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Original article

# Anti-hepatitis B virus and anti-cancer activities of novel isoflavone analogs

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ABSTRACT

We have synthesized a series of novel isoflavone analogs and evaluated their anti-HBV and anti-cancer activities *in vitro*. The bioassays showed that the majority of the resultant compounds exerted inhibitory effects on HBsAg and HBeAg levels, HBV DNA replication, as well as the growth of four human cancer cell lines to various extents, which supported the rationale of the design. In particular, compound **8f** showed the highest activity against HBV infection and HBV-related liver cancer. Compound **7l** ($IC_{50}$ = 0.47 μM) also exerted remarkable inhibitory effect on the growth lung cancer cell line A-549.



## 1. Introduction

The hepatitis B virus (HBV), a hepatotropic member of the hepadnavirus family is a principal causative agent of acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) {Dienstag 2008 NEJM, Liang 2009 Hepatology, National Institutes of Health Consensus Development Conference Statement 2009}. HCC accounts for 80–90% of all primary liver cancers and is the third leading cause of cancer death worldwide {El-Serag 2011 NEJM}. This type of cancer usually occurs in patients with chronic liver disease. Infection with HBV or HCV is the leading cause of HCC, with each virus increasing the risk of cancer for more than 10-fold [1]. Despite drugs such as adefovir dipivoxil and 5-fluorouracil (5-Fu) have been used to combat HBV infection and HBV-mediated HCC, respectively, a versatile drug with inhibitory effects on both viral infection and HCC has not been discovered yet. In addition, drug resistance, toxicity and side effects have made current therapeutic regimens suboptimal. Therefore, it is a pressing need to develop innovative, multi-target drugs that could be used not only to cure patients with HBV infection but also to prevent and treat HBV-induced HCC.

Phytoestrogen, also known as "dietary estrogen", is a naturally occurring nonsteroidal xenoestrogen extracted from plants. This class of compounds could mildly mimic estrogen (Fig. 1) because of their similarities at the molecular level. Isoflavones (Fig. 1), which are found in high concentration in soy bean and soy bean products, represent the most active subfamily of phytoestrogen and have been shown to possess anti-viral, anti-cancer, anti-oxidative, anti-hyperglycemic, anti-inflammatory, insecticidal, anti-fungal and anti-microbial activities [2–8]. Isoflavones exert their effects primarily through binding to estrogen receptors (ER). They also entangle with gamma-aminobutyric acid (GABA) receptors, peroxisome proliferator-activated receptors (PPARs), nuclear factor κB (NF-κB), serotonin (5-HT), dopamine (DA), etc. [9–12]

Our design protocol was shown in Scheme 1. In our previous studies, certain isoflavone analogs (**II**) derived from isoflavone (**I**) have been synthesized and evaluated for their anti-HBV and cytotoxic activities. What encouraged us was that a few synthesized analogs were found to possess potent efficacies against HBV infection and HCC [13]. In view of the novel structural template and to discover more selective and effective anti-HBV or anticancer agents, we intended to synthesize additional analogs of isoflavone and determine their structure–activity relationships (SARs). Acrylic acid derivatives have been reported to be of high importance in therapeutics due to their antitumor, antioxidant and antibacterial activities [14–16]. According to the design proposal, in this study, we introduced the acrylic amide pharmacophore (**III**) to keep the nitrogen atom and conjugation system, and also prolonged the

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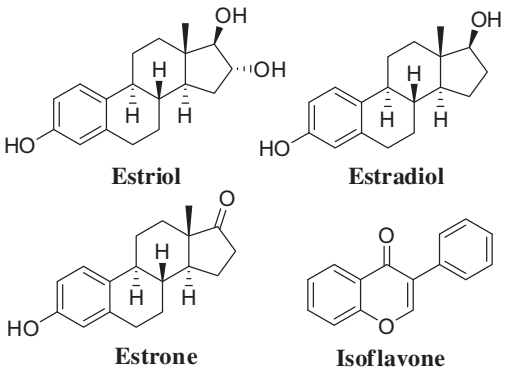



**Fig. 1.** The three major naturally occurring estrogens and the isoflavone.

distance between the benzene ring B and nitrogen atom with one or two carbon atom (**IV**) and (**V**). Herein, SAR studies on anti-HBV and cytotoxic activities were then investigated in this paper.

## 2. Chemistry

The synthetic route of compound **5** was depicted in Scheme 2 according to a reported procedure [13]. Compound **5** was treated with $AlCl_3$ in toluene to obtain compound **6** in 93% yield. Coupling of compound **5** (**6**) with substituted acrylamide by Heck reaction to produce compound **7a**–**7w** (**8a**–**8i**) in 36–73% yield, as illustrated in Scheme 3. In order to establish a robust synthetic procedure of resultant compounds, the Heck reaction conditions were optimized. Microwave heating method was employed and various solvents, bases, metal catalysts, temperatures and heating methods were explored. The DMF was found to be the best choice, with triethylamine as the base and $Pd(OAc)_2$ as the metal catalyst. The optimal microwave power, reaction temperature and time were 500 w, 100 °C and 7 min, respectively. The chemical structures of these compounds were confirmed by HRMS, NMR spectroscopy and elemental analyses. The purity of final compounds was assessed by analytical HPLC (≥95%). (See Supplementary data).

Analyses of $^1$H NMR spectrum of all the final products suggested a doublet of substituted acrylamide at around $\delta$ 7.4 and $\delta$ 7.7, respectively (**7a**–**7w** with $CDCl_3$ as solvent) or around $\delta$ 7.2 and $\delta$ 7.4, respectively (**8a**–**8i** with DMSO-$d_6$ as solvent). The coupling constant ($J$ = 15.0–15.6 Hz) of the two olefinic protons confirmed the trans-double bond.

## 3. Pharmacology

*In vitro* cytotoxicities of all target compounds in HepG2 2.2.15 cells were assessed by MTT assays. Effects on HBsAg and HBeAg levels by these compounds were evaluated in cultured HepG2 2.2.15 cells by enzyme linked immunosorbent assay (ELISA). Effects on HBV DNA replication were evaluated by fluorescence PCR.

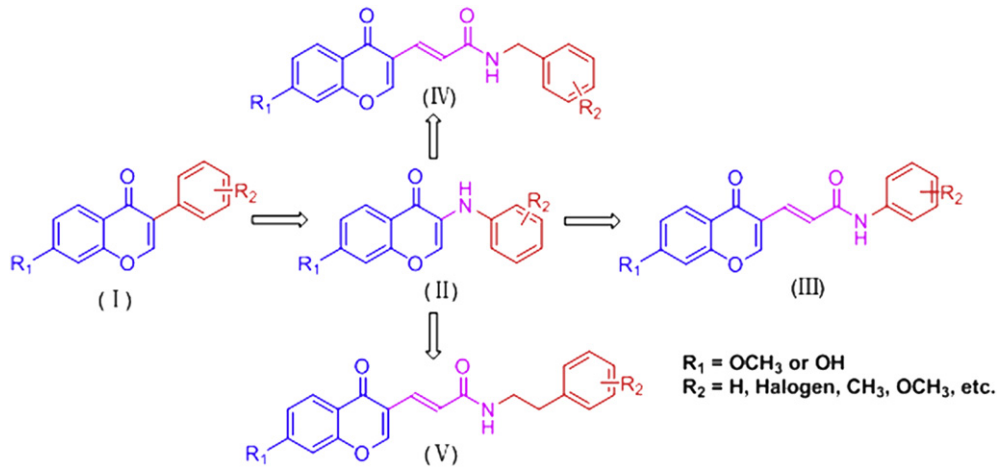
In the cytotoxicity assays, all the synthesized compounds (**7a**–**7w**, **8a**–**8w**) were evaluated for their *in vitro* cytotoxic activities against one human normal cell line and four human cancer cell lines, including L02 (normal human liver cell line), QGY-7703 (human hepatoma cell line), Huh-7 (human hepatoma cell line), HeLa (human cervical carcinoma cell line) and A-549 (human lung adenocarcinoma cell line). Human cervical carcinoma cell line was recruited because of the important estrogen-mimic function of isoflavones in reproductive system; human lung adenocarcinoma cell line was studied to provide another cancer type to oversee the efficacies of the compounds. In all studies, 5-Fluorouracil (5-Fu) was used as a positive control.

## 4. Results and discussion

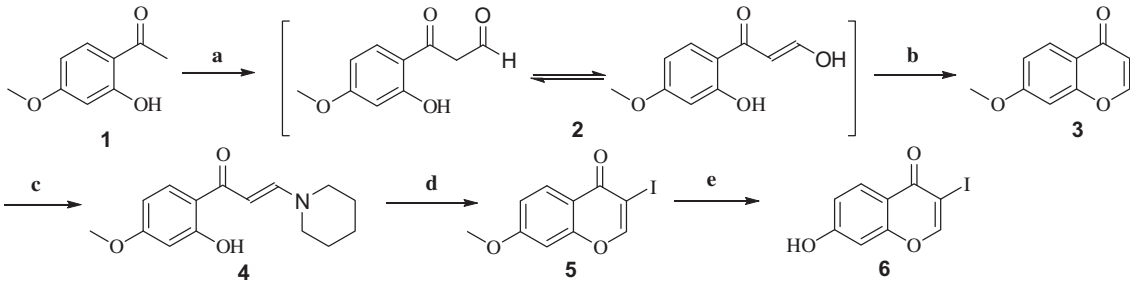
### 4.1. In vitro virological assays of anti-HBV activities

The potential anti-HBV activities and cytotoxicities of the isoflavone analogs were studied in HepG2 2.2.15 cells, with adefovir applied as a positive control drug. The results were summarized in Table 1. The anti-HBV activity of each compound was expressed as the concentration of compound that achieved 50% inhibition ($IC_{50}$) of HBsAg, HBeAg, and HBV DNA replication. The cytotoxicity of each compound was manifested as the concentration of the compound required to kill 50% ($CC_{50}$) of the HepG2 2.2.15 cells. The selectivity index (SI), an important pharmaceutical parameter that facilitates the estimation of possible future clinical development, was determined as the ratio of $CC_{50}$ value to $IC_{50}$ value. The bioactivity of each compound was evaluated by a combination of its $IC_{50}$ value and corresponding SI.

Compounds **7a**–**7m** had different patterns of substitution on the phenyl ring attached to the nitrogen atom of acrylamide. As shown in Table 1, these compounds generally exhibited poor to none anti-HBV activity, except compounds **7a**, **7b**, **7m** (with higher inhibitory effect against HBsAg and HBeAg than adefovir) and **7h** (with moderate effect against HBV DNA replication). Nevertheless, compound **7h** had high $CC_{50}$ value against HepG2 2.2.15 cells, which led to a moderate SI value (SI = 50.38).




**Scheme 1.** Schematics of the design for the target compounds.



Reagents and conditions: (a) Na, Ethyl Formate, Ethyl Ether, 0°C-r.t., 18h; (b) HOAc/HCl, reflux, 30min;(c) Piperidine, $CH_3OH$, reflux, 3h; (d) $I_2$, Pyridine, $CH_2Cl_2$, r.t., 20h (e) $AlCl_3$, Toluene, 100°C, Overnight

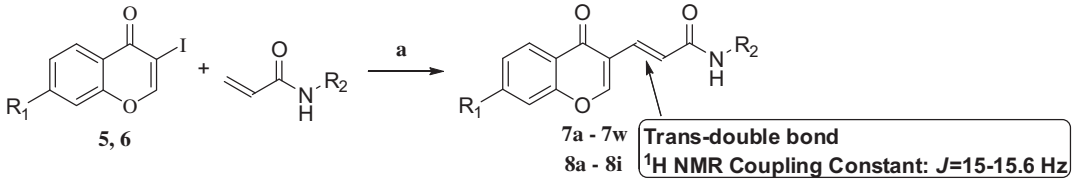
**Scheme 2.** Synthesis of the key intermediates.

Significantly increased anti-HBV activity was observed with the replacement of the substituted phenyl groups of compounds by the substituted benzyl groups (compounds **7n**–**7t**). It is noteworthy that almost all compounds in this subseries showed moderate inhibitory effects on HBV DNA replication and moderate to strong effect against HBsAg, except compound **7n**, of which HBsAg and HBeAg inhibiting effect was not observed. Therefore, these demonstrated that substitutions on the phenyl ring might facilitate antiviral activities. Although compounds **7p** and **7t** showed better activities against HBsAg ($IC_{50}$ value were 13.97 and 14.29 μM, respectively) and HBeAg ($IC_{50}$ value were 14.41 and 14.82 μM, respectively) than the control drug did, their relatively high cytotoxicity ($CC_{50}$ value were 43.01 and 79.25 μM, respectively) led to a low value of SI.

The introduction of substituted phenylethyl groups on the N atom resulted in the loss of anti-HBV DNA replication activity (compounds **7u**–**7w**). However, compound **7w** maintained high activity against both HBsAg and HBeAg, making it more potent than adefovir.

Based on the biological data above, eight compounds (**7a**, **7b**, **7h**, **7n**, **7o**, **7p**, **7s** and **7w**) were selected for further modifications, of which 7-$OCH_3$ were replaced by 7-OH (**8a**–**8i**). It is worthy noting that some modified compounds exhibited increased anti-HBV activity and decreased cytotoxicity. Particularly in the case of compound **8f**, which gained good activities against HBsAg ($IC_{50}$ = 10.22 μM) and HBV DNA replication ($IC_{50}$ = 4.07 μM), excellent activity against HBeAg ($IC_{50}$ = 2.34 μM) and very low toxicity ($CC_{50}$ = 717.95 μM). Likewise, compound **8e** also showed high anti-HBV activity which is only lower than compound **8f**, indicating that the methoxyl at position 4 of the benzyl or methyl group was important for the antiviral activity. Although compound **8i** showed good activity against both HBsAg and HBeAg, its relatively high cytotoxicity resulted in a low value of SI.

According to the data analysis above, preliminary structure–activity relationship (SAR) could be concluded. Firstly, benzyl analogs appeared to be more active than phenyl and phenylethyl analogs. Secondly, different substitution position on the benzene




Reagents and conditions: (a) $Pd(OAc)_2$, $Et_3N$, DMF, Microwave, 100°C, 500w, 7min

| Compd. | $R_1$ | $R_2$ | Compd. | $R_1$ | $R_2$ | Compd. | $R_1$ | $R_2$ |
|---|---|---|---|---|---|---|---|---|
| **7a** | -OMe | -Ph | **7l** | -OMe | -3'-Cl-Ph | **7w** | -OMe | -$CH_2CH_2$-4'-F-Ph |
| **7b** | -OMe | -2'-Me-Ph | **7m** | -OMe | -4'-Cl-Ph | **8a** | -OH | -Ph |
| **7c** | -OMe | -3'-Me-Ph | **7n** | -OMe | -$CH_2$Ph | **8b** | -OH | -2'-Me-Ph |
| **7d** | -OMe | -4'-Me-Ph | **7o** | -OMe | -$CH_2$-4'-Me-Ph | **8c** | -OH | -4'-OMe-Ph |
| **7e** | -OMe | -2',5'-$Me_2$-Ph | **7p** | -OMe | -$CH_2$-4'-OMe-Ph | **8d** | -OH | -$CH_2$Ph |
| **7f** | -OMe | -2'-OMe-Ph | **7q** | -OMe | -$CH_2$-2'-F-Ph | **8e** | -OH | -$CH_2$-4'-Me-Ph |
| **7g** | -OMe | -3'-OMe-Ph | **7r** | -OMe | -$CH_2$-3'-F-Ph | **8f** | -OH | -$CH_2$-4'-OMe-Ph |
| **7h** | -OMe | -4'-OMe-Ph | **7s** | -OMe | -$CH_2$-4'-F-Ph | **8g** | -OH | -$CH_2$-4'-F-Ph |
| **7i** | -OMe | -4'-OEt-Ph | **7t** | -OMe | -$CH_2$-2',4'-$F_2$-Ph | **8h** | -OH | -$CH_2$-2',4'-$F_2$-Ph |
| **7j** | -OMe | -4'-F-Ph | **7u** | -OMe | -$CH_2CH_2$-2'-F-Ph | **8i** | -OH | -$CH_2CH_2$-4'-F-Ph |
| **7k** | -OMe | -2'-Cl-Ph | **7v** | -OMe | -$CH_2CH_2$-3'-F-Ph | | | |

**Scheme 3.** Synthesis of the title compounds.

**Table 1**
*In vitro* anti-HBV activity of the target compounds.

| Compd. | $CC_{50}$ (μM)[a] | HBsAg | | HBeAg | | DNA replication | |
|---|---|---|---|---|---|---|---|
| | | $IC_{50}$ ± SEM (μM)[b] | SI[c] | $IC_{50}$ ± SEM (μM)[b] | SI[c] | $IC_{50}$ ± SEM (μM)[b] | SI[c] |
| **7a** | 522.55 | 15.98 ± 0.63 | 32.70 | 16.92 ± 1.38 | 30.85 | >100 | [d] |
| **7b** | 59.70 | 14.93 ± 2.85 | 4.00 | 15.82 ± 2.33 | 3.77 | >100 | [d] |
| **7c** | 553.73 | >100 | [d] | >100 | [d] | 34.93 ± 2.55 | 15.85 |
| **7d** | 978.97 | >100 | [d] | >100 | [d] | 28.22 ± 0.21 | 34.69 |
| **7e** | 1098.65 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7f** | 1045.34 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7g** | 1125.22 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7h** | 717.95 | >100 | [d] | >100 | [d] | 14.25 ± 0.88 | 50.38 |
| **7i** | 459.56 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7j** | 743.36 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7k** | 1007.04 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7l** | 1104.90 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7m** | 271.66 | 91.70 ± 4.00 | 2.96 | 20.95 ± 0.59 | 12.97 | 67.46 ± 1.68 | 4.03 |
| **7n** | 376.12 | >100 | [d] | >100 | [d] | 56.81 ± 1.65 | 6.62 |
| **7o** | 257.24 | 82.61 ± 0.31 | 3.11 | >100 | [d] | 53.45 ± 0.91 | 4.81 |
| **7p** | 43.01 | 13.97 ± 1.45 | 3.08 | 14.41 ± 1.02 | 2.98 | 24.27 ± 2.34 | 1.77 |
| **7q** | 145.89 | 52.15 ± 1.98 | 2.80 | >100 | [d] | 68.56 ± 3.22 | 2.13 |
| **7r** | 475.18 | 83.80 ± 0.99 | 5.67 | >100 | [d] | 75.44 ± 2.31 | 6.30 |
| **7s** | 133.06 | 58.81 ± 3.01 | 2.26 | >100 | [d] | 11.24 ± 4.27 | 11.84 |
| **7t** | 79.25 | 14.29 ± 2.13 | 5.55 | 14.82 ± 0.19 | 5.35 | 37.03 ± 1.36 | 2.14 |
| **7u** | 457.06 | 47.87 ± 0.96 | 9.55 | >100 | [d] | >100 | [d] |
| **7v** | 1030.46 | >100 | [d] | >100 | [d] | >100 | [d] |
| **7w** | 786.29 | 14.66 ± 3.95 | 53.64 | 14.71 ± 5.01 | 53.45 | >100 | [d] |
| **8a** | 820.85 | 45.54 ± 0.21 | 18.02 | 13.43 ± 0.45 | 61.12 | 29.02 ± 0.34 | 28.29 |
| **8b** | 374.00 | >100 | [d] | >100 | [d] | >100 | [d] |
| **8c** | 975.84 | >100 | [d] | >100 | [d] | 7.01 ± 2.34 | 139.21 |
| **8d** | 435.69 | >100 | [d] | >100 | [d] | >100 | [d] |
| **8e** | 500.72 | 16.54 ± 0.78 | 30.27 | 19.14 ± 3.67 | 26.16 | 14.18 ± 1.69 | 35.31 |
| **8f** | 717.95 | 10.22 ± 0.82 | 70.25 | 2.34 ± 3.34 | 306.82 | 4.70 ± 1.03 | 152.76 |
| **8g** | 357.45 | >100 | [d] | >100 | [d] | 33.46 ± 0.84 | 10.68 |
| **8h** | 469.86 | 14.53 ± 2.11 | 32.34 | 12.35 ± 1.33 | 38.05 | 23.31 ± 0.91 | 20.16 |
| **8i** | 102.41 | 14.56 ± 0.92 | 7.03 | 15.43 ± 1.27 | 6.64 | >100 | [d] |
| Adefovir | 540 | 305 | 1.77 | 286 | 1.89 | 0.517 | 1044.5 |

[a] $CC_{50}$ is 50% cytotoxicity concentration in HepG2 2.2.15 cells.
[b] $IC_{50}$ is 50% inhibitory concentration.
[c] Selectivity index (SI = $CC_{50}/IC_{50}$).
[d] No SI can be obtained.

ring of the side chain could produce different bioactivity to some extent. Either electron-withdrawing or electron-donating groups at position-4 had better activities than other synthesized compounds, with the exception of compound **7i**, which means small-volume *para*-substituted group at benzene ring was the best choice for anti-HBV activity. However, substitution at position-2 could increase the compound's cytotoxicity. Thirdly, demethylation of position-7 could not only increase activity but reduce cytotoxicity.

Overall, our preliminary SAR studies demonstrated that the most potent anti-HBV compounds in this category would contain a position-4 substituted benzyl ring attached to the nitrogen atom of acrylamide, accompanied by a hydrogen group on the benzene ring of the benzopyran-4-one. Further optimizations are being carried on to discover optimized compounds.

### *4.2. In vitro cytotoxic effects of the compounds in various cancer cell lines*

The results of *in vitro* cytotoxic analyses in various cancer cell lines are summarized in Table 2. The $IC_{50}$ value is defined as the concentration of a compound that corresponds to a 50% growth inhibition. We showed that the majority of the synthesized compounds exerted antitumor activities against the four human cancer cell lines to various extents and low toxicity to human normal liver cell line.

For the reason that the main role of these synthesized compounds is to against the HBV, we first discussed cytotoxic effect based on the anti-HBV activity. Among them, compound **8f**, identified to be a promising HBV antiviral drug candidate, also showed strong inhibitory effect against the growth of both QGY-7703 and Huh-7 cells, with $IC_{50}$ values of 10.01 μM and 8.43 μM, respectively, which were comparable to those of 5-Fu. More impressively, compound **8f** had no effect against the growth of either HeLa or A-549 cells, implicating the hepatoma cell line-specific activity of this compound. In addition, compounds **7a**, **7o**, **7p**, **7r**, **7s**, **7v**, **8a**, **8b** and **8f** all showed moderate to strong growth inhibitory effect against the growth of both of the human hepatoma cell lines, particularly in the case of compound **8a**, which exerted a 4-fold higher effect in inhibiting Huh-7 cells than the positive control and all these compounds had good anti-HBV activity except compounds **7v** and **8b**. In contrast, compound **7i** showed no activity against HBV and poor effect in three of the cancer cell lines. However, it exerted a strongest inhibitory effect against the growth of Huh-7 cells among all the tested compounds, implying that this compound may have unique mechanisms of action in Huh-7 cells.

Our results also showed that compounds **7b**, **7g**, **7k**, **7r**, **7v** and **8a** had broad anti-tumor activity against all four cancer cell lines (Table 2). Unexceptionally, these compounds possess substituted groups at either the position-2 or position-3 of the benzene ring. Five of them possess a structural feature of with a benzene ring directly attached to the N atom, suggesting that the benzyl ring is not necessary for the broad anti-cancer features. We also found that compound **7b** was the most potent compound in inhibiting the growth of HeLa cells, with $IC_{50}$ value of 7.27 μM, that is even lower than that of 5-Fu ($IC_{50}$ = 16.32 μM). A possible mechanism for these

**Table 2**
*In vitro* anti-cancer activity of target compounds.

| Compd. | Cytotoxicity $IC_{50}$ ± SEM (μM)[b] | | | | |
|---|---|---|---|---|---|
| | L02[a] | QGY-7701[a] | Huh-7[a] | HeLa[a] | A-549[a] |
| **7a** | >200 | 14.02 ± 0.47 | 9.05 ± 1.45 | >100 | 51.00 ± 1.87 |
| **7b** | >200 | 22.45 ± 0.099 | 26.88 ± 2.11 | 7.27 ± 0.64 | 23.40 ± 2.22 |
| **7c** | >200 | >100 | 15.47 ± 0.92 | >100 | >100 |
| **7d** | >200 | 39.29 ± 6.08 | >100 | 24.13 ± 2.14 | 17.54 ± 0.15 |
| **7e** | >200 | >100 | 11.12 ± 9.11 | 58.42 ± 3.28 | 15.47 ± 1.79 |
| **7f** | >200 | 17.62 ± 5.18 | 32.27 ± 3.13 | >100 | >100 |
| **7g** | >200 | 39.09 ± 0.44 | 11.13 ± 2.18 | 19.23 ± 0.031 | 12.80 ± 3.95 |
| **7h** | >200 | >100 | >100 | 13.03 ± 2.56 | 22.98 ± 4.09 |
| **7i** | >200 | >100 | 3.23 ± 1.13 | >100 | >100 |
| **7j** | >200 | >100 | 33.85 ± 5.23 | 9.68 ± 0.55 | 26.77 ± 0.65 |
| **7k** | >200 | 22.97 ± 0.66 | 58.42 ± 7.50 | 22.42 ± 3.45 | 29.94 ± 3.32 |
| **7l** | >200 | >100 | >100 | 11.85 ± 3.34 | 0.47 ± 0.058 |
| **7m** | >200 | >100 | 37.10 ± 4.73 | >100 | >100 |
| **7n** | >200 | >100 | 13.58 ± 0.86 | 33.39 ± 1.41 | 11.82 ± 1.45 |
| **7o** | >200 | 10.23 ± 0.36 | 14.32 ± 1.65 | >100 | >100 |
| **7p** | >200 | 15.67 ± 1.56 | 7.57 ± 2.22 | >100 | 22.49 ± 1.76 |
| **7q** | >200 | 33.99 ± 0.49 | 8.45 ± 0.091 | >100 | >100 |
| **7r** | >200 | 28.57 ± 5.18 | 10.07 ± 1.60 | 17.58 ± 0.58 | 26.88 ± 5.34 |
| **7s** | >200 | 7.96 ± 1.33 | 20.95 ± 0.73 | >100 | >100 |
| **7t** | >200 | >100 | >100 | >100 | 13.33 ± 1.13 |
| **7u** | >200 | 81.67 ± 8.22 | >100 | 12.23 ± 2.56 | 10.30 ± 0.33 |
| **7v** | >200 | 23.44 ± 0.02 | 8.96 ± 2.66 | 15.29 ± 2.86 | 9.77 ± 0.44 |
| **7w** | >200 | 24.84 ± 4.55 | 29.18 ± 4.09 | >100 | 18.86 ± 5.67 |
| **8a** | >200 | 17.94 ± 0.49 | 3.88 ± 0.023 | 9.70 ± 1.01 | 24.38 ± 3.10 |
| **8b** | >200 | 9.32 ± 1.93 | 11.12 ± 3.32 | 8.67 ± 0.40 | >100 |
| **8c** | >200 | >100 | >100 | >100 | 12.82 ± 2.34 |
| **8d** | >200 | 62.96 ± 8.33 | 4.69 ± 0.38 | >100 | >100 |
| **8e** | >200 | 24.86 ± 2.47 | >100 | >100 | 17.43 ± 1.49 |
| **8f** | >200 | 10.01 ± 1.09 | 8.43 ± 0.21 | >100 | >100 |
| **8g** | >200 | >100 | >100 | >100 | >100 |
| **8h** | >200 | >100 | >100 | >100 | 6.61 ± 0.18 |
| **8i** | >200 | >100 | >100 | >100 | 6.84 ± 0.67 |
| 5-Fu[c] | 46.57 ± 0.68 | 14.30 ± 0.055 | 15.85 ± 0.21 | 16.32 ± 0.36 | 5.64 ± 0.55 |

[a] Abbreviations: L02: normal human liver cell line; QGY-7701: human hepatoma cell line; Huh-7: human hepatoma cell line; HeLa: human cervical carcinoma cell line; A-549: human lung adenocarcinoma cell line.
[b] The $IC_{50}$ value was defined as the concentration at which 50% survival of cells was observed.
[c] Used as a positive control.

findings is that the estrogen-like structure of the isoflavone analogs may contribute to the potencies of the compounds.

As shown in Table 2, compound **7l** exerted a 12-fold stronger effect against A-549 cells ($IC_{50}$ = 0.47 μM) than the reference drug ($IC_{50}$ = 5.64 μM), making it an ideal candidate for the development of anti-lung cancer new drugs, which are worthwhile for further investigation.

Preliminary SAR studies showed that the inhibitory effect against HCC was basically in agreement with the results of anti-HBV effect. Moreover, neither benzyl nor *para*-substituted benzene ring in the side chain appeared to be good for the broad anti-cancer features. And even without any substitution at benzene ring, compound **8a** exhibited best broad anti-tumor spectrum. Finally, what excited us most is that these features about anti-HBV and anti-HCC are not contradictory.

## 5. Conclusion

In this study, we designed and synthesized a series of isoflavone analogs and evaluated their anti-HBV and anti-cancer activities *in vitro*. Among them, compound **8f** seems to be the most promising drug candidate, due to its potent anti-HBV activity and relatively low toxicity in HepG2 2.2.15 and L02 cell lines. This compound also exerted strong inhibitory effect on the growth of two human hepatoma cell lines (QGY-7703 and Huh-7) further supporting the rationale of the design. As such, compound **8f**, a representative isoflavone analog, seems like a promising drug candidate for the treatment of both HBV infection and HBV-related HCC. In addition, compound **7l** exhibited high activity in inhibiting the growth of A-549 cells *in vitro*, suggesting that it could be a candidate for the exploration of novel anti-lung cancer drugs, which is worthwhile for further study and optimization. Overall, the phenotypes exerted new vistas of research for treatment of both HBV and HBV-induced HCC. Further studies are needed for us to better understand the nature and mechanisms of these compounds.

## 6. Experimental procedures

Melting points were measured with an uncorrected X-5 digital melting point apparatus (Gongyi City Yuhua Instrument Co., Ltd.; China). $^1$H NMR and $^{13}$C NMR spectra were recorded with a BRUKER AVANCE 300 spectrometer (Bruker Company, Germany), with TMS used as an internal standard and $CDCl_3$ (99.8% D, Cambridge Isotope Laboratories) or DMSO-$d^6$ (99.9% D, Cambridge Isotope Laboratories) as solvents. Chemical shifts ($\delta$ values) and coupling constants ($J$ values) were given in ppm and Hz, respectively. Elemental analyses were performed with a MOD-1106 instrument and were consistent with theoretical values within 0.4% range. The mass spectra were recorded with an Esquire 3000 LC-MS mass spectrometer. Purity analysis was determined using an Agilent 1100 Serial HPLC (Agilent Company, US); A $C_{18}$ column (ZORBAX(tm) 4.6 × 150 mm, 5 μm, PN: 993967906 SN: USRK042041). TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). Silica gel thin-layer chromatography was performed with Silica gel 60 G (Qindao Haiyang Chemical, China). All solvents and reagents were analytically pure, and no further purification was needed. All starting materials were commercially available.

### 6.1. 3-Iodo-7-methoxy-4H-chromen-4-one (**5**)

Color: Yellow; m.p.: 158.5–159.2 °C; Yield: 92%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.91 (s, 3H, $CH_3$–O), 6.84 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.01 (dd, 1H, $J_1$ = 9.0 Hz, $J_2$ = 2.4 Hz, Ar–H), 8.15 (d, 1H, $J$ = 9.0 Hz, Ar–H), 8.23 ppm (s, 1H, O–CH=C); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 56.16, 86.93, 100.54, 114.92, 115.38, 127.03, 157.52, 158.59, 163.93, 171.96 ppm; HRMS ($m/z$): 302.95 $(M + 1)^+$; Anal. ($C_{10}H_7IO_3$): C, H.

### 6.2. 7-Hydroxy-3-iodo-4H-chromen-4-one (**6**)

To a solution of 3-iodo-7-methoxy-4*H*-chromen-4-one (33 mmol, 10 g) in dry toluene (250 ml) was added $AlCl_3$ (50 mmol, 6.6 g) with stirring at 100 °C for 10 h under $N_2$ protection. The reaction mixture was concentrated under reduced pressure to remove toluene. Then the reaction residue was diluted with EtOAc and poured into 10% HCl ice–water with stirring. The organic layer was separated and the aqueous layer was extracted with EtOAc (200 ml) twice. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure to dryness to provide compound **6**. Color: Yellow; m.p.: 192.2–193.5 °C; Yield: 93%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 6.85 (d, 1H, $J$ = 2.1 Hz, Ar–H), 6.94 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$ = 2.1 Hz, Ar–H), 7.89 (d, 1H, $J$ = 8.7 Hz), 8.67 (s, 1H, O–CH = C), 10.95 (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 68.9, 101.5, 104.6, 114.2, 131.1, 150.5, 158.5, 186.5 ppm; HRMS ($m/z$): 288.93 $(M + 1)^+$; Anal. ($C_9H_5IO_3$): C, H.

### 6.3. General procedure for the synthesis of compounds **7** and **8**

To a solution of compound **5** (**6**) (3 mmol) and substituted acrylamide (3.3 mmol) in dry DMF was added $Pd(OAc)_2$ (3%) and triethylamine (6 mmol). The reaction mixture was stirred under 100 °C via microwave (500 w) for 7 min. Then the mixture was poured into 10% HCl ice water (200 ml) slowly with stirring. The suspension was filtered through a filter and filter cake was collected and dried. The crude product was purified by chromatography over silica gel to give compounds **7** or **8**.

#### 6.3.1. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-phenylacrylamide (**7a**)

Color: Yellow; m.p.: 222.5–223.9 °C; Yield: 79%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.93 (s, 3H, $CH_3$–O), 6.87 (d, 1H, $J$ = 3.0 Hz, Ar–H), 7.02–7.05 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar–H), 7.30–7.43 (m, 5H, Ar–H, O=C–CH=C), 7.72 (d, 1H, $J$ = 12.0 Hz, O=C–C=CH), 8.09 (s, 1H, O–CH=C), 8.19 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 55.83, 100.16, 114.95, 116.48, 119.56, 120.36, 121.01, 127.55, 128.32, 128.64, 133.86, 137.20, 149.64, 157.26, 157.77, 164.74; HRMS ($m/z$): 322.11 $(M + 1)^+$; Anal. ($C_{19}H_{15}NO_4$): C, H, N.

#### 6.3.2. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-o-tolylacrylamide (**7b**)

Color: Yellow; m.p.: 213.1–215.7 °C; Yield: 69%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.32 (s, 3H, $CH_3$), 3.94 (s, 3H, $CH_3$–O), 6.89 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.02–7.06 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar–H), 7.11 (d, 1H, $J$ = 6.9 Hz, Ar–H), 7.19–7.28 (m, 2H, Ar–H), 7.41 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.77 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.04–8.07 (m, 1H, Ar–H), 8.09 (s, 1H, O–CH=C), δ8.20 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 18.33, 55.85, 100.20, 114.05, 114.45, 116.84, 118.06, 121.76, 125.09, 127.57, 128.74, 129.26, 130.86, 131.94, 133.62, 140.95, 148.55, 157.78, 164.26, 167.86, 172.17; HRMS ($m/z$): 336.12 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_4$): C, H, N.

#### 6.3.3. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-m-tolylacrylamide (**7c**)

Color: Yellow; m.p.: 199.8–200.6 °C; Yield: 65%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 2.35 (s, 3H, $CH_3$), 3.92 (s, 3H, $CH_3$–O), 6.87 (d, 1H, $J$ = 2.4 Hz, Ar–H), 6.93 (d, 1H, $J$ = 7.5 Hz, Ar–H), 7.00–7.03 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar–H), 7.19–7.24 (t, 1H, Ar–H), 7.37 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.48 (d, 2H, $J$ = 12.9 Hz, Ar–H), 7.71 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.07 (s, 1H, O–CH=C), 8.18 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 21.40, 55.83, 100.17, 115.07, 116.84, 118.01, 119.06, 120.36, 125.03, 125.26, 127.57, 128.74, 133.08, 137.96, 138.83, 157.16, 157.78, 164.26 ppm; HRMS ($m/z$): 336.12 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_4$): C, H, N.

#### 6.3.4. (E)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)-N-p-tolylacrylamide (**7d**)

Color: Yellow; m.p.: 242.2–243.3 °C; Yield: 54%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 2.33 (s, 3H, $CH_3$), 3.93 (s, 3H, $CH_3$–O), 6.87 (d, 1H, $J$ = 2.1 Hz, Ar–H), 7.01–7.04 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar–H), 7.15 (d, 1H, $J$ = 8.1 Hz, Ar–H), 7.37 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.50 (d, 2H, $J$ = 8.4 Hz, Ar–H), 7.69 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.07 (s, 1H, O–CH=C), 8.19 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 21.36, 55.88, 100.24, 115.07, 118.02, 119.10, 121.64, 122.84, 127.31, 128.96, 133.23, 135.94, 141.52, 150.65, 157.14, 164.01, 164.34, 176.54; HRMS ($m/z$): 336.12 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_4$): C, H, N.

#### 6.3.5. (E)-N-(2,5-Dimethylphenyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7e**)

Color: Yellow; m.p.: 248.7–249.0 °C; Yield: 60%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 2.26 (s, 3H, $CH_3$), 2.34 (s, 3H, $CH_3$), 3.93 (s, 3H, $CH_3$–O), 6.87–6.91 (t, 2H, Ar–H), 7.01–7.09 (m, 2H, Ar–H), 7.14 (s, 1H, Ar–H), 7.39 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.75 (d, 1H, $J$ = 14.7 Hz, O=C–C=CH), 8.08 (s, 1H, O–CH=C), 8.19 ppm (d, 1H, $J$ = 8.7 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 17.41, 21.13, 55.90, 100.24, 115.18, 118.07, 119.10, 123.31, 125.14, 125.77, 127.56, 130.22, 133.26, 135.62, 136.47, 157.24, 157.90, 164.34, 175.95 ppm; HRMS ($m/z$): 350.13 $(M + 1)^+$; Anal. ($C_{21}H_{19}NO_4$): C, H, N.

#### 6.3.6. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-(2-methoxyphenyl)acrylamide (**7f**)

Color: Yellow; m.p.: 196.6–198.5 °C; Yield: 66%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.94 (s, 3H, $CH_3$–O), 3.97 (s, 3H, $CH_3$–O), 6.90 (d, 2H, $J$ = 7.5 Hz, Ar–H), 6.93 (d, 1H, $J$ = 7.2 Hz, Ar–H), 7.01–7.14 (m, 3H, Ar–H), 7.37 (d, 1H, $J$ = 15.3 Hz, O=C–CH=C), 7.77 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.01 (s, 1H, O–CH=C), 8.20 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 55.56, 55.85, 100.17, 109.80, 115.13, 118.02, 119.09, 119.75, 120.99, 123.67, 125.55, 127.49, 127.85, 132.86, 147.88, 157.17, 157.83, 164.25, 175.88 ppm; HRMS ($m/z$): 352.12 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_5$): C, H, N.

#### 6.3.7. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-(3-methoxyphenyl)acrylamide (**7g**)

Color: Yellow; m.p.: 188.9–189.1 °C; Yield: 63%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.85 (s, 3H, $CH_3$–O), $\delta$: 3.93 (s, 3H, $CH_3$–O), 6.86 (d, 1H, $J$ = 2.4 Hz, Ar–H), 6.93 (d, 1H, $J$ = 7.5 Hz, Ar–H), 7.00 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.03 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.18–7.24 (t, 1H, Ar–H), 7.37 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.41 (d, 2H, $J$ = 3.9 Hz, Ar–H), 7.48 (d, 2H, $J$ = 15.0 Hz), 7.72 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.07 (s, 1H, O–CH=C), 8.18 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 55.55, 55.85, 100.15, 109.80, 114.65, 117.00, 118.61, 118.97, 121.16, 123.67, 126.94, 129.44, 136.55, 139.28, 142.06, 150.48, 157.17, 160.61, 166.03, 167.64, 176.12 ppm; HRMS ($m/z$): 352.11 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_5$): C, H, N.

#### 6.3.8. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-(4-methoxyphenyl)acrylamide (**7h**)

Color: Yellow; m.p.: 236.3–236.8 °C; Yield: 74.6%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.81 (s, 3H, $CH_3$–O), 3.93 (s, 3H, $CH_3$–O), 6.87–6.90 (m, 2H, Ar–H), 7.02 (dd, 2H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar–H), 7.36

(s, 1H, Ar–H), 7.36 (d, 1H, $J$ = 14.7 Hz, O=C–CH=C), 7.54 (d, 1H, $J$ = 9.0 Hz, Ar–H), 7.70 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.06 (d, 1H, $J$ = 3.0 Hz, O–CH=C), 8.19 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 56.74, 57.17, 101.50, 115.45, 116.40, 122.75, 126.51, 128.90, 134.16, 158.50, 159.04, 165.59 ppm; HRMS ($m/z$): 352.11 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_5$): C, H, N.

*6.3.9. (E)-N-(4-Ethoxyphenyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7i**)*

Color: Yellow; m.p.: 213.3–213.9 °C; Yield: 44%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 1.39–1.43 (t, 3H, $CH_3$–$CH_2$), 3.93 (s, 3H, $CH_3$–O), 4.04 (dd, 2H, $J_1$ = 6.9 Hz, $J_2$ = 6.9 Hz, $CH_2$–$CH_3$), 6.86–6.89 (m, 2H, Ar–H), 7.02 (dd, 2H, $J_1$ = 2.1 Hz, $J_2$ = 2.1 Hz, Ar–H), 7.36 (d, 1H, $J$ = 15.3 Hz, O=C–CH=C), 7.40 (s, 1H, Ar–H), 7.52 (d, $J$ = 15.0 Hz, Ar–H), 7.70 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.07 (s, 1H, O–CH=C), 8.19 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 15.10, 56.71, 57.15 100.95, 115.45, 116.61, 122.84, 126.88, 129.11, 134.55, 158.94, 159.16, 165.64, 166.56, 177.26 ppm; HRMS ($m/z$): 366.13 $(M + 1)^+$; Anal. ($C_{21}H_{19}NO_5$): C, H, N.

*6.3.10. (E)-N-(4-Fluorophenyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7j**)*

Color: Yellow; m.p.: 240.1–240.8 °C; Yield: 73%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.93 (s, 3H, $CH_3$–O), 6.88 (d, 2H, $J$ = 2.4 Hz, Ar–H), 7.03 (dd, 2H, $J_1$ = 1.2 Hz, $J_2$ = 2.1 Hz, Ar–H), 7.06 (d, 1H, $J$ = 7.2 Hz, Ar–H), 7.37 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.58 (dd, 2H, $J_1$ = 5.1 Hz, $J_2$ = 5.4 Hz, Ar–H), 7.70 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.08 (s, 1H, O–CH=C), 8.19 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 55.65, 100.03, 115.18, 117.23, 118.12, 121.30, 122.51, 125.65, 125.95, 126.32, 128.66, 133.81, 134.76, 156.89, 157.81, 166.24, 175.55; HRMS ($m/z$): 340.08 $(M + 1)^+$; Anal. ($C_{19}H_{14}FNO_4$): C, H, N.

*6.3.11. (E)-N-(2-Chlorophenyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7k**)*

Color: Yellow; m.p.: 205.5–205.8 °C; Yield: 54%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.93 (s, 3H, $CH_3$–O), 6.89 (d, 1H, $J$ = 2.1 Hz, Ar–H), 7.03–7.08 (m, 2H, Ar–H), 7.27–7.31 (m, 1H, Ar–H), 7.38 (s, 1H, Ar–H) 7.41 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.76 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 7.80 (s, 1H, Ar–H), 8.10 (s, 1H, O–CH=C), 8.19 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 55.64, 100.10, 114.65, 117.48, 119.54, 121.24, 122.51, 124.48, 127.44, 129.07, 132.71, 141.61, 149.69, 157.45, 164.15, 167.15, 175.50 ppm; HRMS ($m/z$): 357.06 $(M + 1)^+$; Anal. ($C_{19}H_{14}ClNO_4$): C, H, N.

*6.3.12. (E)-N-(3-Chlorophenyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7l**)*

Color: Yellow; m.p.: 222.8–223.1 °C; Yield: 43%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.93 (s, 3H, $CH_3$–O), 6.88 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.01–7.08 (m, 1H, Ar–H), 7.11 (dd, 1H, $J_1$ = 6.0 Hz, $J_2$ = 6.0 Hz, Ar–H), 7.23–7.28 (m, 1H, Ar–H), 7.38 (d, 1H, $J$ = 14.7 Hz, O=C–CH=C), 7.43–7.47 (m, 2H, Ar–H) 7.72 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.09 (s, 1H, O–CH=C), 8.18 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 55.64, 100.10, 114.66, 117.88, 119.01, 121.55 122.56, 124.84, 127.54, 129.41, 132.75, 141.66, 149.41, 157.45, 164.18, 167.15, 175.50 ppm; HRMS ($m/z$): 357.06 $(M + 1)^+$; Anal. ($C_{19}H_{14}ClNO_4$): C, H, N.

*6.3.13. (E)-N-(4-Clorophenyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7m**)*

Color: Yellow; m.p.: 213.9–214.1 °C; Yield: 65%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.92 (s, 3H, $CH_3$–O), 6.88 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.06 (dd, 2H, $J_1$ = 7.5 Hz, $J_2$ = 8.7 Hz, Ar–H), 7.27–7.33 (m, 1H, Ar–H), 7.40 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.38–7.43 (m, 1H, Ar–H), 7.77 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 7.85 (s, 1H, Ar–H), 8.11 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 55.65, 100.03, 114.95, 117.82, 118.75, 121.34, 122.51, 124.30, 124.54, 127.41, 128.76, 133.81, 134.60, 156.96, 157.81, 164.15, 175.55 ppm; HRMS ($m/z$): 357.06 $(M + 1)^+$; Anal. ($C_{19}H_{14}ClNO_4$): C, H, N.

*6.3.14. (E)-N-benzyl-3-(7-methoxy-4-oxo-4H-chromen-3-yl) acrylamide (**7n**)*

Color: Yellow; m.p.: 257.1–257.5 °C; Yield: 82%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.93 (s, 3H, $CH_3$–O), 4.53 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 5.96 (s, 1H, NH), 6.85–6.90 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar–H), 6.98–7.03 (m, 1H, Ar–H), 7.28–7.37 (m, 6H, Ar–H, O=C–CH=C), 7.74 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.03 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 43.01, 55.87, 100.20, 115.20, 116.45, 119.11, 125.74, 126.01, 127.82, 129.33, 129.44, 130.05, 135.54, 150.65, 157.85, 164.54, 166.25, 175.79 ppm; HRMS ($m/z$): 336.12 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_4$): C, H, N.

*6.3.15. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-(4-methylbenzyl)acrylamide (**7o**)*

Color: Yellow; m.p.: 192.1–193.4 °C; Yield: 55%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 2.33 (d, 3H, $J$ = 6.0 Hz, $CH_3$–Ar), 3.92 (s, 3H, $CH_3$–O), 4.53 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 5.92 (s, 1H, NH), 6.86 (d, 1H, $J$ = 3.0 Hz, Ar–H), 7.00 (dd, 2H, $J_1$ = 2.1 Hz, $J_2$ = 2.4 Hz, Ar–H), 7.18 (dd, 2H, $J_1$ = 8.1 Hz, $J_2$ = 7.5 Hz), 7.31 (d, 1H, $J$ = 15.3 Hz, O=C–CH=C), 7.54 (d, 1H, $J$ = 14.7 Hz, O=C–C=CH), 8.03 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 29.65, 43.02, 55.87, 100.19, 115.32, 119.05, 124.47, 127.54, 129.33, 129.44, 132.62, 133.97, 157.19, 157.58, 164.26, 166.25, 175.83 ppm; HRMS ($m/z$): 350.13 $(M + 1)^+$; Anal. ($C_{21}H_{19}NO_4$): C, H, N.

*6.3.16. (E)-3-(7-Methoxy-4-oxo-4H-chromen-3-yl)-N-(4-methoxybenzyl)acrylamide (**7p**)*

Color: Yellow; m.p.: 219.4–220.6 °C; Yield: 36%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.81 (s, 3H, $CH_3$–O), 3.93 (s, 3H, $CH_3$–O), 4.50 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 5.92 (s, 1H, NH), 6.91 (d, 2H, $J$ = 3.0 Hz, Ar–H), 7.02–7.05 (m, 1H, Ar–H), 7.29–7.41 (m, 2H, Ar–H, O=C–CH=C), 7.55 (d, 2H, $J$ = 8.7 Hz, Ar–H), 7.70 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.08 (s, 1H, Ar–H), 8.20 ppm (d, 1H, $J$ = 9.0 Hz, O–CH=C); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 43.02, 55.87, 57.05, 100.19, 116.11, 119.05, 125.23, 127.55, 129.24, 131.45, 132.58, 135.82 157.19, 157.58, 165.49, 166.25, 175.83 ppm; HRMS ($m/z$): 366.13 $(M + 1)^+$; Anal. ($C_{21}H_{19}NO_5$): C, H, N.

*6.3.17. (E)-N-(2-Fluorobenzyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7q**)*

Color: Yellow; m.p.: 228.6–229.3 °C; Yield: 77%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.92 (s, 3H, $CH_3$–O), 4.63 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 6.03 (d, 1H, $J$ = 5.1 Hz, NH), 6.86 (d, 1H, $J$ = 5.1 Hz, Ar–H), 6.99–7.13 (m, 2H, Ar–H), 7.23 (d, 1H, $J$ = 1.5 Hz, Ar–H), 7.30 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.35–7.40 (m, 1H, Ar–H), 7.54 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.03 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 12.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 38.32, 55.88, 100.09, 113.53, 115.99, 117.38, 119.32, 125.32, 126.53, 127.55, 128.84, 129.11, 130.92, 144.53, 149.84, 158.89, 162.43, 163.28, 165.44, 176.03 ppm; HRMS ($m/z$): 354.11 $(M + 1)^+$; Anal. ($C_{20}H_{16}FNO_4$): C, H, N.

*6.3.18. (E)-N-(3-Fluorobenzyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7r**)*

Color: Yellow; m.p.: 98–100 °C; Yield: 76%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.93 (s, 3H, $CH_3$–O), 4.58 (d, 1H, $J$ = 6.0 Hz, $CH_2$–Ar), 6.03 (s, 1H, NH), 6.74 (s, 1H, Ar–H), 6.87 (d, 1H, $J$ = 2.4 Hz, Ar–H), 6.94–7.00 (m, 2H, Ar–H), 7.09 (d, 1H, $J$ = 7.5 Hz, Ar–H), 7.28–7.36 (m, 2H,

Ar–H, O=C–CH=C), 7.58 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.05 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 44.53, 55.32, 100.10, 113.43, 116.33, 118.96, 119.83, 120.32, 122.58, 127.07, 127.76, 140.43, 143.32, 149.99, 160.22, 162.48, 163.02, 165.89, 175.88 ppm; HRMS ($m/z$): 354.11 $(M + 1)^+$; Anal. ($C_{20}H_{16}FNO_4$): C, H, N.

*6.3.19. (E)-N-(4-Fluorobenzyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7s**)*

Color: Yellow; m.p.: 241.6–243.1 °C; Yield: 53%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.92 (s, 3H, $CH_3$–O), 4.55 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 5.92 (s, 1H, NH), 6.86 (d, 1H, $J$ = 2.4 Hz, Ar–H), 6.99–7.05 (m, 3H, Ar–H), 7.32 (d, 1H, $J$ = 15.3 Hz, O=C–CH=C), 7.28–7.34 (m, 2H, Ar–H), 7.54 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.04 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 43.53, 55.32, 100.10, 114.49, 115.88, 116.59, 119.48, 123.08, 126.18, 129.99, 133.54, 143.15, 149.46, 155.87, 160.48, 165.11, 175.88 ppm; HRMS ($m/z$): 354.11 $(M + 1)^+$; Anal. ($C_{20}H_{16}FNO_4$): C, H, N.

*6.3.20. (E)-N-(2,4-Difluorobenzyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7t**)*

Color: Yellow; m.p.: 130.6–131.3 °C; Yield: 65%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 3.92 (s, 3H, $CH_3$–O), $\delta$ 4.58 (d, 2H, $J$ = 6.3 Hz, $CH_2$–Ar), 6.02 (m, 1H, NH), 6.82–6.87 (m, 3H, Ar–H), 6.99–7.03 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$ = 2.4 Hz, Ar–H), 7.25–7.42 (m, 2H, Ar–H, O=C–CH=C), 7.53 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.03 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 36.16, 55.17, 101.25, 104.22, 111.16, 114.98, 116.34, 119.51, 121.65, 125.18, 127.46, 130.87, 131.94, 157.92, 159.32, 161.81, 163.87, 165.48, 175.87 ppm; HRMS ($m/z$): 372.10 $(M + 1)^+$; Anal. ($C_{20}H_{15}F_2NO_4$): C, H, N.

*6.3.21. (E)-N-(2-Fluorophenethyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7u**)*

Color: White; m.p.: 250.2–250.8 °C; Yield: 80%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 2.91–2.96 (m, 2H, $CH_2$–Ar), 3.63–3.69 (m, 2H, $CH_2$–N), 3.92 (s, 3H, $CH_3$–O), 5.72 (s, 1H, NH), 6.86 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.00–7.11 (m, 3H, Ar–H), 7.19–7.25 (m, 2H, Ar–H), 7.28 (d, 1H, $J$ = 15.3 Hz, O=C–CH=C), 7.45 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.03 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 9.0 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 35.26, 40.75, 55.38, 100.10, 114.75, 115.23, 116.88, 119.04, 121.87, 124.98, 127.61, 127.95, 132.80, 141.17, 149.04, 158.00, 163.76, 166.90, 177.70 ppm; HRMS ($m/z$): 368.13 $(M + 1)^+$; Anal. ($C_{21}H_{18}FNO_4$): C, H, N.

*6.3.22. (E)-N-(3-Fluorophenethyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7v**)*

Color: White; m.p.: 252.6–253.0 °C; Yield: 72%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 2.86–2.91 (m, 2H, $CH_2$–Ar), 3.65–3.67 (m, 2H, $CH_2$–N), 3.92 (s, 3H, $CH_3$–O), 5.62 (s, 1H, NH), 6.86 (d, 1H, $J$ = 2.4 Hz, Ar–H), 6.86–6.95 (m, 2H, Ar–H), 6.99–7.03 (m, 2H, Ar–H), 7.26 (d, 1H, $J$ = 15.3 Hz, O=C–CH=C), 7.27 (s, 1H, Ar–H), 7.45 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.03 (s, 1H, O–CH=C), 8.16 ppm (d, 1H, $J$ = 8.7 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 35.31, 40.39, 55.74, 100.07, 113.48, 114.93, 115.30, 115.58, 117.94, 118.96, 124.25, 124.51, 127.40, 130.02, 132.12, 141.16, 157.07, 157.40, 164.13, 166.21, 175.72 ppm; HRMS ($m/z$): 368.13 $(M + 1)^+$; Anal. ($C_{21}H_{18}FNO_4$): C, H, N.

*6.3.23. (E)-N-(4-Fluorophenethyl)-3-(7-methoxy-4-oxo-4H-chromen-3-yl)acrylamide (**7w**)*

Color: Yellow; m.p.: 177.5–178.5 °C; Yield: 83%; $^1$H NMR: (300 MHz, $CDCl_3$) $\delta$: 2.83–2.87 (m, 2H, $CH_2$–Ar) 3.60–3.66 (m, 2H, $CH_2$–N), 3.92 (s, 3H, $CH_3$–O), 5.70 (s, 1H, NH), 6.86 (d, 1H, $J$ = 2.1 Hz, Ar–H), 6.99–7.03 (m, 3H, Ar–H), 7.15–7.20 (m, 2H, Ar–H), 7.27 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.45 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.03 (s, 1H, O–CH=C), 8.15 ppm (d, 1H, $J$ = 8.7 Hz, Ar–H); $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 35.30, 40.75, 55.40, 100.11, 114.99, 115.13, 116.45, 119.03, 123.35, 127.54, 131.00, 135.53.53, 141, 156.32, 158.44, 161.78, 166.32, 177.83 ppm; HRMS ($m/z$): 368.13 $(M + 1)^+$; Anal. ($C_{21}H_{18}FNO_4$): C, H, N.

*6.3.24. (E)-3-(7-Hydroxy-4-oxo-4H-chromen-3-yl)-N-phenylacrylamide (**8a**)*

Color: Yellow; m.p.: 282.8–229.1 °C; Yield: 72%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 6.86 (d, 1H, $J$ = 2.1 Hz, Ar–H), 6.93 (dd, 1H, $J_1$ = 2.1 Hz, $J_2$ = 2.1 Hz, Ar–H), 7.01 (dd, 1H, $J_1$ = 2.1 Hz, $J_2$ = 2.1 Hz, Ar–H), 7.04–7.28 (m, 2H, Ar–H), 7.33 (s, 1H, Ar–H), 7.65 (d, 1H, $J$ = 7.5 Hz), 7.30 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.77 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 8.03 (d, 1H, $J$ = 8.7 Hz, Ar–H), 8.81 (s, 1H, O–CH=C), 10.30 (s, 1H, NH), 10.97 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 102.38, 115.59, 116.37, 118.22, 119.27, 123.20, 124.65, 127.25, 128.70, 132.67, 139.47, 156.87, 159.18, 162.90, 164.18, 174.76 ppm; HRMS ($m/z$): 308.10 $(M + 1)^+$; Anal. ($C_{18}H_{13}NO_4$): C, H, N.

*6.3.25. (E)-3-(7-Hydroxy-4-oxo-4H-chromen-3-yl)-N-o-tolylacrylamide (**8b**)*

Color: White; m.p.: 316.8–317.3 °C; Yield: 52%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 2.22 (s, 1H, $CH_3$–Ar), 6.90 (d, 1H, $J$ = 8.7 Hz, Ar–H), 6.92–6.96 (m, 2H, Ar–H), 7.03 (d, 1H, $J$ = 7.5 Hz, Ar–H), 7.23–7.28 (m, 2H, Ar–H, O=C–CH=C), 7.60 (d, 1H, $J$ = 15.0 Hz, O=C–C=CH), 7.94 (d, 1H, $J$ = 8.7 Hz, Ar–H), 8.71 (s, 1H, O–CH=C), 9.52 (s, 1H, NH), 10.92 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 18.26, 102.40, 114.65, 115.60, 116.21, 118.14, 120.03, 123.35, 125.01, 127.25, 128.88, 129.32, 130.11, 139.15, 156.55, 160.44, 162.39, 164.20, 175.76 ppm; HRMS ($m/z$): 322.10 $(M + 1)^+$; Anal. ($C_{19}H_{15}NO_4$): C, H, N.

*6.3.26. (E)-3-(7-Hydroxy-4-oxo-4H-chromen-3-yl)-N-(4-methoxyphenyl)acrylamide (**8c**)*

Color: Yellow; m.p.: 324.1–324.5 °C; Yield: 48%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 3.91 (s, 3H, $CH_3$–O), 6.91–7.05 (m, 2H, Ar–H), 7.11–7.31 (m, 3H, Ar–H), 7.42 (d, 1H, $J$ = 15.0 Hz, O=C–CH=C), 7.67 (d, 1H, $J$ = 8.7 Hz, Ar–H), 7.76 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 8.07 (d, 1H, $J$ = 9.0 Hz, Ar–H), 8.23 (s, 1H, O–CH=C), 10.02 (s, 1H, NH), 10.93 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 55.05, 102.33, 114.67, 115.60, 116.40, 119.43, 122.58, 123.22, 127.31, 129.53, 140.01, 158.14, 158.89, 159.20, 162.90, 164.19, 174.77 ppm; HRMS ($m/z$): 328.10 $(M + 1)^+$; Anal. ($C_{19}H_{15}NO_5$): C, H, N.

*6.3.27. (E)-N-Benzyl-3-(7-hydroxy-4-oxo-4H-chromen-3-yl) acrylamide (**8d**)*

Color: Yellow; m.p.: 352.6–253.5 °C; Yield: 77%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 4.38 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 6.88 (d, 1H, $J$ = 2.1 Hz, Ar–H), 6.95 (dd, 1H, $J_1$ = 2.1 Hz, $J_2$ = 2.1 Hz, Ar–H), 7.21 (s, 1H, Ar–H), 7.23 (d, 1H, $J$ = 2.4 Hz, Ar–H), 7.23–7.35 (m, 4H, Ar–H, O=C–CH=C), 7.40 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 7.96 (d, 1H, $J$ = 8.7 Hz, Ar–H), 8.63 (s, 1H, O–CH=C), 8.73 (s, 1H, NH), 10.89 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 42.22, 102.31, 115.50, 116.32, 118.29, 124.36, 126.71, 127.22, 128.25, 131.31, 139.54, 156.87, 158.41, 162.80, 165.44, 174.68 ppm; HRMS ($m/z$): 322.09 $(M + 1)^+$; Anal. ($C_{19}H_{15}NO_4$): C, H, N.

*6.3.28. (E)-3-(7-Hydroxy-4-oxo-4H-chromen-3-yl)-N-(4-methylbenzyl)acrylamide (**8e**)*

Color: Yellow; m.p.: 279.6–281.2 °C; Yield: 63%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 2.25 (s, 3H, $CH_3$–Ar), 4.32 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 6.87 (d, 1H, $J$ = 2.1 Hz, Ar–H), 6.95 (dd, 1H, $J_1$ = 2.4 Hz, $J_2$ = 2.1 Hz, Ar–H), 7.07–7.14 (m, 4H, Ar–H), 7.22 (d, 1H, $J$ = 15.6 Hz,

O=C–CH=C), 7.39 (d, 1H, $J$ = 15.6 Hz, O=C–C=CH), 7.96 (d, 1H, $J$ = 8.7 Hz, Ar–H), 8.62 (s, 1H, O–CH=C), 8.66 (s, 1H, NH), 10.89 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 20.75, 42.11, 102.43, 115.64, 116.43, 118.41, 124.48, 127.36, 128.92, 131.40, 135.91, 136.56, 157.01, 158.43, 162.93, 165.57, 174.85 ppm; HRMS ($m/z$): 336.12 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_4$): C, H, N.

*6.3.29. (E)-3-(7-Hydroxy-4-oxo-4H-chromen-3-yl)-N-(4-methoxybenzyl)acrylamide (**8f**)*

Color: Yellow; m.p.: 270.0–270.3 °C; Yield: 61%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 3.71 (s, 3H, $CH_3$–O), 4.29 (d, 2H, $J$ = 5.7 Hz, $CH_2$–Ar), 6.83–6.88 (m, 2H, Ar–H), 6.92–7.00 (m, 3H, Ar–H), 7.13 (d, 1H, $J$ = 15.6 Hz, O=C–CH=C), 7.17–7.24 (m, 1H, Ar–H), 7.38 (d, 1H, $J$ = 15.6 Hz, O=C–C=CH), 7.92–7.97 (m, 1H, Ar–H), 8.62 (s, 1H, O–CH=C), 8.66 (s, 1H, NH), 10.89 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 41.69, 55.03, 102.31, 113.66, 115.49, 116.33, 118.32, 124.48, 127.20, 128.60, 131.19, 131.50, 156.88, 158.17, 158.33, 162.80, 165.31, 174.69 ppm; HRMS ($m/z$): 352.11 $(M + 1)^+$; Anal. ($C_{20}H_{17}NO_5$): C, H, N.

*6.3.30. (E)-N-(4-Fluorobenzyl)-3-(7-hydroxy-4-oxo-4H-chromen-3-yl)acrylamide (**8g**)*

Color: Brown; m.p.: 297.7–298.2 °C; Yield: 73%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 4.25 (d, 2H, $J$ = 6.0 Hz, $CH_2$–Ar), 6.83–7.08 (m, 5H, Ar–H), 7.10–7.27 (m, 2H, Ar–H, O=C–CH=C), 7.50 (d, 1H, $J$ = 15.6 Hz, O=C–C=CH), 8.46 (d, 1H, $J$ = 9.0 Hz, Ar–H), 8.75 (s, 1H, O–CH=C), 8.77 (s, 1H, NH), 10.88 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 35.81, 102.33, 111.23, 115.55, 116.33, 118.30, 122.21, 127.20, 128.26, 132.11, 156.43, 149.65, 158.50, 159.11, 162.71, 165.58, 177.68 ppm; HRMS ($m/z$): 340.10 $(M + 1)^+$; Anal. ($C_{19}H_{14}FNO_4$): C, H, N.

*6.3.31. (E)-N-(2,4-Difluorobenzyl)-3-(7-hydroxy-4-oxo-4H-chromen-3-yl)acrylamide (**8h**)*

Color: Yellow; m.p.: 268.7–269.1 °C; Yield: 47%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 4.36 (d, 2H, $J$ = 5.4 Hz, $CH_2$–Ar), 6.88 (d, 1H, $J$ = 2.1 Hz, Ar–H), 6.95–7.08 (m, 2H, Ar–H), 7.17–7.24 (m, 2H, Ar–H, O=C–CH=C), 7.38 (d, 1H, $J$ = 15.6 Hz, O=C–C=CH), 7.42 (d, 1H, $J$ = 6.6 Hz, Ar–H), 7.96 (d, 1H, $J$ = 5.7 Hz, Ar–H), 8.62 (s, 1H, O–CH=C), 8.73 (s, 1H, NH), 10.89 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 35.72, 102.31, 103.59, 111.10, 111.40, 115.50, 116.33, 118.24, 122.32, 122.53, 124.05, 127.20, 130.86, 131.56, 156.87, 158.51, 162.82, 165.58, 174.68 ppm; HRMS ($m/z$): 358.08 $(M + 1)^+$; Anal. ($C_{19}H_{13}F_2NO_4$): C, H, N.

*6.3.32. (E)-N-(4-Fluorophenethyl)-3-(7-hydroxy-4-oxo-4H-chromen-3-yl)acrylamide (**8i**)*

Color: Brown; m.p.: 217.9–218.3 °C; Yield: 77%; $^1$H NMR: (300 MHz, DMSO-$d_6$) $\delta$: 2.72–2.85 (m, 2H, $CH_2$–Ar), 3.35–3.47 (m, 2H, $CH_2$–N), 6.87 (d, 1H, $J$ = 2.1 Hz, Ar–H), 7.07–7.14 (m, 3H, Ar–H), 7.19–7.25 (m, 2H, Ar–H), 7.29 (d, 1H, $J$ = 15.6 Hz, O=C–CH=C), 7.45 (d, 1H, $J$ = 15.3 Hz, O=C–C=CH), 7.96 (s, 1H, O–CH=C), 8.46 (d, 1H, $J$ = 1.5 Hz, Ar–H), 8.65 (s, 1H, NH), 10.88 ppm (s, 1H, OH); $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 34.23, 40.90, 102.40, 115.17, 115.65, 116.38, 118.35, 124.53, 127.16, 127.29, 128.36, 130.53, 135.72, 156.99, 158.41, 162.97, 165.57, 174.84 ppm; HRMS ($m/z$): 354.11 $(M + 1)^+$; Anal. ($C_{20}H_{16}FNO_4$): C, H, N.

### 6.4. Pharmacology methods

*6.4.1. HBsAg, HBeAg, and HBV DNA inhibition assays*

The inhibitory effects on HBV antigens and viral DNA replication by various compounds were evaluated in cultured HepG2 2.2.15 cells. Nine days after treatment with various dosages of the compounds, culture supernatants were collected, and levels of HBV antigens were determined by ELISA. Levels of HBV-DNA were quantified by fluorescence PCR on an MJ Research PTC-200 instrument as previously described [17]. PCR primers used were as follows: forward, 5′-TGTCCTGGTTATCGCTGG-3′; reverse, 5′-CAAACGGGCAACATA-CCTT-3′. Probe used was 5′-(FAM)-TGTGTCTGCGGCGTTTTATCAT-(TAMRA)-3′. Data were analyzed by Opticon Monitor software (version 2.01). The antiviral activities of various compounds are summarized in Table 1.

*6.4.2. In vitro cytotoxicity study of target compounds*

Cytotoxicity of all synthesized compounds was assessed by MTT assays as previously described [18]. The compounds, dissolved in DMSO, were serially diluted in cell culture media and then added onto cell monolayers. HepG2 2.2.15 cells were maintained in 96-well tissue culture plates for 48 h before being treated with compounds. The cells were subsequently incubated at 37 °C for 9 days. Cells with media alone were used as the blank control, while adefovir was used as positive controls. MTT reagents were added at a concentration of 5 g/L at 4 h before the cells were harvested and lysed with 10% sodium dodecyl sulfate (SDS) and 50% *N*,*N*-dimethylformamide (DMF), pH 7.2. The OD values of cell lysates were read at a wavelength of 570 nm, and the percentages of cell death were determined.

*6.4.3. Screening of the in vitro anti-cancer activities*

*In vitro* anti-cancer activity was determined by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and manifested by $IC_{50}$ of the compounds [18–20]. Various cancer cell lines were obtained from Shanghai Institute of Biological Sciences (SIBS), CAS. $IC_{50}$ calculation was done according to the National Committee for Clinical Laboratory Standards (NCCLS). All compounds were dissolved in DMSO with the stock concentration of 10 g/L (stored at 4 °C), and diluted with fresh medium promptly before drug administration. Cells were seeded in 96-well plates at the density of 8000 cells/well. 24 h later, compounds at various concentrations were applied in duplicate to cells, which were incubated at 37 °C in a humidified incubator with 5% $CO_2$. After 48 h, 20 μL of MTT (Sigma) at 5 mg/ml in PBS (filter sterilized, light protected, and stored at 4 °C) was added to each well. After 4 h of incubation at 37 °C, MTT was converted to a blue formazan product by mitochondrial succinate dehydrogenase. The yielding product was eluted from cells by adding 150 ml of DMSO. The absorbance at 570 nm wavelength was determined with an ELX800 microplate spectrophotometer. The $IC_{50}$ value was defined as the concentration under which 50% of the cells could survive.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.09.017.

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