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ARTICLE

A facile synthesis and antioxidant evaluation of conjugated 8-azacoumarins based on DFT parameters

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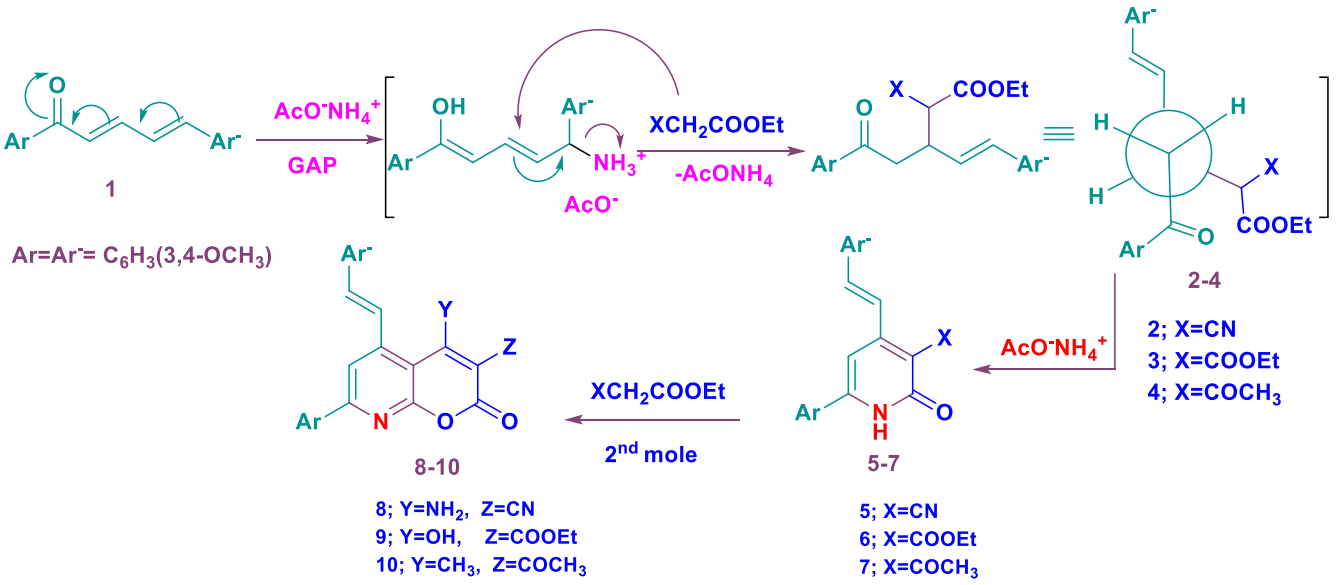
Abstract

A series of 8-azacoumarin, naphthyridine, and dioxazphenalene derivatives bearing conjugated moieties were intended and prepared by a concise and smooth approach using grinding and ultrasonic irradiation. A sustainable one pot reactions have emerged and progressed smoothly as an efficient and eco-safe methods in solvent less organic synthesis. The desired products were obtained in high yields, and the structures of all products were established on the basis of spectroscopic data and elemental analysis. Using the density functional theory (DFT) realized the electronic and structure characterization of synthesized azacoumarin. These heterocyclic compounds are chemically stable and are tested as antioxidants. Antioxidant potential was assessed using ABTS, and anti-hemolytic activity was evaluated by induced peroxide hemolysis. They gave good antioxidant results that showed potency ranging from good azacoumarin analogues **8**, **9**, **13**, **17**, and **20**, among compounds, showed much potency than that of commercial ascorbic acid.

1 | INTRODUCTION

Rapid attention toward clean environment and safeness has concerned universal efforts to promote green eco-friendly remarkable technique [1–3]. Thus, ultrasound corroborative synthesis in solvent less synthesis[4,5] had appeared and gradually utilized in organic synthesis because of more environmentally safe, readily controlled, production of higher yield, relatively low reaction time, and moderate reaction condition. More proper and faster synthetic processes that are energy saving became strongly desired in grinding MCR.[6–8] Green chemical procedure is excessively utilized for the time being and became important in combinatorial chemistry. Antioxidants have great attention owing to their important function in vital and manufacturing processes. Flavonoids are still one of the major naturally occurring antioxidants, more over their presence in green leaves, fruits, olive, purple grapes, dark chocolate, and green tea.[9–11] They possess interesting biological applications especially as antioxidant agents.[12–20] The predominant mechanisms are suggested to illustrate the preventive function for antioxidant agents; the first one is the shift of H-atom, and the second one is a one-electron transmit mechanism. The radicals emerging from both reactions ($ArO^{\bullet}$ and $ArOH^{\bullet+}$) should be settled to stop the chain reactions[21–25] or as active center of flavonoid free radical scavenging.[26] Furthermore, structure-activity relationship (SAR) study has been carried out on the antioxidant activity of chalcone derivatives.[27–29] Substitution of the CH of aryl group by N in position 8 of coumarin, ie, 8-azacoumarin is the most effective planning not only to enhance the hydrophilicity but also to upgrade metabolic stabilization of the antioxidant material. So, they got up enough interest reasonable category of biological applicants.[30] Specially, 8-azacoumarins display noticeable usage like ascorbic. Insufficient synthetic studies for 8-azacoumarin pathways have been totally progressed, with regard to the preparation of them.[31] Currently, a novel procedure has afforded to prepare them with highly expand mentioned via ultrasonic technique.[32–40] While, only few 8-azacoumarins were obtained before



SCHEME 1 Outline the reaction progress by using mechano-chemical grinding and mechanism of the two-component reaction of 8-azacoumarin synthesis [Colour figure can be viewed at wileyonlinelibrary.com]

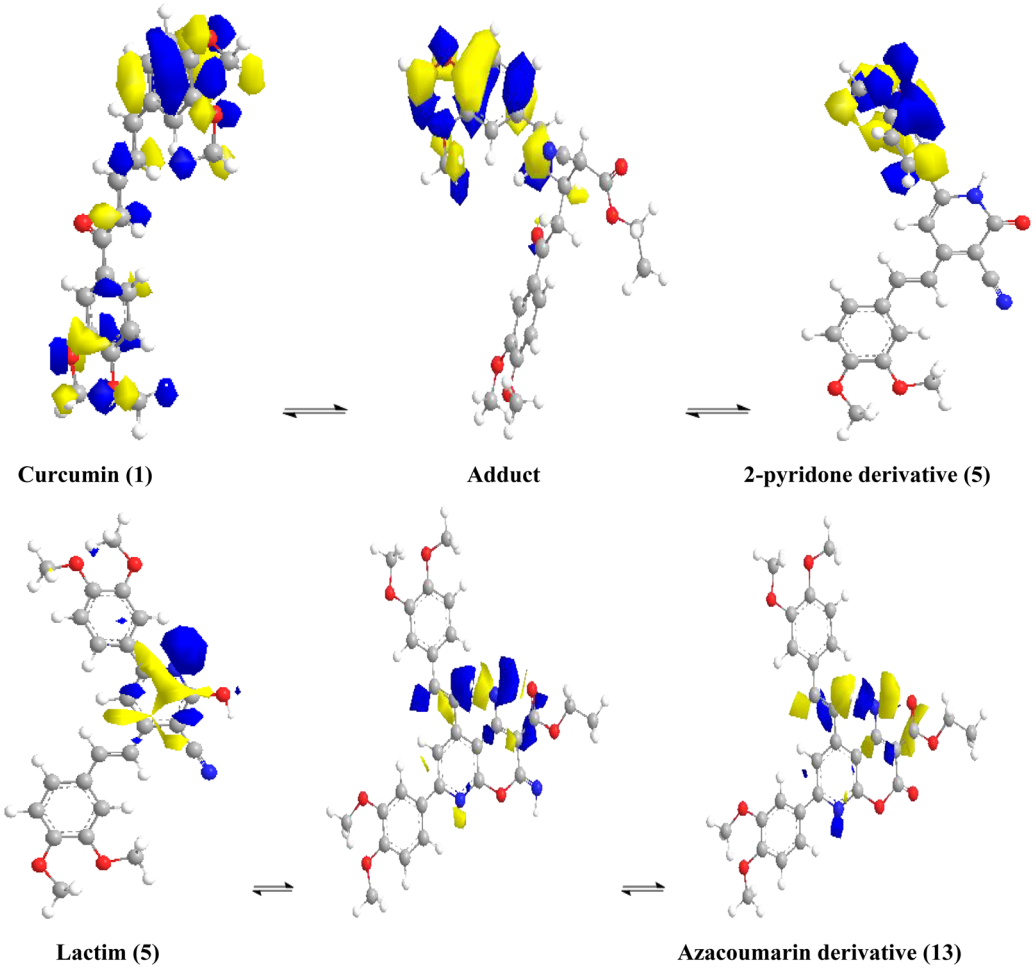
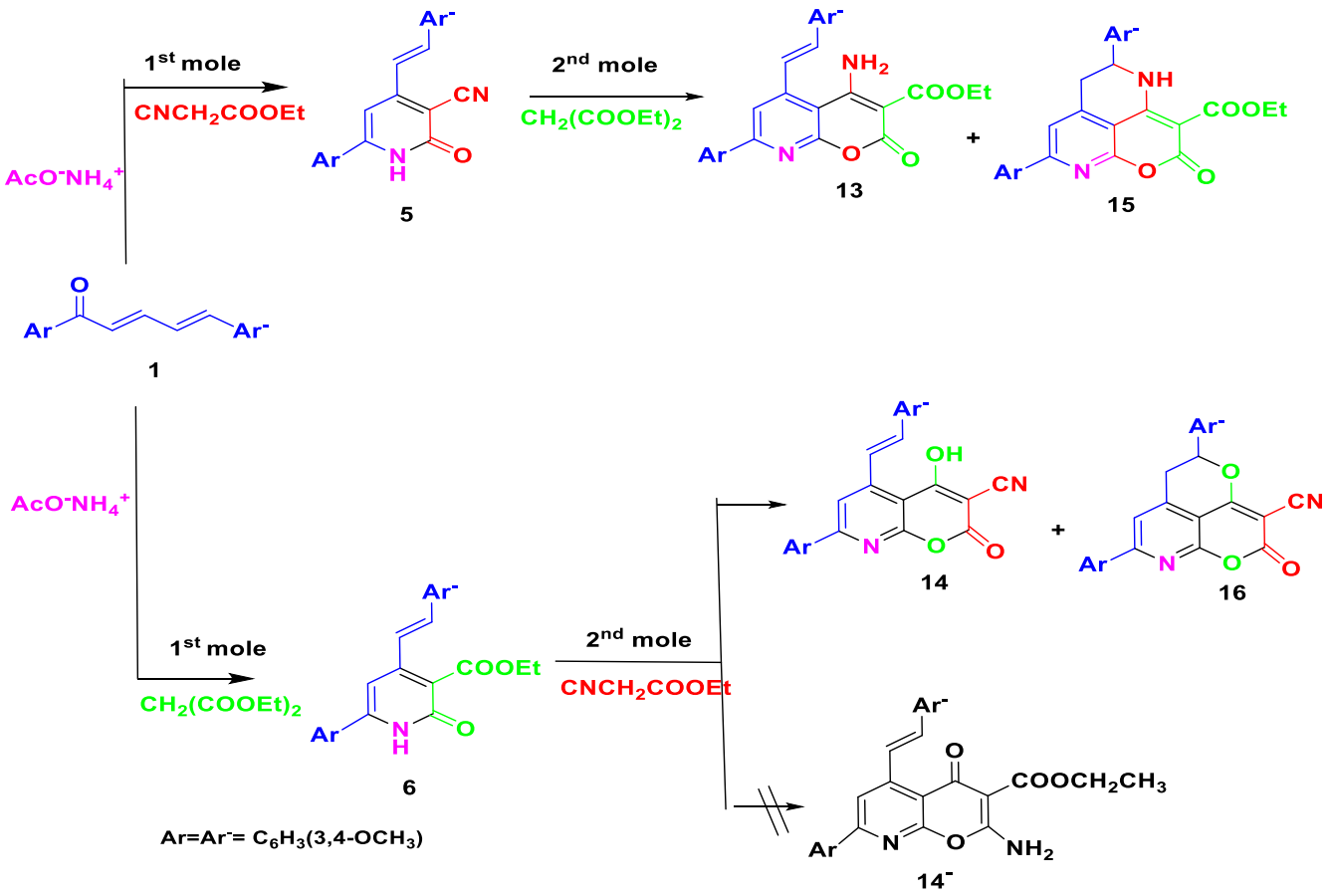


FIGURE 1 Mechanistic description by density functional theory (DFT) in convert curcumin to 8-azacoumarin 13 via ultrasonic irradiation [Colour figure can be viewed at wileyonlinelibrary.com]



SCHEME 2 Elucidate ultrasonic MCR of chalcone 1, ethylcyanoacetate, and diethyl malonate through mechanism for the three-component strategy [Colour figure can be viewed at wileyonlinelibrary.com]

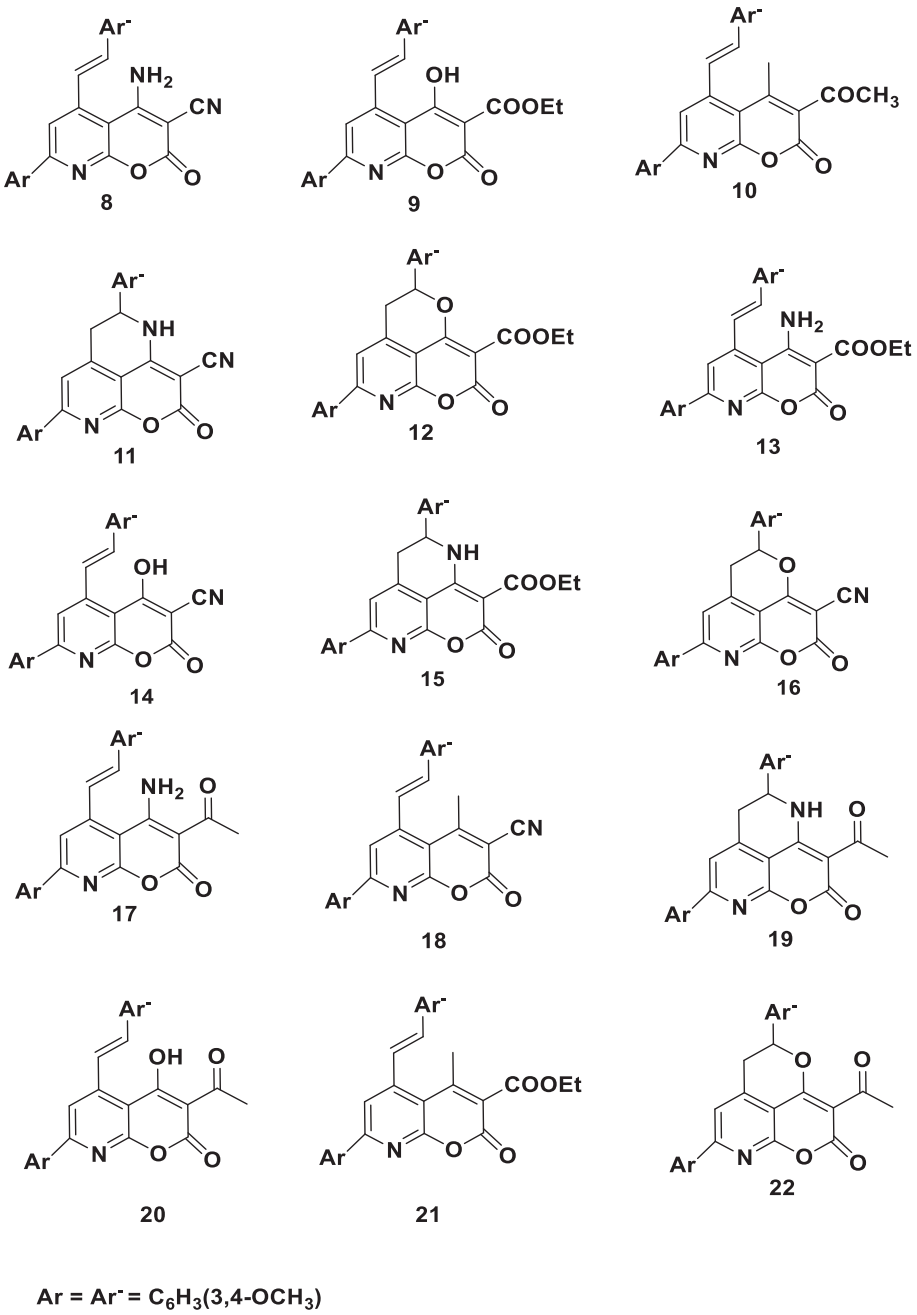
because of the ingrained weak nucleophilicity of pyridine ring, which indicates the proper technique lead to the desired products. In continuation of our work on MCRs, synthesis of 8-azacoumarin extended the fused heterocycles and significant biological actions[38–40].

2 | RESULTS AND DISCUSSION

Curcumin **1**, 2-substituted ethyl acetate, and ammonium acetate were subjected to react with each other under multicomponent reaction condition (MCR) by grinding all of them without utilizing any organic solvent for 15 to 20 minutes and gave crude red solid products (**8-10**) through 2-pyridone **5-7** that enhanced by ammonium acetate as group-assisted purification (GAP)[41–43] (Scheme 1).

Extending GAP rules in the produced materials can considerably minimize the production of waste, lowering operating costs (energy, eluent, silica gels) and formation of pure adducts **2-4** followed by ring closure using ammonium acetate to afford the pyridine-2-one intermediate **5-7.** See more in Figure 1, which outline the DFT mechanism to afford the target product of azacoumarin **8** via 2-pyridone intermediate.

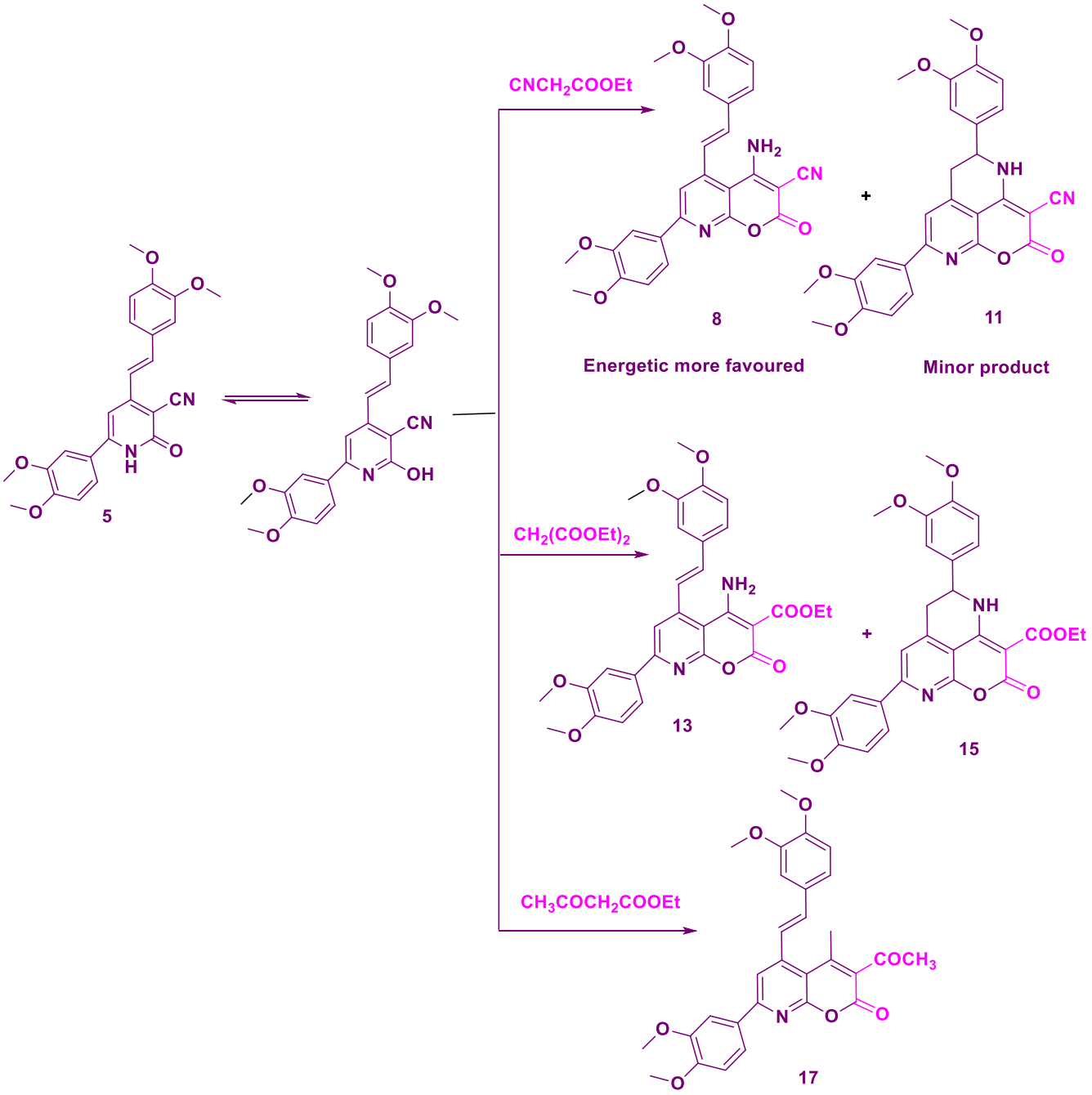
In the mechanochemical grinding condition, the second mole of 2-substituted ethyl acetate, eg, ethyl cyanoacetate, ethyl acetoacetate, or diethyl malonate, was reacted with pyridine-2-one derivatives **5-7** via two-component reactions (when using two moles of the same compound), afforded the 8-azacoumarin derivatives **8-10** in addition to pyrano-2,7-naphthyridine **11** and 3, 6-dioxa-7-azaphenalen-5-one **12**, respectively. Nevertheless, when chalcone **1** was submitted to interact with two moles of 2-substituted ethyl acetate, eg, ethyl cyanoacetate and diethylmalonate, via three-component reaction (using a mixture of two different compounds) afforded the azacoumarin derivatives **13** and **14** as major product in each case in addition to pyrano-2,7-naphthyridine **15** and 3,6-dioxa-7-azaphenalen-5-one **16**, respectively (Scheme 2). Both of aforementioned techniques afforded the desired products in good to excellent yields in addition to normal



SCHEME 3 Outline all the produced compounds

and moderate reaction conditions. Furthermore, the other case when curcumin **1** was subjected to react with ethyl cyanoacetate in the presence of ethyl acetoacetate via three component reactions, two products of azacoumarin derivatives **17** and **18** were afforded in addition to pyrano-2,7-naphthyridine **19**, respectively. In the same way, to the latter reaction with ethyl cyanoacetate and diethyl malonate, chalcone **1** afforded two azacoumarins **20**, **21** and 3, 6-dioxa-7-azaphenalen-5-one **22**, respectively**.** From Table 1, we can conclude from the percentage yield of reaction without the isolation of the chromone product **14**$^{-}$ inverses the reactivity of the ethyl cyanoacetate much more than diethyl malonate precursor and the all structural products **8**-**22** formed outlined in (Scheme 3).

However, in ultrasonic irradiation of the 3-cyano-2-pyridone derivatives **5**-**7** (that isolated under thermal reaction condition[44,45] isomerized to the reactive lactim intermediate form that is proceeding with substituted ethyl acetate under two- or three-component reactions afforded the energetically favorable azacoumarin **8**-**10** as a major product (Scheme 4). Some comparative data are tabulated in Table 2 concerning the yield, reaction time, yield economy (YE), atom economy (AE), and reaction mass efficiency (RME) of both procedures. The results can be indisputable for the sustainability green synthesis



SCHEME 4 Outline the reaction of isolated 2-pyridone with substituted ethyl acetate [Colour figure can be viewed at wileyonlinelibrary.com]

of the novel azacoumarin derivatives. The YE basically measures how much yield (%) of the desired product is obtained over a certain reaction time (ie, yield (%)/reaction time [min]). A higher YE is therefore indicative of a higher level of conversion, a much more efficient chemical process and more economical reaction.[46,47] So, values of YE in the mechanochemical and conventional reactions were 4.86 and 0.08, respectively, reveal larger difference, and the gain status of the former approach. A mechanistic illustration of the 3CR approach for azacoumarin formation was illustrated as before [38-40]. Structures of all synthesized compounds were established on the basis of spectroscopic data and elemental analysis. The IR spectra, exhibited strong absorption bands at 1747-1700 cm^{-1} corresponding to stretching frequency of carbonyl (vC═O) lactone, ester and acetyl groups of all 8-azacoumarin (**8**, **9**, **10**, **13**, **14**, **17**, **18**, **20**, and **21**) and disappeared (vC═O) at 1667-1660 of curcumin.

Moreover, they exhibited appearance of broad bands at 3440 to 3300 cm^{-1} corresponding to NH_2 and sharp band at 2221 cm^{-1} corresponding to C≡N, which prove the construction of products **8**, **11**, **14**, **16**, and **18**. The ^{1}H-

TABLE 1 Outline two and three component reactions

			2CR			3CR					
			XCH_2COOEt	Product		XCH_2COOEt					
Chalcone	Grinding, T m	Ultrasonic T m Irradiation,	(2 moles)	Yield m.p.		1 mole	1 mole	Product Yield m.p.			
1	15	25	$CH_2(CN)COOEt$	8	11	$CH_2(CN)CO_2Et$	$CH_2(COOC_2H_5)_2$	13	14	15	16
				72%	20%			40%	16%	22%	10%
				220-222	168-170			170-172	120-122	198-200	128-130
1	20	25	$CH_2(COOC_2H_5)_2$	9	12	$CH_2(CN)CO_2Et$	CH_3COCH_2COOEt	17	18	19	--
				76%	16%			42%	17%	29%	
				192-194	146-148			182-184	134-136	210-212	
1	20	30	$CH_2(COCH_3)COOEt$	10	--	$CH_2(COOEt)_2$	$CH_3COCH_2CO_2Et$	20	21	22	--
				71%				35%	20%	20%	
				232-234				210-212	176-178	142-144	

TABLE 2 Ultrasonic and grinding characterization of azacoumarins **8**, **9**, **10**, **13**, **14**, **17**, **18**, **20**, **21**, pyrano-2,7-naphthyridine **11**, **15**, **19**, and 3,6-dioxa-7-azaphenalen-5-one **12, 16, 22** via reaction of isolated 2-pyridone **5-7** with active methylene

					Yield %			Yield %		YE	%AE	
Pyrid-2-one	2^{nd} substituted ethylacetate	8-Azacoumarin	Michael Adduct	Ultrasonic Irradiation T min.	8Aza.	MA	Grinding T min.	8 Aza.	MA	Aza	Aza	RME
5	$CNCH_2COOEt$	8	11	15	73	20	10	70	15	4.86	90.30	0.90
5	**$CH_2(COOEt)_2$**	**13**	**15**	**15**	**70**	**22**	**10**	**64**	**19**	**4.67**	**91.18**	**0.91**
5	**CH_3COCH_2COOEt**	**17**	**19**	**10**	**68**	**29**	**15**	**65**	**21**	**6.80**	**90.44**	**0.90**
6	**$CH_2(COOEt)_2$**	**9**	**12**	**15**	**76**	**18**	**15**	**76**	**16**	**5.06**	**83.83**	**0.84**
6	**$CNCH_2COOEt$**	**14**	**16**	**15**	**67**	**20**	**15**	**60**	**17**	**4.46**	**82.37**	**0.82**
6	**CH_3COCH_2COOEt**	**20**	**22**	**15**	**65**	**22**	**15**	**60**	**18**	**4.34**	**82.93**	**0.83**
7	**CH_3COCH_2COOEt**	**10**	-	**15**	**78**	-	**15**	**70**	-	**5.20**	**87.42**	**0.87**
7	**$CNCH_2COOEt$**	**18**	-	**15**	**75**	-	**15**	**72**	-	**5.00**	**87.00**	**0.87**
7	**$CH_2(COOEt)_2$**	**21**	-	**15**	**71**	-	**15**	**70**	-	**4.73**	**88.13**	**0.88**

TABLE 3 Parameters of the quantum chemical computation for the synthesized 8-azacoumarins

Compound	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	I (eV)	A (eV)	χ (eV)	η (eV)	σ eV^{-1}	ΔN	μ (Debye)	A_{molec} (nm^2)
8	−8.572	−5.524	3.01	9.09	0.38	4.74	4.65	0.1068	0.26	8.790	297.443
9	−8.130	−7.525	0.59	8.88	0.83	4.86	4.03	0.1300	0.34	11.558	542.608
13	−5.537	−5.207	0.33	8.66	0.66	4.66	4.00	0.1491	0.38	12.139	590.401
17	−3.475	−2.724	0.751	8.135	1.239	4.69	3.45	0.1281	0.31	9.395	519.866
20	−3.764	2.204	1.56	8.432	1.143	4.36	3.48	0.1180	0.27	9.065	372.768

Abbreviations: *I*, Ionization potential; *A*, Electron affinity; *X*, electronegativily; *η*, hardness; *σ*, softness; *ΔN*, nucleophilicity index; *μ*, dipole moment; *Amolec*, surface area.

NMR spectra of novel 8-azacoumarin derivatives **8** and **9** revealed characteristic singlet signals at δ 9.02 and 9.63 ppm corresponding to NH and OH groups, respectively. Similarly, ^{1}H-NMR spectra of aza-Michael adducts **11**, **15**, and **19** showed signals at δ 6.47 to 6.75 ppm agreeing to NH protons, δ 6.87 to 8.34 ppm (m, ArH, and 1H py) occurred, which clearly established their molecular structures.

2.1 | DFT-based characterization

The quantum mechanical fully optimized minimum energy is used for the molecular parameters for the most potent antioxidant azacoumarin compounds **8**, **9**, **13**, **17**, and **20** that are listed in **Table** 3 (see more in the Supporting Information).

Proceeds, DFT-based anti-hemolytic and radical scavenger of such compounds supported their high antioxidant activity. The high E_{HOMO} indicated a strong donating electron and so the low values of the energy gap ($\Delta E = E_{LUMO} - E_{HOMO}$) will render good inhibition efficiencies, because the energy needed to remove an electron from the last occupied orbital will be low.[48–50] Hard molecules are characterized by larger values of ΔE and E_{HOMO} energy levels through the results of antioxidant activity. The HOMO energy (more negative values) of such 8-azacoumarin, the greater the trend of accepting electrons, and the energy gap (ΔE) are directly proportional with increasing the antioxidant efficiency.

E_{HOMO} parameter is direct proportional to the ionization potential (I) and susceptible towards attacks by electrophiles. HOMOs are distributed over the aryl and arylidene units, whereas LUMOs are focused on the azacoumarin moiety displayed in Table 3 and Supporting Information. The greater polarizable molecules will leave from solvent bulk to absorb radical or oxidized surface to form a protective film to become more inhibitor. Therefore, 8-azacoumarin precursors become most active cores for electron transfer either accept or donor electron, and so, it has considered as antioxidant agents.

The most azacoumarin additive were as follows: **13 > 9 > 17 > 20 > 8**, which were consistent with order of higher E_{HOMO} values of increasing inhibition efficiency. The scavenging ability toward positive hole, radical, and oxygen removable not only depended upon E_{HOMO} values, but also, the number of heteroatoms, electron distributions, surface area, and lipophilicity must be considered. The dipole moment, hardness, softness, and surface area (nm^2) for azacoumarin carrying hydrophobic groups were agreed to be an excellent correlation between oxidation inhibition efficiencies. On the other hand, the Ionization potential (I, eV), transferred electrons and Charge density distribution (ΔN) indicate the greater value of 0.38 for Azacoumarin **13** indicates the maximum transfer of electron. This is coincided with the experimental results,it has powerful antioxidant due to the greater tendency of scavenging radicals. Also, hardness (ή, eV/mol) and softness (σ, eV^{-1}) confirm that the soft molecule is more reactive than a hard molecule because a soft molecule has a lower energy gap. From Table 3, softness is decreased along 8-azacoumarin compounds **13 > 9 > 17 > 20 > 8** with values 0.1491 > 0.1300 > 0.12810 > 0.1180 > 0.1068 eV^{-1}, respectively, with the same trend for antioxidant efficiency.

2.2 | Antioxidant assays

2.2.1 | Anti-hemolytic activity

The oxidative erythrocyte membrane lipids and proteins may be responsible for hemolysis accompanying with several factors, viz, hemoglobinopathies, oxidative drugs, excess of transition metals, various radiation, and deficiencies in erythrocyte antioxidant coordination.[51] The magnitude of hemolysis was appeared to be much more overwhelming, when red blood cells were exposed to any toxicant like hydrogen peroxide.[52] Erythrocytes lysis

TABLE 4 Outline the erythrocyte hemolysis of the synthesized compounds

		Erythrocyte Hemolysis	
Entry	Compounds	A/B × 100 Absorbance of Samples (A)	% Hemolysis
Absorbance of H_2O (B)		0.850	---
Vit - C		0.031	3.64%
1	**1**	0.043	5.05%
2	**2**	0.043	4.98%
3	**3**	0.048	5.64%
4	**4**	0.048	5.64%
5	**5**	0.046	5.41%
6	**6**	0.042	4.94%
7	**7**	0.044	5.14%
8	**8**	0.041	4.88%
9	**9**	0.029	3.20%
10	**10**	0.048	5.64%
11	**11**	0.044	5.17%
12	**12**	0.046	5.41%
13	**13**	0.021	2.60%
14	**14**	0.043	5.06%
15	**15**	0.051	6.00%
16	**16**	0.052	6.11%
17	**17**	0.030	3.52%
18	**18**	0.042	4.94%
19	**19**	0.045	5.29%
20	**20**	0.039	4.64%
21	**21**	0.043	5.06%
22	**22**	0.031	3.60%

TABLE 5 Outline the ABTS scavenging power of the synthesized compounds

	Method	ABTS	
		Abs (control) − Abs (test)/Abs (control) × 100	
Entry	Compounds Control of ABTS Ascorbic-acid	Absorbance of samples 0.525 0.061	% inhibition 0 88.4%
1	**1**	0.404	23.0%
2	**2**	0.409	22.1%
3	**3**	0.262	50.1%
4	**4**	0.281	46.5%
5	**5**	0.412	21.5%
6	**6**	0.406	22.7%
7	**7**	0.408	22.3%
8	**8**	0.169	67.8%
9	**9**	0.043	91.7%
10	**10**	0.410	21.9%
11	**11**	0.363	30.8%
12	**12**	0.384	26.8%
13	**13**	0.034	93.5%
14	**14**	0.419	20.2%
15	**15**	0.101	80.8%
16	**16**	0.406	22.7%
17	**17**	0.054	89.8%
18	**18**	0.133	74.6%
19	**19**	0.408	22.3%
20	**20**	0.095	81.9%
21	**21**	0.384	26.8%
22	**22**	0.406	22.7%

was showed to be decreased with an increase in concentration of extract or fraction. This experiment was aimed to assess whether A. hydaspica prevented oxidative damages to erythrocyte membrane or not. The present experiment results occur the primary antioxidants, which possess anti-hemolytic effect; % hemolysis is increased along 8-azacoumarins **13 < 9 < 17 < Ascorbic < 20 < 8** that agreed with the DFT study (Table 4).

2.2.2 | ABTS radical scavenging assay

The attained result designated that compounds **9** and **17** and their derived fractions scavenge the ABTS radicals. Among the fractions, lowest EC50 values for radical ABTS scavenging as compound **17** (98.0 ± 0.1 μg/ml) while highest EC50 values were recorded for compound **9** (>500 ± 0.26 μg/ml) as shown in Table 5, that is, significantly higher than ascorbic acid (61 ± 0.2 μg/ml). Table 5 outlines the % inhibition decreased in ordered along the synthesized heterocyclic derivatives **9 > 17 > ascorbic > 20 > 8** that approximately agreed with antihemolytic and their DFT study.

3 | CONCLUSION

A sustainable green synthesis via MCRs for some 8-azacoumarin derivatives and their antioxidant evaluations are presented in this work. The most potent derivatives **8**, **9**, **13**, **17**, and **20** showed much better activities were produced via simple, two-step, and eco-friendly

synthetic protocols. Qualified study was concerning the result atom, YE, time, or RME has thru on grinding and ultrasound-assisted tools. All azacoumarin structures can be elucidated by elemental and spectral data. Preliminary structure activity relationship indicated that introduction of diverse functional groups in one molecule improved the possibility of binding with oxidative surface in addition to hydroxy (OH) amino (NH_2) and ester (COOEt) groups, which, in turn, favored the antioxidant activities.

4 | EXPERIMENTAL

Degree of melting points in open-glass capillaries stay uncorrected. The IR spectra (υ_{max} cm^{-1}) KBr pellets were reported on FT-IR Shimadzu-8400S Spectrophotometer (New York, New York). ^{1}H-NMR and ^{13}C-NMR spectra DMSO-d_6, TMS as internal standard, were chronicled on JEOL-AL 300 spectrophotometer (Rheinstetten, Germany, 400 and 100 MHz, Respectively. The mass spectra were done under the electron ionization 70 ev on Shimadzu GCMS-QP-1000 EX mass spectrometer (Kyoto, Japan). CHN analyses were verified at Central forced armed (CFA), Cairo, Egypt. Ultrasonication was achieved in a Toshcon SW 4 cleaner model (300 KHz/200 W. TLC (silica gel [6-120] mesh/UV light) is used to check the compounds' purity. All common reagents and solvents are distilled and pure. Chalcone (**1**) and pyridine-2-one (**5-7**) were synthesized by described method.[38]

4.1 | General procedure for the synthesis of azacoumarin derivatives (8, 9, 10, 13, 14, 17, 18, 20, and 21), pyrano-2,7-naphthyridine (11, 15, and 19), and 3,6-dioxa-7-azaphenalen-5-one (12, 16, and 22

4.1.1 | Method (i)

Curcumin (Chalcone) **1** (0.01 mol), $CNCH_2COOEt$, CH_3COCH_2COOEt or $CH_2(COOEt)_2$ (0.01 mol), and $CH_3COO^-NH_4^+$ (0.04 mol) were organized grinding in a mortar and transferred into a 250-mL round bottom flask with 50-mL ethanol. The reaction flask was situated in an ultrasonic cleaning bath (at optimum maximum energy at which maximum surface disturbance arises). The bath temperature was controlled by adding H_2O at 30°C. The reaction progress was checked by TLC using C_6H_6: EtOAc v/v 95:5 as solvent system. Sonication was continued until starting reactants disappeared as indicated by TLC. A yellow solid product was obtained within 25 to 30 minutes of irradiation (Table 1). After the completion of the reaction, the mixture was poured into crushed ice with constant stirring to obtain a yellow solid mass, which was dried and recrystallized from 95% ethanol.

4.1.2 | Method (ii)

Curcumin **1** or pyridine-2-one **5-7** (0.01 mol), $CNCH_2COOEt$, CH_3COCH_2COOEt or $CH_2(COOEt)_2$ (0.01 mol), and $CH_3COO^-NH_4^+$ (0.04 mol) were mechano-grinded in mortar for 15 to 20 minutes. The colorless reaction mixture changes to light yellow. The reaction progress was observed by TLC using C_6H_6: EtOAc 90:10, left overnight, a yellow solid crude product was formed and recrystallized from 95% aq. ethanol.

*6-(3,4-Dimethoxyphenyl)-4-(3,4-dimethoxystyryl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**5**)*

Yield white solid, IR (ν, cm^{-1}): 3284 (NH), 3050 (ArH), 2216 (C≡N), 1667 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.58-3.60 (s, 12H, 4OMe), 6.22 (s, 1H, PyH), 6.34-6.36 (d, 1H, ═CHAr, J = 15.88 Hz), 6.55-6.57 (d, 1H, PyCH═, J = 15.88 Hz), 7.38-7.79 (m, 6H, ArH), 11.62 (s, 1H, NH, exchangeable D_2O), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 61.3, 62.8, 103.9, 115.1, 123.3, 123.9, 126.4 (2), 128.4, 129.1 (2), 130.2, 131.7, 134.2 (2), 134.5, 135.2, 137.4, 144.2, 145.9, 156.1, 163.5; MS (m/z) [M^+] 418, Anal. Cal. for $C_{24}H_{22}N_2O_5$: C 68.89, H 5.30, N 6.69. Found: C 68.56, H 5.05, N 6.41.

*Ethyl-6-(3,4-dimethoxyphenyl)-4-(3,4-dimethoxystyryl)-2-oxo-1,2-dihydropyridine-3-carboxylate (**6**)*

White solid, IR (ν, cm^{-1}): 3362 (NH), 3045 (ArH), 1743, 1662 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.10-1.12 (t, 3H, $OCH_2\underline{CH_3}$, J = 7.54 Hz), 3.59-3.64 (s, 12H, 4OMe), 4.04-4.07 (q, 2H, $O\underline{CH_2}CH_3$, J = 7.54 Hz), 6.21 (s, 1H, PyH), 6.30-6.31 (d, 1H, ═CHAr, J = 15.54 Hz), 6.46-6.47 (d, 1H, PyCH═, J = 15.54 Hz), 7.38-7.79 (m, 6H, ArH), 11.62 (s, 1H, NH, exchangeable D_2O), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 59.1, 60.2, 105.5, 121.6, 122.7, 127.4, 127.9, 129.1, 131.3, 132.6, 134.2, 134.4, 134.7, 138.1, 141.6, 143.0, 145.2, 146.7, 160.5, 168.65; MS (m/z) [M^+] 465, Anal. Cal. for $C_{26}H_{27}NO_7$: C 67.09, H 5.85, N 3.01. Found: C 66.83, H 5.62, N 2.77.

*3-Acetyl-6-(3,4-dimethoxyphenyl)-4-(3,4-dimethoxystyryl)pyridin-2(1H)-one (**7**)*

White solid, IR (ν, cm^{-1}): 3360 (NH), 3060 (ArH), 1684, 1670 (C═O). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.63 (s, 3H, CH_3CO), 3.57-3.60 (s, 12H, 4OMe), 6.12 (s, 1H, PyH), 6.21-6.26 (d, 1H, ═CHAr, J = 15.20 Hz), 6.42-6.45 (d, 1H, PyCH═, J = 15.20 Hz), 7.38-7.79 (m, 6H, ArH), 11.62 (s, 1H, NH, exchangeable D_2O), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 60.9, 61.7, 99.7, 103.7, 123.0, 123.5, 126.4 (2), 127.9,

129.1, 131.0, 132.4, 134.2, 134.5, 134.8, 136.4, 144.7, 146.2, 158.9, 164.2, 195.6; MS (*m*/*z*) [M^+] 435, Anal. Cal. for $C_{25}H_{25}NO_6$: C 68.95, H 5.79, N 3.22. Found: C 68.72, H 5.51, N, 3.00.

*4-Amino-7-(3,4-dimethoxyphenyl)-5-(3,4-dimethoxystyryl)-8-azacoumarin-3-carbonitrile (**8**)*

Yellow crystal, IR (ν, cm^{-1}): 3320, 3265, (NH_2), 3063 (CH), 2221 (CN), 1738 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.58-3.65 (bs, 12H, 4OMe), 5.62 (s, 2H, NH_2, exchangeable D_2O), 6.52 (s, 1H, PyH), 6.74-6.76 (d, 1H, ═CHAr, J = 14.92 Hz), 6.87-6.89 (d, 1H, PyCH═, J = 14.92 Hz), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 58.6, 60.3, 103.2, 108.7 123.7, 123.9, 127.4, 127.7, 129.7, 130.5, 131.8, 133.4, 134.2, 134.6, 138.1, 160.6 161.7, 165.8; MS (*m*/*z*) [M^+] 485, Anal. Cal. for $C_{27}H_{23}N_3O_6$: C 66.80, H 4.78, N 8.66. Found: C 66.56, H 4.57, N 8.31.

*Ethyl-7-(3,4-dimethoxyphenyl)-5-(3,4-dimethoxystyryl)-4-hydroxy-8-azacoumarin-3-carboxylate (**9**)*

Yellow solid, IR (ν, cm^{-1}): 3436 (OH), 3070 (ArH), 1749, 1737 (C═O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.07-1.09 (t, 3H, $OCH_2\underline{CH_3}$, J = 7.61 Hz), 3.49-3.62 (bs, 12H, 4OMe), 4.20-4.22 (q, 2H, $O\underline{CH_2}CH_3$, J = 7.61 Hz), 6.54 (s, 1H, PyH), 6.72-6.74 (d, 1H, ═CHAr, J = 15.11 Hz), 6.86-6.87 (d, 1H, PyCH═, J = 15.11 Hz), 7.38-7.79 (m, 6H, ArH), 13.71 (s, 1H, OH, exchangeable D_2O), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.32, 52.0, 58.3, 61.2, 97.1, 104.6, 116.8, 123.2, 123.8, 127.4, 127.7, 129.1 (2), 131.1, 132.5, 134.3, 134.5, 134.8, 138.3, 146.3, 146.9, 159.8, 165.1; MS (*m*/*z*) [M^+] 533, Anal. Cal. for $C_{29}H_{27}NO_9$: C 65.29, H 5.10, N 2.63. Found: C 65.08, H 4.88, N 2.38.

*3-Acetyl-7-(3,4-dimethoxyphenyl)-5-(3,4-dimethoxystyryl)-4-methyl-8-azacoumarin (**10**)*

Yellow solid, IR (ν, cm^{-1}): 3090 (ArH), 1740, 1692 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.58 (s, 3H, CH_3CO), 3.11 (s, 3H, CH_3), 3.57-3.79 (d, 12H, 4OMe), 6.62 (s, 1H, PyH), 6.91-6.93 (d, 1H, ═CHAr, J = 15.20 Hz), 7.12-7.15 (d, 1H, PyCH═, J = 15.20 Hz), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.2, 22.4, 57.6, 58.6, 60.8, 101.2, 104.9, 123.3, 123.79, 127.4, 127.5, 129.8, 131.6, 132.8, 133.8, 134.2, 134.7, 138.5, 144.6, 146.4, 159.1, 164.0, 165.4, 194.1; MS (*m*/*z*), [M^+] 501. Anal. Found: C 66.78, H 4.32, N 6.09. Cal. for $C_{29}H_{27}NO_7$: C 66.88, H 4.71, N 6.23.

*5,8-bis(3,4-dimethoxyphenyl)-2-oxo-2,4,5,6-tetrahydropyrano[2,3,4-ij][2,7] naphthyridine-3-carbonitrile (**11**)*

Pale yellow solid, IR (ν, cm^{-1}): 3320 (NH), 3087 (ArH), 2241 (CN), 1738 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.67-3.72 (bs, 12H, 4OMe), 3.94-3.96 (dd, 2H, CH_2Py, diastereotopic protons, J = 15.65, 14.33 Hz), 4.17-4.19 (dd, 1H, CHAr, stereogenic proton, J = 15.65, 14.33 Hz), 4.62 (bs, 1H, NH, exchangeable D_2O), 6.52 (s, 1H, PyH), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.9, 21.8, 33.4, 57.3, 59.4, 71.5, 104.8, 116.3, 123.4, 123.9, 127.4, 127.8, 129.1, 131.4, 132.8, 134.6, 134.8, 135.1, 136.5, 146.3, 146.9, 155.6, 165.9, 169.6; MS (*m*/*z*) [M^+] 485. Anal. Cal. for $C_{27}H_{23}N_3O_6$: C 66.80, H 4.78, N 8.66. Found: C 66.51, H 4.57, N 8.41.

*Ethyl-2,8-bis(3,4-dimethoxyphenyl)-5-oxo-1,5-dihydro-2H-3,6-dioxa-7-azaphenalene-4-carboxylate (**12**)*

Pale yellow solid, IR (ν, cm^{-1}): 3062, 2894 (CH), 1753, 1737 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.07-1.09 (t, 3H, $OCH_2\underline{CH_3}$, J = 7.61 Hz), 3.58-3.64 (bs, 12H, 4OMe), 3.72-3.74 (dd, 2H, CH_2Py, diastereotopic protons, J = 15.44, 13,76 Hz), 4.01-4.05 (dd, 1H, CHAr, stereogenic proton, J = 15.44, 13.64 Hz), 4.18-4.21 (q, 2H, $O\underline{CH_2}CH_3$, J = 7.61 Hz), 6.72 (s, 1H, PyH), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.9, 21.3, 24.8, 35.8, 58.7, 60.4, 62.8, 103.9, 111.2, 121.3, 124.1, 126.4, 128.3, 129., 131.6, 133.4, 134.6, 134.8, 135.8, 137.5, 145.9, 147.0, 160.2, 162.8, 163.9, 167.0; MS (*m*/*z*) [M^+] 533. Anal. Cal. for $C_{29}H_{27}NO_9$: C 65.29, H 5.10, N 2.63. Found: C 65.33, H 4.87, N 2.45.

*Ethyl--4-amino-7-(3,4-dimethoxyphenyl)-5-(3,4-dimethoxystyryl)-8-azacoumarin-3-carboxylate (**13**)*

Yellow solid, IR (ν, cm^{-1}): 3275, 3211)NH_2), 3051 (ArH), 1752, 1740 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.07-1.09 (t, 3H, $OCH_2\underline{CH_3}$, J = 7.61 Hz), 3.53-3.60 (bs, 12H, 4OMe), 4.20-4.22 (q, 2H, $O\underline{CH_2}CH_3$, J = 7.61 Hz), 5.71-5.75 (bs, 1H, NH_2, exchangeable D_2O), 6.54 (s, 1H, PyH), 6.65-6.67 (d, 1H, ═CHAr, J = 15.11 Hz), 6.75-6.77 (d, 1H, PyCH═, J = 15.11 Hz), 7.22-7.68 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.5, 59.3, 60.6, 76.4, 103.9, 116.3, 121.7, 124.2, 125.8, 127.4, 127.7, 131.5, 133.3, 134.3, 135.1, 136.9, 144.4, 145.7, 157.7, 163.5, 181; MS (*m*/*z*) [M^+] 532, Anal. Cal. for $C_{29}H_{28}N_2O_8$: C 65.41, H 5.30, N 5.26. Found: C 65.11, H 5.00, N 4.91.

*7-(3,4-Dimethoxyphenyl)-5-(3,4-dimethoxystyryl)-4-hydroxy-8-azacoumarin-3-carbonitrile (**14**)*

Yellow solid, IR (ν, cm^{-1}): 3443 (OH), 3055 (CH), 2223 (CN), 1738 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.52-3.73 (bs, 12H, 4OMe), 6.54 (s, 1H, PyH), 6.74-6.76 (d, 1H, ═CHAr, J = 14.95 Hz), 6.87-6.89 (d, 1H, PyCH═, J = 14.95 Hz), 7.38-7.79 (m, 6H, ArH), 14.88 (s, 1H, OH, exchangeable D_2O), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 57.9, 58.9, 60.3, 103.2, 108.7 123.7, 123.9, 127.4, 127.7, 129.7, 130.5, 131.8, 133.4, 134.2, 134.6, 138.1, 145.5, 147.4, 160.6,

163.7, 170.8, 197.5; MS (m/z) [M^+] 486, Anal. Cal. for $C_{27}H_{22}N_2O_7$: C 66.66, H 4.56, N 5.76. Found: C 66.34, H 4.27, N 5.51.

*Ethyl-5,8-bis(3,4-dimethoxyphenyl)-2-oxo-2,4,5,6-tetrahydropyrano[2,3,4-ij][2,7] naphthyridine-3-carboxylate (**15**)*

Pale yellow solid, IR (ν, cm^{-1}): 3316 (NH), 3070 (ArH), 1749, 1735 (C═O); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.07-1.09 (t, 3H, $OCH_2\underline{CH_3}$, J = 7.61 Hz), 3.85-3.64 (bs, 12H, 4OMe), 3.72-3.74 (dd, 2H, CH_2Py, diastereotopic protons, J = 15.44, 13,76 Hz), 4.01-4.05 (dd, 1H, CHAr, stereogenic proton, J = 15.44, 13.64 Hz), 4.18-4.21 (q, 2H, $O\underline{CH_2}CH_3$, J = 7.61 Hz), 5.38 (s, 1H, NH, exchangeable D_2O), 6.72 (s, 1H, PyH), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.2, 22.0, 58.7, 59.7, 97.1, 104.6, 116.8, 123.2, 123.8, 127.4, 127.7, 129.1, 131.1, 132.5, 134.3, 134.5, 134.8, 138.3, 146.3, 146.9, 156.8, 165.1, 170.2; MS (m/z) [M^+] 532, Anal. Cal. for $C_{29}H_{28}N_2O_8$: C 65.41, H 5.30, N 5.26. Found: C 65.12, H 5.08, N 5.00.

*2,8-Bis(3,4-dimethoxyphenyl)-5-oxo-1,5-dihydro-2H-3,6-dioxa-7-azaphenalene-4-carbonitrile (**16**)*

Pale yellow solid, IR (ν, cm^{-1}): 3090 (ArH), 2219 (CN), 1742 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.65-3.82 (d, 12H, 4OMe), 3.97-3.99 (dd, 2H, CH_2Py, diastereotopic protons, J = 16.05, 14.73 Hz), 4.30-4.32 (dd, 1H, CHAr, stereogenic proton, J = 16.05, 14.73 Hz), 6.52 (s, 1H, PyH), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.2, 22.4, 58.4, 60.8, 101.2, 104.9, 123.3, 123.79, 127.4, 127.5, 129.8, 131.6, 132.8, 133.8, 134.2, 134.7, 138.5, 144.6, 146.4, 159.1, 164.0, 165.4, 174.1; MS (m/z), [M^+] 486. Anal. Cal. for $C_{27}H_{22}N_2O_7$: C 66.66, H 4.56, N 5.76. Found: C 66.38, H 4.32, N 5.49.

*3-Acetyl-4-amino-7-(3,4-dimethoxyphenyl)-5-(3,4-dimethoxystyryl)-8-azacoumarin (**17**)*

Yellow solid, IR (ν, cm^{-1}): 3320, 3267 (NH), 3087 (ArH), 1738, 1690 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.58 (s, 3H, CH_3CO), 3.56-3.79 (bs, 12H, 4OMe), 5.38 (s, 2H, NH_2, exchangeable D_2O), 6.62 (s, 1H, PyH), 6.91-6.93 (d, 1H, ═CHAr, J = 15.20 Hz), 7.12-7.15 (d, 1H, PyCH═, J = 15.20 Hz), 7.38-7.79 (m, 6H, ArH),^{13}C NMR (100 MHz, $CDCl_3$) δ: 25.4, 59.8,61.2, 101.2, 104.9, 123.3, 123.79, 127.4, 127.5 (2), 129.8, 131.6, 132.8, 133.8, 134.2, 134.7, 138.5, 144.6, 146.4, 159.1, 164.0, 165.4, 194.1; MS (m/z) [M^+] 502. Anal. Cal. for $C_{28}H_{26}N_2O_7$: C 66.92, H 5.22, N 5.57. Found: C 66.51, H 4.97, N 5.21.

*7-(3,4-Dimethoxyphenyl)-5-(3,4-dimethoxystyryl)-4-methyl-8-azacoumarin-3-carbonitrile (**18**)*

Yellow solid, IR (ν, cm^{-1}): 3050, 2913 (CH), 2222 (CN), 1737 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 3.04 (s, 3H, CH_3), 3.65-3.72 (bs, 12H, 4OMe), 6.52 (s, 1H, PyH), 6.74-6.76 (d, 1H, ═CHAr, J = 14.92 Hz), 6.87-6.89 (d, 1H, PyCH═, J = 14.92 Hz), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 37.3, 57.5, 58.9, 60.3, 103.2, 108.7 123.7, 123.9, 127.4, 127.7, 129.7, 130.5, 131.8, 133.4, 134.2, 134.6, 138.1, 160.6 161.7, 165.8; MS (m/z) [M^+] 484. Anal. Cal. for $C_{28}H_{24}N_2O_6$: C 69.41, H 4.99, N 5.78. Found: C 69.13, H 4.72, N 5.45.

*3-Acetyl-5,8-bis(3,4-dimethoxyphenyl)-5,6-dihydropyrano[2,3,4-ij][2,7] naphthyridin-2(4H)-one (**19**)*

Pale yellow crystal, IR (ν, cm^{-1}): 3315, 3175)NH_2), 3051 (ArH), 1740, 1695 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.74 (s, 3H, CH_3CO), 3.56-3.64 (bs, 12H, 4OMe), 3.72-3.74 (dd, 2H, CH_2Py, diastereotopic protons, J = 15.44, 13,76 Hz), 4.01-4.05 (dd, 1H, CHAr, stereogenic proton, J = 15.44, 13.64 Hz), 5.56 (s, 1H, NH, exchangeable D_2O), 6.72 (s, 1H, PyH), 7.38-7.79 (m, 6H, ArH),^{13}C NMR (100 MHz, $CDCl_3$) δ: 23.5, 58.7, 60.3, 61.3, 76.4, 103.9, 116.3, 121.7, 124.2, 125.8, 127.4, 127.7, 131.5, 133.3, 134.3, 135.1, 136.9, 144.4, 145.7, 157.7, 163.5, 181; MS (m/z) [M^+] 502, Anal. Cal. for $C_{28}H_{26}N_2O_7$: C 66.92, H 5.22, N 5.57. Found: C 66.71, H 5.00, N 5.21.

*3-Acetyl-7-(3,4-dimethoxyphenyl)-5-(3,4-dihydroxystyryl)-4-hydroxy-8-azacoumarin (**20**)*

Yellow solid, IR (ν, cm^{-1}): 3503 (OH), 3090 (ArH), 1742, 1699 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.82 (s, 3H, CH_3CO), 3.57-3.64 (bs, 12H, 4OMe), 6.54 (s, 1H, PyH), 6.72-6.74 (d, 1H, ═CHAr, J = 15.11 Hz), 6.86-6.87 (d, 1H, PyCH═, J = 15.11 Hz), 7.38-7.79 (m, 6H, ArH), 12.51 (s, 1H, OH, exchangeable D_2O), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 22.4, 58.8, 60.8, 101.2, 104.9, 123.3, 123.79, 127.4, 127.5, 129.8 (2), 131.6, 132.8, 133.8, 134.2, 134.7, 138.5, 144.6, 146.4, 159.1, 164.0, 165.4, 174.1; MS (m/z), [M^+] 503. Anal. Cal. for $C_{28}H_{25}NO_8$: C 66.79, H 5.00, N 2.78. Found: C 66.45, H 4.72, N 2.49.

*Ethyl-7-(3,4-dimethoxyphenyl)-5-(3,4-dihydroxystyryl)-4-methyl-8-azacoumarin-3-carboxylate (**21**)*

Yellow solid, IR (ν, cm^{-1}): 3087 (ArH), 1758, 1740 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.07-1.09 (t, 3H, $OCH_2\underline{CH_3}$, J = 7.61 Hz), 3.24 (s, 3H, CH_3), 3.68-3.83 (d, 12H, 4OMe), 4.20-4.22 (q, 2H, $O\underline{CH_2}CH_3$, J = 7.61 Hz), 6.54 (s, 1H, PyH), 6.72-6.74 (d, 1H, ═CHAr, J = 15.11 Hz), 6.86-6.87 (d, 1H, PyCH═, J = 15.11 Hz), 7.38-7.79 (m, 6H, ArH), ^{13}C NMR (100 MHz, $CDCl_3$) δ: 19.9, 21.8, 61.5, 104.8, 116.3, 123.4, 123.9, 127.4, 127.8, 129.1, 131.4, 132.8, 134.6, 134.8, 135.1, 136.5, 146.3, 146.9, 155.6, 165.9, 169.6; MS (m/z) [M^+] 531. Anal. Cal. for $C_{30}H_{29}NO_8$: C 67.79, H 5.50, N 2.64. Found: C 67.51, H 5.27, N 2.41.

*4-Acetyl-2,8-bis(3,4-dimethoxyphenyl)-1,2-dihydro-5H-3,6-dioxa-7-azaphenalen-5-one (**22**)*

Pale yellow solid, IR (ν,cm^{-1}): 3064, 2899 (CH), 1737, 1694 (C═O), ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.74 (s, 3H, CH_3CO), 3.58-3.64 (bs, 12H, 4OMe), 3.72-3.74 (dd, 2H, CH_2Py, diastereotopic protons, J = 15.44, 13,76 Hz), 4.01-4.05 (dd, 1H, CHAr, stereogenic proton, J = 15.44, 13.64 Hz), 6.72 (s, 1H, PyH), 7.38-7.79 (m, 6H, ArH),^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.8, 24.4, 37.8, 58.5, 60.3, 62.8, 103.9, 111.2, 121.3, 124.1, 126.4, 128.3, 129.5, 131.6, 133.4, 134.6, 134.8, 135.8, 137.5, 145.9, 147.0, 160.2, 162.8, 163.9, 167.0; MS (*m*/*z*) [M^+] 503. Anal. Cal. for $C_{28}H_{25}NO_8$: C 66.79, H 5.00, N 2.78. Found: C 66.43, H 4.72, N 2.45.

4.2 | Calculation details

Materials Studio 6.0 (MS6.0) software from Accelrys was working DMol3 module to achieve DFT calculations using LDA and DND source. The calculated parameters involved Frontier molecular orbitals (FMOs), ionization potential, electron affinity, electronegativity, dipole moment, hardness, softness, and Mullikan atomic charges. FMOs for each molecule contain HOMO and LUMO. Molecular dynamics (MD) simulation was approved to demonstrate the adsorption of the 8-azacoumarin studied on the oxidized surface at the molecular level. The crystals of adsorbent azacoumarin derivatives were forced to duck the trouble atoms during process of simulation.

4.3 | Antioxidant experiment

4.3.1 | Antihemolytic activity

Extract of the anti-hemolytic was observed by spectrophotometric procedure as described previously.[53] Five milliliters of blood from a healthy person was quiet in EDTA vials. Filter supernatant for 5 minutes at 1000 × g and were unconcerned (0.2M, pH 7.4) hanging in saline solution (0.5 %); 0.5 mL of the fractions (100-1000 μg/mL in PBS) was distributed to 1 mL of suspended erythrocyte and incubated at room temperature (20 min). Next, add 0.5 mL of H_2O_2 solution made in buffered saline to the reaction mixture for irritating oxidative degradation of the membrane lipids. Then, the samples were centrifuged at 1000 × g for 10 minutes, and the absorbance of supernatant was well-known spectrophotometrically at 540 nm. Hemolysis was measured in assessment with the H_2O_2 hemolysis (negative control), which was set as 100%. For positive control, phosphate buffer saline was used. Performed triplicate experiments and different fractions were calculated the inhibitory activity expressed as percent hemolysis inhibition.

4.3.2 | ABTS test

Test of ABTS was working to appraise the prospective of antioxidant biological fluids, synthetic complexes, and natural tissues. The cation radical $ABTS^+$ creation encouraged by hydrogen peroxide and metmyoglobin is restrained by hitherto start protocol.[54] Reaction of ABTS (7mM) in dark with $K_2S_2O_7$ (2.45mM) for 12 hours to get a dark shaded radical cation of ABTS solution. The ABTS solution castoff the examiner was ready by diluting it with methanol (50 %) to attain an absorbance of around 0.70 at 745 nm. Quenching ABTS radical probable was arbitrated by adding 1.0 mL of ABTS solution in 100 μL. The absorbance was verified precisely after 1 minutes, then at the third minute and last reading recorded at the sixth minute. The calculate percentage inhibition: Quenching ability = Control abs_s/abs_c × 100. Ascorbic acid as a standard control in ABTS reaction potassium persulfate in antioxidant outcomes afforded $ABTS^+$ blue/green chromophores spectrophotometrically determined at an absorbance of 745 nm. This antioxidant action is equally pertinent to hydrophilic and lipophilic classes as flavonoids, hydroxycinnamates, carotenoids, and antioxidants in the plasma.[55]

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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