

Synthesis, Characterization and Biological Activity of some 2,4,6 tri substituted Pyrimidine Derivatives

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Abstract:

A series of novel compounds were synthesized and characterized using appropriate spectroscopic techniques. All synthesized compounds were evaluated for their antibacterial activity against various bacterial pathogens using standard in vitro assays. The results of antibacterial screening revealed that all titled compounds exhibited significant activity against the tested bacterial strains. Notably, compounds SB-4, SB-5, SB-8, and SB-9 demonstrated pronounced antibacterial potency that was comparable to or exceeded that of standard reference antibiotics, suggesting superior therapeutic potential. Structure-activity relationship analysis indicated that the enhanced antibacterial efficacy of these lead compounds could be attributed to the presence of electron-donating groups, particularly the nitro group positioned at the 6th position of the parent molecular scaffold. The electron-donating characteristics of the nitro moiety appeared to favorably influence the compound's interaction with bacterial targets, resulting in improved antimicrobial efficacy. To further elucidate the structural features responsible for the observed biological activity and to optimize the lead compounds, subsequent investigations will involve quantitative structure-activity relationship (QSAR) modeling and molecular docking studies. These computational approaches will provide valuable insights into the binding mechanisms and physicochemical properties that correlate with antibacterial activity, thereby facilitating the rational design and development of more potent antibacterial agents from this chemical series.

Keywords: Antibacterial agents, novel synthesized compounds, in vitro antibacterial activity, structure-activity relationship, nitro substituent at C-6, electron-donating groups, lead compounds SB-4 SB-5 SB-8 SB-9, QSAR modeling, molecular docking, rational drug design.

Introduction

Heterocyclic compounds are organic compounds containing at least one atom of carbon and at least one element other than carbon, such as sulfur, oxygen or nitrogen within a ring structure. Since in hetrocycles non-carbons usually are considered to replace carbon atoms, they are called heteroatoms. The heterocyclic compounds usually possess a stable ring structure which does not readily hydrolyzed or depolymerized

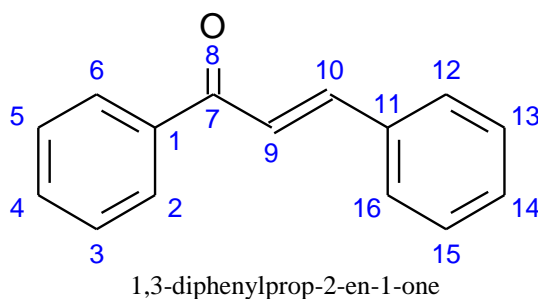
Chalcones

Chalcones are α,β -unsaturated ketones are promising candidates in the new era of medicines on account of their wide spectrum of different activities. Due to reactive keto vinyl moiety these compound may interfere with normal function of the cell membrane of fungi and molds and exhibit static properties against pathogens. Besides antimicrobial activity, chalcones also exhibit anti-cancer, anti-protzoal, enzyme inhibitory properties anti-inflammatory and antiulcer agent.

Chemistry of Chalcones

In Claisen-Schmidt condensation reaction for synthesizing Chalcones, aromatic aldehydes can be condensed with aliphatic or aromatic ketones in the presence of aqueous alkali to form α,β unsaturated ketones called chalcones. In this mechanism, the first step is condensation of the aldol type involving the nucleophilic addition of carbanion derived from the aryl ketones to carbonyl carbon of the aromatic aldehydes. Dehydration of the hydroxyl ketones to form the conjugated α,β unsaturated ketones or chalcones.

The structure of parent molecule of chalcones consist of two phenyl rings and one α,β unsaturated double bond. The ring A must contain an electron deficient moiety like ethyl, methyl or alkyl groups for better activity. The ring B must contain the hydrophobic groups like halogens, nitro and cyano for better activity. The unsaturated double bond plays an important role for the activity but marginal modifications in this bond don't have much effect on the activity. Para position of the ring B is important for the activity. The ortho position of ring B also enhances the activity but in comparison with para position it is low. 3D QSAR studies of chalcone proved all these facts.



The group $-\text{CH}=\text{CHC}(=\text{O})-$ is known as the chalcone functionality/moiety or ketoethylenic group, so chalcones are also called α,β -unsaturated carbonyl/ketone systems. All the α,β -unsaturated ketones are not necessarily be chalcones but all the chalcones are α,β -unsaturated. eg: Mesityl oxide (it is an

unsaturated ketone but not a chalcone). Chalcones belong to flavonoid family. Structurally, chalcones are open-chain flavonoids, which were derived by the cleavage of the C ring in the flavonoids as shown. The basic molecular structure of chalcones includes two aromatic rings linked by an unsaturated three carbon bridge. The source of chalcones are mainly from edible plants or can be readily synthesized by the Claisen-Schmidt condensation method.^[23-26]

Materials and Methods

The following experimental methods were used for the characterization of the synthesized compounds.

- The synthesized compounds were subjected to TLC (pre coated silica gel plates).
- Infrared spectra ($\nu\text{-cm}^{-1}$) were recorded on a SHIMADZU FT-IR 4000.
- ^1H NMR spectra were taken on BRUKER AV-III 500MHz FT-NMR Spectrophotometer using TMS as internal standard.

Instruments used:

- Denver single pan electronic balance
- Heating mantle
- Desiccator
- Magnetic stirrer
- Hot air oven

Table 1: List of Chemicals and their structures used for the synthesis

S.N	Compounds	Structure
1	Vanillin	
2	Veratraldehyde	
3	Furfuraldehyde	
4	Anisaldehyde	

5	4-methoxy acetophenone	
6	P-nitro acetophenone	
7	4-methyl acetophenone	
8	4-hydroxy acetophenone	
9	Methanol	$\text{H}_3\text{C}-\text{OH}$
10	Guanidine	
11	Hydrochloric acid	HCl
12	Sodium hydroxide	NaOH

General procedure:

Procedure for Synthesis of some 2,4,6 tri substituted pyrimidine derivatives can be followed by two steps.

First step is synthesis of chalcone.

Second step is condensation of chalcone with compound containing guanidine moiety to obtain the final product.

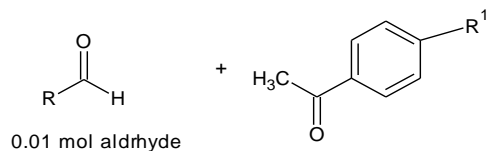
Step-1:

- Aromatic Aldehyde and acetophenone (0.1M) was dissolved in rectified spirit containing beaker, equipped with magnetic stirrer.
- NaOH (20%) solution was added drop wise to the reaction mixture on vigorous stirring for 0.5 hrs.
- Then the solution becomes turbid, the temperature should be maintained between 20-25°C. Stirr the mixture in cold water bath for 4-5 hrs on the magnetic stirrer.
- Reaction mixture was neutralized by 0.1-0.2 N HCl, then the product was precipitated, filter it.
- The obtained crude chalcone was collected, air dried and then recrystallized by using ethanol.

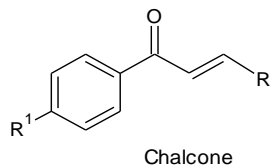
Step-2:

- Equimolar quantities of formed chalcone and compound containing guanidine moiety in methanol was taken.
- Double the quantity of NaOH dissolved in water and added to the reaction mixture.
- Then reflux for 6 hrs, poured in water and recrystallized.

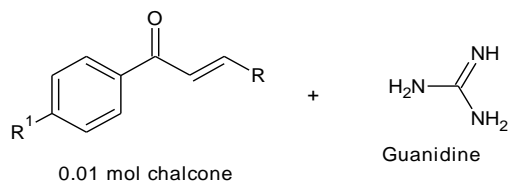
Step 1



NaOH, Ethanol
Stirr for 4 to 5 hrs



Step 2



Methanol(25ml)
HCl(0.5ml)
Reflux 6hrs

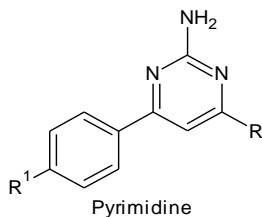
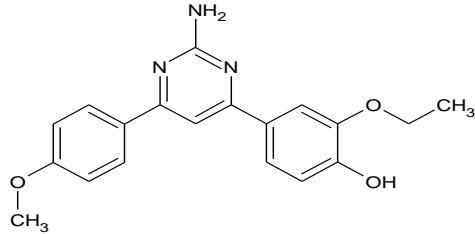
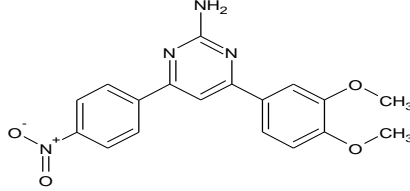
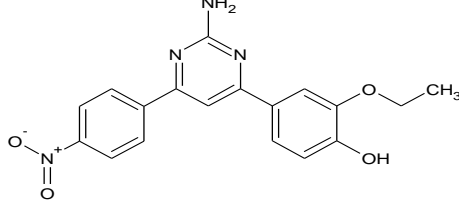
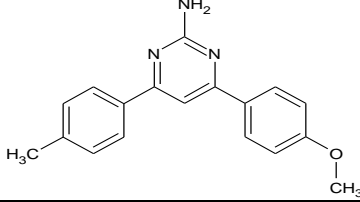
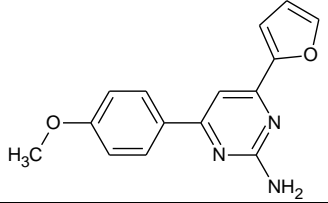
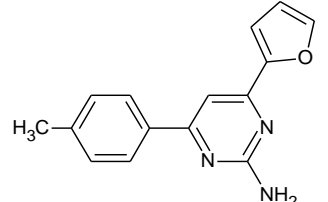
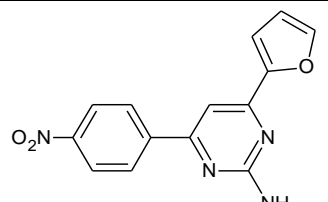


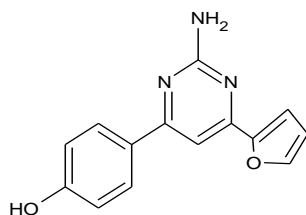
Table.no: 3 Structures and IUPAC Nomenclature of Some Derived Pyrimidines

Compound code	Structure	IUPAC name
SB-1		4-[2-amino-6-(furan-2-yl)pyrimidin-4-yl]phenol
SB-2		4-[2-amino-6-(4-hydroxyphenyl)pyrimidin-4-yl]-2-ethoxyphenol

SB-3		4-[2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-2-ethoxyphenol
SB-4		4-(3,4-dimethoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-amine
SB-5		4-[2-amino-6-(4-nitrophenyl)pyrimidin-4-yl]-2-ethoxyphenol
SB-6		4-(4-methoxyphenyl)-6-(4-methylphenyl)pyrimidin-2-amine
Sb-7		4-(furan-2-yl)-6-(4-methoxyphenyl)pyrimidin-2-amine
SB-8		4-(furan-2-yl)-6-(4-methylphenyl)pyrimidin-2-amine
Sb-9		4-(furan-2-yl)-6-(4-nitrophenyl)pyrimidin-2-amine

IR, ¹H NMR Spectral details of Synthesized Compound (SB-1)

4-[2-amino-6-(furan-2-yl)pyrimidin-4-yl] phenol



IR, ¹H NMR Spectral details of Synthesized Compound (SB-3)
4-[2-amino-6-(4-methoxyphenyl)pyrimidin-4-yl]-2-ethoxyphenol

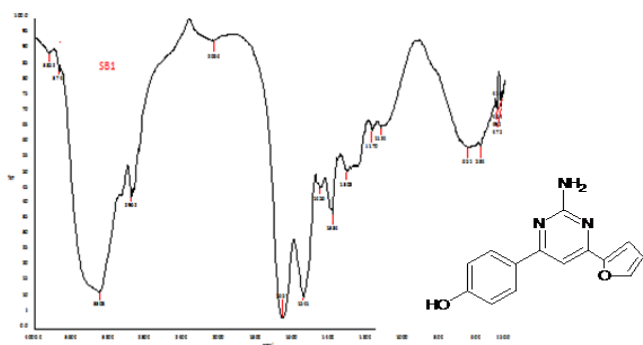


Fig 1: IR Spectral details of Synthesized Compound (SB-1)

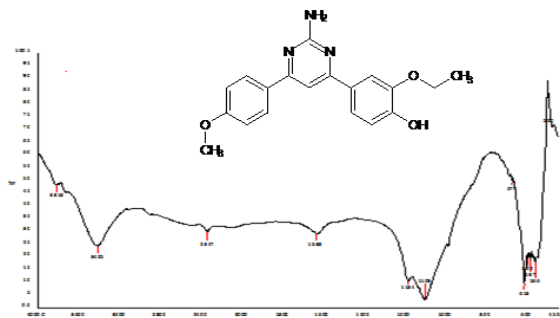


Fig 5: IR, Spectral details of Synthesized Compound (SB-3)

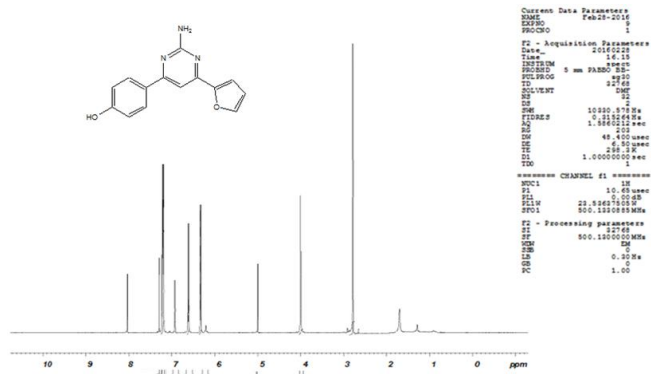


Fig 2: ¹H NMR Spectral details of Synthesized Compound (SB-1)

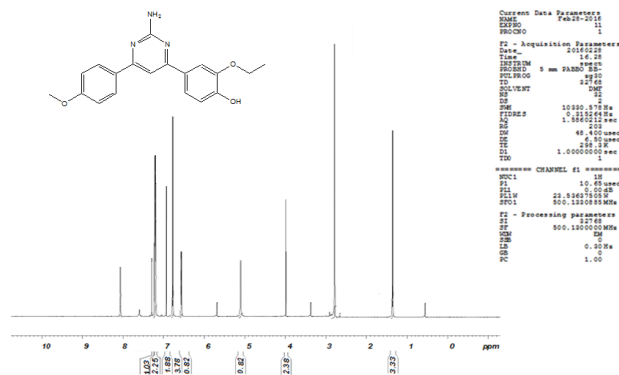


Fig 6: ¹H NMR Spectral details of Synthesized Compound (SB-3)

IR, ¹H NMR Spectral details of Synthesized Compound (SB-2)
4-[2-amino-6-(4-hydroxyphenyl)pyrimidin-4-yl]-2-ethoxyphenol

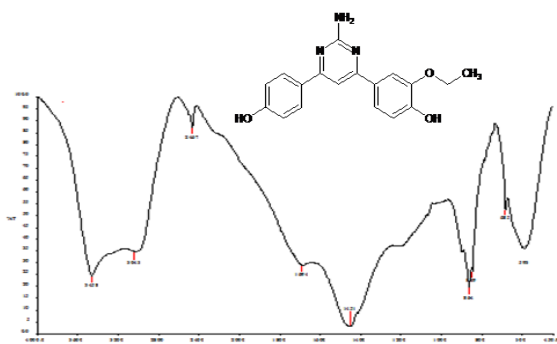


Fig 3: IR spectral details of Synthesized Compound (SB-2)

IR, ¹H NMR Spectral details of Synthesized Compound (SB-4)
4-(3,4-dimethoxyphenyl)-6-(4-nitrophenyl)pyrimidin-2-amine

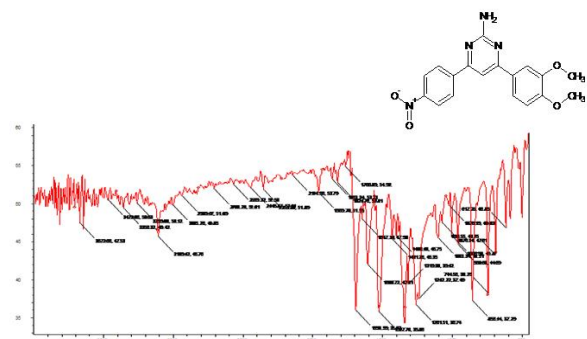


Fig 7: IR Spectral details of Synthesized Compound (SB-4)

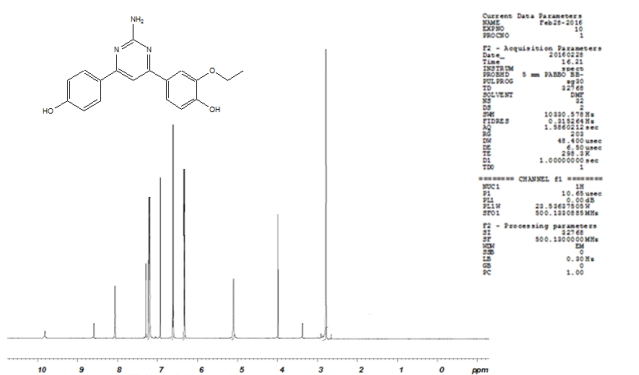


Fig 4: ¹H NMR Spectral details of Synthesized Compound (SB-2)

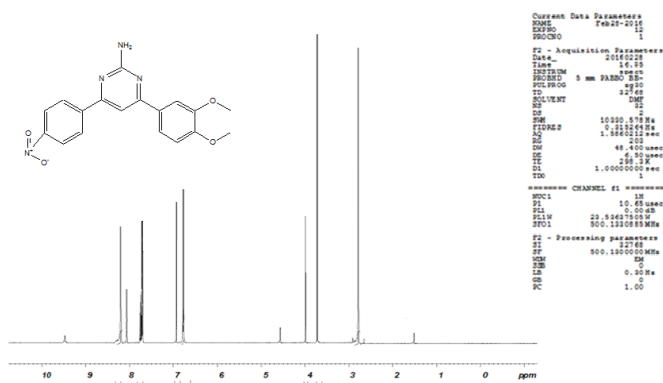


Fig 8: ¹H NMR Spectral details of Synthesized Compound (SB-4)

IR, ¹H NMR Spectral details of Synthesized Compound (SB-5)
4-[2-amino-6-(4-nitrophenyl)pyrimidin-4-yl]-2-ethoxyphenol



Fig :9 IR Spectral details of Synthesized Compound (SB-5)

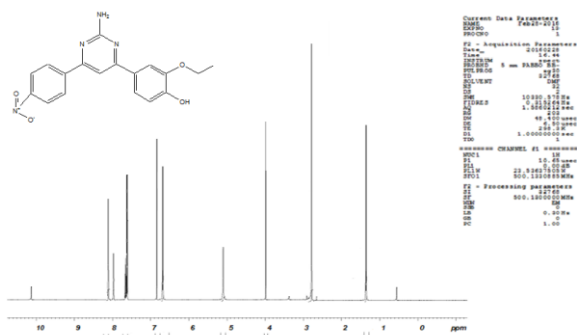


Fig: 10 ¹H NMR Spectral details of Synthesized Compound (SB-5)

Evaluation of Antibacterial Activity

Strains of bacteria used

Gram Positive bacteria: Streptococcus aureus

Gram negative bacteria: Escherichia coli

Standard drug used: Ciprofloxacin

Experimental work:

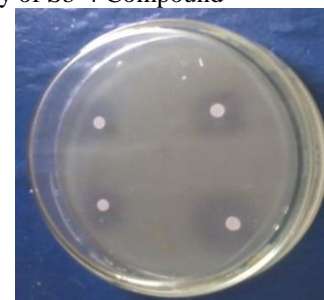
1. Clean and sterilized Petri plates were taken

2. Nutrient agar media was prepared according to following formula
 - Peptone: 0.5gms
 - Yeast extract: 0.3gms
 - Nacl : 0.5gms
 - Nutrient agar: 2gms
 - Distilled water: 100ml
3. The nutrient agar media was sterilized from autoclave
4. After sterilization the nutrient agar media was poured into different bacterial culture petri plates. Mixed well and solidified the culture medium.
5. The solidified petri plates were incubated at 37⁰C for 24hrs.
6. After 24hrs the bacterial culture was developed.
7. The sterilized whatmann filter paper discs were soaked in different test samples as well as standard and dried for 15-20 minutes
8. The dried filter paper discs were placed on the surface of the culture media
9. The standard antibiotic disc was used as reference
10. Again the petri plates were incubated at 37⁰C for 24hrs
11. The diameter of the zone of inhibition was measured and recorded in millimeters.

Anti-Microbial Activity of Sb-4 Compound



E.coli
Fig. No. 59



Staphylococcus aureus
Fig. No. 60

Results and Discussion

A facile method has been devised to synthesize the title compounds where the pharmacophores amino group at 2nd position in the pyrimidine nucleus. The methods include mild conditions and the yields were satisfactory. The course of the proposed reaction was confirmed by TLC. All the synthesized compounds were characterized by FT-IR and ¹H-NMR spectral studies and the structures were established. All the synthesized compounds were predicted for biological properties by using PASS computer programme and screened for Anti-Bacterial activity in *Staphylococcus aureus* & *E.coli* by using Ciprofloxacin as standard (Table No.13).

Table No. 13: Comparison of antibacterial activity with standard

S.No.	<i>Staphylococcus aureus</i> (Gram +ve)				<i>E.coli</i> (Gram -ve)			
	10µg/ml	30µg/ml	40µg/ml	50µg/ml	10µg/ml	30µg/ml	40µg/ml	50µg/ml
SB-1	2	7	11	17	9	16	19	22
SB-2	4	6	9	11	7	10	13	15
SB-3	3	4	6	11	7	11	15	17
SB-4	9	11	17	22	12	18	24	26
SB-5	5	8	13	19	11	18	22	23
SB-6	2	5	9	16	8	12	15	18
SB-7	4	6	8	12	10	11	15	19
SB-8	6	7	9	14	11	13	16	22
SB-9	7	9	10	16	10	14	18	20
STD	Ciprofloxacin sensitive at 10 µg/ml for <i>E.coli</i> is 32mm and <i>Staphylococcus aureus</i> is 27mm.							

From the results of Anti-bacterial activity, it was clear that the compounds SB-4, SB-5, SB-8 & SB-9 exhibit significant activity in comparison with that of standard. This may be due to the presence of electron donating groups like Nitro group at position -6 of the parent molecule.

S.no	Compound	Molecular formula	Molecular weight	Composition	Percentage yield	R _f value	Melting point (°C)
1	SB-1	C ₁₄ H ₁₁ N ₃ O ₂	253.25	C(66.40%) H(4.38%) N(16.59%) O(12.63%)	71	0.676	270
2	SB-2	C ₁₈ H ₁₇ N ₃ O ₃	323.34	C(66.86%) H(5.30%) N(13.00%) O(14.84%)	68	0.803	262
3	SB-3	C ₁₉ H ₁₉ N ₃ O ₃	337.37	C(67.64%) H(5.68%) N(12.46%) O(14.23%)	68	0.816	286
4	SB-4	C ₁₈ H ₁₆ N ₄ O ₄	352.34	C(61.36%) H(4.58%) N(15.90%) O(18.16%)	69	0.796	254
5	SB-5	C ₁₈ H ₁₆ N ₄ O ₄	352.34	C(61.36%) H(4.58%) N(15.90%) O(18.16%)	66	0.786	260
6	SB-6	C ₁₈ H ₁₇ N ₃ O	291.34	C(74.20%) H(5.88%) N(14.42%) O(5.49%)	65	0.696	246
7	SB-7	C ₁₅ H ₁₃ N ₃ O ₂	267.28	C(67.40%) H(4.90%) N(15.72%) O(11.97%)	58	0.712	218
8	SB-8	C ₁₅ H ₁₃ N ₃ O	251.28	C(71.70%) H(5.21%) N(16.72%) O(6.37%)	56	0.724	184
9	SB-9	C ₁₄ H ₁₀ N ₄ O ₃	282.25	C(59.57%) H(3.57%) N(19.85%) O(17.01%)	68	0.623	236

Conclusion

All the titled compounds were synthesized, characterized and screened for their anti-bacterial activity. The results of Anti-bacterial activity revealed that all the titled compounds exhibit significant activity. The compounds SB-4, SB-5, SB-8 & SB-9 exhibit significant activity in comparison with that of standard. This may be due to the presence of electron donating groups like Nitro group at position -6 of the parent molecule. Further studies involves QSAR modeling and Docking studies of titled compounds.

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