

Anti-HIV properties of cationic fullerene derivatives

Silvia Marchesan,^a Tatiana Da Ros,^{a,*} Giampiero Spalluto,^a
Jan Balzarini^b and Maurizio Prato^a

^a*Dipartimento di Scienze Farmaceutiche, Università di Trieste, Piazzale Europa 1, I-34127 Trieste, Italy*

^b*Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

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Abstract—A series of regioisomeric bis-fulleropyrrolidines bearing two ammonium groups have been synthesized and their activities against HIV-1 and HIV-2 have been evaluated. Two trans isomers have been endowed with interesting antiviral properties, confirming the importance of the relative positions of the substituent on the C₆₀ cage. In addition, reduced amphiphilicity of molecules to other compounds previously reported decreases their cytotoxicity in CEM cell cultures. None of the compounds showed any inhibitory activity against a variety of DNA and RNA viruses other than HIV.

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1. Introduction

There is ample evidence to suggest that fullerene and its derivatives possess properties which hint at their use in biomedicine, such as DNA photocleavage,^{1,2} neuroprotection,^{3,4} and inhibition of apoptosis.^{5,6} Moreover, Wudl and co-workers demonstrated that the HIV protease (HIV-P) could be complexed and inhibited by C₆₀, which perfectly fits into the catalytic site.^{7,8} However, lack of solubility of fullerenes in polar solvents and biological fluids restricts their use in pharmacological studies.⁹ For this reason in the last decade many efforts have been made to overcome this problem, obtaining promising results.^{10,11}

Exploration of anti-HIV properties of fullerene derivatives has been performed by different research groups,^{12–16} and up to now, the best result has been that of dendrofullerene **1** (Fig. 1), prepared by Brettreich and Hirsch,¹⁰ which has an EC₅₀ of 0.22 μM in human lymphocytes acutely infected by HIV-1_{LAI}.¹⁷

Recently, our group has reported a series of di-substituted water-soluble C₆₀ derivatives capable of inhibiting HIV-1 infection in cells at low micromolar concentrations.¹⁸ Derivative **2**, the trans-2 isomer, proved to be the most interesting compound of this series in terms of activity.

This study allowed the identification of some structural requirements necessary for bearing anti-HIV-1 properties. In fact, from this evaluation, it has been possible to underline the fact that specific relative positions of the two substituents (trans-2) and positive charges close to the carbon cage are prerequisite for antiviral activity. In contrast, bulky polar chains on a C₆₀ sphere induce cytotoxicity and seem to reduce potency, suggesting a significant steric control. The toxic action could be attributed to the amphiphilic character of this class of compounds, as recently reported.^{19,20}

Taking into account these preliminary observations, we synthesized, as potential anti-HIV agents, a series of C₆₀ derivatives (**3–7**, Fig. 2) bearing two ammonium salts close to the carbon cage, with the aim of defining an optimal combination of substituents on the C₆₀, which could lead to good potency and low cytotoxicity. The trans-2 and trans-4, and the mixture of isomers have already been used by other laboratories to perform biological studies on growth inhibition of *Escherichia coli* (mixture of regioisomers)²¹ and the respiratory chain inhibition (trans-2 and trans-4).²²

2. Results and discussion

Derivatives **3–7** have been prepared by the [3+2] dipolar cycloaddition of azomethine ylides to C₆₀, as briefly summarized in Scheme 1.²³

Keywords: Fullerene; Fulleropyrrolidine; Bis-adducts; Anti-HIV activity.

* Corresponding author. Tel.: +39 040 5583110; fax: +39 040 52572; e-mail: daros@units.it

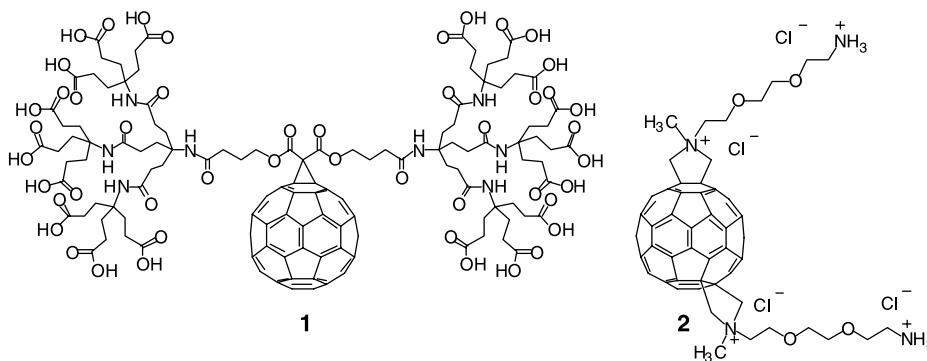


Figure 1. Structures of compounds 1 and 2.

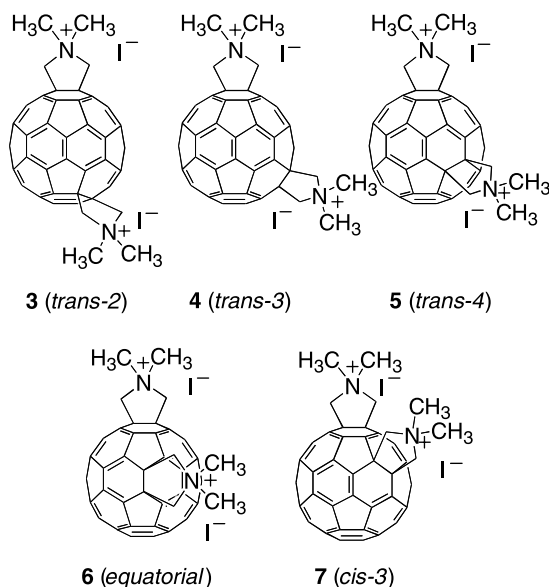


Figure 2. Structures of compounds 3–7.

The reaction of C_{60} with formaldehyde and *N*-methylglycine in refluxing toluene afforded a mixture of mono-adduct and bis-adducts. After the monoadduct was isolated, the isomeric bis-adducts **8–12** were purified by flash chromatography and then by preparative HPLC with a purity >99%.^{24,25} Compounds **3–7** were obtained by methylation of derivatives **8–12** by methyl iodide and their purity was checked by ES-MS.

The fullerene derivatives were administered to lymphocyte CEM cell cultures infected with HIV-1(III_B) or

Table 1. EC₅₀ and CC₅₀ assay results for compounds 3–7

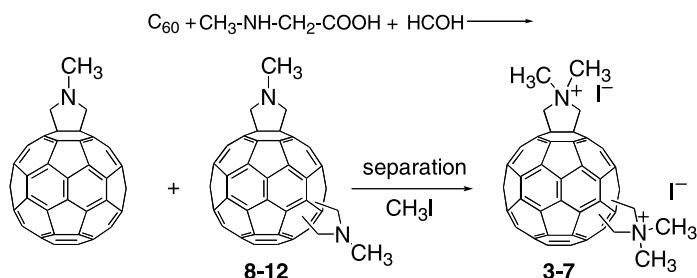
Compound	EC ₅₀ , μM ^a		CC ₅₀ , μM ^a CEM cells
	HIV-1	HIV-2	
3	0.21 (±0.07)	0.2 to 1.0	2.93 (±1.20)
4	0.35 (±0.07)	0.70 (±0.42)	9.04 (±0.18)
5	1.08 (±0.57)	2.50 (±1.90)	12.5 (±7.54)
6	>25	>25	>125
7	2.50 (±0.71)	>10	28.7 (±1.27)

^a Values are means of three experiments; standard deviation is given in parentheses.

HIV-2(ROD), as previously described.^{26,27} The cytostatic activity was also evaluated in CEM cell cultures (Table 1).

As shown in Table 1, compounds **3** and **4** showed interesting anti-HIV properties in the high nanomolar range, confirming the observations previously reported¹⁸ regarding the preferred position of the substituent on the carbon cage of C_{60} . In fact, the trans series (**3–5**) has proved to be more potent than the corresponding isomer cis-3 (**7**) (about 2- to 10-fold), while **6** (equatorial) turned out to be totally inactive.

Analyzing the trans series, it seemed that compounds **3** and **4** showed the best biological profiles (0.21 and 0.35 μM, respectively), while a shift in the pyrrolidine ring to the trans-4 position led to a 5-fold decrease of potency against HIV-1. Furthermore, the peculiar activity versus HIV-2 should be noticed. In fact, while compound **2** was almost inactive against this subtype (>4 μM), compounds **3** and **4** showed an interesting activity against both virus subtypes.



Scheme 1. Synthesis of compounds 3–7.

A different analysis on toxicity should be performed, considering the different profiles between substituent position and toxicity with respect to the anti-HIV properties. In fact, considering both anti-HIV and cytotoxicity properties, the best compounds of this series were the trans-3 isomer, which presents a CC_{50}/EC_{50} ratio of 26, which was higher than reference compound **2** ($CC_{50}/EC_{50} = 12$).

Preliminary computational studies were performed on compounds **2** and **3** utilizing MOE. The dipolar moments were 8.8 D for **2** and 3.2 for **3**. The relative hydrophobic surface, expressed as (hydrophobic surface/total surface) $\times 100$, has also been calculated and in the case of **2** it is 76%, while for **3** it is 92%, demonstrating a significant reduction in the amphiphilic character of the *N,N*-dimethylfulleropyrrolidine trans-2 with respect to **2**. Therefore, differences in toxicity between the two considered compounds could be attributed to differences in amphipathicity, a reason these compounds could be considered useful probes for biological studies. These results are in agreement with our recent study on toxicity and on the hemolytic effect of C_{60} derivatives, from which the toxic effect also seems to be related to the amphiphilic character of the molecules.¹⁹

None of the compounds were active against a variety of DNA and RNA viruses in cell culture, including herpes simplex virus type 1 (HSV-1), HSV-2, vaccinia virus, and vesicular stomatitis virus (VSV) in HEL cell cultures; parainfluenza-3, reovirus-1, Sindbis, Coxsackie B4, and Punta Toro virus in Vero cell cultures; VSV, Coxsackie B4, and respiratory syncytial virus in HeLa cell cultures; and Moloney murine sarcoma virus in C3H/3T3 cell cultures. The assays were performed as previously described.²⁷

3. Conclusions

This study confirms and increases further our knowledge about the structure–activity relationship of C_{60} derivatives as anti-HIV agents. In particular, patterns of substitutions have been found for having improved action against HIV-1 and HIV-2 strains, and a significant increase in the CC_{50}/EC_{50} ratio was also obtained, leading to a better selectivity of antiviral inhibition. In fact, derivative **3** presents an EC_{50} of 0.21 μ M for HIV-1 (as **1**) and also an interesting inhibition of HIV-2 (EC_{50} 0.2–1.0 μ M).

Recently, Mashino et al. reported HIV reverse transcriptase (HIV-RT) inhibition by **3**, **4**, and **5** in the micromolar range,²⁸ but we cannot attribute the action of **3–7** versus the HIV infection to a specific mechanism, HIV-P and/or HIV-RT mediated. Further studies are in progress in our laboratories to understand better the mechanism of action, to increase antiviral potency and, most importantly, to improve the CC_{50}/EC_{50} ratio.

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