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Synthesis, antitumor activity and mechanism of action of novel 1,3-thiazole derivatives containing hydrazide-hydrazone and carboxamide moiety

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Abstract: A series of novel 2,4,5-trisubstituted 1,3-thiazole derivatives containing hydrazide-hydrazine, and carboxamide moiety including 46 compounds **T** were synthesized, and evaluated for their antitumor activity *in vitro* against a panel of five human cancer cell lines. Eighteen title compounds **T** displayed higher inhibitory activity than that of 5-Fu against MCF-7, HepG2, BGC-823, Hela, and A549 cell lines. Especially, **T1**, **T26** and **T38** exhibit best cytotoxic activity with IC_{50} values of 2.21 μg/mL, 1.67 μg/mL and 1.11 μg/mL, against MCF-7, BCG-823, and HepG2 cell lines, respectively. These results suggested that the combination of 1,3-thiazole, hydrazide-hydrazone, and carboxamide moiety was much favorable to cytotoxicity activity. Furthermore, the flow cytometry analysis revealed that compounds **T1** and **T38** could induce apoptosis in HepG2 cells, and it was confirmed **T38** led the induction of cell apoptosis by S cell-cycle arrest.

Keywords: 1, 3-thiazole, carboxamide, hydrazide-hydrazone, antitumor activity, apoptosis.

1,3-thiazole scaffold have attracted considerable attentions for decades due to their remarkable spectrum of biological activity, such as distinctive antifungal,[1,2] antimicrobial,[3-6] antitubercular[7,8] activity. Moreover, several 1,3-thiazole scaffolds were documented to contribute to a variety of antineoplastic potentials being employed as anticancer,[9-13] anti-angiogenic,[14] antiproliferative,[15,16] tubulin polymerization inhibiting,[17] and cytotoxic[18,19] agents. Recently, it was reported that the chemotherapeutic activity of 1,3-thiazoles derivatives are augmented by the discovery of tiazofurin (natural antineoplastic antibiotic) (**Fig 1, A**),[20] and S3U937 (**Fig 1, B**),[21] which exhibited potential antitumor activity against various cancer types.[22] The documented DNA minor groove binding property of 1,3-thiazole-netropsin, and thiazotropsin A (**Fig 1, C** and **D**) are also payed attention.[23,24] Another 1,3-thiazole derivative dasatinib (**Fig 1, E**) was reported to possess potential tyrosine kinase inhibitory activity, and proved to be efficient in the treatment of imatinib resistant mutants.[25] Most of antitumor agents in **Fig 1** are distinguished by containing substituted 1,3-thiazole ring, and carboxamide groups. Furthermore, recently literatures revealed that the hydrazide-hydrazone (-CO-NH-N=CH-) moiety as a pharmacophore played a significant antitumor activity in some antitumor agents.[26-29] We noticed that some reported pharmacologically active 1,3-thiazole derivatives exhibited a narrow activity spectrum,[23,30] and their preparation method is not so effective, and easily due to lower yields, long reaction time or under microwave irradiation.[31] It seemed to be reasonable to improve the synthetic method, and find new 1,3-thiazole derivatives with broad-spectrum antitumor activity.

In view of the above mentioned facts, we report herein the synthesis, *in vitro* cytotoxic evaluation of some novel 1,3-thiazole derivatives based on pharmacophores hybridization. The corresponding carboxamide moiety, and hydrazide-hydrazone pharmacophore were integrated

with structural unit of 1,3-thiazole into the novel structure **T** as shown in **Fig 2**, it was thought that these novel skeleton **T** bearing three pharmacological groups would be well synergistic antitumor effects, and broad-spectrum antitumor activity. Therefore a series of novel 2-(substituted(*N*-(methylcarbamoyl) benzamide))-4-substituted-5-(substituted(*N*-benzylideneacetohydrazide))1,3-thiazole derivatives **T** were designed and synthesized. It was expected that broad-spectrum antitumor activity and higher synergistic effect could be achieved by chemical modifications of R^1, R^2, and R^3 at the skeleton of 2,4,5-trisubstituted 1,3-thiazole derivatives **T**. We are here to report the synthesis, and evaluated for their potential cytotoxic effects against human cancer cell lines of **T**. The possible mechanism of antitumor activity for the highly potential compounds were also examined and discussed.

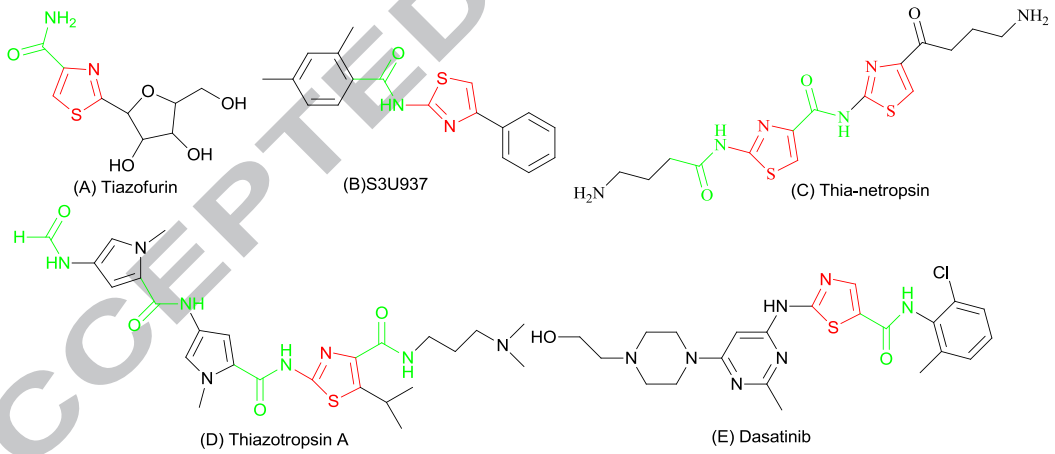


Fig 1. Antitumor agents containing 1,3-thiazole ring and carboxamide moiety.

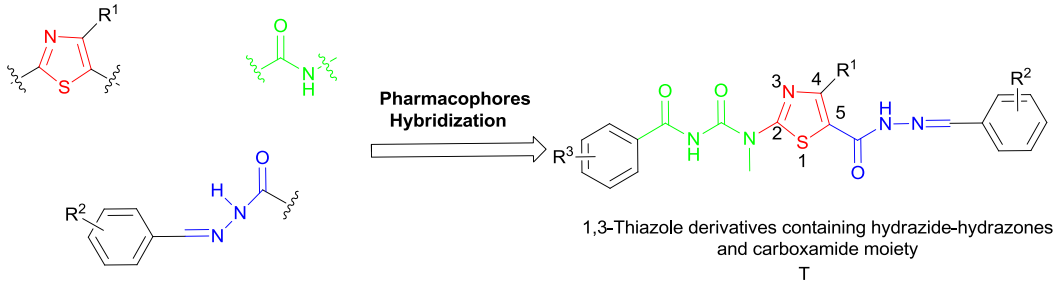
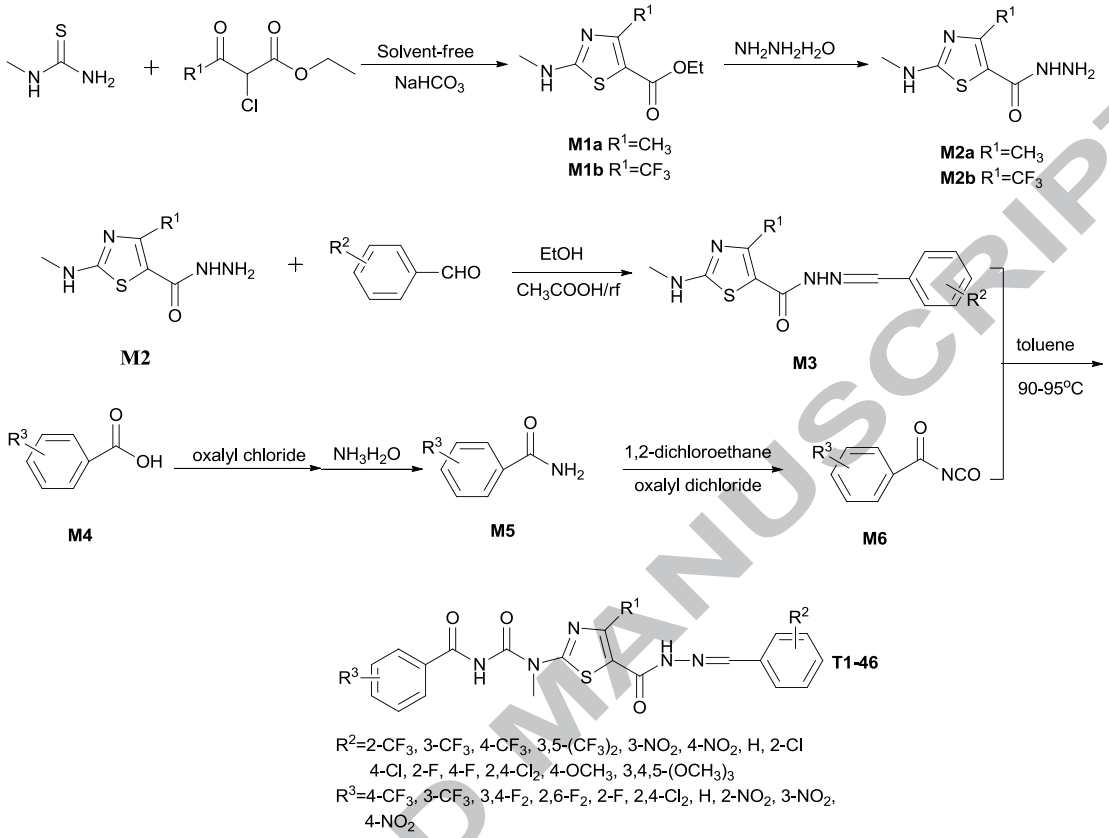


Fig 2. Design of **T** as antitumor agents.

The synthetic route of title compounds **T** is shown in **Scheme 1**.



Scheme 1. Synthesis of title compounds **T1-46**

The key skeleton of 1,3-thiazole ring was constructed by preparing ethyl 4-methyl(or 4-trifluoro methyl)-2-(methylamino)1,3-thiazole-5-carboxylate **M1** which is a key intermediate for the synthesis of title compounds **T**. The preparation of **M1** has been reported by several literatures.[31,32] However these methods are not effective, and need solvent, long reaction time or under microwave irradiation. In our study, we could prepare **M1a** and **M1b** with 98% and 90% yield just in 20 minutes under solvent-free condition. 4-Methyl(or trifluoromethyl)-2-methylamino-1,3-thiazole-5-carbohydrazide **M2** was prepared by hydrazinolysis of **M1** using hydrazine hydrate according to the method in literatures.[33-37] Then **M2** further reacted with substituted benzaldehyde via the nucleophilic addition-eliminate reaction to produce key intermediate **M3**, constructing the basic skeleton of 1,3-thiazole containing hydrazide-hydrazone moiety. The intermediate **M6** could

be easily obtained according to the method in literatures.[33-37] Subsequently, intermediate **M3** reacted with substituted benzoylisocyanates **M6** by the nucleophilic addition-eliminate reaction to produce title compounds **T1-46**.

In order to find novel 2,4,5-trisubstituted 1,3-thiazole derivatives **T** with higher antitumor activity, various substituents, especially, fluoro groups as R^1, R^2, and R^3 were introduced into the parent structure of **M1**, **M3,** and title compounds **T** to examine their effect on antitumor activity against human breast cancer (MCF-7), hepatocellular liver carcinoma (HepG2), gastric cancer (BGC-823), cervical carcinoma (Hela), and lung cancer (A549) cell lines by MTT method.[38] 5-Fluorouracil (5-Fu) was chosen as positive control due to its availability, and widespread using. The inhibitory potency against five human cancer cell lines is showed in **Fig 3**. On the basis of preliminary bioassay, the IC_{50} values of **T1-38, M1a-b** and **M3a-h** against five human cancer cell lines were further tested to compare the effect of R^1, R^2, and R^3 on cytotoxicity. The structure and cytotoxicity (IC_{50}) of **T1-38** and **M3a-h** are listed in **Table 1**.

As shown in **Fig 3A**, **M1** with the skeleton of 2-methylamino-1,3-thiazole-5-carboxylate only showed <10% inhibitory potency against all tested cell lines. Although the cytotoxicity activity could be enhanced by intruding hydrazide-hydrazone moiety into the skeleton of 1,3-thiazole **M1** to produce **M3**, however **M3** still showed <30% inhibitory potency. It was found that all title compounds **T** except **T3** exhibited much higher inhibitory potency against five human cancer cell lines than that of corresponding **M3** by the combination of 1,3-thiazole, hydrazide-hydrazone, and carboxamide pharmacophore. Most title compounds **T** showed noticeable inhibitory activity against BCG-823, and better than 5-Fu. **T38** exhibited best selectively cytotoxic activity with 90% inhibitory potency against HepG2 and its activity higher than 5-Fu. Above results indicated that

the combination of three pharmacophore including 1,3-thiazole, hydrazide-hydrazone, and carboxamide was much favorable to cytotoxicity activity.

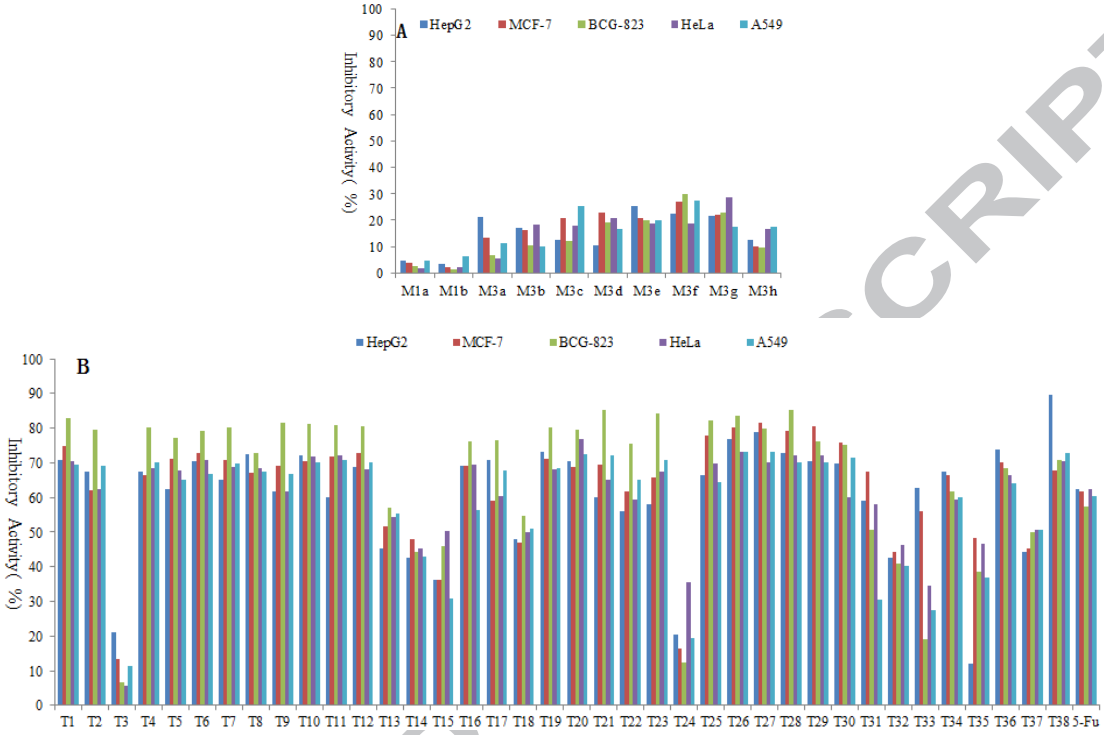
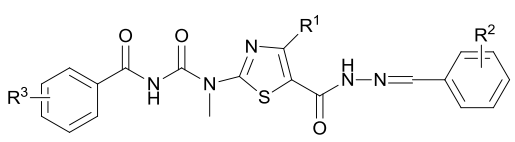


Fig 3. Inhibitory potency (%) of **T1~38, M1** and **M3** against five human cancer cell lines at 20μg/mL.

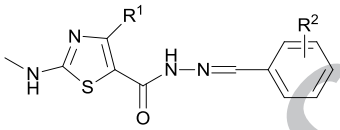
The data in **Table 1** showed that all **T1-38** exhibited moderate to excellent cytotoxic activity against five tested human cancer cell lines with the IC_{50} values ranging from 1.11 to 65.46 μg/mL. All the title compounds **T**, which combining of 1,3-thiazole, hydrazide-hydrazone, and carboxamide pharmacophore, exhibited much higher inhibitory potency against five human cancer cell lines than that of 1,3-thiazole1 **M1,** and 1,3-thiazole containing hydrazide-hydrazone moiety **M3**, except **T3** and **T24** . Most of **T** displayed better or comparable inhibitory activity to 5-Fu among thirty-eight tested compounds. It was found that eighteen compounds including **T1**, **T2**, **T4**, **T6~8**, **T10~12**, **T16**, **T19**, **T20**, **T26~30** and **T38** displayed higher inhibitory activity against all five human cancer cell than that of 5-Fu. Moreover, nine compounds including **T5**, **T9**, **T16**, **T17**, **T21~23**, **T25** and **T36** exhibited better inhibitory activity against two or four human cancer

cell than that of 5-Fu. The results confirmed our hypothesis: incorporation of the hydrazide-hydrazone, and carboxamide moiety into 1,3-thiazole scaffold could extend the antitumor activity spectrum compared with these reported 1,3-thiazole.[30]

Table 1. Structure and cytotoxic activity of **T1-38** and **M3** against various cell lines



T



M3

Compd	Substituents			*In vitro* cytotoxicity IC_{50} (μg/mL)[a]					
	R^1	R^2	R^3	MCF-7[b]	HeLa[b]	Hep-G2[b]	BCG-823[b]	A549[b]	HL-7702[b]
T1	CF_3	2- CF_3	4-CF_3	2.21±0.9	3.76±0.17	3.64±0.26	2.53±0.82	6.68±0.42	12.43±3.09
T2	CF_3	3- CF_3	4-CF_3	8.95±1.67	6.2±0.76	7.95±0.61	1.84±0.44	6.66±0.58	17.99±0.95
T3	CF_3	4- CF_3	4-CF_3	>100	>100	>100	>100	>100	NT[d]
T4	CF_3	3,5-$(CF_3)_2$	4-CF_3	7.37±1.67	5.78±1.47	8.35±1.52	1.77±0.1	5.90±0.53	NT[d]
T5	CF_3	2- CF_3	3- CF_3	7.69±1.72	7.37±0.41	10.41±0.38	7.29±2.31	12.09+1.44	NT[d]
T6	CF_3	3- CF_3	3- CF_3	3.70±0.96	4.81±0.75	5.79±1.23	4.60±1.61	8.16±0.65	NT[d]
T7	CF_3	4- CF_3	3- CF_3	6.50±1.51	7.35±1.36	7.71±0.87	4.22±0.66	6.20±0.26	NT[d]
T8	CF_3	3,5-$(CF_3)_2$	3- CF_3	6.08±0.29	5.34±1.62	5.38±0.88	5.23±1.96	8.20±0.78	NT[d]
T9	CF_3	2- CF_3	3,4-F_2	8.21±1.64	9.24±1.28	10.38+2.11	3.66±0.47	6.96±0.34	NT[d]
T10	CF_3	3- CF_3	3,4-F_2	4.15±0.9	4.21±0.70	5.60±0.67	4.46±0.63	6.52±1.01	23.47±1.01
T11	CF_3	4- CF_3	3,4-F_2	5.93±1.11	5.10±1.16	6.26±0.76	3.85±0.70	6.07±0.75	13.24±1.66
T12	CF_3	3,5-$(CF_3)_2$	3,4-F_2	2.61±0.6	4.45±0.62	5.47±1.03	3.51±0.91	5.47±1.07	20.06±0.39
T13	CF_3	2- CF_3	2,6-F_2	20.43±1.28	19.56±1.98	26.09±4.51	16.95±0.92	17.88+2.88	NT[d]
T14	CF_3	3- CF_3	2,6-F_2	21.78±5.92	21.59±2.01	27.87+6.91	25.86±0.93	27.58+1.68	NT[d]
T15	CF_3	4- CF_3	2,6-F_2	35.33±6.29	30.59±3.12	49.59±6.35	34.88±6.83	40.12+3.56	NT[d]
T16	CF_3	3,5-$(CF_3)_2$	2,6-F_2	6.85±1.12	5.62±1.02	6.28±2.37	5.28±0.52	17.3+1.79	NT[d]
T17	CF_3	2- CF_3	2-F	12.23±1.44	10.12±0.98	5.39±1.56	4.67±1.12	6.32±1.23	NT[d]
T18	CF_3	3- CF_3	2-F	20.54±5.73	19.12±1.32	20.34±2.42	16.34±1.45	19.45+2.32	NT[d]
T19	CF_3	4- CF_3	2-F	4.98±1.1	3.97±0.98	4.56±0.62	3.87±1.02	5.68±2.31	NT[d]
T20	CF_3	3,5-$(CF_3)_2$	2-F	6.16±0.46	4.98±1.11	4.47±1.23	4.23±0.78	5.43±1.09	NT[d]
T21	CF_3	2- CF_3	2,4-Cl_2	9.12±2.69	9.00±0.46	10.65+0.86	5.73±1.91	15.31+3.69	NT[d]
T22	CF_3	3- CF_3	2,4-Cl_2	10.7±2.22	9.6±1.62	11.18±1.03	6.11±1.65	10.55+2.56	NT[d]
T23	CF_3	4- CF_3	2,4-Cl_2	8.42±2.12	7.99±0.66	12.41±0.56	3.08±0.54	9.40±0.82	NT[d]
T24	CF_3	3,5-$(CF_3)_2$	2,4-Cl_2	>100	>100	>100	>100	>100	NT[d]
T25	CF_3	2- CF_3	2,6-Cl_2	6.24±3.1	5.23±0.34	8.95±1.66	4.12±0.65	12.00+1.21	NT[d]
T26	CF_3	3- CF_3	4-NO_2	4.39±0.16	4.12±0.65	4.73±0.91	1.67±0.43	5.42±0.53	NT[d]
T27	CF_3	2- CF_3	3-NO_2	4.73±0.93	4.15±0.76	4.80±1.43	3.97±0.61	5.45±0.89	NT[d]
T28	CF_3	4-NO_2	3,4-F_2	4.81±0.69	5.08±0.22	6.23±1.45	2.99±0.45	5.78±0.87	NT[d]
T29	CF_3	4-NO_2	2,4-Cl_2	6.74±1.20	5.12±0.78	6.85±1.70	2.13±0.45	6.44±1.09	NT[d]

T30	CF_3	3-NO_2	2,4-Cl_2	7.51±0.38	6.56±0.45	7.98±0.52	4.87±0.79	6.34±0.78	NT[d]
T31	CF_3	3-NO_2	H	15.4±3.37	11.23±1.56	14.3±3.57	13.6±1.66	20.67+2.12	NT[d]
T32	Me	3- CF_3	2,6-F_2	28.15±5.21	26.78±2.11	31.57±5.17	32.65±3.12	30.32±1.89	NT[d]
T33	Me	4- CF_3	2,6-F_2	30.57±6.67	40.55±6.21	>100	65.46±7.35	60.45+5.33	NT[d]
T34	Me	3,5-$(CF_3)_2$	2,6-F_2	11.63±2.61	12.21±1.11	11.36±1.50	14.08±1.60	12.98+1.01	NT[d]
T35	Me	4- CF_3	2,4-Cl_2	24.52±3.57	20.32±2.34	>100	37.99±8.84	30.21+2.12	NT[d]
T36	CF_3	4-NO_2	4-NO_2	9.24±1.87	8.42±1.12	12.34_1.70	7.45±0.98	8.98±1.23	NT[d]
T37	CF_3	4-NO_2	2-NO_2	21.23±9.55	18.45±2.32	25.24±2.37	15.67±2.43	17.65+3.89	NT[d]
T38	Me	2- CF_3	2,6-F_2	7.34±2.00	6.51±1.23	1.11±0.26	7.88±2.74	5.43±1.56	9.43±0.74
M3-a	Me	2- CF_3	-	>100	>100	>100	>100	>100	NT[d]
M3-b	Me	3- CF_3	-	>100	>100	>100	>100	>100	NT[d]
M3-c	Me	4- CF_3	-	>100	>100	>100	>100	>100	NT[d]
M3-d	Me	3,5-$(CF_3)_2$	-	>100	>100	>100	>100	>100	NT[d]
M3-e	CF_3	2- CF_3	-	>100	>100	>100	>100	>100	NT[d]
M3-f	CF_3	3- CF_3	-	>100	>100	>100	>100	>100	NT[d]
M3-g	CF_3	4- CF_3	-	>100	>100	>100	>100	>100	NT[d]
M3-h	CF_3	3,5-$(CF_3)_2$	-	>100	>100	>100	>100	>100	NT[d]
5-Fu[c]	-	-	-	10.8±1.02	10.72±1.79	9.23±0.67	10.89±0.93	10.5±0.78	14.53±1.97

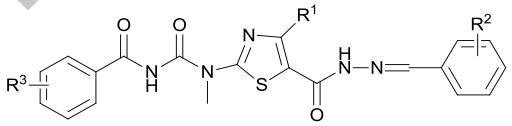
[a]IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control (72 h after treatment). [b]MCF-7: human breast cancer cell lines; HepG2: hepatocellular liver carcinoma cell lines; BGC-823: gastric cancer cell lines; Hela: cervical carcinoma cell lines; A549:lung cancer cell lines; HL-7702: Human normal liver cell lines. [c]5-Fu: 5-Fluorouracil, used as a positive control. [d]NT: Not Test.

As shown in **Table 1**. The cytotoxic activity could be remarkably enhanced by optimizing the structure of R^1, R^2, and R^3. On the basis of 2,4,5-trisubstituted 1,3-thiazole ring, the introduction of a trifluoromethyl group as R^1 has a favourable effect on cytotoxic activity. Such as **T14~16** and **T23** with CF_3 as R^1 exhibited higher cytotoxic activity than that of corresponding compounds **T32~35** with CH_3 as R^1. However, when 2-CF_3 as R^2, and 2,6-F_2 as R^3 were kept same, **T38** with Me as R^1 had much higher cytotoxic activity than **T13** with CF_3 as R^1. In addition **T38** showed higher cytotoxic activity against all five human cancer cells than that of 5-Fu and it was found to be the most potent compound with specificity against HepG2 (IC_{50}=1.11 μg/mL). These results

showed that the antitumor activity of **T** was not only depends on R^1, but also depends on R^2, and R^3. The structures of R^3, and R^2 are critical for antitumor activity.

The screening results also testified that the antitumor activity was highly dependent upon the structure and position of substituent R^2. **T38** was found to be the most potent compound with IC_{50} values 1.11 μg/mL against HepG2. Thus the structure of **T38** was chosen for further modification to explore the effect of R^2 on cytotoxic activity against HepG2. On the basis of the structure of **T38**, Me as R^1, 2,6-F_2 as R^3 were kept same, H, 2-Cl, 4-Cl, 2-F, 4-F, 2,4-Cl_2, 3,4,5-$(OCH_3)_3$ or 4-OCH_3 as R^2 were introduced. The results were listed in **Table 2**. It was found that the cytotoxic activity of compounds (such as **T39~46**) against HepG2 was very weak or almost lost (IC_{50}=22.66~70.1 μg/mL or > 100 μg/mL) when 2-CF_3 as R^2 was changed. This indicated that 2-CF_3 as R^2 was beneficial for antitumor activity. Hence, *para*, *ortho*, *meta* trifluoromethyl or 3,5-ditrifluoromethyl as R^2 was kept, further modification was focused on the substituents of R^3.

Table 2. Cytotoxic activity of **T38-46** against HepG2



Compd.	Substituents			*In vitro* cytotoxicity
	R^1	R^2	R^3	IC_{50} (μg/mL)[a]
T38	Me	2- CF_3	2,6-F_2	1.1±0.26
T39	Me	H	2,6-F_2	54.28
T40	Me	2-Cl	2,6-F_2	>100
T41	Me	4-Cl	2,6-F_2	>100
T42	Me	2-F	2,6-F_2	>100
T43	Me	4-F	2,6-F_2	>100
T44	Me	2,4-Cl_2	2,6-F_2	70.01
T45	Me	3,4,5-$(OCH_3)_3$	2,6-F_2	>100
T46	Me	4-OCH_3	2,6-F_2	22.66
5-Fu[b]				9.23±0.67

[a]IC_{50} is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control

(72 h after treatment). [b]5-Fu: 5-Fluorouracil, used as a positive control

SAR analyses showed that substituent R^3 played a very important role in the antitumor activity of **T**. When CF_3 as R^1 was kept, the substitutions of 3-CF_3, 4-CF_3 (except 4-CF_3 as R^2), 2-F (except 2-CF_3 or 3-CF_3 as R^2) or 3,4-F_2 as R^3 was very promotive for antitumor activity. For example, **T1**, **T2**, **T4**, **T6~8**, **T10~12**, **T19** and **T20** displayed higher antitumor activity against all five human cancer cell than that of positive control 5-Fu. Whereas, when CF_3 as R^1, the introduction of 2,6-F_2 as R^3, such as **T13~15** led to a sharp decrease in antitumor activity, irrespective of *ortho*, *para*, or *meta* trifluoromethyl as R^2, respectively. We noticed that when CF_3 as R^1 compound with 2,6-F_2 as R^3, 3,5-$(CF_3)_2$ as R^2 or 2,6-Cl_2 as R^3, 2-CF_3 as R^2 the antitumor activity was effectively increased, such as, **T16** and **T25** showed better inhibition than 5-Fu against the four cell lines except A549 and much better than that of **T13**, **T14** and **T15**. Above observation indicated that the cytotoxic activity also depends on reasonable combination of R^2 and R^3.

These interesting results indicated that improvement of antitumor activity required a reasonable combination of R^1, R^2, and R^3. **T1** (R^1=CF_3, R^2=2-CF_3, R^3=4-CF_3), **T26** (R^1=CF_3, R^2=3-CF_3, R^3=4-NO_2) and **T38** (R^1=Me, R^2=2-CF_3, R^3=2,6-F_2) were found to be the most promising compounds with wide spectrum, and good activity against five tested human cancer cell lines (IC_{50}=1.11~7.88 μg/mL), their activity were much better than that of positive control 5-Fu (IC_{50}=9.23~10.89 μg/mL). They might be used to develop novel lead scaffolds for potential anticancer agents. In addition, some activity compounds against cancer cell lines were selected for testing their toxicity against human normal liver cell (HL-7702). The data in **Table 1** showed that **T10** (IC_{50}=23.47

μg/mL) and **T12** (IC_{50}=20.06 μg/mL) showed weak toxicity on normal cell line compared with 5-Fu (IC_{50}=14.53 μg/mL).

To determine whether the observed cell death induced by 1,3-thiazole derivatives (**T**) was due to apoptosis or necrosis, the interaction of HepG2 cells with **T1** and **T38** was further investigated using an AnnexinV-FITC/PI (Propidium iodide) dual staining assay[39]. All data obtained in this study are presented in **Fig 4**, as a percentages of intact cells at lower-left quadrant, FITC(－)/PI(－); early apoptotic cells at lower-right quadrant, FITC(+)/PI(－); late apoptotic or necrotic cells at upper-right quadrant, FITC(+)/PI(+); necrotic cells at upper-left quadrant, FITC(−)/PI(+). The apoptotic cell rates were determined for HepG2 cells that were treated with **T1** and **T38** at the concentration of 1.82 μg/mL (0.5IC_{50} value), 3.64 μg/mL (IC_{50} value) and 0. 55 μg/mL (0.5IC_{50} value), IC_{50} 1.1 μg/mL (IC_{50} value) for 24h, respectively. It can be observed from **Fig 4F** that **T1** and **T38** significantly caused the early apoptosis in terms of FITC(+)PI(-) staining. **T1** displayed 7.8% and 18.6% of apoptosis at 0.5 IC_{50} and IC_{50} concentrations, respectively. **T38** displayed 7.0% and 16.5% of apoptosis at 0.5 IC_{50} and IC_{50} concentrations, respectively. However, the control (0.1% DMSO) displayed 2.5% of apoptosis. The results showed that **T1** and **T38** induced cell death in HepG2 cells at least partly (initially) by apoptosis.

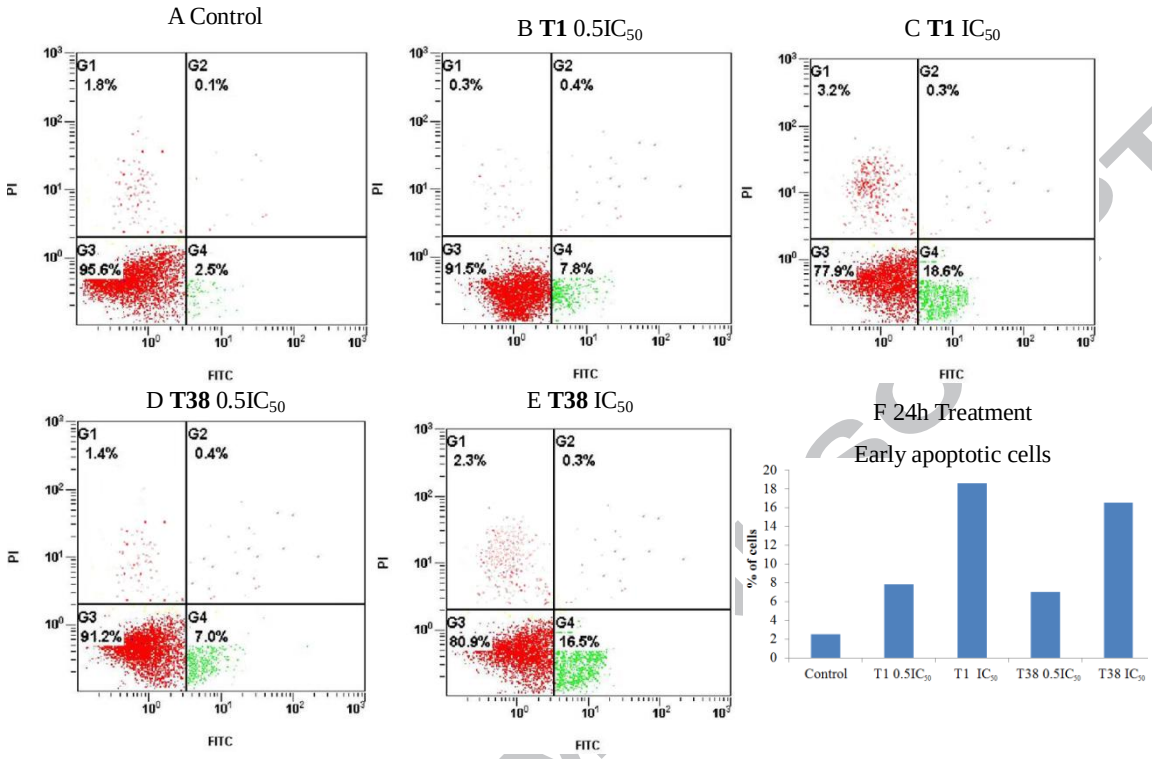


Fig 4. Flow cytometric analysis of HepG2 cells treated with synthetic compounds **T1** and **T38**. Cells were stained with Annexin V-FITC/PI and quantitated by flow cytometry. A) Not treated with compound, as control; B) Treated with **T1** at 1.82 µg/mL (0.5IC_{50} value); C) Treated with **T1** at 3.64 µg/mL (IC_{50} value); D) Treated with **T38** at 0. 55 µg/mL (0.5IC_{50} value) ; E) Treated with **T38** at 1.1 µg/mL (IC_{50} value) for 24h; F) Apoptotic effect of compounds **T1** and **T38** was evaluated after 24 h treatment; bar graphs represent mean ± SD in at least three independent experiments.

The cell cycle is the series of proceedings that occur in a cell resulting in its division and duplication. The cell cycle involves four distinct phases: G1 phase, S phase (synthesis), G2 phase (collectively known as interphase), and M phase (mitosis). The G1 phase is the preparation of energy, and material for DNA replication. The S phase is DNA replication. The G2 phase is the preparation for the M phase. The M phase is "mitosis", and is nuclear and cytoplasmic division. To determine whether the suppression of cancer cell growth using **T38** was caused by a cell cycle

progression, a cell-cycle cytotoxicity assay was performed by treating HepG2 cells with **T38** at the concentration of 0. 55 μg/mL ($0.5IC_{50}$ value) and 1.1μg/mL (IC_{50} value) for 24h respectively. Antitumor activity of **T38** can be accompanied by cell cycle arrest in any of the four phases (sub-G1, G1, S, and G2). As shown in **Fig 5**, cells in the G1 phase decreased from 57.68% as control to 41.02% and 11.83% respectively. A decrease of population in G2 phase from 6.89% as control to 2.11% and 0 respectively was also found when the concentration was increased to $0.5IC_{50}$ and IC_{50}. As control, the cell population in the S phase of the cell cycle was 35.42%, which could be increased to 56.87% at the concentration of $0.5IC_{50}$ and 88.17% at the concentration of IC_{50} respectively. Meanwhile, as obviously increase of cell number in the sub-G1 phase was observed from 0 to 34.48% and 66.72% after treatment with **T38** at the concentration of $0.5IC_{50}$ value and IC_{50} value. Hence, **T38** induced a markedly S phase arrest in a concentration manner and subsequent cell death.

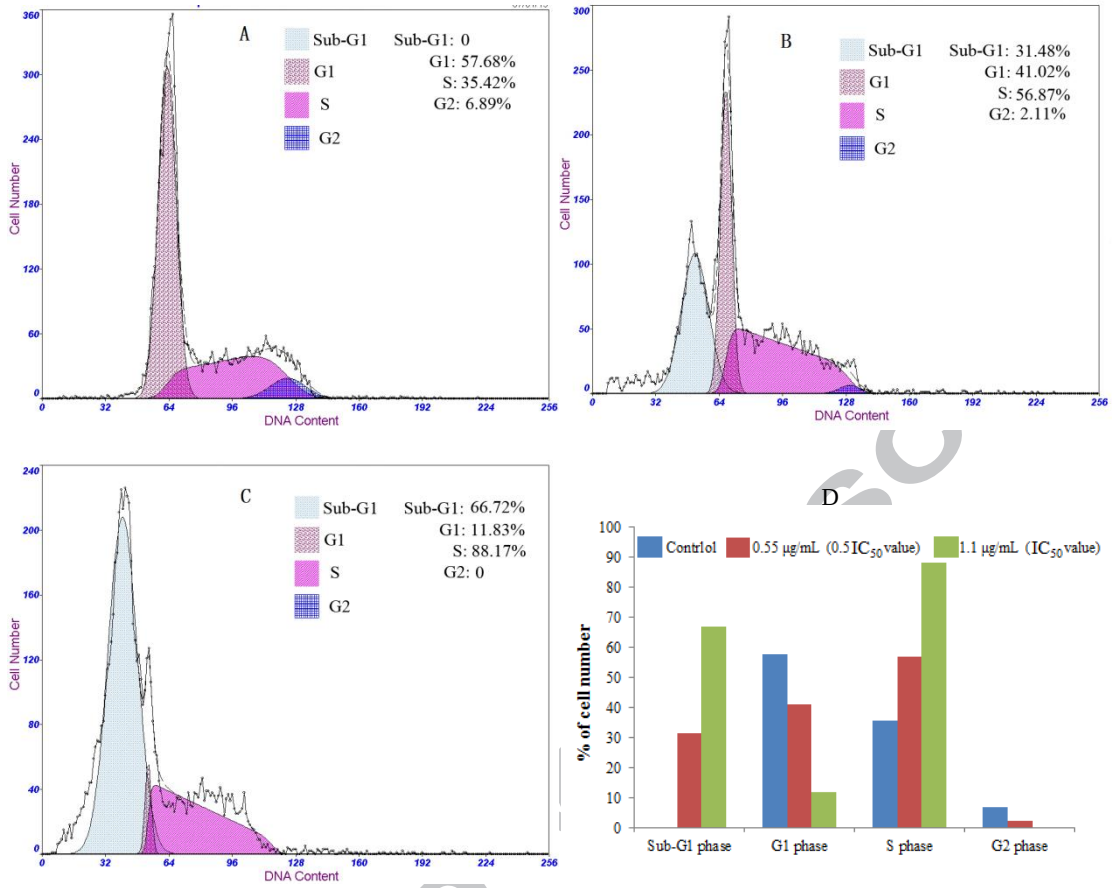


Fig 5. Cell-cycle analysis of **T38** by flow cytometry in HepG2 cells. A) Not treated with **T38** as control at for 48h; B, C) treatment with **T38** at 0.55 μg/mL (0.5IC_{50} value) and 1.1 μg·mL (IC_{50} value) for 48h, respectively. D) The numbers of sub-G1, G1, S, G2 phase cells were expressed as a percentage of the total cell number.

In summary, a series of novel 2,4,5-trisubstituted 1,3-thiazole derivatives containing hydrazide-hydrazone, and carboxamide moiety including 46 compounds were designed and synthesized. The SAR analyses indicated that the satisfactory cytotoxic activity required a reasonable combination of R^1, R^2, and R^3 in parent structure **T**. Antitumor activity could be increased greatly by optimizing R^2, and R^3. Eighteen compounds displayed higher inhibitory activity against all five human cancer cells than that of 5-Fu. **T1**, **T26** and **T38** exhibit best cytotoxic activity with IC_{50} values of 2.21 μg/mL, 1.67 μg/mL and 1.11 μg/mL, against MCF-7, BCG-823 and HepG2 cell lines, respectively. Especially, **T1**, **T26** and **T38** were found to be the

most promising compounds with wide spectrum and much higher activity (IC_{50}=1.11~7.88 μg/mL) than that of positive control 5-Fu (IC_{50}=9.23~10.89 μg/mL) against all tested cell lines. The results showed that we could obtain compounds with good antitumor activity and extend the activity spectrum by the introduction of the hydrazide-hydrazone, and carboxamide moiety into 1,3-thiazole scaffold to form 2,4,5-trisubstituted 1,3-thiazole derivatives. In addition, further studies revealed that **T1** and **T38** could induce apoptosis in HepG2 cells and it was confirmed **T38** led the induction of cell apoptosis through S cell-cycle arrest. This suggested that the scaffold of 2,4,5-trisubstituted 1, 3-thiazole containing hydrazide-hydrazone and carboxamide moiety could be used as a lead structure for further optimization to find more potent antitumor agents.

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Synthesis, antitumor activity and mechanism of action of novel 1,3-thiazole derivatives containing hydrazide-hydrazone and carboxamide moiety

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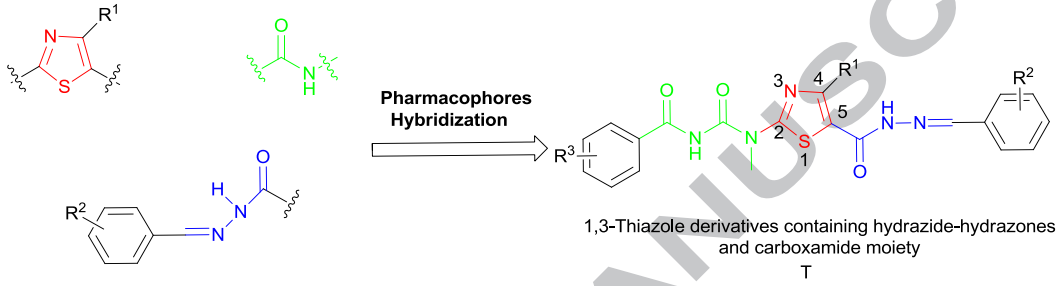
Graphical Abstract



Most of design compounds **T** displayed significantly inhibition activityu against human breast cancer (MCF-7), hepatocellular liver carcinoma (HepG2), gastric cancer (BGC-823), cervical carcinoma (Hela) and lung cancer (A549) cell lines with the control 5-fluorouracil. Especially, **T38** could induce apoptosis in HepG2 cells by S cell-cycle arrest.