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Synthesis, Anti-Inflammatory, Antioxidant Activity And Insilico Evaluation of 3-(Pyridin-4-Yl)-[1,3,4] Oxadiazino[6,5-B] Indoles For Anticancer And Covid-19

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ABSTRACT

Oxadizine moieties are pharmacologically active and are being used for various diseases and disorders. For the synthesis of a series of novel derivatives, an efficient and easy approach has been designed and 3-(pyridin-4-yl)-[1, 3, 4]oxadiazino[6, 5-b]indole derivatives have been synthesized. The synthesized compounds were characterized by elemental analysis of IR, NMR, and Mass spectroscopy. The *in-vitro* anti-inflammatory activity was performed by Cayman's COX(ovine) colorimetric inhibitor screening assay kit and compounds with COX inhibitory activity in *in-vitro* were subjected to *in-vivo* anti-inflammatory activity by carrageenan induced rat paw edema method by Diclofenac as standard drug. *In-vitro* antioxidant activity of newly synthesized compounds was determined by the α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method by using Ascorbic acid as standard drug. Among all the compounds IXf (R=5-NO_2) and IXk (R=7-F) showed efficient anti-inflammatory activity and antioxidant activity respectively. Insilico studies are performed on SARS-CoV-2 main protease (pdb: 7BZ5) and Cancer main protease (pdb: 5OTF). Among them compounds IXf (R=5-NO_2) and IXg (R=7-NO_2) on SARS-CoV-2 main protease (pdb:7BZ5) showed good binding score of -8.7Kcal/mol and -8.6Kcal/mol respectively and Compounds IXn (R=7-$COOCH_3$) and IXg (R=7-NO_2) on Cancer main protease (pdb:5OTF) showed good binding score of -9.1 Kcal/mol and -9.0 Kcal/mol respectively.

Keywords: Isatin, Oxadiazine, Anti-inflammatory, Antioxidant, COX assay, proteases

INTRODUCTION

Heterocyclic moiety Oxadiazine occupies a unique position in heterocyclic chemistry, due to its large number of biological activities[1]. It exists in different isomeric forms and 1,3,4-oxadiazine is a core molecule for the design and synthesis of various medicinal compounds[2]. Inflammation is a tissue reaction to injection, injury, irritation or a foreign substance. It is a part of the host defense mechanism. Aging is also considered to be an inflammatory response. Inflammatory responses mainly include release of histamine, prostaglandins and bradykinin which play a major role in tissue repair[3]. The development of non-steroidal anti-inflammatory agents in recent years has contributed a lot in overcoming the human suffering such as arthritis and in understanding the tissue mechanisms of inflammation. The Non-steroidal anti-inflammatory drugs are mainly used as first choice of drug for relieving aches and pain[4].

An antioxidant is an agent that stops molecules within a cell from oxidizing. It removes electrons or hydrogen from a material. During the biological oxidation reaction, free radicals are formed. Because radicals are reactive, they start the chain reaction simultaneously[5]. This can cause cellular damage or even death. Antioxidants have the ability to stop a chain reaction by removing free radical intermediates[6]. As a result, they are also known as free radical scavengers. Antioxidants are free radical scavengers that neutralize reactive oxygen species (ROS) generated during aerobic cellular metabolism, including superoxide ($O2^-$), hydrogen peroxide ($H2O2^-$), and peroxynitrite ($OONO^-$). Also, antioxidants exert protective effects on cells against the deleterious effects of ROS on cell membranes, mitochondria, DNA, lipids or proteins.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site[7]. Characterization of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes. It is a method which predicts the preferred orientation of one molecule to a second when a ligand and a target are bound to each other to form a stable complex[8]. Here Insilico studies were performed on Cancer Protease (5OTF) and SARS-CoV-2 Main Protease (7BZ5) as a Drug Targets[9].

MATERIALS AND METHOD:

All the chemicals and solvents used were obtained from Merck and Himedia. The synthesized compounds were preliminarily confirmed by thin layer chromatography (TLC) plates and UV chamber. Melting points were determined by using capillary tubes and digital melting point apparatus. The final derivatives were characterized by spectral methods such as Fourier transform infrared (FTIR), 1H-NMR and Mass spectroscopy.

Softwares: PDB(protein data bank), PYREX, AutoDock and AutoDock Vina, rDock, FlexAID, Molecular Operating Environment, Glide and DockThor.

Chemistry:
STEP-1: Synthesis of Indole-2,3-diones (Isatins):

a) Isonitrosoacetanilide: 1200 ml of water and 0.54 mol of chloral hydrate were put in a 5 lit. R.B. flask. Then, crystalline sodium sulfate (1300gm) and a solution of a suitable aromatic amine in 300ml of water and strong hydrochloric acid (0.52mol) were added to this mixture. In the end, 500 ml of hydroxylamine HCl (1.58 mol) solution was added. A Mecker burner heated the flask's contents over a wire gauge to the point where violent boiling started in about 45 minutes. The reaction was finished after one to two minutes of strong boiling. The isonitrosoacetanilide crystals began to separate even throughout the heating process. The entire product solidified after cooling in the water current. It was filtered under suction, air dried and purified by recrystallization from suitable solvent[9].

b) Indole-2,3-diones: In a one-liter RB flask equipped with an effective mechanical stirrer, 326 ml of sulphuric acid were heated to 50 °C. Then, 0.46 mol of suitable isonitrosoacetanilide, which had been finely powdered, were added at a rate that would keep the temperature between 60-70°C, but not higher. The reaction was completed by raising the solution's temperature to 80°C and holding it there for 10 minutes after the isonitroso compound had been added. The reaction mixture was then stirred and put onto crushed ice after cooling to room temperature. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried[10]. Purification of the compound was effected by the recrystallization from methanol. Various derivatives of indole-2,3-diones were prepared by using different aromatic amines and were confirmed by TLC.

STEP-2: Synthesis of Isoniazid (Isonicotinohydrazide): (VII): 10 grams of 4-picoline were oxidized with 45 grams of potassium permanganate. Filtered it, evaporated the combined filtrate, washed it to about 150 mL, and then added strong hydrochloric acid until isonicotinic acid precipitated. Allow the mixture to slowly solidify. Suction filter the crude isonicotinic acid, then rinse with water and dry at 100°C[11]. When isonicotinic acid recrystallizes from hot water, the resultant chemical is isonicotinic acid. Take the above mixture and add 2% Sulphuric acid, methanol as a solvent, and 8gm of hydrazine hydrate. The reaction mixture was then kept at 100oC for 4 hours, after which the solvent was evaporated and the Isonicotinic acid hydrazide precipitate was extracted and dried[12].

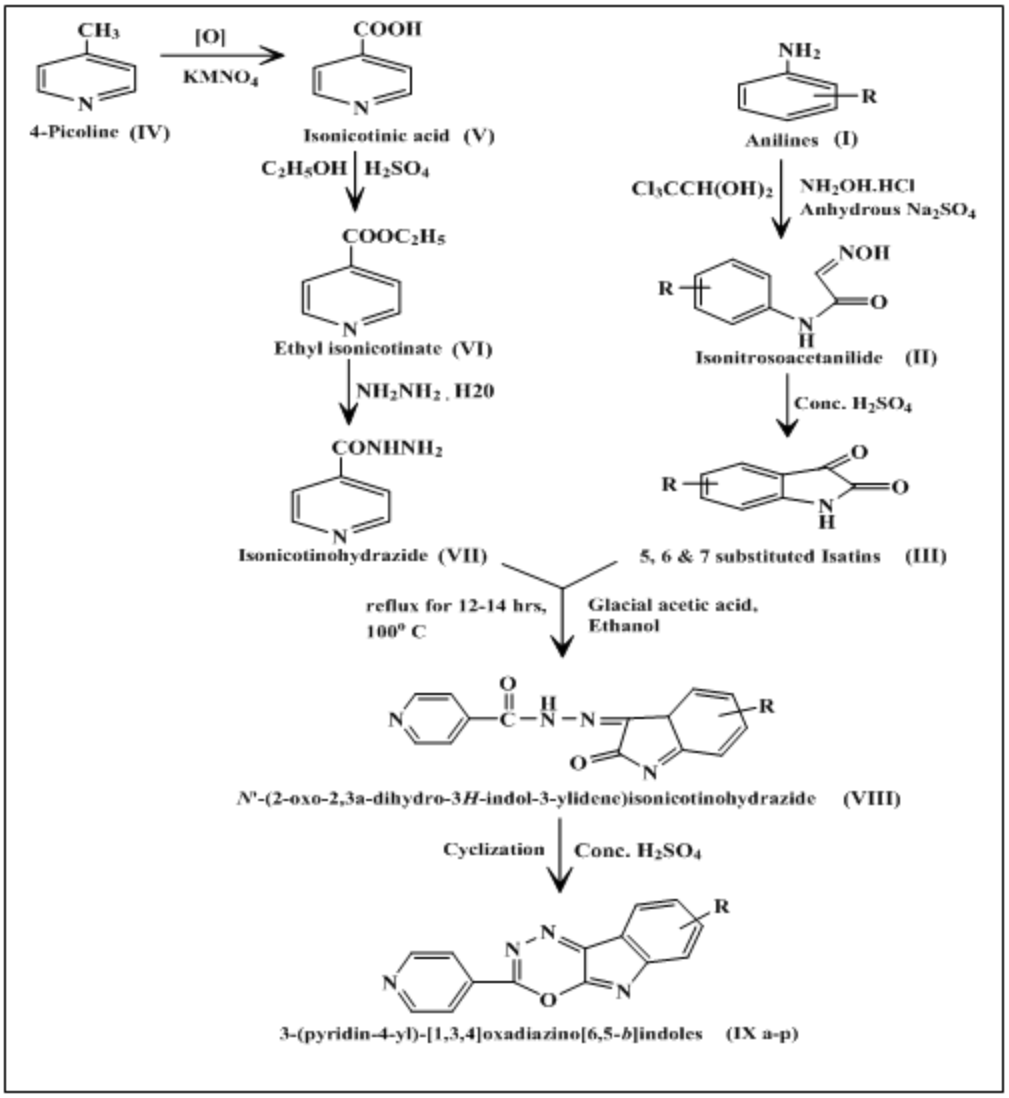

Figure 1: Scheme of Synthesis of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives(IXa-IXp)

STEP-3: Synthesis of N'-(2-oxo-2,3a-dihydro-3H-indol-3ylidene)isonicotinohydrazides (VIII a-p): In a RBF, an equimolar mixture of isoniazid **(VII)** (0.002 mol) and 4,5,6-substituted isatins **(III a-p)** (0.002 mol) and Glacial acetic acid (4–5 drops) in ethyl alcohol (40 ml) were refluxed for 12-14 hrs on the water bath at 100°C[13]. The obtained mixture was poured into crushed ice and stirred vigorously under cold condition and kept aside for overnight and then separated by filtration. The product was washed with little methanol, dried and recrystallized from activated charcoal.

STEP-4: Synthesis of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indoles (IXa-p): A pure compound of N'-(2-oxo-2,3a-dihydro-3H-indol-3-ylidene)isonicotinohydrazide was treated with 4 ml of concentrated sulphuric acid and stirred until dissolved under cold condition and kept aside for overnight[14]. The mixture was allowed to achieve room temperature and transferred into ice-cold water. The resulting product was separated and excess sulphuric acid was neutralized by sodium bicarbonate solution[15]. The new compound was purified by recrystallization from aqueous ethanol. The procedure is repeated for all the remaining intermediated.

Table 1: Physical data of the synthesized compounds (IXa - IXp):

S.No	Compound No.	R	Molecular formula	Molecular weight	yield	R_f Value	Melting point
1.	IXa	H	$C_{14}H_8N_4O$	248.25	89%	0.63	145-148℃
2.	IXb	5-CH_3	$C_{15}H_{10}N_4O$	262.27	87%	0.78	218- 220℃

3.	IXc	7-CH_3	$C_{15}H_{10}N_4O$	262.27	90%	0.76	231-235℃
4.	IXd	5-Cl	$C_{14}H_7ClN_4O$	282.69	87%	0.68	134-137℃
5.	IXe	7-Cl	$C_{14}H_7ClN_4O$	282.69	94%	0.66	136-139℃
6.	IXf	5-NO_2	$C_{14}H_7N_5O_3$	293.24	92%	0.72	171-174℃
7.	IXg	7-NO_2	$C_{14}H_7N_5O_3$	293.24	89%	0.75	175-178℃
8.	IXh	5-Br	$C_{14}H_7BrN_4O$	327.14	93%	0.80	164-186℃
9.	IXi	6-Br	$C_{14}H_7BrN_4O$	327.14	87%	0.75	184-188°C
10.	IXj	7-Br	$C_{14}H_7BrN_4O$	327.14	92%	0.78	152-156℃
11.	IXk	7-F	$C_{14}H_7FN_4O_3$	266.24	84%	0.61	176-180℃
12.	IXl	5-$COOC_2H_5$	$C_{17}H_{12}N_4O_3$	320.31	93%	0.79	160-165℃
13.	IXm	5-COOH	$C_{15}H_8N_4O_3$	292.25	80%	0.70	224-228℃
14.	IXn	7-$COOCH_3$	$C_{16}H_{10}N_4O_3$	306.28	82%	0.65	158-162℃
15.	IXo	6-Cl, 5-F	$C_{14}H_6ClFN_4O$	300.68	94%	0.75	178-182℃
16.	IXp	5-Cl, 7-F	$C_{14}H_6ClFN_4O$	300.68	94%	0.73	174-177℃

SPECTRAL DATA:

Compound IXa- 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}):3071.66-(C-H, Aromatic, str.), 1591.45-(C=N, str.), 1510.81-(C=C, Aromatic, str.), 1124.24-(C-O-C, str). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.5[t, 1H, Ar-H], δ7.6[t, 1H, Ar-H], δ7.8[d, 1H, Ar-H], δ7.8[d, 1H, Ar-H], δ7.9[d, 2H, Pyridine Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 161.65(1C), 151.57(1C), 151.09(2C), 150.12(1C), 144.61(1C), 132.04(1C), 131.52(1C), 126.80(1C), 125.37(1C), 122.58(1C), 121.31(2C), 119.89(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 248(m/z).

Compound IXb- 8-methyl-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}):3023.12-(C-H, Aromatic, str.), 1601.42-(C=N, str.), 1509.81-(C=C, Aromatic, str.), 1133.08-(C-O-C, str), 2833.56-(Methyl C-H). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ2.4[s, 3H, Methyl-H] δ7.25[d, 1H, Ar-H] δ7.28[d, 1H, Ar-H] δ7.7[s, 1H, Ar-H] δ7.9[d, 2H, Pyridine Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 160.60(1C), 158.8(1C), 158.26(1C), 149.56(2C), 145.8(1C), 137.4(1C), 136.2(1C), 131.6(1C), 129.3(1C), 125.9(1C), 124.3(1C), 123.8(2C), 21.6(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 262(m/z).

Compound IXc- 6-methyl-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}):3067.64-(C-H, Aromatic, str.), 1589.24-(C=N, str.), 1507.52-(C=C, Aromatic, str.), 1129.63-(C-O-C, str), 2841.18-(Methyl C-H). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ2.3[s, 3H, Methyl-H] δ7.27[d, 1H, Ar-H] δ7.40[t, 1H, Ar-H] δ7.71[d, 1H, Ar-H] δ7.9[d, 2H, Pyridine Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 160.8(1C), 158.7(1C), 156.2(1C), 150.4(1C), 149.6(2C), 139.7(1C), 135.7(1C), 133.1(1C), 128.5(1C), 127.3(1C), 125.8(1C), 124.2(2C), 19.1(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 262(m/z).

Compound IXd- 8-chloro-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}):3054.32-(C-H, Aromatic, str.), 1586.23-(C=N, str.), 1514.73-(C=C, Aromatic, str.), 1130.16-(C-O-C, str), 744.24-(C-Cl).**^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.2[d, 1H, Ar-H] δ7.5[d, 1H, Ar-H] δ7.81[s, 1H, Ar-H] δ7.89[d, 2H, Pyridine Ar-H] δ8.65[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 160.7(1C), 158.6(1C), 155.2(1C), 149.5(2C), 147.5(1C), 137.4(1C), 132.9(1C), 131.3(1C), 130.3(1C), 126.9(1C), 126.1(1C), 124.2(2C). **Mass Spectrum (EI-MS):** M+ peak observed at 282 and M+2 observed at 284(m/z).

Compound IXe- 6-chloro-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}):3071.66-(C-H, Aromatic, str.), 1591.45-(C=N, str.), 1510.81-(C=C, Aromatic, str.), 1124.24-(C-O-C, str), 748.57-(C-Cl). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.41[t, 1H, Ar-H] δ7.53[d, 1H, Ar-H] δ7.69[d, 1H, Ar-H] δ7.9[d, 2H, Pyridine Ar-H] δ8.67[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 160.9(1C), 158.7(1C), 156.4(1C), 149.7(1C), 149.4(2C), 138.4(1C), 132.5(1C), 132.1(1C), 128.9(1C), 128.2(1C), 126.1(1C), 124.2(2C). **Mass Spectrum (EI-MS):** M+ peak observed at 282 and M+2 observed at 284(m/z).

Compound IXf- 8-nitro-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}): 3018.42-(C-H, Aromatic, str.), 1593.6-(C=N, str.), 1514.58-(C=C, Aromatic, str.), 1536.24-(N-O str.), 1353.1-2(N-O str.), 1129.63-(C-O-C, str). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.6[d, 1H, Ar-H], δ7.8[d, 2H, Ar-H], δ8.3[d,1H, Ar-H] δ8.53[d, 1H, Pyridine Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 161.1(1C), 158.8(1C), 156.4(1C), 155.9(1C), 149.4(2C), 146.4(1C), 138.6(1C), 127.9(1C), 125.3(1C), 124.2(2C), 123.9(1C), 123.6(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 293(m/z).

Compound IXg- 6-nitro-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: **IR (KBR, cm^{-1}):** 3012.45-(C-H, Aromatic, str.), 1591.41-(C=N, str.), 1511.02-(C=C, Aromatic, str.), 1541.12-(N-O str.), 1362.37-(N-O str.), 1124.65-(C-O-C, str). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.7[t, 1H, Ar-H], δ7.8[d, 2H, Pyridine Ar-H], δ8.1[d,1H, Ar-H] δ8.2[d, 1H, Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 159.18(1C), 151.44(1C), 151.09(2C), 142.80(1C), 139.95(1C), 134.56(1C), 132.04(1C), 127.81(1C), 127.45(1C), 126.42(1C), 125.26(1C), 121.31(2C). **Mass Spectrum (EI-MS):** M+ peak observed at 293(m/z).

Compound IXh- 8-bromo-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}): 3021.63-(C-H, Aromatic, str.), 1593.52-(C=N, str.), 1513.11-(C=C, Aromatic, str.), 1129.34-(C-O-C, str), 671.18-(C-Br).**^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.2[d, 1H, Ar-H], δ7.6[d, 1H, Ar-H], δ7.89[s, 1H, Ar-H] δ7.97[d, 2H, Pyridine Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:**160.8(1C), 158.7(1C), 156.1(1C), 151.9(1C), 149.5(2C), 138.4(1C), 132.6(1C), 130.3(1C), 125.4(1C), 125.1(1C), 124.2(2C), 123.6(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 327 and M+2 observed at 329(m/z).

Compound IXi- 7-bromo-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}): 3019.68-(C-H, Aromatic, str.), 1589.54-(C=N, str.), 1508.14-(C=C, Aromatic, str.), 1130.61-(C-O-C, str), 676.59-(C-Br). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm: :** δ7.5[s, 1H, Ar-H], δ7.6[d, 1H, Ar-H], δ7.7[d, 1H, Ar-H] δ7.9[d, 2H, Pyridine Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:**160.8(1C), 158.9(1C), 156.5(1C), 152.8(1C), 149.4(2C), 138.4(1C), 135.3(1C), 129.8(1C), 128.3(1C), 126.9(1C), 124.1(2C), 112(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 327 and M+2 observed at 329(m/z).

Compound IXj- 6-bromo-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}): 3014.36-(C-H, Aromatic, str.), 1590.43-(C=N, str.), 1510.14-(C=C, Aromatic, str.), 1125.63-(C-O-C, str, 670.26-(C-Br).**^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:**δ7.2[t, 1H, Ar-H], δ7.48[d, 1H, Ar-H], δ7.68[d, 1H, Ar-H] δ7.7[d, 2H, Pyridine Ar-H] δ8.6[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:**160.8(1C), 158.7(1C), 156.4(1C), 152.9(1C), 149.4(2C), 138.4(1C), 135.3(1C), 129.5(1C), 128.3(1C), 126.9(1C), 124.2(2C), 111(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 327 and M+2 peak at 329(m/z).

Compound IXk- 6-fluoro-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}): 3013.8-(C-H, Aromatic, str.), 1594.27-(C=N, str.), 1513.18-(C=C, Aromatic, str.), 1423.48(C-F str.), 1120.63-(C-O-C, str). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.3[d, 1H, Ar-H], δ7.58[t, 1H, Ar-H], δ7.6[d, 1H, Ar-H], δ7.7[d, 2H, Pyridine Ar-H] δ8.6[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:**160.8(1C), 158.9(1C), 156.3(1C), 154(1C), 149.4(2C), 138.3(1C), 136.6(1C), 128.9(1C), 126.3(1C), 126.1(1C), 124.1(2C), 120.7(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 266 and M+1 peak at 268(m/z).

Compound IXl- Ethyl 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole-8-carboxylate: IR (KBR, cm^{-1}): 3019.08-(C-H, Aromatic, str.), 2863.52-(C-H str.), 1833.34-(C=O str.), 1588.56-(C=N, str.), 1516.22-(C=C, Aromatic, str.), 1125.34-(C-O-C, str). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ1.3[t, 3H, Methyl H], δ4.3[q, 2H, Methylene], δ7.4[d, 1H, Ar-H], δ7.8[d, 2H, Pyridine Ar-H], δ8.2[d,1H, Ar-H] δ8.5[s, 1H, Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}CNMR($CDCl_3$.D)δppm:** 166(1C), 160.9(1C), 158.6(1C), 156.3(1C), 153.4(1C), 149.4(2C), 138.4(1C), 133.5(1C), 130.4(1C), 129.8(1C), 124.7(1C), 124.1(2C), 122.3(1C), 61(1C), 14(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 320(m/z).

Compound IXm- 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole-8-carboxylic acid: IR (KBR, cm^{-1}): 3216.83-(O-H str.),3012.63-(C-H, Aromatic, str.), 1714.12-(C=O str.), 1590.58-(C=N, str.), 1509.32-(C=C, Aromatic, str.), 1122.14-(C-O-C, str). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.5[d, 1H, Ar-H], δ7.8[d, 2H, Pyridine Ar-H], δ8.37[d, 1H, Ar-H] δ8.6[s, 1H, Ar-H] δ8.8[d, 2H, Pyridine Ar-H] δ12.4[s, 1H, Carboxylic Acid-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 170(1C), 161(1C), 158.7(1C), 156.4(1C), 154.8(1C), 149.4(2C), 138.4(1C), 133.9(1C), 131(1C), 128.8(1C), 124.6(1C), 124.1(2C), 122(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 292(m/z).

Compound IXn- Methyl 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole-8-carboxylate: IR (KBR, cm^{-1}): 3017.09-(C-H, Aromatic, str.), 2834.27-(C-H str.), 1593.68-(C=N, str.), 1513.61-(C=C, Aromatic, str.), 1122.61-(C-O-C, str). **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ3.8[s, 3H, Methyl-H], δ7.6[t, 1H, Ar-H], δ7.8[d, 2H, Pyridine Ar-H], δ8.02[d, 1H, Ar-H] δ8.2[d, 1H, Ar-H] δ8.7[d, 2H, Pyridine Ar-H] . **^{13}CNMR($CDCl_3$.D)δppm:** 164(1C), 160.9(1C), 158.8(1C), 156.3(1C), 150.7(1C), 149.5(2C), 138.4(1C), 134.8(1C), 130(1C), 127(1C), 124.6(1C), 124(2C), 116(1C), 51(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 306(m/z).

Compound IXo- 7-chloro-8-fluoro-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}): 3036.54-(C-H, Aromatic, str.), 1596.32-(C=N, str.), 1514.58-(C=C, Aromatic, str.), 1426.34-(C-F str.), 1141.51-(C-O-C, str), 772.03-(C-Cl str.) . **^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.4[s, 1H, Ar-H], δ7.8[s, 1H, Ar-H], δ7.9[d,2H, Pyridine Ar-H] δ8.7[d, 2H, Pyridine Ar-H]. **^{13}C NMR($CDCl_3$.D)δppm:** 161(1C), 160(1C), 158.7(1C), 156.3(1C), 149.5(2C), 146.6(1C), 138.5(1C), 124.6(1C), 124.4(1C), 124.3(1C), 124.1(2C), 116(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 300 and M+4 observed at 304(m/z).

Compound IXp- 8-chloro-6-fluoro-3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole: IR (KBR, cm^{-1}): 3059.15-(C-H, Aromatic, str.), 1625.44-(C=N, str.), 1512.05-(C=C, Aromatic, str.), 1433.71-(C-F str.), 1175.41-(C-O-C, str), 780.97-(C-Cl str.).**^{1}H NMR ($CDCl_3$, 400MHz)δ ppm:** δ7.2[s, 1H, Ar-H], δ7.5[s, 1H, Ar-H], δ7.8[d, 2H, Pyridine Ar-H] δ8.6[d, 2H, Pyridine Ar-H]**. ^{13}C NMR($CDCl_3$.D)δppm:** 155.75(1C), 153.30(1C), 151.39(2C), 151.09(1C), 136.92(1C), 134.51(1C), 132.04(1C), 131.14(1C), 127.78(1C), 121.31(2C), 118.92(1C), 117.64(1C). **Mass Spectrum (EI-MS):** M+ peak observed at 300.2 and M+4 observed at 304(m/z).

Pharmacological Activity:

***In-vitro* Anti-inflammatory Activity:** *In vitro* anti-inflammatory activity was performed using colorimetric COX(ovine) inhibitor screening assay kit (Cyaman chemical, MI,USA).The colorimetric COX(ovine) inhibitor screening assay utilizes the peroxidase activity of ovine cyclooxygenase to oxidize the colorimetric substrate, N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD). Enzyme assays were carried out using 220µl volumes. The mixture in background wells, 100% initial activity well and inhibitor wells were prepared according to instructions provided in the kit and pre incubated for five minutes at 25^{o}C .The reaction was initiated by addition of 20µl of TMPD solution followed by arachidonic acid (10µl) in all wells. The assay mixture was shaken and incubated at 25^{o}C for 5 min. The enzyme activity was calculated by UV- Spectrophotometer at 590nm.

***In-Vivo* Anti-inflammatory Activity:** Albino rats of either sex (150-200g) were divided into different groups containing six animals each. Animals were fasted for 12hrs before experiment and only water was allowed. The first group was a control one and received vehicle (Tween 80 in propylene glycol(10%V/V), 0.5ml per rat), the second group received vehicle.

Acute toxicity studies: All new isatin derivatives i.e., which have got very good COX-2 inhibitory activity were subjected to acute toxicity studies before doing *in-vivo* anti-inflammatory studies. All six isatin derivatives employed have been found to be free from toxicity as well as toxic symptoms even at high dose of 1000 mg/kg body weight when administered orally as suspensions in 0.1% CMC. The activity data in terms of % inhibition of rat paw volume was presented in Tables-4 using Diclofenac as a standard drug at the dose of 31.4 µg/ml(100mg)/kg body weight.

***In-Vitro* Antioxidant Activity:** The antioxidant activity of the synthesized 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-p) was determined using Ascorbic acid as the standard drug and the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging assay method. DPPH in ethanol exhibits a prominent absorption band at 517 nm and appears deep violet in color. As the DPPH radical is scavenged by the antioxidant's supplied hydrogen, the absorbance lowers according to stoichiometry. 0.5 mL of DPPH solution (0.2 mM) was mixed with 0.1 mL of test drug concentrations (12.5M, 25M, 50M, 100M, 200M, 400M) and 1.5 mL ethanol was added. The mixture was kept at room temperature in the dark for 30 minutes before analyzing the absorbance at 517 nm against a blank. The percentage reduction in free radical concentration with different concentrations of test compounds was calculated and compared to ascorbic acid as a standard. The reduction in absorbance is calculated as percentage inhibition.

$$\text{DPPH Scavenged (\%)} = \frac{\text{Absorbance of Blank} - \text{Absorbance of Test}}{\text{Absorbance of Blank}} \times 100$$

Evaluation:

***In-vitro* Anti-inflammatory Activity:** Data of Figure 2 reveal the anti-inflammatory activity of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp). Data show that the anti-inflammatory activity ranges from 42.18 to 68.42%.

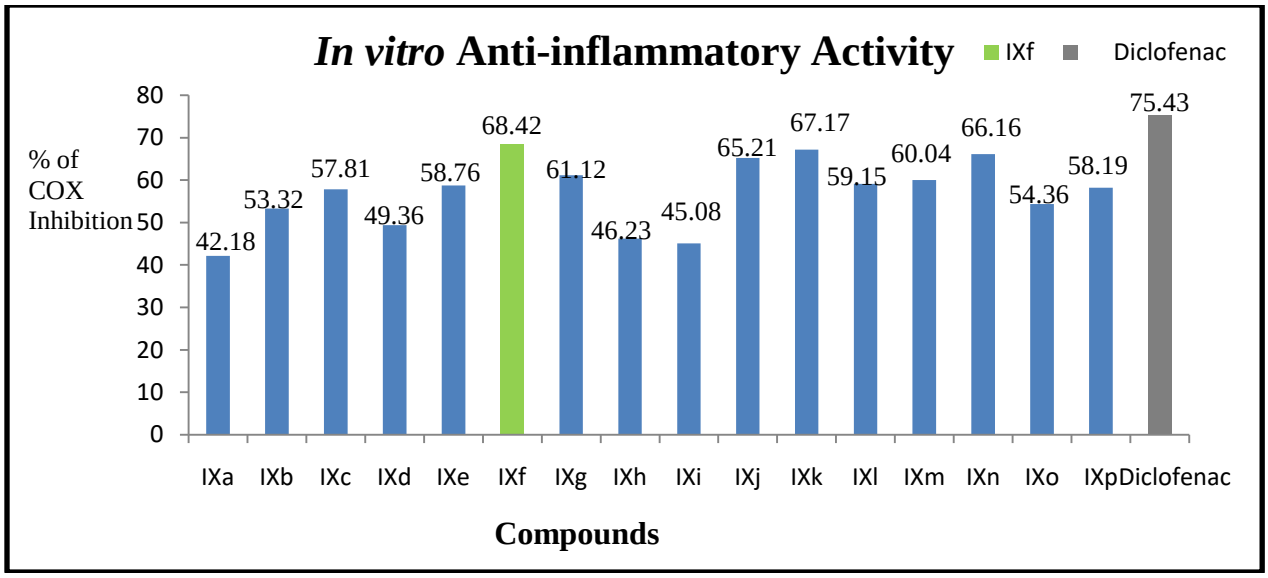

Figure 2: *In-vitro* anti-inflammatory activity data of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp)

***In-vivo* Anti-Inflammatory Activity:** Among the compounds which have shown best *in-vitro* anti-inflammatory activity, 6 compounds evaluated for *in-vivo* anti-inflammatory activity at a dose range of 100mg/kg body weight by carrageenan induced rat paw edema method. The results of *in-vivo* anti-inflammatory activity of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp) (IX f, g, j, k, m, n) are presented in Table 2&3 and Figure 3.

Table 2: *In-vivo* anti-inflammatory activity data of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b] indole derivatives (IXa-IXp)

S. No	Compound (100 mg /kg body weight)	R	Mean Paw Edema Volume in ml ± SD			
			1h	2h	3h	4h
1	IXf	5-NO_2	0.32±0.042	0.29±0.052	0.25±0.084	0.21±0.031
2	IXg	7-NO_2	0.53±0.050	0.49±0.021	0.42±0.075	0.38±0.032
3	IXj	7-Br	0.46±0.080	0.39±0.038	0.36±0.043	0.31±0.020
4	IXk	7-F	0.51±0.043	0.46±0.040	0.38±0.031	0.28±0.052
5	IXm	5-COOH	0.49±0.076	0.43±0.031	0.41±0.056	0.39±0.054
6	IXn	7-$COOCH_3$	0.43±0.030	0.38±0.083	0.32±0.032	0.29±0.045
7	**Control Group (carrageenan induced)**		0.57±0.090	0.64±0.054	0.72±0.024	0.81±0.090
8	**Diclofenac (100mg/kg Body wt)**		0.34±0.070	0.31±0.120	0.27±0.060	0.19±0.051

Table 3: *In-vivo* anti-inflammatory activity data 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp)

S. No	Compound(100mg /kg body weight)	R	% Inhibition of Paw Edema			
			1h	2h	3h	4h
1	IXf	5-NO_2	32.86	41.97	57.79	70.15
2	IXg	7-NO_2	5.35	22.22	40.85	52.5
3	IXj	7-Br	17.86	38.1	49.3	61.25

4	IXk	7-F	23.21	39.68	54.93	66.75
5	IXm	5-COOH	12.5	31.75	42.25	51.25
6	IXn	7-$COOCH_3$	8.92	26.98	46.48	63.24
7	**Diclofenac (100 mg/kg body wt)**		36.5	46.32	63.34	76.4

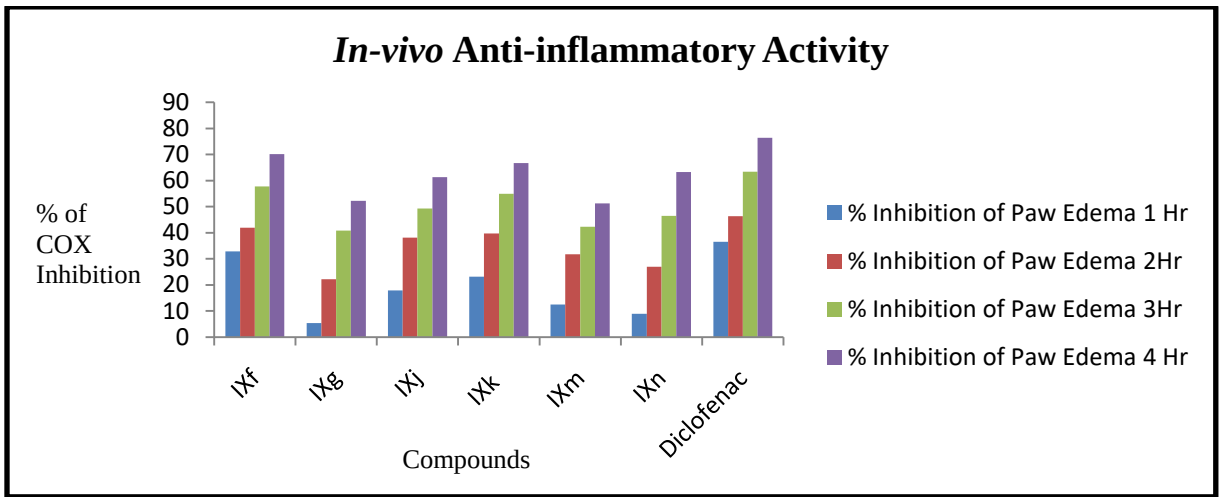

Figure 3: *In-vivo* anti-inflammatory activity data of3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp)

***In-Vitro* Antioxidant Activity**: Compounds of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp) have been evaluated for antioxidant activity by DPPH method. The IC_{50} values of the test compounds are presented in Figure-4. Compounds (IXa-IXp) showed antioxidant activity in the range of 10.09 to 16.75 µM. Compound IXk (R=7-F) showed comparatively more antioxidant activity with IC_{50} value of 10.09 µM when comparable to IC_{50} value of ascorbic acid IC_{50} 5.87 µM.

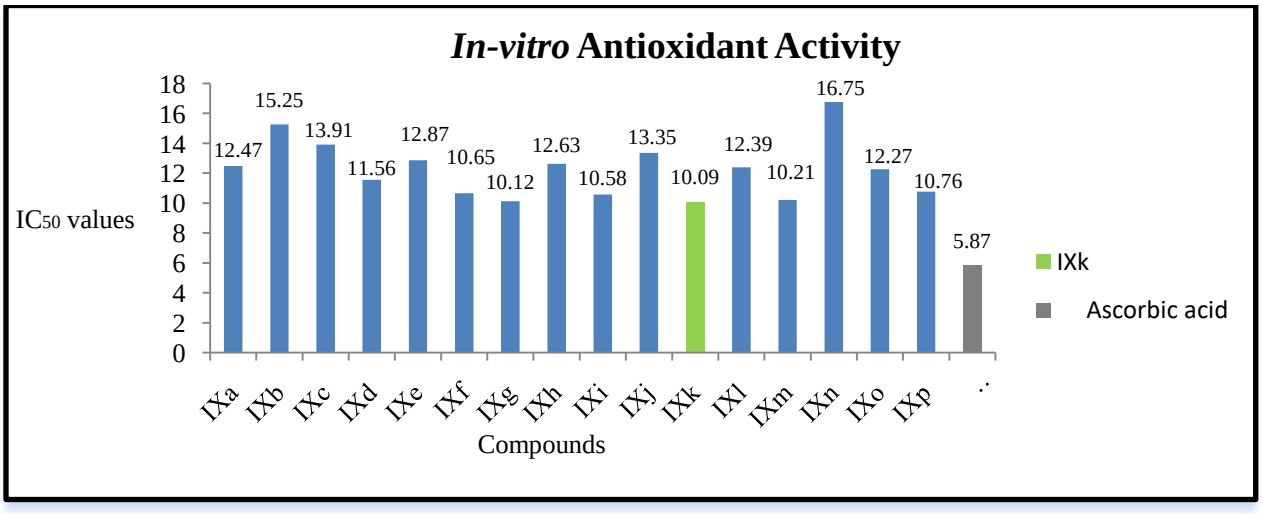

Figure 4: *In-vitro* antioxidant activity data of3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp)

Molecular docking study

Lipinski's Rule

Lipinski rule of five, for parameters like CLogP, molecular weight, number of hydrogen bond

acceptors (HBA), number of hydrogen bond donors (HBD)were checked through, online servers Molinspiration (Molinspiration Cheminformatics, Nova Ulica, Slovak Republic) and OSIRIS (Organic Chemistry, Switzerland) property calculator. All the calculated values were given in Table 6.

Table 6: Lipinski's Rule of Five of synthesized compounds (IXa-IXp)

S.No.	Compound No	Molecular Weight (g/mol)<500	Lipophilicity (CLogP)<5	HBD<5	HBA<10	Rule Violations<2	Lipinski's Rule
1.	IXa	248.25	2.01	0	5	0	Yes
2.	IXb	262.27	1.59	0	5	0	Yes
3.	IXc	262.27	1.48	0	5	0	Yes
4.	IXd	282.69	2.89	0	5	0	Yes
5.	IXe	282.69	2.51	0	5	0	Yes
6.	IXf	293.24	1.03	0	7	0	Yes
7.	IXg	293.24	1.08	0	7	0	Yes
8.	IXh	327.14	2.95	0	5	0	Yes
9	IXi	327.14	3.02	0	5	0	Yes
10.	IXj	327.14	2.76	0	5	0	Yes
11.	IXk	266.24	2.13	0	5	0	Yes
12.	IXl	320.31	1.04	0	7	0	Yes
13.	IXm	292.25	2.10	1	7	0	Yes
14.	IXn	306.28	1.11	0	7	0	Yes
15.	IXo	300.68	2.84	0	5	0	Yes
16	IXp	300.68	2.97	0	5	0	Yes

Docking result for 5OTF against cancer and 7BZ5 against for covid-19:

Table7: Binding free energy and predicted inhibitory constant values of the synthesized compounds (IXa-IXp) with 5OTF protein

Compound No	Confirmation number	Binding free energy (Kcal/mole)	Inhibitory constant (μM)	Best binding pose	No. of Hydrogen bonds	Rank(based on binding energy)
IXa	2	-8.2	2.230	ASP170	0	12
IXb	3	-8.3	1.130	ASP204	0	9
IXc	7	-8.4	2.101	ALA217	1	5
IXd	4	-8.2	1.310	GLU154	1	12
IXe	3	-8.3	0.210	VAL90	0	9
IXf	6	-8.7	2.340	GLU124	2	3
IXg	4	-9.0	2.010	THR137	2	2
IXh	1	-8.0	0.101	MET153	0	15
IXi	6	-8.3	2.830	PHE370	0	9
IXj	7	-8.2	0.254	LEU207	1	12
IXk	8	-8.4	1.307	ILE82	1	5
IXl	3	-7.7	2.101	ILE82	0	16
IXm	7	-8.7	0.460	ASP371	1	3
IXn	4	-9.1	2.301	ASP218	1	1
IXo	3	-8.4	2.540	GLU124	0	5
IXp	2	-8.4	0.401	GLU154	1	5

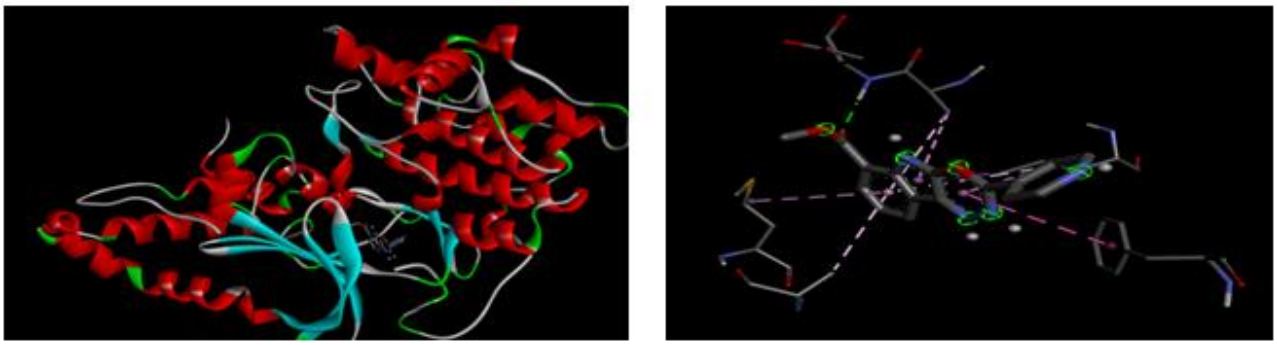

Figure 5: Structural view of binding interaction of protein (5OTF) and ligand (IXn)

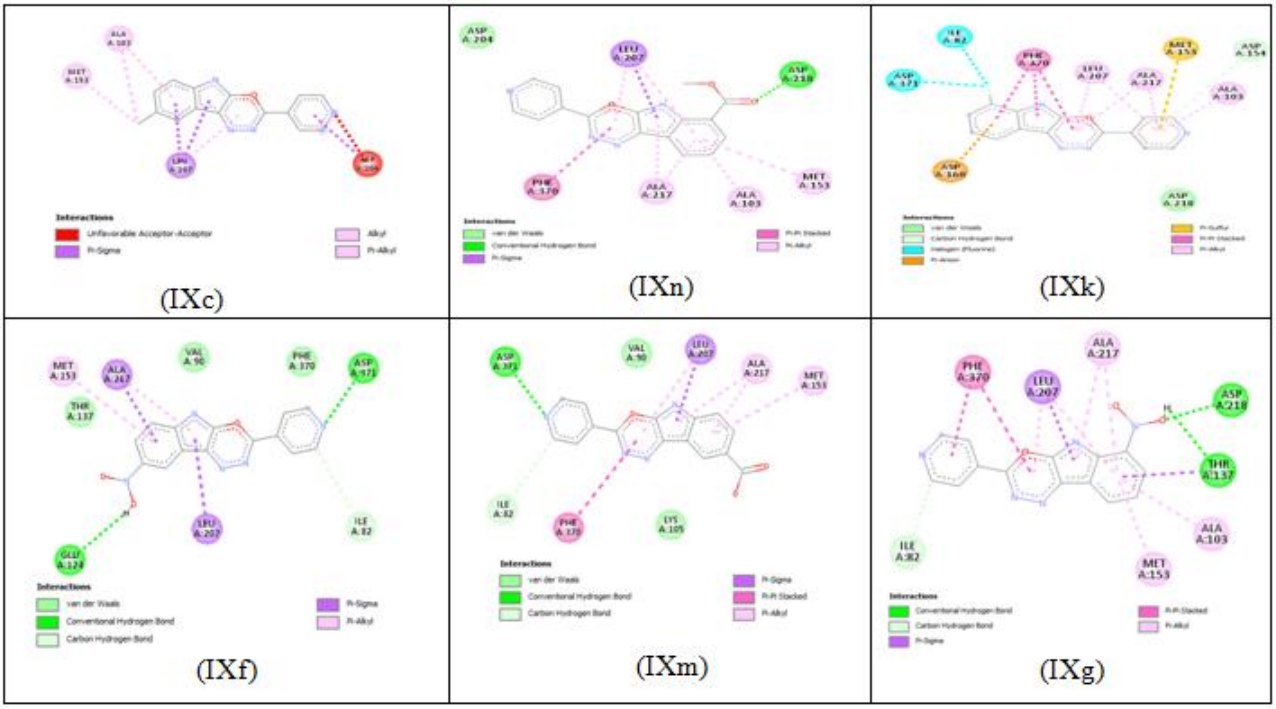

Figure 6: 2D interaction of protein (5OTF) and ligands

Table 8:Binding free energy and predicted inhibitory constant values of the synthesized compounds(IXa-IXp) with 7BZ5 protein

Compound No	Confirmation number	Binding free energy (Kcal/mole)	Inhibitory constant (µM)	Best binding pose	No. of Hydrogen bonds	Rank(based on binding energy)
IXa	6	-8.2	1.407	THR85	2	9
IXb	7	-8.4	2.230	GLY41	2	4
IXc	3	-8.1	1.101	LYS104	0	11
IXd	4	-8.4	1.310	TYR32	1	4
IXe	2	-8.1	0.420	ILE118	0	11
IXf	1	-8.7	2.237	LYS42	1	1
IXg	6	-8.6	2.025	ASN82	1	2
IXh	5	-8.4	1.415	THR85	2	4
IXi	5	-8	2.305	PHE10	0	14
IXj	4	-8	0.268	PHE10	0	14
IXk	2	-8.1	1.409	LYS42	0	11

IXl	3	-8.4	1.145	GLY41	1	4
IXm	1	-7.4	0.560	ILE472	3	16
IXn	8	-8.3	2.201	ALA127	1	8
IXo	5	-8.2	0.540	SER9	0	9
IXp	2	-8.5	2.150	VAL92	0	3

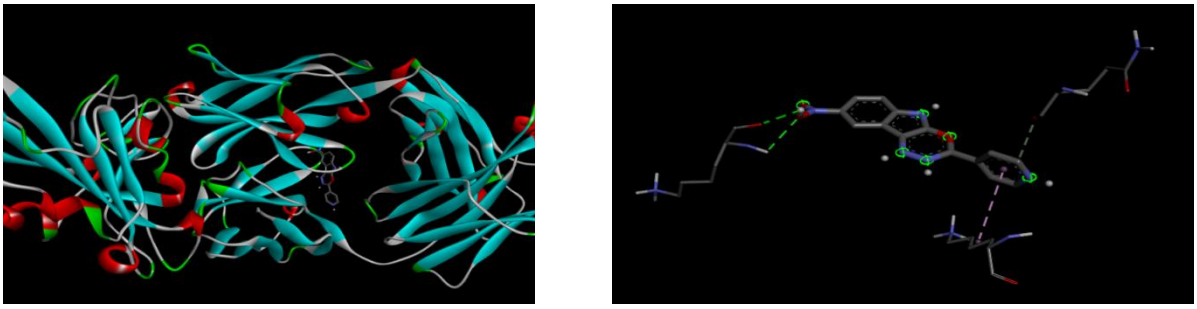

Figure 7: Structural view of binding interaction of protein (7BZ5) and ligand (IXf)

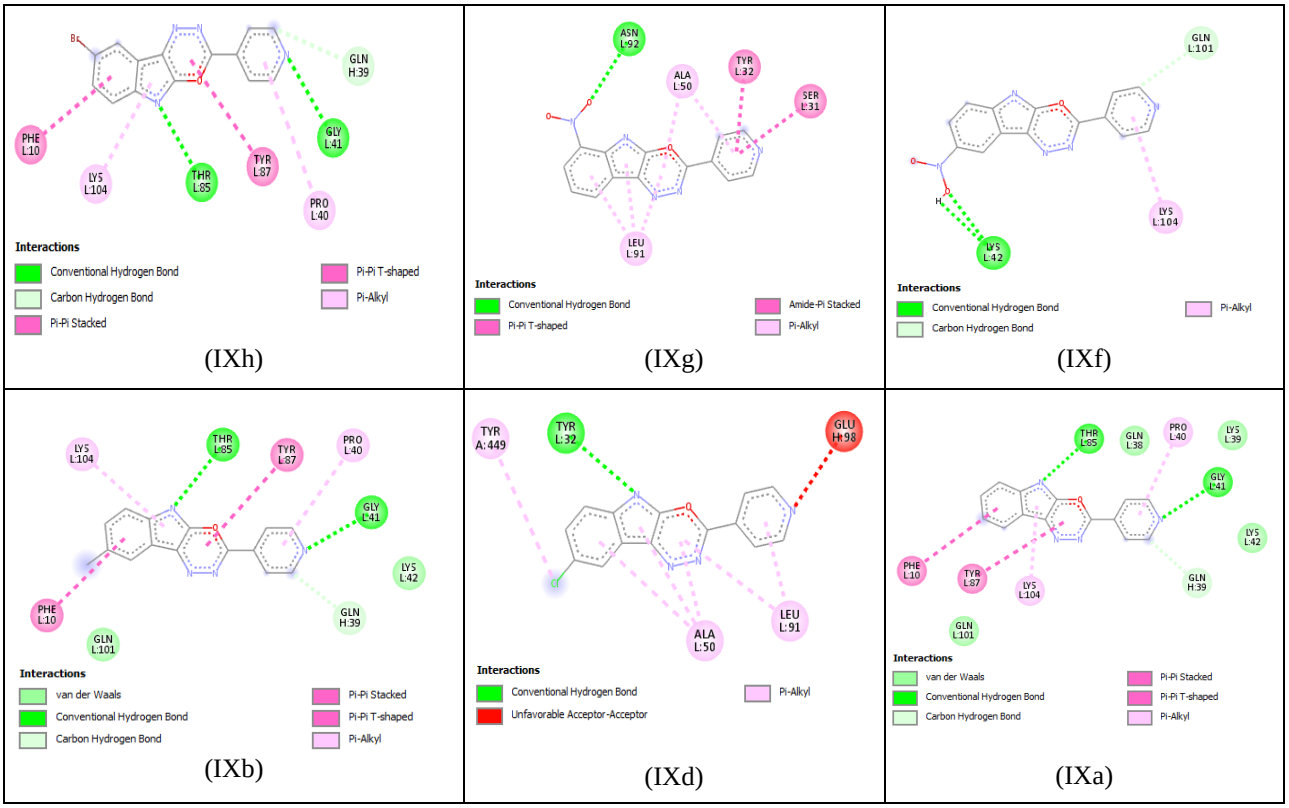

Figure 8: 2D interaction of protein (7BZ5) and Ligands

RESULTS AND DISCUSSION

Some of the new isatin derivatives **3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp)** were synthesized and Physical data of all the synthesized compounds are shown in Table 1.

Anti-inflammatory activity-

In-vitro **anti-inflammatory activity:** The *in-vitro* anti-inflammatory activity data of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp)are presented in Figure 2. The data reveals that the anti-

inflammatory activity is observed in the range of 42.18 to 68.42%. Among the test compounds, the compound **IXf (R=5-NO_2)** showed more anti-inflammatory activity with 68.42%. Compound IXk (R=7-F), Compound IXn (R=7-COOCH3) showed 67.17 and 66.16% COX inhibitory activity respectively with standard Diclofenac by 75.43%. Among all the compounds, R= 5-NO_2 and R= 7-F are showing more *in-vitro* anti-inflammatory activity.

***In-vivo* anti-inflammatory activity:** Among the compounds which have shown best *in-vitro* anti-inflammatory activity, 6 Compounds evaluated for *in-vivo* anti-inflammatory activity at a dose range of 100mg/kg body weight by carrageenan induced rat paw edema method. The results of *in-vivo* anti-inflammatory activity of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp) (IX f, g, j, k, m, n) are presented in Table 3 and Figure 3. Compound **IXf (R= 5-NO_2)** showed comparatively good *in-vivo* anti-inflammatory activity with 70.15% Diclofenac was showing 76.4% rat paw edema. Compound IXk (R= 7-F), compound IXn (R=7-$COOCH_3$) showed *in-vivo* anti-inflammatory activity with 66.75, 63.24, respectively. From the obtained results it is evident that the compound with R= 5-NO_2 is found to be more potent compound. The anti-inflammatory activities showed by the compounds in both the methods are comparable.

***In-vitro* antioxidant activity:** Compounds of 3-(pyridin-4-yl)-[1,3,4]oxadiazino[6,5-b]indole derivatives (IXa-IXp) have been evaluated for antioxidant activity by DPPH method. The IC_{50} values of the test compounds are presented in Figure 4. Compounds (IXa-IXp) showed antioxidant activity in the range of 10.09 to 16.75 µM. Compound **IXk (R=7-F)** showed comparatively more antioxidant activity with IC_{50} value of 10.09 µM. Compound IXg(R=7-NO_2), IXm(R=5-COOH) showed moderate antioxidant activity with IC_{50} values 10.12 and 10.21 µM respectively when compared to IC_{50} value of ascorbic acid 5.87µM. From the obtained results it is clear that R=7-F derivative showed more antioxidant activity among all the test compounds.

Molecular docking results: In this study, sixteen new synthesized molecules (IXa-IXp) were made to interact with selected COVID-19 main protease (pdb:7BZ5) and Cancer main protease (pdb:5OTF). According to the results all synthesized compounds (IXa-IXp) showed satisfactory and better docking results. Among them compounds **IXf(R=5-NO_2)** and **IXg(R=7-NO_2)**on COVID-19 main protease**(pdb:7BZ5)** showed good binding score of **-8.7Kcal/mol**and **-8.6Kcal/mol** respectively and Compounds **IXn(R=7-$COOCH_3$)** and **IXg(R=7-NO_2)**on Cancer main protease **(pdb:5OTF)**showed good binding score of **-9.1 Kcal/mol** and **-9.0 Kcal/mol** respectively.

CONCLUSION

All the desirable new isatin derivatives were synthesized, characterized by physical, analytical and spectral data. Compounds (IXa-IXp)were evaluated and screened for their *in-vitro*, *in-vivo* anti-inflammatory and *in-vitro* antioxidant activities. The data of *in-vitro* anti-inflammatory activity of the compounds reveals that the compounds IXf (R=5-NO_2) showed that more anti-inflammatory activity with 68.42% activity when compared to standard drug Diclofenac 75.43%. The data of *in-vivo* anti-inflammatory activity of the compounds reveals that the compounds IXf (R=5-NO_2) showed that efficient anti-inflammatory activity with 70.15% activity when compared to standard drug Diclofenac 76.4%. Compounds IXk(R=7-F), were showing good antioxidant activity with IC_{50} value of 24.65 µM. From Insilico studies it is found that compounds IXf(R=5-NO_2) and IXg(R=7-NO_2) on COVID-19 main protease (pdb:7BZ5) showed good binding score of -8.7Kcal/mol and -8.6Kcal/mol respectively and Compounds IXn(R=7-$COOCH_3$) and IXg(R=7-NO_2) on Cancer main protease (pdb:5OTF) showed good binding score of -9.1 Kcal/mol and -9.0 Kcal/mol respectively.

ACKNOWLEGDEMENT

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