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Synthesis, characterisation and cytotoxic activity evaluation of new metal-salen complexes based on the 1,2-bicyclo[2.2.2]octane bridge

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A B S T R A C T

(*R*)-1,2-Diaminobicyclo[2.2.2]octane was used as a starting material for the preparation, in solution or in a ball mill, of a salen ligand. Five metal salen complexes were prepared in high yield and their cytotoxic activities were evaluated against the Human Colon cancer HCT116 cell lines. The original manganese salen complex displayed the highest activity with a potency 16 fold higher than that of cisplatin, demonstrating the benefit of the bridging backbone compared to other salen systems. An alternative preparation route for this complex by mechanochemistry was also performed.

Keywords:

Salen metal complex

Manganese

Mechanochemistry

Cytotoxicity

HCT116

Introduction

Metal-based anticancer drugs have received increasing attention since the discovery of cisplatin in the mid-1960s, a successful metallodrug still considered nowadays as part of a standard chemotherapy [1]. However, cisplatin and related platinum-based treatments have been associated with numerous side effects due to oxidative stress and high toxicity [2]. Therefore, the development of novel metal-based compounds with potential anticancer activity is of high importance. Several factors can affect the therapeutic activity of such compounds including the nature of the metal and the structure of the organic ligands.

Among those ligands, salen ligands bear particularly attractive features. Besides their widely known catalytic properties in organic synthesis [3,4], metal-salen complexes have been extensively studied in bioinorganic and medicinal chemistry [5]. The popularity of tetradentate N₂O₂ bis-Schiff base ligands (salen) is mostly explained by their ease of synthesis and ability to coordinate a high diversity of transition metals in various oxidation states and geometries. Due to their catalytic properties and their rather amenable structural and photophysical characteristics, metal-salen complexes provide high ligating properties and have numerous applications as enzyme mimics [6,7], potential therapeutics [8] or biosensors for diagnostics [9,10]. Known to act as oxidation cata-

lysts in synthesis [11], metallosalen species also display cytotoxic activities and have been proved to cause oxidative damage to DNA leading to apoptosis. DNA binding and cleavage were reported using diverse salen complexes with Ni [12], Cr [13], Mn [14], Cu [15], Co [16], or Fe [17] leading to potential application as anti-cancer and antimicrobial agents.

Recent work in our institute led to the development of the original chiral diamine **1** based on a bicyclo[2.2.2]octane scaffold (Fig. 1) [18]. Based on this diamine, diverse ligands and the corresponding copper complexes were synthesised and proved to be efficient catalysts in stereoselective metal-catalysed transformations [19].

We report herein the use of enantiopure diamine (*R*)-1,2-diaminobicyclo[2.2.2]octane **1** in the synthesis of metal-salen complexes involving metals other than copper and their characterisation. The cytotoxicity of these new molecules in the proliferation of HCT116 cancer cells was then evaluated. A mechanosynthesis of the most promising ligand and metal complex was also developed.

Results and discussion

Synthesis of the ligand

The salen ligand used in this study was derived from the bicyclic diamine **1** obtained as an optically pure diacetate salt *via* Diels-Alder cycloaddition, chiral resolution and subsequent Hofmann

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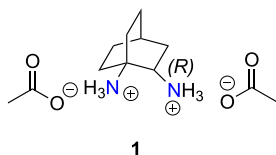


Fig. 1. (R)-1,2-Diaminobicyclo[2.2.2]octane diacetate.

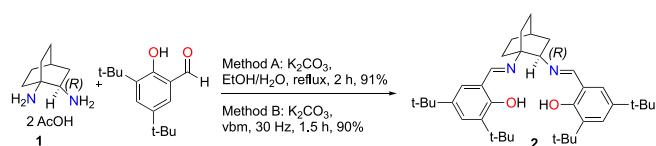
rearrangement of the corresponding bicyclic β -amino acid as described previously [18]. The condensation of diamine **1** and 3,5-di-*tert*-butylsalicylaldehyde at reflux in a mixture of ethanol and water allowed the formation of Schiff base **2** in very high yield (Scheme 1, Method A) [19].

In parallel to the solution synthesis, we also considered a solvent-free mechanochemical route [20,21] for the preparation of the metal complexes. Indeed, it was demonstrated in recent years that ligands and metallic complexes could be efficiently accessed by employing mechanochemistry [22–24]. In most cases, the synthetic procedures for the formation of metallic complexes are faster, simplified and easily scalable in comparison to the solution approach [25–30]. More specifically, it has been reported that salen ligands and the corresponding metal complexes could be mechanothesized [31–33].

First, we decided to test this approach for the preparation of ligand **2**: 2 eq. of 3,5-di-*tert*-butylsalicylaldehyde, 1 eq. of diamine **1** and 1 eq. of K_2CO_3 were mixed in a vibratory ball mill (vbm) at a frequency of 30 Hz for 1.5 h. The reaction mixture was recovered from the milling jar with water and washed with ethanol to provide the expected product with 90% isolated yield. It should be noted that no organic solvent was used to carry out the reaction and only water and EtOH were employed to recover pure product **2**, with analytical data in agreement with those obtained from the solution synthesis of **2**.

Synthesis of the metal-complexes

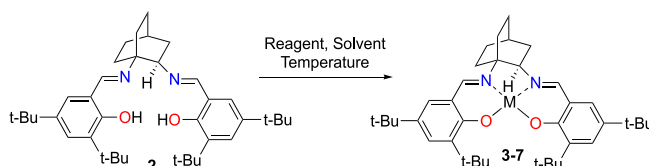
Metal-salen complexes **3** to **7** were synthesised in solution using different metal sources and conditions (Table 1). The procedures and reagents used in these syntheses were inspired by previous work with other types of salen ligands [34]. These novel



Scheme 1. Synthesis of salen ligand **2**.

Table 1

Reaction conditions for the synthesis of metal-salen complexes.



Reagents	Solvents	T (°C)	Product	Yield (%)
$Pd(OAc)_2$	MeOH	65	3 (M = Pd)	98
$Cu(OAc)_2$	MeOH	65	4 (M = Cu)	90
$Co(OAc)_2$	MeOH/ CH_2Cl_2	r.t.	5 (M = Co)	62
	MeOH	65	6 (M = Ni)	97
	MeOH/Toluene	r.t.	7 (M = Mn(Cl))	82

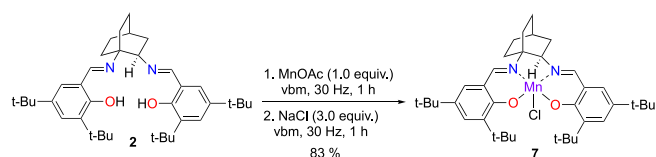
complexes were obtained as high-melting solids in good to very good yields. It is worth noting that the manganese complex spontaneously oxidizes into the Mn(III) complex upon exposure to air as previously reported with other ligands [34,35]. Treatment with brine, opened to air, allowed complete oxidation to the Mn(III) complex replacing the acetate counter ion with a chlorine anion. Complexes **3–7** were characterised using infrared analysis as well as high resolution mass spectrometry. Crystals of Pd(II), Cu(II), Co (II) and Ni(II) complexes (**3** to **6**) were grown by the slow addition of methanol into a solution of the complex in dichloromethane or acetonitrile. However, despite the efforts invested, no suitable crystals of Mn(III) complex **7** have been obtained.

The mechanochemical synthesis of the most promising (*vide infra*) Mn-based complex was also tested in the ball mill, by mixing ligand **2** with $Mn(OAc)_2 \cdot 4H_2O$ for 1 h, followed by the addition of NaCl and further grinding for 1 h (Scheme 2). Gratifyingly, complex **7** was obtained in 83% yield. In sharp contrast to the synthesis in solution, which required an excess of the metallic salt, only an equimolar amount of the ligand and the Mn salt were needed in the case of the mechanochemical approach. Crystals could once again not be obtained for complex **7**. Its existence was asserted by High Resolution Mass Spectrometry and its structure was confirmed by X-ray Photoelectron Spectroscopy (XPS) (see ESI). Complex **7** exhibits a Mn2p3 core level energy at 642.14 eV and a Cl2p at 198.50 eV. This is in agreement with the presence of a Mn(III) species stabilized as a salen complex and chloride ligand as reported previously in the literature [36].

Crystal structures

Compound **2** crystallized as yellow needles and its structure has been confirmed by X-ray diffraction (Fig. 2) [19]. The phenol rings were almost perpendicular and each hydroxyl group formed a strong intramolecular hydrogen bond with the adjacent nitrogen atom (see ESI). These 6-membered pseudo cycles stabilised the imine bonds, giving long term bench-top stability for ligand **2**.

Crystals of M(II) complexes **3** to **6** were isomorphous with respect to each other (Fig. 3). The monoclinic asymmetric-unit contained a dimer of complexes and two molecules of solvent (dichloromethane or acetonitrile). The bond lengths and angles around the metal atoms were comparable to those observed in similar chiral-metal salen complexes (see ESI). The M(II) centers had a square planar geometry in a more or less distorted tetrahedral configuration (see ESI). In all cases, the distortion was more pronounced in one of the two independent molecules and the two phenol rings were nearly coplanar. The square planar geometry is of importance to allow a potential intercalation or minor groove binding to cancer cells DNA, a possible mode of biological action previously described for salen-metal complexes, together with redox proper-



Scheme 2. Mechanochemistry of Mn(III) salen complex **7**.

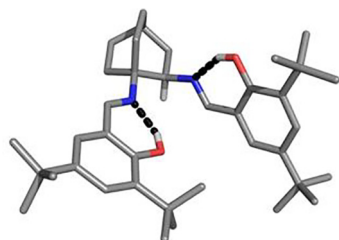


Fig. 2. Crystal structure of salen ligand **2**. H atoms are omitted for clarity.

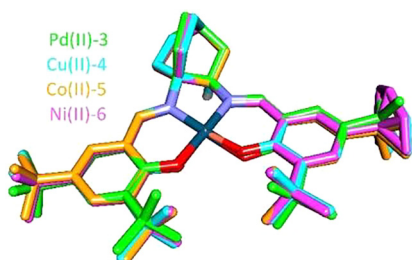


Fig. 3. Superimposition of salen metal-complexes Pd(II)-**3**, Co(II)-**5** and Ni(II)-**6**. H atoms are omitted for clarity.

Table 2

Cytotoxicity of the new metal-salen complexes on the HCT116 cell line (data given as % of toxicity).

Complex	10 ⁻⁵ M	10 ⁻⁶ M
3	7 ± 1	1 ± 0.1
4	16 ± 2	0
5	21 ± 3	0
6	15 ± 1	0
7	100 ± 0.1	97 ± 0.1

ties [5]. Nevertheless, one can expect a modulation of these activities, including the mode of action, due to the presence of the bicyclic diamine as a bridge. Steric effects could indeed hamper an adequate intercalation.

Biological testing

The *in vitro* anticancer potential of **3–7** was established by evaluating their ability to inhibit the cell growth of a human carcinoma cell line, HCT116 (colorectal cancer) [37]. Cisplatin and doxorubicin were used as positive controls to assess the cytotoxicity of the tested compounds. Preliminary data for the primary screening was collected as a percentage of cell toxicity and reported in Table 2 for two different concentrations of each complex (10⁻⁵ M and 10⁻⁶ M). Of note, ligand **2** was tested in the same assay and did not display activity even at 10 μM. None of the M(II) complexes **3** to **6** exhibited any higher activity than 21% cell toxicity at 10⁻⁵ M and the activity was completely lost after diluting the compounds ten times. Therefore, the IC₅₀ values of these compounds (>10 μM) were not evaluated. However Mn(III) complex **7** induced full cellu-

lar death at 10⁻⁵ M and 97% cell toxicity was also maintained at 10⁻⁶ M. These preliminary results proved promising and further investigation was carried out on the activity of compound **7**.

The IC₅₀ value of Mn(III) complex **7** on the HCT116 cell line was determined by measuring the effect on the cell proliferation at different concentrations (see ESI). Compound **7** was found to possess high anti proliferative properties with an IC₅₀ of 336 (±10) nM relative to the HCT116 cell line. Under the same conditions, the extensively used chemotherapy drugs Doxorubicin and cisplatin possess an IC₅₀ value of 810 (±110) nM and 5370 (±150) nM, respectively. By comparison, the manganese-salen complex **7** is more active. It should be emphasized that comparison with cisplatin should be made with caution. Indeed, while cisplatin displayed cytotoxic properties on the HCT116 cell line, it was already shown to be ineffective, with side effects, in the treatment of colon cancer [38]. It remains difficult to perform a direct comparison with results obtained in previously reported work, but typically the manganese salen complexes are, at best, of the same order of magnitude as cisplatin for their cytotoxic activity [39–41]. The activity of the complexes reported herein is strongly dependent on the nature of the metal. Cu, Pd, Co and Ni complexes did not display relevant activities, in sharp contrast to the Mn complex. It is known that reported Mn(III) salen complexes are acting as mimetics of enzymes such as superoxide dismutase (SOD) or catalase [42,43]. Consequently, the higher activity displayed by complex **7**, compared to other metals, suggests a mechanism of biological action involving the complex in an oxido-reduction reaction. It was also demonstrated that these types of complexes could present DNA binding/cleaving properties. However, an increase in the steric hindrance on the bridge of the ligand (such as phenyl groups for example) strongly decreased the possibility of an interaction/reaction with DNA [44]. Ligand **2** containing a bicyclic structure is more encumbered than a simple 1,2-ethane bridge, making it difficult to intercalate into DNA. Consequently, one hypothesis is that Mn(III) complex **7** is acting as a SOD mimetic, generating activated oxygen and triggering apoptosis of the cells [5].

Conclusion

A bridged bicyclic diamine was successfully used to synthesise a series of novel metal-salen complexes with different oxidation states. Mechanochemistry was applied to the synthesis of ligand **2** and one of the metal complexes (Mn-based, **7**), highlighting that this strategy is efficient and could be used in further synthetic developments for application in medicinal mechanochemistry [45–48]. Mn(III)-salen complex **7** proved to be a potent cytotoxic agent (2.4 times more efficient than doxorubicin) on the proliferation of the human cancer cell line HCT116. The results obtained with the other metal salen complexes suggest that a SOD mimetic mode of action, rather than intercalation, might be involved.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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