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Catalyst-Free, Room-Temperature Accessible Regioselective Synthesis of Spiroquinolines and Their Antioxidant Study

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Cite This: *ACS Omega* 2023, 8, 444−456

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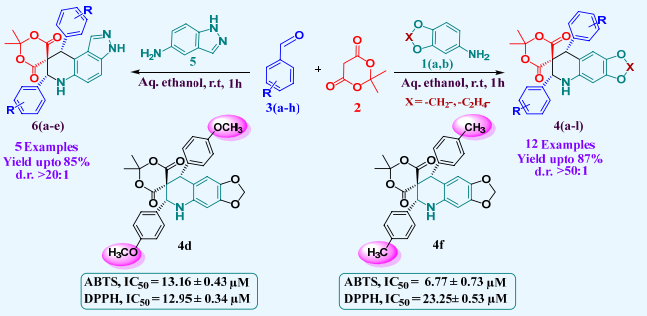
ABSTRACT: An efficient, regioselective, and environmentally benign approach was established using the multicomponent reaction-based synthesis of novel antioxidant spiroquinoline derivatives such as spiro[dioxolo[4,5-*g*]quinoline], spiro[dioxino-[2,3-*g*]quinoline], and spiro[pyrazolo[4,3-*f*]quinoline] by reaction of aryl aldehyde, Meldrum's acid, and amine derivatives under an additive-free reaction in aqueous ethanol. Here, two asymmetric carbon centers, three new C−C bonds, and one C−N bond are developed in the final motif. This synthetic methodology offers excellent yields with an easy workup procedure, high diastereoselectivity [d.r. >50:1 (*cis*/*trans*)], admirable atom economy, and low *E*-factor values. Synthesized spiro compounds were investigated for their in vitro antioxidant activity by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assays. In the ABTS radical scavenging assay, compounds **4d**, **4f**, and **4l** exhibit excellent potency, and in the DPPH radical scavenging assay, compounds **4a**, **4d**, **4f**, and **4g**, exhibit excellent potency.

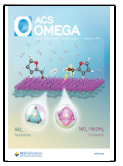


1. INTRODUCTION

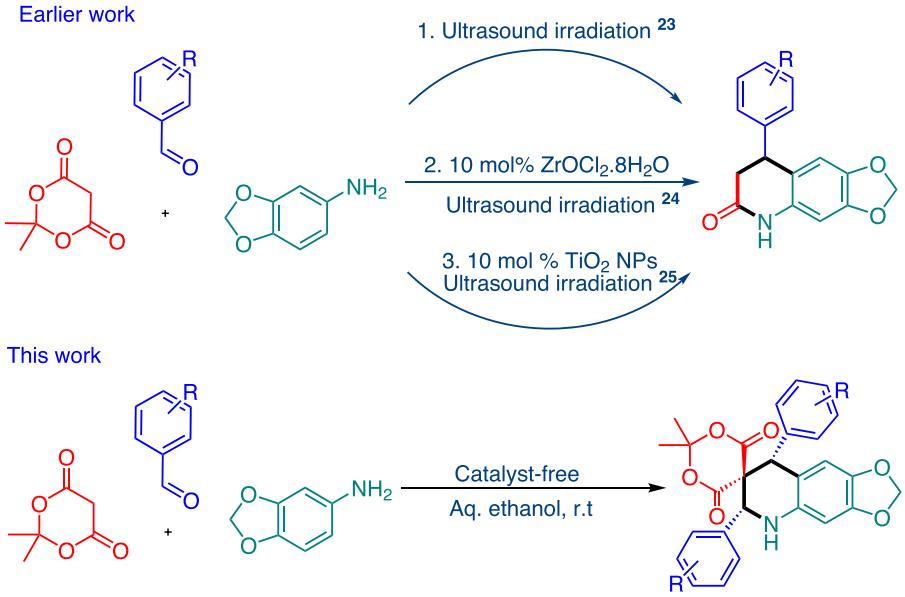
In present days, the development of novel heterocycles through a sustainable synthetic route is of much-growing interest.[1−3] Especially, multicomponent reactions provide excellent atom economy (AE) and synthetic efficiency to construct different heterocycles of biological interest via multiple C−C bond formation without isolation of an intermediate. In such cases, the challenging task is maintaining the sustainability of the procedure because of the higher sensitivity of functional groups attached to the substrate at a higher temperature. Due to the operational simplicity and better reaction efficiency, MCRs are more susceptible than conventional multistep synthesis.[4−10] The toxicity of some highly volatile and hazardous organic solvents poses a threat to the workers if they pass into the atmosphere. Highly volatile solvents often cause fires and/or detonations, resulting in destruction. Therefore, the attempt made to explore organic synthesis in aqueous solvents, deep eutectic solvents, and ionic liquids are in high demand.[11−17] The reactions which lead to successful conversion in aqueous media are gaining much more attention in synthetic organic chemistry not only because water is abundant in nature but due to extensive hydrogen bonding, inexpensive, environmentally benign, high dielectric constant, non-flammability, eco-compatibility, and selectivity in many organic reactions than conventional organic solvents.[18−20]

In the past few years, researchers have developed different multicomponent reactions for the synthesis of small heterocycles of biological and chemical interest.[21,22] For example, the multicomponent synthesis of 7,8-dihydro-[1,3]dioxolo[4,5-*g*]quinolin-6(5*H*)-ones using 5-amino-1,3-benzodioxole, aldehyde, and Meldrum's acid. In 2012, Azarifar and Sheikh have developed an ultrasound-assisted multicomponent reaction of 5-amino-1,3-benzodioxole, aldehyde, and isopropylidene malonate (Meldrum's acid) under the neat condition at ambient temperature for the synthesis of 7,8-dihydro-[1,3]dioxolo[4,5-*g*]quinolin-6(5*H*)-ones.[23] In 2013, the same authors reported an efficient synthesis of 8-aryl-7,8-dihydro[1,3]dioxolo[4,5-*g*]quinolin-6-(5*H*)-ones and 4-aryl-3,4-dihydroquinolin-2-(1*H*)ones using Zirconyl chloride octahydrate catalyst.[24] In 2019, the same MCR was performed by Bhardwaj et al. using TiO_2-based nanoparticles in water and achieved the same motif (Scheme 1).[25] These derivatives are formed because Meldrum's acid commonly holds the exclusive ring-seized malonic acid part, which is generated by the loss of an acetone

Received: August 6, 2022
Accepted: November 30, 2022
Published: December 19, 2022



Scheme 1. Multicomponent Reaction of Meldrum's Acid, Aldehyde, and Amine



molecule, while the reaction with nucleophiles can be convoyed by the liberation of carbon dioxide.[26−28]

Antioxidant activity of dihydro and tetrahydroquinolines were reported rarely; however, quinoline-based alkaloids show excellent activity.[29] As the free radical causes harmful effects on biological organs called oxidative stress which is arisen from an imbalance between free radicals and naturally producing antioxidants.[30,31] Antioxidant substances that are present in the cell at low concentrations significantly inhibit or eliminate oxidative stress.[32] Generally, the human body has created numerous defenses against oxidative stress by manufacturing antioxidants naturally and using antioxidants provided by food.[33] Quinoline and its reduced forms show a broad spectrum of biological properties such as an anticancer,[34] antibacterial,[35] antioxidant,[36] anthelmintic,[37] antiglaucoma,[38] and antimalarial[39] agents. Some aromatic, phenolic, and heterocyclic compounds, especially N−H bond-containing heterocyclic compounds have potent antioxidant activity.[40] The antioxidant properties of 2-oxo-1,2-dihydroquinoline-4-carboxylates has been examined using radical scavenging [(2,2-diphenyl-1-picryl-hydrazyl-hydrate) (DPPH)] assay, ferric reducing (FRAP) power assay, and β-carotene kinetic blanching assays by Sebbar et al.[41] Some quinoline-based compounds that exhibit excellent antioxidant properties are shown in Figure 1.[36,42−44]

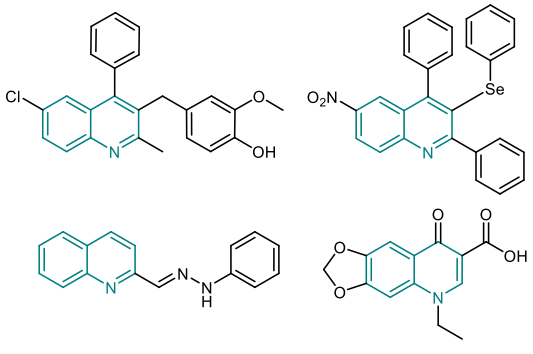


Figure 1. Quinoline derivatives exhibiting antioxidant activity.

With our continued efforts for the development of novel spiro heterocycles, herein, we wish to explore an unforeseen result obtained from the one-pot reaction of Meldrum's acid, aldehyde, and 5-amino-1,3-benzodioxole or 1,4-benzodioxin-6-amine or 5-aminoindazole under catalyst-free reaction conditions in aqueous ethanol (Scheme 2). This protocol offers excellent diastereoselectivity and regioselectivity.

2. RESULTS AND DISCUSSION

2.1. Chemistry. Initially, we choose 3,4-methylenedioxyaniline **1a**, Meldrum's acid **2**, and *p*-chlorobenzaldehyde **3b** as our template substrates to investigate the viability of multicomponent reactions. At first, the reaction was performed using water as reaction media at room temperature with no catalyst, the sticky mass formation was seen in this experiment (Table 1, entry 1). To resolve this problem, we replaced water with ethanol and achieved successful transformation **4b**. Next, we utilized ethanol:water system in different volumetric ratios (1:9, 3:7, and 1:1 v/v) as the reaction medium. Here, an ethanol:water system with a 1:1 v/v ratio enables excellent reaction transformation (87% yield) (Table 1, entry 4). It is observed that an increase in the volumetric amount of water in the ethanol/water system leads to sticky reaction mixture formation (Table 1, entries 2−3).

To achieve a higher yield of the product, we optimized this reaction using different polar and non-polar solvents such as water, ethanol, methanol, butanol, acetic acid, acetonitrile, DCM, and *n*-hexane (Table 1, entry 1, 5−11). We have seen that all solvents provide good isolated yields of product (**4b**); nevertheless, this required purification of product, noteworthily, the aqueous ethanol (1:1, v/v) is the best solvent candidate for this reaction as it shows complete reaction transformations with respect to aldehyde substrate (87% yield) (Table 1, entry-4) and no sticky reaction mass formation observed. Therefore, we opt for this reaction condition for the synthesis of 6′,8′-bis(4-chlorophenyl)-2,2-dimethyl-5′,8′-dihydro-6′*H*-spiro[[1,3]dioxane-5,7′-[1,3]dioxolo[4,5-*g*]-quinoline]-4,6-dione **4b**. With optimized reaction parameters in hand, we explore a substrate scope using different amine

https://doi.org/10.1021/acsomega.2c05020
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Scheme 2. MCRs of Meldrum's Acid, Amine Derivatives, and Aldehydes

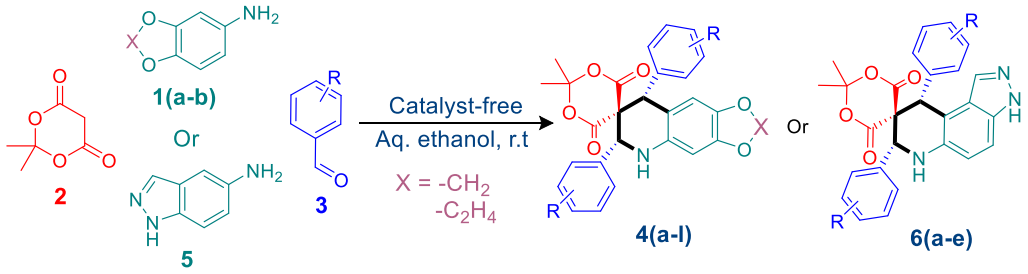
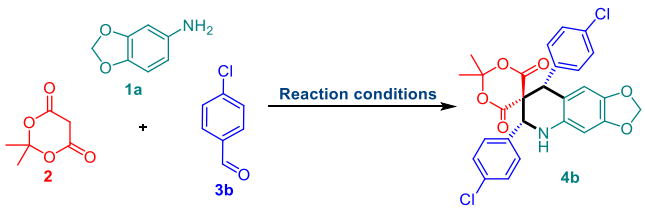


Table 1. Optimization of Reaction Parameters[a]



entry	solvent system	temp. (°C)	time (min)	% yield[b]
1[c]	water	RT	120	–
2[c]	ethanol/water(1:9)	RT	120	–
3[c]	ethanol/water(3:7)	RT	90	–
4	**ethanol/water(1:1)**	**RT**	**60**	**87**
5	ethanol	RT	60	80
6	butanol	RT	90	75
7	DCM	RT	90	50
8	methanol	RT	120	68
9	acetic acid	RT	100	64
10	acetonitrile	RT	60	80
11[c]	*n*-hexane	RT	75	–

[a]Reaction condition: 3,4-methylenedioxyaniline (**1a**, 1.0 mmol), Meldrum's acid (**2**, 1.0 mmol), and *p*-chloro benzaldehyde (**3b**, 2.0 mmol), 5 mL solvent, room temperature (25−30 °C). [b]Isolated yield. [c]Sticky reaction mass.

derivatives (3,4-methylenedioxyaniline (**1a**) and 6-amino-1,4-benzodioxan (**1b**)) and functionalized aldehydes.

The MCRs of 3,4-methylenedioxyaniline **1a**, Meldrum's acid **2**, and various aryl aldehyde **3** flow smoothly under optimal reaction parameters. The results are summarized in Table 2. The aldehyde-bearing ring deactivating functionalities such as *p*-chlorobenzaldehydes **3b** and *p*-bromo benzaldehyde **3c** are tolerated well and form desired product in excellent yields (87% of **4b** and 81% **4c**). Aldehydes having ring-activating functionalities such as *p*-methoxy-, *p*-phenyl- and *p*-methyl-benzaldehydes are also tolerated well and form corresponding products in excellent yields.

Encouraged by these results, we replace 3,4-methylenedioxyaniline **1a** with 6-amino-1,4-benzodioxan **1b** and 5-aminoindazole **5**. We performed the reaction of these amines with Meldrum's acid **2** and aryl aldehyde **3** to construct 2,2-dimethyl-7′,9′-diaryl-2′,3′,6′,9′-tetrahydro-7′*H*-spiro[[1,3]-dioxane-5,8′-[1,4]dioxino[2,3-*g*]quinoline]-4,6-diones **4(g−l)** and 2′,2′-dimethyl-6,8-diphenyl-1,5,6,8-tetrahydrospiro-[pyrazolo[3,4-*g*]quinoline-7,5′-[1,3]dioxane]-4′,6′-diones **6(a−e)**, respectively (Tables 2 and 3). To our delight, all these reactions proceed smoothly and produce the desired products with excellent yields (69−85%). All synthesized compounds were purified by washing them with aqueous ethanol.

A plausible reaction pathway for the synthesis of dihydrospiro[dioxolo[4,5-*g*]quinoline], tetrahydrospiro-[dioxino[2,3-*g*]quinoline] and tetrahydrospiro[pyrazolo[4,3-*f*]quinoline] shown in Figure 2. It involves the initial Knoevenagel condensation reaction of Meldrum's acid **2** with aldehyde **3** to form the Knoevenagel adduct (K). This adduct (K) undertakes a Michael-type addition reaction with 3,4-methylenedioxyaniline **1a**/1,4-benzodioxane-6-amine **1b**/5-aminoindazole **5** (C−H activation step) to produce a reaction intermediate (L) and [L′] respectively. Now this intermediate forms benzylidene type derivatives (M) and [M′] by reaction with 2 equiv of aldehyde. Afterward, this derivative endures the intramolecular ring closing step and furnishes the desired products with high diastereoselectivity.

Green metrics is a vital tool to evaluate synthetic process from a green chemistry viewpoint. To highlight the present work from a sustainability point of view, we perform the calculation of "Green metrics" such as the *E*-factor, AE, reaction mass efficiency (RME), and optimum efficiency (OE). Among all green metrics parameters, *E*-factor is frequently used to highlight the eco-compatibility of the synthetic procedure. The reaction is more eco-friendly when the *E*-factor is lower. The *E*-factor value range from 0.54 to 2.22 confirm the same. As shown in Figure 3, the remarkable values of AE, RME, and OE (up to 94.54, 81.42, and 87.00 respectively) also validate the same.

All synthesized compounds were structurally elucidated by ^{1}H NMR, ^{13}C NMR, and mass spectroscopic methods. Furthermore, we developed a single crystal of compound **4b**, and it was examined by single-crystal X-ray diffraction (XRD) analysis. The result of the analysis confirms the molecular structure of **4b**. It exhibits a triclinic crystal system with a P1 space group. The triclinic crystal system's unit cell constants are: a = 13.5982(5) Å, b = 13.6868(5) Å, c = 13.6868(5) Å, α = 71.4840(10)°, β = 66.0180(10)°, γ = 64.6920(10)°, and volume = 2511.7 Å^3. It is based on refinement, which was carried out with the help of SHELXL-97.[45] We deposited XRD data of compound **4b** online to Cambridge Crystallographic Data Centre (CCDC) with a CCDC deposition number 1985091, which contains the Supporting Information crystallographic data for this paper. The 3D view of compound **4b** is shown in Figure 4, which indicates that **4b** was obtained in the dimer form.

The ^{1}H NMR spectrum shows the most downfield signals for two aryl ring protons that appear as a multiplate at δ_H 7.26−7.34 and 7.08−7.11. Moreover, the two most shielded protons were observed at 0.75 and 0.59 for the two methyl groups. Whereas one doublet of a doublet at δ_H 5.85 appears for dioxane (-OCH$_2$O- protons), and the molecule also possesses two singlets at δ_H 6.34 and 6.20 observed aromatic protons for H-16 and H-10, respectively. The spectrum

https://doi.org/10.1021/acsomega.2c05020
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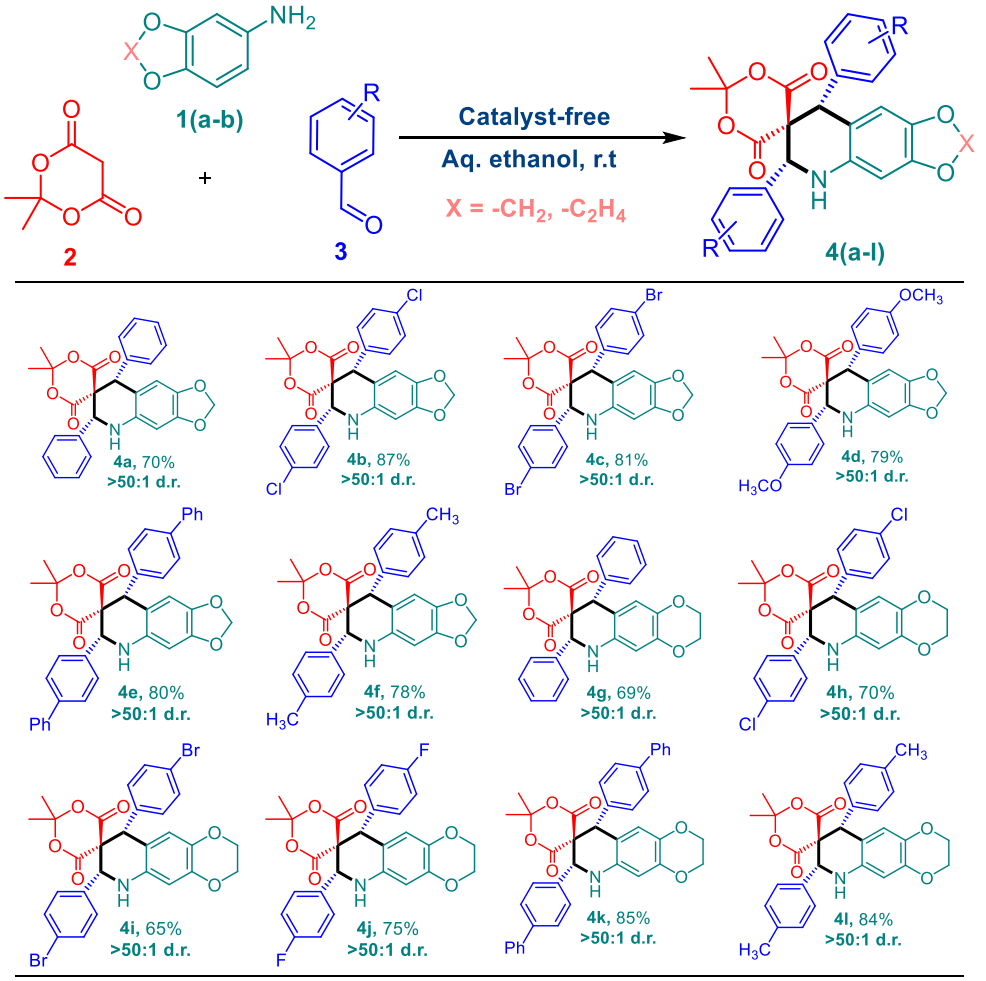
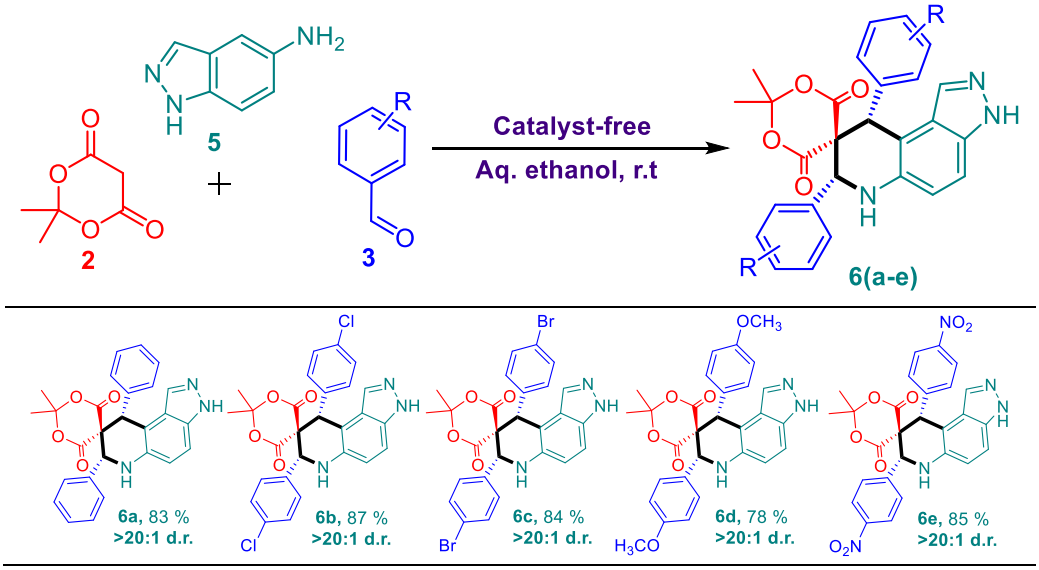
Table 2. Synthesis of Dihydrospiro[dioxolo[4,5-g]quinoline] 4(a–f) and Tetrahydrospiro[dioxino-[2,3-g]quinoline] 4(g–l)[a,b]



[a]Reaction conditions: **1**(**a**–**b**) (1.0 mmol), **2** (1.0 mmol), and **3** (2.0 mmol), in ethanol/water (1:1 v/v) (5 mL) at room temperature (25–30 °C) for 1 h. [b]Isolated yields of the product; the diastereomeric ratio were determined by ^{1}H NMR analysis.

Table 3. Synthesis of Tetrahydrospiro[pyrazolo[3,4-g]quinolines 6(a–e)[a,b]



[a]Reaction conditions: **5** (1.0 mmol), **2** (1.0 mmol), and **3** (2.0 mmol) in ethanol/water (1:1 v/v) (5 mL) at room temperature (25–30 °C) for—1 h. [b]Isolated yields of the product; the diastereomeric ratio were determined by ^{1}H NMR analysis.

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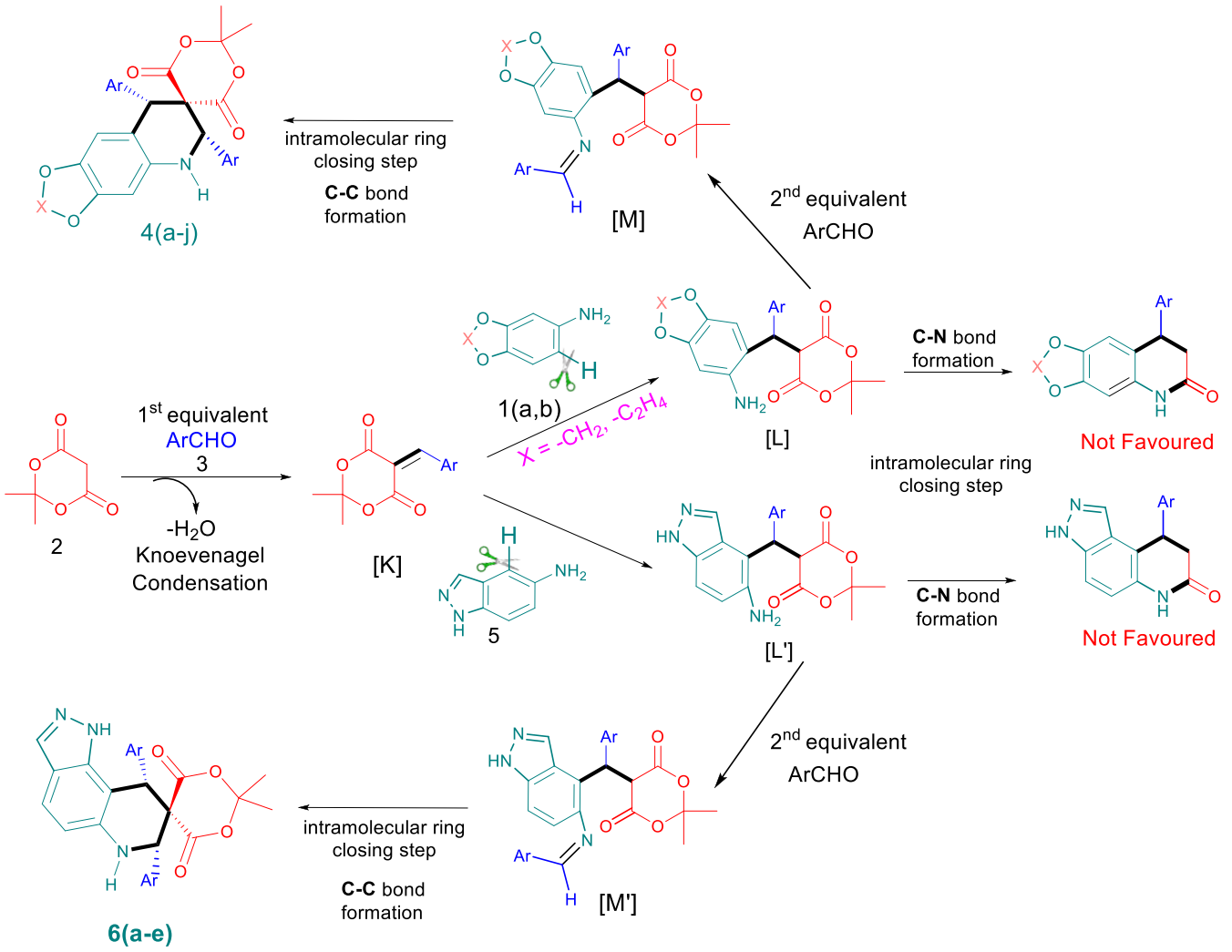

Figure 2. Plausible reaction pathways for spiroquinolines **4(a–l)** and **6(a–e)**.

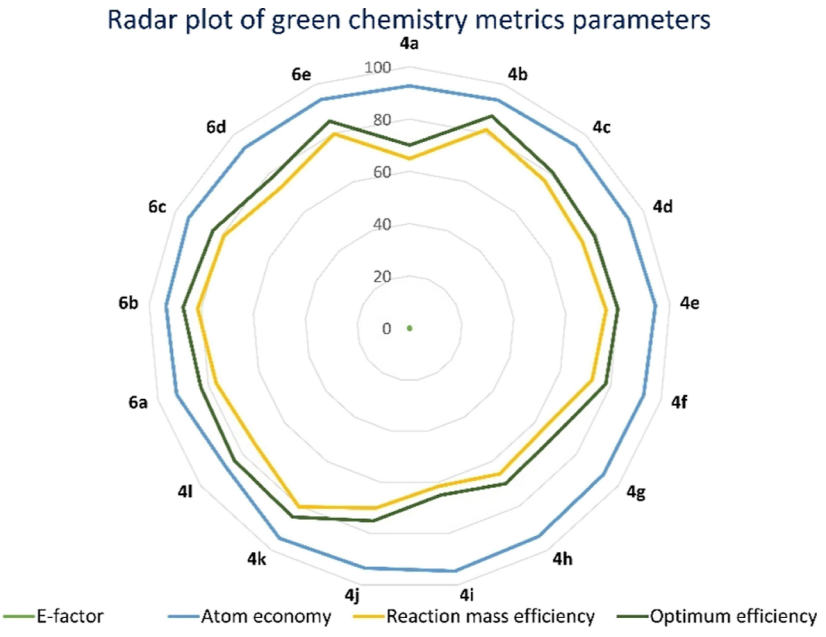

Figure 3. Green metrics for spiroquinolines **4(a–l)** and **6(a–e)**.

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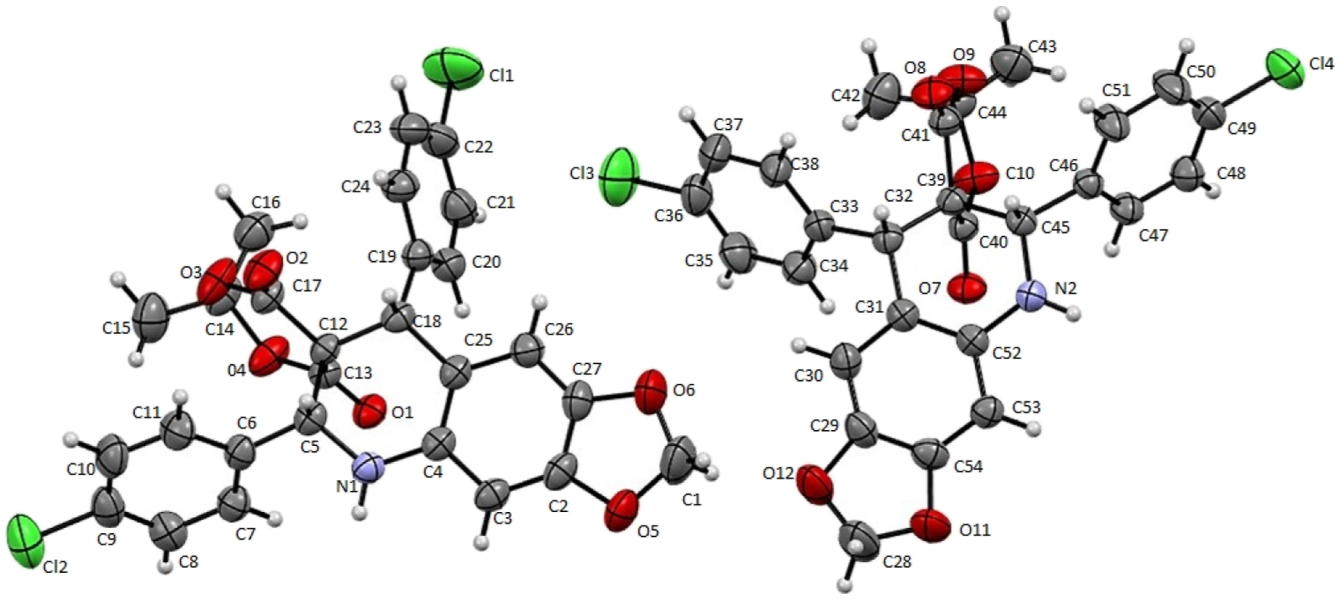

Figure 4. 3D view (dimer) of **4b** compound (CCDC: 1985091).

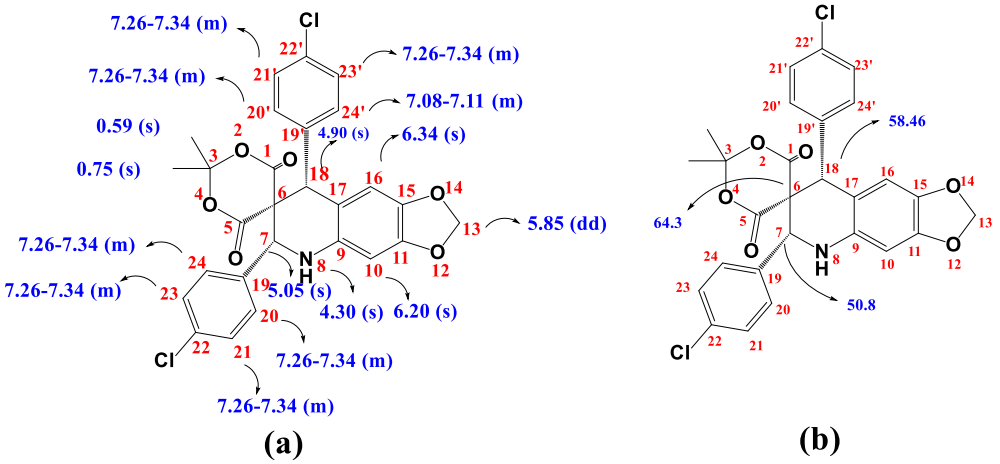

Figure 5. (a) ^{1}H NMR chemical shift of compound **4b**. (b) ^{13}C NMR chemical shift of compound **4b**.

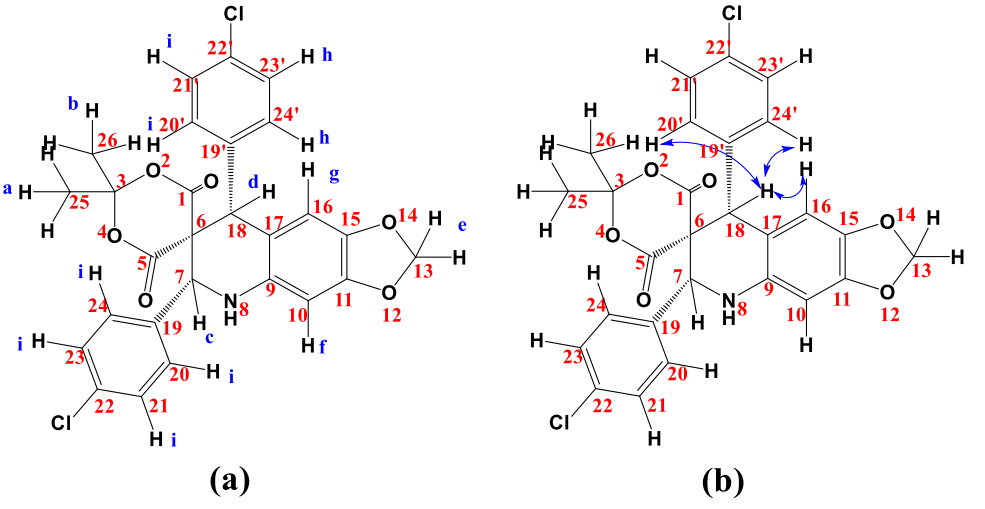

Figure 6. (a) H−C hetero correlation of **4b**. (b) H−H homo correlation **4b**.

showed the most significant signals at δ_H 5.05 and 4.90 of two protons of dihydro spiro quinolines moiety Figure 5a.

In the ^{13}C NMR spectra of compound **4b**, a total of 25 signals appear. Sixteen signals correspond to eighteen aromatic

https://doi.org/10.1021/acsomega.2c05020
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carbons at δ_C 105.94−147.20 and two carbonyl carbon signals appear at δ_C 161.69 and 167.96. Two signals were observed at δ_C 97.86 and 100.97 for C-13 and C-3, respectively. The most significant ^{13}C NMR signals for chiral center carbon (C-7 and C-18) appear at δ_C 50.80 and 58.46, respectively, while one signal shows at 64.32 for spiro carbon (C-6), as shown in Figure 5b. In the MM-APCI spectrum, molecular ion $[M^+]$ peaks observed at m/z 526.20 correlates to the molecular formula ($C_{27}H_{21}Cl_2NO_6$) of the **4b** compound.

The 2D NMR spectra of the HSQC and COSY correlations are useful in the signal assignment of **4b**, and various characteristic signals are shown in Figure 6a,b. In HSQC NMR analysis of compound **4b**, two chiral center protons correlate with carbon at δ 50.80 and 64.25 which corresponds to carbon C-7 and C-18, respectively. One CH_2 and two CH_3 protons correlate with carbon at δ 100.87, 28.02, and 29.18, which corresponds to carbon C-13, C-25, and C-26, respectively. In addition, other characteristic peaks were found at δ 133.16, 131.11, 129.56, 129.35, 129.12, 128.91, 97.78, and 107.99 which correlate to aromatic carbons (Figure S41).[46] In COSY NMR analysis of compound **4b**, it shows that H-18 is correlated with H-16, H-20, and H-24′ (Figure S42).

The stereochemistry of **4g** was established on the basis of NOESY experiments.[47] In the NOESY experiment, it is possible to observe signals corresponding to the strong interaction between the C-1 and C-2. The observation is pertinent in that it is consistent with the proton at C-1 and C-2 being in a cis relationship with respect to one another (see Figure 7).

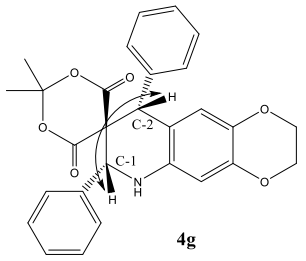

Figure 7. Characteristic NOEs observed for **4g**.

2.2. In Vitro Antioxidant Activity. To establish antioxidant properties of newly synthesized spiroquinolines **4(a−l)** and **6(a−e)**, in vitro 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and DPPH radical scavenging activities of spiroquinolines were performed. Easy operation, rapidity, sensitivity, and utilization of stable radicals, DPPH and ABTS are the best-known and frequently employed techniques for estimating antioxidant activity.[48] Results of these activities in IC_{50} values of **4(a−l)** and **6(a−e)** are summarized in Table 4. Antioxidant ascorbic acid was used as standard. It displayed IC_{50} values of 41.84 ± 0.25 and 90.10 ± 0.74 μM for ABTS and DPPH radical scavenging activities, respectively.

2.2.1. In Vitro ABTS Radical Scavenging Activity. Results of in vitro ABTS radical scavenging activity of **4(a−l)** and **6(a−e)** show that all spiroquinoline derivatives show potent activities as compared to standard ascorbic acid. With IC_{50} of 6.77 ± 0.73 μM displaying the most potent activity among **4(a−l)** and **6(a−e)**. Furthermore, compounds **4l** and **4d** also displayed excellent activity with IC_{50} values 11.02 ± 0.88 and 13.16 ± 0.43 μM, respectively.

Table 4. In Vitro ABTS and DPPH Radical Scavenging Activities of Spiroquinolines 4(a−l) and 6(a−e)

	ABTS radical scavenging activity	DPPH radical scavenging activity
code	IC_{50} ± SEM (μM)[a]	IC_{50} ± SEM (μM)[a]
	Category "A"	
4a	25.22 ± 0.48	34.47 ± 0.88
4b	21.12 ± 0.79	130.97 ± 0.75
4c	22.94 ± 0.11	108.00 ± 1.11
4d	13.16 ± 0.43	**12.95 ± 0.34**
4e	14.66 ± 0.39	113.07 ± 0.85
4f	**6.77 ± 0.73**	23.25 ± 0.53
	Category "B"	
4g	15.31 ± 0.91	77.68 ± 0.19
4h	22.13 ± 0.85	201.53 ± 0.12
4i	24.25 ± 0.21	145.79 ± 0.17
4j	18.34 ± 0.37	266.53 ± 0.18
4k	21.54 ± 0.52	210.79 ± 0.16
4l	11.02 ± 0.88	143.02 ± 0.72
	Category "C"	
6a	27.76 ± 0.88	336.08 ± 0.16
6b	15.58 ± 1.01	224.05 ± 0.79
6c	16.88 ± 1.10	263.42 ± 0.11
6d	27.65 ± 0.69	254.28 ± 0.14
6e	19.74 ± 0.54	280.13 ± 0.09
ascorbic acid[b]	41.84 ± 0.25	90.10 ± 0.74

[a]SEM (standard error mean). [b]Standard for ABTS and DPPH radical scavenging activity. Bold values show the lowest IC_{50}s.

2.2.2. In Vitro DPPH Radical Scavenging Activity. The result of in vitro DPPH radical scavenging activity of **4(a−l)** and **6(a−e)** shows that all spiroquinoline derivatives have DPPH radical scavenging activity. **4a**, **4d**, **4f**, and **4g** show excellent activity as compared to ascorbic acid. The IC_{50} of **4d** (12.95 ± 0.34 μM) displayed most potent activity among **4(a−l)** and **6(a−e)**. Overall, **4d** and **4f** have excellent scavenging potential of ABTS and DPPH radicals.

2.3. Structure−Activity Relationship. All the compounds show good to excellent antioxidant activity (ABTS and DPPH). There are three categories: A, B, and C. They were classified into three groups: 1,3-dioxolane, 1,4-dioxane, and pyrazole respectively, which were fused with spiroquinoline derivatives. The lead radical scavenging activity (ABTS and DPPH) of **4a**, **4d**, **4f**, **4g**, and **4l** compounds are mentioned in Figure 6. From category A, methoxy- and methyl-substituted group at para positions of compound **4d** (ABTS, IC_{50} = 13.16 ± 0.43 μM; DPPH, IC_{50} = 12.95 ± 0.34 μM) and **4f** (ABTS, IC_{50} = 6.77 ± 0.73 μM; DPPH, IC_{50} = 23.25 ± 0.53 μM) were the excellent radical scavengers against both DPPH and ABTS as compared to the standard ascorbic acid (ABTS, IC_{50} = 41.84 ± 0.25 μM; DPPH, IC_{50} = 90.10 ± 0.74 μM). Without any para substitution, compound **4a** shows moderate antioxidant activity (ABTS, IC_{50} = 25.22 ± 0.48 μM; DPPH, IC_{50} = 34.47 ± 0.88 μM), while the electron-withdrawing group at para position compound **4b** (ABTS, IC_{50} = 21.12 ± 0.79 μM; DPPH, IC_{50} = 130.97 ± 0.75 μM), **4c** (ABTS, IC_{50} = 22.94 ± 0.11 μM; DPPH, IC_{50} = 108.00 ± 1.11 μM), and **4e** (ABTS, IC_{50} = 14.66 ± 0.39 μM; DPPH, IC_{50} = 113.07 ± 0.85 μM) were weak radical scavengers as compared to compound **4d** and **4f**. The results of category B shows that the compound bearing the electron-releasing group at the para position exhibited good radical scavenger activity as

https://doi.org/10.1021/acsomega.2c05020
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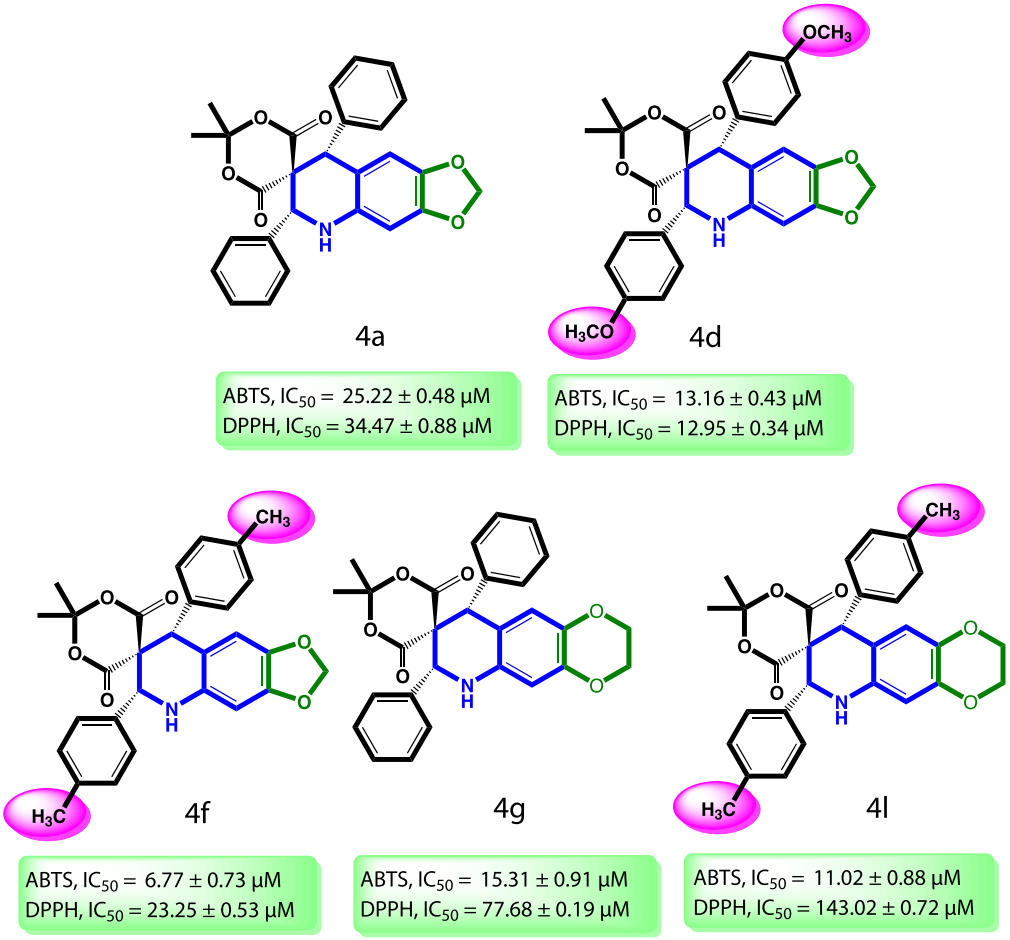

Figure 8. Lead radical scavenging activity (ABTS and DPPH) of **4a**, **4d**, **4f**, **4g**, and **4l** compounds.

compared to the electron-withdrawing group at the para position (Table 4). Compounds of category C were the least radical scavenger as compared to categories A and B; compounds **4a**, **4g**, and **6a** showed ABTS (IC_{50} = 25.22 ± 0.48 μM, 15.31 ± 0.91 μM, and 27.76 ± 0.88 μM, respectively) and DPPH (IC_{50} = 34.47 ± 0.88 μM, 77.68 ± 0.19 μM, and 336.08 ± 0.16 μM, respectively) radical scavenging activities. Compound **6a** belongs to category C (Figure 8).

3. CONCLUSION

In conclusion, we successfully designed an eco-compatible and multicomponent reaction-based protocol for spiroquinolines in aq ethanol under catalyst-free conditions. These spiroquinolines show high diastereoselectivity [d.r. >50:1 (*cis*/*trans*)] and regioselectivity. This protocol offers several noteworthy benefits such as a simple operating procedure, mild reaction conditions, excellent product yield with purity (HPLC) up to 99%, and good agreements with green metrics parameters. All synthesized spiroquinolines are examined for the radical scavenging (DPPH and ABTS) assay as compared to standard ascorbic acid. The results of in vitro radical scavenging (DPPH and ABTS) assay show that compounds **4d**, **4f**, and **4l** in ABTS radical scavenging assay, and compounds **4a**, **4d**, **4f**, and **4g** in DPPH radical scavenging assay were revealed to be the most potent antioxidants. The structure−activity relationship (SAR) highlights that compounds bearing methyl and methoxy groups at the para position are excellent radical scavengers. Altogether, compounds **4d** and **4f** were discovered as having high scavenging potency with DPPH and ABTS radicals.

4. EXPERIMENTAL SECTION

4.1. Materials and Apparatus. All reagents used in this synthesis were purchased from commercially available sources and used without any further purification. Melting points were resolute using the open capillary tube method and were uncorrected. NMR spectra (^{1}H NMR & ^{13}C NMR) were recorded on Bruker 500 MHz NMR spectrometer using solvent peak as $CDCl_3$/DMSO-d_6 solvent. LCMS analyses were performed on an MS-Agilent 6120 quadrupole spectrometer and HRMS was determined on Waters Micromass Q-Tof Micro 4000 quadrupole spectrometer. TLC analyses were performed on aluminum plates precoated with F254 silica gel 60. Single-crystal was analyzed using a Bruker X8 Kappa APEX II diffractometer.

4.2. General Procedure for Synthesis of Spiroquinolines 4(a−l) and 6(a−e). Amine derivatives (**1** & **5**, 1.0 mmol), Meldrum's acid (**2**, 1.0 mmol), and aldehyde (**3**, 2.0 mmol) were mixed in 5 mL of aqueous ethanol into an oven-dried round-bottomed flask. After mixing, the reaction mass was stirred at room temperature for 60−90 min (Table 1). The reaction progress was monitored through periodic TLC analysis (using *n*-hexane/ethyl acetate (7:3) as the mobile phase). After completion of the reaction (monitored by TLC), 5 mL of distilled water was added and stirred at room temperature for complete solidification of the product. The

https://doi.org/10.1021/acsomega.2c05020
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solid mass was filtered off and washed with 5 mL ethanol to yield the pure form. All newly synthesized compounds were characterized by spectral analysis such as ^{1}H NMR, ^{13}C NMR, HRMS or LCMS, and HPLC.

*4.2.1. 2,2-Dimethyl-6′,8′-diphenyl-5′,8′-dihydro-6′H-spiro[[1,3]dioxane-5,7′-[1,3]dioxolo[4,5-g]quinoline]-4,6-dione (**4a**).* Off white solid (80%), % purity (HPLC) = 99.5%, mp 204−206 °C; ^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.36 (s, 5H, ArH), 7.26−7.32 (m, 4H, ArH), 7.15−7.18 (m, 1H, ArH), 6.36 (s, 1H, ArH), 6.28 (s, 1H, ArH), 5.85 (dd, J = 2 Hz, J = 12 Hz, 2H, CH_2), 5.07 (s, 1H, CH), 4.95 (s, 1H, CH), 4.29 (s, 1H, NH), 0.64 (s, 3H, CH_3), 0.47 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 168.22, 161.90, 147.01, 140.87, 138.63, 138.03, 136.46, 132.00, 129.70, 129.64, 129.31, 128.94, 128.44, 127.75, 114.46, 108.38, 105.73, 100.85, 97.8, 64.80, 58.49, 51.64, 28.95, 27.98; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{27}H_{23}NO_6$, 456.15; found, 456.20.

*4.2.2. 6′,8′-Bis(4-chlorophenyl)-2,2-dimethyl-5′,8′-dihydro-6′H-spiro[[1,3]dioxane-5,7′-[1,3]dioxolo[4,5-g]quinoline]-4,6-dione (**4b**).* White solid (85%), % purity (HPLC) = 98.8% mp 208−210 °C; ^{1}H NMR (500 MHz,$CDCl_3$) (δ, ppm): 7.26−7.34 (m, 7H, ArH), 7.08−7.11 (m, 1H, ArH), 6.34 (s, 1H, ArH), 6.20 (s, 1H, ArH), 5.85 (dd, J = 1.5 Hz, J = 14.5 Hz, 2H, CH_2), 5.05 (s, 1H, CH), 4.90 (s, 1H, CH), 4.30 (s, 1H, NH), 0.75 (s, 3H, CH_3), 0.59 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 167.97, 161.69, 147.20, 141.09, 137.86, 137.11, 135.58, 134.79, 134.47, 133.25, 131.17, 129.67, 129.44, 129.20, 129.01, 114.10, 108.09, 105.94, 100.98, 97.86, 64.33, 58.46, 50.81, 29.29, 28.21; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{27}H_{21}Cl_2NO_6$, 524.07; found, 524.20.

*4.2.3. 6′,8′-Bis(4-bromophenyl)-2,2-dimethyl-5′,8′-dihydro-6′H-spiro[[1,3]dioxane-5,7′-[1,3]dioxolo[4,5-g]quinoline]-4,6-dione (**4c**).* Light brown solid (84%), % purity (HPLC) = 97.8%, mp 210−212 °C; ^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.44−7.53 (m, 4H, ArH), 7.26 (s, 1H, ArH), 7.24 (m, 1H, ArH), 7.21 (dd, J = 2.5 Hz, J = 8.0 Hz, 1H, ArH), 7.06 (dd, J = 2 Hz, J = 8.5 Hz, 1H, ArH), 6.36 (s, 1H, CH), 6.22 (s, 1H, CH), 5.87 (dd, J = 1.5 Hz, J = 13.5 Hz, 2H, CH_2), 5.06 (s, 1H, CH), 4.91 (s, 1H, CH), 4.27 (s, 1H, NH), 0.77 (s, 3H, CH_3), 0.61 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 167.73, 161.43, 147.01, 140.90, 137.60, 137.39, 135.07, 133.35, 132.40, 132.23, 131.82, 131.31, 129.25, 123.50, 122.36, 113.77, 107.89, 107.87, 105.76, 100.79, 97.61, 97.58, 64.14, 58.15, 50.67, 29.09, 27.91; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{27}H_{21}Br_2NO_6$, 611.97; found, 612.00.

*4.2.4. 6′,8′-Bis(4-methoxyphenyl)-2,2-dimethyl-5′,8′-dihydro-6′H-spiro[[1,3]dioxane-5,7′-[1,3]dioxolo[4,5-g]quinoline]-4,6-dione (**4d**).* White solid (79%), % purity (HPLC) = 96.8%, mp 190−192 °C;^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.21−7.28 (m, 3H, ArH), 7.06−7.08 (m, 1H, ArH), 6.80−6.89 (m, 4H ArH) 6.33 (s, 1H, CH), 6.27 (s, 1H, CH), 5.84 (dd, J = 2 Hz, J = 13 Hz, 2H, CH_2), 5.00 (s, 1H, CH), 4.88 (s, 1H, CH), 4.23 (s, 1H, NH), 3.76 (d, J = 8.5 Hz, 6H, $2CH_3$), 0.74 (s, 3H, CH_3), 0.58 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 168.47, 162.16, 160.43, 159.55, 146.90, 140.74, 131.14, 133.05, 130.76, 130.55, 128.97, 128.56, 115.09, 114.48, 113.71, 108.34, 105.67, 100.79, 97.65, 64.30, 58.77, 55.45, 50.89, 29.17, 28.17; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{29}H_{27}NO_8$, 516.17; found, 516.20.

*4.2.5. 6′,8′-Di([1,1′-biphenyl]-4-yl)-2,2-dimethyl-5′,8′-dihydro-6′H-spiro[[1,3]dioxane-5,7′-[1,3]dioxolo[4,5-g]quinoline]-4,6-dione (**4e**).* Off white solid (80%), % purity (HPLC) = 99.3%, mp 206−208 °C; ^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.61−7.63 (m, 2H, ArH), 7.54−7.57 (m, 6H, ArH), 7.41−7.47 (m, 7H, ArH), 7.26−7.38 (m, 3H, ArH), 6.41 (s, 1H, CH), 6.38 (s, 1H, CH), 5.88 (dd, J = 1.5 Hz, J = 11.2 Hz, 2H, CH_2), 5.17 (s, 1H, CH), 5.04 (s, 1H, CH_3), 4.36 (s, 1H, NH), 0.70 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 168.05, 161.78, 146.89, 142.32, 141.07, 140.77, 140.20, 139.96, 137.85, 137.42, 135.16, 132.21, 129.90, 128.82, 128.76, 128.00, 127.80, 127.68, 127.64, 127.46, 127.21, 126.90, 126.84, 114.46, 108.19, 105.64, 100.68, 97.64, 64.38, 58.40, 51.15, 28.84, 27.86; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{39}H_{31}NO_6$, 608.21; found, 608.20.

*4.2.6. 2,2-Dimethyl-6′,8′-di-p-tolyl-5′,8′-dihydro-6′H-spiro[[1,3]dioxane-5,7′-[1,3]dioxolo[4,5-g]quinoline]-4,6-dione (**4f**).* Off white solid (78%), % purity (HPLC) = 98.5%, mp 190−192 °C; ^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.18−7.28 (m, 5H, ArH), 7.13 (d, J = 8.5 Hz, 2H, ArH), 7.06−7.08 (m, 1H, ArH), 6.37 (s, 1H, CH), 6.31 (s, 1H, CH), 5.86 (d, J = 1.5 Hz, J = 13.5 Hz, 2H, CH_2), 5.05 (s, 1H, CH), 4.93 (s, 1H, CH), 2.33 (d, J = 15 Hz, 6H, $2CH_3$), 0.72 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 168.24, 161.95, 146.84, 140.74, 139.37, 138.03, 138.01, 135.47, 133.48, 131.80, 129.94, 129.73, 129.40, 129.27, 127.54, 114.99, 108.30, 105.33, 100.69, 97.72, 64.53, 58.44, 51.31, 28.86, 27.96, 21.13, 21.05; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{29}H_{27}NO_6$, 484.18; found, 484.20.

*4.2.7. 2,2-Dimethyl-7′,9′-diphenyl-2′,3′,6′,9′-tetrahydro-7′H-spiro[[1,3]dioxane-5,8′-[1,4]dioxino[2,3-g]quinoline]-4,6-dione (**4g**).* White solid (82%), % purity (HPLC) = 99.2%, mp 218−220 °C; ^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.29−7.40 (m, 9H, ArH), 7.22−7.24 (m, 1H, ArH), 6.38 (s, 2H, ArH), 5.09 (s, 1H, CH), 4.99 (s, 1H, CH), 4.16−4.25 (m, 4H, $2CH_2$), 0.67 (s, 3H, CH_3), 0.51 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 168.05, 161.84, 142.89, 138.48, 137.65, 136.60, 136.51, 131.97, 129.65, 129.46, 129.19, 129.14, 128.78, 128.26, 127.76, 116.11, 105.58, 103.94, 64.70, 64.65, 64.27, 58.67, 51.20, 28.89, 27.88; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{28}H_{25}NO_6$, 470.17; found, 470.30.

*4.2.8. (7′R,9′R)-7′,9′-Bis(4-chlorophenyl)-2,2-dimethyl-2′,3′,6′,9′-tetrahydro-7′H-spiro[[1,3]dioxane-5,8′-[1,4]dioxino[2,3-g]quinoline]-4,6-dione (**4h**).* White solid (70%), % purity (HPLC) = 97.5%, mp 218−220 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36−7.23 (m, 7H, ArH), 7.15 (dd, J = 9.0, 2 Hz, 1H, ArH), 6.34 (s, 1H, ArH), 6.27 (s, 1H, ArH), 5.06 (s, 1H, CH), 4.92 (s, 1H, CH), 4.29−4.10 (m, 4H, $2CH_2$), 0.77 (s, 3H, CH_3), 0.61 (s, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.82, 161.64, 143.07, 137.41, 136.97, 136.73, 135.44, 134.93, 134.32, 133.21, 131.14, 130.92, 129.82, 129.54, 129.29, 129.20, 128.85, 116.63, 115.49, 105.80, 104.05, 64.69, 64.20, 58.64, 50.37, 29.23, 27.99. ESI HRMS: $[M + H]^+$ calcd for $C_{28}H_{23}Cl_2NO_6$, 540.0975; found, 540.0961.

*4.2.9. (7′R,9′R)-7′,9′-Bis(4-bromophenyl)-2,2-dimethyl-2′,3′,6′,9′-tetrahydro-7′H-spiro[[1,3]dioxane-5,8′-[1,4]dioxino[2,3-g]quinoline]-4,6-dione (**4i**).* White solid (65%), % purity (HPLC) = 98.5%, mp 214−216 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54−7.48 (m, 2H), 7.45 (td, J = 8.4, 7.8, 2.2 Hz, 2H), 7.24−7.16 (m, 3H), 7.10 (dd, J = 8.9, 2.3 Hz, 1H), 6.33 (s, 1H), 6.27 (s, 1H), 5.04 (s, 1H), 4.92 (s, 1H), 4.31−4.04 (m, 4H), 0.77 (s, 3H), 0.62 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$): δ 167.78, 161.59, 143.10, 137.46, 137.37, 136.74, 135.42, 134.93, 133.53, 132.48, 132.29, 131.87, 131.48, 129.46, 123.56, 122.42, 116.63, 115.37, 105.82, 103.99, 64.70,

https://doi.org/10.1021/acsomega.2c05020
ACS Omega 2023, 8, 444−456

64.22, 58.52, 50.43, 29.22, 27.99. ESI HRMS: $[M + H]^+$ calcd for $C_{28}H_{23}Br_2NO_6$, 627.9965; found, 627.9997.

*4.2.10. 7′,9′-Bis(4-fluorophenyl)-2,2-dimethyl-2′,3′,6′,9′-tetrahydro-7′H-spiro[[1,3]dioxane-5,8′-[1,4]dioxino[2,3-g]-quinoline]-4,6-dione (**4j**).* White solid (85%), % purity (HPLC) = 96.1%, mp 202−204 °C; ^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.39 (dd, J = 5.5 Hz, J = 10.7 Hz, 2H, ArH), 7.21−7.31 (m, 2H, ArH), 7.01−7.10 (m, 4H, ArH), 6.35 (s, 1H, ArH), 6.30 (s, 1H, CH), 5.09 (s, 1H, CH), 4.97 (s, 1H, CH), 4.16−4.25 (m, 4H, $2CH_2$), 0.78 (s, 3H, CH_3), 0.62 (s, 3H, CH_3);^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 167.97, 161.77, 161.77, 143.02, 137.49, 136.64, 134.25, 134.23, 133.50, 132.38, 131.47, 129.62, 116.66, 116.17, 115.00, 115.76, 115.52, 115.35, 105.71, 103.90, 77.24, 64.70, 64.03, 58.92, 50.27, 29.16 27.99; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{28}H_{23}F_2NO_6$, 506.14; found, 506.20.

*4.2.11. 7′,9′-Di([1,1′-biphenyl]-4-yl)-2,2-dimethyl-2′,3′,6′,9′-tetrahydro-7′H-spiro[[1,3]dioxane-5,8′-[1,4]-dioxino[2,3-g]quinoline]-4,6-dione (**4k**).* Off white solid (79%), % purity (HPLC) = 98.5%, mp 210−212 °C;^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.56−7.65 (m, 8H, ArH), 7.33−7.50 (m, 10H, ArH), 6.45 (s, 1H, ArH), 6.41 (s, 1H, ArH), 5.18 (s, 1H, CH), 5.08 (s, 1H, CH), 4.22−4.28 (m, 2H, CH_2), 4.19−4.27 (m, 4H, $2CH_2$), 0.72 (s, 3H, CH_3), 0.58 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 168.11, 161.91, 142.98, 142.42, 141.14, 140.43, 140.13, 137.65, 137.46, 136.62, 135.49, 132.38, 130.05, 128.93, 128.85, 128.20, 127.92, 127.77, 127.72, 127.52, 127.29, 127.02, 126.96, 116.88, 116.03, 105.73, 103.98, 77.24, 64.73, 64.43, 64.29, 58.76, 50.89, 28.99, 27.96; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{40}H_{33}NO_6$, 622.22; found, 622.30.

*4.2.12. 2,2-Dimethyl-7′,9′-di-p-tolyl-2′,3′,6′,9′-tetrahydro-7′H-spiro[[1,3]dioxane-5,8′-[1,4]dioxino[2,3-g]quinoline]-4,6-dione (**4l**).* Off white solid (78%), % purity (HPLC) = 99.1%, mp 200−202 °C;^{1}H NMR (500 MHz, $CDCl_3$) (δ, ppm): 7.09−7.27 (m, 8H, ArH), 6.36 (s, 1H, ArH), 6.34 (s, 1H, CH), 5.03 (s, 1H, CH) 4.94 (s, 1H, CH), 4.13−4.27 (m, 4H, $2CH_2$), 2.33 (d, J = 17.5 Hz, 6H, $2CH_3$), 0.71 (s, 3H, CH_3), 0.56 (s, 3H, CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) (δ, ppm): 168.17, 161.99, 142.80, 139.33, 137.92, 137.70, 136.46, 135.35, 133.64, 131.83, 129.93, 129.69, 129.42, 129.21, 127.60, 116.85, 116.41, 105.50, 103.86, 77.25, 64.71, 64.43, 64.26, 58.67, 50.88, 28.90, 27.94, 21.13, 21.05; MS (MM-APCI) m/z: $[M - H]^+$ calcd for $C_{30}H_{29}NO_6$, 498.19; found, 498.20.

*4.2.13. 2′,2′-Dimethyl-7,9-diphenyl-3,6,7,9-tetrahydrospiro[pyrazolo[4,3-f]quinoline-8,5′-[1,3]dioxane]-4′,6′-dione (**6a**).* White solid (83%), % purity (HPLC) = 99.7%, mp 298−300 °C; ^{1}H NMR (500 MHz, DMSO-d_6) (δ, ppm): 12.69 (s, 1H, NH), 7.42−7.46 (m, 3H, ArH), 7.40 (s, 1H, ArH), 7.29−7.38 (m, 4H, ArH), 7.21−7.24 (m, 1H, ArH), 7.04−7.06 (d, J = 9 Hz, 1H), 6.75−6.77 (m, 1H, ArH), 6.63 (s, 1H, ArH), 6.11 (s, 1H, ArH), 5.22 (s, 1H, CH), 4.85 (s, 1H, CH), 0.62 (s, 3H, CH_3), 0.46 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) (δ, ppm): 167.50, 160.48, 138.45, 138.16, 136.23,130.69, 129.92, 129.07, 128.69, 128.53, 128.38, 128.11, 127.85, 121.42, 118.41,108.97, 104.87, 64.50, 59.03, 49.18, 28.98, 28.38; ESI HRMS: $[M + H]^+$ calcd for $C_{27}H_{23}N_3O_4$, 454.1767; found, 454.1780.

*4.2.14. 7,9-Bis(4-chlorophenyl)-2′,2′-dimethyl-3,6,7,9-tetrahydrospiro[pyrazolo[4,3-f]quinoline-8,5′-[1,3]dioxane]-4′,6′-dione (**6b**).* White solid (87%), % purity (HPLC) = 99.2%, mp 280−282 °C; ^{1}H NMR (500 MHz, DMSO-d_6) (δ, ppm): 12.76 (s, 1H, NH), 7.56−7.57 (m, 2H, ArH), 7.31−7.43 (m, 5H, ArH), 7.03 (d, J = 8.5 Hz, 5H, ArH), 6.71−6.73 (m, 2H, ArH), 6.17 (s, 1H, ArH), 5.27 (s, 1H, CH), 4.86 (s, 1H, CH), 0.73 (s, 3H, CH_3), 0.58 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) (δ, ppm): 167.27, 160.36, 138.11, 137.35, 135.07, 135.00, 133.74, 132.88, 132.47, 131.75, 131.20, 129.73, 128.63, 128.56, 121.25, 118.32, 109.38, 108.57, 105.06, 63.96, 59.09, 48.17, 28.60, 26.99; ESI HRMS: $[M + H]^+$ calcd for $C_{27}H_{21}Cl_2N_3O_4$, 522.0982; found, 522.1014.

*4.2.15. 7,9-Bis(4-bromophenyl)-2′,2′-dimethyl-3,6,7,9-tetrahydrospiro[pyrazolo[4,3-f]quinoline-8,5′-[1,3]dioxane]-4′,6′-dione (**6c**).* Off white solid (84%), % purity (HPLC) = 99.1%, mp 280−282 °C;^{1}H NMR (500 MHz, DMSO-d_6) (δ, ppm): 12.77 (s, 1H, NH), 7.69 (dd, J = 2 Hz, J = 7 Hz, 2H, ArH), 7.46 (dd, J = 2 Hz, J = 8.5 Hz, 1H, ArH), 7.28−7.36 (m, 4H, ArH), 7.03 (d, J = 9 Hz, 1H, ArH), 6.72 (s, 1H, ArH), 6.66 (dd, J = 2 Hz, J = 8.5 Hz, 1H, ArH), 6.17 (s, 1H, ArH), 5.25 (s, 1H, CH), 4.84 (s, 1H, CH), 0.73 (s, 3H, CH_3), 0.58 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) (δ, ppm): 167.25, 160.33, 138.11, 137.72, 135.39, 134.37, 132.78, 132.07, 131.64, 131.51, 131.47, 131.38, 130.01, 122.25, 121.30, 121.21, 118.35, 108.46, 105.07, 64.02, 58.97, 48.22, 28.59, 26.97; ESI HRMS: $[M + H]^+$ calcd for $C_{27}H_{21}Br_2N_3O_4$, 609.9972; found, 609.9996.

*4.2.16. 7,9-Bis(4-methoxyphenyl)-2′,2′-dimethyl-3,6,7,9-tetrahydrospiro[pyrazolo[4,3-f]quinoline-8,5′-[1,3]dioxane]-4′,6′-dione (**6d**).* Off white solid (78%), % purity (HPLC) = 98.8%, mp 268−270 °C; ^{1}H NMR (500 MHz, DMSO-d_6) (δ, ppm): 12.66 (s, 1H, NH), 7.24−7.28 (m, 3H, ArH), 7.00−7.03 (m, 4H, ArH), 6.81 (dd, J = 3 Hz, J = 9 Hz, 1H, ArH), 6.64−6.67 (m, 1H, ArH), 6.50 (s, 1H, ArH), 6.15 (s, 1H, ArH), 5.14 (s, 1H, CH), 4.76 (s, 1H, CH), 3.74 (d, J = 11 Hz, ArH), 0.71 (s, 3H, CH_3), 0.55 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) (δ, ppm): 167.75, 160.74, 159.76, 158.89, 138.16, 134.99, 131.73, 131.46, 130.95, 130.25, 128.98, 128.17, 121.51, 118.33, 114.18, 113.82, 113.53, 109.46, 108.92, 104.81, 64.00, 59.29, 55.18, 55.02, 48.41, 28.55, 27.14; ESI HRMS: $[M + H]^+$ calcd for $C_{29}H_{27}N_3O_6$, 514.1973; found, 514.1995.

*4.2.17. 2′,2′-Dimethyl-7,9-bis(4-nitrophenyl)-3,6,7,9-tetrahydrospiro[pyrazolo[4,3-f]quinoline-8,5′-[1,3]dioxane]-4′,6′-dione (**6e**).* Light yellow solid (85%), % purity (HPLC) = 98.3%, mp 294−296 °C; ^{1}H NMR (500 MHz, DMSO-d_6) (δ, ppm): 12.84 (s, 1H, NH), 8.13−8.38 (m, 3H, ArH), 7.70−7.72 (m, 1H, ArH), 7.64 (d, J = 9 Hz, 2H, ArH), 7.38 (d, J = 9 Hz, 1H, ArH), 7.07 (d, J = 8.5 Hz, 1H, ArH), 6.96−6.99 (m, 2H, ArH), 6.19 (s, 1H, CH), 5.52 (s, 1H, CH), 5.07 (s, 1H, CH), 0.67 (s, 3H, CH_3), 0.52 (s, 3H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) (δ, ppm): 166.85, 160.12, 147.99, 147.20, 145.95, 143.06, 138.05, 135.18, 132.16, 131.41, 131.13, 129.53, 124.01, 123.70, 123.51, 121.10, 118.32, 109.81, 108.01, 105.36, 64.15, 59.00, 48.19, 28.8, 26.97; ESI HRMS: $[M + H]^+$ calcd for $C_{27}H_{21}N_5O_8$, 544.1463; found, 544.1480.

4.3. ABTS Radical Scavenging Assay. The ABTS free radical cation scavenging activity of the compounds was performed using a standard method.[49,50] First, a 7 mM concentrated solution of ABTS was prepared, and then, a 2.45 mM concentrated solution of potassium persulfate was added to the ABTS solution. This mixture was kept in a dark place at room temperature for 14−16 h. The test compound sample solutions were prepared in absolute alcohol at concentrations ranging from 0.01 to 1 mg/mL. The test sample was added to the ABTS solutions and incubated for 30 min at 37 °C. The absorbance was measured at a wavelength of 734 nm, and the procedure was repeated for ascorbic acid as a reference

https://doi.org/10.1021/acsomega.2c05020
ACS Omega 2023, 8, 444−456

standard. The % inhibition of radical scavenging activity was determined using the given formula.

$$\% \text{ inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}}$$

4.4. DPPH Radical Scavenging Assay. The free radical scavenging activity of DPPH was tested using a standard procedure.[49,50] 0.3 mM DPPH concentrated solution in ethanol incubated for 30 min at 37 °C with various test samples. The absorption of the sample was taken at 517 nm. The same procedure was followed for ascorbic acid as the standard. The % inhibition of radical scavenging activity was determined using the given formula.

$$\% \text{ inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}}$$

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c05020.

^{1}H NMR and ^{13}C NMR spectral data for all synthesized compounds and 2D NMR of **4b** (HSQC and COSY), **4g**, and **6a** (NOESY); LCMS spectral data for **4a−g** and **4j−l** and HRMS spectral data for **4h−I** and **6a−e**; ORTEP diagram of compound **4b**; and green matrix factors, *E*-factor, AE, RME, and OE calculation, for representative all the synthesized (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

P.J.P., R.M.V., D.B.U., S.G.P., and H.M.P. are grateful to the Department of Chemistry, Sardar Patel University, for providing chemicals and lab facilities. P.J.P. is grateful to UGC New Delhi, India, for financial support (file no. 16-9(June 2019)/2019 (NET/CSIR); dated 10/06/2020). All authors are thankful to Manoj Mangukiya, Associate Analytical Scientist, Aether Industries Ltd., Surat, for the LC−MS experiments. This work is dedicated to my adored parents.

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