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Synthesis of novel pyrazole carboxamide derivatives and discovery of modulators for apoptosis or autophagy in A549 lung cancer cells

Xiao-Ling Ding [a,c,†], Hai-Yan Zhang [b,†], Lei Qi [b], Bao-Xiang Zhao [a,*], Song Lian [a], Hong-Shui Lv [a], Jun-Ying Miao [b,*]

[a] Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, PR China
[b] Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, PR China
[c] College of Advanced Professional Technology, Qingdao University, Qingdao 266061, PR China

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ABSTRACT

A series of novel 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxamide derivatives **3a–l**, were synthesized by the reaction of 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carbonyl chloride with substituted amine in excellent yields. The compounds **3e–h** could suppress A549 lung cancer cell growth. More interestingly, compounds **3e** and **3f** might inhibit the A549 cell growth by inducing apoptosis; whereas compounds **3g** and **3h** with fluorine group might inhibit the A549 cell growth by inducing autophagy.

Lung cancer is one of the leading causes of death worldwide.[1] The discovery and development of new, more active, more selective, and less toxic compounds for the treatment of malignancy are one of the most important goals in medicinal chemistry. The understanding of the biology of cancer has undoubtedly improved in recent years and has strongly impacted both experimental and clinical tumor therapy.[2] Apoptosis, or programmed cell death, is a critical cellular process in normal development and homeostasis of multicellular organisms. Inappropriate regulation of apoptosis is associated with many human diseases, including cancer, and it is now recognized that one hallmark of cancer cells is their compromised ability to undergo apoptosis.[3,4] However, non-apoptotic forms of cell death, such as autophagy and extrinsic senescence, have also been shown to contribute to the overall tumor response. Non-apoptotic forms of programmed cell death are targets for novel approaches in anticancer therapy. Autophagy is a process in which subcellular membranes undergo dynamic morphological changes that lead to the degradation of cellular proteins and cytoplasmic organelles. Recently, interest in autophagy has been renewed among oncologists, because different types of cancer cells undergo autophagy after various anticancer therapies.[5–9] Targeting critical apoptosis or autophagy regulators with a goal to promote apoptosis or autophagy in cancer cells is an attractive new cancer therapeutic strategy.

Many pyrazole derivatives are known to exhibit a wide range of biological properties such as cannabinoid type-1 (CB1) receptor antagonists;[10,11] inhibitors of coactivator associated arginine methyltransferase 1 (CARM1) that has been shown to be up-regulated during the progression of prostate cancer;[12,13] inhibitor of CDK2, with good activity against a range of human tumor cell lines;[14] EP1 receptor antagonists;[15] trypsin-like serine protease factor Xa inhibitors;[16,17] HIV-1 integrase inhibitors;[18] inhibitors of tissue-nonspecific alkaline phosphatase (TNAP);[19] non-steroidal selective glucocorticoid receptor (GR) agonists and potent PPARa activators.[20,21] In our effort to discover and develop apoptosis or autophagy inducers as potential new anticancer agents, we reported a series of novel pyrazole and fused-pyrazole derivatives such as ethyl 1-(2′-hydroxy-3′-aroxypropyl)-3-aryl-1*H*-pyrazole-5-carboxylate derivatives, ethyl 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carboxylate derivatives, 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide derivatives, and 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives.[22–26] The evaluation of biological activity showed that these compounds can inhibit A549 lung cancer cell growth. Thus, it is important to modify the structure of pyrazole derivatives and identify the interaction mechanism of small molecules with targets. Herein, we would like

* Corresponding authors. Tel.: +86 531 88366425; fax: +86 531 88564464 (B.-X.Z.).
E-mail addresses: bxzhao@sdu.edu.cn, sduzhao@hotmail.com (B.-X. Zhao), miaojy@sdu.edu.cn (J.-Y. Miao).
† Equal contribution.

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to report the synthesis of 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxamide and the discovery of their biological activities in suppressing the growth of A549 lung cancer cells by inducing apoptosis or autophagy.

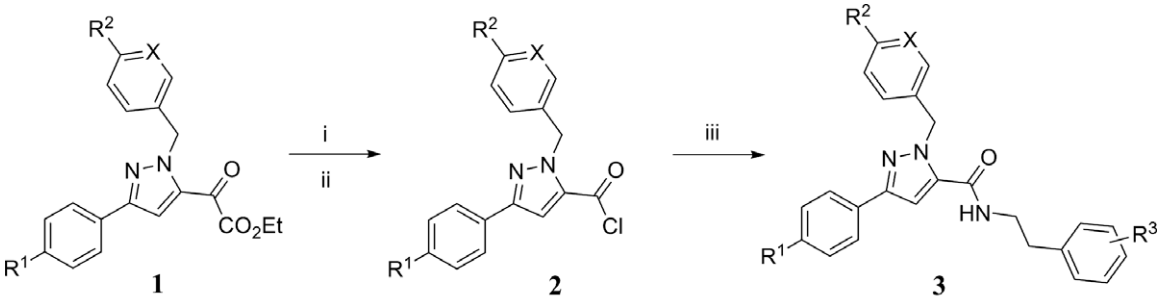
Chemistry. The synthesis of 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxamide derivatives (**3**) has been accomplished as outlined in Scheme 1 starting from ethyl 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxylate (**1**) that can be synthesized as described in our previous paper.[26] Briefly, 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carbonyl chloride (**2**) was synthesized by alkali hydrolysis of ethyl 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxylate (**1**) followed by the reaction with thionyl chloride. 3-Aryl-1-arylmethyl-1*H*-pyrazole-5-carbonyl chloride (**2**) reacts with substituted amine in the presence of triethylamine in dichloromethane at room temperature to afford 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxamide (**3**) in 86–97% yield. The structures of **3a**–**i** were determined by IR, ^{1}H NMR and HRMS spectroscopy.[27]

Inhibition of the compounds on the growth of A549 lung cancer cells. In order to evaluate the inhibitory effects on the growth of A549 cells we carried out the MTT assay with synthesized compounds. The compounds **3a**–**h** have suitable solubility in cell culture medium, however, compounds **3i**–**l** are difficulty to dissolve in cell culture medium. The data obtained by MTT assay showed that compounds **3e**–**h** had inhibitory effects on the growth of A549 cells in dosage- and time-dependent manners. As typically shown in Figure 1, exposure of cells to **3e**–**f** at 10 μM for 24 h resulted in cell viability decrease from 100% to 59.32%–45.52%. When the exposure continued on to 48 h, compared with the control group, the cell viability reduced more significantly from 100% to 44.46%–24.07%. Further, exposure of cells to **3e**–**g** at 20 μM for 48 h, the cell viability reduced more significantly from 100% to 40.44–10.43%, respectively. Taken together, compound **3f** was the most effective compound in suppressing A549 cell growth.

The compounds induced the changes of A549 cell morphology concomitant with cell growth inhibition induced by them (Fig. 2). When A549 cells were treated with compounds **3e** and **3f**, the cells became round and detached from the bottom of cell culture dish, indicating that the compounds might induce A549 cell death by apoptosis. When treated with compound **3g** and **3h**, the cells vacuolated gradually as the concentration increased and the time elongated, indicating that the compounds might induce A549 cell death by autophagy. It was confirmed by the analysis of nuclear and acidic compartment morphology as well as LDH activity assay.

Modulation of the compounds **3e**–**h** *for apoptosis or autophagy in A549 lung cancer cells.* DNA fragmentation, chromatin condensation, cell shrinkage, and membrane blebbing are the characteristics of apoptotic cells. The chromatin condensation and DNA fragmentation in the cells were observed by Hoechst 33258 staining under a Nikon fluorescence microscope. The results showed that nuclear DNA fragmentation occurred in the cells treated with 20 μM of compounds **3e** and **3f** for 48 h (Fig. 3). In the case of **3g** and **3h**, the volume of the cellular acidic compartment, as a marker of autophagy,[25,28,29] was visualized by acridine orange staining. As shown in Figure 4 compounds **3g** and **3h** at 20 μM promoted autophagy dramatically. The data suggested that the compounds **3e** and **3f** might inhibit A549 cell growth through modulating apoptosis while the compounds **3g** and **3h** might inhibit A549 cell growth through modulating autophagy.

To detect whether compounds resulted in necrosis of A549 cells, LDH activity in cell culture medium was measured. As shown in Figure 5, there was no significant difference (p >0.05) in LDH release between the cells of control group and the cells treated with the compounds **3e**–**h** at 10 and 20 μM for 48 h. The results indicated that the compounds at the test range of concentration did not cause necrosis in A549 cells.



a: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = H
b: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = 3-OMe
c: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = 4-F
d: R^1 = H, R^2 = *tert*-Bu, X = C, R^3 = 2-F
e: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = H
f: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = 3-OMe
g: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = 4-F
h: R^1 = Cl, R^2 = *tert*-Bu, X = C, R^3 = 2-F
i: R^1 = Cl, R^2 = Cl, X = N, R^3 = H
j: R^1 = Cl, R^2 = Cl, X = N, R^3 = 3-OMe
k: R^1 = Cl, R^2 = Cl, X = N, R^3 = 4-F
l: R^1 = Cl, R^2 = Cl, X = N, R^3 = 2-F

Scheme 1. Synthesis of 3-aryl-1-arylmethyl-1*H*-pyrazole-5-carboxamide derivatives **3a**–**l**. Reagents and conditions: (i) KOH/EtOH, reflux, 4 h, then HCl aq; (ii) $SOCl_2$, reflux, 3 h; (iii) CH_2Cl_2, Et_3N, $ArCH_2CH_2NH_2$, rt, 2 h.

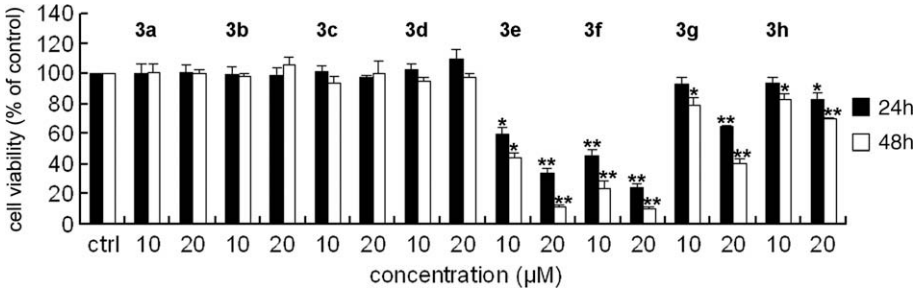


Figure 1. Effects of the compounds **3a**–**h** on cell viability at 24 h (black bar) and 48 h (white bar).

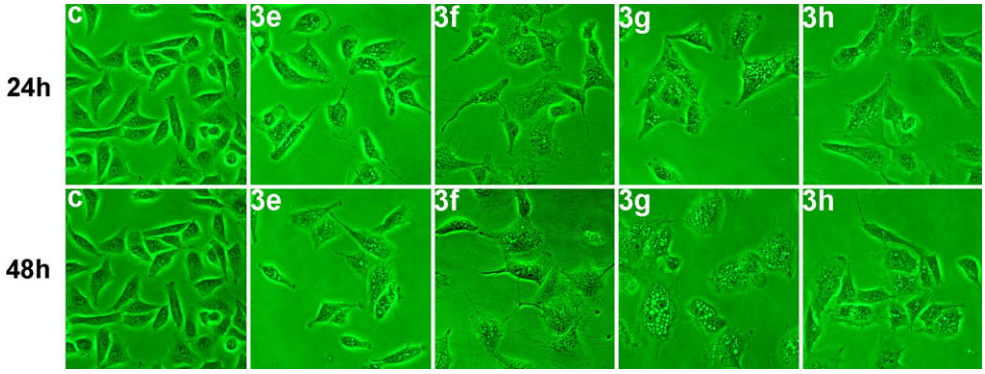


Figure 2. Morphological changes induced by compounds **3e**–**h** at 20 μM. Microscopic photographs (200×) were taken under an inverted phase contrast microscope (Nikon).

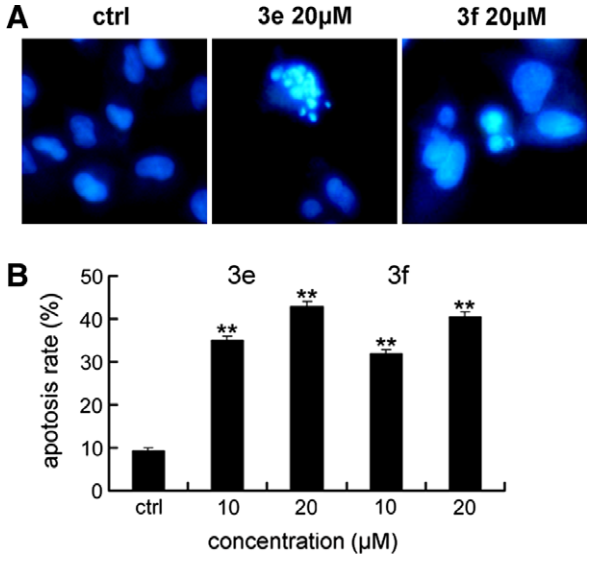


Figure 3. The compounds **3e** and **3f** induced apoptosis in A549 cells at 48 h (p <0.01 vs control, n = 3). Microscopic photographs (200×) were taken under a fluorescent microscope (Nikon).

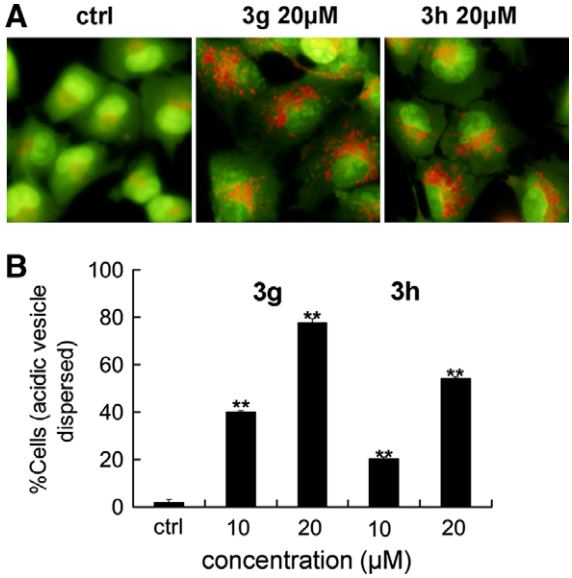


Figure 4. The compounds **3g** and **3h** increased the acidic vesicle level in the cells at 24 h (p <0.01 vs control, n = 3). Microscopic photographs (200×) were taken under a fluorescent microscope (Nikon).

Structure–activity relationships. In our previous papers, we reported that 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide and 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives could inhibit the A549 cell growth obviously and the cytotoxic potency was highly dependent on the substitution types and patterns on the aryl ring, for example, replacing the hydrogen at the 4-position of 1-aryl ring with a bulkier *tert*-butyl group resulted in a significant activity increasing. In present study, we observed that the nature and the position of substituent on the molecule improve biological functions. Indeed, compounds **3e**–**h** with *tert*-butylbenzyl in *N*-1 position and *p*-chlorophenyl group in 3-position were proved to be the most active member with a unique antitumor potency against A549 cell lines. More interestingly, compounds **3e** and **3f** in which R^3 is hydrogen or methoxyl group might inhibit the A549 cell growth by inducing apoptosis, whereas compounds **3g** and **3h** with fluorine group might inhibit the A549 cell growth by inducing autophagy.

In summary, we have described that a series of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carboxamide derivatives were read-

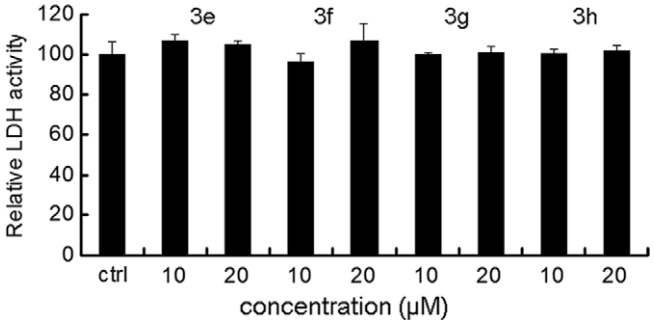


Figure 5. The compounds **3e**–**h** did not induce necrosis in the cells at 48 h (p >0.05 vs control, n = 4).

ily synthesized from ethyl 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carboxylate and substituted amine. The biological evaluation of these compounds on A549 lung cancer cell growth showed that the compounds **3e**–**h** with *tert*-butylbenzyl in *N*-1 position and *p*-chlorophenyl group in 3-position inhibited much more proliferation of A549 cell. More interestingly, compounds **3e** and **3f** might inhibit the A549 cell growth by inducing apoptosis; whereas compounds **3g** and **3h** might inhibit the A549 cell growth by inducing autophagy. The novel compounds certainly deserve further careful investigation in terms of their possible mechanism. Currently, investigations are underway to elucidate the mechanism and the results will be published in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.131.

References and notes

1. Jemal, A.; Siegel, R.; Ward, E.; Hao, Y.; Xu, J.; Murray, T.; Thun, M. J. *CA Cancer J. Clin.* **2008**, *58*, 71.
2. Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57.
3. Lowe, S. W.; Lin, A. W. *Carcinogenesis* **2000**, *21*, 485.
4. Reed, J. C. *Nat. Rev. Drug Disc.* **2002**, *1*, 111.
5. Jin, S.; White, E. *Autophagy* **2007**, *3*, 28.
6. Mizushima, N. *Genes Dev.* **2007**, *21*, 2861.
7. Arthur, C. R.; Gupton, J. T.; Kellogg, G. E.; Yeudall, W. A.; Cabot, M. C.; Newsham, I.; Gewirtz, D. A. *Biochem. Pharmacol.* **2007**, *74*, 981.
8. Levine, B. *Nature* **2007**, *446*, 745.
9. Karantza-Wadsworth, V.; White, E. *Autophagy* **2007**, *3*, 610.
10. Menozzi, G.; Fossa, P.; Cichero, E.; Spallarossa, A.; Ranise, A.; Mosti, L. *Eur. J. Med. Chem.* **2008**, *43*, 2627.
11. Silvestri, R.; Cascio, M. G.; La Regina, G.; Piscitelli, F.; Lavecchia, A.; Brizzi, A.; Pasquini, S.; Botta, M.; Novellino, E.; Di Marzo, V.; Corelli, F. *J. Med. Chem.* **2008**, *51*, 1560.
12. Purandare, A. V.; Chen, Z.; Huynh, T.; Pang, S.; Geng, J.; Vaccaro, W.; Poss, M. A.; Oconnell, J.; Nowak, K.; Jayaraman, L. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4438.
13. Allan, M.; Manku, S.; Therrien, E.; Nguyen, N.; Styhler, S.; Robert, M.-F.; Goulet, A.-C.; Petschner, A. J.; Rahil, G.; MacLeod, A. R.; Déziel, R.; Besterman, J. M.; Nguyen, H.; Wahhab, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1218.
14. Wyatt, P. G.; Woodhead, A. J.; Berdini, V.; Boulstridge, J. A.; Carr, M. G.; Cross, D. M.; Davis, D. J.; Devine, L. A.; Early, T. R.; Feltell, R. E.; Lewis, E. J.; McMenamin, R. L.; Navarro, E. F.; O'Brien, M. A.; O'Reilly, M.; Reule, M.; Saxty, G.; Seavers, L. C. A.; Smith, D.-M.; Squires, M. S.; Trewartha, G.; Walker, M. T.; Woolford, A. J.-A. *J. Med. Chem.* **2008**, *51*, 4986.
15. Hall, A.; Billinton, A.; Bristow, A. K.; Brown, S. H.; Chowdhury, A.; Cutler, L.; Giblin, G. M. P.; Goldsmith, P.; Hayhow, T. G.; Kilford, I. R.; Naylor, A.; Passingham, B.; Rawlings, D. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4027.
16. Qiao, J. X.; King, S. R.; He, K.; Wong, P. C.; Rendina, A. R.; Luettgen, J. M.; Xin, B.; Knabb, R. M.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 462.
17. Qiao, J. X.; Cheney, D. L.; Alexander, R. S.; Smallwood, A. M.; King, S. R.; He, K.; Rendina, A. R.; Luettgen, J. M.; Knabb, R. M.; Wexler, R. R.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4118.
18. Langford, H. M.; Williams, P. D.; Homnick, C. F.; Vacca, J. P.; Felock, P. J.; Stillmock, K. A.; Witmer, M. V.; Hazuda, D. J.; Gabryelskic, L. J.; Schleif, W. A. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 721.
19. Sidique, S.; Ardecky, R.; Su, Y.; Narisawa, S.; Brown, B.; Millán, J. L.; Sergienko, E.; Cosford, N. D. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 222.
20. Barnett, H. A.; Coe, D. M.; Cooper, T. W. J.; Jack, T. I.; Jones, H. T.; Macdonald, S. J. F.; McLay, I. M.; Rayner, N.; Sasse, R. Z.; Shipley, T. J.; Skone, P. A.; Somers, G. I.; Taylor, S.; Uings, I. J.; Woolven, J. M.; Weingarten, G. G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 158.
21. Alvarado, M.; Goya, P.; Macías-González, M.; Pavón, F. J.; Serrano, A.; Jagerovic, N.; Elguero, J.; Gutiérrez-Rodríguez, A.; García-Granda, S.; Suardíaz, M.; Rodríguez de Fonseca, F. *Bioorg. Med. Chem.* **2008**, *16*, 10098.
22. Xia, Y.; Dong, Z.-W.; Zhao, B.-X.; Ge, X.; Meng, N.; Shin, D.-S.; Miao, J.-Y. *Bioorg. Med. Chem.* **2007**, *15*, 6893.
23. Xia, Y.; Fan, C.-D.; Zhao, B.-X.; Zhao, J.; Shin, D.-S.; Miao, J.-Y. *Eur. J. Med. Chem.* **2008**, *43*, 2347.
24. Wei, F.; Zhao, B.-X.; Huang, B.; Zhang, L.; Sun, C.-H.; Dong, W.-L.; Shin, D.-S.; Miao, J.-Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6342.
25. Fan, C.-D.; Zhao, B.-X.; Wei, F.; Zhang, G.-H.; Dong, W.-L.; Miao, J.-Y. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3860.
26. Ding, X.-L.; Meng, N.; Xia, Y.; Wang, L.-J.; Zhang, X.-F.; Zhao, B.-X.; Miao, J.-Y. *Chin. J. Org. Chem.* **2007**, *27*, 1542. (in Chinese).
27. *Spectral data of compounds* **3e**–**h**: *1-(4-tert-butylbenzyl)-3-(4-chlorophenyl)-N-phenethyl-1H-pyrazole-5-carboxamide* ***3e***: mp 116–118 °C; IR (KBr) v: 1641 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.27 (s, 9H), 2.89 (t, 2H, J = 6.8 Hz), 3.72 (q, 2H, J = 6.8 Hz), 5.76 (s, 2H), 6.59 (t, 1H, J = 5.6 Hz), 7.18–7.33 (m, 10H), 7.39 (d, 2H, J = 8.4 Hz), 7.78 (d, 2H, J = 8.4 Hz); HRMS (ESI) calcd for $[M+Cl]^+$ $C_{29}H_{30}Cl_2FN_3O$: 506.1766, found: 506.1768.
1-(4-tert-Butylbenzyl)-3-(4-chlorophenyl)-N-(3-methoxyphenethyl)-1H-pyrazole-5-carboxamide **3f**: mp 107–109 °C; IR (KBr) v: 1640 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.26 (s, 9H), 2.87 (t, 2H, J = 6.8 Hz), 3.71 (q, 2H, J = 6.8 Hz), 3.78 (s, 3H), 5.76 (s, 2H), 6.61 (t, 1H, J = 5.6 Hz), 6.76–6.79 (m, 3H), 7.21 (t, 1H, J = 8.0 Hz), 7.26 (s, 1H), 7.27–7.33 (m, 4H), 7.40 (d, 2H, J = 6.8 Hz), 7.78 (d, 2H, J = 6.8 Hz); HRMS (ESI) calcd for $[M+Cl]^+$ $C_{30}H_{32}Cl_2N_3O_2$: 536.1872, found: 536.1866.
1-(4-tert-Butylbenzyl)-3-(4-chlorophenyl)-N-(4-fluorophenethyl)-1H-pyrazole-5-carboxamide **3g**: mp 126–128 °C; IR (KBr) v: 1641 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.27 (s, 9H), 2.83 (t, 2H, J = 6.4 Hz), 3.61 (q, 2H, J = 6.4 Hz), 5.75 (s, 2H), 5.93 (t, 1H, J = 5.6 Hz), 6.61 (s, 1H), 6.97 (t, 2H, J = 8.4 Hz), 7.08 (dd, 1H, J = 5.6, 8.4 Hz), 7.25 (d, 2H, J = 8.4 Hz)), 7.32 (d, 2H, J = 8.4 Hz), 7.35 (d, 2H, J = 8.4 Hz), 7.71 (d, 2H, J = 8.4 Hz); HRMS (ESI) calcd for $[M+Cl]^+$ $C_{29}H_{29}Cl_2N_3O$: 524.1672, found: 524.1667.
1-(4-tert-Butylbenzyl)-3-(4-chlorophenyl)-N-(2-fluorophenethyl)-1H-pyrazole-5-carboxamide **3h**: mp 119–120 °C; IR (KBr) v: 1643 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.27 (s, 9H), 2.94 (t, 2H, J = 6.8 Hz), 3.72 (q, 2H, J = 6.8 Hz), 5.76 (s, 2H), 6.62 (t, 1H, J = 5.6 Hz), 7.01–7.07 (m, 2H), 7.14–7.25 (m, 2H), 7.26 (s, 1H), 7.27 (d, 2H, J = 8.4 Hz), 7.32 (d, 2H, J = 8.4 Hz), 7.40 (d, 2H, J = 8.4 Hz),7.79 (d, 2H, J = 8.4 Hz); HRMS (ESI) calcd for $[M+Cl]^+$ $C_{29}H_{29}Cl_2N_3O$: 524.1672, found: 524.1751.
28. Paglin, S.; Hollister, T.; Delohery, T.; Hackett, N.; McMahill, M.; Sphika, E. *Cancer Res.* **2001**, *61*, 439.
29. Arthur, C. R.; Gupton, J. T.; Kellogg, G. E.; Yeudall, W. A.; Cabot, M. C.; Newsham, I. F. *Biochem. Pharmacol.* **2007**, *74*, 981.