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Original article

Novel N-phenyl dichloroacetamide derivatives as anticancer reagents: Design, synthesis and biological evaluation

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ABSTRACT

A current study shows that sodium dichloroacetate (DCA) can induce cancer cell apoptosis and inhibit tumor growth, but its cytotoxic activity is low ($IC_{50} > 1000$ μM for A549). In this paper, a variety of DCA derivatives were synthesized, and their cytotoxic activities were evaluated. The result showed that the N-phenyl-2,2-dichloroacetamide analogues had satisfactory potencies. Among them, N-(3-iodophenyl)-2,2-dichloroacetamide (**3e**), an optimized lead compound, has an IC_{50} against A549 as low as 4.76 μM. Furthermore, it can induce cancer cell apoptosis and has a low toxicity in mice ($LD_{50} = 1117$ mg/kg).

1. Introduction

In 1973, Whitehouse et al. discovered that sodium dichloroacetate (DCA, Fig. 1) could activate mitochondrial pyruvate dehydrogenase [1,2]. Since then, DCA has been studied as an inhibitor of pyruvate dehydrogenase kinase [2–8] (PDK) with efficacies on treating diabetes [9–11], ischemia [12], endotoxic shock [13] acute hepatitis [14], cardiac insufficiency [3], lactic acidosis [15–19] and so on. Clinical studies [16,18,19] have shown that DCA is a lactate-lowering drug which can be employed for treating various diseases caused by acquired and congenital lactic acidosis, with an oral dose of 25–100 mg/kg body weight.

Recently, another great success has been reported [20,21]. DCA can induce cancer cell apoptosis, decrease proliferation and inhibit tumor growth without apparent toxicity [20]. In DCA-treated cancer cells, mitochondrial PDK is inhibited, mitochondrial membrane potential is decreased and the expression of the K^+ channel Kv1.5 is increased. DCA upregulates Kv1.5 through an NFAT1-dependent mechanism, in which DCA reverses the metabolism of cancer cells from glycolysis to glucose oxidation, increases mitochondrial H_2O_2, activates Kv channels, depolarizes mitochondrial membrane and decreases mitochondrial membrane potential (MMP), which opens mitochondrial transition pores (MTP). In the meantime, cytochrome C and AIF are released from the mitochondria to the cytoplasm, activating caspases and inducing apoptosis [20]. Very recent reports have showed that DCA could also induce apoptosis in endometrial cancer cells [22] and help to radiate prostate cancer cells [23] in vitro by a similar mechanism.

This news is so encouraging [20–24] that many dying cancer patients opted for the unapproved drug [24] because they had no time to wait for a long-term clinical study. Embarrassingly, the patients had to adopt the oral dosage for lactic acidosis because so far there is no clinical study for cancer. However, compared to other anticancer drugs in the market, the dosage is very high. Our study also discloses that DCA's cytotoxic activity is indeed not ideal, and the value of IC_{50} for A549 cancer cell is more than 1000 μM. Therefore it is necessary to modify DCA to enhance its cytotoxic activity.

In this paper, a variety of DCA derivatives have been designed, synthesized, and biologically evaluated. We found that a potential compound N-3-iodophenyl-2,2-dichloroacetamide (**3e**) possesses

Abbreviations: DCA, sodium dichloroacetate; SAR, structure-activity relationship; IC_{50}, 50% inhibitory concentration; MTP, mitochondrial transition pores; MMP, mitochondrial membrane potential; AIF, apoptosis-inducing factor; NFAT1, nuclear factor of activated T cells 1.

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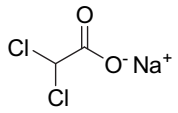


sodium dichloroacetate(DCA)

Fig. 1. Structure of sodium dichloroacetate (DCA).

high activity, low toxicity and could be used as a candidate compound for preclinical study.

2. Chemistry

Synthesis of N-phenyl-2,2-dichloroacetamide derivatives is outlined in Scheme 1. Substituted aniline and 1.3 mol equiv dichloroacetyl chloride reacted in dry toluene, affording the product N-phenyl-2,2-dichloroacetamide derivatives after refluxing about 1–5 h, with yields of 90–99%. **3l** and **4h** were oxygenated [25] for 48 h by AcOH/30% H_2O_2 (v:v/2:1), generating **3m** and **4i**.

3. Pharmacology

3.1. Cytotoxic activity and SAR study

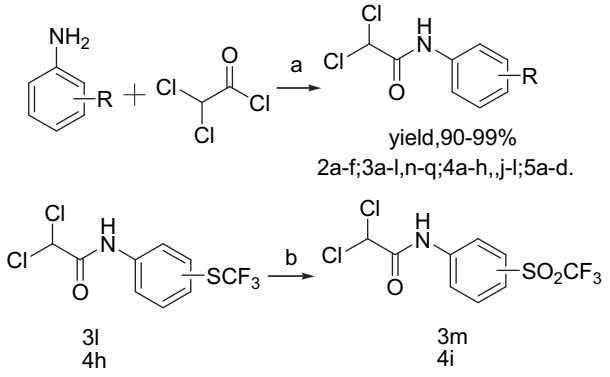
The cytotoxicity of all the synthesized compounds was measured in vitro by MTT assay [26,27] using human tumor cell lines derived from gastric carcinoma (BGC-823), oral epidermoid carcinoma (KB), nonsmall cell lung cancer (A549), and liver carcinoma (BEL-7402). As DCA derivatives on the A549 cell line generally had higher activities, the IC_{50} on A549 was selected as the activity index involved in the SAR discussion. The cytotoxicity of DCA derivatives against the four tumor cell lines will be discussed later.

DCA is a metal-salt that probably cannot penetrate the biomembrane due to its low liposolubility. Accordingly, we designed and synthesized a mass of DCA prodrugs (more than 60) to enhance the liposolubility, including metal-salts, ammonium salts, esters and amides (Figs. 2 and 3). Unexpectedly, most of the compounds could not show better potencies, while N-phenyl-2,2-dichloroacetamide (**1**) displayed a 10-fold increase in IC_{50}. Subsequently, we synthesized a homologous series of N-phenyl-2,2-dichloroacetamide (Table 1). Biological evaluation showed that N-benzyl-2,2-dichloroacetamide and 2,2-dichloro-N-(naphthalen-4-yl)acetamide had no improvement in potency, while substituent N-phenyl-2,2-dichloroacetamides presented satisfactory potency.

Thereafter, we were acutely aware that dichloroacetyl was the active group, and the aniline ring was the essential factor in increasing potency. Optimization of the aniline ring has been accomplished by systematically varying the substituents at ortho-, meta-, and para-positions. These studies showed that the cytotoxic activities of N-phenyl-2,2-dichloroacetamide analogues were completely dependent on the characters and positions of the substituents on the benzene ring (Table 1). Comparing to meta- and para-positions, substitution at ortho-positions obviously had a lower potency. For example, the IC_{50} of **2d** was lower than that of **3d** and **4d**. It was proposed that the ortho-substituent was close to the pharmacophore (2,2-dichloroacetyl) and may block the interaction between the pharmacophore and the receptor. The meta-substituted structures had the best potencies, followed by the ones with para-substituents. For example, the IC_{50} of N-(3-iodophenyl)-2,2-dichloroacetamide (**3e**) was 4.76 μM, and the IC_{50} of N-(4-iodophenyl)-2,2-dichloroacetamide (**4e**) was 12.54 μM. To further study the influence of substituted positions, we synthesized compounds **5a**–**d**. Compared to **3c**, these compounds possessed another Cl atom in 2-, 3-, 4- or 6-position beside the 3-position. The results showed that ortho-Cl reduced the activity (e.g., **5a**, **5d**), while para-Cl (**5b**) and meta-Cl (**5c**) helped to improve the activity, and meta-Cl (**5c**) was the best.

Hence, we focused on the optimization of the meta-position. A series of meta-substituent analogues (**3a**–**q**) have been synthesized and studied. The compounds possessing electron-donating substituents (e.g., **3a**, **3i**) had lower potencies, while those with strong electron-withdrawing substituents (e.g., **3h**, **3j**, **3k**) showed higher activities. However, the ones with moderate electron-withdrawing substituents (e.g., **3e**) exhibited the best potencies, with a 27-fold increase in activity compared to N-phenyl-2,2-dichloroacetamide (**1**). On the other hand, the compounds with hydrophobic substituents (e.g., **3d**–**e**, **3j**–**m**) enhanced the activity, and, with hydrophilic groups, decreased or even lost the activity (e.g., **3n**–**p**). In conclusion, the moderate electron-withdrawing and hydrophobic substituents at the meta-position were beneficial to the activity.

On the whole, DCA derivatives presented powerful potencies in inhibiting not only A549, but also BEL-7402 and KB. The values of IC_{50} against BEL-7402, KB and A549 were almost 5- to 10-fold more potent than against BGC-823. For example, the IC_{50} of N-(3-iodophenyl)-2,2-dichloroacetamide (**3e**) against BEL-7402, KB and A549 was about 5 μM, and 58.83 ± 23.68 μM against BGC-823. Therefore, N-(3-iodophenyl)-2,2-dichloroacetamide (**3e**) had a potential value for drug development.



Scheme 1. General synthetic N-phenyl-2,2-dichloroacetamide derivatives. Reagents and conditions: (a) toluene, reflux, 1–5 h; (b) AcOH/30% H_2O_2 (v:v/2:1), 35 °C, 48 h.

3.2. Annexin V-FITC/propidium iodide analysis of apoptosis

In order to study the mode of cell death (apoptosis or necrosis), compound **3e** was used to induce A549 cells apoptosis by Annexin V-FITC/PI assay (Fig. 4). In early apoptotic cells, membrane phospholipid phosphatidylserine (PS) was translocated from the inner to the outer leaflet of the plasma membrane, exposing PS to the external cellular environment [28]. Annexin V, a Ca^{2+}-dependent phospholipid binding protein with high affinity for PS, binded to cells with exposed PS. With a combination of Annexin V and PI staining, the early apoptosis could be determined. Fig. 4 shows that the percentages of apoptosis (Q2 + Q4) of A549 cells treated with compound **3e** in 10 μM, 15 μM, 30 μM for 24 h were 19.4%, 19.5%, 35.5% respectively, and when the time increased to 48 h, the percentages were 25.4%, 31.3%, 46.2% respectively. Compared to the control assay, compound **3e** could obviously induce apoptosis, and the apoptosis percentage of A549 cells (Q2 + Q4) increased with the concentration of **3e** and treatment time. For example, the apoptosis percentage of cells treated with 30 μM of **3e** for 24 h was 35.5% compared to 19.4% in 10 μM, and 46.4% in 30 μM when treated for 48 h was more than 35.5% at 24 h. The results revealed

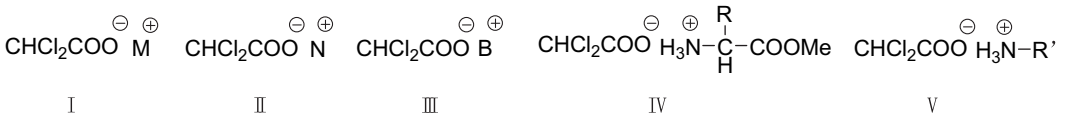


Fig. 2. Structure of DCA salt derivatives. Explanation: I, M = metallic ion; II, N = natural nucleoside and derivatives; III, B = base in nucleoside and derivatives; IV, R = the R substituting group in natural amino acid; V, R′ = alkyl.

a good time-dependent and dose-dependent relationship of **3e** induced A549 cell apoptosis.

3.3. Acute toxicity

The median lethal dose (LD_{50}) in Kunming mice for the compounds with high cytotoxic activities (**3c**, **3e**) were evaluated by intragastric administration for 14 days. The LD_{50} values of **3c** and **3e** were 4549 mg/kg and 1117 mg/kg respectively. The results indicate that the two compounds exhibit low toxicity and are well tolerated by experimental animals.

4. Conclusion

More than 100 DCA derivatives were designed, synthesized, cytotoxic activity screened and SAR studied. The results show that N-phenyl-2,2-dichloroacetamide analogues have satisfactory potencies, and compound **3e** has the highest potency. An apoptosis and acute toxicity study on **3e** showed that it could induce cancer cell apoptosis and had lower toxicity (LD_{50} for mice as 1117 mg/kg). Therefore, it was significant that **3e** would be used as a candidate compound for preclinical research. At the same time, we have offered a novel strategy to modify the classic small molecule drug (DCA), while there has been no report about DCA derivatives since its bioactivity was discovered.

5. Experimental protocols

5.1. Chemistry

Toluene was distilled over calcium hydride, and other reagents, including aniline or substituted aniline and dichloroacetyl chloride, were obtained from commercial suppliers and used without further purification. Proton and carbon magnetic resonance spectra (^{1}H NMR and ^{13}C NMR, JOEL JNM-ECA) were recorded with tetramethylsilane or solvent resonance as the internal standard (^{1}H NMR: TMS at 0.00 ppm, $CDCl_3$ at 7.26 ppm, DMSO-d_6 at 2.50 ppm; ^{13}C NMR: $CDCl_3$ at 77.23 ppm, DMSO-d_6 at 39.51 ppm). Mass spectra were recorded with a Bruker ESQUIRE-LC ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to m/z 6000. HR-MS were obtained using a Bruker Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. TLC analyses were carried out on silica gel F254, and the spots were examined with UV light. All final products had a purity of ≥95%. The purity of final products was determined by HPLC (LabTech). Method: Diamonsil C18 column (4.6 mm × 250 mm, 5 μm); mobile phase: methanol (90%), H_2O (10%); flow rate = 1.0 mL/min; λ = 254 nm.

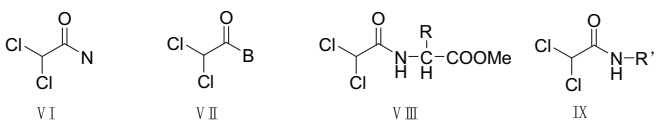
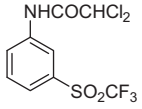


Fig. 3. Structure of DCA amide and ester derivatives. Explanation: VI, N = natural nucleoside and derivatives; VII, B = base in nucleoside and derivatives; VIII, R = the R substituting group in natural amino acid; IX, R′ = alkyl.

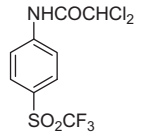
The general procedure for the preparation of N-phenyl-2,2-dichloroacetamide derivatives was as follows: a mixture of aniline or substituted aniline (3 mmol) and dichloroacetyl chloride (3.9 mmol, 1.3 equiv) in dry toluene (20 mL) was stirred under reflux in a round-bottom flask. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 1:1 to 4:1). After 1–5 h, the solvent and excess dichloroacetyl chloride was allowed to evaporate under vacuum. The residual powder was N-phenyl-2,2-dichloroacetamide derivatives, with a 90–99% yield.

5.2. General procedure for the synthesis of *3m* and *4i*



5.2.1. N-(3-(trifluoromethylsulfonyl)phenyl)-2,2-dichloroacetamide *3m*

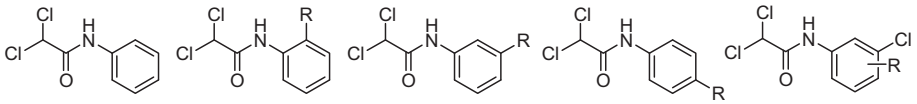
AcOH 20 mL, 30% H_2O_2 10 mL and N-(3-(trifluoromethylthio)-phenyl)-2,2-dichloroacetamide (**3l**) 0.496 g (1.6 mmol) were added in a round-bottom flask, and the mixture was stirred at 35 °C for 48 h. The progress of the reaction was monitored by TLC (petroleum ether/ethyl acetate 4:1). When the reaction finished, the solvent was evaporated under vacuum. The residue was dissolved in 20 mL CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and saturated brines (20 mL). The organic phases were dried with Na_2SO_4 and concentrated. The residue was purified by column chromatography (petroleum ether/ethyl acetate 8:1) to afford the product. Yield: 209 mg (38%), Light yellow solid, mp 98–100 °C; Rf (petroleum ether/ethyl acetate 4:1): 0.50. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.34 (s, 1H), 8.19 (s, 1H), 8.17 (d, J = 8.9, 1H), 7.88 (d, J = 7.6, 1H), 7.72 (dd, J = 7.6, 8.9, 1H), 6.08 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.79, 138.36, 132.27, 131.15,128.25,127.45, 122.02, 119.84 (q, J = 325.8), 66.62; ESI-MS: 287.0 $(M - H)^-$; HR-MS calculated for $C_9H_6Cl_2F_3NO_3S$ $(M - H)^-$ 333.9325, found 333.9331.



5.2.2. N-(4-(trifluoromethylsulfonyl)-phenyl)-2,2-dichloroacetamide *4i*

Procedure for synthesis of **4i** followed **3m**'s steps, N-(4-(trifluoromethylthio)-phenyl)-2,2-dichloroacetamide (**4h**) 0.668 g (2.2 mmol), Yield: 226 mg (31%), light yellow solid, mp 137–139 °C; R_f (petroleum ether/ethyl acetate 4:1): 0.50. ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.36 (s, 1H), 8.07 (d, J = 8.9, 2H), 7.91 (d, J = 8.9, 2H), 6.08 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.67, 144.23, 132.55, 126.74, 120.69, 119.91 (q, J = 326.6), 66.62; ESI-MS: 335.4 $(M - H)^-$; HR-MS calculated for $C_9H_6Cl_2F_3NO_3S$ $(M - H)^-$ 333.9325, found 333.9335.

Table 1
Structure and in vitro activity data for N-phenyl-2,2-dichloroacetamide analogues.



1 2a-f 3a-q 4a-l 5a-d

Entry	R	IC_{50} (μM ± error)[a]			
		BGC-823	KB	A549	BEL-7402
DCA		1051 ± 360	1424 ± 148	1011 ± 300	860 ± 271
1		–[b]	–	130 ± 44	–
2a	Me	–	–	–	–
2b	F	–	–	87.53 ± 25.25	–
2c	Cl	–	–	50.15 ± 3.54	–
2d	Br	–	67.87 ± 20.46	36.99 ± 25.33	38.17 ± 28.59
2e	NO_2	–	253.30 ± 96.92	197.56 ± 58.18	194.1 ± 24.83
2f	$NHCOCHCl_2$	–	–	130.94 ± 6.16	–
3a	Me	–	–	41.05 ± 12.37	–
3b	F	–	–	–	–
3c	Cl	–	–	14.78 ± 4.75	44.45 ± 28.8
3d	Br	95.30 ± 22.81	8.18 ± 7.66	7.80 ± 5.01	7.72 ± 8.52
3e	I	58.83 ± 23.68	5.25 ± 3.25	4.76 ± 2.81	5.39 ± 4.30
3f	CN	–	60.88 ± 10.02	66.53 ± 8.90	48.35 ± 15.12
3g	C≡CH	171.82 ± 41.22	6.26 ± 2.17	11.22 ± 7.08	4.74 ± 3.99
3h	NO_2	122.55 ± 43.90	44.71 ± 23.14	18.04 ± 11.28	15.74 ± 10.45
3i	OMe	179.37	57.69 ± 33.59	25.78 ± 9.97	54.80 ± 6.13
3j	CF_3	142.54	8.86 ± 1.28	12.18 ± 10.25	5.97 ± 1.77
3k	OCF_3	142.14	7.41 ± 2.17	15.05 ± 5.38	10.28 ± 4.44
3l	SCF_3	122.88	4.84 ± 1.85	14.07 ± 4.67	6.54 ± 3.44
3m	SO_2CF_3	63.98 ± 13.39	20.55 ± 18.95	6.53 ± 1.52	9.01 ± 2.57
3n	COOMe	–	–	–	–
3o	$NHCOCHCl_2$	–	–	–	–
3p	$OCOCHCl_2$	–	–	–	–
3q	SO_2Ph(m-$NHCOCHCl_2$)	198.35	23.96 ± 15.08	5.89 ± 2.87	15.88
4a	Me	–	–	48.92 ± 4.29	–
4b	F	–	–	68.41 ± 22.11	–
4c	Cl	–	60.83 ± 12.29	20.37 ± 15.32	49.55 ± 16.51
4d	Br	–	21.70 ± 7.51	13.74 ± 7.00	9.77 ± 4.84
4e	I	221.90 ± 77.50	66.17 ± 77.54	12.54 ± 3.78	11.92 ± 6.04
4f	NO_2	190.00 ± 78.44	60.57 ± 23.16	34.42 ± 32.80	29.50 ± 16.72
4g	OMe	–	277.69 ± 100.14	80.88 ± 41.11	192.18 ± 120.64
4h	SCF_3	203.35 ± 164.98	2.08 ± 1.12	10.56 ± 9.73	3.61 ± 3.72
4i	SO_2CF_3	146.50	9.71 ± 3.36	8.50 ± 4.51	72.82 ± 3.20
4j	COOMe	–	–	–	–
4k	$NHCOCHCl_2$	–	–	–	–
4l	$OCOCHCl_2$	–	–	–	–
5a	2Cl	–	216.98 ± 180.78	–	180.45 ± 0.63
5b	4Cl	176.90 ± 102.43	7.05 ± 3.87	5.09 ± 0.51	7.37 ± 7.06
5c	5Cl	60.05 ± 67.14	4.53 ± 1.65	4.40 ± 0.50	4.65 ± 3.15
5d	6Cl	–	11.53 ± 7.30	12.38 ± 7.34	18.16 ± 14.00

[a] IC_{50} is 50% inhibitory concentration.
[b] '–' means IC_{50} could not be determined (IC_{50} > 400 μM).

*5.2.3. N-phenyl-2,2-dichloroacetamide **1***

White solid, mp 121–122 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.11 (s, 1H), 7.56 (d, J = 8.4, 2H), 7.39 (t, J = 7.2, 2H), 7.21 (t, J = 6.9, 1H), 6.04 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.14, 136.39, 129.39, 125.91, 120.56, 67.05; ESI-MS: 205.6 $(M + H)^+$.

*5.2.4. N-(2-methylphenyl)-2,2-dichloroacetamide **2a***

White solid, mp 140–141 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.09 (s, 1H), 7.77 (d, J = 7.9, 1H), 7.25 (t, J = 7.6, 1H), 7.23 (d, J = 7.9, 1H), 7.15 (t, J = 7.2, 1H), 6.06 (s, 1H), 2.32 (s, 3H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.18, 134.15, 130.91, 130.10, 127.16, 126.62, 123.14, 67.21, 17.59; ESI-MS: 216.0 $(M - H)^-$.

*5.2.5. N-(2-fluorophenyl)-2,2-dichloroacetamide **2b***

White solid, mp 108–109 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.41 (s, 1H), 8.26 (m, 1H), 7.16 (m, 3H), 6.06 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 161.87, 153.05 (d, J = 242.3), 126.11 (d, J = 7.7), 124.99, 124.95, 121.83, 115.33 (d, J = 18.9), 66.75; ESI-MS: 220.0 $(M - H)^-$.

*5.2.6. N-(2-chlorophenyl)-2,2-dichloroacetamide **2c***

White solid, mp 115–116 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.82 (s, 1H), 8.32 (dd, J = 8.3, 1.4, 1H), 7.43 (dd, J = 8.0, 1.4, 1H), 7.33 (td, J = 7.9, 1.4, 1H), 7.14 (td, J = 7.9, 1.4, 1H), 6.07 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 161.84, 133.34, 129.46, 128.10, 126.20, 123.98, 121.58, 67.08; ESI-MS: 238.0 $(M - H)^-$.

*5.2.7. N-(2-bromophenyl)-2,2-dichloroacetamide **2d***

White solid, mp 113–115 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.82 (s, 1H), 8.29 (dd, J = 8.2, 1.4, 1H), 7.59 (dd, J = 8.0, 1.4, 1H), 7.37 (t, J = 7.2, 1H), 7.07 (t, J = 7.0, 1H), 6.07 (s, 1H); ^{13}C NMR (75

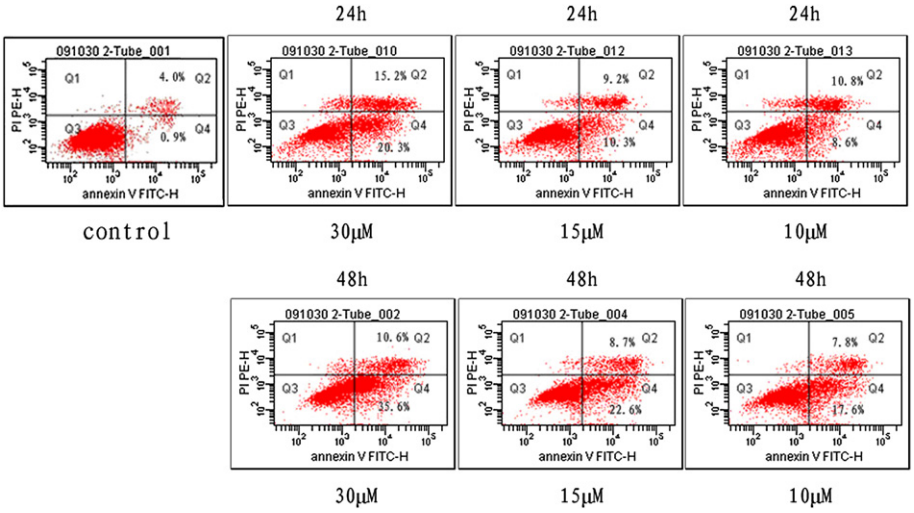


Fig. 4. Flow cytometric analysis of phosphoatidylserine externalization (Annexin V binding) and cell membrane integrity (PI staining). A549 cells were treated with compound **3e** at 10, 15 and 30 μM for 24 and 48 h. Early apoptotic cells were presented in the Q4 quadrant, and late apoptotic cells were presented in the Q2 quadrant.

MHz, CHLOROFORM-d) δ 161.92, 134.45, 132.70, 128.74, 126.67, 121.87, 114.43, 67.14; ESI-MS: 281.8(M − H)⁻.

5.2.8. N-(2-nitrophenyl)-2,2-dichloroacetamide ***2e***

Yellow solid, mp 79–81 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.01 (s, 1H), 8.04 (d, J = 8.1, 1H), 7.78 (t, J = 7.6, 1H), 7.69 (d, J = 7.7, 1H), 7.49 (t, J = 7.7, 1H), 6.78 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.87, 143.26, 134.84, 130.25, 127.30, 126.53, 125.75, 67.25; ESI-MS: 248.0(M − H)⁻.

5.2.9. N-(2-(2,2-dichloroacetamido)phenyl)-2,2-dichloroacetamide ***2f***

White solid, mp 214–216 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.08 (s, 2H), 7.51 (dd, J = 5.9, 3.6, 2H), 7.32 (dd, J = 6.0, 3.5, 2H), 6.65 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.54, 130.02, 126.69, 125.61, 67.02; ESI-MS: 328.9(M − H)⁻.

5.2.10. N-(3-methylphenyl)-2,2-dichloroacetamide ***3a***

White solid, mp 105–106 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.06 (s, 1H), 7.39 (s, 1H), 7.35 (d, J = 8.1, 1H), 7.26 (t, J = 7.7, 1H), 7.02 (d, J = 7.4, 1H), 6.03 (s, 1H), 2.37 (s, 3H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.05, 139.44, 136.30, 129.20, 126.71, 121.13, 117.61, 67.10, 21.6; ESI-MS: 216.0 (M − H)⁻.

5.2.11. N-(3-fluorophenyl)-2,2-dichloroacetamide ***3b***

White solid, mp 133–134 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.19 (s, 1H), 7.52 (s, 1H), 7.52 (dd, J = 8.9, 8.9, 1H), 7.08 (d, J = 8.3, 1H), 7.06 (d, J = 8.9, 1H), 6.05 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.14, 160.38 (d, J = 244.5), 132.39, 122.49 (d, J = 7.9), 116.20(d, J = 22.2), 66.94; ESI-MS: 220.0 (M − H)⁻.

5.2.12. N-(3-chlorophenyl)-2,2-dichloroacetamide ***3c***

White solid, mp 104–105 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.09 (s, 1H), 7.68 (s, 1H), 7.42 (d, J = 8.0, 1H), 7.31 (t, J = 8.0, 1H), 7.19 (d, J = 7.7, 1H), 6.04 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.02, 137.52, 135.18, 130.44, 126.01, 120.58, 118.42, 66.88; ESI-MS: 238.0 (M − H)⁻.

5.2.13. N-(3-bromophenyl)-2,2-dichloroacetamide ***3d***

White solid, mp 107–108 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.11 (s, 1H), 7.81 (s, 1H), 7.48 (d, J = 7.5, 1H), 7.34 (d, J = 7.7, 1H), 7.23 (t, J = 7.9, 1H), 6.04 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.10, 137.62, 130.69, 128.94, 123.46, 123.00, 118.99, 66.87; ESI-MS: 281.7 (M − H)⁻.

5.2.14. N-(3-iodophenyl)-2,2-dichloroacetamide ***3e***

White solid, mp 116–117 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.74 (s, 1H), 8.07 (s, 1H), 7.56 (d, J = 8.2, 1H), 7.52 (d, J = 8.0, 1H), 7.18 (t, J = 8.0, 1H), 6.58 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.87, 138.91, 133.18, 130.96, 127.99, 119.09, 94.58, 67.16; ESI-MS: 328 (M − H)⁻.

5.2.15. N-(3-cyanophenyl)-2,2-dichloroacetamide ***3f***

Yellow solid, mp 149–150 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.27 (s, 1H), 7.99 (s, 1H), 7.87–7.70 (m, 1H), 7.60–7.42 (m, 2H), 6.07 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.28, 138.43, 130.45, 128.19, 124.48, 122.60, 118.39, 111.91, 67.13; ESI-MS: 227.2 (M − H)⁻.

5.2.16. N-(3-ethynylphenyl)-2,2-dichloroacetamide ***3g***

Yellow solid, mp 111–113 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.11 (s, 1H), 7.69 (s, 1H), 7.58 (d, J = 3.5, 1H), 7.35–7.31 (m, 2H), 6.04 (s, 1H), 3.11 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.45, 136.39, 129.57, 129.39, 124.12, 123.31, 121.16, 82.83, 78.34, 66.91; ESI-MS: 226.2 (M − H)⁻.

5.2.17. N-(3-nitrophenyl)-2,2-dichloroacetamide ***3h***

Yellow solid, mp 103–105 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.15 (s, 1H), 8.59 (t, J = 2.1, 1H), 8.00 (dd, J = 8.2, 2.3, 1H), 7.96 (dd, J = 7.8, 1.6, 1H), 7.68 (t, J = 8.2, 1H), 6.64 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.40, 147.97, 138.75, 130.49, 125.83, 119.15, 114.05, 67.15; ESI-MS: 246.8 (M − H)⁻.

5.2.18. N-(3-methoxyphenyl)-2,2-dichloroacetamide ***3i***

White solid, mp 79–82 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.12 (s, 1H), 7.34–7.21 (m, 2H), 7.04 (d, J = 8.0, 1H), 6.75 (dd, J = 8.3, 2.4, 1H), 6.04 (s, 1H), 3.83 (s, 3H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.51, 160.27, 137.49, 130.01, 112.92, 111.73, 106.48, 67.07, 55.45; ESI-MS: 256.0 (M + Na)⁺.

5.2.19. N-(3-(trifluoromethyl)-phenyl)-2,2-dichloroacetamide ***3j***

Pink solid, mp 80–82 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.24 (s, 1H), 7.86 (s, 1H), 7.78 (d, J = 7.7, 1H), 7.52 (t, J = 7.9, 1H), 7.47 (d, J = 7.8, 1H), 6.06 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.86, 136.92, 131.81 (q, J = 32.7), 130.00, 123.90, 123.78 (q, J = 270.3),122.60, 117.60, 66.82; ESI-MS: 271.3 (M − H)⁻.

5.2.20. N-(3-(trifluoromethoxy)-phenyl)-2,2-dichloroacetamide ***3k***

White solid, mp 76–77 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.93 (s, 1H), 7.74 (s, 1H), 7.57 (d, J = 8.3, 1H), 7.51 (t, J = 8.0, 1H), 7.15 (d, J = 7.1, 1H), 6.61 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.17, 148.57, 139.29, 130.74, 120.08(q, J = 254.6), 118.49, 116.67, 112.00, 67.20; ESI-MS: 287.3 $(M-H)^-$; HR-MS calculated for $C_9H_6Cl_2F_3NO_2$ $(M-H)^-$ 285.9655, found 285.9661.

5.2.21. N-(3-(trifluoromethylthio)-phenyl)-2,2-dichloroacetamide ***3l***

White solid, mp 88–89 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.19 (s, 1H), 7.87 (s, 1H), 7.75 (d, J = 7.8, 1H), 7.50 (d, J = 7.8, 1H), 7.44 (t, J = 7.8, 1H), 6.06 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.68, 137.34, 133.47,130.36, 129.59 (q, J = 308.5), 127.94, 125.67, 123.03, 66.83; ESI-MS: 302.2 $(M-H)^-$; HR-MS calculated for $C_9H_6Cl_2F_3NOS$ $(M-H)^-$ 301.9427, found 301.9434.

5.2.22. N-(3-methoxycarbonylphenyl)-2,2-dichloroacetamide ***3n***

White solid, mp 108–110 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.54 (s, 1H), 8.13 (s, 1H), 7.92 (d, J = 7.9, 1H), 7.87 (d, J = 7.6, 1H), 7.47 (t, J = 7.8, 1H), 6.12 (s, 1H), 3.92 (s, 3H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 166.70, 162.54, 136.76, 131.15,129.52,126.81, 125.12, 121.65, 66.91, 52.58; ESI-MS: 262 $(M+H)^+$; HR-MS calculated for $C_{10}H_9Cl_2NO_3$ $(M-H)^-$ 259.9887, found 259.9893.

5.2.23. N-(3-(2,2-dichloroacetamido)phenyl)-2,2-dichloroacetamide ***3o***

Brown solid, mp 199–201 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.76 (s, 2H), 8.03 (s, 1H), 7.38 (d, J = 1.2, 3H), 6.60 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.79, 138.12, 129.62, 115.98,111.16,67.29; ESI-MS: 329.0 $(M-H)^-$.

5.2.24. N-(3-(2,2-dichloroacetoxy)phenyl)-2,2-dichloroacetamide ***3p***

White solid, mp 156–158 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.49 (s, 1H), 9.55 (s, 1H), 7.16 (s, 1H), 7.14 (t, J = 8.3, 1H), 6.98 (d, J = 7.9, 1H), 6.55 (dd, J = 2.0, 8.3, 1H), 6.54 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.54, 157.81, 138.59, 129.78, 111.84, 110.47, 106.83, 67.40; ESI-MS: 330.4 $(M-H)^-$; HR-MS calculated for $C_{10}H_7Cl_4NO_3$ $(M-H)^-$ 327.9107, found 327.9113.

*5.2.25. N-(3-(3-(2,2-dichloroacetamido)-phenylsulfonyl)phenyl)-2,2-dichloroacetamid (****3q****)*

Light yellow solid, mp 205–206 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.07 (s, 2H), 8.27 (s, 2H), 7.88 (d, J = 8.0, 2H), 7.73 (d, J = 7.9, 2H), 7.65 (t, J = 7.9, 2H), 6.60 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.29, 141.47, 138.78, 130.85, 124.60, 123.24, 117.94, 67.17; ESI-MS: 470.0 $(M-H)^-$; HR-MS calculated for $C_{16}H_{12}Cl_4N_2O_4S$ $(M-H)^-$ 466.9199, found 466.9204.

5.2.26. N-(4-methylphenyl)-2,2-dichloroacetamide ***4a***

White solid, mp 159–160 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.54 (s, 1H), 7.48 (d, J = 8.6, 2H), 7.18 (d, J = 8.6, 2H), 6.57(s, 1H), 6.28 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.78, 137.66, 137.40, 121.93, 88.62, 67.25; ESI-MS: 327.9 $(M-H)^-$.

5.2.27. N-(4-fluorophenyl)-2,2-dichloroacetamide ***4b***

White solid, mp 134–135 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.10 (s, 1H), 7.53 (dd, J = 8.8, 4.6, 2H), 7.08 (t, J = 8.5, 2H), 6.04 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.07, 160.38 (d, J = 243.8), 132.38, 122.52(d, J = 8.3), 116.19(d, J = 22.5), 66.93; ESI-MS: 220.9 $(M-H)^-$.

5.2.28. N-(4-chlorophenyl)-2,2-dichloroacetamide ***4c***

White solid, mp 141–142 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.11 (s, 1H), 7.52 (d, J = 8.8, 2H), 7.35 (d, J = 8.8, 2H), 6.04 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.10, 134.95, 131.15, 129.48, 121.80, 66.91; ESI-MS: 238.0 $(M-H)^-$.

5.2.29. N-(4-bromophenyl)-2,2-dichloroacetamide ***4d***

White solid, mp 148–150 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.76 (s, 1H), 7.57 (d, J = 9.7, 4H), 6.58 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.37, 137.50, 132.37, 122.31, 117.03, 67.80; ESI-MS: 281.8 $(M-H)^-$.

5.2.30. N-(4-iodophenyl)-2,2-dichloroacetamide ***4e***

White solid, mp 173–175 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.74 (s, 1H), 7.72(d, J = 8.6, 2H), 7.44 (d, J = 8.6, 2H), 6.58 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.78, 137.66, 137.40, 121.93, 88.62, 67.25; ESI-MS: 327.9 $(M-H)^-$.

5.2.31. N-(4-nitrophenyl)-2,2-dichloroacetamide ***4f***

Yellow solid, mp 130–132 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.24 (s, 1H), 8.29(d, J = 9.2, 2H), 7.88 (d, J = 9.2, 2H), 6.65 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.46, 143.70, 143.26, 125.02, 119.78, 67.14; ESI-MS: 247.9 $(M-H)^-$.

5.2.32. N-(4-methoxyphenyl)-2,2-dichloroacetamide ***4g***

White solid, mp 136–137 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.07 (s, 1H), 7.46 (d, J = 9.0, 2H), 6.90 (d, J = 9.0, 2H), 6.04 (s, 1H), 3.81 (s, 3H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.03, 157.61, 129.36, 122.39, 114.55, 67.06, 55.69; ESI-MS: 231.9 $(M-H)^-$.

5.2.33. N-(4-(trifluoromethylthio)-phenyl)-2,2-dichloroacetamide ***4h***

White solid, mp 124–125 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.20 (s, 1H), 7.91–7.49 (m, 4H), 6.05 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.42, 138.96, 137.68, 129.60 (q, J = 308.3), 121.07, 66.85; ESI-MS: 302.2 $(M-H)^-$; HR-MS calculated for $C_9H_6Cl_2F_3NOS$ $(M-H)^-$ 301.9427, found 301.9434.

5.2.34. N-(4-Methoxycarbonylphenyl)-2,2-dichloroacetamide ***4j***

White solid, mp 141–143 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.98 (s, 1H), 7.98 (d, J = 8.8, 2H), 7.75 (d, J = 8.7, 2H), 6.62 (s, 1H), 3.84 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.64, 162.15, 141.95, 130.43, 125.36, 119.35, 67.23, 52.00; ESI-MS: 261.7 $(M+H)^+$; HR-MS calculated for $C_{10}H_9Cl_2NO_3$ $(M-H)^-$ 259.9887, found 259.9891.

5.2.35. N-(4-(2,2-dichloroacetamido)phenyl)-2,2-dichloroacetamide ***4k***

White solid, mp 263–265 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.68 (s, 2H), 7.61 (s, 4H), 6.58 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.58, 134.11, 120.44, 67.32; ESI-MS: 329.1 $(M-H)^-$.

5.2.36. N-(4-(2,2-dichloroacetoxy)phenyl)-2,2-dichloroacetamide ***4l***

White solid, mp 140–142 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.38 (s, 1H), 9.39 (s, 1H), 7.38 (d, J = 8.8, 2H), 6.75 (d, J = 8.8, 2H), 6.53 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.16, 154.46, 129.06, 121.60, 115.41, 67.46; ESI-MS: 330.4$(M+H)^+$; HR-MS calculated for $C_{10}H_7Cl_4NO_3$ $(M-H)^-$ 327.9107, found 327.9112.

5.2.37. N-(2,3-dichloropheny)-2,2-dichloroacetamide ***5a***

White solid, mp 140–141 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.47 (s, 1H), 7.62 (dd, J = 8.0, 1.3, 1H), 7.56 (dd, J = 8.1, 1.4, 1H), 7.42 (t, J = 8.1, 1H), 6.81 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.62, 135.36, 132.19, 128.27, 128.14, 126.44, 125.31, 66.66; ESI-MS: 271.5 $(M-H)^-$.

5.2.38. N-(3,4-dichloropheny)-2,2-dichloroacetamide ***5b***

White solid, mp 148–149 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.95 (s, 1H), 7.95 (d, J = 2.4, 1H), 7.64 (d, J = 8.8, 1H), 7.54 (dd, J = 8.8, 2.4, 1H), 6.60 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.14, 137.65, 131.26, 130.92, 126.33, 121.11, 119.92, 67.12; ESI-MS: 271.6 $(M-H)^-$.

5.2.39. N-(3,5-dichlorophenyl)-2,2-dichloroacetamide ***5c***

White solid, mp 145–146 °C, ^{1}H NMR (300 MHz, CHLOROFORM-d) δ 8.11 (s, 1H), 7.54 (d, J = 1.4, 2H), 7.20 (s, 1H), 6.03 (s, 1H); ^{13}C NMR (75 MHz, CHLOROFORM-d) δ 162.10, 138.19, 135.81, 125.94, 118.73, 66.72; ESI-MS: 295.9 $(M + Na)^+$.

5.2.40. N-(2,5-dichlorophenyl)-2,2-dichloroacetamide ***5d***

White solid, mp 150–151 °C, ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.42 (s, 1H), 7.80 (d, J = 2.4, 1H), 7.60 (d, J = 8.7, 1H), 7.38 (dd, J = 8.6, 2.5, 1H), 6.83 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.72, 134.67, 131.78, 131.13, 127.32, 125.85, 125.60, 66.58; ESI-MS: 271.5 $(M - H)^-$.

5.3. Cell culture and cytotoxic activity assays

Human gastric carcinoma cell line (BGC-823), human oral epidermoid carcinoma cell line (KB), human nonsmall cell lung cancer cell line (A549), and Human liver carcinoma cell Line (BEL-7402), were obtained from ATCC. BGC-823, KB, and BEL-7402 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2. A549 cells were maintained in F12-k supplemented with 10% fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2.

Cytotoxic activity was assessed by standard MTT assay. A suspension of cells (3000/well in 180 μL) were seeded in 96-well plates and cultured for 24 h. 10 different concentrations of drugs ranging from 4×10^{-4} to 1×10^{-7} M (the concentration of DCA was magnified 10-fold) were added to the corresponding plates with 20 μL/well, and the plates were incubated for 72 h. Then, 20 μL of MTT solution (5 mg/mL) was added to each well. After 4 h of incubation at 37 °C, 50 μL of lysing buffer (20% w/v sodium dodecyl sulfate; 10% v/v isobutanol and 0.024 M HCl) was added to each well to dissolve the formazan crystals, and the solutions were incubated overnight. The absorbance of the wells was read with a test wavelength of 570 nm. The results were expressed as IC_{50} values, which were tested three times independently.

5.4. Annexin V-FITC/propidium iodide analysis of apoptosis

After 24 h or 48 h of drug treatment (30 μM, 15 μM, 10 μM and 0 μM), A549 cells were trypsinized and resuspended in PBS. Cells from plates were collected and centrifuged, and the pellets were used for the flow cytometric apoptosis analysis by ANNEXIN V-FITC/PI kit according to the manufacturer's protocol.

5.5. Determination of acute toxicity (LD_{50})

Kunming mice, half male and half female (18–20 g), purchased from the Experimental Animal Center of Shandong University, were used for determination of the intragastric LD_{50} of two compounds (**3c**, **3e**). The drugs were dispersed in 0.5% sodium carboxymethyl cellulose (CMC) of 10 mg/mL to obtain a homogeneous suspension. 50 animals were divided randomly into 5 groups. Compound **3c** was given orally by intragastric administration, according to 0.4 mL/10 g, in doses of 3132, 3684, 4335, 5100, 6000 mg/kg and **3e** in doses of 633, 844, 1125, 1500, 2000 mg/kg. Changes in appearance, behavior and mortality in mice were monitored for 14 days after administration. LD_{50} values were calculated according to the Kaerber method.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the on-line version, at doi:10.1016/j.ejmech.2010.06.032.

References

[1] S. Whitehouse, P.J. Randle, Biochem. J. 134 (1973) 651–653.
[2] S. Whitehouse, R.H. Cooper, P.J. Randle, Biochem. J. 141 (1974) 761–774.
[3] P.W. Stacpoole, Metabolism 38 (1989) 1124–1144.
[4] M. Kato, J. Li, J.L. Chuang, D.T. Chuang, Structure 15 (2007) 992–1004.
[5] T.D. Aicher, R.C. Anderson, J.P. Gao, S.S. Shetty, G.M. Coppola, J.L. Stanton, et al., J. Med. Chem. 43 (2000) 236–249.
[6] E. Kolobova, A. Tuganova, I. Boulatnikov, K.M. Popov, FEBS Lett. 419 (1997) 197–200.
[7] T.D. Aicher, R.C. Anderson, G.R. Bebernitz, G.M. Coppola, C.F. Jewell, D.C. Knorr, et al., J. Med. Chem. 42 (1999) 2741–2746.
[8] G.R. Bebernitz, T.D. Aicher, J.L. Stanton, J.P. Gao, S.S. Shetty, et al., J. Med. Chem. 43 (2000) 2248–2257.
[9] P.J. Blackshear, P.A.H. Holloway, K.G.M.M. Alberti, Biochem. J. 142 (1974) 279–286.
[10] P.W. Stacpoole, G.W. Moore, D.M.N. Kornhauser, New. Engl. J. Med. 298 (1978) 526–530.
[11] P.G. Wells, G.W. Moore, D. Rabin, G.R. Wilkinson, J.A. Oates, Diabetologia 19 (1980) 109–113.
[12] R.M. Bersin, P.W. Stacpoole, Am. Heart J. 134 (1997) 841–855.
[13] J.C. Preiser, D. Moulart, J.L. Vincent, Circ. Shock 30 (1990) 221–228.
[14] G.A.H. Johnson, K.G.M.M. Alberti, Biochem. Soc. Trans. 5 (1977) 1387–1388.
[15] G.N. Henderson, S.H. Curry, H. Derendorf, E.C. Wright, P.W. Stacpoole, J. Clin. Pharmacol. 37 (1997) 416–425.
[16] P.W. Stacpoole, D.S. Kerr, C. Barnes, S.T. Bunch, P.R. Carney, et al., Pediatrics 5 (2006) 1519–1531.
[17] P.W. Stacpoole, N.V. Nagaraja, A.D. Hutson, J. Clin. Pharmacol. 43 (2003) 683–691.
[18] P.W. Stacpoole, G.N. Henderson, Z.M. Yan, R. Cornett, M.O. James, Drug Metab. Rev. 3 (1998) 499–539.
[19] P.W. Stacpoole, L.R. Gilbert, R.E. Neiberger, P.R. Carney, E. Valenstein, D.W. Theriaque, J.J. Shuster, Pediatrics 5 (2008) 1223–1228.
[20] S. Bonnet, S.L. Archer, J. Allalunis-Turner, A. Haromy, E.D. Michelakis, et al., Cancer Cell. 11 (2007) 37–51.
[21] E.D. Michelakis, L. Webster, J.R. Mackey, Br. J. Cancer 99 (2008) 989–994.
[22] J.Y.Y. Wong, G.S. Huggins, M. Debidda, N.C. Munshi, I.D. Vivo, Gynecol. Oncol. 109 (2008) 394–402.
[23] W.G. Cao, S. Yacoub, K.T. Shiverick, K. Namiki, Y. Sakai, S. Porvasnik, C. Urbanek, C.J. Rosser, Prostate 11 (2008) 1223–1231.
[24] H. Pearson, Nature 446 (2007) 474–475.
[25] L. Porre`s, O. Mongin, C. Katan, M. Charlot, T. Pons, J. Mertz, M. Blanchard-Desce, Org. Lett. 1 (2004) 47–50.
[26] M. Huang, H.Y. Gao, Y. Chen, H. Zhu, J. Ding, et al., Clin. Cancer Res. 4 (2007) 1298–1307.
[27] Y. Yang, Q.J. Lv, Q.Y. Du, B.H. Yang, R.X. Lin, S.Q. Wang, World J. Gastroenterol. 16 (2005) 2491–2496.
[28] S.J. Martin, C.E.M. Reutelingsperger, A.J. McGahon, J.A. Rader, R.C.A.A. van Schie, D.M. LaFace, D.R. Green, J. Exp. Med. 182 (1995) 1545–1556.