Identification of RO4597014, a glucokinase activator studied in the clinic for the treatment of type 2 diabetes

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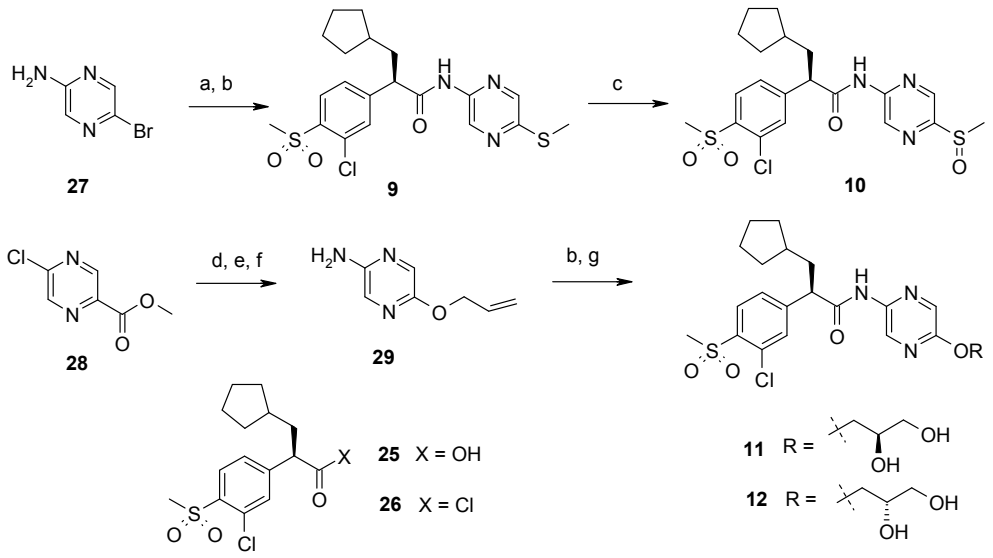
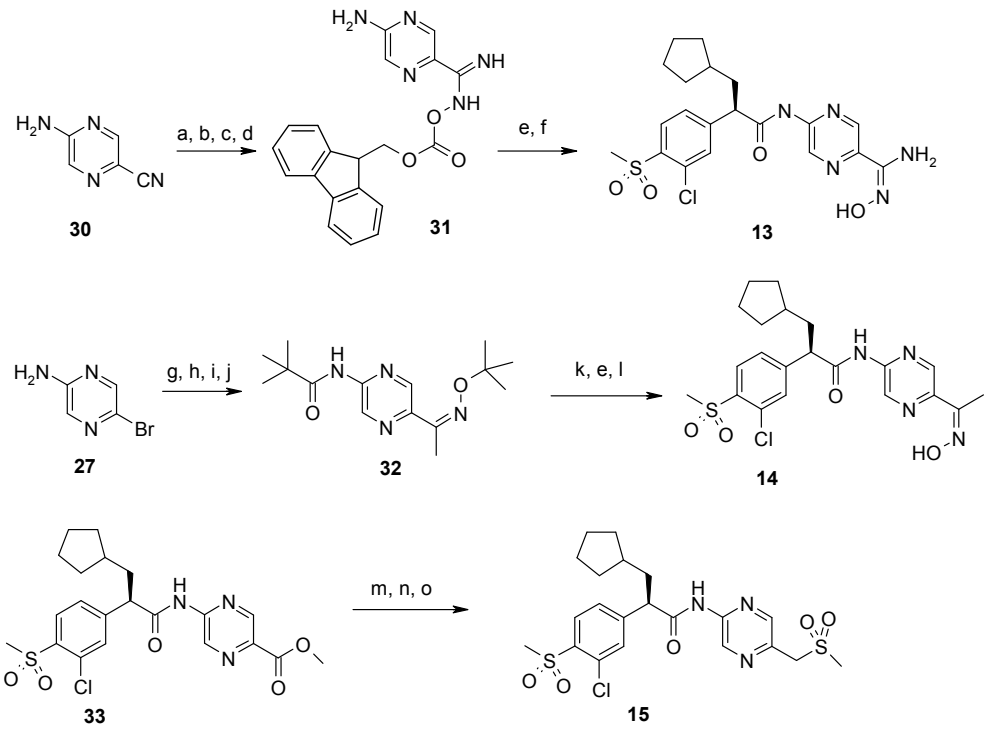
Supporting Information

Scheme S1. Preparation of compounds 9-12



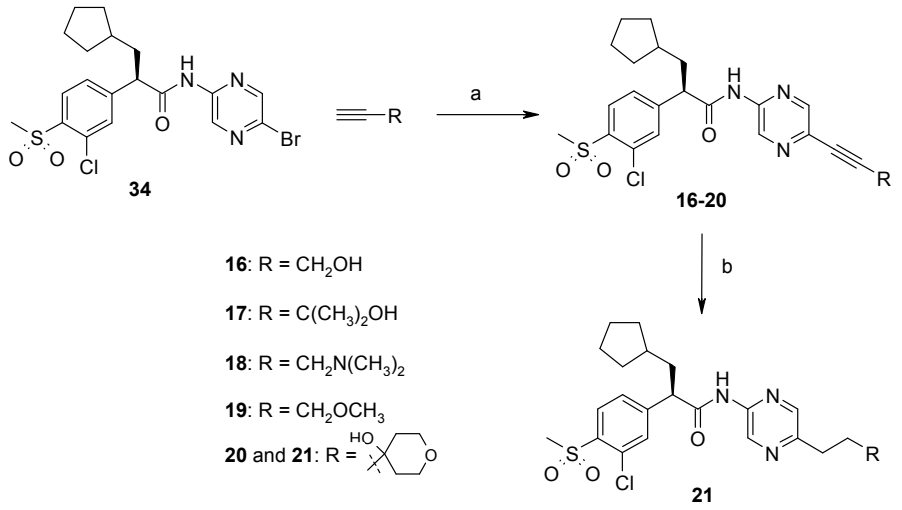
Reagents and conditions: (a) NaSMe, $Pd(PPh_3)_4$ (cat), DMF, 60 °C; (b) **26**, pyridine, CH_2Cl_2; (c) sodium *meta*-periodate, H_2O/THF; (d) allyl alcohol, KOH, 95 °C; (e) diphenylphosphoryl azide, DMF; (f) (i) *tert*-butanol, reflux; (ii) CH_2Cl_2/TFA; (g) $K_3Fe(CN)_6$, K_2CO_3, hydroquinine 1,4-phthalazinediyl diether $(DHQ)_2PHAL$ (cat) or hydroquinidine 1,4-phthalazinediyl diether $(DHQD)_2PHAL$ (cat), OsO_4 (cat), *t*-BuOH/H_2O, 0 °C to rt.

Scheme S2. Synthesis of compounds 13-15



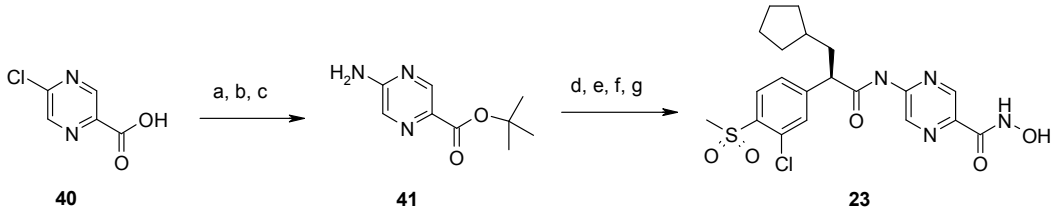
Reagents and conditions: (a) Di-*tert*-butyl dicarbonate, DMAP, *N,N,N',N'*-tetramethylethylenediamine, dioxane; (b) hydroxylamine hydrochloride, piperidine, DMSO; (c) 9-fluorenylmethyl chloroformate, pyridine; (d) CF_3COOH, CH_2Cl_2; (e) **26**, pyridine; (f) triethylamine, pyridine; (g) pivaloyl chloride, pyridine, CH_2Cl_2, (h) 1-ethoxyvinyltributyltin, $PdCl_2(PPh_3)_2$, toluene, reflux; (i) 5% HCl (aq), 0 $^{\circ}$C to rt; (j) O-(*tert*-butyl)hydroxylamine, methanol, reflux; (k) hydrazine hydrate, dioxane, reflux; (l) CF_3COOH, CH_2Cl_2; (m) $NaBH_4$, methanol; (n) PPh_3, CBr_4, THF; (o) sodium methanesulfinate, acetone

Scheme S3. Synthesis of compounds 16-21



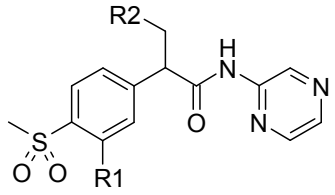
Reagents and conditions: (a) CuI (cat), $PdCl_2(PPh_3)_2$ (cat), $EtN(i\text{-}Pr)_2$, toluene, rt; (b) H_2 (30 psi), 10% Pd/C (cat)

Scheme S4. Preparation of compound 23

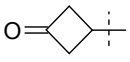
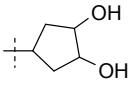


Reagents and conditions: (a) $Cl_3CC{=}NH(OBu^t)$, BF_3/Et_2O, THF, cyclohexane; (b) AgF, acetonitrile; (c) NH_3, THF; (d) compound **26**, pyridine, CH_2Cl_2; (e) CF_3COOH, CH_2Cl_2, rt; (f) (i) oxalyl chloride, CH_2Cl_2; (ii) NH_2OBu^t, pyridine, THF/CH_2Cl_2; (g) CF_3COOH, CH_2Cl_2, 40 °C

Table S1: GK activation potency of compounds 42-49.



42 - 49

Compounds	R1	R2	GK $SC_{1.5}$ (μM)
42	H	cyclopentyl	1.8
43	OH	cyclopentyl	3.0
44	CN	cyclopentyl	0.46
45	CH_3	cyclopentyl	0.67
46	Cl	*iso*-propyl	1.6
47	Cl		>1,000
48	Cl		5.4
49	Cl		200

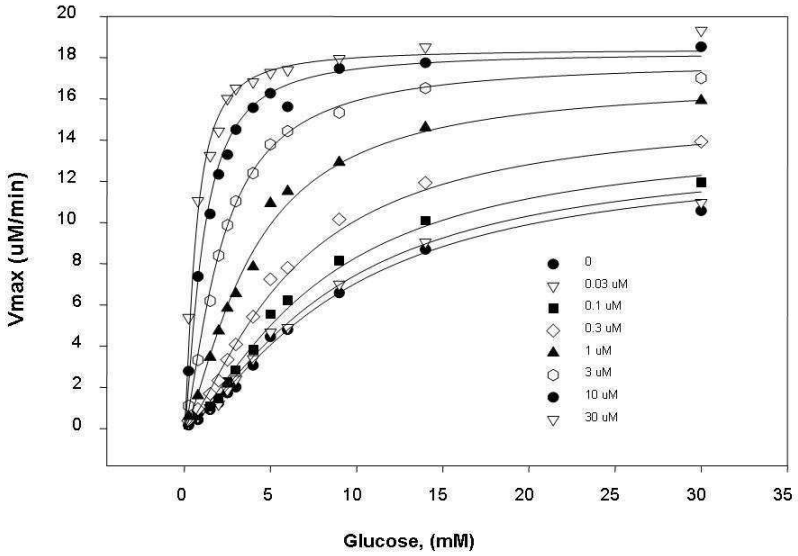


Figure S1. Dose-dependent effects of compound **4** on activating GK enzyme activity

Table S2. **PK-PD relationship of 4 in DIO mice**

4 (po)	Glucose % Change (vs. Vehicle)				Exposures (ng/mL)				AUC	Cmax
Dose	1 hr	2 hr	4 hr	8 hr	1 hr	2 hr	4 hr	8 hr	ng*hr/mL	ng/mL
7.5 mg/kg	-17.4	-26.6	-16.8	-6.3	1590	3030	2190	213	13100	3030
15 mg/kg	-12.9	-32.9	-31.7	-12.7	3180	6190	3840	469	24900	6190
30 mg/kg	-27.4	-46.1	-46.4	-36.5	12600	14500	12500	2110	76100	14500

Table S3. **Metabolite profile of ^{14}C-labeled 2 in primary hepatocytes (in percentage)**

species	m1	m2	m3	m4	m5	parent	Total metabolism (%)
male SD rat	1.8	4.6	0.8	2.3	0.7	89.8	10.2
beagle dog	3.2	3.4	0.9	5.9	1.5	85.1	14.9
cynomolgus monkey	2.5	3.2	0.9	18.0	1.8	73.6	26.4
pooled male and female human	0.4	0.4	0.8	35.0	0.2	63.2	36.8

m1: hydroxylation of **3** on the cyclopentyl ring; m2: hydroxylation of parent **2** on the cyclopentanone ring; m3: amide cleavage of parent **2**; m4: compound **3** (from ketone reduction of **2**); m5: isomer of m1

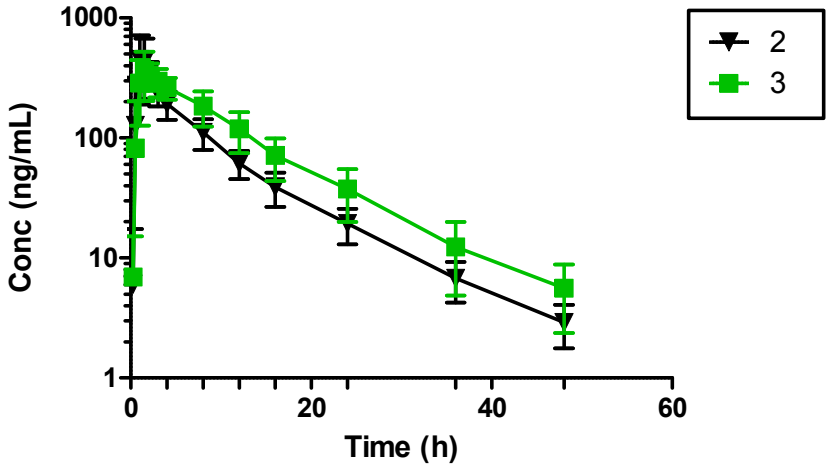
Table S4. Metabolite profile of ^{14}C-labeled 4 in primary hepatocytes (in percentage)

species	M1	M2	M3	M4	parent	Total metabolism (%)
male SD rat	2.3	2.3	1.4		94.0	6.0
male beagle dog	1.3	1.8	1.0		96.0	4.0
male cynomolgus monkey	5.7	10.1	3.7	1.2	79.3	20.7
pooled male and female human	1.5	2.4	1.1		95.0	5.0

Table S5. Metabolite profile of ^{14}C-labeled 4 in liver microsomes (in percentage)

species	M1	M2	M3	M4	M5	M6	parent	Total metabolism (%)
male CD1 mouse	15.8	8.7	2.3	2.5	2.5	1.7	66.3	33.7
male SD rat	26.8	16.5	ND	3.4	2.6	1.8	48.8	51.2
male beagle dog	3.6	5.8	1.3	1.4	1.8		86.2	13.8
male cynomolgus monkey	16.5	42.2	6.0	7.4	3.3	1.7	22.8	77.2
M & F pooled human	15.3	18.2	3.3	2.1	1.4	2.3	57.4	42.6

(A)



(B)

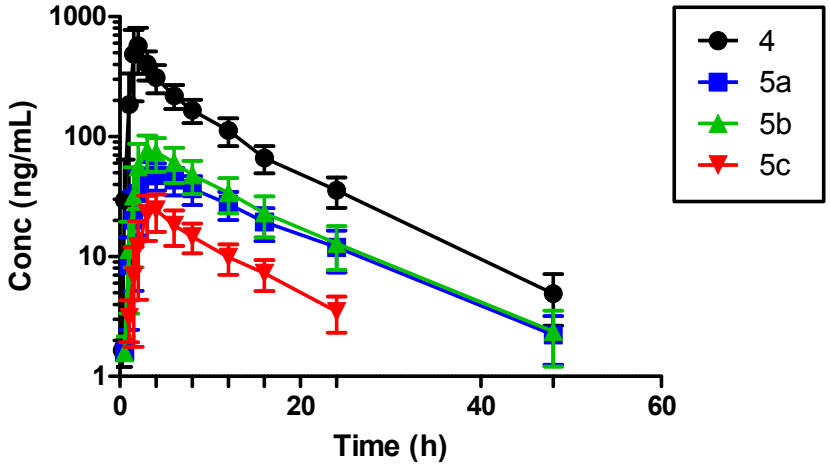


Figure S2. (A) Plasma concentration profile of parent drug and metabolite of compound **2**; (B) Plasma concentration profile of parent drug and metabolites of compound **4**. Both **2** and **4** were dosed at 100 mg orally to type 2 diabetic patients (data from the dose on the first day). Figures show mean and SEM, n= 8.

Table S6. Comparison of mean (%CV) plasma PK parameters of compounds 2 and 4 along with their major metabolites following dosing of 100 mg on day 1 in type 2 diabetic patients (n =8).

Compound	C_{max} (ng/mL)	AUC_{inf} (h*ng/mL)	Metabolite/Parent[a] C_{max} Ratio	Metabolite/Parent[a] AUC_{inf} Ratio
2	582 (38)	2723 (23)	NC[b]	NC[b]
3	435 (28)	3815 (35)	0.74 (33)	1.39 (29)
4	608 (36.4)	4110 (21.8)	NC	NC
5a	50.0 (25.9)	819 (27.9)	0.08 (31.1)	0.19 (24.8)
5b	80.2 (31.4)	1040 (31.5)	0.13 (33.9)	0.24 (26.6)
5c	25.1 (34.0)	290 (28.5)	0.04 (35.2)	0.07 (25.1)

[a]Molar ratio of metabolite/parent drug; [b]NC = not calculated

Experimental

All reactions were carried out under an argon atmosphere. Solvents were purchased from commercial sources and used without further drying. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with Mercury 300 and Unityplus 400 MHz spectrometers. All compounds were analyzed by LC/MS (liquid chromatography/mass spectrometry) using a Waters ZQ mass detector and Waters LC system. Ionization was generally achieved via electrospray (ES). The LC fraction detection consisted of both diode array detector and evaporative light scattering detector and all tested compounds had purity greater than 98%. Optical rotation was measured using a Perkin Elmer polarimeter (Model 241, Na lamp). Analytical chiral HPLC was carried out using a Daicel Chiralpak OD column (250 x 4.6 mm) under isocratic conditions with both diode array detection (wave length of 214 nm and 260 nm) and circular dichroism detection (wave length of 250 nm). Melting points were recorded using a Buchi-510 melting point instrument without calibration. Compound **1** and intermediate **25** were prepared according to procedures published previously.[12]

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-(5-methylsulfanyl-pyrazin-2-yl)-propionamide (9). A mixture of tetrakis(triphenylphosphine)palladium (3.32 g, 2.87 mmol) and 2-amino-5-bromopyrazine (5.00 g, 28.73 mmol) in *N,N*-dimethylformamide (144 mL) was treated with 95% sodium thiomethoxide (4.24 g, 57.47 mmol). The resulting reaction mixture was heated at 60 °C for 10 h. The reaction mixture was allowed to cool to 25 °C and then was poured into a saturated aqueous sodium bicarbonate solution (500 mL). The product was extracted with ethyl acetate (5x200 mL). The combined organic layers were washed with a saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Following flash column chromatography purification (3/7 ethyl acetate/hexanes), 5-methylsulfanyl-pyrazin-2-ylamine was obtained (1.66 g, 40.9%) as an orange solid. Mp 65-67 °C; EI-HRMS (m/e) calcd for $C_5H_7N_3S$ (M^+) 141.0361, found 141.0357.

A solution of 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionic acid (2.34 g, 7.08 mmol) and *N,N*-dimethylformamide (5 drops) in methylene chloride (15 mL) was cooled to 0°C. The reaction mixture was then treated with oxalyl chloride (1.24 mL, 14.16 mmol). The reaction mixture was stirred at 0 °C for 15 min and then at 25 °C for 2 h. The solution was concentrated *in vacuo*, and the yellow semi-solid was dissolved in methylene chloride (8 mL). The resulting solution was added dropwise via an addition funnel at 0 °C to a solution

of 5-methylsulfanyl-pyrazin-2-ylamine (1.0 g, 7.08 mmol) in methylene chloride (5 mL) and pyridine (0.86 mL, 10.6 mmol). The reaction mixture was stirred at 0 °C for 2 h and then at 25 °C overnight. The reaction mixture was quenched with a 1N aqueous citric acid solution (10 mL) and was stirred for 10 min. The mixture was diluted with water (50 mL), methylene chloride (100 mL), and a 1N aqueous citric acid solution (25 mL). The layers were separated, and the organic layer was then washed with a saturated aqueous sodium bicarbonate solution (50 mL) and a saturated aqueous sodium chloride solution (50 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Following flash column chromatography purification (1/1 ethyl acetate/hexanes), compound **9** was obtained (1.75 g, 54%) as a white foam. ES-HRMS (m/e) calcd for $C_{20}H_{24}ClN_3O_3S_2$ $(M+H)^+$ 454.1021, found 454.1026; ^1H NMR ($CDCl_3$) δ ppm 0.97-1.27 (m, 2H), 1.38-2.00 (m, 8H), 2.10-2.36 (m, 1H), 2.59 (s, 3H), 3.27 (s, 3H), 3.48-3.78 (m, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.63 (s, 1 H), 7.92-8.28 (m, 3 H), 9.39 (s, 1 H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-(5-methanesulfinyl-pyrazin-2-yl)-propionamide (10). A solution of 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-(5-methylsulfanyl-pyrazin-2-yl)-propionamide (0.20 g, 0.441 mmol) in THF (3 mL) was added dropwise to a solution of sodium-*meta*-periodate (0.189 g, 0.882 mmol) in water (1.5 mL). The resulting reaction mixture was stirred at 25 °C for 72 h. The reaction mixture was then concentrated *in vacuo*, and the residue was diluted with chloroform (25 mL). The organic layer was washed with water (25 mL), dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Following flash column chromatography purification (2/1 ethyl acetate/hexanes), compound **10** was obtained (96 mg, 46%) as a white foam. ES-HRMS (m/e) calcd for $C_{20}H_{24}ClN_3O_4S_2$ $(M+H)^+$ 470.0970, found 470.0976; ^1H NMR (DMSO-d_6) δ ppm 1.16 (br. s., 2H), 1.37-1.94 (m, 8H), 2.15 (br. s., 1H), 2.82 and 2.85 (2 x s, 3H), 3.35 (br. s., 3H), 4.15 (t, *J* = 7.4 Hz, 1H), 7.64 (d, *J* = 7.7 Hz, 1H), 7.75 (br. s., 1H), 8.03 (d, *J* = 7.7 Hz, 1H), 8.78 (br. s., 1H), 9.36 (br. s., 1H), 11.55 (br. s., 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-2(*R*),3-dihydroxy-propoxy)-pyrazin-2-yl]-propionamide (11). A mixture of methyl 5-chloropyrazine-2-carboxylate (1.7 g, 10 mmol) and allyl alcohol (10 mL, 147 mmol) was heated with stirring to 95 ^oC and then treated with pulverized potassium hydroxide (1.3 g, 23 mmol). Within 10 min, a thick paste developed. The heating was continued for 2 h. The reaction mixture was concentrated to dryness. The residue was treated with toluene (2x50 mL) and further

concentrated to dryness to give 5-allyloxy-pyrazine-2-carboxylic acid potassium salt (2.2 g). This salt (10 mmol) was combined with diphenylphosphoryl azide (2.8 mL, 12.99 mmol) in N,N-dimethylformamide (75 mL), and the suspension was stirred at 25 ^{o}C for 18 h. The reaction mixture was concentrated under reduced pressure, and the residue was diluted with ethyl acetate (50 mL) and water (35 mL). The aqueous phase was back-extracted with ethyl acetate (2x50 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated. The residue was suspended in *tert*-butyl alcohol (25 mL), and then heated under reflux until no gas was observed. The mixture was concentrated and the residue was purified by flash column chromatography to afford 5-allyloxypyrazin-2-yl-carbamic acid *tert*-butyl ester (480 mg, 19%). This material (400 mg, 1.59 mmol) in methylene chloride (2 mL) was treated with a 25% solution of trifluoroacetic acid in methylene chloride (5 mL). The mixture was stirred at rt for 90 min, poured with stirring into a saturated aqueous sodium bicarbonate solution (50 mL) and then sodium chloride (3 g) was added. The resulting mixture was extracted with methylene chloride (3x25 mL), and each of the organic extracts was washed with a small portion of sodium chloride solution. The combined organic extracts were dried over potassium carbonate, filtered, and concentrated to afford 5-allyloxy-pyrazin-2-ylamine (240 mg, 100%) as pale yellow crystals. LRMS calcd for $C_7H_9N_3O$ (m/e) 151, obsd 152, (M+H, ES^+).

This material (237 mg, 1.57 mmol) was coupled to **25** (510 mg, 1.54 mmol) using the same method described in the preparation of **9** to afford N-(5-allyloxy-pyrazin-2-yl)-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide as a white foam (574 mg, 80%). LRMS calcd for $C_{22}H_{26}ClN_3O_4S$ (m/e) 463, obsd 464.1 (M+H, ES^+).

A mixture of potassium ferricyanide (430 mg, 1.3 mmol), potassium carbonate (180 mg, 1.3 mmol), and $(DHQ)_2PHAL$ (8 mg, 0.010 mmol) was treated with a solution of water/*tert*-butyl alcohol (10 mL, 1:1), and the reaction mixture was stirred at rt for 5 min. The mixture was cooled to 0 ^{o}C, and then treated with a 0.2M solution of osmium tetroxide in toluene (20 μL, 0.004 mmol) followed by a mixture of *N*-(5-allyloxypyrazin-2-yl)-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide (210 mg, 0.452 mmol) in water/*tert*-butyl alcohol (3 mL, 1:1). The heterogeneous mixture was stirred for 5 min, the cooling bath was removed, and the stirring continued for 2 h. The mixture was then treated while stirring with ethyl acetate (20 mL) and sodium metabisulfite (150 mg, 0.79 mmol), and the stirring continued for 30 min. The phases were separated, and the aqueous layer was diluted with

water (25 mL) and extracted with ethyl acetate (3x50 mL). The extracts were washed with a saturated aqueous sodium chloride solution (20 mL). The combined organic phases were dried over sodium sulfate, filtered, and concentrated. Purification by flash column chromatography (ethyl acetate/hexanes) afforded **11** (110 mg, 49%) as a white foam. HRMS (m/e) calcd for $C_{22}H_{28}ClN_3O_6S$ $(M+H)^+$ 498.1460, found 498.1462 (ES^+); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 1.07 - 1.20 (m, 2H), 1.37 - 1.80 (m, 8H), 2.07 - 2.16 (m, 1H), 3.33 (s, 3H), 3.42 (t, *J* = 5.7 Hz, 2H), 3.74 - 3.83 (m, 1H), 4.04 - 4.16 (m, 2H), 4.28 (dd, *J* = 10.9, 4.0 Hz, 1H), 4.66 (t, *J* = 5.7 Hz, 1H), 4.94 (d, *J* = 5.3 Hz, 1H), 7.63 (d, *J* = 8.2 Hz, 1H), 7.73 (s, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 8.08 (s, 1H), 8.82 (s, 1H), 10.91 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(2(*S*),3-dihydroxy-propoxy)-pyrazin-2-yl]-propionamide (12). This compound was prepared from the dihydroxylation of *N*-(5-allyloxypyrazin-2-yl)-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide under the same conditions described in the synthesis of **11**, except $(DHQD)_2PHAL$ was used. HRMS (m/e) calcd for $C_{22}H_{28}ClN_3O_6S$ $(M+H)^+$ 498.1460, found 498.1468 (ES^+); ^1H NMR (400 MHz, DMSO-d_6) δ ppm 1.07 - 1.20 (m, 2H), 1.37 - 1.80 (m, 8H), 2.06 - 2.17 (m, 1H), 3.34 (s, 3H), 3.43 (t, *J* = 5.7 Hz, 2H), 3.74 - 3.84 (m, 1H), 4.08 (t, *J* = 7.5 Hz, 1H), 4.15 (dd, *J* = 10.9, 6.6 Hz, 1H), 4.28 (dd, *J* = 10.9, 4.3 Hz, 1H), 4.67 (t, *J* = 5.7 Hz, 1H), 4.94 (d, *J* = 5.3 Hz, 1H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.74 (s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 8.09 (s, 1H), 8.82 (s, 1H), 10.91 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(E)-(*N*-hydroxycarbamimidoyl)-pyrazin-2-yl]-propionamide (13). A solution of 2-amino-5-cyanopyrazine (500.0 mg, 4.16 mmol) in 1,4-dioxane (8.3 mL) was treated with 4-(dimethylamino)pyridine (305.1 mg, 2.5 mmol), *N,N,N′,N′*-tetramethylethylenediamine (241.8 mg, 2.08 mmol), and di-*tert*-butyl dicarbonate (2.9 mL, 12.49 mmol). The reaction was stirred at 25 °C for 20 h and then concentrated *in vacuo*. Flash column chromatography purification (1/9 ethyl acetate/hexanes) afforded 5-[[*bis*[(1,1-dimethylethoxy)carbonyl]]amino]-2-pyrazinecarbonitrile as a white solid (1.87 g). Mp 67-68 °C; ES-HRMS (m/e) calcd for $C_{15}H_{20}N_4O_4$ $(M+Na)^+$ 343.1377, found 343.1379.

A solution of 5-[[*bis*[(1,1-dimethylethoxy)carbonyl]]amino]-2-pyrazinecarbonitrile (1.83 g, 5.72 mmol) in dimethyl sulfoxide (35 mL) was treated with hydroxylamine hydrochloride (2.0 g, 28.8 mmol) and piperidine (3.0 mL, 30.3 mmol). The reaction was stirred at 25 °C for 50 min and then was partitioned between ethyl acetate (500 mL) and water (250 mL). The

organic layer was washed with water and brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (1/3 ethyl acetate/hexanes) afforded the 5-[[bis[(1,1-dimethylethoxy)carbonyl]]amino]-*N*-hydroxy-2-pyrazinecarboximidamide as a white solid (1.12 g, 55%). Mp 185-186 °C; ES-HRMS (m/e) calcd for $C_{15}H_{23}N_5O_5$ $(M+H)^+$ 354.1772, found 354.1775.

A solution of the 5-[[*bis*[(1,1-dimethylethoxy)carbonyl]]amino]-*N*-hydroxy-2-pyrazinecarboximidamide (770.0 mg, 2.18 mmol) in pyridine (20 mL) at 25 °C was treated with 9-fluorenylmethyl chloroformate (680 mg, 2.63 mmol). The reaction mixture was stirred at 25 °C for 45 min. The mixture was then diluted with ethyl acetate, water, and a saturated aqueous sodium chloride solution. The mixture was shaken and separated. The aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography (1/9 to 1/1 ethyl acetate/hexanes) afforded the desired 5-[[*bis*[(1,1-dimethylethoxy)carbonyl]]amino]-*N*-[[[(9*H*-fluoren-9-yl)methoxy]carbonyl]oxy]-2-pyrazine carboximidamide. This material was dissolved in methylene chloride (5 mL). The solution was cooled to 0 °C and then was treated with trifluoroacetic acid (1.6 mL, 20.8 mmol). The reaction mixture was stirred at 0 °C for 30 min and at rt for 30 min. The mixture was then treated with additional trifluoroacetic acid (20 mL, 260 mmol) and stirred at rt for 4 h. The resulting solution was diluted with methylene chloride, washed three times with a saturated aqueous sodium bicarbonate solution, dried over anhydrous sodium sulfate, filtered, concentrated *in vacuo*, and then dried under high vacuum to afford 5-amino-*N*-[[[(9*H*-fluoren-9-yl)methoxy]carbonyl]oxy]-2-pyrazinecarboximidamide (366 mg, 44.9%) as a white solid. LRMS calcd for $C_{20}H_{17}N_5O_3$ (m/e) 375, obsd 376 $(M+H)^+$. This material (210 mg, 0.56 mmol) was coupled with **25** (140 mg, 0.42 mmol) using the same method described in the preparation of **9** to afford (*R*)-3-chloro-α-(cyclopentylmethyl)-*N*-[2-[[[[[(9*H*-fluoren-9-yl)methoxy]carbonyl]oxy]-amino]iminomethyl]-5-pyrazinyl]-4-(methylsulfonyl)benzeneacetamide (151.9 mg, 52.2%) as an off-white foam. LRMS calcd for $C_{35}H_{34}ClN_5O_6S$ (m/e) 687, obsd 688 $(M+H)^+$.

A solution of (*R*)-3-chloro-α.-(cyclopentylmethyl)-*N*-[2-[[[[[(9*H*-fluoren-9-yl)methoxy]carbonyl]oxy]amino]iminomethyl]-5-pyrazinyl]-4-(methylsulfonyl)benzeneacetamide (130.0 mg, 0.189 mmol) in pyridine (2 mL) was treated with triethylamine (0.26 mL, 1.865 mmol) and stirred at rt for 2 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate. The organic layer was

washed with a 0.1N aqueous hydrochloric acid solution followed by an aqueous copper(II) sulfate solution, dried over anhydrous sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (2/1 ethyl acetate/hexanes) afforded compound **13** (42.1 mg, 47.8%) as a white solid. Mp 117-121 °C; ES-HRMS (m/e) calcd for $C_{20}H_{24}ClN_5O_4S$ $(M+H)^+$ 466.1311, found 466.1302; ^{1}H NMR (DMSO-d_6) δ ppm 1.15 (br. s., 2H), 1.28-1.88 (m, 8H), 2.14 (dt, *J* = 13.6, 6.9 Hz, 1H), 3.35 (s, 3 H), 4.14 (t, *J* = 7.3 Hz, 1H), 5.87 (br. s., 2H), 7.64 (d, *J* = 8.4 Hz, 1H), 7.75 (s, 1 H), 8.03 (d, *J* = 8.4 Hz, 1H), 8.78 (s, 1H), 9.26 (s, 1H), 10.01 (s, 1H), 11.27 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(1-(Z)-hydroxyimino-ethyl)-pyrazin-2-yl]-propionamide (14). A solution of 2-amino-5-bromopyrazine (10.00 g, 57.47 mmol) and pyridine (5.6 mL, 68.96 mmol) in methylene chloride (144 mL) was cooled to 0 °C and then was treated slowly with trimethylacetyl chloride (8.6 mL, 69.82 mmol). The resulting reaction mixture was stirred at 0 °C for 30 min and then was allowed to warm to rt where it was stirred for 18 h. At this point, the reaction mixture still contained the starting material. The mixture was treated with an additional amount of trimethylacetyl chloride (4.3 mL, 34.48 mmol) and then stirred at rt for 4 h. The mixture was concentrated *in vacuo* and the resulting residue was diluted with ethyl acetate (700 mL). The organic layer was washed with a 1N aqueous hydrochloric acid solution (2x200 mL) and brine (200 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (1/9 ethyl acetate/hexanes) afforded *N*-(5-bromo-pyrazin-2-yl)-2,2-dimethyl-propionamide (12.19 g, 82%) as a white solid. Mp 122-124 °C; ES-HRMS (m/e) calcd for $C_9H_{12}BrN_3O$ $(M+H)^+$ 258.0237, found 258.0240; ^{1}H NMR ($CDCl_3$) δ ppm 1.35 (s, 9H), 7.94 (br. s., 1H), 8.33 (s, 1H), 9.37 (s, 1H).

A slurry of *N*-(5-bromo-pyrazin-2-yl)-2,2-dimethyl-propionamide (1.30 g, 5.04 mmol) and dichlorobis(triphenylphosphine)palladium(II) (35.3 mg, 0.05 mmol) in toluene (10 mL) was treated with tributyl-(1-ethoxyvinyl)tin (2.00 g, 5.54 mmol). The reaction slurry was then heated under reflux, resulting in a homogeneous yellow solution. After heating under reflux for 15 h, the resulting black mixture was cooled to 0 °C with an ice-water bath. The cooled reaction mixture was treated slowly with a 5% aqueous hydrochloric acid solution (8.4 mL), stirred at 0 °C for 30 min and rt for 24 h. The resulting two layers were separated, and the aqueous layer was extracted with ethyl acetate (100 mL). The organic layer was then diluted with a 10% aqueous ammonium fluoride solution (100 mL), and the resulting mixture was

stirred at rt for 5 h. The solids were filtered, and the filtrate layers were separated. The organic layer was washed with brine (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (15% ethyl acetate in hexanes) afforded *N*-(5-acetyl-pyrazin-2-yl)-2,2-dimethyl-propionamide (1.07 g, 96%) as a white solid. Mp 173-174 °C ; ES-HRMS (m/e) calcd for $C_{11}H_{15}N_3O_2$ $(M+H)^+$ 222.1237, found 222.1240.

A solution of *N*-(5-acetyl-pyrazin-2-yl)-2,2-dimethyl-propionamide (800.0 mg, 3.62 mmol) in methanol (9 mL) and pyridine (9 mL) was treated with *O*-(*tert*-butyl)hydroxylamine hydrochloride (681.1 mg, 5.42 mmol). The resulting mixture was heated under reflux for 30 min and then concentrated *in vacuo*. The resulting residue was diluted with ethyl acetate (50 mL). The organic layer was washed with a 1N aqueous hydrochloric acid solution (50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (1/9 ethyl acetate/hexanes) afforded *N*-[5-(1-*tert*-butoxyimino-ethyl)-pyrazin-2-yl]-2,2-dimethyl-propionamide (1.04 g, 98%) as a white solid. Mp 123-124 °C; EI-HRMS (m/e) calcd for $C_{15}H_{24}N_4O_2$ (M^+) 292.1899, found 292.1901.

A solution of *N*-[5-(1-*tert*-butoxyimino-ethyl)-pyrazin-2-yl]-2,2-dimethyl-propionamide (563.4 mg, 1.93 mmol) in dioxane (5.8 mL) and hydrazine monohydrate (9.6 mL) was heated under reflux for 48 h. The reaction mixture was allowed to cool to rt and then diluted with ethyl acetate (100 mL). The organic layer was washed with brine (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (3/7 ethyl acetate/hexanes) afforded 1-(5-amino-pyrazin-2-yl)-ethanone *O*-*tert*-butyl-oxime (408.4 g, quant.) as a light yellow solid. Mp 113-115 °C; EI-HRMS (m/e) calcd for $C_{10}H_{16}N_4O$ (M^+) 208.1324, found 208.1325. This material (150.2 mg, 0.72 mmol) was coupled with **25** (262.9 mg, 0.79 mmol) to afford the *N*-[5-(1-*tert*-butoxyimino-ethyl)-pyrazin-2-yl]-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide (216.5 mg, 58%) as a white foam. ES-HRMS (m/e) calcd for $C_{25}H_{33}ClN_4O_4S$ $(M+H)^+$ 521.1984, found 521.1994.

A solution of *N*-[5-(1-*tert*-butoxyimino-ethyl)-pyrazin-2-yl]-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide (195.5 mg, 0.38 mmol) in methylene chloride (1.4 mL) was treated with trifluoroacetic acid (2.8 mL). The resulting mixture was stirred at 60 °C overnight and then cooled to rt, diluted with ethyl acetate (50 mL). The mixture was washed with a saturated aqueous sodium bicarbonate solution (2x50 mL), brine

(50 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (1/2 ethyl acetate/hexanes) afforded **14** (110.0 mg, 63%) as an off-white powder. ES-HRMS (m/e) calcd for $C_{21}H_{25}ClN_4O_4S$ $(M+H)^+$ 465.1358, found 465.1363; ^{1}H NMR (DMSO-d_6) δ ppm 1.15 (br. s., 2H), 1.35-1.96 (m, 8H), 2.00-2.38 (m, 1H), 2.17 (br. s., 3H), 3.34 (br. s., 3H), 4.13 (br., 1H), 7.65 (d, J = 7.3 Hz, 1H), 7.75 (br. s., 1H), 8.03 (d, J = 7.3 Hz, 1H), 8.78 (br. s., 1H), 9.27 (br. s., 1H), 11.24 (br. s., 1H), 11.65 (br. s., 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-(5-methanesulfonylmethyl-pyrazin-2-yl)-propionamide (15). A solution of methyl 5-chloropyrazine-2-carboxylate (5.00 g, 28.97 mmol) in acetonitrile (290 mL) was treated with a new bottle of silver(I) fluoride (11.00 g, 86.70 mmol). The reaction setup was covered with aluminum foil, and the reaction mixture was heated at reflux overnight. The mixture was filtered through a pad of Celite, and rinsed with acetonitrile. The filtrate was concentrated *in vacuo*. Flash column chromatography purification (1/3 ethyl acetate/hexanes) afforded 5-fluoro-pyrazine-2-carboxylic acid methyl ester (2.98 g, 66%) as an off-white solid upon cooling. An analytical sample was obtained by trituration with petroleum ether to afford a white crystalline solid. Mp 55.6-56.7 °C; EI-HRMS (m/e) calcd for $C_6H_5FN_2O_2$ (M^+) 156.0335, found 156.0331.

A large steel reaction vessel was charged with a solution of 5-fluoro-pyrazine-2-carboxylic acid methyl ester (17.45 g, 111.78 mmol) in tetrahydrofuran (200 mL). The reaction solution was cooled to 0 °C and was saturated with ammonia gas over 2-3 h. The vessel was then tightly sealed. The reaction was then mechanically agitated and allowed to warm to rt overnight. The vessel was then cooled to -78 °C for 15-20 min and carefully vented, and the contents of the vessel were diluted with diethyl ether (100 mL). The resulting precipitate was isolated via filtration, rinsed with petroleum ether (2x100 mL), and air-dried to afford 5-amino-pyrazine-2-carboxylic acid methyl ester (16.97 g, 99%) as an off-white solid. Mp 229-231 °C; EI-HRMS (m/e) calcd for $C_6H_7N_3O_2$ (M^+) 153.0538, found 153.0537. This material (100 mg, 0.65 mmol) was coupled with compound **25** to provide 5-[2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionylamino]-pyrazine-2-carboxylic acid methyl ester (225.9 mg, 74%) as an white foam. ES-HRMS (m/e) calcd for $C_{21}H_{24}ClN_3O_5S$ $(M+H)^+$ 466.1198, found 466.1204.

A suspension of 5-[2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionylamino]-pyrazine-2-carboxylic acid methyl ester (200 mg, 0.43 mmol) in methanol

(2 mL) was cooled to 0 °C and then treated with sodium borohydride (49.2 mg, 1.29 mmol). The reaction mixture was stirred at 0 °C for 5 min and then at rt for 1.5 h. The reaction was cooled to 0 °C and quenched with water. The mixture was diluted with ethyl acetate (75 mL), washed with a 1N aqueous hydrochloric acid solution (3x75 mL) and brine (75 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification afforded 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-(5-hydroxymethyl-pyrazin-2-yl)-propionamide (110.4 mg, 59%) as a white foam. Mp 78-81 °C (foam to gel); ES-HRMS (m/e) calcd for $C_{20}H_{24}ClN_3O_4S$ $(M+H)^+$ 438.1249, found 438.1252. A solution of 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-(5-hydroxymethyl-pyrazin-2-yl)-propionamide (457.1 mg, 1.04 mmol) in tetrahydrofuran (10 mL) was treated with triphenylphosphine (573.5 mg, 2.19 mmol) and carbon tetrabromide (726.2 mg, 2.19 mmol). The reaction solution was stirred at rt for 6 h and then was concentrated *in vacuo*. Flash column chromatography purification (1/2 ethyl acetate/hexanes) afforded *N*-(5-bromomethyl-pyrazin-2-yl)-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide (337.2 mg, 65%) as a purple foam. ES-HRMS (m/e) calcd for $C_{20}H_{23}BrClN_3O_3S$ $(M+H)^+$ 500.0405, found 500.0410. This material (102.2 mg, 0.20 mmol) in acetone (1 mL) was cooled to 0 °C and then treated with the sodium salt of methanesulfinic acid (31.8 mg, 0.30 mmol). The reaction was stirred at 0 °C for 1 h and then at rt for 4 h. A second aliquot of the sodium salt of methanesulfinic acid (24.0 mg, 0.24 mmol) was added, and the reaction was further stirred at rt overnight. The mixture was concentrated and the residue was diluted with ethyl acetate (50 mL), washed with water (2x25 mL) and brine (25 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (1/1 ethyl acetate/hexanes) afforded **15** (74.9 mg, 73%) as a white foam. ES-HRMS (m/e) calcd for $C_{21}H_{26}ClN_3O_5S_2$ $(M+H)^+$ 500.1075, found 500.1080; ^{1}H NMR (DMSO-d_6) δ ppm 1.14 (d, J = 8.3 Hz, 2H), 1.31-1.88 (m, 8H), 2.02-2.23 (m, 1H), 3.00 (s, 3H), 3.34 (s, 3H), 4.13 (t, J = 7.3 Hz, 1H), 4.68 (s, 2H), 7.64 (d, J = 8.3 Hz, 1H), 7.74 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 8.46 (s, 1H), 9.32 (s, 1 H), 11.31 (s, 1 H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(3-hydroxy-prop-1-ynyl)-pyrazin-2-yl]-propionamide (16). A solution of *N*-(5-bromo-pyrazin-2-yl)-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide (486 mg, 1.0 mmol, prepared from compound **25** and 2-amino-5-bromopyrazine) and propargyl alcohol (84 mg, 1.5 mmol) in toluene (6 mL) was treated with copper(I) iodide (19.2 mg, 0.10 mmol),

dichlorobis(triphenylphosphine)palladium(II) (36 mg, 0.05 mmol), and *N*,*N*-diisopropylethylamine (2 mL). The resulting mixture was stirred at rt overnight and the top clear layer was decanted. The oily residue was treated with 3 mL of toluene followed by 4 mL of hexanes. The solution was discarded and the residue was extracted with dichloromethane and 0.2N hydrochloric acid. The organic layer was washed with a saturated aqueous sodium chloride solution, dried, and concentrated. Purification by flash column chromatography (1/1 ethyl acetate/hexanes) afforded 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(3-hydroxy-prop-1-ynyl)-pyrazin-2-yl]-propionamide (275 mg, 59.5%) as a pale yellow solid. $[\alpha]_D^{20}$ = -60.3 (c = 0.3, $CHCl_3$); HRMS (m/e) calcd for $C_{22}H_{24}ClN_3O_4S$ $(M+H)^+$ 462.1249, found 462.1252 (ES^+); ^1H NMR (DMSO-d_6) δ ppm 1.04 - 1.24 (m, 2H), 1.35 - 1.83 (m, 8H), 2.04 - 2.20 (m, 1H), 3.34 (s, 3H), 4.12 (t, *J* = 7.3 Hz, 1H), 4.34 (d, *J* = 5.1 Hz, 2H), 5.47 (t, *J* = 5.7 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.73 (s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 8.48 (s, 1H), 9.28(s, 1H), 11.31 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-N-[5-(3-hydroxy-3-methyl-but-1-ynyl)-pyrazin-2-yl]-propionamide (17). This compound was prepared under the same conditions described for **13** (84% yield, white fluffy powder). $[\alpha]_D^{20}$ = -67.3 (c = 0.3, $CHCl_3$); HRMS (m/e) calcd for $C_{24}H_{28}ClN_3O_4S$ $(M+H)^+$ 490.1562, found 490.1553 (ES^+); ^1H NMR (DMSO-d_6) δ ppm 1.03 - 1.24 (m, 2H), 1.34 - 1.85 (m, 8H), 1.47 (s, 6H), 2.05 - 2.20 (m, 1H), 3.34 (s, 3H), 4.12 (t, *J* = 7.3 Hz, 1H), 5.63 (br. s., 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.73 (s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 8.45 (s, 1H), 9.27 (s, 1H), 11.31(s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(3-dimethylamino-prop-1-ynyl)-pyrazin-2-yl]-propionamide (18). A solution of N-(5-bromo-pyrazin-2-yl)-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide (486 mg, 1.0 mmol) and 1-dimethylamino-2-propyne (830 mg, 10.0 mmol) in toluene (6 mL) was treated with *N,N*-diisopropylethylamine (1.5 mL), copper(I) iodide (19.2 mg, 0.10 mmol), and dichlorobis(triphenylphosphine)palladium(II) (36.0 mg, 0.05 mmol). The resulting reaction mixture was stirred at rt for 24 h. At this time, the reaction mixture was concentrated and the residue was extracted with methylene chloride and water. The organic layer was dried and concentrated. Purification by flash column chromatography (4/1 ethyl acetate/methanol) afforded 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(3-dimethylamino-prop-1-ynyl)-pyrazin-2-yl]-propionamide (360 mg, 74%) as a pale brown solid. $[\alpha]_D^{20}$ = -56.2 (c = 0.4, $CHCl_3$); HRMS (m/e) calcd for $C_{24}H_{29}ClN_4O_3S$ $(M+H)^+$ 489.1722, found

489.1725 (ES^+); ^{1}H NMR (DMSO-d_6) δ ppm 1.03 - 1.25 (m, 2H), 1.32 - 1.85 (m, 8H), 2.02 - 2.17 (m, 1H), 2.28 (br. s., 6H), 3.32 (s, 2H), 3.33 (s, 3H), 4.11 (t, *J* = 7.1 Hz, 1H), 7.62 (d, *J*=8.1 Hz, 1H), 7.72 (s, 1H), 8.01 (d, *J* = 8.1 Hz, 1H), 8.50 (s, 1H), 9.26 (s, 1H), 11.31 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(3-methoxy-prop-1-ynyl)-pyrazin-2-yl]-propionamide (19). This compound was prepared using the same procedure described in the preparation of compound **16** (69.5% yield) as a white fluffy solid. $[\alpha]_D^{20}$ = -53.8 (c = 0.7, EtOAc); HRMS (m/e) calcd for $C_{23}H_{26}ClN_3O_4S$ $(M+H)^+$ 476.1406, found 476.1405 (ES^+); ^{1}H NMR (DMSO-d_6) δ ppm 1.04 - 1.24 (m, 2H), 1.35 - 1.85 (m, 8H), 2.05 - 2.19 (m, 1H), 3.34 (s, 6H), 4.12 (t, *J* = 7.3 Hz, 1H), 4.37 (s, 2 H), 7.64 (d, *J* = 8.1 Hz, 1 H), 7.73 (s, 1H), 8.03 (d, *J* = 8.1 Hz, 1H), 8.54 (s, 1H), 9.29 (s, 1H), 11.35 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(4-hydroxy-tetrahydropyran-4-yl-ethynyl)-pyrazin-2-yl]-propionamide (20). A solution of tetrahydro-4H-pyran-4-one (1.25 g, 12.5 mmol) in tetrahydrofuran (10 mL) was cooled to 0 ^oC and then treated with a 0.5M solution of ethynylmagnesium bromide in tetrahydrofuran (40 mL, 20 mmol). The mixture was stirred at 0 ^oC for 2 h and at rt for 4 h. The resulting mixture was cooled to 0 ^oC and then diluted with methanol (10 mL). The solvents were concentrated and the residue was extracted with a saturated aqueous ammonium chloride solution and ethyl acetate. The organic layer was washed with a saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered, and concentrated. The residue was dried *in vacuo* to afford a solid as 4-ethynyl-tetrahydro-pyran-4-ol (1.2 g, 76.2%). This material (252 mg, 2.0 mmol) was coupled with *N*-(5-bromo-pyrazin-2-yl)-2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionamide (486 mg, 1.0 mmol) using the same procedure described for the preparation of compound **16** to afford 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(4-hydroxy-tetrahydropyran-4-yl-ethynyl)-pyrazin-2-yl]-propionamide (474 mg, 89.3%) as a fluffy solid. $[\alpha]_D^{20}$ = -51.2 (c = 0.7, EtOAc); HRMS (m/e) calcd for $C_{23}H_{30}ClN_3O_5S$ $(M+H)^+$ 532.1668, found 532.1675 (ES^+); ^{1}H NMR (DMSO-d_6) δ ppm 1.04 - 1.21 (m, 2H), 1.34 - 1.79 (m, 10H), 1.81 - 1.93 (m, 2H), 2.04 - 2.18 (m, 1H), 3.34 (s, 3H), 3.48 - 3.60 (m, 2H), 3.70 - 3.81 (m, 2H), 4.12 (t, *J* = 7.1 Hz, 1H), 5.89 (br. s., 1H), 7.62 (d, *J* = 8.3 Hz, 1H), 7.72 (s, 1H), 8.01 (d, *J* = 8.3 Hz, 1H), 8.50 (s, 1H), 9.26 (s, 1H), 11.33 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(4-hydroxy-tetrahydropyran-4-yl-ethyl)-pyrazin-2-yl]-propionamide (21). Compound **20** (200 mg, 0.376 mmol) in methanol (30 mL) was treated with 10% palladium on activated carbon (39

mg). The reaction mixture was then placed on a Parr shaker under a hydrogen pressure of 30 psi for 4 h. The mixture was filtered, and solvents were concentrated. The residue was purified by flash column chromatography (100% ethyl acetate) to afford 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(4-hydroxy-tetrahydropyran-4-yl-ethyl)-pyrazin-2-yl]-propionamide (169 mg, 84%) as a fluffy solid. $[\alpha]_D^{20}$ = -57.0 (c = 0.6, $CHCl_3$); HRMS (m/e) calcd for $C_{26}H_{34}ClN_3O_5S$ $(M+H)^+$ 536.1981, found 536.1988 (ES^+); ^1H NMR (DMSO-d_6) δ ppm 1.03 - 1.21 (m, 2H), 1.36 - 1.81 (m, 14H), 2.04 - 2.19 (m, 1H), 2.78 (t, *J* = 6.8 Hz, 2H), 3.34 (s, 3H), 3.51 - 3.67 (m, 4H), 4.11 (t, *J* = 7.3 Hz, 1H), 4.32 (br. s., 1H), 7.64 (d, *J* = 8.1 Hz, 1H), 7.72 (s, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 8.28 (s, 1H), 9.18 (s, 1H), 11.04 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(1(*S*),2-dihydroxyethyl)-pyrazin-2-yl]-propionamide (4). A mixture of *N*-(5-bromo-pyrazin-2-yl)-2,2-dimethyl-propionamide (29.67 g, 114.94 mmol), dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloromethane adduct (0.95 g, 1.16 mmol), triethylamine (17.6 mL, 126.27 mmol), and potassium vinyltrifluoroborate (19.25 g, 143.71 mmol) in ethanol (245 mL) was heated at 100 ^oC for 90 min. The reaction mixture was allowed to cool to rt and then concentrated under reduced pressure. The resulting orange slurry was diluted with methylene chloride (200 mL). The organic layer was washed with a 1N aqueous hydrochloric acid solution (2x200 mL), a saturated aqueous sodium bicarbonate solution (200 mL), and a saturated aqueous sodium chloride solution (200 mL). The combined aqueous layers were back-extracted with methylene chloride (200 mL). The combined organic layers were dried over magnesium chloride and decolorizing carbon, filtered, and concentrated *in vacuo*. After flash column chromatography (0% to 10% ethyl acetate in hexanes), 2,2-dimethyl-*N*-(5-vinyl-pyrazin-2-yl)-propionamide (21.64 g, 92%) was obtained as an off-white solid. Mp 80.4-81.8 ^oC; EI-HRMS (m/e) calcd for $C_{11}H_{15}N_3O$ (M^+) 205.1215, found 205.1214; ^1H NMR ($CDCl_3$) δ ppm 1.35 (s, 9H), 5.52 (d, *J* = 11.0 Hz, 1H), 6.23 (d, *J* = 17.4 Hz, 1H), 6.79 (dd, *J* = 17.4, 11.0 Hz, 1H), 7.94 (br. s., 1H), 8.24 (s, 1H), 9.52 (s, 1H).

A mixture of potassium ferricyanide (148.74 g, 450 mmol), potassium carbonate (62.25 g, 450 mmol), and $(DHQ)_2PHAL$ (2.6 g, 3.34 mmol) was treated with a solution of water/*tert*-butyl alcohol (2 L, 1:1), and the reaction mixture was stirred at rt for 15 min. The mixture was cooled to 5 ^oC, treated with a 0.2M solution of osmium tetroxide in toluene (7.5 mL, 1.5

mmol), and then treated with 2,2-dimethyl-*N*-(5-vinyl-pyrazin-2-yl)-propionamide (30.8 g, 150 mmol) which was partially dissolved in water/*tert*-butyl alcohol (150 mL, 1:1). The mixture was stirred at 4.0-5.0 ^{o}C for 18 h using a Neslab Endocal cooling system to control the temperature. While stirring at 4.0 - 5.0 ^{o}C, the mixture was slowly treated with sodium metabisulfite (35 g, 184 mmol) which resulted in effervescence. The cooling bath was removed, and the stirring continued for 15 min. The layers were separated, and the aqueous layer was extracted with ethyl acetate (600 mL). Each organic layer was washed with a saturated aqueous sodium chloride solution (500 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford *N*-[5-(1(*S*),2-dihydroxyethyl)-pyrazin-2-yl]-2,2-dimethyl-propionamide (46 g, crude) as a red oil which was used without further purification. A small amount of this crude material was purified by flash column chromatography (30% to 100% ethyl acetate/hexanes) to give a colorless oil, which was checked by chiral HPLC and showed two enantiomers with a ratio of 14/1. A solution of *N*-[5-(1(*S*),2-dihydroxyethyl)-pyrazin-2-yl]-2,2-dimethyl-propionamide (46 g, slightly wet with solvent, about 150 mmol) in tetrahydrofuran (275 mL) was treated with 2,2-dimethoxypropane (225 mL, 1.88 mol) and p-toluenesulfonic acid monohydrate (3.4 g, 17.9 mmol). The reaction mixture was stirred at rt for 16.5 h and then concentrated *in vacuo*. The residue was dissolved in methylene chloride (600 mL). The organic layer was washed with a saturated aqueous sodium chloride solution (250 mL) and a saturated aqueous sodium bicarbonate solution (250 mL). Each aqueous layer was back-extracted with methylene chloride (250 mL). The combined organic layers were stirred with sodium sulfate (35 g) and Norit A Charcoal (8 g) and then filtered through a pad of Celite. The filtrate was concentrated *in vacuo* to a weight of about 250 g. The material was treated with diethyl ether (300 mL), and the mixture again was concentrated *in vacuo* to a weight of about 350 g, at which time, crystallization began. The mixture was stored in a refrigerator (4 ^{o}C) for 4 h and filtered. The solids were dried in a vacuum oven at 30 ^{o}C for 16 h to afford white crystals (27.6 g, 66%). Mp 144.0-144.5 ^{o}C. Collection of an additional crop from the mother liquor afforded white crystals (9.5 g, 23%) which were comparable in purity to the first crop. HPLC analysis with a chiral column indicated both crops were pure enantiomer (100% ee) as compared to a racemic sample. The two crops were combined to afford the desired *N*-[5-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-pyrazin-2-yl]-2,2-dimethyl-propionamide. ^{1}H NMR ($CDCl_3$) δ ppm 1.33

(s, 9H), 1.50 (s, 3H), 1.54 (s, 3H), 4.01 (dd, *J* = 8.3, 6.4 Hz, 1H), 4.45 (dd, *J* = 8.7, 6.8 Hz, 1H), 5.23 (t, *J* = 6.4 Hz, 1H), 7.97 (br. s., 1H), 8.43 (s, 1H), 9.49 (s, 1H).

A mixture of *N*-[5-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-pyrazin-2-yl]-2,2-dimethyl-propionamide (8.4 g, 30.7 mmol) and potassium carbonate (4.32 g, 31.2 mmol) in methanol (150 mL) was stirred at rt for 16.5 h, at which time, thin layer chromatography suggested partial conversion to a more polar product. Solvent was removed under reduced pressure. The resulting residue was again concentrated *in vacuo* from THF (50 mL). The material was purified by flash column chromatography (ethyl acetate). The early fractions collected allowed for the recovery of the unreacted starting material as a white solid (2.0 g, 24%). The later fractions were concentrated *in vacuo* to provide 5-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-pyrazin-2-ylamine (3.7 g, 63%) as a pale yellow oil. HPLC analysis with a chiral column indicated 100% ee as compared to a racemic sample.

A solution of 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionic acid (6.29g, 19.01 mmol) and N,N-dimethylformamide (2 drops) in methylene chloride (70 mL) was stirred at 2 ^{o}C and then treated with oxalyl chloride (4.15 mL, 45.7 mmol). The mixture was stirred at 2 ^{o}C for 5 min and at rt for 15 min. The reaction mixture was then concentrated *in vacuo*. The residue was dissolved in benzene (25 mL), and the evaporation was repeated. The resulting intermediate **26** was dissolved in methylene chloride (40 mL), cooled under ice bath, and then treated with a solution composed of 5-((S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-pyrazin-2-ylamine (3.65 g, 18.7 mmol), pyridine (4.6 mL, 56.9 mmol) and methylene chloride (40 mL). The mixture was stirred for 16 h without replenishing the cooling bath. The reaction mixture was then treated with a 1N aqueous hydrochloric acid solution (100 mL). The layers were separated, and the aqueous layer was extracted with methylene chloride (75 mL). The organic layers were washed with a saturated aqueous sodium bicarbonate solution (100 mL) and a saturated aqueous sodium chloride solution. The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. After flash column chromatography (1/1 ethyl acetate/hexanes), 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-pyrazin-2-yl]-propionamide (8.9 g, 92%) was obtained as a white foam. ES-HRMS (m/e) calcd for $C_{24}H_{30}ClN_3O_5S$ $(M+H)^+$ 508.1668, found 508.1671.

(1) A solution of 2(*R)*-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-((*S*)-2,2-dimethyl-[1,3]dioxolan-4-yl)-pyrazin-2-yl]-propionamide (8.85 g, 17.4 mmol) in

tetrahydrofuran (50 mL) was treated with a 1N aqueous hydrochloric acid solution (50 mL). The resulting milky mixture was stirred at rt for 16 h. The reaction was concentrated *in vacuo*, and the residue was extracted with methylene chloride (2x100 mL). Each organic extract was washed with a saturated aqueous sodium bicarbonate solution (50 mL) and a saturated aqueous sodium chloride solution (50 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated *in vacuo*. After flash column chromatography (50% to 100% ethyl acetate in hexanes), 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(1(*S*),2-dihydroxy-ethyl)-pyrazin-2-yl]-propionamide (7.15 g, 88%) was obtained as a colorless foam. Analysis by chiral HPLC indicated >99.5% de. This amorphous material was crystalized from a super saturated solution containing non-chemically reactive lipid to give a white crystalline compound as described in the literature (Albano, A. A.; Choi, D. S.; Phuapradit, W.; Radinov, R. N.; Shah, N. H. Crystallization of glucokinase activators. Patent WO2008/074694, 2008). Mp 149.0-150.0 ^{o}C; ES-HRMS (m/e) calcd for $C_{21}H_{26}ClN_3O_5S$ $(M+H)^+$ 468.1355, found 468.1360; ^{1}H NMR (DMSO-d_6) δ ppm 1.05 - 1.25 (m, 2H), 1.33 - 1.87 (m, 8H), 2.03 - 2.22 (m, 1H), 3.34 (s, 3H), 3.52 (dt, *J* = 11.2, 5.9 Hz, 1H), 3.58 - 3.71 (m, 1H), 4.11 (t, *J* = 7.4 Hz, 1H), 4.61 (q, *J* = 5.0 Hz, 1H), 4.68 (t, *J* = 5.9 Hz, 1H), 5.53 (d, *J* = 4.9 Hz, 1H), 7.64 (d, *J* = 7.9 Hz, 1H), 7.73 (s, 1H), 8.02 (d, *J* = 8.3 Hz, 1H), 8.42 (s, 1H), 9.21 (s, 1H), 11.13 (s, 1H); ^{13}C NMR (DMSO-d_6) 24.5, 31.8, 32.1, 37.6, 42.5, 50.0, 65.5, 73.1, 127.3, 130.5, 130.9, 131.1, 134.3, 136.6, 140.5, 147.0, 147.8, 152.6, 171.3.

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(1(*S*),2-dihydroxy-2-methyl-propyl)-pyrazin-2-yl]-propionamide (22). A suspension of magnesium (2.64 g, 110 mmol) in dry tetrahydrofuran (60 mL) was treated with a small amount of iodine and then 1-bromo-2-methylpropene (13.5 g, 100 mmol) in tetrahydrofuran (30 mL) was added in several portions. The mixture was heated under reflux for 3 min. The mixture was cooled to rt and then treated with iodomethane (0.2 mL, 3.0 mmol). The reaction mixture was stirred at rt for 30 min and then heated under reflux for 2 h until all the magnesium was consumed. The mixture was cooled to rt, then treated with a solution of tributyltin chloride (27 mL, 100 mmol) in tetrahydrofuran (30 mL). The mixture was heated under reflux for 19 h and then cooled to rt. The solution was extracted with diethyl ether and a saturated aqueous ammonium chloride solution. The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo* to afford isobutenyl-tri-n-butyltin (31.95 g) as a crude oil. The ^{1}H-NMR data of the crude oil indicated 58% purity of the desired isobutenyl-tri-n-butyltin.

A mixture of the crude isobutenyl-tri-n-butyltin (6.90 g, 58% purity), 2-amino-5-bromopyrazine (1.92 g, 11 mmol), and *N,N*-diisopropylethylamine (5 mL) in *N,N*-dimethylformamide (50 mL) was treated with lithium chloride (2.0 g) and tetrakis(triphenylphosphine)palladium(0) (381 mg, 0.33 mmol). The mixture was stirred at 130 ^{o}C for 4 h until the complete consumption of the starting material. The mixture was concentrated *in vacuo*. The residue was treated with a saturated aqueous potassium fluoride solution and then extracted with ethyl acetate. The organic layer was washed with a saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered, and concentrated *in vacuo*. After flash column chromatography (1.5/1 hexanes/ethyl acetate), 2-amino-5-(2,2-dimethylvinyl)-pyrazine was obtained (420 mg, 26%). This material (420 mg, 2.82 mmol) was coupled with **25** (930 mg, 2.82 mmol) to afford 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(2-methylpropenyl)-pyrazin-2-yl]-propionamide as a light yellow foam (1.10 g, 85%).

A mixture of potassium ferricyanide (738 mg, 2.24 mmol), potassium carbonate (310 mg, 2.24 mmol), and $(DHQ)_2PHAL$ (11.7 mg, 0.015 mmol) was treated with a solution of water/*tert*-butyl alcohol (15 mL, 1:1) and stirred at rt to give a clear solution. The reaction mixture was then treated with a 0.2M solution of osmium tetroxide in toluene (37.4 μL). The reaction mixture was cooled to 0 ^{o}C and then treated with 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-(2-methylpropenyl)-pyrazin-2-yl]-propionamide (345 mg, 0.748 mmol) followed by the addition of methane sulfonamide (71 mg, 0.747 mmol). The mixture was stirred at 0 ^{o}C for 18 h until all the olefin was consumed. The mixture was diluted with ethyl acetate (30 mL) and treated with sodium sulfite (1.0 g). The solution was extracted with ethyl acetate and water. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. After flash column chromatography (1/5 hexanes/ethyl acetate), compound **22** was obtained as an off-white foam (255 mg, 69%). HPLC analysis with a chiral column indicated 89.5% de (18/1 ratio). ES-HRMS (m/e) calcd for $C_{23}H_{30}ClN_3O_5S$ $(M+H)^+$ 496.1668, found 496.1657; ^{1}H NMR ($CDCl_3$) δ ppm 1.08 - 1.21 (m, 2H), 1.14 (s, 3H), 1.25 (s, 3H), 1.42 - 1.85 (m, 7H), 1.86 - 1.98 (m, 1H), 2.17 - 2.31 (m, 1H), 3.28(s, 3H), 3.66 (t, *J* = 7.5 Hz, 1H), 4.54 (s, 1H), 7.50 (d, *J* = 8.1 Hz, 1H), 7.63 (s, 1H), 8.01 (br. s., 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.33 (s, 1H), 9.45 (s, 1H).

5-[2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionylamino]-pyrazine-2-carboxylic acid hydroxyamide (23). A solution of 5-chloro-pyrazine-2-carboxylic acid

(10.00 g, 63.07 mmol) in tetrahydrofuran (126 mL) was treated with a solution of *tert*-butyl 2,2,2-trichloroacetimidate (23 mL, 126.14 mmol) in cyclohexane (126 mL). The reaction was stirred at rt for 5 min and then was treated with boron trifluoride dimethyl etherate (3.2 mL, 25.23 mmol). The resulting reaction mixture was stirred at rt for 16 h and then was diluted with ethyl acetate (200 mL), washed with a saturated aqueous sodium bicarbonate solution (200 mL) and water (200 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (1/9 ethyl acetate/hexanes) afforded 5-chloro-pyrazine-2-carboxylic acid *tert*-butyl ester (12.73 g, 94%) as a colorless oil. EI-HRMS (m/e) calcd for $C_9H_{11}ClN_2O_2$ (M^+) 214.0502, found 214.0510. This material was converted to 5-amino-pyrazine-2-carboxylic acid *tert*-butyl ester using the same procedure described in the preparation of **15** to afford a white powder. Mp 190-193 °C; EI-HRMS (m/e) calcd for $C_9H_{13}N_3O_2$ (M^+) 195.1008, found 195.1009. Coupling of 5-amino-pyrazine-2-carboxylic acid *tert*-butyl ester (0.71 g, 3.64 mmol) with 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionic acid (1.00 g, 3.02 mmol) afforded 5-[2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionylamino]-pyrazine-2-carboxylic acid *tert*-butyl ester (0.80 g, 52%) as a white foam. Mp 107-111 °C (foam to gel); ES-HRMS (m/e) calcd for $C_{24}H_{30}ClN_3O_5S$ $(M+H)^+$ 508.1668, found 508.1666.

A solution of 5-[2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionylamino]-pyrazine-2-carboxylic acid *tert*-butyl ester (3.29 g, 6.48 mmol) in methylene chloride (30 mL) was treated with trifluoroacetic acid (60 mL) and stirred at rt for 65 min. The reaction solution was then concentrated *in vacuo*. The resulting oil was diluted with ethyl acetate (500 mL), washed with water (2x250 mL) and brine (5x250 mL), dried over sodium sulfate, treated with decolorizing carbon, filtered through a pad of Celite, and concentrated *in vacuo* to afford the 5-[2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionylamino]-pyrazine-2-carboxylic acid (2.89 g, 99%) as a light yellow foam. ES-HRMS (m/e) calcd for $C_{20}H_{22}ClN_3O_5S$ $(M+H)^+$ 452.1042, found 452.1046. This material (401.6 mg, 0.889 mmol) in methylene chloride (4.4 mL) was cooled to 0 °C. The reaction mixture was then treated with oxalyl chloride (310 µL, 3.554 mmol) and *N,N*-dimethylformamide (2 drops). The reaction mixture was stirred at 0 °C for 45 min and then slowly warmed to rt over 1 h 45 min. The solution was then concentrated *in vacuo*. The residue was dissolved in methylene chloride (3.4 mL) and cooled to 0 °C. The resulting solution was then treated with a mixture of *O*-(*tert*-butyl)hydroxylamine hydrochloride (133.3 mg, 1.06 mmol) and pyridine (180 µL, 2.3

mmol) in tetrahydrofuran (4.4 mL), followed by a methylene chloride rinse (1 mL). The reaction mixture was stirred at 0 °C for 45 min and then stirred at rt for 2 h. The reaction was partitioned between ethyl acetate (300 mL) and a 1N aqueous citric acid solution (250 mL), and the layers were separated. The organic layer was washed with a saturated aqueous sodium bicarbonate solution (250 mL), water (250 mL), and brine (250 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Flash column chromatography purification (2/3 to 1/1 ethyl acetate/hexanes) afforded 5-[2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-propionylamino]-pyrazine-2-carboxylic acid *tert*-butoxy-amide (338.8 mg, 73%) as an off-white foam. Mp 128-131 °C (foam to gel); ES-HRMS (m/e) calcd for $C_{24}H_{31}ClN_4O_5S$ $(M+H)^+$ 523.1777, found 523.1782. This material (318.3 mg, 0.609 mol) in methylene chloride (2.3 mL) was treated with trifluoroacetic acid (4.6 mL) and stirred at rt overnight, then at 40 °C for 11 h, followed by stirring again at rt overnight. The reaction solution was concentrated *in vacuo*. Reverse phase HPLC purification afforded **23** (90.0 mg, 32%) as a pink foam. ES-HRMS (m/e) calcd for $C_{20}H_{23}ClN_4O_5S$ $(M+H)^+$ 467.1151, found 467.1155; ^{1}H NMR (DMSO-d_6) δ ppm 0.94-1.24 (m, 2H), 1.29-1.89 (m, 8H), 2.03-2.25 (m, 1H), 3.35 (s, 3H), 4.15 (t, J = 7.6 Hz, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.74 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H), 8.86 (s, 1H), 9.14 (br. s., 1H), 9.29 (s, 1H), 11.48 (s, 1H), 11.52 (s, 1H).

2(*R*)-(3-Chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-[5-1(*R*),2-dihydroxy-ethyl)-pyrazin-2-yl]-propionamide (24). A mixture of potassium ferricyanide (365 mg, 1.10 mmol), potassium carbonate (155 mg, 1.12 mmol), and $(DHQD)_2PHAL$ (7 mg, 0.00898 mmol) was treated with a solution of water/*tert*-butyl alcohol (10 mL, 1:1), and the reaction mixture was stirred at rt for 5 min. The reaction mixture was cooled to 0 ^{o}C and then treated with a 0.2M solution of osmium tetroxide in toluene (17 μL, 0.0034 mmol) followed by a mixture of 2(*R*)-(3-chloro-4-methanesulfonyl-phenyl)-3-cyclopentyl-*N*-(5-vinyl-pyrazin-2-yl)-propionamide (175 mg, 0.405 mmol, prepared with the same method described in the preparation of **4**) in water/*tert*-butyl alcohol (2 mL, 1:1). The heterogeneous mixture was stirred for 10 min, the cooling bath was removed, and the stirring was continued for 18 h. The mixture was then treated while stirring with ethyl acetate (20 mL) and sodium metabisulfite (150 mg, 0.79 mmol), and the stirring continued for 15 min. The phases were separated, and the organic layer was washed with a saturated aqueous sodium chloride solution (2x25 mL). Each aqueous phase was back-extracted with ethyl acetate (25 mL). The combined organic layers were dried over sodium sulfate, filtered, and concentrated under reduced pressure.

After flash column chromatography (1/3 ethyl acetate/hexanes to 100% ethyl acetate), compound **24** was obtained as a colorless foam (65 mg, 34%). ES-HRMS (m/e) calcd for $C_{21}H_{26}ClN_3O_5S$ $(M+H)^+$ 468.1355, found 468.1359; ^{1}H NMR (DMSO-d_6) δ ppm 1.05 - 1.22 (m, 2H), 1.35 - 1.83 (m, 8H), 2.07 - 2.20 (m, 1H), 3.35 (s, 3H), 3.55 (dt, *J* = 11.2, 5.9 Hz, 1H), 3.67 (dt, *J* = 10.4, 5.5 Hz, 1H), 4.12 (t, *J* = 7.4 Hz, 1H), 4.62 (q, *J* = 5.1 Hz, 1H), 4.71 (t, *J* = 5.9 Hz, 1H), 5.54 (d, *J* = 5.1 Hz, 1H), 7.65 (d, *J* = 8.2 Hz, 1H), 7.75 (s, 1 H), 8.04 (d, *J* = 8.1 Hz, 1H), 8.43 (s, 1H), 9.22 (s, 1 H), 11.14 (s, 1 H).

Determination of metabolites

Metabolites M1, M2 and M3 from compound **4** metabolism profiling isolated from monkey liver microsome incubation were matched with the synthetic samples of **5a**, **5b** and **5c** by LC/MS and further characterized by ^{1}H-NMR in comparison with the synthesized samples. The synthesis of **5a**, **5b** and **5c** was carried out with the same method as the synthesis of **4** except the left hand molecule cyclopentane was replaced with cyclopentanone. The left hand carboxylic acid with the cyclopentanone fragment was prepared according to reference 12. Reduction of the ketone provided cis- and trans-isomers of the cyclopentanol derivative. Matching the isolated metabolites with the synthetic samples by LC/MS (retention time) and ^{1}H-NMR indicated M1 as the cis-isomer and M2 as the trans-isomer.

Metabolite M1 isolated from monkey microsomal inhibition (**5a**):
^{1}H-NMR (400 MHz, METHANOL-*d*4) δ ppm 1.24 (m, 1H, H_k), 1.46 (m, 1H, H_n), 1.62, 1.72 (2 x m, 2H, H_m), 1.77 (m, 1H, H_j), 1.81 (m, 1H, H_n), 1.91 (m, 1H, H_i), 2.14 (m, 1H, H_k), 2.29 (m, 1H, H_i), 3.27 (s, 3H, SO_2CH_3), 3.68 - 3.87 (2 x m, 2H, H_g), 3.97 (m, 1H, H_h),

4.18 (m, 1H, H_l), 4.76 (m, 1H, H_f), 7.63 (m, 1H, H_a), 7.75 (m, 1H, H_c), 8.07 (d, *J*=8.2 Hz, 1H, H_b), 8.46 (m, 1H, H_e), 9.29 (m, 1H, H_d).

Metabolite M2 isolated from monkey microsomal inhibition (**5b**):
^{1}H-NMR (400 MHz, METHANOL-*d*4) δ ppm 1.23 (m, 1H, H_n), 1.41 (m, 1H, H_k), 1.52 (m, 1H, H_m), 1.81 (m, 1H, H_k), 1.84 (m, 1H, H_i), 1.95 (m, 1H, H_m), 1.98 (m, 1H, H_n), 2.06 (m, 1H, H_j), 2.22 (m, 1H, H_i), 3.27 (s, 3 H, SO_2CH_3), 3.70 - 3.85 (2 x m, 2H, H_g), 3.97 (m, 1H, H_h), 4.26 (m, 1H, H_l), 4.76 (m, 1H, H_f), 7.63 (dd, J_o=8.2 Hz, J_m=1.7 Hz, 1H, H_a), 7.75 (d, J_m=1.7 Hz, 1H, H_c), 8.06 (d, *J*=8.2 Hz, 1H, H_b), 8.47 (m, 1H, H_e), 9.30 (m, 1H, H_d).

Metabolite M3 isolated from monkey microsomal inhibition (**5c**):

^{1}H-NMR (400 MHz, METHANOL-*d*4) δ ppm 1.57-1.68 (m, 1H, H_i), 1.86-2.45 (m, 8H, H_i, H_j, H_m, H_l, H_k), 3.27 (s, 3 H, SO_2CH_3), 3.72 and 3.83 (2 x m, 2H, H_g), 4.01 (m, 1H, H_h), 4.76 (m, 1H, H_f), 7.65 (m, 1H, H_a), 7.78 (d, J_m=1.5 Hz, 1H, H_c), 8.09 (d, J_o=8.4 Hz, 1H, H_b), 8.47 (d, J_p=1.1 Hz, 1H, H_e), 9.30 (m, 1H, H_d).

GK in vitro enzyme assay. The procedures used to determine GK activator potency and effects on enzyme kinetics were described in literature.[3, 12] Compounds (10 nM-30 μM) were assayed in triplicate under a fixed concentration of glucose (5 mM) to determine the compound concentration that leads to an increase in enzyme activity by 1.5 fold ($SC_{1.5}$). To calculate the effect of compound **2** on enzyme kinetic parameters, the activator at various concentrations (0-30 μM) was assayed in the presence of different concentrations of glucose (0-30 μM).[3, 12]

Patch clamp hERG inhibition assay. The detailed procedure for the patch clamp hERG inhibition assay has been described in the literature (Qian, Y. et al. *J. Med. Chem.* **2011**, *54*, 2433-2446). Compounds were tested at various concentrations (0.3 to 100 μM). The inhibition of the hERG current at each concentration was normalized to that recorded in the vehicle control, and fitted with Hill equation to calculate IC_{20} and/or IC_{50}.

Time-dependent Inactivation Assay for CYP3A4/5. To evaluate the time-dependent inactivation of CYP3A4/5, gender pooled human liver microsomes were pre-incubated with compounds (10 μM) at different time periods (0, 3, 6, 12 and 24 minutes) before being exposed to the CYP3A4/5 probe substrate midazolam. The formation of 1'-hydroxymidazolam was used to indicate CYP3A4/5 activity. The amount of 1'-hydroxymidazolam was determined using an LC-MS/MS method employing an internal standard. The enzyme activity of the solvent control samples was used as a reference to calculate the percent enzyme activity remaining in the presence of the inhibitor. The incubation of CYP3A4/5 was conducted using 15 mL polypropylene test tubes (Corning Life Sciences, Acton, MA). The pre-incubation mixture contained a final concentration of 100 mM potassium phosphate buffer (pH 7.4), 3 mM $MgCl_2$, 1 mM EDTA, 1 mg/mL gender pooled human liver microsomes, 1 mM NADPH and 10 μM compound. After 3 minutes of warming at 37°C, the pre-incubation reactions were initiated by adding NADPH. At selected time intervals (0, 3, 6, 12, and 24 min), a 100 μL aliquot of the pre-incubation mixture was transferred to 900 μL of the pre-warmed substrate incubation mixture containing 100 mM

potassium phosphate buffer (pH 7.4), 3 mM $MgCl_2$, 1 mM EDTA, 10 μM midazolam (for CYP3A4/5) and 1 mM NADPH (final concentration in 1000 μL for CYP3A4/5 incubation). The substrate incubations were run for 10 minutes at 37°C. The reactions were then quenched using 1 mL of 0.4% (v/v) acetic acid in acetonitrile. A 100 μL aliquot of 2.5 μM deuterated 1'-hydroxymidazolam was added, as internal standard. After vortexing for 5 minutes and centrifuging for 10 minutes (at 3,500 rpm), a 300 μL aliquot of supernatant was transferred into a 96-well polypropylene block, diluted with 200 μL of water, vortex mixed and injected into the LC-MS/MS for analysis.

Metabolism profiling of 4 and 2 in human hepatocytes. To determine the *in vitro* metabolic profile of compounds **2** and **4**, ^{14}C-labelled compounds were incubated with cryopreserved hepatocytes (pooled male and female human). Incubation conditions are the following: 1.0 million viable hepatocytes per well, 20 μM ^{14}C-labelled compounds or positive control midazolam, three h incubation at 37 ^{o}C. The cryopreserved primary hepatocytes were obtained from In Vitro Technologies (Baltimore, MD). The cells were thawed and washed as recommended by the supplier. Cells were suspended in phenol red-free Williams E medium (supplemented with glutamine and 2% fetal bovine serum (FBS), but not penicillin/streptomycin/gentamicin). Cell aliquots (0.5 mL = 1.0 million cells) were added to a 24-well plate and 500 μL of test compound (or positive control) was added to each well (final substrate concentration = 20 μM). Cells were incubated for 3 h at 37 °C in a humidified, 5% CO_2 atmosphere. After three hours, the reactions were terminated by adding 1 mL of methanol to each well. Samples were vigorously mixed, centrifuged at 2000 x g and the supernatant was transferred to an HPLC vial for UV and radio chromatographic and mass spectrometry analysis. Incubation with midazolam was performed with the same set of cryopreserved hepatocytes to check the viability and metabolic activity of cells. Metabolite structural identification was accomplished by LC/MS/MS using a Micromass Quattro Ultima and Q-Tof mass spectrometer and by comparison with the available synthetic reference compounds.

In vivo studies in C57 mice. Studies were conducted using C57Bl/6J mice (Jackson Laboratories, Bar Harbor, ME). Both normal C57 mice (2 month old, n = 6 per group) and DIO mice (6 month old, C57 mice on high fat diet from the age of 2 months, n = 9 per group) were used for the efficacy studies. Mice were fasted for 2 h prior to the administration of test compound or vehicle and food was withheld for an additional 8 h during the course of the

experiment. The formulation used for the *in vivo* study consisted Gelucire 44/14 (66%), PEG400 (30%) and ethanol (4%). Compounds were administered to mice via oral gavage (5 mL/kg). Blood samples (20 μL) were collected from the tail vein at the indicated time points and placed into a heparinized hematocrit tube. Blood glucose was measured with YSI Mode 2700 Biochemistry Analyzer. Statistical significance was determined by Student's *t* test.

Clinical Pharmacokinetics. A phase I, single-center, randomized, double-blind, placebo-controlled, ascending multiple-dose study was conducted in type II diabetic (T2D) patients. Four escalating oral doses of 100, 300, 600 and 800 mg QD on Day 1 and BID from Days 3 to 8 were administered. Each of the treatment groups were composed of ten patients with eight randomized to receive active drug (compound **4**), and two subjects randomized to receive placebo.