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Chapter 1: Tryptanthrin incorporated N/S/O heterocycles–synthesis and biological properties

Ani Deepthi,* Sruthi S. L., Devika Krishnan

Abstract: The chapter describes the synthesis and biological properties of spiroheterocycles derived from tryptanthrin, a natural quinazoline alkaloid possessing numerous biological properties. Spiroheterocycles were prepared through multicomponent reactions following green protocols and were evaluated for antibacterial and anticancer properties. The noticeable potential of these molecules warrants further attention to natural quinazoline alkaloid derivatives as sources for lead compounds.

Keywords: antibacterial, anticancer, quinazoline, spiroheterocycles, tryptanthrin.

1 Introduction

Tryptanthrin and its derivatives are naturally occurring indolo[2,1-*b*]quinazoline alkaloids that have attracted considerable interest owing to their broad spectrum of biological and pharmaceutical activities (Jao et al., 2008; Bandekar et al., 2010; Yang et al., 2013; Jun et al., 2015). The history of tryptanthrin (6,12-dihydro-6,12-dioxoindolo[2,1-*b*]quinazoline) can be traced back to early 19th century, however first literature paper seems to be that published in 1879 (von Sommaruga, 1879) and all the earlier reports were associated with the isolation of the compound from natural and synthetic indigo (O'Neill, 1892; Perkin, 1906; Bloxam, 1905; Friedländer, 1915).

Ani Deepthi
Assistant Professor, Department of Chemistry, University of Kerala, Kerala, India.
anideepthi@gmail.com
Sruthi S. L.
Department of Chemistry, University of Kerala, Kerala, India.
Devika Krishnan

Tryptanthrin possess several biological properties including antifungal, antibacterial, antiviral, anticancer, antiangiogenic, anti-inflammatory, anti-leishmanial, antimalarial among others (Kaur et al., 2017). Pharmacokinetic profile of tryptanthrin is quite promising due to its good oral absorption and tissue distribution and its ability to cross blood-brain barrier (Jähne et al., 2016). Figure 1 shows the structure of tryptanthrin and selected quinazoline alkaloids possessing biological activity.

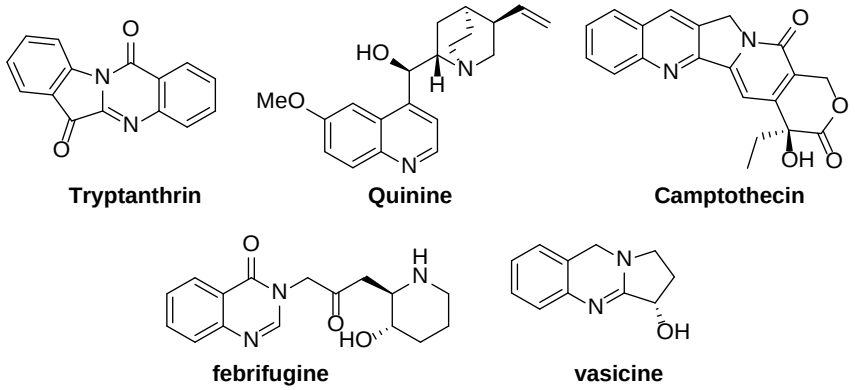


Fig. 1 Biologically active quinazoline alkaloids

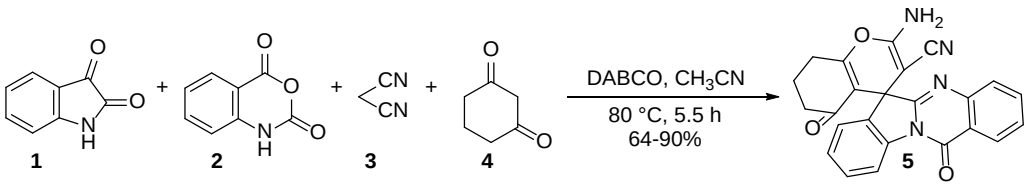
The interesting properties of tryptanthrin encouraged chemists to isolate the compound from various sources which includes plants (*Strobilanthes cusia*, *Isatis tinctoria*, *Couroupita guianensis*, *Strobilanthes formosanus* and *Wrightia tinctoria*), fungi (*Schizophyllum commune* and *Leucopaxillus cerealis*), yeast *Candida lipolytica* and mammals (urine of the Asian elephant, and from the wing sac liquid of *Saccopteryx bilineata* (Tucker & Grundt, 2012). Albeit, various methods have also been developed to synthesize tryptanthin in labs (Witt & Bergman, 2003; Wang et al., 2007; Jahng, 2013). Reviews on tryptanthrin mainly focussing on its synthesis, physicochemical and biological properties and its structure-activity relationships (SAR) were published in literature from time to time. Tucker and co-workers have reviewed the synthesis of tryptanthrin and related compounds (Tucker & Grundt, 2012). Very recently its biological importance was reviewed by Kaur in 2017 (Kaur et al., 2017), Brandão in 2022 (Brandão, 2022) and more specifically the anticancer properties of tryptanthrin were reviewed by Zhou in 2024 (Zhou 2024).

The current chapter focus on the anticancer and antibacterial properties of tryptanthrin and its derivatives, specially the spiroheterocycles derived from them. As is well-known antimicrobial resistance (AMR) is a public threat of late and it is estimated that globally 5 million people are dying of AMR related conditions in a year (O'Neill, 2014). The chief reasons behind AMR are limiting uptake of drug, drug inactivation

and drug target alterations as per WHO. This silent pandemic is expected to kill 10 million people every year by 2050 if urgent means are not adopted to tackle it (Murray et al., 2022). In view of this, we would like to highlight the efficacy of tryptanthrin compounds as antibacterial agents in addition to their anticancer properties. The following sections will discuss about the various spiroheterocycles containing tryptanthrins.

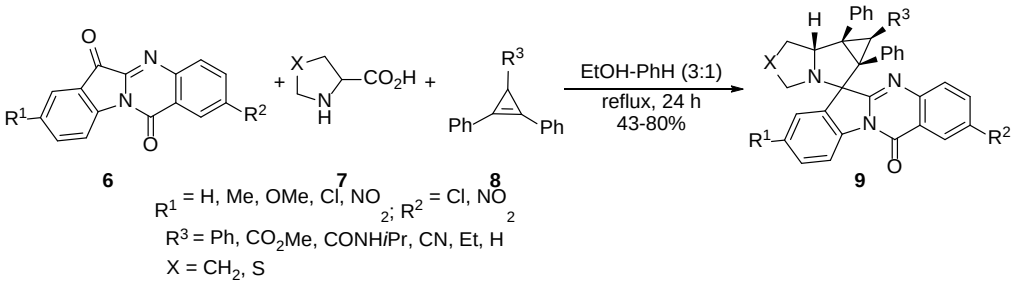
2 Literature review

Earlier research on tryptanthrin was mainly focussed on its isolation, synthesis and biological evaluation. The carbonyl group of tryptanthrin mostly resembles *C*-3 carbonyl of isatin and so many of its reactions have close similarity to that of isatins. The synthesis of γ-spirolactones from tryptanthrins using triphenylphosphine and dimethyl acetylene dicarboxylate was reported in 2004 (Azizian et al., 2004). In another report, nitrile imines (generated from the corresponding hydrazonyl chlorides) could efficiently add to tryptanthrin leading to tryptanthrin-oxadiazole hybrids (Yavari et al., 2019). Hasaninejad and co-workers in 2017, described an efficient four-component reaction of spiroindoloquinazolines in presence of DABCO (1,4-diazabicyclo[2.2.2]octane) (Beyrati & Hasaninejad, 2017). In this reaction, tryptanthrin was generated *in situ* from isatin **1** and isatoic anhydride **2** in presence of DABCO which subsequently underwent Knoevenagel condensation with malononitrile to yield the corresponding ylidene. Michael addition of the enolate formed from carbonyl compound **4** to the ylidene followed by cyclization yielded the final product **5** (Scheme 1).



Scheme 1 Synthesis of spiropyrans

In 2019, Filatov et al., explored the use of natural alkaloid tryptanthrin and its derivatives for the azomethine ylide generation for the first time (Filatov et al., 2019). The *in situ* generated azomethine ylide from tryptanthrin **6** and α-aminoacid **7** underwent 1,3-dipolar cycloaddition with various cyclopropene derivatives **8** yielding the corresponding spiro derivatives **9** (Scheme 2). The spiroadducts displayed activities around or below 10 μmol/L in the *in vitro* MTS-assay of erythroleukemia (K562), cervical carcinoma (HeLa) and colon carcinoma (CT26) cell lines.



Scheme 2 Synthesis of fused cyclopropenes

3 Methods and materials

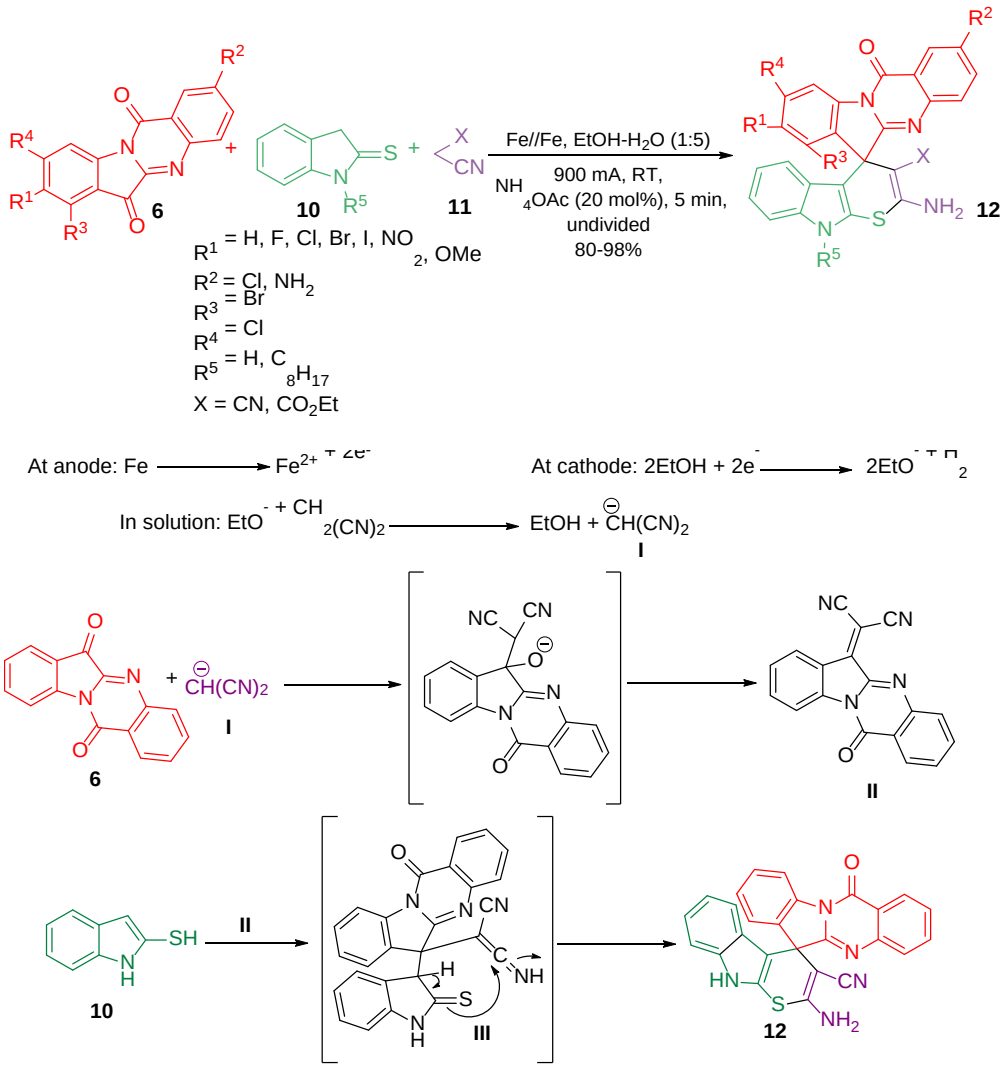
All solvents and reagents used were procured from commercial chemical suppliers in India and were used as such without further purification. Analytical thin layer chromatographic technique (TLC) was used to monitor reactions and TLC plates were visualized using ultraviolet (UV) light of wavelength 366 nm. Gravity column chromatography was used with silica gel (100–200 mesh) as stationary phase, and mixtures of hexane and ethyl acetate as mobile phase. NMR spectra were recorded using Bruker AVANCE III HD 400 MHz spectrometer. Chemical shifts are given in ppm with respect to tetramethylsilane (TMS) which was the internal standard. Infrared (IR) spectra were recorded using Agilent Cary 630 Fourier transform infrared (FTIR) spectrometer.

4 Results and Discussion

4.1 Tryptanthrin incorporated N/S heterocycles

Work in our lab, led to the synthesis of tryptanthrin-thiopyrano[2,3-*b*]indole hybrids of the type **12** under electrochemical conditions, and the compounds were studied for their antibacterial and anticancer properties (Leena et al., 2022). Tryptanthrin **6**, indoline-2-thione **10** and malononitrile **3**/**11** were reacted under electrochemical conditions using ammonium acetate as supporting electrolyte in ethanol-water solvent mixture by passing 900 mA current which resulted in the desired thiopyran compound **12** (Scheme 3). Out of the 24 compounds synthesized, the nitro substituted tryptanthrin appended thiopyran was found to be the lead molecule against *S. aureus* ATCC 29213, demonstrating a high selectivity index and synergized with linezolid. The compound also exhibited good metabolic stability, and promising ADMET properties. Furthermore, *in vitro* anticancer studies revealed that nitro substituted tryptanthrin

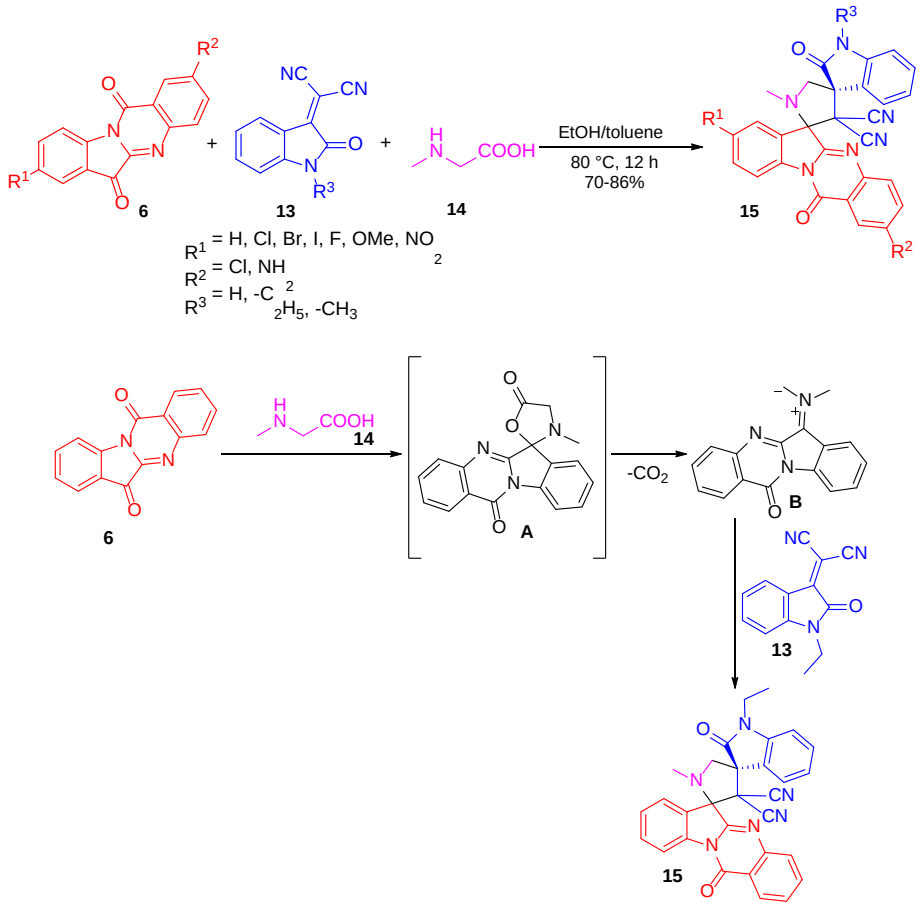
appended thiopyran exhibited strong activity against HCT116 cells with an IC_{50} of 1.94 μg/mL. These results indicate that nitro substituted tryptanthrin appended thiopyran has a great therapeutical advantage and can be progressed to further evaluation. The possible mechanism for formation of the product is explained in the following lines. Initially, reduction of ethanol at cathode forms ethoxide anion through deprotonation of ethanol, which picks a proton from active methylene group in malononitrile to yield dicyanomethanide **I**. Later, Knoevenagel condensation of tryptanthrin with **I** generates ylidene **II,** which simultaneously undergoes Michael addition with enol form of indoline-2-thione **10** to yield ketimine intermediate **III**, which finally cyclizes to give final product **12**.



Scheme 3 Synthesis of tryptanthrin-thiopyrano[2,3-*b*]indole hybrids

4.2 Tryptanthrin incorporated spiropyrrolidines

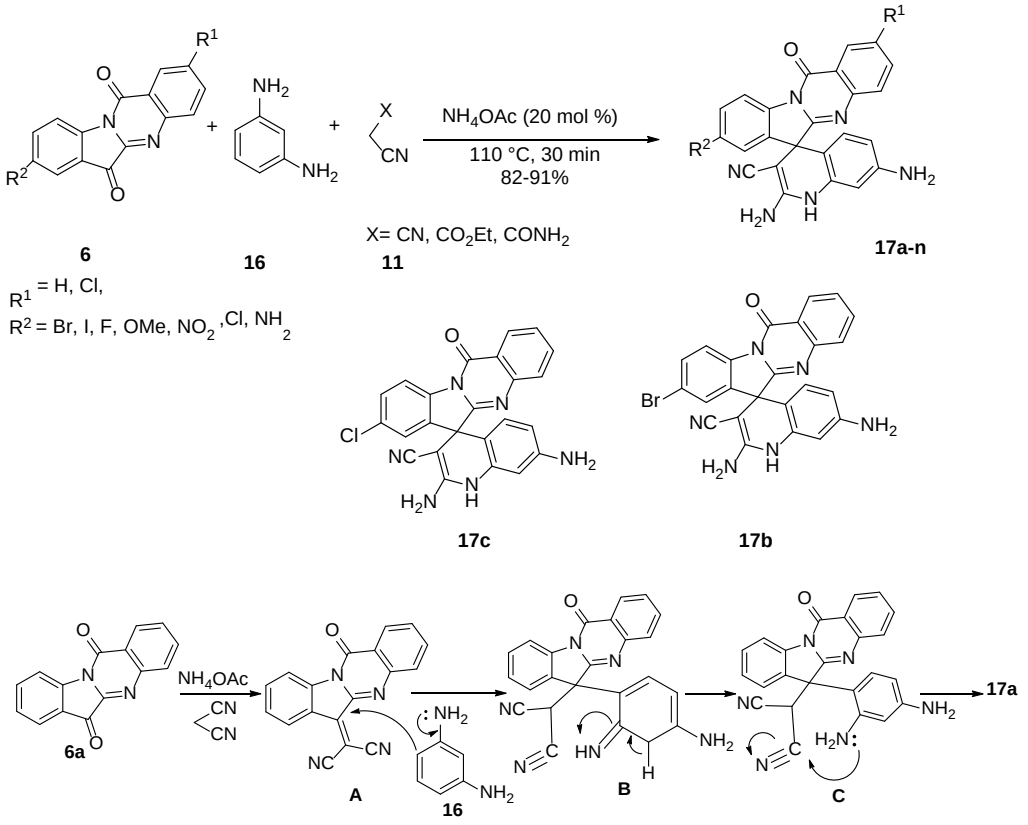
Synthesis of dispiropyrrolidines **15** from azomethine ylides (generated from tryptanthrin **6** and *α*-amino acids **14**), by the classical [3+2] cycloaddition reaction with isatilidene malononitriles **13** (Scheme 4) was also reported recently from our group (Leena et al., 2023). The biological evaluation of these dispiropyrrolidines **15** revealed excellent antibacterial and good anticancer activity. Out of the 16 derivatives synthesized the bromo-substituted dispiropyrrolidine, showed effectiveness against MRSA/VRSA strains and demonstrated a concentration-dependent bactericidal effect. *In vitro* studies of chloro and bromo-substituted dispiropyrrolidines against human colon cancer cells (HCT116) indicated significant cytotoxicity, establishing these derivatives as promising drug candidates. Mechanistically, the azomethine ylide (generated *via* the decarboxylative condensation of tryptanthrin **6** with sarcosine **14**) underwent 1,3-dipolar cycloaddition reaction with isatilidene malononitrile **13**, leading to the formation of the desired regioisomer **15**.



Scheme 4 Synthesis of dispiropyrrolidines

4.3 Tryptanthrin incorporated spiropiperidines

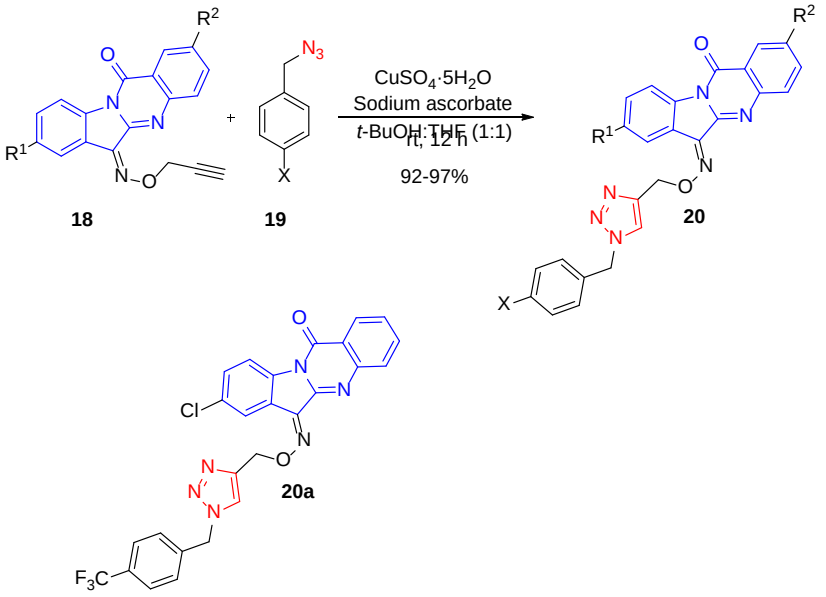
A green method for the synthesis of tryptanthrin-appended spiropiperidines (Scheme 5) using ammonium acetate (solvent free method) was reported by our group (Al-Sharifi et al., 2024). The synthesized compounds were isolated using a simple solvent extraction method. The detailed antibacterial evaluation of the selected compounds showed that, the bromo derivative **17b** and the chloro derivative **17c** have lowest minimum inhibitory concentration (MIC) vs *S. aureus* ATCC 29213. Chloro derivative exhibited a high selectivity index (SI >25) against Vero cells. Both the compounds exhibited equipotent activity against clinically relevant methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA) isolates. A plausible approach for the synthesis of tryptanthrin appended 4-spiropiperidines is shown in scheme 5. Mechanistically the ylidene formed by the reaction of tryptanthrin **6** and malononitrile, in the presence of NH_4OAc, reacts with *m*-phenylene diamine **16** to yield an intermediate, which finaly cyclizes to the product **17a** (*via* proton transfer and intramolecular nucleophilic attack of the amine lone pair on the nitrile group).



Scheme 5 Synthesis of spiropiperidines

4.4 Tryptanthrin appended 1*H*-1,2,3 triazoles

A green synthetic approach for the preparation of tryptanthrin–triazole hybrid molecules **20** was achieved *via* Cu(I)-catalyzed azide–alkyne [3+2] cycloaddition (CuAAC) between tryptanthrin oxime *O*-propargyl ether **18** and various aromatic azides **19**, as illustrated in scheme 6 (Meenakshy et al., 2024). The triazoles were screened for *in vitro* anticancer activity against the A549 lung cancer cell line using the MTT assay. Compound **20a** was found to be the best with IC_{50} 40.8 μM.



Scheme 6 Synthesis of tryptanthrin appended 1*H*-1,2,3 triazoles

Conclusions

In conclusion, the current chapter describes the synthesis of tryptanthrin derived spiroheterocyclic compounds and explores their antibacterial and anticancer properties. The detailed biological evaluation of the novel tryptanthrin hybrids indicate superior activity for certain derivatives, particularly against ESKAPE pathogens and HCT-116 cancer cell lines. The results pinpoint to the potential therapeutic applications of tryptanthrin and paves way for the future synthesis of potential drug molecules from it.

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