Supporting Information

Simplified Analogs of Immucillin-G Retain Potent Human Purine Nucleoside Phosphorylase Inhibitory Activity

Teresa Semeraro,[a] Andrea Lossani,[b] Maurizio Botta,[a] Chiara Ghiron,[c] Reinaldo Alvarez,[b] Fabrizio Manetti,[a] Claudia Mugnaini,[a] Silvia Valesin,[c] Federico Focher,*[,b] Federico Corelli*[,a]

[a]*Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via A. De Gasperi 2, 53100 Siena, Italy*

[b]*Istituto di Genetica Molecolare, CNR, via Abbiategrasso 207, 27100 Pavia, Italy*

[c]*SienaBiotech S.p.a., Via Fiorentina 1, 53100 Siena, Italy*

Synthetic Procedures and Analytical Data

Compounds prepared *via* Mannich reaction according to general method A

2-Amino-1,5-dihydro-7-[[(2*R*)-2-(hydroxymethyl)-1-pyrrolidinyl]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (1b). Yield 35%; mp 152-154°C (dec); $[\alpha]^{20}_{D}$ +13.3 (*c* 1.5, MeOH); ^{1}H NMR (CD_3OD): _ 7.48 (s, 1H), 4.48 (d, *J*= 13.7 Hz, 1H), 4.32 (d, *J*= 13.7 Hz, 1H) 3.69 (m, 4H), 3.38 (m, 1H), 2.02 (m, 4H), 1.92 (s, 6H); IR (nujol) $_{max}$ 1674, 1634, 1580, 1525, 1199, 1121 cm^{-1}; MS (electrospray) *m/z:* 264 [M+1]; Anal. ($C_{12}H_{17}N_5O_2$ _2 CH_3COOH) C, H, N.

2-Amino-1,5-dihydro-7-[[(2*S*)-2-(methoxymethyl)-1-pyrrolidinyl]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (1c). Yield 25%; mp:>260°C (dec); $[\alpha]^{20}_{D}$ -65.5 (*c* 1, MeOH); ^{1}H NMR (CD_3OD): _ 7.20 (s, 1H), 3.98 (d, *J*= 13.7 Hz, 1H), 3.67 (d, *J*=

13.7 Hz), 3.41 (m, 2H), 3.32 (s, 3H), 2.90 (m, 2H), 2.44 (m, 1H), 1.72 (m, 4H); IR (nujol) _ $_{max}$ 1681, 1633, 1568, 1199, 1124 cm^{-1}; MS (electrospray) *m/z:* 278 [M+1]; Anal. ($C_{13}H_{19}N_5O_2$) C, H, N.

2-Amino-1,5-dihydro-7-[[(2*S*,4*R*)-2-(hydroxymethyl)-4-hydroxy-1-pyrrolidinyl]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one(1d). Yield 15%; syrup; $[\alpha]^{20}_D$ -42.66 (*c* 0.75, MeOH); ^{1}H NMR (CD_3OD): _ 7.38 (s, 1H), 4.42 (m, 1H), 4.43 (d, *J*= 13.6 Hz, 1H), 4.27 (d, *J*= 13.6 Hz, 1H), 3.73 (m, 3H), 3.46 (m, 1H), 3.24 (m, 1H), 2.03 (m, 2H); IR (nujol) _ $_{max}$ 1697, 1646, 1525, 1105 cm^{-1} ; MS (electrospray) *m/z:* 280 [M+1]; Anal. ($C_{12}H_{17}N_5O_3$) C, H, N.

2-Amino-1,5-dihydro-7-[[[*N*-[2-(hydroxy)ethyl]-*N*-methyl]amino]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (2a). Yield 33%; mp: 89-90 °C; ^{1}H NMR (CD_3OD): _ 7.44 (s, 1H), 4.32 (s, 2H), 3.88 (t, *J*= 5.1 Hz, 1H), 3.22 (t, *J*= 5.1 Hz, 2H), 2.79 (s, 3H), 1.92 (s, 6H); IR (nujol) _ $_{max}$ 1683, 1558, 1106 cm^{-1} ; MS (electrospray) *m/z:* 238 [M+1]; Anal. ($C_{10}H_{15}N_5O_2$ _2 CH_3COOH) C, H, N.

2-Amino-1,5-dihydro-7-[[*N*-[ethyl]-*N*-[2-(hydroxy)ethyl]amino]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (2b). Yield 10%; syrup; ^{1}H NMR (CD_3OD): _ 7.24 (s, 1H), 3.92 (s, 2H), 3.74 (t, *J*= 5.9 Hz, 2H), 2.92 (t, *J*= 5.9 Hz, 2H), 2.76 (q, *J*= 6.9 Hz, 2H), 1.92 (s, 6H), 1.22 (t, *J*= 6.9 Hz, 3H) MS (electrospray) *m/z:* 252 [M+1]; Anal.($C_{11}H_{17}N_5O_2$ _2 CH_3COOH) C, H, N

2-Amino-1,5-dihydro-7-[[*N*,*N*-bis-[2-(hydroxy)ethyl]amino]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (2c). Yield 27%; mp: 140-141 °C; ^{1}H NMR (CD_3OD): _ 7.36 (s, 1H), 4.27 (s, 2H), 3.85 (t, *J*= 5.2, 4H), 3.13 (t, *J*= 5.2, 4H), 1.92 (s, 6H); MS (electrospray) *m/z:* 268 [M+1]; Anal. ($C_{11}H_{17}N_5O_3$ _2 CH_3COOH) C, H, N.

2-Amino-1,5-dihydro-7-[[3-(hydroxymethyl)-1-piperidinyl]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (3). Yield 35%; mp 91-93°C; ^{1}H NMR (CD_3OD): _ 7.41 (s, 1H), 4.17 (s, 2H), 3.42 (m, 2H), 2.66 (m, 4H), 1.92 (s, 6H), 1.78 (m, 3H), 1.2 (m, 2H); IR (nujol) λ_{max} 1686, 1635, 1560, 1533, 1131, 1039 cm^{-1};MS (electrospray) *m/z:* 278 [M+1]; Anal. ($C_{13}H_{19}N_5O_2$ _2 CH_3COOH) C, H, N.

Compounds prepared *via* reductive amination according to general method B

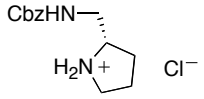
2-Amino-1,5-dihydro-7-[[(2*S*)-2-(benzyloxycarbonylaminomethyl)-1-pyrrolidinyl]methyl]-4*H*-pyrrolo[3,2-d]pyrimidin-4-one (7). Yield 38%; ^{1}H NMR (CD_3OD): _ 7.30 (m, 5H), 7.13 (s, 1H), 5.07 (s, 2H), 3.93 (d, *J*= 13.5 Hz, 1H), 3.52 (d, *J*= 13.5 Hz, 1H), 3.35 (m, 3H), 2.90 (m, 1H), 2.65 (m, 1H), 2.32 (m, 1H), 1.82 (m, 1H), 1.59 (m, 2H); MS (electrospray) *m/z:* 397 [M+1]; Anal. ($C_{20}H_{24}N_6O_3$) C, H, N.

Synthesis of *(S)*-2-(benzyloxycarbonylaminomethyl)pyrrolidine hydrochloride. To a solution of *(S)*-2-aminomethyl-*N*-*tert*-butoxycarbonylpyrrolidine (80 mg, 0.4 mmol) in 20% aqueous Na_2CO_3 (2 mL) and MeOH (5 mL) at 0 °C benzyl chloroformate (63 _L, 0.44 mmol) was added dropwise; the mixture was stirred at 0 °C for 1 h and then at room temperature for 12 h. The solvent was removed under reduced pressure, water was added and the solution was extracted with CH_2Cl_2 (3 x 5 mL); the combined extracts were washed with brine, dried over Na_2SO_4 and concentrated. The crude product was dissolved in MeOH , 12 N HCl (185 _L) was added dropwise and the solution was stirred for 12 h. The reaction mixture was then concentrated under reduced pressure to give the title compound as hydrochloride salt (yield 95%); ^{1}H NMR (CD_3OD): _ 7.32 (m, 5H); 5.08 (s, 2H); 3.69 (m, 1H); 3.43 (m,

2H); 2.06 (m, 4H); 1.73 (m, 2H); MS (electrospray) *m/z:* 235 [M+1]. Anal. ($C_{13}H_{18}N_2O_2$ • HCl) C, H, N.

Elemental Analyses

Compd	Formula	Calcd.			Found		
		C, %	H, %	N, %	C, %	H, %	N, %
1a	$C_{12}H_{17}N_5O_2$ _2 CH_3COOH	50.12	6.57	18.27	50.33	6.49	18.11
1b	$C_{12}H_{17}N_5O_2$ _2 CH_3COOH	50.12	6.57	18.27	50.40	6.66	18.09
1c	$C_{13}H_{19}N_5O_2$	56.30	6.91	25.25	56.51	6.80	25.35
1d	$C_{12}H_{17}N_5O_3$	51.60	6.14	25.08	51.45	6.20	51.22
1e	$C_{12}H_{18}N_6O$	54.95	6.92	32.04	55.14	6.84	32.21
2a	$C_{10}H_{15}N_5O_2$ _2 CH_3COOH	47.05	6.49	19.60	47.29	6.33	19.47
2b	$C_{11}H_{17}N_5O_2$ _2 CH_3COOH	48.51	6.78	18.86	48.80	6.62	18.58
2c	$C_{11}H_{17}N_5O_3$ _2 CH_3COOH	46.51	6.50	18.08	46.83	6.40	17.95

2d	$C_9H_{13}N_5O_2$ _2 CH_3COOH	45.48	6.17	20.40	45.28	6.30	20.23
3	$C_{13}H_{19}N_5O_2$ _2 CH_3COOH	51.38	6.86	17.62	51.50	6.74	17.49
6	$C_{16}H_{21}N_5O_4$	55.32	6.09	20.16	55.11	6.17	54.92
7	$C_{20}H_{24}N_6O_3$	60.59	6.10	21.20	60.71	6.19	21.00
	$C_{13}H_{19}ClN_2O_2$	57.67	7.07	10.35	57.53	7.15	10.22