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Article

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A Novel Series of Dihydropyridinone P2X7 Receptor Antagonists

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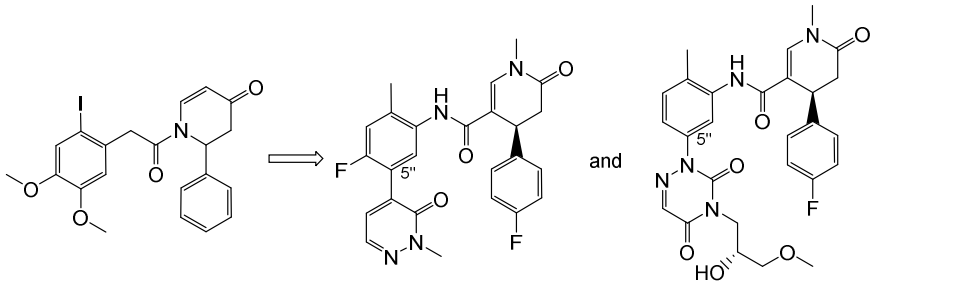
Hoffmann-La Roche Inc, 340 Kingsland St, Nutley, NJ 07110, USA

ABSTRACT: Identification of singleton P2X7 inhibitor **1** from HTS gave a pharmacophore that eventually turned into potential clinical candidates **17** and **19**. During development, a number of issues were successfully addressed, such as metabolic stability, plasma stability, GSH adduct formation and aniline mutagenicity. Thus, careful modification of the molecule, such as conversion of the 1,4-dihydropyridinone to the 1,2-dihydropyridinone system, proper substitution at C-5", and in some cases addition of fluorine atoms to the aniline ring allowed for the identification of a novel class of potent P2X7 inhibitors suitable for evaluating the role of P2X7 in inflammatory, immune, neurologic or musculoskeletal disorders.



1
Singleton from HTS
FLIPR IC_{50} 104 nM
Metabolically labile

17
HWB IC_{50} 13 nM
Aniline negative in Ames assay
Rat F 64%, IV CL 17 mL/min/kg

19
HWB IC_{50} 17 nM
Aniline negative in Ames assay
Rat F 20%, IV CL 23 mL/min/kg

INTRODUCTION

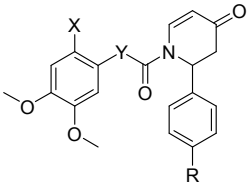
The P2X7 receptor (P2X7R) is an ATP ligand-gated ion channel expressed in a wide variety of cells involved in inflammatory and immune responses, and which mediates a major cellular pathway for the processing and release of the pro-inflammatory cytokines IL-1β and IL-18.[1] Studies of human tissue or mouse models suggest that P2X7 may play important roles in a number of inflammatory, immune, neurologic or musculoskeletal disorders, such as multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, Huntington's disease, cancer, ischemia, neuropathic and inflammatory pain, rheumatoid arthritis, glomerulonephritis, pulmonary fibrosis, and graft-versus-host disease.[2] Based on these findings several P2X7R antagonists have been advanced into clinical trials for rheumatoid arthritis, however, these efforts failed to demonstrate efficacy in early proof of concept clinical studies.[3] Nonetheless, because the P2X7R is implicated in a number of other disorders (*vide supra*), new potent and selective P2X7R antagonists remain of high value.[2] During the course of the investigations, several P2X7R antagonists have been reported, some of them containing the adamantyl group or similar lipophilic groups.[4] In this report, we present our efforts on the identification and characterization of potent and selective P2X7 antagonists based on novel chemical templates with no large lipophilic groups, and with good physicochemical properties and a good safety profile. These

novel P2X7 antagonists may be useful for further biological exploration into the role of P2X7 in inflammation, neurologic, musculoskeletal or immune-mediated disorders.[2]

RESULTS AND DISCUSSION

The Roche screening library was assayed in 1321N1 cells expressing human P2X7R stimulated with BzATP using a FLIPR Ca^{2+} flux assay. One of the hits from this screen was the singleton 1,4-dihydropyridinone **1** (Table 1) with an IC_{50} of 104 nM. However, after initial optimization, it was found these aryl acetamides had low metabolic stability, e.g., **1** and **2** HLM CL_{int} were 168 and 153 μL/min/mg protein, respectively. Structure modification to the corresponding ureas considerably improved metabolic stability while maintaining potency, e.g., **3** and **4** had HLM CL_{int} of 22 and 8.2 μL/min/mg protein, with IC_{50} values of 39 and 12 nM, respectively. When testing the separated enantiomers, potency was found to reside only in the (S)-enantiomer (Table 2). The X-Ray crystal structure of **9** is shown,[5] in which the fluorophenyl ring is pseudoaxial, and the dihedral angle between the urea carbonyl and the N1-C6 bond is 11°.

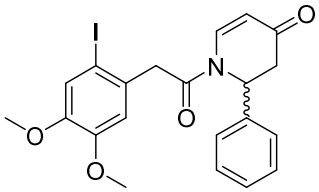
Table 1. **Potency and Metabolic Stability of 1,4-Dihydropyridinones 1-4**[a]



Entry[1]	X	Y	R	IC_{50}[b,c] (nM)	HLM CL_{int}[c] (μL/min/mg protein)	RLM CL_{int}[c] (μL/min/mg protein)
1	I	CH_2	H	104	168	548
2	Br	CH_2	H	92	153	490
3	Br	NH	H	39	22	78
4	Me	NH	F	12	8.2	25

[a]Compounds **1-3** are racemic, and **4** is the (S)-enantiomer (*vide infra)*; [b]FLIPR Ca^{2+} flux assay using 1321N1 cells expressing human P2X7R stimulated with BzATP. [c]Mean value of $n \geq 2$ in presence of a positive control.

Table 2. Potency of (R) and (S)-Enantiomers for Compounds 5-9, and X-Ray Crystal Structure of Compound 9[5]



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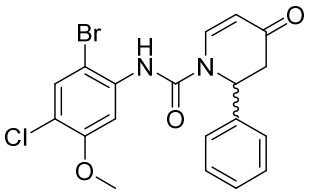
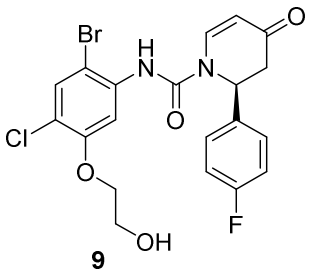
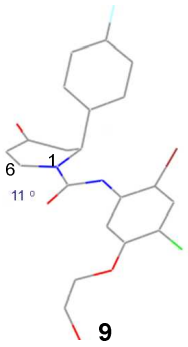


7-8



9



9

Entry	Configuration	IC_{50} (nM)[a]
5	S	38
6	R	3800
7	S	33
8	R	>10000
9	S	40

[a]Mean value of $n \geq 2$ in presence of a positive control.

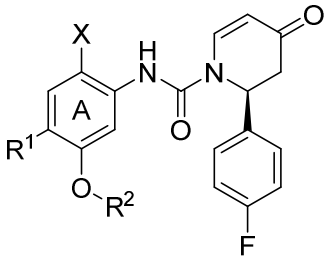
Unfortunately, during DMPK profiling of compounds from this series, it became apparent that the urea compounds were both positive in the glutathione (GSH) trapping assay (e.g., **10**, Table 3) and unstable in human plasma (e.g., **13**). In the GSH trapping assay, the resulting adduct contained the glutathione moiety as part of ring A (Table 3) in both human and rat liver microsomes. For the characterization of the GSH adduct, the assay for compound **10** showed a major chromatographic peak (Figure 1) due to the GSH adduct. In the figure, unreacted **10** elutes at 16.58 min. The major GSH adduct (**GSH-1**) has retention time of 10.74 minutes and shows $[M+H]^+$ ion at *m/z* 740, corresponding formally to a loss of a CH_2 (- 14 Da) plus 2 hydrogens (-2

Da) and addition of a GSH (+307 Da). The peak at 14.8 minutes is due to a minor des-methyl metabolite. A second, minor GSH adduct elutes at 10.2 minutes. The other peaks are background. Figure 2 shows the MS/MS spectrum of **GSH-1**. A characteristic from compounds of this urea series is that they all show a fragmentation to give an isocyanate (left-side of the molecule) and an aryl-pyridinone (right-side of the molecule). For **GSH-1**, the corresponding isocyanate $[M+H]^+$ ion is at *m/z* 549, a loss of 191 Da (i.e., loss of the fluorophenyl-pyridinone). The other ion species are due to classic[6] GSH adduct fragmentation with loss of glycine (75 Da) from the intact precursor ion to give *m/z* 665 or from the intermediate *m/z* 549 to give *m/z* 474, or loss of pyroglutamate (129 Da) from the intact precursor ion to give *m/z* 611, or from the intermediate *m/z* 549 to give *m/z* 420. Our hypothesis for the formation of **GSH-1** is shown in Figure 3. The first step is cytochrome P450 oxidative demethylation. This common reaction is supported by observation of minor amounts of the des-methyl metabolite in the assay mixture. This intermediate is setup for further oxidative dehydrogenation to give the reactive quinone-imine, which by addition of GSH gives the corresponding adduct with the observed molecular weight. The regiochemistry is not proven by this data, other isomeric adducts are possible. For all compounds tested, the observed results on the GSH adduct show that in no case was there any evidence for addition of GSH to unmetabolically-activated molecules, e.g., compound **14** (Figure 4), in which no GSH addition to the 1,4-pyridinone ring was observed.

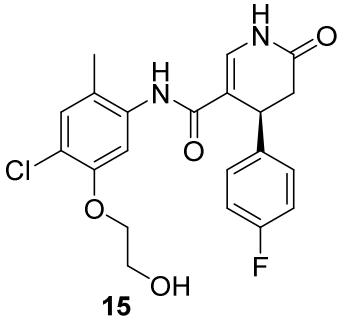
Hypothesizing that replacement of the methoxy group *para* to the anilide nitrogen could reduce the glutathione adduct formation, halide compounds **11** and **12** were prepared and tested in the GSH assay (Table 3).[7] However, fluoride **11** gave no improvement in the extent of GSH adduct formation, whereas chloride **12** showed only 50% reduction in its relative formation.[8] More elaborate compound **13** with an improved cLogP of 2.76 with respect to **12** (cLogP 4.1)

had further reduced formation of the GSH adduct in both human and rat liver microsomes (4 and 5% relative UV peak area in HLM and RLM, respectively). However, while changes in the aniline portion of the molecule showed potential for circumventing the GSH liability, the plasma instability, which could be attributed to a facile enzymatic hydrolysis of the urea group, needed to be addressed by modification of the urea linkage.[9] To this end compound **15** (Table 3), in which the 1,4-dihydropyridinone was modified to a 1,2-dihydropyridinone system, was prepared and assessed in the plasma stability assay. Gratifyingly, compound **15** was not only completely stable in both human and rat plasma, but importantly potency was maintained and the GSH adduct formation was now below detectable levels (Table 3). Since the responsible group for trapping the GSH was still present in compound **15** (*vide supra*), the undetectable GSH adduct formation could be due to a further reduction of cLogP, from 2.76 for **13** to 1.97 for **15**, which presumably reduces binding to metabolizing enzymes.[10]

Table 3. **Relative Glutathione Adduct Formation and Plasma Stability**



10-13



15

Entry	X	R^1	R^2	IC_{50}[b] (nM)	% Relative GSH UV peak area[a] HLM	RLM	Plasma stability[b]
10	Br	MeO	Me	9	28	33	Not Determined
11	Br	F	Me	32	27	37	Not Determined
12	Br	Cl	Me	37	15	16	Not Determined

13	Me	Cl	$(CH_2)_2OH$	9	4	5	50% degradation in human plasma at 4h
15	-	-	-	23	BQL	BQL	Stable in both human and rat plasma

[a]% Relative GSH adduct UV peak area with respect to the total area of the GSH adduct, other metabolites and parent compound, [b]Mean value of $n \geq 2$ in presence of a positive control.

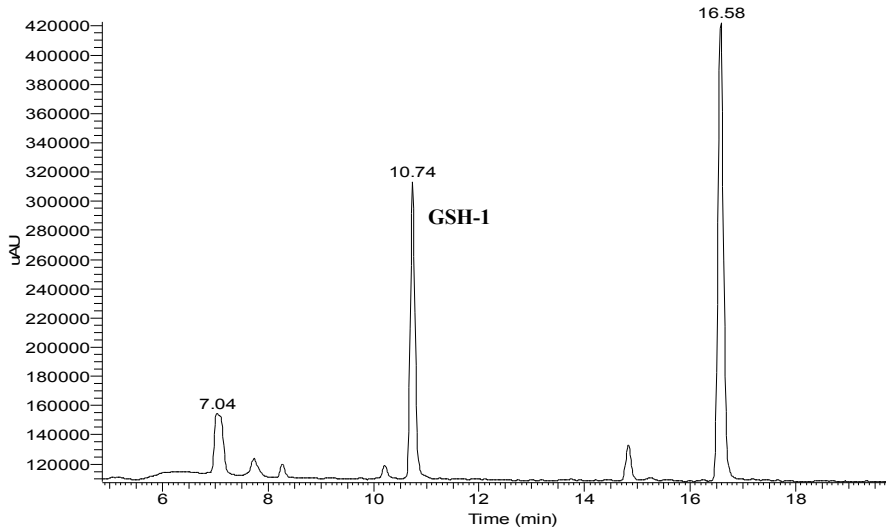


Figure 1. LC/UV chromatogram of the GSH trapping assay on compound **10**.

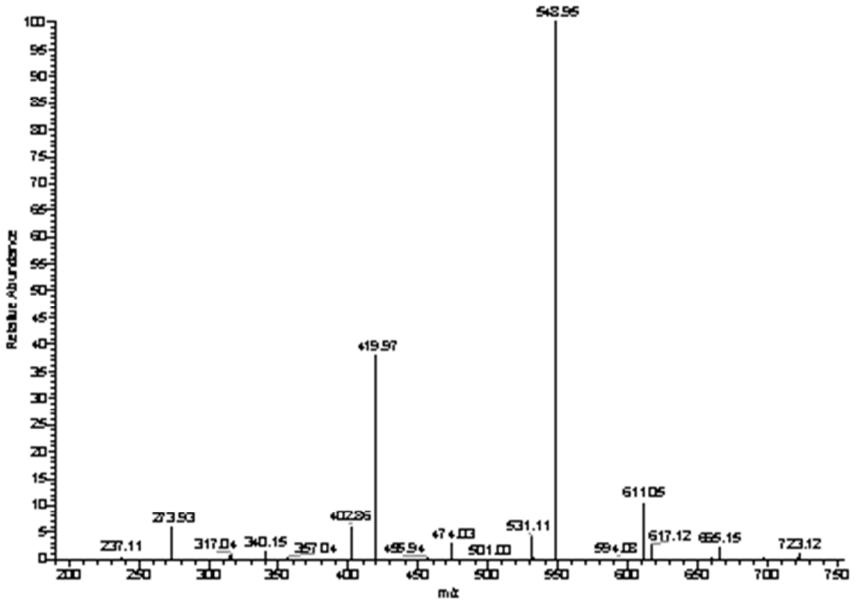


Figure 2. Collision induced decomposition MS/MS spectrum of **GSH-1**.

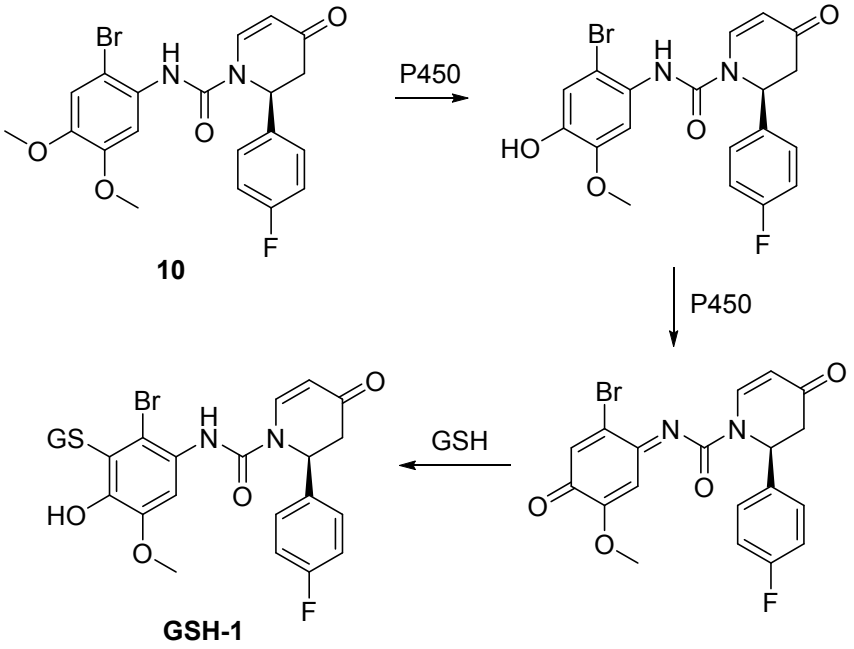


Figure 3. Hypothesis for formation of **GSH-1**.

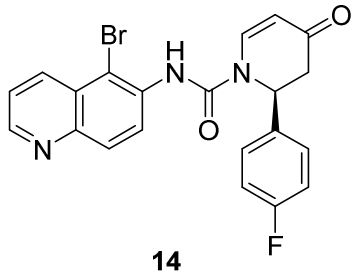


Figure 4. Compound **14** showed no GSH adduct formation.

This interesting new scaffold was profiled further. A summary of SAR for the new 1,2-dihydropyridinone series, from which **15** was identified, is presented in Figure 5, and representative cases are exemplified in Table 4.[11] Thus, the dihydropyridinone ring nitrogen can be substituted with relatively small alkyl groups, such as methyl or hydroxyethyl (e.g., **17**, Table 4); replacement of C-5 with NH is tolerated (e.g., **20**); the phenyl group at C-4 only allows small substituents with 3'-fluoro (e.g., **16**) or 4'-fluoro substitution (e.g., **15**) being optimal. Only halides or small alkyl or alkoxy groups are tolerated at positions 2", 3" and 4" (e.g., **16-18, 21**[12]),

whereas groups of different size at position 5" are allowed (e.g., **15**, **17-20**), and even fusion of the 4"-5" bond to form a bicyclic system (e.g., **22**) is acceptable. The finding that a variety of groups are acceptable at C-5" allowed the modulation of physicochemical properties of compounds to achieve desired parameters, for example for attaining acceptable rat SDPK or overcoming the mutagenic liability (*vide infra*).

These analogs proved to be active in a human whole blood (HWB) IL-1β assay (Table 4). However, consistent with other published P2X7 antagonists,[13] the 1,2-dihydropyridinone compounds showed no correlation of potency between rat and human. For example, the IC_{50} of **15** at the human P2X7 receptor was 23 nM (FLIPR Ca^{2+} flux assay), whereas the IC_{50} at the rat P2X7 was greater than 10,000 nM. This behavior precluded development of a robust *in vivo* pharmacology package in rodent models, for example in models of inflammatory/rheumatoid arthritis. However, in a cynomologous non-human primate (NHP) *ex vivo* PK/PD assay (Figure 6), sustained inhibition of the pro-inflammatory cytokine IL-1β was observed to correlate with total plasma concentration of **15**, providing evidence for P2X7 receptor target engagement *in vivo* and ability to mediate inhibition of IL-1β.

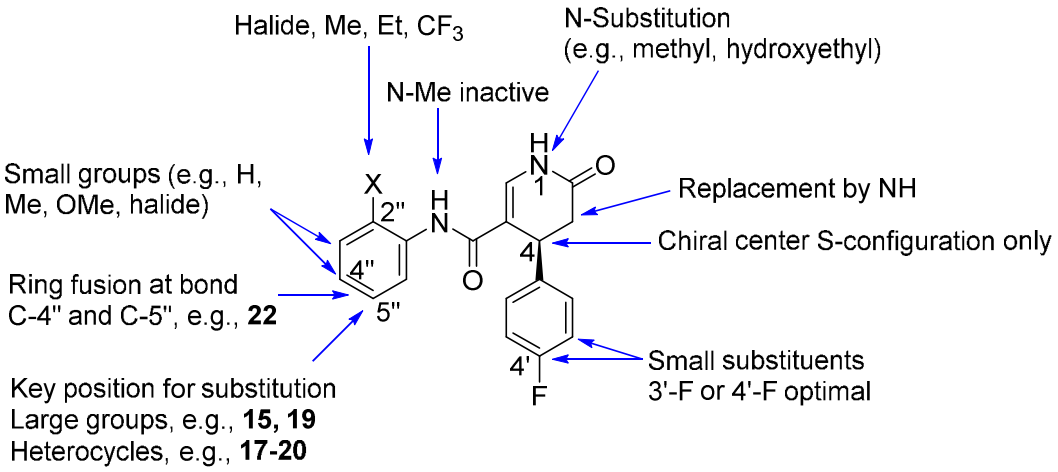
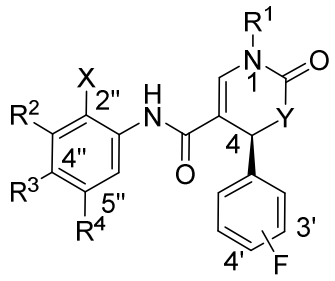
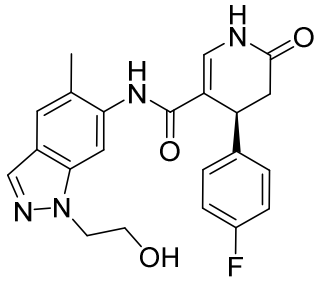


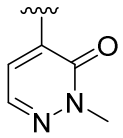
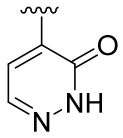
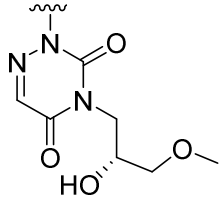
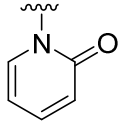
Figure 5. SAR for 1,2-dihydropyridinone series. Shown are replacements or substitutions that maintain potency FLIPR IC_{50} within 3 times, unless otherwise indicated.

Table 4. **Inhibition of P2X7 Activity by the 1,2-Dihydropyridinone Series**





22

Entry	X	Y	R^1	R^2	R^3	R^4	F	FLIPR IC_{50} (nM)[a]	HWB IC_{50} (nM)[a]
15	Me	CH_2	H	H	Cl	OCH_2CH_2OH	C-4’	23	60
16	Br	CH_2	H	H	OMe	OMe	C-3’	9	107
17	Me	CH_2	Me	H	F		C-4’	8	13
18	F	CH_2	Me	H	H		C-4’	26	27
19	Me	CH_2	Me	H	H		C-4’	30	17
20	Me	NH	Me	H	H		C-4’	22	33
21	Me	CH_2	H	Me	H	OMe	C-4’	9	-
22	-	-	-	-	-	-	-	23	47

[a]Mean value of $n \geq 2$ in presence of a positive control.

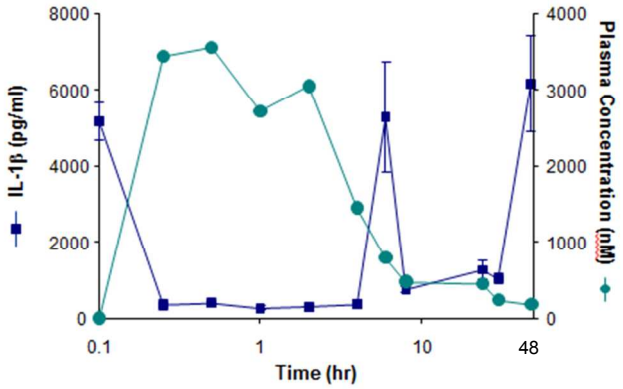


Figure 6. Representative NHP *ex vivo* PK/PD assay performed with compound **15** at 10 mg/kg.[14] Each point indicates mean value of $n \geq 2$. *Ex vivo* NHP WB IL-1β IC_{50} 300 nM.

Table 5 presents further profiling of compound **15**. As noted above, **15** is highly potent in the FLIPR Ca^{2+} flux assay as well as in the HWB IL-1β assay (IC_{50} 60 nM). Rat single dose pharmacokinetics showed excellent absorption and systemic exposure (F 74% and AUC/dose 1550 ng*h/ml/mg/kg); and, as predicted from the glutathione assay (*vide supra*), the HLM and RLM covalent binding (CVB) assays were negative. In addition, compound **15** showed excellent selectivity against other P2X receptors (Table 5), and no hits over 33% inhibition at 10 uM were found from a panel of 78 receptors as performed by the commercial receptor scan at CEREP (see Supporting Information for detailed report).

Table 5. Compound 15 Rat SDPK (iv 0.5, po 2 mg/kg), CVB and P2X Selectivity[a]

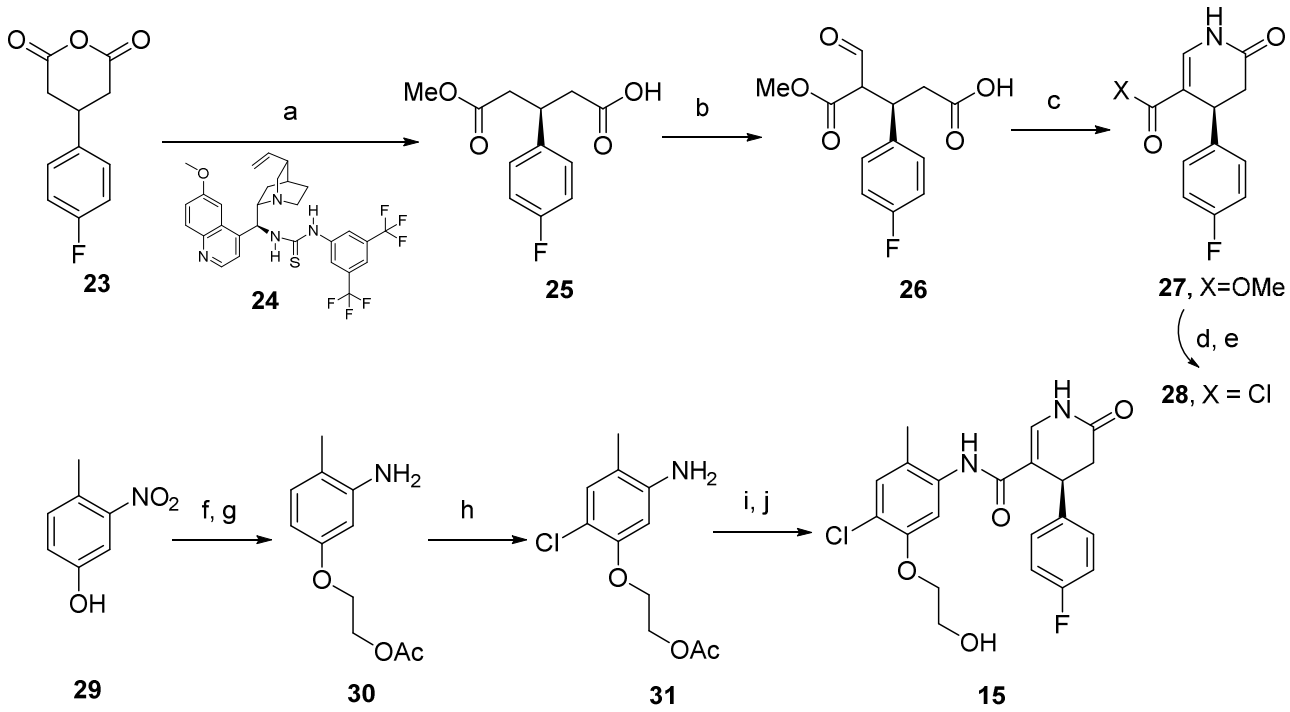
	HWB 1L-1β IC_{50}(nM)	F	po AUC/dose (ng*h/mL/mg/kg)	po Cmax (ng/mL)	IV CL (mL/min/kg)	Vdss (L/kg)	$t_{1/2}$ (h)	CVB[b] RLM/HLM	P2X[c] Selectivity FLIPR IC_{50}
15	60	74%	1550	330	8.4	2.2	4.0	Negative	> 10 μM

[a] $n \geq 2$ per time point, [b]CVB results with absence and presence of NADPH: RLM 2.6 and 38; and HLM 2.8 and

18 pmole-Eqs/mg protein, respectively. Internal Guideline: Flag above 100 pmole-Eqs/mg protein, [c]P2X selectivity against the P2X1, P2X2, P2X3, P2X2/3, P2X4, P2X5 receptors.

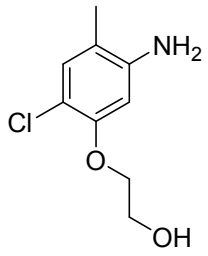
An enantioselective synthesis of **15** is presented in Scheme 1. Thus, pyrandione **23**[15] was subjected to an enantioselective methanolysis using organocatalyst **24** to obtain chiral monoacid **25** in 97% ee.[16] Regioselective formylation of **25** with LDA and methyl formate at -45 ^{o}C furnished **26**, which was cyclized smoothly with ammonium acetate in the presence of acetic acid to obtain 1,2-dihydropyridinone **27** with good overall yield from pyrandione **23** (48% yield for 3 steps) and preserving the enantiomeric purity from **25**. Hydrolysis of **27** by the action of lithium hydroxide in water/methanol, followed by treatment with thionyl chloride afforded acid chloride **28**. The aniline portion was prepared starting with 4-methyl-3-nitro-phenol **29**, which was alkylated and reduced to aniline **30** with 86% overall yield. Aniline **30** was then selectively chlorinated at C-4 with *N*-chlorosuccinimide to obtain **31** in 70% yield. Treatment of aniline **31** with acid chloride **28**, followed by methanolysis of the acetyl protecting group gave desired **15** with 89% yield for 2 steps.

Scheme 1. Enantioselective Synthesis of Compound 15[a]



[a]Reagents and conditions: a) MeOH (10 eq), 2 mol% **24**, 2-Me-THF, rt, then treatment with toluene/hexane, 97% ee; b) LDA, HCO_2Me, THF, -45 ^oC; c) NH_4OAc, HOAc, 80 ^oC, 48% 3 steps, 97% ee; d) LiOH, H_2O, MeOH, 86%, 96% ee; e) $SOCl_2$, DMF, DCM; f) $Br(CH_2)_2OAc$, K_2CO_3, DMA; g) H_2, 5% Pd/C, EtOH, 5 psi, 86% 2 steps ; h) NCS, CH_3CN, 70%; i) **28**, 2,6-lutidine, Me-THF; j) cat. NaOMe, MeOH, 89% 2 steps.

Although compound **15** was observed to be stable in both human plasma (4 h) and blood (3 h); and *in vitro* metabolism (HLM and HH) and *in vivo* metabolic studies [NHP plasma and urine (10 mg/kg)] did not show any signs of hydrolysis of the amide bond, a one month stability assessment showed slow degradation of **15** in the solid phase to produce free aniline **32**. This was also observed in formulations at different pH systems. Because of this finding, aniline **32** was assessed in the Ames mutagenicity assay,[17] which was positive. Even though parent compound **15** was Ames negative, we considered that it would not be possible to control the level of aniline present in the final drug product to the required level. As a consequence further development of compound **15** was discontinued.



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Our focus shifted to molecules that contained anilines devoid of mutagenic liability. It is known that mutagenic activity can be diminished or even eliminated by addition of fluorine atoms in the aniline ring,[18] as often predicted by the *in silico* tools DEREK and MCASE.[19] Thus, compounds **33** and **34** (Figure 7), which had excellent HWB potency but contained Ames positive anilines, were modified to fluoro-analogs **17** and **18**. Gratifyingly, potency was maintained, and not only had the corresponding anilines been predicted to be negative in both DEREK and MCASE, but also were shown to be negative in the Ames mutagenicity assay (Figure 7).

During our efforts to identify additional non-mutagenic anilines, we found that some, although predicted to be positive in both DEREK and MCASE, were negative in the Ames assay. Examples of compounds containing such anilines are presented in Figure 8.

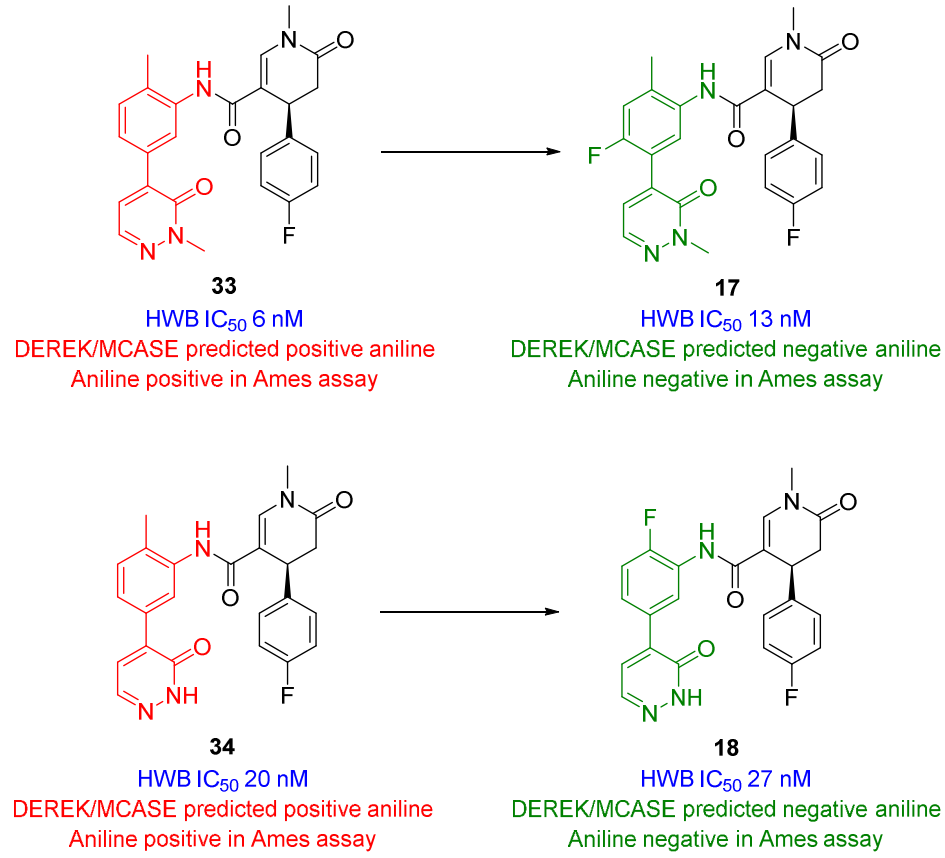


Figure 7. Addition of fluorine retains potency and gives Ames negative anilines.

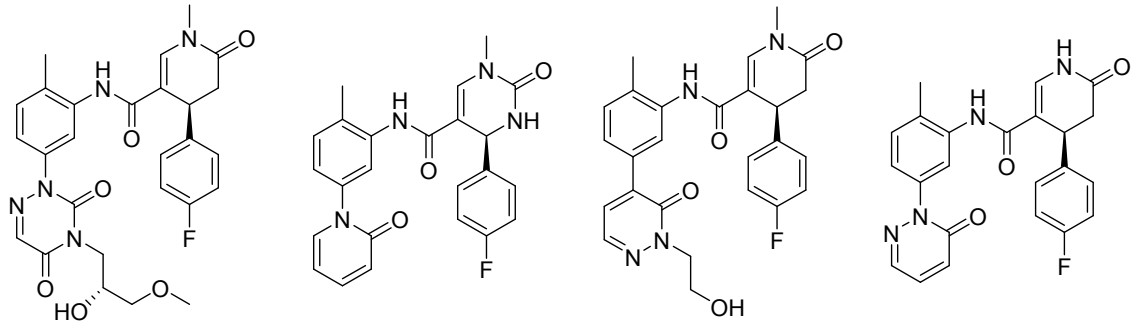


Figure 8. Compounds containing anilines predicted to be positive in DEREK and MCASE, but negative in the Ames mutagenicity assay.

The knowledge gained from compounds with Ames negative anilines allowed the selection of **17** and **19** as potential clinical candidates. Both compounds had excellent potency in the HWB

assay, good solubility, good overall rat PK profile, and good safety as measured by CYP inhibition, GSH adduct formation, and the aniline Ames mutagenicity assay (Table 6).

Table 6. **Potency, Solubility, CYP Inhibition, GSH Adduct formation, Aniline Ames Mutagenicity Results, and Rat Pharmacokinetic Parameters for Compounds 17 and 19**[a]

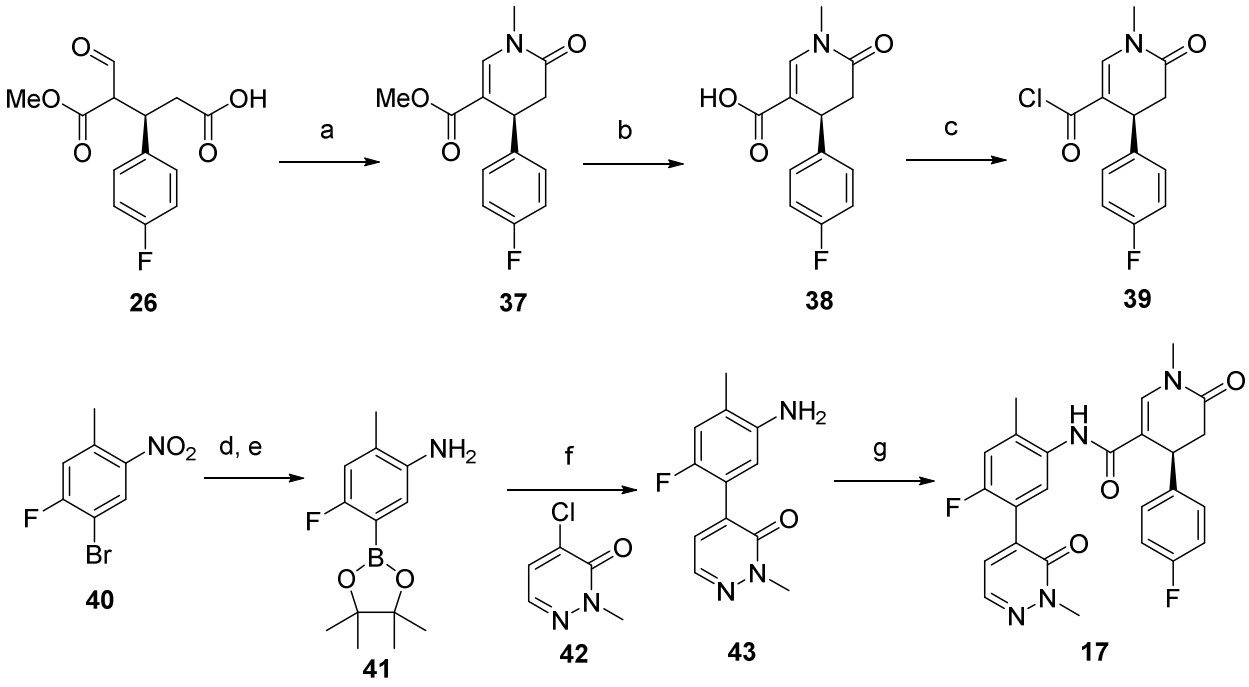
	cLogP/ PSA[b]	FLIPR IC_{50} (nM)	HWB IC_{50} (nM)	Solub[c] (μg/mL)	CYP Inh[d] (μM)	GSH	Aniline Ames	Rat PK IV 0.5 mg/kg PO 2 mg/kg
17	2.5/82	8	13	322	>32	Trace[e]	Negative	F 64%, CL[f] 17, $t_{1/2}$ 1.0 h, Vss[g] 1.0, AUC[h] 1121
19	0.9/131	33	17	1025	>50	Negative	Negative	F 20%, CL[f] 23, $t_{1/2}$ 4.0 h, Vss[g] 1.2, AUC[h] 279

[a]Experimental values are an average of $n \geq 2$, [b]cLogP and PSA (polar surface area) values were obtained from ChemBioDraw Ultra 13.0, [c]Solubility was obtained using LYSA (lyophilization solubility assay),[20] [d]A fluorescence-based CYP inhibition assay was utilized to generate IC_{50} values for inhibition of CYP3A4, CYP2D6, and CYP2C9,[21] [e]0.2% HLM/RLM relative UV peak area, [f]mL/min/kg, [g]L/kg, [h]PO ng*h/mL

Schemes 2 and 3 present the preparation of **17** and **19**, respectively. Thus, chiral acid **26** (*vide supra)* was subjected to cyclization in the presence of methylamine to furnish N-methyl-dihydropyridinone **37** in 100% ee after crystallization from MTBE.[16b] Ester hydrolysis, followed by treatment with thionyl chloride gave acid chloride **39**. For the aniline, 1-bromo-2-fluoro-4-methyl-5-nitro-benzene **40** was converted to the corresponding boronate ester, which was reduced to aniline **41** in 58% yield. Suzuki coupling with chloropyridazinone **42** furnished **43** in 93% yield. Final coupling of aniline **43** with acid chloride **39** furnished desired amide **17**. For the preparation of **19** (Scheme 3), 4-methyl-3-nitro-aniline **44** was transformed to its diazonium salt, which by treatment with bis-ethoxycarbonyl-malonamide **45** afforded triazinedione ester **46.** Hydrolysis with aqueous sodium hydroxide followed by decarboxylation with mercaptoacetic acid at 180 ^{o}C gave **47** in 58% overall yield for 4 steps. Alkylation with (*R*)-glycidylmethyl ester,

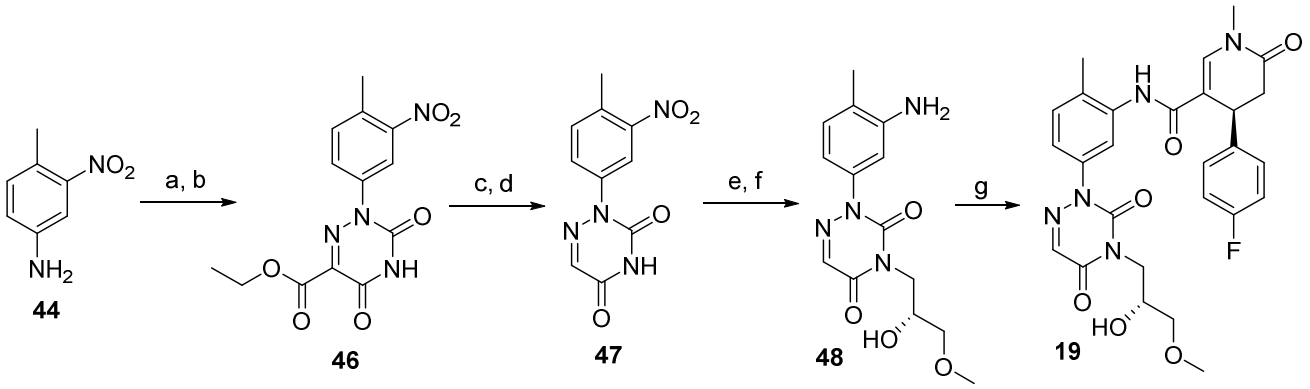
followed by reduction of the nitro group furnished aniline **48** with 54% yield for 2 steps. Final coupling of **48** with acid **38** in the presence of HATU/DIPEA afforded desired amide **19**.

Scheme 2. Enantioselective Synthesis of Compound 17[a]



[a]Reagents and conditions: a) $MeNH_2$/water (40% weight, 3.0 eq), AcOH, 65 ^oC, then recrystallization from MTBE, 44% overall, 100% ee; b) LiOH, H_2O, MeOH, 86%; c) $SOCl_2$ (2 eq), DCM, DMF (1 drop); d) bis(pinacolato)diboron (1.2 eq), KOAc (3.0 eq), $Pd(dppf)Cl_2$ (0.5 mol %) dioxane, 100 ^oC, 91%; e) H_2, Pd/C, THF, 58%; f) **42** (1.0 eq), Cs_2CO_3 (3 eq), $Pd(Ph_3P)_4$ (10 mol %), DMF/H_2O, microwave, 120 ^oC, 93%; g) **39** (1.25 eq), pyridine (1.25 eq), DCM, 43%

Scheme 3. Preparation of Compound 19[a]



[a]Reagents and conditions: a) $NaNO_2$ (1.05 eq), HCl (conc, 8 eq), $CH_2(CONHCO_2Et)_2$ (**45**, 1.0 eq), H_2O, -5 ^oC; b) aq KOH, THF/EtOH; c) NaOH, EtOH/H_2O; d) $HSCH_2CO_2H$, 180 ^oC, 58% overall 3 steps; e) (*R*)-(-)-glycidylmethyl ether (3.0 eq), silica gel (1.5 g/ 1.0 g **47**, 200-400 mesh), DMF, 80 ^oC, 64%; f) Fe (5 eq), NH_4Cl (5 eq), EtOH/H_2O, 85 ^oC, 84%; g) **38** (1.0 eq), HATU (1.0 eq), DIPEA (2.0 eq), DMF, 80 ^oC, 39%.

CONCLUSION

In summary, identification of a singleton P2X7 inhibitor **1** from HTS revealed a pharmacophore that was eventually turned into potential clinical candidates **17** and **19**. During development, a number of different properties were optimized and issues circumvented, such as metabolic stability, plasma stability, GSH adduct formation and aniline mutagenicity. Thus, iterative modifications of the molecule, such as conversion of the 1,4-dihydropyridinone to the 1,2-dihydropyridinone, proper substitution at C-5", and addition of fluorine atoms to the aniline ring allowed the identification of potent and selective P2X7 antagonists that may be useful for efforts aimed at evaluating the biology of P2X7 and its role in inflammatory, immune, neurologic or musculoskeletal disorders.[2]

EXPERIMENTAL SECTION

General Methods. All reagents and solvents were purchased from commercial sources and used without further purification. ^1H NMR spectra were measured using a Bruker NMR Avance 400 MHz or Bruker NMR Avance 300 MHz spectrometer, and chemical shifts were expressed in

δ (ppm) units using tetramethylsilane as an internal standard. Mass spectra were recorded on an Agilent AG1 Single Quad G6140A spectrometer.

Purity values for all tested compounds were found to be above 95% from the HPLC analyses. In general, the nomenclature used in this section is based on IUPAC as noted above, or, alternatively, based on ChemDraw.

The preparation of compound 1 represents a typical procedure used for the synthesis of 4-phenyl-2,3-dihydro-1H-pyridine-4-one amides. 1-[2-(2-Iodo-4,5-dimethoxy-phenyl)-acetyl]-2-phenyl-2,3-dihydro-1H-pyridin-4-one (1). To a solution of (2-iodo-4,5-dimethoxyphenyl)-acetic acid (2.03 g, 6.3 mmol) in DCM (20 mL) was added oxalyl chloride (0.6 mL, 6.93 mmol) under argon. One drop of DMF was added and the resulting mixture was stirred at rt for 3.5 h. The mixture was evaporated to dryness. Dichloroethane was added and the resulting solution was evaporated to dryness. This was repeated and the resulting residue was dried under vacuum to afford 2.2 g of (2-iodo-4,5-dimethoxyphenyl)-acetyl chloride.

To a solution of 4-methoxypyridine (305.7 mg, 2.8 mmol) in THF (6 mL) was added at -25 to -30° C phenyl magnesium bromide (3M in ether, 1.03 mL, 3.08 mmol) under an argon atmosphere. After 5 minutes, (2-iodo-4,5-dimethoxyphenyl)-acetyl chloride (953.9 mg, 2.8 mmol) was added slowly keeping the temperture between – 25 to -30 °C. The reaction mixture was allowed to stir at the same temperature for 2.5 h. An aqueous solution of HCl (2N) was added to the reaction mixture at - 30°C and the resulting mixture was allowed to warm to rt and stirred at rt for 10 minutes. The mixture was extracted with EtOAc (2x). The combined organic extract was washed with saturated sodium chloride solution, dried over Na_2SO_4 and concentrated in vacuo. Purification by flash chromatography (6:4 hexane/EtOAc) provided 1 (869 mg, 65%). LCMS (M + H)+ = 478. 1H NMR (300 MHz, DMSO-d6) δ ppm 2.73 (dt, J=16.44, 1.67 Hz, 1

H) 3.17 (dd, J=16.48, 7.06 Hz, 1 H) 3.71 (s, 3 H) 3.75 (s, 3 H) 3.88 - 4.00 (m, 1 H) 4.22 (d, J=16.95 Hz, 1 H) 5.32 (dd, J=8.48, 1.51 Hz, 1 H) 5.96 (d, J=6.97 Hz, 1 H) 6.90 (s, 1 H) 7.17 - 7.41 (m, 6 H) 8.21 (dd, J=8.29, 1.32 Hz, 1 H).

The preparation of compound 4 represents a typical procedure used for the synthesis of 4-phenyl-2,3-dihydro-1H-pyridine-4-one ureas. (2S)-N-(4,5-dimethoxy-2-methyl-phenyl)-2-(4-fluorophenyl)-4-oxo-2,3-dihydropyridine-1-carboxamide (4). Step 1. [(1R,5R)-5-methyl-2-(1-methyl-1-phenyl-ethyl)cyclohexyl] (2S)-2-(4-fluorophenyl)-4-oxo-5-triisopropylsilyl-2,3-dihydropyridine-1-carboxylate. Following Comins' procedure,[22] to a solution of 4-methoxy-3-triisopropylsilanyl-pyridine (7.11 g, 26.8 mmol) in toluene (193 mL) at −25 °C. was added a solution of [(1R,5R)-5-methyl-2-(1-methyl-1-phenyl-ethyl)cyclohexyl] carbonochloridate (7.9 g, 26.8 mmol) in toluene (160 mL). After 15 minutes the mixture was cooled to −78 °C. and 1.0M 4-fluorophenylmagnesium bromide in THF (30.6 mL, 30.6 mmol) was added slowly. The mixture was stirred at −78 °C. for 1 hour. 2N HCl was added and the mixture was allowed to warm to room temperature and was stirred for additional 15 minutes. The mixture was extracted with ethyl acetate, washed with water and brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification of the residue by flash chromatography (hexane/ethyl acetate 9:1) gave [(1R,5R)-5-methyl-2-(1-methyl-1-phenyl-ethyl)cyclohexyl] (2S)-2-(4-fluorophenyl)-4-oxo-5-triisopropylsilyl-2,3-dihydropyridine-1-carboxylate (13.14 g, 81%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.54 (br. s., 1 H) 0.75 (d, J=6.40 Hz, 3 H) 0.77 - 0.83 (m, 1 H) 0.98 (dd, J=7.35, 1.70 Hz, 18 H) 1.16 (s, 3 H) 1.21-1.45 (m, 5H) 1.29 (s, 3 H) 1.47 - 1.65 (m, 2 H) 1.75 (d, J=12.81 Hz, 1 H) 1.97 - 2.12 (m, 1 H) 2.34 (d, J=15.07 Hz, 1 H) 2.86 (dd, J=15.82, 7.54 Hz, 1 H) 4.38 (br. s., 1 H) 4.73 (td, J=10.64, 4.33 Hz, 1 H) 6.88 - 7.11 (m, 4 H) 7.14 - 7.24 (m, 1 H) 7.24 - 7.36 (m, 4 H) 7.84 (s, 1 H).

Step 2 (S)-2-(4-Fluoro-phenyl)-2,3-dihydro-1H-pyridin-4-one. To a solution of [(1R,5R)-5-methyl-2-(1-methyl-1-phenyl-ethyl)cyclohexyl] (2S)-2-(4-fluorophenyl)-4-oxo-5-triisopropylsilyl-2,3-dihydropyridine-1-carboxylate (13.14 g, 21.9 mmol) in methanol (160 mL) was added 25% sodium methoxide in methanol (49.6 mL, 218.6 mmol). The mixture was heated at reflux for 16 h. After cooling to room temperature, oxalic acid (78 g, 867 mmol) was added and the mixture was stirred for 2 h. The solvent was evaporated to dryness. The crude residue was partitioned between ethyl acetate and water. The organic layer was washed with water, then brine, dried over $MgSO_4$, filtered and concentrated to dryness. Purification by flash chromatography (EtOAc/ hexane gradient, 0 – 95%) gave (S)-2-(4-fluorophenyl)-2,3-dihydro-1H-pyridin-4-one (2.34 g, 56%, ee 98.9%)[23] as a yellow solid. LCMS $(M + H)^+ = 192$. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.45 - 2.58 (m, 1 H) 2.63 - 2.80 (m, 1 H) 4.75 (dd, J=14.51, 5.09 Hz, 1 H) 5.08 (br. s., 1 H) 5.13 (d, J=7.54 Hz, 1 H) 7.02 - 7.15 (m, 2 H) 7.20 - 7.31 (m, 1 H) 7.32 - 7.44 (m, 2 H).

Step 3. (2S)-N-(4,5-dimethoxy-2-methyl-phenyl)-2-(4-fluorophenyl)-4-oxo-2,3-dihydropyridine-1-carboxamide (4). To a solution of 4,5-dimethoxy-2-methyl-aniline (84 mg, 0.5 mmol) in 10 mL of toluene was added pyridine (0.6 mL) and DMAP (61 mg) followed by phosgene (0.34 mL of 20% in toluene). The mixture was stirred at room temperature for 2 h. The solution was filtered and the filtrate concentrated in vacuo. The residue was diluted with DCM and concentrated in vacuo again to provide 1-isocyanato-4,5-dimethoxy-2-methyl-benzene (96 mg, 99%) as a white crystalline solid.

(S)-2-(4-Fluoro-phenyl)-2,3-dihydro-1H-pyridin-4-one (80 mg, 0.42 mmol) was dissolved in 5 mL of dry THF and cooled to – 40° C. A solution of n-BuLi (0.22 mL of 2.5 M, 0.55 mmol) was added and the mixture was allowed to stir for 30 min. 1-isocyanato-4,5-dimethoxy-2-methyl-

benzene (96 mg, 0.50 mmol) was added and the mixture was stirred for 1 h and then allowed to warm to room temperature and stir at room temperature for 2 h. The reaction was quenched with saturated NH_4Cl (aq.) and water. The mixture was extracted with EtOAc. The organic extracts were dried over Na_2SO_4 and concentrate in vacuo. The residue was purified by flash chromatography (silica, 1:1 hexane: EtOAc) to afford (2S)-N-(4,5-dimethoxy-2-methyl-phenyl)-2-(4-fluorophenyl)-4-oxo-2,3-dihydropyridine-1-carboxamide **4** (95 mg, 54%) as a yellow foam. LCMS $(M + H)^+ = 385$. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.93 (s, 3 H) 2.70 (d, J=16.48 Hz, 1 H) 3.22 (dd, J=16.48, 7.32 Hz, 1 H) 3.69 (s, 3 H) 3.72 (s, 3 H) 5.11 - 5.32 (m, 1 H) 5.85 (d, J=6.71 Hz, 1 H) 6.75 (s, 1 H) 6.79 (s, 1 H) 7.12 - 7.33 (m, 4 H) 8.24 (dd, J=8.54, 1.22 Hz, 1 H) 9.08 (s, 1 H).

2-(3-Amino-4-methyl-phenoxy)ethyl acetate (30). A 12-L 3-neck round bottom flask flushed with nitrogen was charged with 4-methyl-3-nitro-phenol (350 g, 2.29 mol) followed by an addition funnel over a period of 1 h. K_2CO_3 (325 mesh, 441.5 g, 3.20 mol) and DMA (700 mL). To the mixture was added 2-bromoethyl acetate (438.9 g, 2.63 mol) via After the addition was complete, the reaction mixture was stirred at 65 °C for 24 h, and then cooled to 15 °C. To the reaction mixture was then added slowly 2.7 L of MTBE and 3.8 L of water. The mixture was stirred for 10 min, and the organic layer was separated and washed with 4 X 2.1 L of water. The organic layer was then distilled at atmospheric pressure to a final volume of ca. 1 L. To this residue was added 800 mL of EtOH and the atmospheric distillation was continued to remove additional 750 mL of the distillate with the final internal temperature of 82 °C. This solution was used as is in the next step.

Note: A small aliquot of the ethanolic solution was concentrated to record a ^{1}H NMR of the intermediate 2-(4-methyl-3-nitro-phenoxy)ethyl acetate in $CDCl_3$ (300 MHz): 7.54 (1H, d, J= 3

Hz), 7.23 (1H, s), 7.09 (1H, d, J1= 3 Hz, J2= 2.0 Hz), 4.44 (2H, d, J= 6 Hz), 4.21 (2H, d, J= 6Hz), 2.53 (3H, s), 2.11 (3H, s).

A 25-L reactor, flushed with 3 cycles of vacuum and nitrogen, was charged with 55 g of 20% $Pd(OH)_2$ on carbon and then flushed again with 3 cycles of vacuum and nitrogen. To this vessel was charged ethanolic solution of intermediate 2-(4-methyl-3-nitro-phenoxy)ethyl acetate from above. The mixture so obtained was then stirred under hydrogen atmosphere (3–4 psi) at 45–50 °C for 8 h. Upon complete conversion, the mixture was cooled to 20 °C, purged with nitrogen and filtered through a pad of 200 g of Solka-Floc® and the filtercake was washed with 1.3 L of ethanol. The combined filtrate and washes were concentrated at 40 °C under vacuum to remove ca. 1.5 L of ethanol to obtain a slurry, which was cooled to 0 °C and then aged for 1 h. The solids were filtered on a sintered glass funnel and washed with 1 L of ice-cold ethyl acetate. The wet cake was dried in a vacuum oven at 40 °C for 18 h to obtain 409.9 g (85% yield) of 2-(3-amino-4-methyl-phenoxy)ethyl acetate, **30,** as a beige colored solid (Mp: 98–100 °C).

2-(5-Amino-2-chloro-4-methyl-phenoxy)ethyl acetate (31). To an ice-cold (0–7 °C) slurry of **30** (500 g, 2.39 mol) in 2.5 L of acetonitrile was added in three equal portions N-chlorosuccinimide (NCS, 367 g, 2.75 mol). After ca. 2–3 h, a solution of Na_2SO_3 (173 g, 1.38 mol) in water (1.0 L) was added such that the reaction temperature was maintained below 10 °C. The solution so obtained was then added slowly to 12 L of ice-cold water over ca. 2 h such that the reaction temperature was maintained below 10 °C. The resulting slurry was stirred at 4–10 °C for 1 h and then filtered on a sintered glass funnel. The wet cake was washed with 2x 2.5 L of cold water followed by 2x 1.0 L of hexanes, and then dried in a vacuum oven at ca. 45 °C for 18 h to obtain 530 g (91% crude yield) of 2-(5-amino-2-chloro-4-methyl-phenoxy)ethyl acetate,

31. The material was recrystallized from 8 volumes of 1:2 ethyl acetate/ hexanes with ca. 80% recovery.

^{1}H NMR (300 MHz, $CDCl_3$): 7.01 (1H, s), 6.30 (1H, s), 4.43 (2H, t, J= 6 Hz), 4.16 (2H, t, J= 6 Hz), 3.60 (2H, broad s), 2.10 (3H, s), 2.08 (3H, s).

(4S)-N-[4-chloro-5-(2-hydroxyethoxy)-2-methyl-phenyl]-4-(4-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carboxamide (15). To a suspension of (4S)-4-(4-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carboxylic acid,[16] (10 g, 42.5 mmol) in 50 mL of dichloromethane at ambient temperature was added DMF (30μL, 387 μmol) followed by slow addition of $SOCl_2$ (6.21 mL, 85.1 mmol). The resulting mixture was stirred at ambient temperature for at 2 h, during which time the gas evolution had ceased and the reaction mixture had turned homogenous. The reaction mixture was concentrated under reduced pressure at 50 °C to a minimum volume. The concentrated mixture was further evaporated with 2 x 54 mL of toluene to obtain the (4S)-4-(4-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carbonyl chloride, **28,** as a thick orange oil, which was dissolved in 54 mL of MeTHF.

In separate flask, 2-(5-amino-2-chloro-4-methyl-phenoxy)ethyl acetate, **31** (10.8 g, 40.4 mmol) was dissolved in 54 mL of MeTHF, and to this solution was added 2,6-dimethylpyridine (5.2 mL, 44.7 mmol). To this solution, cooled to 0–4 °C with an ice/water bath, was added via an addition funnel the MeTHF solution **28** from above. After the addition was complete (ca. 15 min), the addition funnel was rinsed with 2 mL of MeTHF and the rinse was added to the reaction mixture, which was then stirred for ca. 2 h to obtain a slurry. The reaction mixture was allowed to warm to ambient temperature and to the reaction flask was slowly added 15 mL of 1:2 mixture of brine:1N HCl to obtain a biphasic mixture. The organic layer was separated, and then washed twice with 15 mL 1:1, brine:water followed by 15 mL of 1.05 M sodium carbonate and

finally with 15 mL of brine. The organic layer was concentrated under vacuum at 40 °C, and residue so obtained was azeotroped with MeOH such that a solution of acetylated **15** in ca. 45 mL of MeOH was obtained. To this methanol solution was added via a syringe pump 25 weight % methanolic solution of sodium methoxide (4.45 mL, 19.46 mmol). After ca. 3 h at ambient temperature, 145 mL of water was added at a rate of 76 mL/h such that internal temperature was < 35 °C. After the water addition was complete the slurry so obtained was stirred at ambient temperature for at 2 h, and then filtered on a sintered glass funnel. The wet cake was washed with 35–40 mL of 15% (v/v) methanol/water, then dried in vacuum oven at 60 °C for 18 h providing 14.93 g (89–91% yield) of (4S)-N-[4-chloro-5-(2-hydroxyethoxy)-2-methyl-phenyl]-4-(4-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carboxamide, **15**. MS (M+H) = 419. ^{1}H NMR (300 MHz, DMSO-d_6): 9.88 (1H, broad s), 9.08 (1H, broad s), 7.29–7.22 (3H, m), 7.17–7.10 (3H, m), 4.83 (1H, bs), 4.27 (1H, d, J= 6.6 Hz), 4.06–3.95 (2H, m), 3.72-3.60 (2H, m), 3.00 (1H, dd, J1= 6 Hz, J2= 6 Hz), 2.42 (1H, d, J= 3 Hz), 1.94 (3H, s).

^{13}C NMR (75 MHz, DMSO-d_6): 169.03, 164.12, 160.96 (d, J_{C-F}= 242 Hz), 151.62, 138.14, 138.10, 136.07, 133.03, 130.57, 128.51, 128.41, 125.80, 117.46, 115.35, 115.07, 112.35, 111.55, 70.64, 59.28, 35.08, 16.46.

The preparation of compound 16 represents a general procedure used for the synthesis of (4S)-2-oxo-4-aryl-3,4-dihydro-1H-pyridine-5-carboxamides. Step 1. Preparation of 4-(3-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carboxylic acid. To a solution of 6-chloro-nicotinic acid (3.2 g, 20 mmol) in 16 mL of THF was added (3-fluorophenyl)magnesium bromide (16 mL of 1M in THF) at 0 °C over 30 min. The resulting reaction mixture was stirred at room temperature for 48 h, before cooling to -10 °C. To the cooled mixture was added 30 mL of acetic acid. The reaction mixture was warmed to room temperature and 100 mL of water was

added. The mixture was stirred at room temperature for 2h and extracted with DCM (3 X 300 mL). The combined organic layer was concentrated, washed with hexane:DCM(9:1, 100 mL) and decanted. The solids were dried under vacuum to afford 1.8 g of 4-(3-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carboxylic acid. LCMS $(M+H)^+$ = 236, $(M-H)^+$ = 234;. ^{1}H NMR (300 MHz, Methanol-d_4) δ ppm 2.64 (dd, J=16.77, 1.70 Hz, 1 H) 3.03 (dd, J=16.58, 8.67 Hz, 1 H) 4.17 (d, J=7.16 Hz, 1 H) 6.85 - 6.97 (m, 2 H) 7.04 (d, J=7.91 Hz, 1 H) 7.21 - 7.35 (m, 1 H) 7.54 (s, 1 H).

Step 2. Preparation of (4S)-N-(2-bromo-4,5-dimethoxy-phenyl)-4-(3-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carboxamide (16). A solution of 4-(3-fluorophenyl)-2-oxo-3,4-dihydro-1H-pyridine-5-carboxylic acid (150 mg, 0.64 mmol) in DCM (10 mL) was treated sequentially with 3 drops DMF and 2 equiv. oxalyl chloride. The resulting reaction mixture was stirred for 30 min. before concentration under vacuum. The residue was re-dissolved in DCM and to the resulting solution was added 2-bromo-4,5-dimethoxy-aniline (150 mg, 0.65 mmol) and 2 equiv. TEA. The reaction mixture was stirred at room temperature for 24 h. Chromatography (30 – 100% EtOAc/ hexane gradient) provided crude product, which was further purified by chiral HPLC to provide 80 mg of a front-running, more active isomer, assigned as the desired product, (4S)-N-(2-bromo-4,5-dimethoxyphenyl)-4-(3-fluorophenyl)-2-oxo-3,4- dihydro-1H-pyridine-5-carboxamide, **16**. LCMS $(M + H)^+$ = 449, 451. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.75 (d, J=15.64 Hz, 1 H) 3.13 (dd, J=16.58, 8.48 Hz, 1 H) 3.81 (s, 3 H) 3.87 (s, 3 H) 4.18 (d, J=6.97 Hz, 1 H) 6.89 (s, 1 H) 6.94 - 7.06 (m, 2 H) 7.12 (d, J=7.91 Hz, 1 H) 7.29 - 7.39 (m, 1 H) 7.42 (s, 1 H) 7.57 (d, J=5.27 Hz, 1 H) 7.99 (s, 1 H) 8.44 (d, J=5.09 Hz, 1 H).

(4S)-4-(4-Fluorophenyl)-1-methyl-2-oxo-3,4-dihydropyridine-5-carboxylic acid (38). To a solution of methyl (4S)-4-(4-fluorophenyl)-1-methyl-2-oxo-3,4-dihydropyridine-5-carboxylate

37[16b] (7.18 g, 27 mmol) in 65 mL of methanol at room temperature under argon was added lithium hydroxide hydrate (1.5 g, 35 mmol) followed by water (11 mL). The resulting reaction mixture was warmed at 60 °C for 18 h, then cooled to room temperature and concentrated under vacuum. Water and ether were added. The aqueous phase was washed with ether and the ethereal layer discarded. The aqueous layer was then acidified to pH 2 with 6 N HCl. The aqueous phase was extracted with ether (2X). The combined ethereal extracts were washed with brine, dried over $MgSO_4$, filtered and evaporated in vacuo to provide the title compound, **38** (6.27 g, 93%) as a white foam. LCMS $(M + H)^+ = 250$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (s, 1H), 7.08-7.22 (m, 2H), 6.89-7.05 (m, 2H), 4.13 (d, J=8.59 Hz, 1H), 3.23 (s, 3H), 3.00 (dd, J=8.34, 16.42 Hz, 1H), 2.79 (dd, J=1.52, 16.67 Hz, 1H).

4-Fluoro-2-methyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenylamine (41). Step 1. A mixture of 4-bromo-5-fluoro-2-nitrotoluene, **40** (4.9 grams, 0.02moles, 1equiv.), bis(pinacolato) diboron (6.38 grams, 0.0251 moles, 1.2 equiv.), potassium acetate (6.17 grams, 0.062 moles, 3.0 equiv.) and [1,1'-bis(diphenyphosphino)ferrocene]-dichoropalladium(II) (1:1) complex with dichoromethane (0.85 g, 0.0001 moles) in anhydrous dioxane (25 ml) was heated in sealed reaction vessel under argon for 1 hour at 100 °C. To the reaction mixture was added 50 ml water and the resultant mixture was filtered through pad of celite, washed and extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, filtered and concentrated under vacuum. Chromatography on silica gel, eluting with 20% ethyl acetate in hexanes afforded 5.36 grams of 2-(2-fluoro-4-methyl-5-nitrophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.0191 moles, 91%). mp 95-96 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.37 (s, 12 H) 2.63 (s, 3 H) 7.01 (d, J=9.60 Hz, 1 H) 8.43 (d, J=5.56 Hz, 1 H).

Step 2. To a solution of 2-(2-fluoro-4-methyl-5-nitrophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (2.95 grams, 0.0105 moles) in 50 mL THF was added 100 mg of 10% palladium on carbon under nitrogen. The reaction mixture was evacuated under vacuum, then filled with hydrogen gas via balloon, and stirred under the hydrogen atmosphere at room temperature for 4 hours. After the reaction was complete, the reaction mixture was filtered through celite, and purified by chromatography on silica, eluting with 20% ethyl acetate in hexanes to give 1.54 g 4-fluoro-2-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl amine, **41** (0.006 moles, 58%). mp 91-93 $^{\circ}$C. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.36 (s, 12 H) 2.17 (s, 3 H) 3.53 (br. s., 2 H) 6.78 (d, J=9.82 Hz, 1 H) 7.00 (d, J=4.91 Hz, 1 H).

4-Chloro-2-methyl-2H-pyridazin-3-one (42). Step 1. A solution of 4,5-dichloro-2-methyl-2H-pyridazin-3-one, (5 g, 28 mM) in 20 ml ethanol was added dropwise to anhydrous hydrazine (3.5 ml, 112 mM) that was stirring in 20 ml ethanol at 3 °C. The reaction mixture was stirred at 0 °C an additional 3 hours, then filtered and the precipitate washed with ether to provide 0.68 g slightly impure product. The filtrate was cooled in the freezer overnight, filtered and washed with ether to provide an additional 2.37 g of product. The collected solids were combined to provide 3.05 g of impure 4-chloro-5-hydrazino-2-methyl-2H-pyridazin-3-one, which was used as is for the next step. LCMS $(M + H)^+ = 175$.

Step 2. A solution of 4-chloro-5-hydrazino-2-methyl-2H-pyridazin-3-one (2.5 g, 14.3 mM) and $CuSO_4.5H_2O$ (20 g) in 100 ml H_2O was heated at 90 °C for 45 minutes. The reaction mixture was allowed to cool and extracted into DCM. The combined organic extracts were washed with water, then brine and dried over sodium sulfate and concentrated in vacuo. Chromatography on silica gel using a 0% - 20% EtOAc/Hexane gradient afforded 0.74 g (35%)

of 4-chloro-2-methyl-2H-pyridazin-3-one, **42**. LCMS $(M + H)^+$ = 145. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 3.86 (s, 3 H) 7.38 (d, J=4.52 Hz, 1 H) 7.67 (d, J=4.14 Hz, 1 H).

4-(5-Amino-2-fluoro-4-methylphenyl)-2-methyl-2H-pyridazin-3-one (43). A microwave vial was charged with a mixture of 4-fluoro-2-methyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-phenylamine, **41** (0.104 g, 0.415 mmol), 4-chloro-2-methyl-2H-pyridazin-3-one, **42**, (0.06 g, 0.415 mmol), cesium carbonate (0.405 g, 1.245 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.048 g, 0.041 mmol) in a 1:1 solution of DMF-water (3 mL). The vial was sonicated while argon was bubbled into the solution for 10 min. Then the vial was heated under microwaves at 120 °C for 20 min. After cooling to room temperature, the mixture was extracted with ethyl acetate (3x15 mL), combined organics were washed with brine (3x5 mL), dried over magnesium sulfate, filtered and concentrated. The residue was purified by flash chromatography eluting with a 0 to 100% ethyl acetate /hexanes gradient to yield the title compound, **43** (0.109 g, 93%). LCMS: $(M+H)^+$ = 234; ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.20 (s, 3 H) 3.87 (s, 3 H) 6.89 (dd, J=11.12, 0.51 Hz, 1 H) 7.10 (d, J=6.57 Hz, 1 H) 7.34 (dd, J=4.29, 1.77 Hz, 1 H) 7.80 (d, J=4.04 Hz, 1 H).

(S)-4-(4-Fluorophenyl)-1-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylic acid [4-fluoro-2-methyl-5-(2-methyl-3-oxo-2,3-dihydro-pyridazin-4-yl)-phenyl]-amide (17). To a solution of (S)-4-(4-fluorophenyl)-1-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylic acid, **38** (0.12 g, 0.48 mmol) in dichloromethane (2 mL), containing a catalytic amount of DMF (1 drop), was added dropwise thionyl chloride (0.07 mL, 0.962 mmol) and the mixture stirred at room temperature for 2 h. Then the solvents were evaporated under vacuum, residue suspended in toluene (10 mL) and concentrated again (twice) and residue dried in high vacuum for 30 min to afford acid chloride intermediate **39**, which was used as is. The residue was dissolved in

dichloroethane (10 mL) and added to 4-(5-amino-2-fluoro-4-methyl-phenyl)-2-methyl-2H-pyridazin-3-one (0.109 g, 0.384 mmol), followed by addition of pyridine (0.04 mL, 0.481 mmol) and the solution stirred at room temperature for 18 h. Then solvents were evaporated under vacuum, and the residue purified by preparative TLC eluting with 100:10:1; dichloromethane:methanol:ammonium hydroxide to give the title compound (0.077 g, 43.2 %) as a white foam. LCMS: $(M+H)^+ = 465$; ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 - 7.85 (m, 2H), 7.52 (s, 1H), 7.17 - 7.33 (m, 3H), 7.12 (s, 1H), 6.97 - 7.08 (m, 2H), 6.87 (d, J = 10.58 Hz, 1H), 4.12 (dd, J = 2.08, 8.50 Hz, 1H), 3.84 (s, 3H), 3.22 (s, 3H), 3.07 (dd, J = 8.50, 16.43 Hz, 1H), 2.75 (dd, J = 2.27, 16.24 Hz, 1H), 1.82 (s, 3H).

Bis-ethoxycarbonylmalonamide (45). Ethyl carbamate (17.8 g, 0.2 moles) and malonic acid (10.4 g, 0.1 mole) were combined and stirred with heating to 65 °C under a stream of N_2. To the mixture was then added acetic anhydride (18.9 mL, 0.2 mol) in one portion and the reaction was heated at 75 °C under a stream of N_2 for 3 h. After cooling to room temperature, benzene was added (200 mL). The mixture was stirred, filtered and the solid washed with benzene and dried under vacuum to provide bis-ethoxycarbonylmalonamide, **45** (11.43 g, 43%). LCMS: $(M+H)^+ = 247$, $(M+Na)^+ = 269$.

Ethyl N-[2-(4-methyl-3-nitro-phenyl)-3,5-dioxo-1,2,4-triazine-6-carbonyl]carbamate (46). A mixture of 4-methyl-3-nitroaniline, **44** (6.2 g, 41 mmol) in water (200 mL) and concentrated HCl (27 mL) was heated at 70 °C under N_2 for 30 min. The mixture was then cooled to – 5 °C and $NaNO_2$ (3g, 43 mmol) in 20 mL of water was added by dropwise addition. The resultant mixture was stirred at this temperature for 20 min. before addition of sodium acetate trihydrate (42 g, 310 mmol) in 50 mL of water was added. The reaction was stirred for an additional 20 min. The resulting solution was added at -2 °C to a stirred (mechanical stirring) solution of **45**

(10 g, 41 mmol) and sodium acetate trihydrate (73 g, 540 mmol) in 1.5 L of water at -2 °C. The mixture was stirred at 0 °C for 90 min. The solid was collected by filtration and washed with water to afford intermediate ethyl N-[3-(ethoxycarbonylamino)-2-[(4-methyl-3-nitro-phenyl)hydrazono]-3-oxo-propanoyl]carbamate as a wet filtercake.

The wet filtercake from above was placed in ethanol (200 mL) and THF (200 mL) in a beaker and stirred at room temperature. To that was added 10% aqueous KOH (120 mL). The mixture was stirred for 40 minutes, then was poured into ice containing 25 mL conc. HCl. The mixture was extracted with EtOAc (2X400 mL). To the combined organic extracts was added 3 large scoops of Norit®. The mixture was filtered through celite®, then evaporated to approx. 100 mL volume. An equal volume of hexane was added with stirring. A precipitate formed, which was collected by filtration, washed with hexane and dried under vacuum to provide the title compound, **46** (8.03 g). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.23 (t, J=7.18 Hz, 3 H) 2.58 (s, 3 H) 4.16 (q, J=7.05 Hz, 2 H) 7.64 (d, J=8.31 Hz, 1 H) 7.75 - 7.88 (m, 1 H) 8.20 (d, J=2.27 Hz, 1 H) 11.47 (s, 1 H).

2-(4-Methyl-3-nitro-phenyl)-1,2,4-triazine-3,5-dione (47). To a stirred solution of **46** (8 g, 22 mmol) in ethanol (150 mL) and water (75 mL) was added 10% aqueous NaOH (75 mL). The mixture was stirred at room temperature for 3.5 h. An additional 150 mL of water was added and the mixture was poured onto ice and made acidic with conc. HCl. The resulting slurry was stirred and filtered. The filtercake was washed with water and dried in a vacuum oven at 35 °C for 36 h. to provide 6.7 g of crude acid intermediate. This solid was combined with mercaptoacetic acid (30 mL) in a flask and the flask was lowered into an oil bath, that was pre-heated to 180 °C under a N_2 stream. After 25 min. the flask was removed from the bath and allowed to cool. The mixture was poured into ice water (250 mL), stirred and filtered. The

filtercake was washed with water and dried in the vacuum oven at 45 °C overnight to provide the titled compound, **47** (5.84g, 58% yield from **44**). Used as is without further purification. LCMS $(M - H)^+ = 247$. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.57 (s, 3 H) 7.62 (d, J=8.69 Hz, 1 H) 7.70 (s, 1 H) 7.80 (dd, J=8.31, 2.27 Hz, 1 H) 8.18 (d, J=2.27 Hz, 1 H) 12.43 (br. s., 1 H).

2-(3-Amino-4-methylphenyl)-4-[(2R)-2-hydroxy-3-methoxy-propyl]-1,2,4-triazine-3,5-dione (48). 2-(4-Methyl-3-nitro-phenyl)-1,2,4-triazine-3,5-dione, **47** (3.0 g, 12 mmol), (R)-(-)-glycidyl methyl ether (3.3 mL, 36 mmol) and 4.5 g SiO_2 were combined in 15 mL DMF. The mixture was stirred at 80 °C for 60 h. The reaction was allowed to cool to room temperature, diluted with EtOAc (600 mL). The SiO_2 was filtered off and washed with EtOAc. Combined filtrate and wash was washed with sat. $NaHCO_3$ (aq.), dried over Na_2SO_4 and concentrated in vacuo. Chromatography (silica, 50% EtOAc/hexane eluent) provided intermediate, 4-[(2R)-2-hydroxy-3-methoxy-propyl]-2-(4-methyl-3-nitro-phenyl)-1,2,4-triazine-3,5-dione (2.6 g, 64%), which (2.6 g, 7.7 mmol) was combined with ammonium chloride (2.1 g, 39 mmol) in ethanol (30 mL) and water (30 mL). Electrolytic iron (2.2 g, 39 mmole) was added. The reaction was vigorously stirred at 85 °C for 45 minutes until LCMS showed that reaction was complete. The reaction mixture was cooled to room temperature, filtered, washed with ethanol and the combined filtrates and washes were concentrated in vacuo to afford the crude product. The crude product was dissolved in water and EtOAc. The layers were separated. The aqueous phase was extracted with EtOAc (1x). The combined organics were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was taken up in EtOAc and solid filtered off to afford title compound, **48** (504 mg). Filtrate was purified by chromatography (SiO_2, 100% EtOAc eluant) to provide an additional 1.47g of **48.** Total yield, 1.97g, 84%. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.20 (s, 3 H) 3.41 (s, 3 H) 3.44 - 3.56 (m, 2 H) 4.02 - 4.09 (m, 1 H) 4.13

(dq, J=8.22, 4.12 Hz, 1 H) 4.33 (dd, J=13.18, 8.41 Hz, 1 H) 6.82 (d, J=5.77 Hz, 2 H) 7.13 (d, J=8.53 Hz, 1 H) 7.56 (s, 1 H).

(4S)-4-(4-Fluorophenyl)-N-[5-[4-[(2R)-2-hydroxy-3-methoxy-propyl]-3,5-dioxo-1,2,4-triazin-2-yl]-2-methyl-phenyl]-1-methyl-2-oxo-3,4-dihydropyridine-5-carboxamide (19). To (S)-4-(4-Fluorophenyl)-1-methyl-6-oxo-1,4,5,6-tetrahydro-pyridine-3-carboxylic acid, **38** (1.7 g, 6.6 mmol, Eq: 1.0) in DMF (20 mL) was added DIEA (2.23 ml, 13.3 mmol, Eq: 2.0), followed by HATU (2.5 g, 6.6 mmol, Eq: 1.0). The solution stirred for 30 min. The solution was cooled to 0 °C. 2-(3-Amino-4-methyl-phenyl)-4-((R)-2-hydroxy-3-methoxy-propyl)-2H-[1,2,4]triazine-3,5-dione (2.0 g, 6.6 mmol, Eq: 1.0) was dissolved in DCM (20 ml). The aniline solution was slowly added to the cooled acid solution. The reaction mixture was warmed to room temperature, and then heated to 80 °C for 18 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The crude material was purified by flash chromatography (silica gel, 100% EtOAc) to give (S)-4-(4-Fluoro-phenyl)-1-methyl-6-oxo-1,4,5,6-tetrahydro-pyridine-3-carboxylic acid {5-[4-((R)-2-hydroxy-3-methoxy-propyl)-3,5-dioxo-4,5-dihydro-3H-[1,2,4]triazin-2-yl]-2-methyl-phenyl}-amide (3.2 g) of an impure material. The compound was further purified using SFC chromatography to give 1.4 g of clean material (39 %) as a white solid. LCMS: (M+H)+ = 538; 1H NMR (400 MHz, CDCl3) δ 8.21 (d, J = 1.52 Hz, 1H), 7.57 (d, J = 10.61 Hz, 2H), 7.24 – 7.28 (m, 2H), 7.13 - 7.18 (m, 2H), 7.02 - 7.12 (m, 2H), 6.82 (s, 1H), 4.31 (dd, J = 8.59, 13.14 Hz, 1H), 4.01 - 4.08 (m, 2H), 3.43 - 3.58 (m, 2H), 3.40 (s, 3H), 3.25 (s, 3H), 3.12 (dd, J = 8.59, 16.17 Hz, 1H), 2.76 (dd, J = 2.53, 16.17 Hz, 1H), 2.60 (d, J = 7.07 Hz, 1H), 1.75 (s, 3H).

The preparation of compound 20 represents a typical procedure used for the synthesis of (6S)-6-(4-fluorophenyl)-3-methyl-2-oxo-N-phenyl-1,6-dihydropyrimidine-5-carboxamides.

Step 1 Ethyl 6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylate. Ethyl 6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylate was prepared using the Biginelli reaction. To a solution of p-fluorobenzaldehyde (15 mL) in 400 mL dry acetonitrile was added ethyl 3-ethoxyacrylate (21.4 mL), methyl urea (10.0 g) and freshly dried SiO_2-$NaHSO_4$ (7.4 g). The solution was heated at reflux for 12 hours, then cooled and poured into ice water. The precipitate was recovered by filtration and air-dried to yield ethyl 6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylate ester (38.9 g, 83%).

Step 2. (6S)-6-(4-Fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylic acid. Ethyl 6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylate ester (10.0 g) was suspended in 200 mL MeOH and 10% aq. NaOH (75 mL) was added. The mixture was heated at reflux for 1 hour, then cooled and filtered. The filtrate was diluted with water (300 mL) and acidified with cone. HCl. The resulting precipitate was recovered by filtration and air dried to yield 6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylic acid (6.5 g). The racemic 6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylic acid was chromatographed on an R.R. Whelk-0 (Regis Technologies) chiral 30 mm supercritical fluid chromatography column, 20% MeOH in liquid CO_2, at 70 mL/minute. The (S) enantiomer (first fraction, $alpha_{[D]}$ = +142.1) was collected to provide (6S)-6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylic acid. LCMS $(M + H)^+$ = 251; LCMS $(M - H)^+$ = 249; ^1H NMR (400 MHz, DMSO-d_6) δ ppm 3.10 (s, 3 H) 5.14 (d, J=2.76 Hz, 1 H) 7.09 - 7.23 (m, 2 H) 7.31 (dd, J=8.53, 5.52 Hz, 2 H) 7.50 (s, 1 H) 7.79 (d, J=2.76 Hz, 1 H) 12.01 (br. s., 1 H).

Step 3. 1-(3-Amino-4-methyl-phenyl)pyridin-2-one. In a 100 mL round-bottomed flask, 4-iodo-1-methyl-2-nitrobenzene (1.2g, 4.6 mmol), 2-hyroxypyridine (0.65g, 6.8 mmol), potassium carbonate (0.94 g, 6.8 mmol) and 8-hydroxyquinoline (66 mg, 0.45 mmol) were combined in

DMF (18 mL). Reaction was degassed by bubbling argon through the reaction, then copper(I) chloride (23 mg, 0.23 mmol) was added. The reaction mixture was heated at 120 ^{o}C with stirring for 20 h. Allowed to cool to room temperature. The reaction mixture was filtered through a hydromatrix cartridge® and washed with several volumes of DCM until no more product eluted. The combined DCM washes were poured onto H_2O and extracted with DCM (3X). The organic layers were combined, dried over Na_2SO_4 and concentrated in vacuo. Purification by chromatography (silica, 30% - 70% EtOAc in hexanes gradient) afforded intermediate, 1-(4-methyl-3-nitro-phenyl)pyridin-2-one (604 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 2.68 (s, 3 H) 6.31 (td, J=6.80, 1.13 Hz, 1 H) 6.68 (d, J=9.44 Hz, 1 H) 7.34 (dd, J=6.99, 1.70 Hz, 1 H) 7.44 (ddd, J=9.25, 6.80, 2.08 Hz, 1 H) 7.49 (d, J=8.31 Hz, 1 H) 7.63 (dd, J=7.93, 2.27 Hz, 1 H) 8.06 (d, J=2.27 Hz, 1 H).

In a 100 mL round-bottomed flask fitted with a reflux condenser was placed 580 mg (2.5 mmol) of the 1-(4-methyl-3-nitro-phenyl)pyridin-2-one and 700 mg NH_4Cl (13 mmol) in 15 mL water and 15 mL ethanol. The resulting slurry was warmed to 50 ^{o}C. Electrolytic iron powder (700 mg, 12.5 mmol) was added with vigorous stirring. The reaction mixture was heated at reflux for 2.5 hour until complete, then allowed to cool to room temperature. Celite® was added to the reaction mixture and the mixture was stirred vigorously to get a uniform suspension. The resulting dark suspension was filtered through Celite® and the filtercake washed several times with ethanol. The combined filtrate and washes were concentrated to 1-2 mL and EtOAc and water were added. The mixture was extracted with EtOAc (4X) and DCM (2X). The organic layers were combined, dried over Na_2SO_4 and concentrated in vacuo. Purification by chromatography (silica, 1% - 1.5% MeOH/DCM gradient) afforded 1-(3-amino-4-methyl-phenyl)pyridin-2-one (398 mg, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 2.19 (s, 3 H) 3.74 (br.

s., 2 H) 6.20 (td, J=6.69, 1.26 Hz, 1 H) 6.65 (td, J=5.18, 3.28 Hz, 2 H) 6.69 (d, J=2.02 Hz, 1 H) 7.14 (d, J=7.58 Hz, 1 H) 7.31 (dd, J=7.07, 2.02 Hz, 1 H) 7.37 (ddd, J=9.22, 6.69, 2.27 Hz, 1 H).

Step 4. (6S)-6-(4-Fluorophenyl)-3-methyl-N-[2-methyl-5-(2-oxo-1-pyridyl)phenyl]-2-oxo-1,6-dihydropyrimidine-5-carboxamide (20). To a solution of (6S)-6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carboxylic acid (prepared in step 2, 425 mg, 1.7 mmol) in dry DCM (10 mL) and DMF (4 drops) at 0 °C under argon was added oxalyl chloride (0.161 mL, 1.9 mmol). The ice bath was removed and the reaction was stirred at room temperature for 1.5 h before concentration in vacuo. The crude residue was re-dissolved in DCM and concentrated in vacuo. The resulting residue of crude (6S)-6-(4-fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carbonyl chloride (1.7 mmol) was dissolved in 6.8 mL of DCE to provide a 0.25 M solution of the acid chloride.

1-(3-Amino-4-methyl-phenyl)pyridin-2-one (prepared in step 3, 82 mg, 0.41 mmole) and pyridine (41 μl, 0.5 mmol) in 1.5 mL of dichloroethane was placed in a sealed tube, put under argon and treated with a 0.25M solution of (6S)-6-(4-Fluorophenyl)-3-methyl-2-oxo-1,6-dihydropyrimidine-5-carbonyl chloride in dichloroethane (1.8 mL, 0.44 mmol). The tube was sealed and warmed at 60 ^{o}C for 18 h. Chromatography (1% - 5% methanol in CH_2Cl_2) followed by trituration with EtOAc and washing with ether afforded 45 mg (6S)-6-(4-fluorophenyl)-3-methyl-N-[2-methyl-5-(2-oxo-1-pyridyl)phenyl]-2-oxo-1,6-dihydropyrimidine-5-carboxamide, , **20**. Mp 160-165 ^{o}C; LCMS: $(M+H)^+ = 433$, $(M+Na)^+ = 455$; ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 2.05 (s, 3 H) 3.09 (s, 3 H) 5.40 (d, J=3.02 Hz, 1 H) 6.27 (td, J=6.70, 1.32 Hz, 1 H) 6.44 (d, J=9.06 Hz, 1 H) 7.07 (dd, J=8.12, 2.45 Hz, 1 H) 7.11 - 7.22 (m, 2 H) 7.24 - 7.38 (m, 4 H) 7.47 (ddd, J=9.16, 6.70, 1.89 Hz, 1 H) 7.52 - 7.58 (m, 2 H) 7.80 (d, J=3.02 Hz, 1 H) 9.14 (s, 1 H).

ASSOCIATED CONTENT

Supporting Information

Additional experimental procedures for all compounds, and P2X7 assay information. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTOR INFORMATION

Corresponding Author

*E-mail: ftapia@cc.hawaii.edu

Notes

The authors declare no competing financial interest.

ABREVIATIONS USED

HTS, high throughput screening; GSH, glutathione; UV, ultraviolet; Vdss, volume of distribution at steady state; CL, clearance; CL_{int}, intrinsic clearance; HWB, human whole blood; IV, intravenous; LYSA, lyophilization solubility assay; NHP, non-human primate; BQL, below limit of quantitation; FLIPR, fluorescent imaging plate reader; CVB, covalent binding; HLM, human liver microsomes; RLM, rat liver microsomes; HH, human hepatocytes; CYP, cytochrome P450; DMPK, drug metabolism and pharmacokinetics; PK, pharmacokinetics; SDPK, single dose pharmacokintetics; F, bioavailability; AUC, area under the curve; po, oral dose; Cmax, maximum concentration; PK/PD, pharmacokinetics/pharmacodynamics; PSA, polar surface area; DEREK, deductive estimation of risk from existing knowledge; MCASE, multi computer automated structure evaluation; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak; Ac, acetyl; Atm, atmospheres; Boc, *tert*-butoxycarbonyl; Bn, benzyl; Bu, butyl; CBZ, benzyloxycarbonyl; dba, dibenzylidineacetone; THF, tetrahydrofuran; MeTHF or 2-MeTHF, 2-methyltetrahydrofuran; MTBE, methyl *tert*-butyl ether DCM, dichloromethane; DIBAL or DIBAL-H, di-*iso*-butylaluminumhydride; DIPEA, di-*iso*-propylethylamine; DMAP,

4-N,N-dimethylaminopyridine; DMF, N,N-dimethylformamide; DMSO, dimethyl sulfoxide; dppf, 1,1'bis-diphenylphosphino)ferrocene; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; LDA, lithium diisopropylamide EDTA ethylenediaminetetraacetic acid; NCS, N-chlorosuccinimide; Et, ethyl; EtOAc, ethyl acetate; EtOH ethanol; Et_2O, diethyl ethe; HATU, O-(7-azabenzotriazole-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate acetic acid; HOBT, 1-N-hydroxybenzotriazole; HPLC, high pressure liquid chromatography; LCMS, liquid chromatography-mass spectrometry; MeOH, methanol; mp or MP, melting point; Me, methyl; ms or MS, mass spectrum; NMM, N-methylmorpholine; NMP N-methylpyrrolidone; Ph, phenyl; Pr, propyl; *i*-Pr, iso-propyl; pyr, pyridine; rt or RT, room temperature; TEA or Et_3N, triethylamine; TFA, trifluoroacetic acid; TLC, thin layer chromatography.. Conventional nomenclature including the prefixes *normal* (*n*), *iso* (*i*-), *secondary* (*sec*-) *tertiary* (*tert*-), and *neo*- have their customary meaning when used with an alkyl moiety.

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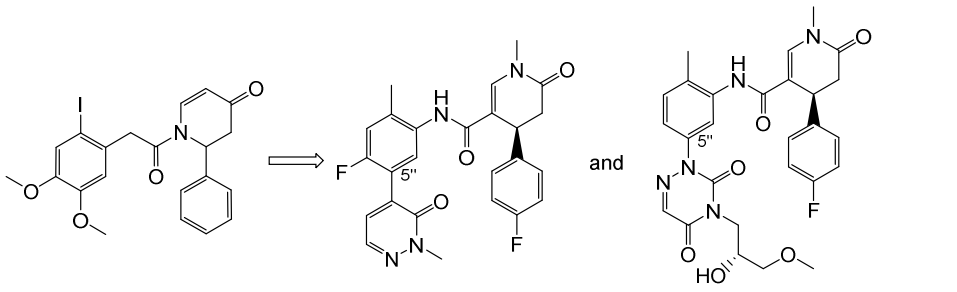
Table of Contents Graphic



1
Singleton from HTS
FLIPR IC_{50} 104 nM
Metabolically labile

17
HWB IC_{50} 13 nM
Aniline negative in Ames assay
Rat F 64%, IV CL 17 mL/min/kg

19
HWB IC_{50} 17 nM
Aniline negative in Ames assay
Rat F 20%, IV CL 23 mL/min/kg