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Anti-HIV properties of cationic fullerene derivatives

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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 C_{60} 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.

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 C_{60}, 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 EC_{50} 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 C_{60} 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 C_{60} 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 C_{60} 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 C_{60}, 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 C_{60}, as briefly summarized in Scheme 1.[23]

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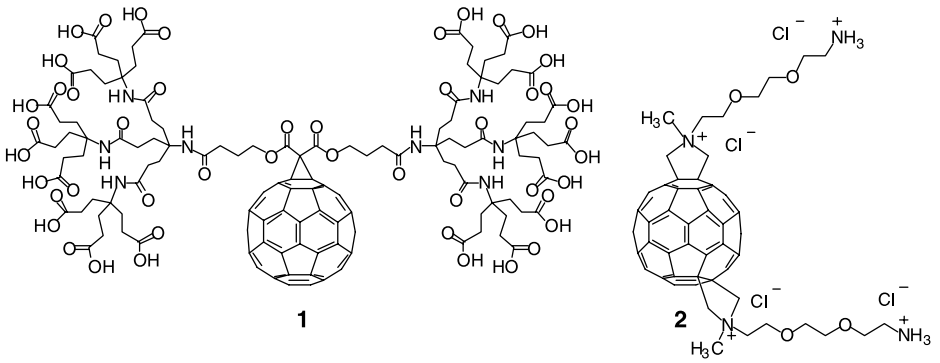


Figure 1. Structures of compounds **1** and **2**.

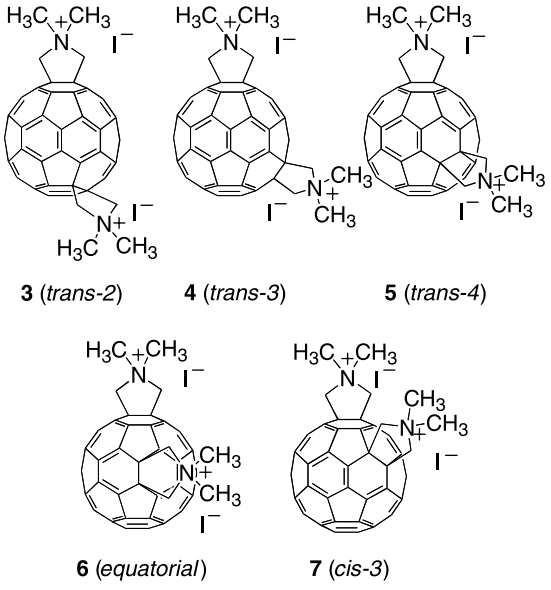


Figure 2. Structures of compounds **3**–**7**.

The reaction of C_{60} with formaldehyde and *N*-methylglycine in refluxing toluene afforded a mixture of monoadduct 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 HIV-2(ROD), as previously described.[26,27] The cytostatic activity was also evaluated in CEM cell cultures (Table 1).

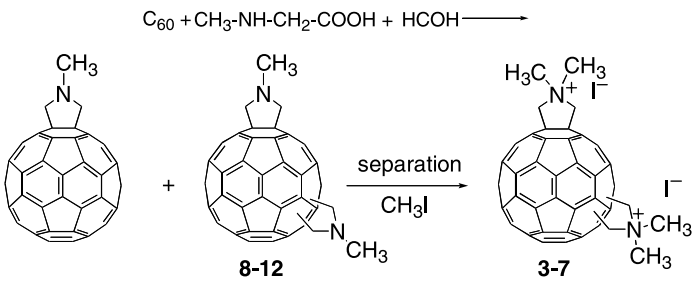
Table 1. EC_{50} and CC_{50} assay results for compounds **3**–**7**

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.

As shown in Table 1, compounds **3** and **4** showed interesting anti-HIV properties in the high nanomolar range, confirming the observations previously reported[18] 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) × 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 μM for HIV-1 (as **1**) and also an interesting inhibition of HIV-2 (EC_{50} 0.2–1.0 μM).

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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