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Synthesis and antitumor activity of methanofullerenes equipped with norbornadiene and quadricyclane moieties

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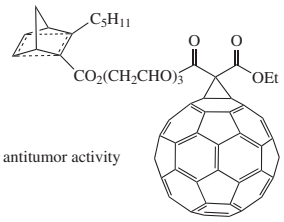
Hybrids composed of 3-pentylnorbornadiene-2-carboxylic acid or its quadricyclane isomer molecules linked to methano-C_{60} fullerene *via* triethylene glycol spacer were synthesized by the Bingel–Hirsch reaction. The quadricyclane derivative possesses markedly higher apoptosis-inducing activity against the Jurkat tumor cells than the norbornadiene one.



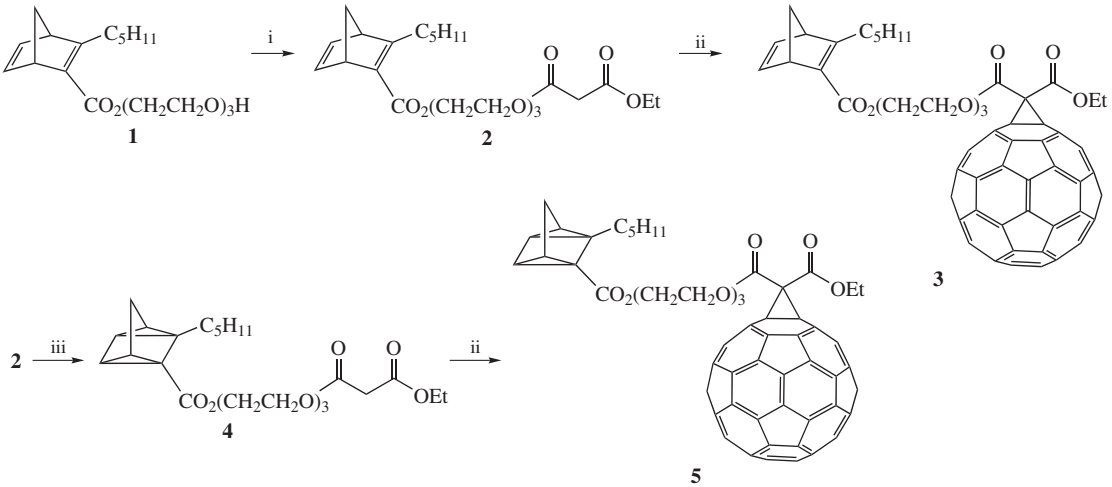
Keywords: C_{60} fullerene, norbornadiene, quadricyclane, methanofullerenes, cytotoxic activity.

Recently,[1,2] we put forward the idea that fullerene-containing quadricyclanes in combination with cisplatin may be efficient antitumor agents. The idea was based on the ability of hybrid fullerene-containing quadricyclanes to cleave DNA due to the presence of the fullerene moiety[3–5] with simultaneous thermal action on tumor cells upon carbon–carbon bond breaking in quadricyclanes under the action of Pt or Pd ions.[6–9] Preliminary *in vitro* experiments on human T-lymphoblastic leukemia (Jurkat) cells showed a significant dose-dependent increase in the number of dead cells upon treatment with that quadricyclane-containing fullerene in combination with cisplatin as compared with the control cells treated with the single components.[2]

In view of the prospects and practical value of quadricyclane-containing fullerenes as new-generation anticancer agents and in order to elucidate the influence of the structure of these compounds on their cytotoxic activity, we synthesized herein two hybrid quadricyclane- and norbornadiene-containing methanofullerenes in which the polycycloalkane moieties are linked to C_{60} fullerene *via* the triethylene glycol spacer. The apoptosis-inducing activity of the hybrid methanofullerenes was studied in detail, in particular, in combination with cisplatin.

The goal set for this study was addressed, first, by synthesizing 3-pentylnorbornadiene-2-carboxylic acid triethylene glycol ester **1** which was then condensed with ethyl hydrogen malonate to give triester **2** (Scheme 1). On exposure to UV radiation (310 nm) in acetonitrile solution, compound **2** was isomerized to quadricyclane ester **4** in almost quantitative yield. The Bingel–Hirsch reactions[9,10] of esters **2** and **4** with C_{60} fullerene in the presence of CBr_4 and DBU afforded methanofullerenes **3** and **5** in 58 and 63% yields, respectively.

Study of the stability of hybrid molecule **5** demonstrated that the long triethylene glycol spacer between quadricyclane and methano-



Scheme 1 *Reagents and conditions*: i, $HO_2CCH_2CO_2Et$, DCC, DMAP, CH_2Cl_2; ii, fullerene C_{60}, CBr_4, DBU, *o*-xylene, room temperature; iii, UV irradiation, MeCN, 1 h.

fullerene moieties decreases the stability of compound **5** in solution, namely, quadricyclane moiety is completely opened within 72 h to give compound **3**. Meanwhile, compound **5** isolated from the reaction mixture as a dry powder retains its structure at room temperature for more than 30 days.

Presumably, the three-membered cycles of quadricyclane **5** being electron reservoirs would form intramolecular donor–acceptor complexes with the fullerene core, which can induce carbon–carbon bond breaking in cyclopropanes to give thermodynamically more stable norbornadiene isomer **3**. It is noteworthy that the hybrid quadricyclane-containing methanofullerenes with ethylene glycol spacer synthesized in our earlier study[11] proved to be more stable both in solution and in the solid state, probably, due to steric factors hampering intramolecular interactions.

To elucidate the apoptosis-inducing activity of methanofullerenes **3** and **5**, we prepared stable water-soluble complexes of these hybrid molecules with polyvinylpyrrolidone by a reported procedure.[12] In view of the fact that quadricyclane-containing methanofullerene **5** would undergo cyclopropane ring opening in solution also in the absence of cisplatin, it appeared of interest to study the antitumor activity of this compound in the absence of Pt ions.

Treatment of cells with aqueous solutions of the polyvinylpyrrolidone complexes of methanofullerenes **3** and **5** in various concentrations induces a statistically reliable dose-dependent increase in the number of dead cells in each group, formed according to the amount of added hybrid, in comparison with the control (Figure 1).

Using the cytotoxicity data for methanofullerenes **3** and **5**, we analyzed the apoptosis-inducing properties of these compounds towards Jurkat tumor cells. The application of annexin V, a recombinant protein possessing a high affinity to phosphatidylserine conjugated with Alexa Fluor 488 fluorochrome, makes it possible to detect cell apoptosis. Furthermore, the use of annexin V together with 7AAD enables simultaneous identification of living cells (annexin V and 7AAD negative), early apoptotic cells (annexin V positive and 7AAD negative), and late apoptotic or necrotic cells (annexin V and 7AAD positive).

The highest percentage of apoptosis upon addition of compound **3** to Jurkat cell culture amounted to 63.18% and was detected at the compound concentration of 0.06 μM (Figure 2). A similar situation was observed for compound **5**, however the concentration of this compound needed to initiate late apoptosis in 70% cells was much higher (~1 μM).

As a continuation of this study, we also showed that compounds **3** and **5** exhibited low cytotoxic activity against normal fibroblasts in comparison with that against the Jurkat tumor cell line taken

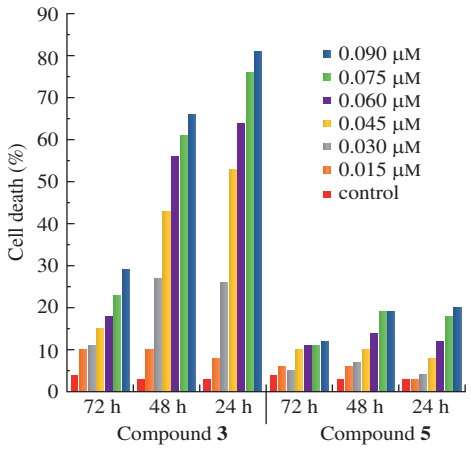


Figure 1 Diagram of the cytotoxic activity of methanofullerenes **3** and **5** against Jurkat cells.

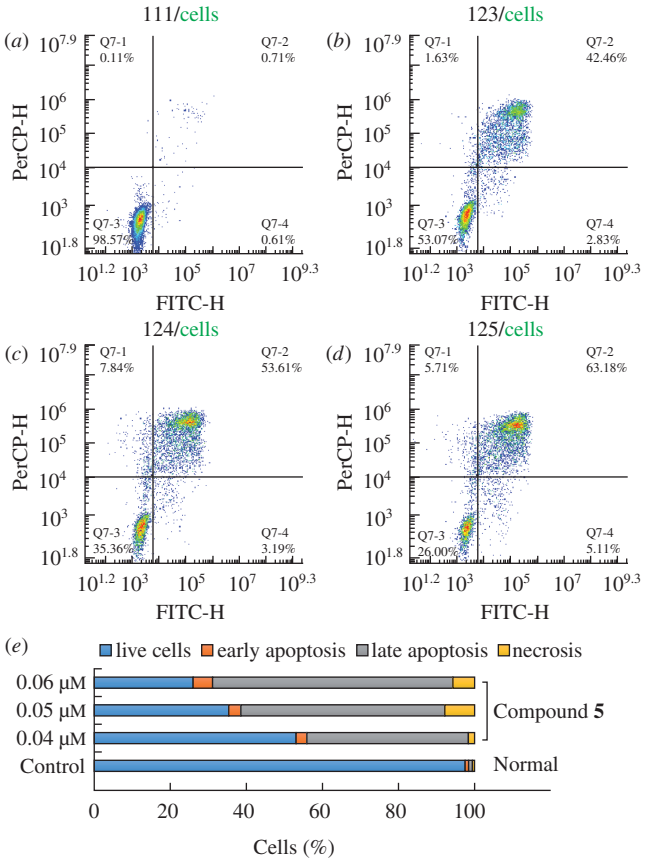


Figure 2 Jurkat tumor cells treated with compound **3** in different concentrations and stained with annexin V/7AAD. Flow cytometry data: (*a*) control; (*b*) **3** (0.040 μM); (*c*) **3** (0.05 μM); (*d*) **3** (0.06 μM). Q7-1, necrotic cells; Q7-2, late apoptotic cells; Q7-3, living cells; Q7-4, early apoptotic cells. (*e*) The histogram shows the apoptosis rate (%) for compound **5**; $^{**}P<0.05$ when compared with the control group ($n = 3$).

in the experiment, with high selectivity indices (SI = fibroblast IC_{50} / tumor cell IC_{50}), which were 7 and 12 for compounds **3** and **5**, respectively.

In conclusion, employing the Bingel–Hirsh reaction, we synthesized hybrid methanofullerenes containing norbornadiene and quadricyclane moieties. Estimation of the cytotoxic effect of the obtained hybrid molecules on human T-lymphoblastic leukemia cells (Jurkat cells) has revealed that water-soluble polyvinylpyrrolione complex of methanofullerene containing a quadricyclane addend induces a statistically reliable dose-dependent increase in the number of dead cells as compared with the control. We have also demonstrated that the synthesized compounds induce apoptosis of Jurkat tumor cells. The quadricyclane-containing methanofullerene is more active than the norbornadiene isomer.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.03.006.

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