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# Antimycobacterial Activity of Ionic Fullerene Derivatives

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**Abstract**—Positively charged fullerene derivatives, moderately soluble in water:DMSO 9:1, have been tested using three strains of *Mycobacterium* spp. Some compounds inhibit the growth of *Mycobacterium tubercolosis*, a human clinical isolate, particularly virulent and resistant, at doses as low as 5 μg/mL. 

The study of the biological properties of fullerenes and fullerene derivatives has developed widely in recent years.[1,2] After the first account on inhibition of HIV-protease,[3,4] several interesting reports have dealt with potential activity in photodynamic therapy,[5] neuroprotection[6] and apoptosis.[1,2] However, a main issue when dealing with fullerenes is the absolute lack of solubility in any polar solvent for biological evaluation. For this reason fullerenes have to be chemically modified in such a way that they acquire solubility and versatility.[7,8] We have recently shown that the covalent attachment of solubilizing chains to [60] fullerene brings about the formation of water-soluble fulleropyrrolidines.[9] This enabled us to carry out preliminary experiments on biological properties of these compounds. It was found that, at concentrations of 260 μg/mL, water-soluble fulleropyrrolidine **3** (Scheme 1) inhibited the growth of a strain of *Mycobacterium avium*, a mycobacterium peculiar to birds but dangerous for immunodepressed humans.

It should be noted that these microorganisms are resistant to most antimicrobial drugs, and that, in general, tubercolosis is a tough adversary.[10] The exact nature of the action of fulleropyrrolidines is not known, but it is likely that the fullerene derivatives fit inside the tidy structure of the mycobacterial cell-wall, causing its disarrangement and death of the microorganism. These encouraging results have stimulated us to further investigate this field. First of all, it was desirable to test the activity of fulleropyrrolidines against human strains of mycobacteria. In addition, new fullerene derivatives should be considered to improve the interactions with the mycobacterial structure. In this paper we report the synthesis, solubility and antibacterial activity (against human strains) of a series of fulleropyrrolidinium salts **4**–**6** (Scheme 1).[11] Singly charged fullerene derivatives seemed very appealing substrates for the interaction with the cell-wall structure. The simultaneous presence of a charged species together with the hydrophobic spheroid offers the potential advantage of driving the compounds toward the charged double layer and then allowing the spheroid to enter the hydrophobic membrane. For comparison, we have prepared and tested all the model compounds and concluded that the biological activity is to be attributed entirely to the fullerene spheroid.

## Synthesis

Fulleropyrrolidines **1**–**3** were prepared according to Scheme 1.[9,12,13] Compounds **1**–**3** are characterized by the presence of triethylene glycol monomethyl ether chains either on nitrogen and/or on carbon 2 of the pyrrolidine ring. All the compounds were dissolved in DMSO and diluted with water. Neutral derivatives **1**–**3** exhibited solubility values in water:DMSO 90:10 in the low $10^{-5}$ M range (Table 1). The same compounds were subjected to methylation using methyl iodide to obtain the three corresponding fulleropyrrolidinium salts **4**–**6** (Scheme 1).

As expected, the solubility of the salts in DMSO:$H_2O$ 1:9 was found to be higher as compared to the neutral compounds (Table 1).

For comparison, the corresponding model pyrrolidines **7**–**12** have been synthesized (Scheme 2).

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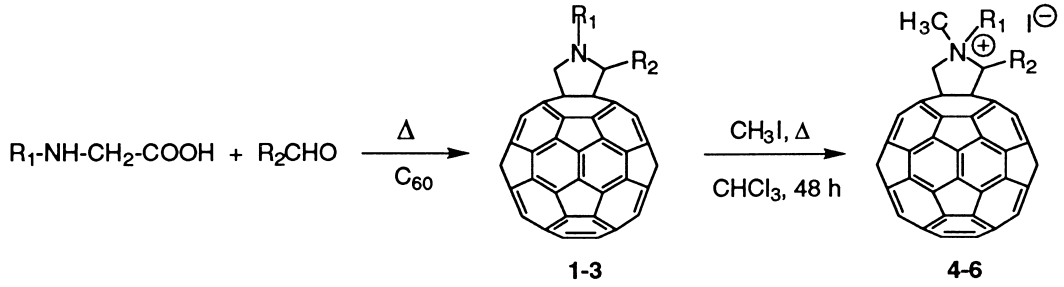



**Scheme 1.** **1,4**: $R_1 = CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$; R2 = H; **2,5**: $R_1 = CH_3$; $R_2 = CH_2OCH_2CH_2OCH_2CH_2OCH_3$; **3,6**: $R_1 = CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$; $R_2 = CH_2OCH_2CH_2OCH_2CH_2OCH_3$.

**Table 1.** Solubility of compound **1–6** in DMSO:$H_2O$ 1:9

| Compound | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| M | $1.5\times10^{-5}$ | $1.5\times10^{-5}$ | $3.0\times10^{-5}$ | $4.3\times10^{-5}$ | $1.5\times10^{-4}$ | $9.5\times10^{-5}$ |

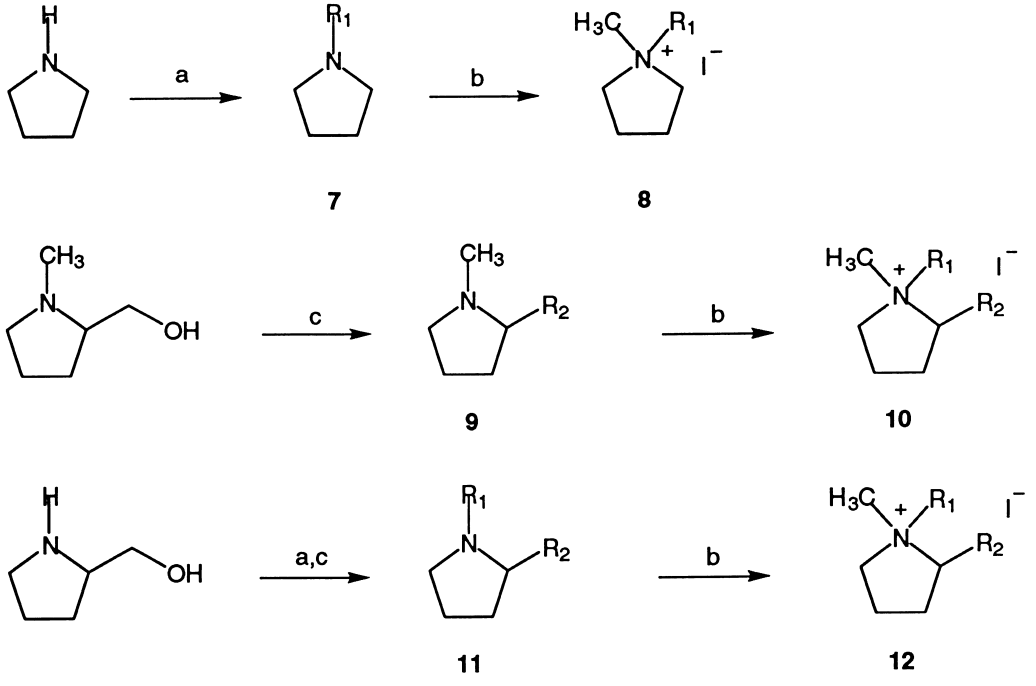
## Biological tests

Two strains of *M. tuberculosis*: strain H37Rv, reference strain, and strain H6/99, a recent clinical isolate, obtained from a pneumological clinic in Trieste, particularly virulent and resistant, and a strain of *M. avium*, strain 485, were chosen to test the salts and the most soluble fulleropyrrolidine **3**.

Each compound (5 mg) was dissolved in 10 mL of DMSO (solution A). Dilutions from solution A gave solution B (50 μg/mL), and solution C (5 μg/mL), both in DMSO:$H_2O$ 1:9. Sterile Eppendorf vials were filled with 150 μL of each fullerene solution, and with 10 μL of each different mycobacterial suspension containing $10^3$ bacteria in PBS/serum 10%.

The vials were then positioned in incubator at 37 °C for 90 min and then their content was spread on the Petri dishes filled with Mycobacteria 7H11 Agar (Difco). After 3 weeks, the Petri dishes were inspected: the results of three independent determinations are reported in Table 2. Data refer to 100% inhibition.

As already reported, compound **3** inhibits the growth of *M. avium* at moderate concentrations. However, the same compound did not show appreciable biological activity with respect to the human strains H37Rv and H6/99. Conversely, the ionic compounds **4–6**, though less active against *M. avium*, are very good inhibitors of the human strains, especially the clinical isolate H6/99. It should also be noted that compounds **7–12** were completely inactive under the same experimental conditions (Table 2), thus allowing attribution of the biological




**Scheme 2.** Reagents and conditions: (a) $CH_3OCH_2CH_2OCH_2CH_2OCH_2CHO$, $NaCNBH_3$, $CH_3OH$; (b) $CH_3I$, $CHCl_3$, 80 °C; (c) $CH_3OCH_2CH_2OCH_2CH_2Cl$, KOH, DMSO. **7,8**: $R_1 = CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$; $R_2$ = H. **9,10**: $R_1 = CH_3$; $R_2 = CH_2OCH_2CH_2OCH_2CH_2OCH_3$. **11,12**: $R_1 = CH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$; $R_2 = CH_2OCH_2CH_2OCH_2CH_2OCH_3$.

**Table 2.** Antimycobacterial activity[14] of compounds **3**–**12** and standard references[a]

| Compound | Avium | H37Rv | H6/99 |
|---|---|---|---|
| **3** | 50 | 500 | 500 |
| **4** | 500 | 50 | 500 |
| **5** | 500 | 50 | 5 |
| **6** | 50 | 50 | 5 |
| **7**–**12** | >500 | >500 | >500 |
| Isoniazide (5 μg disk) | R | S | R |
| Rifampicin (30 μg disk) | R | S | R |

[a]Minimum inhibitory concentration, MIC, μg/mL, S = sensitive, R = resistant.

activity of **4**–**6** to the fullerene core.[15] Since fullerenes become cytotoxic under irradiation, the influence of light was also examined. Before incubation, the suspension containing the *Mycobacteria* and the fullerene derivatives were irradiated for 90 min with a 100 W tungsten lamp placed at a distance of 15 cm. No difference was observed with tests performed in the dark.

At the present stage it is not clear why compound **4** does not show similar behavior as **5** and **6**. The presence of the oligoethylene glycol chain in position 2 of the pyrrolidine ring seems to play a role. Also the mechanism of action is not well understood. Both aspects are currently being investigated in our laboratories.

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15. In addition, compound **5** showed relative low toxicity in in vivo experiments. We thank Dr. F. Moussa and Prof. R. Ceolin, Faculty of Pharmacy, Paris, for disclosing to us these preliminary results.