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Research Article

Synthesis and Biological Evaluation of 1,4-Dihydropyridine Derivatives as Anti-inflammatory Agents

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Abstract

1,4-dihydropyridine nucleus has been used as an anti-inflammatory. Derivatives designed by using Swiss Target Prediction. This study revealed maximum COX-II receptor binding activity. The six most promising derivatives of 1,4-Dihydropyridine were synthesized by the substitution of aromatic benzaldehyde with ethyl acetoacetate. Subsequently, spectral analyses were conducted utilizing UV and IR spectrophotometry. The In-Vivo assessments (biological evaluations) of all synthesized compounds were executed employing the paw edema model in rats. The anti-inflammatory efficacy of the synthetic compounds is in the following order: 3d>3b>3e>3c>3a>3f. Due to the presence of the electron-donating chlorine group and the electron-withdrawing methoxy group in the 1,4-dihydropyridine derivatives, compound 3d demonstrated superior activity relative to the other compounds. These two functional groups enhance the anti-inflammatory activity observed in the rat model.

Introduction

Non-steroidal anti-inflammatory medicines (NSAIDs) are the most often used treatment for inflammation, while there are other options as well[1,2]. Around the world, millions of people take NSAIDs every day[3]. Both acute and chronic inflammation can be treated with them, and they are usually very successful[4,5]. There are more than 40 known NSAIDs, and they are frequently categorized into many classes according to their structure and anticipated risk[6,7].Although there are a number of NSAIDs developed and available in market but rigorous search for new molecular is on wheels[8]. This is due to variety of side effect like gastric ulceration, inhibitions of platelet function, alteration of renal function, etc[9,10]. Our research explores enhanced 1,4 dihydropyridine (1,4-DHP) chemical structures that exhibit stronger effects while decreasing harmful side effects[11].1,4-Dihydropyridine forms a nitrogen-based six membered hydrocarbon ring[12]. The nucleus, both when considered in isolation from other chemical entities and in conjunction with an array of other molecular components, has been extensively documented in scientific literature to exhibit a diverse range of pharmacological and biological activities, including but not limited to, its notable capabilities in combating bacterial infections, mitigating inflammatory responses, and regulating hypertensive conditions, among various other significant therapeutic effects[13].The unsubstituted free NH group in the DHP ring is essential for its improved activity with regard to any therapeutic characteristic, according to thorough literature survey reports on the structure activity relationship (SAR) investigation of the 1,4-DHP structure[14]. Furthermore, key structural elements for a noticeable biological action include the presence of methyl groups at positions 2nd and 6th, ester groups at positions 3rd and 5th, and an aryl ring at position 4th[15].Therefore, our aim is to synthesize different deivatives of 1,4 - Dihydropyridine as anti-inflammatory agent by substitution of different electron donating and electron withdrawing group and evaluate them for their anti-inflammatory activity.

2. Experiment

2.1 Materials

These chemicals were all commercially purchased from Loba Chem Ltd. and were of synthetic grade. and S.D, Sigma-Aldrich. Chem Fine. Ltd (Indian) Business. Before using any solvents, we dried and distilled them all. Using iodine chamber developed spots and selected solvents, we used thin layer chromatography to monitor the progress of the reaction. Using a Veego apparatus, the melting points of the manufactured compounds were measured. To confirm the structure, the synthesized compounds were subjected to spectral analysis. A UV Shimadzu 1700s Spectrophotometer was used to analyze the UV spectra of the synthesized compounds. The IR Shimadzu instrument was used to perform the IR spectral analysis at the RGPV Bhopal.

2.2 Methods: Synthesis of compounds

The scheme of the synthesis of derivatives 1,4-dihydropyridine (DHP) is shown in figure 2

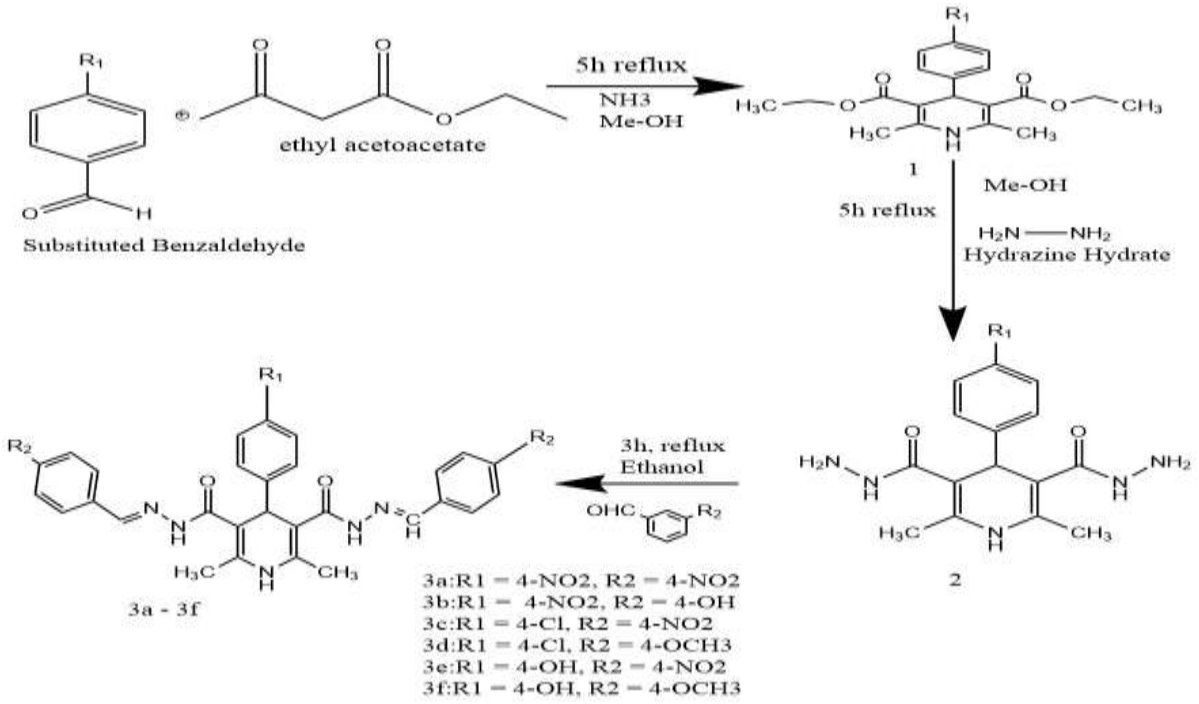

Figure 2.2 Synthesis of 1,4-dihydropyridine derivatives

Synthesis of 1,4-dihydro-2,6 dimethyl-4-nitrophenyl-3,5-dicarboxylic acid ethyl ester (1).

Substituted benzaldehyde (0.01mol) and ethyl-acetoacetate (3.82ml. 0.03mol) in methanol (20ml.) was treated with 0.465 ml. ammonia solution (25%, 0.02mol) and refluxed for 5hour. Than mixture was cooled over an ice bath to produce crude crystal once the reaction was finished. After being separated, the solid material was filtered, cleaned with methanol, and dried. Recrystallization was done by using methanol and water[16].

% Yield of derivatives was found to be 70%. Melting point of derivatives was found to be 210-211ºC. TLC Rf value derivatives was found to be 0.57 in solvent system of acetic acid: ethanol: water (2:4:4).

Synthesis of 1,4-dihydro-2,6 dimethyl-4-nitrophenyl-pyridine-3,5-dicarbohydrazide (2).

To the solution of analogue 1 (1.85gm, 0.005mol) in 20ml. methanol, 5ml. of hydrazine hydrate was added and refluxed for 5h. After completion of reaction, methanol was removed and crystals of hydrazide derivatives were formed immediately. Water was used to wash the crystals before they were recrystallized from methanol.

% Yield of derivatives was found to be 88%. Melting point of derivatives was found to be 236-237ºC. TLC Rf value derivatives was found to be 0.46.

Synthesis of N'3,N'5-di((E)-benzylidene)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarbohydrazide (3).

A mixture of compound 3 (0.003mol) and substituted benzaldehyde (0.006mol) in ethanol was refluxed for 3hr. The mixture was cooled and filtered then recrystallized from ethanol[17].

Synthesis of 2,6 dimethyl-4-nitrophenyl-1,4-dihydropyridine-3,5-diyl) bis (N'-4 nitro benzylidene) formic hydrazide (3a).

% Yield 79%; Melting point (°C): 220-221; Rf value: 0.45; UV λmax (nm):305; IR (cm^{-1}): 1517(N-O bending(S)), 29788(C-H stretching(M)), 3300(N-H stretching(M)), 1701(C=O stretching(S)), 1213(C-C stretching(M)), 1489(C=C stretching(W)), 3100(C-H stretching(M)).

Figure 2.1.1: UV spectrum of synthesized compound 3a

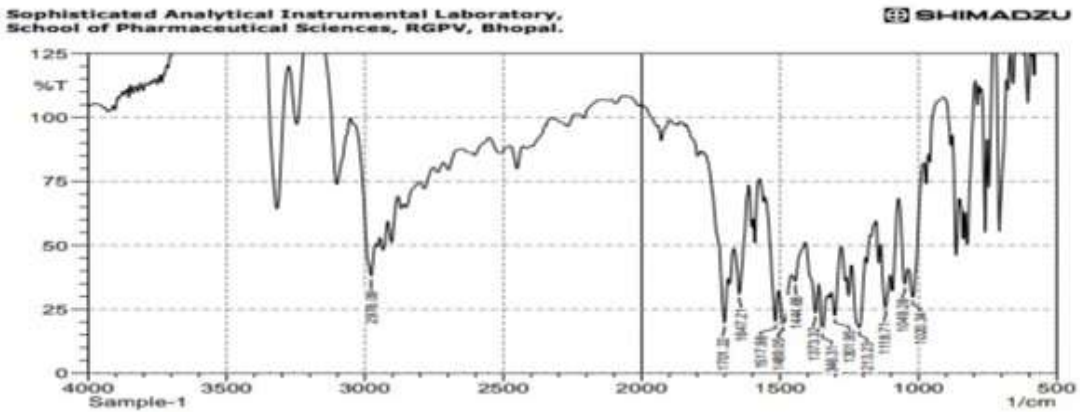

Figure 2.2.1: IR spectrum data of synthesized compounds 3a

Synthesis of 2,6-dimethyl-4-nitrophenyl-1,4-dihydropyridine-3,5-diyl) bis (N'-(4-Hydroxy benzylidene) formic hydrazide (3b).

% Yield 67%; Melting point (ºC): 228-239; Rf value: 0.38; UV λmax (nm): 324; IR (cm^{-1}): 2978(O-H Stretching (S)), 1571(N-H Bending (S)), 3300(N-H Stretching(M)), 1600(C=O Stretching(S)), 1487(C=C Stretching(W)), 1649(C=N Stretching(M)), 1301(C-N Stretching(M))

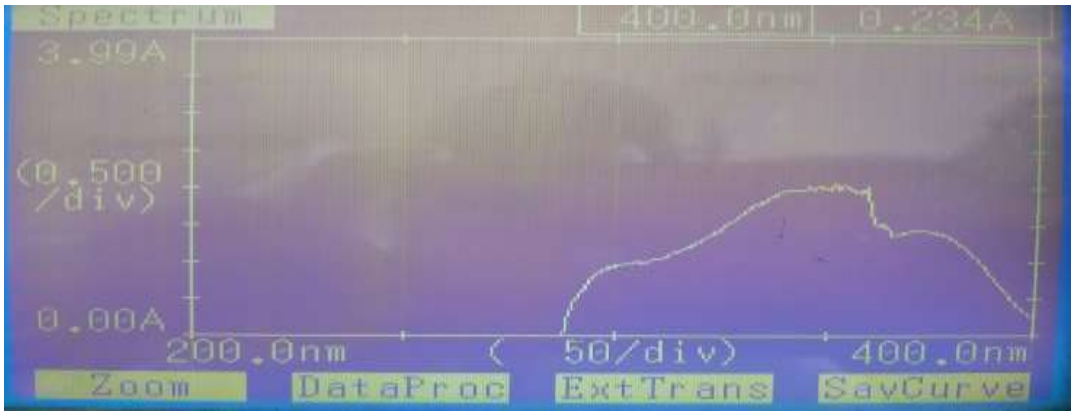

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Figure 2.1.2: UV spectrum of synthesized compound 3b

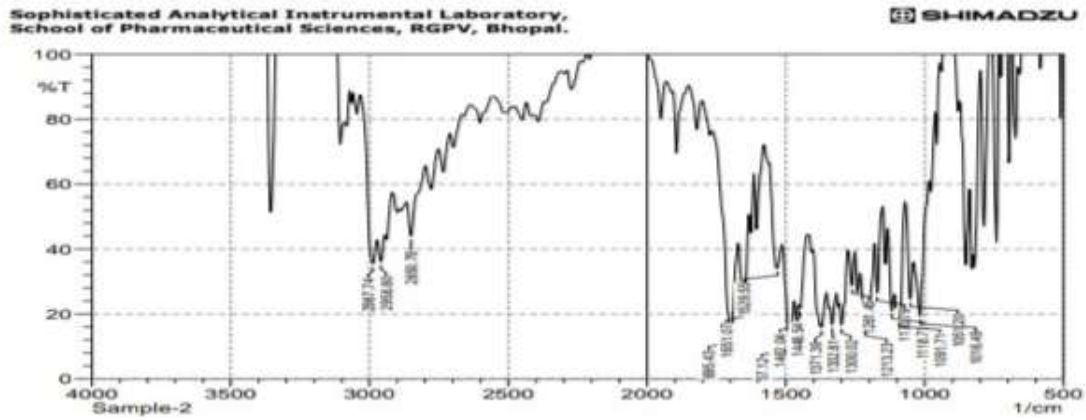

Figure 2.2.2 IR spectrum data of synthesized compounds 3b.

Synthesis of 2,6-dimethyl-4-chlorophenyl-1,4-dihydropyridine-3,5-diyl) bis (N'-(4-nitro benzylidene) formic hydrazide (3c)

% Yield 63%; Melting point (ºC): 150-151; Rf value: 0.44; UV λmax (nm): 320; IR (cm^{-1}): 720(C-Cl Stretching (S)), 1529(N-O Bending (S)), 3350(N-H Stretching(M)), 1600(C=O Stretching(S)), 3100(C-H Stretching(M)),1487(C=C Stretching(W)), 1651(C=N Stretching(M)), 1332(C-N Stretching(M)).

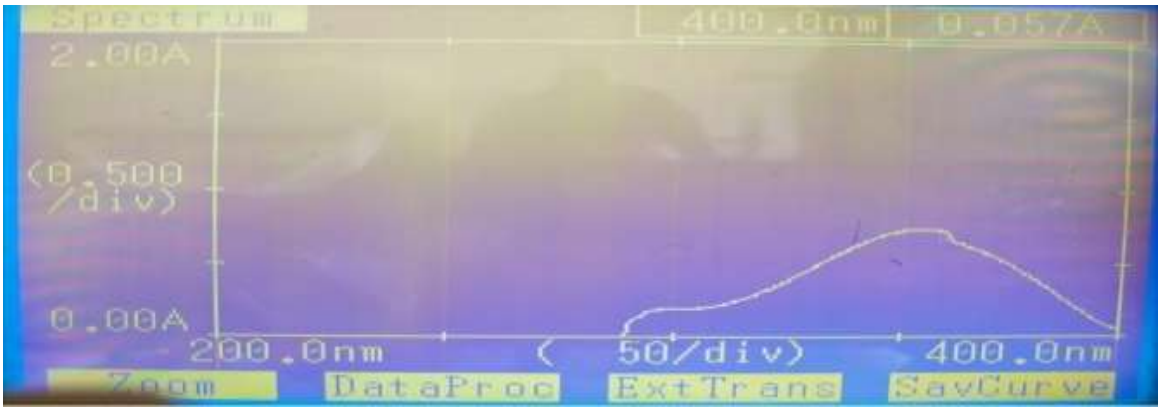

Figure 2.1.3 UV spectrum of synthesized compound 3c

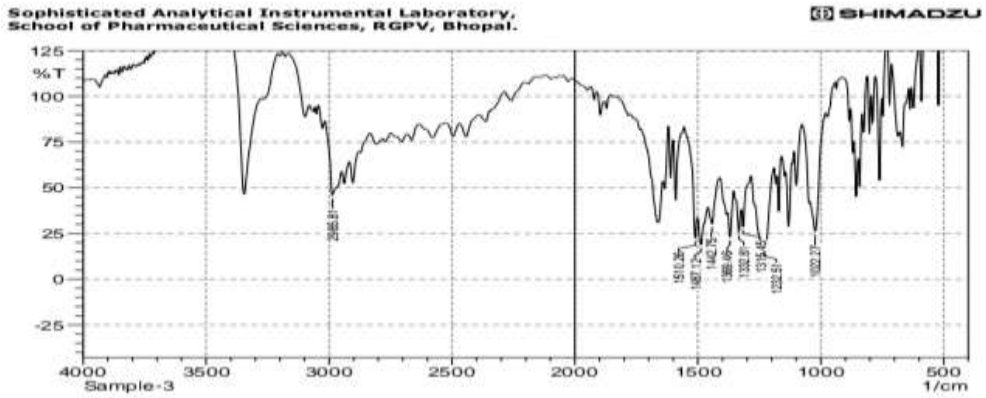

Figure 2.2.3 IR spectrum data of synthesized compounds 3c

Synthesis of 2,6-dimethyl-4-chlorophenyl-1,4-dihydropyridine-3,5-diyl) bis (N'-(4-methoxy benzylidene) formic hydrazide (3d).

% Yield 69%; Melting point (°C): 157-158; Rf value: 0.32; UV λmax (nm): 333; IR (cm^{-1}): 720(C-Cl Stretching (S)), 3100(C-H Bending (S)), 1228(C-O Stretching(S)), 1600(C=O Stretching(S)), 3350(N-H Stretching(M)),1487(C=C Stretching(W)), 1651(C=N Stretching(M)), 1332(C-N Stretching(M)).

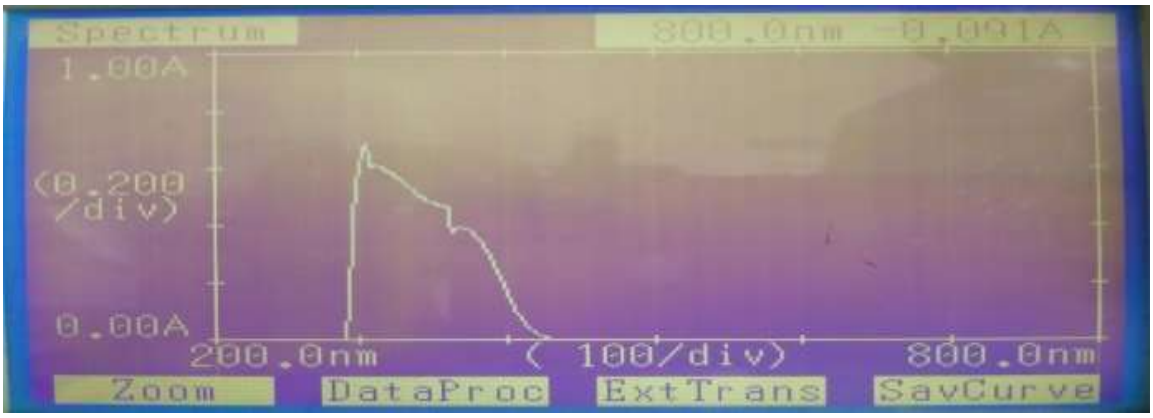

Figure 2.1.4: UV spectrum of synthesized compound 3d

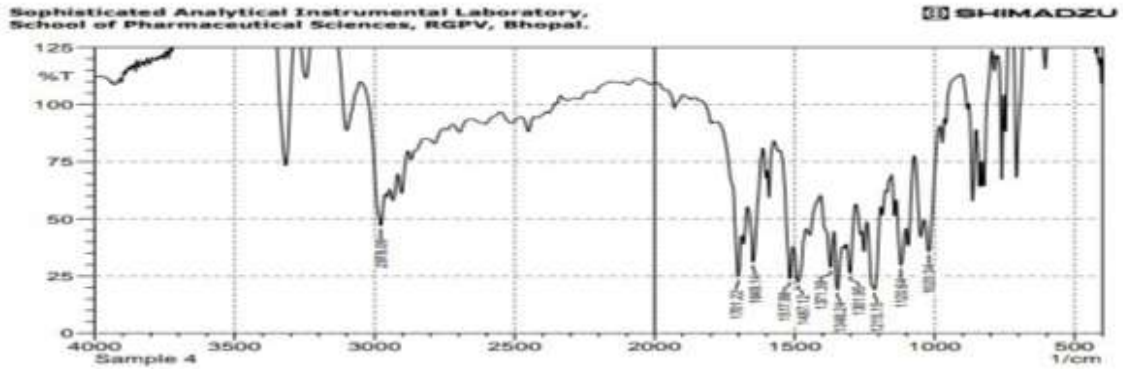

Figure: 2.2.4:IR Spectrum data of synthesized compound 3d

Synthesis of 2,6-dimethyl-4-hydroxyphenyl-1,4-dihydropyridine-3, diyl) bis (N'-(4-nitro benzylidene) formic hydrazide (3e).

% Yield 73%; Melting point (°C): 120-121; Rf value: 0.55; UV λmax (nm): 348; IR (cm^{-1}): 2985(O-OH Stretching (S)), 1510(N-O Stretching(S)), 3100(C-H Stretching(M)), 3350(N-H Stretching(M)), 1600(C=O Stretching(S)),1487(C=C Stretching(W)), 1651(C=N Stretching(M)), 1332(C-N Stretching(M)).

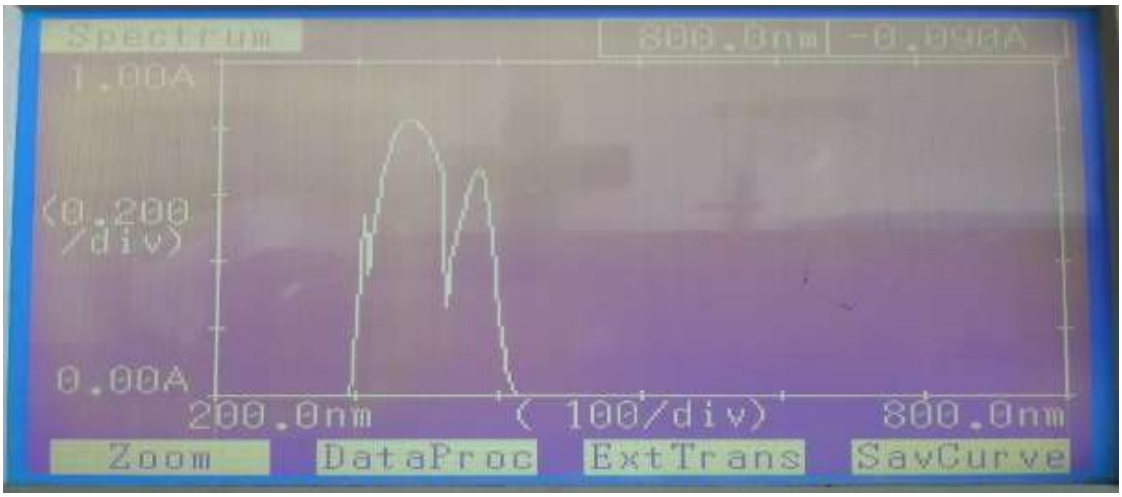

Figure 2.1.5: UV spectrum of synthesized compound 3e

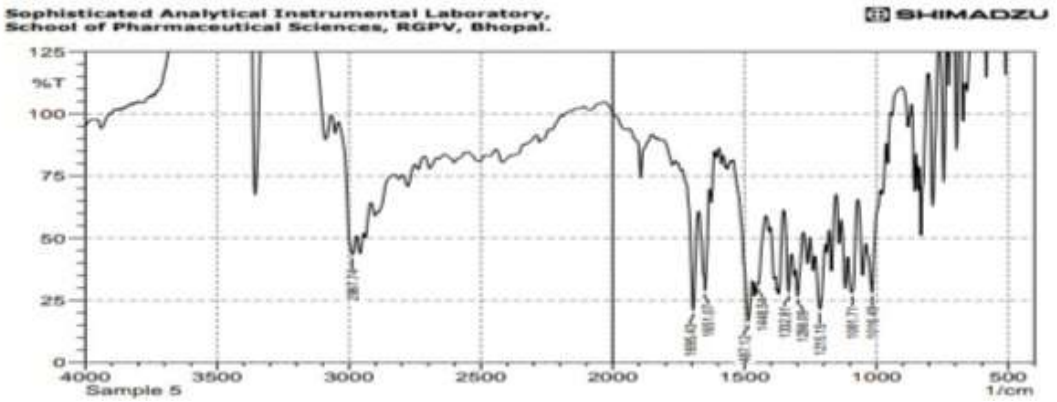

Figure: 2.2.5:IR Spectrum data of synthesized compound 3e

Synthesis of 2,6-dimethyl-4-hydroxyphenyl-1,4-dihydropyridine-3,5-diyl) bis (N'-(4-methoxy benzylidene) formic hydrazide (3f).

% Yield 56%; Melting point (°C): 130-131; Rf value: 0.57; UV λmax (nm): 351; IR (cm^{-1}): 2985(O-OH Stretching (S)), 3100(C-H Stretching(M)), 1200(C-O Stretching(S)), 3350(N-H Stretching(M)), 1600(C=O Stretching(S)), 1487(C=C Stretching(W)), 1651(C=N Stretching(M)), 1332(C-N Stretching(M)).

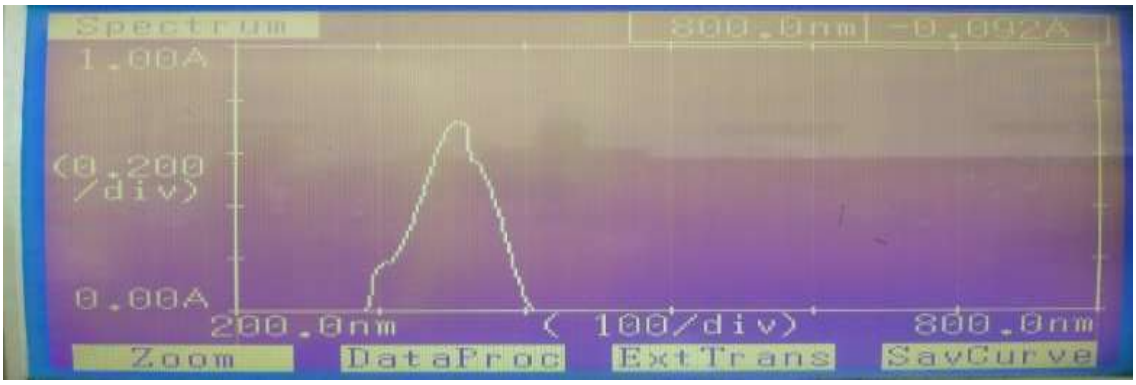

Figure 2.1.6: UV spectrum of synthesized compound 3f

Figure: 2.2.6:IR Spectrum data of synthesized compound 3f

3. BIOLOGICAL EVALUATION

3.1 Swiss target prediction: Swiss target prediction is a Webserver for target prediction of bio-active small molecules in human. Many proteins such as specific kinase or Phosphatase, hundreds of small molecules ligands have been identified[18,19]. The figure are shown in 3.1.

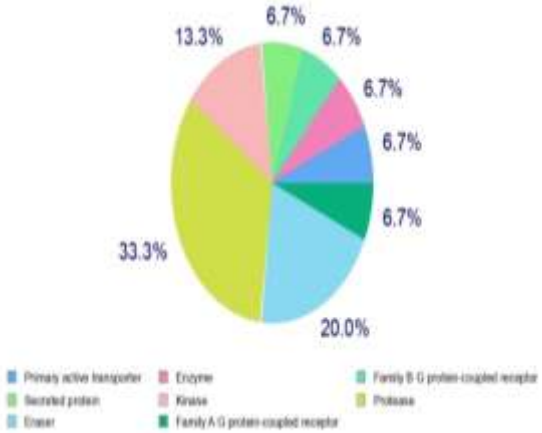

Figure 3.1: Swiss prediction

Data of synthesized compound 3d

3.2 *IN-VIVO* Study

Anti-inflammatory activities of synthesized compounds have been done by using formalin induced hind (Lower left) paw edema model. All studies conducted by the Institutional Animal Ethical Committee VNS faculty of pharmacy Bhopal (Approval no. PH/IAEC/VNS/2K19/08).

Anti-inflammatory activity by formalin induced hind (Lower left) paw edema model

In this method, rat hind (Lower left) paw edema induced by the sub-planter injection of 2.5% formalin solution and than paw volume was measured in Swiss albino rat taken randomly with weight 168-235 gram and divided into the following 8 group of 6 animals each. It consequently causes oedema to build in the area. A Plethysmometer was used to measure the rat's paw edema's volume every hour (The figure is shown in 3.2). The following formula was used to calculation and compare the percentage (%) of increase in paw volume with time[20,21]. The results are shown in table 3.2.1 & 3.2.2.

Figure 3.2: Induction of paw edema by using 2.5% formalin solution

Table 3.2.1: Anti-inflammatory activity of synthesized compounds by formalin-induced by rat hind (Lower left) paw edema model.

Groups	**Dose (mg/kg)**	**Before formalin (V_0)**	**After 3hrs. (Vt)**	**Vc Or V_t**	**%Inhibition [1-(Vt/V_c)] *100**
Control	----	2.16±0.176	4.85±0.91	2.69	-----
Diclofenac sodium (Std.)	25	2.36±0312	3.15±0.232	0.79	70.63*
3a.	25	2.0±0.173	4.09±0.190	2.09	22.30*
3b	25	2.32± 0.234	3.88±0.131	1.56	42.00*
3c	25	2.15±0.143	3.66±0.123	1.51	40.92*
3d	25	2.09±0.173	3.31±0.190	1.22	54.79*
3e	25	2.10± 0.234	3.84±0.131	1.74	35.31*
3f	25	2.24±0.182	4.42±0.281	2.18	18.95*

Values are represented Mean ± SEM n=6 albino rats per groups; *p<0.01 as compared with control group

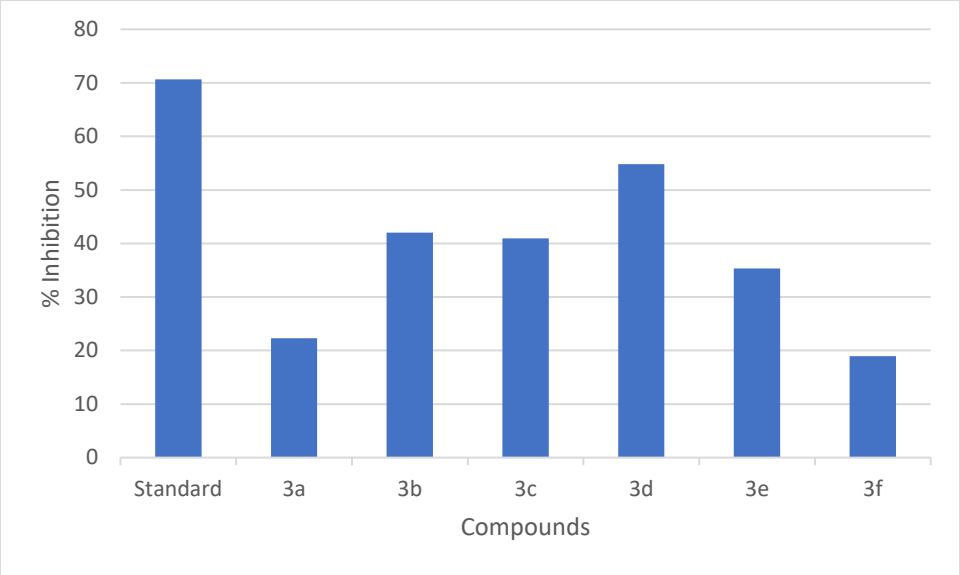

Figure 3.2.2. Graph shows the percentage inhibitions of the synthesized compounds

4. Result and Discussion

The synthesis of 1,4-dihydropyridine derivatives with enhanced anti-inflammatory activity was the main goal of this study. Three steps were used to complete the synthesis of derivatives using a proton transfer mechanism and cyclization reaction. This reaction yields six derivatives: 3a, 3b, 3c, 3d, 3e, and 3f. were synthesized.

All of the synthesized compounds had melting points between 120 and 222 degrees Celsius. Using silica gel-G as an absorbent and a solvent system consisting of acetic acid, ethanol, and water (2:4:4), TLC was performed on all synthesized compounds. The solubility study's findings indicate that all synthesized compounds have a maximum solubility in benzene and chloroform and are insoluble in water.

All synthetic derivatives of organic and inorganic compounds are analyzed both quantitatively and qualitatively using UV spectroscopy. All synthesized compounds' wavelength (λmax nm) is represented by UV spectroscopy.

IR spectroscopy is used for both qualitative and quantitative analysis to determine the functional group. The group of all synthesized compounds that validates their functional group is represented by the peak values. The representative functional group that is present in each compound exhibits a distinct peak value.

The Swiss target prediction webserver was used to make the target prediction. According to this study, all of the synthesized derivatives (3a–3f) provide accurate cyclooxygenase target predictions. The compound 3d exhibits the highest Probability of target COX-2 inhibition (0.125).

The synthesized compounds were also tested in-vivo using the formalin-induced rat hind paw edema method. The synthetic compounds' anti-inflammatory activity is arranged as follows:

Synthesized compound 3d,3b,3c,3e,3a,3f, showed 54%, 42%,40%,35%,22%,18% inhibition of paw edema, in turn. Comparing compound 3d to other compounds, it demonstrated the highest level of activity. The fourth position of the phenyl ring in the most active compound, 3d, contains an electron-withdrawing group and an electron-donating group methoxy is present 3 &

5 positions indicating that both electron-withdrawing and donating group give maximum activity in 1,4-dihydropyridine derivatives.

5. Conclusion

he synthesized derivatives are known to be 1,4 dihydropyridine derivatives. so, it is concluded that both electron withdrawing and electron donating group enhances the anti-inflammatory activity at different position of 1,4-dihydropyridine. This result can be explored for further study in this field.

6. Acknowledgement

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Conflict of Interest: None
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Ethics Approval: All studies conducted by the Institutional Animal Ethical Committee VNS faculty of pharmacy Bhopal (Approval no. PH/IAEC/VNS/2K19/08).

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