Dalton Transactions

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal | View Issue

Check for updates

Cite this: *Dalton Trans.*, 2021, **50**, 6423

Received 21st January 2021,
Accepted 18th March 2021

DOI: 10.1039/d1dt00219h

rsc.li/dalton

Synthesis of asymmetrical diaminobis(alkoxo)-bisphenol compounds and their C_1-symmetrical mono-ligated titanium(IV) complexes as highly stable highly active antitumor compounds†

Gilad Nahari and Edit Y. Tshuva *

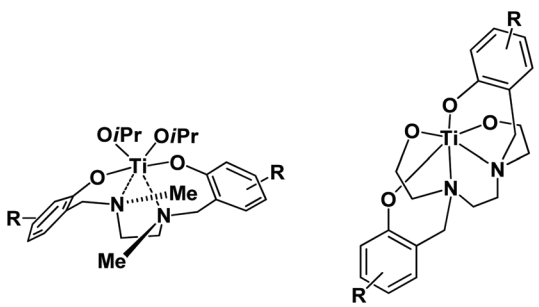
Asymmetrical 2,2′-((ethane-1,2-diylbis((2-hydroxyethyl)azanediyl)) bis(methylene))diphenol substituted compounds and their C_1-symmetrical diaminobis(phenolato)-bis(alkoxo) titanium(IV) complexes were synthesized, with one symmetrical analogue. X-ray crystallography corroborated tight ligand binding. Different substitutions on the two aromatic rings enabled fine-tuning of the complex properties, giving enhanced solubility, high anticancer activity (IC_{50} < 4 μM), and significant hydrolytic stability.

Titanium(IV) complexes play a significant role in anticancer chemotherapy, whereby the biofriendly titanium-based compounds serve as safe alternatives to the toxic platinum-based drugs.[1–8] In fact, the thermodynamic hydrolysis product of titanium(IV) complexes, TiO_2, is an inert non-toxic supplement in food, drugs, and cosmetics.[8,9] Titanium(IV) compounds that include diketonato and cyclopentadienyl ligands were presented in the 1980s as active against various cancer cell lines;[10–12] nevertheless, they failed clinical trials due to rapid hydrolysis that leads to formation of undefined clusters;[13–20] this behavior has motivated the design of ligands appropriate for stabilizing the titanium(IV) core in biological environments for sufficient periods to demonstrate defined activity, before the safe, yet inactive, TiO_2 is formed.

Our group has introduced hydrolytically stable anticancer titanium(IV) complexes based on phenolato ligands (Scheme 1).[3,21–28] The first complexes employed the tetradentate diaminobis(phenolato) salan ligand and demonstrated both *in vitro* and *in vivo* activity, alongside enhanced stability.[21–24,29,30] Structure–activity studies revealed that the substituents on the aromatic rings affect the cytotoxic activity and hydrolytic stability of the Ti(IV)-salan complexes.[22,29,31,32] Particularly, NO_2 substituents diminish the hydrolytic stability whereas *ortho* halogenation increases it, especially for Cl and Br substitutions, with reduced impact for F substitutions.[29] A subsequent study explored C_1-symmetrical Ti(IV)-salan complexes by utilizing different substitutions on the two phenolato moieties; these complexes exhibited improved cytotoxicity for most derivatives relative to the analogous C_2-symmetrical Ti(IV)-salan complexes.[33,34] Following studies promoted the hexadentate diaminobis(phenolato)-bis(alkoxo) ligands for the formation of mono-ligated octahedral Ti(IV) complexes with no labile ligands (Scheme 1).[35] PhenolaTi (Scheme 1, right: R = *m*,*p*-di-Me) featured high cytotoxicity toward a wide range of cancer cell lines, including those in the NCI-60 panel, alongside high efficacy in *in vivo* trials, in addition to remarkable hydrolytic stability.[35–37]

This paper presents C_1-symmetrical Ti(IV) complexes, based on asymmetrical diaminobis(phenolato)-bis(alkoxo) ligands. Aiming to fine-tune the complex properties, we developed a stepwise synthetic procedure for development of ligands with different substituted aromatic rings. Highly cytotoxic and hydrolytically stable complexes with enhanced solubility are reported.

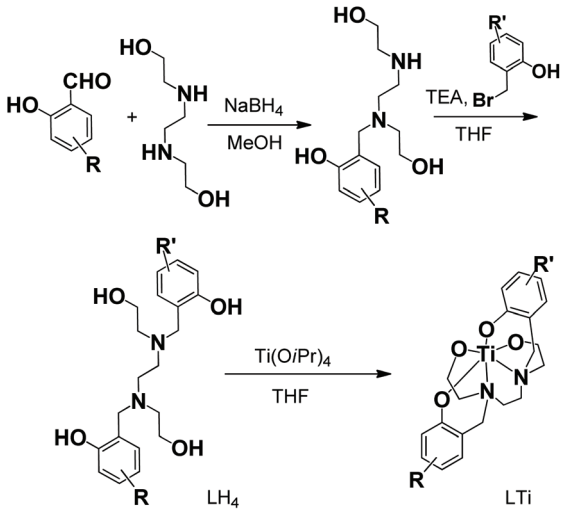
Four asymmetrical diaminobis(phenolato)-bis(alkoxo) ligands were synthesized: three with one nitrated ring and one bis-halogenated ring (Scheme 2, $L^{1–3,4}H_4$) and one with two



Scheme 1 Ti(IV) complexes with bis(phenolato) salan ligand (left), and with bis(phenolato)-bis(alkoxo) ligand (right).

The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem 9190401, Israel. E-mail: edit.tshuva@mail.huji.ac.il

†Electronic supplementary information (ESI) available: Detailed experimental procedures, ligands and complex characterization, NMR spectra, and crystal data of $L^{3,4}$Ti. CCDC 2053342. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d1dt00219h



$L^{1,4}$: R = o,p-F, R' = p-NO_2 $L^{2,3}$: R = o,p-Cl, R' = o,p-Br
$L^{2,4}$: R = o,p-Cl, R' = p-NO_2 $L^{2,2}$: R = o,p-Cl, R' = o,p-Cl
$L^{3,4}$: R = o,p-Br, R' = p-NO_2 $L^{4,4}$: R = p-NO_2, R' = p-NO_2[16]

Scheme 2 Synthesis of asymmetrical diaminobis(phenolato)-bis(alkoxo) ligands and their Ti(IV) complexes.

differently bis-halogenated rings (Scheme 2, $L^{2,3}H_4$). For comparison, a representative symmetrical *o*,*p*-chlorinated ligand was also synthesized (Scheme 2, $L^{2,2}H_4$), joining the symmetrical *p*-nitrated ligand previously reported (Scheme 2, $L^{4,4}H_4$).[35] The asymmetrical ligands were prepared in 16–44% yields *via* a two-step procedure: (a) reductive amination of halogenated salicylaldehyde and 2,2′-(ethane-1,2-diylbis(azanediyl))diethanol; (b) substitution reaction between the product and differently substitute benzylbromide (Scheme 2). The symmetrical ligand $L^{2,2}H_4$ was synthesized in 22% yield by a Mannich condensation similarly to published procedures.[35]

The ^{1}H NMR spectra of $L^{1-3,4}H_4$ (Fig. S1–S3†) and $L^{2,3}H_4$, (Fig. S4†) exhibited five and four distinct signals in the aromatic region, respectively, alongside two singlets of the protons in the different benzyl positions, confirming that C_s-symmetrical ligands had formed. Contrary, the spectrum of $L^{2,2}H_4$ featured two aromatic signals and single set of signals in the aliphatic region as expected (Fig. S5†). The ligands were also characterized by HRMS and/or elemental analysis, confirming that the desired compounds had been obtained.

Reaction of the hexadentate ligands with $Ti(OiPr)_4$ overnight under inert conditions, followed by decantation of the precipitates from the THF solution yielded the corresponding octahedral Ti(IV) complexes (Scheme 2). The ^{1}H NMR spectra verified formation of the four desired C_1-symmetrical products ($L^{1-3,4}$Ti, $L^{2,3}$Ti, Fig. S6–S9†) with different signals in the aromatic region similarly to those of the ligands as discussed above, and with distinct signals of AB system couplings for each of the benzylic protons; additionally, a symmetrical complex ($L^{2,2}$Ti, Fig. S10†), featured only two signals in aromatic region and a corresponding set of aliphatic signals. Further analysis of a representative complex, $L^{2,4}$Ti, was performed by ^{1}H–^{13}C HSQC NMR (Fig. S11†). The ^{1}H NMR spectrum features up to 0.64 ppm difference in the chemical shifts of aliphatic protons that bind the same carbon. In contrast, protons binding to different but parallel carbons possess indistinguishable difference in chemical shifts. Accordingly, these protons are characterized by a set of multiplets with integration corresponding to two protons. These findings indicate that the spatial arrangement of the ligand around the metal center influence the chemical environment of the aliphatic moiety more than the feature of C_1-symmetry; hence, evincing that the overall structure of the Ti(IV) complex is similar to those of the corresponding C_2-symmetrical titanium compounds.[35]

A representative complex $L^{3,4}$Ti crystallized from dichloromethane at −30 °C, and the single crystals were analyzed crystallographically. The X-ray structure (Fig. 1) featured a C_1-symmetrical Ti(IV) octahedral complex of a single chelating hexadentate ligand, confirming the NMR data. Moreover, the two O^{Ar} moieties bound in a *cis*-configuration, whereas the aliphatic O-donors bound in a *trans*-configuration, similarly to C_2-symmetrical titanium(IV) analogues,[35] supporting the ^{1}H–^{13}C HSQC NMR analysis. In addition, similar bond lengths and angles (Fig. 1) were obtained, with 1.87–1.88 Å for the covalent Ti–O and 2.20–2.24 Å for the coordinative Ti–N bond lengths. Interestingly, both Ti–O and the Ti–N bonds proximate to the nitrated ring were slightly longer than the corresponding bonds to the donors on the halogenated ring side, in accordance with the electron withdrawal nature of the NO_2 substituent.

The hydrolytic stability of the complexes was evaluated by ^{1}H NMR, monitoring the signals of the Ti(IV) complexes in DMSO-d_6 solution comprising 10% D_2O, based on procedures reported previously.[35] Whereas the nitrated/fluorinated complex $L^{1,4}$Ti demonstrated a moderate hydrolytic stability with $t_{1/2}$ of 25 h (Table S1†), all other complexes exhibited a high stability with less than 25% hydrolysis following 72 h from D_2O addition (Table S1†). These results are notable as the corresponding nitrated C_2-symmetrical titanium(IV) complex $L^{4,4}$Ti was not evaluated due to insufficient solubility.[35] The results are also in agreement with previous reports on the reduced stabilizing effect of fluoro substitution in salan-type complexes.[29] The nitro substituent very slightly

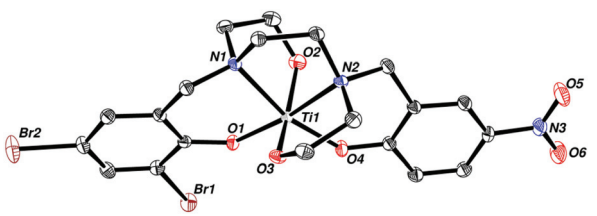


Fig. 1 ORTEP drawing of $L^{3,4}$Ti with 50% probability ellipsoids; H atoms and solvent were omitted for clarity. Selected bond lengths (Å) and angels (°): Ti(1)–O(1) 1.871(2), Ti(1)–O(2) 1.876(2), Ti(1)–O(3) 1.880(2), Ti(1)–O(4) 1.886(2), Ti(1)–N(1) 2.205(2), Ti(1)–N(2) 2.241(2); O(1)–Ti(1)–O(4) 107.19(7), O(2)–Ti(1)–O(3) 157.76(8), N(1)–Ti(1)–N(2) 81.33(7).

decreased the hydrolytic stability of the complex relatively to solely halogenated complexes and to a symmetrical methylated derivative.[35] This observation confirms that the hexadentate bis(phenolato)-bis(alkoxo) ligand binding is the main contributor to the complex stability.

The cytotoxic activity of the Ti(IV) complexes was analyzed toward human colon HT-29, human ovarian A2780, and human ovarian cisplatin-resistant A2780cp cancer cell lines. The viability of the cells was measured by the methylthiazolyldiphenyltetrazolium (MTT) assay, as previously published (Fig. 2).[38] All titanium(IV) complexes exhibited a marked cytotoxicity against the analyzed cancer cell lines, with IC_{50} values <4 μM (Fig. 2), indicating activity higher than that of cisplatin toward HT-29 and A2780cp cells (cisplatin: 13 and 33 μM, respectively).[35] Moreover, the complexes demonstrated high cytotoxic activity against the cisplatin-resistant ovarian A2780cp cancer cells, supporting a different mechanism of action of these titanium compounds than that of platinum drugs.[2,39] Notably, although activity is similar for asymmetrical and symmetrical complexes, the mono-nitrated C_1-symmetrical complexes reached somewhat higher maximal inhibition (MI) of HT-29 cell growth (~67–80%), unlike the 60% MI obtained for the corresponding C_2-symmetrical bis-nitrated complex,[35] presumably due to enhanced solubility (with higher MI values for the ovarian lines; Fig. 2). Particularly, $L^{2,4}Ti$ exhibited the highest MI value (80%) compared with those of $L^{1,4}Ti$ (67%) and $L^{3,4}Ti$ (74%), differences that are mostly attributed to different solubilities. In fact, the mono-nitrated complexes exhibited solubility in medium *ca.* 4-fold higher compared with the corresponding value of the reported bis-nitrated complex.[35] Thus, fine tuning the complex properties yielded higher solubility/lipophilicity, which improved the accessibility of the complex to its cellular target.

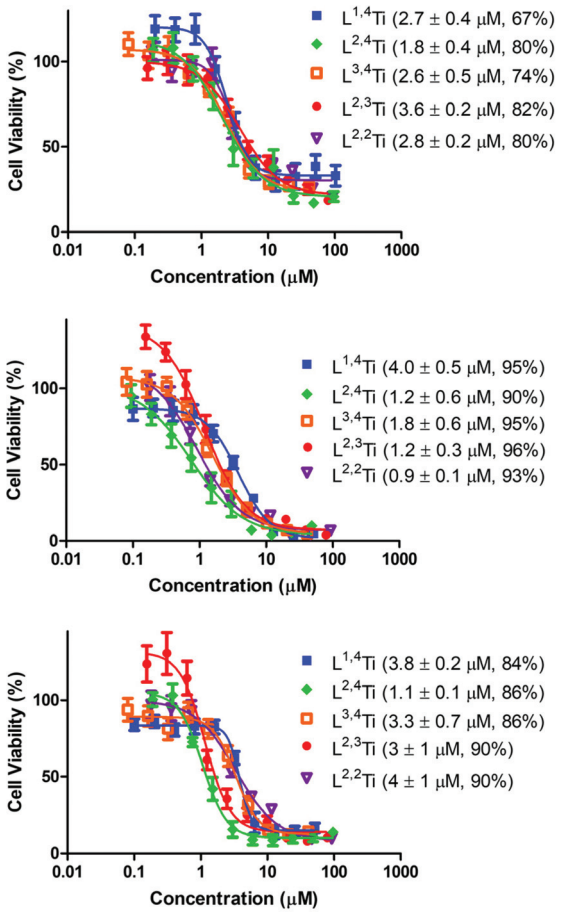


Fig. 2 Dependence of HT-29 (top), A2780 (middle), and A2780cp (bottom) cell viability based on the MTT assay following a three day incubation period with administered concentration of LTi; in parenthesis: relative IC_{50} values (μM), maximal cell growth inhibition (MI) (%).

Conclusions

In conclusion, this paper presents a synthetic procedure for a family of asymmetrical 2,2′-((ethane-1,2-diylbis((2-hydroxyethyl)azanediyl))bis(methylene))diphenol compounds, and their C_1-symmetrical Ti(IV) complexes as characterized crystallographically. Such organic and inorganic compounds with wider possibilities of steric and electronic characteristics may be employed for various applications, from catalysis to medicine. Particularly, fine tuning of complex features by incorporation of different substituents on the two aromatic rings identified complexes of enhanced solubility in biologically-relevant environments that are highly stable in the presence of water and highly cytotoxic on colon and ovarian, cisplatin-sensitive and -resistant lines. These results highlight the advantages of the diaminobis(phenolato)-bis(alkoxo) ligand system for the stabilization of titanium(IV) metal centers and expand the opportunities for their use in anticancer therapy, as well as in various other fields.

Author contributions

Gilad Nahari: Conceptualization, methodology, validation, formal analysis, investigation, writing – original draft, writing – review & editing, visualization Edit Y. Tshuva: Conceptualization, resources, writing – review & editing, visualization, supervision, project administration, funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

We thank Dr Benny Bogoslavsky for crystallography. Funding was received from the European Research Council (ERC) under the European Union's Horizon 2020 research (grant agreements #681243).

Notes and references

1 E. Meléndez, *Crit. Rev. Oncol. Hematol.*, 2002, **42**, 309–315.
2 M. Cini, T. D. Bradshaw and S. Woodward, *Chem. Soc. Rev.*, 2017, **46**, 1040–1051.
3 E. Y. Tshuva and M. Miller, in *Metallo-Drugs: Development and Action of Anticancer Agents*, ed. A. Sigel, H. Sigel, E. Freisinger and R. K. O. Sigel, De Gruyter, Berlin, 2018, pp. 219–249.
4 L. Fernández-Vega, V. A. R. Silva, T. M. Domínguez-González, S. Claudio-Betancourt, R. E. Toro-Maldonado, L. C. C. Maso, K. S. Ortiz, J. A. Pérez-Verdejo, J. R. González, G. T. Rosado-Fraticelli, F. P. Meléndez, F. M. B. Santiago, D. A. Rivera-Rivera, C. M. Navarro, A. C. B. Chardón, A. O. Vera and A. D. Tinoco, *Inorganics*, 2020, **8**, 10.
5 I. Ott and R. Gust, *Arch. Pharm.*, 2007, **340**, 117–126.
6 Y. Ellahioui, S. Prashar and S. Gómez-Ruiz, *Inorganics*, 2017, **5**, 4.
7 W. A. Wani, S. Prashar, S. Shreaz and S. Gómez-Ruiz, *Coord. Chem. Rev.*, 2016, **312**, 67–98.
8 S. A. Loza-Rosas, M. Saxena, Y. Delgado, K. Gaur, M. Pandrala, A. D. Tinoco, M. Lankosz, W. Piekoszewski, J. Baumgart, G. Sass, D. J. Kerr, S. Seeber, E. Thiel and W. E. Berdel, *Metallomics*, 2017, **9**, 346–356.
9 K. M. Buettner and A. M. Valentine, *Chem. Rev.*, 2012, **112**, 1863–1881.
10 P. Köpf-Maier and H. Köpf, *Chem. Rev.*, 1987, **87**, 1137–1152.
11 B. K. Keppler, C. Friesen, H. G. Moritz, H. Vongerichten and E. Vogel, in *Bioinorganic Chemistry*, Springer, Berlin, Heidelberg, 1991, pp. 97–127.
12 H. Köpf and P. Köpf-Maier, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 477–478.
13 N. Muhammad and Z. Guo, *Curr. Opin. Chem. Biol.*, 2014, **19**, 144–153.
14 F. Caruso, M. Rossi and C. Pettinari, *Expert Opin. Ther. Pat.*, 2001, **11**, 969–979.
15 N. Kröger, U. R. Kleeberg, K. Mross, L. Edler and D. K. Hossfeld, *Oncol. Res. Treat.*, 2000, **23**, 60–62.
16 T. Schilling, K. B. Keppler, M. E. Heim, G. Niebch, H. Dietzfelbinger, J. Rastetter and A. R. Hanauske, *Invest. New Drugs*, 1995, **13**, 327–332.
17 G. Lümmen, H. Sperling, H. Luboldt, T. Otto and H. Rübben, *Cancer Chemother. Pharmacol.*, 1998, **42**, 415–417.
18 K. Strohfeldt and M. Tacke, *Chem. Soc. Rev.*, 2008, **37**, 1174–1187.
19 P. M. Abeysinghe and M. M. Harding, *Dalton Trans.*, 2007, 3474–3482.
20 F. Caruso and M. Rossi, in *Metal Ions in Biological Systems*, Bentham Science Publishers Ltd., 2004, vol. 42, pp. 353–384.
21 M. Shavit, D. Peri, C. M. Manna, J. S. Alexander and E. Y. Tshuva, *J. Am. Chem. Soc.*, 2007, **129**, 12098–12099.
22 D. Peri, S. Meker, M. Shavit and E. Y. Tshuva, *Chem. – Eur. J.*, 2009, **15**, 2403–2415.
23 E. Y. Tshuva and J. A. Ashenhurst, *Eur. J. Inorg. Chem.*, 2009, **2009**, 2203–2218.
24 E. Y. Tshuva and D. Peri, *Coord. Chem. Rev.*, 2009, **253**, 2098–2115.
25 D. Peri, C. M. Manna, M. Shavit and E. Y. Tshuva, *Eur. J. Inorg. Chem.*, 2011, **2011**, 4896–4900.
26 S. Meker, K. Margulis-Goshen, E. Weiss, S. Magdassi and E. Y. Tshuva, *Angew. Chem., Int. Ed.*, 2012, **51**, 10515–10517.
27 A. Tzubery and E. Y. Tshuva, *Inorg. Chem.*, 2012, **51**, 1796–1804.
28 S. Meker, O. Braitbard, K. Margulis-Goshen, S. Magdassi, J. Hochman and E. Y. Tshuva, *Molecules*, 2015, **20**, 18526–18538.
29 D. Peri, S. Meker, C. M. Manna and E. Y. Tshuva, *Inorg. Chem.*, 2011, **50**, 1030–1038.
30 C. M. Manna, O. Braitbard, E. Weiss, J. Hochman and E. Y. Tshuva, *ChemMedChem*, 2012, **7**, 703–708.
31 T. A. Immel, U. Groth and T. Huhn, *Chem. – Eur. J.*, 2010, **16**, 2775–2789.
32 T. A. Immel, U. Groth, T. Huhn and P. Öhlschläger, *PLoS One*, 2011, **6**, e17869.
33 H. Glasner and E. Y. Tshuva, *Inorg. Chem.*, 2014, **53**, 3170–3176.
34 H. Glasner and E. Y. Tshuva, *J. Am. Chem. Soc.*, 2011, **133**, 16812–16814.
35 S. Meker, O. Braitbard, M. D. Hall, J. Hochman and E. Y. Tshuva, *Chem. – Eur. J.*, 2016, **22**, 9986–9995.
36 N. Ganot, O. Briaitbard, A. Gammal, J. Tam, J. Hochman and E. Y. Tshuva, *ChemMedChem*, 2018, **13**, 2290–2296.
37 G. Nahari, O. Braitbard, L. Larush, J. Hochman and E. Y. Tshuva, *ChemMedChem*, 2021, **16**, 108–112.
38 N. Ganot, S. Meker, L. Reytman, A. Tzubery and E. Y. Tshuva, *J. Visualized Exp.*, 2013, e50767.
39 M. Miller, A. Mellul, M. Braun, D. Sherill-Rofe, E. Cohen, Z. Shpilt, I. Unterman, O. Braitbard, J. Hochman, E. Y. Tshuva and Y. Tabach, *iScience*, 2020, **23**, 101262.