Pure Appl. Chem., Vol. 76, No. 5, pp. 931–939, 2004.

Search for noncompetitive 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid receptor (AMPAR) antagonists: Synthesis, pharmacological properties, and computational studies*

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Abstract: The development of new 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA) receptor (AMPAR) negative modulators has received considerable interest due to their crucial role in specific neurological diseases.

In recent years, our research group has been engaged in the development of new AMPAR ligands and chemical and biological studies of various 2,3-benzodiazepin-4-(thi)ones (CFMs) and their analogous cyclofunctionalized have been reported. Electrophysiological experiments confirmed that their effects are mediated through the AMPAR complex in a selective and noncompetitive fashion. Moreover, we carried out computational studies which suggested the possible binding site for noncompetitive antagonists; we also developed a 3D ligand-based pharmacophore model in order to map common structural features of highly potent compounds. Our hypothesis was successfully used as a framework for the design of a new class of allosteric modulators containing a tetrahydroisoquinoline skeleton and led to the discovery of a very potent AMPAR antagonist with marked antiepileptic effects.

INTRODUCTION

Central nervous system (CNS) diseases are increasing, and there is a significant unmet medical need for new, effective, and safe drugs. There is considerable evidence that ion channel glutamate receptors (iGluRs), identified as NMDA, 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and KA receptors, play important roles in a number of neurological disorders; consequently, they are potential targets for therapeutic intervention and ligands that interact with them are of significant interest.

Considering the pivotal role of AMPA receptors (AMPARs) in physiological and pathological processes, significant effort has been focused on the synthesis of specific ligands as a source of potential anticonvulsant and neuroprotective agents. In particular, the search was addressed in the field of noncompetitive antagonists because they do not bind at the same binding site of glutamate and therefore remain efficacious independently of the level of the endogenous agonist reached during an ischemic or epileptic attack. Furthermore, their prolonged use in neuroprotective treatments might not influence the normal glutamatergic activity [1].

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*Lecture presented at the Polish–Austrian–German–Hungarian–Italian Joint Meeting on Medicinal Chemistry, Kraków, Poland, 15–18 October 2003. Other presentations are published in this issue, pp. 907–1032.
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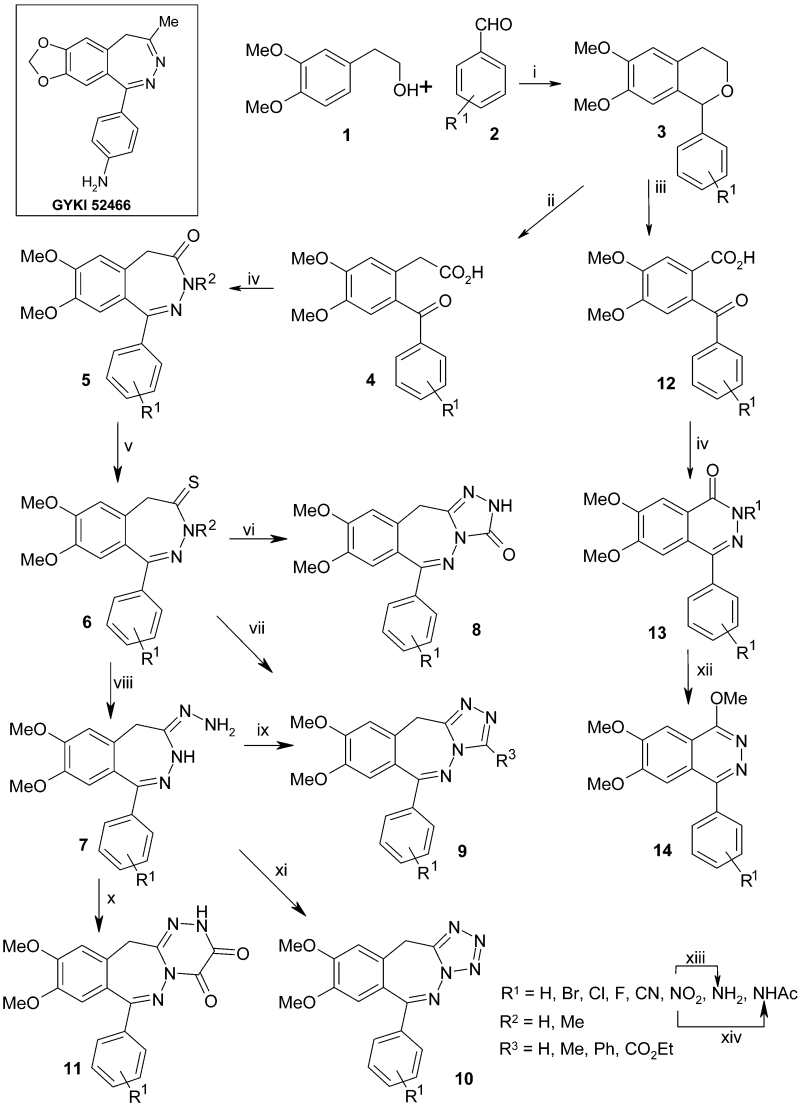
NONCOMPETITIVE AMPA RECEPTOR ANTAGONISTS

In the last decade, our research group has been working in the development of new AMPAR negative modulators and has reported chemical and biological studies of various 2,3-benzodiazepin-4-ones (CFMs) and thiocarbonyl analogs as marked antiepileptic agents.

We chose as lead compound GYKI 52466, the first noncompetitive AMPA receptor antagonist showing cerebroprotective and anticonvulsant, but not hypnotic–sedative effects. Considering GYKI 52466 as template, we planned a series of modifications on the 2,3-benzodiazepine skeleton in order to obtain more potent, less toxic, and longer-lasting derivatives (Scheme 1).

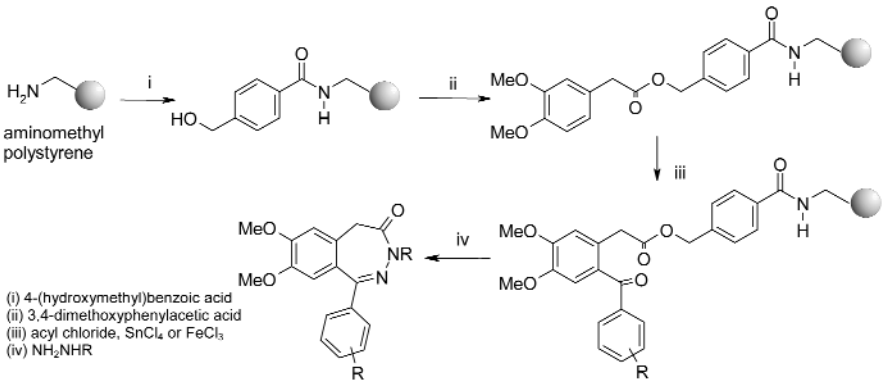


Scheme 1

The synthesis of 1-aryl-3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones (**5**) and 4-thiones (**6**) [2–5] was performed according to the reported Scheme 1 or by solid-phase approach (Scheme 2). In the last

procedure the key steps were a Friedel–Crafts acylation of a resin-bound 3,4-dimethoxyphenylacetate and a concomitant hydrazine-mediated cleavage and ring closure [6].

The synthesis of annelated 2,3-benzodiazepine (**8–11**) derivatives (Scheme 1) was performed starting from 3,5-dihydro-4*H*-2,3-benzodiazepin-4-ones (**5**), or the corresponding activated thiocarbonyl (**6**) derivatives. The reaction of thiocarbonyl derivatives with hydrazine hydrate furnished corresponding 2,3-benzodiazepin-4-ylhydrazine (**7**) intermediates, which were treated with sodium nitrite in acidic medium to afford 11*H*-tetrazolo[1,5-*c*][2,3]benzodiazepines (**10**) [14] or with oxalyl chloride to give the tricyclic 2,12-dihydro-9,10-dimethoxy[1,2,4]triazino[4,3-c][2,3]benzodiazepines (**11**) [15] or with ethyloxalyl chloride to furnish the 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]-benzodiazepines (**9**, $R^3 = CO_2Et$) [16]. By refluxing 3,5-dihydro-4*H*-2,3-benzodiazepine-4-thiones with suitable hydrazides or ethyl carbazate, 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines (**9**) [17], and 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]-benzodiazepin-3(2*H*)-ones (**8**) were prepared [15,18].



Scheme 2

We also synthesized the 4-aryl-6,7-dimethoxyphthalazin-1(2*H*)-ones (**13–14**) (Scheme 1), wherein the phthalazine six-membered framework substitutes the diazepine nucleus, whereas some structural features such as the dimethoxybenzene moiety and lactam functionality were kept [19].

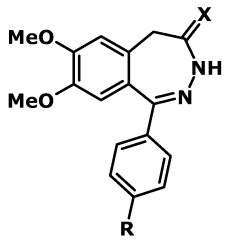
Compounds **5–6** were tested for anticonvulsant activity against audiogenic, PTZ- and MES-induced seizures and compared with GYKI 52466. They showed significant anticonvulsant activity also against seizures induced by AMPA, thus suggesting an involvement of AMPA receptor complex. The results obtained showed that some derivatives, that we named CFM, were more potent than the lead compound and showed longer-lasting activity and less toxicity. In particular, 1-(4-aminophenyl)-3,5-dihydro-4*H*-2,3-benzodiazepine-4-thione (CFM-2S) derivative was 2.5- and 6-fold more active than the corresponding carbonyl isostere and GYKI 52466, respectively (Table 1).

The longer-lasting activity of the thione derivatives (**6**) could be explained by taking into account the influence of biotransformation reactions on molecular properties and the consequences of such changes as demonstrated by high-performance liquid chromatography (HPLC) assay on rat plasma [7–9].

In order to define the nature of the AMPA receptor block we carried out electrophysiological experiments, which confirmed a noncompetitive mechanism at the doses studied [2,3].

The anticonvulsant effects of our compounds were compared with competitive and noncompetitive AMPAR antagonists as well as with diazepam, a conventional antiepileptic drug. Our 2,3-benzodiazepine derivatives (**5–6**) showed an excellent broad spectrum of anticonvulsant properties [10–12]. Furthermore, the activity of our derivatives is positively influenced by some metabotropic glutamate agonists of group III such as (*R*,*S*)-PPG and ACPT-1, which given i.c.v. enhanced the anticonvulsant properties and prolonged the time course of compound CFM-2 [13].

Table 1



CFM-1 R = H, X = O
CFM-2 R = NH_2, X = O
CFM-2S R = NH_2, X = S

	ED_{50} µmol/kg				TD_{50} µmol/kg	
	Sound-induced seizures	MES-induced seizures	PTZ-induced seizures	AMPA-induced seizures	Rotarod test	TI TD_{50}/ED_{50}
GYKI 52466	35.7	35.7	68.3	57.5	76.1	2.1
CFM-1	33.9	35.8	68.2	66.0	142	4.2
CFM-2	15.0	15.9	22.6	32.1	56.8	3.8
CFM-2S	6.30	7.75	15.4	17.1	42.6	6.8

The anticonvulsant properties of the tricyclic derivatives were evaluated against audiogenic seizures to test the effect of cyclofunctionalization on the pharmacological profile. 11*H*-Tetrazolo[1,5-*c*][2,3]benzodiazepines (**10**), 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepines (**9**) and 3-ethoxycarbonyl-11*H*-[1,2,4]triazolo[4,5-*c*][2,3]-benzodiazepines (**9**, $R^3 = CO_2Et$) showed generally anticonvulsant effects weaker than those of their parent compounds. On the contrary, the biological results of 11*H*-[1,2,4]triazolo[4,5-*c*][2,3]benzodiazepin-3(2*H*)-ones (**8**) revealed that the introduction of the triazolone nucleus on the diazepine skeleton leads to compounds with higher anticonvulsant potency (i.e., ED_{50} = 3.65 µmol/kg) [15,18].

Structure–activity relationship (SAR) studies furnished significant information about the main structural requirements for the anticonvulsant effects. In particular, it was observed that: (i) the increase of lipophilicity is favorable to pharmacological profile; (ii) the cyclofunctionalization of the diazepine nucleus influences the anticonvulsant potency depending on the nature of the fused heterocyclic ring; (iii) the unsubstituted (thio)lactam moiety plays a pivotal role in the case of both bicyclic and annelated 2,3-benzodiazepines; and (iv) the phthalazine derivatives are less potent than the corresponding benzodiazepines.

Despite the insightful interest for allosteric modulators of AMPAR, a comprehensive study of the structural features affecting potency and selectivity was lacking. Furthermore, no information was available about the location and composition of the AMPAR negative allosteric ligand binding region. In the absence of such structure-based information, we attempted to identify the hypothetical 3D ligand-based pharmacophore model by using the hypothesis generation approach HipHop/Catalyst, which finds common features among a set of highly active compounds [20]. The goal of our work was the identification of the 3D structural requirements that are relevant in a molecule in order to noncompetitively interact with AMPAR. Fourteen molecules were selected as the training set, representing the most interesting classes of noncompetitive AMPAR antagonists (Fig. 1). These compounds are characterized by significant anticonvulsant effects, and their interaction with AMPAR was confirmed by biological tests.

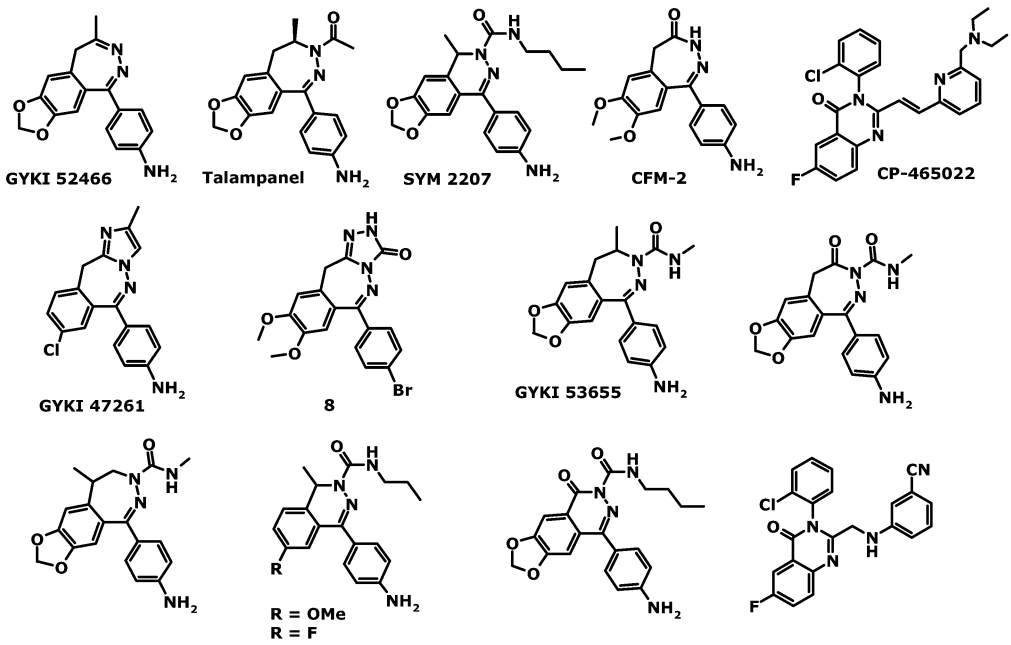


Fig. 1 Chemical structures of noncompetitive AMPA receptor antagonists.

The minimal structural requirements for AMPA interaction were identified to be two hydrophobic groups, one aromatic region and one hydrogen bond acceptor in a specific 3D arrangement. Compound CFM-2 mapped well onto our hypothesis (Fig. 2), suggesting some structural modifications that could lead to new selective negative allosteric AMPAR modulators.

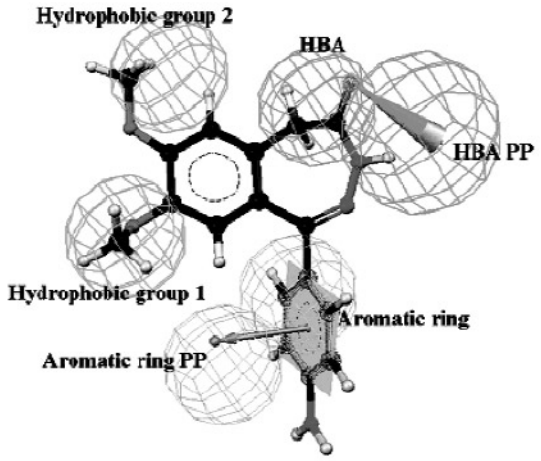
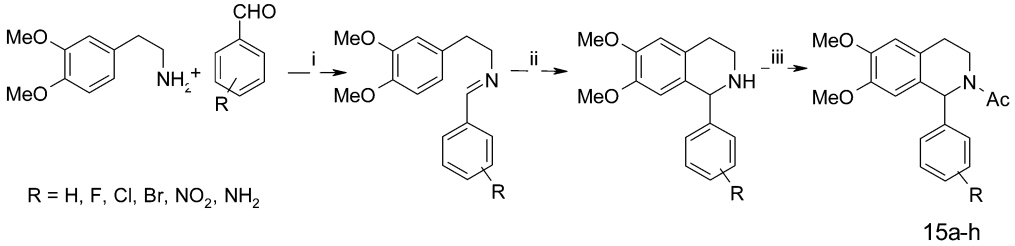


Fig. 2

In particular, the simple replacement of diazepine ring with tetrahydropyridine system directed the synthesis toward the 2-acetyl-1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines [20]. We decided to keep the 4-aryl substituent and dimethoxybenzene moiety by analogy with CFM-2; furthermore, we chose to include the *N*-acetyl functional group in the compounds to be synthesized in order

to meet the hydrogen bond acceptor feature described in the 3D model, improving the chance of obtaining active molecules.

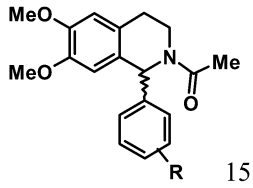
The designed 1-aryl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolines were easily synthesized as a racemic mixture via Pictet–Spengler synthetic approach with good yields and were further subjected to reaction with acetic anhydride to afford *N*-acetyl derivatives [20,21].



Scheme 3

The anticonvulsant effects were evaluated (Table 2), and pharmacological screening showed that some of these molecules were efficacious against sound-induced seizures, and in particular the 4-chlorophenyl derivative (**15c**) proved to be more potent than GYKI 52466, CFM-2 and talampanel, a noncompetitive AMPA receptor antagonist currently being investigated in phase III trials as an antiepileptic agent. Our derivative demonstrated anticonvulsant activity with ED_{50} values an order of magnitude less than those of GYKI 52466 and was 3-fold more active than talampanel and CFM-2 [21].

Table 2



15 a-h

		ED_{50} values μmol/kg	
Cpd	R	Clonus	Tonus
a	H	33.9	31.8
b	4-F	36.8	18.0
c	4-Cl	4.18	2.39
d	4-Br	43.1	16.5
e	4-NO_2	>100	>100
f	3-NO_2	37.2	12.8
g	4-NH_2	32.1	21.1
h	3-NH_2	29.9	17.5

In addition, to define the mechanism of action and confirm the hypothesis suggested by molecular modeling studies, electrophysiological experiments were carried out.

In the presence of this compound, AMPA responses were consistently abolished, and the results confirmed a noncompetitive-type blocking mechanism (Fig. 3). It is worth noting that to obtain a similar response in the same assay, it is necessary to use 100-fold higher dose of GYKI 52466 and some other 2,3-benzodiazepines.

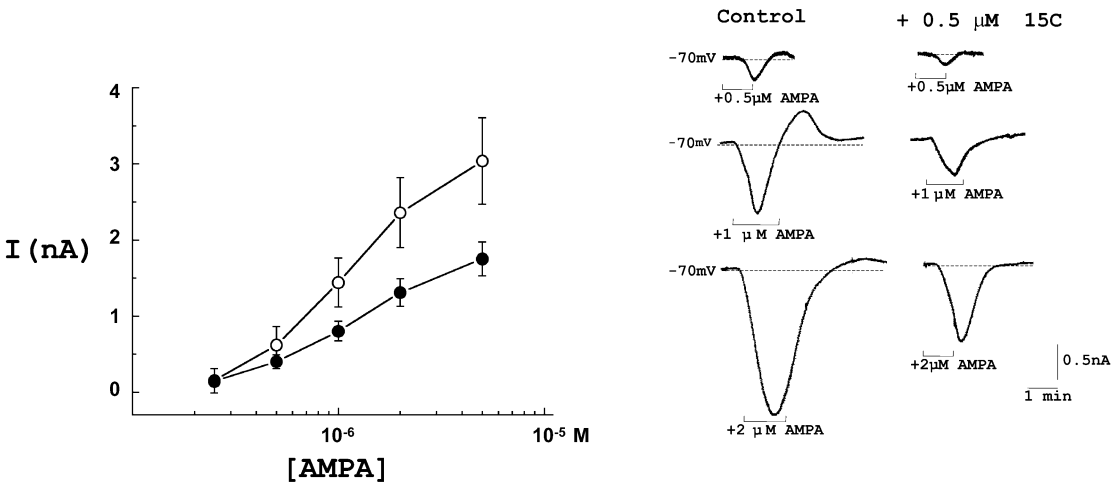


Fig. 3

The alignment of 1-(4-chlorophenyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**15c**) with the 3D-hypothesis showed that the features of the proposed model were well matched by the chemical groups of the molecule, indicating a preference for the *S*-enantiomer (Fig. 4). This compound can be thus considered the lead template of a new class of negative modulators of AMPAR.

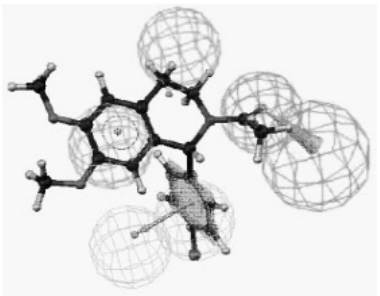


Fig. 4

The four-point pharmacophore hypothesis was also directly used as a query for virtual screening, looking for additional candidates for biological testing. A subset of molecules was chosen and overlaid with the 3D-pharmacophore. Eight compounds were selected for anticonvulsant testing, and some of them demonstrated anticonvulsant effects, confirming the strength of our pharmacophore modeling approach [20].

Finally, we tried to understand where these ligands might bind. There was considerable evidence that all GluRs have a modular architecture characterized by the presence of two segments (S1 and S2) homologous to LAOBP and an *N*-terminal segment homologous to LIVBP-like. While the S1-S2 has unequivocally been shown to contain the binding site for agonists and competitive antagonists, the significance of LIVBP domain was much less understood.

Recently, it has been reported that the LIVBP domain in the NMDA receptors (in NR2 subunits) contains the binding site for noncompetitive antagonists [22]. On the basis of the structural and functional similarity between AMPA and NMDA receptors, we advanced the hypothesis that a similar binding mechanism could also work in the case of noncompetitive modulators of AMPAR. In addition, considering that NR2B is endowed with a folding similar to that of mGluR1 and that the crystal structure of the ligand-binding domain of mGluR1 was reported, the sequences of the amino terminal domains of AMPA (LIVBP-like region), mGluR1 and NMDA (NR2B, LIVBP-like region) were aligned.

Using the results of the multiple alignment and the ligand-binding domain (LBD) of mGluR1 as template, a homology model of the LIVBP-like domain of AMPAR was obtained. Once having generated a reliable model of the LIVBP-like region of AMPAR, our objective was to identify the correct binding mode of noncompetitive AMPA antagonists at the modeled *N*-terminus region [23].

We thus selected the most representative compounds and carried out an automated docking procedure. All the studied compounds had a single preferred disposition in the identified binding site and presented a common binding mode (Fig. 5). CFM-2, selected as example, is involved in hydrogen bonds and in π-π interaction with some amino acids, in agreement with the reported pharmacophore model [20].

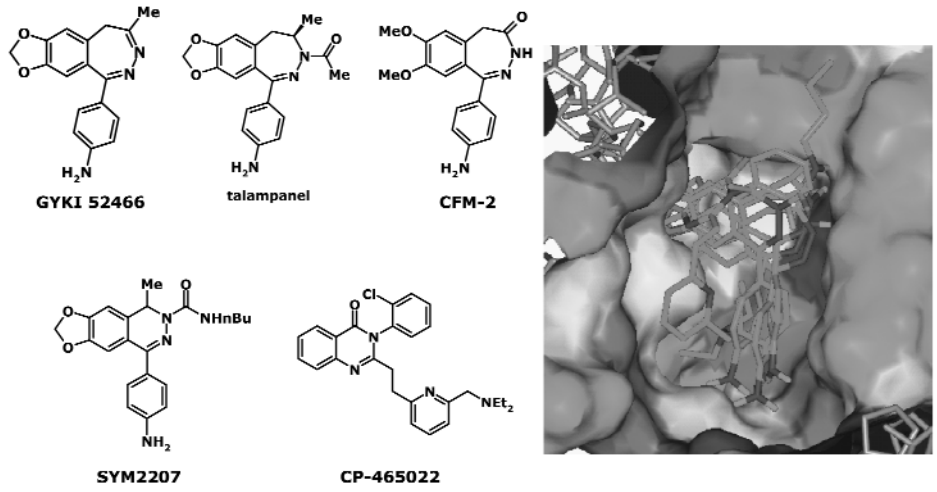


Fig. 5

In conclusion, our research led to: (i) the discovery of a different series of highly potent in vitro and in vivo AMPAR antagonists; (ii) the first solid-phase synthesis of 2,3-benzodiazepines; (iii) the generation of a 3D pharmacophore model; and (iv) the individuation of the possible binding site for AMPAR negative allosteric modulators.

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