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Research Article

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

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Abstract

The new 1,4-dihydropyridine drug series emerged from Swiss Target Prediction's design process. Testing showed these compounds had peak binding ability with COX-II receptor. The research team created six top-performing 1,4-Dihydropyridine variations through ethyl acetoacetate and aromatic benzaldehyde replacements. Our team used UV and IR instruments to study the basic structure of each compound. All tested compounds underwent paw edema testing using rats to measure their biological effects. New compounds showed the highest anti-inflammatory performance first (3d) followed by 3b, 3e and 3c. These effects placed better than 3a and 3f. The substance 3d displayed superior anti-inflammatory results because its extra strength comes from both the methoxy electron withdrawing site and the chlorine electron donating group found in 1,4 dihydropyridines. Each of these groups works to make rats less inflamed. ***@2024 IJPHI All rights reserve***

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Introduction

NSAIDs become the most widespread treatment for inflammation because they remain the go-to option for practitioners. Throughout the world millions of people consume NSAIDs daily[1,2]. These medications show good results against both short-term and long-term inflammation and help many patients recover[3-5]. Researchers have identified NSAIDs while categorizing them into multiple groups according to their chemical design and medical consequences. Although there are a number of NSAIDs developed and available in market but rigorous search for new molecular is on wheels[6-8]. 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. 1,4-Dihydropyridine forms a nitrogen-based six-member hydrocarbon ring type structure with four atoms arranged in a circle. Studies confirm that both single and combined versions of the moiety show numerous pharmacological activities including anti-inflammation and blood pressure control[11-13]. Research shows that a free NH group in a 1,4-DHP system requires no chemical modifications to perform well as a medicine. Research shows that the methylation of positions 2 and 6 plus esterification of 3 and 5 and an aryl ring at position 4 form the base architecture for effective biological action. Therefore, by substituting various electron-donating and electron-withdrawing groups, we hope to synthesise several 1,4-Dihydropyridine derivatives as anti-inflammatory drugs and assess their anti-inflammatory efficacy.[14-15]

Experimental

Materials

Four different chemical producers provided synthetic grade raw materials to our team. We distilled and dried all the solvents prior to use. We checked reaction progress using a thin layer chromatography method with chosen solvents and iodine gas developed spots. The fabricated compounds' melting points measured melting point device. Our team did spectroscopic testing on manufactured compounds for complete structure verification. We employed a UV Shimadzu 1700s instrument to verify the light absorption patterns of our manufactured mixtures. At RGPV Bhopal team used Shimadzu technology to run IR spectroscopy tests.

Methods: synthesis of compounds

The scheme of the synthesis of 1,4-dihydropyridine derivatives is shown in figure 1

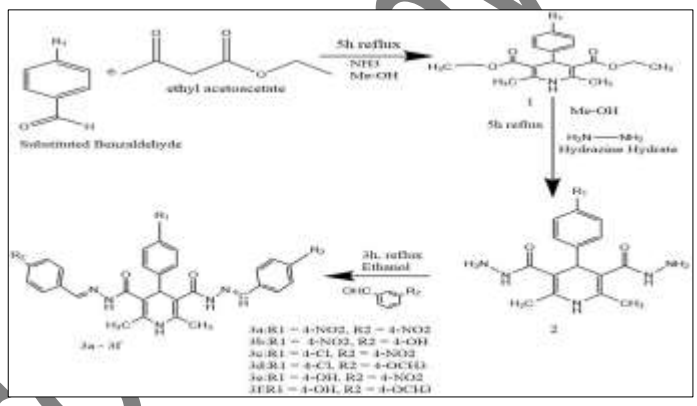

Figure 1.Synthesis of 1,4-dihydropyridine derivatives

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 5h. After the completion of the reaction, the mixture was cooled over ice bath to obtain crude crystal. After filtering the solids, we cleaned them multiple times with methanol before removing the remaining solvent and moisture. The researchers purified the solid material through multiple steps in methanol and water[16]. % Yield of derivatives was found to be 70%. Melting point of derivatives was found to be 210211ºC. 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,

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methanol was removed and crystals of hydrazide derivatives were formed immediately. The crystals were washed with water and recrystallized from methanol.

% Yield of derivatives was found to be 88%. Melting point of derivatives was found to be 236237°C. TLC Rf value derivatives was found to be 0.46[16].

Synthesis of N'3, N'5-di((E)-benzylidene)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5dicarbohydrazide (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(NO 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.UV spectrum of synthesized compound

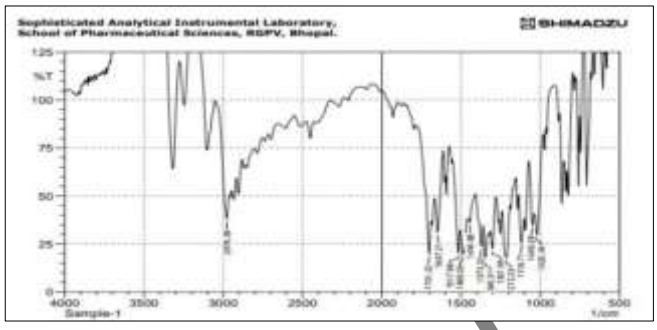

Figure 3. IR spectrum data of synthesized compounds

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 (°C): 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)).

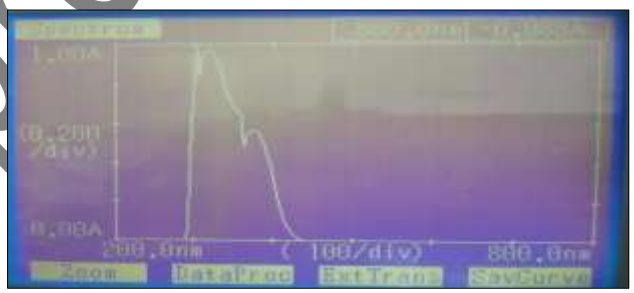

Figure 4. UV spectrum of synthesized compound

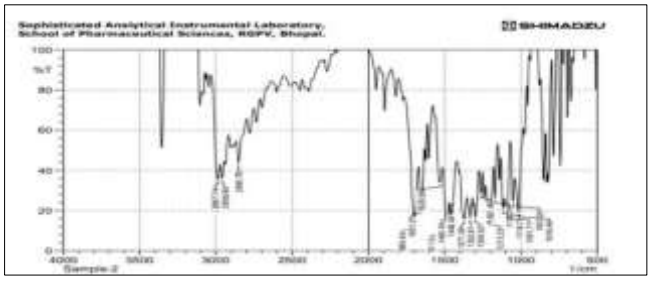

Figure 5. IR spectrum data of synthesized compounds

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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 (°C): 150-151; Rf value: 0.44; UV λmax (nm): 320; IR (cm^{-1}): 720(CCl 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 6. UV spectrum of synthesized compound

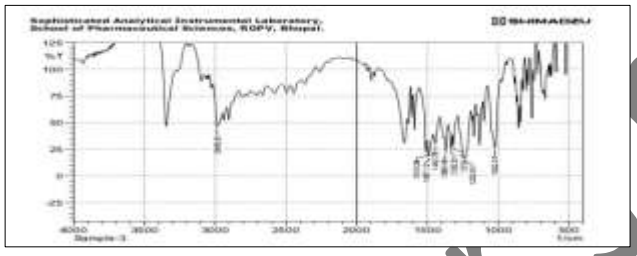

Figure 7. IR spectrum data of synthesized compounds

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

Figure 8. UV spectrum of synthesized compound

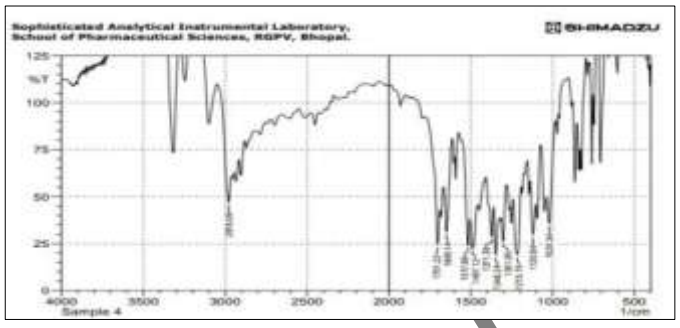

Figure 9. IR Spectrum data of synthesized compound

% Yield 69%; Melting point (°C): 157-158; Rf value: 0.32; UV λmax (nm): 333; IR (cm^{-1}): 720(CCl 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)).

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)).

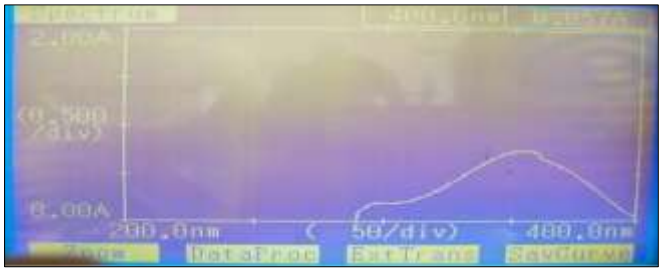

Figure 10. UV spectrum of synthesized compound

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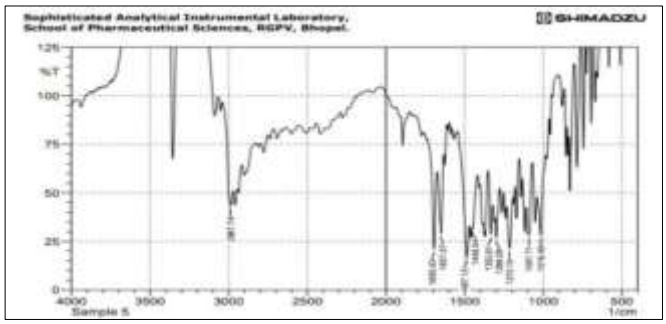

Figure 11. IR Spectrum data of synthesized compound

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

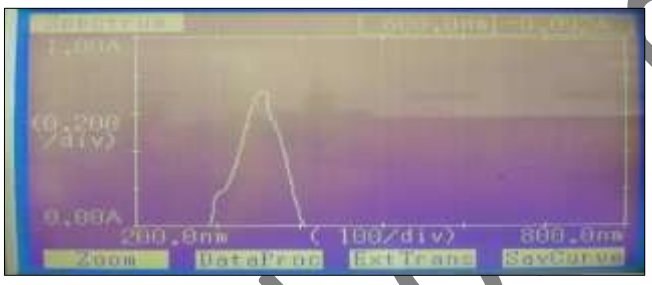

Figure 12. UV spectrum of synthesized compound

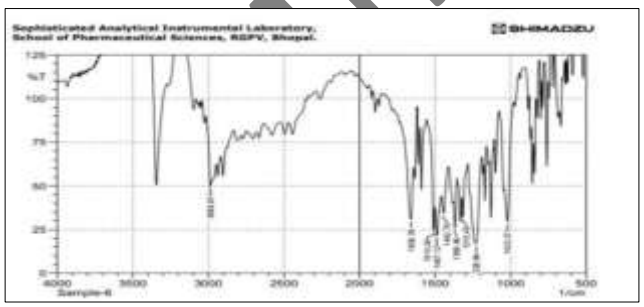

Figure 13. IR Spectrum data of synthesized compound

Biological evaluation

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 14.

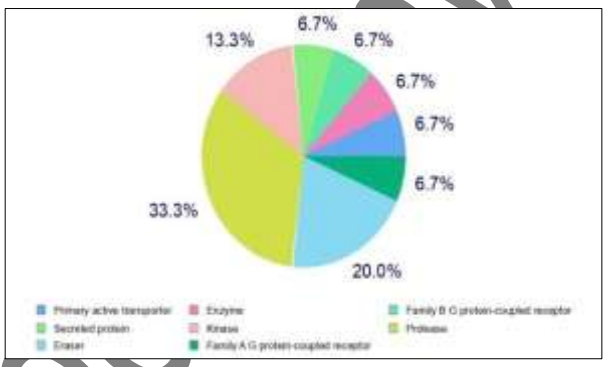

Figure 14. Swiss prediction Data of synthesized compound

IN-VIVO STUDY

The formalin-induced left hind paw oedema method has been used to test the anti-inflammatory properties of synthetic substances. 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 left hind paw edema method

Using this technique, Swiss albino rats weighing between 168 and 235 grammes were randomly selected, their paw volumes were measured, and they were split into eight groups of six animals each. The rats' hind paw oedema was caused by a supplanter injection of 2.5° formalin solution. It consequently causes oedema to build in the area. A Plethymometer 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 calculate and compare the percentage (%) of increase in

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paw volume with time[20,21]. The results are shown in table 3.2.1 & 3.2.2.

Figure 15 Induction of paw edema by using 2.5% formalin solution

Table 1 Anti-inflammatory activity of synthesized compounds by formalin-induced by rat hind paw edema method

Groups	Dose (mg/kg)	Before formalin (V_0)	After 3hrs. (Vt)	V c O r Vt	%Inhibition [1-(Vt/V_0)] *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

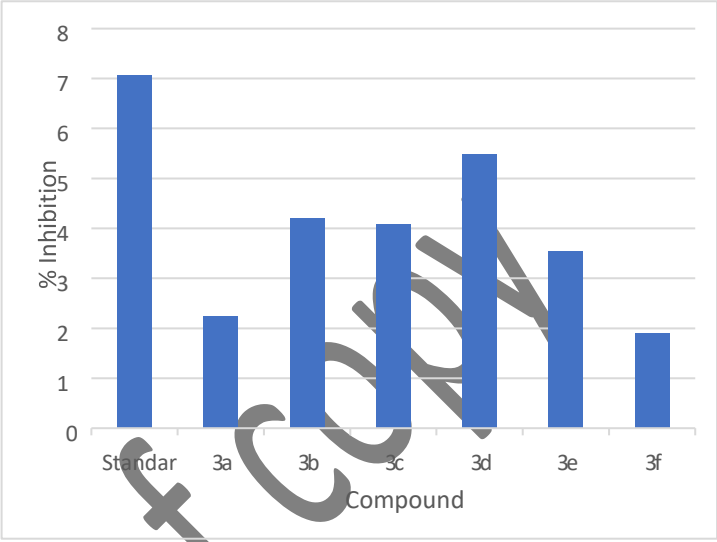

Figure 16 Graph shows the percentage inhibitions of the synthesized compounds

Result and Discussion

Our research cantered on developing better anti-inflammatory 1,4-dihydropyridine compounds through synthesis. Our team did three procedural steps and made the chemical connections work through proton transfers. Through this approach our team produced six finished compounds (3a, 3b, 3c, 3d, 3e, and 3f). All produced materials showed melting points from 120 to 222 degrees Celsius. TLC of all synthesized compounds was done by using silica gel-G as an absorbent and (acetic acid: ethanol: water) (2:4:4) solvent system. Our solubility tests show all our synthesized products do not dissolve in water but have high solubility in chloroform and benzene.

UV spectroscopy is used for the qualitative and quantitative analysis of organic and inorganic compounds in all synthesized derivatives. The UV spectroscopy represents the λmax (nm) of all synthesized compounds.

IR spectroscopy used to identify the functional group, qualitative and quantitative analysis. The peak values represent the group of all synthesized compound that confirm the functional group of all synthesized

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compound. The peak value of the representative group that is currently present in the compounds is displayed by every compound.

The target prediction was done by Swiss target prediction webserver. This study shows that all synthesized derivatives (3a-3f), gives good target prediction on cyclooxygenase. The compound 3d shows maximum probability (0.125) of target COX -2 inhibition.

The formalin-induced rat hind paw oedema method was also used to examine the synthesised compounds in vivo. The following is the order of the synthetic chemicals' anti-inflammatory activity:

New compound 3d reduced inflammation in paws by 54% and 42% respectively through 3b, 3c, and 3e but dropped to 40% with 3a before reaching 18% with 3f. The drug compound 3d demonstrated its best effectiveness among all tested compounds. The most active compound 3d contain an electron withdrawing group chlorine at 4th position of phenyl ring and an electron donating group methoxy is present 3 & 5 position indicated that both electron withdrawing and donating group gives maximum activity in 1,4-dihydropyridine derivatives.

Conclusion

As we know that the synthesized compounds are 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.

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Conflict of Interest: None

Funding Source: Nil

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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