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Rational design, synthesis, and structure–activity relationship of benzoxazolones: New potent mglu5 receptor antagonists based on the fenobam structure

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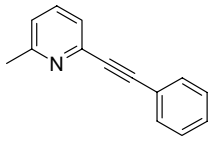
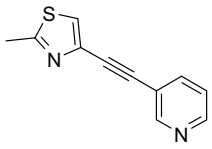
Abstract—A novel class of potent and stable mGlu5 receptor antagonists was developed by combining information from a high-throughput screening campaign with the structure of the known anxiolytic fenobam. Representative compounds from this class show favorable pharmacokinetic properties and are active in an in vivo model of anxiety.

The metabotropic glutamate receptors (mGlus) are a class of G-protein coupled receptors which respond to the excitatory neurotransmitter glutamate.[1] Eight subtypes of mGlus have been identified, which are divided into several subclasses based on sequence similarity, second messenger coupling, and pharmacology.[2] The mGlu5 receptor belongs to group I mGlus and upon binding of glutamate activates phospholipase C, resulting in the mobilization of intracellular calcium. It has been shown that selective modulation of mGlu5 is useful in the treatment of various pain states[3] and pre-clinical evidence supports the use of mGlu5 antagonists in the treatment of mood disorders, in particular anxiety and depression.[4] Additional evidence suggests the potential utility of mGlu5 modulation in the treatment of a wide range of psychiatric and neurological disorders, including schizophrenia, Parkinson's disease, cognitive dysfunction, epilepsy, and drug addiction.[5] mGlu5 possesses a unique allosteric site in the transmembrane region, which makes it a particularly attractive target for pharmacological modulation, since it allows for selectivity against other mGlus.[4]

The limitations of currently available anxiolytics support a continuing interest in novel targets for the treatment of anxiety states. In this respect, the mGlu5 receptor appears to be an attractive target, due to the validated proof of concept[6] and the potential for selectivity and chemical tractability offered by the allosteric site. The attention of the pharmaceutical industry for this target is testified by the growing body of patents[7] and scientific literature[8] detailing the development of allosteric mGlu5 modulators. Among these, MPEP (**1**) and MTEP (**2**) constitute the most advanced and documented non-competitive mGlu5 receptor antagonists and have reached the status of prototypical pharmacological tools for this target.[9]



1, MPEP



2, MTEP

Porter et al. recently reported the finding that fenobam **3**, a clinically tested anxiolytic agent developed by McNeil more than 30 years ago,[10] is also a non-competitive mGlu5 antagonist,[11] showing in our hands a

Keywords: mGluR5; Fenobam; Anxiety; MPEP; Benzoxazolone.
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binding K_i of 61 nM[12] and a functional IC_{50} of 38 nM.[13] Fenobam is reported to have extensive in vivo metabolism,[14] possibly leading to the poor efficacy and high variability in exposure observed in clinical studies.[10] Attempts to identify more stable fenobam analogs were faced with the unyielding structure–activity relationship of related creatinine ureas, as reported by Wållberg et al.[15] and confirmed by our own efforts.[16]

Creatinine ureas like fenobam **3** exist mainly in the tautomer form B (Fig. 1), stabilized by an intramolecular hydrogen bond.[16] Upon screening of our compound collection aiming at the identification of novel mGlu5 allosteric inhibitors, we identified salicylamides such as **4**, showing a binding K_i at the MPEP site comparable to fenobam. The intramolecular hydrogen bond which is also a characteristic of the salicylamides led to the hypothesis that this structural feature could be a key pharmacophoric element for mGlu5 antagonism.[17] Other hit compounds, like quinazoline **5**, which can be seen as an analog of **4** where the H-bond is substituted by a permanent covalent bond, seemed to further support this notion. While neither salicylamides nor quinazolines were considered promising starting points, the observation that the carbonyl group in the fenobam structure and the phenol ring in the salicylic amides occupy different and complementary positions with respect to the amide bond led to the design of structure **6**, combining both elements in a single compact molecule (Fig. 1) which, despite its structural simplicity, had not been described before. Gratifyingly, already the first examples synthesized, benzoxazolones **6** and **7**, showed a binding affinity and potency comparable to fenobam (Fig. 1 and Table 1). The chlorine in position 3 of the phenyl ring, as present in the hit salicylamide **4**, did not show any noticeable influence on potency.

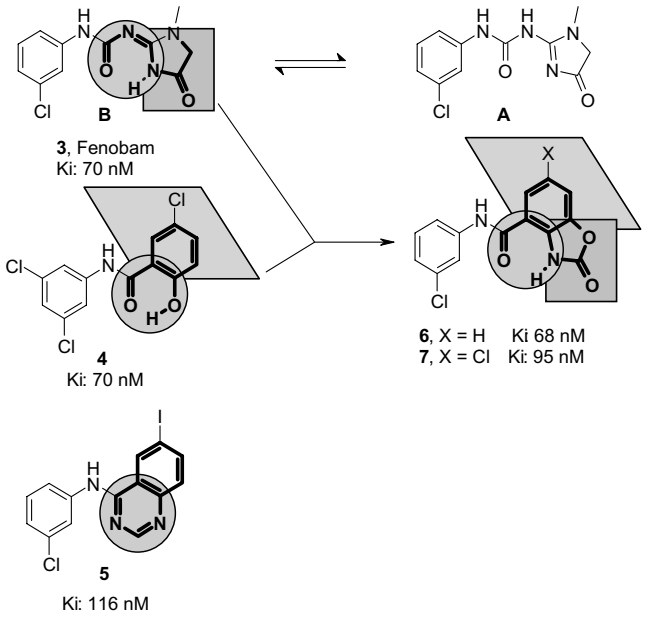
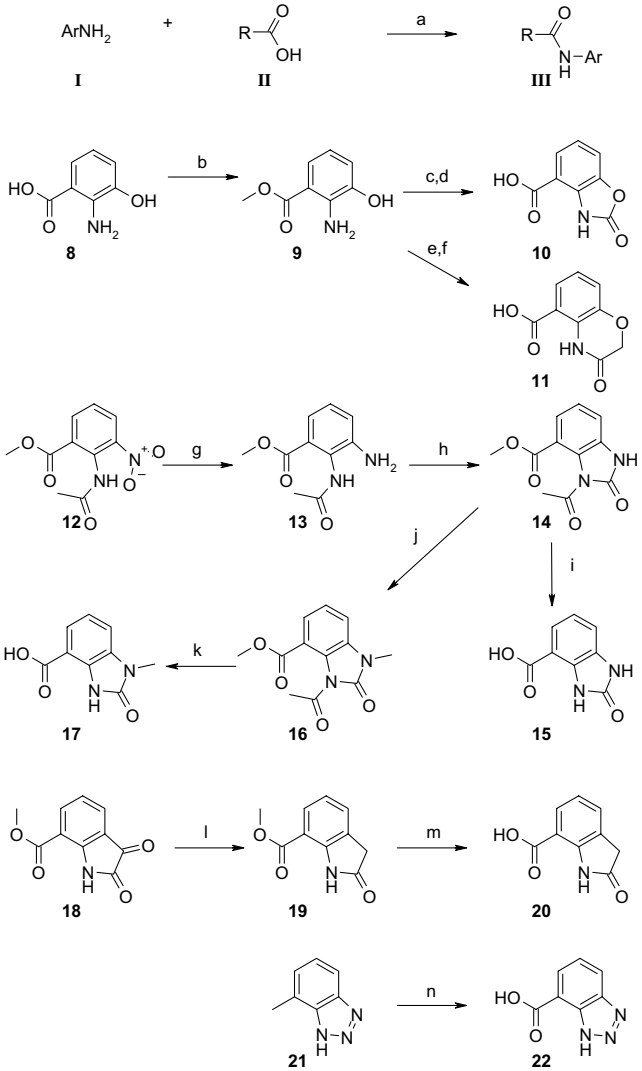


Figure 1. Comparison between the most stable tautomeric form of fenobam **3**, the salicylamide **4**, the quinazoline **5**, and the benzoxazolones **6** and **7**, highlighting the elements which have been combined to generate the new structures.

In the following, SAR, properties, and efficacy in an in vivo anxiety model of this new class of mGlu5 receptor antagonists will be presented.

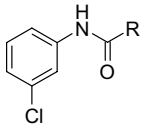
Synthesis of the compounds described in this communication and of related analogs was performed by simple amide coupling between various anilines **I** and arylbenzoic acids **II** according to standard methodology (Scheme 1). The functionalized arylbenzoic acids were either commercially available or synthesized as illustrated in Scheme 1.

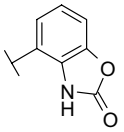
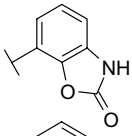
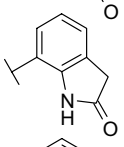
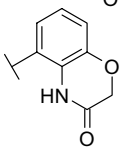
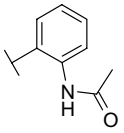
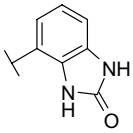
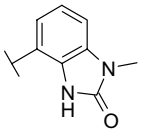
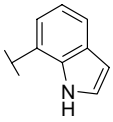
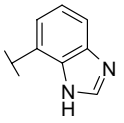
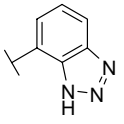
2-Oxo-2,3-dihydro-benzooxazole-4-carboxylic acid **10** was synthesized from the corresponding open amino-



Scheme 1. Synthesis of the non-commercial arylbenzoic acid used to generate the compounds described in this paper. Reagents and conditions: (a) coupling agent (EDC/HBTO, TBTU or CDI), base (DIPEA, pyridine, TEA or no base), DMF, rt; (b) MeOH, H_2SO_4, reflux, 1.5 h; (c) $COCl_2$ 20% in toluene, Py, rt, 20 h; (d) NaOH 0.5 N, rt, 4 h; (e) $ClCH_2COCl$, $BnEt_3NCl$, $NaHCO_3$, $CHCl_3$, 55 °C, 5 h; (f) NaOH 0.5 N, rt, 4 h; (g) Pd/C, H_2, EtOH, rt, 4 h; (h) $COCl_2$ 20% in toluene, Py, rt, 20 h; (i) NaOH 1 N, rt, 4 h; (j) MeI, K_2CO_3, CH_3CN, 50 °C, 2 h; (k) NaOH 1 N, rt 4 h; (l) i. H_2, Pd/C, EtOH; ii. H_2, Pd/C, EtOH, HCl; (m) NaOH, MeOH; (n) $KMnO_4$, KOH/H_2O, 100 °C, 24 h.

Table 1. Binding constants of analogs of compound **6** where the benzoxazolone is replaced by other benzocondensed heterocycles



Compound	R	K_i (nM)
6		68[a]
23		>10000
24		397
25		2900
26		>10000
27		3900
28		>10000
29		3900
30		>10000
31		>10000

[a] Functional assay: Ca^{2+} efflux IC_{50} (FLIPR): 195 nM.

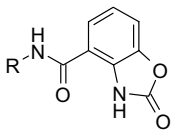
phenol **9** via cyclization with phosgene followed by ester hydrolysis. From the same aminophenol **9**, the six-membered ring analog **11** could be accessed by treatment with chloroacetyl chloride and sodium hydrogencarbonate in the presence of a phase transfer catalyst. Synthesis of the cyclic urea derivative **15** was accomplished starting from the 2-acetylamino-3-nitro-benzoic acid methyl ester **12** through reduction of the nitro group and cyclization to the monoprotected urea **14** with phosgene, followed by deprotection and hydrolysis. The protected intermediate **14** can also be monomethylated to prepare the methylated analog **17**. Lactame **20**, where the oxygen of the benzoxazolone is substituted by a methylene group, was prepared from the commercially available dioxoindoline **18** via selective reduction and hydrolysis. Benzotriazole **22** was prepared from the corresponding 7-methyl benzotriazole **21** by oxidation of the methyl group with potassium permanganate.

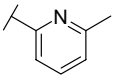
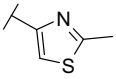
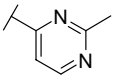
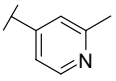
Table 1 illustrates binding constants of analogs of compound **6** where the benzoxazolone is replaced by other ring systems. The hypothesis that an intramolecular hydrogen bond involving the amide carbonyl is a key element for mGlu5 inhibition in this series is supported by the fact that the inverted benzoxazolone isomer **23** shows no binding affinity. The indolinone **24**, despite being a closer analog to the fenobam creatinine ring, shows a reduced K_i. This is also the case for the homolog **25** of benzoxazolone **6**, having a six-membered oxazinone ring. The open-chain analog **26**, surprisingly, is completely inactive. Other H-bond forming heterocyclic analogs, like the benzimidazolinones **27** and **28**, and the aromatic systems **29**–**31** show a very reduced or no binding affinity at all.

On the aniline side (Table 2), the analogy between the SAR of the benzoxazolones and that of the MPEP-type compounds and fenobam was soon apparent.[16] As observed for several classes of mGlu5 allosteric inhibitors, a single spherical lipophilic substituent in the meta position brings the highest binding affinity and functional activity, as shown in Table 2 (compounds **33**–**37**). The simple unsubstituted phenyl, as in **32**, as well as compounds with ortho or para substitution and doubly substituted compounds (examples **38**–**40**, Table 2) show a substantially reduced binding affinity. In Table 2 are also shown binding affinities of a few selected heteroarylamine derivatives. Also in this set, analogies to the known SAR of other classes of mGlu5 receptor antagonists are found, with the MPEP substituent 6-methylpyridin-2-yl (**42**) showing the highest affinity and functional activity. The MTEP-type substituent 2-methyl-1,3-thiazol-5-yl (**43**), as well as the 2-pyridyl derivative **41**, retain a binding affinity of 0.25 μM. Other heterocyclic amide derivatives, as for example, **44** and **45**, show much reduced affinity. Any attempt to substitute the amide NH with small alkyl chains led to completely inactive compounds (unreported data).

In view of its high functional activity, reasonable solubility (30 mg/L),[18] and good foreseen permeation behavior (PAMPA Pe: 7.13 cm/s × 10^{-6}),[19] compound **42** was selected for in vivo characterization. Single dose rat pharmacokinetics showed, contrary to what was reported for fenobam,[14] a very promising profile, with a clearance of 8.2 ml/min/kg and an oral bioavailability of 64%. Volume of distribution was very low (0.18 L/kg), leading to a relatively short half-life (0.22 h). A prelimin-

Table 2. SAR of benzoxazolone derivatives at the aniline site



Compound	R	K_i (nM)
6	3-Cl–Ph	68
32	Ph	2200
33	3-Br–Ph	56[a]
34	3-CN–Ph	327
35	3-CF_3–Ph	485
36	3-CH_3–Ph	109[b]
37	3-MeO–Ph	3900
38	2-Cl–Ph	>10000
39	4-Cl–Ph	3800
40	3,5-Cl_2–Ph	>10000
41		251
42		95[c]
43		250
44		1970
45		3100

[a] Functional assay: Ca^{2+} efflux IC_{50} (FLIPR): 94 nM.
[b] Functional assay: Ca^{2+} efflux IC_{50} (FLIPR): 104 nM.
[c] Functional assay: Ca^{2+} Efflux IC_{50} (FLIPR): 60 nM.

ary evaluation of blood/brain partitioning revealed a relatively low brain/plasma ratio of 0.06 three hours after administration, though a measurable concentration of compound was present in the cerebro-spinal fluid (1% of plasma concentration). Compound **42** showed dose dependent efficacy in the stress induced hyperthermia (SIH) model of anxiety in NMRI mice (Fig. 2),[4a] with a minimum effective dose of 10 mg/kg po (no effect on T1 at either dose).

In conclusion, the notion that the known anxiolytic fenobam is a mGlu5 allosteric inhibitor and comparison of its structure with that of salicylamide **4** (which was found as a hit after screening our compound library for mGlu5 binders at the allosteric site) led to the hypothesis that an intramolecular hydrogen bond might be a pharmacophoric feature of this type of mGlu5 allosteric inhibitors. Based on this concept, compound **6** was designed combining features of fenobam **3** and salicylamide **4**. Despite the limited options for structural variation, SAR exploration led to 2-oxo-2,3-dihydrobenzooxazole-4-carboxylic acid (6-methyl-pyridin-2-yl)-amide **42**, which showed good pharmacokinetic properties and efficacy in an in vivo model of anxiety. The small size and promising profile of **42** make the

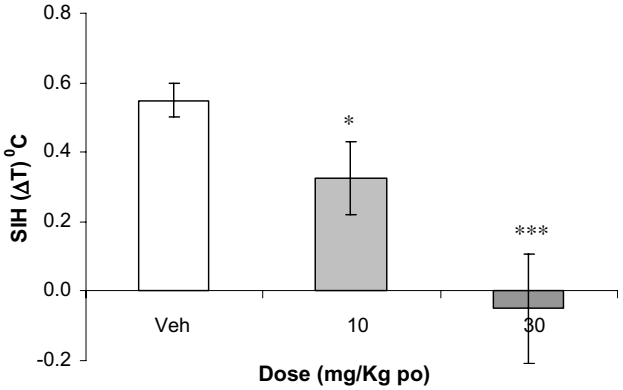


Figure 2. Effect of compound **42** on ΔT in the stress induced hyperthermia (SIH) model of anxiety in NMRI mice.[4a] Vehicle: 0.3% Tween 80 in 0.9% NaCl. Pre-treatment time: 60 min. Statistics: $^{*}p < 0.05$ compared to vehicle with a *T*-test independent by group; $^{***}p < 0.001$ compared to vehicle with a *T*-test independent by group.

benzoxazolone class an attractive option for the development of anxiolytics targeting mGlu5. Intramolecular hydrogen bonding, moreover, could be used as a leading concept for the rational design of novel and diverse mGlu5 allosteric inhibitors.

Acknowledgments

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