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Bicyclo((aryl)methyl)benzamides as inhibitors of GlyT1

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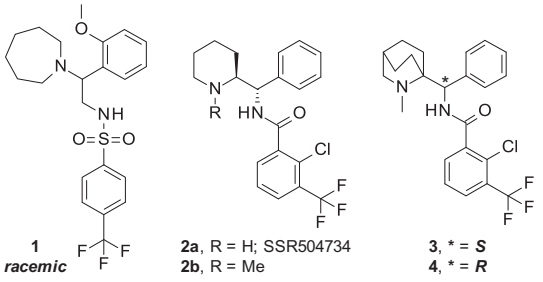
ABSTRACT

A series of isoquinuclidine benzamides as glycine uptake inhibitors for the treatment of schizophrenia are described. Potency, lipophilicity, and intrinsic human microsomal clearance were parameters for optimization. Potency correlated with the nature of the ortho substituents of the benzamide ring, and reductions in lipophilicity could be achieved through heteroatom incorporation in the benzamide and pendant phenyl moieties. Improvements in human CL_{int} were achieved through changes in ring size and the *N*-alkyl group of the isoquinuclidine itself, with des-alkyl derivatives (**40**–**41**, **44**) demonstrating the most robust microsomal stability. Dimethylbenzamide **9** was tested in a mouse MK801 LMA assay and had a statistically significant attenuation of locomotor activity at 3 and 10 μmol/kg compared to control.

The *N*-methyl-D-aspartate receptor (NMDAr) hypofunction hypothesis of schizophrenia correlates disease symptomology with glutamatergic neurotransmission dysfunction.[1–4] Therefore, inhibition of glycine transporter 1 (GlyT1) to elevate synaptic levels of glycine and potentiate NMDAr signaling is a promising therapy for this disease. Current treatments for schizophrenia are associated with unwanted side effects and do not provide sufficient relief across all symptom domains.[2,5–7] Additionally, FDA-approved GlyT1 inhibitors for schizophrenia remain elusive, with Hoffman-La Roche clinical candidate bitopertin (RG1678) being one of the most advanced to date. Having reached Phase III, the compound was ultimately withdrawn from development after failing to achieve statistical significance in the improvement of negative symptoms compared to control.[1] As such, treatments for the positive, negative, and cognitive symptoms of schizophrenia remain a significant unmet medical need.



We previously reported a series of azepane sulfonamides as GlyT1 inhibitors.[8] Identified through a virtual screening campaign and exemplified by **1**, these compounds were characterized by notable glycine uptake efficiency[9] (GlyT1 IC_{50} for **1**: 37 ± 6 nM), low aqueous solubility,[10] and favorable brain-to-plasma ratios (1.6–2.1). As previously noted,[8] **1** is structurally similar to Sanfoi's withdrawn clinical candidate SSR504734[1,11] (**2a**) and the more potent related N-Me analog **2b**.[12] We speculated that by capitalizing on our learnings from development of the former we might increase the potency of **2b** through an increase in piperidine lipophilicity. However, rather than evoke a ring expansion to form an azepane, we proposed that conversion to an isoquinuclidine, such as in **3**,[13] might also provide the desired outcome. While

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quinuclidine isomers of **2b** similar to **3** have been reported,[14] **3** also had the potential advantage of having a single chiral center. We were optimistic that this might increase synthetic tractability and facilitate exploration of our hypothesis.

As shown in Table 1, a comparison of **2b** and **3** in a glycine uptake inhibition assay[9] revealed slightly more than a ten-fold decrease in potency. While this did not confirm our hypothesis, we were gratified that such a change still afforded a compound with appreciable in vitro activity. Even more interesting, enantiomer **4** *did* demonstrate activity comparable to **2b**. This was surprising, because the chirality of the benzylic position of **4** (*R* enantiomer) is opposite that of the same position in **2b** (*S*,*S*). Suspecting an error in stereochemical assignment, we validated the chirality of both **3** and **4** by vibrational circular dichroism (VCD) and X-ray crystallography (discussed below). A molecular overlay (recreated in MOE)[15] reinforced the concept that similar potencies between **2b** and **4** were not unreasonable as there is good overlap of benzamide, aryl, and heterocyclic regions (Fig. 1).

We were interested in modifying **4** to be a probe suitable for evaluation in a murine MK801 locomotor activity (LMA) assay.[16]

Table 1
Initial assessment of isoquinuclidine enantiomers.

	2b (*S*,*S*)	**3 (*S*)**	**4 (*R*)**
Uptake IC_{50} (nM)[a]	3	41	6
Sol (μM)[b]	124	>500	>482
clog*P*	5.1	5.4	5.4
log*D*[c]	2.9	2.7	2.7
PSA (Å^2)	34	34	34
hu CL_{int} (μL/min/mg)[d]	223	103	109

[a] Glycine uptake inhibition assay.[9]
[b] Equilibrium solubility (pH 7.4).[10]
[c] Empirically determined partitioning ratio: octanol/water (pH 7.4).
[d] Rate of clearance as measured in human liver microsomes.

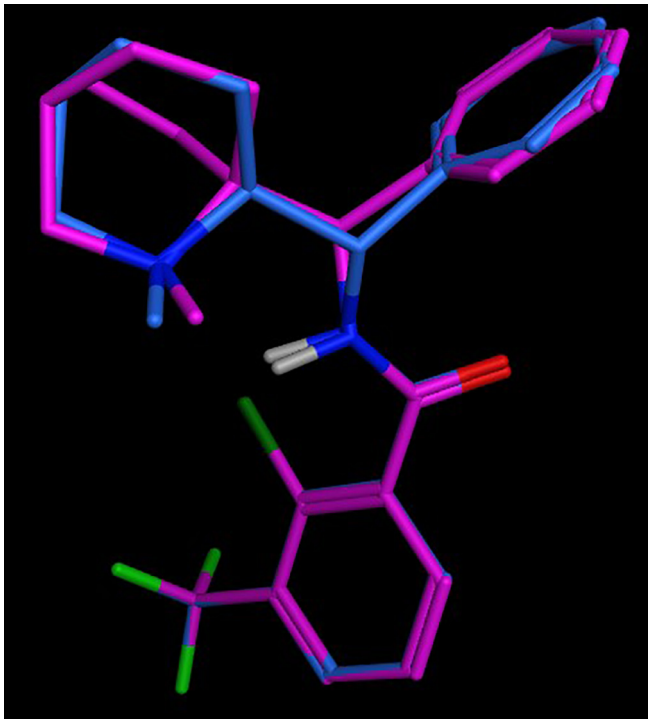


Fig. 1. Overlay of **2b** (blue) with **4** (magenta) using MOE (Chemical Computing Group) software.[15]

While the potency and solubility (typically high for the compounds described herein) of **4** were acceptable, both lipophilicity, as measured by clog*P*, and stability on incubation with human liver microsomes were higher than desired. Reasoning that reductions to the former might also attenuate the latter, we sought to reduce clog*P* (<4) in a variety of ways. This included a) heteroaromatic and substituent changes to the benzamide aromatic ring, b) conversion of the unsubstituted phenyl moiety into various heterocycles, and c) ring contraction of the isoquinuclidine itself.

In the course of elucidating benzamide SAR to attenuate lipophilicity, simple heterocyclic replacements (Not shown: 1-methyl-imidazol-4-yl, thiazol-4-yl; GlyT1 uptake IC_{50} > 20 μM) of the aryl ring were insufficient to retain potency. For benzamides themselves, potency was found to directly depend on the nature and number of ortho substituents. Specifically, linear free energy relationship analysis (LFER) identified both the ortho substituent parameter (E_s; inverse correlation)[17] and hydrophobic nature (π Hammett constant; positive correlation) of the benzamide *ortho*-substituents to be good predictors of glycine uptake inhibition. Where such information was not available, a torsion angle analysis could be used to roughly bin compounds according to potency (Fig. 2). In other words, hydrophobic *ortho*-substituents that perturbed the planarity of amide-aryl conjugation generally were more active at inhibiting glycine uptake.

Armed with this knowledge, we looked at ways to reduce the clog*P* of **4** through nitrogen incorporation while still maintaining potency via installation of ortho substituents such as chloro, methyl, bromo, and thiomethyl (Table 2). In general, pyridine analogs were less active (**6** vs **7**; **9** vs **10**; **14** vs **15**) than their simple phenyl counterparts, with 3-pyridyl amides evoking the most dramatic potency reductions (**10** & **13**). The most active compounds, however, were those containing chloro, methyl, or thiomethyl ortho substituents (**6**–**9**), and, in particular, 4-pyridyl analog **7** reinforced the concept that clog*P* could be reduced in select cases with only a slight decrease in uptake efficiency.

Despite reductions in lipophilicity, we saw no improvement in predicted human clearance (**6** vs **7**; **14** vs **15**) for pyridine matched pairs, and all compounds contained a low to moderate efflux liability. Improvements in metabolic stability were only observed in a very small number of benzamide modifications that involved introduction of polar ortho substituents such as for **16** and **17** (hu CL_{int} <4 μL/min/mg). While very potent, both compounds carried additional liabilities such as concerns around potentially mutagenic anilines (**16**) or excessively high efflux (**17**).

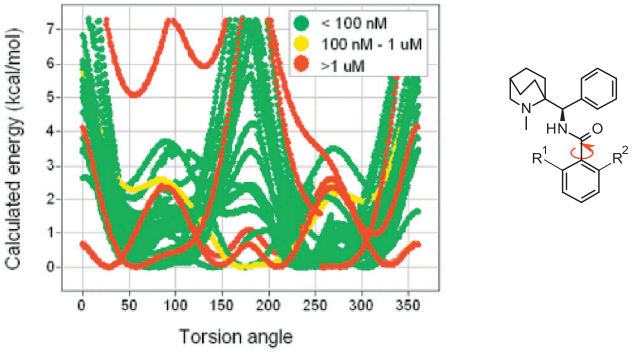
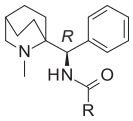
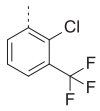
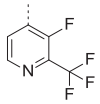
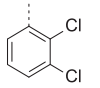
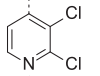
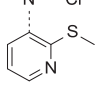
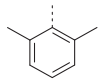
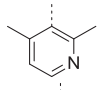
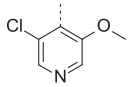
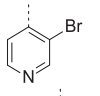
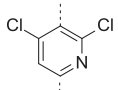
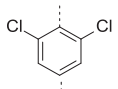
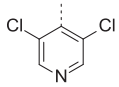
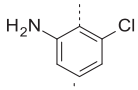
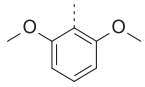


Fig. 2. Benzamide aryl-carbonyl (red arrow) torsion angle analysis color coded according to GlyT1 uptake IC_{50} potency. Green: <100 nM; yellow: 100 nM to 1 μM; red: >1 μM. Aryl rings with hydrophobic *ortho*-substituents (R^1 & R^2) that hindered free rotation were generally predisposed to be more potent at inhibiting glycine uptake, with the preferred conformation trending towards one in which the aromatic ring is perpendicular to the plane of the amide itself.

Table 2
Reduction of lipophilicity through benzamide modifications.



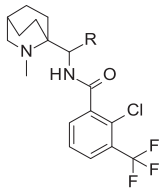
#	R	Uptake IC_{50} (nM)[a]	Sol (μM)[b]	*clogP*	logD[c]	hu CL_{int} (μL/min/mg)[d]	MDR1 efflux ratio[e]
4		6	>482	5.4	2.7	109	>1.9
5		92	>500	4.4	2.4	197	2.2
6		1	493	5.2	2.2	57	1.5
7		6	498	4.3	2.2	95	1.5
8		3	>500	4.0	1.3	76	6.3
9		1	414	4.8	1.9	121	6.2
10		494	373	4.0	0.8	–	–
11		474	>500	3.8	1.0	45	–
12		75	>500	3.7	–	50	2.9
13		809	>500	3.6	1.1	–	–
14		23	>500	4.5	1.9	91	3.1
15		45	481	3.6	1.4	97	6.3
16		8	462	4.2	1.3	<4	3.4
17		6	495	4.4	0.7	<4	>29

[a] Glycine uptake inhibition assay.[9]
[b] Equilibrium solubility (pH 7.4).[10]
[c] Empirical logD measured as a ratio of compound partitioned between octanol and water (pH 7.4).
[d] Rate of clearance as measured in human liver microsomes.
[e] Transporter efflux assay using canine kidney cells expressing P-glycoprotein.

A similar exercise aimed at reducing lipophilicity and/or exploring areas for scaffold elaboration was conducted through modification of the pendant phenyl ring using racemic material for ease of accessibility. Unfortunately, glycine uptake inhibition decreased in nearly all cases compared to racemic **4b** (Table 3). Only data for meta substitution (**20** & **21** vs **4b**) suggested that there was room for a potency increase, and this was accompanied by a commensurate increase in lipophilicity. Furan **26** was also promising as this was the first example of a phenyl replacement that appeared to retain a significant degree of potency while lowering *clogP*; disappointingly this had an adverse impact on metabolic stability. Generally, efflux ratios were not investigated for the majority of these samples as these were racemates.

It is worth noting that 4-pyridine analog **27** is the only chiral compound in Table 3 and so must be compared to chiral **4** rather than **4b**. There was roughly a threefold decrease in potency upon

Table 3
Profiling phenyl modifications for racemic analogs of **4**.



#	R	Uptake IC_{50} (nM)[a]	*clogP*	logD[b]	hu CL_{int} (μL/min/mg)[c]
4b	Ph	39	5.4	2.7	94
18	2-OMe-Ph	340	5.2	–	81
19	2-Me-Ph	514	5.9	3.0	247
20	3-Br-Ph	9	6.3	3.3	58
21	3-Me-Ph	29	5.9	3.0	119
22	3-OMe-Ph	177	5.2	–	102
23	4-Me-Ph	1703	5.9	–	69
24	4-OMe-Ph	1759	5.2	2.7	73
25	*n*-Butyl	3362	5.5	–	–
26	Furan-2-yl	78	4.6	2.0	128
27	Pyridin-4-yl[d]	18	3.9	2.4	119
28	Pyridin-3-yl	211	3.9	2.3	56
29	Pyridin-2-yl	904	3.9	1.6	55

[a] Glycine uptake inhibition assay.[9]
[b] Empirical logD measured as a ratio of compound partitioned between octanol and water (pH 7.4).
[c] Rate of clearance as measured in human liver microsomes.
[d] Chiral, single enantiomer, absolute configuration not determined.

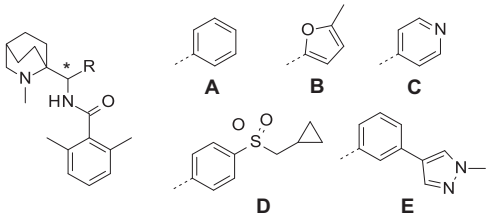
incorporation of a nitrogen at the 4-position of the phenyl ring, no significant change in metabolic liability despite a 1.5 log unit drop in *clogP*, and efflux liability persisted (efflux ratio: 4.7).

We opted to prepare a small number of additional analogs to finish our exploration of phenyl ring SAR using the more potent scaffold of 2,6-dimethylbenzamide **9** (Table 4). Encouraged by the data for **26**, 2-methylfuran **30** was prepared in an attempt to mitigate any potential metabolism associated with the 2-position of the furan ring itself. While potency was retained and lipholicity and human CL_{int} decreased, efflux was unchanged. Similar potency and efflux results were obtained with 4-pyridine **31**. The last two compounds of Table 4 involved scaffold modifications similar to those described by Sanfoi[11b] and Taisho Pharmaceuticals[18] respectively. While we synthesized a number of potent sulfones (and a few sulfonamides) such as **32**, all suffered from high predicted human clearance and efflux liabilities. Lastly, pyrazole **33**, despite the promise suggested by bromide **20**, failed to realize any gain through expansion off the meta position of the phenyl ring.

What remained elusive within the isoquinuclidines were structural modifications leading to a reduction in both clearance and efflux. We suspected that *N*-dealkylation was at least partly to blame, and, through a combination of changes in the nitrogen substituent and isoquinuclidine itself, we were able to demonstrate this conclusively (Table 5).

Thus, for des-methyl analog **34**, in vitro microsomal clearance decreased by fourfold (34 vs 121 μL/min/mg) compared to **9**. Removal of a methylene spacer to afford a series of azabicyclo [2.2.1]heptanes[19] (ABHs; **35**–**41**) reduced *clogP* by roughly half a

Table 4
Select phenyl ring modifications of benzamide **9**.



#	R	Uptake IC_{50} (nM)[a]	*clogP*	logD[b]	hu CL_{int} (μL/min/mg)[c]	MDR1 efflux ratio[d]
9	A	1	4.8	1.9	121	6.2
30[e]	B	1	4.4	1.5	76	6.0
31[e]	C	1	3.3	1.4	–	6.3
32[e]	D	1	4.1	2.0	167	14
33[e]	E	946	4.8	2.1	–	–

[a] Glycine uptake inhibition assay.[9]
[b] Empirical logD measured as a ratio of compound partitioned between octanol and water (pH 7.4).
[c] Rate of clearance as measured in human liver microsomes.
[d] Transporter efflux assay using canine kidney cells expressing P-glycoprotein.
[e] Single enantiomer; absolute chirality not determined.

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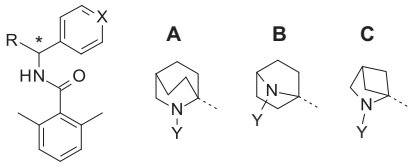
Table 5
Exploration of isoquinuclidine modifications of benzamide **9**.



#	R	X	Y	Uptake IC_{50} (nM)[a]	*clogP*	logD[b]	hu CL_{int} (μL/min/mg)[c]	MDR1 efflux ratio[d]
9	A	CH	Me	1	4.8	1.9	121	6.2
31[e]	A	N	Me	1	3.3	1.4	–	6.3
34[e]	A	CH	H	3	4.2	1.3	34	11.8
35[e]	B	CH	Me	3	4.2	1.9	96	2.9
36[e]	B	CH	Et	4	4.7	2.1	145	6.2
37[e]	B	CH	iPr	1	5.0	1.9	39	4.3
38[e]	B	N	iPr	10	3.5	–	43	–
39[e]	B	CH	cPr	2	4.6	1.1	142	1.1
40[e]	B	CH	H	58	3.6	1.4	22	4.7
41[e]	B	N	H	45	2.1	0.9	15	20
42[e]	C	CH	Me	7	3.6	1.1	43	7.4
43[e]	C	CH	Et	3	4.2	1.1	40	8.3
44[e]	C	CH	H	168	3.1	0.8	<4	6.6

[a] Glycine uptake inhibition assay.[9]
[b] Empirical logD measured as a ratio of compound partitioned between octanol and water (pH 7.4).
[c] Rate of clearance as measured in human liver microsomes.
[d] Transporter efflux assay using canine kidney cells expressing P-glycoprotein.
[e] Single enantiomer; absolute chirality not determined.

log unit and afforded comparable potency (**9** vs **35**). As before, removal of the *N*-alkyl substituent within the ABHs (**40**) reduced predicted human clearances by roughly fivefold. Similarly, decreasing ring size further to establish a series of azabicyclo[2.1.1]hexanes[20] (ABXs; **42**–**44**) was accompanied by another drop in lipophilicity and potency relative to parent isoquinuclidines, however, this series also had overall lower liabilities from a microsomal stability perspective. Consistent with isoquinuclidine and ABH compounds, des-methyl ABX **44** had the most favorable result in this regard but also the largest drop-off in glycine uptake inhibition.

We also explored various nitrogen substituents with an aim toward modulating sterics and basicity within the ABH series. No glycine uptake inhibition was detected with non-basic bicycles (data not shown). Isopropyl **37** was the best compromise between desired on-target effect and clearance, however, efflux was still problematic. We did see a favorable shift in the latter with cyclopropyl **39**, but microsomal stability was dramatically worse. Lastly, as with pyridine **31**, reduction in *clogP* through nitrogen incorporation was unsuccessful. This change adversely impacted potency (**38**) and efflux (**41**) respectively.

Table 6
IV and PO DMPK parameters for **9** in Sprague Dawley rats.[a]

Route	Parameter	**9**
in vitro	CL_{int} (μL/min/mg)[b]	182
	PPB (% free)[c]	14
IV	Clearance (mL/min/kg)	32
	VD_{ss} (L/kg)	1.8
	$t_{1/2}$ (h)	3.1
	Brain/plasma[d]	0.2
PO	C_{max} (nM)	2452
	T_{max} (h)	1.0
	F (%)	105

[a] Dose: IV = 3 μmol/kg; PO = 9 μmol/kg.
[b] Rate of clearance as measured in rat liver microsomes.
[c] Determined by equilibrium dialysis.
[d] Brain-to-plasma ratio; brain concentrations not corrected for 2–3% vascular contamination.

Intravenous and oral pharmacokinetic profiles of dimethylbenzamide **9** were determined in Sprague Dawley rats (Table 6). Compared to in vitro results, a lower than expected in vivo clearance was observed, which may be indicative of an underlying saturation mechanism. Consequently, bioavailability was also high while volume of distribution was low. Consistent with MDR1 efflux results, the brain-to-plasma ratio was low in rat but, for reasons not well understood, higher in mouse (brain/plasma: 0.4; CD-1 mice dosed s.c.). This may be at least partially reflective of the lower plasma-protein binding observed in the latter species (mu PPB: 32% free).

Additionally, when profiling **9** for off-target pharmacology, only five out of 100 targets demonstrated moderate activity in an MDS Pharma panel (% inhib @ 10 μM): human: α_{2B} (54), RXR- α (−5 4) & $5HT_{2a}$ (62); rat: Ca^{2+} channel L-type (65) & Na^{+} channel site-2 (70). Minimal inhibition (<20%) was observed for related targets such as adenosine, DAT, NET, and SERT transporters and the NMDA receptor. Activity against glycine transporter-2 (GlyT2) was also low (GlyT2 IC_{50} 3.8 μM).

Compound **9** was tested in a mouse MK801 locomotor activity assay to model the treatment of positive and negative symptoms of schizophrenia.[16] Compound **9** showed dose-dependent reversal of MK801-induced locomotor activity and a statistically significant dose response at 3 and 10 μmol/kg ($p < 0.01$). Furthermore, potency at 3 μmol/kg for **9** was comparable to **2b** at a higher dose (10 μmol/kg) (Fig. 3).

Compounds described herein were prepared using the general route described in Scheme 1, which involved addition of an aryl or heteroaryl Grignard or lithium alternative to a chiral or racemic sulfinamide[21] followed by deprotection and acylation. Isoquinuclidine/done, ABH, or ABX core aldehydes were prepared from their respective acid or ester precursors, and these syntheses, along with those of the fully elaborated final products have been previously reported.[22–24]

An example synthesis of isoquinuclidine **9** is shown in Scheme 2. Amide **45**[22a] was alkylated with iodomethane and then reduced at low temperature using lithium aluminum hydride[25] to afford alde-

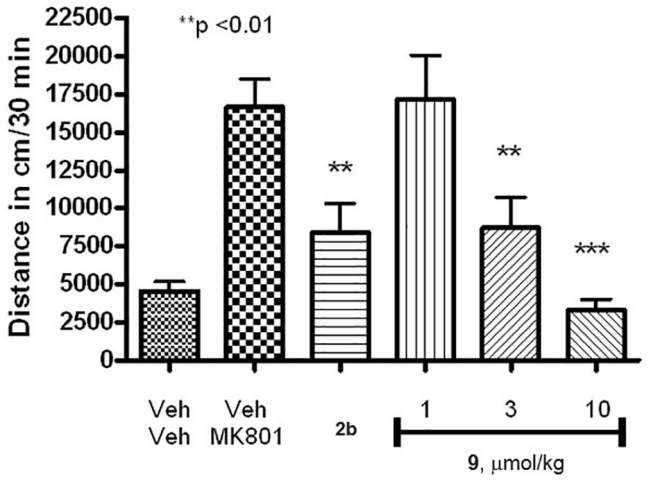
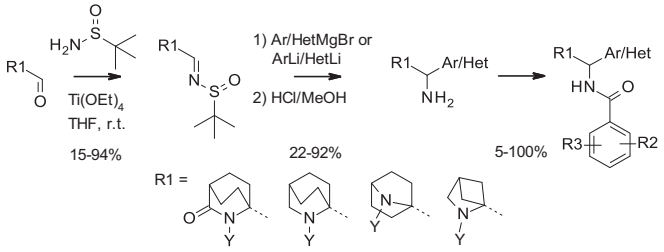
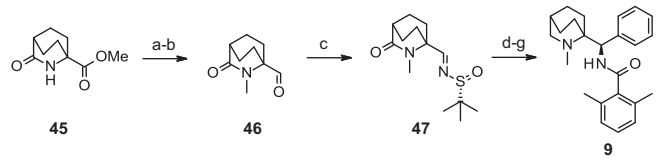


Fig. 3. Dose-response of **9** in a mouse MK801 LMA assay. MK801 and **2b** were dosed at 1.4 and 10 μmol/kg, respectively. Male CD-1 mice were dosed subcutaneously using a formulation of 20% sulfobutylether-β-cyclodextrin (SBECD).



Scheme 1. General synthetic route & yields to bicyclo((aryl)methyl)benzamides. Y is either alkyl, *tert*-butylcarbonate (Boc), or allyl. Allyl groups enabled late stage diversification of nitrogen alkyl substituents following Pd-mediated deprotection.[22b]



Scheme 2. a) NaH, MeI, DMF, 81%; b) LAH, THF, −78 °C then 1 N aq HCl, 85%; c) (*R*)-*t*BuSONH$_2$, Ti(OEt)$_4$, THF, 68%; d) PhLi in Et$_2$O, AlMe$_3$, THF, −78 °C; e) RhH(CO)(PPh$_3$)$_4$, Ph$_2$SiH$_2$, THF, 68% over 2 steps; f) 4 M HCl/diox, MeOH, quant. g) TBTU, HOBt monohydrate, DIPEA, DMF, 90%.

hyde **46** directly. Lewis acid-mediated sulfinamide formation to give **47** was followed by trimethylaluminum-assisted[26] addition of phenyllithium (98% d.e.), amide reduction using catalytic RhH(CO)(PPh$_3$)$_4$,[22a] and deprotection using methanolic HCl. Acid coupling then provided **9**.[22b] Final products derived from racemic analogs of **47** (isoquinuclidines, ABHs, or ABXs) could be resolved under chiral chromatography conditions using supercritical fluid chromatography (SFC) or reverse phase chiral chromatography as a final step.[27]

For chiral isoquinuclidine final compounds, stereochemistry was assigned based on a comparison of intermediate calculated VCD[28] spectra (*R*, red/upper; *S*, green/lower) with that derived experimentally from the amine (prior to amide coupling) which afforded more active **4** (blue).[29]

In summary, we have described modifications to a series of isoquinuclidine benzamides as part of an effort to develop a novel in vivo probe for inhibiting glycine uptake in an MK801 LMA assay. In addition to potency, lipophilicity and human microsomal clearance were parameters for optimization. Heteroatom incorporation into the benzamide portion of **4** led to equipotent derivatives (**7**, **8**) with reduced *clogP*. Conversion of the pendant phenyl ring into a 2-methylfuran or pyridine (**30**–**31**) was less well tolerated but still superior from a potency perspective to all but changes to the meta position. Modest improvements in predicted human CL$_{int}$ could be had through changes in ring size and the *N*-alkyl group of the isoquinuclidine itself, with ABH and ABX bicycles lacking an alkylated nitrogen (**40**–**41**, **44**) demonstrating the largest improvement in microsomal stability. Simultaneously achieving a low efflux ratio remained elusive. Dimethylbenzamide **9**, after evaluation for rat pharmacokinetics, was tested in a mouse MK801 LMA assay and had a statistically significant attenuation of locomotor activity at 3 and 10 μmol/kg compared to control, with favorable comparison to **2b**. The results of further optimization, an investigation of clearance mechanisms, and conditioned avoidance response and novel object recognition data will be reported in due course.

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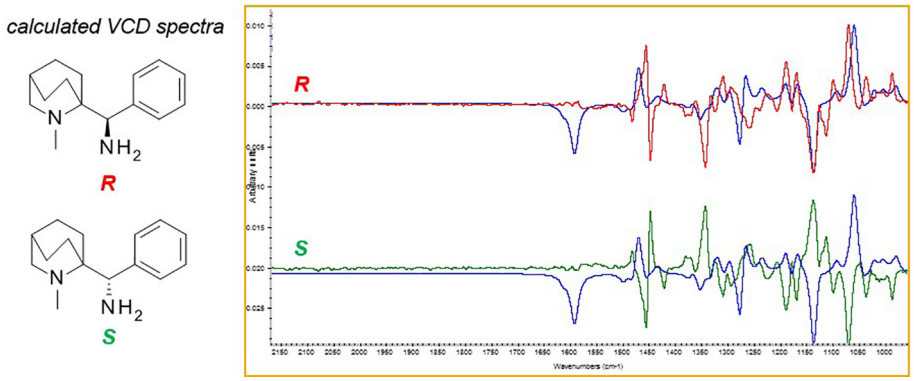

Fig. 4. Overlay of the experimental (blue) VCD spectrum of the benzyl amine precursor of **4** with those predicted for *R* (red, upper) and *S* (green, lower) amines.

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