Heterotropic Activation of the Midazolam Hydroxylase Activity of P450 3A by a Positive Allosteric Modulator of Metabotropic Glutamate Receptor 5: *In Vitro* to *In Vivo* Translation and Potential Impact on Clinically Relevant Drug-Drug Interactions

Anna L. Blobaum, Thomas M. Bridges, Frank W. Byers, Mark. L. Turlington, Margrith E. Mattmann, Ryan D. Morrison, Claire Mackie, Hilde Lavreysen, José M. Bartolomé, Gregor J. MacDonald, Thomas Steckler, Carrie K. Jones, Colleen M. Niswender, P. Jeffrey Conn, Craig W. Lindsley, Shaun R. Stauffer and J. Scott Daniels*

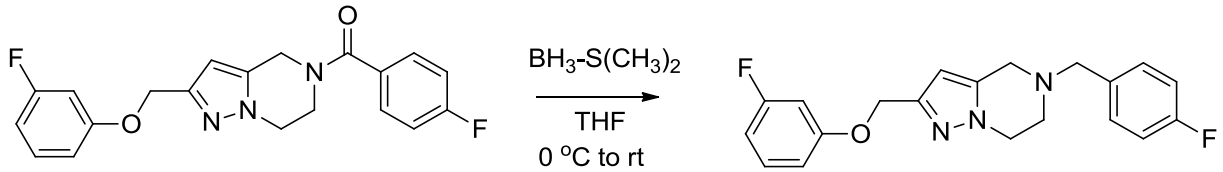
Vanderbilt University Medical Center, Vanderbilt Center for Neuroscience Drug Discovery, Department of Pharmacology, Nashville, TN, 37232 (A.L.B., T.M.B., F.W.B., M.L.T., M.E.M., R.D.M., C.K.J., C.M.N., P.J.C., C.W.L., S.R.S., J.S.D.), CREATe ADME/Tox, Janssen Research and Development, Turnhoutseweg 30, B-2340, Beerse, Belgium (C.M.), Neuroscience, Janssen Research and Development, Turnhoutseweg 30, B-2340, Beerse, Belgium (H.L., G.J.M., T.S.), Neuroscience, Janssen Research and Development, Jarama 75, 45007-Toledo, Spain (J.M.B.)

Supplementary Material

VU0464797 and VU0448187 Synthesis

General. All NMR spectra were recorded on a Bruker 400 MHz instrument. ^{1}H chemical shifts are reported in δ values in ppm downfield from TMS as the internal standard in $CDCl_3$. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, bs = broad singlet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, m = multiplet), integration, coupling constant (Hz). Low resolution mass spectra were obtained on an Agilent 1200 series 6130 mass spectrometer. High resolution mass spectra were recorded on a Waters Q-TOF API-US. Analytical thin layer chromatography was performed on Analtech silica gel GF 250 micron plates. Analytical HPLC was performed on an HP1100 with UV detection at 214 and 254 nm along with ELSD detection, LC/MS (J-Sphere80-C18, 3.0 x 50 mm, 4.1 min gradient, 5%[0.05%TFA/CH_3CN]:95%[0.05%TFA/H_2O] to 100%[0.05%TFA/CH_3CN]. Preparative RP-HPLC purification was performed on a custom HP1100 automated purification system with collection triggered by mass detection or using a Gilson Inc. preparative UV-based system using a Phenomenex Luna C18 column (50 x 30 mm I.D., 5 μm) with an acetonitrile (unmodified)-water (0.1% TFA) custom gradient. Normal-phase silica gel preparative purification was performed using an automated Combi-flash companion from ISCO. Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased from Aldrich Chemical Co. and were used without purification. All polymer-supported reagents were purchased from Argonaut Technologies and Biotage.

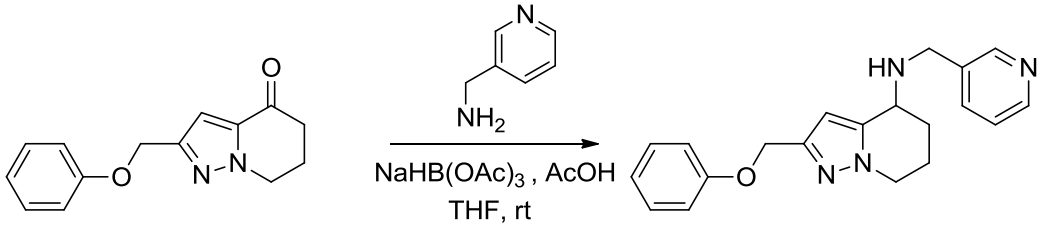
Scheme 1. Synthesis of VU0448187



1.1[1] **VU0448187**

5-(4-Fluorobenzyl)-2-((3-fluorophenoxy)methyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyrazine (VU0448187): To a 0 ^{o}C solution of 1.1, (2-((3-fluorophenoxy)methyl)-6,7-dihydropyrazolo[1,5-a]pyrazin-5(4H)-yl)(4-fluorophenyl)methanone[1] (333 mg, 0.90 mmol) in THF (9 mL) was added dropwise borane dimethyl sulfide (10 M, 9 mL, 9 mmol). Upon completion the mixture was allowed to warm to rt while stirring. Upon disappearance of starting material as judged by LC-MS (1.5 h) the mixture was cooled to 0 ^{o}C and MeOH added intermittently dropwise until gas evolution ceased. Excess MeOH was added (10 mL) and vessel heated under reflux for 1.5h. The mixture was concentrated and the resulting residue purified by automated silica gel chromatography (EtOAc/hexanes) to give desired product as a white low melting semi-solid (70% yield): ^{1}H NMR δ 7.32 (2H, m), 7.21 (1H, q, *J* = 8.3 Hz), 7.03 (2H, t, *J* = 8.9 Hz), 6.77 (1H, dd, *J* = 8.3, 2.2 Hz), 6.71 (1H, dt, *J* = 11.0, 2.4 Hz), 6.65 (1H, td, *J* = 8.3, 1.7 Hz), 6.04 (1H, s), 5.02 (2H, s), 4.18 (2H, t, *J* = 5.6 Hz), 3.68 (2H, s), 3.65 (2H, s), 2.93 (2H, t, *J* = 5.4 Hz); LC/MS (>98%) R_t = 0.638, *m/z* = 356.1 [M+H].

Scheme 2. Synthesis of VU0464797.



2.1[2] **VU0464797**

2-(phenoxymethyl)-N-(pyridin-3-ylmethyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyridin-4-amine (VU0464797):

To a solution of ketone 2.1[2] (200 mg, 0.83 mmol) in DCE (4 mL) was added 3-picolylamine (0.1 mL, 0.99 mmol). $NaHB(OAc)_3$ (280 mg), 1.32 mmol) and acetic acid (0.094 mL, 1.65 mmol) were then added and the mixture was stirred at room temperature for 1 day. After the reaction was judged to be complete by LC-MS the reaction was quenched with aqueous NaOH (2 mL, 1.0 M) and extracted with EtOAc (3 x 10 mL). The organic layer was dried with Na_2SO_4, concentrated, and the resulting oil was purified by reverse-phase HPLC chromatography 10%[CH_3CN/0.1%TFA]—90%[CH_3CN/0.1%TFA] to yield a brown oil (84% yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (1H, bs), 8.52 (1H, d, *J* = 4.1 Hz), 7.73 (1H, d, *J* = 7.7 Hz), 7.28 (3H,

m), 7.01 (2H, d, *J* = 8.3 Hz), 6.95 (1H, t, *J* = 7.3 Hz), 6.33 (1H, s), 5.05 (2H, s), 4.14 (2H, t, *J* = 6.2 Hz), 3.94 (1H, m), 3.93 (2H, s), 2.27 (1H, m), 2.13(1H, m), 1.96 (1H, m), 1.76 (1H, m), 1.66 (1H, bs); LC/MS (>98%), R_t = 0.382, *m/z* = 335.2 [M+H].

REFERENCES

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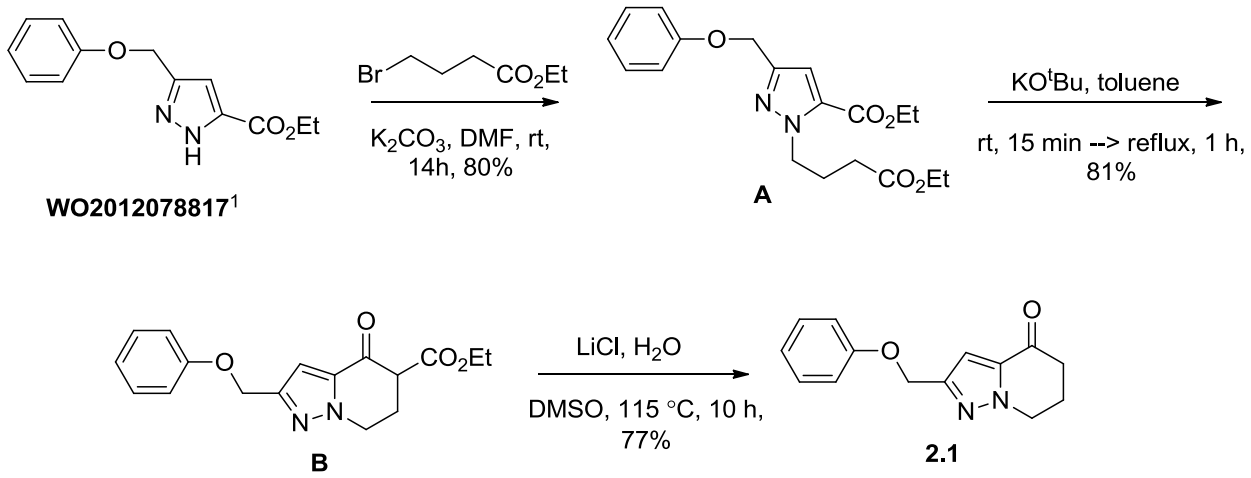
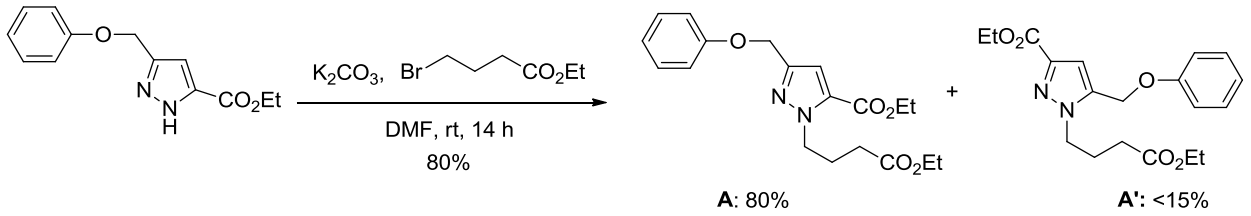
2) Turlington, M. L.; Noetzel, M. J.; Vinson, P. N.; Bridges, T. M.; Steckler, T.; Lavreysen, H.; Mackie, C.; Bartolomé, J. M.; MacDonald, G. J.; Daniels, J. S.; Jones, C. K., Niswender, C. M.; Conn, P. J.; Lindsley, C. W.; Stauffer, S. R. Discovery and SAR of a novel series of highly efficient metabotropic glutamate receptor 5 positive allosteric modulators. *Bioorg. Med. Chem. Lett*., **2013**, *23*, *in preparation* (separate supporting material attached as addendum for review purposes).

SI addendum: Route to prepare ketone precursor **2.1**.



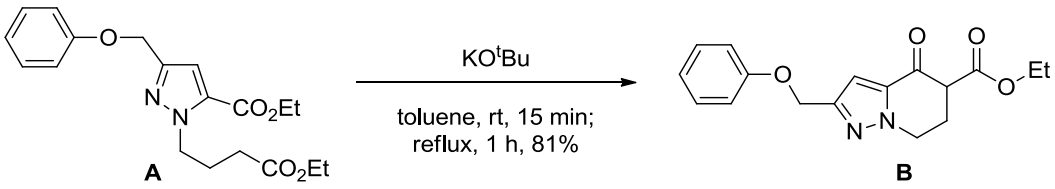
a. Step 1: Preparation of Ethyl 1-(4-ethoxy-4-oxobutyl)-3-(phenoxymethyl)-1H-pyrazole-5-carboxylate, A.



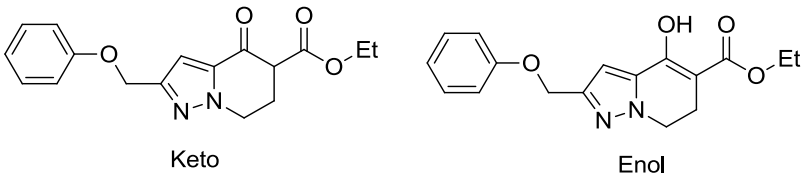
To a solution of ethyl 3-(phenoxymethyl)-1H-pyrazole-5-carboxylate (1g, 4.06 mmol, 1 equiv) in DMF (40 mL, 0.1M) was added K_2CO_3 (0.84 g, 6.99 mmol, 1.5 equiv) and ethyl 4-bromobutyrate (0.87 mL, 6.09 mmol, 1.5 equiv). The reaction was stirred for 14 h at rt, after which the starting material was observed to be consumed by TLC and LC/MS. Two products are observed: the less polar desired regioisomer **A** (TLC: r_f = 0.5, 1:1 Hex/EtOAc; LC/MS: rt = 0.811 min) and the more polar, undesired regioisomer **A'** (TLC: r_f = 0.4, 1:1 Hex/EtOAc; LC/MS: rt = 0.760). The reaction mixture was diluted with EtOAc (125 mL) and washed with H_20 (3 x 50 mL). The aqueous layer was back extracted with EtOAc (1 x 50 mL), and the organic layers were combined, dried with $MgSO_4$, and concentrated. The mixture of regioisomers was purified by silica gel column chromatography eluting with Hex/EtOAc (100:0 Hex/EtOAc to 60:40 Hex/EtOAc) with the desired regioisomer **A** eluting at 15% Hex/EtOAc (**A'**

eluting at 30% Hex/EtOAc) to yield 1.17 g (80% yield) of a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (m, 2H), 6.98 (m, 3H), 6.94 (s, 1H), 5.07 (s, 2H), 4.62 (t, 2H, J = 6.8 Hz), 4.32 (q, 2H, J = 7.1 Hz), 4.12 (q, 2H, J = 7.2 Hz), 2.32 (t, 2H, J = 7.3 Hz), 2.19 (p, 2H, J = 7.1 Hz), 1.36 (t, 3H, J = 7.1 Hz), 1.25 (t, 3H, J = 7.2 Hz). LC/MS (215/254 nm): single peak, *m/z* = 361.3 ([M+H]).

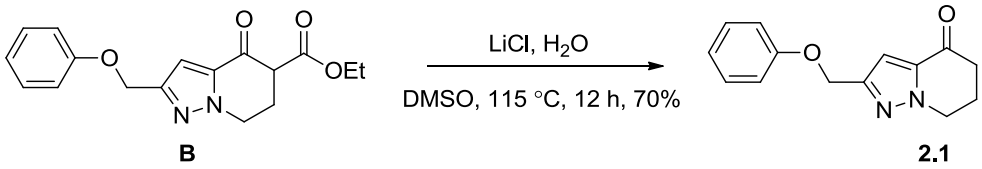
b. Step 2: Preparation of Ethyl 4-oxo-2-(phenoxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-5-carboxylate, B.



To a solution of ethyl 1-(4-ethoxy-4-oxobutyl)-3-(phenoxymethyl)-1H-pyrazole-5-carboxylate, **A**, (1.15 g, 3.19 mmol, 1 equiv) in toluene (31 mL, 0.1 M) was added KO^{t}Bu (0.43 g, 3.8 mmol, 1.2 equiv). The reaction was stirred at rt for 15 minutes, and then warmed to reflux for 1 h, upon which the starting material was consumed by TLC. The solution was cooled to room temperature and diluted with CH_2Cl_2 (25 mL) and H_20 (25 mL). The aqueous layer was acidified with dilute aqueous HCl to neutral pH, and then extracted with CH_2Cl_2 (3 x 25 mL). The organic layer was dried with $MgSO_4$, concentrated, and purified by silica gel column eluting with Hex/EtOAc (100:0 Hex/EtOAc to 60:40 Hex/EtOAC). The product **B** eluted at 30% Hex/EtOAc to yield 0.806 g (81% yield) of clear oil which solidifies to a white solid under high vacuum. *Note: Product exists as 1:2 mixture of keto/enol tautomers as evidenced by ^{1}H NMR.* Keto tautomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (m, 2H), 7.00 (m, 3H), 6.72 (s, 1H), 5.10 (s, 2H), 4.53 (m, 1H), 4.40 (m, 1H), 4.26 (m, 2H), 3.64 (m, 1H), 2.74 (m, 1H), 2.56 (m, 1H), 1.29 (t, 3H, J = 7.1 Hz). Enol tautomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (m, 2H), 7.00 (m, 3H), 6.72 (s, 1H), 5.09 (s, 2H), 4.30 (q, 2H, J = 7.1 Hz), 4.23 (t, 2H, J = 7.4 Hz), 2.87 (t, 2H, J = 7.4 Hz), 1.35 (t, 3H, J = 7.1 Hz). *Two peaks are also present in the LC/MS with both corresponding to the correct mass.* LC/MS (215/254 nm): two peaks, R_t = 0.697 min, 0.800, *m/z* = 315.1 ([M+H]).



c. Step 3: Preparation of 2-(phenoxymethyl)-6,7-dihydropyrazolo[1,5-a]pyridin-4(5H)-one, 2.1.



Ethyl 4-oxo-2-(phenoxymethyl)-4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-5-carboxylate, **B** (0.6 g, 2.39 mmol, 1 equiv) was dissolved in DMSO (19 mL, 0.1M), and LiCl (0.38 g, 11.3 mmol, 4.75 equiv) and H_2O (0.14 mL, 9.6 mmol, 4 equiv) was added. The solution was heated to 115 °C for 12 h, upon which the starting material was observed to be consumed by TLC and LC/MS. The reaction mixture was diluted with EtOAc (100 mL) and washed with H_20 (3 x 50 mL). The aqueous layer was back extracted with EtOAc (1 x 50 mL), and the organic layers were combined, dried with $MgSO_4$, and concentrated. The crude product was purified by silica gel column chromatography eluting with Hex/EtOAc (100:0 Hex/EtOAc to 50:50 Hex/EtOAC) with the desired product **2.1** eluting at 40% Hex/EtOAc to yield 0.44 g (77% yield) of a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (m, 2H), 6.98 (m, 3H), 6.93 (s, 1H), 5.09 (s, 2H), 4.38 (t, 2H, J = 8.5 Hz), 2.68 (t, 2H, J = 6.7 Hz), 2.37 (p, 2H, J = 5.9 Hz). LC/MS (215/254 nm): single peak, R_t = 0.617 min, *m/z* = 243.1 ([M+H]).