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Fullerenyl-1,2,3-triazoles: Synthesis and Cytotoxic Activity †

Zarema Sadretdinova *, Arslan Akhmetov, Lilya U. Dzhemileva, Vladimir A. D'yakonov, Airat Tuktarov and Usein Dzhemilev

Institute of Petrochemistry and Catalysis of RAS, Ufa 450075, Russia; e-mail@e-mail.com (A.A.); e-mail@e-mail.com (L.U.D.); e-mail@e-mail.com (V.A.D.); e-mail@e-mail.com (A.T.); e-mail@e-mail.com (U.D.)

* Correspondence: zarema.hairullina18@yandex.ru

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Abstract: By the reaction of fullerenyl azide with terminal acetylenes previously undescribed 1-butyl-2-triazolylfullerenes were synthesized for the first time, in which the heterocyclic fragment is directly attached to the fullerene backbone. Water-soluble complexes of the synthesized adducts of fullerene with polyvinylpyrrolidone showed high cytotoxic activity towards tumor cells of the Jurkat, K562, U937 lines.

Keywords: fullerenyl azide; terminal alkynes; click-reaction; triazolofullerenes; cytotoxic activity

1. Introduction

N-containing heterocycles such as triazoles and tetrazoles are known pharmacophores and are widely used in drug development. Thus, it is known that 1,2,3-triazoles have a wide spectrum of biological action [1,2] and exhibit high antitumor, antiviral, antibacterial, antifungal and other activity. An increase in the biological activity of organic compounds after the introduction of tri- and tetrazole fragments into them is associated with a moderate dipole character of the heterocycle, the possibility of additional hydrogen bonds formation, resistance to redox reactions and acid or alkaline hydrolysis [3].

Fullerenes and their derivatives are of particular practical interest from a medical point of view. The biological activity of fullerenes is based on three properties: electron deficiency, lipophilicity, and the ability to react with free radicals. Currently a great number of published papers describing various fullerene derivatives with various activity [4–9]. Despite this, there is practically no information in the literature on the synthesis of biologically active fullerenes containing triazole fragments. For example, it is known [10] that the conjugate of fullerene with doxorubicin (DOX) exhibits an antiproliferative effect in comparison with unconjugated DOX upon incubation with MCF-7 cancer cells. In this case, hybrid fullerene molecules with biologically active diene acids containing triazole fragments in their structure exhibit a higher selectivity of action with respect to a wide range of tumor cells [11]. In turn, hexamethanofullerene with six triazole cycles was found to have high antiviral activity in an infectious model against the Ebola pseudovirus [12]. All of the above examples of triazole-containing fullerenes are characterized by a significant removal of the heterocyclic fragment from the fullerene framework, and therefore, their mutual influence on each other is leveled.

In this work, we discuss the synthesis of new triazole-containing fullerenes, in which the heterocyclic substituent is directly bonded to the core of the carbon molecule, and provide preliminary data on their cytotoxic activity.

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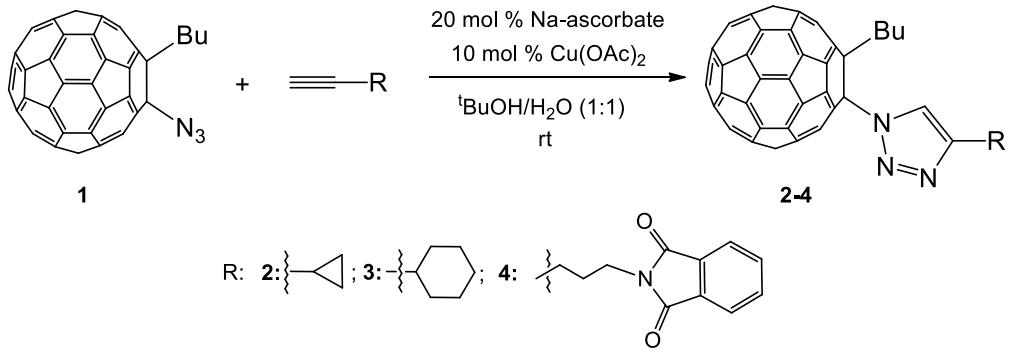
2. Results and Discussion

The synthesis of the previously undescribed fullerenyltriazoles **2**-**4** was carried out under the conditions of the alkyne-azide method. Fullerenyl azide 1, which was previously synthesized for the first time in our laboratory [13], and a number of terminal acetylenes containing cyclopropyl, cyclohexyl, and isoindoldione substituents, which are part of a large number of drugs, were used as precursors.



Scheme 1. Synthesis of 1-butyl-2-triazolyl-dihydrofullerenes **2-4**.

The structure of the synthesized compounds was reliably determined applying modern physicochemical methods of analysis such as NMR and MALDITOF/TOF mass spectrometry.

We carried out preliminary experiments antitumor effect in vitro of an aqueous solution of the polyvinylpyrrolidone complex of synthesized fullerene adduct **2-4** containing triazole fragments, using K562, U937, Jurkat cell lines including the determination of IC_{50} using flow cytofluorimetry (Table 1).

Table 1. Cytotoxic activities in vitro of compounds **2-4** measured on tumor cell cultures (Jurkat, K562, U937) (μM).

Comp.	Jurkat (IC_{50}, μM)	U937 (IC_{50}, μM)	K562 (IC_{50}, μM)
2	0.05 ± 0.01	0.16 ± 0.01	0.19 ± 0.02
3	0.04 ± 0.01	0.04 ± 0.01	0.15 ± 0.01
4	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.01

The experimental data prove that the synthesized novel fullerene adducts **2-4** exhibit cytotoxic effect with respect to the selected tumor cell lines in the range IC_{50} = 0.02–0.19 μM, the most active is triazolylcontaining fullerene **4**.

Currently, the Laboratory of Molecular Design and Biological Screening of Candidate Substances for the Pharmaceutical Industry at the Institute of Petrochemistry and Catalysis of RAS is conducting more detailed studies of the antitumor activity of synthesized new fullerene derivatives using a wide range of cancer cells as examples.

3. Materials and Methods

All reactions were performed under an argon atmosphere and in anhydrous solvent. The solvents and reagents were dried or refined according to the literature procedures. The reaction products were analyzed on a HPLC chromatograph Shimadzu SPD-20A (Japan) equipped with the UV detector at 313 or 340 nm. The mixtures were separated on a preparative column Cosmosil Buckyprep Waters (250 × 10 mm) at ~20 °C. Toluene was used as eluent, the flow rate was 3.0 mL·min^{-1}. The ^{1}H and ^{13}C NMR spectra were run on a Bruker Avance-500 spectrometer. The mass spectra were obtained on an UltraFlex III TOF/TOF (Bruker Daltonik GmbH, Germany) operating in linear (TOF) and reflection

(TOF/TOF) positive and negative ion modes. S_8 and DCTB (trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenyliden]malononitrile) were used as the matrix.

Procedure for the synthesis of triazolylcontaining fullerenes **2**-**4**.

Triazolylcontaining fullerenes were obtained by alkyne-azide addition in the presence of a copper (I) catalyst $Cu(OAc)_2$ using the «click» reaction method. In a two-necked flask, 1-azido-2-butyl(C_{60}-*I*$_h$)[5,6]fullerene (1) (0.05 g, 0.061 mmol) was dissolved in chlorobenzene (10 mL) with vigorous stirring, acetylene (0.061 mmol) was added, in the presence of $Cu(OAc)_2$ (0.005 mmol) and Na ascorbate (0.009 mmol), then tert-butanol and water were added in a 1:1 ratio. The reaction mixture was stirred for 12 h at room temperature. 50 mL of water was added to the reaction mass, the organic layer was separated and passed through a Schott filter, an individual compound was isolated using high performance liquid chromatography. The yields of the synthesized compounds ranged from 75 to 81%.

1-Butyl-2-(4′-cyclopropyl-1*H*-1′,2′,3′-triazol-1′-yl)(C_{60}- I$_h$)[5,6]fullerene **2**

Brown powder; yield 81%. IR (KBr, neat, cm^{-1}): 2950, 2920, 2852, 1637, 1508, 1025, 525. UV-Vis ($CHCl_3$), λ_{max}, nm: 258, 327, 427. ^{1}H NMR (500 MHz, $CDCl_3$:CS_2 1:5) δ 1.00 (t, *J* 7 Hz, 3H, CH_3), 1.13–1.18 (m, 4H, 2CH_2), 1.46–1.51 (m, 2H, CH), 1.98–2.08 (m, 2H, CH_2), 2.24–2.29 (m, 1H, CH), 2.77–2.99 (m, 2H, CH_2), 8.40 (s, 1H, CH). ^{13}C NMR (125 MHz, $CDCl_3$) δ 7.2, 8.3, 14.1, 23.5, 31.3, 38.3, 65.9, 81.2, 123.1, 139.6, 139.9, 141.3, 142.4, 142.8, 142.9, 143.1, 143.2, 144.5, 144.9, 145.4, 145.7, 145.8, 146.2, 146.3, 146.4, 146.6, 146.8, 147.8, 148.4, 149.8, 154.7. HRMS (MALDI TOF/TOF), *m*/*z*: calcd. for $C_{69}H_{15}N_3$ 885.1467; found 885.1463.

1-Butyl-2-(4′-cyclohexyl-1*H*-1′,2′,3′-triazol-1′-yl)(C_{60}-I$_h$)[5,6]fullerene **3**

Brown powder; yield 75%. IR (KBr, neat, cm^{-1}): 2922, 2850, 1620, 1461, 1180, 1024, 751, 525. UV-Vis ($CHCl_3$), λ_{max}, nm: 254, 314, 429. ^{1}H NMR (500 MHz, $CDCl_3$:CS_2 1:5) δ 1.00 (t, *J* 7 Hz, 3H, CH_3), 1.2–1.28 (m, 2H, CH_2), 1.44–1.49 (m, 3CH, 2CH_2), 1.56–1.63 (m, 1H, CH_2), 1.67–1.74 (m, 2H, CH_2), 1.86–1.90 (m, 1H, CH_2), 1.95–2.03 (m, 3H, 2CH_2), 2.30–2.37 (m, 2H, CH_2), 2.80–2.90 (m, 2H, CH_2), 3.54–3.57 (m, 1H, CH), 8.41 (s, 1H, CH). ^{13}C NMR (125 MHz, $CDCl_3$:CS_2 1:5) δ 14.1, 23.6, 26.5, 29.9, 31.3, 33.4, 38.4, 41.5, 66.0, 81.2, 122.7, 139.6, 139.9, 141.3, 141.4, 141.5, 141.9, 142.3, 142.4, 142.7, 142.83, 142.88, 143.1, 143.2, 144.4, 144.6, 144.9, 145.3, 145.4, 145.5, 145.7, 145.8, 146.3, 146.40, 146.44, 146.5, 146.6, 146.7, 146.9, 147.8, 154.64. HRMS (MALDI TOF), *m*/*z*: calcd. for $C_{72}H_{21}N_3$ 927.1788; found 928.1783.

1-Butyl-2-(4′-isoindol-1,3-dione-1*H*-1′,2′,3′-triazol-1′-yl)(C_{60}-I$_h$)[5,6]fullerene **4**

Brown powder; yield 78%. IR (KBr, neat, cm^{-1}): 3417, 2951, 2852, 1712, 1385, 1033, 720, 526. UV-Vis ($CHCl_3$), λ_{max}, nm: 257, 327, 426. ^{1}H NMR (500 MHz, $CDCl_3$:CS_2 1:5) δ 0.98 (t, *J* 7 Hz, 3H, CH_3), 1.28 (s, 2H, CH_2), 1.44–1.50 (m, 1H, CH), 1.98–2.03 (m, 2H, CH_2), 2.30–2.38 (m, 2H, CH_2), 2.80–2.88 (m, 2H, CH_2), 3.10 (t, 2H, CH_2, *J* = 5.0 Hz), 7.7–7.82 (m, 1H, CH), 7.90–7.94 (m, 1H, CH), 8.62 (s, 1H, CH). ^{13}C NMR (125 MHz, $CDCl_3$:CS_2 1:5) δ 14.1, 23.3, 28.6, 31.3, 37.2, 38.2, 56.4, 66.0, 81.3, 123.3, 124.8, 132.2, 134.0, 137.8, 138.2, 139.5, 139.9, 141.8, 141.9, 142.4, 142.5, 142.8, 142.8, 143.1, 143.2, 144.9, 145.4, 145.7, 145.9, 146.2, 146.3, 146.4, 146.6, 146.8, 147.8, 148.4, 155.6. HRMS (MALDI TOF/TOF), *m*/*z*: calcd. for $C_{78}H_{21}N_3O_2$ 1032.0541; found 1032.0548.

4. Conclusions

For the first time we synthesized fullerenyltriazoles by the reaction of azidofullerene with terminal acetylenes, in which the heterocyclic fragment is directly attached to the fullerene core. Furthermore, it was demonstrated that the synthesized fullerene adducts exhibit a high antitumor potential in relation to K562, U937 and Jurkat tumor cells.

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References

1. Wamhoff, H. *1,2,3-Triazoles and their Benzo Derivatives Comprehensive Heterocyclic Chemistry*; Pergamon Press: Oxford, UK, 1984; Volume 5, pp. 670–732.
2. Bonandi, E.; Christodoulou, M.S.; Fumagalli, G.; Perdicchia, D.; Rastelli, G.; Passarella, D. The 1,2,3-triazole ring as a bioisostere in medicinal chemistry. *Drug Discov. Today* **2017**, *22*, 1572–1581.
3. Senger, G.D.; Da Silva, M.R.; Ferreira F. de C.; Silva, V.F.; Paes F. 1-Phenyl-1H- and 2-phenyl-2H-1,2,3-triazol derivatives: Design, synthesis and inhibitory effect on alpha-glycosidases *Eur. J. Med.Chem.* **2014**, *74*, 461–476.
4. Castro, E.; Garcia, A.H.; Zavala, G.; Echegoyen, L. Fullerenes in Biology and Medicine. *J. Mater. Chem. B Mater. Biol. Med.* **2017**, *5*, 6523–6535.
5. Bakry R.;Vallant, R.M.; Najam-ul-Haq, M.; Rainer, M.; Szabo, Z.; Huck Ch, W.; Bonn, G.K. Medicinal applications of fullerenes**.** *Int. J. Nanomed*. **2007**, *2*, 639–649.
6. Bogdanović, G.; Djordjević, A. Carbon nanomaterials: Biologically active fullerene derivatives. *Srp. Arh. Celok. Lek*. **2016**, *144*, 222–231.
7. Lin, C.M.; Lu, T.Y. C60 fullerene derivatized nanoparticles and their application to therapeutics. *Recent Pat. Nanotechnol*. **2012**, *6*, 105–13.
8. Anilkumar, P.; Lu, F.; Cao, L.; Luo, P.G.; Liu, J.H.; Sahu, S.; Tackett, K.N.; Wang, Y.; Sun, Y.P. Fullerenes for applications in biology and medicine. *Curr. Med. Chem*. **2011**, *18*, 2045–2059.
9. Yang, X.; Ebrahimi, A.; Li, J.; Cui, Q. Fullerene-biomolecule conjugates and their biomedicinal applications. *Int. J. Nanomed*. **2014**, *9*, 77–92.
10. Magoulas, G.E.; Bantzi, M.; Messari, D.; Voulgari, E.; Gialeli, C.; Barbouri, D.; Giannis, A.; Karamanos, N.K.; Papaioannou, D.; Avgoustakis, K. Synthesis and Evaluation of Anticancer Activity in Cells of Novel Stoichiometric Pegylated Fullerene-Doxorubicin Conjugates. *Pharm. Res.* **2015**, *32*, 1676–1693.
11. Tuktarov, A.R.; Khuzina, L.L.; Akhmetov, A.A.; Tuktarova, R.A.; Ishmukhametova, S.R.; Dzhemileva, L.U.; D'yakonov, V.A.; Dzhemilev, U. M. Hybrid Molecules Based on Fullerene C_{60} and 5*Z*,9*Z*-dienoic Acids: Synthesis and Cytotoxic Activity. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127289.
12. Nierengarten, J.-F.; Schneider, J.P.; Trinh Th, M.N.; Joosten, A.; Holler, M.; Lepage, M.L.; Bodlenner, A.; García-Moreno, M.I.; Mellet, C.O.; Compain, P. Giant Glycosidase Inhibitors: First- and Second-Generation Fullerodendrimers with a Dense Iminosugar Shell. *Chem. A Eur. J.* **2018**, *24*, 2483-2492.
13. Akhmetov, A.R.; Yarullin, I.R.; Tuktarov, A.R.; Dzhemilev, U.M. Reactions of fullerene C_{60} with organometallic azides. *Tetrahedron Lett.* **2014**, *55*, 3747.