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ABSTRACT: A ferrocene containing ortho-aminoanilide, N^1-(2-aminophenyl)-N^8-ferrocenyloctanediamide, 2b (Pojamide) displayed nanomolar potency vs. HDAC3. Compared to RGFP966, a potent and selective HDAC3 inhibitor, Pojamide displayed superior activity in HCT116 colorectal cancer cell invasion assays; however, TCH106 and Romidepsin, potent HDAC1 inhibitors, outperformed Pojamide in cellular proliferation and colony formation assays. Together, these data suggest that HDAC 1 & 3 inhibition is desirable to achieve maximum anti-cancer benefits. Additionally, we explored Pojamide-induced redox-pharmacology. Indeed, treating HCT116 cells with Pojamide, SNP (sodium nitroprusside) and glutathione (GSH) led to greatly enhanced cytotoxicity and DNA damage attributed to activation to an Fe(III) species.

Introduction

Histone deacetylases (HDACs) are key targets in cancer and neurodegeneration^1. Their overexpression leads to increased deacetylated lysine/arginine levels and a condensed chromatin state resulting in transcriptional silencing. Clinically useful HDAC inhibitors (HDACis) include SAHA^2 (Fig. 1) and Romidepsin^3, which trigger growth arrest and apoptosis via histone hyperacetylation by pan-inhibition principally of Class I and II HDACs and other targets (e.g. p53).

Our increased knowledge of HDAC biology has emphasised the need for isoform-selective histone deacetylase inhibitors (HDACis)^4. Selective inhibitors of HDAC3, (e.g. the benzamides TCH106 and RGFP966) (Fig. 1)^5-6, are particularly attractive in CNS applications^7 and cancer^8. However, recent studies using HDAC3-overexpressing HCT116 cells show that silencing individual HDACs (1-3) through RNAi is insufficient to achieve similar levels of growth arrest and apoptosis induced by generic HDACis such as Trichostatin A^9. It is therefore likely that the inhibition of specific combinations of HDAC isoforms, HDACs 1-3 in particular, may help to achieve the full benefits of HDAC inhibition in colorectal carcinomas and possibly other cancer cells.

![Figure 1. SAHA, and known HDACis.](image-url)
Results and Discussion

Transition metal-based anticancer agents are interesting due to their novel ligand exchange and redox chemistry, the ability of a heavy metal atom to facilitate phasing in protein x-ray crystallography and the availability of geometries and oxidation states unachievable with carbon-based therapeutics10-13. Ferrocene-based JAHAs and other metal-based analogues, many containing a hydroxamic acid zinc-binding group (ZBG), are effective HDACis with good activity vs. Class I HDACs14-25. Guided by docking studies of a standard “cap-linker-ZBG” arrangement, we wished to extend the chemistry of JAHAs to ortho-anilide analogues, anticipating that this may lead to HDAC3-selectivity and alleviate toxicity issues documented for hydroxamate ZBGs26. Hence, compounds 1a and 2a were readily made by standard coupling reactions and were characterised in the solid state by x-ray crystallography (Figure 2)27.

To explore the binding modes of 1b and 2b (Pojamide) in HDAC3 we performed docking studies using the structure of HDAC3 bound to co-repressor and inositol tetraphosphate (Figure 3A,B)29. We found that Pojamide bound to the zinc active site forming hydrogen bonds between the N-H of the amide and the carbonyl of Asp93, the benzamide amide N-H and the nitrogen of His134, and the carbonyl of Asp170 as well as the characteristic benzamide-zinc interaction.

The docked structure of 1b is docked slightly shifted from Pojamide. It forms hydrogen bonds with Asp93 and Gly143. However, the substitution of the 6-carbon aliphatic chain for a 4-carbon chain results in a rotation of the benzamide group leading to a loss of key interactions, namely, hydrogen bonding with His134 and Asp170 as well as forcing zinc coordination from the benzamide aniline to the amide carbonyl (Figure 3A,B). Comparison of the active sites of HDAC3 and 8 reveals that ferrocenyl substitution for the archetypal aryl cap in HDAC inhibitors in Pojamide clashes with Tyr100 and Lys33 in HDAC8; assuming a similar binding mode to that of Pojamide in HDAC3. Thus it is assumed that ferrocenyl substitution in Pojamide and 1b is responsible for HDAC3 selectivity (Figure 3C).

In addition to structural evidence supporting selective HDAC3-binding, we confirmed Pojamide’s cell permeation and HDAC3-specificity using Xenopus laevis embryos (2-cell to stage 14) as a model system for deacetylase activity. Xenopus laevis embryos were incubated with 1b and Pojamide in order to test their bioavailability and JAHAs, a broad HDAC inhibitor, was used as a positive control10. As expected Pojamide and 1b, which have some selectivity towards HDAC3 (vide infra), did not affect α-tubulin acetylation, whereas JAHAs increased acetylation of α-tubulin (Figure S1A). Acetylated H4K12 (H4K12ac) has, however, been shown to be a target of HDAC3 and was expected to increase if these HDAC3is were able to function in the whole organism31. Compound 1b gave no sign of affecting H4K12 acetylation levels unlike Pojamide, which demonstrated a concentration-dependent accumulation of H4K12ac (Figure S1B). In three separate experiments the level of H4K12ac, as detected by western blotting, increased in developing embryos treated with Pojamide; however, embryo development was severely affected and many died (as low as 10% survival rates). For this reason, it was impossible to obtain a clear concentration-dependency for Pojamide, nonetheless we conclude that Pojamide is highly likely to be cell-permeable as an HDAC3 inhibitor, but that compound 1b is not.

Figure 2. Ferrocene-based HDACis and precursors.

Figure 3. A,B) Docking poses of 1b and Pojamide in HDAC3 (PDB code: 4A69). Top docking poses of 1b (teal, A) and Pojamide (slate, B) in HDAC3. C) Superposition of the active sites of HDAC3 (gray) and HDAC828 (PDB code: 1T69, green) showing key residues overlaid with Pojamide top docking pose in HDAC3. Color Scheme: Hydrogen bonds shown in green dashed lines, π-π interactions shown in orange dashed lines.
Next, we tested Pojamide on a panel of HDACs vs. 1b, SAHA and HDAC3is (Table 1). Indeed both 1b and Pojamide displayed ca. 11- and 22-fold selectivity respectively towards HDAC3 over HDACs 1/2 and significantly greater selectivity over HDACs 4-8. Pojamide’s profile was on par with other ortho-anilide (benzamide) inhibitors, being most similar to that of TCH106, yet was outperformed by RGFP966 with respect to HDAC3-selectivity. Only SAHA displayed activity vs. HDAC8 (Table 1).

With *in vitro* validation of the anti-HDAC3 activity of these ferrocene-analogues, we sought to explore their inhibitory activity in HDAC3-overexpressing cervical and colorectal cancer cell lines. HeLa, HT-29 and HCT116 cells are tumor-forming cell lines that have been used previously as model systems to characterise HDACis9,33-35. We confirm in this study that compared to hTERT immortalised Retinal Pigment Epithelium (RPE) cells – cells with a longer lifespan, but incapable of forming tumors36 – the malignant cancer cell lines HeLa, HT-29 and HCT116 all showed significantly elevated HDAC3 expression levels (Figure 4).

![Figure 4](image-url) Western blot analysis of HDAC3 and α-Tubulin (Tub) in Retinal Pigment Epithelium (RPE), HeLa, HT-29 and HCT116 cells.

<table>
<thead>
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<th>Table 1. Biochemical evaluation of HDAC isoforms 1 – 8.</th>
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<td>IC50 (µM)</td>
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<tr>
<td>HDAC</td>
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<td>------</td>
</tr>
<tr>
<td>1</td>
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<td>2</td>
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<tr>
<td>3</td>
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<td>4</td>
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<td>5</td>
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[a]Profiling done in duplicate, n=8. All others n=4; [b] Slow, tight-binding inhibitor, with inverted IC50 and Ki values, causes IC50 value to drop over longer pre-incubation periods. The IC50 value was defined as the amount of compound that caused 50% reduction in HDAC activity in comparison with DMSO-treated control and was calculated using GraphPad Prism version 6 software.

Based on these data, this small panel of cell lines was treated with increasing concentrations of 1b, Pojamide and the control HDACis RGFP966 and TCH106. Despite RGFP966 showing 6.5-fold greater potency than Pojamide in blocking HDAC3 activity (Table 1), we found that RGFP966 and Pojamide are equipotent at inhibiting HCT116 cellular proliferation, displaying GC50 values of 8.9 and 8.6 µM respectively (Figure 5A, Table 2). RPE cells on the other hand were as sensitive to Pojamide as HCT116 cells, but were not inhibited by RGFP966 (Figure 5B, Table 2). TCH106 displayed superior potency against all cell lines with GC50 values ranging from 1 – 2 µM and completely blocked colony formation at 10 µM in HCT116 colony formation assays compared to 75 and 60% inhibition of colony formation by Pojamide and RGFP966 respectively (Figures 5A-E, S2 A-C). Interestingly, TCH106 is 1.6- and 2.8-fold more potent toward HDAC1 inhibition than RGFP966 and Pojamide respectively (Table 1) and proliferating RPE cells have been shown to overexpress HDACs 1, 2 and 537, suggesting that HDAC1 inhibition is in part responsible for the anti-proliferative effects of Pojamide and TCH106 in these cell lines. In fact, cellular proliferation and colony formation assays using Romidepsin, a potent and exquisitely selective HDAC1/2 inhibitor (reported IC50 values of 36 and 47 nM respectively38), revealed a GC50 value of 0.52 ± 0.02 nM and near complete inhibition of colony formation at 0.5 nM; clearly the most potent anti-proliferative HDACi tested in this study (Figures 5F, S3 A,B). HeLa and HT-29 cells were only mildly inhibited by Pojamide and RGFP966 showing maximal growth inhibition of ~40 and 30% at 10 µM respectively, and in all cases compound 1b was ineffective at inhibiting cellular proliferation (Figure 5A-D). This result was mirrored in *Xenopus laevis* embryo development assays, whereby Pojamide caused a concentration-dependent increase in acetylated H4K12 levels and 1b did not (Figure S1).
To establish the anti-invasive properties of **Pojamide** against known HDACis, we investigated the effects of **RGFP966**, **TCH106** and **Pojamide** on HCT116 cellular invasion. In this assay, we decided to test compounds at 1x and 0.1x of their GC50 value determined using the HCT116 cellular proliferation assay. At the lowest concentrations tested, only **Pojamide** demonstrated robust inhibition of invasion, with about 70% inhibitory activity; however, at the 1x concentrations both **RGFP966** and **Pojamide**, exhibiting ca. 40 and 11-fold selectivity for HDAC3 inhibition respectively compared to HDAC1 (Table 1), inhibited invasion by about 90% compared to 70% for **TCH106**, which showed only 7-fold selectivity for HDAC3 vs. HDAC1 inhibition (Figure 5G-I). Based on these data and taking into consideration the activity of these compounds in the cellular proliferation assay, we conclude that HDAC isoform synergistic effects can be exploited using HDAC1- and HDAC3-selective HDACis; proliferation being attenuated more so by HDAC1, and possibly HDAC2, inhibition and invasion blocked more robustly through HDAC3 inhibition.

**Table 2.** Cellular characterization of HDACis

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<th>Cell Line</th>
<th>GC50 (µM) or (nM)[b]</th>
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<tr>
<td>RPE</td>
<td>9.0 ± 0.7</td>
</tr>
<tr>
<td>HCT116</td>
<td>8.6 ± 1.2</td>
</tr>
<tr>
<td>HeLa</td>
<td>na</td>
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<tr>
<td>HT-29</td>
<td>1.5 ± 0.2</td>
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[a]The GC50 value was defined as the amount of compound that caused 50% reduction in cellular proliferation in comparison with DMSO-treated control and was calculated using GraphPad Prism version 6 software; na = not applicable and nd = not determined.

**Pojamide** appears more efficacious in preventing HCT116 cellular invasion, particularly at lower concentrations (i.e. 1 µM). In addition, we wanted to explore the possibility that engaging a different oxidation state of the iron atom in the ferrocene moiety might offer a unique advantage at targeting cancer cells. Seminal studies with Ehrlich ascites tumor (EAT) cells and HPB (human leukemic T lymphocytes) showed that incubation with ferrocenium salts (e.g. Fe(III)Cp2PF6) inhibited tumor growth, whereas their ferrocene counterparts were ineffective. Indeed, ferrocenium’s toxicity involves the generation of ·OH radicals and the rapid induction of DNA-damage; repeated later in MCF7 and MCF10A cells.

In order to take advantage of ferrocenium cytotoxicity, we generated the ferrocenium species 3b (Fe(III)-Poj) through standard means by reacting **Pojamide** with nitrosonium (NO⁺) tetrafluoroborate (Scheme 1), which was confirmed by cyclic voltammetry (Figure S4). Hence, we hypothesised that intracellular generation of NO⁺ in the presence of **Pojamide** might also lead to Fe(III)-Poj in cells.

**Scheme 1. Synthetic ferrocenium Pojamides.**

It has previously been reported that sodium nitroprusside (SNP) leads to intracellular NO⁺ release, yet studies in neuronal PC12 cells have shown that SNP alone triggers apoptosis at concentrations greater than 30 µM. To develop a cell-based assay utilising SNP as an NO⁺ donor, we conducted cytotoxicity studies using the colony formation assay (Figures S5A). At 25 µM SNP, just shy of the concentration that triggers apoptosis in PC12 cells, HCT116 colony formation is reduced by about 30%; however, addition of 500 µM GSH completely eliminated SNP cytotoxicity (Figure S5A,B). GSH is a free radical scavenger, detoxifies H₂O₂ in a glutathione peroxidase-1 dependent manner and its cytoprotective effects were demonstrated in our assay at concentrations of 50 and 500 µM, whereby GSH treatments, in the presence of...
SNP, enhanced colony formation by about 4 and 10% respectively (Figure S5A,B). The reaction of SNP with GSH to form innocuous NO may further reduce SNP cytotoxicity.\(^{44}\)

Figure 6. A-C) Western analysis of pH2AX and α-tubulin (Tub) in HCT116 cells treated for 3 d with (A) RGFP966, (B) Pojamide and (C) Pojamide + SNP/GSH. D) The pH2AX/Tub ratio was determined via densitometry and the average ratio normalised to DMSO control was plotted as the mean ± S.D. E) Western analysis of pH2AX and α-tubulin (Tub) in HCT116 cells treated for 6 d as indicated above. F,G) NAD+ fold-induction (F), total NAD+/NADH (F) and reactive oxygen species (ROS) (G) levels normalised to the DMSO control; the average (n=10) was plotted ± S.D. The t-test statistical module of Prism 6.0 was used to determine p-values (ns (not statistically significant): P > 0.05; *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001).

After identifying the optimal conditions for SNP/GSH treatment, we treated HCT116 cells with increasing concentrations of inhibitor in the presence and absence of SNP/GSH. Generation of Fe(III)-Poj was monitored by blotting for the DNA-damage marker phosphoγH2AX (pH2AX)\(^{45}\) (Figure 6 A-D).

Without SNP/GSH, increasing concentrations of RGFP966 and Pojamide reduced pH2AX levels, but addition of SNP/GSH to cells treated with Pojamide led to an initial decrease in pH2AX, with recurrence of the DNA-damage marker at concentrations greater than 2 \(\mu M\) (Figure 6A-D). In a longer time-course (6 d) with 4 \(\mu M\) inhibitor (+ SNP/GSH), only the Pojamide/SNP/GSH combination led to significant DNA-damage nearly tantamount to the levels of DNA-damage caused by cisplatin (4 \(\mu M\)) (Figure 6E).

To further support that our SNP/GSH treatment in the presence of Pojamide leads to production of Fe(III)-Poj, we assessed the levels of NAD\(^+\) in cells using the NAD/NADH-Glo Assay. Early studies on the one-electron transfer from NADH to ferrocenium oxidants such as Fe(III)Cp\(_2\)PF\(_6\) demonstrated that ferrocenium salts can successfully oxidise NADH to NAD\(^+\) at physiologic pH in a phosphate buffer in vitro\(^{46}\). To recapitulate this conversion in cells, we treated HCT116 cells with DMSO/SNP/GSH and 2.5 \(\mu M\) Pojamide with and without SNP/GSH. For each condition, total NAD+/NADH levels remained the same, but Pojamide in the presence of SNP/GSH led to an increase in NAD\(^+\) levels; similar to those obtained with synthetic Fe(III)-Poj. In contrast, Pojamide treatment alone decreased NAD\(^+\) levels (Figure 6F).

In order to correlate this increase in NAD\(^+\) with oxidative stress, reactive oxygen species (ROS) levels were determined in HCT116 cells treated with Pojamide, the non-transition metal-based HDAC3i RGFP966, and other transition metal-based compounds JAHA and ruthenocene. Indeed, without SNP/GSH, both ferrocene-ligands Pojamide and JAHA caused an induction in ROS; an effect documented with other ferrocene-ligands such as ferrocifens and aminoferrocene prodrugs, which produce quinone methides, and in the latter case, ferrocium catalysts for ROS production, in the absence of SNP\(^{47,48}\). DMSO, RGFP966 and ruthenocene were assayed and showed no ROS induction without SNP/GSH. In the presence of SNP/GSH, ROS levels increased by 2.5-fold with and without RGFP966 and, interestingly, all other conditions with SNP/GSH, Pojamide, JAHA and ruthenocene, led to greater ROS levels with Pojamide displaying the most significant increase, 3.3-fold, despite co-treatment with excess GSH (Figure 6G).

Figure 7. A) HCT116 colony formation assays; % Colony formation (B) (normalised to DMSO control) was quantitated manually and the
average ± S.D. was plotted. The t-test statistical module of Prism 6.0 was used to determine p-values (ns (not statistically significant): P > 0.05; *: P ≤ 0.05; **: P ≤ 0.01; ***: P ≤ 0.001).

**Figure 8.** Model for the enhanced redox-triggered cytotoxicity of Pojamide.

With cellular validation of Fe(III)-Poj generation, we tested our system in the HCT116 colony formation assay (Figures 7A,B). Pojamide alone caused a significant decrease in colony formation, which was enhanced by addition of SNP/GSH; 27 and 53% inhibition respectively. At 4 µM, RGFP966, with and without SNP/GSH, displayed a 10 – 15% reduction in colony formation; however, like Pojamide, both ferrocenium salts were less effective without SNP/GSH. In combination with SNP/GSH, Fe(III)-Poj and Fe(III)Cp2PF6 caused a slight, about 10%, reduction in colony formation (Figure 7A,B). Interestingly, colonies resulting from treatment with ferrocenium salts and SNP/GSH were larger, but overall fewer colonies formed; a result similar to RGFP966 treatment. Although the cytotoxicity of ferrocenium salts is well documented, their activity is ~100-fold reduced compared to Pojamide59, perhaps due to limited membrane permeability, and explains their meagre inhibitory activities at the 4 and 20 µM concentrations tested.

**Conclusions**

In summary, the SNP/GSH combination treatment is an ideal system for increasing the intracellular ferrocenium concentration from a Pojamide precursor. Also, when Fe(II)-Poj is reduced back to the Fe(II) species, it would be available to act upon HDACs. Due to having two separate modes of action, one which is SNP/GSH-dependent, Pojamide is advantageous compared to other similarly potent HDACis: its cytotoxicity is enhanced by its facile conversion to the cytotoxic Fe(III) species in cells, whilst the reduced species inhibits cellular invasion through potently targeting HDAC3 and proliferation, to a lesser extent, due to its low micromolar HDAC1 inhibitory activity (Figure 8). Lastly, cotreatments of intravenous SNP injection along with Pojamide administration might offer a highly efficacious strategy for managing some colon carcinomas; a strategy that might have broader, generalizable applications when used with pharmacologically distinct Fe(II)Cp2-containing drugs (e.g. aminooferrocenes, ferrocenamines, ferroquine).47-50 Current studies are looking at ruthenium-based HDACis and will be reported in due course.

Experimental

Solvents and reagents were purchased from commercial suppliers and were used without purification. Ferrocenylamine was purchased from TCI, UK, and used as such. All reactions were performed in a fume hood. NMR spectra were recorded on Varian 500 MHz or 400 MHz spectrometers and chemical shifts are reported in ppm, usually referenced to TMS as an internal standard. LCMS were performed by Shimadzu LCMS-2020 equipped with a Gemini® 5µm C18 110Å column and percentage purities were run over 30 minutes in water/acetonitrile with 0.1% formic acid (5 min at 5%, 5%-95% over 20 min, 5 min at 95%) with the UV detector at 254 nm. High-resolution mass spectrometry (HRMS) was performed by the EPSRC National Mass Spectrometry Facility, University of Swansea. Elemental analyses were conducted by Stephen Boyer (London Metropolitan University). FT-IR were recorded on a PerkinElmer Spectrum Version 10.03.06.

**N’-(2-Aminophenyl)-N’-ferrocenyldipamidamide 1b.** The previous compound, tert-butyl-2-(6-oxo-6-(phenylamino)hexanamido)ferrocenylcarbamate, (520 mg, 1.00 mmol, 1 equiv.) was suspended in dichloromethane (40 mL) and MeOH (4 mL). To this mixture 4N HCl/dioxane (8 mL) was added and the mixture was stirred at room temperature overnight. The volatiles were removed in vacuo, then sat. Na2CO3 (aq.) was added to the residue and the mixture was sonicated. The precipitate was collected by suction and washed on the frit with water, dried, triturated with CH2Cl2 to give the title compound as a brown solid (318 mg, 76%). 1H NMR (DMSO-d6): δ = 9.44 (1H, s, NH), 9.22 (1H, s, NH), 7.53 (1H, d, J=7.8 Hz, CHAr), 7.41 (1H, d, J=7.8 Hz, CHAr), 7.12-7.04 (1H, m, CHAr), 7.06 (1H, d, J=7.8 Hz, CHAr), 4.57 (2H, s, 2CH (Cp)), 4.08 (5H, s, unsubst. Cp), 3.93 (2H, s, 2CH (Cp)), 2.37 (2H, d, J=8.3 Hz, CH2), 2.19 (2H, t, J=6.1 Hz, CH3), 1.65-1.56 (4H, m, 2CH2), 1.45 (9H, s, 3CH3). 13C NMR (DMSO-d6, 126 MHz): δ = 171.0, 153.5, 130.1, 125.4, 125.3, 124.3, 124.1, 96.1, 79.8, 69.2, 64.1, 61.1, 36.2, 28.7, 28.5, 25.3.

**Tert-butyl-2-(8-oxo-8-(phenylamino)octanamido) ferrocenylcarbamate 2a.** Methyl-8-oxo-8-(ferrocenylamino)octanoic acid (215 mg, 0.6 mmol, 1 equiv.) and N-Boc-o-phenylenediamine (137.4 mg, 0.66 mmol, 1.1 equiv.) were dissolved in dichloromethane (7.7 mL). To this triethylamine (0.5 mL, 3.6 mmol) was added and the mixture was cooled in an ice bath. Next, propane phosphonic acid anhydride (T3P) (50% solution in DMF, 1.38 mL, 1.1 mmol) was added and the reaction mixture was allowed to warm up to room temperature overnight. Then the mixture was poured into a saturated solution of K2CO3, stirred for 30 min. and extracted into CH2Cl2 (DCM). The organic layer was dried (MgSO4), filtered and evaporated in vacuo. The residue was purified by trituration with DCM to give an orange solid (576.3 mg, 74%). Crystallization by solvent evaporation of DCM provided yellow crystals. 1H NMR (DMSO-d6, 500 MHz): δ = 9.44 (1H, s, NH), 9.22 (1H, s, NH), 8.31 (1H, s, NH), 7.53 (1H, d, J=7.8 Hz, CHAr), 7.41 (1H, d, J=7.8 Hz, CHAr), 7.12-7.04 (1H, m, CHAr), 7.06 (1H, d, J=7.8 Hz, CHAr), 4.57 (2H, s, 2CH (Cp)), 4.08 (5H, s, unsubst. Cp), 3.93 (2H, s, 2CH (Cp)), 2.37 (2H, d, J=8.3 Hz, CH2), 2.19 (2H, t, J=6.1 Hz, CH3), 1.65-1.56 (4H, m, 2CH2), 1.45 (9H, s, 3CH3). 13C NMR (DMSO-d6, 126 MHz): δ = 171.0, 153.5, 130.1, 125.4, 125.3, 124.3, 124.1, 96.1, 79.8, 69.2, 64.1, 61.1, 36.2, 28.7, 28.5, 25.3.
71%). Crystallization by solvent evaporation of DCM provided yellow crystals. 1H NMR (DMSO-d6): 9.40 (1H, s, NH), 9.15 (1H, s, NH), 8.26 (1H, s, NH), 7.54-7.48 (1H, m, CHAr), 7.42-7.35 (1H, m, CHAr), 7.13-7.09 (1H, m, CHAr), 7.08-7.03 (1H, m, CHAr), 4.55 (2H, s, 2CH (Cp)), 4.06 (5H, s, unsubst. Cp), 3.91 (2H, s, 2CH (Cp)), 2.33 (2H, t, J=7.4 Hz, CH2), 2.14 (2H, t, J=7.4 Hz, CH2), 1.62-1.54 (4H, m, 2CH2), 1.44 (9H, s, 3CH3), 1.38-1.26 (4H, m, 2CH2). 13C NMR (DMSO-d6, 126 MHz): δ = 171.6, 171.2, 142.3, 126.1, 125.7, 124.1, 116.6, 116.3, 96.1, 69.1, 64.1, 61.0, 39.7, 39.5, 36.4, 36.2, 29.0, 28.9, 25.7, 25.6.

Cp), 3.91 (2H, s, 2CH (Cp)), 2.33 (2H, t, J=7.4 Hz, CH2), 2.14 (2H, t, J=7.4 Hz, CH2), 1.62-1.54 (4H, m, 2CH2), 1.44 (9H, s, 3CH3), 1.38-1.26 (4H, m, 2CH2). 13C NMR (DMSO-d6, 126 MHz): δ = 171.6, 171.2, 142.3, 126.1, 125.7, 124.1, 116.6, 116.3, 96.1, 69.1, 64.1, 61.0, 39.7, 39.5, 36.4, 36.2, 29.0, 28.9, 25.7, 25.6. HRMS -ESI (m/z): found for [C24H30FeN3O2]+ 448.1675, calc. for [C24H30FeN3O2]+ 448.1682. Anal. Calcd (%): C, 64.44; H, 6.53; N, 9.39. Found (%): C, 64.23; H, 6.60; N, 9.29.

N1-(2-Aminophenyl)-N8-ferrocenylcarbamate 2b (Pojamide)

**Tert-butyl-2-(8-oxo-8-(phenylamino)octanamido) ferroceniumcarbamate**

Tert-butyl-2-(8-oxo-8-(phenylamino)octanamido) ferroceniumcarbamate (136.8 mg, 0.25 mmol, 1 equiv.) was suspended in dichloromethane (10 mL) and MeOH (1 mL). To this mixture 4N HCl/dioxane (2 mL) was added and the mixture was stirred in vacuo. To this mixture carbamate (136.8 mg, 0.25 mmol, 1 equiv.) was suspended in dichloromethane (10 mL) and MeOH (1 mL). To this mixture terbutyl-2-(8-oxo-8-(phenylamino)octanamido)ferrocenylcarbamate (109.5 mg, 0.2 mmol, 1 equiv.) was suspended in dry DCM (5 mL). To this mixture NOBF4 (37.4 mg, 0.32 mmol, 1.6 equiv.) was added. The color of the solution changed from yellow to dark brown. Filtration afforded the title compound as a dark brown solid (78 mg, 73%). 19F NMR (DMSO-d6): -29.53. 11B NMR (DMSO-d6): -1.34. C2H5BF4FeN4O2: C, 66.97; H, 5.84; N, 9.31. FTIR (cm-1): 1038 (BF4). Found (%): C, 64.23; H, 6.60; N, 9.29.

Tert-butyl-2-(8-oxo-8-(phenylamino)octanamido) ferroceniumcarbamate tetrafluoroborate, 3b (Fe(III)-Poj).


