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Synthesis and antiproliferative evaluation of piperazine-1-carbothiohydrazide derivatives of indolin-2-one

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ABSTRACT

By varying the substituents (R^1) at the indolin-2-one scaffold, a series of indolin-2-one derivatives bearing 4-phenylpiperazine-1-carbothiohydrazide moiety at the C3-position were synthesized and evaluated for their antiproliferative activity against three human cancer cell lines. We further selected the 5-chloroindolin-2-one moiety for the extension to another series of compounds by varying the substituents (R^2) at the phenyl group connected with the piperazine ring. Among all the compounds synthesized, **6d** and **6l** were most potent with IC_{50} values of 3.59 and 5.58 μM, respectively against A549 lung cancer cells, while **5f** and **6l** possessed IC_{50} values of 3.49 and 4.57 μM, respectively against HCT-116 colon cancer cells which were comparable to that of Sunitinib, an indolin-2-one derivative in cancer therapy.

Indolin-2-one has been shown to be an attractive scaffold of a class of new antitumor agents,[1] most of which appear to act as inhibitors of various protein kinase families, particularly receptor tyrosine kinases (RTKs) and serine/threonine-specific protein kinases such as the cyclin-dependent kinases (CDKs).[2] For example, Sunitinib (SU11248, Fig. 1) is the first kinase inhibitor of the indolin-2-one type targeting at multiple kinases, which has been approved for the treatment of renal cell carcinoma and gastrointestinal stromal tumor.[3] On the other hand, thiosemicarbazone derivatives are known to possess a potent antitumor activity,[4–7] which have attracted considerable attention in the development of antitumor agents. For example, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Fig. 1) is a potent ribonucleotide reductase inhibitor that has entered phase II clinical trials for the treatment of a number of cancer, including non-small-cell lung cancer and renal carcinoma.[8,9] Recently, the incorporation of thiosemicarbazone moiety into the C3-position of indolin-2-one has proven to be an efficient strategy to design novel antitumor agents.[10–14] In additon, we have synthesized a number of 2-methyl-4-oxoquinazoline or 2,4-diaminoquinazoline derivatives bearing 4-substituted-piperazine-1-carbodithioate side chains (**I**, **II**, Fig. 1).[15,16] Some of the synthesized compounds significantly inhibited the proliferation of human tumor cells in culture, suggesting that the piperazine-1-carbodithioate moiety might contribute to the cytotoxic activity. In view of these observations, we integrated the structural features of indolin-2-one, thiosemicarbazone and piperazine-1-carbodithioate to design and synthesize a new class of piperazine-1-carbothiohydrazide derivatives of indolin-2-one, aiming at the identification of novel small molecules with potent antiproliferative effects on tumor cells.

As shown in Scheme 1, methyl hydrazinecarbodithioate **1** was prepared by reaction of 85% hydrazine hydrate, carbon disulfide and iodomethane in isopropanol/water in the presence of potassium hydroxide, according to the procedure reported by Klayman et al.[17] Reaction of **1** with 1-phenylpiperazine **2a** in ethanol at reflux afforded 4-phenylpiperazine-1-carbothiohydrazide **3a**, which was condensed with various indoline-2,3-diones **4a**–**l** to give the first series of target compounds **5a**–**l**. The results of cell proliferative inhibition assay showed that compound **5f** was the most potent among this series (Table 1). Consequently, reaction of **1** with various 1-substituted piperazines **2b**–**m** gave intermediates **3b**–**m**, which reacted with 5-chloroindoline-2,3-dione **4f**, respectively, to furnish the second series of target compounds **6a**–**l**. (For synthetic procedures and spectroscopic data of compounds **3a**–**m**, **5a**–**l** and **6a**–**l**, see Supplementary data.)

The target compounds may exist as either the Z or E-isomers due to the exocyclic C=N double bond at the C3 position of indolin-2-one.[18] The ^{1}H NMR spectra of **5a**–**l** and **6a**–**l** displayed the chemical shifts of the NH protons in carbothiohydrazide moiety

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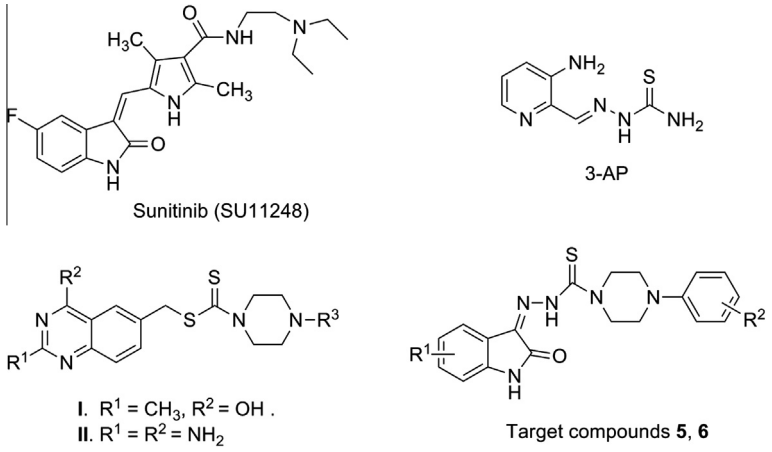
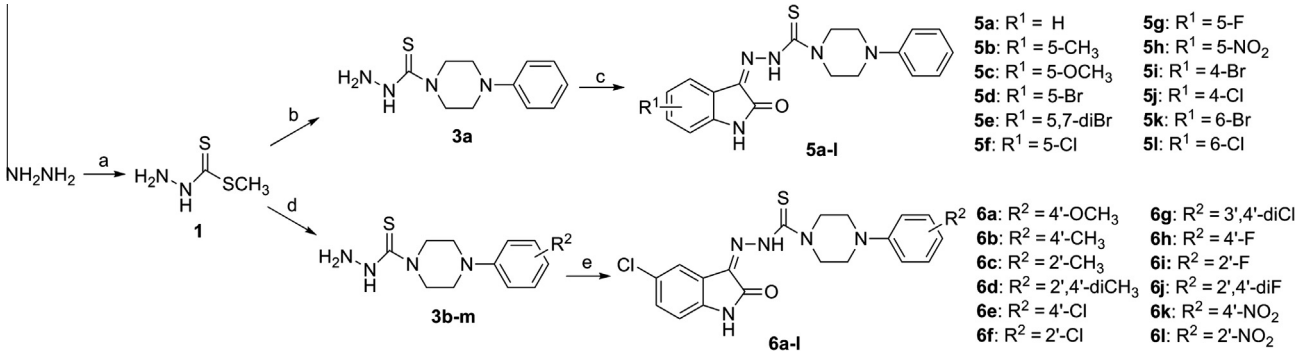


Figure 1. Structures of Sunitinib, 3-AP, compounds **I**, **II** and target compounds **5**, **6**.



Scheme 1. Reagents and conditions: (a) CS_2, CH_3I, KOH, *i*-PrOH/H_2O; 0 °C, 2 h; (b) 1-phenylpiperazine (**2a**), EtOH, reflux, 10 h; (c) indoline-2,3-diones **4a**–**l**, EtOH, reflux, 10 h; (d) 1-substituted-piperazines **2b**–**m**, EtOH, reflux, 5–10 h; (e) 5-chloroindoline-2,3-dione (**4f**), EtOH, reflux, 10 h.

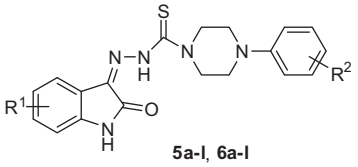
ranging from 13.06 to 13.31 ppm as single singlets, indicating that these compounds were obtained as pure geometrical isomers. Compared to the chemical shifts of the NH protons in intermediates **3a**–**m** (δ 9.12–9.21 ppm), the downfield shift of the NH protons in carbothiohydrazide moiety of **5a**–**l** and **6a**–**l** (δ 13.06–13.31 ppm) was due to the deshielding effect of the adjacent carbonyl group (Fig. 2). Therefore, we believe that compounds **5a**–**l** and **6a**–**l** exist as the thermodynamically stable Z-isomers forming a six-membered ring with an intramolecular hydrogen bond between the NH proton and the C2 carbonyl oxygen atom of indolin-2-one (Fig. 2), and this configuration is in agreement with the crystal structure of the similar compounds reported by Hall et al.[14]

The MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyl-tetrazolium bromide] cell proliferation assay was used to evaluate the antiproliferative activity of the synthesized compounds against three human cancer cell lines including A549 (lung cancer), MCF-7 (breast adenocarcinoma) and HCT-116 (colorectal cancer) cell lines (see Supplementary data). The inhibition of cell proliferation was determined 72 h after cells were exposed to the tested compounds at a concentration of 100 μM. The compounds with 50% or more inhibition compared with vehicle-treated cells were considered active. Inhibition of cell proliferation by these active compounds at various concentrations were further measured, and their IC_{50} (the concentration that causes 50% of cell proliferation inhibition) values were determined and summarized in Table 1. Sunitinib was used as a positive control.

To explore the structure–activity relationship and optimize the structures of piperazine-1-carbothiohydrazide derivatives of indolin-2-one, we first varied the substituents at the indolin-2-one ring while maintaining the phenyl ring at the N4-position of piperazine to synthesize a series of target compounds **5a**–**l** (Scheme 1) and evaluate their antiproliferative activity. As shown in Table 1, the unsubstituted compound **5a** exhibited moderate inhibitory activity against the proliferation of A549, MCF-7 and HCT-116 cell lines with IC_{50} values of 18.56, 24.86 and 11.13 μM, respectively. Introduction of an electron-donating group of methyl (**5b**) or methoxyl (**5c**) into the 5-position of indolin-2-one ring led to an obvious increase in antiproliferative activity against A549 and MCF-7 cells, and a slight increase against HCT-116 cells, in comparison to the parent compound **5a**. Compounds **5g** and **5h** bearing a strong electron-withdrawing group of a fluoro or nitro group, respectively, at the 5-position of indolin-2-one ring exhibited a decreased or similar antiproliferative activity compared to compound **5a**. However, introduction of a weak electron-withdrawing substituent of bromo (**5d**) into the 5-position of indolin-2-one ring resulted in an increase in the antiproliferative activity. Further introduction of a bromo substituent into the 7-position of indolin-2-one ring of **5d** resulted in compound **5e**, which possessed higher antiproliferative activity against three cell lines than **5d**. Interestingly, compound **5f** possessing a chloro substituent at the 5-position of indolin-2-one ring yielded comparable potency to **5d** against A549 and MCF-7 cells, but more potent than **5d** against HCT-116 cells with an IC_{50} value of 3.49 μM. These results suggest that an electron-donating group, a weak or medium electron-withdrawing group is more favorable for enhancing the activity than a strong electron-withdrawing group.

Compared to 4-substituted compounds **5i** (R^1 = 4-Br) and **5j** (R^1 = 4-Cl), 6-substituted compounds **5k** (R^1 = 6-Br) and **5l** (R^1 = 6-Cl) were more active, suggesting that substituents at the

Table 1
Antiproliferative activity of compounds **5a–l** and **6a–l** against three human cancer cell lines



5a-l, **6a-l**

Compound	R^1	R^2	IC_{50}[a] (μM) ± SD		
			A549	MCF-7	HCT-116
5a	H	H	18.56 ± 1.70	24.86 ± 0.28	11.13 ± 2.89
5b	5-CH_3	H	9.88 ± 0.82	14.86 ± 1.14	9.48 ± 0.17
5c	5-OCH_3	H	7.52 ± 0.54	14.41 ± 0.17	9.53 ± 0.73
5d	5-Br	H	12.25 ± 1.42	20.50 ± 3.21	10.39 ± 0.99
5e	5,7-diBr	H	6.11 ± 1.87	11.55 ± 0.94	6.32 ± 0.56
5f	5-Cl	H	7.34 ± 2.18	10.39 ± 1.84	3.49 ± 1.26
5g	5-F	H	20.73 ± 4.25	24.30 ± 5.05	12.35 ± 0.11
5h	5-NO_2	H	17.91 ± 2.97	23.33 ± 5.41	15.09 ± 0.68
5i	4-Br	H	53.97 ± 4.95	40.69 ± 0.47	33.60 ± 5.74
5j	4-Cl	H	55.93 ± 5.26	52.07 ± 2.23	42.14 ± 2.67
5k	6-Br	H	15.84 ± 2.82	19.65 ± 2.56	14.51 ± 1.53
5l	6-Cl	H	27.65 ± 2.78	22.98 ± 0.62	17.86 ± 0.10
6a	5-Cl	4-OCH_3	7.68 ± 1.28	13.79 ± 1.99	7.74 ± 0.89
6b	5-Cl	4-CH_3	10.13 ± 0.82	17.13 ± 2.30	6.39 ± 0.81
6c	5-Cl	2-CH_3	6.52 ± 1.24	10.90 ± 2.36	4.91 ± 0.49
6d	5-Cl	2,4-diCH_3	3.59 ± 0.77	10.83 ± 1.86	7.18 ± 0.39
6e	5-Cl	4-Cl	8.18 ± 0.42	13.10 ± 0.35	5.39 ± 1.09
6f	5-Cl	2-Cl	7.92 ± 0.59	11.73 ± 1.30	4.91 ± 0.36
6g	5-Cl	2,4-diCl	7.95 ± 0.52	10.82 ± 0.18	5.82 ± 0.67
6h	5-Cl	4-F	8.90 ± 0.49	12.32 ± 0.54	6.35 ± 0.10
6i	5-Cl	2-F	10.51 ± 0.83	15.23 ± 1.30	8.29 ± 1.23
6j	5-Cl	2,4-diF	11.38 ± 0.81	20.06 ± 1.56	5.89 ± 1.11
6k	5-Cl	4-NO_2	11.77 ± 0.90	20.86 ± 1.68	12.0 ± 0.86
6l	5-Cl	2-NO_2	5.58 ± 0.22	7.49 ± 1.06	4.57 ± 0.44
Sunitinib			2.44 ± 0.22	6.29 ± 0.22	4.71 ± 0.32

[a] The concentration that causes 50% of cell proliferation inhibition. Data are expressed as means ± SD of three separate experiments.

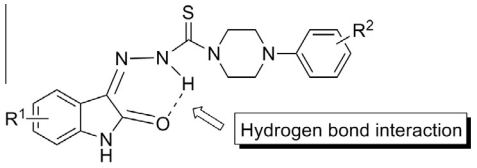


Figure 2. Compounds **5a–l** and **6a–l** adopt the *Z*-configuration due to the formation of a hydrogen bond.

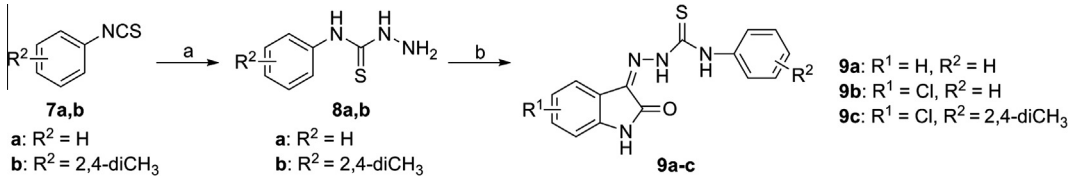
6-position of indolin-2-one ring are favorable for the antiproliferative activity. Nevertheless, both compounds **5i** (R^1 = 4-Br) and **5k** (R^1 = 6-Br) were generally less active than compound **5d** (R^1 = 5-Br). Similarly, compounds **5j** (R^1 = 4-Cl) and **5l** (R^1 = 6-Cl) were less potent than compound **5f** (R^1 = 5-Cl). These results indicate that the presence of a substituent at the 5-position of indolin-2-one ring is important for antitumor activity. We thus selected 5-chloroindolin-2-one moiety to serve as a template for further investigation of structure–activity relationship.

By maintaining the 5-chloro substituent at the indolin-2-one moiety, we introduced various substituents into the phenyl ring at the N4-position of piperazine ring to generate the second series of target compounds **6a–l** (Scheme 1) and evaluated for their antiproliferative activity. As shown in Table 1, the IC_{50} values of this series of compounds against three cell lines were generally lower than 20 μM, furthermore, these compounds were more active than the parent compound **5a**. In particular, the antiproliferative activity of compounds **6a–l** against A549 cells were obviously more potent than the first series of compounds **5a–l** (IC_{50}, 3.59–11.77 μM vs 7.34–55.93 μM), with compound **6d** being the most potent with an IC_{50} value of 3.59 μM. In fact, the second series of compounds were generally more potent against HCT-116 cells (IC_{50}, 4.57–12.0 μM) than the first series of compounds (IC_{50}, 3.49–42.14 μM) as well, of which compounds **6c** (IC_{50}, 4.91 μM), **6f** (IC_{50}, 4.91 μM) and **6l** (IC_{50}, 4.57 μM) exhibited comparable activity to the positive control Sunitinib (IC_{50}, 4.71 μM).

As compared with compound **5f** with no substituent at the phenyl ring, the antiproliferative activity of compounds **6a**, **6b**, **6e**, **6h**, and **6k** bearing a methoxyl, methyl, chloro, fluoro, or nitro group at the 4′-position of the phenyl ring were in general less active against three cell lines. However, compounds **6c**, **6f** and **6l** bearing a methyl, chloro or nitro group at the 2′-position of the phenyl ring were more potent than their 4-substituted counterparts **6b**, **6e** and **6k** against three tumor cell lines and comparable to compound **5f**, whereas **6i** (R^2 = 2′-F) was less potent than **6h** (R^2 = 4′-F). In addition, introduction of two substituents into both the 2′- (or 3′- for **6g**) and 4′-position of the phenyl ring resulted in compounds **6d**, **6g** and **6j**, of which only **6d** containing 2,4-dimethylphenyl group was more active against A549 cells (IC_{50}, 3.59 μM) than compound **5f** (IC_{50}, 7.34 μM), whereas **6g** (3,4-dichlorophenyl) and **6j** (2,4-difluorophenyl) exhibited comparable or weaker activity against three cell lines compared to **5f**. These results suggest that the presence of a bulky group at the 2′-position of the phenyl ring is favorable for retaining the potency, and the electronic effect of substituents was not important for the antiproliferative activity.

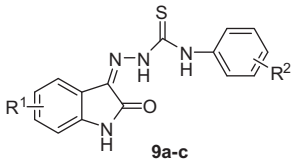
In order to investigate the effect of the piperazine moiety on antiproliferative activity, we synthesized compounds **9a–c** that are the analogs having no piperazine moiety of representative compounds **5a**, **5f** and **6d**. As illustrated in Scheme 2, treatment of 1-isothiocyanatobenzene **7a** or 1-isothiocyanato-2,4-dimethylbenzene **7b** with 85% hydrazine hydrate to give *N*-substituted hydrazinecarbothioamides **8a**,**b**, which were condensed with indoline-2,3-dione **4a** or 5-chloroindoline-2,3-dione **4f**, respectively to furnish **9a–c** (see Supplementary data). Unfortunately, the reaction of 1-isothiocyanato-2-nitrobenzene with 85% hydrazine hydrate did not take place at room temperature or even at reflux, so that we failed to prepare the counterpart of the active compound **6d**.

It can be seen from Table 2 that compounds **9a** and **9c** were inactive against the proliferation of A549, MCF-7 and HCT-116 cells (IC_{50} > 100 μM), whereas their analogs **5a** and **6d** exhibited moderate or strong antiproliferative activity against three cell lines (IC_{50}, 3.49–24.86 μM). Compound **9b** showed moderate antiproliferative



Scheme 2. Reagents and conditions: (a) 85% hydrazine hydrate, *i*-PrOH, rt, 1-5 h; (b) indoline-2,3-diones **4a**,**f**, EtOH, acetic acid, reflux, 10 h.

Table 2
Antiproliferative activity of compounds **9a–c** against three human cancer cell lines



9a-c

Compound	R^1	R^2	IC_{50} (μM) ± SD		
			A549	MCF-7	HCT-116
9a	H	H	>100[a]	>100	>100
9b	5-Cl	H	33.14 ± 1.04	35.00 ± 0.90	30.76 ± 0.21
9c	5-Cl	2,4-diCH_3	>100	>100	>100

[a] IC_{50} >100 μM indicates that cell proliferation inhibition is lower than 50% at the concentration of 100 μM.

activity against A549, MCF-7 and HCT-116 cells (IC_{50}, 33.14, 35.0 and 30.76 μM), however it was less potent than its counterpart **5f** (IC_{50}, 7.34, 10.39 and 3.49 μM) and most of compounds listed in Table 1. Although other indolin-2-one derivatives bearing *N*-phenylhydrazinecarbothioamide moiety also showed antitumor activities,[13,14] our results indicate that the presence of piperazine moiety between the hydrazinecarbothioamide moiety and phenyl ring is essential for generating or improving the antiproliferative activity.

In conclusion, we synthesized a set of piperazine-1-carbothiohydrazide derivatives of indole-2-one and evaluated for their antiproliferative activity against A549, MCF-7 and HCT-116 cell lines. Varying the substituents (R^1) at the indolin-2-one scaffold showed that the presence of a substituent at the 5-position is important for the antiproliferative activity, with 5-chloro substituted compound **5f** being the most potent with IC_{50} values of 7.34, 10.39 and 3.49 μM against A549, MCF-7 and HCT-116 cells, respectively. Moreover, introduction of a substituent (R^2) into the 2′-position, instead of 4′-position, of the phenyl group connected with piperazine ring was favorable for retaining the activity and the steric effect of substituents was more important for the potency than the electronic effect. Thus, compounds **6c** (R^2 = 2′-CH_3), **6f** (R^2 = 2′-Cl) and **6l** (R^2 = 2′-NO_2) exhibited comparable antiproliferative activity against HCT-116 cells to Sunitinib, while compound **6d** (R^2 = 2′,4′-diCH_3) was the most potent against A549 cells. Further studies to investigate the possible mechanism of action of the active compounds are currently in progress.

Acknowledgements

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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.2013.03.099.

References and notes

1. Prakash, C. R.; Raja, S. *Mini-Rev. Med. Chem.* **2012**, *12*, 98.
2. Vine, K. L.; Matesic, L.; Locke, J. M.; Ranson, M.; Skropeta, D. *Anti-Cancer Agents Med. Chem.* **2009**, *9*, 397.
3. Chow, L. Q.; Eckhardt, S. G. *J. Clin. Oncol.* **2007**, *25*, 884.
4. Krishnan, K.; Prathiba, K.; Jayaprakash, V.; Basu, A.; Mishra, N.; Zhou, B. S.; Hub, S.; Yen, Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6248.
5. Lessa, J. A.; Mendes, I. C.; da Silva, P. R. O.; Soares, M. A.; dos Santos, R. G.; Speziali, N. L.; Romeiro, N. C.; Barreiro, E. J.; Beraldo, H. *Eur. J. Med. Chem.* **2010**, *45*, 5671.
6. Yu, Y.; Kalinowski, D. S.; Kovacevic, Z.; Siafakas, A. R.; Jansson, P. J.; Stefani, C.; Lovejoy, D. B.; Sharpe, P. C.; Bernhardt, P. V.; Richardson, D. R. *J. Med. Chem.* **2009**, *52*, 5271.
7. Zeglis, B. M.; Divilov, V.; Lewis, J. S. *J. Med. Chem.* **2011**, *54*, 2391.
8. Giles, F. J.; Fracasso, P. M.; Kantarjian, H. M.; Cortes, J. E.; Brown, R. A.; Verstovsek, S.; Alvarado, Y.; Thomas, D. A.; Faderl, S.; Garcia-Manero, G.; Wright, L. P.; Samson, T.; Cahill, A.; Lambert, P.; Plunkett, W.; Sznol, M.; DiPersio, J. F.; Gandhi, V. *Leuk. Res.* **2003**, *27*, 1077.
9. Ma, B.; Goh, B. C.; Tan, E. H.; Lam, K. C.; Soo, R.; Leong, S. S.; Wang, L. Z.; Mo, F.; Chan, A. T.; Zee, B.; Mok, T. A. *Invest. New Drugs* **2008**, *26*, 169.
10. Karali, N. *Eur. J. Med. Chem.* **2002**, *37*, 909.
11. Solomon, V. R.; Hua, C. K.; Lee, H. *Bioorg. Med. Chem.* **2010**, *18*, 1563.
12. Karki, S. S.; Kulkarni, A.; Teraiya, N.; Clercq, E. D.; Balzarini, J. *Med. Chem. Res.* **2011**, *20*, 1229.
13. Hall, M. D.; Salam, N. K.; Hellawell, J. L.; Fales, H. M.; Kensler, C. B.; Ludwig, J. A.; Szakacs, G.; Hibbs, D. E.; Gottesman, M. M. *J. Med. Chem.* **2009**, *52*, 3191.
14. Hall, M. D.; Brimacombe, K. R.; Varonka, M. S.; Pluchino, K. M.; Monda, J. K.; Li, J. Y.; Walsh, M. J.; Boxer, M. B.; Warren, T. H.; Fales, H. M.; Gottesman, M. M. *J. Med. Chem.* **2011**, *54*, 5878.
15. Cao, S. L.; Feng, Y. P.; Jiang, Y. Y.; Liu, S. Y.; Ding, G. Y.; Li, R. T. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1915.
16. Cao, S. L.; Han, Y.; Yuan, C. Z.; Wang, Y.; Xiahou, Z. K.; Liao, J.; Gao, R. T.; Mao, B. B.; Zhao, B. L.; Li, Z. F.; Xu, X. *Eur. J. Med. Chem.* **2013**. http://dx.doi.org/10.1016/j.ejmech.2013.04.017.
17. Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. *J. Med. Chem.* **1979**, *22*, 855.
18. Abadi, A. H.; Abou-Seri, S. M.; Abdel-Rahman, D. E.; Klein, C.; Lozach, O.; Meijer, L. *Eur. J. Med. Chem.* **2006**, *41*, 296.