European Journal of Medicinal Chemistry 54 (2012) 813–822

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

One-pot synthesis of novel isoindoline-1,3-dione derivatives bearing 1,2,4-triazole moiety and their preliminary biological evaluation

Pei-Liang Zhao [a,1], Wei-Feng Ma [b,1], An-Na Duan [a], Min Zou [a], Yi-Chen Yan [a], Wen-Wei You [a,*], Shu-Guang Wu [a,*]

[a] Department of Chemistry, School of Pharmaceutical Science, Southern Medical University, Guangzhou 510515, PR China
[b] Department of Microbiology, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou 510515, PR China

ARTICLE INFO

Article history:
Received 24 April 2012
Received in revised form
19 June 2012
Accepted 21 June 2012
Available online 3 July 2012

Keywords:
Isoindoline-1,3-dione
1,2,4-Triazole
One-pot synthesis
Antifungal
Antitumor activity

ABSTRACT

A series of novel isoindoline-1,3-diones containing 1,2,4-triazole moiety were synthesized *via* a one-pot reaction. Bioassay indicated that compounds **33**, **35**, **37** and **39** exhibited much higher activities against *Botryodiplodia theobromae* than commercial fungicide triadimefon at the dosage of 150 mg/L. Most interestingly, compounds **36**, **37** and **45** displayed much stronger antitumor activities against four human cell lines than positive control Fluorouracil. Particularly, compound **37** had four-fold improvement compared to Fluorouracil in inhibiting A549 and HepG2 cell proliferation with IC_{50} values of 6.76 and 9.44 μM, respectively. Further flow-activated cell sorting analysis revealed that compound **37** displayed apoptosis-inducing effect on HepG2 cells in a dose-dependent manner. These encouraging results could be helpful for the development of new antitumor compounds.

1. Introduction

Recently, increasing interests have been focused on the cyclic imides because of their broad spectrum of biological activities [1–6]. Among the existing various N-substituted cyclic imides, isoindoline-1,3-dione derivatives have been identified as one of the most promising scaffold, which contain the general structure –CO–N(R)–CO–, so that they are hydrophobic and neutral, and can therefore cross biological membranes *in vivo* [7]. N-substituted isoindoline-1,3-dione has been a very interesting and hot research area due to its broad structural diversity and broad-spectrum biological activities [8–12]. As shown in Fig. 1, by introduction of nitrogen-containing heterocycle into the isoindoline-1,3-diones, compounds **1** and **2** have been found to exhibit potent antibacterial and antifungal activities [7,13]. While halogen-substituted compound **3** has been proved to be effective in treating prostate cancer [14], which is the most common malignancy in American men and the second leading cause of cancer mortality [15]. More recently compound **4** has emerged as potential antitumor agent inhibiting cyclin-dependent kinases (CDKs) [16].

It is well-known that the derivatives of 1,2,4-triazole always possess a wide range of biological activities including insecticidal [17], antifungal [18], antimicrobial [19], antiviral [20], antitumor [21], and anti-inflammatory [22] properties. Some of 1,2,4-triazole derivatives have also been widely used in agriculture and medicine. For example, bromuconazole (**5**) and fluconazole (**6**) have been used as commercial antifungicides for many years [23,24] (Fig. 2), while anastrozole (**7**) and vorozole (**8**) have been developed as anticancer drugs recently [25,26]. Therefore, as a part of our research work on the development of novel bioactive nitrogen-containing heterocycles [27–29], as shown in Fig. 3, we are very interested in the design and efficient synthesis of isoindoline-1,3-diones bearing 1,2,4-triazole substructure, which will be expected to exhibit interesting features due to the co-existence of two kinds of pharmacophore. In this paper, we firstly described the one-pot synthesis and biological activity of a series of new isoindoline-1,3-dione derivatives containing 1,2,4-triazol moiety **9**–**46** (Fig. 3), which have, to our knowledge, not been reported so far.

2. Chemistry

Due to the different substituents on the 1,2,4-triazole core, two methods were used to synthesize the 4-amino-5-substituted-4H-

* Corresponding authors. Tel./fax: +86 (0)20 61648196.
E-mail addresses: youww@smu.edu.cn (W.-W. You), shuguang@smu.edu.cn (S.-G. Wu).
[1] These authors contributed equally to this work.

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http://dx.doi.org/10.1016/j.ejmech.2012.06.041

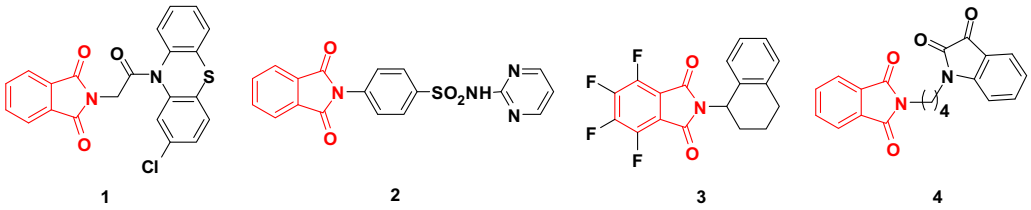


Fig. 1. Structures of some bioactive isoindoline-1,3-dione.

1,2,4-triazole-3-thiols **47** with the starting materials of their corresponding carboxylic acids according to our previously reported method [29,30]. Meanwhile, a variety of phenacyl bromides were readily afforded by the bromination reaction of commercially available acetophenones with 1.02 equiv. N-bromosuccinimide (NBS) and catalytic amount of 2,2′-azo-bisisobutyronitrile (AIBN) in anhydrous carbon tetrachloride, respectively. The expected target isoindoline-1,3-dione derivatives (**9**–**46**) were prepared by equimolar amounts of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiol (**47**), isobenzofuran-1,3-dione (**48**) and excess amount of halogenated compounds (1.2 equiv) with equimolar amount of zinc bromide and 2.0 equimolar amounts of triethylamine (TEA) in absolute toluene (Scheme 1). It should be noted that the substituents at the 5-position of 1,2,4-triazole ring such as CH_3, CH_3CH_2, $CH_3CH_3CH_2$, and 3,4,5-trimethoxyphenyl, have practically no obvious effect on the yields.

The structures of the prepared isoindoline-1,3-dione derivatives **9**–**46** were characterized by ^{1}H NMR, ^{13}C NMR, ESI-MS spectrum and elemental analysis, and the results are presented in the Experimental section. In addition, the representative compound **37** was further confirmed by single-crystal X-ray diffraction. As shown in Fig. 4, the dihedral angle of the 1,2,4-triazole ring and the adjacent pyrrolidine plane shows a gauche conformation with an angle of 88.32° that is affected by the bulky 4-methylphenacylthio group.

3. Pharmacology results and discussion

3.1. Antifungal activity

The *in vitro* antifungal activities of compounds **9**–**46** against six fungi including *Botryodiplodia theobromae*, *Fusarium oxysporum* f. sp. Niveum, *Fusarium oxysporum* f. sp. Cubense, *Colletotrichum musae* (Berk & Curt) Arx, *Phyricularia oryzae* Cav. and *Colletotrichum gloeosporioides* Penz. were tested. The results are listed in Table 1, in which the inhibition percentage was expressed as the mean of values obtained in three independent experiments. Triadimefon, a commercial fungicide, was used as a positive control. For the convenience of structure–activity relationship analysis, compounds **9**–**32** and **33**–**46** were defined as alkyl-triazole derivatives and aryl-triazole derivatives, respectively. Although it seems impossible to extract an obvious structure–activity relationship from the data shown in Table 1, we can conclude clearly that nearly all compounds exhibit significant antifungal activities against *B. theobromae*, *F. oxysporum* f. sp. Niveum, *C. gloeosporioides* Penz. and *P. oryzae* Cav. at the concentration of 150 mg/L. In most cases, aryl-triazole derivatives displayed higher fungicidal activity against *B. theobromae* and *F. oxysporum* f. sp. Niveum than alkyl-triazole derivatives, for example, compounds **33** and **18** (R = CH_2CH_2 = CH_2), **35** and **17** (R = $CH_2CH{\equiv}CH$), **37** and **12** (R = 4-$MeC_6H_4COCH_2$), **39** and **10** (R = $EtCO_2CH_2$). However, when aryl-triazole derivatives contain benzyl group, the antifungal activity against *B. theobromae* decreased dramatically, such as compounds **34** (R = 2-$ClC_6H_4CH_2$) and **40** (R = $C_6H_5CH_2$), which exhibited much lower antifungal activity than the corresponding alkyl-triazole derivatives **16** and**19**, respectively.

It is showed in Table 1 that, within the series of alkyl-triazole derivatives, antifungal activities were influenced by the S-substituents on the 1,2,4-triazole ring and phenacyl substitution always resulted in higher activities against *F. oxysporum* f. sp. Cubense and *P. oryzae* Cav., and only compound **10** (R = 4-$ClC_6H_4COCH_2$) displayed good antifungal activities (≥80%) against *C. gloeosporioides* Penz. at the concentration of 150 mg/L. While most compounds of aryl-triazole derivatives **33**, **35**, **37** and **39** displayed excellent antifungal activities (≥90%) against *B. theobromae*, which are superior to that of Triadimefon, a commercial fungicide. Interestingly, compounds **33** and **37** also displayed similar potent antifungal activities (≥80%) against *C. gloeosporioides* Penz. in comparison with reference drug. Meanwhile, compound **33** was also found to display fungicidal activity against other fungi. These results indicated that compound **33** showed a broad spectrum antifungal activity and further investigations are necessary to optimize the potentially leading compound as more efficacious antifungal agents.

3.2. Antitumor activity

The *in vitro* antitumor activities of the synthesized compounds **9**–**46** against four human cancer cell lines, including HepG2, A549, PC-3M and MKN45, were assayed by MTT method. Fluorouracil was used as the reference drug and the results expressed as IC_{50} (μM) were summarized in Table 2. Here, the IC_{50} value represents the concentration of one compound resulting in a 50% inhibition in cell growth after 48 h incubation, and is the average of three independent experiments.

As indicated in Table 2, the results indicated that only compounds **36**, **37** and **41**–**46** were found to be active. Among them, three representative compounds **36**, **37** and **45** displayed much higher antitumor activities against HepG2, A549, PC-3M and

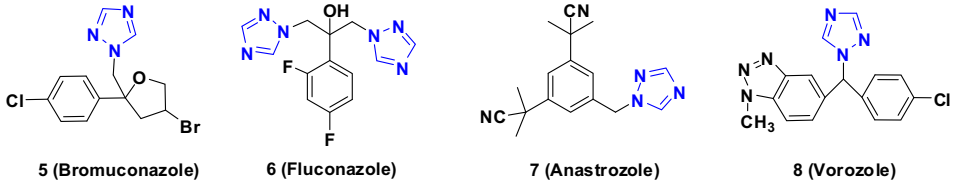


Fig. 2. Structures of some commercial 1,2,4-triazole derivatives.

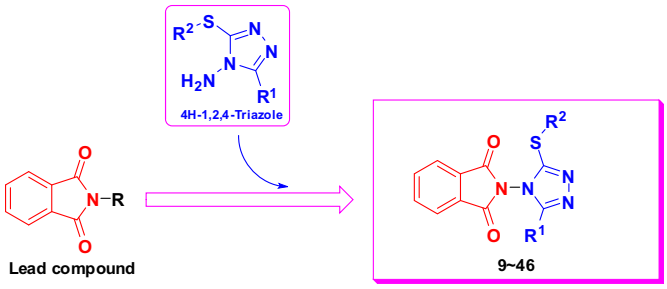


Fig. 3. Design strategy of the title compounds.

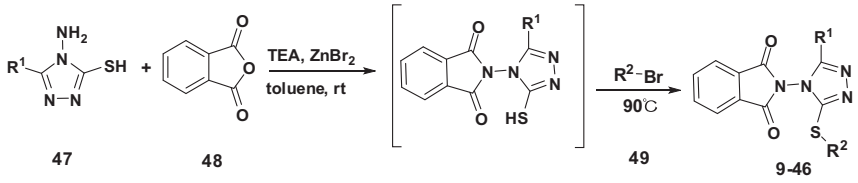
MKN45 cell lines than positive control Fluorouracil. Especially, compound **37** showed pretty high potency against A549, HepG2 cell lines with IC_{50} values of 6.76, 9.44 μM, respectively, which was found to be four times more potent than Fluorouracil. These encouraging results could be helpful for the development of new antitumor compounds.

Further analysis on the structure–activity relationship investigated the effects of several substituents on 1,2,4-triazole ring. In most cases, compounds bearing 3,4,5-trimethoxyphenyl group at the 5-position of 1,2,4-triazole ring (**36**, **37** and **41**–**46**) displayed obviously higher cytotoxic activity than those of the corresponding compounds with methyl, ethyl or n-propyl group at the same position (**9**–**32**). Nevertheless, within the series of compounds **33**–**46** bearing 3,4,5-trimethoxyphenyl group, anticancer activities were influenced by the C-3 substituents on the 1,2,4-triazole ring and phenacyl substitution always gave higher activities (**36**, **37** and **45**). Replacement of phenacyl group with methyl, benzyl, allyl, propargyl and ester group (**33**–**35** and **38**–**40**) led to a dramatic decrease in antitumor activity. It can be concluded that 3,4,5-trimethoxyphenyl group at the 5-position of 1,2,4-triazole ring and substituted phenacyl group at the 3-position play a crucial role in modulating the antitumor activity.

To study the effect of the synthesized compounds on cell cycle progression, flow-activated cell sorting analysis was performed. The most promising compound **37** was tested against HepG2 cell lines at given concentrations (4, 8, 16 μM). Fig. 5 and Table 3 show the results after 24 h treatment with **37**. As can be seen in Fig. 5 and Table 3, compound **37** had no obvious effect on the cell cycle arrest. However, cells accumulated dramatically in sub-G0 phase at 15.05–32.58%, suggesting that compound **37** might possess apoptosis-inducing effect on HepG2 cells in a dose-dependent manner.

4. Conclusion

In summary, a series of novel isoindoline-1,3-diones bearing 1,2,4-triazole ring were designed and efficiently synthesized *via* a one-pot, three-component reaction of isobenzofuran-1,3-dione, 4-amino-5-substituted-4H-1,2,4-triazole-3-thiol, and various halogenated compounds. Subsequent biological evaluation indicated that four compounds **33**, **35**, **37** and **39** exhibited more potent *in vitro* antifungal activities than the reference drug against *B. theobromae*. Among them, compound **33** was identified as the most promising antifungal candidate for further study, due to its broad spectrum and the highest antifungal activity. Most interestingly, three representative compounds **36**, **37** and **45** displayed much higher antitumor activities against HepG2, A549, PC-3M and



Scheme 1. One-pot synthesis of compounds **9**–**46**.

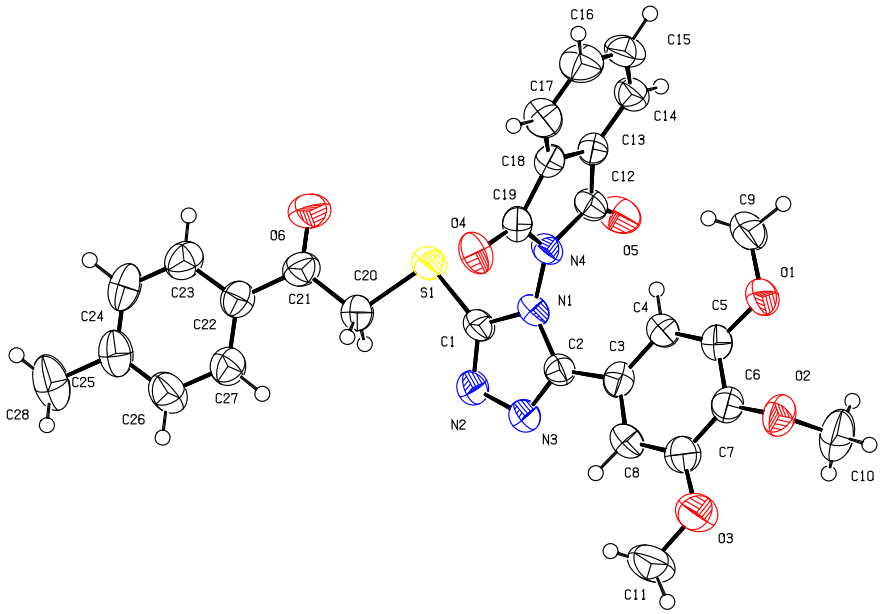
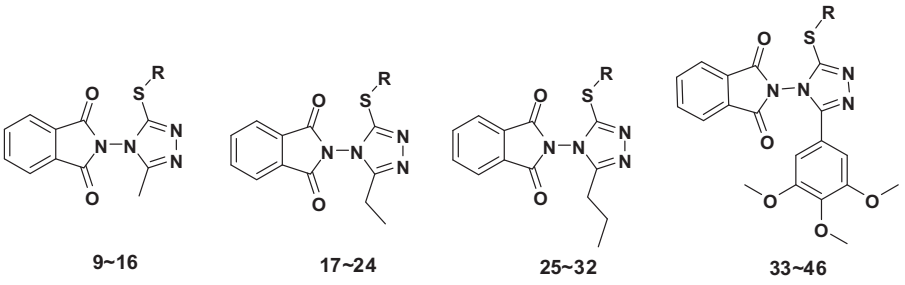


Fig. 4. Molecular structure of compound **37**.

Table 1
Structures and antifungal activities of compounds **9**–**46**.



Comp.	R	150 mg/L (*in vitro*)[b]					
		BT[a]	FN[a]	FC[a]	CA[a]	PC[a]	CG[a]
9	$C_6H_5CH_2$	−	++	−	−	−	−
10	$EtCO_2CH_2$	−	++	−	−	−	++
11	4-$ClC_6H_4COCH_2$	−	++	−	−	++	+++
12	4-$MeC_6H_4COCH_2$	++	++	−	−	+	+
13	$C_6H_5COCH_2$	+	++	−	−	−	+
14	$HC{\equiv}CCH_2$	+	++	−	−	++	+
15	Me	−	++	−	−	−	+
16	2-$ClC_6H_5CH_2$	++	++	−	−	++	+
17	$HC{\equiv}CCH_2$	++	+	−	+	++	++
18	$H_2C{=}CHCH_2$	+	++	−	−	−	++
19	$C_6H_5CH_2$	++	++	−	−	+	+
20	$C_6H_5COCH_2$	+	++	−	−	−	+
21	4-$ClC_6H_4COCH_2$	−	+	−	−	−	++
22	4-$MeC_6H_4COCH_2$	−	++	−	−	−	++
23	Me	−	−	−	−	−	−
24	$EtCO_2CH_2$	−	+	−	−	+	−
25	$H_2C{=}CHCH_2$	++	++	−	+	−	++
26	$C_6H_5CH_2$	++	+	−	−	−	+
27	4-$ClC_6H_4COCH_2$	−	++	−	−	−	++
28	4-$MeC_6H_4COCH_2$	+	++	−	−	+++	+
29	$HC{\equiv}CCH_2$	+	++	−	−	−	++
30	2-$ClC_6H_5CH_2$	+	++	−	−	−	−
31	$C_6H_5COCH_2$	−	++	−	+	−	++
32	Me	−	++	−	−	−	−
33	$H_2C{=}CHCH_2$	++++	+++	++	++	++	+
34	2-$ClC_6H_5CH_2$	−	++	−	−	−	−
35	$HC{\equiv}CCH_2$	++++	++	−	−	−	++
36	4-$ClC_6H_4COCH_2$	−	++	−	−	−	−
37	4-$MeC_6H_4COCH_2$	++++	+++	−	+	−	−
38	Me	−	++	−	+	+	++
39	$EtCO_2CH_2$	++++	++	−	++	−	++
40	$C_6H_5CH_2$	+	++	−	−	−	−
41	$C_6H_5COCH_2$	+	+		+	++	
42	4-$MeOC_6H_4COCH_2$	+++	−	+	−	−	−
43	2-$MeC_6H_4COCH_2$	++	++	+	−	−	−
44	4-$FC_6H_4COCH_2$	−	++	−	−	−	++
45	4-$BrC_6H_4COCH_2$	++	+++	−	−	++	−
46	3,4-$F_2C_6H_4COCH_2$	+	++	−	++	−	−
Triadimefon		+++	++++	++	++++	+++	+++

[a] BT for *Botryodiplodia theobromae*, FN for *Fusarium oxysporum* f. sp. Niveum, FC for *Fusarium oxysporum* f. sp. Cubense, CA for *Colletotrichum musae* (Berk & Curt) Arx, PC for *Phyricularia oryzae* Cav., CG for *Colletotrichum gloeosporioides* Penz.
[b] Rating system for the inhibition percentage: ++++, ≥90%; +++, ≥80%; ++, ≥60%; +, ≥50%; −, <50%.

MKN45 cell lines than positive control Fluorouracil. Particularly, compound **37** was four-fold improvement compared to Fluorouracil in inhibiting A549 and HepG2 cell proliferation with IC_{50} values of 6.76 and 9.44 μM, respectively.

These results indicated that compound **37** could be used as a lead for further developing new isoindoline-1,3-diones derivatives possessing both antifungal and antitumor activities. Flow-activated cell sorting analysis revealed that compound **37** displayed apoptosis-inducing effect on HepG2 cells in a dose-dependent manner. Further structural optimization and fungicidal and antitumor activities about the isoindoline-1,3-dione analogs are well under way.

5. Experimental protocols

5.1. Chemistry

^1H NMR and ^{13}C NMR spectra were recorded on a Mercury-Plus 400 spectrometer in $CDCl_3$ or DMSO-d_6 solution and chemical shifts were recorded in parts per million (ppm) with TMS as the internal reference. MS spectra were determined using a Micromass ZQ 4000 mass spectrometry, and signals were given in *m*/*z*. Elementary analyses were performed on a Vario EL III elemental analysis instrument. Melting points (mp) were taken on a Buchi B-545 melting point apparatus and uncorrected. Unless otherwise

Table 2
Cytotoxic activities of compounds **36**, **37** and **41**–**46** against human tumor cells.

Comp.	R	*In vitro* cytotoxicity IC_{50} (μM)[a]			
		HepG2	A549	PC-3M	MKN45
36	4-$ClC_6H_4COCH_2$	22.03	**9.03**	20.69	26.64
37	4-$MeC_6H_4COCH_2$	**9.44**	**6.76**	**11.71**	**9.62**
41	$C_6H_5COCH_2$	170.71	>200	>200	>200
42	4-$MeOC_6H_4COCH_2$	28.73	30.03	32.80	37.23
43	2-$MeC_6H_4COCH_2$	>200	143.87	>200	>200
44	4-$FC_6H_4COCH_2$	>200	>200	>200	126.40
45	4-$BrC_6H_4COCH_2$	27.71	16.05	22.58	22.32
46	3,4-$F_2C_6H_4COCH_2$	>200	>200	163.02	>200
Fluorouracil		46.83	35.41	22.63	41.73

[a] The IC_{50} values of all the other compounds that were not listed, were estimated to be greater than 200 μM.

noted, reagents were purchased from commercial suppliers and used without further purification while all solvents were redistilled before use.

5.1.1. General procedure for the one-pot reactions of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiol, isobenzofuran-1,3-dione, and various halogenated compounds

A mixture of 4-amino-5-substituted-4H-1,2,4-triazole-3-thiol (1.0 mmol), isobenzofuran-1,3-dione (1.0 mmol, 0.163 g), zinc bromide (1.0 mmol, 0.225 g) and triethylamine (2.0 mmol, 0.202 g) in 3.0 mL of dry toluene was stirred at room temperature for about 0.5 h. Then halogenated compound (1.2 mmol) was added. The reaction mixture was heated at 90 °C for another 3–8 h. After the reaction was complete according to the TLC detection, solvent was removed under reduced pressure and the residue was purified by column chromatography using a mixture of petroleum ether and ethyl acetate (10:1) as an eluent to give the target compounds in yields of 62–81%.

*5.1.1.1. 2-(3-(Benzylthio)-5-methyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**9**).* Yield, 74%; mp 111–113 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (s, 3H, triazole–CH_3), 4.36 (s, 2H, SCH_2), 7.22–7.28 (m, 5H, ArH), 7.89 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H, ArH), 7.99 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.0, 38.2, 124.8, 127.8, 128.6, 129.0, 129.1, 135.4, 135.7, 150.7, 152.5, 162.1. ESI-MS: m/z = 373.25 $[M + Na]^+$. Anal. Calcd. for $C_{18}H_{14}N_4O_2S$: C 61.70, H 4.03, N 15.99, S 9.15. Found C 61.47, H 3.71, N 16.16, S 9.46.

*5.1.1.2. Ethyl 2-(4-(1,3-dioxoisoindolin-2-yl)-5-methyl-4H-1,2,4-triazol-3-ylthio)acetate (**10**).* Yield, 76%; mp 105–106 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (t, J = 7.0 Hz, 3H, $OCH_2\underline{CH_3}$), 2.33 (s, 3H, triazole-CH_3), 3.94 (s, 2H, SCH_2), 4.13 (dd, J = 7.0 Hz, J = 14.2 Hz, 2H, $O\underline{CH_2}CH_3$), 7.91 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H, ArH), 8.01 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.9, 13.9, 35.8, 62.1, 124.9, 129.1, 135.7, 149.7, 152.7, 162.1, 167.1. ESI-MS: m/z = 370.22 $[M + Na]^+$, 347.08 $[M + 1]^+$. Anal. Calcd. for $C_{15}H_{14}N_4O_4S$: C 52.02, H 4.07, N 16.18, S 9.26; Found C 52.26, H 4.28, N 16.36, S 9.52.

*5.1.1.3. 2-(3-(2-(4-Chlorophenyl)-2-oxoethylthio)-5-methyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**11**).* Yield, 74%; mp 115–117 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (s, 3H, triazole-

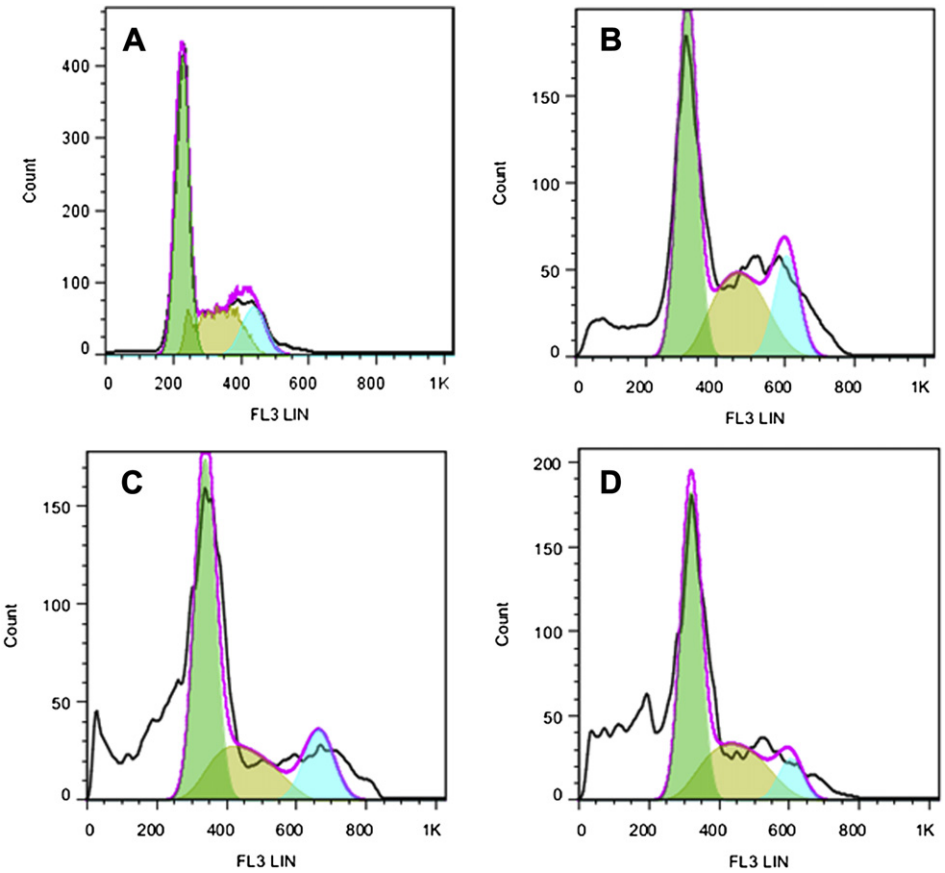


Fig. 5. Effect of compound **37** on cell cycle and apoptosis in HepG2 cells. Flow cytometry analysis of HepG2 cells treated with **37** for 24 h. (A) Control; (B) 37, 4 μM; (C) 37, 8 μM; (D) 37, 16 μM.

Table 3
Effect of compound **37** on cell cycle distribution in HepG2 cells.

Concentration	Sub-G_0	G_0/G_1	S	G_2/M
0 μM	2.48	54.65	29.84	16.38
4 μM	15.05	40.73	25.41	14.40
8 μM	29.22	37.45	17.50	11.38
16 μM	32.58	36.70	21.07	6.31

CH_3), 4.83 (s, 2H, SCH_2), 7.42 (d, J = 8.4 Hz, 2H, ArH), 7.88–7.92 (m, 4H, ArH), 8.00 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.9, 42.7, 124.9, 129.1, 129.2, 129.8, 133.0, 135.8, 140.6, 150.2, 152.7, 162.1, 191.6. ESI-MS: 413.09 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{13}ClN_4O_3S$: C 55.28, H 3.17, N 13.57, S 7.77. Found C 55.59,
H 3.53, N 13.74, S 7.65.

*5.1.1.4. 2-(3-Methyl-5-(2-oxo-2-p-tolylethylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**12**).* Yield, 65%; mp 114–116 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.33 (s, 3H, triazole-CH_3), 2.39 (s, 3H, Ar-CH_3), 4.86 (s, 2H, SCH_2), 7.36 (d, J = 8.0 Hz, 2H, ArH), 7.62–7.71 (m, 3H, ArH), 7.91–7.96 (m, 3H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.9, 21.7, 43.2, 124.9, 128.5, 129.2, 129.5, 132.2, 135.7, 145.2, 150.6, 152.6, 162.1, 192.3. ESI-MS: 393.16 $[M + 1]^+$. Anal. Calcd. for $C_{20}H_{16}ClN_4O_3S$: C 61.21, H 4.11, N 14.28, S 8.17. Found C 61.53, H 4.49, N 14.58, S 8.39.

*5.1.1.5. 2-(3-Methyl-5-(2-oxo-2-phenylethylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**13**).* Yield, 63%; mp 183–185 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (s, 3H, triazole-CH_3), 4.89 (d, J = 4.8 Hz, 2H, SCH_2), 7.45 (t, J = 8.0 Hz, 2H, ArH), 7.56 (t, J = 4.2 Hz, 1H, ArH), 7.89–7.95 (m, 4H, ArH), 7.98–8.01 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 9.9, 43.2, 125.0, 128.5, 128.8, 129.2, 134.1, 134.7, 135.8, 150.5, 152.7, 162.2, 192.7. ESI-MS: m/z = 401.34 $[M + Na]^+$, 379.42 $[M + 1]^+$. Anal. Calcd. for $C_{20}H_{16}ClN_4O_3S$: C 60.31, H 3.73, N 14.81, S 8.47. Found C 60.69, H 3.99, N 14.51, S 8.30.

*5.1.1.6. 2-(3-Methyl-5-(prop-2-ynylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**14**).* Yield, 75%; mp 96–98 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.21 (t, J = 2.6 Hz, 1H, ≡CH), 2.35 (s, 3H, triazole–CH_3), 3.81 (d, J = 2.4 Hz, 2H, SCH_2), 7.92 (dd, J = 3.0 Hz, J = 5.4 Hz, 2H, ArH), 8.01 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.0, 22.7, 73.3, 77.5, 124.9, 129.1, 135.8, 149.3, 152.9, 162.2. ESI-MS: m/z = 299.09 $[M + 1]^+$. Anal. Calcd. for $C_{14}H_{10}N_4O_2S$: C 56.37, H 3.38, N 18.78, S 10.75. Found C 56.70, H 3.62, N 18.58, S 10.48.

*5.1.1.7. 2-(3-Methyl-5-(methylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**15**).* Yield, 78%; mp 113–115 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.32 (s, 3H, triazole–CH_3), 2.61 (s, 3H, SCH_3), 7.91 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH), 8.00 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 9.6, 15.4, 124.6, 128.8, 135.6, 151.6, 152.2, 162.0. ESI-MS: m/z = 297.20 $[M + Na]^+$, 275.15 $[M + 1]^+$. Anal. Calcd. for $C_{12}H_{10}N_4O_2S$: C 52.54, H 3.67, N 20.43, S 11.69. Found C 52.37, H 3.76, N 20.15, S 11.38.

*5.1.1.8. 2-(3-(2-Chlorobenzylthio)-5-methyl-4H-1,2,4-triazol-4-yl) isoindoline-1,3-dione (**16**).* Yield, 73%; mp 134–136 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (s, 3H, triazole–CH_3), 4.42 (s, 2H, SCH_2), 7.15–7.19 (m, 2H, ArH), 7.27 (t, J = 2.8 Hz, 1H, ArH), 7.39 (d, J = 2.4 Hz, 1H, ArH), 7.90 (dd, J = 3.2 Hz, J = 5.2 Hz, 2H, ArH), 7.98 (d, J = 3.0 Hz, J = 5.4 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.0, 36.1, 124.8, 127.0, 129.1, 129.4, 129.5, 131.4, 133.5, 134.1, 135.7, 150.3, 152.6, 162.1. ESI-MS: m/z = 385.34 $[M + 1]^+$. Anal. Calcd. for $C_{18}H_{13}ClN_4O_2S$: C 56.18, H 3.40, N 14.56, S 8.33. Found C 55.95, H 3.64, N 14.35, S 8.15.

*5.1.1.9. 2-(3-Ethyl-5-(prop-2-ynylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**17**).* Yield, 71%; mp 128–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (t, J = 7.6 Hz, 3H, $CH_2\underline{CH_3}$), 2.20 (t, J = 2.6 Hz, 1H, ≡CH), 2.61 (dd, J = 7.4 Hz, J = 15.0 Hz, 2H, $\underline{CH_2}CH_3$), 3.79 (d, J = 2.8 Hz, 2H, SCH_2), 7.90–7.93 (m, 2H, ArH), 7.98–8.01 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.8, 18.0, 22.8, 73.3, 77.6, 124.9, 129.1, 135.8, 149.3, 157.2, 162.3. ESI-MS: m/z = 335.19 $[M + Na]^+$, 313.26 $[M + 1]^+$. Anal. Calcd. for $C_{15}H_{12}N_4O_2S$: C 57.68, H 3.87, N 17.94, S 10.27. Found C 57.95, H 4.16, N 17.89, S 10.08.

*5.1.1.10. 2-(3-(Allylthio)-5-ethyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**18**).* Yield, 81%; mp 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.26 (t, J = 7.6 Hz, 3H, $-CH_2\underline{CH_3}$), 2.59 (dd, J = 7.2 Hz, J = 14.8 Hz, 2H, $-\underline{CH_2}CH_3$), 3.68 (d, J = 6.8 Hz, 2H, SCH_2), 5.07 (d, J = 10.0 Hz, 1H, =CH_a), 5.17 (d, J = 16.8 Hz, 1H, =CH_b), 5.78–5.84 (m, 1H, $\underline{CH}$=), 7.90 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH), 8.00 (dd, J = 3.0 Hz, J = 5.0 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.9, 18.0, 36.8, 119.5, 124.9, 129.2, 131.9, 135.7, 150.3, 156.8, 162.4. ESI-MS: m/z = 337.23 $[M + Na]^+$, 315.21 $[M + 1]^+$. Anal. Calcd. for $C_{15}H_{14}N_4O_2S$: C 57.31, H 4.49, N 17.82, S 10.20. Found C 57.58, H 4.75, N 17.56, S 10.43.

*5.1.1.11. 2-(3-(Benzylthio)-5-ethyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**19**).* Yield, 66%; mp 87–89 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.30 (t, J = 7.6 Hz, 3H, $CH_2\underline{CH_3}$), 2.63 (dd, J = 7.6 Hz, J = 15.2 Hz, 2H, $-\underline{CH_2}CH_3$), 4.37 (s, 2H, $\underline{SCH_2}$), 7.22–7.30 (m, 5H, ArH), 7.90 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH), 7.99 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.9, 17.9, 38.3, 124.8, 127.8, 128.6, 129.0, 129.1, 135.5, 135.7, 150.7, 156.7, 162.3. ESI-MS: m/z = 387.27 $[M + Na]^+$, 365.26 $[M + 1]^+$. Anal. Calcd. for $C_{19}H_{16}N_4O_2S$: C 62.62, H 4.43, N 15.37, S 8.80. Found C 62.41, H 4.13, N 15.65, S 8.53.

*5.1.1.12. 2-(3-Ethyl-5-(2-oxo-2-phenylethylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**20**).* Yield, 67%; mp 158–159 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (t, J = 7.6 Hz, 3H, $CH_2\underline{CH_3}$), 2.61 (dd, 2H, J = 7.2 Hz, J = 15.2 Hz, $-\underline{CH_2}CH_3$), 4.88 (s, 2H, $\underline{SCH_2}$), 7.42–7.47 (t, J = 7.6 Hz, 2H, ArH), 7.56 (d, J = 7.6 Hz, 1H, ArH), 7.89–7.94 (m, 4H, ArH), 7.99 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.8, 17.8, 43.1, 124.8, 128.3, 128.7, 129.0, 134.0, 134.6, 135.7, 150.3, 156.9, 162.2, 192.6. ESI-MS: m/z = 415.31 $[M + Na]^+$, 393.47 $[M]^+$. Anal. Calcd. for $C_{20}H_{16}N_4O_3S$: C 61.21, H 4.11, N 14.28, S 8.17. Found C 61.35, H 4.31, N 14.15, S 8.39.

*5.1.1.13. 2-(3-(2-(4-Chlorophenyl)-2-oxoethylthio)-5-ethyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**21**).* Yield, 72%; mp 170–171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (t, J = 7.6 Hz, 3H, $CH_2\underline{CH_3}$), 2.61 (dd, J = 7.6 Hz, J = 15.2 Hz, 2H, $\underline{CH_2}CH_3$), 4.82 (s, 2H, SCH_2), 7.42 (dd, J = 2.8 Hz, J = 8.8 Hz, 2H, ArH), 7.86–7.92 (m, 4H, ArH), 7.98–8.00 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.8, 17.8, 42.6, 124.8, 129.0, 129.1, 129.8, 133.0, 135.7, 140.5, 150.0, 156.9, 162.2, 191.5. ESI-MS: m/z = 449.26 $[M + Na]^+$, 427.23 $[M + 1]^+$. Anal. Calcd. for $C_{20}H_{15}ClN_4O_3S$: C 56.27, H 3.54, N 13.12, S 7.51. Found C 56.40, H 3.80, N 13.41, S 7.79.

*5.1.1.14. 2-(3-Ethyl-5-(2-oxo-2-p-tolylethylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**22**).* Yield, 69%; mp 130–131 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (t, J = 3.6 Hz, 3H, $CH_2\underline{CH_3}$), 2.38 (s, 3H, Ar–CH_3), 2.62 (dd, J = 7.6 Hz, J = 14.8 Hz, 2H, $\underline{CH_2}CH_3$), 4.86 (s, 2H, SCH_2), 7.24 (t, J = 4.0 Hz, 2H, ArH), 7.83 (d, J = 8.0 Hz, 2H, ArH), 7.90 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H, ArH), 7.99 (dd, J = 3.0 Hz, J = 5.6 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 10.9, 17.9, 21.7, 43.3, 124.9, 128.5, 129.2, 129.5, 132.3, 135.7, 145.2, 150.5, 156.9, 162.3, 192.3. ESI-MS: m/z = 430.09 $[M + Na]^+$, 407.13 $[M + 1]^+$. Anal. Calcd. for $C_{21}H_{18}N_4O_3S$: C 62.05, H 4.46, N 13.78, S 7.89. Found C 62.16, H 4.56, N 13.56, S 7.98.

*5.1.1.15. 2-(3-Ethyl-5-(methylthio)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**23**).* Yield, 73%; mp 82–83 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 (t, J = 3.6 Hz, 3H, $-CH_2\underline{CH_3}$), 2.61 (dd, J = 4.8 Hz, J = 7.2 Hz, 2H, $-\underline{CH_2}CH_3$), 2.65 (s, 3H, SCH_3), 7.91 (dd, J = 3.2 Hz, J = 5.6 Hz, 2H,

ArH), 8.01 (dd, $J = 3.2$ Hz, $J = 5.6$ Hz, 2H, ArH). ^{13}C NMR (150 MHz, DMSO-d_6): $\delta = 11.0, 15.7, 17.9, 124.9, 129.2, 135.7, 151.8, 156.7, 162.4$. ESI-MS: $m/z = 311.12$ $[M + Na]^+$, 289.20 $[M + 1]^+$. Anal. Calcd. for $C_{13}H_{12}N_4O_2S$: C 54.15, H 4.20, N 19.43, S 11.12. Found C 53.98, H 4.48, N 19.20, S 11.43.

*5.1.1.16. Ethyl 2-(4-(1,3-dioxoisoindolin-2-yl)-5-ethyl-4H-1,2,4-triazol-3-ylthio)acetate (**24**).* Yield, 69%; mp 110–111 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (t, $J = 7.2$ Hz, 3H, $OCH_2\underline{CH_3}$), 1.28 (t, $J = 7.6$ Hz, 3H, $-CH_2\underline{CH_3}$), 2.60 (dd, $J = 7.6$ Hz, $J = 15.2$ Hz, 2H, $-\underline{CH_2}CH_3$), 3.94 (s, 2H, SCH_2), 4.13 (dd, $J = 7.2$ Hz, $J = 14.2$ Hz, 2H, $O\underline{CH_2}CH_3$), 7.90–7.93 (m, 2H, ArH), 8.00 (dd, $J = 3.0$ Hz, $J = 5.4$ Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 10.6, 13.7, 17.7, 35.5, 61.9, 124.7, 128.9, 135.7, 149.5, 156.9, 162.1, 167.5$. ESI-MS: $m/z = 384.26$ $[M + Na]^+$, 361.21 $[M + 1]^+$. Anal. Calcd. for $C_{16}H_{16}N_4O_4S$: C, 53.32; H, 4.47; N, 15.55; S, 8.90. Found C 53.47, H 4.68, N 15.09, S 8.78.

*5.1.1.17. 2-(3-(Allylthio)-5-propyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**25**).* Yield, 64%; mp 125–127 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (t, $J = 7.4$ Hz, 3H, $-CH_2CH_2\underline{CH_3}$), 1.71 (dd, $J = 7.6$ Hz, $J = 15.2$ Hz, 2H, $-CH_2\underline{CH_2}CH_3$), 2.55 (t, $J = 7.8$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 3.69 (d, $J = 7.2$ Hz, 2H, SCH_2), 5.076–5.20 (m, 2H, $CH{=}\underline{CH_2}$), 5.78–5.87 (m, 1H, $\underline{CH}{=}CH_2$), 7.90–7.93 (m, 2H, ArH), 7.98–8.02 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.6, 19.9, 26.2, 36.8, 119.4, 124.9, 129.1, 131.9, 135.7, 150.1, 155.7, 162.4$. ESI-MS: $m/z = 351.31$ $[M + Na]^+$, 329.22 $[M+1]^+$. Anal. Calcd. for $C_{16}H_{16}N_4O_2S$: C 58.52, H 4.91, N 17.06, S 9.76. Found C 58.60, H 5.15, N 17.10, S 9.93.

*5.1.1.18. 2-(3-(Benzylthio)-5-propyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**26**).* Yield, 67%; mp 106–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (t, $J = 7.4$ Hz, 3H, $-CH_2CH_2\underline{CH_3}$), 1.70–1.76 (m, 2H, $-CH_2\underline{CH_2}CH_3$), 2.57 (t, $J = 7.8$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 4.36 (s, 2H, SCH_2), 7.20–7.28 (m, 5H, ArH), 7.89 (dd, $J = 3.2$ Hz, $J = 5.6$ Hz, 2H, ArH), 7.99 (dd, $J = 3.2$ Hz, $J = 5.6$ Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 13.6, 20.0, 26.1, 38.3, 124.9, 127.9, 128.6, 129.0, 129.1, 135.4, 135.7, 150.7, 155.7, 162.3$. ESI-MS: $m/z = 412.27$ $[M + Na]^+$, 379.46 $[M + 1]^+$. Anal. Calcd. for $C_{20}H_{18}N_4O_2S$: C 63.47, H 4.79, N 14.80, S 8.47. Found C 63.62, H 4.81, N 14.62, S 8.83.

*5.1.1.19. 2-(3-(2-(4-Chlorophenyl)-2-oxoethylthio)-5-propyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**27**).* Yield, 65%; mp 91–92 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, $J = 7.2$ Hz, 3H, $-CH_2CH_2\underline{CH_3}$), 1.69–1.75 (m, 2H, $-CH_2\underline{CH_2}CH_3$), 2.56 (t, $J = 7.6$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 4.83 (s, 2H, SCH_2), 7.42 (d, $J = 8.4$ Hz, 2H, ArH), 7.87–7.93 (m, 4H, ArH), 8.00 (dd, $J = 3.2$ Hz, $J = 5.6$ Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$ + DMSO-d_6): $\delta = 11.8, 18.1, 23.5, 38.1, 126.3, 127.0, 128.1, 128.4, 128.7, 130.0, 132.1, 133.2, 137.2, 148.1, 154.4, 165.3, 165.7, 190.3$. ESI-MS: $m/z = 441.09$ $[M + 1]^+$. Anal. Calcd. for $C_{21}H_{17}ClN_4O_3S$: C 57.21, H 3.89, N 12.71, S 7.27. Found C 57.49, H 3.67, N 12.59, S 7.60.

*5.1.1.20. 2-(3-(2-Oxo-2-p-tolylethylthio)-5-propyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**28**).* Yield, 70%; mp 98–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (t, $J = 7.2$ Hz, 3H, $CH_2CH_2\underline{CH_3}$), 1.70–1.76 (m, 2H, $CH_2\underline{CH_2}CH_3$), 2.39 (s, 3H, Ar–CH_3), 2.57 (t, $J = 7.2$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 4.88 (s, 2H, SCH_2), 7.25 (d, $J = 7.2$ Hz, 2H, ArH), 7.84 (d, $J = 8.4$ Hz, 2H, ArH), 7.91 (dd, $J = 2.8$ Hz, $J = 5.2$ Hz, 2H, ArH), 8.00 (dd, $J = 3.2$ Hz, $J = 5.6$ Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 13.6, 20.1, 21.7, 26.2, 43.3, 125.0, 128.6, 128.7, 129.3, 129.6, 132.3, 135.8, 145.2, 150.6, 155.9, 162.3, 192.4$. ESI-MS: $m/z = 434.26$ $[M + Na]^+$, 421.13 $[M + 1]^+$. Anal. Calcd. for $C_{22}H_{20}N_4O_3S$: C 62.84, H 4.79, N 13.32, S 7.63. Found C 62.75, H 4.59, N 13.12, S 7.82.

*5.1.1.21. 2-(3-(Prop-2-ynylthio)-5-propyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**29**).* Yield, 77%; mp 98–99 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (t, $J = 7.4$ Hz, 3H, $-CH_2CH_2\underline{CH_3}$), 1.74 (dd, $J = 7.6$ Hz, $J = 15.2$ Hz, 2H, $-CH_2\underline{CH_2}CH_3$), 2.21 (t, $J = 2.6$ Hz, 1H, ≡CH), 2.58 (t, $J = 7.6$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 3.82 (d, $J = 2.8$ Hz, 2H, SCH_2), 7.92 (dd, $J = 3.0$ Hz, $J = 5.4$ Hz, 2H, ArH), 8.02 (dd, $J = 3.0$ Hz, $J = 16.0$ Hz, 2H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6): $\delta = 13.5, 19.8, 20.4, 25.2, 74.8, 79.3, 128.1, 130.0, 130.5, 130.8, 132.1, 134.8, 149.0, 156.3, 167.1, 167.5$. ESI-MS: $m/z = 350.06$ $[M + Na]^+$, 327.13 $[M + 1]^+$. Anal. Calcd. for $C_{16}H_{14}N_4O_2S$: C 58.88, H 4.32, N 17.17, S 9.82. Found C 59.06, H 4.52, N 16.96, S 9.49.

*5.1.1.22. 2-(3-(2-Chlorobenzylthio)-5-propyl-4H-1,2,4-triazol-4-yl) isoindoline-1,3-dione (**30**).* Yield, 66%; mp 91–93 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.93 (t, $J = 7.6$ Hz, 3H, $-CH_2CH_2\underline{CH_3}$), 1.71 (m, 2H, $-CH_2\underline{CH_2}CH_3$), 2.54 (t, $J = 7.6$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 4.39 (s, 2H, SCH_2), 7.13–7.17 (m, 2H, ArH), 7.25 (t, $J = 4.8$ Hz, 1H, ArH), 7.38 (d, $J = 2.0$ Hz, 1H, ArH), 7.89 (dd, $J = 3.2$ Hz, $J = 5.2$ Hz, 2H, ArH), 7.97 (dd, $J = 2.8$ Hz, $J = 5.2$ Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 13.6, 19.9, 26.2, 36.2, 124.9, 127.1, 129.2, 129.4, 129.6, 131.5, 133.6, 134.2, 135.7, 150.3, 155.9, 162.3$. ESI-MS: $m/z = 415.26$ $[M + 1]^+$. Anal. Calcd. for $C_{20}H_{17}ClN_4O_2S$: C 58.18, H 4.15, N 13.57, S 7.77. Found C 58.46, H 4.38, N 13.43, S 7.98.

*5.1.1.23. 2-(3-(2-Oxo-2-phenylethylthio)-5-propyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**31**).* Yield, 64%; mp 96–97 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.93 (t, $J = 7.4$ Hz, 3H, $-CH_2CH_2\underline{CH_3}$), 1.67 (m, 2H, $-CH_2\underline{CH_2}CH_3$), 2.69 (t, $J = 7.0$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 4.92 (d, $J = 23.6$ Hz, 2H, SCH_2), 7.56 (t, $J = 7.8$ Hz, 3H, ArH), 7.69 (t, $J = 3.8$ Hz, 2H, ArH), 7.75 (dd, $J = 1.2$ Hz, $J = 7.6$ Hz, 1H, ArH), 7.94 (dd, $J = 0.8$ Hz, $J = 7.6$ Hz, 1H, ArH), 8.02 (t, $J = 4.2$ Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$ + DMSO-d_6): $\delta = 11.8, 18.1, 23.6, 38.3, 126.3, 126.6, 126.9, 128.2, 128.7, 128.8, 130.0, 131.8, 133.3, 133.6, 148.3, 154.4, 165.3, 165.8, 191.2$. ESI-MS: $m/z = 430.06$ $[M + Na]^+$, 407.13 $[M + 1]^+$. Anal. Calcd. for $C_{21}H_{18}N_4O_3S$: C 62.05, H 4.46, N 13.78, S 7.89. Found C 62.23, H 4.64, N 13.62, S 7.73.

*5.1.1.24. 2-(3-(Methylthio)-5-propyl-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**32**).* Yield, 67%; mp 77–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (t, $J = 7.2$ Hz, 3H, $-CH_2CH_2\underline{CH_3}$), 1.67–1.77 (m, 2H, $CH_2\underline{CH_2}CH_3$), 2.55 (t, $J = 7.2$ Hz, 2H, $-\underline{CH_2}CH_2CH_3$), 2.62 (s, 3H, SCH_3), 7.89–7.94 (m, 2H, ArH), 7.99–8.03 (m, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 13.5, 15.6, 19.9, 26.0, 124.8, 129.1, 135.7, 151.7, 155.6, 162.4$. ESI-MS: $m/z = 325.13$ $[M + Na]^+$, 303.32 $[M + 1]^+$. Anal. Calcd. for $C_{14}H_{14}N_4O_2S$: C 55.61, H 4.67, N 18.53, S 10.61. Found C 55.57, H 4.94, N 18.26, S 10.83.

*5.1.1.25. 2-(3-(Allylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**33**).* Yield, 73%; mp 72–74 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 3.83 (d, $J = 7.2$ Hz, 2H, $=CH_2$), 5.16 (dd, $J = 0.6$ Hz, $J = 9.8$ Hz, 1H, triazole–SCH_a), 5.28 (dd, $J = 1.2$ Hz, $J = 16.8$ Hz, 1H, triazole–SCH_b), 5.87–5.95 (m, 1H, CH=), 6.95 (s, 2H, ArH), 7.89 (dd, $J = 3.2$ Hz, $J = 5.6$ Hz, 2H, ArH), 7.96 (dd, $J = 3.2$ Hz, $J = 5.6$ Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 36.5, 56.0, 60.8, 105.0, 119.7, 119.8, 124.8, 129.1, 131.8, 135.8, 140.1, 152.0, 153.5, 155.2, 162.7$. ESI-MS: m/z (%): $m/z = 475.04$ $[M + Na]^+$, 453.08 $[M + 1]^+$. Anal. Calcd. for $C_{22}H_{20}N_4O_5S$: C 58.40, H 4.46, N 12.38, S, 7.09. Found C 58.15, H 4.74, N 12.50, S 6.83.

*5.1.1.26. 2-(3-(2-Chlorobenzylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**34**).* Yield, 62%; mp 76–77 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 3.70 (s, 6H, 2 × OCH_3), 3.79 (s, 3H, OCH_3), 4.53 (s, 2H, SCH_2), 6.79 (s, 2H, ArH), 7.18–7.31 (m, 3H, ArH), 7.50 (t, $J = 4.6$ Hz, 1H, ArH), 7.87 (dd, $J = 3.0$ Hz, $J = 6.0$ Hz, 2H, ArH), 7.94 (dd, $J = 3.0$ Hz, $J = 5.4$ Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 35.7, 56.0, 60.7, 104.9, 119.7, 124.7, 127.0, 128.9, 129.4, 129.5, 131.5, 133.4, 134.1, 135.8, 140.0, 152.2, 153.5, 155.2, 162.5$. ESI-

MS: *m*/*z* (%): *m*/*z* = 559.10 $[M + Na]^+$, 537.12 $[M + 1]^+$. Anal. Calcd. for $C_{26}H_{21}CN_4O_5S$: C 58.15, H 3.94, N 10.43, S, 5.97. Found C 58.45, H 4.23, N 10.67, S 6.16.

*5.1.1.27. 2-(3-(Prop-2-ynylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**35**).* Yield, 69%; mp 80–81 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 2.25 (s, 2H, ≡CH), 3.70 (s, 6H, 2 × OCH_3), 3.79 (s, 3H, OCH_3), 3.93 (s, 2H, SCH_2), 6.79 (s, 2H, ArH), 7.88–7.91 (m, 2H, ArH), 7.94–7.97 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* = 22.4, 55.9, 60.7, 104.9, 119.5, 124.8, 128.8, 135.9, 140.1, 151.0, 153.4, 155.4, 162.5. ESI-MS: *m*/*z* (%): *m*/*z* = 473.01 $[M + Na]^+$, 451.01 $[M + 1]^+$. Anal. Calcd. for $C_{22}H_{18}N_4O_5S$: C 58.66, H 4.03, N 12.44, S, 7.12. Found C 58.88, H 3.87, N 12.31, S 7.41.

*5.1.1.28. 2-(3-(2-(4-Chlorophenyl)-2-oxoethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**36**).* Yield, 78%; mp 79–80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 4.94 (s, 2H, SCH_2), 6.79 (s, 2H, ArH), 7.45 (d, *J* = 8.8 Hz, 2H, ArH), 7.88–7.97 (m, 6H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 42.2, 55.8, 60.6, 104.7, 119.4, 124.7, 128.7, 128.9, 129.7, 132.9, 135.7, 139.9, 140.4, 151.8, 153.3, 155.1, 162.4, 191.2. ESI-MS: *m*/*z* = 587.09 $[M + Na]^+$, 565.13 $[M + 1]^+$. Anal. Calcd. for $C_{27}H_{21}ClN_4O_6S$: C 57.40, H 3.75, N 9.92, S, 5.68. Found C 57.12, H 3.98, N 9.71, S 5.97.

*5.1.1.29. 2-(3-(2-Oxo-2-p-tolylethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**37**).* Yield, 69%; mp 197–198 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 2.40 (s, 3H, Ar–CH_3), 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 4.97 (s, 2H, CH_2), 6.79 (s, 2H, ArH), 7.26 (t, *J* = 8.0 Hz, 2H, ArH), 7.87–7.89 (m, 4H, ArH), 7.96 (dd, *J* = 3.2 Hz, *J* = 5.6 Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 21.7, 43.0, 56.0, 60.7, 104.9, 119.6, 124.8, 128.5, 129.0, 129.5, 132.2, 135.8, 140.0, 145.2, 152.3, 153.5, 155.3, 162.5, 192.1. ESI-MS: *m*/*z* = 567.20 $[M + Na]^+$, 545.21 $[M + 1]^+$. Anal. Calcd. for $C_{28}H_{24}N_4O_6S$: C 61.75, H 4.44, N 10.29, S 5.89. Found C 61.79, H 4.72, N 9.99, S 6.27.

*5.1.1.30. 2-(3-(Methylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**38**).* Yield, 72%; mp 77–79 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 2.70 (s, 3H, SCH_3), 3.71 (s, 6H, 2 × OCH_3), 3.79 (s, 3H, OCH_3), 6.79 (s, 2H, ArH), 7.89 (dd, *J* = 2.8 Hz, *J* = 5.2 Hz, 2H, ArH), 7.96 (dd, *J* = 3.2 Hz, *J* = 5.6 Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 15.4, 55.9, 60.7, 104.9, 119.7, 124.8, 128.9, 135.8, 140.0, 153.4, 153.5, 155.1, 162.6. ESI-MS: *m*/*z* (%): *m*/*z* = 449.02 $[M + Na]^+$, 427.06 $[M + 1]^+$. Anal. Calcd. for $C_{20}H_{18}N_4O_5S$: C 56.33, H 4.25, N 13.14, S 7.52. Found C 56.68, H 4.16, N 13.21, S 7.90.

*5.1.1.31. Ethyl 2-(4-(1,3-dioxoisoindolin-2-yl)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-3-ylthio)acetate (**39**).* Yield, 66%; mp 59–61 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 1.24 (t, *J* = 7.2 Hz, 2H, $OCH_2\underline{CH_3}$), 3.69 (d, *J* = 3.2 Hz, 6H, 2 × OCH_3), 3.78 (s, 3H, OCH_3), 4.05 (s, 2H, SCH_2), 4.17 (dd, *J* = 7.2 Hz, *J* = 14.2 Hz, 2H, $O\underline{CH_2}CH_3$), 6.77 (d, *J* = 2.0 Hz, 2H, ArH), 7.88 (dd, *J* = 2.8 Hz, *J* = 5.2 Hz, 2H, ArH), 7.96 (dd, *J* = 3.2 Hz, *J* = 5.6 Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 13.9, 35.3, 55.9, 60.7, 62.1, 104.9, 119.5, 124.8, 128.9, 135.8, 140.1, 151.4, 153.4, 155.2, 162.4, 167.5. ESI-MS: *m*/*z* = 521.12 $[M + Na]^+$, 499.09 $[M + 1]^+$. Anal. Calcd. for $C_{23}H_{22}N_4O_7S$: C 55.41, H 4.45, N 11.24, S 6.43. Found C 55.58, H 4.70, N 11.17, S 6.28.

*5.1.1.32. 2-(3-(Benzylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**40**).* Yield, 79%; mp 77–78 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.70 (s, 6H, 2 × OCH_3), 3.79 (s, 3H, OCH_3), 4.45 (s, 2H, SCH_2), 6.79 (s, 2H, ArH), 7.25 (t, *J* = 3.8 Hz, 3H, ArH), 7.31 (dd, *J* = 1.2 Hz, *J* = 7.6 Hz, 2H, ArH), 7.86 (dd, *J* = 3.0 Hz, *J* = 4.4 Hz, 2H, ArH), 7.93 (dd, *J* = 3.0 Hz, *J* = 4.4 Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 37.9, 56.0, 60.8, 104.9, 119.7, 124.7, 127.9, 128.6, 128.9, 129.0, 135.3, 135.8, 140.1, 152.5, 153.5, 155.1, 162.5. ESI-MS: *m*/*z* = 525.13 $[M + Na]^+$, 503.06 $[M + 1]^+$. Anal. Calcd. for $C_{26}H_{22}N_4O_5S$: C 62.14, H 4.41, N 11.15, S 6.38. Found C 62.40, H 4.68, N 10.87, S 6.71.

*5.1.1.33. 2-(3-(2-Oxo-2-phenylethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**41**).* Yield, 73%; mp 161–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 5.01 (s, 2H, SCH_2), 6.80 (s, 2H, ArH), 7.48 (t, *J* = 7.6 Hz, 2H, ArH), 7.61 (t, *J* = 7.4 Hz, 1H, ArH), 7.88–7.91 (m, 2H, ArH), 7.95–8.00 (m, 4H, ArH). ESI-MS: *m*/*z* = 553.3 $[M + Na]^+$, 531.4 $[M + 1]^+$. ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 42.6, 55.8, 60.6, 104.7, 119.4, 124.6, 128.2, 128.6, 128.7, 133.9, 134.5, 135.7, 140.0, 152.0, 153.3, 155.0, 162.4, 192.3. Anal. Calcd. for $C_{27}H_{22}N_4O_6S$: C 61.12, H 4.18, N 10.56, S 6.04. Found C 60.90, H 3.91, N 10.72, S 6.31.

*5.1.1.34. 2-(3-(2-(4-Methoxyphenyl)-2-oxoethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**42**).* Yield, 86%; mp 187–188 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 4.98 (s, 2H, SCH_2), 6.79 (s, 2H, Ar), 6.93 (d, *J* = 8.8 Hz, 2H, ArH), 7.89 (dd, *J* = 3.0 Hz, *J* = 5.4 Hz, 2H, ArH), 7.94–7.98 (m, 4H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 12.7, 55.4, 55.9, 60.7, 104.9, 114.0, 119.6, 124.8, 127.7, 128.9, 130.8, 135.8, 140.0, 152.3, 153.5, 155.1, 162.5, 164.2, 190.9. ESI-MS: *m*/*z* = 583.2 $[M + Na]^+$, 561.3 $[M+1]^+$. Anal. Calcd. for $C_{28}H_{24}N_4O_7S$: C 59.99, H 4.32, N 9.99, S 5.72. Found C 59.78, H 4.59, N 10.02, S 5.92.

*5.1.1.35. 2-(3-(2-Oxo-2-o-tolylethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**43**).* Yield, 69%; mp 170–172 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 2.49 (s, 3H, Ar–CH_3), 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 4.95 (s, 2H, SCH_2), 6.80 (s, 2H, ArH), 7.26–7.32 (m, 2H, ArH), 7.42 (t, *J* = 7.2 Hz, 1H, ArH), 7.83 (d, *J* = 7.6 Hz, 1H, ArH), 7.89 (dd, *J* = 3.2 Hz, *J* = 5.6 Hz, 2H, ArH), 7.95–7.98 (m, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 21.6, 44.9, 56.0, 60.8, 105.0, 119.6, 124.8, 126.0, 129.0, 129.6, 132.3, 132.7, 134.5, 135.8, 139.6, 140.1, 152.3, 153.5, 155.3, 162.5, 195.2. ESI-MS: *m*/*z* = 567.4 $[M + Na]^+$, 545.5 $[M + 1]^+$. Anal. Calcd. for $C_{28}H_{24}N_4O_6S$: C 61.75, H 4.44, N 10.29, S 5.89. Found C 61.50, H 4.27, N 10.05, S 6.20.

*5.1.1.36. 2-(3-(2-(4-Fluorophenyl)-2-oxoethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**44**).* Yield, 79%; mp 168–169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 4.96 (s, 2H, SCH_2), 6.79 (s, 2H, ArH), 7.15 (t, *J* = 8.6 Hz, 2H, ArH), 7.89 (dd, *J* = 3.2 Hz, *J* = 5.2 Hz, 2H, ArH), 7.97 (dd, *J* = 3.0 Hz, *J* = 5.4 Hz, 2H, ArH), 8.03 (dd, *J* = 5.4 Hz, *J* = 8.6 Hz, 2H, ArH). ^{13}C NMR (150 MHz, $CDCl_3$): *δ* = 42.4, 55.9, 60.6, 104.8, 115.8, 116.0, 119.4, 124.7, 128.8, 131.1, 131.2, 135.8, 140.0, 152.0, 153.4, 155.1, 162.4, 165.2, 166.9, 190.9. ESI-MS: *m*/*z* = 587.4 $[M + K]^+$, 549.5 $[M + 1]^+$. Anal. Calcd. for $C_{27}H_{21}FN_4O_6S$: C 59.12, H 3.86, N 10.21, S 5.85; Found C 59.06, H 3.99, N 10.03, S 6.04.

*5.1.1.37. 2-(3-(2-(4-Bromophenyl)-2-oxoethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**45**).* Yield, 76%; mp 189–190 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.71 (s, 6H, 2 × OCH_3), 3.80 (s, 3H, OCH_3), 4.96 (s, 2H, SCH_2), 6.79 (s, 2H, ArH), 7.63 (d, *J* = 8.4 Hz, 2H, ArH), 7.86–7.91 (m, 4H, ArH), 7.97 (dd, *J* = 3.0 Hz, *J* = 5.4 Hz, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* = 42.4, 56.0, 60.7, 104.9, 119.5, 124.8, 128.9, 129.4, 129.9, 132.4, 133.4, 135.8, 140.1, 151.9, 153.5, 155.3, 162.5, 191.6. ESI-MS: *m*/*z* = 611.3 $[M + Na]^+$, 609.2 $[M + 1]^+$. Anal. Calcd. for $C_{27}H_{21}BrN_4O_6S$: C 53.21, H 3.47, N 9.19, S 5.26. Found C 53.10, H 3.66, N 9.32, S 5.48.

*5.1.1.38. 2-(3-(2-(3,4-Difluorophenyl)-2-oxoethylthio)-5-(3,4,5-trimethoxyphenyl)-4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (**46**).* Yield, 64%; mp 166–168 °C; ^{1}H NMR (400 MHz, $CDCl_3$) *δ*: 3.70 (s, 6H, 2 × OCH_3), 3.79 (s, 3H, OCH_3), 4.89 (s, 2H, SCH_2), 6.77

(s, 2H, ArH), 7.26 (t, $J = 8.0$ Hz, 1H, ArH), 7.79–7.84 (m, 2H, ArH), 7.88–7.91 (m, 2H, ArH), 7.94–7.97 (m, 2H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 41.9, 55.9, 60.6, 104.8, 117.6, 117.7, 117.8, 119.3, 124.7, 125.6, 125.7, 128.8, 131.7, 131.8, 135.8, 140.0, 149.4, 149.5, 151.1, 151.2, 151.7, 153.1, 153.2, 153.4, 154.8, 154.9, 155.2, 162.4, 190.1. ESI-MS: $m/z = 589.3$ $[M + Na]^+$, 567.4 $[M + 1]^+$. Anal. Calcd. for $C_{27}H_{20}F_2N_4O_6S$: C 57.24, H 3.56, N 9.89, S 5.66. Found C 57.51, H 3.79, N 10.06, S 5.91.

5.2. Crystallographic analysis

Colorless blocks of **37** were mounted on a quartz fiber. Cell dimensions and intensities were measured at 293 K on a Bruker SMART CCD area detector diffractometer with graphite monochromated Mo Kα radiation ($\lambda = 0.71073$ Å); $\theta_{max} = 27.48$; 13,548 measured reflections; 6130 independent reflections ($R_{int} = 0.0514$) of which 3868 had $|F_o| > 2|F_o|$. Data were corrected for Lorentz and polarization effects and for absorption ($T_{min} = 0.4415$; $T_{max} = 0.6353$). The structure was solved by direct methods using SHELXS-97 [31]; all other calculations were performed with Bruker SAINT System and Bruker SMART programs [32]. Full-matrix least-squares refinement based on F^2 using the weight of $1/[\sigma^2(F_o^2) + (0.1230P)^2 + 8.0218P]$ gave final values of $R = 0.067$, $\omega R = 0.206$, and GOF(F) = 0.760 for 356 variables and 5896 contributing reflections. Maximum shift/error = 0.000(3), max/min residual electron density = 0.21/−0.16 e Å^{-3}. Hydrogen atoms were observed and refined with a fixed value of their isotropic displacement parameter.

Crystallographic data for the structure **37** has been deposited with the Cambridge Crystallographic Data Center (CCDC) under the number 866294. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk or at www.ccdc.cam.ac.uk].

5.3. Pharmacology evaluation

5.3.1. Antifungal activity

The fungicidal activities of the compounds **9**–**46** were tested *in vitro* against *C. musae*, *F. oxysporum* f. sp. Niveum, *C. gloeosporioides* Penz., *B. theobromae*, *F. oxysporum* f. sp. Cubense and *P. oryzae* Cav. and their relative inhibitory ratio (%) had been determined by using the mycelium growth rate method [33,34]. Briefly, each tested sample was dissolved in DMF, added to the thawed potato glucose agar culture medium (PDA) under 50 °C, mixed to homogeneity and transferred to sterile Petri dishes to solidify. For primary screenings, compounds were used at a concentration of 150 mg/L. At the same time, Triadimefon (a commercial fungicide) and 1 equiv of DMF were used as drug and blank controls, respectively. After the dishes were cooled, the solidified plates were incubated with 5 mm mycelium disk, inverted, and incubated at 26 ± 2°C for 48 h. Three replicates of each test were carried out. The mycelial elongation radius (mm) of fungi settlements was measured after 48 h of culture. The growth inhibition rates were calculated with the following equation:

$I = [(C - T)/C] \times 100\%$ where I is the growth inhibition rate (%), C is the average diameter of mycelia in the blank test, and T is the average diameter of mycelia in the presence of those compounds. The inhibition ratio of those compounds at the dose of 150 mg/L was summarized in Table 2.

5.3.2. Antitumor activity

The antitumor activities of compounds **9**–**46** were evaluated with HepG2, A549, PC-3M, and MKN45 cell lines by the standard MTT assay [35] *in vitro*. The cancer cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Cells were passaged at 70–80% confluence, about twice a week by trypsinization.

Exponentially growing cells were plated in 96-well plates (1×10^4 cells/well for HepG2 cells, 1.6×10^4 cells/well for A549, PC-3M and MKN45 cells) and incubated at 37 °C for 24 h for attachment. Test compounds were prepared by dissolving in dimethyl sulfoxide (DMSO) at 10 mM and diluted with the medium. Then, culture medium was changed, and cells grew in medium with the test compounds. DMSO (0.1%) was used as negative control. Cells were incubated at 37 °C for 48 h. Then the medium was replaced with MTT solution (5 mg/ml, 100 μL) followed by incubation for another 3 h. The medium was then aspirated and formazan crystals were dissolved in DMSO (100 μL) for about 10 min. The absorbance at 570 nm (Abs) of the suspension was measured by an enzyme-linked immunosorbent assay (ELISA) reader. The inhibition percentage was calculated using the following formula: % inhibition = $(Abs_{control} - Abs_{compound})/Abs_{control} \times 100\%$. The IC_{50} values of the test compounds and Fluorouracil were measured by treating cells with drugs of varying concentrations, and analyzed by use of the prism statistical package (GraphPad Software, San Diego, CA, U.S.A.).

5.3.3. Flow-activating cell sorting analysis (FACS)

The effect of compound **37** on cell cycle phase distribution of human hepatoma HepG2 was assessed using flow cytometry. When the cells were grown to about 70% confluence in 6-well microplates and treated with compound **37** at given concentrations (4, 8, 16 μM). After 24 h, cells were harvested by trypsinization, washed in PBS, and fixed in 70% ice cold (4 °C) ethanol overnight. Then, they were washed with PBS, incubated with RNase (50 μg/mL final concentration) at 37 °C for 30 min, stained with propidium iodide (50 μg/mL final concentration), and analyzed by flow cytometry (Beckman Coulter).

Acknowledgments

This work was supported by National Natural Science Foundation of China (21102069 and 81101732), Natural Science Foundation of Guangdong Province (10451051501004725), and the project of science and technology new star in Zhujiang Guangzhou city (No. 2012J2200051).

Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2012.06.041.

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