Synthesis and Pharmacological Evaluation of *N,N′*-Diarylguanidines as Potent Sodium Channel Blockers and Anticonvulsant Agents†

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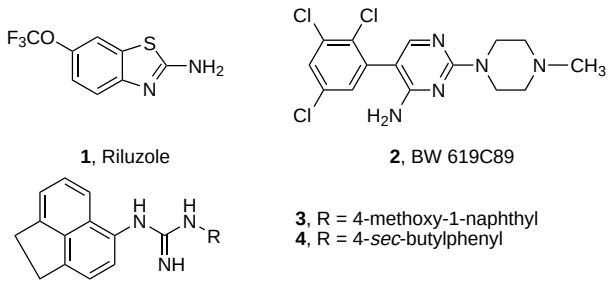
Synthesis and structure−activity relationships (SAR) are described for a series of *N,N′*-diarylguanidines related to *N*-acenaphth-5-yl-*N′*-(4-methoxynaphth-1-yl)guanidine (**3**) as anticonvulsants through blockade of sodium channels. SAR studies on compound **3** led to several simpler diphenylguanidines with improved in vitro and in vivo activity. Compounds were screened for blockade of sodium channels in a veratridine-induced [^{14}C]guanidinium influx assay (type IIA sodium channels) and for anticonvulsant activity in the audiogenic DBA/2 mouse model. Results indicated that *N,N′*-diphenylguanidines substituted with flexible and moderate size lipophilic groups were preferred over aryl and/or hydrophilic groups for biological activity. Among the compounds studied, *n*-butyl- and/or *n*-butoxy-containing guanidines showed superior biological activity. A possible relationship between in vitro and in vivo activity of this compound series and their measured/calculated lipophilicities was investigated. Compounds of this series showed only weak NMDA ion channel-blocking activity indicating that the anticonvulsant activity of these compounds is unlikely to be mediated by NMDA ion channels but, more likely, by acting at voltage-gated sodium channels.

Introduction

Voltage-dependent Na^+-channel blockers have been developed in the past as local anesthetics[1,2] and antiarrhythmic agents,[3] but in recent years these Na^+-channels have been recognized as targets for developing anticonvulsant drugs[4] (e.g., phenytoin, carbamazepine, lamotrigine) and neuroprotective agents[5,6] (e.g., lifarizine, lubeluzole) to treat acute ischemia and/or neurodegenerative conditions. Unlike other neuroprotective agents, Na^+-channel blockers prevent injury from hypoxia in isolated optic nerve, a model of ischemic white matter injury, while glutamate antagonists are only active in gray matter.[7−11] Since axons lack a significant number of glutamate receptors, ischemic damage in white matter has important mechanistic differences from damage in gray matter. It was also demonstrated that voltage-dependent Na^+-channel blockers can reduce neuropathic pain due to trigeminal neuralgia, diabetic neuropathy, postherpetic neuralgia, neuromas, and phantom limb syndrome.[12] Some recently developed voltage-gated Na^+-channel blockers with potential clinical utility include an aminothiazole derivative, riluzole (**1**), and an aminopyrimidine derivative, BW 619C89 (**2**), and its congeners (Chart 1).[13] We have previously reported[14] an acenaphthylguanidine derivative **(3)** as an effective inhibitor of voltage-gated sodium channels and a potential neuroprotective agent.

In this paper, the synthesis and systematic structure−activity studies (SAR) based on lead compound **3,** to generate compounds with improved Na^+-channel activity, are reported. The studies were focused on various para-substituted *N,N′*-diphenylguanidines as blockers of voltage-gated sodium channels and anticonvulsant agents. Compounds were screened for their inhibition of veratridine-induced [^{14}C]guanidinium flux in a CHO cell line expressing cloned type IIA sodium channels[15] and for anticonvulsant activity (in vivo) in the audiogenic DBA/2 mouse model.[16]

Chart 1



Chemistry

The symmetrical *N,N′*-diarylguanidines were prepared (Scheme 1) by the reaction of 2 equiv of aromatic amine with 1 equiv of cyanogen bromide in ethanol or toluene at reflux to produce guanidines in nearly quantitative yields.[17] The unsymmetrical guanidines were prepared by reacting arylcyanamides with corresponding aniline hydrochlorides in toluene or chlorobenzene at reflux.[17] The requisite arylcyanamides were prepared from anilines and cyanogen bromide in diethyl ether at low to room temperature.

Compound **3**, with an IC_{50} of 1.6 μM in the guanidine flux assay, showed only moderate in vivo activity in the audiogenic DBA/2 mouse model (see Table 2). Its weak NMDA ion channel activity (K_i = 2500 nM) indicated

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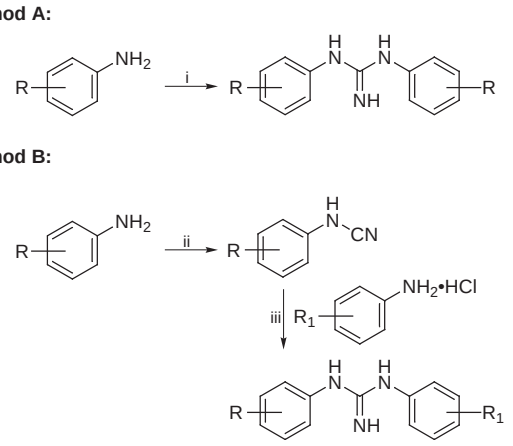
Scheme 1[a]



[a] (i) BrCN, ethanol or toluene, reflux; (ii) BrCN, ether, 0° C−rt; (iii) toluene, reflux.

that its sodium channel activity might be responsible for in vivo activity. Since NMDA ion channel blockers exhibit severe behavioral side effects that are different in nature from those described for voltage-gated ion channel blockers,[18] we explored SAR studies on compound **3** in order to generate molecules that show potent anticonvulsant activity in the audiogenic DBA/2 mouse model through blockade of neuronal sodium channels. In preliminary SAR studies[19] of compound **3**, we found that the 4-methoxy-1-naphthyl substituent can be replaced with certain substituted phenyl groups without adversely affecting in vitro activity. One of the typical compounds of this series, *N*-acenaphth-5-yl-*N*′-(4-*sec*-butylphenyl)guanidine **(4)**, was found to be more potent in the guanidinium flux assay than compound **3**. These results prompted us to investigate the SAR of para-substituted diphenylguanidines for sodium channel blockade and anticonvulsant activity.

Biological Testing

Compounds in this study were tested for their ability to block sodium channels as determined in a functional assay employing a Chinese hamster ovary (CHO) cell line expressing cloned type IIA sodium channels derived from rat brain.[20] The assay entails measuring percent block of [^{14}C]guanidinium flux through veratridine-stimulated, tetrodotoxin-sensitive sodium channels. The affinity of compounds to displace [^{3}H]MK-801 radioligand in rat brain membrane suspensions is reported as their K_i for the NMDA receptor ion channel sites.[21] The in vivo activity of compounds was measured as their effectiveness in preventing seizures induced by loud high-frequency sounds in the DBA/2 mouse.[16] Mice were injected intraperitoneally with compound solution or with vehicle control 30 min prior to being placed in a bell jar and initiating the auditory stimulus. Percentage inhibition of seizures was determined with reference to vehicle controls.

Biological Results and Discussion

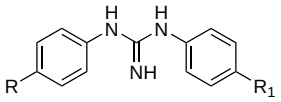
Since compound **3** was found to be a good sodium channel blocker with moderate anticonvulsant activity, we explored the replacement of the 4-methoxy-1-naphthyl group with substituted phenyl groups. The first compound of this series, the 4-*sec*-butylphenyl analogue (**4**), led to an increase in sodium channel activity with an IC_{50} of 0.68 μM in the guanidine flux assay compared to 1.64 μM for compound **3**. Subsequently we replaced the acenaphthyl substituent of compound **4** with another 4-*sec*-butylphenyl group to give a symmetrical guanidine (**8**) which showed improved anticonvulsant activity (69% inhibition @ 20 mg/kg) in the DBA/2 mouse model compared to compound **3** or **4**. These results led us to investigate in more detail the effects of para-substituent on *N*,*N*′-diphenylguanidines in in vitro and in vivo studies. The biological results of a number of diarylguanidines along with reference compounds (**1**−**3)** are reported in Table 2.

The simplest analogue, *N*,*N*′-bis(4-tolyl)guanidine (**5**), showed weak sodium channel activity (IC_{50}, 9.21 μM) compared to *sec*-butyl analogue **8**. The ethyl (**6**) and isopropyl (**7**) analogues showed improved sodium channel activity with IC_{50} values of 1.39 and 0.46 μM, respectively. The greater in vitro activity of the *n*-butyl analogue **10** compared with that of the *sec*-butyl analogue **8** and the *tert*-butyl analogue **9** (IC_{50} values of 0.13 vs 0.8 vs 1.54 μM, respectively) suggests that branching of alkyl chains may be disadvantageous. Similarly the anticonvulsant activity measured in the audiogenic mouse model was also increased as the size of the straight-chain alkyls was increased. However, the higher homologue of the *n*-butyl group was not favored as *N*,*N*′-bis(*n*-hexylphenyl)guanidine (**12**) showed decreased sodium channel activity (IC_{50}, 0.83 μM) compared with compound **10**. We replaced the flexible and more bulky *n*-hexyl group with a phenyl group to investigate the effect of planarity on biological activity. The *N*,*N*′-bis(4-biphenyl)guanidine (**13)** showed reduced sodium channel activity, and further, its poor solubility hampered testing in the anticonvulsant model. The phenoxy analogue **14,** prepared for potentially improved solubility, showed enhanced sodium channel activity compared to compound **13,** but weak in vivo activity compared to the *n*-hexyl analogue **12**, indicating a preference of flexible *n*-alkyl groups for in vivo activity. The mercaptophenyl analogue **15** was found to be similar to the phenoxy compound **14**, in vitro.

At this point, the most active compound of the series was *N*,*N*′-bis(*n*-butylphenyl)guanidine (**10**) with potent sodium channel-blocking activity and excellent anticonvulsant activity (85% of inhibition of seizures at a dose of 25 mg/kg). The *N*,*N*′-bis(*n*-butoxyphenyl)guanidine (**11**), investigated for the effect of H-bond acceptor oxygen atom, showed comparable in vivo activity to that of di-*n*-butyl analogue **10**.

We also studied unsymmetrical guanidines carrying a benzyloxyphenyl group (extended phenoxy analogue) on one nitrogen and an alkylphenyl group on another nitrogen of the guanidine. Compounds of this type, **16** and **17,** showed improved in vitro and in vivo activity compared to the phenoxy derivative **14**. These results further supported the preference for flexible alkyl or aralkyl groups over rigid phenyl or phenoxy groups. The moderate in vivo activity of these compounds compared to that of compound **10** also suggests that *n*-alkyl substituents are preferred over aralkyl groups. We also investigated the preference of lipophilic groups over hydrophilic substituents. *N*,*N*′-Bis(4-hydroxybutylphenyl)guanidine (**19**) showed very weak sodium channel

Table 1. Physical Data for the *N,N′*-Diphenylguanidine Analogues



compd[a]	R	R_1	mp,°C	% yield[b]	anal. C, H, N[c]
4[d]			*e*	50	$C_{23}H_{26}N_3{\cdot}CH_3SO_3H$
5	$-CH_3$	$-CH_3$	168−70	20	$C_{15}H_{17}N_3$
6	$-C_2H_5$	$-C_2H_5$	136−8	38	$C_{17}H_{21}N_3$
7	$-i$-C_3H_7	$-i$-C_3H_7	137−9	27	$C_{19}H_{25}N_3$
8	$-s$-C_4H_9	$-s$-C_4H_9	113−5	32	$C_{21}H_{29}N_3{\cdot}CH_3SO_3H{\cdot}3.0H_2O$[f]
9	$-t$-C_4H_9	$-t$-C_4H_9	160−2	40	$C_{21}H_{29}N_3{\cdot}0.1\ H_2O$
10	$-n$-C_4H_9	$-n$-C_4H_9	120−2	20	$C_{21}H_{29}N_3{\cdot}CH_3SO_3H$
11	$-n$-OC_4H_9	$-n$-OC_4H_9	88	27	$C_{21}H_{29}N_3O_2{\cdot}HBr{\cdot}1.0\ H_2O$[g]
12	$-n$-C_6H_{13}	$-n$-C_6H_{13}	90−2	68	$C_{25}H_{37}N_3{\cdot}CH_3SO_3H{\cdot}0.2\ H_2O$
13	$-C_6H_5$	$-C_6H_5$	254−5	8	$C_{25}H_{21}N_3{\cdot}HBr$
14	$-OC_6H_5$	$-OC_6H_5$	127−8	38	$C_{25}H_{21}N_3O_2{\cdot}HBr$
15	$-SC_6H_5$	$-SC_6H_5$	153	14	$C_{25}H_{21}N_3S_2{\cdot}HBr{\cdot}0.5\ H_2O$
16	$-OCH_2C_6H_5$	$-t$-C_4H_9	143−4	53	$C_{24}H_{27}N_3O{\cdot}HCl$
17	$-OCH_2C_6H_5$	$-n$-C_4H_9	112	44	$C_{24}H_{27}N_3O{\cdot}HCl$
18	$-n$-OC_4H_9	$-(CH_2)_4$-OH	108	67	$C_{21}H_{29}N_3O_2{\cdot}CH_3SO_3H{\cdot}1.0H_2O$[h]
19	$-(CH_2)_4$-OH	$-(CH_2)_4$-OH	143−4	79	$C_{21}H_{29}N_3O_2{\cdot}HBr{\cdot}0.5\ H_2O$

[a] All new compounds were analyzed for C, H, and N analyses or by high-resolution mass spectroscopy and HPLC. [b] Yields based on the purified material. [c] Compounds **5**−**7** are reported in ref 17. [d] Please see Chart 1 for structure. [e] Not crystalline. [f] H: calcd, 8.3; found, 7.25. [g] N: calcd, 9.25; found, 10.36. [h] H: calcd, 7.51; found, 6.92.

Table 2. Summary of Results from the in Vitro and in Vivo Studies[a]

compd	IC_{50} (μM) vs [^{14}C]guanidinium ion,[b] $IC_{50} \pm$ SEM (n)	K_i (nM) vs [^{3}H]MK-801[c] $IC_{50} \pm$ SEM (n)	% inhibition of seizure in DBA/2 mice[d] @ 20 mg/kg (ip)	log $D_{7.4}$[e]
1 (riluzole)	4.73 ± 1.79 (4)	nt	92	3.43*
2 (BW 619C89)	2.55 ± 0.05 (2)	nt	66	3.39 *
3	1.64 ± 0.40 (5)	2490 ± 1010 (2)	50 @ 40	2.70*
4	0.68 ± 0.28 (2)	nt	33	3.31
5	9.21 ± 1.00 (6)	10200 ± 2600 (4)	43	0.70*
6	1.39 ± 0.41 (3)	7910 ± 470 (4)	100	1.70*
7	0.46 ± 0.11 (2)	17900 ± 7600 (6)	85	2.72
8	0.8 ± 0.08 (3)	10100 ±1000 (2)	69	3.94*
9	1.54 ± 0.83 (2)	>7700 (2)	60	3.65
10	0.13 ± 0.03 (3)	3320 ± 160 (2)	82 @ 25	4.10*
11	0.19 ± 0.01 (2)	3480 ± 90 (2)	52	3.52
12	0.83 ± 0.20 (2)	7790 ± 1300 (2)	79	6.07
13	0.85 ± 0.11 (2)	nt	nt	3.13
14	0.43 ± 0.25 (6)	nt	21	3.55*
15	0.84 ± 0.17 (3)	nt	nt	4.18
16	0.11 ± 0.02 (2)	2190 ± 160 (2)	27	3.46*
17	0.14 ± 0.01 (2)	4010 ± 570 (2)	40	
18	1.78 ± 0.82 (4)	nt	11	1.72
19	8.19 ± 1.98 (4)	nt	0	0.047*

[a] Each biological experiment was run in triplicate. [b] See Blaustein, M. P.; et al. *J. Physiol.* **1985**, *361*, 251−258. [c] See ref 21. [d] See ref 16. [e] Measured at pH 7.4 by the shake-flask method (asterisk) or calculated from log k_w. nt, not tested.

activity (IC_{50}, 8.9 μM) and no anticonvulsant activity relative to compounds with lipophilic alkyl or alkoxy groups. The in vitro activity was increased by more than 4-fold when one of the hydroxybutyl groups of compound **19** was replaced with a more lipophilic butoxy (**18**) substituent. It was evident from these studies that polar H-bond donor hydroxyl groups on diphenylguanidines are detrimental for biological activity.

We have investigated the relationship of in vitro and in vivo activity of these compounds with their measured/calculated lipophilicities. Partition coefficient was measured for these compounds at physiological pH (octanol/buffer, log $D_{7.4}$) in order to probe the role of overall lipophilicity in binding to sodium channels and in transport to the site of action in the CNS as measured by anticonvulsant activity in the audiogenic DBA/2 mouse model. Values of log k_w (log k' extrapolated to 100% aqueous mobile phase)[22,23] obtained by reverse-phase HPLC at pH 7.4 were found to correlate well with log $D_{7.4}$ measured by the "shake-flask" method[24] for seven para-substituted diphenylguanidines. This relationship was then utilized to calculate log $D_{7.4}$ from log k_w for the remaining compounds in this structural class. In addition to reducing the time needed for the lipophilicity determinations, use of log k_w also permitted an estimation of the log D value for the extremely lipophilic compound **12**.

As can be seen in Table 2, there is no clear dependence of either in vivo activity or sodium channel blockade on lipophilicity for this set of compounds. This may be a consequence of over half of the compounds having log D values in a rather narrow range (3−4). There is also no correlation between in vivo activity and in vitro activity. The results for some individual compounds are of interest, however. Compound **18** has a very similar affinity to that of **6,** and both possess a log D near the value of 2 considered to be optimal for CNS activity.[25] The much weaker in vivo activity for

18 would therefore not be predicted on this basis, but the presence of a hydroxyl group in this compound would be expected to substantially increase H-bond donation, which has been reported to reduce brain uptake.[26] Compound **12** is highly potent in vivo and also highly lipophilic, suggesting that other mechanisms may contribute to its high antiseizure activity. One possibility is membrane activity, which is likely for cationic compounds with long alkyl side chains. Changes in membrane fluidity have been reported to be induced by some anticonvulsant drugs.[27−29] Additional parameters that could affect in vivo activity and may also be dependent on lipophilicity include solubility and protein binding in plasma, absorption, and clearance.

Conclusion

A series of *N*,*N*′-diarylguanidines were synthesized and studied for voltage-gated sodium channel blockade in the guanidinium flux assay and anticonvulsant activity in the audiogenic DBA/2 mouse model. Among the substituents studied on the diphenylguanidines, simple *n*-alkyl or *n*-alkoxy groups were favored over phenyl or hydroxyalkyl groups. The more potent compound in vitro that is also highly active in the in vivo model is *N*,*N*′-bis(*n*-butylphenyl)guanidine (**10**) with an IC_{50} of 0.13 μM in the guanidinium flux assay and 85% inhibition of seizures at 25 mg/kg in the audiogenic DBA/2 mouse model. All of the compounds of the series showed only weak NMDA receptor ion channel activity. It appears that this series of *N*,*N*′-diarylguanidines is active in the audiogenic DBA/2 mouse model partly through their blockade of neuronal sodium channels. An investigation into the relationship of in vitro and in vivo activity of this compound series with their measured/calculated lipophilicities suggested that other physicochemical parameters including electronic effects, p$K_{a/b}$, may also be relevant for in vivo activity. This compound series may generate agents of potential therapeutic utility for a range of neurodegenerative disorders.

Experimental Section

Chemistry. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Thin-layer chromatography was performed on Merck silica gel 60 F_{254} (0.2 mm) or Baker-flex 1B2-F silica gel plates. Guanidines were visualized on TLC with 254-nm UV light or as a blue spot with bromcresol spray reagent (Sigma Chemical Co.). Preparative TLC was performed on Analtech GF precoated silica gel (1000 μM) glass-backed plates (20 × 20 cm). The IR and NMR spectra of all compounds were consistent with their assigned structures. NMR spectra were recorded on a General Electric QE-300 spectrometer, and the chemical shifts are reported in ppm (δ) relative to the residual signal of the deuterated solvent ($CHCl_3$, δ 7.26; CHD_2OD, δ 3.30). Infrared spectra were recorded in $CHCl_3$ (unless otherwise noted) on a Nicolet 5DXB FT-IR or Perkin-Elmer model 1420 spectrometer. All new compounds were analyzed either for C, H, and N elemental analyses or for exact mass. Elemental analyses were performed by either Desert Analytics (Tucson, AZ) or Galbraith Laboratories (Knoxville, TN). High-resolution mass spectra (HRMS) were recorded on a Finnegan MAT 90 instrument. HPLC analyses were performed on a C18 reverse-phase column using 50:50 water/acetonitrile with 0.1% TFA as the mobile phase.

The general experimental details for the synthesis of symmetrical and unsymmetrical *N*,*N*′-diarylguanidines have been described previously.[17]

Determination of log $D_{7.4}$ Values. Solutions of compounds at a concentration of 100 μg/mL in methanol were injected (20 μL) onto a Phase Sep Spherisorb C-8 column (3 μM, 15 cm × 4.6 mm) and eluted with varying percentages of MeOH (60−90%, 3−4 concentrations/compound) in 15 mM HEPES/0.15% triethylammonium phosphate buffer at pH 7.4 with a flow rate of 1 mL/min (at ambient temperature, with detection at 220 nm). Values of log k' were obtained from log $k' = \log[(t - t_o)/t_o]$, where t is the retention time of the compound and t_o is that of MeOH. Linear extrapolation of log k' to 0% MeOH yielded values of log k_w.

Values of log $D_{7.4}$ were also measured for representative compounds using the "shake-flask" method (in octanol/15 mM Hepes/135 mM NaCl at pH 7.4). Using the regression equation $\log D_{7.4} = 1.22 \log k_w - 0.51$ ($r = 0.993$) derived from these data, log $D_{7.4}$ values were then calculated from log k_w for the remaining para-substituted diphenylguanidines.

Biological Methods. Veratridine-Induced [^{14}C]Guanidine Flux Assay: CNaIIA-I cells were obtained from the Catterall Lab (University of Washington, Seattle, WA). The cells were plated at 3×10^6 cells/96-well plate up to passage 20 and fed 24 h before use.

Compounds were dissolved in DMSO and MeOH and diluted for dose−response curves from 50 to 0.006 μM in preincubation buffer (5.4 mM KCl, 0.8 mM $MgSO_4$, 50 mM HEPES, 130 mM choline chloride, 0.1 mg/mL BSA, 1.0 mM guanidine-HCl, and 5.5 mM D-glucose). Veratridine was dissolved at 400 mM in MeOH and diluted in preincubation buffer (final assay concentration of 200 μM). [^{14}C]Guanidine was also prepared in preincubation buffer (final assay concentration 0.125 μCi/well).

Cultures are rinsed with and allowed to equilibrate in preincubation buffer for at least 10 min before flux was initiated with the addition of drugs, veratridine, and [^{14}C]-guanidine and incubated for 1 h at room temperature. Flux was terminated by rinsing with ice-cold wash buffer (163 mM choline chloride, 0.8 mM $MgSO_4$, 1.8 mM $CaCl_2$, 5.0 mM HEPES, and 1.0 mg/mL BSA). The plates are then aspirated dry, 100 μL of Optiphase "HiSafe" (Wallac) scintillation fluid was added per well, and the plates were sealed and counted on a Wallac Microbeta 1450 scintillation counter. IC_{50} values were calculated using nonlinear regression.

Audiogenic DBA/2 Mouse Model: Testing Procedure— Mice (Jackson Labs, ME; weight range 6.5−12 g, 20−23 days of age) were placed individually in a glass jar (25 cm i.d.) and exposed to pure tone sound of 12 kHz and 120 dB for 45 s. Animals were injected ip with the drug or vehicle (0.3 M mannitol, in a volume of 10 mL/kg of body weight) 30 min prior to exposure to the sound, unless otherwise noted. All experiments were done in a fully blinded fashion and took place between 11 a.m. and 5 p.m., and % response inhibition = (MRS control − MRS treatment)/MRS control × 100. Mean response scores (MRS) were calculated as the average seizure score of a test group of mice on our scale from 0 to 4. The Jonckheere nonparametric trend test, one-sided, was used to determine the lowest effective dose in dose−response studies. Otherwise, the Kruskal−Wallis nonparametric test was used, with Dunn's post-hoc test.

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