Synthesis and evaluation of mutual prodrug of selected NSAIIDs with paracetamol

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Abstract : The gastrointestinal adverse effects of NSAIDs can be mitigated and the therapeutic efficacy enhanced through carboxyl group modification. The selection of naproxen and mefenamic acid, both anti-inflammatory medications, was made with the purpose of developing mutual prodrugs in combination with paracetamol. The chemicals were produced with the objective of attaining a synergistic outcome and mitigating the stomach irritation associated with nonsteroidal anti-inflammatory drugs (NSAIDs). The compounds were verified using the process of spectrum characterisation. The experimental procedure encompassed the esterification reaction between the carboxyl functional group of specific nonsteroidal anti-inflammatory drugs (NSAIDs) and the hydroxyl functional group of paracetamol. The hydrolysis research of mutual prodrugs was conducted in both an acidic medium and a phosphate buffer. The study determined that compounds exhibit regulated hydrolysis in the environment. Consequently, it was concluded that a mutual prodrug strategy can be successfully employed to enhance the efficacy of non-steroidal anti-inflammatory drugs (NSAIDs) in two distinct manners. The first involves masking the carboxyl group of NSAIDs, while the second strategy involves utilizing paracetamol as a pro moiety to achieve synergistic effects. The mutual prodrugs have demonstrated the ability to maintain their anti-inflammatory properties while significantly reducing gastrointestinal irritation.

Keywords: NSAIDs; Paracetamol; Esters; Hydrolysis; In vivo assessment.

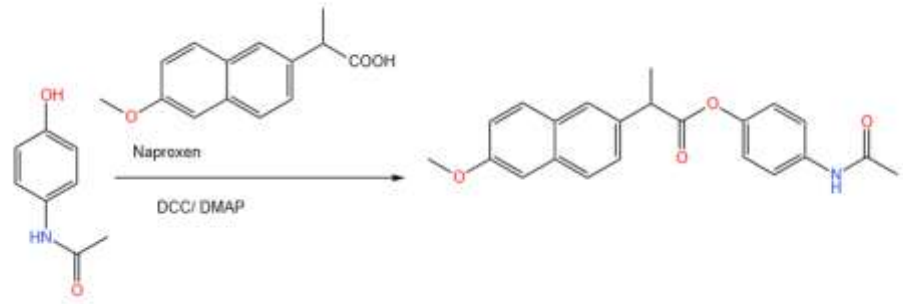
Introduction

A significant number of nonsteroidal anti-inflammatory drugs (NSAIDs) now in use are linked to adverse effects that are deemed unsatisfactory. Nonsteroidal anti-inflammatory medicines (NSAIDs) have been found to present challenges due to their prevalent side effects, including gastrointestinal irritation and ulcers, which impose restrictions on their utilization.[1] Nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively utilized for the management of pain and inflammation associated with various medical disorders, such as osteoarthritis (OA) and rheumatoid arthriti(RA).[2] The primary constraints associated with utilization pertain to gastrointestinal (GI) adverse effects, particularly peptic ulcers, bleeding, and perforation. The observed phenomenon can be attributed to a localized effect resulting from the direct interaction between the medication and the gastric mucosa[3]. The involvement of the carboxyl group in nonsteroidal anti-inflammatory drugs (NSAIDs)

is significant in the pathogenesis of stomach irritation and ulcers[4].Various strategies have been documented in the literature to mitigate the adverse effects associated with a given intervention. The mutual prodrug notion encompasses the amalgamation of two pharmacologically active substances, wherein one drug functions as a promoiety[3,10]. The utilization of this method has been employed in order to mitigate gastrointestinal (GI) toxicity by transiently concealing the carboxyl group of nonsteroidal anti-inflammatory drugs (NSAIDs) with the phenolic hydroxyl group of paracetamol. The enhancement of absorption values is also observed in this context. Medicinal chemists find prodrugs of anti-inflammatory drugs particularly intriguing due to the ease with which the carboxyl group can be derivatized, resulting in the creation of versatile derivatives[6-8]. Furthermore, there have been reports of prodrugs for certain non-steroidal anti-inflammatory drugs (NSAIDs) as well[9]. In this study, the researchers created prodrugs of naproxen and mefenamic acid by conjugating them with paracetamol as a promoiety. In the current study, the carboxyl group of nonsteroidal anti-inflammatory drugs (NSAIDs) is chemically linked to the phenolic hydroxyl group of paracetamol via an esterification reaction.

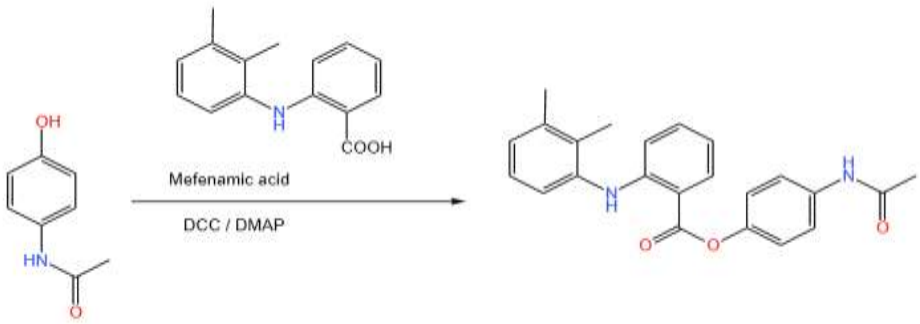
Material and method

The synthesis of mutual prodrugs of naproxen and mefenamic acid with paracetamol was achieved through the application of the Steglich esterification reaction. The experimental procedure involves employing dicyclohexylcarbodiimide (DCC) as a coupling reagent and 4-dimethyl aminopyridine (DMAP) as a catalyst in order to achieve esterification. The reaction mechanism comprises of two distinct phases. During the initial stage, the carboxylic acid underwent a reaction with DCC, resulting in the formation of an O-acyl isourea. This intermediate exhibited higher reactivity compared to the free acid. Subsequently, in a second step, the phenolic hydroxyl group of paracetamol initiated an assault on this intermediate, leading to the formation of DCU and the corresponding ester [16].



Paracetamol Prodrug NP

Figure 1 Synthesis of prodrug NP



Paracetamol Prodrug MP

Figure 2. Synthesis of prodrug MP

Materials

Bluecross Laboratories Ltd. in Nashik provided mefenamic acid and paracetamol as a gift sample. The source of naproxen was Holden Pharmaceuticals in Sinnar, Nashik. Standard sources such as SD Fine, Qualigen, Fischer Scientific, Loba Chemie, and others are the source of all other chemicals and reagents.

General Procedure

The reaction was conducted by combining 2 millimoles of the medication, which was afterwards dissolved in 20 milliliters of dichloromethane (DCM). To this solution, 1 millimole of dicyclohexylcarbodiimide (DCC) grams was introduced. The resulting combination was then stirred for a duration of 4 hours. Subsequently, DMAP (40 mg) was introduced into the mixture, A quantity of 2 millimoles (0.302 grams) of Paracetamol was introduced into the mixture, which was thereafter subjected to stirring for a duration of 24 hours. The reaction was monitored through the utilization of thin-layer chromatography (TLC), employing a mobile phase consisting of a mixture ofpetroleum ether and ethyl acetate in a ratio of 2:1.

The ester that was generated underwent extraction and chromatographic separation. This process involved adding 50 ml of distilled water to the crude mixture in a separating funnel. The organic layer was subjected to a drying process utilizing magnesium sulfate (MgSO4). The organic layer was subsequently subjected to evaporation under lower pressure utilizing the rotary evaporator device. The stationary phase employed in the experiment was silica gel, while the drug was separated from the stationary phase using the mobile phase consisting of a mixture of petroleum ether and ethyl acetate in a ratio of 2:1.

The physicochemical properties were determined and shown in **Table 1**.

Table 1. Physicochemical properties of synthesized prodrug.

Prodrug Code	**Molecular formula**	**Mol. wt.**	**Colour**	**M.P.(°C)**	**% Yield**	**Rf value**	**Log P**
NP	$C_{22}H_{21}NO_4$	363.4	White	158-160	82	0.60	5.68
MP	$C_{26}H_{25}NO_4$	415.4	White	180-182	72	0.26	5.50

In-vitro Hydrolysis Studies of Mutual Prodrug

The hydrolysis of the pro drugs NP and MP was conducted at pH levels of 1.2 and 7.2, which correspond to the acidity levels seen in the stomach, large intestine, and small intestine of the human body. The hydrolysis process was monitored using a UV spectrophotometer, specifically measuring the absorbance at λmax values of 256 nm and 259 nm for NP, and 258 nm and 262 nm for MP, respectively. The hydrolysis reaction was conducted at the specified pH level after preparing a solution of 100 ml in a volumetric flask. The reaction was carried out at a temperature of 37 ± 0.1℃. A 2.4 ml aliquot of the 10 ppm stock solution derived from the prodrug is combined with 0.6 ml of Acetonitrile at the desired pH for experimental investigation. The initial absorbance (A) was recorded before to immersing the samples in a water bath at time zero (0). Subsequently, the absorbance was measured at a time interval of 30 minutes at a temperature of 37℃, over a total duration of 500 minutes for each sample. The solution used in this experiment consists of 0.6 ml of Acetonitrile and 2.4 ml of buffer solution, which is then compared to the sample solution.The absorbance of the prodrugs was measured at the maximum wavelength (λmax).
Table 2 represents the results of study

Table 2. Kinetic parameters for hydrolysis of mutual prodrugs NP at 37 ℃

Prodrug code	K obs (a)	t1/2 (h)a	K obs (b)	t1/2 (h)b
NP	0.1440	4.81	0.1928	3.59
MP	0.1534	4.51	0.1873	3.69

a In 0.1N HCl (pH-1.2), b In Phosphate buffer (pH- 7.2)

Pharmacological activity

Anti- inflammatory activity

The synthesized compounds were subjected to anti-inflammatory screening using the carrageenan-induced paw edema method in Wistar rats weighing between 120-160 g. In this approach, Naproxen (25 mg/kg p.o.) and Mefenamic acid (37 mg/kg p.o.) were employed as standard drugs for comparative purposes[17]. The drug and test chemicals were administered orally by creating a suspension in a 0.5% carboxymethyl cellulose (CMC) solution. The injection of carrageenan was performed by preparing a freshly made aqueous suspension with a concentration of 1% weight/volume, and a volume of 0.1 ml. The suspension was administered by injection into the right hind paw of each rat. The approach employed in this study involved the utilization of mercury displacement equipment, specifically a plethysmograph. This particular method is frequently employed in comparison to alternative approaches due to its reliance on the capacity of anti-inflammatory drugs to impede the development of oedema in the hind paw of rats subsequent to injection.

Procedure

- The animals were divided into groups, 6 rats in each group. One group of animals allotted to control and two group for standard drug (Naproxen and Mefenamic acid). Rest of the group was allotted to the test compounds.
- 0.5 % CMC as control, Naproxen, Mefenamic acid as standard, and test compound were given orally by preparing with 0.5% CMC suspension to group (standard, control and test compound respectively).
- After 30 min, 0.1mL of 1% freshly prepared carrageenan suspension in 0.9% NaCl solution was injected subcutaneously into the planter aponeurosis of the right hind paw and volume was measured.
- The paw volume was measured at 15,30,60 and 120 minutes, the mean increase in the paw volume in each group was calculated.
- The paw volume was measured by a plethysmometer apparatus. The difference in volume given the amount of oedema developed.
- The percent inhibition value calculated by formula given below

% anti-inflammatory activity = [1-Dt / Dc]*100

Dt and Dc are paw volumes of oedema is tested and control groups respectively

Table 3 Anti-inflammatory effect of synthesizes compound on carragenan - induced paw edema in rats using Ibuprofen as standard drug (50 mg/kg)

Treatment	**Increase in Paw volume (ml) (% inhibition of paw oedema)**			
	15 min	**30 min**	**60 min**	**120 min**
Vehicle (0.5 %	0.21 ± 0.011	0.21 ± 0.112	0.21 ± 0.87	0.22 ± 0.49

CMC)				
Carragenan	0.35 ± 0.161	0.44 ± 0.554	0.48 ± 0.113	0.48 ± 0.248
Naproxen (25 mg/kg p.o.)	0.25± 0.014 (28.41%)	0.24 ± 0.03 (44.76%)	0.24±0.529 (48.87%)	0.23±0.0245 (52.98%)
Compound NP (100 mg/kg p.o.)	0.32 ± 0.214 (8.6%)	0.31 ±0.124 (28%)	0.29±0.663 (28.5%)	0.27 ± 0.361 (45.8 %)
Mefenamic acid (37 mg/kg p.o.)	0.23±0.381 (34.38%)	0.25±0.268 (42.98 %)	0.25±0.275 (48.25%)	0.22±0.297 (46.18%)
Compound MP (100mg/kg p.o.)	0.31± 0.224 (12%)	0.31 ± 0.04 (30.9 %)	0.28 ± 0.12 (40.4%)	0.28 ± 0.669 (41%)

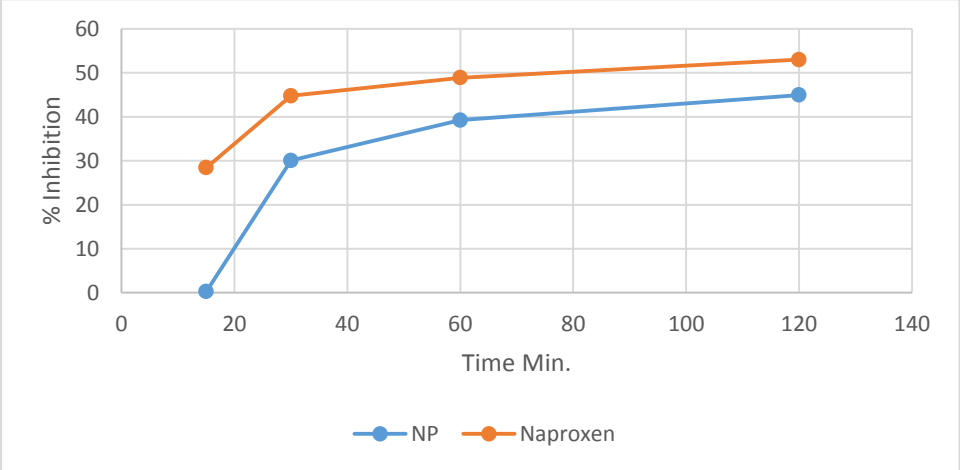


Fig.3 Comparative % Paw volume inhibition by Naproxen and NP

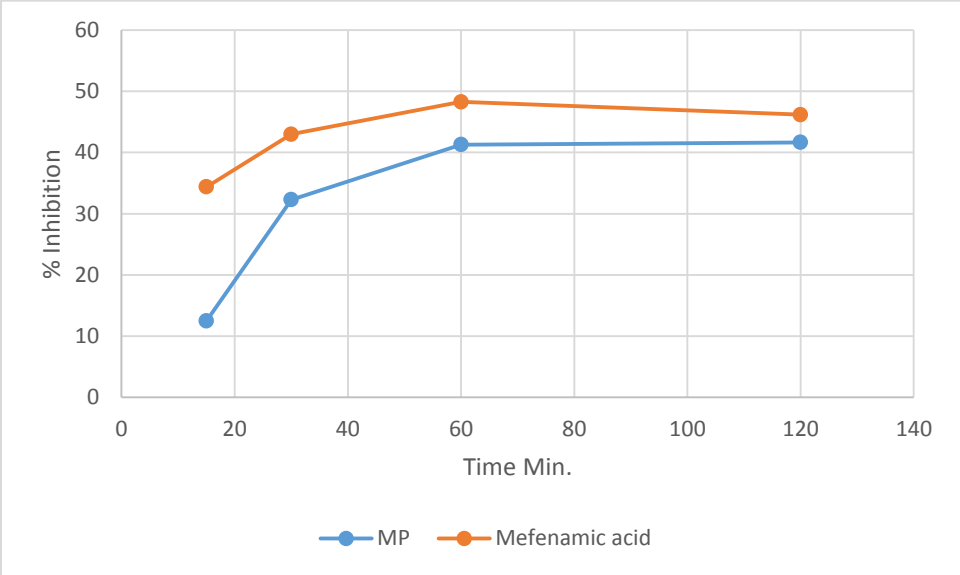


Fig.4 Comparative % Paw volume inhibition by Mefenamic acid and MP

Gastric irritant Activity

The gastric irritation test was conducted using the protocol outlined by Kunchandy et al. in 19. The Wistar rats were partitioned into five groups, each including five individuals. Prior to the medication delivery, a fasting period of 12 hours was observed for all animal subjects.

The initial group was designated as the control group and was administered the vehicle (0.5% carboxymethylcellulose) orally. The participants in Group II were administered Ibuprofen as reference standard only by taking it as reference drug for comparison . Equivalent doses of prodrugs NP and MP were administered to groups III and IV, respectively. The test medicines and vehicle control were orally administered to thrice a day to rats for three consecutive days.All of the rats underwent a fasting period of 4 hours following the administration of their last dose. The animal was subjected to an excessive amount of anesthetic during the sacrificial procedure. The stomach was surgically excised, thereafter dissected along the larger curvature, and carefully rinsed using a continuous flow of flowing water. The gastrointestinal mucosa of the rat was analyzed through tissue sampling using a microscope, and subsequently compared to the condition observed following the administration of ibuprofen. The mucosal injury of each stomach was evaluated using the subsequent grading system: The scoring system used to assess the effects on the stomach is as follows: a score of 0.0 indicates a normal coloration, a score of 1 indicates mild effects, a score of 2 indicates moderate effects, and a score of 3 indicates severe symptoms. The subsequent findings are derived from the conducted study.

Table 4 Gastric irritation study results

Observations	**Vehicle**	**Ibuprofen**	**Test NP**	**Test MP**
Mucosal desquamation	0	3	2	3
Infilteration cells inflammatory propria	0	2	1	2
Odema in lamina propria	0	2	2	1

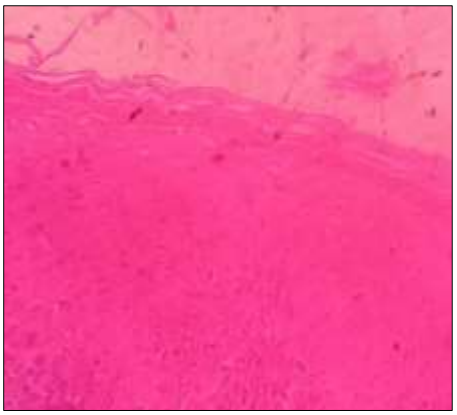


Figure 5 Histopathological Examination (Vehicle)

Inference: The mucosal anatomy appeared to be within normal parameters, with no presence of inflammatory cells or signs of oedema.

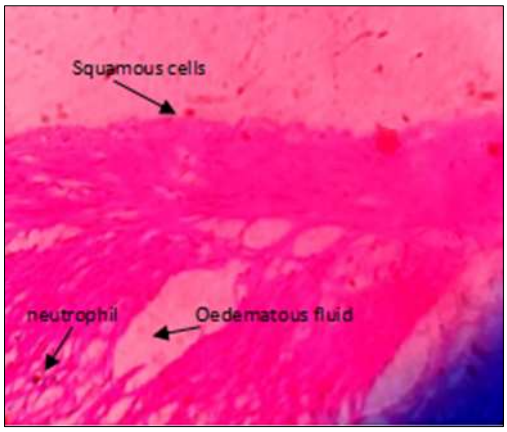


Figure 6 Histopathological Examination (Ibuprofen)

Inference: The presence of pronounced desquamation in the mucosal layer was found. Moderate observations were made regarding the severe infiltration of inflammatory cells and the accumulation of oedemateous fluid in the lamina propria.

Figure 7 Histopathological Examination (NP)

Inference:

The presence of moderate desquamation was noticed in the mucosal layer. A moderate presence of inflammatory cells and the accumulation of oedematous fluid in the lamina propria were noted.

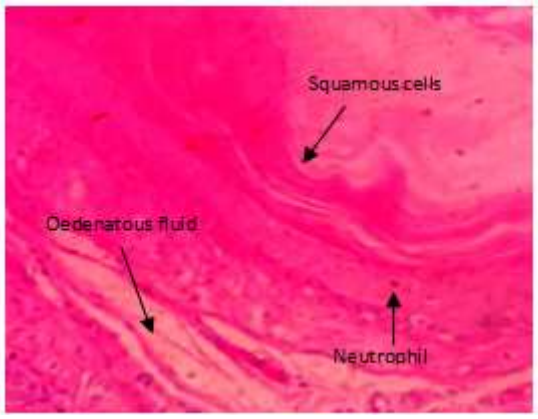


Figure 8 Histopathological Examination (MP)

Inference:

The presence of pronounced desquamation inside the mucosal layer was noticed. A moderate presence of inflammatory cells and the accumulation of oedematous fluid in the lamina propria were noted.

Discussion

The present study presents a novel strategy for addressing the undesirable characteristics associated with nonsteroidal anti-inflammatory drugs (NSAIDs) through the development of a mutual prodrug design. The synthesis of a mutual prodrug combining paracetamol with naproxen and mefenamic acid was successfully accomplished. The synthesis and characterization of the target compounds NP and

MP were achieved through the conversion of specific anti-inflammatory drugs into their respective esters using paracetamol. The hydroxyl group attached to the phenolic ring of paracetamol functions as a masking agent for the carboxyl group present in certain nonsteroidal anti-inflammatory drugs (NSAIDs). The reaction's advancement was observed by thin-layer chromatography (TLC) employing a mixture of petroleum ether and ethyl acetate in a 2:1 ratio. The generated compounds underwent purification using the process of recrystallization. The spectrum data obtained exhibits a high level of concordance with the produced molecules. The infrared spectra exhibited the presence of C=O stretching vibrations within the distinctive band region of 1716-1747 cm-1, as well as C-O stretching vibrations within the range of 1225-1232 cm-1. These findings provided confirmation of the ester formation. The 1H NMR spectra of the produced compounds exhibit chemical modifications, wherein the lack of the carboxylic acid proton signal serves as an indicator of the successful occurrence of the reaction. The mass spectra of the synthesized derivatives exhibit an initial peak that corresponds to the molecular weight of the aforementioned substances. A study was conducted to investigate the hydrolytic kinetics of the prodrugs at both acidic and basic pH levels in order to ascertain the destiny of the prodrugs. Based on the available literature, it appears that a crucial factor for the effective utilization of prodrugs is the acid stability of the compounds, which is necessary to prevent direct interaction with the gastric mucosa and the subsequent suppression of prostaglandins. The produced prodrug compounds were assessed under suitable conditions of acidic and basic pH. The UV study conducted on the kinetics of hydrolysis revealed that the prodrug derivatives undergo chemical decomposition following a first-order kinetic model, leading to a complete conversion into the parent medicines. The produced prodrugs exhibited significant stability when exposed to acidic conditions, suggesting that upon oral administration, the chemical remained unhydrolyzed as it traversed the stomach. On the other hand, the capacity of prodrugs to undergo hydrolysis at alkaline pH levels suggests their vulnerability to hydrolysis in the fluid present in the intestines. The synthesized prodrugs were subjected to in-vivo evaluation, which demonstrated an enhancement in the therapeutic index of the original parent drugs.

Conclusion

Research findings indicate that the utilization of a mutual prodrug strategy can effectively enhance the therapeutic effectiveness of specific non-steroidal anti-inflammatory drugs (NSAIDs) through two distinct mechanisms. Firstly, by concealing the carboxyl group through esterification, and secondly, by employing established NSAIDs to elicit a synergistic impact. Based on empirical observations, it may be inferred that NP, MP prodrugs have anti-inflammatory properties in comparison to the drugs Naproxen and Mefenamic acid. Prodrugs exhibit a diminished level of stomach irritating activity in comparison to Ibuprofen; nonetheless, it is important to note that none of these prodrugs are completely devoid of gastric irritation effects. These compounds may be regarded as mutual prodrugs exhibiting enhanced characteristics. Hence, the utilization of a mutual prodrug

strategy enables medicinal chemists to enhance the clinical and therapeutic effectiveness of a drug that possesses unfavorable characteristics, thereby restricting its practical application in clinical settings.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

The present study has obtained ethical approval.
Animal studies are currently conducted at the Pharmacology Department of Bhupal Nobles Institute of Pharmaceutical Sciences in Udaipur, Rajasthan, India. These studies are conducted with the approval of the Institutional Animal Ethics Committee (IAEC), which operates in accordance with the guidelines set forth by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The CPCSEA's website can be accessed at http://cpcsea.nic.in.

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