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Triazole-based, optically-pure metallosupramolecules; highly potent and selective anticancer compounds†

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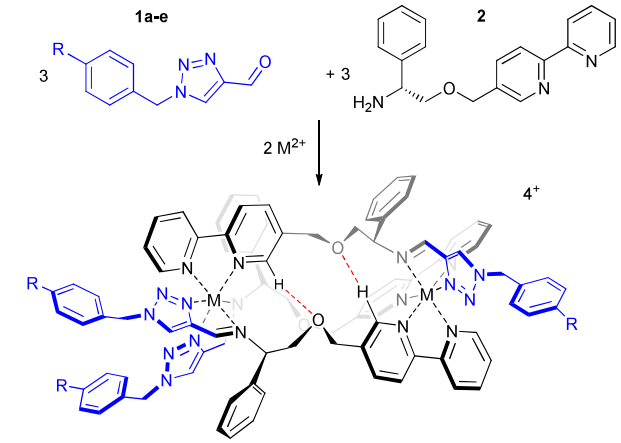
Hualong Song,[a] Nicola Rogers,*[,a] Viktor Brabec,*[,c] Guy Clarkson,[a] James Coverdale,[a] Hana Kostrhunova,[c] Roger M. Phillips,[b] Miles Postings, [a] Samantha L. Shepherd[b] and Peter Scott*[,a]

Functionalised triazole aldehydes are used in the highly selective self-assembly of water-compatible, optically pure, low symmetry Fe(II)- and Zn(II)-based metallohelices. Sub-micromolar antiproliferative activity is observed against various cancerous cell lines, accompanied by excellent selectivity versus non-cancerous cells and potential for synergistic combinatorial therapy with cisplatin.

Pyridines and other heterocyclic donor ligands are ubiquitous in multinuclear metal coordination structures[1-7] despite the often cumbersome syntheses of the ligands and the lack of functional diversity. The 1,2,3-triazoles, available in various forms via copper(I)-catalysed alkyne-azide cycloaddition/click (CuAAC) chemistry,[8] have recently been employed as ligands in a range of potential applications.[9-15] However, since these heterocycles are both weaker σ-donors and π-acceptors than e.g. pyridines,[12] the metal complexes are inherently less stable to hydrolysis or competition with coordinating counter-ions such as chloride. In this context, few triazole-derived metallo-supramolecules have been reported. Petitjean and co-workers synthesized a family of M_2L_3 (M = $Fe^{2+}$and Ni^{2+}) helicates based on pyridyl-1,2,3-triazoles.[16, 17] Crowley and co-workers' Fe(II) systems[18] necessarily have weakly-coordinating counter-ions, so there is little if any solubility in water, and while matters were addressed successfully by using inert metals,[19,20] no significant biological activity was found. As with most helicates, all the above systems are racemic.

We noticed that the triazole aldehydes such as **1** (Scheme 1) had been little exploited in ligand synthesis,[21-23] and to our knowledge not at all in the metallo-supramolecular arena. The compounds **1a-e** were thus synthesised from propargyl alcohol and the respective benzyl azides using CuAAC, followed by



(R_c,Δ_{Fe})-HHT-[$Fe_2L^{3a}_3$]Cl_4 (R = H)
-[$Fe_2L^{3b}_3$]Cl_4 (R = CN)
-[$Fe_2L^{3c}_3$]Cl_4 (R = OCH_3)
-[$Fe_2L^{3d}_3$]Cl_4 (R = F)
-[$Fe_2L^{3e}_3$]Cl_4 (R = COOH)

(S_c,Λ_{Fe})-HHT-[$Fe_2L^{3a}_3$]Cl_4 (R = H)
-[$Fe_2L^{3b}_3$]Cl_4 (R = CN)
-[$Fe_2L^{3c}_3$]Cl_4 (R = OCH_3)
-[$Fe_2L^{3d}_3$]Cl_4 (R = F)
-[$Fe_2L^{3e}_3$]Cl_4 (R = COOH)

(R_c,Δ_{Zn})-HHT-[$Zn_2L^{3a}_3$][ClO_4]$_4$ (R = H)
-[$Zn_2L^{3b}_3$][ClO_4]$_4$ (R = CN)
-[$Zn_2L^{3c}_3$][ClO_4]$_4$ (R = OCH_3)
-[$Zn_2L^{3d}_3$][ClO_4]$_4$ (R = F)

Scheme 1 Synthesis of triazole-imine/bipyridine derived triplex metallohelices. Inter-strand H-bonds indicated by red dashed lines.

oxidation; MnO_2 was a more effective in our hands than pyridinium chloro-chromate,[24] 2-iodoxy-benzoic acid,[25] or Jones reagents.[26]

Given the challenges noted above in the coordination chemistry of triazoles we were surprised to find that mixing aldehydes **1** with enantiomers of 2-([2,2'-bipyridin]-5-ylmethoxy)-1-phenylethan-1-amine[6] (**2**) in appropriate proportions with divalent metal cations directly gave the new optically pure assemblies shown in Scheme 1; reactions were essentially quantitative according to ^{1}H NMR experiments. Water-soluble enantiomers [$Fe_2L^{3a-e}_3$]Cl_4 were synthesised using Fe(II) chloride, while Zn(II) perchlorate gave the isostructural analogues [$Zn_2L^{3a-d}_3$][ClO_4]$_4$.

[a] Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK.
[b] School of Applied Sciences, University of Huddersfield, Huddersfield, HD1 3DH.
[c] The Czech Academy of Sciences, Institute of Biophysics, Kralovopolska 135, CZ-61265 Brno, Czech Republic.

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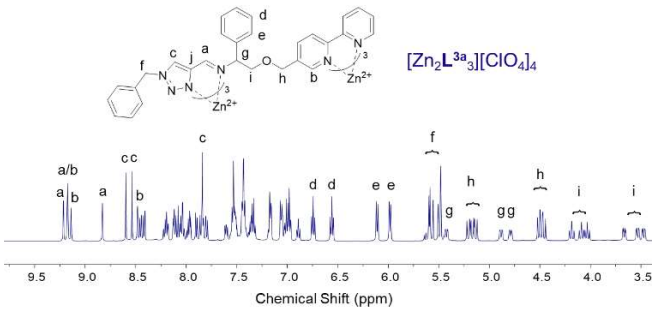

Fig. 1 ^{1}H (500 MHz, CD_3CN, 298 K) NMR spectrum of of (R_c,Δ_{Zn})-HHT-$[Zn_2\mathbf{L^{3a}}_3][ClO_4]_4$

The presence of three unique ligand environments in the ^{1}H spectra (e.g. Fig. 1; Fig. S1-S11, ESI) indicates the formation in each case of a *single* antiparallel (head-to-head-to-tail, HHT) i.e. "triplex"[6] configuration of the new triazole-containing metallohelices, with no detectable HHH species or other diastereomers. Given that components **2** are optically pure, we know that the new self-assembled compounds are single enantiomers. This selectivity is thus even greater than that observed (*ca* 99% dr) in the related architectures of this kind based solely on pyridines.[6] Signals for the imine protons H^a and triazole H^c were observed at low fields (9.4-7.8 ppm). Two bpy protons H^b, assigned by 2D NMR experiments, also appear in this region as a result of inter-strand H-bonding as shown in Scheme 1. Two sets of phenyl ring protons H^d and H^e are also at unusual chemical shifts (6.8–5.8 ppm) as a result of strong through-space shielding from the bpy unit of an adjacent ligand.

Methanolic solutions of $[Fe_2\mathbf{L^{3a}}_3]Cl_4$ enantiomers were analysed using circular dichroism absorption spectroscopy, which show equal and opposite differential extinction coefficients, typical of enantiomeric pairs. (Fig.S18 ESI).

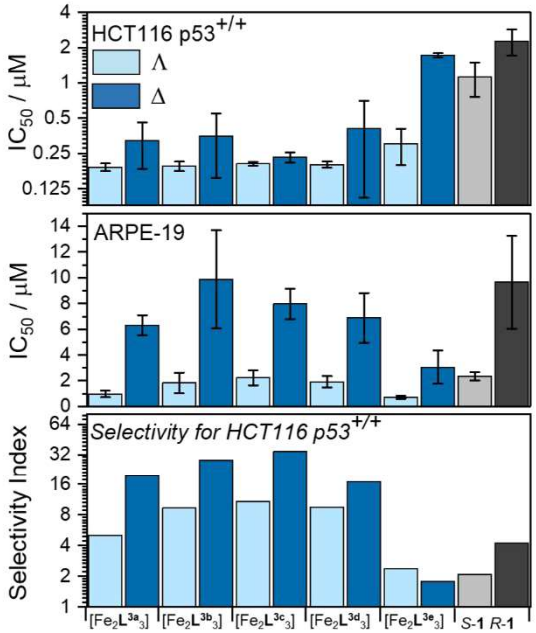

Fig. 2 IC_{50} concentrations of metallohelices $[Fe_2\mathbf{L^{3a-e}}_3]Cl_4$ and ligand precursor (*S*/*R*)-**1** against (a) HCT116 p53^{++} cancer cell line; (b) ARPE-19 (noncancerous cell line). (c) Selectivity indices i.e. [mean IC_{50} (ARPE-19) / mean IC_{50} (HCT116 p35$^{+/+}$).

While some of our metallohelices have high antimicrobial potency,[7, 27] here no significant activity against *S. Aureus* (ATCC29213) and *E. coli* (ATCC25922) was observed (minimum inhibitory concentration ≥ 128 μg mL^{-1} for enantiomers of $[Fe_2\mathbf{L^{3a}}_3]Cl_4$). Pleasingly, the same compounds were also inactive in red blood cell haemolysis at concentrations up to 256 μg mL^{-1} (Table S3, S4, ESI).

The panel of ten Fe(II) compounds (Scheme 1) were initially screened for cancer antiproliferative potency against the human colorectal cancer cells with wild-type p53 (HCT116 p53$^{+/+}$), and for comparison against non-cancerous human epithelial retinal pigment cells (ARPE-19). We observed very high activity against HCT116 p53$^{+/+}$ with IC_{50} < 500 nM (*cf.* IC_{50} of cisplatin = 3.5±1.5 μM) for most complexes, with the sole exception of the tricarboxylic acid enantiomer Δ-$[Fe_2\mathbf{L^{3e}}_3]Cl_4$. The Λ enantiomers were marginally more active than Δ, and the most potent compound was Λ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ with IC_{50} 191 ± 10 nM.

In the above screens we noted that complexes $[Fe_2\mathbf{L^{3a}}_3]Cl_4$ were surprisingly stable in water and biological media. In pH 1.5 buffer the compound has $t_{1/2}$ of *ca* 4.3 h (Fig. S20, ESI) comparable with that for Hannon's "cylinder" of *ca* 1.4 h at pH ~1.[6] At neutral pH, time-dependent photoabsorbance measurements of Λ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ solutions (100 μM) reveal a loss of only 18% intensity at 480 nm (MLCT absorption) over one week (Fig. S19, ESI). We further tested the IC_{50} of Δ/Λ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ complexes that had been pre-incubated in cell medium for 24 h (37 °C) and observed identical responses to fresh solutions (Table S2, ESI). The potencies of the ligand precursors **1a** and **2,** as well as $FeCl_2$ against HCT116 p53^{++} were measured; we found that **1a** has minimal activity (IC_{50} > 70 μM) and $FeCl_2$ induced no measurable antiproliferative response in concentrations ≤ 100 μM (Table S1 and Fig. S21, ESI), whilst the two precursor amine enantiomers **2** are both less potent than their respective metallohelix (by a factor of *ca* 2 per mole of ligand).

Significant enantiomeric differences in potency were observed in the ARPE-19 cells; the Λ enantiomers (average IC_{50} = 1.5 μM) were more potent than the Δ enantiomers (average IC_{50} = 6.8 μM) (Fig. 2b). The ligand precursor **1a** has modest antiproliferative activity (IC_{50} > 89 μM) towards ARPE-19 cells while the amine enantiomers **2** have IC_{50} < 10 μM, and both are more potent than their respective Δ/Λ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ metallohelix, per mole of ligand.

Selectivity index (*SI*), defined as the mean IC_{50} of ARPE-19 divided by that of HCT116 p53^{++}, are shown in Fig. 2(c). Excluding for the moment the $[Fe_2\mathbf{L^{3e}}]Cl_4$ compounds, we see that the Δ enantiomers exhibit substantially better selectivity (*SI* 17-34) than Λ enantiomers (*SI* 5-10). Notably the compound Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ has *five times* the selectivity of its amine precursor (*R*)-**2**. In contrast, enantiomers $[Fe_2\mathbf{L^{3e}}]Cl_4$ are poorly selective; the three carboxylic acid groups present in these compounds will be substantially deprotonated at cell pH. These observations suggest that the cationic charge is important for the overall selectivity

Due to the high potency and high selectivity Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ was selected for screening against a variety of cancer cell lines of different tissue origins. As seen in Table 1, Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$

showed excellent activity throughout the panel of employed cell lines with nanomolar IC_{50} values.

Table 1 Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$, and cisplatin were tested with the MTT assay to assess the compounds' antiproliferative effect (IC_{50}, μM) on cancerous and non-cancerous cells. The cells were treated for 72 hours. The results are expressed as mean ± SD from at least three independent experiments. [a] The cells were treated for 96h

	Cell line	IC_{50} (μM) Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$	cisPt
Cancer	HCT116 $p53^{++}$ *(colon)*	0.5 ± 0.2	9 ± 1
	MDA-MB-231 *(breast)*	0.24 ± 0.05	29 ± 8
	MCF-7 *(breast)*	0.15 ± 0.01	15 ± 1
	HeLa *(cervical)*	0.8 ± 0.2	18 ± 4
	A2780 *(ovarian)*	0.33 ± 0.01	3.2 ± 0.5
	A2780cisR *(ovarian)*	0.36 ± 0.02	21 ± 2
Non-cancer	MRC-5 pd30 *(lung)*	3.7 ± 0.9	10 ± 3
	[a]ARPE-19 *(retinal)*	6.3 ± 0.8	6.4 ± 0.9

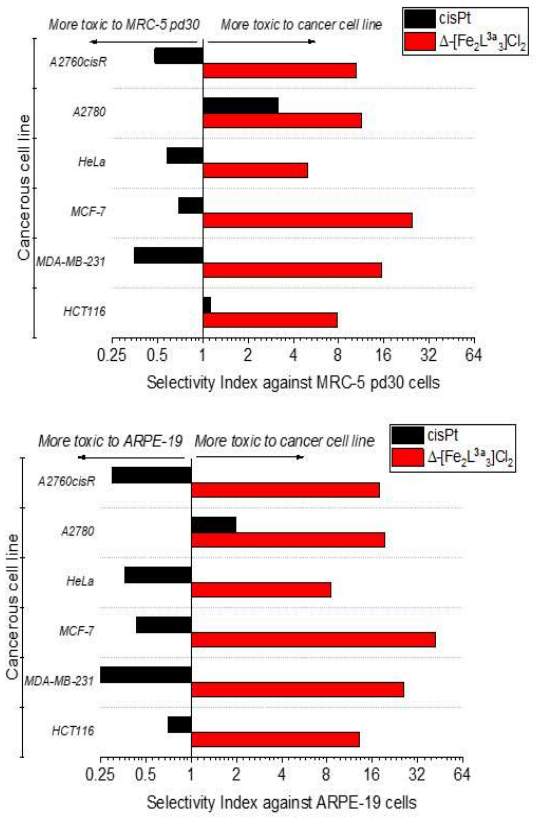

Fig. 3 Selectivity Indices of Δ-$[Fe_2L^{3a}_3]Cl_4$ for six cancerous cell lines *vs* two non-cancerous cell lines – i.e. [mean IC_{50} (non-cancer cell line)] / [mean IC_{50} (cancer cell line), compared with cisplatin.

Table 2 Combination of Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$, and cisplatin (molar ratio 1:1) were tested with the MTT assay to assess the antiproliferative effect of this combination (IC_{50}, μM) on cancerous cells. The cells were treated for 72 hours. The results are expressed as the mean along with the range of the combination indices (CIs, in parentheses) from at least three independent experiments. CIs were calculated using the CompuSyn software (Combo SynInc, City, State, USA).

Cancerous cell line	Mean CI (range) Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$+cisplatin (1:1)
HCT116 $p53^{++}$ *(colon)*	0.47 (0.39-0.55)
MDA-MB-231 *(breast)*	0.61 (0.27-0.74)
MCF-7 *(breast)*	0.69 (0.46-0.96)
HeLa *(cervical)*	0.75 (0.43-1.08)

The most sensitive cell line was MCF-7 (breast cancer), with IC_{50} as low as 151 nM. Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ is 20 times more active than cisplatin against HCT116 $p53^{+/+}$ (colon cancer) and HeLa (cervical cancer), and 100 times more active than cisplatin against both MCF-7 and MDA-MD-231 breast cancer cell lines.

The selectivity indices of the six cancerous cell lines in Table 1 with respect to the non-cancerous cell lines ARPE-19 and MRC-5 pd30 are plotted in Fig. 3. The metallohelix Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ far outperforms cisplatin across the board, ranging from 5 (HeLa *vs* MRC-5 pd30) to 42 (MCF-7 *vs* ARPE-19) for Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$, compared with $SI < 1$ for the majority of the cell lines screened with cisplatin.

Cisplatin is an important agent in the treatment of numerous malignancies, but its clinical use is limited by side effects and intrinsic or acquired resistance. Therefore, there is need for alternatives to cisplatin or for the development of new treatment procedures, such as combined therapy with other compounds[28, 29] The new metallohelix Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ exhibited almost identical activity towards cisplatin-sensitive ovarian cancer cell line A2780 and its cisplatin-resistant partner A2780cisR (Table 1), whereas, the resistance factor for cisplatin is 6.6. Thus, we investigated the effect of combined treatment of cisplatin and Δ-$[Fe_2\mathbf{L^{3a}}_3]Cl_4$ in four different cell lines (Table 2), at equimolar concentrations (1:1). The data analysis to determine the combination index (CI) of the used combination treatment was carried out using the CompuSyn software (Combo SynInc, City, State, USA). CI < 0.9 is considered as synergism, CI > 1.1 is considered as antagonism, and CI within the range (0.9 – 1.1) indicates additive effect. In all probed cell lines, the mean combination index ranged from 0.47 to 0.75 indicating synergism. In HCT116 $p53^{++}$ and MDA-MB-231 all values showed significant synergism while in MCF-7 and HeLa, the individual CI values fell within the range from synergism to additive effect.

In this context we make two further mechanistic observations. First, Real-time Cell Analysis,[30] which has previously been employed to characterize the overall effect of small compounds on cell growth and morphology, shows a very different profile for $[Fe_2\mathbf{L^{3a}}_3]Cl_4$ to that of cisplatin in A2780 cells (Fig. S22, ESI). Secondly, preliminary linear dichroism studies indicate that the enantiomers $[Fe_2\mathbf{L^{3a}}_3]Cl_4$ are rare examples of DNA groove-binding metallohelices ($logK_{app}$ *ca* 6) (Fig. S23), but that the orientations of the two enantiomers are different (Fig.

S24). This could be the origin of the large enantiomer effects in selectivity described above.

Overall we conclude that the new metallohelices reported here have a number of appealing features. The systems self-assemble with exceptionally high stereoselectivity to single enantiomers, whilst the water-soluble Fe compounds are surprisingly stable to hydrolysis, thus enabling reliable testing in the biological media, and they are also available with a range of functional groups. The chemical diversity available via the triazole aldehydes **1** is far more readily accessible than the equivalent pyridines or other similar heterocycles, and we hope that this will allow rapid development of new functionalised metallo-supramolecular architectures. The current metallohelices are exceptionally potent against cancer cell lines, with IC_{50} values comfortably sub-micromolar, and there is pronounced selectivity *versus* the non-cancer cells tested. The combination therapy tests demonstrate that Δ-[Fe_2**L**$^{3a}_3$]Cl_4 and cisplatin act synergistically; this could decrease the drug dosage whilst maintaining/increasing efficacy, attenuating toxicity and slowing down the development of drug resistance.

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Conflicts of interest

There are no conflicts to declare.

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