



Article

Platinum(IV) Prodrugs Incorporating an Indole-Based Derivative, 5-Benzyloxyindole-3-Acetic Acid in the Axial Position Exhibit Prominent Anticancer Activity

Angelico D. Aputen ¹, Maria George Elias ^{1,2}, Jayne Gilbert ³, Jennette A. Sakoff ³, Christopher P. Gordon ¹, Kieran F. Scott ^{2,4} and Janice R. Aldrich-Wright ^{1,*}

¹ School of Science, Western Sydney University, Sydney, NSW 2751, Australia; a.aputen@westernsydney.edu.au (A.D.A.); m.elias3@westernsydney.edu.au (M.G.E.); c.gordon@westernsydney.edu.au (C.P.G.)

² Ingham Institute, Sydney, NSW 2170, Australia; kieran.scott@westernsydney.edu.au

³ Calvary Mater Newcastle Hospital, Newcastle, NSW 2298, Australia; jayne.gilbert@newcastle.edu.au (J.G.); jennette.sakoff@newcastle.edu.au (J.A.S.)

⁴ School of Medicine, Western Sydney University, Sydney, NSW 2751, Australia

* Correspondence: j.aldrich-wright@westernsydney.edu.au; Tel.: +61-2462-03218

Abstract: Kinetically inert platinum(IV) complexes are a chemical strategy to overcome the impediments of standard platinum(II) antineoplastic drugs like cisplatin, oxaliplatin and carboplatin. In this study, we reported the syntheses and structural characterisation of three platinum(IV) complexes that incorporate 5-benzyloxyindole-3-acetic acid, a bioactive ligand that integrates an indole pharmacophore. The purity and chemical structures of the resultant complexes, **P-5B3A**, **5-5B3A** and **56-5B3A** were confirmed via spectroscopic means. The complexes were evaluated for anticancer activity against multiple human cell lines. All complexes proved to be considerably more active than cisplatin, oxaliplatin and carboplatin in most cell lines tested. Remarkably, **56-5B3A** demonstrated the greatest anticancer activity, displaying GI₅₀ values between 1.2 and 150 nM. Enhanced production of reactive oxygen species paired with the decline in mitochondrial activity as well as inhibition of histone deacetylase were also demonstrated by the complexes in HT29 colon cells.

Keywords: chemotherapy; platinum(II); cisplatin; **56MESS**; platinum(IV); cytotoxicity; ROS; mitochondria; HDAC



Citation: Aputen, A.D.; Elias, M.G.; Gilbert, J.; Sakoff, J.A.; Gordon, C.P.; Scott, K.F.; Aldrich-Wright, J.R. Platinum(IV) Prodrugs Incorporating an Indole-Based Derivative, 5-Benzyloxyindole-3-Acetic Acid in the Axial Position Exhibit Prominent Anticancer Activity. *Int. J. Mol. Sci.* **2024**, *25*, 2181. <https://doi.org/10.3390/ijms25042181>

Academic Editor: Bernhard Biersack

Received: 29 November 2023

Revised: 7 February 2024

Accepted: 8 February 2024

Published: 11 February 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The classical cytotoxic platinum(II) drug, *cis*-diammineplatinum(II) dichloride (*cis*-platin) and its direct analogues, *trans*-L(1*R*,2*R*-diaminocyclohexane) oxalatoplatinum(II) (oxaliplatin) and *cis*-diammine(1,1-cyclobutanecarboxylato)platinum(II) (carboplatin) (Figure 1), are a class of square planar platinum(II) anticancer drugs, which have irrevocably evolved as working pillars of chemotherapy [1–5]. The potency of platinum(II) drugs is demonstrated through their ability to induce deoxyribonucleic acid (DNA) lesions by covalently binding to DNA [6]. Consequently, DNA replication and transcription are effectively ceased, which induces apoptosis [7]. It is also noteworthy that while DNA is the therapeutic target of these drugs, inevitably, they also exhibit non-specific binding with phospholipids, thiols, ribonucleic acid, and other biomolecules that contain nitrogen, sulfur, and oxygen atoms [8–10]. Unfortunately, this singularity reflects the non-selectivity of the drugs resulting in decreased therapeutic efficacy and increased incidence of pernicious side effects, which patients commonly experience when receiving platinum-based regimens [11–15]. Furthermore, drug resistance is another clinical limitation associated with the use of platinum(II) drugs, which often develops when treatment is prolonged [16,17]. Because of this, combinatorial treatment is employed, wherein therapeutic agents are administered together with platinum(II) drugs in an attempt to enhance treatment effectivity [18]. However, this

type of regimen can complicate patient care and interfere with therapeutic outcomes since each co-administered agent has a unique pharmacokinetic index [19,20].

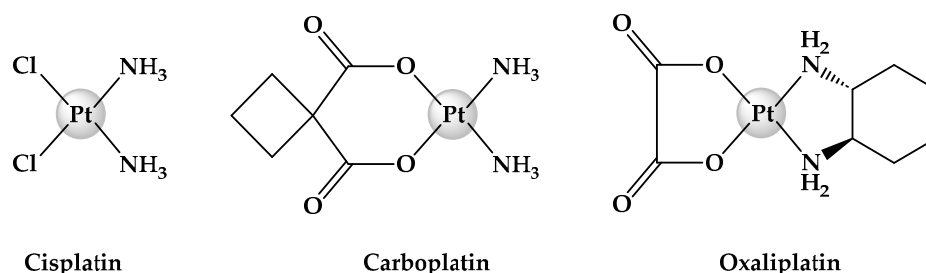


Figure 1. Molecular structures of standard platinum(II) drugs with worldwide approval for cancer treatment.

Cisplatin, oxaliplatin and carboplatin are effective anticancer agents. Regrettably, their disadvantageous traits proved to be burdensome to patients, and thus it is only ethical to develop safer chemotherapeutic alternatives that can improve cancer treatment experience. Many platinum-based complexes have been previously synthesised and investigated in the hope of finding another chemotherapeutic agent that is as potent as cisplatin, but without the toxic side effects, and has the potential to circumvent chemoresistance [21–24]. Some examples include zeniplatin [25], spiroplatin [26], enloplatin [27], aroplatin [28] and miboplatin [29] (Figure 2). These third-generation platinum(II) complexes were in clinical trials, but were eventually withdrawn either because of demonstrated severe toxicity or limited antitumour activity.

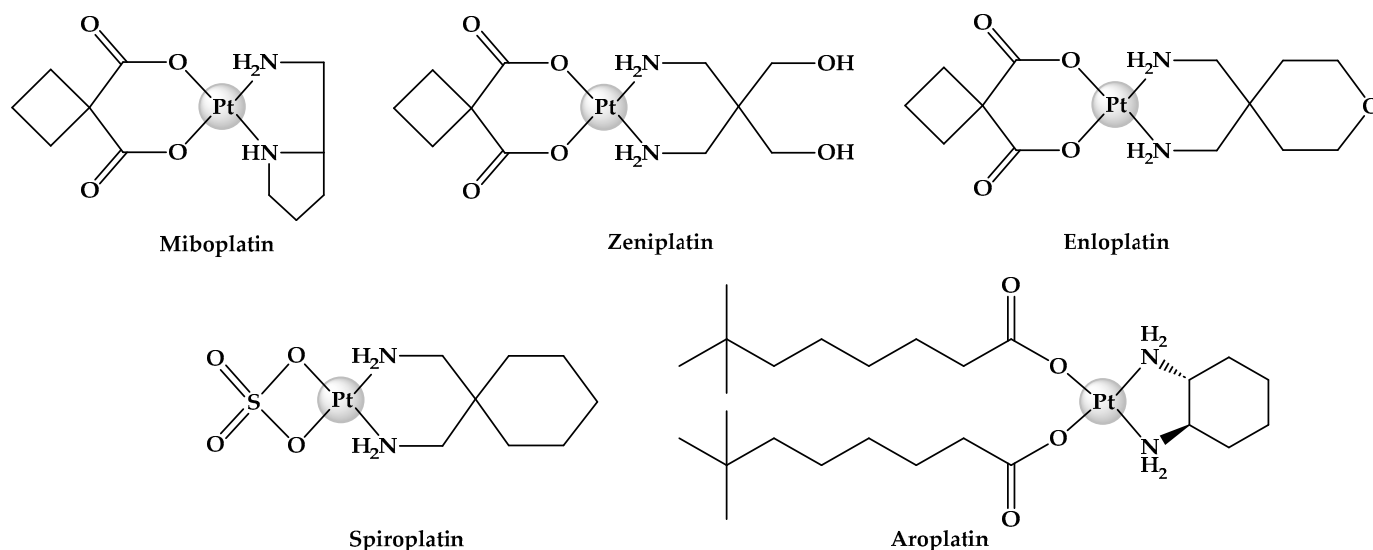


Figure 2. Molecular structures of third-generation platinum(II) complexes that were clinically investigated.

A collection of structurally related platinum(II) complexes have also been investigated and numerous studies have confirmed their impressive biological activity against multiple human cell lines [30–35]. The chemical structures of these complexes are represented as $[Pt^{II}(H_L)(A_L)]^{2+}$, where H_L is a bulky heterocyclic ligand (i.e., 1,10-phenanthroline (phen), 5-methyl-1,10-phenanthroline (5-Mephen) or 5,6-dimethyl-1,10-phenanthroline (5,6-Me₂phen)) and A_L is a chiral ancillary ligand (i.e., 1*S*,2*S*-diaminocyclohexane (SS-DACH or DACH)). $[Pt^{II}(\text{phen})(SS\text{-DACH})]^{2+}$ (**PHENSS**), $[Pt^{II}(5\text{-Mephen})(SS\text{-DACH})]^{2+}$ (**5MESS**) and $[Pt^{II}(5,6\text{-Me}_2\text{phen})(SS\text{-DACH})]^{2+}$ (**56MESS**) are three notable examples from this class (Figure 3). **56MESS** has proven to be the most potent and has been reported to promote epigenomic changes by interfering with the cytoskeleton, cell-cycle proteins, mitochondria, and nuclear DNA [36,37]. Despite the remarkable in vitro activity of **56MESS**,

preliminary in vivo studies indicated that the drug was ineffective in suppressing tumour on BD-IX rats with peritoneal carcinoma and caused nephrotoxicity [38]. Intriguingly, when **PHENSS** was administered on mice with prostate tumour, no toxicity was detected and the rate of tumour regression was comparable to cisplatin [32]. To further elucidate this irregularity, the equivalent platinum(IV) derivatives of the complexes were synthesised and investigated, with preliminary evidence showing an enhancement in pharmacokinetic and pharmacological properties [39,40].

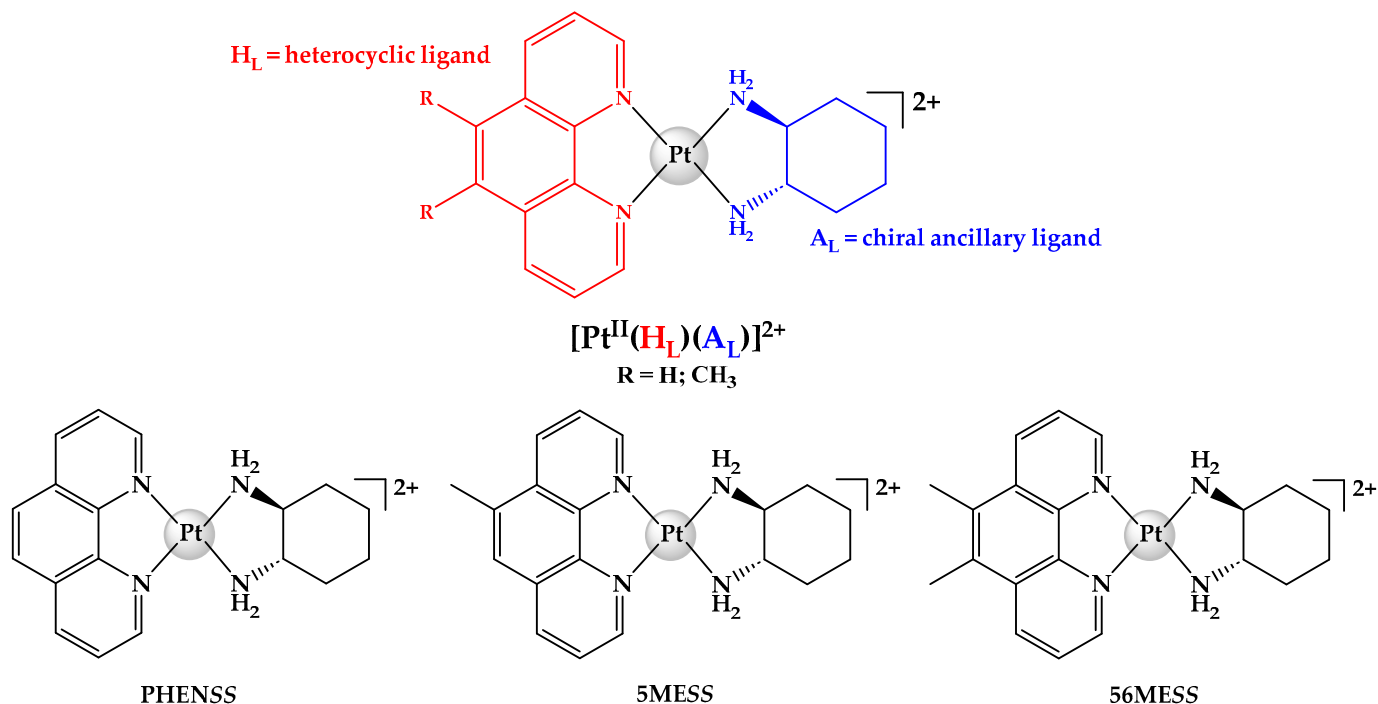


Figure 3. Molecular structures of unconventional platinum(II) complexes featuring a bulky heterocyclic ligand and a chiral ancillary ligand.

Researchers envisioned that designs based on kinetically inert octahedral platinum(IV) complexes would deliver safer, well-tolerated and effective anticancer treatment [41–45]. It is generally accepted that platinum(IV) complexes are the likely chemotherapeutic replacements for their equivalent platinum(II) parent complexes because of their kinetic inertness and potential for bioactive functionalisation. Commonly, platinum(IV) complexes are prepared by oxidation of the platinum(II) precursors with hydrogen peroxide [46,47]. This synthetic step generates the platinum(IV) scaffold, incorporating two axial positions with two hydroxides coordinated and readily available for substitution by bioactive ligands. While cisplatin, carboplatin and oxaliplatin are being exploited as precursor cores for new platinum(IV) complexes [48–51], we contribute to this approach by utilising the potent platinum(II) complexes, **PHENSS**, **5MESS** and **56MESS**. With the vast number of bioactive ligands available to coordinate to platinum(IV), the indole-based derivative, 5-benzyloxyindole-3-acetic acid (5B3A) caught our interest. We have selected 5B3A as axial ligand because it incorporates an indole pharmacophore, which has been used in drug design and discovery [52,53]. The bicyclic nature of the compound exhibits multiple therapeutic properties [54,55]; and for this reason, it is found in many drugs, specifically those that target cancer [56]. Recent studies have also shown the effective use of the indole scaffold in creating new metal-based anticancer drugs with improved activity [57,58]. We expect that by coordinating 5B3A to the platinum(IV) form of **PHENSS**, **5MESS** and **56MESS**, we will enhance the anticancer properties by providing an additional mode of action against cancer cells.

Herewith, we report the syntheses and structural characterisation of three platinum(IV) complexes of type $[\text{Pt}^{\text{IV}}(\text{H}_L)(\text{A}_L)(\text{X})(\text{OH})]^{2+}$, where **X** is the indole-based axial ligand, 5B3A: $[\text{Pt}^{\text{IV}}(\text{phen})(\text{SS-DACH})(5\text{B3A})(\text{OH})]^{2+}$ ($[\text{PHENSS}(\text{IV})(5\text{B3A})(\text{OH})]^{2+} = \text{P-5B3A}$), $[\text{Pt}^{\text{IV}}(5\text{-Mephen})(\text{SS-DACH})(5\text{B3A})(\text{OH})]^{2+}$ ($[\text{5MESS}(\text{IV})(5\text{B3A})(\text{OH})]^{2+} = \text{5-5B3A}$), and $[\text{Pt}^{\text{IV}}(5,6\text{-Me}_2\text{phen})(\text{SS-DACH})(5\text{B3A})(\text{OH})]^{2+}$ ($[\text{56MESS}(\text{IV})(5\text{B3A})(\text{OH})]^{2+} = \text{56-5B3A}$) (Figure 4). The purity and chemical structures of the complexes were confirmed through high-performance liquid chromatography (HPLC), nuclear magnetic resonance ($^1\text{H-NMR}$; two-dimensional correlation spectroscopy (2D-COSY); heteronuclear multiple quantum correlation ($^1\text{H-}^{195}\text{Pt-HMQC}$)), ultraviolet–visible (UV), circular dichroism (CD) and elemental microanalysis experiments. All complexes were purified via a flash chromatography system. To determine the anticancer activity of the resultant complexes, they were screened against twelve human cell lines including HT29 colon, U87 glioblastoma, MCF-7 breast, A2780 ovarian, H460 lung, A431 skin, Du145 prostate, BE2-C neuroblastoma, SJ-G2 glioblastoma, MIA pancreas, the cisplatin-resistant ADDP ovarian variant, and the non-tumour-derived MCF10A breast line. In addition to solubility, stability and lipophilicity studies, the reduction behaviour of **P-5B3A**, **5-5B3A** and **56-5B3A** upon treatment with ascorbic acid (AsA) was also determined with the aid of $^1\text{H-NMR}$ and one-dimensional $^{195}\text{Pt-NMR}$ (1D- $^{195}\text{Pt-NMR}$). The capacity of the complexes to generate reactive oxygen species (ROS), alter mitochondrial activity via causing mitochondrial membrane potential (MtMP) changes, and inhibit histone deacetylase (HDAC) were also investigated to determine how these parameters may affect their overall biological activity.

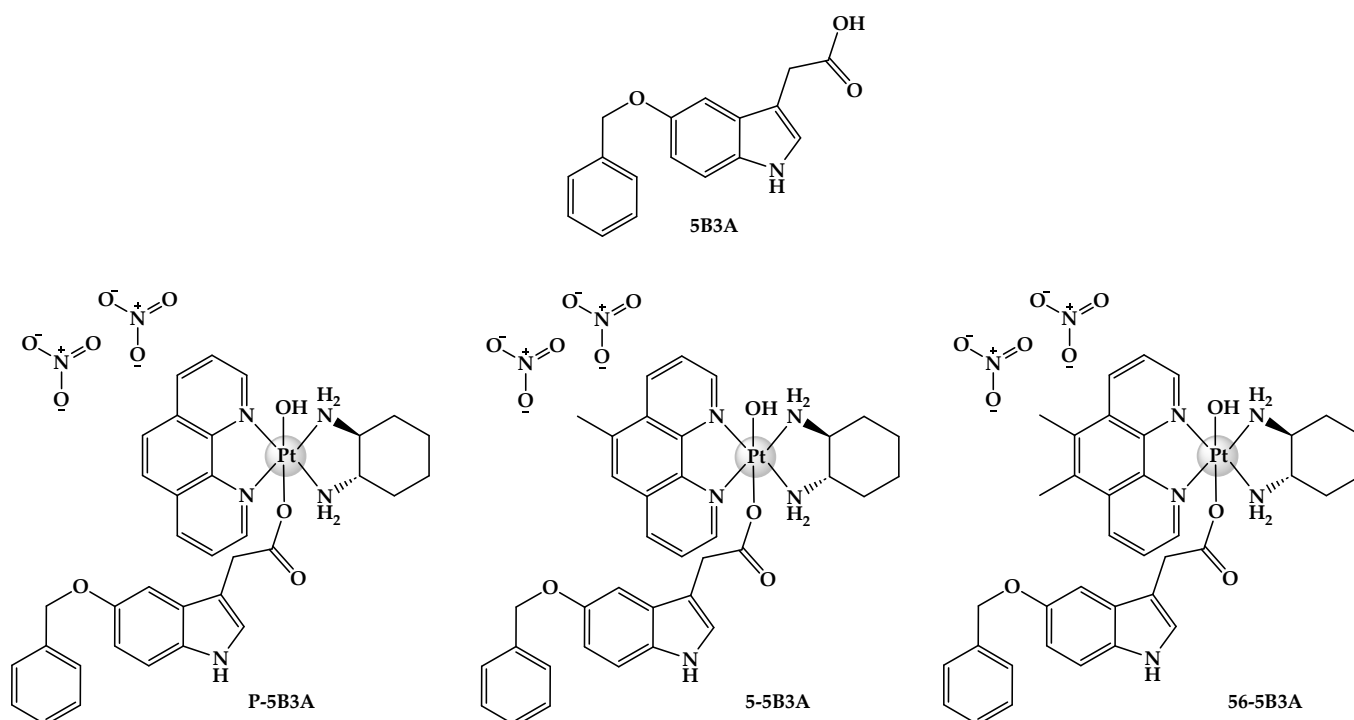


Figure 4. Molecular structures of the indole-based axial ligand, 5B3A, and the synthesised platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A**.

2. Results and Discussion

2.1. Syntheses and Characterisation

The platinum(II) precursors and platinum(IV) scaffolds of $[\text{Pt}^{\text{II}}(\text{H}_L)(\text{A}_L)]^{2+}$ and $[\text{Pt}^{\text{IV}}(\text{H}_L)(\text{A}_L)(\text{OH})_2]^{2+}$ were prepared as described in the literature without modifications [59]. The NHS ester of 5B3A was also prepared as described in the literature [60]; and to confirm its synthesis, HPLC, $^1\text{H-NMR}$ and 2D-COSY experiments were undertaken (Figures S1–S3). The $^1\text{H-NMR}$ and 2D-COSY spectra of the uncoordinated axial ligand, 5B3A were also acquired for reference and used to assist in assigning the protons for its NHS ester deriva-

tive, NHS-5B3A (Figures S4 and S5). To synthesise the proposed platinum(IV) complexes, $[\text{Pt}^{\text{IV}}(\text{H}_\text{L})(\text{A}_\text{L})(\text{X})(\text{OH})]^{2+}$ (**P-5B3A**, **5-5B3A** and **56-5B3A**) (Figure 5), established protocols were adapted [61]. The resultant complexes were purified via a flash chromatography system to acquire purity of above 95%. The purity and chemical structures of **P-5B3A**, **5-5B3A** and **56-5B3A** were confirmed by HPLC (Figures S6–S8), ^1H -NMR (Figures S9–S11), 2D-COSY (Figures S12–S14), ^1H - ^{195}Pt -HMQC (Figures S15–S20), UV (Figures S21–S23), CD (Figures S24–S26) and elemental microanalysis experiments.

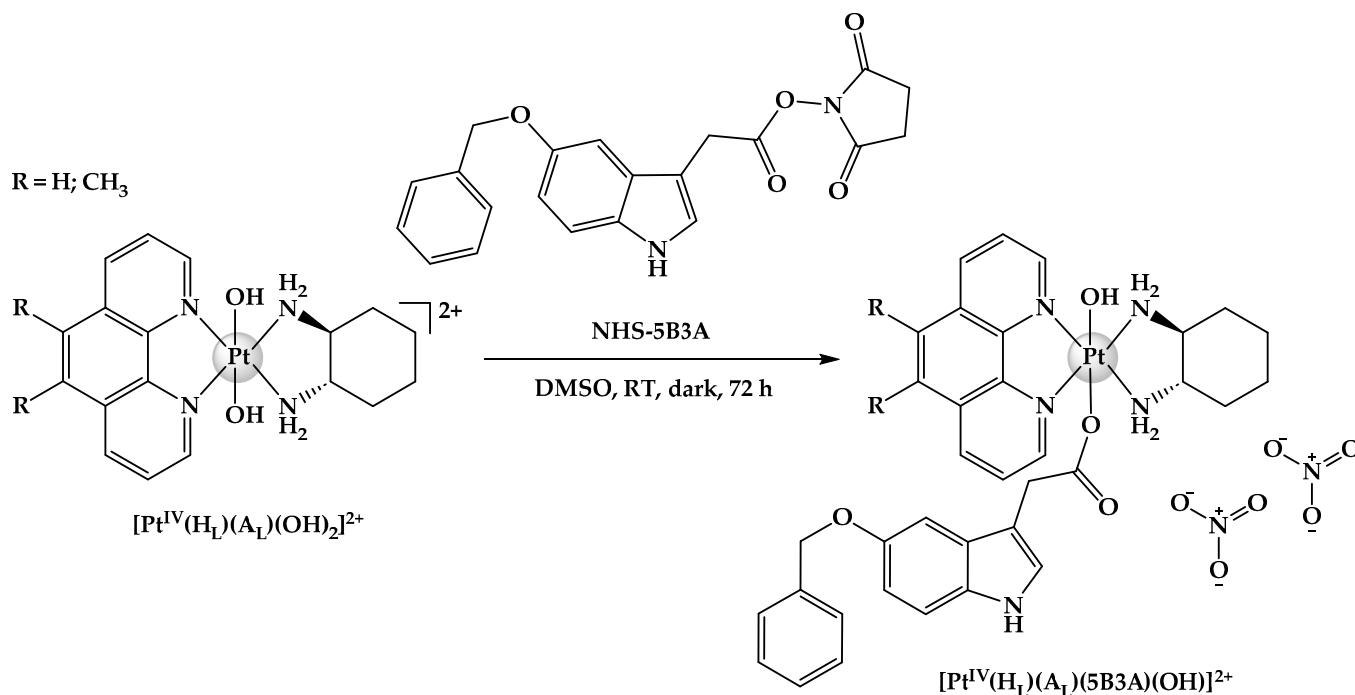


Figure 5. Synthetic scheme of **P-5B3A**, **5-5B3A** and **56-5B3A**.

2.1.1. NMR Assignments

NMR techniques including ^1H -NMR, 2D-COSY and ^1H - ^{195}Pt -HMQC were applied to confirm the chemical structures of the synthesised platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A** (Figures S9–S20). The ^1H -NMR and ^1H - ^{195}Pt -HMQC assignments of **P-5B3A**, **5-5B3A** and **56-5B3A** are summarised in the Materials and Methods section. Because DMSO- d_6 was used for the NMR experiments, amine proton resonances were observed in the aromatic region (8–10 ppm) (Figures S9–S11). The acquired NMR data are comparable to published data [40,61–63].

As depicted in the ^1H -NMR spectrum of **P-5B3A** (Figure S9), the protons of the heterocyclic ligand, phen, resonated between 8 and 10 ppm. The most deshielded or downfield phen protons, H2 and H9, were assigned to the doublets at 9.40 and 9.38 ppm, which exhibit an equal J -coupling constant of 5.4 Hz. The doublet of doublets at 9.06 ppm was assigned to H4 and H7 protons, and both have J -coupling constants of 4.8 and 8.1 Hz, respectively. Subsequently, a multiplet was recorded at 8.32 ppm which was induced by H3 and H8 protons. The remaining protons in the phen ligand, H5 and H6, were assigned to the sharp singlet at 8.26 ppm. It is also noteworthy that the broad signals adjacent to the signals of H4, H7, H3, H8, H2 and H9 protons were caused by the amines of the chiral ancillary ligand, SS-DACH (Figure S9).

When comparing the multiplicities demonstrated for **P-5B3A** in the aromatic region with the multiplicities for its corresponding platinum(II) precursor and platinum(IV) scaffold, **PHENSS** and **PHENSS(IV)(OH) $_2$** , slight variations are evident as shown in Figure 6. Upon coordinating 5B3A to the platinum(IV) scaffold, **PHENSS(IV)(OH) $_2$** , an upfield shift was detected for the following phen protons: H4, H7, H3, H8, H5 and H6 (Figure 6). This phenomenon may have been caused by the possible π - π stacking interactions between the

aromatic rings of 5B3A and the phen, and this upfield movement was also observed by structurally related complexes in the literature [59,61]. Overall, the variations in multiplicity reported for **P-5B3A** is primarily influenced by the coordination of the axial ligand, 5B3A.

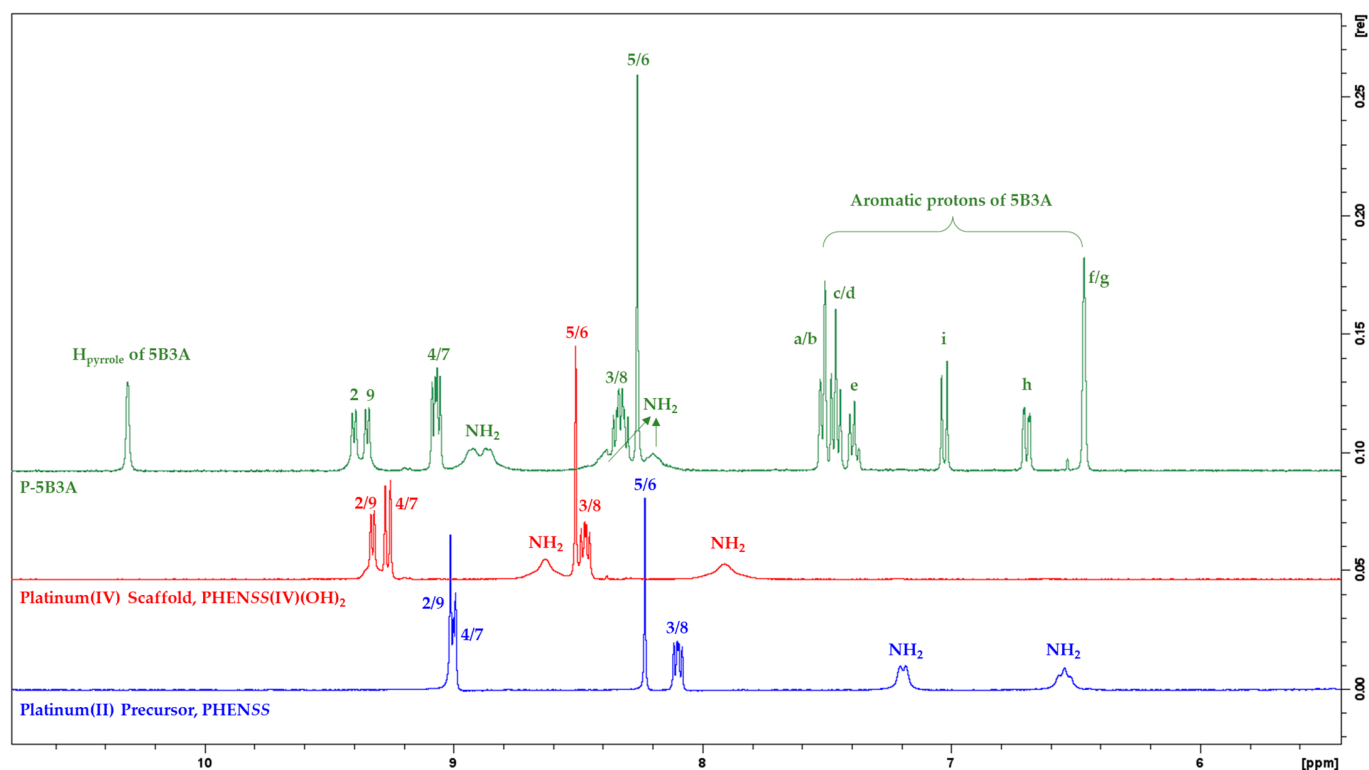


Figure 6. Expanded ^1H -NMR spectra of **P-5B3A** and its equivalent platinum(II) precursor and platinum(IV) scaffold, **PHENSS** and **PHENSS(IV)(OH)₂**, respectively, in DMSO-d_6 , showing the multiplicity variations and shifts in chemical resonances.

Notable differences in chemical multiplicity were also demonstrated by the protons of 5B3A originating from the benzene and indole bicyclic rings (benzene fused with pyrrole) (Figures 6 and S9). The pyrrole proton ($\text{H}_{\text{pyrrole}}$) proved to be the most deshielded as it resonated furthest downfield as a singlet at 10.3 ppm, as shown in Figures 6 and S9. The distorted multiplicities between 7.37–7.53 ppm were assigned to the benzene protons, a, b, c, d and e (Figures 6 and S9). The irregular doublet and doublet of doublets at 7.02 and 6.69 ppm were assigned to the indole protons, i and h, respectively. As for the remaining indole protons, f and g, a singlet at 6.46 ppm was recorded, which indicates overlapping. Of further note, the 2D-COSY of **P-5B3A** was also analysed to elucidate the assignment of the indole protons of 5B3A (Figure S12). As shown in Figure S12, f and h proved to be the only protons coupled to $\text{H}_{\text{pyrrole}}$. Proton h was found to be coupled to protons g and i (Figure S12). Furthermore, the methylene protons of 5B3A, β and α , was assigned to the singlet and the multiplet at 4.91 and 3.23 ppm, respectively (Figure S9). Within the aliphatic region (1–3 ppm), the recorded resonances exhibited by the protons of the **SS-DACH** (Figure S9) are also consistent with literature data [40,63,64].

^1H - ^{195}Pt -HMQC experiments were also conducted for **P-5B3A** at 400 and -2800 ppm (Figures S15–S20), to further validate the coordination of 5B3A to **PHENSS(IV)(OH)₂**. The platinum(II) precursors, $[\text{Pt}^{\text{II}}(\text{H}_\text{L})(\text{A}_\text{L})]^{2+}$ typically resonate at -2800 ppm while their equivalent platinum(IV) scaffolds, $[\text{Pt}^{\text{IV}}(\text{H}_\text{L})(\text{A}_\text{L})(\text{OH})_2]^{2+}$ resonate within 400 ppm [40,59,63]. The ^1H - ^{195}Pt -HMQC spectrum of **P-5B3A** highlights the correlation of protons H2 and H9 (9.12 ppm) and of H3 and H8 (8.10 ppm) with the platinum peaks at 498 ppm (Figure S15). No peak was found at -2800 ppm (Figure S16), which confirmed the absence of platinum(II) species. This further supports that not only the sample used for the NMR

experiments are free of platinum(II) species denoting purity, the 5B3A ligand was also successfully coordinated to **PHENSS(IV)(OH)₂** and occupied one axial position.

The ¹H-NMR assignment of multiplicities for the spectra of **5-5B3A** and **56-5B3A** (Figures S10 and S11) followed the same rationale to that discussed for **P-5B3A**. With respect to the ¹H-NMR spectra of **5-5B3A** and **56-5B3A** (Figures S10 and S11), some distinctions in multiplicity were evident when compared to those observed for **P-5B3A**, most specifically in the aromatic region. While these distinctions are mostly attributed to the coordination 5B3A ligand, another factor that contributes to these is the methylation of the heterocyclic rings of **5-5B3A** and **56-5B3A**. This phenomenon has been observed previously with similar complexes in the literature [59,61,65]. Finally, the ¹H-¹⁹⁵Pt-HMQC spectra of **5-5B3A** and **56-5B3A** (Figures S17–S20) also confirmed the successful coordination of 5B3A to the respective platinum(IV) scaffolds, indicating the axial ligand only occupying one axial position.

In addition to NMR, elemental microanalysis was performed on an Elementar Vario MICRO (Elementar Analysensysteme GmbH) to further confirm the chemical composition and purity of **P-5B3A**, **5-5B3A** and **56-5B3A**. The resulting elemental microanalysis is consistent with the calculated values for the prodrugs as nitrates with co-crystallised H₂O molecules, as summarised in the Materials and Methods section for **P-5B3A.2H₂O**, **5-5B3A.3H₂O** and **56-5B3A.2H₂O**.

2.1.2. UV and CD Spectral Analysis

The molecular electronic transitions exhibited by **P-5B3A**, **5-5B3A** and **56-5B3A** in the UV measurements are comparable to published data (Figures S21–S23) [40,61,62,66]. The acquisition of UV spectra was achieved as previously described [59,61,65,67]. Experiments were obtained in triplicates; three separate stock solutions were prepared for each complex. All spectra were baseline corrected. Mean extinction coefficients (ε) were determined with standard deviation and errors based on the constructed plot curves. CD measurements were also undertaken to confirm that the chirality of the ancillary ligand, **SS-DACH**, was retained by **P-5B3A**, **5-5B3A** and **56-5B3A** during syntheses. Furthermore, the absorption bands exhibited in the CD spectra of **P-5B3A**, **5-5B3A** and **56-5B3A** (Figures S24–S26) are also comparable to published data [40,61,62,66]. Due to the structural similarities of the complexes, no significant differences were observed in their UV or CD spectra. All characteristic peaks exhibited by **P-5B3A**, **5-5B3A** and **56-5B3A** in their UV and CD spectra are summarised in Table 1.

Table 1. A summary of the peak maxima in the UV and CD spectra of **P-5B3A**, **5-5B3A** and **56-5B3A**.

Platinum(IV) Complexes	UV λ _{max} nm (ε/M.cm ^{−1} ± SD × 10 ⁴)	CD λ _{max} nm (Δε/M.cm ^{−1} × 10 ¹)
P-5B3A	304 (0.73 ± 3.15), 279 (2.16 ± 1.63), 204 (8.08 ± 1.70)	202 (−463), 203 (−485), 205 (−551), 228 (−58.9), 239 (−99.6), 263 (+31), 269 (+5.15), 274 (+21.3), 285 (−16.5), 313 (−32.2)
5-5B3A	310 (1.01 ± 0.73), 286 (3.63 ± 0.33), 204 (13.5 ± 3.61)	202 (−433), 203 (−460), 204 (−490), 230 (−35.1), 240 (−65.1), 257 (+16.9), 263 (+28.6), 270 (+30.7), 286 (−53.5)
56-5B3A	315 (0.59 ± 0.94), 291 (2.02 ± 1.78), 250 (1.31 ± 2.53), 206 (8.22 ± 1.75)	202 (−376), 204 (−384), 208 (−425), 230 (−6.55), 235 (−25.4), 239 (+7.34), 248 (+73.4), 257 (+101), 275 (+80.7), 288 (+0.91)

The anticipated metal-to-ligand charge transfer interactions and π–π* transitions were exhibited in the UV spectra of the complexes (Figures S21–S23), and are consistent with similar complexes in the literature [40,61,62,66]. These interactions are the effect of the heterocyclic ligands of the complexes, phen, 5-Mephen and 5,6-Me₂phen which exhibit ligand-centred π–π* transitions. The patterns observed in the UV spectra of **P-5B3A**, **5-5B3A** and **56-5B3A** (Figures 7 and S21–S23), especially the bathochromic shift, are heavily influenced by their heterocyclic ligands. This observation corresponds to published data [40,61,62,66].

Prominent peaks were detected at the wavelengths ~ 200 and ~ 279 – 289 nm for all complexes, as shown in Figure 7.

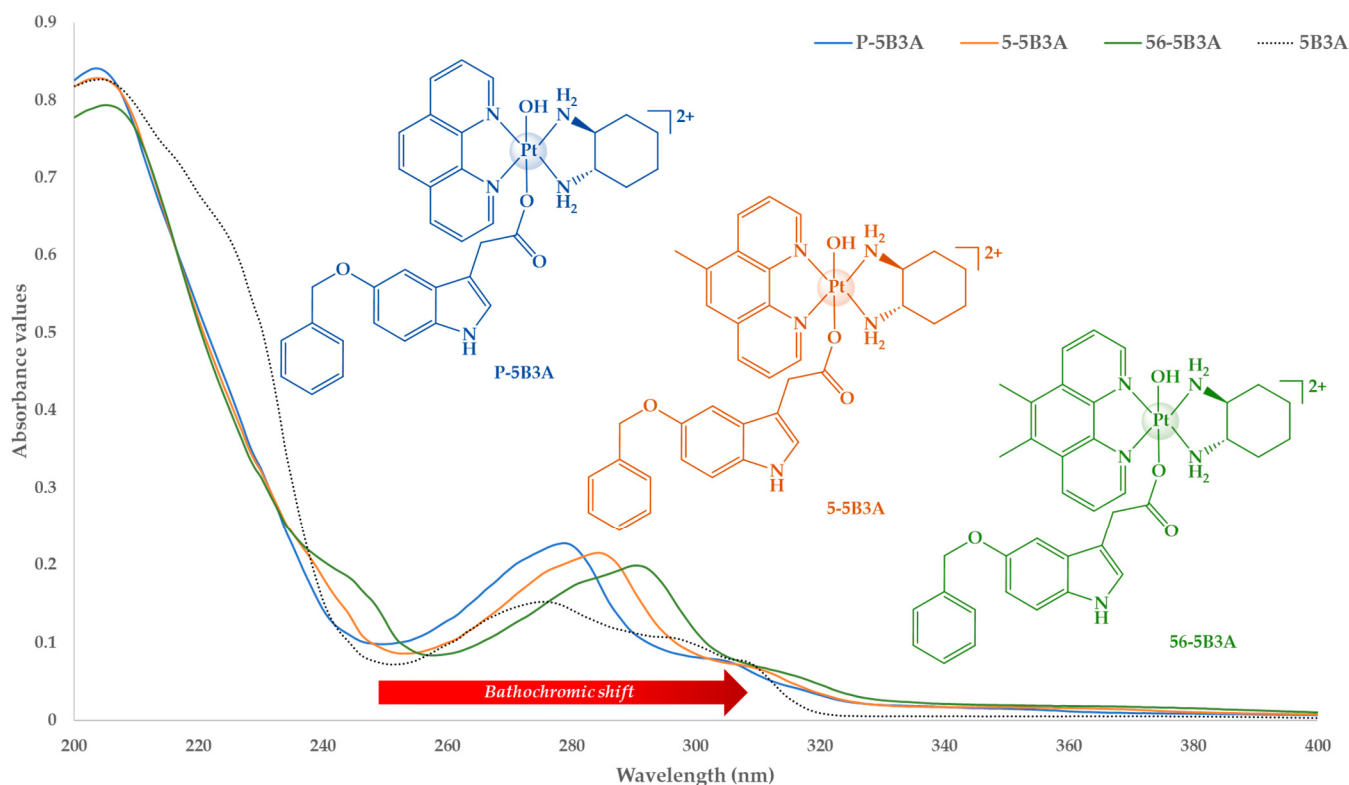


Figure 7. UV spectra of **P-5B3A**, **5-5B3A**, **56-5B3A** and the **5B3A** ligand, obtained at 298 K, displaying the recorded UV absorption bands at various wavelengths. Inset: colour-coded structures of **P-5B3A**, **5-5B3A** and **56-5B3A**.

Based on the UV spectra of **P-5B3A** (Figures 7 and S21), a prominent absorption band was detected at 204 nm, which was followed by a dip between ~ 240 and 250 nm, denoting a hypochromic shift. Subsequently, a hyperchromic shift was observed when a less prominent peak appeared at 279 nm, which was followed by a weak band between 300–310 nm, as shown in Figure 7. The absorption bands detected for **P-5B3A** were also evident in the UV spectra of **5-5B3A** and **56-5B3A** (Figures 7, S22 and S23). Moreover, the absorption bands shifted towards a longer wavelength, which indicates bathochromic shift. Interestingly, a characteristic band was observed for **56-5B3A** between ~ 240 and 250 nm which appears like a weak shoulder. Finally, it is also noteworthy that the characteristic absorption bands for uncoordinated **5B3A** are evident within the absorption bands for the complexes (Figure 7). This provides further evidence of the contributions of **5B3A** when coordinated to platinum. Overall, the UV spectra demonstrated by **P-5B3A**, **5-5B3A**, **56-5B3A** also show evidence of the presence or lack of methyl groups on the heterocyclic ligands.

CD measurements were also performed to confirm that the chirality of the chiral ancillary ligand, *SS*-DACH was retained during syntheses. Chirality is an essential feature that directly contributes to the potency of the studied complexes, and this has been the subject of previous studies [33–35,68]. The CD spectra of **P-5B3A**, **5-5B3A** and **56-5B3A** (Figures S24–S26), demonstrated no significant differences. Strong peaks were evident at lower wavelengths, which is consistent with previously reported structurally similar platinum(IV) complexes [40,61,62,66]. The CD spectra of **P-5B3A** (Figure S24), exhibited prominent negative absorption bands (200–240 nm), followed by weaker positive absorption bands between 250 and 280 nm. This pattern was also observed in the CD spectra of **5-5B3A** (Figure S25) and **56-5B3A** (Figure S26), although **56-5B3A** exhibited slightly more prominent positive absorption bands between 250 and 280 nm.

2.2. Physicochemical Investigations

2.2.1. Lipophilicity

The order of complexes by increasing lipophilicity is **P-5B3A** < **5-5B3A** < **56-5B3A** (Table 2 and Figure S27). It can be deduced that increasing methylation on the heterocyclic ligand increased lipophilicity. This trend agrees with published data [59,61,65,66,69]. The determined $\log k_w$ values are consistent and within the range of the $\log k_w$ values determined for similar complexes reported in the literature [59,61]. Furthermore, cisplatin has a reported $\log k_w$ value of -2.19 [70,71], which is significantly lower than the acquired $\log k_w$ values of **P-5B3A**, **5-5B3A** and **56-5B3A** (Table 2). This suggests that **P-5B3A**, **5-5B3A** and **56-5B3A** are expected to have better absorption properties than cisplatin.

Table 2. Calculated $\log k_w$ values of **P-5B3A**, **5-5B3A** and **56-5B3A**.

Platinum(IV) Complexes	$\log k_w$ Values
P-5B3A	1.82
5-5B3A	1.91
56-5B3A	2.11

2.2.2. Preliminary Reduction Measurements

^1H -NMR and $1\text{D-}^{195}\text{Pt}$ -NMR spectroscopy were utilized to investigate the reduction activity of the platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A**, using previously reported methods [59,61,65]. The biological reductant selected for this study is AsA. Briefly, sequential ^1H -NMR experiments were performed for 1 h at 37°C , proceeded by $1\text{D-}^{195}\text{Pt}$ -NMR at 400 and -2800 ppm (30 min per region). It is noteworthy that the purity of each complex was examined prior the reduction experiments ensuring that no traces of platinum(II) species were evident in the samples used. All complexes were dissolved in PBS and $\text{DMSO-}d_6$, without the reductant. Preliminary $1\text{D-}^{195}\text{Pt}$ -NMR measurements were conducted at 37°C at 400 and -2800 ppm. This was completed to ensure that the PBS and $\text{DMSO-}d_6$ do not interfere with the complexes by causing unwanted reduction. From the preliminary $1\text{D-}^{195}\text{Pt}$ -NMR spectra, it was confirmed that the PBS and $\text{DMSO-}d_6$ did not cause the complexes to reduce (Figures S28–S30). Upon treatment of the platinum(IV) complexes with AsA, there was considerable reduction observed in the ^1H -NMR and $1\text{D-}^{195}\text{Pt}$ -NMR spectra acquired (Figures S31–S36). Notable changes in chemical multiplicity were recorded in the ^1H -NMR reduction spectra of **P-5B3A**, **5-5B3A** and **56-5B3A** (Figures S31, S33 and S35), especially along the aromatic region. The estimated reduction time points for **P-5B3A**, **5-5B3A** and **56-5B3A** are also outlined in Table 3.

Table 3. The approximate time points in min for **P-5B3A**, **5-5B3A** and **56-5B3A** at which 50 and 100% reductions occurs in the presence of AsA.

Platinum(IV) Complexes	Approximate Reduction Time Points (min)	
	$T_{50\%}$	$T_{100\%}$
P-5B3A	10–15	60
5-5B3A	5–10	30
56-5B3	0–5	10

$T_{50\%}$: Time at which 50% of reduction occurs. $T_{100\%}$: Time at which 100% of reduction occurs.

The expanded ^1H -NMR reduction spectra for **P-5B3A** at different time intervals showing distinct changes in chemical multiplicity are highlighted in Figure 8. The separate doublets exhibited by H2 and H9 protons and the multiplet exhibited by H4 and H7 protons (in the platinum(IV) form) resolved into a doublet (Figure 8). Subsequently, an upfield shift was observed for H3, H8, H5 and H6 protons. Notably, the H_{pyrrole} of the axial ligand, 5B3A appeared further downfield at 10.8 ppm upon dissociating from platinum. Another

notable change in multiplicity was demonstrated by the indole protons, f and g. As shown in Figure 8, these protons resonated as a singlet at 6.46 ppm (in the platinum(IV) form), but eventually resolved into two separate singlets as it dissociated from the platinum. These changes in multiplicity show that the platinum(IV) complex, **P-5B3A** successfully reduced to its core platinum(II) precursor, **PHENSS**. To further confirm this, 1D- ^{195}Pt -NMR measurements were performed at 37 °C at 400 and −2800 ppm immediately after the final ^1H -NMR measurement. At approximately 1.5 h, the 1D- ^{195}Pt -NMR reduction spectra of **P-5B3A** was acquired and no platinum(IV) peak was found at 400 ppm, as shown in Figure S32. However, at −2800 ppm, 30 min after the first 1D- ^{195}Pt -NMR reduction spectra were acquired, a prominent peak was recorded, which indicates the presence of the platinum(II) precursor, **PHENSS** (Figure S32). The changes in multiplicity observed in the ^1H -NMR reduction spectra of **P-5B3A** (Figures 8 and S31) were comparable to those observed for **5-5B3A** and **56-5B3A** (Figures S33 and S35). **5-5B3A** and **56-5B3A** were also confirmed to have successfully reduced to their core platinum(II) precursors, **5MESS** and **56MESS**, respectively, based on the acquired 1D- ^{195}Pt -NMR reduction spectra (Figures S34 and S36). Because **P-5B3A**, **5-5B3A** and **56-5B3A** are reduced to their core platinum(II) precursors, this further confirms that they are prodrugs.

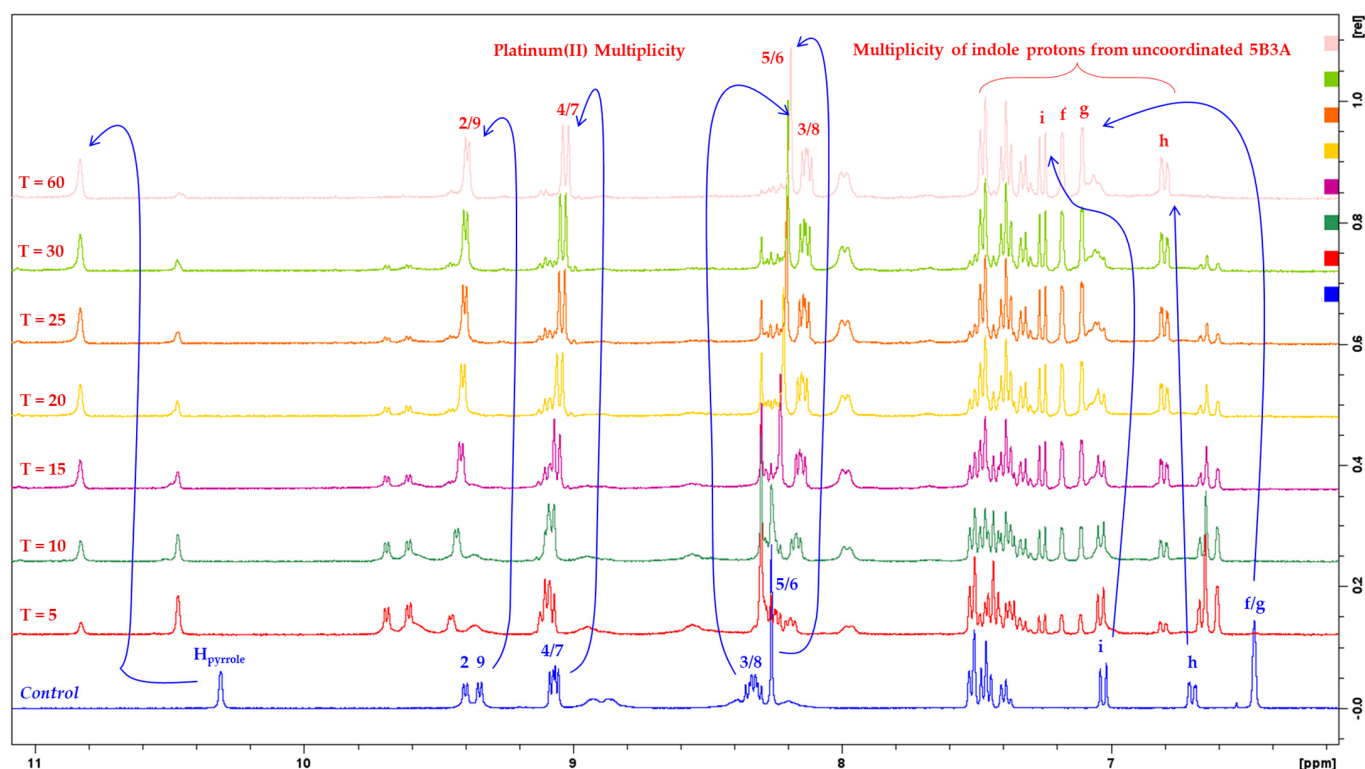


Figure 8. The expanded ^1H -NMR spectra of **P-5B3A** treated with PBS and AsA in DMSO-d_6 at 37 °C, highlighting the variations in chemical multiplicity in the aromatic region depicted by the arrows. T is time in min. The expanded ^1H -NMR spectrum of the untreated complex (control) was also included for comparison.

With respect to the estimated reduction time points in Table 3, there was a notable difference in the rate of reduction exhibited by the complexes (Table 3). **P-5B3A** was the only complex to have reduced more slowly, with 50% reduction occurring between 10 and 15 min and maximal reduction at 60 min (Figures 8 and S31). In comparison, **5-5B3A** reduced by 50% between 5 and 10 min, while maximal reduction occurred at 30 min (Figure S33). Interestingly, **56-5B3A** was confirmed to have the fastest rate of reduction with maximal reduction at 10 min (Figure S36). This indicates that the differences in reduction rates are primarily regulated by the heterocyclic ligands rather than the coordinated

5B3A ligand. This trend has also been observed previously with similar complexes in the literature [59,61]. These preliminary results provided insight into how these complexes reduce in the presence of a reducing agent like AsA. We expect the complexes to reduce differently under physiological conditions (i.e., in the presence of human serum or plasma).

2.2.3. Stability Measurements

The stability of **P-5B3A**, **5-5B3A** and **56-5B3A** was monitored via analytical HPLC. Surprisingly, **P-5B3A** and **5-5B3A** did not show any signs of significant hydrolysis for up to 1 week according to the HPLC chromatograms obtained (Figures S37 and S38), only minimal peak traces at ~5 min denoting the presence of the equivalent platinum(II) precursors. While **56-5B3A** did not completely hydrolyse in solution, a trace platinum(II) peak was also recorded at ~6 min which gradually became more prominent over the week (Figures S37 and S38). Overall, the platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A** proved to be adequately stable in aqueous solution for up to 1 week at 37 °C without any significant hydrolysis involved.

2.3. Biological Investigations

2.3.1. In Vitro Anticancer Activity

The in vitro anticancer activity of the synthesised platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A**, including the uncoordinated axial ligand, 5B3A was determined in multiple human cell lines such as HT29 colon, U87 glioblastoma, MCF-7 breast, A2780 ovarian, H460 lung, A431 skin, Du145 prostate, BE2-C neuroblastoma, SJ-G2 glioblastoma, MIA pancreas, the cisplatin-resistant ADDP ovarian variant, and the non-tumour-derived MCF10A breast line using the MTT assay [59,61,65,72]. The cisplatin-resistant ADDP ovarian variant was included for this study to evaluate whether the complexes can circumvent resistance. All cytotoxicity values are expressed as GI₅₀. The cytotoxicity values for 5B3A, **P-5B3A**, **5-5B3A** and **56-5B3A**, together with cisplatin, oxaliplatin and carboplatin, are summarised in Table 4. The cytotoxicity values of cisplatin, oxaliplatin and carboplatin were acquired from our previous studies [59,61,65], and have been included for comparison purposes. Additionally, the cytotoxicity values for platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**) are also summarised in Table 5 for reference [59,61,65].

Table 4. The in vitro cytotoxicity values of 5B3A, **P-5B3A**, **5-5B3A** and **56-5B3A** in multiple human cell lines, expressed as GI₅₀ (nM). For comparison, the GI₅₀ values of cisplatin, oxaliplatin and carboplatin were also included [59,61,65]. Significant increase in potency was identified by a paired T-test with a two-tailed distribution ($n = 3$). Group differences were considered statistically significant if * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared with cisplatin.

Cell Lines	GI ₅₀ (nM)						
	5B3A	P-5B3A	5-5B3A	56-5B3A	Cisplatin	Oxaliplatin	Carboplatin
HT29	>50,000 ***	200 ± 27 **	18 ± 0.67 **	4.4 ± 1.6 **	11,300 ± 1900	900 ± 200	>50,000
U87	>50,000 ***	1900 ± 150 *	240 ± 23	88 ± 7 *	3800 ± 1100	1800 ± 200	>50,000
H460	>50,000 ****	430 ± 50 *	30 ± 3	7.8 ± 2.2 *	900 ± 200	1600 ± 100	14,000 ± 1000
A431	>50,000 ****	1200 ± 100 **	93 ± 8 **	33 ± 8.4 **	2400 ± 300	4100 ± 500	24,000 ± 2200
Du145	>50,000 ****	190 ± 23 **	10 ± 2 **	1.2 ± 0.6 **	1200 ± 100	2900 ± 400	15,000 ± 1200
BE2-C	>50,000 ****	13,000 ± 5000 **	340 ± 40	150 ± 24 **	1900 ± 200	900 ± 200	19,000 ± 1200
SJ-G2	>50,000 ****	1300 ± 200	200 ± 40 *	110 ± 20	400 ± 100	3000 ± 1200	5700 ± 200
MIA	>50,000 ****	310 ± 26 **	31 ± 3 **	12 ± 3 **	7500 ± 1300	900 ± 200	>50,000
A2780	>50,000 **	470 ± 18 **	42 ± 4 **	18 ± 6.1 **	1000 ± 100	160 ± 100	9200 ± 2900
ADDP	>50,000 ****	330 ± 8.8 ****	23 ± 0.88 ****	6 ± 3 ****	28,000 ± 1600	800 ± 100	>50,000
MCF-7	>50,000 **	5400 ± 2800 **	200 ± 21	68 ± 10 **	6500 ± 800	500 ± 100	>50,000
MCF10A	44,000 ± 3000 **	280 ± 39 **	19 ± 1.5 **	3.3 ± 0.9 **	5200 ± 520	Not determined	>50,000
Mean GI ₅₀	49,500 ± 3000	2084 ± 703	104 ± 12	42 ± 7	5842 ± 610	1463 ± 320	32,242 ± 1450

Table 5. The in vitro cytotoxicity values of platinum(II) complexes (PHENSS, 5MESS and 56MESS) and their corresponding platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), expressed as GI₅₀ (nM) [59,61,65]. Significant increase in potency was identified by a paired T-test with a two-tailed distribution ($n = 3$). Group differences were considered statistically significant if * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$ compared with cisplatin (see Table 6 for the GI₅₀ values of cisplatin).

Cell Lines	GI ₅₀ (nM)					
	PHENSS	5MESS	56MESS	PHENSS(IV)(OH) ₂	5MESS(IV)(OH) ₂	56MESS(IV)(OH) ₂
HT29	160 ± 45 **	33 ± 4 **	10 ± 1.6 **	710 ± 300 **	60 ± 6 **	36 ± 7 **
U87	980 ± 270 *	320 ± 26 *	35 ± 6.4 *	4900 ± 610	900 ± 58	190 ± 23 *
H460	360 ± 35 *	41 ± 5 *	21 ± 2 *	1700 ± 200	60 ± 5 *	190 ± 150 *
A431	480 ± 170 **	120 ± 25 **	29 ± 1 **	4300 ± 530 *	360 ± 58 **	120 ± 22 **
Du145	100 ± 38 **	22 ± 3 **	4.6 ± 0.4 **	310 ± 92 **	41 ± 5 **	15 ± 2.6 **
BE2-C	380 ± 46 **	270 ± 38 **	59 ± 4 **	3000 ± 530	1400 ± 300	240 ± 22 **
SJ-G2	330 ± 66	220 ± 10	66 ± 22 **	1700 ± 350 **	640 ± 70	210 ± 45 *
MIA	200 ± 57 **	48 ± 2 **	13 ± 2 **	3400 ± 2200	160 ± 29 **	43 ± 2.5 **
A2780	230 ± 30 **	61 ± 10 **	76 ± 57 **	800 ± 84	240 ± 9 **	59 ± 7 **
ADDP	190 ± 47 ****	34 ± 2 ****	13 ± 2 ****	1300 ± 350 ****	130 ± 22 ****	170 ± 120 ****
MCF-7	1500 ± 500 **	200 ± 12 **	93 ± 44 **	16,000 ± 4500	1200 ± 390 **	480 ± 140 **
MCF10A	300 ± 58 **	30 ± 2 **	16 ± 1 **	1700 ± 200	130 ± 19 **	61 ± 7 **
Mean GI ₅₀	434 ± 110	117 ± 12	36 ± 10	3318 ± 880	443 ± 81	151 ± 50

Table 6. The SCI of P-5B3A, 5-5B3A, 56-5B3A and cisplatin in all cancerous cell lines.

Cell Lines	Platinum Complexes			
	P-5B3A	5-5B3A	56-5B3A	Cisplatin
HT29	1.40	1.06	0.75	0.46
U87	0.15	0.08	0.04	1.37
MCF-7	0.05	0.10	0.05	0.80
H460	0.65	0.63	0.42	5.78
A431	0.23	0.20	0.10	2.17
Du145	1.47	1.90	2.75	4.33
BE2-C	0.02	0.06	0.02	2.74
SJ-G2	0.22	0.10	0.03	13.00
MIA	0.90	0.61	0.28	0.69
A2780	0.60	0.45	0.18	5.20
ADDP	0.85	0.83	0.55	0.19

SCI = GI₅₀ (normal cancer cell line, MCF10A)/GI₅₀ (cancer cell lines).

P-5B3A, 5-5B3A and 56-5B3A proved to be potent in most cell lines (Table 4), although P-5B3A was least active in the BE2-C neuroblastoma cell line, with a GI₅₀ value of 13,000 ± 5000 nM. 5-5B3A and 56-5B3A were found to be more active than cisplatin, oxaliplatin and carboplatin in all the cell lines, as shown in Table 4. The average GI₅₀ values of the complexes were calculated from all cell lines and with the order of increasing cytotoxicity: P-5B3A < 5-5B3A < 56-5B3A. The complexes incorporating the methylated heterocyclic ligands, 5-5B3A and 56-5B3A demonstrated greater anticancer potential compared to P-5B3A. This trend in cytotoxicity was anticipated, as this has been previously observed with the equivalent platinum(II) precursors (PHENSS, 5MESS and 56MESS) and platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂).

(Table 5), as well as other similar complexes in the literature [31,38,59,61,62,65,66,69,73]. These results corroborate the notion that increasing methylation of the heterocyclic ligand enhances the biological activity of the complexes.

P-5B3A exhibited an average GI_{50} value of 2084 ± 703 nM, which is 20- and 50-fold larger than the average GI_{50} values calculated for its derivatives, **5-5B3A** (104 ± 12 nM) and **56-5B3A** (42 ± 7 nM), respectively (Table 4). **P-5B3A** was more potent than cisplatin, oxaliplatin and carboplatin in most cell lines; for example, **P-5B3A** was at least 57-, 5- and 250-fold more potent than cisplatin ($11,300 \pm 1900$ nM) (** $p < 0.01$), oxaliplatin (900 ± 200 nM) and carboplatin ($>50,000$ nM), respectively, in the HT29 colon cell line (Table 4). In the cisplatin-resistant ADDP ovarian variant cell line, **P-5B3A** (330 ± 8.8 nM) was at least 85-fold more potent than cisplatin ($28,000 \pm 1600$ nM) (**** $p < 0.0001$), and 152-fold more potent than carboplatin ($>50,000$ nM) (Table 4). **5-5B3A** also demonstrated potency in most cell lines, most notably in the Du145 prostate, HT29 colon and ADDP. In the Du145 prostate cell line, **5-5B3A** was 120-, 290- and 1500-fold more potent than cisplatin (1200 ± 100 nM) (** $p < 0.01$), oxaliplatin (2900 ± 400 nM) and carboplatin ($15,000 \pm 1200$ nM), respectively (Table 4). Moreover, **5-5B3A** elicited a GI_{50} value of 18 ± 0.67 nM in the HT29 colon cell line as shown in Table 4, which makes cisplatin almost 630-fold less effective in this cell line (** $p < 0.01$). Of further note, **5-5B3A** was also found to be almost 1220-fold more effective at inhibiting ADDP ovarian variant cells compared to cisplatin (**** $p < 0.0001$) (Table 4). **56-5B3A** proved to be the most biologically active platinum(IV) derivative, its potency was significant in all cell lines with GI_{50} values ranging between 1.2 and 150 nM (Table 4). Remarkably, **56-5B3A** (1.2 ± 0.6 nM) was 1000-fold more potent than cisplatin (** $p < 0.01$), and at least 2400- and 12,500-fold more potent than oxaliplatin and carboplatin, respectively, in the Du145 prostate cell line. In addition, **56-5B3A** also produced impressive cytotoxicity on the following cell lines: HT29 colon (4.4 ± 1.6 nM), H460 lung (7.8 ± 2.2 nM) and MIA pancreas (12 ± 3 nM), as shown in Table 4.

The coordination of 5B3A in the axial position of the platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**) resulted in enhanced overall biological activity of the complexes (**P-5B3A**, **5-5B3A** and **56-5B3A**). For example, the average GI_{50} value of **PHENSS(IV)(OH)₂** in all cell lines was 3318 ± 880 nM (Table 5) and this decreased by almost 1.6 upon coordinating 5B3A to form the platinum(IV) derivative, **P-5B3A** (2084 ± 703 nM). The average GI_{50} values of **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂** also decreased by 4 and 3.6 fold when compared to the GI_{50} values of their platinum(IV) derivatives, **5-5B3A** and **56-5B3A**, respectively (Tables 4 and 5). Furthermore, the cytotoxicity of the platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and their platinum(IV) derivatives (**P-5B3A**, **5-5B3A** and **56-5B3A**) in most cell lines are comparable. Intriguingly, **P-5B3A** was less potent than **PHENSS** in all cancer cell lines (Table 4). In the MCF10A normal breast cell line, only a 0.9-fold difference in cytotoxicity was reported between **PHENSS** (300 ± 58 nM) and **P-5B3A** (280 ± 39 nM) (Tables 4 and 5). Whereas **5-5B3A** and **56-5B3A** were found to be marginally more potent than their platinum(II) precursors, **5MESS** and **56MESS**, respectively, in most cell lines. **5-5B3A** has an average GI_{50} value of 104 ± 12 nM, which is approximately 0.9-fold lower than the average GI_{50} value determined for **5MESS** (117 ± 12 nM). Lastly, **56-5B3A** was confirmed to be slightly more effective than **56MESS** in inhibiting HT29 colon, Du145 prostate and ADDP cell populations (Tables 4 and 5). **56-5B3A** was 0.4-, 0.3- and 0.5-fold more potent than **56MESS** in these cell lines.

The selectivity cytotoxicity index (SCI) of the synthesised platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A**, and cisplatin was also calculated by dividing their GI_{50} in the normal breast cancer cell line (MCF10A) to their GI_{50} in all cancer cell lines (Table 6). Generally, a higher SCI corresponds to improved selectivity towards cancer cells [74,75]. The complexes demonstrated a degree of selectivity towards the HT29 colon, Du145 prostate and the cisplatin-resistant ADDP cell lines relative to cisplatin (Table 6). This also corresponds to the low GI_{50} values elicited by the complexes in these cell lines (Table 4). In the

HT29 colon cell line, **P-5B3A** and **5-5B3A** had a calculated SCI of 1.40 and 1.06, respectively, while for **56-5B3A**, the calculated SCI was 0.75. In the same cell line, cisplatin was less selective with a calculated SCI of 0.46. Moreover, while it was expected that cisplatin had a lower SCI (0.19) in the cisplatin-resistant ADDP cell line, the SCI of **P-5B3A**, **5-5B3A** and **56-5B3A** ranged between 0.55 and 0.85 (Table 6). Interestingly, cisplatin had a higher SCI (4.33) compared to the complexes (1.47–2.75) in the Du145 prostate cell line, as shown in Table 6. What is also noteworthy is that cisplatin had higher SCI in most of the reported cell lines (Table 6).

Despite the impressive in vitro cytotoxicity exhibited by **P-5B3A**, **5-5B3A** and **56-5B3A** in most cell lines, it is important to note that these complexes are not selective (Table 6). For example, in the MCF10A normal breast cell line, the complexes were significantly more toxic than in the MCF-7 cancerous breast cell line (Table 4). Additionally, the prodrugs, and their corresponding platinum(IV) scaffolds and platinum(II) precursors, exhibited decreased cytotoxicity in the BE2-C neuroblastoma and SJ-G2 glioblastoma cell lines compared to the other cell lines tested (Tables 4 and 5). This decreased cytotoxicity may be associated with efflux resistance mechanisms by the cell lines. P-glycoproteins are multidrug resistance proteins that are upregulated in the BE2-C neuroblastoma and SJ-G2 glioblastoma cell types [76], and these may prevent the adequate accumulation of the complexes in these cell lines. Nevertheless, the complexes demonstrated a degree of selectivity towards the HT29 colon, Du145 prostate and the cisplatin-resistant ADDP ovarian variant cell lines, which has been a consistent trend observed with similar complexes in the literature [59,61,65,66,69]. Their capacity to overcome resistance was confirmed based on their responsiveness to the cisplatin-resistant ADDP ovarian variant cell line. The coordination of 5B3A to the platinum(IV) cores of **PHENSS**, **5MESS** and **56MESS** enhanced the biological activity of the resultant complexes (**P-5B3A**, **5-5B3A** and **56-5B3A**) in most cell lines tested (Tables 4 and 5).

2.3.2. Drug-Induced ROS Production as a Treatment Strategy

ROS embody a variety of chemically reactive free and non-free radicals which are by-products of aerobic metabolism [77]. These metabolic intermediates are mostly generated in the mitochondria [78,79]. ROS are highly versatile for having important functions in cell signaling and other physiological cascades that contribute to maintaining cellular life [80,81]. Additionally, ROS levels are tightly regulated and constantly maintained at basal levels in the normal cell environment [82,83]. Regulation is critical to prevent excessive release of ROS from inducing oxidative stress, causing damage to cell membranes, proteins, ribonucleic acid, and DNA [84,85]. Other sources that can also elevate levels of ROS are exogenous substances such as drugs, xenobiotics, and radiation pollutants [85].

Elevated ROS levels are disadvantageous for normal healthy cells because oxidative stress would transpire. In contrast, this is advantageous for cancer cells to an extent as cancer cells are equipped with greater levels of antioxidant enzymes to neutralise excessive amount of ROS during proliferation [86]. Elevated levels of ROS can promote cancer development and progression. However, cancer cells also become vulnerable to different death-inducing pathways when there is a disproportional increase in ROS levels especially when exposed for prolonged periods [87]. This can be achieved by introducing anticancer drugs. Most chemotherapeutics can increase ROS intracellularly, and this treatment approach has the potential to enhance cancer-killing effects [87–90]. We hypothesised that **P-5B3A**, **5-5B3A** and **56-5B3A** would produce higher levels of ROS than their equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**) because of the coordinated 5B3A axial ligand. The indole core of 5B3A has garnered synthetic interest because of the numerous pharmacological potencies it offers [52,53,55], and one of them is the generation of ROS [91,92].

The ROS generation capacity of **P-5B3A**, **5-5B3A** and **56-5B3A**, 5B3A, and cisplatin was determined in HT29 colon cells at different time intervals: 24, 48 and 72 h (Table 7

and Figures 9, S39 and S40). All values are expressed as relative fluorescence units (RFU). With reference to the control, a greater RFU corresponds to elevated ROS production (increased fluorescence of DCF). Previously published RFU values of the platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**) were also included in Table 7 and Figure 9, for reference [59,61,65]. The HT29 colon cell line was nominated for testing because **P-5B3A**, **5-5B3A** and **56-5B3A** displayed a degree of selectivity in this cell line (Tables 4 and 6).

Table 7. ROS production upon treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, **5B3A**, and cisplatin in HT29 colon cells at 24, 48 and 72 h. The RFU values reported for platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**) were acquired from the literature for comparison [59,61,65].

Compounds and Platinum Complexes	ROS Production * at Different Time Intervals (RFU)		
	24 h	48 h	72 h
Control	60 ± 3	63 ± 4	85 ± 6
Cisplatin	265 ± 19	299 ± 22	364 ± 18
(Tert-butyl hydroperoxide) TBHP	614 ± 21	477 ± 23	311 ± 18
PHENSS	174 ± 2	172 ± 9	176 ± 7
5MESS	204 ± 4	205 ± 3	188 ± 3
56MESS	240 ± 5	218 ± 3	255 ± 4
PHENSS(IV)(OH)₂	144 ± 5	273 ± 4	303 ± 1
5MESS(IV)(OH)₂	234 ± 1	323 ± 9	335 ± 2
56MESS(IV)(OH)₂	259 ± 3	356 ± 11	438 ± 7
5B3A	138 ± 8	153 ± 6	164 ± 4
P-5B3A	156 ± 16	214 ± 12	246 ± 11
5-5B3A	160 ± 11	230 ± 9	315 ± 14
56-5B3A	274 ± 9	418 ± 15	474 ± 10

* Data are the mean ± SEM of three independent experiments, each performed in triplicate.

Relative to the control, the synthesised platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A** caused a notable increase in ROS levels by 24 h, and progressively continued until completion of the experiments at 72 h in the HT29 colon cell line (Table 7 and Figures 9 and S39). **P-5B3A** and **5-5B3A** displayed similar RFU values at 24 and 48 h. At 24 h, **P-5B3A** incurred a slightly lower RFU value (156) compared to **5-5B3A** (160) (Table 7). Subsequently, at 48 h, the RFU value reported for **P-5B3A** increased to 214, which is slightly lower than the RFU value reported for **5-5B3A** (230), as shown in Table 7. At 72 h, the RFU values reported for **P-5B3A** and **5-5B3A** considerably increased to 246 and 315, respectively. The most potent platinum(IV) derivative, **56-5B3A** caused the greatest ROS production compared to **P-5B3A** and **5-5B3A** (Table 7 and Figures 9, S39 and S40 (compared to cisplatin)). At 24 and 48 h, **56-5B3A** elicited RFU values of 274 and 418, respectively, which are greater than the RFU values reported for **P-5B3A** and **5-5B3A** in that time period, as shown in Table 7. Peak ROS production was demonstrated at 72 h for **56-5B3A**, with an RFU value of 474 (Table 7). The **5B3A** ligand alone generated ROS (RFU values of 138, 153 and 164) (Table 7 and Figures 9, S39 and S40), but to a lesser extent when compared to **P-5B3A**, **5-5B3A** and **56-5B3A**. It is also noteworthy that there may be a link between ROS production at 72 h (Table 7 and Figures 9, S39 and S40) and in vitro cytotoxicity, in the HT29 colon cell line (Table 4). At 72 h, the least potent platinum(IV) derivative (**P-5B3A**) incurred an RFU value of 246, while the more potent platinum(IV) derivative (**5-5B3A**) incurred an RFU value of 315 (Table 7). Moreover, the most potent platinum(IV) derivative (**56-5B3A**) caused the greatest ROS production at 72 h with a reported RFU value of 474, as shown in

Table 7. This trend was also observed with similar complexes in the literature [59,65]. It is noteworthy that the GI_{50} values for each complex were used for the ROS experiments, so the same concentration of each complex is not directly compared. With the exception of **56-5B3A**, the reduced effect of the prodrugs, **P-5B3A** and **5-5B3A**, as well as the platinum(II) precursors at 72 h may have been due to their cytostatic effects. Overall, the findings indicate that **P-5B3A**, **5-5B3A** and **56-5B3A** are effective at causing elevated levels of ROS for up to 72 h. While there were no significant differences observed between these platinum(IV) complexes cisplatin in terms of ROS production (Figure S43), the increase in ROS from 24, 48 to 72 h for **56-5B3A** was still greater than that of cisplatin. Nevertheless, through this mechanism, the reported platinum(IV) complexes are still expected to trigger oxidative stress inside cancer cells, hence effectively ceasing cancer development. Our findings are also comparable to the results obtained in a similar study undertaken by Tolan et al. [92], where they have demonstrated the coordination of a ROS-inducing indole-based ligand to the core of a platinum complex (cisplatin), produced prodrugs with enhanced anticancer killing effects via the effective production of ROS.

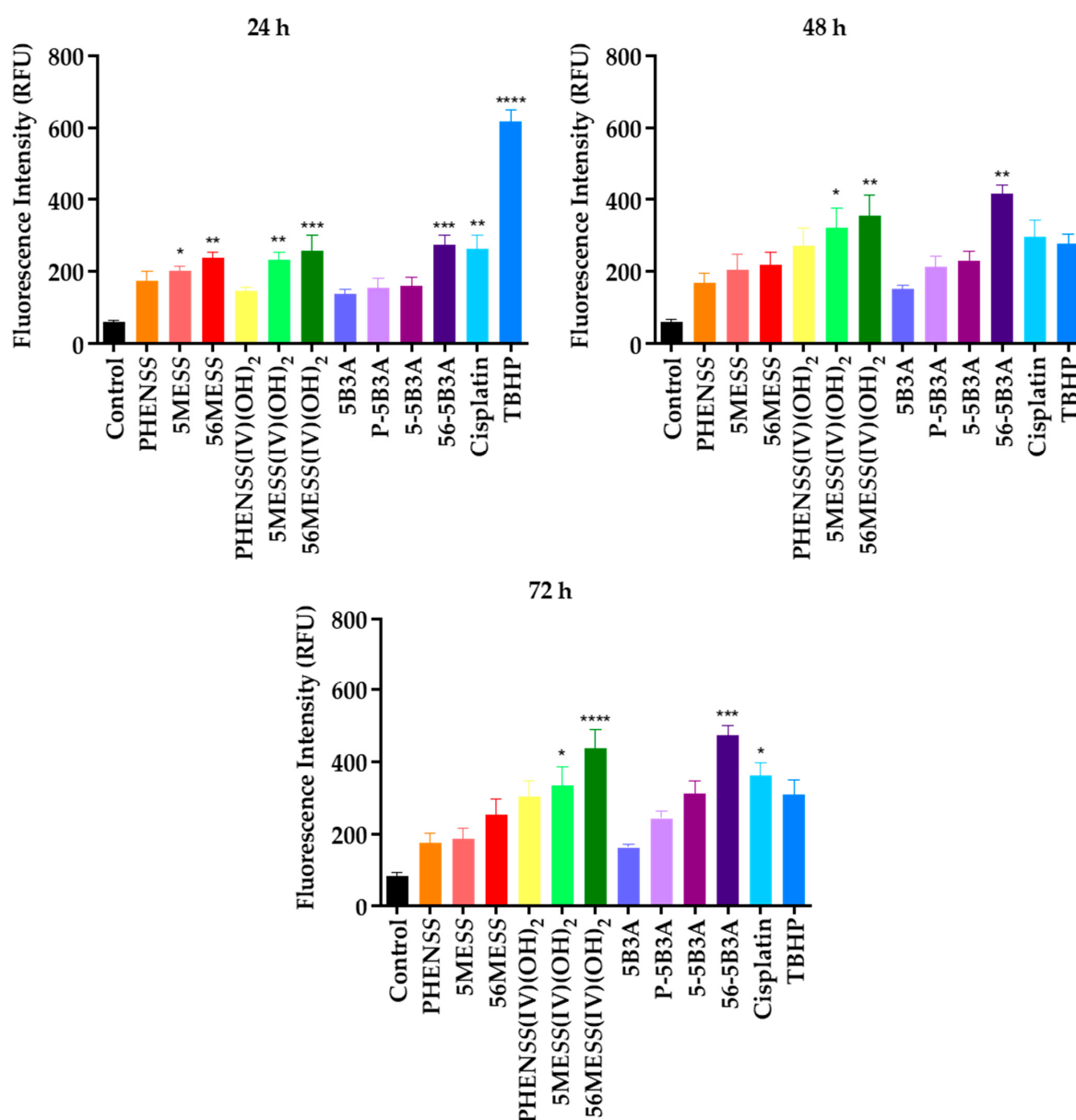


Figure 9. ROS generation in HT29 colon cells after treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, **5B3A**, the equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds

(PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), cisplatin and TBHP at 24, 48 and 72 h. Data are expressed in RFU values. The RFU values of the platinum(II) precursors (PHENSS, 5MESS and 56MESS) and the platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂) were included in the diagrams for comparison [59,61,65]. Data points signify the mean $\pm$ SEM. $n = 3$ from three independent tests where samples were achieved in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons. Group differences were considered statistically significant if * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared with control, as measured by one-way ANOVA.

2.3.3. Drug-Induced Mitochondrial Dysfunction as a Treatment Strategy

The mitochondria are a key feature of in cell types and are primarily responsible for producing energy required for metabolism [93,94]. While it is often described as a powerhouse, the mitochondria are also susceptible to oxidative stress [95,96]. Several drug classes, including chemotherapy drugs have been identified to promote disturbance in mitochondrial functions [97,98]. When this occurs, the mitochondrial membrane undergoes permeabilisation, which in turn creates an imbalance in MtMP [99,100]. MtMP is a parameter that reflects mitochondrial homeostasis, clearly because it is involved in the energy storage process during oxidative phosphorylation [101,102]. Fluctuations in MtMP are a consequence of normal physiological activity; however, prolonged fluctuations, either a significant increase or decrease in MtMP, prove to be deleterious to the mitochondria as this effect can trigger the release of apoptotic factors that lead to cell death [103–105].

Because the majority of ROS is generated in the mitochondria [78,106], it was appropriate to assess any mitochondrial activity changes upon treatment with the synthesised platinum(IV) complexes, P-5B3A, 5-5B3A and 56-5B3A. This was achieved by determining if the overproduction of ROS induced by these complexes impacts mitochondrial activity by altering MtMP. Briefly, there is evidence showing a strong correlation between increased levels of ROS and mitochondrial impairment [106–110]. We determined MtMP changes induced by P-5B3A, 5-5B3A and 56-5B3A, 5B3A, the platinum(II) precursors (PHENSS, 5MESS and 56MESS), the platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), and cisplatin in HT29 colon cells at different time intervals: 24, 48 and 72 h (Table 8 and Figures 10 and S41). The MtMP changes recorded are measured as RFU. Relative to the control, a lower or greater RFU is indicative of mitochondrial dysfunction caused by unstable MtMP.

In summary, the platinum(IV) complexes, P-5B3A, 5-5B3A, 56-5B3A and the 5B3A ligand, together with their equivalent platinum(II) precursors (PHENSS, 5MESS and 56MESS) and platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), and cisplatin caused a progressive decline in mitochondrial activity for up to 72 h, as reflected by the incurred RFU values (Table 8 and Figures 10 and S41). Interestingly, the pattern observed here may be linked to the elevated ROS levels detected for these complexes and the 5B3A ligand (Table 7 and Figures 9, S39 and S40). Our findings serve as another layer of evidence supporting the notion that elevated levels of ROS can promote mitochondrial impairment [106–110]. Furthermore, the RFU values reported for P-5B3A, 5-5B3A and 56-5B3A, and their equivalent platinum(II) precursors (PHENSS, 5MESS and 56MESS) were almost equivalent at 24, 48 and 72 h (Table 8 and Figures 10 and S41). This suggests that the coordinated 5B3A ligand did not play a significant role in altering MtMP. The changes in MtMP detected were predominantly caused by the core platinum(II) precursors (PHENSS, 5MESS and 56MESS) of the complexes. The results indicate that the platinum(II) precursors of the studied platinum(IV) prodrugs produced more pronounced impairment in the mitochondria. This phenomenon may be linked to the reduction potential of the prodrugs in the mitochondria and to their mechanism of action with a comparable delayed response to that of their platinum(II) precursors. A more pronounced effect of the prodrugs may be observed at a timeframe of 96 h, possibly reaching the same potential as their platinum(II) precursors in causing mitochondrial impairment.

Table 8. The detected MtMP changes upon treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, **5B3A**, the platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and the platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**), and cisplatin in HT29 colon cells at 24, 48 and 72 h.

Compounds and Platinum Complexes	MtMP Changes * at Different Time Intervals (RFU)		
	24 h	48 h	72 h
Control	542 ± 4	507 ± 11	449 ± 10
Cisplatin	232 ± 4	230 ± 4	192 ± 7
Carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP)	234 ± 3	151 ± 7	84 ± 5
PHENSS	321 ± 7	305 ± 5	257 ± 6
5MESS	283 ± 4	261 ± 9	189 ± 11
56MESS	277 ± 5	211 ± 7	151 ± 7
PHENSS(IV)(OH)₂	328 ± 7	314 ± 6	234 ± 11
5MESS(IV)(OH)₂	311 ± 3	291 ± 9	228 ± 7
56MESS(IV)(OH)₂	291 ± 9	221 ± 5	188 ± 5
5B3A	489 ± 6	415 ± 5	327 ± 9
P-5B3A	332 ± 7	317 ± 7	263 ± 5
5-5B3A	324 ± 9	280 ± 12	184 ± 4
56-5B3A	321 ± 11	267 ± 8	148 ± 7

* Data are the mean ± SEM of three independent experiments, each performed in triplicate.

2.3.4. Histone Deacetylase (HDAC) Inhibition Studies

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) are critical transcriptional enzymes that play a role in regulating gene expression [111]. Histones are chromatin-regulating proteins which serve as scaffolding that maintain the structural integrity of chromosomal DNA [112]. HATs are responsible for adding acetyl (–COCH₃) to the highly charged lysine residues of histones [113]. This process eliminates the charge of the lysine residues, which decreases the affinity between histones and the DNA. As a result, the chromatin conforms into a relaxed state and this promotes gene activation by enhancing transcription [114]. Conversely, the primary function of HDACs is to remove acetyl from lysine residues of hyperacetylated histones and this forces the chromatin to condense, thus preventing further transcription [115].

Interestingly, HDACs are overexpressed in multiple cancer types and this overexpression aids in cell proliferation and survival [116]. For this reason, HDACs are considered valuable therapeutic targets in cancer therapy. Over the years, the development of HDAC inhibitors (HDACis) for cancer treatment has been a fruitful area of research [117,118]. To date, vorinostat, panobinostat, belinostat, romidepsin and chidamide are the only HDACis currently marketed for cancer treatment [119]. Several HDACis are also in clinical trials [120]. Other clinically investigated HDACis also include smaller molecules like valproic acid (VPA) [121,122] and 4-phenylbutyric acid (PhB) [123], as well as a range of indole-based derivatives [124,125]. Of further note, HDACis are reported to reverse the tumour-related phenomenon, Warburg effect [126,127]. Unfortunately, treatment with HDACis alone have limited success in cancer therapy due to their poor pharmacokinetics (i.e., limited absorption, distribution, metabolism and excretion) [128], susceptibility to resistance [129,130], and low potency towards solid tumours [131]. Combination therapy is proposed to achieve the full potential of HDACis [132,133]. Another approach that can be employed is by coordinating HDACis to a platinum(IV) scaffold. For example, PhB has been coordinated to platinum(IV) complex with a cisplatin core which produced derivatives with

enhanced antiproliferative effects and are capable of overcoming resistance [48,134]. This demonstrated a synergism between PhB and cisplatin [48]. At a molecular level, HDACis (i.e., PhB) can expose the DNA to platination, meaning that the affinity of DNA-binding agents (i.e., cisplatin) to DNA is increased [135].

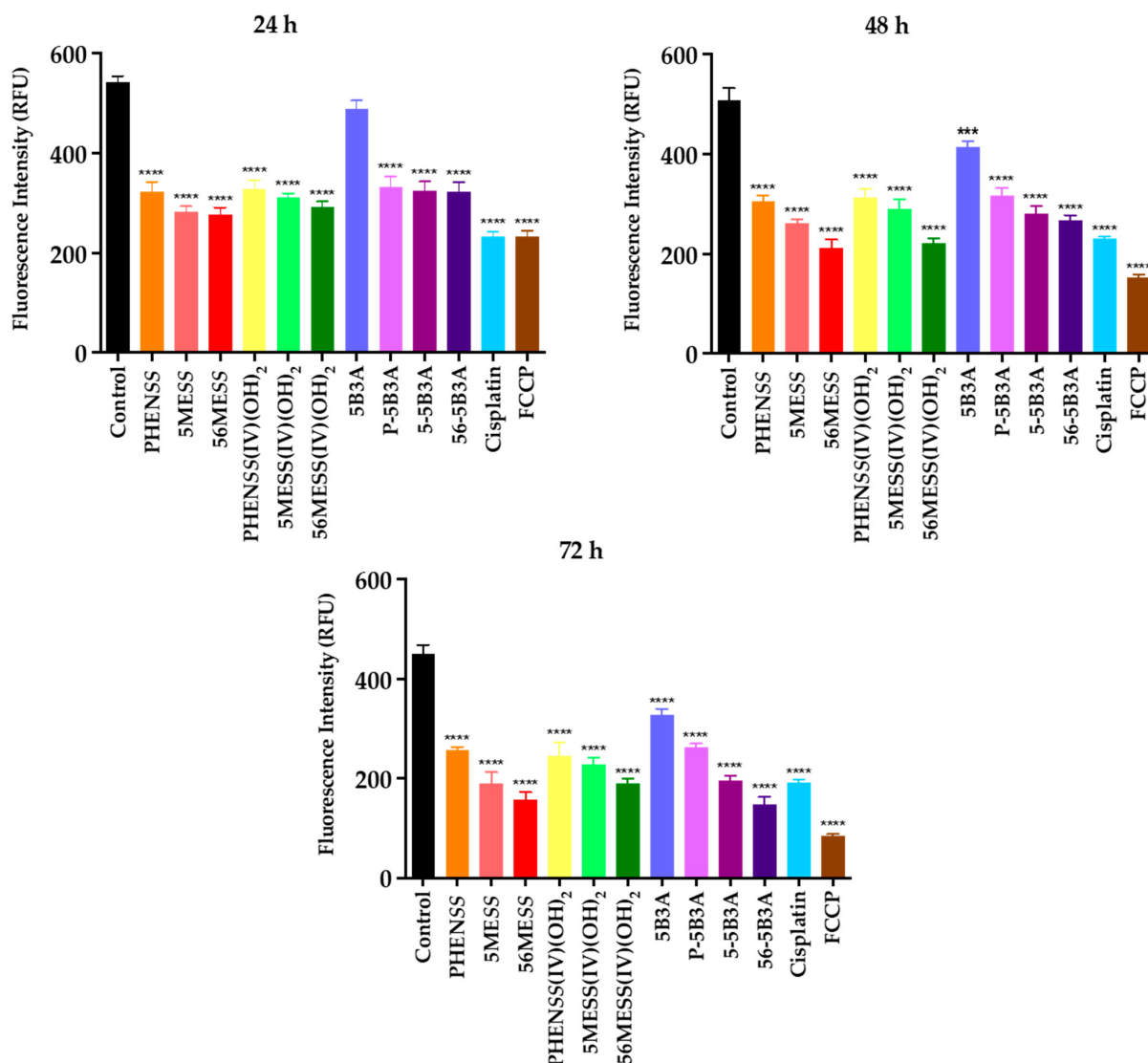


Figure 10. The detected MtMP changes in HT29 colon cells after treatment with P-5B3A, 5-5B3A and 56-5B3A, 5B3A, the equivalent platinum(II) precursors (PHENSS, 5MESS and 56MESS) and platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), cisplatin and FCCP at 24, 48 and 72 h. Data are expressed in RFU values. Data points signify the mean $\pm$ SEM. $n = 3$ from three independent tests where samples were achieved in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons. Group differences were considered statistically significant if *** $p < 0.001$ and **** $p < 0.0001$ compared with control, as measured by one-way ANOVA.

We determined the inhibition of HDAC activity by P-5B3A, 5-5B3A and 56-5B3A, 5B3A, the equivalent platinum(II) precursors (PHENSS, 5MESS and 56MESS) and platinum(IV) scaffolds (PHENSS(IV)(OH)₂, 5MESS(IV)(OH)₂ and 56MESS(IV)(OH)₂), together with cisplatin in HT29 colon cells at 72 h (Table 9 and Figures 11 and S42). All values are expressed as RFU. Relative to the HT29 extract (control), a lower RFU value corresponds to a decline in HDAC activity.

Table 9. HDAC activity inhibition upon treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, 5B3A, the platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**), and cisplatin in HT29 colon cells at 72 h.

Compounds and Complexes	HDAC Inhibition * at 72 h
HT29 extract	5154 ± 40
PHENSS	3733 ± 37
5MESS	2124 ± 21
56MESS	1847 ± 42
PHENSS(IV)(OH)₂	4107 ± 19
5MESS(IV)(OH)₂	3119 ± 9
56MESS(IV)(OH)₂	3011 ± 23
5B3A	3942 ± 28
P-5B3A	3620 ± 35
5-5B3A	2791 ± 72
56-5B3A	2550 ± 57
Cisplatin	3698 ± 59
No Enzyme Control	617 ± 3
HeLa Nuclear Extract	6697 ± 55
Trichostatin A (TSA)	749 ± 8

* Data are the mean ± SEM of three independent experiments, each performed in triplicate.

Peak HDAC inhibition was demonstrated by the potent HDAC inhibitor, TSA with a reported RFU value of 749 (Table 9 and Figure 11 and Figure S42). The studied platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A**, the 5B3A ligand, the equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**), the platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**), and cisplatin exhibited considerable HDAC inhibition in HT29 colon cells at 72 h, relative to the HT29 extract (control) (Table 9 and Figures 11 and S42). Interestingly, **PHENSS**, **PHENSS(IV)(OH)₂**, **P-5B3A**, the 5B3A ligand and cisplatin caused HDAC inhibition to a similar degree, with minor differences in the reported RFU values (Table 9). In comparison, **5MESS**, **56MESS**, **5MESS(IV)(OH)₂**, **56MESS(IV)(OH)₂**, **5-5B3A** and **56-5B3A** displayed greater HDAC inhibition when compared to their counterparts and cisplatin (Table 9 and Figures 11 and S42). Noticeably, the complexes bearing the methylated heterocyclic ligands (**5MESS**, **56MESS**, **5MESS(IV)(OH)₂**, **56MESS(IV)(OH)₂**, **5-5B3A** and **56-5B3A**) are more potent HDAC inhibitors than the unmethylated complexes (**PHENSS**, **PHENSS(IV)(OH)₂** and **P-5B3A**) (Table 9 and Figures 11 and S42). This pattern of HDAC inhibition further corroborates that methylation of the heterocyclic ligand is critical to overall biological activity. Moreover, this pattern can also be associated with the increase in ROS levels (Table 7 and Figure 9) and decrease in mitochondrial activity (Table 8 and Figure 10) observed with these complexes.

Surprisingly, **5MESS** and **56MESS** displayed the greatest HDAC inhibition amongst all the platinum complexes in this study with reported RFU values of 2124 and 1847 (Table 9), respectively. It is not clear why the other platinum(II) derivative, **PHENSS** was not able to cause the same degree of HDAC inhibition. However, it may be implied that the methylation at the heterocyclic ligands of **5MESS** and **56MESS** may have aided the complexes to have better inhibitory response towards HDAC. We also believe that this phenomenon may also be related to the mode of action of the complexes. Although the mode of action of **5MESS** has not been investigated before, we can only speculate that it parallels the proposed mode of action of **56MESS** due to their structural similarity. **56MESS** is reported to interfere with cytoskeletal networks [36,37]. Briefly, there are 18 HDACs present in humans [115], and one of them is a cytoplasmic enzyme called HDAC-6 that

is responsible for regulating cytoskeletal assembly [136–138]. HDAC-6 is overexpressed in HT29 colon cells [139,140]. Based on this information, it may be concluded that the strong inhibitory response of **56MESS** towards HDAC is a consequence of its ability to inhibit the cytoskeleton. Of further note, while it was anticipated that the platinum(IV) complexes, **5-5B3A** and **56-5B3A** would display better HDAC inhibition than their platinum(II) counterparts this was not reflected in the results. The weaker HDAC inhibitory response of **5-5B3A** and **56-5B3A** may be due to non-additive or antagonistic effect with the coordinated ligand, **5B3A**.

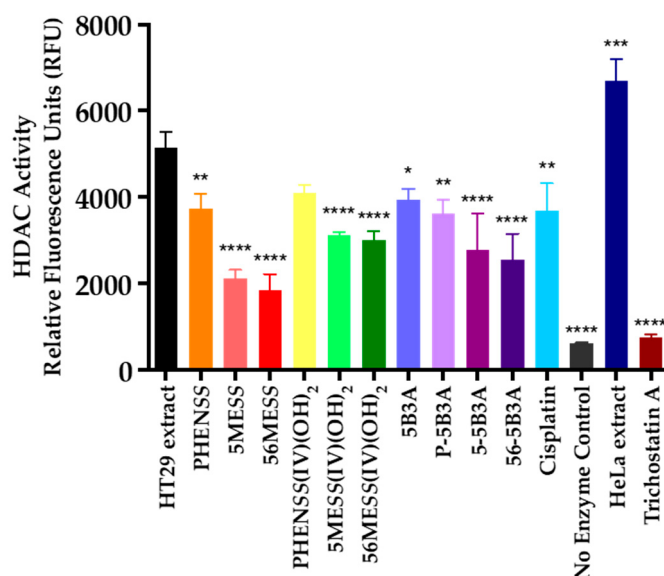


Figure 11. HDAC activity upon treatment with **P-5B3A**, **5-5B3A** and **56-5B3A**, **5B3A**, the equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**), and cisplatin at 72 h. Basal fluorescence was measured every min for 60 min. HeLa nuclear extract (ab156064) was used as positive control for activity, Trichostatin A (TSA) as inhibitor control for HDAC activity, and a No enzyme control (kit buffer and developers ab156064). The representative bar graph denotes the RFU for the end point. Data points signify the mean $\pm$ SEM. $n = 3$ from three independent experiments where samples were conducted in triplicate. Significance was identified by Tukey's multiple comparisons test for multiple comparisons. Group differences were considered statistically significant if * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ compared with HT29 extract (control), as measured by one-way ANOVA.

3. Materials and Methods

3.1. Materials

All reagents used were of spectroscopic grade. The deionised water (d.i.H₂O) used in the experimentations was acquired from a MilliQ™ system (Millipore Australia Pty Ltd., Sydney, NSW, Australia). Phen, 5-Mephen, 5,6-Me₂phen, **5B3A**, acetonitrile (CH₃CN), dimethyl sulfoxide (DMSO), N-hydroxysuccinimide, AsA, *N,N'*-dicyclohexylcarbodiimide (DCC) and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich, Sydney, NSW, Australia. Deuterated DMSO (DMSO-d₆) was purchased from Cambridge Isotope Laboratories, Andover, MA, USA. Methanol (MeOH) was obtained from Honeywell Research Chemicals, NJ, USA. Acetone (C₃H₆O) and diethyl ether (Et₂O) were purchased from ChemSupply Australia, Gillman, SA, Australia. Additional reagents used in the experiments were purchased from commercial sources. The cell lines HT29 colon, U87 glioblastoma, MCF-7 breast, H460 lung, Du145 prostate, BE2-C neuroblastoma, MIA pancreas, the cisplatin-resistant ADDP ovarian variant and the non-tumour-derived MCF10A breast were sourced from the American Type Culture Collection (ATCC). The A431 skin and A2780 ovarian cell lines were purchased from the European Collection of Authenticated

Cell Cultures (ECACC). The SJ-G2 glioblastoma was provided by Dr. Mary Danks of St. Jude Children's Research Hospital, Memphis, TN, USA. All cell lines were authenticated by CellBank Australia, (part of Children's Medical Research Institute, Westmead, Sydney, NSW, Australia).

3.2. Chemistry

3.2.1. Synthesis Route of NHS-5B3A

NHS-5B3A was prepared according to established protocols [60]. 5B3A was reacted with 1 mol eq. of NHS and DCC in C_3H_6O . The reaction solution was left stirring at room temperature for 24 h. The precipitated by-product, dicyclohexylurea (DCU), was removed through syringe filtration. The filtrate was collected and reduced to dryness through rotary evaporation. The NHS ester was used without further purification.

NHS-5B3A—Yield: 320 mg; 84%. 1H -NMR (400 MHz, $DMSO-d_6$, δ): 10.9 (s, $H_{pyrrole}$, 1H), 7.48 (d, a and b, 2H, $J = 7.6$ Hz), 7.40 (t, c and d, 2H, $J = 7.6$ Hz), 7.33 (m, e and f, 2H, $J = 7.8$ Hz), 7.28 (d, i, 1H, $J = 8.8$ Hz), 7.18 (s, g, 1H), 6.83 (dd, h, 1H, $J_1 = 1.9$ Hz, $J_2 = 8.7$ Hz), 5.10 (s, β , 2H), 4.13 (s, α , 2H), and 2.83 (s, j and k, 4H). HPLC λ_{max} nm, T_R : 254 nm, 11.8 min.

3.2.2. Syntheses of Platinum(II) Precursors and Platinum(IV) Scaffolds

All platinum(II) precursors of type $[Pt^{II}(H_L)(A_L)]^{2+}$ and platinum(IV) scaffolds of type $[Pt^{IV}(H_L)(A_L)(OH)_2]^{2+}$ were prepared as reported [59].

3.2.3. Syntheses of Platinum(IV) Derivatives Incorporating 5B3A (**P-5B3A**, **5-5B3A** and **56-5B3A**)

$[Pt^{IV}(H_L)(A_L)(X)(OH)]^{2+}$ were synthesised according to established protocols [61]. The appropriate precursor, $[Pt^{IV}(H_L)(A_L)(OH)_2]^{2+}$, was reacted with 2 mol eq. of NHS ester of 5B3A in $DMSO$ (1–2 mL) for 72 h at room temperature, in the dark. The reaction solution was washed with excess Et_2O and vigorously mixed using a plastic pipette, followed by centrifugation to afford a colourless supernatant and an oily brown layer. The colourless supernatant was discarded while the oily brown layer was collected and dissolved in methanol (1–2 mL), followed by the addition of excess Et_2O to induce precipitation. Centrifugation was undertaken to collect the final precipitate. Excess C_3H_6O was mixed with the precipitate and sonicated, affording a pure and solidified yellow or green precipitate that was collected through centrifugation.

P-5B3A—Yield: 64 mg; 80%. 1H -NMR (400 MHz, $DMSO-d_6$, δ): 10.3 (s, $H_{pyrrole}$, 1H), 9.40 (d, H2, 1H, $J = 5.4$ Hz), 9.38 (d, H9, 1H, $J = 5.4$ Hz), 9.06 (dd, H4 and H7, 2H, $J_1 = 4.8$ Hz, $J_2 = 8.1$ Hz), 8.26 (s, H5 and H6, 2H), 8.32 (m, H3 and H8, 2H), 7.51 (d, a and b, 2H, $J = 7.5$ Hz), 7.45 (t, c and d, 2H, $J = 7.4$ Hz), 7.38 (t, e, 1H, $J = 7.1$ Hz), 7.02 (d, i, 1H, $J = 8.7$ Hz), 6.69 (dd, h, 1H, $J_1 = 2.0$ Hz, $J_2 = 8.6$ Hz), 6.46 (s, f and g, 2H), 4.91 (s, β , 2H), 3.23 (d, α , 2H, $J = 6.4$ Hz), 2.98 (s, H1' and H2', 2H), 2.23 (m, H3' and H6' eq., 2H), 1.64 (m, H4' and H5' eq.; H3' and H6' ax., 4H), and 1.23 (m, H4' and H5' ax., 2H). 1H - ^{195}Pt -HMQC (400 MHz, $DMSO-d_6$, δ): 9.40/498 ppm; 9.38/498 ppm; 8.32/498 ppm; 3.24/498 ppm. HPLC λ_{max} nm, T_R : 254 nm, 8.08 min. UV λ_{max} nm ($\epsilon/M.cm^{-1} \pm SD \times 10^4$, d.i. H_2O): 304 (0.73 ± 3.15), 279 (2.16 ± 1.63), 204 (8.08 ± 1.70). CD λ_{max} nm ($\Delta\epsilon/M.cm^{-1} \times 10^1$, d.i. H_2O): 202 (−463), 203 (−485), 205 (−551), 228 (−58.9), 239 (−99.6), 263 (+31), 269 (+5.15), 274 (+21.3), 285 (−16.5), 313 (−32.2). Elemental microanalysis: *calculated* for $C_{35}H_{37}N_7O_{10}Pt$: C: 44.40%; H: 4.36%; N: 10.36%, found: C: 44.12%; H: 4.38%, N: 10.15%. Solubility: 7 mg/mL.

5-5B3A—Yield: 76 mg; 77%. 1H -NMR (400 MHz, $DMSO-d_6$, δ): 10.3 (d, $H_{pyrrole}$, 1H, $J = 1.8$ Hz), 9.33 (m, H2 and H9, 2H), 9.05 (dd, H4, 1H, $J = 7.8$ Hz), 8.91 (dd, H7, 1H, $J_1 = 4.4$ Hz, $J_2 = 8.2$ Hz), 8.30 (m, H3 and H8, 2H), 7.52 (d, a and b, 2H, $J = 7.2$ Hz), 7.46 (t, c and d, 2H, $J = 7.3$ Hz), 7.39 (t, e, 1H, $J = 7.2$ Hz), 7 (d, i, 1H, $J = 8.7$ Hz), 6.68 (d, h, 1H, $J = 8.7$ Hz), 6.42 (d, f, 1H, $J = 2.2$ Hz), 6.38 (s, g, 1H), 4.86 (s, β , 2H), 3.20 (m, α , 2H), 2.99 (s, H1' and H2', 2H), 2.88 (s, CH_3 , 3H), 2.24 (m, H3' and H6' eq., 2H), 1.66 (m, H4' and H5' eq.; H3' and H6' ax., 4H), and 1.25 (m, H4' and H5' ax., 2H). 1H - ^{195}Pt -HMQC (400 MHz, $DMSO-d_6$, δ): 9.33/499 ppm; 9.05/499 ppm; 8.91/499 ppm; 3.20/499 ppm. HPLC λ_{max} nm,

T_R : 254 nm, 8.38 min. UV λ_{max} nm ($\epsilon/M.cm^{-1} \pm SD \times 10^4$, d.i.H₂O): 310 (1.01 ± 0.73), 286 (3.63 ± 0.33), 204 (13.5 ± 3.61). CD λ_{max} nm ($\Delta\epsilon/M.cm^{-1} \times 10^1$, d.i.H₂O): 202 (−433), 203 (−460), 204 (−490), 230 (−35.1), 240 (−65.1), 257 (+16.9), 263 (+28.6), 270 (+30.7), 286 (−53.5). Elemental microanalysis: *calculated* for C₃₆H₃₉N₇O₁₀Pt: C: 44.17%; H: 4.63%; N: 10.02%, found: C: 44.22%; H: 4.44%, N: 9.92%. Solubility: 6 mg/mL.

56-5B3A—Yield: 58 mg; 88%. ¹H-NMR (400 MHz, DMSO-d₆, δ): 10.3 (d, H_{pyrrole}, 1H, $J = 1.9$ Hz), 9.31 (d, H₂, 1H, $J = 5.4$ Hz), 9.25 (d, H₉, 1H, $J = 5.4$ Hz), 9.08 (dd, H₄ and H₇, 2H, $J = 7.9$ Hz), 8.27 (m, H₃ and H₈, 2H), 7.52 (d, a and b, 2H, $J = 7.1$ Hz), 7.47 (t, c and d, 2H, $J = 7.3$ Hz), 7.42 (t, e, 1H, $J = 7.2$ Hz), 6.95 (d, i, 1H, $J = 8.7$ Hz), 6.64 (dd, h, 1H, $J_1 = 2.4$ Hz, $J_2 = 8.7$ Hz), 6.36 (d, f, 1H, $J = 2.2$ Hz), 6.30 (d, g, 1H, $J = 2.2$ Hz), 4.80 (s, β , 2H), 3.18 (d, α , 2H, $J = 4.8$ Hz), 2.99 (m, H_{1'} and H_{2'}, 2H), 2.79 (m, 2 $\times$ CH₃, 6H), 2.25 (d, H_{3'} and H_{6'} eq., 2H), 1.67 (m, H_{4'} and H_{5'} eq.; H_{3'} and H_{6'} ax., 4H), and 1.25 (m, H_{4'} and H_{5'} ax., 2H). ¹H-¹⁹⁵Pt-HMQC (400 MHz, DMSO-d₆, δ): 9.31/485 ppm; 9.25/485 ppm; 3.18/485 ppm. HPLC λ_{max} nm, T_R : 254 nm, 8.54 min. UV λ_{max} nm ($\epsilon/M.cm^{-1} \pm SD \times 10^4$, d.i.H₂O): 315 (0.59 ± 0.94), 291 (2.02 ± 1.78), 250 (1.31 ± 2.53), 206 (8.22 ± 1.75). CD λ_{max} nm ($\Delta\epsilon/M.cm^{-1} \times 10^1$, d.i.H₂O): 202 (−376), 204 (−384), 208 (−425), 230 (−6.55), 235 (−25.4), 239 (+7.34), 248 (+73.4), 257 (+101), 275 (+80.7), 288 (+0.91). Elemental microanalysis: *calculated* for C₃₇H₄₁N₇O₁₀Pt: C: 45.58%; H: 4.65%; N: 10.06%, found: C: 45.77%; H: 4.28%, N: 10.33%. Solubility: 4 mg/mL.

3.3. Laboratory Instrumentation

Equipment utilised to verify the purity and confirm the chemical structures of the studied platinum(IV) complexes including relevant protocols are outlined below.

3.3.1. Flash Chromatography

A Biotage Isolera™ One flash chromatography system (Shimadzu, Sydney, NSW, Australia) equipped with a Biotage® Sfar C18 D (Duo 100 Å 30 μ m 30 g) (Shimadzu, Sydney, NSW, Australia) was employed to purify the platinum(IV) complexes. The mobile phase consisted of solvents, A (d.i.H₂O) and B (MeOH). The samples were dissolved in d.i.H₂O/MeOH (50:50) and eluted through the column with a 0–40% linear gradient for 40 min with a flow rate of 4 mL.min^{−1}, collected within the set wavelengths of 200–400 nm.

3.3.2. High-Performance Liquid Chromatography (HPLC)

An Agilent (Melbourne, VIC, Australia) Technologies 1260 Infinity instrument equipped with a Phenomenex Onyx™ Monolithic C₁₈ reverse-phase column (100 $\times$ 4.6 mm, 5 μ m pore size) (Sydney, NSW, Australia) was utilised for the complexes. The mobile phase consisted of solvents, A (0.06% TFA in d.i.H₂O) and B (0.06% TFA in CH₃CN/d.i.H₂O (90:10)). An injection volume of 5 μ L was utilised and eluted with a 0–100% linear gradient over 15 min with a flow rate of 1 mL.min^{−1}, at the set wavelengths of 214 and 254 nm. An Agilent ZORBAX RX-C₁₈ column (100 $\times$ 4.6 mm, 3.5 μ m pore size) (Sydney, NSW, Australia) was utilised for the NHS ester of 5B3A using the same method described above.

3.3.3. Nuclear Magnetic Resonance (NMR) Spectroscopy

¹H-NMR, 2D-COSY, ¹H-¹⁹⁵Pt-HMQC and 1D-¹⁹⁵Pt-NMR were carried out on a 400 MHz Bruker (Melbourne, VIC, Australia) Avance spectrometer at 298 K. All complexes were prepared to a concentration of 10 mM in 600 μ L using D₂O. DMSO-d₆ was utilised for 5B3A, NHS ester of 5B3A, and the platinum(IV) complexes, **P-5B3A**, **5-5B3A** and **56-5B3A**. ¹H-NMR was set to 10 ppm and 16 scans with a spectral width of 8250 Hz and 65,536 data points. 2D-COSY was acquired using a spectral width of 3443 Hz for both ¹H nucleus, F1 and F2 dimensions, with 256 and 2048 data points, respectively. ¹H-¹⁹⁵Pt-HMQC was carried out using a spectral width of 214,436 Hz and 256 data points for ¹⁹⁵Pt nucleus, F1 dimension, also a spectral width of 4808 Hz with 2048 data points for ¹H nucleus, F2 dimension. 1D-¹⁹⁵Pt was measured using a spectral width of 85,470 Hz and 674 data points. All resonance recorded, were presented as chemical shifts in parts per million (δ ppm)

with *J*-coupling constants reported in Hz. For spin multiplicity: s (singlet); d (doublet); dd (doublet of doublets); t (triplet); q (quartet) and m (multiplet). All spectroscopic data gathered were generated and plotted using TopSpin 4.1.3 analysis software.

3.3.4. Ultraviolet–Visible (UV) Spectroscopy

An Agilent (Melbourne, VIC, Australia) Technologies Cary 3500 UV–Vis Multicell Peltier spectrophotometer was utilised to perform the UV spectroscopy experiments. UV spectroscopic experiments were completed at room temperature in the range of 200–400 nm with a 1 cm quartz cuvette. All complexes were prepared in d.i.H₂O, while 5B3A was prepared in CH₃CN. A stock solution of each complex or compound (1 mM) was prepared, and absorption spectra were recorded at a series of different concentrations by titrating 9 × 3 µL aliquots into a cuvette containing d.i.H₂O (3000 µL). Experiments were repeated in triplicate. All spectra were baseline corrected by the instrument—a baseline containing d.i.H₂O was acquired first, and automatically subtracted from each experiment. Average extinction coefficients (ε) were determined with standard deviation and errors based on the generated plot curves.

3.3.5. Circular Dichroism (CD) Spectroscopy

A Jasco (Easton, PA, USA) J-810 CD spectropolarimeter was used to measure the CD spectra of the complexes. The samples were prepared in d.i.H₂O, with similar concentrations (~0.05 mM) using a 1 mm optical glass cuvette or 1 cm quartz cuvette. CD experiments were undertaken at room temperature in the wavelength range of 200–400 nm (30 accumulations) with a bandwidth of 1 nm, data pitch of 0.5 nm, a response time of 1 s and a 100 nm.min^{−1} scan speed. The flowrate of nitrogen gas was 6 L.min^{−1}. The HT (photomultiplier) level remained below 500 V for all experiments. A CD simulation tool (CDToolX—Windows 10 Version) was used to process the spectra.

3.3.6. Elemental Microanalysis

Elemental analysis was performed on Elementar Vario MICRO (Elementar Analysensysteme GmbH, Frankfurt, Germany). The instrument hardware was configured for the analysis of 4 elements (C, H, N and S). An amount of 1.2 ± 0.2 mg of sample material was loaded into a tin foil crucible and combusted at 1150 °C with oxygen dosing time of 80 s and total O₂ flow rate of 30 mL/min. Ultra-high purity grade helium (BOC, 99.999%) and oxygen (BOC, 99.995%) were employed as working fluids in all cases. Pure sulfanilamide was employed as a standard with quality control samples added into the workflow every 10–20 runs. The follow-up data analysis was performed using a custom peak picking and integration algorithm written in Python 3.11.

3.4. Physicochemical Measurements

3.4.1. Solubility

The solubility of **P-5B3A**, **5-5B3A** and **56-5B3A** was tested in d.i.H₂O at room temperature. Small aliquots of d.i.H₂O were titrated into an Eppendorf tube containing 1 mg of each complex until full dissolution. For every titration, each sample solution was vortexed and sonicated.

3.4.2. Stability

The stability of **P-5B3A**, **5-5B3A** and **56-5B3A** were measured at 254 nm. Each complex was incubated in aqueous phosphate-buffered saline (PBS) solution (pH ~7.4) (total sample concentration, ~10 mM) at 37 °C for one week. Analytical HPLC was utilised to monitor the experiments.

3.4.3. Lipophilicity

Lipophilicity measurements were completed via analytical HPLC, as previously reported [61].

3.4.4. Reduction

The reduction behaviour of **P-5B3A**, **5-5B3A** and **56-5B3A** was investigated using ^1H -NMR and $1\text{D-}^{195}\text{Pt}$ -NMR spectroscopy, using previously established methods [61].

3.5. Biological Measurements

3.5.1. Cell Viability Assays

Cell viability assays were completed at the Calvary Mater Newcastle Hospital, NSW, Australia, as previously described [61,72]. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was used to measure the drug concentration at which cell growth was inhibited by 50% or GI_{50} . **P-5B3A**, **5-5B3A**, **56-5B3A** and **5B3A** were tested against the following cell lines: HT29 colon, U87 glioblastoma, MCF-7 breast, A2780 ovarian, H460 lung, A431 skin, Du145 prostate, BE2-C neuroblastoma, SJ-G2 glioblastoma, MIA pancreas, the cisplatin-resistant ADDP ovarian variant, and the non-tumour-derived MCF10A breast line. The SCI for **P-5B3A**, **5-5B3A** and **56-5B3A** was also calculated by dividing the GI_{50} values of the complexes in the normal breast cell line MCF10A by their GI_{50} in all cancerous cell lines. Generally, the higher the SCI the more selective a drug is towards cancer cells [74,75].

3.5.2. Drug-Induced Reactive Oxygen Species (ROS) Production

To evaluate the presence of ROS in treated cells, a DCFDA/ H_2DCFDA -cellular ROS Assay Kit (Abcam, Cambridge, MA, USA) was used, as previously described [61,141,142]. A total of 25,000 cells/mL of HT29 cells in DMEM were seeded in 96-well plates. Cells were washed with 1X kit buffer, and then stained with $25\text{ }\mu\text{M}$ 2',7'-dichlorofluorescein diacetate (DCFH-DA) and incubated for 45 min. DCFH-DA was then removed, and cells were then re-washed with 1X kit buffer, after which phenol red free media was added. Cells were then treated with a GI_{50} drug concentration for each complex. The plates were directly scanned to measure fluorescence (relative fluorescence units (RFU)) at the time points indicated the Glo-Max[®]-Multimode microplate reader (Promega Corporation, Alexandra, VIC, Australia) at an excitation/emission of 485/535 nm, which is directly correlated to the DCFH-DA that is deacetylated by cellular esterases and then oxidised by ROS to the fluorescent compound DCF. To generate the positive control ($20\text{ }\mu\text{M}$ *tert*-butyl hydroperoxide (TBHP)), cells were washed with 1X kit buffer, and stained with DCFDA ($25\text{ }\mu\text{M}$) for 45 min; this was removed and TBHP was added in phenol red free media, and the resulting solution was scanned as described above.

3.5.3. Mitochondrial Membrane Potential (MtMP) Changes

To investigate the MtMP changes in HT29 treated cells, a TMRE-MtMP Assay Kit (Abcam, Cambridge, MA, USA) was used, as previously described [36,61]. In 96-well plates, 25,000 cells/mL of HT29 colon cells in DMEM were seeded. Cells were treated with a GI_{50} drug concentration for each complex. At 24, 48 or 72 h, cells were washed with PBS and stained with tetramethylrhodamine, ethyl ester (TMRE) ($1\text{ }\mu\text{M}$) and incubated for 30 min. TMRE was then removed, and cells were then re-washed with PBS (0.2% BSA), after which phenol red free media was added. The plates were directly scanned to measure fluorescence (expressed as RFU), using the Glo-Max[®]-Multimode microplate reader (Promega Corporation, Alexandra, VIC, Australia) at an excitation/emission of 549/575 nm. For the positive control, $20\text{ }\mu\text{M}$ carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) was added to the cells and incubated for 10 min. The cells were washed with PBS (0.2% BSA) and stained with TMRE ($1\text{ }\mu\text{M}$) for 30 min; this was removed, and phenol red free media was added, and the final solution was scanned as mentioned above.

3.5.4. Histone Deacetylase (HDAC) Inhibition Measurements

To study the HDAC activity in HT29 treated cells, a Fluorometric HDAC Activity Assay Kit ab156064 (Abcam, Cambridge, MA, USA) was used and all sample preparations were made based on the manufacturer's protocol. A total of 10^6 cells/mL of HT29 cells

were seeded in DMEM with serum deprivation for 18 h to synchronise the cells. Then, cells were treated with GI₅₀ drug concentration of each complex or compound for 72 h. Cells were then washed with cold PBS and resuspended in lysis buffer (10 mM Tris HCl (pH 7.5), 10 mM NaCl, 15 mM MgCl₂, 250 mM sucrose, 0.5% NP-40 and 0.1 mM EGTA) and lysed on ice for 20 min. Cells were centrifuged through 4 mL of sucrose cushion (30% sucrose, 10mM Tris HCl (pH 7.5), 10 mM NaCl and 3mM MgCl₂) at 1300× g for 10 min at 4 °C. Supernatant was discarded and the pellet was washed and resuspended in extraction buffer (50 mM HEPES-KOH (pH 7.5), 420 mM NaCl, 0.5 mM EDTA 2Na, 0.1 mM EGTA and 10% glycerol) and then sonicated for 30 s. Nuclei were lysed for 30 min on ice and centrifuged at 20,000× g for 30 min. The supernatant was collected, and the samples were prepared for the fluorometric assay according to the manufacturer's protocol (Histone Deacetylase (HDAC) Activity Assay Fluorometric Kit ab156064, Abcam). The changes in the relative fluorescence intensity (expressed as RFU) were recorded each minute over 60 min using the Glo-Max[®]-Multimode microplate reader (Promega Corporation, Alexandra, VIC, Australia) at an excitation/emission of 355/460 nm.

4. Conclusions

Three platinum(IV) complexes incorporating the indole-based ligand, 5B3A (**P-5B3A**, **5-5B3A** and **56-5B3A**) were successfully synthesised in good yields. HPLC, NMR, UV and CD, as well as elemental microanalysis experiments confirmed the purity and chemical structures of the resultant complexes. The reduction behaviour of the complexes in PBS and AsA were monitored via NMR at 37 °C, with results indicating the ability to reduce to the equivalent platinum(II) precursors. From this, it is proposed that they can be defined as prodrugs. Additionally, the complexes were adequately stable in PBS for up to one week at 37 °C, suggesting their resistance towards significant hydrolysis in aqueous solutions. The rank of increasing lipophilicity of the complexes, as determined by analytical HPLC, was **P-5B3A** < **5-5B3A** < **56-5B3A**.

The MTT assay was used to measure the anticancer activity of **P-5B3A**, **5-5B3A** and **56-5B3A**. The findings indicated that the complexes were considerably more potent than cisplatin, oxaliplatin and carboplatin in most cell lines tested. This means that the coordination of the 5B3A ligand to our platinum(IV) scaffolds enhanced the biological activity of the resultant platinum(IV) complexes to an extent. The rank of increasing cytotoxicity was determined as **P-5B3A** < **5-5B3A** < **56-5B3A**. This also relates with the rank of increasing lipophilicity. Notably, the most lipophilic complex, **56-5B3A** displayed the greatest growth inhibitory response in the Du145 prostate and HT29 colon cell lines with reported GI₅₀ values of 1.2 ± 0.6 nM and 4.4 ± 1.6 nM, respectively. Unfortunately, **P-5B3A**, **5-5B3A** and **56-5B3A** were found to be significantly toxic towards the MCF10A normal breast cell line, denoting their limited selectivity. While this is the case, it should be noted that this outcome may not be translated in vivo. Despite this, the complexes were still significantly more potent than cisplatin in the cisplatin-resistant ADDP ovarian variant cell lines, thus confirming their ability to overcome resistance mechanisms by cancer cells.

A sustained increase in ROS production was also observed in HT29 colon cells, upon treatment with **P-5B3A**, **5-5B3A** and **56-5B3A** for up to 72 h. The order by which the complexes displayed cumulative ROS production at 24, 48 and 72 h was determined to be **P-5B3A** < **5-5B3A** < **56-5B3A**. This trend in ROS production also relates to the trend in cytotoxicity, further confirming that the presence of the 5B3A ligand in the axial position aided the complexes to produce ROS. Moreover, the observed ROS production was also in agreement with the decrease in mitochondrial activity induced by the complexes. **P-5B3A**, **5-5B3A** and **56-5B3A** caused a progressive decrease in MtMP in HT29 colon cells for up to 72 h, which translates to mitochondrial dysfunction. These results strongly suggest that ROS production and mitochondrial dysfunction via decreasing MtMP may be additional cytotoxic elements that contribute to the overall potency of the complexes.

The HDAC inhibition of **P-5B3A**, **5-5B3A** and **56-5B3A** was also investigated in HT29 colon cells. We anticipated that the 5B3A ligand would enhance the inhibitory response

of the resultant complexes towards HDAC, because of its indole pharmacophore which has been reported to inhibit HDAC. Unexpectedly, the results indicated otherwise. **P-5B3A** proved to be the least active against HDAC. While the other derivatives, **5-5B3A** and **56-5B3A** displayed a better inhibitory response against HDAC, the degree of inhibition was significantly inferior compared to TSA. Clearly, the 5B3A ligand did not enhance the activity of **P-5B3A**, **5-5B3A** and **56-5B3A** against HDAC. For the first time, we also reported the HDAC inhibition of their equivalent platinum(II) precursors (**PHENSS**, **5MESS** and **56MESS**) and platinum(IV) scaffolds (**PHENSS(IV)(OH)₂**, **5MESS(IV)(OH)₂** and **56MESS(IV)(OH)₂**) in the same cell line. Amongst the complexes, **5MESS** and **56MESS** proved somewhat active against HDAC. Particularly for **56MESS**, we speculate that its ability to inhibit HDAC significantly is closely associated with one of its mode of actions, that is its interference with the cytoskeleton.

5. Patents

This work is part of Australian PCT application (**PCT/AU2023/050027**), Platinum(IV) complexes, 20 February 2023, Western Sydney University, Sydney, Australia.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms25042181/s1>.

Author Contributions: Conceptualization, A.D.A. and J.R.A.-W.; data curation, A.D.A., M.G.E., J.G. and J.A.S.; formal analysis, A.D.A., M.G.E., J.G. and J.A.S.; investigation, A.D.A., M.G.E., J.G. and J.A.S.; methodology, A.D.A. and M.G.E.; project administration, A.D.A. and J.R.A.-W.; supervision, C.P.G., K.F.S. and J.R.A.-W.; validation, A.D.A., M.G.E., J.G., J.A.S. and J.R.A.-W.; visualization, A.D.A.; writing—original draft, A.D.A.; writing—review and editing, A.D.A., M.G.E., C.P.G., J.A.S., K.F.S. and J.R.A.-W. All authors have read and agreed to the published version of the manuscript.

Funding: The authors thank Western Sydney University for financial support. Angelico D. Aputen and Maria George Elias were supported through a Western Sydney University Australian Postgraduate Award. The School of Science, Western Sydney University, has supported this work through research funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data relevant to the publication are included.

Acknowledgments: The authors gratefully acknowledge the scientific and technical support at the Biomedical Magnetic Resonance Facility, Western Sydney University. Angelico D. Aputen, Christopher P. Gordon and Janice R. Aldrich-Wright thank Jayne Gilbert and Jennette A. Sakoff for their continuous collaboration with the research team. The authors also thank Mary Danks for providing the SJ-G2 glioblastoma cell line. Maria George Elias and Janice R. Aldrich-Wright thank Kieran F. Scott from the School of Medicine, Western Sydney University, and the Ingham Institute. Angelico D. Aputen also thank Ashley Jurisinec for his insights on the NMR assignments. Angelico D. Aputen and Janice R. Aldrich-Wright also like to acknowledge the Macquarie University Chemical Analysis and Biotechnology Centre (MUCAB) for access to its instrumentation, and the staff.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Rosenberg, B.; Van Camp, L.; Krigas, T. Inhibition of cell division in *Escherichia coli* by electrolysis products from a platinum electrode. *Nature* **1965**, *205*, 698–699. [\[CrossRef\]](#)
2. Kelland, L.R.; Sharp, S.Y.; O'Neill, C.F.; Raynaud, F.I.; Beale, P.J.; Judson, I.R. Mini-review: Discovery and development of platinum complexes designed to circumvent cisplatin resistance. *J. Inorg. Biochem.* **1999**, *77*, 111–115. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Galanski, M.; Jakupec, M.A.; Keppler, B.K. Update of the preclinical situation of anticancer platinum complexes: Novel design strategies and innovative analytical approaches. *Curr. Med. Chem.* **2005**, *12*, 2075–2094. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Lokich, J.; Anderson, N. Carboplatin versus cisplatin in solid tumours: An analysis of the literature. *Ann. Oncol.* **1998**, *9*, 13–21. [\[CrossRef\]](#) [\[PubMed\]](#)

5. Taamma, A.; Cvitkovic, E.; Raymond, E.; Chaney, S.G. Oxaliplatin: A review of preclinical and clinical studies. *Ann. Oncol.* **1998**, *9*, 1053–1071.
6. Schoch, S.; Gajewski, S.; Rothfuß, J.; Hartwig, A.; Köberle, B. Comparative study of the mode of action of clinically approved platinum-based chemotherapeutics. *Int. J. Mol. Sci.* **2020**, *21*, 6928. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Rocha, C.R.R.; Silva, M.M.; Quinet, A.; Cabral-Neto, J.B.; Menck, C.F.M. DNA repair pathways and cisplatin resistance: An intimate relationship. *Clinics* **2018**, *73* (Suppl. S1), e478s. [\[CrossRef\]](#)
8. Mezencev, R. Interactions of cisplatin with non-DNA targets and their influence on anticancer activity and drug toxicity: The complex world of the platinum complex. *Curr. Cancer Drug Targets* **2015**, *14*, 794–816. [\[CrossRef\]](#)
9. Wang, J.; Tao, J.; Jia, S.; Wang, M.; Jiang, H.; Du, Z. The protein-binding behavior of platinum anticancer drugs in blood revealed by mass spectrometry. *Pharmaceuticals* **2021**, *14*, 104. [\[CrossRef\]](#)
10. Allassadi, S.; Pisani, M.J.; Wheate, N.J. A chemical perspective on the clinical use of platinum-based anticancer drugs. *Dalton Trans.* **2022**, *51*, 10835–10846. [\[CrossRef\]](#)
11. Goldstein, R.S.; Mayor, G.H. The nephrotoxicity of cisplatin. *Life Sci.* **1983**, *32*, 685–690. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Kidera, Y.; Kawakami, H.; Sakiyama, T.; Okamoto, K.; Tanaka, K.; Takeda, M.; Kaneda, H.; Nishina, S.; Tsurutani, J.; Fujiwara, K.; et al. Risk factors for cisplatin-induced nephrotoxicity and potential of magnesium supplementation for renal protection. *PLoS ONE* **2014**, *9*, e101902. [\[CrossRef\]](#) [\[PubMed\]](#)
13. McSweeney, K.R.; Gadanec, L.K.; Qaradakhi, T.; Ali, B.A.; Zulli, A.; Apostolopoulos, V. Mechanisms of cisplatin-induced acute kidney injury: Pathological mechanisms, pharmacological interventions, and genetic mitigations. *Cancers* **2021**, *13*, 1572. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Rogers, B.B.; Cuddahy, T.; Briscella, C.; Ross, N.; Olszanski, A.J.; Denlinger, C.S. Oxaliplatin. *Clin. J. Oncol. Nurs.* **2019**, *23*, 68–75. [\[PubMed\]](#)
15. Fotopoulou, C. Limitations to the use of carboplatin-based therapy in advanced ovarian cancer. *Eur. J. Cancer Suppl.* **2014**, *12*, 13–16. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Coffetti, G.; Moraschi, M.; Facchetti, G.; Rimoldi, I. The challenging treatment of cisplatin-resistant tumours: State of the art and future perspectives. *Molecules* **2023**, *28*, 3407. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Amable, L. Cisplatin resistance and opportunities for precision medicine. *Pharmacol. Res.* **2016**, *106*, 27–36. [\[CrossRef\]](#)
18. Bayat Mokhtari, R.; Homayouni, T.S.; Baluch, N.; Morgatskaya, E.; Kumar, S.; Das, B.; Yeager, H. Combination therapy in combating cancer. *Oncotarget* **2017**, *8*, 38022–38043. [\[CrossRef\]](#)
19. Tolcher, A.W.; Mayer, L.D. Improving combination cancer therapy: The CombiPlex® development platform. *Future Oncol.* **2018**, *14*, 1317–1332. [\[CrossRef\]](#)
20. Boshuizen, J.; Peeper, D.S. Rational cancer treatment combinations: An urgent clinical need. *Mol. Cell* **2020**, *78*, 1002–1018. [\[CrossRef\]](#)
21. Wheate, N.J.; Walker, S.; Craig, G.E.; Oun, R. The status of platinum anticancer drugs in the clinic and in clinical trials. *Dalton Trans.* **2010**, *39*, 8113–8127. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Johnstone, T.C.; Park, G.Y.; Lippard, S.J. Understanding and improving platinum anticancer drugs—Phenanthriplatin. *Anticancer Res.* **2014**, *34*, 471–476. [\[PubMed\]](#)
23. Johnstone, T.C.; Suntharalingam, K.; Lippard, S.J. The next generation of platinum drugs: Targeted Pt(II) agents, nanoparticle delivery, and Pt(IV) prodrugs. *Chem. Rev.* **2016**, *116*, 3436–3486. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Ndagi, U.; Mhlongo, N.; Soliman, M.E. Metal complexes in cancer therapy—An update from drug design perspective. *Drug Des. Devel. Ther.* **2017**, *11*, 599–616. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Markman, M.; DeMarco, L.C.; Birkhofer, M.; Budman, D.; Hakes, T.; Reichman, B.; Rubin, S.; Jones, W.; Barakat, R.; Curtin, J.; et al. Phase II trial of zeniplatin (CL 286 558), a new platinum compound, in patients with advanced ovarian cancer previously treated with organoplatinum-based therapy. *J. Cancer Res. Clin. Oncol.* **1993**, *119*, 234–236. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Tanis, B.C.; Vermorken, J.B.; ten Bokkel Huinink, W.W.; van Oosterom, A.T.; Splinter, T.A.; Vendrik, K.J.; Sleijfer, D.T.; van der Burg, M.E.; van der Putten, E.; Pinedo, H.M. Multicenter phase II study of spiroplatin. *Oncology* **1992**, *49*, 99–103. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Kudelka, A.P.; Siddik, Z.H.; Tresukosol, D.; Edwards, C.L.; Freedman, R.S.; Madden, T.L.; Rastogi, R.; Hord, M.; Kim, E.E.; Tornos, C.; et al. A phase II and pharmacokinetic study of enloplatin in patients with platinum refractory advanced ovarian carcinoma. *Anti-Cancer Drugs* **1997**, *8*, 649–656. [\[CrossRef\]](#)
28. Dragovich, T.; Mendelson, D.; Hoos, A.; Lewis, J.; Kurtin, S.; Richardson, K.; Von Hoff, D. 268 A phase II trial of aroplatin (L-NDDP), a liposomal DACH platinum, in patients with metastatic colorectal cancer (CRC)—A preliminary report. *Eur. J. Cancer Suppl.* **2003**, *1*, S82–S83. [\[CrossRef\]](#)
29. Lebwohl, D.; Canetta, R. Clinical development of platinum complexes in cancer therapy: An historical perspective and an update. *Eur. J. Cancer* **1998**, *34*, 1522–1534. [\[CrossRef\]](#)
30. Brodie, C.R.; Collins, J.G.; Aldrich-Wright, J.R. DNA binding and biological activity of some platinum(II) intercalating compounds containing methyl-substituted 1,10-phenanthrolines. *Dalton Trans.* **2004**, *8*, 1145–1152. [\[CrossRef\]](#)
31. Wheate, N.J.; Taleb, R.I.; Krause-Heuer, A.M.; Cook, R.L.; Wang, S.; Higgins, V.J.; Aldrich-Wright, J.R. Novel platinum(II)-based anticancer complexes and molecular hosts as their drug delivery vehicles. *Dalton Trans.* **2007**, *43*, 5055–5064. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Fisher, D.M.; Fenton, R.; Aldrich-Wright, J. In vivo studies of a platinum(II) metallointercalator. *Chem. Comm.* **2008**, *43*, 5613–5615. [\[CrossRef\]](#) [\[PubMed\]](#)

33. Kemp, S.; Wheate, N.J.; Buck, D.P.; Nikac, M.; Collins, J.G.; Aldrich-Wright, J.R. The effect of ancillary ligand chirality and phenanthroline functional group substitution on the cytotoxicity of platinum(II)-based metallointercalators. *J. Inorg. Biochem.* **2007**, *101*, 1049–1058. [[CrossRef](#)] [[PubMed](#)]
34. Fisher, D.M.; Bednarski, P.J.; Grünert, R.; Turner, P.; Fenton, R.R.; Aldrich-Wright, J.R. Chiral platinum(ii) metallointercalators with potent in vitro cytotoxic activity. *Chem. Med. Chem.* **2007**, *2*, 488–495. [[CrossRef](#)]
35. Krause-Heuer, A.M.; Grünert, R.; Kuhne, S.; Buczkowska, M.; Wheate, N.J.; Le Pevelen, D.D.; Boag, L.R.; Fisher, D.M.; Kasparkova, J.; Malina, J.; et al. Studies of the mechanism of action of platinum(II) complexes with potent cytotoxicity in human cancer cells. *J. Med. Chem.* **2009**, *52*, 5474–5484. [[CrossRef](#)] [[PubMed](#)]
36. Kostrhunova, H.; Zajac, J.; Novohradsky, V.; Kasparkova, J.; Malina, J.; Aldrich-Wright, J.R.; Petruzzella, E.; Sirota, R.; Gibson, D.; Brabec, V. A subset of new platinum antitumour agents kills cells by a multimodal mechanism of action also involving changes in the organization of the microtubule cytoskeleton. *J. Med. Chem.* **2019**, *62*, 5176–5190. [[CrossRef](#)] [[PubMed](#)]
37. Garbutcheon-Singh, K.B.; Myers, S.; Harper, B.W.; Ng, N.S.; Dong, Q.; Xie, C.; Aldrich-Wright, J.R. The effects of 56MESS on mitochondrial and cytoskeletal proteins and the cell cycle in MDCK cells. *Metallomics* **2013**, *5*, 1061–1067. [[CrossRef](#)] [[PubMed](#)]
38. Moretto, J.; Chauffert, B.; Ghiringhelli, F.; Aldrich-Wright, J.R.; Bouyer, F. Discrepancy between in vitro and in vivo antitumour effect of a new platinum(II) metallointercalator. *Investig. N. Drugs* **2011**, *29*, 1164–1176. [[CrossRef](#)]
39. Harper, B.W.J.; Petruzzella, E.; Sirota, R.; Faccioli, F.F.; Aldrich-Wright, J.R.; Gandin, V.; Gibson, D. Synthesis, characterization and in vitro and in vivo anticancer activity of Pt(IV) derivatives of [Pt(1S,2S-DACH)(5,6-dimethyl-1,10-phenanthroline)]. *Dalton Trans.* **2017**, *46*, 7005–7019. [[CrossRef](#)]
40. Macias, F.J.; Deo, K.M.; Pages, B.J.; Wormell, P.; Clegg, J.K.; Zhang, Y.; Li, F.; Zheng, G.; Sakoff, J.; Gilbert, J.; et al. Synthesis and analysis of the structure, diffusion and cytotoxicity of heterocyclic platinum(IV) complexes. *Chemistry* **2015**, *21*, 16990–17001. [[CrossRef](#)]
41. Marotta, C.; Giorgi, E.; Binacchi, F.; Cirri, D.; Gabbiani, C.; Pratesi, A. An overview of recent advancements in anticancer Pt(IV) prodrugs: New smart drug combinations, activation and delivery strategies. *Inorganica Chim. Acta* **2023**, *548*, 121388. [[CrossRef](#)]
42. Xu, Z.; Wang, Z.; Deng, Z.; Zhu, G. Recent advances in the synthesis, stability, and activation of platinum(IV) anticancer prodrugs. *Coord. Chem. Rev.* **2021**, *442*, 213991–214023. [[CrossRef](#)]
43. Ponte, F.; Scoditti, S.; Mazzone, G.; Sicilia, E. The current status in computational exploration of Pt(IV) prodrug activation by reduction. *Phys. Chem. Chem. Phys.* **2023**, *25*, 15586–15599. [[CrossRef](#)] [[PubMed](#)]
44. Wong, D.Y.Q.; Ang, W.H. Development of platinum(IV) complexes as anticancer prodrugs: The story so far. *Cosmos* **2012**, *8*, 121–135. [[CrossRef](#)]
45. Zhong, Y.; Jia, C.; Zhang, X.; Liao, X.; Yang, B.; Cong, Y.; Pu, S.; Gao, C. Targeting drug delivery system for platinum(IV)-based antitumour complexes. *Eur. J. Med. Chem.* **2020**, *194*, 112229. [[CrossRef](#)] [[PubMed](#)]
46. Hall, M.D.; Mellor, H.R.; Callaghan, R.; Hambley, T.W. Basis for design and development of platinum(IV) anticancer complexes. *J. Med. Chem.* **2007**, *50*, 3403–3411. [[CrossRef](#)] [[PubMed](#)]
47. Ravera, M.; Gabano, E.; McGlinchey, M.J.; Osella, D. Pt(IV) antitumour prodrugs: Dogmas, paradigms, and realities. *Dalton Trans.* **2022**, *51*, 2121–2134. [[CrossRef](#)]
48. Raveendran, R.; Braude, J.P.; Wexselblatt, E.; Novohradsky, V.; Stuchlikova, O.; Brabec, V.; Gandin, V.; Gibson, D. Pt(IV) derivatives of cisplatin and oxaliplatin with phenylbutyrate axial ligands are potent cytotoxic agents that act by several mechanisms of action. *Chem. Sci.* **2016**, *7*, 2381–2391. [[CrossRef](#)]
49. Li, Z.; Wang, Q.; Li, L.; Chen, Y.; Cui, J.; Liu, M.; Zhang, N.; Liu, Z.; Han, J.; Wang, Z. Ketoprofen and loxoprofen platinum(IV) complexes displaying antimetastatic activities by inducing dna damage, inflammation suppression, and enhanced immune response. *J. Med. Chem.* **2021**, *64*, 17920–17935. [[CrossRef](#)]
50. Han, W.; He, W.; Song, Y.; Zhao, J.; Song, Z.; Shan, Y.; Hua, W.; Sun, Y. Multifunctional platinum(IV) complex bearing HDAC inhibitor and biotin moiety exhibits prominent cytotoxicity and tumour-targeting ability. *Dalton Trans.* **2022**, *51*, 7343–7351. [[CrossRef](#)]
51. Karmakar, S.; Kostrhunova, H.; Ctvrtlikova, T.; Novohradsky, V.; Gibson, D.; Brabec, V. Platinum(IV)-estramustine multi-action prodrugs are effective antiproliferative agents against prostate cancer cells. *J. Med. Chem.* **2020**, *63*, 13861–13877. [[CrossRef](#)] [[PubMed](#)]
52. Teraiya, N.; Agrawal, K.; Patel, T.M.; Patel, A.; Patel, S.; Shah, U.; Shah, S.; Rathod, K.; Patel, K. A review of the therapeutic importance of indole scaffold in drug discovery. *Curr. Drug Discov. Technol.* **2023**, *20*, e050523216584. [[CrossRef](#)] [[PubMed](#)]
53. Taliani, S.; Da Settimo, F.; Martini, C.; Laneri, S.; Novellino, E.; Greco, G. Exploiting the indole scaffold to design compounds binding to different pharmacological targets. *Molecules* **2020**, *25*, 2331. [[CrossRef](#)] [[PubMed](#)]
54. Devi, N.; Kaur, K.; Biharee, A.; Jaitak, V. Recent development in indole derivatives as anticancer agent: A mechanistic approach. *Anticancer Agents Med. Chem.* **2021**, *21*, 1802–1824. [[CrossRef](#)] [[PubMed](#)]
55. Dhuguru, J.; Skouta, R. Role of indole scaffolds as pharmacophores in the development of anti-lung cancer agents. *Molecules* **2020**, *25*, 1615. [[CrossRef](#)] [[PubMed](#)]
56. Dhiman, A.; Sharma, R.; Singh, R.K. Target-based anticancer indole derivatives and insight into structure–Activity relationship: A mechanistic review update (2018–2021). *Acta Pharm. Sin. B.* **2022**, *12*, 3006–3027. [[CrossRef](#)] [[PubMed](#)]

57. Oberhuber, N.; Ghosh, H.; Nitzsche, B.; Dandawate, P.; Höpfner, M.; Schobert, R.; Biersack, B. Synthesis and anticancer evaluation of new indole-based tyrphostin derivatives and their (p-cymene)dichloridoruthenium(II) complexes. *Int. J. Mol. Sci.* **2023**, *24*, 854. [\[CrossRef\]](#)
58. Bresciani, G.; Cervinka, J.; Kostrhunova, H.; Biancalana, L.; Bortoluzzi, M.; Pampaloni, G.; Novohradsky, V.; Brabec, V.; Marchetti, F.; Kasparkova, J. N-indolyl diiron vinyliminium complexes exhibit antiproliferative effects in cancer cells associated with disruption of mitochondrial homeostasis, ROS scavenging, and antioxidant activity. *Chem. Biol. Interact.* **2023**, *385*, 110742. [\[CrossRef\]](#)
59. Aputen, A.D.; Elias, M.G.; Gilbert, J.; Sakoff, J.A.; Gordon, C.P.; Scott, K.F.; Aldrich-Wright, J.R. Potent chlorambucil-platinum(IV) prodrugs. *Int. J. Mol. Sci.* **2022**, *23*, 10471–10499. [\[CrossRef\]](#)
60. Barré, A.; Tintas, M.; Levacher, V.; Papamichael, C.; Vincent, G. An overview of the synthesis of highly versatile n-hydroxysuccinimide esters. *Synthesis* **2017**, *49*, 472–483.
61. Aputen, A.D.; Elias, M.G.; Gilbert, J.; Sakoff, J.A.; Gordon, C.P.; Scott, K.F.; Aldrich-Wright, J.R. Versatile platinum(IV) prodrugs of naproxen and acetaminophen as chemo-anti-inflammatory agents. *Cancers* **2023**, *15*, 2460. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Deo, K.M.; Sakoff, J.; Gilbert, J.; Zhang, Y.; Aldrich Wright, J.R. Synthesis, characterisation and potent cytotoxicity of unconventional platinum(IV) complexes with modified lipophilicity. *Dalton Trans.* **2019**, *48*, 17217–17227. [\[CrossRef\]](#) [\[PubMed\]](#)
63. Khoury, A.; Elias, E.; Mehanna, S.; Shebaby, W.; Deo, K.M.; Mansour, N.; Khalil, C.; Sayyed, K.; Sakoff, J.A.; Gilbert, J.; et al. Novel platinum(II) and platinum(IV) antitumour agents that exhibit potent cytotoxicity and selectivity. *J. Med. Chem.* **2022**, *65*, 16481–16493. [\[CrossRef\]](#) [\[PubMed\]](#)
64. McGhie, B.S.; Sakoff, J.; Gilbert, J.; Aldrich-Wright, J.R. Synthesis and characterisation of platinum(IV) polypyridyl complexes with halide axial ligands. *Inorganica Chim. Acta* **2019**, *495*, 118964–118974. [\[CrossRef\]](#)
65. Aputen, A.D.; Elias, M.G.; Gilbert, J.; Sakoff, J.A.; Gordon, C.P.; Scott, K.F.; Aldrich-Wright, J.R. Bioactive platinum(IV) complexes incorporating halogenated phenylacetates. *Molecules* **2022**, *27*, 7120. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Khoury, A.; Sakoff, J.A.; Gilbert, J.; Scott, K.F.; Karan, S.; Gordon, C.P.; Aldrich-Wright, J.R. Cyclooxygenase-inhibiting platinum(IV) prodrugs with potent anticancer activity. *Pharmaceutics* **2022**, *14*, 787–808. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Aputen, A. Novel platinum(IV) prodrugs. Master's Thesis, Western Sydney University, Campbelltown, NSW, Australia, 2019.
68. Garbutcheon-Singh, K.B.; Leverett, P.; Myers, S.; Aldrich-Wright, J.R. Cytotoxic platinum(II) intercalators that incorporate 1R,2R-diaminocyclopentane. *Dalton Trans.* **2013**, *42*, 918–926. [\[CrossRef\]](#)
69. Khoury, A.; Sakoff, J.A.; Gilbert, J.; Karan, S.; Gordon, C.P.; Aldrich-Wright, J.R. Potent platinum(IV) prodrugs that incorporate a biotin moiety to selectively target cancer cells. *Pharmaceutics* **2022**, *14*, 2780. [\[CrossRef\]](#)
70. Dasari, S.; Tchounwou, P.B. Cisplatin in cancer therapy: Molecular mechanisms of action. *Eur. J. Pharmacol.* **2014**, *740*, 364–378. [\[CrossRef\]](#)
71. Chen, T.-Y.; Tsai, M.-J.; Chang, L.-C.; Wu, P.-C. Co-delivery of cisplatin and gemcitabine via viscous nanoemulsion for potential synergistic intravesical chemotherapy. *Pharmaceutics* **2020**, *12*, 949. [\[CrossRef\]](#)
72. Tarleton, M.; Gilbert, J.; Robertson, M.J.; McCluskey, A.; Sakoff, J.A. Library synthesis and cytotoxicity of a family of 2-phenylacrylonitriles and discovery of an estrogen dependent breast cancer lead compound. *Med. Chem. Comm.* **2011**, *2*, 31–37. [\[CrossRef\]](#)
73. Deo, K.M.; Sakoff, J.; Gilbert, J.; Zhang, Y.; Aldrich Wright, J.R. Synthesis, characterisation and influence of lipophilicity on cellular accumulation and cytotoxicity of unconventional platinum(IV) prodrugs as potent anticancer agents. *Dalton Trans.* **2019**, *48*, 17228–17240. [\[CrossRef\]](#) [\[PubMed\]](#)
74. López-Lázaro, M. Two preclinical tests to evaluate anticancer activity and to help validate drug candidates for clinical trials. *Oncoscience* **2015**, *2*, 91–98. [\[CrossRef\]](#) [\[PubMed\]](#)
75. López-Lázaro, M. A simple and reliable approach for assessing anticancer activity in vitro. *Curr. Med. Chem.* **2015**, *22*, 1324–1334. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Pilotto Heming, C.; Muriithi, W.; Wanjiku Macharia, L.; Niemeyer Filho, P.; Moura-Neto, V.; Aran, V. P-glycoprotein and cancer: What do we currently know? *Heliyon* **2022**, *8*, e11171. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Pizzino, G.; Irrera, N.; Cucinotta, M.; Pallio, G.; Mannino, F.; Arcoraci, V.; Squadrito, F.; Altavilla, D.; Bitto, A. Oxidative stress: Harms and benefits for human health. *Oxid. Med. Cell. Longev.* **2017**, *2017*, 8416763. [\[CrossRef\]](#) [\[PubMed\]](#)
78. Murphy, M.P. How mitochondria produce reactive oxygen species. *Biochem J.* **2009**, *417*, 1–13. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Zhao, R.Z.; Jiang, S.; Zhang, L.; Yu, Z.B. Mitochondrial electron transport chain, ROS generation and uncoupling (Review). *Int. J. Mol. Med.* **2019**, *44*, 3–15. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Checa, J.; Aran, J.M. Reactive oxygen species: Drivers of physiological and pathological processes. *J. Inflamm. Res.* **2020**, *13*, 1057–1073. [\[CrossRef\]](#)
81. Szechyńska-Hebda, M.; Ghalami, R.Z.; Kamran, M.; Van Breusegem, F.; Karpiński, S. To be or not to be? are reactive oxygen species, antioxidants, and stress signalling universal determinants of life or death? *Cells* **2022**, *11*, 4105. [\[CrossRef\]](#)
82. Redza-Dutordoir, M.; Averill-Bates, D.A. Activation of apoptosis signalling pathways by reactive oxygen species. *Biochim. Biophys. Acta Mol. Cell Res.* **2016**, *1863*, 2977–2992. [\[CrossRef\]](#) [\[PubMed\]](#)
83. Villalpando-Rodriguez, G.E.; Gibson, S.B. Reactive oxygen species (ROS) regulates different types of cell death by acting as a rheostat. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 9912436. [\[CrossRef\]](#) [\[PubMed\]](#)
84. Nakamura, H.; Takada, K. Reactive oxygen species in cancer: Current findings and future directions. *Cancer Sci.* **2021**, *112*, 3945–3952. [\[CrossRef\]](#)

85. Juan, C.A.; Pérez de la Lastra, J.M.; Plou, F.J.; Pérez-Lebeña, E. The chemistry of reactive oxygen species (ros) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies. *Int. J. Mol. Sci.* **2021**, *22*, 4642. [\[CrossRef\]](#)
86. George, S.; Abrahamse, H. Redox potential of antioxidants in cancer progression and prevention. *Antioxidants* **2020**, *9*, 1156. [\[CrossRef\]](#) [\[PubMed\]](#)
87. Galadari, S.; Rahman, A.; Pallichankandy, S.; Thayyullathil, F. Reactive oxygen species and cancer paradox: To promote or to suppress? *Free Radic. Biol. Med.* **2017**, *104*, 144–164. [\[CrossRef\]](#)
88. Jia, P.; Dai, C.; Cao, P.; Sun, D.; Ouyang, R.; Miao, Y. The role of reactive oxygen species in tumour treatment. *RSC Adv.* **2020**, *10*, 7740–7750. [\[CrossRef\]](#)
89. Aggarwal, V.; Tuli, H.S.; Varol, A.; Thakral, F.; Yerer, M.B.; Sak, K.; Varol, M.; Jain, A.; Khan, M.A.; Sethi, G. Role of reactive oxygen species in cancer progression: Molecular mechanisms and recent advancements. *Biomolecules* **2019**, *9*, 735. [\[CrossRef\]](#)
90. Perillo, B.; Di Donato, M.; Pezone, A.; Di Zazzo, E.; Giovannelli, P.; Galasso, G.; Castoria, G.; Migliaccio, A. ROS in cancer therapy: The bright side of the moon. *Exp. Mol. Med.* **2020**, *52*, 192–203. [\[CrossRef\]](#)
91. Estevão, M.S.; Carvalho, L.C.; Ribeiro, D.; Couto, D.; Freitas, M.; Gomes, A.; Ferreira, L.M.; Fernandes, E.; Marques, M.M.B. Antioxidant activity of unexplored indole derivatives: Synthesis and screening. *Eur. J. Med. Chem.* **2010**, *45*, 4869–4878. [\[CrossRef\]](#)
92. Tolan, D.; Gandin, V.; Morrison, L.; El-Nahas, A.; Marzano, C.; Montagner, D.; Erxleben, A. Oxidative stress induced by Pt(IV) pro-drugs based on the cisplatin scaffold and indole carboxylic acids in axial position. *Sci. Rep.* **2016**, *6*, 29367–29379. [\[CrossRef\]](#) [\[PubMed\]](#)
93. Javadov, S.; Kuznetsov, A.V. Mitochondria: The cell powerhouse and nexus of stress. *Front. Physiol.* **2013**, *4*, 207. [\[CrossRef\]](#)
94. McBride, H.M.; Neuspiel, M.; Wasiak, S. Mitochondria: More than just a powerhouse. *Curr. Biol.* **2006**, *16*, R551–R560. [\[CrossRef\]](#) [\[PubMed\]](#)
95. Pistritto, G.; Trisciuglio, D.; Ceci, C.; Garufi, A.; D’Orazi, G. Apoptosis as anticancer mechanism: Function and dysfunction of its modulators and targeted therapeutic strategies. *Aging* **2016**, *8*, 603–619. [\[CrossRef\]](#) [\[PubMed\]](#)
96. Shokolenko, I.; Venediktova, N.; Bochkareva, A.; Wilson, G.L.; Alexeyev, M.F. Oxidative stress induces degradation of mitochondrial DNA. *Nucleic Acids Res.* **2009**, *37*, 2539–2548. [\[CrossRef\]](#) [\[PubMed\]](#)
97. Sahu, K.; Langeh, U.; Singh, C.; Singh, A. Crosstalk between anticancer drugs and mitochondrial functions. *Curr. Res. Pharmacol. Drug Discov.* **2021**, *2*, 100047. [\[CrossRef\]](#) [\[PubMed\]](#)
98. Kuretu, A.; Arineitwe, C.; Mothibe, M.; Ngubane, P.; Khathi, A.; Sibiya, N. Drug-induced mitochondrial toxicity: Risks of developing glucose handling impairments. *Front. Endocrinol.* **2023**, *14*, 1123928. [\[CrossRef\]](#)
99. Vieira, H.L.A.; Haouzi, D.; El Hamel, C.; Jacotot, E.; Belzacq, A.S.; Brenner, C.; Kroemer, G. Permeabilization of the mitochondrial inner membrane during apoptosis: Impact of the adenine nucleotide translocator. *Cell Death Differ.* **2000**, *7*, 1146–1154. [\[CrossRef\]](#)
100. Chipuk, J.E.; Bouchier-Hayes, L.; Green, D.R. Mitochondrial outer membrane permeabilization during apoptosis: The innocent bystander scenario. *Cell Death Differ.* **2006**, *13*, 1396–1402. [\[CrossRef\]](#)
101. Li, L.; Qi, R.; Zhang, L.; Yu, Y.; Hou, J.; Gu, Y.; Song, D.; Wang, X. Potential biomarkers and targets of mitochondrial dynamics. *Clin. Transl. Med.* **2021**, *11*, e529. [\[CrossRef\]](#)
102. Zorova, L.D.; Popkov, V.A.; Plotnikov, E.J.; Silachev, D.N.; Pevzner, I.B.; Jankauskas, S.S.; Zorov, S.D.; Babenko, V.A.; Zorov, D.B. Functional significance of the mitochondrial membrane potential. *Biochem. (Mosc.) Suppl. A Membr.* **2018**, *12*, 20–26. [\[CrossRef\]](#)
103. Vasan, K.; Clutter, M.; Fernandez Dunne, S.; George, M.D.; Luan, C.-H.; Chandel, N.S.; Martínez-Reyes, I. Genes involved in maintaining mitochondrial membrane potential upon electron transport chain disruption. *Front. Cell Dev. Biol.* **2022**, *10*, 781558. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Teodoro, J.S.; Palmeira, C.M.; Rolo, A.P. Mitochondrial Membrane Potential ($\Delta\Psi$) Fluctuations Associated with the Metabolic States of Mitochondria. In *Mitochondrial Bioenergetics: Methods and Protocols*; Palmeira, C.M., Moreno, A.J., Eds.; Springer New York: New York, NY, USA, 2018; pp. 109–119.
105. Hüser, J.; Blatter, L.A. Fluctuations in mitochondrial membrane potential caused by repetitive gating of the permeability transition pore. *Biochem. J.* **1999**, *343*, 311–317. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Kuznetsov, A.V.; Margreiter, R.; Ausserlechner, M.J.; Hagenbuchner, J. The complex interplay between mitochondria, ros and entire cellular metabolism. *Antioxidants* **2022**, *11*, 1995. [\[CrossRef\]](#) [\[PubMed\]](#)
107. Murphy, M.P. Mitochondrial Dysfunction Indirectly Elevates ROS Production by the Endoplasmic Reticulum. *Cell Metab.* **2013**, *18*, 145–146. [\[CrossRef\]](#) [\[PubMed\]](#)
108. Suski, J.; Lebiecinska, M.; Bonora, M.; Pinton, P.; Duszynski, J.; Wieckowski, M.R. Relation Between Mitochondrial Membrane Potential and ROS Formation. In *Mitochondrial Bioenergetics: Methods and Protocols*; Palmeira, C.M., Moreno, A.J., Eds.; Springer: New York, NY, USA, 2018; pp. 357–381.
109. Sullivan, L.B.; Chandel, N.S. Mitochondrial reactive oxygen species and cancer. *Cancer Metab.* **2014**, *2*, 17. [\[CrossRef\]](#) [\[PubMed\]](#)
110. Zorov, D.B.; Juhaszova, M.; Sollott, S.J. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol. Rev.* **2014**, *94*, 909–950. [\[CrossRef\]](#) [\[PubMed\]](#)
111. Yang, X.J.; Seto, E. HATs and HDACs: From structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* **2007**, *26*, 5310–5318. [\[CrossRef\]](#)
112. Prado, F.; Jimeno-González, S.; Reyes, J.C. Histone availability as a strategy to control gene expression. *RNA Biol.* **2017**, *14*, 281–286. [\[CrossRef\]](#)

113. Marmorstein, R.; Roth, S.Y. Histone acetyltransferases: Function, structure, and catalysis. *Curr. Opin. Genet. Dev.* **2001**, *11*, 155–161. [\[CrossRef\]](#)
114. Dueva, R.; Akopyan, K.; Pederiva, C.; Trevisan, D.; Dhanjal, S.; Lindqvist, A.; Farnebo, M. Neutralization of the positive charges on histone tails by rna promotes an open chromatin structure. *Cell Chem. Biol.* **2019**, *26*, 1436–1449. [\[CrossRef\]](#) [\[PubMed\]](#)
115. Seto, E.; Yoshida, M. Erasers of histone acetylation: The histone deacetylase enzymes. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, a018713. [\[CrossRef\]](#) [\[PubMed\]](#)
116. Yang, H.; Salz, T.; Zajac-Kaye, M.; Liao, D.; Huang, S.; Qiu, Y. Overexpression of histone deacetylases in cancer cells is controlled by interplay of transcription factors and epigenetic modulators. *FASEB J.* **2014**, *28*, 4265–4279. [\[CrossRef\]](#) [\[PubMed\]](#)
117. Ho, T.C.S.; Chan, A.H.Y.; Ganesan, A. Thirty years of HDAC inhibitors: 2020 insight and hindsight. *J. Med. Chem.* **2020**, *63*, 12460–12484. [\[CrossRef\]](#) [\[PubMed\]](#)
118. Moran, B.; Davern, M.; Reynolds, J.V.; Donlon, N.E.; Lysaght, J. The impact of histone deacetylase inhibitors on immune cells and implications for cancer therapy. *Cancer Lett.* **2023**, *559*, 216121. [\[CrossRef\]](#) [\[PubMed\]](#)
119. Uba, A.I.; Zengin, G. In the quest for histone deacetylase inhibitors: Current trends in the application of multilayered computational methods. *Amino Acids* **2023**, *55*, 1709–1726. [\[CrossRef\]](#) [\[PubMed\]](#)
120. Bondarev, A.D.; Attwood, M.M.; Jonsson, J.; Chubarev, V.N.; Tarasov, V.V.; Schiöth, H.B. Recent developments of HDAC inhibitors: Emerging indications and novel molecules. *Br. J. Clin. Pharmacol.* **2021**, *87*, 4577–4597. [\[CrossRef\]](#) [\[PubMed\]](#)
121. Göttlicher, M.; Minucci, S.; Zhu, P.; Krämer, O.H.; Schimpf, A.; Giavara, S.; Sleeman, J.P.; Lo Coco, F.; Nervi, C.; Pelicci, P.G.; et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J.* **2001**, *20*, 6969–6978. [\[CrossRef\]](#)
122. Phiel, C.J.; Zhang, F.; Huang, E.Y.; Guenther, M.G.; Lazar, M.A.; Klein, P.S. Histone Deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen *. *J. Biol. Chem.* **2001**, *276*, 36734–36741. [\[CrossRef\]](#)
123. He, B.; Moreau, R. Lipid-regulating properties of butyric acid and 4-phenylbutyric acid: Molecular mechanisms and therapeutic applications. *Pharmacol. Res.* **2019**, *144*, 116–131. [\[CrossRef\]](#)
124. Moinul, M.; Amin, S.A.; Khatun, S.; Das, S.; Jha, T.; Gayen, S. A detail survey and analysis of selectivity criteria for indole-based histone deacetylase 8 (HDAC8) inhibitors. *J. Mol. Struct.* **2023**, *1271*, 133967. [\[CrossRef\]](#)
125. Jiang, B.-E.; Hu, J.; Liu, H.; Liu, Z.; Wen, Y.; Liu, M.; Zhang, H.-K.; Pang, X.; Yu, L.-F. Design, synthesis, and biological evaluation of indole-based hydroxamic acid derivatives as histone deacetylase inhibitors. *Eur. J. Med. Chem.* **2022**, *227*, 113893. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Yang, J.; Jin, X.; Yan, Y.; Shao, Y.; Pan, Y.; Roberts, L.R.; Zhang, J.; Huang, H.; Jiang, J. Inhibiting histone deacetylases suppresses glucose metabolism and hepatocellular carcinoma growth by restoring FBP1 expression. *Sci. Rep.* **2017**, *7*, 43864. [\[CrossRef\]](#) [\[PubMed\]](#)
127. Nguyen, T.T.T.; Zhang, Y.; Shang, E.; Shu, C.; Torrini, C.; Zhao, J.; Bianchetti, E.; Mela, A.; Humala, N.; Mahajan, A.; et al. HDAC inhibitors elicit metabolic reprogramming by targeting super-enhancers in glioblastoma models. *J. Clin. Investig.* **2020**, *130*, 3699–3716. [\[CrossRef\]](#) [\[PubMed\]](#)
128. Shanmugam, G.; Rakshit, S.; Sarkar, K. HDAC inhibitors: Targets for tumour therapy, immune modulation and lung diseases. *Transl. Oncol.* **2022**, *16*, 101312. [\[CrossRef\]](#) [\[PubMed\]](#)
129. Islam, S.; Espitia, C.M.; Persky, D.O.; Carew, J.S.; Nawrocki, S.T. Resistance to histone deacetylase inhibitors confers hypersensitivity to oncolytic reovirus therapy. *Blood Adv.* **2020**, *4*, 5297–5310. [\[CrossRef\]](#) [\[PubMed\]](#)
130. Fantin, V.R.; Richon, V.M. Mechanisms of resistance to histone deacetylase inhibitors and their therapeutic implications. *Clin. Cancer Res.* **2007**, *13*, 7237–7242. [\[CrossRef\]](#)
131. Blaszcak, W.; Liu, G.; Zhu, H.; Barczak, W.; Shrestha, A.; Albayrak, G.; Zheng, S.; Kerr, D.; Samsonova, A.; La Thangue, N.B. Immune modulation underpins the anti-cancer activity of HDAC inhibitors. *Mol. Oncol.* **2021**, *15*, 3280–3298. [\[CrossRef\]](#)
132. Suraweera, A.; O'Byrne, K.J.; Richard, D.J. Combination therapy with histone deacetylase inhibitors (hdaci) for the treatment of cancer: Achieving the full therapeutic potential of HDACi. *Front. Oncol.* **2018**, *8*, 92. [\[CrossRef\]](#)
133. Vancurova, I.; Uddin, M.M.; Zou, Y.; Vancura, A. Combination therapies targeting HDAC and IKK in solid tumours. *Trends Pharmacol. Sci.* **2018**, *39*, 295–306. [\[CrossRef\]](#)
134. Mendrina, T.; Poetsch, I.; Schueffl, H.; Baier, D.; Pirker, C.; Ries, A.; Keppler, B.K.; Kowol, C.R.; Gibson, D.; Grusch, M.; et al. Influence of the fatty acid metabolism on the mode of action of a cisplatin(iv) complex with phenylbutyrate as axial ligands. *Pharmaceutics* **2023**, *15*, 677. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Diyabalanage, H.V.; Granda, M.L.; Hooker, J.M. Combination therapy: Histone deacetylase inhibitors and platinum-based chemotherapeutics for cancer. *Cancer Lett.* **2013**, *329*, 1–8. [\[CrossRef\]](#) [\[PubMed\]](#)
136. Zhang, Y.; Li, N.; Caron, C.; Matthias, G.; Hess, D.; Khochbin, S.; Matthias, P. HDAC-6 interacts with and deacetylates tubulin and microtubules in vivo. *EMBO J.* **2003**, *22*, 1168–1179. [\[CrossRef\]](#) [\[PubMed\]](#)
137. Balmik, A.A.; Chinnathambi, S. Inter-relationship of histone deacetylase-6 with tau-cytoskeletal organization and remodeling. *Eur. J. Cell Biol.* **2022**, *101*, 151202. [\[CrossRef\]](#) [\[PubMed\]](#)
138. Boyault, C.; Sadoul, K.; Pabion, M.; Khochbin, S. HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. *Oncogene* **2007**, *26*, 5468–5476. [\[CrossRef\]](#)
139. Xu, G.; Niu, L.; Wang, Y.; Yang, G.; Zhu, X.; Yao, Y.; Zhao, G.; Wang, S.; Li, H. HDAC6-dependent deacetylation of TAK1 enhances siL-6R release to promote macrophage M2 polarization in colon cancer. *Cell Death Dis.* **2022**, *13*, 888. [\[CrossRef\]](#) [\[PubMed\]](#)

140. Zhang, S.L.; Zhu, H.Y.; Zhou, B.Y.; Chu, Y.; Huo, J.R.; Tan, Y.Y.; Liu, D.L. Histone deacetylase 6 is overexpressed and promotes tumour growth of colon cancer through regulation of the MAPK/ERK signal pathway. *Onco Targets Ther.* **2019**, *12*, 2409–2419. [[CrossRef](#)]
141. Mehanna, S.; Mansour, N.; Daher, C.F.; Elias, M.G.; Dagher, C.; Khnayzer, R.S. Drug-free phototherapy of superficial tumours: White light at the end of the tunnel. *J. Photochem. Photobiol. B* **2021**, *224*, 112324. [[CrossRef](#)]
142. Elias, M.G.; Mehanna, S.; Elias, E.; Khnayzer, R.S.; Daher, C.F. A photoactivatable chemotherapeutic Ru(II) complex bearing bathocuproine ligand efficiently induces cell death in human malignant melanoma cells through a multi-mechanistic pathway. *Chem. Biol. Interact.* **2021**, *348*, 109644. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.