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Design, synthesis and biological evaluation of novel 3-alkylsulfanyl-4-amino-1,2,4-triazole derivatives

Pei-Liang Zhao [a, *, #], Peng Chen [a, #], Qiu Li [a], Meng-Jin Hu [a], Peng-Cheng Diao [a], En-Shan Pan [b], Wen-Wei You [a,*]

[a] *Guangdong Provincial Key Laboratory of New Drug Screening, School of Pharmaceutical Science, Southern Medical University, Guangzhou 510515, P.R.China*

[b] *School of Traditional Chinese Medicine, Southern Medical University, Guangzhou 510515, P.R. China*

ABSTRACT: Based on our previous work, a series of novel 3-alkylsulfanyl-4-amino-1,2,4-triazole derivatives were designed, synthesized and evaluated for their antiproliferative activities. The results indicated that some compounds possessed significant antiproliferative activities against four cancer cell lines, HepG2, HCT116, PC-3, and Hela. Particularly, the most promising compound **8d** displayed 184-, 18-, and 17-fold improvement compared to fluorouracil in inhibiting HCT116, Hela and PC-3 cell proliferation with IC_{50} values of 0.37, 2.94, and 31.31 μM, respectively. Most interestingly, the compound did not affect the normal human embryonic kidney cells, HEK-293. Moreover, mechanistic investigation showed that the representative compound **8d** induced apoptosis and blocked cell cycle in G_2/M phase in Hela cells in a dose-dependent manner. These findings suggest that compound **8d** may have potential to be developed as a promising lead for the design of novel anticancer small-molecule drugs.

Keywords: 1,2,4-Triazole; Alkylsulfanyl; Synthesis; Antiproliferative activity.

[*] Corresponding author. Tel./fax: +86(0)20 61648196.

[*] Corresponding author. E-mail: plzhao@smu.edu.cn (P.-L. Zhao), youww@smu.edu.cn (W.-W. You)

[#] These authors contributed equally to this work.

In recent years, 1,2,4-triazole scaffold has attracted increasing attentions and has been found in abundance in the biologically active compounds such as pharmaceuticals and agrochemicals.[1-5] Among the structurally diverse 1,2,4-triazole derivatives, mercapto-substituted 1,2,4-triazoles have been a very interesting and hot research area due to their important chemopreventive and chemotherapeutic effects on cancer.[6-13] For example, as shown in Fig. 1, 3-cyclopentylthio-1,2,4-triazole **1** has been identified as a new potent and selective valosine-containing protein (VCP) inhibitor with an IC_{50} of 24 nM and possesses submicromolar antiproliferative activity on HCT116 cell lines.[14] While acetophenone and acetamide derivatives of 1,2,4-triazole **2-4** have emerged as potential therapeutic reagents in cancer.[15-17] Most interestingly, Westwell et al. reported that 4-amino-1,2,4-triazole derivative **5** exhibited submicromolar IC_{50} values in Bcl-2 expressing human cancer cell lines.[18] More recently, additional 4-amino-3-ylthio-1,2,4-triazole **6** was also proved to exhibit potential antitumor activity inhibiting mushroom tyrosinase.[19]

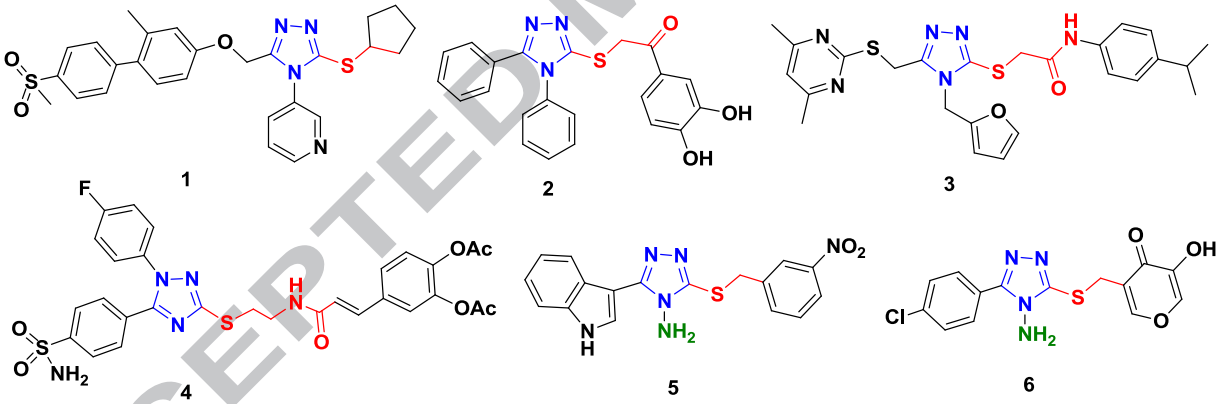


Fig. 1. Selected 3-alkylsulfanyl-1,2,4-triazoles with antitumor activity

In our early work, a series of 1,2,4-triazole derivatives containing isoindoline-1,3-diones moiety were synthesized *via* a one-pot reaction, and found to possess significant antiproliferative activity.[20] Derivative **7** (Fig. 2) was the most active as an inhibitor of tumor cell growth, with IC_{50} values of 6.76−11.71μM against a panel of four cancer cell lines, which encourages us to carry out further study on this scaffold. In a view of the above mentioned prominence of 1,2,4-triazole derivatives bearing acetophenone, acetamide and 4-amino moieties, and in prolongation of our ongoing research on developing novel tumor growth inhibitors,[21-25] we aspired to design and synthesis a series of novel 1,2,4-triazole derivatives using $–NH_2$ to replace isoindoline-1,3-dione ring. Meanwhile, we replaced acetophenone of compound **7** with *N*-substituted acetamide group (Fig. 2).

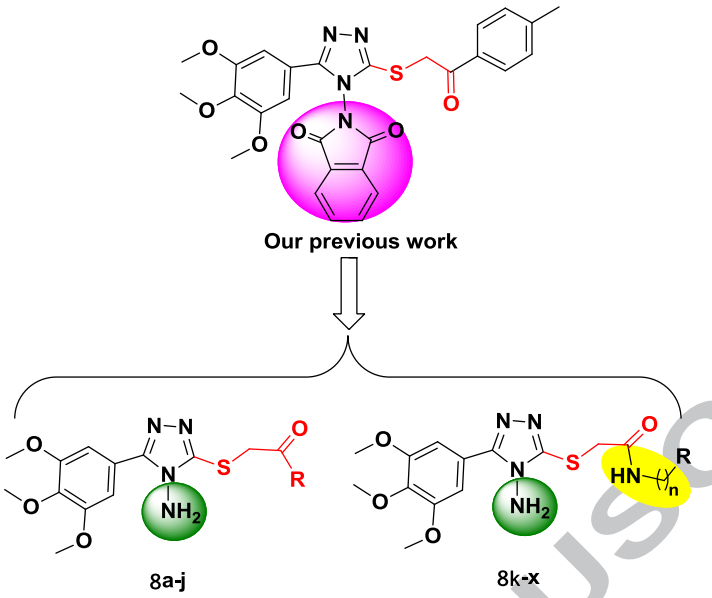
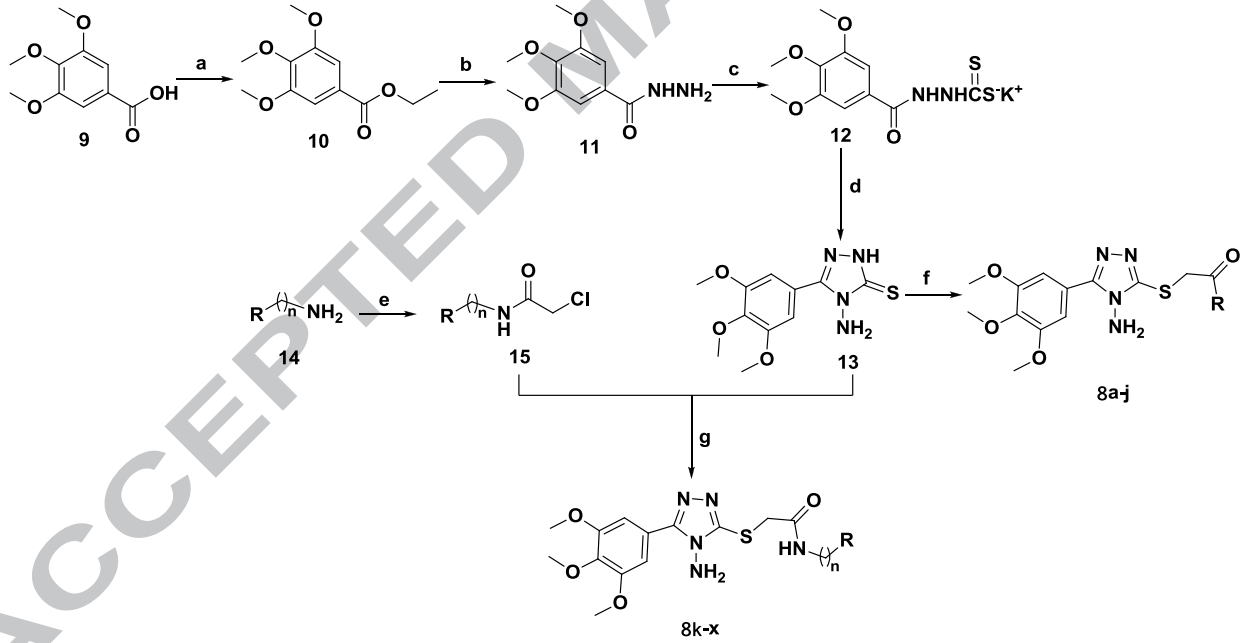


Fig. 2. Design strategy of the title compounds **8a-x**.



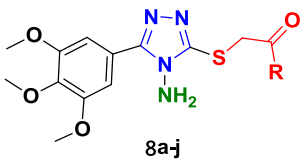
Scheme 1. General synthetic route for target compounds **8a-x.** Reagents and conditions: (a) con. H_2SO_4, ethanol, reflux; (b) 60% $NH_2NH_2{\cdot}H_2O$, ethanol, reflux; (c) KOH, CS_2, ethanol, rt.; (d) 60% $NH_2NH_2{\cdot}H_2O$, H_2O, reflux, HAc; (e) chloroacetyl chloride, Et_3N, CH_2Cl_2, r.t.; (f) K_2CO_3, acetone, substituted phenacyl bromides, r.t.; (g) K_2CO_3, ethanol, reflux.

The reaction sequence employed for the synthesis of the title compounds was shown in Scheme 1. According to our recently reported approach,[22] the key intermediate 4-amino-3-(3,4,5-trimethoxyphenyl)-1H-1,2,4-triazole-5(4H)-thione (**13**) was prepared from 3,4,5-trimethoxybenzoic acid, *via* a four-step operation empolying esterification, hydrazidation, salt formation, and cyclization. Diverse chloro acetamides (**15**) were obtained through a biphasic acylation of appropriate aniline with chloroacetyl chloride in the presence of triethylamine and

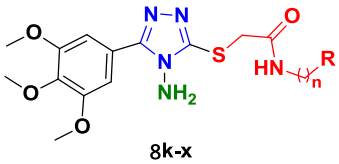
dichloromethane with excellent yields.[26] Subsequently, the intermediate 1,2,4-triazole derivative (**13**) was substituted by various commercial phenacyl bromides using K_2CO_3 as base in anhydrous acetone at room temperature to generate the target compounds **8a-j** in moderate to good isolated yields ranging from 56% to 88%. Meanwhile compound (**13**) was treated with 2-chloro-*N*-sbustituted acetamide derivatives (**15)** by refluxing in anhydrous ethanol in the presence of K_2CO_3 to afford the desired compounds **8k-x** in moderate to good yields. The structures of the final compounds were characterized by ^{1}H NMR, ^{13}C NMR and HRMS spectroscopic techniques, and the spectral data agree with the proposed structures.

The *in vitro* antiproliferative activities of the synthesized compounds **8a-x** against four human cancer cell lines, including HepG2 (human hepatoma cells), HCT116 (human colon cancer cell lines), PC-3 (human prostate cancer cell lines), and Hela (human cervical cancer cells), were evaluated through MTT screening assay. Meanwhile five selected compounds were evaluated cytotoxic activity against a representative normal cell line HEK-293 (human embryonic kidney cells). For comparison, fluorouracil (5-Fu) was selected as a positive control and the results expressed as IC_{50} (μM) were summarized in Table 1. Here, the IC_{50} value represents the concentration of one compound resulting in a 50% inhibition in cell growth after a 48 h incubation, and is the average of three independent experiments.

Table 1 Cytotoxic activities of compounds 8a-x against human tumor cells.



8a-j



8k-x

Comp.	n	R	*In vitro* cytotoxicity IC_{50}(μM) [a]				
			HepG2	**HCT 116**	**PC-3**	**Hela**	**HEK-293** [b]
8a	/	C_6H_5	7.80	53.01	>100	>100	NT [c]
8b	/	4-ClC_6H_4	>100	7.50	>100	13.11	NT
8c	/	4-BrC_6H_4	92.50	9.23	>100	10.38	NT
8d	/	4-$CH_3C_6H_4$	>100	**0.37**	31.31	2.94	>100
8e	/	4-$CF_3C_6H_4$	>100	16.70	>100	3.38	NT
8f	/	4-FC_6H_4	90.41	3.23	>100	20.85	NT
8g	/	3-BrC_6H_4	56.02	5.32	>100	77.47	NT
8h	/	3,4-$F_2C_6H_3$	>100	10.43	>100	>100	NT
8i	/	2-FC_6H_4	22.58	10.76	43.09	80.12	NT
8j	/	4-$CH_3OC_6H_4$	>100	**1.24**	97.90	17.41	>100
8k	0	C_6H_5	49.87	1.73	37.85	8.28	NT

8l	0	2-$CH_3C_6H_4$	>100	>100	>100	>100	NT
8m	0	4-$CH_3C_6H_4$	>100	3.45	43.05	4.77	79.35
8n	0	3-$CH_3OC_6H_4$	>100	**0.68**	>100	4.02	>100
8o	0	2-FC_6H_4	18.66	**1.16**	36.66	38.24	>100
8p	0	3,4,5-$(CH_3O)_3C_6H_2$	>100	>100	>100	>100	NT
8q	1	4-ClC_6H_4	>100	>100	>100	>100	NT
8r	1	4-$CH_3C_6H_4$	>100	>100	92.8	>100	NT
8s	1	3,4-$(CH_3O)_2C_6H_3$	>100	>100	>100	>100	NT
8t	2	4-ClC_6H_4	>100	39.61	>100	79.92	NT
8u	2	4-$CH_3C_6H_4$	>100	>100	41.26	>100	NT
8v	2	4-FC_6H_4	>100	>100	56.05	66.47	NT
8w	2	4-$CH_3OC_6H_4$	>100	>100	73.59	>100	NT
8x	2	C_6H_5	>100	>100	80.47	>100	NT
		5-Fu	46.83	68.71	57.04	57.17	NT

[a] IC_{50} values are presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%. [b] Normal human embryonic kidney (HEK-293) cell lines. [C] NT: not tested.

For the sake of convenience, according to the structure of 3-alkylsulfanyl of triazole ring, compounds **8a-j** and **8k-x** were named as acetophenone and acetamide derivatives, respectively, throughout the text. As can be seen in Table 1, almost all of the acetophenone derivatives **8a-j** showed significant antiproliferative activities against Hela and HCT116 cell lines, while most of the acetamide derivatives **8k-x** were ineffective (IC_{50} >100 μM) for Hela, HepG2 and HCT116 cell lines. These results clearly demonstrate that the in most cases, acetophenone derivatives displayed higher cytotoxic activities than acetamide derivatives. Especially, the most promising compound **8d** displayed 184-, 18-, and 17-fold improvement compared to fluorouracil in inhibiting HCT116, Hela and PC-3 cell proliferation with IC_{50} values of 0.37, 2.94 and 31.31 μM, respectively. It was worth noting that, the compound did not affect the normal human embryonic kidney cells, HEK-293.

Further analysis on the role of the aryl groups (R) and linker-length of acetamide (n) were carried out to explore the requirements for activity. Within the series of acetophenone derivatives, introduction of electron donating substitutes on the phenyl group (R) leads to dramatical enhancement of antiproliferative activities against HCT116, PC-3 and Hela cell lines (**8a** vs **8d**, **8a** vs 8**j**). However, among the series of acetamide derivatives **8k-x**, electron donating substitutes on the phenyl group (R) results in significant decrease of activities against HepG2 and PC-3 cell lines (**8k** vs **8l**, **8k** vs **8m**, **8k** vs **8n**, **8k** vs **8p**). These results suggest that electronic effect of substituents on phenyl group plays a crucial role on antitumor activities. Besides, linker-length of acetamide (n) has also profound effects on inhibitory activity. It was interesting to note that the longer acetamide

linkage substituted derivatives (n = 2), such as **8t** and **8u** exhibited better antiproliferative activities than the corresponding shorter acetamide derivatives (n = 1), including **8q** and **8r**.

To study the effect of the synthesized compounds on cell cycle progression, flow-activated cell sorting analysis was performed. The most potent compound **8d** was tested against Hela cell lines at given concentrations (2, 4, 8 μM). As shown in Fig. 3 and Table 2, compound **8d** displayed obvious apoptosis-inducing effect on Hela cells. Meanwhile, The G2/M peak significantly increased from 16.67% to 32.55% (2 μM), 70.18% (4 μM), and 78.81% (8 μM) after 12 h of treatment. These data suggest that compound **8d** induced significant cell cycle arrest in the G_2/M phase in a dose-dependent manner, compared to untreated cells.

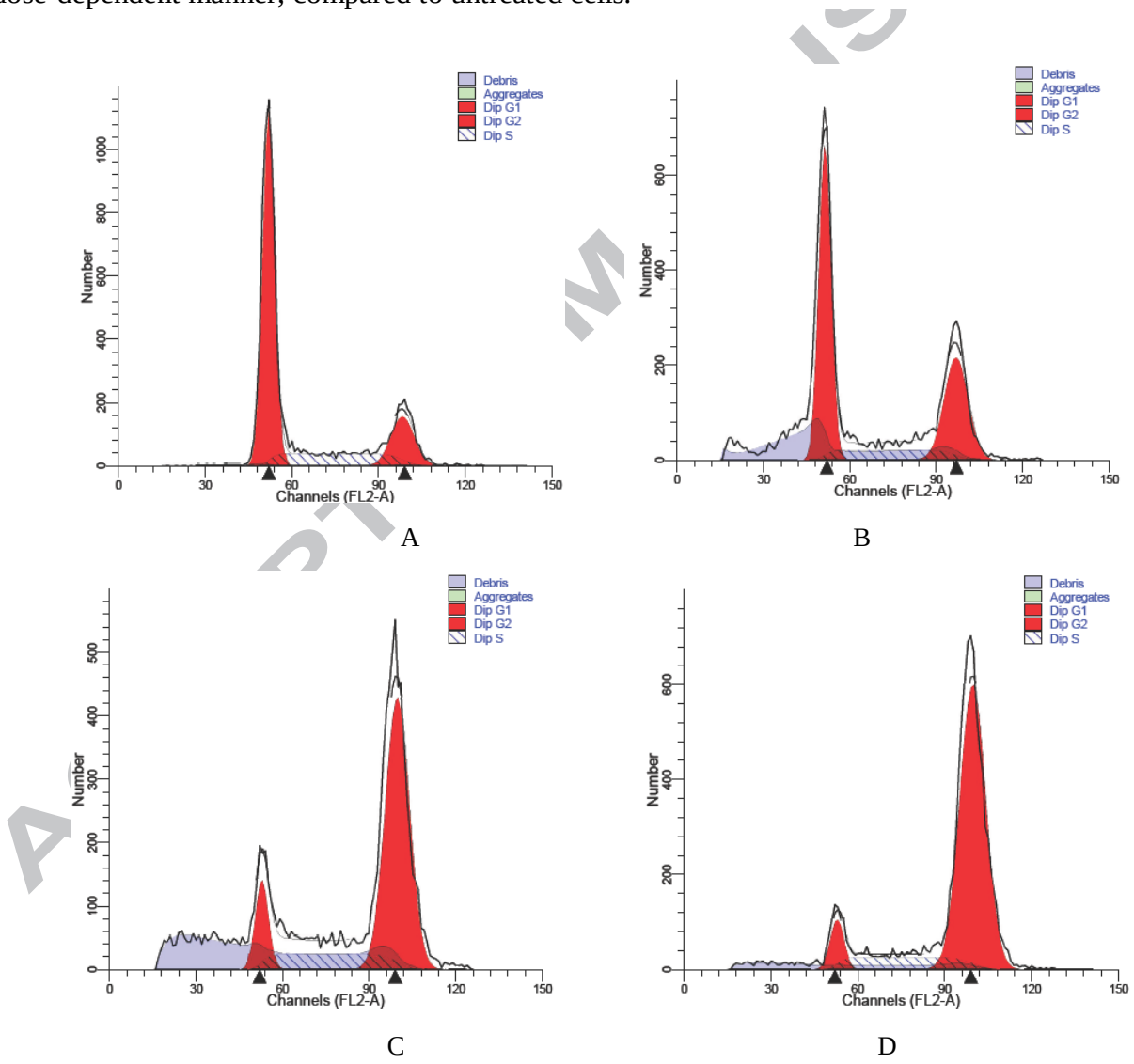


Fig. 3. Effect of compound **8d** on cell cycle and apoptosis in Hela cells. Flow cytometry analysis of Hela cells treated with **8d** for 18 h. (A) Control; (B) **8d** , 2 μM; (C) **8d**, 4 μM; (D) **8d**, 8 μM.

Table 2 Effect of compound 8d on cell cycle distribution in Hela cells.

Concentration	G_0/G_1(%) [a]	S(%) [b]	G_2/M(%) [c]
0μM	64.31	19.02	16.67
2μM	53.39	14.07	32.55
4μM	12.41	17.42	70.18
8μM	7.37	13.81	78.81

[a] G0/G1: to prepare the cell for DNA synthesis. [b] S: DNA is manufactured during the phase. [c] G2/M: is the phase in wich DNA replication completed, start to mitosis.

In conclusion, based on our previously reported antiproliferative compounds, a series of novel 3-alkylsulfanyl-4-amino-1,2,4-triazole derivatives exhibiting significantly antitumor activities were successfully identified. The most promising compound **8d** showed more potent *in vitro* cytotoxic activities against HCT116, Hela and PC-3 with IC_{50} values of 0.37, 2.94 and 31.31 μM, respectively, which represented 184-, 18-, and 17-fold improvement in activity compared to the chemotherapy drug fluorouracil. Most interestingly, the compound did not affect the normal human embryonic kidney cells, HEK-293. Additionally, in mechanistic studies, the representative compound **8d** was found to induce apoptosis and G2/M phase cell cycle arrest in a dose-dependent manner in Hela cells. Further research on the mechanisms of these compounds and modification is underway.

Acknowledgments

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Supplementary data

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

References and notes

1. Ayati, A.; Emami, S.; Foroumadi, A. *Eur. J. Med. Chem.* **2016**, *109*, 380.
2. Papadopoulou, M.V.; Bloomer, W.D.; Lepesheva, G.I.; Rosenzweig, H.S.; Kaiser, M.; Aguilera-Venegas, B.; Wilkinson, S.R.; Chatelain, E.; Ioset, J.R. *J. Med. Chem.* **2015**, *58*, 1307.
3. Li, B.L.; Li, B.; Zhang, R.L.; Zhao, J.J.; Wang, X.F.; Liu, Y.M.; Shi, Y.P.; Liu, J.B.; Chen, B.Q. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1279.
4. Li, W.J.; Li, Q.; Liu, D.L.; Ding, M.W. *J. Agric. Food Chem.* **2013**, *61*, 1419.
5. Hahn, H.G.; Choi, J.S.; Lim, H.K.; Lee, K.I.; Hwang, I.T. *Pestic. Biochem. Physiol.* **2015**, *125*, 78.
6. Plech, T.; Kaproń, B.; Paneth, A.; Kosikowska, U.; Malm, A.; Strzelczyk, A.; Stączek, P.; Świątek, L.; Rajtar, B.; Polz-Dacewicz, M. *Eur. J. Med. Chem.* **2015**, *97*, 94.
7. Ünver, Y.; Sancak, K.; Çelik, F.; Birinci, E.; Küçük, M.; Soylu, S.; Burnaz, N.A. *Eur. J. Med. Chem.* **2014**, *84*, 639.
8. Hassan, G.S.; El-Messery, S.M.; Al-Omary, F.A.; Al-Rashood, S.T.; Shabayek, M.I.; Abulfadl, Y.S.; Habib, El-SE.; El-Hallouty, S.M.; Fayad, W.; Mohamed, K.M.; El-Menshawi, B.S.; El-Subbagh, H.I. *Eur. J. Med. Chem.* **2013**, *66*, 135.
9. SitaRam; Celik, G.; Khloya, P.; Vullo, D.; Supuran, C.T.; Sharma, P.K. *Bioorg. Med. Chem.* **2014**, *22*, 1873.
10. Kücükgüzel, S.G.; Cıkla-Süzgün, P. *Eur. J. Med. Chem.* **2015**, *97*, 830.
11. Sahu, J.K.; Ganguly, S.; Kaushik, A. *Chin. J. Nat. Med.* **2013**, *11*, 456.
12. Yadagiri, B.; Gurrala, S.; Bantu, R.; Nagarapu, L.; Polepalli, S.; Srujana, G.; Jain, N. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 2220.
13. Khan, I.; Zaib, S.; Ibrar, A.; Rama, N.H.; Simpson, J.; Iqbal, J. *Eur. J. Med. Chem.* **2014**, *78*, 167.
14. Polucci, P.; Magnaghi, P.; Angiolini, M.; Asa, D.; Avanzi, N.; Badari, A.; Bertrand, J.; Casale, E.; Cauteruccio, S.; Cirla, A.; Cozzi, L.; Galvani, A.; Jackson, P.K.; Liu, Y.; Magnuson, S.; Malgesini, B.; Nuvoloni, S.; Orrenius, C.; Sirtori, F.R.; Riceputi, L.; Rizzi, S.; Trucchi, B.; O'Brien, T.; Isacchi, A.; Donati, D.; D'Alessio, R. *J. Med. Chem.* **2013**, *56*, 437.
15. Park, H.; Bahn, Y.J.; Ryu, S.E. *Bioorg. Med. Chem. Lett.* **2009**, *29*, 4330.

16. Reddy, T.R.; Li, C.; Guo, X.; Fischer, P.M.; Dekker, L.V. *Bioorg. Med. Chem.* **2014**, *22*, 5378.

17. Cai, H.; Huang, X.; Xu, S.; Shen, H.; Zhang, P.; Huang, Y.; Jiang, J.; Sun, Y.; Jiang, B.; Wu, X.; Yao, H.; Xu, J. *Eur. J. Med. Chem.* **2015**, *108*, 89.

18. Hamdy, R.; Ziedan, N.; Ali, S.; El-Sadek, M.; Lashin, E.; Brancale, A.; Jones, A.T.; Westwell, A.D. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2391.

19. Xie, W.; Zhang, J.; Ma, X.; Yang, W.; Zhou, Y.; Tang, X.; Zou, Y.; Li, H.; He, J.; Xie,S.; Zhao, Y.; Liu, F. *Chem. Biol. Drug Des*. **2015**, *86*, 1087.

20. Zhao, P.L.; Ma, W.F.; Duan, A.N.; Zou, M.; Yan, Y. C.; You, W.W.; Wu, S.G. *Eur. J. Med. Chem.* **2012**, *54*, 813.

21. Zhang, B.; Li, Y.H.; Liu, Y.; Chen, Y.R; Pan, .E.S.; You, W.W.; Zhao, P.L. *Eur. J. Med. Chem*. **2015**, *103*, 335.

22. Zhao, P.L.; Duan, A.N.; Zou, M.; Yang, H.K.; You, W.W.; Wu, S.G. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 4471.

23. Yang, H.K.; Xu, W.F.; Duan, A.N.; You, W.W.; Zhao, P. L. *Chem. J. Chinese U*. **2014**, *35*, 555.

24. Hu, M.J.; Zhang, B.; Yang, H.K.; Liu, Y. ; Chen, Y.R. ; Ma, T.Z.; Lu, L.; You, W.W.; Zhao, P.L. *Chem. Biol. Drug Des*. **2015**, *86*, 1491.

25. Ma, W.F.; Yang, H K.; Hu, M.J.; Li, Q.; Ma, T.Z.; Zhou, Z.Z.; Liu, R.Y.; You, W.W.; Zhao, P.L. *Eur. J. Med. Chem*. **2014**, *84*, 127.

26. Gao, M.; Wang, M.; Zheng, Q.H. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1371.

Graphical abstract