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Original article

Synthesis and structure–activity relationships of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives as potential agents against A549 lung cancer cells

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Abstract

A series of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives were synthesized and the effects of all the compounds on A549 cell growth were investigated. The results showed that all compounds had almost inhibitory effects on the growth of A549 cells. The study on structure–activity relationships and prediction of lipophilicities of compounds showed that compounds with Log *P* values in the range of 4.12–6.80 had inhibitory effects on the growth of A549 cells, and among of them the hydrazone derived from salicylaldehyde had much more inhibitory effects.

Keywords: Arylpyrazole carbohydrazide; Hydrazone; X-ray crystallography; A549 cells; Growth inhibitory; Structure–activity relationship

1. Introduction

A number of hydrazide–hydrazone derivatives have been claimed to possess interesting bioactivity such as antibacterial–antifungal [1], anticonvulsant [2], antiinflammatory [3], antimalarial [4], analgesic [5,6], antiplatelets [7], antituberculosis [8] and anticancer activities [9]. Aroylhydrazide–hydrazones containing hetero-ring such as pyridine [3,10], indole [11], 1,2,4-oxadiazole [5], 1,2,3-triazole [6] and imidazo[2,1-*b*][1,3,4]thiadiazole ring [8] have attracted special attention. A few of pyrazole carbohydrazide hydrazone derivatives have also been reported [12,13]. However, there has been no report in the literature on the synthesis and biological evaluation of 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives. In a previous paper we described the synthesis and the effects of 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide derivatives on A549 cell growth [14]. The study on structure–activity relationships and prediction of lipophilicities of compounds showed that compounds with Log *P* values in the range of 3.12–4.94 had more inhibitory effects on the growth of A549 cells. With the aim of obtaining new anticancer compounds we synthesized a series of novel 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives and evaluated effects of these compounds on A549 cell growth inhibitory.

2. Results and discussion

2.1. Chemistry

The synthesis of 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone (**II**) has been accomplished as outlined in Scheme 1 starting from 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide (**I**) that can be synthesized as described in

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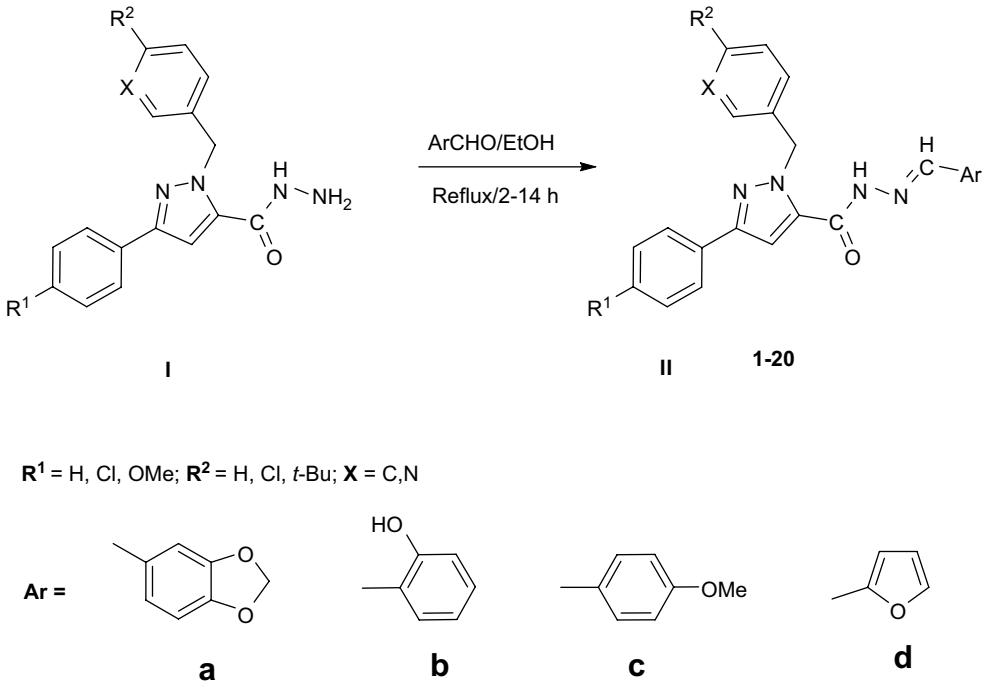

Scheme 1. Synthesis of compounds **1**–**20**.

our previous paper [14]. For example, (*E*)-*N*′-(benzo[*d*][1,3]-dioxolo-5-ylmethylene)-1-benzyl-3-(4-chlorophenyl)-1*H*-pyrazole-5-carbohydrazide (**1**) was synthesized in 82% yield by the reaction of 1-benzyl-3-(4-chlorophenyl)-1*H*-pyrazole-5-carbohydrazide with piperonal in ethanol over a 4 h reflux period. The structures of hydrazone derivatives **II** were determined by IR, ^{1}H NMR and Mass spectroscopy. Thus, for example **1**, obtained as white crystal, gave an [M + H]-ion peak at *m*/*z* 459.5 in the ESI-MS, in accord with the molecular formula $C_{25}H_{19}ClN_4O_3$. In the IR spectra, the carbonyl group absorptions in hydrazide moiety and NH bands in CONH were observed in the 1651 cm^{-1} and 3203–2892 cm^{-1} regions, respectively. The ^{1}H NMR spectra indicated the chemical shift of the NH at $\delta = 11.18$ ppm in the form of singlet peak. The chemical shift of the N=CH appeared at $\delta = 8.23$ ppm in the form of singlet peak. Two *ortho*-aromatic protons signals in 4-chlorobenzene moiety appeared at the range of $\delta = 7.39$ and 7.78 ppm as doublet peaks ($J = 8.4$ Hz). Two *ortho*-aromatic protons signals in piperonyl moiety appeared at the range of $\delta = 6.82$ and 7.09 ppm as doublet peaks ($J = 8.1$ Hz). Three singlet signals appeared at $\delta = 5.85$, 6.02 and 7.17 ppm are consistent with methylene protons in benzyl, piperonyl groups and pyrazole moiety, respectively. Furthermore, the structure of (*E*)-*N*′-(2-hydroxybenzylidene)-1-(4-*tert*-butylbenzyl)-3-phenyl-1*H*-pyrazole-5-carbohydrazide **16** was confirmed by the X-ray diffraction as shown in Fig. 1.

2.2. Evaluation of biological activity

2.2.1. Effects of the compounds on the viability of A549 lung cancer cells

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay has been widely accepted as a reliable way to measure the cell proliferation rate, and conversely when metabolic events lead to apoptosis or necrosis. The data obtained by MTT assay showed that compounds **II** had inhibitory effects on the growth of A549 cells in dosage- and time-dependent manners except compounds **1**, **5**, **7**, **9** and **11** as indicated by the results in Fig. 2. As typically shown in Fig. 3, exposure of cells to compounds **4**, **8** and **12** at 10 μM for 24 h resulted in cell viability decrease from 100% to 69, 61 and 80%, respectively.

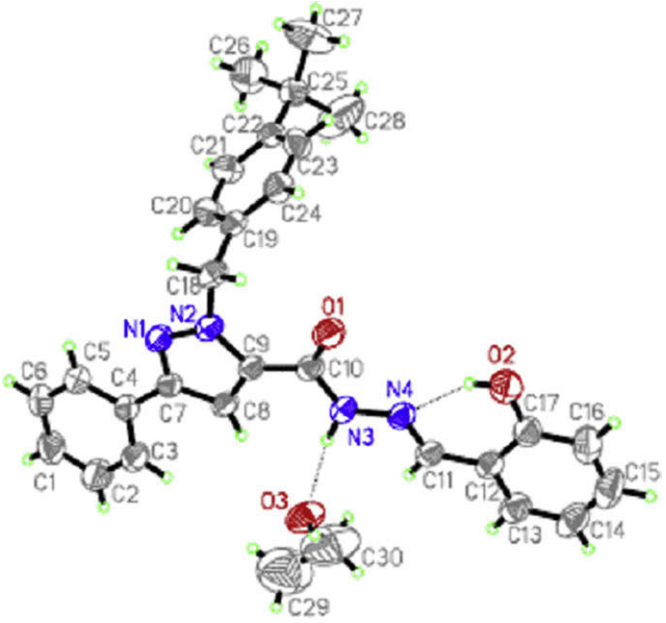

Fig. 1. X-ray crystal structures of compound **16**. Displacement ellipsoids are drawn at 50% probability level.

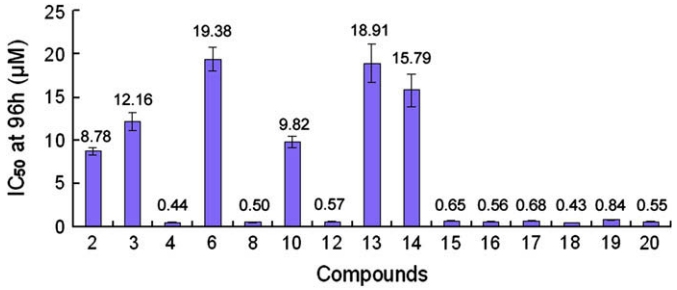

Fig. 2. Growth inhibitory properties IC_{50} (μM) for the compounds at 96 h.

Continuing the exposure for 48 h resulted in cell viability decrease from 100% to 41, 32 and 45%, respectively. When the exposure continued on to 96 h, compared with the control group, the cell viability reduced more significantly from 100% to 6, 7 and 10% ($p < 0.01$). Further, exposure of cells to compounds **4**, **8** and **12** at 20 μM for 48 h, the cell viability reduced more significantly from 100% to 38, 18 and 34%, respectively. The compounds **15**–**20** showed the similar dosage- and time-dependent manners as shown in Fig. 3B and C.

Further investigation of the concentration- and time-dependent viability of A549 cells exposed to compounds **4**, **8**, **12** and **15**–**20** was conducted. The exposure of A549 cells to compounds **4**, **8**, **12** and **15**–**20** for the duration of 24, 48, and 96 h resulted in significant increase in the cell proliferation inhibitory percentage.

2.2.2. Structure–activity relationships

Growth inhibitory properties (IC_{50}) are listed. The data suggested that compounds could almost inhibit the cell growth obviously in the concentrations of 0.5–20 μM after 96 h of the treatment except compounds **1**, **5**, **7**, **9** and **11**. In our previous paper, we reported that 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide derivatives, which are the starting material in this study, could inhibit the A549 cell growth obviously in the concentrations of 20–80 μM after 48 h of the treatment 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. Regarding the Ar substituted-hydrazone structure variants **a**, **b**, **c** and **d**, the antitumor activity of these compounds appears to be closely related to

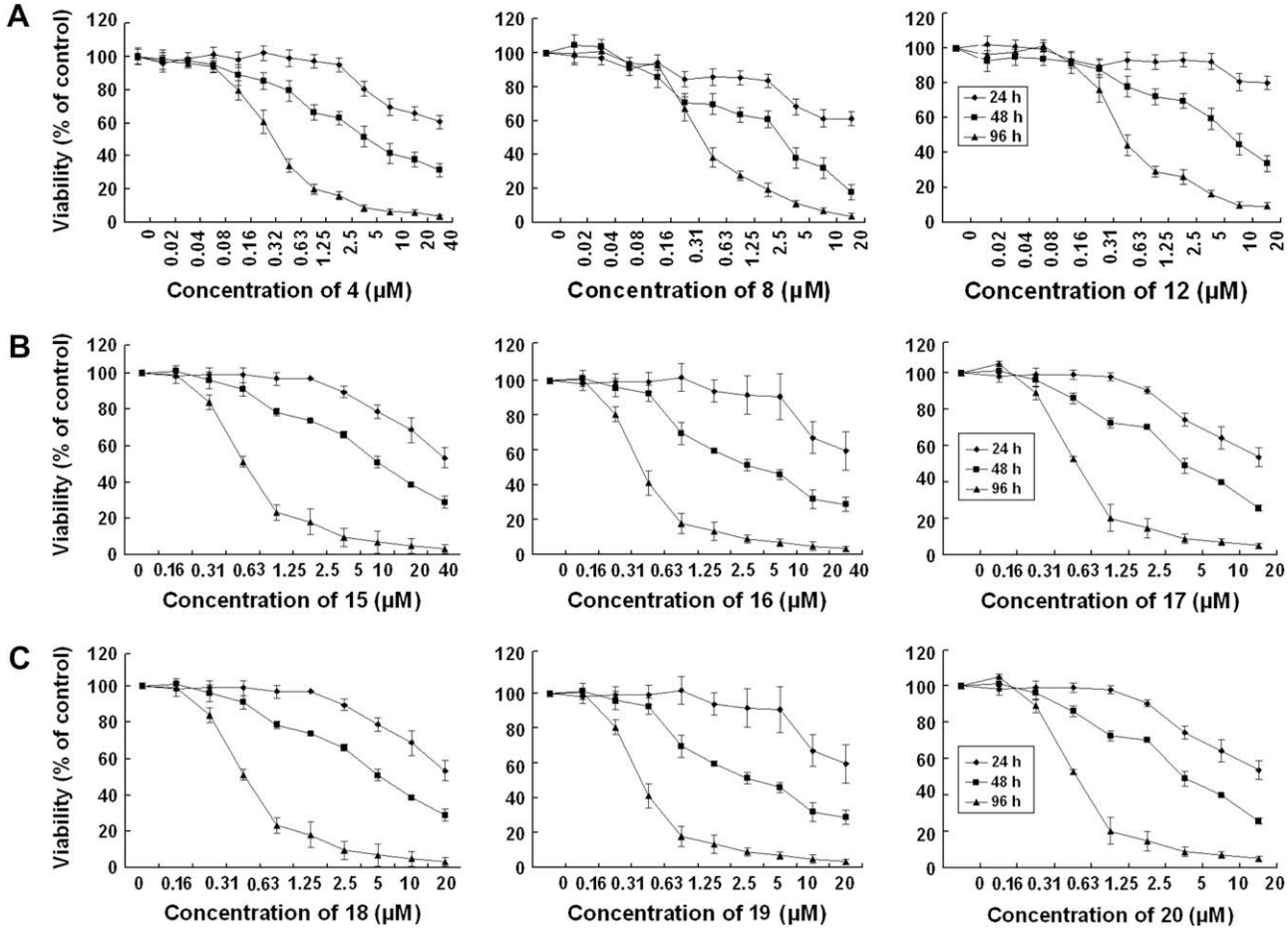

Fig. 3. (A) Effects of the compounds **4**, **8** and **12** on the viability of A549 lung cancer cells. (B) Effects of the compounds **15**–**17** on the viability of A549 lung cancer cells. (C) Effects of the compounds **18**–**20** on the viability of A549 lung cancer cells.

the nature of the Ar group. Compounds **4**, **8**, **12** and **15**–**20** with **b** group were proved to be the most active member with a unique antitumor potency against A549 cell lines. Furthermore, replacement of the benzyl group with a *t*-butylbenzyl moiety as in **16**, **18** and **20**, resulted in a higher growth inhibitory effect.

When Ar group was **a** and **c**, the compounds were difficult to dissolve in RPMI 1640 medium so that the activity of these compounds could not be examined in suitable concentration. Here, it should be pointed out that the solubility data (Log *S*) obtained by calculating were not concordant with the solubility in RPMI 1640 medium examined by the experimental method as shown in Table 1. When Ar was **d**, the growth inhibitory properties of the compounds were inferior to the compounds with **b** group, although they had a good solubility in RPMI 1640 medium.

These results revealed the crucial role of the *o*-hydroxybenzene moiety in the hydrazone cytotoxic activities. It should be raised from the possibility of compounds chelating with some ions in cell.

Compared the growth inhibitory properties (IC_{50}) of compounds **I** and **II**, it was obvious that compounds **II** were more effective. For example, after 48 h of the treatment, the IC_{50} for compound **18** and **20** was 3.33 and 4.80 μM, however, the IC_{50} for corresponding parent compound, 1-(4-*tert*-butylbenzyl)-3-(4-chlorophenyl)-1*H*-pyrazole-5-carbohydrazide and 1-(4-*tert*-butylbenzyl)-3-(4-methoxyphenyl)-1*H*-pyrazole-5-carbohydrazide were 18.52 and 23.86 μM, respectively.

The lipophilicity of the compounds is well known to play an important role in the penetration of these compounds into cells. The partition coefficient Log *P* is a parameter which describes the manner in which a drug partitions between polar and non-polar phases, and it has been demonstrated to be an indispensable tool in predicting the transport and activity of drugs [15–18]. Prediction of lipophilicity (Log *P*) and aqueous solubility (Log *S*) of compounds **1**–**20** was calculated using ALOGPS 2.1 software [19,20]. Assuming that the issue of penetration is even more crucial for compound's activity against cell, our results demonstrated that simply increasing the lipophilic character of compounds increased the activity, as shown with Log *P* values of the synthesized compounds hydrazone (4.12–6.80) (Table 1) were much more than the parent compound hydrazide (2.52–4.94).

Table 1
The IC_{50}, Log *P*, Log *S* and solubility in 1640 of the compounds **1**–**20**

Compound	R^1	R^2	X	Ar	Smax in 1640 (μM)	Log *S* (μM)	Log *P* (μM)	IC_{50} (μM)	
								48 h	96 h
1	Cl	H	C	**a**	8.0	4.92	4.96	Nd	Nd
2	Cl	H	C	**d**	12.5	49.80	4.76	Nd	8.78
3	H	Cl	N	**a**	17.0	15.83	4.35	Nd	12.16
4	H	Cl	N	**b**	45.0	12.13	4.86	5.83	0.44
5	H	Cl	N	**c**	15.0	5.36	4.58	Nd	Nd
6	H	Cl	N	**d**	50.0	72.59	4.12	26.13	19.38
7	Cl	Cl	N	**a**	2.0	8.48	4.79	Nd	Nd
8	Cl	Cl	N	**b**	22.5	6.80	5.38	3.68	0.50
9	Cl	Cl	N	**c**	3.3	3.41	5.17	Nd	Nd
10	Cl	Cl	N	**d**	70.0	60.08	4.65	18.65	9.82
11	OMe	Cl	N	**a**	7.5	15.04	4.36	Nd	Nd
12	OMe	Cl	N	**b**	70.0	13.62	4.87	8.21	0.57
13	OMe	Cl	N	**c**	17.5	5.67	4.80	Nd	18.91
14	OMe	Cl	N	**d**	30.0	74.54	4.20	Nd	15.79
15	H	H	C	**b**	44.0	10.70	4.88	10.76	0.65
16	H	*t*-Bu	C	**b**	47.0	1.08	6.25	6.24	0.56
17	Cl	H	C	**b**	25.0	4.64	5.48	4.87	0.68
18	Cl	*t*-Bu	C	**b**	36.0	0.60	6.80	3.33	0.43
19	OMe	H	C	**b**	23.0	8.86	4.86	7.69	0.84
20	OMe	*t*-Bu	C	**b**	22.0	1.06	6.25	4.80	0.55

Nd: not determined.

3. Conclusion

The biological evaluation of 1-arylmethyl-3-aryl-1*H*-pyrazole-5-carbohydrazide hydrazone derivatives **1**–**20** on A549 lung cancer cell growth showed that the hydrazone compounds derived from salicylaldehyde inhibited much more proliferation of A549 cell. It was suggested that the potency of these compounds against A549 lung cancer cell not only related with the lipophilicity of the compound but also depended on the capacity of compound chelating metal ions because it was recognized that metal ions play an important role in the cell growth. As potent chelators of ions that effectively inhibit tumor cell growth, 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.

4. Experimental

Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plates (Merck KGaA). ^{1}H NMR spectra were recorded on a Bruker Avance 300 (300 MHz) spectrometer, using $CDCl_3$ or DMSO as solvents and tetramethylsilane (TMS) as internal standard. Melting points were determined on an XD-4 digital micro melting point apparatus and are uncorrected. IR spectra were recorded with an IR spectrophotometer Avtar 370 FT-IR (Termo Nicolet). MS spectra were recorded on a Trace DSQ mass spectrograph. Log *P* and Log *S* were calculated using ALOGPS 2.1 software.

*4.1. General procedure for the synthesis of 1-arylmethyl-3-aryl-1H-pyrazole-5-carbohydrazide hydrazone derivatives (**1**–**20**)*

To a stirred solution of 1 mmol of derivatives **I** in 10 ml of ethanol, there was added an equimolar amount of the appropriate aldehyde derivative. The reaction mixture was maintained under reflux for 2–14 h, until TLC indicated the end of reaction. After this time, the reaction mixture stood over night and the solid formed was collected by filtration and washed with ethanol and recrystallized from ethanol to afford crystals. As a result of this process the compounds **II** (**1**–**20**) were prepared in yield of 82–98%.

*4.1.1. (E)-N′-(benzo[d][1,3]dioxolo-5-ylmethylene)-1-benzyl-3-(4-chlorophenyl)-1H-pyrazole-5-carbohydrazide (**1**)*

White solid, yield 82%, mp 233–236 °C; IR (KBr) *ν*: 3211–2892 (NH), 1651 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + DMSO) *δ*: 5.85 (s, 2H, CH_2), 6.02 (s, 2H, O–CH_2), 6.82 (d, *J* = 8.1 Hz, 1H, ArH), 7.09 (d, *J* = 8.1 Hz, 1H, ArH), 7.17 (s, 1H, 4-H), 7.26–7.35 (m, 3H, ArH), 7.39 (d, *J* = 8.4 Hz, 2H, ArH), 7.42–7.46 (m, 3H, ArH), 7.78 (d, *J* = 8.4 Hz, 2H, ArH), 8.23 (s, 1H, =CH), 11.18 (s, 1H, NH); ESI-MS: 459.5 $(M+H)^+$.

*4.1.2. (E)-1-benzyl-3-(4-chlorophenyl)-N′-(furan-2-ylmethylene)-1H-pyrazole-5-carbohydrazide (**2**)*

White solid, yield 92%, mp 249–251 °C; IR (KBr) *ν*: 3198–3062 (NH), 1651 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) *δ*: 5.84 (s, 2H, CH_2), 6.50–6.51 (m, 1H, FuH), 6.84 (d, *J* = 2.1 Hz, 1H, FuH), 7.20 (s, 1H, 4-H), 7.22–7.29 (m, 3H, ArH), 7.35–7.42 (m, 4H, ArH), 7.54 (s, 1H, FuH), 7.77 (d, *J* = 8.4 Hz, 2H, ArH), 8.29 (s, 1H, =CH), 11.51 (s, 1H, NH); ESI-MS: 405.5 $(M+H)^+$.

*4.1.3. (E)-N′-(benzo[d][1,3]dioxolo-5-ylmethylene)-1-((6-chloropyridin-3-yl)methyl)-3-phenyl-1H-pyrazole-5-carbohydrazide (**3**)*

Yellow solid, yield 87%, mp 213–215 °C; IR (KBr) *ν*: 3173–2952 (NH), 1662 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO) *δ*: 5.81 (s, 2H, CH_2), 6.10 (s, 2H, O–CH_2), 7.01 (d, *J* = 8.0 Hz, 1H, ArH), 7.20 (d, *J* = 8.0 Hz, 1H, ArH), 7.30 (s, 1H, 4-H), 7.37–7.51 (m, 5H, ArH), 7.73 (dd, *J* = 2.1 Hz, 8.4 Hz, 1H, PyH), 7.81 (d, *J* = 7.6 Hz, 2H, ArH), 8.33 (s, 1H, =CH), 8.37 (d, *J* = 2.1 Hz, 1H, PyH), 11.90 (s, 1H, NH); ESI-MS: 460.5 $(M+H)^+$.

*4.1.4. (E)-1-((6-chloropyridin-3-yl)methyl)-N′-(2-hydroxybenzylidene)-3-phenyl-1H-pyrazole-5-carbohydrazide (**4**)*

Yellow solid, yield 89%, mp 99–100 °C; IR (KBr) *ν*: 3429–2959 (NH), 1664 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ*: 5.78 (s, 2H, CH_2), 6.89–7.02 (m, 3H, 4-H, ArH), 7.19–7.43 (m, 7H, ArH), 7.73–7.74 (m, 3H, ArH, PyH, NH), 8.38 (s, 1H,=CH), 8.43 (s, 1H, PyH), 9.50 (s, 1H, OH); ESI-MS: 432.5 $(M+H)^+$.

*4.1.5. (E)-1-((6-chloropyridin-3-yl)methyl)-N′-(4-methoxybenzylidene)-3-phenyl-1H-pyrazole-5-carbohydrazide (**5**)*

White solid, yield 98%, mp 213–215 °C; IR (KBr) *ν*: 3441–2838 (NH), 1651 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) *δ*: 3.85 (s, 3H, OMe), 5.83 (s, 2H, CH_2), 6.94–7.02 (m, 3H, ArH), 7.23 (s, 1H, 4-H), 7.35–7.42 (m, 3H, ArH, PyH), 7.59–7.79 (m, 5H, ArH, PyH), 8.14 (s, 1H, =CH), 8.50 (s, 1H, PyH), 9.21 (s, 1H, NH); ESI-MS: 446.5 $(M+H)^+$.

*4.1.6. (E)-1-((6-chloropyridin-3-yl)methyl)-N′-(furan-2-ylmethylene)-3-phenyl-1H-pyrazole-5-carbo-hydrazide (**6**)*

White solid, yield 98%, mp 210–212 °C; IR (KBr) *ν*: 3204–3049 (NH), 1649 (C=O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + DMSO) *δ*: 5.86 (s, 2H, CH_2), 6.51 (s, 1H, FuH), 6.87 (s, 1H, FuH), 7.24 (s, 1H, 4-H), 7.27–7.43 (m, 5H, ArH, PyH, FuH), 7.55–7.83 (m, 3H, ArH, PyH), 8.31 (s, 1H, =CH), 8.50 (s, 1H, PyH), 11.57 (s, 1H, NH); ESI-MS: 406.6 $(M+H)^+$.

*4.1.7. (E)-N′-(benzo[d][1,3]dioxolo-5-ylmethylene)-3-(4-chlorophenyl)-1-((6-chloropyridin-3-yl)methyl)-1H-pyrazole-5-carbohydrazide (**7**)*

White solid, yield 94%, mp 256–258 °C; IR (KBr) *ν*: 3165–2955 (NH), 1660 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO) *δ*: 5.80 (s, 2H, CH_2), 6.10 (s, 2H, O–CH_2), 7.01 (d, *J* = 8.0 Hz, 1H, ArH), 7.20 (d, *J* = 8.0 Hz, 1H, ArH), 7.30 (s, 1H, ArH), 7.47 (s, 1H, 4-H), 7.50 (d, *J* = 8.4 Hz, 1H, PyH), 7.54 (d, *J* = 8.4 Hz, 2H, ArH), 7.74 (dd, *J* = 2.1 Hz, 8.4 Hz, 1H, PyH), 7.82 (d, *J* = 8.4 Hz, 2H, ArH), 8.32 (s, 1H, =CH), 8.37 (d, *J* = 2.1 Hz, 1H, PyH), 11.91 (s, 1H, NH); ESI-MS: 494.3 $(M+H)^+$.

*4.1.8. (E)-3-(4-chlorophenyl)-1-((6-chloropyridin-3-yl)methyl)-N′-(2-hydroxybenzylidene)-1H-pyrazole-5-carbohydrazide (**8**)*

Yellow solid, yield 96%, mp 231–233 °C; IR (KBr) *ν*: 3615 (OH), 3216–3059 (NH), 1677 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) *δ*: 5.79 (s, 2H, CH_2), 6.82–6.88 (m, 2H, ArH), 7.20–7.28 (m, 2H, ArH), 7.33–7.36 (m, 4H, ArH, PyH, 4-H), 7.71–7.73 (m, 3H, ArH, PyH), 8.36 (s, 1H, =CH), 8.49 (s, 1H, PyH), 11.10 (s, 1H, OH), 11.99 (s, 1H, NH); ESI-MS: 466.4 $(M+H)^+$.

*4.1.9. (E)-3-(4-chlorophenyl)-1-((6-chloropyridin-3-yl)methyl)-N′-(4-methoxybenzylidene)-1H-pyrazole-5-carbohydrazide (**9**)*

Yellow solid, yield 94%, mp 235–237 °C; IR (KBr) *ν*: 3173–2961 (NH), 1653 (C=O), 1267 (O–C) cm^{-1}; ^1H NMR (400 MHz, DMSO) *δ*: 3.82 (s, 3H, OMe), 5.81 (s, 2H, CH_2), 7.04 (d, *J* = 8.4 Hz, 2H, ArH), 7.47 (s, 1H, 4-H), 7.50 (d, *J* = 8.4 Hz, 1H, PyH), 7.53 (d, *J* = 8.4 Hz, 2H, ArH), 7.69 (d, *J* = 8.4 Hz, 2H, ArH), 7.74 (dd, *J* = 2.2 Hz, 8.4 Hz, 1H, PyH), 7.82 (d, *J* = 8.4 Hz, 2H, ArH), 8.35 (s, 1H, =CH), 8.38 (d, *J* = 2.2 Hz, 1H, PyH), 11.88 (s, 1H, NH); ESI-MS: 480.4 $(M+H)^+$.

*4.1.10. (E)-3-(4-chlorophenyl)-1-((6-chloropyridin-3-yl)methyl)-N′-(furan-2-ylmethylene)-1H-pyrazole-5-carbohydrazide (**10**)*

White solid, yield 81%, mp 178–180 °C; IR (KBr) *ν*: 3419–3051 (NH), 1654 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO) *δ*: 5.80 (s, 2H, CH_2), 6.66 (s, 1H, FuH), 6.99 (d, *J* = 3.1 Hz, 1H, FuH), 7.45 (s, 1H, 4-H), 7.50 (d, *J* = 8.6 Hz, 1H, PyH), 7.53 (d, *J* = 8.4 Hz, 2H, ArH), 7.73 (d, *J* = 8.6 Hz, 1H, PyH), 7.83 (d, *J* = 8.4 Hz, 2H, ArH), 7.88

(s, 1H, FuH), 8.28 (s, 1H, =CH), 8.37 (s, 1H, PyH), 11.95 (s, 1H, NH); ESI-MS: 440.5 $(M+H)^+$.

*4.1.11. (E)-N′-(benzo[d][1,3]dioxolo-5-ylmethylene)-1-((6-chloropyridin-3-yl)methyl)-3-(4-methoxyphenyl)-1H-pyrazole-5-carbohydrazide (**11**)*

Yellow solid, yield 92%, mp 208–209 °C; IR (KBr) ν: 3176–2957 (NH), 1652 (C=O), 1260 (O–C) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ: 3.79 (s, 3H, OMe), 5.79 (s, 2H, CH_2), 6.10 (s, 2H, O–CH_2), 7.00 (d, $J=8.0$ Hz, 1H, ArH), 7.02 (d, $J=8.4$ Hz, 2H, ArH), 7.19 (d, $J=8.0$ Hz, 1H, ArH), 7.30 (s, 1H, 4-H), 7.37 (s, 1H, ArH), 7.50 (d, $J=8.0$ Hz, 1H, PyH), 7.72 (dd, $J=2.2$ Hz, 8.4 Hz, 1H, PyH), 7.73 (d, $J=8.4$ Hz, 2H, ArH), 8.32 (s, 1H =CH), 8.36 (d, $J=2.2$ Hz, 1H, PyH), 11.87 (s, 1H, NH); ESI-MS: 490.4 $(M)^+$.

*4.1.12. (E)-1-((6-chloropyridin-3-yl)methyl)-N′-(2-hydroxybenzylidene)-3-(4-methoxyphenyl)-1H-pyrazole-5-carbohydrazide (**12**)*

Yellow solid, yield 98%, mp 100–102 °C; IR (KBr) ν: 3560 (OH), 3209–2833 (NH), 1680 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) δ: 3.77 (s, 3H, OMe), 5.76 (s, 2H, CH_2), 6.81–6.92 (m, 5H, ArH, OH), 7.20–7.22 (m, 4H, ArH, PyH, 4-H), 7.66 (d, $J=8.4$ Hz, 2H, ArH), 7.68 (dd, $J=2.2$ Hz, 8.4 Hz, 1H, PyH), 8.35 (d, $J=2.2$ Hz, 1H, PyH), 8.45 (s, 1H, =CH), 11.85 (s, 1H, NH); ESI-MS: 462.4 $(M+H)^+$.

*4.1.13. (E)-1-((6-chloropyridin-3-yl)methyl)-N′-(4-methoxybenzylidene)-3-(4-methoxyphenyl)-1H-pyrazole-5-carbohydrazide (**13**)*

Yellow solid, yield 91%, mp 205–206 °C; IR (KBr) ν: 3174–2837 (NH), 1654 (C=O), 1263 (O–C) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ: 3.79 (s, 3H, OMe), 3.82 (s, 3H, OMe), 5.79 (s, 2H, CH_2), 7.02 ($J=8.4$ Hz, 2H, ArH), 7.03 (d, $J=8.4$ Hz, 2H, ArH), 7.37 (s, 1H, 4-H), 7.49 (d, $J=8.4$ Hz, 1H, PyH), 7.68 (d, $J=8.4$ Hz, 2H, ArH), 7.71–7.75 (m, 3H, ArH, PyH), 8.35 (s, 1H =CH), 8.37 (s, 1H, PyH), 11.83 (s, 1H, NH); ESI-MS: 476.4 $(M+H)^+$.

*4.1.14. (E)-1-((6-chloropyridin-3-yl)methyl)-N′-(furan-2-ylmethylene)-3-(4-methoxyphenyl)-1H-pyrazole-5-carbohydrazide (**14**)*

Yellow solid, yield 91%, mp 200–202 °C; IR (KBr) ν: 3211–2927 (NH), 1655 (C=O) cm^{-1}; ^1H NMR (400 MHz, DMSO) δ: 3.79 (s, 3H, OMe), 5.78 (s, 2H, CH_2), 6.64–6.66 (m, 1H, FuH), 6.98 (d, $J=3.0$ Hz, 1H, FuH), 7.02 (d, $J=8.4$ Hz, 2H, ArH), 7.35 (s, 1H, 4-H), 7.50 (d, $J=8.4$ Hz, 1H, PyH), 7.72 (dd, $J=2.0$, 8.4 Hz, 1H, PyH), 7.74 (d, $J=8.4$ Hz, 2H, ArH), 7.87 (s, 1H, FuH), 8.29 (s, 1H, =CH), 8.36 (d, $J=2.0$ Hz, 1H, PyH), 11.91 (s, 1H, NH); ESI-MS: 436.5 $(M+H)^+$.

*4.1.15. (E)-N′-(2-hydroxybenzylidene)-1-benzyl-3-phenyl-1H-pyrazole-5-carbohydrazide (**15**)*

White solid, yield 82%, mp 234–236 °C; IR (KBr) ν: 3246–2931 (NH), 1660 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) δ: 5.74 (s, 2H, CH_2), 6.77 (t, $J=8.0$ Hz, 1H, ArH), 6.87 (d, $J=8.4$ Hz, 1H, ArH), 7.10–7.32 (m, 11H, 4-H, ArH), 7.72 (d, $J=8.0$ Hz, 2H, ArH), 8.34 (s, 1H, =CH), 11.23 (s, 1H, OH), 11.52 (s, 1H, NH); ESI-MS: 397.5 $(M+H)^+$.

*4.1.16. (E)-N′-(2-hydroxybenzylidene)-1-(4-tert-butylbenzyl)-3-phenyl-1H-pyrazole-5-carbohydrazide (**16**)*

White solid, yield 97%, mp 106–108 °C; IR (KBr) ν: 3480–2957 (NH), 1664 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) δ: 1.19 (s, 9H, 3Me), 5.74 (s, 2H, CH_2), 6.81–6.88 (m, 2H, ArH), 7.18–7.29 (m, 9H, ArH, 4-H, OH), 7.34 (t, $J=7.6$ Hz, 2H, ArH), 7.75 (d, $J=7.6$ Hz, 2H, ArH), 8.45 (s, 1H, =CH), 11.86 (s, 1H, NH); ESI-MS: 453.6 $(M+H)^+$.

*4.1.17. (E)-N′-(2-hydroxybenzylidene)-1-benzyl-3-(4-clorophenyl)-1H-pyrazole-5-carbohydrazide (**17**)*

White solid, yield 98%, mp 208–209 °C; IR (KBr) ν: 3234–3019 (NH), 1649 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) δ: 5.77 (s, 2H, CH_2), 6.80–6.90 (m, 2H, ArH), 7.14–7.28 (m, 8H, ArH, 4-H,), 7.32 (d, $J=8.4$ Hz, 2H, ArH), 7.72 (d, $J=8.4$ Hz, 2H, ArH), 8.43 (s, 1H, =CH), 11.21 (s, 1H, OH), 11.86 (s, 1H, NH); ESI-MS: 431.5 $(M+H)^+$.

*4.1.18. (E)-N′-(2-hydroxybenzylidene)-1-(4-tert-butylbenzyl)-3-(4-chlorophenyl)-1H-pyrazole-5-carbohydrazide (**18**)*

White solid, yield 92%, mp 206–208 °C; IR (KBr) ν: 3173–2866 (NH), 1657 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) δ: 1.19 (s, 9H, 3Me), 5.73 (s, 2H, CH_2), 6.81–6.89 (m, 2H, ArH), 7.18–7.29 (m, 7H, ArH, 4-H), 7.33 (d, $J=8.4$ Hz, 2H, ArH), 7.72 (d, $J=8.4$ Hz, 2H, ArH), 8.44 (s, 1H =CH), 11.20 (s, 1H, OH), 11.89 (s, 1H, NH); ESI-MS: 487.5 $(M+H)^+$.

*4.1.19. (E)-N′-(2-hydroxybenzylidene)-1-benzyl-3-(4-methoxyphenyl)-1H-pyrazole-5-carbohydrazide (**19**)*

White solid, yield 90%, mp 206–208 °C; IR (KBr) ν: 3201–2828 (NH), 1657 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) δ: 3.76 (s, 3H, OMe), 5.76 (s, 2H, CH_2), 6.81–6.89 (m, 4H, ArH), 7.11–7.26 (m, 8H, ArH, 4-H), 7.58 (s, 1H, OH), 7.67 (d, $J=8.4$ Hz, 2H, ArH), 8.44 (s, 1H, =CH), 11.84 (s, 1H, NH); ESI-MS: 427.5 $(M+H)^+$.

*4.1.20. (E)-N′-(2-hydroxybenzylidene)-1-(4-tert-butylbenzyl)-3-(4-methoxyphenyl)-1H-pyrazole-5-carbohydrazide (**20**)*

Yellow solid, yield 92%, mp 158–160 °C; IR (KBr) ν: 3523–2962 (NH), 1659 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$ + DMSO) δ: 1.18 (s, 9H, 3Me), 3.76 (s, 3H, OMe), 5.73 (s, 2H, CH_2), 6.80–6.93 (m, 4H, ArH), 7.09 (s, 1H, 4-H), 7.16–7.22 (m, 7H, ArH, OH), 7.67 (d, $J=8.4$ Hz, 2H, ArH), 8.40 (s, 1H, =CH), 11.67 (s, 1H, NH); ESI-MS: 483.5 $(M+H)^+$.

4.2. Biological activity assay

4.2.1. Materials

RPMI 1640 was obtained from Gibco BRL Co. (Grand Island, USA) and bovine calf serum was from Beijing DingGuo Biotechnology Co. (China). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco.

4.2.2. Cell culture

A549 lung cancer cells were cultured in RPMI 1640 medium, supplemented with 10% (v/v) newborn calf serum at 37 °C in 5% CO_2, and 95% air. The cells were routinely seeded at the density of 3125/cm^2 into 96-well plates or other appropriate dishes containing the medium.

4.2.3. MTT assay for cell viability

The compounds were dissolved in DMSO. The final concentration of DMSO was below 0.1% in the culture medium (v/v) (DMSO at these final concentrations did not affect the viability of the cells). Cells were seeded in 96-well plates and treated with compounds **1**–**20** at 0.1–40 μM for 24, 48 and 96 h, respectively. The cell viability was determined by the MTT assay following the procedure described by Price and McMillan [21]. The light absorptions were measured at 570 nm using SpectraMAX 190 microplate spectrophotometer (GMI Co., USA).

4.2.4. Determination of solubility of compounds in RPMI 1640 medium

The compounds were dissolved in supposed range of concentrations at an interval of 0.5 μM in RPMI 1640 medium, supplemented with 10% (v/v) Bovine Calf Serum, at 37 °C. Then the medium was added into 24-well plates in which had been seeded A549 cells at a density of 3125/cm^2. The plates were incubated at 37 °C in 5% CO_2, and 95% air, in an incubator. The compound was considered to be dissolved completely if there were no visible crystals observed under Phase Contrast Microscope (Nikon, Japan) in the medium at 24 h. The maximum concentration at which the compound dissolved completely was considered the dissolvability of the compound in the medium. We got the dissolvability of the compounds from three independent experiments.

4.2.5. Statistical analyses

Data were expressed as means ± SE, accompanied by the number of experiments performed independently, and analyzed by *t*-test. Differences at $p < 0.05$ were considered statistically significant.

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