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Polycarboxylic fullerene derivatives as protein tyrosine phosphatase inhibitors

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The study of the inhibition of human protein tyrosine phosphatases by water-soluble [60]fullerene derivatives bearing five organic addends loaded with carboxylic groups demonstrated that a fullerene core is responsible for interactions with the enzymes while organic addends play a minor role modifying the selectivity of the inhibition.

Protein tyrosine phosphatases (PTPases) play an important role in cellular signaling processes and act on cell growth, development, differentiation, migration and apoptosis. These enzymes inhibit intracellular signal transmission through the dephosphorylation of phosphotyrosine amino acid residues of cellular proteins. The excessive activity of protein tyrosine phosphatases causes various diseases;[1] therefore, a search for effective inhibitors of PTPases may provide an efficient approach to the development of new drugs.[2] The protein tyrosine phosphatase CD45, also known as a leukocyte common antigen, is a transmembrane glycoprotein found in the membranes of hematopoietic cells, except mature erythrocytes and their immediate predecessors.[3] This enzyme participates in the formation of immune responses through the dephosphorylation of Src family kinases (Lck and Fyn) and, as a consequence, regulates the activity of TCRζ and ZAP-70.[4] CD45 is a therapeutic target for the treatment of IgE-mediated allergic diseases[5] and autoimmune disorders and for the prevention of graft rejection.[6] Protein tyrosine phosphatase 1B is an intracellular enzyme, which is able to dephosphorylate tyrosine residues of the insulin receptor and thereby promotes the development of type 2 diabetes.[7] A negative role of this enzyme in the development of obesity is supposed to be a result of the dephosphorylation of Janus kinase 2 (Jak2).[8] The abnormal activity of PTP1B is also associated with the appearance of breast and ovarian cancer.[9] Therefore, PTP1B is a promising therapeutic target in the treatment of diseases.[10] SHP2 is an important protein tyrosine phosphatase, which is involved in the regulation of PI 3-K/Akt and Jak2/STAT and various cellular pathways. The increased activity of SHP2 was observed in more than 50% of patients with Noonan syndrome and various cancers, including the cancer of stomach, breast cancer and leukemia.[11]

Recently, we have demonstrated that functionalized fullerene derivatives can inhibit human protein tyrosine phosphatases.[12,13] The compounds of this class are of interest as potential inhibitors of PTP1B and CD45. The aim of this work was to reveal a relationship between the molecular structures of eight polycarboxylic fullerene derivatives and their activities as inhibitors of the therapeutically important protein tyrosine phosphatases.

We have reported recently[12] that a fullerene derivative with five residues of phenylacetic acid attached to the [60]fullerene cage inhibits CD45, PTP1B and SHP2 displaying IC_{50} values in the low micromolar range. The test compounds have very similar molecular structures (Figure 1). Fullerene derivatives **1**–**8** comprising the residues of phenylacetic, phenylpropionic, phenylbutyric, naphthylacetic, biphenylacetic, phenoxyacetic, phenoxypropionic and phenoxybutyric acids, respectively, demonstrated valuable biological activities, in particular, the ability to inhibit viruses such as Herpes Simplex Virus, Cytomegalovirus and HIV.[14–16] These compounds were synthesized using previously developed procedures.[16,17] In general, chlorofullerene $C_{60}Cl_6$ was applied as a precursor in the Friedel–Crafts arylation reactions involving the esters of corresponding acids (Scheme 1). Pure esters were isolated by column chromatography. In some cases, the remaining chlorine atom was replaced with hydrogen using the treatment of the fullerene derivatives with triphenylphosphine.[18] The purified esters were hydrolyzed under acidic conditions (AcOH and HCl mixtures). The resulting carboxylic fullerene derivatives were converted to corresponding water-soluble potassium salts. All synthesized compounds were characterized by NMR spectroscopy and mass spectrometry (see Online Supplementary Materials).

Among the test enzymes, CD45, PTP1B and SHP2 were very sensitive to the fullerene-based inhibitors; therefore, these PTPases were chosen for further investigations with the use of *p*-nitrophenyl phosphate (*p*-NPP) as an enzyme substrate. The concentrations of *p*-NPP were 5 (CD45), 2 (PTP1B) and 7 mmol dm^{-3} (SHP2). The reaction mixture also contained 50 mM Bis-Tris (pH 7.2), 1 mM DTT, 0.2 mM EDTA, 100 mM NaCl and 1% DMSO. Enzyme activity was assessed spectrophotometrically by measuring the absorbance of *p*-nitrophenol (product of the substrate hydrolysis) at 410 nm. The apparent Michaelis constants (K_m) were 1.7 (PTP1B), 4.8 (CD45) and 7.5 mM (SHP2).

The artificial *p*-NPP substrate was stable under incubation with the fullerene derivatives in control experiments without the enzymes. The experimental kinetic curves of inhibition were linear. The inhibitory activities of all of the tested compounds were calculated based on the results of a dose-dependent study. The value of IC_{50} was defined as a fullerene derivative concentration that reduces the enzyme activity by 50% after 5 min of incubation of the reaction mixture (Table 1).

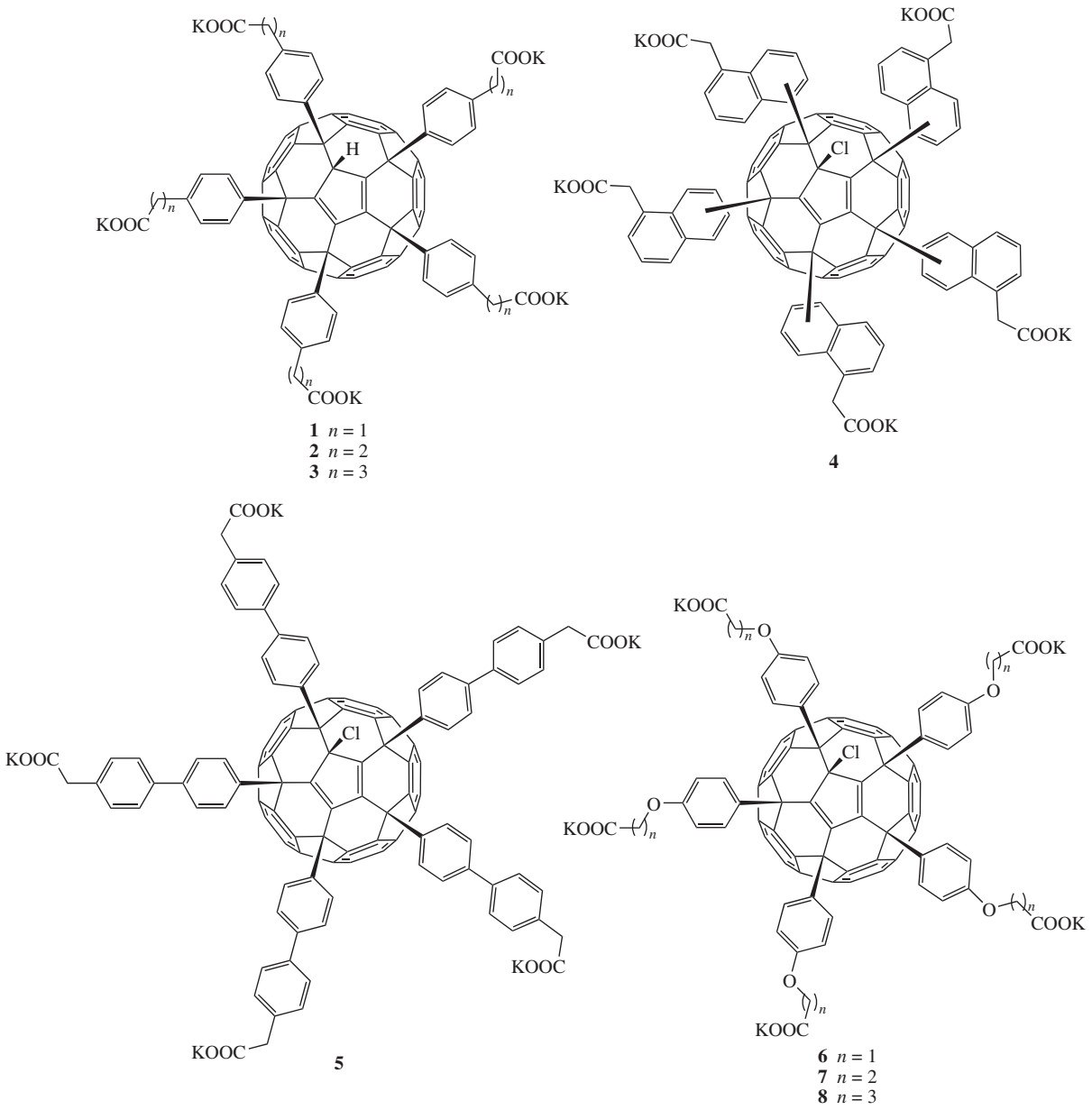


Figure 1 Molecular structures of water-soluble polycarboxylic fullerene derivatives.

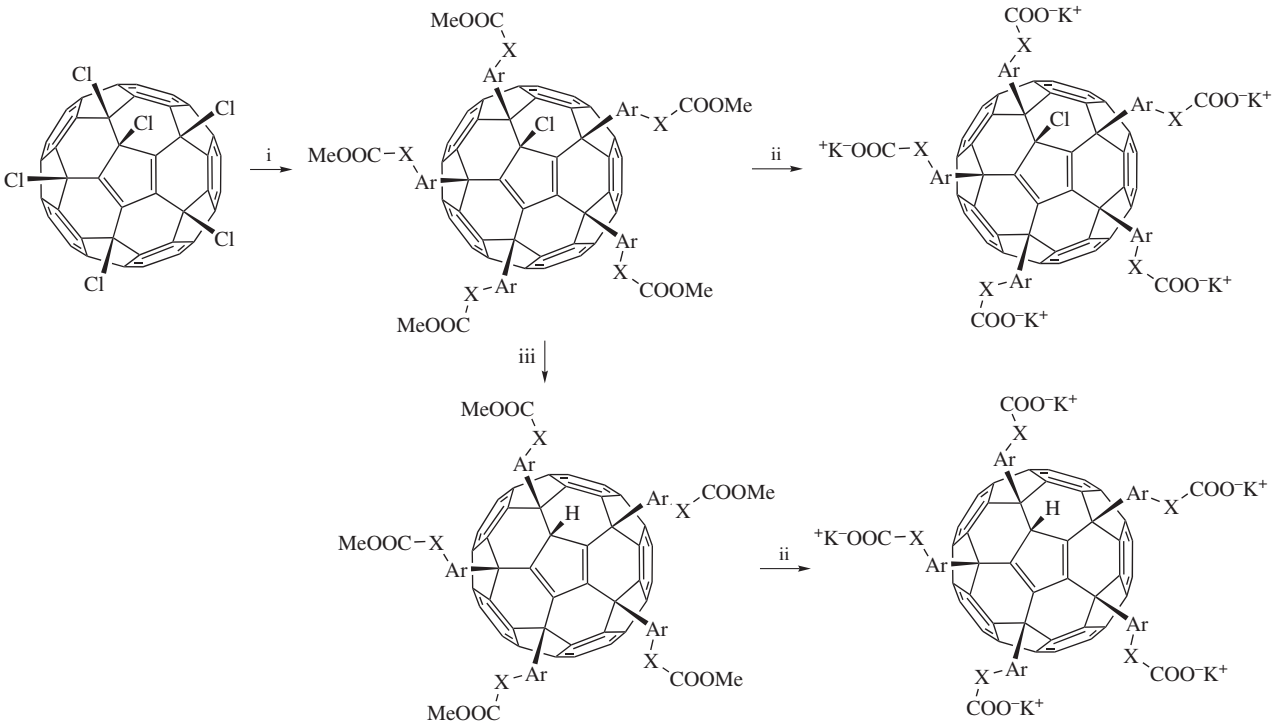
Table 1 IC_{50} values (μmol dm^{-3}) of polycarboxylic fullerene derivatives **1**–**8** as inhibitors of protein tyrosine phosphatases.[a]

Inhibitor	CD45	PTP1B	SHP2
1	0.12	0.27	0.42
2	0.68	0.89	1.92
3	0.17	0.24	1.83
4	0.05	0.32	0.38
5	0.20	1.06	7.54
6	0.79	0.94	1.24
7	1.26	2.20	2.18
8	0.12	0.97	2.67

[a] IC_{50} values are the mean of three assays with a standard deviation within 30% of the mean.

Table 1 shows that the test compounds exhibited high affinity for CD45 being less efficient inhibitors of PTP1B and SHP2. The CD45 was the most efficiently inhibited by fullerene derivative **4**, whereas compound **8** bearing phenoxybutyric acid residues was more selective. The IC_{50} value of 0.12 μmol dm^{-3} reflecting the inhibition activity of **8** with respect to the CD45 is considerably lower, as compared to the values obtained for PTP1B and SHP2 (8- and 20-fold, respectively). All investigated fullerene derivatives as inhibitors of CD45 demonstrated 10–70-fold selectivity over TC-PTP and PTPβ (data not shown). Compounds **1**, **2**, **6** and **7** manifested comparable inhibition activities with respect to CD45, PTP1B and SHP2. On the contrary, fullerene derivatives **4**, **5** and **8** showed more than 5–8-fold selectivity for CD45 over PTP1B and 7–35-fold selectivity over SHP2.

We tried to correlate the observed variations in the enzyme inhibition activities of the fullerene derivatives with their molecular structures. Considering the rows of isotypical compounds **1**, **2** and **3** on the one hand and **6**, **7** and **8** on the other, we can notice that fullerene derivatives comprising $CH_2CH_2COO^-$ fragments (**2** and **7**) demonstrated weaker inhibition activity against CD45 and PTB1B. At the same time, the efficacies of the CD45 inhibition by the fullerene derivatives bearing the residues of phenylacetic (**1**), naphthylacetic (**4**) and biphenylacetic (**5**) acids were comparable to each other thus suggesting that modification of the aromatic part of the addend has no big influence for this enzyme. On the contrary, the replacement of phenyl with biphenyl reduced noticeably the inhibition activity with respect to the SHP2 enzyme. These examples show that organic addends have some (though rather limited) effect on the enzyme inhibi-



Scheme 1 *Reagents and conditions*: i, Ar–X–COOMe, $FeCl_3$ or $SnCl_4$, $PhNO_2$, 90 °C; ii, HCl–HOAc, toluene, reflux, then K_2CO_3; iii, PPh_3.

tion activities of fullerene derivatives **1**–**8** and, most importantly, modified the selectivity of their action on different PTPases. However, considering the data in Table 1, we conclude that the major contribution to the enzyme inhibition efficiency is provided by the fullerene core, which interacts with the phosphatase CD45 and other protein tyrosine phosphatases.[12]

In conclusion, we found that polycarboxylic [60]fullerene derivatives behave as good inhibitors of CD45 and other PTPs. The selectivity of compounds **4**, **5** and **8** bearing five naphthylacetic, biphenylacetic or phenoxybutyric acid residues was observed with respect to CD45, as compared to PTP1B and SHP2. Thus, the results are expected to stimulate further research on the design of efficient nanoscale inhibitors of PTPases with possible therapeutic significance.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.mencom.2015.05.013.

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