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Studies on Inclusion Complexes of 2-(Benzothiazolyl-2')-hydrazono-3-phenyl-5-arylidene-4-thiazolidinone Derivatives with β-Cyclodextrin

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A series of 2-(benzothiazolyl-2')-hydrazono-3-phenyl-5-arylidene-4-thiazolidinone derivatives have been synthesized starting from 2-hydrazinobenzothiazole.To enhance the solubility and bioaccessibility of these synthesized pharmacophores the inclusion complexes have been prepared with β-cyclodextrin. The synthesis of compounds and their inclusion complexes have been ascertained from their changes in physical, thermal and spectral characteristics (UV, IR and NMR). The stability constant and free energy change of the inclusion complexes have also been determined. The study suggests that inclusion complexes are appreciably stable and thermodynamically allowed. The synthesized compounds and their inclusion complexes have been screened for their possible antibacterial and antioxidant activities. The results revealed that compounds show better antibacterial and antioxidant activities after inclusion complex formation.

Keywords: 4-Thiazolidinone, β-Cyclodextrin, Phase solubility study, Inclusion complexes, Antibacterial, Antioxidant study.

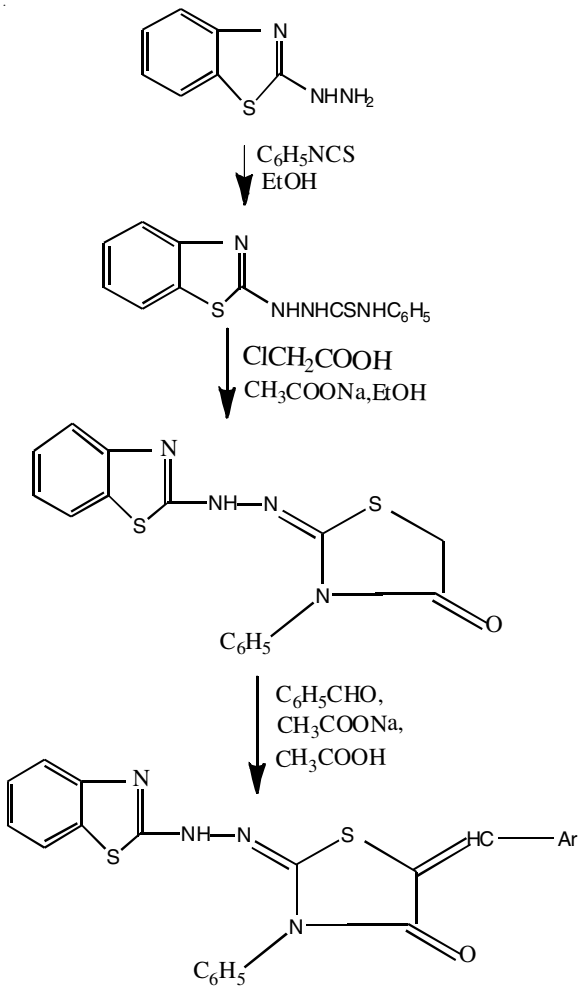
INTRODUCTION

Due to the need of present society, the research has been carried out with the important heterocycles with active pharmacophore moiety. Thiazolidinones and their derivatives have importance in the field of medicinal chemistry due to their wide range of chemotherapeutic uses. The substituted thiazolidinones are well known for their biological activities such as antimicrobial [1,2], antioxidant [3], anti-HIV [4], antihistaminic [5], anticonvulsant [6,7], anti-inflammatory [8-10]. The present work has emphasized on the synthesis of a series of 2-(benzothiazolyl-2')hydrazono-3-phenyl-5-arylidene-4-thiazolidinone derivatives starting from 2-hydrazinobenzothiazoles. Due to the poor solubility of the compounds, they are unable to show significant bioaccessibility. The drugs are encapsulated in the hydrophobic cavity of cyclodextrin [11] and this leads to an increase in the biological activities. The cavity shape of the cyclodextrins are considered as the most appropriate synthetic model host cavities, which make available water insoluble guests for encapsulation, thereby making them water soluble [12]. Among various cyclodextrins, β-cyclodextrin is the suitable one for inclusion complex formation because the cavity size of the β-cyclodextrin is appropriate for heterocyclic compounds [13]. Inclusion complexes are prepared by encapsulating the synthesized (2-benzothiazolyl-2')-hydrazono-3-phenyl-5-arylidene-4-thiazolidinone derivatives with β-cyclodextrin. The development of the compounds and their inclusion complexes have been established by the study of their physical, thermal and spectral data (UV, IR and NMR).The thermodynamic stability constants and the free energy change of the inclusion complexes are determined. The impact of naked compounds and their inclusion complexes on antibacterial and antioxidant activities have also been studied.

EXPERIMENTAL

All the chemicals used are procured with analytical reagent grade. Double distilled water is used as the solvent for dilution was prepared in the laboratory. The electronic spectra were recorded on Shimadzu UV-1700 Spectrophotometer while IR-spectra were recorded in KBr pellets in 4000-400 cm^{-1} region on a Shimadzu 8400 FTIR Spectrophotometer. ^1H NMR spectra ($CDCl_3$) are scanned on a DRX-300 (300 MHz) spectrophotometer using TMS as internal standard and chemical shifts are expressed in δ scale. Purity of synthesized compounds has been checked by sulphur detection and homogeneity has been checked by TLC using silica gel. Melting points are recorded by open capillary method and are uncorrected.

Synthesis of compounds: The compounds were synthesized as describe by Garnaik and Mishra [14] (**Scheme-I**).



Ar = phenyl(compound- **A**)
= *p*-chloro phenyl(compound -**B**)
= *p*-methoxy phenyl(compound-**C**)

Scheme-I

Step-I: 1-(Benzothiazolyl-2')-4-phenyl thiosemicarbazide: A mixture of 2-hydrazinobenzothiazole (1.65 g, 10 mmol) in ethanol (10 mL), phenyl isothiocyanate (1.35 g, 10 mmol) was added with stirring during a period of 5 min. The resulting solution was refluxed with stirring with 0.5 h and cooled. The crystals obtained were filtered and recrystallized from ethanol. m.p. 179 °C, yield 2.1 g (70 %), (Found S, 21.2 %, $C_{14}H_{22}N_4S_2$ requires S, 21.4 %)

Step-II: 2-(Benzothiazolyl-2')-hydrazono-3-phenyl- 4-thiazolidinone: A mixture of 1-(benzothiazolyl-2')-4-phenyl thiosemicarbazide (0.06 g, 2 mmol), monochloroacetic acid (0.25 g, 2 mmol) and anhydrous sodium acetate (0.2 g) in absolute ethanol (10 mL) were refluxed for 3 h. The excess of solvent was removed and poured into cold water. The solid obtained was filtered, washed with hot water, dried and recrystallized from ethanol, m.p. 166 °C, yield 0.3 g (44 %), (Found: S, 18.80 % $C_{16}H_{12}N_4OS_2$ requires S,18.95 %). IR (KBr, ν_{max}, cm^{-1}): 746.45 (C-S str), 1487.12 (C=C str) 1556.55 (C=N str), 1714.72 (C=O str), 3030.17 (Ar-H str), 3226.91 (N-H str).

Step-III: 2-(Benzothiazolyl-2')-hydrazono-3-phenyl-5-arylidene-4-thiazolidinone (Compound-A): A mixture of 2-(benzothiazolyl-2')-hydrazono-3-phenyl-4-thiazolidinone (0.35 g, 2 mmol), benzaldehyde (0.22 g, 2 mmol), fused sodium acetate (1 g) in glacial acetic acid (15 mL) was heated under reflux for 4 h. The pale yellow solution after cooling to room temperature was poured into ice cold water when yellow solids separated out. The crude product was filtered, washed with water and recrystallized from ethanol, m.p. 122 °C, yield-0.28 g (63 %). (Found: S, 14.07 % $C_{23}H_{17}N_4OS_2$ requires S, 14.91 %). Similarly the other compounds of the series were prepared by using *p*-chloro benzaldehyde (compound **B**) and *p*-methoxy benzaldehyde (compound **C**), respectively.

Aqueous phase solubility study: The aqueous phase solubility of all the compounds have been studied with different concentration of β-cyclodextrin (0-10 mM) with accurately weighed compound in different conical flasks as per Higuchi Connors method [15].

Synthesis of inclusion complexes: The inclusion complexes of the synthesized compounds (**A**, **B** and **C**) with β-cyclodextrin were prepared as per co-precipitation method [16-18].

Evaluation of antibacterial activity: As per cup-plate method [19,20] the antibacterial activity of compounds was carried out. Dimethyl sulphoxide at 500 µg/mL were taken and solutions of synthesized compounds and inclusion complex is prepared with a conc. of 500 µg/mL. Three types of bacteria namely *E. coli*, *S. aureus* and *P. vulgaris* were inoculated into 100 mL of the sterile nutrient broth and incubated for 24 h at at 37 °C. Uniform diameter of agar plates were used to inoculate them one by one with the test organisms aseptically. With the help of micropipette drug and test compounds solution is taken and then plates were placed in the refrigerator at 8-10 °C for right dispersal of drug into the media. The Petri plates were transferred to incubator after 2 h and maintained at 38 °C for 22 h. Then the Petri plates were observed for zone of inhibition by using vernier scale and the data were observed.

Evaluation of antioxidant activity: As suggested by Tagashira and Ohtake [21], the antioxidant activity of the synthesized compounds was studied. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) method is used for screening the compound. Ethanolic DPPH having 100 µg/mL concentration is used to prepare sample solution for test. The mixture is incubated for 10 min at room temperature After vortexing with 100 µg/mL concentration. The absorbances of the samples are calculated at 517 nm. The difference of absorbance between a test sample and a control is the activity of the sample. Here the reference substance used is butylated hydroxyl toluene (BHT).

RESULTS AND DISCUSSION

The encapsulation of the compounds in the cavity of β-cyclodextrin is confirmed from the changes in melting point, colour and spectral characteristics with respect to their compounds (Tables 1 and 2). From the melting point data it is observed that inclusion complexes are having higher value than that of compounds due to the fact that it requires an additional thermal energy to carry the compounds from the cavity of the β-cyclodextrin.

TABLE-1
PHYSICAL PROPERTIES OF COMPOUNDS AND THEIR INCLUSIONS

Compound/Complex	Substituent	Colour	m.p. (°C)	Yield (%)
Compound A	Phenyl	Yellow	122	63
Compound A with β-cyclodextrin		Yellowish white	145	45
Compound B	*p*-Chloro phenyl	Brown	190	65
Compound B with β-cyclodextrin		Pale brown	205	50
Compound C	*p*-Methoxy phenyl	Yellow	201	63
ompound C with β-cyclodextrin		Yellowish white	225	48

TABLE-2
SPECTRAL DATA OF SYNTHESIZED COMPOUNDS AND THEIR INCLUSIONS

Compound/complexes	UV λ_{max}	IR (KBr, ν_{max}, cm^{-1})	^1H NMR
Compound-A	275	742.59 (C-S str), 1492.60 (C=C str), 1589.34 (C=N str), 1645.28 (C=O str), 3194.12 (N-H str)	^1H NMR ($CDCl_3$): δ 6.8-8.2 (d, 6H, Ar-H), 4.2 (s, 1H, C-NH), 7.58 (s, 1H, C-H), 7.3-7.6 (t, 8H, Ar-H)
Compound-A with β-CD	278	746.45 (C-S str), 1494.83 (C=C str), 1581.83 (C=N str), 1714.12 (C=O str), 3224.34 (N-H str)	^1H NMR ($CDCl_3$): δ 6.1-7.8 (d, 6H, Ar-H), 3.8 (s, 1H, C-NH), 7.11 (s, 1H, C-H), 6.8-7.2 (t, 8H, Ar-H)
Compound-B	265	692.44 (C-Cl str), 744.52 (C-S str), 1487.12 (C=C str), 1583.56 (C=N str), 2916.37 (Ar-H str) 1701.22, 1645.28 (C=O str), 3197.89 (N-H str)	^1H NMR ($CDCl_3$): δ 6.95-8.6 (d, 6H, Ar-H), 4.4 (s, 1H, C-NH), 7.80 (s, 1H, C-H), 7.56-7.9 (t, 8H, Ar-H)
Compound-B with β-CD	267	692.44 (C-Cl str), 746.45 (C-S str), 1489.05 (C=C str), 153 9.20 (C=N str), 1714.72 (C=O str), 3030.17 (Ar-H str), 3325.28, 3194.12 (N-H str)	^1H NMR ($CDCl_3$): δ 6.3 -7.45 (d, 6H, Ar-H), 3.9 (s, 1H, C-NH), 7.25 (s, 1H, C-H), 6.9-7.3 (t, 8H, Ar-H)
Compound-C	296	748.38 (C-S str), 1425.40 (C=N str), 1494.83 (C=C str), 1593.20 (C=N str), 1712.97 (C=O), 3062.96 (Ar-H str)	^1H NMR ($CDCl_3$): δ 6.6-8.5 (d, 6H, Ar-H), 4.3 (s, 1H, C-NH), 7.65 (s, 1H, C-H), 7.3-7.6 (t, 8H, Ar-H), 3.95 (s, 3H, OCH_3)
Compound-C with β-CD	299	748.38 (C-1417.60 (C-N str), 1456.26 (C=C str) 1508.33 (C=N str), 1699.29, 1637.56 (C=O str), 3331.07 (N-H str)	^1H NMR ($CDCl_3$): δ 6.1-7.8 (d, 6H, Ar-H), 3.7 (s, 1H, C-NH), 7.5 (s, 1H, C-H), 6.6-7.1 (t, 8H, Ar-H) 3.65 (s, 3H, OCH_3)

In case of IR data of compound **A** it is seen that the IR frequencies are found at 742.59 (C-S str), 1492.60 (C=C str), 1589.34 (C=N str), 1645.28 (C=O str), 3194.12 (N-H str) indicating the presence of C-S, C=C, C=N, C=O and N-H in the compound as expected and similarly the IR data of inclusion complexes of compound **A** show characteristics absorption at 746.45 (C-S str), 1494.83 (C=C str), 1581.83 (C=N str) 1714.12 (C=O str), 3224.34 (N-H str) indicating the presence of C-S, C=C, C=N, C=O and N-H in the compounds. Absorption at suitable characteristic frequencies of IR data of compounds **B**, **C** and their inclusion complexes are shown in Table-2. For compounds **C**, the IR frequency of carbonyl group (C=O) shifted towards lower energy side. For all inclusion complexes there is a remarkable change in the IR spectrum (broader and smoother) as the compounds are encapsulated into the cavity of β-cyclodextrin. The host and guest molecules are interacted with each other through H-bonding and van der Waals forces [22]. It can be concluded that the δ values of the inclusion complexes are having low value as compare to their respective compounds. This means PMR signals are shifted towards up field in the inclusion complex due to the notable shielding factor which arise through encapsulation within the cavity of β-cyclodextrin.

From the graphs of aqueous phase solubility studies, it can be concluded that there is a linear increase in solubility of the compounds with respect to concentration of β-cyclodextrin (Fig. 1). The stoichiometry of these complexes may be 1:1 as the values of all the slopes of plots were less than unity. In order to calculate the thermodynamic stability constants (K_T) of inclusion complexes, Benesi-Hilderband relation is used [20].

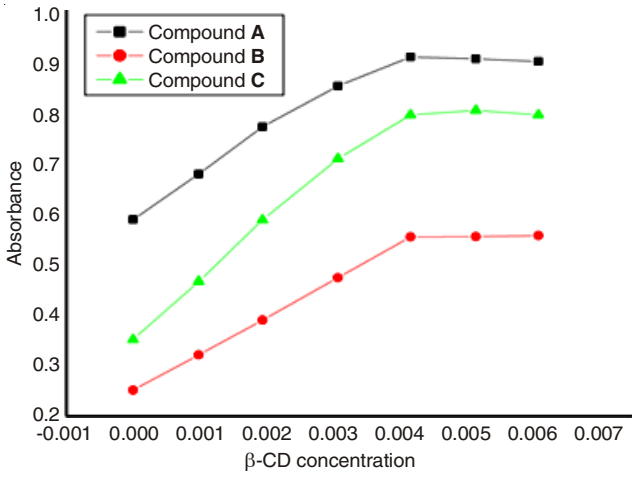


Fig. 1. Variation of absorption with concentration

$$1/\Delta A = 1/\Delta\epsilon + 1/K_T\,[Guest]_o\,\Delta\epsilon \cdot [\beta\text{-}CD]_o$$

where ΔA is change in absorbance, Δϵ is change in molar extension coefficient, $[Guest]_o$ is concentration of compound in inclusion complex and $[\beta\text{-}CD]_o$ is molar concentration of β-cyclodextrin.

Plot of $1/\Delta A$ *versus* $1/[\beta\text{-}CD]_o$ for compounds show appreciable linear correlations (Fig. 2). Using the equation K_T = Intercept/Slope, the values of thermodynamic stability constant K_T are determined. The K_T values of the inclusion complexes of compounds with β-cyclodextrin were found to be 526.13, 399.66 and 533.94 M^{-1}, respectively (Table-3). All the data were remaining within the ideal range of 100 to 1000 M^{-1} signifying stabilities for the inclusion complexes through weak molecular interactions [23-25].

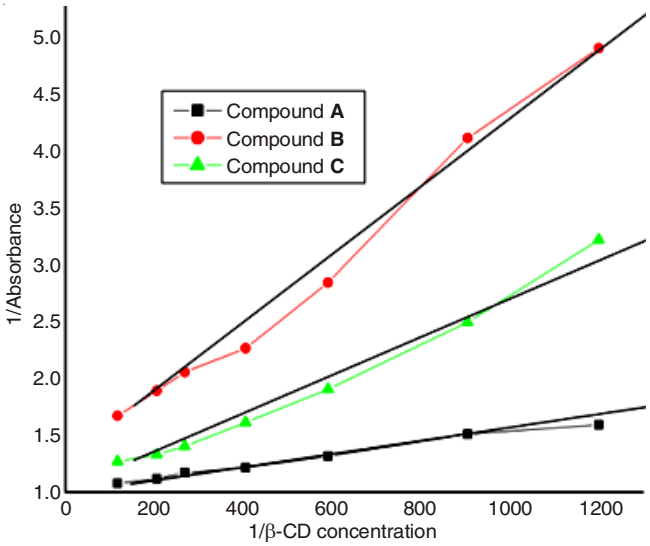


Fig. 2. Variation of 1/absorption with 1/concentration

TABLE-3
EQULIBRIUM CONSTANT AND FREE ENERGY CHANGE OF INCLUSION COMPLEXES

Inclusion complex of compound	Equlibrium constant (K_T)	ΔG (kJ/mol)
Inclusion complex **A**	526.123	-15.627
Inclusion complex **B**	399.666	-14.941
Inclusion complex **C**	533.941	-15.756

The ΔG value can be calculated at 298 K using the equation:

$$\Delta G = -2.303RT \log K$$

The value of free energy of activation has been calculated and found to be -15.627, -14.941 and -15.756 kJ/mol (Table-3) for the inclusion complexes of Compound **A**, **B** and **C**, respectively. In all cases we obtained negative values of ΔG, which make the process thermodynamically favourable.

From the graphs of antibacterial studies against three bacterial strains namely *E. coli*, *S. aureus* and *P. vulgaris*, it is found that the diameter of the zone of inhibition of inclusion complexes noticeably high as compare to the compounds shown in Figs. 3-5. Among the tested substances the inclusion

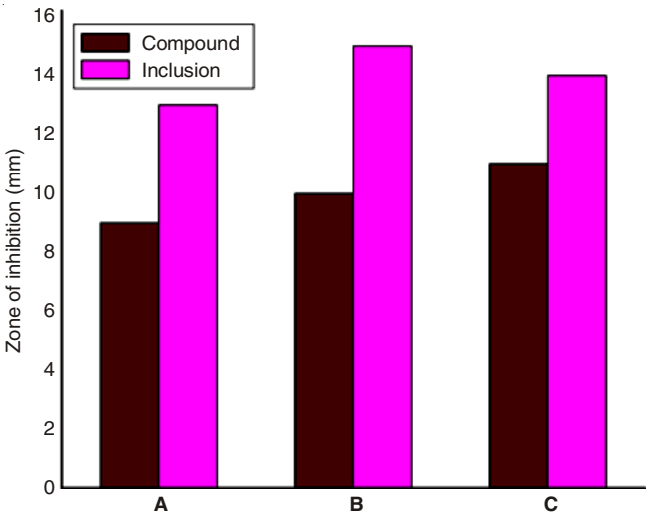


Fig. 3. Antibacterial activity of the compound and inclusion complex against *S. aureus*

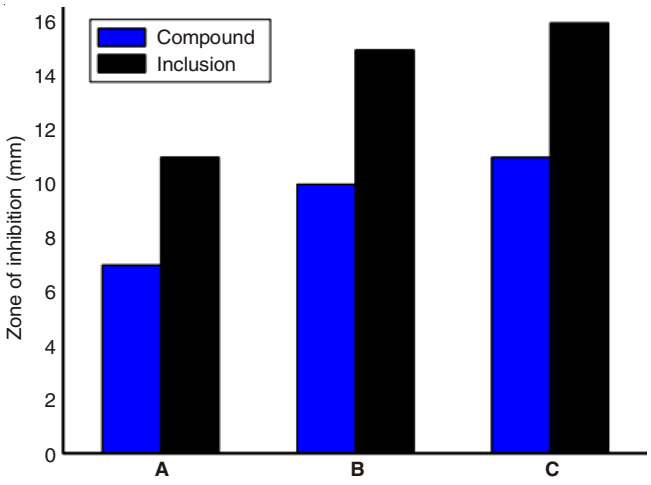


Fig. 4. Antibacterial activity of the tested compounds and their inclusions against *E. coli*

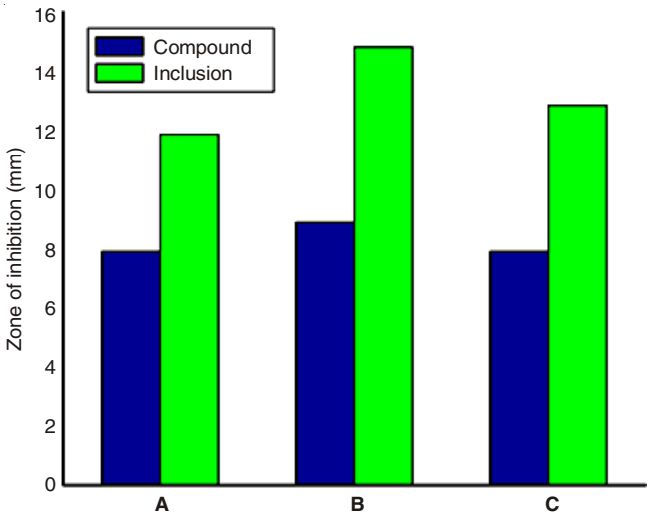


Fig. 5. Antibacterial activity of the compound and inclusion complex against *P. vulgaris*

complex of compound **C** exhibited maximum activity against *S. aureus* than that of other complexes whereas compound **B** shows maximum activity against *E. coli* and *P. vulgaris* with respect to other complexes. The remarkable enhancement of antibacterial activity of the inclusion complexes due to their solubility in the aqueous medium which makes them more bio-accessible and effective towards specific tissues thereby increasing drug efficiency. After encapsulation, the radical scavenging activity of the compound increases significantly (Table-4). This can be associated with higher solubility

TABLE-4
ANTIOXIDANT ACTIVITY OF SYNTHESIZED COMPOUNDS AND THEIR INCLUSIONS

Compound/Complex	Conc. (500 µg/mL) % of inhibition	Conc. (100 µg/mL) % of inhibition
Compound-A	34.6	28.80
Inclusion with β-CD	47.8	35.87
Compound-B	28.7	22.60
Inclusion with β-CD	44.4	31.80
Compound-C	36.5	27.60
Inclusion with β-CD	51.6	36.80
Ascorbic acid	90.0	74.30

of the compounds due to inclusion complex formation there by increasing the bioaccessibility. The free radical capturing capacity and interaction with reactive oxygen species of the compounds improved with the increase in bio-accessibility thereby increasing antioxidant activity of the compounds [26].

Conclusion

From the above experimental observation, it is accomplished that the solubility and bio-accessibility of the synthesized compounds are increased appreciably by the formation of inclusion complexes with β-cyclodextrin which can be used as an important tool to increase therapeutic potential of the synthesized drugs. The formation of inclusion complexes are thermodynamically allowed and having higher stability. The antibacterial and antioxidant activities of the compounds have been appreciably enhanced after formation of the inclusion complex.

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