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A β -Fluoroamine Inhibitor of Purine Nucleoside Phosphorylase

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Abstract

The potent immucillin purine nucleoside phosphorylase (PNP) inhibitors F-DADMe-ImmH [(3*S*, 4*S*)-**3**], and [(3*R*, 4*R*)-**3**] are synthesized in seven steps. Cycloaddition to a fluoroalkene and an enzymic resolution are the key features of the construction of the fluoropyrrolidines **11**, from which the immucillins are assembled by use of a three-component Mannich reaction. Slow-onset binding constants (K_i^*) for [(3*S*, 4*S*)-**3**] and [(3*R*, 4*R*)-**3**] with human PNP are 0.032 and 1.82 nM, respectively. F-DADMe-ImmH [(3*S*, 4*S*)-**3**] exhibits oral availability in mice at doses as low as 0.2 mg/kg.

Introduction

Purine nucleoside phosphorylase (PNP) is a purine-metabolizing enzyme that catalyzes the phosphorolysis of purine nucleosides to ribose or deoxyribose 1-phosphate and a nucleobase.(1) Individuals with a congenital deficiency of PNP exhibit an unusual and characteristic impairment of their immune system in that they have little or no T-cell immunity but retain normal B-cell function.(2) The biochemical link between PNP and T-cell deficiencies is the failure to degrade deoxyguanosine and its conversion to deoxyguanosine triphosphate (dGTP) in activated T-cells.(3, 4) Such T-cells require DNA synthesis for cell division and excess dGTP acts to inhibit ribonucleotide-diphosphate reductase, causing unbalanced deoxynucleotide pools and the induction of apoptosis.(5, 6) Therefore, PNP inhibitors are potential therapeutics for the control of T-cell proliferation without affecting humoral immunity. We have reported the design and synthesis of a series of transition state analogue inhibitors of PNP.(7-10) Two of these, Immucillin-H (**1**, ImmH, Forodesine)(11, 12) and DADMe-Immucillin-H (**2**, DADMe-ImmH, BCX-3408, R-3421)

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Supporting Information Available

Experimental details for compounds ($\pm$)-**8**, ($\pm$)-**9**, ($\pm$)-**10**, ($\pm$)-**12**, (3*S*, 4*R*)-**8**, (3*R*, 4*S*)-**9**, (3*S*, 4*S*)-**10**, (3*S*, 4*S*)-**12**, (3*R*, 4*R*)-**10**, and (3*R*, 4*R*)-**12**; ¹H NMR spectra of ($\pm$)-**8**, ($\pm$)-**9**, ($\pm$)-**12**, (3*S*, 4*R*)-**8**, (3*R*, 4*S*)-**9**, (3*S*, 4*S*)-**10**, (3*S*, 4*S*)-**12**, (3*R*, 4*R*)-**10**, (3*R*, 4*R*)-**12**, ($\pm$)-**3**, (3*S*, 4*S*)-**3**, and (3*R*, 4*R*)-**3**; HPLC data for ($\pm$)-**3**, (3*S*, 4*S*)-**3**, and (3*R*, 4*R*)-**3**; details of X-ray crystallographic structure determination of (3*R*, 4*R*)-**10**; details of inhibition assays; bioavailability of DADMe-ImmH (**2**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

(13) (Figure 1), are currently in clinical trials against T-cell cancers and autoimmune diseases.(14, 15)

Since the 1980s, fluorine substituents have become widespread and important drug components.(16) Organofluorine affects most adsorption, distribution, metabolic, and excretion properties as well as potency at the site of interest.(17) In the case of amines, the effects of β - or γ -fluorine substitution are largely the result of a change in the degree of protonation at physiological pH because such substitution lowers basicity.(18) For example, the pK_a s of protonated ethylamines are: 10.7 for ethylamine, 9.0 for 2-fluoroethylamine, and 5.7 for trifluoroethylamine.(16) Also, fluorine substitution at the 3-position of a piperidine ring lowers the pK_a by over 2 units.(19) There are many studies connecting the decrease in pK_a associated with β -fluorination of aliphatic amines with changes in biological properties. Some examples include: reduced affinity of fluoroisoquinolines for the α_2 -adrenoceptor,(20) increased activity of lung *N*-methyltransferase for fluoroalkylarylamines,(21) changes in affinity and activity of propranolols for cytochrome oxidase enzymes,(22, 23) increased oral absorption of fluorinated piperidine and piperazine indoles,(24) and changes in metabolism and tissue distribution of amphetamines.(25)

As part of our ongoing program of synthesis of transition-state analogue inhibitors of *N*-ribosylphosphorylases and hydrolases and the use of these compounds as therapeutic agents, (9, 10) we now report the synthesis of fluoro-DADMe-Immucillin-H [(3*S*,4*S*)-**3**], and its enantiomer.

Results and Discussion

DADMe-Immucillin-H (**2**) is most easily assembled by means of a three-component Mannich reaction between 9-deazahypoxanthine, formaldehyde, and pyrrolidine **4**.(26) This method, which is tolerant of variations in both the amine and 9-deazapurine, has provided us with many variants of **2** and so was an obvious choice for our synthesis of **3**.(9, 27) We chose to initially pursue a synthesis of the racemate, with the required fluoropyrrolidine ($\pm$) -**11** (Scheme 1) to be constructed through a cycloaddition of azomethine ylid **6** to the known fluoroacrylate **7**.(28, 29)

The 1,3-dipolar cycloaddition between azomethine ylids and alkenes is a potent method for the construction of substituted pyrrolidines with defined regio- and stereochemistry. Such a cycloaddition is the key step in one of the routes to diol **4**,(30, 31) and a plethora of other examples can be found in the literature.(32-34) The cycloaddition reaction is accelerated by electron withdrawing substituents on the alkene,(35),and so fluoroalkenes are suitable substrates for synthesis of 3- and 4-fluoropyrrolidines. However, this approach has only recently received attention.(36)

Z-Ethyl 3-acetoxy-2-fluoroacrylate (**7**) was synthesized, following the literature method, from ethyl fluoroacetate.(28) *Z*-configuration was assigned on the basis of the H-F coupling constant ($^3J_{H,F} = 18$ Hz).(29, 37) Cycloaddition between ester **7** and the azomethine ylid **6**, generated in situ from *N*-methoxymethyl-*N*-(trimethylsilylmethyl)benzylamine (**5**), gave pyrrolidine ester ($\pm$)-**8** in good yield.(30) A *cis* arrangement of the fluoro and acetoxy

substituents in pyrrolidine ($\pm$)–**8**, as would be expected from a concerted cycloaddition to enol acetate **7**, was assumed at this point and was later confirmed by crystal structure (vide infra).

Reduction of ester ($\pm$)–**8** (LiBH_4 , 80%) gave crystalline diol ($\pm$)–**10**, which was converted to *N*-Boc-pyrrolidinol ($\pm$)–**12** in quantitative yield by hydrogenolysis in the presence of di-*tert*-butyl dicarbonate. Pyrrolidine ($\pm$)–**11**, produced either by hydrogenolysis of ($\pm$)–**10** or hydrolysis of ($\pm$)–**12**, participated in a three-component Mannich reaction with 9-deazahypoxanthine and formaldehyde to give the target compound ($\pm$)–**3** in moderate yield.

We then turned our attention to the synthesis of the enantiomerically pure forms of **3**. An enzymic transacetylation is a key step in one of the published syntheses of nonracemic pyrrolidine diol **4**, and so we applied this method to the 4-fluoropyrrolidines (3*S*,4*S*)– and (3*R*,4*R*)–**11**.(38) Pyrrolidine ($\pm$)–**8** was first deacetylated, in moderate yield, with sodium ethoxide to give secondary alcohol ($\pm$)–**9**. Acetylation catalyzed by an immobilized form of lipase B of *Candida antarctica* (CAL-B) gave moderate yields (31%) of alcohol (+)–**9** [(3*R*,4*S*)–**9**, vide infra] and acetate (+)–**8** [(3*S*,4*R*)–**8**]. The enantiomeric excess (ee) of alcohol (+)–**9** was determined to be 90% by HPLC. Reduction of (+)–**8** with lithium borohydride gave crystalline *N*-benzylpyrrolidinol (3*R*,4*R*)–**10**. A crystal structure (Figure 2) determined both the absolute stereochemistry of this compound and the *cis* relationship of the fluoro and hydroxy substituents that had been established during the initial cycloaddition.(39)

Alcohol (3*R*,4*S*)–**9** was also reduced with lithium borohydride to *N*-benzylpyrrolidinol (3*S*,4*S*)–**10**. Both benzylpyrrolidinols were purified by crystallization from ethyl acetate–hexanes. The *N*-Boc-pyrrolidinols (3*S*,4*S*)– and (3*R*,4*R*)–**12**, synthesized from these crystals by hydrogenolysis in the presence of di-*tert*-butyl dicarbonate, both had ee greater than 95%; some enhancement of ee having occurred upon crystallization. Deprotection followed by Mannich reaction gave the target compounds (3*S*,4*S*)– and (3*R*,4*R*)–**3**.

Inhibition Studies

F-DADMe-ImmH [(3*S*,4*S*)–**3**] is a potent inhibitor of human purine nucleoside phosphorylase, having a slow-onset dissociation constant (K_i^*) of 32 pM, binding 3-fold weaker than DADMe-ImmH (**2**) (Table 1). As expected, the enantiomer [(3*R*,4*R*)–**3**] does not bind as tightly to the enzyme, but it is still a nanomolar inhibitor and, by comparison of its to the K_i of L-DADMe-ImmH, is about five times less potent than its nonfluorinated counterpart. *Plasmodium falciparum*(malaria) PNP binds these compounds less well, but this is not surprising because this enzyme has repeatedly shown relatively poor affinity for immucillins.(41)

Bioavailability

F-DADMe-ImmH [(3*S*,4*S*)–**3**] was administered to mice orally or by intraperitoneal (ip) injection with a single dosage of 14 nmol. Complete inhibition of PNP catalytic activity was observed in the ip injection group within 30 min, yielding a $t_{1/2}$ of less than 15 min for the onset of inhibition (Figure 3). For the orally treated group, the $t_{1/2}$ of onset was 20 h,

suggesting that F-DADMe-ImmH was slowly absorbed from the mouse gastrointestinal tract. Despite slow uptake, 80% inhibition of blood PNP with a single oral dose of 0.2 mg/kg is adequate for oral delivery. Both ip and oral groups regained 50% PNP catalytic activity at about 70–75 h, demonstrating long-term action for both routes of administration. In control experiments, mice were treated by oral and ip administration with 14 nmol of DADMe-ImmH (**2**). The $t_{1/2}$ of PNP inhibition for oral administration of **2** was 90 min and no regaining of catalytic activity was observed after 80 h for either administration route (Supporting Information). Thus, the bioavailability and duration of action for F-DADMe-ImmH [(3*S*,4*S*)-**3**] is less than that of DADMe-ImmH (**2**).

Conclusions

We have synthesized F-DADMe-ImmH [(3*S*,4*S*)-**3**] in seven steps. Cycloaddition to a fluoroalkene and an enzymic resolution are the key features of the construction of the fluoropyrrolidine **11**, from which the target is assembled by use of a three-component Mannich reaction. F-DADMe-ImmH [(3*S*,4*S*)-**3**] is a potent inhibitor of human PNP. It is orally available in mice at 0.2 mg/kg but has decreased oral availability and duration of action relative to DADMe-ImmH (**2**).

Experimental Section

cis-1-((9-Deazahypoxanthin-9-yl)methyl)-4-fluoro-4-hydroxymethylpyrrolidin-3-ol [(±)-**3**]

HCl (37%, 0.2 mL) was added to a solution of pyrrolidinol (±)-**10** (31 mg, 0.13 mmol) in methanol (5 mL), and the whole was concentrated to dryness under reduced pressure. The residue was suspended in methanol and again concentrated to dryness. Water (1.5 mL), dioxane (0.3 mL), NaOAc (16.2 mg, 1.5 equiv), 9-deazahypoxanthine(44) (36 mg, 2 equiv), and formaldehyde solution (37%, 22 μ L, 2 equiv) were added, and the mixture was heated at 100 °C overnight. Chromatography of the residue obtained when this solution was evaporated to dryness, using methanolic ammonia (2 M, 35%) in CH₂Cl₂ as eluant, gave the crude title compound (±)-**3** as a cream-colored foam (15 mg, 40%). ¹H NMR (of hydrochloride) (D₂O) δ 8.31 (s, 1H), 7.79 (s, 1H), 4.60 (s, 2H), 4.45 (m, 1H), 3.9–3.7 (m, 5H), 3.49 (m, 1H). ¹³C NMR (of hydrochloride) (D₂O) δ 155.2, 144.3, 141.8, 132.7, 118.7, 104.5, 100.5 (d, J = 186 Hz), 69.6 (d, J = 17 Hz), 61.3 (d, J = 27 Hz), 57.8 (d, J = 25 Hz), 56.2, 49.7. ¹⁹F NMR (D₂O) δ -176. HRMS for C₁₂H₁₆N₄O₃F [M + H]⁺ calcd, 283.1206; found, 283.1208. This material was further purified by HPLC.

(3*S*,4*S*)-1-((9-Deazahypoxanthin-9-yl)methyl)-4-fluoro-4-hydroxymethyl pyrrolidin-3-ol [(3*S*,4*S*)-**3**]

(3*S*,4*S*)-**12** (10 mg, 0.043 mmol) was deprotected and coupled with 9-deazahypoxanthine(44) as described for the racemate to give crude title compound (3*S*,4*S*)-**3** (8 mg, 67%), which was further purified by HPLC. ¹H NMR (of hydrochloride) identical to the racemate. HRMS for C₁₂H₁₆N₄O₃F [M + H]⁺ calcd, 283.1206; found, 283.1201.

(3*R*,4*R*)-1-((9-Deazahypoxanthin-9-yl)methyl)-4-fluoro-4-hydroxymethyl pyrrolidin-3-ol [(3*R*,4*R*)-3]

(3*R*,4*R*)-**12** (10 mg, 0.043 mmol) was deprotected and coupled with 9-deazahypoxanthine(44) as described for the racemate to give crude title compound (3*R*,4*R*)-**3** (8 mg, 67%), which was further purified by HPLC. ¹H NMR (of hydrochloride) identical to the racemate. HRMS for C₁₂H₁₆N₄O₃F [M + H]⁺ calcd, 283.1206; found, 283.1204.

Bioavailability of F-DADMe-ImmH [(3*S*,4*S*)-3]

Two male BALB/c mice (~25 g, purchased from the National Cancer Institute) were fasted overnight. One mouse was treated orally with a single dosage of 14 nmol of F-DADMe-ImmH [(3*S*,4*S*)-**3**], and the same dosage was administered by intraperitoneal (ip) injection to the other mouse. Blood samples (5 µL) were taken at intervals, mixed with the same volume of 1% heparin and 0.3% Triton X-100 in PBS, and stored at 4 °C before assays. Erythrocyte PNP catalytic activity was monitored spectrophotometrically by adding 1 µL of the blood sample to the reaction mixture containing 50 mM potassium phosphate (pH 7.4), 1 mM inosine, and 60 mU of xanthine oxidase. PNP catalytic activity of each sample was normalized for protein concentration. The normalized rate at each time point was divided by the normalized rate prior to inhibitor administration and plotted against time. The time (*t*_{1/2}) required for 50% of the PNP catalytic activity to be lost and the time (*t*₂) required for 50% regain of PNP catalytic activity were interpolated from the plot.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structures of some immucillins and a precursor amine.

Figure 2.
ORTEP(40) drawing of (3R,4R)-10.

Figure 3. Bioavailability of F-DADMe-ImmH in mice. Enzyme activity was assayed following administration of 14 nmol by oral treatment (■) or intraperitoneal injection (◆).

Scheme 1.

Synthesis of the Racemate and Enantiomers of F-DADMe-ImmH [(±)-3, (3*S*,4*S*)-3, and (3*R*,4*R*)-3]^a

a Reagents (a) TFA, CH₂Cl₂, 0 °C, 64% from **7**; (b) LiBH₄, MeOH, Et₂O, 0 °C then HCl, 50 °C, 80%; (c) H₂, Pd–C, (Boc)₂O, MeOH, 100%; (d) HCl, then 9-deazahypoxanthine, CH₂O, NaOAc, water-dioxane, 100 °C, 40%; (e) NaOEt, EtOH, rt, 55%; (f) CAL-B, vinyl acetate, *tert*-BuOMe, 50 °C, 31% [(3*S*,4*R*)–**8**] and 30% [(3*R*,4*S*)–**9**].

Table 1

Inhibition of human and plasmodial PNP by the racemate and enantiomers of F-DADMe-ImmH [(±)-**3**, (3*S*, 4*S*)-**3** and (3*R*,4*R*)-**3**]

	Human PNP		<i>P. falciparum</i> PNP	
	K_i (nM)	K_i^* (nM) ^a	K_i (nM)	K_i^* (nM)
(±)- 3	0.50 ± 0.07	0.066 ± 0.009	23.3 ± 1.4	3.6 ± 0.5
(3 <i>S</i> ,4 <i>S</i>)- 3	0.33 ± 0.11	0.032 ± 0.010	19 ± 4	2.63 ± 0.18
(3 <i>R</i> ,4 <i>R</i>)- 3	18 ± 2	1.82 ± 0.08	2070 ± 160	260 ± 20
DADMe-ImmH (2)	1.10 ± 0.12 ^b	0.011 ± 0.001 ^b	0.50 ± 0.04 ^c	
L-DADMe-ImmH	0.38 ± 0.03 ^d		1700 ± 300 ^e	80 ± 7 ^e

^a K_i is the dissociation constant for the first step in $E + I \leftrightarrow EI \leftrightarrow EI^*$, the two-step binding characteristic of slow-onset, tight-binding inhibition. K_i^* is the overall dissociation constant for $E + I \leftrightarrow EI^*$. In cases where only K_i is reported, no slow-onset inhibition was observed.

^b Ref 42.

^c Ref 41.

^d Ref 43.

^e Ref 38.