Titanocene / cyclodextrin supramolecular systems: a theoretical approach

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Abstract

Background: Recently, various metallocenes were synthesized and analyzed by biological activity point of view (such as antiproliferative properties): ruthenocenes, cobaltoceniums, titanocenes, zirconocenes, vanadocenes, niobocenes, molibdocenes etc. Two main disadvantages of metallocenes are the poor hydrosolubility and the hydrolytic instability. These problems could be resolved in two ways: synthetically modifying the structure or finding new formulations with enhanced properties. The aqueous solubility of metallocenes with cytostatic activities could be enhanced by molecular encapsulation in cyclodextrins, as well as the hydrolytic instability of these compounds could be reduced.

Results: This study presents a theoretical approach on the nanoencapsulation of a series of titanocenes with cytotoxic activity in α-, β-, and γ-cyclodextrin. The HyperChem 5.11 package was used for building and molecular modelling of titanocene and cyclodextrin structures, as well as for titanocene/cyclodextrin complex optimization. For titanocene/cyclodextrin complex optimization experiments, the titanocene and cyclodextrin structures in minimal energy conformations were set up at various distances and positions between molecules (molecular mechanics functionality, MM+). The best interaction between titanocene structures and cyclodextrins was obtained in the case of β- and γ-cyclodextrin, having the hydrophobic moieties oriented to the secondary face of cyclodextrin. The hydrophobicity of titanocenes (logP) correlate with the titanocene-cyclodextrin interaction parameters, especially with the titanocene-cyclodextrin interaction energy; the compatible geometry and the interaction energy denote that the titanocene/β- and γ-cyclodextrin complex can be achieved. Valuable quantitative structure-activity relationships (QSARs) were also obtained in the titanocene class by using the same logP as the main parameter for the in vitro cytotoxic activity against HeLa, K562, and Fem-x cell lines.

Conclusions: According to our theoretical study, the titanocene/cyclodextrin inclusion compounds can be obtained (high interaction energy; the encapsulation is energetically favourable). Further, the most hydrophobic compounds are better encapsulated in β- and γ-cyclodextrin molecules and are more stable (from energetically point of view) in comparison with α-cyclodextrin case. This study suggests that the titanocene / β- and γ-cyclodextrin complexes (or synthetically modified cyclodextrins with higher water solubility) could be experimentally synthesized and could have enhanced cytotoxic activity and even lower toxicity.

Keywords: Metallocenes, Titanocenes, Cyclodextrins, Supramolecular systems, Molecular modelling, QSAR

Background

Cancer is a generic name comprises a great number of medical affections, having various locations and symptoms [1-5]. Even this disease is studied more than fifty years, the cause and action mechanisms are not completely elucidated [3,6,7]. Chemotherapy is widely used in order to cure this disease, by using various cytostatic or cytotoxic compounds: alkylating agents, antimetabolites, hormones, immunostimulating agents, antibiotics, alkaloids, all of them with higher toxicity [1,8].

Organometallic compounds is an important class used in chemotherapy and the main groups studied are metallocenes (compounds which contains two cyclopentadienyl anions bound to a metal centre in the oxidation state II), ruthenium-, osmium-, iridium half-sandwich complexes, rhenium organometallics, metal N-heterocyclic carbene complexes, metal carbonyl complexes, or miscellaneous organometallic compounds [9]. The actual trend in cancer
treatment is to replace some of the more toxic drugs such as cisplatin with less toxic compounds. Organometallic compounds are widely studied from cytotoxic point of view. Ferrocene was one of the first organometallic compounds from the first group evaluated for its anti-proliferative properties [9,10]. Ferrocene derivatives were obtained as antimalarial or cytostatic drugs and drug candidates [9]. Recently, similar metalloccenes were synthesized and analyzed by biological activity point of view: ruthenocenes, cobaltoceniums, titanocenes, zirconocenes, vanadocenes, niobocenes, molybdocenes etc. [9-23]. The titanocene compounds are promising such drugs, but the hydrolytic instability and slightly water solubility conduct to a lower cytotoxic activity (approximately ten fold lower than cisplatin). Further, in the titanocene series cytotoxic activity against HeLa, K562, and Fem-x cell lines increases with the overall hydrophobicity of compounds [16,17,22]. On the other hand, increasing the hydrophobicity of titanocenes conducts to a more lower water solubility and reducing the transport capacity in aqueous layers (even the transport capacity through lipid layers are increased) [16,17]. Despite of the resemblance of titanocene dichloride derivatives with cisplatin, seems that the mode of action as anti-cancerigene is different: binding to DNA and apoptosis of the cancer cell for the cisplatin and binding to DNA phosphate group, with additional interaction stabilizing the binding to DNA, for titanocenes. Two main problems of the titanocene dihalides are the poor hydrosolubility and the hydrolytic instability [22].

These problems could be resolved in two ways: synthetically modifying the titanocene structure (laborious, other physico-chemical and biological analyses needed) or finding new formulations with enhanced properties [11,13,15,19,24-27]. Natural or chemically modified cyclodextrins (cyclic oligosaccharides with hydrophobic inner cavities and hydrosolubilizing outer groups) are widely used for protection, enhancing water solubility, and controlled release properties of bioactive compounds [28-34]. The aqueous solubility of metalloccenes (i.e. titanocenes) with cytostatic or cytotoxic activities could be enhanced by molecular encapsulation in cyclodextrins, as well as the hydrolytic instability of these compounds could be reduced (by reducing the access of water molecules to the metalloccene halide reaction centre) [13,19,25,27].

This study presents a theoretical approach on the molecular encapsulation of a series of titanocenes with cytostatic activity in α-, β-, and γ-cyclodextrin, in order to obtain supramolecular systems with enhanced stability and bioavailability. Further, a quantitative structure-biological activity relationships (QSAR) studies were performed in order to evaluate the main parameters which influencing the in vitro cytostatic activity.

Results and discussion
Quantitative structure-activity relationships (QSARs)
Molecular modelling and conformational analysis of titanocenes were performed by using the default parameters of MM+ functionality from the HyperChem 5.11 package [35]. This method is appropriate especially for organic molecules, but in our organometallic series the “inner” metal did not have a major influence on the overall geometry of compounds. Titanocenes with cytotoxic activity indicate a reduced number of stable conformations, especially for structures with a lower flexible bonds (codes 01TC, 02TC, 08TC to 10TC); structures having dimethylvinyl-silyl-ethyl or trivinyl-silyl-ethyl moieties at the Si, or pyridinium-methyl moiety at the cyclopentadienyl rings have a higher number of stable conformers; the most stable conformations are partially superimposed, especially at the titanocene skeleton. The most stable conformations have all flexible substituents (γ, R, and R′ from the general structure, Table 1 and Scheme 1) oriented close to a pseudoplan formed by Ti and the gravity centres of the two cyclopentadiene rings (Figure 1) [see Additional file 1].

In order to evaluate the importance of titanocene structure/conformation to the overall cytotoxic activity, the most important steric, electronic, and hydrophobic descriptors were evaluated by using QSAR Properties functionality. Molecular van der Waals surface and volume, refractivity, and polarizability for titanocenes in minimum energy conformations were evaluated from sterical descriptor class (Table 2), but some intercorrelation exists, especially in the case of $S^{vdW}$ – $V^{vdW}$ and $Rf$ – Pol pairs (intercorrelation coefficient of 0.87 and 1.0, respectively, Table 3). This intercorrelation is poor for $S^{vdW}/V^{vdW}$ and $Rf/Pol$. Hydration energy has no correlation with descriptors from the other classes, but the transport parameter, logP, correlates with $Rf$ and Pol parameters with higher intercorrelation coefficients (inverse correlation of −0.85 and −0.87, respectively, Table 3).

Taking into account these intercorrelations, only $logP$, $Rf$, and $Pol$ reveals statistically significant quantitative structure – cytotoxic activity relationships (QSARs) [36,37], which could drive molecular changes in this titanocene class in order to obtain new compounds with higher biological activity. In the case of HeLa cell line, in vitro cytotoxic activity of titanocenes correlates with the $logP$ parameter (Eq. 1), having a correlation coefficient of 0.80, which is higher than in the other cases; the correlation coefficient for the case of K562 is little bit lower (Eq. 2), but statistically significant, as well as in the case of Fem-x cell line (Eq. 3). A significant correlation was also obtained if the polarizability, Pol, was used as independent parameter (Eq. 4); for example, an inverse correlation exists between cytotoxic activity against K562 cells (expressed as $pIC_{50}$) and polarizability ($r = 0.80$), but this parameter is intercorrelated with the
hydrophobic one. As a result, if the polarizability is lower, the hydrophobicity is increased and the transport, the interaction of titanocene with the hydrophobic cell membrane is favoured, and further the cytotoxic activity is increased. The predictive accuracy was evaluated by cross-validation method (leave-half-out method), all models having cross-validation correlation coefficients \( q^2_{cv} > 0.75 \) [see Additional file 1]. The predicted \( pA \) activities, calculated with equations Eq. 1 to Eq. 4, as well as the differences between experimental and predicted activities, are presented in Table 4 [see Additional file 1].

QSAR results (Table 4 and Eqs. 1–4) indicate very good predictions of cytotoxic activity against HeLa and Fem-x cell lines, especially in the case of compounds 01TC-11TC (\( |\Delta pA| < 0.11 \), except compound 09TC for HeLa cytotoxic activity; this compound contains Ge as the second metal atom, in comparison with the other titanocene compounds, which contain only Si instead). Higher differences were observed in the case of pyridinium-methyl derivatives which have no Si as the second metal atom; the presence of Si atom in the structure stabilizes the overall titanocene structure (compounds 23TC, 24TC, and 26TC). Good results were obtained for prediction of the cytotoxic activity against K562 cell lines for models obtained with \( \log P \) and \( Rf \) as structural parameters (Eqs. 2 and 4). The difference between experimental and predicted activity was lower than 0.2 in almost all titanocene cases (except compound 18TC, having trivinyl-silyl moiety and the highest \( Rf \) value) (Table 4). However, a correlation between predicted activities against K562 cell lines obtained with Eqs. 2 and 4 exists (\( r = 0.67 \)); this result is sustained by the \( \log P - Rf \) intercorrelation coefficient which are involved in Eqs. 2 and 4 (Table 3).

\[
\begin{align*}
\Delta pA_1 &= 3.40(\pm 0.17) + 0.69(\pm 0.17) \cdot \log Pi \quad (1) \\
n &= 11; \quad r = 0.80; \quad F = 16; \quad q^2_{cv} = 0.75 \\
pA_{21} &= 3.54(\pm 0.23) + 0.69(\pm 0.30) \cdot \log Pi \quad (2) \\
n &= 8; \quad r = 0.70; \quad F = 5.5; \quad q^2_{cv} = 0.88 \\
pA_{31} &= 3.63(\pm 0.12) + 0.39(\pm 0.16) \cdot \log Pi \quad (3) \\
n &= 7; \quad r = 0.74; \quad F = 5.9; \quad q^2_{cv} = 0.80 \\
pA_{22} &= 5.27(\pm 0.37) - 0.014(\pm 0.004) \cdot Rf \quad (4) \\
n &= 11; \quad r = 0.80; \quad F = 10.5; \quad q^2_{cv} = 0.75 
\end{align*}
\]

Geometry optimization of titanocene / cyclodextrin supramolecular systems

It is known that the cytotoxic activity of titanocene dichloride is different from cisplatin action (the last being more toxic, but with higher cytotoxic activity); titanocenes conduct to adduct with DNA and prevent the replication and/or transcription, resulting in cell death [22]. Thus, the transport of titanocene to the DNA is very important, but the lower water solubility

### Table 1 Titanocene structures (see Scheme 1) and in vitro cytotoxic activities

| No | Code | Structure | \( pA_1 \) | \( pA_2 \) | \( pA_3 \) |
|----|------|-----------|------------|------------|------------|
| 1  | 01TC | M: Si; X: CH\(_3\); Y: CH=CH\(_2\); R: all H; R': all CH\(_3\) | 4.10 | 4.20 | 3.87 |
| 2  | 02TC | M: Si; X: CH\(_3\); Y: H; R: all CH\(_3\); R': all CH\(_3\) | 3.96 | 4.23 | 3.93 |
| 3  | 03TC | M: Si; X: CH\(_3\); Y: (CH\(_3\))\(_2\)Si(CH=CH\(_2\))CH=CH\(_2\); R: all H; R': all CH\(_3\) | 3.72 | 3.81 | 3.70 |
| 4  | 08TC | M: Si; X: CH\(_3\); Y: CH\(_3\); R: all H; R': all CH\(_3\) | 3.87 | 4.18 | 4.02 |
| 5  | 09TC | M: Ge; X: CH\(_3\); Y: CH\(_3\); R: all H; R': all CH\(_3\) | 3.81 | 4.14 | 3.97 |
| 6  | 10TC | M: Si; X: CH\(_3\); Y: H; R: all CH\(_3\); R': all CH\(_3\) | 3.96 | 4.23 | 3.94 |
| 7  | 11TC | M: Si; X: CH\(_3\); Y: CH\(_3\); R: 3-CH\(_2\)(3-pyridinium); R': all CH\(_3\) | 3.93 | 4.06 | 4.00 |
| 8  | 18TC | M: Si; X: CH\(_3\); Y: (CH\(_3\))\(_2\)Si(CH=CH\(_2\))\(_2\); R: all H; R': all CH\(_3\) | 3.70 | 3.70 | 3.81 |
| 9  | 23TC | M, X, Y: none; R: 3-CH\(_3\)(3-pyridinium); R': 3-CH\(_3\)(3-pyridinium) | 3.70 | 3.70 | 3.81 |
| 10 | 24TC | M, X, Y: none; R: 3-CH\(_3\)(4-pyridinium); R': 3-CH\(_3\)(4-pyridinium) | 4.25 | 4.25 | 4.87 |
| 11 | 26TC | M, X, Y: none; R: 3-CH\(_3\)(4-pyridinium); R': 3-CH\(_3\)(4-pyridinium) | 4.97 | 4.97 | 4.97 |

\(^{a}\) cytotoxic activity was expressed as the logarithm of the inverse inhibitory concentration 50%, \( pIC_{50} = \log(1/IC_{50}) \); \( pA_1 \), \( pA_2 \), \( pA_3 \) were used for in vitro cytotoxic activity against HeLa, K562, and Fem-x cell lines, respectively.

**Scheme 1** General structure of titanocene compounds.
and instability (hydrolysis of chloride ligands) reduces the access to the target - biomacromolecules; on the other hand, if the hydrophobicity is increased (the QSAR study indicates a higher in vitro biological activity for more hydrophobic titanocenes), the titanocene transfer in aqueous layer is decreased (especially in the case of the in vivo experiments). Thus, the increase of the hydrophobicity in order to enhance the in vitro cytotoxic activity conducts to a less water solubility and a harder transport of the titanocenes to the target. The water solubility of hydrophobic molecules could be realized by molecular encapsulation in matrices such as cyclodextrins. Among the increasing of water solubility, the advantage of this procedure is to protect of easier hydrolysable titanocenes against degradation and controlled release to the target (i.e. DNA); further, the higher hydrophobicity of modified titanocenes conduct to a better interaction with the hydrophobic inner cavity of cyclodextrins.

Fully geometry optimization of titanocene / cyclodextrin supramolecular systems or docking of organometallic compounds in cyclodextrins [38,39] could provide information on the stability of complexes and suggest chemical modifications for new titanocenes with higher cytotoxic activity. In our theoretical experiments of fully geometry optimization of titanocene / cyclodextrin supramolecular systems in vacuum (the default HyperChem molecular mechanics MM+ force field was used), only the interaction of the hydrophobic moiety of titanocene with the inner cavity of cyclodextrins from the secondary face was efficient (higher stability of the complex). The main interactions which stabilize the titanocene / cyclodextrin complex in vacuum were bond stretching energy, the angle bending energy, the torsional energy, and the energy arising from van der Waals interactions of non-bonded pairs of atoms. Due to the fact that all titanocene compounds from the studied series are chemically similar molecules, we have an internal consistency from the force field point of view [15,40-49].

In the case of α-cyclodextrin, the interaction of titanocenes with the secondary face is poor, even with structures having thin hydrophobic chains (e.g. 03TC, 18TC, 23TC, 24TC, and 26TC; Figure 2) [see Additional file 1]; the worst interaction was observed for the case of titanocene oriented to the primary face of α-cyclodextrin or with the titanocene moiety in the front of both cyclodextrin rings. Theoretical interaction energy between titanocenes and α-cyclodextrin was in the range of 11.9 kcal/mole to 19.7 kcal/mole (Table 5). The best interactions of titanocenes with α-cyclodextrin were obtained in the case of structures having compounds monosubstituted at the Si (02TC and 10TC, $E_{\text{int}}$ 19.7 kcal/mole and 17.0 kcal/mole, respectively) or having flexible chain connected to this atom (03TC and 18TC, $E_{\text{int}}$ 18.0 kcal/mole and 17.0 kcal/mole, respectively). Lower interaction energies between titanocenes and α-cyclodextrin were obtained for compounds di-substituted at the Si atom with short chain moieties (methyl or vinyl) and for those substituted at the cyclopentadienyl rings with pyridinium-methyl moieties (Table 5). These low interaction energies are especially due to the geometric compatibilities, which are close to the limits and the interactions with the hydrophobic inner cavity of α-
Cyclohexane are reduced. This is the reason that the hydrophobicity (expressed as logP) is not important (r < 0.5) in relation with the titanocene / α-cyclodextrin interaction energy, while the hydration energy (Ehydro) weak correlates with this interaction energy (Eq. 5).

\[
E_{\text{int,αCD,i}} = 15.54(±0.57) \\
+ 0.356(±0.137)E_{\text{hydro,αCD,i}} \\
\]

n = 11; r = 0.656; F = 6.8

The superior homologues, β- and γ-cyclodextrins, are more proper for molecular encapsulation of the studied titanocenes (Figures 3 and 4). In all β- and γ-cyclodextrin complexes the mono- or disubstituted silyl moiety, the bulky moiety (dimethyl-vinyl-silyl or trivinylsilyl substituents) from the edge of the corresponding substituent, or the pyridinium-methyl substituent from the cyclopentadienyl ring are almost completely encapsulated in the cyclodextrin cavities [see Additional file 1]. In these cases the hydrophobicity of titanocenes (quantified by the logarithm of the 1-octanol/water partition coefficient) is important for the molecular encapsulation process and the interaction energy correlates with logP in a statistically significant way (Eq. 6 and Eq. 7). Thus, better interactions were obtained with the titanocenes having pyridinium-methyl substituents on the cyclopentadienyl rings (codes 2TC and 26TC), the interaction energy being 23.7-24.3 kcal/mole and 27.3-28.1 kcal/mole for β- and γ-cyclodextrin, respectively (Table 5). Higher interaction energies were obtained also for titanocenes having monosubstituted silyl moieties, 02TC and 10TC, especially in the case of γ-cyclodextrin complexes (28.4 kcal/mole and 28.6 kcal/mole, respectively; Table 5). Furthermore, a statistically significant correlation between titanocene / β- and γ-cyclodextrin interaction energies was observed (r = 0.82) [see Additional file 1].

\[
E_{\text{int,βCD,i}} = 16.24(±1.64) \\
+ 4.459(±1.687)\log P_{βCD,i} \\
\]

n = 11; r = 0.661; F = 7.0

\[
E_{\text{int,γCD,i}} = 21.46(±1.99) \\
+ 4.347(±2.047)\log P_{γCD,i} \\
\]

n = 11; r = 0.578; F = 4.1

The stability of the titanocene / cyclodextrin supramolecular system is observed from Figure 5 for β- and γ-cyclodextrin cases: the better stability is obtained in the case of γ-cyclodextrin, followed by β-cyclodextrin case, and finally for α-cyclodextrin complex after more than double number of iterations. This variation of the energy interaction reveals the possibility of forming the titanocene/cyclodextrin complexes and the stability of these supramolecular systems [see Additional files 1 and 2].

Conclusions

In our study the importance of the hydrophobic parameter (logP – the logarithm of the octanol/water partition coefficient) in both of in vitro cytotoxic activity against HeLa, K362, and Fem-x cell lines, as well as in the cyclodextrin nano encapsulation of the titanocene compounds were demonstrated.

Our theoretical studies demonstrate that the molecular encapsulation of titanocenes in natural cyclodextrins could resolves some of cytotoxic titanocene disadvantages: a more hydrophobic titanocene, which have higher cytotoxic activity (the in vitro cytotoxic activity against HeLa, K362, and Fem-x cell lines is increased with the logP of the titanocene compound; r > 0.7), is better encapsulated in cyclodextrins and could be transported through the aqueous layers (cyclodextrin inclusion compounds are water soluble) and protected against hydrolysis. Our theoretical study on the titanocene / cyclodextrin complexes indicate that the hydrophobic biologically active compounds (with higher cytotoxic activity) are better encapsulated in β- and γ-cyclodextrin; the highest

| Table 2 Values of the structural descriptors(a) for minimum energy conformations of titanocenes |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| No   | Code | SvdW (Å²) | VvdW (Å³) | Ehydro (kcal/mole) | logP | Rf   | Pol   |
|-----|-----|-----------|-----------|-------------------|------|------|-------|
| 1   | 01TC | 340       | 317       | 1.68              | 0.86 | 77.7 | 33.4  |
| 2   | 02TC | 359       | 351       | 3.57              | 0.80 | 87.7 | 37.3  |
| 3   | 03TC | 446       | 414       | 3.10              | 0.36 | 98.5 | 43.1  |
| 4   | 08TC | 327       | 302       | 2.97              | 0.73 | 74.0 | 31.8  |
| 5   | 09TC | 310       | 297       | 2.72              | 1.06 | 75.5 | 32.5  |
| 6   | 10TC | 506       | 958       | 3.55              | 0.80 | 87.7 | 37.3  |
| 7   | 11TC | 492       | 897       | 3.32              | 0.81 | 78.6 | 33.6  |
| 8   | 18TC | 470       | 438       | 0.70              | 0.62 | 106.0| 46.3  |
| 9   | 23TC | 305       | 273       | 3.02              | 0.51 | 74.6 | 30.4  |
| 10  | 24TC | 401       | 361       | 7.32              | 1.63 | 103.0| 41.7  |
| 11  | 26TC | 401       | 361       | 7.85              | 1.63 | 103.0| 41.7  |

(a) van der Waals molecular surface (SvdW, Å²), van der Waals molecular volume (VvdW, Å³), hydration energy (Ehydro, kcal/mole), logarithm of the octanol/water partition coefficient (logP), refractivity (Rf, Å³), and polarizability (Pol, Å³).
Titanocene / β- and γ-cyclodextrin complex interaction energies of ~24 kcal/mole and ~28 kcal/mole was obtained in vacuum for bis(pyridinium-methyl)-silyl derivatives, respectively. This study indicate that the titanocene compound can be controlled released to the target from the cyclodextrin complex; this complex allow to transfer the more hydrophobic titanocene through lipid layers, to increase the concentration of bioactive compound to the DNA phosphoester groups and further to form the titanocene-DNA adducts. Due to this process the inhibition of DNA transcription and/or replication appears (cytotoxicity).

According to our theoretical study, these titanocene/ cyclodextrin inclusion compounds can be obtained (the encapsulation process is energetically favourable for β- and γ-cyclodextrin complexes). Further, the most hydrophobic compounds are better encapsulated in β- and γ-cyclodextrin molecules and are more stable (from energetically point of view) in comparison with α-cyclodextrin case. This study suggests that the titanocene/β- and γ-cyclodextrin complexes (or synthetically modified cyclodextrins with higher water solubility) could be experimentally synthesized and could have enhanced cytotoxic activity and even lower toxicity.

### Methods

**Titanocene structure selection and cytotoxic activity**

Titanocenes with potential anticarcinogenic properties were recently synthesized by Gómez-Ruiz et al. [16,17] and Potter et al. [22] and have structural variability at the cyclopentadienyl moieties. All these new titanocene structures (eleven organometallic compounds) with cytotoxic activity against cervical carcinoma cell line

| No | Code | A1 | A2 | A3 | pA1 | pA2 | pA3 | ΔpA1 | ΔpA2 | ΔpA3 |
|----|------|----|----|----|-----|-----|-----|------|------|------|
| 1  | 01TC | 79.2±6.9 | 63.7±9.5 | 134.3±18.1 | 4.10 | 4.20 | 3.87 | 3.99 | 4.13 | 3.97 | 4.18 | 0.11 | 0.07 | 0.10 | 0.02 |
| 2  | 02TC | 108.6±8.6 | 59.4±8 | 116.3±8.7 | 3.96 | 4.23 | 3.93 | 3.95 | 4.09 | 3.94 | 4.04 | 0.01 | 0.14 | 0.01 | 0.19 |
| 3  | 03TC | 189±13.1 | 155.2±8.7 | 200 | 3.72 | 3.81 | 3.70 | 3.65 | 3.79 | 3.77 | 3.89 | 0.07 | 0.02 | 0.07 | 0.08 |
| 4  | 04TC | 135±6 | 66±6 | 96±4 | 3.87 | 4.18 | 4.02 | 3.90 | 4.04 | 3.92 | 4.23 | −0.03 | 0.14 | 0.11 | 0.05 |
| 5  | 05TC | 154±4 | 73±1 | 106±5 | 3.81 | 4.14 | 3.97 | 4.13 | 4.27 | 4.04 | 4.21 | −0.32 | −0.13 | −0.07 | −0.07 |
| 6  | 06TC | 109±9 | 59±8 | 116±9 | 3.96 | 4.23 | 3.94 | 3.95 | 4.09 | 3.94 | 4.04 | 0.01 | 0.14 | −0.00 | 0.19 |
| 7  | 07TC | 117±3 | 88±4 | 101±9 | 3.93 | 4.06 | 4.00 | 3.96 | 4.10 | 3.95 | 4.17 | −0.03 | −0.04 | 0.05 | −0.11 |
| 8  | 08TC | 200 | 200 | 200 | 3.70 | 3.70 | 3.83 | 3.97 | 3.79 | −0.13 | −0.27 | −0.09 |
| 9  | 09TC | 114.2±57 | 3.94 | 3.75 | 0.19 |
| 10 | 10TC | 55.9±16.2 | 4.25 | 4.53 | −0.28 |
| 11 | 11TC | 10.8±0.6 | 4.97 | 4.53 | 0.45 |

**Table 4 QSAR results for cytotoxic activity of titanocenes against HeLa, K562, and Fem-x cell lines (experimental activities – A and pA, predicted activities – pA(pred), and the differences between experimental and predicted activities, ΔpA)**

(a) Compounds No 1–3 are selected from reference [16], compounds No 4–8 from [17], and compounds No 9–11 from [22].

(b) A (IC50, μM) – the in vitro cytotoxic activity (±SD) of titanocenes selected from references [16,17,22]; pA – the logarithm of the inverse inhibitory concentration 50%, pIC50 = log1/IC50; pA(pred) – the predicted cytotoxic activity (as the logarithm of the predicted inverse inhibitory concentration 50%, pIC50(pred) = log(1/IC50(pred)); ΔpA – the difference between experimental and predicted activities (as the logarithm of the inverse inhibitory concentration 50%), ΔpA = pA – pA(pred).
HeLa, human myelogenous leukemia cell line K562, and human malignant carcinoma cell line Fem-x were considered in this theoretical study. Cytotoxic activity was expressed as the logarithm of the inverse inhibitory concentration 50%, \( p_{IC_{50}} = \log(1/I_{C_{50}}) \); \( pA_1 \), \( pA_2 \), \( pA_3 \) were used for cytotoxic activity against HeLa, K562, and Fem-x, respectively (Table 1).

Table 5 Energies (resulted from the MM+ molecular modeling and titanocene/cyclodextrin optimization experiments) for cyclodextrins (\( E_{CD} \), \( \alpha \), \( \beta \), and \( \gamma \)-cyclodextrin, codes aCD, bCD, and gCD), titanocenes (\( E_{TC} \), codes xTC, where \( x = 01-03, 08-11, 18, 23, 24, \) and \( 26 \)), the sum of titanocene and cyclodextrin energies, with no interaction (\( E_{TC+CD} \)), the energies of the TC-CD complex (\( E_{TC-CD \text{ complex}} \)), and the TC-CD interaction energies (\( E_{int} \)), determined as the difference between the TC+CD energy, with no interaction, and the energy of the TC-CD complex

| No | Code   | \( E_{CD} \) (kcal/mole) | \( E_{TC} \) (kcal/mole) | \( E_{TC+CD} \) (kcal/mole) | \( E_{TC-CD \text{ complex}} \) (kcal/mole) | \( E_{int} \) (kcal/mole) |
|----|--------|--------------------------|--------------------------|----------------------------|---------------------------------|--------------------------|
| 1  | 01TC_aCD | 572.70                    | 642.05                   | 626.0                      | 16.08                           |
| 2  | 02TC_aCD | 601.41                    | 670.76                   | 651.1                      | 19.67                           |
| 3  | 03TC_aCD | 567.76                    | 637.11                   | 619.1                      | 17.97                           |
| 4  | 08TC_aCD | 571.92                    | 641.27                   | 624.8                      | 16.43                           |
| 5  | 09TC_aCD | 686.73                    | 756.08                   | 740.3                      | 15.79                           |
| 6  | 10TC_aCD | 601.53 (69.35)            | 670.88                   | 653.8                      | 17.04                           |
| 7  | 11TC_aCD | 575.11                    | 644.46                   | 631.6                      | 12.88                           |
| 8  | 18TC_aCD | 571.08                    | 640.43                   | 623.4                      | 16.98                           |
| 9  | 23TC_aCD | 557.15                    | 626.50                   | 613.3                      | 13.19                           |
| 10 | 24TC_aCD | 566.55                    | 635.90                   | 624.0                      | 11.90                           |
| 11 | 26TC_aCD | 566.44                    | 635.79                   | 621.5                      | 14.28                           |
| 12 | 01TC_bCD | 572.70                    | 652.50                   | 637.9                      | 14.64                           |
| 13 | 02TC_bCD | 601.41                    | 681.21                   | 660.1                      | 21.11                           |
| 14 | 03TC_bCD | 567.76                    | 647.56                   | 630.8                      | 16.73                           |
| 15 | 08TC_bCD | 571.92                    | 651.72                   | 632.2                      | 19.51                           |
| 16 | 09TC_bCD | 686.73                    | 766.53                   | 746.0                      | 20.50                           |
| 17 | 10TC_bCD | 601.53 (79.80)            | 681.33                   | 660.8                      | 20.49                           |
| 18 | 11TC_bCD | 575.11                    | 654.91                   | 634.9                      | 19.97                           |
| 19 | 18TC_bCD | 571.08                    | 650.88                   | 629.1                      | 21.76                           |
| 20 | 23TC_bCD | 557.15                    | 636.95                   | 617.3                      | 19.68                           |
| 21 | 24TC_bCD | 566.55                    | 646.35                   | 622.0                      | 24.32                           |
| 22 | 26TC_bCD | 566.44                    | 646.24                   | 622.6                      | 23.65                           |
| 23 | 01TC_gCD | 572.70                    | 663.09                   | 640.2                      | 23.78                           |
| 24 | 02TC_gCD | 601.41                    | 692.70                   | 664.3                      | 28.36                           |
| 25 | 03TC_gCD | 567.76                    | 659.05                   | 635.6                      | 23.44                           |
| 26 | 08TC_gCD | 571.92                    | 663.21                   | 637.1                      | 26.09                           |
| 27 | 09TC_gCD | 686.73 (91.29)            | 778.02                   | 752.4                      | 25.61                           |
| 28 | 10TC_gCD | 601.53                    | 692.82                   | 664.2                      | 28.59                           |
| 29 | 11TC_gCD | 575.11                    | 666.40                   | 639.6                      | 26.83                           |
| 30 | 18TC_gCD | 571.08                    | 662.37                   | 640.9                      | 21.43                           |
| 31 | 23TC_gCD | 557.15                    | 648.44                   | 629.3                      | 19.10                           |
| 32 | 24TC_gCD | 566.55                    | 657.84                   | 629.7                      | 28.12                           |
| 33 | 26TC_gCD | 566.44                    | 657.73                   | 630.4                      | 27.34                           |

Molecular modelling
Molecular modelling of titanocene molecules as well as \( \alpha \), \( \beta \), and \( \gamma \)-cyclodextrins was performed by using the molecular mechanics MM+ functionality from the HyperChem 5.11. The MM+ molecular mechanics force field with a RMS of 0.005 kcal/mole, a number of maximum cycles to limit the search directions of fifteen...
times the number of atoms, and a Polak-Ribiere algorithm (a gradient method using one-dimensional searches) were used in the molecular modelling process. Bond dipole was used to calculate all nonbonded electrostatic interactions. In the MM+ calculations potential energy depends on bond lengths, bond angles, torsion angles, and nonbonded interactions (van der Waals forces, electrostatic interactions, and hydrogen bonds) [50,51].

Conformational analysis
In order to find the most stable conformation even for titanocenes or cyclodextrins, a conformational analysis by using Conformational Search functionality (HyperChem 5.11) was performed. In titanocene structures only some side chains have flexible bonds. On the other hand, the flexible bonds in cyclodextrins were only those corresponding to the hydroxymethyl from C5 position of glucopyranose unit; the flexible rings were all glucopyranose rings and the corresponding macrocyclic ring. The following conditions were set up for conformational search: variation of the flexible torsion angles ±60° ÷ ±180°, energy criterion for acceptance of the conformation 4 kcal/mole above minimum, all conformations with atomic distances lower than 0.5 Å, and differences between torsion angles lower than 15° were not considered as well as conformations with energy differences lower than 0.05 kcal/mole (duplicates); the maximum number of optimization and iterative calculations was 1000 and maximum 20 conformations were retained. The hydrogen atoms were neglected.

Geometry optimization of titanocene / cyclodextrin supramolecular systems
The geometry optimization of titanocene (the most stable conformations) / cyclodextrin (α-, β-, and γ-cyclodextrin) complexes was realized by using the molecular mechanics interactions of the host-guest molecules in vacuum. The titanocene and cyclodextrin structures in minimal energy conformations were set up at distances of ~8Å between the gravity centres of the host-guest molecules, and the titanocene structure was oriented with the hydrophobic side chain in front of the primary (A) or secondary (B) face of cyclodextrin (the principal axis corresponding to the biocompound or side chain moiety was perpendicular to the A or B plan of cyclodextrin). The complex was modeled in absence of water molecules by using the same MM+ functionality and the interaction was stopped when the RMS gradient was lower than 0.005 kcal/mole. The titanocene-cyclodextrin interaction energy was evaluated as the difference between the overall energies of these two molecules and the energy of the complex.

Structural parameters, correlations, and QSARs
The main molecular descriptors of titanocenes were evaluated by using QSAR Properties functionality from the HyperChem 5.11 package. The following descriptors were calculated and were used as structural parameters for obtaining correlations with titanocene-cyclodextrin interaction energy or quantitative structure-activity relationships (QSARs): van der Waals molecular surface ($S^{vdW}$, Å²; van der Waals surface area was carried out by an approximate developed by Still and co-workers [52,53]), van der Waals molecular volume ($V^{vdW}$, Å³; the grid method described by Bodor et al. [54] was used for van der Waals volume calculation. The QSAR Properties functionality uses the atomic radii of Gavezotti [55] for this method), hydration energy ($E_{hydr}$ kcal/mole; the method of calculation is developed by Ooi et al. [56]. The calculation is based on exposed surface area, and employs the surface area as computed by the approximate method,
weighted by atom type), logarithm of the octanol/water partition coefficient (logP; it was calculated by means of atomic contributions [57,58]. Metal atoms were excepted from calculation; this approximation did not significantly affect the final results due to the “hidden” positions of these metal atoms), refractivity (Rf, Å$^3$; the refractivity is estimated by the same method as logP, presented by Ghose and Crippen [59]), and polarizability (Pol, Å$^3$; this parameter was estimated according to an additive method of Miller [60], where the different increments are associated with different atom types).

Monolinear correlations (Eqs. 8a and 8b) in theoretical titanocene-cyclodextrin interaction experiments and QSARs were evaluated by using interaction energy between titanocene and cyclodextrin molecules ($E_{\text{int}}$) or the above mentioned pA cytotoxic activity and structural parameters ($P$).

$$E_{\text{int},i} = a_0 + b \cdot P_i;$$  \hspace{1cm} (8a)  

$$pA_i = a_0 + b \cdot P_i;$$  \hspace{1cm} (8b)
Additional files

Additional file 1: Molecular modelling and complex optimization of titanocene / cyclodextrin systems. Description: In this additional file the molecular modelling of titanocenes (A1), the quantitative structure-activity relationships (QSARs), which contain correlations between experimental and predicted activities, as well as the cross-validation data (leave-half-out method) for QSARs (A2), and complex optimization of titanocene/cyclodextrin complexes (starting positions and the most stable titanocene / cyclodextrin supramolecular systems; variation of the titanocene / cyclodextrin interaction energy in the complexation process; titanocene / cyclodextrin interaction energies correlations) (A3) are presented. These data supports all considerations presented in the manuscript.

Additional file 2: Titanocene / cyclodextrin interaction energies. Description: In this additional file the interaction energy versus the number of cycles data from MM+ optimization experiments for titanocene / cyclodextrin complexation process is presented; this supports all considerations presented in the manuscript.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
NGH and DIH carried out all theoretical experiments and prepared the final manuscript. AR and ZG help to discuss the theoretical results. All authors read and approved the final manuscript.

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References
1. Avendaño C, Menéndez JC. Medicinal chemistry of anticancer drugs. Amsterdam: Elsevier; 2008:1–3.
2. Quintans-Júnior L, Fagundes da Rocha R, Freitas Caregnato F, Fonseca Moreira K, Amaral da Silva F, Antunes de Souza Araújo A, Almeida dos Santos JP, Santos Melo M, Perigoent de Sousa D, Rigoldi Bonjardim L, Pens Gélain D. Antinociceptive Action and Redox Properties of Citronellol, an Essential Oil Present in Lemongrass. J Med Food 2011, 14:630–639. doi: 10.1089/jmf.2010.0125.
3. Clapp RW, Jacobs MM, Loechler EL. Environmental and occupational causes of cancer. Lowell: The Lowell Center for Sustainable Production, University of Massachusetts; 2007:24–28.
4. Vogelstein B, Kinzler KW. The Genetic Basis of Human Cancer. New York: McGraw-Hill, Medical Publication Division; 2002.
5. Gass G, Ott I, Metzler-Noite N. Organometallic anticancer compounds. J Med Chem 2011, 54:3–25. doi: 10.1021/jm100020w.
6. Veselkov Á, Plamont M-A, Cabestang C, Claffey J, Deckmann S, Hogan M, Müller-Bunz H, Strohfeldt K, Tacke M. Proliferative and anti-proliferative effects of titanium- and iron-based metalloocene anti-cancer drugs. J Organomet Chem 2009, 694:674–679. doi: 10.1016/j.organchem.2008.11.071.
7. Ashton PR, Babzai V, Clemente-Leon M, Colonna B, Credi A, Jayaraman N, Raymo FM, Stoddart JF, Venturi M. Ferrocene-containing carbohydrate dendrimers. Chem Eur J 2002, 8:673–684. doi: 10.1002/1521-3765(200201)8:3<673::AID-CEID673>3.0.CO;2-D.
8. Campbell KS, Dillon CT, Smith SJ, Harding MM. Radiotracer studies of the antitumor metalloocene molybdenocene dichloride with biomolecules. Polyhedron 2007, 26:456–459. doi: 10.1016/j.poly.2006.07.004.
9. Casas-Solvas JM, Ortiz-Salmeron E, Fernandez J, Garcia-Fuentes L, Santoyo-Gonzalez F, Vargas-Berenguel A. Ferrocene-β-cyclodextrin conjugates: synthesis, supramolecular behavior, and use as electrochemical sensors. Chem Eur J 2009, 15:8146–8162. doi: 10.1002/chem.200900593.
10. Chohan ZH, Sumra SH, Yousoufi MH, Hadda TB. Metal based biologically active compounds: Design, synthesis, and antibacterial/antifungal/cytotoxic properties of triazole-derived Schiff bases and their oxovanadium(IV) complexes. J Med Chem 2010, 45:2739–2747. doi: 10.1021/jm100253z.
11. Fey N. Organometallic molecular modelling - the computational chemistry of metalloccenes: a review. J Chem Technol Biotechnol 1999, 74:852–862.
12. Gómez-Ruiz S, Kaluderovic GN, Pol-Clorón D, Prashar S, Fajardo M, Zitak Z, Juranić ZD, Sabo TJ. Study of the cytotoxic activity of alkenyl-substituted ansa-titanocene complexes. J Inorg Chem Commun 2007, 10:748–752. doi: 10.1016/j.jinorgchemcomm.2007.03.016.
13. Gómez-Ruiz S, Kaluderovic GN, Prashar S, Pol-Clorón D, Fajardo M, Zitak Z, Sabo TJ, Juranić ZD. Cytotoxic studies of substituted titanocene and ansa-titanocene anticancer drugs. J Inorg Biochem 2008, 102:1558–1570. doi: 10.1016/j.injbio.2008.02.001.
14. Immel TA, Martin JT, Durr CJ, Groth U, Huhn T. Dimethyl titanium ylane: A valuable precursor for libraries of cytotoxic titanocene derivatives. J Inorg Biochem 2010, 104:863–867. doi: 10.1016/j.injbio.2010.04.003.
15. Liu Y, Zhong R-Q, Zhang H-Y, Song H-B. A unique tetramer of 4: 5 β-cyclodextrin-ferrocene in the solid state. Chem Commun 2005, 19:221–223. doi: 10.1039/b412202k.
16. Lu Z, Lu C, Ren X, Meng Q. New metalloocene-bridged cyclodextrin dimer: A stable derivative of the antitumor drug titanocene dichloride and its potent cytotoxic activity against human breast cancer (MCF-7) cells. J Organomet Chem 2006, 691:5859–5869. doi: 10.1016/j.orrochem.2006.09.052.
17. Napoli M, Sarumio C, Sirignano E, Popolo A, Pinto A, Longo P. Synthesis, characterization and cytotoxicity studies of methoxy alkyl substituted metallocenes. Eur J Med Chem 2011, 46:122–128. doi: 10.1016/j.ejmech.2010.10.021.
18. Potter GD, Baird MC, Cole SP. A new series of titanocene dichloride derivatives bearing cyclic alkylammonium groups: Assessment of their cytotoxic properties. J Organomet Chem 2007, 692:3508–3518. doi: 10.1016/j.organomet.2007.04.024.
19. Wallis D, Claffey J, Gleeson B, Hogan M, Müller-Bunz H, Tacke M. Novel zirconocene anticancer drugs? J Organomet Chem 2009, 694:828–833. doi: 10.1016/j.orrochem.2008.08.020.
20. Braga SS, Almeida Paz FA, Pillinger M, Sexas JD, Romão CC, Gonçalves IS. Structural studies of β-cyclodextrin and permethylated β-cyclodextrin inclusion compounds of cyclopentadienyl metal carbonyl complexes. Eur J Inorg Chem 2006, 2006:1662–1669. doi: 10.1002/ejic.200501006.
21. Morales Á, Weber BT, Melendez E. Spectroscopic and thermal characterization of the host-guest interactions between α, β, and γ-cyclodextrins and vanadocene dichloride. Appl Organomet Chem 2008, 22:440–450. doi: 10.1002/acid.1420.
22. Singh R, Bhati N, Madan J, Hirmarn SM. Characterization of cyclodextrin inclusion complexes - a review. J Pharm Sci Technol 2010, 2:171–183.
23. Sokolov VI. Cyclodextrin-metallocene inclusion complexes. In Supramolecular stereochemistry. Edited by Siegel JS. Dordrecht: Kluwer Academic Publishers; 1995:239–245. doi: 10.1007/978-94-011-0533-4_30.
24. Brewster ME, Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Deliv Rev 2007, 59:465–466. doi: 10.1016/j.addr.2007.05.012.
25. Hadadragi D, Hadaugă NG, Bandur GN, Isegerad H-D. Water content of flavonoid/cyclodextrin nanoparticles: relationship with the structural
descriptors of biologically active compounds. Food Chem 2012, 132:1651–1659. doi: 10.1016/j.foodchem.2011.06.004.

30. Hăldăruș D, Hăldăruș NG, Bandur GN, Riviș A, Costescu C, Ordoci V, Ardelean A. Berberis vulgaris extract/β-cyclodextrin nanoparticles: synthesis and characterization. Rev Chem 2010, 61:659–675.

31. Hăldăruș D, Hăldăruș NG, Butnaru G, Tatu C, Gruia A. Bioactive microparticles (10): thermal and oxidative stability of nicotine and its complex with β-cyclodextrin. J Incl Phenom Macrocycl Chem 2010, 68:155–164. doi: 10.1007/s10847-010-9761-0.

32. Hăldăruș NG, Hăldăruș D, Plănescu V, Tatu C, Ordoci VL, Bandur GN, Lupea AX. Bioactive nanoparticles (6). thermal stability of linoleic acid / α- and β-cyclodextrin complexes. Food Chem 2006, 99:500–508. doi: 10.1016/j.foodchem.2005.08.012.

33. Szejtli J, Szente L. Elimination of bitter, disgusting tastes of drugs and food by using cyclodextrins. J Am Chem Soc 1989, 111:7272–7274. doi: 10.1021/ja00354a007.

34. Bowen JP, Allinger NL. Molecular Mechanics: The Art and Science of Modelling molecular structures. Chichester: John Wiley & Sons, 2001.

35. Allinger NL, Zhou X, Bergsma J. Molecular mechanics and crystal structure analysis in drug discovery. J Chem Inf Comp Sci 2010, 50:920–925. doi: 10.1021/ji9003011.

36. Viswanadhan VN, Ghose AK, Revankar GR, Robbins RK. Atomic physicochemical parameters for three dimensional structure directed quantitative structure-activity relationships II: modeling hydrophobic interaction. J Comput Chem 1988, 9:90–90. doi: 10.1002/jcc.540090111.

37. Doman TN, Lollis TK, Bosnich B. Molecular modeling of inorganic compounds. Weinheim: Wiley-VCH, 2001.

38. Menger FM, Sherrod MJ. π-analysis. A method for the correlation of antibiotics. J Chem Inf Comp Sci 1992, 32:1721–1725. doi: 10.1021/ci010086n.

39. Sherrod MJ. Exploration of cycloalanto-oligoasarharide (cyclodecin) chemistry with molecular mechanics: docking calculations on the complexation of ferrocenes with cyclodextrins. Carbohydr Res 1989, 192:11–32. doi: 10.1016/0008-6215(89)81614-1.

40. Duchamp DJ. Molecular mechanics and crystal structure analysis in drug design. In Computer-Assisted Drug Design. Volume 112. Edited by Olston EC, Christofferson RE. Washington, DC: American Chemical Society; 1979:79–102. doi: 10.1012/bk-1979-0112.2003.

41. Ulrich B, Allinger NL. Molecular mechanics: Washington: American Chemical Society; 1982.

42. Leach AR. Molecular modeling. Principles and Applications. Harlow: Pearson Education Limited, 2001.

43. Hinchliffe A. Modelling molecular structures. Chichester: John Wiley & Sons, Ltd., 2000.

44. Bowen JP, Allinger NL. Molecular Mechanics: The Art and Science of Parameterization. In Reviews in Computational Chemistry. Volume 2. Edited by Lipkowitz KB, Boyd DB, Hoboken, NJ: John Wiley & Sons, Inc; 2007. doi: 10.1002/978047025793.3.

45. Allinger NL, Zhou X, Bergsma J. Molecular mechanics parameters. J Mol Struct (THEOCHEM) 1994, 312:69–83. doi: 10.1016/0166-2236(94)85008-0.

46. Jianu C. Synthesis of nonionic-anionic colloidal systems based on alkaline and ammonium β-nonylphenol polyethylenoxylene (n = 3--20) propionates / dodecylbenzenesulfonates with prospects for food hygiene. Chem Cent J 2012, 95. doi: 10.1186/1752-153X-6-95.

47. Doman TN, Landis CR, Bosnich B. Molecular mechanics force fields for linear metalloenes. J Am Chem Soc 1992, 114:7264–7272. doi: 10.1021/ja00349a042.

48. Doman TN, Lollis TK, Bosnich B. Molecular mechanics force fields for bent metalloenes of the type [M(Cp)(CO)]. J Am Chem Soc 1995, 117:1352–1368. doi: 10.1021/ja00190a020.

49. Timofeeva TV, Lii JH, Allinger NL. Molecular mechanics explanation of the metalloocene bent sandwich structure. J Am Chem Soc 1995, 117:452–7459. doi: 10.1021/ja00133a018.

50. Compa P, Hambly TW. Molecular Modeling of Inorganic Compounds. Weinheim: Wiley-VCH, 2001.

51. Yao O, Shoji T, Iwamoto Y, Kamei E. Consideration of an activity of the metalloocene catalyst by using molecular mechanics, molecular dynamics and Qsar. Comput Theor Polym Sci 1999, 6:41–46. doi: 10.1016/S1089-3156(98)00051-8.