Bioorganic & Medicinal Chemistry Letters 20 (2010) 184–188

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Piperidyl amides as novel, potent and orally active mGlu5 receptor antagonists with anxiolytic-like activity

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ARTICLE INFO

Article history:
Received 6 October 2009
Revised 30 October 2009
Accepted 1 November 2009
Available online 5 November 2009

Keywords:
Metabotropic glutamate receptor
mGluR5 antagonist
Piperidylamides

ABSTRACT

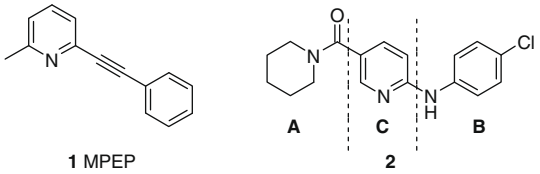
High throughput screening led to the identification of nicotinamide derivative **2** as a structurally novel mGluR5 antagonist. Optimization of the modular scaffold led to the discovery of **16m**, a compound with high affinity for mGluR5 and excellent selectivity over other glutamate receptors. Compound **16m** exhibits a favorable PK profile in rats, robust anxiolytic-like effects in three different animal models of fear and anxiety, as well as a good PK/PD correlation.

Glutamate is the main excitatory neurotransmitter in the mammalian central nervous system and as such involved in a variety of physiological and pathophysiological functions. Glutamate receptors are classified into ionotropic (iGlu) receptors and metabotropic glutamate (mGlu) receptors. The iGlu receptors form ligand-gated ion channels (AMPA-, kainate-, and NMDA-type) which mediate fast excitatory synaptic transmission. Metabotropic glutamate receptors are G-protein-coupled receptors[1] linked to multiple second messenger systems modulating, for example, ion channel functions in the neurons.[2] Currently, eight distinct metabotropic receptor subtypes are known (mGluR1–mGluR8), which are divided into three groups based on sequence homology, pharmacological profiles, and signal transduction pathways. mGlu5 receptors are highly expressed in brain regions thought to mediate and modulate emotions like fear and anxiety (amygdala, prefrontal cortex, hippocampus, and basal ganglia).[3] mGlu5 receptors are predominantly postsynaptically located and have been shown to play a role in regulating glutamatergic transmission via potentiation of NMDA receptor activity. Excessive glutamatergic transmission has been proposed to play a role in psychiatric diseases like anxiety disorders and depression.[4] Additionally, mGlu5 receptor antagonism has been proposed as a potential approach for the treatment of pain, obesity, Parkinson's disease and drug abuse.[5] Therefore, the development of potent and selective mGlu5 receptor antagonists as potential therapeutic agents has been the focus of significant research in our laboratories.

Our previous research efforts for mGlu5 receptor antagonists had been focused on alkyne derivatives such as MPEP (**1**) (Fig. 1) which proved to be potent and highly selective mGlu5 receptor antagonist with in vivo activity in animal models of anxiety.[6] In the course of our search for novel structural leads, compound **2** was identified in a high-throughput screen, based on the inhibition of agonist induced elevation of the intracellular Ca^{2+} concentration.[7a]

In the present Letter, a detailed structure–activity relationship (SAR) study will be discussed as well as the biological evaluation of an optimized candidate in different animal models of anxiety.

Modifications of all three parts of scaffold **2**, namely amide **A**, aniline **B**, and 'core' **C**, have been explored. In a first round, each region was optimized separately. Then, in a second round, optimal combinations were identified and further fine-tuned. The target molecules can be synthesized by a convenient two-step synthetic process starting from readily available building blocks



1 MPEP **2**

Figure 1. mGluR5 antagonists.

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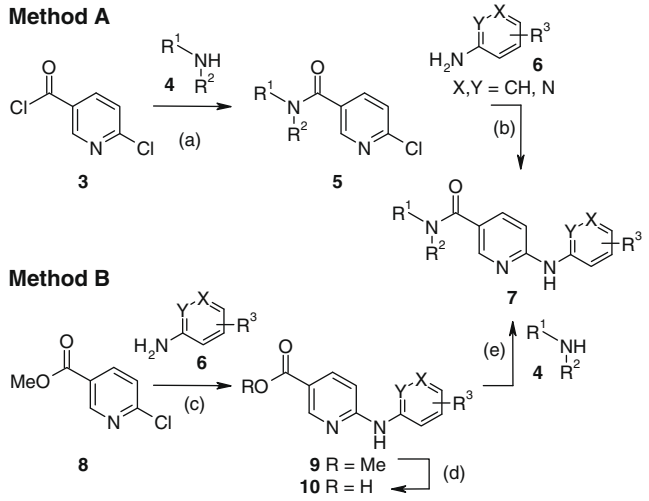
doi:10.1016/j.bmcl.2009.11.001



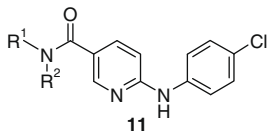
Scheme 1. Reagents and conditions: (a) NEt_3, DCM, 0 °C, 1 h; (b) $AcOH/H_2O$ 3:7, 95 °C, 18 h; (c) $Pd(OAc)_2$, BINAP, K_2CO_3, toluene, 70 °C, 1.5 h; (d) 2 M NaOH, MeOH, 60 °C, 0.5 h; (e) HATU, DIPEA, DME, rt, 6 h.

(Scheme 1). In a first step, 6-chloronicotinoyl chloride (**3**) was reacted with an amine **4**. Subsequent S_NAr reaction of the 6-chloro function of corresponding amide **5** with an aniline **6** afforded the desired product **7** (method A). Alternatively, the reaction sequence can be reversed: First, a Buchwald reaction of nicotinic ester **8** with aniline **6** followed by amide formation of intermediate **9** via corresponding acid **10** and an amine **4** resulted in the formation of **7** (method B).

SAR evaluation started with variation of the amide functionality. Tertiary amides carrying lipophilic substituents were clearly preferred, in particular if R^1 and R^2 together form a ring. Small substituents at the cyclohexyl ring (**11e–g**) were tolerated, however potency of the racemic derivatives did not further improve. Secondary amides resulted in compounds with significantly decreased potency against the mGlu5 receptor (Table 1).

In a second sub-library, the influence of different aniline and heteroarylamine moieties (fragment **B**) on the potency was studied (Table 2). A very steep SAR was observed, with substitution of the aromatic system only being tolerated in *para*-position. Best results were obtained with small lipophilic fragments (e.g., Me, Cl). Interestingly, the phenyl group could be replaced by a more polar 3-pyridyl substituent which gave the most potent derivative **12k** of this set. The corresponding 2,2′-dipyridylamines **12i–j** that relate

Table 1
Optimization of amide part **A**: in vitro hmGluR5 antagonism



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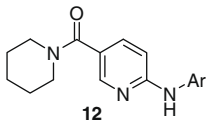
Compound	R^1	R^2	Method[a]	IC_{50}[b] (μM)
2			A	0.31
11a			A	5.40
11b			B	0.25
11c			B	2.30
11d	Et	Et	A	0.46
11e			A	0.43
11f			A	0.53
11g			B	2.30
11h		H	B	>10

[a] See Scheme 1.
[b] hmGluR5 Ca^{2+} flux[7] using glutamate as agonist, data are geometric means of $n \geqslant 2$.

Table 2
Optimization of aniline fragment **B**: in vitro hmGluR5 antagonism



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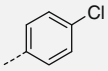
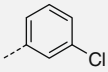
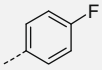
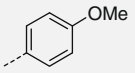
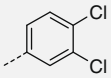
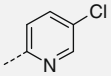
Compound	Ar	Method[a]	IC_{50}[b] (μM)
2		A	0.31
12a	Ph	A	~10
12b	Cyclohexyl	B	>10
12c		B	>10
12d		A	>10
12e		B	2.30
12f		A	2.30
12g		A	1.80
12h		A	>10
12i		B	~10
12j		B	>10
12k		B	0.42

[a] See Scheme 1.
[b] hmGluR5 Ca^{2+} flux[7] using glutamate as agonist, data are geometric means of $n \geqslant 2$.

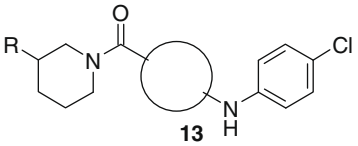
to a previously published class of potent mGluR5 antagonists[8] exhibited surprisingly low potency.

Modification of the 6-substituted nicotinamide, 'core' **C** showed that potency could be gained by introduction of an electron-withdrawing substituent in 5-position (**13b**). Benzamide **13a**, pyridazine-carboxamide **13d**, and pyrimidine-5-carboxamide **13e** were only moderately active, whereas pyrazine-2-carboxamide **13f** and pyrazole-3-carboxamide **13g** were completely inactive (Table 3).

Finally, the importance of the hydrogen bond donor on the nitrogen bridge between the two aromatic rings was studied. Loss of activity for the N-methylated derivative **14** and ether **15** in direct comparison to **2** and **11f**, respectively, confirmed the need for a secondary amine functionality (Fig. 2).

Combination of optimal fragments identified for each region led to the highly potent derivative **16a** (Table 4). Since in the first optimization round, potency could not be improved by modification of fragment **A**, the potential for substitution of the piperidine ring (R^1, R^2) was re-investigated, aiming at an improved potency as well as a good balance of aqueous solubility at pH 6.8 and an acceptable intrinsic clearance in human and rat liver microsomes (Table 4). Whereas for substituent R^2, chain length and stereochemistry had not much influence on potency, the situation was different for substituent R^1, where the (*R*)-forms generally were more potent in two functional assays on hmGluR5 than the corresponding (*S*)-forms. In general, both solubility and microsomal stability decreased with increasing size of the substituents. The best balance between potency, solubility and microsomal stability was achieved for analogue **16m**, bearing a (*R*)-2-ethyl-piperidineamide as fragment **A**.

Table 3
Optimization of 'core' **C**: in vitro hmGluR5 antagonism



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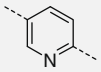
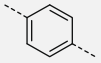
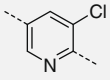
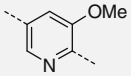
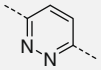
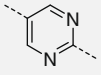
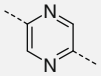
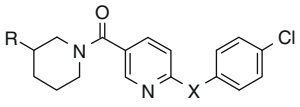
Compound	R	'Core'	Method[a]	IC_{50}[b] (μM)
2	H		A	0.31
13a	Me		A	1.60
13b	Me		B	0.095
13c	H		A[9]	2.10
13d	Me		B	0.72
13e	Me		B	0.98
13f	H		A	>10
13g	Me		B[10]	>10

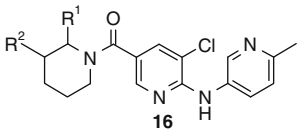
[a] See Scheme 1.
[b] hmGluR5 Ca^{2+} flux[7] using glutamate as agonist, data are geometric means of $n \geqslant 2$.



			hmGluR5 Ca^{2+} flux IC_{50} [μM]
2:	R = H	X = NH	0.31
14:	R = H	X = NMe	>10
11f:	R = Me	X = NH	0.53
15:	R = Me	X = O	>10

Figure 2. Secondary amines **2**, **11f** and tertiary amine **14** and ether **15**.

Table 4
Substitution of the piperidine ring: in vitro hmGluR5 antagonism, aqueous solubility at pH 6.8 and intrinsic clearance in human and rat liver microsomes



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Compd	R^1	R^2	IC_{50} (nM)[d] Ca^{2+}flux/PI	Solubility[e] pH 6.8	CLint[f] (h/r)
16a[a]	H	H	87/258	150	33/48
16b[a]	H	*rac*-Me	105/310	—	60/38
16c[a]	H	*rac*-Et	65/48	15	171/417
16d[a]	H	*rac*-Pr	190/140	—	–/–
16e[a]	H	*R*-Et	40/69	95	322/643
16f[b]	H	*S*-Et	40/110	<3	174/687
16g[b]	*S*-Me	H	1400/–	499	39/68
16h[b]	*R*-Me	H	240/430	216	44/73
16i[b]	*rac*-Et	H	110/90	122	138/220
16j[b]	*rac*-Pr	H	90/16	<3	224/885
16k[b]	*rac*-Bu	H	136/5	<3	227/637
16l[c]	*S*-Et	H	390/360	56	216/190
16m[c]	*R*-Et	H	32/36	58	89/87

[a] Synthesis method A.
[b] Synthesis method B.
[c] via separation of racemate **16i** by chiral HPLC (Chiralpak AD-H, 30 × 250 mm, hexane, 50% ethanol).
[d] hmGluR5: Ca^{2+} flux using glutamate and phosphoinositol turnover using quisqualate as agonist,[7] data are geometric means of $n \geqslant 2$, IC_{50} (nM).
[e] Solubility (mg/L) measured in a dissolution template potentiometric titration approach.
[f] CLint: intrinsic clearance (μL/min/mg protein).

In vitro radioligand displacement assays utilizing [^{3}H]-ABP688[11] showed that **16m** is a high-affinity ligand at the previously characterized allosteric binding site located in the membrane-spanning region of mGlu5 receptors[12] with a K_i at the human recombinant receptor of 38 nM.[13] In addition, **16m** showed a high degree of selectivity over representatives of groups I–III metabotropic glutamate receptor subtypes (IC_{50} >10 μM for hmGluR1, -2, -7) and ionotropic glutamate receptors (IC_{50} >10 μM).[14]

A single-dose pharmacokinetic study of **16m** in rats (Table 5) revealed that after an oral dose of 30 μmol/kg, the mean maximal

Table 5
Pharmacokinetic parameters[a] of **16m**

Absorption[a] (30 μmol/kg po)	Disposition (10 μmol/kg iv)
C_{max} = 1230 pmol/mL	$t_{1/2}$ 1;2= 0.3; 0.9 h (54%)
T_{max} = 0.25 h	MRT = 0.9 h
AUC = 7230 pmol h/mL	CL = 37.2 mL/min/kg
F = 54%	V_{ss} = 2 L/kg
Brain/plasma-ratio = 1.3	AUC = 4480 pmol h/mL
	brain/plasma-ratio = 1.36

[a] *n* = 3 Sprague-Dawley rats/group.

plasma concentration was reached quickly post-dose. A second increase in plasma concentration between 4 and 8 h was observed which points towards either a second absorption phase or entero-hepatic recirculation. Absolute oral bioavailability was estimated to 54%. After an iv bolus dose of 10 μmol/kg, the first and second elimination half-lives amounted to 0.3 h and 0.9 h ($t_{1/2,2}$: 55% of AUC), respectively. Compound **16m** was moderately cleared in rats and moderately distributed to tissues. After both oral and intravenous dosages, the penetration of **16m** into the brain was significant with brain/plasma AUC ratios of 1.30 and 1.36, respectively.

Given the good in vitro potency and pharmacokinetic profile in rats, **16m** was evaluated in three standard in vivo models for assessing anxiolytic-like activity. In stress-induced hyperthermia, exaggerated response of the autonomic nervous system to stress is recorded using body-temperature measurements in mice.[15] Oral administration of **16m** (1, 3, and 10 mg/kg, 1 h before T1) significantly reduced stress-induced hyperthermia at 3 and 10 mg/kg, while having no effect on baseline temperature (Fig. 3).

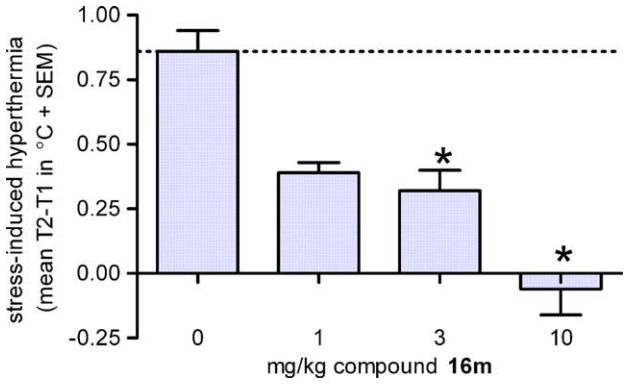

Figure 3. Stress-induced hyperthermia: individually housed mice (n = 14/group) were orally treated and, 1 h later, the initial temperature (T1) was determined via a rectal thermistor. Fifteen minutes later core-temperature were re-determined (T2) and SIH was defined as T2 − T1. Depicted are means (T2 − T1) and SEM. Statistics: Kruskal–Wallis ANOVA on ranks: H = 39.9, p <0.001; *p <0.05 (Dunn's post-hoc test vs 0 mg/kg).

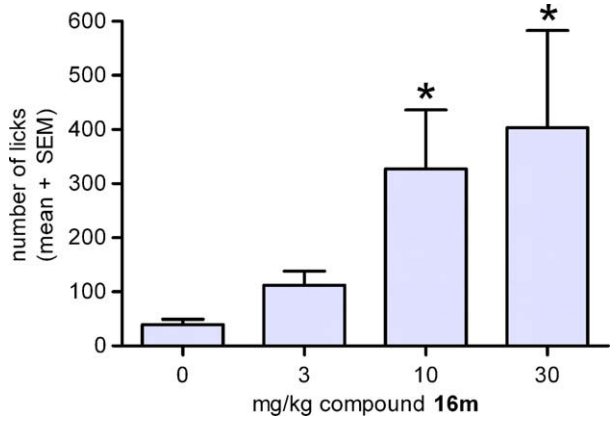

Figure 4. Vogel conflict test (punished drinking): after a 24 h water-deprivation, rats were re-exposed to the boxes and had free access to the drinking spout and then returned back to the home-cage where they had access to water for an additional 30 min period. After another 24 h period of water deprivation rats were orally treated (n = 12/group) and, 1 h later re-exposed to the boxes and now every lick was punished by a shock (0.5 mA; 0.6 s duration). The number of punished licks throughout this 10 min session was used as experimental parameter and is depicted in the figure. Statistics: Kruskal–Wallis ANOVA on ranks: H = 22.4, p <0.001; *p < 0.05 (Dunn's post-hoc test vs 0 mg/kg).

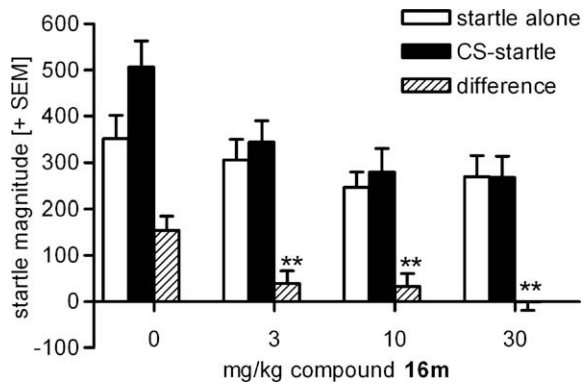

Figure 5. Expression of fear-potentiated and baseline startle response (3–30 mg/kg compound **16m**): rats were fear-conditioned by 20 pairings of a light stimulus (5 s) and an electric foot shock (0.5 mA, 0.5 s) on two successive days. On day three, animals were orally treated (n = 10/group) and, 1 h later, the startle magnitude to a noise burst was measured in the presence of the light stimulus as well as without (12 presentations each). Depicted are mean startle magnitudes and SEMs of startle stimulus alone and CS-startle stimulus trials, respectively, as well as the difference. Statistics: multifactorial ANOVA: interaction treatment × trial type: F3,36 = 5.69, p = 0.001; **p <0.01 (Dunnett post-hoc test vs 0 mg/kg).

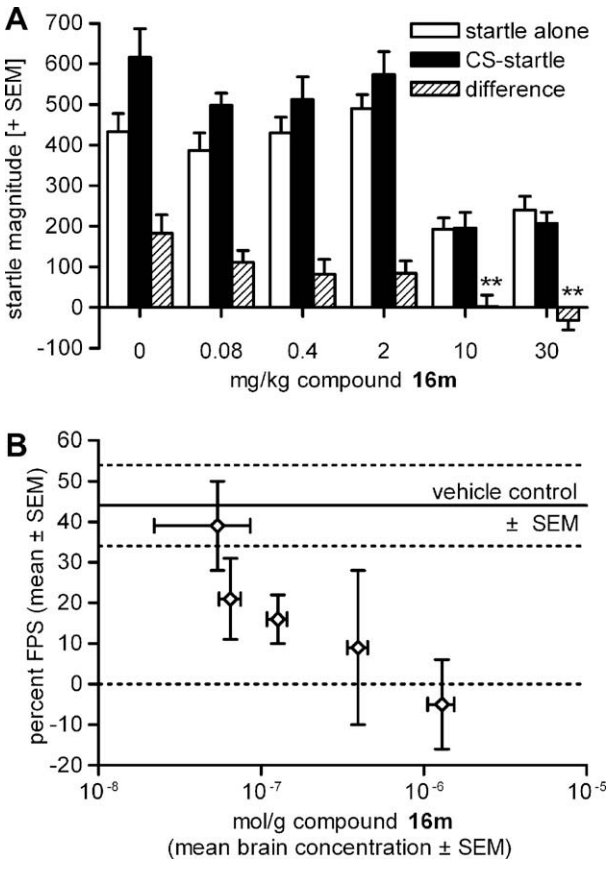

Figure 6. (A) Expression of fear-potentiated and baseline startle response (0.08–30 mg/kg compound **16m**), for methods see Fig. 5. Multifactorial ANOVA: interaction treatment x trial type: F5,54 = 5.50, p <0.001, p = 0.001; **p <0.01 (Dunnett post-hoc test vs 0 mg/kg). (B) Immediately after the behavioral test, the animals were sacrified and the brains were removed for HPLC analysis of compound × concentration in the brain. Depicted are the mean (±SEM) brain concentration of compound **16m** in the different treatment groups as well as the mean percent startle difference (±SEM). Compound **16m** brain levels are nicely correlated with the expression of fear-potentiated startle (sigmoid fit: r2=0.85).

Compound **16m** was further evaluated in the Vogel conflict test[16] in which drinking is punished in water-deprived rats by a mild electrical shock. Compound **16m** (3, 10, and 30 mg/kg, oral administration 1 h before test), significantly increased the number of punished licks at doses of 10 or 30 mg/kg but not at 3 mg/kg, (Fig. 4), suggesting a strong anxiolytic-like effect at the two doses.

In the fear-potentiated startle test (FPS), the effects of **16m** were evaluated on the expression of FPS in rats.[17] Vehicle-treated animals displayed a potentiated startle magnitude in the presence of the light stimulus which had previously been paired with an electric footshock, indicating the presence of conditioned fear under control conditions. Rats, following an oral pre-treatment (−1 h) at doses of 3, 10, and 30 mg/kg showed a significant and dose-dependent decrease of FPS. The baseline startle response was not modified at any dose tested (Fig. 5).

In conclusion, robust efficacy of **16m** in three different animal models of fear and anxiety was observed. We were further interested whether brain and plasma levels of compound **16m** can be correlated with its anxiolytic-like activity of compound **16m**. This was investigated in a further experiment using the FPS model (Fig. 6).

Analysis of plasma and brain concentrations of **16m** showed a good dose-proportionality from 0.4 to 30 mg/kg in plasma and brain. Furthermore, a clear relationship between plasma and brain levels and the behavioral effects could be observed. The half-maximal behavioral effect for **16m** could be observed at a dose of 2 mg/kg, corresponding to a brain concentration of 127 ± 56 pmol/g.

In conclusion, high-throughput screening led to the identification of **2** as a structurally novel mGlu5 receptor antagonist with modest in vitro potency. Optimization of the modular lead scaffold led to the discovery of **16m**, a compound showing high affinity at mGluR5 and selectivity over other glutamate receptors. Characterization in vivo revealed a good pharmacokinetic profile in rats, robust anxiolytic-like effects in three different animal models of fear and anxiety as well as a good PK/PD correlation. In view of the robust anxiolytic-like properties in different animal models, compound **16m** was considered for further development.

Acknowledgments

The excellent technical assistance of Christian Boesch, Hugo Bürki, Riccardo Canova, Stefan Imobersteg, David Kolarik, Nicole Reymann, Patrick Seitzer, Christine Stierlin, Engin Tasdelen, Roland Wermuth, Peter Wipfli, Francis Risser, Pierrette Guntz, and Valerie Cordier is gratefully acknowledged.

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