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Discovery of BMS-986235/LAR-1219: A Potent Formyl Peptide Receptor 2 (FPR2) Selective Agonist for the Prevention of Heart Failure

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Fuji[†], Kazunori Fukuchi[†], Ricardo Garcia[‡], Mei-Yin Hsu[‡], Junichi Ishiyama[†], Bruce Ito[#],
Ellen Kick[‡], John Lupisella[‡], Shingo Matsushima[†], Kohei Ohata[†], Jacek Ostrowski[‡],
Yoshifumi Saito[†], Kosuke Tsuda[†], Francisco Villarrea[#], Hitomi Yamada[†], Toshikazu
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4 KEYWORDS: Formyl Peptide Receptor, Resolution of Inflammation, Heart Failure,
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7 Conformational Restriction, GPCR Agonist
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12 ABSTRACT: Formyl Peptide Receptor 2 (FPR2) agonists can stimulate resolution of
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15 inflammation and may have utility for treatment of diseases caused by chronic
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18 inflammation, including heart failure. We report the discovery of a potent and selective
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21 FPR2 agonist, and its evaluation in a mouse heart failure model. A simple linear urea with
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24 moderate agonist activity served as the starting point for optimization. Introduction of a
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27 pyrrolidinone core accessed a rigid conformation that produced potent FPR2 and FPR1
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30 agonists. Optimization of lactam substituents led to the discovery of the FPR2 selective
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33 agonist **13c**, BMS-986235/LAR-1219. In cellular assays **13c** inhibited neutrophil
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36 chemotaxis and stimulated macrophage phagocytosis, key endpoints to promote
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39 resolution of inflammation. Cardiac structure and functional improvements were
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42 observed in a mouse heart failure model following treatment with BMS-986235/LAR-
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55 Introduction

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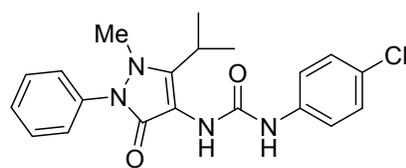
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4 Formyl peptide receptors (FPRs) constitute a G-protein coupled chemotactic receptor
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6 (GPCR) family, which are mainly expressed in neutrophils and monocytes and play an
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8 important role in host defense and inflammation. In humans, three subtypes (FPR1, 2,
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11 3) have been identified.¹⁻³ FPR1 initiates inflammatory responses such as neutrophil
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14 chemotaxis, degranulation, respiratory burst, and cytokine release when activated with
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17 ligands such as *N*-formyl-methionyl-leucyl-phenylalanine (fMLF) produced by bacteria.⁴
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21 FPR2 is involved in both initiation and resolution of inflammation. FPR2 peptide ligands,
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24 such as serum amyloid A (SAA), are associated with production of pro-inflammatory
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27 cytokine interleukin (IL)-8,⁵ while ligands such as Lipoxin A4 and Annexin A1 have been
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31 shown to inhibit migration of neutrophils, differentiate macrophages into pro-wound
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34 healing phenotypes and upregulate anti-inflammatory cytokines such as IL-10.^{6,7} Thus,
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37 FPR2 activates both inflammatory and pro-resolution responses depending on the ligand
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41 and its binding regions.^{8,9}

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48 Since the first small-molecule FPR agonist was reported in 2004,¹⁰ research groups in
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51 academia and industry have reported a diverse set of FPR agonists and antagonists.¹¹⁻¹⁴
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55 Representative FPR agonists include pyrazolone ureas (Compound 43),^{15,16}
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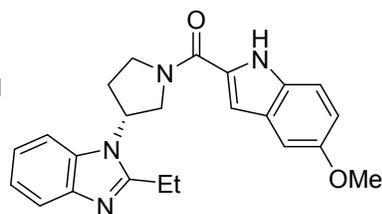
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3 benzimidazoles,¹⁷ bridged spiro[2.4]heptanes¹⁸ and aminotriazoles (ACT-389949)¹⁹
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7 (Figure 1). Among these compounds, dual FPR2/FPR1 Compound 43 has been used for
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10 proof of concept in vivo studies, including the preservation of cardiac function and
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13 prevention of adverse remodeling in rodent heart failure models.^{8,16,20} FPR2 agonist ACT-
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17 389949 was dosed in Phase 1 clinical trials and induced plasma biomarker changes (e.g.
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20 IL-10 and leukocyte levels) in humans after one dose; however, the authors reported
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23 tachyphylaxis in the multiday dosing study.²¹ Although there are no compounds launched
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27 as therapeutic agents to date, FPR2 remains a promising target to resolve chronic
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30 inflammation and promote the wound healing processes.
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35 Based on reported FPR biology, we focused on the discovery of FPR2 agonists with
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38 pro-resolution activity and selectivity over FPR1. We selected simple urea derivative AG-
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42 26 (1)²² as a promising starting point with multiple options for future structural
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45 transformation.
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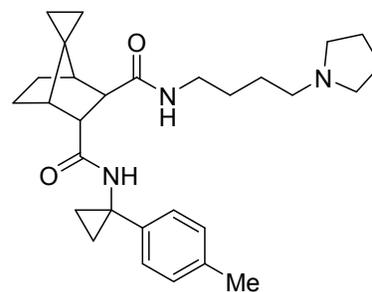
53 **Figure 1.** Structures of selected reported FPR2 agonists
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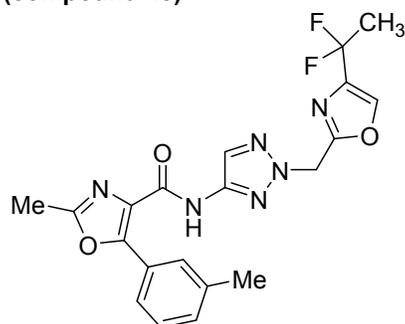
Amgen (2006)
Pyrazolone Urea
(compound 43)



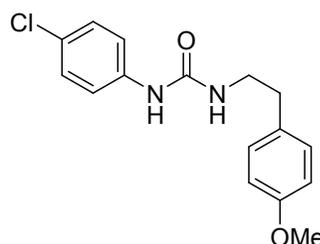
Amgen (2007)
Benzimidazole



Actelion (2013)
Bridged spiro[2,4]heptane



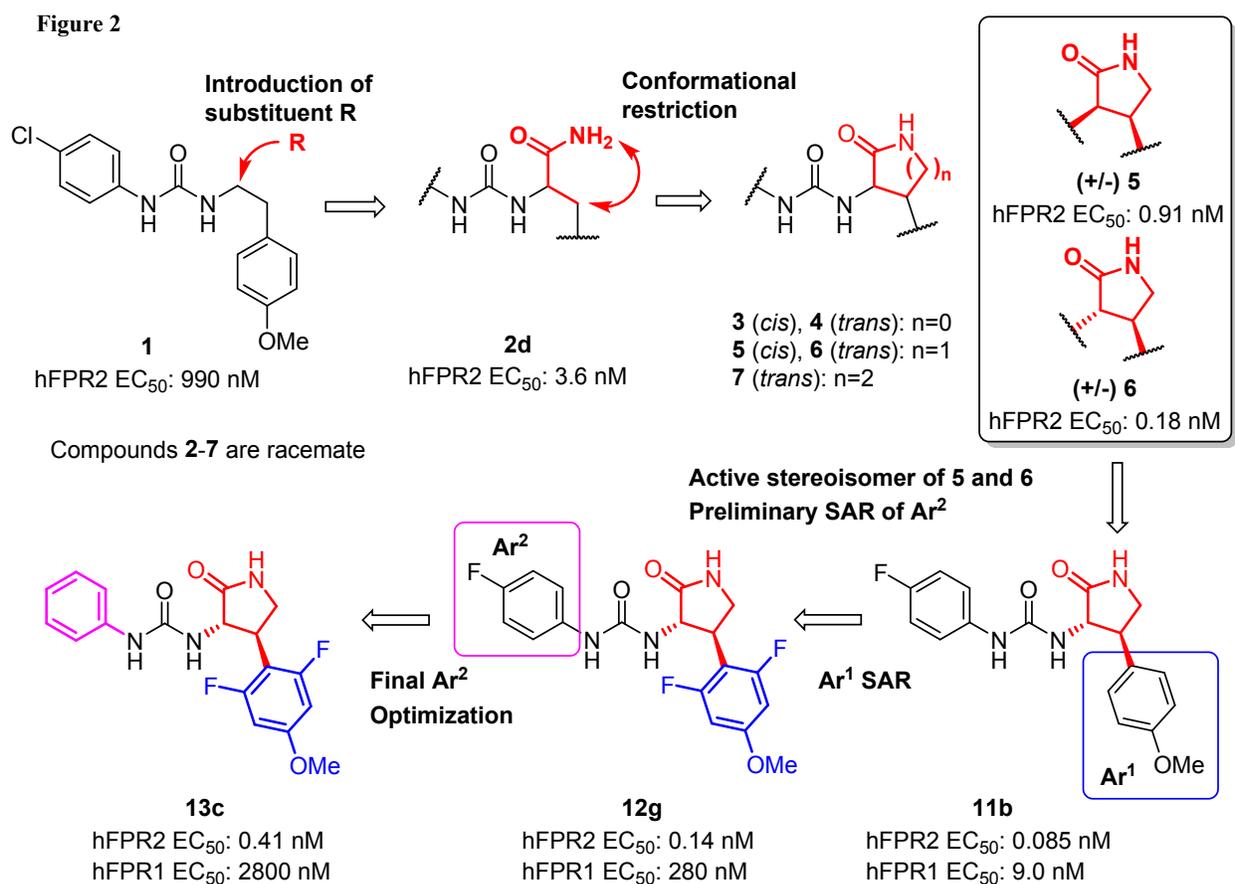
Actelion (2010)
Aminotriazole
(ACT-389949)



AG-26 (1)

Investigation of substituents on the 2-(4-methoxyphenyl)ethyl moiety of compound **1** resulted in carbamoyl derivative **2d** with enhanced FPR2 activity (Figure 2). In order to investigate conformationally constrained analogs of **2d**, four to six membered lactam derivatives **3–7** were prepared. Among these, 5-membered pyrrolidinone derivatives **5** and **6** showed significantly improved activity. Of four optical isomers of **5** and **6**, the (3*S*,4*R*)-form was confirmed to be the most potent FPR2 agonist. Preliminary investigation of the Ar² group led to 4-fluorophenyl (3*S*,4*R*) analog **11b**, which exhibited better activity than the 4-chlorophenyl (3*S*,4*R*) analog. Optimization of the Ar¹ group, led

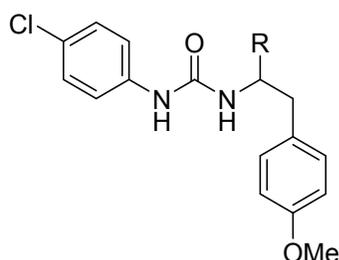
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3 to **12g** bearing the 2,6-difluoro-4-methoxyphenyl group which dramatically improved
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7 selectivity over FPR1 without decreasing FPR2 activity. Finally, reoptimization of the Ar²
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10 group to further improve FPR2 selectivity resulted in the selection of clinical candidate
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14 **13c**. Details of this research are described below.
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Figure 2. Structure Activity Relationship (SAR) Summary of Pyrrolidinone Urea FPR2**Agonist****Results and Discussions**

All synthesized compounds were evaluated for agonistic activity to induce Ca²⁺ mobilization in HEK293 cells over-expressing hFPR2 and HEK293 cells over-expressing hFPR1.

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3 First, the hFPR2 agonistic activity of acyclic urea derivatives **1** and **2a–d** were evaluated
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7 to determine the effect of substitution on the 4-methoxyphenylethyl moiety (Table 1).
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10 Compound **1** showed weaker hFPR2 activity than that described in the literature, which
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14 may be due to assay conditions and expression levels of the receptor in the reporter cell
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17 lines. By contrast, compounds **2a–d** exhibited 32- to 275-fold more potent hFPR2 activity
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20 than **1**. Carbamoyl derivative **2d** exhibited the strongest hFPR2 activity ($EC_{50} = 3.6$ nM)
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24 and was selected as a template for structural modifications.
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28 **Table 1.** Human FPR2 Agonist Activity for Acyclic Analogs
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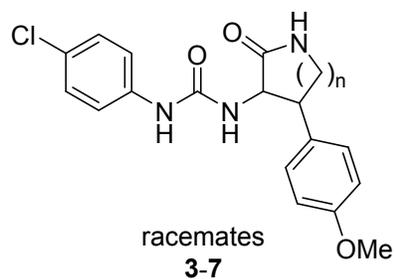
comp	R	hFPR2 EC_{50}^a (nM)
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2a	Me	23
2b	CH ₂ OH	6.8
2c	COOH	31
2d	CONH ₂	3.6

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8 $^{a}EC_{50}$ values were determined by Ca^{2+} mobilization in HEK293 cells over-expressing
9 hFPR2.
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14 We designed 4–6 membered lactam derivatives **3–7** to constrain the conformation of
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17 **2d** without changing the steric bulk of the carbamoyl moiety and the lipophilicity of the
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20 molecule. In addition to the hFPR2 activity of compounds **3–7**, the agonist activity for
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23 hFPR1 was measured to evaluate receptor selectivity (Table 2). The template compound
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26 **2d** had excellent hFPR2 selectivity (hFPR1/2 ratio >2800). The hFPR2 activity of *cis*- and
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29 *trans*-oriented azetidinone urea derivatives **3** and **4** were 3- to >28-fold less potent than
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31
32 *trans*-oriented pyrrolidinone urea derivatives **3** and **4** were 3- to >28-fold less potent than
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35 **2d**. By contrast, *cis*- and *trans*-oriented piperidinone urea derivatives and *trans*-oriented
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38 piperidinone derivative **5–7** were 4- to 33-fold more potent for hFPR2 over **2d**. However,
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41 hFPR1 activity of **5–7** also increased, and hFPR1/2 selectivity of these compounds was
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44 lower than that of **2d**.
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51 **Table 2.** Activity of **3–7** in HEK293 Cells Expressing Human FPR2 and FPR1
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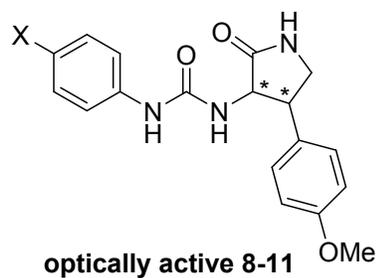
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comp		EC ₅₀ (nM) ^a		ratio
		hFPR2	hFPR1	
2d		3.6	>10000	>2800
3		12	>10000	>830
4		>100	>10000	NC ^c
5		0.91	100	110
6		0.18	31	170
7		0.11	<10	<91

48 ^aEC₅₀ values were determined by Ca²⁺ mobilization in HEK293 cells over-expressing
49 hFPR2 or hFPR1. ^cNot calculated.
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4 Based on the results described above, we selected pyrrolidinone derivatives **5** and **6**
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7 for further optimization since they offered the best combination of potency and selectivity.
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10 The four optically active isomers **8–10** and **11a** were assayed to ascertain the most active
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13 stereoisomer (Table 3). In the *cis*-oriented isomers, (+)-enantiomer **9** had 5-fold more
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16 potent hFPR2 activity than (-)- **8**. In the *trans*-oriented isomers, (3*S*,4*R*)-enantiomer **11a**
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19 was 360-fold more potent for hFPR2 than the (3*R*,4*S*)-enantiomer **10**. Comparing the
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22 hFPR2 activity of diastereomers **9** and **11a**, **11a** was 10-fold more active than **9**,
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25 demonstrating that the (3*S*,4*R*)-isomer was the most active stereoisomer. However, **11a**
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28 was only moderately selective over FPR1 (42x). Compound **11b** bearing a 4-fluorophenyl
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31 group had similar hFPR2 activity and selectivity to **11a**, so we selected **11b** as the lead
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35 compound for detailed structure activity relationship (SAR) studies to identify a candidate
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42 compound.

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49 **Table 3.** Activity of **8–11** in HEK293 Cells Expressing Human FPR2 and FPR1
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comp		X	EC ₅₀ (nM) ^a		ratio
			hFPR2	hFPR1	
8		Cl	4.6	950	210
	(-)- <i>cis</i>				
9		Cl	0.90	81	90
	(+)- <i>cis</i>				
10		Cl	34	910	27
11a		Cl	0.092	3.9	42
11b		F	0.085	9.0	110

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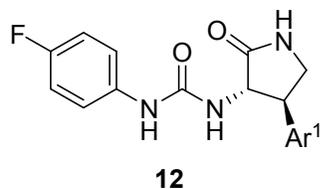
^aEC₅₀ values were determined by Ca²⁺ mobilization in HEK293 cells over-expressing hFPR2 or hFPR1.

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After the core pyrrolidinone was selected, optimization of the 4-methoxyphenyl group was carried out (Table 4). Introduction of a fluorine atom to the 2- or 3-position of the 4-

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3 methoxyphenyl group (**12a**, **12b**) resulted in 5- to 10-fold decreased hFPR2 activity
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7 relative to **11b** and did not improve hFPR2 selectivity. Furthermore, 2-chloro (**12c**), 2-
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10 methyl (**12d**) or 4-chloro (**12e**) analogs also had nanomolar hFPR2 activity. By contrast,
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14 compounds **12f** and **12g** with an additional fluorine atom at position 5 or 6 of **12a**
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17 maintained picomolar hFPR2 activity. Remarkably, a dramatic decrease in FPR1 activity
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20 was observed in the compound **12g** bearing 2,6-difluoro-4-methoxyphenyl group as Ar¹.
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24 Compound **12g** showed excellent hFPR2 activity (EC₅₀ = 0.14 nM) and high hFPR2
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27 selectivity (FPR1/2 ratio = 2000). Whereas the 3-fluoro-5-methoxypyridin-2-yl group
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31 (**12h**) maintained FPR2 activity, the selectivity over hFPR1 was reduced relative to **12g**.
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35 Furthermore, replacement of the 4-methoxy group of **12g** with an ethoxy (**12i**), an ethyl
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38 (**12j**), a cyano (**12k**) or a methylamino (**12l**) group resulted in a 10- to 700-fold decreased
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42 hFPR2 activity over **12g**.
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49 **Table 4.** Activity of **12** in HEK293 Cells Expressing Human FPR2 and FPR1
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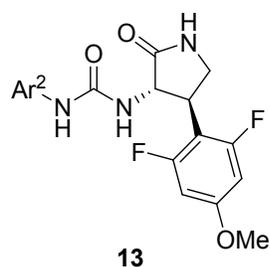
comp	Ar ¹	EC ₅₀ (nM) ^a		ratio	comp	Ar ¹	EC ₅₀ (nM) ^a		ratio
		FPR2	FPR1				FPR2	FPR1	
11b		0.085	9.0	110	12f		0.15	17	110
12a		0.45	86	190	12g		0.14	280	2000
12b		0.90	72	80	12h		0.36	190	530
12c		4.0	410	100	12i		14	NT ^b	NC ^c
12d		1.6	76	48	12j		5.8	1500	260
12e		3.1	29	9.4	12k		9.7	40000	4100
					12l		1.4	1900	1400

^aEC₅₀ values were determined by Ca²⁺ mobilization in HEK293 cells over-expressing hFPR2 or hFPR1. ^bNot tested. ^cNot calculated.

We next evaluated the substituent effect of Ar² for a series of 4-(2,6-difluoro-4-methoxyphenyl)pyrrolidinone derivatives **13a–q** (Table 5). The 3-fluorophenyl and 2-

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3 fluorophenyl derivative **13a** and **13b** showed 7- and 310-fold less hFPR2 activity than
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7 **12g**. On the other hand, the phenyl, 4-chlorophenyl, and 4-bromophenyl derivatives **13c-e**
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10 showed comparable hFPR2 activity to **12g**. Among these compounds, phenyl derivative
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12
13 **13c** showed higher hFPR2 selectivity ($FPR_{1/2} = 6800$) than **12g**. Other 4-position
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15
16 substituents, including cyano, methyl and methoxy derivatives **13f-h** were 6- to 23-fold
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18
19 less active than **12g**. The 3,4-disubstituted phenyl derivatives **13i-l** showed comparable
20
21
22 hFPR2 activity to **12g**. Among these compounds 3,4-difluorophenyl derivative **13i** and 3-
23
24
25 hydroxy-4-methylphenyl derivative **13l** showed higher hFPR2 selectivity ($hFPR_{1/2} = 5100$
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27
28 and 13000, respectively) than **12g**. Although, the aromatic heterocycle derivatives **13m-q**
29
30
31 showed 2- to 32-fold reduced activity compared to **12g**, 5-chlorothiophen-2-yl derivative
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34 **13n** showed good hFPR2 activity ($EC_{50} = 0.29$ nM), and higher hFPR2 selectivity ($FPR_{1/2}$
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37 = 4100) than **12g**.
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48 **Table 5.** Activity of **13a-q** in HEK293 Cells Expressing Human FPR2 and FPR1
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comp	Ar ²	EC ₅₀ (nM) ^a		ratio	comp	Ar ²	EC ₅₀ (nM) ^a		ratio
		FPR2	FPR1				FPR2	FPR1	
12g		0.14	280	2000	13i		0.16	820	5100
13a		0.95	1700	1800	13j		0.087	72	830
13b		43	NT ^b	NC ^c	13k		0.050	40	800
13c		0.41	2800	6800	13l		0.078	1000	13000
13d		0.11	120	1100	13m		4.5	6900	1500
13e		0.19	96	510	13n		0.29	1200	4100
13f		0.78	350	450	13o		1.9	NT ^b	NC ^c
13g		2.0	3200	1600	13p		0.32	290	910
13h		3.2	1000	310	13q		0.76	3000	3900

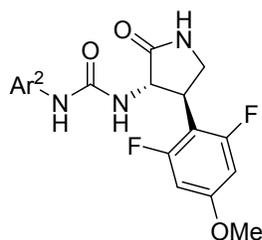
^aEC₅₀ values were determined by Ca²⁺ mobilization in HEK293 cells over-expressing hFPR2 or hFPR1. ^bNot tested. ^cNot calculated.

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4 Finally, to select a candidate compound, in vivo efficacy and CYP3A4 inhibition were
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7 evaluated for 5 analogs, **12g**, **13c**, **13i**, **13l**, and **13n**, which had potent in vitro hFPR2
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10 activity ($EC_{50} < 0.50$ nM) and high hFPR2 selectivity ($hFPR1/2 > 2000$). In vivo efficacy
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13 was evaluated in a mouse inflammation model by measuring inhibition of neutrophil
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16 infiltration into lung after oral administration at 1 mg/kg (Table 6). The 4-fluorophenyl
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19 derivative **12g**, phenyl derivative **13c** and 5-chlorothiophen-2-yl derivative **13n** showed
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22 excellent in vivo efficacy (inhibition $>80\%$), while the disubstituted phenyl derivatives **13i**
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25 and **13l** showed weaker in vivo efficacy.
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32 Among the three compounds that inhibited neutrophil infiltration in mice, selection for
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35 advanced in vivo studies relied on several factors. Analog **13n** was excluded as a
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37
38 candidate compound due CYP3A4 inhibition. Higher FPR2 selectivity in the Ca^{2+} flux
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41 assay was measured for **13c** versus **12g** (6800 vs 2000 fold, Table 5); in addition, only
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44 oxidative metabolites were observed after hepatocyte incubation with **13c**, while oxidative
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47 defluorination was observed with **12g** (data not provided). Based on these differences,
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52 **13c** was progressed to additional studies.
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Table 6. Effect of Selected Compounds on LPS-induced Neutrophil Infiltration in Mouse

Lungs

**12g, 13c,i,l,n**

compd	Ar ²	FPR2 EC ₅₀ (nM) ^a		In vivo inhibition (%) ^b	CYP inhibition IC ₅₀ (μM) ^c
		human	mouse		
12g		0.14	1.5	97	>20
13c		0.41	3.4	88	>20
13i		0.16	8.5	4.9	>20
13l		0.078	0.63	55	>20
13n		0.29	1.4	84	5.6

^aEC₅₀ values were determined by Ca²⁺ mobilization in HEK293 cells over-expressing hFPR2 or mFPR2. ^bInhibition (%) was determined by measurement of neutrophils in BALF of LPS-dosed mice after oral dosing of 1 mg / kg. ^cIC₅₀ values were determined with recombinant human CYP3A4.

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3 Additional in vitro assays examined more detailed cellular signaling of FPR2 agonist
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7 **13c** (Table 7). Inhibition of cAMP levels was observed in cells over-expressing either
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9
10 hFPR2 or hFPR1, consistent with G_i coupling, with an 80x selectivity for hFPR2. β -Arrestin
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12
13 recruitment was also observed. Potency was right shifted in both assays compared to calcium
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15 mobilization, which could be due to different assay conditions, including the level of receptor
16
17 expression in the cell lines. Confirming cross species selectivity, **13c** demonstrated 1000x
18
19 selectivity for FPR2 over FPR1 in the cAMP assay using CHO cells over-expressing the mouse
20
21 receptors. The impact on inflammatory cells was measured in two key cellular assays: Compound
22
23 **13c** inhibited chemotaxis of a neutrophil-like cell line against a gradient of SAA and stimulated
24
25 phagocytosis by mouse macrophages at low nanomolar potency. These assays show the potential
26
27 of FPR2 agonists to prevent pro-inflammatory neutrophil infiltration, and the ability to promote a
28
29 wound healing phenotype in macrophages, which are key characteristics of compounds that
30
31 stimulate resolution of inflammation.
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37 **Table 7.** Mechanistic in vitro assays **13c**
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Assay	EC ₅₀ or IC ₅₀ ^a (nM)
hFPR2 cAMP	5.0
hFPR1 cAMP	400
mFPR2 cAMP	0.5
mFPR1 cAMP	500
β -Arrestin Recruitment	130
Chemotaxis	57

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3 Phagocytosis <1
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10 ^aEC₅₀ values for cAMP were determined in Chinese Hamster Ovary cells (CHO) over-
11 expressing hFPR1, hFPR2, mFPR1 or mFPR2 receptors. β-Arrestin values were
12 measured in a DiscoverX Pathhunter® cell line. Phagocytosis assays used mouse
13 peritoneal macrophages. IC₅₀ value for the chemotaxis assays used a HL-60 cell line.
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21 In vivo pharmacokinetic (PK) data and in vitro intrinsic clearance values in liver
22 microsomes and hepatocytes for compound **13c** are listed in Table 8. Compound **13c**
23
24 had high clearance in rat and mouse, but moderate clearance in dog and monkey with
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26 good bioavailability. The clearance values in human microsomes and human
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28 hepatocytes were about 10-fold lower than those of rat, dog and monkey, suggesting that
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38 **13c** is more metabolically stable in humans than in these animal species.
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45 **Table 8.** Cross Species Comparison of In Vivo PK Parameters and In Vitro Intrinsic CL of
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48 **13c**
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Species	i.v.			p.o.					Liver microsome int.CL ^f	Hepatocyte int.CL ^g
	Dose ^a	V _{dss} ^b	CL _{tot} ^c	Dose ^a	C _{max} ^d	T _{1/2} (h)	AUC _{0-inf} ^e	BA (%)		
Mouse	0.5	3.5	90	1.0	160	0.68	120	24	NT ^h	NT ^h
Rat	1.0	3.0	49	1.0	240	3.8	640	67	0.021	15
Dog	1.0	0.98	6.6	1.0	820	2.5	5700	50	0.042	50
Monkey	1.0	1.2	12	3.0	800	5.0	5800	49	0.030	28
Human	NT ^h	NT ^h	NT ^h	NT ^h	NT ^h	NT ^h	NT ^h	NT ^h	0.0033	3.5

^aDose is expressed in mg/kg. ^bV_{dss} is expressed in L/kg. ^cCL_{tot} is expressed in mL/min/kg. ^dC_{max} is expressed in nmol/L. ^eAUC_{0-inf} is expressed in nmol/L · h. ^fCL is expressed in mL/min/mg protein. ^gCL is expressed in mL/min/kg. ^hNot tested

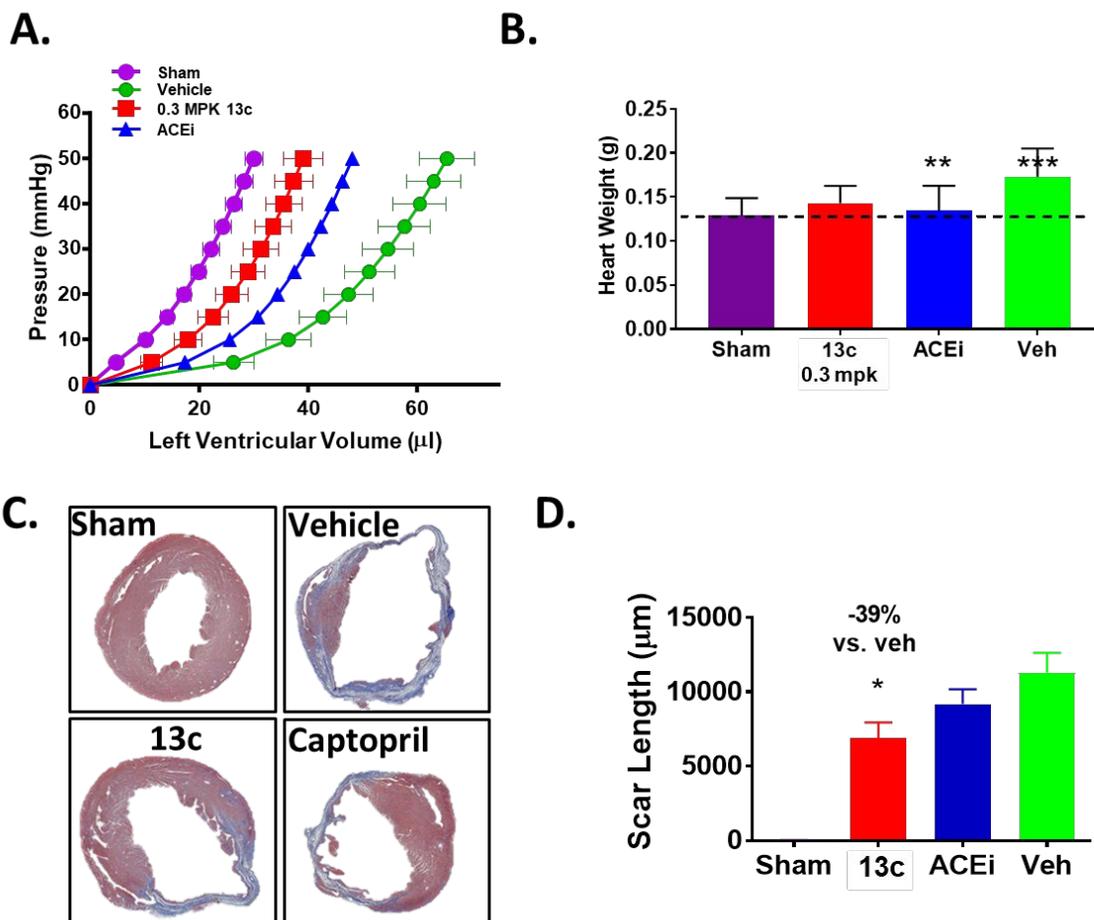
A major cause of heart failure is adverse cardiac remodeling post myocardial infarction (MI) during a period characterized by dysregulated chronic inflammation. FPR2 agonists have the potential to resolve this inflammation and improve cardiac structure and function. To investigate the role of FPR2 agonism in the setting of heart failure, the selective FPR2 agonist **13c** was evaluated in a mouse model of myocardial infarction (MI), which leads to heart failure development. In these studies, the left anterior descending (LAD) artery was permanently occluded with a surgical suture. Non-infarcted surgical sham mice had sutures placed around the LAD artery but not tightened. The lowest dose of **13c** that showed a significant effect on IL-10 stimulation (0.3 mg/kg) in mice treated with LPS was selected for testing in the myocardial infarction model. Vehicle and **13c** were dosed daily

1
2
3 by oral gavage for 24 days starting 4 days post MI. The angiotensin converting enzyme
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7 inhibitor (ACEi), captopril, was provided in the drinking water (~ 100 mg/kg/day) to
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10 compare a standard of care therapy for acute MI.
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14 After 28 days, infarct scar expansion and left ventricular chamber remodeling were
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17 evaluated. The passive mechanics of the left ventricle (LV) were analyzed using a
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20 modified Langendorff apparatus. A deflated balloon attached to a pressure transducer
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23 was inserted into the LV cavity. The passive compliance of the left ventricle was evaluated
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26 following three inflation and deflation cycles of the balloon. Figure 4A shows mean left
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29 ventricular pressure–volume (P–V) curves obtained from the various treatment groups as
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32 well as surgical sham mice. Treatment with vehicle yielded a right-shifted P-V curve
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35 relative to non-infarcted sham hearts shown at the left of the plot. The results indicate that
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42 infarcted mouse hearts treated with vehicle have dilated LV chambers at each measured
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45 pressure. By contrast, the P-V curve obtained with the FPR2 agonist **13c** is left shifted
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48 relative to vehicle, indicating smaller LV chambers with treatment. Treatment with
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52 captopril also yielded a left shifted P-V curve relative to vehicle, but to a lesser degree
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56 than that obtained with compound **13c**. The findings suggest that LV chamber remodeling
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3 is attenuated with both compound **13c** and captopril treatments post MI. Heart weights
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7 for compound **13c** and captopril treatment groups were reduced relative to the vehicle
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10 group (Figure 4B). These findings indicate that compound **13c** can attenuate left ventricle
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13 and global cardiac remodeling.
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22 **Figure 4:** Effect of **13c** and captopril (ACEi) treatment on myocardial structure and
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24 function post LAD in mice. A) Left ventricular pressure-volume curves. B) Heart weights
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28 28 days post MI. C) Representative heart cross-sections by histology depicting the degree
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31 of myocardial infarction. Non-infarcted surgical sham animals are shown for comparisons.
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36 D) Scar length 28 days post MI. Doses: **13c**, 0.3 mg/kg/d; captopril, 100 mg/kg/d.
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Cross sections of the left ventricle were analyzed for histomorphometric assessments of infarct structure. Representative images showing the size of the MI and LV chambers are shown for all groups (Figure 4C). Infarct length was quantified for all groups as an indicator of overall scar expansion. As shown in Figure 4D, vehicle-treated mice yielded the largest scar lengths. By contrast, treatment with compound **13c** reduced infarct length by 39% relative to vehicle ($P < 0.05$). Despite the decreases in heart weight and decreased

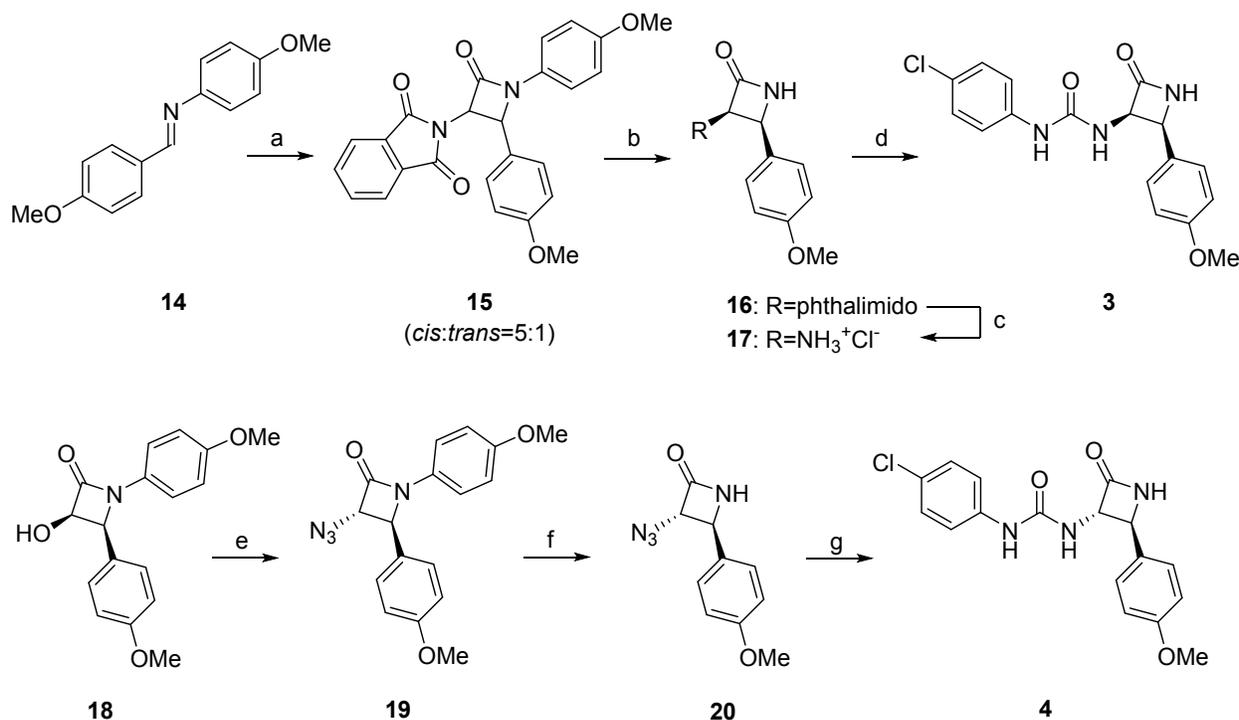
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4 LV chamber areas noted in Figure 4A and B, treatment with captopril was unable to
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7 reduce scar length to a degree similar to that obtained with compound **13c**.
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11 12 13 14 **Chemistry** 15

16
17 The synthesis of racemic *cis*- and *trans*-oriented azetidine urea derivatives **3** and **4** are
18
19 shown in Scheme 1. Reaction of imine **14** with 2-(1,3-dioxoisindolin-2-yl)acetyl chloride
20
21 in the presence of triethylamine, gave **15**²³ as the mixture of *cis* and *trans* isomers.
22
23
24 Removal of the 4-methoxyphenyl group of **15** with cerium (IV) ammonium nitrate (CAN)
25
26 provided *cis* isomer **16**. Treatment of **16** with hydrazine monohydrate, followed by
27
28 treatment with HCl, gave **17**. Reaction of **17** with 4-chlorophenyl isocyanate in the
29
30 presence of saturated aqueous sodium hydrogen carbonate provided *cis*-oriented **3**.
31
32
33 Azidation of a hydroxyl group of *cis*-isomer **18**,²⁴ followed by deprotection of the 4-
34
35 methoxyphenyl group of *trans*-**19** with CAN, gave **20**. A Staudinger reaction followed by
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37 treatment with 4-chlorophenyl isocyanate, provided *trans*-oriented **4**. The stereochemistry
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39 of the C-3 and C-4 positions of all compounds was determined by comparison of coupling
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constant of the C-3 and C-4 hydrogen atom in the ^1H NMR ($J_{3,4} > 4.0$ Hz for the *cis* isomer, $J_{3,4} < 3.0$ Hz for the *trans* isomer).²⁵

Scheme 1. Synthesis of Azetidinone Urea Derivatives **3** and **4**^a



Compounds **3**, **4**, **15-20** are racemic.

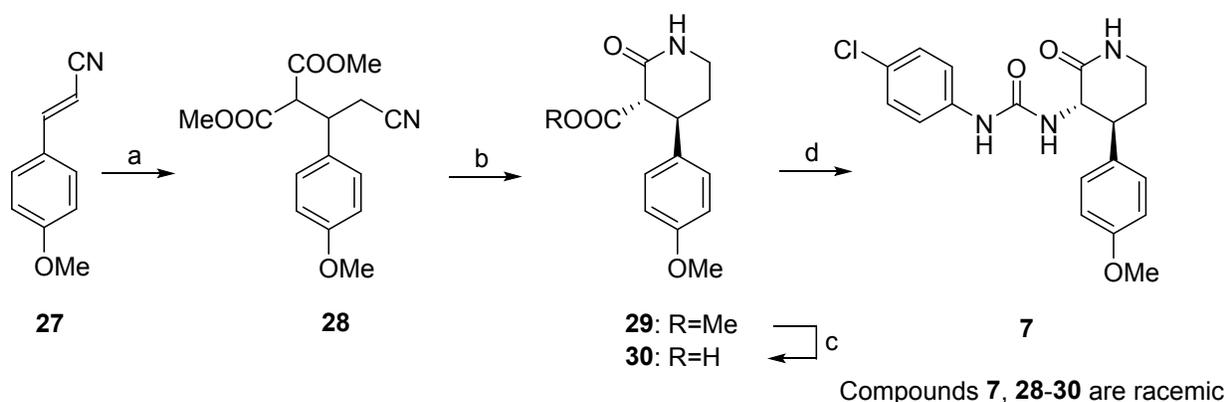
^aReagents and conditions: (a) 2-(1,3-dioxisoindolin-2-yl)acetyl chloride, Et₃N, CH₂Cl₂, 28%; (b) CAN, MeCN-THF-H₂O, 62%; (c) (1) hydrazine monohydrate, dioxane, (2) HCl, 53%; (d) 4-chlorophenyl isocyanate, sat NaHCO₃ aq., AcOEt, 63%; (e) (1) MsCl, Et₃N, (2) NaN₃, DMF, 89%; (f) CAN, MeCN, 85%; (g) (1) PPh₃, THF (2) H₂O (3) 4-chlorophenyl isocyanate, 48% (three steps) .

The synthesis of racemic *cis*- and *trans*-oriented pyrrolidinone urea derivatives **5** and **6** (Scheme 2) began with the reaction of **21**²⁶ and *tert*-butyl (2,2,2-trifluoroacetyl)glycinate

TiCl₃, NaOAc, MeOH-H₂O 63% (two steps); (e) 1 M aq. NaOH, MeOH; (f) 4-chlorophenyl isocyanate, EtOAc, 4% (two steps for **5**), 44% (2 steps for **6**).

Racemic *trans*-oriented piperidinone urea derivative **7** (Scheme 3) was synthesized by conjugate addition of dimethyl malonate to compound **27** in the presence of sodium methoxide,²⁸ followed by reduction of the cyano group in **28** using nickel boride to give piperidinone **29**. After hydrolysis of ester **29** under basic conditions, treatment of **30** with diphenylphosphoryl azide (DPPA) in the presence of triethylamine, followed by the reaction with 4-chloroaniline provided piperidinone **7**.

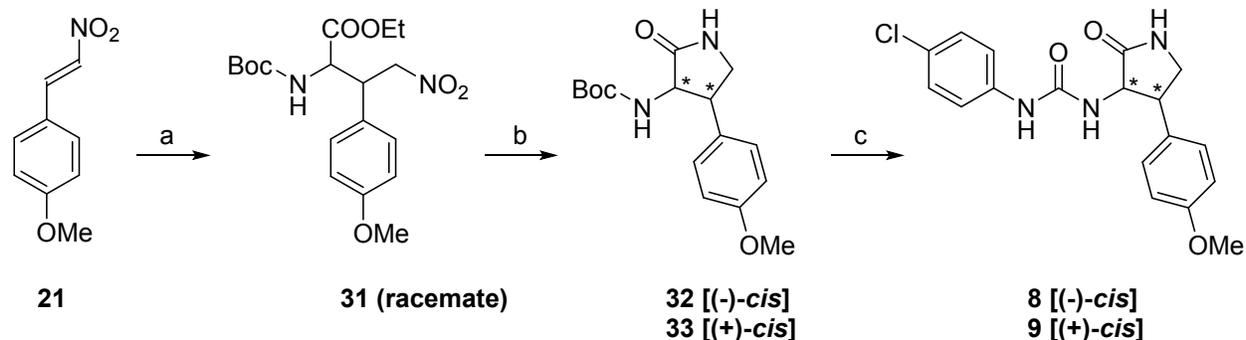
Scheme 3. Synthesis of Piperidinone Urea Derivative **7**^a



^aReagents and conditions: (a) Dimethyl malonate, NaOMe, MeOH, 24%; (b) NaBH₄, NiCl₂·6H₂O, MeOH, 65%; (c) 1 M NaOH, MeOH, 92%; (d) DPPA, Et₃N, toluene then 4-chloroaniline (6.5%).

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3 The synthesis of optically active *cis*-oriented pyrrolidinone urea derivatives **8** and **9** is
4 shown in Scheme 4. After the reaction of **21** with ethyl 2-
5
6 [(diphenylmethylene)amino]acetate in the presence of lithium diisopropylamide (LDA),
7
8 removal of the diphenylmethylene group under acidic condition, followed by treatment
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10 with di-*tert*-butyl dicarbonate (Boc₂O) in the presence of saturated aqueous sodium
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12 hydrogen carbonate gave **31**. Reduction of the nitro group with nickel boride, followed by
13
14 isolation of enantiomers using HPLC chromatography (CHIRALPAK ID), gave (-)-*cis*-
15
16 isomer **32** and (+)-*cis*-isomer **33**. Deprotection of the Boc group of **32** and **33** under acidic
17
18 conditions, followed by treatment with 4-chlorophenyl isocyanate in the presence of
19
20 saturated aqueous sodium hydrogen carbonate, gave (-)-*cis*-isomer **8** and (+)-*cis*-isomer
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Scheme 4. Synthesis of Optically Active *Cis*-oriented Pyrrolidinone Urea Derivatives **8** and **9**^a

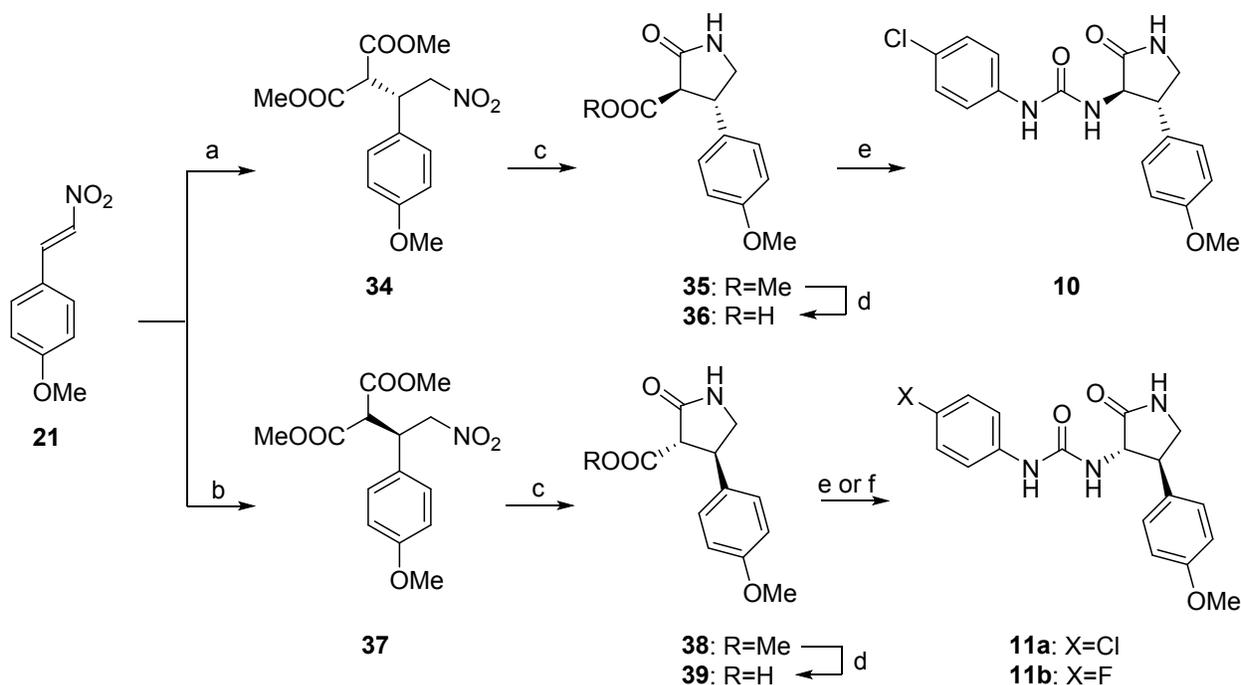


^aReagents and conditions: (a) (1) ethyl 2-[(diphenylmethylene)amino]acetate, LDA, THF, (2) AcOH, H₂O, (3) Boc₂O, NaHCO₃ aq., THF, 63% (three steps); (b) (1) NaBH₄, NiCl₂·6H₂O, MeOH, 84% (2) HPLC separation, CHIRALPAK ID, 35% (for **32**), 43% (for **33**); (c) (1) TFA, CH₂Cl₂, (2) 4-chlorophenyl isocyanate, sat NaHCO₃, THF, 56% (two steps for **8**), 56% (two steps for **9**).

The synthesis of *trans*-oriented (3*R*,4*S*)- and (3*S*,4*R*)-pyrrolidinone urea derivatives **10** and **11** is shown in Scheme 5. Enantioselective Michael addition²⁹ of dimethyl malonate to **21** using Ni(II)-*bis*[(*R,R*)-*N,N*-dibenzylcyclohexane-1,2-diamine]bromide^{29a} followed by reduction of the nitro group using nickel boride, gave (3*R*,4*S*)-isomer **35**. After hydrolysis of ester **35** under basic condition, reaction of acid **36** with DPPA in the presence of triethylamine, followed by treatment with 4-chloroaniline, provided (3*R*,4*S*)-isomer **10**. The (3*S*,4*R*)-isomer **38** was synthesized in the same manner as described for **35** using Ni(II)-*bis*[(*S,S*)-*N,N*-dibenzylcyclohexane-1,2-diamine]bromide as the catalyst. After hydrolysis of ester **38** under basic condition, reaction with DPPA in the presence of

triethylamine, followed by the treatment with 4-chloroaniline or 4-fluoroaniline, gave **11a** and **11b** respectively.

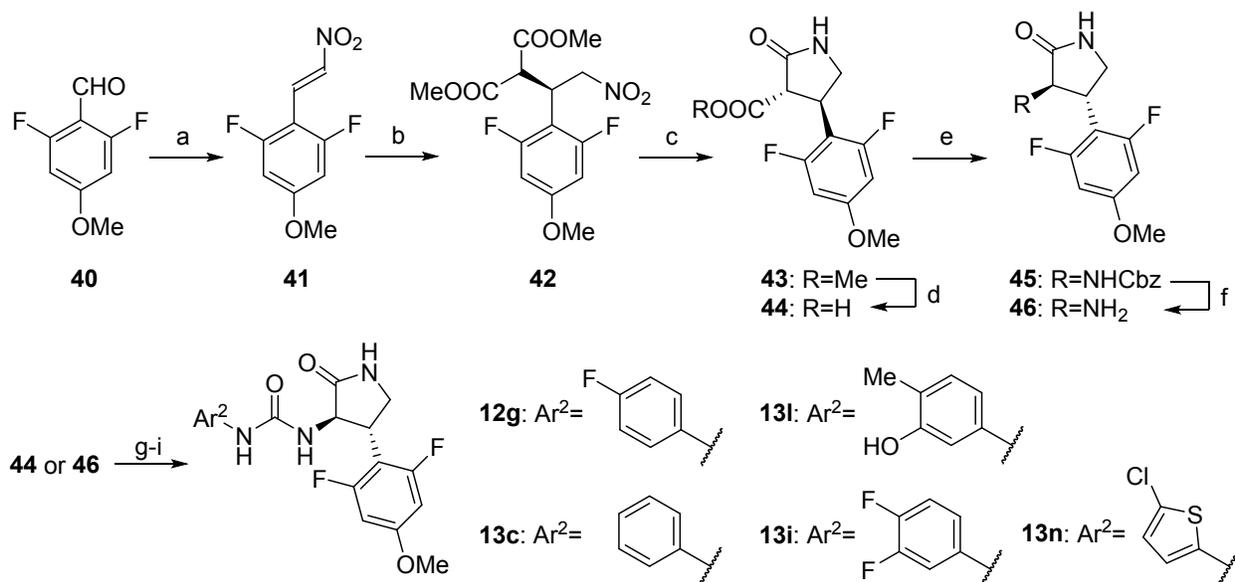
Scheme 5. Synthesis of Optically Active *Trans*-oriented Pyrrolidinone Ureas **10** and **11a**^a



^aReagents and conditions: (a) Dimethyl malonate, Ni(II)-*bis*[(*R,R*)-*N,N*-dibenzylcyclohexane-1,2-diamine]bromide (3 mol%), toluene; (b) Dimethyl malonate, Ni(II)-*bis*[(*S,S*)-*N,N*-dibenzylcyclohexane-1,2-diamine]bromide (3 mol%), toluene; (c) NaBH₄, NiCl₂·6H₂O, MeOH, 73% (two steps for **35**), 68% (two steps for **38**); (d) 1 M NaOH aq., MeOH, 88%, (for **36**), 98% (for **39**); (e) DPPA, Et₃N, toluene then 4-chloroaniline, 53% (for **10**), 24% (for **11a**); (f) DPPA, Et₃N, toluene then 4-fluoroaniline, 18% (for **11b**).

The synthesis of the selected compounds **12g**, **13c**, **13i**, **13l** and **13n** is shown in Scheme 6. Treatment of **40** with MeNO₂ in the presence of ammonium acetate³⁰, gave nitrostyrene **41**, that was converted to carboxy derivative **44** in the same manner as described for **39**. Conversion of **44** to *N*-Cbz derivative **45** by a Curtius rearrangement, and removal of the Cbz group by catalytic hydrogenation, gave amino derivative **46**. Reaction of **44** with DPPA in the presence of triethylamine, followed by treatment with 4-fluoroaniline or 5-amino-2-methylphenol, gave **12g** and **13l** respectively. Reaction of **46** with phenyl isocyanate or 2,4-difluorophenyl isocyanate, gave **13c** and **13i** respectively. Treatment of **46** with 2-chlorothiophene-5-carboxylic acid in the presence of DPPA and triethylamine, gave **13n**.

Scheme 6. Synthesis of Selected Compounds **12g**, **13c**, **13i**, **13l** and **13n**^a



^aReagents and conditions: (a) MeNO₂, ammonium acetate, AcOH, 95%; (b) dimethyl malonate, Ni(II)-bis[(*S,S*)-*N,N'*-dibenzylcyclohexane-1,2-diamine]bromide (3 mol%), toluene, 95%; (c) NaBH₄, NiCl₂·6H₂O, MeOH, 55%; (d) 1 M NaOH aq., MeOH, 99%; (e) DPPA, Et₃N, toluene then BnOH, 47%; (f) H₂, 10% Pd-C, EtOH, 100%; (g) **44**, DPPA, Et₃N, toluene then 4-fluoroaniline or 5-amino-2-methylphenol, 55% (for **12g**), 65% (for **13l**); (h) **46**, phenyl isocyanate or 3,4-difluorophenyl isocyanate, THF, 76% (for **13c**), 86% (for **13i**); (i) **46**, 2-chlorothiophene-5-carboxylic acid, DPPA, Et₃N, toluene, 38% (for **13n**).

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3 Other analogs were synthesized using analogous chemistry and details are provided in the
4 supporting information section.
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10 **Conclusion**

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12 Conformational restriction of a simple phenylethyl urea FPR2 agonist led to the
13 discovery of a pyrrolidinone core, which was further optimized to produce potent FPR2
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15 agonists with selectivity over FPR1. Evaluation of lead compounds in a lung LPS model
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17 and metabolic profiling assays led to the identification of compound **13c** (BMS-
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19 986235/LAR-1219) as a clinical candidate. Compound **13c** showed robust efficacy on
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21 both structure and function endpoints in a mouse myocardial infarction heart failure
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23 model after oral dosing for 28 days. These findings suggest that this selective, orally
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25 bioavailable small molecule FPR2 agonist could prevent adverse pathological remodeling
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27 that leads to heart failure.
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47 **Experimental Section**

54 **Chemistry**

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4 Melting points were measured on an OptiMelt automated melting point system
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7 MPA100 without correction. Infrared spectra (IR) were recorded with a ParkinElmer
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9
10 Spectrum 100 spectrometer. Measurements of mass spectra (MS) and high resolution
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13 MS (HRMS) were performed with a JEOL JMS SX-102A or a JEOL JMS-T100LP mass
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15
16 spectrometer. Proton nuclear magnetic resonance (^1H NMR) spectra were measured
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18
19 with a JEOL JMN-EX400 (400 MHz) or a JEOL JMN-ECA-400 (400 MHz) spectrometer.
20
21
22 The chemical shifts are expressed in parts per million (δ value) downfield from
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24
25 tetramethylsilane, using tetramethylsilane ($\delta = 0$) and/or residual solvents such as
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27
28 chloroform ($\delta = 7.26$) as an internal standard. Splitting patterns are indicated as follows:
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31 s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br; broad peak. Specific optical
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34 rotations were measured on a JASCO P-1000 polarimeter. Purity data were collected by
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37 an Agilent 1100 HPLC with Agilent G1315B diode array detector. The column was used
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40 was a RP-AQUA (50 mm \times 2.1 mm i.d., 2.6 μm , ChromaNik Technologies Inc., Japan)
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43 with a temperature of 45 $^\circ\text{C}$ and a flow rate of 0.5 mL/min. Mobile phase A and B were a
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46 mixture of 0.05% formic acid in water, and 0.05% formic acid in MeCN, respectively. The
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49 ratio of mobile phase was increased lineally from 5% to 95% over 5 min, 95% over the
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3 next 3 min. The data for elemental analysis were within $\pm 0.4\%$ of the theoretical values
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7 and were determined by a Yanaco micro corder JM11. Column chromatography was
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10 carried out with silica gel [silica gel 60 (Kanto)] as an absorbent. Merck precoated thin
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13 layer chromatography (TLC) plates (silica gel 60 F₂₅₄, 0.25 mm, Art 5715) were used for
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16 the TLC analysis. All purities for final compounds are $\geq 95.0\%$ and were measured using
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21 HPLC.
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24 **2-[1,2-Bis(4-methoxyphenyl)-4-oxoazetidin-3-yl]isoindoline-1,3-dione (15).** A solution
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26
27 of 2-(1,3-dioxisoindolin-2-yl)acetyl chloride (2.00 g, 8.94 mmol) in CH₂Cl₂ was added
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29
30 dropwise to a mixture of **14** (1.44 g, 5.87 mmol), triethylamine (2.7 mL, 19 mmol), and
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32
33 CH₂Cl₂ (65 mL) under cooling with NaCl-ice, and the mixture was stirred at room
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35
36 temperature for 38 h, and concentrated in vacuo. Flash chromatography (hexane/AcOEt
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38
39 = 2:1) of the residue gave **15** (718 mg, 28%). ¹H NMR (CDCl₃) δ : 3.66 (s, 2.5H, OCH₃ of
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41
42 major isomer), 3.74 (s, 0.5H, OCH₃ of minor isomer), 3.75 (s, 0.5H, OCH₃ of minor
43
44
45 isomer), 3.79 (s, 2.5H, OCH₃ of major isomer), 5.26 (d, $J = 2.4$ Hz, 0.2H, C-4H of minor
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47
48 isomer), 5.30 (d, $J = 3.0$ Hz, 0.2H, C-3H of minor isomer), 5.40 (d, $J = 5.4$ Hz, 0.8H, C-4H
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51 of major isomer), 5.62 (d, $J = 5.4$ Hz, 0.8H, C-3H of major isomer), 6.65 (d, $J = 8.5$ Hz,
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3 0.3H, ArH of minor isomer), 6.70–6.76 (m, 1.7H, ArH of major isomer), 6.81 (d, $J = 9.1$
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7 Hz, 0.3H, ArH of minor isomer), 6.86 (d, $J = 9.1$ Hz, 1.7H, ArH of major isomer), 6.91 (d,
8
9
10 $J = 8.5$ Hz, 0.3H, ArH of minor isomer), 7.18 (d, $J = 8.5$ Hz, 1.7H, ArH of major isomer),
11
12
13 7.27–7.31 (m, 0.3H, ArH of minor isomer), 7.39 (d, $J = 9.1$ Hz, 1.7H, ArH of major isomer),
14
15
16
17 7.63–7.67 (m, 1.7H, ArH of major isomer), 7.69–7.72 (m, 1.7H, ArH of major isomer),
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21 7.76 (dd, $J = 5.5, 3.0$ Hz, 0.3H, ArH of minor isomer), 7.88 (dd, $J = 5.5, 3.0$ Hz, 0.3H, ArH
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23
24 of minor isomer).
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28 **2-[*cis*-2-(4-Methoxyphenyl)-4-oxoazetidin-3-yl]isoindoline-1,3-dione (16)**. A solution of
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31 ammonium cerium(IV) nitrate (2.33 g, 4.25 mmol) in water (14 mL) was added to a mixture
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34 of **15** (608 mg, 1.42 mmol), MeCN (28 mL) and THF (7 mL) under cooling with NaCl-ice,
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37 and the mixture was stirred at same temperature for 2 h, and then at room temperature
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41 for 2 h. After quenching the reaction addition of water, the mixture was extracted with
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44 AcOEt (3 × 40 mL). The combined extracts were washed with saturated aqueous
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48 NaHCO₃ solution, 10% aqueous NaHSO₃ solution, and brine, dried over anhydrous
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51 Na₂SO₄, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt =
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55 2:1 → 1:2) of the residue gave **16** (283 mg, 62%) as a pale yellow amorphous solid. ¹H
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3 NMR (CDCl₃) δ : 3.68 (s, 3H), 5.13 (d, J = 5.4 Hz, 1H), 5.56 (dd, J = 4.8, 1.8 Hz, 1H), 6.44
4
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6
7 (br s, 1H), 6.75 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 8.5 Hz, 2H), 7.63–7.66 (m, 2H), 7.68–7.72
8
9
10 (m, 2H). MS (FI⁺) m/z : 322 (M⁺). HRMS (FI⁺) for C₁₈H₁₄N₂O₄ (M⁺): calcd, 322.09536;
11
12
13 found, 322.09543. IR (ATR) cm⁻¹: 3280, 1760, 1717.
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17 ***cis*-3-Amino-4-(4-methoxyphenyl)azetidin-2-one Hydrochloride (17)**. A solution of
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19 hydrazine monohydrate (0.050 mL, 1.0 mmol) in MeOH (0.3 mL) was added to a solution
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21 of **16** (250 mg, 0.776 mmol) in 1,4-dioxane (3.1 mL), the mixture was stirred at room
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28 temperature for 1 h, and concentrated in vacuo. After addition of MeOH (3.0 mL) and 1
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31 M HCl (1.5 mL) to the residue, the mixture was stirred at 40 °C for 2 h, and concentrated
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38 in vacuo. After addition of water to the residue, the insoluble materials were filtered off,
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4 **1-(4-Chlorophenyl)-3-[*cis*-2-(4-methoxyphenyl)-4-oxoazetidin-3-yl]urea (3).** To a
5
6
7 mixture of **17** (89.0 mg, 0.389 mmol), AcOEt (1.6 mL), and saturated aqueous NaHCO₃
8
9
10 solution was added 4-chlorophenyl isocyanate (56.0 mg, 0.365 mmol) under cooling with
11
12
13 ice and the mixture was stirred at same temperature for 2 h. After quenching the reaction
14
15
16 by the addition of water, the mixture was extracted with AcOEt (2 × 10 mL). The combined
17
18
19 extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered, and then
20
21
22 concentrated in vacuo. Trituration of the residue with AcOEt–Et₂O gave **3** (85 mg, 63%)
23
24
25 as a white powder. Mp: 184–186 °C ¹H NMR (DMSO-*d*₆) δ: 3.73 (s, 3H), 4.86 (d, *J* = 4.8
26
27
28 Hz, 1H), 5.29 (dd, *J* = 9.7, 4.8 Hz, 1H), 6.27 (d, *J* = 9.7 Hz, 1H), 6.93–6.97 (m, 2H),
29
30
31 7.13–7.16 (m, 2H), 7.22–7.25 (m, 2H), 7.28–7.31 (m, 2H), 8.49 (s, 1H), 8.61 (s, 1H). MS
32
33
34 (FD⁺) *m/z*. 345 (M⁺). HRMS (FD⁺) for C₁₇H₁₆ClN₃O₃ (M⁺): calcd, 345.08802; found,
35
36
37 345.08767. IR (ATR) cm⁻¹: 3278, 1771, 1627. HPLC purity: 98.1%.
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45 ***trans*-3-Azide-1,2-bis(4-methoxyphenyl)azetidin-2-one (19).** To a mixture of **18** (530
46
47
48 mg, 1.77 mmol), triethylamine (0.37 mL, 2.7 mmol), and THF (8.9 mL) was added
49
50
51 methanesulfonyl chloride (0.17 mL, 2.2 mmol) under cooling with ice; the mixture was
52
53
54
55
56 stirred at same temperature for 1.5 h. After quenching the reaction by the addition of
57
58
59
60

1
2
3 ice-water, the mixture was extracted with CH₂Cl₂ (3 × 20 mL). The combined extracts
4
5
6 were washed with brine, dried over anhydrous Na₂SO₄, filtered, and then concentrated in
7
8
9 vacuo. A mixture of the residue, NaN₃ (690 mg, 10.6 mmol), and DMF (8.9 mL) was
10
11
12 stirred at 90 °C for 74 h. After quenching the reaction by the addition of ice-water, the
13
14
15 mixture was extracted with AcOEt (2 × 20 mL). The combined extracts were washed with
16
17
18 brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Flash
19
20
21 chromatography (hexane/AcOEt = 3:1) of the residue gave **19** (510 mg, 89%) as a pale
22
23
24 yellow solid. ¹H NMR (CDCl₃) δ: 3.74 (s, 3H), 3.81 (s, 3H), 4.46 (d, *J* = 1.8 Hz, 1H), 4.77
25
26
27 (d, *J* = 1.8 Hz, 1H), 6.76–6.80 (m, 2H), 6.90–6.93 (m, 2H), 7.19–7.23 (m, 2H), 7.24–7.28
28
29
30 (m, 2H). MS (ESI⁺) *m/z*: 325 (M⁺ + H). HRMS (ESI⁺) for C₁₇H₁₇N₄O₃ (M⁺ + H): calcd,
31
32
33 325.13006; found, 325.12938. IR (ATR) cm⁻¹: 2122, 1758.
34
35
36
37
38
39
40

41
42 ***trans*-3-Azide-4-(4-methoxyphenyl)azetidin-2-one (20)**. The compound **20** (285 mg,
43
44
45 85%) was prepared from **19** (500 mg, 1.54 mmol) by the same method as that used for
46
47
48 **16**. ¹H NMR (CDCl₃) δ: 3.83 (s, 3H), 4.36 (d, *J* = 1.8 Hz, 1H), 4.55 (d, *J* = 1.8 Hz, 1H),
49
50
51 6.13 (br s, 1H), 6.92–6.95 (m, 2H), 7.27–7.30 (m, 2H). MS (FD⁺) *m/z*: 218 (M⁺). HRMS
52
53
54
55
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57
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60

(FD⁺) for C₁₀H₁₀N₄O₂ (M⁺): calcd, 218.08038; found, 218.08038. IR (ATR) cm⁻¹: 3079, 2101, 1772, 1733.

1-(4-Chlorophenyl)-3-[*trans*-2-(4-methoxyphenyl)-4-oxoazetidin-3-yl]urea (4). To a mixture of **20** (100 mg, 0.46 mmol) and THF (4.6 mL) was added triphenylphosphine (136 mg, 0.519 mmol), and the mixture was stirred at room temperature for 100 min. After addition of water (0.04 mL) to the resulting mixture, the reaction mixture was stirred at 50 °C for 5 h. To the resulting mixture was added 4-chlorophenyl isocyanate (48.0 mg, 0.31 mmo), and the mixture was stirred at room temperature for 70 min. To the resulting mixture was added 4-chlorophenyl isocyanate (15.0 mg, 0.098 mmo), and the mixture was stirred at room temperature for 10 min. After quenching the reaction by the addition of water, the mixture was extracted with AcOEt (2 × 10 mL). The combined extracts were washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Trituration of the residue with AcOEt–MeOH gave **4** (76.0 mg, 48%) as a white powder. Mp: 182–183 °C. ¹H NMR (DMSO-*d*₆) δ: 3.75 (s, 3H), 4.37 (dd, *J* = 7.9, 2.4 Hz, 1H), 4.56 (d, *J* = 2.4 Hz, 1H), 6.93–6.97 (m, 2H), 7.01 (d, *J* = 7.9 Hz, 1H), 7.26–7.34 (m, 4H), 7.41–7.45 (m, 2H), 8.53 (s, 1H), 8.88 (s, 1H). MS (FD⁺) *m/z*: 345 (M⁺). HRMS (FD⁺) for

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2
3
4 $C_{17}H_{16}ClN_3O_3$ (M^+): calcd, 345.08802; found, 345.08802. IR (ATR) cm^{-1} : 3364, 1741,
5
6
7 1691. HPLC purity: 99.0%.
8
9

10 ***tert*-Butyl 3-(4-Methoxyphenyl)-4-nitro-2-(2,2,2-trifluoroacetamido)butanoate (22).**
11
12

13
14 Lithium *bis*(trimethylsilyl)amide (1.6 M THF solution, 44 mL, 70 mmol) was added
15
16 dropwise to a solution of *tert*-butyl (2,2,2-trifluoroacetyl)glycinate (6.33 g, 27.9 mmol) in
17
18 THF (50 mL) at -78 °C, and the mixture was stirred at same temperature for 30 min. To
19
20 the resulting mixture was added dropwise a solution of **21** (5.00 g, 27.9 mmol) in THF (30
21
22 mL) at -78 °C, and then the mixture was stirred at room temperature for 30 min. After
23
24 quenching the reaction by the addition of 1 M HCl, the mixture was extracted with AcOEt.
25
26
27
28
29
30
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33
34
35 The extract was washed with brine, dried over anhydrous Na_2SO_4 , filtered, and
36
37 concentrated in vacuo. Flash chromatography of the residue (hexane/AcOEt = 4:1) gave
38
39 **22** (10.0 g, 88%) as a pale yellow oil. 1H NMR ($CDCl_3$) δ : 1.38 and 1.47 [each s, total 9H,
40
41 $C(CH_3)_3$ of major and minor isomers), 3.79 and 3.80 (each s, total 3H, OCH_3 of major and
42
43 minor isomers), 3.96 (q, $J = 7.3$ Hz, 0.7H, C-3H of major isomer), 4.20–4.26 (m, 0.3H,
44
45 C-3H of minor isomer), 4.63–4.69 (m, 0.3H, C-2H of minor isomer), 4.76–4.87 [m, 2H
46
47 (C-4H of major and minor isomers)+0.7H (C-3H of major isomer)], 6.74–6.89 [m, 2H
48
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1
2
3 (ArH of major and minor isomers)+1H (NH of major and minor isomers)], 7.05–7.10 (m,
4
5
6
7 2H, ArH of major and minor isomers).
8
9

10 **3-(4-Methoxyphenyl)-4-nitro-2-(2,2,2-trifluoroacetamido)butanoic Acid (23)**. A mixture
11
12 of **22** (10.0 g, 24.6 mmol) and trifluoroacetic acid (15 mL) was stirred at room temperature
13
14 for 3 h and then concentrated in vacuo. A mixture of the resulting residue, AcOEt, and
15
16
17 water was extracted with saturated aqueous NaHCO₃ solution. The aqueous layer was
18
19
20 acidified with 1 M HCl, and then the mixture was extracted with AcOEt. The extract was
21
22
23 washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and then
24
25
26 concentrated in vacuo to obtain **23** (5.0 g, 58%). Compound **23** was used for next step
27
28
29 without further purification.
30
31
32
33
34
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37

38 **Methyl 3-(4-Methoxyphenyl)-4-nitro-2-(2,2,2-trifluoroacetamido)butanoate (24)**. To a
39
40 solution of **23** (5.00 g, 14.3 mmol) in DMF (15 mL) was added KHCO₃ (2.86 g, 28.6 mmol)
41
42 and iodomethane (1.78 mL, 28.6 mmol), and the mixture was stirred at 50 °C for 2 h.
43
44
45 After quenching the reaction by the addition of water, the mixture was extracted with
46
47
48 AcOEt. The extract was washed with water and brine, dried over anhydrous Na₂SO₄,
49
50
51 filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt = 4:1)
52
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1
2
3 of the residue gave **24** (4.8 g, 92%) as an off-white solid. $^1\text{H NMR}$ (CDCl_3) δ : 3.73 (s, 2H,
4
5
6
7 OCH_3 of major isomer), 3.80 and 3.81 (each s, total 4H, OCH_3 of major and minor
8
9
10 isomers), 4.00 (dd, $J = 13.9, 7.9$ Hz, 0.7H, C-3H of major isomer), 4.26–4.32 (m, 0.3H,
11
12
13 C-3H of minor isomer), 4.68 (dd, $J = 13.9, 8.5$ Hz, 0.3H, C-4H of minor isomer),
14
15
16
17 4.85–4.94 [m, 2.1H (C-2H and C-4H of major isomer)+0.3H (C-2H of minor isomer)],
18
19
20
21 5.01–5.07 (m, 0.3H, C-4H of minor isomer), 6.68–6.80 (br, 1H, NH of major and minor
22
23
24 isomers), 6.88 (d $J = 9.1$ Hz, 2H, ArH of major and minor isomers), 7.00–7.03 (m, 2H,
25
26
27 ArH of major and minor isomers). MS (FI^+) m/z : 364 (M^+). HRMS (FI^+) for $\text{C}_{14}\text{H}_{15}\text{F}_3\text{N}_2\text{O}_6$
28
29
30
31 (M^+): calcd, 364.08822; found, 364.08839. IR (ATR) cm^{-1} : 3266, 1734, 1706.
32
33

34
35 **2,2,2-Trifluoro-*N*[4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]acetamide (25)**. To a
36
37
38 solution of **24** (4.78 g, 13.1 mmol) in MeOH (130 mL) were added a solution of NH_4Cl
39
40
41 (7.00 g, 131 mmol) in water (30 mL) and zinc powder (8.50 g, 131 mmol), and the mixture
42
43
44
45 was stirred at room temperature for 30 min. The reaction mixture was diluted with AcOEt
46
47
48 and the insoluble materials were filtered off. After addition of saturated aqueous NaHCO_3
49
50
51
52 solution to the filtrate, the mixture was extracted with AcOEt. The extract was washed
53
54
55
56 with water and brine, dried over anhydrous Na_2SO_4 , filtered, and then concentrated in
57
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3 vacuo. To a solution of the residue in MeOH (65 mL) was added a solution of sodium
4
5
6 acetate (13.0 g, 159 mmol) in water (43 mL) and 20% aqueous titanium(III) chloride
7
8
9 solution (17 mL), and then the mixture was stirred at room temperature for 2 h. After
10
11
12 quenching the reaction by addition of water, the mixture was extracted with AcOEt. The
13
14
15 extract was washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and then
16
17
18 concentrated in vacuo. Flash chromatography (hexane/AcOEt = 1:2) of the residue gave
19
20
21 **25** (2.5 g, 63%) as a pale yellow oil. ¹H NMR (CDCl₃) δ: 3.43 (t, *J* = 9.7 Hz, 0.3H, C-5H
22
23
24 of minor isomer), 3.61–3.68 (m, 1H, C-5H of major and minor isomers), 3.73 (t, *J* = 9.7
25
26
27 Hz, 0.3H, C-4H of minor isomer), 3.79 and 3.81 (each s, total 3H, OCH₃ of major and
28
29
30 minor isomers), 3.93 (dd, *J* = 10.3, 6.7 Hz, 0.7H, C-5H of major isomer), 4.04 (t, *J* = 6.7
31
32
33 Hz, 0.7H, C-4H of major isomer), 4.72 (dd, *J* = 10.9, 8.5 Hz, 0.3H, C-3H of minor isomer),
34
35
36 4.77 (t, *J* = 6.7 Hz, 0.7H, C-3H of major isomer), 6.31 (br s, 1H, NH of major and minor
37
38
39 isomers), 6.34–6.42 (br, 0.7H, NH of major isomer), 6.84 (d, *J* = 9.1 Hz, 1.3H, ArH of
40
41
42 major isomer), 6.91 (d, *J* = 8.5 Hz, 0.7H, ArH of minor isomer), 7.00 (d, *J* = 7.9 Hz, 0.3H,
43
44
45 NH of minor isomer), 7.07 (d, *J* = 8.5 Hz, 1.3H, ArH of major isomer), 7.22 (d, *J* = 8.5 Hz,
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0.7H, ArH of minor isomer). MS (FI⁺) *m/z*: 302 (M⁺). HRMS (FI⁺) for C₁₃H₁₃F₃N₂O₃ (M⁺): calcd, 302.08783; found, 302.08781. IR (ATR) cm⁻¹: 3296, 3235, 1722, 1696, 1674.

1-(4-Chlorophenyl)-3-[*cis*-4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (**5**) and 1-(4-Chlorophenyl)-3-[*trans*-4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (**6**). To a solution of **25** (60.0 mg, 0.199 mmol) in MeOH (3.0 mL) was added 1 M aqueous NaOH solution (0.5 mL), and the mixture was stirred at 70 °C for 2 h. After neutralization (pH 7) of the reaction mixture by addition of 1 M HCl, the mixture was concentrated in vacuo. To the mixture of residue (**26**) and AcOEt (3.0 mL), 4-chlorophenyl isocyanate (30.0 mg, 0.195 mmol) was added, and the mixture was stirred at room temperature for 10 min. After dilution of the reaction mixture with AcOEt, the mixture was washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt → AcOEt → AcOEt/MeOH) of the residue gave **5** (3.00 mg, 4.2%) and **6** (31.0 mg, 44%).

26: ¹H NMR (CDCl₃) δ: 3.20–3.27 (m, 1H), 3.36 (d, *J* = 9.7 Hz, 1H), 3.58–3.74 (m, 2H), 3.81 (s, 3H), 5.91 (br s, 1H), 6.86–6.92 (m, 2H), 7.17–7.24 (m, 2H). MS (FI⁺) *m/z*: 206 (M⁺). HRMS (FI⁺) for C₁₁H₁₄N₂O₂ (M⁺): calcd, 206.10553; found, 206.10598.

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4 **5.**: White powder. Mp: 211–213 °C. ¹H NMR (DMSO-*d*₆) δ: 3.25–3.30 (m, 1H),
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6
7 3.68–3.72 (m, 5H), 4.59 (t, *J* = 7.3 Hz, 1H), 5.80 (d, *J* = 7.3 Hz, 1H), 6.83–6.87 (m, 2H),
8
9
10 7.03–7.06 (m, 2H), 7.21–7.25 (m, 2H), 7.30–7.34 (m, 2H), 8.10 (s, 1H), 8.73 (s, 1H). MS
11
12
13 (ESI⁺) *m/z*. 360 (M⁺ + H). HRMS (ESI⁺) for C₁₈H₁₉CIN₃O₃ (M⁺ + H): calcd, 360.11149;
14
15
16
17 found, 360.11120. IR (ATR) cm⁻¹: 3303, 1699. HPLC purity: 99.0%.

19
20
21 **6.**: White powder. Mp: 151–154 °C. ¹H NMR (DMSO-*d*₆) δ: 3.13–3.20 (m, 1H),
22
23
24 3.43–3.50 (m, 2H), 3.72 (s, 3H), 4.48 (dd, *J* = 10.9, 9.1 Hz, 1H), 6.47 (d, *J* = 8.5 Hz, 1H),
25
26
27 6.86–6.90 (m, 2H), 7.22–7.26 (m, 2H), 7.28–7.32 (m, 2H), 7.37–7.41 (m, 2H), 7.92 (s,
28
29
30 1H), 8.68 (s, 1H). MS (ESI⁺) *m/z*. 360 (M⁺ + H). HRMS (ESI⁺) for C₁₈H₁₉CIN₃O₃ (M⁺ + H):
31
32
33 calcd, 360.11149; found, 360.11142. IR (ATR) cm⁻¹: 3387, 1729, 1660. HPLC purity:
34
35
36
37
38 99.0%.

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40
41 **Dimethyl 2-[2-Cyano-1-(4-methoxyphenyl)ethyl]malonate (28).** To a 1 M solution of
42
43
44 sodium methoxide in MeOH [prepared from sodium (460 mg, 20.0 mmol) and MeOH (20
45
46
47 mL)] was added dimethyl malonate (3.00 mL, 26.3 mmol), and the mixture was stirred at
48
49
50 room temperature for 10 min. To the reaction mixture was added **27** (2.90 mL, 20.0
51
52
53 mmol), and the mixture was heated under reflux for 18 h. After quenching the reaction
54
55
56
57
58
59
60

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3
4 by the addition of 1 M HCl (50 mL) under ice cooling, the mixture was extracted with
5
6
7 AcOEt (2 × 100 mL). The combined extracts were washed with brine (2 × 50 mL), dried
8
9
10 over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. After suspension of the
11
12
13 residue in MeOH (50 mL), the insoluble materials were filtered off, and the filtrate was
14
15
16 concentrated in vacuo. Flash chromatography (hexane/AcOEt = 10:1 → 1:2) of the
17
18
19 residue gave **28** (1.43 g, 24%) as a white solid. ¹H NMR (CDCl₃) δ: 2.83 (dd, *J* = 17.0,
20
21 4.8 Hz, 1H), 2.89 (dd, *J* = 16.3, 7.3 Hz, 1H), 3.53 (s, 3H), 3.67–3.73 (m, 1H), 3.786 (s,
22
23 3H), 3.795 (s, 3H), 3.85 (d, *J* = 9.7 Hz, 1H), 6.85–6.89 (m, 2H), 7.18–7.22 (m, 2H). MS
24
25
26 (EI⁺) *m/z*: 291 (M⁺). IR (ATR) cm⁻¹: 2248, 1750, 1734.

27
28
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35 **Methyl 4-(4-Methoxyphenyl)-2-oxopiperidine-3-carboxylate (29)**. To a mixture of **28**
36
37
38 (1.31 g, 4.50 mmol) and nickel(II) chloride hexahydrate (1.07 g, 4.50 mmol) in MeOH (45
39
40
41 mL) was added portionwise NaBH₄ (1.02 g, 27.0 mmol) under cooling with ice, and the
42
43
44 mixture was stirred at room temperature for 1 h. After quenching the reaction by adding
45
46
47 1 M HCl (30 mL), the mixture was extracted with AcOEt (2 × 100 mL). The combined
48
49
50 extracts were washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and
51
52
53 concentrated in vacuo. Flash chromatography (hexane/AcOEt = 1:2) of the residue gave
54
55
56
57
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59
60

1
2
3 **29** (765 mg, 65%) as a white solid. $^1\text{H NMR}$ ($\text{DMSO}-d_6$) δ : 1.80 (br d, $J = 13.5$ Hz, 1H),
4
5
6
7 1.97 (dq, $J = 12.7, 5.5$ Hz, 1H), 3.14–3.31 (m, 3H), 3.44 (s, 3H), 3.52 (d, $J = 11.5$ Hz, 1H),
8
9
10 3.72 (s, 3H), 6.84–6.87 (m, 2H), 7.16–7.19 (m, 2H), 7.89 (d, $J = 2.4$ Hz, 1H). MS (ESI⁺)
11
12
13
14 m/z : 264 ($\text{M}^+ + \text{H}$). IR (ATR) cm^{-1} : 3191, 1740, 1656.

15
16
17 **4-(4-Methoxyphenyl)-2-oxopiperidine-3-carboxylic Acid (30)**. To a solution of **29** (710
18
19
20 mg, 2.70 mmol) in MeOH (5.4 mL) was added 1 M aqueous NaOH solution (5.40 mL,
21
22
23 5.40 mmol) and then the mixture was stirred at 50 °C for 1 h. After removal of MeOH in
24
25
26
27 vacuo, the resulting mixture was adjusted to pH 1 by addition of 1 M HCl under cooling
28
29
30
31 with ice, and the resulting precipitate was collected by filtration. The filtered precipitate
32
33
34
35 was washed with water and hexane, and dried in vacuo to give **30** (618 mg, 92%) as a
36
37
38 white solid. $^1\text{H NMR}$ ($\text{DMSO}-d_6$) δ : 1.79 (br d, $J = 13.3$ Hz, 1H), 1.92 (dq, $J = 12.7, 5.5$
39
40
41 Hz, 1H), 3.11–3.30 (m, 3H), 3.36 (d, $J = 11.5$ Hz, 1H), 3.72 (s, 3H), 6.84–6.68 (m, 2H),
42
43
44
45 7.17–7.21 (m, 2H), 7.80 (d, $J = 3.0$ Hz, 1H), 12.2 (br s, 1H). MS (FD⁺) m/z : 249 (M^+). IR
46
47
48 (ATR) cm^{-1} : 3305, 1740.

49
50
51
52 **1-(4-Chlorophenyl)-3-[*trans*-4-(4-methoxyphenyl)-2-oxopiperidin-3-yl]urea (7)**.
53

54
55
56 Diphenylphosphoryl azide (0.17 mL, 0.760 mmol) and triethylamine (0.11 mL, 0.789
57
58
59
60

1
2
3 mmol) were added to a mixture of **30** (150 mg, 0.602 mmol) in toluene (3.0 mL) and MeCN
4
5
6
7 (0.3 mL), the mixture was stirred at room temperature for 1 h, and then at 100 °C for 1 h.

8
9
10 To the reaction mixture was added 4-chloroaniline (155 mg, 1.22 mmol), the mixture was
11
12
13 stirred at 100 °C for 6.5 h, and concentrated in vacuo. Flash chromatography (AcOEt →

14
15
16
17 AcOEt/MeOH = 4:1) of the residue gave **7** (14.6 mg, 6.5%) as a white powder. Mp:

18
19
20 254–260 °C. ¹H NMR (DMSO-*d*₆) δ: 1.85 (br d, *J* = 12.1 Hz, 1H), 2.01 (dq, *J* = 12.1, 5.5

21
22
23 Hz, 1H), 3.12–3.21 (m, 2H), 3.26 (dt, *J* = 11.5, 4.2 Hz, 1H), 3.69 (s, 3H), 4.13 (dd, *J* =

24
25
26
27 11.5, 9.1 Hz, 1H), 6.24 (d, *J* = 9.1 Hz, 1H), 6.82 (d, *J* = 8.5 Hz, 2H), 7.17 (d, *J* = 8.5 Hz,

28
29
30 2H), 7.20 (d, *J* = 9.1 Hz, 2H), 7.34 (d, *J* = 9.1 Hz, 2H), 7.61 (s, 1H), 8.59 (s, 1H). MS

31
32
33 (ESI⁺) *m/z*: 374 (M⁺ + H). HRMS (ESI⁺) for C₁₉H₂₁ClN₃O₃ (M⁺ + H): calcd, 374.12714;

34
35
36 found, 374.12805. IR (ATR) cm⁻¹: 3322, 1678, 1647. Anal calcd for

37
38
39 C₁₉H₂₀ClN₃O₃·0.8H₂O: C, 58.78; H, 5.61; N, 10.82. Found: C, 58.84; H, 5.34; N, 10.74.

40
41
42
43
44
45 **Ethyl 2-[(*tert*-butoxycarbonyl)amino]-3-(4-methoxyphenyl)-4-nitrobutanoate (31).**

46
47
48 Lithium diisopropylamide (1.09 M THF solution, 18.3 mL, 20.0 mmol) was added dropwise

49
50
51 to a solution of ethyl 2-[(diphenylmethylene)amino]acetate (5.35 g, 20.0 mmol) in THF (56

52
53
54
55 mL) at -78 °C, and the mixture was stirred at -78 °C for 1 h. To the reaction mixture was

1
2
3 added dropwise a solution of **21** (3.00 g, 16.7 mmol) in THF (16 mL) at -78 °C, and the
4
5
6
7 mixture was stirred for 4.5 h with gradually warming to room temperature. The reaction
8
9
10 mixture was poured into saturated aqueous NH₄Cl solution (150 mL), and the resulting
11
12
13 mixture was extracted with AcOEt (2 × 30 mL). The combined extracts were washed with
14
15
16
17 brine, dried over anhydrous Na₂SO₄, filtered, and then concentrated in vacuo. Flash
18
19
20 chromatography (hexane/AcOEt = 4:1) of the residue gave ethyl 2-
21
22
23 [(diphenylmethylene)amino]-3-(4-methoxyphenyl)-4-nitrobutanoate (8.47 g). A mixture of the
24
25
26
27 compound obtained (8.47 g), acetic acid (45 mL) and water (9 mL) was stirred at 45 °C
28
29
30 for 4 h, and then at 50 °C for 2 h. After addition of 0.5 M HCl (70 mL) and diethyl ether
31
32
33
34 (20 mL) to the reaction mixture, the aqueous layer was separated and washed with diethyl
35
36
37
38 ether (2 × 20 mL). The resulting mixture was neutralized with saturated aqueous NaHCO₃
39
40
41
42 solution, and extracted with AcOEt (2 × 50 mL). The combined extracts were washed
43
44
45
46 with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Di-*tert*-
47
48
49 butyl dicarbonate (4.02 g, 18.4 mmol) and THF (42 mL) were added to the residue and
50
51
52 the reaction mixture was stirred at room temperature for 6 h. To the reaction mixture were
53
54
55
56 added AcOEt (30 mL) and water (100 mL). The organic layer was separated and the
57
58
59
60

1
2
3 aqueous layer was extracted with AcOEt (2 × 20 mL). The combined extracts were
4
5
6
7 washed with brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo.
8
9
10 Flash chromatography (hexane/AcOEt = 95:5 → 4:1) of the residue gave **31** (4.00 g, 63%).
11
12
13 ¹H NMR (CDCl₃) δ: 1.08 (br, 3H), 1.46 (s, 9H), 3.78 (s, 3H), 3.79–3.85 (m, 1H), 4.01 (d,
14
15 *J* = 6.7 Hz, 2H), 4.53–4.57 (m, 1H), 4.77 (dd, *J* = 13.4 Hz, 9.2 Hz, 1H), 4.85 (dd, *J* = 13.4
16
17 Hz, 5.5 Hz, 1H), 5.11 (br, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 2H). MS
18
19
20
21
22
23
24 (ESI⁺) *m/z*: 383 (M⁺ + H). HRMS (ESI⁺) for C₁₈H₂₇N₂O₇ (M⁺ + H): calcd, 383.18183; found,
25
26
27 383.18131. IR (ATR) cm⁻¹: 3401, 1732, 1697.
28
29
30

31 **(-)-*tert*-Butyl [4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]carbamate (32) and (+)-*tert*-**
32
33
34 **Butyl [4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]carbamate (33).** To a mixture of **31** (4.00
35
36 g, 10.5 mmol) and nickel(II) chloride hexahydrate (2.50 g, 10.5 mmol) in MeOH (210 mL)
37
38 was added portionwise NaBH₄ (2.38 g, 63.0 mmol) under cooling with ice, the mixture
39
40
41 was stirred at room temperature for 45 min, and then at 40 °C for 2 h. The reaction
42
43
44 mixture was poured into saturated aqueous NH₄Cl solution, and the resulting mixture was
45
46
47 extracted with AcOEt (500 mL). The extract was washed with brine, dried over anhydrous
48
49
50 Na₂SO₄, filtered, and then concentrated in vacuo. Flash chromatography (hexane/AcOEt
51
52
53
54
55
56
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58
59
60

1
2
3
4 = 3:2) of the residue gave the racemic *tert*-butyl [4-(4-methoxyphenyl)-2-oxopyrrolidin-
5
6
7 3-yl]carbamate (2.68 g). Chiral separation of the racemate by HPLC using CHIRALPAK
8
9
10 AD column (hexane/EtOH/*tert*-butyl methyl ether =45:30:25) gave **32** (1.14 g, 35%) and
11
12
13
14 **33** (1.40 g, 43%).

15
16
17 **32.** ¹H NMR (CDCl₃) δ: 1.37 (s, 9H), 3.55 (d, *J* = 9.8 Hz, 1H), 3.80 (s, 3H), 3.82–3.92
18
19
20 (m, 2H), 4.58 (br s, 2H), 5.75 (br s, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 2H).
21
22
23
24 MS (ESI⁺) *m/z*: 307 (M⁺ + H). HRMS (ESI⁺) for C₁₆H₂₃N₂O₄ (M⁺ + H): calcd, 307.16578;
25
26
27 found, 307.16561. IR (ATR) cm⁻¹: 3363, 3287, 1708, 1684. [α]²²_D -88.2 (*c*0.104, EtOH).
28
29
30

31
32 **33.** ¹H NMR (CDCl₃) δ: 1.37 (s, 9H), 3.56 (d, *J* = 9.8 Hz, 1H), 3.80 (s, 3H), 3.82–3.97
33
34 (m, 2H), 4.58 (br s, 2H), 5.73 (br s, 1H), 6.83 (d, *J* = 8.6 Hz, 2H), 7.10 (d, *J* = 8.6 Hz, 2H).
35
36
37
38 MS (ESI⁺) *m/z*: 307 (M⁺ + H). HRMS (ESI⁺) for C₁₆H₂₃N₂O₄ (M⁺ + H): calcd, 307.16578;
39
40
41 found, 307.16615. IR (ATR) cm⁻¹: 3363, 3287, 1708, 1684. [α]²²_D +98.4 (*c*0.101, EtOH).
42
43
44

45 **(-)-1-(4-Chlorophenyl)-3-[*cis*-4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (8).**

46
47
48 Trifluoroacetic acid (0.5 mL) was added to a solution of **32** (42.9 mg, 0.140 mmol) in
49
50
51 CH₂Cl₂ (1 mL) at 0 °C, and the mixture was stirred at room temperature for 75 min. After
52
53
54
55 concentration of the reaction mixture in vacuo, a mixture of the residue, saturated
56
57
58
59
60

1
2
3 aqueous NaHCO₃, THF (1 mL), and 4-chlorophenyl isocyanate (23.6 mg, 0.154 mmol)
4
5
6
7 was stirred at room temperature for 1 h. To the reaction mixture was added 4-
8
9
10 chlorophenyl isocyanate (11 mg, 0.070 mmol), and the mixture was stirred at room
11
12
13 temperature for 40 min, and concentrated in vacuo. Flash chromatography of the residue
14
15
16 (CHCl₃/MeOH = 10:1) gave **8** (28.0 mg, 56%) as a white powder. Mp: 212–214 °C. ¹H
17
18 NMR (DMSO-*d*₆) δ: 3.25–3.31 (m, 1H), 3.68–3.72 (m, 5H), 4.59 (t, *J* = 7.3 Hz, 1H), 5.81
19
20
21 (d, *J* = 6.7 Hz, 1H), 6.83–6.87 (m, 2H), 7.03–7.06 (m, 2H), 7.21–7.25 (m, 2H), 7.30–7.34
22
23
24 (m, 2H), 8.10 (s, 1H), 8.74 (s, 1H). MS (ESI⁺) *m/z*: 360 (M⁺ + H). HRMS (ESI⁺) for
25
26
27 C₁₈H₁₉ClN₃O₃ (M⁺ + H): calcd, 360.11149; found, 360.11194. IR (ATR) cm⁻¹: 3301, 1664.
28
29
30
31 Enantiomeric excess was determined by HPLC analysis with CHIRALPAK ID column
32
33
34 (30:70 hexane:EtOH, 1.0 mL/min, 254 nm); major enantiomer *t*_r = 11.2 min; 100% ee;
35
36
37
38
39
40
41
42 [α]²⁵_D -260 (*c* 0.101, EtOH). HPLC purity 100%

43
44
45 (+)-1-(4-Chlorophenyl)-3-[*cis*-4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (**9**). The
46
47
48 compound **9** (26.6 mg, 56%) was prepared from **33** (40.3 mg, 0.132 mmol) by the same
49
50
51 method as that used for **8**. White powder. Mp: 211–214 °C. ¹H NMR (DMSO-*d*₆) δ:
52
53
54 3.25–3.31 (m, 1H), 3.68–3.72 (m, 5H), 4.59 (t, *J* = 7.3 Hz, 1H), 5.81 (d, *J* = 7.3 Hz, 1H),
55
56
57
58
59
60

1
2
3
4 6.83–6.87 (m, 2H), 7.03–7.06 (m, 2H), 7.21–7.25 (m, 2H), 7.30–7.34 (m, 2H), 8.10 (s,
5
6
7 1H), 8.74 (s, 1H). MS (ESI⁺) *m/z*: 360 (M⁺ + H). HRMS (ESI⁺) for C₁₈H₁₉ClN₃O₃ (M⁺ +
8
9
10 H): calcd, 360.11149; found, 360.11186. IR (ATR) cm⁻¹: 3304, 1684, 1666. Enantiomeric
11
12
13 excess was determined by HPLC analysis with CHIRALPAK ID column (30:70
14
15 hexane:EtOH, 1.0 mL/min, 254 nm); major enantiomer *t*_r = 12.5 min; 100% ee; [α]²⁵_D
16
17 +235 (*c* 0.104, EtOH). HPLC purity 99.9%.
18
19
20
21
22
23

24 **Methyl (3*R*,4*S*)-4-(4-Methoxyphenyl)-2-oxopyrrolidine-3-carboxylate (35)**. To a solution
25
26
27 of **21** (12.0 g, 64.3 mmol) in toluene (64 mL) was added dimethyl malonate (7.50 mL, 64.3
28
29 mmol) and nickel(II)-*bis*[(*R,R*)-*N,N*-dibenzylcyclohexan-1,2-diamine]bromide (1.56 g,
30
31 1.93 mmol), and the mixture was stirred at room temperature for 28 h. The reaction
32
33
34 mixture was washed with 0.1 M HCl (50 mL) and water (50 mL), dried over anhydrous
35
36
37 Na₂SO₄, filtered, and then concentrated in vacuo to give dimethyl (*S*)-2-[1-(4-
38
39 methoxyphenyl)-2-nitroethyl]malonate (**34**) as a white solid. To a mixture of crude **34**
40
41
42 and nickel(II) chloride hexahydrate (17.2 g, 70.7 mmol) in MeOH (322 mL) was added
43
44
45 portionwise NaBH₄ (14.9 g, 386 mmol) under cooling with ice, and then the mixture was
46
47
48 stirred at room temperature for 2.5 h. To the reaction mixture was added saturated
49
50
51
52
53
54
55
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60

1
2
3 aqueous NH_4Cl (800 mL) and AcOEt (1200 mL) under cooling with ice, and the mixture
4
5
6
7 was stirred at room temperature for 2 h, and then the organic layer was separated. The
8
9
10 organic layer was washed with saturated aqueous NH_4Cl (500 mL), water (500 mL), and
11
12
13 brine (500 mL), dried over anhydrous Na_2SO_4 , filtered, and concentrated in vacuo.
14
15
16
17 Trituration of the residue with EtOH -diisopropyl ether gave **35** (11.6 g, 73%) as a white
18
19
20
21 solid.

22
23
24 **34.** $^1\text{H NMR}$ (CDCl_3) δ : 3.58 (s, 3H), 3.76 (s, 3H), 3.78 (s, 3H), 3.83 (d, $J = 9.1$ Hz, 1H),
25
26
27 4.19 (dt, $J = 9.1, 4.8$ Hz, 1H), 4.83 (dd, $J = 12.7, 9.1$ Hz, 1H), 4.89 (dd, $J = 12.7, 4.8$ Hz,
28
29
30 1H), 6.82–6.86 (m, 2H), 7.13–7.16 (m, 2H). Enantiomeric excess was determined by
31
32
33
34
35 HPLC analysis with CHIRALPAK ID column (90:10 hexane:EtOH, 1.0 mL/min, 254 nm);
36
37
38 minor enantiomer $t_r = 21.5$ min, major enantiomer $t_r = 19.8$ min; 92.4% ee; $[\alpha]_D^{25} +5.33$ (c
39
40
41 1.121, CHCl_3).

42
43
44
45 **35.** $^1\text{H NMR}$ (CDCl_3) δ : 3.40 (t, $J = 8.5$ Hz, 1H), 3.54 (d, $J = 9.7$ Hz, 1H), 3.71–3.74 (m,
46
47
48 1H), 3.78 (s, 3H), 3.80 (s, 3H), 4.09 (q, $J = 8.5$ Hz, 1H), 5.72 (br s, 1H), 6.86–6.90 (m,
49
50
51 2H), 7.17–7.20 (m, 2H). MS (FI^+) m/z : 249 (M^+). HRMS (FI^+) for $\text{C}_{13}\text{H}_{15}\text{NO}_4$ (M^+): calcd,
52
53
54
55 249.10011; found, 249.10051. IR (ATR) cm^{-1} : 3226, 1718, 1693. Enantiomeric excess
56
57
58
59
60

1
2
3 was determined by HPLC analysis with CHIRALPAK ID column (80:20 hexane:EtOH, 1.0
4 mL/min, 254 nm); minor enantiomer $t_r = 14.4$ min, major enantiomer $t_r = 17.0$ min; 99.1%
5
6
7 ee; $[\alpha]^{25}_D +154$ (c 0.164, EtOH).
8
9
10
11
12

13
14 **(3*R*,4*S*)-4-(4-Methoxyphenyl)-2-oxopyrrolidine-3-carboxylic Acid (36)**. To a solution of
15
16
17 **35** (200 mg, 0.802 mmol) in MeOH (4.0 mL) was added 2 M NaOH solution (0.80 mL, 1.6
18 mmol), and the mixture was stirred at 60 °C for 1.5 h. After addition of 1 M HCl (1.8 mL)
19
20
21 to the reaction mixture, water (25 mL) was added, and the mixture was extracted with
22
23
24 AcOEt (2 × 35 mL). The combined extracts were washed with brine, dried over anhydrous
25
26
27 Na₂SO₄, filtered, and concentrated in vacuo to give **36** (166 mg, 88%). ¹H NMR
28
29
30 (DMSO-*d*₆) δ : 3.16 (t, $J = 9.1$ Hz, 1H), 3.41 (br d, 1H), 3.55 (t, $J = 8.5$ Hz, 1H), 3.72 (s,
31
32 3H), 3.79 (q, $J = 8.5$ Hz, 1H), 6.88 (d, $J = 8.5$ Hz, 2H), 7.24 (d, $J = 8.5$ Hz, 2H), 8.01 (s,
33
34 1H), 12.6 (br s, 1H). MS (FD⁺) m/z : 235 (M⁺). HRMS (FD⁺) for C₁₂H₁₃NO₄ (M⁺): calcd,
35
36 235.08446; found, 235.08379. IR (ATR) cm⁻¹: 3239, 1686. $[\alpha]^{25}_D +146$ (c 0.192, EtOH).
37
38
39
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47
48

49 **1-(4-Chlorophenyl)-3-[(3*R*,4*S*)-4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (10)**. To
50
51
52 a mixture of **36** (80.0 mg, 0.340 mmol) in toluene (1.7 mL) was added triethylamine (61.9
53
54
55 μ L, 0.442 mmol) and diphenylphosphoryl azide (80.6 μ L, 0.374 mmol), and the mixture
56
57
58
59
60

1
2
3 was stirred at room temperature for 4.5 h and then at 60 °C for 45 min. To the reaction
4
5
6
7 mixture was added 4-chloroaniline (87.6 μ L, 0.680 mmol), and the mixture was stirred at
8
9
10 90 °C for 2.5 h. AcOEt (3.0 mL) and 1 mol/L HCl (2.0 mL) were added to the reaction
11
12
13
14 mixture and the organic layer was separated. The organic layer was washed with 1 M
15
16
17 HCl, saturated aqueous NaHCO₃ solution, and brine, dried over anhydrous Na₂SO₄,
18
19
20
21 filtered, and concentrated in vacuo. Flash chromatography (AcOEt \rightarrow AcOEt/MeOH =
22
23
24 20:1) of the residue gave **10** (65 mg, 53%) as a white powder. Mp: 191–193 °C. ¹H NMR
25
26
27 (DMSO-*d*₆) δ : 3.13–3.20 (m, 1H), 3.42–3.50 (m, 2H), 3.72 (s, 3H), 4.47 (dd, *J* = 10.9, 9.1
28
29
30 Hz, 1H), 6.49 (d, *J* = 9.1 Hz, 1H), 6.86–6.90 (m, 2H), 7.22–7.26 (m, 2H), 7.28–7.32 (m,
31
32
33 2H), 7.37–7.41 (m, 2H), 7.92 (s, 1H), 8.70 (s, 1H). MS (ESI⁺) *m/z*: 360 (M⁺ + H). HRMS
34
35
36 (ESI⁺) for C₁₈H₁₉ClN₃O₃ (M⁺ + H): calcd, 360.11149; found, 360.11120. IR (ATR) cm⁻¹:
37
38
39 3303, 1664. Enantiomeric excess was determined by HPLC analysis with CHIRALPAK
40
41
42 ID column (50:50 hexane:EtOH, 1.0 mL/min, 254 nm); minor enantiomer *t*_r = 7.1 min,
43
44
45 major enantiomer *t*_r = 11.0 min; 99.9% ee; [α]_D²⁵ +145 (*c* 0.298, EtOH). HPLC purity
46
47
48
49
50
51
52 99.5%.
53
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1
2
3 **Methyl (3*S*,4*R*)-4-(4-Methoxyphenyl)-2-oxopyrrolidine-3-carboxylate (38).** Compound

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5
6
7 **38** (19.2 g, 68%) was prepared from **21** (20.0 g, 112 mmol) and nickel(II)-*bis*[(*S,S*)-*N,N*-
8
9
10 dibenzylcyclohexan-1,2-diamine]bromide (2.56 g, 3.28 mmol) by the same method as that
11
12
13
14 used for **35**.

15
16
17 **37.** White solid. $^1\text{H NMR}$ (CDCl_3) δ : 3.57 (s, 3H), 3.76 (s, 3H), 3.78 (s, 3H), 3.83 (d, J
18
19
20 = 9.1 Hz, 1H), 4.19 (d t, J = 9.1, 5.4 Hz, 1H), 4.82 (dd, J = 13.3, 9.1 Hz, 1H), 4.89 (dd, J
21
22 = 13.3, 5.5 Hz, 1H), 6.82–6.86 (m, 2H), 7.12–7.16 (m, 2H). Enantiomeric excess was
23
24
25 determined by HPLC analysis with CHIRALPAK ID column (90:10 hexane:EtOH, 1.0
26
27 mL/min, 254 nm); minor enantiomer t_r = 20.4 min, major enantiomer t_r = 21.9 min; 92.5%
28
29
30 ee; $[\alpha]_D^{25}$ -7.25 (c 1.230, CHCl_3).

31
32
33
34
35 **38.** Pale brown solid. $^1\text{H NMR}$ (CDCl_3) δ : 3.40 (t, J = 9.1 Hz, 1H), 3.54 (d, J = 10.3 Hz,
36
37
38 1H), 3.76–3.80 (s \times 2 + m, total 7H), 4.09 (q, J = 8.5 Hz, 1H), 5.75 (br s, 1H), 6.86–6.90
39
40
41 (m, 2H), 7.17–7.20 (m, 2H). MS (FI^+) m/z : 249 (M^+). HRMS (FI^+) for $\text{C}_{13}\text{H}_{15}\text{NO}_4$ (M^+):
42
43
44
45 calcd, 249.10011; found, 249.09991. IR (ATR) cm^{-1} : 3226, 1738, 1718, 1692.
46
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51
52 Enantiomeric excess was determined by HPLC analysis with CHIRALPAK ID column
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(80:20 hexane:EtOH, 1.0 mL/min, 254 nm); minor enantiomer $t_r = 16.3$ min, major enantiomer $t_r = 13.6$ min; 99.1% ee; $[\alpha]^{25}_D -145$ (c 0.187, EtOH).

(3*S*,4*R*)-4-(4-Methoxyphenyl)-2-oxopyrrolidine-3-carboxylic Acid (39). Compound **39** (1.85 g, 98%) was prepared from **38** (2.00 g, 8.02 mmol) by the same method as that used for **36**. Pale brown solid. $^1\text{H NMR}$ (DMSO- d_6) δ : 3.16 (t, $J = 9.1$ Hz, 1H), 3.42 (d, $J = 10.9$ Hz, 1H), 3.55 (t, $J = 8.5$ Hz, 1H), 3.72 (s, 3H), 3.79 (q, $J = 9.1$ Hz, 1H), 6.88 (d, $J = 8.5$ Hz, 2H), 7.24 (d, $J = 9.1$ Hz, 2H), 8.03 (s, 1H), 12.5 (br s, 1H). MS (ESI $^+$) m/z : 236 ($M^+ + H$). HRMS (ESI $^+$) for $\text{C}_{12}\text{H}_{14}\text{NO}_4$ ($M^+ + H$): calcd, 236.09228; found, 236.09242. IR (ATR) cm^{-1} : 3240, 1686. $[\alpha]^{26}_D -139$ (c 0.148, EtOH).

1-(4-Chlorophenyl)-3-[(3*S*,4*R*)-4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (11a). Compound **11a** (40 mg, 24%) was prepared from **39** (110 mg, 0.47 mmol) by the same method as that used for **10**. White powder. Mp: 192–195 °C. $^1\text{H NMR}$ (DMSO- d_6) δ : 3.13–3.20 (m, 1H), 3.43–3.50 (m, 2H), 3.72 (s, 3H), 4.48 (dd, $J = 10.9, 9.1$ Hz, 1H), 6.47 (d, $J = 9.1$ Hz, 1H), 6.86–6.90 (m, 2H), 7.22–7.26 (m, 2H), 7.28–7.32 (m, 2H), 7.37–7.41 (m, 2H), 7.92 (s, 1H), 8.68 (s, 1H). MS (ESI $^+$) m/z : 360 ($M^+ + H$). HRMS (ESI $^+$) for $\text{C}_{18}\text{H}_{19}\text{ClN}_3\text{O}_3$ ($M^+ + H$): calcd, 360.11149; found, 360.11076. IR (ATR) cm^{-1} : 3333, 1669.

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3
4 Enantiomeric excess was determined by HPLC analysis with CHIRALPAK ID column
5
6
7 (50:50 hexane:EtOH, 1.0 mL/min, 254 nm); minor enantiomer $t_r = 11.1$ min, major
8
9
10 enantiomer $t_r = 7.1$ min; 99.7% ee; $[\alpha]^{28}_D -130$ (c 0.101, EtOH). HPLC purity 97.4%.

11
12
13
14 **1-(4-Fluorophenyl)-3-[(3*S*,4*R*)-4-(4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (11b).**

15
16
17 Compound **11b** (44 mg, 18%) was prepared from **39** (170 mg, 0.773 mmol) and 4-
18
19
20 fluoroaniline (140 μ L, 1.45 mmol) by the same method as that used for **10**. White powder.

21
22
23
24 Mp: 203–204 °C. $^1\text{H NMR}$ (DMSO- d_6) δ : 3.13–3.20 (m, 1H), 3.42–3.50 (m, 2H), 3.72 (s,
25
26
27 3H), 4.47 (dd, $J = 10.9, 9.1$ Hz, 1H), 6.41 (d, $J = 8.5$ Hz, 1H), 6.86–6.90 (m, 2H), 7.01–7.07
28
29
30 (m, 2H), 7.28–7.32 (m, 2H), 7.33–7.39 (m, 2H), 7.91 (s, 1H), 8.55 (s, 1H). MS (ESI $^+$) m/z :
31
32
33 344 ($M^+ + H$). HRMS (ESI $^+$) for $C_{18}H_{19}FN_3O_3$ ($M^+ + H$): calcd, 344.14104; found,
34
35
36 344.14061. IR (ATR) cm^{-1} : 3345, 1673. $[\alpha]^{28}_D -156$ (c 0.100, EtOH). HPLC purity: 99.6%.

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41 **(*E*)-2,6-Difluoro-4-methoxy-(2-nitrovinyl)benzene (41).** Ammonium acetate (11.2 g, 145
42
43 mmol) and nitromethane (22.9 mL, 427 mmol) were added to a solution of **40** (14.7 g, 85.4 mmol)
44
45 in acetic acid (85 mL), the mixture was stirred at 100 °C for 6 h, and concentrated in vacuo. Water
46
47 (50 mL) was added to the residue and the resulting precipitates were collected by filtration. The
48
49 precipitates were washed with water and dried in vacuo to give **41** (17.5 g, 95%) as a yellow solid.
50
51
52 $^1\text{H NMR}$ (CDCl_3) δ : 3.87 (s, 3H), 6.56 (d, $J = 10.3$ Hz, 2H), 7.77 (d, $J = 13.9$ Hz, 1H), 8.11 (d, J

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3 = 13.9 Hz, 1H). MS (EI⁺) *m/z*: 215 (M⁺). HRMS (EI⁺) for C₉H₇F₂NO₃ (M⁺): calcd, 215.03940;
4
5 found, 215.03889. IR (ATR) cm⁻¹: 1624, 1571, 1509.
6
7

8 **Dimethyl (*R*)-[1-(2,6-Difluoro-4-methoxyphenyl)-2-nitroethyl]malonate (42).** Compound **42**

9 (22.5 g, 95%) was prepared from **41** (14.7 g, 68.3 mmol) by the same method as that used for **37**.

10 White solid. ¹H NMR (CDCl₃) δ: 3.57 (s, 3H), 3.77 (s, 3H), 3.80 (s, 3H), 3.92 (d, *J* = 10.4 Hz,
11
12 1H), 4.66 (dt, *J* = 9.8, 4.9 Hz, 1H), 4.81 (dd, *J* = 12.8, 9.8 Hz, 1H), 4.91 (dd, *J* = 12.8, 4.9 Hz, 1H),
13
14 6.41–6.47 (m, 2H). MS (FI⁺) *m/z*: 347 (M⁺). HRMS (FI⁺) for C₁₄H₁₅F₂NO₇ (M⁺): calcd,
15
16 347.08166; found, 347.082160. IR (ATR) cm⁻¹: 1726, 1551. [α]_D²⁴ -25.0 (*c* 0.106, EtOH).
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23 **Methyl (3*S*,4*R*)-4-(2,6-Difluoro-4-methoxyphenyl)-2-oxopyrrolidine-3-carboxylate (43).**

24 Compound **43** (10.5 g, 55%) was prepared from **42** (23.3 g, 67.0 mmol) by the same method as
25
26 that used for **38**. White solid. ¹H NMR (CDCl₃) δ: 3.51 (t, *J* = 9.2 Hz, 1H), 3.67 (dt, *J* = 9.2, 1.2
27
28 Hz, 1H), 3.78–3.81 (m, 7H), 4.46 (q, *J* = 9.2 Hz, 1H), 6.24 (br, 1H), 6.47 (d, *J* = 10.4 Hz, 2H).
29
30 MS (ESI⁺) *m/z*: 286 (M⁺ + H). HRMS (ESI⁺) for C₁₃H₁₄F₂NO₄ (M⁺ + H): calcd, 286.08909; found,
31
32 286.08922. IR (ATR) cm⁻¹: 3219, 1742, 1706. [α]_D²⁴ -120 (*c* 0.106, EtOH).
33
34
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36
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38 **(3*S*,4*R*)-4-(2,6-Difluoro-4-methoxyphenyl)-2-oxopyrrolidine-3-carboxylic Acid (44).**

39 Compound **44** (9.85 g, 100%) was prepared from **43** (10.4 g, 36.4 mmol) by the same method as
40
41 that used for **39**. White solid. ¹H NMR (DMSO-*d*₆) δ: 3.25 (t, *J* = 9.2 Hz, 1H), 3.43 (d, *J* = 10.4
42
43 Hz, 1H), 3.56 (t, *J* = 9.2 Hz, 1H), 3.76 (s, 3H), 4.14 (q, *J* = 9.2 Hz, 1H), 6.73–6.79 (m, 2H), 8.20
44
45 (s, 1H), 12.8 (br, 1H). MS (ESI⁺) *m/z*: 272 (M⁺ + H). HRMS (ESI⁺) for C₁₂H₁₂F₂NO₄ (M⁺ + H):
46
47 calcd, 272.07344; found, 272.07428. IR (ATR) cm⁻¹: 3263, 1726, 1638. [α]_D²³ -121 (*c* 0.100,
48
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53 EtOH).
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Benzyl [(3*S*,4*R*)-4-(2,6-difluoro-4-methoxyphenyl)-2-oxopyrrolidin-3-yl]carbamate (45).

To a solution of **44** (3.00 g, 11.1 mmol) in toluene (157 mL) and MeCN (42 mL) were added triethylamine (1.70 mL, 12.2 mmol) and diphenylphosphoryl azide (3.00 mL, 13.4 mmol), and the mixture was stirred at room temperature for 2 h. Benzyl alcohol (5.70 mL, 55.1 mmol) was added to the reaction mixture, and the mixture was stirred at 80 °C for 30 min and then 110 °C for 2 h. After removal of MeCN in vacuo, the resulting mixture was washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. A mixture of the residue and benzyl alcohol (11.3 mL, 109 mmol) was stirred at 110 °C for 23 h. Flash chromatography (hexane/AcOEt = 4:1 → 1:1 → AcOEt) of the resulting mixture gave **45** (1.93 g, 47%). ¹H NMR (CDCl₃) δ: 3.48–3.62 (m, 2H), 3.79 (s, 3H), 3.80–3.96 (m, 1H), 4.67–4.75 (m, 1H), 5.05 (s, 2H), 5.37 (br d, 1H), 6.46 (br d, 2H), 6.60 (s, 1H), 7.26–7.36 (m, 5H). MS (ESI⁺) *m/z*: 377 (M⁺ + H). HRMS (ESI⁺) for C₁₉H₁₉F₂N₂O₄ (M⁺ + H): calcd, 377.13129; found, 377.13184. [α]_D²⁴ -107 (*c* 0.102, EtOH).

(3*S*,4*R*)-3-Amino-4-(2,6-difluoro-4-methoxyphenyl)pyrrolidin-2-one (46). A suspension of **45** (811 mg, 2.15 mmol) and 10% Pd–C (wetted with ca. 55% water, 81.0 mg) in EtOH (30 mL) was stirred at room temperature for 2 h under H₂ atmosphere. After the insoluble materials were filtered off, the filtrate was concentrated in vacuo. Flash chromatography (hexane/AcOEt = 1:1 → AcOEt → AcOEt/MeOH = 5:1) of the residue gave **46** (525 mg, 100%). ¹H NMR (DMSO-*d*₆) δ: 1.78 (br s, 2H), 3.22 (t, *J* = 8.6 Hz, 1H), 3.34–3.48 (m, 3H), 3.76 (s, 3H), 6.74 (d, *J* = 11.0 Hz, 2H), 7.88 (br s, 1H). MS (ESI⁺) *m/z*: 243 (M⁺ + H). HRMS (ESI⁺) for C₁₁H₁₃F₂N₂O₂ (M⁺ + H): calcd, 243.09451; found, 243.09492. [α]_D²⁴ -90.1 (*c* 0.110, EtOH).

1-[(3*S*,4*R*)-4-(2,6-Difluoro-4-methoxyphenyl)-2-oxopyrrolidin-3-yl]-3-(4-fluorophenyl)urea

(12g). To a mixture of **44** (3.00 g, 11.1 mmol), toluene (110 mL) and MeCN (30 mL) was added triethylamine (1.69 mL, 12.2 mmol) and diphenylphosphoryl azide (2.72 mL, 12.2 mmol), and the mixture was stirred at room temperature for 1 h and then at 80 °C for 30 min. To the reaction mixture was added 4-fluoroaniline (2.11 mL, 22.0 mmol), and the mixture was stirred at 110 °C for 2 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo. To the residue was added 1 M HCl, and the mixture was extracted with AcOEt. The extract was washed with water and brine, dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 1:1 → AcOEt → AcOEt/MeOH = 95:5) of the residue gave **12g** (2.29 g, 55%) as a white powder. Mp: 194–197 °C ¹H NMR (DMSO-*d*₆) δ: 3.31 (t, *J* = 9.7 Hz, 1H), 3.45 (t, *J* = 9.1 Hz, 1H), 3.76 (s, 3H), 3.80 (q, *J* = 10.9 Hz, 1H), 4.58 (dd, *J* = 10.9 8.5 Hz, 1H), 6.47 (d, *J* = 7.9 Hz, 1H), 6.75 (d, *J* = 10.3 Hz, 2H), 6.99–7.06 (m, 2H), 7.31–7.37 (m, 2H), 8.07 (s, 1H), 8.68 (s, 1H). MS (ESI⁺) *m/z*: 380 (M⁺ + H). HRMS (ESI⁺) for C₁₈H₁₇F₃N₃O₃ (M⁺ + H): calcd, 380.12220; found, 380.12171. IR (ATR) cm⁻¹: 3316, 1711, 1638. [α]_D²⁸ -156 (*c* 0.101, EtOH). HPLC purity 99.4%.

1-[(3*S*,4*R*)-4-(2,6-Difluoro-4-methoxyphenyl)-2-oxopyrrolidin-3-yl]-3-phenylurea (13c). A mixture of **46** (74.3 mg, 0.306 mmol) and phenyl isocyanate (33.0 μL, 0.306 mmol) in THF (3.1 mL) was stirred at room temperature for 15 min, and concentrated in vacuo. Flash chromatography (hexane/AcOEt = 4:1 → AcOEt) of the residue gave **13c** (83.8 mg, 76%) as a white powder. Mp: 211–213 °C. ¹H NMR (DMSO-*d*₆) δ: 3.31 (t, *J* = 9.1 Hz 1H), 3.46 (t, *J* = 9.1 Hz, 1H), 3.76 (s, 3H), 3.80 (q, *J* = 9.1 Hz, 1H), 4.58 (dd, *J* = 10.9, 8.5 Hz, 1H), 6.47 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 10.9 Hz, 2H), 6.85–6.89 (m, 1H), 7.16–7.27 (m, 2H), 7.31–7.35 (m, 2H), 8.07 (s, 1H), 8.63 (s, 1H). MS (ESI⁺) *m/z*: 362 (M⁺ + H). HRMS (ESI⁺) for C₁₈H₁₈F₂N₃O₃ (M⁺ + H): calcd, 362.13162;

found, 362.13145. IR (ATR) cm^{-1} : 3315, 1709, 1638. $[\alpha]_{\text{D}}^{27} -162$ (*c* 0.103, EtOH). HPLC purity 98.1%.

1-[(3*S*,4*R*)-4-(2,6-difluoro-4-methoxyphenyl)-2-oxopyrrolidin-3-yl]-3-(3,4-difluorophenyl)urea (13i). Compound **13i** (56.6 mg, 86%) was prepared from **46** (40.0 mg, 0.165 mmol) and 3,4-difluorophenyl isocyanate (20.0 μL , 0.165 mmol) by the same method as that used for **13c**. White amorphous solid. ^1H NMR (DMSO- d_6) δ : 3.31 (t, *J* = 9.7 Hz, 1H), 3.46 (t, *J* = 9.1 Hz, 1H), 3.76 (s, 3H), 3.82 (q, *J* = 9.7 Hz, 1H), 4.57 (dd, *J* = 10.9, 8.5 Hz, 1H), 6.59 (d, *J* = 8.5 Hz, 1H), 6.75 (d, *J* = 10.9 Hz, 2H), 6.99–7.04 (m, 1H), 7.22–7.29 (m, 1H), 7.54 (ddd, *J* = 10.3, 7.9, 3.0 Hz, 1H), 8.08 (s, 1H), 8.90 (s, 1H). MS (ESI⁺) *m/z*: 398 (M^+ + H). HRMS (ESI⁺) for $\text{C}_{18}\text{H}_{16}\text{F}_4\text{N}_3\text{O}_3$ (M^+ + H): calcd, 398.11278; found, 398.11214. IR (ATR) cm^{-1} : 3303, 1666, 1638. $[\alpha]_{\text{D}}^{27} -138$ (*c* 0.100, EtOH). HPLC purity 99.5%.

1-[(3*S*,4*R*)-4-(2,6-Difluoro-4-methoxyphenyl)-2-oxopyrrolidin-3-yl]-3-(3-hydroxy-4-methylphenyl)urea (13l). Compound **13l** (105 mg, 65%) was prepared from **44** (130 mg, 0.387 mmol) and 5-amino-2-methylphenol (95.0 mg, 0.771 mmol) by the same method as that used for **12g**. White amorphous solid. ^1H NMR (DMSO- d_6) δ : 1.99 (s, 3H), 3.30 (t, *J* = 9.7 Hz, 1H), 3.45 (t, *J* = 9.7 Hz, 1H), 3.72–3.80 (m, 4H), 4.58 (dd, *J* = 10.9, 8.5 Hz, 1H), 6.32 (d, *J* = 8.5 Hz, 1H), 6.56 (dd, *J* = 7.9, 2.4 Hz, 1H), 6.74 (d, *J* = 10.9 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.99 (d, *J* = 2.4 Hz, 1H), 8.07 (s, 1H), 8.41 (s, 1H), 9.10 (s, 1H). MS (ESI⁺) *m/z*: 392 (M^+ + H). HRMS (ESI⁺) for $\text{C}_{19}\text{H}_{20}\text{F}_2\text{N}_3\text{O}_4$ (M^+ + H): calcd, 392.14219; found, 392.14299. IR (ATR) cm^{-1} : 3302, 1638. $[\alpha]_{\text{D}}^{25} -151$ (*c* 0.151, EtOH). HPLC purity 97.9%.

1-(5-Chlorothiophen-2-yl)-3-[(3*S*,4*R*)-4-(2,6-difluoro-4-methoxyphenyl)-2-oxopyrrolidin-3-yl]urea (13n). To a mixture of 2-chlorothiophene-5-carboxylic acid (60.0 mg, 0.369 mmol), triethylamine (51.0 μL , 0.369 mmol) in toluene (3.7 mL) was added diphenylphosphoryl azide

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3 (83.0 μ L, 0.369 mmol), and the mixture was stirred at 40 °C for 1 h, 50 °C for 1 h, and then at 100
4 °C for 1 h. After addition of **46** (44.8 mg, 0.185 mmol), the mixture was stirred at 100 °C for 15
5 min. After addition of **46** (26.6 mg, 0.110 mmol), the mixture was stirred at 100 °C for 3 h, and
6 concentrated in vacuo. Flash chromatography (hexane/AcOEt = 3:1 \rightarrow AcOEt) of the residue gave
7 **13n** (45.0 mg, 38%) as a white powder. Mp: 180–183 °C. ¹H NMR (DMSO-*d*₆) δ : 3.31 (t, *J* =
8 9.7 Hz, 1H), 3.46 (t, *J* = 9.1 Hz, 1H), 3.76 (s, 3H), 3.86 (q, *J* = 9.7 Hz, 1H), 4.54 (dd, *J* = 10.9, 8.5
9 Hz, 1H), 6.22 (d, *J* = 4.2 Hz, 1H), 6.72–6.79 (m, 4H), 8.08 (s, 1H), 9.95 (s, 1H). MS (ESI⁺) *m/z*:
10 402 (M⁺ + H). HRMS (ESI⁺) for C₁₆H₁₅ClF₂N₃O₃S (M⁺ + H): calcd, 402.04907; found,
11 402.04877. IR (ATR) cm⁻¹: 3275, 1713, 1664, 1638. [α]_D²⁷ -135 (*c* 0.105, EtOH). HPLC purity
12 99.8%.
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30 Pharmacology and Biology

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33 Animal studies were carried out in accordance with American Association for
34 Accreditation of Laboratory Animal Care guidelines and protocols were approved by
35 Bristol-Myers Squibb and University of California San Diego Animal Care and Use
36 committees.
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47 **Calcium Mobilization Assays.** HEK293 cells were obtained from NIHS (JCRB; Cell
48 number JCRB9068) and maintained in Dulbecco's Modified Eagle Medium (Thermo
49 Fisher Scientific, Waltham, MA) supplemented with 10% heat-inactivated fetal bovine
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3 serum and 100 U/mL penicillin/streptomycin at 37°C under 5% CO₂. HEK293 cells were
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5
6
7 transfected with plasmids encoding human FPR1, human FPR2, or mouse FPR2 together
8
9
10 with Gα15 using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's
11
12
13 protocol. After 24 h incubation, the HEK293 cells expressing FPRs/Gα15 were re-seeded
14
15
16
17 in Biocoat Poly-D-Lysine 96-well plates (7 x 10⁴ cells/well) and further incubated for an
18
19
20 additional day. Changes in intracellular Ca²⁺ were measured with a FlexStation III
21
22
23 scanning fluorometer (Molecular Devices Co., Ltd.) using a Fluo-4 NW Calcium Assay Kit
24
25
26
27 (Invitrogen). Briefly, cell culture medium was replaced to dye loading solution (90 μL/well)
28
29
30 and incubated for 45 min at 37°C under 5% CO₂. After removing dye loading solution, 90
31
32
33 μL/well of assay buffer was added to each well, and plates were mounted in the
34
35
36
37 FlexStation III. The fluorescence intensity (Excitation at 485 nm, and Emission at 525 nm)
38
39
40 was measured for 80 sec at 1.5 sec interval after adding the assay solutions including
41
42
43 test compounds (10 μL/well) to cells. The value was quantified as the maximal peak
44
45
46
47 height, which was calculated automatically by subtracting the basal value from the
48
49
50 maximal value during each measurement. Curve fitting and calculation of EC₅₀ were
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52
53 performed by nonlinear regression analysis of the dose–response curves generated using
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3 Prism 4 (GraphPad Software, Inc., San Diego, CA). Compound 43 (Figure 1) was used
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5
6
7 as an internal standard: hFPR2 $EC_{50} = 8.4$ nM (SD = 7.4, n = 173), hFPR1 $EC_{50} = 9.0$ nM
8
9
10 (SD = 7.1, n = 75).
11
12

13
14 **FPR2 and FPR1 Cyclic Adenosine Monophosphate (cAMP) Assays.** A mixture of
15
16 forskolin (5 μ M final for FPR2 or 10 μ M final for FPR1) and IBMX (200 μ M final) were
17
18 added to 384-well Proxiplates (Perkin-Elmer) pre-dotted with test compounds in DMSO
19
20
21 (1% final) at final concentrations in the range of 1.7 nM to 100 μ M. Chinese Hamster
22
23
24
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26
27
28 Ovary cells (CHO) overexpressing human FPR1 or human FPR2 receptors were cultured
29
30
31 in F-12 (Ham's) medium supplemented with 10% qualified FBS, 250 μ g/ml zeocin and
32
33
34
35 300 μ g/ml hygromycin (Life Technologies). Reactions were initiated by adding 2,000
36
37
38 human FPR2 cells per well or 4,000 human FPR1 cells per well in Dulbecco's PBS (with
39
40
41 calcium and magnesium) (Life Technologies) supplemented with 0.1% BSA (Perkin-
42
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45 Elmer). The reaction mixtures were incubated for 30 min at room temperature. The level
46
47
48
49 of intracellular cAMP was determined using the HTRF HiRange cAMP assay reagent kit
50
51
52 (Cisbio) according to manufacturer's instruction. Solutions of cryptate conjugated anti-
53
54
55
56 cAMP and d2 fluorophore-labelled cAMP were made in a supplied lysis buffer separately.
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58
59
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4 Upon completion of the reaction, the cells were lysed with equal volume of the d2-cAMP
5
6
7 solution and anti-cAMP solution. After a 1-h room temperature incubation, time-resolved
8
9
10 fluorescence intensity was measured using the Envision (Perkin-Elmer) at 400 nm
11
12
13 excitation and dual emission at 590 nm and 665 nm. A calibration curve was constructed
14
15
16 with an external cAMP standard at concentrations ranging from 1 μ M to 0.1 pM by plotting
17
18
19 the fluorescent intensity ratio from 665 nm emission to the intensity from the 590 nm
20
21
22 emission against cAMP concentrations. The potency and activity of a compound to inhibit
23
24
25 cAMP production was then determined by fitting to a 4-parametric logistic equation from
26
27
28 a plot of cAMP level versus compound concentrations Values are an average of at least
29
30
31
32
33
34
35 2 test occasions.

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38 **Chemotaxis Assay.** HL-60 cells were differentiated for 5 days in 1.2% DMSO. Assay
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41 media was phenol red free RPMI with 0.2% fatty acid free BSA. Approximately 1×10^6
42
43
44 cells were added to the upper chamber of a transwell-plate (Corning#3387). Migration
45
46
47 was induced by placing chemoattractant in the bottom chamber and the dHL-60 cells in
48
49
50 the top chamber. Following migration, dHL-60 cells in the lower chamber (migrated
51
52
53 fraction) were quantitated using a luminescence cell viability assay (Promega, G7571).
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3 **Phagocytosis.** Mice were injected i.p. with 1ml of 2% BioGel P100 solution (BioRad,
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6
7 Inc.). After 4 days, the peritoneum was lavaged with PBS/2mM EDTA. Residual BioGel
8
9
10 particles were removed by passing the exudate through a 40 μm strainer. Cells were
11
12
13 washed with PBS and then seeded into 96-well plates (Costar 3904) at a density of
14
15
16
17 1.2×10^5 cells/well. After 90min, non-adherent cells were removed by washing with PBS
18
19
20 and the macrophages were incubated overnight in Macrophage-SFM media
21
22
23 (ThermoFisher, Inc.). Macrophages were treated with test compound for 15 minutes and
24
25
26
27 then fed opsonized FITC labeled zymosan (1:8 ratio of cells:zymosan) for 45 min at 37°C.
28
29
30
31 Cells were washed and extracellular fluorescence was quenched with 0.025% Trypan
32
33
34 Blue. Phagocytosis was measured using a SpectraMAX Gemini EM plate reader
35
36
37
38 (Molecular Devices, Inc.).
39
40
41

42 **Pharmacokinetic study in animals.** Male mice (BALB/cCrSlc for oral administration,
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45
46 Crl:CD1(ICR) for intravenous administration), male rats (Crl:CD(SD)), male dogs
47
48
49 (beagle), and male monkeys (cynomolgus) were used. The **13c** suspensions in 0.5% MC
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51
52
53 were orally administered. The **13c** solution in DMSO/PEG400/saline (5/47.5/47.5) was
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3 administered from the vein. The blood was collected to a micro haematocrit capillary tube
4
5
6
7 (EDTA 2K). After the centrifugation, the plasma samples were stored in a freezer until
8
9
10 analysis. The plasma samples were mixed with methanol/acetonitrile (1:1) and the
11
12
13 internal standard solution. After the centrifugation, the supernatant was mixed with
14
15
16 purified water. The concentration of **13c** was determined by a liquid chromatography
17
18
19 tandem mass spectrometry (LC-MS/MS) method.
20
21
22
23

24 The area under plasma concentration curve from 0 to infinity was calculated using
25
26
27 trapezoidal rule. Extrapolation to infinity was accomplished using the elimination rate
28
29
30 constant (k_{el}) calculated from the terminal phase of the plasma concentration-time curve.
31
32
33

34 The half-life ($t_{1/2}$) was calculated as $\ln 2 / k_{el}$. The bioavailability (BA) was determined using
35
36
37 the equation of $BA = (AUC_{0-inf, p.o.} / dose, p.o.) / (AUC_{0-inf, i.v.} / dose, i.v.) \times 100$.
38
39
40

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42 **Metabolic stability of 13c in human and animal liver microsomes.** The suspension of
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44
45 liver microsomes with NADPH regenerating systems was pre-incubated at 37°C for 5 min.
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47
48 By adding **13c** standard solution, the metabolic reaction was initiated. Final
49
50
51 concentrations of **13c** and microsomal protein were 0.1 $\mu\text{mol/L}$ and 1 mg protein/mL,
52
53
54 respectively. After the incubation at 37°C for 0, 2, 5, 10, 15, 20, 30 and 60 min, an aliquot
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3 of the reaction mixture was mixed with methanol/acetonitrile (1/1) and the internal
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5
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7 standard solution, followed by the centrifugation. The supernatant was diluted with
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9
10 purified water. An aliquot of the diluted supernatant was injected into the LC-MS/MS
11
12
13
14 system to determine **13c** concentration. All procedures were run in triplicate. The
15
16
17 elimination rate constant (k_{el}) was calculated from the residual ratio–time curve using five
18
19
20 time points from 5 min to 30 min. Intrinsic hepatic clearance ($CL_{int, in vitro}$) was calculated
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22
23 using the equation of $CL_{int, in vitro}$ (mL/min/mg protein) = k_{el} (min⁻¹) / microsomal protein
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26
27 concentration (mg protein/mL).
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31 **Metabolic stability of 13c in human and animal hepatocytes.** The suspension of
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34 hepatocytes (1×10^6 cells/mL) in Krebs–Henseleit Buffer Modified was pre-incubated at
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37
38 37°C for 5 min. By adding **13c** standard solution to the mixture and mixing gently, the
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40
41 metabolic reaction was initiated. Final concentration of **13c** was 0.1 μ mol/L. After the
42
43
44 incubation at 37°C for 0, 1, 2, 4 and 6 h, an aliquot of the reaction mixture was mixed with
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48 methanol/acetonitrile (1/1), followed by the centrifugation. The supernatant was mixed
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52 with internal standard solution and purified water. An aliquot of the mixture was injected
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3 into the LC-MS/MS system to determine **13c** concentration. All procedures were run in
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6
7 duplicate.

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10 The elimination rate constant (k_{el}) was calculated from the residual ratio–time curve
11
12
13 using three time points from 0 to 2 h. Intrinsic hepatic clearance ($CL_{int, in vitro}$) was
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15
16 calculated using the equation of $CL_{int, in vitro}$ (mL/min/kg) = k_{el} (h⁻¹) / 60 / (hepatocytes
17
18
19 concentration (cells/mL) / hepatocellularity numbers (cells/g liver) / liver weight (g
20
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25 liver/kg)).

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28 **Mouse Myocardial Infarction Studies.** Male C57BL/6 mice were purchased from the
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31 Jackson Laboratory and were 10-12 weeks of age at the time of surgery. Myocardial
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34 infarction was induced by permanent ligation of the left anterior descending coronary
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37 artery (LAD) using two procedures. For 28-day studies mice were anesthetized with a
38
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40 mixture of ketamine (100mg/kg) and xylazine (8mg/kg) given via the intraperitoneal route
41
42
43 followed by oral intubation with a modified endotracheal tube. Mice were placed in a right
44
45
46 lateral position on a circulating-water heating pad for maintenance of normal body
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48
49 temperature and mechanically ventilated at a tidal volume of ~ 0.5ml and ~105
50
51
52 respirations per min. The neck and chest areas were shaved and prepped with a 70%
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4 isopropyl alcohol followed by betadine solution. Following draping for aseptic surgery the
5
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7 heart was exposed via a left thoracotomy in the fourth intercostal space, and the
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9
10 pericardium incised. The descending left coronary artery was regionally located (*ramus*
11
12
13 *interventricularis paraconalis*). Animals were randomized into treatment groups. The
14
15
16 coronary artery was permanently ligated with a silk suture with a tapered curved 6mm
17
18
19 needle. Complete coronary occlusion was confirmed visually by noting the prompt and
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21
22 sustained pallor of the anterior wall distal to the ligation site. For animals designated as
23
24
25 Sham, the snare was placed but not tightened. The chest was closed in layers and the
26
27
28 pneumothorax evacuated. For pain management a local anesthetic (bupivacaine) was
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31 infiltrated subcutaneously along the edges of the incision sites, and buprenorphine (0.05-
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34 0.1 mg/kg) administered immediately post-operatively. Animals were returned to their
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37 cage, given water and standard rodent chow ad libitum, and monitored daily until the
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40 terminal procedure. Mice were dosed daily (QD) with compound **13c**, captopril or with
41
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43 vehicle for 28 days. Dosing was initiated 24 h post MI. Dosing route with compound **13c**
44
45
46 and vehicle was oral (PO) via gavage at a dose volume of 5ml/kg. Treatment with the
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3 angiotensin converting enzyme inhibitor captopril was provided in the drinking water (~
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7 100 mg/kg/day).
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11 **Ex Vivo Passive Mechanics.** Following exposure of the heart via thoracotomy, the heart
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14 was arrested in diastole by rapid intracardiac infusion of ice-cold cardioplegic solution
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16
17 containing high potassium and 2,3 butanedione monoxime. The heart was excised, the
18
19
20 aorta cannulated and the heart perfused with cardioplegia to flush out residual blood. The
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22
23 atrial appendages were trimmed, the hearts weighed and a cardiac balloon inserted into
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25
26 the left ventricle (LV) across the mitral valve. The hearts were mounted onto a pressure-
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29 volume (PV) measurement stand and multiple epicardial surface markers placed onto the
30
31
32 anterior wall. A digital camera was positioned to enable capture of video images of the
33
34
35 anterior surface of the heart. PV curves were obtained by digitally acquiring left ventricular
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38 pressure and volume during balloon inflations. There were 2 to 3 conditioning runs
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41 followed by 2 to 3 data acquisition runs with maximal LV pressure of ~50mmHg.
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49 Synchronized video images were obtained during each data run. Following acquisition of
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52
53 the PV data, the hearts were perfusion-fixed for 5 to 10 minutes by infusion of 10%
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3 formalin into the aortic cannula while maintaining LV balloon pressure at 10 to 15 mm Hg.
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7 Hearts were stored in 10% formalin at room temperature for subsequent histologic
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10 processing
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13 14 15 ASSOCIATED CONTENT

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19 **Supporting Information.** The following files are available free of charge.
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21

22
23 Experimental procedures for compounds **2a–d**, **12a–f,h–l**, **13a,b,d–h,j,k,m,o–q** (PDF),
24
25

26 Molecular Formula Strings for all compound included in the article.
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55 56 Author Contributions

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4 The manuscript was written through contributions of all authors. All authors have given
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7 approval to the final version of the manuscript.
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11 ABBREVIATIONS USED

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13 ACEi, angiotensin-converting enzyme inhibitor; BALF, Bronchoalveolar lavage fluid;
14 cAMP, Cyclic adenosine monophosphate; CHO, Chinese hamster ovary; CL, Clearance;
15 CYP, cytochrome P; DPPA, Diphenyl Phosphorylazide; fMLP aka fMPF, *N*-formyl-
16 methionyl-leucyl-phenylalanine; FPR, formyl peptide receptor; IL, interleukin; LAD, left
17 anterior descending artery; LV, left ventricle; MI, myocardial infarction; P-V, pressure
18 volume; SAA, serum amyloid A
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28 REFERENCES

- 29
30
31 (1) Ye, R. D.; Boulay, F.; Wang, J. M.; Dahlgren, C.; Gerard, C.; Parmentier, M.; Serhan, C. N.;
32
33 Murphy, P. M. International union of basic and clinical pharmacology. LXXIII. Nomenclature
34 for the formyl peptide receptor (FPR) family. *Pharmacol. Rev.* **2009**, *61*, 119–161.
35
36
37 (2) Bao, L.; Gerard, N. P.; Eddy Jr., R. L.; Shows, T. B.; Gerard, C. Mapping of genes for the
38 human C5a receptor (C5AR), human FMLP receptor (FPR), and two FMLP receptor
39 homologue orphan receptors (FPRH1, FPRH2) to chromosome 19. *Genomics* **1992**, *13*,
40 437–440.
41
42
43 (3) Le, Y.; Murphy, P. M.; Wang, J. M. Formyl-peptide receptors revisited. *Trends Immunol.*
44
45 **2002**, *23*, 541–548.
46
47
48 (4) Yang, S. C.; Hwang, T. L. The potential impacts of formylpeptide receptor 1 in inflammatory
49
50
51
52
53
54
55
56
57
58
59
60 diseases. *Front. Biosci., Elite Ed.* **2016**, *8*, 436–449.

- 1
2
3 (5) He, R.; Sang, H.; Ye, R. D. Serum amyloid A induced IL-8 secretion through a G protein-
4 coupled receptor, FPRL1/LXA4R. *Blood* **2003**, *101*, 1572–1581.
5
6
7
8 (6) Chiang, N.; Serhan, C. N.; Dahlén, S-E.; Drazen, J. M.; Hay, D. W. P.; Rovati, G. E.; Shimizu,
9 T.; Yokomizo, T.; Brink, C. The lipoxin receptor ALX: Potent ligand-specific and
10 stereoselective actions in vivo. *Pharmacol. Rev.* **2006**, *58*, 463–489.
11
12
13
14 (7) Perretti, M.; Chiang, N.; La, M.; Fierro, I. M.; Marullo, S.; Getting, S. J.; Solito, E.; Serhan,
15 C. N. Endogenous lipid- and peptide derived anti-inflammatory pathways generated with
16 glucocorticoid and aspirin treatment activate the lipoxin A4 receptor. *Nat. Med.* **2002**, *8*,
17 1296–1302.
18
19
20
21
22
23 (8) Bena, S.; Brancaleone, V.; Wang, J. M.; Perretti, J. M.; Flower, R. J. Annexin A1 interaction
24 with the FPR2/ALX receptor. *J. Biol. Chem.* **2012**, *287*, 24690–24697.
25
26
27
28 (9) Cattaneo, F.; Parisi, M.; Ammendola, R. Distinct signaling cascades elicited by different
29 formyl peptide receptor 2 (FPR2) agonists. *Int. J. Mol. Sci.* **2013**, *14*, 7193–7230.
30
31
32
33 (10) Nash, N.; Scully, A.; Gardeli, L.; Olsson, R.; Gustafsson, M. Use of the Lipoxin Receptor,
34 FPRL1, as a Tool for Identifying Compounds Effective in the Treatment of Pain and
35 Onflammation. PCT Int. Appl. WO 2005/047899, 2005.
36
37
38
39 (11) Schepetkin, I. A.; Khlebnikov, A. I.; Giovannoni, M. P.; Kirpotina, L. N.; Cilibrizzi, A.;
40 Quinn, M. T. Development of small molecule non-peptide formyl peptide receptor (FPR)
41 ligands and molecular modeling of their recognition. *Curr. Med. Chem.* **2014**, *21*, 1478–1504.
42
43
44
45 (12) Corminboeuf, O.; Leroy, X. FPR2/ALXR agonists and the resolution of inflammation. *J.*
46
47
48
49
50
51 (13) Tsai, Y-F.; Yang, S-C.; Hwang, T-L. Formyl peptide receptor modulators: a patent review
52 and potential applications for inflammatory diseases (2012-2015). *Expert Opin. Ther. Pat.*
53
54
55
56
57
58
59
60

- 1
2
3 **2016**, *26*, 1139–1156.
4
- 5 (14) Schepetkin, I. A.; Khlebnikov, A. I.; Kirpotina, L. N.; Quinn, M. T. Antagonism of human
6 formyl peptide receptor 1 with natural compounds and their synthetic derivatives. *Int.*
7
8 *Immunopharmacol.* **2016**, *37*, 43–58.
9
- 10 (15) Bürli, R. W.; Xu, H.; Zou, X.; Muller, L.; Golden, J.; Frohn, M.; Adlam, M.; Plant, M. H.;
11
12 Wong, M.; McElvain, M.; Regal, K.; Viswanadhan, V. N.; Tagari, P.; Hungate, R. Potent
13
14 hFPRL1 (ALXR) agonists as potential anti-inflammatory agents. *Bioorg. Med. Chem. Lett.*
15
16 **2006**, *16*, 3713–3718.
17
- 18 (16) García, R.; Ito, B.; Lupisella, J.; Carson, N.; Hsu, M.; Fernando, G.; Heroux, M.; Bouvier,
19
20 M.; Dierks, E.; Kick, E.; Gordon, D.; Chen, J.; Mintier, G.; Carrier, M.; Onge, S.; Shah, H.;
21
22 Towne, J.; Bucardo, M.; Ma X.; Ryan, C.; Wurtz, N.; Ostrowski, J.; Villarreal, F. Preservation
23
24 of post-infarction cardiac structure and function via long-term oral formyl peptide receptor
25
26 agonist treatment. *J. Am. Coll. Cardiol. Basic Trans. Science* **2019**, *4*, 905-920.
27
28
- 29 (17) Frohn, M.; Xu, H.; Zou, X.; Chang, C.; McElvain, M.; Plant, M. H.; Wong, M.; Tagari, P.;
30
31 Hungate, R.; Bürli, R. W. New ‘chemical probes’ to examine the role of the hFPRL1 (or
32
33 ALXR) receptor in inflammation. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6633–6637.
34
35
- 36 (18) Corminboeuf, O.; Cren, S. 1-(p-Tolyl)cyclopropyl Substituted Bridged Spiro[2.4]heptane
37
38 Derivatives as ALX Receptor Agonists. PCT Int. Appl. WO 2013/171687, 2013.
39
- 40 (19) Bur, D.; Corminboeuf, O.; Cren, S.; Grisostomi, C.; Leroy, X.; Richard-Bikdstein S.
41
42 Fluorinated Aminotriazole Derivatives. PCT Int. Appl. WO2010/143116, 2010.
43
44
- 45 (20) Qin, C. X.; May, L. T.; Li, R.; Cao, N.; Rosli, S.; Deo, M.; Alexander, A. E.; Horlock, D.;
46
47 Bourke, J. E.; Yang, Y. H.; Stewart, A. G.; Kaye, D. M.; Du, X-J.; Sexton, P. M.; Christopoulos,
48
49 A.; Gao, X-M.; Ritchie, R. H. Small-molecule-biased formyl peptide receptor agonist
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3 compound 17b protects agonist myocardial ischaemia-reperfusion injury in mice. *Nat.*
4
5 *Commun.* **2017**, *8*, 14232.
6
7
8 (21) Stalder, A. K.; Lott, D.; Strasser, D. S.; Cruz, H. G.; Krause, A.; Groenen, P. M. A.;
9
10 Dingemanse, J. Biomarker-guided clinical development of the first-in-class anti-inflammatory
11
12 FPR2/ALX agonist ACT-389949. *Br. J. Clin. Pharmacol.* **2017**, *83*, 476–486.
13
14
15 (22) Kirpotina, L. N.; Khlebnikov, A. I.; Schepetkin, I. A.; Ye, R. D.; Rabiet, M.-J.; Jutila, M.
16
17 A.; Quinn, M. T. identification of novel small-molecule agonists for human formyl peptide
18
19 receptors and pharmacophore models of their recognition. *Mol. Pharmacol.* **2010**, *77*,
20
21 159–170.
22
23
24 (23) (a) Zarei, M. A straightforward approach to 2-azetidinones from imines and carboxylic
25
26 acids using dimethylsulfoxide and acetic anhydride. *Tetrahedron Lett.* **2014**, *55*, 5354–5357.
27
28 (b) Zarei, M. β -Lactam preparation via Staudinger reaction with activated dimethylsulfoxide.
29
30 *J. Hetrocycl. Chem.* **2017**, *54*, 1161–1166.
31
32
33 (24) Guo, M.; Min, J.; Li, R.; Zhang, L. Studies on monocyclic β -lactams. (I). Synthesis of 3-
34
35 hydroxy-4-substituted-2-azetidinones. *Gaodeng Xuexiao Huaxue Xuebao* **1992**, *13*, 919–922.
36
37
38 (25) Li, B.; Wang, Y.; Du, D.-M.; Xu, J. Notable and obvious ketene substituent-dependent
39
40 effect of temperature on the stereoselectivity in the Staudinger reaction. *J. Org. Chem.* **2007**,
41
42 *72*, 990–997.
43
44
45 (26) Gairaud, C.; Lappin, G. R. The synthesis of ω -nitrostyrenes. *J. Org. Chem.* **1953**, *18*, 1–3.
46
47
48 (27) Yamada, K.; Kishikawa, K.; Yamamoto, M. Stereospecificity of the photorearrangement
49
50 of nitronate anions and its utilization for stereospecific cleavage of cyclic compounds. *J. Org.*
51
52 *Chem.* **1987**, *52*, 2327–2330.
53
54 (28) Ronsen B.; Upadiyaya, S. P. Process for Preparing Arylpiperidine Carbinol Intermediates
55
56
57
58
59
60

1
2
3 and Derivatives. PCT Int. Appl. WO2000/037443, 2000.
4

- 5 (29) (a) Evans, D. A.; Mito, S.; Seidel, D. Scope and mechanism of enantioselective Michael
6 additions of 1,3-dicarbonyl compounds to nitroalkenes catalyzed by nickel(II)-diamine
7 complexes. *J. Am. Chem. Soc.* **2007**, *129*, 11583–11592. (b) Okino, T.; Hoashi, Y.; Furukawa,
8 T.; Xu, X.; Takemoto, Y. Enantio- and diastereoselective Michael reaction of 1,3-dicarbonyl
9 compounds to nitroolefins catalyzed by a bifunctional thiourea. *J. Am. Chem. Soc.* **2005**, *127*,
10 119–125. (c) Li, H.; Wang, Y.; Tang, L.; Deng, L. Highly enantioselective conjugate addition
11 of malonate and β -ketoester to nitroalkenes: Asymmetric C–C bond formation with new
12 bifunctional organic catalysts based on cinchona alkaloids. *J. Am. Chem. Soc.* **2004**, *126*,
13 9906–9907.
14
15
16
17
18
19
20
21
22
23
24
25
26 (30) Hynes, P. S.; Stuppel, P. A.; Dixon, D. J. Organocatalytic asymmetric total synthesis of
27 (R)-Rolipram and formal synthesis of (3S,4R)-Paroxetine. *Org. Lett.* **2008**, *10*, 1389–1391.
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
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