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Novel Approach Synthesis, Molecular Docking and Cytotoxic Activity Evaluation of N-phenyl-2,2-dichloroacetamide Derivatives as Anticancer Agents

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Abstract

Dichloroacetate (DCA) as a small, cheap and available anticancer agent, is a pyruvate mimetic compound that stimulates the activity of pyruvate dehydrogenase (PDH) enzyme through inhibition of pyruvate dehydrogenase kinases (PDHK1-4). DCA turns on programed cell death (apoptosis) which suppressed in tumor cells and therefore inhibits tumor growth. DCA also interferes with the glucose uses of cancer cell, ravenous the cell of energy, but it does not starve normal cells of glucose. In the present study, using a novel synthetic method, we synthesized a series of N-phenyl-2,2-dichloroacetamide derivatives and evaluated their cytotoxic activities against various human cancer cell lines including NCI-H460 (lung cancer), HCA-7 (colon cancer) and MCF-7 (breast cancer). Toxicity risk factors of each compound were calculated. Docking studies were also done to find their binding site to PDHK receptor. The result showed that all synthesized compounds had an acceptable anti-cancer activity. Among them, the best compound was 2,2-dichloro-N-(3-((trifluoromethyl)sulfonyl) phenyl) acetamide (25) which had an IC50 of 6.5 μM against NCI-H460 cells, 10.5 μM against HCA-7 cells and 9.4 μM against MCF-7 cells. Toxicity risk factors studies have also implied that this compound is the best one in this series. Therefore, compound 25 might have a potential value for further study in drug development.

Keywords: Dichloroacetate; N-phenyl-2,2-dichloroacetamide; Cytotoxic activity; Docking; Toxicity risk factors.

Introduction

Recent researches in the fields of oncogenic regulation of metabolism and mitochondrial function have attracted scientists' interest into the tumor metabolism and the Warburg effect [1]. Some metabolic pathways with a great role in tumor growth are recently

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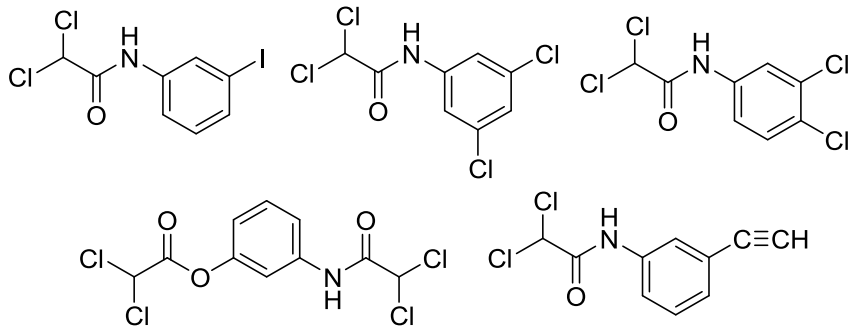


Scheme 1. Structure of some active *N*-phenyl-2,2-dichloroacetamide derivatives.

being explored as novel targets for anticancer drug development [2, 3]. Extensive investigations have been focused on strategies which selectively induce programmed cell death (apoptosis) in cancer cells [1, 2]. It is well demonestrated that defect in apoptosis have a great role in the development of cancers [4] and tumor cells have been evolved a complex process to refrain from engagement of cell death. Many glycolytic enzymes and some oncoproteins which could induce the expression of these enzymes have been also identified to control apoptosis.

Pyruvate dehydrogenase complex (PDC) is one of the major regulator of mitochondrial function. At the center of aerobic carbohydrate metabolism, PDC is localized in the matrix of mitochondria where it catalyzes irreversible oxidative decarboxylation of pyruvate entering the organelle to produce NADH, acetyl-CoA, and CO_2. Accordingly, it links and regulates the flow of energy in the cells by determining when pyruvate should be used for oxidative phosphorylation or "neutralized" to lactic acid to allow continued glycolysis. The activity of PDC is regulated by reversible phosphorylation of three serine residues on the E1α subunit which could be phosphorylated by PDH kinases (PDK) There are four known isoforms for PDKs that are distributed in a different manner in tissues. Their expressions are regulated by various factors like hypoxia, starvation and employment of glucose and fatty acids in various tissues. The main role of PDK (1–4) is inhibition of PDC activity [2, 5].

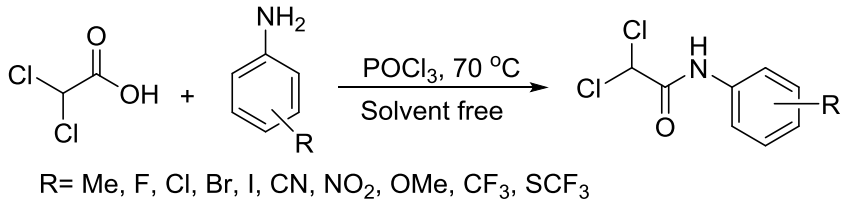
Current studies has been showed that dichloroacetate (DCA), a synthetic analog of pyruvate, could act as a pyruvate dehydrogenase activator through stimulating PDC activity. DCA is a lactate-lowering drug which has been in use for many years to treat various diseases such as lactic acidosis with inborn errors in mitochondrial function [6]. Bonnet et al. discovered that DCA could induce cell death in human lung, breast and brain cancer cells embedded into rats with no cytotoxic effect on healthy cells [7]. DCA also inhibits cell growth of many other types of tumor cells including endometrial [8], prostate [9], pediatric [10], pancreatic [11], cervical [12] and colorectal [13] cancer cells through promoting mitochondria-regulated apoptosis and decreasing proliferation.

In a recent study, yang et al. synthesized a series of *N*-phenyl dichloroacetamide derivatives (Scheme 1) and evaluated their cytotoxic activities against human gastric carcinoma (BGC-823), human oral epidermoid carcinoma (KB), human nonsmall cell lung cancer (A549), and human liver carcinoma (BEL-7402) cell lines [14]. In the present study, we used a new synthetic method to produce the same compounds with a less toxic synthetic pathway, mild condition and more purified products. Moreover, the cytotoxic activity of these products was evaluated against different human cancer cell lines such as human lung (NCI-H460), colon (HCA-7) and endometrial (MCF-7) cancer cell lines. Toxicity risk factors and molecular dockings of these compounds were also conducted and promising results were obtained.

Materials and Methods

Chemistry

All chemicals were obtained from Aldrich or Merck chemical companies. The Progress of the reactions was followed by TLC using silica gel polygrams SIL G/UV 254 plates or by GC using a Bruker gas chromatograph 450-GC, equipped with a flame ionization detector (FID) and a 3-meter length capillary column CP-SIL 5CB and nitrogen as the carrier gas. IR spectra were run on a Bruker's VERTEX 70 Series FT-IR Spectrometers. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 300 FT-NMR spectrometer (^{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). All yields refer to the isolated products. Evaporation of solvents was performed at reduced



Scheme 2. Synthesis of *N*-phenyl-2,2-dichloroacetamide derivatives.

pressure, with a Buchi rotary evaporator. General melting point were recorded on an electrothermal digital melting point apparatus. Mass spectra were recorded on a Finnigan MAT 8430 mass spectrometer operating at an electron energy of 70 eV.

Typical procedure for N-Phenyl-2,2-dichloroacetamide derivatives (compounds 1-24) synthesis

N-Phenyl-2,2-dichloroacetamide analogues were synthesized from the respective aniline derivatives, DCA and phosphorusoxychloride. In a flask, DCA (5 mmol) and phosphorus oxychloride ($POCl_3$, 7.5 mmol, 1.5 equiv) were mixed together slowly to obtain a clear mixture. Then aniline derivatives (5 mmol) were added with constant stirring (Scheme 2) under solvent free condition. The mixture was slowly warmed to expel the hydrochloric acid formed. The progress of reaction was monitored by TLC (*n*-hexane/ethyl acetate). The excess of $POCl_3$ was hydrolyzed by adding cold water. The produced hydrochloric acid was removed by treating with excess of 2N sodium hydroxide solution (NaOH). The crude products were separated, washed with distilled water and dried. Then it was recrystallized from ethanol. The melting point and ^{1}H-NMR of all the compounds were obtained. The time required for each reaction and the isolated yields are indicated in Table 1.

Typical procedure for compounds (25) and (26) synthesis

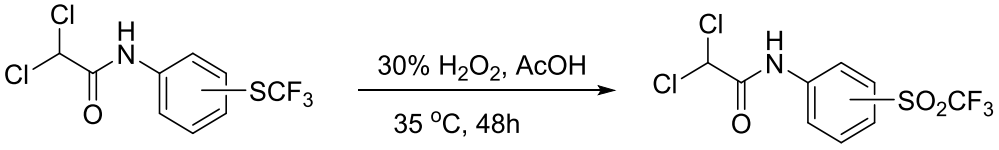
As it was shown in Scheme 2, compounds 25, 26 were prepared according to Yang et al study [14]. To a flask containing 20 mL acetic acid (AcOH) and 10 mL H_2O_2 30%, *N*-(3-(trifluoromethylthio)-phenyl)-2,2-dichloroacetamide (23) 0.496 g (1.6 mmol) were mixed and stirred at 35 ^{o}C. TLC analysis (*n*-hexane /ethyl acetate 4:1) of the reaction mixture showed the completion of the reaction after 48 hours (Scheme 3). After the reaction was finished, the solvent was evaporated under vacuum and the residue was dissolved in 20 ml dichloromethane and washed with saturated aqueous $NaHCO_3$ and saturated brines. The organic phases were dried with calcium chloride ($CaCl_2$) and concentrated. Chromatography on a short column of silica gel using *n*-hexane/ ethyl acetate (8/1) as eluent gave, the purified compound 25.

N-(2-methylphenyl)-2,2-dichloroacetamide (1)

White solid, mp 140-141 ^{o}C. IR (KBr, cm^{-1}) ν_{max}: 3250 (N-H), 1677 (C = O), 1611 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.09 (s, 1H, NH-amide), 7.77 (d, *J* = 7.9, 1H, H $_6$ -2-methylphenyl), 7.25 (t, *J* = 7.6, 1H, H $_5$ -2-methylphenyl), 7.23 (d, *J* = 7.9, 1H, H $_3$ -2-methylphenyl), 7.15 (t, *J* = 7.2, 1H, H $_4$ -2-methylphenyl), 6.06 (s, 1H, -$CHCl_2$), 2.32 (s, 3H, CH_3-phenyl) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.18, 134.15, 130.91, 130.10, 127.16, 126.62, 123.14, 67.21, 17.59 ppm; ESI-MS *m/z*: 217.01 $[M]^+$.

N-(3-methylphenyl)-2,2-dichloroacetamide (2)

White solid, mp 105-106 ^{o}C. IR (KBr, cm^{-1}) ν_{max}: 3240 (N-H), 1671 (C = O), 1605 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.06 (s, 1H, NH-amide), 7.39 (s, 1H, H $_2$ -3-methylphenyl), 7.35 (d, *J* = 8.1, 1H, H $_6$ -3-methylphenyl), 7.26 (t, *J* = 7.7, 1H, H $_5$ -3-methylphenyl), 7.02 (d, *J* = 7.4, 1H, H $_4$ -3-methylphenyl), 6.03 (s, 1H, -$CHCl_2$), 2.37 (s, 3H, CH_3-phenyl) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.05, 139.44, 136.30, 129.20, 126.71, 121.13, 117.61, 67.10,



Scheme 3. Synthesis of 2,2-dichloro-*N*-(3 or 4-((trifluoromethyl)sulfonyl)phenyl)acetamide (25 or 26)

21.6 ppm; ESI-MS *m/z*: 217.01 $[M]^+$.

N-(4-methylphenyl)-2,2-dichloroacetamide (3)

White solid, mp 159-160 °C. IR (KBr, cm^{-1}) ν_{max}: 3240 (N-H), 1669 (C = O), 1601 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ= 10.54 (s, 1H, NH-amide), 7.48 (d, *J* = 8.6, 2H, H $_{2,6}$ -4-methylphenyl), 7.18 (d, *J* = 8.6, 2H, H $_{3,5}$ -4-methylphenyl), 6.57(s, 1H, -$CHCl_2$), 6.28 (s, 3H, CH_3-phenyl) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ= 161.78, 137.66, 137.40, 121.93, 88.62, 67.25 ppm; ESI-MS *m/z*: 217.01 $[M]^+$.

N-(2-fluorophenyl)-2,2-dichloroacetamide (4)

White solid, mp 108-109 °C. IR (KBr, cm^{-1}) ν_{max}: 3254 (N-H), 1675 (C = O), 1599 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.41 (s, 1H, NH-amide), 8.26 (m, 1H, H $_6$ -2-fluorophenyl), 7.16 (m, 3H, H $_{3,4,5}$ -2-fluorophenyl), 6.06 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 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 ppm; ESI-MS *m/z*: 220.98 $[M]^+$.

N-(3-fluorophenyl)-2,2-dichloroacetamide (5)

White solid, mp 133-134 °C. IR (KBr, cm^{-1}) ν_{max}: 3262 (N-H), 1674 (C = O), 1603 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.19 (s, 1H, NH-amide), 7.52 (s, 1H, H $_2$ -3-fluorophenyl), 7.52 (dd, *J* = 8.9, 8.9, 1H, H $_5$ -3-fluorophenyl), 7.08 (d, *J* = 8.3,1H, H $_6$ -3-fluorophenyl), 7.06 (d, *J* = 8.9, 1H, H $_4$ -3-fluorophenyl), 6.05 (s, 1H, -$CHCl_2$) ppm ; ^{13}C NMR (75 MHz, $CDCl_3$) 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 ppm; ESI-MS *m/z*: 220.98 $[M]^+$.

N-(4-fluorophenyl)-2,2-dichloroacetamide (6)

White solid, mp 134-135 °C. IR (KBr, cm^{-1}) ν_{max}: 3279 (N-H), 1669 (C = O), 1608 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.10 (s,1H, NH-amide), 7.53 (dd, *J*= 8.8, 4.6, 2H, H $_{2,6}$ -4-fluorophenyl), 7.08 (t, *J*= 8.5, 2H, H $_{3,5}$ -4-fluorophenyl), 6.04 (s,1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 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 ppm; ESI-MS *m/z*: 220.98 $[M]^+$.

N-(2-chlorophenyl)-2,2-dichloroacetamide (7)

White solid, mp 115-116 °C. IR (KBr, cm^{-1}) ν_{max}: 3255 (N-H), 1678 (C = O), 1593 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.82 (s, 1H, NH-amide), 8.32 (dd, *J* = 8.3, 1.4, 1H, H $_6$ -2-chlorophenyl), 7.43 (dd, *J* = 8.0, 1.4, 1H, H $_3$ -2-chlorophenyl), 7.33 (td, *J* = 7.9, 1.4, 1H, H $_5$ -2-chlorophenyl), 7.14 (td, *J* = 7.9, 1.4, 1H, H $_4$ -2-chlorophenyl), 6.07 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 161.84, 133.34, 129.46, 128.10, 126.20, 123.98, 121.58, 67.08 ppm; ESI-MS *m/z*: 238.95 $[M]^+$.

N-(3-chlorophenyl)-2,2-dichloroacetamide (8)

White solid, mp 104-105 °C. IR (KBr, cm^{-1}) ν_{max}: 3269 (N-H), 1677 (C = O), 1609 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.09 (s, 1H, NH-amide), 7.68 (s, 1H, H $_2$ -3-chlorophenyl), 7.42 (d, *J* = 8.0, 1H, H $_6$ -3-chlorophenyl), 7.31 (t, *J* = 8.0, 1H, H $_5$ -3-chlorophenyl), 7.19 (d, *J* = 7.7, 1H, H $_4$ -3-chlorophenyl), 6.04 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) d 162.02, 137.52, 135.18, 130.44, 126.01, 120.58, 118.42, 66.88 ppm; ESI-MS *m/z*: 238.95 $[M]^+$.

N-(4-chlorophenyl)-2,2-dichloroacetamide (9)

White solid, mp 141-142 °C. IR (KBr, cm^{-1}) ν_{max}: 3277 (N-H), 1676 (C = O), 1617 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.11 (s, 1H, NH-amide), 7.52 (d, *J* = 8.8, 2H, H $_{2,6}$ -4-chlorophenyl), 7.35 (d, *J* = 8.8, 2H, H $_{3,5}$ -4-chlorophenyl), 6.04 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.10,134. 95,131.15, 129.48, 121.80, 66.91; ESI-MS *m/z*: 238.95 $[M]^+$.

N-(2-bromophenyl)-2,2-dichloroacetamide (10)

White solid, mp 113-115 °C. IR (KBr, cm^{-1}) ν_{max}: 3263 (N-H), 1671 (C = O), 1589 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.82 (s, 1H, NH-amide), 8.29 (dd, *J* = 8.2, 1.4, 1H, H $_6$ -2-bromophenyl), 7.59 (dd, *J* = 8.0, 1.4, 1H, H $_3$ -2-bromophenyl), 7.37 (t, *J* = 7.2, 1H, H $_5$ -2-bromophenyl), 7.07 (t, *J* = 7.0, 1H, H $_4$ -2-bromophenyl), 6.07 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75MHz, $CDCl_3$) δ= 161.92, 134.45, 132.70, 128.74, 126.67, 121.87, 114.43, 67.14 ppm; ESI-MS *m/z*: 282.90 $[M]^+$.

N-(3-bromophenyl)-2,2-dichloroacetamide (11)

White solid, mp 107-108 °C. IR (KBr, cm^{-1}) ν_{max}: 3266 (N-H), 1679 (C = O), 1606 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.11 (s, 1H, NH-amide), 7.81 (s, 1H, H $_2$ -3-bromophenyl), 7.48 (d, *J* = 7.5, 1H, H $_6$ -3-bromophenyl), 7.34 (d, *J* = 7.7, 1H, H $_5$ -3-bromophenyl), 7.23 (t, *J* = 7.9, 1H, H $_4$ -3-bromophenyl), 6.04 (s,1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.10, 137.62, 130.69, 128.94, 123.46, 123.00, 118.99, 66.87 ppm; ESI-MS *m/z*: 282.90 $[M]^+$.

N-(4-bromophenyl)-2,2-dichloroacetamide (12)

White solid, mp 148-150 °C. IR (KBr, cm^{-1}) ν_{max}: 3273 (N-H), 1674 (C = O), 1607 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ= 10.76 (s, 1H, NH-amide), 7.57 (d, *J* = 9.7, 4H, H $_{2,3,5,6}$ -4-bromophenyl), 6.58 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ=

162.37, 137.50, 132.37, 122.31, 117.03, 67.80 ppm; ESI-MS *m/z*: 282.90 $[M]^+$.

N-(3-cyanophenyl)-2,2-dichloroacetamide (13)

Yellow solid, mp 149-150 °C. IR (KBr, cm^{-1}) ν_{max}: 3261 (N-H), 2245 (C≡N), 1669 (C = O), 1601 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.27 (s, 1H, NH-amide), 7.99 (s, 1H, H_2 -3-cyanophenyl), 7.87-7.70 (m, 1H, H_6 -3-cyanophenyl), 7.60-7.42 (m, 2H, $H_{4,5}$ -3-cyanophenyl), 6.07 (s, 1H, -$CHCl_2$) ppm; ^{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 *m/z*: 227.99 $[M]^+$.

N-(3-iodophenyl)-2,2-dichloroacetamide (14)

White solid, mp 116-117 °C. IR (KBr, cm^{-1}) ν_{max}: 3267 (N-H), 1677 (C = O), 1603 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ= 10.74 (s, 1H, NH-amide), 8.07 (s, 1H, H_2 -3-iodophenyl), 7.56 (d, *J* = 8.2, 1H, H_6 -3-iodophenyl), 7.52 (d, *J* = 8.0, 1H, H_5 -3-iodophenyl), 7.18 (t, *J* = 8.0, 1H, H_4 -3-iodophenyl), 6.58 (s, 1H, -$CHCl_2$) ppm; ^{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 ppm; ESI-MS *m/z*: 328.89 $[M]^+$.

N-(4-iodophenyl)-2,2-dichloroacetamide (15)

White solid, mp 173-175 °C. IR (KBr, cm^{-1}) ν_{max}: 3269 (N-H), 1670 (C = O), 1602 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ=10.74 (s, 1H, NH-amide), 7.72(d, *J* = 8.6, 2H, $H_{2,6}$ -4-iodophenyl), 7.44 (d, *J* = 8.6, 2H, $H_{3,5}$ -4-iodophenyl), 6.58 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ=161.78, 137.66, 137.40, 121.93, 88.62, 67.25 ppm; ESI-MS *m/z*: 328.89 $[M]^+$.

N-(2-nitrophenyl)-2,2-dichloroacetamide (16)

Yellow solid, mp 79-81 °C. IR (KBr, cm^{-1}) ν_{max}: 3266 (N-H), 1673 (C = O), 1591 (C = C), 1543, 1362 (N-O). ^{1}H NMR (300 MHz, DMSO-d_6) δ= 11.01 (s, 1H, NH-amide), 8.04 (d, *J* = 8.1, 1H, H_6 -2-nitrophenyl), 7.78 (t, *J* = 7.6, 1H, H_5 -2-nitrophenyl), 7.69 (d, *J* = 7.7, 1H, H_3 -2-nitrophenyl), 7.49 (t, *J* = 7.7, 1H, H_4 -2-nitrophenyl), 6.78 (s, 1H, -$CHCl_2$) ppm; ^{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 ppm; ESI-MS *m/z*: 247.98 $[M]^+$.

N-(3-nitrophenyl)-2,2-dichloroacetamide (17)

Yellow solid, mp 103-105 °C. IR (KBr, cm^{-1}) ν_{max}: 3261 (N-H), 1669 (C = O), 1601 (C = C), 1539, 1358 (N-O). ^{1}H NMR (300 MHz, DMSO-d_6) δ= 11.15 (s, 1H, NH-amide), 8.59 (t, *J* = 2.1, 1H, H_2 -3-nitrophenyl), 8.00 (dd, *J* = 8.2, 2.3, 1H, H_3 -3-nitrophenyl), 7.96 (dd, *J* = 7.8, 1.6, 1H, H_6 -3-nitrophenyl), 7.68 (t, *J* = 8.2, 1H, H_5 -3-nitrophenyl), 6.64 (s, 1H, -$CHCl_2$) ppm; ^{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 ppm; ESI-MS *m/z*: 247.98 $[M]^+$.

N-(4-nitrophenyl)-2,2-dichloroacetamide (18)

Yellow solid, mp 130-132 °C. IR (KBr, cm^{-1}) ν_{max}: 3259 (N-H), 1670 (C = O), 1593 (C = C), 1545, 1361 (N-O). ^{1}H NMR (300 MHz, DMSO-d_6) δ= 11.24 (s, 1H, NH-amide), 8.29(d, *J* = 9.2, 2H, $H_{2,6}$ -4-nitrophenyl), 7.88 (d, *J* = 9.2, 2H, $H_{3,5}$ -4-nitrophenyl), 6.65 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ= 162.46, 143.70, 143.26, 125.02, 119.78, 67.14 ppm; ESI-MS *m/z*: 247.98 $[M]^+$.

N-(3-methoxyphenyl)-2,2-dichloroacetamide (19)

White solid, mp 79-82 °C. IR (KBr, cm^{-1}) ν_{max}: 3267 (N-H), 1675 (C = O), 1608 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.12 (s, 1H, NH-amide), 7.34-7.21 (m, 2H, $H_{2,6}$ -3-methoxyphenyl), 7.04 (d, *J* = 8.0, 1H, H_4 -3-methoxyphenyl), 6.75 (dd, *J* = 8.3, 2.4, 1H, H_5 -3-methoxyphenyl), 6.04 (s, 1H, -$CHCl_2$), 3.83 (s, 3H, 3-OCH_3-phenyl) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.51, 160.27, 137.49, 130.01, 112.92, 111.73, 106.48, 67.07, 55.45 ppm; ESI-MS *m/z*: 232.9 $[M]^+$.

N-(4-methoxyphenyl)-2,2-dichloroacetamide (20)

White solid, mp 136-137 °C. IR (KBr, cm^{-1}) ν_{max}: 3264 (N-H), 1674 (C = O), 1601 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.07 (s, 1H, NH-amide), 7.46 (d, *J* = 9.0, 2H, $H_{2,6}$ -4-methoxyphenyl), 6.90 (d, *J* = 9.0, 2H, $H_{3,5}$ -4-methoxyphenyl), 6.04 (s, 1H, -$CHCl_2$), 3.81 (s, 3H, 4-OCH_3-phenyl) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.03, 157.61, 129.36, 122.39, 114.55, 67.06, 55.69 ppm; ESI-MS *m/z*: 232.9 $[M]^+$.

N-(3-(trifluoromethyl)-phenyl)-2,2-dichloroacetamide (21)

Pink solid, mp 80-82 °C. IR (KBr, cm^{-1}) ν_{max}: 3266 (N-H), 1670 (C = O), 1598 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.24 (s, 1H, NH-amide), 7.86 (s, 1H, H_2 -3-CF_3-phenyl), 7.78 (d, *J* = 7.7, 1H, H_6 -3- CF_3-phenyl), 7.52 (t, *J* = 7.9, 1H, H_5 -3-CF_3-phenyl), 7.47 (d, *J* = 7.8, 1H, H_4 -3-CF_3-phenyl), 6.06 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) 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 ppm; ESI-MS *m/z*: 270.98 $[M]^+$.

N-(3-(trifluoromethoxy)-phenyl)-2,2-dichloroacetamide (22)

White solid, mp 75-77 °C. IR (KBr, cm^{-1}) ν_{max}: 3263

(N-H), 1667 (C = O), 1591 (C = C). ^{1}H NMR (300 MHz, DMSO-d_6) δ= 10.93 (s, 1H, NH-amide), 7.74 (s, 1H, H $_2$ -3-OCF_3-phenyl), 7.57 (d, *J* = 8.3, 1H, H $_6$ -3-OCF_3-phenyl), 7.51 (t, *J* = 8.0, 1H, H $_5$ -3-OCF_3-phenyl), 7.15 (d, *J* = 7.1, 1H, H $_4$ -3-OCF_3-phenyl), 6.61 (s, 1H, -$CHCl_2$) ppm; ^{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 ppm; ESI-MS *m/z*: 286.97 $[M]^+$.

N-(3-(trifluoromethylthio)-phenyl)-2,2-dichloroacetamide (23)

White solid, mp 87-89 ^{o}C. IR (KBr, cm^{-1}) ν_{max}: 3261 (N-H), 1674 (C = O), 1602 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.19 (s, 1H, NH-amide), 7.87 (s, 1H, H $_2$ -3-SCF_3-phenyl), 7.75 (d, *J* = 7.8, 1H, H $_6$ -3-SCF_3-phenyl), 7.50 (d, *J* = 7.8, 1H, H $_4$ -3-SCF_3-phenyl), 7.44 (t, *J* = 7.8, 1H, H $_5$ -3-SCF_3-phenyl), 6.06 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.68, 137.34, 133.47,130.36, 129.59 (q, *J* = 308.5), 127.94, 125.67, 123.03, 66.83 ppm; ESI-MS *m/z*: 302.95 $[M]^+$.

N-(4-(trifluoromethylthio)-phenyl)-2,2-dichloroacetamide (24)

White solid, mp 123-125 ^{o}C. IR (KBr, cm^{-1}) ν_{max}: 3268 (N-H), 1669 (C = O), 1599 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.20 (s, 1H, NH-amide), 7.91-7.49 (m, 4H, H $_{2,3,5,6}$ -4-SCF_3-phenyl), 6.05 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.42, 138.96, 137.68, 129.60 (q, *J* ¼ 308.3), 121.07, 66.85; ESI-MS *m/z*: 302.95 $[M]^+$.

N-(3-(trifluoromethylsulfonyl)-phenyl)-2,2-dichloroacetamide (25)

Light yellow solid, mp 98-100 ^{o}C. IR (KBr, cm^{-1}) ν_{max}: 3267 (N-H), 1671 (C = O), 1602 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.34 (s, 1H, NH-amide), 8.19 (s, 1H, H $_2$ -3-SO_2CF_3-phenyl), 8.17 (d, *J* = 8.9, 1H, H $_6$ -3-SO_2CF_3-phenyl), 7.88 (d, *J* = 7.6, 1H, H $_4$ -3-SO_2CF_3-phenyl), 7.72 (dd, *J* = 7.6, 8.9, 1H, H $_5$ -3-SO_2CF_3-phenyl), 6.08 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.79, 138.36, 132.27, 131.15,128.25,127.45, 122.02, 119.84 (q, *J* = 325.8), 66.62 ppm; ESI-MS *m/z*: 334.94 $[M]^+$.

N-(4-(trifluoromethylsulfonyl)-phenyl)-2,2-dichloroacetamide (26)

light yellow solid, mp 137-139 ^{o}C. IR (KBr, cm^{-1}) ν_{max}: 3268 (N-H), 1669 (C = O), 1601 (C = C). ^{1}H NMR (300 MHz, $CDCl_3$) δ= 8.36 (s, 1H, NH-amide), 8.07 (d, *J* = 8.9, 2H, H $_{2,6}$ -4-SO_2CF_3-phenyl), 7.91 (d, *J* = 8.9, 2H, H $_{3,5}$ -4-SO_2CF_3-phenyl), 6.08 (s, 1H, -$CHCl_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ= 162.67, 144.23, 132.55, 126.74, 120.69, 119.91 (q, *J* = 326.6), 66.62 ppm; ESI-MS *m/z*: 334.94 $[M]^+$.

Toxicity risk factors

Recently Hughes, J.D. et al. discovered that high ClogP and low total polar surface area (TPSA) are two risk factors linked to a propensity for toxicity of a chemical compound [15]. Compounds with both risk factors present (ClogP > 3 and TPSA < 75) are 2.5 times more likely to be toxic as to be clean. These properties of our synthesized compounds were analyzed based on Jurica Levatić et al. reports [16]. Toxicity risk factors of the synthesized compounds were calculated by the use of The BioZyne P-gp server [17] to show whether a compound is likely to be a substrate of the P-gp drug efflux pump.

Docking procedure

The docking simulations were carried out by means of an *in house* batch script (DOCKFACE) for automatic running of AutoDock 4.2 [18] in a parallel mode using all system resources. In all experiments genetic algoritm search method was used to find the best pose of each ligand in the active site of the target enzyme. The three dimensional crystal structure of pyruvate dehydrogenase kinase 2 (PDB ID: 2BU8) were retrieved from protein data bank [19]. Co-crystal ligand molecules were excluded from the structures and the PDBs were checked in terms of missing atom types by modeller 9.12 [20]. For Lamarckian GA method; 27,000 maximum generations; 2500000 maximum No. of evaluations, 150 population size, mutation rate of 0.02; and a crossover rate of 0.8 were used. A grid box of 50×50×50 points in x, y, and z direction with a grid spacing of 0.375 Å was built. No. of points in x, y and z was 50, 41 and 82 respectively.

Biological assay

Cell lines and cell culture

Human lung cancer cell line (NCI-H460), human colon cancer cell line (HCA-7) and human breast cancer cell line (MCF-7) were obtained from National Cell Bank of Iran (NCBI, Pasteur Institute, Tehran, Iran). All cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and pencilin-streptomycin at 37 ^{o}C in humidified CO_2 incubator.

Cytotoxic activity of all the synthesized compounds was assessed by standard 3-(4,5-dimethylthiazol-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The cells were harvested and plated in 96-well microplates at a density of 1×10^4 cells per well in 180 µl complete culture media. After 24 h incubation, each cell was

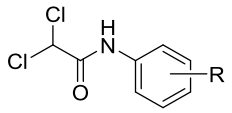
treated with five different concentrations of the compounds ranging from 4×10^{-4} to 1×10^{-7} M. After 72h, media were replaced with 150 µl media containing 0.5 (mg/ml) of MTT solution. Then media containing MTT were discarded and 150 µl dimethylsulfoxide was added to each well to dissolve the formazan crystals. The solutions were incubated overnight. The absorbance in individual wells was determined at 570 nm by a Bio-Rad microplate reader (Model 680). The results were expressed as IC50 values which were tested four times for each compound. Data are demonstrated as mean ± SEM.

Results

Cytotoxic activity and SAR studies

In this paper, we executed a novel synthetic method for synthesizing *N*-phenyl dichloroacetamide derivatives which recently has been synthesized by Yang et al [14]. In Yang et al method amidation reaction was done with dichloroacetyl chloride and an amine group. The reaction was proceed with liberation of hydrochloride acid (HCl). Moreover, their reaction has been carried out in toluene as the solvent and in reflux temperature without any purification to give the final compounds. Without any purification, liberated HCl from reaction could form hydrochloride salt on amine group in final products. Therefore, the final products are mixture of free base and hydrochloride salt. Our proposed synthetic method ignored the use of toluene as a toxic solvent and carried out in a solvent free conditions and without using of high reflux temperature and the final product was just free base. Therefore, cytotoxicity assay was done on pure compound not mixture of the free base and salt. Having synthesized *N*-phenyl dichloroacetamide derivatives, then in-vitro cytotoxicity of all the synthesized compounds was carried out by MTT assay on human lung cancer cell line (NCI-H460), human colon cancer cell line (HCA-7) and human breast cancer cell line (MCF-7).

Table 1. Chemical structure and cytotoxic activity of *N*-phenyl-2,2-dichloroacetamide derivatives.



Name	R	Time (h)	Yield[a] (%)	IC50 (µM ± error)		
				NCI-H460	HCA-7	MCF-7
DCA	-	-	-	>400	>400	>400
1	2-Me	3	73	>400	>400	>400
2	3-Me	2.5	88	66 ± 5.2	187 ± 13.6	112 ± 8.5
3	4-Me	2.5	91	79 ± 5.2	>400	>400
4	2-F	3.5	80	106 ± 12.6	>400	>400
5	3-F	3	93	63 ± 10.5	>400	>400
6	4-F	3	87	89 ± 9.7	>400	>400
7	2-Cl	4	87	69 ± 11.3	198 ± 9.6	139 ± 13.8
8	3-Cl	3.5	94	18 ± 2.7	94 ± 6.3	81 ± 7.8
9	4-Cl	4	91	23 ± 7.4	91 ± 11.2	66 ± 5.9
10	2-Br	4	85	47 ± 5.2	101 ± 6.9	87 ± 9.1
11	3-Br	3.5	86	11 ± 3.7	68 ± 3.8	38 ± 5.7
12	4-Br	3	95	9 ± 1.9	49 ± 1.7	27 ± 4.1
13	3-CN	4	89	69 ± 8.1	149 ± 14.5	71 ± 12
14	3-I	3.5	92	8.3 ± 1.7	41 ± 4.5	19.5 ± 2.6
15	4-I	3.5	94	17.7 ± 3.6	89 ± 10.5	37.5 ± 6.6
16	2-NO_2	5	72	86 ± 7.5	>400	>400
17	3-NO_2	3.5	86	31 ± 3.3	129 ± 12.7	79 ± 5.9
18	4-NO_2	3	85	59 ± 7.8	182 ± 9.8	96 ± 7.3
19	3-OMe	3.5	88	74 ± 5.9	97 ± 8.2	61 ± 4.7
20	4-OMe	3.5	93	107 ± 8.5	>400	149 ± 11.6
21	3-CF_3	4	91	7.5 ± 1.2	15 ± 2.6	11.4 ± 3.4
22	3-OCF_3	4	90	11.2 ± 3.8	21 ± 6.8	14.3 ± 2.9
23	3-SCF_3	4.5	91	8.9 ± 2.2	11 ± 3.5	9.5 ± 1.6
24	4-SCF_3	4	92	7.5 ± 3.1	13 ± 4.1	11.5 ± 2.4
25	3-SO_2CF_3	48[b]	57	6.5 ± 2.7	10.5 ± 3.9	9.1 ± 4.9
26	4-SO_2CF_3	48[b]	55	11.6 ± 3.8	26 ± 5.5	24 ± 6.4

[a] Isolated Yield, [b] Time of step 2

Table 2. Docking binding energy and toxicity risk factors of *N*-arylphenyl-2,2-dichloroacetamide analogues

Name	Binding Energy (kcal/mol)	Toxicity risk factors	
		Total polar surface area	XlogP[a]
DCA	-3.63	37.30	0.57
1	-4.28	29.10	3.02
2	-5.37	29.10	3.23
3	-5.22	29.10	3.23
4	-4.84	29.10	2.65
5	-5.18	29.10	2.65
6	-5.13	29.10	2.65
7	-5.22	29.10	2.79
8	-5.58	29.10	2.79
9	-5.27	29.10	2.79
10	-5.51	29.10	3.13
11	-5.61	29.10	3.13
12	-5.65	29.10	3.13
13	-5.39	52.89	2.04
14	-5.68	29.10	3.33
15	-5.29	29.10	3.33
16	-5.71	66.40	3.31
17	-6.16	66.40	2.46
18	-5.53	66.40	2.46
19	-5.16	38.33	2.25
20	-4.93	38.33	2.25
21	-5.67	29.10	3.81
22	-5.5	38.33	3.50
23	-5.37	54.40	4.06
24	-5.71	54.40	4.06
25	-5.56	72.62	2.34
26	-5.43	72.62	2.84

[a] Octanol-water partition coefficient

As it was shown in Table 1, HCA-7 cells are more resistant to DCA and *N*-phenyl-2,2-dichloroacetamide derivatives (for example compounds **7**-15) than NCI-H460 and MCF-7 cells. DCA derivatives generally had better cytotoxic activities on the NCI-H460 cell line. So, the IC50 on NCI-H460 was chosen as the activity index involved in the SAR discussion.

Cytotoxic activities of *N*-phenyl-2,2-dichloroacetamide analogues on studied cell lines were entirely dependent on the position of the substituents on benzene ring as well as electron donating or electron withdrawing nature substitution. These results are closely related to the previous reported cytotoxicity evaluation of these compounds on the other cell lines [14]. Substitution to *meta* and *para*-positions, had a greater potency compared to *ortho*-positions. For example, the IC50 of compound **7** was lower than that of 8 and 9. These should be because of steric hindrance of *ortho*-positions which may block the interaction between the pharmacophore and the receptor.

Typically, the *meta*-substituted structures had the best potencies, for example, the IC50 of 2,2-dichloro-N-(3-fluorophenyl)acetamide (5) was 63 μM, and the IC50 of 2,2-dichloro-*N*-(4-fluorophenyl)acetamide (6) was 89 μM. However in some cases such as compounds 12 and 24, the *para*-substituent had greatest potencies, for example, the IC50 of 2,2-dichloro-*N*-(3-bromophenyl)acetamide (11) was 11 μM, and the IC50 of 2,2-dichloro-*N*-(4-bromophenyl)acetamide (12) was 9 μM. Therefore, we focused on the optimization of the *meta* and *para*-positions.

A series of *meta* and *para*-substituent derivatives were synthesized and studied. The compounds possessing electron-donating substituents (e.g., 19, 20) had lower potencies, than strong electron-withdrawing substituents (e.g., 17, 21-26). Among halogen substituents, iodo and bromo exhibited greater potencies and the fluoro had the lowest cytotoxic activity. As a result, the moderate electron-withdrawing and hydrophobic substituents at the *para* and specially *meta*-positions were advantageous to the cytotoxic activity of these class of DCA derivatives.

In addition, *N*-phenyl-2,2-dichloroacetamide derivatives exhibited great potencies in inhibiting H460, HCA-7 and MCF-7 cell lines. The IC50 against H460 and MCF-7 was greater than HCA-7. For example, the IC50 of 2,2-dichloro-*N*-(3-cyanophenyl)acetamide (13) against H460 and MCF-7 was about 69 μM, and 71 μM,

respectively whereas it was about 149 μM against HCA-7.

Among the studied compounds, 2,2-dichloro-*N*-(3-((trifluoromethyl)sulfonyl)phenyl)acetamide (25) had a greatest cytotoxic activity against different cancer cell lines (6.5 μM for H460, 10.5 μM for HCA-7 and 9.1 μM for MCF-7) and therefore it may have a potential value for drug development.

Toxicity risk factors prediction

Table 2 shows toxicity risk factors of our synthesized compounds. Recently, Jurica Levatić et al. found that the principal determinants of a compound's recognition by the P-gp are the size of molecule (expressed as the number of atoms), and the specific atomic volume (reciprocal density, in $Å^3$/atom) [16]. As shown in Table 2, total polar surface area of compound **25** was 72.62 and XlogP was 2.34. So as it was mentioned, compounds with ClogP < 3 and TPSA > 75 are more likely to be clean drug candidates, this compound might be the best compound in this series based on the toxicity risk factors and it had another potential value for drug candidate. The specific atomic volume versus the size of molecule for compound 25 , together with a set of P-gp substrates and non-substrates are displayed in Figure 1.

Molecular docking study

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to determine the affinity and activity of the small molecule. Hence docking plays a great role in the rational design of drugs. DCA stimulates the activity of the enzyme PDH through inhibition of the PDKs enzymes. So the crystal structure of PDK2 in complex with DCA was acquired. It showed that DCA indwells the pyruvate binding site in the N-terminal regulatory (R) domain [2].

In this work, docking studies were carried out on our ligands to find their binding site, binding modes and the best direction base on of their binding energy. The docking simulations were done by means of an *in house* batch script (DOCKFACE) for automatic running of AutoDock 4.2 in a parallel mode using all system resources. After completing the docking process, the protein–ligand complex was then analyzed to investigate the type of interactions. Top ranked binding energies (kcal/mol) in AutoDock dlg output file were considered as a response in each run. Docking results were mostly supported by high cluster populations. Conformation with the lowest binding energy was considered as the best docking result in each case.

The docking biding energies for the derivatives were obtained. As shown in Table 2, all the synthesized compounds had a greater binding energy to the PDHK receptor than the co-crystal ligand, DCA. For example the docking binding energy of compound 17 was -6.16, whereas it was -3.63 for DCA. Figure 2 shows that there

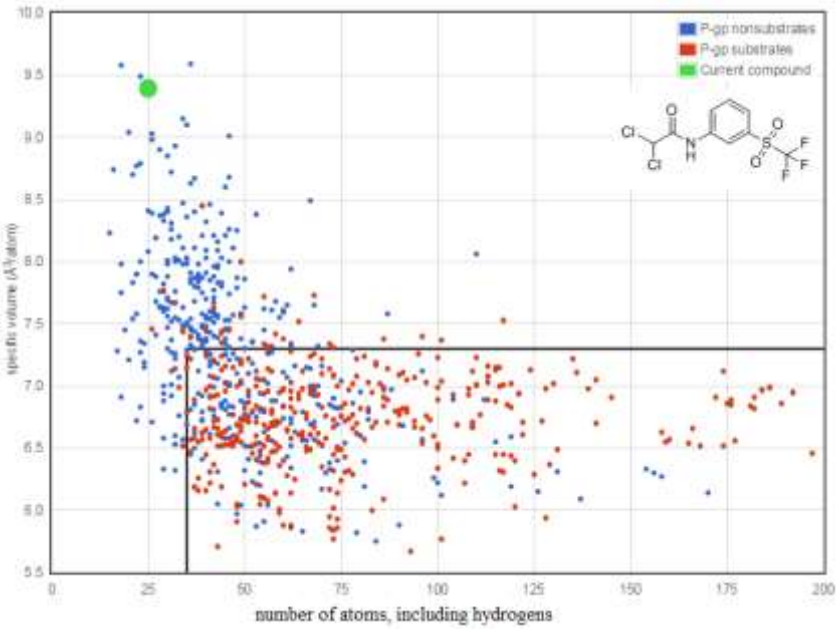


Figure 1. Plot of specific atomic volume versus the size of molecule for compound 25.

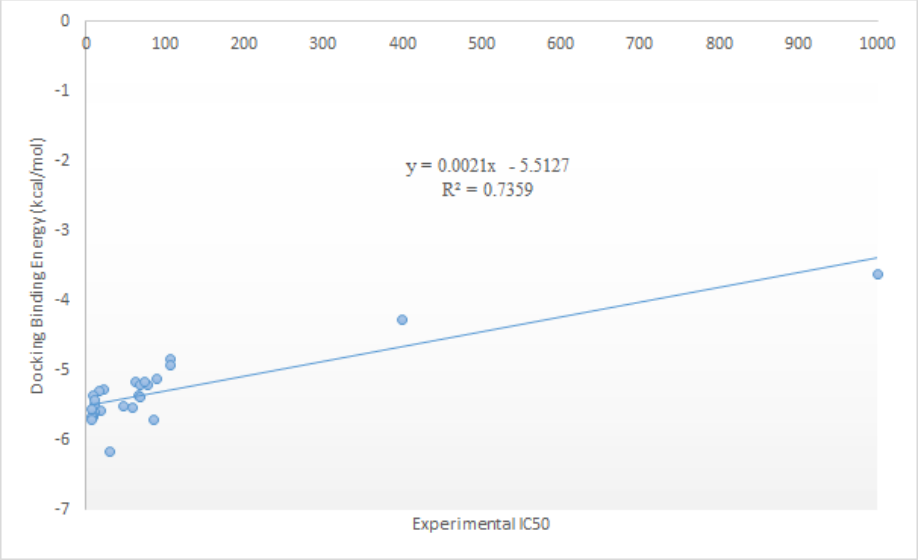


Figure 2. Plot of docking binding energy (kcal/mol) against experimental IC50 on NCI-H460 cell line.

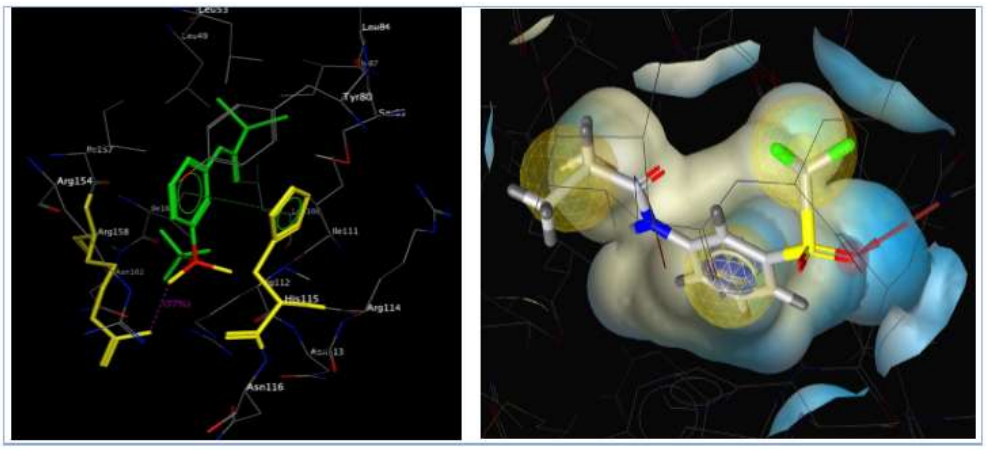


Figure 3. Interactions of compound **25** with the residues in the binding site of PDHK receptor

is a good correlation between the data from docking binding energy and experimental IC50 on the NCI-H460 cell line.

In addition, interaction type of compounds to their target was also explored. The result for each ligand was compared to its corresponding co-crystal ligand, DCA. Hydrogen bindings between docked potent agents such as 25 and PDHK receptor were analyzed using Autodock tools program (ADT, version 1.5.6), ligplot version 4.5.3 [21] and LigandScout version 3.12 [22]. As shown in Figure 3, a hydrogen bond acceptor interaction exists between oxygen of sulfone group of compound 25 with Arg158 in the receptor. There is also an arene-arene interaction between phenyl group of this compound with imidazole ring of His115 in the receptor.

Discussion

In this study, 26 DCA analogues were synthesized by a new less toxic method and their cytotoxic activities of them were also evaluated against three different cancerous cell lines. The results showed that *N*-phenyl-2,2-dichloroacetamide analogues have adequate cytotoxic activities, and among them, compound 25 had the highest potency. The IC50 of this compound was 6.5 μM against NCI-H460 cells, 10.5 μM against HCA-7 cells and 9.4 μM against MCF-7 cells. The cytotoxic

activity evaluations and toxicity risk factors calculations of our synthesized analogues suggest that compound 25 could be a potentially valuable candidate for drug development and a promising anticancer agent. According to our results (shown in Table 1), it seems that the colorectal cancer cellsare more resistant to DCA and *N*-phenyl-2,2-dichloroacetamide derivatives comparing to lung and breast cancer cells. The docking study revealed that, there is an arene-arene interaction between phenyl group of our ligands with imidazole ring of His115 in the receptor.Based on the substituents on phenyl group such as trifluoro(methylsulfonyl)methane, a hydrogen bond interaction might be existed between the oxygen of this substituent with Arg158 in the receptor and these interactions probably are responsible for the PDHK inhibitions of compound 25.

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