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SYNTHESIS & IN-VITRO PROTEIN DENATURATION SCREENING OF 2-[(1, 5-DISUBSTITUTEDPHENYL-4,5-DIHYDRO-1H-PYRAZOL-3-YL)OXY]BENZOIC ACID DERIVATIVES

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Abstract

Novel 2-[(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)oxy]benzoic acid (2a) & 2-[5-(2-hydroxyphenyl)-1-Phenyl-4,5-dihydro-1H-pyrazol-3-yl]benzoic acid (2b) were produced and examined for their in-vitro protein denaturation activities. It was discovered that compound 2b showed promise and had more potency than acetylsalicylic acid (NSAID) in inhibiting denaturation of bovine serum albumin. Docking research also supports this. The compound 2b has the highest docking scores with COX1(PDB ID 3N8Z), COX2 (PDB ID 4PH9), and TNF (PDB ID 2AZ5), respectively, of Etotal -233.75, -256.48, and -255.83. TLC and elemental tests were used to determine the compounds' purity. All of the generated molecules' analytical and spectral data (1H NMR, FTIR, and MS) were entirely consistent with the proposed structures.

Keyterms: Pyrazoline, BSA, Protein Denaturation Activity, Docking, Anti-Inflammatory Activity

INTRODUCTION

Heterocyclic compounds with five members that have a wide range of biological action. Pyrazole, which has two nitrogen atoms, has a history of biological activity. Acetylsalicylic acid (Aspirin) has been documented to display a range of biological actions and is a very avluable component in the disciplines of medical and pharmaceutical chemistry.Chalcones and their analogues with an unsaturated carbonyl system are extremely flexible substrates for the development of different reactions and chemical products with biological activity7.PhNHNH2, H2O reacts with various chalcones of the acetylsalicylic acid derivative to produce 2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl) benzoic acids (2a,b) with the objective of creating some novel heterocyclic systems, we thought it would be interesting to synthesise four distinct yet pharmacologically compatible compounds in light of the pharmacological profiles of these two chemical moieties as mentioned above.

MATERIALS AND METHODS

Molecular Modeling Studies:

Molecular modeling studies have been carried out using HEX 5.1 (Grid-based Ligand Docking with Energetics) software workspace was used for all the steps involved in ligand preparation, protein preparation and docking. ACD Chem Sketch is chemical drawing software developed by ACD LAB. The software is user-friendly, provides all

details of drawn structures and helped to calculate chemical properties, design professional reports and presentations.

Ligand Preparation:

The ligands used in this study were prepared using ARGUS LAB (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization.

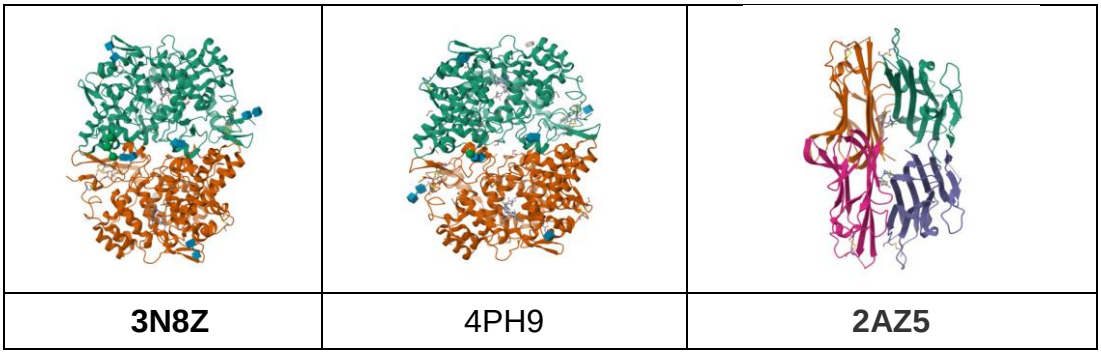
Protein Preparation:

The X-ray crystal structures retrieved from PDB database as raw could not be suitable for molecular docking studies. A typical PDB structure consists only of heavy atoms, waters, Cofactors, metal ions and can be of multimeric. These structures do not have the information about bond orders, topologies or formal atomic charges. So, the raw PDB structure should be prepared in a suitable manner for docking. ARGUS LAB (Optimized Potential Liquid Simulations for All Atoms) force fields for energy minimization.

Lipinski Rule of Five Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules.[15] It predicts high probability of success or failure due to drug likeness for molecules complying with 3 or more of the following rules

- Molecular mass less than 500 Dalton
- High lipophilicity (expressed as LogP less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130.

Figure No.1

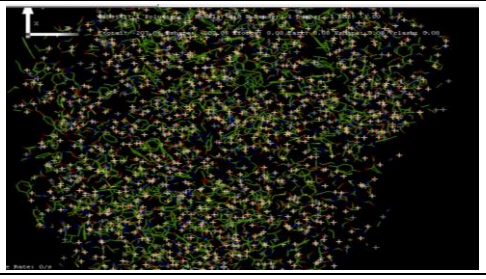
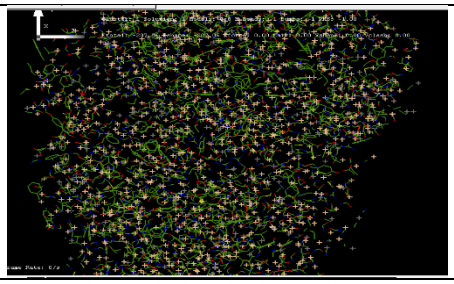
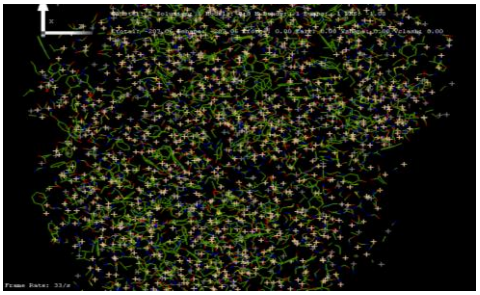
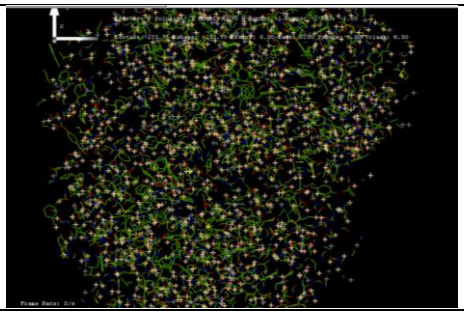
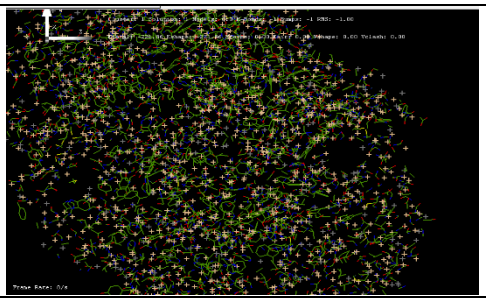
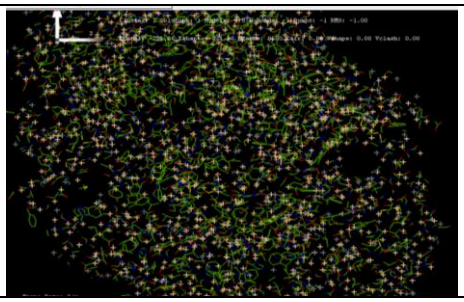
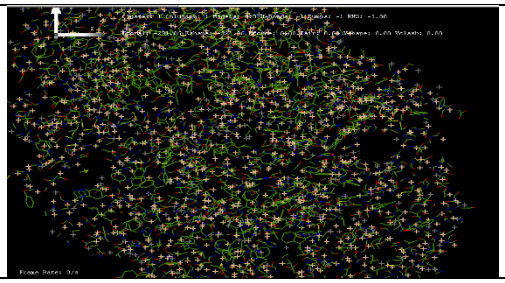
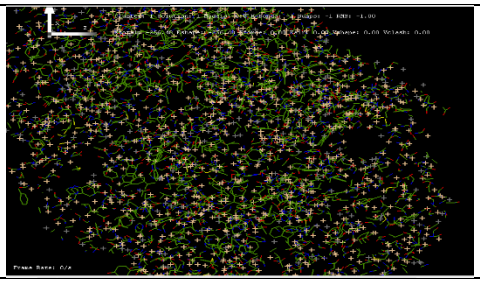
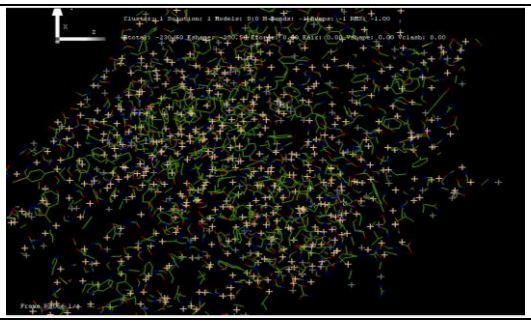
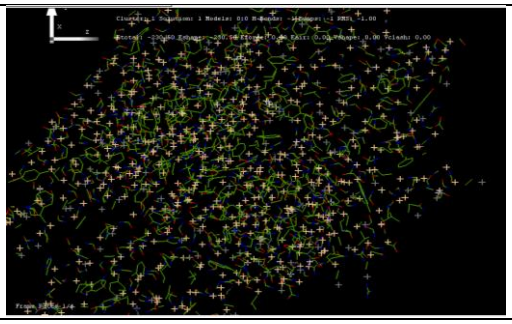


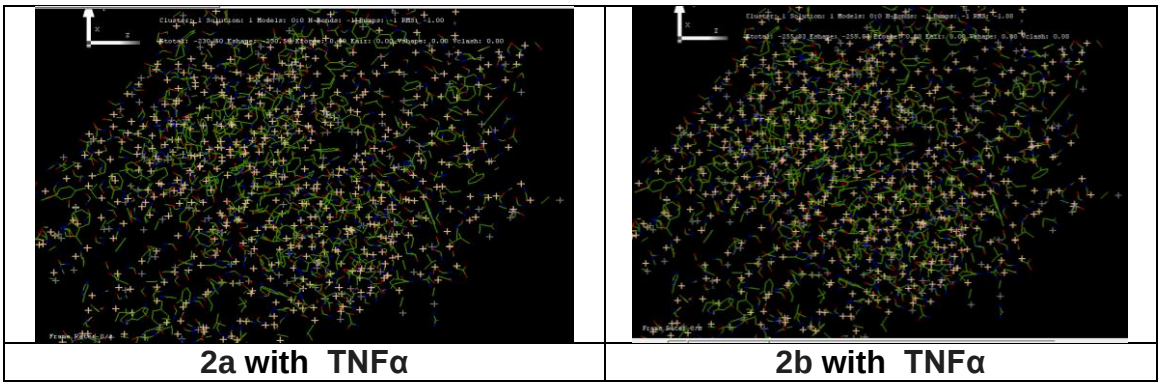
3N8Z	4PH9	**2AZ5**

Table No.1: Physiochemical properties and docking score

Compound Name	miLogP	TPSA	natoms	MW	nON	nOHNH	nViolations	nrotb	Volume	Rule of 5	E. total Cox1	E. total Cox2	E. total TNFα
1a	3.58	63.60	20	268.27	4	1	0	5	237.84	Followed	-207.05	-221.86	-230.50
1b	3.34	83.83	21	284.27	5	2	0	5	285.46	Followed	-207.06	-221.86	-230.50
2a	5.28	62.13	27	358.40	5	1	1	5	321.68	Followed	-207.06	-221.86	-230.50
2b	5.22	82.36	28	374.40	6	2	1	5	329.70	Followed	-233.75	-256.48	-255.33

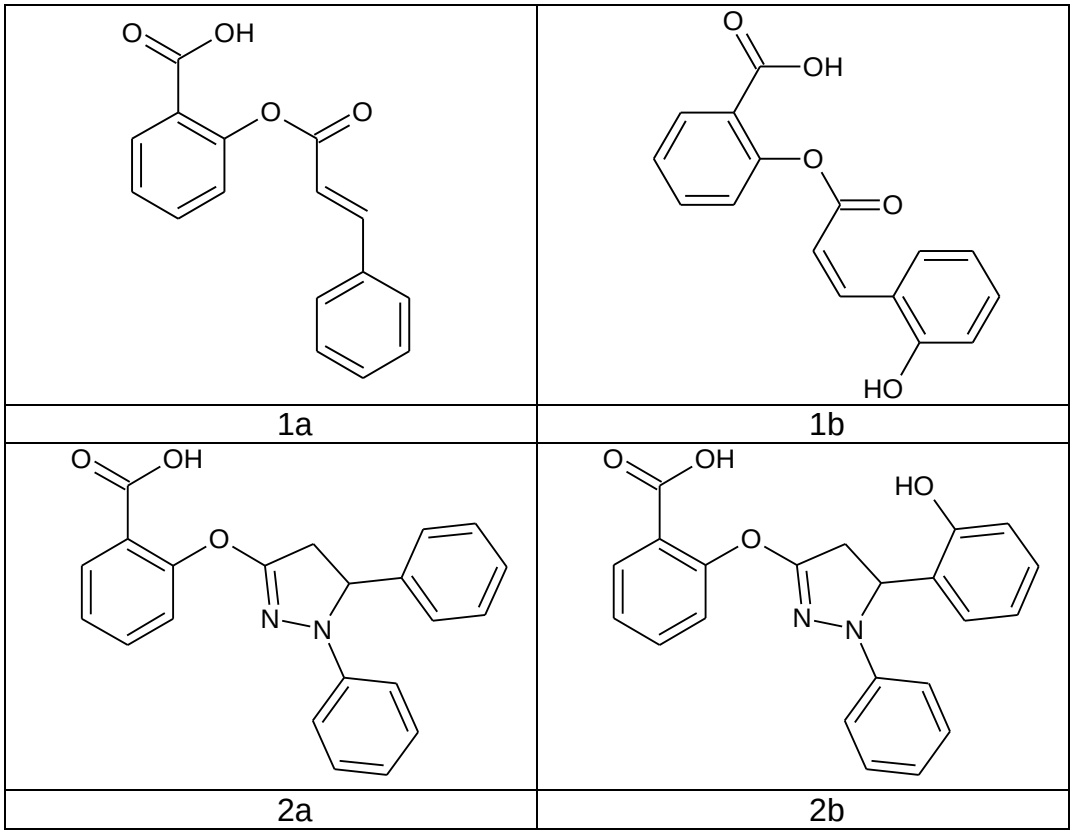
Figure No.2: Docking image of compounds with Cox1 (PDB ID 3n8z), Cox2 (PDB ID 4ph9) and TNFα (PDB ID 2az5)

	
1a with Cox1	**1b with Cox1**
	
2a with Cox1	**2b with Cox1**
	
1a with Cox2	**1b with Cox2**
	
2a with Cox2	**2b with Cox2**
	
1a with TNFα	**1b with TNFα**



2a with TNFα	2b with TNFα

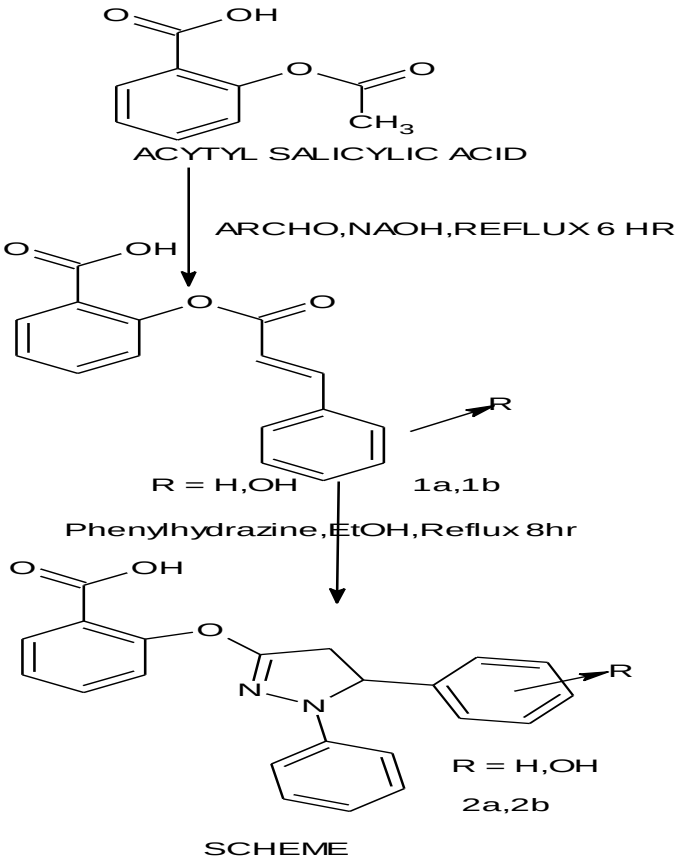
Figure No.3: Structure of synthesized compounds



1a	1b
2a	2b

Experimental:

Melting points were determined on a Tempstar apparatus and are uncorrected. Electronics spectra were recorded in double beem uv-vis spetrophotometry (V-600,Jasco),Infrared spectra were recorded on a Jasco (410) FT-infrared spectrophotometer, measured as KBr disks.1H NMR were recorded on a Bruker DPX-300 MHz spectrometer in deuteriochloroform with trimethyl silane as internal standard (chemical shift in δ ppm). The mass spectral data were obtained with a Perkin-Elmer Hitachi RMU-6L MS-30 spectrometer at 70 ev and a 90^0C inlet temperature. Purity of all the compounds was checked on silica gel plates and spots were located in iodine vapours. Elemental analysis was performed on EURO EA (Italy) analyzer and the results were within $\pm$ 0.4 % of calculated values. Physical data of the synthesized compounds are presented in Table No.2.



SCHEME

General method for the preparation of 2-{-3-phenylprop-2-enoyloxy} benzoic acid (1a):

Acetylsalicylic acid (0.01 m) and benzaldehyde (0.01 m) were mixed in ethanol in an equimolar ratio. 30 minutes were spent adding 10ml of a 2% sodium hydroxide solution drop by drop while swirling continuously in a magnetic stirrer. After 17 hours of stirring, the reaction mixture was refluxed for 6 hours. The reaction mixture was then added to some ice-cold water. Filtered, aqueous ethanol was used to wash the separated material before it was recrystallized from a 50:50 mixture of ethanol and water. Analogously, compound 1b was produced.

2-{-3-phenylprop-2-enoyloxy} benzoic acid (1a)

IR (KBr): 3076(Ar-CH), 1732 (C=O), 1670 (C=O), 1609 (C=C), MS: (m/z) 252(M+); 1H NMR (CDCl3): 11.69 (s, 1H, COOH), 7.61(d,CH,CH=CH), 7.68(d,CH,CH=CH),8.06 (m, 9H, ArH), 6.02-7.58 (m, 4H, ArH);

2-[3-(2-hydroxyphenyl) prop-2-enoyl] benzoic acid (1b)

IR (KBr): 3470 (OH), 3072(Ar-CH), 1730 (C=O), 1672 (C=O), 1602 (C=C), MS: (m/z) 268(M+); 1H NMR (CDCl3) : 11.68 (s, 1H, COOH), 7.66(d,1H,CH=CH), 7.74(d,1H,CH=CH),8.06 (m, 9H, ArH), 6.02-7.58 (m, 4H, ArH),5.69(s,IH,OH);

General method for the preparation of 2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)benzoic acids (2a)

In ethanol, equimolor amounts of 1a and phenylhydrazine were combined. 8 hours were spent refluxing the reaction mixture. The reaction mixture was then added to

some ice-cold water. Filtering, washing with aqueous ethanol, and recrystallization from water and ethanol (50:50) were all steps in the preparation of compound 2b.

2-[(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)oxy]benzoic acid (2a)

IR (KBr): 3075(Ar-CH), 1731(C=O), 1687 (C=O), 1609 (C=N), MS: (m/z) 358 (M+); 1H NMR (CDCl3) : 11.70 (s, 1H, COOH), 5.69(t,1H,CH), 3.28(d,2H,CH2), 6.08-8.17 (m, 4H, ArH);

2-[5-(2-hydroxyphenyl)-1-Phenyl-4,5-dihydro-1H-pyrazol-3-yl]benzoic acid (2b)

IR (KBr): 3470 (OH), 3076(Ar-CH), 1729 (C=O), 1688 (C=O), 1615 (C=N), MS: (m/z) 374(M+); 1H NMR (CDCl3) : 11.72 (s, 1H, COOH), 5.61(t,1H,CH), 3.31(d,2H,CH2), 6.08-8.27 (m, 4H, ArH),5.3(s,IH,OH);

Table No. 2: Physical data of synthesized compounds 1a-b & 2a-b

Compd.	R	Molecular Formula	m.p (C0)	Yield (%)	% Analysis Calc.(Found) C H N		
1a	H	$C_{16}H_{12}O_3$	99	69	76.18 (76.22)	4.79 (4.76)	--------
1b	OH	$C_{16}H_{12}O_4$	96	59	71.64 (71.53)	4.51 (4.48)	--------
2a	H	$C_{22}H_{18}N_2O_3$	159	73	73.73 (73.78)	5.06 (5.08)	7.82 (7.78)
2b	OH	$C_{22}H_{18}N_2O_4$	152	75	70.58 (70.71)	4.85 (4.76)	7.48 (7.53)

Experimental model:

Inhibition of protein denaturation:

The test substance (0.05ml) had a final concentration of 100 and 250 mg/ml and was combined with 0.45ml bovine serum albumin (5% aqueous solution) to form the reaction mixtures (0.5ml). Using a little quantity of IN HCl, the pH was set at 6.3. 3 minutes were spent incubating the samples at 370C. 6.5 pH phosphate buffer saline was added to each tube after the samples had cooled. Utilising spectrophotometry, turbidity was determined at 660 nm. Bovine serum albumin was absent from control experiments, which employed 0.05 ml of pure water instead of a synthetic product. Following are the calculations used to get the % inhibition of protein denaturation.

$$100- \frac{(O.D.of\ test - O.D.of\ product\ control)}{O.D.of\ control} x100$$

Table No.3. In vitro anti-inflammatory screening of synthesized compounds by bovine serum albumin denaturation

Compound	Absorbance value (660nm)*	Inhibition of Protein Denaturation (%)
2a	0.190	29
2b	0.173	44
Acetyl Salicylic Acid	0.149	66
Control	0.111	00

(*Average of three readings.)

RESULTS AND DISCUSSION

Chemical synthesis:

By combining aspirin and a substituted benzaldehyde, 2-[3-(2-hydroxyphenyl)prop-2-enoyl]benzoic acid (1a) and 2-[3-(2-phenylprop-2-enoyl)oxybenzoic acid (1b) were created. Compounds 1a,b were condensed with phenyl hydrazine in ethanol to obtain the corresponding pyrazole (2a 2-[(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)oxy]benzoic acid , 2-{[1-(2-hydroxyphenyl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl]oxy}benzoic acid 2b) derivatives in 73-75% yields (Table 1).

By using their elemental analyses, FT-IR, 1H NMR, and mass spectroscopy, all the synthesised compounds were identified. The IR spectra of 1a, for instance, has an absorption band at 1729 cm-1 that corresponds to the stretching vibration of the COOH group and one at 1674 cm-1 that corresponds to the distinctive keto group of chalcone. The Imino(C=N) groups, which are respectively present at the Pyrazole moiety, have an absorption band at 1614 cm-1 in the IR spectra of compound 2a.

The 1H NMR spectrum of 2a had two singlets at 5.66 and 3.11 ppm that were assigned to the CH and CH2 groups, respectively. The 1H NMR of 2a revealed the lack of the signal for the CH=CH group. At the anticipated chemical shifts, all of the pyrazole moieties' aromatic protons were present. Additionally, the mass spectrum of 1a and 2a, which displays molecular ion peaks (M+) at m/z 252 and m/z 358, supported the structures of the ions in those atoms.

Biological activity:

Using the BSA assay method8 and bovine serum albumin, several 2-(1,5-disubstituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)benzoic acids (2a-b) were evaluated for their ability to inhibit protein denaturation activity. Aspirin, a reference medication, and the compounds' percentage protein denaturation activity are compared in Table No. 3. Two of the two synthesised molecules showed notable activity. Therefore, it can be inferred that the size of the hydroxyl substituents at the pyrazole moiety is crucial for the anti-inflammatory effect in vitro.

Protein denaturation activity: It is widely known that one of the causes of RA is protein denaturation. In some rheumatic disorders, the production of autoantigens may be triggered by the denaturation of proteins in culture. The modification of electrostatic, hydrogen, hydrophobic, and disulphide boarding is likely the process of denaturation. Based on the findings of the current investigation, it can be concluded that synthetic pyrazole analogues have the ability to regulate the formation of autoantibodies brought on by in-vivo protein denaturation in rheumatic disease.

This discovery supports the product's value in the management and treatment of diseases like arthritis that are characterised by inflammation. Based on the findings of the current studies, it can be said that a novel 2-(1,5-disubstituted phenyl-4,5-dihydro-1H-pyrazol-3-yl)benzoic acid (2a-b) acid has significant anti-arthritic and anti-inflammatory activity.

CONCLUSION

In this study, a collection of four new compounds with pyrrazole cores made of benzoic acid-based moieties were synthesised, and their spectral and elemental analyses were used to characterise them.To investigate their binding affinities and understand how they function to reduce inflammation, all recently synthesised compounds were also subjected to in silico docking studies against the target enzymes COX1(PDBID:3n8z), COX2(PDBID:4ph9), and TNF(PDBID:2az5).It was also tested whether each newly created chemical could in vitro denaturate bovine serum albumin.The chemical 2b has a fairly strong inhibitory activity in comparison to the standard therapy.

The most physiologically active molecule, as determined by in silico and in vitro tests, was compound 2b (2-[5-(2-hydroxyphenyl)-1-Phenyl-4,5-dihydro-1H-pyrazol-3-yl]benzoic acid). Consequently, it might act as a foundation for later optimisation to create potent molecules that target diseases with inflammation. Among the newer derivatives, compound 2b showed a promising activity in the test. It is conceivable that these derivatives showing invitro Protein Denaturation activity can be further modified to achieve NSAID agents with antiarthritis activity. SAR studies and evaluation of more potent analogues are continuing.

Acknowledgements

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Conflict of interest:

All authors declare that there is no competing interest.

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