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Synthesis of exotic polycycles such as cyclooctatrienes and fenestrenes with differential pro-apoptotic activities on human TRAIL-resistant metastatic cell lines

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ABSTRACT

New cyclooctatrienes were prepared by semihydrogenation of trienynes followed by 8π electrocyclization. Cyclooctatrienes and original fenestrenes previously reported were tested for their pro-apoptotic activities on two human cancer cell lines (THP-1 and SW620). Among the 20 new compounds tested, two compounds presented specific activities on the colon carcinomas TRAIL-resistant metastatic cell SW620, but a minor action on the monocytic leukemia THP-1 cell line. Six other compounds showed cell type specific activities: four induced apoptosis only in THP-1 cells and two only in SW620. Such differential pro-apoptotic activities suggest that these molecules could serve as potent pharmacological tools to study TRAIL associated cellular mechanisms.

Eight-membered rings are present in more than 100 natural products, particularly in terpenoids, such as steganone[1] and ophiobolin C.[2] For the past 30 years, the family of cyclooctanoids has attracted much attention due to their exceptional biological activity.[3–6] The discovery of the taxol,[7] an anticancer drug, highlighted the synthetic and biological interest of such complex polycycles. Some remarkable syntheses of cyclooctanoids were reported, in spite of the fact that unfavorable entropic and enthalpic factors render the synthesis of eight-membered rings challenging.[3–5] Thus, the introduction of novel methods that access new cyclooctanoids in an efficient way is important to exploit their promising therapeutic potential.

Our aim is to develop new syntheses of cyclooctanoids, and then to evaluate their biological activities against cancer. We reported recently[8] the synthesis of cyclooctatrienes **6a–g** (Scheme 1).

Our approach involved the formation of these structures by a cascade reaction, based on two consecutive transformations starting from the seven-membered ring trienynes **2a–g**: an initial mild hydrogenation using a P-2 Nickel catalyst at room temperature, giving the intermediate tetraene **4**, followed by a conrotatory 8π electrocyclization. When the semihydrogenation of the six-membered ring trienyne **1a** was carried out under thermal conditions, the desired cyclooctatriene **7a** was obtained.

Surprisingly, when the same reaction starting from six-membered ring trienynes **1a–d,g** was conducted at room temperature, the intermediate cyclooctatriene **9** was obtained with an opposite torquoselectivity. Then a disrotatory 6π electrocyclization and a final spontaneous oxidation with molecular oxygen led to [4.6.4.6]fenestrenes **10a–d,g**.[9] An explanation for this unusual reactivity has already been reported.[8] These highly strained molecules belong to the family of fenestranes, which are tetracyclic compounds containing a tetracoordinate carbon shared by four rings.[10–12] Found in natural products, laurenene[13,14] and penifulvins A–E,[15,16] these fascinating molecules have attracted much theoretical and synthetic interest since 1970. The synthesis of these novel fenestrenes **10a–d,g** from simple starting material with the minimum of steps thus represents a real challenge in the discovery of new potentially drugs.

In this Letter, we report the synthesis of new cyclooctatrienes **8a–f** starting from acyclic trienynes **3a–f**, and the biological activity of all cyclooctatrienes **6a–g**, **7a**, **8a–f** and [4.6.4.6]fenestrenes **10a–d,g** against cancer (Scheme 1). Cancer is a hyperproliferative disorder that in a first step involves cellular transformation, dysregulation of apoptosis, uncontrolled cellular proliferation and later invasion, angiogenesis, and metastasis.[17] Therefore, induction of apoptosis is one of the mechanisms of chemotherapeutic agents against cancer. Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) is an important member of the TNF superfamily.

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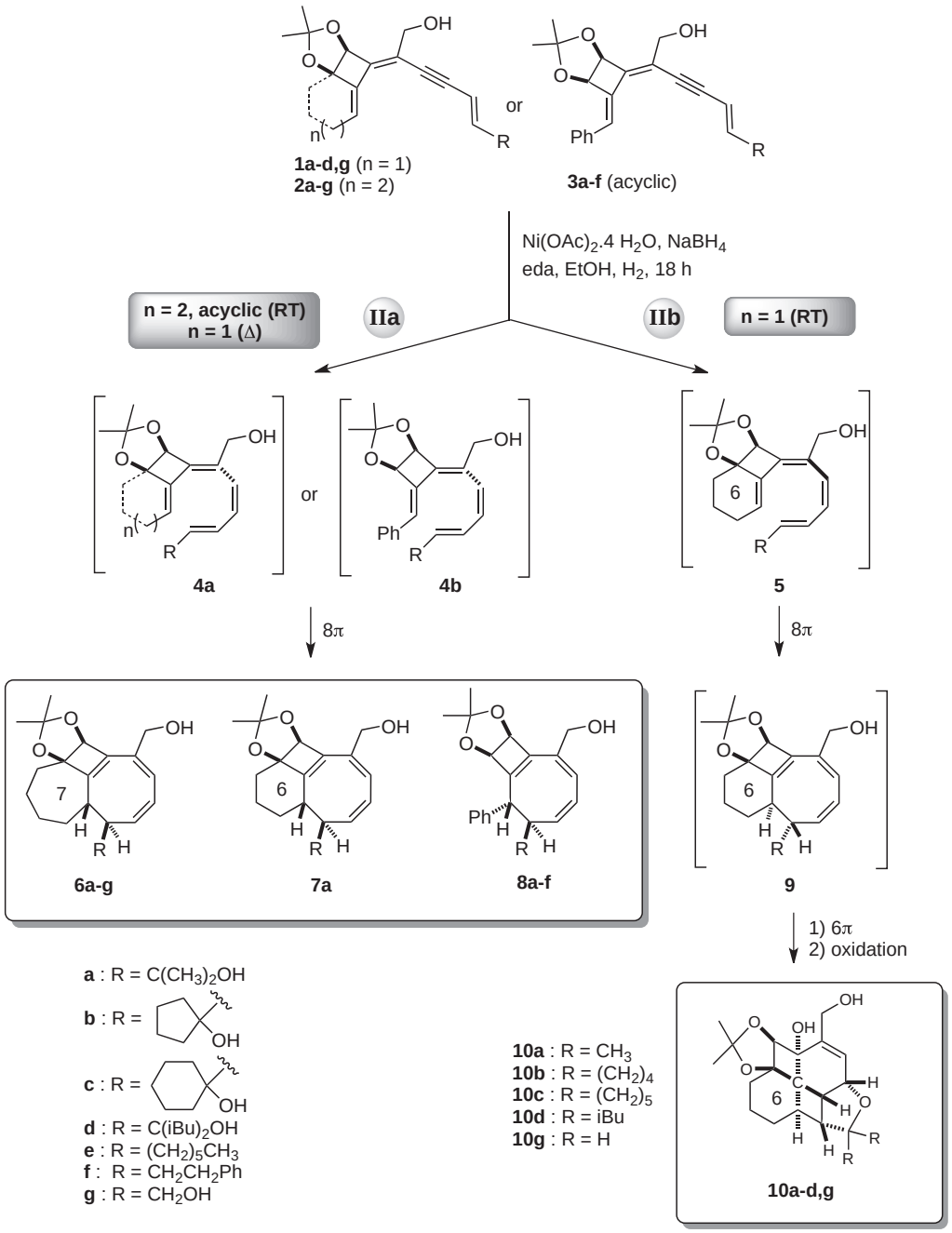
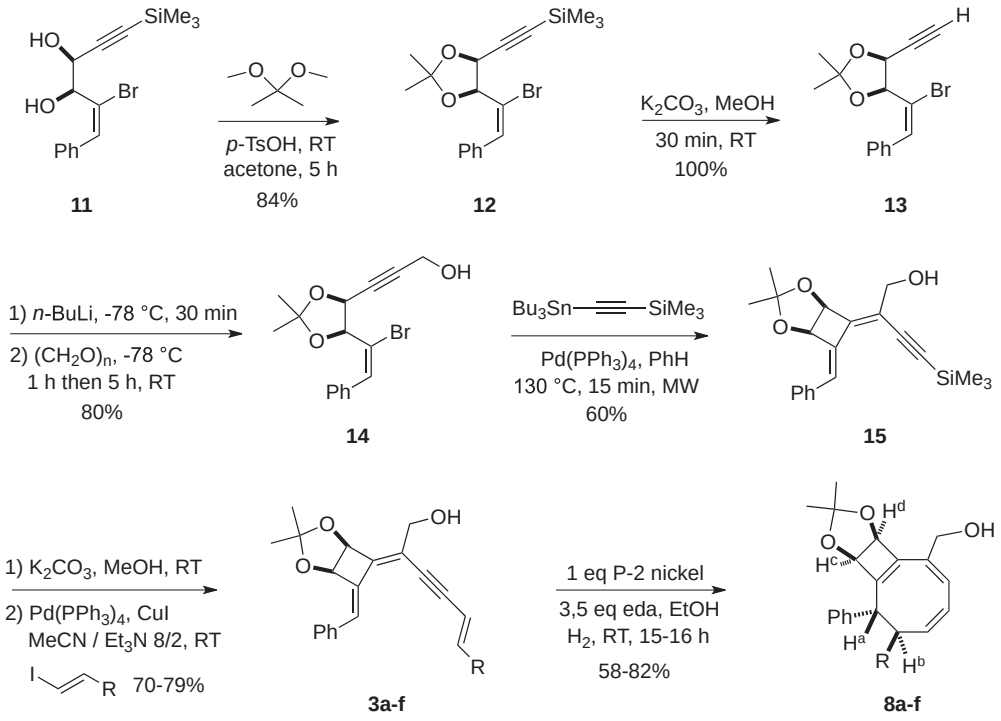


Scheme 1. Synthesis of cyclooctatrienes **6a–g**, **7a**, **8a–f** and [4.6.4.6]fenestrenes **10a–d,g** (eda = ethylenediamine).

TRAIL selectively induces the apoptosis of a wide variety of cancer and transformed cells without damaging most normal cells. However, the clinical application of TRAIL in cancer therapy is limited as many cancer cells have been identified as resistant to the cytotoxicity of TRAIL.[18] Although TRAIL has strong apoptosis activation properties, some cancer cells are resistant to TRAIL-mediated programmed death. Thus we choose to monitor the potential of the newly synthesized cyclooctatrienes and fenestrenes to induce apoptosis with two different human cancer cell lines: the THP-1 monocytic leukemia and the TRAIL-resistant colon carcinomas SW620.

The synthesis of cyclooctatrienes **6a–g** and **7a** and [4.6.4.6]fenestrenes **10a–d,g** from trienynes **1a–d,g** and **2a–g** was described in our previous papers.[8,9] Cyclooctatrienes **8a–f** were synthesized according to the same procedure (Scheme 2).

Starting *anti* diol **11** was prepared by addition of a suitably protected metallated propargylic alcohol to (2*E*)-2-bromo-3-phenylacrylaldehyde, followed by deprotection and chromatographic separation of the *anti* and *syn* diastereomers.[19] After the protection of the diol functionality of **11** with 2,2-methoxypropane and removal of the trimethylsilyl group, the free alkyne **13** was metallated with *n*-butyllithium in THF at −78 °C, followed by addition of paraformaldehyde. The 4-*exo*-dig cyclocarbopalladation/Stille cross coupling was then conducted on the propargylic alcohol **14** in the presence of [$Pd(PPh_3)_4$] (10 mol %) as catalyst under microwave irradiation in benzene at 130 °C. These conditions afforded the compound **15** in 60% yield after 15 min of irradiation. Desilylation followed by Sonogashira cross coupling with a number of substituted vinyl iodides, [$Pd(PPh_3)_4$] and CuI gave compounds **3a–f** in 70–79% yields in two steps. Semihydrogenation of these



Scheme 2. Syntheses of cyclooctatrienes **8a–f**.

trienynes **3a–f** with P-2 nickel catalyst[20] under one atmosphere of H_2 finally led to desired cyclooctatrienes **8a–f**.

Structure **8a** was unambiguously confirmed by single crystal X-ray diffraction analysis (Fig. 1). The relative configuration between the Ph group and the R group are *anti* in all new compounds **8a–f**, as indicated by the similar coupling constant between the two vicinal protons H^a and H^b (10.5–11.1 Hz), in accordance with the structure of **8a** determined by X-ray crystallographic analysis.[25] The relative stereochemistry of H^a and H^b (Scheme 2) was also confirmed by a series of 2D NMR experiments (COSY, NOESY, HMBC, HSQC) that clearly shown that H^a is on the same side than dioxolane protected diol and opposite to the two protons H^b and H^c.

When tested for their properties of inducing apoptosis in two human cancerous cell lines (cf. Table 1 and Fig. 2), several molecules showed high potentials as compared to Celastrol, an immunosuppressive triterpenoid well described today as a potent apoptosis inducer.[21–23] For example, **8d–f** were all effective in inducing apoptosis in either colon carcinoma[24] or monocytic leukemia cell lines. More interestingly, **6d** and **6e** were only efficient on the colon carcinomas TRAIL-resistant metastatic cell SW620 with less activity on the monocytes of THP-1 cell line. When checked for caspase 8 activity, the later molecules were capable of inducing more than 90 ± 5% of inhibition in SW620 cells ($n = 4$, data not shown) in a manner similar to Celastrol (90 ± 6%).

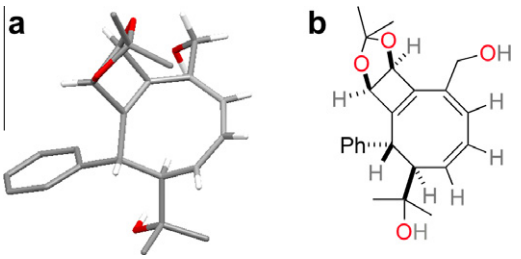


Figure 1. Crystal structure of compound **8a** (a) and stereochemistry (b) The majority of H is omitted for clarity.

Table 1
Pro apoptotic activities tested on two cell lines

% Apoptotic cells	SW620	sd ($n = 4$)	THP-1	sd ($n = 4$)
Cells	7	6	3	1
Cells + reagents	9	8	3	0
Cells + reagents + DMSO 0.5%	8	8	2	2
Celastrol	99	1	99	1
6a	16	3	2	2
6b	22	21	2	1
6c	17	8	2	1
6d	68	18	36	6
6e	70	33	20	11
6f	23	4	10	6
6g	11	3	2	1
7a	12	6	2	1
8a	15	14	2	1
8b	12	9	3	1
8c	10	6	3	2
8d	66	6	97	2
8e	70	13	97	3
8f	58	13	98	2
10a	4	1	29	10
10a′	5	2	6	2
10b	5	3	15	4
10c	5	2	5	2
10d	13	10	83	4
10g	4	3	94	4

Results are expressed as percent of apoptotic cells, that is, cells presenting AnnexinV-PE tagged phosphatidyl-serine on the cell surface, viability being assessed by 7-AAD exclusion, all compounds tested at 50 μM final concentration after a 24 h incubation period.

If TRAIL is involved, these preliminary results highlight the interest of **6d** and **6e**, circumpassing the TRAIL resistance, in being putative tools to decipher the TRAIL-resistance mechanism in this cell line. Considering that **6d,e** and **8d–f** present a common scaffold, the putative molecular target could be identical, **6d** and **6e** being able to 'reactivate' their molecular target. Another tool to decipher such mechanism is the molecular family of **10d** and **10g** with potent activity in inducing apoptosis, but only in the TRAIL-

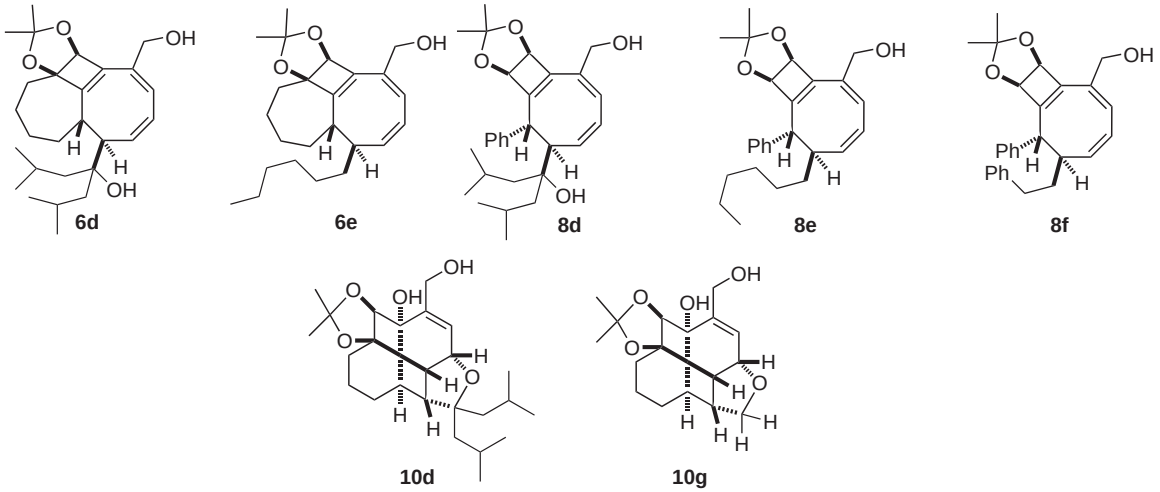


Figure 2. Derivatives showing pro-apoptotic activities on different cancer cell lines.

sensitive cell line THP-1. In this case, the molecular target could be related to the TRAIL receptor. Further competitions experiments will help to determine the exact mechanism.

The pro-apoptotic activities of the most active compounds (**8d**–**f**) on THP-1 cells did not present any drastic change on EC_{50} values (induction of 50% of apoptotic cells). When compared to our reference molecule Celastrol (EC_{50}: 15 μM), all three compounds proved to be potent anticancer drug candidates (EC_{50}: **8d**, 32.4 μM; **8e**, 52.9 μM; **8f**, 49.3 μM).

Considering the SAR information available from these results, it appears from compounds **6d** and **6e** that the presence of the seven-membered ring seems to be important for potent pro-apoptotic activity in colon carcinomas TRAIL-resistant metastatic cells SW620. When this region of the molecule is not present, for example, in **8d**–**f**, the activities becomes less specific, displaying activities also in THP-1 and SW620 with a higher activity on THP-1. For the fenestrene series, both **10d** and **10g** have a high selective activity in THP-1 and the SAR considerations are more difficult to establish at this stage. Other compounds have to be synthesized, then examined in these biological tests in order to obtain more insight into their SAR. But at first, for at least six compounds (**6d**, **6e**, **8d**–**f** and **10d**), it is important to note that activity is observed when the R group is either an *iso*-butyl or a long carbon chain in the α-position of the H^a proton (Scheme 2, formula 8a–f) indicating that lipophilic interactions are needed in that region.

Among the 20 new compounds tested, two compounds presented specific activities on the colon carcinomas TRAIL-resistant metastatic cell SW620, but a minor action on the monocytic leukemia THP-1 cell line. Such differential pro-apoptotic activities point out these two molecules as potent pharmacological tools to study TRAIL associated cellular mechanisms.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.094.

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25. CCDC 707563 for **8a** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB21EZ, UK; Tel.: +44 1223 336408; or deposit@ccdc.cam.ac.uk).