Supporting Information

Piperazinyl-Glutamate-Pyridines as Potent Orally Bioavailable P2Y₁₂ Antagonists for Inhibition of Platelet Aggregation

John J. Parlow,^{a,*} Mary W. Burney,^b Brenda L. Case,^a Thomas J. Girard,^b Kerri A. Hall,^b Peter K. Harris,^b Ronald R. Hiebsch,^b Rita M. Huff,^b Rhonda M. Lachance,^b Deborah A. Mischke,^a Stephen R. Rapp,^b Rhonda S. Woerndle,^a Michael D. Ennis^a

^a*Department of Medicinal Chemistry, Pfizer Global Research & Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017*

^b*Department of Biology, Pfizer Global Research & Development, 700 Chesterfield Parkway West, Chesterfield, MO 63017*

e-mail address: john.j.parlow@pfizer.com

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Table 1. Binding and PRP Activity Data for a Representative Set of Substituted Piperazine Analogs **20**

Cpd	R ₁	K _i ^a (nM)	IC ₅₀ ^b (μM)
18	H	>5,680	>100
20a	Pr	>3,800	>100
20b	<i>i</i> -Bu	>3,800	>100
20c	Pent	>3,590	>100
20d	Hex	>2,710	>100
20e	COMe	>3,590	>100
20f	COEt	>3,590	>100
20g	CO <i>i</i> Pr	>3,590	>100
20h	COPr	3,100	>100
20i	COBu	1,430	>100
20j	COCH ₂ OMe	>3,590	>100
20k	COCH ₂ NMe ₂	>3,590	>100
20l	COPh	1,880	>100
20m	SO ₂ Et	>3,590	>100
20n	SO ₂ Pr	>3,590	>100
20o	SO ₂ Bu	>3,590	>100
20p	SO ₂ Pent	>3,590	>100
20q	SO ₂ Ph	842	>100
20r	CONHEt	288	80
20s	CONHPr	277	>100
20t	CONHBu	613	>100
20u	CONHPent	55	>100
20v	CONHHex	54	>100
20w	CONHPh	842	>100
20x	CSNHEt	9	39
20y	CSNHPr	42	81
20z	CSNHBu	8	56
20aa	CSNHPent	6	42

^a Membranes from CHO cells expressing recombinant human P2Y₁₂ receptors incubated with ³³P ADP and compound. K_i values are corrected from IC₅₀ using Cheng and Prusoff equation and are the geometric mean of n=2 or greater.

^b IC₅₀ values are from human PRP incubated with 20 μM ADP.

(4S)-5-Oxo-4-[[[(6-phenylpyridin-2-yl)carbonyl]amino]-5-(4-propylpiperazin-1-yl)pentanoic acid (20a). General Procedure C afforded 37.2 mg (34%) of product **20a**. HRMS calcd for C₂₄H₃₀N₄O₄ (M⁺ + H) 439.2340, found 439.2300.

(4S)-5-(4-Isobutylpiperazin-1-yl)-5-oxo-4-[[[(6-phenylpyridin-2-yl)carbonyl]amino]pentanoic acid (20b). General Procedure C afforded 65.8 mg (58%) of product **20b**. HRMS calcd for C₂₅H₃₂N₄O₄ (M⁺ + H) 453.2496, found 453.2430.

(4S)-5-Oxo-5-(4-pentylpiperazin-1-yl)-4-[[[(6-phenylpyridin-2-yl)carbonyl]amino]pentanoic acid (20c). General Procedure C afforded 101.8 mg

(88%) of product **20c**. HRMS calcd for $C_{26}H_{34}N_4O_4$ ($M^+ + H$) 467.2652, found 467.2353.

(4S)-5-(4-Hexylpiperazin-1-yl)-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20d). General Procedure C afforded 80.5 mg (68%) of product **20d**. HRMS calcd for $C_{27}H_{36}N_4O_4$ ($M^+ + H$) 481.2809, found 481.2810.

(4S)-5-(4-Acetylpiperazin-1-yl)-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20e). General Procedure C afforded 23.3 mg (35%) of product **20e**. HRMS calcd for $C_{23}H_{26}N_4O_5$ ($M^+ + H$) 439.1976, found 439.1950.

(4S)-5-Oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}-5-(4-propionylpiperazin-1-yl)pentanoic acid (20f). General Procedure C afforded 19.7 mg (29%) of product **20f**. HRMS calcd for $C_{24}H_{28}N_4O_5$ ($M^+ + H$) 453.2132, found 453.2105.

(4S)-5-(4-Isobutyrylpiperazin-1-yl)-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20g). General Procedure C afforded 27.2 mg (40%) of product **20g**. HRMS calcd for $C_{25}H_{30}N_4O_5$ ($M^+ + H$) 467.2289, found 467.2262.

(4S)-5-(4-Butyrylpiperazin-1-yl)-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20h). General Procedure C afforded 25.4 mg (36%) of product **20h**. HRMS calcd for $C_{25}H_{30}N_4O_5$ ($M^+ + H$) 467.2289, found 467.2273.

(4S)-5-Oxo-5-(4-pentanoylpiperazin-1-yl)-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20i). General Procedure C afforded 13.5 mg (19%) of product **20i**. HRMS calcd for $C_{26}H_{32}N_4O_5$ ($M^+ + H$) 481.2445, found 481.2443.

(4S)-5-[4-(Methoxyacetyl)piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20j). General Procedure C afforded 22.4 mg (32%) of product **20j**. HRMS calcd for $C_{24}H_{28}N_4O_6$ ($M^+ + H$) 469.2082, found 469.2049.

(4S)-5-[4-(N,N-Dimethylglycyl)piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20k). General Procedure C afforded 28.3 mg (39%) of product **20k**. HRMS calcd for $C_{25}H_{31}N_5O_5$ ($M^+ + H$) 482.2398, found 482.2397.

(4S)-5-(4-Benzoylpiperazin-1-yl)-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20l). General Procedure C afforded 15.5 mg (21%) of product **20l**. HRMS calcd for $C_{28}H_{28}N_4O_5$ ($M^+ + H$) 501.2132, found 501.2107.

(4S)-5-[4-(Ethylsulfonyl)piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20m). General Procedure C afforded 167.3 mg (100%) of product **20m**. HRMS calcd for $C_{23}H_{28}N_4O_6S$ ($M^+ + H$) 489.1802, found 489.1793.

(4S)-5-Oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}-5-[4-(propylsulfonyl)piperazin-1-yl]pentanoic acid (20n). General Procedure C afforded

180.4 mg (100%) of product **20n**. HRMS calcd for $C_{24}H_{30}N_4O_6S$ ($M^+ + H$) 503.1959, found 503.1939.

(4S)-5-[4-(Butylsulfonyl)piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20o). General Procedure C afforded 156.3 mg (100%) of product **20o**. HRMS calcd for $C_{25}H_{32}N_4O_6S$ ($M^+ + H$) 517.2115, found 517.2134.

(4S)-5-Oxo-5-[4-(pentylsulfonyl)piperazin-1-yl]-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20p). General Procedure C afforded 150 mg (100%) of product **20p**. HRMS calcd for $C_{26}H_{34}N_4O_6S$ ($M^+ + H$) 531.2272, found 531.2303.

(4S)-5-Oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}-5-[4-(phenylsulfonyl)piperazin-1-yl]pentanoic acid (20q). General Procedure C afforded 25.5 mg (32%) of product **20q**. HRMS calcd for $C_{27}H_{28}N_4O_6S$ ($M^+ + H$) 537.1802, found 537.1810.

(4S)-5-[4-[(Ethylamino)carbonyl]piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20r). General Procedure C afforded 62.9 mg (72%) of product **20r**. HRMS calcd for $C_{24}H_{29}N_5O_5$ ($M^+ + H$) 468.2241, found 468.2237.

(4S)-5-Oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}-5-[4-[(propylamino)carbonyl]piperazin-1-yl]pentanoic acid (20s). General Procedure C afforded 63.5 mg (71%) of product **20s**. HRMS calcd for $C_{25}H_{31}N_5O_5$ ($M^+ + H$) 482.2398, found 482.2403.

(4S)-5-[4-[(Butylamino)carbonyl]piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20t). General Procedure C afforded 62.1 mg (68%) of product **20t**. HRMS calcd for $C_{26}H_{33}N_5O_5$ ($M^+ + H$) 496.2554, found 496.2524.

(4S)-5-Oxo-5-[4-[(pentylamino)carbonyl]piperazin-1-yl]-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20u). General Procedure C afforded 54.3 mg (58%) of product **20u**. HRMS calcd for $C_{27}H_{35}N_5O_5$ ($M^+ + H$) 510.2711, found 510.2684.

(4S)-5-[4-[(Hexylamino)carbonyl]piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20v). General Procedure C afforded 70.7 mg (74%) of product **20v**. HRMS calcd for $C_{28}H_{37}N_5O_5$ ($M^+ + H$) 524.2867, found 524.2872.

(4S)-5-[4-(Anilino)carbonyl]piperazin-1-yl]-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20w). General Procedure C afforded 51.1 mg (66%) of product **20w**. HRMS calcd for $C_{28}H_{29}N_5O_5$ ($M^+ + H$) 516.2241, found 516.2245.

(4S)-5-{4-[(Ethylamino)carbonothioyl]piperazin-1-yl}-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20x). General Procedure C afforded 63.8 mg (71%) of product **20x**. HRMS calcd for C₂₄H₂₉N₅O₄S (M⁺ + H) 484.2013, found 484.1994.

(4S)-5-Oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}-5-{4-[(propylamino)carbonothioyl]piperazin-1-yl}pentanoic acid (20y). General Procedure C afforded 50.6 mg (55%) of product **20y**. HRMS calcd for C₂₅H₃₁N₅O₄S (M⁺ + H) 498.2170, found 498.2162.

(4S)-5-{4-[(Butylamino)carbonothioyl]piperazin-1-yl}-5-oxo-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20z). General Procedure C afforded 58.9 mg (63%) of product **20z**. HRMS calcd for C₂₆H₃₃N₅O₄S (M⁺ + H) 512.2326, found 512.2341.

(4S)-5-Oxo-5-{4-[(pentylamino)carbonothioyl]piperazin-1-yl}-4-[[6-phenylpyridin-2-yl]carbonyl]amino}pentanoic acid (20aa). General Procedure C afforded 55.8 mg (58%) of product **20aa**. HRMS calcd for C₂₇H₃₅N₅O₄S (M⁺ + H) 526.2483, found 526.2467.

Table 2. Binding and PRP Activity Data for a Representative Set of 4-Amide Pyridine Analogs **75**

Cpd	R	Ki ^a (nM)	IC ₅₀ ^b (μM)	Relative Potency to AZD6140 ^c
75a	Me	6.2	>10	-
75b	CF ₃	9.1	8.2	8.2
75c		8.5	>10	-
75d		3.0	>10	-
75e		2.3	4.3	4.1
75f	CH ₂ OMe	25	>10	-
75g	CH ₂ NHMe	5.7	>10	-
75h	CH ₂ NMe ₂	4.1	5.6	6.4

^a Membranes from CHO cells expressing recombinant human P2Y₁₂ receptors incubated with ³³P ADP and compound. Ki values are corrected from IC₅₀ using Cheng and Prusoff equation and are the geometric mean of n=2 or greater.

^b IC₅₀ values are from human PRP incubated with 20 μM ADP.

^c IC₅₀ value of compound/IC₅₀ value of AZD-6140 control from same plate = normalized IC₅₀ ratio.

(4S)-4-([4-(Acetylamino)-6-phenylpyridin-2-yl]carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (75a). General Procedure O afforded 85 mg (94%) of product **75a**. HRMS calcd for C₂₉H₃₇N₅O₇ (M⁺ + H) 568.2766, found 568.2745.

(4S)-5-Oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}-4-[(6-phenyl-4-(trifluoroacetyl)amino]pyridin-2-yl]carbonyl]amino}pentanoic acid (75b). General

Procedure O afforded 19 mg (25%) of product **75b**. HRMS calcd for $C_{29}H_{34}F_3N_5O_7$ ($M^+ + H$) 622.2488, found 622.2454.

(4S)-5-Oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}-4-[(6-phenyl-4-[(tetrahydro-2H-pyran-4-ylcarbonyl)amino]pyridin-2-yl)carbonyl]amino]pentanoic acid (75c).

General Procedure O afforded 100 mg (89%) of product **75c**. HRMS calcd for $C_{33}H_{43}N_5O_8$ ($M^+ + H$) 638.3184, found 638.3196.

(4S)-4-[(4-[(Morpholin-4-ylacetyl)amino]-6-phenylpyridin-2-yl)carbonyl]amino]-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (75d). General

Procedure O afforded 103 mg (81%) of product **75d**. HRMS calcd for $C_{33}H_{44}N_6O_8$ ($M^+ + H$) 653.3293, found 653.3272.

(4S)-4-[(4-[(4-Methylpiperazin-1-yl)acetyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino]-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (75e). General

Procedure O afforded 94 mg (63%) of product **75e**. HRMS calcd for $C_{34}H_{47}N_7O_7$ ($M^+ + H$) 666.3610, found 666.3609.

(4S)-4-[(4-[(Methoxyacetyl)amino]-6-phenylpyridin-2-yl)carbonyl]amino]-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (75f). General

Procedure O afforded 41 mg (41%) of product **75f**. HRMS calcd for $C_{30}H_{39}N_5O_8$ ($M^+ + H$) 598.2871, found 598.2834.

(4S)-4-[(4-[(N-Methylglycyl)amino]-6-phenylpyridin-2-yl)carbonyl]amino]-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (75g). General

Procedure O afforded 110 mg (94%) of product **75g**. HRMS calcd for $C_{30}H_{40}N_6O_7$ ($M^+ + H$) 597.3031, found 597.2998.

(4S)-4-[(4-[(N,N-Dimethylglycyl)amino]-6-phenylpyridin-2-yl)carbonyl]amino]-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (75h). General

Procedure O afforded 108 mg (91%) of product **75h**. HRMS calcd for $C_{31}H_{42}N_6O_7$ ($M^+ + H$) 611.3188, found 611.3182.

Table 3. Binding and PRP Activity Data for a Representative Set of 4-Urea Pyridine Analogs **76**

Cpd	R'	R''	K _i ^a (nM)	IC ₅₀ ^b (μM)	Relative Potency to AZD6140 ^c
76a	H	<i>i</i> -Pr	2.7	>10	-
76b	H		1.4	5.6	3.8
76c	H	CH ₂ CONMe ₂	4.7	3.4	3.9
76d			5.8	>10	-
76e			3.2	11	7.2
76f			9.2	5.5	6.1
76g			43	4.5	5.2

^a Membranes from CHO cells expressing recombinant human P2Y₁₂ receptors incubated with ³³P ADP and compound. K_i values are corrected from IC₅₀ using Cheng and Prusoff equation and are the geometric mean of n=2 or greater.

^b IC₅₀ values are from human PRP incubated with 20 μM ADP.

^c IC₅₀ value of compound/IC₅₀ value of AZD-6140 control from same plate = normalized IC₅₀ ratio.

(4S)-4-([(4-[(Isopropylamino)carbonyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (76a). General Procedure P afforded 66 mg (91%) of product **76a**. HRMS calcd for C₃₁H₄₂N₆O₇ (M⁺ + H) 611.3188, found 611.3150.

(4S)-5-Oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}-4-([(6-phenyl-4-([(piperidin-4-ylmethyl)amino]carbonyl)amino)pyridin-2-yl]carbonyl)amino)pentanoic acid (76b). General Procedure P afforded 73 mg (100%) of product **76b**. HRMS calcd for C₃₄H₄₇N₇O₇ (M⁺ + H) 666.3610, found 666.3585.

(4S)-4-([(4-([(2-(Dimethylamino)-2-oxoethyl]amino)carbonyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (76c). General Procedure P afforded 53 mg (97%) of product **76c**. HRMS calcd for C₃₂H₄₃N₇O₈ (M⁺ + H) 654.3246, found 654.3224.

(4S)-5-Oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}-4-([(6-phenyl-4-[(piperidin-1-ylcarbonyl)amino]pyridin-2-yl)carbonyl]amino)pentanoic acid (76d). General Procedure P afforded 61 mg (92%) of product **76d**. HRMS calcd for C₃₃H₄₄N₆O₇ (M⁺ + H) 637.3344, found 637.3342.

(4S)-4-([(4-[(4-Methylpiperazin-1-yl)carbonyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (76e). General Procedure P afforded 69 mg (82%) of product **76e**. HRMS calcd for C₃₃H₄₅N₇O₇ (M⁺ + H) 652.3453, found 652.3464.

(4S)-4-([(4-[(4-Methoxypiperidin-1-yl)carbonyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (76f). General Procedure P afforded 61 mg (98%) of product **76f**. HRMS calcd for C₃₄H₄₆N₆O₈ (M⁺ + H) 667.3450, found 667.3421.

(4S)-4-([(4-[(4-(Methoxymethyl)piperidin-1-yl)carbonyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (76g). General Procedure P afforded 68 mg (97%) of product **76g**. HRMS calcd for C₃₅H₄₈N₆O₈ (M⁺ + H) 681.3606, found 681.3616.

Table 4. Binding and PRP Activity Data for a Representative Set of 4-Carbamate Pyridine Analogs **77**

Cpd	R	Ki ^a (nM)	IC ₅₀ ^b (μM)	Relative Potency to AZD6140 ^c
77a	Et	11	9.3	15
77b	(CH ₂) ₂ OMe	1.4	>10	-
77c	(CH ₂) ₃ NH ₂	1.9	5.7	3.9
77d		1.7	5.8	3.7

^a Membranes from CHO cells expressing recombinant human P2Y₁₂ receptors incubated with ³³P ADP and compound. Ki values are corrected from IC₅₀ using Cheng and Prusoff equation and are the geometric mean of n=2 or greater.

^b IC₅₀ values are from human PRP incubated with 20 μM ADP.

^c IC₅₀ value of compound/IC₅₀ value of AZD-6140 control from same plate = normalized IC₅₀ ratio.

(4S)-4-([(4-[(Ethoxycarbonyl)amino]-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (77a). General Procedure P afforded 76 mg (100%) of product **77a**. HRMS calcd for C₃₀H₃₉N₅O₈ (M⁺ + H) 598.2871, found 598.2854.

(4S)-4-([(4-[(2-Methoxyethoxy)carbonyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (77b). General Procedure P afforded 130 mg (90%) of product **77b**. HRMS calcd for C₃₁H₄₁N₅O₉ (M⁺ + H) 638.2977, found 628.2975.

(4S)-4-([(4-[(3-Aminopropoxy)carbonyl]amino)-6-phenylpyridin-2-yl)carbonyl]amino)-5-oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}pentanoic acid (77c). General Procedure P afforded 53 mg (95%) of product **77c**. HRMS calcd for C₃₁H₄₂N₆O₈ (M⁺ + H) 627.3137, found 627.3166.

(4S)-5-Oxo-5-{4-[(pentyloxy)carbonyl]piperazin-1-yl}-4-[(6-phenyl-4-[(piperidin-4-ylmethoxy)carbonyl]amino)pyridin-2-yl)carbonyl]amino}pentanoic acid (77d). General Procedure P afforded 78 mg (100%) of product **77d**. HRMS calcd for C₃₄H₄₆N₆O₈ (M⁺ + H) 667.3450, found 667.3473.