Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 2590

www.rsc.org/obc

PAPER

Synthesis and biological profiling of tellimagrandin I and analogues reveals that the medium ring can significantly modulate biological activity†

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Received 10th January 2012, Accepted 30th January 2012
DOI: 10.1039/c2ob25065a

A novel synthesis of the ellagitannin natural product tellimagrandin I and a series of medium ring analogues is described. These compounds were all subsequently screened for redox activity, ability to precipitate protein and cellular phenotype in HeLa cells. From this we have shown that all properties can be modulated independently by varying ring size and by moving the ester out of conjugation with the biaryl ring system. Increasing ring size increased redox activity and cytotoxicity, leading to the identification of a compound (**10**) which was significantly more cytotoxic. In addition compounds identified with a redox active scaffold and low cytotoxicity may be employed as a new class of redox probes.

Introduction

Tellimagrandin I belongs to the family of ellagitannin natural products, which are bioactive plant polyphenols found widely in fruits, nuts and vegetables.[1] Tellimagrandin I is composed of a hexahydroxydiphenoyl (HHDP) unit and a D-glucopyranosyl core to which two galloyl units are appended (Fig. 1). This class of polyphenolic compounds has been employed in a plethora of diverse functional roles, providing resistance against bacterial[2] and viral infections,[3] affording anti-herbivore activity[4,5] and protecting against DNA damaging solar radiation.[6] Many herbal remedies used in traditional oriental medicine contain plant extracts, whose active ingredients have been identified as polyphenolic compounds. The therapeutic value of this compound class has received increasing attention from the academic community, particularly over the last decade. The medicinal worth of this compound class has been attributed largely to its antioxidant properties; they act as scavengers of reactive oxygen species (ROS) such as hydroxyl radicals, singlet oxygen and free radical species.[7] However, in addition to their antioxidant properties, plant polyphenols can also act as prooxidants. In the presence of metal ions, such as iron(III) or copper(II), their prooxidant activity leads to the generation of ROS which can cause DNA lesions and produce highly electrophilic *ortho*-quinone species which are subject to nucleophilic attack from amino acids and biomolecules.[8] There is increasing evidence to suggest that ellagitannins in particular may be beneficial in cancer prevention and treatment; this may be attributed to their prooxidant activity, promoted by higher levels of oxidative stress in cancer cells.[9] Ellagitannins are the most redox active of all tannins[5,10] and it is this activity and their ability to precipitate proteins which are thought to contribute to their broad bioactivity profile.[1c,6] In a comparative study on the oxidative activity of hydrolysable tannins, Moilanen and Salminen discovered that the presence of biaryl systems, such as the HHDP or NHTP unit, increases the oxidative activity of the compound class, whilst the sugar and galloyl sub-units do not make a significant contribution.[11]

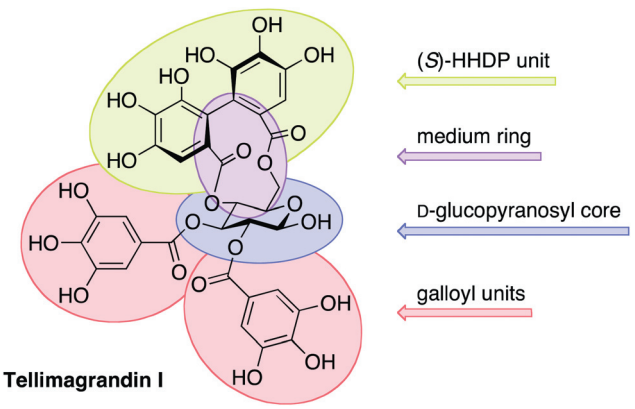

Fig. 1 Tellimagrandin I and its constituent components.

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†Electronic supplementary information (ESI) available: Details of the preparation of the compounds discussed and the methods of biological evaluation. See DOI: 10.1039/c2ob25065a

Protein precipitation by tannins has been assessed using several assays,[12] all of which have demonstrated that increasing the number of galloyl units increases the protein precipitation capability. Despite significant efforts in the field, no unified study relating redox activity, ability to precipitate protein and biological phenotype exists.

The aim of this work was to explore the redox activity and protein precipitation capability of ellagitannins and to determine the effect on cellular phenotype, as well as establish structure activity relationships (SARs) for different ellagitannin components. We chose to focus particularly on the medium ring, as we predicted that varying the size and electronic properties of this unit would have a significant effect on biological properties. Tellimagrandin I was initially chosen as a model system of ellagitannin bioactivity and its precursors in the synthetic route could be used to expand the SAR (Fig. 1).

Synthesis of tellimagrandin I and analogues

The first total synthesis of tellimagrandin I was published by Feldman *et al.* in 1994,[13] and subsequently several total syntheses of other ellagitannins[13,14] or their permethylated derivatives[15] have been reported. The key chiral biaryl unit can be preformed before conducting a diesterification reaction on substituted glucose,[16] or constructed in a late step by diastereoselective oxidative coupling.[17] The biaryl coupling has often been accomplished by employing costly and toxic metal catalysts. Our group has recently reported the total synthesis of the ellagitannin natural product sanguiin H-5[14e] using a copper-mediated oxidation of an organozinc halide to form the key biaryl bond.[14e] The coupling methodology utilized a halogen–metal exchange followed by copper salt mediated transmetalation and finally organocuprate oxidation with concomitant biaryl bond formation. We envisaged that this strategy could be applied to the total synthesis of tellimagrandin I (Scheme 1). Our route began from the glucose derivative **1**, which was synthesized in three steps from D-glucose, according to literature procedures,[13] and benzyl protected gallic acid (Scheme 1).[14e] The diol **1** was esterified, followed by hydrolysis of the benzylidene acetal and further esterification to furnish the cyclisation precursor **2** in 59% yield over 3 steps. Bromine–zinc exchange with Rieke® zinc gave the requisite organozinc intermediate for copper-catalysed oxidative biaryl coupling. The reaction proceeded with complete atropdiastereoselectivity and in good isolated yield (68%, α : β anomer ratio 2.2 : 1).[18] To complete the total synthesis, deprotection by hydrogenolysis followed by filtration through Celite® furnished tellimagrandin I quantitatively.[19] The spectroscopic data obtained matched that reported previously.[13]

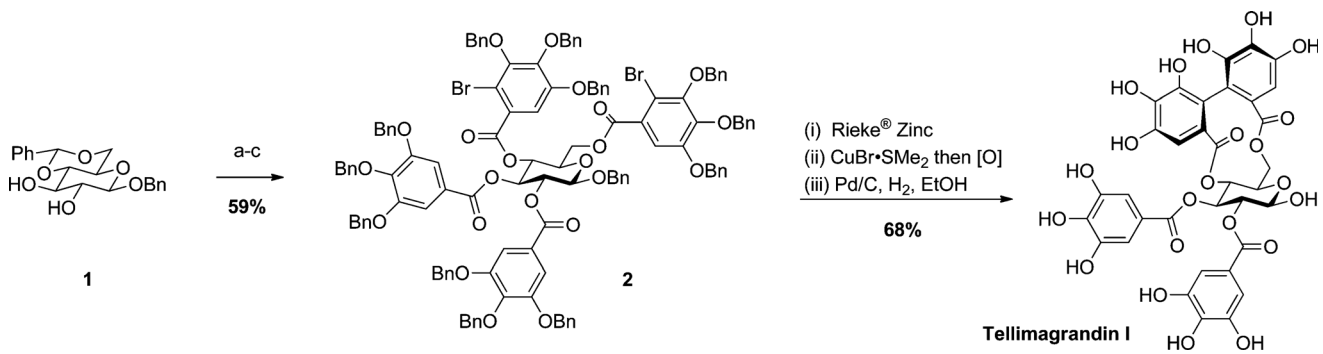
Following the completion of the total synthesis, we began SAR studies with the aim of: (1) investigating which fragments of tellimagrandin I play a key role in its biological activity, (2) identifying whether the medium ring significantly affected this activity by synthesising analogues containing one HHDP unit and linkers of different lengths, and (3) observing whether the cellular activity of these compounds could be attributed to their redox activity and/or ability to precipitate protein *in vitro*. The analogues synthesized included deprotected intermediates from the total synthesis of tellimagrandin I (Fig. 2, **3** and **4**),[20] as well as HHDP units with different linker lengths (**5**–**10**).[21] We were also interested in observing whether moving the ester out of conjugation with the aromatic ring would influence biological activity (**12** and **13**), and if opening the medium ring would have a significant effect (**3**, **4** and **11**).

The biological activity of tellimagrandin I and analogues was determined by high content analysis (HCA) using a Cellomics Arrayscan. HCA is an automated microscope-based approach that enables several parameters to be assessed simultaneously at the single cell level. HeLa cells were selected as an established cervical cancer cell line known to be well suited to HCA. Cells were stained with a DNA dye (Hoechst) to measure cell number to assess growth inhibition, and with an antibody against cleaved poly(ADP-ribose) polymerase (C-PARP) to determine induction of apoptosis (Fig. 3). Additionally, all compounds were assayed for their redox activity, by testing their ability to catalyse the reduction of resazurin to resorufin by dithiothreitol, using a modified protocol based on work by Schwartz and co-workers.[22] The ability of these compounds to precipitate protein was also assessed using bovine serum albumin (BSA) as a surrogate protein.[12c] The percentage of precipitated protein was measured by HPLC.

From this analysis several trends were observed (Table 1):

• Cytotoxicity increased with increasing ring size correlating generally with redox activity but not protein precipitation.

• Placing the ester out of conjugation with the aromatic rings, increased redox activity without increasing cytotoxicity, exemplifying the possibility of modulating this property with small structural changes.



Scheme 1 Synthesis of tellimagrandin I. (a) 3,4,5-Tribenzylgallic acid, DCC, DMAP, CH_2Cl_2, 83%; (b) I_2, MeOH, 100%; (c) 2-bromo-3,4,5-tribenzylgallic acid, DCC, DMAP, CH_2Cl_2, 71%. Bn = benzyl; DCC = *N*,*N*′-dicyclohexylcarbodiimide; DMAP = 4-dimethylaminopyridine; [O] = 1-(3,5-dinitrobenzoyl)-4-methylpiperazine.

• Analogues lacking the medium/large ring were less cytotoxic and redox active, but did not affect protein precipitation.

Tellimagrandin I was significantly more cytotoxic than its precursors **3** and **4**, which lacked the medium ring, and its IC_{50} was consistent with the value previously reported.[23] This suggests that the medium ring unit is important for the cytotoxicity of this natural product class. Opening the medium ring (**3**, **4** and **11**) reduced cytotoxicity, further supporting the hypothesis regarding the importance of this subunit. Modifications of ring size showed that cytotoxicity generally increased with ring size. Moving the ester out of conjugation with the biaryl moiety did not significantly affect its cytotoxic potential (**12** and **13**). One medium ring analogue (**10**) displayed significantly greater cytotoxicity than tellimagrandin I (TI). Considering the large (10-fold) increase in potency with the simple addition of one carbon in the macrocycle, we predict that this compound may be acting by an alternative mechanism to the others in the series. A computational target identification strategy was employed to predict possible targets for this molecule. A similarity ensemble approach (SEA)[24] identified arachidonate-12-lipoxygenase as a potential target. Inhibition of this protein causes cell death by apoptosis and it is known to be sensitive to redox active compounds.[25] The investigation of **10** as a potential inhibitor of this protein is ongoing.[26]

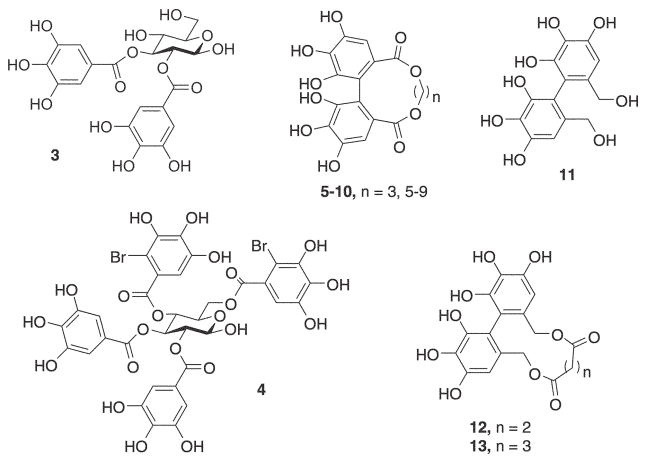


Fig. 2 Analogues used in biological evaluation.

An increase in apoptosis, as measured by the appearance of cleaved PARP, was observed when cells were treated with the most cytotoxic compounds (Table 1 and Fig. 3). This was confirmed by direct visualisation of the cells, which revealed that cleaved PARP positive cells also exhibited pyknotic and often fragmented nuclei (Fig. 3), which are characteristic of apoptosis. However, induction of apoptosis required higher compound concentrations than those required to cause cytotoxicity, suggesting that the two may occur through independent mechanisms.

Compounds that precipitated protein were generally not cytotoxic with the exception of tellimagrandin I. However, this did not preclude them from having potent redox activity (**12** and **13**). Both **12** and **13** contain ester functionalities that are out of conjugation with the aromatic ring, which increases their redox activity compared to the conjugated analogue (**6**), due to an increased ability to donate electrons. Given the very low cytotoxicity of these compounds we were pleased to see that redox activity could be significantly increased with a very small

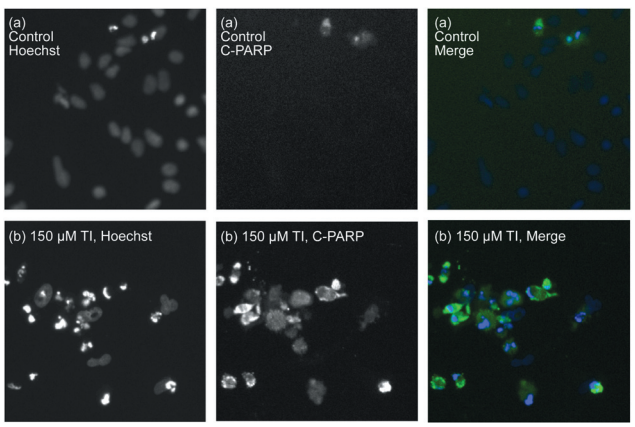


Fig. 3 Example of apoptotic cells induced by ellagitannins. (a) Control HeLa cells, (b) HeLa cells treated with 150 μM TI for 72 h show increased cell death and induction of apoptosis, blue = Hoechst, green = cleaved poly(ADP-ribose) polymerase (C-PARP). TI = tellimagrandin I.

Table 1 Biological profiling of tellimagrandin I (TI) and analogues[a]

Cmpd code	Ring size	Growth inhibition IC_{50} (μM)	Induction of C-PARP (apoptosis) EC_{50} (μM)	Redox activity EC_{50} (μM)	% Protein precipitation
TI	11	45 ± 6	109 ± 17	61 ± 3	56 ± 3
3	N/A[b]	>300	>300	>400	12 ± 2
4	N/A[b]	251 ± 26	>300	>400	51 ± 3
5	11	>300	>300	312 ± 6	5 ± 1
6	13	282 ± 75	>300	>400	N/D[c]
7	14	163 ± 5	>300	388 ± 18	10 ± 1
8	15	120 ± 8	>300	281 ± 12	N/D[c]
9	16	98 ± 4	275 ± 14	52 ± 3	N/D[c]
10	17	11 ± 2	180 ± 25	71 ± 3	N/D[c]
11	N/A[b]	>300	>300	69 ± 8	66 ± 4
12	12	>300	>300	35 ± 2	100 ± 5
13	13	185 ± 11	>300	11 ± 2	40 ± 2

[a] Cell growth and induction of apoptosis were assessed using a high content assay; redox activity was assessed by the compounds' ability to catalyse the reduction of resazurin to resofurin; and protein precipitation ability was assessed by the percentage BSA precipitated in the presence of excess compound. [b] N/A = not applicable, no medium/large ring present. [c] N/D = not determined, due to insufficient compound solubility. TI = tellimagrandin I.

structural and synthetic change. This novel compound class may find use as redox probes. For compounds **6–10** redox activity generally increased with increasing ring size, which mirrored the cytotoxicity data. From this we suggest that cytotoxicity may occur at least partially from redox activity in ellagitannins, but not necessarily in other compound classes where the HHDP is not fully conserved. Compounds **3** and **4**, which lack the medium/large ring, did not show any redox activity in accordance with literature predictions.[5,8a] Increasing the number of phenolic oxygens increased protein precipitation ability, consistent with literature data, but the presence of a medium/large ring did not significantly affect this. Increasing the size of the medium/large ring appeared to reduce the ability to precipitate protein, however solubility problems prevented further evaluation. Given that the concentration of compound required to obtain pronounced protein precipitation was high (150 times the BSA concentration), we predict that the effects observed are not physiologically relevant. This may also explain the lack of correlation with the cytotoxicity data. However, larger and polymeric ellagitannins may be more adept at efficiently forming a complex with, and precipitating, protein *in vivo*.

Conclusions

In conclusion, we have shown that tellimagrandin I can be synthesized in five linear steps with an overall yield of 38% using an atropdiastereoselective oxidative biaryl coupling as the key step. Tellimagrandin I and analogues were used to explore the SAR of ellagitannin components. We have adapted several rapid assays for benchmarking ellagitannin activity in two of its most commonly associated biological properties and shown how this translates into a cellular setting. Cytotoxicity increased with ring size and was lost with the removal of the medium ring. This data correlated with the redox activity but not with the ability to precipitate protein, suggesting the importance of the former in ellagitannin bioactivity. Additionally, a new analogue (**10**) was identified which was significantly more potent than tellimagrandin I but structurally and synthetically simpler. Our data suggests that the biological activity of ellagitannins can be optimised for the required properties and that complex scaffolds may not be required to exert this activity. We have uncovered a novel class of redox modulating agents (**12** and **13**) which are only weakly cytotoxic at high concentrations and which may find applications as redox probes. The synthetic methodology for ellagitannin synthesis and the screening cascade outlined are extremely valuable in profiling other members of the ellagitannin and related families of natural products, which will be reported in due course.

Acknowledgements

We would like to thank Cancer Research UK, EPSRC, BBSRC, MRC, Wellcome Trust, and Francis and Augustus Newman Foundation for funding.

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