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Design, synthesis, molecular docking and cytotoxic activity evaluations of novel piperidine and piperazine derivatives of dichloroacetate as potential anticancer agents

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Dichloroacetate (DCA) as a small and active anticancer agent through inhibition of pyruvate dehydrogenase kinases (PDKs), prevents proliferation of tumor growth. In this research, a series of novel piperidine and piperazine derivatives of DCA have been designed and subjected to molecular docking studies. Based on the docking results, nine compounds with the lowest binding energy and better interaction with PDKs isoenzymes have been selected and synthesized. The cytotoxic activities of the synthesized compounds have been evaluated against HT-29 and MCF7 human cancer cell lines. These compounds show moderate potencies with much higher anticancer activity than DCA. The most active of the series, **f1**, showed IC_{50} value of 7.79 μM against HT-29 cell line.

Keywords: Dichloroacetate (DCA), cytotoxic activity, molecular docking, pyruvate dehydrogenase kinase inhibitors, synthesis $CDCl_3$

Dichloroacetate (DCA) is a simple and inexpensive chemical compound with promising anticancer activity. Its salts and derivatives[1] may serve as a viable treatment to many forms of cancer *via* inhibition of pyruvate dehydrogenase kinase[2-5]. Also DCA, has several therapeutic applications for example in ischemia[6], diabetes[7], endotoxic shock[8], acute hepatitis[9] and cardiac insufficiency[10].

Researches has shown that DCA can prevent tumor growth *via* enforcing of cell death (apoptosis) without any significant toxicity[11] in endometrial[12], prostate[13], pediatric[14], pancreatic[15], cervical[16] and colorectal[17] cancer cells.

Main path of apoptosis in cells widespread is adjusted by mitochondrial function. Dysfunction and activity of mitochondria is an advantage for proliferation of cancer cells in comparison with normal cells. Mitochondria is adjusted energy production by oxidation of pyruvate and lipid. Glucose oxidation is initiated with insertion of pyruvate in mitochondria. finally, function of mitochondria in glucose oxidation is involved in apoptosis[18].

Pyruvate dehydrogenase complex (PDC) is regulated mitochondrial function. The four isozymes known for PDKs (2BU8, 3D2R, 1Y8O and 2Q8H)[19] have been distributed in different tissues[20]. DCA as a mitochondrial kinase inhibitor which can inactivated pyruvate dehydrogenase (PDH) through inhibition of pyruvate dehydrogenase kinases (PDKs), Hence pyruvate insertion to the mitochondria is limited and finally the tumor growth is stopped[21].

Recently, new researches had been reported dichloroacetamide derivatives which showed moderate to high potencies against different cancer cell lines with higher cytotoxic activities than DCA as the parent molecule[1,22,23].

In this article, a series of 2,2-dicholoroa-cetylpiperidine and 2,2-dicholoroacetylpiperazine derivatives as an anticancer agents have been synthesized and fully characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The cytotoxic activities of them have been evaluated against human breast (MCF7) and human colon (HT-29) cancer cell lines. Molecular docking studies of them were also conducted to find their types of interaction with PDKs isoenzymes.

Materials and Methods

Molecular Docking

In this study, at first, 100 structures of piperazine and piperidine derivatives of DCA were designed based on Scheme I. The two dimensional structures of them were drawn using Chem BioDraw Ultra 13.0. The ligands were subjected to minimization procedures by means of an in house TCL script[20, 22, 24] using Hyperchem (Version 8, Hypercube Inc., Gainesville, FL, USA). The three dimensional crystal structure of PDKs (2BU8, 3D2R, 1Y8O and 2Q8H) were obtained from protein data bank[25]. Ligand – receptor interactions were performed *via* Dockface software[26, 27]. A grid box of 50 ×50×50 points in x, y, and z direction with a grid spacing of 0.375 Å was made with X center, Y center and Z center 56.344, 44.674 & 80.946 for 2BU8; 1.439, 38.929 &-9,933 for 2Q8H;-63.421, 4.375 & 75.947 for 1Y8O and-25,-6.8 and 6 for 3D2R respectively[28-30]. The lowest docking binding energies for the synthesized compounds were shown in Table I.

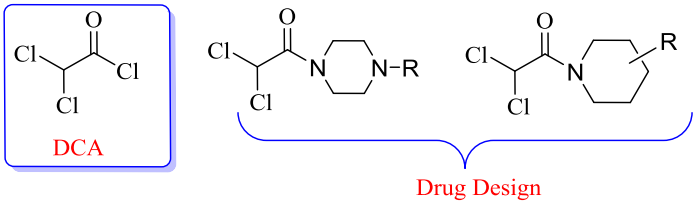
Experimental Section

All of the compounds were purchased from Sigma Aldrich or Merck chemical companies. The FT-IR spectra were obtained on a Bruker's VERTEX 70. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 250 instrument.

Synthesis of 2,2-dicholoroacetylpiperazine and 2,2-dicholoroacetylpiperidine derivatives is shown in Scheme II and Scheme III, respectively.

General procedure for the synthesize of N-dichloroacetyl piperazine

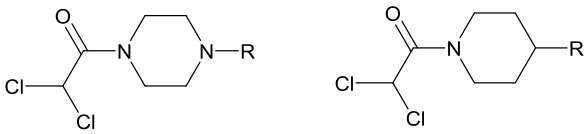
A mixture of piperazine or substituted piperazine (3 mmol) and dichloroacetyl chloride (3.5 mmol) in dry toluene or chloroform (15 mL) was stirred for 1-4 hours in a round-bottom flask. Then, 10 mL saturated aqueous $NaHCO_3$ was added to the reaction mixture in a separatory funnel, the organic layer was separated and the solvent was allowed to evaporate. The residual powder N-dichloroacetylpiperazine or its derivatives, were purified with ethanol.



R: Phenyland substituted phenyl with EDG and EWG, alkyl, acetyland halid

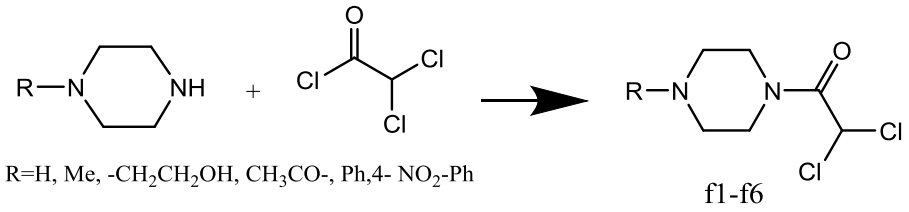
Scheme I — Chemical structure of 2,2-dicholoroacetylpiperazine and 2,2-dicholoroacetylpiperidine derivatives used in molecular docking study

Table I — Docking binding Energy (kcal/mol) of synthesized compounds on PDK1-4 isoenzymes

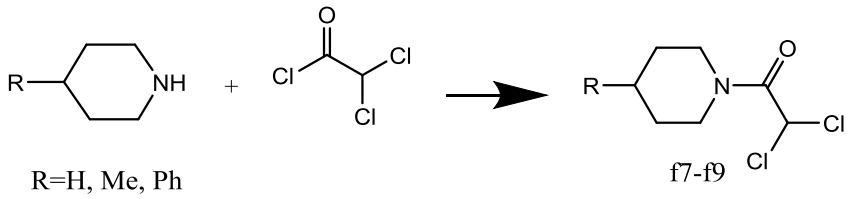


f1-f6 f7-f9

Name	Lowest Binding Energy ΔG_b(Kcal/mol)			
Receptor	2BU8	3D2R	1Y8O	2Q8H
DCA	-4.27	-4.08	-3.87	-4.08
f1	-5.28	-4.19	-5.68	-4.77
f2	-5.54	-4.50	-6.81	-5.67
f3	-4.07	-3.46	-4.68	-4.19
f4	-4.31	-3.71	-6.59	-4.63
f5	-4.78	-4.65	-9.77	-5.31
f6	-4.81	-4.10	-5.21	-4.99
f7	-5.49	-4.68	-5.31	-6.11
f8	-5.76	-4.73	-5.56	-6.48
f9	-5.78	-4.92	-6.85	-6.02



Scheme II — Synthesis of 2,2-dicholoroacetylpiperazine derivatives



Scheme III — Synthesis of 2,2-dicholoroacetylpiperidine derivatives

General procedure for the synthesis N-dichloroacetyl piperidine

Dichloroacetyl chloride (3.5 mmol) and 3 mmol piperidine and substituted piperidine in a 25 mL flask in 15 mL dry solvent (chloroform or toluene) was stirred under reflux for 4 hours. Then, washed with 10 mL saturated aqueous $NaHCO_3$. The organic layer was separated by decanter, after vaporization of the solvent the crude product was recrystallized by ethanol.

1, 1'-(Piperazine-1,4-diyl)bis(2,2-dichloroethan-1-one), f1: White solid. Yield 90%. m.p.216°C. IR (KBr): 1667.04, 1245.17, 648.45 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 6.20 (s, 2H), 4.38 – 3.04 (m, 8H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 163.28, 66.56, 46.84, 43.63.

2,2-Dichloro-1-(4-phenylpiperazin-1-yl) ethan-1-one, f2: White solid. Yield 65%. m.p.131-132°C. IR (KBr): 1662, 1445-1594, 3027, 2811, 2906, 763, 1220.58 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.33 – 6.90 (m, 5H), 6.23 (s, 1H), 3.91-3.80 (dt, *J* = 22.1, 5.3 Hz, 4H), 3.29 – 3.01 (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 163.09, 151.06, 130.31, 121.84, 117.77, 66.66, 50.33, 50.19, 47.33, 44.11.

2,2-Dichloro-1-(4-(2-hydroxyethyl) piperazin-1-yl) ethan-1-one, f3: White solid. Yield 85%. m.p up to 165°C. IR (KBr): 1676.24, 1251.76, 2937, 3336.61, 1445, 652 cm^{-1}; ^1H NMR (250 MHz, D_2O): δ 6.80 (s, 1H), 4.66 (s, 2H), 3.85 (q, *J* = 5.4 Hz, 4H), 3.42 (m, 2H), 3.28 (m, 4H); ^{13}C NMR (63 MHz, D_2O): δ 165.46, 65.44, 59.1, 55.79, 52.01, 43.91, 41.04.

2,2-Dichloro-1-(4-methylpiperazin-1-yl) ethan-1-one, f4: White solid. Yield 75%. m.p. up to 165°C. IR (KBr): 1673, 2949, 1456, 1263, 655 cm^{-1}; ^1H NMR (250 MHz, D_2O): δ 6.75 (s, 1H), 4.47 (d, *J* = 13.5 Hz, 2H), 4.17 (d, *J* = 15.1 Hz, 2H), 3.49 (d, *J* = 11.4 Hz, 2H), 3.23 – 2.93 (m, 2H), 2.81 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 165.52, 65.37, 53.42, 44.14, 43.87, 41.31.

2,2-Dichloro-1-(4-(4-nitrophenyl) piperazine-1-yl) ethan-1-one, f5: Yellow solid. Yield 96%. m.p. 166-168°C. IR (KBr): 1662.68, 1323,159, 1234.19, 655.07 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 8.16 (d, *J* = 9.5 Hz, 2H), 6.86 (d, *J* = 9.6 Hz, 2H), 6.22 (s, 1H), 4.04 – 3.75 (m, 4H), 3.67 – 3.40 (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 162.22, 154.26, 139.44, 125.91, 113.28, 77.21, 65.57, 46.87, 46.59, 45.51, 42.57.

1-(4-Acetylpiperazin-1-yl)-2,2-dichloroethan-1-one, f6: White solid. Yield 58%. m.p. 115-116°C. IR (KBr): 1637.11, 1670.95, 1435.99, 1245, 804 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 6.20 (s, 1H), 4.19 – 3.17 (m, 8H), 2.13 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 169.20, 162.39, 65.73, 65.43, 46.26, 46.10, 45.59, 43.11, 40.89, 40.73, 21.30.

2,2-Dichloro-1-(piperidin-1-yl) ethan-1-one, f7: White solid. Yield 61%. m.p. 41-42°C. IR (KBr): 1667.04, 1443, 1245, 648.45 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$) δ 6.22 (s, 1H), 3.96 – 3.23 (m, 4H), 1.93 – 1.25 (m, 6H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 161.82, 65.78, 47.4, 44.38, 25.85, 25.30, 24.08.

2,2-Dichloro-1-(4-methylpiperidin-1-yl) ethan-1-one, f8: White solid. Yield 90%. m.p. 44-45°C. IR (KBr): 1661.96, 2932, 1451, 1253, 654 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 6.22 (s, 1H), 4.45 (d, *J* = 13.5 Hz, 1H), 4.13 (d, *J* = 13.6 Hz, 1H), 3.09 (t, *J* = 12.1 Hz, 1H), 2.68 (t, *J* = 11.8 Hz, 1H), 1.69 (t, *J* = 12.3 Hz, 4H), 1.23 (ddd, *J* = 17.0, 12.6, 6.3 Hz, 1H), 0.95 (d, *J* = 6.2 Hz, 3H); ^{13}C NMR (63 MHz,

$CDCl_3$): δ 162.24, 66.23, 47.11, 44.19, 34.42, 33.88, 31.12, 21.94.

2,2-Dichloro-1-(4-phenylpiperidin-1-yl) ethan-1-one, f9: White solid. Yield 95%. m.p. 74-75°C. IR (KBr): 1652, 1451.87, 755.31, 699.78 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.90 – 6.77 (m, 5H), 6.25 (s, 1H), 4.96 – 4.44 (m, 1H), 4.59 – 4.12 (m, 1H), 3.53 – 3.04 (m, 1H), 2.99 – 2.55 (m, 2H), 2.20 – 1.68 (m, 4H); ^{13}C NMR (63 MHz, $CDCl_3$): δ 162.97, 145.62, 129.17, 127.68, 66.89, 48.07, 45.14, 43.32, 34.25, 33.62.

Biological Assay

The cytotoxicity of all the compounds was determined *in vitro* by standard MTT assay. Human colon (HT-29) and human breast (MCF7) cancer cell lines were obtained from National Cell Bank of Iran (NCBI, Pasteur Institute, Tehran, Iran). HT-29 cell line was cultured in DMEM culture medium and MCF-7 cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin and incubated at 37°C in humidified CO_2 incubator.

Cytotoxic activity of all synthesized compounds was checked by standard 3-(4,5-dimethylthiazol-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay as described in our previous publications[31-33]. Briefly, the cells were harvested and plated in 96-well micro plates at a density of 1×10^4 cells per well in 100 µL complete culture medium (containing FBS and antibiotics). After 24h incubation, each cell was treated with different concentrations of each compound (from 2×10^{-4} to 1×10^{-7} M) in triplicate manner. Different concentrations of DCA were also used as positive controls. Three untreated wells were considered as negative controls. After 72h, media were completely removed and replaced with 100 µL media containing 0.5 mg/mL of MTT solution and incubated for 3-4 hours. Then, media containing MTT were discarded and 150 µL dimethylsulfoxide was added to each well to dissolve the formazan crystals and were incubated for more 3 hours. After 30 min, the absorbance in individual wells was determined at 570 nm by a Bio-Rad microplate reader (Model 680). Data were calculated and expressed as the 50% inhibitory concentrations (IC_{50}). Each experiment was independently repeated three times. Data are presented as mean ± SEM. The result were shown in Table II.

Results and Discussion

In this research, nine piperazine and piperidine analogues of DCA were synthesized. In order to determine the binding sites and binding orientation of the synthesized compounds to PDKs isoenzymes, molecular docking were applied. According to the results of Table I, docking binding energy of all compounds were less than DCA. The best docking binding energy (the most negative) on two isozymes 2BU8& 3D2R was related to compound **f9** and compound **f8** had the lowest binding energy with 2Q8H.

The interactions of the synthesized compounds with four isozymes of PDK were investigated. As it was depicted in Figure 1, in binding to 2Q8H (PDK1) receptor, compound **f5** had interactions *via* nitro group, phenyl group and chlorine atom with Met 159, Ile 155 and Arg 154 respectively. It had also existed interactions *via* oxygen atom of its nitro group with NH of Arg 188 as a H-acceptor and oxygen atom of carbonyl group with N atom of Asn 196 as a H-acceptor. Meanwhile interactions *via* oxygen atom of carbonyl group with N of Arg 112 as a H-acceptor, oxygen atom of nitro group with N atom of Met159 in binding to 2BU8 (PDK2) receptor were observed. The most important residues in binding to 1Y8O (PDK3)

Table II — Chemical structure and IC_{50} of the synthesized compounds

Name	R	Time (h)	Yield[a] (%)	IC_{50} ± SD (µM)	
				MCF7	HT-29
DCA	-	-	-	>200	>200
F1	$CHCl_2CO^*$	4	99	197.84±0.90	7.79±2.90
F2	Ph	1	50	>200	147.28±0.80
F3	CH_2CH_2OH-	3	85	42.08±1.84	88.24±2.06
F4	Me	3	75	140.90±3.98	123.56±1.5
F5	4-NO_2-Ph	4	96	>200	13.81±0.77
F6	CH_3CO-	4	58	197±0.90	11.99±4.00
F7	H	4	61	145.18±5.25	10.64±1.20
F8	Me	4	90	>200	>200
F9	Ph	4	99	191.06±2.96	>200

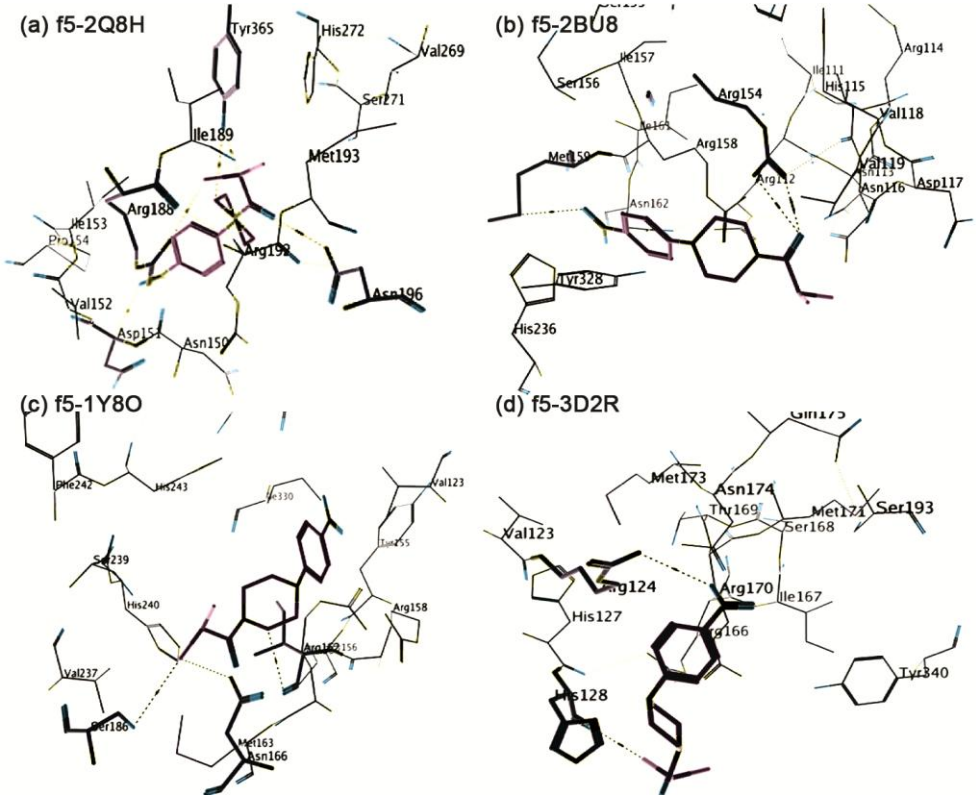


Figure 1 — Interactions of compound **f5** with the residues in the binding site of PDK isoenzymes: (a) PDK1 (2Q8H), (b) PDK2 (2BU8), (c) PDK3 (1Y8O), (d) PDK4 (3D2R)

target was carbon atom of piperazine ring with oxygen of Ile 159 as a H-donor, chlorine atom with O atom of Ser 186 as a H-donor. The most important residues in binding to 3D2R (PDK4) waschlorine atom with O of His 128 as a H-donor, oxygen atom of nitro group with NH of Arg 124 as a H-acceptor.

Cytotoxic activities of the synthesized compounds were showed the great cytotoxicity, against MCF7 cancer cell lines in compared to the others. Compound **f1** had the great cytotoxic activity with IC_{50} of 7.79 against HT-29 cancer cell line.

Compounds **f7, f6** and **f5** were also showed good anti-proliferative activity with IC_{50} of 10.64, 11.99 and 13.81 against HT-29 respectively.

* Piperazine reacts *via* two nitrogen atoms with DCA.

Conclusion

In this research, with the respect of docking results, nine compounds of piperazine and piperidine derivatives of DCA have been synthesized and their biological activity were evaluated on HT-29 and MCF7 cancer cell lines. IC_{50} of these compounds have been calculated. Generally, all of the compounds have been showed the better results in compared with DCA and piperazine compounds have been obtained more suitable IC_{50}.

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