DOI 10.1007/s11094-020-02172-4
Pharmaceutical Chemistry Journal, Vol. 54, No. 2, May, 2020 (Russian Original Vol. 54, No. 2, February, 2020)

DESIGN, SYNTHESIS, MOLECULAR DOCKING AND BIOLOGICAL ACTIVITY OF NEW PIPERIDINE AND PIPERAZINE DERIVATIVES OF DICHLOROACETATE AS POTENTIAL ANTICANCER AGENTS

Elham Manouchehrizadeh,[1,2] Azar Mostoufi,[2,3,*] Elham Tahanpesar,[2] and Masood Fereidoonnezhad[4]

Original article submitted November 30, 2019.

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

Keywords: dichloroacetate; cytotoxic activity; molecular docking, pyruvate dehydrogenase kinase inhibitors.

1. INTRODUCTION

Dichloroacetate (DCA) salts and derivatives [1] may serve a viable treatment to many forms of cancer via inhibition of pyruvate dehydrogenase kinase [2 – 4]. In addition, DCA has several therapeutic applications, e.g., for the treatment of ischemia [5], diabetes [6], endotoxic shock [7], acute hepatitis [8], and cardiac insufficiency [9]. Investigations showed that DCA can prevent tumor growth via enforcing cell death (apoptosis) without any significant toxicity [10] in endometrial [11], prostate [12], pediatric [13], pancreatic [14], cervical [15] and colorectal [16] cancer cells. The main path of apoptosis in cells widespread is adjusted by mitochondrial malfunction. Dysfunction and activity of mitochondria favors the proliferation of cancer cells in comparison to normal cells. Mitochondria induce energy production by oxidation of pyruvate and lipids. Glucose oxidation is initiated by insertion of pyruvate in mitochondria. Finally, the function of mitochondria in glucose oxidation is involved in apoptosis [17].

Pyruvate dehydrogenase complex (PDC) is regulated mitochondrial function. The four isozymes known for PDKs (2BU8, 3D2R, 1Y8O and 2Q8H) [18] have been distributed in different tissues [19]. 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 [20].

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, 21, 22].

In this work, a series of 2,2-dicholoroacetylpiperidine and 2,2-dicholoroacetylpiperazine derivatives as anticancer agents have been synthesized and structurally characterized by FT-IR, ^{1}H NMR and ^{13}C NMR spectroscopy. The cytotoxic activity of these compounds has been evaluated against human breast (MCF7) and human colon (HT-29) cancer cell lines. Molecular docking studies were also conducted to find their types of interaction with PDK isoenzymes.

[1] Department of Chemistry, Khuzestan Science and Research Branch, Islamic Azad University, Ahvaz, Iran.
[2] Department of Chemistry, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran.
[3] Marine Pharmaceutical Science Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[4] Department of Medicinal Chemistry, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
[*] e-mail: mostoufi-a@ajums.ac.ir

2. MATERIALS AND METHODS

2.1. Molecular Docking

At first stage of this study, 100 structures of piperazine and piperidine derivatives of DCA were designed based on Scheme 1. The two dimensional structures of them were drawn using ChemBioDraw Ultra 13.0. The ligands were subjected to minimization procedures by means of an *in house* TCL script [19, 21, 23] 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 [24]. Ligand – receptor interactions were performed via Dockface software [25, 26]. 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 [27 – 29]. The lowest docking binding energies for the synthesized compounds are shown below in Table 1.

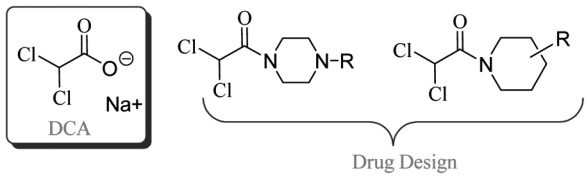
2.2. Chemistry

All chemicals were purchased from Sigma Aldrich or Merck companies. The FT-IR spectra (in KBr) were obtained on a Bruker's VERTEX 70. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker's 250 in $CDCl_3$ or D_2O at 250 MHz and 63 MHz, respectively. The structures and synthesis of 2,2-dicholoroacetylpiperazine and 2,2-dicholoroacetylpiperidine derivatives are shown in Schemes 1 – 3, respectively.

General procedure for the synthesis 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 h 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 of N-dichloroacetyl piperazine or its derivatives, was purified by recrystallization from ethanol.

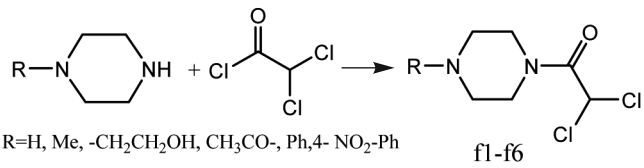
General procedure for the synthesis N-dichloroacetyl piperidine. A mixture of 3.5 mmol dichloroacetyl chloride and 3 mmol piperidine or substituted piperidine in 25 mL flask with 15 mL dry solvent (chloroform or toluene) was stirred under reflux for 4 h, washed with 10 mL saturated aqueous $NaHCO_3$. The organic layer was separated by decanter, the solvent was evaporated, and the crude product was recrystallized from ethanol.

1,1′-(Piperazine-1,4-diyl)bis(2,2-dichloroethan-1-one) (f1). White solid; yield, 0.265 g (90%); m.p. 216°C; IR spectrum, ν_{max}, cm^{-1}: 1667.04 (C = O), 1245.17 (C – N), 648.45 (C – Cl). ^{1}H NMR spectrum (chloroform-d_3) δ, ppm: 6.20 (s, 2H, $CHCl_2$), 4.38 – 3.04 (m, 8H, NCH_2CH_2N; ^{13}C NMR spectrum (chloroform-d_3) δ, ppm: 163.28, 66.56, 46.84, 43.63.

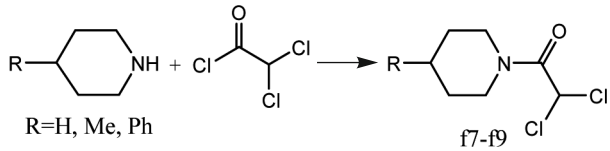


R: Phenyland substituted phenyl with EDG and EWG, alkyl, acetyland halid

Scheme 1. Chemical structure of 2,2-dicholoroacetylpiperazine and 2,2-dicholoroacetylpiperidine derivatives used in molecular docking study.



Scheme 2. Synthesis of 2,2- dicholoroacetylpiperazine derivatives.



Scheme 3. Synthesis of 2, 2- dicholoroacetylpiperidine derivatives.

2,2-Dichloro-1-(4-phenylpiperazin-1-yl)ethan-1-one (f2). White solid; yield, 0.532 g (65%); m.p. 131 – 132°C; IR spectrum, ν_{max}, cm^{-1}: 1662 (C=O), 1445 – 1594 (Aryl C = C), 3027(Aryl C–H), 2811 (aliphatic C–H), 1220.58 (C–N). ^{1}H NMR spectrum (chloroform-d_3) δ, ppm: 7.33 – 6.90 (m, 5H, arom. H), 6.23 (s, 1H, $CHCl_2$), 3.91 – 3.80 (dt, J = 22.1, 5.3 Hz, 4H, CH_2NCH_2), 3.29 – 3.01 (m, 4H, CH_2NCH_2). ^{13}C NMR spectrum (chloroform-d_3) δ, ppm: 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, 0.614 g (85%); m.p up to 165°C; IR spectrum, ν_{max}, cm^{-1}: 1676.24 (C=O), 1251.76 (C–N), 2937(C–H stretching), 3336.61 (O–H), 1445 (CH_2 bending), 652 (C–Cl). ^{1}H NMR spectrum (deuterium oxide) δ, ppm: 6.80 (s, 1H, $CHCl_2$), 4.66 (s, 2H, CH_2OH), 3.85 (q, J = 5.4 Hz, 4H, CH_2NCH_2), 3.42 (m, 2H, CH_2NCH_2), 3.28 (m, 4H, CH_2N). ^{13}C NMR spectrum (deuterium oxide) δ, ppm: 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, 0.474g (75%); m.p. up to 165°C; IR spectrum, ν_{max}, cm^{-1}: 1673 (C=O), 2949 (C–H aliphatic), 1456 (CH_2 bending), 1263 (C–N), 655 (C–Cl). ^{1}H NMR

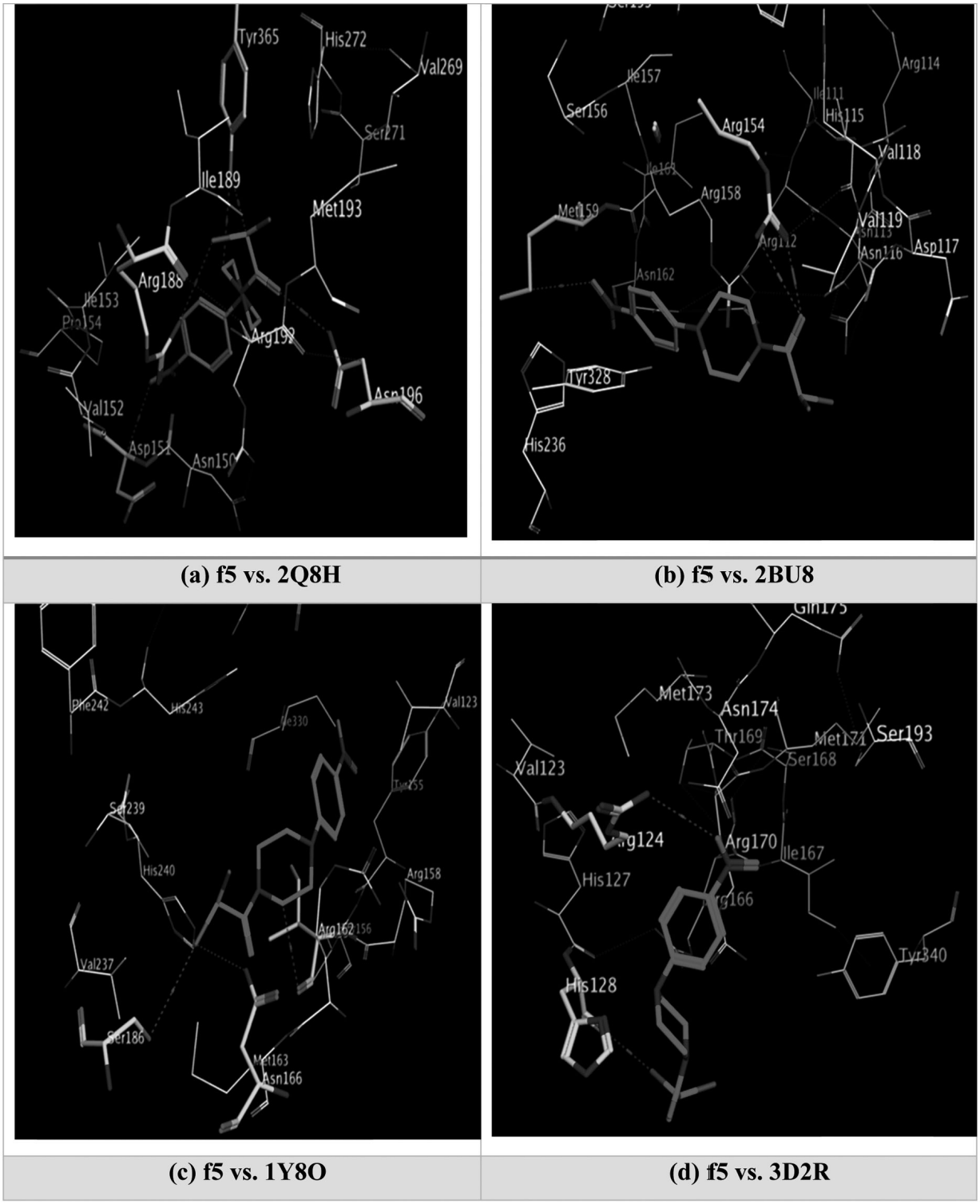


Fig. 1. Interactions of compound **f5** with residues in the binding site of PDK isoenzymes: (a) PDK1 (2Q8H); (b) PDK2 (2BU8); (c) PDK3 (1Y8O); and (d) PDK4 (3D2R).

spectrum (deuterium oxide) δ, ppm: 6.75 (s, 1H, $CHCl_2$), 4.47 (d, J = 13.5 Hz, 2Haxial, CH_2NCH_2), 4.17 (d, J = 15.1 Hz, 2H equatorial, CH_2NCH_2), 3.49 (d, J = 11.4 Hz, 2H axial, CH_2NCH_2), 3.23 – 2.93 (m, 2H equtorial, CH_2NCH_2), 2.81 (s, 3H). ^{13}C NMR spectrum (chloroform-d_3) δ, ppm: 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, 0.915 g (96%); m.p.

166 – 168°C; IR spectrum, ν_{max}, cm^{-1}: 1662.68 (C=O), 1323,159, 1234.19(C–N), 655.07 (C–Cl). ^1H NMR spectrum (chloroform-d_3) δ, ppm: 8.16 (d, J = 9.5 Hz, arom. 2H.), 6.86 (d, J = 9.6 Hz, arom. 2H), 6.22 (s, 1H, $CHCl_2$), 4.04 – 3.75 (m, 4H, CH_2NCH_2), 3.67 – 3.40 (m, 4H, CH_2NCH_2). ^{13}C NMR spectrum (chloroform-d_3) δ, ppm: 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, 0.415 g (58%); m.p. 115 – 116°C; IR spectrum, ν_{max}, cm^{-1}: 1637.11 (CH_3-C=O), 1670.95 (C=O), 1435.99 (bending CH_2), 1245 (C–N). ^1H NMR spectrum (chloroform-d_3) δ, ppm: 6.20 (s, 1H, $CHCl_2$), 4.19 – 3.17 (m, 8H, 2 NCH_2CH_2N), 2.13 (s, 3H, CH_3). ^{13}C NMR (chloroform-d_3) δ, ppm: 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, 0.358 g (61%); m.p. 41 – 42°C; IR spectrum, ν_{max}, cm^{-1}: 1667.04 (C=O), 1443 (bending CH_2), 1245(C–N), 648.45 (C–Cl). ^1H NMR spectrum (chloroform-d_3) δ, ppm: 6.22 (s, 1H, $CHCl_2$), 3.96 – 3.23 (m, 4H, CH_2NCH_2), 1.93 – 1.25 (m, 6H, $CH_2CH_2CH_2$). ^{13}C NMR spectrum (chloroform-d_3) δ, ppm: 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, 0.567 g (90%); m.p. 44 – 45°C. IR spectrum, ν_{max}, cm^{-1}: 1661.96(C=O), 2932(stretching CH_2), 1451 (bending CH_2), 1253 (C–N), 654 (C–Cl). ^1H NMR spectrum (chloroform-d_3) δ, ppm: 6.22 (s, 1H, $CHCl_2$), 4.45 (d, J = 13.5 Hz, 1H axial, NCH), 4.13 (d, J = 13.6 Hz, 1H axial, NCH), 3.09 (t, J = 12.1 Hz, 1H equtorial, NCH), 2.68 (t, J = 11.8 Hz, 1H equtorial, NCH,), 1.69 (t, J = 12.3 Hz, 4H, $2CH_2$), 1.23 (ddd, J = 17.0, 12.6, 6.3 Hz, 1H, $CHCH_3$), 0.95 (d, J = 6.2 Hz, 3H, CH_3). ^{13}C NMR spectrum (chloroform-d_3) δ, ppm: 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, 0.775 g (95%); m.p. 74 – 75°C; IR spectrum, ν_{max}, cm^{-1}: 1652 (C=O), 1451.87 (aryl C=C), 699.78 (C–Cl). ^1H NMR spectrum (chloroform-d_3) δ, ppm: 7.90 – 6.77 (m, arom. 5H), 6.25 (s, 1H, $CHCl_2$), 4.96 – 4.44 (m, 1H axial, NCH), 4.59 – 4.12 (m, 1H axial, NCH), 3.53 – 3.04 (m, 1H, ph-CH), 2.99 – 2.55 (m, 2H equtorial, NCH), 2.20 – 1.68 (m, 4H, $2CH_2$). ^{13}C NMR spectrum (chloroform-d) δ, ppm: 162.97,145.62, 129.17, 127.68, 66.89, 48.07, 45.14, 43.32, 34.25, 33.62.

2.3. Biological Activity

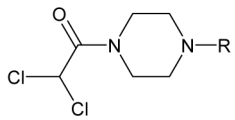
The cytotoxicity of all compounds was studied *in vitro* by standard 3-(4,5-dimethylthiazol-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Human colon (HT-29) and human breast (MCF7) cancer cell lines for this test were obtained from National Cell Bank of Iran (NCBI, Pasteur Institute, Tehran, Iran). 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 atmosphere.

The cytotoxic activity of synthesized compounds was checked by MTT assay as described previously [30 – 32]. Briefly, the cells were harvested and plated in 96-well microplates at a density of 1×10^4 cells per well in 100 μL complete culture medium (containing FBS and antibiotics). After 24 h incubation, each cell was treated with different concentrations of each compound (from 2×10^{-4} to 1×10^{-7} M) in triplicate. Various concentrations of DCA were also used as positive control. 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 MTT solution and incubated for 3 – 4 h.. Then, MTT containing medium was discarded, 150 μL dimethylsulfoxide was added to each well so as to dissolve formazan crystals, and the plates were incubated for another 3 h. After 30 min, the absorbance of individual wells at 570 nm was measured by Bio-Rad Model 680 microplate reader. Data were processed and expressed as 50% inhibitory concentrations (IC_{50}). Each experiment was independently repeated three times. The results are shown in Table 2, where all values are presented as mean ± SEM.

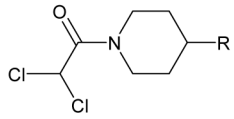
3. RESULTS AND DISCUSSION

In this study, nine piperazine and piperidine analogs of DCA were synthesized. In order to determine the binding

TABLE 1. Docking Binding Energy (kcal/mol) of Synthesized Compounds on PDK1-4 Isoenzymes



f1-f6



f7-f9

Compound	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

TABLE 2. Chemical Structures and IC_{50} Values of the Synthesized Compounds against HT-29 and MCF7 Cell Lines

Compound	R	Time (h)	Yield[a] (%)	IC_{50} (μM ± SD)	
				HT-29	MCF7
DCA	–	–	–	>200	>200
f1	$CHCl_2CO$*	4	99	7.79 ± 2.90	197.84 ± 0.90
f2	Ph	1	50	147.28 ± 0.80	>200
f3	CH_2CH_2OH-	3	85	88.24 ± 2.06	42.08 ± 1.84
f4	Me	3	75	123.56 ± 1.5	140.90 ± 3.98
f5	4-NO_2-Ph	4	96	13.81 ± 0.77	>200
f6	CH_3CO-	4	58	11.99 ± 4.00	197 ± 0.90
f7	H	4	61	10.64 ± 1.20	145.18 ± 5.25
f8	Me	4	90	>200	>200
f9	Ph	4	99	>200	191.06 ± 2.96

* Piperazine reacts with DCA via two nitrogen atoms.

sites and binding orientation of the synthesized compounds to PDK isoenzymes, molecular docking method was applied. According to the results presented in Table 1, the docking binding energies of all compounds were less than those of DCA. The best docking result (the most negative binding energy) on two isozymes (2BU8 and 3D2R) was observed for compound **f9**, while compound **f8** had the lowest binding energy with 2Q8H.

Interactions of the synthesized compounds with four isozymes of PDK were investigated. As can be seen from Fig. 1, compound **f5** binding to 2Q8H (PDK1) receptor had interactions via nitro group, phenyl group, and chlorine atom with Met 159, Ile 155 and Arg 154 respectively. There 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 H-acceptor. In addition, 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) target were carbon atom of piperazine ring with oxygen of Ile 159 as H-donor and chlorine atom with O atom of Ser 186 as a H-donor. The most important residues in binding to 3D2R (PDK4) were chlorine atom and O atom of His 128 as H-donor, and oxygen atom of nitro group with NH group of Arg 124 as H- acceptor.

Cytotoxic activities of the synthesized compounds were evaluated against two cancer cell lines: HT-29 and MCF7. As can be seen from data in Table 2, piperazine derivatives of DCA had generally higher cytotoxic activities compared to piperidine derivatives. For example, the IC_{50} values of 2,2-dichloro-1-(4-(2-hydroxyethyl)piperazin-1-yl)ethan-1on e (**f3**) for these cells were 42.08 and 88.24 μM, respectively, whereas for 2,2-dichloro-1-(4-methylpiperidin-1-yl)ethan-1-one (**f8**) against MCF-7 and HT-29 cell lines these values were >200 and >200, respectively.

It should be noted that the cytotoxic activities against HT-29 were better than those for MCF7 cancer cell line. Compound **f3** showed higher cytotoxicity against MCF7 cancer cell lines in comparison to other compounds. Compound **f1** had greater cytotoxic activity with IC_{50} of 7.79 μM against HT-29 cancer cell line. Compounds **f7, f6** and **f5** also showed good anti-proliferative activity with IC_{50} of 10.64, 11.99, and 13.81 against HT-29, respectively.

Generally, all synthesized compounds showed better cytotoxic activity test results as compared to DCA, but piperazine derivatives showed more suitable IC_{50} values.

ACKNOWLEDGEMENTs

The authors would like to thank the Department of Medicinal Chemistry, Ahvaz Jundishapur University of Medical Sciences and the Marine Pharmaceutical Science Research Center, Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran) for support and help in performing this research.

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