C13), 21.7 (C14), 152.7 (C15), 129.1 (C16), 130.5 (C17,21), 127.9 (C18,20), 127.4 (C19).

M5 IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl 2-hydroxybenzoate: Yield (87%); White Semi solid. Molecular formula: $C_{21}H_{17}N_3O_6S_2$ and molecular weight: 471.51gm/mol. Elemental analysis (calculated) for $C_{21}H_{17}N_3O_6S_2$: C, 53.49; H, 3.63; N, 8.91; O, 20.36; S, 13.60 (found) C, 53.49; H, 3.63; N, 8.91; O, 20.36; S, 13.60, FT-IR v (cm-1), 2984 (C−H), 1716 (C=O), 1684 (C=N), 1473 (CH=CH), 1149 (C−N), 3363, (N−H), 3649(O-H), ^{1}H NMR (DMSO, ppm) δ, 2.25s (3H, CH_3), 1.99s (3H, CH_3), 7.04 s, (1H, CH), 7.39-7.40t, 7.41-7.42 d, (4H, CH), 9.78 s, (1H, NH), 7.06-7.07d, 7.43-7.44d , (4H, CH), 5.30 s(1H, OH), ^{13}C NMR (DMSO, ppm) δ: 125.5 (C1), 128.7 (C2), 131.6 (C3), 119.3 (C4), 129.7(C5), 111.1(C6), 148.7 (C7), 128.6 (C8), 170.9 (C9), 172.7 (C10), 115.5 (C11), 134.7 (C12), 13.9 (C13), 21.8(C14), 153.2 (C15), 123.4 (C16), 110.0 (C17,21), 115.9 (C18,20), 174.7 (C19).

M6 IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl 2-amino-4-methylpentanoate: Yield (85%); Yellow Powder M. P. 210 °C. Molecular formula: $C_{20}H_{24}N_4O_5S_2$ and molecular weight: 464.56 gm/mol. Elemental analysis (calculated) for $C_{20}H_{24}N_4O_5S_2$: C, 51.71; H, 5.21; N, 12.06; S, 13.80 (found) C, 51.80; H, 5.15; N, 12.15; S, 13.85, FT-IR v (cm-1), 2957, 2870 (C−H), 1608 (CH=CH), 1179 (C−N), 1269 (C−O), 3293 (N−H), ^{1}H NMR (DMSO, ppm) δ, 2.22s (3H, CH_3), 1.97s (3H, CH_3), 7.43 s, (1H, CH), 7.40-7.41t, 7.42-7.44m, (4H, CH), 8.72 s, (1H, NH), 0.77-0.78 t, 0.80-0.83 t,(6H, CH_3), 1.62-1.66m,(1H, CH), 1.85-1.89t, (2H, CH_2), 3.35-3.39 m (1H, CH),4.37 s, (2H,NH_2) ^{13}C NMR (DMSO, ppm) δ: 124.5 (C1), 129.2 (C2), 131.6 (C3), 122.3 (C4), 129.7(C5), 111.1(C6), 148.7 (C7), 128.6 (C8), 171.9 (C9), 174.7 (C10), 112.9 (C11), 133.4 (C12), 10.6 (C13), 21.5(C14), 153.2 (C15), 30.5 (C16), 30.3 (C17), 21.6 (C18), 21.5 (C19), 17.8(C20).

M7, IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl pyrrolidine-2-carboxylate: Yield (80%); White semi solid. Molecular formula: $C_{19}H_{20}N_4O_5S_2$ and molecular weight: 448.52 gm/mol. Elemental analysis (calculated) for $C_{19}H_{20}N_4O_5S_2$: C, 50.88; H, 4.49; N, 12.49; S, 14.30 (found) C,

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50.80; H, 4.55; N, 12.40; S, 14.38, FT-IR v (cm-1), 3035, 2978, 2937, (C−H), 1701(C=O), 1660 (C=N), 1593 (CH=CH), 1157 (C−N), 1259 (C−O), 3360 (N−H),

^{1}H NMR (DMSO, ppm) δ, 2.24s (3H, CH_3), 1.97s (3H, CH_3), 7.03 s, (1H, CH), 7.62-7.64 t, 7.78-7.79m, (4H, CH), 8.02 s, (1H, NH), 3.37.3.38 t, (1H, CH), 1.86-1.89 m(2H, CH_2), 1.85-1.89t, (2H, CH_2), 1.92-1.94 m, (2H, CH_2), 4.37 s, (1H,NH) ^{13}C NMR (DMSO, ppm) δ: 123.6 (C1), 129.4 (C2), 129.5 (C3), 120.1 (C4), 127.7(C5), 110.4(C6), 135.4 (C7), 128.6 (C8), 172.1 (C9), 172.0 (C10), 112.9 (C11), 132.3 (C12), 10.6 (C13), 21.5(C14), 174.5.2 (C15), 55.3 (C16), 30.2 (C17), 21.6 (C18), 54.9 (C19)

M8, IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl 2-amino-4-(methylthio) butanoate: Yield (80%); Grey semi solid. Molecular formula: $C_{19}H_{22}N_4O_5S_3$ and molecular weight: 482.60gm/mol. Elemental analysis (calculated) for $C_{19}H_{22}N_4O_5S_3$: C, 47.29; H, 4.59; N, 11.61; S, 19.93 (found) C, 47.25; H, 5.05; N, 11.60; S, 19.87, FT-IR v (cm-1), 3067, 3034, 2978, 2937 (C−H), 1701 (C=O), 1660 (C=N), 1593 (CH=CH), 1157 (C−N), 1259 (C−O), 3360 (N−H), ^{1}H NMR (DMSO, ppm) δ, 2.24s (3H, CH_3), 1.97s (3H, CH_3), 7.30 s, (1H, CH), 7.39-7.40t, 7.43-7.44m, (4H, CH), 8.78 s, (1H, NH), 2.29 s,(3H, CH_3), 3.26-3.27t,(2H, CH_2), 3.45-3.47 m(2H, CH_2),4.31-4.32 t(1H, CH), 4.37 s, (2H, NH_2), ^{13}C NMR (DMSO, ppm) δ: 125.5 (C1), 130.2 (C2), 132.6 (C3), 120.3 (C4), 128.5 (C5), 109.1(C6), 146.2 (C7), 129.2 (C8), 162.9 (C9), 171.9 (C10), 113.9 (C11), 130.4 (C12), 10.6 (C13), 21.5(C14), 170.9 (C15), 30.5 (C16), 30.3 (C17), 21.6 (C18), 21.5 (C19).

M9, IUPAC Name: 2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl 2-aminoacetate: Yield (82%); White semi solid. Molecular formula: $C_{16}H_{16}N_4O_5S_2$ and molecular weight: 408.45gm/mol. Elemental analysis (calculated) for $C_{16}H_{16}N_4O_5S_2$: C, 47.05; H, 3.95; N, 13.72; S, 15.70; (found) C, 47.10; H, 4.05; N, 13.65; S, 15.75, FT-IR v (cm-1), 3032, 2976 (C−H), 1735 (C=O), 1660 (C=N), 1593 (CH=CH), 1157 (C−N), 1259 (C−O), 3386 (N−H). ^{1}H NMR (DMSO, ppm) δ, 2.21s (3H, CH_3), 1.95s (3H, CH_3), 7.03 s, (1H, CH), 7.40-7.42t, 7.44-7.45m, (4H, CH), 8.02 s, (1H, NH), 4.37 s, (2H, CH_2), 1.35 s, (2H,NH_2) ^{13}C NMR (DMSO, ppm) δ: 122.5 (C1), 128.2 (C2), 131.8 (C3), 121.3 (C4), 127.5 (C5), 109.1 (C6), 148.7 (C7), 128.6 (C8), 171.9 (C9), 174.7 (C10), 112.9 (C11), 133.4 (C12), 12.6 (C13), 21.5(C14), 157.2 (C15), 31.5(C16).

M10, IUPAC Name: bis (2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl) oxalate: Yield (80%); White Semi solid. Molecular formula: $C_{30}H_{24}N_6O_{10}S_4$ and molecular weight: 756.81 gm/mol. Elemental analysis (calculated) for $C_{30}H_{24}N_6O_{10}S_4$: C, 47.61; H, 3.20; N, 11.10; S, 16.95 (found) C, 47.55; H, 3.25; N, 11.15; S, 16.85, FT-IR v (cm-1), 1733, 1697 (C=O), 1653 (C=N), 1593 (CH=CH), 1130 (C−N), 1251 (C−O), 3380 (N−H). ^{1}H NMR (DMSO, ppm) δ, 2.24s (6H, CH_3), 1.95s (6H, CH_3), 6.50 s, (2H, CH), 7.40-7.41t, 7.42-7.44m, (8H, CH), 7.47 s, (2H, NH), ^{13}C NMR (DMSO, ppm) δ: 126.4 (C1,1'), 128.1 (C2,2'), 131.1 (C3,3'), 128.4 (C4,4'), 129.0 (C5,5'), 119.1 (C6,6'), 143.7 (C7,7'), 128.6 (C8,8'), 172.1 (C9,9'), 174.6 (C10,10'), 119.1 (C11,11'), 134.3 (C12,12'), 10.5 (C13,13'), 21.5 (C14,14'), 174.7 (C15,15').

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M11, IUPAC Name: bis (2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl) phthalate: Yield (88%); White semisolid. Molecular formula: $C_{36}H_{28}N_6O_{10}S_4$ and molecular weight: 832.90 gm/mol. Elemental analysis (calculated) for $C_{36}H_{28}N_6O_{10}S_4$: C, 51.91; H, 3.39; N, 10.09; S, 15.40 (found) C, 51.80; H, 3.45; N, 10.15; S, 15.48, FT-IR v (cm-1), 2980, (C−H), 1732, 1716, 1698 (C=O), 1647 (C=N), 1473 (CH=CH), 1152 (C−N), 3353 (N−H), ^{1}H NMR (DMSO, ppm) δ, 2.14s (6H, CH_3), 2.01s (6H, CH_3), 6.93 s, (2H, CH), 7.31-7.32t, 7.44-7.45m, (8H, CH), 8.62 s, (2H, NH), 7.23-7.24d, 7.27-7.28t, 7.37-7.38d (4H, CH), ^{13}C NMR (DMSO, ppm) δ: 124.1 (C1,1'), 127.4 (C2,2'), 129.3 (C3,3'), 122.7 (C4,4'), 127.0 (C5,5'), 111.9 (C6,6'), 147.1 (C7,7'), 127.9 (C8,8'), 172.2 (C9,9'), 173.5 (C10,10'), 112.9 (C11,11'), 134.4 (C12.12'), 11.6 (C13,13'), 21.5(C14,14'), 157.2 (C15,22), 130.5 (C16,21), 128.4 (C17,20), 127.6 (C18,19).

M12, IUPAC Name: bis (2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl) succinate: Yield (85%); White powder M. P. 280°C. Molecular formula: $C_{32}H_{28}N_6O_{10}S_4$ and molecular weight: 784.86 gm/mol. Elemental analysis (calculated) for $C_{32}H_{28}N_6O_{10}S_4$: C, 48.97; H, 3.60; N, 10.71; S, 16.34 (found) C, 48.90; H, 3.65; N, 10.60; S, 16.40, FT-IR v (cm-1), 2981 (C−H), 1716, 1698 (C=O), 1653, 1635 (C=N), 1473 (CH=CH), 1175 (C−N), 1266 (C−O), 3196 (N−H), ^{1}H NMR (DMSO, ppm) δ, 2.12s (6H, CH_3), 2.07s (6H, CH_3), 6.82 s, (2H, CH), 7.38-7.40t, 7.42-7.44m, (8H, CH), 8.32 s, (2H, NH), 2.79-2.80 m(4H, CH_2), ^{13}C NMR (DMSO, ppm) δ: 124.7 (C1,1'), 128.9 (C2,2'), 132.2 (C3,3'), 121.3 (C4,4'), 128.7(C5,5'), 109.1(C6,6'), 147.5 (C7,7'), 127.6 (C8,8'), 162.1 (C9,9'), 163.7 (C10,10'), 112.2 (C11,11'), 132.6 (C12,12'), 10.5 (C13,13'), 21.7(C14,14), 156.2 (C15,15'), 30.5 (C16,16'),

M13, IUPAC Name: bis (2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl) 2-aminopentanedioate. Yield (85%); Yellow Powder, M. P. 290 °C. Molecular formula: $C_{33}H_{31}N_7O_{10}S_4$ and molecular weight: 813.90 gm/mol. Elemental analysis (calculated) for $C_{33}H_{31}N_7O_{10}S_4$: C, 48.70; H, 3.84; N, 12.05; S, 15.76, (found) C, 48.80; H, 3.80; N, 12.15; S, 15.70, FT-IR v (cm-1), 2971 (C−H), 1717, 1698 (C=O), 1653, 1636 (C=N), 1473 (CH=CH), 1171 (C−N), 1262 (C−O), 3356, (N−H), ^{1}H NMR (DMSO, ppm) δ, 2.12s (6H, CH_3), 1.95s (6H, CH_3), 6.67 s, (2H, CH), 7.34-7.36t, 7.47-7.49m, (8H, CH), 8.32 s, (2H, NH), 3.35-3.39 t (1H, CH), 1.56-1.57 m(2H, CH_2), 3.01-3.02m(2H, CH_2), 4.37 s, (2H,NH_2) ^{13}C NMR (DMSO, ppm) δ: 125.6 (C1,1'), 129.8 (C2,2'), 131.5 (C3,3'), 123.3 (C4,4'), 129.1 (C5,5'), 111.5 (C6,6'), 146.5 (C7,7'), 127.9 (C8,8'), 161.3 (C9,9'), 164.7 (C10,10'), 115.9 (C11,11'), 133.2 (C12,12'), 10.2 (C13,13'), 21.7(C14,14'), 159.2 (C15), 42.5 (C16), 30.3 (C17), 25.6 (C18), 161.5 (C19)

M14, IUPAC Name: bis(2-methyl-3-((5-methylthiazol-2-yl)carbamoyl)-1,1-dioxido-2H-benzo[e][1,2]thiazin-4-yl) 2,3-dihydroxysuccinate: Yield (90%); White Crystalline Powder, M. P. 230°C. Molecular formula: $C_{32}H_{28}N_6O_{12}S_4$ and molecular weight: 816.86 gm/mol. Elemental analysis (calculated) for $C_{32}H_{28}N_6O_{12}S_4$: C, 47.05; H, 3.45; N, 10.29; S, 15.70 (found) C, 47.01; H, 3.35; N, 10.36; S, 15.80, FT-IR v (cm-1), 2809 (C−H), 1698 (C=O), 1653 (C=N), 1615 (CH=CH), 1188 (C−N), 1250 (C−O), 3337, 3275 (N−H), 3562

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(O-H), ^{1}H NMR (DMSO, ppm) δ, 2.24s (6H, CH_3), 1.98s (6H, CH_3), 6.98 s, (2H, CH), 7.44-7.45t, 7.49-7.50m, (8H, CH), 7.82 s, (2H, NH), 4.44-4.43d(2H, CH), 3.17 s, (2H,OH) ^{13}C NMR (DMSO, ppm) δ: 126.1 (C1,1'), 127.2 (C2,2'), 131.4 (C3,3'), 120.9 (C4,4'), 128.7(C5,5'), 111.5 (C6,6'), 151.2 (C7,7'), 128.4 (C8,8'), 169.1 (C9,9'), 165.7 (C10,10'), 114.6 (C11,11'), 135.4 (C12,12'), 10.2 (C13,13'), 29.5(C14,14'), 167.2 (C15,15'), 67.5 (C16,16').

M15, IUPAC Name: bis (2-methyl-3-((5-methylthiazol-2-yl) carbamoyl)-1, 1-dioxido-2H-benzo[e] [1, 2] thiazin-4-yl) 2-aminosuccinate: Yield (90%); Grey Semi Solid. Molecular formula: $C_{32}H_{29}N_7O_{10}S_4$ and molecular weight: 799.87gm/mol. Elemental analysis (calculated) for $C_{32}H_{29}N_7O_{10}S_4$: C, 48.05; H, 3.65; N, 12.26; S, 16.04 (found) C, 48.10; H, 3.70; N, 12.20; S, 16.10, FT-IR v (cm-1), 3067, 3034, 2978, 2937 (C−H), 1720, 1701 (C=O), 1660 (C=N), 1593, (CH=CH), 1157 (C−N), 1259 (C−O), 3362 (N−H), ^{1}H NMR (DMSO, ppm) δ, 2.14s (6H, CH_3), 1.87s (6H, CH_3), 6.77 s, (2H, CH), 7.45-7.46 t, 7.52-7.53 m, (8H, CH), 7.72 s, (2H, NH), 3.11-3.12 t (2H, CH_2), 3.69-3.70 m (1H, CH), 4.37 s, (2H,NH_2) ^{13}C NMR (DMSO, ppm) δ: 125.1 (C1,1'), 129.8 (C2,2'), 131.8 (C3,3'), 124.3 (C4,4'), 131.7 (C5,5'), 113.1 (C6,6'), 153.7 (C7,7'), 129.9 (C8,8'), 165.4 (C9,9'), 163.1 (C10,10'), 115.2 (C11,11'), 135.7 (C12,12'), 10.5 (C13,13'), 21.2 (C14,14'), 158.2 (C15), 45.5 (C16), 35.6 (C17), 168.2 (C18).

In Vitro Biological study

Brine Shrimp Cytotoxicity Assay

Brine shrimp cytotoxicity assay was performed on 96 well microplate. Artemia salina eggs were put for incubation till hatched. Incubation time was 2days at 30^{o}C in sea water having 38g/L supplement and 6mg/L yeast. The above solution was pre-saturated with oxygen. For this objective, a special tank was designed having one large and one small compartment divided by a porous wall. Eggs were placed in the large compartment covered with aluminum foil and the lamp was placed in small compartment to keep it constantly illuminated. After hatching of larvae, these larvae moved through the pores, and, with use of Pasteur pipette, calculated into petri dish, and transferred into 96 well microplate which contains the solution of sea water and DMSO with concentration less than 1%. These synthesized derivatives were tested in three different concentration 40, 20 and 10 5µg/ml. DMSO is used as negative control and doxorubicin was used as positive control at concentration of 0.4mg/ml. The extent of fatality was determined after 24hrs of compound addition. The number of surviving larvae were counted. Curve 2Dv5.11 was used for determination of median lethal concentration LC50 with mortality ≥50% (24).

Inhibition Assay of α-amylase

Inhibition of α-amylase of synthesized derivatives was determined. 25µl α-amylase (0.14 U/ml) in phosphate buffer of pH 6.8 (15µl), 40µl of potassium phosphate having concentration of 2mg/ml with starch solution, 10µl sample of 1mg/ml DMSO concentration was put for incubation in 96well plate at temperature 50°C for 30minutes. To cease the

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reaction, 20µl of 1M HCl was added into the reaction mixture. Subsequently followed by the addition of 90µl of iodide reagent (iodine 5mMolar and potassium iodide 5mMolar). Blank solution was prepared by mixing phosphate buffer with DMSO. Negative control has only DMSO and positive control has acarbose 250µM. Absorbance of reaction mixture was determined at 540nm (25). The % inhibition of α-amylase was determined by following formula.

$$\% \text{ α-amylase inhibition} = (O_s - O_n)/ (O_b - O_n) \times 100$$

Where, $\mathbf{O_s}$ = sample Absorbance, $\mathbf{O_n}$**=** negative control absorbance & $\mathbf{O_b}$ = blank absorbance (26)

Urease Inhibition Activity

For the Quantification of enzyme urease and ammonia, indophenol method is used. The absorbance process is used for determination of urease inhibition activity (27). In short, 40 µl buffer having pH 8.2 (EDTA 1mM, urea 100 mM, $K_2HPO_4$0.01 M, 0.01 M $LiCl_2$), 10 µl of enzyme (5U/ml) and 10µl of test compound were incubated in a 96 well plate at 37^oC for 10 min. Furthermore, solutions of 40µl of alkali reagent (NaOH 0.5% w/v, NaOCl 0.1%) and 40µl of phenol reagent having sodium nitroprusside 0.005% w/v and phenol 1% w/v was added to each well. Thiourea was used as standard inhibitors of urease. Experiments were performed in triplicate. Absorbance was checked at 625nm. Bio-TekELx 800 microplate reader is used to perform these analysis and percent inhibition was calculated by using following equation.

100 - (OD test well/ OD control) * 100.

The results were calculated using PRISM 0.5 (GraphPad, San Diego, CA, USA).

Insilico studies

Protein Structure Preparation

From Protein Data Bank, the crystal structure of urease enzyme (PDB ID: 1E9Y) was obtained (28). Already present ligand in crystal structure was removed from protein. Protein was modified by adding polar hydrogens and Kollman charges, and water molecules were removed (29). Final files were stored in PDBQT format to facilitate analysis in a later stage of simulation (30).

Ligand Preparation

The structures of all derivatives were built and their geometries were optimized by using the semi-empirical quantum mechanical method PM3. All the derivatives were prepared for docking by building their 3D conformers by ChemDraw 12.0 in the SDF format. For docking, converted SDF to PDBQT format. The Pyrex 0.08 programmed imported all tested compounds into Open Babel (31), where they were subjected to energy minimization. The energy was reduced via conjugate gradient approach by using Universal force field and energy difference less than 0.01 kcal/mol. For further analysis, the compounds having less energy were then converted to PDBQT format to carry out

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the docking simulation

3. RESULTS

All the newly synthesized compounds were checked for their yield, physical state, solubility and melting points. Esters are polar molecules, and these were soluble in organic solvents. In IR spectra of products, the presence of characteristic bands was checked in series (M1-M15) and the presence of carbonyl functional group indicated the formation of esters.

Biological Evaluation

Brine Shrimp Cytotoxicity

Cytotoxicity results of 15 synthesized derivatives along with parent drug meloxicam are listed in Table 1.

Table 1: Brine shrimp cytotoxicity assay of derived esters %mortality and LC-50

Compound	% Mortality	LC50
M	20	--
M1	60	21.93
M2	70	19.08
M3	20	--
M4	20	--
M5	30	--
M6	40	--
M7	40	--
M8	40	--
M9	10	--
M10	50	11.82
M11	20	--
M12	10	--
M13	40	--
M14	30	--
M15	50	9.624
Positive (Doxorubicin)	80	--
Negative (DMSO)	20	--

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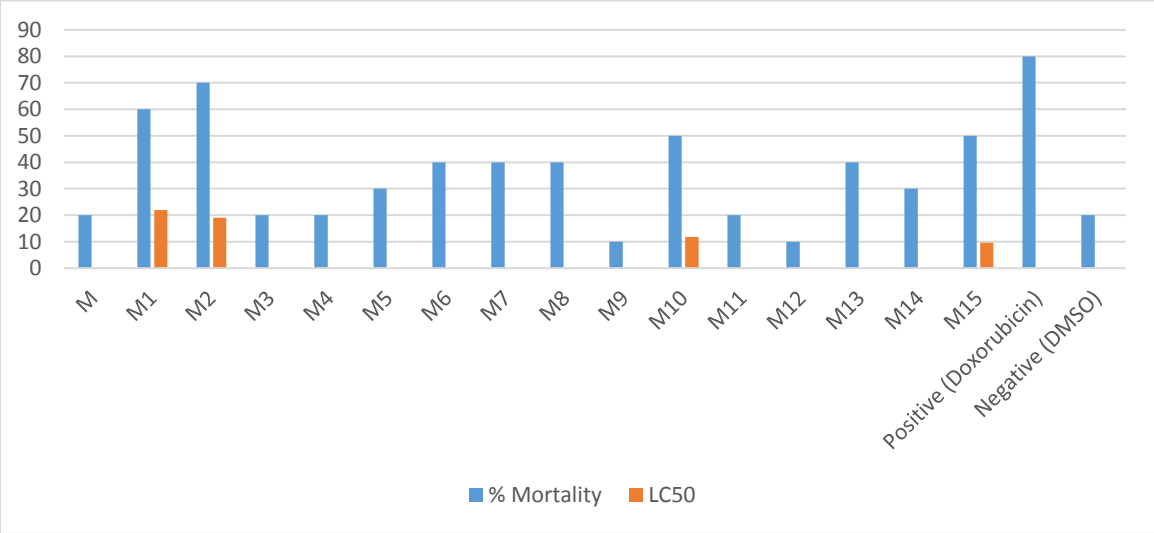
Graph 1: Brine shrimp cytotoxicity assay



α- Amylase Inhibition Assay

All the 15 ester derivatives of meloxicam were subjected to standard chromogenic α-amylase inhibition Assay and the results are prepared in Table 2.

Table 2: α- amylase percent inhibition

Compound	Percent Inhibition
M1	--
M2	--
M3	--
M4	1.361
M5	2.041
M6	3.741
M7	5.102
M8	--
M9	1.61
M10	1.360
M11	1.531
M12	--
M13	--
M14	--
M15	1.871
Positive (Acarbose)	6.102
Negative (DMSO)	--

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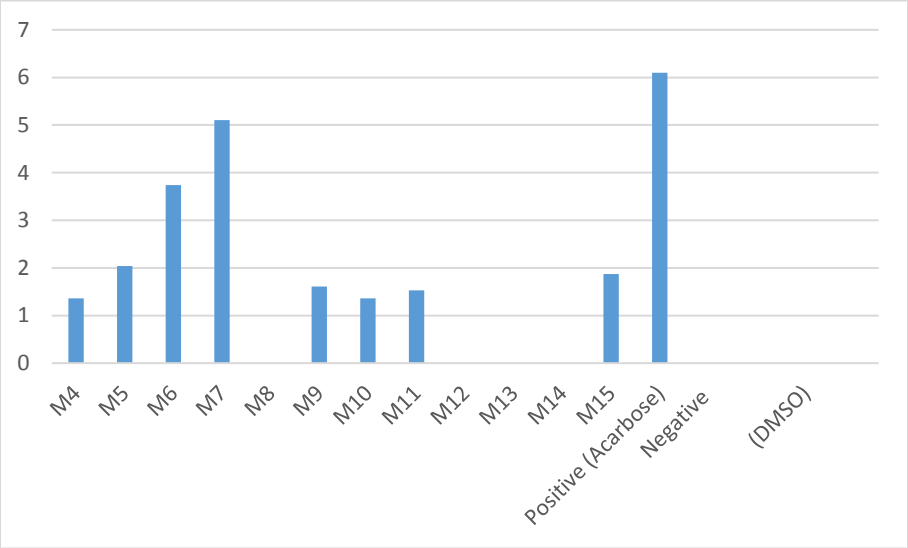
Graph 2: α amylase percent inhibition



In-vitro Anti-Urease Activity

All 15 derivatives were tested for anti-urease enzyme activity at 0.1 mM and results are listed in table 3.

Table 3: Anti-urease Enzyme Activity

Compound	IC50±SEM (μM)
M	>200
M1	14.8±1.37
M2	>200
M3	1.78±0.12
M4	28.8±2.37
M5	>200
M6	>200
M7	5.13±1.07
M8	4.86±0.97
M9	5.88±1.01
M10	4.98±0.17
M11	>200
M12	>200
M13	1.08±0.04
M14	>200
M15	1.98±0.11
Thiourea	4.24±0.13

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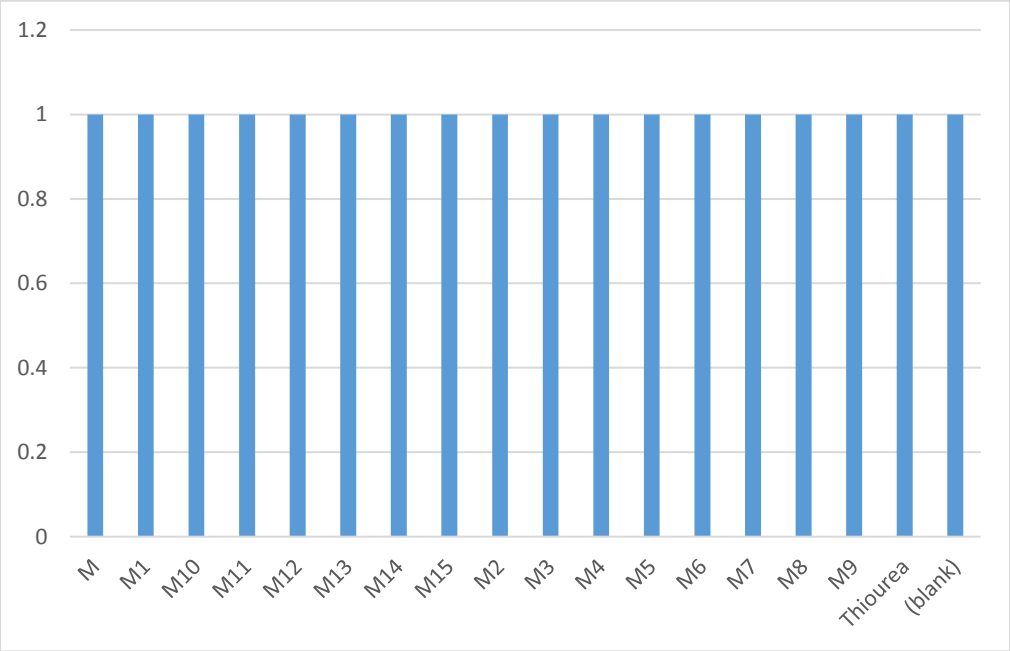
Graph 3: anti-urease enzyme IC50



Molecular Docking Analysis

Molecular docking study was done on all derivatives and their binding energy and urease inhibition constant are listed in Table 4. Moreover, interaction of ester derivatives with helicobacter pylori urease enzyme are listed in Table 5.

Table 4: Binding energy and urease inhibition constant Ki

Compound	Binding energy	Urease Inhibition constant Ki μM
M	-10.45	0.03684
M1	-0.69	0.97980
M2	-0.83	0.41028
M3	-5.72	0.06376
M4	-5.32	0.12585
M5	-8.49	0.0124
M6	-10.79	0.00221
M7	-4.10	0.31392
M8	-3.04	0.59121
M9	-3.62	0.59651
M10	1.58	----
M11	-5.22	0.15003
M12	-4.86	0.27485
M13	-4.81	0.29759
M14	-5.83	0.5319
M15	-2.54	0.1366

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Table 5: Interaction of esters with helicobacter pylori urease enzyme

Target	Compound	Residues forming H bonds	Distance between H bonds (Å)	Residues participating in Hydrophobic interactions	Distance between hydrophobic interactions (Å)	Residues participating in ELECTROSTATIC/ π-Stacking/other	Distance between Electrostatic interactions (Å)
Urease Enzyme (1E9Y) Meloxicam Ester Derivative	**Meloxicam**	HIS 136	3.14	THR 171	3.44	HIS 136	4.46
		THR 171	1.96	ALA 246	3.91	HIS 221	4.51
		ILE 220	2.94	HIS 248	3.91	HIS 221	4.28
		HIS 248	2.30				
		HIS274	2.76				
	M 1	ASP 67	3.10	ASP 90	3.64		
		GLY 452	3.23	PHE 454	3.77		
		ASP 488	2.65				
	M 2	GLN 471	2.94	TYR 474		MET 12	
		VAL 473	2.83				
	M 3	ALA37	2.88	TYR32	3.49		
		LYS445	2.84	VAL33	3.73		
		GLN 471	2.81	VAL36	3.09		
		VAL473	3.26	PRO472	3.89		
				TYR474	3.32		
	M 4	GLY 453	2.93	PHE454	3.74	GLY 453	3.09
				455 ILE	3.15	ILE 450	2.93
						LEU 121	2.70
	M 5	LYS445	2.39	PRO472	3.83	TYR 474	3.69
		GLN 471	2.62				
	M 6	PHE139	3.39	ILE 137	3.90	PHE139	4.32
		SER 141	3.05	PRO142	3.61		
				144GLN	3.54		
				145 ILE	3.31		
				189 TYR	3.34		
				193 LEU	3.74		
	M 7	GLU 393	2.69	Lys 394	3.90	LYS 403	4.19
		LYS 394	2.11	Asn399	3.39	GLU 393	
		ASN 397	1.70				
		PHE 400	2.45				
		LYS 403	2.28				
	M 8	SER 151	3.60	THR 147	3.49	TYR 475	5.15
		TYR 475	3.50	ALA 150	3.27		
				LYS 445	3.44		
				TYR 474	3.58		
				TYR 475	3.26		
	M 9	ASP 67	3.20	ASP67	3.84		
		GLY 91	2.10	LEU 68	2.93		
				GLU 119	3.93		
				PHE 454	3.41		
	M 10			SER466	3.34		
				ILE467	3.33		
	M 11	THR469	3.99	GLN 471	3.87		
	M 12	LYS 445	3.17	TYR 474	3.51		
		GLNN 471	2.80				
		VAL 473	2.85				

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	M 13	LYS 92	1.98	GLU 421	3.68	TYR 422	4.87
		GLU 421	2.36	LEU 514	3.77	LYS 92	5.16
		VAL 431	3.61				
	M 14	GLN 471	2.95	LYS 445	3.59		
		VAL 473	2.28				
	M15	ALA 497	3.63	TYR 241	3.72		
				ASP 242	3.32		
				GLN 496	3.22		
				ALA 497	3.77		

4. DISCUSSION

Mostly synthesized derivatives were soluble in polar solvents. Melting points of esters was enhanced as compared to parent drug meloxicam.

Almost all the compounds were biologically active. Meloxicam parent compound exhibited 20% of mortality rate as compared to positive control which exhibited 80% mortality rate. The most active derived compounds M1, M2 M10 and M15 have shown 60, 70, 50 and 50% mortality rate respectively comparable to the positive control. These four compounds exhibited LC50 as 21.93, 19.08, 11.82 and 9.624 respectively (Table1).

α- amylase inhibitory effect of all the synthesized derivatives and parent molecules was observed. Among the synthesized derivatives M4, M5, M6, M7, M10 and M11 had shown α amylase inhibitory effect as 1.361, 2.041, 3.741, 1.61, 1.360, 1.531 and 5.102 respectively. (Table 2)

Some of the derivatives have shown significantly high enzyme inhibition activity like M5 and M6 (0.01224 and 0.00221 µM respectively). Some derivatives have exhibited the moderate significant activity as compared to the parent compound meloxicam. Among moderately significant active compounds are M4, M7, M11, M12, M13 and M14 as 0.06376, 0.01285, 0.31392, 0.59121, 0.59651, 0.15003, 0.27485, 0.29759 and 0.5319 µM respectively. The other derivatives M1, M2, M10 and M15 have shown insignificant activity as compared to meloxicam. Urease inhibition constant Ki of Meloxicam is 0.03684 µM (Table 4).

From molecular docking, it was concluded that the derived esters of meloxicam having aliphatic side chain are less active in contrary to aromatic side chain. Among aromatic side chain, unsubstituted aromatic side chain have shown significantly better inhibitory activity than the substituted aromatic side chain.The urease inhibition activity was further confirmed by using computational molecular docking of all the derivatives. Meloxicam being parent molecule have binding energy -10.14 kcal. Among the ester derivatives, M5 and M6 have shown significant binding energy as -8.45 and -10.79 kcal/mol, respectively. M6 binding energy is highest than the Meloxicam. The other compounds M3, M4, M7, M8 M9, M11, M12, M13 and M14 have shown low binding energy as compared to the parent molecule. M1.M2 and M10 have shown less affinity towards the Helicobacter pylori urease protein.

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Meloxicam has shown different interaction with docked protein. These interactions include H-bonding, Hydrophobic interaction and π-Stacking. It has shown that hydrogen bond with residues HIS-136A, THR-171A, ILE-220A, HIS 248A and HIS-274A of bond length 3.14, 1.96, 2.94, 2.30 and 2.74 Å. All these bond lengths are less and made strong interaction with protein molecule. The strong H-bonding is due to enolic oxygen. Meloxicam also exhibited hydrophobic interaction THR 171, ALA 246 and HIS 248 π stacking with HIS 136, HIS 221 and HIS 221. M1, the derived ester of meloxicam, have also exhibited hydrogen bonding interaction with amino acid residues ASP 67 GLY 452 and ASP 488. It also shows strong interaction but less than meloxicam due to absence of enolic oxygen. Less binding energy of M1 was due to absence of π stacking because of the absence of aromatic side chain. In M1, two molecules of meloxicam are attached with oxygen of oxalic acid and made interaction with pocket residues and aromatic side chain of parent molecule could not form any interaction with residues.

M2 is cinnamic acid derived ester of meloxicam. It has shown H-bonding interaction with two residues of amino acid side chain GLN 471 and VAL 473. The aliphatic side chain of cinnamic acid renders the interaction of ester to pocket the atoms and this is the reason of less activity of M2 ester derivatives.

M3 also showed the H-bonding higher than the M2 due to polar hydroxyl group attached with aromatic side chain. This polar hydroxyl side chain renders the π-stacking with the side chain residue. The H-bond interaction forming residues are ALA37, LYS445, GLN 471 and VAL473 having bond length 2.88, 2.84, 2.8 and 3.26Å respectively.

M4 also exhibited medium range bonding interaction with protein residues GLY 473 of 2.93Å bond length with active amino group that is attached with aromatic ring. Being electron donating group, free amino group helped in formation of hydrophobic and π-stacking with amino acid residues PHE454 and ILE455 (hydrophobic) GLY 453 ILE 450 and LEU 121 π stacking.

M5 have exhibited significant invitro activity because it forms strong H-bonding with two amino acid residues LYS445 2.39Å and GLN 471 2.62Å. The hydrophobic side chain benzene ring helped in formation of hydrophobic interaction with PRO-472 and π-stacking interaction with aromatic ring of benzene.

M6 has exhibited less H-bonding interaction due to hydrophobic nature of esters and it also exhibited more binding energy among all the derived esters. In vitro activity has shown the highest inhibitory constant. All these are due to the strong hydrophobic interaction with amino acid residues like ILE 137(3.90 Å), PRO14 (3.61 Å), GLN 144 (3.54 Å), ILE145 (3.31 Å), TYR 189 (3.34 Å) and LEU 193 (3.74 Å).

M7, M8 and M9 are moderately active esters and exhibited moderately significant inhibitory effect. All these are active due to aromatic side chain and exhibited hydrophobic interaction with in range of 2.93Å to 3.93Å bond length.

M10 inactive compound against the helicobacter pylori urease enzyme.

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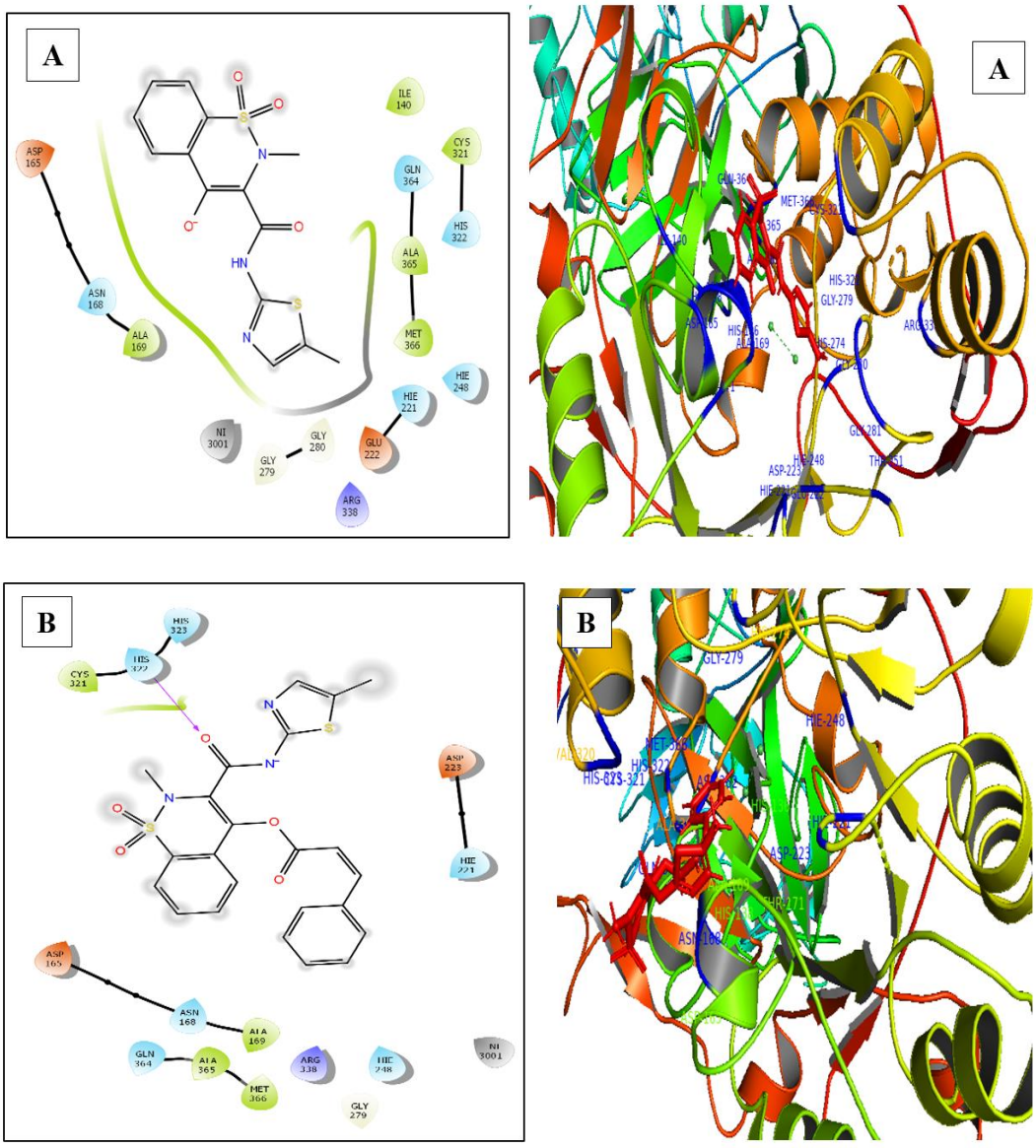
M11, M12, M13 and M14 are moderately active compound because these have H-bond interaction and hydrophobic interactions. These compounds did not exhibited π-stacking due to aliphatic side chain molecule. M13 exhibited strong H- bond interaction with amino acid residue LYS-92. The bond length is minimum about 1.92Å. This strong hydrogen bonding is due to free amino group attached with aliphatic side chain.

M15 has one H-bond interaction with ALA-497 with bond length 3.67Å and TYR 241, ASP 242, GLN 496 and ALA 497 hydrophobic interaction. But there is no π stacking amino acid residue due to absence of aromatic side chain.



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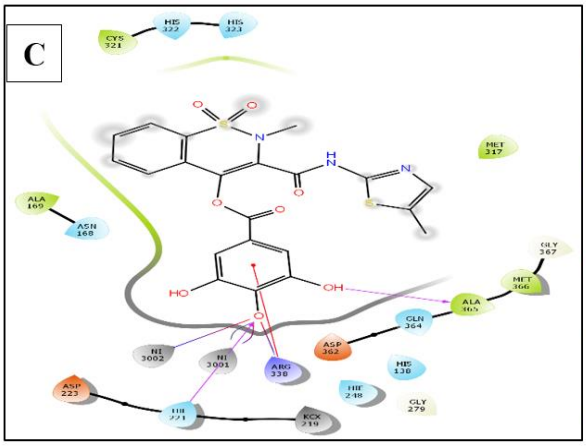

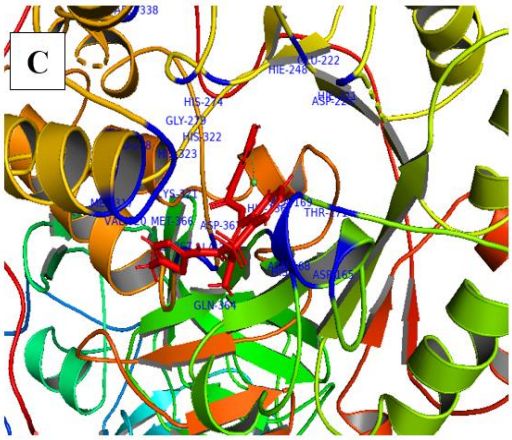

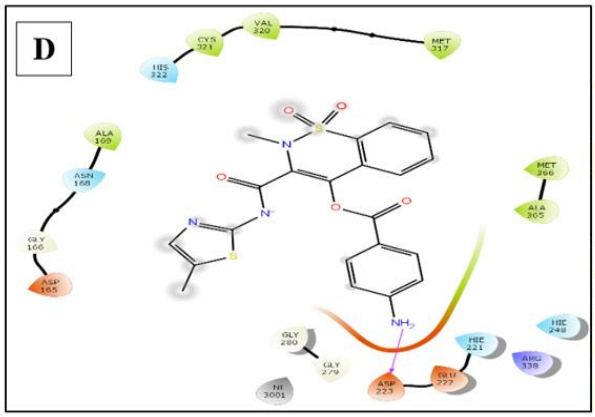

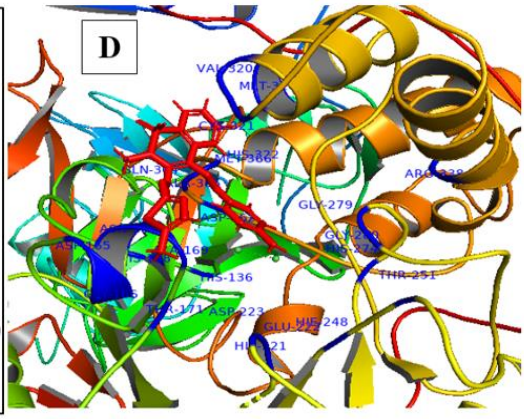

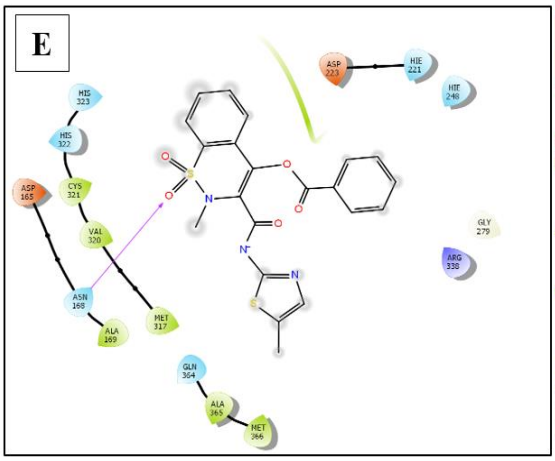

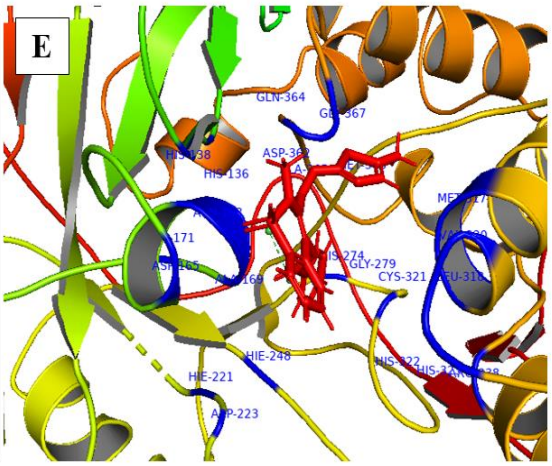

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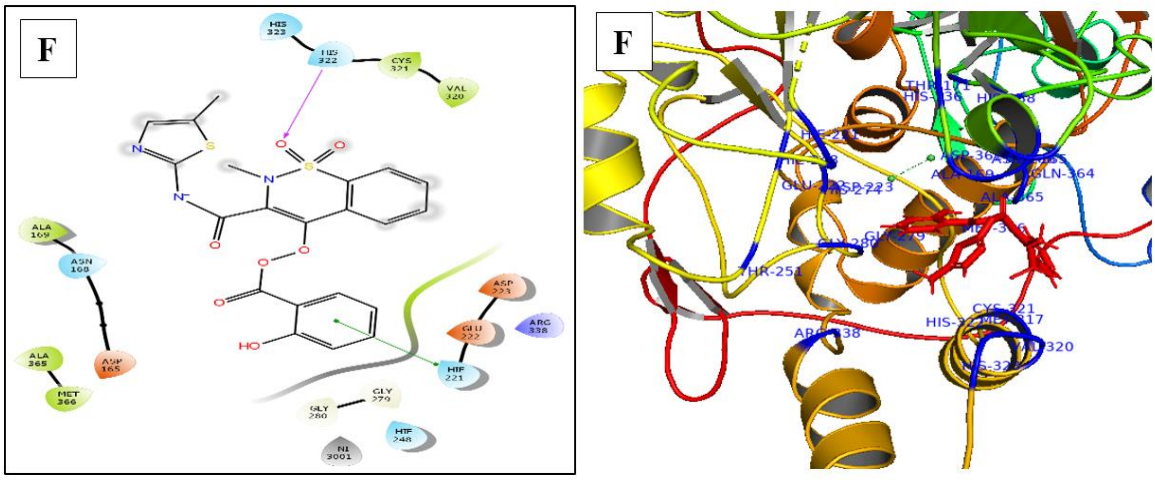

Fig 4: Derivatives Interaction with Protein (1E9Y) residue, (A) Meloxicam, (B) Derivative M2

(C) Derivative M3, (D) Derivative M4, (E) Derivative M6, (F) Derivative M7

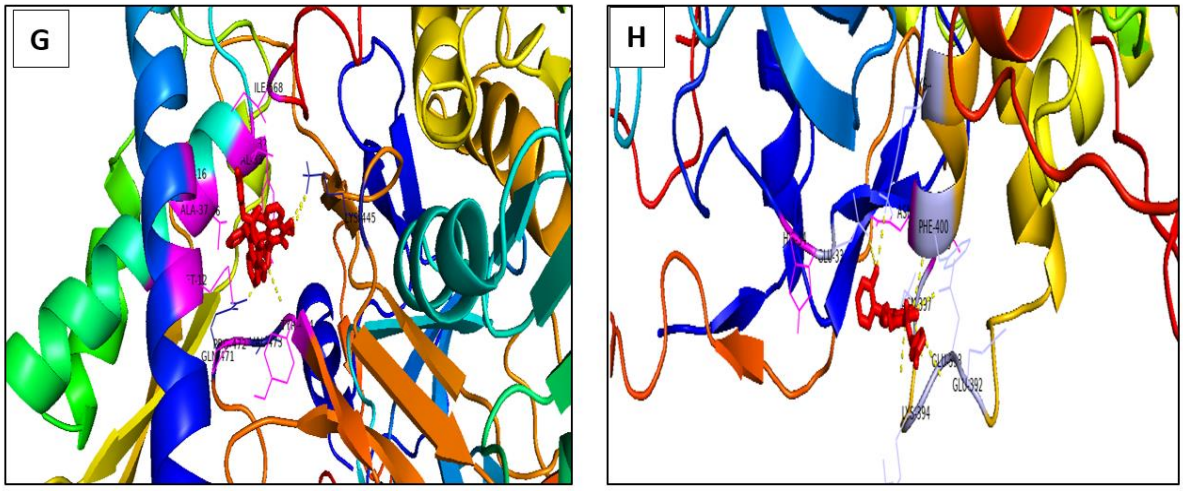

Figure 5: 3D View of Derivatives interaction with Protein IE9Y residue (G) Derivatives M5, (H) Derivative M7

5. CONCLUSION

Synthesis of meloxicam derivatives was carried out by condensation of enolic group of meloxicam with available carboxylic acid moieties. All the synthesized compounds were physically and chemically characterized and in-vitro studies like brine shrimp toxicity assay, α amylase inhibition and urease inhibition activity was performed. We concluded that the synthesized esters of meloxicam are biologically active compound. M5 and M6 have shown potential activity against α amylase and helicobacter pylori urease inhibitory

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effect. We also calculated binding energy of these compounds. We concluded the coherence between anti-urease screening results and computational analysis. Our results suggest that synthesized derivatives exhibited more urease enzyme inhibition potential as compared to parent drug meloxicam. These derivatives may be used as potential anti-helicobacter Pylori agents for the treatment of gastric mucosal infections caused by NSAIDS. We also calculated binding energy of all derivatives. Further research may be carried out for optimization of these compounds in search of potent Helicobacter-Pylori urease inhibitors.

Structure Activity Relationship of Meloxicam

The SAR of oxicams has been extensively explored for optimization of anti-inflammatory activity, mainly during the first decades when the class of NSAIDs was introduced (32, 33). The bitterness of meloxicam drug is due to its phenolic -OH group. So phenolic -OH group is substituted to mask its bitter taste.

Consent for Publication

Not available

Conflict of Interest

The authors declare no conflict of interest financial or otherwise.

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