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Synthesis and anticonvulsant activity of some 1,4-dihydropyridine derivatives

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A series of asymmetrical 4-alkyl/aryl-2,6-dimethyl-3-N-(aryl/heteroaryl)-carbamoyl-5-ethoxycarbonyl-1, 4-dihydropyridines **3a-d** and symmetrical 4-alkyl/aryl-2,6-dimethyl-3,5-bis-(ethoxycarbonyl)-1,4-dihydropyridines **4a** and **4b** have been prepared by the condensation of various benzaldehydes, ethylacetoacetate, 2-aminopyridine or *p*-toludine in ethanol (Hantzch method). The structures of all the synthesized 1,4-dihydropyridine derivatives have been confirmed by spectral data (IR, ^{1}H NMR) and elemental analysis. Compounds **3a-c**, **4a** and **4b** (10 mg/kg) have been evaluated for their anticonvulsant effect against pentylenetetrazole- induced convulsions with phenytoin (4 mg/kg) as the standard. The anticonvulsant potential of the newly synthesized compounds have been assessed on the basis of increase in latency (onset time) to induce convulsions; decrease in number of convulsions and increase in latency of death compared to control and standard.

Keywords: 1,4-Dihydropyridine, Hantzsch method, pentylenetetrazole, anticonvulsant, synthesis

Convulsion is where the body muscles contract and unwind quickly and over and again, bringing about a wild shaking of the body[1]. In 1950's Bromide was introduced as first true antiepileptic drug (AED). The usage of Bromide has decreased in twentieth century when Phenobarbitone was accidentally discovered to be effective in suppressing seizures. Due to the side effects, toxicity and teratogenic effects of current antiepileptic drugs in the treatment of epilepsy, calcium channel blockers as antiepileptic agents have recently been considered[2]. There are considerable evidences that calcium is an important factor for the induction of epilepsy. Specifically, interesting seizure-instigating administrators or frameworks cause a quick intraneuronal union of calcium particles[3]. In particular, unique seizure-inciting operators or systems cause a fast intraneuronal convergence of calcium particles, which is easily identified with the ensuing epileptiform movement[4]. Conversely, calcium channel inhibitors (1,4-dihydopyridines) are effective against the whole range of convulsive procedures including electro, pentylene tetrazole, sound and pressure-induced seizures. Nifedipine and other dihydropyridine derivatives such as nimodipine, nitradipine, and nisoldipine (Figure 1) are potent blockers of the calcium channels of smooth muscles and also bind with high affinity to the brain membranes, hence can be employed as antiepileptic agents[5-8]. Considering the anticonvulsant potential of 1,4-dihydropyridines and in continuation to our work[9-14] on this scaffold herein we report the synthesis and anticonvulsant activity of 4-alky/aryl-2,6-dimethyl-3-N-(aryl/heteroaryl)-carbomo yl-5-ethoxycarbonyl-1,4-dihydropyridines **3a-d** and 4-alkyl/aryl-2,6-dimethyl-3,5-bis-(ethoxycarbonyl)-1,4-dihydropyridines **4a** and **4b** (Scheme I).

Results and Discussion

N-(aryl/heteroaryl)acetoacetamide **2** was synthesized from the reaction of *p*-toludine/2- aminopyridine and ethylacetoacetate **1** using conventional and microwave irradiation methods. In both the methods there was an increase in yield with increase in concentration of ethylacetoacetate up to 1:1.8 (*p*-toludine/ 2-aminopyridine: ethylacetoacetate), beyond which it decreased. Hence this ratio where highest yield was

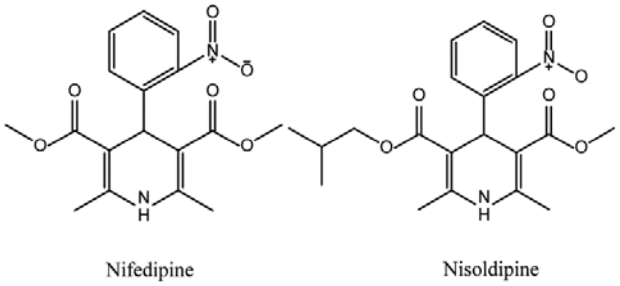
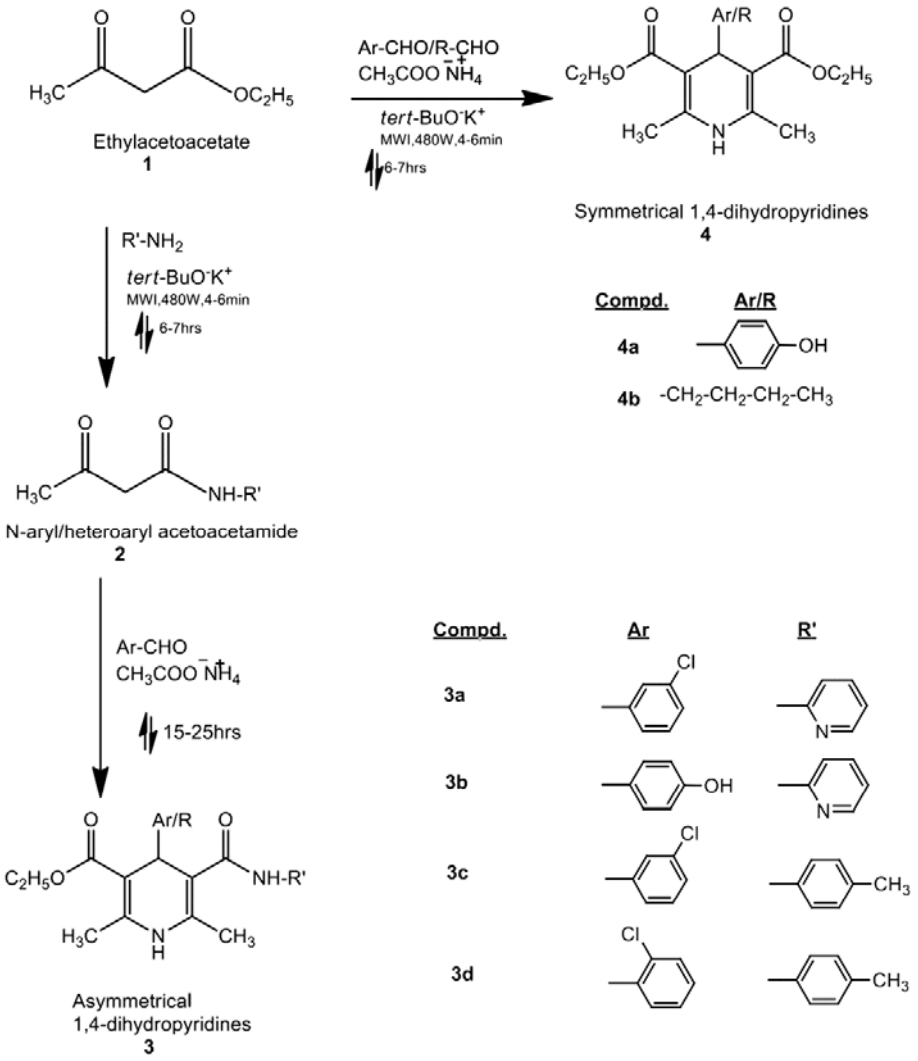


Figure 1 — Potent calcium channel blockers



Scheme I — Synthetic route for 1,4-dihydropyridine derivatives

obtained was chosen to synthesize the N-(aryl/heteroaryl) acetoacetamide needed for preparing 1,4-DHPs **3a-d**. Reaction of compound **2** with appropriate aromatic aldehydes in presence of ammonium acetate produced the unsymmetrical dihydropyridines **3a-d**. Symmetrical 1,4-dihydropyridines **4a** and **4b** were synthesized from the one-pot three component reaction of ethylacetoacetate, ammonium acetate and *p*-hydroxyphenyl or butyrl aldehyde using conventional and microwave irradiation methods.

Spectral Data

Ethyl-4-(3-chlorophenyl)-2,6-dimethyl-5-(pyridin-2-yl-carbamoyl)-1,4-dihydropyridine- 3-carboxylate, 3a: Brown solid. Yield 58%. m.p. 125-130°C. IR: 3494 (N-H str), 2981 (C-H str), 1694 (-C=O str, Amide), 1579 (C=C str), 770 cm^{-1} (C-Cl str); ^{1}H NMR (DMSO-d_6): δ 1.09-1.13 (t, 3H, -CH_2-CH_3), 2.11-2.25 (q, 2H, -CH_3), 2.48 (s, 6H, 2× –CH_3), 4.82 (s, 1H, H4-D*H*P), 4.93 (s, 1H, N*H*-DHP), 7.11-8.34 (m, 8H, Ar-*H*), 10.69 (s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6): δ 14.2, 18.8, 19.1, 43.5, 61.6, 103.5, 114.6, 118.2, 126.2, 129.4, 133.6, 142.9, 150.5, 165.2, 167.2; LCMS: *m/z* 411.88 (M^+), 413.13 (M + 2). Anal. Calcd for $C_{22}H_{22}ClN_3O_3$: C, 64.15; H, 5.38; N, 10.20. Found: C, 64.18; H, 5.40; N, 10.18%.

Ethyl-4-(4-hydroxyphenyl)-2,6-dimethyl-5-(pyridine-2yl-carbamoyl)-1,4- dihydropyridine-3-carboxylate, 3b: Brown solid. Yield 60%. m.p. 120-123°C. IR: 3370 (O-H str broad), 3295 (N-H str), 3105 (C-H str, aromatic), 2960 (C-H str, aliphatic), 1745 (C=O str, ester), 1660 (C=O str, amide), 1570 (C=C str) 1100 cm^{-1}

(C-O str); ^{1}H NMR (DMSO-d_6): δ 1.31 (t, 3H, -C*H*$_3$), 2.28 (s, 6H, 2× -C*H*$_3$), 4.18 (q, 2H, -C*H*$_2$), 4.78 (s, 2H, H4 + N*H*-DHP), 5.35 (s, 1H, -OH), 6.53-6.65 (m, 4H, Ar-*H*), 7.19-7.82 (m, 4H, Ar-*H*), 9.15 (s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6): δ 14.3, 18.7, 19.0, 43.5, 61.6, 102.7, 114.2, 115.9, 118.2, 130.6, 137.0, 138.3, 149.5, 150.1, 156.7, 163.1, 167.2; LCMS: *m/z* 393.44 (M^+). Anal. Calcd for $C_{22}H_{23}N_3O_4$: C, 67.16; H, 5.89; N, 10.68. Found: C, 67.18; H, 5.93; N, 10.65%.

Ethyl-4-(3-chlorophenyl)-2,6-dimethyl-5-(*p*-tolylcarbamoyl)-1,4-dihydropyridine-3- carboxylate, 3c: Brown solid. Yield 58%, m.p. 128-133°C. IR: 3293 (N-H str), 2984 (C-H str), 1635 (C=N str), 1750 (C=O, str, ester), 1590 (-C=O str, amide), 1561 (C=C str), 1428 (C-H bend), 770 cm^{-1} (C-Cl str); ^{1}H NMR (DMSO-d_6): δ 1.11 (t, 3H, -CH$_2$-C*H*$_3$), 2.22 (s, 6H, 2× -C*H*$_3$), 2.48 (s, 3H, Ar-C*H*$_3$), 3.34 (q, 2H, -C*H*$_2$), 4.8 (s, 1H, H4-DHP), 7.04-7.32 (m, 9H, Ar-*H*, N*H*-DHP), 8.4 (s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6): δ 14.5, 18.6, 19.2, 21.4, 43.8, 61.7, 102.8, 121.6 125.8, 129.2, 136.2, 138.3, 142.4, 149.8, 163.1, 167.4; LCMS: *m/z* 424.92 (M^+), 426.15 (M + 2). Anal. Calcd for $C_{24}H_{25}ClN_2O_3$: C, 67.84; H, 5.93; N, 6.59. Found: C, 67.85; H, 5.95; N, 6.62%.

Ethyl-4-(2-chlorophenyl)-2,6-dimethyl-5-(*p*-tolylcarbamoyl)-1,4-dihydropyridine-3- carboxylate, 3d: Brown solid. Yield 50%, m.p. 126-129°C. IR: 3315 (N-H str), 3115 (C-H str, aromatic), 2995 (C-H str, aliphatic), 1760 (C=O, str, ester), 1645 (C=O str, amide), 1585 (C=C str), 1076 (C-O bend), 785 cm^{-1} (C-Cl str); ^{1}H NMR (DMSO-d_6): δ 1.28 (t, 3H, -C*H*$_3$), 2.26 (s, 6H, 2× –C*H*$_3$, DHP), 2.43 (s, 3H, -C*H*$_3$), 4.23 (q, 2H, -C*H*$_2$), 4.78 (s, 1H, *H*4-DHP), 5.15 (s, 1H, N*H*-DHP), 7.17-7.65 (m, 8H, Ar-H), 10.16 (s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6): δ 14.5, 18.6, 19.0, 21.3, 39.2, 61.5, 102.5, 102.8, 121.5, 126.7, 128.7, 131.4, 137.8, 143.4, 150.1, 163.1, 167.2; LCMS: *m/z* 424.92 (M^+), 426.15 (M + 2). Anal. Calcd for $C_{24}H_{25}ClN_2O_3$: C, 67.84; H, 5.93; N, 6.59. Found: C, 67.85; H, 5.95; N, 6.54%.

Diethyl-4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 4a: Brown solid. Yield 65%, m.p. 115-118°C. IR: 3365 (O-H str, broad), 3010 (C-H str, aromatic), 2827 (C-H str, aliphatic), 1656 (C=O, str), 1555 (C=C str), 1225 (C-N str), 1095 cm^{-1} (C-Ostr); ^{1}H NMR (DMSO-d_6): δ 1.31 (t, 6H, 2× -C*H*$_3$), 2.26 (s, 6H, 2× -C*H*$_3$, DHP), 4.25 (q, 4H, 2× -C*H*$_3$), 4.82 (s, 2H, H4-DHP + NH-DHP), 5.38 (s, 1H, -OH), 6.75-6.98 (m, 4H, Ar- *H*); ^{13}C NMR (DMSO-d_6): δ 14.8, 19.5, 42.9, 62.5, 103.1, 116.4, 131.3, 137.0, 151.6, 157.2, 168.9; LCMS: *m/z* 345.16 (M^+). Anal. Calcd for $C_{19}H_{23}NO_5$: C, 66.07; H, 6.71; N, 4.06. Found: C, 66.10; H, 6.74; N, 4.02%.

Diethyl-4-butyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate, 4b: Brown solid. Yield 48%, m.p. 93-96°C. IR: 3254 (N-H str), 2917 (C-H str), 1646 (C=O str), 1525 (C=C str), 1210 (C-N str), 1080 cm^{-1} (C-O str); ^{1}H NMR (DMSO-d_6): δ 0.92 (t, 6H, 3× -C*H*$_3$), 1.10 (q, 2H, –C*H*$_2$), 1.32-1.35 (m, 10H, 2×- -C*H*$_3$ + 2× -C*H*$_2$),2.26 (s, 6H, 2× -C*H*$_3$, DHP), 3.51 (t, 1H, H4-DHP), 4.12 (s, 1H, NH-DHP), 4.35 (q, 4H,2×- C*H*$_2$-CH$_3$); ^{13}C NMR (DMSO-d_6): δ 14.5, 19.1, 23.8, 27.9, 33.2, 34.5, 61.5, 109.3, 150.6, 167.8; LCMS: *m/z* 309.19 (M^+). Anal. Calcd for $C_{17}H_{27}NO_4$: C, 65.99; H, 8.80; N, 4.53. Found: C, 66.01; H, 8.78; N, 4.55%.

Anticonvulsant effect of some synthesized 1,4-dihydropyridine derivatives in mice using PTZ induced seizures

The six test compounds (**3a-d, 4a & 4b**) were evaluated for anticonvulsant activity against PTZ induced seizures using Phenytoin as standard (Table I). All the tested compounds (**3a-d, 4a** and **4b**) were found to possess anticonvulsant activity. The test compounds showed increase in latency to induce convulsions,

Table I — Anticonvulsant effect of some synthesized 1,4-dihydropyridine derivatives in rats using PTZ induced seizures

Compd	Dose (mg/kg)	Latency to induce convulsions (min)	No. of convulsions	No. of Animals Died/used
Control(PTZ)	80	6.333	9	4/6
Phenytoin	4	10± 1	3	2/6
3a	10	8.33± 0.5	6	3/6
3b	10	10± 0.9	5	5/6
3c	10	11.83± 1.2	4	0/6
3d	10	10± 1.2	5	0/6
4a	10	10± 1	4	0/6
4b	10	11.5± 1	3	0/6

decline in number of convulsions and increase in latency of death compared to control and standard. Compounds **3c, 4b** (10mg/kg body weight) were found to be more potent than the standard, Phenytoin (4mg/kg body weight).

Experimental Section

Chemicals used in synthetic work were 2-aminopyridine, *p*-toludine, ethylacetoacetate, and potassium-*tert*-butoxide, various benzaldehydes such as 2-chlorobenzaldehyde, 3-chlorobenzaldehyde, 4-hydroxybenzaldehyde, butyraldehyde, and ethanol. Chemicals used for biological activity were phenytoin, pentylenetetrazole, DMSO. All the reactions were performed in dried borosil glass beakers, round bottom flasks, conical flasks. Domestic LG microwave oven was used for synthesizing compounds by microwave irradiation method. Precoated silica gel plates (MERCK) were used for TLC to monitor the progress of the reaction. Compounds melting points were determined by capillary method and are uncorrected. JASCO UV Chamber was used for detection of spots in TLC. IR Spectra were recorded on BRUKER FTIR Spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded on Bruker spectrometer using DMSO-d6 as solvent and TMS as internal standard. Mass spectra were obtained on Shimadzu LCMS 2010 spectrophotometer. Elemental analysis was performed on Perkin-Elmer 204B analyzer.

General procedure for the synthesis of N-(aryl/heteroaryl) acetoacetamide, 2

(a) Conventional method

A mixture of aryl/heteryl amine and ethylacetoacetate in 1:1.8 ratio and catalytic amount of potassium-*tert*-butoxide was taken into a 250 mL RB flask and dissolved in 25 mL of ethanol. The reaction mixture was heated under reflux for 6 to 7hrs, while monitoring the progress of the reaction by TLC. The solvent was removed from the reaction mixture to a possible extent, under reduced pressure and the residue was cooled and triturated with dry ether. The product was filtered and washed with small portions of dry ether. Purification was effected by recrystallization from ethanol to obtain colorless crystalline solid[13].

(b) Microwave irradiation

A mixture of aryl/heteryl amine, ethylacetoacetate in 1:1.8 ratio and a catalytic amount of potassium-*tert*-butoxide was taken into a 250 mL Pyrex beaker with an inverted glass funnel and irradiated in a domestic microwave oven for 4 to 6 min with 10 sec pulses at 480W while monitoring the progress of the reaction by TLC. On completion of the reaction, the reaction mixture was cooled and triturated with ice-cold ether. The product separated was filtered, washed with small portions of ice-cold ether and dried. Purification by recrystallization from ethanol afforded a colorless crystalline solid[13].

General procedure for the synthesis of 4-alky/aryl-2,6-dimethyl-3-N-(aryl/heteroaryl)- carbomoyl-5-ethoxycarbonyl-1,4-dihydropyridines, 3a-d

A mixture of N-(aryl/heteroaryl)acetoacetamide (**2** 0.01mol), ethylacetoacetate (0.01mol), an appropriate aldehyde (0.01mol) and urea (0.01mol) in ethanol (25 mL) was heated under reflux, on a water-bath for 15-25hrs while monitoring the reaction by TLC. On completion of the reaction, the solvent was removed to the possible extent by distillation under reduced pressure and the residue was cooled. The product was filtered, washed with small portions of ice-cold ethanol and dried. Further purification was effected by column chromatography by gradient elution technique[14].

General procedure for the synthesis of 4-alkyl/aryl-2,6-dimethyl-3,5-bis- (ethoxycarbonyl)-1,4-dihydropyridines 4a and 4b

(a) Conventional method

A mixture of ethylacetoacetate (0.02mol), an appropriate aldehyde (0.01mol) ammonium acetate (0.01mol) and catalytic amount of potassium-*tert*-butoxide in ethanol (25 mL) was heated under reflux, on a water-bath for 6-7hrs while monitoring the reaction by TLC. On completion of the reaction, the solvent was removed to the possible extent by distillation under reduced pressure and the residue was cooled. The product was filtered, washed with small portions of ice-cold ethanol and dried. Further purification was effected by recrystallization from ethanol to obtain colorless crystalline solid[14].

(b) Microwave irradiation

A mixture of ethylacetoacetate (0.02mol), an appropriate aldehyde (0.01mol) ammonium acetate (0.01mol) and catalytic amount of potassium-*tert*-butoxide was taken into a 250 mL Pyrex beaker with an inverted glass funnel and irradiated in a domestic microwave oven for 4 to 6 min with 10 sec pulses at

480 W while monitoring the progress of the reaction by TLC. On completion of the reaction, the reaction mixture was cooled and triturated with ice-cold ether. The product separated was filtered, washed with small portions of ice-cold ether and dried. Purification by recrystallization from ethanol afforded a colorless crystalline solid[14].

Evaluation of anticonvulsant activity

PTZ induced Seizures

Animals: Wister Albino rats of male sex, weighing 150-200 gm were used. They were purchased from Mahaveer enterprises, Hyderabad and housed in different groups consisting of six animals in each group; in plastic cages under good hygienic conditions bedding of rice husk was replaced twice in a week so as to maintain good hygienic conditions. Ambient temperature of 25±1 ºC, relative humidity of 45-55% and 12 hrs light: 12 hrs dark cycles were maintained in animal house. The animals had free access to water and standard pelleted diet, except during experimentation when food and water were withheld. All animals were routinely examined for infections, disorders and injuries and they were treated ethically and humanly. Thirty minutes before the experiment, animals were selected randomly and transferred into individual cages and allowed to acclimatize before injection of drugs or vehicle. The experimental protocol was approved by the Animal Ethical Committee (IAEC), Vaagdevi College of Pharmacy, Warangal (Application No: 2014/11/4/3).

Preparation of doses: The drugs and chemicals were freshly prepared. Pentylenetetrazole (80 mg/kg, i.p.), a stock solution containing 8 mg/mL was prepared by dissolving it in distilled water. Phenytoin (4 mg/kg, i.p.), a stock solution containing 0.2 mg/mL was prepared by dissolving it in distilled water. The doses of test compounds were 10 mg/kg, i.p. and 15 mg/kg, i.p. respectively and stock solutions of test compounds containing 1 mg/mL and 1.5 mg/ mL were prepared by dissolving the test compounds in dimethylsulphoxide. The injection volume was 1mL/100 gm body weight of animal[1].

Procedure: The animals were first weighed and were selected for the experiment depending on weight. The animals were then divided into seven groups, of six animals each. One group was used for studying the effects of Pentylenetetrazole alone (Control) and the other for studying the protective effects of Phenytoin (Standard). The remaining five groups were used for studying the effects of synthesized compounds (Test). Pentylenetetrazole (80 mg/kg) was administered intraperitoneally to induce convulsions in control and the onset and severity of convulsions, and mortality was noted. PTZ was administered half an hour after administration of phenytoin or test compounds[4]. In phenytoin treated animals, either delay or complete abolition of convulsions was noted. The test group animals were observed for onset of convulsions and number of convulsions. The drug treated (standard/test) animals were observed following PTZ injection up to one hour. The anticonvulsant potential of newly synthesized compounds was evaluated on the basis of increase in latency (onset time) to induce convulsions; decrease in number of convulsions and increase in latency of death compared to control and standard[1].

Conclusion

Compounds **3c, 4b** (10mg/kg body weight) were found to be more potent than the standard, Phenytoin (4mg/kg body weight). Hence, in view of the anticonvulsant potency exhibited by the newly synthesized 1,4-dihydropyridine derivatives (**3a-d, 4a-b**), there is a need to carry out further studies to explore their potential as novel anticonvulsants.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/60.

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