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Design, Synthesis and Stability Studies of Mutual Prodrugs of NSAID's

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Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen and indomethacin have been conjugated with naturally occurring and synthetic phenolic and alcoholic antioxidants with the objective of obtaining NSAIDs-antioxidant prodrugs as gastrosparing NSAIDs with improving therapeutic efficacy by masking of carboxylic group chemically. Promoieties like vanilline, and chalcone were selected with the aim of getting synergistic effect and antioxidant property. *In silico* prediction of solubility and partition coefficient was performed using calculated physicochemical parameters. All the prodrugs were found to be highly stable at acidic pH while undergoes hydrolysis at neutral and alkaline pH as indicated by their $t_{1/2}$ values. Synthesized prodrug derivatives showed increased anti-inflammatory activity that might be attributed to synergistic effect as ibuprofen and indomethacin conjugates to antioxidants that are natural analgesics.

Keywords: NSAID; Mutual prodrug; Antioxidant; Hydrolysis kinetics; Anti-inflammatory

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Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications in the world, owing to their analgesic, anti-inflammatory and antipyretic properties [1]. However, the use of "traditional" NSAIDs results in serious upper gastrointestinal (GI) adverse events e.g. indomethacin and ibuprofen [2]. The pharmacological activity of NSAIDs is related to their ability to inhibit the activity of the enzyme cyclooxygenases (COXs) involved in the biosynthesis of prostaglandin H_2 (PGH_2) [3]. It is now well known that COX exists in two isoforms, namely COX-I and COX-II, which are regulated differently. COX-I is constitutively expressed in stomach to provide cytoprotection in the GIT. COX-II is inducible and plays a major role in prostaglandin biosynthesis in inflammatory cells. Since most of the NSAIDs used clinically inhibit both isoforms, there is enough evidence that inhibition of COX-I rather than that of COX-II underlies gastric ulcer formation [3,4]. However, long term uses of these agents have shown some potential limitation, including ulcer exacerbation in high-risk patients, delayed GI ulcer healing, kidney toxicity and cardiovascular side effects. Hence safety of these agents is questionable on their long-term use and some of these agents have been withdrawn from the market. Thus need of safer NSAID still remains.

Prodrug design is a choice of approach in solving many of the stability, solubility, permeability and targeting problems that plague drug discovery and development. The prodrug approach has the ability to keep promising new drug candidates alive through development and improving the safety and efficacy of existing drug products. It is effective for drugs suffering from undesirable side effects. A mutual prodrug normally comprises of two biologically active agents coupled together so that each acts as a pro-moiety for the other agent [5,6]. The carrier may have synergistic effect or it may have some additional pharmacological properties lacking in the parent drug. In Benorylate, aspirin was coupled with paracetamol which helped in minimizing ulceration caused by aspirin. In this drug, hydroxyl group of paracetamol was coupled with carboxylic group of aspirin by an ester bond [7,8].

Esters are the most common prodrugs used, and it is estimated that approximately 49% of all marketed prodrugs are activated by enzymatic hydrolysis. Ester prodrugs are most often used to enhance the lipophilicity, and thus the passive membrane permeability, of water soluble drugs by masking charged groups such as carboxylic acids and phosphates. The synthesis of an ester prodrug is often straightforward. Once in the body, the ester bond is readily hydrolysed by ubiquitous enzyme esterases found in the blood, liver and other organs and tissues, including carboxyl esterases, acetylcholinesterases, butyrylcholinesterases, paraoxonases and arylesterases [9]. Indomethacin was conjugated with PEG or TEG by an ester or amide linkage [10]. Mefenamic acid was conjugated with β-cyclodextrin via ester bond. After

oral administration, cyclodextrins are not hydrolysed during their transit time through the stomach, but its hydrolysis occurs only in colon by colonic micro flora. Hence, this approach can be used for colon targeting and to avoid the exposure of free drug to the stomach [11].

Literature reveals that many efforts have been made to synthesize prodrugs of ketoprofen, aceclofenac, diclofenac, flurbiprofen, naproxen, ibuprofen, etc., via masking the carboxylic acid group by forming ester and amide prodrugs using various amino acids, dextran and sulpha drug [12-22]. Glucosamine hydrochloride, an amino sugar, is being used as anti arthritic agents, was used to mask COOH group of flurbiprofen [23]. It has been well known that reactive oxygen species (ROS) plays a significant role in the formation of gastric ulceration associated with NSAID therapy. Co-administration of antioxidants with NSAIDs in formulated dosage forms have shown decrease the risk of NSAIDs induced GI toxicity and ulcerogenic side effects. These observations indicate that antioxidants may be used to prevent NSAIDs induced gastric ulcers [24-27].

During the past few decades, a large number of naturally occurring compounds have been identified as antioxidants and anti-inflammatory such as vanillin, and chalcone (phenyl styryl ketone) and which are viewed as promising therapeutic agents for treating free radical mediated diseases including NSAID induced peptic ulcers. Based on these observations, it has been suggested that co-administration of antioxidants and NSAID's in formulated dosage form may possibly decrease the risk of NSAIDs induced gastrointestinal side effects [28].

Thus introduction of mutual prodrugs in human therapy had been successful in overcoming the undesirable properties like poor absorption, poor bioavailability, non-specificity and GIT toxicity. In the view of this background, the present study was conducted to design, synthesis, and preliminary kinetics study of mutual prodrugs of NSAIDs with different antioxidants to get NSAIDs with lesser ulcerogenic side effects while retaining the anti-inflammatory and analgesic activity.

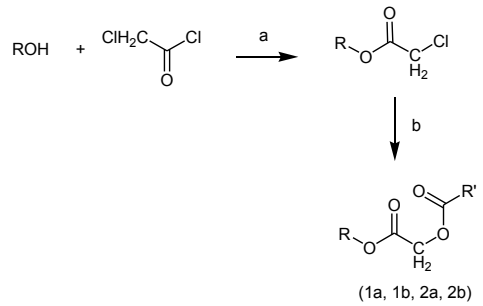
Experimental

Molecular modelling

Indomethacin and ibuprofen were drawn and minimized using steepest descent method with MMFF force field. The prodrug structures were processed in similar manner. Descriptors were calculated to find the physicochemical characteristics. MDS 3.0 was used for the calculation of descriptors [29]. Antiulcer activity prediction was performed using PASS (Prediction of Activity Spectrum for Substance) software [30].

Chemistry

All reagents and anhydrous solvents were of analytical grade and were used as received from the commercial supplier. NSAIDs (ibuprofen and indomethacin) were obtained as gift samples from Micro HC Labs, Bangalore. Plasma sample was obtained from Kashibai Navale Hospital, Pune. Melting Points were determined with Veego VMP-D digital melting point apparatus and are uncorrected. Thin layer chromatography (TLC) was run on precoated silica gel G plates (Whatman) to check the purity of the products as well as monitoring the progress of reactions. FT-IR spectra were recorded by using Jasco FT-IR 4100 spectrophotometer and the determination of the spectra were performed by using KBr discs. NMR of samples was carried out at Pune University. Jasco UV-Visible Double beam spectrophotometer of model V-630 was used with UV 2075 plus as detector. The title compounds described in this study were prepared as outlined in scheme given in Figure 1.



a=TEA, Stirring 1 hr, b=R'COOH, TEA, NaI, stirring 24 hr at 25 °C.

Compound	R	R′
1a	CHO, OCH_3 — Vanilline part	H_3CO, Cl, H_3C, O — Indomethacin part
1b	Cl, O — Chalcone part	H_3CO, Cl, H_3C, O — Indomethacin part
2a	CHO, OCH_3 — Vanilline part	CH_3, CH_3, H_3C — Ibuprofen part
2b	Cl, O — Chalcone part	CH_3, CH_3, H_3C — Ibuprofen part

Figure 1 Scheme of Synthesis.

Synthesis of antioxidant-chloroacetyl derivative

A mixture of an appropriate antioxidant (0.01 mole) and TEA (0.01 mole) in dichloromethane (25 ml) was cooled in an ice salt mixture to 10˚C. To this reaction mixture, chloroacetylchloride (0.01 mole) in chloroform (25 ml) was added drop wise with constant stirring over a period of 1 hr, maintaining the temperature constant. The reaction mixture was stirred over night at room temperature, washed with 5% HCl (3 × 50 ml), 5% NaOH (3 × 50 ml) and finally with brine solution (2 × 25 ml). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed under reduced pressure to obtain the corresponding antioxidant-chloroacetyl derivative. This general procedure was

used with different antioxidants (Vanilline and Chalcone) to prepare corresponding chloroacetyl derivative. These derivatives were recrystallized from petroleum ether and ethyl acetate.

Synthesis of NSAIDs-antioxidant mutual prodrugs

A mixture of appropriate antioxidant- chloroacetyl derivatives (0.01 mole), NSAID (ibuprofen, indomethacin) (0.01 mole), TEA (0.01 mole) and sodium iodide (0.01 mole) in DMF (25 ml) was stirred over night at room temperature. The reaction mixture was poured into finely crushing ice with stirring and extracted with chloroform (4 × 25 ml). The combined organic layer was washed with 2% sodium thiosulphate (3 × 50 ml), 5% HCl (3 × 50 ml), 5% NaOH (3 × 50 ml) and finally with brine solution (2 × 25 ml). The organic layer was dried over anhydrous sodium sulphate, filtered and the solvent was removed under reduced pressure to obtain the NSAIDs-antioxidant mutual prodrugs.

Chemical and enzymatic hydrolysis

Hydrolysis at pH 1.2, pH 7.4 and pH 9.5 (chemical stability): The hydrolysis of the prodrugs was studied in aqueous HCl, phosphate buffer and NaOH solution of pH 1.2, pH 7.4 and pH 9.5 at 37˚C. The total buffer concentration was 0.1M. The samples were centrifuged for 3000 rpm for 15 min and the supernatant was taken for further analysis. The rate of hydrolysis was followed spectrophotometrically by recording the decrease in the absorbance of prodrugs accompanying the hydrolysis.

The reactions were initiated by adding 1 ml of stock solutions (1 mg/ml) of the derivatives in ethanol to preheated buffer solution to give final concentration of derivatives 0.02 mg/ml. The solutions were kept in a water bath at 37˚C and samples (3 ml) were withdrawn at appropriate time interval (15, 30, 60, 120, 240 min) and the absorbances were recorded.

Pseudo first-order rate constants (K_{obs}) for the individual reactions were calculated with the help of equation, K_{obs}=2.303/t *[log (a/a-x)], Where, 'a' is initial concentration, 'x' is the amount of drug hydrolyzed and 't' is time in minutes. The corresponding half-life ($t_{1/2}$) was then obtained from the equation: $t_{1/2}$=0.693/K_{obs} [31].

hydrolysis in plasma (enzymatic stability): The hydrolysis rates of prodrugs were studied in 80% human plasma diluted with isotonic phosphate buffer (pH 7.4) at 37˚C. The reactions were initiated by adding 0.5 ml of stock solutions (1 mg/ml) of the prodrugs in ethanol to preheated diluted plasma to give final concentration of derivatives 0.02 mg/ml. Samples (3 ml) were withdrawn at appropriate time interval (15, 30, 60, 120, 240 min), after each incubation time sample was centrifuged and the supernatant was analyzed by UV spectrophotometer.

Biological Evaluation

Anti-inflammatory activity: Anti-inflammatory activity of ibuprofen, indomethacin and prodrugs was done by using carrageenan-induced rat paw edema model [32,33]. Group I served as control and received only vehicle (0.5% w/v carboxymethylcellulose). Group II received ibuprofen (20 mg/kg) while III, IV and V, VI received prodrugs in dose molecularly equivalent to ibuprofen and indomethacin. All compounds were administered through oral gavage. After 30 min of compound administration, 0.1 ml of 1% carrageenan in normal saline was injected into the sub planter region of left hind paw and the oedema volume was measured before injection (V_0) and at the interval of every hour up to 4 hr. The percentages of swelling inhibition was calculated by formula,

% Inhibition={((V_t-V_0) control-(V_t-V_0) treated)/(V_t-V_0) control}*100.

Where, V_0 and V_t are the average volume in the hind paw of the rats before and after treatment respectively.

Results and Discussion

Molecular Modelling

Before synthesis the structures of prodrugs were drawn and minimized using MMFF force field and their physicochemical properties were calculated using Schrödinger software.

Table 1 shows calculated molecular descriptors such as H-bond acceptor, H-bond donor, molecular weight, number of rotatable bonds, calculated logP. Using these descriptors Lipinski's rule of five was verified for drug likeliness of the designed prodrugs. There is increase in rotatable bonds, H-bond acceptor count of prodrugs, while H-bond donor property of podrugs was abolished. There is also increase in polarity and logP of the prodrug molecules. The calculated log P values indicated that prodrugs are more lipophilic than the parent drugs. Thus molecules have balance of polarity and lipophilicity. Since all the five parameters of Lipinski's rule are satisfied, prodrugs show drug likeliness. Calculated values for solubility (logS) and partition coefficient (XlogP) of prodrugs were

Table 1 Calculated physicochemical descriptors of prodrugs.

Compounds	Mol.Wt.	Volume	H-Acceptor Count	H-Donor Count	Rotatable Bond Count	XlogP	Polarizability	logS
Indomethacin	357.79	303.25	4	1	8	4.226	37.36	-5.2
1a	549.96	464.83	8	0	15	5.555	55.80	-5.38
1b	656.51	552.99	7	0	15	8.577	68.29	-9.32
Ibuprofen	206.28	211.48	2	1	8	3.481	23.94	-3.5
2a	398.45	373.25	6	0	15	4.81	42.35	-4.76
2b	505.00	461.15	5	0	15	7.832	54.86	-8.17

Table 2 Characterisation of synthesized compounds.

Compound	Molecular formula	Description	% Yield	Melting point ˚C	Rf value*
1a	$C_{29}H_{24}ClNO_8$	White	56	63-65	0.76
1b	$C_{36}H_{25}Cl_2NO_7$	Yellow	68	150-152	0.62
2a	$C_{23}H_{26}O_6$	White	45	70-72	0.89
2b	$C_{30}H_{27}ClO_5$	Yellow	77	112-115	0.90

*Solvent system used: Chloroform:Methanol (4.5: 0.5)

Synthesis of 1a:

FT-IR (KBr) cm^{-1}: 2833.88(C-H), 1765 (C=O) ester, 1684 (C=O) amid (indomethacin), 1599 and 1422 (C=C), 1H NMR ($CDCl_3$ d ppm) 3.0 (S, 6H, 2-OCH_3), 7-8 (m, 10H, -Ar), 10.0 (S, 1H, -CHO), 2.4 (S, 2H, ester)

Synthesis of 2a:

FT-IR (KBr) cm^{-1}: 2868.59(C-H), 1747.19 (C=O) ester, 1684 (C=O) , 1504 and 1463 (C=C), 1H NMR ($CDCl_3$ d ppm) 3.0 (S, 3H, -OCH_3), 7.0 - 7.6 (m, 7H, -Ar), 10.0(S, 1H, -CHO), 2.5 (S, 2H, ester), 1.6 (S, 3H, R-CH_3)

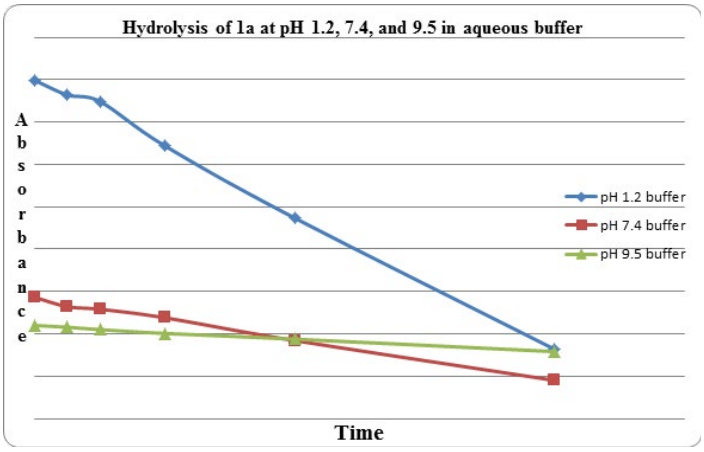
Synthesis of 1b:

FT-IR (KBr) cm^{-1}: 1750 (C=O) ester, 1644 (C=O), 1573 and 1438 (C=C), 1H NMR ($CDCl_3$ d ppm) 3.0 (S, 3H, -OCH_3), 7.0 - 8.0 (m, 15H, -Ar), 2.3 (S, 2H, ester),

Synthesis of 2b:

FT-IR (KBr) cm^{-1}: 1748 (C=O) ester, 1644 (C=O), 1574 (C=C), 1H NMR ($CDCl_3$ d ppm) 7.0 - 7.6 (m, 12H, -Ar), 2.4 (S, 2H, ester), 1.6 (S, 3H, R-CH_3)

Table 3 Hydrolysis of 1a at pH 1.2, 7.4, and 9.5 in aqueous buffer.

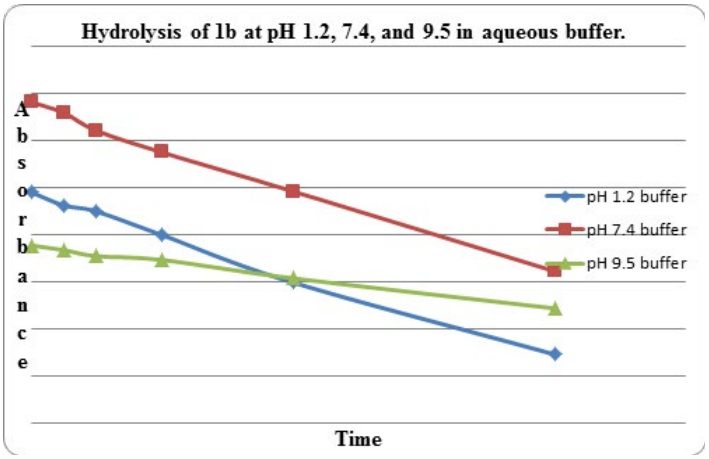


Time (min)	pH 1.2 buffer	pH 7.4 buffer	pH 9.5 buffer
0	0.399	0.143	0.1099
15	0.3819	0.132	0.1078
30	0.3743	0.129	0.105
60	0.3219	0.119	0.1005
120	0.2368	0.0921	0.0937
240	0.082	0.0451	0.079

higher than their parent NSAID compounds.

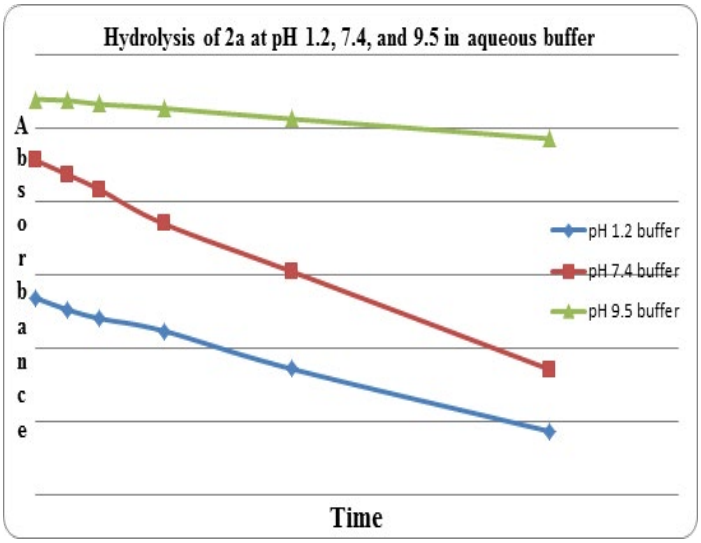
The structure of novel compounds was elucidated by spectroscopic measurements (IR and 1H NMR) and elemental analysis. Thin layer chromatography (TLC) was used throughout to optimize the reaction

Table 4 Hydrolysis of 1b at pH 1.2, 7.4, and 9.5 in aqueous buffer.



Time(min)	pH 1.2 buffer	pH 7.4 buffer	pH 9.5 buffer
0	0.049	0.0682	0.0377
15	0.0462	0.0659	0.0367
30	0.0449	0.0621	0.0354
60	0.0399	0.0575	0.0346
120	0.0298	0.0492	0.0307
240	0.0145	0.0322	0.0243

Table 5 Hydrolysis of 2a at pH 1.2, 7.4, and 9.5 in aqueous buffer.



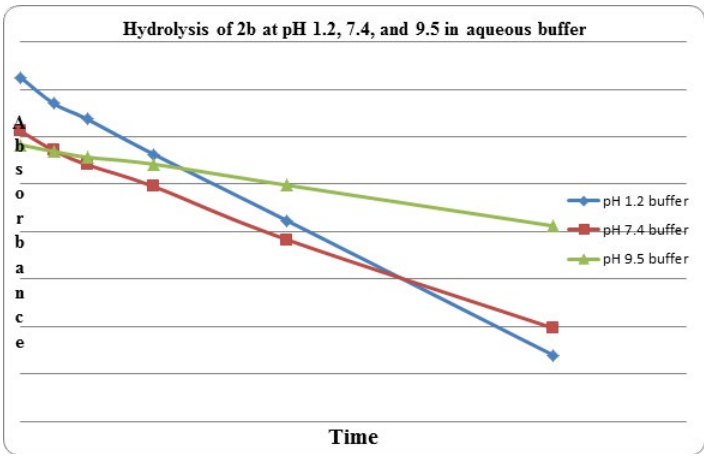
Time(min)	pH 1.2 buffer	pH 7.4 buffer	pH 9.5 buffer
0	0.269	0.4568	0.5401
15	0.253	0.4373	0.5383
30	0.241	0.4167	0.5334
60	0.224	0.3701	0.5275
120	0.172	0.3046	0.5131
240	0.0872	0.1717	0.4862

for purity and completion along with physical and elemental analyses data for titled compounds are summarized in **Table 2**.

Chemical and enzymatic hydrolysis evaluation

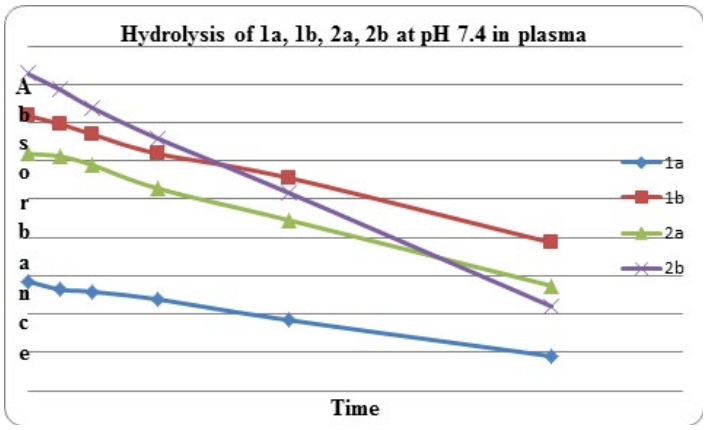
Hydrolysis kinetics study of prodrugs was determined in acidic, neutral and alkaline pH to determine the fate of prodrugs.

Table 6 Hydrolysis of 2b at pH 1.2, 7.4, and 9.5 in aqueous buffer.



Time (min)	pH 1.2 buffer	pH 7.4 buffer	pH 9.5 buffer
0	0.0725	0.0612	0.0583
15	0.067	0.0571	0.0569
30	0.0637	0.0541	0.0557
60	0.0563	0.0495	0.0542
120	0.0423	0.0383	0.0498
240	0.0139	0.0196	0.0412

Table 7: Hydrolysis of 1a, 1b, 2a, 2b at pH 7.4 in plasma.



	Absorbance			
Time (min)	1a	1b	2a	2b
0	0.143	0.3592	0.3093	0.414
15	0.132	0.3481	0.3058	0.393
30	0.129	0.3344	0.2943	0.369
60	0.119	0.3093	0.2638	0.329
120	0.092	0.2776	0.2224	0.258
240	0.045	0.1933	0.137	0.11

It has been reported that the essential pre-requisite for success in the use of prodrugs is that the masked compounds should be acid stable to prevent the direct contact effects with the gastric mucosa as well as the local inhibition of the prostaglandins. Therefore, pH values ranging acidic, neutral and alkaline were selected to mimic the appropriate clinical range. **Tables 3-6** shows absorbance reading obtained by UV

Table 8 Results of rate constant of hydrolysis of 1a, 1b, 2a, 2b in aqueous buffer at pH 1.2, 7.4 and 9.5.

Compound	pH of buffer	Kobs (min^{-1})	$t_{1/2}$ (min)
1a	7.4	$4.2*10^{-2}$	150.93
	9.5	$4.2*10^{-2}$	50.37
2a	7.4	$7.2*10^{-2}$	99.62
	9.5	$6.7*10^{-2}$	40.2
1b	7.4	$4.3*10^{-2}$	100.89
	9.5	$4.9*10^{-2}$	70.24
2b	7.4	$4.3*10^{-2}$	183.75
	9.5	$7.0*10^{-2}$	54.18

Table 9 Results of rate constant of hydrolysis of 1a, 1b, 2a, 2b, in plasma at pH 7.4.

Compound	Kobs(min^{-1})	$t_{1/2}$(min)
1a	$5.5*10^{-2}$	118.6
1b	$5.02*10^{-2}$	71.84
2a	$5.4*10^{-2}$	86.71
2b	$4.02*10^{-2}$	102.2

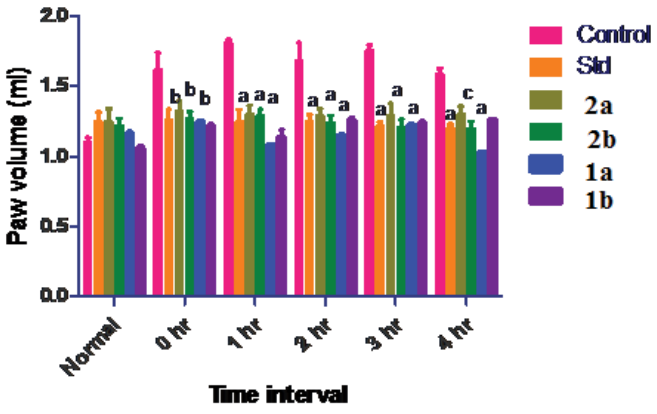


Figure 2 Anti-inflammatory activity of 1a, 1b, 2a, 2b.

spectrophotometrically due to hydrolysis of 1a, 1b, 2a, 2b at pH 1.2, 7.4, and 9.5 in aqueous buffer respectively. **Table 7** shows absorbance reading obtained by UV spectrophotometrically due to hydrolysis of 1a, 1b, 2a, 2b at pH 7.4 in plasma respectively. Tables show plot of Time Vs absorbance for values obtained.

The results of rate constant of hydrolysis of 1a, 1b, 2a, 2b in aqueous buffer at pH 1.2, 7.4 and 9.5 are shown in **(Table 8)**, while **(Table 9)** shows results of rate constant of hydrolysis of 1a, 1b, 2a, 2b, in plasma at pH 7.4. The chemical degradation of ester prodrugs of ibuprofen followed first order kinetics, as revealed by UV analysis. In the acidic buffer solution of pH 1.2, all prodrugs (1a, 1b, 2a, 2b) showed high chemical stability, which implied that the compounds passed unhydrolyzed through the stomach on oral administration. While at neutral pH 7.4 their ($t_{1/2}$) ranging from 99 min to 183 min. Furthermore,

Table 10 Anti-inflammatory activity of mutual prodrugs of ibuprofen and indomethacin.

Compound	(% Inhibition)[a]			
	1 hr	2 hr	3 hr	4 hr
Indomethacin	32.93 ± 0.64	34.0 ± 1.99	23.59 ± 2.74	23.72 ± 3.77
1a	36.08 ± 2.04	36.68 ± 3.01	27.15 ± 2.82	27.30 ± 2.89
1b	31.55 ± 1.31	34.37 ± 1.51	25.63 ± 3.27	26.25 ± 4.11
Ibuprofen	30.77 ± 4.90	31.64 ± 2.71	23.59 ± 2.74	23.72 ± 3.77
2a	28.40 ± 2.92	25.59 ± 5.18	19.42 ± 4.24	18.15 ± 1.70
2b	28.52 ± 1.80	31.603 ± 79	23.96 ± 3.67	24.19 ± 3.43

[a]Data represented as mean ± SEM, n=6, p<0.001 with respect to control.

the degradation of all three prodrugs at alkaline pH 9.5 showed comparative less half lives than at neutral pH with $t_{1/2}$ ranging from 40 to 55 min indicating hydrolysis pattern at alkaline condition. Values of the rate parameter K_{obs} for hydrolysis of prodrugs at different pH and 37˚C are listed in **Table 8** along with the half-lives ($t_{1/2}$). Thus, it confirms that the possibility for gastric irritation produced by ibuprofen and indomethacin is reduced. The mutual prodrug may have devoid of gastric irritation in GIT due to unionized form in stomach and ionized form in intestine and may have synergistic effect that can also be attributed to anti-inflammatory activity data.

From the above study, therefore, the prepared mutual prodrugs of ibuprofen and indomethacin fulfilled the requirement since they showed good stability at acidic pH and thus, antioxidants suits to be the best promoieties for ibuprofen and indomethacin.

Anti-inflammatory activity

Anti-inflammatory screening for parent and prodrugs is indicated by percentage inhibition of swelling in carrageenan-induced edema in rat paw. All the experimental protocols were carried out with the permission of the Institutional Animal EtFhics Committee (IAEC). The prodrugs showed better anti-inflammatory activity with percentage inhibition of 28% to 36% as compared to 30% when studied up to 4 hr at the interval of every hour, as tabulated in **Table 10**. The compound 1a and 1b showed enhanced anti-inflammatory activity than parent drug. Statistical significance testing using one way analysis of variance showed that activity of the parent and prodrugs were effective in comparison with the control group. It is also evident that improvement in anti-inflammatory potency may be due to the increased lipophilic character of ester as compared to parent drug as increased lipophilicity increases cell membrane permeability (to a certain extent) and, ultimately, improves bioavailability. This increased anti-inflammatory might be due to the resultant synergistic effect by conjugation with natural antioxidants. Figure 2 shows plot of rat paw volume Vs Time interval.

Prediction of antiulcer activity

Antiulcer activity and gastroprotective properties were predicted using PASS software. **Table 11** shows prediction of antiulcer activity of the compounds using PASS software. PASS (Prediction of Activity Spectra for Substances) is a software tool for evaluating the general biological potential of an organic drug-like molecule.

Table 11 Prediction of antiulcer activity of compounds using PASS software.

Compound	Pa value	Pi value	Difference
Indomethacin	0.246	0.148	0.098
1a	0.294	0.103	0.191
1b	0.697	0.005	0.692
Ibuprofen	0.296	0.102	0.194
2a	0.417	0.039	0.378
2b	0.697	0.005	0.692

Pa: probability "to be active, Pi: probability "to be inactive"

Leave-one-out cross-validation (LOO CV) procedure is performed using the whole PASS training set for validation of prediction quality. Biological activity spectrum is predicted for each compound using the structure-activity relationships calculated from the data for all other compounds. The prediction result is compared with known experimental data for the studied compound. The procedure is repeated for all compounds from the PASS training set. Based on error of prediction (EP) obtained, the average Invariant Accuracy of Prediction (IAP=1-EP) values are calculated for each biological activity and for all biological activities. It estimates Pa (probability "to be active") and Pi (probability "to be inactive") as a chance that the studied compound is belonging to the sub-class of active or inactive compounds in PASS training set respectively. If the Pa value (probability "to be active") is greater than Pi value (probability "to be inactive") for all the prodrugs indicating that probability of antiulcer activity is more than parent NSAID compounds. The predicted values indicate that software passes the test for prodrugs.

Conclusion

The mutual prodrugs of ibuprofen and indomethacin employing vanillin and chalcone were successfully synthesized and characterized by spectral (UV, IR, NMR) data. Calculated logP values indicated that the prodrugs are more lipophilic than the parent drugs. The mutual prodrugs were hydrolysed at pH 7.4 and pH 9.5 but were resistant at pH 1.2 indicating that the conjugates are resistant to acidic condition and both were found to be showing enhanced anti-inflammatory activity than the parent drug. The prodrugs were significantly hydrolysed in 80% diluted plasma at pH 7.4. Hence vanillin and chalcone could be used as promoities for ibuprofen and indomethacin. Mutual prodrug approach therefore gives an opportunity in medicinal chemistry to improve the clinical and therapeutic effectiveness of a drug that is suffering from some undesirable properties hindering its clinical usefulness.

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Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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