Communications to the Editor

Structure-Based Design of Sulfonamide-Substituted Non-Peptidic HIV Protease Inhibitors

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Received August 28, 1995

The rapid and alarming spread of acquired immune deficiency syndrome (AIDS) has sparked an intense worldwide search for drugs to combat the causative agent of this disease, human immunodeficiency virus (HIV). The HIV protease enzyme is a particularly attractive target in this regard since it plays a key role in viral maturation.[1] Although a number of interesting compounds are in development, they are primarily peptide based and therefore have pharmacokinetic drawbacks (either poor bioavailability or relatively rapid clearance) and complicated syntheses.[2-4] Using a high-throughput screening approach, we identified phenprocoumon (**1**) as a novel lead template possessing weak HIV protease enzyme inhibitor activity ($K_i = 1\ \mu M$) but superior pharmacokinetics.[5] Other workers have also reported the discovery of warfarin analogues as competitive inhibitors of HIV protease.[6-10] The coumarin template **1** was developed into the first-generation pyrone clinical candidate **2** (U-96988) which maintained excellent pharmacokinetic properties ($C_{max} > 50\ \mu M$ and $t_{1/2} > 4$ h following a single oral dose of 10 mg/kg in dogs), but had increased enzyme potency ($K_i = 38$ nM) and appreciable antiviral activity in cell culture ($IC_{50} = 3\ \mu M$).[5] Compound **2** has two chiral centers but is readily prepared as a mixture of four compounds in a simple three-step synthesis.[5]

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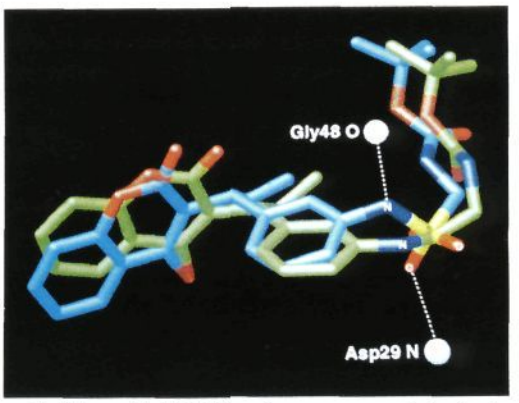
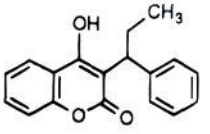


Figure 1. Shown in light blue is an energy minimized model (in the HIV-2 protease binding site) of an SO_2 analogue of compound **3**, in which two hydrogen bonds to the protein are formed. This model was constructed from the green compound (compound **3**) which, in an early stage of refinement as shown here, did not form the same hydrogen bonds. Oxygen atoms are shown in red, nitrogens in dark blue, and sulfur in yellow.



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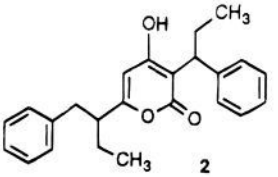

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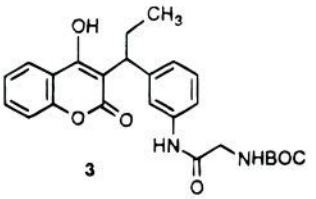
We have been interested in preparing additional compounds with improved potency and potentially reduced complexity. Examination of the crystal structure of closely related analogues of **1** bound to HIV protease overlayed with structures of peptide-based inhibitors suggested that addition of an amide linkage could improve the potency of the coumarin-based inhibitors.[11] This did prove to be the case, at least in terms of enzyme inhibition. For example, compound **3** with a BOC-glycine side chain was about 6-fold more potent than compound **1**.[11] Further modeling based on the crystal structure of **3** bound to HIV-2 protease suggested that replacement of the amide carbonyl with an SO_2 group might result in additional hydrogen bonds that were not observed with the parent compound (Figure 1).[12] While in general the coumarin derivatives are only modest enzyme inhibitors, a significant improvement in enzyme potency was noted with the saturated analog, in particular the cyclooctylpyranone inhibitor **4** ($K_i = 15$ nM).[13] Compound **4** has good pharmacokinetics and is relatively easy to prepare; however, its activity in cell culture was disappointing ($IC_{50} = 57\ \mu M$).[13] As with the coumarins, addition of the carboxamide side chain provided an increase in enzyme inhibitor potency.[14]



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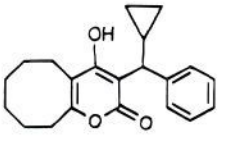

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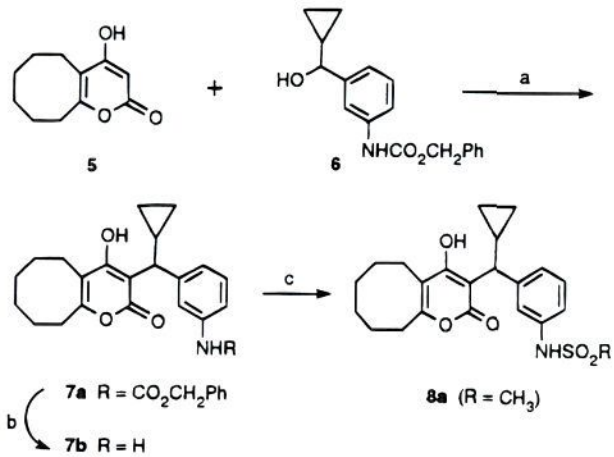
Scheme 1[a]



[a] Reagents: (a) *p*-TsOH, toluene, 62%; (b) Pd/C, cyclohexene, 100%; (c) RSO_2Cl, pyridine, CH_2Cl_2, 65–80%.

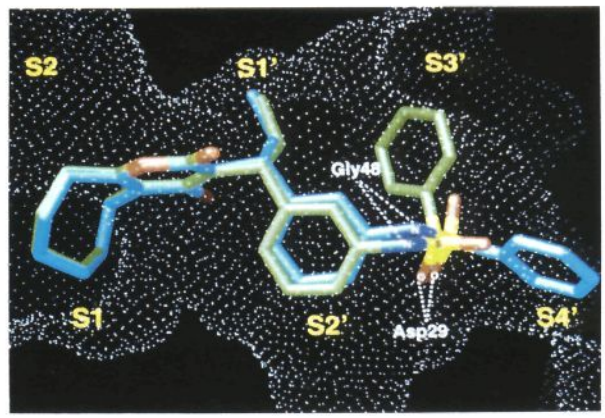
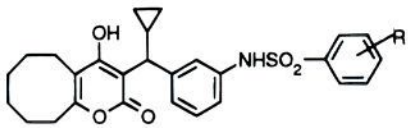


Figure 2. Two working models of compound **8b**, developed from a Monte Carlo/molecular mechanics procedure, in which the terminal phenyl was predicted to occupy either the S3′ or S4′ pocket in the enzyme. The models were constructed from a crystal structure of compound **4** (ref 13). The surface of the HIV-2 protease substrate binding site is shown in white.

Table 1



compd	R	K_i (nM)[a]	IC_{50} (μM)[b]
8b	H	3	5
8c	*o*-CH_3	9	>10
8d	*m*-CH_3	5	4.0
8e	*p*-CH_3	3	3.6
8f	*p*-CN	0.8	1.5

[a] Enzyme inhibition as determined by the method in ref 5. [b] Antiviral potency as determined in ref 18.

Thus, we became interested in preparing sulfonamide-substituted cyclooctylpyranone analogues. The compounds were prepared in a straightforward manner (five steps from cyclooctanone) in a process which parallels that used to prepare amide-substituted analogues of **4** and involves the acid-mediated coupling of the known cyclooctylpyranone **5** with **6**[11] in the key step (Scheme 1).[14] The initial racemic cyclooctylpyranonesulfonamide prepared, **8a** (K_i = 11 nM),[15] with a simple methanesulfonamide substituent was at least as potent as compound **4**. Molecular modeling starting with crystal structures of the parent cyclooctylpyranone suggested that larger attachments to the sulfonamide group could be accommodated (Figure 2). In particular, the model suggested that a phenyl group could be easily tolerated and predicted that the sulfonamide group would form hydrogen bonds to Gly-48 of Asp-29 of the protease. Of the many possible positionings of the phenyl ring, two models were favored, in which the phenyl occupied the S3′ or S4′ sites.[16]

As indicated in Table 1, the benzenesulfonamide **8b** is indeed a potent enzyme inhibitor with K_i = 3 nM against HIV-1. On the basis of the working model which positioned the phenyl group in the S4′ site, a number of analogues were prepared with the sulfonamide NH replaced with an alkyl or substituted alkyl side chain in an attempt to fill the S3′ site. However, these were uniformly less active (data not shown), suggesting that the working model with the phenyl group extended into the S3′ pocket might be the correct one. Subsequently, the crystal structure of **8b** bound to HIV-1 protease was solved and clearly showed the binding of the phenyl group in the S3′ pocket. Furthermore, the predicted hydrogen bonds to Gly-48 and Asp-29 were confirmed. In addition, the crystal structure revealed a favorable interaction between the phenyl ring of **8b** and Arg-8 of the enzyme, which in turn formed a salt bridge with Asp-29. Modification of the terminal phenyl group of **8b** showed that ortho substituents (e.g. as in **8c**) were disfavored whereas meta (**8d**) and para (**8e**) substitution with small groups were tolerated (Table 1). Especially interesting was the *p*-cyano derivative **8f** (U-103017) with a K_i = 0.8 nM.[17] The para position on the phenyl ring has room for nonbulky substituents, and this was confirmed with the crystal structure of **8f** bound to HIV protease (Figure 3). The presence of the *p*-cyano group, however, causes the benzenesulfonamide moiety to shift its position somewhat, weakening the interaction of the NH with Gly-48.

In addition to increasing enzyme inhibition, substitution of the cyclooctylpyranrone moiety with arene-

Table 2. Selected Pharmacokinetic Parameters for **8f** Calculated form Time-Course Plasma Concentrations (Mean ± SD)[a]

species	dose (mg/kg)/route	CL_T (L/h/kg)	V_{ss} (L/kg)	$t_{1/2}$ (h)	C_{max} (μM)	t_{max} (h)	F (%)
rat	2.5/iv	0.075 ± 0.014	0.11 ± 0.014	3.0 ± 0.18			
	5/po				24.2 ± 9.99	0.25–1	41.9 ± 3.15
dog	5/iv	0.044 ± 0.008	0.084 ± 0.018	6.3 ± 1.8			
	10/po				156 ± 46.1	0.67–1	76.7 ± 4.1

[a] The plasma concentration–time data were analyzed by noncompartmental methods, and the distribution rate constant (β) and half-life ($t_{1/2}$) were estimated. The total body clearance (CL_T), steady-state volume of distribution (V_{ss}), maximum concentration (C_{max}), time of maximum concentration (t_{max}), and bioavailability (F) were also calculated. Compound **8f** was administered orally as a single dose in an aqueous sodium hydroxide solution adjusted to pH 10.

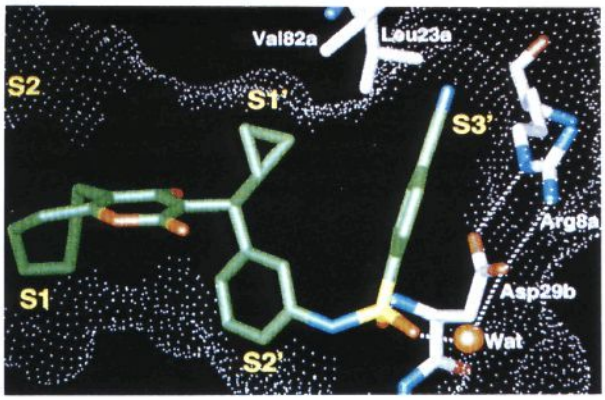


Figure 3. Crystal structure of the more active enantiomer (*S*-configuration at the chiral center) of **8f** bound to HIV-1 protease. The carbon backbone of **8f** is shown in green. The hydrogen bond between NH of Asp-29 and the oxygen of the sulfonyl group is partially obscured in this view.

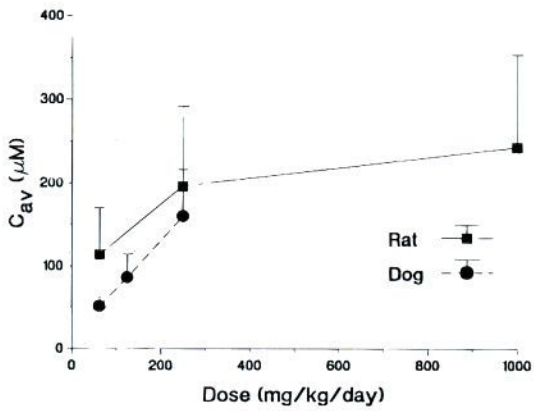


Figure 4. Comparison of average steady-state concentrations (C_{av}) in rats and dogs after oral administration of **8f** as a solution formulation, twice daily, for 28 days.

sulfonamide groups provided improvement in antiviral activity. The vast majority of coumarins and pyrone analogues (including, interestingly, most of the amide-substituted analogues) showing potent enzyme inhibition demonstrated relatively little antiviral activity in cell culture. In contrast, substituting the cyclooctylpyranones with a wide variety of arenesulfonamides led to analogues with markedly enhanced antiviral activity. This was not because of an increase in toxicity with such compounds; for example, compound **8f** (IC_{50} = 1.5 μM, $TCID_{50}$ = 100 μM) showed a 70-fold selectivity index in MT4 cells acutely infected with HIV-1_{IIIB}.[18] Resolution via chiral HPLC[19] of the cyclooctylpyranones such as **8f** revealed at most a 2−4 fold difference in potency between the two enantiomers either at the enzyme level or in cell culture, with the more potent enantiomer having a K_i = 0.6 nM and an antiviral IC_{50} = 1 μM.

Further work with compound **8f** revealed that it was roughly equipotent against both HIV-1 (K_i = 0.8 nM) and HIV-2 (K_i = 3.2 nM) protease but did not have significant activity against a panel of human aspartyl proteases (renin, pepsin, gastricsin, cathepsin D, and cathepsin E).[20] Furthermore, it was active against all clinical viral isolates evaluated (median IC_{50} = 5 μM, range 1−8 μM).[21] Pharmacokinetic properties in rats and dogs were very promising (Table 2). Oral bioavailability ranged from 42% (rat) to 77% (dog), and the oral half-life in the dog was 6 h. Particularly impressive were the concentrations of the drug in animals which could be safely achieved and maintained with multiple dosing, typically above hundred micromolar (Figure 4). Because of its subnanomolar enzyme inhibition, potent anti-HIV activity, favorable pharmacokinetic properties, and relative ease of synthesis, compound **8f** (U-103017) was selected for development and has been entered into phase I clinical testing.

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(15) In vitro inhibition of HIV-1 protease was measured as described in ref 5.

(16) S_n to $S_{n'}$ refer to nomenclature for enzyme binding pockets as described in the following: Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.

(17) Compound **8f**: mp 183–183.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84–7.81 (m, 2H), 7.73–7.70 (m, 2H), 7.26–7.23 (m, 4H), 7.13 (s, 1H), 7.0–6.9 (m, 1H), 6.79 (s, 1H), 6.34 (s, 1H), 3.85–3.83 (d, 1H), 2.61 (m, 2H), 2.49 (m, 2H), 1.8–1.7 (m, 2H), 1.65–1.3 (m, 5H), 1.75–1.55 (m, 2H), 0.45–0.4 (m, 1H), 0.25–0.15 (m, 1H); HRMS found 504.1710, calcd 504.1709. Anal. Calcd for $C_{28}H_{28}N_2O_5S$: C, 66.64; H, 5.59; N, 5.55; S, 6.35. Found: C, 66.76; H, 5.68; N, 5.38; S, 6.30.

(18) Antiviral activity and cytotoxicity were measured as described in the following: Chong, K.-T.; Pagano, P. J.; Hinshaw, R. R. Bisheteroarylpiperazine Reverse Transcriptase Inhibitor in Combination with 3′-Azido-3′-Deoxythymidine or 2′,3′-Dideoxycytidine Synergistically Inhibits Human Immunodeficiency Virus Type 1 Replication In Vitro. *Antimicrob. Agents Chemother.* **1994**, *38*, 288–293.

(19) (*R,R*) Whelk-O I chiral stationary phase of 75% hexane, 25% isopropyl alcohol, and 0.05% acetic acid; flow rate of 1.0 mL/min; UV detector at 288 nM.

(20) Our thanks to Dr. Alfredo G. Tomasselli for renin inhibition data and Professor Ben M. Dunn, University of Florida, Gainesville, FL, for the remaining enzyme inhibition data.

(21) Experiments (12 clinical isolates) performed at SRA Technologies, Inc., Rockville, MD.

JM950638Z