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## Accepted Article

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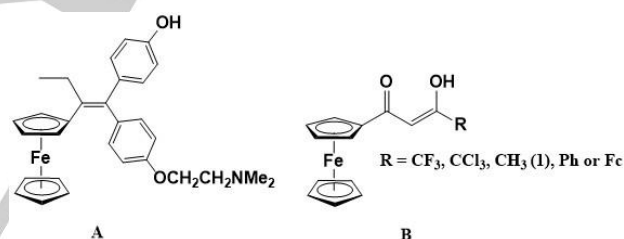
# $\beta$ -diketonate versus $\beta$ -ketoiminate: the importance of a ferrocenyl moiety on improving the anticancer potency

Matthew Allison,<sup>[a]</sup> Daniel Wilson,<sup>[a]</sup> Christopher M. Pask,<sup>[a]</sup> Patrick C. McGowan<sup>[a]\*</sup> and Rianne M. Lord<sup>[b,c]\*</sup>

**Abstract:** Herein we present a library of fully characterized  $\beta$ -diketonate and  $\beta$ -ketoiminate compounds which are functionalized with a ferrocenyl moiety. Their cytotoxic potential has been determined by screening against human breast adenocarcinomas (MCF-7 and MDA-MB-231), human colorectal carcinoma p53-wildtype (HCT116 p53<sup>wt</sup>) and normal human prostate (PNT2). The ferrocenyl  $\beta$ -diketonate compounds are > 18x more cytotoxic than the ferrocenyl  $\beta$ -ketoiminate analogues. Against MCF-7, the compounds functionalized in the *meta* position are up to 9x more cytotoxic than when functionalized in the *para* position. The ferrocenyl  $\beta$ -diketonate compounds have increased selectivity towards MCF-7 and MDA-MB-231 when compared to carboplatin, with several complexes having selectivity index (SI) values which are > 9x (MCF-7) and > 6x (MDA-MB-231). The stability of these compounds in dimethylsulfoxide (DMSO) and dimethylformamide (DMF) has been assessed by NMR spectroscopy and mass spectrometry studies, and show no oxidation of the iron center from +2 to +3. Cytotoxicity screening was performed in both DMSO and DMF, highlighting no significant differences difference in their potency.

Cisplatin, *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>],<sup>[1]</sup> is the most well-known metal-based compound which is used widely in the clinic for the treatment of cancer.<sup>[2,3]</sup> Since its success, there have been a variety of platinum-based therapeutics which have been designed and tested,<sup>[4,5]</sup> however, alternatives are being developed due to tumors developing platinum resistance, which have rendered the platinum-based drugs ineffective. Due to the inability of the platinum compounds to target the cancerous cells over normal cells, high levels of toxicity are observed, leading to severe side-effects such as nephrotoxicity.<sup>[6]</sup> There has been a surge in research towards different metal-based therapeutics,<sup>[7–11]</sup> including organometallic compounds. Tamoxifen compounds

which are functionalized with ferrocenyl, are known as ferrocifens, and are amongst the earliest organometallic selective estrogen receptor modulators (SERM).<sup>[12,13]</sup> Ferrocenyl hydroxytamoxifen (Fc-OH-Tam, **Figure 1A**)<sup>[14]</sup> is one of the leading compounds of this class, and possesses anti-proliferation against both hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cells. This compound was shown to induce strong senescence in MDA-MB-231 cells and exhibits low apoptosis.<sup>[15]</sup> Additionally, Fc-OH-Tam, has been shown to significantly inhibit *in vivo* growth of MDA-MB-231 xenografted tumors in mice when formulated in lipid nanocapsules (LNCs).<sup>[16,17]</sup> The dual effect of targeting both MCF-7 and MDA-MB-231 is very significant, since the organic compound hydroxytamoxifen (OH-Tam) shows activity against MCF-7 only. This highlights the importance the ferrocenyl moiety on the increased activity against breast cancers.<sup>[18]</sup>



**Figure 1** Chemical structures of **A.** ferrocenyl hydroxytamoxifen (Fc-OH-Tam)<sup>[14]</sup> and **B.** ferrocenyl  $\beta$ -diketonate compounds.<sup>[19]</sup>

Although the mechanism of action is still not fully understood, it was found that compounds such as Fc-OH-Tam can generate hydroxyl radicals in physiological conditions, and this Fenton-type reaction is thought to lead to DNA damage.<sup>[18,20]</sup> Since this discovery, there have been many ferrocenyl derived compounds which have been synthesized and screened for their cellular activities.<sup>[21–24]</sup> Electron transfer processes between the ferrocenyl and ferrocenium states are fast and reversible, and the ferrocenyl moiety will exist as a mixture of the neutral ferrocenyl and cationic radical ferrocenium species.<sup>[25–27]</sup> It was therefore suggested that such compounds can be administered in either the reduced ferrocenyl or oxidized ferrocenium state, providing the formal reduction potential of the ferrocenyl group is low enough to allow ferrocenyl oxidation inside a cell. Many researchers have shown that this oxidation from Fe(II) to Fe(III) can cause the compounds to undergo chemical oxidation to give quinone methides (QM)<sup>[28,29]</sup> which were able to strongly inhibit *in vitro* thioredoxin reductase (TrxR).<sup>[29,30]</sup>

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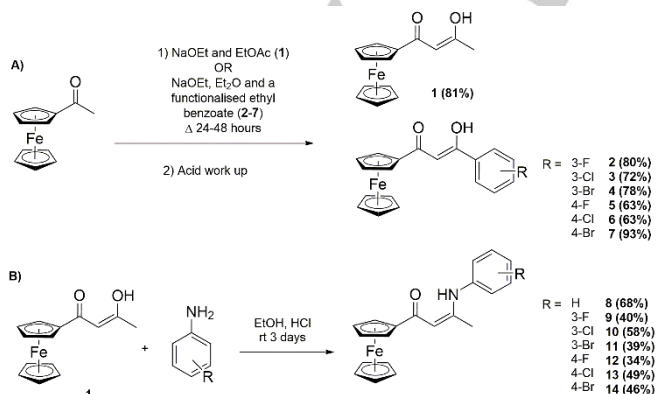
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Swarts and co-workers developed and screened ferrocenyl  $\beta$ -diketonate compounds, and also provided evidence that halides had a significant effect on the compound's cytotoxicity (**Figure 1B**).<sup>[19]</sup> The compound functionalized with a  $\text{CF}_3$  moiety exhibited low  $\mu\text{M}$  activity against colorectal adenocarcinoma (CoLo 320DM), and was more cytotoxic than cisplatin. We have previously synthesized a range of  $\beta$ -diketonate and  $\beta$ -ketoiminate ligands, which we have used for complexation reactions with metals such as Ti, Ru and Ir.<sup>[31–33]</sup> However, the ligands exhibit no toxicity, with  $\text{IC}_{50}$  values  $> 100 \mu\text{M}$ . Herein, we have functionalized these ligands with ferrocenyl and extended the library published by Swarts et al. (**Figure 1B**). We report an increase in cytotoxicity against human carcinomas, highlighting the ferrocenyl  $\beta$ -diketonate ligands to be more cytotoxic than the ferrocenyl  $\beta$ -ketoiminate analogues. The stability of these compounds has been assessed by NMR and mass spectroscopy in DMSO and DMF over 4 days, showing no oxidation of the iron centers, but the possibility of new species in solution. Additionally, the compounds were screened against a colorectal carcinoma cell line, HCT116  $p53^{+/+}$ , after being dissolved in DMF. The results are similar to those obtained in DMSO, unlike the clinical platinum compounds, where carboplatin and oxaliplatin become less cytotoxic in DMF.

A simple acid-catalyzed Friedel-Crafts acylation was used to synthesize acetyl ferrocene, which was purified by column chromatography, followed by a Claisen condensation with a functionalized acetophenone (1 eq.). Complex **1** was synthesized by refluxing acetyl ferrocene (1 eq.), sodium ethoxide (2 eq.) and ethyl acetate for 3 hours. After an acid work up and recrystallization from hexane, red crystals were obtained in an 81% yield (**Scheme 1A**). Complexes **2–7** were synthesized by refluxing acetyl ferrocene (1 eq.), sodium ethoxide (1 eq.) and a functionalized ethyl benzoate (1 eq.) in diethyl ether for 3 hours (**Scheme 1A**). These complexes were purified by column chromatography and obtained in yields of 63–93%. Complexes **8–14** were synthesized by addition of compound **1** (1 eq.), to a functionalized aniline (excess) and a catalytic amount of concentrated hydrochloric acid. The reaction mixture was stirred at room temperature for 3 days, and after removal of the solvent and purification, the compounds were obtained in 34–68% yields (**Scheme 1B**). All compounds were fully characterized by  $^1\text{H}$  NMR and  $^{13}\text{C}\{^1\text{H}\}$  NMR spectroscopy, elemental analysis, high resolution mass spectrometry and single crystal X-ray diffraction where possible.



**Scheme 1** Synthesis of **A)** ferrocenyl  $\beta$ -diketonate compounds **1–7** and **B)** ferrocenyl  $\beta$ -ketoiminate compounds **8–14**.

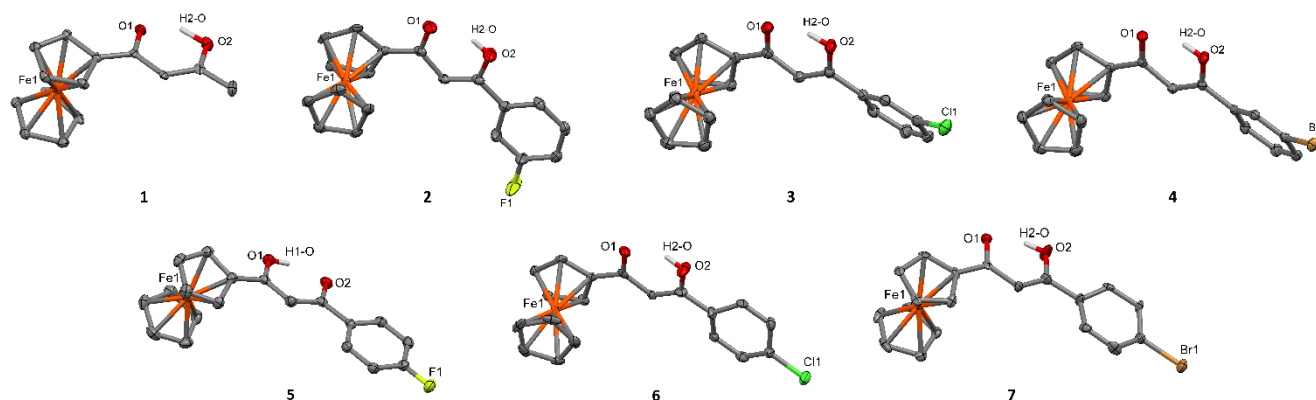
Crystal structures of compounds **1–13** are reported herein, and the crystal structure of compound **14** has previously been published.<sup>[34]</sup> Red/orange single crystals suitable for X-ray crystallographic analysis were obtained for compounds **1–7**, by slow evaporation of an acetonitrile solution (**Figure 2**). Compound **1** has previously been published, however, this is the first time a crystal structure has been obtained. The ferrocenyl  $\beta$ -diketonate compounds all crystallized in either an orthorhombic or monoclinic cell, and structures were solved in space groups  $P2_12_12_1$  (**1**),  $P2_1/n$  (**2, 6, 7**),  $C2/c$  (**3, 4**) or  $P2_1/c$  (**5**). The cyclopentadienyl (Cp) substituents of the ferrocenyl adopt an eclipsed conformation in all cases, with the exception of compound **7**, which has a disordered staggered cyclopentadienyl rings. The eclipsed arrangement has been postulated to be the energetically preferred conformer.<sup>[35,36]</sup> The  $\beta$ -diketonate section of the compounds are all planar, with O1-C11-C12-O2 angles of  $119\text{--}122^\circ$  (**Table S3**) and carbonyl bond lengths of  $1.2\text{--}1.3 \text{ \AA}$  (**Table 1**). Short intramolecular hydrogen bonding interactions are observed between O(1)–H...O(2), at a distance of  $2.4\text{--}2.5 \text{ \AA}$  (D...A) in all cases, which is characteristic for such acetylacetone molecules in their enol formation.

Red/orange single crystals suitable for X-ray crystallographic analysis were obtained for compounds **8–13**, by slow cooling from hot ethanol then storing at  $-20^\circ\text{C}$  or by slow evaporation from acetone (**Figure 3**). The ferrocenyl  $\beta$ -ketoiminate compounds crystallized in either a monoclinic or orthorhombic cell, with structures solved in the space groups  $P2_1/c$  (**8, 10, 11** and **13**),  $P2_1/n$  (**12**), or  $Pca2_1$  (**9**). In the case of the  $\beta$ -ketoiminate compounds, the Cp moiety adopts an eclipsed conformation in all cases, and have short intramolecular hydrogen bonding, with O1...H–N1 distances of  $2.58\text{--}2.64 \text{ \AA}$ . All crystallographic data are stated in **Table S1a–b** (**1–7**) and **Table S2a–b** (**8–13**).

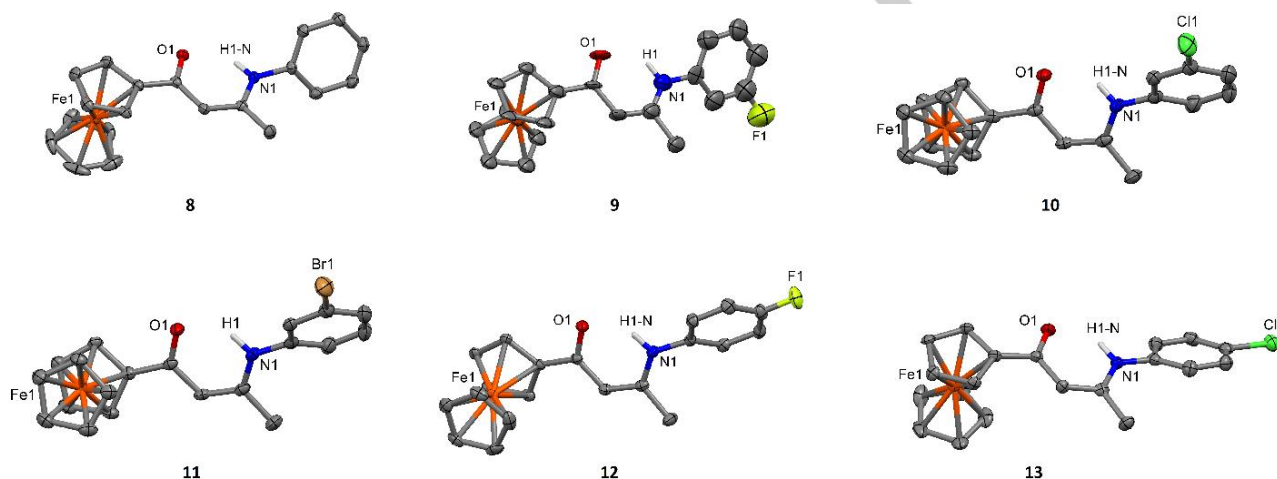
**Table 1** Selected bond lengths for ferrocenyl  $\beta$ -diketonate compounds **1–7** and ferrocenyl  $\beta$ -ketoiminate compounds **8–13**.

Bond lengths (Å)	C11–O1	C11–C12	C12–C13	C13–O2 or C13–N1
<b>1</b>	1.284(6)	1.412(8)	1.371(8)	1.320(7)
<b>2</b>	1.266(3)	1.449(4)	1.366(4)	1.331(3)
<b>3</b>	1.260(5)	1.441(5)	1.363(5)	1.329(5)
<b>4</b>	1.262(2)	1.437(3)	1.358(3)	1.334(2)
<b>5</b>	1.302(2)	1.397(3)	1.400(3)	1.294(2)
<b>6</b>	1.274(7)	1.433(8)	1.355(8)	1.332(7)
<b>7</b>	1.276(9)	1.425(10)	1.359(11)	1.320(10)
<b>8</b>	1.2560(18)	1.433(2)	1.373(2)	1.3561(10)
<b>9</b>	1.25(5)/ 1.25(4)	1.29(5)/ 1.56(5)	1.35(6)/ 1.30(6)	1.34(6)/ 1.32(5)
<b>10</b>	1.258(2)	1.429(3)	1.372(3)	1.346(3)
<b>11</b>	1.260(3)	1.425(3)	1.378(4)	1.341(3)
<b>12</b>	1.24(5)/ 1.20(4)	1.44(5)/ 1.47(6)	1.37(7)/ 1.35(6)	1.29(6)/ 1.36(5)
<b>13</b>	1.267(5)	1.438(5)	1.374(6)	1.358(5)

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**Figure 2** Molecular structures of ferrocenyl  $\beta$ -diketonate compounds 1-7. Displacement ellipsoids are at the 50% probability level and hydrogen atoms and disordered atoms are omitted for clarity.

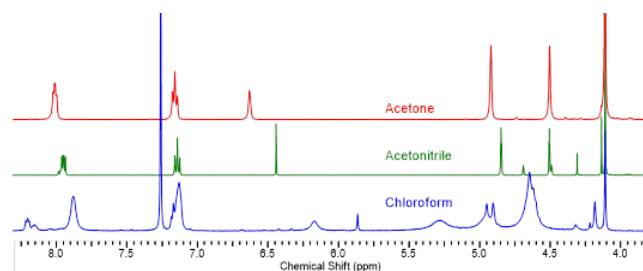


**Figure 3** Molecular structures of ferrocenyl  $\beta$ -ketoiminate compounds 8-13. Displacement ellipsoids are at the 50% probability level and hydrogen atoms and disordered atoms are omitted for clarity.

The ferrocenyl  $\beta$ -diketonate compounds are distinguishable by the presence of the methine singlet at  $\sim 5.5$ - $6.5$  ppm, and in some solvents the appearance of the OH resonance at  $\sim 16.0$  ppm. Upon synthesis of the ferrocenyl  $\beta$ -ketoiminate compounds, the methine resonances undergo a minor shift and the disappearance of the OH resonance and the appearance of a new aniline NH resonance at 11-13 ppm verifies the successful synthesis. NMR samples were first prepared in  $\text{CDCl}_3$ , however, the obtained spectra showed broad peaks (Figure 4, blue). This was attributed partly to the fluctuating nature of the ligands, as they can undergo tautomerisation between the cyclic enol and diketo forms.<sup>[37–39]</sup> The use of more polar solvents, i.e. acetonitrile- $d_3$ , stabilized the enol system through hydrogen bonding interactions, producing sharp peaks in the NMR spectra (Figure 3, green).

To address the stability of these compounds prior to cell screening,  $^1\text{H}$  NMR spectroscopy was used to monitor any changes in the unfunctionalized ferrocenyl  $\beta$ -diketonate compound 1 and unfunctionalized ferrocenyl  $\beta$ -ketoiminate compound 8. The compounds (5 mg) were dissolved in  $\text{DMSO}-d_6$  and an initial spectrum taken, and then recorded at varying time intervals between initial and 4 days. Selected time intervals are shown for compounds 1 and 8, in Figure 5 and Figure 6, respectively. The

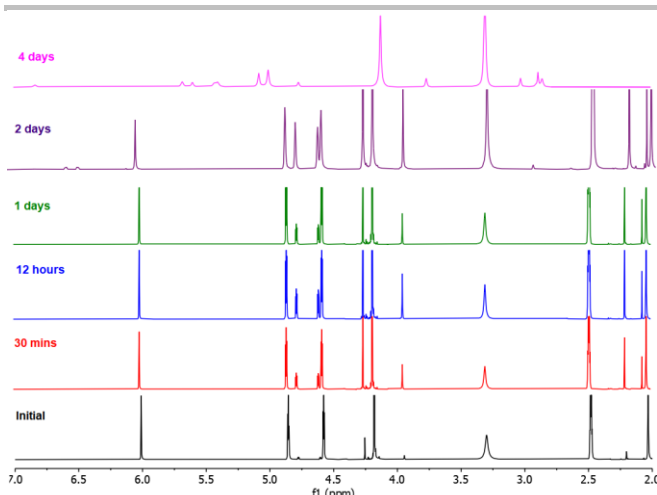
compounds show the appearance of new resonances which are also similar to those observed with the 4 day NMR of acetyl ferrocene (Figure S1 and Figure S2, respectively), showing there is likely some rearrangement of the compounds in solution, and no decomposition to acetyl ferrocene or ferrocene. Although the solutions turn darker in color over time (Figure S5 and Figure S6), the NMR resonances do not over time and there is no evidence of paramagnetic resonances, indicating no oxidation from  $\text{Fe(II)}$  to  $\text{Fe(III)}$ .



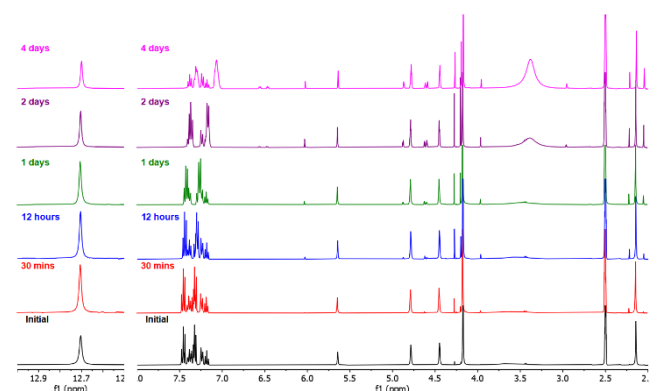
**Figure 4**  $^1\text{H}$  NMR spectra of unfunctionalized ferrocenyl  $\beta$ -diketonate compound 1 in  $\text{CDCl}_3$  (blue), acetonitrile- $d_3$  (green) and acetone- $d_6$  (red) (400 MHz, 298K).



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**Figure 5**  $^1\text{H}$  NMR spectra of ferrocenyl  $\beta$ -diketonate compound **1** in DMSO at selected time intervals between initial to 4 days (298K, 400 MHz).

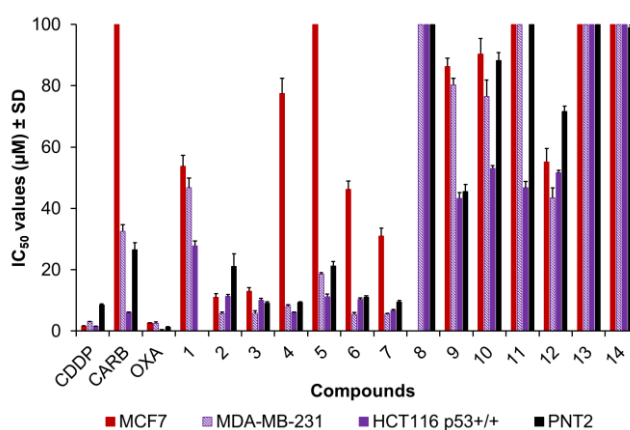


**Figure 6**  $^1\text{H}$  NMR spectra of ferrocenyl  $\beta$ -ketoiminate compound **8** in DMSO at selected time intervals between initial to 4 days (298K, 400 MHz).

The stability of compounds **1** and **8** were also assessed by dissolving 5 mg in  $\text{DMF-d}_7$  and  $^1\text{H}$  NMR spectra recorded at the same time intervals as previously discussed (Figures S3 and S4). Although DMF solutions of compound **1** appear to darken over 2 hours (Figure S3 and S5), the spectra showed no significant changes until day 4. In comparison, compound **8**, which exhibited significant changes in the aromatic region (7.0–7.5 ppm) after only 30 mins, which differs from the DMSO studies, where compound change slowly over time (Figure 6 and Figure S4). These changes were surprising, as compound **8** did not experience darkening of the solution when dissolved in DMF for 2 hours (Figure S6). As with the DMSO results, the NMR resonances in DMF do not appear to broaden and there is again no evidence of paramagnetic resonances, indicating no oxidation from Fe(II) to Fe(III). In attempts to establish the structures in solution, mass spectrometry was performed after samples were dissolved in DMSO or DMF over a period of 4 days. Although there is evidence of each compound in solution over time, there are multiple new mass peaks which could not be assigned, and we were unable to elucidate the structures or rearrangement products of these compounds (Table S4).

The range of compounds were screened for their cytotoxicity against human breast adenocarcinomas MCF-7 and MDA-MB-231 and human colorectal carcinoma  $p53^{+/+}$ , HCT116  $p53^{+/+}$ . Stock solutions in DMSO (100 mM) were made fresh on each day of testing, and were immediately (< 5 mins) plated with the cell lines for 96 h (DMSO < 0.1% v/v) before performing an MTT assay. The clinical drugs cisplatin (**CDDP**), oxaliplatin (**OXA**) and carboplatin (**CARB**) were screened for comparison. The results are shown in Table 2 and Figure 7, and across all of the cell lines tested, there is a general trend whereby the ferrocenyl  $\beta$ -diketonate compounds **1–7** are more cytotoxic than the analogous ferrocenyl  $\beta$ -ketoiminate compounds **8–14**. The most significant differences are observed against the triple negative breast adenocarcinoma, MDA-MD-231, where the ferrocenyl  $\beta$ -diketonate compounds are > 18x more cytotoxic than the ferrocenyl  $\beta$ -ketoiminate compounds (i.e. **6** compared to **13**, see Figure S7). This is contrary to our previously investigated Ru(II) and Ir(III) complexes, in which the  $\beta$ -ketoiminate complexes were more cytotoxic than those with a  $\beta$ -diketonate ligand, where the latter were generally non-toxic.<sup>[31–33]</sup>

The unfunctionalized ferrocenyl  $\beta$ -diketonate compound **1** is moderately cytotoxic towards all cell lines, and generally the toxicity increases when the compound is functionalized with a halide substituent. Compounds **2–4** are functionalized with halide groups in the *meta* position of the arene ring, and 3'-F (**2**) has increased cytotoxicity when compared to 3'-Cl (**3**) and 3'-Br (**4**), following the order **2** > **3** > **4**. Using the same substituents in the *para* position (**5–7**), the activity is generally reversed and the 4'-Br compound **7** is more cytotoxic than 4'-F (**5**) and 4'-Cl (**6**), following the order **7** > **6** > **5**. This highlights the importance of the position of the halide substituent on the compound's *in vitro* activity. The *meta* substituted compounds are up to 9x more cytotoxic than the *para* substituted compounds against MCF-7 (i.e. **2** cf. **5**). The ferrocenyl  $\beta$ -diketonate compounds have an increased sensitivity towards the MDA-MD-231 cell line, with compounds **2**, **3**, **5** and **7** having  $\text{IC}_{50}$  values ranging from 5.4–5.8  $\mu\text{M}$  and comparable activity to **CDDP** ( $p > 0.05$ ), yet are >5x more cytotoxic than **CARB** ( $p < 0.05$ ).



**Figure 7** Bar-chart of  $\text{IC}_{50}$  values against MCF7, MDA-MB-231, HCT116  $p53^{+/+}$  and PNT2 for cisplatin (**CDDP**), carboplatin (**CARB**), oxaliplatin (**OXA**), ferrocenyl  $\beta$ -ketoiminate compounds **1–7** and ferrocenyl  $\beta$ -ketoiminate compounds **8–14**.

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**Table 2** IC<sub>50</sub> values (μM) ± SD after 96 h incubation with MCF-7, MDA-MB-231, HCT116 p53<sup>+/+</sup> (DMSO and DMF) and PNT2. All values are averages of duplicate of triplicate repeats. The values in parentheses are the selectivity indices (SI) when compared to the normal cell lines PNT2.

Compounds	IC <sub>50</sub> values (μM) ± SD				
	MCF-7	MDA-MB-231	HCT116 p53 <sup>+/+</sup> DMSO	HCT116 p53 <sup>+/+</sup> DMF	PNT2
<b>CDDP</b>	1.5 ± 0.2 (5.7)	3.07 ± 0.02 (2.8)	1.5 ± 0.1 (5.7)	2.3 ± 0.1	8.5 ± 0.4
<b>CARB</b>	>100 (<0.27*)	33 ± 2 (0.8)	6.0 ± 0.2 (4.5)	45 ± 2	27 ± 2
<b>OXA</b>	2.6 ± 0.2 (0.5)	2.6 ± 0.4 (0.5)	0.445 ± 0.002 (2.9)	1.6 ± 0.1	1.3 ± 0.2
<b>1</b>	54 ± 4 (1.9)	47 ± 3 (2.1)	28 ± 1 (3.6)	32 ± 1	>100
<b>2</b>	11 ± 1 (1.9)	5.7 ± 0.5 (3.7)	11.3 ± 0.7 (1.9)	9.3 ± 0.4	21 ± 4
<b>3</b>	13 ± 1 (0.7)	5.8 ± 0.9 (1.6)	10.1 ± 0.5 (0.9)	5.8 ± 0.2	9.0 ± 0.5
<b>4</b>	77 ± 5 (0.1)	8.1 ± 0.4 (1.2)	6.0 ± 0.2 (1.6)	4.45 ± 0.09	9.4 ± 0.1
<b>5</b>	>100 (<0.2*)	18.6 ± 0.5 (1.1)	11.2 ± 0.9 (1.9)	10.7 ± 0.6	21 ± 2
<b>6</b>	46 ± 3 (0.2)	5.4 ± 0.6 (2.1)	10.3 ± 0.6 (1.1)	4.8 ± 0.6	11.1 ± 0.4
<b>7</b>	31 ± 3 (0.3)	5.6 ± 0.2 (1.7)	6.7 ± 0.4 (1.4)	5.6 ± 0.5	9.4 ± 0.6
<b>8</b>	>100 (n.d.)	>100 (n.d.)	>100 (n.d.)	59 ± 2	>100
<b>9</b>	86 ± 3 (0.5)	80 ± 2 (0.6)	43 ± 2 (1.1)	52 ± 2	46 ± 2
<b>10</b>	90 ± 5 (1.0)	77 ± 5 (1.1)	53.1 ± 0.9 (1.7)	43 ± 2	88 ± 3
<b>11</b>	>100 (n.d.)	>100 (n.d.)	47 ± 2 (>2.1*)	41 ± 2	>100
<b>12</b>	55 ± 4 (1.3)	43 ± 3 (1.7)	51.6 ± 0.7 (1.4)	56 ± 2	72 ± 2
<b>13</b>	>100 (n.d.)	>100 (n.d.)	>100 (n.d.)	86 ± 2	>100
<b>14</b>	>100 (<1.0*)	>100 (<1.0*)	>100 (<1.0*)	84 ± 2	99 ± 1

\* denotes the minimum SI value, as at least one of the IC<sub>50</sub> values is >100 μM

n.d. denotes the values which cannot be determined, as both of the IC<sub>50</sub> values are >100 μM

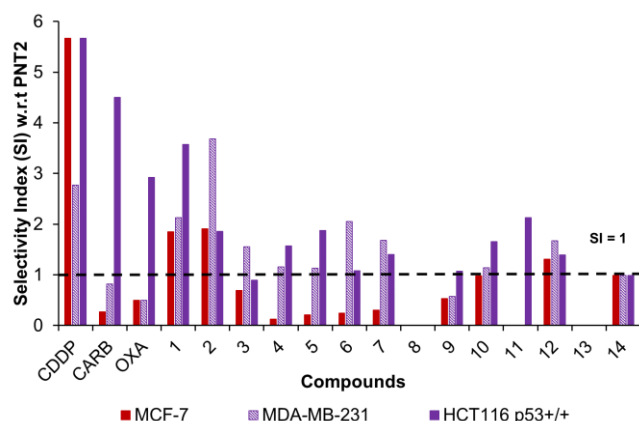
The unfunctionalized ferrocenyl β-ketoiminate compound **8** is non-toxic against all cell lines (IC<sub>50</sub> > 100 μM), and is up to 3.5x less cytotoxic than the analogues β-diketonate compound **1** (HCT116 p53<sup>+/+</sup>). There are no general trends observed when these compounds are functionalized in the *meta* position of the aniline ring, although the 3'-Br compound **11** has the lowest toxicity. When comparing the halide functionalization in the *para* position, unlike the ferrocenyl β-diketonate compounds **6** and **7**, the 4'-Cl (**13**) and 4'-Br (**14**) β-ketoiminate compounds are non-toxic against all cell lines (IC<sub>50</sub> > 100 μM) and are up to 18.5x less toxic than their β-diketonate analogues (i.e. **6** cf. **13**, **Figure S7**). The 4'-F compound **12** is the only *para* substituted β-ketoiminate compound to show moderate cytotoxicity, with values ranging between 43–55 μM, and is up to 2.3x more cytotoxic than compounds **13** and **14** (MDA-MB-231, *p* < 0.05).

In order to assess the compounds' selectivity towards cancerous cells, the compounds and clinical drugs were screened against normal prostate epithelium cells, PNT2 (**Table 2** and **Figure 8**). Generally, the IC<sub>50</sub> values show the ferrocenyl β-diketonate compounds **1–7** are between 2.2x (*cf.* **9**) to 10.6x (*cf.* **11**) more potent towards normal cells, when compared to the cytotoxicity of the ferrocenyl β-ketoiminate compounds **8–14**. The IC<sub>50</sub> values against PNT2 were divided by the IC<sub>50</sub> values against either of the

cancerous cell lines, to give a selectivity index (SI). The values are shown in parentheses of **Table 2** and displayed as a bar-chart in **Figure 8**. SI values > 1 indicate a selectivity for the cancerous cell line, and highlight the potential to overcome issues of toxicity towards healthy cells. The clinical compound **OXA** has no selectivity for cancerous cells, with all SI values < 1. However, **CDDP** shows the highest selectivity, with all SI values between 2.8–5.7, whilst **CARB** is only selective towards HCT116 p53<sup>+/+</sup> (SI = 4.5).

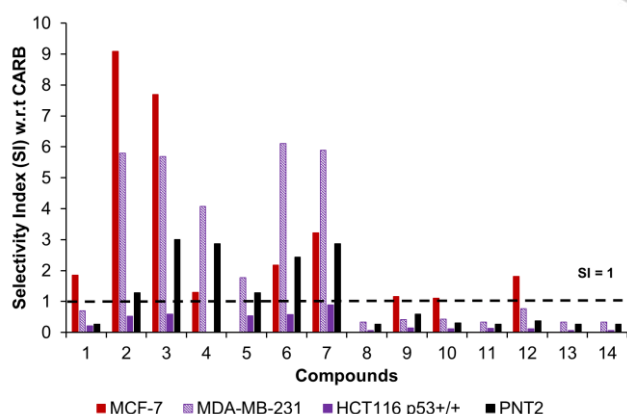
The ferrocenyl compounds **1–14** generally do not have a selectivity towards MCF-7, except for compounds **1** and **2**, which have SI values of 1.9. The compounds are moderately selective towards MDA-MB-231 and HCT116 p53<sup>+/+</sup>, with values ranging from 0.6–3.7 and 0.9–3.6, respectively. The ferrocenyl β-diketonate compounds **1–7** are generally more selective than the analogous ferrocenyl β-ketoiminate compounds **8–14**. In particular, compounds **1** and **2** have the highest selectivity against all cell lines. On comparing compounds **1** with **8** (R = H) and **2** with **9** (R = 3'-F), compound **1** is at least 3.6x more selective than **8** (HCT116 p53<sup>+/+</sup>), whilst **2** is at least 6.4x more selective than **9** (MDA-MB-231).

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**Figure 8** Selectivity Index (SI) for **CDDP**, **CARB**, **OXA**, compounds **1-14** when comparing the  $IC_{50}$  values against cancerous and PNT2. SI > 1 shows selectivity for the cancer cell lines, SI = 1 shows equitoxicity for cancerous and normal cell lines, and SI < 1 shows selectivity for the PNT2.

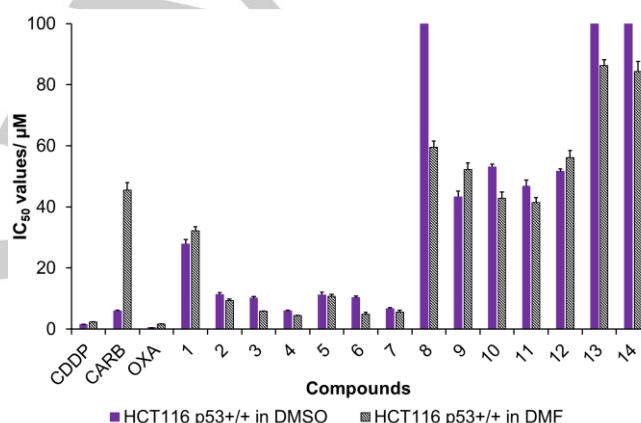
The SI values were also calculated for compounds **1-14** in comparison with **CDDP**, **CARB** and **OXA**. The results for **CDDP** and **OXA** show no selectivity, and these clinical compounds outperform our library or ferrocenyl compounds (**Figure S8** and **Figure S9**, respectively). However, the ferrocenyl  $\beta$ -diketonate compounds are generally more selective for breast cancer cell lines MCF-7 and MDA-MB-231, when compared to **CARB** (**Figure 9**). On comparison of **CARB** and compounds **2** and **3**, these compounds have increased selectivity against MCF-7, with SI values of 9.1 and 7.8, respectively. Whilst compounds **2-7** are all more selective than **CARB** against MDA-MB-231, with SI values ranging from 1.8 (**5**) to 6.1 (**6**). As with the other cytotoxicity results, the ferrocenyl  $\beta$ -diketonate compounds **1-7** are generally more selective than ferrocenyl  $\beta$ -ketoiminate compounds **8-14** when compared to **CARB**, with SI values up to 18.5x higher (i.e. **6** cf. **13** against MDA-MB-231).



**Figure 9** Selectivity Index (SI) values for compounds **1-14** when comparing the  $IC_{50}$  values with **CARB**. SI > 1 shows selectivity for the ferrocenyl compounds, SI = 1 shows equitoxicity and SI < 1 shows selectivity for **CARB**.

Due to the differences observed in the NMR spectra of the compounds in DMSO- $d_6$  and DMF- $d_7$  (**Figure 4-5** and **Figure S3-S6**), and the previously reported cytotoxicity differences observed

when iridium compounds were screened in DMSO and DMF,<sup>[40]</sup> the compounds were screened against HCT116  $p53^{+/+}$  after being dissolved in DMF. As with the DMSO screening, 100 mM stock solutions of compounds **1-14** and the clinical drugs (**CDDP**, **OXA**, **CARB**) were prepared in DMF and cells were incubated for 96 h (DMF < 0.1% v/v) before performing an MTT assay. The  $IC_{50}$  values for **CDDP** are comparable ( $p > 0.05$ ), however, the values for **OXA** and **CARB** significantly decrease in DMF (**Table 2** and **Figure 10**), by 3.6 and 7.6-fold, respectively. On comparison of the  $IC_{50}$  values of compounds **1-14** in DMSO and DMF, with the exception of compound **8** (1.6-fold decrease), all compounds have similar activities in both DMSO and DMF, indicating that neither of these solvents has a significant effect on the overall cytotoxicity. Yi and Bae highlight the decrease in cytotoxicity of the clinical platinum compounds against human ovarian carcinoma (A2780) when changing solvents from DMSO to DMF,<sup>[40]</sup> whilst Gasser and co-workers have shown the cytotoxicity of iridium compounds and **CDDP** generally increased in DMF (A2780 and HeLa).<sup>[40]</sup>



**Figure 10** Bar-chart of  $IC_{50}$  values against HCT116  $p53^{+/+}$  when compounds were dissolved in either DMSO (purple) or DMF (grey).

In conclusion, we report the synthesis of seven ferrocenyl  $\beta$ -diketonate compounds (**1-7**) and seven ferrocenyl  $\beta$ -ketoiminate compounds (**8-14**), including single crystal X-ray analysis of thirteen new compounds.  $^1H$  NMR studies were used to assess the compounds' stabilities in DMSO- $d_6$  and DMF- $d_7$ , and results indicate no oxidation of the metal center from Fe(II) to Fe(III) over a 4 day period. Additional mass spectrometry analysis was conducted to understand the possible structures in DMSO and DMF over time, yet no conclusions could be drawn to the possible speciation. The library of compounds was screened against human breast carcinoma (MCF-7, MDA-MB-231), human colorectal adenocarcinoma,  $p53$ -wildtype (HCT116  $p53^{+/+}$ ) and a normal human prostate cell line (PNT2). Generally, the ferrocenyl  $\beta$ -diketonate compounds show a significant increase in cytotoxicity when compared to the analogues ferrocenyl  $\beta$ -ketoiminate compounds. Several compounds are more cytotoxic when compared to **CARB**, particularly against MCF-7 (SI > 9x) and MDA-MB-231 (SI > 6x). When comparing the results against normal prostate cells, PNT2, the ferrocenyl  $\beta$ -diketonate compounds are less selective than the clinical platinum drugs against MCF-7, however, they exhibit greater selectivity towards MDA-MB-231 and HCT116  $p53^{+/+}$ . Additional chemosensitivity



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studies were conducted after stock solutions were made in DMF, and the results highlight only small changes are observed in the compounds potency, contrary to the clinical platinum drugs which exhibit lower toxicity when screened in DMF against HCT116 p53<sup>+/+</sup>.

## Experimental Section

The general experimental details, X-ray crystallography details and cell culture protocols can be found in the *Supplementary Information*.

**Synthesis of [C<sub>14</sub>H<sub>14</sub>FeO<sub>2</sub>] (1):** Acetyl ferrocene (2.80 g, 12.3 mmol) was dissolved in ethyl acetate (25 mL) and stirred before the addition of sodium ethoxide (1.70 g, 25.0 mmol). The solution was stirred at reflux for 3 hours forming a yellow solid which was filtered and washed with ethyl acetate. The yellow solid was then dissolved in distilled water (150 mL) and acidified with 10% hydrochloric acid until pH 5 which caused a red solid to precipitate. Recrystallization from hexane gave red crystals (the resonances for enol are stated, however, a small amount of the diketone resonance form is observed). **Yield:** 2.70 g, 81%; **<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>, δ):** 5.63 (s, 1H, methine CH), 4.77 (br. t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 6.8 Hz, Cp-CH), 4.50 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 4.1 Hz, Cp-CH), 4.19 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.00 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, CDCl<sub>3</sub>, δ):** 192.5 (Q, CO), 186.4 (Q, CO), 98.2 (methine CH), 77.7 (Q, Cp), 73.1 (Cp-CH), 72.1 (Cp-CH), 70.3 (Cp-C<sub>5</sub>H<sub>5</sub>), 70.1 (Cp-CH), 68.7 (Cp-CH), 24.2 (CH<sub>3</sub>); **Analysis:** **Calc. for C<sub>14</sub>H<sub>14</sub>FeO<sub>2</sub>:** C 62.25, H 5.22%, **Found:** C 62.28, H 5.10%; **HRMS [ES<sup>+</sup>]:** 271.041 [MH<sup>+</sup>]

**Synthesis of compounds 2-7:** Acetyl ferrocene (1 eq.) was dissolved in diethyl ether (20 mL) and with stirring sodium ethoxide (1 eq.) and a functionalized benzoate (1 eq.) were added, and the mixture refluxed for 24 hours. The solid precipitate was isolated by filtration, dissolved in distilled water (150 mL) and acidified with 10% hydrochloric acid until pH 5 which caused a red solid to precipitate. The solid was filtered and dried overnight under vacuum before purification.

**[C<sub>19</sub>H<sub>15</sub>FFeO<sub>2</sub>] (2):** The product was purified by column chromatography, eluting with 83:17 v/v hexane/ethyl acetate to give a red solid. **Yield:** 2.00 g, 80%; **<sup>1</sup>H NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 7.77 (br. d, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.8 Hz, phenyl-CH), 7.66 (br. d, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 10.1 Hz, phenyl-CH), 7.44 (dt, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.2, 6.0 Hz, phenyl-CH), 7.22 (td, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.4 and <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.8 Hz, phenyl CH), 6.68 (s, 1H, methine CH), 4.95 (br. s, 2H, Cp-CH), 4.52 (br. s, 2H, Cp-CH), 4.11 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 196.0 (Q, CO), 178.37 (Q, CO), 163.9 (d, Q, CF, <sup>1</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 243.9 Hz), 138.6 (d, Q phenyl-C, <sup>3</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 7.8 Hz), 131.5 (d, phenyl-CH, <sup>3</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 8.3 Hz), 123.6 (d, phenyl-CH, <sup>4</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 2.1 Hz), 119.4 (d, , phenyl-CH, <sup>2</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 21.8 Hz), 114.2 (d, , phenyl-CH, <sup>2</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 23.9 Hz), 95.1 (methine CH), 79.0 (Q, Cp), 73.5 (Cp-CH), 71.2 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.9 (Cp-CH); **Analysis:** **Calc. for C<sub>19</sub>H<sub>15</sub>FFeO<sub>2</sub>:** C 65.17, H 4.32%, **Found** C 65.10, H 4.30%; **HRMS [ES<sup>+</sup>]:** 349.032 [M-H<sup>+</sup>]

**[C<sub>19</sub>H<sub>15</sub>ClFeO<sub>2</sub>] (3):** The product was purified by column chromatography, eluting with 90:10 v/v hexane/ethyl acetate to give a red solid. **Yield:** 1.89 g, 72 %; **<sup>1</sup>H NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 7.92 (t, 1H, <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.8 Hz, phenyl-CH), 7.88 (dt, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.8 and <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.3 Hz, phenyl-CH), 7.48 (dq, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.8 and <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.0 Hz, phenyl-CH), 7.42 (t, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.8 Hz, phenyl-CH), 6.70 (s, 1H, methine CH), 4.95 (t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 2.0 Hz, Cp-CH), 4.52 (t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 2.0 Hz, Cp-CH), 4.12 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 196.0 (Q, CO), 178.3 (Q, CO), 138.2 (Q, phenyl-C), 135.2 (Q, C-Cl), 132.4 (phenyl-CH), 131.3 (phenyl-CH), 127.4 (phenyl-CH), 126.1 (phenyl-CH), 95.1 (methine CH), 79.0 (Q, Cp), 73.5 (Cp-CH), 71.2 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.9 (Cp-CH); **Analysis:** **Calculated for C<sub>19</sub>H<sub>15</sub>ClFeO<sub>2</sub>:** C 62.25, H 4.12%, **Found:** C 62.18, H 4.13%; **HRMS [ES<sup>+</sup>]:** 366.010 [MH<sup>+</sup>]

**[C<sub>19</sub>H<sub>15</sub>BrFeO<sub>2</sub>] (4):** The product was purified by column chromatography, eluting with 90:10 v/v hexane/ethyl acetate to give a red solid. **Yield:** 2.32 g, 78 %; **<sup>1</sup>H NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 8.07 (t, 1H, <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.6 Hz, phenyl-CH), 7.93 (dt, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.8 and <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.1 Hz, phenyl-CH), 7.62 (dt, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.0 and <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 0.8 Hz, phenyl-CH), 7.36 (t, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.9 Hz, phenyl-CH), 6.69 (s, 1H, methine CH), 4.94 (t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.8 Hz, Cp-CH), 4.52 (t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.8 Hz, Cp-CH), 4.11 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 196.0 (Q, CO), 178.3 (Q, CO), 138.4 (Q, phenyl-C), 135.4 (phenyl-CH), 131.5 (phenyl-CH), 130.3 (phenyl-CH), 126.5 (phenyl-CH), 123.3 (Q, C-Br), 95.1 (methine CH), 79.0 (Q, Cp), 73.5 (Cp-CH), 71.2 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.9 (Cp-CH); **Analysis:** **Calculated for C<sub>19</sub>H<sub>15</sub>BrFeO<sub>2</sub>:** C 55.52, H 3.68%, **Found:** C 55.54, H 3.77%; **HRMS [ES<sup>+</sup>]:** 409.960 [MH<sup>+</sup>]

**[C<sub>19</sub>H<sub>15</sub>FFeO<sub>2</sub>] (5):** The product was purified by column chromatography, eluting with 80:20 v/v hexane/ethyl acetate to give a red solid. **Yield:** 1.59 g, 63 %; **<sup>1</sup>H NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 8.10 (m, 2H, phenyl-CH), 7.29 (t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.7 Hz, phenyl-CH), 6.59 (s, 1H, methine CH), 5.00 (t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.8 Hz, Cp-CH), 4.66 (t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.8 Hz, Cp-CH), 4.26 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 195.0 (Q, CO), 179.7 (Q, CO), 165.8 (d, Q, C-F, <sup>1</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 250.2 Hz), 132.6 (d, Q, phenyl-C, <sup>4</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 2.1 Hz), 130.3 (d, phenyl-CH, <sup>3</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 9.3 Hz), 116.4 (d, phenyl-CH, <sup>2</sup>J(<sup>13</sup>C-<sup>19</sup>F) = 21.8 Hz), 94.4 (methine CH), 73.5 (Q, Cp), 73.2 (Cp-CH), 71.1 (Cp-C<sub>5</sub>H<sub>5</sub>), 70.8 (Cp-CH), 70.6 (Cp-CH), 69.8 (Cp-CH); **Analysis:** **Calculated for C<sub>19</sub>H<sub>15</sub>FFeO<sub>2</sub>:** C 65.17, H 4.32 %, **Found:** C 65.00, H 4.40 %; **HRMS [ES<sup>+</sup>]:** 349.330 [M-H<sup>+</sup>]

**[C<sub>19</sub>H<sub>15</sub>ClFeO<sub>2</sub>] (6):** The product was purified by column chromatography, eluting with 83:17 v/v hexane/ethyl acetate to give a red solid. **Yield:** 1.65 g, 63 %; **<sup>1</sup>H NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 7.95 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.7 Hz, phenyl-CH), 7.41 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.7 Hz, phenyl-CH), 6.36 (d, 1H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 5.5 Hz, methine CH), 4.92 (br. t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.7 Hz, Cp-CH), 4.51 (br. t, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.7 Hz, Cp-CH), 4.11 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 195.7 (Q, CO), 178.9 (Q, CO), 134.9 (Q, phenyl-C), 131.4 (Q, C-Cl), 129.7 (phenyl-CH), 129.3 (phenyl-CH), 94.8 (methine CH), 79.1 (Q, Cp), 73.4 (Cp-CH), 71.2 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.8 (Cp-CH); **Analysis:** **Calculated for C<sub>19</sub>H<sub>15</sub>ClFeO<sub>2</sub>:** C 62.25, H 4.12, Cl 9.67%, **Found:** C 62.30, H 4.10, Cl 9.50%; **HRMS [ES<sup>+</sup>]:** 366.011 [MH<sup>+</sup>]

**[C<sub>19</sub>H<sub>15</sub>BrFeO<sub>2</sub>] (7):** The product was purified by column chromatography, eluting with 90:10 v/v hexane/ethyl acetate to give a red solid. **Yield:** 2.76 g, 93%; **<sup>1</sup>H NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 7.87 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.0 Hz, phenyl-CH), 7.58 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.1 Hz, phenyl-CH), 6.66 (s, 1H, methine CH), 4.92 (br. s, 2H, Cp-CH), 4.51 (br. s, 2H, Cp-CH), 4.11 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, CDCl<sub>3</sub>, δ):** 194.3 (Q, CO), 178.5 (Q, CO), 134.2 (Q, phenyl-C), 131.9 (phenyl-CH), 128.2 (phenyl-CH), 126.5 (Q, C-Br), 93.6 (methine CH), 78.0 (Q, Cp), 72.4 (Cp-CH), 70.4 (Cp-C<sub>5</sub>H<sub>5</sub>), 68.8 (Cp-CH); **Analysis:** **Calculated for C<sub>19</sub>H<sub>15</sub>BrFeO<sub>2</sub> (+0.75 DCM):** C 49.96, H 3.50%, **Found:** C 50.00, H 3.20%; **HRMS [ES<sup>+</sup>]:** 409.960 [MH<sup>+</sup>]

**Synthesis of complexes 8-14:** 1-Ferrocenylbutane-1,3-dione (compound 1, 1 eq.) was dissolved in ethanol (20 mL) followed by the addition of a functionalized aniline (2 eq.) and concentrated hydrochloric acid (2 mL). The reaction was stirred at room temperature for three days. The solution was filtered and the solvent removed *in vacuo*.

**[C<sub>20</sub>H<sub>19</sub>FeNO] (8):** The crude solid was dissolved in ethyl acetate/hexane (1:4) and filtered through a silica plug. The solvent was removed, leaving a red solid. Red crystals were obtained upon slow evaporation from acetone. **Yield:** 0.86g, 68%; **<sup>1</sup>H NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 12.86 (s, 1H, NH), 7.50-7.34 (m, 2H, phenyl-CH), 7.31 – 7.14 (m, 3H, phenyl-CH), 5.65 (s, 1H, methine CH), 4.86-4.75 (m, 2H, m, Cp-CH), 4.47-4.37 (m, 2H, Cp-CH), 4.17 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.16 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 193.00 (Q, CO), 159.15 (Q, phenyl-C), 139.32 (Q, CNH), 129.22 (phenyl-CH), 125.18 (phenyl-CH), 124.33 (phenyl-CH), 95.68 (methine CH), 82.45 (Q, Cp), 71.05 (Cp-CH), 69.94 (Cp-C<sub>5</sub>H<sub>5</sub>), 68.60 (Cp-CH), 20.58 (CH<sub>3</sub>). **HRMS [ES<sup>+</sup>]:** 346.0915 [M-H<sup>+</sup>] (calculated 346.0889)



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**[C<sub>20</sub>H<sub>18</sub>FFeNO] (9):** The crude solid was dissolved in ethyl acetate/hexane (3:7) and filtered through a silica plug. The solvent was removed, and the crude product was dissolved in an ethanol/ether mixture, and cooled to -20°C overnight, yielding red crystals. **Yield:** 0.49 g, 40%; **<sup>1</sup>H NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 12.89 (s, 1H, NH), 7.42 (m, 1H, phenyl-CH), 7.07 (m, 2H, phenyl-CH), 6.94 (m, 1H, phenyl-CH), 5.69 (s, 1H, methine CH), 4.85–4.77 (m, 2H, Cp-CH), 4.49–4.40 (m, 2H, Cp-CH), 4.17 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.22 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (101 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 192.81 (Q, CO), 157.80 (Q, phenyl-C), 142.51 (Q, CNH), 139.24 (phenyl-CH), 130.72 (phenyl-CH), 119.08 (Q, C-Fl), 110.92 (phenyl-CH), 110.10 (phenyl-CH), 96.56 (methine CH), 83.67 (Q, Cp), 70.87 (Cp-CH), 70.27 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.50 (Cp-CH), 19.56 (CH<sub>3</sub>); **HRMS [ES<sup>+</sup>]:** 364.0845 [M-H<sup>+</sup>]

**[C<sub>20</sub>H<sub>18</sub>ClFeNO] (10):** The crude solid was dissolved in ethyl acetate/hexane (1:4) and filtered through a silica plug. The solvent was removed, leaving a red solid. Red crystals were obtained upon slow evaporation from acetone. **Yield:** 0.82 g, 58%; **<sup>1</sup>H NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 12.87 (s, 1H, NH), 7.46–7.34 (m, 1H, phenyl-CH), 7.29 (t, 1H, <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 2.0, phenyl-CH), 7.20 (dd, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 7.9 and <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.9, phenyl-CH), 5.69 (s, 1H, methine CH), 4.81 (s, 2H, Cp-CH), 4.43 (2H, Cp-CH), 4.17 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.21 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 192.85 (Q, CO), 157.80 (Q, phenyl-C), 141.17 (Q, CNH), 134.21 (phenyl-CH), 130.58 (phenyl-CH), 124.23 (phenyl-CH), 123.02 (phenyl-CH), 121.78 (phenyl-CH), 96.61 (methine CH), 82.38 (Q, Cp), 70.90 (Cp-CH), 69.63 (Cp-C<sub>5</sub>H<sub>5</sub>), 68.51 (Cp-CH), 19.51 (CH<sub>3</sub>); **Analysis Calculated for C<sub>20</sub>H<sub>18</sub>ClFeNO:** C 63.27 H 4.78 N 3.69 Cl 9.34%; **Found:** C 62.90 H 4.80 N 3.50 Cl 9.10%; **HRMS [ES<sup>+</sup>]:** 380.0507 [M+H]<sup>+</sup>

**[C<sub>20</sub>H<sub>18</sub>BrFeNO] (11):** The crude solid was dissolved in ethyl acetate/hexane (1:4) and filtered through a silica plug. The solvent was removed, leaving a red solid. Red crystals were obtained upon slow evaporation from acetone. **Yield:** 0.62 g, 39%; **<sup>1</sup>H NMR (300 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 12.86 (s, 1H, NH), 7.44 (br. t, 1H, phenyl-CH), 7.39–7.18 (m, 2H, phenyl-CH), 7.29–7.21 (m, 1H, phenyl-CH), 5.69 (s, 1H, methine CH), 4.85–4.77 (m, 2H, Cp-CH), 4.48–4.40 (m, 2H, Cp-CH), 4.17 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.20 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 192.83 (Q, CO), 157.76 (Q, phenyl-C), 157.37 (Q, CNH), 141.31 (phenyl-CH), 130.83 (phenyl-CH), 127.21 (phenyl-CH), 125.94 (phenyl-CH), 122.22 (phenyl-CH), 96.62 (methine CH), 82.39 (Cp-CH), 70.89 (Cp-CH), 70.06 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.63 (Cp-CH), 19.48 (CH<sub>3</sub>); **Analysis Calculated for C<sub>20</sub>H<sub>18</sub>BrFeNO:** C 56.64 H 4.28 N 3.30%; **Found:** C 56.10 H 4.30 N 3.00%; **HRMS [ES<sup>+</sup>]:** 425.9996 [M+H]<sup>+</sup>

**[C<sub>20</sub>H<sub>18</sub>FFeNO] (12):** After addition of HCl, the reaction was heated to 80 °C and stirred overnight. The solution was filtered and the remaining solid washed with toluene and petroleum ether. The solvent was removed, leaving a red-brown oil, before being purified by column chromatography, eluting with 80:20 dichloromethane/petroleum ether to give an orange solid. **Yield:** 0.49g, 34%; **<sup>1</sup>H (300 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 12.73 (s, 1H, NH), 7.29 (dd, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 9.0 and <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 4.9, phenyl-CH), 7.23 (br. tt, 2H, phenyl-CH), 5.65 (s, 1H, methine CH), 4.79 (t, 2H, <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.9, Cp-CH), 4.41 (t, 2H, <sup>4</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 1.9, Cp-CH), 4.16 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.10 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (500 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 193.3 (Q, CO), 162.0 (Q, C-Cl), 159.8 (phenyl-CH), 136.6 (Q, CNH), 126.9 (phenyl-CH), 116.6 (phenyl-CH), 96.3 (methine CH), 83.6 (Cp-CH), 72.8 (Cp-CH), 70.9 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.4 (Cp-CH), 20.1 (CH<sub>3</sub>); **Analysis Calculated for C<sub>20</sub>H<sub>18</sub>FFeNO:** C 67.65, H 5.00, N 3.86%; **Found:** C 64.90, H 5.10, N 5.70%; **HRMS [ES<sup>+</sup>]:** 364.0798 [M+H]<sup>+</sup>

**[C<sub>20</sub>H<sub>18</sub>ClFeNO] (13):** The reaction mixture was stirred at room temperature overnight. TLC showed the presence of 1-ferrocenylbutane-1,3-dione but no aniline. A further 0.93 g (7.4 mmol) of 4-chloroaniline was added and the reaction mixture stirred for another 2 days. The solvent was removed *in vacuo* and the resulting solid purified by column chromatography, eluting with ethyl acetate/hexane (1:9). The resulting solid was recrystallized from hot ethanol, giving red crystals. **Yield:** 0.69 g, 49%; **<sup>1</sup>H (300 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 12.87 (s, 1H, NH), 7.45 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.8, phenyl-CH), 7.30 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.8, phenyl-CH), 5.71 (s,

1H, methine CH), 4.90–4.80 (m, 2H, Cp-CH), 4.52–4.42 (m, 2H, Cp-CH), 4.20 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.21 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (100 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 193.54 (Q, CO), 159.00 (Q, CNH), 139.42 (Q, phenyl-C), 130.04 (phenyl-CH), 125.94 (phenyl-CH), 97.09 (methine CH), 83.41 (Q, Cp), 71.70 (Cp-CH), 70.50 (Cp-C<sub>5</sub>H<sub>5</sub>), 69.36 (Cp-CH), 20.30 (CH<sub>3</sub>). **Analysis Calculated for C<sub>20</sub>H<sub>18</sub>ClFeNO:** C 63.27, H 4.78, N 3.69, Cl 9.34%; **Found:** C 63.00, H 4.90, N 3.40, Cl 9.25%. **HRMS [ES<sup>+</sup>]:** 380.0510 [M-H<sup>+</sup>]

**[C<sub>20</sub>H<sub>18</sub>ClFeNO] (14):** After addition of HCl, the mixture was stirred for 48 hours at room temperature. The solvent was removed *in vacuo* and the resulting solid was dissolved in ethyl acetate/hexane (1:4), and filtered through a silica plug. The solvent was removed and the red solid recrystallized from hot ethanol yielding red crystals. **Yield:** 0.72 g, 46%; **<sup>1</sup>H (300 MHz, (CD<sub>3</sub>)<sub>2</sub>CO, δ):** 12.83 (s, 1H, NH), 7.55 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.8, phenyl-CH), 7.21 (d, 2H, <sup>3</sup>J(<sup>1</sup>H-<sup>1</sup>H) = 8.8, phenyl-CH), 5.68 (s, 1H, methine CH), 4.84–4.76 (m, 2H, Cp-CH), 4.47–4.39 (m, 2H, Cp-CH), 4.17 (s, 5H, Cp-C<sub>5</sub>H<sub>5</sub>), 2.18 (s, 3H, CH<sub>3</sub>); **<sup>13</sup>C{<sup>1</sup>H} NMR (300 MHz, DMSO-d<sub>6</sub>, δ):** 192.72 (Q, CO), 158.51 (Q, CNH), 138.80 (Q, phenyl-C), 132.50 (phenyl-CH), 125.65 (phenyl-CH), 117.16 (phenyl-CH), 96.73 (methine CH), 82.52 (Q, Cp), 71.39 (Cp-CH), 70.02 (Cp-C<sub>5</sub>H<sub>5</sub>), 68.82 (Cp-CH), 20.41 (CH<sub>3</sub>); **Analysis Calculated for C<sub>20</sub>H<sub>18</sub>ClFeNO:** C 56.64, H 4.28, N 3.30, Br 18.84%; **Found:** C 56.50, H 4.35, N 3.10, Br 18.70%. **HRMS [ES<sup>+</sup>]:** 425.999 [M-H<sup>+</sup>]

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**Keywords:** β-diketonate ligands • β-ketoiminate ligands • Bioinorganic chemistry • Cancer • Ferrocenyl compounds

- [1] B. Rosenberg, L. Van Camp, T. Krigas, *Nature* **1965**, *205*, 698–699.
- [2] A. Bergamo, R. Gagliardi, V. Scarcia, A. Furlani, E. Alessio, G. Mestroni, G. Sava, *J. Pharmacol. Exp. Ther.* **1999**, *289*, 559–564.
- [3] S. Dasari, P. Bernard Tchounwou, *Eur. J. Pharmacol.* **2014**, *740*, 364–378.
- [4] P. Comella, R. Casaretti, C. Sandomenico, A. Avallone, L. Franco, *Ther. Clin. Risk Manag.* **2009**, *5*, 229–238.
- [5] D. J. Stewart, *Crit. Rev. Oncol. Hematol.* **2007**, *63*, 12–31.
- [6] J. T. Hartmann, H.-P. Lipp, *Expert Opin. Pharmacother.* **2003**, *4*, 889–901.
- [7] M. J. Clarke, F. Zhu, D. R. Frasca, *Chem. Rev.* **1999**, *99*, 2511–2534.
- [8] P. Köpf-Maier, *Eur. J. Clin. Pharmacol.* **1994**, *47*, 1–16.
- [9] A. M. Evangelou, *Crit. Rev. Oncol. Hematol.* **2002**, *42*, 249–265.
- [10] C.-H. Leung, H.-J. Zhong, D. S.-H. Chan, D.-L. Ma, *Coord. Chem. Rev.* **2013**, *257*, 1764–1776.
- [11] B. Bertrand, A. Casini, *Dalton Trans* **2014**, *43*, 4209–4219.
- [12] S. Top, A. Vessières, C. Cabestaing, I. Laios, G. Leclercq, C. Provot, G. Jaouen, *J. Organomet. Chem.* **2001**, *637*–639, 500–506.
- [13] S. Top, A. Vessières, P. Pigeon, M.-N. Rager, M. Huché, E. Salomon, C. Cabestaing, J. Vaissermann, G. Jaouen, *ChemBioChem* **n.d.**, *5*, 1104–1113.
- [14] S. Top, J. Tang, A. Vessières, D. Carrez, C. Provot, G. Jaouen, *Chem. Commun.* **1996**, *0*, 955–956.
- [15] A. Vessières, C. Corbet, J. M. Heldt, N. Lories, N. Jouy, I. Laios, G. Leclercq, G. Jaouen, R.-A. Toillon, *J. Inorg. Biochem.* **2010**, *104*, 503–511.
- [16] E. Allard, C. Passirani, E. Garcion, P. Pigeon, A. Vessières, G. Jaouen, J.-P. Benoit, *J. Controlled Release* **2008**, *130*, 146–153.
- [17] E. Allard, N. T. Huynh, A. Vessières, P. Pigeon, G. Jaouen, J.-P. Benoit, C. Passirani, *Int. J. Pharm.* **2009**, *379*, 317–323.

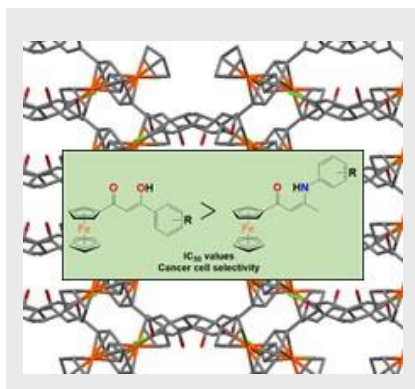
## COMMUNICATION

- [18] G. Jaouen, A. Vessi res, S. Top, *Chem Soc Rev* **2015**, *44*, 8802–8817.
- [19] J. C. Swarts, T. G. Vosloo, S. J. Cronje, W. C. Du Plessis, C. E. J. Van Rensburg, E. Kreft, J. E. Van Lier, *Anticancer Res.* **2008**, *28*, 2781–2784.
- [20] H. Tamura, M. Miwa, *Chem. Lett.* **1997**, *26*, 1177–1178.
- [19] S. Sansook, E. Lineham, S. Hassell-Hart, G. J. Tizzard, S. J. Coles, J. Spencer, S. J. Morley, *Moecules.* **2018**, *23*(9), 2126
- [22] S. Peter, B. A. Aderibigbe, *Molecules* **2019**, *24*, 3604.
- [23] G. Gasser, I. Ott, N. Metzler-Nolte, *J. Med. Chem.* **2011**, *54*, 3–25.
- [24] J. Hess, J. Keiser, G. Gasser, *Future Med. Chem.* **2015**, *7*, 821–830.
- [25] R. Epton, M. E. Hobson, G. Marr, *J. Organomet. Chem.* **1978**, *149*, 231–244.
- [26] J. R. Pladziewicz, M. J. Carney, *J. Am. Chem. Soc.* **1982**, *104*, 3544–3545.
- [27] J. R. Pladziewicz, A. J. Abrahamson, R. A. Davis, M. D. Likar, *Inorg. Chem.* **1987**, *26*, 2058–2062.
- [28] D. Hamels, P. M. Dansette, E. A. Hillard, S. Top, A. Vessi res, P. Herson, G. Jaouen, D. Mansuy, *Angew. Chem. Int. Ed.* **2009**, *48*, 9124–9126.
- [29] A. Citta, A. Folda, A. Bindoli, P. Pigeon, S. Top, A. Vessi res, M. Salmain, G. Jaouen, M. P. Rigobello, *J. Med. Chem.* **2014**, *57*, 8849–8859.
- [30] V. Scalcon, M. Salmain, A. Folda, S. Top, P. Pigeon, H. Z. Shirley Lee, G. Jaouen, A. Bindoli, A. Vessi res, M. P. Rigobello, *Metallomics* **2017**, *9*, 949–959.
- [31] R. M. Lord, J. J. Mannion, A. J. Hebden, A. E. Nako, B. D. Crossley, M. W. McMullon, F. D. Janeway, R. M. Phillips, P. C. McGowan, *ChemMedChem* **2014**, *9*, 1136–1139.
- [32] R. M. Lord, A. J. Hebden, C. M. Pask, I. R. Henderson, S. J. Allison, S. L. Shepherd, R. M. Phillips, P. C. McGowan, *J. Med. Chem.* **2015**, *58*, 4940–4953.
- [33] R. M. Lord, J. J. Mannion, B. D. Crossley, A. J. Hebden, McMullon, J. Fisher, R. M. Phillips, P. C. McGowan, *ChemistrySelect* **2016**, *1*, 6598–6605.
- [34] C. Valdebenito, M. T. Garland, M. Fuentealba, A. H. Klahn, C. Manzur, *Acta Crystallogr. Sect. E* **2010**, *E66*, m1655.
- [35] F. Wang, S. Islam, V. Vasilyev, *Mater. Basel Switz.* **2015**, *8*, 7723–7737.
- [36] J. D. Bourke, M. T. Islam, S. P. Best, C. Q. Tran, F. Wang, C. T. Chantler, *J. Phys. Chem. Lett.* **2016**, *7*, 2792–2796.
- [37] G. Alagona, C. Ghio, *Int. J. Quantum Chem.* **2008**, *108*, 1840–1855.
- [38] T. Tsukahara, K. Nagaoka, K. Morikawa, K. Mawatari, T. Kitamori, *J. Phys. Chem. B* **2015**, *119*, 14750–14755.
- [39] A. Matwijczuk, D. Karcz, R. Walkowiak, J. Furso, B. G dyszewska, S. Wybraniec, A. Niewiadomy, G. P. Karwasz, M. Gago , *J. Phys. Chem. A* **2017**, *121*, 1402–1411.
- [40] H. Huang, N. Humbert, V. Bizet, M. Patra, H. Chao, C. Mazet, G. Gasser, *J. Organomet. Chem.* **2017**, *839*, 15–18.

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We report the synthesis of new  $\beta$ -diketonate and  $\beta$ -ketoiminate compounds, which have been functionalized with a ferrocenyl moiety. The ferrocenyl  $\beta$ -diketonate compounds have high  $IC_{50}$  values and are more selective towards cancerous cells, in particular human breast carcinomas. Some compounds are up to 9x more cytotoxic and selective when compared to carboplatin.



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**$\beta$ -diketonate versus  $\beta$ -ketoiminate:  
the importance of a ferrocenyl moiety  
on improving the anticancer potency**