Synthesis and characterization of some novel 5-[(4-hydroxy-2-oxo-2H-1-benzopyran-3yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives and investigation of their biological activities with molecular docking studies.

Megha G V[a], Yadav D. Bodke[a]*.

[a]Department of PG Chemistry, Surana College, Autonomous, South-end road, Bangalore-560004 Karnataka, India.
[a*]Department of PG Studies and Research in Chemistry, Jnana sahyadri, Kuvempu University, Shankaraghatta-577451, Karnataka, India.

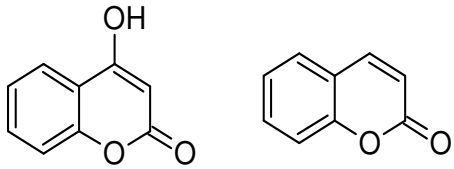
Abstract

Herein, an efficient and convenient method for the Synthesis and characterization of some novel 5-[(4-hydroxy-2-oxo-2H-1-benzopyran-3yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives and investigation of their biological activities with molecular docking studies.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 anti-TB activity against the H37RV strain of tuberculosis (TB). Among the compounds tested, compound **4b** displayed notable anti-TB activity, boasting a minimum inhibitory concentration (MIC) value of 25 µg/mL. Further, all The compounds synthesized **(4a-j)** underwent assessment for their ability to scavenge free radicals using the DPPH method, as depicted in Figure 3. In comparison to the standard Ascorbic acid, all these compounds exhibited varying degrees of free radical scavenging capabilities. Among the tested compounds, **4b** emerged as the most potent antioxidant, displaying an IC_{50} value of 34. 66±2. 43 µg/mL, Further, the binding capability for the synthesized compounds (**4a-j**) was analyzed by molecular docking studies using the receptors of Human Peroxiredoxin 5 to evaluate their antioxidant activity with compounds **4(a-j),** it was observed that all these compounds formed strong bonds with specific amino acids within the receptor's active pocket.

Keywords: 4-hydroxy Coumarin; anti-TB; antioxidant; molecular docking.

1. Introduction

Oxygen-containing heterocycles are largely distributed in natural and synthetic compounds. Coumarins are among the most famous heterocycles which possess one oxygen atom in their rings [**1**]. Coumarin compounds represent an important type of naturally occurring and synthetic oxygen-containing heterocycles with typical benzopyrone framework. They are naturally plant-derived and synthetically taken polyphenolic substances, presenting a wide variety of biological activities and behaviours, supporting their use as therapeutic agents for multiple diseases. Their structural characteristics correlated to physicochemical properties seem to define the extent of the biological activity **[2.3].** Coumarins have been known as fragrance materials in perfumes for a rather long time, because of their sweet smell. Naturally occurring coumarins are known in about 700 structures in more than 100 plant families [**4**] and the number of structures still increases.



(1) **(2)**

Fig. 1. Structures of 4-hydroxycoumarin **(1)** and Coumarin **(2).**

The first important research about coumarins and its derivatives were about their potential toxicities to both animals and plants, such as phototoxic, photosensitizing. But these properties are also considered as therapeutic potentials. **[5].** 4-Hydroxycoumarins are among the most important coumarin derivatives, because they contain a wide range of biological activities. They can be anticoagulant,[**6]** antibacterial**,[7]** anti-HIV active,[**8**] and anti-tumoral.[**9**] The

nucleus of 4- hydroxycoumarin is very susceptible to electrophilic substitution,[**10**] so they are very easy synthesized and substituted by other functional groups to enhance their biological activities. apart from their medicinal applications, Coumarins are widely used in pharmaceutics because of their biological activities. Additionally, coumarin derivatives have been used as antioxidant reagents,[**11**] dyes,[**12**] insecticides,[**13**] sensitizers,[**14**] herbicides, food additives,. perfumes, and cosmetics (**Fig. 1).** 4-Hydroxy coumarin (2*H*-1benzopyran- 2-ones) is one of the versatile and important synthons for the synthesis of a wide variety of heterocyclic pharmacophores. The importance of these precursors in the field of organic synthesis has been amplified, not only because it's substantial synthetic end products but also constitute one of the major structurally important nuclei of many natural products **[15].**

In 4-hydroxy coumarin, it is found that the molecule possesses both electrophilic and nucleophilic assets. [**16,17**].The carbon atom at the third position of the 4-hydroxy coumarin is more susceptible to many reactions of the significant nucleophilicity which arises mainly due to the presence of hydroxyl group (electron–donating) as well as carbonyl oxygen (electron-withdrawing) atom present at the adjacent second and fourth position respectively. These special applications of 4-hydroxycoumarin have motivated considerable interest in this class of compounds for number of researchers across the globe [**18-20].**

Tuberculosis (TB), commonly known as 'white plaque,' is a potentially severe infectious disease. It is caused by various members of the Mycobacterium tuberculosis (MTB) complex, which includes MTB itself, M. africanum, M. bovis, M. caprae, M. microti, M. pinnipedii, and M. canettii. MTB primarily affects the lungs, referred to as pulmonary TB **[21].** According to the World Health Organization (WHO), approximately one-third of the global population, around 2 billion people, carry latent MTB infections. These infections can remain dormant for many years,

with approximately 5-10% of latent cases progressing to active TB. Although first-line anti-TB drugs like isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA) are highly effective, they are not ideal due to the increasing threat of TB, especially in developing nations. Current TB treatment regimens require long-term therapy, often lasting 6-12 months, which can lead to poor patient compliance. Furthermore, drug-resistant forms of TB, including multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB),have become alarmingly prevalent in recent decades **[22].**

Another contributing factor to the resurgence of TB is its co-infection with HIV, which makes patients more susceptible to reinfection by drug-susceptible or drug-resistant TB strains. TB is a leading cause of death among people living with HIV/AIDS, worsening the TB situation globally **[23,24].** Therefore, there is an urgent need to develop new anti-TB drugs with unique mechanisms of action, high tolerability, effectiveness against both drug-susceptible and drug-resistant MTB strains, low toxicity, and shorter therapy durations to combat this public health challenge.

The interest in synthesizing 4-hydroxy coumarin derivatives has been aroused mainly because of the remarkable pharmacological significance, such as anti-coagulant, antimicrobial, antitumor, anti-inflammatory, and HIV protease inhibitor activity, and also associated with some physiological applications[**25**]. The coumarin skeleton has been used as a pharmacophore in the development of anti-TB drugs because of the afore mentioned advantageous characteristics. Numerous coumarin-containing derivatives have been synthesized and tested for their anti-TB activity in searching for novel anti-TB medicines. Some of the importance of 4-hydroxy coumarin-based compounds are available in the market as shown in **Fig. 2.** Some reported

biologically important heterocyclic compounds and 4-hydroxy coumarin-containing derivatives have been discussed below.

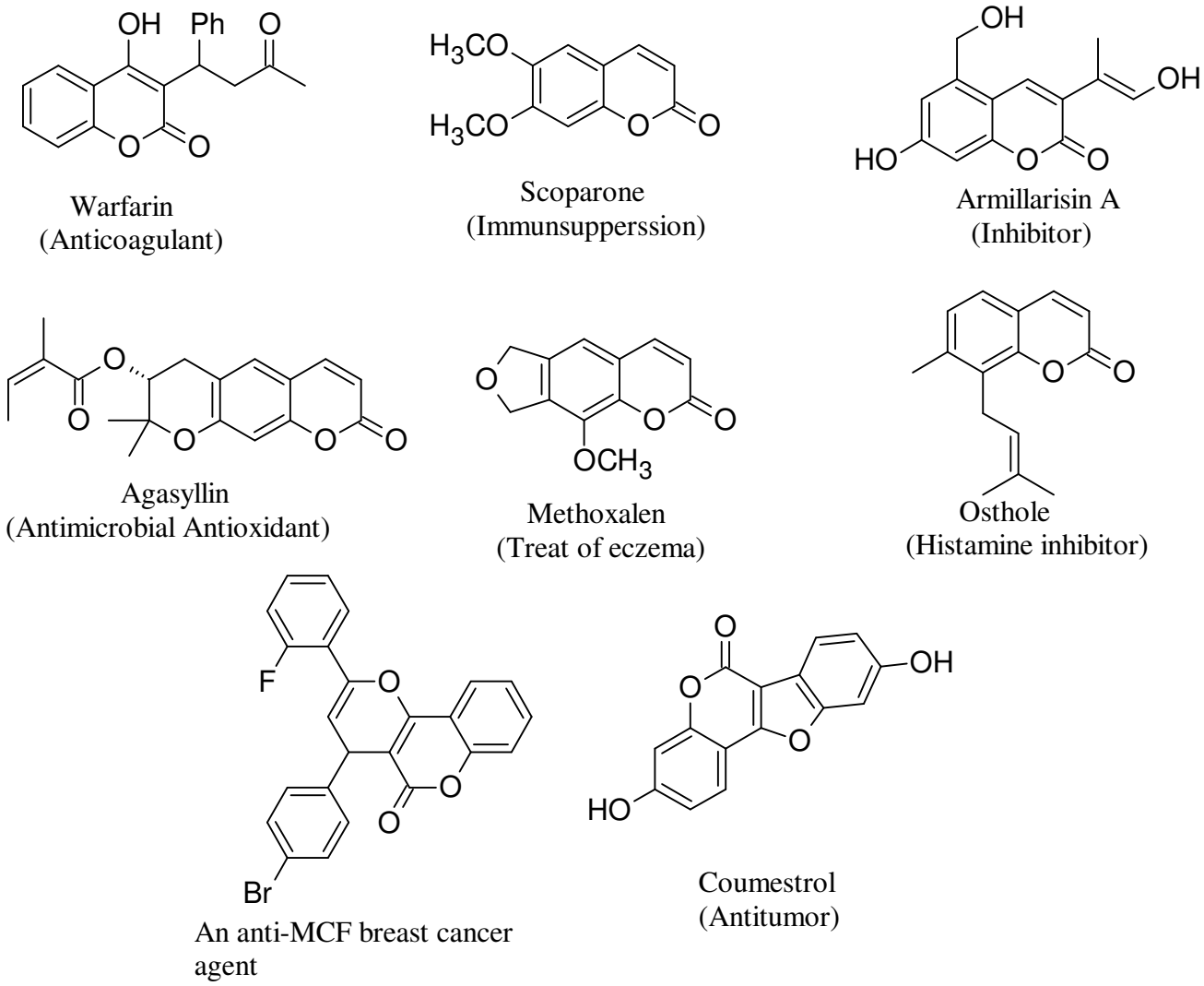
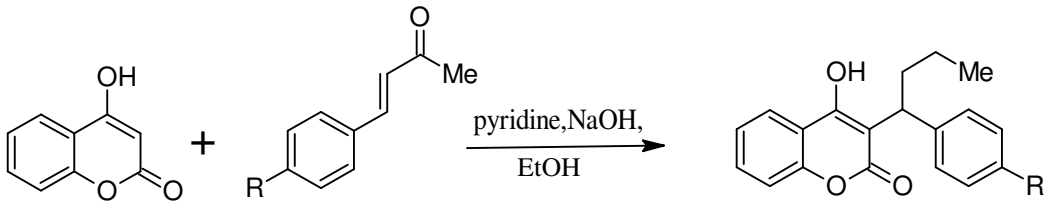


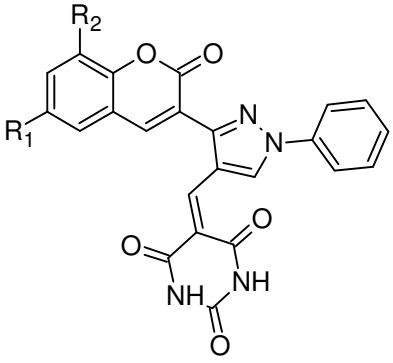
Fig.2. Some of the drugs containing 4-hydroxy coumarin nucleus.

4-Hydroxy coumarin derivatives were synthesized in 1999, according to Danchev N.D. *et al.* **[26].** A potential lead molecule among their 4-hydroxy coumarin derivatives is 3,3'-(4-chlorophenylmethylene)-bis-(4-hydroxy-2H-1-benzopyran-2-one), which has a low toxicity (1).

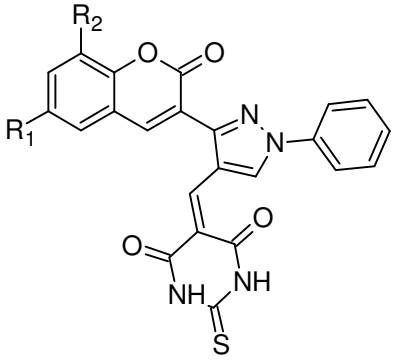


R=H, Halogen, Nitro **(1)**

In 2012,S. Vijaya Laxmi and co-workers reported a synthesis of coumarin pyrazole barbiturate and thiobarbituric acid derivatives [**27**]. and evaluated their antimicrobial and anti-fungal activity. Among them compounds **(2)** and **(3)** exhibited good anti-fungal activity against *Aspergillus niger*.



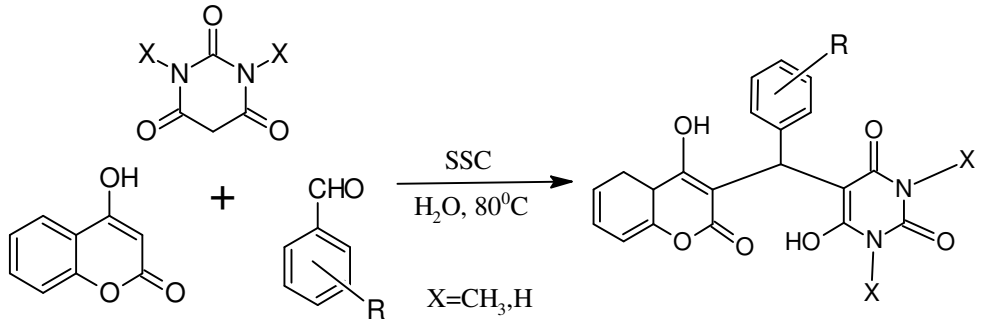
(2)



(3)

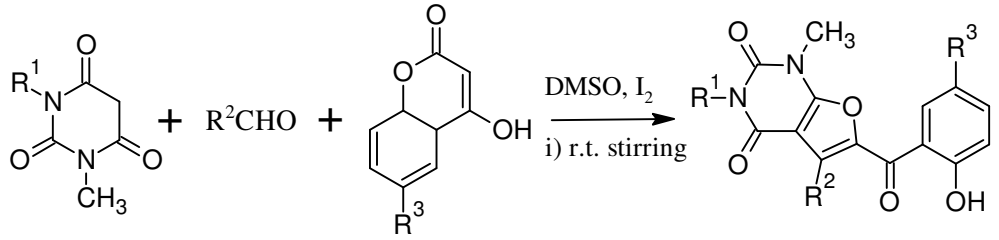
In 2015, Khalil Eskandari *et al.* published a study detailing the synthesis of innovative benzylbarbituro coumarin derivatives using silica sodium carbonate as a catalyst in water **[28].** They employed a single-step environmentally friendly method to create these novel heterocyclic compounds, utilizing SSC as a solid base catalyst. This process yielded promising biologically

active compounds (4). Notably, this approach's advantage lies in its ability to produce novel organic compounds with high yields, employing an eco-friendly catalyst and solvent.



(4)

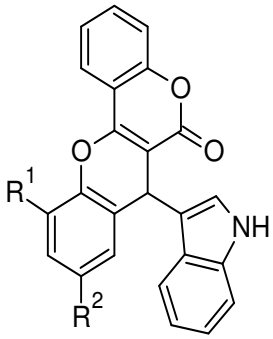
In 2016, Leema Dutta *et al.,* reported the synthesis of novel functionalized furo[2, 3-d]pyrimidines through a one-pot, three-component reaction. This reaction involved 1, 3-dimethyl barbituric acids, aldehydes, and derivatives of 4-hydroxy coumarins **[29].** The process was catalyzed by iodine and conducted in dimethyl sulfoxide (DMSO). It comprised a sequence of steps, including condensation and Michael addition, followed by lactone ring opening and intermolecular cyclization. This method yielded the desired product in both high quantity and good quality (5).



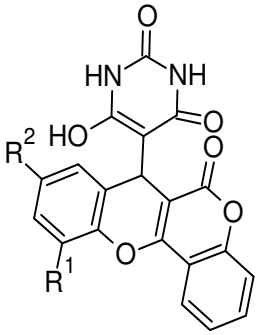
(5)

In 2015, H. M. Kasralikar *et al*. reported a synthesis and molecular docking study of novel chromenochromenones as anti-HIV-1 NNRT inhibitors **[30]**. The one-pot, three-component condensation reaction of salicylaldehydes, 4-hydroxy coumarin, and indole or barbituric acid.

The simple procedure and good to excellent yields (78-90%) are notable features of the method. The obtained derivatives were examined for their molecular docking study. All the screened compounds exhibited excellent Anti-HIV-1 activity **(6,7).**

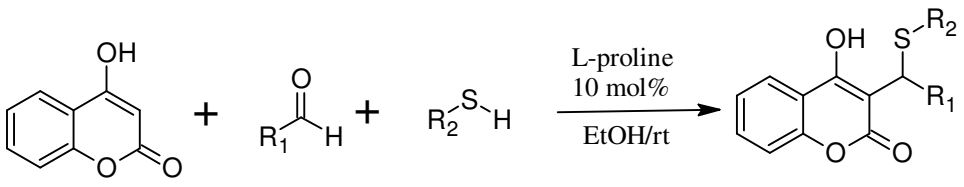


(6)



(7)

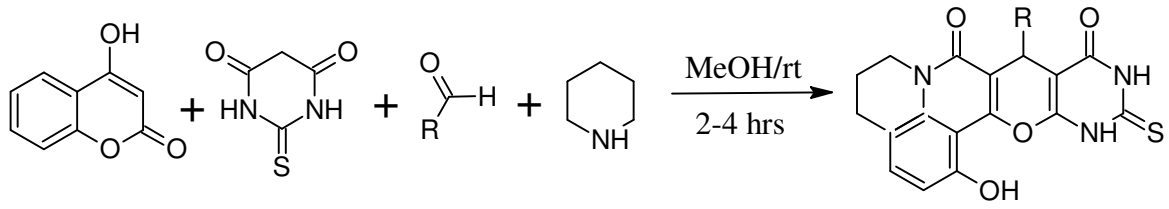
Ajaz A. Dar *et.al*., reported the L-proline catalyzed synthesis of coumarin derivatives **(8)** owing thioether at 3rd position via a Knovengel condensation between 4- hydroxyl coumarin and aldehyde followed by Michael addition of thiol. This protocol involves milder reaction conditions, green solvent, easy workup procedure, and less amount of catalyst **[31].**



(8)

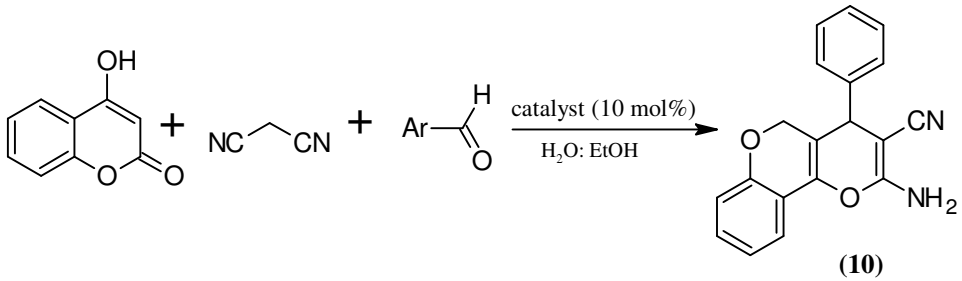
In 2016, Naik, M. D. and colleagues accomplished a straightforward one-pot synthesis of derivatives of 1H-pyrano[2, 3-d]pyrimidin-4(5H)-ones (9). They subsequently assessed the biological activities of these compounds, revealing their remarkable DNA cleavage capabilities. Surprisingly, all the ligands completely cleaved the Ct-DNA strand. Additionally, these synthesized compounds exhibited potency against various bacterial strains and displayed efficacy in inhibiting inflammation. Furthermore, the compounds effectively prevented protein

denaturation in bovine serum albumin and stabilized the HRBC membrane at minimal concentrations. Moreover, a significant number of these compounds efficiently inhibited the growth of both gram-positive and gram-negative bacteria **[32].**



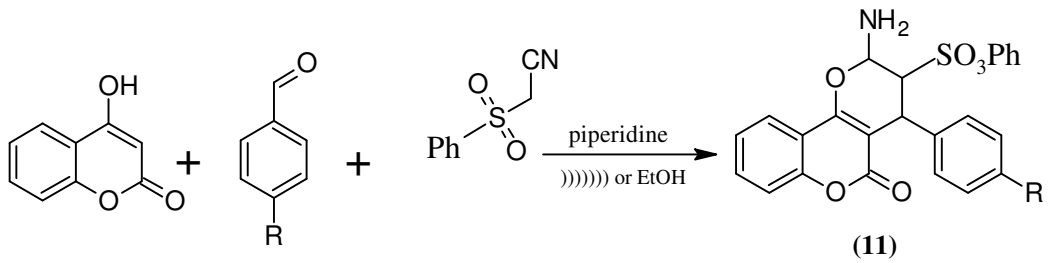
(9)

S.A. Mohammadi *et al.* described the diammonium hydrogen phosphate catalyzed an efficient one-pot synthesis of novel series of 3,4-dihydropyrano[c]chromenes **(10)** via one pot, three-component reaction involving 4-hydroxy coumarin, malononitrile, and aromatic aldehyde in aqueous media **[33].**



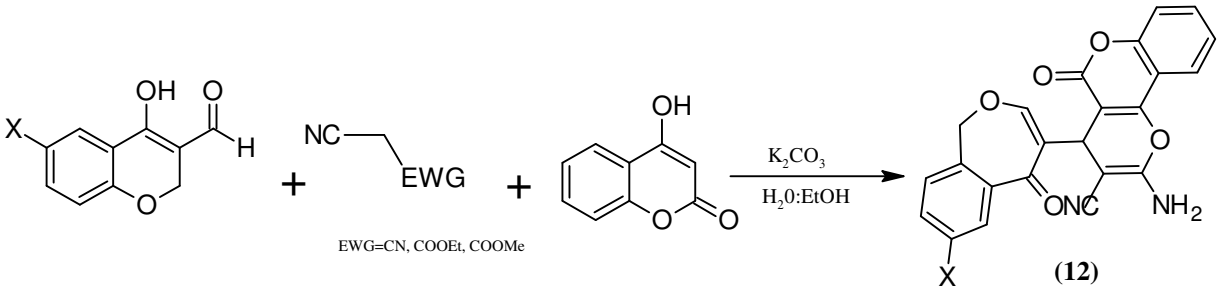
(10)

A.S. Al-bogami *et al.* developed an eco-friendly method by utilizing ultrasonic irradiation for the synthesis of a new series of pyrano [3,2-c]coumarin derivatives **(11).** The three-component reaction involves 4-hydroxy coumarin, substituted aromatic aldehydes, and sulfone derivatives catalyzed by piperidine. The reaction was performed under both conventional and microwave irradiation reactions and as a result, the product obtained with good yield under ultrasonic irradiation compared to the conventional method **[34].**



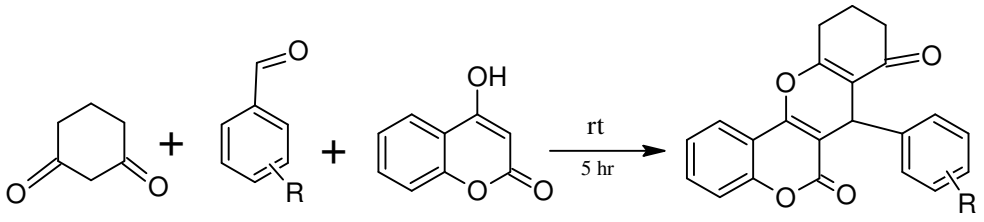
(11)

H.R. Bijanzadesh *et al.* introduced a K_2CO_3-mediated reaction for the synthesis of functionalized heterocycles **(12)** comprising chromone skeleton. Three-component reaction involving 3-formyl coumarin, malonitrile, and 4-hydroxy Coumarin (active methylene compounds) was carried out to get the target compounds. The reaction strategy provides a good yield of the product, easy work, and also an eco-friendly method **[35].**



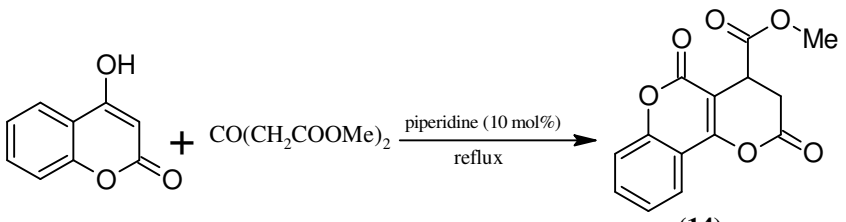
(12)

Abbas Shafiee *et al.* reported an efficient synthesis of a novel series of benzopyrano[3,2-c]chromene-6,8-diones **(13)** using 4-hydroxy coumarin, substituted aromatic aldehydes and 1,3 cyclohexadiene **[36].**



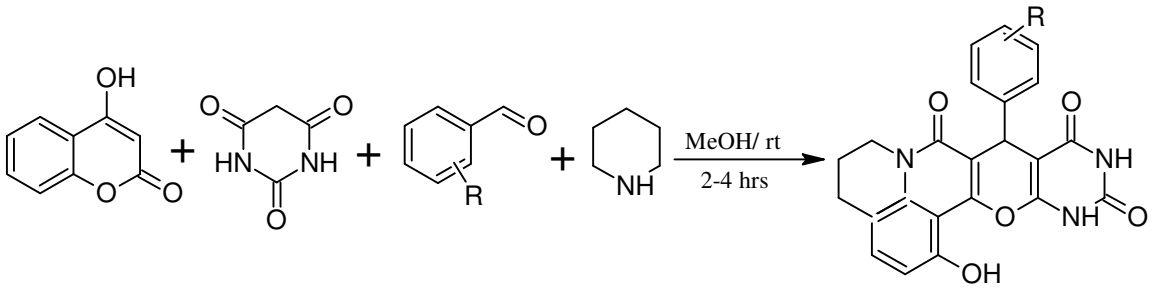
(13)

Sirisha *et al.,* developed a facial solvent-free method for the synthesis of pyrano-benzopyrano derivatives **(14).**The reaction involves the condensation of 4-hydroxy coumarin with active methylene esters in the presence of piperidine as a catalyst **[37].**



(14)

In the year 2020, Naik and their team reported an efficient multicomponent synthesis method for producing derivatives of 1H-pyrano[2, 3-d]pyrimidine-2, 4(3H, 5H)-diones (15). This synthesis process was remarkably straightforward and involved multiple components. Subsequently, all the newly synthesized compounds underwent evaluation for their potential to inhibit in vitro α-amylase and α-glucosidase enzymes. The results of these assays unveiled varying enzyme inhibition activities among the compounds. Notably, it was observed that the concentration required to inhibit enzyme activity was lower for α-glucosidases compared to α-amylases. In other words, the synthesized compounds exhibited greater potency in inhibiting α-glucosidase enzyme activity. Importantly, this method yielded the desired product with a commendable yield when compared to conventional approaches **[38].**



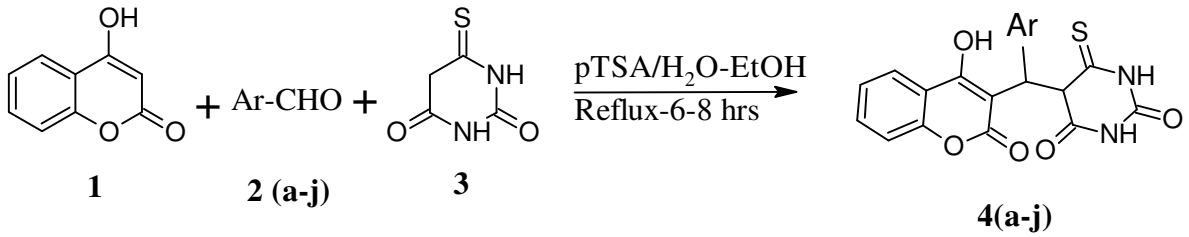
(15)

Based on the above investigations, we reported the synthesis of a series of some novel 5-[(4-hydroxy-2-oxo-2H-1-benzopyran-3-yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives. and examined their biological activities like anti-oxidant, anti-TB, and in silico docking studies.

1.2. Present work

The concept of enhancing molecular diversity through the fusion of small heterocycles has garnered significant attention in the realm of drug discovery. Multicomponent reactions (MCRs) have emerged as a prominently explored and widely embraced methodology for uncovering the most potent bioactive pharmacophores. MCRs stand out as exceptionally advantageous synthetic techniques in organic chemistry due to their economic and eco-friendly nature, shorter reaction times, and higher yields.

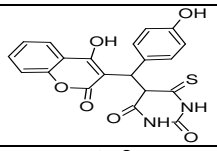
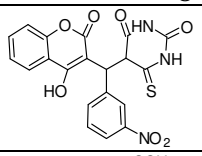
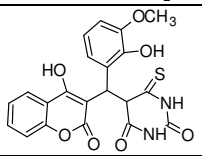
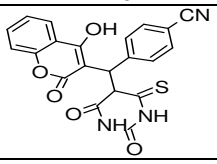
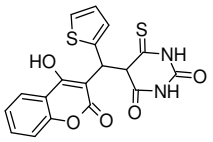
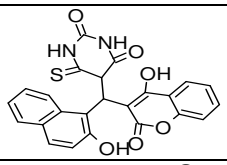
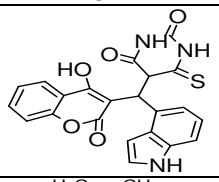
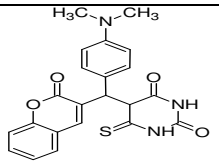
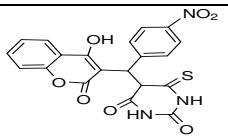
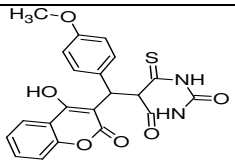
In this context, coumarin nuclei have emerged as extensively researched core heterocyclic structures, primarily owing to their profound medicinal relevance. Recognizing the pivotal role of MCRs as an ideal approach for multi-step synthesis, it was decided to embark on the synthesis of fused scaffolds that incorporate coumarin synthesis into a single framework. The synthetic strategy employed to obtain the compounds designated as **4(a-j)** is elucidated in **Scheme 5.**



1 **2 (a-j)** **3** **4(a-j)**

Scheme 5. Synthesis of a series of some novel 5-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives **4 (a-j).**

The physical data of synthesized compounds have been given below in **Table 1.**

sample	colour	M.P.(^{0}C)	Mol.wt	Yield %	compound	Mol. formula
4a	Yellowish solid	189-190	410	81		$C_{20}H_{14}N_2O_6S$
4b	Yellow-orange	185-187	439	78		$C_{20}H_{13}N_3O_8$
4c	Pale yellow	193-194	440	82		$C_{21}H_{16}N_2O_7$
4d	Creamy white	198-200	419	79		$C_{21}H_{13}N_3O_5S$
4e	White solid	196-197	400	88		$C_{18}H_{12}N_2O_5S_2$
4f	Yellow solid	195-196	460	91		$C_{24}H_{16}N_2O_6S$
4g	Pale reddish	186-187	433	79		$C_{22}H_{15}N_3O_5S$
4h	White solid	193-194	421	86		$C_{22}H_{19}N_3O_4S$
4i	Creamy solid	186-188	423	79		$C_{20}H_{13}N_3O_8$
4j	Pale yellow solid	198-200	424	84		$C_{21}H_{16}N_2O_6S$

The plausible mechanism for the formation of new 5-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives has been proposed in **Fig. 2**

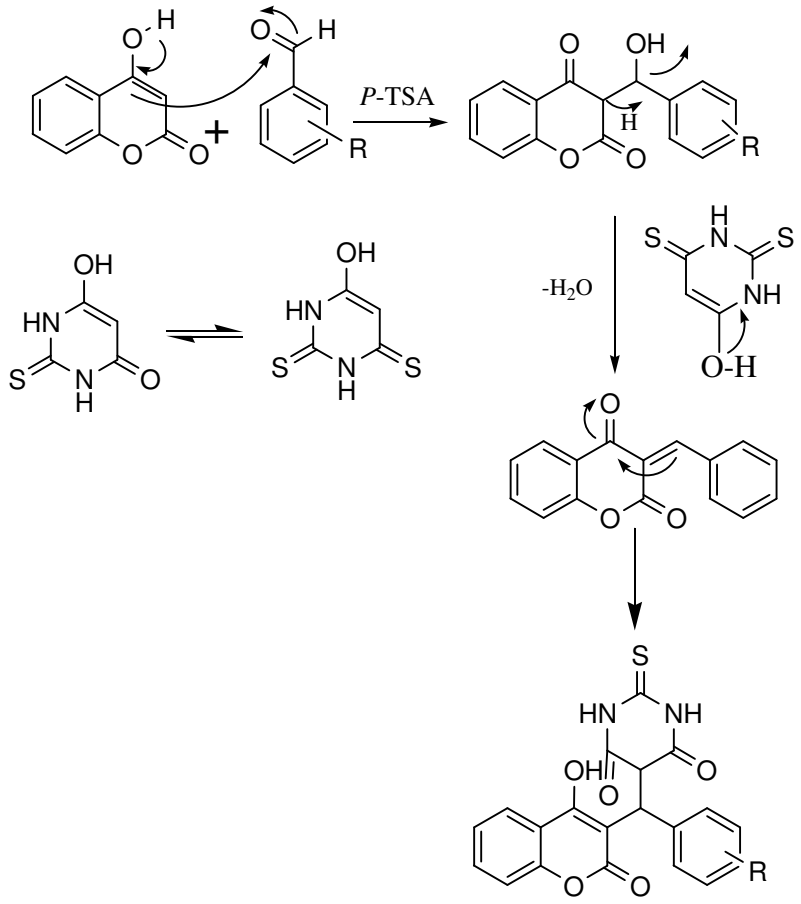
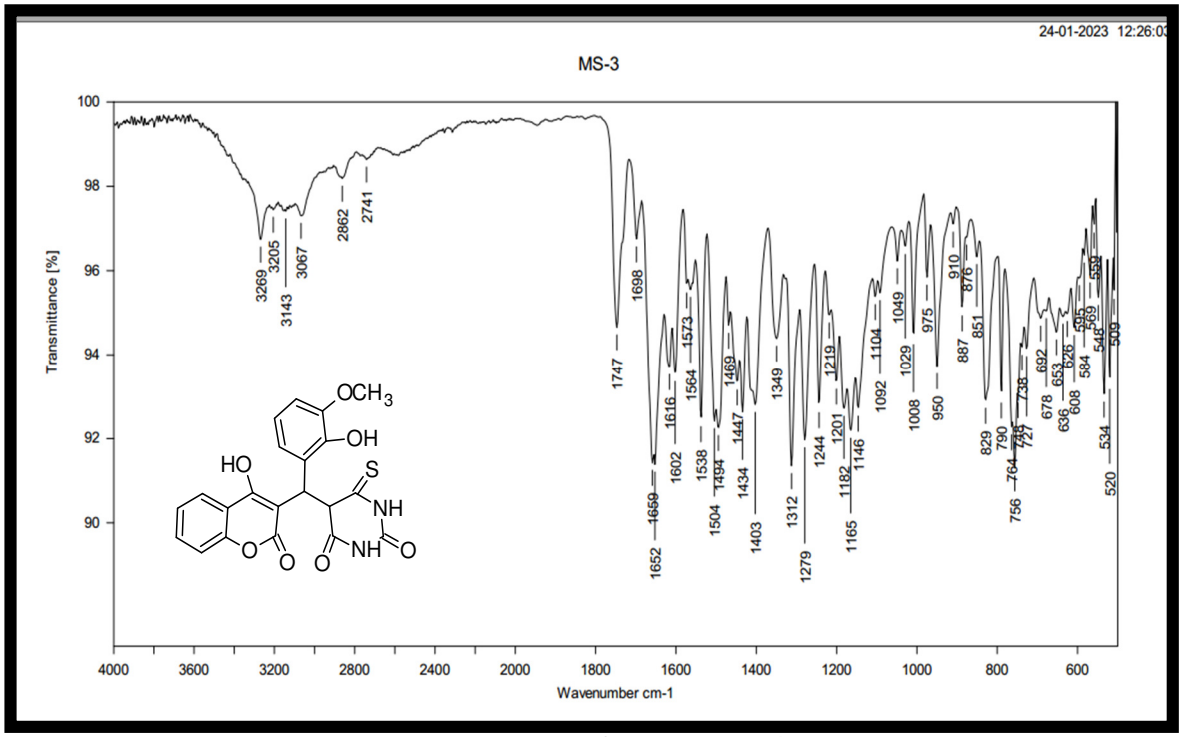


Fig 2: plausible mechanism of the formation of target compounds.

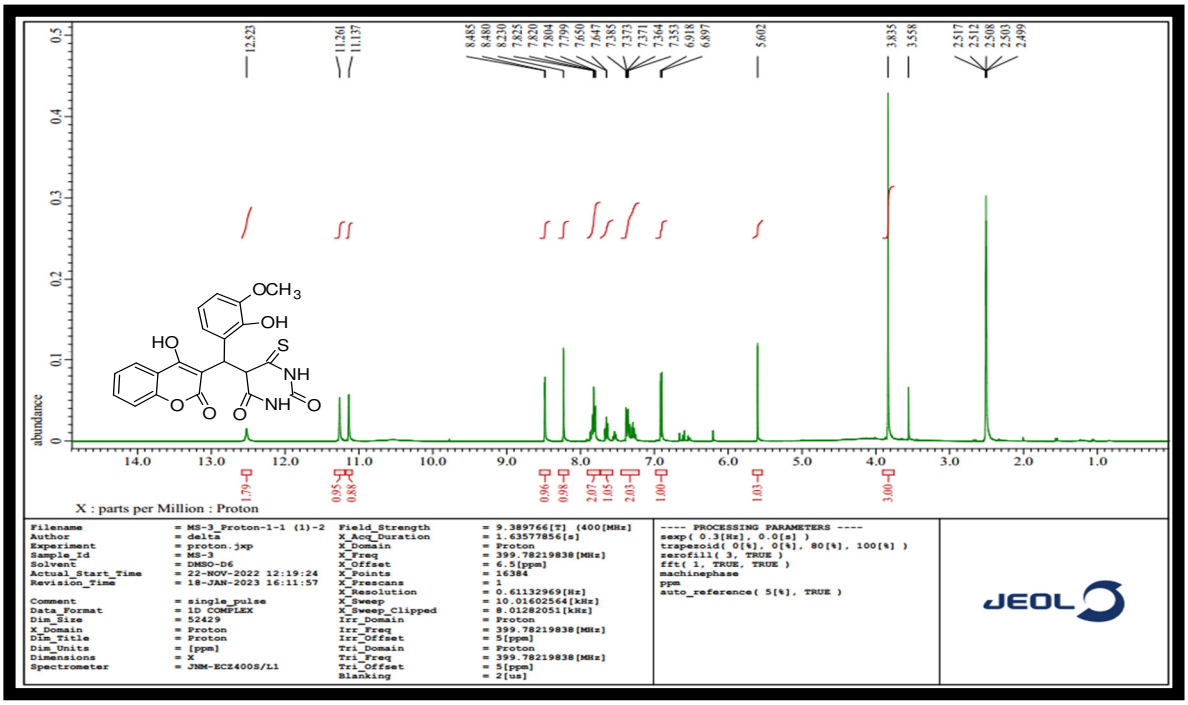
Initially, we studied the effect of a catalyst on the reaction. In the previous reports, the same reaction was carried out in the presence of different catalysts such as pyridine, silica, sodium carbonate, iodine, L-proline, hydrogen phosphate, and piperidine, and also in the absence of a catalyst. We have carried out the reaction using p-Toluene sulfonic acid (**PTSA** or pTsOH) as a catalyst to determine the progress of the reaction as well as the product yield of the **4a**

compound. By using this catalyst the result was very encouraging with a short reaction time and good yield. Consequently, to study the effect of temperature on synthesized compound **4a,** we carried out a reaction at room temperature, 50°C, and 80°C. As a result, an increase in the reaction temperature decreased the reaction time from 60 to 20 and 20 to 10 min, respectively, but the product yield was not affected by the change in temperature.

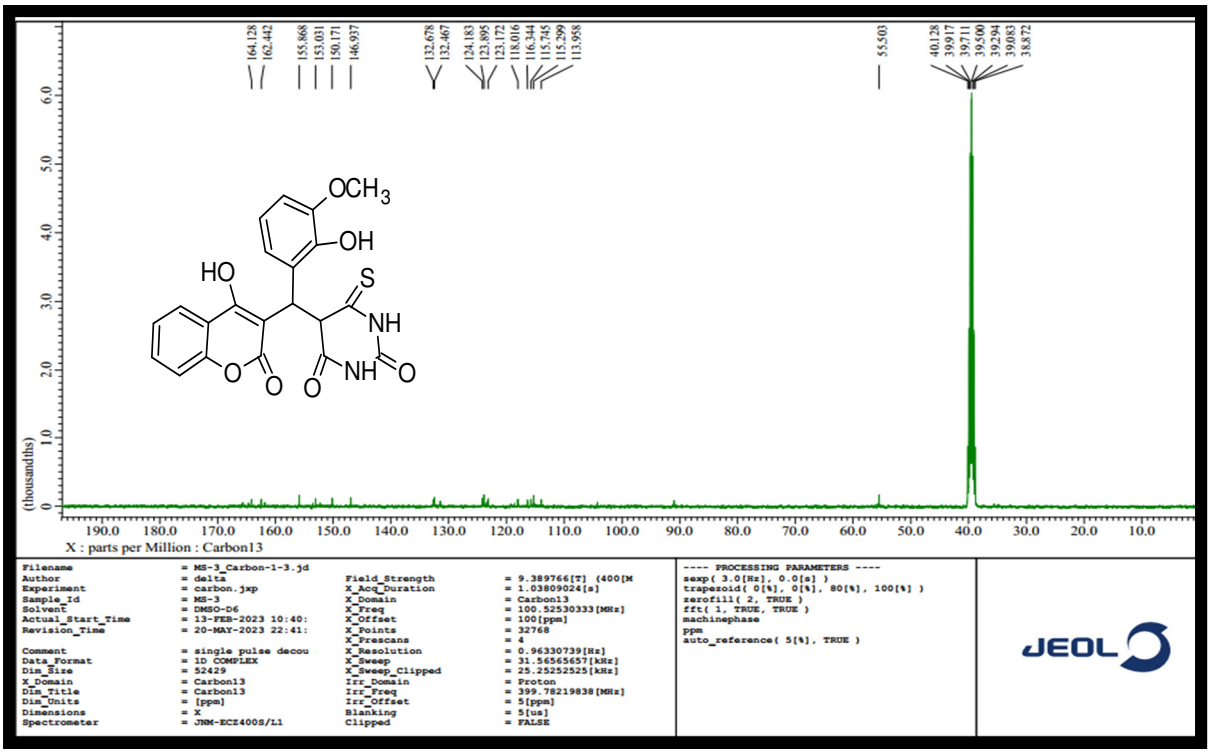
Further, the structures of the intended 5-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives **4(a-j)** were confirmed by IR, ^{1}H NMR,^{13}C NMR, and HRMS spectral data. The IR spectrum of compound **4c** showed the two peaks at 3143 and 3269 cm^{-1} which attribute to the NH stretch group respectively. A broad band at 3205 cm-1 corresponds to the hydroxyl functionality a band at 1740 cm^{-1} is stretching vibration of the carbonyl group (C=O). ^{1}H NMR spectrum, compound **4c** exhibited two singlet at δ 11.13 and 11.26 ppm, which corresponds to two NH protons of coumarin nucleus (s, 2H, NH) and another singlet at δ 12.52 ppm due to OH proton (s, 1H, CH). A signal at δ 8.48 ppm corresponds to one aromatic proton (S, 1H, Ar-H), and at δ 8.23 ppm corresponds to one aromatic proton (S, 1H, Ar-H), δ 7.82-7.79 ppm due to two aromatic proton (d,d. *J*= 12 Hz, 2H, Ar-H). δ 7.65-7.64 ppm due to one aromatic proton (d, *J*= 8 Hz, 1H, Ar-H). Multiple peaks were observed in the range of δ 7.38-7.35 ppm, which corresponds to two aromatic protons (m, 2H, Ar-H), δ 6.91-7.79 ppm, corresponds to one aromatic proton (d, *J*= 8Hz, 1H, Ar-H). Singlet peak at δ 5.60 ppm due to CH proton (s, 1H, CH). finally δ 3.8 ppm due to O-CH_3 methoxy proton (s, 1H, O-CH_3). In addition, the ^{13}C NMR spectrum of compound **4c** exhibited peaks at δ 162.44 and 164.12 ppm, which corresponds to the carbonyl carbon.The mass spectrum showed a molecular ion peak [M^+] at m/z at 441.11 which corresponds to the molecular weight of compound **4c.**



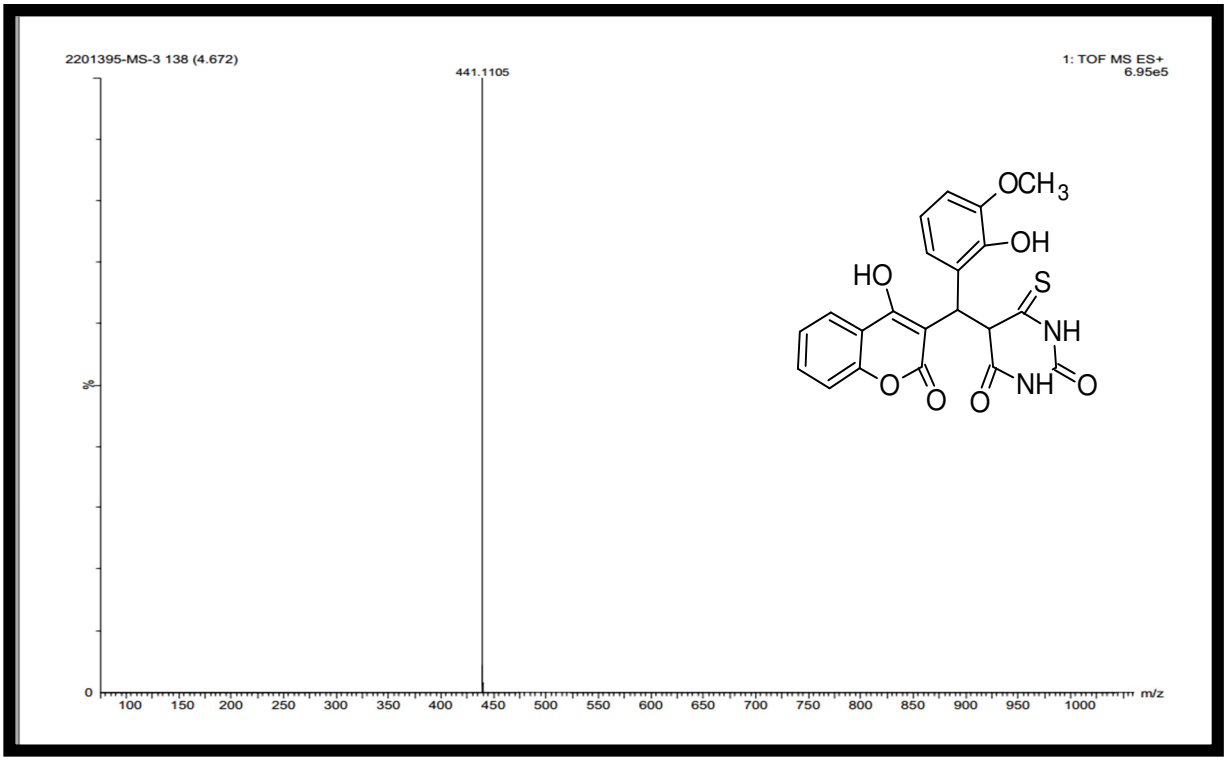
FT-IR spectrum of compound 4c



^{1}H NMR spectrum of compound 4c



^{13}C NMR spectrum of compound 4c



Mass spectrum of compound 4c

1.2.3. Biological studies

1.2.3.1. Anti-tubercular activity

The synthesized compounds were screened for their effectiveness against the H37RV strain of tuberculosis (TB). Among the compounds tested, compound **4b** displayed notable anti-TB activity, boasting a minimum inhibitory concentration (MIC) value of 25 µg/mL. This enhanced efficacy can be attributed to the presence of the nitro group within the compound. Conversely, the remaining compounds exhibited moderate activity, with MIC values falling within the range of 50-100 µg/mL when compared to standard anti-TB drugs (as shown in Table 2 and Figure 2). It was observed that the presence of an electron-withdrawing group in the para position proved highly advantageous for inhibiting the growth of Mycobacterium tuberculosis (MTB). In contrast, electron-donating groups rendered the compounds either inactive or less effective in inhibiting growth. Consequently, the growth inhibition occurred at higher concentrations in comparison to the standard drugs, owing to this specific reason **[39].**

Figure 2. Anti-tubercular activity results of the synthesized compounds (**4a-j**)

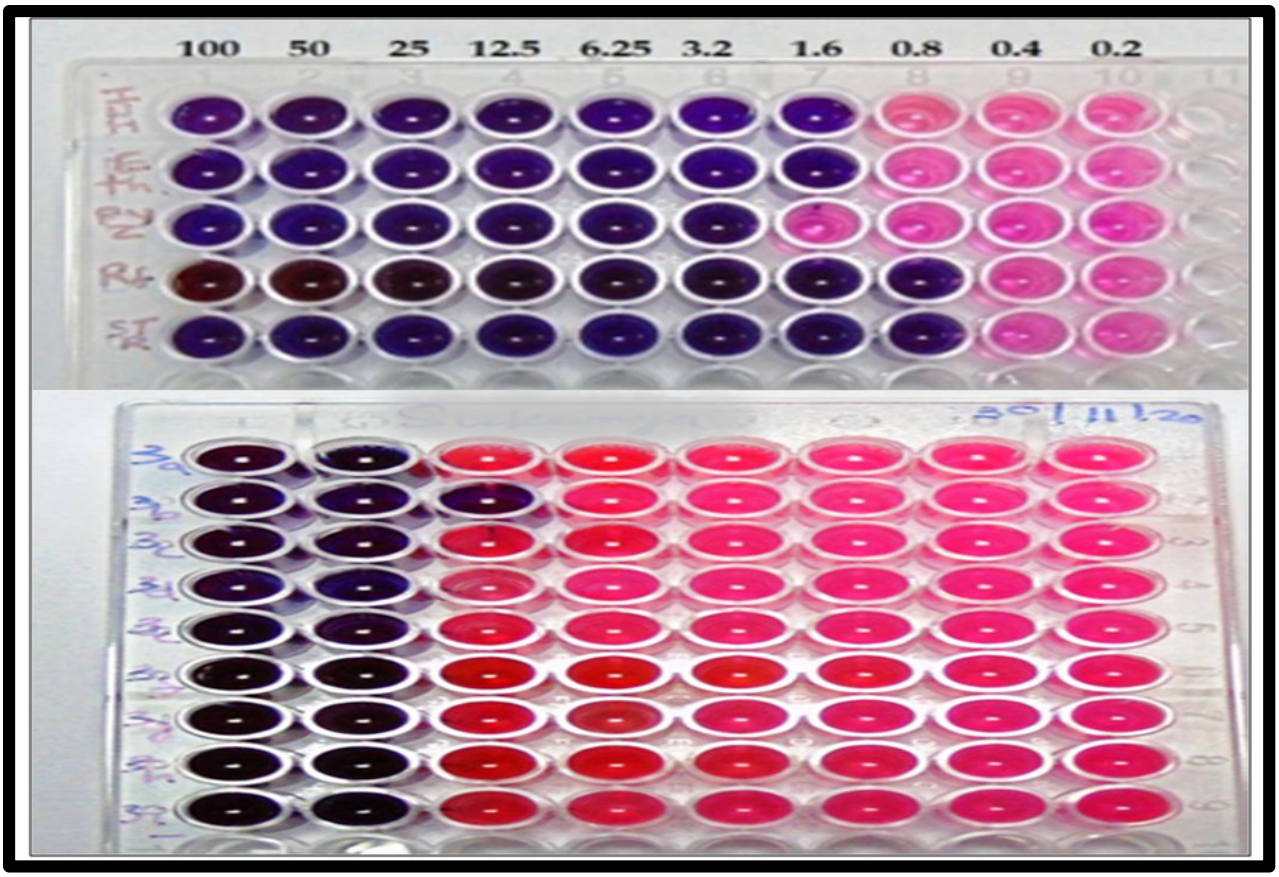


Table 2. Anti-tubercular activity results of synthesized compounds **4(a-j)**

sample	100 µg/mL	50 µg/mL	25 µg/mL	12.5 µg/mL	6.25 µg/mL	3.12 µg/mL	1.6 µg/mL	0.8 µg/mL
4a	S	S	R	R	R	R	R	R
4b	S	S	S	R	R	R	R	R
4c	S	S	R	R	R	R	R	R
4d	S	S	R	R	R	R	R	R
4e	S	S	R	R	R	R	R	R
4f	S	S	R	R	R	R	R	R
4g	S	S	R	R	R	R	R	R
4h	S	S	R	R	R	R	R	R
4i	S	S	R	R	R	R	R	R
4j	S	S	S	S	S	S	S	R
std[a]	S	S	S	S	S	S	S	R
std[b]	S	S	S	S	S	S	R	R

std[c]	S	S	S	S	S	S	S	S
std[d]	S	S	S	S	S	S	S	S

1.2.3.2. Anti-oxidant activity

The compounds synthesized **(4a-j)** underwent assessment for their ability to scavenge free radicals using the DPPH method, as depicted in Figure 3. In comparison to the standard Ascorbic acid, all these compounds exhibited varying degrees of free radical scavenging capabilities. Among the tested compounds, **4b** emerged as the most potent antioxidant, displaying an IC_{50} value of 34. 66±2. 43 µg/mL, albeit still less effective than the reference standard drug with an IC_{50} of 8. 87±1. 19 µg/mL. Compounds **4a, 4c, 4f,** and **4j** demonstrated promising antioxidant activity with IC_{50} values ranging from 41. 54 to 44. 11 µg/mL, while the remaining compounds exhibited moderate scavenging activity according to the DPPH method. This enhanced antioxidant efficacy of the synthesized compounds can be attributed to the presence of the electron-donating group (OH) located at the para position of the phenyl ring **[40].** Detailed results are provided in **Table 3.**

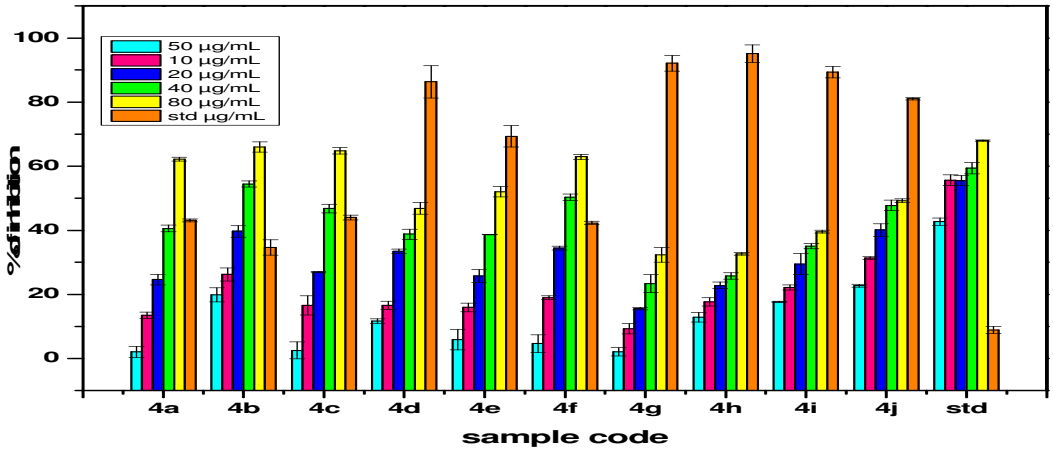


Figure 3. Anti-oxidant activity results of the synthesized compounds (**4a-j**)

Table 3. DPPH radical scavenging activity of the synthesized compounds **4(a-j)**

Compound	DPPH radical scavenging activity %of inhibition.					
	Concentration in µg /mL					
	5	10	20	40	80	IC_{50}
4a	2.054±1.71	13.47±1.00	24.63±1.62	40.59±1.05	62.20±0.60	43.11±0.50
4b	19.88±2.19	26.23±2.04	39.70±1.79	54.44±0.92	66.00±1.61	34.66±2.43
4c	2.523±2.65	16.60±2.99	26.95±0.23	46.80±1.38	64.79±1.00	44.02±0.77
4d	11.68±0.66	16.59±1.28	33.46±0.68	38.77±1.60	46.87±1.84	86.36±5.02
4e	5.913±3.22	15.91±1.37	25.75±2.00	38.64±0.04	52.05±1.61	69.33±3.37
4f	4.648±2.71	18.99±0.60	34.39±0.60	50.33±1.00	62.94±0.79	42.30±0.52
4g	2.125±1.28	9.296±1.65	15.53±0.39	23.37±2.79	32.40±2.30	92.1±12.5
4h	12.88±1.50	17.66±1.28	22.84±1.00	25.76±1.00	32.66±0.39	95.1.±2.8
4i	17.66±0.22	22.17±0.82	29.48±3.32	35.06±0.79	39.57±0.46	89.37±1.85
4j	22.76±0.46	31.34±0.46	40.09±1.96	47.80±1.59	49.23±0.60	40.02±0.34
Standard	42.76±1.15	55.64±1.61	55.51±1.61	59.36±1.73	68.01±0.22	8.87±1.19

1.2.3.3. *in silico* Molecular docking study

When docking the receptors of Human Peroxiredoxin 5 to evaluate their antioxidant activity with compounds **4(a-j),** it was observed that all these compounds formed strong bonds with specific amino acids within the receptor's active pocket. Notably, interactions occurred with amino acids such as Thr44, Gly46, Cys47, Ile119, Ile194, Gly96, Leu197, Ser94, Ala198, Arg127, and Thr147. The results of the antioxidant activity docking simulations indicated that compounds

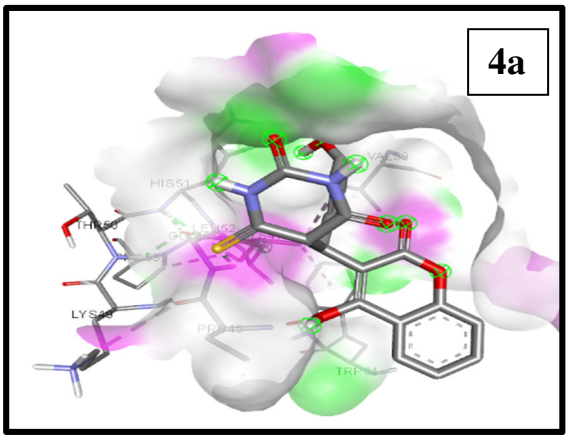
4(a-j) exhibited significant binding modes, with docking scores ranging from -7. 6 to -8. 3 kcal/mol, along with 2-3 hydrogen bonds formed, in contrast to the reference standard Pramipexole, which had a docking score of -4. 1 kcal/mol. Among these compounds, **4h** displayed the lowest binding energy of -8. 3 kcal/mol, indicating a superior binding affinity when compared to the reference standard Pramipexole (-4. 1 kcal/mol). These findings are summarized in **Table 4** and illustrated in **Figure 4.**

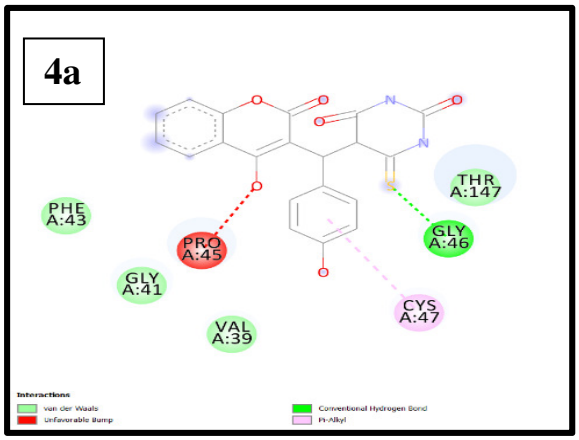
Table 4. *in silico* molecular docking results for anti-oxidant activity of synthesized compounds 4(a-j) and standard drug (Pramipexole).

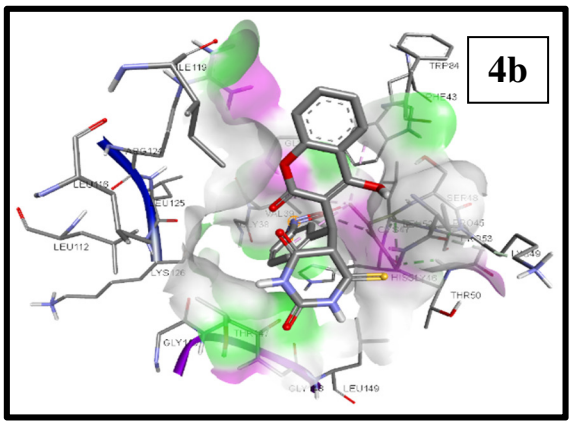
Ligand	Binding affinity (kcal/mol)	Hydrogen bond interaction	Hydrogen Bond length in Å	Electrostatic interaction	Hydrophobic and Other interactions
4a	-8.2	GLY46	2.15	-	CYS47, PRO45, THR147, HIS57, PHE43, VAL39
4b	-8.1	GLY46	2.32	-	CYS47, PRO45, THR147, HIS57, PHE43, VAL39
4c	-7.9	GLY46	2.93	-	HIS57, PHE43, VAL39, CYS47, PRO45, THR147
4d	-8.2	CYS47	2.35, 2.13	-	PRO40, THR44, PRO45, CYS47, PHE120, ILE119, THR50, LEU153, HIS51
4e	-7.6	CYS47	2.25	ARG127	LEU149, PRO40, PRO45, PHE120, GLY46

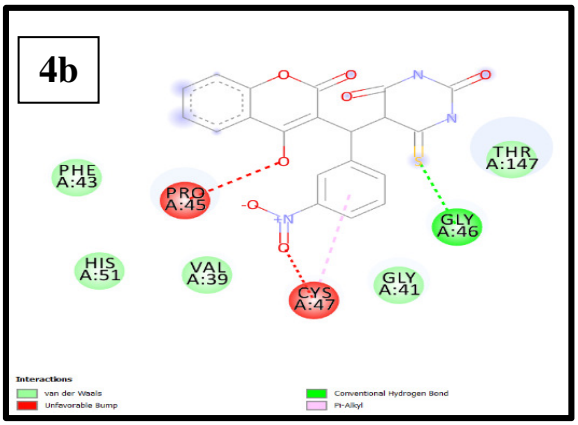
4f	-7.9	THR44, THR147	2.12, 2.98	-	PRO40, CYS47, VAL39, GLY148, PHE120, GLY46
4g	-8.0	PRO45, THR147, GLY46	2.69, 2.54, 2.17	-	CYS47, THR44, GLY148, THR150, LYS49, ILE119
4h	-7.9	PRO45, THR147, GLY46	2.88, 2.36, 2.58	-	CYS47, THR44, GLY148, THR150, LYS49, ILE119
4i	-8.3	GLY46	2.71, 2.99	-	VAL39, PRO45, PRO40, GLY41, SER48, GLY148
4j	-8.1	GLY46	2.99, 2.84	ARG127	VAL39, PRO45, PRO40, GLY41, SER48, GLY148
Pramipexole	-4.1	THR147, ARG127, GLY46	2.55, 6.08, 3.09	-	PHE120, PRO45, GLY148, LEU149

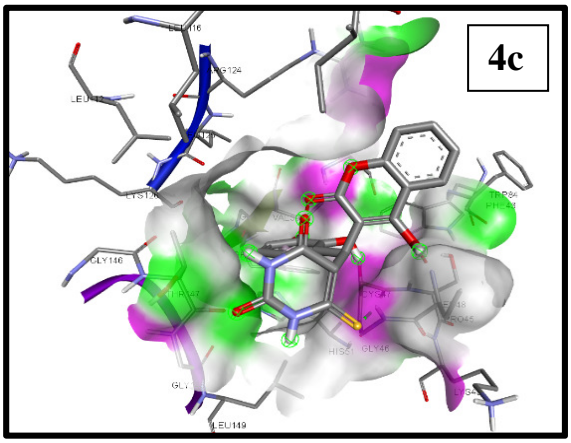
Protein Name: Crystal structure of Human peroxiredoxin 5 (PDB ID: 1OC3)

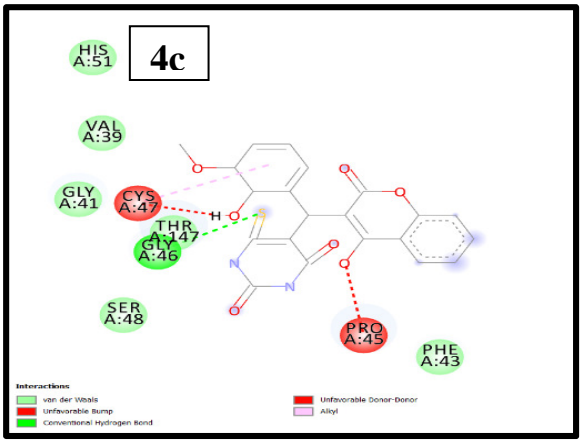

4a

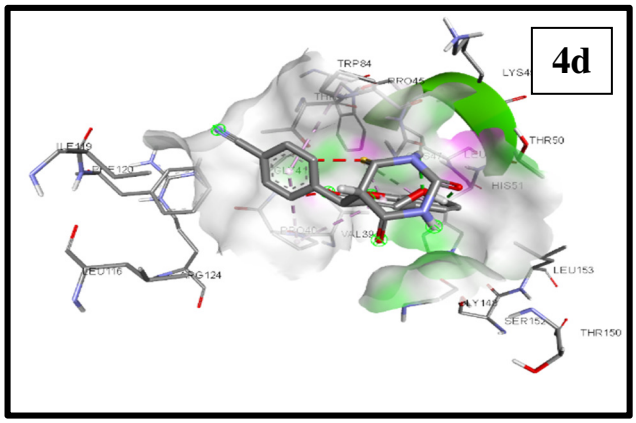

4a
PHE A:43
PRO A:45
GLY A:41
VAL A:39
CYS A:47
GLY A:46
THR A:147
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Pi-Alkyl

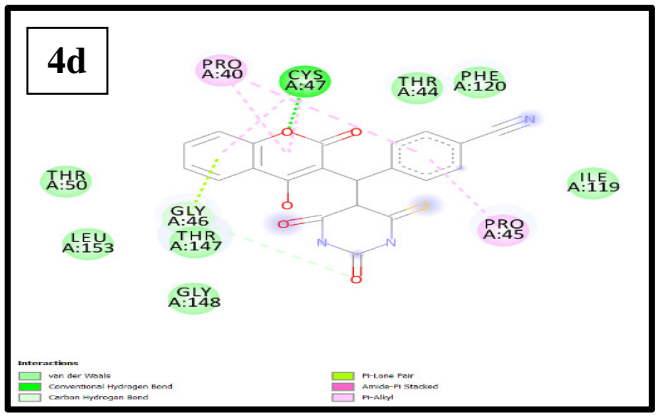

4b

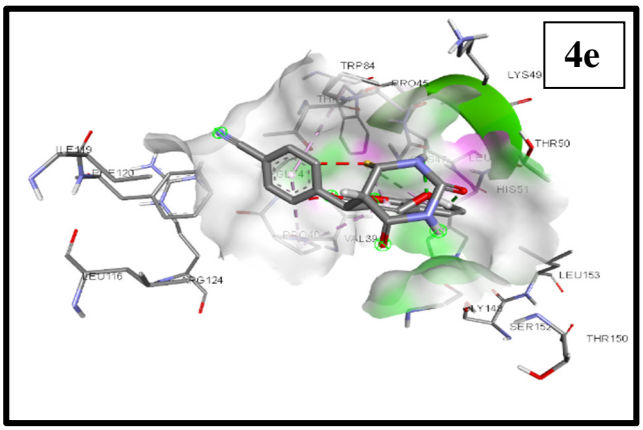

4b
PHE A:43
PRO A:45
HIS A:51
VAL A:39
CYS A:47
GLY A:41
GLY A:46
THR A:147
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Pi-Alkyl

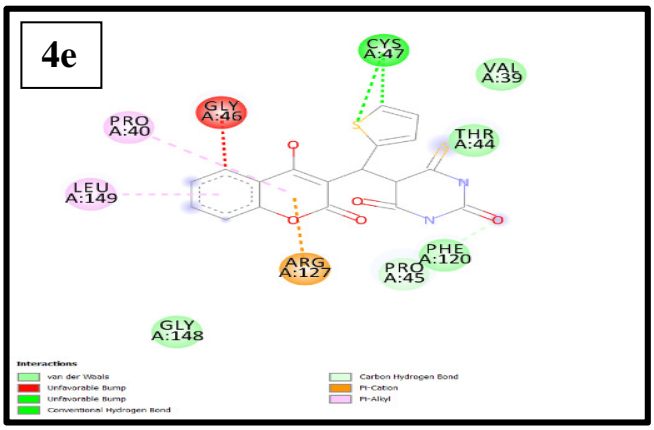

4c

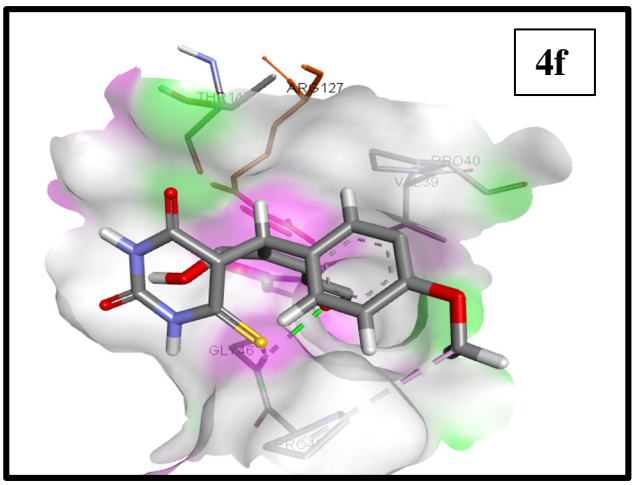

4c
HIS A:51
VAL A:39
GLY A:41
CYS A:47
THR A:147
GLY A:46
SER A:48
PRO A:45
PHE A:43
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Unfavorable Donor-Donor
Alkyl

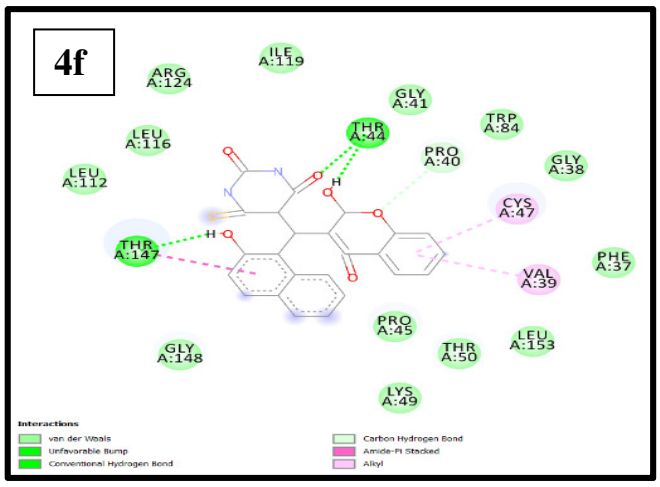

4d
TRP84
LYS49
THR50
HIS51
LEU153
THR150

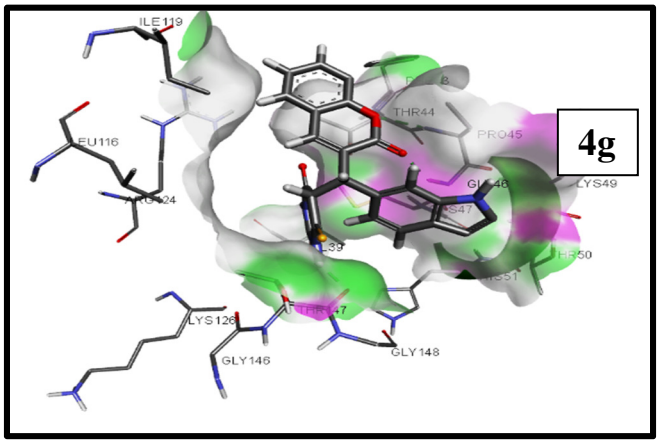

4d
PRO A:40
CYS A:47
THR A:44
PHE A:120
THR A:50
ILE A:119
GLY A:46
LEU A:153
THR A:147
PRO A:45
GLY A:148
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Lone Pair
Amide-Pi Stacked
Pi-Alkyl

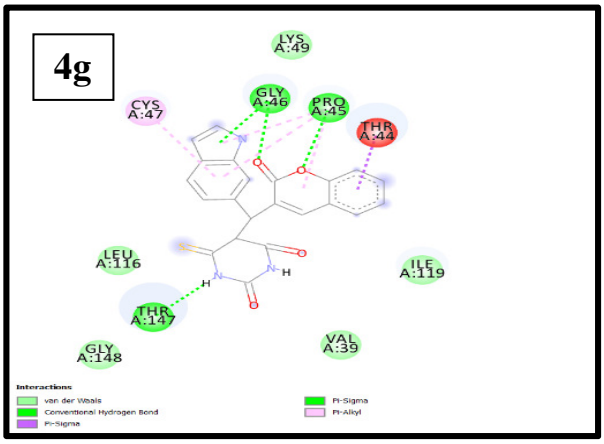

4e
TRP84
LYS49
THR50
HIS51
LEU153
THR150

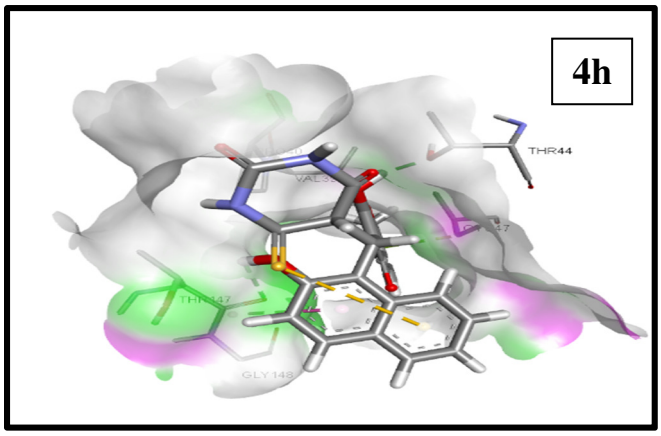

4e
CYS A:47
VAL A:39
GLY A:46
PRO A:40
THR A:44
LEU A:149
ARG A:127
PHE A:120
PRO A:45
GLY A:148
Interactions
van der Waals
Unfavorable Bump
Unfavorable Bump
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Cation
Pi-Alkyl

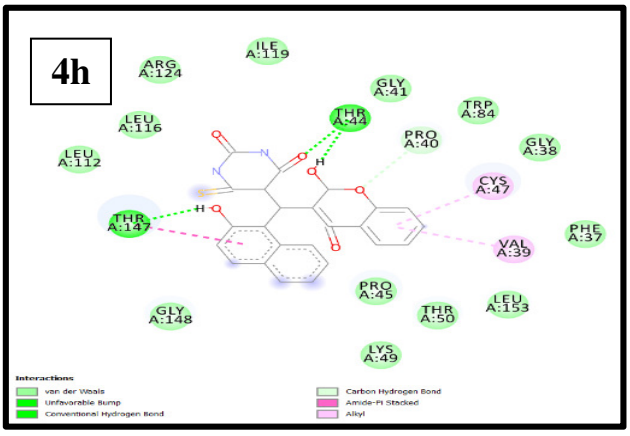

4f
ARG127
PRO40
GLY46

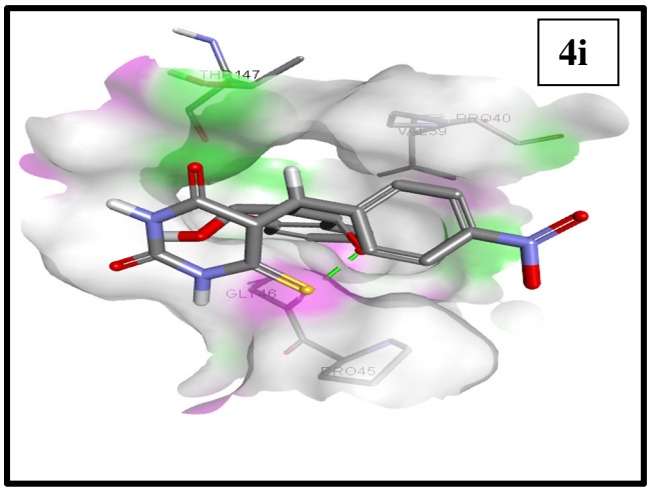

4f
ILE A:119
ARG A:124
GLY A:41
TRP A:84
LEU A:116
THR A:44
PRO A:40
GLY A:38
LEU A:112
CYS A:47
THR A:147
PHE A:37
VAL A:39
PRO A:45
LEU A:153
GLY A:148
THR A:50
LYS A:49
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Amide-Pi Stacked
Alkyl

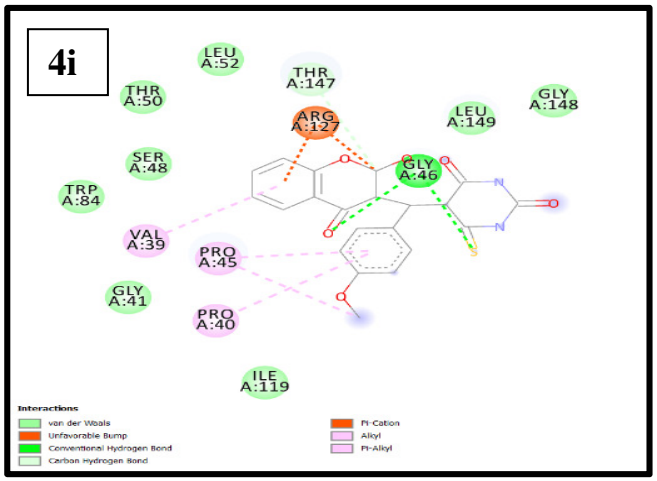

4g
ILE119
THR44
PRO45
LYS49
LYS126
GLY146
GLY148


4g
LYS A:49
CYS A:47
GLY A:46
PRO A:45
THR A:44
LEU A:116
ILE A:119
THR A:147
VAL A:39
GLY A:148
Interactions
van der Waals
Conventional Hydrogen Bond
Pi-Sigma
Pi-Alkyl


4h
THR44
GLY148


4h
ILE A:119
ARG A:124
GLY A:41
TRP A:84
LEU A:116
THR A:44
PRO A:40
GLY A:38
LEU A:112
CYS A:47
THR A:147
PHE A:37
VAL A:39
PRO A:45
GLY A:148
THR A:50
LEU A:153
LYS A:49
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Amide-Pi Stacked
Alkyl


4i
THR147
PRO40
PRO45


4i
LEU A:52
THR A:147
THR A:50
GLY A:148
ARG A:127
LEU A:149
SER A:48
GLY A:46
TRP A:84
VAL A:39
PRO A:45
GLY A:41
PRO A:40
ILE A:119
Interactions
van der Waals
Unfavorable Bump
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Pi-Cation
Alkyl
Pi-Alkyl

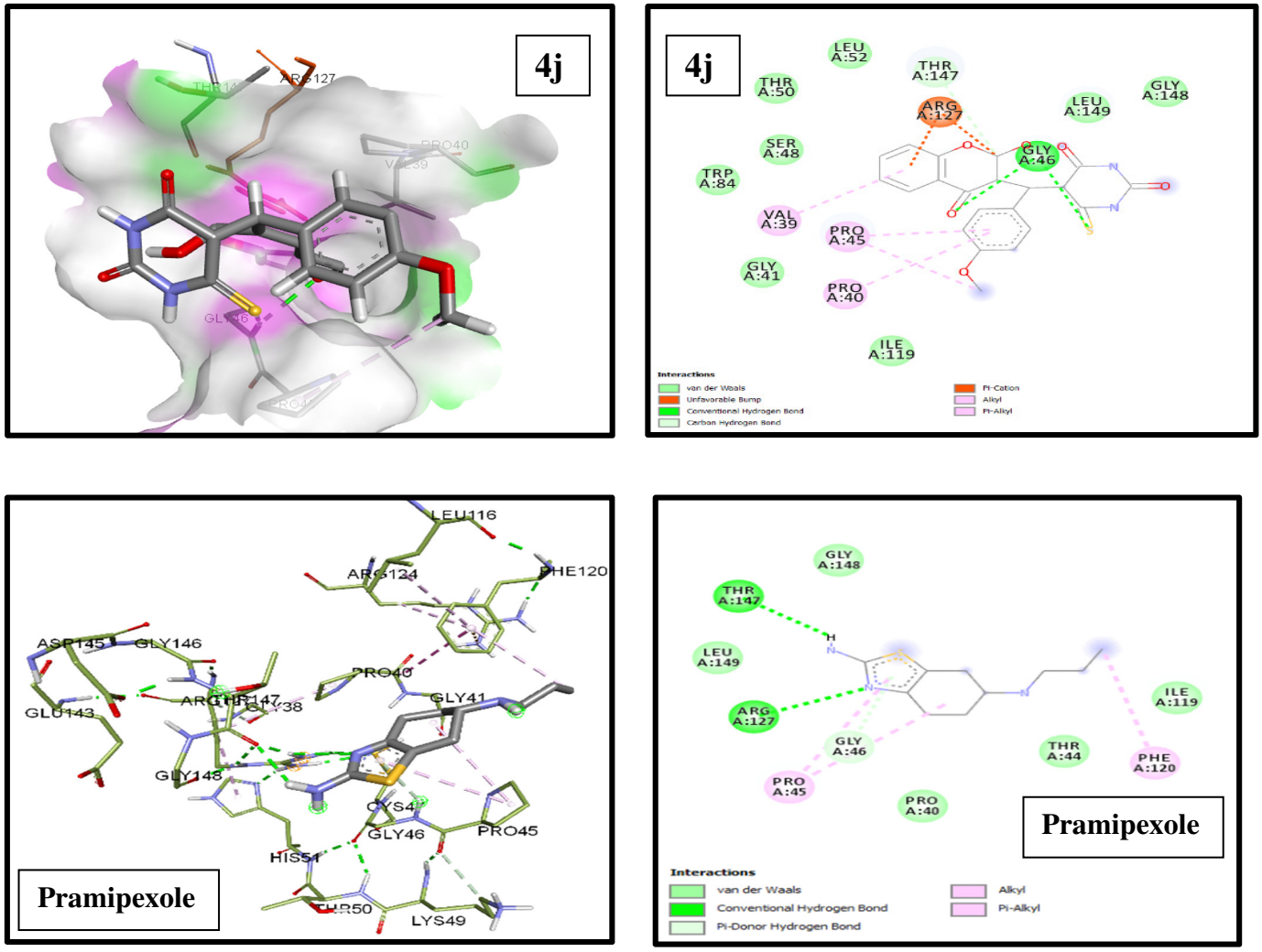


Fig 4. 2D and 3D interaction of synthesized compounds 4(a-j) and reference standard (Pramipexole) with Human peroxiredoxin 5.

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.

1.3.2. General Procedure for synthesis of 5-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives 4(a-j):

An equimolar mixture of 4-hydroxy coumarin (**1**, 1 mmol), substituted aromatic aldehydes **(2,** 1 mmol), and thiobarbituric acid (**3**, 1 mmol) was placed in a 100 mL round-bottom flask. To this mixture, 20 mL of ethanol and 5 mL of distilled water were added. As a positive catalyst, 8 mmol of p-Toluenesulfonic acid was introduced. The reaction mixture was subjected to reflux for approximately 6-8 hours. The completion of the reaction was monitored by thin-layer chromatography (TLC) using an ethyl acetate:petroleum ether in ratio of 1:4. Upon completion of the reaction, the solid compound that formed was isolated by filtration, thoroughly washed, and subsequently recrystallized from absolute ethanol to obtain pure compounds. .

Dihydro-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(4-hydroxyphenyl)methyl)-6-thioxopyrimidine-2,4(1H,3H)-dione (4a)

Yellowish, solid, yield 81%, MP 189-190 ^{0}C; FTIR (KBr υ cm^{-1}): 1218 (C=S), 1694 (C=O), 1716 (C=O), 3188 (NH), 3268 (NH); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 5.60 (s, 1H, CH), 6.60 (s, IH, Ar-H), 6.87-6.90 (d, *J*=8H$_Z$, 2H, Ar-H), 7.35-7.36 (d,*J*=4H$_Z$, 1H, Ar-H), 7.38-7.35 (t,*J*=12Hz, Ar-H, 1H), 7.65 (s, 1H, Ar-H), 7.84-7.82 (d.d,*J*=8H$_Z$, 2H, Ar-H), 8.22 (s, 1H, Ar-H), 8.34-8.3290 (d, *J*=8H$_Z$, 2H, Ar-H), 10.77 (s, 1H, OH), 11.12 (s, 1H, NH), 11.25 (s,1H, NH), 12.51 (s, 1H, alde OH); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ ppm):91.01, 114.20, 115.51, 115.80, 116.36, 123.20, 123.80, 123.91, 132.69, 138.29, 150.22, 153.52, 155.52, 161.89, 162.29, 163.02, 164.11, 165.63; LCMS: m/z 411.30[M^{+}]. Anal.Calcd.for $C_{20}H_{14}N_2O_6S$: C, 58.53 %; H, 3.44%; N, 6.83%. O, 23.39, S, 7.81. Found: C, 587.02 %; H, 4.66%; N, 5.56%.

Dihydro-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(3-nitrophenyl)methyl)-6-thioxopyrimidine-2,4(1H,3H)-dione (4b)

Light orange, yield 78%, MP 185-187 ^{0}C; FTIR (KBr υ cm^{-1}): 1218 (C=S), 1694 (C=O), 1716 (C=O), 3188 (NH), 3268 (NH); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 6.22 (s, 1H, Ar-H), 7.32-7.30 (d, IH, Ar-H), 7.54-7.48 (M, 4H, Ar-H), 7.87-7.79 (M,4H, Ar-H), 10.02 (s,2H,OH), 10.15 (s, 1H,NH); ^{13}C-NMR (100 MHz, DMSO-d$_6$, δ ppm):115.58, 121.06, 123.96, 133.78; LCMS: m/z 439.05[M^{+}]. Anal.Calcd.for $C_{20}H_{13}N_3O_7S$: C, 54.67 %; H, 2.98%; N, 9.56%. O, 25.49, S, 7.30. Found: C, 53.02 %; H, 1.66%; N, 8.56%.

Dihydro-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(2-hydroxyphenyl)methyl)-6-thioxopyrimidine-2,4(1H,3H)-dione (4c)

Pale yellow solid, yield 82%, MP 193-194 ^{0}C; FTIR (KBr υ cm^{-1}): 1218 (C=S), 1740 (C=O),2862 (OCH_3), 3143 (NH), 3205 (OH), 3269 (NH); ^{1}H NMR (400 MHz, DMSO-d$_6$, δ ppm): 3.8 (s, 3H, OCH_3), 5.60 (s, IH, CH), 6.91-6.89 (d, *J*=8H$_Z$, 1H, Ar-H), 7.38-7.35 (M,2H, Ar-H), 7.65-7.64 (d,*J*=8Hz, Ar-H, 1H), 7.82-7.79 (d, *J*=8H$_Z$, 2H, Ar-H), 8.23 (s, 1H, Ar-H),8.48 (s, 1H, Ar-H), 11.26 (s, 1H, NH), 11.13 (s, 1H, NH), 12.52 (s, 1H, OH); ^{13}C-NMR (100 MHz,

DMSO-d_6, δ ppm):55.50, 113.95, 115.29, 115.74, 116.34, 118.01, 123.17, 123.89, 124.18, 132.46, 132.67, 146.93, 150.17, 153.03, 155.86, 162.44, 164.12; LCMS: m/z 441.11[M^+]. Anal.Calcd.for $C_{21}H_{16}N_2O_8$: C, 57.27 %; H, 3.66 %; N, 6.36 %. O, 25.43, S, 7.28. Found: C, 56.02 %; H, 4.66%; N, 5.56%.

4-(hexahydro-2,4-dioxo-6-thioxopyrimidin-5-yl)(4-hydroxy-2-oxo-2H-chromen-3-yl)methyl)benzonitrile (4d).

creamy white solid, yield 79%, MP 198-200^{0}C; FTIR (KBr υ cm^{-1}): 1221 (C=S), 1732 (C=O), 2228(C=N); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.33 (s, 1H, Ar-H), 7.36-7.26 (M, 5H, Ar-H), 7.68-7.64 (t, 2H, Ar-H), 7.84-7.83 (d,2H, Ar-H), 10.60 (s, 1H, OH), 11.34 (s, 1H,NH), 11.29(s, 1H, NH);^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm):102.92, 115.68, 119.09, 123.22, 123.77, 124.07, 127.80, 131.42, 131.72, 131.82, 152.45, 164.41, 167.09; LCMS: m/z 419.05[M^+]. Anal.Calcd.for $C_{21}H_{13}N_3O_5S$: C, 60.14 %; H, 3.12%; N, 10.02%. O, 19.07, S, 7.65. Found: C, 59.02 %; H, 4.66%; N, 8.56%.

Dihydro-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(thiophen-2-yl)methyl)-6-thioxopyrimidine-2,4(1H,3H-dione (4e)

white solid, yield 81%, MP 196-197 ^{0}C; FTIR (KBr υ cm^{-1}): 1218 (C=S), 1740 (C=O), 2831(CH), 3079 (NH), 3269 (NH); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.33 (s, 1H, Ar-H), 7.36-7.26 (M, 5H, Ar-H), 7.68-7.64 (t, 2H, Ar-H), 7.84-7.83 (d,2H, Ar-H), 10.60 (s, 1H, OH), 11.34 (s, 1H, NH), 11.29 (s, 1H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm):131.28, 140.43, 150.19; LCMS: m/z 400.02[M^+]. Anal.Calcd.for $C_{18}H_{14}N_2O_5S_2$: C, 53.99 %; H, 3.02%; N, 7.00 %. O, 19.98, S, 16.02. Found: C, 54.02 %; H, 4.66%; N, 8.56%.

Dihydro-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(2-hydroxynapthalen-1-yl)methyl)-6-thioxopyrimine-2,4(1H,3H)-dione (4f)

yellow solid, yield 91%, MP 195-196 ^{0}C; FTIR (KBr υ cm^{-1}): 1716 (C=O), 1242(C=S), 1676(C=O), 3086 (OH), 3169 (OH); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 6.04 (s, 1H, Ar-H), 7.34-7.31 (s, IH, Ar-H), 7.56-7.41 (M, 6H, Ar-H), 7.93-7.90 (q,2H, Ar-H), 11.1(s, NH, 1H), 7.93-7.90 (q, 2H, Ar-H), 11.1(s, 1H, NH),10.83 (s, 1H, NH), 12.05 (s, 1H, OH), 12.02 (s, 1H, OH), and ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm):115.99, 116.43, 123.91, 124.93, 127.19, 128.66, 129.10, 130.82, 132.06, 149.53, 152.08; LCMS: m/z 460.10[M^{+}]. Anal.Calcd.for$C_{24}H_{16}N_2O_6S$: C, 62.60 %; H, 3.50 %; N, 6.08%. O, 20.85 S, 7.61. Found: C, 63.02 %; H, 4.66%; N, 5.56%.

Dihydro-5-((4-hydroxy-2-oxo-2H-chromen-3-yl)(1H-indol-4-yl)methyl)-6-thioxopyrimidine-2,4(1H,3H)-dione (4g).

Pale reddish solid, yield 79%, MP 186-187 ^{0}C; FTIR (KBr υ cm^{-1}): 1357(C=S), 1680 (C=O), 3156 (NH), 3424(OH); ^{1}H NMR (400 MHz, DMSO-d_6, δ ppm): 5.58 (s, 1H, CH), 7.34-7.32 (q, 4H, Ar-H), 7.61-7.59 (d, *J*=8Hz, 2H, Ar-H), 7.89-7.87 (t,2H, Ar-H), 8.72(s, 1H, Ar-H,), 9.58-9.57 (d, *J*=8Hz, 1H, Ar-H), 12.21(s, 1H, NH),12.91 (s, 1H, OH), 12.16 (s, 2H, NH); ^{13}C-NMR (100 MHz, DMSO-d_6, δ ppm):91.00, 108.73, 112.44, 113.38, 116.36, 117.87, 123.11, 123.20, 123.92, 124.06, 129.07, 132.70, 136.64, 141.10, 144.61, 153.52, 160.99, 161.92, 162.83, 165.65, 177.72; LCMS: m/z 433.43[M^{+}]. Anal.Calcd.for $C_{22}H_{15}N_3O_5S$: C, 60.96 %; H, 3.49 %; N, 9.69%. O, 18.46, S, 7.40. Found: C, 56.02 %; H, 4.66%; N, 5.56%**.**

1.4.3. Biological studies

1.4.1. Tuberculosis Inhibitory Activity

The anti-tubercular activity was assessed by Microplate Alamar Blue assay (MABA) method. against M. tuberculosis (H37RV strain**) [40].** This methodology is non-toxic, uses thermally

stable reagents, and shows a good correlation with proportional and BACTEC radiometric methods. Briefly, 200µL of sterile deionized water was added to the outer perimeter wells of the sterile 96 well plates to minimize evaporation of medium in the test wells during incubation. The 96 well plates received 100 µL of the Middle brook 7H9 broth and serial dilution of compounds was made directly on a plate **[41].** The final drug concentrations tested were 0.2 to 100 µg/mL and plates were covered and sealed with Parafilm and incubated at 37ºC for five days. After this time, 25µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10% between 80 was added to the plate and incubated for 24 h. Compounds at eight different concentrations (0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 & 100 µg/mL) were used for the analysis. Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, and Streptomycin were used as standard drugs for comparison.The blue color in the well was interpreted as no bacterial growth, and the pink color was scored as growth. The MIC was defined as the lowest drug concentration which prevented the color change from blue to pink.

1.4.2. Antioxidant Activity

The synthesized compounds (**4a-j**) were screened for DPPH scavenging activity, and followed according to the procedure M.N. Joy, *et al*., in this DPPH method was carried out according to the reported procedure [**42**]. Compounds at different concentrations (5µg/mL, 10 µg/mL, 20 µg/mL, 40 µg/mL, 80 µg/mL) were used for analysis. Ascorbic acid was chosen for comparison as a standard drug and Radical scavenging activities were calculated using the formula :

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance of the synthesized compound. IC_{50} value was calculated using the formula: $IC_{50} = [(C/\Sigma I) \times 50]$, where ΣC is the sum of synthesized compound concentrations used to test and ΣI is the sum of % of inhibition at different concentrations. Each value is expressed as mean ± SD of three replicates.

1.4.3. *in Silico* Molecular Docking Study

in silico molecular docking was 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, employing 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 **[43].** These compounds served as ligands, while the docking receptors were represented by the Human peroxiredoxin 5 with PDB ID: 1OC3.

Preparation of Ligands:

The ligands were designed 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 **[44,45].**

Preparation of the Receptor:

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, hydrogen atoms were added, non-polar hydrogens were merged, charges were assigned, and the protein was energy-minimized using the AMBER force field through the USCF Chimera tool. The prepared proteins were then converted into PDB format **[46.47].**

Binding Site Identification:

A binding pocket around the active site CYS47 amino acid residue was defined based on the co-crystal ligand, facilitating target selection.

Docking and Visualization:

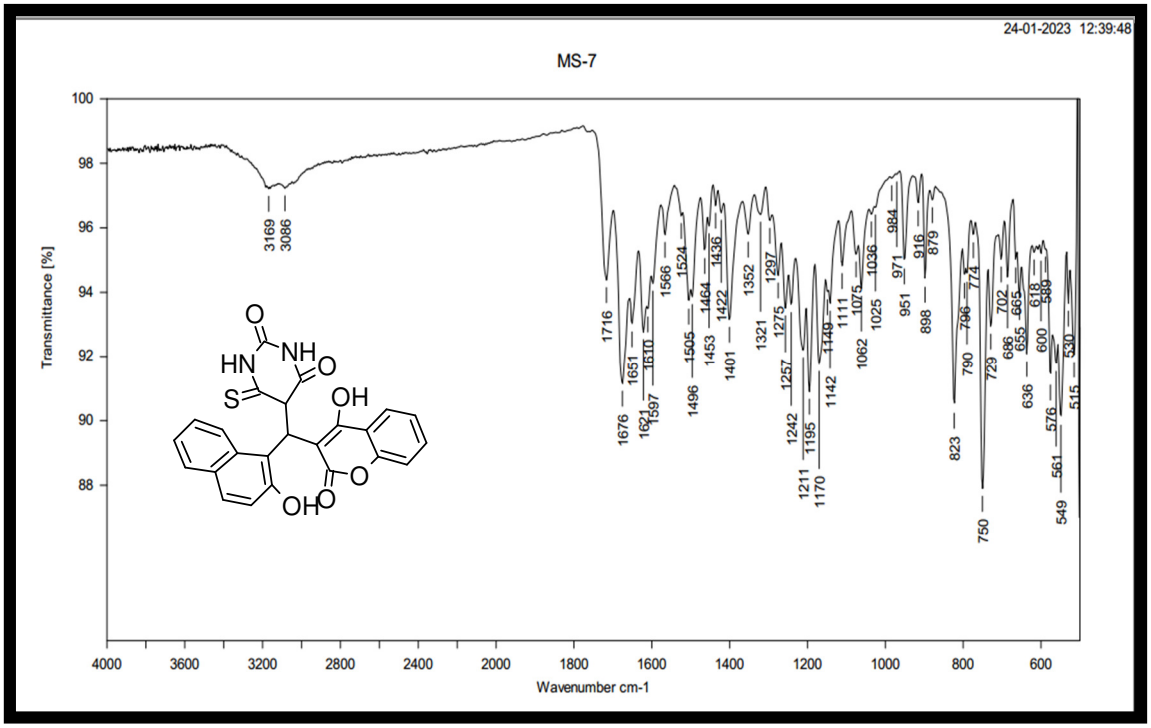
Grid boxes were set around the active sites of the proteins, and the designed ligands were docked against the receptor using Autodock Vina within the PyRX work station. The best-docked ligand and target conformation were determined based on the ligand with the lowest binding affinity. The docked protein and target were converted into PDB format using Schrödinger PyMol, and interactions were visualized using Biovia Discovery Studios **[48-53].**

1.5. Conclusion

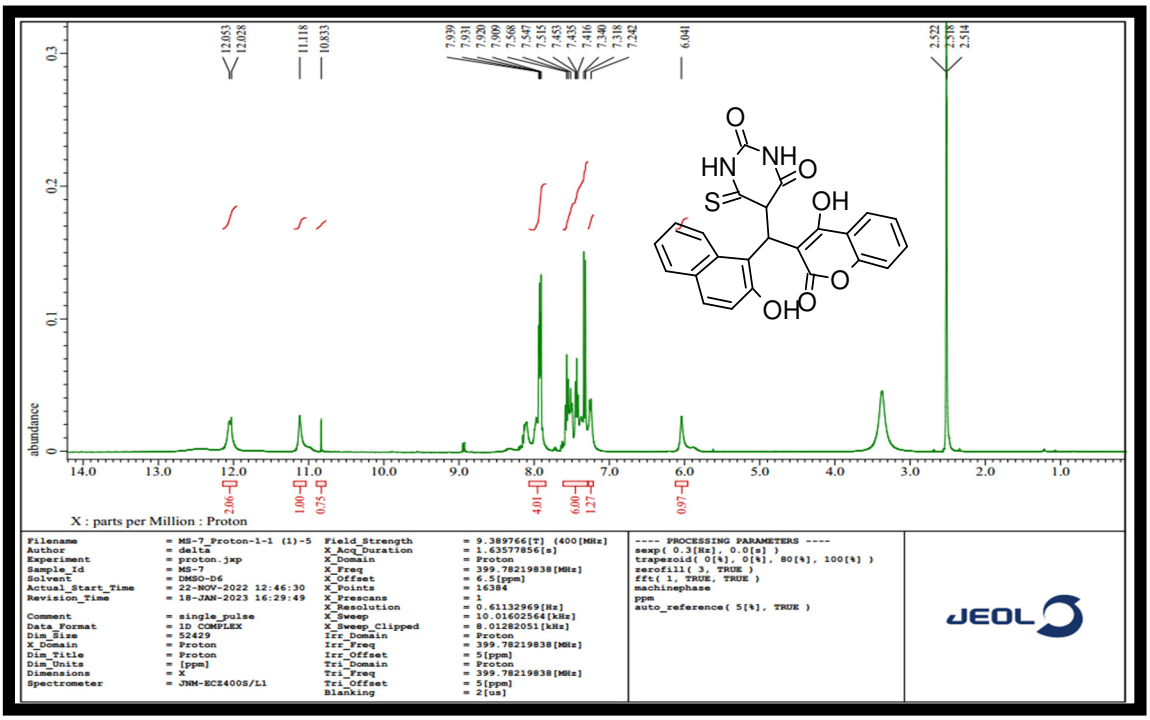
In this chapter, we developed a simple and efficient method for the synthesis of 5-[(4-hydroxy-2-oxo-2*H*-1-benzopyran-3-yl)(phenyl)methyl]-6-sulfanylidene-1,3-diazinane-2,4-dione derivatives **4(a-j)** as a potent anti-oxidant and anti-TB agents. It is a facile synthetic approach for simple steps, mild reaction conditions, essay work-up procedure, shorter reaction time, and excellent yield. Also, activity results suggested that **4b** exhibited the most effective anti-oxidant efficacy with an IC_{50} value of 34.66±2.43 µg/mL as compared to the reference standard drug

(IC_{50} 8.87±1.19 µg/mL). The compounds **4a**, **4c**, **4f,** and **4j** show an IC_{50} value range of 41.54-44.11 µg/mL have showed promising anti-oxidant activity and the rest of the compounds showed moderate scavenging activity by the DPPH method. Anti-TB activity results suggested that compound **4b** exhibited more potent efficacy with a MIC value of 25 µg/mL. Moreover, docking results for the anti-oxidant activity of synthesized compounds revealed that compound **4i** showed the least binding energy of -8.3 kcal/mol.Obtained results are compared to dock scores with the reference standards Pramipexole (-4.1 kcal/mol) could act as a better binder than target molecules respectively. Therefore we conclude that these compounds are more potent anti-oxidant and anti-TB agents.

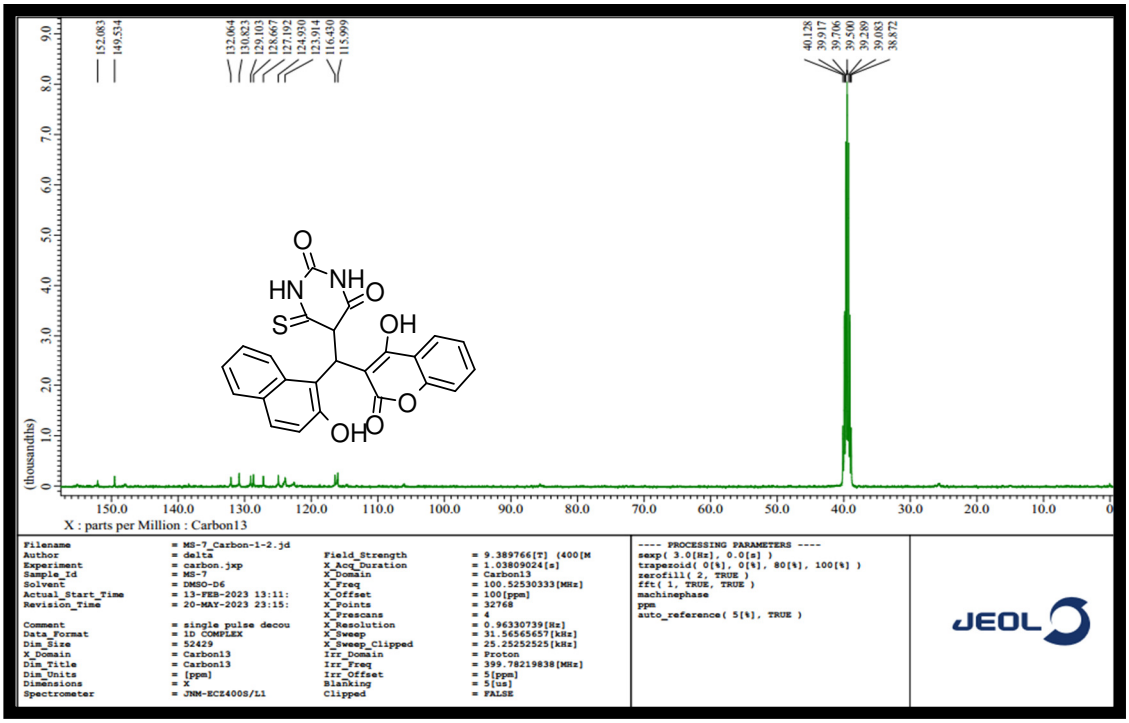
1.6. spectra of selected compounds:



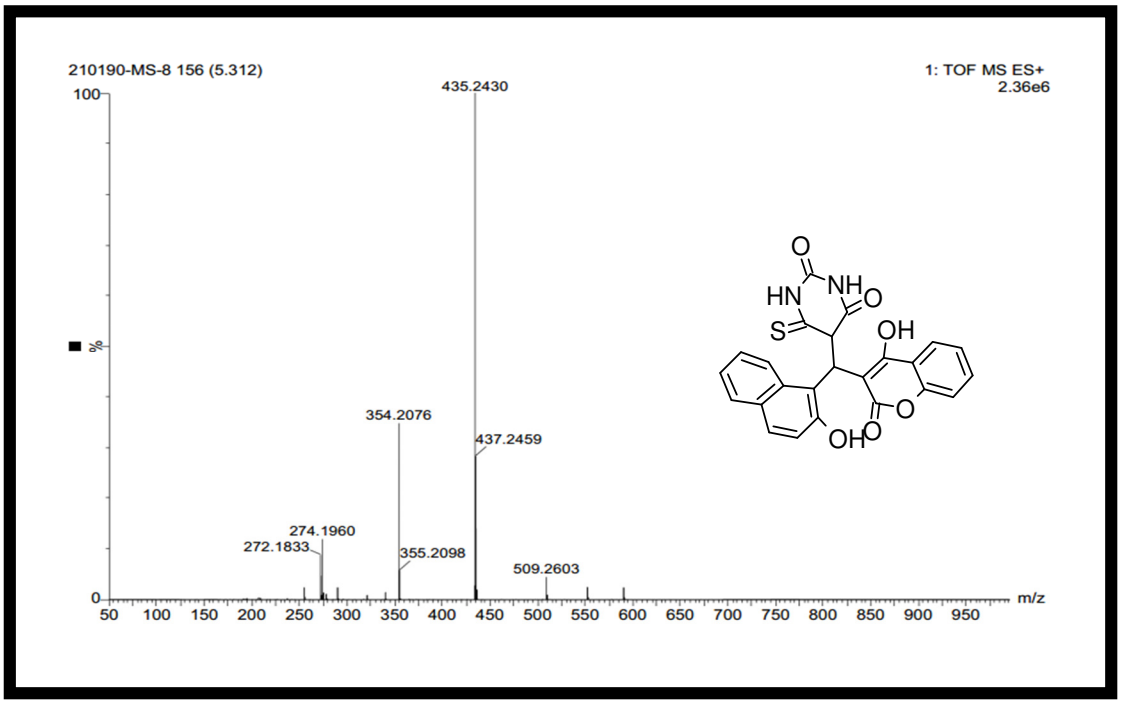
IR spectrum of compound 4f



^{1}H NMR spectrum of compound 4f



^{13}C NMR spectrum of compound 4f



Mass spectrum of compound 4f

1.7. References

1. M.S. Tolba, M.A. Abd ul-Malik , A.M. Kamal El-Dean , A.A. Geiesc, S.M. Radwanc ,R.M. Zakic, M. Sayeda , S.K. Mohamedd, and A. Shaban, AbdelRaheemf, *Current Chemistry Letters* 11 , **2022,** 29-42.
2. Peng, Xin-Mei, L.V. Damu, Guri, *Current Pharmaceutical Design*,19, **2013**, 3884-3930.
3. A.M. Katsori, D. Hadjipavlou-Litina, Coumarin derivatives: an updated patent review, *Expert Opinion on Therapeutic Patents.*, 12, **2014,** 1323-1347.
4. K.N. Venugopala, V. Rashmi, B. Odhav, *BioMed Res. Int.,* **2013**, 1-14.
5. J. R. S, Hoult. M. Paydt, *Gen. Pharmac.,* 27, 4, **1996**, 713-722.
6. I. Rengel, A.R. Friedrich, *Vet. Res. Commun.,* 17 , 6, **1993**, 421- 427.
7. J. Nawrot-Modranka, E. Nawrot, and J. Graczyk, *Eur. J. Med. Chem.,* 41, 11, **2006**, 1301–1309.
8. S. Thaisrivongs, M.N. Janakiraman, T.C. Chong, P.K. Tomich, L.A. Dolak, S.R. Turner, J.W. Strohbach, J.C. Lynn, M.M. Horng, R.R. Hinshaw, K.D. Watenpaugh, *J. Med. Chem.,* 39, 12, **1996**, 2400-2410.
9. B. Thati, A, Noble, B.S. Creaven, M. Walsh, M. McCann, K. Kavanagh, M, Devereux, D.A. Egan, *Cancer Lett.,* 248 ,2, **2007,** 321-331.
10. W.M. Lauer, *J. Am. Chem. Soc.,* 82, 19, **1960,** 5259-5260.
11. M.M. Garazd, O.V. Muzychka, A.I. Vovk, I.V. Nagorichna, A.S.Ogorodniichuk, *Chem. Nat. Compd.,* 43, 1, **2007**, 19-23.
12. V.F. Traven, T.A. Chibisova, A.V. Manaev, *Dyes Pigm.,* 58, 1, **2003,** 41–46.
13. D.L. Dreyer, K.C. Jones, L. Jurd, B.C. Campbell, *J. Chem. Ecol.,* 13, 4, **1987**, 925-930.
14. Y.A. Vladimirov, V.S. Sharov, E.S. Driomina, A.V. Reznitchenko, S.B. Gashev, *Free Radical Biol. Med.,* 18, 4, **1995**, 739-745.
15. Z.N. Siddiqui, A. Mohammad, *Ind J Chem*, 45, **2006**, 2704-2709
16. J.C. Jung, O.S. Park, *Molecules.,***2019,**14, 4790.
17. M.M. Abdou, R.A.El-Saeed, S. Bondock, *Arabian J. Chem.,***2019**, 12, 88.
18. M. Chandel, S.M. Roy, D. Sharma, S.K. Sahoo, A. Patel, P. Kumari, U.D. Patil, *J. Lumin.* 154, **2014,** 515–519.
19. A.O. Gerasov, M.P. Shandura, Y.P. Kovtun, *Dyes Pigments,* 79, **2008**, 252–258.

20. A.O. Gerasov, M.P. Shandura, Y.P. Kovtun, *Dyes Pigments*, 77, **2008**, 598–607.

21. R.S. Keri, S.A. Patil, *Biomed. Pharmacother.*68, **2014**, 1161-1175.

22. WHO/HTM/TB/2016.10, World Health, Organization, Global Tuberculosis Report,10, **2016.**

23. D. Sriram, P. Senthilkumar, M. Dinakaran, P. Yogeeswari, A. China, V. Nagaraja, *J. Med. Chem.*, **2007,** 50, 6232-6239.

24. A. Lilienkampf, J. Mao, B. Wan, Y. Wang, S.G. Franzblau, A.P. Kozikowski, *J. Med. Chem.,* **2009,** 52, 2109-2118.

25. M.M. Abdou, R.A. El-Saeed, S. Bondock. *Arabian J.Chem*.,**2019**, 12, 974.

26. I. Manolov, Danchev, N.D. *Arch. Pharm. Pharm. Med. Chem.,* **1999**, 332, 243-248.

27. S. Vijaya Laxmi, B. Suresh Kuarm, B. Rajitha. *Med Chem Res.,***2012.**

28. B. Karami, M. Farahi, V. Mouzari, K. Eskandari. *Tetrahedron,.***2015**, 15, 0040-4039.

29. Leema Dutta ,J. Pulak, Bhuyan. *ChemistrySelect,.***2016**, 1, 5944-5947.

30. H. M. Kasralikar, S. C. Jadhavar, R. Sudhakar Bhusare. *Synlett,.***2015,** 26, 1969-1972.

31. A. Ajaz Dar, S. Hussain, D. Dutta, K. Parameswar, Iyer, and T. Abu, Khan, *RSC Adv.,***2015**, 5, 57749.

32. M.D, Naik,Y.D. Bodke, Prashantha. *J. Chem Sci*.,**2021**, 17, 133.

33. S.A. Mohammadi, S. Balalaie, *Tetra.Lett.,***2007,** 48, 3299.

34. S.A. Abdullah. *Res.Chem. intermed*.,**2013**, 41, 93.

35. H.R. Bijanzadesh, S. Mehrparvar, S. Balalaie, *J.Iran.Chem.,***2015**, 12, 1859.

36. A. Shafiee, R. Motamedi, O. Firuzi, S. Meili, A.R. Mehdipour, R. Miri, *Med.Chem.Res.,* **2010,** 20, 466.

37. P. Sirisha, P. Suhas, RK Anant, N. Minal, *J.Chem. Sci.,***2011**, 123, 667.

38. MD. Naik, Y.D. Bodke, J.K. Naik, *Journal of Chemical Research.,***2021,** 45, 228-236.

39. K.N. Venugopala, C. Tratrat, M. Pillay, S. Chandrashekharappa, M. Attimarad, O.I. Alwassil, A.B. Nair, N. Sreeharsha, R. Venugopala, M.A. Morsy, M. Kumalo, B. Odhav, K. Mlisana, H.M. Haroun, *Drug Des. Dev. Ther.,* **2020,** 14, 1027-1039.

40. M.C.S, Lourenco, M.V.N, de Souza, A.C. Pinheiro, M.L. Ferreira, R.S.B, Gonçalves, T.C.M. Nogueira, M.A. Peralta, *ARKIVOC,* **2007,** 181-191.

41. O. Trott, A.J. Olson, *J. Comput. Chem.*, **2010,** 31, 455-461.

42. M.N. Joy, Y.D. Bodke, S. Telkar, *Bulg. Chem. Commun.,* **2020,** 52, 237-244

43. A. Pandith, A. Kumar, H.S. Kim, *RSC Adv.,* **2015,** 5, 81808-81816.

44. B.N. Nippu, R. Sandeep Kumar Jain, A. Rahman, H.M. Kumaraswamy, N.D. Satyanarayan, *J. Mol. Struct.,* **2022**, 133103.

45. G.C. Anjan Kumar, Y.D. Bodke, B. Manjunatha, N.D. Satyanarayan, B.N. Nippu, Joy, *Chim. Techno Acta ,* 9, **2022**, 9104.

46. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin*, J. Comput. Chem.,* 25, **2004,** 1605-1612.

47. R.P. Gurav, R.D. Nalawade, S.D. Sawant, N.D. Satyanarayan, S.A. Sankpal, S.P. Hangirgekar, *Appl. Organomet. Chem*., 36, **2022,**

48. Gaillard, *J. Chem. Inf. Model.* **2018,** 58, 1697-1706.

49. S. Dallakyan, A.J. Olson, *Methods Mol. Biol.,* **2014**, 243-250.

50. R.E. Rigsby, A.B. Parker, *Biochem. Mol. Biol. Educ,* **2016,** 44, 433-437.

51. A. Rahman, N. Prashanth, B. N. Nippu, H.M. Kumaraswamy, A.N. Rajeshwara, N.D. Satyanarayan, *J. Mol. Struct.,* **2022,**1264, 133211.

52. B.N. Nippu, R. Sandeep Kumar Jain, A. Rahman, H.M. Kumaraswamy, K.M. Mahadevan, N.D. Satyanarayan*, J. Mol. Struct.,***2022,**134829.

53. P. Patil, A. Yadav, L. Bavkar, B.N. Nippu, N.D. Satyanarayan, Ananda Mane, A.Gurav, S.Hangirgekar, S. Sankpal, *Mol. Struct.,***2021,** 1264, 130672