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Functionalized ferrocenes: The role of the para substituent on the phenoxy pendant group

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Abstract

Six ferrocenecarboxylates with phenyl, 4-(1*H*-pyrrol-1-yl)phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-bromophenyl, 4-iodophenyl as pendant groups were synthesized and fully characterized by spectroscopic, electrochemical and X-ray diffraction methods. The anti-proliferative activity of these complexes were investigated in hormone dependent MCF-7 breast cancer and MCF-10A normal breast cell lines, to determine the role of the para substituent on the phenoxy pendant group. The 4-fluorophenyl ferrocenecarboxylate is inactive in both cell lines while 4-(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylate is highly cytotoxic in both cell lines. 4-chlorophenyl and 4-bromophenyl ferrocenecarboxylates have moderate to good anti-proliferative activity in MCF-7 and low anti-proliferative activity on normal breast cell line, MCF-10A whereas the 4-iodophenyl analog is highly toxic on normal breast cell line. The phenyl ferrocenecarboxylate has proliferative effects on MCF-7 and is inactive in MCF-10A. Docking studies between the complexes and the alpha-estrogen receptor (ER_α) were performed to search for key interactions which may explain the anti-proliferative activity of 4-bromophenyl ferrocenecarboxylate. Docking studies suggest the anti-proliferative activity of these ferrocenecarboxylates is attributed to the cytotoxic effects of the ferrocene group and not to anti-estrogenic effects.

Keywords

Ferrocenecarboxylate; MCF-7; MCF-10A; Anti-cancer; Estrogen receptor; Docking

1. Introduction

The design and synthesis of functionalized ferrocenes has become a very active research area for many investigators, in particular for the development of metal-based anti-cancer agents [1–4]. This increasing interest on ferrocenes is derived from the leading experiments

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of Köpf-Maier and co-workers, which in 1984 reported for the first time the anti-cancer properties of ferrocenium in Ehrlich ascite tumor [5]. It became clear that besides the applications in organic synthesis, catalysis and material science [6–9], ferrocene had opened a new area of research in the design of metal-based anti-cancer drugs.

We have recently reported the cytotoxic activity of functionalized ferrocene esters with estrogens (estradiol, estrone) and Vitamin D₂ vectors (pendant groups), in order to make target specific drugs to nuclear receptors [10]. Our *in vitro* studies in the hormone dependent MCF-7 breast cancer cell line revealed that ferrocenoyl 17 β -hydroxy-estra-1,3,5(10)-trien-3-olate (ferrocene ester containing estradiol pendant group) has a remarkable IC₅₀, in the low micromolar range. There are only few ferrocenes reported by Jaouen and coworkers with IC₅₀ values in this range and currently they are considered good anti-cancer drugs [11–16]. The anti-proliferative activity of ferrocenoyl 17 β -hydroxy-estra-1,3,5(10)-trien-3-olate was attributed to the presence of the estradiol vector which is recognized by the estrogen nuclear receptor, producing a target specific drug [10]. Due to the low IC₅₀ value, this complex has great potential to undergo further development as an anti-cancer drug. However, it is also known that ferrocene derivatives have antitumor activity as a result of its redox property and the facile formation of ferrocenium ion. This redox property is responsible for the formation of oxygen free radical species inducing oxidative damage to DNA [17,18]. In order to investigate the role of the pendant group on ferrocene, we synthesized phenyl ferrocenecarboxylate, 4-fluorophenyl ferrocenecarboxylate, 4-chlorophenyl ferrocenecarboxylate, 4-bromophenyl ferrocenecarboxylate, 4-iodophenyl ferrocenecarboxylate and its analog replacing the halide with a redox active species pyrrol (4(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylate) and investigated their anti-proliferative properties in the MCF-7 breast cancer and MCF-10 normal breast cell lines. Herein we report our findings.

2. Results

2.1. Synthesis and characterization

The esterification of ferrocene was achieved by activating the ferrocenecarboxylic acid with oxalyl chloride and resulting ferrocenecarbonyl chloride was reacted with the corresponding phenols to yield the corresponding esters, Fig. 1, as described previously [10,19].

The IR spectra showed $\nu(\text{C}=\text{O})$ broad bands in the 1730–1740 cm⁻¹ corresponding to the carbonyl groups of the ferrocene esters. In the ¹³C NMR spectra, the ferrocene esters (ferrocenecarboxylates) showed a signal about 170 ppm attributed to the carbonyl carbons of the ester groups. In the ¹H NMR spectra, the complexes showed one signal (singlet) at about 4.3 ppm corresponding to the unsubstituted Cp rings. Two set of overlapping doublets between 4.4 and 5 ppm, corresponding to the four Cp protons of the functionalized Cp rings are observed. These multiplets showed an AA'BB' splitting pattern typical of the mono-substituted Cp ring. The proton signals of the phenoxy pendant groups are shifted downfield when compared to the free phenols. Based on these spectral features, the formation of the ferrocene esters are confirmed, as shown in Fig. 1.

To further corroborate their structures, the solid state structures of these ferrocene esters were investigated by single crystal X-ray diffraction. The solid state structural details of 4-chlorophenyl ferrocenecarboxylate and 4-bromophenyl ferrocenecarboxylate are presented in Table 1. Both complexes crystallize with the same crystal system, monoclinic and the same space group with two symmetry independent, but chemically identical molecules in the crystal lattice. Figs. 2 and 3 present only one molecule for 4-chlorophenyl ferrocenecarboxylate and 4-bromophenyl ferrocenecarboxylate respectively. Table 2 presents selected bonding lengths and angles.

In 4-bromophenyl ferrocenecarboxylate structure, the Cp rings are positioned nearly eclipsed and parallel, with Cp stagger and Cp bent angles of 1.74° and 0.48° for molecule 1 and 1.24° and 1.11° for molecule 2. The twist angles between the Cp ring and the carboxylate group (Fig. 4a) are 7.97° and 9.03° for 1 and 2 respectively, suggesting the pendant groups induce the slight tilt (bent angle) between the Cp rings of 0.48° and 1.11° . In both molecules, the Fe–C bond distances of the substituted carbons are shorter than the remaining. The geometry of the carbonyl carbons is distorted trigonal planar and the twist angles between the Cp plane and the aromatic ring (Fig. 4b) are 84.11° and 81.26° for molecules 1 and 2 respectively.

The 4-chlorophenyl ferrocenecarboxylate structure is also very similar to the 4-bromophenyl derivative with the Cp rings nearly eclipsed and parallel, with Cp stagger and Cp bent angles of 2.95° and 0.54° for molecule 1 and 1.19° and 1.25° for molecule 2. In the molecule 1, the aromatic ring is rotated (twist angle, Fig. 4b) by 87.58° from the Cp plane while for 2 is 78.06° . Again, the geometry around the carbonyl carbons is distorted trigonal planar. The twist angle between the Cp ring and the carboxylate group is 6.75° and 10.15° for molecules 1 and 2 respectively. The Cp stagger and Cp bent angles are similar to those reported for other mono-substituted ferrocenoyl complexes [20].

2.2. Electrochemical characterization

The redox behavior of functionalized ferrocenes is of fundamental importance since the facile oxidation to ferrocenium cation is attributed to be a key step inducing genotoxicity [17,18]. Ferrocene and ferrocenium species express their anti-proliferative effects by generating reactive oxygen species (ROS) [17,18]. Furthermore, pyrrol is an organic molecule used in biosensors due to its capacity of producing a conducting polymer (polypyrrol) via electrodeposition [21]. We recently published the 4-(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylate redox properties, forming a conductive poly(pyrrole-*N*-ferrocene-pyrrole) copolymer which was used as a mediator free glucose biosensor [21]. Thus, the subject complexes where the para substituent on the phenyl ring is changed were investigated by cyclic voltammetry under pseudo physiological conditions, using 1:1 methanol/DMEM cell culture media with TBAPF₆. It is the objective of this study to investigate the redox capabilities of these ferrocenes as they are known to generate ROS upon oxidation, leading to genotoxic effects. The E_{pa} , E_{pc} are presented in Table 3 and compared to Fc/Fc⁺ redox couple.

Cyclic voltammetric studies of the ferrocenecarboxylates showed they have much higher oxidation and redox potentials than Fc/Fc⁺ (Supplementary material). Thus, the oxidation

potentials of ferrocenecarboxylates are higher than ferrocene due to the inductive effects of the ester groups. In addition, the 4-(1*H*-pyrrol-1-yl) phenyl ferrocenecarboxylate has the higher oxidation potential for the metal center (Fc/Fc⁺) and also exhibited a second oxidation process corresponding to the pendant group (4-(1*H*-pyrrol-1-yl) phenolate) at higher potentials than ferrocenoyl moiety. Therefore, this species has an additional redox active group which may contribute to its anti-proliferative activity by generating reactive oxygen species (ROS), potentially causing genotoxic DNA damage. However, this redox potential was measured in the organic solvent CH₃CN, under non-physiological conditions. The low solubility of the 4-(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylate in buffer solutions at physiological pH hindered its electrochemical study under pseudo-physiological conditions.

For the remaining ferrocene ester complexes, we were able to study them under pseudo-physiological conditions using 1:1 methanol/DMEM cell medium, pH 7.4 with TBAPF₆ as supporting electrolyte. Phenyl ferrocenecarboxylate has the higher oxidation potential and the para-halogen-phenyl ferrocenecarboxylates follow a pattern. That is, as we increase the electronegativity of the para substituent, the oxidation potential decreases. This pattern is interesting since we may expect that fluoro being the most electronegative substituent would not get engaged in resonance with the phenyl ring as much as chloro, bromo or iodo and this in turn will make the electrons on the metal complex less available for oxidation. Instead we observe that as we increase the polarizability of the halogen, the ferrocene (metal center) gets more resistant to oxidation. In general terms, this data is used as a guide to predict ferrocenyls cytotoxic behaviors and it is clear that the 4-(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylate has two redox active groups. With this in mind we have investigated the anti-proliferative activity of the ferrocenecarboxylates containing different substituent on the para position of the phenyl ring.

2.3. In vitro anti-proliferative activity

The subject complexes were tested on two commercial cell lines; hormone-dependent MCF-7 breast cancer and MCF-10A normal breast cell lines using MTT assay [22,23]. A particular characteristic of the MCF-7 cell line is the high expression of estrogen receptor (ER) and it is highly sensitive to estrogens for proliferation. This ER receptor mediates the transport of estrogen inside the proliferating cells and can be used as a target for breast cancer. Drugs that interact with a specific receptor in cancer cells are known as target specific drugs and are frequently used to treat breast and other hormonal cancers [1,2,11–16].

The half maximal inhibitory concentration (IC₅₀) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. In this study it was utilized as a measure of cell proliferation. The IC₅₀ values for MCF-7 breast cancer cell and MCF-10A normal breast cell lines are listed in Table 4. The *in vitro* anti-proliferative studies revealed the 4-fluorophenyl substituent has no cytotoxic effect on both cell lines, while the phenyl group has proliferative effects on MCF-7 and it is inactive in MCF-10A cell line. 4-iodophenyl ferrocenecarboxylate has low anti-proliferative activity on breast cancer cells whereas in normal breast cell is highly active. Evidently, these properties are detrimental and

further use of 4-iodophenyl ferrocenecarboxylate as a metal-based drug is not encouraged. Conversely, 4-chlorophenyl and 4-bromophenyl ferrocenecarboxylates have moderate to good anti-proliferative behavior in MCF-7 and low anti-proliferative activity on normal breast cell line, MCF-10A. It is important to point out that compounds with good anti-proliferative activity on cancer cells and low activity in normal cells are desired and could have potential to be developed as drugs since this should minimize the well-known toxic side effects encountered in most of the anti-cancer drugs.

The effect of lipophilicity in the ferrocene's anti-proliferative activity has been reported previously [24]. Thus, replacing the halide on the phenyl ring by a redox active group (pyrrol) increases the lipophilic character and enhances anti-proliferative activity on MCF-7 breast cancer cell line but render detrimental in normal cells (MCF-10A), which also exhibited high cytotoxic activity. Thus, 4-(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylate does not have the cytotoxic properties to become a potential anti-cancer drug, since it displayed no selectivity between breast cancer cells and normal cells and could have severe toxic side effects.

Particular attention should be given to 4-bromophenyl ferrocenecarboxylate due to its anti-proliferative activity on MCF-7 cells, with low IC₅₀ value and low anti-proliferative effects on normal cells, MCF-10A. As stated above, this low IC₅₀ value for MCF-7 has been determined on estrogen vectorized ferrocene, ferrocenoyl 17 β -hydroxy-estra-1,3,5(10)-trien-3-olate complex [10]. Docking studies between the ferrocenoyl 17 β -hydroxy-estra-1,3,5(10)-trien-3-olate and the estrogen receptor (ER α) revealed that its good anti-proliferative activity was correlated to the appended group on the Cp ring (estradiol hormone) which targets the estrogen receptor alpha (ER α), facilitating the entry inside the cell by docking in the ligand (estradiol) binding site and subsequently impairing the ER α . On the other hand, Jaouen and co-workers reported a series of ferrocifens, ferrocenes analog of tamoxifen a selective receptor estrogen modulator (SERM) drug with excellent anti-proliferative activities on MCF-7 cell line [11–16]. Using molecular modeling methods they proposed that the ferrocifens dock in the ligand binding site and induce a conformational change in the protein impairing its function. But the 4-bromo ferrocenecarboxylate has no hormone appended to the Cp ring, although has a phenyl group which could be correlated to the A-ring of the estradiol. Since one of the differences between MCF-7 and MCF-10A is the high content of ER on MCF-7, we performed ER α -ferrocene docking studies to look for an explanation and what interactions possibly are responsible for their anti-proliferative effects.

2.4. Docking studies

The molecular modeling (docking) studies of 4-fluoro, 4-chloro, 4-bromo, 4-iodo and 4-(1*H*-pyrrol-1-yl)phenyl ferrocenecarboxylates on the ligand binding domain/binding pocket of the ER α were performed using VINA Program [25]. It is known that receptor agonist must fit the ligand binding pocket of the receptor whereas the antagonist may reside either in the pocket or in other location but must induce a conformational change in the protein structure, inactivating the receptor. Three set of docking studies were performed. In the first study the ferrocenecarboxylates structures used for docking were flexible and the amino acids in the

binding pocket were fixed in the conformation adopted by estradiol. This permits the ferrocene to accommodate within the confined space of the bonding pocket with the lowest energy conformation. Secondly, using flexible ferrocenecarboxylate structures, the lowest conformation was calculated setting the amino acids in the binding pocket at a distance of 4.5 Å flexible. The third study, ferrocenecarboxylate structures and the amino acids in the binding pocket at a distance of 5.0 Å were flexible. The most stable conformations were selected to those with the highest affinity (kcal/mol). Table 5 summarizes the amino acid residues surrounding the complexes within 4 Å (the most stable conformation) which correspond mainly to hydrophobic and van der Waals interactions with the fixed binding pocket and their binding affinities (kcal/mol). As reference, the calculated binding affinity of the estradiol in this binding pocket using the conformation provided by the crystal structure (1A52 pdb) is -9.8 kcal/mol.

The first study revealed that all the ferrocenecarboxylates dock in the same region as estradiol does but the orientations of the ferrocenecarboxylate complexes vary with the substitution on the phenyl ring with exception the 4-bromo and 4-iodo (Fig. 5). The 4-iodo and 4-bromo reside on the same position with the same orientation with respect to estradiol. Second, their binding affinities are lower than ER α -estradiol (-9.8 kcal/mol). Third, the phenyl rings with the substituent on the 4-position point in the same direction as the hydroxyl group on the hormone A ring (phenol) but, in contrast to estradiol, the halides are not engaged in hydrogen bonding with GLU 353 and ARG 394. The ferrocenecarboxylates dock inside the ligand binding pocket without the need of changing the protein conformation because they are smaller than estradiol. Thus, the introduction of these ferrocenecarboxylates in the ligand binding pocket (LBP) should not encounter any steric hindrance. In general the Cp rings in all the complexes get involved in hydrophobic interactions with the same set of amino acid residues the estradiol does. Noteworthy, the Cp rings are positioned in the region where the C and D rings of the estradiol reside. The Cp rings on the 4-F, 4-Br, 4-I and 4-pyrrol are above and below the C and D rings with some variation in the orientation of the Cp rings while 4-Cl is sideways and both Cp rings are below the C and D steroid rings. The 4-iodo and 4-bromophenyl derivatives get engaged in hydrogen bonding with the ferrocenecarboxylate O(carbonyl) and the HO(β -hydroxyl) of TRH 347. Interestingly, the 4-Br and 4-I have the same type of interactions inside the estradiol binding pocket (Fig. 5a) but their anti-proliferative properties are quite different. Thus, the docking positions and interactions of the species within the ligand binding domain (LBD) cannot explain the differences in the anti-proliferative properties of these complexes. It becomes clear that these species do not have anti-estrogenicity and the anti-proliferative activity is correlated to the cytotoxic effects of ferrocene. However, other factors such as size, charge distribution and polarizability of the halogen apparently may affect their anti-proliferative activity.

The second and third docking studies showed the following, Table 6. The fluoro derivative showed better docking affinity when the amino acids were flexible at 4.5 Å (-10.7 kcal/mol), see Fig. 6. That involved repositioned of LEU 346, LEU 387, LEU 391, ILE 424 and LEU 525 residues in the binding pocket. In this conformation, the complex does not get engaged in hydrogen bonding with the pocket amino acids. Although the binding affinity of the ER α -4-fluorophenyl ferrocenecarboxylate is better than the ER α - estradiol, it has no

effect on the anti-proliferative activity since this complex is inactive in both cell lines, MCF-7 and MCF-10A. Likewise, the chloro derivative has the most stable docking conformation at 5.0 Å (−10.5 kcal/mol). The amino acids surrounding this pocket as well as the ferrocenecarboxylate are repositioned in comparison with the docking conformation adopted in the fixed binding pocket (Fig. 5b). But, similar to the fluoro derivative, there is no hydrogen bonding with the binding pocket amino acids. For the iodo and bromo derivatives, the flexibility of the amino acids (at 4.5 and 5.0 Å) does not improve the stability of the protein-complex (docking) interactions and the lack of hydrogen bondings in these conformations suggest that the most stable docking conformations are the ones with the fixed amino acids in the binding pocket (first study) as discussed previously. Therefore, even though the ferrocenecarboxylates can fit into the estradiol binding pocket with binding affinities similar to estradiol, we cannot identify a particular interaction that can explain the good anti-proliferative activity of 4-bromophenyl ferrocenecarboxylate on MCF-7 over the remaining complexes. As stated previously, this study strongly suggests that the anti-proliferative activity of these ferrocenecarboxylate corresponds to the cytotoxic effects of the ferrocene group and not to anti-estrogenic effects.

3. Discussion

The synthesis of ferrocene esters was achieved by a well established procedure via the ferrocenecarbonyl chloride intermediate [10,19]. This synthetic route allowed us to prepare esters with different electrochemical properties. In particular, the incorporation of 4-(1*H*-pyrrol-1-yl) phenolate, allows us to study the role of redox active groups on ferrocene on MCF-7 and MCF-10A cell lines.

We have compared our results with Jaouen and co-workers electrochemical studies on ferrocifen complexes [15]. Ferrocifens, the tamoxifen derivatives of ferrocene, have exhibited high anti-proliferative activity on MCF-7 cell line [1,2,11–15]. In particular, the 1,1-bis(4'-hydroxyphenyl)-2-ferrocenyl-but-1-ene showed high anti-proliferative activity in MCF-7 with an IC₅₀ of 0.7 μM [15]. According to Jaouen et al., this high anti-proliferative activity is related to the ability of electron transfer from the phenol to the ferrocenyl to form the quinine methide radical species, which subsequently activates the redox process on the cell [15]. It is known that the main mechanism of action of ferrocene is by increasing activity of ROS formation [17,18]. Based on the above arguments, we could explain the high anti-proliferative activity of 4-(1*H*-pyrrol-yl)phenyl ferrocenecarboxylate on both cell lines as a result of having two redox active moieties which could be coupled increasing the production of ROS and stimulating the redox signaling process in the cell, combined with an increase in lipophilic character brought by the 4-(1*H*-pyrrol-yl)phenyl group. However, the fact that this species is cytotoxic in both cancer and normal cell lines, a non-desired effect, rule out the potential use of this complex as an anti-cancer drug. Thus, the overall effect of para substitution by the 1*H*-pyrrol-1-yl is detrimental.

Highly active ferrocene complexes with chalcone pendant group in MCF-7 breast cancer cell line have been reported. For instance, (E)-cyclopenta-2,4-dienyl(2-(3-(6-hydroxy-4-methoxybenzofuran-5-yl)-3-oxoprop-1-enyl)(cyclopentadienyl) iron exhibited IC₅₀ of 17.24 μM on MCF-7 [26]. Likewise, 1-*S*-(2,3,4,6-tetra-*O*-acetyl-1-*D*-deoxy-1-thio-*D*-

glucopyranose)-1-ferrocene carboxylate has an IC_{50} of 87 μM on HTB-129 human breast cancer but data on MCF-7 cell line is not available [27]. Furthermore, [bis-[(*p*-methoxybenzyl)cyclopentadienyl]iron(II)] (Ferrocene Y) was tested on MCF-7 and MDA-MB-231 [28]. These cell lines are used to study the estrogenicity (MCF-7) and the cytotoxicity (MDA-MB-231) of the ferrocenyl complexes. At low concentration (1 μM) Ferrocene Y has no proliferative effect and it is slightly cytotoxic at 10 μM (only 18% at this concentration) [28], demonstrating the lack of estrogenicity of this ferrocenyl. The 4-bromophenyl ferrocenecarboxylate has anti-proliferative activity ($IC_{50} = 9.2 \mu M$) that exceed those above-mentioned complexes. However, in a similar manner to Ferrocene Y, it has no anti-estrogenic effects. Therefore, this implies that 4-bromo ferrocenecarboxylate anti-proliferative activity comes from the cytotoxic effects of ferrocene. Conversely, although 4-bromo ferrocenecarboxylate has low toxic effects in normal cells, its anti-proliferative behavior does not exceed the anti-proliferative activity of 1,1-bis(4'-hydroxyphenyl)-2-ferrocenyl-but-1-ene which possess both, anti-estrogenic and cytotoxic effects [15].

Many halogenated (F, Cl, Br, I) organic compounds possess medicinal properties, in particular in marine natural products [29]. Interestingly, although the concentration of chloride in sea water is higher, the bromo containing compounds are more abundant. Many medicinal chemists have used the strategy of incorporating halogen in the drug's candidate to enhance bioactivity [30,31]. Halogens, with exception of fluorine, have unique properties which allow them to stabilize direct interaction between the drugs and their protein targets. They are useful for filling small hydrophobic cavities present in many protein targets and get engaged in halogen bonding with electronegative atoms, such as oxygen or nitrogen [31,32]. Due to the anisotropy of charge distribution around the halogen atom, halogens have regions which remain positively charged called sigma-holes, which are responsible for the directionality and stabilizing character of halogen bonding with other electronegative atoms (N, O) within the protein [31,32]. Upon examination of the docking studies, we cannot detect any halogen bonding with N or O of the nearby amino acid residues, with a distance less than the addition of their van der Waals radius. Therefore, we cannot propose the halogen bonding as a key interaction between the ER_{α} protein and the 4-X-phenyl ferrocenecarboxylates and that the binding of these species to ER_{α} is not responsible for their anti-proliferative activity. This also suggests that ER_{α} is not the target for the ferrocenecarboxylates.

4. Concluding remarks

Six ferrocene esters were synthesized and allowed us to study the role of the para substituent on the ferrocene's anti-proliferative activity and establish structure-activity relationship. In summary, the *in vitro* study revealed the following. The fluoro substituent is completely inactive perhaps due to its size and electronegativity. Iodo substituent is toxic in normal cell lines and has low anti-proliferative activity on cancer cells, thus ruling out its potential development as anti-cancer drug. The chloro analog has moderate and low anti-proliferative activities in MCF-7 and MCF-10A respectively. Particularly notable, the bromo substituent has the size, electronegativity and charge distribution to display low toxicity in normal cells and high anti-proliferative activity on breast cancer cell line with high expression of ER_{α} . In this regard, the anti-proliferative activity does not correspond to its anti-estrogenic properties

and it is correlated to the cytotoxic effects of ferrocene. It is clear that ER_{α} is not the target for this species. Therefore, it is likely that the bromo substituent plays an important role in determining the biological activity of the corresponding ferrocene complex.

5. Experimental section

5.1. General procedure

All reactions were performed under an atmosphere of dry nitrogen using Schlenk glassware or a glovebox, unless otherwise stated. Reaction vessels were flame dried under a stream of nitrogen, and anhydrous solvents were transferred by oven-dried syringes or cannula. CH_2Cl_2 was dried and deoxygenated by distillation over CaH_2 under nitrogen. Infrared spectra were recorded on a Bruker Vector-22 spectrometer with the samples as compressed KBr discs. The NMR spectra were obtained on a 500 MHz Bruker spectrometer. Electrochemical characterizations were carried out on a BAS CV-50W voltammetric analyzer. Elemental analyses were obtained from Atlantic Microlab Inc. Silica gel was heated at about 200 °C while a slow stream of dry nitrogen was passed through it prior to use. 4-(1*H*-Pyrrol-1-yl)phenol, 4-bromophenol, 4-fluorophenol, 4-chlorophenol, 4-iodophenol, phenol and ferrocenecarboxylic acid were purchased from Aldrich and used as received.

The breast adenocarcinoma cell line MCF-7 was purchased from American Type Culture Collection and was grown in a growth chamber at 37 °C and 95% Air/5% CO_2 . Growth medium for MCF7 was Eagle's Minimum Essential Media supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) antibiotic/antimycotic, non-essential amino acids and 0.01 mg/mL bovine insulin. MTT and Triton X-100 used for the cytotoxic assay were obtained from Sigma. All MTT manipulations were performed in a dark room.

5.2. Synthesis and characterization

5.2.1. General synthesis of ferrocene esters—In a 50 mL three necks round bottom flask and under nitrogen atmosphere, 1 mmol of ferrocenecarboxylic acid was dissolved in 15 mL of dry dichloromethane at room temperature. To this solution, 170–200 μ L of oxalyl chloride was added dropwise and stirred overnight. The solution changed from orange to dark red. The reaction mixture was filtered in a fritted funnel and the filtrate collected. In a separate 100 mL three necks round flask under nitrogen, 1.0 mmol of the 4-X-phenol and 1.0 mmol of pyridine were dissolved in 10–15 mL of dichloromethane. To this solution, the ferrocenecarbonyl chloride solution prepared previously was added dropwise. The solution was stirred 6–12 h in the dark, under nitrogen atmosphere, and at room temperature. Thin layer chromatography (TLC) was used to monitor the reaction. After the reaction was finished, the mixture was filtered in a fritted funnel with a pad of celite. The filtrate collected was washed with 3 $\times$ 5 mL 1 N HCl to remove pyridine and other by-products. The organic layer containing the compound was purified by column chromatography, using Silica gel and eluted with dichloromethane and isolated in 40–45% yield.

5.2.1.1. 4-(1H-Pyrrol-1-yl)phenyl ferrocenecarboxylate: ^1H NMR (500 MHz, CDCl_3): δ (ppm) 6.37 [d, 2H, $^3J(\text{H}, \text{H}) = 2.0$ Hz], 7.09 [d, 2H, $^3J(\text{H}, \text{H}) = 2.0$ Hz], 7.45 [d, 2H, $^3J(\text{H}, \text{H}) = 8.8$ Hz], 7.46 [d, 2H, $^3J(\text{H}, \text{H}) = 8.8$ Hz], 4.98 [overlapping doublets, 2H, $^3J(\text{H}, \text{H}) = 1.7$ Hz, AA'BB'; Cp], 4.53 [overlapping doublets, 2H, $^3J(\text{H}, \text{H}) = 1.7$ Hz; AA'BB'; Cp], 4.32 [s, 5H, Cp]. ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 170.6 (C=O), 151.1, 138.6, 123.0, 121.8, 119.8, 110.7, 72.3, 70.9, 70.2. IR (KBr, cm^{-1}): 3151, 3091, 1736, 1558, 1526, 1453, 1409, 1372, 1329, 1269, 1221, 1176, 1112, 1068, 1008, 914, 853, 813, 794, 726. Anal. Calc. for $\text{C}_{21}\text{H}_{17}\text{O}_2\text{NFe}$: C, 67.94; H, 4.67. Found: C, 67.67; H, 4.43.

5.2.1.2. 4-Bromophenyl ferrocenecarboxylate: ^1H NMR (500 MHz, CDCl_3): δ (ppm) 8.82 [d, 2H, $^3J(\text{H}, \text{H}) = 8.84$ Hz], 8.77 [d, 2H, $^3J(\text{H}, \text{H}) = 8.84$ Hz], 4.961 [overlapping doublets, 2H, $^3J(\text{H}, \text{H}) = 1.7$ Hz, AA'BB'; Cp], 4.52 [overlapping doublets, 2H, $^3J(\text{H}, \text{H}) = 1.7$ Hz; AA'BB'; Cp], 4.31 [s, 5H, Cp]. ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 170.3 (C=O), 150.2, 132.6, 123.7, 118.8, 72.3, 70.9, 70.2, 69.9. IR (KBr, cm^{-1}): 3110, 3090, 1737, 1483, 1452, 1407, 1395, 1372, 1271, 1227, 1213, 1193, 1160, 1107, 1093, 1060, 1009, 909, 864, 830, 823, 760. Anal. Calc. for $\text{C}_{17}\text{H}_{13}\text{O}_2\text{BrFe}$: C, 53.03; H, 3.40. Found: C, 52.75; H, 3.26.

5.2.1.3. Phenyl ferrocenecarboxylate: ^1H NMR (500 MHz, CDCl_3): δ (ppm) 5.00 (t, 2H, $^3J(\text{H}, \text{H}) = 1.7$ Hz, A_2B_2 ; Cp), 4.54 (t, 2H, $^3J(\text{H}, \text{H}) = 1.7$ Hz; A_2B_2 ; Cp), 4.34 (s, 5H, Cp), 6.367 (d, 2H, $^3J(\text{H}, \text{H}) = 2.0$), 7.21 (d, 2H, $^3J(\text{H}, \text{H}) = 8.2$), 7.29 (t, 2H, $^3J(\text{H}, \text{H}) = 7.6$), 7.46 (t, 2H, $^3J(\text{H}, \text{H}) = 7.6$). ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 170.2 (C=O), 150.9, 138.7, 124.2, 89.7, 72.3, 70.9, 70.2. FTIR (KBr, cm^{-1}): 3102, 1716, 1454, 1376, 1276, 1190, 1108, 746, 504. Anal. Calc. for $\text{C}_{17}\text{H}_{14}\text{O}_2\text{Fe}$: C, 66.70; H, 4.61. Found: C, 67.61; H, 4.63.

5.2.1.4. 4-Fluorophenyl ferrocenecarboxylate: ^1H NMR (500 MHz, CDCl_3): δ (ppm) 4.97 (t, 2H, $^3J(\text{H}, \text{H}) = 1.9$ Hz, A_2B_2 ; Cp), 4.52 (t, 2H, $^3J(\text{H}, \text{H}) = 1.9$ Hz; A_2B_2 ; Cp), 4.31 (s, 5H, Cp), 7.18–7.12 (m, 4H). ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 170.6 (C=O), 70.2, 72.2, 116.2, 116.4, 123.3, 146.9, 159.3, 161.3. FTIR (KBr, cm^{-1}): 3108, 3068, 1728, 1502, 1448, 1374, 1270, 1180, 1098, 912, 490, 484. Anal. Calc. for $\text{C}_{17}\text{H}_{13}\text{O}_2\text{FFe}$: C, 63.00; H, 4.05. Found: C, 63.03; H, 4.03.

5.2.1.5. 4-Chlorophenyl ferrocenecarboxylate: ^1H NMR (500 MHz, CDCl_3): δ (ppm) 4.96 (t, 2H, $^3J(\text{H}, \text{H}) = 1.8$ Hz, A_2B_2 ; Cp), 4.52 (t, 2H, $^3J(\text{H}, \text{H}) = 1.8$ Hz; A_2B_2 ; Cp), 4.30 (s, 5H, Cp), 7.14 (d, 2H, $^3J(\text{H}, \text{H}) = 8.8$), 7.49 (d, 2H, $^3J(\text{H}, \text{H}) = 8.8$). ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 170.4 (C=O), 70.2, 70.9, 72.3, 123.3, 129.7, 131.1, 149.6. FTIR (KBr, cm^{-1}): 3092, 1730, 1486, 1452, 1372, 1268, 1192, 1094, 908, 830, 502. Anal. Calc. for $\text{C}_{17}\text{H}_{13}\text{O}_2\text{ClFe}$: C, 59.95; H, 3.85. Found: C, 59.73; H, 3.92.

5.2.1.6. 4-Iodophenyl ferrocenecarboxylate: ^1H NMR (500 MHz, CDCl_3): δ (ppm) 4.52 (t, 2H, $^3J(\text{H}, \text{H}) = 1.9$ Hz, A_2B_2 ; Cp), 4.95 (t, 2H, $^3J(\text{H}, \text{H}) = 1.9$ Hz; A_2B_2 ; Cp), 4.30 (s, 5H, Cp), 6.96 (d, 2H, $^3J(\text{H}, \text{H}) = 8.5$), 7.73 (d, 2H, $^3J(\text{H}, \text{H}) = 8.5$). ^{13}C NMR (125 MHz, CDCl_3): δ (ppm) 170.2 (C=O), 69.8, 70.2, 70.9, 72.3, 89.7, 124.2, 138.7, 151.0. FTIR (KBr, cm^{-1}): 3084, 3080, 1736, 1714, 1482, 1454, 1270, 1190, 1164, 1094, 1004, 910, 824, 760, 534, 506. Anal. Calc. for $\text{C}_{13}\text{H}_{13}\text{O}_2\text{IFe}$: C, 47.26; H, 3.03. Found: C, 47.06; H, 2.94.

5.3. In vitro anti-proliferative studies

Biological activity was determined using the MTT assay originally described by Mossman [22,23] but using 10% Triton in isopropanol as a solvent for the MTT formazan crystals [22,23]. HT29 and MCF7 cells were maintained at 37 °C and 95% Air/5% CO₂ in a growth chamber. Cells were grown using McCoy's 5A (ATCC) complete medium, which had been supplemented with 10% (v/v) fetal bovine serum (ATCC) and 1% (v/v) antibiotic/antimycotic (Sigma). Asynchronously growing cells were seeded at 1.5×10^4 cells per well in 96-well plates containing 100 μ L of complete growth medium, and allowed to recover overnight. Various concentrations of the complexes (10–1300 μ M) dissolved in 5% DMSO/95% Medium were added to the wells (eight wells per concentration; experiments performed in quadruplicate plates). The complexes' solutions were prepared first by dissolving the corresponding ferrocene in DMSO and then Medium was added to a final composition of 5% DMSO/95% Medium. In addition to the cells treated with the ferrocenes, two controls experiments were run: one without any addition of solvent mixture (5% DMSO/95% Medium) and one adding 5% DMSO/95% Medium to the cells. Both control experiments behaved identical within the experimental error, showing that 5% of DMSO in the Medium did not render toxic to these types of cells. The cells were incubated for an additional 70 h. After this time, MTT dissolved in complete growth medium was added to each well to a final concentration of 1.0 mg/mL and incubated for two additional hours. After this period of time, all MTT containing medium was removed, cells were washed with cold PBS and dissolved with 200 μ L of a 10% (v/v) Triton X-100 solution in isopropanol. After complete dissolution of the formazan crystals, well absorbances were recorded in triplicates on a 340 ATTC Microplate Reader (SLT Lab Instruments) at 570 nm with background subtraction at 630 nm. Concentrations of compounds required to inhibit cell proliferation by 50% (IC₅₀) were calculated by fitting data to a four-parameter logistic plot by means of SigmaPlot software from SPSS.

5.4. Electrochemistry

Cyclic voltammetric experiments were performed in deoxygenated CH₃CN solution of ferrocene complexes with 1 M of NBu₄⁺PF₆⁻ as supporting electrolyte and ferrocene complex concentration of 2×10^{-3} M. The three electrodes used were platinum disk as the working electrode, Ag/AgCl as a reference electrode, and Pt wire as an auxiliary electrode. The working electrode was polished with 0.05 μ m alumina slurry for 1–2 min, and then rinsed with double-distilled and deionized water. This cleaning process is done before each cv experiment and a sweep between 0 and 2000 mV is performed on the electrolyte solution to detect any possible deposition of ferrocene on the electrode surface.

5.5. Docking studies

Docking studies between the ER α and the complex were performed using VINA program. The ER α dimeric structure (code:1A52 pdb) initially occupied by β -estradiol was selected but only half of it was used for the studies. The β -estradiol inside the binding pocket was replaced by ferrocenecarboxylates. The best ten structures were calculated to obtain the minimum energy conformation of the ligand–receptor by adjusting the following parameters. In the first docking study, rotation of the ester group relative to the Cp ring was flexible but

the amino acid residues within the binding cavity were fixed. This permits the ferrocene to accommodate within the confined space of the binding pocket with the lowest energy conformation. In the second docking study, rotation of the ester group relative to the Cp ring was flexible and the amino acid residues within 4.5 Å in the binding cavity were flexible. In the third docking study, rotation of the ester group relative to the Cp ring was flexible and the amino acid residues within 5.0 Å in the binding cavity were flexible. For the calculation, the parameter *exhaustiveness* was set to 15 and the program determines the ten most probable conformations. The conformation with the most exothermic binding affinity value (kcal/mol) is selected as the stable docking conformation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary material

CCDC 949002 and CCDC 949003 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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Fig. 1. Formation of ferrocenyl esters, R = phenyl. X = H, 1*H*-pyrrol-1-yl, fluoro, chloro, bromo, iodo.

Fig. 2.
Solid-state structure of 4-bromophenyl ferrocenecarboxylate. Only one of two chemically equivalent molecules is presented.

Fig. 3.
Solid-state structure of 4-chlorophenyl ferrocenecarboxylate. Only one of two chemically equivalent presented.

Fig. 4. Twist angle between the Cp ring and the carboxylate group (a) and the twist angles between the Cp plane and the aromatic ring (b).

Fig. 5.
Docking representations of 4-X-phenyl ferrocenecarboxylates with ER $_{\alpha}$ ligand binding domain. a) Br, I b) Br, Cl c) Br, F d) Br, pyrrol.

Fig. 6.
Docking representation of 4-fluorophenyl ferrocenecarboxylate with ER α ligand binding domain. Black lines represent the new position of the amino acid residues.

Table 1

Crystal data and structure refinement for 4-bromophenyl and 4-chlorophenyl ferrocenecarboxylate.

Empirical formula	C ₁₇ H ₁₃ Br Fe O ₂	C ₁₇ H ₁₃ Cl Fe O ₂
Formula weight	385.03	340.56
Temperature	100(2) K	100(2) K
Wavelength	0.71073 Å	0.71073 Å
Crystal system	Monoclinic	Monoclinic
Space group	<i>P2(1)/c</i>	<i>P2(1)/c</i>
Unit cell dimensions	<i>a</i> = 7.5400(4) Å <i>b</i> = 22.6220(14) Å <i>c</i> = 17.4291(10) Å β = 102.491(2)°	<i>a</i> = 7.4929(2) Å <i>b</i> = 22.6129(5) Å <i>c</i> = 17.3025(4) Å β = 102.0290(10)°
Volume	2902.5(3) Å ³	2867.30(12) Å ³
<i>Z</i> , <i>Z'</i>	8, 2	8, 2
Density (calculated)	1.762 g/m ³	1.578 g/cm ³
Absorption coefficient	3.792 mm ⁻¹	1.238 mm ⁻¹
Crystal size	0.20 × 0.10 × 0.10 mm ³	0.30 × 0.25 × 0.20 mm ³
Crystal color	Orange	Orange
Goodness-of-fit on F ²	1.079	1.023
Final <i>R</i> indices [<i>I</i> > 2σ(<i>I</i>)]	<i>R</i> 1 = 0.0291, w <i>R</i> 2 = 0.0660	<i>R</i> 1 = 0.0256, w <i>R</i> 2 = 0.0592
<i>R</i> indices (all data)	<i>R</i> 1 = 0.0372, w <i>R</i> 2 = 0.0704	<i>R</i> 1 = 0.0303, w <i>R</i> 2 = 0.0610

Table 2Selected Bond lengths [$\text{\AA}$] and angles [$^{\circ}$].

Molecule 1	Molecule 2		
4-Chlorophenyl ferrocenecarboxylate			
Bond lengths [Å]			
Fe-C _{av} (Cp)	2.046	2.043	
Fe-C _{av} (Cp) subt.	2.044	2.043	
Fe(1)–C(8)	2.0259(18)	Fe(2)–C(25)	2.0175(18)
Fe(1)–C(16)	2.0397(18)	Fe(2)–C(24)	2.0395(19)
Fe(1)–C(3)	2.0436(18)	Fe(2)–C(21)	2.0410(17)
Fe(1)–C(2)	2.0463(18)	Fe(2)–C(26)	2.0423(18)
Fe(1)–C(5)	2.0484(19)	Fe(2)–C(20)	2.0429(18)
Fe(1)–C(7)	2.0490(18)	Fe(2)–C(18)	2.0440(19)
Fe(1)–C(4)	2.0510(18)	Fe(2)–C(19)	2.0442(19)
Fe(1)–C(1)	2.0525(19)	Fe(2)–C(22)	2.0453(17)
Fe(1)–C(17)	2.0536(17)	Fe(2)–C(23)	2.0532(18)
Fe(1)–C(6)	2.0603(18)	Fe(2)–C(27)	2.0552(18)
O(1)–C(9)	1.201(2)	O(3)–C(28)	1.376(2)
O(2)–C(9)	1.368(2)	O(3)–C(29)	1.411(2)
O(2)–C(10)	1.413(2)	O(4)–C(28)	1.199(2)
Angles [°]			
O(1)–C(9)–O(2)	123.45(17)	O(4)–C(28)–O(3)	123.29(16)
O(1)–C(9)–C(8)	125.56(17)	O(4)–C(28)–C(25)	126.11(17)
O(2)–C(9)–C(8)	110.99(15)	O(3)–C(28)–C(25)	110.60(15)
Bent angle between Cp rings [°]			
0.54		1.25	
Twist angle between Cp and carboxylate [°]			
6.75		10.15	
Twist angle between Cp plane and the aromatic ring [°]			
87.58		78.06	
Cp stagger angle [°]			
2.95		1.19	
4-bromophenyl ferrocenecarboxylate			
Bond lengths [Å]			
Fe-C _{av} (Cp)	2.046	2.042	
Fe-C _{av} (Cp) substituted	2.044	2.040	
Fe(1)–C(6)	2.026(2)	Fe(2)–C(23)	2.015(2)
Fe(1)–C(7)	2.037(2)	Fe(2)–C(24)	2.037(3)
Fe(1)–C(1)	2.043(3)	Fe(2)–C(27)	2.039(3)
Fe(1)–C(2)	2.045(3)	Fe(2)–C(18))	2.040(2)
Fe(1)–C(4)	2.046(3)	Fe(2)–C(20)	2.042(3)

Molecule 1		Molecule 2	
4-Chlorophenyl ferrocenecarboxylate			
Fe(1)–C(3)	2.048(3)	Fe(2)–C(22)	2.043(3)
Fe(1)–C(5)	2.048(3)	Fe(2)–C(19)	2.043(3)
Fe(1)–C(10)	2.049(3)	Fe(2)–C(21)	2.044(3)
Fe(1)–C(8)	2.053(2)	Fe(2)–C(25)	2.052(3)
Fe(1)–C(9)	2.055(3)	Fe(2)–C(26)	2.056(3)
O(1)–C(11)	1.203(3)	O(3)–C(28)	1.202(3)
O(2)–C(11)	1.363(3)	O(4)–C(28)	1.377(3)
O(2)–C(12)	1.407(3)	O(4)–C(29)	1.407(3)
Angles [°]			
O(1)–C(11)–O(2)	123.2(2)	O(3)–C(28)–O(4)	122.9(2)
O(1)–C(11)–C(6)	125.8(2)	O(3)–C(28)–C(23)	126.4(2)
O(2)–C(11)–C(6)	111.0(2)	O(4)–C(28)–C(23)	110.7(2)
Bent angle between Cp rings [°]			
0.48		1.24	
Twist angle between Cp and carboxylate [°]			
7.97		9.03	
Twist angle between Cp plane and the aromatic ring [°]			
87.58		78.06	
Cp stagger angle [°]			
1.74		1.24	

Table 3

Cyclic Voltammetric Studies. Oxidation and reduction peaks for ferrocene derived complexes tested for cytotoxicity. Scan rate of 100 mV/s and platinum disk as the working electrode, Ag/AgCl as a reference electrode, and Pt wire as an auxiliary electrode.

	Oxidation potential (mV)	Reduction potential (mV)
Fc	304	131
FcCO ₂ -Ph	653	559
Fc-CO ₂ -Ph-4-F	566	488
Fc-CO ₂ -Ph-4-Cl	570	476
Fc-CO ₂ -Ph-4-Br	580	484
Fc-CO ₂ -Ph-4-I	589	502
Fc-Ph-4-Py	Fc _{ox} = 728/Py _{ox} = 1554	649

Electrochemical data presented on this table was obtained under pseudo-physiological conditions using 1:1 methanol/DMEM media with TBAPF₆.

*Acetonitrile/TBAPF₆ was used for Fc-Ph-4-Py instead of 1:1 methanol/DMEM media with TBAPF₆.

Table 4

Cytotoxicities of ferrocene ester complexes studied on MCF-7 breast cancer and MCF-10A normal breast cell lines at 72 h, as determined by MTT assay. IC₅₀ values are the average of four independent measurements with their standard deviations (). n/a = not active under the concentrations studied.

Complex	MCF-7 IC ₅₀ (μM)	MCF-10A IC ₅₀ (μM)
Fc- CO₂-Ph	Proliferative effect	No effect
Fc- CO₂-Ph-4-F	No effect	No effect
Fc- CO₂-Ph-4-Cl	33(±4)	500(±80)
Fc- CO₂-Ph-4-Br	9.2(±0.8)	353(±8)
Fc- CO₂-Ph-4-I	256(±20)	7(±1)
Fc-Ph-4-Py	1.4 (±0.1)	1.8 (±0.1)

Table 5

Amino acid residues surrounding the ferrocenecarboxylates (**Fc-CO₂-Ph-4-X**) in the ER_α ligand binding domain at a distance of 4 Å. In parenthesis the binding affinity in kcal/mol. Computational conditions: Fixed amino acids in the binding pocket. The binding affinity of estradiol in the binding pocket calculated using the crystallographic co-ordinates is −9.8 kcal/mol.

X = F (−9.3)	X = Cl (−9.0)	X = Br (−8.4)	X = I (−8.3)	X = pyrrol (−7.4)
Cps	Cps	Cps	Cps	Cps
MET 343	MET 343	MET 343	MET 343	THR 347
LEU 346	LEU 346	LEU 346	LEU 346	MET 421
PHE 404	THR 347	THR 347	THR 347	ILE 424
MET 421	MET 348	TRP 383	THR 383	PHE 425
		LEU 384	LEU 384	
		MET 421	MET 421	
ILE 424	ALA 350	ILE 424	ILE 424	GLY 521
PHE 425	TRP 383	PHE 425	PHE 425	HIS 524
GLY 521	PHE 404	LEU 428	PHE 425	LEU 525
HIS 524	MET 421	GLY 521	GLU 521	
LEU 525	ILE 424	HIS 524	HIS 524	
	GLY 521	LEU 525	LEU 525	
	HIS 524			
Phenyl ring	Phenyl ring	Phenyl ring	Phenyl ring	Phenyl ring
LEU 349	LEU 349	ALA 350	ALA 350	LEU 346
	ALA 350	GLU 353	GLU 353	LEU 349
GLU 353	GLU 353	MET 388	TRP 383	ALA 350
LEU 387	LEU 387	LEU 387	LEU 387	GLU 353
MET 388	MET 388	LEU 391	LEU 391	LEU 387
ARG 394	ARG 394	ARG 394	ARG 394	MET 388
PHE 404	PHE 404	PHE 404	PHE 404	LEU 391
		Hydrogen bond	Hydrogen bond	ARG 394
		FcCO(carbonyl)—HO(THR 347)	FcCO(carbonyl)—HO(THR 347)	PHE 404

Table 6

Amino acid residues surrounding the ferrocenecarboxylates in the ER $_{\alpha}$ ligand binding domain. In parenthesis the binding affinity in kcal/mol. Computational conditions; Flexible amino acids in the binding pocket in Å.

<u>X = F (−10.7)</u> <u>At 4.5 Å</u>	<u>X = Cl (−10.5)</u> <u>At 5.0 Å</u>	<u>X = Br (−6.4)</u> <u>At 5.0 Å</u>	<u>X = I (−8.4)</u> <u>At 5.0 Å</u>
Cps	Cps	Cps	Cps
TRP 383	MET 343	MET 388	LEU 387
LEU 384	LEU 346	LEU 391	MET 388
LEU 387	THR 347	PHE 404	LEU 391
MET 388	LEU 384	MET 421	PHE 404
LEU 391	MET 388	ILE 424	MET 421
PHE 404	LEU 391	PHE 425	ILE 424
MET 421	LEU 402	LEU 428	PHE 425
ILE 424	PHE 404	GLY 521	LEU 428
PHE 425	LEU 428	HIS 524	
LEU 428			
Phenyl ring	Phenyl ring	Phenyl ring	Phenyl ring
MET 343	ALA 350	MET 343	MET 343
LEU 346	GLY 521 (Ph-CO)	LEU 346	LEU 346
THR 347	HIS 524	THR 347	THR 347
ALA 350	LEU 525	ALA 350	ALA 350
GLY 521 (Ph-CO)	LEU 540	TRP 383	TRP 383
HIS 524		LEU 384	LEU 384
LEU 525		LEU 387	LEU 387
		GLY 521 (Ph-CO)	GLY 521 (Ph-CO)
LEU 540		LEU 525	LEU 525
		LEU 540	LEU 540
			VAL 534