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Expedited SAR study of an mGluR5 antagonists: generation of a focused library using a solution-phase Suzuki coupling methodology

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Abstract—The SAR of the lead compounds **2a** and **2b** was rapidly explored. Utilizing a parallel solution-phase Suzuki coupling approach, in tandem with strong cation exchange resin (SCX) purification afforded the desired focused library. The library was evaluated in vitro, a ninefold potency increase was achieved and the preference for *ortho* substitution of moderate steric bulk of the fourth, phenyl ring was identified. In addition, dimethylisoxazole, as a heterocyclic replacement for the phenylic ring of the lead compound, was also identified by this approach.

1. Introduction

The excitatory neurotransmitter glutamate activates both ionotropic receptors and G protein-coupled metabotropic glutamate (mGlu) receptors. To date eight mGlu receptors have been identified and these are categorized as follows: Group I includes mGlu1 and mGlu5 receptors, Group II comprises mGlu2 and mGlu3 receptors and Group III encompasses the mGlu4 and mGlu6-8 subtypes.[1] The Group I receptors activate phospholipase C and this results in the mobilization of intracellular calcium.[2]

The inhibition of mGlu5 receptors represents a novel approach for the treatment of diseases that affect the central nervous system.[2] Research showing potential for the treatment of psychiatric and neurological disorders via the modulation of mGlu5 receptors is compelling. Thus mGlu5 receptor antagonists[3,4] such as **1a** have been shown to be effective in animal models of mood disorders such as anxiety.[3] Gene expression data from humans indicate that mGlu5 receptor modulation may be useful for the treatment of schizophrenia.[5] The potential for treatment of movement disorders such as Parkinson's disease by the modulation of mGlur5 has also been suggested.[6] Finally, studies using the mGlu5 receptor antagonist MPEP and mGlu5 receptor knock-out mice have shown that inhibition of mGlu5 receptors may be useful for the treatment of drug dependence[7] (Fig. 1).

In our ongoing efforts to investigate mGlu5 receptor antagonists, the novel compounds 2-(2-biphenyl-3-yl-2*H*-tetrazol-5-yl)pyridine pyridine **2a** and 2-[2-(5-fluorobiphenyl-3-yl)-2*H*-tetrazol-5-yl]pyridine **2b**, derived from tetrazole derivatives **1a** and **1b**, were identified,[4] and **1a** has been shown to be potent and selective[8] mGlu5 receptor antagonist (mGlu5 Ca^{2+} flux = 73 nM). In an effort to advance the SAR of this series, we applied

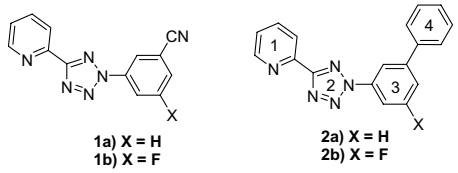

Figure 1. mGluR5 antagonists.

Keywords: Metabotropic glutamate; Antagonist; Tetrazole; Suzuki coupling; Library.
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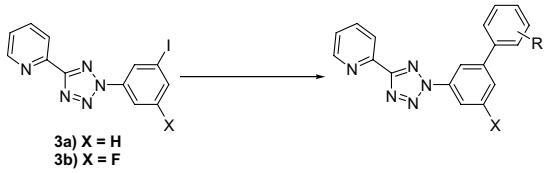
high throughput parallel synthesis and purification towards the synthesis of a focused library in order to explore the SAR of the fourth ring of our scaffold (**2a**) and (**2b**), by looking at substituents on the ring and heterocyclic replacements for the fourth ring with the goal of improving the potency of the lead scaffolds. The use of solid and solution phase chemistry for the generation of non-peptidic small molecule libraries has become common practice in both industry and academia.[9] Utilizing these techniques to produce small focused libraries to rapidly explore the SAR of drug leads has become an important factor in the chain of drug discovery.

2. Chemistry

As the iodide derivatives are readily available from synthesis,[4] and the purification of Suzuki reactions utilizing strong cation exchange resin (SCX) to remove unwanted impurities for compound containing tertiary amines is known in the literature,[10,11] we felt this solution-phase Suzuki reaction/ion exchange purification process could be readily adapted to pyridine containing compounds. With this in mind, we expedited the SAR of the series with emphasis on the fourth ring (Scheme 1).

The parallel chemistries are described herein. The library synthesis was performed on an Argonaut Trident Synthesizer in conjunction with a Zinzer workstation. Purification using a resin capture approach was accomplished by utilizing the Zinzer workstation.

Stock solutions of the iodides (0.08 mmol/mL), boronic acids (0.33 mmol/mL) and Pd $(Ph_3)_4$ (0.008 mmol/mL) were prepared in degassed THF. The trident liquid handler was used to transfer 1 mL of the iodide solution, 1 mL of the boronic acid solution and 0.25 mL of the 1 M KOH (aqueous) solution to the appropriated 4 mL reaction vessel in a trident cassette. The solutions were then degassed for 10 s. The $Pd(Ph_3)_4$ was then added (0.5 mL) and the solutions were degassed for 3 s. The reactions were then heated to 65 °C and shaken on deck, for 16 h. The reactions were allowed to cool to room temperature. The Zinzer workstation was then used to transfer the solutions from the reaction cassettes to Varian Chem Elut columns (3 mL capacity, diatomaceous earth). The organics were eluted off of the columns (4×4 mL of DCM). The reaction mixtures were concentrated; the resulting residue was taken in methanol/acetic acid (10%) and loaded onto SCX columns (1.5 g of Argonaut MP-TsOH resin, 4.07 mmol/g). Subsequently, the columns were washed with 4×4 mL methanol/acetic acid (10%), followed by elution of the columns with 4×4 mL 1 N NH_3 in methanol, afforded the desired products. Of the 192 reactions carried out 72% were better than 90% pure by LC/MS, yields ranged from 50% to 70%. Only the compounds that had greater than 90% purity, based on LC/MS, were used in this study. In addition, a series of randomly selected products were subjected to ^1H NMR in order to confirm the final structure and corroborate the purity.



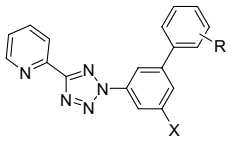
Scheme 1. Reagents: $RB(OH)_2$, $Pd(Ph_3)_4$, KOH, DMF/H_2O.

3. Results and discussions

A summary of the results of the library are shown in Table 1. Using this library approach, a number of analogues, which are more potent or comparable to the initial leads, **2a** and **2b** were identified **4**, **5**, **6**, **7**, **8**, **27**, **28**, **29**, **30** and **42**. In addition, by virtue of this parallel approach, we were able to quickly scope out the SAR of ring four, and demonstrate that substitution patterns with in the fourth ring exhibit a preference for *ortho* substitution for both **2a** and **2b**. The phenyl ring is capable of accommodating *ortho* substituents the size of a methyl group as exemplified by 4 and 29, which more active than **1a** and **1b** by a factor of 2–3. *ortho* Substitution with chloride (**5**, **27**) and fluoride (**32**) indicate there is no clear electronic preference for substitution at this position on the ring as the electron with drawing chloride roughly equivalent in activity to methyl substitution. Also the strongly electron withdrawing trifluromethyl (**8**) is roughly equivalent to hydroxyl (**9**, **30**) and trifluoromethoxy (**13**, **34**) is roughly equivalent to methoxy (**10**, **17**, **31**). Furthermore methoxy carbonyl (**7**), acetyl (**14**) and nitro substitution (**33**) are less potent in activity compared to **4** or are comparable to **2a**,**b** in the calcium flux assay. When the fourth ring has a larger *ortho* substituent than a methyl group, such as ethyl (**19**, **41**) ethoxy (**11**, **38**), Acetyl (**14**), formyl (**18**, **35**), or phenoxy (**15**, **37**), phenyl (**21**, **40**) benzyloxy (**16**) and the amide (**36**) the activity is decreased as compared to **4** and **29**, as is the case when the substituent is small (**32**). There appears to be a correlation between activity of the *ortho* substituent and Meyer's steric descriptor[12] V^a as can be seen from Table 1. The activity increases when V^a is between 2 and 3. Substitution by groups larger than hydrogen at *meta* or *para* positions is less tolerated as illustrated by examples methyl substituents **24**, **25**, **45**, **47**; chloride **23**, **26**, **44**, **48**, and when the substituent is the small fluoride **32**, **42**, **46** *ortho*, *meta* and *para* are all equivalent in activity to **2b**. Compounds **6** and **28** show there is tolerance for di-*ortho* substitution for the small fluorine, but dimethyl substitution (**20**, **39**) caused a significant loss of activity.

In further elaborating the SAR of the fourth ring beyond substituted phenyls, we prepared a second iteration focused library directed towards the incorporation of heterocyclic moieties as other publications from this laboratory have demonstrated that by substituting the fourth ring with a 3-pyridinyl moiety the activity in the calcium flux assay is increased, as shown by compound 2-[2-(3-fluoro-5-pyridin-3-ylphenyl)-2*H*-tetrazol-5-yl]pyridine[13] **56**. Most notable was the identification

Table 1. In vitro data for mGlur5 receptor antagonists[a]



Compd	X =	R =	mGlur5 Ca^{2+} flux IC_{50} (nM)[b,c]	V[a,d]
2a	H	H	334	
4	H	2-Me	113	2.84
5	H	2-Cl	150	2.54
6	H	2,6-F	151	
7	H	2-CO_2Me	223	
8	H	2-CF_3	229	3.54
9	H	2-OH	288	
10	H	2-OMe–5-Me	312	
11	H	2-OEt	316	
12	H	2,5-F	342	
13	H	2-OCF_3	376	
14	H	2-COMe	418	
15	H	2-OPh	445	
16	H	2-OCH_2Ph	451	
17	H	2-OMe	461	3.39
18	H	2-COH	497	
19	H	2-Et	664	4.31
20	H	2,6-Me	949	
21	H	2-Ph	982	6.1
22	H	3-CO_2Me	337	
23	H	3-Cl	966	
24	H	3-Me	1171	
25	H	4-Me	1546	
26	H	4-Cl	3000	
2b	F	H	247	
27	F	2-Cl	108	2.54
28	F	2,6-F	108	
29	F	2-Me	120	2.84
30	F	2-OH	201	
31	F	2-OMe	271	3.39
32	F	2-F	287	1.22
33	F	2-NO_2	307	
34	F	2-OCF_3	316	
35	F	2-COH	359	
36	F	2-$CONH_2$	389	
37	F	2-OPh	418	
38	F	2-OEt	451	
39	F	2,6-Me	875	
40	F	2-Ph	1000	6.1
41	F	2-Et	3000	4.31
42	F	3-F	217	
43	F	3-CO_2Me	318	
44	F	3-Cl	824	
45	F	3-Me	3000	
46	F	4-F	335	
47	F	4-Me	1467	
48	F	4-Cl	2059	

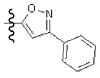
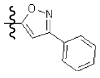
[a] Only activity for compounds less than or equal to 500 nM have been included except for **19**, **20**, **21**, **23**, **24**, **25**, **26**, **39**, **40**, **41**, **44**, **45**, **47**, **48**, **52**, **53**, **54**, **60** and **61**, which were added for the sake of completion.

[b] Ca^{2+} flux assay using glutamate (10 μM) as agonist. Concentration–response curves were performed using 12 concentrations, performed in duplicate wells in two or more separate experiments.[6]

[c] Average standard deviation ± 28%.

[d] Meyer's steric descriptor.[11]

Table 2. In vitro data for mGlur5 receptor antagonists with heterocyclic ring replacements

Compd	X =	R =	mGlur5 Ca^{2+} flux IC_{50} (nM)[a,b]
49	H		63
50	H		251
51	H		309
52	H		882
53	H		3000
54	H		2102
55	F		28
56	F		54
57	F		75
58	F		94
59	F		201
60	F		575
61	F		806

[a] Ca^{2+} flux assay using glutamate (10 μM) as agonist. Concentration–response curves were performed using 12 concentrations, performed in duplicate wells in two or more separate experiments.[6]

[b] Average standard deviation ± 28%.

of compound **55**, which is 10 times more potent than the initial lead **2b**. Compounds **55**, **57** and **58** show that substitution of the fourth ring with an isoxazole or 2-methoxy pyrimidinyl is tolerated in the fluorinated scaffold, but not in the des fluoro scaffold **50**, **52**, **51** as is moving the pyridinyl nitrogen from the 3- to the 4-position (**54**, **61**). Adding a second nitrogen to the ring **59** decreases the activity in the calcium flux assay for the fluoro scaffold, as does the replacement by furan **53**, **60** (Table 2).

4. Conclusion

In summary, though the use of high throughput organic synthesis (HTOS) we were able to improve the potency and rapidly examine the SAR of our lead compounds **2a** and **2b**, and identified the preference for *ortho* substitution of moderate steric bulk of the fourth phenyl ring.

In addition we identified dimethylisoxazole as a potential heterocyclic replacement for the phenylic ring moiety.

References and notes

1. O'Brien, J. A.; Lemaire, W.; Chen, T. B.; Chang, R. S.; Jacobson, M. A.; Ha, S. N.; Lindsley, C. W.; Schaffhauser, H. J.; Sur, C.; Pettibone, D. J.; Conn, P. J.; Williams, D. L. *J. Mol. Pharmacol.* **2003**, *3*, 731.
2. Spooren, W. P. J. M.; Gasparini, F.; Salt, T. E.; Kuhn, R. *Trends Pharmacol. Sci.* **2001**, *22*, 331.
3. Cosford, N. D. P.; Tehrani, L.; Roppe, J.; Schweiger, E.; Smith, N. D.; Anderson, J.; Bristow, L.; Brodkin, J.; Jiang, X.; McDonald, I.; Rao, S.; Washburn, M.; Varney, M. A. *J. Med. Chem.* **2003**, *46*(2), 204–206, and references cited therein.
4. Roppe, J.; Smith, N. D.; Huang, D.; Tehrani, L.; Wang, B.; Anderson, J.; Brodkin, J.; Chung, J.; Jiang, X.; King, C.; King, C.; Munoz, B.; Varney, M. A.; Prasit, P.; Cosford, N. D. P. *J. Med. Chem.* **2004**, *47*, 4645–4648.
5. Ohnuma, T.; Augood, S. J.; Arai, H.; McKenna, P. J.; Emson, P. C. *Mol. Brain Res.* **1998**, *56*, 207.
6. Spooren, W. P. J. M.; Gasparini, F.; Bergmann, R.; Kuhn, R. *Eur. J. Pharmacol.* **2000**, *406*, 403; Awad, H.; Hubert, G. W.; Smith, Y.; Levey, A. I.; Conn, P. I. *J. Neurosci.* **2000**, *20*, 7871.
7. Chiamulera, C.; Epping-Jordan, M. P.; Zocchi, A.; Marcon, C.; Cottiny, C.; Tacconi, S.; Corsi, M.; Orzi, F.; Conquet, S. *Nature Neurosci.* **2001**, *4*, 873.
8. The selectivity of the prototypical tetrazole **2** was determined by extensive profiling against a battery of in vitro assays (MDS Pharma Services screen). In addition, **2** is highly selective for mGlu5 over mGlu1 (mGlu1 Ca^{2+} flux $IC_{50} > 10\,\mu M$).
9. (a) Bhattacharyya, S. *Curr. Med. Chem.* **2001**, *8*, 1383–1404; (b) Lou, B. *Drug Discovery Today* **2001**, *6*, 1288–1294; (c) Sammelson, R. E.; Kurth, M. J. *Chem. Rev.* **2001**, *101*, 137–202; (d) Baldwin, J. J. *Comb. Chem. Mol. Diversity Drug Discovery* **1998**, 181–188.
10. Organ, M.; Arvanitis, E.; Dixon, C.; Lavorato, D. *J. Comb. Chem.* **2001**, *3*, 473.
11. Organ, M.; Mayhew, D.; Cooper, J.; Dixon, C.; Lavorato, D.; Kaldor, S.; Siegel, M. *J. Comb. Chem.* **2001**, *3*, 64.
12. Meyer, A. *J. Chem. Soc., Perkin. Trans. 2* **1986**, 1567.
13. (a) Poon, S. F.; Eastman, B. W.; Chapman, D. F.; Chung, J.; Cramer, M.; Holtz, G.; Cosford, N. D. P.; Smith N. D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, in press. doi:10.1016/j.bmcl.2004.09.011; (b) Smith, N. D.; Poon, S. F.; Huang, D.; Green, M.; King, C.; Tehrani, L.; Roppe, J. R.; Chung, J.; Chapman, D. P.; Cramer, M.; Cosford, N. D. P. *Bioorg. Med. Chem. Lett*. **2004**, *14*, in press. doi:10.1016/j.bmcl.2004.09.018.