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SUBSTITUTED ALKYLPYRIDINES AS P_3' LIGANDS FOR THE HYDROXYETHYLPIPERAZINE CLASS OF HIV-1 PROTEASE INHIBITORS: IMPROVED PHARMACOKINETIC PROFILES

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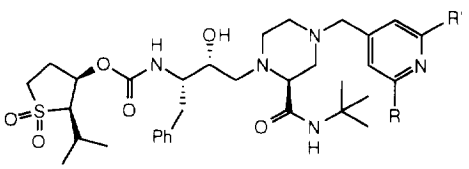
Abstract: As a systematic approach to develop HIV-1 protease inhibitors exhibiting desirable pharmacokinetic profiles, hydroxyethylpiperazine series of inhibitors containing various mono- or dialkyl-substituted pyridylmethyl groups have been examined. Very high enzyme inhibitory potency and antiviral activity in a whole cell assay were observed with these inhibitors and, when administered orally to dogs, selected compounds in this series exhibited prolonged half-lives compared to the non-substituted pyridylmethyl compound **1**.

HIV-1 protease (HIV PR) plays a critical role in the viral life cycle by participating in the maturation of the viral particles and thus has been considered one of the most attractive targets for AIDS chemotherapy.[2] A number of HIV PR inhibitors have been reported from many laboratories with vast structural diversity[3] and several of them are being evaluated in clinical trials.[4] However, despite tremendous efforts in the development of HIV PR inhibitors, only a handful of the known inhibitors are reported to be orally bioavailable in animal models.[4] Recently we have disclosed a series of highly potent and orally bioavailable HIV PR inhibitors such as **1** possessing the hydroxyethylpiperazine-2-carboxamide structure.[5] We have also demonstrated that extremely potent inhibitors against the spread of virus in a whole cell assay could be prepared in this series by incorporating bicyclic thienylmethyl derivatives at the N(4)-position of the piperazine ring.[6]



1 (R=R'=H)

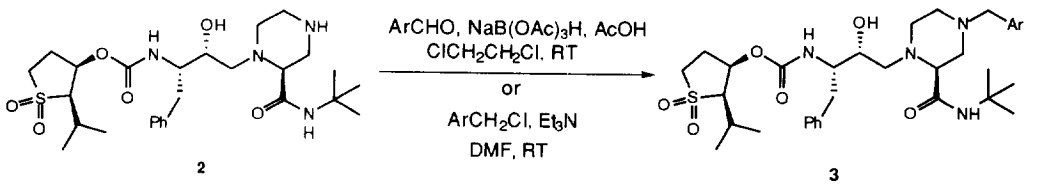
As a part of our continuing effort to develop potent HIV PR inhibitors suitable for a clinical application,[7] our focus was turned to the short half-lives that most of the hydroxyethylpiperazine class of compounds exhibit when they were examined in animal models such as dogs. In the case of compound **1**, the pyridyl group was found initially to help enhance the oral absorption of the compound but later contributes to a rapid clearance through extensive metabolism.[8] We initiated a systematic investigation where the possible metabolic pathways are blocked by replacing the simple pyridyl group of inhibitor **1** by 2- or 2,6-alkyl substituted pyridine derivatives.

Herein we report structure-activity studies of such inhibitors vs. HIV PR, their antiviral activities in cells and the results of pharmacokinetic investigation in dogs for some selected compounds.

Chemistry

Inhibitors possessing various alkyl-substituted pyridylmethyl moieties at the 4-position of the piperazine-2-carboxamide of compound type **1** were prepared through either reductive alkylation or S_N2 type alkylation of the free amine **2** with the corresponding aryl aldehydes or arylmethyl halides, respectively, as shown in Scheme 1.[5]

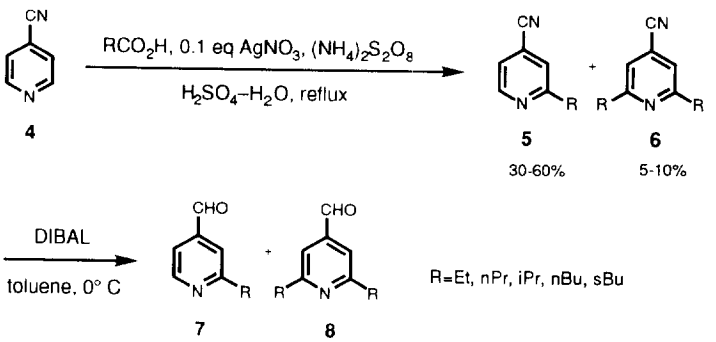
Scheme 1



2 **3**

A key step in the preparation of 2-alkyl- and 2,6-dialkyl-substituted 4-formylpyridine was a radical substitution reaction of 4-cyanopyridine.[9] As depicted in Scheme 2, treatment of 4-cyanopyridine (**4**) with ammonium persulfate and a catalytic amount of silver nitrate in the presence of an aliphatic carboxylic acid provided a mixture of mono- and dialkylated pyridines **5** and **6** in 30-60% and 5-10% yields, respectively. This mixture of alkylated cyanopyridines was reduced with DIBAL in toluene to provide the corresponding aldehydes **7** and **8** in 70-80% yields.[10] The mono- and dialkylated aldehydes thus obtained were easily separated by silica gel column chromatography. Using this two step sequence, preparation of ethyl, propyl, isopropyl, n-butyl, and sec-butyl substituted pyridine-4-carboxaldehydes was easily accomplished. Aldehydes thus obtained were coupled to amine **2** through reductive alkylation pathways as shown in Scheme 1.

Scheme 2



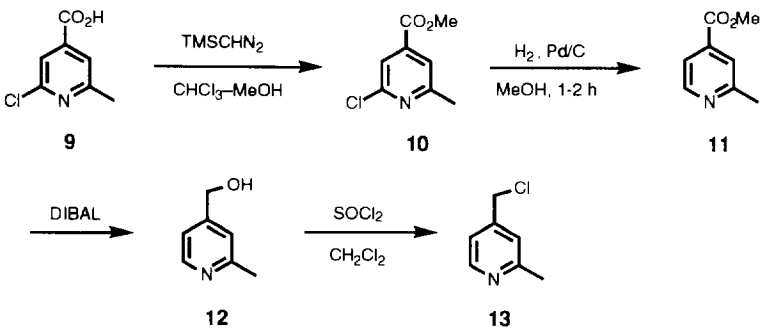
4 **5** 30-60% **6** 5-10%

7 **8**

In the case of 2-methyl- and 2,6-dimethyl-substituted pyridine derivatives, however, the sequence described in Scheme 2 employing acetic acid yielded a complex mixture of products. Therefore alternative routes were devised to provide the desired compounds.

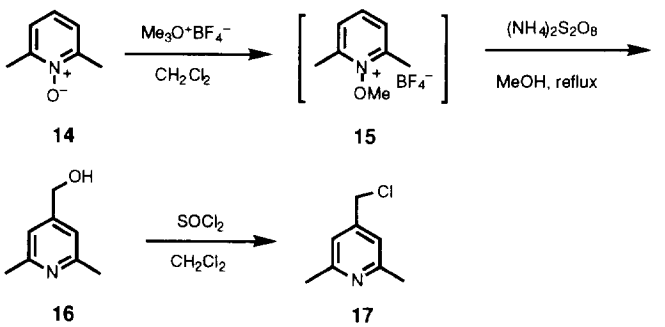
For the preparation of 2-methyl-4-chloromethylpyridine, utilization of readily available 2-chloro-6-methyl-4-pyridinecarboxylic acid (**9**) as a starting material was explored. As depicted in Scheme 3, esterification of the acid **9** followed by reduction under catalytic hydrogenation conditions provided the desired des-chloro compound **11** in 85% yield. Reduction of the ester **11** to alcohol **12** and subsequent treatment of **12** using thionyl chloride provided chloromethyl compound **13** in a good yield (70-80%), which was readily coupled to the piperazine **2**.

Scheme 3



The synthesis of 2,6-dimethyl-4-chloromethylpyridine (**17**) was accomplished following a two step sequence depicted in Scheme 4. One pot conversion of 2,6-lutidine-N-oxide (**14**) to 2,6-dimethyl-4-hydroxymethylpyridine (**16**) via methylation of the N-oxide followed by radical-mediated substitution was accomplished in good yields (60-70%).[11] Treatment of **16** with thionyl chloride furnished the corresponding chloride **17**.

Scheme 4

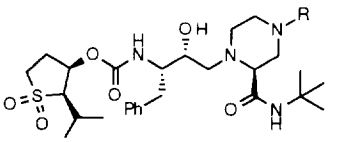


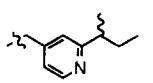
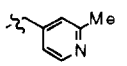
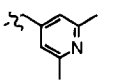
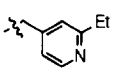
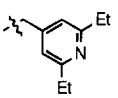
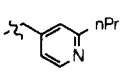
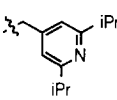
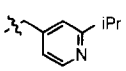
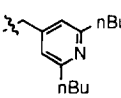
Results and Discussion

Inhibitors thus obtained which contained alkyl-substituted 4-pyridylmethyl derivatives were tested in both an *in vitro* enzyme inhibition assay[12] and a viral spread assay in MT4 human lymphoid cells infected with the IIIb isolate.[13] As documented in Table 1, compounds in this class exhibited high potencies against the enzyme (IC_{50}=1-2 nM) and excellent antiviral activities (CIC_{95}=12-200 nM) were also observed in the cell-based assay. It appears that mono- or dialkyl substitution on the pyridine boosts the antiviral potency of this series of

compounds compared to the non-substituted compound **1**, although no considerable increase in *in vitro* potency was observed. The increased antiviral potency of alkylated pyridine derivatives in the whole cell assay may be due to the enhanced cell penetration. Both mono- and dialkyl substituted pyridyl derivatives seem to serve as efficient ligands for HIV PR inhibitors in both assays. Among the compounds examined, n-propyl substituted compound **21** showed the highest potency in the cell-based assay.

Table 1. SAR Studies of Inhibitors Containing Mono- or Dialkylpyridylmethyl Derivatives[14]



Compound	R	IC_{50} (nM)	CIC_{95} (nM)[a]	Compound	R	IC_{50} (nM)	CIC_{95} (nM)[a]
1		1.3	200	**23**[c]		1.2	25
19		1.4	75±35 (n=5)	**24**		2.5	50
20		1.5	81±80 (n=4)	**25**		1.8	42±14 (n=3)
21		1.1	12 (n=2)	**26**		2.3	50, 100 (n=2)
22		-[b]	67±29 (n=3)	**27**		0.95	25 (n=2)

[a] Number in parentheses denotes repetitions. [b] Not determined. [c] A 1:1 mixture of diastereomers at *sec*-butyl.

Selected compounds were examined in the pharmacokinetic experiment in dogs. Compounds were administered orally to two dogs as a 0.05 M citric acid solution and time-course plasma levels were followed by HPLC analysis.[15] As presented in Figure 1, it is clear that compound **1** was absorbed well ($Cmax_{ave}$=6.68 μM) immediately after administration (Tmax=25 min). However, no appreciable concentration of **1** was found in plasma after 3 h. Then a series of mono-and dialkyl-substituted pyridyl derivatives (compounds **19~21**, **24** and **25**) were examined and their pharmacokinetic properties are tabulated in Table 2. While pharmacokinetic properties of compounds **21**, **24**, and **25** did not show much improvement over those of compound **1**, monomethyl- and monoethyl-substituted compounds **19** and **20** exhibited considerably improved half-lives (121 and 91 min, respectively). Figure 1 clearly demonstrates the extended half-lives of these two compounds over compound **1**. It is particularly noteworthy that both compounds showed concentration levels above their CIC_{95} values through the 8 h time period. Compound **19**, when administered i.v. at 2 mg/kg, showed $t_{1/2}$ ~70 min and

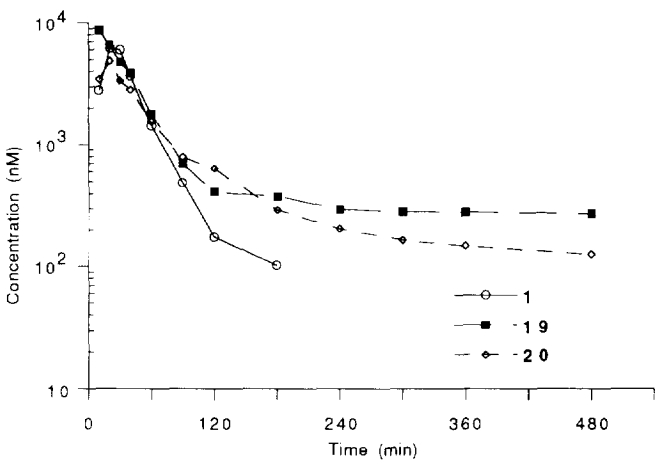
its oral bioavailability was found to be 41%. Further evaluation of compounds **19** and **20** is currently in progress and the results will be reported in due course.

Table 2. Pharmacokinetic properties of various inhibitors in dogs after oral administration (10 mpk) as a 0.05 M citric acid solution[a]

Compound	Cmax (μM)	Tmax (min)	$t_{1/2}$ (min)	AUC (μMxhr)
1	6.7	25	24	4.3
19	8.6	10	**121**	7.4
20	4.1	20	**91**	5.4
21[b]	2.3	25	21	1.5
24[b]	3.6	40	28	4.5
25	3.0	25	36	2.9

[a] Average values from two dogs are presented. [b] Dosed 8 mg/kg instead of 10 mg/kg.

Fig. 1. Plasma Levels of Compounds 1, 19 and 20 After Oral Administration to Dogs (0.05 M citric acid, 10 mpk)



Summary: HIV PR inhibitors possessing various 2-alkyl- or 2,6-dialkyl-4-pyridylmethyl groups as $P_{3'}$ ligands were prepared for the purpose of obtaining better pharmacokinetic properties than the parent compound **1**. These inhibitors exhibited good to excellent antiviral potencies. Among them, monomethyl- and monoethyl-substituted inhibitors **19** and **20** showed prolonged pharmacokinetic half-lives when administered orally to dogs.

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14. 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.
15. For the dog pharmacokinetic study protocol, see reference 7a.

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