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# Synthezise, Spectral, Antimicrobial and Antioxidant Studies of Mannich Bases of B-Naphthol and Gabapentin

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**Abstract:** Present study deals with synthesis of novel Mannich bases from β-Naphthol and gabapentin by using Mannich condensation reaction. The Mannich bases 2-[1-({[(3-hydroxynaphthalen-2-yl)methyl] amino}methyl) cyclohexyl]acetic acid (TNGF) and 2-[1-({(3-hydroxynaphthalen-2-yl)(phenyl)methylamino} methyl)cyclohexyl]acetic acid (TNGB) were characterized by UV-Vis, FT-IR, $^{1}$HNMR, $^{13}$CNMR, MASS and Elemental analysis. The synthesized Mannich bases were subjected to the antimicrobial assay using standard microbiological method against pathogens. The antioxidant activity analyzed by standard methods and ascorbic acid used as standard. All the synthesized compounds TNGF and TNGB show significant inhibition to the tested pathogens and also have moderate antioxidant activity.
**Keywords**: Mannich base, β-Naphthol, Gabapentin, Antimicrobial activity, Antioxidant activity.

## Introduction

The Mannich reaction is an organic reaction used to synthesize of natural compounds like nucleotides, peptides and alkaloids. It is also used for the synthesis of medicinal drugs, agro chemicals, polymers and catalysts. The Mannich bases are interested in the pharmacological field because of their wide range of applications, specifically antimicrobial [1], antimalarial [2], antimicrobial, anti-HIV [3], anti-tubercular [4], analgesics, anti-cancer [5], anti-inflammatory [6], vasorelaxing, antitumor [7], cytotoxic activities and antioxidant agents [8]. In this series mannich reaction is places most important role to synthesis of new types of biologically active drugs. Mannich bases are also used as active alkylating agents. Besides of the active pharmacological interest of the Mannich bases were synthesized using various active hydrogen and different amine compounds.

Gabapentin is used as anticonvulsants in pain treatment. The profile of anticonvulsant and pharmacokinetic activity suggests that gabapentin differs pharmacologically from other commonly studied and used anticonvulsants. Gabapentin Compounds showed good anticonvulsant activity with no neurological toxicity. It demonstrated good antioxidant activity also. The SAR studies reveal that, both linkage and substituent on phenyl ring are responsible for anticonvulsant and antioxidant activities of these classes of agents [9]. From the profile which emerges from the literature, gabapentin can be considered an emergent solution for the "pain riddle", even if some aspects of the drug must be clarified. Starting from this point, more randomized, double-blind studies, comparing traditional analgesic drugs with gabapentin, may be relevant to identifying the first choice therapy for acute and chronic pain relief [10]. The presence of more electron enriched alkoxy, amino and phenolic groups on the heterocycle shows excellent activity [11].

This research work was an attempt to synthesize some of new Mannich bases of gabapentin and 2-naphthol then study their antimicrobial and antioxidant studies. The Mannich reaction is three component condensation reaction which a compound with active hydrogen atom is react to aldehydes and a primary or secondary amine with liberation of water. In Mannich base reaction the active hydrogen is replaced by an aminomethyl group. This Mannich reaction both C–C and C–N bonds are formed by aminomethylation process which makes the reaction is an extremely useful synthetic transformation. Mannich bases have wide range of application in the area of pharmaceuticals and macromolecular chemistry [12]. Mannich bases have antihistamines, anti-inflammatory[13], and antimicrobials properties. Amide derivatives of Mannich bases have antiepileptic, anticonvulsive [14], fungicidal [15], and many biological and pharmacological activities [16].

## Materials and Methods

All the chemicals and solvents were used this synthesis were obtained from Aldrich and Merck chemical companies and used without further purification. The Pre-coated silica gel plates (Kieselgel 60 F254, Merck) were used for monitoring the reactions and the spot visualized using UV lamps. Melting points were determined in open capillary tubes by using Elico instrument and readings were uncorrected. Ultraviolet-visible (UV-Vis) absorption spectra recorded in Systronics 2202 Spectrophotometer at the wavelength of maximum absorption ($\lambda_{max}$). IR spectra were recorded in a Perkin-Elmer FTIR spectrophotometer with KBr disc. Nuclear Magnetic Resonance spectra were recorded in a Bruker Advance DPX 300 MHz Ultra-Shield FT-NMR Spectrophotometer using $CDCl_3$ as a solvent and tetramethylsilan (TMS) as reference standard. GC-MS analysis carried out by Agilent-7890A GC instrument coupled with MS-5975 inert MSD with triple axis mass selective ion detector. The micro-elemental analyses were done in Vario-EL instrument. The human pathogenic bacterial species were purchased from MTCC Chandigarh, India. The bacteria (*Escherichia coli* MTCC No 433, *Salmonella typhi* MTCC No 733, *Staphylococcus aureus* MTCC No 1430 and *Vibrio cholerae* MTCC No 3940) and fungal species (*Aspergillus* flavus MTCC No 4613 and *Aspergillus fumigatus* MTCC No 343) were used for the antimicrobial studies.

## Experimental

## Synthesis

### 2-[1-({[(3-hydroxynaphthalen-2-yl)methyl]amino}methyl)cyclohexyl] acetic acid (TNGF):

Gabapentin (1.71g, 0.01M) dissolved in a minimum quantity of water and add saturated ethanolic solution of β-naphthol (1.44g,0.01M) with constant stirring. Then add formaldehyde (1 Ml, 0.01M) very slowly with continuous stirring. The mixture was stirred at room temperature for 24 h. The ethanol was then removed under vacuum then creamy white colour precipitate obtained (Fig.1). This was kept for a 48 hrs to complete the reaction. The precipitate washed several times with water and acetonitrile. Then recrystalize using chloroform.

**2-[1-({(3-hydroxynaphthalen-2-yl)(phenyl)methylamino}methyl)cyclohexyl]acetic acid (TNGB):**

Gabapentin (1.71g, 0.01M) dissolved in a minimum quantity of water and add saturated ethanolic solution of β-naphthol (1.44g, 0.01M) with constant stirring. Then add benzaldehyde (1 Ml, 0.01M) very slowly with continuous stirring. The mixture was stirred at room temperature for 24 h. The ethanol was then removed under vacuum. Then we get a brown colour precipitate (Fig.2). Then stand that for a week to complete the reaction. We got the pale yellow powder then washed several times with water and acetonitrile. Then recrystalize using chloroform.

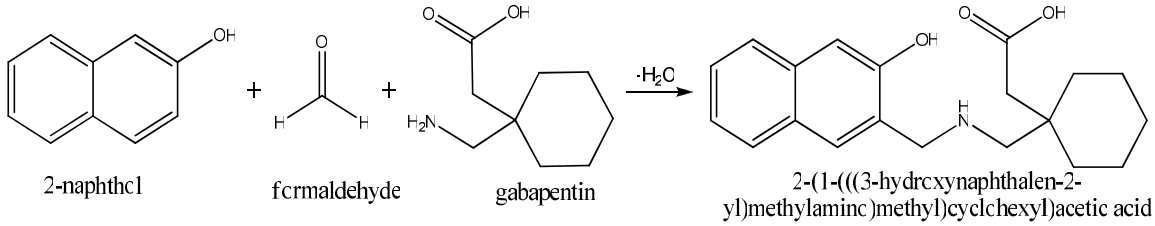


**Figure 1: 2-[1-({[(3-hydroxynaphthalen-2-yl)methyl]amino}methyl)cyclohexyl] acetic acid**

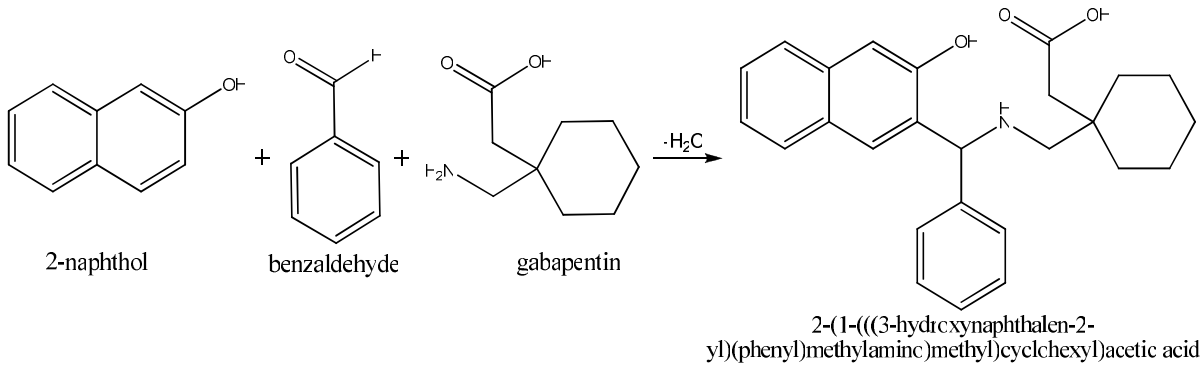


**Figure 2: 2-[1-({(3-hydroxynaphthalen-2-yl)(phenyl)methylamino}methyl) cyclohexyl]acetic acid**

## Spectral Data

**2-[1-({[(3-hydroxynaphthalen-2-yl)methyl]amino}methyl)cyclohexyl] acetic acid (TNGF):**

The reaction was monitored by TLC until the reaction was complete. Mol. F: $C_{20}H_{25}NO_3$, Yield: 57.83%, Appearance: creamy white, Mol. wt: 327, M.P: 145-150°C. FT-IR (KBr, υ in $cm^{-1}$): 3280 (N-H), 3050 (C-H), 2925 (O-H), 1630 (C=O), 1150 (C-N-C). $^1$H-NMR ($CDCl_3$, 300 MHz): δ 12.80 (s, br, OH), 10.08 (s, 1H, OH), 7.93 (d, NH), 7.21-7.80 (m, 6H, Ar-H), 4.82 (s, 1H, CH), 3.38 (s, 2H, $CH_2$), 2.29 (s, 2H, $CH_2$), 1.43-1.62 (s, 10H, cyclohexyl ring). $^{13}$C-NMR ($CDCl_3$, 300 MHz): δ 172.4 (s, 1C), 154.0 (s, 1C), 113.1-133.2 (m, 10C), 56.9 (s, 1C), 22.3-35.9 (m, 6C). EI-MS (positive mode) *m/z*: 326.9 ($C_{20}H_{25}NO_3^{+}$), 309.1 ($C_{20}H_{24}NO_2^{+\cdot}$), 206.9 ($C_{15}H_{15}N^{+\cdot}$), 156 ($C_{11}H_9N^{+\cdot}$). Elemental analysis: $C_{20}H_{25}NO_3$ %: C 73.37, H 7.70, N 4.28, O 14.66. Found %: C 72.95, H 7.82, N 4.35.

**2-[1-({(3-hydroxynaphthalen-2-yl)(phenyl)methylamino}methyl)cyclohexyl]acetic acid (TNGB):**

The reaction was monitored by TLC until the reaction was complete. Mol. F: $C_{26}H_{29}NO_3$, Yield: 88.0%, Appearance: pale yellow, Mol. wt: 403, M.P: 120-125°C. FT-IR (KBr, υ in $cm^{-1}$): 3350 (N-H), 3034 (C-H), 2923 (O-H), 1649 (C=O), 1178 (C-N-C). $^1$H-NMR ($CDCl_3$, 300 MHz): δ 13.0 (s, br, OH), 9.72 (s, 1H, OH), 7.98 (d, NH), 7.07-7.52 (m, 11H, Ar-H), 6.88 (d, 1H, CH), 3.50 (s, 2H, $CH_2$), 2.70 (s, 2H, $CH_2$), 1.25-1.52 (s, 10H, cyclohexyl ring). $^{13}$C-NMR ($CDCl_3$, 300 MHz): δ 173.2 (s, 1C), 163.3 (s, 1C), 109.1-141.4 (m, 16C), 67.1

(s, 1C), 21.0-40.5 (m, 6C). EI-MS (positive mode) *m/z*: 403.2 ($C_{26}H_{29}NO_3^{+\cdot}$), 258.0 ($C_{18}H_{16}NO^{+\cdot}$), 207.0 ($C_{15}H_{15}N^{+\cdot}$), 181.0 ($C_{13}H_9O^{+\cdot}$). Elemental analysis: $C_{26}H_{29}NO_3$ %: C 77.39, H 7.24, N 3.47, O 11.90. Found %: C 77.51, H 7.02, N 3.45.

## Biological Evaluation

### *In vitro* Antimicrobial activity

The synthesized compounds were evaluated for its *in vitro* antimicrobial potential by conventional agar well diffusion method against pathogenic bacteria and fungus. The strains include some of gram negative bacteria The bacteria (*Escherichia coli*, *Salmonella typhi* and *Vibrio cholerae* and Gram positive bacteria (*Staphylococcus aureus*). The anti-fungal activity was tested against *Aspergillus flavus*, *Aspergillus fumigatus.* The Minimum Bacterial Concentration (MBC) and Minimum Inhibitory Concentration (MIC) were analyzed. The pathogens were activated by inoculating of strain in broth (20 mL) to a 100 mL Erlenmeyer flask and incubated at 37°C on a rotary shaker for 24 hrs. Fresh inoculum of 0.1 mL was spread on the surface of disinfected agar plates using a sterilized glass spreader and wells were made on the seeded plates. The compounds (50µl of 20 mg/mL concentration) were dispended into the well, and the plates were incubated aerobically at 37°C for pathogens. The negative control well was made with only 4% DMSO separately streptomycin (25 µg disc) and ketoconazole (50 µg disc) used as positive control. The zones of inhibition (mm) of the compounds were examined after 24 hrs. The plates were observed in zone formation around the wells and MIC was determined.

### *In vitro* antioxidant activity

The antioxidant and free radical scavenging activity of the Mannich bases were measured using various *in vitro* antioxidant assays. Hydrogen peroxide scavenging activity method used to analyze the ability to scavenge the radicals [17]. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical, which was widely accepted tool for estimating free radical scavenging activities of antioxidants. DPPH free radical scavenging activity usually involves hydrogen atom transfer reaction [18]. The reducing power of Mannich bases were determined by using the standard method [19]. For the above studies ascorbic acid was used as reference material.

## Results and Discussion

The Mannich bases were synthesized by condensation method and its structures were elucidated by analytical and spectroscopic methods. The synthesized ligands characterized by elemental analysis, UV-Vis, $^1$H NMR, $^{13}$C NMR spectral studies. The UV spectra indicates the presence of conjugated aromatic ring, alcoholic groups and secondary amine in it. This moieties further also confirmed by using IR spectrum studies. The sharp bands at 3280 & 3350 $cm^{-1}$ indicates the N-H group, 3050 & 3034 $cm^{-1}$ shows C-H, 2925 & 2923 $cm^{-1}$ shows O-H, 1630 & 1649 $cm^{-1}$ shows C=O, and 1150 & 1178 $cm^{-1}$ indicates C-N-C functional groups present in TNGF & TNGB respectively. The structural assignments further made using $^1$H NMR spectra. It indicates signal at δ 12.80 & 13.0 broad singlet signal shows the COOH, δ 10.08 & 9.72 singlet shows the OH, 7.93 & 7.98 doublet shows NH, δ 7.21-7.80 & 7.07-7.52 multiplet shows aromatic protons, δ 4.82 & 3.50 singlet shows CH protons, δ 1.43-1.62 & 1.25-1.52 shows 10H of cyclohexyl ring of TNGF & TNGB respectively. In $^{13}$C NMR spectra shows δ 172.4 & 173.2 singlet of C=O, δ 154.0 & 163.3 shows C=O, δ 113.1-133.2 & 109.1-141.4 multiplet shows aromatic carbon, δ 22.3-35.9 & 21.0-40.5 shows cyclohexyl carbons in it. Based upon the spectroscopic data of ligands 3-(phenyl(p-tolylamino)methyl)naphthalene-2-ol (TNPTB) and 3-((1H-benzo[d]imidazole-1-yl)methyl)naphthalene-2-ol are good agreement with the proposed structure.

The synthesized Mannich bases TNGF and TNGB ligands were subjected to the antimicrobial assay. The TNGB showed activity in all the tested strains (>12 mm) and the TNGF only showed activity in *S.aureus* and *S.typhi* (the zone of inhibition >10) other tested pathogens are resistant. Minimum Inhibitory Concentration (MIC) were also analyzed. Among the tested ligands, TNGB showed profound MIC (0.010-0.027 mg / mL) against tested fungal pathogens followed by bacteria (0.76-0.90 mg / mL). The observed data on the

antibacterial and antifungal activity of the synthesized compounds are given in Tables 1&2. For control experiment, streptomycin and ketoconazole showed activity in the zone of inhibition (> 8 mm for bacteria and > 12 mm for fungus) against tested pathogens. This study confirms TNGF and TNGB ligands having the good antimicrobial property. The Mannich base compounds exhibited significant antimicrobial activities due to the presence of electron donating substituent OH group present in naphthols [20]. In all synthesized compounds are active because of more electrons enriched amino and phenolic group in it [21]. In TNGB, the aromatic group of benzaldehyde may enriched the antimicrobial activity. Furthermore, presence of aromatic ring and hetero atoms may be a potent reason for the significant activity in both compounds.

The synthesized naphthols and gabapentin derivatives analysed for their inhibition efficiency with the standard antioxidant methods. Hydrogen peroxide scavenging activity of the compounds decreases in the following order: L ascorbic acid > TNGB > TNGF at concentration of 80 µg/mL, respectively. The potential of L-ascorbic acid to scavenge Hydrogen peroxide is directly proportional to the concentration (Fig. 3). The DPPH assay shows free radical scavenging activity of compounds increases with increasing the concentration (Fig. 4). TNGB possessed the efficient free radical scavenging activity compared to TNGF. The reducing power of the compounds increased in following order: L ascorbic acid > TNGB > TNGF with concentration respectively (Fig. 5). The presence of electron donating group on benzene ring in naphthol is the enriched antioxidant activity of both compounds [21]. Moreover, the phenolic and naphtholic compounds were effective antioxidants by scavenging radicals through the mode of a chain breaking path.

**Table-1: *In vitro* antibacterial activity of Mannich bases**

| Mannich base (mg) | *E.Coli* | | *S.Typhi* | | *V.Cholerae* | | *S.Aureus* | |
|---|---|---|---|---|---|---|---|---|
| | ZI | MIC | ZI | MIC | ZI | MIC | ZI | MIC |
| TNGF | 10 | 0.92 | 11 | 0.87 | MD | -- | MD | -- |
| TNGB | 12 | 0.85 | 13 | 0.83 | 9 | 0.90 | 15 | 0.79 |

**Table-2: *In vitro* antifungal activity of Mannich bases**

| Mannich base (mg) | *A.Flavus* | | *A.Fumigutus* | |
|---|---|---|---|---|
| | ZI | MIC | ZI | MIC |
| TNGF | MD | -- | MD | -- |
| TNGB | 23 | 0.027 | 30 | 0.023 |

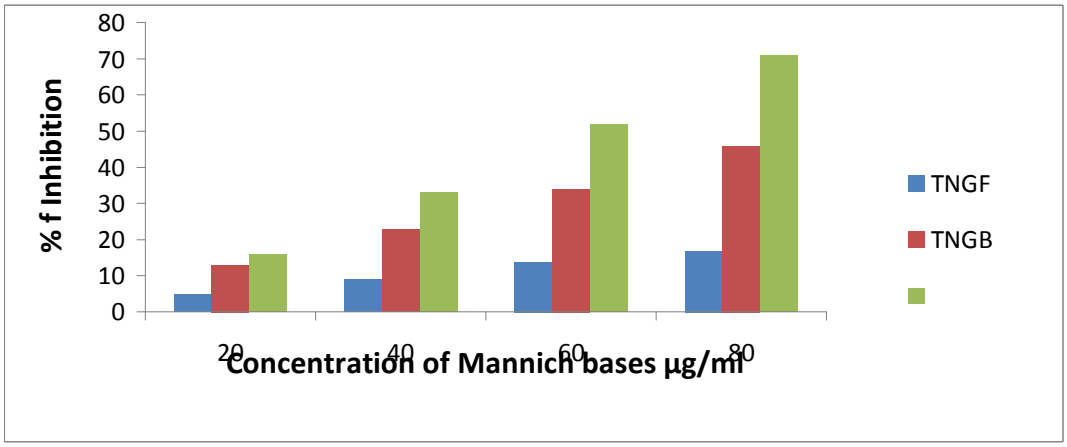


**Figure 3: Hydrogen peroxide scavenging activity of synthesized compounds**

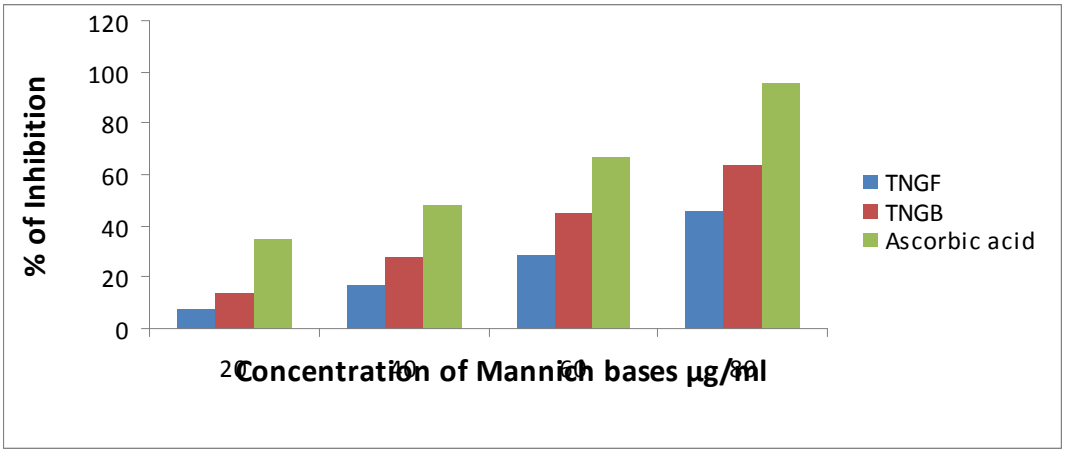


**Figure 4: Free radical scavenging activity of Mannich base by DPPH method**

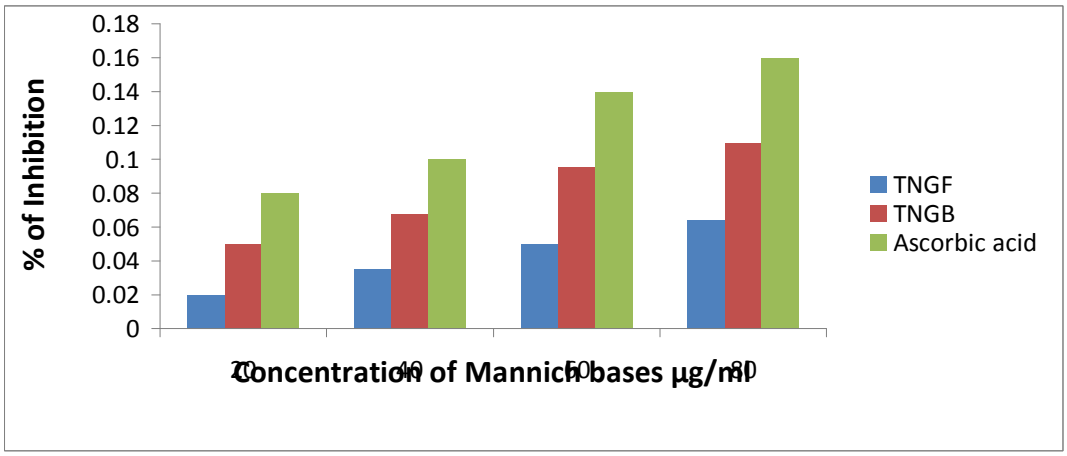


**Figure 5: Reducing power activity of synthesized compounds**

## Conclusion

The novel Mannich bases derived from β-naphthol and gabapentin by condensation reactions were characterized by analytical and spectroscopic methods. The elemental analysis results were coincident with the analytically calculated C, H, N percentage in it. The $^{1}H$ NMR and $^{13}C$ NMR spectrum confirms the position of H & C and structural details of the both compounds. The IR spectroscopy result confirms the presence of functional groups in the proposed compounds. The characterised results were confirming the novel compounds TNGF and TNGB. Both the synthesized compounds shows moderate antimicrobial and anti-oxidant activities. The anti-bacterial, anti-fungal and anti-oxidant assay concludes the TNGB shows the good and significant activities compared to the TNGF. The structural and biological activities of TNGB may be due to presence of aromatic group in benzaldehyde.

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