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Discovery of heterocyclic substituted dihydropyrazoles as potent anticancer agents

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ABSTRACT

In this work, a series of novel heterocyclic substituted dihydropyrazole derivatives have been prepared, and *in vitro* anticancer activity against a panel of human tumor cell lines by SRB were evaluated. The results indicated that piperazine substituted dihydropyrazole derivatives exhibited superior anticancer activity than that of other compounds. Especially, compounds **4g**, **4h**, **4l**, **4m**, **4o**, **6g**, **6j** and **6l** showed potent antitumor activity. Further mechanism study demonstrated that compound **4o** could induce G2/M arrest in HCC1806 cell and p21 accumulation significantly.

Cancer is a large family of diseases resulting from uncontrolled cell growth, which has gradually become the leading cause of deaths around the world. The latest data from IARC revealed that cancer burden rised to 19.3 million new cases and 10.0 million cancer deaths in 2020.[1] Although a great many of manpower and material resources are consumed, there is no currently safe and effective agent treating the cancers. Therefore, cancer is a serious and persistent threat to human health, and the development of new anticancer agents and effective therapy strategies for cancer has been given more constant attention.

Natural dihydropyrazoles and derivatives are a class of important *N*-containing heterocycles, which exhibit a variety of biological activities, such as antioxidant, anti-inflammatory, antiviral and anticancer.[2–5] In recent years, many reports indicate that dihydropyrazole derivatives can be used as selective inhibitors to kill cancer cells,[6–7] so many researchers pay more attention to these compounds. Liu *et al* have discovered a series of 4,5-dihydro-2*H*-pyrazole 2-hydroxyphenyl derivatives showing good anticancer activity as BRAF inhibitors.[8] Cox *et al* has reported 4,5-dihydropyrazole derivatives as a potent, selective inhibitor of Kinesin spindle protein.[9] Wu *et al* have found that 1-(3-substituted-5-phenyl-4,5-dihydropyrazol-1-yl)-2-thioethanone derivatives showed anti-proliferative activity against several cell lines as potential telomerase inhibitors.[10] In our previous work, a series of 3-aryl-5-furanyl-4, 5-dihydropyrazole derivatives were synthesized exhibiting good anticancer activity against A549, Hela and SGC7901.[11]

Heterocyclic compounds, especially *N*-containing heterocycles, a class of important small organic molecules, which have attracted more and more interests due to their various properties in organic chemistry and pharmacology. For instance,

piperazine, imidazole and triazole are introduced in pharmaceutical molecules as the bioactive core units, such as fluconazole, floxacins, ketoconazole and so on. In former work, we have reported the synthesis of a series of heterocyclic substituted chalcones and benzofurans bearing potent anticancer activity.[12–15] Considering the potent anticancer activity of dihydropyrazoles as well as the broad activities of *N*-containing heterocycles, we were interested in designing and synthesizing a kind of novel heterocyclic substituted 4, 5-dihydropyrazole derivatives (Scheme 1),[16] and *in vitro* antitumor activity against a panel of human tumor cell lines as well as preliminary mechanism have been evaluated.

The general synthetic route is outlined in Scheme 2. Heterocyclic substituted chalcones (**2**) were prepared according to our former work,[17] Then, treatment of compound **2** with hydrazine hydrate in reflux EtOH gave the heterocyclic substituted dihydropyrazoles (**3**), which were purified by recrystallization. Finally, 46 new heterocyclic substituted dihydropyrazole derivatives (**4**–**6**) were prepared in excellent yields by reaction of key intermediate **3** with the corresponding acyl chloride or sulfonyl chloride in presence of K_2CO_3. To probe the

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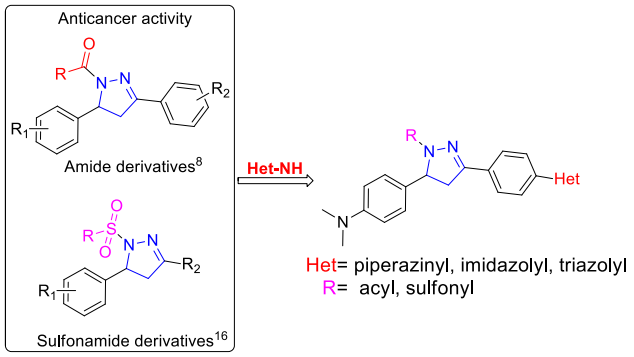


Scheme 1. Design of new heterocyclic substituted dihydropyrazoles.

influence of types of groups (electron donating and electron withdrawing) on antitumor activity and investigate the structure–activity relationships (SAR), various acyl and sulfonyl bearing substituents (X, alkyl, alkoxy, CN and CF_3, etc) were introduced into the derived groups of the title derivatives. Comparative data for new dihydropyrazole compounds with respective to structures, formula, melting point and yields were shown in Table 1. All of the synthesized compounds were characterized by ^{1}H NMR and ^{13}C NMR, and some representative compounds were characterized by HRMS analysis.

Anticancer activity: Initially, preliminary anticancer effect of new synthesized dihydropyrazole derivatives were evaluated on human breast cancer cell line (HCC1806), human breast cancer cell line (MDA-MB-231) and human lung cancer cell line (NCI-H1975) by sulforhodamine B (SRB). The inhibition rate of cancer cell lines under the dose of 10 μM were summarized in Table 2.

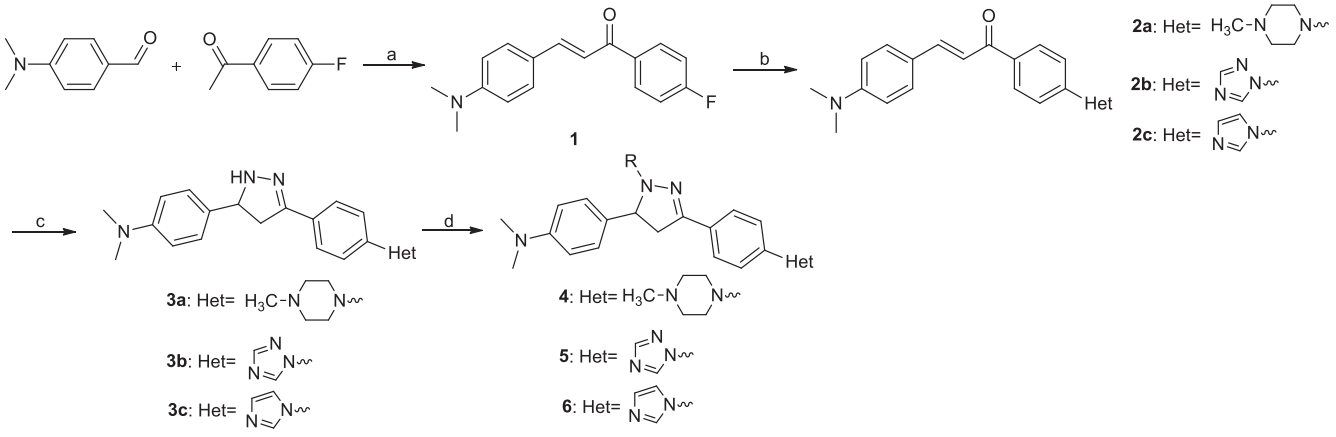
As shown in Table 2, some compounds showed good anticancer activity at a dose of 10 μM. In general, piperazinyl dihydropyrazole derivatives (**4**) displayed the better cytotoxic activity against three cancer cells than that of triazolyl (**5**) and imidazolyl products (**6**). Among all derivatives, compounds **4g**, **4h**, **4l**, **4m**, **4o** and **6j** displayed the potential cytotoxic activity against HCC1806, compounds **4g**, **4o** and **4j** showed good antitumor activity against MDA-MB-231, compounds **4g**, **4h**, **4l**, **4m**, **4o**, **6g**, **6j** and **6l** displayed selective inhibition activity against NCI-H1975 (inhibition rate > 50%, respectively). Especially, the inhibition rate of compound **4g** was 93.2% against NCI-H1975, **4l** was over 90% against HCC1806 and NCI-H1975, and **4o** was up to 100% against three cancer cell lines, respectively. Besides, other compounds displayed low cytotoxic activity against three cancer cells (inhibition rate < 50%, respectively). The preliminary result indicated that heterocycle of title compounds had obvious influence on anticancer activity, and piperazine unit played a vital role in activity. Furthermore, there were two series of dihydropyrazole derivatives including amide and sulfamide. Among them, sulfamides showed better cytotoxic activity than amides. Especially, derivative **4c** exhibited the worst anticancer activity against three cell lines at a dose of 10 μM (inhibition rate = -8.3%, −0.4% and −2.2%, respectively), derivative **4o** exhibited the best anticancer activity against three cell lines (inhibition rate = 99.8%, 99.8% and 100%, respectively).

To explore the structure–activity relationship (SAR) towards potent anticancer activity of heterocyclic substituted dihydropyrazoles against three cancer cell lines, various groups including EWG and EDG on the phenyl ring as well as aliphatic of the derivatization functionality were introduced. We observed that compounds containing electron-withdrawing substituents and halogen contributed to potential antitumor activity, such as CF_3, CN, F and Br. However, EDG led to poor cytotoxic activity.

In addition, in order to contrast the anticancer effect of potent compounds (**4g**, **4h**, **4l**, **4m** and **4o**) on other cancer cells, the cytotoxic activity against human colorectal cancer cell line (HCT116), cervical carcinoma cell line (HeLa) and lung cancer cell line (A549) were carried out. As shown in Table 3, the result showed that five compounds displayed selective inhibition against four cancer cell lines (IC_{50} < 10 μM). Especially, compound **4o** showed the best potent antitumor activity (IC_{50} < 3 μM). We further evaluated the time-dependent efficacy of compound **4o**. Compound **4o** showed max efficacy after treatment for 48 h through the evaluation of cell inhibition rate in different time points after treatment in HCC1806 cells (Fig. 1). Then, we carried out the cell cycle arrest effects of compound **4o** in HCC1806 cells. After treated with compound **4o**, cells of the G2/M phase were increased, while cells of the G1 and S phase were decreased (Fig. 2).

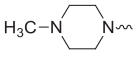
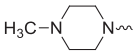
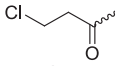
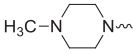
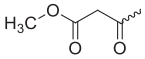
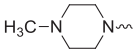
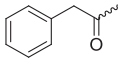
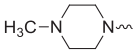
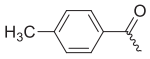
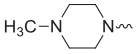
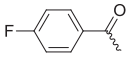
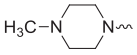
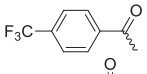
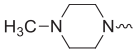
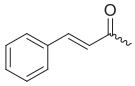
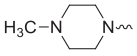
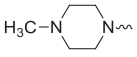
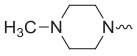
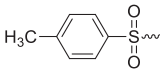
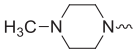
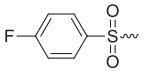
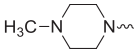
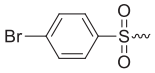
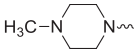
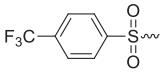
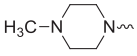
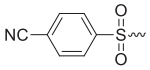
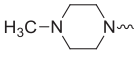
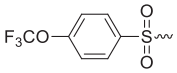
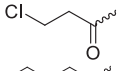
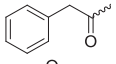
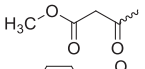
Furthermore, $p21^{Waf1/Cip1}$ is cyclin-dependent kinase inhibitor, which can arrest cell cycle progression in response to many stimuli, including antitumor compounds. p21 promote cell cycle arrest in G1 and G2/M phase through inhibiting CDK4,6/cyclin-D and CDK2/cyclin-E,[18] respectively. Therefore, we detected the change of p21 protein level after treatment with indicated compound **4o** for 12 h in HCC1806 cells. Western blot showed that compound **4o** induced p21 accumulation significantly (Fig. 3).

In conclusion, a series of heterocyclic substituted dihydropyrazoles have been designed and synthesized. We evaluated the *in vitro* anticancer activities of derivatives against three human cancer cell lines. The results indicated that piperazinyl dihydropyrazole compounds **4g**, **4h**, **4l**, **4m**, **4o**, **6g**, **6j** and **6l** showed the better cytotoxic activity, especially compound **4o** displayed showed the best potent antitumor activity. Further mechanism study demonstrated that compound **4o** could induce G2/M arrest in HCC1806 cell and p21 accumulation significantly, which could be considered as a potent anticancer agent,



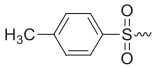
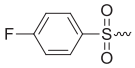
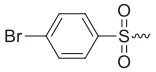
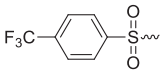
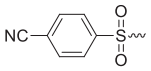
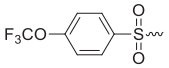
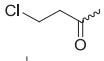
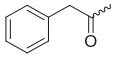
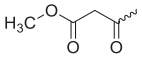
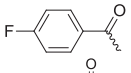
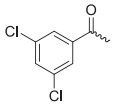
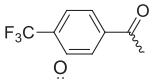
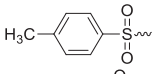
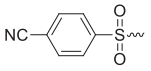
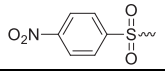
Scheme 2. Synthetic routes of dihydropyrazole derivatives. *Conditions*: (a) 20% KOH, EtOH, rt, 6 h; (b) K_2CO_3, Het-NH, DMF, 110 °C, 12–24 h; (c) Hydrazine hydrate, EtOH, reflux, 12 h; (d) RCOCl or RSO_2Cl, K_2CO_3, DCM, rt, 2–5 h.

Table 1
Structures and yields of title compounds.

Compound	Het	R	Molecular formula	M. p (°C) [a]	Yields (%) [b]
4a			$C_{24}H_{31}N_5O$	191–193	92
4b			$C_{25}H_{32}ClN_5O$	182–184	86
4c			$C_{26}H_{33}N_5O_3$	190–192	83
4d			$C_{30}H_{35}N_5O$	198–200	84
4e			$C_{30}H_{35}N_5O$	205–207	85
4f			$C_{29}H_{32}FN_5O$	193–195	87
4g			$C_{30}H_{32}F_3N_5O$	179–181	87
4h			$C_{31}H_{35}N_5O$	173–175	82
4i			$C_{23}H_{31}N_5O_2S$	157–159	80
4j			$C_{25}H_{33}N_5O_2S$	144–146	84
4k			$C_{29}H_{35}N_5O_2S$	177–179	86
4l			$C_{28}H_{32}FN_5O_2S$	180–182	84
4m			$C_{28}H_{32}BrN_5O_2S$	201–203	78
4n			$C_{29}H_{32}F_3N_5O_2S$	172–174	80
4o			$C_{29}H_{32}N_6O_2S$	165–167	83
4p			$C_{29}H_{32}F_3N_5O_3S$	156–158	88
5a			$C_{21}H_{22}N_6O$	213–215	90
5b			$C_{22}H_{23}ClN_6O$	175–177	82
5c			$C_{27}H_{26}N_6O$	187–189	84
5d			$C_{23}H_{24}N_6O_3$	162–164	79
5e			$C_{26}H_{23}FN_6O$	184–186	84
5f			$C_{26}H_{22}Cl_2N_6O$	206–208	82
5g			$C_{27}H_{23}F_3N_6O$	211–213	80
5h			$C_{28}H_{26}N_6O$	167–169	83
5i			$C_{20}H_{22}N_6O_2S$	154–156	78
5j			$C_{26}H_{26}N_6O_2S$	196–198	81

(*continued on next page*)

Table 1 (*continued*)

Compound	Het	R	Molecular formula	M. p (°C) [a]	Yields (%) [b]
					
5k			$C_{25}H_{23}FN_6O_2S$	201–203	84
5l			$C_{25}H_{23}BrN_6O_2S$	224–226	86
5m			$C_{26}H_{23}F_3N_6O_2S$	209–211	80
5n			$C_{26}H_{23}N_7O_2S$	224–226	85
5o			$C_{26}H_{23}F_3N_6O_3S$	185–187	89
6a			$C_{22}H_{23}N_5O$	230–232	88
6b			$C_{23}H_{24}ClN_5O$	176–178	78
6c			$C_{24}H_{25}N_5O$	202–204	76
6d			$C_{28}H_{27}N_5O$	183–185	85
6e			$C_{24}H_{25}N_5O_3$	161–163	80
6f			$C_{27}H_{24}FN_5O$	209–212	83
6g			$C_{27}H_{23}Cl_2N_5O$	220–222	81
6h			$C_{28}H_{24}F_3N_5O$	171–173	82
6i			$C_{21}H_{23}N_5O_2S$	172–174	72
6j			$C_{27}H_{27}N_5O_2S$	159–161	84
6k			$C_{27}H_{24}N_6O_2S$	194–196	81
6l			$C_{26}H_{24}N_6O_4S$	168–170	79

[a] Uncorrected temperature.
[b] Isolated yields.

Table 2
In vitro inhibition rate of compounds against cancer cell lines under the dose of 10 μM.

Compound	Inhibition rate (%) [a]		
	HCC1806	MDA-MB-231	NCI-H1975
3a	31.2	11.4	2.4
3b	6.8	7.0	−3.3
3c	−20.9	7.6	0.1
4a	−0.8	−1.0	−1.9
4b	1.7	2.4	−0.2
4c	−8.3	−0.4	−2.2
4d	1.0	1.9	−2.8
4e	−2.5	−0.7	−0.4
4f	31.8	3.5	32.2
4g	**84.1**	**55.4**	**93.2**
4h	**71.0**	35.9	**81.0**
4i	−18.5	0.6	−1.3
4j	4.7	3.7	0.2
4k	32.2	10.7	38.1
4l	**92.3**	15.7	**95.7**
4m	**87.1**	23.7	**70.8**
4*n*	27.0	12.1	18.9
4o	**99.8**	**99.8**	**100.0**
4p	70.8	10.6	26.5
5a	11.0	7.4	−0.3
5b	−3.4	3.7	−0.7
5c	−6.9	2.9	−4.0
5d	−7.8	6.8	1.5
5e	15.6	12.5	−1.1
5f	5.3	9.4	8.4
5g	7.0	16.7	11.5
5h	−3.7	8.5	−2.3
5i	10.8	7.8	−3.1
5j	27.7	20.7	20.0
5k	24.1	12.1	−0.5
5l	26.3	10.4	−0.6
5m	37.0	15.3	5.5
5n	33.1	32.8	22.6
5o	25.2	18.5	11.1
6a	−9.7	5.0	−2.1
6b	−5.2	2.1	−2.5
6c	1.6	4.3	−3.7
6d	2.0	7.2	−5.5
6e	2.1	2.3	−0.1
6f	7.1	9.4	−3.5
6g	44.5	48.8	**58.7**
6h	28.0	17.9	15.9
6i	11.3	12.0	−3.8
6j	**66.0**	**50.8**	**50.5**
6k	4.7	7.1	0.7
6l	46.6	41.5	**53.0**

[a] Each value was reproduced in triplicate.

Table 3
In vitro cell proliferation inhibition of potent compounds.

Compound	IC_{50}, μM			
	HCC1806	HCT116	HeLa	A549
4g	5.4 ± 0.73	>10	>100	>10
4h	9.05 ± 0.75	>10	>10	>10
4l	6.1 ± 0.79	>10	>10	9.6 ± 0.98
4m	8.65 ± 0.44	>10	>10	>10
4o	2.99 ± 0.48	3.6 ± 0.55	3.0 ± 0.48	3.6 ± 0.56

and the correlative research is currently in progress.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

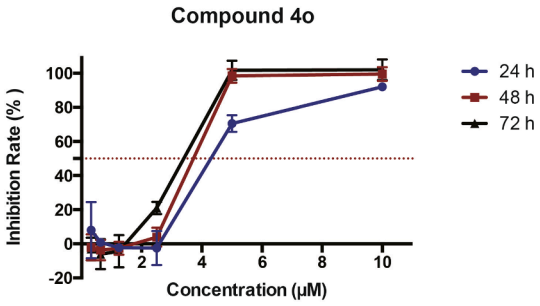


Fig. 1. Compound **4o** showed max efficacy after treatment for 48 h. SRB assay evaluate cell proliferation inhibition rate, and HCC1806 cells were treated with indicated compound **4o** for 24 h, 48 h, 72 h. Error bar represents the SD of experimental triplicates.

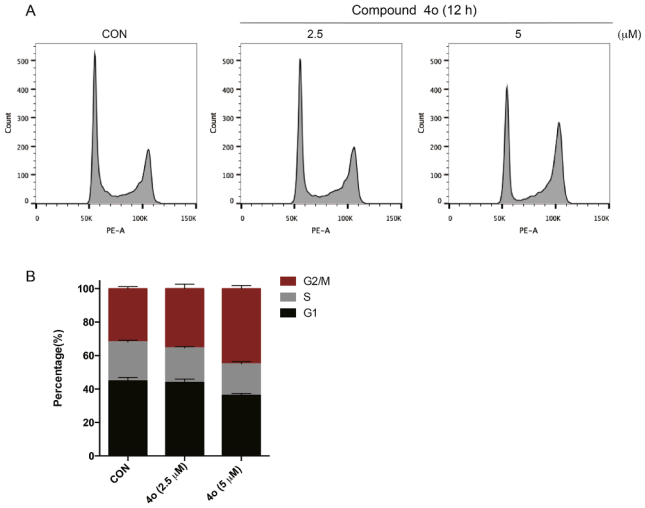


Fig. 2. Compound **4o** induced G2/M arrest. A. Cell cycle analysis of HCC1806 cells after treated with indicated compound **4o** for 12 h by flow cytometry. B is quantification of A. In B, error bar represents the SD of experimental duplicates.

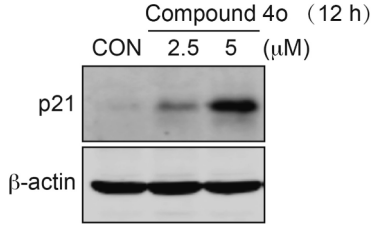


Fig. 3. Compound **4o** induced p21 accumulation significantly. HCC1806 cells were exposed with indicated compound **4o** for 12 h. Protein level were detected by immunoblotting, β-actin was used as loading control.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.128233.

References

1 International Agency for Reaearch on Cancer.Latest global cancer data: Cancer burden rises to 19.3million new cases and 10.0 million cancer deaths in 2020 [R]. Lyon: IARC, 2020.
2 George RF, Samir EM, Abdelhamed MN, Abdel-Aziz HA, Abbas S. E-S. *Bioorg. Chem.* 2019;83:186.
3 Qiu, H. Y., Wang, P. F., Li, Z., Ma, J. T., Wang, X. M., Yang, Y. H., Zhu, H. L. Pharmacol Res; 2016, 104, 86.
4 Fioravanti, R., Desideri, N., Carta, A., Atzori,E. M., Delogu, L., Collu, G., Loddo, R. Eur J Med Chem; 2017, 141, 15.
5 Chen Z, Wang ZC, Yan XQ, et al. *Bioorg Med Chem Lett.* 1947;2015:25.
6 Havrylyuk D, Zimenkovsky B, Vasylenko O, Zaprutko L, Gzella A, Lesyk R. *Eur J Med Chem.* 2009;44:1396.
7 Shaharyar M, Abdullah MM, Bakht MA, Majeed J. *Eur J Med Chem.* 2010;45:114.
8 Liu JJ, Zhang H, Sun Y, et al. *Bioorg Med Chem.* 2012;20:6089.
9 Cox CD, Torrent M, Breslin MJ, et al. *Bioorg Med Chem Lett.* 2006;16:3175.
10 Wu XQ, Huang C, Jia YM, Song BA, Li J, Liu XH. *Eur J Med Chem.* 2014;74:717.
11 Mao Z, Liu B, Zhu P, et al. *Chin J Org Chem.* 2018;38:2167.
12 Mao ZW, Zheng X, Lin YP, Hu CY, Wang XL, Wan CP, Rao GX. *Bioorg Med Chem Lett.* 2016;26:3421.
13 Gao H, Zhang X, Pu XJ, et al. *Bioorg Med Chem Lett.* 2019;29:806.
14 Mao Z, Zheng X, Qi Y, et al. *RSC Adv.* 2016;6:7723.
15 Ma, Y. L., Zheng, X., Gao, H. Wan, C., Rao, G., Mao, Z. Molecules; 2016, 21, 1684.
16 Liu XH, Ruan BF, Liu JX, et al. *Bioorg Med Chem Lett.* 2011;21:2916.
17 Mao ZW, Zheng X, Lin YP, et al. *Heterocycles.* 2016;92:1102.
18 Karimian A, Ahmadi Y, Yousefi B. *DNA Repair.* 2016;42:63.