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AN EFFECTIVE NOVEL RED BRICK POWDER CATALYSED SYNTHESIS, IN-SILICO AND IN-VITRO ANTI-INFLAMMATORY STUDIES OF IMIDAZOL-5-ONE DERIVATIVES

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ABSTRACT

In-silico studies for targets were carried out using PyRx and various softwares. The compounds with highest binding affinities were selected for synthesis and biological evaluation. Oxazolin-5-ones were prepared by condensation of hippuric acid and various substituted benzaldehydes. Imidazole-5-ones were synthesized by the reaction between various oxazolin-5-ones with substituted anilines, sulfanilamide, sulfamethoxazole, and 4-amino antipyrine in the presence of red brick powder as a green catalyst. *In-vitro* anti-inflammatory activity was performed by modified protein denaturation (Bovine serum albumin) assay method for the compounds. Compounds SW-7 and SW-8 showed significant activity when compared with standard diclofenac sodium.

KEYWORDS: Oxazolin-5-one, Imidazol-5-one, Green catalyst, Red Brick powder, anti-inflammatory.

1. INTRODUCTION

Inflammation can be defined as the local response produced by living mammalian tissues to injury due to any agent. It is a defense reaction of living body in order to eliminate or reduce the spread of injurious agent, followed by removal of the dead/infected cells and tissues. The agents causing inflammation can be broadly classified into infective agents (bacteria, viruses and their toxins), immunological agents (cell mediated and antigen-antibody reactions), physical agents (heat, cold, radiation, and mechanical trauma), chemical agents (organic and inorganic poisons) and inert materials such (foreign bodies).[1] The activated endothelium, macrophages, and mast cells present in the tissues quickly recruit neutrophils in the inflammatory areas in response to tissue damage by secreting particular mediators. Neutrophils are the first effectors of the inflammatory response, and they are recruited through a four-step process that includes L-, P, and E-selectin-mediated activation to promote cell rolling along the vascular endothelium, leukocyte integrin activation, neutrophil immobilization on the vascular endothelium, and transmigration to inflammatory sites.[2] When macrophages are activated, they produce growth factors and cytokines that recruit various types of inflammatory cells to the inflamed area. All of these inflammatory response effectors play a role in maintaining the body's defense against harm. The inflammatory response is self-limiting, and its duration is controlled by a number of substances that have both pro-inflammatory and antiinflammatory properties. TGF (transforming growth factor) is an anti-inflammatory mediator that is released in response to the phagocytosis of apoptotic cells and aids to the resolution of inflammation by allowing inflammatory cells to be cleared quickly. If the inflammatory response lasts too long, chronic inflammation develops, which is marked by the existence of lymphocytes and macrophages with aberrant morphology that secrete growth factors and cytokines on a regular basis. By creating a microenvironment that promotes cell proliferation and predisposes to cancer, continuous production of inflammatory mediators can lead to tissue and DNA damage.[3]

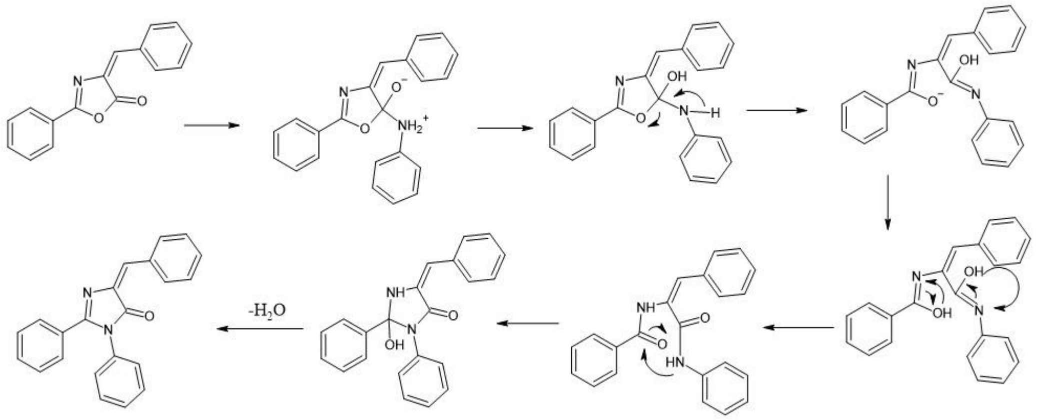


Fig 1: Mechanism of synthesis of imidazol-5-one from oxazol-5-one

Imidazoles

Imidazoles are organic heterocyclic compounds that possess two nitrogen atoms in their ring system. The presence of Imidazole skeleton in living organisms in the form of histamine, histidine, biotin, alkaloids and nucleic acids performs various biological activities. They constitute a very important class among the medicinal compounds and also present in many natural products like pilocarpine, allantoin. The benzimidazole nucleus appears in vitamin B12 (Cobalamin).[4] Imidazoles have huge and wide therapeutic application in medical field like analgesic[5], anti-allergic[6], antibacterial[7], anti-cancer[8], anti-diabetic[9], anti-fungal[10], anti-inflammatory[11], anti-leishmanial[12], anti-oxidant[13], antipyretic[14], anti-tubercular[15] and anti-viral[16] agents. Imidazol-5-one is a planner five membered heterocyclic ring system having nitrogen atoms at 1 and 3 positions and a carbonyl group at 5-position.

Red brick powder as catalyst

Catalysis is the process of increasing the rate and yield of the reaction via using various chemicals substances and the substances used for catalysis are known as catalysts. Majorly there are two type of catalysts, homogeneous and heterogeneous catalysts, classified based upon the phase (solid/liquid) in which the catalyst is present with respect to the reaction medium. Catalysis can help to improve the atom economy, in addition to that most of heterogeneous catalysts can be recyclable and reduces the power consumption via decreasing the time to complete the reaction, hence they are cost effective in nature.[17] It has been proven that clay based materials are effective in many chemical reactions by catalyzing the reactions and these catalysts are greener in nature (non-hazardous). Usually clay materials are acidic, with high surface area exhibit better catalytic property via better ion-exchange properties. Hence, there is a constant need to develop selective, cost effective, ecofriendly and recyclable clay material. Red brick clay is a prominent example for the clay based catalyst because it contains various minerals like SiO_2, Fe_2O_3, Al_2O_3 as well as Ca, Mg and K in small proportions which act as catalyst. So in this research work, we are utilizing red brick clay powder as novel catalyst in the synthesis of imdazol-5-ones from oxazol-5-ones via amidification followed by dehydration.[18]

2. MATERIALS AND METHOD

2.1 Chemistry

All the required chemicals were procured from SPECTRUM, CENTRAL DRUG HOUSE (CDH), S.D. Fine Chem. Ltd. Melting points of the synthesized compound was detected by MEPA melting point apparatus by LABINDIA. Purity of the compounds was checked by thin layer chromatography (TLC) using Silica gel 60 F_{254} pre-coated plates by Merck and combination of mobile phase. The spots resolved were visualized by using UV and iodine chamber. The IR spectra of the synthesized compounds were recorded in FT-IR Bruker Alpha

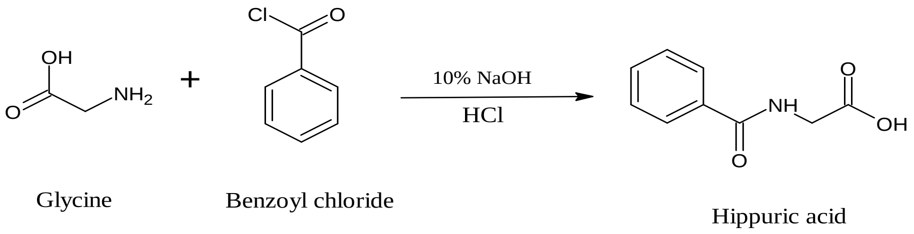
2 in RLJ Pharma Solutions, in the ranges of 400-4000 and the values of Vmax were reported in cm^{-1}. ^{1}H-NMR spectra were recorded in Bruker 400MHz using $CDCl_3$ and chemical shifts (δ) are reported in parts per million downfield from internal reference tetramethylsilane (TMS) in Department of Science and Technology-SAIF, Dharwad.

Synthesis of Imidazole derivatives.
Synthesis of imidazole derivatives was done in 3 steps.
Step 1: Synthesis of hippuric acid.
Step 2: Synthesis of oxazol-5-ones from hippuric acid.
Step 3: Synthesis of imidazole-5-ones from oxazol-5-ones.

2.1.1. Synthesis of hippuric acid (Y)

Dissolve glycine (0.01mol) in 10% NaOH solution in a flask and add benzoyl chloride (0.012mol) to it. Plug the mouth of flask with cotton plug and shake vigorously until smell of benzoyl chloride can no longer be detected. Then add 1- 2 drops of conc. HCl to make the mixture acidic. Filter the product, wash with water and recrystallize the dried product from boiling water.

Yield: 76%, Appearance: White Crystal Solid, MP: 187-192°C, Rf value (chloroform: ethyl acetate): 0.6, FTIR cm^{-1}: 3332 (NH), 2986(Ar-CH), 1740 (C=O), 1603(C=O-NH), 1545 (C=C), 1300(C-N).



Step 1: Synthesis of Hippuric acid (Y)

2.1.2. Synthesis of 4-benzylidene-2-phenyl-1,3-oxazol-5-one (1B).

A mixture of hippuric acid (0.01 mol), benzaldehyde (0.01 mol), 10 mL of acetic anhydride and anhydrous sodium acetate (0.02mol) was heated with constant shaking in a round bottomed flask. As soon as the mixture was liquefied completely, it was refluxed on a heating mantle. The reaction mixture was monitored by TLC. After the completion of reaction, the reaction mixture was cooled down to room temperature, 5 mL of ethanol was added slowly into the flask, the reaction mixture was allowed to stand overnight in a refrigerator, later poured into ice cold water, filtered, washed with hot water and recrystallized from chloroform.

Yield: 64%, MP: 165-167°C, Rf value (chloroform: ethyl acetate): 0.72, FTIR cm^{-1}: 1794 (C=O, Azlactone), 1647(C=C), 1516 (C=N), 1153(C-O), 1481 (C-N), 3109(Ar-CH).

2.1.3 Synthesis of 4-(3-nitrophenyl)-2-phenyl-1,3-oxazol-5-one (2B).

A mixture of hippuric acid (0.01 mol), 3-nitro benzaldehyde (0.01 mol), 10 mL of acetic anhydride and anhydrous sodium acetate (0.02mol) was heated with constant shaking in a round bottomed flask. As soon as the mixture was liquefied completely, it was refluxed on a heating mantle. The reaction mixture was monitored by TLC. After the completion of reaction, the reaction mixture was cooled down to room temperature, 5 mL of ethanol was added slowly into the flask, the reaction mixture was allowed to stand overnight in a refrigerator, later poured into ice cold water, filtered, washed with hot water and recrystallized from chloroform.

Yield: 83%, MP: 175-177°C, Rf value (chloroform: ethyl acetate): 0.75, FTIR cm^{-1}: 1790 (C=O, Azlactone), 1652(C=C), 1525 (C=N), 1164(C-O), 1483(C-N), 1339(-NO_2), 3090(Ar-CH).

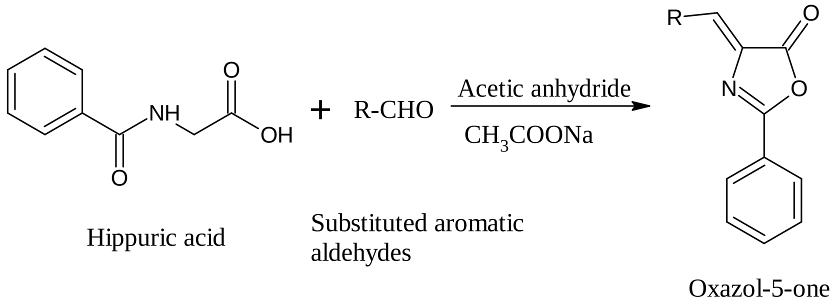
2.1.4 Synthesis of 4-(4-dimethylamino) phenyl-methylidene-2-phenyl-1,3-oxazol-5-one (3B):

A mixture of hippuric acid (0.01 mol), N,N-dimethyl amino benzaldehyde (0.01 mol), 10 mL of acetic anhydride and anhydrous sodium acetate (0.02mol) was heated with constant shaking in a round bottomed flask. As soon as the mixture was liquefied completely, it was refluxed on a heating mantle. The reaction mixture was monitored by TLC. After the completion of reaction, the reaction mixture was cooled down to room temperature, 5 mL of ethanol was added slowly into the flask, the reaction mixture was allowed to stand overnight in a refrigerator, later poured into ice cold water, filtered, washed with hot water and recrystallized from chloroform.

Yield: 85%, MP: 210-215°C, Rf value (chloroform: ethyl acetate): 0.76, FTIR cm^{-1}: 1750 (C=O, Azlactone), 1647(C=C), 1517 (C=N), 1153(C-O), 1374 (C-N), 3112(Ar-CH).

2.1.5 Synthesis of 4-(4-methoxyphenyl) methylidene-2-phenyl-1,3-oxazol-5-one (4B): A mixture of hippuric acid (0.01 mol), 4-methoxy benzaldehyde (0.01 mol), 10 mL of acetic anhydride and anhydrous sodium acetate (0.02mol) was heated with constant shaking in a round bottomed flask. As soon as the mixture was liquefied completely, it was refluxed on a heating mantle. The reaction mixture was monitored by TLC. After the completion of reaction, the reaction mixture was cooled down to room temperature, 5 mL of ethanol was added slowly into the flask, the reaction mixture was allowed to stand overnight in a refrigerator, later poured into ice cold water, filtered, washed with hot water and recrystallized from chloroform.

Yield: 71%, MP: 150-152°C, Rf value (chloroform: ethyl acetate): 0.76, FTIR cm^{-1}: 1772 (C=O, Azlactone), 1646(C=C), 1554 (C=N), 1153(C-O), 1435(C-N), 2975($-OCH_3$) 3109(Ar-CH).



R ⟶ C_6H_5, $3\text{-}NO_2\text{-}C_6H_5$, $4\text{-}N(CH_3)_2\text{-}C_6H_5$, $4\text{-}OCH_3\text{-}C_6H_5$

Step 2: Synthesis of oxazol-5-ones from hippuric acid (1B - 4B)

2.1.6 Synthesis of 4-benzylidene-1,2-diphenyl-imidazol-5-one (SW-1): 4-benzylidene-2-phenyl-1,3-oxazol-5-one (1B) (0.005 mol), aniline (0.005 mol) and red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 3-5 hours. The reaction mixture was monitored by TLC. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

Yield: 87%. MP: 228°C. Rf value (n-hexane: ethyl acetate): 0.64, FTIR cm^{-1}: 1749(C=O), 1642(C=C), 1599(C=N), 1444(C-N), 2975(Ar-CH). ^1H-NMR, $CDCl_3$, 400 MHz: δ 8.40-8.28 (m, 1H, Ar-H), 7.85 (d, 2H, Ar-H), 7.57 (s, 3H, Ar-H), 7.48-7.41 (m, 5H, Ar-H), 7.33-7.30 (m, 4H, Ar-H), 7.19 (m, 1H, C=CH).

2.1.7 Synthesis of 4-benzylidene-1-(4-nitrophenyl),2-phenyl-imidazol-5-one (SW-2): 4-benzylidene-2-phenyl-1,3-oxazol-5-one (1B) (0.005 mol), 4-nitro aniline (0.005 mol) and red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 3-5 hours. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

Yield: 71%. MP: 159.5°C. Rf value (n-hexane: ethyl acetate): 0.65, FTIR cm^{-1}: 1792(C=O), 1644(C=C), 1599(C=N), 1448(C-N), 1338($N0_2$), 2974(Ar-CH). ^{1}H-NMR, $CDCl_3$, 400 MHz: δ 8.29-8.26 (m, 4H, Ar-H), 8.15 (m, 1H, Ar-H), 7.81 (m, 1H, Ar-H), 7.65 (m, 1H, Ar-H), 7.54 (d, 2H, Ar-H), 7.47 (m, 3H, Ar-H), 7.40-7.33 (m, 3H, Ar-H, C=CH).

2.1.8 Synthesis of 4-(4-methoxybenzylidene)-1-(4-nitrophenyl)-2-phenyl-imidazol-5-one (SW-3): 4-(4-methoxyphenyl) methylidene-2-phenyl-1,3-oxazol-5-one (4B) (0.005 mol), aniline (0.005 mol) and red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 3-5 hours. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

Yield: 76%. MP: 180°C. Rf value (n-hexane: ethyl acetate): 0.62, FTIR cm^{-1}: 1712(C=O), 1639(C=C), 1594(C=N), 1442(C-N), 1256(C-O-C), 2920(O-CH_3), 2974(Ar-CH).^{1}H-NMR, $CDCl_3$, 400 MHz: δ8.28-8.26 (d, 2H, Ar-H), 7.549 (d, 2H, Ar-H), 7.40-7.37 (m, 1H, Ar-H), 7.35-7.26 (m, 5H, Ar-H), 7.13 (d, 2H, Ar-H), 7.00 (d, 2H, Ar-H, C=CH), 3.88 (s, 3H, -OCH_3).

2.1.9 Synthesis of 4-(4-dimethylamino)-benzylidene]-1,2-diphenyl-imidazole-5-one (SW-4): 4-(4-dimethylamino) phenyl-methylidene-2-phenyl-1,3-oxazol-5-one (3B) (0.005 mol), aniline (0.005 mol) and red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 3-5 hours. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

Yield: 70%. MP: 163°C. Rf value (n-hexane: ethyl acetate): 0.62, FTIR cm^{-1}: 1794(C=O), 1696(C=C), 1595(C=N), 1445(C-N), 1073(C-N, 3° Amide), 2974(Ar-CH). ^{1}H-NMR, $CDCl_3$, 400 MHz: δ8.23 (d, 2H, Ar-H), 7.54 (t, 2H, Ar-H), 7.43-7.36 (m, 5H, Ar-H), 7.29 (t, 2H, Ar-H), 7.19 (d, 2H, Ar-H), 6.75 (d, 2H, Ar-H, C=CH), 3.08 (d, 6H, $N(CH_3)_2$).

2.1.10 Synthesis of 4-benzylidene-5-oxo-2-phenylimidazol-1-yl-benzene-sulfonamide (SW-5): 4-benzylidene-2-phenyl-1,3-oxazol-5-one (1B) (0.005 mol), sulfanilamide (0.0075 mol) and red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 8-9 hours. The reaction mixture was monitored by TLC. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

Yield: 64%. MP: 143°C. Rf value (n-hexane: ethyl acetate): 0.49. FTIR cm^{-1}: 3316, 3204(NH_2), 1698(C=O), 1632(C=C), 1596(C=N), 1443(C-N), 1352(S=O), 3070(Ar-CH). ^{1}H-NMR, $CDCl_3$, 400 MHz: δ 8.29 (d, 2H, Ar-H), 7.99 (t, 2H, Ar-H), 7.55 (d, 2H Ar-H), 7.48 (t, 4H, Ar-H), 7.40-7.33 (m, 5H Ar-H, C=CH), 4.82 (s, 2H, NH_2).

2.1.11 Synthesis of 4-(*p*-methoxyphenyl) methylene]-5-oxo-2-phenyl-2-imidazolin-1-yl-benzene-sulfonamide (SW-6): 4-(4-methoxyphenyl) methylidene-2-phenyl-1,3-oxazol-5-one (4B) (0.005 mol), sulfanilamide (0.0075 mol) and Red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 8-9 hours. The reaction mixture was monitored by TLC. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

Yield: 72%. MP: 180°C. Rf value (n-hexane: ethyl acetate): 0.48. FTIR cm^{-1}: 3315, 3206 (NH_2), 1698(C=O), 1637(C=C), 1596(C=N), 1442(C-N), 1347(S=O), 2900 (OCH_3), 1257(C-O-C), 3069(Ar-CH). ^{1}H-NMR, $CDCl_3$, δ 8.28 (d, 2H, Ar-H), 7.97 (d, 2H, Ar-H), 7.54-7.46 (m, 3H, Ar-H), 7.36 (dd, 5H, Ar-H), 7.00 (d, 2H, Ar-H, C=CH), 4.80 (s, 2H, NH_2), 3.89 (s, 3H, OCH_3).

2.1.12 Synthesis of 4-Phenylmethylene-1-[p-(5-methyl-3-isoxazolylaminosulfonyl) phenyl]-2-phenyl-2-imidazolin-5-one (SW-7): 4-benzylidene-2-phenyl-1,3-oxazol-5-one (1B) (0.005 mol), sulphamethaoxazole (0.0075 mol) and Red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 8-10 hours. The reaction mixture was monitored by TLC. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

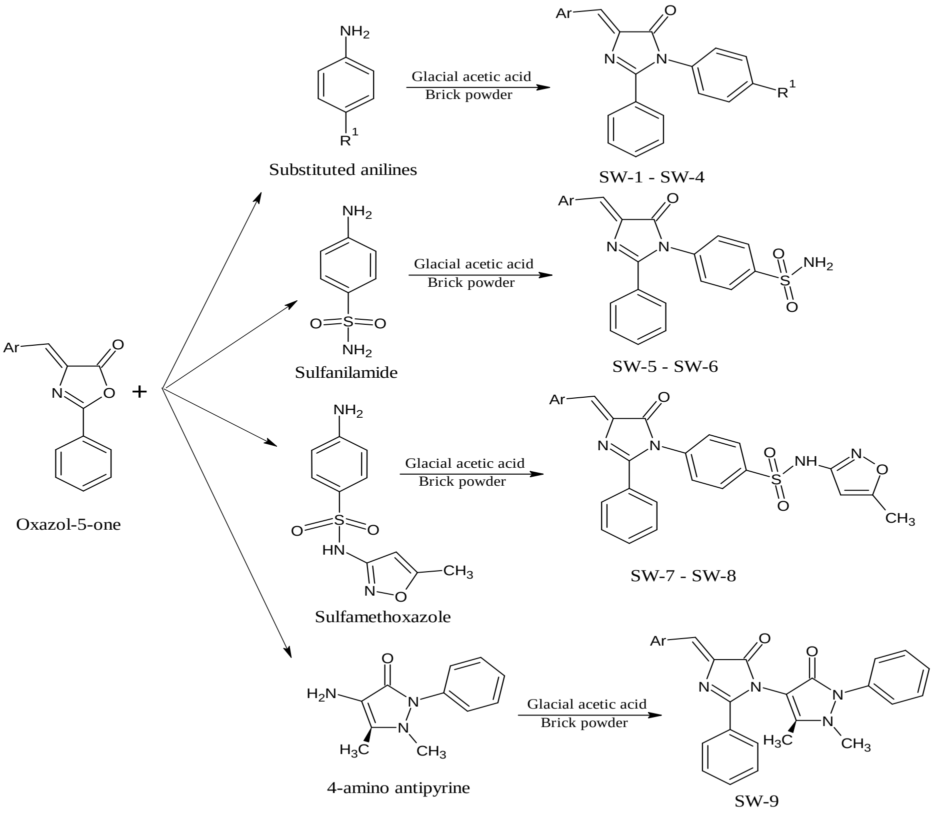
Yield: 75%. MP: 126°C. Rf value (n-hexane: ethyl acetate): 0.83. FTIR cm^{-1}: 1716 (C=O), 1490 (C=N), 1604 (C=C), 1458 (C-N), 1370 (S=O), 2983 (Ar-CH). ^{1}H-NMR, $CDCl_3$, δ 8.56-8.19 (m, 3H, Ar-H), 7.87 (d, 2H, -NH, Ar-H), 7.46 (q, 5H, Ar-H), 7.37-7.29 (m, 6H, Ar-H), 6.25 (d, 1H, -CH), 2.38 (s, 3H, $-CH_3$).

2.1.13 Synthesis of 4-[(4-methoxyphenyl) methylene]-1-[4-(5-methylisoxazol-3-yl aminosulfonyl) phenyl]-2-phenyl-2-imidazolin-5-one (SW-8): 4-(3-nitrophenyl)-2-phenyl-1,3-oxazol-5-one (2B) (0.005 mol), sulphamethaoxazole (0.0075 mol) and Red brick powder were taken in a two necked flask, 30mL of glacial acetic acid was added. The above mixture was refluxed on a magnetic stirrer for 8-10 hours. The reaction mixture was monitored by TLC. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

Yield: 80%. MP: 160°C. Rf value (n-hexane: ethyl acetate): 0.80. FTIR cm^{-1}: 1731 (C=O), 1681 (C=C), 1596 (C=N), 1476 (C-N), 1390(S=O), 1349 (NO_2) 2982 (Ar-CH). ^{1}H-NMR, $CDCl_3$, δ 8.44 (s, 1H, Ar-H), 8.30-8.11 (m, 3H, Ar-H), 8.01-7.86 (m, 3H, Ar-H), 7.74-7.51 (m, 4H, Ar-H), 7.47-7.30 (m, 4H, Ar-H), 6.26 (s, 1H, CH), 2.40-2.36 (m, 3H, $-CH_3$).

2.2.14. Synthesis of 4-[4-Phenylmethylene-5-oxo-2-phenyl-2-imidazolin-1-yl]-1-methyl-5-methyl-2-phenyl-1,2-dihydro-pyrazol-3-one (SW-9): 4-benzylidene-2-phenyl-1,3-oxazol-5-one (1B) (0.005 mol), 4-amino antipyrine (0.0075 mol) and red brick powder were taken in a two necked flask, 20mL of glacial acetic acid was added. The above mixture was refluxed on a heating mantle for 10-12 hours. The reaction mixture was monitored by TLC. After the reaction was completed, the reaction mixture was cooled, filtered to separate the red brick powder and filtrate was then poured into a beaker containing ice-cold water. Solid product obtained was filtered, washed with water and recrystallized from chloroform.

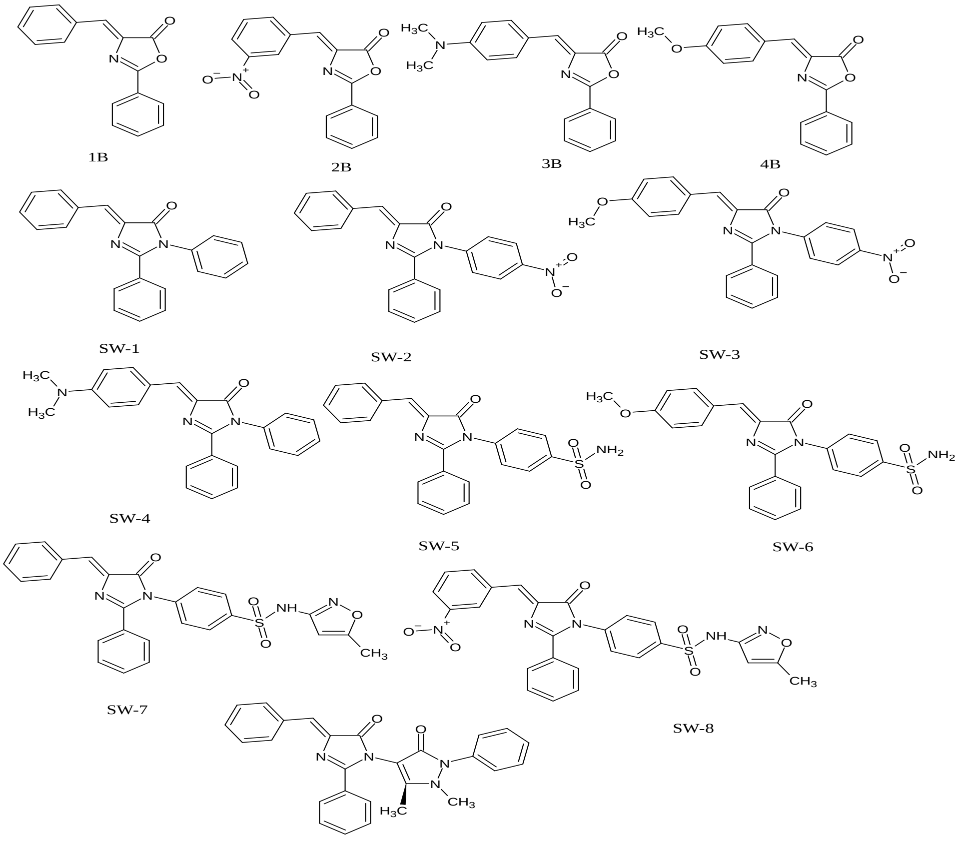
Yield: 69%. MP: 169°C. Rf value (n-hexane: ethyl acetate): 0.80. FTIR cm^{-1}: 1590 (C=O), 1522 (C=C), 1486 (C=N), 1443 (C-N), 2916 (Ar-CH). ^{1}H-NMR, $CDCl_3$, δ 8.27 (d, 2H, Ar-H), 7.88 (d, 2H, Ar-H), 7.46 (t, 5H, Ar-H), 7.40 (q, 4H, Ar-H), 7.36-7.30 (m, 3H, Ar-H), 3.18 (s, 3H, $N-CH_3$), 2.13 (s, 3H, $-CH_3$).



Ar $\longrightarrow$ C_6H_5 , 3-NO_2-C_6H_5, 4-OCH_3-C_6H_5, N$(CH)_3$-C_6H_5

R^1 $\longrightarrow$ H, NO_2

Step 3: Synthesis of imidazol-5-one from oxazol-5-one with various aromatic/heterocyclic amines (SW-1 - SW-9)


1B 2B 3B 4B

SW-1 SW-2 SW-3

SW-4 SW-5 SW-6

SW-7 SW-8

SW-9

Fig 2: Structures of oxazolin-5-ones and imidazol-5-ones

2.2.Molecular docking study

The molecular docking study was done using free docking software PyRx version 0.8 to study the binding interactions of the ligands with targets (COX) enzymes. The literature survey was carried out to analyze the binding of the ligands with the target to check whether the ligands are interacting in the active site or any other part of the protein. The crystal structures of the investigational targets (1PXX/3LN1) are downloaded in 3D format from RCSB protein data bank. The downloaded protein structures were then processed in Biovia discovery studio visualizer tool version 16.1.0. by removing previously present heterocyclic ligands and adding polar hydrogens, later saved in PDB format. The structures of the compounds were drawn in Chemsketch software and saved in .mol file format, these targets were then converted into their .pdbqt format in PyRx using open babel option. The grid was drawn around the target protein. The ligands are then allowed to dock with the protein and the results obtained were visualized in Biovia discovery studio, the protein interactions were saved in 2D format and the binding energies were saved into an excel sheet.[19]

2.3.In-vitro anti-inflammatory studies by bovine serum albumin (BSA) denaturation assay

Denaturation of proteins is one of the causes of inflammation is well documented. Production of auto-antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. In the present study, the selected compounds were evaluated for their in-vitro anti-inflammatory activity by bovine serum albumin (BSA) denaturation assay with slight modifications.[20]

Chemicals and Reagents used

A. Albumin solution**:** A Stock solution of 1mM was prepared by dissolving albumin in phosphate buffer (0.2M, pH 7.4).

B. Dimethyl formamide (DMF), distilled

C. Phosphate Buffer (0.2M, pH 7.4).

D. Preparation of test and standard solutions: Concentrations ranging from (250-50 µg/mL) was prepared by dissolving in a minimum amount of DMF and diluted with phosphate buffer (0.2M, pH 7.4). Final concentration of DMF in all solutions was less than 2.5%.

E. Standard: Diclofenac sodium.

Procedure

Test and Standard solutions containing different concentrations of drug was mixed with 1mL of 1mM albumin solution in phosphate buffer and incubated at 27± 1°C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 80 ±1°C in a water bath for 10 minutes. The control solution was prepared by the same above method without adding test or standard solution. After cooling the turbidity was measured at 660 nm (UV-1660-Shimadzu Spectrophotometer). Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken.[21]

Formula. % Inhibition of BSA denaturation = (absorbance of control - absorbance of sample / absorbance of control) * 100.

3. RESULTS AND DISCUSSION

The synthesis of substituted imidazoles is carried out in three steps, in which the first step is based on Schotten-Baumann reaction, second step is based on Erlenmeyer azlactone synthesis and third step is based on amidification reaction followed by dehydration. The schemes and the structures are given in materials and methods section.

Table No.1: Drug likeness and non-drug likeness of investigational ligands.

Sl. No	Ligand	MW	HBD	HBA	LOG P	MR	TPSA	GIA	BBB
1.	SW-1	324.38	0	2	4.55	107.59	32.67	High	Yes
2.	SW-2	369.37	0	4	4.51	116.41	78.49	High	No
3.	SW-3	399.40	0	5	4.57	122.90	87.72	High	No
4.	SW-4	367.44	0	2	4.65	121.79	35.91	High	Yes
5.	SW-5	403.45	1	5	3.25	118.58	101.21	High	No
6.	SW-6	433.48	1	6	3.30	125.07	110.44	High	No
7.	SW-7	484.53	1	6	4.15	139.73	113.25	High	No
8.	SW-8	529.52	1	8	4.08	148.55	159.07	High	No
9.	SW-9	434.49	0	3	4.36	137.4	59.60	High	Yes

MW: Molecular weight; HBD: Hydrogen bond donor; HBA: Hydrogen bond acceptor; LOG P: Lipophilicity; MR: Molar refractivity; TPSA: Tropical polar surface area; GIA: Gastrointestinal absorption; BBB: Blood brain barrier permeation.

Table No. 2: Binding energies of investigational selected ligands with appropriate proteins.

Sl. No.	**LIGAND**	**1PXX**		**3LN1**	
		Binding Affinity (Kcal/mol)	**H-bond**	**Binding Affinity (Kcal/mol)**	**H-bond**
1	SW-1	-10.1	ARG 44 (π-donor H)	-8.4	SER 129 (C-H)
2	SW-2	-9.1	Hydrophobic	-9.7	ARG 319
3	SW-3	-9.2	CYS 47	-9.9	ARG 319
4	SW-4	-8.9	SER 530 (π-donor H)	-8.6	SER 129 (C-H)
5	SW-5	-9.3	CYS 47, GLN 461, GLU 465	-9.7	CYS 32, ASN 243
6	SW-6	-8.5	ARG 44, 61	-9.9	CYS 26 (C-H)
7	SW-7	-10.9	Hydrophobic	-11.6	CYS 32, TRP 309, GLN 313,
8	SW-8	-9.6	ARG 44	-10.4	ASN 24, CYS 32, GLN 447, TYR 122
9	SW-9	-10.1	HIS 207	-9.5	GLY 121
10	Diclofenac	-8.5	ASN 39	-7.5	ASN 19, CYS 32

In the first step, the glycine was reacted with benzoyl chloride in 10% NaOH via Schotten-Baumann reaction to afford hippuric acid. In second step, hippuric acid was cyclized by condensing with various substituted aromatic aldehydes in the presence of sodium acetate to form corresponding oxazolin-5-ones. In third step, substituted oxazolin-5-ones were condensed with substituted anilines, sulfanilamide, sulfamethoxazole and 4-amino antipyrine in the presence of red brick powder to yield corresponding imidazol-5-ones. Sulfanilamide, sulfamethoxazole and 4-amino antipyrine were taken excess to increase the rate of reaction and can be easily removed by water wash due to their solubility in water. All the final compounds were identified by analytical and spectral techniques such as thin layer chromatography, melting point, Fourier transform infrared (FTIR) and ^{1}H-NMR spectroscopy.

In all the oxazol-5-ones, the C-O stretching can be seen at 1150 cm^{-1}, a sharp band around 1790 cm^{-1} indicates the carbonyl group stretching of azalactone (oxazole-5-one), and in imidazol-5-one the same carbonyl group show the stretching around 1700 cm^{-1} and absence of C-O stretch the graph shows the conversion of azalactone to imidazole-5-one. The structures and purity of the final compounds were confirmed by proton NMR spectroscopy, where all the aromatic protons were observed between 9-7 ppm, and the =CH of the basic imidazole was found merged with the aromatic protons, due to factors that affect the chemical shift a) presence of electron withdrawing group, i.e., aromatic group and b) due to the conjugation (alternative pi-sigma bonds combination) can be observable inside the imidazole nucleus and also into the aromatic system through =CH.

Table No. 3: The study of anti-inflammatory activity by bovine serum albumin denaturation assay.

Sl.no	Compounds	% Inhibition of BSA Denaturation				
		50 µg/ml	**100 µg/ml**	**150 µg/ml**	**200 µg/ml**	**250 µg/ml**
1.	**STD**	26.45	39.10	54.86	63.79	72.56
2.	**SW-1**	32.58	39.30	51.60	58.40	71.70
3.	**SW-2**	25.02	38.78	49.76	62.25	75.94
4.	**SW-3**	36.50	60.09	75.66	80.53	80.90
5.	**SW-4**	41.8	49.56	56.56	62.22	74.89
6.	**SW-5**	29.89	35.89	48.45	61.78	75.34
7.	**SW-6**	21.07	36.23	47.96	60.90	79.45
8.	**SW-7**	28.59	38.50	48.60	72.89	89.25
9.	**SW-8**	24.78	35.89	42.96	66.56	82.59
10.	**SW-9**	23.88	34.42	43.62	51.10	76.92

The drug-likeness of investigational ligands are given in the table No. 1. The molecular docking studies were performed for the selection of the compounds for the synthesis and activity part of research, the selected best results are given in table No. 2. The target protein used for the study of interactions are COX 2 enzymes, as this is the enzyme responsible for the conversion of arachidonic acid to prostaglandins, these prostaglandins are responsible of the inflammation. Hence, inhibiting this enzyme in the system will lead to inhibition of the production of the prostaglandins, which in turn reduced the inflammation. Many of the marketed drugs inhibit the COX enzyme to show their anti-inflammatory activity. The two COX 2 proteins (1PXX and 3LN1) were selected from literature survey and the possible ligand structures were drawn and made to interact with each other. The site of binding of the various imidazole-5-ones with protein is comparable with standard marketed drug (diclofenac) as the binding pockets of both are found to be at similar site in the protein. Among the selected compounds with protein (1PXX) the strong conventional hydrogen bonding indicates a strong interaction between the ligand and protein, however only SW-1, 3, 5, 6, 8 (binding affinities: 8.5-10.9 Kcal/mol) are having hydrogen bond at residues ARG44, CYS47, CYS47, ARG44, ARG44 respectively, their active binding pockets are similar to that of diclofenac at ASN39. With the protein (3LN1) only SW-5,6,7,8 (binding affinities: 8.4-11.6 Kcal/mol) are having hydrogen bond at residues CYS26, CYS32, CYS26, ASN24 respectively, their active binding pockets are similar to that of diclofenac at ASN39.

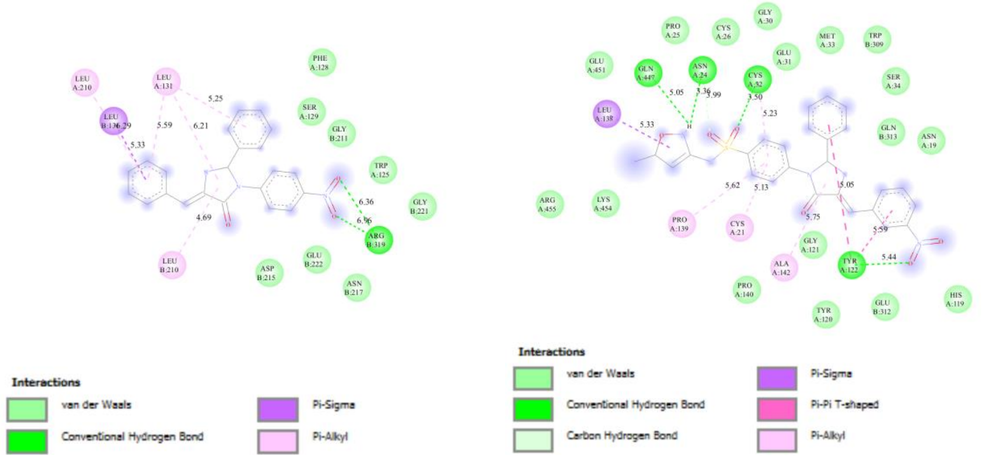


Fig 3: 2D interaction diagrams of SW-2 and SW-8 with protein (PDB ID: 3LN1).

The hydrogen bond is forming mainly with the carbonyl (C=O) group of imidazole nucleus but if in case the molecule contains NO_2, SO_2, NH_2 the hydrogen bond prioritizes more there. Even the compounds which don't have the hydrogen bonding interaction also have a good binding affinity, this is mainly due to the van der waals forces acting upon. The compounds containing nitro group has shown the best consistent binding affinity towards the protein. The compounds that have better binding affinity and parameters were selected for synthesis and anti-inflammatory evaluation.

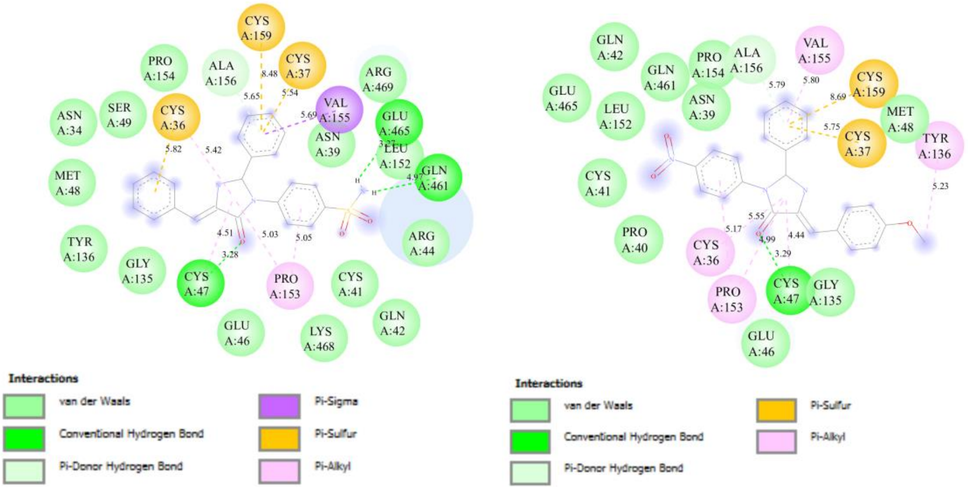


Fig 4: 2D interaction diagrams of SW-5 and SW-3 with protein (PDB ID: 1PXX).

The selected compounds were screened for the in-vitro anti-inflammatory activity by bovine serum albumin denaturation assay method given in table No. 3, the synthesized compounds have shown more significant/equal activity when compared to the standard diclofenac sodium. The compounds SW-7 and SW-8 have shown better activity among the synthesized compounds maybe due to the presence of oxazole moiety along with imidazole nucleus. Also the compounds with other heterocyclic nucleus substitution i.e., SW-5, 6 and 9 also shown significant activity.

4. CONCLUSION

Hence, the substituted imidazol-5-one derivatives have notable anti-inflammatory activity and further research should be conducted on lead optimization to enhance the biological property. Further synthesis of few more derivatives, characterization and *in-vivo* activities to be carried out for the future perspective.

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