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Synthesis and characterization of novel natural product-Gd(III) MRI contrast agent conjugates

Eleni K. Efthimiadou [a], Maria E. Katsarou [a], Michael Fardis [b], Christos Zikos [a], Emmanuel N. Pitsinos [a,*], Athanasios Kazantzis [a], Leondios Leondiadis [c], Marina Sagnou [d], Dionisios Vourloumis [a,*]

[a] Chemical Biology Program, Institute of Physical Chemistry, NCSR 'DEMOKRITOS', PO Box 60228, GR-153 10 Aghia, Paraskevi, Greece
[b] Institute of Material Sciences, NCSR 'DEMOKRITOS', Greece
[c] Mass Spectrometry and Dioxin Analysis, Institute of Radiodiagnostics and Radiopharmaceuticals, NCSR 'DEMOKRITOS', Greece
[d] NMR Studies of Biomolecules and Pharmaceuticals, Institute of Biology, NCSR 'D', Greece

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ABSTRACT

Several novel gadolinium chelates conjugated with paclitaxel, colchicine and thyroxine have been prepared as MRI contrast agents targeted to tubulin and thyroxine-binding globulin, respectively.

Magnetic resonance imaging (MRI) is a non-invasive imaging technique, extensively used in the clinic, which allows for high-definition pictures of a whole-organism to be obtained over extended periods of time. The technique is often enhanced by the use of contrast agents (CAs). Among them, paramagnetic gadolinium chelates hold a prominent position.[1] In general, these agents are injected systemically and serve to passively and non-specifically enhance the signal by increasing the longitudinal relaxation rate ($1/T_1$) of surrounding water molecules. Furthermore, binding to a macromolecule increases concentration and retention of the CA at the binding site and affords an increase in relaxivity, resulting from a decrease in the rotational correlation time (τ_r).[2] As the contrast gained is still limited, relatively high doses of CA are required for sufficient contrast enhancement. There are two major ways to decrease the unwanted and potentially toxic high CA administered dose: (i) by employing new Gd(III) complexes endowed with much higher relaxivities; (ii) by improvement of the biodistribution of CAs. Recently, the development of 'smart' CAs that will accumulate at a specific tissue, or sense a particular biological event, has attracted considerable attention.[3] Thus, gadolinium chelates have been tagged with various targeting elements, including natural products. Conjugates with peptides,[4] steroids,[5] carbohydrates,[6] and an anticancer anthracycline[7] have been prepared, with the aim to guide the gadolinium label towards the biological target of the natural product, to increase the lipophilicity of the CA or to facilitate pharmacokinetic studies. In this context, it should be noted that the delivery of sufficient quantity of the paramagnetic label and/or the ability of the conjugates to cross cellular membranes is a challenge.[8]

In the frame of a research program aiming at the development of novel, targeted MRI contrast agents, we have designed conjugates of paclitaxel, colchicine and thyroxine with the known, and approved for clinical use as MRI-CA, Gd(III) complexes with diethylenetriaminepentacetic acid bis-methyl amide ([Gd(DTPA-BMA)], Omniscan™) and/or 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid ([Gd-DOTA], Dotarem™) (Fig. 1). We report herein

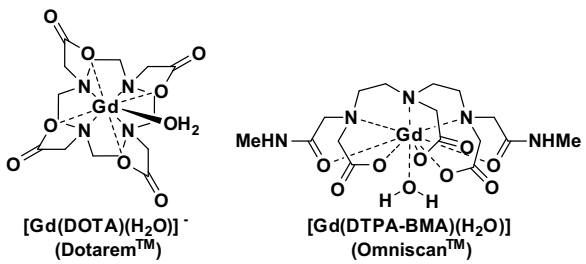

Figure 1. Structures of some clinically used Gd-chelates.

* Corresponding authors. Tel.: +30 2106503624; fax: +30 2106511766.
E-mail address: vourloumis@chem.demokritos.gr (D. Vourloumis).

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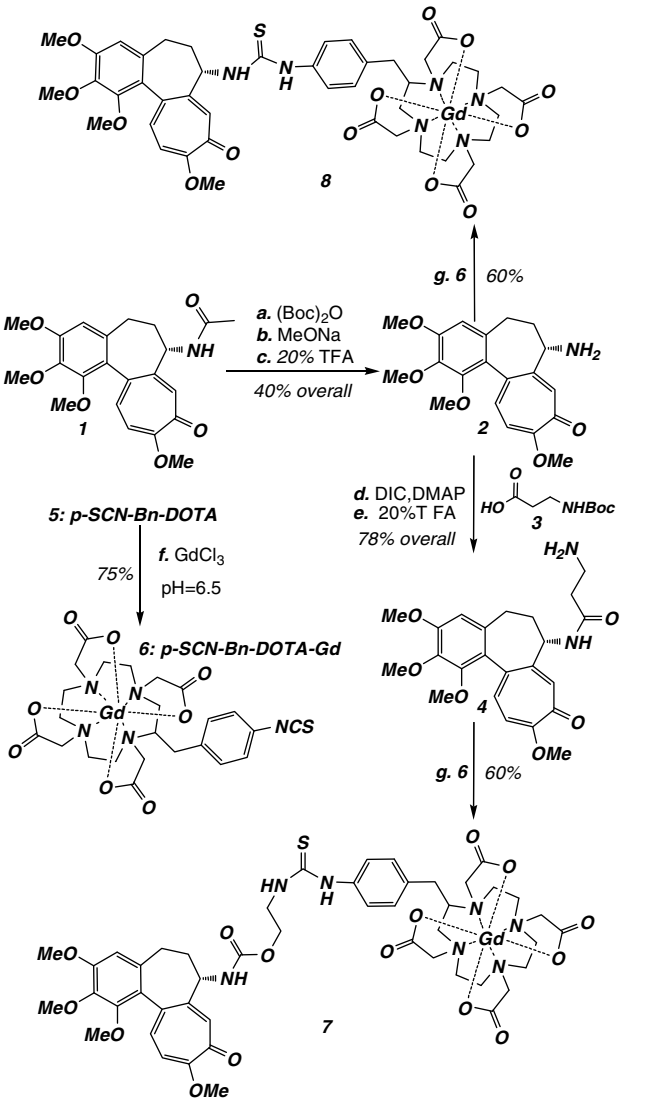
the synthesis of the targeted conjugates along with their relevant toxicities and magnetic properties.

Microtubules are long stiff polymers formed from molecules of tubulin (heterodimer of α- and β-form). They are critical for internal cell organelle trafficking and vital for mitotic spindle formation during cell division.[9] Paclitaxel, a natural product clinically used for the therapy of several carcinoma lines (Taxol™), binds to microtubules with high affinity ($K_d \sim 10^{-8}$ M) and a maximum stoichiometry of one ligand per heterodimer, promoting tubulin polymerization.[10] On the other hand, each molecule of the alkaloid colchicine, which is clinically utilized for the treatment of autoinflammatory diseases and gout, binds tightly to one tubulin molecule and prevents its polymerization.[11] The obvious complementarity of the two has inspired the synthesis of several paclitaxel[12] and colchicine derivatives,[11] to study intracellular drug distribution and their interference with the, otherwise, finely balanced assembly/disassembly dynamics of microtubules. Based on the above mentioned literature evidence, paclitaxel and colchicine conjugates are expected to target the CA to tissues of high tubulin content, such as sites of actively propagating tumours. In addition, they may facilitate the prescription of these clinically used drugs on a personalized medicine basis, or the identification of any possible, previously undetected, preference in their distribution in the body.

Thyroxine (T_4) is one of the hormones produced by the thyroid gland. Only a minute fraction (<0.03%) of T_4 circulating in the blood is free and thus available to tissues. Most of it is present in the biologically inactive state, bound to transport proteins, mainly thyroxine-binding globulin (TBG) but also transthyretin (TTR) and albumin (HSA).[13] These proteins sequester one (TBG, TTR) or several (HSA) hormone molecules. Although albumin is present in higher concentration (40,000 mg/L vs 16 mg/L of TBG), TBG has the highest affinity ($K_a = 1 \times 10^{10}$ M^{-1}) and carries most (75%) of the serum's T_4.[14] Binding of T_4 by these proteins safeguards the body from the effects of abrupt fluctuations in hormone secretion. Interestingly, in the case of TBG, selective hormone release at sites of inflammation has also been reported.[15] Consequently, thyroxine conjugates are expected to exhibit increased retention time in the blood-stream, compared with the untagged CA, and thus improved performance in cardiovascular MRI. Furthermore, T_4 conjugates might allow selective MRI of inflammation sites.

For the synthesis of colchicine-Gd(III) conjugates the natural product **1** was deacetylated, as previously described, to yield amine **2** in 40% overall yield (Scheme 1).[11b] In order to address the possibility of the gadolinium-complex interfering with binding of the natural product to its biological target, a short, 4-atom linker, was introduced through a coupling reaction with **3** mediated by DIC, followed by liberation of the corresponding amine through acidic treatment. In parallel, complexation of $GdCl_3$ with commercially available *p*-SCN-Bn-DOTA was accomplished under carefully controlled pH conditions,[5b] producing complexed isothiocyanate **6** in 75% yield, spectroscopically identical to the one reported previously.[5b] Thus, no further attempts for the determination of Gd(III)-concentration in the complex were performed. Mixing of the two colchicine analogues **2** and **4** with **6** in DMSO, in the presence of triethylamine, furnished the corresponding conjugates **7** and **8** in 60% yield, after HPLC purification.

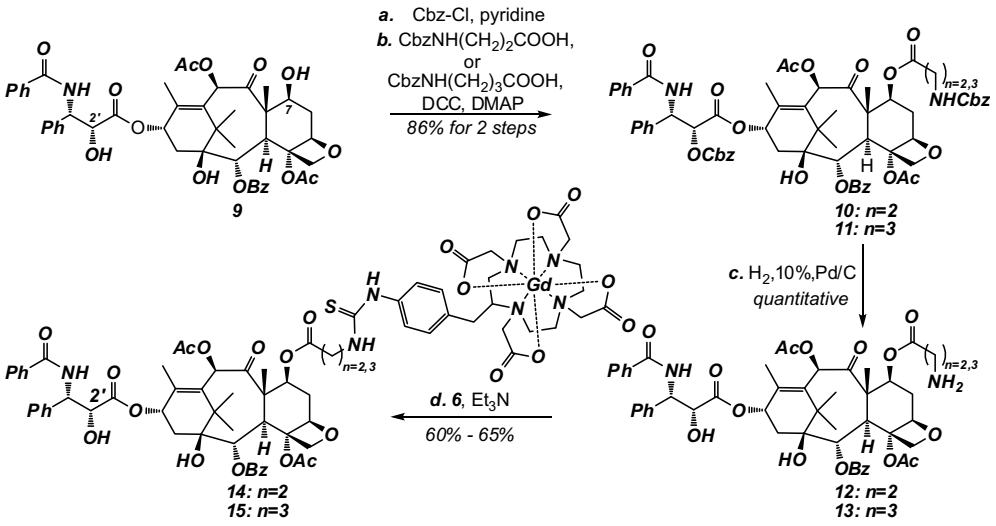
Similarly, taking advantage of the differential reactivity of the hydroxyls at 2′- and 7-positions, paclitaxel was selectively protected in the form of a Cbz-carbonate (Scheme 2).[12a] Introduction of an attachment linker was performed by DCC mediated coupling of protected γ- or δ-aminoacids to furnish, upon subsequent simultaneous removal of the Cbz protecting groups by catalytic hydrogenation, **12** or **13** in 95% yield. Attachment of the complexed isothiocyanate **6** was performed as before, yielding conjugates **14** and **15** in 60% and 65% yield, respectively.



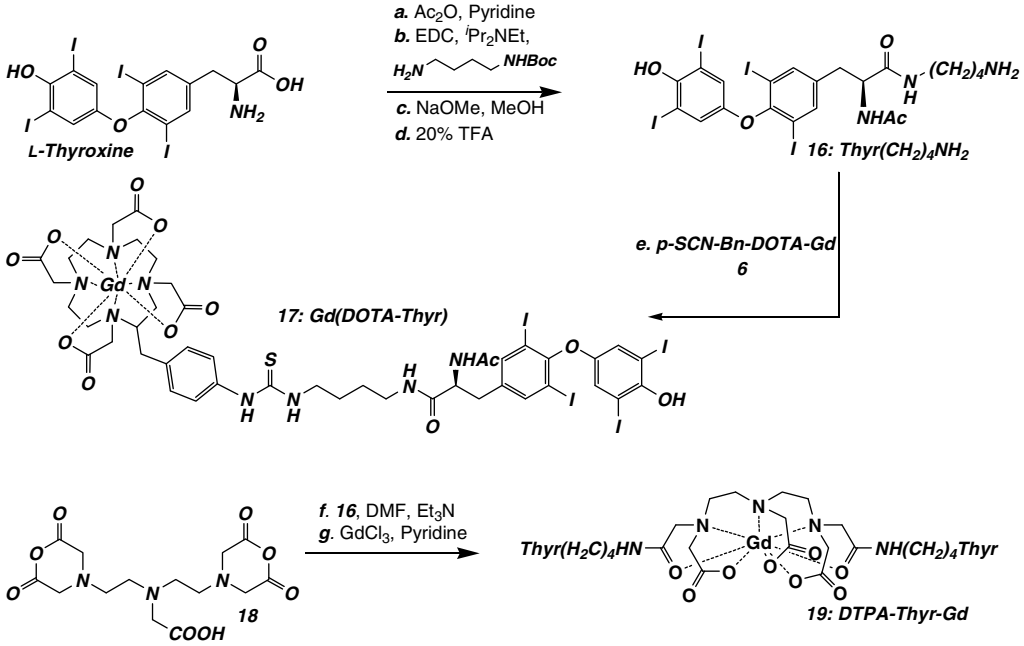
Scheme 1. Synthesis of colchicine derivatives **7** and **8**. Reagents and conditions: (a) $(Boc)_2O$ (5.5 equiv), DMAP (1.1 equiv), Et_3N (11.0 equiv), 12 h, 0 °C, 60%; (b) NaOMe (3.5 equiv), MeOH (0.20 M), 30 min, 0 °C, 75%; (c) 20% TFA, CH_2Cl_2, pentamethylbenzene (4.0 equiv), 8 h, 40 °C, 90%; (d) **3** (2.1 equiv), DIC (1.5 equiv), DMAP (0.01 equiv), 3 h, 0 → 25 °C, 86%; (e) 20% TFA, CH_2Cl_2, pentamethylbenzene (4.0 equiv), 8 h, 40 °C, 90%; (f) $GdCl_3$ (1.0 equiv), phosphate buffer, pH 6.5, 30 min, 75%; (g) **6**, Et_3N (1.5 equiv), DMSO (0.05 M), 24 h, 25 °C, 60% for **7** and **8**. Boc = *t*-butoxycarbonyl; DIC = *N*,*N*′-diisopropylcarbodiimide; DMAP = 4-(dimethylamino)pyridine; TFA = trifluoroacetic acid; DMSO = dimethyl sulfoxide.

For the synthesis of thyroxine conjugates, peracetylation of the natural product was required to allow the selective coupling of the carboxyl-moiety with an appropriately protected diamine-linker. Subsequent saponification of the acetate ester followed by removal of the Boc protective group, furnished amine **16** in 75% overall yield. Attachment of the Gd(III) complex **6** was performed as before, yielding conjugate **17** in quantitative yield. Reaction of **16** with commercially available dianhydride **18** produced, after complexation with $GdCl_3$ in pyridine, dimeric compound **19** in 65% overall yield (Scheme 3).

The effect of compounds **7**, **8**, **14**, **15**, **17**, and **19** on the viability of two human cancer cell lines, HeLa and MCF-7 was assessed, using the MTT assay.[16] Complex **6** was also tested in various concentrations (in the μM range). The IC_{50} values were estimated from the % viability versus concentration plots, using sigmoidal fit and are presented in Table 1. In general, no significant cytotoxicity



Scheme 2. Reagents and conditions: (a) Cbz-Cl (30.0 equiv), CH_2Cl_2 (0.02 M), pyridine (100 equiv), 25 °C, 24 h, 90%; (b) CbzNH-CH_2CH_2COOH or CbzNH-$CH_2CH_2CH_2$COOH (4.4 equiv), DCC (4.4 equiv), DMAP (1.0 equiv), 23 °C, 18 h, 95% for **10** and 95% for **11**; (c) H_2, 10% Pd/C (5% wt), MeOH (0.015 M), 23 °C, 2 h, quantitative for **12** and **13**; (d) *p*-SCN-Bn-DOTA-Gd, **6** (1.08 equiv), Et_3N (1.5 equiv), DMSO (0.05 M), 23 °C, 24 h, 60% for **14** and 65% **15**. CbzCl = benzyl chloroformate; DCC = *N*, *N*′- dicyclohexylcarbodiimide.



Scheme 3. Synthesis of thyroxine derivatives **17** and **19**. Reagents and conditions: (a) Ac_2O (2.5 equiv), pyridine (0.3 M), 0 → 23 °C, 12 h; (b) EDC (1.1 equiv), $^iPr_2NEt$ (1.2 equiv), CH_2Cl_2/DMF (1/1, 0.04 M), 0 → 23 °C, 12 h; (c). NaOMe (0.1 equiv), MeOH (0.05 M), 0 → 23 °C, 12 h, 75% over three steps; (d) 20% TFA in CH_2Cl_2, 30 min, 0 → 23 °C, 100%; (e) *p*-SCN-Bn-DOTA-Gd, **6** (1.08 equiv), Et_3N (1.5 equiv), DMSO (0.02 M), 23 °C, 24 h, 100%; (f) **16** (2.0 equiv), DMF (0.05 M), Et_3N (6.0 equiv), 23 °C, 24 h, 96%; (g) $GdCl_3{\cdot}6H_2O$ (1.1 equiv, 1.0 M in H_2O), pyridine (0.04 M), 70 °C, 4 h, 68%. Ac = acetyl; EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide; THF = tetrahydrofuran; DMF = dimethyl formamide.

was observed for almost all the compounds tested (micromolar range). This is highly encouraging since we are aiming the development of relatively non-toxic bioconjugates with CA properties to ensure the systemic safety of the imaging process. From all compounds tested, **15** exerted relatively enhanced toxicity against both cell lines. Also compound **7** had a cytotoxic effect against MCF-7 cells with an IC_{50} value of 90 μM. Interestingly, compound **17** decreased the viability of human breast cancer line, MCF-7 (IC_{50} = 114 μM), a fact that may be related to the hormone-dependent nature of the cells.[17] All other compounds tested did not have any significant effect on the viability of either HeLa or MCF-7 cells.

The relaxivities of the new CAs prepared were measured based on the equation $(1/T_{1s}) = (1/T_{1c}) + r_1[C]$, where T_{1s} and T_{1c} are the relaxation times with and without CA, respectively, *C* is the concentration of CA in mM and r_1 is the relaxivity expressed in $mM^{-1}\ s^{-1}$. Gadolinium complex solutions of at least three different concentrations, ranging from 0.5 to 4.0 mM, were employed. Methanol or DMSO was added as co-solvent (50%) in order to facilitate solubilization of the complexes. The results are presented in Table 1. Compounds **8**, **14**, and **15** exhibit comparable relaxivities in the expected range (r = 3.5–5).[1,2,6c] The higher values observed for **7**, **17**, and **19** may be due to the hydrophobic nature of colchicine and thyroxine molecules, rendering the corresponding complexes amphiphilic enough to aggregate, resulting in an increase in the rotational correlation time, and consequently an increase in relaxivity.[5a,5b]

Table 1
Cytotoxic effects and relaxivities of new contrast agents

Compound	HeLa IC_{50}[a] (μM)	MCF-7 IC_{50}[a] (μM)	Relaxivity[b,c] (mM^{-1} s^{-1})
6	>400	>400	N/A
7	>400	90	13
8	>400	>400	3.8
14	>400	110	3.7
15	37	117	4.7
17	>400	114	13
19	>400	>400	8.0

[a] All compounds were added as 1:1 DMSO/H_2O or EtOH/H_2O solutions and were incubated for 24 h.
[b] pH = 7.0; r^2 = 0.99–0.96.
[c] Measurements were performed on a Bruker MSL-100 spectrometer, equipped with a superconducting magnet operating at 2.35 Tesla and yielding a ^1H NMR resonance frequency of 100.17 MHz. All measurements have been made at room temperature (about 25 °C).

In conclusion, several novel gadolinium chelates conjugated with paclitaxel, colchicine and thyroxine have been prepared. They exhibit useful relaxivities which are expected to improve in the presence of their biological targets (tubulin or TBG). The cytotoxicities observed for some of the compounds could indicate their ability to cross the cell membrane under the conditions tested. Further studies to validate their performance in the presence of their target proteins and their cellular uptake are underway.

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Supplementary data

Detailed synthetic procedures and spectroscopic data for final products and selected intermediates, cell proliferation assay and relaxometric measurements. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.10.027.

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