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Review

New Antitumor Organotitanium Complexes with a Pendant Biologically Active Diazo Group

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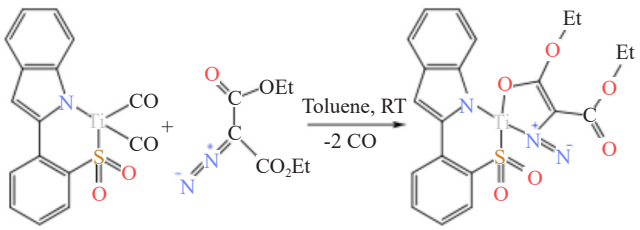
Abstract: The ligand of antitumor organotitanium or other metallodrug complexes plays a pivotal role in determining the mechanism of their cytotoxic action. Although the specific contribution of several ligands is generally well established, our understanding of the overall mechanism of the cytotoxic action of the complexes themselves is limited and incomplete in most cases, except perhaps in the case of cis-platin. A strategy to monitor the mode of cytotoxic action of candidate antitumor complexes requires tagging with bioactive side chains like a diazo group, for in-cell site-specific labelling. In this review we discuss new methods for the preparation of potential antitumor organotitanium complexes with a pendant diazo group, aiming at better understanding their mode of cytotoxic action. By introducing this new class of titanium-based potential antitumor agents, we hope to contribute to the world-wide effort in this important area of medicinal chemistry research, for an ultimate usable titanium-based antitumor drug. The following figure represents a model reaction depicting the methodology, for the formation of a bioactive Titanium complex with a pendant diazo group. Moreover, in this case, a scheme is required that represents all the possibilities of formation of the bioactive complex of titanium with a pendant diazo group.



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1. Introduction

Antitumor drugs act by interfering with molecular processes in the cell replication cycle. The success of cis-platin

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and its FDA-approved derivatives,[1] widely and effectively used in chemotherapy even today, are attributed to targeting and damaging the DNA of tumor cells. Cisplatin reaches the tumor cell intact, crosses the cell membrane, arriving at areas of low concentration of Cl- ions. Subsequently, it undergoes hydrolysis, by replacing one or two Cl- ions and thus forming intra- or inter-strand Pt-DNA bonds, mainly involving guanine N (7) sites. This DNA transformation causes cells to arrest at either the G1, S, or G2 phase of the cell cycle. Eventually, the cells undergo an aberrant mitosis followed by apoptosis.[2] Nevertheless, Pt^{II} drugs are not specific, have numerous side effects, a limited spectrum of activity, and cellular resistance during extended use.

The modus operandi of cis-platin on tumor cells appears to be almost perfect in terms of our present understanding of its therapeutic applications. At our current level of knowledge, no other proposed anticancer metallodrug, including a number of titanium-based candidates, appear to follow any similar or closely related mechanism of action in such detail. Several reported structural modifications of DNA or other cellular targets by organotitanium complexes are currently poorly understood.

Only scattered information on the role of individual organotitanium complexes in cancer treatment is available. Titanocene dichloride Cp_2TiCl_2, entered in phase II clinical trials as an anticancer drug, interacts with human serum transferrin and with Adenosine Triphosphate (ATP)[3] under physiological conditions of pH 7.4. Ti^{IV} partially replaces the Fe^{III} of transferrin, thus providing a route for entry of Ti^{IV} into tumor cells via the transferrin receptor. It is not clear how this "replacement" or Ti^{IV}/Fe^{III} ion exchange occurs, since Cp_2TiCl_2 looses first the chlorides and then the Cp ligands to hydrolysis at the physiological aqueous environment. Also, the high affinity of Ti^{IV} for phosphate groups may be important for its biological activity.

Hydrolysis of the organotitanium complex may be prevented by choosing appropriate ligands, such as naphthaline-2,3-diolate,[4] and their solubility in the aqueous environment may be substantially enhanced by the incorporation of sulfonate groups on the ligand. In this case, the hydrolytically stable complex is unable to deliver Ti^{IV} to the human transferrin, and though it can associate with DNA, the DNA bound complex does not display any cleavage activity.

Several other hydrolytically stable organotitanium compounds have been proposed, among them Ti^{IV} citrares,[5] Cp-substituted titanocene Y,[6] including oxali-titanocene Y,[7] Ti^{IV} deferasirox,[8] six-coordinate salen and salan complexes,[9,10] complexes of chiral diaminobis(phenolato) ligands, claimed to provide the best combination of hydrolytic stability and biological activity.[11] Although in each case the special role of the ligand in the hydrolytic stability and cytotoxicity is well recognized, the mechanism of cytotoxic action for each complex is mostly ambiguous.

Since the choice of ligand in the organotitanium complex plays a pivotal role in therapeutic applications, we have searched for ligands incorporating biologically active functional groups, aiming at improving the overall chemoselectivity of the complex. Diazo-tagged side-chains[12] or in general diazo compounds,[13] well known as versatile tools for chemical biology, appear to perfectly fit this description. A number of naturally occuring antibiotics containing a diazo group, like 6-diazo-5-oxo-L-norleucine (DON),[14,15] kinamycins,[16] O-diazo acetyl-L-serine (azaserine),[17] inhibit glutamine metabolism, preventing growth of several tumors such as breast, liver, kidney and T-cell leukemia. Moreover, there are diazo-forming enzymes in cremeomycin biosynthesis,[18] and diazo compounds have been applied in bioreversible esterification of proteins.[19] And perhaps for the first time, a precise installation of a diazo-tagged side-chain on proteins enables in vitro and in-cell site specific labelling.[12]

Aiming at similar precisely targeted biochemical applications or bioconjugations, we explain in the present mini review new organotitanium complexes with pendant diazo groups, expecting that they would follow reaction pathways similar to those established for naturally occuring diazo antibiotics, vide supra. At this state of our research effort, our approach follows well established experimental pathways, with great probability of success in experimental verification. Except from a brief mention in a previous short article,[1] organotitanium or organozirconium compounds featuring a pendant diazo group have not been reported or considered as anticancer drug candidates.

2. Discussion

The preparation of organotitanium complexes featuring a pendant diazo group involves a combination of new and established chemistry. The foundation of the new chemistry originates from our serendipitous discovery of direct metallation of 2-phenyl indole with $TiCl_4$ or $ZrCl_4$ in toluene at 105 °C, either at the ortho position of the pendant

phenyl group or at the 3-position of the indole scaffold, depending on the molar ratio of 2-phenyl indole to the metal chloride.[20,21] This one-pot, single step efficient reaction, complete in less than 30 min, may be expanded to several related ligands, for example 2-phenyl benzoxazole, yielding three main types of complexes, I, II, and III, Figure 1.

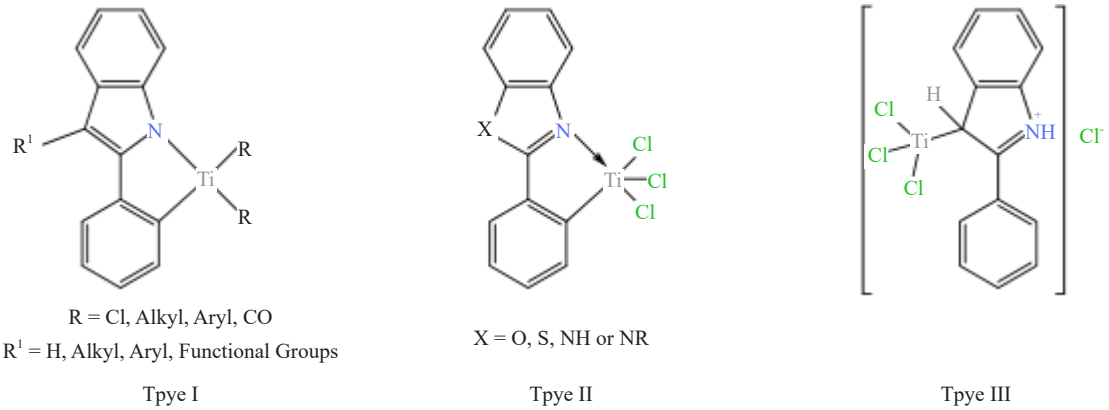


Figure 1. Types of organotitanium complexes-starting materials for potential new anticancer drug formulations

In Type I, Ti is four-coordinated, with the double bond of the pyrrole ring in the 2-3 position. In Type II, Ti is five-coordinated, with the double bond in the 1-2 position. Type III is an unsaturated ammonium salt, and titanation occurs at the 3-position of the indole scaffold. This complex may be neutralized with stronger amines like Et_3N.

Our initial preparative approach utilized a Type I, formally Ti^{II} dicarbonyl or di-trimethylphosphine derivative, after insertion into the Ti-C(phenyl) σ-bond of a small molecule like SO_2 or CO_2, for stabilization and improved solubility purposes,[1] see complexes 1 and 2 (Figure 2). Insertion of small molecules like CO_2, SO_2, SO, COS, NO_2, N_2O, azides (RN_3), ketones, aldehydes, nitriles, isonitriles, etc., into the active Ti-C σ-bond of the complexes is an integral part of our methodology.

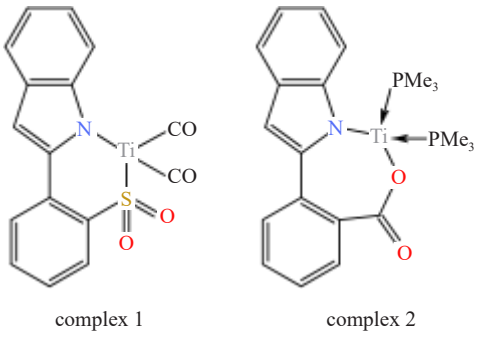
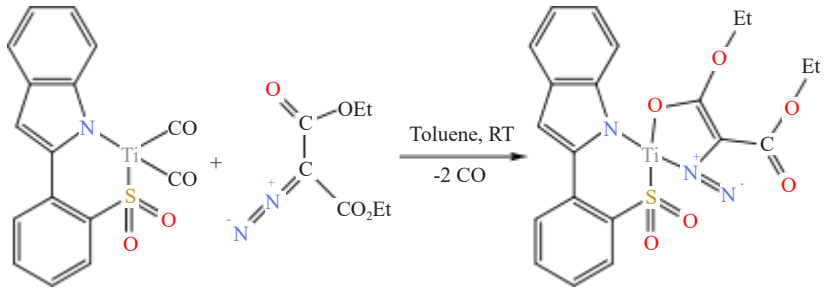


Figure 2. Starting reagents for a diazo complex preparation

Either one of the two complexes 1 or 2 are capable of undergoing a slow, room temperature reaction in toluene with diethyl- 3 or diphenyldiazomalonate, with loss of two equivalents of CO, to form complex 4, stable to heat and to air, Figure 3.



complex 1 diethyl diazomalonate 3 complex 4

Figure 3. Decarbonylation of complex 1 with diethyl diazomalonate 3, forming the candidate anticancer drug 4, having a pendant diazo group

The above reaction Scheme was originally reported in 1982[22] by Gambarotta and coworkers, Figure 4.

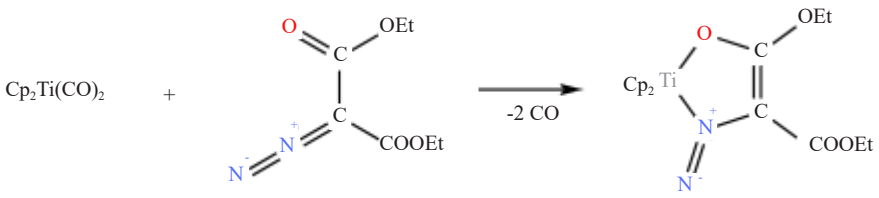
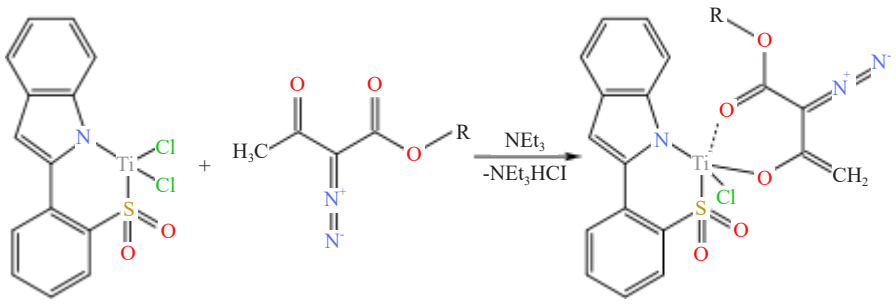


Figure 4. Reaction of $Cp_2Ti(CO)_2$ with diethyl diazomalonate

A second important preparative method would be the reaction of any dichloro Type I complex, for example 5, with the α-diazo β-ketoester 6 in the presence of a strong base (NEt_3) to generate the Ti enolate 7, having two active functional groups, diazo and methylene,[21] available for possible bioactive interactions, Figure 5. Substitution of the Cl in 7 with myristic acid will help cell penetration.



complex 5 diazo derivative 6 complex 7

Figure 5. Reaction of Type I complex 5 with the α-diazo β-ketoester 6 in the presence of NEt_3 to generate the anticancer candidate 7, featuring two active functionalities, a diazo group and a conjugated methylene group

Substitution of the Cl in 7 with myristic acid will help cell penetration.

A cysteine-selective complex may be generated by the reaction of 1 with the biocompatible diazo derivative 8 yielding 9 by CO elimination, Figure 6. The olefinic double bond of the side chain in 9 will react the -SH site of cysteine in protein.[12]

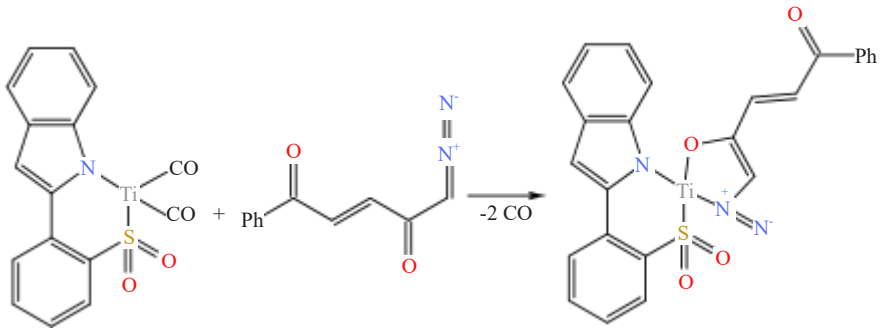


Figure 6. Reaction of complex 1 with the biocompatible diazo derivative 8

Widely investigated titanocene Y,[6] has been used in initial clinical trials against breast and renal-cell cancer. The dicarbonyl derivative 10 may be modified now by incorporating a diazo compound like 8, Figure 7.

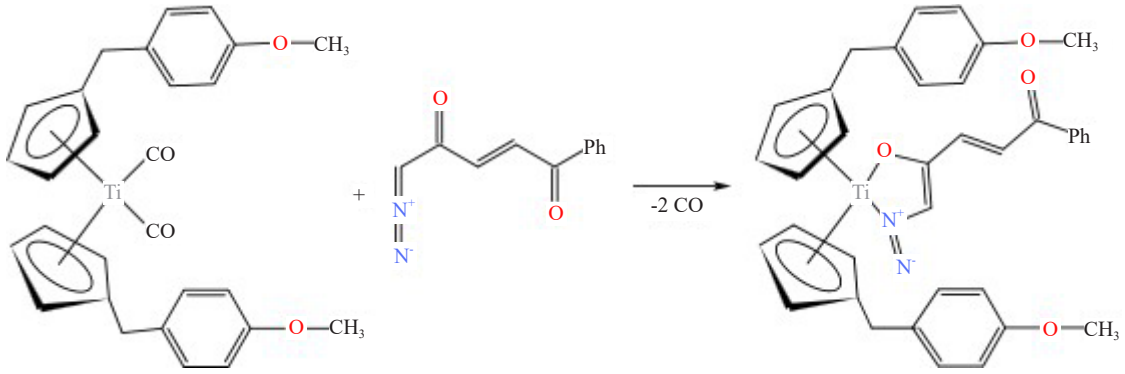


Figure 7. An example of Titanocene Y modification, incorporating new bioactive functionalities

It could be further modified by the methodology of Figure 5, or by introduction of any other diazo derivative, vide infra. It will require tedious work to determine the cytotoxic effect of these Titanocene Y transformations.

The above modifications of prior considered anticancer complexes are obviously not restricted to titanocene Y alone, but may include a universe of other complexes, like complex 12D and 13, Figure 8.

Complex 12D comprises a cyclohexyl-based diamino bis-phenolato derivative[11] with very good hydrolytic stability and biological activity. The diazo chain will enhance its biological accessibility. The proposed preparation procedure of 12D is depicted in Figure 9. The SALAN derivative 12A reacts with $TiCl_4$ in toluene at the 1:1 molar ratio in the presence of NEt_3 to form the dichloride 12B, followed by transformation of the dichloro product 12B to the dicarbonyl 12C, by reaction with Mg under CO pressure. The dicarbonyl derivative 12C will react with 8 to yield 12D by decarbonylation.

The preparation of the deferasirox Ti complex 13[23] would follow a similar procedure, starting with the corresponding bis-phenolato ligand. Complex 13 is expected to have high aqueous solubility and stability. It can rapidly bind Fe^{III} via a transmetallation process, and could eventually result in the delivery of Ti^{IV} to intracellular target sites.

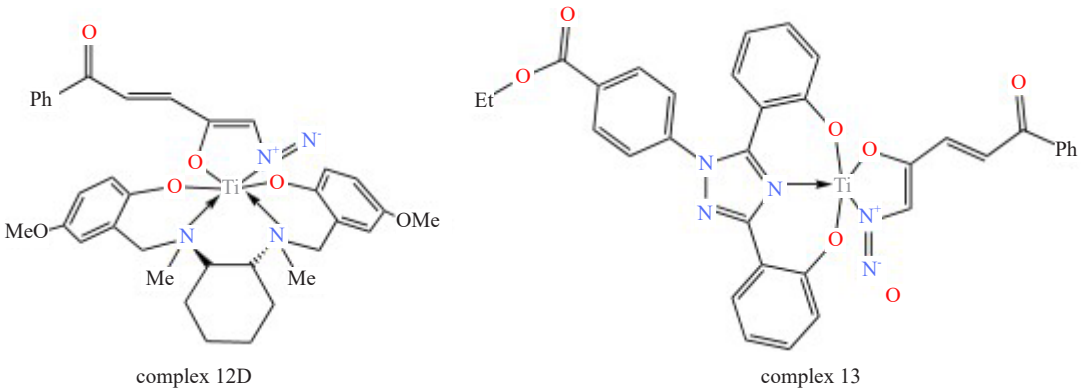


Figure 8. Anticancer drugs 12D and 13, ligated with the diazo compound 8

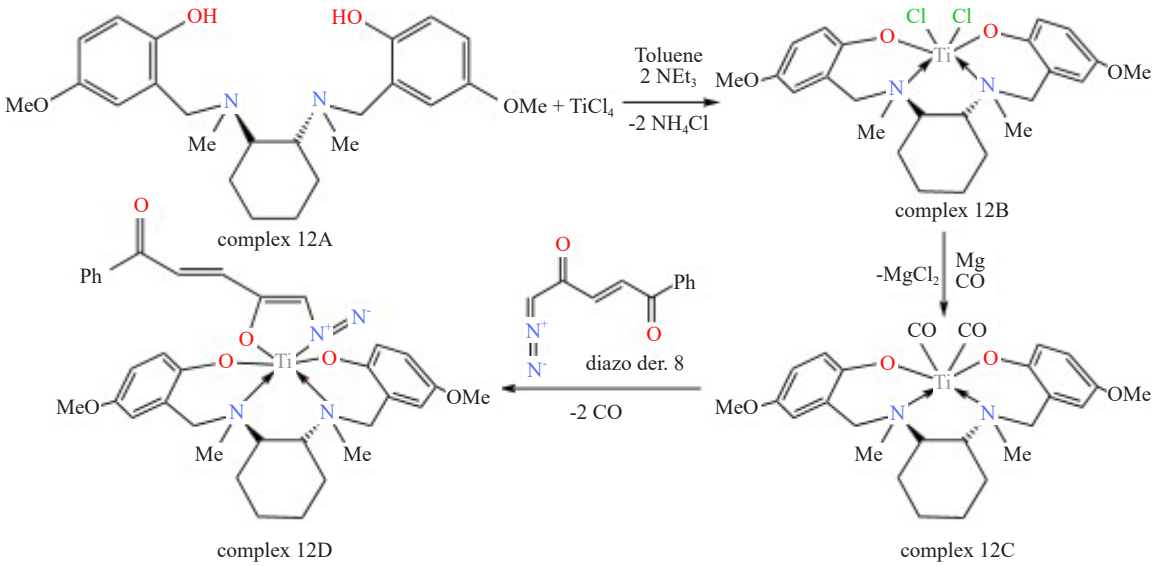


Figure 9. Preparation procedure for complex 12D

The diazo compound 15 (Figure 10), prepared from 4,5-diazafluorenone, reacts with copper nitrate at a molar ratio of 2:1 to form complex bis(9-diazo-4,5-diazofluorene)copper(II) nitrate.[24] The latter represents a potential model for the action of Kinamycin antitumor antibiotics, used to monitor biological activity through DNA cleavage essays.

In a similar fashion, the diazo compound 15 will react with the type I dichloro complex 14 or any other related synthon to produce complex 16 (Figure 10), anticipating a mimicking role to natural antitumor antibiotics.

The plethora of known diazocarbonyl and related compounds,[25] several of them commercially available, present an opportunity to expand the search for suitable organotitanium complexes with pendant diazo functionality, for consideration as antitumor agents in a spectrum of targeted therapeutic applications. The possibility of engaging any of the three types of organotitanium complexes mentioned above, along with an expanded choice of small molecules, for insertion reactions into the active Ti-C σ-bond, aiming at controlling the stability and aqueous solubility of the antitumor candidates, gives rise to a great number of possible effective cytotoxic complexes.

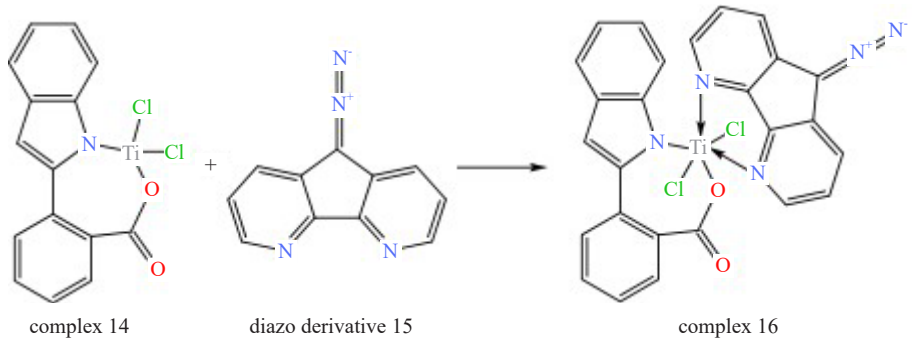


Figure 10. Diazo compound 15 reacting with a Type I complex 14

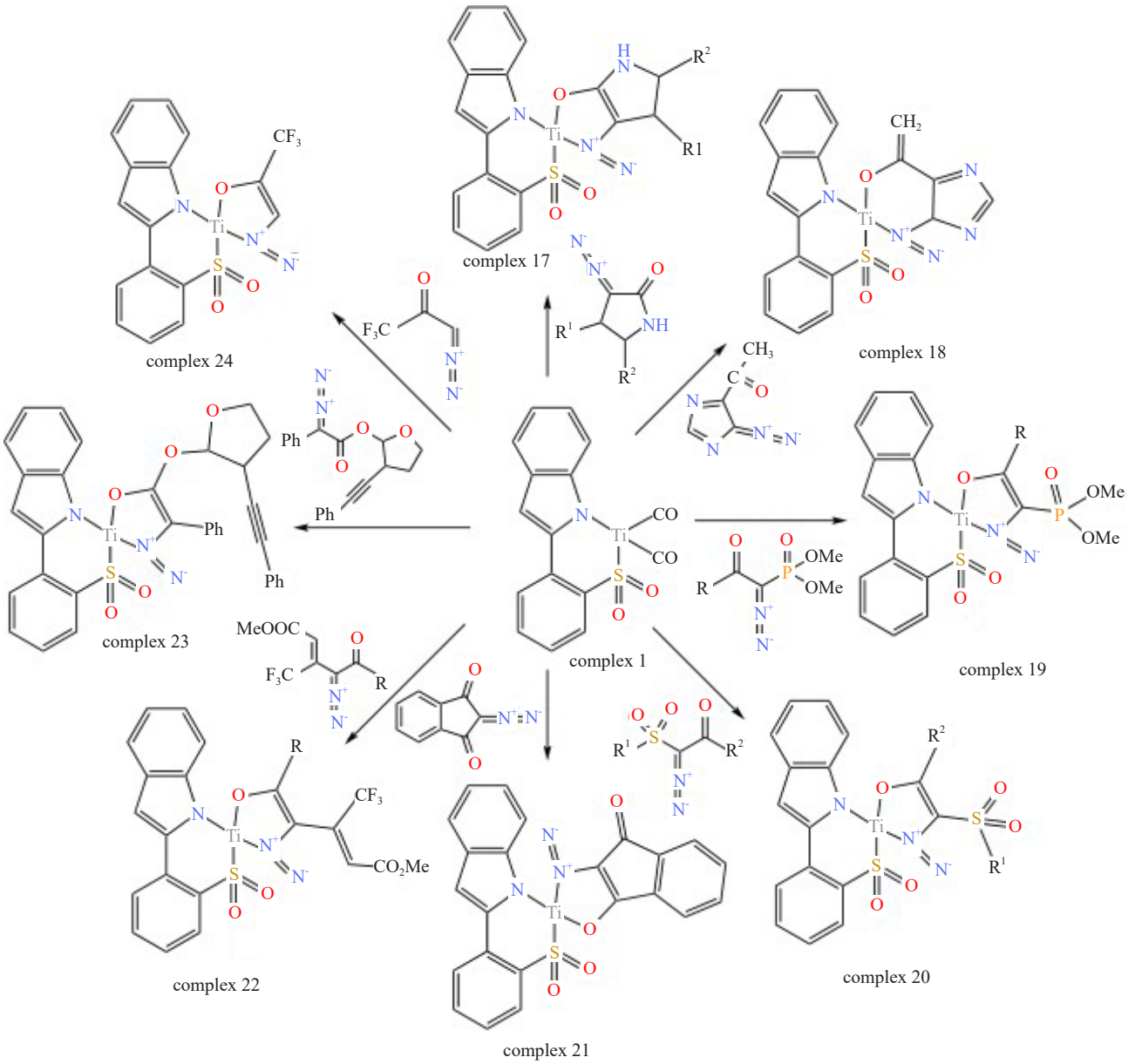


Figure 11. Reactions of the dicarbonyl Type I complex with several diazo derivatives

An illustrative example of the wide range of possibilities springing from our methodology is the collection of diverse organotitanium complexes 17 through 24, each with a pendant diazo group, originating from complex 1, presented in Figure 11. Besides their common diazo group, each complex carries a different bioactive ligand, expected to play a unique role in biocompatibility and chemoselectivity. Featured moieties include a pyrrole ring (complex 17), an imidazole ring and a conjugated methylene group (complex 18), a phosphate group (complex 19), a second sulfoxide (complex 20), an indonyl group (complex 21), CF_3 groups (complexes 22 and 24), and a phenyl acetylene moiety (complex 23).

In Figures 3, 6, 7 and 11, the proposed anticancer candidates are reaction products of a Type I complex (for example 1, 2, 5, 14), with an equimolar amount of a diazocarbonyl derivative, bonding at the two reactive coordination sites of a Type I complex, forming a tetracoordinated compound. In Figure 5, a single chloride is substituted by the reaction with α-diazo β-ketoester resulting in a pentacoordinated complex 7. Finally, in Figure 10 the diazo ligand 15 affords the hexacoordinated complex 16, with two chlorides still available for further substitutions. The above variety of complexes clearly demonstrates that our methodology provides multiple routes for preparing diazo-containing organotitanium complexes.

Moreover, expanded possibilities exist by utilizing the sulfonated Type II 2-phenyl benzoxazole titanium trichloride complex 25 to prepare a diazo derivative. One of several approaches is to first react one mole equivalent of 3-hydroxypyran-4-one 26 with complex 25 to produce 27 (Figure 12). The dicarbonyl complex 28 is obtained by treatment with Mg under an atmosphere of CO, or in the presence of PMe_3. Final addition of the diazo derivative 29 will afford the six-coordinated titanium with NSNOOO heteroatoms complex 30, expected to have high hydrolytic stability and aqueous solubility under biological conditions.

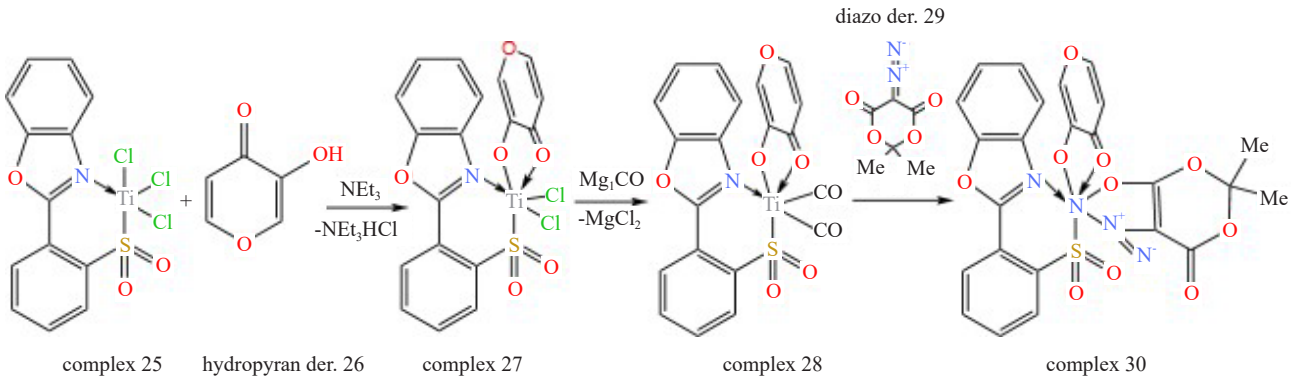


Figure 12. Type II complex 25, incorporating two new ligands 26 and 29, forming the hexacoordinated complex 30, with pendant diazo group

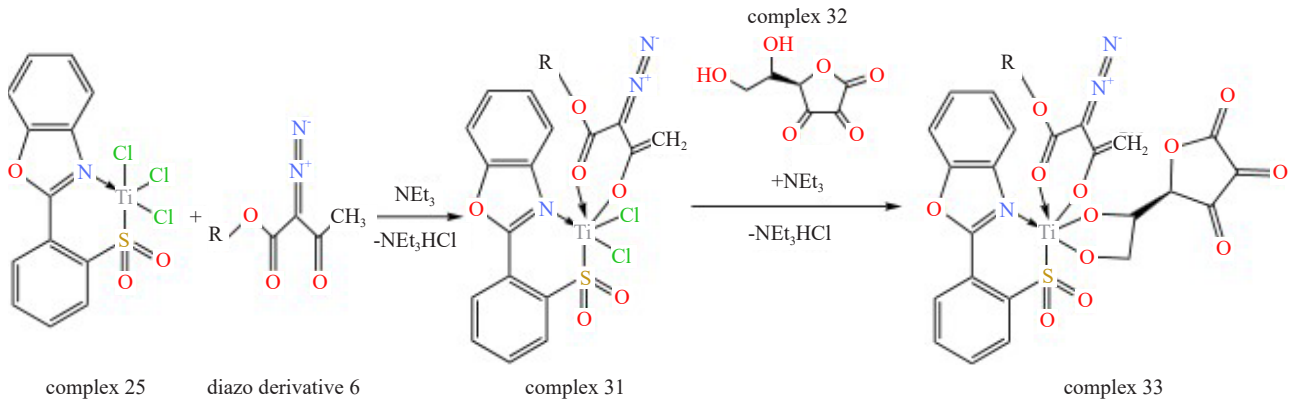


Figure 13. Type II complex 25 incorporating diazo compound 6 and dehydroascorbic acid 32, affording a new hexacoordinated complex 33, with pendant diazo group

Another available option is to reverse the order of addition in the reaction sequence of the type II complex 25 with two new ligands, adding the diazo-containing ligand first, Figure 13. Product 31 will now react with one mol equivalent of dehydroascorbic acid 32 to yield the titanium with heteroatoms NSOOOO six-coordinated complex 33, with anticipated good hydrolytic stability and aqueous solubility. Alternatively, in the last step we may apply the dilithiated form of the dehydroascorbic acid, avoiding the use of NEt_3.

An iconic example of a six coordinated titanium complex stabilized by biological ligands would be compound 35, with a suggested preparation from dicarbonyl titanium complex 28 and the N-acetyldiazomannosamine (Ac4ManDiaz) 34.[26] It is reasonable to assume that the presence of the diazo group in the Ac4ManDiaz ligand of 35 will provide opportunities for chemoselective orthogonal reactivity.

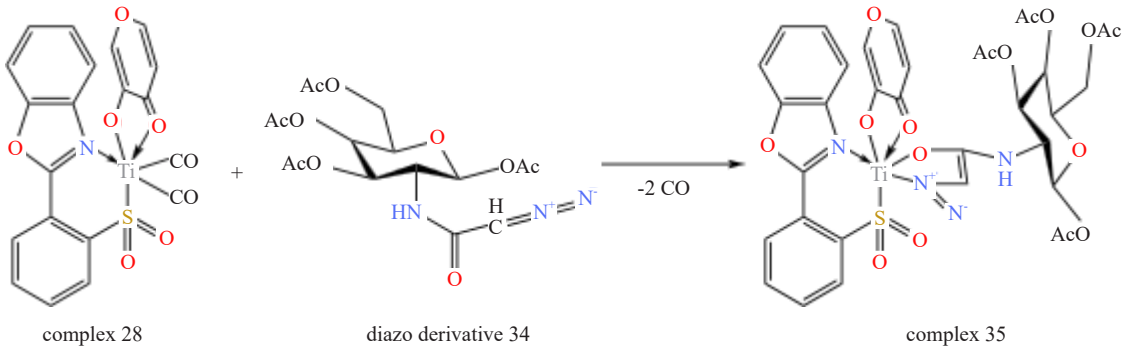


Figure 14. Intermediate 28 reacting with Ac4ManDiaz 34 to create the iconic complex 35

3. Conclusions

The above proposed preparative schemes illustrate the available possibilities in our methodology, to prepare organotitanium complexes, and possible anticancer drug candidates. Simply starting with the three reagent Types in Figure 1, followed by the stabilizing insertion of a small molecule into the active Ti-C σ-bond, and finally incorporating a diazo moiety, applying formerly established reaction pathways, as in Figures 1, 3, 5, and 10.

Through the introduction of a diazo group in our organotitanium complexes they assume the role of reporters for biological discovery.[27] Several of the established interactions of diazo groups in chemical biology, applied to a number of our complexes carrying the diazo group, may open up new avenues for our understanding of the mechanism of their cytotoxic action and efficacy. In some instances, diazo-tagging[27] may monitor biological activity through DNA cleavage.[24] Even the hypothesis has been advanced that the diazo moiety is the active pharmacophore.[13]

Conflict of interest

The author declares that he has no conflict of interest.

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