Synthesis and Biological Activities of 1-Azaaurone Derivatives

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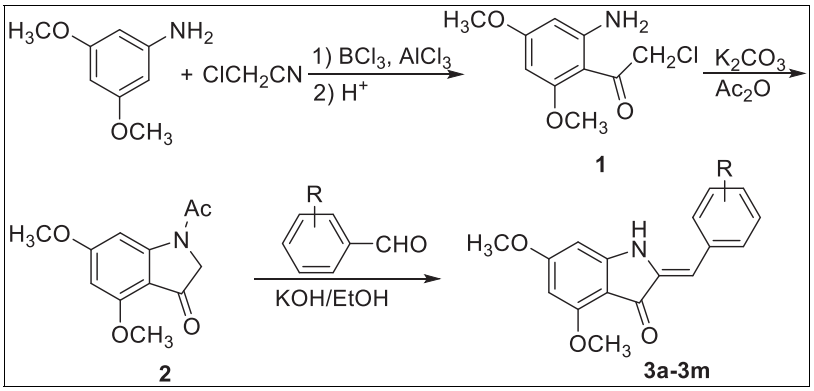

A series of 1-azaaurone derivatives were designed and synthesized from 3,5-dimethoxyaniline and 2-chloroacetonitrile. Their structures were characterized by melting point, ^{1}H NMR, IR, and elemental analysis, as well as ^{13}C NMR. The target compounds were evaluated for antitumor activities against human hepatocellular liver carcinoma cell line (HepG-2) and human cervix carcinoma cell line (Hela) using methyl thiazolyl tetrazolium method. The results revealed that several 1-azaaurones exhibited strong proliferation inhibition efficacy against HepG-2 and Hela with an IC_{50} range of 5.6–8.8 μg/mL without damaging normal cell.

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INTRODUCTION

Aurones, a subclass of naturally occurring flavonoids, are met in vegetables, flowers, and fruits where they contribute to the yellow or orange color [1]. They were also used by plants to resist pathogens [2], pests [3], and competitive weeds [4,5]. Recently, increasing attention has been focused on aurones because both natural aurones and synthetic analogs exhibited excellent antitumor [6,7], antioxidant [8,9], antibacterial, and anti-inflammatory [10,11] activities, as well as acted as potent multifunctional defense agents against Alzheimer's disease [12,13]. In addition, aurones have been proved to be valuable inhibitors of human tyrosinase to prevent hyperpigmentation-related abnormality [14,15]. These studies indicate that aurone has evolved to be an excellent leading scaffold for the development of various analogs with significant biological activity.

1-Azaaurone, an isosteric equivalent replacement of aurone by replacing intracyclic O atom at 1-position with NH group (Fig. 1), has been found to display important bioactivity [7,16,17]. Moreover, some azaaurones exhibited much stronger bioactivity than the parent aurones. For example, Souard *et al.* reported that azaaurone analogs demonstrated better antiplasmodial activity in comparison with corresponding aurones [16]. In one of our previous studies [18], it was found that 6-substituted aurones possessed excellent antitumor effects against HepG-2, Hela, and MCF-7 cancer cell lines, and the variations of substituents at the A-ring and/or B-ring could cause remarkable changes in the activity. On the other hand, some examples support that the presence of methoxy moieties at C-4 and C-6 positions of aurones may lead to a substantial increase in the activity [4,19,20]. Collectively, it is of interest to synthesize 1-azaaurones bearing methoxyl groups at C-4 and C-6 positions and possessing diverse substituents at the B-ring, to evaluate their preliminary antitumor activity and to explore the structure–activity relationship.

RESULTS AND DISCUSSION

Synthesis. The synthetic pathway of title compounds was summarized in Scheme 1. 1-(2-Amino-4,6-dimethoxyphenyl)-2-chloroethan-1-one (**1**) was synthesized from 3,5-dimethoxyaniline and 2-chloroacetonitrile in the presence of trichloroborane and dry aluminum trichloride, followed by hydrolysis according to the reported procedure [21]. Treatment of **1** with acetic anhydride in the presence of potassium carbonate produced *N*-acetyl-4,6-dimethoxyindolin-3-one (**2**) in

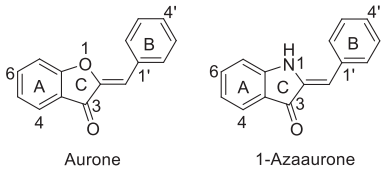
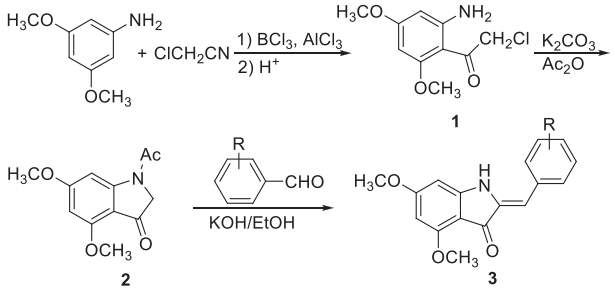


Figure 1. Basic structures of aurone and 1-azaaurone.

Scheme 1. Synthetic route of the title compounds.



3a: R = H; **3b**: R = 2'-F; **3c**: R = 3'-F; **3d**: R = 4'-F;
3e: R = 2'-CH_3; **3f**: R = 3'-CH_3; **3g**: R = 4'-CH_3;
3h: R = 2'-OCH_3; **3i**: R = 3'-OCH_3; **3j**: R = 4'-OCH_3;
3k: R = 3'-CF_3; **3l**: R = 3',4'-$(OCH_3)_2$; **3m**: R = 4'-OH-3'-OCH_3

good yield. The target 1-azaaurone derivatives **3a**–**3m** were obtained by the condensation of **2** with various arylaldehydes in H_2O/EtOH under alkali condition. Six of them (**3d**, **3e**, **3g**, and **3k**–**3m)** are new compounds. The target compounds were confirmed by ^{1}H NMR, IR, and elemental analysis, as well as ^{13}C NMR. These 1-azaaurones were identified exclusively as *Z* stereoisomers on the basis of the ^{1}H chemical shifts of exocyclic vinyl proton (═CH─), which appeared at δ 6.38–6.84 ppm in ^{1}H NMR, corresponding to the known values (ca. 6.70 ppm) for (*Z*)-aurones [4,8,12].

Biological activity. The preliminary antitumor activities of all synthetic 1-azaaurones against HepG-2 and Hela were evaluated using methyl thiazolyl tetrazolium (MTT) assay [18]. As demonstrated in Table 1, most of the compounds were active toward HepG-2 and Hela at the concentration of 10 μg/mL. For example, compounds **3f**, **3l**, and **3m** displayed an inhibition rate of higher than 60% against both HepG-2 and Hela. Regarding antitumor activity against HepG-2, the structure–activity relationship (Table 1) confirmed that the presence of electron-withdrawing group at the B-ring led to less active compounds (**3c**, **3d**, and **3k** vs **3a**), while those with a donating-electron group at the B-ring showed a little better inhibitory effect (**3e**, **3f** vs **3a**). Further, two electron-donating groups at the B-ring led to a dramatic increase in the activity (**3l**, **3m** vs **3a**). It was also found that hydroxyl group at C-4′ position was highly beneficial when **3l** was compared with **3m**. With regard to the activity against Hela, the presence of electron-donating group at the B-ring was highly advantageous (**3f**, **3h**–**3j** vs **3a**–**3d**, **3k**); further, two methoxyl groups at C-3′ and C-4′ positions led to the most potent compound (**3l** vs **3i**, **3j**). However, when the methoxy moiety at C-4′ position was replaced by hydroxyl group, the activity decreased slightly (**3m** vs **3l**). Taken together, possessing the electron-donating groups at the B-ring is beneficial to the antitumor activity of 1-azaaurones but is disadvantageous to that of aurones [18], which is probably correlated to the difference of O atom and NH group at 1-position.

On the basis of the screening results, compounds **3f**, **3j**, **3l**, and **3m** were selected to further evaluate their IC_{50} values against cancer cell lines HepG-2 and Hela, as well as normal human hepatic cell line (WRL-68) with **Aurone A** as a reference control, which is the most active aurone derivative in our previous work [18]. As shown in Table 2, each of them was active with IC_{50} value below 10 μg/mL. The most active compound was **3m** with IC_{50} values of 5.6 and 6.3 μg/mL against HepG-2 and Hela comparative to IC_{50} values of **Aurone A** (8.4 and 3.3 μg/mL), respectively. More interestingly, compounds **3l** and **3m** were proved to be ineffective against WRL-68 with IC_{50} values of 74.1 and 62.4 μg/mL, respectively. These results implied that 1-azaaurone derivatives selectively inhibited cancer cell lines without damaging normal cell. Moreover, these 1-azaaurones displayed much higher inhibition toward Hela than the aurones described in the literature [22], which might also

Table 1

Antiproliferative activities of 1-azaaurones **3a**–**3m** (inhibition% at 10 μg/mL).

Compound	HepG-2	Hela	Compound	HepG-2	Hela
3a	55.4	NA	**3h**	51.6	54.8
3b	55.3	31.8	**3i**	46.7	54.4
3c	43.6	8.75	**3j**	50.5	60.9
3d	16.8	NA	**3k**	36.3	16.5
3e	60.3	17.7	**3l**	63.7	80.0
3f	63.2	65.5	**3m**	82.6	71.7
3g	50.5	22.7			

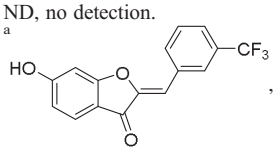
NA, no activity.

Table 2

In vitro antitumor activities of some target compounds (IC_{50}, μg/mL).

Compound	HepG-2	Hela	WRL-68
3f	7.5	7.8	ND
3j	8.0	6.7	ND
3l	7.4	8.8	74.1
3m	5.6	6.3	62.4
Aurone A[a]	8.4	3.3	ND

ND, no detection.

[a]  , reported in our previous work [18].

be attributed to the replacement of the intracyclic O atom by the NH group.

In conclusion, a series of 1-azaaurones were designed and synthesized, and their antitumor activities against cancer cell lines HepG-2, Hela, and normal cell WRL-68 were evaluated. The preliminary bioassay demonstrated that some 1-azaaurones selectively inhibited cancer cell lines without damaging normal cell. The structure–activity relationship revealed that the substituents at the B-ring played an important role on the activity. The present investigation brings essential elements, which will help to design and synthesize more potent azaaurones.

EXPERIMENTAL

IR spectroscopy were recorded on a Nicolet Nexus 470 spectrometer (Nicolet Instrument Corporation, Madison, WI) as KBr pellets. Melting points were uncorrected and obtained using an X-4 digital melting point apparatus. NMR spectra were recorded on a Bruker AVANCEII 400 MHz instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C). Elemental analysis was carried out with a FLASH1112A analyzer (CE Instruments Ltd, Milan, Italy).

Synthesis of compound 1. 1-(2-Amino-4,6-dimethoxyphenyl)-2-chloroethan-1-one (**1**) was prepared basing on the procedure [21] from the reaction of 3,5-dimethoxyaniline (3.06 g, 20.0 mmol) and 2-chloroacetonitrile (23.6 mmol) in dry dichloromethane (20 mL), in the presence of trichloroborane (23.6 mmol) and anhydrous aluminum trichloride (2.93 g, 22.0 mmol) at 0°C. After working up, the reaction solution was treated with hydrochloric acid solution (2 mol/L), followed by heating at 80°C for 0.5 h. The resulting solution was extracted three times with CH_2Cl_2, washed with water, dried with Na_2SO_4, and followed by removing the solvent to give a yellow solid, yield 70%, mp 90–92°C.

Synthesis of compound 2. *N*-Acetyl-4,6-dimethoxyindolin-3-one (**2**) was prepared according to a reported method [21]. A solution of intermediate **1** (1.15 g, 5.0 mmol), K_2CO_3 (0.83 g, 6.0 mmol) in acetone (30 mL) was stirred at room temperature till all solid was dissolved. Then, the solution was treated with acetic anhydride (1.08 g, 10.6 mmol) and heated under reflux until completion of the reaction (monitored via thin-layer chromatography). The resulting solution was poured into ice water after cooling. The formed solid was filtered off, washed with water, dried, and recrystallized from EtOH to afford product **2** in 60% yield, mp 93–95°C.

General synthetic procedure for 1-azaaurones 3a–3m. Oxindole **2** (0.24 g, 1.0 mmol) was dissolved in EtOH (5 mL) then treated with KOH solution (10%, 5 mL). The corresponding arylaldehyde (1.2 mmol) was added to the mixture. Then, the solution was heated at 50°C for 3–5 h. EtOH was evaporated after the reaction was finished by thin-layer chromatography monitoring. Ice water was added to the residue, followed by neutralizing with hydrochloric acid solution (2 mol/L). The precipitate was collected by filtration, washed with water three times, and purified by recrystallization from EtOH to yield compounds **3a**–**3m**.

(Z)-2-Benzylidene-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3a). Compound **3a** has been reported previously [23,24]. Yellow solid, yield 83.0%, mp 134–136°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.86 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 5.93 (d, J = 1.6 Hz, 1H, ArH), 6.06 (d, J = 2.0 Hz, 1H, ArH), 6.75 (s, 1H, =CH), 6.84 (br s, 1H, NH), 7.29–7.34 (m, 1H, ArH), 7.39–7.45 (m, 2H, ArH), 7.49–7.53 (m, 1H, ArH). IR (KBr) v: 3404, 3068, 1672, 1624, 1597, 1585, 1464, 1451, 1398, 1364, 1263, 1223, 1211, 1159, 1126, 1050 cm^{-1}. *Anal*. Calcd for $C_{17}H_{15}NO_3$: C 72.58, H 5.37, N 4.98. Found: C 72.26, H 5.53, N 5.20.

(Z)-2-(2-Fluorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3b). Compound **3b** has been reported in the literature [19]. Orange solid, yield 68.4%, mp 110–113°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.01 (d, J = 2.0 Hz, 1H, ArH), 6.19 (d, J = 2.0 Hz, 1H, ArH), 6.44 (s, 1H, =CH), 7.25–7.33 (m, 2H, ArH), 7.35–7.41 (m, 1H, ArH), 7.76–7.82 (m, 1H, ArH), 9.76 (br s, 1H, NH). IR (KBr) v: 3362, 3088, 2978, 1667, 1626, 1577, 1516, 1513, 1467, 1438, 1372, 1296, 1252, 1219, 1198, 1153, 1119,1017 cm^{-1}. *Anal*. Calcd for $C_{17}H_{14}FNO_3$: C 68.22, H 4.71, N 4.68. Found: C 68.46, H 4.53, N, 5.05.

(Z)-2-(3-Fluorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3c). Compound **3c** has been reported in the literature [25]. Orange solid, yield 66.3%, mp 95–96°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.82 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.01 (d, J = 1.6 Hz, 1H, ArH), 6.20 (d, J = 1.6 Hz, 1H, ArH), 6.38 (s, 1H, =CH), 7.11–7.18 (m, 1H, ArH), 7.43–7.54 (m, 3H, ArH), 9.79 (br s, 1H, NH). IR (KBr) v: 3412, 3036, 1627, 1590, 1511, 1458, 1380, 1242, 1218, 1156, 1120, 1050, 1009 cm^{-1}. *Anal*.

Calcd for $C_{17}H_{14}FNO_3$: C 68.22, H 4.71, N 4.68. Found: C 67.91, H 4.93, N 4.97.

(Z)-2-(4-Fluorobenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3d). Orange solid, yield 68.9%, mp 108–110°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 5.99 (d, J = 1.6 Hz, 1H, ArH), 6.19 (d, J = 1.6 Hz, 1H, ArH), 6.40 (s, 1H, =CH), 7.24–7.31 (m, 2H, ArH), 7.67–7.75 (m, 2H, ArH), 9.73 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.4, 55.6, 88.6, 90.6, 103.0, 105.9, 115.7 (d, J = 21.4 Hz, 2C), 130.9 (d, J = 3.2 Hz), 131.4 (d, J = 8.0 Hz, 2C), 135.1, 157.0, 159.6, 161.3 (d, J = 245.3 Hz), 167.9, 181.3. IR (KBr) v: 3420, 3032, 1626, 1595, 1507, 1462, 1380, 1262, 1217, 1162, 1128 cm^{-1}. *Anal*. Calcd for $C_{17}H_{14}FNO_3$: C 68.22, H 4.71, N 4.68. Found: C 68.01, H 4.90, N 4.89.

(Z)-2-(2-Methylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3e). Yellow solid, yield 82.6%, mp 213–214°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.38 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.90 (d, J = 2.0 Hz, 1H, ArH), 6.00 (d, J = 1.6 Hz, 1H, ArH), 6.81 (br s, 1H, NH), 6.84 (s, 1H, =CH), 7.17–7.25 (m, 3H, ArH), 7.47–7.51 (m, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 20.0, 55.7, 55.8, 88.1, 91.0, 105.1, 107.5, 126.2, 127.9, 128.0, 130.7, 133.8, 136.8, 138.1, 156.5, 160.4, 168.5, 181.9. IR (KBr) v: 3420, 3095, 2966, 1674, 1607, 1517, 1462, 1370, 1257, 1217, 1200, 1154, 1123, 1033, 1001 cm^{-1}. *Anal*. Calcd for $C_{18}H_{17}NO_3$: C 73.20, H 5.80, N 4.74. Found: C 73.01, H 5.95, N 4.50.

(Z)-2-(3-Methylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3f). Compound **3f** has been reported previously [24]. Yellow solid, yield 65.1%, mp 168–170°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.38 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.91 (d, J = 1.6 Hz, 1H, ArH), 6.08 (d, J = 2.0 Hz, 1H, ArH), 6.71 (s, 1H, =CH), 7.01 (br s, 1H, NH), 7.08–7.12 (m, 1H, ArH), 7.28–7.33 (m, 3H, ArH). IR (KBr) v: 3244, 2970, 2848, 1675, 1605, 1511, 1458, 1430, 1370, 1262, 1216, 1158, 1119, 1046 cm^{-1}. *Anal*. Calcd for $C_{18}H_{17}NO_3$: C 73.20, H 5.80, N 4.74. Found: C 73.54, H 5.62, N 4.90.

(Z)-2-(4-Methylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3g). Yellow solid, yield 84.2%, mp 187–189°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.37 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.91 (d, J = 1.6 Hz, 1H, ArH), 6.07 (d, J = 2.0 Hz, 1H, ArH), 6.74 (s, 1H, =CH), 6.91 (br s, 1H, NH), 7.21 (d, J = 8.0 Hz, 2H, ArH), 7.40 (d, J = 8.0 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 21.3, 55.7(2C), 88.3, 91.1, 105.0, 109.9, 129.2(2C), 129.7(2C), 132.1, 135.8, 138.2, 156.5, 160.3, 168.4, 182.4. IR (KBr) v: 3285, 3100, 2998, 1683, 1630, 1610, 1590, 1508, 1463, 1383, 1319, 1254, 1214, 1158, 1115, 1042 cm^{-1}. *Anal*. Calcd for $C_{18}H_{17}NO_3$: C 73.20, H 5.80, N 4.74. Found: C 72.95, H 6.11, N 4.94.

(Z)-2-(2-Methoxylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3h). Compound **3h** has been reported in the literature [25]. Yellow solid, yield 67.1%, mp 214–216°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.85 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 5.88 (d, J = 1.6 Hz, 1H, ArH), 6.00 (d, J = 2.0 Hz, 1H, ArH), 6.84 (s, 1H, =CH), 6.93–7.03 (m, 2H, ArH), 7.27–7.34 (m, 2H, ArH + NH), 7.46 (dd, J = 7.6, 1.2 Hz, 1H, ArH). IR (KBr) v: 3377, 2933, 2840, 1671, 1630, 1589, 1511, 1489, 1463, 1434, 1381, 1264, 1246, 1218, 1157, 1117, 1029 cm^{-1}. *Anal*. Calcd for $C_{18}H_{17}NO_4$: C 69.44, H 5.50, N 4.50. Found: C 69.65, H 5.44, N, 4.24.

(Z)-2-(3-Methoxylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3i). Compound **3i** has been reported previously [24]. Orange-yellow solid, yield 85.0%, mp 187–189°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.91 (d, J = 1.6 Hz, 1H, ArH), 6.05 (d, J = 2.0 Hz, 1H, ArH), 6.69 (s, 1H, =CH), 6.84 (dd, J = 8.0, 2.0 Hz, 1H, ArH), 6.99 (br s, 1H, NH), 7.00–7.03 (m, 1H, ArH), 7.07–7.12 (m, 1H, ArH), 7.29–7.35 (m, 1H, ArH). IR (KBr) v: 3426, 3077, 2942, 1667, 1626, 1592, 1516, 1463, 1385, 1256, 1216, 1158, 1120, 1051 cm^{-1}. *Anal*. Calcd for $C_{18}H_{17}NO_4$: C 69.44, H 5.50, N 4.50. Found: C 69.67, H 5.26, N 4.74.

(Z)-2-(4-Methoxylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3j). Compound **3j** has also been reported in the literature [24]. Yellow solid, yield 87.3%, mp 213–215°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 5.90 (d, J = 1.6 Hz, 1H, ArH), 6.08 (d, J = 1.6 Hz, 1H, ArH), 6.73 (s, 1H, =CH), 6.93 (d, J = 8.8 Hz, 2H, ArH), 6.97 (br s, 1H, NH), 7.46 (d, J = 8.8 Hz, 2H, ArH). IR (KBr) v: 3421, 3089, 2929, 1630, 1591, 1509, 1467, 1366, 1299, 1253, 1217, 1176, 1160, 1119, 1029 cm^{-1}. *Anal*. Calcd for $C_{18}H_{17}NO_4$: C, 69.44; H 5.50, N 4.50. Found: C 69.35, H 5.66, N 4.35.

(Z)-2-(3-Trifluoromethylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3k). Yellow solid, yield 77.4%, mp 160–162°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.86 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.92 (d, J = 0.8 Hz, 1H, ArH), 6.11 (d, J = 0.8 Hz 1H, ArH), 6.69 (s, 1H, =CH), 7.10 (br s, 1H, NH), 7.49–7.54 (m, 2H, ArH), 7.64–7.69 (m, 1H, ArH), 7.70–7.73 (m, 1H, ArH). IR (KBr) v: 3444, 3036, 1683, 1599, 1471, 1386, 1328, 1279, 1219, 1160, 1117, 1070 cm^{-1}. *Anal*. Calcd for $C_{18}H_{14}F_3NO_3$: C 61.89, H 4.04, N 4.01. Found: C 61.65, H 4.31, N 4.24.

(Z)-2-(3,4-Dimethoxylbenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3l). Yellow solid, yield 83.6%, mp 137–139°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.83 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 5.90 (d, J = 1.6 Hz, 1H, ArH), 6.08 (d, J = 1.6 Hz, 1H, ArH), 6.68 (s, 1H, =CH), 6.87 (d, J = 8.0 Hz, 1H), 7.00 (d, J = 1.6 Hz, 1H, ArH), 7.06 (br s, 1H, NH), 7.12 (dd, J = 8.4, 1.6 Hz, 1H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 55.4, 55.5, 55.6(2C), 88.5, 90.5, 103.3, 108.1, 111.9, 113.3, 122.6, 127.1,

134.2, 148.8, 148.9, 156.9, 159.4, 167.6, 181.3. IR (KBr) *v*: 3432, 3020, 1667, 1627, 1590, 1510, 1465, 1369, 1271, 1249, 1216, 1160, 1116, 1025 cm^{-1}. *Anal*. Calcd for $C_{19}H_{19}NO_5$: C 66.85, H 5.61, N 4.10. Found: C 66.95, H 5.33, N 3.86.

(Z)-2-(4-Hydroxy-3-methoxybenzylidene)-4,6-dimethoxy-2,3-dihydro-1H-indol-3-one (3m). Yellow solid, yield 85.3%, mp 259–260°C. ^1H NMR (400 MHz, DMSO-d_6) δ: 3.81 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 5.97 (d, J = 2.0 Hz, 1H, ArH), 6.20 (d, J = 2.0 Hz, 1H, ArH), 6.39 (s, 1H, =CH), 6.84 (d, J = 8.4 Hz, 1H, ArH), 7.13–7.21 (m, 2H, ArH), 9.42 (s, 1H, OH), 9.51 (br s, 1H, NH). IR (KBr) *v*: 3363, 3015, 2921, 1665, 1625, 1577, 1513, 1470, 1445, 1372, 1296, 1252, 1219, 1198, 1153, 1119, 1042, 1019 cm^{-1}. *Anal*. Calcd for $C_{18}H_{17}NO_5$: C 66.05, H 5.23, N 4.28. Found: C 66.30, H 5.21, N 4.44.

Biological screening. The potential toxicity of the title compounds against HepG-2, Hela, and WRL-68 cells were performed using MTT assay [18] as follows: Cells were seeded into 96-multiwell plate (4×10^3 cells/well) and incubated for 24 h. Different concentrations of each test compound were added to the cells triplicate wells. The cells without test compounds were used as negative controls. Following the exposure of 48 h to the test compound, MTT solution (100 μL/well, 1 mg/mL) was added to each well and left for another 4 h. After removing MTT, dimethyl sulfoxide was added to the cell to dissolve the precipitate, and the absorbance (Abs) of each well was measured at 570 nm using the ELISA reader (Spectra MAX 190, Molecular Devices Corporation, USA). The inhibitory effect was calculated as follows: Inhibition (%) = $(1 - Abs_{compound}/Abs_{control}) \times 100$. IC_{50} was obtained for each test compound using SPSS 16.0 version (SPSS Inc., Chicago, USA).

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