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Chemical Characterization and Pharmacological Evaluation of Phytophenols-Etodolac Mutual Prodrugs

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Abstract

Etodolac is the non-steroidal anti-inflammatory agent. It is the commonly used drug for combating pain and inflammation, still the drug has prominent side effect of GI-lesion or erosion, that is due to presence of free carboxylic acid in the molecule. Structurally etodolac belongs to the chemical class of indole acetic acid derivative. It is used as non-selective COX-inhibitor. Here the ester linked mutual prodrugs of etodolac with phytophenols like vanillin, carvacrol, umbelliferone, guaiacol, sesamol and syringaldehyde were synthesized. These prodrugs were synthesized using steglich esterification by utilizing parent drug, dicyclohexyl carbodiimide, dimethyl amino pyridine, aromatic phenols (phytophenol) and dichloromethane (solvent) at lower temperature. All the prodrugs were characterized by IR-spectroscopy, 1H-NMR, 13C-NMR and mass spectrometry. The hydrolysis studies were performed at acidic and basic pH. The hydrolysis data of synthesized prodrugs revealed that major part of the prodrugs got hydrolyzed in basic medium which was clearly indicating that the synthesized prodrugs were less ulcerogenic the result also satisfy the same. The synthesized prodrugs were screened for analgesic, anti-inflammatory and ulcerogenic activities. Acetic acid induced writhing model was used for analgesic activity and carrageenan induced model was used for anti-inflammatory activity. Among the synthesized prodrugs, the Eto-van, Eto-umbe, Eto-sesa and Eto-syr showed improved analgesic and anti-inflammatory activity than etodolac. Eto-syr depicted utmost pharmacological actions among the synthesized compounds. It showed 70.27 % inhibition during analgesic activity and 81.94 % inhibition during anti-inflammatory activity. While on observing ulcerogenic activity, All the synthesized prodrugs showed less ulcerogenic side effects compared to etodolac. The reason behind significant pharmacological activities may be due to the phytophenols which were conjugated with the parent drug. These phytophenols are also playing active role directly or indirectly in inhibiting reactive oxygen species formation which may be the cause of inflammation and GI lesion.

Keywords: Analgesic, Anti-inflammatory, Etodolac, Mutual prodrugs, Phytophenol, Ulcerogenic.

التوصيف الكيميائي والتقييم الدوائي للعقاقير الأولية المتبادلة فيتوفينول-إيتودولاك

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الخلاصة

إيتودولاك هو عامل مضاد للالتهابات غير الستيرويدية .وهو دواء شائع الاستخدام لمكافحة الألم والالتهابات، ولا يزال للدواء آثار جانبية بارزة تتمثل في آفة الجهاز الهضمي أو التآكل، وذلك بسبب وجود حمض الكربوكسيل الحر في الجزيء .من الناحية الهيكلية، ينتمي إيتودولاك إلى الفئة الكيميائية من مشتقات حمض الأسيتيك الإندول .يتم استخدامه كمثبط غير انتقائي لـ COX. هنا تم تصنيع العقاقير الأولية المتبادلة للإيتودولاك مع الفيتوفينول مثل الفانيلين، كارفاكرول، أومبيليفيرون، جواياكول، سيسامول وسيرينجالدهيد .تم تصنيع هذه العقاقير الأولية باستخدام استرة ستيجليش باستخدام الدواء الأصلي، ثنائي سيكلوهكسيل كاربوديميد، ثنائي ميثيل أمينو بيريدين، الفينولات العطرية) فيتوفينول (وثنائي كلورو ميثان)مذيب (عند درجة حرارة منخفضة .تم تشخيص جميع العقاقير الأولية بواسطة مطيافية الأشعة تحت الحمراء،1H-NMR ، 13C-NMR ومطيافية الكتلة .تم إجراء دراسات التحلل المائي عند درجة الحموضة الحمضية والأساسية .أظهرت بيانات التحلل المائي للعقاقير الأولية المحضرة أن الجزء الأكبر من العقاقير الأولية قد تم تحلله في الوسط القاعدي مما يشير بوضوح إلى أن العقاقير الأولية المحضرة كانت أقل تقرحا والنتيجة مرضية أيضا .تم فحص العقاقير الأولية المصنعة بحثًا عن أنشطة مسكنة ومضادة للالتهابات ومسببة للقرحة .تم استخدام نموذج التلويح الناجم عن حمض الأسيتيك في النشاط المسكن وتم استخدام نموذج الكاراجينان في النشاط المضاد للالتهابات .من بين العقاقير الأولية المصنعة، أظهرت Eto-van و Eto-umbeو Eto-sesaو Eto-syrنشاطًا مسكنًا ومضادًا للالتهابات أفضل من etodolac. يصور إيتو-سير أقصى التأثيرات الدوائية بين المركبات المصنعة .أظهر تثبيطًا بنسبة 70.27% أثناء النشاط المسكن وتثبيطًا بنسبة 81.94% أثناء النشاط المضاد للالتهابات .أثناء ملاحظة النشاط التقرحي، أظهرت جميع العقاقير الأولية المحضرة تأثيرات جانبية تقرحية أقل مقارنة بالإيتودولاك .قد يكون السبب وراء الأنشطة الدوائية الهامة يرجع إلى الفيتوفينول الذي كان مترافقًا مع الدواء الأصلي .تلعب هذه الفيتوفينول أيضًا دورًا نشطًا بشكل مباشر أو غير مباشر في تثبيط تكوين أنواع الأكسجين التفاعلية التي قد تكون سببًا للالتهاب وآفة الجهاز الهضمي.

الكلمات المفتاحية : مسكن، مضاد للالتهابات، إيتودولاك، عقاقير أولية متبادلة، فيتوفينول، مسبب للقرحة

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Introduction

The non-steroidal anti-inflammatory drugs (NSAIDs),a group of drugs with diverse chemical structure are frequently used in pain, inflammation, arthritis and fever [1-3].The drugs that are selective COX-2 inhibitors suffered with cardiovascular risk while the non-selective COX–inhibitors suffered with gastro-intestinal lesion [4] . Although hepatotoxicity is another serious problem that is some time observed with NSAIDs use [5] . There are several attempts were made to reduce the GI-damage by converting them into hydrogel form, prodrug, codrug, encapsulated liposomal form and pegylation by using polyethylene glycol to improve pharmacokinetic properties and sustained release as well as intestinal targeting [6-10]. Apart from prodrugs preparation of NSAIDs there is prodrugs of doxorubicin with vitamin-E succinate encapsulated in hyaluronic acid to overcome the doxorubicin resistance and release of drugs into tumor microenvironment [11]. Some other approaches in the prodrug development are conversion of active chemical entity into ionic liquid form, photoresponsive hybrid prodrug that contain photoremovable protecting group [12]. Among the NSAIDs, etodolac is the drug of choice which is frequently used in osteo- and rheumatoid arthritis [13]. Chemically etodolac belongs to the class indole acetic acid derivative and it is non-selective COX-inhibitor but preferentially COX-2 inhibitor [14]. It is having the side effect of gastric ulceration which is due to free carboxylic acidic functionality and inhibition of COX-1. COX-1 plays a housekeeping role and catalyzes the synthesis of prostraglandins and prostacycline [15]. Prostacycline promotes the formation of gastric mucous layer [16]. The NSAIDs including etodolac are non-selective COX-inhibitor having a common side effect of gastric ulceration [9-10), (17] Therefore, there is need to reduce the gastric ulceration associated with their use and it is achieved by making their esters or amides. Here, the esterification of parent drug was done with phytophenols. As the phytophenols like resveratrol, carvacrol, vanillin, thymol, sesamol etc. are found to have good antioxidant activity. Literature supported that phytophenols like vanillin, umbelliferone, carvacrol, syringaldehyde, sesamol, and guaiacol are widely used for making the esters and amino acids like glycine, tryptophan, glutamine cysteine etc. are used for amide prodrug formation [18-22]. The combination of NSAIDs with phytophenols acts as mutual prodrug. As both the entities or moieties have their own pharmacological actions [23]. It can be depicted by observing mutual prodrugs of naproxen and mefenamic acid with phytophenols. The rationale behind the use of phytophenols is that they are directly or indirectly result to inhibit the formation of ROS (anti-oxidant) which are formed at the site of pain and inflammation [24]. When these phytophenols conjugated with NSAIDs the newer molecule would going to have greater effect than NSAIDs alone as well as there would be reduction in gastric ulcer. The synthesized mutual prodrugs have less GI-irritancy with improved therapeutic effects [25- 26]. The main objectives of the development of prodrugs are to ameliorate the stability, improve solubility profile, decrease side effects, enhance the bioavailability, enhanced penetration and permeability which lead to increase therapeutic potential [27-28].

Materials and Methods

Materials

Syringaldehyde, sesamol and umbelliferone were obtained from M/s Sigma-Aldrich, Mumbai, India and etodolac, carvarol, guaiacol and vanillin obtained from Yarrow Chem products Mumbai. All other solvents and reagents used were of analytical grade. The melting points were recorded by thiele's tube method and are uncorrected. The UV-Visible spectra was recorded using Shimadzu UV-1800. The infrared spectra of the synthesized prodrugs were recorded on Shimadzu FTIR Affinity 1 and mass spectra of the synthesized compound were determined by Waters Q-TOF Micromass (ESI-MS). The ^{1}H spectra were recorded by Bruker Avance Neo 500 MHz using $CDCl_3$ as the solvent.

Synthetic procedure of prodrugs

The prodrugs were synthesized by taking all the reagents in stoichiometric amount of 0.01 mole. First of all, etodolac of 0.01 mole transferred into a 100 mL flat bottom round flask containing 25 mL of dichloromethane (DCM)DCM, the mixture was stirred for 10 -20 min until the solution become uniform. Afterwards phytophenol (0.01 mole) which was previously dissolved in 15 mL of DCM with continuous stirring was added. Then after 20 min duration 4- Dimethyl amino pyridine (4-DMAP) and N, N' Dicyclohexyl carbodiimide (DCC) were added into the above mixture and temperature was maintained at -5 to 0 ˚C with continuous stirring. After completion of reaction which is confirmed by TLC (solvent system n-hexane: ethyl acetate) the reaction mixture is filtered and the filtrate is washed with 0.1N HCl and then with 5% w/v sodium bicarbonate solution. The excess solvent was removed by placing the washed filtrate into a china dish in a desiccator containing anhydrous calcium chloride. The crude solid product obtained is purified by washing with cold water and finally recrystallized with ethanol (Figure. 1 and Figure. 2). The melting point is determined by thiele tube method containing liquid paraffin which were observed in a range and uncorrected [29-32].

Characterization of prodrugs

The various physicochemical properties such as R_f – value, melting point, partition coefficient, molecular wt. and color of the synthesized prodrugs were determined and presented in the table: 1.

Thin-layer chromatohraphy

The thin layer chromatography was performed on pre coated silica gel G plate and using iodine chamber for spot detection. The solvent system is n-hexane: ethyl acetate (8:2) was taken.

Melting point determination

Melting point of the synthesized compound was determined by thiele tube method and which were uncorrected.

Molecular weight determination

The molecular weight of the compounds was determined by interpretation of mass spectrum obtained from Waters Q-TOF Micromass (ESI-MS) mass spectrometer.

Partition coefficient

For the determination of partition coefficient, the 100mg of synthesized prodrugs were weighed and transferred to separating funnel flask containing the 10mL mixture of 5mL n-octanol and 5mL phosphate buffer (pH 7.4). The flask was shaken for 24 hours and set aside for 1 hour. After that the aqueous layer was collected in a 10mL volumetric flask and extracted with 10mL chloroform. The chloroform layer was analysed by UV-spectrophotometer and amount of drug was calculated with the help of calibration curve of parent drug that was drawn in between of 2 to 16 µg/mL concentration, the straight-line equation found to be, where y = mx + c.
m = 0.067 (Slope) and c = 0.05 (Intercept) [25], [33].

Hydrolysis study in SGF and SIF

For the hydrolysis study in simulated gastric fluid (SGF) i.e. acidic hydrolysis, there is usage of 0.1N HCl (pH 1.2) and for hydrolysis study in simulated intestinal fluid (SIF) i.e. basic hydrolysis study there is usage of phosphate buffer (pH 7.4). [15], [33-36].

The synthesized prodrugs weighed 100 mg and transferred to single rotatory paddle type dissolution apparatus containing either of 900 mL 0.1N HCl or phosphate buffer and maintained the static temperature at 37 ± 0.5 ℃ with paddle speed of 100 rpm. At an interval of 30 minutes 5mL of aliquots were withdrawn and the same quantity of 0.1N HCl or phosphate buffer were added to maintain the constant volume upto 900 mL. The aliquots were extracted with chloroform and collected in 10mL of volumetric flask and left overnight for solvent evaporation. After all the chloroform was evaporated then added 10 mL of methanol in each volumetric flask and observed under UV-spectrophotometer at a wavelength of 280nm.

Comparative Pharmacological screening of synthesized prodrugs with standard drugs

For the pharmacological screening of the synthesized compound, the animal selected were wistar albino rats for anti-inflammatory and ulcerogenic activity and swiss albino mice for analgesic activity. Before performing the experiment, all the animals divided into groups of 6 animals and housed in acrylic cages provided standard environmental conditions of relative humidity 45-55% at a temperature of 25 ± 2 ℃, in a proper ventilated room with 12 hr: 12 hr light/dark cycle, and fed with standard diet and water ad libitum. The above protocol was approved by institutional animal ethical committee, Institute of Pharmaceutical Research, GLA University, Mathura (1260/PO/Re/S/09/CPCSEA).

Anti-inflammatory effect

For evaluation of anti-inflammatory effects, the method chosen was carrageenan induced hind paw oedema. For this screening the wistar albino rats weighing between 100-200g were used and divided into 3 groups, having 6 animals in each group as vehicle control, test drug control and standard drug control (etodolac). The initial size of the hind paws was measured. The test drugs and standard drug suspension in 1% w/v CMC was prepared and administered per oral one hour before the 0.1mL of 1% w/v carrageenan suspension as a phlogistic agent [36-39] . For every 2-hour interval and up to 8 hours the size of paws was measured and percentage inhibition was calculated by using the formula:

% Decrease in edema = $[(M_C – M_t) / M_C] \times 100$
Where, M_C = Mean edema in vehicle control;
M_t = Mean edema in test group.
The data of the anti-inflammatory activity represented in Table:3

Analgesic effect

For the analgesic screening of prodrugs, the acetic acid induced writhing method was chosen and the swiss albino mice weighing between 20 - 25g were selected as experimental animal. The suspension of test compound and standard drug (etodolac) were prepared in 1% w/v CMC and 1% CMC used as a vehicle. The per oral administration of test drugs, standard drugs and vehicle to mice was accompanied before one-hour intraperitoneal administration of 0.1mL 6% acetic acid as an analgesic agent. All the animals were placed in glass chamber and the number of writhes were counted for 10 minute [14-15], [23], [37]

% Decrease in writhes = $[(N_C – N_t) / N_C] \times 100$
Where, N_C = Mean writhes in vehicle control;
N_t = Mean writhes in test group.
The data of the analgesic activity represented in Table 2.

Ulcerogenic screening

For the evaluation of anti-inflammatory effects, the wistar rats were selected and divided into three groups viz. vehicle control, test drug control and standard drug control. The suspension of drug and prodrugs were prepared in 1.0% w/v CMC solution, which is used as a vehicle. Each groups contained 6 rats and fasted for 24 hours with continuous free access to water ad libitum before challenge for ulcer. The animals were sacrificed 12 hours after they dosed with prodrugs, standard drug and vehicle (1% w/v CMC). The stomach was isolated and opened through its greater curvature, washed with saline and observed for ulcers. Comparative measurement was done between the groups. The ulcer index was calculated by scoring the ulcer as: 0 = no observable damage to GI-mucosa; 1 = superficially damage to GI-mucosa; 2 = inside deep ulcer; and 3 = perforations [14), (40-42)]. The ulcer index was calculated as

$UI = [U_N + U_S + U_P] \times 10^{-1}$

Where, U_N = Average number of ulcers per animal;

U_S = Average of severity scores;

U_P = Percentage of animals with ulcers.

The data of the ulcerogenic activity represented in Table 2.

Results and Discussion

Characterization of etodolac prodrugs

The synthesized prodrugs of etodolac conjugated with different phytophenols were evaluated physiochemically and all the data presented in Table:1.

The results of thin layer chromatography revealed a single spot, confirming the product formation as well as complete reaction between the reactants [(37)]. All the synthesized prodrugs have R_f-value ranges from 0.64 to 0.82. Also, all the prodrugs have partition coefficient between n-octanol and phosphate buffer range from 4.09 to 6.19 which exhibit the synthesized compounds are lipophilic in nature and having better absorption property across the intestinal membrane or lipoidal membrane. All the synthesized compounds are subjected to infrared spectroscopy and there is presence of –C-O- stretching peaks and absence of –O-H- stretching. The –C-O- stretching bands appear in the region of 1253.4, 1193.25, 1140.42, 1257.59, 1095.15 and 1311.59 cm^{-1} for Eto-van, Eto-car, Eto-sesa, Eto-gua, Eto-umbe and Eto-syr respectively. The presence of sharp -C-O- stretching and absence of –O-H- stretching confirm the product formation. There is sharp –N-H- stretching at 3390.15 cm^{-1} 3385.07 cm^{-1}, 3395.35 cm^{-1}, 3336.71 cm^{-1}, 3367.71 cm^{-1}, 3340.34 cm^{-1} and 3327.21 cm^{-1} for above prodrugs and –C=O stretching was observed at 1735.93 cm^{-1}, 1720.35 cm^{-1}, 1740.71 cm^{-1}, 1753.29 cm^{-1}, 1739.35 cm^{-1} and 1716.71 cm^{-1} for the above compound respectively which also revealed the product formed was an ester (Table 4).

The proton spectra of the synthesized prodrugs of etodolac were determined by dissolving them into $CDCl_3$ and tetramethylsilane as internal standard. The ^{1}H-NMR spectra show the different values of singlet peak of –NH- proton, it is due to different chemical structure or environment. There is absence of singlet peaks of phenolic proton as well as carboxylic group proton which are 11.0 ppm and 12.05 ppm.

Hydrolysis study of etodolac prodrug

Hydrolysis studies of the prodrugs were carried out *in-vitro* in simulated gastric fluid and simulated intestinal fluid. The SGF having the pH value is 1.2 and SIF having pH 7.4[(29)]. The hydrolysis data revealed that all the prodrugs of etodolac have better tolerability in acidic environment than in basic medium. The release of free drug concentration is rapid in basic medium compare with acidic environment in addition maximum absorption from intestine and reduction in gastric lesion occurred. The data of hydrolysis presented in Tables 5.1 and 5.2.

The reported literature confirmed that the results obtained in the manuscript mimic the data and confirmed that the synthesized prodrugs are stable at acidic pH and would not harm the acidic lining of stomach and finally absorb at intestinal pH [(43)].

Pharmacological screening of etodolac prodrugs

The anti-inflammatory, analgesic and ulcerogenic screening of all prodrugs were performed. For anti-inflammatory activity the model used is carrageenan used hind paw edema in which carrageenan is used as phlogistic agent and for analgesic activity acetic acid induced writhing method is used.

The anti-inflammatory effects of the synthesized prodrugs were observed after 1 hour of the per oral administration up to 8 hours. Except Eto-car and Eto-gua, all the prodrugs have anti-inflammatory more than the etodolac. The data of anti-inflammatory activity was present in the tabulated form. All the prodrugs show anti-inflammatory effects between 68.33% to 81.944%. Among the synthesized compound Eto-car (68.33% inhibition) and Eto-gua (70.277% inhibition) are less potent than etodolac (75.27% inhibition). When synthesized compound are screened for analgesic effect then the five compounds show more analgesic effect than etodolac but only one compound (Eto-gua) are less potent than the standard etodolac. The reason for better analgesic and anti-inflammatory effect is that the more amount of drug reach to systemic circulation and very less amount metabolized in stomach pH environment. Phytophenols have tendency either to suppress or prevent the formation of ROS and or scavenge them from the site of action [(29)]. The electrogenicity study of the prodrugs and standard etodolac were

performed and it is found that mean ulcer index for etodolac is 10.96, while all the prodrugs exhibit comparatively less electrogenicity. The minimized electrogenicity of the prodrugs are due to masking of free carboxylic acidic group of etodolac as well as ROS scavenging effect of phytophenols [44]. Free carboxylic acid group is responsible for lesions on gastric mucosa by ion trapping mechanism which is minimized by esterification of acidic functionality with phytophenols and the greater amount of free drug release in the intestinal pH compared to stomach pH [43].

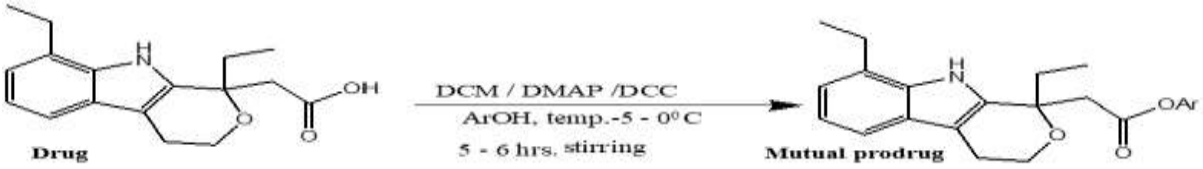


Figure 1. Synthetic scheme
Ar = Phytophenols

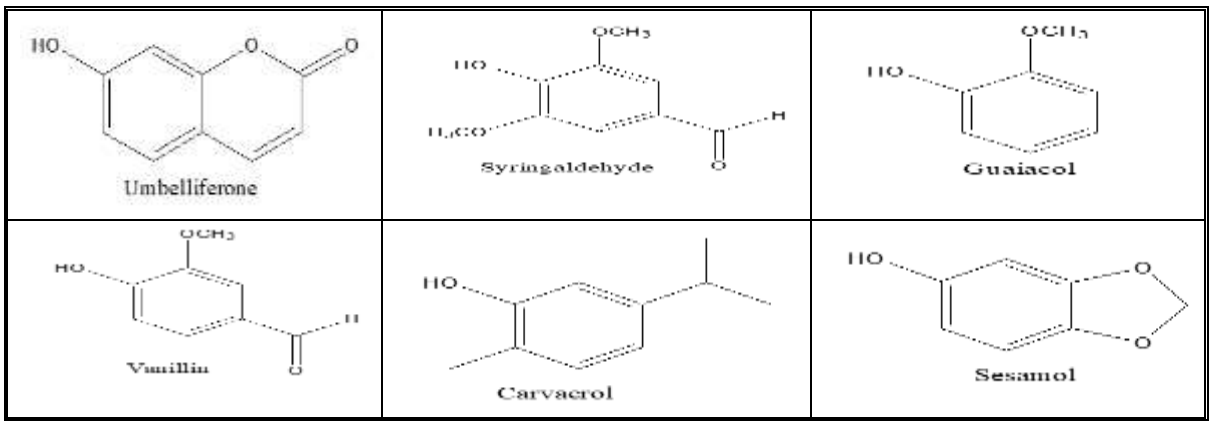


Table 1. Physiochemical Characterization

Prodrug/molecular formula	Mol. Wt	Melting point	Yield (%)	R_f value	Partition coefficient	Colour
Eto-van $C_{25}H_{27}NO_5$	421.5	158 – 160 ˚C	68	0.68	4.09	White
Eto-car $C_{27}H_{33}NO_3$	419.56	156 – 158 ˚C	56	0.72	6.19	White
Eto-sesa $C_{24}H_{25}NO_5$	407.47	166 – 168 ˚C	64	0.78	4.25	Pale brown
Eto-gua $C_{24}H_{27}NO_4$	393.49	162 -164 ˚C	74	0.82	4.34	White
Eto-umbe $C_{26}H_{25}NO_5$	431.49	216 – 218 ˚C	62	0.74	4.26	Pale brown
Eto-syr $C_{26}H_{29}NO_6$	451.52	186 – 188 ˚C	58	0.64	4.96	White

Table 2. Data of Analgesic and ulcerogenic activity

Group	Number of writhes	% Inhibition	Ulcer Index
Vehicle	51.38±0.806		
Etodolac	18.72±0.407	63.56	10.96± 0.03
Eto-van	16.55±0.364	67.78*	4.23±0.42
Eto-umbe	16.16±0.192	68.54*	4.81±0.37
Eto-syr	15.27±0.343	70.27*	4.66±0.71
Eto-sesa	15.99±0.190	68.87*	5.23±0.71
Eto-gua	18.44±0.242	64.10*	4.08±0.41
Eto-car	18.77±0.223	63.46	4.24±0.97

***P < 0.05 compared to the standard drug (etodolac) are significant, and data were given in mean ± SE of %inhibition and analyzed by ANOVA**

Table 3. Data of anti-inflammatory activity

Group	Time				% Inhibition			
	2hr.	**4 hr.**	**6 hr.**	**8 hr.**	**2hr.**	**4 hr.**	**6 hr.**	**8 hr.**
Vehicle	0.316± 0.030	0.500± 0.036	0.500± 0.089	0.400± 0.076				
Etodolac	0.233± 0.009	0.330± 0.009	0.205± 0.003	0.127± 0.005	26.380± 3.670	32.770± 2.160	59.810± 1.220	72.770± 1.270
Eto-van	0.205± 0.005	0.289± 0.010	0.188± 0.004	0.083± 0.006	37.037± 1.850	40.640± 3.330	61.110± 1.110	81.110± 2.160*
Eto-umbe	0.205± 0.014	0.288± 0.011	0.188± 0.005	0.094± 0.011	33.790± 2.816	42.315± 1.766	61.759± 1.031	80.278± 2.778*
Eto-syr	0.194± 0.015	0.288± 0.011	0.188± 0.005	0.083± 0.009	29.630± 2.018	41.944±1.951	62.500± 1.156	81.944± 1.944*
Eto-sesa	0.194± 0.014	0.244± 0.005	0.144± 0.005	0.100± 0.009	39.352± 3.610	49.352±1.306	70.648± 0.925	79.259± 1.766*
Eto-gua	0.277± 0.005	0.316± 0.009	0.271± 0.005	0.133± 0.009	26.851± 1.851	35.000±1.924	42.770± 1.272	70.277± 2.169
Eto-car	0.200± 0.0192	0.305± 0.011	0.205± 0.005	0.150± 0.009	35.640± 6.819	36.110±2.770	56.660± 1.380	68.330± 2.169

***P < 0.05 compared to the standard drug (etodolac) are significant, and data were given in mean ± SE of %inhibition and analyzed by ANOVA.**

Table 4. Spectral analysis

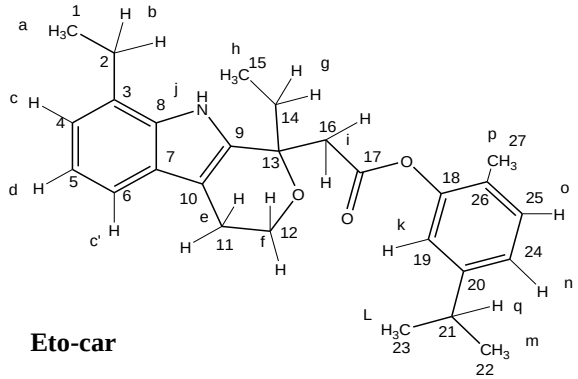
Prodrug	Spectral analysis
Eto-van	IR: 3390.15 cm^{-1} (N-H) stretching of amine, 1735.93 cm^{-1} (C=O) Stretching of esters, 1253.45 cm^{-1} (C-O) stretching of esters; ^{1}H-NMR: A=1.09-1.16(t), b=2.14-2.18(qua), c=7.03-7.04(d), c'=6.99-7.00(d), d=7.34-7.36(t), e=2.85-2.88(t), f=2.93-3.03(t), g=1.98-2.02(qua), h=0.88-0.92(t), i=2.93(s), j=9.23(s), k=7.250-7.251(d), L=7.25-7.26(d), m=9.82(s), n=7.46(s), o=3.15(s); ^{13}C-NMR: δ = 173.27 (C-17), 135.91 (C-9), 134.48(C-8), 126.62 (C-24), 126.18 (C-18) Mass (m/z): 422.49(M+1), 270.14($C_{17}H_{19}NO_2$)
Eto-umbe	IR: 3340.34 cm^{-1} (N-H) Stretching of amine, 1739.35 cm^{-1} (C=O) Stretching of esters, 1095.15 cm^{-1} (C-O) stretching; ^{1}H-NMR: a=1.29-1.32(t), b=2.04-2.09(qua), c=7.00-7.01(d), d=7.05-7.08(t), e=6.53-6.54(d), f=3.22-3.25(t), g=3.12-3.16(t), h=2.17-2.21(t), i=2.72(s), j=9.26(s), k=6.72-6.82(d), L=7.02-7.16(d), m=6.83-6.86(d), n=6.76-6.78(d), o=7.42(s); Mass (m/z): $M^{+\cdot}$ =431.38, M+1=432, 270.14($C_{17}H_{19}NO_2$)
Eto-syr	IR: 3327.21 cm^{-1} (N-H) stretching of amine, 1716.71 cm^{-1} (C=O) stretching of esters, 1311.59 cm^{-1} (C-O) stretching of esters.; ^{1}H-NMR: a=1.29-1.32(t), b=2.04-2.09(qua), c=7.00-7.01(d), d=7.05-7.08(t), e=6.53-6.54(d), f=3.22-3.25(t), g=3.12-3.16(t), h=0.89-0.92(t), i=2.93(s), j=9.82(s), k=3.42(s), L=7.26(s), m=9.62(s), n=7.26(s), o=3.62(s); Mass (m/z): 452.52(M +1), 270 ($C_{17}H_{19}NO_2$)
Eto-sesa	IR: 3395.35 cm^{-1} (N-H) stretching of amine, 1740.71 cm^{-1} (C=O) stretching of ester, 1140.42 cm^{-1} (C-O) stretching of ester; ^{1}H-NMR: a=1.29-1.32(t), b=2.04-2.09(qua), c=7.00-7.01(d), d=7.05-7.08(t), e=6.53-6.54(d), f=3.22-3.25(t), g=3.12-3.16(t), h=2.17-2.21(qua), i=0.88-0.91(t), j=2.98(s), k=6.76-6.78(d), L=7.36-7.37(d), m=7.25(s), n=5.89(s), o=8.89(s); ^{13}C-NMR: δ = 171.83 (C-17), 148.15 (C-23), 145.68 (C-21), 144.54 (C-18), 135.53 (C-9), 134.51 (C-8), 60.73 (C-13), 43.11 (C-12); Mass (m/z): 408.46 (M+1), 270=($C_{17}H_{19}NO_2$);
Eto-gua	IR: 3367.71 cm^{-1} (N-H) Stretching of amine, 1753.29 cm^{-1} (C=O) stretching of ester, 1257.59 cm^{-1} (C-O) stretching of ester; ^{1}H-NMR: a=1.22-1.25(t), b=2.80-2.83(qua), c&c'=7.36-7.37(d), d=7.23-7.24(t), e=3.30-3.35(t), f=3.15-3.19(t), g=2.73-2.77(qua), h=0.92-0.95(t), i=1.66(s), j=9.17(s), k=7.235-7.239(d), L&m=7.25-7.29(t), n=7.37(d), o=3.83(s); ^{13}C-NMR: δ = 170.69 (C-17), 150.85 (C-18),139.38 (C-23), 135.97 (C-9), 134.5 (C-8), 60.71 (C-13), 55.86 (C-12), 43.04 (C-24); Mass (m/z): 394.48 (M+1), 270 ($C_{17}H_{19}NO_2$);
Eto-car	IR: 3385.07 cm^{-1}(N-H) stretching of amine, 1720.35 cm^{-1} (C=O) stretching of ester, 1193.25 cm^{-1} (C-O) stretching of ester. ^{1}H-NMR: a&h=1.29-1.32(t), b=2.15-2.21(q), c,=7.36-7.37(d), c'=7.00-7.01(d), d=7.05-7.08(t), e=3.22-.3.25(t), f=3.11-3.15(t), g=2.04-2.11(q), i=1.67(s), j=8.77(s) k=6.53(s), L&m=0.88-0.91(d), n=6.45-6.47(d), o=6.76-6.78(d), p=1.25(s), q=2.76-2.88(septate). Mass (m/z): 420.25(M+1), 270 ($C_{17}H_{19}NO_2$)

Table 5.1. Acidic hydrolysis of mutual prodrugs (pH 1.2)

Drugs	Amount of etodolac released (mg) in different 30 min. interval						
	0.0 hr.	**0.5 hr.**	**1.0 hr.**	**1.5 hr.**	**2.0 hr.**	**2.5 hr.**	**3.0 hr.**
Eto-van	0.9	1.8	3.3	4.9	6.2	6.8	9.4
Eto-gua	0.76	2.01	3.15	5.1	6.12	6.71	7.9
Eto-car	0.8	1.74	2.87	4.06	5.74	7.60	9.17
Eto-umbe	0.91	1.59	2.72	3.62	6.26	7.45	9.02
Eto-syr	0.88	1.71	3.45	4.63	6.15	7.31	7.98
Eto-sesa	0.91	1.89	3.47	5.55	7.60	8.20	9.40

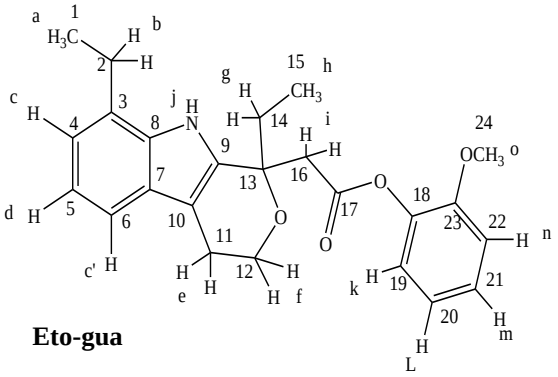
Table 5.2. Basic hydrolysis of mutual prodrugs (pH 7.4)

Drugs	Amount of etodolac released (mg) in different 30 min. interval						
	0.0 hr.	**0.5 hr.**	**1.0 hr.**	**1.5 hr.**	**2.0 hr.**	**2.5 hr.**	**3.0 hr.**
Eto-van	1.15	3.35	6.44	10.94	12.41	13.94	18.05
Eto-gua	1.15	2.31	4.95	7.95	10.92	13.65	16.18
Eto-car	1.00	2.45	5.10	8.56	12.41	15.67	15.97
Eto-umbe	1.00	3.05	6.74	9.09	12.56	17.16	18.94
Eto-syr	1.44	4.84	6.44	10.94	13.90	15.73	17.90
Eto-sesa	0.80	2.60	4.66	7.96	9.40	12.16	17.16



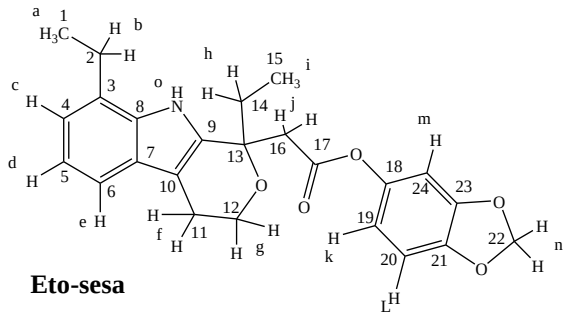
Eto-car

IUPAC Name: (5-isopropyl-2-methylphenyl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate)



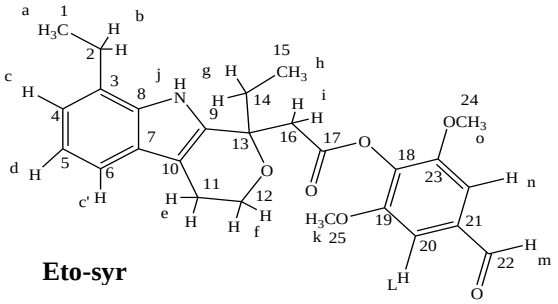
Eto-gua

IUPAC Name: 2-methoxyphenyl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate



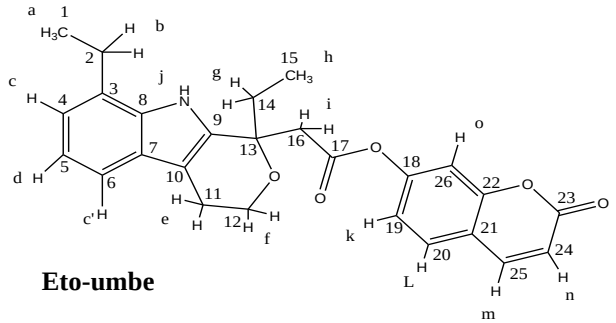
Eto-sesa

IUPAC Name: benzo[d][1,3]dioxol-5-yl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate



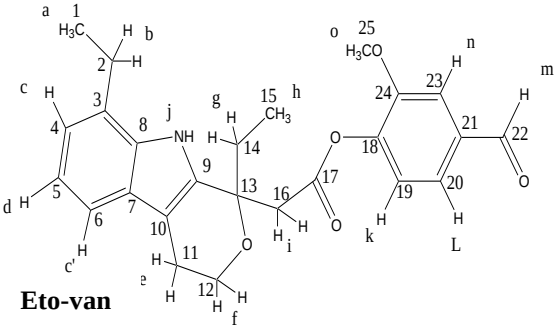
Eto-syr

IUPAC Name: 4-formyl-2,6-dimethoxyphenyl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate



Eto-umbe

IUPAC Name: 2-oxo-2H-chromen-7-yl 2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate



Eto-van

IUPAC Name: 4-formyl-2-methoxyphenyl2-(1,8-diethyl-1,3,4,9-tetrahydropyrano[3,4-b]indol-1-yl)acetate

Figure 2. Structures of mutual prodrugs

Conclusion

The prodrugs of etodolac were synthesized by following the Steglich esterification reaction. All the prodrugs have appropriate lipophilicity, and chemically stable. Hydrolysis study of prodrugs showed the ester linkage prodrugs were hydrolysed faster in comparison with acidic hydrolysis, in that all the prodrugs hydrolysed slowly. Relatively slower hydrolysis in acidic environment and faster hydrolysis in basic medium revealed the greater amount of drug absorb from intestine and also it reduces the ulcerogenic potential of the parent molecule. The synthesized compounds had higher analgesic and anti-inflammatory effects only except Eto-gua and Eto-car. All of the six compounds were free from ulcerogenic effect which was much more with etodolac. The main conclusion of the study was that mutual prodrugs were more effective than parent chemical entity and masking of the acidic functionality of NSAIDs with phytophenols make them more effective and reduction in the side effects like masking of gastric ulceration.

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Conflicts of Interest

There is no conflict of interest in the manuscript

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Ethics Statements

The authors have used animals for study, so taken approval from Institutional Animal Ethical Committee, Institute of Pharmaceutical Research, GLA University, Mathura, UP, India. (1260/PO/Re/S/09/CPCSEA).

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Kamal Shah.; analysis, interpretation of results, draft manuscript preparation: Gaurav Krishna. All authors reviewed the results and approved the final version of the manuscript.

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