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Synthesis and Antimicrobial Activity of Novel Quinazolone Derivatives

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Received February 14, 1983, from the **Pharmaceutical Chemistry Department, Faculty of Pharmacy, University of Alexandria, Egypt* and the ‡*College of Pharmacy, Florida A&M University, Tallahassee, FL 32307.* Accepted for publication May 11, 1983.

Abstract □ Three novel series of 4-oxoquinazoline derivatives were prepared and evaluated as potential antimicrobial agents. Evaluation of the antimicrobial activity of a variety of 4-substituted-1-thiosemicarbazides, 3,4-disubstituted thiazolines, and 3-substituted-5-thiazolidones reveals that the majority possess significant *in vitro* activity against Gram-positive organisms. Some derivatives also exhibited antifungal activity.

Keyphrases □ 4-Oxoquinazoline analogues—synthesis, antimicrobial activity, *in vitro* screen □ Antimicrobial agents—potential, 4-oxoquinazoline analogues, synthesis

There has been considerable interest in various 4-oxoquinazoline analogues. Some have shown a wide spectrum of biological effects (1–5) including antitubercular (6), antibacterial (7), and antifungal (8) activities. Thiazoline analogues were also reported to exhibit antitubercular (9) and antibacterial (10) activities. These observations prompted us to synthesize a variety of compounds containing the thiazoline nucleus and examine them for antimicrobial activity (11). Recently, the antimicrobial activities of some 4-oxoquinazoline thiosemicarbazides and thiazoline derivatives were investigated (12).

In a continuing effort to develop new antimicrobial agents, 1- [4- (2-methyl-4-oxoquinazoline-3-yl)benzoyl] -4-alkyl-, -aryl-, and -aralkyl-3-thiosemicarbazides (VII–X), 3,4-disubstituted thiazoline-2-oxo[4-(2-methyl-4-oxoquinazoline-3-yl)benzoyl]hydrazones (XI–XIV), and 3-substituted 5-thiazolidone-2-oxo-[4-(2-methyl-4-oxoquinazoline-3-yl)-benzoyl]hydrazones (XV–XVIII) were synthesized and evaluated as potential antimicrobial agents.

RESULTS AND DISCUSSION

Chemistry—Treatment of 2-substituted-4-oxoquinazolines, with hydrazine hydrate results in hydrazinolysis of the quinazolone ring to give 3-amino-2-substituted-4-oxoquinazolines (13). In the present investigation, as shown in Scheme I, heating 3-(4-carbethoxyphenyl)-2-methyl-4-oxoquinazolone (I) with hydrazine hydrate resulted in the formation of three products: *p*-aminobenzoic acid hydrazide (II), 4-(2-methyl-4-oxoquinazoline-3-yl)benzoic acid hydrazide (III) (as a minor product), and 3-amino-2-methyl-4-oxoquinazoline (IV). Compounds II and IV were identified by TLC, IR, and mixed melting point comparisons with authentic samples prepared by the previously reported methods (14, 15), while III was identified by IR spectrometry and elemental analysis. The formation of these three different products might indicate that hydrazine attacks first the ester side chain of I, then the quinazolone nucleus. This appears to be true since the treatment of III with hydrazine, resulted in the formation of II and IV. More evidence was obtained when we found that the hydrazine hydrate can replace substituted hydrazine from the quinazolone nucleus. This was shown by the hydrazinolysis of 3-(4-carbethoxyphenylamino)-2-methyl-4-oxoquinazoline (V) into IV and *p*-hydrazinobenzoic acid hydrazide (VI) (Scheme I).

In the present work, the intermediate 4-(2-methyl-4-oxoquinazoline-3-yl)benzoic acid hydrazide (III) was prepared in a higher yield, under carefully controlled reaction conditions by treating hydrazine hydrate with I at room temperature for 1 h. As outlined in Scheme II, treatment of III with the selected alkyl-, aryl-, or aralkylisothiocyanate in refluxing ethanol afforded the desired thiosemicarbazides (VII–X) (Table I). Reaction of the formed thiosemicarbazides with phenacyl bromide or ethyl bromoacetate gave the thiazoline (XI–XIV) or thiazolidone (XV–XVIII) derivatives, as shown in Table II and Table III, respectively. The products were identified by elemental

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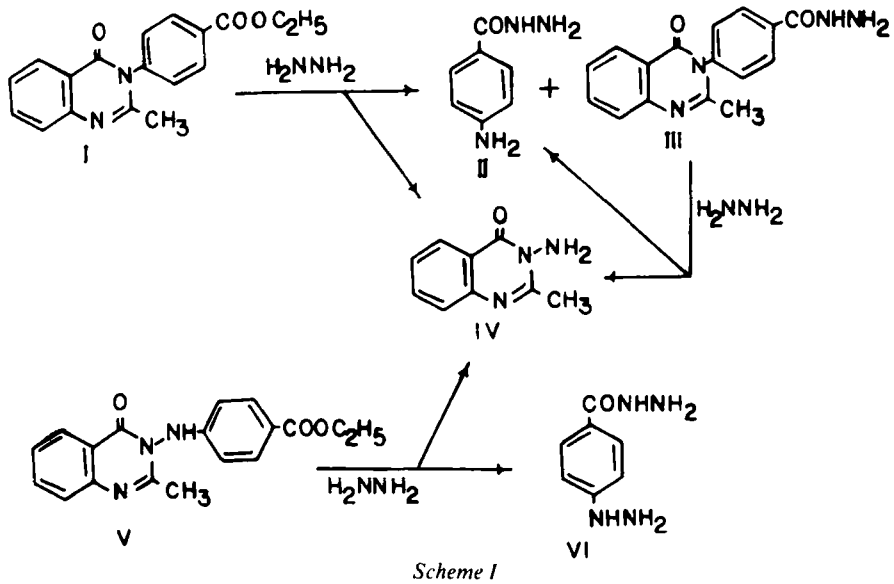


Scheme I

analyses, IR, and for some representative examples by H^1-NMR and MS. The H^1-NMR spectrum for thiazoline derivative XIV showed a singlet at δ 5.7 ppm characteristic for the thiazoline proton, while the spectrum for XVIII showed a singlet at δ 3.85 ppm characteristic of the thiazolidone protons. The mass spectra of both derivatives XIV and XVIII showed molecular ion, at *m/z* 543 and 483, respectively, and a base peak at *m/z* 91 corresponding to the tropylium ion.

Antimicrobial Activity—Compounds VII–XVIII were tested for antimicrobial activity by the agar diffusion method (16). The test organisms used were *Staphylococcus aureus* NCTC 4163, *Escherichia coli* NCTC 5933, and *Candida albicans* 3501[1]. The preliminary results obtained (Tables I–III) indicate that most of the tested compounds possess considerable antimicrobial activity against Gram-positive bacteria (*S. aureus*) and showed no activity against Gram-negative bacteria (*E. coli*). In addition, many of the tested compounds showed inhibitory activity against *C. albicans*.

EXPERIMENTAL SECTION[2]

3-(4-Carbethoxyphenyl)-2-methyl-4-oxoquinazoline (I) and 3-(4-Carbethoxyphenylamino)-2-methyl-4-oxoquinazoline (V)—Compounds I and V were prepared according to a previously reported method (17); I (80% yield), mp 169–170°C [lit. (17) mp 169–170°C]; V (72% yield) mp 149–150°C [lit. (17) mp 149–150°C].

Reaction of Hydrazine Hydrate with 2-(4-Carbethoxyphenyl)-2-methyl-4-oxoquinazoline (I)—A mixture of hydrazine hydrate (5 mL) and I (0.92 g, 3 mmol) in ethanol (15 mL) was refluxed for 30 min, diluted with water, and extracted with ethyl acetate. The organic phase was washed with water then dried over anhydrous sodium sulfate. The residue obtained after evaporation of the solvent to dryness, showed three spots on TLC. This mixture was chromatographed on a silica gel column [chloroform–ethanol (9:1)]. The first fraction afforded 3-amino-2-methyl-4-oxoquinazoline (IV) as white needle crystals from aqueous ethanol in 53% yield mp 149–151°C [lit. (15) mp 151–152°C]. The second fraction furnished *p*-aminobenzoic acid hydrazide (II) as white needles from ethanol in 35% yield, mp 221–223°C [lit. (14) mp 222°C]. The third fraction gave 4-(2-methyl-4-oxoquinazoline-3-yl)benzoic acid hydrazide (III) as white prisms from chloroform–benzene (10% yield), mp 220–221°C; IR: 3380–3350 (NH), 1670, and 1650 cm^{-1} (C=O).

Anal.—Calc. for $C_{16}H_{14}N_4O_2$: C, 65.31; H, 4.76; N, 19.05. Found: C, 65.20; H, 4.80; N, 19.30.

Reaction of Hydrazine Hydrate with III—A mixture of III (0.29 g, 0.001 mol) and hydrazine hydrate (2 mL) in ethanol (5 mL) was heated under reflux for 30 min, diluted with water, and extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous sodium sulfate, and the solvent was evaporated to dryness. The residue was chromatographed on silica gel [chloroform–ethanol (9:1)]. The initial fraction afforded IV, while the later fractions afforded II, identical in all respects with the aforementioned specimens.

Reaction of Hydrazine Hydrate with 3-(4-Carbethoxyphenylamino)-2-methyl-4-oxoquinazoline (V)—A mixture of V (0.32 g, 0.001 mol), hydrazine hydrate (2 mL), and ethanol (10 mL) was refluxed for 30 min. The mixture was treated in a manner similar to that above. The initial fraction afforded IV as white needles from aqueous ethanol (65% yield), mp 150–152°C [lit. (15) 151–152°C]. The later eluate furnished *p*-hydrazinobenzoic acid hydrazide (VI) as white needles from methanol (30% yield), mp 163–165°C [lit. (18) mp 165°C].

4-(2-Methyl-4-oxoquinazoline-3-yl)benzoic Acid Hydrazide (III)—Hydrazine hydrate (5 mL) was added to a solution of 3-(4-carbethoxyphenyl)-2-methyl-4-oxoquinazoline (I) (0.92 g, 3 mmol) in benzene (9 mL) and ethanol (1 mL). The mixture was stirred for 1 h at room temperature, then diluted with water. The remaining ester I was removed by extraction with benzene. The aqueous layer was then adjusted pH 6 with HCl and then extracted with chloroform. The extract was washed with water, dried over anhydrous sodium sulfate, and the solvent was evaporated *in vacuo* at room temperature. The residue was crystallized from chloroform–benzene to give III as white prisms; (33% yield), mp 220–221°C; IR: 3380–3350 (NH), 1670, and 1650 cm^{-1} (C=O).

Anal.—Calc. for $C_{16}H_{14}N_4O_2$: C, 65.31; H, 4.76; N, 19.05. Found: C, 65.20; H, 4.80; N, 19.30.

1-[4-(2-Methyl-4-oxoquinazoline-3-yl)benzoyl]-4-alkyl-, aryl-, or -aralkyl-3-thiosemicarbazides (VII–X)—A solution of equimolecular amounts of an alkyl-, aryl-, or aralkylisothiocyanate (2 mmol) and 4-(2-methyl-4-oxoquinazoline-3-yl)benzoic acid hydrazide (III) (0.58 g, 2 mmol) in ethanol (20 mL) was heated under reflux for 15 min. The mixture was concentrated, cooled, and left overnight at room temperature. The precipitate was removed by filtration and recrystallized from ethanol (only VII was recrystallized from aqueous ethanol). The yields and physical constants of the products are listed in Table I; IR: 3270–3140 (NH), 1700–1670 (C=O and C=N), and 1550–1520, 1340–1330, 1070–1050, and 870–830 cm^{-1} [—N—C=S I, II, III, and IV mixed-vibration bands, respectively (19)].

3,4-Disubstituted Thiazoline-2-oxo[4-(2-methyl-4-oxoquinazoline-3-yl)-benzoyl]hydrazones (XI–XIV)—A mixture of the appropriate thiosemicarbazides (VII–X) (0.01 mol), phenacyl bromide (0.01 mol), and anhydrous sodium acetate (0.04 mol) in absolute ethanol (40 mL) was refluxed for 2 h.

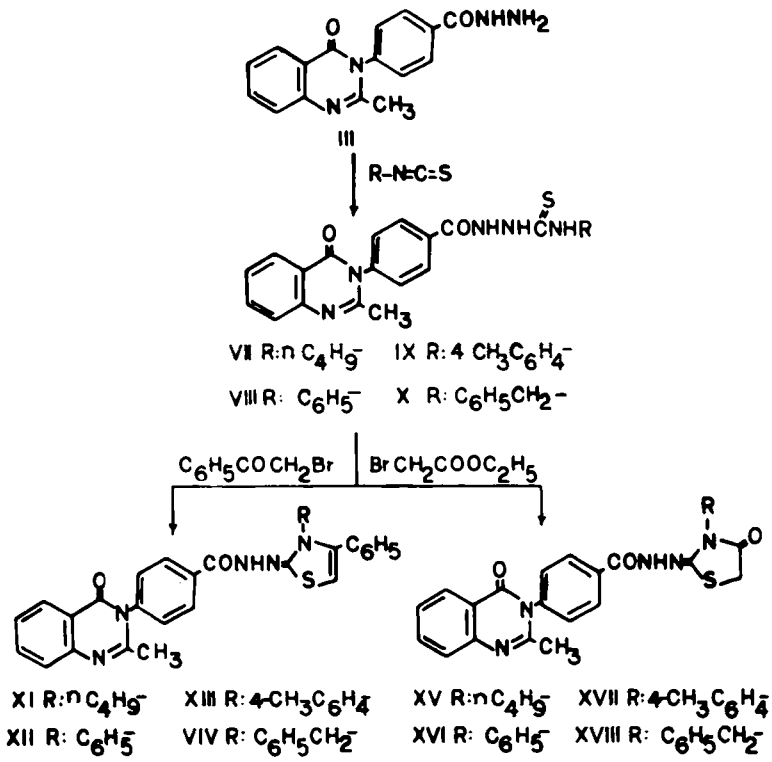
Table I—Properties and Antimicrobial Activity of 1-[4-(2-Methyl-4-oxoquinazoline-3-yl)benzoyl]-4-alkyl-, -aryl- or -aralkyl-3-thiosemicarbazides[a]

Compound	Yield, %	mp, °C	Molecular Formula	Antimicrobial Activity[b] *S. aureus*	*E. coli*	*C. albicans*
VII	70	141–142	$C_{21}H_{23}N_5O_2S$[c]	11	(–)	11
VIII	85	214–215	$C_{23}H_{19}N_5O_2S$	11	(–)	13
IX	85	225–226	$C_{24}H_{21}N_5O_2S$	12	(–)	14
X	75	145–146	$C_{24}H_{21}N_5O_2S$	11	(–)	12

[a] Compounds VII–X were analyzed for C, H, N, and S; unless otherwise noted, all results were within ±0.4% of the theoretical values. [b] Expressed in terms of *in vitro* inhibition zones in millimeters. [c] Calc. for S, 7.82; found, 7.40.

[1] The standard strain was obtained from the Institut of Microbiology, Göttingen, West Germany.

[2] All melting points were determined in open glass capillary tubes and are reported uncorrected. IR spectra were measured in Nujol mulls on a Beckman 4210 spectrophotometer. H^1-NMR spectra were obtained on a Bruker HX-270, and the chemical shift was expressed in ppm downfield from an internal tetramethylsilane standard. Mass spectra were recorded on a Finnigan 4510 GCMS at an electron energy of 70 eV.



Scheme II

Table II—Properties and Antimicrobial Activity of 3,4-Disubstituted Thiazoline-2-oxo[4-(2-methyl-4-oxoquinazoline-3-yl)benzoyl]hydrazones[a]

Compound	Yield, %	Recrystallization Solvent	mp, °C	Molecular Formula	Antimicrobial Activity[b]		
					S. aureus	*E. coli*	*C. albicans*
XI	57	EtOH-H_2O	186-187	$C_{29}H_{27}N_5O_2S$	17	(−)	11
XII	64	$CHCl_3$-pet.ether	260-261	$C_{31}H_{23}N_5O_2S$	11	(−)	11
XIII	51	EtOH-H_2O	287-288	$C_{32}H_{25}N_5O_2S$	11	(−)	(−)
XIV	52	EtOH	120-121	$C_{32}H_{25}N_5O_2S$	13	(−)	10

[a] Compounds XI-XIV were analyzed for C, H, N, and S; all results were within ±0.4% of the theoretical values. [b] Expressed in terms of *in vitro* inhibition zones in millimeters.

Table III—Properties and Antimicrobial Activity of 3-Substituted 5-Thiazolidone-2-oxo[4-(2-methyl-4-oxoquinazoline-3-yl)benzoyl]hydrazones[a]

Compound	Yield, %	Recrystallization Solvent	mp, °C	Molecular Formula	Antimicrobial Activity[b]		
					S. aureus	*E. coli*	*C. albicans*
XV	91	EtOH-H_2O	96-7	$C_{23}H_{23}N_5O_3S$	12	(−)	10
XVI	87	$CHCl_3$-benzene	178-9	$C_{25}H_{19}N_5O_3S$	18	(−)	14
XVII	92	$CHCl_3$-pet.ether	255-6	$C_{26}H_{21}N_5O_3S$[c]	11	(−)	(−)
XVIII	91	benzene	139-40	$C_{26}H_{21}N_5O_3S$	15	(−)	10

[a] Compounds XV-XVIII were analyzed for C, H, N, and S; unless otherwise noted, all results were within ±0.4% of the theoretical values. [b] Expressed in terms of *in vitro* inhibition zones in millimeters. [c] Calc. for N, 14.49; found, 16.00.

The mixture was cooled, diluted with water, and allowed to stand. The solid material was removed by filtration and recrystallized from the appropriate solvent. The yields and physical constants of the products are summarized in Table II; IR: 3300-3200 (NH), 1680-1650 (C=O), 1543-1530 (NH), and 1330-1300 cm^{-1} (C—N); H^1-NMR for XIV ($CDCl_3$): δ 2.24 (s, 3, CH_3), 4.6 (s, 2, benzyl), 5.75 (s, 1, thiazoline proton), 7.39, 7.75, and 8.1 (3m, 19, ArH), and 8.3 ppm (br s, 1, N—H); MS for XIV, *m/z* (relative intensities): M^+ at 543 (0.06), 500 (0.02), 409 (38), 326 (0.29), 279 (2), 263 (12), 143 (24), 131 (2), 105 (13), 91 (100), and 76 (8).

2-Substituted 5-Thiazolidone-2-oxo[4-(2-methyl-4-oxoquinazoline-3-yl)-benzoyl]hydrazones (XV-XVIII)—These compounds were prepared by applying the same molar amounts and solvent volumes as in the preparation of XI-XIV, but using ethyl bromoacetate instead of phenacyl bromide. The yields and physical data of the products are recorded in Table III; IR: 3250-3200 (NH), 1740-1720 (C=O of thiazolidone), 1680-1650 (C=O), 1543-1530 (NH), and 1370-1320 cm^{-1} (C—N); H^1-NMR for XVIII ($CDCl_3$): δ 2.25 (s, 3, CH_3), 3.85 (s. 2, thiazolidone protons), 5.0 (s, 2 benzyl), 7.33, 7.76, and 8.05 (3 m, 13, ArH), and 8.49 ppm (br s, 1, N—H); MS for XVIII, *m/z* (%): M^+ at 483 (13), 409 (7), 279 (4), 263 (59), 236 (4), 143 (50), 121 (6), 116 (4), 105 (3), 91 (100), and 76 (14).

Antimicrobial Testing—Antimicrobial testing was performed by the agar diffusion method (16). The prepared compounds were dissolved in propylene glycol (2 mg/mL). Sterile nutrient agar (oxoid) was incubated with the test organisms (*S. aureus*, *E. coli*, and *C. albicans*). Each 100 mL of the medium received 1 mL of 24-hr broth culture, and 3 drops of the tested compound were placed separately in cups (8-mm diameter) cut in the agar. The plates were incubated at 37°C for 24 h and the resulting inhibition zones were measured (Tables I-III).

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Determination of Acetazolamide in Biological Fluids by Reverse-Phase High-Performance Liquid Chromatography

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Received February 24, 1983, from the **School of Pharmacy, University of Connecticut, Storrs, CT 06268* and the *‡Clinical Pharmacokinetics Laboratory, University of Connecticut Health Center, Farmington, CT 06032.* Accepted for publication May 23, 1983.

Abstract □ A high-performance liquid chromatographic method for the determination of acetazolamide in whole blood, plasma, and urine was developed. Samples of biological fluids containing various concentrations of acetazolamide were spiked with the internal standard, sulfadiazine. Samples were then mixed with a 50% ammonium sulfamate solution. Whole blood samples were heated for 25 s in boiling water. All samples were extracted with ethyl acetate; a phosphate buffer (pH 8.0) was used to wash the extracts. Acetazolamide was back-extracted into a glycine buffer (pH 10.0), which was then washed with ether. Separation of acetazolamide and internal standard from other biological constituents was achieved on a 10-μm C_{18} reverse-phase column using an acetonitrile–methanol–acetate buffer (pH 4.0). The eluant was monitored at 254 nm. All calibration curves were linear, and the results from reproducibility studies were excellent. Application of the method to human pharmacokinetic studies was demonstrated.

Keyphrases □ Acetazolamide—biological fluids, reverse-phase HPLC, pharmacokinetics □ HPLC-acetazolamide, biological fluids, pharmacokinetics

Acetazolamide, a carbonic anhydrase inhibitor widely used in the medical management of glaucoma, lowers intraocular pressure by reducing the rate of aqueous humor formation. Acetazolamide is also used as an antiepileptic drug, where its effect is thought to result from inhibition of brain carbonic anhydrase. There is evidence that effective intraocular hypotensive and anticonvulsant actions are seen at plasma concentrations of 5–20 and 10 μg/mL, respectively (1–4). The drug is concentrated in erythrocytes, a site of action which has received little attention in the clinical literature. In humans, high erythrocyte levels have recently been associated with significant toxicity (4, 5). This is attributed to carbon dioxide retention resulting from inhibition of erythrocyte carbonic anhydrase, an important enzyme that greatly facilitates carbon dioxide exchange and transport in the capillary beds (6). Acetazolamide is completely eliminated unchanged *via* the kidney; determination of its clearance by this organ may prove useful for adjusting acetazolamide plasma concentrations.

Several high-performance liquid chromatographic (HPLC) assays have been published for determining acetazolamide levels in plasma or serum (7–9), and they have been an improvement on the older, less sensitive enzymatic assays (10, 11). A sensitive GC method is available, but it requires an electron-capture detector (12). Some of the recent HPLC procedures require repeated and/or successive extractions and dry-down procedures. None of the HPLC methods can quantitate acetazolamide in whole blood [erythrocytes indirectly (12)] or urine, fluids from which clinically important information may be ascertained.

The present report describes an HPLC method that (*a*) obviates time-consuming solvent evaporation steps, (*b*) displays excellent sensitivity and reproducibility, and (*c*) can be applied to several different biological fluids.

EXPERIMENTAL SECTION

Reagents and Materials—Acetazolamide[1] and sulfadiazine[2] were used as supplied. The solvents used for extraction and chromatography were all HPLC grade[3]. All chemicals were analytical reagent grade[4].

Standards—Separate stock solutions containing 1.0 mg/mL of acetazolamide and sulfadiazine (internal standard) were prepared weekly by dissolving these agents in 0.005 and 0.01 M NaOH, respectively. Aqueous solutions of lower concentrations (*i.e.*, 0.01–0.5 mg/mL) were prepared extemporaneously as needed from the stock solutions. Buffer solutions of 0.032 M glycine (pH 10.0) and 0.1 M phosphate buffer (pH 8.0) were prepared monthly. All solutions were stored at 4°C when not in use.

Extraction—Urine and heparinized whole blood were obtained from drug-free, normal, human volunteers. Plasma was obtained from an aliquot of whole blood using heparin as anticoagulant. Two hundred-microliter aliquots of plasma, whole blood, or urine (diluted 1:10) were added to 13 × 100-mm borosilicate glass tubes.

[1] Sigma Chemical Co., St. Louis, Mo.
[2] ICN Pharmaceuticals Inc., Cleveland, Ohio.
[3] Burdick and Jackson, Muskegon, Mich.
[4] Fisher Scientific, Fair Lawn, N.J.

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