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Araştırma Makalesi / Research Article

The Synthesis and Anticancer Activities of Ferrocenyl Chalcones; Drug-Likeness Calculations

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Abstract

A series of **1-8** different ferrocenyl chalcones were synthesized through the Claisen–Schmidt condensation and the Friedel−Crafts acylation. Their structures were confirmed by means of ^{1}H, ^{13}C NMR and FT−IR. The cytotoxic and antiproliferative activities of the chalcones were screened against HeLa (cervix cancer) and PC3 (prostate cancer) cell lines. They showed excellent anticancer activities especially against PC3 cell even at very low concentration (5 µM). Also, their physicochemical properties, drug-likeness calculations and the rule of five (RO5) were investigated.

Key Words

"*Ferrocene, Chalcone, PC3, HeLa, Anti-cancer, Drug-likeness*"

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1. Introduction

Chalcones can be defined as aromatic enones i.e. 1,3-diphenyl-2-propen-1-one derivative. Due to conjugated and delocalized aromatic π electrons, they are colorful. Thus, they have excellent spectral properties. For example, their fluorescent properties were used in biochemical application (Tomasch at al., 2012). The Claisen–Schmidt condensation is most widely used method for ferrocenyl chalcone synthesis (Smith & Paulsen, 1954). Cross-Coupling reaction is another common method for chalcone synthesis (Zou at al., 2007). Microwave irradiation method can be given as an alternative method for chalcone synthesis (Kakati & Sarma, 2011). Chalcones can also be prepared by the Friedel−Crafts acylation (Shotter at al., 1978).

The inherent cyclization ability of chalcone allows using as precursor for many cyclization reactions such as flavonoids and isoflavonoids (Horborne at al., 1975). Another cyclization undergoes via Michael addition and 1,2 addition reaction afforded 1,5-benzothiazepines and/or 1,4-benzothiazines (Prakash at al., 2007). Also, Michael addition of chalcone is widely studied (Zhuang at al., 2017). Many natural compounds containing chalcone has been reported (fig. 1).

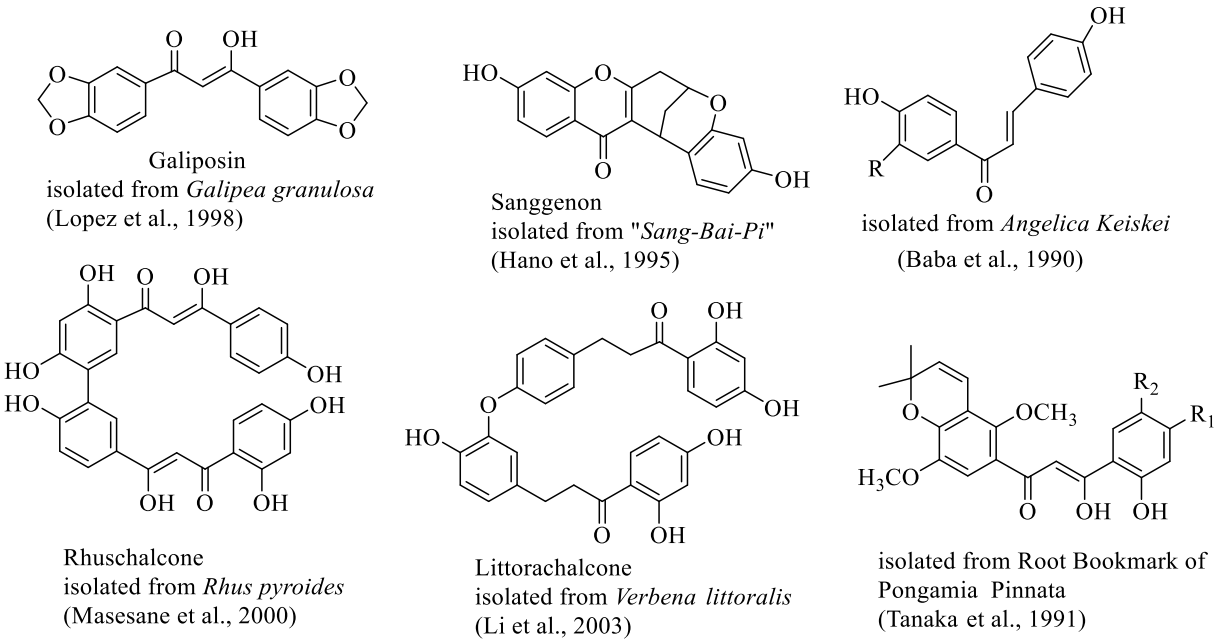


Figure 1. Some natural compounds containing chalcone unit

Given that chalcone containing compounds exhibit a broad range of therapeutic activities, several reports describing biological activities of chalcones have been reported. Boumendjel et al. reviewed the chalcones showing anticancer activities (Boumendjel et al., 2009). It has been observed that some chalcones have multiple important biological activities. For example, Isoliquiritigenin isolated from Nepalese propolis shows anticancer, anti-oxidant, cancer chemopreventer and anti-inflammation (Zhuang et al., 2017).

On the other hand, ferrocenyl chalcone synthesis and their biological evaluations were well studied. Yadav et al. synthesized 16 ferrocenyl chalcone through using microwave in high yields and screened them for antifungal against *Sclerotium rolfsii* and *Alternaria solani* (Yadav et al., 2019). They have found that their chalcones showed good antifungal activity. Similar antifungal activities were reported by Muskinja et al. for different ferrocenyl chalcones (Muskinja et al., 2016). Janka et al. prepared novel ferrocenyl chalcones and screened their anti-cancer activities against HeLa, MCF7, A549 and MDA cells and reported that they have potential antitumor activity (Janka et al., 2015).

Cancer is a large family of diseases caused by uncontrolled cell divisions and the fear of cancer is an ever-present in our daily life. Prostate cancer is the second most common cancer-related death in men. On the other hand, cervical cancer is the second most common cancer in women worldwide (Petignat et al., 2007). About half million patients are newly diagnosed annually and more than 200,000 woman die due to cervical cancer (Parkin et al., 2005).

In this study, firstly a series of 1-8 different ferrocenyl chalcones were synthesized through the Claisen–Schmidt condensation and the Friedel−Crafts acylation and confirmed to their structures by means of ^{1}H, ^{13}C NMR and FT−IR. Secondly, The cytotoxic and antiproliferative activities of the chalcones were screen against HeLa (cervix cancer) and PC3 (prostate cancer) cell lines. Also, their physicochemical properties, drug-likeness calculations and the rule of five (RO5) were investigated.

2. Material Method

2.1. General

All chemicals were supplied from Sigma-Aldrich Company. ^{1}H and ^{13}C NMR spectra of $CDCl_3$ solutions were measured on a Bruker spectrospin Avance DPX-400 Ultra shield instrument at 400 MHz and 100 MHz respectively (standard TMS). FT-IR spectra were obtained on Bruker Platinum ATR-IR instrument and reported in reciprocal centimeters (cm^{-1}). All solvents were dried and distilled prior to use. Products were separated by flash column chromatography on Silica Gel 60 (Merck, 230–400 mesh ASTM). TLC analyses were applied on 250 µm Silica Gel 60 F254 plates. HeLa (human cervical cancer), PC3 (human prostate cancer cell) cancer cell lines (ATCC®, Manassas, VA, USA) were purchased in this study. Cell proliferation BrdU ELISA kits were provided from Roche (Germany), while 5-fluorouracil (5-FU) and others were from Sigma and Merck. The results of investigation in vitro are means ± SD of six measurement. Differences between groups were tested with ANOVA, Duncan. p values of <0.01 were considered as significant. IC_{50} values were determined using ED50plus v1.0.

2.2. General Procedure for Acylation of Ferrocene

1.0 g (5.38 mmol) ferrocene and 0.98 g (5.91 mmol) cinnamoyl chloride were mixed in 6 mL of CH_2Cl_2 at 0 ℃ for 1/2 hours. Next, 16.14 mL of $EtAlCl_2$ (16.14 mmol, 1 M stock solution in hexane) was added by dropwise at 0 ℃ for 15 min. After mixing another 3 hours at same temperature, the solution was poured into 25 mL of sat. NH_4Cl solution. CH_2Cl_2 layer was extracted and dried over $MgSO_4$. The flash column chromatography was applied with the eluent system (7:1, Hex:EtAc) for purification. 0.54 g **1** was obtained (yield, 32%).

2.2.1. Cinnamoyl Ferrocene (1)

(32% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (d, *J* = 15.7 Hz, 1H), 7.59 (dd, *J* = 7.3, 2.0 Hz, 2H), 7.36 (m, 3H), 7.06 (d, *J* = 15.7 Hz, 1H), 4.85 (s, 2H), 4.53 (s, 2H), 4.16 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.7, 140.9, 135.2, 130.1, 129.0, 128.3, 123.0, 80.6, 72.8, 70.1, 69.6. IR (cm^{-1}) 1649, 1593, 1456, 1240, 1079, 979, 759, 687, 484.

2.2.2. Crotonoyl Ferrocene (2)

(82% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (dq, *J* = 13.8, 6.9 Hz, 1H), 6.54 (dd, *J* = 15.2, 1.6 Hz 1H), 4.82 (s, 2H), 4.53 (s, 2H), 4.19 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 193.1, 140.7, 128.1, 80.1, 72.5, 70.0, 69.6, 18.1. IR (cm^{-1}) 1660, 1608, 1458, 1244, 980, 895, 823, 502, 483.

2.3. General Procedure for Claisen-Schmidt Condensation

0,5 g (2.19 mmol) aceytlferrocene and 0.147 g (2.63 mmol) KOH were dissolved in 13 mL ethanol. After mixing 15 min, 0.214 g (2.19 mmol) of p-metoxybenzaldehyde in 13 mL ethanol was added by dropwise into the solution. Next, it was refluxed for 2 hours. Finally, it was hydrolyzed with sat. NH_4Cl solution (15 mL) and extracted with CH_2Cl_2 (25 mL). The product **3** was easily purified with the flash column chromatography (7:1, Hex:EtAc, 0.26 g 30 % yield).

2.3.1. (E)-1-ferrocenyl-3-(4-methoxyphenyl)prop-2-en-1-one (3)

(30% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (d, *J* = 15.6 Hz, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 6.95 (d, *J* = 15.6 Hz, 1H), 6.88 (d, *J* = 8.7 Hz, 1H), 4.84 (s, 2H), 4.50 (s, 2H), 4.14 (s, 5H), 3.79 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 209.9, 161.4, 140.4, 129.9, 120.7, 114.2, 80.8, 72.6, 70.1, 69.7, 55.4. IR (cm^{-1}) 1646, 1580, 1509, 1453, 1239, 1172, 1078, 1021, 995, 826, 504, 483.

2.3.2. (E)-1-ferrocenyl-3-(2-furan-2-yl)prop-2-en-1-one (4)

(56% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.47 (d, *J* = 15.0 Hz, 2H), 6.95(d, *J* = 15.4 Hz, 1H), 6.61(d, *J* = 3.3 Hz, 1H), 6.44 (dd, *J* = 3.1, 1.7 Hz, 1H), 4.83 (s, 2H), 4.51 (s, 2H), 4.14 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.7, 151.8, 144.3, 127.1, 120.7, 115.3, 112.4, 80.7, 72.7, 70.1, 69.7. IR (cm^{-1}) 1649, 1589, 1549, 1449, 1374, 1282, 1072, 973, 817, 756, 668, 506, 483.

2.3.3. (E)-1-ferrocenyl-3-(4-chlorophenyl)prop-2-en-1-one (5)

(26% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 15.7 Hz, 1H), 7.51 (d, *J* = 8.5 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.02 (d, *J* = 15.5 Hz, 1H), 4.84 (s, 2H), 4.53 (s, 2H), 4.14 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 139.4, 129.4, 129.3, 129.2, 123.4, 72.9, 70.1, 70.0, 69.7. IR (cm^{-1}) 1650, 1594, 1456, 1239, 1094, 985, 815, 482.

2.3.4. (E)-1-ferrocenyl-3-(2-thiophen-2-yl)prop-2-en-1-one (6)

(55% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 15.3 Hz, 1H), 7.33 (d, *J* = 5.1 Hz, 1H), 7.28 (d, *J* = 3.5 Hz, 1H), 7.02 (dd, *J* = 5.0, 3.6 Hz, 1H), 6.85 (d, *J* = 15.3 Hz, 1H), 4.83 (s, 2H), 4.51 (s, 2H), 4.14 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.5, 140.7, 133.3, 131.4, 128.3, 127.9, 122.0, 80.6, 72.8, 70.1, 69.7. ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.5, 140.7, 133.3, 131.4, 128.3, 127.9, 122.0, 80.6, 72.8, 70.1, 69.7. IR (cm^{-1}) 1641, 1580, 1456, 1378, 1279, 1075, 972, 827, 705, 479.

2.3.5. (E)-1-ferrocenyl-3-(4-bromophenyl)prop-2-en-1-one (7)

(41% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 15.7 Hz, 1H), 7.47 (m, 4H), 7.03 (d, *J* = 15.6 Hz, 1H), 4.83 (s, 2H), 4.54 (s, 2H), 4.15 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.9, 139.5, 134.1, 132.2, 129.6, 124.2, 123.5, 80.5, 72.9, 70.1, 69.8. IR (cm^{-1}) 1651, 1595, 1456, 1240, 1071, 985, 815, 480.

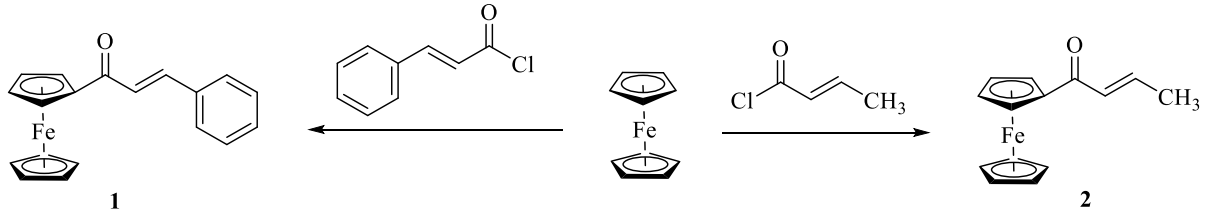
2.3.6. (E)-3-(naphthalen-1-yl)-1-ferrocenylprop-2-en-1-one (8)
(50% yield), ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.56 (d, *J* = 15.4 Hz, 1H), 8.24 (d, *J* = 8.4 Hz, 1H), 7.83 (m, 3H), 7.49 (m, 3H), 4.88 (s, 2H), 4.54 (s, 2H), 4.17 (s, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 192.9, 137.8, 133.8, 132.8, 131.8, 130.4, 128.7, 126.9, 126.3, 125.9, 125.4, 124.8, 123.7, 80.6, 72.9, 70.2, 69.8, 29.7. IR (cm^{-1}) 1639, 1569, 1450, 1345, 1256, 1075, 986, 793, 774, 484.

3. Result and Discussions

Within this work 8 ferrocenyl chalcones were synthesized and tested the anticancer activities against PC3 and HeLa cells. In addition, the drug-likeness and physochemical properties of molecules were calculated.

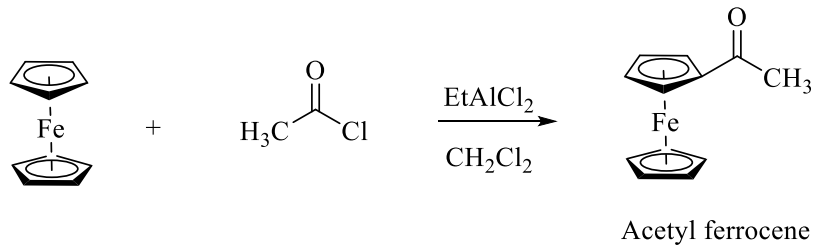
3.1. Chemistry

The synthetic part might be divided into two sections as follows; Claisen–Schmidt condensation and. Friedel–Crafts acylation. In the first part, the chalcones 1 and 2 were readily obtained from ferrocene and corresponding acyl chlorides by Friedel–Crafts acylation (scheme 1) (Dogan et al., 2005).



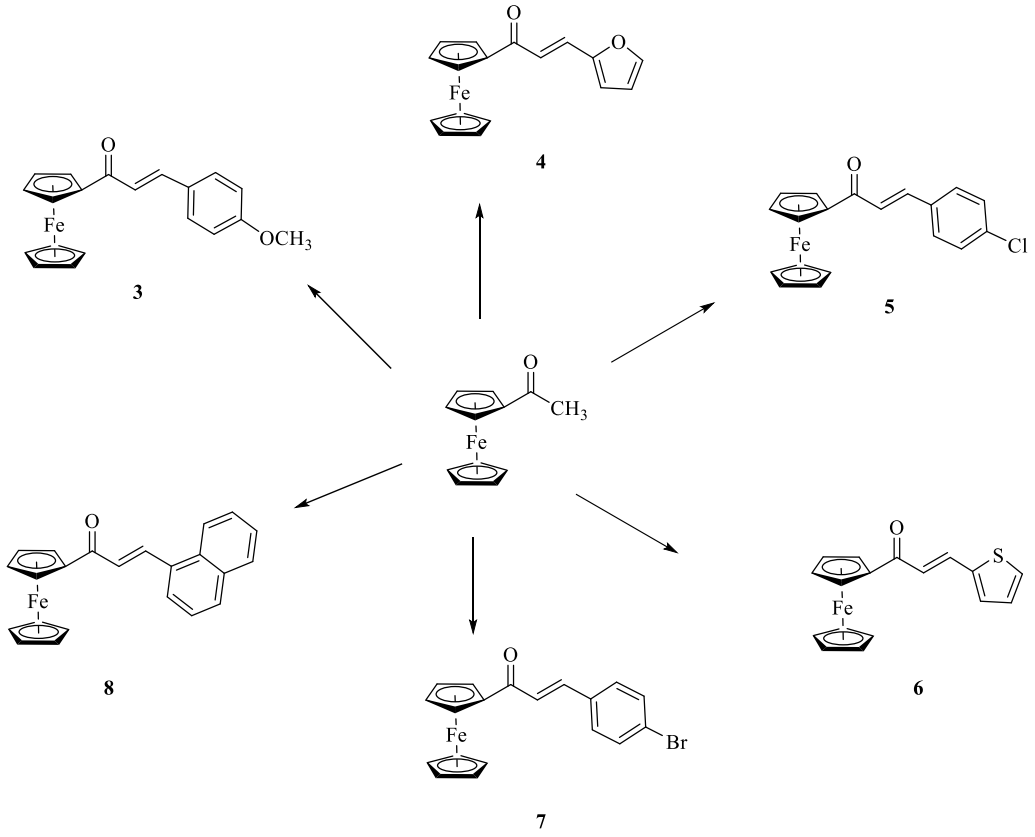
Scheme 1. Friedel–Crafts Ferrocenyl chalcone synthesis

In the second part, acetylferrocene was synthesized from ferrocene and acetyl chloride at 0 ^{0}C mediated with $EtAlCl_2$ (adopted from the Dogan group procedure (Dogan et al., 2005)). The pure and sole product was easily purified through flash column in high yields (up to 99 % yield) (Scheme 2).



Scheme 2. Acetyl ferrocene synthesis in the presence of alkyl Lewis acid, $EtAlCl_2$

Next, acetylferrocene was converted into the chalcones by Claisen–Schmidt condensation using appropriate aldehydes and pure colorful products were easily via flash column in high yields (up to 90 %) (Scheme 3).



Scheme 3. Ferrocenyl chalcone synthesis via Claisen–Schmidt condensation

3.2. Anticancer Activity Results

Anticancer activities of the chalcones (**1-8**) and 5-Florouracil (**5-FU**) against HeLa and PC3 cells were screened at four different concentrations (5, 25, 50 and 100 µM). IC_{50} values of compounds **(1-8)** and **5-FU** against HeLa and PC3 cells are depicted in the Table 1. The data presented in the Tables 1 revealed that all the chalcones (**1-8**) provided better IC_{50} inhibition activities than the standard drug (5-Fu) against PC3 cell. Among the screened the chalcones against PC3 cell, **7** gave the best inhibition with the IC_{50} value of 22.86 µM according to the standard drug with the IC_{50} value of 37.36 µM.

Table 1. IC_{50} values of the compounds **(1-8)** and 5-FU

Sample name	PC3 cell (µM)	HeLa cell (µM)
1	26.38 ± 0.10	2.40 ± .01
2	24.45 ± 0.12	2.29 ± 0.02
3	28.04 ± 0.05	2.50 ± 0.05
4	26.45 ± 0.24	2.46 ± 0.01
5	24.67 ± 0.17	2.53 ± 0.03
6	24.04 ± 0.11	2.42 ± 0.01
7	22.86 ± 0.28	2.45 ± 0.03
8	27.99 ± 0.30	2.81 ± 0.01
5-FU	37.36 ± 0.30	2.50 ± 0.01

Similarly, all the chalcones except 8 had also better anticancer activities than 5FU against HeLa cell (table 1). To illustrate, the IC_{50} value of 2 (2.29 µM) was the lower than 5-FU IC50 value (IC50 = 2.50 µM). As can be seen in Figure 1A and 1B, **1-8** had better anticancer activity than the 5-FU at all concentrations (5, 25, 50 and100 µM) against both PC3 and HeLa cells. Notably, all the chalcones showed much better activity than 5-FU at the low concentration (5 µM) against PC3 cell. Also, the chalcone **1-8** against HeLa cell showed more than 10 times activity than against PC3 (Table 1). In addition, 1, 2 and 4 showed better activity than the 5-FU at the lowest dose (5 µM) against HeLa cell (Figure 2B).

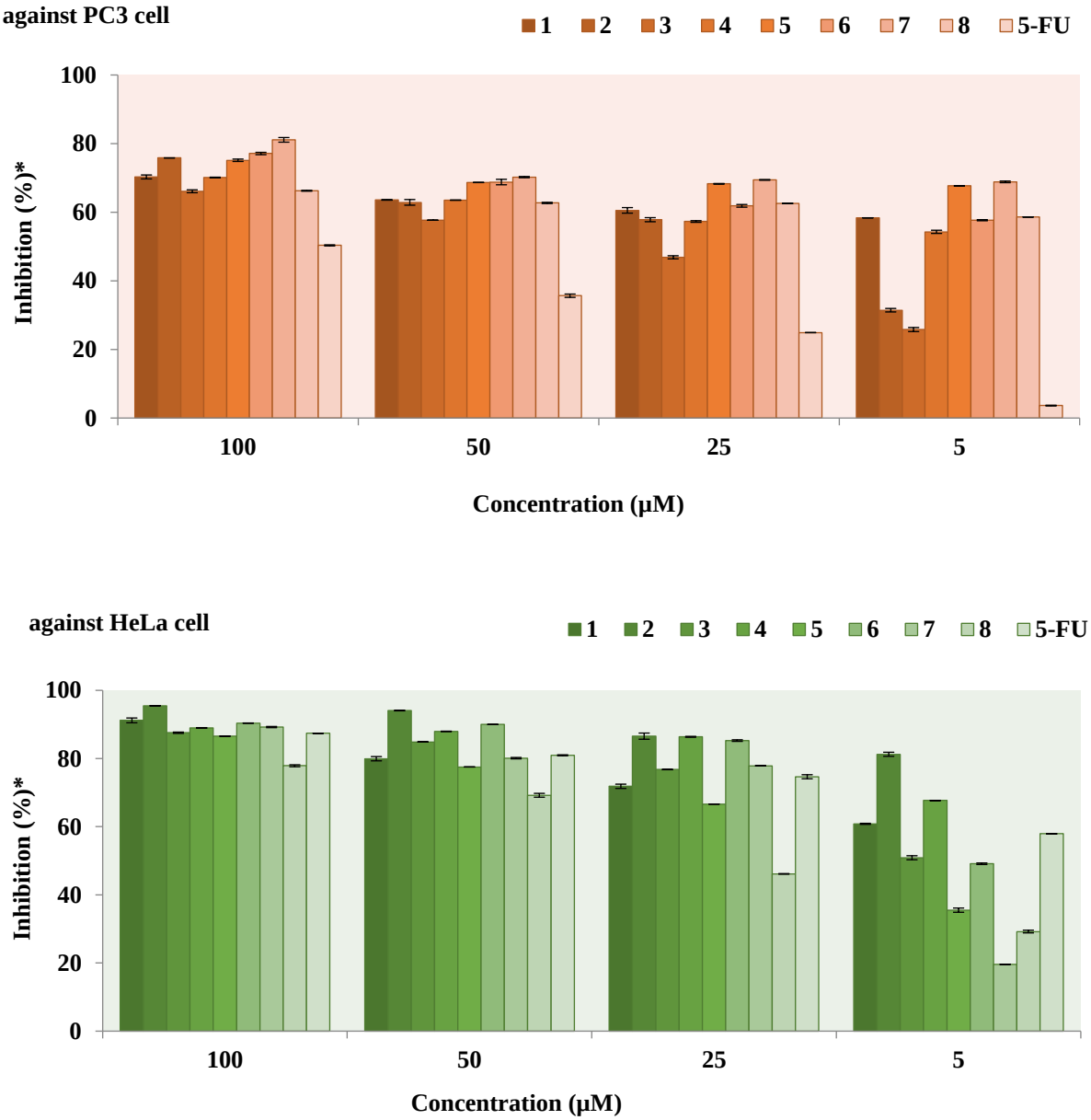


Figure 2. Anticancer activity of **(1-8)** and **5-FU** against PC3 (**A**) and HeLa (**B**) cells lines *Data are presented as mean ± SD (n=6). Statistical significant difference ($p<0.01$) was observed between treatments (ANOVA, Duncan).

3.3. Drug Likeness Properties

Before starting the synthesis or testing process as a drug, some theoretical calculations are made such as the Lipinski rule, physicochemical properties, water solubility, BBB score and lipophilicity. All the chalcones (**1** to **8**) obey the Lipinski's rule as explained below. They have hydrogen bond acceptors (HBA) and donors (HBD) within the Lipinski's rules, n-ON <10 and n-OHNH<5. The calculations of logP proved that they are smaller than 5 that is within the Lipinski's rules (Table 2). According to another Lipinski rule, the molecular weight of compounds should be less than 500 gr/mol. All the chalcones have molecular weights between 254.11 to 395.07 g/mol. The Blood-Brain Barrier (BBB) score is valuable physicochemical information which can avoids toxicities of drugs and it should be less than 6.0. Fortunately, the BBB score of **(1-8)** are ranges between from 4.32 to 4.98. On the other hand, synthetic accessibility and topological polar surface area (TPSA) must be smaller than 10 and 70 $Å^2$ respectively. And it was calculated that the synthetic accessibilities and topological polar surface areas (TPSA) of the chalcones are smaller than 5 and 70 $Å^2$ respectively (table 2 and figure 2). Another important property for the drug-likeness calculation is the solubility and it was found that all the chalcones are either soluble or moderately soluble. The solubilities of the chalcones (**1** to **8**) are between -5.49 and -3.25 (The solubility (log S) scale value ranges between -10 (insoluble), -6 (poorly soluble), -4 (soluble), -2 (very soluble) and 0 (highly soluble)) (table 2). The skin permeation results proved that the chalcones (**1** to **8**) are good anti-inflammatory with the log Kp values between -5.55 and -4.36 cm/s.

According to Lipinski's rule of five; the chalcones **(1-8)** could be a new potential anticancer agent according to calculated data (Table 2 and Table 3). The drug-likeness of **1**-**8** were found to have satisfactory "Bioavailability radar" results which implies they can be can be good candidates as oral drugs (Figure 3).

Table 2. Physicochemical properties, lipophilicity, solubility, pharmacokinetics, drug-likeness and medicinal chemistry of the chalcones (**1-8**) predicted using Swiss ADME

No	Physicochemical properties	Lipophilicity	Water solubility	Pharmacokinetics	Drug likeness	Medicinal Chemistry
1	Formula:C19H16FeO Molecular weight: 316.17 g/mol Num. heavy atoms: 21 Num. arom.heavy atoms: 6 Fraction Csp3:0.11 Num. rotatable bonds: 5 Num. H-bond acceptors: 1 Num. H-bond donors: 0 Molar Refractivity: 83.49 TPSA: 17.07 Å²	Log $P_{o/W}$ (iLOGP): 0.00 Log $P_{o/W}$ (XLOGP3):4.59 Log $P_{o/W}$ (WLOGP):4.44 Log $P_{o/W}$ (MLOGP):3.93 Log $P_{o/W}$ (SILICOS-IT):2.50 Consensus Log $P_{o/W}$: 3.09	Log *S* (ESOL): -4.57 Solubility:8.44e-03 mg/ml; 2.67e-05mol/l Class:Moderately soluble Log *S* (Ali): -4.67 Solubility: 6.72e-03 mg/ml; 2.12e-05mol/l Class: Moderately soluble Log *S*(SILICOS-IT):-3.27 Solubility: 1.69e-01 mg/ml; 5.36e-04mol/l Class: Soluble	GI absorption: High BBB permeant:Yes P-gpsubstrate:No CYP1A2 inhibitor:No CYP2C19 inhibitor:Yes CYP2C9 inhibitor:Yes CYP2D6 inhibitor:No CYP3A4 inhibitor:No Log Kp (skin permeation):-4.97 cm/s	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: No; 1 violation: Heteroatoms< 2 Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 2 alerts: heavy_metal, Michael_acceptor_1 Leadlikeness: No;1 violation: XLOGP3>3.5 Synthetic accessibility: 4.27
2	Formula:C14H14FeO Molecular weight: 254.11g/mol Num. heavy atoms: 16 Num. arom.heavy atoms: 0 Fraction Csp3:0.21 Num. rotatable bonds: 4 Num. H-bond acceptors: 1 Num. H-bond donors: 0 Molar Refractivity: 63.01 TPSA: 17.07 Å²	Log Po/W (iLOGP): 0.00 Log Po/W (XLOGP3):3.33 Log Po/W (WLOGP):3.41 Log Po/W (MLOGP):2.95 Log Po/W (SILICOS-IT):1.33 Consensus Log Po/W: 2.21	Log S (ESOL): -3.25 Solubility:1.43e-01 mg/ml; 5.63e-04mol/l Class:Soluble Log S (Ali): -3.37 Solubility: 1.10e-01 mg/ml; 4.31e-04mol/l Class: Soluble Log S(SILICOS-IT):-1.17 Solubility: 1.74e+01 mg/ml; 6.84e-02mol/l Class: Soluble	GI absorption: High BBB permeant:Yes P-gpsubstrate:No CYP1A2 inhibitor:No CYP2C19 inhibitor:No CYP2C9 inhibitor:No CYP2D6 inhibitor:No CYP3A4 inhibitor:No Log Kp (skin permeation):-5.49 cm/s	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: No;1 violation: Heteroatoms< 2 Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 2 alerts: heavy_metal, Michael_acceptor_1 Leadlikeness: Yes Synthetic accessibility: 4.36

Table 2 (*cont*). Physicochemical properties, lipophilicity, solubility, pharmacokinetics, drug-likeness and medicinal chemistry of the chalcones (**1-8**) predicted using Swiss ADME

No	Physicochemical properties	Lipophilicity	Water solubility	Pharmacokinetics	Drug likeness	Medicinal Chemistry
3	Formula:C20H18FeO Molecular weight: 330.20 g/mol Num. heavy atoms: 22 Num. arom.heavy atoms: 6 Fraction Csp3:0.15 Num. rotatable bonds: 5 Num. H-bond acceptors: 1 Num. H-bond donors: 0 Molar Refractivity: 88.45 TPSA: 17.07 Å²	Log Po/W (iLOGP): 0.00 Log Po/W (XLOGP3):4.96 Log Po/W (WLOGP):4.75 Log Po/W (MLOGP):4.16 Log Po/W (SILICOS-IT):3.01 Consensus Log Po/W: 3.38	Log S (ESOL): -4.88 Solubility:4.31e-03 mg/ml ; 1.31e-05 mol/l Class:Moderately soluble Log S (Ali): -5.06 Solubility: 2.90e-03 mg/ml; 8.78e-06 mol/l Class: Moderately soluble Log S(SILICOS-IT):-3.65 Solubility: 7.39e-02 mg/ml ; 2.24e-04 mol/l Class: Soluble	GI absorption: High BBB permeant:Yes P-gpsubstrate:Yes CYP1A2 inhibitor:No CYP2C19 inhibitor:Yes CYP2C9 inhibitor:Yes CYP2D6 inhibitor:No CYP3A4 inhibitor:No Log Kp (skin permeation):-4.79 cm/s	Lipinski: Yes; 1 violation: MLOGP>4.15 Ghose: Yes Veber: Yes Egan: Yes Muegge: No; 1 violation: Heteroatoms< 2 Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 2 alerts: heavy_metal, Michael_acceptor_1 Leadlikeness: No;1 violation: XLOGP3>3.5 Synthetic accessibility: 4.37
4	Formula:C20H20FeO2 Molecular weight: 348.22 g/mol Num. heavy atoms: 23 Num. arom.heavy atoms: 6 Fraction Csp3:0.25 Num. rotatable bonds: 6 Num. H-bond acceptors: 2 Num. H-bond donors: 0 Molar Refractivity: 90.45 TPSA: 26.30 Å²	Log Po/W (iLOGP): 0.00 Log Po/W (XLOGP3):4.61 Log Po/W (WLOGP):4.67 Log Po/W (MLOGP):3.58 Log Po/W (SILICOS-IT):2.95 Consensus Log Po/W: 3.16	Log S (ESOL): -4.70 Solubility:6.94e-03 mg/ml ; 1.99e-05 mol/l Class: Moderately soluble Log S (Ali): -4.89 Solubility: 4.51e-03 mg/ml ; 1.30e-05 mol/l Class: Moderately soluble Log S(SILICOS-IT): -3.85 Solubility: 4.94e-02 mg/ml; 1.42e-04 mol/l Class: Soluble	GI absorption: High BBB permeant:Yes P-gpsubstrate: Yes CYP1A2 inhibitor:No CYP2C19 inhibitor:Yes CYP2C9 inhibitor:No CYP2D6 inhibitor:No CYP3A4 inhibitor:Yes Log Kp (skin permeation): -5.15 cm/s	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: Yes Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 3 alerts: heavy_metal, isolated_alkene, michael_acceptor_1 Leadlikeness: No; 1 violation: XLOGP3>3.5 Synthetic accessibility: 4.30

Table 2 (*cont*). Physicochemical properties, lipophilicity, solubility, pharmacokinetics, drug-likeness and medicinal chemistry of the chalcones (**1-8**) predicted using Swiss ADME

No	Physicochemical properties	Lipophilicity	Water solubility	Pharmacokinetics	Drug likeness	Medicinal Chemistry
5	Formula:C17H14FeO2 Molecular weight: 306.14 g/mol Num. heavy atoms: 20 Num. arom.heavy atoms: 5 Fraction Csp3:0.12 Num. rotatable bonds: 5 Num. H-bond acceptors: 2 Num. H-bond donors: 0 Molar Refractivity: 75.75 TPSA: 30.21 Å²	Log Po/W (iLOGP): 0.00 Log Po/W (XLOGP3):3.69 Log Po/W (WLOGP):4.03 Log Po/W (MLOGP):2.57 Log Po/W (SILICOS-IT):1.87 Consensus Log Po/W: 2.43	Log S (ESOL): -3.92 Solubility: 3.70e-02 mg/ml; 1.21e-04 mol/l Class: Soluble Log S (Ali): -4.01 Solubility: 2.96e-02 mg/ml ; 9.67e-05 mol/l Class: Moderately soluble Log S(SILICOS-IT): -2.49 Solubility: 9.91e-01 mg/ml; 3.24e-03 mol/l Class: Soluble	GI absorption: High BBB permeant:Yes P-gpsubstrate: No CYP1A2 inhibitor:No CYP2C19 inhibitor:Yes CYP2C9 inhibitor:No CYP2D6 inhibitor:No CYP3A4 inhibitor:No Log Kp (skin permeation): -5.55 cm/s	Lipinski: Yes Ghose: Yes Veber: Yes Egan: Yes Muegge: Yes Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 2 alerts: heavy_metal, michael_acceptor_1 Leadlikeness: No; 1 violation: XLOGP3>3.5 Synthetic accessibility: 4.31
6	Formula: C19H15ClFeO Molecular weight: 350.62 g/mol Num. heavy atoms: 22 Num. arom.heavy atoms: 6 Fraction Csp3:0.11 Num. rotatable bonds: 5 Num. H-bond acceptors: 1 Num. H-bond donors: 0 Molar Refractivity: 88.50 TPSA: 17.07 Å²	Log Po/W (iLOGP): 0.00 Log Po/W (XLOGP3):5.22 Log Po/W (WLOGP):5.10 Log Po/W (MLOGP):4.43 Log Po/W (SILICOS-IT):3.13 Consensus Log Po/W: 3.57	Log S (ESOL): -5.17 Solubility: 2.35e-03 mg/ml; 6.69e-06 mol/l Class: Moderately soluble Log S (Ali): -5.33 Solubility1.65e-03 mg/ml ; 4.72e-06 mol/l Class: Moderately soluble Log S(SILICOS-IT): -3.87 Solubility:4.77e-02 mg/ml ; 1.36e-04 mol/l Class: Soluble	GI absorption: High BBB permeant:Yes P-gpsubstrate: Yes CYP1A2 inhibitor:No CYP2C19 inhibitor:Yes CYP2C9 inhibitor:Yes CYP2D6 inhibitor:No CYP3A4 inhibitor:Yes Log Kp (skin permeation):-4.73 cm/s	Lipinski: Yes; 1 violation: MLOGP>4.15 Ghose: Yes Veber: Yes Egan: Yes Muegge: No; 2 violations: XLOGP3>5, Heteroatoms< 2 Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 2 alerts: heavy_metal, michael_acceptor_1 Leadlikeness: No; 2 violations: MW>350, XLOGP3>3.5 Synthetic accessibility: 4.21

Table 2 (*cont*). Physicochemical properties, lipophilicity, solubility, pharmacokinetics, drug-likeness and medicinal chemistry of the chalcones (**1-8**) predicted using Swiss ADME

No	Physicochemical properties	Lipophilicity	Water solubility	Pharmacokinetics	Drug likeness	Medicinal Chemistry
7	Formula: C17H14FeOS Molecular weight: 322.20 g/mol Num. heavy atoms: 20 Num. arom.heavy atoms: 5 Fraction Csp3:0.12 Num. rotatable bonds: 5 Num. H-bond acceptors: 1 Num. H-bond donors: 0 Molar Refractivity: 81.36 TPSA: 45.31 Å²	Log Po/W (iLOGP): 0.00 Log Po/W (XLOGP3):4.31 Log Po/W (WLOGP):4.50 Log Po/W (MLOGP):3.50 Log Po/W (SILICOS-IT):3.12 Consensus Log Po/W: 3.09	Log S (ESOL): -4.41 Solubility: 1.26e-02 mg/ml; 3.91e-05 mol/l Class: Moderately soluble Log S (Ali): -4.98 Solubility: 3.41e-03 mg/ml; 1.06e-05 mol/l Class: Moderately soluble Log S(SILICOS-IT): -2.54 Solubility: 9.36e-01 mg/ml; 2.90e-03 mol/l Class: Soluble	GI absorption: High BBB permeant:Yes P-gp substrate: No CYP1A2 inhibitor:No CYP2C19 inhibitor:Yes CYP2C9 inhibitor:Yes CYP2D6 inhibitor:No CYP3A4 inhibitor:No Log Kp (skin permeation):-5.21 cm/s	Lipinski: Yes; Ghose: Yes Veber: Yes Egan: Yes Muegge: Yes Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 2 alerts: heavy_metal, michael_acceptor_1 Leadlikeness: No; 1 violation: XLOGP3>3.5 Synthetic accessibility: 4.32
8	Formula: C19H15BrFeO Molecular weight: 395.07 g/mol Num. heavy atoms: 22 Num. arom.heavy atoms: 6 Fraction Csp3:0.11 Num. rotatable bonds: 5 Num. H-bond acceptors: 1 Num. H-bond donors: 0 Molar Refractivity: 91.19 TPSA: 17.07 Å²	Log Po/W (iLOGP): 0.00 Log Po/W (XLOGP3):5.28 Log Po/W (WLOGP):5.20 Log Po/W (MLOGP):4.54 Log Po/W (SILICOS-IT):3.17 Consensus Log Po/W: 3.64	Log S (ESOL): -5.49 Solubility: 1.29e-03 mg/ml; 3.25e-06 mol/l Class: Moderately soluble Log S (Ali): -5.39 Solubility 1.61e-03 mg/ml ; 4.09e-06 mol/l Class: Moderately soluble Log S(SILICOS-IT): -4.07 Solubility: 3.38e-02 mg/ml; 8.55e-05 mol/l Class: Moderately soluble	GI absorption: High BBB permeant:Yes P-gp substrate: Yes CYP1A2 inhibitor:No CYP2C19 inhibitor:Yes CYP2C9 inhibitor:Yes CYP2D6 inhibitor:No CYP3A4 inhibitor:Yes Log Kp (skin permeation):-4.96 cm/s	Lipinski: Yes; 1 violation: MLOGP>4.15 Ghose: Yes Veber: Yes Egan: Yes Muegge: No; 2 violations: XLOGP3>5, Heteroatoms< 2 Bioavailability Score: 0.55	PAINS: = 0 alert Brenk: 2 alerts: heavy_metal, michael_acceptor_1 Leadlikeness: No; 2 violations: MW>350, XLOGP3>3.5 Synthetic accessibility: 4.23

The bioavailability radars of **1**-**8** were demonstrated on Figure 2. The optimal range was drawn as pentagonal pink (lipophilicity: LOGP between −0.7 and +5.0, size: MW between 150 and 500 g/mol, polarity: TPSA between 20 and 130 Å^2, solubility: log S not higher than 6, saturation: fraction of carbons in the sp^3 hybridization not less than 0.25, and flexibility: no more than 9 rotatable bonds). According to the bioavailability radars and the smiles, the chalcones **(1-8)** can be accepted orally bioavailable (figure 2 and table 3).

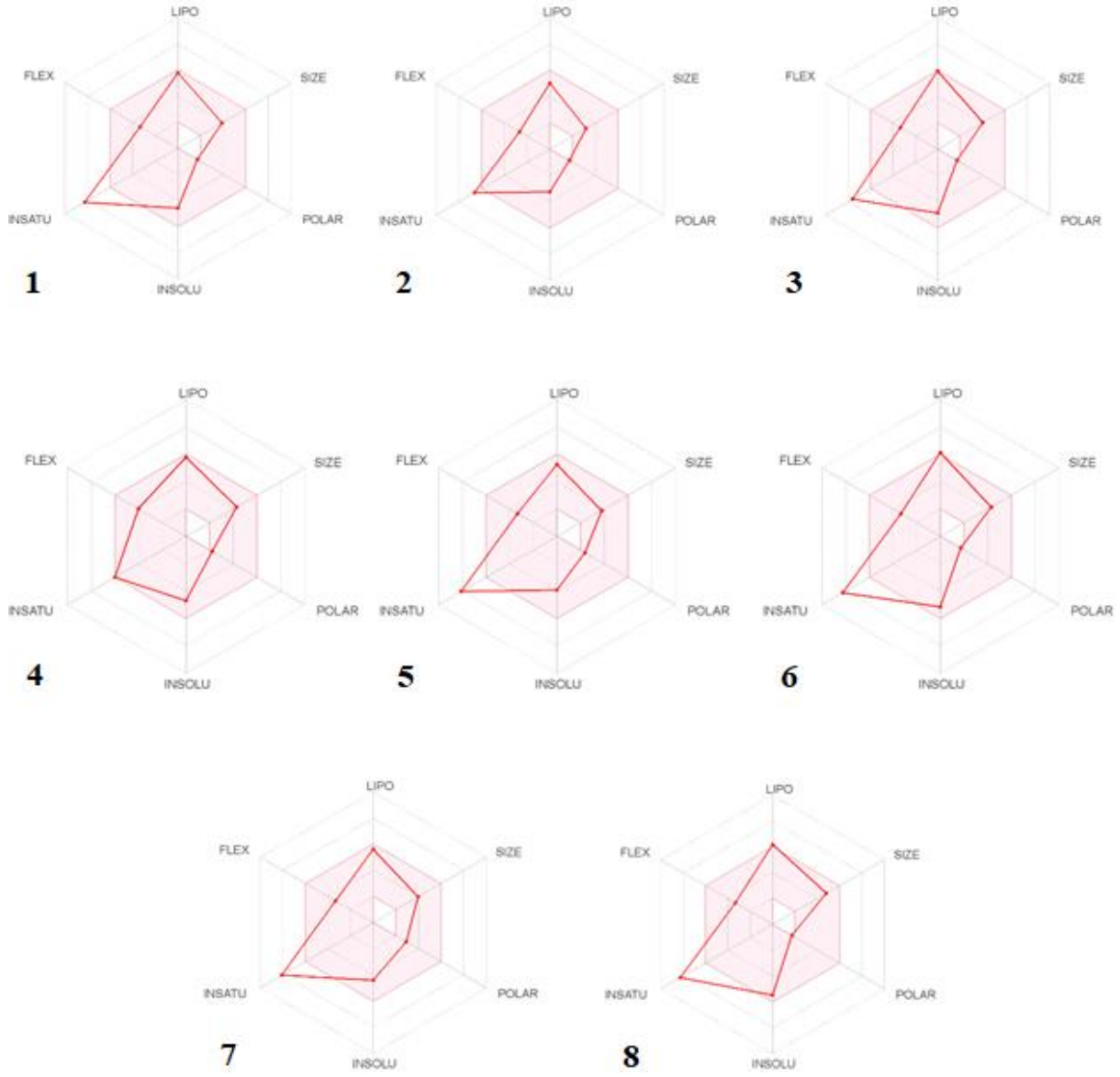


Figure 3. Drug-likeness of the chalcones (**1-8**) were predicted using Bioavailability radar. The pink area represents the optimal range for each properties (Lipo: Lipophilicity, Size: Moleculer weight, POLAR: Total Polar Surface Area, INSOLU: Insolubility, INSATU: Insaturation, FLEX: Flexibility).

Table 3. SMILES, Lipinski rule of five and drug-likeness of the chalcones (**1-8**) predicted using molsoft programe.

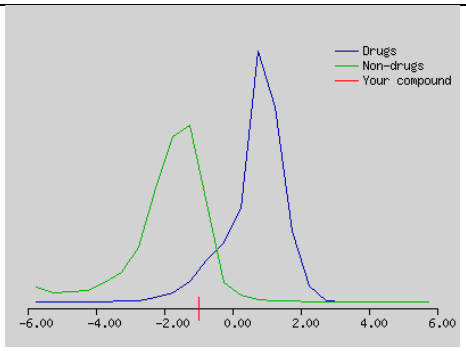
No	SMILES	Moleculer properties	Drug likeliness
1	O=C(\C=C\C1=CC=CC=C1)C1=CC=CC1[Fe]C1C=CC=C1	Moleculer formula:C19 H16 Fe O Moleculer weight: 316.06 Number of HBA: 1 Number of HBD: 0 MolLogP: 4.77 MolLogS: -4.96 (in Log(moles/L)) 3.50 (in mg/L) MolPSA: 14.06 A^2 MolVol: 341.99 A^3 Number of stereo centers: 1 BBB Score: 4.81	 Drug-likeness model score: -0.97

Table 3 (*cont*). SMILES, Lipinski rule of five and drug-likeness of the chalcones (**1-8**) predicted using molsoft programe.

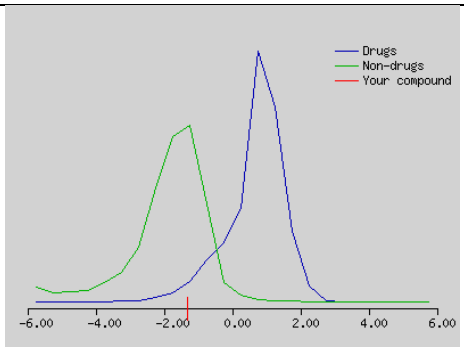
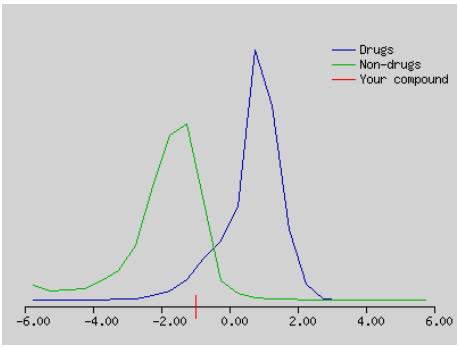
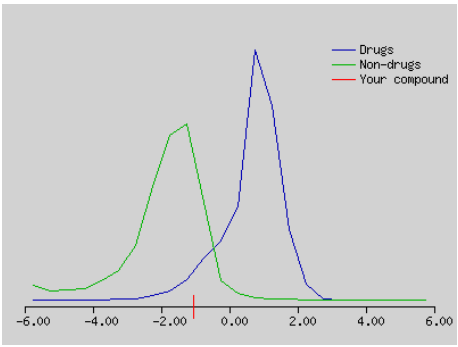
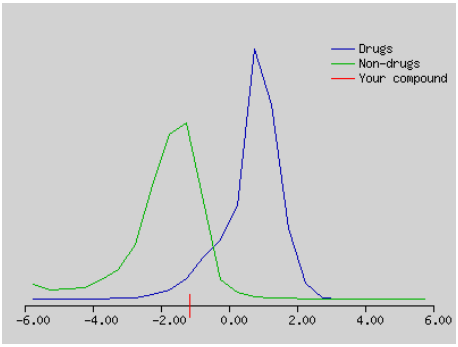
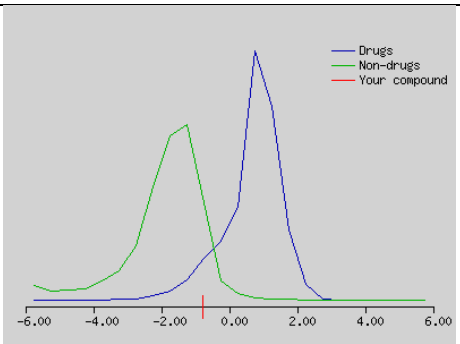
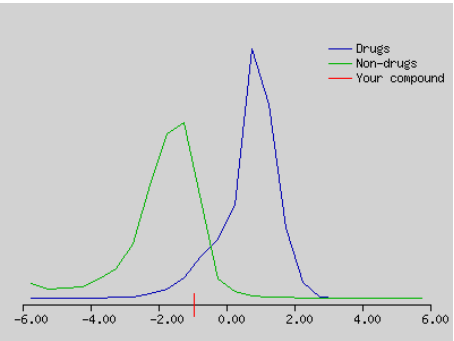
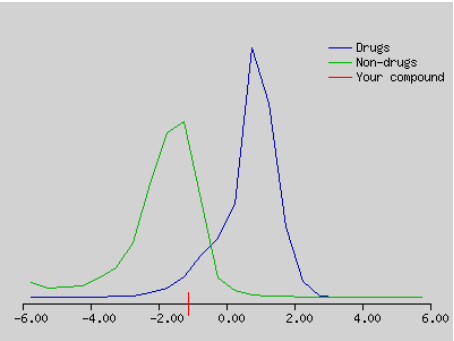
No	SMILES	Moleculer properties	Drug likeliness
2	C\C=C\C(=O)C1=CC=CC1[Fe]C1C=CC=C1	Moleculer formula:C14 H14 Fe O Moleculer weight: 254.04 Number of HBA: 1 Number of HBD: 0 MolLogP: 3.53 MolLogS: -3.57 (in Log(moles/L)) 69.11 (in mg/L) MolPSA: 14.33 A^2 MolVol: 284.80 A^3 Number of stereo centers: 1 BBB Score: 4.37	 Drug-likeness model score: -1.33
3	CC1=CC=C(\C=C\C(=O)C2=CC=CC2[Fe]C2C=CC=C2)C=C1	Moleculer formula: C20 H18 Fe O Moleculer weight: 330.07 Number of HBA: 1 Number of HBD: 0 MolLogP: 5.22 (> 5) MolLogS: -5.24 (in Log(moles/L)) 1.90 (in mg/L) MolPSA: 14.06 A^2 MolVol: 362.93 A^3 Number of stereo centers: 1 BBB Score: 4.78	 Drug-likeness model score: -0.98
4	COC1=CC=C(\C=C\C(=O)C2=CC=CC2[Fe]C2CCC=C2)C=C1	Moleculer formula: C20 H18 Fe O2 Moleculer weight: 346.07 Number of HBA: 2 Number of HBD: 0 MolLogP: 4.73 MolLogS: -4.70 (in Log(moles/L)) 6.86 (in mg/L) MolPSA: 21.60 A^2 MolVol: 373.84 A^3 Number of stereo centers: 1 BBB Score: 4.92	 Drug-likeness model score: -1.05
5	O=C(\C=C\C1=CC=CO1)C1=CC=CC1[Fe]C1C=CC=C1	Moleculer formula: C17 H14 Fe O2 Moleculer weight: 306.03 Number of HBA: 2 Number of HBD: 0 MolLogP: 3.94 MolLogS: - -4.29 (in Log(moles/L)) 15.78 (in mg/L) MolPSA: 21.81 A^2 MolVol: 327.96 A^3 Number of stereo centers: 1 BBB Score: 4.98	 Drug-likeness model score: -1.14

Table 3 (*cont*). SMILES, Lipinski rule of five and drug-likeness of the chalcones (**1-8**) predicted using molsoft programe.

No	SMILES	Moleculer properties	Drug likeliness
6	ClC1=CC=C(\C=C\C(=O)C2=CC=CC2[Fe]C2C=CC=C2)C=C1	Moleculer formula: C19 H15 Cl Fe O Moleculer weight: 350.02 Number of HBA: 1 Number of HBD: 0 MolLogP: 5.37 (> 5) MolLogS: -6.36 (in Log(moles/L)) 0.15 (in mg/L) MolPSA: 14.06 A^2 MolVol: 359.18 A^3 Number of stereo centers: 1 BBB Score: 4.75	 Drug-likeness model score: -0.79
7	O=C(\C=C\C1=CC=CS1)C1=CC=CC1[Fe]C1C=CC=C1	Moleculer formula: C17 H14 Fe O S Moleculer weight: 322.01 Number of HBA: 2 Number of HBD: 0 MolLogP: 4.15 MolLogS: -4.64 (in Log(moles/L)) 7.42 (in mg/L) MolPSA: 15.08 A^2 MolVol: 340.20 A^3 Number of stereo centers: 1 BBB Score: 4.79	 Drug-likeness model score: -0.94
8	BrC1=CC=C(\C=C\C(=O)C2=CC=CC2[Fe]C2C=CC=C2)C=C1	Moleculer formula: C19 H15 Br Fe O Moleculer weight: 393.97 Number of HBA: 1 Number of HBD: 0 MolLogP: 5.61 (> 5) MolLogS: -5.84 (in Log(moles/L)) 0.57 (in mg/L) MolPSA: 14.06 A^2 MolVol: 363.85 A^3 Number of stereo centers: 1 BBB Score: 4.70	 Drug-likeness model score: -1.13

4. Conclusions

1-8 chalcones were obtained via Claisen–Schmidt condensation and the Friedel−Crafts acylation. Their structures were confirmed by means of ^{1}H and ^{13}C NMR and FT-IR spectroscopy. Anti-cancer activity studies of them proved that they have excellent anti-cancer activities against both PC3 and HeLa cells. Notably, **1**, **3**, **4**, **5**, **6**, **7** and **8** showed better activities more than 5 times than the standard drug (5-FU) even at the lowest concentration (5 µM) against PC3 cell. Moreover, the chalcones (1-8) obey Lipinski's rule. Also, they have satisfactory physicochemical properties such as lipophilicity, solubility, pharmacokinetics and bioavailability radar. Thus, it can be deduced from the aforementioned results ferrocenyl chalcones (**1-8**) are promising candidates for design of new drugs.

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