stance K,[74] angiotensin,[75] and the light receptor, rhodopsin,[76] which interacts with the G-protein homologue transducin. As mentioned above, structurally, rhodopsin may be taken as a prototype for the G-protein-linked receptor superfamily. In view of the studies pointing to the importance of the β-adenergic receptor domain C-III for G-protein interactions, it will be of great interest to examine the consequences of altering the homologous domain in the other receptors. In addition, since these receptors are thought to interact with distinct G-proteins,[18-20] it will be important to search for distinct sequences in the C-III domain region that confer G-protein selectivity. Reciprocally, structure–activity studies of the several G-proteins themselves should reveal the G-protein sequences that lead to a selective interaction of these proteins with their activating receptors.

6.0 Summary and Implications for Future Work

As outlined in sections 3.0–5.0, there are now a number of good examples of studies identifying specific receptor sequences involved in the domain functions discussed in section 2.0, and a number of the questions outlined in section 2.0 are, in part, being answered. The implications of these studies are at least 2-fold in terms of future work that can be done using the approaches outlined in this section. First, using site-directed and deletional mutational analysis of receptor sequences, it should be possible to determine with precision the functional role of specific amino acid residues, for instance as has been done for the ATP-binding function of lysine 721 of the EGF-URO receptor and for lysine 1030 of the insulin receptor. Such studies will very likely be complemented in the future by a crystallographic examination of receptor structure, as has been done for a variety of enzymes. Thus, a satisfying picture of the molecular basis of receptor function should emerge. A second implication of the studies relates to the substituents in or near the plasma membrane, with which specific receptors interact. For, once the domains on the receptor and on the interacting protein have been identified (e.g. domain C-III of the adrenergic receptor and a complementary G-protein domain), it may prove possible to design specific reagents related to these sequences that can modulate receptor–effector interactions. In terms of analogous enzyme–peptide substrate interactions of medical significance, one can point to the development of angiotensin converting enzyme inhibitors that are proving of enormous use in the treatment of hypertension. Thus, it may not be overly optimistic to hope for the development of specific compounds that may be able to regulate the interactions of specific cellular substrates with receptor tyrosine kinases. Such compounds might prove of use in controlling the oncogenic process that appears to result from the aberrant overproduction of tyrosine kinase receptor domains (e.g. the erythroleukemia virus erb-B counterpart of the EGF-URO receptor[13-16,58]). In a similar vein, studies of receptor domain function may lead to a better understanding of the interaction of peptide agonists (insulin, EGF-URO, etc.) with their receptors, so as to provide a novel basis for the design of new peptide antagonists and agonists. Overall, one can look forward with excitement to new developments in the area of receptor structure–activity studies, for which it can be said that a new era is just beginning.

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(72) Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. *Nature* **1988**, *335*, 358.
(73) Julius, D.; MacDermott, A. B.; Axel, R.; Jessell, T. M. *Science* **1988**, *241*, 558.
(74) Masu, Y.; Nakayama, K.; Tamaki, H.; Harada, Y.; Kuno, M.; Nakanishi, S. *Nature* **1987**, *329*, 836.
(75) Jackson, T. R.; Blair, L. A. C.; Marshall, J.; Goedert, M.; Hanley, M. R. *Nature* **1988**, *335*, 437.
(76) Dratz, E. A.; Hargrave, P. A. *Trends Biochem. Sci.* **1983**, *8*, 128.

Communications to the Editor

Cyclobut-A and Cyclobut-G: Broad-Spectrum Antiviral Agents with Potential Utility for the Therapy of AIDS

Sir:

In addition to the human immunodeficiency virus (HIV), virtually all adults with the acquired immunodeficiency syndrome (AIDS)[1] have been infected with one or more herpesviruses.[2] Cytomegalovirus (CMV) may threaten up to 25% of AIDS patients with blindness or death,[3] and at autopsy, evidence of active CMV infections is found with frequencies as high as 90%.[4] Although relatively benign in immunocompetent individuals, herpes simplex viruses (HSV-1 and HSV-2) can cause chronic ulcerative lesions in the immunocompromised.[3] Reactivation of varicella-zoster virus (VZV) afflicts many AIDS patients with painful vesicular eruptions.[3] Epstein-Barr virus (EBV) has been associated with AIDS related hairy leukoplakia and non-Hodgkin's lymphomas.[5]

Besides these direct contributions to morbidity and mortality, herpesviruses may play a more insidious role in the pathogenesis of AIDS by enhancing the replication and

(1) *AIDS: Modern Concepts and Therapeutic Challenges*; Broder, S., Ed.; Marcel Dekker: New York, 1987.
(2) (a) Quinnan, G. V.; Masur, H.; Rook, A. H.; Armstrong, G.; Frederick, W. R.; Epstein, J.; Manischewitz, J. F.; Macher, A. M.; Jackson, L.; Ames, J.; Smith, H. A.; Parker, M.; Pearson, G. R.; Parillo, J.; Mitchell, C.; Straus, S. E. *J. Am. Med. Assoc.* **1984**, *252*, 72–77. (b) Quinn, T. C.; Piot, P.; McCormick, J. B.; Feinsod, F. M.; Taelman, H.; Kapita, B.; Stevens, W.; Fauci, A. S. *J. Am. Med. Assoc.* **1987**, *257*, 2617–2621.
(3) Drew, W. L.; Buhles, W.; Erlich, K. S. In *The Medical Management of AIDS*; Sande, M. A., Volberding, P. A., Eds.; W. B. Saunders: Philadelphia 1988; Chapter 22.
(4) Macher, A. M.; Reichert, C. M.; Straus, S. E.; Longo, D. L.; Parrillo, J.; Lane, H. C.; Fauci, A. S.; Rook, A. H.; Manischewitz, J. F.; Quinnan, G. V. *N. Engl. J. Med.* **1983**, *309*, 1454.
(5) Ernberg, I.; Altiok, E. *APMIS* **1989**, *Suppl. 8*, 58–61 and references therein.

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Table I. In Vitro Antiviral Activity of Cyclobut-A and Cyclobut-G against Herpesviruses

	cyclobut-A		cyclobut-G		acyclovir/ganciclovir[a]	
assay[b]	ED_{50}[c]	ID_{50}[d]	ED_{50}	ID_{50}	ED_{50}	ID_{50}
HSV-1[e]						
E-377	1.0	>100	0.05	>100	0.08	>100
HSV-2[e]						
MS	1.6	>100	0.07	>100	0.09	>100
X-79	1.8	>100	0.06	>100	0.10	>100
JEN	2.3	>100	0.05	>100	0.07	>100
HEET	2.2	>100	0.07	>100	0.06	>100
HCMV[e]						
AD169	3.7	>100	6.2	>100	2.7[a]	>100
Davis	0.9	>100	4.9	>100	3.6[a]	>100
EC	2.2	>100	3.9	>100	2.8[a]	>100
LA	1.0	>100	1.2	>100	2.4[a]	>100
CH	0.9	>100			3.1[a]	>100
MCMV[f]						
Smith	0.05	>100	0.10	>100	1.0[a]	>100
VZV[e]						
Ellen	2.1	>100	0.40	>100	2.3	>100
OKA	2.0	>100	0.40	>100	3.7	>100
EBV[g]						
Raji cells			0.01	94	3.8	>100
cell proliferation[h]		7.7		27.0		165/43[f]

[a] Ganciclovir. [b] Mean of two to four assays. [c] Drug concentration (μg/mL) calculated to reduce plaque formation (or antigen production for EBV) in infected cell monolayers to 50% of untreated, infected controls. [d] For antiviral assays, the drug concentration (μg/mL) calculated to reduce uptake of neutral red stain by uninfected cell monolayers to 50% of untreated, uninfected controls; for the cell proliferation assay, the drug concentration (μg/mL) calculated to reduce proliferation of human foreskin fibroblasts to 50% of untreated controls. [e] Plaque reduction assay in human foreskin fibroblasts. [f] Plaque reduction assay in mouse embryo fibroblasts. [g] Inhibition of diffuse early antigen production assayed by immunofluorescent monoclonal antibody. [h] Human foreskin fibroblasts at 25% confluency were incubated in the presence and absence of serial dilutions of drug. After 72 h, the cultures were trypsinized and the number of cells determined with a Coulter counter. The 50% inhibitory dose was calculated from comparison of the number of cells in drug-treated and untreated cultures.

cytopathic effects of HIV.[6] The ability of herpesvirus regulatory proteins to transactivate the HIV long terminal repeat is firmly established and suggests a mechanism by which active HIV replication could be triggered in the clinically latent phase of the disease.[7] In vitro, bidirectional interactions between HIV and CMV[8] and HIV and human herpesvirus type 6 (HHV-6)[9] have been shown to enhance viral replication and accelerate cell death. Indeed, cells dually infected with HIV and CMV have been found in some AIDS patients,[10] and their occurrence appears to correlate with more rapid progression of retinal necrosis.[10b] In the absence of coinfected cells, HIV replication could also be stimulated indirectly by cytokines generated by the immune response to herpesvirus infections.[7]

Given the complex involvement of herpesviruses in the pathogenesis of AIDS, an agent capable of controlling the replication of both HIV and herpesviruses may offer special benefits in AIDS therapy. In response to this need, we report here the synthesis and broad-spectrum antiviral activity of two oxetanocin[11] analogues, (±)-9-[(1β,2α,3β)-2,3-bis(hydroxymethyl)-1-cyclobutyl]adenine ((±)-cyclobut-A, **4**) and (±)-9-[(1β,2α,3β)-2,3-bis(hydroxymethyl)-1-cyclobutyl]guanine ((±)-cyclobut-G, **5**).[12]

As outlined in Scheme I, the known condensation of allene and diethyl fumarate gave ready access to molar quantities of the racemic cyclobutane **1**.[13] Standard functional group manipulations led to the *O*-methyloxime **2** as a mixture of *Z* and *E* isomers. In the key step, re-

(6) Fauci, A. S. *Clin. Res.* **1987**, *35*, 503–510.
(7) Rosenberg, Z. F.; Fauci, A. S. *Aids Res. Human Retroviruses* **1989**, *5*, 1–4.
(8) Skolnik, P. R.; Kosloff, B. R.; Hirsch, M. S. *J. Infect. Dis.* **1988**, *157*, 508–514.
(9) Lusso, P.; Ensoli, B.; Markham, P. D.; Ablashi, D. V.; Salahuddin, S. Z.; Tschachler, E.; Wong-Staal, F.; Gallo, R. C. *Nature* **1989**, *337*, 370–373.
(10) (a) Nelson, J. A.; Reynolds-Kohler, C.; Oldstone, M. B. A.; Wiley, C. A. *Virology* **1988**, 286–290. (b) Skolnik, P. R.; Pomerantz, R. J.; de la Monte, S.; Lee, S. F.; Hsiung, G. D.; Foos, R. Y.; Cowan, G. M.; Kosloff, B. R.; Jirsch, M. R.; Pepose, J. S. *Am. J. Opthalmol.* **1989**, *107*, 361–372.
(11) (a) Shimada, N.; Hasegawa, S.; Harada, T.; Tomisawa, T.; Fugii, A.; Takita, T. *J. Antibiotics* **1986**, *39*, 1623–1625. (b) Nakamura, H.; Hasegawa, S.; Shimada, N.; Fujii, A.; Takita, T.; Iitaka, Y. *J. Antibiotics* **1986**, *39*, 1626–1629.
(12) (a) The synthesis and antiviral properties of cyclobut-A, cyclobut-G, and related compounds were presented in part at the 4th Cyprus Conference on New Methods in Drug Research, Paphos, Cyprus, May 21–27, 1989, and at the 5th International Conference on AIDS, Montreal, Quebec, June 5–9, 1989. (b) Norbeck, D.; Rosenbrook, W.; Plattner, J.; Erickson, J.; Arnett, G.; Shannon, W. *Abstracts of Papers*, 5th International Conference on AIDS, Montreal, Quebec; International Development Research Center: Ottawa, Ontario, 1989; M. C. P. 65. (c) Hayashi, S.; Norbeck, D. W.; Plattner, J.; Broder, S.; Mitsuya, H. *Abstracts of Papers*, 5th International Conference on AIDS, Montreal, Quebec; International Development Research Center: Ottawa, Ontario, 1989; M. C. P. 135. (d) Independent data confirming the anti-herpesvirus activity of these compounds was presented at the 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX, September 17–20, 1989. (e) Slusarchyk, W. A.; Bisacchi, G. S.; Hockstein, D. R.; Young, M. G.; Field, A. K.; McGeever-Rubin, B.; Tuomari, A. V.; Zahler, R. *Abstracts of Papers*, 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, Texas; American Society for Microbiology: Washington, DC, 1989; #1330. (f) Field, A. K.; Tuomari, A. V.; Mazina, K. E.; McGeever-Rubin, B.; Hagen, M. E.; Haffey, M. L.; Zahler, R.; Terry, B. J. *Abstracts of Papers*, 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX; American Society for Microbiology: Washington, DC, 1989; #1331. (g) Braitman, A.; Clark, J. M.; Toumari, A. V.; Lynch, J. S.; McGeever-Rubin, B.; Bonner, D. P.; Field, A. K. *Abstracts of Papers*, 29th Interscience Conference on Antimicrobial Agents and Chemotherapy, Houston, TX; American Society for Microbiology: Washington, DC, 1989; #1332. (h) A nonstereoselective synthesis of cyclobut-A has also been reported: (i) Honjo, M.; Maruyama, T.; Sata, Y.; Horii, T. *Chem. Pharm. Bull.* **1989**, *37*, 1413–1415.
(13) Cripps, H. N.; Williams, J. K.; Sharkey, W. H. *J. Am. Chem. Soc.* **1959**, *81*, 2723–2728.

Table II. In Vitro Antiviral Activity of Cyclobut-A and Cyclobut-G against HIV-1 in ATH8 Cells[a]

	cyclobut-A				cyclobut-G				ddA				ddG				AZT			
concn, μM	1	10	50	100	0.5	1	10	100	2	10	100	200	2	10	100	500	1	5	20	100
protection,[b] %	47	63	48	52	29	100	46	11	69	99	100	99	54	100	99	48	65	96	65	47
cytotoxicity,[c] %	11	32	42	44	8	0	41	83	5	0	8	0	0	0	4	50	3	30	54	54

[a] 2′,3′-Dideoxyadenosine (ddA), 2′,3′-dideoxyguanosine (ddG), and 3′-azido-3′-deoxythymidine (AZT) are shown for reference. ATH8 cells were exposed to HIV-1/IIIB (1000 viral particles/cell; 1.4×10^4 $TCID_{50}$/cell) and cultured in the presence of various concentrations of drug. Control cells were treated similarly but not exposed to the virus. Total viable cells were counted on day 7 by the trypan blue exclusion method as previously described by Mitsuya et al.[19] Each compound was tested more than four times; representative data are shown. [b] The percentage protection was determined by the following formula: 100 × [(number of viable cells exposed to HIV-1 and cultured in the presence of the compound) − (number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]/[(number of viable cells cultured alone) − (number of viable cells exposed to HIV-1 and cultured in the absence of the compound)]. Calculated percentages greater than or equal to 100% are expressed as 100%. [c] The percentage cytotoxicity was determined by the following formula: 100 × [1 − (number of total viable cells cultured in the presence of compound)/(number of total viable cells cultured alone)]. Calculated percentages equal to or less than 0% are expressed as 0%. Percent toxicities less than 10% are not biologically significant in this assay system.

Table III. Activity of Cyclobut-A and Cyclobut-G against HSV-2 Encephalitis in Mice[a]

compound	dose,[b] mg/kg per day	MDD[c]	p value	survivors/treated	p value	ED_{50}, mg/kg per day
cyclobut-A	125			10/10	<0.001	9.7
	31.2	10.0	ns[d]	9/10	<0.001	
	7.8	10.8	ns	5/10	ns	
cyclobut-G	125	16.3	<0.05	8/10	<0.005	39.8
	31.2	11.2	ns	4/10	ns	
	7.8	12.1	ns	5/10	ns	
ara-A	125	11.1	ns	3/10	ns	>125
	31.2	9.3	ns	0/10	ns	
	7.8	7.9	ns	0/10	ns	
control		8.6		0/10		

[a] Infection level: 100 LD_{50} doses (1.3×10^3 PFU/mouse) determined by in vivo titration. [b] Agents administered BID intraperitoneally for 5 days beginning 7 h postinfection. [c] Mean day to death of nonsurvivors. [d] Not significant.

Table IV. Activity of Cyclobut-A and Cyclobut-G against Systemic HSV-1 Infection in Mice[a]

compound	dose,[b] mg/kg per day	MDD[c]	p value	survivors/treated	p value	ED_{50}, mg/kg per day
cyclobut-A	75			10/10	<0.001	<4.7
	18.7			10/10	<0.001	
	4.7	7.5	ns[d]	6/10	<0.05	
cyclobut-G	18.7			10/10	<0.001	1.9
	4.7	9.3	ns	7/10	<0.005	
	1.2	9.3	<0.05	4/10	ns	
ara-A	50			10/10	<0.001	5.2
	12.5	13.5	<0.05	8/10	0.001	
	3.1	6.9	ns	3/10	ns	
acyclovir	200	12.3	0.001	4/10	ns	189.9
	50	8.0	<0.05	0/10	ns	
	12.5	7.8	ns	2/10	ns	
control		6.7		0/10		

[a] Infection level: 10 LD_{50} doses (1.4×10^4 PFU/mouse) determined by in vivo titration. [b] Agents administered BID intraperitoneally for 4 days beginning 7 h postinfection. [c] Mean day to death of nonsurvivors. [d] Not significant.

duction of **2** with sodium (trifluoroacetoxy)borohydride[14] generated the cyclobutylamine **3** with complete stereoselectivity.[15] Adaptations of literature procedures[16] for the annelation of the adenine and guanine bases onto alkylamines provided cyclobut-A and cyclobut-G as white, crystalline solids.[17]

Direct comparisons of the in vitro anti-herpesvirus activities of cyclobut-A and cyclobut-G to those of acyclovir[18] and ganciclovir[18] are summarized in Table I. Cyclobut-G had similar potency to acyclovir against HSV-1 and HSV-2 and was more potent than acyclovir against VZV and EBV. Cyclobut-A was more potent than acyclovir against VZV, but less potent against HSV-1 and HSV-2. Both cyclobut-A and cyclobut-G displayed excellent activity against human and murine CMV, and cyclobut-A appeared to be more potent than ganciclovir against most of the strains examined. The concentrations of cyclobut-A and cyclobut-G required to impair the viability of mouse embryo and human foreskin fibroblast monolayers generally exceeded the effective antiviral concentrations by at least 50-fold; as expected, proliferating cells were more sensitive to inhibition, especially by cyclobut-A.

(14) Umino, N.; Iwakuman, T.; Ikezaki, M.; Itoh, N. *Chem. Pharm. Bull.* **1978**, *26*, 2897–2989.

(15) The relative stereochemistry of the amine **3** was unambiguously established by X-ray crystallographic analysis of the hydrochloride salt of cyclobut-G (**5**). Spanton, S. Unpublished results.

(16) (a) Marquez, V. E.; Lim, M.-I. *Med. Res. Rev.* **1986**, *6*, 1–40. (b) Constant, J. F.; Carden, B. M.; Lhomme, J. *J. Heterocycl. Chem.* **1985**, *22*, 1035–1040.

(17) (a) Cyclobut-A: crystallized from MeOH, mp 181–183 °C; FAB MS, exact mass calcd for $C_{11}H_{16}N_5O_2$ $(M + H)^+$ 250.1304, found 250.1303; analysis for $C_{11}H_{15}N_5O_2$ (C, H, N). (b) Cyclobut-G: crystallized from H_2O, mp >220 °C; FAB MS, exact mass calcd for $C_{11}H_{16}N_5O_3$ $(M + H)^+$ 266.1253, found 266.1248; analysis for $C_{11}H_{15}N_5O_3 \cdot 0.35H_2O$ (C, H, N). 300-MHz ^1H NMR spectra were fully consistent with the proposed structures.

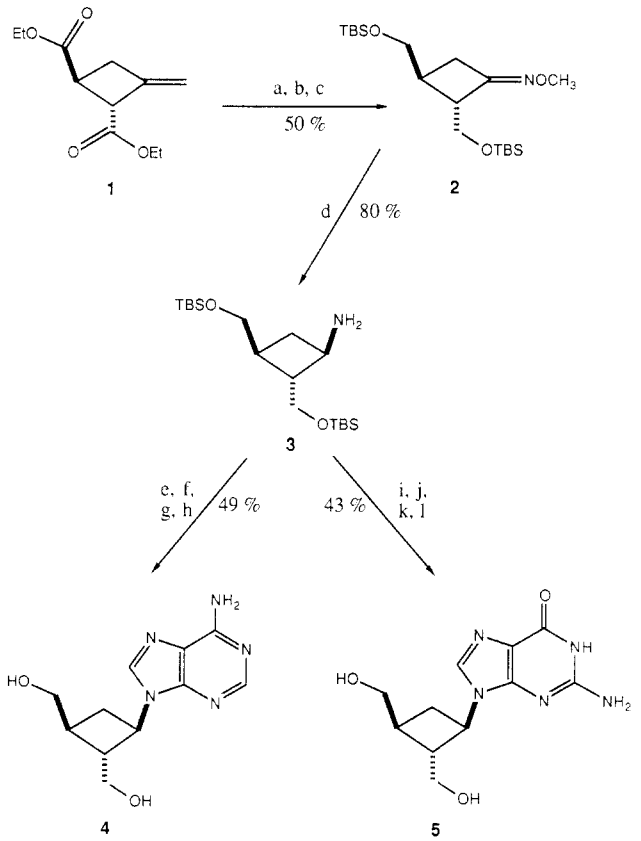
(18) For leading references to acyclovir, ganciclovir, AZT, and ara-A, see: Hayden, F. G.; Douglas, R. G. in *Principles and Practice of Infectious Diseases*, 3rd ed.; Mandell, G. L., Douglas, R. G., Bennet, J. E., Eds.; Churchill Livingstone: New York, 1989; Chapter 34.

Table V. Activity of Cyclobut-A and Cyclobut-G against Systemic MCMV Infection in Mice[a]

compound	dose,[b] mg/kg per day	MDD[c]	*p* value	survivors/treated	*p* value	ED_{50}, mg/kg per day
cyclobut-A	100			15/15	<0.001	2.7
	33.4	5.0	ns[d]	14/15	<0.001	
	11.2	6.0	ns	14/15	<0.001	
	3.8	5.3	ns	4/15	ns	
cyclobut-G	100	3.5	<0.05	13/15	<0.001	2.2
	33.4	4.5	ns	13/15	<0.001	
	11.2	5.8	ns	9/15	0.06	
	3.8	5.9	ns	7/15	ns	
ganciclovir	33.4			15/15	<0.001	<1.9
	11.2			15/15	<0.001	
	1.9	7.6	<0.005	10/15	<0.05	
control		5.7		3/15		

[a] Infection level: 1×10^5 PFU/mouse. [b] Agents administered BID intraperitoneally for 5 days beginning 6 h postinfection. [c] Mean day to death for nonsurvivors. [d] Not significant.

Scheme I. Synthesis of Cyclobut-A and Cyclobut-G[a]



[a] (a) 2.0 equiv of LAH, Et_2O, 0 °C, 1 h; (b) O_3, 4:1 (v/v) CH_2Cl_2/MeOH, −78 °C, 4 h; 10.0 equiv of $(CH_3)_2S$, −78 to 25 °C, 2 h; (c) 1.1 equiv of $MeONH_3^+Cl^-$, pyridine, 25 °C, 1 h; 3.0 equiv of *tert*-butyldimethylsilyl chloride, 25 °C, 18 h; (d) 4.7 equiv of $NaBH_3(O_2CCF_3)$, THF, 25 °C, 16 h; (e) 1.3 equiv of trimethylsilyl chloride, MeOH, 25 °C, 1 h; (f) 1.4 equiv of 5-amino-4,6-dichloropyrimidine, 4.8 equiv of Et_3N, *n*-BuOH, 118 °C, 16 h; (g) 102 equiv of $(EtO)_2CHOAc$, 165 °C, 16 h; 0.05 equiv of *p*-TsOH, MeOH, 25 °C, 1 h; (h) 0.04 M in 1.6:1 (v/v) NH_3/MeOH, 70 °C, 48 h; (i) 1.0 equiv of 2-amino-6-chloro-5-nitro-4(3*H*)-pyrimidinone, 1.5 equiv of Et_3N, DMF, 50 °C, 2 h; (j) 40.0 equiv of Zn, 99% HCO_2H, 25 °C, 1 h; (k) 99% HCO_2H, 180 °C, 2 h; (l) 15 M aq NH_4OH, 25 °C, 30 min.

The in vitro anti-HIV-1 activity[19] of cyclobut-A and cyclobut-G is summarized in Table II.[20] Both agents provided significant protection to ATH8 cells at concentrations of 1 μM, and the anti-HIV activity of cyclobut-G appeared to be comparable to that of AZT.[18] It should be noted, however, that ddA[19] and ddG[19] exerted antiviral activity over a greater range of nontoxic concentrations than did either cyclobut-A or cyclobut-G in this system. Nonetheless, in monocytes/macrophages,[21] both cyclobut-A and cyclobut-G blocked the infectivity and replication of a monocytotrophic HIV-1 strain more efficiently than AZT, and both compounds could in fact completely suppress the production of the HIV p24 antigen in these cells.[20] Furthermore, it should be emphasized that unlike cyclobut-A and cyclobut-G, 2′,3′-dideoxynucleoside derivatives like ddA and ddG have no activity against DNA-containing viruses in vitro.[22]

Both cyclobut-A and cyclobut-G have demonstrated efficacy in murine infection models against HSV-1, HSV-2, and MCMV.[23] In the most stringent test, cyclobut-A and cyclobut-G were administered to mice which had been inoculated intracranially with 100 LD_{50}'s of HSV-2 (Table III). Both agents were curative in this model with ED_{50} values of 9.7 and 39.8 mg/kg per day, respectively, and were superior to the positive control compound, ara-A[18]

(19) For a description of this assay, see: Mitsuya, H.; Matsukura, M.; Broder, S. In *AIDS: Modern Concepts and Therapeutic Challenges*; Broder, S., Ed.; Marcel Dekker: New York, 1987; Chapter 18.

(20) Hayashi, S.; Norbeck, D. W.; Rosenbrook, W.; Fine, R. L.; Matsukura, M.; Plattner, J. J.; Broder, S.; Mitsuya, H. *Antimicrob. Agents Chemother.* **1990**, *34*, in press.

(21) Perno, C. F.; Yarchoan, R.; Cooney, D. A.; Hartman, N. R.; Webb, D. S. A.; Hao, Z.; Mitsuya, H.; Johns, D. G.; Broder, S. *J. Exp. Med.* **1989**, *169*, 933–951.

(22) In vitro activity against EBV has been reported for AZT, but it is inactive against the other common herpesviruses: (a) Lin, J.-C.; Zhang, Z.-X.; Smith, M. C.; Biron, K.; Pagano, J. S. *Antimicrob. Agents Chemother.* **1988**, *32*, 265–267. (b) Furman, P. A.; Barry, D. W. *Am. J. Med.* **1988**, *85*, (2A), 176–181.

(23) In vivo methods. Female CF-1 mice, 8–10 weeks old (Sasco, Inc., Madison, WI), were used for HSV-1 and HSV-2 studies. Female Swiss Webster mice, 3 weeks old (Charles River, Portage, MI), were used for MCMV studies. Systemic HSV-1 (HS-123) infections wre produced by intraperitoneal (ip) inoculation of a 10 LD_{50} dose of virus (1.4×10^4 PFU; 0.5 mL) into groups of 10 mice. An HSV-2 (strain G, B. Roizman, University of Chicago) encephalitis was produced by intracerebral inoculation of a 100 LD_{50} dose (1.3×10^3 PFU; 0.01 mL) into the left cerebral hemisphere of anesthetized mice with a 27-gauge needle. For both HSV infections, ip treatment was initiated 7 h postinfection and then continued twice daily for 4 days. Systemic MCMV (Smith) infections were produced by ip inoculation with 1×10^5 PFU of virus into groups of 15 mice. Ip treatment was initiated 6 h postinfection and then continued for 5 days. All infected animals were checked daily for mortality for 21 days, and then final mortality rates and mean day to death (MDD) were calculated. Differences in mortality were evaluated by using the Fisher exact test. The MDDs were compared by the Mann-Whitney U rank Sum Test. A *p* value of 0.05 or less was considered significant. Median 50% effective doses (ED_{50}'s) were calculated by a trimmed logit method based on cumulative mortality: Hamilton, M. A.; Russo, R. C.; Thurston, R. V. *Environ. Sci. Technol.* **1977**, *11*, 714–719.

(ED_{50} > 125 mg/kg per day). The implied ability of cyclobut-A and cyclobut-G to cross the blood–brain barrier may be important in treating the neurological manifestations of AIDS. Despite its lower in vitro activity against HSV, cyclobut-A was as effective as cyclobut-G in protecting mice from a systemic challenge with 10 LD_{50}'s of HSV-1 (Table IV). In this model, intraperitoneal (ip) therapy with either agent was initiated 7 h postinfection and then continued twice a day for 4 days. Both drugs produced 100% survival at 18.7 mg/kg per day and again appeared to be more potent than ara-A. In comparison, acyclovir produced only 40% survival at 200 mg/kg per day. Cyclobut-A and cyclobut-G were also effective in a murine CMV model (Table V). In this experiment, an ip dose of 16.7 mg/kg of either cyclobut-A, cyclobut-G, or ganciclovir twice daily for 5 days increased survival from 20% in the untreated controls to 93, 87, and 100% respectively.

The broad-spectrum activity of cyclobut-A and cyclobut-G against herpesviruses and HIV warrants further development of these compounds as possible agents for the treatment of AIDS. Preliminary studies have found neither cyclobut-A or cyclobut-G to be acutely toxic to mice at 1000 mg/kg ip. Ongoing investigations will address the mechanisms of action and pharmacodynamics of these promising agents, as well as the biological activities of the individual enantiomers.

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Hydroxyethylamine Analogues of the p17/p24 Substrate Cleavage Site Are Tight-Binding Inhibitors of HIV Protease

Sir:

A key step in the replication of the human immunodeficiency virus (HIV) occurs when HIV-1 protease, the proteolytic enzyme encoded by the retrovirus, cleaves specific amide bonds in precursor *gag* and *pol* proteins to form the mature proteins needed for production of infectious viral particles.[1] Replacement of the catalytically active residues in protein precursors by site-directed mutagenesis techniques leads to the formation of noninfective virions,[2] and for this reason HIV protease is regarded as a potential target for developing agents for the treatment of acquired immunodeficiency syndrome (AIDS) and related diseases. Several X-ray crystal structures have established that the mature HIV protease is an aspartic proteinase that is formed from two identical 99 amino acid peptides, each subunit contributing one Asp-Thr-Gly unit.[3-6] The active site so formed closely resembles the active sites found in other well-characterized aspartic proteinases[7-9] and confirms some of the properties of HIV protease that were predicted on the basis of sequence homology.[10,11]

The discovery that mature HIV protease is an aspartic proteinase suggested to us and others[12a-e] that the general design strategy of replacing the P_1–P_1' cleavage point in substrates with transition-state analogues could be used to design tight-binding inhibitors of HIV protease, in the same way this approach was used to prepare inhibitors of other aspartic proteinases, e.g. pepsin, penicillopepsin, renin, cathepsin D.[13] We report herein the synthesis of hydroxyethylamine (HEA) dipeptidyl isosteres **1** and **2** that were designed to mimic the tetrahedral intermediate (**3**) for hydrolysis of Tyr-Pro, one of the partial substrate sequences cleaved by HIV protease (Figure 1). Incorporation of hydroxyethylamines **1** and **2** in peptides related

(1) Krausslich, H.-G.; Wimmer, E. *Ann. Rev. Biochem.* **1988**, *57*, 701.
(2) Seelmeier, S.; Schmidt, H.; Turk, V.; von der Helm, K. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6612. Mous, J.; Heimer, E. P.; LeGrice, S. F. J. *J. Virol.* **1988**, *62*, 1433.
(3) Wlodawer, A.; Miller, M.; Jaskolski, M.; Sathyanarayana, B. K.; Baldwin, E.; Weber, I. T.; Selk, L. M.; Clawson, L.; Schmeider, J.; Kent, S. B. H. *Science* **1989**, *245*, 616.
(4) Navia, M. A.; Fitzgerald, P.; McKeever, B.; Leu, C-. T.; Heimbach, J.; Herber, W.; Sigal, I.; Darke, P. *Nature* **1989**, *337*, 615.
(5) Lapatto, R.; Blundell, T.; Hemmings, A.; Overington, J.; Wilderspin, A.; Wood, S.; Merson, J. R.; Whittle, P. J.; Danley, D. E.; Geoghegan, K. F.; Hawrylik, S. J.; Lee, S. E.; Scheid, K. G.; Hobart, P. M. *Nature (London)* **1989**, *342*, 299–302.
(6) Miller, M.; Sathyanarayanba, B. K.; Toth, M. V.; Marshall, G. R.; Clawson, L.; Selk, L.; Schneider, J.; Kent, S. B. H.; Wlodawer, A. *Science* **1989**, *246*, 1149.
(7) Bott, R.; Subramanian, E.; Davies, D. R. *Biochemistry* **1982**, *21*, 6956.
(8) James, M. N. G.; Sielecki, A. R.; Salituro, F.; Rich, D. H.; Hofmann, T. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6137.
(9) Blundell, T. L.; Cooper, J.; Foundling, S. I.; Jones, D. M.; Atrash, B.; Szelke, M. *Biochemistry* **1987**, *26*, 5585.
(10) Toh, H.; Ono, M.; Saigo, K.; Miyata, T. *Nature* **1985**, *315*, 691.
(11) Pearl, L. H.; Taylor, W. R. *Nature* **1987**, 328, 482; *Ibid* **1987**, *329*, 351.
(12) (a) Moore, M. L.; Bryan, W. M.; Fakhoury, S. A., Magaard, V. W.; Huffman, W. F.; Dayton, B. D.; Meek, T. D.; Hyland, L.; Dreyer, G. B.; Metcalf, B. W.; Strickler, J. E.; Gorniak, J. G.; Debouck, C. *Biochem. Biophys. Res. Commun.* **1989**, *159*, 420. (b) Dreyer, G. B.; Metcalf, B. W.; Tomaszek, T. A., Jr.; Carr, T. J.; Chandler, A. C., III; Hyland, L.; Fakhoury, S. A.; Magaard, V. W.; Moore, M. L.; Strickler, J. E.; Debouck, C.; Meek, T. D. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 9752–9756. (c) Sigal, I. S.; Huff, J. R.; Darke, P. L.; Vacca, J. P.; Young, S. D.; Desolms, J. S.; Thompson, W. J.; Lyle, T. A.; Graham, S. L.; Ghosh, A. K., European Patent Appln. #0337714, 1988. (d) McQuade, T. J.; Tomasselli, A. G.; Lui, L.; Karacostas, V.; Moss, B.; Sawyer, T. K.; Heinrikson, R. L.; Tarpley, W. G. *Science* **1990**, *247*, 454–456. (e) Billich, S.; Knoop, M.-T.; Hansen, J.; Strop, P.; Sedlacek, J.; Mertz, R.; Moelling, K. *J. Biol. Chem.* **1988**, *263*, 17905–17908.

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