IP3

Chalcone-ligated molybdenum carbonyl complexes: Synthesis and evaluation as quadruplex DNA binders

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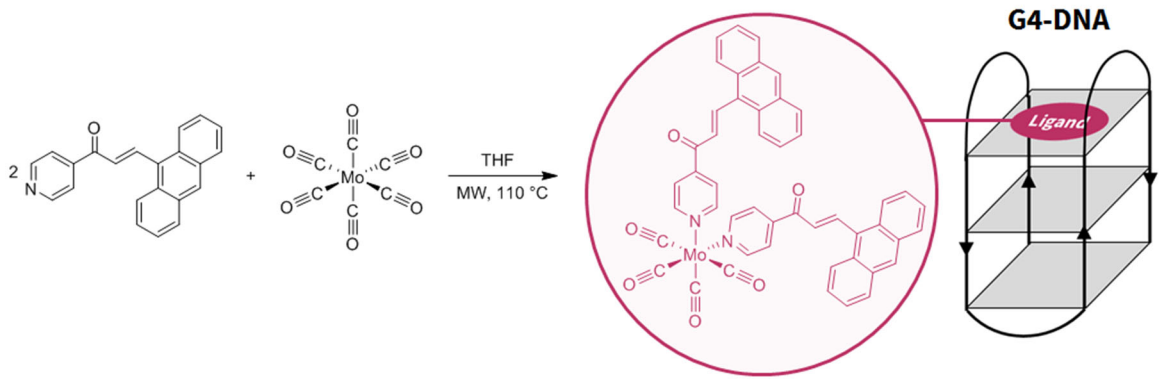
Abstract

The microwave-assisted reaction of $Mo(CO)_6$ with isomeric pyridyl anthryl chalcones **1a-f** afforded six novel molybdenum carbonyl complexes **2a-f**. Complexes **2a-f** have been characterized by infrared and ^1H Nuclear Magnetic Resonance spectroscopies, and the structures of **2a-d** were revealed to be *cis*-disubstituted complexes with general formula $Mo(CO)_4L_2$. The intercalative abilities of **2a-d** towards quadruplex DNA were examined using the Fluorescent Intercalator Displacement (FID) assay.

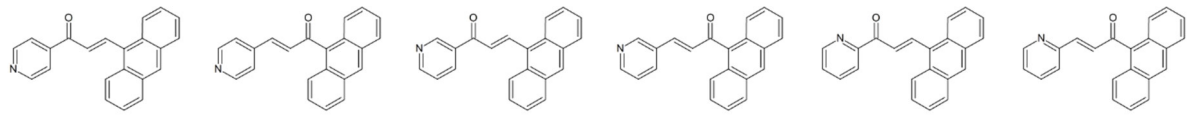


1. Introduction

Recently, a structure known as the G-quadruplex (G4) has emerged as a promising anti-cancer drug target. G4 structures are helical structures formed in guanine-rich regions of RNA or DNA sequences stabilised by sodium or potassium cations [1]. Such structures are found in higher frequency in telomeres and promoters [2], suggesting that they may play a role in cancer regulation. It is hence hypothesised that molecules that can stabilise G4 structures may display anti-cancer activity.

Chalcones are a class of plant secondary metabolites featuring the α,β-unsaturated ketone pharmacophore and display a wide range of biological activity [3]. Pyridyl anthryl chalcones contain the planar anthryl group, which has a large π surface area, potentially allowing it to intercalate between the guanine bases and act as a ligand for G4 structures, enhancing its anti-cancer activity. On the other hand, molybdenum carbonyl complexes have been investigated as Carbon Monoxide Releasing Molecules (CORMs) which can release therapeutic amounts of carbon monoxide to tissues [4]. The attachment of pyridine anthryl chalcones to molybdenum carbonyl complexes may enhance its G4-binding ability due to electrostatic attraction between the metal center and the negatively-charged phosphate backbone of the G4-DNA.

In this work, molybdenum carbonyl complexes **2a**-**f** were synthesised by the reaction of $Mo(CO)_6$ with the anthryl pyridine chalcones **1a**-**f** (Chart 1). The intercalative abilities of **2a**-**d** towards quadruplex DNA were examined to ascertain their potential as anti-cancer agents.



1a **1b** **1c** **1d** **1e** **1f**

Chart 1. Structures of **1a–f**

2. Experimental

*Synthesis of molybdenum complexes **2a-f***

$Mo(CO)_6$ (15 mg, 57 μmol) and the corresponding chalcone (2 molar equivalent) were added to 1 mL of tetrahydrofuran and reacted in a Discover-SP microwave reactor. The solvent was removed under reduced pressure and the residue was washed with hexane and air dried to yield the solid product.

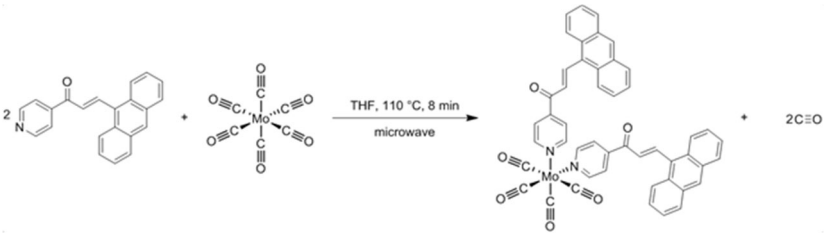
Fluorescent Intercalator Displacement (FID) assay

The FID assay [5] was performed on **2a–d** against G4 quadruplex DNA (HTelo and *c-myc*) as well as duplex DNA (ds26) to obtain their corresponding half-maximal degradation concentration (DC_{50}) values.

3. Results and Discussion

Synthesis and characterization

The reaction of $Mo(CO)_6$ with the respective chalcone **1a–d** afforded the novel *cis*-disubstituted molybdenum carbonyl complexes **2a–d** (Scheme 1) in moderate yields. All complexes were characterised by infrared (IR) and ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopies.



Scheme 1. Synthesis of the *cis*-disubstituted complex **2a**

The IR spectra of **2a**-**d** in the νCO region (Figure 1) resembles those of previously reported *cis*-$Mo(CO)_4(amine)_2$ complexes [6], confirming the identities of **2a**-**d**.

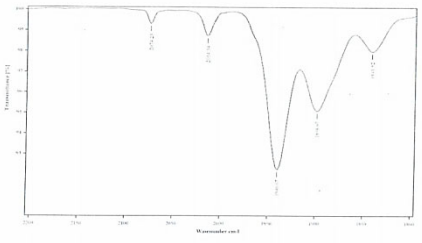


Figure 1. IR spectrum of **2a** in dichloromethane solution

The ^{1}H NMR spectra of all complexes displayed one set of signals, suggesting that the complexes are symmetrical in solution. The assignment of protons on **2a** (Figure 2) is used as an example. The singlet at δ = 8.51 ppm can be assigned to the proton on position 10 on the anthracene ring, while the doublet at δ = 8.87 ppm can be assigned to the protons at the *ortho* position of the pyridine ring. The two sets of doublets at δ = 8.87 ppm and δ = 7.54 ppm with coupling constant J = 16.0 Hz can be assigned to the vinylic protons. The value of the coupling constant suggests that the chalcone exists in the *trans* configuration.

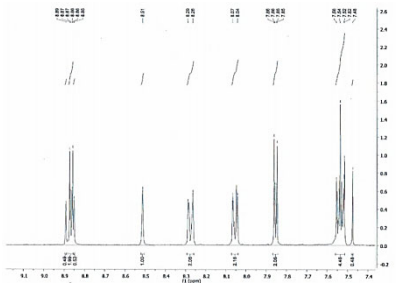


Figure 2. ^{1}H NMR spectrum of **2a** in $CDCl_3$

Fluorescent Intercalator Displacement (FID) Assay

The DC_{50} values of **2a**-**d** were determined using the FID assay (Table 1); a lower DC_{50} value is indicative of better quadruplex intercalative properties.

While $Mo(CO)_6$ and **1a**-**d** did not bind to quadruplex DNA, complexes **2a**-**d** were able to intercalate to HTelo and *c-myc*. This suggests that there might be synergistic effects, such as

electrostatic attraction, which allow **2a**-**d** to intercalate. All four complexes showed greater selectivity for HTelo over *c-myc*. This might be because *c-myc* adopts a parallel topology while HTelo forms a (3+1) hybrid, and the more compact structure of *c-myc* would increase the difficulty of intercalation. However, all complexes showed greatest selectivity for duplex DNA, suggesting an alternative mode of interaction besides intercalation.

Compound	DC_{50} [a]/ μM		
	HTelo	*c-myc*	ds26
2a	44.33 (2.29)	54.33 (1.82)	10.01 (0.18)
2b	39.87 (0.31)	74.77 (0.51)	9.03 (0.09)
2c	55.87 (1.22)	81.85 (3.03)	10.71 (0.34)
2d	39.20 (0.37)	102.88 (4.14)	10.35 (0.21)

[a] Standard errors (N = 3) are shown in parentheses.

Table 1. Affinity and selectivity of **2a**-**d** for oligonucleotides, determined by the FID assay

4. Conclusion

Six novel molybdenum carbonyl complexes with chalcone ligands were synthesized and characterized. FID assays revealed that **2a**-**d** displayed moderate intercalative abilities for quadruplex DNA while having greater selectivity for HTelo over *c-myc*.

5. Future Work

The identities of **2e** and **2f** will be established followed by FID assays on these two complexes, and the brine shrimp lethality (BLT) assay will be carried out on complexes **2a**-**f** to evaluate their cytotoxicity.

6. References

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