Supporting Information

Discovery of Novel Tricyclic Heterocycles as Potent and Selective DPP-4 Inhibitors for the Treatment of Type 2 Diabetes

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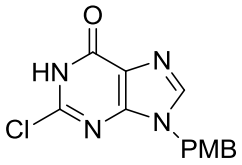
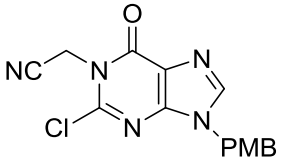
Chemistry. General methods. Where NMR data are presented, ^{1}H spectra were obtained on XL-400 (400 MHz) and are reported as ppm down field from Me_4Si with number of protons, multiplicities, and coupling constants in Hertz indicated parenthetically. Where LC-MS data are presented, analyses were performed using an Applied Biosystems API-100 mass spectrometer and Shimadzu SCL-10A LC column: Altech platinum C18, 3 micron, 33mm x 7mm ID; gradient flow: 0 min – 10% CH_3CN, 5 min – 95% CH_3CN, 7 min – 95% CH_3CN, 7.5 min – 10% CH_3CN, 9 min – stop. The observed parent ion are given. The purity of final compounds was based on UV wavelength of 254 nm. All compounds tested were of greater than 90% purity as

determined by LC-MS and NMR analysis. The HRMS analyses were performed using Waters Acquity UPLC H-Class system and Acquity UPLC BEH C18 1.7 μm column. All commercially available reagents were used without further purification.



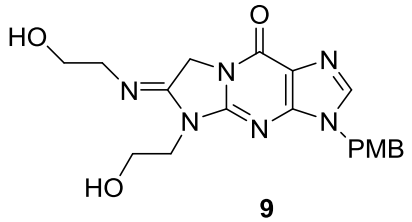
6

2-Chloro-9-(4-methoxybenzyl)-1,9-dihydro-6H-purin-6-one (6). A suspension of 31.0 g (100.6 mmol) of compound **5** in 500 mL of 1 N NaOH solution was stirred at reflux for 2 h and cooled to room temperature. The mixture was neutralized with 300 mL of 2 N HCl in ice water. The precipitate was collected by filtration. The solid was washed with water, and dried over vacuum to give 26.3 g (90%) of compound **6**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (s, 1 H), 7.22 (d, J = 8.8 Hz, 2 H), 6.88 (d, J = 8.8 Hz, 2 H), 5.21 (s, 2 H), 3.79 (s, 3 H). LC-MS $C_{13}H_{11}ClN_4O_2$ m/e = 291 (M+1).



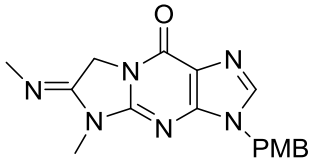
7

2-(2-Chloro-9-(4-methoxybenzyl)-6-oxo-6,9-dihydro-1H-purin-1-yl)acetonitrile (7). A solution of 26.0 g (90 mmol) of compound **6,** 15.6 g (130 mmol) of bromoacetonitrile, and 26.0 g (200 mmol) of diisopropylethylamine in 250 mL of DMF was stirred at 50 ^{o}C for 2 h, and cooled to room temperature. The mixture was diluted with 300 mL of water, and then extracted with three 300 mL portions of dichloromethane. The combined organic extracts were washed with 100 mL of brine, and concentrated. The residue was purified by flash chromatography eluting with a gradient of 5 - 95% ethyl acetate in hexanes to give 16.2 g (55%) of compound **7**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1 H), 7.22 (d, J = 8.8 Hz, 2 H), 6.89 (d, J = 8.8 Hz, 2 H), 5.23 (s, 2 H), 5.21 (s, 2 H), 3.79 (s, 3 H). LC-MS $C_{15}H_{12}ClN_5O_2$ m/e = 330 (M+1).

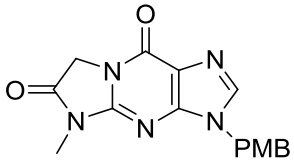


9

E-5-(2-hydroxyethyl)-6-((2-hydroxyethyl)imino)-3-(4-methoxybenzyl)-3,5,6,7-tetrahydro-9H-imidazole[1,2-a]purin-9-one (9). A suspension of 0.033 g (0.1 mmol) of compound **7** and 0.02 g (3.3 mmol) of 2-hydroxyethylamine and 0.03 g (2.3 mmol) of DIEA in 3 mL of DMF in a sealed tube was heated at 120 ^{o}C for 1 h. The mixture was concentrated; the residue was purified by chromatography (4 g of SiO_2, 0 to 7% MeOH in DCM plus 1% NH_4OH) to give 0.026 g (65%) of compound **9**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1 H), 7.20 (d, J = 8.8 Hz, 2 H), 6.85 (d, J = 8.8 Hz, 2 H), 5.12 (s, 2 H), 4.72 (s, 2 H), 4.09 (t, J = 4.4 Hz, 2 H), 3.92 (t, J = 4.4 Hz, 2 H), 3.86 (t, J = 4.8 Hz, 2 H), 3.77 (s, 3 H), 3.42 (t, J = 4.8 Hz, 2 H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 158.7, 155.9, 154.7, 152.6, 149.1, 137.9, 129.3, 127.2, 119.8, 114.4, 62.4, 61.6, 55.3, 52.8, 47.1, 44.7, 44.3. LC-MS $C_{19}H_{22}N_6O_4$ m/e = 399 (M+1).



10

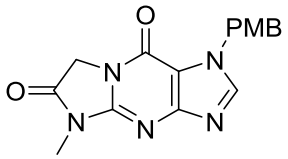


11

(E)-3-(4-methoxybenzyl)-5-methyl-6-(methylimino)-3,5,6,7-tetrahydro-9H-imidazo[1,2-a]purin-9-one (10) and **3-(4-methoxybenzyl)-5-methyl-3,5-dihro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (11).** A suspension of 0.30 g (0.91 mmol) of compound **7** and 0.20 g (2.58 mmol) of 40% $MeNH_2$ in 5 mL of 1,4-dioxane was stirred at room temperature overnight (20 h). The mixture was concentrated; the residue was purified by chromatography (4 g of SiO_2, 0 to 6% MeOH in DCM plus 1% NH_4OH) to give 0.20 g (66 %) of compound **10** and 0.068 g (23 %) of compound **11**. Compound **10**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1 H), 7.21 (d, J = 8.8 Hz, 2 H), 6.84 (d, J = 8.8 Hz, 2 H), 5.15 (s, 2 H), 4.63 (s, 2 H), 3.77 (s, 3 H), 3.27 (s, 3 H), 3.13 (s, 3 H). LC-MS $C_{17}H_{18}N_6O_2$ m/e = 339 (M+1).

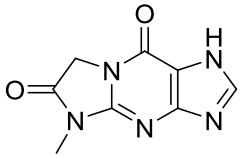
Compound **11:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1 H), 7.22 (d, J = 8.4 Hz, 2 H), 6.86 (d, J = 8.4 Hz, 2 H), 5.17 (s, 2 H), 4.65 (s, 2 H), 3.78 (s, 3 H), 3.31 (s, 3 H). LC-MS $C_{16}H_{15}N_5O_3$ m/e = 326 (M+1).

Alternative route: A suspension of 6.6 g (20 mmol) of compound **7** and 4.0 g (52 mmol) of 40% $MeNH_2$ in water was stirred at room temperature for 18 h. The mixture was concentrated to give crude compound **10,** which was used in the next step without purification. LC-MS $C_{17}H_{18}N_6O_2$ m/e = 339 (M+1).



12

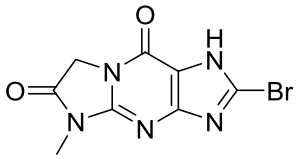
1-(4-Methoxybenzyl)-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (12). A solution of compound **10** mentioned above and 20 mL of 6 N HCl in 80 mL of MeOH was stirred at reflux for 3 h. It was concentrated; the residue was neutralized with 200 mL of saturated $NaHCO_3$ solution. The mixture was extracted with four 200 mL portions of dichloromethane. The combined organic extracts were concentrated to give 6.6 g (100%) of compound **12**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1 H), 7.22 (d, J = 8.8 Hz, 2 H), 6.87 (d, J = 8.8 Hz, 2 H), 5.20 (s, 2 H), 4.56 (s, 2 H), 3.78 (s, 3 H), 3.31 (s, 3 H). LC-MS $C_{16}H_{15}N_5O_3$ m/e = 326 (M+1).



13

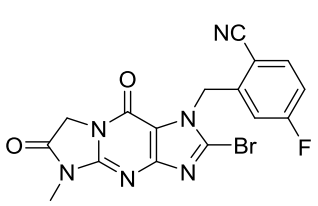
5-Methyl-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (13). A suspension of 0.85 g (2.62 mmol) of compound **12** in 15 mL of TFA in a sealed tube was heated at 100 ^oC in microwave for 5 h. It was concentrated; the residue was triturated with ether and small amount of MeOH, and filtered to give 0.50 g (93%) of compound **13**

(0.50 g, 93%). ^{1}H NMR (400 MHz, d_6-DMSO) δ 7.40 (s, 1 H), 4.06 (s, 3 H), 3.74 (s, 2 H). LC-MS $C_8H_7N_5O_2$ m/e = 206 (M+1).

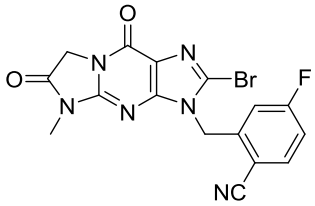


14

2-Bromo-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (14). To a suspension of 0.50 g (2.44 mmol) of compound **13** in 70 mL of MeCN was added 1.0 g (excess) of bromine dropwise. The mixture was stirred at room temperature for 20 h, and concentrated to give 0.78 g of crude compound **14** (0.78 g) as a TFA salt. ^{1}H NMR (400 MHz, d_6-DMSO) δ 4.05 (s, 3 H), 3.72 (s, 2 H). LC-MS $C_8H_6BrN_5O_2$ m/e = 284 (M+1).



15ca

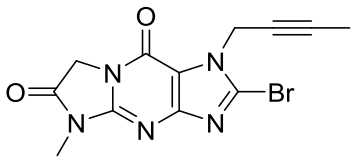


15cb

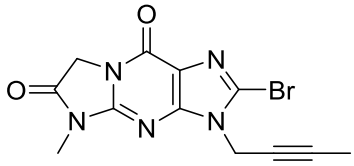
2-((2-Bromo-5-methyl-6,9-dioxo-5,6,7,9-tetrahydro-1H-imidazo[1,2-a]purin-1-yl)methyl-4-fluobenzonitrile (15ca) and **2-((2-Bromo-5-methyl-6,9-dioxo-5,6,7,9-tetrahydro-3H-imidazo[1,2-a]purin-1-yl)methyl-4-fluobenzonitrile (15cb).** A solution of 2.5 g (6.85 mmol) of compound **14** (TFA salt)**,** 1.91 g (8.9 mmol) of 2-cyano-5-fluorobenzyl bromide, and 1.77 g (13.7 mmol) of diisopropylethylamine in 15 mL of DMF was stirred at room temperature for 3 h, and concentrated. The residue was purified by flash chromatography eluting with 0-3% MeOH in CH_2Cl_2 plus 1% NH_4OH to give 0.615 g (22%) of compound **15ca** and 0.676 g (24%) of **15cb**. The regioisomers were assigned on the basis of their relative polarity and eventually confirmed by x-ray of compound **17c**.

15ca: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (m, 2 H), 7.15 (m, 1 H), 6.71 (dd, J = 8.8, 2.4 Hz, 1 H), 5.54 (s, 2 H), 4.58 (s, 2 H), 3.29 (s, 3 H). LC-MS $C_{16}H_{10}BrFN_6O_2$ m/e = 417 (M+1).

15cb: ^{1}H NMR (400 MHz, $CDCl_3$)).δ 7.75 (m, 1 H), 7.11 (m, 1 H), 6.57 (dd, J = 8.8, 2.4 Hz, 1 H), 5.82 (s, 2 H), 4.50 (s, 2 H), 3.33 (s, 3 H). LC-MS $C_{16}H_{10}BrFN_6O_2$ m/e = 417 (M+1).

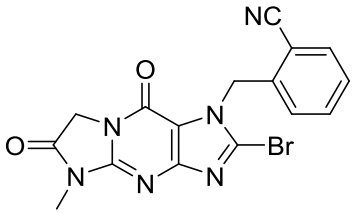


15aa

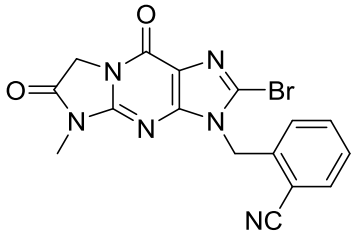


15ab

2-Bromo-1-(but-2-yn-1-yl)-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (15aa) and **2-Bromo-1-(but-2-yn-1-yl)-5-methyl-3,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (15ab).** Compounds **15aa** and **15ab** were prepared analogously as a mixture from compound **14** and 1-bromobut-2-yne. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.24 (s, 2 H), 4.51 (s, 2 H), 3.31 (s, 3 H), 1.79 (s, 3 H); δ 4.82 (s, 2 H), 4.55 (s, 2 H), 3.30 (s, 3 H), 1.78 (s, 3 H). LC-MS $C_{12}H_{10}BrN_5O_2$ m/e = 336 (M+1).



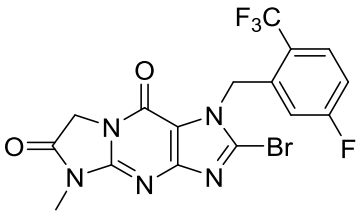
15ba



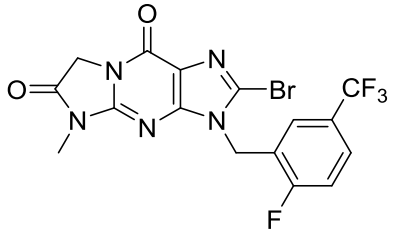
15bb

2-((2-Bromo-5-methyl-6,9-dioxo-5,6,7,9-tetrahydro-1H-imidazo[1,2-a]purin-1-yl)methyl)benzonitrile (15ba) and **2-((2-Bromo-5-methyl-6,9-dioxo-5,6,7,9-tetrahydro-3H-imidazo[1,2-a]purin-3-yl)methyl)benzonitrile (15bb)** Compounds **15ba** and **15bb** were prepared analogously as a mixture from compound **14** and 2-(bromomethyl)benzonitrile. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.3 – 7.7 (m, 3 H), 7.10 (d, J = 8.8 Hz, 1 H), 5.80 (s, 2 H), 4.54 (s, 2 H), 3.29 (s, 3 H); δ 7.3 – 7.7 (m, 3 H), 6.86 (d, J

= 8.8 Hz, 1 H), 5.52 (s, 2 H), 4.48 (s, 2 H), 3.24 (s, 3 H). LC-MS $C_{16}H_{11}BrN_6O_2$ m/e = 399 (M+1).

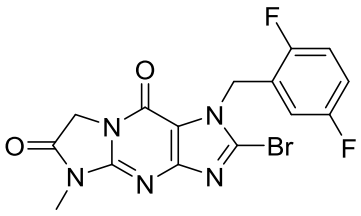


15da

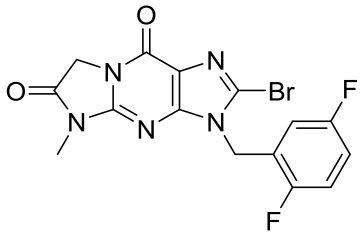


15db

2-Bromo-1-(5-difluoro-2-(trifluoromethyl)benzyl)-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (15da) and **2-Bromo-1-(5-difluoro-2-(trifluoromethyl)benzyl)-5-methyl-3,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (15db)** Compounds **15da** and **15db** were prepared analogously as a mixture from compound **14** and 2-(bromomethyl)-4-fluoro-1-(trifluoromethyl)benzene. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (m, 2 H), 7.04 (m, 1 H), 6.16 (dd, J = 9.6, 2.0 Hz, 1 H), 5.78 (s, 2 H), 4.46 (s, 2 H), 3.30 (s, 3 H); δ 7.74 (m, 2 H), 7.09 (m, 1 H), 6.27 (dd, J = 9.6, 2.4 Hz, 1 H), 5.49 (s, 2 H), 4.56 (s, 2 H), 3.17 (s, 3 H). LC-MS $C_{16}H_{10}BrF_4N_5O_2$ m/e = 460 (M+1).



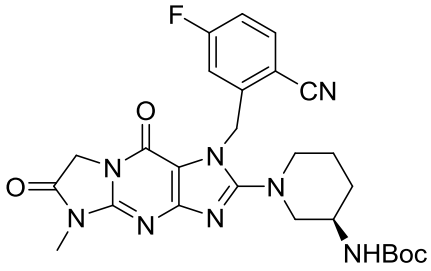
15ea



15eb

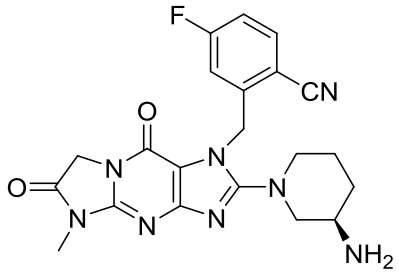
2-Bromo-1-(2,5-difluorobenzyl)-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (15ea) and **2-Bromo-1-(2,5-difluorobenzyl)-5-methyl-3,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (15eb).** Compounds **15ea** and **15eb** were prepared as a mixture analogously from compound **14** and 2-(bromomethyl)-1,4-difluorobenzene. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (m, 2 H), 6.68 (m, 1 H), 5.32 (s, 2

H), 4.53 (s, 2 H), 3.27 (s, 3 H); δ 7.06 (m, 2 H), 6.61 (m, 1 H), 5.60 (s, 2 H), 4.48 (s, 2 H), 3.24 (s, 3 H). LC-MS $C_{15}H_{10}BrF_2N_5O_2$ m/e = 410 (M+1).



16c

tert-Butyl (R)-(1-(1-(2-cyano-5-fluorobenzyl)-5-methyl-6,9-dioxo-5,6,7,9-tetrahydro-1H-imidazo[1,2-a]purin-2-yl)piperidin-3-yl)carbamate (16c). A solution of 0.60 g (1.44 mmol) of compound **15ca,** 0.288 g (1.44 mmol) of (R)-3-(Boc-amino) piperidine, and 0.24 g (1.87 mmol) of diisopropylethylamine in 15 mL of MeCN was stirred at 90 ^{o}C for 18 h, and concentrated. The residue was purified by flash chromatography eluting with 0-5% MeOH in CH_2Cl_2 plus 1% NH_4OH to give a crude product, which was further purified by preparative TLC eluting with 7% MeOH in CH_2Cl_2 to give 0.226 g (29%) of pure compound **16c**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (m, 1 H), 7.04 (m, 1 H), 6.71 (dd, J =8.8, 2.4 Hz, 1 H), 5.56 (s, 2 H), 4.43 (s, 2 H), 3.68 (br, 1 H), 3.68 (m, 1 H), 3.46 (m, 1 H), 3.29 (m, 4 H), 3.02 (m, 1 H), 2.88 (m, 1 H), 1.60-1.90 (m, 3 H), 1.40 (m, 1 H), 1.34 (s, 9 H). LC-MS $C_{26}H_{29}FN_8O_4$ m/e = 537 (M+1).

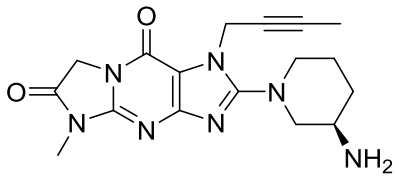


17c

(R)-2-((2-(3-aminopiperidin-1-yl)-5-methyl-6,9-dioxo-5,6,7,9-tetrahydro-1H-imidazo[1,2-a]purin-1-yl)methyl)-4-fluorobenzonitrile (17c). A solution of 0.22 g (0.41 mmol) of compound **16c** and 2.0 g of TFA in 2 mL of CH_2Cl_2 was stirred at room

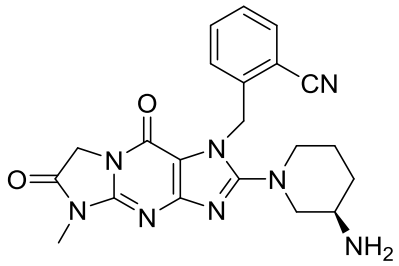
temperature for 20 h, and concentrated. The residue was purified by flash chromatography eluting with 0 -7% MeOH in CH_2Cl_2 plus 1% NH_4OH to give example **17c**, which was treated with HCl in ether to form 0.184 g (95%) of HCl salt. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (m, 1 H), 7.09 (m, 1 H), 6.71 (dd, J =8.8, 2.4 Hz, 1 H), 5.58 (s, 2 H), 4.57 (s, 2 H), 3.45 (m, 1 H), 3.30 (s, 3 H), 3.28 (m, 1 H), 3.01 (m, 2 H), 2.79 (m, 1 H), 1.92 (m, 1 H), 1.60-1.80 (m, 2 H), 1.27 (m, 1 H). LC-MS $C_{21}H_{21}FN_8O_2$ m/e = 437 (M+1). HRMS (M+1) calcd 437.1850, found 437.1860.

Compounds **17a, 17b, 17d, and 17e** were prepared according to the procedures described for compounds **16c** and **17c**, starting from a mixture of regioisomers **15aa** and **15ab**, **15ba** and **15bb**, **15da** and **15db**, **15ea** and **15eb**, respectively. The crude mixtures of regioisomers **16** were not purified but used in the next step directly. The desired final products (less polar regioisomers) were separated by silica gel chromatography.



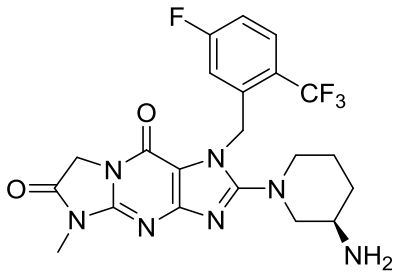
17a

(R)-2-(3-aminopiperidin-1-yl)-1-(but-2-yn-1-yl)-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purin-1-6,9(7H)-dione (17a). The crude product was purified by flash chromatography eluting with 0-8% MeOH in CH_2Cl_2 plus 1% NH_4OH to give compound **17a.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.86 (s, 2 H), 4.47 (s, 2 H), 3.71 (m, 1 H), 3.62 (m, 1 H), 3.25 (s, 3 H), 3.08 (m, 2 H), 2.88 (m, 1 H), 1.95 (m, 1 H), 1.80 (s, 3 H), 1.65 – 1.90 (m, 2 H), 1.33 (m, 1 H). LC-MS $C_{17}H_{21}N_7O_2$ m/e = 356 (M+1). HRMS (M+1) calcd 356.1835, found 356.1849.



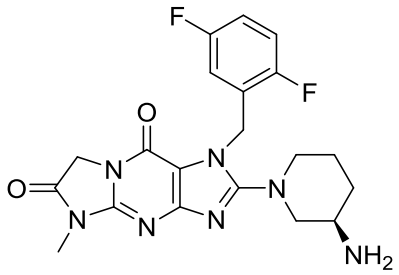
17b

(R)-2-((2-(3-aminopiperidin-1-yl)-5-methyl-6,9-dioxo-5,6,7,9-tetrahydro-1H-imidazo[1,2-a]purin-1-yl)methyl)-benzonitrile (17b). The crude product was purified by flash chromatography eluting with 0-6% MeOH in CH_2Cl_2 plus 1% NH_4OH to give compound **17b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, J = 7.8 Hz, 1 H), 7.50 (t, J = 7.8 Hz, 1 H), 7.38 (t, J = 7.8 Hz, 1 H), 7.00 (d, J = 8.0 Hz, 1 H), 5.60 (s, 2 H), 4.44 (s, 2 H), 3.44 (d, J = 12.4 Hz, 1 H), 3.28 (s, 3 H), 3.25 (m, 1 H), 3.00 (m, 2 H), 2.75 (m, 1 H), 1.89 (s, 1 H), 1.69 (m, 1 H), 1.58 (m, 1 H), 1.23 (m, 1 H). LC-MS $C_{21}H_{22}N_8O_4$ m/e = 419 (M+1). HRMS (M+1) calcd 419.1944, found 419.1953.



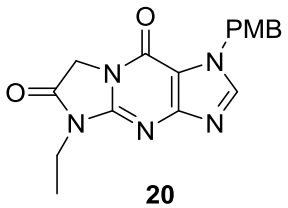
17d

(R)-2-((2-(3-aminopiperidin-1-yl)-1-(5-fluoro-2-(trifluoromethyl)benzyl)-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purin-1-6,9(7H)-dione (17d). The crude product was purified by preparative TLC eluting with 6% MeOH in CH_2Cl_2 plus 1% NH_4OH to give compound **17a**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (m, 1 H), 7.06 (m, 1 H), 6.56 (m, 1 H), 5.56 (s, 2 H), 4.48 (s, 2 H), 3.47 (m, 1 H), 3.31 (s, 3 H), 3.29 (m, 1 H), 2.93 (m, 2 H), 2.74 (m, 1 H), 1.92 (m, 1 H), 1.60-1.80 (m, 2 H), 1.24 (m, 1 H). LC-MS $C_{21}H_{21}F_4N_7O_2$ m/e = 480 (M+1). HRMS (M+1) calcd 480.1771, found 480.1775.



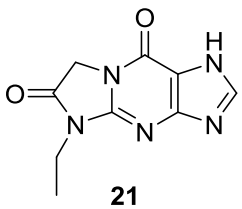
17e

(R)-2-((2-(3-aminopiperidin-1-yl)-1-(2,5-difluorobenzyl)-5-methyl-1,5-dihydro-9H-imidazo[1,2-a]purin-1-6,9(7H)-dione (17e). The crude product was purified by flash chromatography eluting with 0 -8% MeOH in CH_2Cl_2 plus 1% NH_4OH to give compound **17e**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.03 (m, 1 H), 6.93 (m, 1 H), 6.65 (m, 1 H), 5.41 (s, 2 H), 4.46 (s, 2 H), 3.48 (m, 1 H), 3.31 (m, 1 H), 3.30 (s, 3 H), 3.00 (m, 2 H), 2.78 (m, 1 H), 1.92 (m, 1 H), 1.60-1.80 (m, 2 H), 1.29 (m, 1 H). LC-MS $C_{20}H_{21}F_2N_7O_4$ m/e = 430 (M+1). HRMS (M+1) calcd 430.1803, found 430.1808.



20

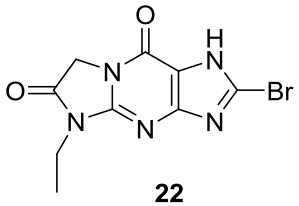
5-Ethyl-1-(4-Methoxybenzyl)-1,5-dihrdro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (20). Using the procedure described for compound **12**, compound **7** was converted to compound **20** using $EtNH_2$ insdeat of $MeNH_2$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (s, 1 H), 7.22 (d, J = 8.6 Hz, 2 H), 6.86 (d, J = 8.6 Hz, 2 H), 5.19 (s, 2 H), 4.54 (s, 2 H), 3.87 (q, J = 7.0 Hz, 2 H), 3.78 (s, 3 H), 1.35 (t, J = 7.0 Hz, 3 H).



21

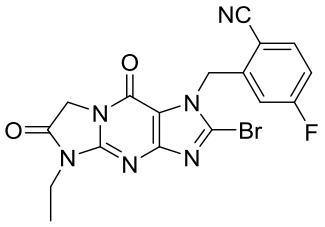
5-Ethyl-1,5-dihrdro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (21). Using the procedure described for compound **13**, compound **20** was converted to compound **21.** ^{1}H

NMR (400 MHz, d_6-DMSO) δ 8.19 (s, 1 H), 4.49 (s, 2 H), 3.66 (q, J = 7.0 Hz, 2 H), 1.19 (t, J = 7.0 Hz, 3 H).

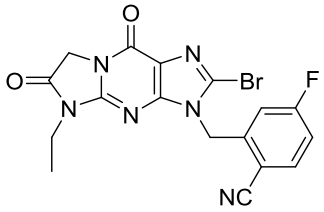


22

2-Bromo-5-ethyl-1,5-dihrdro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (22). Using the procedure described for compound **14**, compound **21** was converted to compound **22.** ^{1}H NMR (400 MHz, d_6-DMSO) δ 4.37 (s, 2 H), 3.60 (q, J = 6.8 Hz, 2 H), 1.35 (t, J = 6.8 Hz, 3 H). LC-MS $C_9H_8BrN_5O_2$ m/e = 298 (M+1).

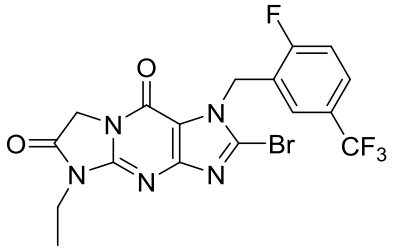


23aa

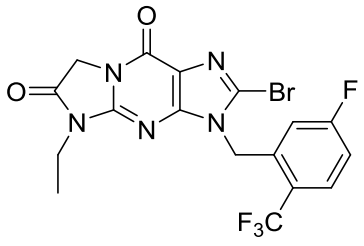


23ab

2-((2-Bromo-5-ethyl-6,9-dioxo-5,6,7,9-tetrahydro-1H-imidazo[1,2-a]purin-1-yl)methyl-4-fluobenzonitrile (23aa) and **2-((2-Bromo-5-ethyl-6,9-dioxo-5,6,7,9-tetrahydro-3H-imidazo[1,2-a]purin-1-yl)methyl-4-fluobenzonitrile (23ab).** Using the procedure described for compounds **15ca** and **15cb**, compound **22** was treated with 2-cyano-5-fluorobenzyl bromide to give compounds **23aa** and **23ab** as a mixture. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (m, 1 H), 7.10 (m, 1 H), 6.75 (dd, J = 8.8, 2.5 Hz, 1 H), 6.55 (dd, J = 8.9, 2.5 Hz, 1 H), 5.76 (s, 2 H), 5.49 (s, 2 H), 4.50 (s, 2 H), 4.45 (s, 2 H), 3.85 (q, J = 7.2 Hz, 2 H), 3.81 (q, J = 7.2 Hz, 2 H), 1.26 (t, J = 7.2 Hz, Hz, 3 H), 1. 18 (t, J = 7.2 Hz, 3 H). LC-MS $C_{17}H_{12}BrFN_6O_2$ m/e = 433 (M+1).

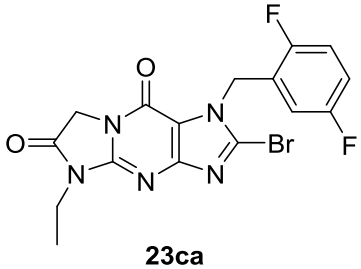


23ba

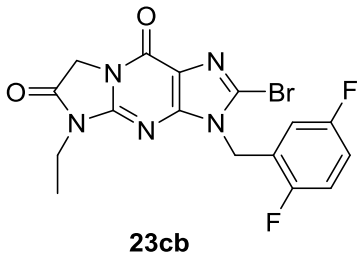


23bb

2-Bromo-5-ethyl-1-(2-fluoro-5-(trifluoromethyl)benzyl)-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (23ba) and **2-Bromo-5-ethyl-3-(5-fluoro-2-(trifluoromethyl)benzyl)-3,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (23bb).** Compounds **23ba** and **23bb** were prepared analogously as a mixture from compound **22** and using 2-(bromomethyl)-4-fluoro-1-(trifluoromethyl)benzene. LC-MS $C_{17}H_{12}BrF_4N_5O_2$ m/e = 476 (M+1).

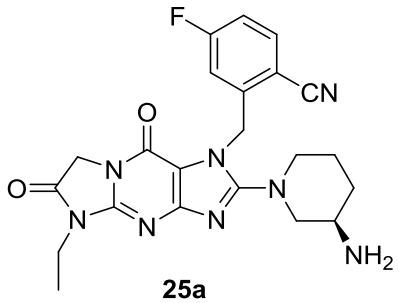


23ca



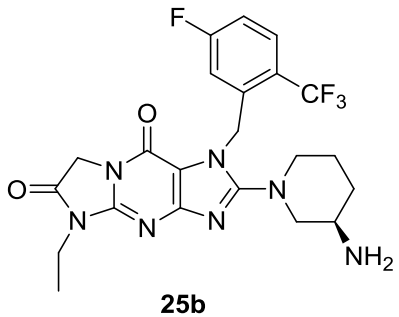
23cb

2-Bromo-1-(2,5-fluorobenzyl)-5-ethyl-1,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (23ca) and **2-Bromo-3-(2,5-fluorobenzyl)-5-ethyl-3,5-dihydro-9H-imidazo[1,2-a]purine-6,9(7H)-dione (23cb)** Compounds **23ca** and **23cb** were prepared analogously as a mixture from compound **22** and 2-(bromomethyl)-1,4-difluorobenzene. LC-MS $C_{16}H_{12}BrF_2N_5O_2$ m/e = 424 (M+1).



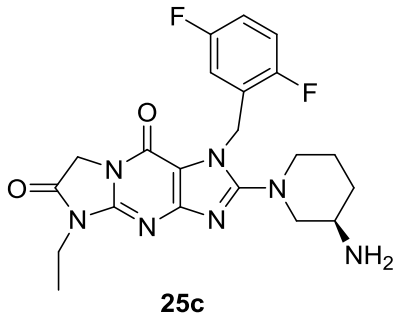
25a

(R)-2-((2-(3-aminopiperidin-1-yl)-5-ethyl-6,9-dioxo-5,6,7,9-tetrahydro-1H-imidazo[1,2-a]purin-1-yl)methyl)-4-fluorobenzonitrile (25a). Using the procedures described for compounds **16c** and **17c**, a mixture of **23aa** and **23ab** was converted to compound **25a,** which was purified by preparative TLC eluting with 6% 7 M NH_3/MeOH in CH_2Cl_2. **.** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, J = 8.6, 5.3 Hz, 1 H), 7.08 (td, J = 8.1, 2.6 Hz, 1 H), 6.73 (dd, J = 9.1, 2.5 Hz, 1 H), 5.57 (s, 2 H), 4.43 (s, 2 H), 3.87 (q, J = 7.2 Hz, 2 H), 3.45 (m, 1 H), 3.25 (d, J = 12.5 Hz, 1 H), 3.07 – 2.97 (m, 2 H), 2.81 (dd, J = 12.2, 8.9 Hz, 1 H), 1.92 (m, 1 H), 1.76 (m, 1 H), 1.64 (m, 1 H), 1.32 (t, J = 7.2 Hz, 3 H), 1.30 (m, 1 H). LC-MS $C_{22}H_{23}FN_8O_2$ m/e = 451 (M+1). HRMS (M+1) calcd 451.2006, found 451.2013.



25b

(R)-2-((2-(3-aminopiperidin-1-yl)-5-ethyl-1-(5-fluoro-2-(trifluoromethyl)benzyl)-1,5-dihydro-9H-imidazo[1,2-a]purin-6,9(7H)-dione (25b). Compound **25b** was prepared from a mixture of **23ba** and **23bb** analogously and purified by preparative TLC eluting with 6% 7 M NH_3/MeOH in CH_2Cl_2. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (dd, J = 8.8, 5.3 Hz, 2 H), 7.06 (s, 1 H), 6.58 (d, J = 9.5 Hz, 1 H), 5.56 (s, 2 H), 4.43 (s, 2 H), 3.88 (q, J = 7.2 Hz, 2 H), 3.50 (dd, 1 H), 3.24 (m, 1 H), 3.02 (m, 1 H), 2.92 (m, 1 H), 2.82 (d, J = 11.1 Hz, 1 H), 1.99 (m, 1 H), 1.70 (m, 1 H), 1.55 (m, 1 H), 1.34 (t, J = 7.2 Hz, 3 H), 1.32 (m, 1 H). LC-MS $C_{22}H_{23}F_4N_7O_2$ m/e = 494 (M+1). HRMS (M+1) calcd 494.1928, found 494.1936.



25c

(R)-2-((2-(3-aminopiperidin-1-yl)-1-(2,5-difluorobenzyl)-5-ethyl-1,5-dihydro-9H-imidazo[1,2-a]purin-6,9(7H)-dione (25c). Compound **25c** was prepared from a mixture of **23ca** and **23cb** analogously and purified by preparative TLC eluting with 6% 7 M NH_3/MeOH in CH_2Cl_2. LC-MS $C_{21}H_{23}F_2N_7O_2$ m/e = 444 (M+1). HRMS (M+1) calcd 444.1960, found 444.1971.

X-Ray Crystallography and docking software

DPP4 protein expression, purification and crystallization, and all crystallographic methods have been previously reported.[1] The structures of DPP-4 in complex with inhibitors **17c** and alogliptin have been determined to atomic resolution. The coordinates have been deposited and are available at the Protein Data Bank, code 5I7U**.** Figures were generated using PyMol.[2]

The docking experiments were done using an in-house method called SQW, which is a modified version of SQ.[3]

Biology. All of the biological assays and experiments with animal models performed in Merck & Co were in accordance with all national or local guidelines and regulations.

***In vitro* Assays**

DPP-4 activity was measured according to a modified literature protocol[4] using the peptide substrate, Gly-Pro-AMC, which is cleaved by DPP-4 to release the fluorescent AMC group. Release of AMC was monitored in real time in a continuous fluorescence intensity measurement (kinetic mode) using the PHERAstar Plus plate reader (BMG Lab. Tech.) at an excitation wavelength of 360 nm and emission wavelength of 460 nm. All assay components and materials were pre-incubated to the assay temperature (37 ºC) prior to starting the reaction. Enzyme, test compound, and substrate working solutions were prepared in assay buffer (100 mM HEPES, 100 mM NaCl, 0.1 mg/ml BSA, pH 7.5). To determine enzyme inhibition potency (IC_{50}), 10 µl DPP4 and 10 µl of test compound (11 concentrations from a 3-fold serial dilution) were mixed in 384-well plate and pre-incubated at 37 ºC for 30 min. Substrate (10 µl) was then added to start the reaction (final assay concentrations: 20 pM DPP4, 50 µM Gly-Pro-AMC, varying concentrations of test compound starting at 10 µM, 1 % DMSO in assay buffer). Initial reaction velocities were monitored for ~50 minutes at 37 ºC.

Recombinant DPP8 activity was measured using the peptide substrate, Ala-Pro-AFC, which is cleaved by DPP8 to release the fluorescent AFC group. Release of AFC was monitored in real time in a continuous fluorescence intensity measurement (kinetic mode) using the PHERAstar Plus plate reader (BMG Lab. Tech.) at an excitation wavelength of 400 nm and emission wavelength of 505 nm. All assay components and materials were pre-incubated to the assay temperature (37 ºC) prior to starting the reaction. Enzyme, test compound, and substrate working solutions were prepared in assay buffer (100 mM HEPES, 100 mM NaCl, 0.1 mg/ml BSA, pH 7.5). To determine enzyme inhibition

potency (IC_{50}), 10 μl DPP8 and 10 μl of test compound (11 concentrations from a 3-fold serial dilution) were mixed in 384-well plate and pre-incubated at 37 ºC for 20 min. 10 μl substrate (300 uM in assay buffer) was then added to start the reaction. Initial reaction velocities were monitored for ~ 40 minutes at 37 ºC.

DPP9 activity was measured using the peptide substrate, Gly-Pro-AMC, which is cleaved by DPP9 to release the fluorescent AMC group. Release of AMC was monitored in real time in a continuous fluorescence intensity measurement (kinetic mode) using the PHERAstar Plus plate reader (BMG Lab. Tech.) at an excitation wavelength of 360 nm and emission wavelength of 460 nm. All assay components and materials were pre-incubated to the assay temperature (37 ºC) prior to starting the reaction. Enzyme, test compound, and substrate working solutions were prepared in assay buffer (100 mM HEPES, 100 mM NaCl, 0.1 mg/ml BSA, pH 7.5). To determine enzyme inhibition potency (IC_{50}), 10 μl DPP9 and 10 μl of test compound (11 concentrations from a 3-fold serial dilution) were mixed in 384-well plate and pre-incubated at 37 ºC for 20 min. 10 μl substrate was then added to start the reaction (final assay concentrations: 30 pM DPP9; 100 μM Gly-Pro-AMC, varying concentrations of test compound starting at 10 uM, 1 % DMSO in assay buffer). Initial reaction velocities were monitored for ~ 50 minutes at 37 ºC.

FAP activity was measured using the substrate, Nle-Pro-AMC, which is cleaved by FAP to release the fluorescent AMC group. Release of AMC was monitored in real time in a continuous fluorescence intensity measurement (kinetic mode) using the PHERAstar Plus

plate reader (BMG Lab. Tech.) at an excitation wavelength of 360 nm and emission wavelength of 460 nm. All assay components and materials were pre-incubated to the assay temperature (37 ºC) prior to starting the reaction. Enzyme, test compound, and substrate working solutions were prepared in assay buffer (100 mM Tris, 100 mM NaCl, 0.1 mg/ml BSA, pH 8). To determine enzyme inhibition potency (IC_{50}), 10 µl FAP solution and 10 µl of test compound solution (11 concentrations from a 3-fold serial dilution starting at 10 µM) were mixed in 384-well plate and pre-incubated at 37 ºC for 20 min. Substrate (10µl of 150 µM) was then added to start the reaction (1 % final DMSO concentration). Initial reaction velocities were monitored for ~ 40 minutes at 37 ºC.

QPP activity was measured using the substrate, Nle-Pro-AMC, which is cleaved by QPP to release the fluorescent AMC group. Release of AMC was monitored in real time in a continuous fluorescence intensity measurement (kinetic mode) using the PHERAstar Plus plate reader (BMG Lab. Tech.) at an excitation wavelength of 360 nm and emission wavelength of 460 nm. All assay components and materials were pre-incubated to the assay temperature (37 ºC) prior to starting the reaction. Enzyme, test compound, and substrate working solutions were prepared in assay buffer (100mM Cacodylate buffer, 0.1 mg/ml BSA, pH 5.5). To determine enzyme inhibition potency (IC_{50}), 10 µl QPP solution and 10 µl of test compound solution (11 concentrations from a 3-fold serial dilution starting at 10 µM) were mixed in 384-well plate and pre-incubated at 37 ºC for 30 min. 10 µl of 60 uM substrate was then added to start the reaction (1 % final DMSO concentration). Initial reaction velocities were monitored for ~ 50 minutes at 37 ºC.

IC_{50} values for DPP4, DPP8, DPP9, FAP, and QPP inhibition were calculated from a non-linear fit of initial reaction velocities versus compound concentration using inhibition dose-response equation (four-parameter; variable slope) using PRISM software (GraphPad). The data are averages of three measurements. Standard deviation is within 10%.

Oral Glucose Tolerance Test (OGTT)

56 male 7-week-old lean C57BL/6N mice from Taconic Farm were used for this study. Mice were group housed in polycarbonate cages in a controlled environment (70±2ºF, 30-70 % relative humidity, 12/12 hours light/dark cycle) and had *ad libitum* access to reverse-osmosis purified water via automatic watering system and standard rodent chow (7012, Teklad, Madison, WI). Animals were acclimated for at least seven days prior to the study. All animal procedures were performed within an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited facility and approved by the Merck Institutional Animal Care and Use Committee (IACUC). Prior to oGTT, mice were fasted overnight by removing food the day before study at 4:00 p.m. On the morning of the study, at 8:00 a.m., blood glucose level (T= -60 min) was measured by tail snipping using OneTouch glucometer (LifeScMilpitas, CA). Mice were weighed and dosed orally with vehicle (0.4% methyl cellulose) or treatment compounds (n=8 per group), and gently put back to home cages. 60 min later, mice were orally challenged with either water (control) or 50% dextrose (5.0 g/kg), immediately after measuring 0 min blood glucose reading. Upon dextrose challenge, ensuing blood glucose measurements at various time points were recorded and analyzed.

Ex vivo Pharmacodynamic assay

Male 6-week-old lean C57BL/6N mice from Taconic Farm were used for this study. Mice were group housed in polycarbonate cages in a controlled environment (70±2ºF, 30-70 % relative humidity, 12/12 hours light/dark cycle) and had *ad libitum* access to reverse-osmosis purified water via automatic watering system and standard rodent chow (7012, Teklad, Madison, WI). Animals were acclimated for at least seven days prior to the study. All animal procedures were performed within an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International-accredited facility and approved by the Merck Institutional Animal Care and Use Committee (IACUC).

Compounds were dissolved in 0.4% hydroxypropyl methylcellulose in 10 ml/kg dosing volume. Mice were weighed and cages were marked with groups/time-points (2, 6 and 24hr). Mice were dosed orally with vehicle (0.4% methylcellulose) or treatment compounds, and gently put back to home cages. 2hr, 6hr and 24hr post dose, mice were euthanized with CO_2 overdose, and blood was collected via cardiocentesis, respectively. Plasma was then submitted for in vitro assay to access DPPIV enzymatic activity.

Hepatocyte clearance assay

Intrinsic clearance (CL_{int}) was evaluated in cryopreserved rat, dog, monkey, and human hepatocytes. Hepatocytes were incubated with compounds at 1 μM for 0, 30, 60, and 120 min. The incubation was terminated by the addition of acetonitrile and the samples

were analyzed by LC-MS/MS. The in vitro CL_{int} for hepatocytes was calculated according to the following formula:

$$CL_{int} = \frac{C_0 - C_{120\,min}}{AUC_{0-120min}} \cdot \frac{V}{N}$$

where C_0 and $C_{120\,min}$ were the concentrations of the compound (μM) at 0 and 120 min, respectively; $AUC_{0\text{-}120\,min}$ was the area under the concentration-time curve from time zero to 180 min; V is the volume of incubation and N is the number of hepatocytes in millions.[5]

CYP enzyme inhibition assay

To assess the potential for inhibition of CYPs (CYP3A4, CYP2D6 and CYP2C9), human liver microsomes were incubated with several concentrations of tested articles (0 to 50 μM), 1 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH) and substrates for various CYPs at 37°C for 10 min.[6] The substrate concentration was kept near the Km value for each CYP reaction. The concentrations of the metabolites formed from each substrate after incubation were determined by LC-MS/MS using a standard curve. The concentrations at which 50% of the initial enzyme activity was inhibited (IC_{50}) were determined from the graph of compound concentrations versus percent of inhibition.

To evaluate metabolism/mechanism-based inhibition, compound was pre-incubated with human liver microsomes for 30 min at 37°C in the presence of NADPH and in the absence of substrates. After the pre-incubation step, the CYP substrates were added at the previously stated concentrations and the reactions were allowed to proceed as indicated in the previous paragraph.

PXR assay

The pregnane X receptor (PXR) assay provides an in vitro model to assess the potential for induction of human CYP gene expression through the PXR pathway.[7] After activation by a xenobiotic, the PXR stimulates the transcription of the CYP3A4 gene in addition to other genes. A PXR activation assay employing a luciferase reporter gene was used to determine whether compounds induce CYP3A4 gene expression.[8] Induction potential was evaluated at concentrations up to 30 μM. Rifampicin was used as the positive control.

hERG assay

The displacement of ^{35}S MK499 binding assay for hERG liability was performed according to the literature protocol.[9]

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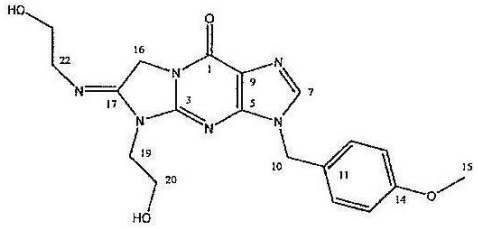
Spectra of compound 9

Compound 9

Reference:

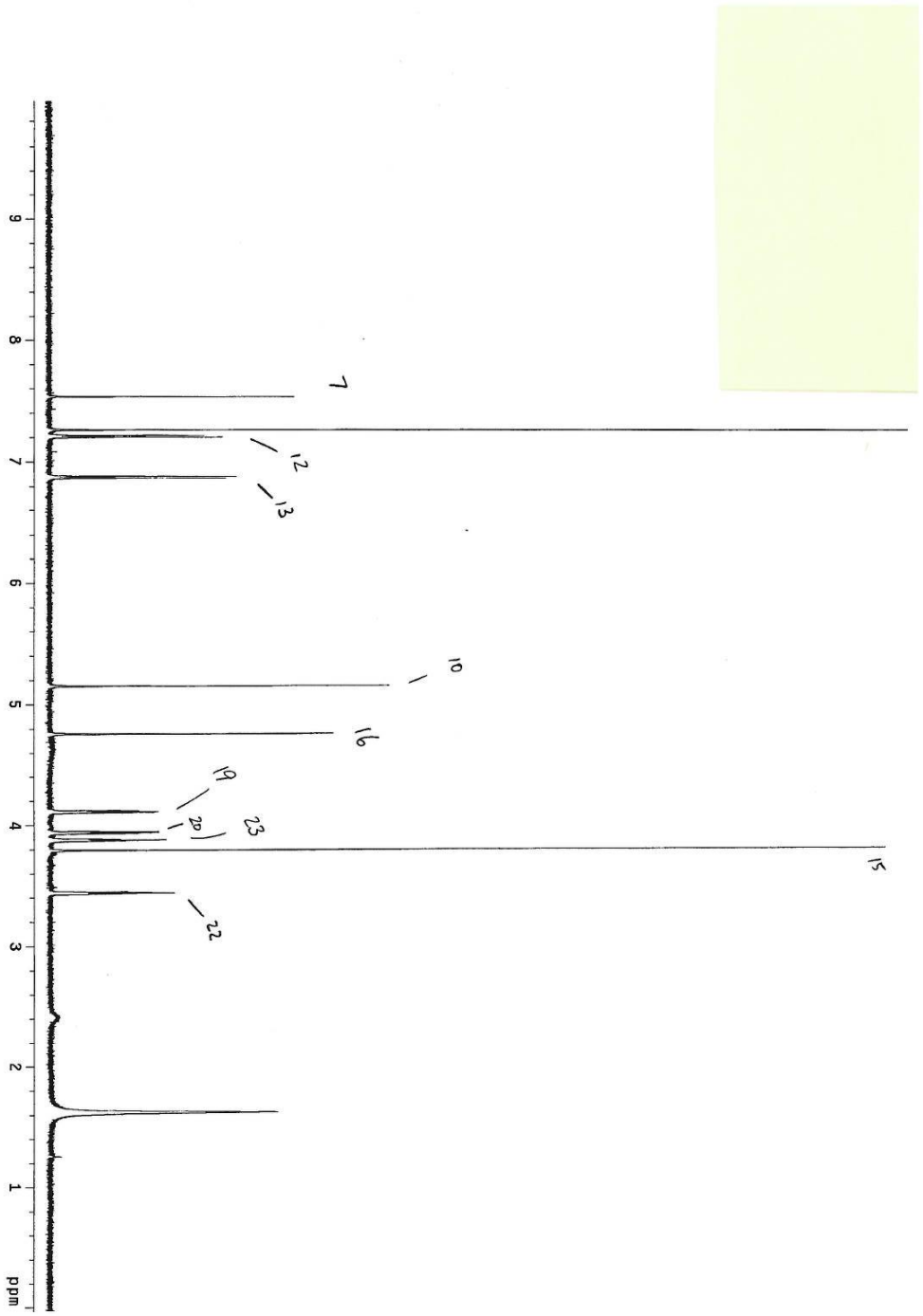


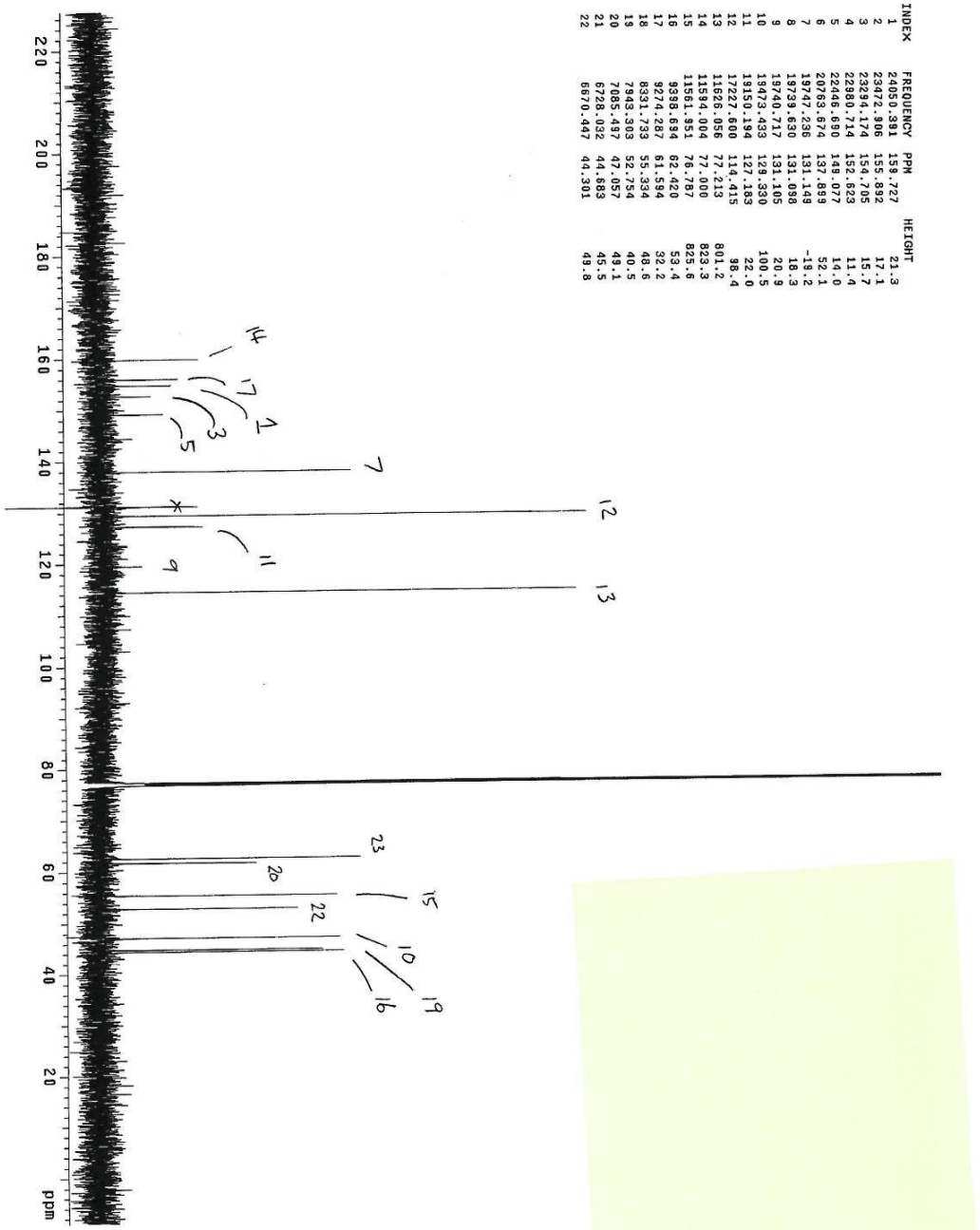
Chemical Formula: $C_{19}H_{22}N_6O_4$
Exact Mass: 398

NMR spectra were taken in $CDCl_3$ on a 600 MHz spectrometer. Proton, Carbon, HSQC, HMBC and NOESY spectra were obtained.

Chemical shifts for C10, C16, C19, C22, C20 and C23 are consistent with structure.

HMBC: H16, H19, H22 to C17 (156 ppm)
H16 abd H19 to C3 (153 ppm)
No correlation from H20 and H23 to any carbons.


9
8
7
6
5
4
3
2
1
ppm
7
12
13
10
1
16
19
20
1
23
15
22


INDEX FREQUENCY PPM HEIGHT
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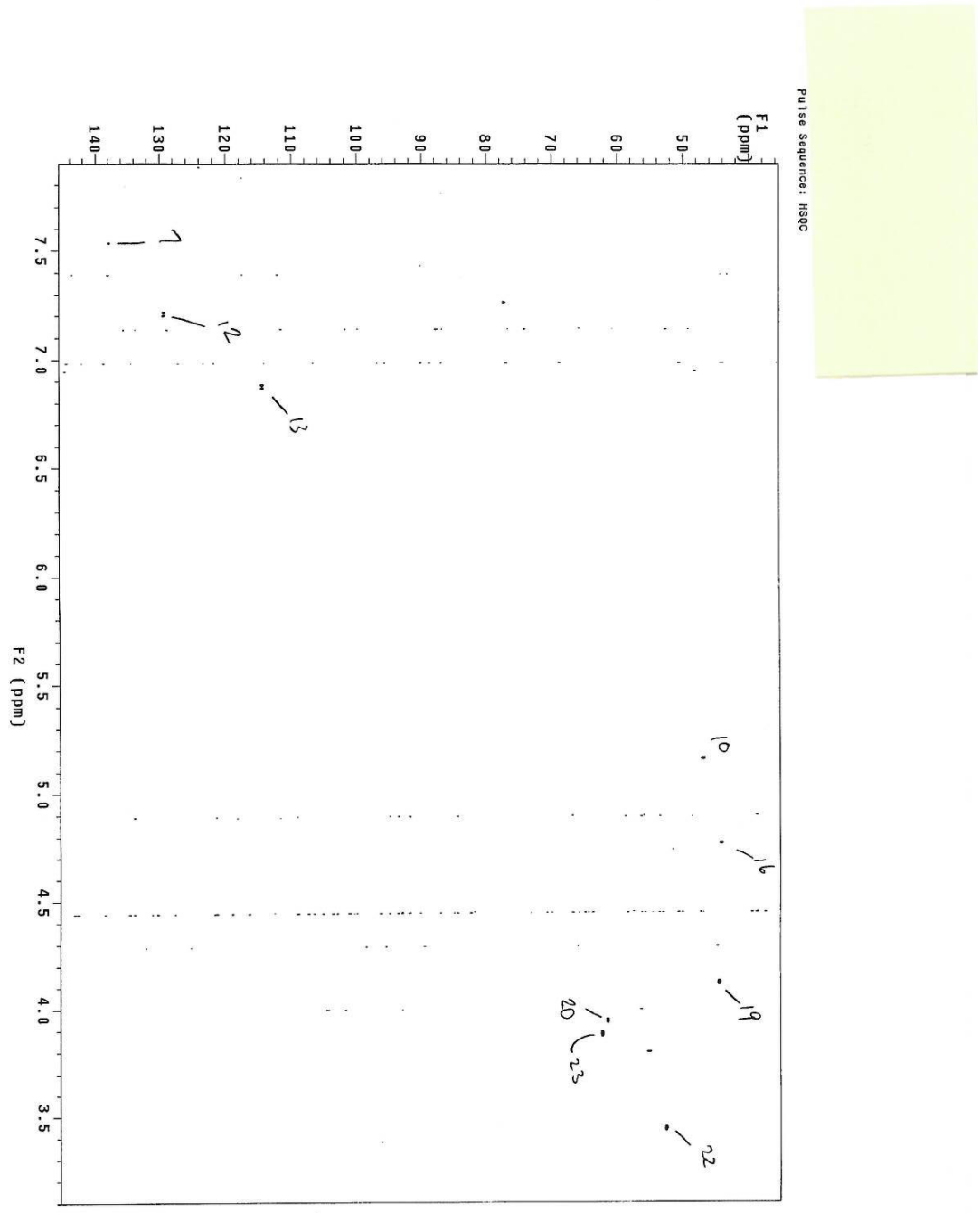
2 23972.906 155.892 17.1
3 23894.174 154.705 15.7
4 22980.714 152.623 11.4
5 22446.690 148.077 14.0
6 20763.674 137.899 52.1
7 19747.236 131.149 -19.2
8 19739.630 131.098 18.3
9 19740.717 131.105 20.9
10 19473.433 129.330 100.5
11 19150.194 127.183 22.0
12 17227.600 114.415 98.4
13 11626.056 77.213 801.2
14 11594.004 77.000 823.3
15 11561.951 76.787 825.6
16 9398.694 62.420 53.4
17 9274.287 61.594 32.2
18 8331.733 55.334 48.6
19 7943.303 52.754 40.5
20 7685.497 47.057 49.1
21 6728.032 44.683 45.5
22 6670.447 44.301 49.8
220
200
180
160
140
120
100
80
60
40
20
ppm
14
17
1
3
5
7
12
11
9
13
23
20
22
15
10
19
16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F1 (ppm)
F2 (ppm)
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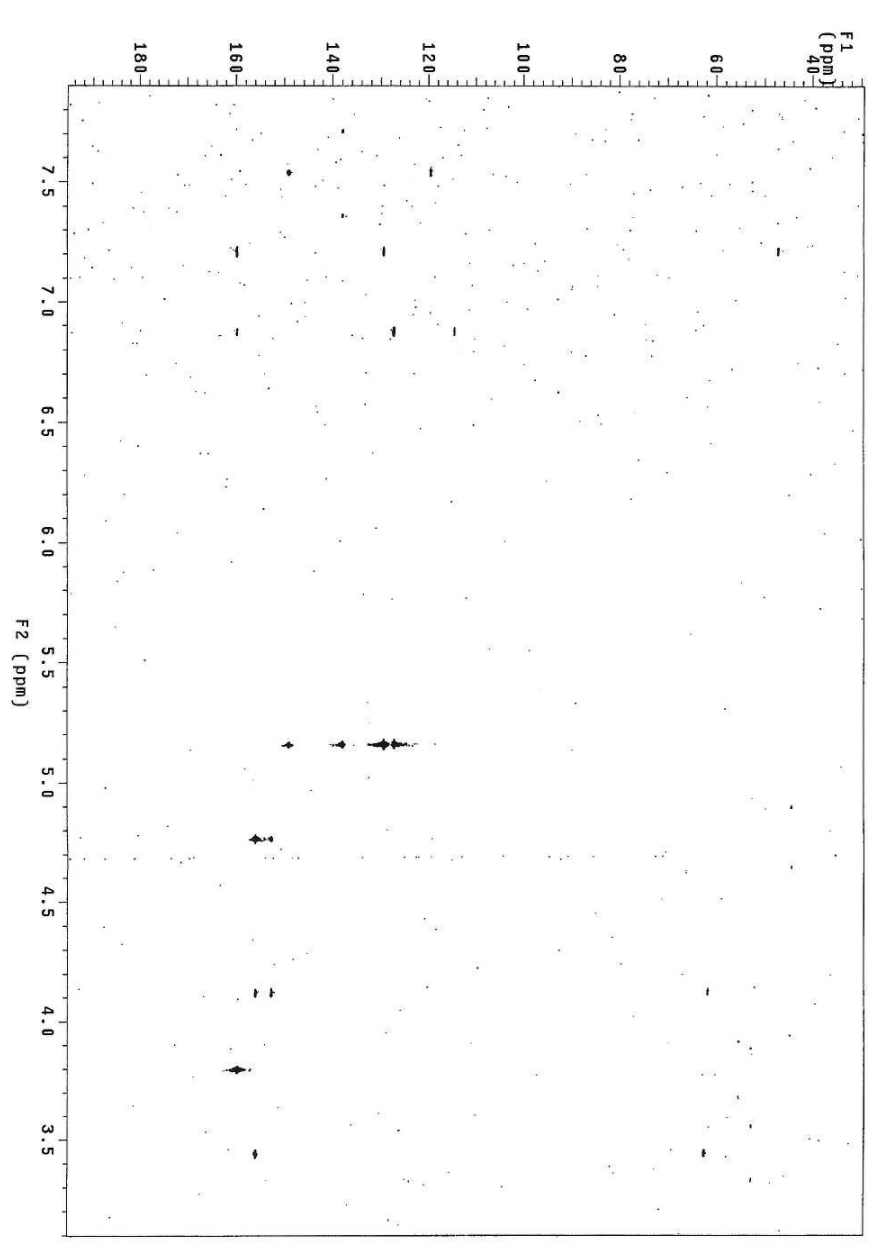
16
19
20
23
22
12
13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F1 (ppm)
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60
80
100
120
140
160
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F2 (ppm)
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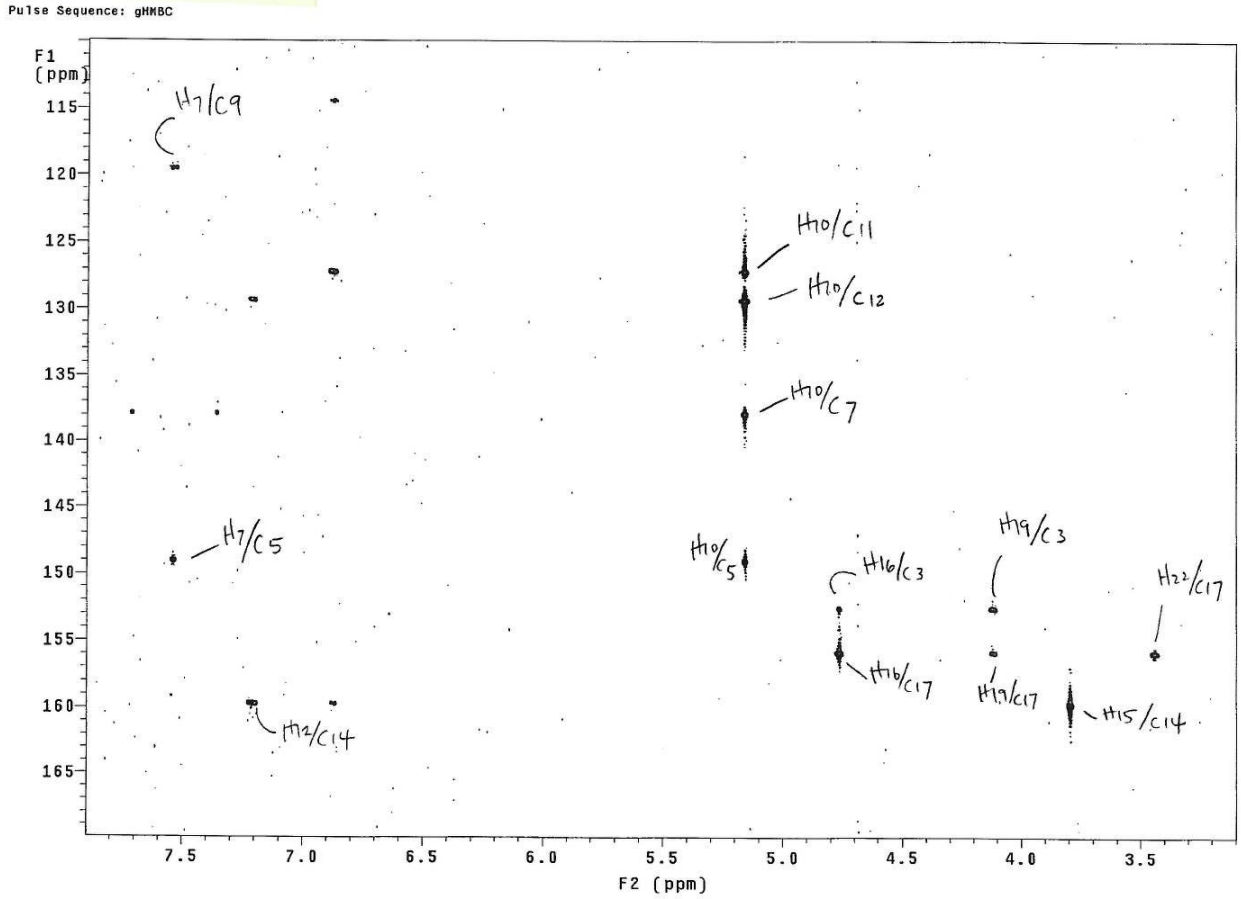
6.0
5.5
5.0
4.5
4.0
3.5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F1 (ppm)
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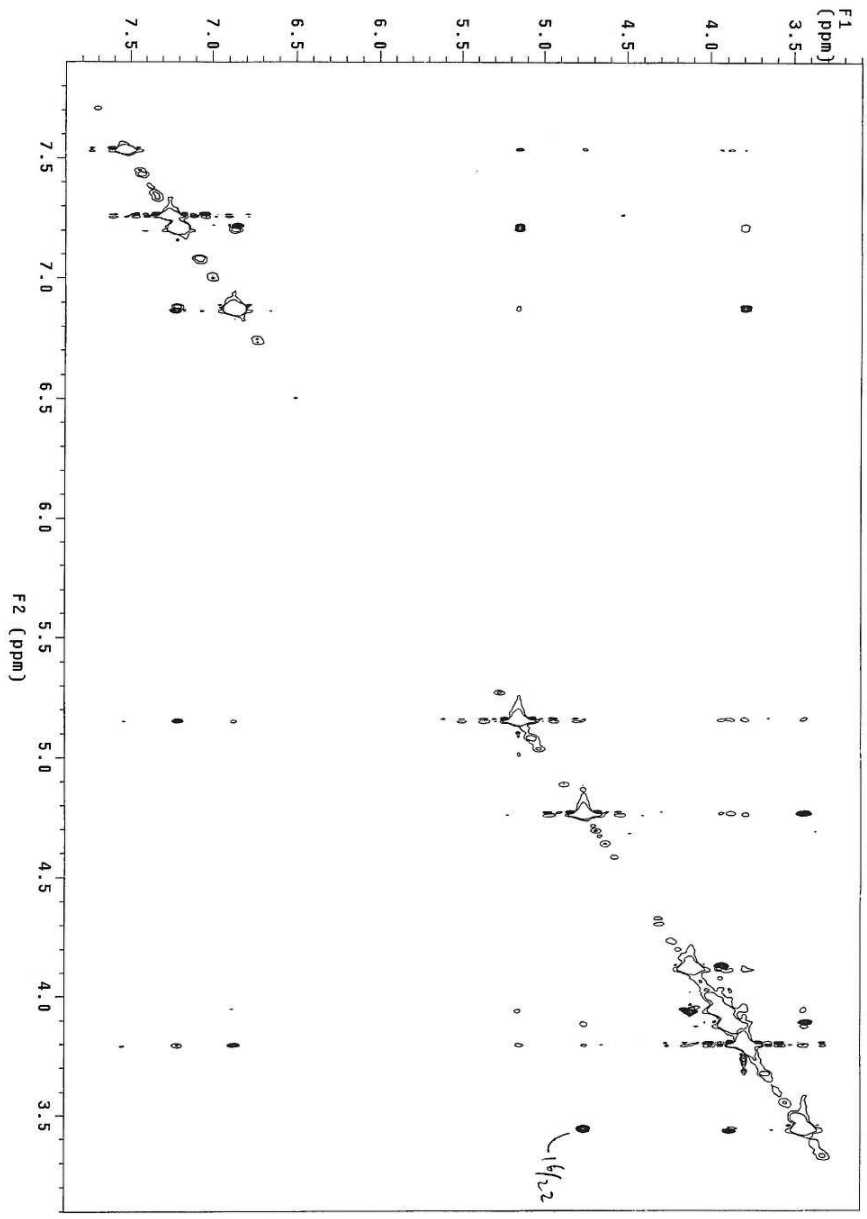
120
125
130
135
140
145
150
155
160
165
H7/C9
H10/C11
H10/C12
H10/C7
H7/C5
H10/C5
H16/C3
H19/C3
H22/C17
H16/C17
H19/C17
H15/C14
H12/C14
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
F2 (ppm)


Pulse Sequence: NOESY
F1 (ppm)
F2 (ppm)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5