Design, synthesis and biological evaluation of novel potent MDM2/p53 small-molecule inhibitors

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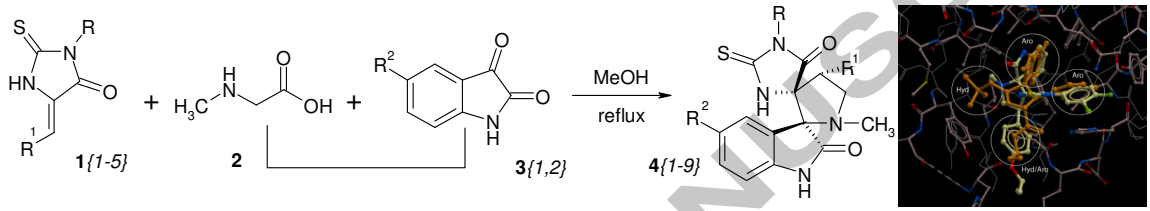

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ABSTRACT

Regioselective synthesis, biological evaluation and 3D-molecular modeling for a series of novel diastereomeric 2-thioxo-5*H*-dispiro[imidazolidine-4,3-pyrrolidine-2,3-indole]-2,5(1*H*)-diones are described. The studied compounds have been tentatively identified as potent small molecule MDM2/p53 PPI inhibitors and can therefore be reasonably regarded as promising anticancer therapeutics.

Spiro-oxindole alkaloids were first isolated from plants of the *Apocynaceae* and *Rubiacae* families.[1] The key structural feature of these compounds is the *spiro* ring fusion at position 3 of the indolinone core, with varying degrees of substitution around the pyrrolidine and indolinone moieties.

Several *spiro*-thiohydantoin derivatives are physiologically active heterocyclic compounds with a promising antitumor activity (Fig. 1).[2-5] They can also be readily used as convenient starting points or intermediates in the synthesis of a wide range of structurally diverse natural-like products.[6] A thorough search across the close structural analogues of the synthesized compounds (*vide infra*) has revealed a range of *spiro*-indolinones with promising anticancer activity (Fig. 2). These compounds were reported as highly active MDM2/p53 protein-protein interaction (PPI) inhibitors. Some of them, e.g. MI-43 and ISA-27, are currently evaluated in preclinical trials,[7,8] MI-773 is now undergoing Phase I clinical trial against cancer.[9] Absolutely brilliant examples of α-helix mimetics can be found among these compounds. For instance, highly active MDM2/p53 inhibitors with IC_{50} values in the range of 24.1 nM - 181 μM were published by Zhao and co-workers.[10] *Dispiro* analogues are described much poorly and include aryl-substituted (3"R)-4,4-dimethyldispiro[cyclohexane-1,2'-pyrrolidine-3',3"-indol]-2"(1"*H*)-ones by Daiichi Sankyo (Fig. 2).[11] These agents inhibited MDM2/p53 PPI with IC_{50} values in the range of 0.001-0.05 μM resulted in an effective blockage of human lung cancer NCI-H460 cell proliferation with wild type p53 (GI_{50}<0.1 μM in MTT assay).

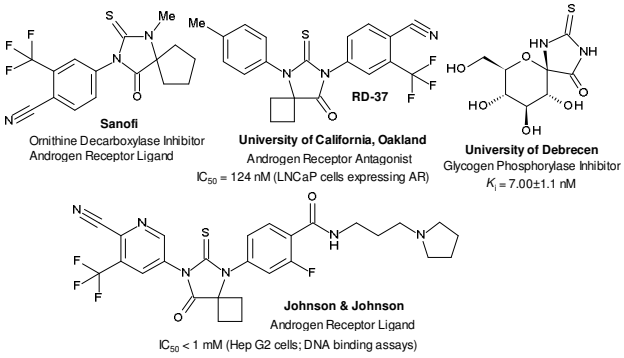


Figure 1.Several examples of physiologically active *spiro*-thiohydantoin derivatives

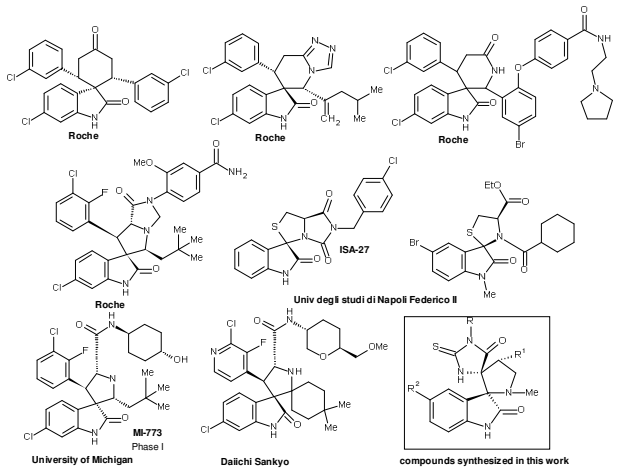


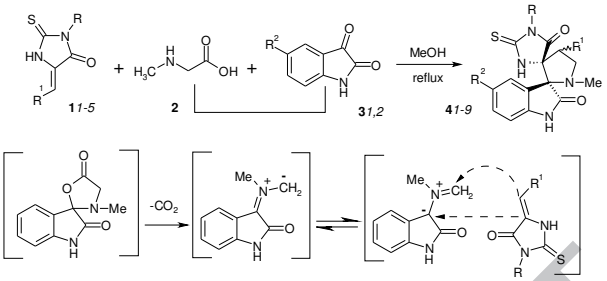
Figure 2. Representative examples of known MDM2/P53 PPI inhibitors and compounds synthesized in this work

Therefore, elegant coupling of two privileged scaffolds (*spiro*-oxindole and 2-thiohydantoin moieties) in the same molecule presumably provides compounds with a wide spectrum of physiological activity, including the most paramount anticancer indication.

Several synthetic routes to novel *dispiro*-indolinones were reported. Recently, Ouyang and co-workers[12] have described a convenient synthetic approach to novel substituted *dispiro*-oxindole derivatives (yields: 82-90%) obtained by the three-component 1,3-dipolar cycloaddition of azomethineylides with the dipolarophile 5-benzylideneimidazolidine-2,4-dione. The intermediate was generated *in situ* by the decarboxylative condensation of isatin and an α-amino acid. Sun and colleagues have recently published a versatile synthesis of novel *dispiro*-oxindole-fused heterocycles (yields: 78-89%) via the three-component reaction on the basis of domino 1,4-dipolar addition and Diels-Alder reaction.[13]

Here we describe an efficient regioselective synthesis of novel substituted 2-thioxo-5*H*-dispiro[imidazolidine-4,3-pyrrolidine-2,3-indole]-2,5(1*H*)-diones by dipolar cycloaddition of azomethineylides generated *in situ* by the decarboxylative condensation of isatin and sarcosine (*N*-methyl substituted glycine) through lactone decomposition with diaryl-substituted 2-thioxoimidazol-4-ones by analogy to the approach described by Ouyang(Scheme 1).[12]

Scheme 1



Cmpd	R	R^1	R^2
4*{1}*	3,4-ClFC$_6$H$_3$	4-O(*i*-C$_3$H$_7$)C$_6$H$_4$	H
4*{2}*	3,4-ClFC$_6$H$_3$	4-ClC$_6$H$_4$	H
4*{3}*	3,4-ClFC$_6$H$_3$	4-O(*i*-C$_3$H$_7$)C$_6$H$_4$	Br
4*{4}*	4-OC$_2$H$_5$C$_6$H$_4$	4-ClC$_6$H$_4$	H
4*{5}*	3,4-ClFC$_6$H$_3$	4-C$_2$H$_5$C$_6$H$_4$	H
4*{6}*	4-OC$_2$H$_5$C$_6$H$_4$	4-ClC$_6$H$_4$	Br
4*{7}*	3,4-ClFC$_6$H$_3$	4-ClC$_6$H$_4$	Br
4*{8}*	3,4-ClFC$_6$H$_3$	4-C$_2$H$_5$C$_6$H$_4$	Br
4*{9}*	Ph	2-Py	Br

According to the approach depicted in Scheme 1, the target compounds **4***{1-9}* can be readily obtained in two-step one-pot format. Thus, the mixture of commercially available 2-thioxoimidazol-4-one **1***{1-5}*, sarcosine (with four-fold excess) and isatin**3**{*1,2}* in methanol was refluxed for 2-4 h. After the reaction was completed (TLC control) the formed precipitate was filtered and washed with methanol. The desired diastereomerically pure *dispiro*-indolinones**4***{1-9}* were obtained in moderate-*to*-high yields (40-90%) comparable to the approaches mentioned above.All the synthesized compounds were sufficiently characterized by ^{1}H NMR, LCMS spectra as well as elemental analysis. Satisfactory analytical data consistent with the shown molecular structures were obtained for all novel compounds described (*see supporting information*).

The reaction presumably proceeded following the reported mechanism of such condensation. Thus, immediately after formation, the intermediate is trapped with Knoevenagel adduct 2-thioxoimidazol-4-one acting as dipolarophile, to afford the desired *dispiro*-indolinones. Although the azomethineylides furnish the chiral center occurred through possible resonance structures, only diastereometric pure products with (*S,S,R*)-configuration was formed on the basis of X-Ray crystallographic analysis (Fig. 3). The detailed description of RSA data is provided in supplementary materials.

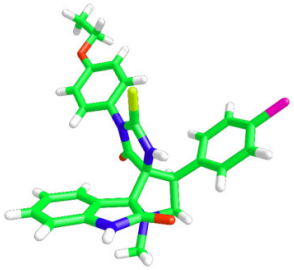


Figure 3. 3D-representation of the obtained X-Ray data for compound **4***{4}*

It may be due to more appropriate transition state for regioselective condensation that is stabilized by planar dipole-dipole interaction observed between NH-moiety of

thioxoimidazole fragment and carbonyl group of indolinone core. Moreover, steric clashes can also be accrued between a relatively bulky phenyl ring of the dipolarophile and indolinone upon dipolar cycloaddition. It can be further speculated that only *cis*-isomer of thioxoimidazole can provide the *spiro*-joint by itself or by Z to E racemization in solution under the applied reaction conditions catalyzed by transition state stabilization. Following the same approach several selenium analogues have also been synthesized using various selenoxo-imidazol-4-ones as a starting point (*this data is not shown*). In turn, the overall aim of this work lies in a comparative study of the obtained compounds against MDM2/p53 PPI.

All the synthesized *dispiro*-indolinones have then been tested on their ability to block the proliferation of the selected model cell lines as well as apoptosis induction. These models included a well-differentiated hepatocellular carcinoma perpetual cell line (HepG2), human embryonic kidney 293 cells (Hek293), breast cancer cell line (MCF-7), human cervical cancer cells (SiHa) and human colon cancer cells (HCT116). The cytotoxicity of the tested substances was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl tetrazolium bromide) assay based on the modified approach reported by Ferrari and colleagues.[14] The detailed methodology of the performed cell-based test is provided in supplementary materials. The resulting cytostatic potency is summarized in Table 1.

Table 1. The activity of the synthesized compounds against different cell lines

Cmpd	IC_{50} (μM)					
	HepG2	Hek	MCF-7	SiHa	HCT116 $P53^{-/-}$	HCT116 $P53^{+/+}$
4*{1}*	49.94±14.39	13.47±0.23	20.22±1.22	na*	7.4±0.1	8.55±0.5
4*{2}*	9.08±1.38	5.68±0.29	4.88±1.5	7.65±1.00	7.33±0.11	7.78±0.23
4*{3}*	34.58±12.06	15.84±0.58	34.88±9.6	na	19.43±0.74	38.75±8.9
4*{4}*	103.06±35.13	9.10±3.24	87.65±15.2	81.68±17.27	8.57±0.11	8.6±0.07
4*{5}*	33.87±5.34	12.53±3.05	18.96±7.9	42.49±26.74	8.45±0.11	8.6±0.48
4*{6}*	42.08±0.71	28.61±8.68	34.90±6.4	na	16.3±6.7	≥50
4*{7}*	11.05±0.10	10.31±4.25	6.84±1.7	12.14±0.37	8.65±0.27	9.9±0.26
4*{8}*	23.02±0.83	15.62±3.34	15.52±2.31	79.13±25.75	33.45±1.49	11.63±0.65

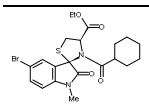
* - not active

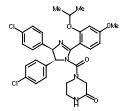
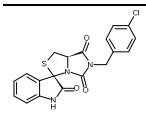
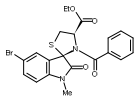
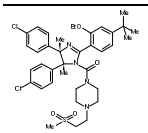
As shown in Table 1, the most potent compound from this series (**4***{2}*) exhibited an IC_{50} value in the range of 4.88-10.46 μM against the panel. However, no relevant selectivity was observed among the cell types. Three compounds **4***{1,3,6}* were found to be selective towards HepG2, Hek and MCF-7 cells with no activity against SiHa cells. Compounds **4***{4,5,7}* showed moderate potency. The negative response of SiHa cells to the treatment with compounds **4***{1,3,6}* can be associated with MDM2/p53 mode of action due to p53 tumor suppressor pathway in this cell type is in most cases disrupted by human papillomavirus (HPV).[15] The activated p53 induces the transcription of MDM2, which can directly interact with transactivation domain of p53 thereby inhibiting its transcription activity by targeting it for polyubiquitination and further proteasome-mediated degradation.[16] In many cancer cells, including HepG2, Hek and MCF-7, the overexpression of MDM2 gene is actually observed resulting in significant apoptosis attenuation. However, some confused outcomes have been obtained. For instance, compound **4***{4}* inhibited SiHa proliferation with an IC_{50} value of 81.68±17.27 μM, in contrast to its close structural analogue, compound **4***{6}*, which showed no activity. To clear the dominate mode of action we further used HCT116 (p53 negative, $p53^{-/-}$) and HCT116 (wild type, $p53^{+/+}$) cells by analogy with the paper published by Shangary and colleagues.[17] This isogenic cell line is commonly applied to prove the p53/MDM2-depended mode of action. Thus, only one compound **4***{8}* showed a statistically significant selectivity against HCT116 ($p53^{+/+}$) over HCT116 ($p53^{-/-}$), with IC_{50}values of 12 and 34 μM, respectively. Under the same conditions Nutlin-3a was found to be more active than the hit molecule and demonstrated IC_{50} values of 3.3±0.13 μM and 35.12±2.65 μM, respectively. In terms of selectivity index (SI) this difference can be expressed as 2.87 (compound **4***{8}*) and 10.64 (Nutlin), respectively. Subsequent structural optimization of the hit compound can markedly enhance the target activity and selectivity. Moreover, the diastereomeric mixture was actually evaluated, therefore we are currently carrying out the separation procedure to isolate individual isomers.

In addition, a proper SAR study can`t be adequately performed because of insufficient data. A protein-based assay specific for MDM2/p53 PPI is strongly needed to confirm the target activity of the synthesized compounds. Interestingly, there are at least two close structural *spiro*-analogues to the compounds described herein. These compounds inhibit two different intramolecular targets. The first one is the reported MDM3/p53 inhibitor, ISA-27 (*see above*), the second molecule, Isorhynchophylline (IsoRh), has recently been found to induce autophagy through Beclin-1 (BECN1) axis, independent of the mTOR pathway, but PI3KIII-stimulated.[18]This compound was also shown to attenuateGSK-3β activity via the activation of Akt kinase by PI3K.[19] In PC12 cells, IsoRh showed a significant protective effect against Aβ 25-35-induced apoptosis through the enhancement of p-CREB expression via PI3K/Akt/GSK-3β signaling route.[20] It was also demonstrated that BECN1 is an essential target for Akt kinase.[21]BECN1 shares at least three common domain recognition elements (DREs), including BH3 domain,which is specifically recognized by anti-apoptotic proteins Bcl-2/Bcl-xL thereby strongly inhibiting the BECN1-dependent autophagy.[22]We have, therefore, concluded that some of the tested compounds, particularly which are not selective towards SiHa cell line, can interact with other targets including Bcl-2/BECN1 PPI because they are hydrophobic *i,i*+4,*i*+7α-helix mimetics as many of P53/MDM2 inhibitors.

We also have summarized the available data outputted from analogous cell-based tests (Dye assay/MTT) performed for known MDM2/p53 inhibitors, with the main focus on *spiro*-indolinone scaffold (Table 2). For example, a rough comparative analysis has shown that the activity of the most potent compound within our series with an IC_{50} value of 4.88±1.5 μM against MCF-7 cells is quite comparable with Nutlin-3a and its direct analogues, while approx. 100 times less potent than two 2-oxo-1,2-dihydrospiro[indole-3,2'-[1,3]thiazolidines with an IC_{50} value of 0.04±0.01μM and 0.06±0.05μM, respectively. Therefore, an extensive *in*-house research program has recently been initiated towards the optimization of the primarily hits.

Table 2. The results of evaluation of several MDM2/p53 inhibitors against the cell lines similar to that used in this work

Structure	IC_{50}* or CC_{50} (μM)			
	HepG2	HEK293	MCF-7	Ref
	IC_{50}=0.14 ±0.06	-	IC_{50}=0.04 ±0.01	23

 Nutlin-3a	IC_{50}=5.1-52.3	-	IC_{50}=2.9 ±0.31	24
 ISA-27	-	CC_{50}=0.44±0.01	-	25
	-	-	IC_{50}=0.06 ±0.05	26
	-	-	CC_{50}=0.94 (Cytotoxicity, 120 h)	27

* mitogenesis inhibition

Currently, more than 50 crystallographic complexes obtained for various small-molecule MDM2/p53 inhibitors, including *spiro*-indolinones, are available within PDB databank.[28] To elucidate the possible binding affinity of the synthesized compounds towards MDM2, a static 3D molecular docking study was performed in ICM-Pro software[29] based on several X-Ray data, including 4MDQ, 4JVR and 4JWR (Fig. 4).

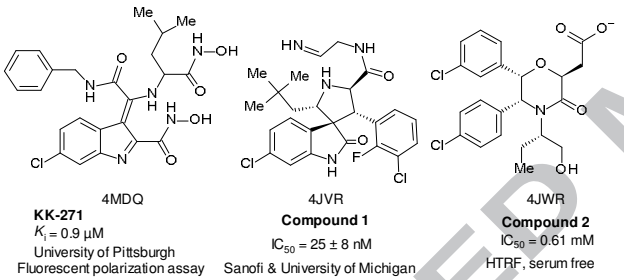


Figure 4. MDM2/p53 inhibitors used for 3D model construction and self-validation

The binding site was re-constructed and compared with the binding mode revealed recently for recombinant p53 binding domain (residues 17-125).[30] Water molecules were removed from the site. The reference compounds were then docked into the constructed model starting form 2D structures without any stereo assignment. The obtained results are well correlated with the RSA data used (av. RMSD=0.2, Fig. 5). All the synthesized compounds were then docked into the static pocket using an extensive range of key force-fields, particularly describing hydrophobic interactions. As shown in Fig. A.5, three template molecules have a very similar binding mode, as revealed by the 3D alignment of the selected crystals. Interestingly, two different binding modes have been proposed for the novel compounds **4***{1-9}*. Thus, the first binding mode is the same as observed for the reference ligands, particularly for *spiro*-indolinone **1** (Fig. B.5). At least one alternative binding mode (Fig.C.5) that was more benefit in terms of the score function applied positioned the indolinone core targeting the area occupied by *tret*-butyl anchor of compound 1, while the methyl group attached to pyrrolidine moiety had a similar location as that observed for oxoindole fragment. All crucial binding points are highlighted with circles and overlap exactly with the core PPI interface (Fig. D.5).

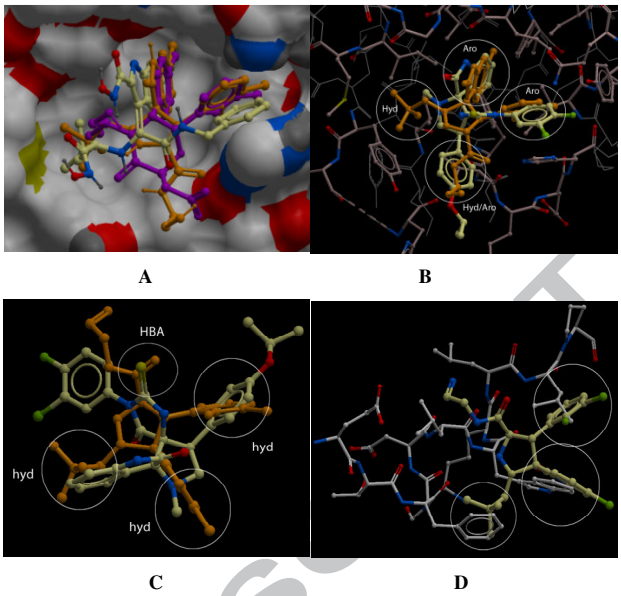


Fig. 5. (**A**) overlapping of KK-271 (*yellow*), Compound **1** (*orange*) and **2** (*violet*) in the P53 binding site of MDM2 (*RSA data*); (**B**) Compound **1** (*orange*, *RSA data*) and compound **4***{1}* (yellow, docking study); (**C**) alternative binding mode for compound **4***{1}*; (**D**) Compound **2** (*yellow*) superposed with P53 domain recognition element (DRE) (*RSA data*) - 3 key hydrophobic binding points, typical α-helix mimetic core

Although the cell-based assay that was carried out in this work did not elucidate the underlying mechanism of action for the evaluated compounds, this scaffold brings novelty and possesses the 3D-pharmacophore elements crucial for binding as compared to the reported MDM2/p53 PPI inhibitors, including *spiro*-indolinones. Indeed, a specific protein-based assay is urgently needed to clear their mode of action. The results obtained from the presented molecular docking study, biological trials as well as 2D-structural similarity analysis put the main focus primarily on MDM2 as the most reliable biological target. This affinity will be disclosed within the following paper.

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References and notes

1. Galliford, C. V.; Scheidt, K. A. Angew. Chem. Int. Ed. 2007, 46, 8748.
2. Somsak, L.; Kovacs, L.; Toth, M.; Osz, E.; Szilagyi, L.; Gyoergydeak, Z.; Dinya, Z.; Docsa, T.; Toth, B.; Gergely, P. J. Med. Chem. 2001, 44, 2843.
3. Sawyers, C. L. U.S. Patent application US8658681B2, 2014.
4. Jung, M. E.; Ouk, S.; Yoo, D.; Sawyers, C. L.; Chen, C.; Tran, C.; Wongvipat, J. J. Med. Chem. 2010, 53, 2779.
5. Smith, N.D. U.S. Patent 2013116258, 2013.
6. Lanman, B. A.; Overman, L. E.; Paulini, R.; White, N. S. J. Am. Chem. Soc. 2007, 129, 12896.
7. Carlson, H. A.; Zhong, H.; Bowman, A.L. Presented at the 231st National Meeting of the American Chemical Society, Atlanta, GA, March 2006. Abstract MEDI 229.
8. Gomez-Monterrey, I.; Bertamino, A.; Porta, A.; Carotenuto, A.; Musella, S.; Aquino, C.; Granata, I.; Sala, M.; Brancaccio, D.; Picone, D.; Ercole, C.; Stiuso, P.; Campiglia, P.; Grieco, P.; Ianelli, P.; Maresca, B.; Novellino, E. J. Med. Chem. 2010, 53, 8319.

9. ClinicalTrials.gov Identifier: NCT01636479. http://clinicaltrials.gov/show/NCT01636479 [date last accessed April 25, 2014].
10. Zhao, Y.; Liu, L.; Sun, W.; Lu, J.; McEachern, D.; Li, X.; Yu, S.; Bernard, D.; Ochsenbein, P.; Ferey, V.; Carry, J. C.; Deschamps, J. R.; Sun, D.; Wang, S. J. Am. Chem. Soc. 2013, 135, 7223.
11. Sugimoto, Y. U.S. Patent application 20130165424A9, 2012.
12. He, J.; Ouyang, G.; Yuan, Z.; Tong, R.; Shi, J.; Ouyang, L. Molecules 2013, 18, 5142.
13. Sun, J.; Sun, Y.; Gong, H.; Xie, Y. J.; Yan, C. G. Org. Lett. 2012, 14, 5172.
14. Ferrari, M.; Fornasiero, M. C.; Isetta, A. M. J. Immunol. Methods 1990, 131, 165.
15. Hietanen, S.; Lain, S.; Krausz, E.; Blattner, C.; Lane, D. P. Activation of p53 in cervical carcinoma cells by small molecules. Proc. Natl. Acad. Sci. USA 2000, 97, 8501.
16. Haupt, Y.; Maya, R.; Kazaz, A.; Oren, M. Nature 1997, 387, 296.
17. Shangary, S.; Qin, D.; McEachern, D.; Liu, M.; Miller, R. S.; Qiu, S.; Nikolovska-Coleska, Z.; Ding, K.; Wang, G.; Chen, J.; Bernard, D.; Zhang, J.; Lu, Y.; Gu, Q.; Shah, R. B.; Pienta, K. J.; Ling, X.; Kang, S.; Guo, M.; Sun, Y.; Yang, D.; Wang. S. Proc. Natl. Acad. Sci. USA. 2008, 105, 3933.
18. Lu, J. H.; Tan, J. Q.; Durairajan, S. S.; Liu, L. F.; Zhang, Z. H.; Ma, L.; Shen, H. M.; Chan, H. Y.; Li, M. Autophagy. 2012, 8, 98.
19. Xian, Y. F.; Mao, Q. Q.; Wu, J. C.; Su, Z. R.; Chen, J. N.; Lai, X. P.; Ip, S. P.; Lin, Z. X. J. Alzheimers. Dis. 2014, 39, 331.
20. Xian, Y. F.; Lin, Z. X.; Mao, Q. Q.; Chen, J. N.; Su, Z. R.; Lai, X. P.; Ip, P. S. Evid. Based. Complement. Alternat. Med. 2013, 2013, 163057.
21. Wang, R. C.; Wei, Y.; An, Z.; Zou, Z.; Xiao, G.; Bhagat, G.; White, M.; Reichelt, J.; Levine, B. Science. 2012, 338, 956.
22. Fu, L. L.; Cheng, Y.; Liu, B. Int. J. Biochem. Cell. Biol. 2013, 45, 921.
23. Bertamino, A.; Soprano, M.; Musella, S.; Rusciano, M. R.; Sala, M.; Vernieri, E.; Di Sarno, V.; Limatola, A.; Carotenuto, A.; Cosconati, S.; Grieco, P.; Novellino, E.; Illario, M.; Campiglia, P.; Gomez-Monterrey, I. J. Med. Chem. 2013, 56, 5407.
24. Endo, S.; Yamato, K.; Hirai, S.; Moriwaki, T.; Fukuda, K.; Suzuki, H.; Abei, M.; Nakagawa, I.; Hyodo, I. Cancer Sci. 2011, 102, 605.
25. Gomez-Monterrey, I.; Bertamino, A.; Porta, A.; Carotenuto, A.; Musella, S.; Aquino, C.; Granata, I.; Sala, M.; Brancaccio, D.; Picone, D.; Ercole, C.; Stiuso, P.; Campiglia, P.; Grieco, P.; Ianelli, P.; Maresca, B.; Novellino, E. J. Med. Chem. 2010, 53, 8319.
26. Bertamino, A.; Soprano, M.; Musella, S.; et al. J Med Chem 2013, 56(13): 5407
27. Tovar, C.; Graves, B.; Packman, K.; Filipovic, Z.; Higgins, B.; Xia, M.; Tardell, C.; Garrido, R.; Lee, E.; Kolinsky, K.; To, K. H.; Linn, M.; Podlaski, F.; Wovkulich, P.; Vu, B.; Vassilev, L. T. Cancer. Res. 2013, 73, 2587.
28. The official website of RCSB Protein Data Bank (PDB): http://www.rcsb.org [date last accessed April 25, 2014].
29. The official website of Molsoft LLC: https://www.molsoft.com [date last accessed April 25, 2014].
30. Anil, B.; Riedinger, C.; Endicott, J. A.; Noble, M. E. ActaCrystallogr. Sect. D 2013, 69, 1358.

Supplementary Material

A sufficient analytical data for the synthesized compounds including ^{1}H NMR, LCMS spectra, X-Ray dataas well as elemental analysis are presented in supplementary materials.