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Synthesis, structure and biological activity of amide-functionalized titanocenyls: Improving their cytotoxic properties

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ABSTRACT

Nine amide-functionalized titanocenyls have been synthesized and characterized by spectroscopic and analytical methods and the solid state structure of $Cp(CpCO\text{-}NH\text{-}C_6H_4\text{-}OCF_3)TiCl_2$ was determined by single crystal X-ray diffraction. X-ray analysis of $Cp(CpCO\text{-}NH\text{-}C_6H_4\text{-}OCF_3)TiCl_2$ showed that titanium is in a pseudo tetrahedral geometry and contains a Ti–O(amide) coordination. In principle, Ti–O coordination should provide more hydrolytic stability to the corresponding titanocenyls than titanocene dichloride. The cytotoxic activities of these amide-functionalized titanocenyls on HT-29 colon cancer cell line were determined by MTT assay to elucidate structure-activity relationship. All complexes were more cytotoxic than titanocene dichloride and there is no correlation between the para substituents on the phenyl ring and their cytotoxicities.

1. Introduction

In 1979, Köpf and Köpf-Maier opened a new chapter in the field of medicinal chemistry with the discovery of the first metallocene-based organometallic anticancer agent, titanocene dichloride, Cp_2TiCl_2 [1]. The initial interest in Cp_2TiCl_2 (Fig. 1) was based on the fact that it possesses antineoplastic properties in cancer cell lines that are insensitive to cis-platin [1–8]. In addition, Cp_2TiCl_2 exhibited less toxic effects than cis-platin [1–8]. Titanocene dichloride reached clinical trials phase I and II but additional trials, necessary for a drug to become approved, were abandoned mainly due to its low hydrolytic stability [9–14].

Surprisingly, 29 years later the scientific community remains interested in titanocene dichloride due to the potential to modify its structure and make more potent and efficient anticancer agents. Our group has worked in the structure modification of titanocene by either replacing chloride with hydrophilic or biologically important ligands or functionalizing the Cp ring, in order to study structure-activity relationships [15–19]. The improvement of titanocene biological activity by these two approaches is not trivial. For instance, careful selection of the functional group and the synthetic route/methodology must be done to achieve the desired complex. We have selected amide functionalization on the Cp ring since, as observed by X-ray crystallography, it provides an additional Ti–O (C=O) bonding interaction which can provide higher stability in water, preventing hydrolysis of the titanocene [20]. In fact, the new titanocenyl complexes containing C=O and N–H groups, in principle could be engaged in hydrogen bonding with biologically important molecules and as a result an improvement in their anticancer activity. In addition, we have investigated titanocenyl amide complexes with different substituents on the para position of the phenyl ring. This allows us to study the role of electronegativity and polarity on the cytotoxic properties. Herein we report the synthesis, solid state structure and cytotoxic properties of titanocenyl amide complexes on colon cancer cell line HT-29.

2. Results and discussion

The synthesis of the amide-functionalized titanocene dichlorides was performed by a published procedure [20]. This involves the activation of the coordinated Cp ring by an acyl chloride, starting from fulvene. The reaction of the titanocene acyl chloride with the corresponding substituted aniline affords the amide-functionalized titanocenyl dichloride, Eq. (1). The complexes are air- and moisture-stable, soluble in DMSO and water and crystallize as methylene chloride solvate, as reported by Gansäuer and co-workers [20]. They were characterized by IR and NMR spectroscopies and elemental analysis.

The synthetic methodology developed by Gansäuer and co-workers allowed us to incorporate a variety of anilines with different substituents on the para position, Eq. (1). In this regard, nine

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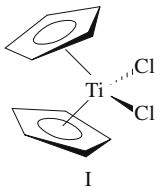

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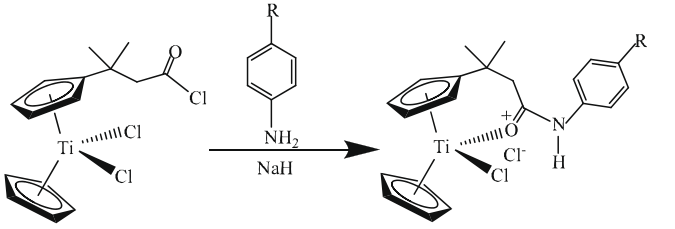
Fig. 1. Structure of titanocene dichloride.

titanocenyl complexes were prepared varying the electron-donating capability of the para substituents on the phenyl ring, in order to investigate structure-activity relationship.



(1)

The IR spectral data of these species showed characteristic carbonyl bands about 1600 cm^{-1} corresponding to the amide groups. In the ^1H NMR spectra, these titanocenyl showed N–H signals between 13 and 14 ppm, corroborating the presence of the amide protons and in the ^{13}C NMR data, the carbonyl carbons appeared at about 170 ppm. The ring signals of the substituted Cp ligand, in both ^1H and ^{13}C NMR spectra, are shifted downfield compared to the unsubstituted Cp ligands. All complexes showed a signal about 5.30 ppm in the ^1H spectra demonstrating the presence of CH_2Cl_2 in the samples. This signal corresponds to the molecule of CH_2Cl_2 co-crystallized with the titanocenyl complex.

To verify the chemical nature of these species, single X-ray diffraction study on $Cp(CpCO\text{-}NH\text{-}C_6H_4\text{-}OCF_3)TiCl_2$ was performed, see Fig. 2. Crystallographic details are included in Table 1 and the crystal structure has been deposited at the Cambridge Crystallographic Data Centre, CCDC 730724. This complex crystallizes as a solvate, containing one molecule of water and one molecule of $CHCl_3$ per titanocenyl dichloride, see Section 4. Although the *R* factor is high, the data has enough resolution to provide evidence of the most important structural features. As evidenced by X-ray analysis, the amide-functionalized titanocenyl dichloride contains a Ti–O(amide) coordination, analogous to previous reported amide–titanocene complexes [20]. The complex is cationic and as mentioned previously contains water molecules in the unit cell. The oxygen coordination by the carbonyl group should provide more hydrolytic stability and the presence of water in the lattice could be an indication of this. Furthermore, the presence of water could be the result of the cationic nature of the complex as well as the presence of the N–H moiety. In fact these titanocenyls, in general, are more stable in DMSO and aqueous solutions than Cp_2TiCl_2.

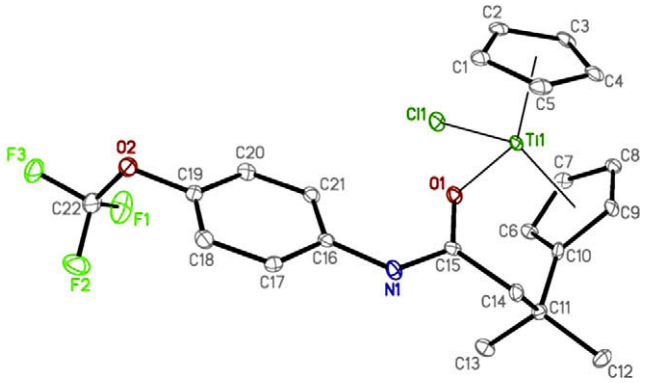


Fig. 2. Solid state structure of **7** drawn 50% thermal ellipsoids. Only cationic species presented. Free chloride ion (courterion) and water and $CHCl_3$ molecules omitted. Selected bond lengths (Å), Ti–O(1), 2.013(4), O(1)–C(15), 1.258(9), C(15)–N(1), 1.329(9), Ti(1)–Cl(1) 2.347(2), Ti(1)–C(3), 2.348(8), Ti(1)–C(9), 2.355(8), Ti(1)–C(8), 2.357(7), Ti(1)–C(5), 2.368(9), Ti(1)–C(7), 2.369(8), Ti(1)–C(4), 2.371(8), Ti(1)–C(1), 2.386(9), Ti(1)–C(6), 2.389(9), Ti(1)–C(2), 2.393(9), Ti(1)–C(10), 2.407(9). Selected angles (°), O(1)–Ti(1)–Cl(1), 92.70(14), C(15)–O(1)–Ti(1), 140.9(4), O(1)–C(15)–N(1), 121.6(6), O(1)–C(15)–C(14), 120.4(6), N(1)–C(15)–C(14), 118.0(6).

The cytotoxicities of the titanocenyl complexes on colon cancer HT-29 cell line were measured using a slightly modified MTT assay at 72 h [21,22]. Since titanocene dichloride has a longer intracellular activation period, the titanocenyls were tested at a time interval of 72 h. As a reference, the cytotoxic activity of Cp_2TiCl_2 was re-tested at 72 h and an IC_{50} value of 413 μM was obtained, as previously reported by our group [18]. In addition, two control experiments were run: 100% Medium and 5% DMSO/95% medium. Both control experiments behaved identically, demonstrating that 5% DMSO in the Medium does not have any cytotoxic effect on these cells.

The objective of this study is to determine the role of the substituents on the phenyl ring and the resulting anticancer properties on colon cancer. Table 2 presents the IC_{50} values on HT-29 colon cancer cell line as determined by MTT assay and Fig. 3 depicts the dose response curve for selected titanocenyls. Upon analysis of Table 2, we can observe that all the functionalized titanocenes are more cytotoxic than titanocene dichloride (IC_{50} = 413 μM) on colon cancer cell line HT-29. Thus, the amide functionalization increases the cytotoxic activity of the titanocenes when compared to Cp_2TiCl_2. Second, we can separate the titanocenyls in two groups: titanocenyl complexes with IC_{50} values over 100 μM and highly active titanocenyl complexes with IC_{50} values below 100 μM. While we were expecting a general trend between para substituent on the phenyl ring (polar or non-polar groups, electronegativity) and cytotoxicity, such correlation cannot be made. For instance, titanocenyls **1** and **8** are remarkably different on their substitution (Br vs. $(CH_2)_2CH_3$) but have very similar cytotoxicity, 11(2) and 12.8(3) μM, respectively.

Table 1
Crystal data and structure refinement for 7.

Empirical formula	$C_{23}H_{23}C_{l5}F_3NO_3Ti$
Formula weight	643.57
Temperature (K)	101(2)
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions	
a (Å)	8.865(3)
b (Å)	11.598(4)
c (Å)	13.698(4)
α (°)	90.300(4)
β (°)	90.293(4)
γ (°)	105.787(4)
V (Å^3)	1355.2(7)
Z	2
Density (calculated) (g/cm^3)	1.577
Absorption coefficient (mm^{-1})	0.856
Crystal size (mm^3)	0.40 × 0.35 × 0.30
Reflections collected	7586
Independent reflections [$R_{(int)}$]	2809 [0.0807]
Absorption correction	Multi-scan
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2809/0/327
Goodness-of-fit (GOF) on F^2	1.079
Final *R* indices [$I > 2\sigma(I)$]	R_1 = 0.0962, wR_2 = 0.2480
R indices (all data)	R_1 = 0.1030, wR_2 = 0.2549

Table 2
Cytotoxicities of the complexes studied on HT-29 colon cancer cell line, as determined by MTT assay. IC_{50} values based on quadruplicate experiments and std in parenthesis.

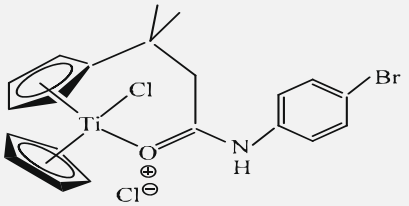
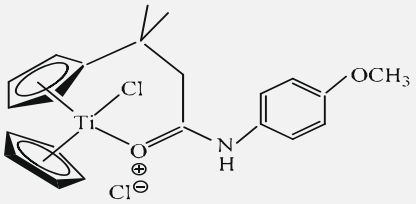
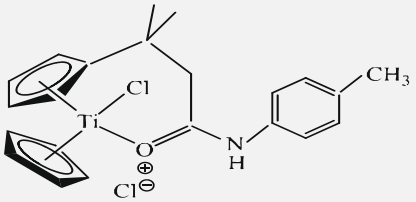
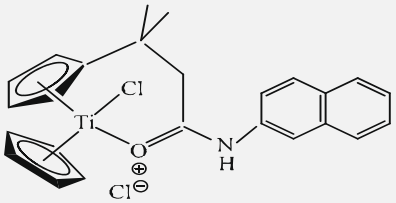
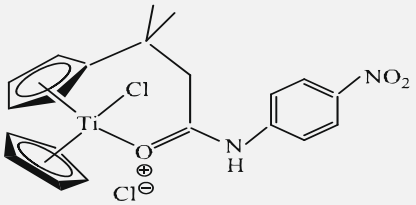
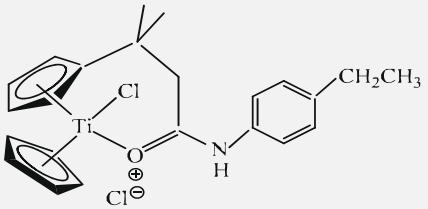
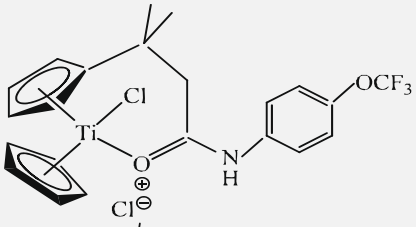
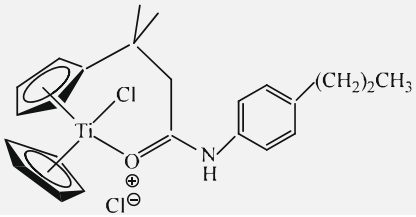
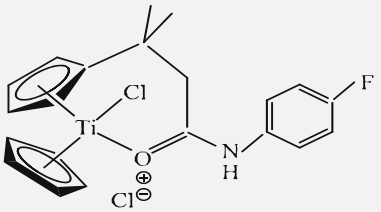
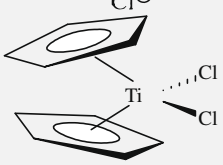
Complex	$IC_{50}\mu M$	Structure
1	11(2)	
2	143(5)	
3	109(3)	
4	65(1)	
5	23.4(1)	
6	152(6)	
7	8.9(4)	
8	12.8(3)	

Table 2 (*continued*)

Complex	$IC_{50}\mu M$	Structure
9	137.0(5)	
Cp_2TiCl_2	413.0(2)	

From the amide–functionalized titanocenes tested in this study, titanocenyl **7** showed to be the most active with an IC_{50} at least one order of magnitude lower than the rest of complexes, except for titanocenyls **1** and **8,** and two orders of magnitude lower than titanocene dichloride. This substantial improvement in the cytotoxicity (with IC_{50} in the micromolar range) deserves to be investigated in other cancer cell lines and pursue its potential applications as chemotherapeutic agent.

Recently, other amide-functionalized titanocenes have been reported with anticancer properties with cytotoxicities in the 10^{-5} M range in six cancer cell lines: BJAB (lymphoma), MelHo and A375 (melanoma), MCF-7 (breast carcinoma) and Nalm-6 and Jurkat (leukemia) [23]. However, their cytotoxic data was obtained by measuring apoptosis (AC_{50}) and not IC_{50} and these results cannot be compared with ours. On the other hand, Tacke and co-workers have developed a synthetic methodology to prepare a series of functionalized titanocenes with high cytotoxic activity, with IC_{50} values in the micromolar range [24–27]. Among them are bis-[*p*-methoxybenzyl)cyclopentadienyl] titanium dichloride (Titanocene Y) and bis-*N,N*-dimethylamino-2-(*N*-methylpyrrolyl)methylcyclopentadienyl titanium dichloride (Titanocene C). The most exciting and promising complex is Titanocene Y, which is active against a wide variety of cancer cell lines, in particular to colon cancer cell lines obtained from biopsied tumor using human tumor cloning assay, with an IC_{50} of 2.1 μM [26]. Our titanocenyl **7** falls in the category with cytotoxic activity similar to Titanocene Y on colon cancer with an IC_{50} of 8.9 μM.

3. Conclusion

We have prepared nine new amide titanocenyl dichlorides applying the synthetic methodology developed by Gansäuer and co-workers [20]. This allowed us to study how the structure of the complexes influences their cytotoxic activity. We managed to synthesize at least four titanocenes with IC_{50} values in the micromolar range. In particular, titanocenyl **7** showed to be superior to the rest of the titanocenyl complexes reported here. Our complexes have a group of structural properties that can be compared to those of Tacke and co-workers. First, in comparison to Titanocene **Y**, titanocenyl **7,** as well as the other reported complexes here, contains a phenyl group [26]. With regard to Titanocene **C**, our complexes possess Ti–O(ketonic) stabilization analogous to the Ti–N(amine) stabilization proposed by Tacke and co-workers [27].

Fluorinated derivatives of Titanocene Y [28] and their vanadocene analogs [29] have been synthesized and tested on LLLC-PK

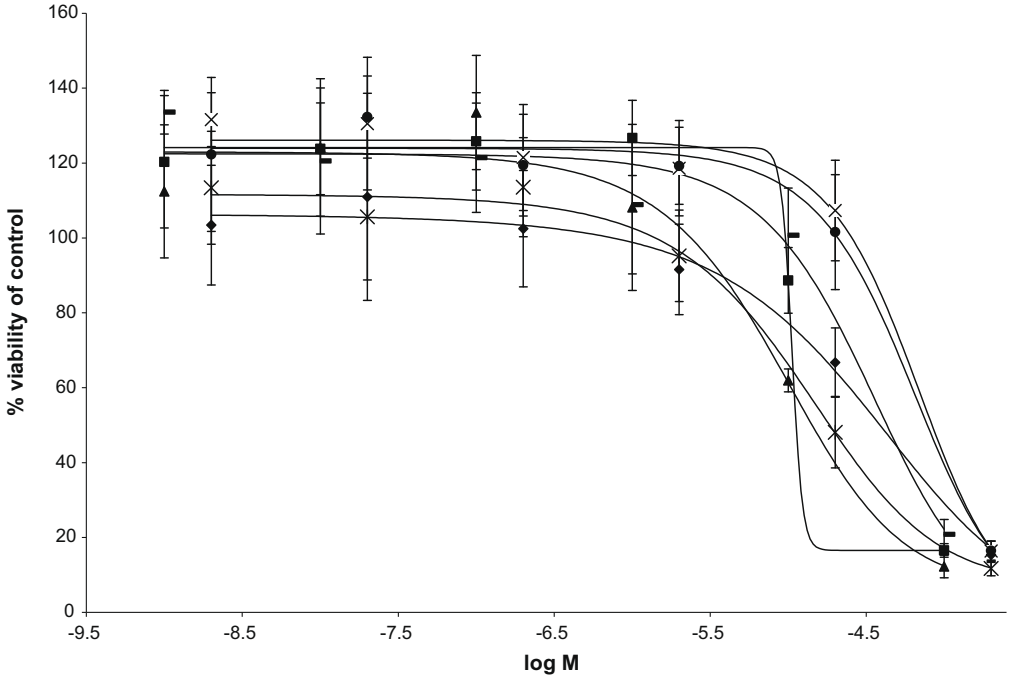


Fig. 3. Dose–response curves for selected amide-functionalized titanocenyls complexes against HT-29 colon cells at 72 h drug exposure. Legend: complex-**1** (squares), complex-**2** (crosshairs), complex-**3** (sunlamps), complex-**5** (linesegments), complex-**6** (diamonds), complex-**7** (triangles), and complex-**8** (asterisk). Experiments run in quadruplicates.

(long-lasting cells-pig kidney) and Caki-1 cell lines. The incorporation of fluorine on the phenyl ring improved substantially their cytotoxicity versus the parent complexes, Titanocene Y and Vanadocene Y [28,29]. In both cases, the enhancement as a result of fluorine substitution on the phenyl ring was clearly demonstrated. In particular for Vanadocene Y, the incorporation of OCF_3 on the phenyl ring demonstrated to produce a highly active cytotoxic complex on Caki-1 cell line, in analogous manner as titanocenyl **7.** Therefore, titanocenyl **7** have structural properties found by this group to be successful in the preparation of highly active anticancer agents. The cytotoxic activity of these amide titanocenyl complexes will be investigated in other cancer cell lines in the future.

4. Experimental details

All reactions were run 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. Tetrahydrofuran was dried and deoxygenated by distillation over K-benzophenone under nitrogen. Infrared spectra were obtained in dried KBr pellets. The NMR spectra were obtained on a DRX-500 MHz Bruker spectrometer. For the samples prepared on $CDCl_3$, chemical shifts were reference relative to $CHCl_3$ at 7.27 ppm (^{1}H NMR) and $CHCl_3$ at 77.00 ppm (^{13}C NMR) as internal standard. Analytical data were obtained from Atlantic Microlab Inc. Thionyl chloride ($\geqslant$99%), dichloromethane (anhydrous, $\geqslant$99.8%), methylene chloride (HPLC, Solvent), sodium hydride, and cyclopentadienyl titanium(IV) trichloride were purchased from Sigma–Aldrich. Lipophilic sephadex LH-20 was purchased from Sigma–Aldrich. Bio-Bead S-X3 (200-400 mesh) was purchased from *Bio-Rad Laboratories, Inc.*

Titanocene acylchloride and its precursor were prepared as described by Gansäuer and co-workers [20].

General procedure for the synthesis of complexes **1-9.**

4.1. Complex *1*

The titanocenyl carboxylate [20] (0.25 mmol, 77.4 mg) was dissolved in $SOCl_2$ (1.0 mL) and stirred for 2 h at r.t. Excess $SOCl_2$ was removed under high-vacuum and dried for 24 h. The precipitate was dissolved in CH_2Cl_2 (2.0 mL), added dropwise to a mixture of NaH (0.75 mmol, 18 mg) and 4-bromoaniline (0.25 mmol, 43 mg) in CH_2Cl_2 (2.0 mL) and stirred for another 20 h. After filtration through Celite the solvent was washed with a mixture of 1 N HCl and NaCl (1.0 g each 10 mL) (2×5.0 mL). The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude product was chromatographed on Bio-Bead S-X3 200–400 mesh (before use, swell the Bio-Bead S-X3 in CH_2Cl_2 for 24 h) eluting with methylene chloride to give 0.112 g (89% yield) of a pale red sold. The product was recrystallized in dichloromethane/hexane at -20 °C and a pale red solid could be obtained. ^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.54 (s, 1H; NH), 7.63 (d, ^3J = 8.5 Hz, 2H; AA'BB', $-C_6H_4-$), 7.52 (d, ^3J = 8.5 Hz, 2H; AA'BB', $-C_6H_4-$), 7.36 (m, 1H; Cp), 7.10 (m, 1H; Cp), 6.68 (s, 5H; Cp), 6.67 (m, 1H; Cp), 6.06 (m, 1H; Cp), 3.61 (d, ^2J = 12.5 Hz, 1H), 3.14 (d, ^2J = 11.0 Hz, 1H), 1.39 (s, 3H), 1.35 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 175.4, 151.2, 134.3, 132.3, 124.9, 123.8, 121.6, 120.8, 120.5, 116.6, 109.9, 48.1, 35.3, 30.6, 25.6. IR (KBr, cm^{-1}): 2963, 2867, 1615, 558, 1489, 1369, 1072, 1009, 825, 689. Anal. Calc. for $C_{21}H_{22}Cl_2BrNOTi^{*}1/2CH_2Cl_2$: C, 47.51; H, 4.27; N, 2.57. Found: C, 47.41; H, 4.64; N, 2.65%.

To prepare complexes **2–9**, the same procedure followed for the synthesis of **1** was used.

4.2. Complex *2*

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.55 (s, 1H; NH), 7.67 (d, ^3J = 9.0 Hz, 2H; AA'BB', $-C_6H_4-$), 6.92 (d, ^3J = 9.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.22 (m, 1H; Cp), 7.05 (m, 1H; Cp), 6.66 (m, 1H; Cp), 6.65 (s, 5H; Cp), 6.07 (m, 1H; Cp), 3.65 (d, ^2J = 14.5 Hz, 1H), 3.12

(d, ^2J = 13.5 Hz, 1H), 1.40 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 173.7, 158.5, 151.1, 128.4, 124.8, 123.7, 121.3, 120.2, 116.4, 114.3, 109.5, 55.5, 47.6, 35.1, 30.3, 25.8. IR (KBr, cm^{-1}): 2962, 2867, 2833, 1610, 1569, 1511, 1443, 1370, 1251, 1010, 828. Yield, 93%. Anal. Calc. for $C_{22}H_{25}Cl_2NO_2Ti^*1/3CH_2Cl_2$: C, 55.59; H, 5.37; N, 2.90. Found: C, 55.37; H, 5.80; N, 2.86%.

4.3. Complex **3**

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.97 (s, 1H; NH), 7.62 (d, ^3J = 8.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.19 (d, ^3J = 9.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.26 (m, 1H; Cp), 7.09 (m, 1H; Cp), 6.67 (s, 5H; Cp), 6.65 (m, 1H; Cp), 6.05 (m, 1H; Cp), 3.63 (d, ^2J = 13.6 Hz, 1H), 3.15 (d, ^2J = 13.0 Hz, 1H), 1.39 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 174.5, 151.1, 137.3, 132.7, 129.7, 124.8, 122.1, 121.5, 120.2, 116.7, 109.7, 48.1, 35.1, 30.5, 25.8, 21.1. IR (KBr, cm^{-1}): 2963, 2867, 1617, 1563, 1512, 1445, 1370, 1012, 821. Yield, 91%. Anal. Calc. for $C_{22}H_{25}Cl_2NOTi^*1/2CH_2Cl_2$: C, 56.21; H, 5.45; N, 2.91. Found: C, 55.96; H, 5.66; N, 2.96%.

4.4. Complex **4**

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.37 (s, 1H; NH), 8.12 (m, 1H; –naph–), 7.87 (m, 1H, –naph–), 7.52 (m, 3H; –naph–), 7.41 (m, 1H; –aph–), 7.52 (m, 1H; Cp), 7.14 (m, 1H; Cp), 6.70 (m, 1H; Cp), 6.19 (s, 5H; Cp), 5.82 (m, 1H; Cp), 3.64 (d, ^2J = 13.5 Hz, 1H), 3.54 (d, ^2J = 12.0 Hz, 1H), 1.47 (s, 3H), 1.31 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 177.1, 149.3, 134.0, 130.7, 128.8, 128.4, 128.2, 127.3, 126.7, 126.3, 125.1, 123.8, 123.3, 121.3, 118.3, 117.5, 45.0, 34.6, 28.7, 27.9. IR (KBr, cm^{-1}): 2963, 2865, 1634, 1583, 1555, 1444, 1368, 1015, 827, 786. Yield, 88%. Anal. Calc. for $C_{25}H_{25}Cl_2NOTi^*1/3CH_2Cl_2$: C, 60.69; H, 5.16; N, 2.79. Found: C, 60.79; H, 5.41; N, 2.69%.

4.5. Complex **5**

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.98 (s, 1H; NH), 8.28 (d, ^3J = 9.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.95 (d, ^3J = 9.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.21 (m, 1H; Cp), 7.11 (m, 1H; Cp), 6.70 (s, 5H; Cp), 6.70 (m, 1H; Cp), 6.12 (m, 1H; Cp), 3.70 (d, ^2J = 13.5 Hz, 1H), 3.10 (d, ^2J = 13.0 Hz, 1H), 1.38 (s, 3H), 1.27 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 177.1, 151.2, 145.9, 140.6, 124.9, 124.8, 122.6, 121.7, 116.5, 109.7, 48.5, 35.4, 29.7, 25.7. IR (KBr, cm^{-1}): 2922, 2851, 1607, 1557, 1510, 1341, 1012, 856, 828, 728. Yield, 75%. Anal. Calc. for $C_{21}H_{22}Cl_2N_2O_3Ti^*1/2CH_2Cl_2$: C, 50.59; H, 4.54; N, 5.49. Found: C, 50.39; H, 4.65; N, 5.30%.

4.6. Complex **6**

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.37 (s, 1H; NH), 7.62 (d, ^3J = 8.5 Hz, 2H; AA'BB', $-C_6H_4-$), 7.22 (d, ^3J = 8.5 Hz, 2H; AA'BB', $-C_6H_4-$), 7.11 (m, 1H; Cp), 7.07 (m, 1H; Cp), 6.65 (s, 5H; Cp), 6.65 (m, 1H; Cp), 6.05 (m, 1H; Cp), 3.64 (d, ^2J = 14.0 Hz, 1H), 3.08 (d, ^2J = 14.0 Hz, 1H), 2.65 (q, ^3J = 8.5 Hz, 2H; $-CH_2CH_3$), 1.41 (s, 3H), 1.38 (s, 3H), 1.21(t, ^3J = 8.5 Hz, 2H; $-CH_2CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 174.3, 151.2, 143.6, 132.9, 128.5, 124.8, 122.2, 121.5, 120.2, 48.0, 35.1, 30.4, 29.7, 25.7, 15.4. IR (KBr, cm^{-1}): 2962, 2925, 2854, 1620, 1567, 1513, 1445, 1412, 1370, 1278, 1009, 827. Yield, 95%. Anal. Calc. for $C_{23}H_{27}Cl_2NOTi^*1/8CH_2Cl_2$: C, 60.11; H, 5.95; N, 3.03. Found: C, 60.06; H, 6.20; N, 3.24%.

4.7. Complex **7**

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.92 (s, 1H; NH), 7.81 (d, ^3J = 9.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.27 (d, ^3J = 9.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.29 (m, 1H; Cp), 7.05 (m, 1H; Cp), 6.65 (s, 5H; Cp), 6.70 (m, 1H; Cp), 6.12 (m, 1H; Cp), 3.71 (d, ^2J = 14.0 Hz, 1H), 3.01 (d, ^2J = 14.0 Hz, 1H), 1.40 (s, 3H), 1.36 (s, 3H), ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 175.5, 151.2, 147.7, 133.8, 124.9, 123.6, 121.7, 121.6, 121.9, 120.5, 119.3, 116.6, 109.9, 48.1, 35.3, 30.5, 29.7, 25.5. IR (KBr, cm^{-1}): 2924, 2852, 1613, 1568, 1509, 1446, 1419, 1372, 1259, 1221, 1200, 1163, 1011, 829. Yield, 96%. Anal. Calc. for $C_{22}H_{22}Cl_2F_3NO_2Ti^*1/4CH_2Cl_2$: C, 50.47; H, 4.28; N, 2.64. Found: C, 50.47; H, 4.08; N, 2.42%.

4.8. Complex **8**

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.42 (s, 1H; NH), 7.62 (d, ^3J = 8.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.23 (d, ^3J = 8.0 Hz, 2H; AA'BB', $-C_6H_4-$), 7.20 (m, 1H; Cp), 7.05 (m, 1H; Cp), 6.63 (s, 5H; Cp), 6.67 (m, 1H; Cp), 6.06 (m, 1H; Cp), 3.65 (d, ^2J = 14.0 Hz, 1H), 3.05 (d, ^2J = 14.0 Hz, 1H), 2.55 (t, ^3J = 8.5 Hz, 2H; $-CH_2CH_2CH_3$), 1.65 (m, 2H; $-CH_2CH_2CH_3$), 1.34 (s, 3H), 1.25 (s, 3H), 0.95(t, ^3J = 8.5 Hz, 3H; $-CH_2CH_3$). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 174.4, 151.1, 142.1, 132.9, 129.1, 124.8, 122.1, 121.5, 120.2, 48.0, 37.6, 35.1, 29.7, 25.7, 24.4, 13.7. IR (KBr, cm^{-1}): 2959, 2925, 2869, 1605, 1565, 1512, 1445, 1411, 1370, 1282, 1010, 826. Yield, 90%. Anal. Calc. for $C_{24}H_{29}Cl_2NOTi^*1/4CH_2Cl_2$: C, 59.86; H, 6.12; N, 2.88. Found: C, 59.43; H, 6.22; N, 2.86%.

4.9. Complex **9**

^{1}H NMR (500 MHz, $CDCl_3$), δ (ppm): 13.53 (s, 1H; NH), 7.72 (m, 2H; AA'BB', $-C_6H_4-$), 7.11 (m, 2H; AA'BB', $-C_6H_4-$), 7.20 (m, 1H; Cp), 7.08 (m, 1H; Cp), 6.65 (s, 5H; Cp), 6.67 (m, 1H; Cp), 6.07 (m, 1H; Cp), 3.62 (d, ^2J = 14.0 Hz, 1H), 3.10 (d, ^2J = 14.0 Hz, 1H), 1.38 (s, 3H), 1.27 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$), δ (ppm): 174.9, 162.3, 160.3, 151.1, 131.3, 131.2, 125.0, 124.2, 124.1, 121.5, 120.4, 116.7, 116.2, 115.9, 109.6, 47.8, 35.2, 30.4, 25.7. IR (KBr, cm^{-1}): 3056, 2924, 2852, 1630, 1569, 1508, 1447, 1404, 1370, 1234, 1212, 1159, 1011, 830, 790, 730. Yield, 91%. Anal. Calc. for $C_{21}H_{22}Cl_2FNOTi^*1/3CH_2Cl_2$: C, 54.60; H, 4.87; N, 2.98. Found: C, 54.75; H, 4.98; N, 2.60%.

4.10. Crystallographic studies

Suitable crystals for X-ray diffraction studies were obtained from a dilute chloroform solution of **7** inside an NMR and upon standing at room temperature for a week. A block type orange crystal with 0.40 × 0.35 × 0.30 mm in size was mounted on a cryo-loop with Paratone® oil. Data was collected in a nitrogen gas stream at 101(2) K on a Bruker Smart system. Data collection was 95.8% complete to 24° in θ. The data was integrated using the Bruker SAINT software program. The structure was solved by direct methods and all non-hydrogen atoms were refined anisotropically by full-matrix least-squares (SHELXL-97).

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Appendix A. Supplementary material

CCDC 730724 contains the supplementary crystallographic data for **7**. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2009.09.016.

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