



Research article

Synthesis, characterization and biological investigations of potentially bioactive heterocyclic compounds containing 4-hydroxy coumarin

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ARTICLE INFO

Keywords:

Organic chemistry
Coumarin
Azo dye
Biological activities
DFT studies

ABSTRACT

In this paper, we have reported the synthesis of a series of heterocyclic azo dyes containing 4-hydroxy coumarin by diazo-coupling reaction. The structural aspect of the newly synthesized compounds was accomplished by various physico-chemical techniques like UV-Visible, FT-IR, NMR, and mass spectrometry. The computational calculations and geometrical optimization of the newly synthesized azo dyes were investigated by using Gaussian software with the help of Density functional theory (DFT)/B3LYP method using 6-31G(d,p) basis set at gaseous phase. Also, the quantum chemical parameters were evaluated to understand the structural activity concept of the dyes. The pharmacological efficacy of the azo dyes was investigated by antimicrobial, antitubercular, DNA cleavage and *in silico* molecular docking studies. All the newly synthesized compounds were able to exhibit significant inhibitory activity against tested microbes. Further, the *in silico* molecular docking showed effective binding properties of the compounds against RpsA target receptor.

1. Introduction

The coumarin derivatives were found to be having innumerable pharmacological properties and exhibit various biochemical and therapeutic applications depending upon the pattern of the substitution [1, 2, 3]. Among the family of the coumarins, 4-hydroxy coumarin have been extensively studied for a wide range of biological activities including anticoagulant, insecticidal, anthelmintic, hypnotic, antifungal, phytoalexin, and HIV protease inhibition activities [4, 5, 6, 7]. These special applications of 4-hydroxycoumarin have motivated considerable interest in this class of compounds for number of researchers across the globe [8, 9, 10, 11].

The recent development in the synthesis of heterocyclic compounds containing azo chromophore has become more important as they exhibit different properties based on effective conjugation and substituent's electronic effect. The coumarin which exhibits brilliant physical, thermal, optical and biological properties can be used as dyes for colouring of fabrics, fluorophores and optical brightening agents [12, 13, 14]. Some of the work reported on the azo group incorporated in the mesogenic core of coumarin has increased the dipole moments and stability of coumarin

azo-ester series as related to the coumarin esters [15]. Alternatively, a molecule which has azo linkage is generally exhibited reversible isomerization transformations upon irradiation with ultraviolet (~365 nm) and visible (~450 nm) light. The molecules containing extended conjugation and more number of electron releasing substituents on the diazo group relatively enhances the electron density of the molecule which in turn intense optical absorption and related optical properties [16, 17, 18]. Also, the azo benzene derivatives found more applications in liquid crystal studies due to its rod-like shape, photosensitive ability and photo induced alignment with reversible cis-trans transformation, dimerization in cross linking material and irreversible photo degradation [19]. The azo dyes containing coumarin nucleus has potential biological, optical and electrochemical properties and therefore it has been extensively studied by number of researchers [20, 21, 22, 23].

From the above observations and extensive findings on the coumarin analogues, in the present work we have described the synthesis of coumarin based azo dyes and their structural characterization. The quantum chemical technique was used to optimize the molecular geometry of the newly synthesized compounds and the biological activities were also carried out to check the inhibitory effect against the organisms.

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Received 18 March 2020; Received in revised form 19 May 2020; Accepted 15 June 2020

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2. Experimental

2.1. Materials and methods

All the chemicals and reagents used are of analytical grade and purchased from Sigma Aldrich Chemical Company and used as such received. Melting points of the compounds were recorded on an electro thermal apparatus and are uncorrected. The electronic absorption spectra were recorded on UV-1800 Shimadzu spectrophotometer in the range of 200–800 nm using 10^{-6} M solution of dimethylsulphoxide (DMSO), N,N-dimethylformamide (DMF), tetrahydrofuran (THF) and dichloromethane (DCM). The FT-IR spectra were obtained using Perkin Elmer- RX-FITIR spectrophotometer using KBr pellets. The ^1H and ^{13}C -NMR spectra were recorded with the aid of Bruker spectrometer 400 MHz and 100 MHz respectively; chemical shifts (δ) were recorded in parts per million (ppm) with respect to tetramethylsilane (TMS). The mass spectra were recorded on LC-MS 2010, SHIMADZU mass spectrometer. The computational studies were carried out by Density functional theory (DFT)/B3LYP method using Gaussian 09W software using 6-31G(d,p) basis set at gas phase. Geometry optimization, vibrational analysis and quantum chemical parameters were analyzed at same basis set level [24].

2.2. General procedure for the synthesis of coumarin based azo dyes (F₂₁–F₂₄)

A well-stirred and ice-cold solution of heterocyclic amines (0.002 mmol) in hydrochloric acid (2 ml of HCl in 3 ml of H₂O) were added drop wise with constant stirring to the solution of sodium nitrite in sulphuric acid (2 mL) at 0–5 °C and stirred for 2 h to get the diazonium salt solution. Then, this diazonium salt solution was added to the ice-cold solution of 4-hydroxy coumarin in aqueous KOH solution and stirred for another 1h. The obtained coloured precipitates were filtered, washed with distilled water until it is free from impurities and recrystallized in ethanol to afford coloured azo dyes (F₂₁–F₂₄).

2.2.1. 4-Hydroxy-3-[(4-nitrophenyl) diazenyl]-2H-chromen-2-one (F₂₁)

Yellow coloured solid, 80% yield, m.p. 182–184 °C. FT-IR (KBr, ν_{max} , cm^{-1}): 3421 (OH), 3072 (Ar-CH), 1660 (C=O), 1461 (N=N), 1421 (C-N). ^1H NMR (DMSO-*d*₆, δ ppm): 16.22 (s, 1H, OH), 8.34 (d, *J*=8 Hz, 1H,

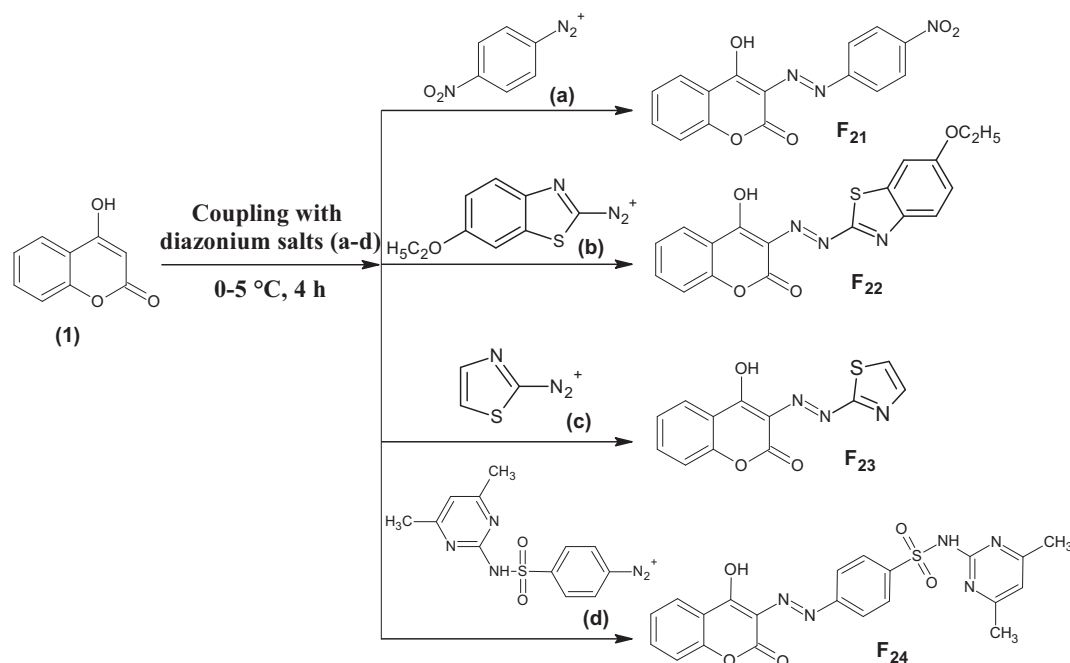
Table 1. The comparative analysis of experimental and calculated vibrational frequencies for the coumarin based azo dyes (F₂₁–F₂₄).

Compounds	Assignments	FT-IR absorption frequencies (cm^{-1})	
		Experimental	Theoretical
F ₂₁	ν_{OH}	3421	3686
	$\nu_{\text{Ar-CH}}$	3072	3185
	$\nu_{\text{C=O}}$	1660	1672
	$\nu_{\text{N=N}}$	1461	1458
	$\nu_{\text{C-N}}$	1421	1409
F ₂₂	ν_{OH}	3449	3569
	$\nu_{\text{Ar-CH}}$	3055	3047
	$\nu_{\text{C=O}}$	1663	1821
	$\nu_{\text{N=N}}$	1491	1515
	$\nu_{\text{C-N}}$	1447	1496
F ₂₃	ν_{OH}	3442	3642
	$\nu_{\text{Ar-CH}}$	2925	3154
	$\nu_{\text{C=O}}$	1728	1814
	$\nu_{\text{N=N}}$	1512	1531
	$\nu_{\text{C-N}}$	1488	1473
F ₂₄	ν_{OH}	3420	3598
	ν_{NH}	3073	3229
	$\nu_{\text{Ar-CH}}$	2928	3052
	$\nu_{\text{C=O}}$	1724	1672
	$\nu_{\text{N=N}}$	1515	1502
	$\nu_{\text{C-N}}$	1444	1458

Ar-H), 8.24 (d, *J*=8 Hz, 1H, Ar-H), 8.08 (d, *J*=7.6 Hz, 1H, Ar-H), 7.99–7.80 (m, 2H, Ar-H), 7.54 (t, *J*=8 Hz, 1H, Ar-H), ^{13}C NMR (DMSO-*d*₆, δ ppm): 195.51 (coumarin C=O), 159.24 (C–OH), 155.33, 147.49, 134.41, 130.24, 128.67, 124.99, 124.52, 118.26, 116.69; LCMS: *m/z* 312 [M+1]. Anal. Calcd for C₁₅H₉N₃O₅: C, 57.88; H, 2.91; N, 13.50, Found: C, 57.75; H, 2.85; N, 13.43.

2.2.2. 3-[(5-Ethoxy-1,3-benzothiazol-2-yl) diazenyl]-4-hydroxy-2H-chromen-2-one (F₂₂)

Reddish coloured solid, 75% yield, m.p. 224–226 °C. FT-IR (KBr, ν_{max} , cm^{-1}): 3449 (OH), 3055 (Ar-CH), 2925 (C–CH₃), 1663 (C=O), 1491



Scheme 1. Synthetic route adopted for the preparation of coumarin based azo dyes (F₂₁–F₂₄).

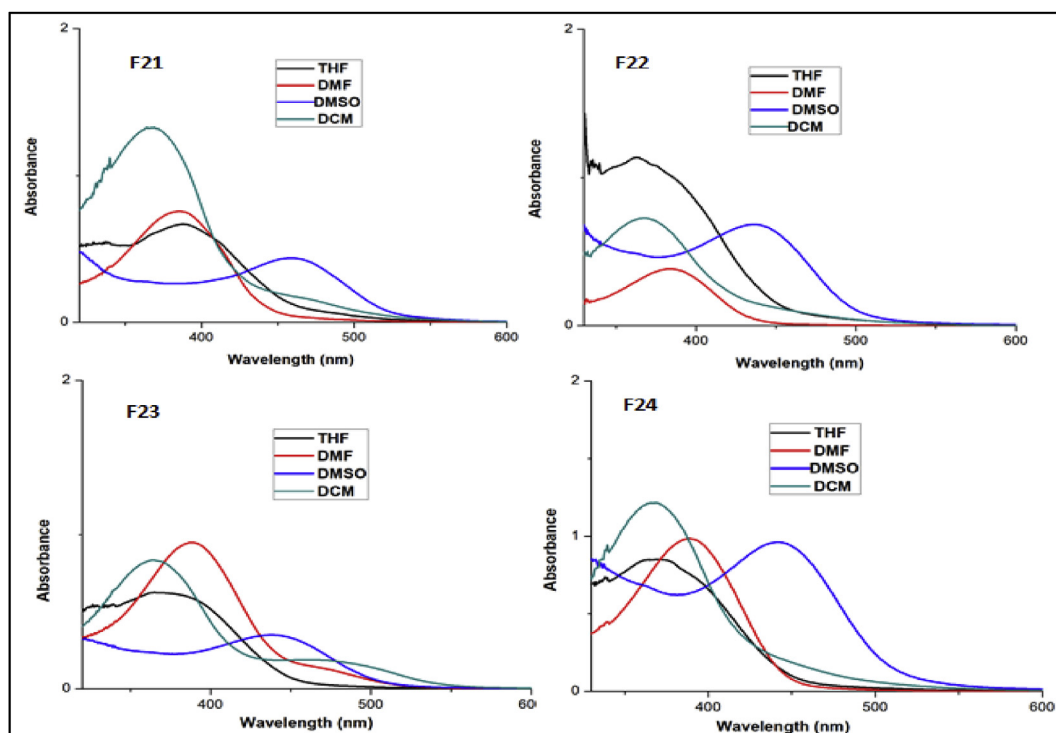


Figure 1. The electronic spectra of the compounds (F₂₁–F₂₄) in various solvents recorded at room temperature at 10^{−6} M concentration.

Table 2. The electronic spectral data of the compounds (F₂₁–F₂₄) obtained indifferent solvents.

Compounds	λ _{max} (nm)				Logε			
	DMSO	DMF	THF	DCM	DMSO	DMF	THF	DCM
F ₂₁	436	382	362	369	5.84	5.57	6.05	5.87
F ₂₂	461	386	389	366	5.64	5.88	5.81	6.12
F ₂₃	443	389	371	367	5.98	5.99	5.93	6.09
F ₂₄	441	388	376	365	5.53	5.99	5.80	5.93

(N=N), 1447 (C–N). ¹H NMR (DMSO-*d*₆, δ ppm): 12.50 (s, 1H, OH), 7.84–7.08 (m, 6H, Ar–H), 6.97 (t, *J* = 5.6 Hz, 1H, Ar–H), 4.14–4.00 (m, 2H, CH₂), 1.39–1.35 (m, 3H, CH₃). ¹³C NMR (DMSO-*d*₆, δ ppm): 166.19 (coumarin C=O), 163.66 (C–OH), 136.43, 135.73, 129.66, 129.57, 126.80, 122.72, 122.34, 119.59, 118.42, 116.30, 116.07, 111.41, 111.35, (CH₂) 43.05, 25.48 (CH₃); LCMS: *m/z* 368 [M+1]. Anal. Calcd

Table 3. The quantum chemical parameters evaluated for the azo dyes (F₂₁–F₂₄) by DFT method at B3LYP/6-31G(d,p).

Electronic parameters	F ₂₁	F ₂₂	F ₂₃	F ₂₄
E _{HOMO} (eV)	-5.52	-4.47	-4.38	-5.96
E _{LUMO} (eV)	-2.51	-1.53	-1.47	-2.70
E _{HOMO} –E _{LUMO} (eV)	3.00	2.97	2.90	3.25
Electronegativity (χ)	4.01	3.00	2.93	1.62
Chemical potential(μ)	-4.01	-3.00	-2.93	-1.62
Hardness(η)	1.50	1.474	1.45	1.62
Electrophilicity index (ω)	5.37	2.035	2.01	1.00
Ionization potential (A)	5.52	4.47	4.38	5.96
Electron affinity (I)	2.51	1.53	1.47	2.70
Dipole moment (D)	1.72	3.02	1.14	0.84

for C₁₈H₁₃N₃O₄S: C, 58.85; H, 3.57; N, 11.44, Found: C, 58.74; H, 3.47; N, 11.33.

2.2.3. 4-Hydroxy-3-[(*E*)-1,3-thiazol-2-ylidiazonyl]-2H-chromen-2-one (F₂₃)

Orange coloured solid, 77% yield, m.p. 194–196 °C. FT-IR (KBr, ν_{max}, cm^{−1}): 3442 (OH), 2925 (Ar–CH), 1728 (C=O), 1512 (N=N), 1488 (C–N). ¹H NMR (DMSO-*d*₆, δ ppm): 11.37 (s, 1H, OH), 8.36–8.10 (m, 1H, Ar–H), 7.93 (d, *J* = 7.6 Hz, 1H, Ar–H), 7.23 (t, *J* = 8.4 Hz, 1H, Ar–H), 7.19 (m, 1H, Ar–H), ¹³C NMR (DMSO-*d*₆, δ ppm): 167.95 (coumarin C=O), 155.19 (C–OH), 138.73, 135.60, 132.18, 128.36, 127.03, 126.49, 126.36, 122.58, 121.00, 118.15; LCMS: *m/z* 274 [M+1]. Anal. Calcd for C₁₂H₇N₃O₃S: C, 52.74; H, 2.58; N, 15.38, Found: C, 52.67; H, 2.46; N, 15.54.

2.2.4. *N*-(4,6-dimethylpyrimidin-2-yl)-4-[(*E*)-(4-hydroxy-2-oxo-2H-chromen-3-yl) diazenyl] benzenesulfonamide (F₂₄)

Light yellow coloured solid, 81% yield, m.p. 215–217 °C. FT-IR (KBr, ν_{max}, cm^{−1}): 3420 (OH), 3073 (NH), 2928 (Ar–CH), 1724 (C=O), 1515 (N=N), 1444 (C–N). ¹H NMR (DMSO-*d*₆, δ ppm): δ 12.53 (s, 1H, OH), 7.84 (q, *J* = 6.4 Hz, 2H, Ar–H), 7.66 (q, *J* = 6.8 Hz, 3H, Ar–H), 7.38 (q, *J* = 6 Hz, 4H, Ar–H), 5.60 (s, 1H, NH), 2.51 (t, *J* = 3 Hz, 6H, 2CH₃). ¹³C NMR (DMSO-*d*₆, δ ppm): 173.16 (coumarin C=O), 162.26 (C–OH), 157.13, 156.63, 144.21, 141.66, 139.84, 135.69, 129.82, 129.80, 129.68, 129.07, 127.04, 122.99, 120.97, 118.50, 117.66, 115.92, 39.72 (C–CH₃);

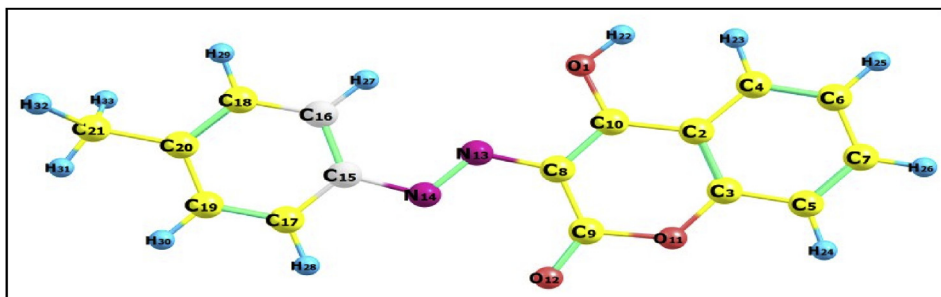


Figure 2. The optimized molecular structure of the compound F₂₁.

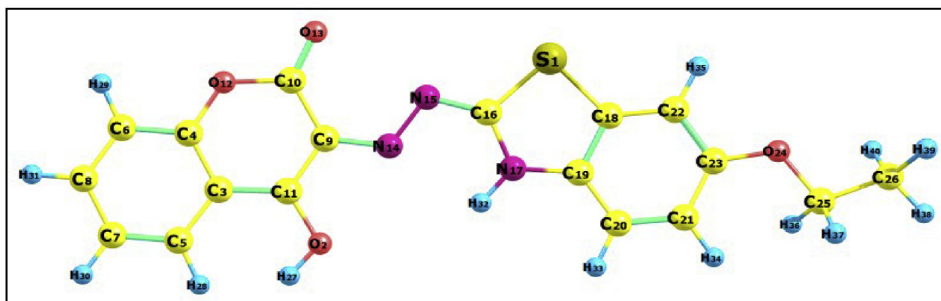


Figure 3. The optimized molecular structure of the compound F₂₂.

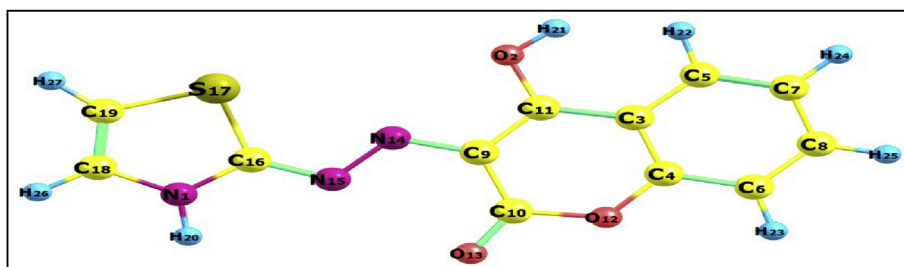


Figure 4. The optimized molecular structure of the compound F₂₃.

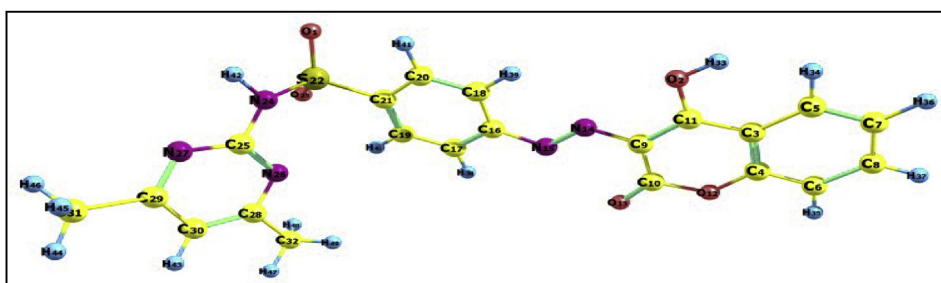


Figure 5. The optimized molecular structure of the compound F₂₄.

LCMS: m/z 452 [M+1]. Mol. Formula: Anal. Calcd for $C_{21}H_{17}N_5O_5S$: C, 55.87; H, 3.80; N, 15.51, Found: C, 52.73; H, 3.72; N, 15.43.

2.3. Biological studies

2.3.1. Antimicrobial activity

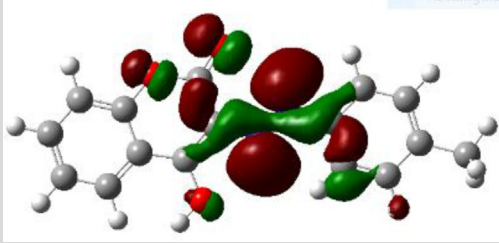
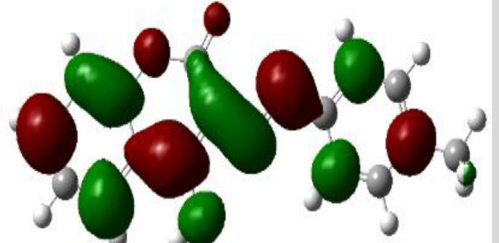
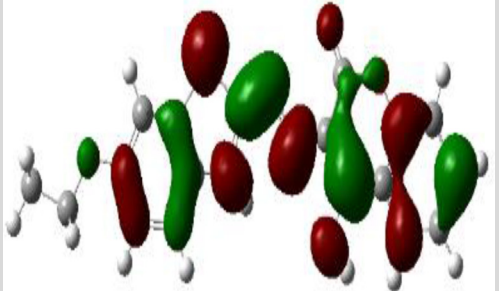
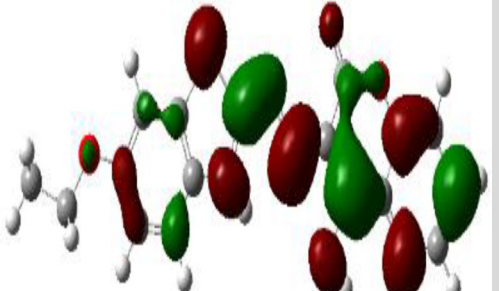
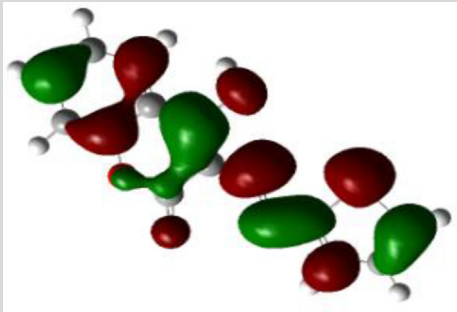
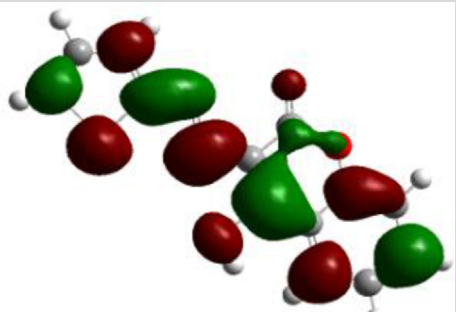
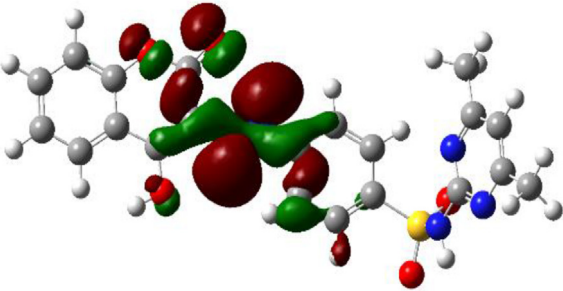
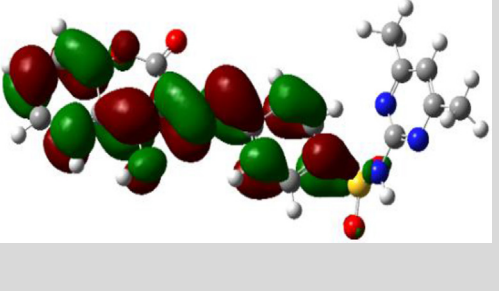
The antimicrobial activity of the coumarin based azo dyes was screened using three bacterial strains *Escherichia coli*, *Pseudomonas aeruginosa*, (gram-negative bacteria), *Enterococcus faecalis* (gram-positive bacteria) and three fungal strains *Candida albicans*, *Aspergillus flavus* and *Aspergillus niger* by tube dilution assay [25,26]. The Brain heart infusion

(BHI) was used as a medium for growing the target microorganisms, the ciprofloxacin and fluconazole were used as a positive control and DMSO as a negative control. Further, the inhibitory effect of the compounds against microbial strains was computed in terms of minimum inhibitory concentration (MIC) along with the standard drugs.

2.3.2. Antitubercular activity

Mycobacterium tuberculosis is a deadly microorganism and causes respiratory diseases to the human beings. Even though, number of drugs are available to reduce the toxic effects of the pathogen, the death rates due to tuberculosis are continuously increasing. It is due to the increasing

Table 4. The structure of the HOMO and LUMO of the compounds (F₂₁–F₂₄) generated from B3LYP method using 6-31G(d,p) basis set.

Compounds	Structure of HOMO	Structure of LUMO
F ₂₁		
F ₂₂		
F ₂₃		
F ₂₄		

of toxicity of the existing drugs and multi drug resistance strain and some other clinical and environmental problems [27]. Therefore, in the present study we have synthesized coumarin based-azo dyes (F₂₁–F₂₄) and studied the antitubercular activity against *M. tuberculosis* by Microplate Alamar Blue Assay (MABA) method [28]. The obtained results were compared with the standard drugs Pyrazinamide, Ciprofloxacin and Streptomycin. The colour change in the microplate from pink to blue suggesting no bacterial growth where as pink colour stipulates the growth.

2.3.3. DNA cleavage

The target compounds (F₂₁–F₂₄) were studied for their cleavage study against super coiled pBR-322 DNA by electrophoresis technique [29]. The experimental technique involves the use of accurately

weighed DNA sample (0.35 µg/test), this was added to the target compounds (F₂₁–F₂₄) and these mixtures were incubated at 37 °C for 30 min. Then, the 250mg of agarose was dissolved in 25 mL of TAE buffer (4.84 g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) and was added to the reaction mixture. When the agarose gel attains ~55 °C, it was poured into the gel cassette fitted with a comb. After solidification, the gel was detached carefully and shifted into the electrophoresis chamber containing TAE buffer. The mixture containing equimolar ratio of bromophenol blue dye and 20 µL of the DNA sample was loaded into the wells along with the standard DNA marker. Later, electricity of 50 V was constantly supplied to the chamber for about 45 min. After this period, the gel was removed from the chamber and stained with ETBR solution (10 µg/ml) for 10–15 min and the bands were observed under UV transilluminator.

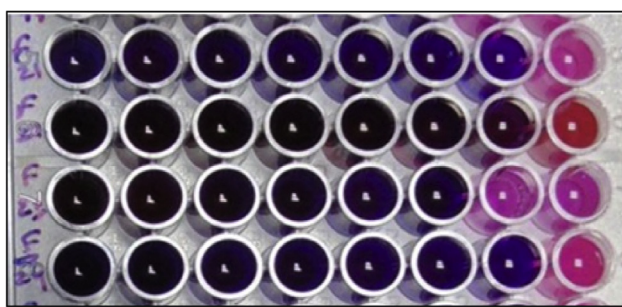
Table 5. Antimicrobial activity results of the azo dyes (F₂₁–F₂₄) in terms of MIC (mg/mL).

Compounds (mg/mL)	100	50	25	12.5	6.25	3.12	1.6	0.8	0.4	0.2
<i>E. coli</i>										
F ₂₁	S	S	S	S	S	S	S	S	S	R
F ₂₂	S	S	S	S	S	S	S	S	R	R
F ₂₃	S	S	S	S	S	S	S	S	R	R
F ₂₄	S	S	S	S	S	S	S	S	S	R
<i>P. aeruginosa</i>										
F ₂₁	S	R	R	R	R	R	R	R	R	R
F ₂₂	S	S	S	R	R	R	R	R	R	R
F ₂₃	S	S	R	R	R	R	R	R	R	R
F ₂₄	S	S	R	R	R	R	R	R	R	R
<i>E. faecalis</i>										
F ₂₁	S	S	S	R	R	R	R	R	R	R
F ₂₂	S	S	R	R	R	R	R	R	R	R
F ₂₃	S	S	S	R	R	R	R	R	R	R
F ₂₄	S	S	S	S	R	R	R	R	R	R
Ciprofloxacin	S	S	S	S	S	S	S	S	S	S
<i>C. albicans</i>										
F ₂₁	S	S	S	S	S	S	R	R	R	R
F ₂₂	S	S	S	S	S	S	R	R	R	R
F ₂₃	S	S	S	R	R	R	R	R	R	R
F ₂₄	S	S	S	S	S	R	R	R	R	R
<i>A. flavus</i>										
F ₂₁	S	S	S	S	S	S	S	S	R	R
F ₂₂	S	S	R	R	R	R	R	R	R	R
F ₂₃	S	S	S	S	S	S	S	S	R	R
F ₂₄	S	S	S	S	S	S	S	S	R	R
<i>A. niger</i>										
F ₂₁	S	S	S	R	R	R	R	R	R	R
F ₂₂	S	S	R	R	R	R	R	R	R	R
F ₂₃	S	S	R	R	R	R	R	R	R	R
F ₂₄	S	R	R	R	R	R	R	R	R	R
Fluconazole	S	S	S	S	S	S	S	S	S	R

Here, S: Sensitive, R: Resistance.

Table 6. The results of the antitubercular activity against *M. tuberculosis* (MIC, mg/mL) of the compounds (F₂₁–F₂₄).

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
F1	S	S	S	S	S	S	S	R
F2	S	S	S	S	S	S	S	R
F3	S	S	S	S	S	S	S	R
F4	S	S	S	S	S	S	R	R

**Figure 6.** Antitubercular activity results of the compounds (F₂₁–F₂₄).

2.3.4. *In silico* molecular docking studies

The antimicrobial activity results of synthesized compounds inspired us to investigate the interaction of the target compounds with the biological receptors theoretically by *in silico* molecular docking using RpsA receptor. The molecular structures of the coumarin derivatives (F₂₁–F₂₄) were optimized by using Chem Bio Draw tool (Chem Bio Office Ultra 14.0 suite) with 2D-orientation and then they were converted into 3D-format with the minimization energy by Schrodinger Maestro. The 2XCT-Protein Data Bank (PDB) was used to get the 3D coordinates of the target receptor and the best docked conformation of the tested structures were obtained on the basis of glide energy, docking score, active hydrogen bonding sites and hydrophobic interactions [30].

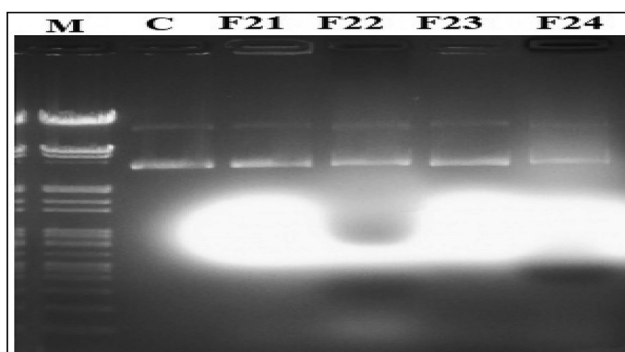


Figure 7. The picture showing cleavage of the supercoiled pBR322 DNA by the azo dyes (F₂₁–F₂₄), here M: marker DNA, C: control DNA, F₂₁–F₂₄: coumarin based azo dyes.

3. Result and discussion

The purpose of this study is to explore the structural and biological properties of the novel coumarin based azo dyes. We adopted the simple and conventional diazo-coupling reaction to synthesize the azo dyes containing 4-hydroxy coumarin nucleus. The reaction pathway involved in getting the target compounds was depicted in Scheme 1.

3.1. IR spectral data

The FTIR spectra of the synthesized compounds (F₂₁–F₂₄) were recorded using KBr pellets in the region 4000–400 cm⁻¹. The important IR bands exhibited by azo dyes were displayed in Table 1. Further, the absorption frequencies of the compounds obtained experimentally were compared with theoretical values calculated by DFT/B3LYP method using 6-31G(d,p) bases set at gaseous state which have been summarized in the Table 1.

A medium intensity broad peak observed in the region between 3449–3420 cm⁻¹ is due to the presence of O–H group attached to the coumarin ring and the corresponding theoretical value was appeared between 3686–3569 cm⁻¹ which were in well agreement with the literature. Another absorption band for the presence of N–H functionality of the sulfamethazine moiety in compound F₂₄ was appeared at 3073 cm⁻¹ and its corresponding theoretical N–H stretching value was found to be at 3229 cm⁻¹. The absorption band appeared in the region 3100–2925 cm⁻¹ was assigned to the aromatic C–H stretching vibrations for all the compounds and its respective theoretical value was observed at 3185–3052 cm⁻¹. The carbonyl (C=O) and –N=N– functionalities exhibited medium intensity bands in the region 1728–1660 and 1515–1461 cm⁻¹ respectively and their corresponding theoretical values obtained in the region 1821–1672 and 1531–1458 cm⁻¹ respectively. Another low intensity absorption band was appeared in the region 1488–1421 cm⁻¹ due

to the C–N stretching vibrations in all the dyes and their corresponding theoretical values lies in the region of 1496–1409 cm⁻¹. Thus, from the above discussion it is clear that the experimentally recorded IR spectra were almost matches with the computational IR spectral data [31,32].

3.2. Electronic absorption spectra

The effect of solvent polarity and the electronic substitution was studied for the title compounds (F₂₁–F₂₄) in four different solvents such as DMSO, DMF, THF and DCM at a concentration of 10⁻⁶ M at ambient temperature and the typical absorption spectra were displayed in Figure 1. The absorption maxima (λ_{max}) and its corresponding logarithmic molar extinction coefficient for all the compounds in studied solvents were obtained from the plot and summarized in the Table 2. The electronic spectra of the synthesized compounds showed broad peaks in the region 461–436, 389–382, 389–362 and 365–369 nm in DMSO, DMF, THF and DCM solvents due to π→π* or n→π* transitions respectively [33]. From the close observation of the spectral data (Table 2), it is clearly evident that as the polarity of the solvent increases, the absorption maxima shift towards longer wavelength so that the bathochromic shift observed in all the compounds. This may be due to the effective interaction between the solvent molecules and the lone pair of electrons present on the diazo component. The presence of electron releasing substituents on the aromatic ring bearing the azo group also contributes to the bathochromic shift. From this study, it concludes that solvent polarity and electronic substitution played very important role in the shift of λ_{max} for all the studied azo dyes [34].

3.3. NMR spectral data

The structural confirmations of the synthesized compounds (F₂₁–F₂₄) were accomplished by recording their ¹H and ¹³C NMR spectra in DMSO-d₆. In ¹H NMR spectra, the hydroxyl proton attached to the coumarin ring present in all the compounds was appeared in the region 16.22–11.37 ppm as singlet. The aromatic protons were resonated as multiplet in the region 8.36–6.79 ppm. A broad peak appeared at δ 5.6 in compound F₂₄ was due to the presence of NH proton. The –CH₂ protons attached to the benzothiazole ring in the compound F₂₂ was appeared as doublet at δ 3.92–3.81 and the methyl protons of compounds F₂₂ and F₂₄ were appeared as singlet in the region 2.50–1.32 ppm. These ¹H NMR spectral results are in close agreement with the proposed structure of the compounds. The ¹³C NMR spectrum of the compound F₂₁ shows signals at δ 195.51, 159.24, 155.33, 147.49, 134.41, 130.24, 128.67, 124.99, 124.52, 118.26 and 116.69 ppm corresponding to the carbon atoms C11, C5, C7, C6, C2, C1, C3, C4, C10, C8 and C9 respectively. The compound F₂₂ displayed the ¹³C NMR signals at δ 166.19, 163.66, 136.41, 135.73, 129.66, 129.57, 126.80, 122.72, 122.34, 119.57, 118.42, 116.30, 116.07, 111.41, 43.05 and 30.98 ppm related to the carbon atoms C16, C5, C1, C2, C3, C4, C6, C7, C8, C9, C10, C11, C12, C13, C14 and C15 respectively. The ¹³C NMR spectrum of compound F₂₃ showed the signals at δ 167.95, 155.19,

Table 7. The results of the *in silico* molecular docking studies of the azo dyes (F₂₁–F₂₄) against RpsA enzyme.

Compounds	Affinity (kcal/mol)	H-bonds	H-bond length (Å)	H-bond with	Hydrophobic interactions
F ₂₁	-5.0	3	3.03	4NNI:Arg357::F21:O2	Tyr280, Lys303, Phe310, Glu318, Arg355, Arg356
			3.07	4NNI:Arg357::F21:O2	
F ₂₂	-5.1	1	2.80	4NNI:Arg357::F22:O4	Lys303, Phe310, Glu318, Arg355
F ₂₃	-5.4	2	2.92	4NNI:Tyr::F23:O2	Lys303, Phe310, Glu318, Arg355
			3.19	4NNI:Arg356::F23:N3	
F ₂₄	-5.9	1	2.85	4NNI:Arg357::F24:O3	Lys303, Phe310, Glu318, Arg355
Pyrazinamide	-5.3	2	3.01	4NNI:Arg357::PYZ:O	Phe307, Leu320, His322
			3.06	4NNI:Arg357::PYZ:O	

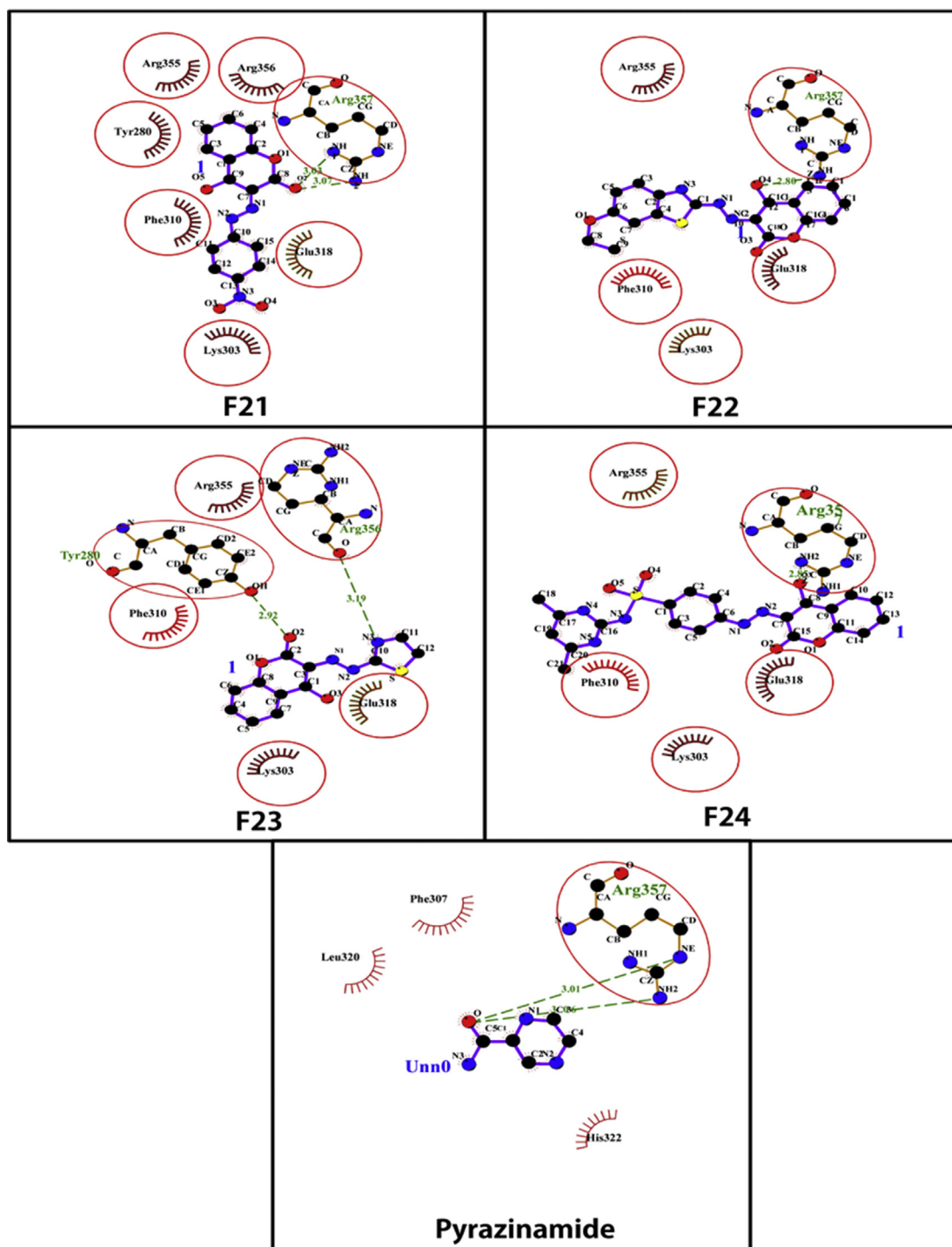


Figure 8. 2D representation of the interaction of compounds F₂₁, F₂₂, F₂₃, F₂₄ and standard drugs with pyrazinamide against RpsA receptor.

138.73, 135.60, 132.16, 128.36, 127.03, 126.49, 126.36, 122.58, 121.00 and 118.15 ppm related to the carbon atoms of C12, C7, C5, C1, C2, C3, C4, C6, C7, C8, C9, C10 and C11. The ¹³C NMR spectrum of the synthesized compound F₂₄ exhibits the signals at δ 173.16, 162.26, 144.21, 141.66, 139.84, 135.69, 129.82, 129.80, 129.68, 129.07, 127.04, 122.99, 120.97, 118.50, 117.66, 39.72 and 36.38 ppm resultant to the carbon atoms of C17, C5, C1, C2, C3, C4, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15 and C16 respectively.

The FT-IR, ¹H NMR, ¹³C NMR and Mass spectra of the compounds (F₂₁–F₂₄) have been given in the supplementary data file (S1–S20).

3.4. Geometrical optimization

In order to understand the molecular properties of the synthesized compounds (F₂₁–F₂₄), we carried out their structural optimization and the energies of the Highly Occupied Molecular Orbital (HOMO) and Least Unoccupied Molecular Orbital (LUMO) were evaluated from the optimized structures. Further, the energies of HOMO and LUMO were utilized to calculate some of the parameters like electronegativity (χ), chemical potential (α), hardness (η), electrophilicity index (ω), electron affinity (I) and ionization potential (A) to understand the reactivity of the

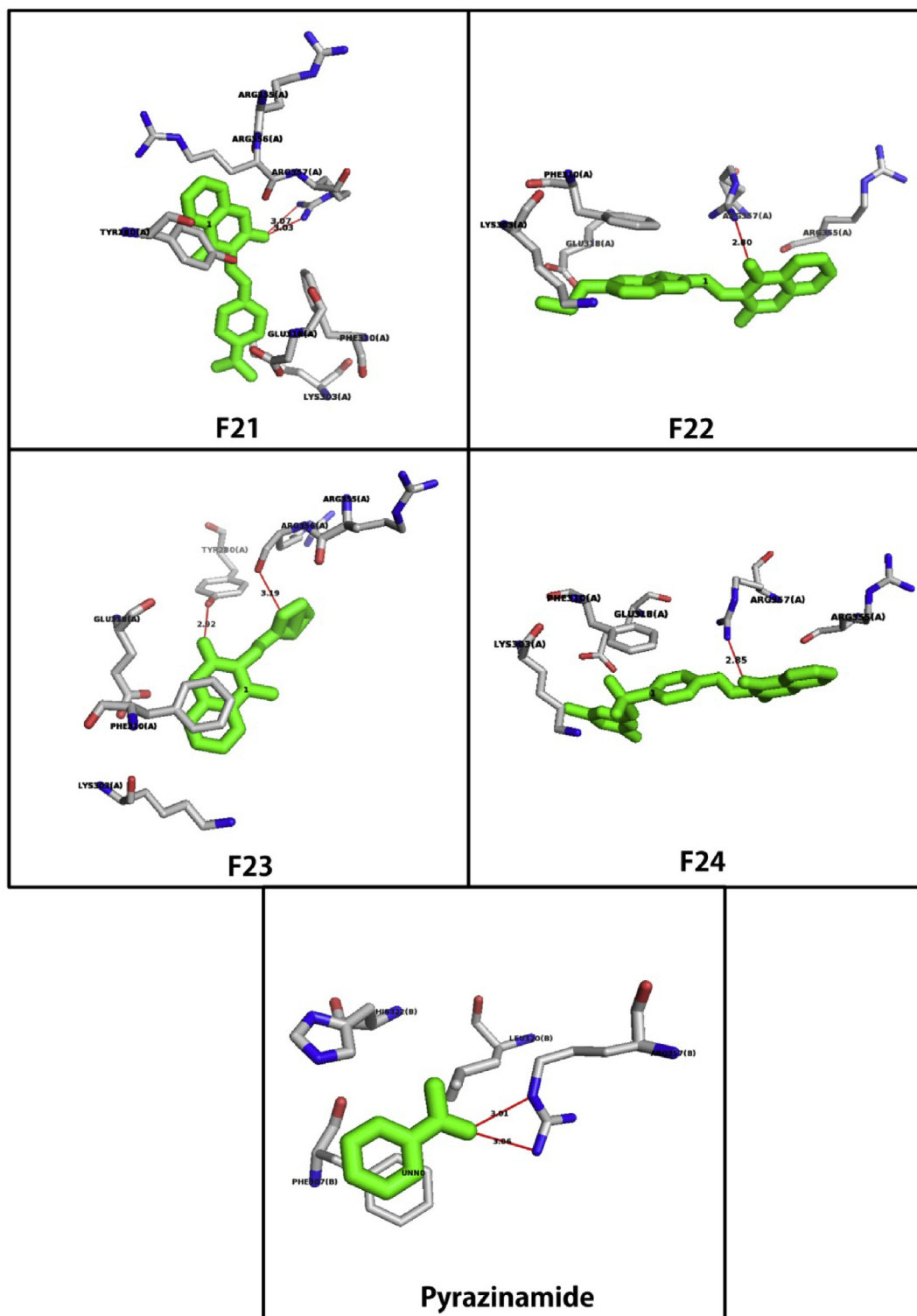


Figure 9. 3D representation of the interaction of compounds F21, F22, F23, F24 and standard drugs with pyrazinamide against RpsA receptor.

studied molecules. The global parameters have been calculated by using the following equations.

$$\text{Energy gap}(\Delta E) = E_{\text{HOMO}} - E_{\text{LUMO}} \quad (1)$$

$$\text{Electronegativity}(\chi) = \frac{(I + A)}{2} \quad (2)$$

$$\text{Chemical potential}(\mu) = -\frac{(I + A)}{2} \quad (3)$$

$$\text{Hardness}(\eta) = \frac{(I - A)}{2} \quad (4)$$

$$\text{Electrophilicity index}(\omega) = \frac{\alpha^2}{2\eta} \quad (5)$$

$$\text{Electron affinity}(I) = -E_{\text{LUMO}} \quad (6)$$

$$\text{Ionization potential}(A) = -E_{\text{HOMO}} \quad (7)$$

The calculated HOMO-LUMO energies and global parameters of the prepared azo molecules (F₂₁–F₂₄) are displayed in Table 3.

The tabulated data reveals that, low energy gap, chemical hardness and electrophilicity index values are responsible for the good biological activity of the azo dyes (F₂₁–F₂₄) [35].

The optimized molecular structures and HOMO-LUMO energy level diagrams of the azo dyes (F₂₁–F₂₄) were shown in Figures 2, 3, 4, and 5 and Table 4. The reactivity of molecules often decided by using density functional theory (DFT) and that is based on the energy differences between the HOMO and LUMO. From the literature review, it was observed that if the difference between the HOMO and LUMO is small, the energy required to excite an electron to higher energy state is less and therefore the molecules become more reactive chemically and biologically [36,37]. If the gap is large, then the promotion of electron becomes difficult and requires lot of energy, so that the molecules become more stable towards any reaction. Thus, from the above discussion it is inferred that the theoretical modeling is most useful in the interpretation of chemical reactivity, kinetic stability, polarizability and biological properties of the molecules [38,39,40].

3.5. Biological evaluation

3.5.1. Antimicrobial activity

A multidrug resistance of bacterial strains remains to be a great challenge due to their biochemical and morphological modifications and essential for the development of novel drugs. Therefore, the heterocyclic molecules having azo chromospheres received significant importance due to their wide spectrum of biological applications [41]. Thus, we have studied *in vitro* antibacterial activities of the synthesized azo dyes by tube dilution method and the results of the study have been summarized in Table 5. The results indicated that all the studied compounds showed efficient antibacterial activity against tested microbial strains. In particular, among the tested pathogens, the compounds exhibited effective inhibition against *E. coli* and *C. albicans*, whereas moderate activity exhibited against rest of the organisms.

The highest antibacterial activity of the synthesized compounds against *E. coli* and *C. albicans* pathogens may be due to the presence of electron donating atoms, delocalization of pi electrons, solubility and dipole moment. It increases the lipophilic environment around the microorganisms and allows the dye molecules to penetrate into the cell and finally an arrest the functions of the cell thereby suppresses the activity.

3.5.2. Antitubercular activity

The antitubercular activity of the compounds (F₂₁–F₂₄) was carried out against *M. tuberculosis* by MABA method and the result of the study was interpreted in terms of MIC depicted in Table 6 and Figure 6. From the results of the study it is inferred that, the synthesized compound can be able to show effective inhibitory action with MIC value equal to 1.6 µg/mL and it is almost equal to the MIC of the standard drugs. Therefore, from our study it is concluded that, the synthesized compounds could be able to show appreciable antitubercular activity against *M. tuberculosis* and they may be used as better agents in the development of antitubercular drugs in future.

3.5.3. DNA cleavage activity

The examination on the cleaving affinity of compounds to DNA is very attractive since it can provide to understand the toxicity mechanism of

them and to develop new artificial nuclease. Thus, the cleavage ability of the newly synthesized compounds (F₂₁–F₂₄) was studied by agarose gel electrophoresis assay against super coiled pBR322 plasmid DNA. The Figure 7 provided below is the gel picture showing cleavage properties of the tested compounds against pBR322 DNA. From the results of the study indicated that, the prepared azo compounds F₂₃ and F₂₄ cleaved Form II DNA and has not cleaved Form I DNA, whereas rest of the compounds (F₂₁ and F₂₂) did not showed much cleavage activity.

3.5.4. Molecular docking studies

Most of the drug design for the treatment of various diseases requires theoretical modelling of the structure of the drug. Nowadays, rather than going to synthesize the suitable compounds for the treatment, researchers chose to design the structure of the drugs by using theoretical modelling. One such method is the molecular docking, in which the interaction of the drugs with the appropriate proteins were checked. The *in silico* molecular docking is one of the most advanced technique to understand the bonding interaction, toxicity and multi drug resistance strain of the new drugs. One such attempt was made in the present work to check the mode of interaction of the synthesized compounds (F₂₁–F₂₄) with target receptor RpsA and the docking results of the studied compounds were depicted in the Table 7.

The 2D and 3D representations the interaction of compounds F₂₁–F₂₄ interacted with the target receptor RpsA have been shown in Figure 8 and Figure 9 respectively.

From the obtained results, it is noticed that the studied compounds were significantly interacted with the amino acids of the target protein RpsA with appreciable binding energy ranging from -5.0 to -5.9 kcal/mol and it is almost equal to the binding energy of the standard drug pyrazinamide. Therefore, our synthesized compounds were potentially efficient in interacting with the target receptor and can be utilised in the designing of potential drugs in the treatment of various diseases.

4. Conclusion

The present work aimed to synthesize novel heterocyclic coumarin based azo molecules by simple diazo-coupling reaction with good yield. The newly synthesized compounds were thoroughly characterized by elemental, FT-IR, UV-Visible, NMR and mass spectrometric studies. The structures of synthesized compounds were also studied by DFT method at B3LYP/6-31G(d,p). Further, these coumarin based azo dyes were screened for their antimicrobial, antitubercular, DNA cleavage and *in silico* molecular docking studies. The results of all the activities indicated that, the bioactive coumarin nucleus in the compounds could be able to show appreciable pharmacological properties against tested organisms. Thus, the newly synthesized azo compounds can be useful in the drug designing.

Declarations

Author contribution statement

Nagaraja O: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Yadav D. Bodke: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Itte Pushpavathi, Ravi Kumar S: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at <https://doi.org/10.1016/j.heliyon.2020.e04245>.

Acknowledgements

One of the authors Mr. Nagaraja O thankful to the Department of Industrial Chemistry for providing laboratory facility. Authors are also grateful to SAIF- Panjab University, Chandigarh for providing spectral data, Maratha Mandal's Central Research Lab, Belagavi and Biogenic, Hubli in getting the biological activity results.

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