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Thiophene Derivatives as Extremely High Affinity $P_{3'}$ Ligands for the Hydroxyethylpiperazine Class of HIV-1 Protease Inhibitors

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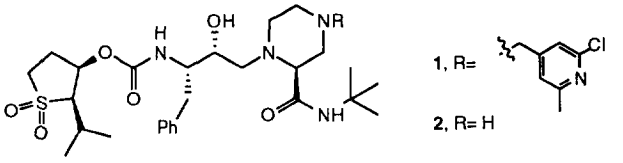
Abstract: A series of hydroxyethylpiperazine HIV-1 protease inhibitors containing various monocyclic or bicyclic thienylmethyl substituents as $P_{3'}$ ligands were prepared. They were found to exhibit extremely high potency in the enzyme inhibition assay. These inhibitors also proved to be highly effective against viral spread in a whole cell assay. Some representative compounds in this series have been examined for oral bioavailability in dogs and the pharmacokinetic properties were found to be somewhat related to their aqueous solubilities.

The life cycle of human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome (AIDS), has been intensely studied and many of its gene products have been identified.[1] Among them, HIV-1 protease (HIV PR) plays a critical role in the maturation of the viral particle.[2] Genetic inactivation of this virally encoded protease results in the production of noninfectious virions.[3] Therefore, HIV-1 protease has been considered as one of the most attractive targets for AIDS chemotherapy. A number of protease inhibitors have been prepared with vast structural diversity.[4]



It has been recently demonstrated that the HIV-1 develops resistant strains by mutating its protease sequences under the pressure of protease inhibitors.[5] Alarmed by these findings, much attention is being focused on how to counter viral resistance to protease inhibitors. One of the viable strategies calls for the development of inhibitors possessing extremely high potency, so that sufficient plasma concentrations of the inhibitors above the range of effective antiviral concentration can be maintained in the initial stage of treatment. Recently we have disclosed a new hydroxyethylpiperazine class of HIV-1 protease inhibitors such as **1** which incorporates a piperazine carboxamide unit as the P_1'-$P_{3'}$ ligand and 3(R)-[2(R)-isopropyl-1,1-dioxotetrahydrothienyl]-oxycarbonyl as a P_2 ligand.[6] Inhibitors in this series showed subnanomolar enzyme inhibitory potency[7] and good to excellent antiviral activity in a cell based assay[8] (e.g. **1**, IC_{50}=0.54 nM, CIC_{95}=25 nM). In a continuing effort to further improve the antiviral potency of this series of inhibitors to meet the aforementioned demand, we have

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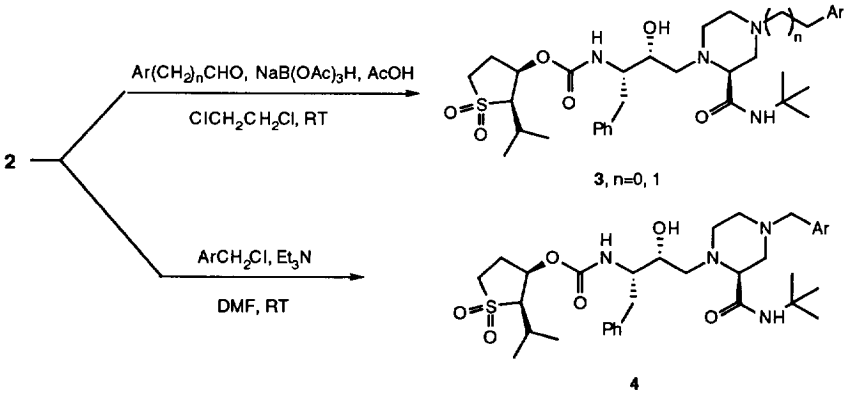
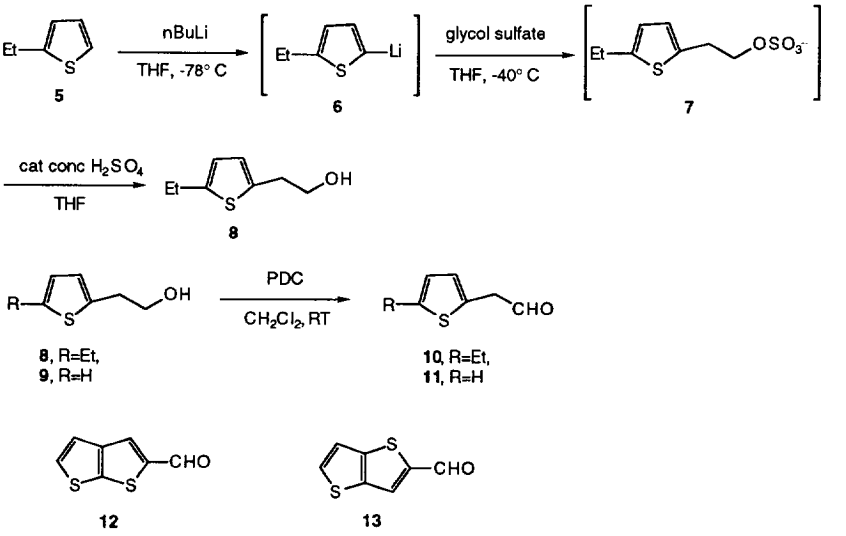
examined a variety of $P_{3'}$ ligands tethered at the 4-amine position of the piperazine unit of **2**. Special attention has been focused on maximizing the van der Waals interactions of the ligands with $S_{3'}$ binding cleft which is constituted mostly with hydrophobic residues.[4] During this study a series of extremely potent HIV-1 protease inhibitors incorporating mono- or bicyclic thienylmethyl derivatives has been developed. Herein we report structure-activity studies of these inhibitors vs. HIV-1 protease and pharmacokinetic investigation of some selected compounds in dogs.

Scheme 1



Inhibitors possessing various thienylmethyl or thienylethyl moieties at the 4-position of the piperazinecarboxamide of compound type **1** were prepared through either reductive alkylation or S_N2 type alkylation of the free amine **2** with the corresponding aldehydes or halides, respectively, as shown in Scheme 1.[6]

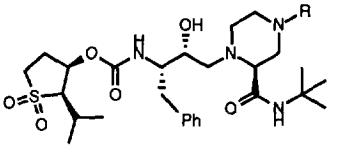
Scheme 2

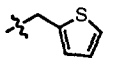
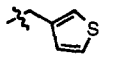
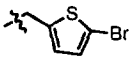
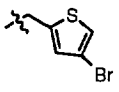
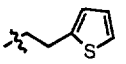
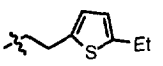
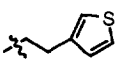


Thienyl aldehydes employed in the reductive coupling reaction in Scheme 1 which were not commercially available were prepared as depicted in Scheme 2. Treatment of 5-ethyl-2-lithiothiophene (**6**), prepared from 2-

ethylthiophene (**5**), with glycol cyclic-sulfate[9] provided the β-sulfate **7**, which upon acid-catalyzed hydrolysis[10] was cleanly converted to the corresponding alcohol **8**. Various 2-thienylethyl alcohols were oxidized by PDC[11] to provide the desired aldehydes (e.g. **10** and **11**). The two fused bicyclic aldehydes, thieno[2,3-*b*]thiophene-2-carboxaldehyde (**12**) and thieno[3,2-*b*]thiophene-2-carboxaldehyde (**13**) were prepared according to published procedures.[12]

Table 1. SAR Studies of Inhibitors Containing Monocyclic Thiophene Derivatives[13]



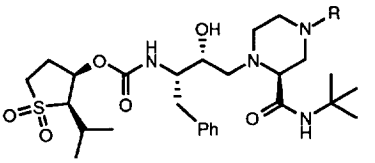
R	Compound	IC_{50} (nM)	CIC_{95} (nM)
	**14**	0.6	25-50
	**15**	3.6	50
	**16**	0.25	100
	**17**	0.18	25
	**18**	0.59	25-50
	**19**	0.76	50
	**20**	1.6	50

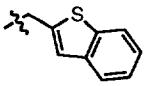
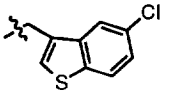
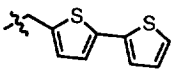
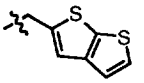
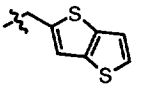
The monocyclic thiophene derivatives were tested in both an *in vitro* enzyme inhibition assay[7] and an antiviral assay in MT4 human lymphoid cells infected with the IIIb isolate.[8] As documented in Table 1, highly potent inhibitors emerged from this class of compounds. All the compounds examined exhibited nanomolar to subnanomolar inhibitory potency towards HIV-PR and high antiviral potency (CIC_{95}=25-100 nM) in the inhibition of viral spread. Between the two monothienylmethyl derivatives (compounds **14** and **15**), the 2-thienylmethyl derivative (**14**) showed much higher inhibitory potency towards the enzyme, although they exhibited comparable antiviral potencies in the cell based assay. In the case of bromine substituted thienylmethyl derivatives, both the 2- and 3-bromo derivatives, **16** and **17**, respectively, exhibited similarly improved affinity for the enzyme compared to the unsubstituted compound **14**. However, this enhancement of binding affinity was not reflected in the antiviral assay especially in the case of compound **16**. Ethylene bridged thiophene derivatives

18–20 proved to be comparable to the methylene bridged compounds in both assays. Although the potencies of these compounds fell within the acceptable range, they did not reach the goal, i.e. improvement over compound **1**.

It was noteworthy that among the compounds examined in Table 1, both 2- and 3-bromo-substituted derivatives **16** and **17** exhibited the highest enzyme inhibition potency. Extending this observation, we decided to examine bicyclic thienyl derivatives including bithienyl or fused thienothienyl groups based on the premise that they are comparable both in size and electronic arrangement to the two bromine-substituted thiophenes. This indeed resulted in a series of extremely potent inhibitors as documented in Table 2. In the case of the benzothiophene derivative (**21**), potency in the enzyme inhibition assay was boosted somewhat (0.1 nM) vs. the parent compound **14**, but the CIC_{95} value (100 nM) was not improved. The bithienylmethyl derivative (compound **23**) exhibited a 40 pM IC_{50} against HIV PR, the highest enzyme affinity observed in this series of inhibitors.[14] Antiviral potency was not similarly enhanced, however. The highest antiviral activities (CIC_{95} = 6-12 nM) in this series were observed with the two fused bicyclic thienothienylmethyl derivatives (**24** and **25**).

Table 2. SAR Studies of Inhibitors Containing Bicyclic Thiophene Derivatives[13]



R	Compound	IC_{50} (nM)	CIC_{95} (nM)
	**21**	0.1	100
	**22**	0.47	100
	**23**	0.04	50
	**24**	0.11	12
	**25**	0.32	6

The pharmacokinetics of two monocyclic (**17** and **18**) and two bicyclic (**24** and **25**) thiophene derivatives from Tables 1 and 2 were evaluated in dogs. Time-course plasma levels in dogs after oral administration of these compounds in 0.05 M citric acid solution were followed and the results are presented in Fig. 1.[15] As can be seen, none of the compounds examined exhibited desirable absorption profiles. Among the four compounds, only compound **18** which has the thienylethyl substitution showed a C_{max} above 1 μM.

Examination of the physical properties of these four compounds to gain an insight into the poor absorption in dogs revealed that these compounds in general exhibit extremely poor water-solubility as shown in Table 3. Compound **18**, which exhibited the highest absorption among the four compounds indeed showed the highest solubility at both pH 5.2 and 7.4. Within this series of inhibitors, absorption in dogs may be related to the aqueous solubility. Efforts are now in progress to incorporate more solubilizing ligands in this inhibitor class while preserving the high-affinity $P_{3'}$ ligands.[16]

Figure 1. Plasma Levels of Compounds 17, 18, 24 and 25 in Dogs (0.05 M Citric Acid Solution)

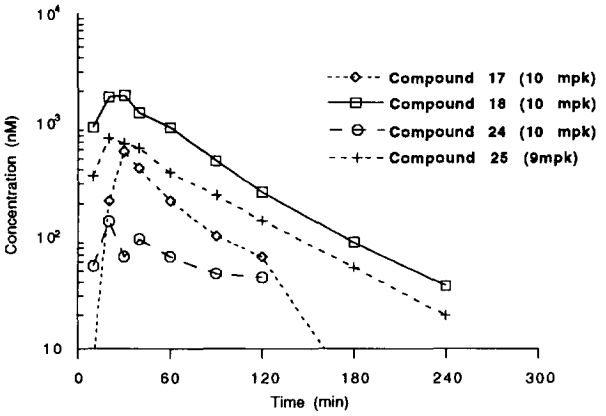


Table 3. Solubilities of Compounds **17**, **18**, **24** and **25** at pH 5.2 and 7.4[17]

Compound	Solubility (mg/mL) at pH=7.4	at pH=5.2
17	<0.00008	0.0372
18	0.0913	1.27
24	0.0001	0.0111
25	<0.00005	0.0069

Summary: Various monocyclic or bicyclic thienylmethyl derivatives proved to be highly efficient $P_{3'}$ ligands for the hydroxyethylpiperazine class of HIV PR inhibitors. By incorporating bicyclic thienothiophene moieties, extremely effective inhibition of the spread of virus in the whole cell assay was realized at as low a concentration as 6 nM. However, perhaps because of their poor water solubility, these inhibitors exhibit rather poor oral absorption profiles. Studies are in progress to improve the pharmacokinetic profile of this extremely potent class of inhibitors by modifying other parts of the inhibitors.

Acknowledgments: We are grateful to Dr. John Prugh for providing key intermediates for [2,3-*b*]- and [3,2-*b*]thienothiophene-2-carboxaldehydes. We also appreciate the support of Dr. H. G. Ramjit and Mr. A. B. Coddington (MS analysis) and Drs. Graham Smith, William Randall, Mr. John Moreau, Mr. Matthew Zrada, Mr. Ken Anderson and Ms. Patrice Ciecko (analytical support). Ms. J. F. Kaysen is gratefully acknowledged for the manuscript preparation.

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8. For assay protocol, see Thompson, W. J.; Fitzgerald, P. M. D.; Holloway, M. K.; Emini, E. A.; Darke, P. L.; McKeever, B. M.; Schleif, W. A.; Quintero, J. C.; Zugay, J. A.; Tucker, T. J.; Schwering, J. E.; Homnick, C. F.; Nunberg, J.; Springer, J. P.; Huff, J. R. *J. Med. Chem.* **1992**, *35*, 1685 and references cited therein.
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13. Proton nmr and ir spectra were consistent with assigned structures. Satisfactory (±0.4%) elemental analyses and/or high resolution MS were obtained for all compounds.
14. This high potency is believed to be due to the maximized hydrophobic interaction of the bithienylmethyl ligand with the S_3' subsite and molecular modeling studies are in progress to confirm this hypothesis.
15. Two dogs per each compound were examined and the average plasma concentrations are shown in Fig. 1. For the dog pharmacokinetic study protocol, see Vacca, J. P.; Dorsey, B. D.; Schleif, W. A.; Levin, R. B.; McDaniel, S. L.; Darke, P. L.; Zugay, J.; Quintero, J. C.; Blahy, O. M.; Roth, E.; Sardana, V. V.; Schlabach, A. J.; Graham, P. I.; Condra, J. H.; Gotlib, L.; Holloway, M. K.; Lin, J.; Chen, I-W. Vastag, K.; Ostovic, D.; Anderson, P. S.; Emini, E. A.; Huff, J. R. *Proc. Natl. Acad. Sci, USA* **1994**, *91*, 4096-4100
16. These efforts include replacing the phenylmethyl group at the P_1-position with a 3- or 4-pyridylmethyl group. For this type of approach in a hydroxypentanamide series of protease inhibitors, see Dorsey, B. D.; McDaniel, S. L.; Levin, R. B.; Vacca, J. P.; Darke, P. L.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Lin, J. H.; Chen, I.-W.; Holloway, M. K.; Anderson, P. S.; Huff, J. R. *Biomed. Chem. Lett.* in press.
17. All the solubilities were determined on amorphous materials.

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