Synthesis of 4-[(4-hydroxy-2-oxo-2H-1-benzopyran-3-yl)(phenyl)methyl]-3-phenyl-1,2-oxazol-5(4H)-one derivatives as potent biological agents

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Abstract

Herein, an efficient and convenient method for the synthesis 4-[(4-hydroxy-2-oxo-2H-1-benzopyran-3-yl)(phenyl)methyl]-3-phenyl-1, 2-oxazol-5(4H)-one derivatives, have been reported using *P*-TSA as catalyst. The structures of synthesized compounds were confirmed using FT-IR, ^{1}H, ^{13}C-NMR and LC-MS spectroscopic techniques. through one-pot reaction and screened for their pharmacological and *in silico* investigations. The synthesized compounds have been evaluated for antibacterial activity against bacterial strains by agar diffusion method at different concentrations. Further, all the targeted compounds were screened for the compound **4h** displayed significant cytotoxic effect on the anti-cancer activity. Further, the binding capability for the synthesized compounds (**4a-j**) was analyzed by molecular docking studies using human peroxiredoxin 5 (PDB ID: 1HD2) and P38 MAP kinase (PDB ID: 1OUK) protein.

Keywords: 4-hydroxy Coumarin; anti-bacterial; anti-cancer; molecular docking.

1.1. Introduction

Multi-component reactions (MCRs) have emerged as a highly valuable tool in the realms of synthetic and medicinal chemistry. They are deemed pivotal for the creation of polyfunctional molecules, offering a distinct advantage over labor-intensive multistep syntheses. Globally, MCRs have garnered significant interest as an efficient means of producing various active pharmaceutical compounds **[1].** Notably, MCRs boast several merits, including the elimination of superfluous purification steps, reduction of waste generation, minimization of byproducts, decreased reliance on toxic reagents and solvents, expedited workup procedures, shorter reaction times, and a commendable atom economy **[2].**

In the domain of organic synthesis and industrial processes, acid catalysts find frequent application. Examples of such catalysts include p-Toluenesulfonic acid, sulfuric acid, and fluoro hydric acids, which are utilized in alkylation, esterification, hydrolysis reactions, among others **[3].** Particularly, p-Toluenesulfonic acid (P-TSA) stands out as a commercially available and cost-effective chemical with notable stability. This catalyst exhibits eco-friendliness across various organic transformations, proving to be non-volatile, recyclable, non-explosive, easily manageable, thermally robust, and highly efficient. Its usage has witnessed a rapid rise **[4].** Importantly, P-TSA demonstrates exceptional catalytic process in water, facilitating the testing of a range of aromatic aldehydes with diverse electron-withdrawing and electron-donating groups using the new water-based method **[5].**

Isoxazole, a pivotal heterocyclic structure, is a recurring motif in numerous pharmacologically active natural products, clinical drugs, and lead compounds **[6].** Isoxazole derivatives have garnered significant attention in the field of heterocyclic chemistry due to their expansive array of

biological activities, encompassing antimicrobial, anticancer, anti-HIV, anti-tuberculosis, anti-inflammatory, neuroprotective, anti-diabetic, and antidepressant properties **[7-14].** In addition to their biological relevance, isoxazole and its derivatives serve as crucial building blocks in organic synthesis **[15, 16]** and find application as materials in optoelectronics **[17, 18].**

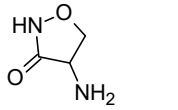
Heterocyclic compounds, given their broad biological significance, play a dominant role in both medicinal and synthetic organic chemistry. Continually caltivating the interest of medicinal chemists and researchers, these compounds are recognized for their multifaceted pharmacological activities **[19, 20].** On an earlier day, the coumarin-associated heterocycles were extracted from plants that have been extensively used as herbal remedies because of their versatile biodynamic activities. About thousands of different coumarin derivatives are isolated from 800plant species and microorganisms and animals **[21].**

4-Hydroxy coumarin is another important constituent of natural products and one of the versatile synthons used in the realm of organic synthesis. It is one of the important molecular precursors with a broad spectrum of pharmacological and physiological properties and finds its application in the synthesis of a variety of heterocycles with remarkable biological activities [**22**].

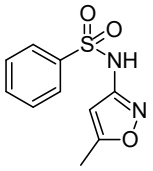
Cancer stands as a global menace, inflicting severe health afflictions and often culminating in fatality. This insidious disease is typically characterized by the rapid and unbridled proliferation of cells, leading to the invasive infiltration of various bodily tissues. Although significant strides have been made in understanding the hereditary aspects of cancer within the medical field, the quest for a definitive cure remains an enduring and formidable challenge for the scientific community **[23].**

Lung cancer, in particular, looms as the primary cause of cancer-related deaths not only in the United States but also in many Western nations. The latest available statistics reveal a concerning number of both male and female fatalities attributed to lung cancer **[24].** Critically, approximately six out of every ten individuals diagnosed with lung cancer succumb to the disease within the first year of diagnosis. This grim statistic damage between the second and third year, with seven to eight out of ten patients meeting the same tragic fate. While some patients may initially respond partially or completely to treatment, the majority eventually experience relapse and, sadly, meet a fatal end **[25].**

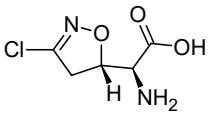
Rationalizing this hypothesis by synthesizing a series of 4-hydroxy coumarin derivatives could result in novel molecules displaying considerable pharmacological potential. Keeping this in mind, we have developed a simple and effective protocol for the synthesis of some bioactive coumarin heterocycles under reflux conditions without the use of toxic organic solvents and reagents. The importance of isoxazole-based compounds is available in the market as shown in **Fig. 1** and some reported biologically important heterocyclic compounds as well as isoxazole-containing derivatives have been discussed below.



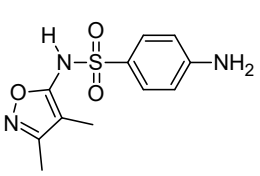
Cycloserine
(Anti TB, antibacterial agent)



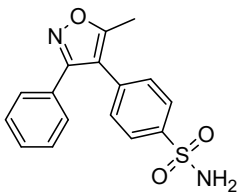
Sulfamethoxazole
(Antitumour, antileishmanial agent)



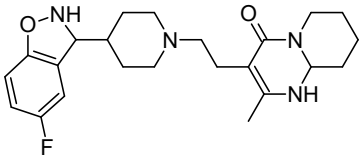
Acivicin
(Antitumour, antileishmanial agent)



Sulfisoxazole
(Antitumour, antileishmanial agent)



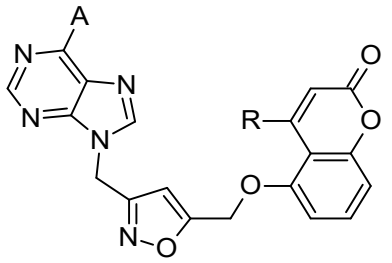
Valdecoxib
(Anti-inflammatory agent)



Risperidone
(Antipsychotic)

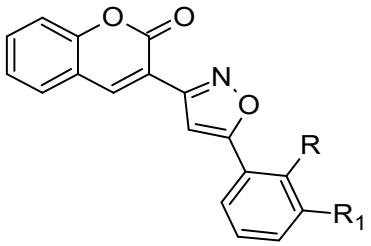
Fig.1. Some of the drugs containing isoxazole nucleus.

In the year 2017, Michael G. Kallitsakis and colleagues conducted a study wherein they detailed the synthesis and subsequent biological assessment of innovative hybrid compounds featuring combinations of purine, coumarin, and either isoxazoline or isoxazole components **[26].** The majority of these synthesized hybrids underwent rigorous screening to evaluate their antioxidant properties, positioning them as potential blueprints for the development of pharmaceuticals aimed at addressing conditions like Alzheimer's disease (AD), which involve the detrimental impact of reactive oxygen species (ROS). The hybrids' collective ability to exhibit anti-lipoxygenase (anti-LO), anti-acetylcholinesterase (anti-AChE), and anti-monoamine oxidase B (anti-MAO-B) activities contributed significantly to their robust antioxidant profile. These research findings align harmoniously with the concept of a novel lead molecule (1).



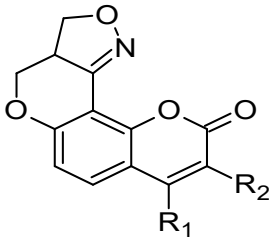
(**1**)

Lekha Prabakaran *et al*. reported the synthesis of novel isoxazole incorporated coumarin derivatives [27**]** and evaluated their potent α-amylase inhibitory potent agents in 2019. Among the tested analogs, most of the derivatives showed potent activity of design of α-amylaseinhibitors and anti- diabetic activity (**2).**



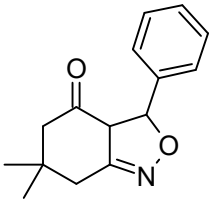
(2)

C. Krishna *et al*. have designed and reported the novel synthesis of pyrano isoxazoline/isoxazoles derivatives **(3).** All the synthesized derivatives were screened for anticancer activity and found that compounds were active against the cell line with IC_{50} value.and all compounds have a certain potential against Colo-205 and Hep G2 cell lines [**28**].



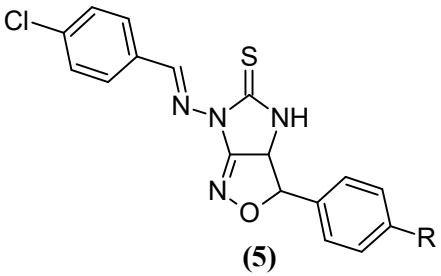
(3)

V.V. Dabholkar and F.Y. Ansari reported a cyclo condensation of fused isoxazole derivatives **(4 and 5)** as a potent antibacterial agent **[29].** In 1995, L. Somogyi *et.al*.,have reported 3,6-dimethyl-4H-pyrano[3,4-d][1,2]oxazol-4-one **(6)** derivatives from the acylhydrazones **[30].**



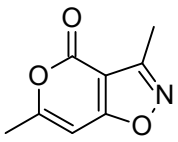
(4)

4a: R=OCH_3; **4b:** R=OH



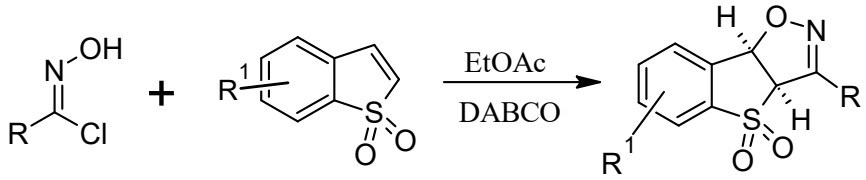
(5)

5a: R=$N(CH_3)_2$



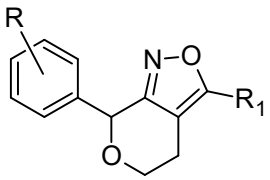
(6)

In 2020, tricyclic isoxazole-fused benzo[b]thiophene 1, 1-dioxide derivatives **(7)** have been reported by K.K. Wang *et. al.,* via 1, 3-dipolar cycloaddition **[31].**



(7)

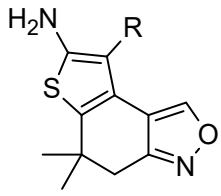
H. J. Kim and co-workers synthesized 4,5-dihydro-7H-pyrano[3,4-c]isoxazole derivatives **(8)** as a fungicidal agent. The compounds **8a** and **8b** showed a higher % of inhibition against all the fungal strains viz *P. Oryzae, R. Solani, B. Cinerea, P. Infestants, P. Recondite* and *E. Graminis* **[32].**



8a: R=o-C_6H_4; R_1=CH_2Br
8b: R=p-C_6H_4; R_1=CH_2Br

(8)

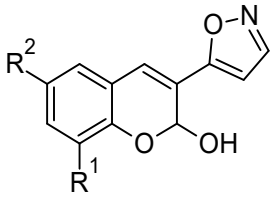
In 2020, R.M. Mohareb *et. al.* synthesized a 5,5-dimethyl-4,5-dihydrothieno[3,2-e][2,1]benzoxazol-7-amines **(9)** as a potent anticancer agent and tyrosine kinase inhibitors. and also exhibits good inhibitions toward Pim-1 kinase **[33].**



9a: R=$COOC_2H_5$

(9)

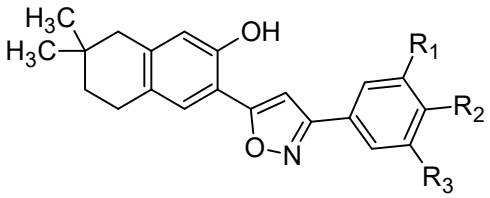
In 2017 Khaled R. A. Abdellatif *et al*. **[34]** reported the synthesis and antioxidant and anticancer Aactivity of new coumarin derivatives linked with thiazole, isoxazole, or pyrazole moiety (**10).**



10a: $R^1=OCH_3, R^2=H$
10b: $R^1=OC_2H_5, R^2=H$
10c: $R^1=H, R^2=Br$
10d: $R^1=OCH_3, R^2=Br$

(10)

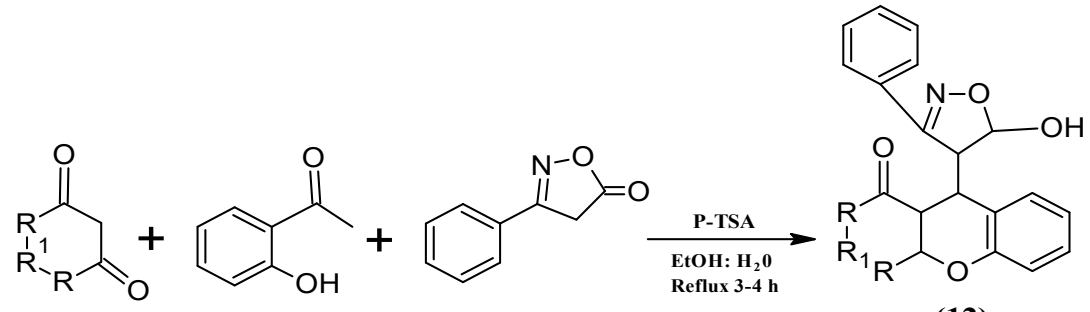
In the year 2015, Gollapalli Naga Raju and co-researchers published a study detailing the production of derivatives involving 3-substituted phenyl-5-(2, 2, -dimethyl, 7-hydroxy chroman) isoxazoles [35]. The researchers subjected all the newly synthesized compounds to in vitro testing for their antibacterial and antifungal activity, employing the broth dilution method. Their evaluation encompassed two Gram-positive bacterial strains, two Gram-negative bacterial strains, and two fungal strains, yielding promising outcomes against these microbial strains. Furthermore, the study identified a range of coumarins combined with isoxazole molecules [11].



(11)

A series of some novel substituted-(5-hydroxy-3-phenylisoxazol-4-yl)-1,3-dimethyl-1H-chromeno[2,3-d]pyrimidine-2,4(3H,5H)-dione/3,3-dimethyl-2H-xanthen-1(9H)-one derivative was reported by S.H. Sukanya *et al.,* in 2021, and all the synthesized derivatives were screened

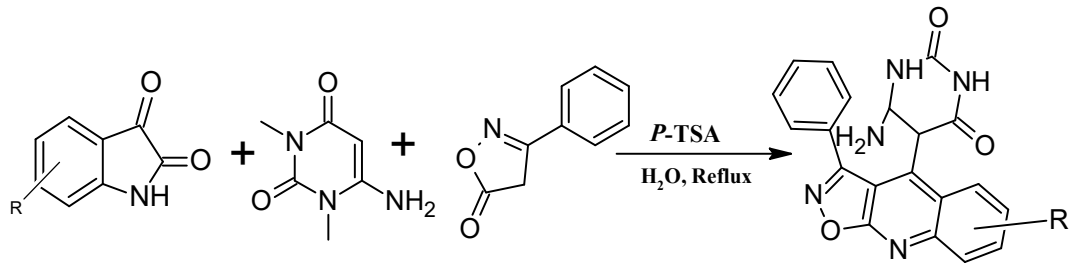
for their pharmacological investigations. **[36]** The compounds showed the best anti-TB efficacy **(12).**



(12)

R=N-CH_3, CH_2
R^1=C=O, $C(CH_3)_2$
R^2=H, 3-OCH_3, C_4H_4, 5-NO_2, 5-Br

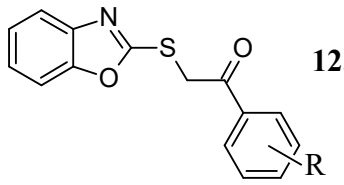
In 2015, isoxazolo[5,4-b]quinolin-4-yl)pyrimidine 2,4(1H,3H)-dione derivatives **(13)** was reported by N. Poomathi and co-authors via cleavage of the isatin C-N bond followed by ring expansion reaction using *p*-TSA as the catalyst **[37].**



(13)

In 2021, M. Staniszewska *et. al.*reported a 2-(1,3-benzoxazol-2-ylsulfanyl)-1-phenylethanoid derivatives **(14)** as an antifungal agent against *C. albicans* and *C. glabrata strains.* Compounds

14a and **14b** exhibited as most active compounds with a % of inhibition value of 64.2 ±10.6 and 53.0 ± 3.5 at 16 µg/mL aginst fungal strains *C. albicans and C. glabrata* respectively **[38].**

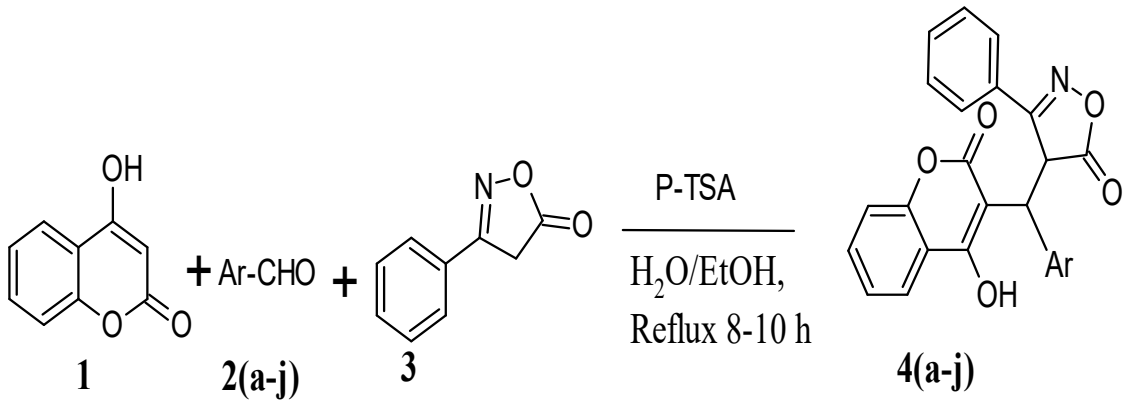


12a: R=2,4,6-Cl_3: **12b:**R= 2,3,4-Cl_3

(14)

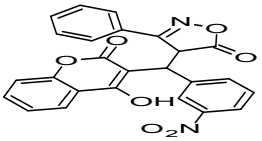
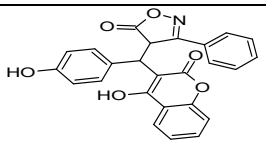
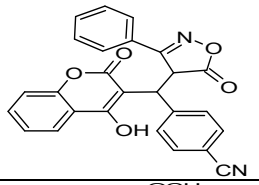
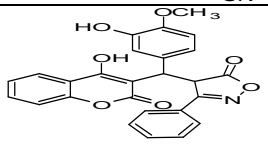
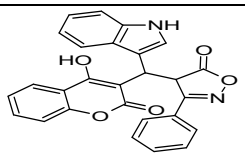
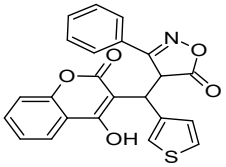
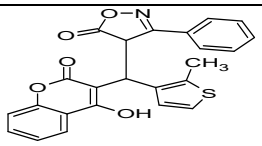
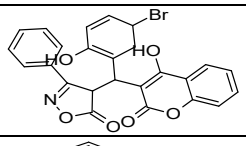
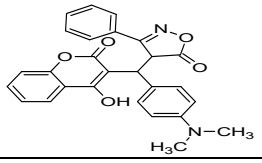
1.2. Present work

In this chapter, a series of some novel have been synthesized A series of some novel 4-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)(phenyl)methyl]-3-phenyl-1,2-oxazol-5(4*H*)-one derivatives **4(a-j)** were reported in this chapter, by the reaction of 4-hydroxy Coumarin (**1**), substituted aldehydes (**2**) **2(a-j)** and 3-phenyl-5-isoxozolone in presence of *P*-TSA in aqueous ethanol. The synthetic pathway of the synthesized compounds has been given in **Scheme 6.**

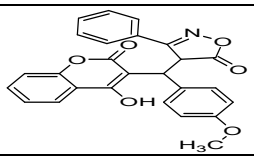


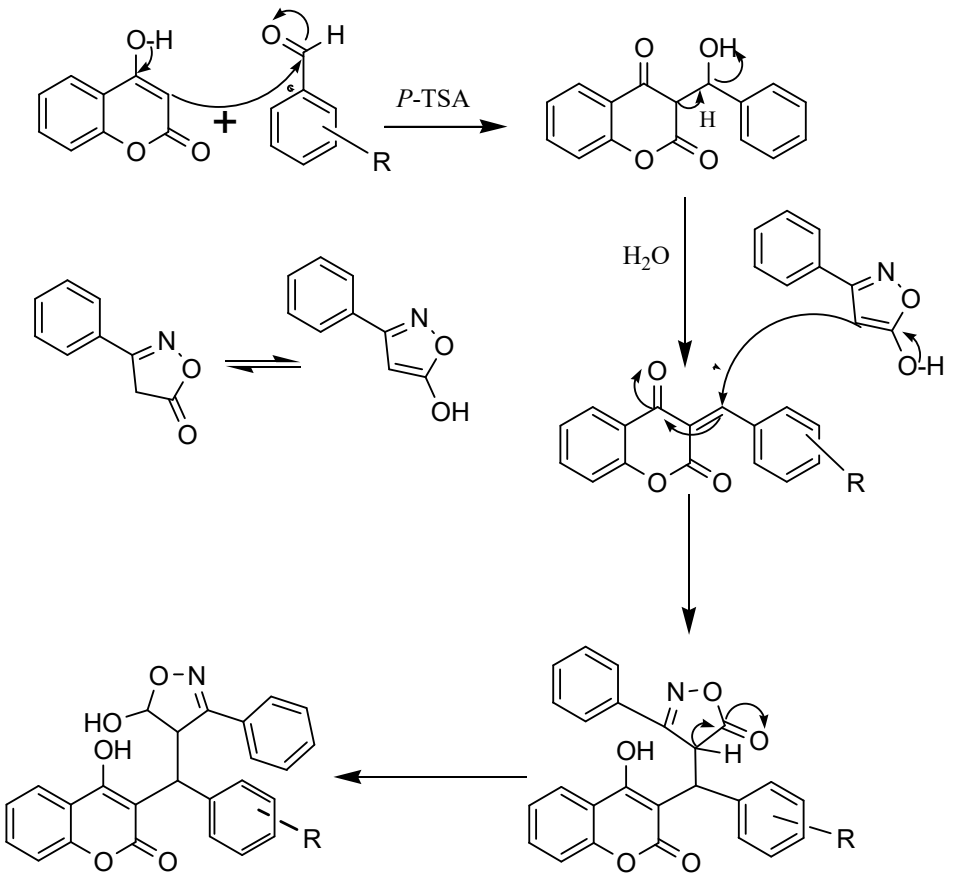
Scheme-6: Synthesis of 4-hydroxy-3-phenyl-1,2-oxazol-5(4*H*)-one derivatives **4(a-j)**

The physical data of synthesized derivatives **4(a-j)**

sample	colour	M.P.(^{0}C)	Mol.wt	Yield %	compound	Mol. formula
4a	Yellowish solid	234-238	456.40	78		$C_{25}H_{16}N_2O_7$
4b	Pale yellow	234-238	427.40	81		$C_{26}H_{16}N_2O_5$
4c	Light orange	228-230	436.41	79		$C_{26}H_{16}N_2O_5$
4d	Yellow solid	180-182	457.43	82		$C_{26}H_{19}NO_7$
4e	white solid	166-168	450.44	88		$C_{27}H_{18}N_2O_5$
4f	Creamy white	220-222	417.13	76		$C_{23}H_{15}NO_5S$
4g	Light orange	204-206	431.46	82		$C_{24}H_{17}NO_5S$
4h	Creamy solid	214-216	508.31	79		$C_{25}H_{18}BrNO_6$
4i	White solid	188-190	454.47	73		$C_{27}H_{22}N_2O_5$

Possible mechanism for the formation of target compounds have been given in **scheme 2.**

4j	Pale yellow solid	198-200	441.43	83		$C_{26}H_{19}N_2O_6$



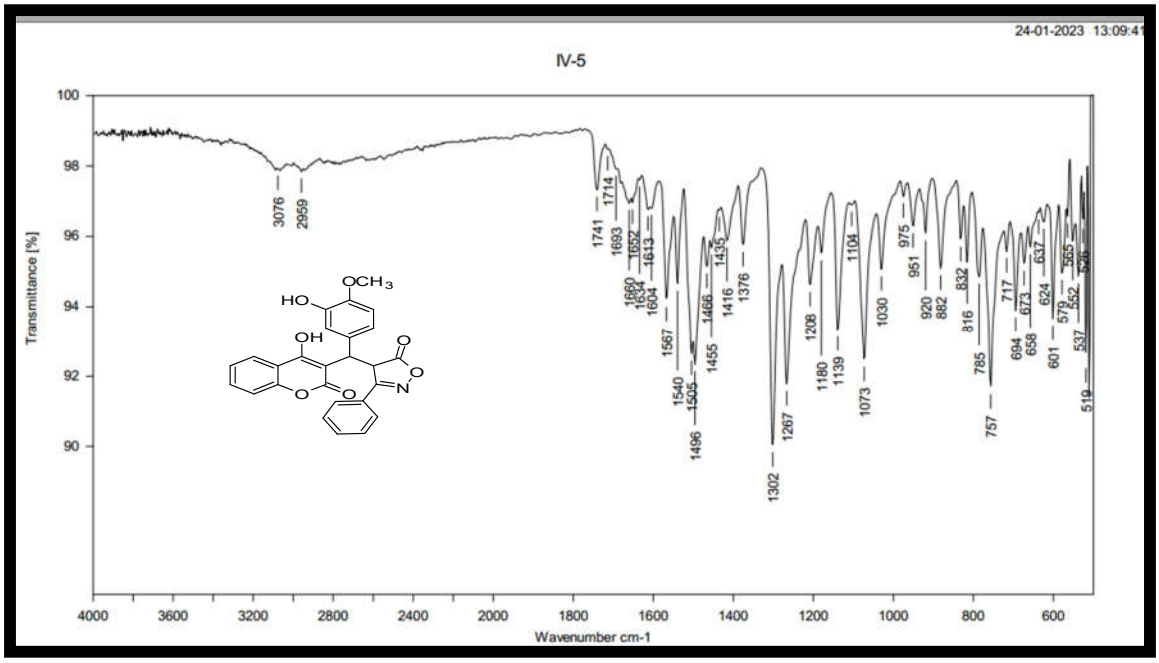
Scheme 2: Possible mechanism of the synthesized compounds **4(a-j).**

The structures of the newly synthesized 4-[(4-hydroxy-2-oxo-2H-1-benzopyran-3-yl)(phenyl)methyl]-3-phenyl-1, 2-oxazol-5(4H)-one derivatives, designated as **4(a-j),** were confirmed through the analysis of their IR, ^{1}H NMR, ^{13}C NMR, and Mass spectra. In the IR spectrum of compound **4d,** notable absorption bands were observed. An absorption band at 3076 cm^{-1} was attributed to the stretching vibration of the OH group, while two absorption bands at

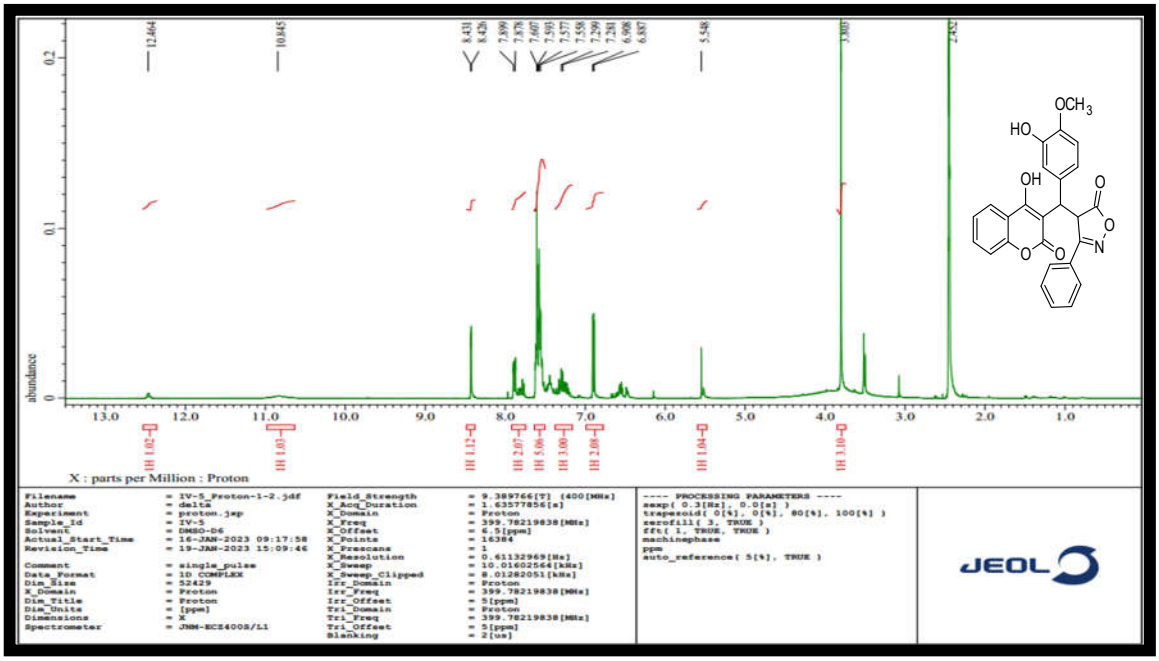
1660 and 1693 cm-1 corresponded to the stretching vibration of the carbonyl group (C=O). Additionally, a stretching vibrational band at 1604 cm^{-1} was associated with the cyano group (C=N), and another absorption band at 2959 cm^{-1} was attributed to the stretching vibration of (OCH_3).

In the ^{1}H NMR spectrum of compound **4d,** distinct peaks were observed. A singlet at δ 5.54 ppm represented the CH proton (s, 1H, CH), while peaks at δ 6. 90-6. 88 ppm indicated two aromatic protons (d, J=8 Hz, 2H, Ar-H). Peaks at δ 7. 29-7. 28 ppm corresponded to three aromatic protons (d, J=8 Hz, 3H, Ar-H), and multiplet peaks at δ 7. 60-7. 55 ppm were associated with five aromatic protons (m, 5H, Ar-H). Furthermore, doublet at δ 7. 89-7. 87 ppm and 8. 43-8. 42 ppm corresponded to 3 aromatic protons (d, J=8 Hz, 3H, Ar-H). Two singlet at δ 10. 84 and δ 12. 46 were observed, which were attributed to OH protons (s, 1H, OH).

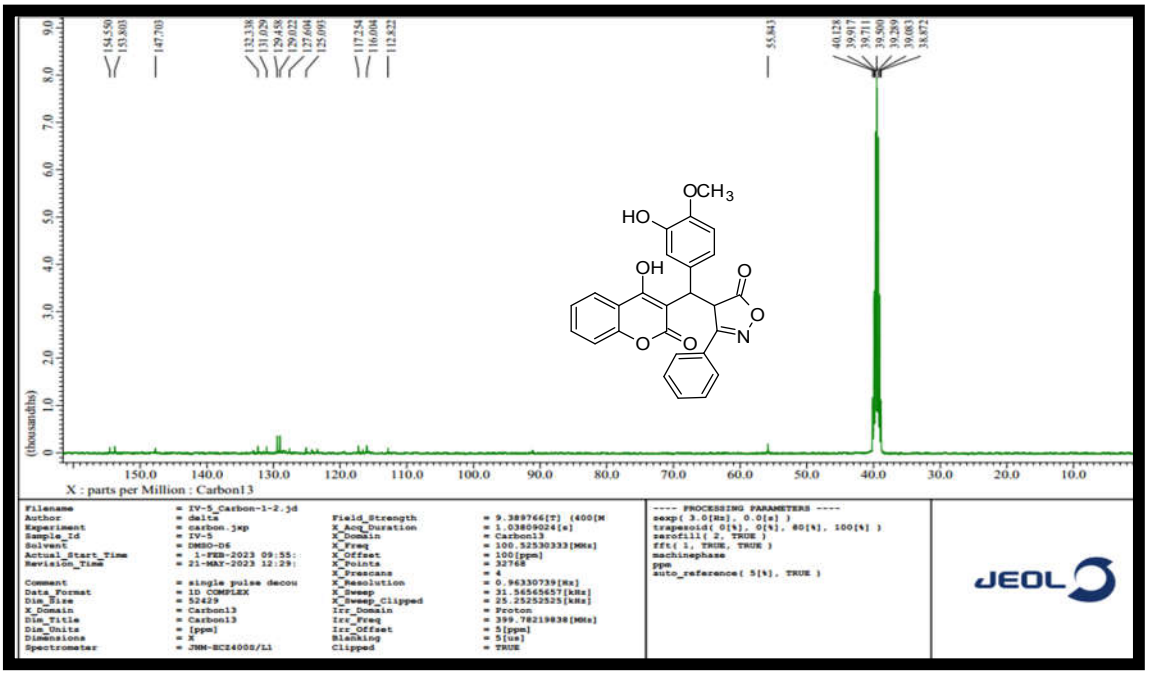
The ^{13}C NMR spectrum of compound **4d** exhibited distinctive peaks at δ 153. 80 and 154. 55 ppm, corresponding to carbonyl carbons. In the mass spectrum, a molecular ion peak with an m/z value of 457 [M+] was observed, indicative of the molecular weight of compound **4d.**



IR spectrum of compound 4d



^{1}H NMR spectrum of compound 4d



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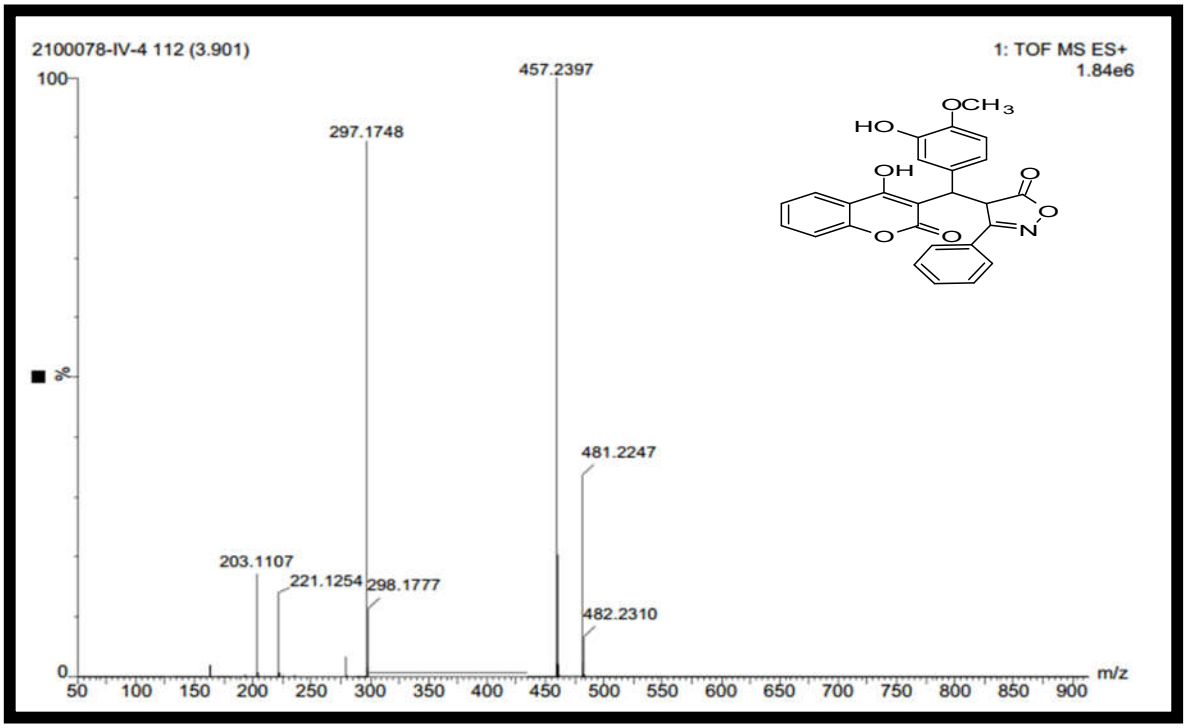


MASS spectrum of compound 4d

1.2.3. Biological studies

1.2.3.1. Antibacterial activity

The antibacterial efficacy of the synthesized compounds (designated as **4a-j**) was assessed through in vitro experiments against four bacterial strains, namely *S. pyogenes, B. substilis, E. coli, and P. aeruginosa.* The concentrations tested included 3, 6, 9, and 12 µg/mL. The results obtained were quantified in terms of both the zone of inhibition and the minimum inhibitory concentrations (MIC), and these outcomes were compared to those of the standard drug Streptomycin. A summary of the findings can be found in **Tables 2** and **3.**

The investigation of antibacterial activity revealed that the tested compounds exhibited remarkable inhibition zones against all four bacterial strains. Notably, among the newly synthesized molecules, compounds **4a, 4c, 4h,** and **4i** displayed equivalent activity against *E. coli, Pseudomonas,* **and** *S. aureus,* with MIC values ranging from 2. 1 to 2. 3 mg/mL. In the context of structure-activity relationships, it was observed that compounds **4e** and **4j,** which featured methoxy groups on the aromatic ring, demonstrated exceptional activity within the series, surpassing the performance of the standard drug. Additionally, compounds **4a, 4c, 4f,** and **4g** exhibited promising activity against *B. subtilis.* and the remaining synthesized compounds demonstrated moderate activity against the selected bacterial strains.

Table2. Antibacterial activity results of synthesized compounds **4(a-j)**

Compound.	***B. subtilis***		***S.pyogenes***		***E. coli***		***P. aeruginosa***	
	25mg/ml	**50mg/ml**	**25mg/ml**	**50mg/ml**	**25mg/ml**	**50mg/ml**	**25mg/ml**	**50 mg/ml**
4a	1.5±0.3	1.9±0.5	1.3±0.12	1.5±0.24	1.2±0.6	1.7±0.8	1.2±0.15	1.6±0.24

4b	2.0±0.12	2.4±0.12	1.8±0.12	2.0±0.21	1.6±0.2	1.8±0.5	2.1±0.25	2.4±0.21
4c	2.0±0.1	2.1±0.4	1.7±0.2	2.1±0.21	1.5±0.22	1.8±0.23	1.8±0.12	1.9±0.26
4d	1.4±0.24	1.7±0.29	1.9±0.23	1.8±0.22	1.8±0.1	2.2±0.30	1.8±0.15	2.1±0.28
4e	1.0±0.21	1.7±0.24	1.0±0.21	2.4±0.25	1.0±0.15	1.4±0.21	1.7±0.2	1.8±0.23
4f	2.0±0.12	2.3±0.21	2.0±0.12	2.3±0.21	1.8±0.23	2.1±0.24	1.6±0.18	2.1±0.23
4g	2.5±0.3	1.9±0.26	2.4±0.25	1.7±0.8	1.6±0.18	2.4±0.12	1.6±0.18	1.7±0.8
4h	1.8±0.12	2.4±0.12	2.1±0.24	1.9±0.26	1.7±0.24	2.2±0.30	1.5±0.22	2.4±0.12
4i	1.5±0.3	1.7±0.2	1.8±0.15	2.2±0.30	1.5±0.22	1.7±0.24	1.9±0.5	1.4±0.21
4j	2.1±0.25	2.2±0.30	1.6±0.2	1.7±0.24	1.8±0.12	2.1±0.24	1.8±0.23	1.6±0.18
Gatifloxacin	2.3±0.32	3.0±0.35	2.5±0.31	2.9±0.35	2.1±0.25	2.5±0.28	2.3±0.27	2.5±0.30

Table 3. MIC results of synthesized compounds **4(a-j)**

MIC µg/mL (mean ± SD)				
Compound	***B. subtilis***	***S.pyogenes***	***E. coli***	***P. aeruginosa***
4a	2.3 ± 0.26	2.4 ± 0.25	2.1 ± 0.25	2.2 ± 0.26
4b	2.4 ± 0.32	2.1 ± 0.29	2.3 ± 0.25	2.2 ± 0.25
4c	2.4 ± 0.24	2.3 ± 0.26	2.1 ± 0.23	2.3 ± 0.24
4d	2.5 ± 0.26	2.3 ± 0.26	2.3± 0.26	2.5 ± 0.29
4e	2.4 ± 0.21	2.1 ± 0.22	2.4 ± 0.26	2.3 ± 0.25
4f	2.5 ± 0.21	2.4 ± 0.23	2.1 ± 0.25	2.3 ± 0.26
4g	2.1 ± 0.25	2.3 ± 0.26	2.3 ± 0.26	2.1 ± 0.25
4h	2.3 ± 0.26	2.2 ± 0.26	2.3 ± 0.26	2.3 ± 0.26
4i	2.3 ± 0.26	2.1 ± 0.25	2.1 ± 0.25	2.3 ± 0.24
4j	2.5 ± 0.26	2.4 ± 0.23	2.3 ± 0.26	2.3 ± 026
Gatifloxacin	2.5 ± 0.30	2.5 ± 0.30	2.2 ± 0.26	2.3 ± 0.28

1.2.3.2. *in vitro* cytotoxicity

The synthesised targets **4(a-j)** appeared to have strong selectivity against the MCF-7 (Human breast cancer) cell line, according to the *in vitro* cytotoxicity data, with IC_{50} values ranging from 21.620.01 to 101.854.60 g/mL. Compound **4h** among them demonstrated a significant cytotoxic effect with the lowest IC_{50} value of 21.62 0.01 g/mL; compounds **4b, 4e,** and **4f** also demonstrated good cytotoxicity with IC_{50} values of 30.29 2.20, 35.76 1.04, and 33.47 0.73 g/mL; and the remaining compounds demonstrated a moderate cytotoxic effect with an IC_{50} value in the range of 36. Results were given in **Table 4** along with the percentage of cell viability and IC_{50} values for the targets. and the images have been displayed in **Fig 1.**

comp	Mean cell viability of MCF-7 (Human breast cancer) concentration in µg /mL							IC_{50} in µg/mL
	NC	3.125	6.25	12.5	25	50	100	
4a	100	95.46±0.40	90.93±1.20	89.23±0.80	85.41±0.20	73.79±1.80	42.06±0.60	101.85±4.60
4b		73.50±0.60	71.81±1.00	62.88±0.79	54.38±0.40	44.61±2.20	40.65±1.40	30.29±2.20
4c		93.34±1.00	81.15±0.60	74.07±0.20	71.24±1.00	44.75±0.40	28.75±0.19	42.90±0.81
4d		87.67±1.00	72.66±0.19	70.96±0.19	64.30±0.80	61.18±2.00	42.63±1.00	52.96±2.79
4e		82.57±0.60	79.88±1.20	78.47±1.20	67.13±0.40	36.68±2.20	24.92±0.79	35.76±1.04
4f		83.28±1.20	70.25±0.80	57.36±0.19	56.51±0.19	55.52±0.80	35.83±1.40	33.47±0.73
4g		94.75±0.99	88.38±0.80	72.09±0.60	67.84±2.20	52.68±0.40	48.01±1.00	59.03±2.38
4h		82.29±2.20	67.70±0.80	64.72±0.20	50.70±1.20	28.89±0.39	25.63±1.00	21.62±0.01

4i		81.15±0.60	62.74±0.20	57.92±1.80	56.08±0.40	54.24±0.20	49.99±3.40	36.38±0.87
4j		81.86±0.79	66.42±0.60	58.77±0.60	56.65±0.79	54.95±0.40	50.98±0.40	39.42±1.65
Std		48.58±0.60	42.77±0.79	40.65±1.40	38.52±0.79	34.84±0.40	33.15±0.41	7.56±0.08

Table 4: % of cell viability against MCF-7 (Human breast cancer) cell line of the synthesized compounds **4(a-j).**

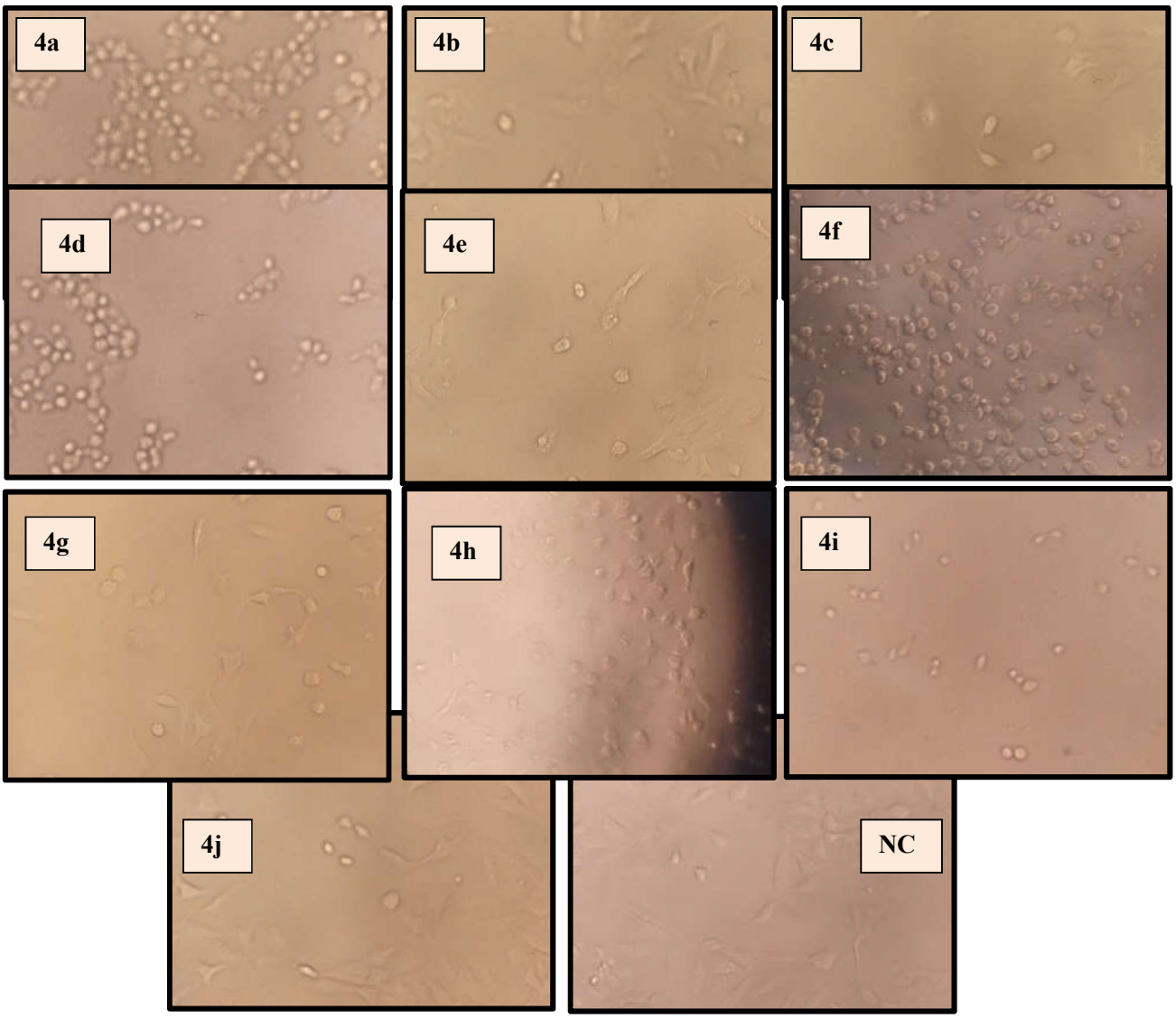


Fig 1. Images of cytotoxicity of the synthesized compounds **4(a-j)** and Negative control (**NC**)

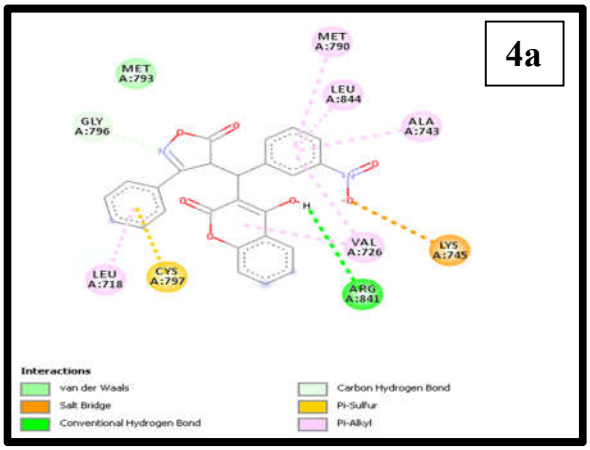
1.2.3.3. in silico molecular docking study

The docking of receptors Human peroxiredoxin 5 for antioxidant activity with **4(a-j)** obtained all compounds **4(a-j)** exhibited well-established bonds with amino acids (Thr44, Gly46, Cys47, Ile119 and Ile194, Gly96, Leu197, Ser94, Ala198, Arg127, and Thr147) in the receptor active pocket. The docking results of antioxidant activity revealed that the compounds **4(a-j)** had significant binding modes, with docking scores ranging from -7.6 to -8.3 kcal/mol and 2-3 hydrogen bonds respectively as compared with the reference standard Pramipexole (-4.1 kcal/mol). In that, the compound **4h** showed the least binding energy -8.3 kcal/mol has almost least with the highest dock score compared with the reference standard Pramipexole (-4.1 kcal/mol) and could act as a better binder than target molecules as shown in **Table 5 and Fig.2**

Table 5. In silico molecular docking results for antioxidant activity of synthesized compounds 4(a-j) and standard drug (Pramipexole). Docking results of 4(a-j) against EGFR

Ligand	Binding affinity (kcal/mol)	Hydrogen bond interaction	Hydrogen Bond length in Å	Hydrophobic and Other interactions
4a	-8.3	ARG881	2.62	LYS745, CYS797, GLY796, ALA743, LEU844, MET790, VAL726, LEU718
4b	-8.6	THR854	2.66	VAL726, LEU718, CYS797, MET793, ALA743, LEU844, MET790, LYS745
4c	-8.4	ARG841,CYS 797	2.95, 3.68	ASP800, LEU718, LEU799, ARG803, ASN842, SER720, ASN842, GLY719, GLY721, ALA722, ASP855, VAL726, THR854, LEU844
4d	-8.3	THR854	4.45	SER720, ARG841, LEU844,

				VAL726, ASP855, ALA743, MET790, LEU788, LYS745, MET793, LEU792, LEU718, GLY796, CYS797, ASP800, GLY719
4e	-8.7	CYS797, MET793	3.87, 5.29	LEU718, GLY796, LEU792, LEU844, ALA743, MET790, LYS745, THR854, ASN842, VAL726, GLY721, ARG841, GLY719, SER720
4f	-8.5	ARG841	5.66	GLY719, GLY796, LEU844, LEU718, VAL726, LYS745, THR854, ASP855, ASN842, GLY721, SER720, LEU799, CYA797
4g	-8.4	ARG841, THR854	6.22, 4.78	SER720, GLY719, MET793, LEU792, GLY796, CYS797, LEU718, ASP800, LYS745, LEU844, ASP855, MET790, ALA743, VAL726
4h	-8.5			ASP800, GLY719, CYS797, LEU718, MET793, GLY796, LEU844, THR854, ASN842, LYS745, ASP855, ARG841, GLY721, VAL726, SER720
4i	-8.7	THR854, MET793	4.50, 3.87	ASP800, LEU718, CYS797, GLY719, SER720, ASN842, LEU844, VAL726, ALA743, ASP855, LYS745, MET790, LEU792, GLY796
4j	-8.6	CYS797	3.52	LEU799, ARG841, ARG803, ASP800, GLY719, ASN842, SER720, GLY721, ALA722, ASP855, VAL726, THR854, LYS745, LEU844, GLY796, LEU718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Salt Bridge
Pi-Sulfur
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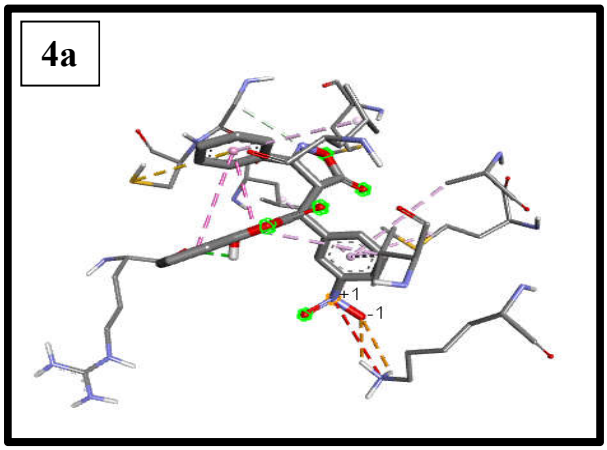
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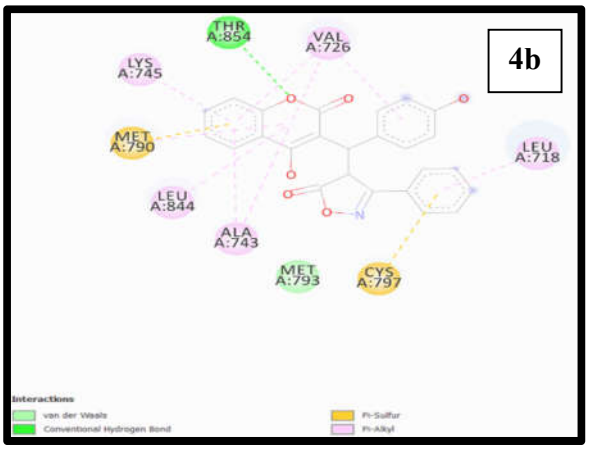
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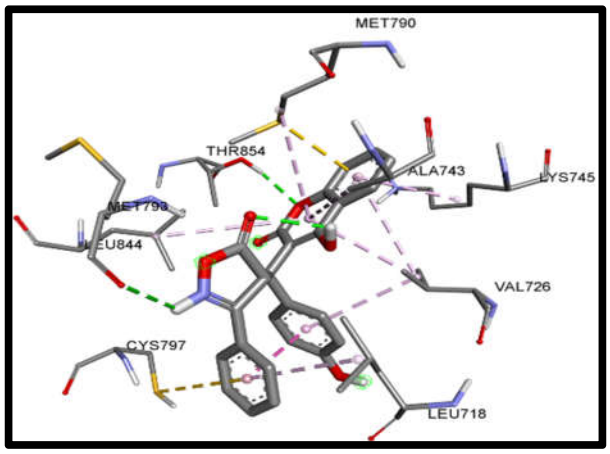
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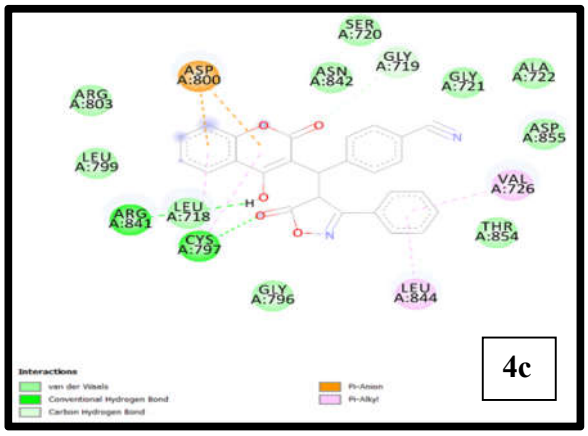
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Interactions
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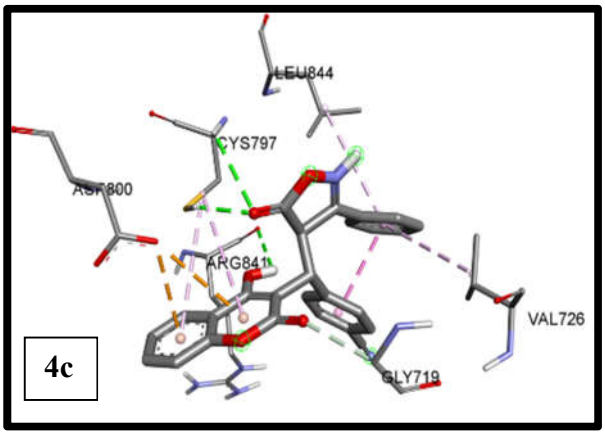
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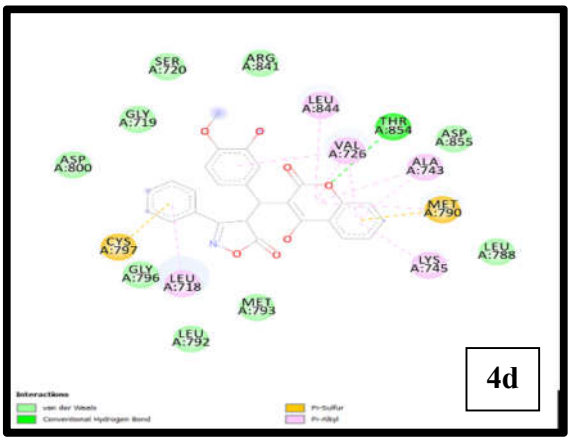
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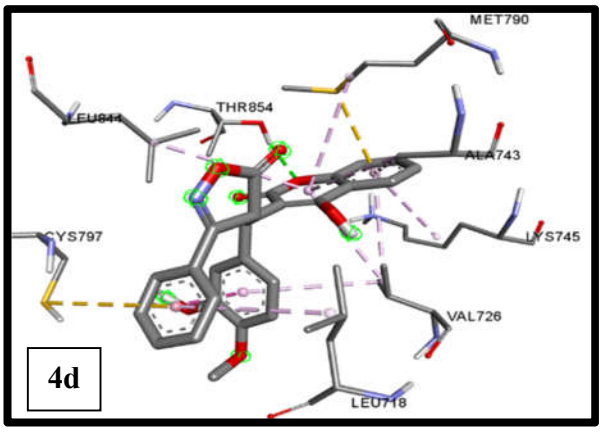
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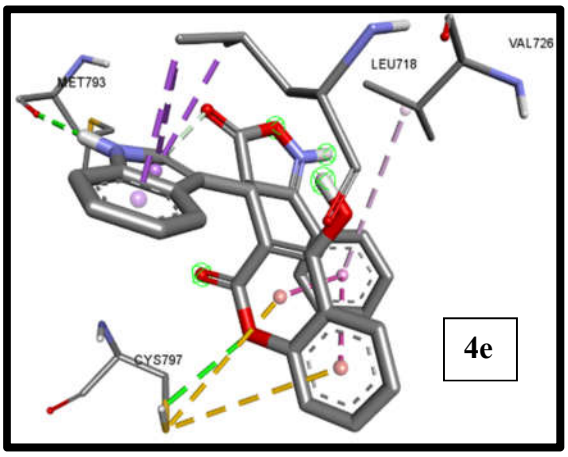
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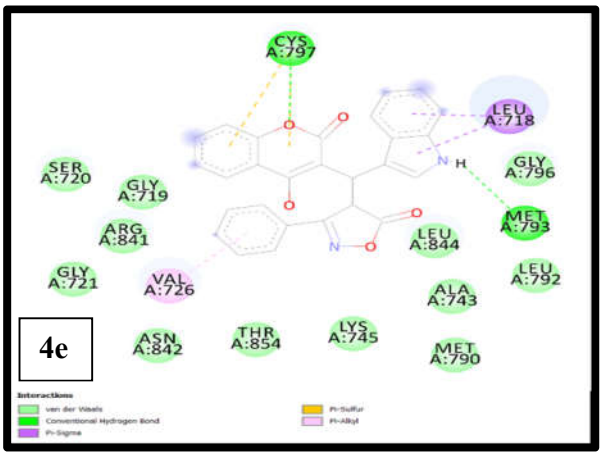
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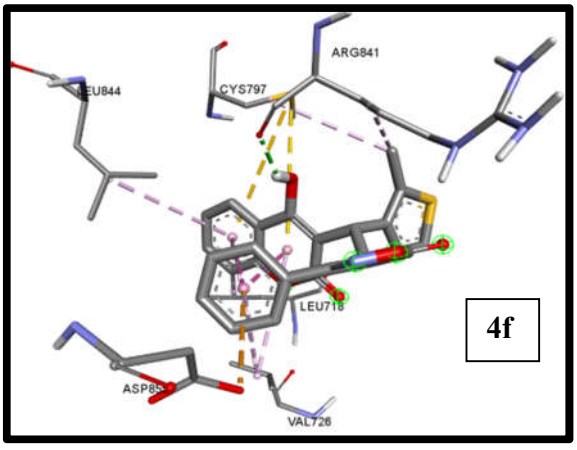
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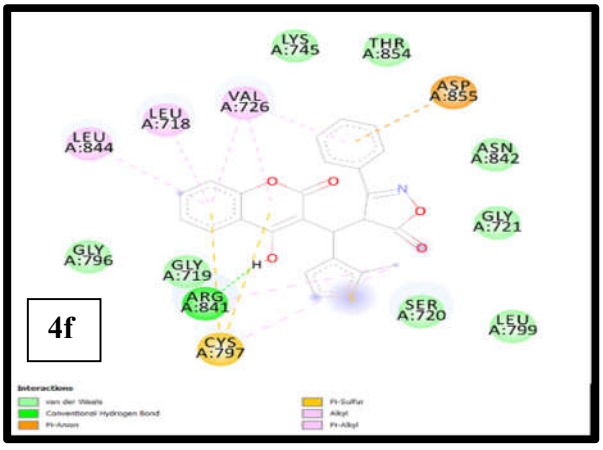
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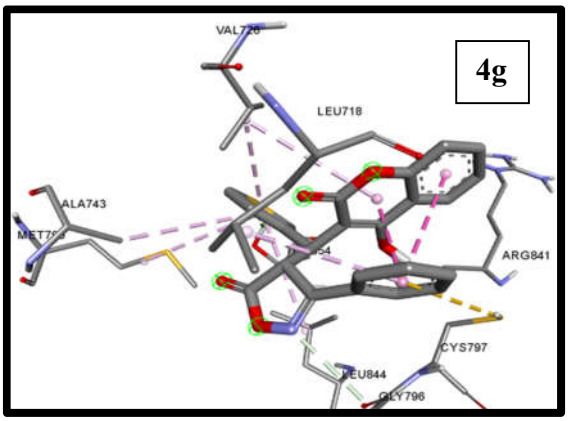
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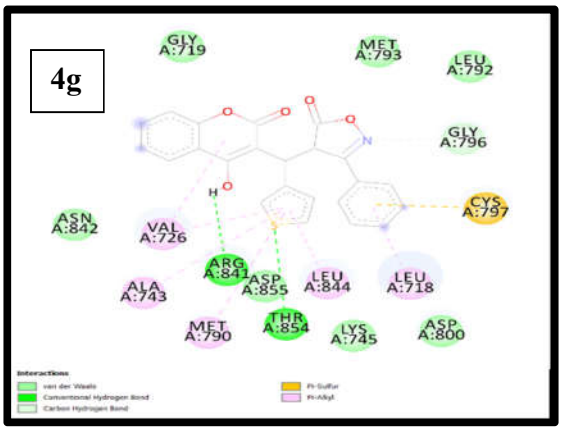
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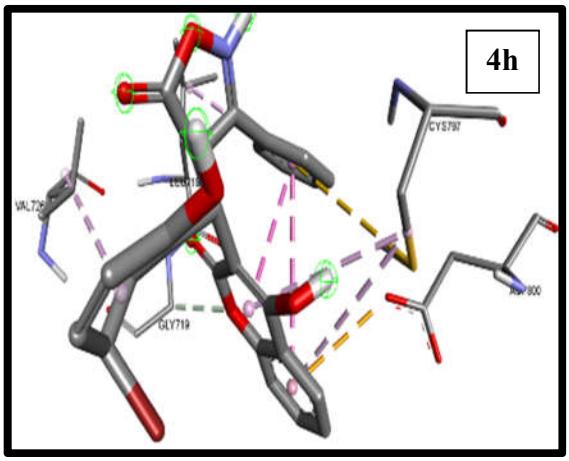
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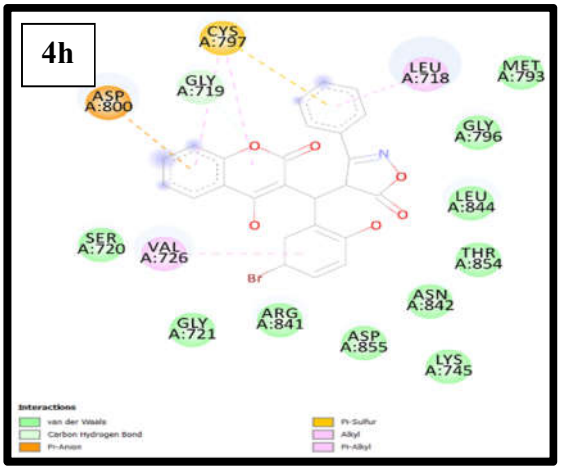
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Interactions
van der Waals
Carbon Hydrogen Bond
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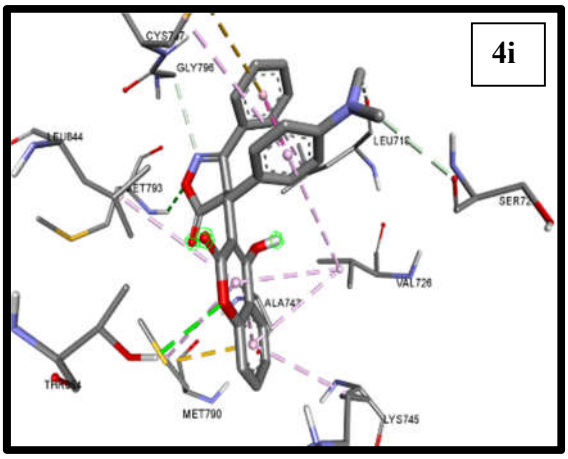
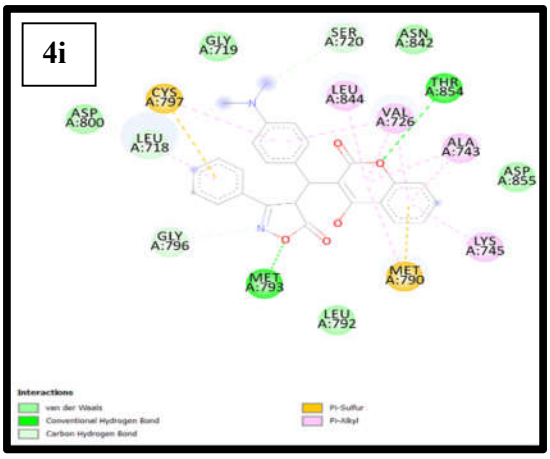
Pi-Sulfur
Alkyl
Pi-Alkyl


4i
GLY796
LEU844
LEU718
MET793
SER720
VAL726
ALA743
MET790
LYS745


4i
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SER A:720
ASN A:842
CYS A:797
LEU A:844
THR A:854
ASP A:800
VAL A:726
LEU A:718
ALA A:743
ASP A:855
GLY A:796
LYS A:745
MET A:793
MET A:790
LEU A:792
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Sulfur
Pi-Alkyl

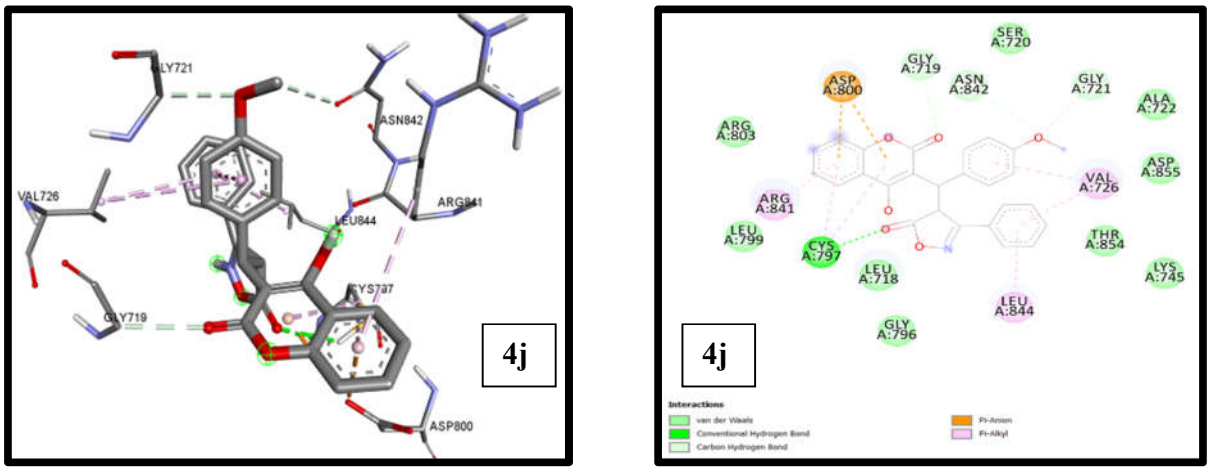


Fig 2. 2D and 3D interaction of synthesized compounds 4(a-j) and reference standard (Pramipexole) with Human peroxiredoxin 5.

1.3. Experimental

1.3.1. Materials and Method

High-purity reagents, solvents, and chemicals purchased from Sigma-Aldrich were used for the synthesis without further purification. Alumina TLC plates were used to check the progress of the reaction using ethyl acetate: hexane (1:4) as a mobile phase. Spots were identified by UV chamber. The melting points were determined by the electro-thermal apparatus using open capillary tubes and are uncorrected. FTIR spectra were recorded on a Bruker spectrophotometer using KBr pellets in the region of 400-4000 cm^{-1}. ^{1}H NMR and ^{13}C NMR spectra were recorded with the aid of a Bruker spectrometer at 400 MHz and 100 MHz respectively; chemical shifts (δ) were recorded in ppm relative to tetramethylsilane. The mass spectra of the compounds were confirmed by LC-MS 2010, SHIMADZU mass analyzer. Elemental analysis was calculated by using the unique elementary method. The anti-cancer and anti-bacterial activities were carried out in the Dept. of

Microbiology, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, Belgaum, Karnataka. And in silico protein-ligand dockingstudies were carried out by the automated docking tool AutodockVina. It was used to study the molecular interactions of the produced compounds as well as binding interaction also.

1.3.2. General procedure for the synthesis 4-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)(phenyl)methyl]-3-phenyl-1,2-oxazol-5(4*H*)-one derivatives.4(a-j):

A mixture of 4-hydroxy coumarin (**1**,1 mmol), substituted aldehydes (**2**, 1 mmol), and p-TSA (10 mol %) in ethanol/water (1:1) was stirred under refluxed condition. After 5 min, 3-phenyl-5-isoxazolone (**3**, 1 mmol) was added to it and refluxed at a temperature with constant stirring for about 8-10 h. simultaneously, the reaction was monitored by TLC (Ethyl acetate and Pet ether). After completion of the reaction, the reaction mixture was cooled to room temperature and poured into the 100 mL ice flake with vigorous stirring to get a solid precipitate. Then, it was filtered, washed, recrystallized from absolute ethanol, and dried to afford pure solid products **4(a-j).**

4-((4-Hydroxy-2-oxo-2H-chromen-3-yl)(3-nitrophenyl)methyl)-3-phenylisoxazol-5(4H)-one (4a)

Yellowish solid, yield: 78%; M.p. 234-238^{o}C; FTIR (KBr υ cm^{-1}): 1607(C=N), 1662 (C=O), 1669(C=O); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.72 (s, 1H, CH), 7.39-7.29 (m, 5H, Ar-H), 7.49-7.47 (d, *J*=8Hz, 2H, Ar-H), 7.52-7.47 (q, 2H, Ar-H), 7.61-7.57 (t, 2H, Ar-H), 7.98-7.89 (d.d. ,5H, Ar-H), 10.13 (s, 1H, OH), ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 116.05, 121.20, 122.17, 123.76, 127.99, 128.14, 128.55, 129.46, 130.18, 132.13, 134.54, 147.53, 152.18, 162.87, 163.33; LCMS: m/z 456 [M^{+}]. Anal.Calcd. for $C_{25}H_{16}N_2O_7$: C 65.01%, H 3.41 %, N 6.32%, O 25.26% Found: C61.57%, H 4.82%, and N 8.39 %.

4-((4-Hydroxy-2-oxo-2*H*-chromen-3-yl)(4-hydroxyphenyl)methyl)-3-phenylisoxazol-5(4*H*)-one(4b)

Pale yellow, yield: 81%; M.p. 234-238^{o}C; FTIR (KBr υ cm^{-1}): 1615 (C=N), 1697 (C=O), 3359 (OH); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.56 (s, 1H, CH), 6.56-6.53 (d, *J*=8Hz 2H, Ar-H), 6.91-6.89 (d, *J*=8Hz, 2H, Ar-H), 7.62-7.56 (m, 6H, Ar-H), 8.41-8.39 (d, *J*=8Hz, 2H, Ar-H), 11.09 (s,1H,OH), 12.48 (s, 1H, OH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 116.119, 128.78, 129.180, 137.99; LCMS: m/z 427 [M^{+}]. Anal.Calcd. for $C_{25}H_{17}NO_6$: C 70.25%, H 4.01 %, N 3.28%,O 22.46, Found: C 71.57%, H 3.82%, and N 5.39 %.

4-((4,5-dihydro-5-oxo-3-phenylisoxazol-4-yl)(4-hydroxy-2-oxo-2*H*-chromen-3-yl)methyl)benzonitrile (4c)

light orange, yield: 79%; M.p. 228-230 ^{o}C; FTIR (KBr υ cm^{-1}): 1607 (C=N), 1661 (C=O), 2228(CN); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.66 (s, 1H, CH), 6.33 (s, 1H, CH), 7.35-7.29 (m, 4H, Ar-H), 7.45-7.43 (d, *J*=8 Hz, 2H, Ar-H), 7.66-7.54 (m, 5H, Ar-H), 7.89-7.87 (d, J=8 Hz, 2H, Ar-H), 10.10 (s, 1H, OH), ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 103.09, 105.08, 108.90, 115.78, 116.03, 118.85, 118.95, 123.37, 123.78, 123.83, 124.06, 127.76, 127.86, 128.21, 128.47, 128.72, 130.48, 131.58, 131.88, 131.97, 132.18, 152.17, 152.45, 163.06, 163.37, 164.46, 166.77, 173.73; LCMS: m/z 436 [M^{+}]. Anal.Calcd. for $C_{26}H_{16}N_2O_5$: C 71.56%, H 3.70 %, N 6.42%, O 18.33; Found: C72.57%, H 4.82%, and N 9.39 %.

4-((4-hydroxy-2-oxo-2*H*-chromen-3-yl)(3-hydroxy-4-methoxyphenyl)methyl)-3-phenylisoxazol-5(4*H*)-one (4d)

Yellow solid, yield: 82%; M.p.= 180-182 ^{o}C; FTIR (KBr υ cm^{-1}): 1604 (C=N), 1660 (C=O), 1693 (C=O), 2959 (OCH_3), 3076(OH); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.54 (s, 1H, CH), 6.90-6.88(d, *J*=8Hz, 2H, Ar-H),7.29-7.28 (d, *J*=8Hz, 3H, Ar-H), 7.60-7.55 (m, 5H, Ar-H), 7.89-7.87 (d, *J*=8Hz, 2H, Ar-H), 8.43-8.42 (d,*J*=8Hz, 1H, Ar-H)), 10.84 (s, 1H, OH), 12.46 (s, 1H, OH);^{13}C-

NMR (100 MHz, DMSO-d_6, δ ppm): 55.84, 112.82, 116.00, 117.25, 125.09, 127.60, 129.02, 129.45, 131.02, 132.33, 147.70, 153.80, 154.55; LCMS: m/z 457 [M^+]. Anal.Calcd. for $C_{26}H_{19}NO_7$: C 68.27%, H 4.19 %, N 3.06 %. Found: C71.57%, H 4.82%, and N 5.39 %.

4-((4-Hydroxy-2-oxo-2*H*-chromen-3-yl)(1*H*-indol-3-yl)methyl)-3-phenylisoxazol-5(4*H*)-one (4e)

white solid, yield: 88%; M.p.= 166-168 °C; FTIR (KBr υ cm^{-1}): 1616 (C=N), 1651 (C=O), 1705 (C=O), 3063 (NH), 3365 (OH); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.59 (s, 1H, CH), 7.35-7.32 (t, 4H, Ar-H), 7.64-7.59 (q, 4H, Ar-H), 7.73-7.71 (m, 3H, Ar-H), 8.57 (s, 1H, Ar-H), 9.59 (s,1H, Ar-H), 12.5 (s, 1H, OH), 12.9(s, 1H, NH);^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 91.13, 107.26, 113.19, 113.59, 116.00, 116.61, 118.36, 123.34, 123.47, 124.28, 124.52, 128.02, 128.32, 128.81, 129.57, 130.91, 133.04, 136.70, 139.64, 141.50, 153.70, 162.45, 164.01, 166.05, 170.87; LCMS: m/z 450 [M^+]. Anal.Calcd. for $C_{27}H_{18}N_2O_5$: C 71.99%, H 4.03%, N 6.22%. Found: C 69.57%, H 4.82%, and N 4.39 %.

4-((4-Hydroxy-2-oxo-2*H*-chromen-3-yl)(thiophen-3-yl)methyl)-3-phenylisoxazol-5(4*H*)-one (4f)

Creamy solid, yield: 76%; M.p.= 220-222°C; ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.58 (s, 1H, CH), 6.45 (s, 1H, Ar-H), 7.39-7.26 (t, 4H, Ar-H), 7.70-7.53 (m, 5H, Ar-H), 8.15 (s, 1H, Ar-H), 8.38-8.28 (q, 2H, Ar-H), 12.50 (s, 1H, OH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm): 103.98, 111.53, 115.69, 118.97, 123.06, 123.29, 123.42, 124.11, 126.24, 127.19, 128.61, 128.97, 129.24, 130.90, 131.49, 136.19, 142.28, 143.23, 144.75, 152.34, 163.35, 164.18, 166.81, 168.75; LCMS: m/z 634.66 [M^+]. Anal.Calcd. for $C_{23}H_{16}NO_5O$: C 66.18 %, H 36.2 %, N 3.36 %. Found: C71.57%, H 34.82%, and N 9.39 %.

1.4. Biological studies

1.4.1. Antibacterial Activity

The antibacterial Activity was assessed by the Kirby-Bauer disc diffusion method **[40].** against a panel of Gram-positive and Gram-negative bacterial strains, including *Bacillus subtilis* (MTCC 5674), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 7410), and *Pseudomonas aeruginosa* (MTCC 1688) at various concentrations **[41.42].** Briefly, all the compounds were dissolved in DMSO in two different concentrations (25 and 50 mg/mL) and to this test solution, previously cultured Mueller Hinton Agar Sabouraud's dextrose agar medium was added and autoclaved at ± 37 °C for about 24 h. gatifloxacin. was used as a standard drug and 10% DMSO used as a negative control. The antimicrobial assay of the title compounds was measured by the formed zone of inhibition against pathogenic strains. The test was performed in triplicate and the average was taken as a final reading. The minimum inhibitory concentration (MIC) was determined by the serial broth-dilution method.

1.4.2. *in vitro* Cytotoxicity Activity

in vitro cytotoxicity was evaluated using the MTT assay method **[43]** against the MCF-7 (Human breast cancer) cell line **[44]**. *in vitro*-Cytotoxicity was assessed by MTT assay by following the procedure of Kumbar *et al* **[45]** against MCF-7 (Breast cancer) cell line. The cells were seeded in a 96-well flat-bottom microplate and maintained at 37 °C in 95% humidity and 5% CO_2 overnight. Different concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL) of samples were treated. The cells were incubated for another 48 h, and the wells were washed twice with PBS. 20 µL of MTT staining solution was added to each well, and the plate was incubated at 37 °C. After 4 h, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded at 570 nm using a microplate reader. The percentage of cell survival was calculated by using the following formula.

$$\%\ \text{of cell survial} = \frac{\text{Mean OD of Test compound}}{\text{Mean OD of Negative control}} \times 100$$

1.4.3. *in Silico* Molecular Docking Study

in silico molecular docking studies were employed to predict the binding affinity of the compounds and assess their orientation within the active pockets of receptors. The antioxidant activity results of the synthesized compounds were subjected to molecular docking studies using Autodock Vina within the PyRX workstation, utilizing a genetic algorithm. The 2D structures of the synthesized compounds were converted into energy-minimized 3D structures and utilized for in silico protein-ligand docking **[46].** These compounds were employed as ligands, and the docking receptors were selected as Human peroxiredoxin 5 (PDB ID: 1OC3).

Ligand Preparation:

The ligands were created using ChemDraw software, and their 3D structures and energies were minimized using the USCF Chimera tool with the AMBER force field. Subsequently, the ligands were converted into PDB format **[47.48].**

Receptor Preparation:

The 3D X-ray crystal structure of HUMAN PEROXIREDOXIN 5 (PDB ID: 1OC3) was obtained from the Protein Data Bank (https://www. rcsb. org). The co-crystal ligands and water molecules were removed from the protein structure. Hydrogen atoms were added, non-polar hydrogens were merged, charges were assigned, and the protein was energy-minimized using the AMBER force field, all performed using the USCF Chimera tool. The prepared protein was then converted into PDB format **[49-54]**

Binding Site Identification:

The active binding pocket of the target was determined based on the location of the CYS47 amino acid residue, which facilitated the placement of a grid box around the active site.

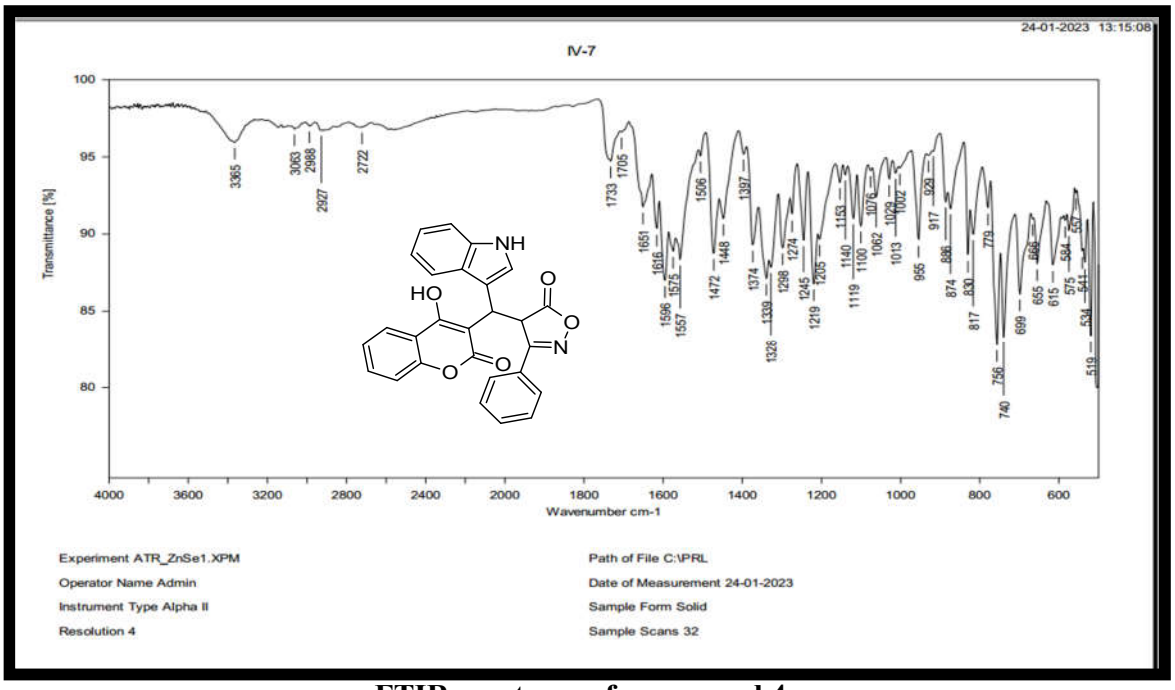
Docking and Visualization:

A grid box was set around the active site of the protein, and the designed ligands were docked against the receptor using Autodock Vina within the PyRX workstation. The ligand with the lowest binding affinity was considered the best conformation. The docked protein and target were converted into PDB format using Schrödinger PyMol, and the interactions were visualized using Biovia Discovery Studios **[55-57]**

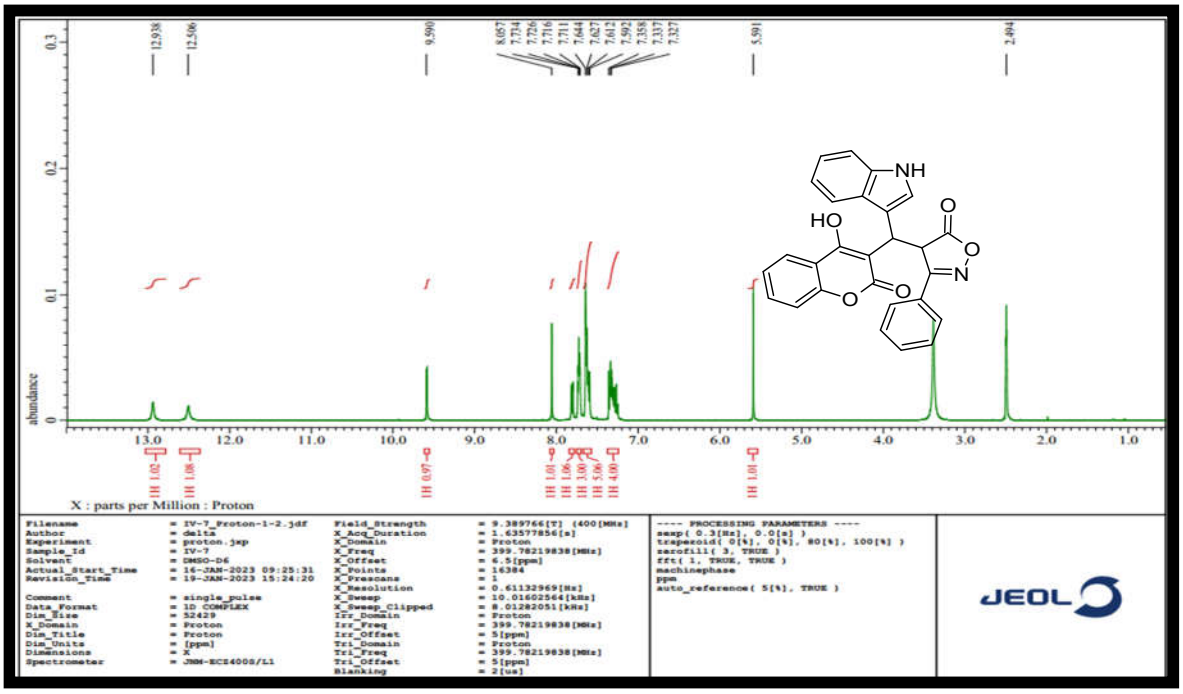
1.5. Conclusion

We have reported an easy and efficient protocol of the synthesized some novel through one-pot reaction and screened for their pharmacological and *in silico* investigations. From the results of anti-bacterial activity it was found that compounds 4a, 4c, 4h, and 4i exhibited equipotent activity against *E.coli*, *Pseudomonas and S.aureus with MIC* 2.1–2.3 mg/ml. From the structure activity relationship studies, it was observed that the compounds 4e and 4j having methoxy groups respectively on the aromatic ring showed excellent activity among the series when compared to the standard drug. Compounds 4a, 4c, 4f, and 4g having -NO_2 and -Cl group respectively displayed promising activity against *B. subtilis*. Rest of the synthesized compounds showed moderate activity against selected bacterial strains.The compound 4h displayed significant cytotoxic effect on the anti -cancer activity.

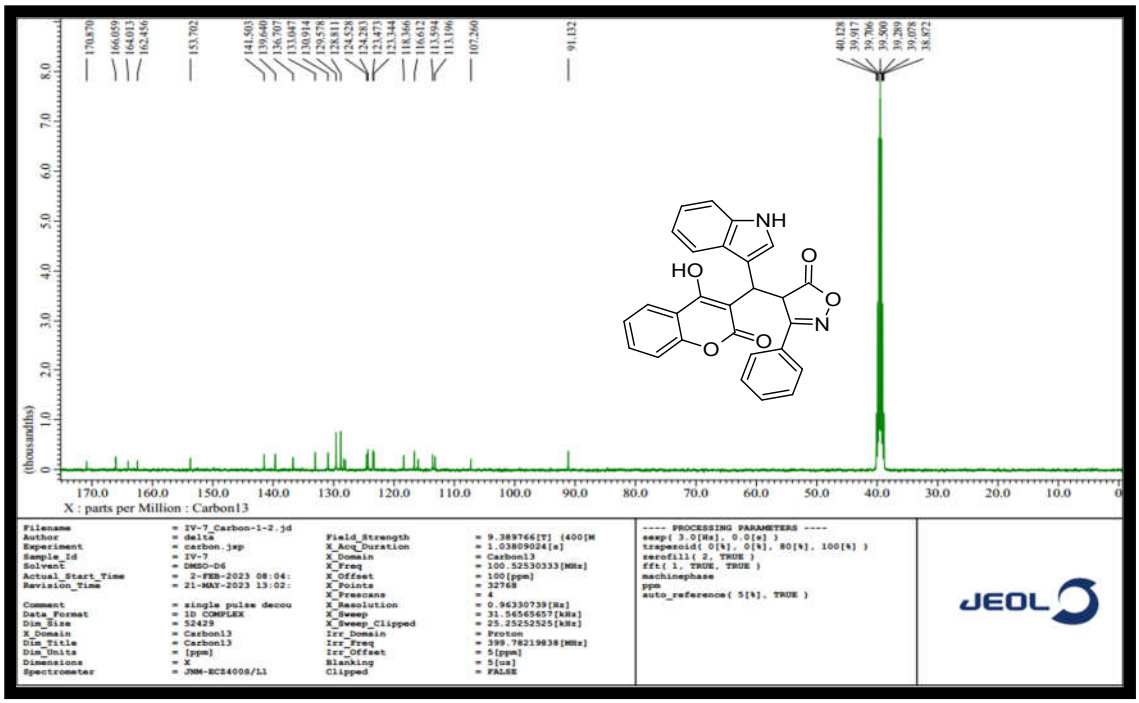
1.6. spectra of selected compounds



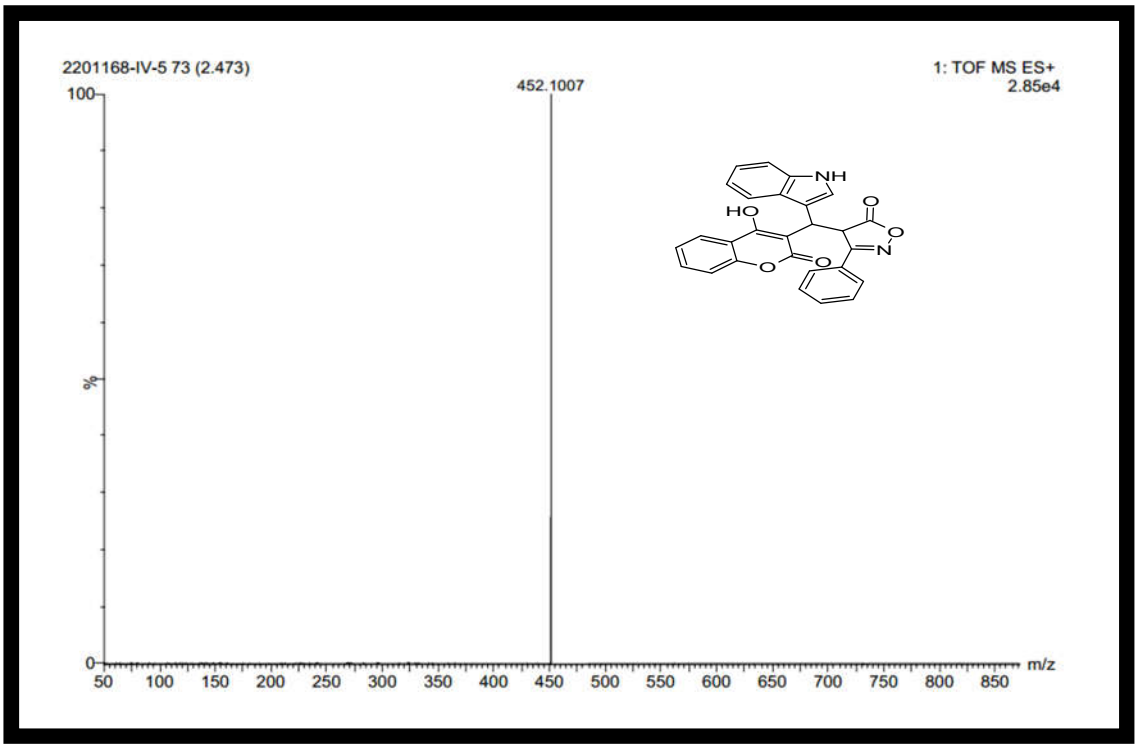
FTIR spectrum of compound 4e



H NMR spectrum of compound 4e



^{13}C NMR spectrum of compound 4e



MASS spectrum of compound 4e

1.7. References

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