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Communications to the Editor

Synthesis of 5,6-Dihydro-4-hydroxy-2-pyrones as HIV-1 Protease Inhibitors: The Profound Effect of Polarity on Antiviral Activity

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Recent advancements in the treatment of HIV infection have received extensive coverage in both the scientific arena and popular media.[1] In particular, the use of HIV protease inhibitors has revolutionized AIDS care and has even raised the specter of a cure for the disease.[1c,2] Often overlooked in these reports are the very real problems associated with the currently marketed protease inhibitors: low bioavailability,[3] significant side effects and drug interactions,[2a,3b,4] inconvenient dosing schedules,[4] and high cost of drug.[4] More ominous is the emergence of HIV strains which are resistant to current therapies.[2,5] Therefore, the search for a new generation of inhibitors which are not cross-resistant to the current agents continues unabated.[1a]

Previous investigations in our laboratories[6] and others[7] have identified the 5,6-dihydro-4-hydroxy-2-pyrones as an exciting new class of non-peptidic protease inhibitors. Initially, high volume screening of a chemical library led to the discovery of pyrone **1** (Figure 1), a micromolar inhibitor of the protease enzyme that showed no activity in antiviral assays.[8] The extensive synthetic modification and structure−activity studies initiated by this early lead resulted in the discovery of the dihydropyrone analogs. Further optimization of the alkyl groups at the C-3 and C-6 positions culminated in the synthesis of compound **13a**, which contains a single chiral center and displays excellent activity against the protease enzyme.[9] X-ray crystallographic examination of selected inhibitors from this series bound to protein revealed that the central core of the dihydropyrone formed hydrogen bonds to the catalytic aspartates as well as to Ile 50/Ile 150; at the same time, the substituents at C-6 and C-3 filled the critical binding pockets at S_1, S_2, S_1', and S_2'. Despite this encouraging crystallographic evidence and the nanomolar potency in vitro, these compounds still exhibited disappointing activity in cell culture.

Therefore, efforts were concentrated on the improvement of antiviral efficacy by the addition of polar groups to the dihydropyrones. A study of structure−activity relationships was undertaken in which the substituents at S_1, S_2, S_1', and S_2' were retained while the optimal polar functional groups (and their relative positions) were determined. Further reductions in lipophilicity were engendered by the judicious replacement of phenyl groups with appropriate alkyl moieties. In this communication, we describe the preparation and activity of a new series of HIV protease inhibitors, of which several derivatives display subnanomolar K_i's, excellent antiviral potency, and superior pharmacokinetics in mice.

Chemistry. The synthesis of target compounds **13** necessitated the preparation of a thiotosylate **11** (to append at the 3-position) and a dihydropyrone core (**12**). The thiotosylates **11** were prepared from the reaction of the appropriate thiol and tosyl bromide;[9,10] the thiol itself was prepared via one of two routes as summarized in Scheme 1. For those cases where Z = CH_2OH, the commercially available 2-*tert*-butyl-5-methylphenol (**2**) was brominated and elaborated into the methyl benzoate **3**. The phenolic hydroxyl group was then converted to the thiocarbamate in order to effect the Newman−Kwart transformation.[11] Reduction of both the ester and the thioamide in **4** yielded the desired

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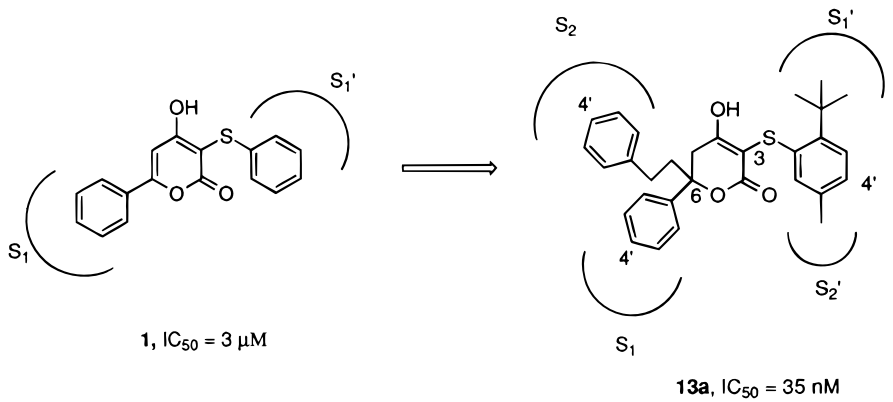
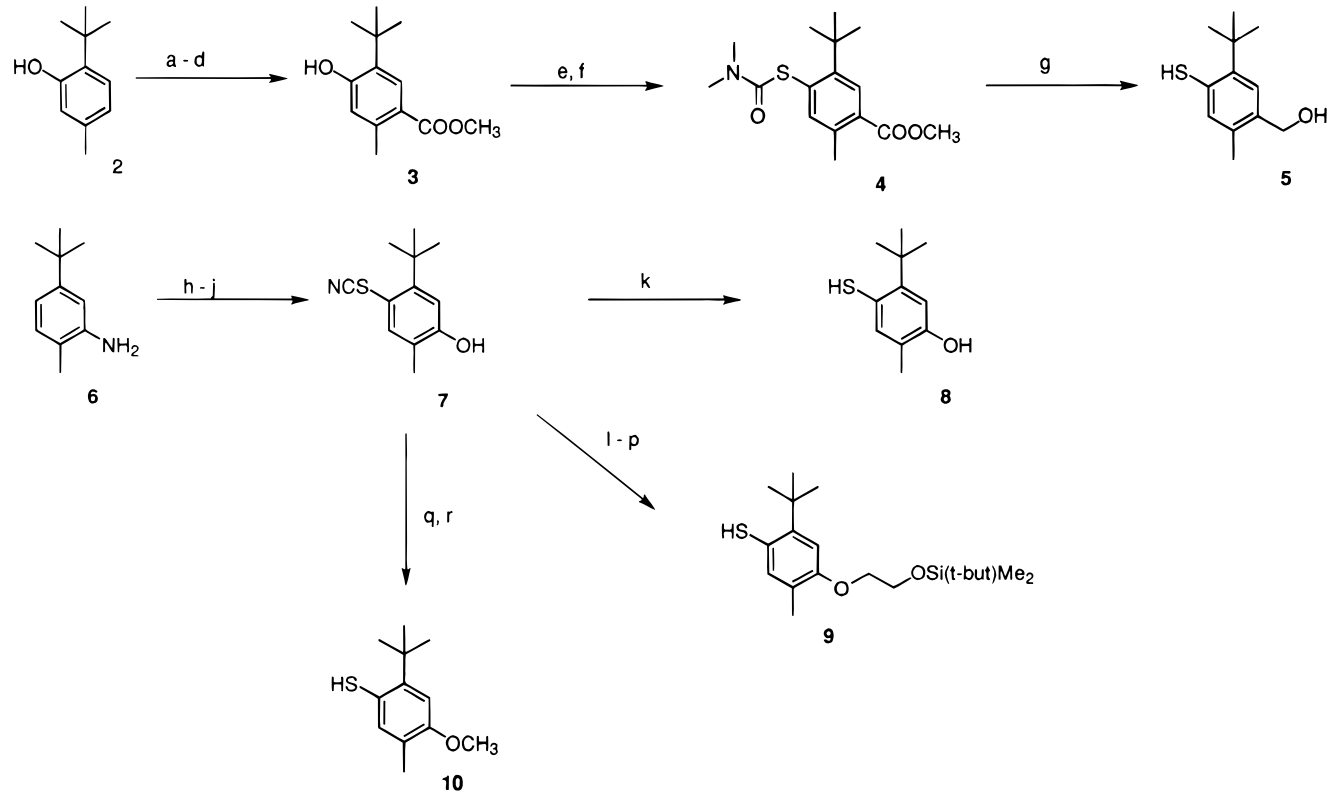


Figure 1. Evolution of 5,6-dihydro-4-hydroxy-2-pyrone lead from 4-hydroxypyrone screening hit.

Scheme 1. Synthesis of Thiophenols[a]



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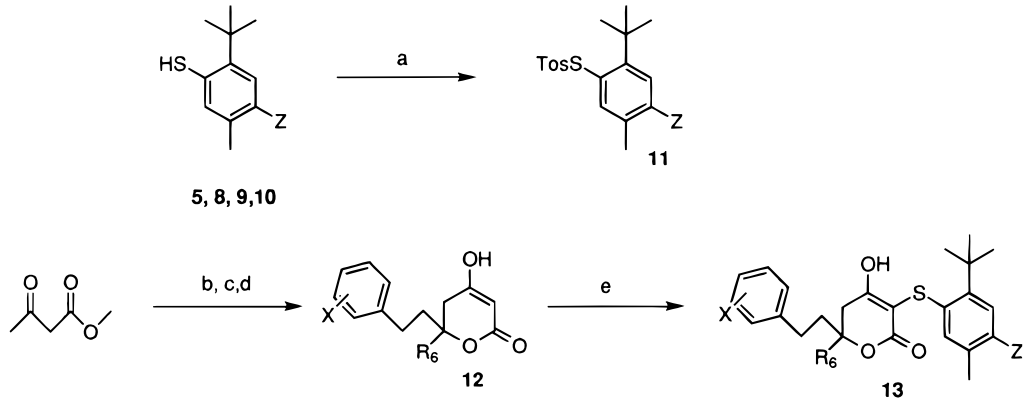
[a] (a) Br_2, CCl_4; (b) NaH, MEM Cl, THF; (c) *n*-BuLi, then CO_2; (d) H_2SO_4, MeOH; (e) NaH, then $ClCSNMe_2$, DMF; (f) 240 °C; (g) DIBAL, CH_2Cl_2; (h) $NaNO_2$; (i) H_2SO_4; (j) NaSCN, NaBr, Br_2; (k) DTT, EtOH, phosphate buffer; (l) $CsCO_3$, NaI, $BrCH_2CO_2CH_3$; (m) $LiAlH_4$; (n) I_2, EtOAc; (o) (*t*-Bu)Me_2SiCl, imidazole, DMF; (p) DTT, EtOH, phosphate buffer; (q) $CsCO_3$, MeI; (r) DTT, EtOH, phosphate buffer.

thiophenol **5**. Synthesis of the Z = OR analogs (where R = H, CH_3, and CH_2CH_2OH) began with conversion of aniline **6** to the corresponding phenol and reaction of that phenol with thiocyanate and bromine. Resulting intermediate **7** was elaborated into a variety of derivatives via O-alkylation of the phenolic group.

Intermediate dihydropyrone **12** was prepared from the appropriate ketone and the dianion of methyl acetoacetate as described previously.[9] Reaction of **12** with a slight excess of thiotosylate **11** in DMF with several equivalents of potassium carbonate gave target compounds **13a**−**y** in good overall yield (Scheme 2; physical data in Tables 1 and 2). Purification of the desired compound was accomplished using either trituration or silica gel chromatography. The inhibition of HIV-1 protease was measured by an HPLC assay at pH 6.2 as reported previously,[12] and antiviral activity was determined in CEM cells using a standard protocol.[13] The enzyme activity (IC_{50}), cellular antiviral activity (EC_{50}), and cellular toxicity (TC_{50}) are summarized in Tables 1 and 2.

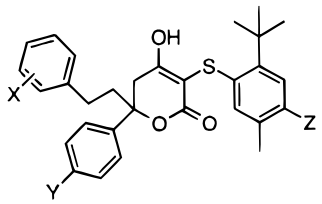
Results and Discussion. In both the pyrone and dihydropyrone series, achievement of cellular antiviral activity has been difficult even though strong in vitro enzyme affinity has been observed. X-ray crystallographic examination of analogs structurally similar to **13a** bound to HIV protease indicated that the four crucial binding pockets were effectively filled by the alkyl or aryl groups at C-3 and C-6 (Figure 1).[9] Since a relationship between enzyme potency and antiviral activity had not been established for this series, any further improvement in the binding between the inhibi-

Scheme 2. Preparation of Target 4-Hydroxy-5,6-dihydropyrones[a]



[a] (a) Tosyl bromide, pyr, CCl_4; (b) NaH, then *n*-BuLi, THF; (c) $R_6CO(CH_2)_2Ph$; (d) NaOH, then H^+; (e) **11**, K_2CO_3, DMF.

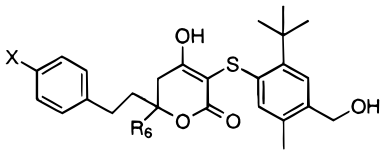
Table 1. Chemical and Biological Data for 6-Phenyl-6-phenethyldihydropyrones



compd	X	Y	Z	mp, °C	analysis[a]	IC_{50} (nM)[b]	EC_{50} (μM)[c]	TC_{50} (μM)[d]
13a	H	H	H	71−72	$C_{30}H_{32}O_3S\cdot0.15H_2O$	35	>55	55
13b	H	H	OH	86−88	$C_{30}H_{32}O_4S\cdot0.7H_2O$	33	>23	23
13c	H	H	$O(CH_2)_2OH$	179−182	$C_{32}H_{36}O_5S\cdot0.2H_2O$	6.8	>20	20
13d	H	H	CH_2OH	173−174	$C_{31}H_{34}O_4S\cdot0.3H_2O$	6.6	2.5	66
13e	H	H	OCH_3	75−77	$C_{31}H_{34}O_4S\cdot0.2H_2O$	15	>33	33
13f	4-OH	H	H	199−201	$C_{30}H_{32}O_4S\cdot0.75H_2O$	11	>69	69
13g	4-NH_2	H	H	211−214	$C_{30}H_{33}NO_3S\cdot0.5H_2O$	24	>54	54
13h	H	OH	H	155−161	$C_{30}H_{32}O_4S$	40	>23	23
13i	H	NH_2	H	157−160	$C_{30}H_{32}NO_3S\cdot0.5H_2O$	32	>67	67
13j	H	$O(CH_2)_2OH$	H	150−151	$C_{32}H_{36}O_5S\cdot0.2H_2O$	12	9.4	23
13k	4-OH	H	CH_2OH	196−199	$C_{31}H_{34}O_5S\cdot0.5H_2O$	1.7	4.7	>100
13l	3-OH	H	CH_2OH	132−135	$C_{31}H_{34}O_5S\cdot0.5H_2O$	2.5	6.1	>100
13m	4-NH_2	H	CH_2OH	218−221	$C_{31}H_{35}NO_4S\cdot0.7H_2O$	3.1	3.7	94
13n	3-NH_2	H	CH_2OH	285−287	$C_{31}H_{35}NO_4S\cdot0.7H_2O$	4.0	3.1	23
13o	H	$O(CH_2)_2OH$	CH_2OH	152 dec	$C_{33}H_{38}O_6S\cdot0.53H_2O$	1.4	4.2	67
13p	H	$O(CH_2)_2OH$	$O(CH_2)_2OH$	190 dec	$C_{34}H_{40}O_7S\cdot0.5H_2O$	6.4	1.8	25
13q	H	$O(CH_2)_2OH$	OH	168−170	$C_{32}H_{36}O_6S\cdot2.0H_2O$	3.7	4.2	27
13r	H	$O(CH_2)_2OH$	CH_2OCH_3	174 dec	$C_{34}H_{40}O_6S\cdot0.25H_2O$	4.5	>18	18
13s	4-OH	OH	CH_2OH	106−108	$C_{31}H_{34}O_6S\cdot1.0H_2O$	120	>21	21
13t	4-OH	OCH_3	CH_2OH	79−82	$C_{32}H_{36}O_6S\cdot1.5H_2O$		2.0	>100

[a] All compounds were analyzed for C, H, N and had results ±0.4% of theoretical values. [b] Enzyme inhibition was determined as described in ref 11. [c] Antiviral activity in HIV-infected CEM cells. EC_{50} refers to the effective concentration at which 50% of cells are protected from cytopathic effects. [d] Toxicity measured in CEM cells in the absence of virus.

Table 2. Chemical and Biological Data for 6-Alkyl-6-phenethyldihydropyrones



compd	X	R_6	mp, °C	analysis[a]	IC_{50} (nM)[b]	EC_5 (μM)[c]	TC_{50} (μM)[d]
13u	OH	cyclohexyl	138−139	$C_{31}H_{40}O_5S\cdot0.84H_2O$	4.1	0.5	75
13v	OH	isopropyl	158−160	$C_{28}H_{36}O_5S\cdot0.46H_2O$	3.6	0.6	>100
13w	OH	methyl	143−145	$C_{26}H_{32}O_5S\cdot0.37H_2O$	4.3	2.5	>100
13x	NH_2	cyclohexyl	214−216	$C_{31}H_{41}NO_4S\cdot0.7H_2O$	3.2	1.4	80
13y	NH_2	isopropyl	139−141	$C_{28}H_{37}NO_4S\cdot0.7H_2O$	2.7	0.5	>100

[a] All compounds were analyzed for C, H, N and had results ±0.4% of theoretical values. [b] Enzyme inhibition was determined as described in ref 11. [c] Antiviral activity in HIV-infected CEM cells. EC_{50} refers to the effective concentration at which 50% of cells are protected from cytopathic effects. [d] Toxicity measured in CEM cells in the absence of virus.

tor and enzyme would not necessarily translate into improved antiviral efficacy. However, the X-ray conformation did suggest several possible sites for further substitution: namely, the 4′-position of the aryl ring at C-3, the 3′- or 4′-position in the phenethyl ring at C-6, or the 4′-position of the phenyl ring at C-6. Modifica-

tions at any of these sites appeared to offer the opportunity to manipulate physical properties without deleterious influence on the enzymatic activity.

Compounds **13b**−**j** show the effect of adding a single polar group to the dihydropyrone skeleton of **13a**. Without exception, the polar analogs were at least equipotent to the parent **13a** (IC_{50} = 35 nM) in the HIV protease assay. The activity of two analogs containing polar groups at C-3—**13c** (Z = OCH_2CH_2OH; IC_{50} = 6.8 nM) and **13d** (Z = CH_2OH; IC_{50} = 6.6 nM)—actually increased 4-fold over that of the parent. However, this boost in enzyme potency correlated with an increase in cellular efficacy for **13d** only (EC_{50} = 2.5 μM.) A similar observation was noted when polarity was introduced into either the phenethyl moiety or the phenyl group at C-6: that is, the enzymatic activity improved slightly (**13f**, IC_{50} = 11 nM; **13j**, IC_{50} = 12 nM) but the cellular potency remained unchanged. Clearly, the enhancement in both enzymatic and cellular potency conferred by the benzyl alcohol moiety warranted further exploration in a series of disubstituted analogs.

Introduction of polar functionalities at both C-3 and C-6 was effected to give derivatives **13k**−**r**. For compounds **13k**−**n**, the 3-position substituent was kept constant as the benzyl alcohol (Z = CH_2OH) and the substituents in the phenethyl moiety were varied (X = OH or NH_2). All compounds in this series showed a substantial enhancement in enzymatic activity; indeed, compound **13k** displayed a 20-fold improvement in potency versus the parent dihydropyrone **13a**. Of more significance, however, was the consistent improvement observed in cellular potency. Dihydropyrones **13k**−**n** possessed low micromolar efficacy in cellular assays, and in all cases save one (**13n**) this activity was clearly differentiated from cellular toxicity. In fact, two compounds—**13k** (Z = CH_2OH, X = 4-OH) and **13l** (Z = CH_2OH, X = 3-OH)—showed no toxicity in the antiviral screen and therefore possessed promising therapeutic indices (EC_{50}/TC_{50}) in excess of 20.

Substitution of the phenyl ring at C-6 with concomitant substitution at C-3 also resulted in an improvement in antiviral activity, as demonstrated by compounds **13o**−**r**. In this series of disubstituted analogs, the Y substituent was kept constant as OCH_2CH_2OH (previously shown to confer good enzyme activity) while the 3-substituent was varied. As observed in the previous disubstituted series, the IC_{50}'s against the protease enzyme improved by a 5−25-fold margin when compared to the parent **13a**, while the cellular EC_{50}'s reached the low micromolar range. However, these derivatives displayed increased cellular toxicities when compared to the series **13k**−**n**, yielding therapeutic indices of only 1 to 14. It is interesting to note that the benzyl alcohol moiety in the aryl ring at C-3 seemed to confer the greatest boost in potency in vitro for this disubstituted series, just as it did in the monosubstituted series (**13b**−**e**).

Therefore, the introduction of appropriate polar functional groups into the dihydropyrone prototype **13a** resulted in substantial improvements in both enzymatic and cellular activities. Specifically, a hydroxyl (or amino) group in the phenethyl ring at C-6 and a benzyl alcohol moiety in the *S*-aryl ring at C-3 seemed to confer the optimal blend of good antiviral activity with low toxicity. Analogs with three polar functional groups (**13t** in particular) also displayed good antiviral activity. At this point, a complementary strategy was devised in which overall polarity was increased by modifying lipophilicity. When the aryl group at C-6 was replaced with the isosteric cyclohexyl moiety (**13u** and **13x**), no significant effect on enzyme activity was observed, although antiviral activity increased dramatically (EC_{50} = 0.5 μM for **13u**). When an even smaller alkyl group—namely, the isopropyl group—was attached to C-6, the same striking enhancements in antiviral activity were obtained with no concomitant toxicity (TC_{50} > 100). Derivatives **13v** (X = OH) and **13y** (X = NH_2) displayed a 10-fold improvement in EC_{50} when compared to their phenyl analogs (**13k** and **13m**, respectively). These 6-alkyldihydropyrone compounds displayed sub-micromolar activity in cell culture (EC_{50} = 0.6 μM for **13v** and 0.5 μM for **13y**) and subnanomolar K_i's against the protease enzyme. (The assay in which K_i's were determined utilized a lower enzyme concentration than that used for the determination of IC_{50}'s. In this system, the IC_{50} for **13v** was 1.1 nM with a K_i of 0.17 nM, while the IC_{50} for **13y** was 2.7 nM with a K_i = 0.43 nM. The K_m for these determinations was 92 mM.)

The potency of **13v** and **13y** in the antiviral assay prompted further testing for pharmacokinetic parameters. Both compounds were dosed orally in mice, using a vehicle of 20% 0.1 NaOH and 80% methylcellulose (pH adjusted to 8.) After administration of a 25 mg/kg dose, compound **13v** had C_{max} = 17.3 μM, $t_{1/2}$ = 1.6 h, and oral bioavailability of 96%; under the same experimental conditions, compound **13y** had a C_{max} = 23 μM, $t_{1/2}$ = 2.2 h, and 80% oral bioavailability. Encouraging results were also observed when compound **13v** was tested against several HIV proteases showing high-level resistance to ritonavir. For example, in an in vitro assay using the V82F/84V mutant, ritonavir displayed a 200-fold increase in K_i while compound **13v** showed no significant loss in potency (less than 4-fold). The potential therefore exists that **13v** and similar derivatives will not be cross-resistant to currently marketed inhibitors.

In conclusion, a series of dihydropyrones substituted with one or more polar groups was synthesized and assayed for antiviral activity. Optimal substituents at C-3 and C-6 were identified as a benzyl alcohol moiety on the *S*-aryl ring at C-3, a hydroxyl or amino group on the phenyl ring of the phenethyl substituent at C-6, and an isopropyl group replacing the phenyl group at C-6. This combination of increased polarity and reduced lipophilicity resulted in the discovery of **13v** and **13y**. These low molecular weight derivatives displayed subnanomolar K_i's against the enzyme, excellent antiviral activity, no appreciable toxicity, and promising bioavailability in mice. Also promising are the ease of synthesis of these compounds and the presence of a single chiral center in the molecule. Preliminary data against ritonavir-resistant mutants suggest that these agents could be effective against protease resistant strains of HIV. The synthesis and activity of the chiral forms of **13v** and **13y**, as well as an extensive SAR study, will be reported shortly as further work in this series continues.

References

(1) (a) Chrusciel, R. A.; Romines, K. R. Recent Developments in HIV Protease Inhibitor Research. *Expert Opin. Ther. Pat.* **1997**, *7*, 111−121. (b) Havlir, D. V.; Richman, D. D. Viral Dynamics of HIV: Implications for Drug Development and Therapeutic Strategies. *Ann. Intern. Med.* **1996**, *124*, 984−994. (c) For coverage in the popular media, see: Leland, J. The End of AIDS. *Newsweek* **1996**, Dec. 2, 65−73.

(2) (a) Deeks, S. G.; Smith, M.; Holodniy, M.; Kahn, J. O. HIV-1 Protease Inhibitors: A Review for Clinicians. *J. Am. Med. Assoc.* **1997**, *277*, 145−153. (b) Moyle, G.; Gazzard, B. Current Knowledge and Future Prospects for the Use of HIV Protease Inhibitors. *Drugs* **1996**, *51*, 701−712.

(3) (a) Plattner, J. J.; Norbeck, D. W. Chapter 5. Obstacles to Drug Development from Peptide Leads. In *Drug Discovery Technologies*; Clark, C. R., Moos, W. H., Eds.; Ellis Horwood: Chichester, England, 1990; pp 92−126. (b) Barry, M.; Gibbons, S.; Back, D.; Mulcahy, F. Protease Inhibitors in Patients with HIV Disease. *Clin Pharmacokinet.* **1997**, *32*, 194−209.

(4) (a) Mascolini, M. Protease Inhibitors and Nucleoside Combinations. *J. Int. Assoc. Physicians AIDS Care* **1996**, *4*, 23−33. (b) Waldholz, M. New Aids Treatment Raises Tough Question of Who Will Get It. *Wall Street Journal*, July 3 1996, 1−2. (c) Waldman, A. When You Want to Live But Cannot Afford It. *Washington Post*, April 27, 1997, C01.

(5) (a) Ridky, T.; Leis, J. Development of Drug Resistance to HIV-1 Protease Inhibitors. *J. Biol. Chem.* **1995**, *270*, 29621−29623. (B) Erickson, J. W. The Not-so-great Escape. *Nature Struct. Biol.* **1995**, *2*, 523−529.

(6) Tummino, P. J.; Vara Prasad, J. V. N.; Ferguson, D.; Nouhan, C.; Graham, N.; Domagala, J. M.; Ellsworth, E.; Gajda, C.; Hagen, S. E.; Lunney, E. A.; Para, K. S.; Tait, B. D.; Pavlovsky, A.; Erickson, J. W.; Gracheck, S.; McQuade, T. J.; Hupe, D. J. Discovery and Optimization of Nonpeptide HIV-1 Protease Inhibitors. *Bioorg. Med. Chem.* **1996**, *4*, 1401−1404.

(7) (a) Romines, K. R.; Chrusciel, R. A. 4-Hydroxypyrones and Related Templates as Nonpeptidic HIV Protease Inhibitors. *Curr. Med. Chem.* **1995**, *2*, 825−838. (b) Thaisrivongs, S.; Skulnik, H. I.; Turner, S. R.; Strohbach, J. W.; Tommasi, R. A.; Johnson, P. D.; Aristoff, P. A.; Judge, T. M.; Gammill, R. B.; Morris, J. K.; Romines, K. R.; Chrusciel, R. A.; Hinshaw, R. R.; Chong, K.-T.; Tarpley, W. G.; Poppe, S. M.; Slade, D. E.; Lynn, J. C.; Horng, M.-M.; Tomich, P. K.; Seest, E. P.; Dolak, L. A.; Howe, W. J.; Howard, G. M.; Schwende, F. J.; Toth, L. N.; Padbury, G. E.; Wilson, G. J.; Shiou, L.; Zipp, G. L.; Wilkinson, K. F.; Rush, B. D.; Ruwart, M. J.; Koeplinger, K. A.; Zhao, Z.; Cole, S.; Zaya, R. M.; Kakuk, T. J.; Janakiraman, M. N.; Watenpaugh, K. D. Structure-Based Design of HIV Protease Inhibitors: Sulfonamide-Containing 5,6-Dihydro-4-hydroxy-2-pyrones as Non-Peptidic Inhibitors. *J. Med. Chem.* **1996**, *39*, 4349−4353.

(8) Vara Prasad, J. V. N.; Para, K. S.; Lunney, E. A.; Ortwine, D. F.; Dunbar, J. B.; Ferguson, D.; Tummino, P. J.; Hupe, D.; Tait, B. D.; Domagala, J. M.; Humblet, C.; Bhat, T. N.; Liu, B.; Guerin, D. M. A.; Baldwin, E. T.; Erickson, Sawyer, T. K. Novel Series of Achiral, Low Molecular Weight, and Potent HIV-1 Protease Inhibitors. *J. Am. Chem. Soc.* **1994**, *116*, 6989−6990.

(9) Tait, B. D.; Domagala, J. M.; Ellsworth, E. L.; Gajda, C.; Hagen, S. E.; Hamilton, H.; Vara Prasad, J. V. N.; Ferguson, D.; Graham, N.; Hupe, D.; Nouhan, C.; Tummino, P. J.; Humblet, C.; Lunney, E. A.; Pavlovsky, A.; Rubin, R. J.; Baldwin, E. T.; Bhat, T. N.; Erickson, J. W.; Gulnik, S.; Liu, B. 4-Hydroxy-5,6-dihydropyrones. 2. Potent Non-Peptide Inhibitors of HIV Protease. *J. Med. Chem.* **1997**, *40*, 3781−3792.

(10) Ranasinghe, M. G.; Fuchs, P. L. A Facile Synthesis of Unsymmetrical Thiosulfonates via Sulfonylation of Mercaptans. *Synth. Commun.* **1988**, *18*, 227−232.

(11) Newman, M. S.; Karnes, H. A. The Conversion of Phenols to Thiophenols via Dialkylthiocarbamates. *J. Org. Chem.* **1966**, *31*, 3980−3984.

(12) Experimental conditions for the enzyme assay at pH 4.7: Tummino, P. J.; Ferguson, D.; Jacobs, C. M.; Tait, B. D.; Hupe, L.; Lunney, E.; Hupe, D. Competitive Inhibition of HIV-1 Protease by Biphenyl Carboxylic Acids. *Arch. Biochem. Biophys.* **1995**, *316*, 523−528. This procedure was modified slightly when run at pH 6.2: the buffer was changed to 1.0 mM dithiothreitol, 80 mM MES, 160 mM NaCl, 1.0 mM EDTA, and 0.1% polyethylene glycol (MW 8000). The final concentration of HIV protease used was 1.5 nM.

(13) Buckheit, R. W., Jr.; Hollingshead, M. G.; Germany-Decker, J.; White, L.; McMahon, J. B.; Allen, L. B.; Ross, L. J.; Decker, W. D.; Westbrook, L.; Shannon, W. M.; Weislow, O.; Bader, J. P.; Boyd, M. R. Thiazolobenzimidazole: Biological and Biochemical Anti-retroviral Activity of a New Nonnucleoside Reverse Transcriptase Inhibitor. *Antiviral Res.* **1993**, *21*, 247−265.

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