Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 6, No. 11, pp. 1253-1256, 1996

0960-894X/96 $15.00 + 0.00

PII: S0960-894X(96)00210-7

ANTI-HUMAN IMMUNODEFICIENCY VIRUS ACTIVITY AND CYTOTOXICITY OF DERIVATIZED BUCKMINSTERFULLERENES

David I. Schuster,[a]* Stephen R. Wilson,[a]* and Raymond F. Schinazi[b]*

[a]*Department of Chemistry, New York University, New York, NY 10003*
[b]*Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine/Veterans Affairs Medical Center, Decatur, GA 30033*

Abstract: Eleven new functional derivatives of C_{60} have been tested for anti-HIV activity in peripheral blood mononuclear cells infected with HIV-1_{LAV}. Nine compounds displayed activity in the low micromolar range (EC_{50}), three of them lower than any fullerene derivative reported to date. With one exception, these compounds displayed no cytotoxicity up to 100 μM. Copyright © 1996 Elsevier Science Ltd

Interesting biological properties of fullerenes and fullerene derivatives have been demonstrated in the last few years. These are summarized in a recent review.[1] Of particular relevance to the present work was the discovery by Friedman et al.[2] that a water-soluble methanofullerene derivative **1**[3] was a competitive inhibitor of recombinant protease specific for human immunodeficiency virus (HIVP) with a K_i of 5.3 μM. This group anticipated that C_{60} should fit nicely into the hydrophobic cavity of HIVP, and they were able to fit a minimized structure of C_{60} into the enzyme active site using the program DOCK3.

Fullerene derivative **1** was tested by Schinazi et al.[4] for antiviral activity in acutely HIV-1 and HIV-2 infected human peripheral blood mononuclear cells (PBMC) and found to have a median effective concentration (EC_{50}) of 7.3 μM (Table 1) and 5.5 μM, respectively. Compound **1** was also active against chronically infected H9 cells (EC_{50} = 10.8 μM); selective activity in these cells is considered a hallmark of all protease inhibitors. Schinazi et al. also reported that **1** had anti-HIVP activity at a concentration comparable to the antiviral activity observed in infected lymphocytes. It was also shown that **1** has direct virucidal activity,[5] and similar antiviral activity against AZT-susceptible, as well as AZT-resistant HIV-1 in acutely infected primary human lymphocytes. No cytotoxicity was observed with **1** up to 100 μM in uninfected slowly dividing PBMC or rapidly dividing H9, Vero, or CEM cells, under conditions where AZT is cytotoxic in all but the first cell line. Furthermore, when **1** was administered intraperitoneally to mice in doses up to 50 mg/kg/day for 6 days, the animals all steadily gained weight and none died up to two months after initial treatment.[5]

Subsequently, the tris-hydroxymethyl methanofullerene derivative **2** was prepared and evaluated for anti-HIV activity by Schinazi, Wudl, and co-workers using the same assays as described above for **1**.[6] Compound **2** was slightly more active than **1** against HIV-1_{LAI} in human PBM cells (see Table 1) and demonstrated no toxicity up to 100 μM in two human (PBM and CEM) and one monkey (Vero) cell lines.

A number of useful thermal and photochemical reactions for functionalizing C_{60} and C_{70} have been developed in the last few years by the New York University Fullerene Group, leading to a host of new derivatives.[7] A representative group of these compounds (Figure 1) were evaluated for anti-HIV activity and for toxicity in different cells. Since stock solutions of the test materials in DMSO are used, most compounds had to be converted into derivatives possessing greater DMSO solubility, although some compounds could be used directly.

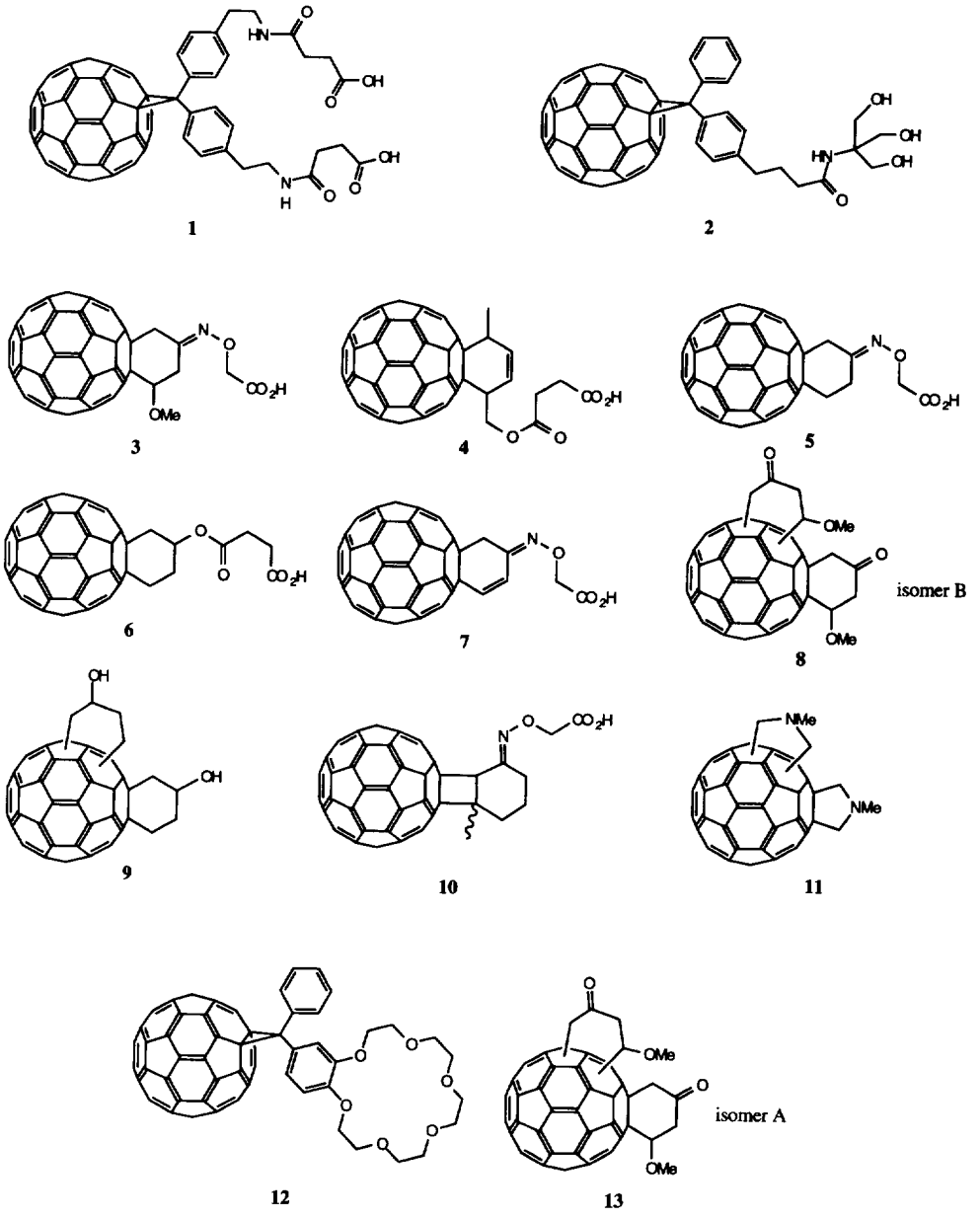

Figure 1.

RESULTS

Compounds **3**–**13** were all synthesized at NYU by Wilson, Schuster, and co-workers (see section at the end regarding preparation and characterization of these materials). These compounds were tested as DMSO/water emulsions against human peripheral blood mononuclear cells infected with HIV-1 strain LAI using procedures previously described.[4,8] As seen in Table 1, all but one of these materials showed antiviral activity as measured by EC_{50} values in the low micromolar range. The three most active fullerene derivatives in this assay were compounds **3**, **4**, and **5** (EC_{50} 0.88, 2.2, and 2.9 μM, respectively). These and all but one of the other test compounds demonstrated no toxicity in rapidly dividing Vero cells (IC_{50} > 100 μM). The mechanism of anti-HIV activity of compounds **3**–**13** has not yet been established using cell-free assays, nor has their activity been measured in acutely *vis a vis* chronically infected cells. Nonetheless, it is obvious that anti-HIV activity and low toxicity seem to be general properties of many types of C_{60} derivatives, although the exact pattern of activity may vary from compound to compound.

Table 1. Anti-HIV Activity and Toxicity of Fullerene Derivatives

Compound	Anti-HIV-1 Activity (EC_{50}, μM)[a]	Toxicity (IC_{50}, μM) PBM cells	Vero Cells
1	7.3[c]	> 100	> 100
2	2.5[d]	> 100	> 100
3	0.9	> 100	> 100
4	2.2	> 100	> 100
5	2.9	> 100	> 100
6	6.3	> 100	> 100
7	7.3	> 100	33.9
8	7.7	> 100	> 100
9	17.6	> 100	> 100
10	21.7	> 100	> 100
11	72.7	> 100	≥ 100
12	137	> 100	> 100
13	> 100	> 100	> 100
AZT	0.004	> 100	29.0

[a]Assays were conducted in human peripheral blood mononuclear cells infected with HIV-1_{LAI}, as previously described.[4,8] All dilutions and assays were carried out in the dark. [b]Toxicity was evaluated in human PBM cells and in rapidly dividing Vero cells (African Green monkeys kidney cells), as previously described.[4,8] [c]Data from references 4 and 5. [d]Data from ref 6.

An important structural feature of most of these new fullerene derivatives is that they are chiral, in contrast to **1** and **2**. Thus, it will be possible to determine the anti-HIV activity of the enantiomers of **3** and **4**, for example, in both cell-based and cell-free assays. The presence or absence of enantiospecifity in these bioassays will shed further light on the nature of the molecular interactions responsible for the observed anti-HIV activity. It will also be interesting to determine if these and other fullerene derivatives have activity against other viruses.

Preparation of Fullerene Derivatives. Compound **3** was prepared by addition of carboxymethoxylamine (CMA) with a methoxy letone from Diels–Alder reaction of Danishefsky's diene to C_{60}.[7e] Compound **4** was prepared by reaction of succinic anhydridewith the Diels–Alder adduct of $CH_3CH{=}CH{-}CH{=}CH_2OH$ with C_{60}.[7e] Elimination of methanol from the methoxy ketone from Diels–Alder reaction of Danishefsky's diene with C_{60}, followed by reaction with carboxymethoxyl-amine (CMA) gave compound **7**. Reduction of this ketone to the cyclohexanone followed by reaction with CMA afforded **5**.[7e] This same ketone can be prepared by addition of $CH_2{=}CH(OSiMe_3){-}CH{=}CH_2$ to C_{60} followed by hydrolysis, as reported by Rubin.[9] Reduction of the ketone to the alcohol with sodium borohydride, followed by reaction with succinic anhydride gave **6**.[7e] Compound **8** with undetermined regiochemistry[10] is a bis-adduct of

Danishefsky's diene and C_{60}. Compound **9** is derived from **8** by treatment with acid and borohydride reduction.[7c] Photoaddition of 3-methylcyclohexenone to C_{60} affords a mixture of *cis*- and *trans*-fused [2+2] adducts,[7a] which gave the mixture of diasteromers **10** by reaction with CMA.[7c] Bis-addition of the azomethine ylide CH_2=N^+Me-CH_2^- (derived from *N*-methylglycine and formaldehyde)[11] gives a mixture of regioisomers **11**[12] which were submitted for testing without further purification. The preparation of crown ether **12** has been previously reported.[7b] Compound **13** with undetermined regiochemistry is the hydrolysis product of the principal bis adduct of Danishefsky's diene and C_{60}. All compounds were characterized by electrospray mass spectrometry, either directly or after tagging with a diazo crown ether reagent,[7b] and by their ^{1}H NMR spectra.

Acknowledgments. We wish to thank A. McMillan, S. Schlueter Wurtz, and A. Juodawlkis for excellent technical assistance. RFS is supported by the Georgia VA Research Center for AIDS and HIV infections. DIS and RSW are grateful to the National Science Foundation for support of fullerene research at NYU.

References

1. Jensen, A. W.; Wilson, S. R.; Schuster, D. I. *Bioorg. Med. Chem.* **1996**, in press.
2. Friedman, S. H.; DeCamp, D. L.; Sijbesma, R. P.; Srdanov, G.; Wudl, F.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6506.
3. Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510.
4. Schinazi, R. F.; Sijbesma, R.; Srdanov, G.; Hill, C. L.; Wudl, F. *Antimicrob. Agents Chemother.* **1993**, *37*, 1707.
5. Schinazi, R. F.; McMillan, A.; Juodawlkis, A.S.; Pharr, J.; Sijbesma, R.; Srdanov, G.; Hummelen, J.-C.; Boudinot, F. D.; Hill, C. L.; Wudl, F. In *Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Ruoff, R. S.; Kadish, K. M., Eds.; The Electrochemical Soc.: Pennington, 1994; pp 689–696.
6. Schinazi, R. F.; Bellavia, C.; Gonzalez, R.; Hill, C. L.; Wudl, F. *Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Ruoff, R. S.; Kadish, K. M., Eds.; The Electrochemical Soc.: Pennington, 1995; pp 696–698.
7. (a) Wilson, S.R.; Kaprinidis, N.A.; Wu, Y.; Schuster, D. I. *J. Am. Chem. Soc.* **1993**, *115*, 8595. (b) Wilson, S. R.; Wu, Y. *J. Chem. Soc., Chem. Commun.* **1993**, *9*, 784. (c) Wilson, S. R.; Lu, Q. *Tetrahedron Lett.* **1993**, *34*, 8043. (d) Wilson, S. R.; Cao, J.; Chin, E.; Saneii, H.; Peterson, M. L.; Healy, E. In *Recent Advances in the Chemistry and Physics of Fullerenes and Related Materials*; Kadish, K. M.; Ruoff, R. S., Eds.; Electrochemical Society Proceedings: Pennington, 1995; pp 22–27. (e) Lu, Q. Ph.D. Dissertation, New York Unversity, May 1995. (f) Wilson, S. R.; Lu, Q. *J. Org. Chem.* **1995**, *60*, 6496; (g) Wilson, S. R.; Lu, Q. *Tetrahedron Lett.* **1995**, *36*, 5707. (h) Wang, Y.; Cao, J.; Schuster, D. I.; Wilson, S. R. *Tetrahedron Lett.* **1995**, *38*, 6843. (h) Wilson, S. R.: Wang, Y.; Cao, J.; Tan, X. *Tetrahedron Lett.* **1996**, *37*, 775; (i) Schuster, D. I.; Cao, J.; Kaprinidis, N.; Wu, Y.; Jensen, A. W.; Wilson, S. R. *J. Am. Chem. Soc.* **1996**, in press.
8. Schinazi, R. F.; Sommadossi, J.-P.; Saalmann, V.; Cannon, D. L.; Xie, M.-Y.; Hart, G. C.; Smith, G. A.; Hahn, E. F. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061; Schinazi, R. F.; McMillan, A.; Cannon, D. C.; Mathis, R.; Lloyd, R. M., Jr.; Peck, A.; Sommadossi, J.-P.; St. Clair, M.; Wilson, J.; Furman, P. A.; Painter, G.; Choi, W-B.; Liotta, D. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 2423.
9. An. Y.-Z.; Anderson, J. L.; Rubin, Y. *J. Org. Chem.* **1993**, *58*, 4799.
10. (a) Hirsch, A.; Lamparth, I.; Karfunkel, H. R. *Angew. Chem.* **1994**, *105*, 453; *Angew. Chem. Intl. Ed. Engl.* **1994**, *33*, 437; (b) Hirsch, A. *The Chemistry of Fullerenes*; G. Thieme Verlag: Stuttgart, New York, 1994.
11. Maggini, M.; Scorrano, G.; Prato, M. *J. Am. Chem. Soc.* **1993**, *115*, 9798; see also ref 10b.
12. Lu, Q. Ph. D. Dissertation, New York University, Feb. 1996.

(*Received in USA* 10 *April* 1996; *accepted* 29 *April* 1996)