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Efficient and Regioselective Synthesis of Novel Functionalized Dispiropyrrolidines and Their Cytotoxic Activities

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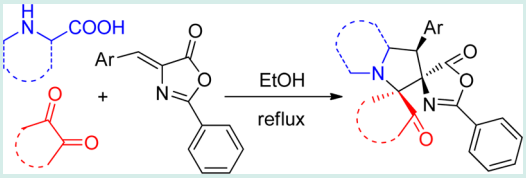
Supporting Information

ABSTRACT: An efficient and regioselective synthesis of novel functionalized dispiropyrrolizidine derivatives via a three-component [3 + 2] cycloaddition reaction of azomethine ylides is described. This protocol has the advantages of high efficiency, mild reaction conditions, a one-pot procedure, and convenient operation. Many of these compounds were evaluated for their antiproliferative properties in vitro against cancer cells and several compounds were found to have good activities.



KEYWORDS: *dispiropyrrolizidine, three-component reaction, azomethine ylide, antitumor activity*

INTRODUCTION

Among one-pot reactions, multicomponent reactions (MCRs) have emerged as powerful synthetic strategies because of their efficiency, atom economy, high selectivity, and convenient construction of multiple new bonds; these characteristics give rapid access to combinatorial libraries of complex organic molecules for efficient lead structure identification and optimization in drug discovery.[1] In the past decade, there have been major developments in three- and four-component reactions, and much effort continues to be devoted to developing new MCRs.[2]

Spiro compounds are an important class of naturally occurring substances and they have pronounced biological properties.[3] Spiropyrrolidines have attracted attention because of their antiviral[4] and local anesthetic[5] activities and as potential antileukemic and anticonvulsant agents.[6] The spiropyrrolidine ring system also occurs in alkaloids,[7] for example, (−)-horsfiline[7a] and spirotryprostatin A (Figure 1).[7b] A number of methods have been reported for the preparation of spirooxindole-fused heterocycles.[8]

The methodology described here incorporates familiar elements in a new and useful way. 1,3-Dipolar cycloaddition reactions play a key role in the synthesis of five-membered heterocycles,[9] and azomethine ylides have been employed for pharmacologically important pyrrolidines and pyrrolizines[10] as well as spiro compounds with high regio- and stereoselectivity.[11] Oxazolone plays a vital role in the manufacture of biologically active agents such as analgesic, anti-inflammatory, antidepressant, anticancer, antimicrobial, antidiabetic, and antiobesity drugs.[12] (*Z*)-4-Benzylidene-2-phenyloxazol-5(4*H*)-ones are readily prepared by the Erlenmeyer−Plöchl reaction, an acetate-catalyzed cyclodehydration−condensation of aldehyde and hippuric acid in dry acetic anhydride.[13] These intermediates contain multiple electrophilic centers (Figure 2), with attack usually occurring at the carbonyl group, often leading to ring-opening.[14] In addition, the exocyclic double bond can operate as a dienophile, and *N*-substituted oxazolones participate in intermolecular Diels−Alder reactions.[15] We

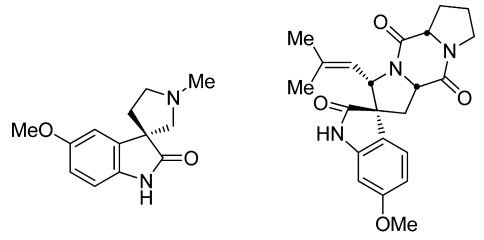


Figure 1. Structures of (−)-horsfiline and spirotryprostatin A.

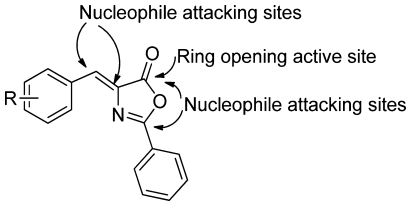


Figure 2. Reaction centers of (*Z*)-4-benzylidene-2-phenyloxazol-5(4*H*)-ones.

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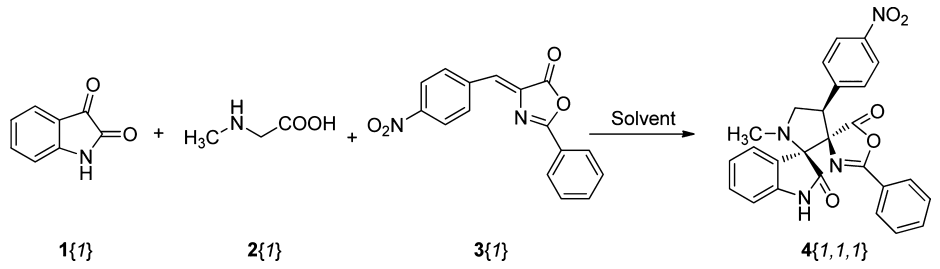
Scheme 1. Model Reaction



recently reported the use of this rich reactive system in a 1,3-dipolar cycloaddition-based preparation of some novel dispiropyrrolidine derivatives.[16] Here we extend this methodology to the facile synthesis of dispiropyrrolidines containing an oxazolone moiety via one-pot, three-component, 1,3-dipolar cycloaddition reactions of (*Z*)-4-benzylidene-2-phenyloxazol-5(4*H*)-ones with azomethine ylides.

RESULTS AND DISCUSSION

We initially evaluated the three-component reaction of isatin **1**{*1*}, sarcosine **2**{*1*}, and (*Z*)-4-(4-nitrobenzylidene)-2-phenyloxazol-5(4*H*)-one **3**{*1*} (Scheme 1). The reaction mixture, which consisted of a 1:1:1 mixture of **1**{*1*}, **2**{*1*}, and **3**{*1*}, was tested under various conditions. The effects of solvents and reaction temperature on this reaction were evaluated; the results are summarized in Table 1. It can be seen from Table 1 that ethanol is the solvent of choice for the reaction, and the desired product is obtained in excellent yield.

Table 1. Optimizing the Reaction Conditions for the Synthesis of 4{*1,1,1*}

entry	solvent	temperature (°C)	time (h)	isolated yield (%)
1	EtOH	reflux	3	87
2	MeOH	reflux	3	78
3	CH_3CN	reflux	3	75
4	THF	reflux	3	54
5	1,4-dioxane	reflux	3	68
6	toluene	reflux	3	70

The optimized reaction conditions were then tested for library construction, using five 1,2-dicarbonyl compounds **1**{*1*−*5*}, two amino acid derivatives **2**{*1*−*2*}, and 11 dipolarophiles **3**{*1*−*11*} (Figure 3). The corresponding functionalized dispiropyrrolizidine derivatives **4** were obtained in good yields at refluxing temperature in ethanol without any catalyst. The results are summarized in Table 2. This protocol was efficient when 1,2-dicarbonyl compounds were used with either isatin or acenaphthenequinone. It was also found that phenyl groups bearing either electron-withdrawing or -donating groups on the dipolarophiles were tolerated under the reaction conditions, leading to the final products in satisfactory yields (68−95%). However, the electronic properties of the substituents of phenyl groups on the dipolarophiles affected the product yields. When the substituents were electron-withdrawing groups (such as NO_2, F, Cl, and Br), the yields were higher and the reaction times were shorter. When the substituents were electron-donating groups (such as CH_3O and CH_3), the yields were lower and the reaction time were longer.

The structures of **4** were characterized using IR, ^{1}H NMR, and ^{13}C NMR spectroscopies, and HRMS analysis. The

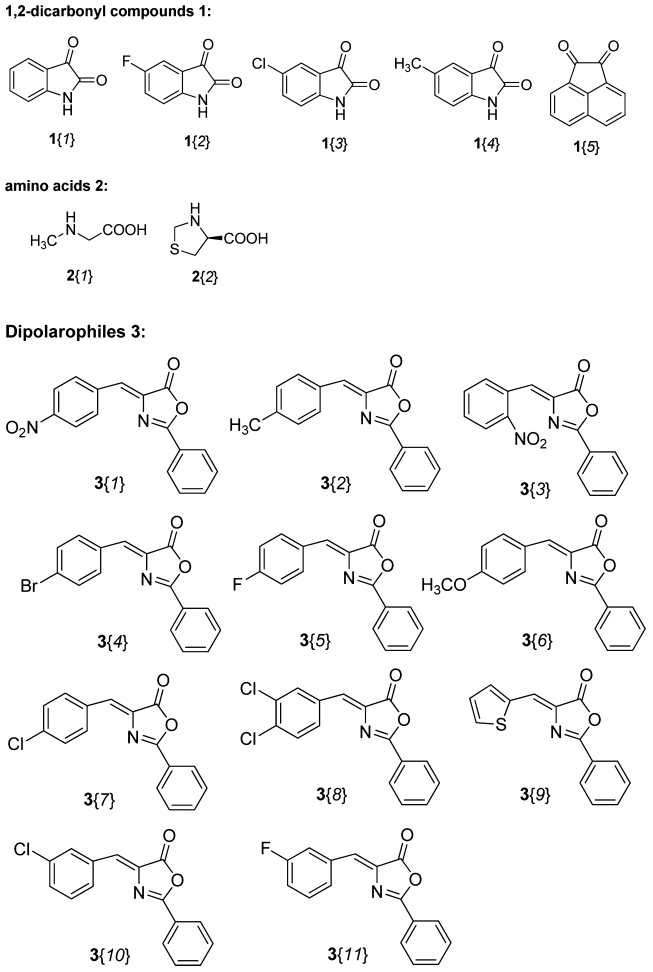


Figure 3. Substrates used in synthesis of dispiropyrrolidine derivatives **4**.

structures of **4**{*5,1,3*} and **4**{*5,2,8*} were further confirmed by X-ray diffraction (see the Supporting Information). The molecular structures of **4**{*5,1,3*} and **4**{*5,2,8*} are shown in Figure 4.

Although the detailed mechanism of the above reaction has not yet been clarified, the formation of **4** can be explained by the possible mechanism presented in Scheme 2. The reaction proceeds through generation of an azomethine ylide (dipole **10**) via condensation of isatin **1** with sarcosine **2**, and decarboxylation. The dipolarophile **3** regioselectively reacts with the azomethine ylide (dipole **10**) in ethanol to give the desired product **4** (Scheme 2, path A). The regioselectivity of the product formation can be explained by considering the secondary orbital interaction (SOI)[17] of the orbital of the carbonyl group of dipolarophile **3** with that of the ylide **10**, as

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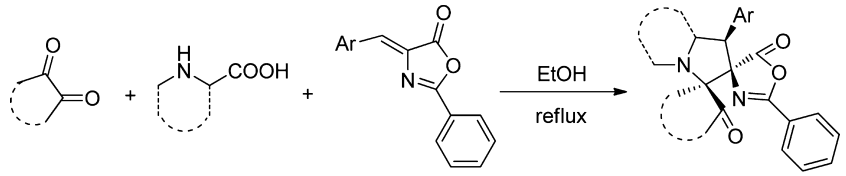
Table 2. Synthesis of Functionalized Dispiropyrrolizidine Derivatives 4



1{*1-5*} **2**{*1-2*} **3**{*1-11*} **4**{*1(1-5),2(1-2),3(1-11)*}

entry	products	time (h)	isolated yield (%)
1	4{*1,1,1*}	3	87
2	4{*1,1,2*}	5	74
3	4{*1,1,3*}	5	78
4	4{*1,2,2*}	5	75
5	4{*1,2,4*}	3	87
6	4{*1,2,5*}	3	84
7	4{*1,2,6*}	5	68
8	4{*1,2,7* }	3	83
9	4{*2,1,5* }	3	86
10	4{*2,1,11* }	3	85
11	4{*3,1,5* }	3	80
12	4{*3,1,11* }	3	84
13	4{*4,1,7* }	3	83
14	4{*4,1,11* }	3	80
15	4{*5,1,1*}	2	91
16	4{*5,1,3*}	3	80
17	4{*5,1,7*}	3	83
18	4{*5,1,8*}	3	85
19	4{*5,1,9*}	4	76
20	4{*5,2,1*}	2	95
21	4{*5,2,4*}	3	87
22	4{*5,2,5*}	3	90
23	4{*5,2,8*}	3	88
24	4{*5,2,9*}	3	80
25	4{*5,2,10*}	3	82

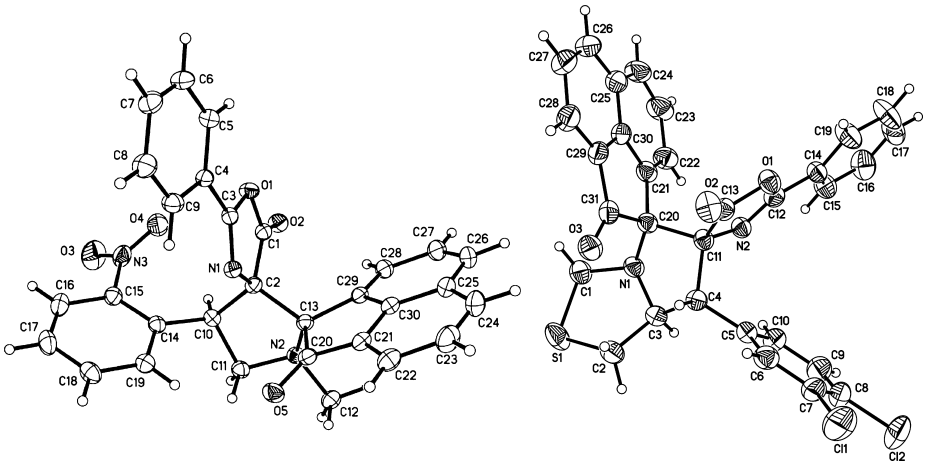


Figure 4. X-ray crystal structures of (left) 4{*5,1,3*} and (right) 4{*5,2,8*}.

shown in Scheme 2. Formation of the observed regioisomer **4** via path A is therefore favorable because of the SOI, which is not possible in path B. Control of the relative stereochemistry at the spiro center was observed. The cycloaddition proceeds via an endo transition state.[18]

Most of the dispiropyrrolizidine derivatives synthesized were tested for their antiproliferative properties against hepatic carcinoma (HepG2) cells in vitro. The inhibitory rates obtained for the compounds are summarized in Figure 5. Some compounds with inhibitory rates greater than 50% were tested further to determine the IC_{50} value. The results are shown in Table 3. The most active compounds were 4{*1,1,3*} and 4{*5,1,3*}, with IC_{50} values of 10.5 ± 0.3 and 11.7 ± 1.4 μM, respectively. Both of these molecules possess a 2-nitrophenyl group at the pyrrole ring, so this is suggested as being important for cytotoxic activity.

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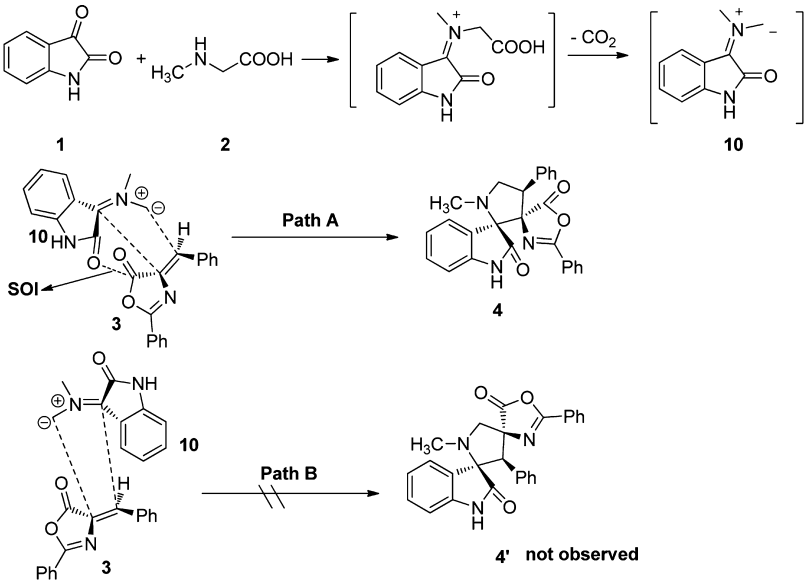
Scheme 2. Proposed Mechanism for the Synthesis of 4



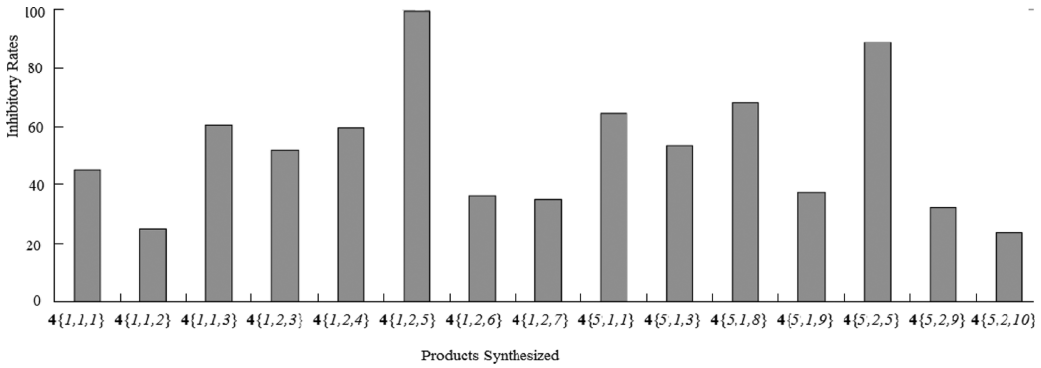


Figure 5. Inhibitory rates of compounds **4** (30 μm) for HepG2 cells.

CONCLUSION

In summary, we have developed an efficient one-pot method for the synthesis of pharmacologically important, highly functionalized dispiropyrrolizidine derivatives by 1,3-dipolar cycloaddition, using (*Z*)-4-benzylidene-2-phenyloxazol-5(4*H*)-ones, which have multiactive centers, as dipolarophiles. The chemo-, regio-, and stereo-selectivity can be controlled. This method has the advantages of high atom economy, excellent yields, mild reaction conditions, and high selectivity. Overall, our study suggests that the dispiropyrrolizidine derivatives presented here have medicinal values and the basic framework of this class of heterocycles is an attractive template for the identification of novel potential anticancer agents.

EXPERIMENTAL SECTION

General Procedure for the Synthesis of Dispiropyrrolizidine Derivatives 4. A dry 50 mL flask was charged with 1,2-dicarbonyl compounds **1**{*1*−*5*} (0.5 mmol), amino acid derivatives **2**{*1*−*2*} (0.5 mmol), dipolarophiles **3**{*1*−*11*} (0.5 mmol), and ethanol (10 mL). The reaction mixture was stirred at reflux temperature for 3−5 h. After completion of the reaction, the solvent was removed under vacuum. The solid was recrystallizated from ethanol, and then dried at 80 °C for 4 h under vacuum to give **4**.

Compound ***4****{1,1,1}*. m.p.: 216−218 °C. IR (KBr, ν, cm^{-1}): 3429, 3163, 3076, 2874, 1813, 1723, 1640, 1469, 1309, 1214, 979, 899, 754. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 3.55 (t, J = 7.6 Hz, 1H, CH−H), 4.12 (t, J = 7.6 Hz, 1H, CH−H), 4.21 (t, J = 9.2 Hz, 1H, CH), 6.69 (d, J = 7.6 Hz, 1H, ArH), 6.99 (t, J = 7.6 Hz, 1H, ArH), 7.19 (t, J = 6.8 Hz, 2H, ArH), 7.25 (t, J = 7.2 Hz, 3H, ArH), 7.40 (t, J = 7.6 Hz, 2H, ArH), 7.51−7.54 (m, 4H, ArH), 10.40 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 39.7, 59.0, 64.8, 82.5, 87.5, 114.9, 127.1, 128.9, 129.7, 131.5, 132.7, 132.8, 133.2, 133.9, 134.5, 135.3, 135.7, 138.8, 141.1, 149.0, 164.9, 180.8, 183.7. HRMS calcd for $C_{26}H_{21}N_4O_5$ $[M + H]^+$: 469.1512, found: 469.1523.

Compound ***4****{1,2,2}*. m.p.: 214−216 °C. IR (KBr, ν, cm^{-1}): 3279, 2900, 1819, 1731, 1648, 1463, 1306, 1188, 976, 785. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.16 (s, 3H, CH_3), 3.00−3.03 (m, 1H, CH−H), 3.13−3.18 (m, 1H, CH−H), 3.65 (d, J = 7.6 Hz, 1H, CH−H), 3.82 (d, J = 8.0 Hz, 1H, CH−H), 4.66 (s, 2H, 2 × CH), 6.76−6.80 (m, 2H, ArH), 7.04 (d, J = 7.6 Hz, 2H, ArH), 7.13−7.21 (m, 3H, ArH), 7.36 (d, J = 7.2 Hz, 1H, ArH), 7.51 (t, J = 7.2 Hz, 2H, ArH), 7.62−7.66 (m, 1H, ArH), 7.83 (d, J = 8.0 Hz, 2H, ArH), 10.82 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 21.2, 36.1, 51.5, 54.4, 73.5, 78.4, 86.3, 110.6, 122.2, 124.7, 125.2, 127.9, 128.2, 129.7, 129.9, 130.0, 130.1, 131.0, 134.2, 138.1, 142. 7, 160.3, 173.4,

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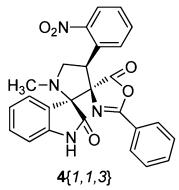
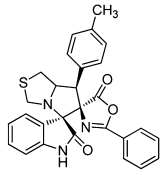
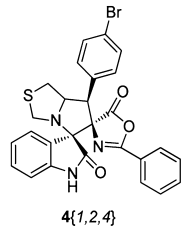
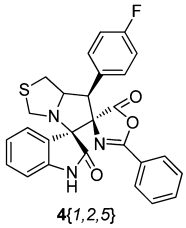
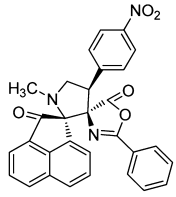
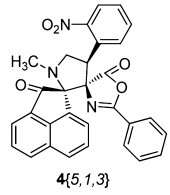
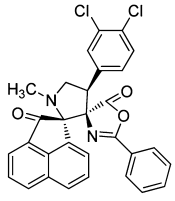
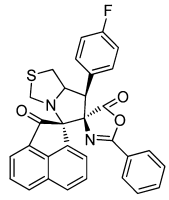
Table 3. IC_{50} Values of Selected Compounds

compound	IC_{50}/μm	compound	IC_{50}/μm	compound	IC_{50}/μm
 4{1,1,3}	10.5 ± 0.3	 4{1,2,2}	> 30	 4{1,2,4}	> 30
 4{1,2,5}	21.9 ± 2.7	 4{5,1,1}	16.4 ± 3.5	 4{5,1,3}	11.7 ± 1.4
 4{5,1,8}	14.8 ± 0.2	 4{5,2,5}	24.3 ± 1.9		

175.4. HRMS calcd for $C_{28}H_{22}N_3O_3S$ $[M - H]^+$: 480.1382, found: 480.1345.

Compound 4{5,1,1}. m.p.: 176−178 °C. IR (KBr, ν, cm^{-1}): 3061, 2947, 2709, 1816, 1719, 1649, 1516, 1339, 1113, 974, 697. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 2.24 (s, 3H, CH_3), 3.78−3.79 (m, 1H, CH−H), 4.30−4.33 (m, 1H, CH−H), 4.43−4.45 (m, 1H, CH), 7.27 (s, 4H, ArH), 7.42−7.44 (m, 1H, ArH), 7.65−7.90 (m, 6H, ArH), 8.01−8.02 (m, 1H, ArH), 8.17−8.18 (m, 3H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 34.7, 52.7, 60.4, 80.9, 83.1, 121.1, 123.2, 123.4, 124.1, 126.8, 127.6, 129.1, 129.3, 129.4, 132.1, 132.4, 142.0, 144.4, 147.2, 160.7, 178.1, 204.3. HRMS calcd for $C_{30}H_{22}N_3O_5$ $[M + H]^+$: 504.1559, found: 504.1583.

Compound 4{5,2,1}. m.p.: 202−204 °C. IR (KBr, ν, cm^{-1}): 3055, 2934, 1817, 1723, 1645, 1510, 1300, 987, 706. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.20−3.22 (m, 2H, CH_2), 3.57−3.60 (m, 1H, CH), 3.86−3.87 (m, 1H, CH), 4.82−4.88 (m, 2H, CH_2), 7.50−7.87 (m, 9H, ArH), 7.89−8.13 (m, 5H, ArH), 8.29−8.31 (m, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 36.2, 51.3, 55.1, 73.1, 81.5, 86.2, 122.0, 123.9, 124.1, 124.5, 126.9, 128.2, 129.0, 129.3, 129.8, 129.9, 130.5, 130.8, 131.2, 133.2, 134.2, 134.3, 134.4, 141.6, 147.8, 160.9, 173.1, 203.5. HRMS calcd for $C_{31}H_{22}N_3O_5S$ $[M + H]^+$: 548.1280, found: 548.1289.

Inhibition of Cell Proliferation. Hepatic carcinoma HepG2 cells were grown in the minimum essential medium Eagles with Earle's balanced salts (MEM-EBSS) medium (Hyclone, Logan, Utah). The medium was supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin and 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere containing 5% CO_2.

For cell proliferation assay, cells were seeded into 96-well plates (4000 cells/well) and incubated at 37 °C in a humidified 5% CO_2 atmosphere. After 24 h, cells were treated with different concentration compounds for 48 h in triplicate to generate dose−response curves. Cell proliferation was determined by the SRB assay as previous described.[19] The IC_{50} value was calculated using SigmaPlot 10.0 software which defined as the inhibitor concentration of 50% cell growth inhibition.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details and spectroscopic characterization for compounds **4** and the X-ray crystallographic information for compounds **4**{*5,1,3*} and **4**{*5,2,8*}. This information is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for the structures of compounds **4**{*5,1,3*} and **4**{*5,2,8*} have been depsited at the Cambridge Crystallographic Data Center, and the deposit numbers are CCDC-970292 and CCDC-970281. Copy of available material can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax +44 1223 336 033; e-mail deposit@ccdc.cam.ac.uk). Crystal data of **4**{*5,1,3*}: molecular formula = $C_{30}H_{21}N_3O_5 \cdot C_2H_5OH$, formula weight = 503.50, (crystal system) triclinic, (space group) $P\bar{1}$, a = 7.8331(13) Å, b = 10.443(2) Å, c = 16.690(3) Å, α = 101.953(19)°, β = 94.064(3)°, γ = 111.538(4)°, V = 1226.1(4) $Å^3$, T = 223(2) K, Z = 2, D_c = 1.364 Mg m^{-3}, μ(Mo Kα) = 0.095 mm^{-1}, 10625 reflection measured, 5505 independent reflections, R_1 = 0.0452, wR_2 = 0.1098. Crystal data of **4**{*5,2,8*}: molecular formula = $C_{31}H_{20}Cl_2N_2O_3S \cdot DMF$, formula weight = 571.45, (crystal system) monoclinic, (space group) $P2_1/n$, a =

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10.912(2) Å, b = 15.206(3) Å, c = 16.341(3) Å, β = 95.182(6)°, V = 2700.3(9) Å^3, T = 293(2) K, Z = 4, D_c = 1.406 Mg m^{-3}, μ(Mo Kα) = 0.355 mm^{-1}, 25982 reflection measured, 4938 independent reflections, R_1 = 0.0776, wR_2 = 0.1671.

AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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