Bioorganic & Medicinal Chemistry Letters 21 (2011) 3346–3348

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and characterisation of a novel tubulin-directed DO3A–colchicine conjugate with potential theranostic features

Nicholas. J. Wardle [a], Tammy Kalber [b], Jimmy D. Bell [b], S. W. Annie Bligh [a,*]

[a] Institute for Health and Research Policy, London Metropolitan University, Holloway Road, London N7 8DB, UK
[b] Metabolic and Molecular Imaging Group, Imaging Sciences Department, MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital, London W12 0NN, UK

ARTICLE INFO

Article history:
Received 25 February 2011
Revised 26 March 2011
Accepted 3 April 2011
Available online 8 April 2011

Keywords:
Magnetic resonance imaging
Contrast agent
Gadolinium
Colchicine
Tubulin

ABSTRACT

Colchicine is a known tubulin binding agent enabling necrosis in tumors. A novel tubulin-directed DO3A–colchicine conjugate and its Gd(III) complex were prepared from *N*-deacetylcolchicine, coupling alkaloid and polyaza-alicyclic functions via a peptide coupling methodology. The longitudinal proton relaxivity of the Gd(III) complex in water at 4.7 T is 2.86 mM^{-1} s^{-1} and a similar efficacy as colchicine towards ovarian carcinoma cells in vitro.

There is increasing interest in the design of theranostics, compounds with both diagnostic capability and therapeutic entity, especially in cancer areas.[1–3] Theranostics are more commonly used in the design of imaging agents in nuclear medicine to monitor the progress of therapeutic intervention. However, the use of theranostic strategies in drug discovery and innovative medicine development has prompted researchers in the magnetic resonance imaging (MRI) field to couple MR contrast agents to molecular-targeted therapeutic drugs for clinical use. Approaches to the design of MRI theranostics have to date focused on attaching the therapeutic to iron oxide nanoparticles,[4] or biopolymers.[5] In such approaches, the drug delivery mechanism of the therapeutic is changed since it is no longer a drug-like molecule. The ideal theranostic should elicit very little change in the efficacy of the drug, precipitate little or no immune response and have an enhanced therapeutic response. In this study we have chosen a potent tubulin binding agent, colchicine, and conjugated it to a simple Gd(III)–DO3A (DO3A: 1,4,7,10-tetraazacyclododecane-N′,N′,N″′-triacetic acid) complex to demonstrate the feasibility of producing a drug-like MRI theranostic.

Colchicine and its analogues are of interest as putative vectors for radiological cancer therapeutics, and conjugation to a MRI contrast agent offers the facility to monitor their physiological distribution and effectiveness as vascular disrupting agents. Efforts have been made to achieve such conjugate structures, exemplified in investigations of a ^{90}Y-labelled DOTA conjugate with trimethylcolchicinic acid and a ^{99m}Tc-labelled ethylenedicysteine conjugate with colchicine itself in nuclear medicine.[6,7]

In recent years the mechanism by which colchicine derives its potent antimitotic properties through disruption of microtubule assembly has been extensively studied.[8–10] Structure–activity relationship studies and thermodynamics studies conducted on the requisite colchicine–tubulin binding interactions have highlighted the negligible latitude for modification of colchicine A-[11] and C-rings, respectively, tolerated for high-affinity tubulin binding.[12,13] This has severely limited the site and manner of colchicine-derivatisation available to generate an effective derivative. However, appropriate substitution of the B-ring N-site (Fig. 1) is not necessarily deleterious in this respect,[14] and has been employed to modify physicochemical and cytotoxicity profiles.[15]

Meanwhile, a conjugate derived from reaction between *p*-SCN-Bn-DOTA–Gd(III) complex and a *N*-(2-aminoethyl)carbamoyl-derivative of colchicine has been investigated in the context of MRI.[16] This latter conjugate introduces a 4-atom linker between alkaloid and thiourea-functionalised DOTA–Gd(III) (**A**, Fig 2) structure with the explicit aim of avoiding steric interactions in binding with the desired physiological target. However, it seems reasonable that such attenuation introduces conformational mobility, which can compromise the diagnostic response in terms of relaxivity at low magnetic fields.[17] However complex **A** did not show significant cytotoxicity on HeLa and MCF-7 cell lines despite keeping the A- and C- rings intact, and gave a longitudinal relaxivities r_1 3.8 mM^{-1} s^{-1} at 2.37 T.

* Corresponding author.
E-mail address: a.bligh@londonmet.ac.uk (S.W.A. Bligh).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.014

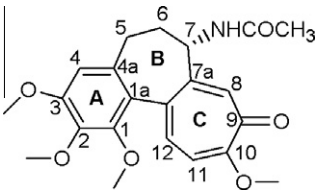


Figure 1. Structure of colchicine.

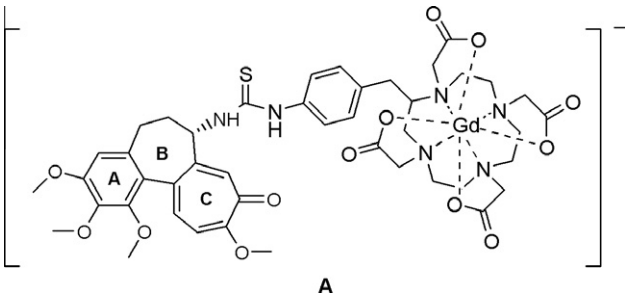


Figure 2. Structure of **A**.

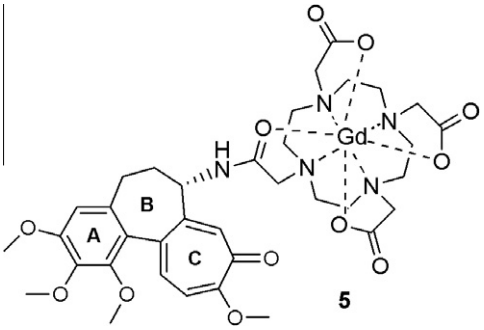


Figure 3. Structure of **5**.

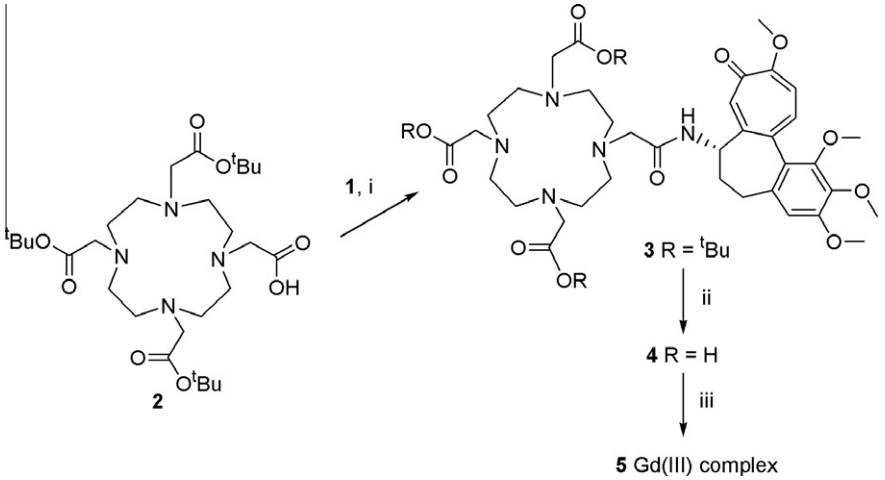
In this work we aim to produce a molecule with both diagnostic and therapeutic capabilities. We kept the molecular weight of the molecule as low as possible, and produced a neutral complex for improved uptake and reduced immuno response. Hence we appended the colchicine to one of the pendant arms in the cyclen to produce a DO3A derivative. We report here the synthesis of a neutral gadolinium(III) complex of 1-(benzo[a]heptalen-9[5H]-one-6,7-dihydro-1,2,3,10-tetramethoxy-7-carbamoyl)methyl-4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecane, (**5**, Fig. 3), characterisation of ligand and complex by NMR and microanalysis, relaxivity (r_1) of the complex, and in vitro cell studies in OVCAR-3 cells.

At the outset, generation of the Gd(III)–DO3A–colchicine-conjugate (**5**) was envisaged via derivatisation of the B-ring amine function of *N*-deacetylcolchicine (**1**, Scheme 1),[16] necessitated by the minimum modification of colchicine A- and C-rings for effective tubulin-binding. A standard peptide-coupling methodology would be employed for this purpose, presenting the polyaza-alicyclic system as the known protected DOTA-based prochelator; 1-carboxymethyl-4,7,10-tris(carbo[1,1-dimethyl]ethoxymethyl)-1,4,7,10-tetraazacyclododecane (DOTA–tris-*t*-Bu ester; **2**).[18]

The method of choice to generate the fully-protected conjugate **3** employed a benzotriazol-1-yloxytris(dimethyl-amino)phosphonium hexafluorophosphate (BOP)-mediated coupling methodology in dichloromethane;[19] a method selected to allow generation of the active ester intermediate from deprotonated carboxylate, an important consideration in the presence of four cyclen-ring N-sites. ^{1}H and ^{13}C NMR, and electrospray ionisation mass spectrometry (ESI-MS) indicated successful coupling to generate **3** (41%), (TLC; eluent—CH_2Cl_2/MeOH 7:1 v/v, R_f = 0.66).

Mild acidolysis with TFA then revealed the requisite DO3A–colchicine conjugate proligand **4** without demethylation of the C-10 tropolone methoxy function (a potential outcome in acidic environments),[20] as indicated by retention of the associated resonances in the ^{1}H NMR spectrum (δ_H = 3.51, 3.58 ppm; 2× s, 3H). Complexation of **4** was carried out at 80 °C in an aqueous suspension of $Gd_2(CO_3)_3$, the reaction progress indicated by gradual disappearance of the suspension. Elemental analysis of **5** was consistent with the anticipated 1:1 Gd(III):ligand stoichiometry confirming the retention of C-10 tropolone methoxy functionality under the conditions employed.

The longitudinal proton relaxivity (r_1) of the neutral [Gd(DO3A–colchicine H_2O)] (**5**) in water was 2.86 mM^{-1} s^{-1}, lower than that of the nonconjugated anionic complex $[Gd(DOTA)(H_2O)]^-$ 3.66 mM^{-1} s^{-1} and the neutral complex $[Gd(DO3A)(H_2O)_2]$ 4.82 mM^{-1} s^{-1}. However comparing the r_1 of **5** with a conjugated complex such as the 'SMART' contrast agent [Gd(GAL-DO3A)] 1.52 mM^{-1} s^{-1}, it is almost double the value of the latter



Scheme 1. Synthesis of a DO3A–colchicine derivative **4** and its neutral Gd(III) complex **5**. Reagents and conditions: (i) **1** (deacetylcolchicine), BOP (benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate), *N*-methylmorpholine, dichloromethane, rt, 24 h; (ii) TFA (trifluoroacetic acid), rt, 4 h; (iii) $Gd_2(CO_3)_3$, H_2O, 80 °C, 20 h.

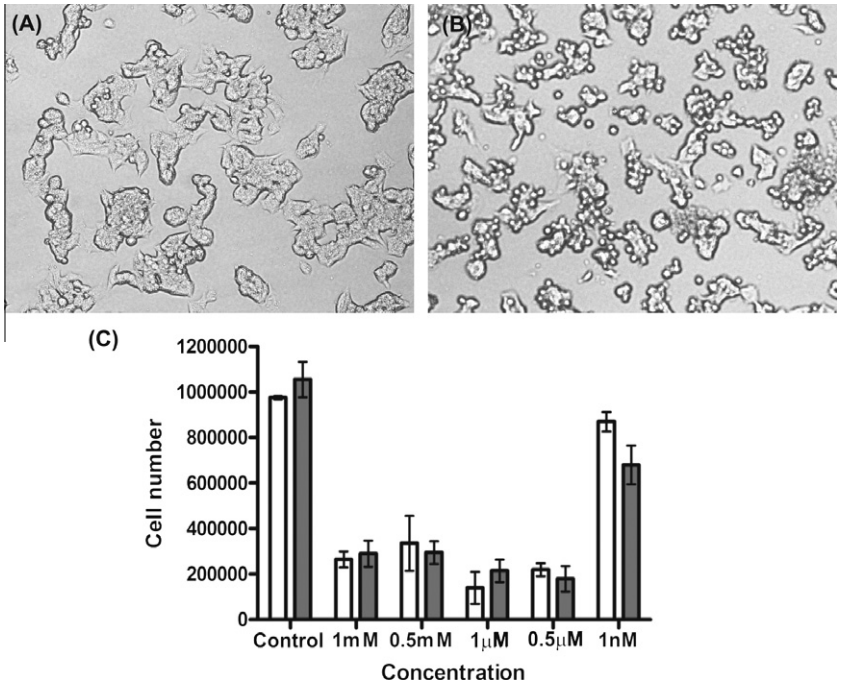


Figure 4. In vitro cell studies of the ligand **4** in OVCAR-3 cells. A histology picture of OVCAR-3 cells, (A) without and (B) with 1 nM of **4** after incubation for 24 h; (C) cell counts for both colchicine (white) and the Gd(III) complex **5** after 24 h at various concentrations (black).

complex.[21] The slight reduction in r_1 of **5** indicates that functionalization of one arm of the DOTA hardly reduced the number of water molecules (q) that were in fast exchange and that the amide carbonyl was coordinated to the Gd(III) ion. The value of q for the complex [Tb-(Gal-DO3A)] was reported to be 0.7 by fluorescence study, and the enzymatically cleaved terbium complex [Tb-(R-DO3A)] was 1.2.[22] Hence it is estimated the value of q to be about 1 as in the $[Gd(DOTA)(H_2O)]^-$ complex.

The efficiency of the ligand **4** for binding tubulin was assessed in an ovarian carcinoma cell line, selected for its rapidly dividing nature and sensitivity to colchicine.[23] In fact the efficiency of **4** at causing cell shape changes and cell death when compared to colchicine, produced very similar results. Cell counts showed that both the Gd(III) complex **5** and colchicine were equally effective at (significantly) reducing cell numbers (Fig. 4). In fact, there were enough cells only at 1 nM which could still be harvested and pelleted for MRI scanning to assess the uptake of **5**. However, at this concentration of **5**, changes in r_1 were too small to be detected.

A novel DO3A–colchicine tubulin-directed ligand and its Gd(III) complex were thereby prepared, the former exhibiting an efficiency similar to that of colchicine as an anti-cancer agent. The r_1 of its Gd(III) complex is similar to that of the nonderivatised DOTA complex. The synthetic strategy in producing a cyclen-based ligand retaining its complexation property, that is, four nitrogen and four oxygen donor atoms, and a drug-like molecule was achieved.

Supplementary data

Supplementary data (detailed synthetic procedures and spectroscopic data of the products **3**–**5**, toxicity studies and relaxation measurements are deposited) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.014.

References and notes

1. Cai, W. B.; Niu, G.; Chen, X. Y. *Curr. Pharm. Des.* **2008**, *14*, 2943.
2. Guthi, J. S.; Yang, S. G.; Huang, G.; Li, S. Z.; Khemtong, C.; Kessinger, C. W.; Peyton, M.; Minna, J. D.; Brown, K. C.; Gao, J. M. *Mol. Pharm.* **2010**, *7*, 32.
3. Jain, T. K.; Foy, S. P.; Erokwu, B.; Dimitrijevic, S.; Flask, C. A.; Labhasetwar, V. *Biomaterials* **2009**, *30*, 6748.
4. Chen, W.; Xu, N. F.; Xu, L. G.; Wang, L. B.; Li, Z. K.; Ma, W.; Zhu, Y. Y.; Xu, C. L.; Kotov, N. A. *Macromol. Rapid Commun.* **2010**, *31*, 228.
5. Franchini, M. C.; Baldi, G.; Bonacchi, D.; Gentili, D.; Giudetti, G.; Lascialfari, A.; Corti, M.; Marmorato, P.; Ponti, J.; Micotti, E.; Guerrini, U.; Sironi, L.; Gelosa, P.; Ravagli, C.; Ricci, A. *Small* **2010**, *6*, 366.
6. Satpati, D.; Korde, A.; Pandey, U.; Dhami, P.; Banerjee, S.; Venkatesh, M. *J. Labelled Compd. Radiopharm.* **2006**, *49*, 951.
7. Zareneyrizi, F.; Yang, D. J.; Oh, C. S.; Ilgan, S.; Yu, D. F.; Tansey, W.; Liu, C. W.; Kim, E. E.; Podoloff, D. A. *Anticancer Drugs* **1999**, *10*, 685.
8. Hastie, S. B. *Pharmacol. Ther.* **1991**, *51*, 377.
9. Brossi, A.; Yeh, H. J. C.; Chrzanowska, M.; Wolff, J.; Hamel, E.; Lin, C. M.; Quin, F.; Suffness, M.; Silverton, J. *Med. Res. Rev.* **1988**, *8*, 77.
10. Ravelli, R. B. G.; Gigant, B.; Curmi, P. A.; Jourdain, I.; Lachkar, S.; Sobel, A.; Knossow, M. *Nature* **2004**, *428*, 198.
11. Andreu, J. M.; Perez-Ramirez, B.; Gorbunoff, M. J.; Ayala, D.; Timasheff, S. N. *Biochemistry* **1998**, *37*, 8356.
12. Mareel, M. M.; Demets, M. *Int Rev Cytol: Surv. Cell Biol.* **1984**, *90*, 125.
13. Muzaffar, A.; Brossi, A.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1990**, *33*, 567.
14. Ray, K.; Bhattacharyya, B.; Biswas, B. B. *J. Biol. Chem.* **1981**, *256*, 6241.
15. Bombuwala, K.; Kinstle, T.; Popik, V.; O Uppal, S.; Olesen, J. B.; Vina, J.; Heckman, C. A. *Beilstein J. Org. Chem.* **2006**, *2*, ARTN 13.
16. Efthimiadou, E. K.; Katsarou, M. E.; Fardis, M.; Zikos, C.; Pitsinos, E. N.; Kazantzis, A.; Leondiadis, L.; Sagnou, M.; Vourloumis, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6058.
17. Caravan, P.; Farrar, C. T.; Frullano, L.; Uppal, R. *Contrast Media Mol. Imaging* **2009**, *4*, 89.
18. Heppeler, A.; Froidevaux, S.; Macke, H. R.; Jermann, E.; Behe, M.; Powell, P.; Hennig, M. *Chem. Eur. J.* **1999**, *5*, 1974.
19. Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.
20. Kocienski, P. J. *Protecting Groups*; Stuttgart: Georg Thieme Verlag, 2005.
21. Wardle, N. J.; Herlihy, A. H.; So, P. W.; Bell, J. D.; Bligh, S. W. A. *Bioorg. Med. Chem.* **2007**, *15*, 4714.
22. Moats, R. A.; Fraser, S. E.; Meade, T. J. *Angew. Chem.* **1997**, *36*, 726.
23. Tozer, G. M.; Kanthou, C.; Baguley, B. C. *Nat. Rev. Cancer* **2005**, *5*, 423.