Novel Bicyclo[3.1.0]hexane Analogs as Antagonists of Metabotropic Glutamate 2/3 Receptors for the Treatment of Depression

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Graphical Abstract

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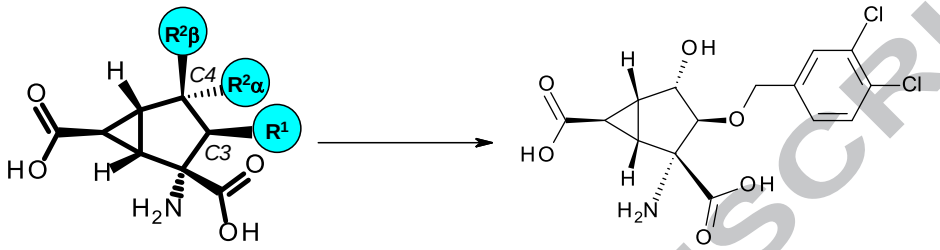

17 Analogs
$hmGlu_2$ IC_{50} = 12.7-1,130 nM
$hmGlu_3$ IC_{50} = 14.3-1,070 nM
R^1 = OCH_2Ar, R^2 = NHCOR, SR, OH, F, NH_2, N_3
Mouse forced swim test
ED_{60} = 0.72-9.5 mg/kg

18
$hmGlu_2$ IC_{50} = 46 ± 14.1 nM
$hmGlu_3$ IC_{50} = 46.1 ± 36.2 nM
ED_{60} = 0.72 mg/kg

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Novel Bicyclo[3.1.0]hexane Analogs as Antagonists of Metabotropic Glutamate 2/3 Receptors for the Treatment of Depression

Bruce A. Dressman*[,a] , Eric G. Tromiczak,[a] Mark D. Chappell,[a] Allie E. Tripp,[a] Steven J. Quimby,[a] Tatiana Vetman,[a] Adam M. Fivush,[a] James Matt,[a] Carlos Jaramillo,[b] Renhua Li,[a] Albert Khilevich,[a] Maria-Jesus Blanco,[a] Stephon C. Smith,[a] Mercedes Carpintero,[b] José Eugenio de Diego,[b] Mario Barberis,[b] Susana García-Cerrada,[b] José F. Soriano,[b] Jeffrey M. Schkeryantz,[a] Jeffrey M. Witkin,[a] Keith A. Wafford,[c] Wesley Seidel,[c] Thomas Britton,[a] Carl D. Overshiner,[a] Xia Li,[a] Xu-Shan Wang,[a] Beverly A. Heinz,[a] John T. Catlow,[a] Steven Swanson,[a] David Bedwell,[a] Paul L. Ornstein[a] and Charles H. Mitch[a]

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ABSTRACT

Negative modulators of metabotropic glutamate 2 & 3 receptors demonstrate antidepressant-like activity in animal models and hold promise as novel therapeutic agents for the treatment of major depressive disorder. Herein we describe our efforts to prepare and optimize a series of conformationally constrained 3,4-disubstituted bicyclo[3.1.0]hexane glutamic acid analogs as orthosteric (glutamate site) $mGlu_{2/3}$ receptor antagonists. This work led to the discovery of a highly potent and efficacious tool compound **18** ($hmGlu_2$ IC_{50} 46 ± 14.2 nM, $hmGlu_3$ IC_{50} = 46.1 ± 36.2 nM). Compound **18** showed activity in the mouse forced swim test with a minimal effective dose (MED) of 1 mg/kg ip. While in rat EEG studies it exhibited wake promoting effects at 3 and 10 mg/kg ip without any significant effects on locomotor activity. Compound **18** thus represents a novel tool molecule for studying the impact of blocking $mGlu_{2/3}$ receptors both *in vitro* and *in vivo*.

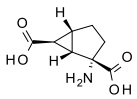
Metabotropic glutamate (mGlu) receptors belong to the class C GPCR family and consist of eight known subtypes which have been historically divided into three groups (Group I: $mGlu_1$ & $_5$; Group II: $mGlu_2$ & $_3$; Group III: $mGlu_4$, $_6$, $_7$ & $_8$) The Group II mGlu receptors are highly expressed in prefrontal cortex, striatum, thalamus, hippocampus, and amygdala, where they act to regulate neuronal excitability via presynaptic, postsynaptic and glial mechanisms. Activation of $mGlu_{2/3}$ receptors is known to inhibit the synaptic release of glutamate, leading to a reduction of synaptic transmission. Accordingly, $mGlu_{2/3}$ agonists (e.g. (1S,2S,5R,6S)-2-aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740), (1R,4S,5S,6S)-4-amino-2-thiabicyclo[3.1.0] hexane-4,6-dicarboxylic acid 2,2-dioxide (LY404039)), Figure 1) produce beneficial effects in rodent models (anxiety, psychosis, pain) thought to be driven by excessive glutamate neurotransmission, and oral prodrugs of these agents ((1S,2S,5R,6S)-2-(L-Alanylamino)bicyclo[3.1.0]hexane-2,6-di carboxylic acid (LY544344), (1R,4S,5S,6S)-4-(L-methionyl amino)-2-thiabicyclo[3.1.0]hexane-4,6-dicarboxylic acid 2,2-dioxide (LY2140023)) have demonstrated efficacy in generalized anxiety disorder[1] and schizophrenia patients,[2] respectively.

Conversely, $mGlu_{2/3}$ receptor antagonists facilitate the presynaptic release of glutamate and thereby enhance synaptic AMPA- and NMDA-receptor activation and neurotransmission under conditions where $mGlu_{2/3}$ receptors are tonically activated. Consistent with this, preclinical studies have demonstrated that $mGlu_{2/3}$ antagonists such as (1S,2S)-2-[(1S)-1-amino-1-carboxy-2-(9H-xanthen-9-yl)ethyl]cyclopropanecarboxylic acid (LY341495) and (1R,2R,3R,5R,6R)-2-amino-3-[(3,4-dichlorobenzyl)oxy]-6-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (MGS0039) (Figure 1) elicit glutamate-driven AMPA receptor-dependent antidepressant[3] and wake-promoting[4] responses in rodents.

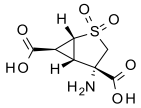
As part of our ongoing research efforts targeting these receptors, we explored the effect of structural modifications at the C3- and C4-positions of LY354740 on functional

$mGlu_{2/3}$-mediated activity. Literature data[18] as well as our own in-house structure activity relationship (SAR) data as exemplified by compound **(+/-)-1**,[5] showed that substitution at the C3-position of LY354740 produced compounds that are functional antagonists. Conversely, we have published an extensive SAR of analogs of LY354740 with a range of C4-postion substituents that exhibit agonist activity.[6,7] In order to further understand the effects of substituents at these two positions on functional activity we set out to develop synthetic methodologies that would enable their dual functionalization. This work has led to the identification of a number of novel potent and selective $mGlu_{2/3}$ receptor antagonists.

$mGlu_{2/3}$ Agonist

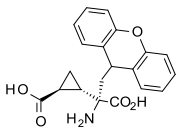


LY354740

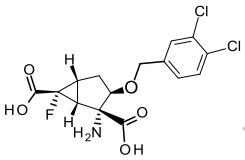


LY404039

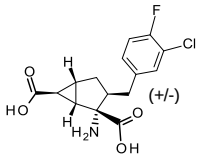
$mGlu_{2/3}$ Antagonist



LY341495



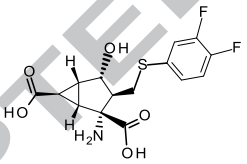
MGS0039



1

$hmGlu_2$ IC_{50} = 312 nM

$hmGlu_3$ IC_{50} = 76.1 nM



LY3020371

Current Work

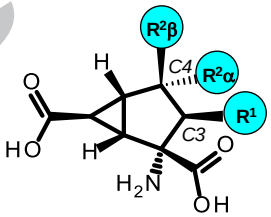
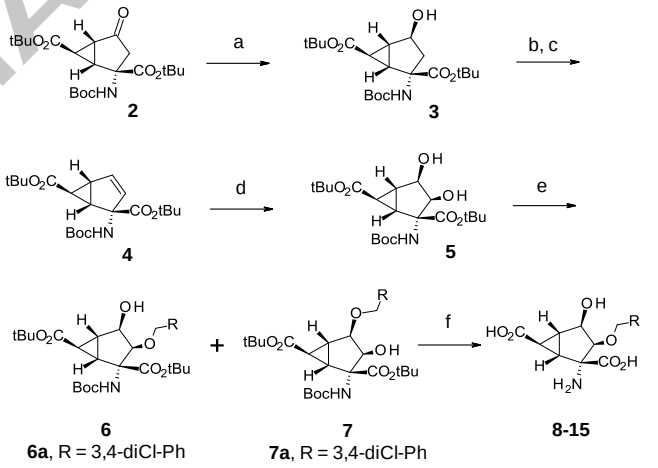


Figure 1: Literature and in-house $mGlu_{2/3}$ receptor ligands.

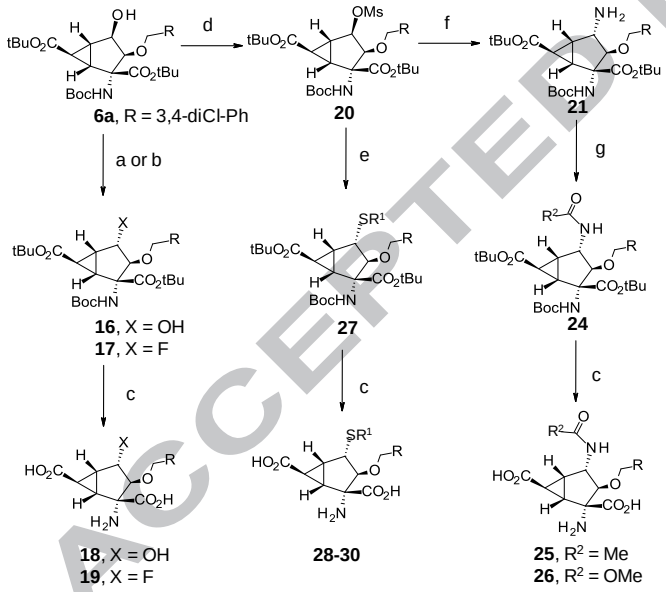
We began our work employing the previously reported enantiomerically pure ketone **2**[6] which can be synthesized on multi-kilogram scale (Scheme 1). Ketone **2** was reduced to give the $C4_\beta$-alcohol **3** in high yield and with excellent diastereoselectivity using L-Selectride®. The alcohol was converted to the corresponding mesylate using methyl sulfonyl chloride (MsCl) and triethyl amine (TEA) and then subjected to tetrabutylammonium fluoride (TBAF) to cleanly give the elimination product **4** in good yield along with unreacted alcohol **3** which could be recovered by normal phase chromatography. Alkene **4** was oxidized using catalytic osmium tetroxide (OsO4) and N-methylmorpholine-N-oxide (NMO) as a co-oxidant to give the expected *cis* $C3_\beta$,$C4_\beta$-diol **5** in high diastereoselective (>50:1) and synthetic yield through oxidant approach from the less sterically hindered face of the bicyclo[3.1.0]hexane ring system. Regioselective alkylation of the C3 carbinol was achieved using silver oxide (Ag_2O) and tetrabutyl ammonium iodine (TBAI) and a benzyl halide to give a mixture of the mono C3 and C4 benzyl ethers **6** and **7** that were separated by normal phase chromatography. In this way, the diol **5** was selectively alkylated with 3,4-dichlorobenyl bromide to give a 3:1 mixture of the 3,4-dichlorobenzyl ether intermediates **6a** and **7a** that were readily separated by normal phase column chromatography to give pure intermediate **6a** in 72% yield. Structural confirmation of **6a** was established by proton NMR.[8] Exhaustive deprotection of intermediate **6a** in glacial acetic acid and water using our previously reported procedure[6] at elevated temperatures in a microwave provided the $C4_\beta$-hydroxyl-$C3_\beta$-3,4-dichlorobenzyl ether **11** as a white solid in 74% yield and in high chemical purity (as determined by proton NMR and LCMS) following concentration of the reaction mixture and trituration of the recovered solid with water and ether. Using a similar process a series of $C4_\beta$-hydroxyl-$C4_\beta$-benzyl ethers **8-15** were prepared and their functional $hmGlu_2$ and $hmGlu_3$ activity was evaluated.



Scheme 1: Reagents and Conditions: (a) L-Selectride®(1M in THF, 1.5 eq.), THF, 0°C then 30% H_2O_2, $NaHCO_3$(quantitative); (b) MsCl, TEA; (c) TBAF, THF, reflux (76% 2 steps); (d) OsO_4 (0.05 eq.), NMO (2.5 eq.), acetone, water [>50:1dr] (91%); (e) RCH_2Br (1.5 eq.), Ag_2O (1.5 eq.), TBAI (1 eq.), DMF [generally 3:1 regioselectivity for **6** vs **7**] (R = 3,4-diCl-Ph, 94%); (f) Acetic acid, water, 140°C, microwave (20-84%).

Based on our initial biochemical results of the $C4_\beta$-hydroxyl-$C4_\beta$-benzyl ethers **8-15** (Table 1), further investigation of the C4 position was conducted using the $C4_\beta$-hydroxyl-$C3_\beta$-3,4-dichlorobenzyl ether intermediate **6a** (Scheme 2). Inversion of the C4 alcohol of **6a** was readily achieved *via* a Mitsunobu reaction using *p*-nitrobenzoic acid followed by hydrolysis of the resulting ester to give the $C4_\alpha$-alcohol **16**. Exhaustive deprotection using aqueous acetic acid as previously described provided the $C4_\alpha$-hydroxyl-$C3_\beta$-benzyl ether **18**. In a similar manner the $C4_\alpha$-fluoro compound **19** was prepared from **6a** in moderate yield using an excess of Deoxo-Fluor®.

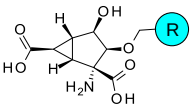
Additional C4-substituted variants were accessible from mesylate **20**, itself prepared from **6a** in good yield. Owing to the high steric congestion of groups around the C4 position as well as the cup-shape of the bicyclo[3.1.0]hexane ring system the approach of nucleophiles to the alpha (concave) face of these molecules is hindered and displacement of the mesylate with various nucleophiles required large excesses of the nucleophile, long reaction times and elevated reaction temperatures to obtain sufficient synthetic yields of products. For example, a large stoichiometric excess (>5 equiv.) of sodium azide and heating in DMF over 6 days with a catalytic amount of 15-crown-5 mesylate **20** was successfully converted to the $C4_{\alpha}$-azide in good yield (71%). Reduction of the azide to the amine was accomplished using trimethylphosphine in wet THF to cleanly give the primary amine intermediate **21**. Global deprotection of intermediate **21** and the corresponding azide intermediate using our standard deprotection conditions successfully provided the $C4_{\alpha}$-primary amine **22** as well as the $C4_{\alpha}$-azide **23** (Table 2; not shown in Scheme 2). Acylation of the primary amine intermediate **21** with acetyl chloride or methyl chloroformate was achieved using a slight excess of acylating agent in dichloromethane with triethylamine as a base to give the acylated amine adducts **24** in good yield. Deprotection as previously described provided the $C4_{\alpha}$-methyl amide and methyl carbamate **25** and **26**. In a similar manner the $C4_{\alpha}$-heteroaryl thio ethers **28-30** were obtained from mesylate **20** in low to moderate synthetic yield using a slight excess of thiol (1.3 eq.) and potassium carbonate in DMF at elevated temperatures over 1-2 days followed by deprotection using our standard conditions.



Scheme 2: Reagents and Conditions: (a) X = OH, (i) *p*-nitrobenzoic acid (2 eq.), PPh_3 (2 eq.), DEAD (2 eq.), THF (88%); (ii) K_2CO_3, MeOH (87%); (b) X = F, Deoxo-Fluor® (2.5 eq.), DCM, -78°C to rt (58%); (c) Acetic acid, water, 140°C, microwave (20-99%); (d) MsCl, TEA, THF, 0°C–rt (92%); (e) R^1SH (1.3 eq.), K_2CO_3 (3 eq.), DMF, 80°C, 1-2 days (11-62%); (f) (i) NaN_3 (5 eq.), cat. 15-crown-5 (0.1 eq.), DMF, 85°C, 6 days (71%); (ii) PMe_3 (1.5 eq.), THF, water, rt (87%); (g) R^2COCl, TEA, DCM (92-99%).

We assessed $hmGlu_2$ and $hmGlu_3$ functional antagonist activity by measuring the ability of test molecules to block $mGlu_{2/3}$ agonist-inhibited, forskolin-stimulated cAMP formation in cells expressing recombinant human $mGlu_2$ or $mGlu_3$ receptors along with the recombinant human glutamate transporter EAAT1. An EC_{90} concentration of the $mGlu_{2/3}$ receptor agonist (1*R*,2*R*)-3-[(1S)-1-amino-2-hydroxy-2-oxo-ethyl]cyclopropane-1,2-dicarboxylic acid (DCG-IV)[9] was used in these experiments.[7]

Using our described synthetic route, we prepared a number of $C3_{\beta}$-benzyl ether analogs and assessed their functional activity with a common $C4_{\beta}$-hydroxyl group (Table 1). For analogs **8-15**, substitution of the aryl ring had only modest effects on antagonist potency (Table 1). For example, 2,3-disubstituted benzyl ether (**13**) and 3,4-disubstituted benzyl ethers (**11, 14, 15**) showed modestly better activity (3 fold) as exemplified by the 2,3-dichlorobenzyl and 3,4-dichlorobenzyl ethers **11** and **13** verses 2,5-disubstituted (**12**), monosubstituted (**9, 10**) or unsubstitued benzyl ethers (**8**). With these data in hand, we next explored the effect of altering substituents at the C4-position while holding the C3 substituent constant as a 3,4-diclorobenzyloxy moiety.

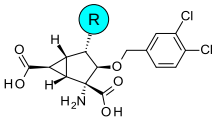


Compds.	R	$hmGlu_2$ IC_{50}(nM) ± SEM[19]	$hmGlu_3$ IC_{50}(nM) ± SEM[19]
8	Ph	306 ± 36.3	239 ± 139
9	3-Cl-Ph	280 ± 142	178 ± 136
10	4-Cl-Ph	210 ± 22.7	145 ± 18.4
11	3,4-diCl-Ph	169 ± 56	153 ± 130
12	2,5-diCl-Ph	387 ± 94.3	185 ± 68.7
13	2,3-diCl-Ph	149 ± 31.4	83.2 ± 80.2
14	3-F-4-Cl-Ph	225 ± 56.9	127 ± 84.8
15	3-Me-4-F-Ph	215 ± 65.4	85.0 ± 16.9

Table 1: cAMP $hmGlu_2$ and $hmGlu_3$ receptor functional activity for $C4_{\beta}$-hydroxyl-$C3_{\beta}$-benzyl ethers.

Our initial focus was to invert the hydroxyl group at the C4 position to the $C4_{\alpha}$-hydroxy analog based on data associated with agonist-associated SAR.[6] Structurally analogous agonist isomers **31** and **32** with a C4 hydroxy group that were devoid of a C3 group showed a slight preference in binding affinity of the α isomer (Ki = 166 ± 28.1 nM) vs the β isomer (Ki = 230 ± 20.2 nM) at $mGlu_2$ receptors (Figure 2). We hypothesized that a similar enhancement in potency of antagonist analogs might also be observed on inversion of OH stereochemistry from β to α. Gratifyingly the $C4_{\alpha}$-hydroxy analog **18** showed a 3.7-fold improvement in $hmGlu_2$ functional antagonist potency compared to the C_{β} isomer **11** (Table 2).

Incorporation of other small groups such as fluoro (**19**) or azide (**22**) at the $C4_{\alpha}$ position led to similar potency gains compared to **11** with the exception of a primary amino group (**23**) which lost approximately 7-fold in potency and displayed only micromolar potency ($hmGlu_2$ IC_{50} = 1130 ± 340 nM, $hmGlu_3$ IC_{50} = 1070 ± 400 nM). Acylation of the amine **23** recaptured and further improved potency and the $C4_{\alpha}$ N-methyl carbamate **26** ($hmGlu_2$ IC_{50} = 18.8 ± 4.95 nM, $hmGlu_3$ IC_{50} = 20.5 ± 20.4 nM) and N-acetyl **25** ($hmGlu_2$ IC_{50} = 26.1 ± 4.69 nM, $hmGlu_3$ IC_{50} = 14.3 ± 9.96 nM) showed excellent antagonist potencies at both receptor subtypes.



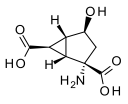
Cpds.	R	$hmGlu_2$ IC_{50}(nM) ± SEM[19]	$hmGlu_3$ IC_{50}(nM) ± SEM[19]
18	-OH	46 ± 14.1	46.1 ± 36.2
19	-F	66.9 ± 15.4	71.0 ± 58.1
22	$-N_3$	34.5 ± 4.68	54.1 ± 19.9
23	$-NH_2$	1130 ± 340	1070 ± 400
25	-NHAc	26.1 ± 4.69	14.3 ± 9.96
26	$-NHCO_2Me$	18.8 ± 4.95	20.5 ± 20.4
28		12.7 ± 3.3	23.5 ± 17.4
29		19.9 ± 3.65	29.6 ± 21.1
30		16.3 ± 11.7	38.5 ± 13.4

Table 2: cAMP $hmGlu_2$ and $hmGlu_3$ receptor functional data for $C4_\alpha$-hydroxyl-$C3_\beta$-benzyl ethers.

Next we assessed the impact of sterically larger C4-substituents on antagonist potency. We prepared the $C4_\alpha$-sulfanyl-1H-1,2,4-triazole **28** based on similar analogs with this C4 group from an agonist SAR effort that showed excellent binding affinity with mixed functional agonist ($mGlu_2$) and partial antagonist ($mGlu_3$) activity.[7] This substituent further improved binding affinity ($hmGlu_2$ Ki = 7.43 ± 4.16 nM; $hmGlu_3$ Ki = 2.70 ± 1.25 nM) which nicely translated to improved antagonist functional potency ($hmGlu_2$ IC_{50} = 12.7 ± 3.3 nM, $hmGlu_3$ IC_{50} = 23.5 ± 17.4 nM). Evaluation of the 1-methyl-1,2,4-triazole **29** as well as the 2-imidazole analog **30** showed slightly less potency compared to the potency observed for the 1H-1,2,4-triazole **28.**

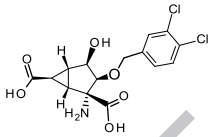
We evaluated all of our compounds for agonist functional activity as it was uncertain what functional activity 3,4-disubstituted analogs would possess (full agonist, partial agonist or full antagonist). At concentrations up to 25 μM, compounds **8-15** and **18-30** were found to be devoid of agonist activity at either $mGlu_2$ or $mGlu_3$ receptors; rather, functional antagonist activity was observed for each of these analogs.

To understand the observed C4 SAR for compounds of the present series, we manually superimposed and minimized the structure of compound **28** with the crystal structure of antagonist (1S,2R,3S,4S,5R,6R)-2-amino-3-[(3,4-difluorophenyl) sulfanylmethyl]-4-hydroxy-bicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY3020371, Figure 1) bound to the human $mGlu_2$ amino terminal domain of the receptor[11] using MOE modeling software.[12] These manual docking studies suggest potential interaction of the C4 triazole group with Arg57 in the receptor binding pocket that is hypothesized to contribute to the excellent potency of this compound (Figure 3). Additionally, having Arg57 proximal to the C4 site explains the relatively poor potency observed with compound **23** which possesses a basic C4 primary ammonium group (pKa = 9.88[13]) which would be expected to be fully protonated at physiological pH and therefore exhibit repulsive interactions with the protonated Arg57 residue.



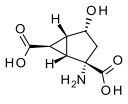
31

Ki = 230 ± 20.2 nM



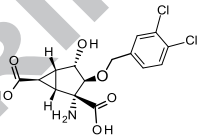
11

Ki = 76 ± 3.99 nM



32

Ki = 166 ± 28.1 nM



18

Ki = 20.1 ± 7.23 nM

Figure 2: Competitive $hmGlu_2$ binding data with [^{3}H]-LY459477[10] and C4-hydroxyl agonist and antagonist compounds.

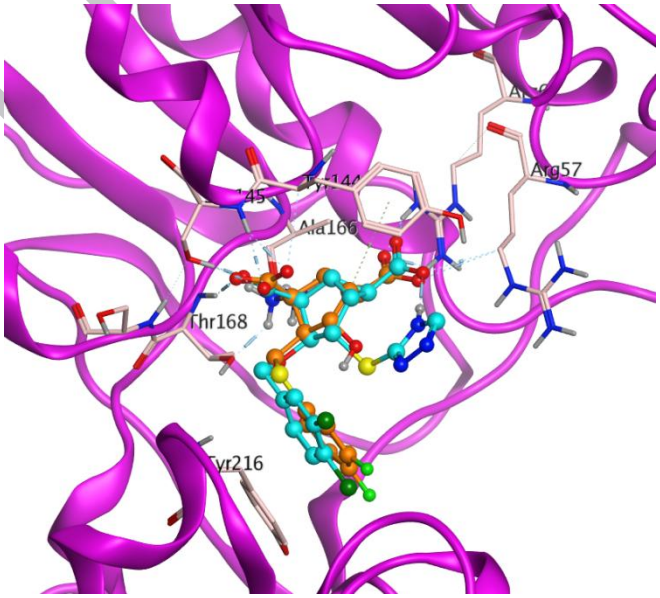


Figure 3: Compound **28** (blue) overlaid with LY3020371(orange) in the $hmGlu_2$ receptor suggest possible interactions of the C4 triazole group with an Arg57 in the binding pocket.

Compound **18** was further profiled for selectivity against the other human metabotropic receptor subtypes using both agonist and antagonist testing modes (Table 3). Testing under conditions to assess antagonist functional activity it showed modest selectivity vs $mGlu_6$ (70-fold) and the $mGlu_8$ (40-fold) receptor subtypes. However, it showed greater than 200-fold selectivity against the other receptor subtypes ($mGlu_1$, $_4$, and $_7$). No functional agonist activity at any of the metabotropic glutamate receptor subtypes was observed at concentrations up to 25 μM.

Broader profiling of **18** revealed it to have no significant activity at over 50 other biologic targets including biogenic amine receptors, ion channels (including ionotropic glutamate receptors 1-6), nuclear hormone receptors and kinases. Importantly, it showed low risk of cardiac toxicities related to

activity at the human *ether-à-go-go*-related gene (hERG) as assessed through competitive binding with [^{3}H]Astemizole in a membrane binding assay (13% inhibition at 100 μM).

Receptor	IC_{50}	EC_{50}
$hmGlu_1$[a]	>12.5 μM	>25 μM
$hmGlu_2$[b]	46 ± 14.1 nM	>25 μM
$hmGlu_3$[b]	46.1 ± 36.2 nM	>25 μM
$hmGlu_4$[a]	>12.5 μM	>25 μM
$hmGlu_5$[a]	>12.5 μM	>25 μM
$hmGlu_6$[b]	3.3 μM	>12.5 μM
$hmGlu_7$[a]	10.5 μM	>25 μM
$hmGlu_8$	1.9 μM[a]	>50 μM[b]

Table 3: Human metabotropic glutamate receptor subtype functional activity of compound **18** in AV12 cell lines expressing the recombinant human receptor. [a]Ca^{2+} FLIPR testing format. [b]cAMP testing format.

In preparation for pharmacokinetic studies, **18** was evaluated for aqueous solubility as well as profiled in a number of *in vitro* assays to assess ADME attributes (Table 4). It showed high aqueous solubility at pH 7.4 (0.778 mg/mL), low rodent, monkey and human microsomal metabolism (<20%) and no significant cytochrome P450 enzyme isoform inhibition (<5% at 10 μM) as well as a high plasma unbound fraction (>40%). It showed moderate permeability (A-B transport, 2%) in a Madin-Darby canine kidney (MDCK) epithelial cell line, an *in vitro* model of blood brain permeability, and it did not act as a substrate or inhibitor of P-glycoprotein (Pgp) efflux in engineered MDCK cell lines expressing the human ABCB1 (*MDR1*) transporter.[14]

Aqueous solubility (pH 7.4)	0.778 mg/mL
MDCK permeability (A-B transport)[a]	2%
% inhibition of human Pgp transport in MDCK cell lines expressing human MDR1[b]	<1%
% loss in microsomes (rodent, dog, monkey and human)[c]	<20%
% inhibition of human Cyp isoforms (3A4, 2C9, 2D6)[d]	<5%
hERG binding Ki[e]	>100 μM
Plasma unbound fraction (rodent, monkey and human)[f]	>40%

Table 4: Solubility, *in vitro* ADME and toxicology attributes of compound **18**. [a]Tested at 20 μM. [b]Tested at 5 and 25 μM using Cimetidine as a positive control. [c]Tested at 4 μM with a 30 minute incubation time. [d]Tested at 10 μM. [e]Determined using [^{3}H]Astemizole. [f]Tested at 1 μM.

The pharmacokinetic profile of compound **18** was assessed in rodent using intravenous (iv), oral and intraperitoneal (ip) administration (Table 5). It showed low bioavailability using oral dosing at 5 mg/kg (F = 6%) and achieved only modest Cmax plasma concentration levels at this dose (262 ± 26 nM). However, it showed low clearance (CL = 3.67 ± 0.225 mL min^{-1} kg^{-1}), a low volume of distribution (Vdss = 0.589 ± 0.020 L/kg), as well as an acceptable half-life ($T_{1/2}$ = 4.61 ± 0.394 h) following iv administration at 1 mg/kg. IP administration of **18** at 10 mg/kg produced a maximal plasma concentration of 48,100 nM and 24 h exposure of 116,000 nM*h. Measured brain to plasma (B/P) concentrations at 30 minutes in mouse for compound **18** following this dose showed a B/P ratio of 0.015, or 447 nM (n = 3). Considering the physicochemical attributes of this molecule that includes high aqueous solubility, low cLogD (-4.66[13]) and high measured free fraction in mouse (56%) it is assumed that most of the drug which partitions into the brain remains in the extracellular compartment where the antagonist binding site resides.

Dose		1 mg/kg[20]	5 mg/kg[20]	10 mg/kg[20]
Parameters	Units	iv, mg ± SD[a]	oral, nM ± SD[a]	ip, nM ± SD[b]
AUC(0-24h)	nMh	32165 ± 2012	1783 ± 471	116000 ± 37300
Co or C_{max}	nM	33892 ± 1026	262 ± 26	48100 ± 4700
T_{max}	h	-	1.67 ± 0.58	0.38 ± 0.13
CL	mL min^{-1} kg^{-1}	3.67 ± 0.225	-	-
V_{dss}	L/kg	0.589 ± 0.020	-	-
$T_{1/2}$	h	4.61 ± 0.394	5.49 ± 2.50	4.48 ± 0.410
Estimated bioavailability	%	-	6	-

Table 5: Mean plasma pharmakinetic attributes of compound **18** in rat following intravenous, oral and intraperitoneal dosing. [a]Mean determination based on n = 3. [b]Average determination based on n = 2.

We next assessed compound **18** in the mouse forced swim test (mFST),[15] a rodent model capable of detecting known antidepressant drugs[16] and previously described $mGlu_{2/3}$ receptor antagonists.[3a] Using ip dosing and a 30 minute pretreatment time, compound **18** demonstrated excellent potency and robust efficacy in the mFST with a calculated ED_{60} value of 0.72 mg/kg and MED of 1 mg/kg (Figure 4). A companion study in mouse to assess plasma level concentrations using the aforementioned B/P ratio of 1.5% showed doses of **18** of 1 mg/kg and 3 mg/kg are predicted to exceed the IC_{50} values for both $mGlu_2$ and $mGlu_3$ in brain (1 mg/kg, C_{30min} = 4811 ± 2051 nM in plasma; calculated brain = 72 nM. 3 mg/kg C_{30min} = 16525 ± 6337 nM in plasma; calculated brain = 248 nM). While the 0.3 mg/kg dose which was ineffective in reducing immobility time in the mFST produced plasma drug levels (C_{30min} = 1288 ± 550 nM) that would not be expected to achieve mGlu2/3 antagonist-relevant concentrations in the central compartment (predicted brain = 19 nM). Testing of compounds **25, 26, 28-30** similarly using ip dosing showed them to be less active in this model (ED_{60} 2.1 – 9.5 mg/kg).[17]

We further evaluated the wake promoting effects of **18** in a rat EEG sleep study. Previous work has demonstrated a wake-

promoting effect elicited by LY341495 (Figure 1) an $mGlu_{2/3}$ antagonist.[4] Using ip administration, compound **18** produced a dose dependent wake promoting effect in rats at 3 and 10 mg/kg as measured by total increases in accumulated wake over 7 hours (41 minutes and 131 minutes respectively *vs* vehicle control) without any significant increases in recorded locomotor activity. There was no significant effect on the percentage of NREM sleep however there was a significant increase in the percentage of REM sleep consistent with previously reported sleep studies with LY341495.[4]

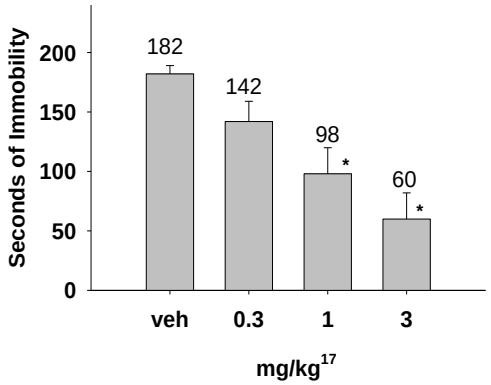


Figure 4: Activity of compound **18** in th mFST following ip administration. *Significant difference *vs* vehicle with a Dunnett's test value < 0.05.

In conclusion, we developed a chemistry route that has allowed for the dual functionalization of both the C3 and C4 positions of the bicyclo[3.1.0]hexane ring system. In particular, our regioselective silver mediated alkylation of diol **5** provided a highly efficient way to place benzyl ethers at the C3 position in a β-face stereochemical orientation, a group and configuration that is known to produce functionally active antagonist in the bicyclo[3.1.0]hexane ring system,[18] while providing a hydroxyl substituent at the C4 position for further elaboration. In the context of the SAR study we successfully leveraged binding data from our agonist research efforts to drive C4 group optimization to obtain antagonists with high potency at both the $mGlu_2$ and $mGlu_3$ receptors. In rodents, an optimized analog from this investigation, **18**, was shown to demonstrate good pharmacokinetic characteristics with low clearance and volume of distribution as well as robust efficacy in in $mGlu_{2/3}$ antagonist-sensitive animal models. Therefore, **18** should be a useful tool for studying $mGlu_{2/3}$ receptor function both *in vitro* and *in vivo*.

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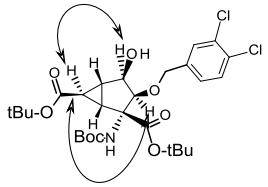

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(20) The following formulations were used for compound dosing: iv, A pH adjusted solution in saline; po, A solution in water; ip, A suspension in water modified with hydroxyethyl-cellulose and antifoam 1510.