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Thieno[2,3-*b*]pyridines as negative allosteric modulators of metabotropic GluR5 receptors: Hit-to-lead optimization

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ABSTRACT

An HTS campaign of our corporate compound library resulted in thieno[2,3-*b*]pyridines derivative hits with mGluR5 negative allosteric modulator effects. During the hit-to-lead development our objective was to improve affinity, and to keep the ligand efficiency values at an acceptable level. After different modifications of the linker resulted in a 2-sulfonyl-thieno[2,3-*b*]pyridines derivative, which fulfilled the lead criteria.

Glutamate, the most prominent excitatory neurotransmitter in human brain and the vertebrate central nervous system (CNS), acts through both ionotropic and metabotropic glutamate receptors.[1] Three types of ionotropic glutamate receptors (NMDA, AMPA, and kainate receptors) are ligand-gated ion channels responsible for fast excitatory transmission.

Metabotropic glutamate receptors (mGluRs) belong to family C of G-protein-coupled receptors (GPCRs) and modulate ion channel activity or influence neurotransmitter release.[2] Eight metabotropic glutamate receptor subtypes (mGluR1–mGluR8) are known to date which have been clustered into three groups (I–III). Group I mGlu receptors (mGluR1 and mGluR5) are positively coupled to phospholipase C; group II mGlu receptors (mGluR2 and mGluR3) and group III mGlu receptors (mGluR4, mGluR6, mGluR7 and mGluR8) are negatively coupled to adenylate cyclase.[3] In the last decade the discovery of small molecules that selectively bind to both group I and group II receptors has significantly facilitated the understanding of their roles in brain physiology and pathophysiology.[4]

Several mGluR5 antagonists have now entered human clinical trials. Before metabotropic glutamate receptor subtypes were cloned, the non-GABAergic agent fenobam (Fig 1.) was investigated in a double-blind, placebo-controlled clinical trial in which it showed efficacy and onset of action comparable with that of diazepam.[5] In 2005 Roche reported that fenobam, a negative allosteric modulator (NAM) of mGluR5[6] provided clinical proof of principle for the mGluR5 approach to the treatment of anxiety. Phase II or III clinical trials with Dipraglurant (Addex), Mavoglurant (Novartis), RG-7090 (Roche, Cugai) and STX-107 (Seaside, Merck, Roche) (Fig. 1) are currently underway for different indications such as fragile X syndrome, gastroesophageal reflux disease, Parkinson's disease levodopa induced dyskinesia, treatment-resistant depression and major depressive disorder.[7]

According to patent reviews,[8] most mGluR5 NAM clinical candidates and in vivo tool compounds are alkyne chemotypes. This crowded intellectual property space subsequently led scientists to look for non-alkyne chemotypes for this molecular target.

High throughput screening (HTS) of our corporate compound collection resulted in several non-alkyne chemotype hit clusters which we optimized as reported in patent applications.[9] Further optimization of two hit clusters resulted in the lead compounds (**1**, **2**) (Fig. 2), as reported in our previous communications.[10] This Letter describes the optimization of another structural family, the thieno[2,3-*b*]pyridines, represented by compounds **3a–c** (Fig. 2, Table 1). The hit cluster was supplemented by analogues **3d** and **3e** (Table 1). The preliminary SAR showed that halogen substitution of the phenyl rings improved both the binding and functional activity, indicating the importance of this moiety for biological activity. Compound **3e** served as an advanced hit for the hit-to-lead optimization which was monitored by calculating ligand efficiency (LE)[13] and lipophilic ligand efficiency metrics (LELP).[14] LE values were calculated using the formula: [$LE[\Delta g] = -RT \log[K_i]/N$ non-hydrogen atoms]. LELP was defined as the log *P*/LE ratio indicating the price of ligand efficiency paid in log *P*. Consequently, the higher the absolute value of LELP the less drug-like the lead compound.

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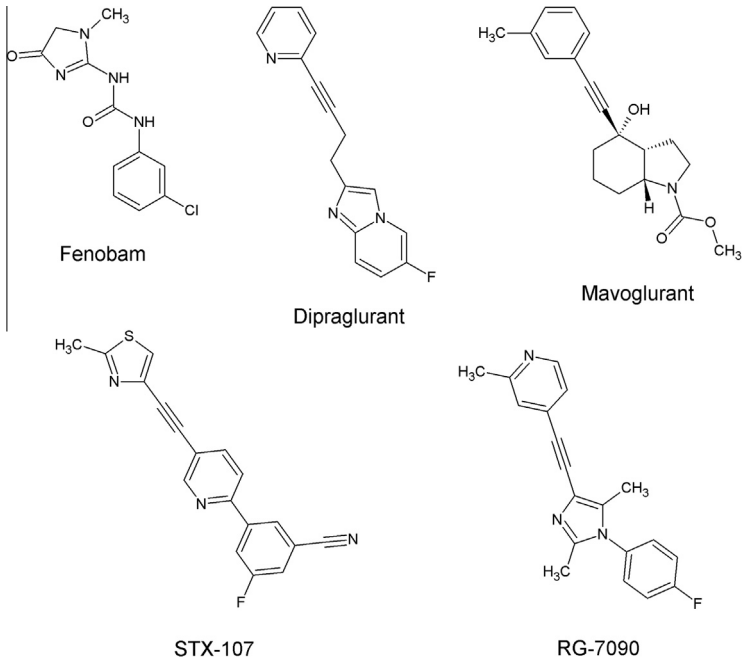


Figure 1. mGluR5 NAMs in clinical trials.

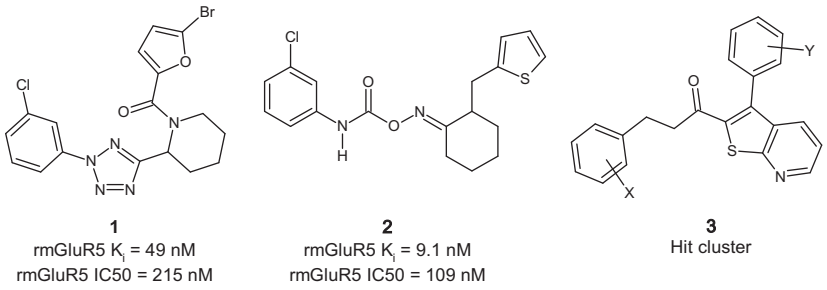
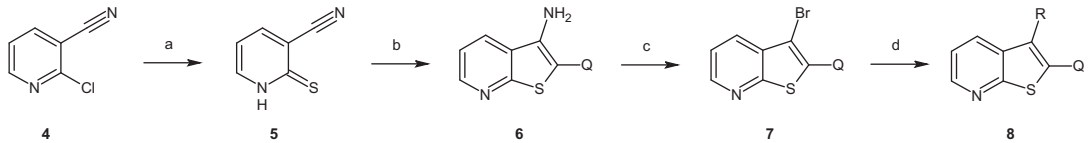


Figure 2. Non-alkyne chemotypes lead compounds (**1**, **2**) and general structure of hit cluster (**3**).

Table 1
Hit cluster

Compd	X	Y	mGluR5		LE[13]	LELP[14]
			K_i (nM)[11]	IC_{50} (μM)[12]		
3a	—	—	730	3.3	0.34	16.3
3b	4-MeO	—	2500	8.0	0.29	18.8
3c	4-F	—	343	2.1	0.34	16.5
3d	3-F	4-F	224	—	0.34	17.1
3e	4-F	4-F	180	1.0	0.35	16.9

The synthesis of thieno[2,3-*b*]pyridines (**8**) is shown in Scheme 1. Reaction of 2-chloro-3-pyridinecarbonitrile (**4**) with thiourea in aqueous ethanol under reflux first afforded 2-mercapto-nicotinonitrile (**5**) which was converted into the amino-thieno[2,3-*b*]pyridines (**6**) by reaction with a suitable halomethyl-derivative in *N*,*N*-dimethylformamide. This amine was then transformed into the bromide (**7**) by reaction with $CuBr_2$ and *tert*-butyl nitrite in acetonitrile. Finally, derivatives of (**8**) were prepared from the appropriate bromide (**7**) and boronic-acid using the well known methods of Suzuki coupling reactions.[9g] (Scheme 1)



where

Q = $CO(CH2)_nAr$, COOAr, CONHAr, CONMeAr, SO_2Ar; Har

R = Ar;

n = 0-2;

Ar = optionally substituted phenyl;

Har = optionally substituted heteroaryl

Scheme 1. Reagents and conditions: (a) thiourea, 50% water/50% ethanol, reflux, 4 h, 90% yield; (b) Cl-CH_2-Q, DMF, 70–100 °C, 1–2 h, 60–80% yield; (c) $CuBr_2$, *tert*-butyl nitrite, CH_3CN, 60–80 °C, 1–2 h, 50–70% yield; (d) R-$B(OH)_2$, Na_2CO_3, 40–50% toluene/50–60% ethanol, $Pd(PPh_3)_4$, 1–2 h, 70–110 °C, 50–70% yield.

Table 2
Modification of the central ring

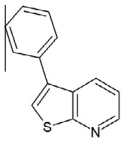
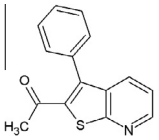
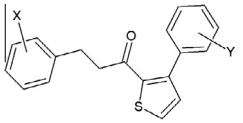
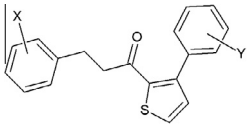
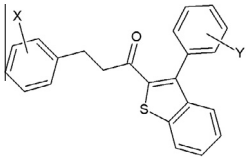
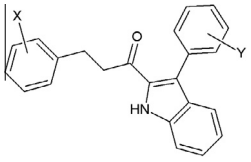
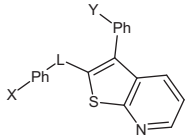
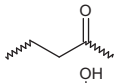
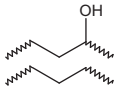
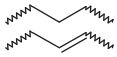
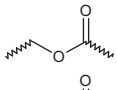
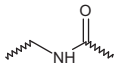
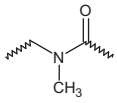
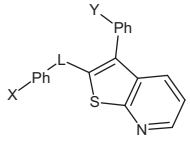
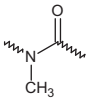
Compd	Structure	X	Y	mGluR5 binding inhib. at 1 μM[11] (%)
9		–	–	0
10		–	–	17
11		–	–	0
12		–	4-F	0
13		4-F	4-F	8
14		4-F	4-F	4

Table 3
Modification of linker

Compd				mGluR5		LE[13]	LELP[14]
				K_i (nM) or bind. inhib. at 1 μM[11]	IC_{50} (nM)[12]		
	X	L	Y				
3e	4-F		4-F	180	1000	0.35	16.9
23	4-F		4-F	3%	–	–	–
24	4-F		4-F	0%	–	–	–
25	4-F		4-F	12%	–	–	–
26	4-F		4-Cl	41	150	0.38	16.3
27	4-F		4-F	311	–	0.34	14.4
28	4-F		4-Cl	13%	–	–	–
29	4-F		4-Cl	15%	–	–	–

(*continued on next page*)

Table 3 (*continued*)

Compd				mGluR5		LE[13]	LELP[14]
				K_i (nM) or bind. inhib. at 1 μM[11]	IC_{50} (nM)[12]		
	X	L	Y				
30	4-F		4-Cl	0%	–	–	–
31	4-F		4-F	19%	–	–	–
32	4-F		4-F	140	–	0.37	14.8
33	4-F		4-Cl	83	350	0.38	15.5
34	4-Cl		4-F	30%	-	-	-
35	4-Cl		4-Cl	12.3	46	0.43	14.0
36	4-F		4-F	52%	–	–	–
37	4-F		4-Cl	0%	–	–	–
38	4-F		4-Cl	24%	–	–	–

The final products were purified by either crystallization or column chromatography to >95% purity (HPLC, ^{1}H, and ^{13}C NMR).[9c]

Multiple objectives were set for our hit-to-lead optimization, the most important of which were identification of the essential moieties and modification of the central ring and propan-1-one linker. First, the need for the phenyl-propyl side chain was checked. Binding results of the fragments (**9**) and (**10**) (Table 2) showed the necessity of the cleaved moiety. The next steps were omission of the pyridine ring from the central core (**11**, **12**), and replacement of the thieno[2,3-*b*]pyridines core by benzo(*b*)thiophene (**13**), or indole (**14**). None of these modifications were tolerated, as shown by their poor affinity for the mGluR5 allosteric binding site (Table 2).

The next change was to modify the propanone spacer. (Table 3) Changing oxo- to a hydroxyl-group (**23**), or omission of the oxo-group from the linker (**24**, **25**) were non-tolerated modifications. Modification of linker's component showed mixed results: thus, the ester linker (**26**) improved activity whereas the simple amide linker (**27**) maintained it and the methyl substituted amide linker (**28**) decreased activity. Shortening the linker also produced mixed results. Analogues with shorter ester or amide linkers (**29**–**31**) lost their affinity to the mGluR5 allosteric binding site but compounds with the ethanone spacer (**32**, **33**) had slightly better activity than that of the advanced hit (**3e**). In parallel with these activity improvements, the LE and LELP data were also slightly better for both compounds. The last step of linker shortening resulted in an inactive compound (**34**).

As these modifications did not significantly improve the biological activity our next step was to replace the ethanone linker with the sulfonyl spacer, or either bioisosteric heterocycles. While compounds with heterocyclic linkers showed only weak affinity (**36**), or lacked activity (**37**–**38**) to the mGluR5 allosteric binding site, the sulfonyl compound (**35**)[15] brought a breakthrough. It resulted in an order of magnitude better binding and functional data, with significantly improved LE and LELP values, than those of the advanced hit (**3e**). As the improved affinity result showed, the sulfonyl group solves as a bioisostere of ethanone linker in case of mGluR5 NAMs. Moreover, compound (**35**)[15] showed significant, dose-dependent activity in the Vogel punished drinking model[16] (Fig. 3), which is a well established and widely accepted anxiolytic method. mGluR5 antagonist reference compound (MPEP) showed robust efficacy in this test.[10b]

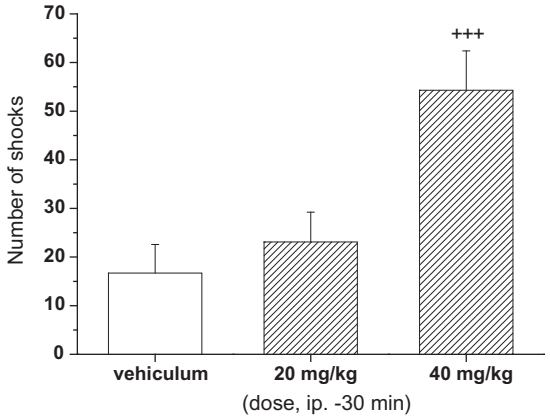


Figure 3. Effect of (**35**) in the Vogel punished drinking test in rats. +++: p <0.001 versus control.

In summary, our HTS campaign identified thieno[2,3-*b*]pyridines derivatives (**3**) as a novel class of mGluR5 negative allosteric modulator. During the hit-to-lead optimization process both fragmentation of the phenyl-propyl side chain at position 2, and

modification of central thieno[2,3-*b*]pyridines core were futile. Focusing on the propanone linker, modification of its elements resulted in more active compounds. Finally, replacing of ethanone by the sulfonyl linker resulted in compound (**35**), which fulfilled the lead criteria of the project.

Acknowledgment

K.N. is indebted to Katalin Koch for preparative assistance.

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11. The mGluR5 receptor binding was determined according to Gasparini et al. (Gasparini, F.; Andres, H.; Flor, P. J.; Heinrich, M.; Inderbitzin, W.; Lingenhöhl, K.; Müller, H.; Munk, V. C.; Omilusik, K.; Stierlin, C.; Stoehr, N.; Vranesic, I.; Kuhn, R. *Bioorg. Med. Chem. Lett.* **2002**, *2*, 407.) with modifications. Rat cerebrocortical membrane preparation was used to determine the binding characteristics of reference compounds and novel compounds to the rat mGluR5. As radioligand [^{3}H]-M-MPEP (2 nM) was used. The non-specific binding was determined in the presence of 10 μM M-MPEP. The ligand displacement by the compounds was determined in duplicates or triplicates. The ligand displacement at 1 μM concentration of ligand were named as binding inhibition at 1 μM. For IC_{50} (K_i) determinations concentration–displacement curves were generated consisting of minimum of six concentrations. K_i values (i.e., inhibition constants) were calculated using the Cheng–Prusoff equation: $K_i = IC_{50}/[1 + (L/K_d)]$, where $[L]$ is the radioligand concentration and K_d the affinity of the labeled ligand for receptor. K_d was determined from the Scatchard plot. Binding inhibition at 1 μM means the displacement.
12. Functional activity at mGluR5 was measured in primary rat neuronal cultures of neocortical origin, taking advantage of the high expression level of mGluR5 in this brain area (Romano, C.; Sesma, M. A.; McDonald, C. T.; O'Malley, K.; Van den Pol, A. N.; Olney, J. W. *J. Comp. Neurol.* **1995**, *355*, 455.) Functional activity at mGluR5 was measured by Ca^{2+}-fluorometry according to Nagy et al. with modifications. Briefly, cells were isolated from E17 rat embryos, seeded in 96-well plates and cultured at least for 5 days before being subjected to Ca^{2+}-measurements. For the Ca^{2+}-measurements cells were loaded with the Ca2+-sensitive dye, fluo-4/AM. Baseline and agonist evoked signals were recorded with a plate reader fluorometer. Agonist was (*S*)-3,5-dihydroxyphenylglycine. For IC_{50} determination sigmoidal (4-parameter) concentration–inhibition curves were fitted to the percent inhibition data derived from at least three independent experiments using GraphPad Prism software.
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14. Keserű, G. M.; Makara, G. M. *Nat. Rev. Drug Disc.* **2009**, *8*, 203.
15. Structure characterization and synthesis of compound **35** is described in patent application (compound **1** and example **3** in Ref. 9g).
16. The Vogel punished drinking test was according to Vogel et al. (Vogel, J. R.; Beer, B.; Clody, D. E. *Psychopharmacology* **1971**, *100*, 138.) with modifications. Rats are deprived of drinking water for 48 h prior to test. 24 h prior to test they are placed into the test chambers equipped with a metal water spout mounted on the wall of the chamber and a metal grid floor for delivering electric shocks. 260 during a 5-min adaptation period, they have free access to the drinking spout. On the day of the measurement, the animals are treated with the test compounds (intraperitoneally administration in solution of 5% Tween/distilled water) then placed into the test chambers where they have free access to drinking water for a 30-s unpunished period. After that, electric shocks (1 mA, 1 s) are applied through the drinking spout following every 10 licks during a 5-min punished period. Number of licks and shocks delivered are recorded and stored in a computer. Anxiolytic or anxiogenic activity is reflected by increased or decreased number of accepted shocks, respectively. Test compounds are studied minimally at three dose levels because of the occurrence of bell-shaped dose–response curves.