Medicinal chemistry with fullerenes and fullerene derivatives

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Received (in Cambridge, UK) 4th December 1998, Accepted 8th February 1999

The study of the biological applications of fullerenes has attracted increasing attention despite the low solubility of the carbon spheres in physiological media. The organic functionalisation of fullerenes has helped solubilisation by covalent attachment of hydrophilic appendages. Therefore, recently synthesised fullerene derivatives reach satisfactory concentrations in water. However, the tendency of the fullerenes to form clusters is enhanced in polar media, where better solubilisation can be achieved by means of multiple functionalisation or using micellar systems. Once homogeneously dissolved, the fullerenes and fullerene derivatives exhibit an interesting range of biological activities, especially promising in the field of photodynamic therapy, HIV, neuroprotection and apoptosis.

The fullerenes, a class of spheroidally shaped molecules made exclusively of carbon atoms, were observed for the first time in 1985[1] and isolated in bulk in 1990.[2] Since then, many research groups have been eagerly looking for practical applications of these novel materials.[3] Their interesting features are at the edge of different scientific disciplines, ranging from non-linear optical properties to superconductivity. Also in the biological field the fullerenes have had a strong impact, as it was discovered that functionalised fullerenes can be used in photodynamic therapy[4] or as inhibitors of the HIV-1 protease.[5,6] However, the general excitement was not followed by a comparable outburst of results. The main reason for the slow progress in this field is generally attributed to the total absence of solubility in aqueous media, a problem that has hampered systematic testing. On the other hand, it is clear that size, hydrophobicity, three-dimensionality and electronic effects make the fullerenes very appealing subjects in medicinal chemistry.[7] For instance, it can be easily conceived that the spheroidal shape of fullerenes is potentially accommodated inside hydrophobic regions of enzymes or cells, with biological applications in different fields.

Two excellent reviews cover the main achievements in the biological application of fullerenes up to 1996.[8,9] In this article, we will provide a general overview of the current state of the art of the medicinal chemistry of fullerenes, with particular emphasis on one hand on the most recent and promising general applicative aspects and on the other hand to our own achievements in this field.

Tatiana Da Ros was born in Palmanova, Italy, in 1969. She received her Laurea degree in Pharmaceutical Chemistry from the University of Trieste in 1995 working with Professor Marco De Amici (magna cum laude). *She then took her PhD in 1999, under the supervision of Professor Maurizio Prato, her dissertation dealing with the biological properties of water-soluble fullerene derivatives. She has a research contract with the University of Trieste and is currently working in the Department of Pharmaceutical Sciences.*

Maurizio Prato took his Laurea degree in Chemistry in Padova in 1978, under the supervision of Professor Gianfranco Scorrano. He then joined the Faculty of Science and the Organic Chemistry Department at the University of Padova. He moved to the University of Trieste in 1992, where he is currently Associate Professor of Organic Chemistry. He spent sabbatical terms at Yale University in 1986–1987 (with Professor Samuel J. Danishefsky) and at the University of California, Santa Barbara in 1991–1992 (with Professor Fred Wudl). He received the Federchimica Prize in 1995. His current research interests focus mainly on functionalised fullerenes for applications in materials science and medicinal chemistry.

Solubility

There have been several attempts to overcome the natural repulsion of fullerenes for water. The most widely used methodologies are: (a) encapsulation or microencapsulation in special carriers; (b) suspension with the help of co-solvents; (c) chemical functionalisation for the introduction of solubilising appendages.

Encapsulation

It has long been known that [60]fullerene can be solubilised in water in the form of complexes with cyclodextrins[10,11] or calixarenes.[12,13] More concentrated solutions (up to 4.1×10^{-5} M) can be achieved using polyvinylpyrrolidone (PVP)[14] or artificial membranes like Triton X-100, DODAB (dimethyldioctadecylammonium bromide), DHP (dihexadecyl hydrogen phosphate) or lecithin.[15,16] Colloidal systems are formed with ammonium halides such as cetyltrimethylammonium bromide or sodium dodecyl sulfate.[17] Also co-polymers such as polyphenyl quinoline/polystyrene form self-assemblying systems with well-organised structures.[18] Incorporation of [60]fullerene and photoactive [60]fullerene derivatives in liposomes suggests that a photoexcited fullerene is capable of initiating a redox cycle that has cytochrome *c* and ubiquinone as mediators.[19]

The combination of fullerenes and lipid membranes has led to very interesting results. Lipid bilayers are dynamically mobile structures, partially ordered and of biopharmaceutical interest for covering biocompatible surfaces or for the controlled release of drugs. Hexakis adducts of [60]fullerene with an octahedral pattern, functionalised with C_{12} or C_{18} chains, have allowed the formation of lipid systems with multilamellar vesicles of dipalmitoyl-*sn*-glycero-3-phosphatidylcholine.[20]

Suspensions

Suspensions of fullerenes can be prepared from saturated benzene solutions poured into THF. The resulting mixture is

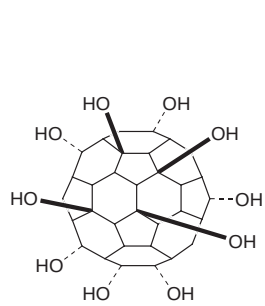
added dropwise to acetone, and then water is slowly added. A yellow suspension is formed, and the solvents are evaporated to a final known volume of water.[21]

Soluble fullerene derivatives

So far, several fullerene derivatives relatively soluble in water or aqueous mixtures have been synthesised. Fullerenols or fullerols **1**,[22] for instance, are very soluble in water, and even though their structures are not well-defined, a lot of biological studies have been carried out (see below). Water soluble polymers, covalently attached to [60]fullerene, are also easily dissolved in water.[23] Well-defined compounds solubilised in water media include **2**–**5**. Among these, compound **4** reaches a maximum concentration of 1.5×10^{-5} M in H_2O–DMSO 9 : 1.[24] The record for water solubility among monofunctionalised fullerenes so far is to be attributed to dendrimer **5**, whose solubilty in water is 34 mg ml^{-1} at pH 7.4 and an outstanding 254 mg ml^{-1} at pH 10.[25]

In conclusion, when a hydrophilic appendage is covalently attached to [60]fullerene, solubilisation in aqueous solvents is ensured. However, addition of only one solubilizing chain appears insufficient to avoid clustering. The hydrophobic carbon spheres will stick together, leaving the hydrophilic chains on the outside of the aggregate.[26,27] More importantly, formation of clusters decreases the lifetime of the excited triplet state by 2–3 orders of magnitude,[28,29] thus affecting the potential of fullerenes in photodynamic therapy (see below). The degree of aggregation can be monitored by UV–VIS spectrophotometry, pulse radiolysis or light-scattering. Clustering seems to cause broadening of the absorption bands with decrease of absorption coefficients and loss of structural features.[16,26,30,31] To avoid aggregation of the fullerene spheres, either calixarenes or cyclodextrins can be used, or surfactants.[16,29] However, the presence of bulky functionalities, able to shield the fullerene spheroid from water, produces sufficiently homogeneous solutions. Dendrimer **5** shows an almost ideal behavior, with formation of very small aggregates whose size decreases with increasing pH.[25]

Multiple functionalisation can also be helpful. By means of controlled additions it is possible to place polar groups around the fullerene spheroid. It appears that bis-functionalisation is sufficient to avoid clustering in reasonably dilute solutions.[27] However, the radical quenching ability and the singlet oxygen production of the multiple adducts may depend on the number of addends. To test this, the reaction of OH• with a series of functionalised fullerenes was studied.[32] It was found that the reaction rates decrease with an increasing number of additions, fast reactions occurring with bisadducts and much slower reactions with fullerenols.[32] On the other hand, singlet oxygen production was found to be independent of the nature of the addends, but it did decrease with an increasing number of saturated double bonds on the spheroid.[33]



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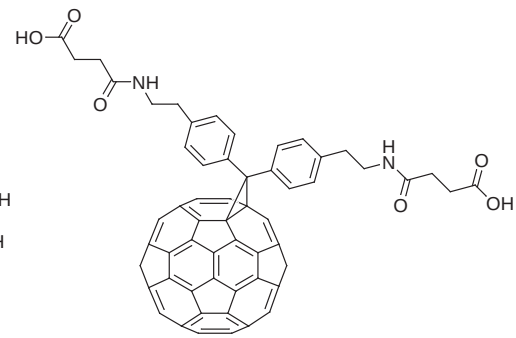

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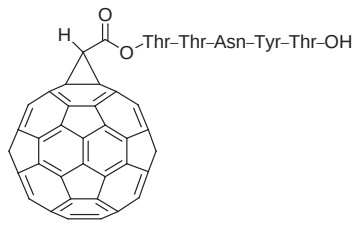

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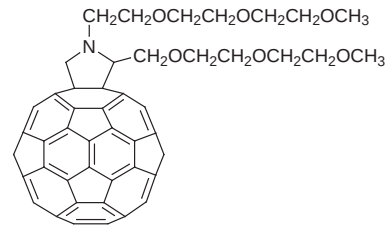

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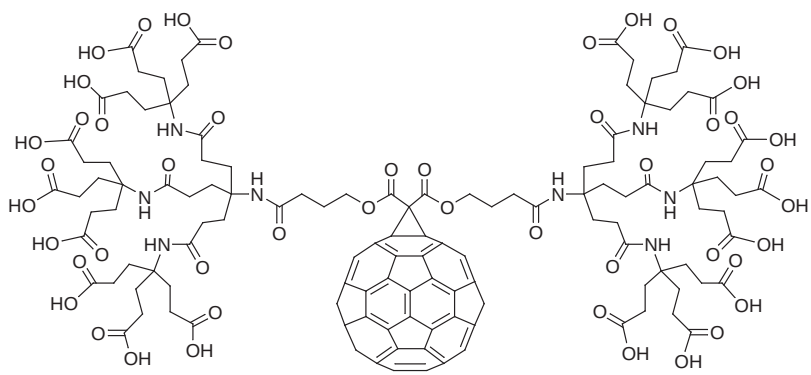
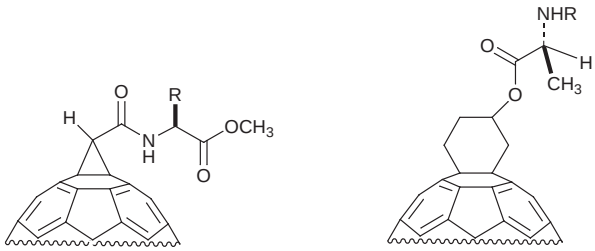


5

Fullerenic amino acids and peptides

Fullerene-based amino acids (*e.g.* **6**–**9**) have been synthesised by several groups.[34–42] The main objective of these studies is the potential incorporation of these non-natural amino acids in natural peptides and proteins.



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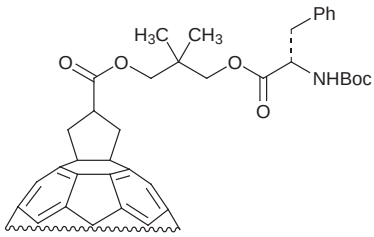

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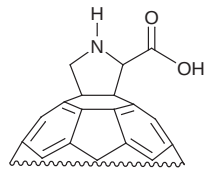
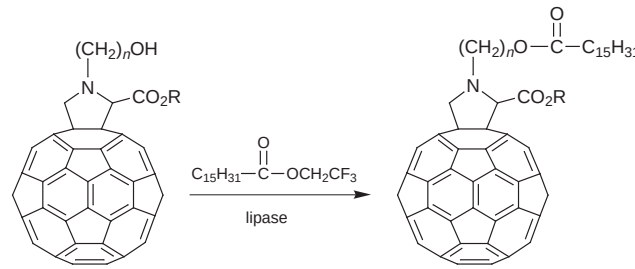


9

Fulleroproline **9** (abbreviated to Fpr using the three-letter code for amino acids), probably the biggest unnatural amino acid,[43] features a proline ring fused with the 6,6 ring junction of [60]fullerene.[37,44] Fpr forms peptides both at the amine and the carboxylic acid ends leading to di- and tri-peptides.[44–46] However, Fpr is usually prepared in racemic form, such that two diastereoisomers are formed when this amino acid is coupled with chiral amino acids. This problem can be overcome by using diastereoselective additions, starting with chiral components.[44,47] Alternatively, the diasteromeric mixtures can be separated by column chromatography or by preparative HPLC. In all cases, enantiopure fulleroprolines can be obtained.[44] Their absolute configuration can be easily established by means of circular dichroism. In all CD spectra of fulleroproline derivatives and peptides, a sharp maximum at about 428 nm was always observed. The sign of this Cotton effect is diagnostic for the determination of the absolute configuration of the chiral C^{α} atom in fulleroproline: a positive maximum is indicative of *R* configuration, whereas the *S* isomer gives rise to a negative maximum.[44]

Naturally encoded proline stands alone in the α-amino acid panorama, as it plays a central role in directing polypeptide folding and in providing conformational rigidifying effects to Pro-containing compounds.[48] Two conformational features contribute to the peculiarity of proline: (1) the propensity to induce a β-turn conformation and (2) the relatively slow rotation around the C–N tertiary amide bond, which results in *trans–cis* isomerism. Both aspects have been examined in fulleroproline peptides. A conformational study in solution has shown that the heterochiral -L-Fpr-D-Ala- sequence is mostly folded in a type-II β-turn conformation, in analogy with the known behavior of the -L-Pro-D-Ala- parent sequence.[45] On the other hand, it was found that the activation parameters for *trans–cis* isomerism in Ac-Fpr-OBu^t are very different from the model Ac-Pro-OBu^t. A difference of *ca*. 7 kcal mol^{-1} in the activation enthalpy makes the conformational interconversion much faster in the fullerene derivative.[46] This was attributed to a low level of availability of the nitrogen lone pair for carbonyl conjugation in fulleroproline.[46] In fact, the nitrogen in pyrrolidine and proline derivatives of [60]fullerene is much less basic than in the corresponding, non-fullerenic, amines.[49]

The ability of some representative hydrolytic enzymes to induce modifications in a series of fulleroprolines was tested with the aim of resolving racemic mixtures and also to obtain detailed structural information on the nature of the interaction.[50] Preliminary results show that, among the many enzymes tested, only lipase B from *Candida antarctica* (CALB) and lipoprotein lipase from *Pseudomonas specie* (LPL) were able to catalyse the acylation of fullerene derivatives, the former being more efficient (Scheme 1). However, even with these active enzymes, no reaction was observed when the functional group to be transformed is close to the fullerene spheroid (Scheme 1).



10

n = 2, 3

11

Scheme 1

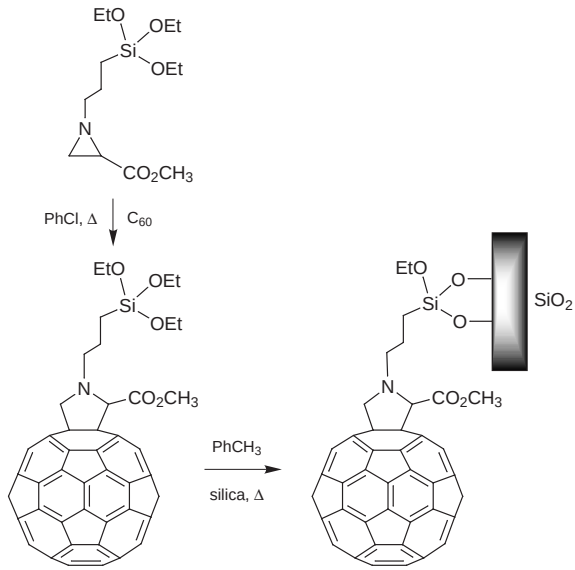
Relatively fast reactions and modest enantioselectivities (up to 45% ee) were observed for reaction centers spatially distant from the carbon sphere. These results seem to indicate that the [60]fullerene spheroid encumbers the productive fitting of the substrate either in the acyl- or alcohol-binding sites when the reaction center is close to the fullerene spheroid. This may also be due to the fact that [60]fullerene derivatives, owing to their size, cannot be easily accommodated inside the lipase active site. However, it looks as though the [60]fullerene spheroid is able to establish hydrophobic interactions with more superficial regions of the enzyme, while the proline moiety approaches the active site cavity of the lipase and the hydroxy group interacts with the catalytic triad.[50]

Other examples of fullerene–peptide conjugates have been reported. The first fulleropeptide reported contained a sequence of five hydrophobic amino acids.[51] On the basis of spectroscopic data, it was concluded that the conjugate possesses the properties of both [60]fullerene and peptide. The secondary structure adopted by the new peptide in solution was found to be the 3_{10}-helix[52] rather than the α-helix, owing to the high AIB content.[51] In a next step, the synthesis of a fullerene–peptide conjugate involving a series of highly hydrophilic amino acids was studied (**3**). The compound is reasonably soluble in water and exhibits interesting biological properties in terms of monocyte chemotaxis and anti-HIV activity (*vide infra*).[53]

More complex peptides such as alamethicin[54] and proteins such as azurin[55] have been covalently attached to [60]fullerene. IgG antibodies have been produced for the first time using [60]fullerene–bovine thyroglobulin conjugates, which could be used in very sensitive immunological procedures.[56]

Given the complexity of the biological systems, a quick way to establish potential interactions of fullerenes with enzymes and proteins would be of great help in preliminary screenings. To this aim, a stationary phase for HPLC was functionalised using a fulleroproline derivative, which, through a silicon alkoxide end group, ensures the chemical linking of the fullerene to the silica matrix (Scheme 2).[57]

The new phase is suitable for analysis in aqueous media, which represents a real practical advantage: tests of interactions of physiological solutions with fullerenes are possible without necessarily solubilizing the carbon cages in water. As a test of efficiency, a series of small peptides containing hydrophobic cavities, simulating a biological environment, was selectively recognised. Peptides characterised by electron-rich hydro-



Scheme 2

phobic clefts, thus giving rise to favourable interactions, were more strongly retained in the chromatographic runs.[57]

Toxicity studies

The absorption, distribution and excretion of fullerenes, together with their potential toxicity, are of fundamental importance in any biological evaluation and, accordingly, have been studied in detail using either unmodified [60]fullerene and water-soluble fullerene derivatives.

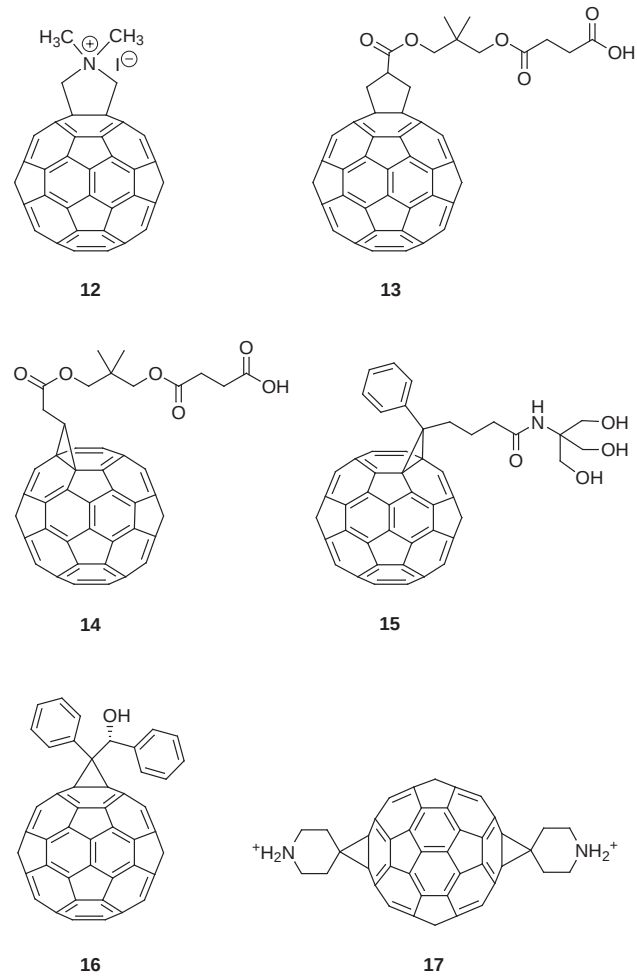
Unmodified [60]fullerene

A water suspension of ^{14}C-labelled [60]fullerene was added to a culture medium in which immortalised human keratinocytes were grown. [60]Fullerene was associated to the cells in a way dependent of time, such that after six hours about 50% of radioactivity was captured by keratinocytes.[21] No effects of [60]fullerene on the proliferation rate of keratinocytes and fibroblasts was observed, since no variations were detected on thymidine incorporation. *In vivo* tests, performed on Swiss mice and using a dispersion of micronised [60]fullerene in water containing surfactants, did not give any lethal effect, toxicity, or growth inhibition. The accumulation of [60]fullerene in fat-storing cells, in which the retinoid concentration is very high, led to an unusual metabolic path.[58] [60]Fullerene reacted *via* Diels–Alder addition, producing vitamin A derivatives. It was also found that [60]fullerene can be phagocytised by human leukocytes[59] and monocytes.[60] However, the associated usual increase of interleukin IL-1β release was not observed. This effect is not clear but it seems to be correlated with the lack of inflammatory response observed after [60]fullerene administration in Swiss mice.[61]

Hemolysis tests performed on sheep-red blood cells showed no effects of a [60]fullerene–PVP suspension.[14] The same suspension was used to study chondrogenesis in cells. Whereas PVP exhibits cytotoxicity, weakly increases chondrogenesis and decreases cell proliferation, the presence of [60]fullerene specifically increases the differentiation of LB cells (rat limb bud cells). The fullerene could then be incorporated in the endoplasmic reticulum and could promote the synthesis of proteoglycan with a mechanism similar to that known for other substances.[62] Damaging effects *in vitro* have been found in mouse MB cells (midbrain cells), since [60]fullerene decreases cell differentiation and proliferation. Embryos of pregnant mice treated *in vivo* with fullerene died with deformations: [60]fullerene was incorporated through maternal flux producing heavy disfunctions to the embryo morphogenesis.[63]

Fullerene derivatives

Pyrrolidinium salt **12** was synthesised using ^{14}C-labelled [60]fullerene.[64] It was possible to see that, after intravenous administration, the compound was rapidly adsorbed and accumulated by the liver. Only 9% of **12** was in the blood one



12 **13** **14** **15** **16** **17**

minute after application, a percentage reduced to 1.5% after 2 h. After five days, 95% of the compound was accumulated in the liver, whereas no traces were found in urine or feces.[64] Partially similar results were found for derivative **13**, differences from **12** probably arising from different hydrophobicities.[65] Oral administration of labelled **13** gave only a trace of radioactivity in the liver after 6 h, whereas 97% was excreted in 48 h, with traces in urine. Intravenous administration was followed by quick localisation in the liver with slow excretion (5.4% in the feces after 160 h). Compound **14** exhibited relative acute toxicity on ddY female mice, when administered intraperitoneally in concentrations of 200–500 mg kg^{-1}, with 5–10% weight loss, but full survival rate after a week.[65] Succinic derivative **2** (15 mg kg^{-1}) was injected intravenously in mice.[66] In this case, 99% was found to bind plasmatic proteins with fast distribution and slow excretion, with accumulation in the liver. Administration of higher doses (25 mg kg^{-1}) led to shortness of breath and death after five minutes.

Toxicity studies of fullerenols carried out intraperitoneally on male ICR mice and Sprague–Dawley rats showed that DL_{50} is 1.2 g kg^{-1} (confidence limits 0.5–2.4 g kg^{-1}). *In vivo* studies suggested that fullerenols can suppress the microsomial enzyme levels and decrease the activities of P450-dependent monooxygenase. These compounds can also inhibit mitochondrial

Mg^{2+}-ATPase activity, in a way that is dependent on fullerenol concentration, with an IC_{50} of 7.1 ± 0.3 μM. It was suggested that fullerenols can behave as artificial electron acceptors, thus decreasing the flux of reducing equivalents from NADPH to oxidase through NADPH-cytochrome P450 reductase.[67]

DNA cleavage and photodynamic therapy

The ground state absorption spectrum of [60]fullerene is characterised by intense absorptions in the UV region with weaker bands extending throughout the visible region up to 700 nm. Upon irradiation, [60]fullerene is excited to a short-lived singlet state (lifetime ≈ 1.3 ns), which converts almost quantitatively into a longer-lived triplet state (lifetime = 50–100 μs). The triplet state transfers energy very efficiently to molecular oxygen, generating singlet oxygen with almost unitary yield.[68] The triplet lifetime is fundamental for photoactivity in cells, whose viscosity is much higher than fluid solutions. In fact, it was suggested that only triplet states with lifetimes longer than 100 μs have enough cytotoxic potential.[69] Compound **14**, which has a triplet lifetime of 40 μs, was not active up to 50 μM concentrations, whereas at higher concentrations inhibited cell growth even in the absence of light.[69] The strongly oxidative properties typical of singlet oxygen are therefore considered to be responsible for a series of biological activities exhibited by fullerene solutions. In fact, the damaging effects are usually inhibited by the presence of singlet oxygen quenchers.

Supercoiled pBR322 DNA, incubated with compound **13** and irradiated by visible light, was cleaved with moderate efficiency.[4] Also membranes of cells grown on the surface of unmodified [60]fullerene were heavily damaged by photoirradiation.[70,71] Immobilised DNA on a monolayer containing [60]fullerene was cleaved.[72] DNA cleavage was improved when an acridine moiety was attached to [60]fullerene, most probably owing to the known affinity of acridine for DNA double helix.[73]

Usually, DNA cleavage occurs selectively at guanine (G) residues, but without differentiation among the various G sites.[4] Specific G residues can be recognised utilizing more complex conjugates. A [60]fullerene derivative, conjugated with 14 deoxynucleotides, was synthesised and found to possess high affinity for both single and double stranded DNA.[74] The observed increased reactivity at specific G sites was tentatively attributed to the action of singlet oxygen. A similar study by Rubin, Foote and collaborators was also reported.[75] A comparison between two identical deoxyoligonucleotide residues linked to [60]fullerene and eosin, respectively, showed a clear difference in that the latter was much less selective, cleaving a number of different G sites. The fullerene derivative gave a marked preference for the cleavage of a specific G site, namely that closest to the fullerene spheroid. The authors concluded that the cleavage cannot be attributed to the activity of singlet oxygen, an easily diffusing species whose action should be wider, but most probably is to be ascribed to a direct electron-transfer between guanosine and excited fullerene.

A direct consequence of DNA cleavage is the observed cytotoxicity of fullerene derivatives. *In vitro* tests performed on tumoral HeLa S3 cells using compound **13** confirmed that cytotoxic activity is present only in the case of irradiation.[4]

Another case of antitumoral activity was observed by Tabata *et al*.[23] Local irradiation of mice affected by fibrosarcoma and treated with a functionalised [60]fullerene derivative gave not only reduction of the tumor mass but also tumor necrosis without skin damage. It was noted that [60]fullerene accumulates in tumoral tissues not for a specific tropism, but, essentially, because of the excellent vascular permeability and the relatively immature lymphatic system of the tumoral tissues.[23]

It has also been shown that [60]fullerene deactivated enveloped viruses (Togaviridae and Rhabdoviridae) owing to the production of singlet oxygen. The deactivation was oxygen-dependent and the efficacy was maintained even in the presence of proteins.[76]

Enzymatic inhibition and anti-HIV activity

Fullerene derivatives (**13** and **14**) exhibited inhibition of cysteine (papain and cathepsin) and serine (trypsin, plasmin, trombine) proteinases.[4,9] The mechanism of action is not yet well understood, but the hydrophobicity and the electrophilicity of the fullerene spheroid seem to be responsible for inhibition.

The active site of the HIV-1 Protease (HIVP) is a quasi-spherical hydrophobic cavity, whose diameter is about 10 Å. On its surface, two amino acid residues, aspartate 25 and aspartate 125, catalyse the hydrolysis of the substrate. On the basis of molecular modeling, Friedman *et al*. were the first to recognise that the [60]fullerene spheroid can be almost perfectly accommodated inside the hydrophobic site.[5] If the interactions are sufficiently strong, inhibition of the catalytic activity of HIVP is to be expected. *In vitro* studies, performed using a 'first generation' water soluble fullerene derivative (**2**), confirmed that inhibition of acutely and cronically affected peripheral blood mononuclear cells (PBMC) indeed occurred with an EC_{50} of 7 μM.[6,77] In addition, no cytotoxic effect was recorded on non-infected PBMC. Although HIVP inhibitors currently considered for therapy are active at nanomolar or even subnanomolar concentrations, the potential use of fullerene derivatives in this field should not be disregarded, because the recent advancements in the functionalisation chemistry of fullerenes can produce novel interesting candidates. For instance, derivatisation of the fullerene at specific positions with groups that may give electrostatic interactions with Asp 25 and 125 should increase the binding constant by up to three orders of magnitude.[5] A guideline for the preparation of more active fullerene derivatives was suggested based on an increase of desolvation of the hydrophobic cavity of the enzyme and the formation of stronger hydrophobic interactions. Inside the cavity, in fact, two symmetric hydrophobic channels are not occupied by **2** because of steric hindrance. The incorporation of appropriate apolar groups on the fullerene derivative might lead to occupancy of those channels with an increase of binding constants. Calculations carried out on derivatives **16** and **17** confirmed this hypothesis, with a binding constant for **16** about fifty times higher than for **2**.[78]

Other fullerene derivatives that have been solubilised and tested as HIVP inhibitors include **15**, **14** and water soluble peptide **3**.[53] Compound **15** showed an EC_{50} of 2.5 μM without any toxicity,[79] whereas the more active derivative **14** displayed a K_i value of 0.32 μM.[9]

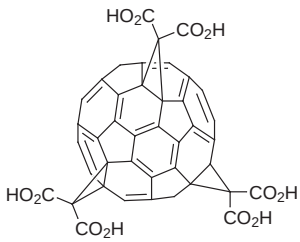
An extensive study of anti-HIVP activity was performed using a variety of functionalised [60]fullerene derivatives. For three of these compounds, an EC_{50} in the range of 0. 9–2.9 μM was observed.[80]

Neuroprotective properties

Many neurodegenerative diseases originate from excess production of superoxide and nitric oxide radicals, whose origin may be due to overexcitation of glutamic acid receptors. It has been shown that compounds that act as radical sponges reduce, though not completely, neuronal death. [60]Fullerene, owing to its antioxidant properties and high reactivity toward free radicals, shows promising behavior in this field.[81] Two derivatives of trisaddition of malonic units to [60]fullerene, **18** and **19**,[82] are highly soluble in water and are excellent radical scavengers. *In vitro* experiments using cultures of neocortical cells showed a dose-dependent decrease of neuronal death, with

derivative **18** being more active than **19**.[83] Compound **18** increases also the lifetime of cells deprived of oxygen and glucose, without antagonism between **18** and NMDA (*N*-methyl-D-aspartate) receptors. Daily administration of **18** (15 mg kg^{-1}) to G93A SOD1 G_1 mice, used as models of amyotrophic lateral sclerosis, led to an increase of lifetime of about ten days with respect to control animals.[83]

Superoxide radical quenching was also observed in a bis-malonic acid derivative of [60]fullerene.[84]



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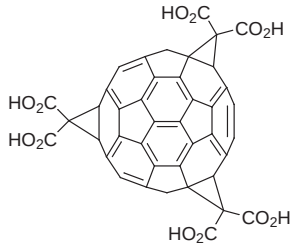

19

Antiapoptotic activity

Serum depletion induces apoptosis associated with an increase of production of free radicals. The same isomers **18** and **19** have been shown to possess antiapoptotic activity. Apoptosis due to amyloyl peptide $A\beta_{1\text{-}42}$ was also inhibited by the same fullerene derivatives.[83] In a strictly connected work, the effects of trisadducts **18** and **19** on apoptosis induced by ceramide were evaluated. In this case isomer **19** is more active.[85]

A study carried out again utilizing derivatives **18** and **19** among other antioxidant agents showed that only the fullerene derivatives inhibit apoptosis of human hepatoma Hep3B cells, induced by transforming growth factor β (TGF-β).[86] The mechanism of action is supposed to be indirect because all other actions induced by TGF-β remain unaltered.

Antibacterial activity

In preliminary tests, water soluble fullerene derivative **4** was found to be active against a variety of microorganisms. Different species of bacteria and different fungal strains were killed in a slightly modified agar diffusion test: two strains, clinical isolates CA1 and Z11, of *Candida albicans*, a fastidious pathogenic eukariote; strain ATCC 6633 of *Bacillus subtilis*, a spore-forming, Gram positive bacterium; strain AB1153 of *Escherichia coli*, a Gram negative enteric bacterium; a clinical isolate, strain 261/6 of *Mycobacterium avium*, an acid fast, emerging pathogen resistant to most antimicrobial drugs. In the latter case, 70% inhibition was observed with a concentration of 26 μg ml^{-1}, whereas complete inhibition was achieved with concentrations 10 times higher.[24]

Miscellaneous uses

Broncoconstriction induced by exsanguination in guinea-pigs was limited by fullerenols, without significant alteration of respiratory functions.[87] Fullerenols could be used in the perfusion of kidneys before transplantation, as they decreased infiltration of inflammatory cells and inhibited tubular swelling and necrosis.[88] Fullerenols were also investigated as anti-proliferative agents in arteriosclerosis, because they inhibited transduction signals.[89]

Macroscopic quantities of endohedral metallofullerenes containing Gd, Ho, Y, Ce were synthesised, in which, upon neutron activation, the inside metals become radioactive, but are still isolated from the outside world by the presence of the carbon cage. As a matter of fact, this novel class of fullerenes might be useful in nuclear medicine, with potential use as radiotracers or radiopharmaceuticals.[90]

Fullerenes can also be used for analytical purposes. Modelling studies have shown that it is possible to form a salt in which the anion is a DNA phosphate and the cation is a pyrrolidinium salt of [60]fullerene. This hybrid nanoarchitecture would be easily detected with transmission electron microscopy, since the fullerene moiety confers the necessary electron density to these complexes.[91]

Conclusions and perspectives

The impact of fullerenes and fullerene derivatives in biology will be substantiated only if their toxicity is found to be sufficiently low. Preliminary toxicological studies have shown that these novel compounds are not carcinogenic when applied on skin. Although [60]fullerene is incorporated inside cells, it does not seem to affect the proliferation rate of keratinocytes, fibroblasts or leucocytes. Studies of acute, subacute and chronic toxicity at reasonable dosing have so far given negative results. However, dose-dependent toxicity has been observed, whereas administration to pregnant mice gave rise to genetic malformations. Different toxicity levels have also been found for different derivatives.

On the other hand, once the problems of solubilisation and clustering are completely solved, and their toxicity fully tested, the fullerenes will probably find extended use in biology. In fact, owing to their ability of cleaving DNA, these carbon spheres appear promising candidates for use in diagnostics, photodynamic therapy, and as useful photoprobes in the study of genetic transcription. As radical scavengers, the fullerenes are to be considered neuroprotectors in neurodegenerative diseases and in the inhibition of apoptosis. The peculiar geometrical shape makes [60]fullerene an ideal inhibitor of HIV protease, especially if additional stabilising interactions, besides hydrophobic affinity, can be obtained.[5]

Maybe the phrase 'medicinal chemistry' used for the title of this Article is a bit premature for this young field. But we hope that this work will attract organic and medicinal chemists, as well as pharmacologists and biologists. Only their combined efforts will help create the new field of the medicinal chemistry of fullerenes.

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Paper 8/09495K