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The Bingel-Hirsch Reaction Is a Universal Key to the Synthesis of Hybrid Molecules Based on C60 Fullerene and 5Z,9Z-Dienoic Acids—Potential Antitumor Drugs †

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Abstract: The data obtained by the author in the field of chemistry of carbon clusters, namely, the chemical binding of C_{60} fullerene with 5Z,9Z-dienoic acids by nucleophilic addition to the carbon cluster of α halocarbanions generated in situ from their malonic acid esters are summarized. Based on ethyl, benzyl, acetylene and triazole esters of malonic acid containing 5Z,9Z-dienoic acids and C_{60} fullerene, new hybrid methanofullerenes were synthesized under the conditions of the Bingel-Hirsch reaction. The synthesized methanofullerenes exhibit high cytotoxic activity towards tumor cells of the Jurkat, K562, U937, HL60 lines, and it has also been established that they are effective inducers of apoptosis, exerting a phase-specific cytotoxic effect on the cell cycle of tumor cells. Next, by the method of intermolecular esterification of malonic acid with α,ω-diols, in the presence of hafnium triflate Hf(OTf)4, previously undescribed macrodiolides containing a 1Z,5Z-diene fragment in the structure were synthesized in high yields and stereoselectivity.Under the conditions of the Bingel-Hirsch reaction, chemical binding of the synthesized macrodiolides with C_{60} fullerene was carried out to obtain the corresponding methanofullerenes. The cytotoxic activity of macrodiolides and methanofullerenes in relation to tumor cell lines Jurkat, K562, U937, HL60 and normal fibroblasts was studied. It was found that the cytotoxicity of the hybrid molecule is higher compared to the original macrodiolide (from 5 to 170 times). In addition, the synthesized hybrid molecules initiate apoptosis by uncoupling oxidation and phosphorylation of the mitochondrial membrane of tumor cells.

Keywords: fullerene C60; Bingel-Hirsch reaction; 5Z,9Z-dienoic acid; triazoles; malonic acid; macrodiolides; cytotoxic activity; tumor cells; cell cycle; apoptosis

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1. Introduction

Low selectivity of drugs is one of the pressing problems of modern pharmacology and medicine. Drugs administered to the body by traditional methods are distributed in it relatively evenly. According to [1], only 1% of the administered dose gets into the target cells. This forces the use of high therapeutic doses of the drug, which often leads to side effects. Accordingly, the solution to this problem is the creation of targeted drug transport systems.

Since the discovery of a new allotropic modification of carbon—fullerene in 1985, interest in its derivatives has grown due to the possibility of using them in various fields of science and technology, and the range of nanosized vectors for the delivery of antitumor drugs has expanded. Due to its size, shape and high lipophilicity, fullerene C60 easily penetrates cell membranes and represents an ideal combination of properties for use as a vector for targeted drug delivery.

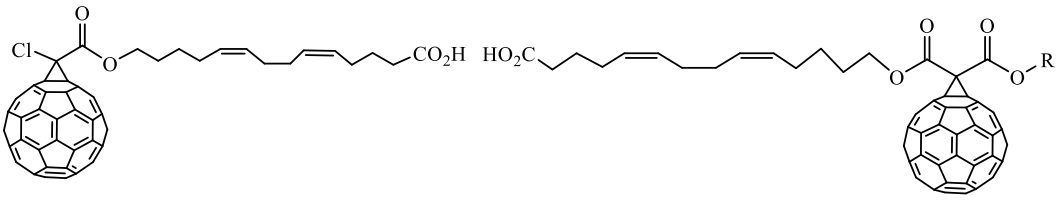
For example, the use of Doxorubicin in the form of a complex with fullerene C60 leads to an increase in the rate of penetration into cancer cells by 20–30%, an increase in the effectiveness of action by 1.5–2 times, reduces the tumor volume by 40–60% and increases the life expectancy of mice by 2.5 times [2,3]. At the same time, an increase in the effectiveness of the conjugate does not lead to an increase in the side effects of the original drug [4,5].

Having summarized the literature data, and also with the aim of developing new hybrid molecules for the effective fight against oncological diseases, we carried out the binding of 5Z,9Z-dienoic acid with fullerene C60, followed by a study of their antitumor activity in vitro.

2. Results and Discussion

The Bingel [6] and Bingel-Hirsch [7] reactions were chosen as the main method for chemically bonding fullerene C60 with diene acids, which involves nucleophilic addition to the carbon cluster of α-halocarbanions generated in situ from malonic or dichloroacetic acid esters.

Thus, we carried out covalent binding of fullerene C60 with 5Z,9Z-dienoic acids to obtain methanofullerenes exhibiting high cytotoxicity in vitro with respect to tumor cell lines Jurkat, U937, HL60 and K562 [8].



1 **2:** R = Et; **3:** R = Bn

Scheme 1. Fullerene derivatives containing 5Z,9Z-dienoic acids.

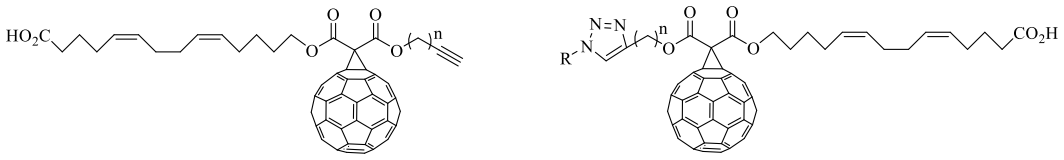
Table 1. Cytotoxic activities in vitro of compounds **1**, **2**, **3**.

Comp.	Jurkat (IC_{50}, μM)	K562 (IC_{50}, μM)	U937 (IC_{50}, μM)	HL60 (IC_{50}, μM)	Fibroblasts (IC_{50}, μM)
1	0.216 ± 0.019	0.249 ± 0.021	0.437 ± 0.019	0.198 ± 0.013	1.957 ± 0.038
2	0.712 ± 0.037	0.678 ± 0.034	0.827 ± 0.041	0.551 ± 0.027	3.217 ± 0.077
3	0.315 ± 0.018	0.421 ± 0.025	0.631 ± 0.022	0.262 ± 0.028	2.076 ± 0.048
Cpt	0.949 ± 0.051	1.067 ± 0.033	1.327 ± 0.049	0.863 ± 0.042	3.389 ± 0.069

The use of malonic acid esters in the Bingel-Hirsch reaction creates conditions for the introduction of other biologically active compounds along with 5Z,9Z-dienoic acids at the free carboxyl group. From the analysis of literature data, we found that 1,2,3-triazole derivatives exhibit antitumor, antiviral, antibacterial, and antifungal activity [9,10]. Therefore, in order to increase cytotoxicity toward tumor cells, we introduced 1,2,3-triazole fragments at the second carboxyl group of malonic acid in the methanofullerene molecule.

The synthesis of 1,2,3-triazole derivatives was carried out using the cycloaddition reaction of azides to acetyl derivatives of malonic acid ("clickchemistry") [11].

Thus, we obtained methanofullerenes containing 5Z,9Z-dienoic acids and triazole or acetylene fragments [12]. The cytotoxic activity of the latter in relation to tumor cells of the Jurkat, K562, U937, HL60 lines was studied. It was established that hybrid methanofullerenes containing acetylene fragments, in contrast to triazole substituents, exhibit higher cytotoxicity, but are characterized by lower selectivity of action in relation to healthy cells.



4: n= 1; **5:** n= 3 **6:** n= 1, R= Bu **7:** n= 1, R= Bn **8:** n= 3, R= Bu **9:** n= 3, R= Bn

Scheme 2. Methanofullerenes with triazole and acetylene groups.

Table 2. Cytotoxic activities in vitro of compounds **4–9** measured on tumor cell cultures.

Comp.	Jurkat (IC_{50}, μM)	K562 (IC_{50}, μM)	U937 (IC_{50}, μM)	HL60 (IC_{50}, μM)	Fibroblasts (IC_{50}, μM)
4	0.022 ± 0.001	0.037 ± 0.003	0.027 ± 0.002	0.021 ± 0.001	0.196 ± 0.015
5	0.037 ± 0.002	0.048 ± 0.004	0.039 ± 0.003	0.033 ± 0.002	0.276 ± 0.021
6	0.034 ± 0.002	0.042 ± 0.003	0.036 ± 0.003	0.030 ± 0.002	0.291 ± 0.023
7	0.032 ± 0.002	0.037 ± 0.004	0.033 ± 0.002	0.028 ± 0.002	0.284 ± 0.025
8	0.041 ± 0.003	0.046 ± 0.004	0.044 ± 0.003	0.036 ± 0.001	0.349 ± 0.028
9	0.031 ± 0.002	0.044 ± 0.003	0.036 ± 0.003	0.031 ± 0.002	0.266 ± 0.022
Cpt	0.812 ± 0.064	0.934 ± 0.071	1.125 ± 0.089	0.781 ± 0.056	3.124 ± 0.218

Hybrid molecules **4–9** exhibit pronounced apoptosis-inducing activity. The highest percentage of late apoptosis (95%) and the lowest percentage of living cells (0.53%) are observed upon addition of compound **5** to the Jurkat cell culture at a concentration of 0.045 μM.

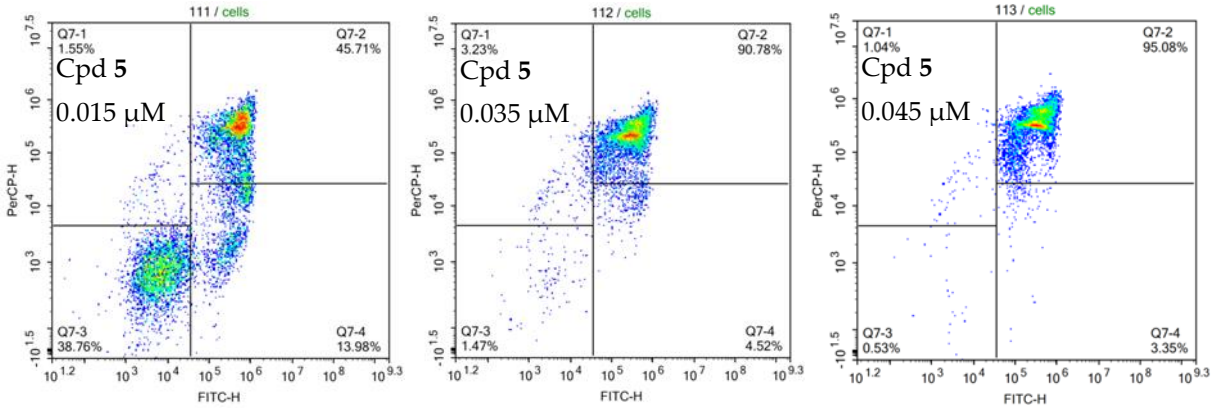
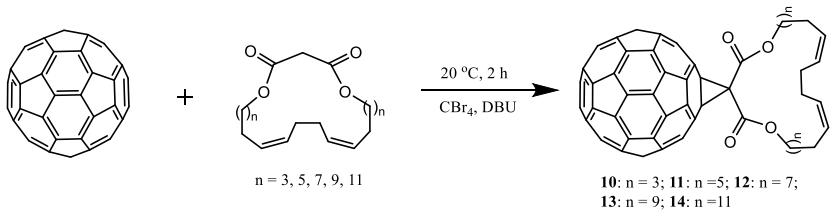


Figure 1. Comparative analysis of the apoptosis-inducing activity of compound 5 at different concentrations.

By the time our studies began, it was known that bis-methylene-separated Z,Z-diene alcohols were capable of entering into a condensation reaction with α,ω-dicarboxylic acids of various structures, yielding the corresponding macrolides, except for malonic and oxalic acids. This limitation complicated the implementation of our idea of the possibility of synthesizing hybrid molecules with antitumor activity based on fullerene C60 under the conditions of Bingel-Hirsch reactions. Experimentally, we found that the technique described in the literature [13] also allows one to obtain macrodiolides based on malonic acid, which, when interacting with C60 fullerene, yield the corresponding methanofullerenes [14].



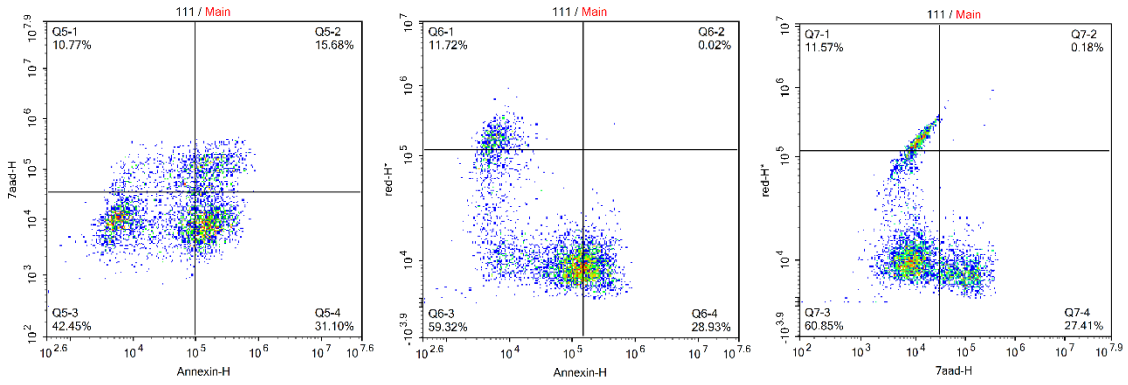
Scheme 3. Interaction of fullerene C60 with macrodiolides.

For all synthesized methanofullerenes containing macrocyclic fragments, as well as the original macrodiolides, we evaluated their antitumor effect in vitro using Jurkat, K562, HL60, U937 cell lines and normal fibroblasts, including determining IC_{50}, studying apoptosis-inducing activity and the effect on the cell cycle using flow cytofluorometry. The experimental data obtained indicate that cytotoxicity significantly decreases with increasing cycle size. Analysis of data on the cytotoxicity of methanofullerenes indicates a marked increase in the cytotoxic effect (from 5 to 170 times) with covalent binding of macrodiolides to the C60 fullerene molecule, compared to the original macrodiolide.

Table 3. Cytotoxic activities in vitro of compounds **10–14** measured on tumor cell cultures (Jurkat, K562, U937, HL60 and Fibroblasts) (μM).

Comp.	Jurkat (IC_{50}, μM)	K562 (IC_{50}, μM)	U937 (IC_{50}, μM)	HL60 (IC_{50}, μM)	Fibroblasts (IC_{50}, μM)
10	0.18 ± 0.02	0.11 ± 0.01	0.14 ± 0.01	0.09 ± 0.01	0.91 ± 0.07
11	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.31 ± 0.03
12	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.96 ± 0.07
13	0.06 ± 0.01	0.03 ± 0.001	0.04 ± 0.01	0.03 ± 0.01	0.47 ± 0.03
14	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.36 ± 0.02

In addition, detection of changes in mitochondrial membrane potential (ΔΨ) and associated early and late apoptosis in Jurkat cells treated with compound **10** (0.18 μM), **12** (0.09 μM) and staurosporine (0.2 μM) showed that the synthesized methanofullerenes act as effective inducers of the mitochondrial apoptotic pathway.



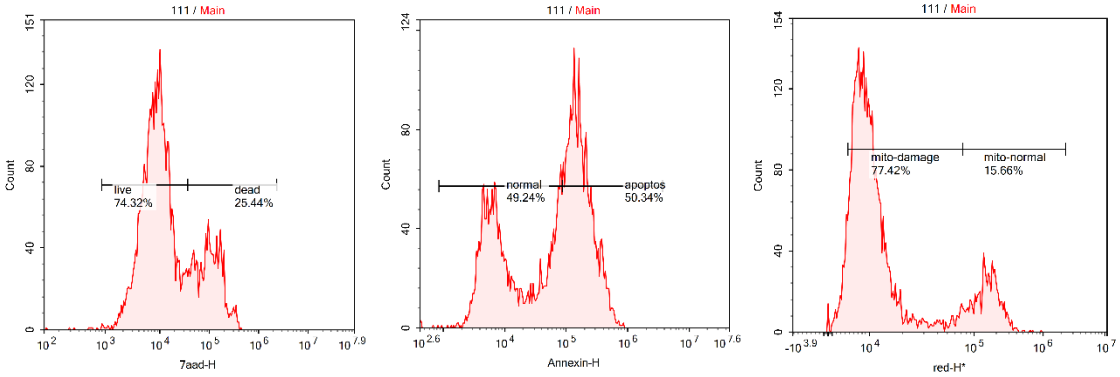


Figure 2. Detection of changes in mitochondrial membrane potential (ΔΨ) in Jurkat cells treated with compound 10. Cells were stained with MitoSenseRed, Annexin V—CF488A and 7-AAD. Incubation time was 3 h.

3. Conclusions

Thus, based on the above, it can be concluded that the Bingel-Hirsch reaction is a universal key to the synthesis of hybrid molecules with antitumor properties, which opens up new horizons and opportunities for the treatment of oncological diseases.

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Conflicts of Interest: The authors declare no conflicts of interest.

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