

Novel ibuprofen prodrugs with improved pharmacokinetics and non-ulcerogenic potential

Valmik D. Dhakane · Hemant V. Chavan ·
Vishnu N. Thakare · Laxman K. Adsul ·
Sadanand N. Shringare · Babasaheb P. Bandgar

Received: 15 March 2013 / Accepted: 20 May 2013
© Springer Science+Business Media New York 2013

Abstract In the present study, we evaluated the anti-inflammatory activity with pharmacokinetic, ulcerogenic properties of various synthesized prodrugs of ibuprofen in experimental animals. Prodrugs **2**, **6**, **9**, **10**, **12**, and **14** were found to possess significant anti-inflammatory activity with almost non-ulcerogenic potential than standard drug ibuprofen **1a** in both normal and inflammation-induced rats. Metabolic stability of prodrugs **2**, **6**, **9**, **10**, **12**, and **14** were also studied in rat liver microsomes and oral bioavailability was determined by estimating area under curve (AUC) and plasma concentration of these prodrugs at various time intervals. The experimental findings elicited higher AUC and plasma concentration at 1 and 2 h indicating improved oral bioavailability as compared to parent ibuprofen. These prodrugs are found to have least gastric ulceration with retain anti-inflammatory activity observed in experimental animals. Therefore, present experimental findings demonstrated significant improvement of various pharmacokinetic properties with least ulcerogenic potential of ester prodrugs of ibuprofen an anti-inflammatory agent

Keywords Prodrug · Ibuprofen · Anti-inflammatory activity · Non-ulcerogenic · Pharmacokinetic properties

Abbreviations

NSAID	Nonsteroidal anti-inflammatory drug
Tris-HCl	Tris(hydroxymethyl)aminomethane hydrochloride
NADPH	Nicotinamide adenine dinucleotide phosphate
HPLC	High performance liquid chromatography
GC	Gas chromatography
RLM	Rat liver microsomes
RP	Rat plasma
API	Active pharmaceutical ingredient
UI	Ulcer index
GI	Gastrointestinal
DMAc	Dimethyl acetamide
PTSA	<i>p</i> -Toluene sulfonic acid
PXRD	Powder X-ray diffraction
RT	Room temperature
TMG	1,1,3,3-Tetramethyl guanidine
18 Crown 6	1,4,7,10,13,16-Hexaoxacyclooctadecane
EP	European pharmacopoeia
Ar	Aromatic
BDL	Below detection limit

Introduction

Ibuprofen, a well-known nonsteroidal anti-inflammatory drug (NSAIDs) most widely used and represents a mainstay in the therapy of acute and chronic pain and inflammation viz. osteoarthritis and rheumatoid arthritis (Coruzzi *et al.*, 2007; Kawail *et al.*, 2005; Klasser and Epstein, 2005). The

V. D. Dhakane · H. V. Chavan · L. K. Adsul ·
S. N. Shringare · B. P. Bandgar (✉)
Medicinal Chemistry Research Laboratory, School of Chemical
Sciences, Solapur University, Solapur 413 255,
Maharashtra, India
e-mail: bandgar_bp@yahoo.com

H. V. Chavan
e-mail: hemantchavan.sus@rediffmail.com

V. N. Thakare
Department of Pharmacology, Sinhgad Institute
of Pharmaceutical Sciences, Kusgaon (Bk),
Lonavala 410 401, Maharashtra, India

anti-inflammatory activity of ibuprofen is mainly due to its inhibitory effect on prostaglandin synthase (cyclooxygenase) which is involved in the formation of various inflammatory prostaglandins. However, the use of ibuprofen is frequently associated with an adverse effect, in particular its gastritis associated with ulceration, which are related to inhibiting prostaglandin synthesis. These adverse effects are likely due to the presence of carboxylic group and inhibition of protective cyclooxygenase-1 (COX-1) enzyme in stomach (Rainsford, 1988; Bhosale *et al.*, 2006). Inhibition of cyclooxygenase-2 (COX-2) enzyme is desirable with ibuprofen for its anti-inflammatory activity is concern; however, simultaneously the COX-1 inhibition is documented with ibuprofen which is undesired. Selective inhibition of COX-2 with coxibs (rofecoxib, valdecoxib) are of great importance in inflammation. However, their serious cardiovascular effects (stroke) observed from clinical studies and post marketing surveillance, forcing the pharma companies to withdraw these coxibs from the market (Bresalier *et al.*, 2005; Nussmeier *et al.*, 2005). Precondition for the prodrugs possesses good pharmacological (anti-inflammatory) effect, differentiated pharmacokinetic property (appropriate aqueous solubility and lipophilicity) and no ulcerogenic action. Various ester prodrugs of NSAIDs were found to possess potent anti-inflammatory activity with lesser ulcerogenic property on gastrointestinal (GI) mucosa Perioli *et al.*, 2004; Halen *et al.*, 2009; Shanbhag *et al.*, 1992; Chen *et al.*, 2009; Borhade *et al.*, 2012.

The majority of NSAID prodrugs have been produced by masking of carboxylic functional group. The esters have dominated prodrug research due to their ideal characteristic of exhibiting reasonable in vitro chemical stability, allowing them to formulate with desired $t_{1/2}$. Because of its ability to function as esterase substrates, esters are suitably liable in vivo condition (Bundgaard, 1986, 1989). Therefore, by the use of prodrug approach, one can mask the carboxylic acid functional group of NSAID, which on hydrolysis, an active parent drug to be released in vivo for its anti-inflammatory activity along with reduction in ulcerogenic property (Gairola *et al.*, 2005; Wallace, 1994; Kim *et al.*, 2006).

Toxicological and pharmacological profiles of ibuprofen guaiacol ester (Cioli *et al.*, 1980), synthesized ester prodrugs using *N*-hydroxymethylsuccinimide and *N*-hydroxymethylisatin as promoieties to reduce their GI toxicity and improve bioavailability (Mahfouz *et al.*, 1999), 2-formylphenyl esters (Abordo *et al.*, 1998) was studied. However, their release characteristic is found to exhibit variation. And most of the prodrugs of NSAIDs reported in the literatures have poor or limited hydro solubility (Nielsen and Bundgaard, 1988; Shaaya *et al.*, 2003).

The various studies on prodrugs suggested that, nitric oxide (NO) release elicits cytoprotective activity and retained

anti-inflammatory activity (Huang *et al.*, 2011). However, the aim of synthesis of prodrugs of NSAIDs is to prevent the adverse effect by protecting GI damage and this effect can be masked by producing inert promoieties. Because there may be a chance of other side effect that might be associated with NO on the other organ system apart from GI.

Based on the scientific reports on ibuprofen prodrugs which are of great interest of reducing GI toxicity and improved biopharmaceutical properties further experiments are required to design and identify prodrugs of ibuprofen, which would be suitable for clinical practice in terms of stability, metabolism, toxicology and side effects. Ibuprofen is presently marketed as a racemic mixture of active substance for its anti-inflammatory and analgesic actions. Therefore, racemic mixture of ibuprofen is taken for the preparation of prodrugs.

The present research work is an attempt to develop prodrugs of ibuprofen **1–15**, using novel promoieties **2a–o** having high in vivo enzymatic conversion rate, anti-inflammatory, physicochemical and non-ulcerogenic properties.

Experimental section

Materials and methods

All reactions were monitored by thin layer chromatography (TLC) with 0.2 mm Merck silica gel F₂₅₄ plates and GC. NMR spectra were recorded on Bruker DRX FT NMR at 400 MHz spectrometer in CDCl₃ using TMS as internal standard (chemical shifts are expressed as δ values relative to TMS as internal standard). IR spectra were recorded on a FTIR Perkin-Elmer model RXI spectrophotometer. Mass spectra were recorded on an Agilent GCMS-5975C VL MSD spectrophotometer. HPLC analysis was performed using a Shimadzu LC 2010 CHT with UV detector. All prodrugs **1–15** were analyzed by HPLC, and their purity was confirmed to be excess of 98 %. GC analysis was performed on Shimadzu GC-2014 instrument, and purity of promoieties **2a–o** was confirmed to be in excess of 97 %. In vivo study was performed using experimental protocols reviewed and approved by the Institutional Animal Ethics Committee (SIPS/IAEC/2011-12/17) and conform to the Indian National Science Academy Guidelines for the use and care of experimental animals in research. The animal house Registration No. with Government of India is 962/c/06/CPCSEA.

General procedure for the preparation of prodrugs (**1–15**)

Ibuprofen (2 g, 9.67 mmol) was dissolved in dimethyl acetamide (25 mL) at room temperature (25 °C). To this solution,

potassium carbonate (4 g, 28.9 mmol) under nitrogen atmosphere was added. The reaction mass was stirred for 20 min at room temperature (25 °C). Then, to the above reaction mass, promoiety **2a–2o** (8.7 mmol) was added under stirring and maintained the reaction under stirring for 4–5 h at room temperature. The reaction progress was monitored by TLC and GC. After completion, the reaction mass was added under stirring into the mixture of ethyl acetate (60 mL) and water (100 mL). The organic layer was separated and aqueous layer extracted with ethyl acetate twice (60 mL \times 2). Combined organic layer washed with 20 % solution of sodium bicarbonate (100 mL \times 2) followed by brine wash (10 % NaCl, 100 mL). Organic layer was dried over anhydrous sodium sulfate, distilled off the solvent under vacuum and desired product collected as viscous oil.

Spectral data of synthesized compounds

Ethyl 2-(2-(4-isobutylphenyl)propanoyloxy)-3-oxobutanoate (1) Chromatographic purity (HPLC) 99.78 %; bp 298 °C; MS (ESI+) m/z = 334 [M^+]; IR (KBr, cm^{-1}): 2957, 2871, 1745, 1633, 1609, 1514, 1369, 1156, 852; ^1H NMR (CDCl_3 , 400 MHz) δ 0.89 (d, J = 6.8 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.23 (t, J = 7.2 Hz, 3H, OCH_2CH_3); 1.57 (m, 3H, ArCHCH_3), 1.84 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.08 (s, 3H, COCH_3), 2.45 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.90 (q, J = 7.2 Hz, 2H, OCH_2CH_3), 4.24 (q, J = 7.2 Hz, 1H, ArCHCH_3), 5.43 (s, 1H, OCHCO), 7.10 (d, J = 8 Hz, 2H, ArH), 7.25 (d, J = 8 Hz, 2H, ArH).

4-Acetoxybutyl-2-(4-isobutylphenyl)propanoate (2) Chromatographic purity (HPLC) 99.60 %; bp > 300 °C; MS (ESI+) m/z = 320 [M^+]; IR (KBr, cm^{-1}): 2945, 2870, 1743, 1614, 1515, 1460, 1151, 843; ^1H NMR (CDCl_3 , 400 MHz) δ 0.88 (d, J = 6.8 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.49 (d, J = 7.2 Hz, 3H, ArCHCH_3), 1.54–1.68 (m, 4H, $\text{CH}_2\text{CH}_2\text{CH}_2\text{O}$), 1.84 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.02 (s, 3H, COCH_3), 2.45 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.69 (q, J = 7.2 Hz, 1H, ArCHCH_3), 4.01 (t, J = 6 Hz, 2H, $\text{CH}_2\text{COOCH}_3$), 4.08 (t, J = 6 Hz, 2H, CH_2OCOCH), 7.09 (d, J = 8 Hz, 2H, ArH), 7.20 (d, J = 8 Hz, 2H, ArH).

1-Methoxy-1-oxopropan-2-yl-2-(4-isobutylphenyl)propanoate (3) Chromatographic purity (HPLC) 99.69 %; bp 276 °C; MS (ESI+) m/z = 292 [M^+]; IR (KBr, cm^{-1}): 2955, 2870, 1744, 1614, 1514, 1455, 1160, 1101, 848; ^1H NMR (CDCl_3 , 400 MHz) δ 0.90 (d, J = 6.4 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.42 (d, J = 7.2 Hz, 1H, OCHCH_3), 1.53 (d, J = 7.2 Hz, 3H, ArCHCH_3), 1.84 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.45 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.62 (s, 3H, COOCH_3), 3.79 (q, J = 7.2 Hz, 1H, ArCHCH_3), 5.08 (q, J = 7.2 Hz, 1H, OCHCH_3), 7.10 (d, J = 8 Hz, 2H, ArH), 7.22 (d, J = 8 Hz, 2H, ArH).

2-(Cyclohexyloxy)-2-oxoethyl-2-(4-isobutylphenyl)propanoate (4) Chromatographic purity (HPLC) 99.91 %; bp > 300 °C; MS (ESI+) m/z = 346 [M^+]; IR (KBr, cm^{-1}): 2938, 2865, 1746, 1614, 1514, 1455, 1150, 845; ^1H NMR (CDCl_3 , 400 MHz) δ 0.86 (d, J = 6.4 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.19–1.34 (m, 6H, $3 \times \text{CH}_2$), 1.60–1.71 (m, 4H, $2 \times \text{CH}_2$), 1.41 (d, J = 7.2 Hz, 3H, ArCHCH_3), 1.80 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.42 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.85 (q, J = 7.2 Hz, 1H, ArCHCH_3), 4.61 (s, 2H, OCH_2CO), 4.69 (m, 1H, COOCH), 7.12 (d, J = 8 Hz, 2H, ArH), 7.20 (d, J = 8 Hz, 2H, ArH).

2-(2-Ethylbutoxy)-2-oxoethyl-2-(4-isobutylphenyl)propanoate (5) Chromatographic purity (HPLC) 99.78 %; bp 291 °C; MS (ESI+) m/z = 348 [M^+]; IR (KBr, cm^{-1}): 2962, 2875, 1747, 1513, 1463, 1152, 847; ^1H NMR (CDCl_3 , 400 MHz) δ 0.84–0.88 (m, 12H, $4 \times \text{CH}_3$), 1.23–1.34 (m, 3H, $\text{CHCH}_2\text{CH}_3/\text{CHCH}_2\text{CH}_3$), 1.50 (qu, 2H, CHCH_2CH_3), 1.41 (d, J = 7.2 Hz, 3H, ArCHCH_3), 1.81 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.42 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.85 (q, J = 7.2 Hz, 1H, ArCHCH_3), 4.05 (t, J = 6.4 Hz, 2H, COOCH_2CH), 4.64 (s, 2H, OCH_2CO), 7.11 (d, J = 8 Hz, 2H, ArH), 7.20 (d, J = 8 Hz, 2H, ArH).

2-(2-Methoxyethoxy)-2-oxoethyl-2-(4-isobutylphenyl)propanoate (6) Chromatographic purity (HPLC) 99.77 %; bp 288 °C; MS (ESI+) m/z = 322 [M^+]; IR (KBr, cm^{-1}): 2955, 2870, 1746, 1513, 1458, 1157, 847; ^1H NMR (CDCl_3 , 400 MHz) δ 0.88 (d, J = 6.6 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.54 (d, J = 7.2 Hz, 3H, ArCHCH_3), 1.84 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.45 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.36 (s, 3H, OCH_3), 3.56 (m, 2H, CH_2OCH_3), 3.81 (q, J = 7.2 Hz, 1H, ArCHCH_3), 4.28 (m, 2H, COOCH_2), 4.59 (s, 2H, OCH_2CO), 7.11 (d, J = 8 Hz, 2H, ArH), 7.22 (d, J = 8 Hz, 2H, ArH).

2-Isopropoxy-2-oxoethyl-2-(4-isobutylphenyl)propanoate (7) Chromatographic purity (HPLC) 98.34 %; bp 266 °C; MS (ESI+) m/z = 306 [M^+]; IR (KBr, cm^{-1}): 2976, 2870, 1741, 1516, 1462, 1373, 1105, 845; ^1H NMR (CDCl_3 , 400 MHz) δ 0.84 (d, J = 6.8 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.14 (d, J = 6.4 Hz, 6H, $\text{OCH}(\text{CH}_3)_2$), 1.41 (d, J = 7.2 Hz, 3H, ArCHCH_3), 1.81 (m, 1H, $\text{CH}_2\text{CH}(\text{CH}_3)_2$), 2.42 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.85 (q, J = 7.2 Hz, 1H, ArCHCH_3), 4.59 (s, 2H, OCH_2CO), 4.91 (sept, J = 6.4 Hz, 1H, $\text{OCH}(\text{CH}_3)_2$), 7.12 (d, J = 8 Hz, 2H, ArH), 7.23 (d, J = 8 Hz, 2H, ArH).

2-Butoxy-2-oxoethyl-2-(4-isobutylphenyl)propanoate (8) Chromatographic purity (HPLC) 99.67 %; bp 275 °C; MS (ESI+) m/z = 320 [M^+]; IR (KBr, cm^{-1}): 2958, 2871, 1747, 1514, 1458, 1153, 846; ^1H NMR (CDCl_3 , 400 MHz) δ 0.80–0.86 (m, 11H, $3 \times \text{CH}_3$, CH_2CH_3), 1.25 (qu, J = 6.8 Hz, 2H, OCH_2CH_2), 1.41 (d, J = 7.2 Hz, 3H, ArCHCH_3), 1.80 (m, 1H, $\text{CH}(\text{CH}_3)_2$), 2.42 (d, J = 7.2 Hz, 2H, ArCH_2CH), 3.85

(q, $J = 7.2$ Hz, 1H, ArCHCH₃), 3.98 (m, 2H, OCH₂CH₂), 4.65 (s, 2H, OCH₂CO), 7.11 (d, $J = 8$ Hz, 2H, ArH), 7.20 (d, $J = 8$ Hz, 2H, ArH).

2-(Benzyloxy)-2-oxoethyl-2-(4-isobutylphenyl)propanoate (9) Chromatographic purity (HPLC) 98.81 %; bp > 300 °C; MS (ESI+) $m/z = 354$ [M⁺]; IR (KBr, cm⁻¹): 2954, 2848, 1747, 1608, 1514, 1455, 1150, 847, 750, 698; ¹H NMR (CDCl₃, 400 MHz) δ 0.85–0.90 (m, 6H, CH(CH₃)₂), 1.52 (d, $J = 7.2$ Hz, 3H, ArCHCH₃), 1.84 (m, 1H, CH(CH₃)₂), 2.43 (d, $J = 7.2$ Hz, 2H, ArCH₂CH), 3.82 (q, $J = 7.2$ Hz, 1H, ArCHCH₃), 4.54 (s, 2H, COOCH₂CO), 4.05–4.10 (m, 2H, OCH₂Ph), 7.11 (d, $J = 8$ Hz, 2H, ArH), 7.22 (d, $J = 8$ Hz, 2H, ArH), 7.28–7.40 (m, 5H, ArH).

Cyclohexyl-2-(2-(4-isobutylphenyl)propanoyloxy)butanoate (10) Chromatographic purity (HPLC) 98.88 %; bp > 300 °C; MS (ESI+) $m/z = 374$ [M⁺]; IR (KBr, cm⁻¹): 2941, 2869, 1748, 1615, 1512, 1458, 1153, 844; ¹H NMR (CDCl₃, 400 MHz) δ 0.81–0.94 (m, 9H, 3×CH₃), 1.25–1.39 (m, 6H, 3×CH₂), 1.53 (m, 3H, ArCHCH₃), 1.65–1.74 (m, 4H, 2×CH₂), 1.76–1.87 (m, 3H, CH(CH₃)₂/CHCH₂CH₃), 2.43 (d, $J = 7.2$ Hz, 2H, ArCH₂CH), 3.79 (q, $J = 7.2$ Hz, 1H, ArCHCH₃), 4.75–4.90 (m, 2H, OCHCO/OCH(CH₂)₂), 7.09 (d, $J = 8$ Hz, 2H, ArH), 7.24 (d, $J = 8$ Hz, 2H, ArH), 7.28–7.40 (m, 5H, ArH).

2-Ethylbutyl-2-(2-(4-isobutylphenyl)propanoyloxy)butanoate (11) Chromatographic purity (HPLC) 99.78 %; bp > 300 °C; MS (ESI+) $m/z = 376$ [M⁺]; IR (KBr, cm⁻¹): 2966, 2877, 1748, 1514, 1460, 1151, 848; ¹H NMR (CDCl₃, 400 MHz) δ 0.73–0.85 (m, 15H, 5×CH₃), 1.17–1.30 (m, 4H, CH(CH₂CH₃)₂), 1.46 (m, 1H, OCH₂CH), 1.66–1.82 (m, 3H, CH(CH₃)₂/CHCH₂CH₃), 1.40 (d, $J = 7.2$ Hz, 3H, ArCHCH₃), 2.42 (d, $J = 7.2$ Hz, 2H, ArCH₂CH), 3.79–3.95 (m, 2H, OCH₂CH), 3.96–4.06 (m, 1H, ArCHCH₃), 4.85 (m, 1H, OCHCO), 7.11 (d, $J = 8$ Hz, 2H, ArH), 7.20 (d, $J = 8$ Hz, 2H, ArH).

2-Methoxyethyl-2-(2-(4-isobutylphenyl)propanoyloxy)butanoate (12) Chromatographic purity (HPLC) 99.81 %; bp > 300 °C; MS (ESI+) $m/z = 350$ [M⁺]; IR (KBr, cm⁻¹): 2955, 2880, 1743, 1514, 1458, 1383, 1199, 1161, 848; ¹H NMR (CDCl₃, 400 MHz) δ 0.80–0.94 (m, 9H, 3×CH₃), 1.54 (m, 3H, ArCHCH₃), 1.79–1.88 (m, 3H, CH(CH₃)₂/CH₃CH₂CH), 2.43 (d, $J = 7.2$ Hz, ArCH₂CH), 3.36 (s, 3H, OCH₃), 3.47–3.58 (m, 2H, CH₂OCH₃), 3.80 (m, 1H, ArCHCH₃), 4.21–4.30 (m, 2H, COOCH₂), 4.90–4.95 (m, 1H, OCHCO), 7.08–7.11 (m, 2H, ArH), 7.21–7.26 (m, 2H, ArH).

Isopropyl-2-(2-(4-isobutylphenyl)propanoyloxy)butanoate (13) Chromatographic purity (HPLC) 99.97 %; bp 296 °C; MS (ESI+) $m/z = 334$ [M⁺]; IR (KBr, cm⁻¹): 2978,

2937, 2870, 1740, 1513, 1465, 1376, 1103, 847; ¹H NMR (CDCl₃, 400 MHz) δ 0.81–0.94 (m, 9H, 3×CH₃), 1.11 (d, $J = 6.4$ Hz, 3H, CH₃CH), 1.20 (d, $J = 6.4$ Hz, 3H, CH₃CH), 1.51 (d, $J = 7.2$ Hz, 3H, ArCHCH₃), 1.55–1.86 (m, 3H, CH(CH₃)₂/CH₃CH₂CH), 2.43 (d, $J = 7.2$ Hz, ArCH₂CH), 3.79 (m, 1H, ArCHCH₃), 4.81–4.88 (m, 1H, OCH(CH₃)₂), 4.95–5.07 (m, 1H, OCHCO), 7.07–7.10 (m, 2H, ArH), 7.22–7.26 (m, 2H, ArH).

Butyl-2-(2-(4-isobutylphenyl)propanoyloxy)butanoate (14) Chromatographic purity (HPLC) 99.80 %; bp > 300 °C; MS (ESI+) $m/z = 348$ [M⁺]; IR (KBr, cm⁻¹): 2960, 2872, 1746, 1514, 1457, 1154, 847; ¹H NMR (CDCl₃, 400 MHz) δ 0.76 (t, $J = 7.2$ Hz, 3H, CH₃CH₂), 0.82–0.89 (m, 9H, 3×CH₃), 1.27 (m, 2H, CH₃CH₂CH₂), 1.40 (d, $J = 7.2$ Hz, 3H, ArCHCH₃), 1.50 (m, 2H, OCH₂CH₂CH₂), 1.68–1.82 (m, 3H, CH(CH₃)₂/CHCH₂CH₃), 2.42 (d, $J = 7.2$ Hz, ArCH₂CH), 3.81–3.99 (m, 2H, OCH₂CH₂), 4.01–4.11 (m, 1H, ArCHCH₃), 4.83 (m, 1H, OCHCO), 7.11 (d, $J = 8$ Hz, 2H, ArH), 7.22 (d, $J = 8$ Hz, 2H, ArH).

Benzyl-2-(2-(4-isobutylphenyl)propanoyloxy)butanoate (15) Chromatographic purity (HPLC) 99.59 %; bp > 300 °C; MS (ESI+) $m/z = 382$ [M⁺]; IR (KBr, cm⁻¹): 2955, 2870, 1740, 1608, 1514, 1456, 1383, 1197, 1107, 847, 750, 698; ¹H NMR (CDCl₃, 400 MHz) δ 0.73 (t, $J = 7.2$ Hz, 3H, CH₃CH₂), 0.82 (m, 6H, CH(CH₃)₂), 1.38 (t, $J = 7.2$ Hz, 3H, ArCHCH₃), 1.66–1.81 (m, 3H, CH(CH₃)₂, CH₃CH₂CH), 2.41 (d, $J = 7.2$ Hz, 2H, ArCH₂CH), 3.83 (q, $J = 7.2$ Hz, 1H, ArCHCH₃), 4.90 (m, 1H, OCHCO), 5.09 (s, 2H, OCH₂Ph), 7.09 (d, $J = 8$ Hz, 2H, ArH), 7.18 (d, $J = 8$ Hz, 2H, ArH), 7.27–7.38 (m, 5H, ArH).

Preparation of promoieties

Ethyl-2-chloro-3-oxobutanoate (2a)

2-Chloro-3-oxobutanoic acid (20 g, 0.15 mol) was treated with ethanol (9.2 g, 0.2 mol) in the presence of toluene (100 g, 1.08 mmol) as a solvent and PTSA (0.5 gm, 0.0026 mmol) as a catalyst. The reaction mass was maintained at reflux for 2 h. Reaction progress was monitored on GC. After completion, reaction mass cooled to 30 °C and washed twice with 200 mL 20 % sodium bicarbonate solution in water followed by brine and water wash. Organic layer dried over anhydrous sodium sulfate and distilled off the solvent under reduced pressure and pure ethyl 2-chloro 3-oxobutanoate was collected as colorless oil.

Chromatographic purity (HPLC) 97.73 %; bp 200 °C; MS (ESI+) $m/z = 164$ [M⁺], 166 [M+2]; IR (KBr, cm⁻¹): 2985, 1732, 1645, 1621, 1257, 1032; ¹H NMR (CDCl₃, 400 MHz) δ 1.34 (t, $J = 7.6$ Hz, 3H, CH₂CH₃), 2.39 (s,

3H, COCH₃), 4.30 (q, $J = 7.6$ Hz, 2H, CH₂CH₃), 4.79 (s, 1H, CHCl).

4-Bromobutyl acetate (2b)

4-Bromobutyric acid (20 g, 0.12 mol), methanol (9.5 g, 0.29 mol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 99.08 %; bp 211 °C; MS (ESI+) $m/z = 194$ [M⁺], 196 [M+2]; IR (KBr, cm⁻¹): 2963, 1739, 1438, 1237, 1042; ¹H NMR (CDCl₃, 400 MHz) δ 1.81 (qu, $J = 6.4$ Hz, 2H, CH₂CH₂Br), 1.93 (qu, $J = 6.4$ Hz, 2H, CH₂CH₂O), 2.06 (s, 3H, COCH₃), 3.44 (t, $J = 6.4$ Hz, 2H, CH₂Br), 4.10 (t, $J = 6.4$ Hz, 2H, COOCH₂).

Methyl-2-bromopropanoate (2c)

2-Bromopropionic acid (20 g, 0.13 mol), methanol (9.5 g, 0.29 mol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 97.72 %; bp 145 °C; MS (ESI+) $m/z = 166$ [M⁺], 168 [M+2]; IR (KBr, cm⁻¹): 2968, 2878, 1736, 1458, 1270, 1156; ¹H NMR (CDCl₃, 400 MHz) δ 1.84 (d, $J = 7.6$ Hz, 3H, CH₂CH₃), 3.78 (s, 3H, OCH₃), 4.40 (q, $J = 7.6$ Hz, 1H, CHCH₃).

Cyclohexyl-2-bromoacetate (2d)

Bromoacetic acid (20 g, 0.14 mmol), cyclohexanol (12.9 g, 0.12 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.80 %; bp 242 °C; MS (ESI+) $m/z = 221$ [M⁺]; IR (KBr, cm⁻¹): 2965, 2877, 1736, 1451, 1233, 1163; ¹H NMR (CDCl₃, 400 MHz) δ 1.42–1.57 (m, 6H, 2×CH₂), 1.72–1.88 (m, 4H, CH(CH₂)₂), 3.81 (s, 2H, COCH₂Br), 4.82 (m, 1H, CH(CH₂)₂).

2-Ethylbutyl-2-bromoacetate (2e)

Bromoacetic acid (20 g, 0.14 mmol), 2-ethyl butanol (12.86 g, 0.12 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.60 %; bp 261 °C; MS (ESI+) $m/z = 223$ [M⁺]; IR (KBr, cm⁻¹): 2969, 2876, 1742, 1458, 1265, 1162; ¹H NMR (CDCl₃, 400 MHz) δ 0.90 (t, $J = 7.4$ Hz, 6H, (CH₂CH₃)₂), 1.37 (qu, $J = 7.4$ Hz,

4H (CH₂CH₃)₂), 1.56 (septet, $J = 6$ Hz, 1H, CH(CH₂)₃), 3.84 (s, 2H, COCH₂Br), 4.11 (d, $J = 6$ Hz, 2H, OCH₂CH).

2-Methoxyethyl-2-bromoacetate (2f)

Bromoacetic acid (20 g, 0.14 mmol) 2-methoxyethanol (9.16 g, 0.12 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.74 %; bp 213 °C; MS (ESI+) $m/z = 198$ [M+2]; IR (KBr, cm⁻¹): 2955, 2891, 2823, 1739, 1452, 1286, 1128, 1034; ¹H NMR (CDCl₃, 400 MHz) δ 3.40 (s, 3H, OCH₃), 3.63 (t, $J = 4.4$ Hz, 2H, OCH₂CH₂), 3.89 (s, 2H, COCH₂Br), 4.33 (t, $J = 4.4$ Hz, 2H, COOCH₂CH₂).

Isopropyl-2-bromoacetate (2g)

Bromoacetic acid (20 g, 0.14 mmol), isopropanol (7.56 g, 0.12 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 95.89 %; bp 274 °C; MS (ESI+) $m/z = 179$ [M⁺]; IR (KBr, cm⁻¹): 2968, 2881, 1741, 1461, 1272, 1155; ¹H NMR (CDCl₃, 400 MHz) δ 1.28 (d, $J = 6.4$ Hz, 6H, CH(CH₃)₂), 3.80 (s, 2H, COCH₂Br), 5.06 (septet, $J = 6.4$ Hz, 1H, CH(CH₃)₂).

Butyl-2-bromoacetate (2h)

Bromoacetic acid (20 g, 0.14 mmol), n-bromobutanol (9.60 g, 0.12 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 97.30 %; bp 92 °C; MS (ESI+) $m/z = 195$ [M⁺]; IR (KBr, cm⁻¹): 2937, 2875, 1738, 1458, 1283, 1170; ¹H NMR (CDCl₃, 400 MHz) δ 0.94 (t, $J = 7.6$ Hz, 3H, CH₂CH₃), 1.40 (sextet, $J = 7.6$ Hz, 2H, CH₂CH₂CH₃), 1.65 (qu, $J = 7.6$ Hz, 2H, CH₂CH₂CH₃), 3.83 (s, 2H, COCH₂Br), 4.18 (t, $J = 6.8$ Hz, 2H, OCH₂CH₂).

Benzyl-2-bromoacetate (2i)

Bromoacetic acid (20 g, 0.14 mmol), Benzyl alcohol (13.72 g, 0.12 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 96.82 %; bp 287 °C; MS (ESI+) $m/z = 228$ [M⁺]; IR (KBr, cm⁻¹): 3033, 2960,

1738, 1455, 1278, 1156, 971; ^1H NMR (CDCl_3 , 400 MHz) δ 3.84 (s, 2H, COCH_2Br), 5.18 (s, 2H, OCH_2Ph), 7.35 (m, 5H, ArH).

2-Bromo-butyric acid cyclohexyl ester (**2j**)

Bromobutyric acid (20 g, 0.14 mmol), cyclohexanol (10.70 g, 0.10 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 99.00 %; bp 280 °C; MS (ESI+) m/z = 249 [M^+]; IR (KBr, cm^{-1}): 2939, 2860, 1732, 1455, 1262, 1161; ^1H NMR (CDCl_3 , 400 MHz) δ 1.04 (t, J = 7.2 Hz, 3H, CH_3CH_2), 1.27–1.56 (m, 6H, $3 \times \text{CH}_2$), 1.70–1.88 (m, 4H, $2 \times \text{CH}_2$), 1.85–2.15 (m, 2H, CH_2CHBr), 4.14 (t, J = 7.2 Hz, 1H, COCHBr), 4.83 (m, 1H, COOCH).

2-Bromo-butyric acid 2-ethyl-butyl ester (**2k**)

2-Bromobutyric acid (20 g, 0.11 mmol), 2-ethyl butanol (10.11 g, 0.09 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.88 %; bp 292 °C; MS (ESI+) m/z = 251 [M^+]; IR (KBr, cm^{-1}): 2965, 2878, 1741, 1460, 1268, 1156; ^1H NMR (CDCl_3 , 400 MHz) δ 0.90 (t, J = 7.6 Hz, 6H, $2 \times \text{CH}_3\text{CH}_2$), 1.02 (t, J = 7.6 Hz, 3H, CH_3CH_2), 1.38 (qu, J = 7.6 Hz, 4H, $2 \times \text{CH}_2\text{CH}_3$), 1.56 (sept, J = 7.6 Hz, 1H, $\text{CH}(\text{CH}_3)_2$), 1.97–2.14 (m, 2H, CH_2CHBr), 4.06–4.18 (m, 3H, COOCH_2 , CH_2CHBr).

2-Bromo-butyric acid 2-methoxy-ethyl ester (**2l**)

2-Bromobutyric acid (20 g, 0.11 mmol), 2-methoxy ethanol (7.5 g, 0.09 mol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.46 %; bp 181 °C; MS (ESI+) m/z = 225 [M^+]; IR (KBr, cm^{-1}): 2975, 2881, 1743, 1457, 1262, 1158; ^1H NMR (CDCl_3 , 400 MHz) δ 1.04 (t, J = 7.2 Hz, 3H, CH_3CH_2), 1.96–2.15 (m, 2H, CH_2CHBr), 3.39 (s, 3H, OCH_3), 3.61 (t, J = 4.8 Hz, 2H, $\text{CH}_2\text{CH}_2\text{O}$), 4.21 (t, J = 7.2 Hz, 1H, CH_2CHBr), 4.32 (t, J = 4.8 Hz, 2H, $\text{COOCH}_2\text{CH}_2$).

2-Bromo-butyric acid isopropyl ester (**2m**)

2-Bromobutyric acid (20 g, 0.11 mmol), Isopropanol (5.94 g, 0.09 mmol) in the presence of toluene (100 g, 1.08 mmol)

and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.19 %; bp 213 °C; MS (ESI+) m/z = 208 [M^+]; IR (KBr, cm^{-1}): 2981, 2939, 2880, 1738, 1458, 1272, 1161, 1105; ^1H NMR (CDCl_3 , 400 MHz) δ 1.02 (t, J = 7.6 Hz, 3H, CH_3CH_2), 1.26 (d, J = 6.4 Hz, 6H, $\text{CH}(\text{CH}_3)_2$), 1.96–2.13 (m, 2H, CH_2CHBr), 4.12 (t, J = 7.6 Hz, 1H, CH_2CHBr), 5.06 (sept, J = 6.4 Hz, 1H, $\text{CH}(\text{CH}_3)_2$).

2-Bromo-butyric acid butyl ester (**2n**)

2-Bromobutyric acid (20 g, 0.11 mmol), n-butanol (7.3 g, 0.09 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.80 %; bp 255 °C; MS (ESI+) m/z = 223 [M^+]; IR (KBr, cm^{-1}): 2963, 2877, 1740, 1458, 1271, 1157; ^1H NMR (CDCl_3 , 400 MHz) δ 0.94 (t, J = 7.6 Hz, 3H, $\text{CH}_3\text{CH}_2\text{CH}_2$), 1.03 (t, J = 7.2 Hz, 3H, $\text{CH}_3\text{CH}_2\text{CHBr}$), 1.40 (sextet, J = 7.6 Hz, 2H, $\text{CH}_3\text{CH}_2\text{CH}_2$), 1.66 (qu, J = 7.6 Hz, 2H, OCH_2CH_2), 1.95–2.16 (m, 2H, CH_2CHBr), 4.13–4.22 (m, 3H, OCH_2 , CH_2CHBr).

2-Bromo-butyric acid benzyl ester (**2o**)

2-Bromobutyric acid (20 g, 0.11 mmol), benzyl alcohol (10.72 g, 0.09 mmol) in the presence of toluene (100 g, 1.08 mmol) and PTSA (0.5 g, 0.0026 mmol) refluxed for 2 h. Work up of reaction carried out as per the procedure given for **2a**.

Chromatographic purity (HPLC) 98.45 %; bp 295 °C; MS (ESI+) m/z = 256 [M^+]; IR (KBr, cm^{-1}): 2973, 1741, 1455, 1266, 1150; ^1H NMR (CDCl_3 , 400 MHz) δ 1.00 (t, J = 7.2 Hz, 3H, CH_3CH_2), 1.97–2.15 (m, 2H, CH_2CHBr), 4.20 (t, J = 7.2 Hz, 1H, CH_2CHBr), 5.20 (s, 2H, OCH_2Ph), 7.36 (m, 5H, ArH).

Anti-inflammatory activity evaluation in animal models

Oral acute toxicity study

Acute toxicity of various prodrugs was carried out in Swiss albino mice (20–25 g) as per the OECD guidelines (2006). Different doses of various prodrugs were administered to groups (n = 3) up to 2,000 mg/kg orally and animals were observed for a period of 72 h for behavioral changes, toxic reactions, and mortality if any.

Carrageenan-induced hind paw edema in rats

Dose response study Rats were divided into various groups (n = 6) and allowed to free access to water ad libitum.

Different groups of rats administered with ibuprofen (100 mg/kg, b.w.) and various prodrugs (100 mg/kg, v) orally. One group of rats served as a control and administered with gum acacia (1 %, w/v; 10 mL/kg, b.w., p.o.). One hour after the drug administration, to all rats, hind paw edema was induced by the method of winter et al. (1962) by injecting 0.1 mL of 1 % (w/v) solution of carrageenan subcutaneously into the subplanter region of hind paw.

Time course study The hind paw edema volume was measured by volume displacement method using plethysmometer (Orchid scientific; 112, India) by immersing the paw till the level *lateral malleolus* at various time intervals (1, 3 and 6 h) after carrageenan injection.

Cotton pellets-induced granuloma formation in rats

Two sterilised cotton pellets (10 mg) were implanted on ventral regions of rats, procedure described by winter and porter (Winter and Porter, 1957) and divided into various groups (6 rats/group). Different groups of rats administered with ibuprofen (100 mg/kg) and various prodrugs (100 mg/kg) orally for a duration of 8 days. Control group received gum acacia (2 %, 10 mL/kg, p.o.). On the day 9th, rats were sacrificed with excess ether anesthesia. The cotton pellets were removed and freed from extraneous tissue and used for granular tissue formation by recording wet (immediately) and dry weight of pellets. The granular tissue formation was studied by drying cotton pellets at 55 °C for 6 h or till the weight of pellets remains constant. The dry weight was calculated after deducting cotton pellet weight and taken as a granular tissue formation.

Measurement of ulcer index

Ulcer index were evaluated in 2 experimental conditions (a) For the normal 24 h fasted rats were taken and were administered with test compounds orally at 400 mg/kg and 24 h after the drug administration, animals were scarified and stomach were isolated and observed for number of ulcers, (b) in cotton pellets-induced granuloma in rats- Immediately after cotton removal in above experiment, stomach was dissected out, incised along the greater curvature and mucosa were rinsed with cold normal saline solution (pH 7.2) to remove blood contaminant if any. The tissue were kept overnight in 10 % formalin solution. Next day, ulcers were examined under the 10× lens using method described in the literature (Ganguly and Bhatnagar, 1973) on the scale [0 = no ulcer; 1 = hemorrhagic patch; 2 = ulcer <2 mm; 3 = ulcer >2 mm or <4 mm; 4 = ulcer >4 mm]. The ulcer index was calculated as mean ± SEM of 6 rats in each group.

In vivo metabolic stability study (plasma drug concentration determination)

Metabolic stability is an important property of drug candidates, as it affects parameters such as plasma clearance, half-life, and bioavailability. A successful prodrug candidate is expected to undergo rapid, complete conversion to parent compound in the plasma or microsomes within 1–3 h for its desired pharmacological action. The metabolic stability study was carried out in the following method. The male Sprague-Dawley rats were used to compare the bioavailability of prodrugs (**2**, **6**, **9**, **10**, **12**, and **14**) with that of ibuprofen (**1a**) following oral administration. Overnight fasted rats were divided into various groups ($n = 6$), and they were administered prodrugs (**2**, **6**, **9**, **10**, **12**, and **14**) at the dose of 100 mg/kg, orally. Blood sample from each rats were withdrawn through retro orbital plexus in eppendorf tube (containing EDTA 5 % as anticoagulant) at various time intervals viz; 0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h after the drug administration and centrifuged at 5000 rpm for 10 min for plasma separation. To 200 µL of plasma, 200 µL of acetonitrile solution was added and vortexed for 2 min for the precipitation of protein which was separated by centrifugation. The clear supernatant obtained and used for determination of concentration by HPLC technique. The area under curve (AUC) is calculated by

$$\text{AUC} = C_0 + \frac{C_1}{2} \times \text{Time}_{1-0}$$

where, C_0 represents the peak area at time t_0 and C_1 represents the peak area at time t_1 .

Microsomal stability

Materials Nicotinamide adenine dinucleotide phosphate (NADPH) (Sigma, lot no. N 6674); acetonitrile (HPLC grade, Merck, India); Tris-HCl (S.D.Fine-Chem, India); rat liver microsomes (isolated in pharmacology laboratory); prodrugs; and standard ibuprofen.

Requirement Required items were as follows: rat liver microsomes (10 mg/mL protein concentration), NADPH (10 mM solution), Tris-HCl buffer (pH 7.4), Eppendorf tubes, and solutions of various prodrugs of ibuprofen (5 mM).

Experimental conditions Incubation period, 60 min; incubation conditions, 37 °C with 60 rpm shaking; protein precipitation solvent, acetonitrile.

Procedure In 50 µL of rat liver microsomes, 100 µL Tris-HCl buffer was added, with 50 µL of NADPH solution (10 mM), and were mixed thoroughly and vortexed

subsequently for 10 s. To this mixture, 5 μ L prodrug solution (5 mM) was injected and vortexed well. The 75 μ L sample was withdrawn immediately and transferred to the centrifuge tube containing 75 μ L ice cold acetonitrile, vortexed, and centrifuged at 14,000 rpm for 10 min. Aliquots of the supernatant was separated and used for HPLC analysis. Then the assay mixture was incubated in a water bath at 37 °C for 60 min. At specific time points (30 and 60 min), the 75 μ L assay mixture was withdrawn and vortexed in equal volume of cold acetonitrile and centrifuged again at 14,000 rpm for 10 min. Aliquots of the supernatant were separated and used for analysis by HPLC. The percentage of prodrug remaining in rat microsomes was calculated by:

$$\text{Prodrug remaining (\%)} = \frac{\text{Peak area at respective time (min)} \times 100}{\text{Peak area at 0 min}}$$

Aqueous solubility

The solubility of prodrugs **2**, **6**, **9**, **10**, **12**, and **14** was determined using boric acid buffer (pH 9.0) at 40 °C, 0.2 mM phosphate buffer (pH 7.4), 0.1 mM phosphate buffer (pH 5.0), 0.1 mM citric acid buffer (pH 3.0), and 0.2 M hydrochloric acid (pH 1.0). The various prodrugs of ibuprofen (5 mg) in buffer solution (10 mL) were incubated at 40 °C for 4 h at 400 rpm on a mechanical shaker. The solution was filtered through 0.20 μ M membrane filter and the filtrate was analyzed quantitatively by HPLC at a wavelength of 254 nm for its solubility and hydrolysis.

Metabolic stability

For the HPLC analysis system; water alliance 2695-separation module with 2996 PDA detector, C₁₈ Column, ODS 3 v, 250 mm \times 4.6 mm, 5 μ M, and mobile phase was A-10 mM potassium dihydrogen phosphate, wavelength, 265 nm and for B-acetonitrile at various ratios (90:10, 30:70, 10:90 and 70:30).

Results and discussion

Chemistry

A new series of ibuprofen ester prodrugs **1–15** (Fig. 1) were readily prepared in good yields by the condensation of ibuprofen **1a** with appropriate promoieties **2a–o** (Fig. 2), in the presence of potassium carbonate in dimethyl acetamide (DMAc) at room temperature under nitrogen atmosphere

(Scheme 1). The structures of all prodrugs were established by IR, ¹H NMR, mass spectrometry, and their purity in excess of 99 % was confirmed by HPLC analysis.

The syntheses of promoieties **2a–c** were outlined in Scheme 2. The promoieties **2d–i** were obtained by refluxing 2-bromoacetic acid with respective alcohols in the presence of toluene and catalytic amount of PTSA (Scheme 3). Similarly, promoieties **2j–o** were obtained by refluxing 2-bromobutanoic acid with respective alcohols in the presence of toluene and catalytic amount of PTSA (Scheme 4). The structures of all promoieties were established by IR, ¹H NMR, and mass spectrometry, and their purity in excess of 97 % was confirmed by HPLC analysis.

Pharmacological evaluation

The therapeutic use of NSAIDs is often restricted by the necessity to deliver the drug to specific sites of target organ or tissue. The use of NSAIDs is also limited by their irritant side effects on the gastro-enteric mucous and by their poor water solubility. A series of ester prodrugs **1–15** of ibuprofen **1a** was synthesized with the aim of minimizing these side effects and to enhance the oral bioavailability.

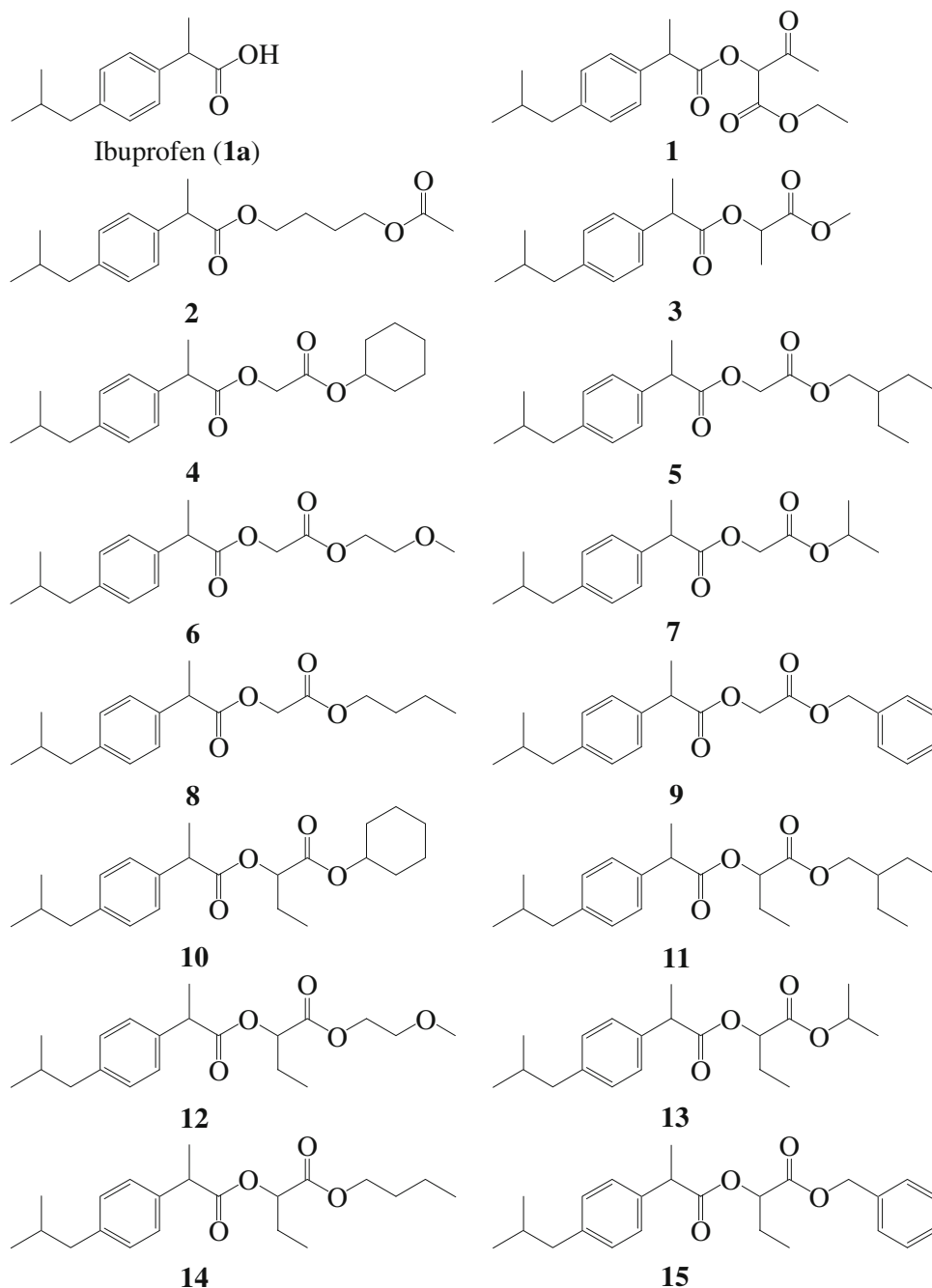
Acute toxicity study

There were no significant behavioral changes observed with all the employed doses of prodrugs (**1–15**). To all the mice, neither toxic reaction nor mortality observed. Therefore, 2,000 mg/kg, body weight (b.w.), per oral (p.o.) dose was considered as maximum tolerated dose. Based on acute toxicity study, we have selected 100 mg/kg for anti-inflammatory activity evaluation of various prodrugs (**1–15**).

Anti-inflammatory activities evaluation

In a dose response study in carrageenan edema, there was significant ($p < 0.01$) increase in paw volume of carrageenan control rats when compared with normal rats. All prodrugs significantly inhibited the edema formation ($p < 0.01$) than the parent drug ibuprofen. However, the extent of edema inhibition in rats treated with **2**, **6**, **9**, **10**, **12**, and **14** was much greater at 3 and 6 h than other prodrugs when compared to carrageenan control rats (Table 1). Inhibition of paw edema formation at 6 h is

Fig. 1 Chemical structures of ibuprofen (**1a**) and produgs **1–15**



greater due to the inhibition of prostaglandins, which is known to be released at 4–6 h after the carrageenan injection (Crunchon and Meacock, 1971). Prodrugs elicited greater paw edema inhibition (**2**, **6**, **9**, **10**, **12**, and **14**) was taken for further evaluation.

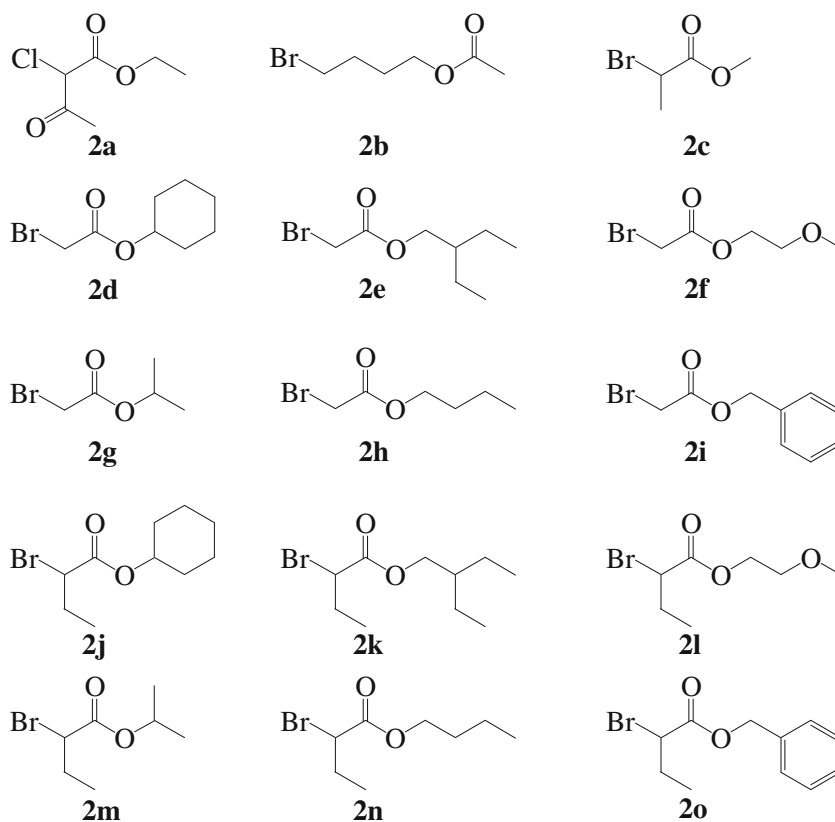
In cotton pellet-induced granuloma in rats, all the selected prodrugs **2**, **6**, **9**, **10**, **12**, and **14** were significantly inhibited ($p < 0.01$) both the exudatory and granulatory phases of inflammation than the parent drug ibuprofen (**1a**). However, greater degree inhibition of these phases was

found with prodrugs **2**, **12** and **14** when compared with control rats. Ibuprofen treatment also showed similar inhibitory effects on both the phases of inflammation (Table 2).

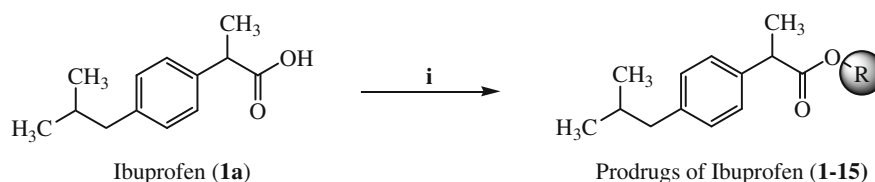
Ulcerogenic assay

Ulcer index in rats during cotton pellet-induced granuloma In this model, lesser degree of ulcers was observed in

Fig. 2 Chemical structures of ethyl-2-chloro-3-oxobutanoate (**2a**), 4-bromobutyl acetate (**2b**), methyl-2-bromopropanoate (**2c**), cyclohexyl-2-bromoacetate (**2d**), 2-ethylbutyl-2-bromoacetate (**2e**), 2-methoxyethyl-2-bromoacetate (**2f**), isopropyl-2-bromoacetate (**2g**), butyl-2-bromoacetate (**2h**), benzyl-2-bromoacetate (**2i**), 2-bromo-butyl-2-bromoacetate cyclohexyl ester (**2j**), 2-bromo-butyl-2-bromoacetate 2-ethyl-butyl ester (**2k**), 2-bromo-butyl-2-bromoacetate 2-methoxy-ethyl ester (**2l**), 2-bromo-butyl-2-bromoacetate isopropyl ester (**2m**), 2-bromo-butyl-2-bromoacetate butyl ester (**2n**), 2-bromo-butyl-2-bromoacetate benzyl ester (**2o**)



Scheme 1 Synthesis of ibuprofen ester prodrugs **1–15**. Reagents and conditions: (i) promoieties **2a–o**, K_2CO_3 , DMAc, rt

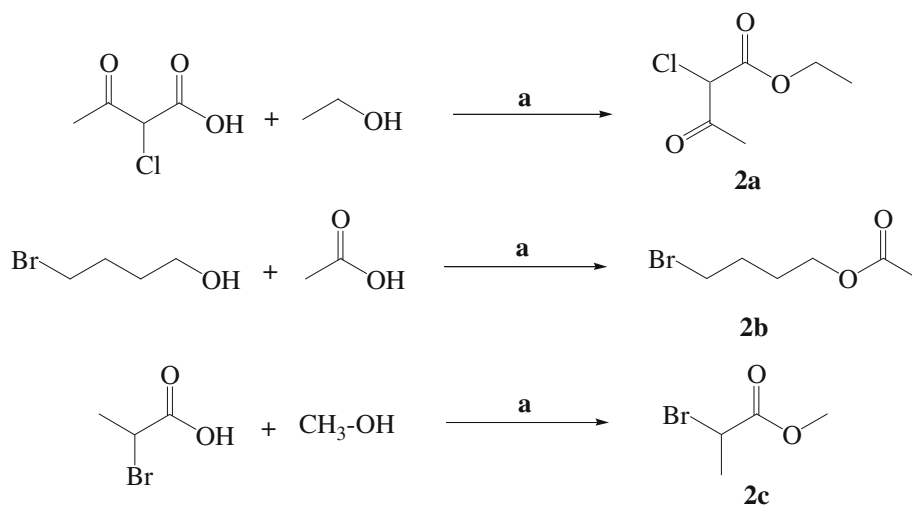


- | | |
|-------------------------------------|--|
| (1) $R = CH(COCH_3)COOCH_2CH_3$ | (8) $R = CH_2COO(CH_2)_3CH_3$ |
| (2) $R = CH_2CH_2CH_2CH_2OCOCH_3$ | (9) $R = CH_2COOCH_2Ph$ |
| (3) $R = CH(CH_3)COOCH_3$ | (10) $R = CH(CH_2CH_3)COOC_6H_{11}$ |
| (4) $R = CH_2COOC_6H_{11}$ | (11) $R = CH(CH_2CH_3)COOCH_2CH(CH_2CH_3)_2$ |
| (5) $R = CH_2COOCH_2CH(CH_2CH_3)_2$ | (12) $R = CH(CH_2CH_3)COOCH_2CH_2OCH_3$ |
| (6) $R = CH_2COOCH_2CH_2OCH_3$ | (13) $R = CH(CH_2CH_3)COOCH(CH_3)_2$ |
| (7) $R = CH_2COOCH(CH_3)_2$ | (14) $R = CH(CH_2CH_3)COO(CH_2)_3CH_3$ |
| | (15) $R = CH(CH_2CH_3)COOCH_2Ph$ |

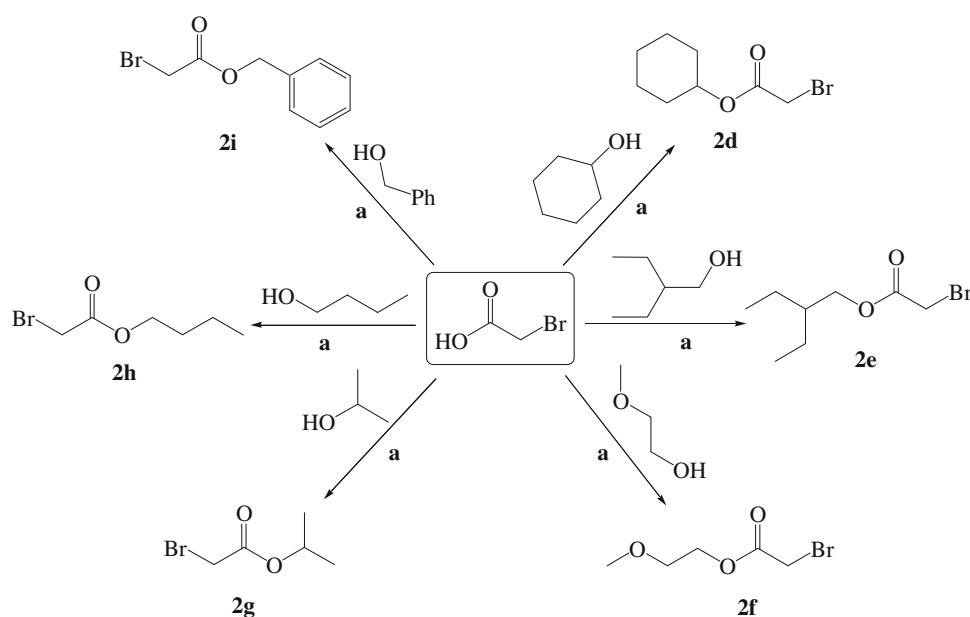
rats treated with prodrugs **2**, **6**, **9**, **10**, **12**, and **14** when compared to standard ibuprofen (Table 3). Administration of ibuprofen **1a** and prodrugs **2**, **6**, **9**, **10**, **12** and **14** (100 mg/kg, b.w., p.o.) daily for 7 days in rats implanted with cotton pellet-induced ulcers. As the ibuprofen was found to produce irritation and ulceration of the GI mucosa, has arisen in

clinical trials (Ashraf *et al.*, 1999; Kean and Buchanan, 2005). The GI ulceration in both the experimental conditions may be due to the presence of carboxylic acid functional group present in ibuprofen, inhibition of protective prostaglandins and inhibition of mucus production. Rats treated with standard drug ibuprofen **1a** had ulcer index of about

Scheme 2 Reagents and conditions: (a) PTSA, toluene, reflux, 2 h



Scheme 3 Reagents and conditions: (a) PTSA, toluene, reflux, 2 h



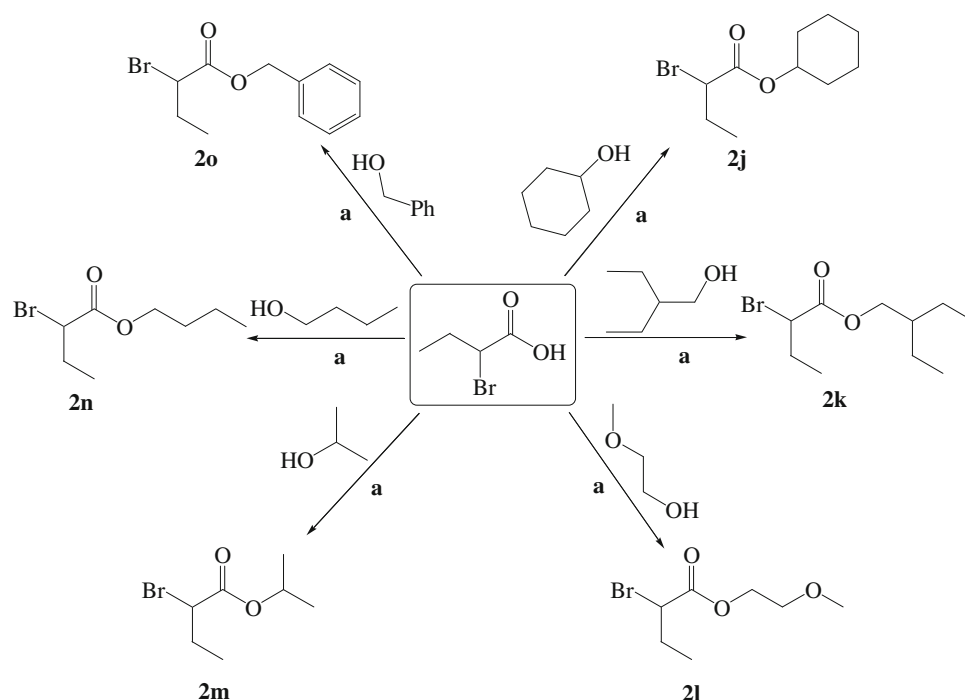
6 mm lesion and found to be much greater than all the synthesized prodrugs (Fig. 3). Rats treated with **2** (0.1 ulcer), **12** (0.1 ulcer) and **14** (0.1 ulcer) found to developed no ulcerogenic lesions as same that of ibuprofen **1a** (6 ulcer). These experimental findings revealed that the presence of functional groups like, 4-bromobutyl acetate, 2-methoxy ethyl-2-bromobutanoate, and butyl-2-bromo-butanoate on carboxylic acid to **2**, **12**, and **14** respectively. However, other prodrugs, viz. **6**, **9**, and **10** also showed lesser degree of ulcer formations but comparatively greater than **2**, **12**, and **14**. Therefore, **2**, **12**, and **14** are most potent anti-inflammatory prodrugs with no or less ulcerogenic potential as compared to parent ibuprofen.

Ulcer index in normal rats Experimental findings demonstrated that rats administered with ibuprofen **1a** at

400 mg/kg showed high incidence of ulcers when compared with normal group; however, the lesser degree of ulcer formation was observed in test prodrugs **2**, **6**, **9**, **10**, **12**, and **14** as compared to standard ibuprofen **1a** (Table 4). Rats treated with standard drug ibuprofen **1a** had ulcer index of about 10.2 and found to be much greater than the entire group of tested prodrugs (Fig. 4). This gives clear indication that ibuprofen is highly ulcerogenic than other ester prodrugs. Furthermore, compounds **2**, **12**, and **14** were found to produce least ulcer in normal rats at 400 mg/kg, indicating significant anti-inflammatory activity with no ulcer formation.

The present experimental findings demonstrated that 4-bromobutyl acetate, 2-methoxy ethyl-2-bromobutanoate, and butyl-2-bromo-butanoate ester prodrugs of ibuprofen protected the gastric mucosal damage induced by ibuprofen. In this respect, our findings shed new chemical requisite to

Scheme 4 Reagents and conditions: (a) PTSA, toluene, reflux, 2 h



design new agents of ibuprofen with no ulcerogenic effects on GI system.

In vivo metabolic stability study (plasma drug concentration determination)

The hydrolysis studies were also studied in male Wistar rats. All selected ester prodrugs (**2**, **6**, **9**, **10**, **12**, and **14**) were quantitatively hydrolyzed to the parent drug ibuprofen by enzymatic conversion in vivo; their respective release characteristics were observed (Fig. 5). The C_{\max} (maximum concentration) of standard ibuprofen **1a** was 91.2 mg/L and $(AUC)_{0-t}$ was 239, whereas C_{\max} of **2**, **6**, **9**, **10**, **12**, and **14** were 70.1, 73.2, 78.0, 80.2, 92.3, 115.2 mg/L respectively with AUC_{0-t} of **2**, **6**, **9**, **10**, **12**, and **14** were 188, 244, 241, 237, 192 and 335 respectively (Table 5).

Bioavailability was measured by calculating the AUC of the drug concentration time profile. The plasma concentration of compound **14** was found to maximum as compared to parent drug ibuprofen indicating improved bioavailability. Prodrug **10** showed slow released rate than other compounds which indicate that slow enzymatic conversion of prodrug moiety into parent compound. Similarly, compound **12** also showed same characteristic release rate in vivo with C_{\max} of 92.3 with higher bioavailability compared to all other prodrugs except **14** indicate fast release of prodrug into parent ibuprofen for its pharmacological action.

Table 1 Effects of various prodrugs **1–15** on carrageenan-induced paw edema in rats

Treatment and dose (100 mg/kg p.o.)	Paw volume (mL) (Mean \pm SEM) at various time interval		
	1 h	3 h	6 h
Normal group	0.40 \pm 0.04	0.35 \pm 0.04	0.38 \pm 0.06
Carrageenan control	0.94 \pm 0.05 [#]	1.17 \pm 0.10 [#]	1.69 \pm 0.08 ^{##}
Ibuprofen (1a)	0.84 \pm 0.05	1.00 \pm 0.07	0.73 \pm 0.05 ^{**}
1	1.20 \pm 0.04	0.96 \pm 0.10	1.26 \pm 0.10
2	1.03 \pm 0.10	0.98 \pm 0.10	0.84 \pm 0.10 ^{**}
3	1.12 \pm 0.06	0.97 \pm 0.05	1.20 \pm 1.10
4	1.29 \pm 0.10	1.11 \pm 0.10	1.01 \pm 0.10 [*]
5	0.88 \pm 0.10	0.98 \pm 0.10	1.19 \pm 0.10
6	1.03 \pm 0.10	1.00 \pm 0.10	0.89 \pm 0.10 ^{**}
7	1.11 \pm 0.04	1.10 \pm 0.03	1.18 \pm 0.20
8	1.10 \pm 0.04	1.10 \pm 0.03	1.18 \pm 0.20
9	1.26 \pm 0.15	0.68 \pm 0.03 ^{**}	0.75 \pm 0.10 ^{**}
10	0.76 \pm 0.10	0.95 \pm 0.10 [*]	0.82 \pm 0.10 ^{**}
11	0.93 \pm 0.10	0.89 \pm 0.10 [*]	1.00 \pm 0.10 [*]
12	1.04 \pm 0.10	0.93 \pm 0.1 [*]	0.22 \pm 0.10 ^{**}
13	1.09 \pm 0.10	0.85 \pm 0.05 [*]	0.98 \pm 0.03 [*]
14	0.80 \pm 0.10	1.05 \pm 0.10	0.81 \pm 0.01 ^{**}
15	1.03 \pm 0.05	0.96 \pm 0.10	1.00 \pm 0.05 [*]

[#] $p < 0.05$; ^{##} $p < 0.01$ when compared with normal control group.

^{**} $p < 0.01$; ^{*} $p < 0.05$ when compared with carrageenan control group

Table 2 Effects of various prodrugs on cotton pellet-induced granuloma in rats

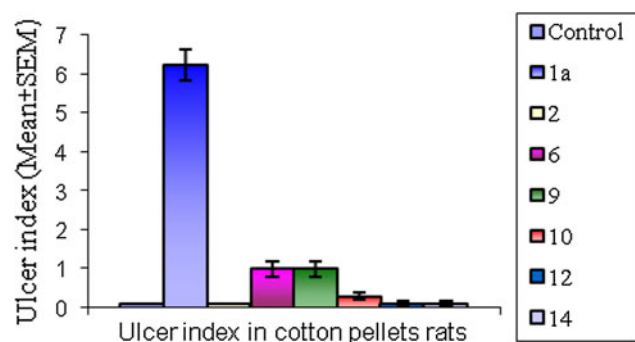
Treatment and dose (100 mg/kg p.o.)	Weight (mg) (Mean \pm SEM)	
	Wet	Dry
Normal control	256 \pm 11.2	153 \pm 9.9
Ibuprofen (1a)	148 \pm 11.5**	76 \pm 4.9
2	174 \pm 8.5**	104 \pm 3.9
6	170 \pm 13.8**	90.4 \pm 5.9
9	165 \pm 13.2**	92.8 \pm 4.6
10	194 \pm 4.2*	122.4 \pm 3.9
12	178 \pm 5.08**	77.4 \pm 7.2
14	161 \pm 3.9**	79 \pm 1.9

** $p < 0.01$; * $p < 0.05$ when compared with normal control group
 # $p < 0.05$; ## $p < 0.01$ when compared with normal control group
 ** $p < 0.01$; * $p < 0.05$ when compared with vehicle treated control group

Table 3 Ulcer index of various prodrugs in cotton pellets-induced granuloma in rats

Treatment and dose (400 mg/kg p.o.)	Number of ulcer (Mean \pm SEM)	Ulcer index (Mean \pm SEM)
Normal control	0.00 \pm 0.0	0.10 \pm 0.01
Ibuprofen (1a)	5.1 \pm 0.20##	6.0 \pm 0.30##
2	0.2 \pm 0.03**	0.1 \pm 0.02**
6	0.7 \pm 0.01**	1.0 \pm 0.01**
9	0.8 \pm 0.04**	1.0 \pm 0.01**
10	0.9 \pm 0.01**	1.1 \pm 0.07**
12	0.2 \pm 0.01**	0.1 \pm 0.02**
14	0.2 \pm 0.01**	0.1 \pm 0.01**

$p < 0.05$; ## $p < 0.01$ when compared with normal control group
 ** $p < 0.01$; * $p < 0.05$ when compared with ibuprofen group

**Fig. 3** Ulcer index of various prodrugs in cotton pellets-induced granuloma in rats

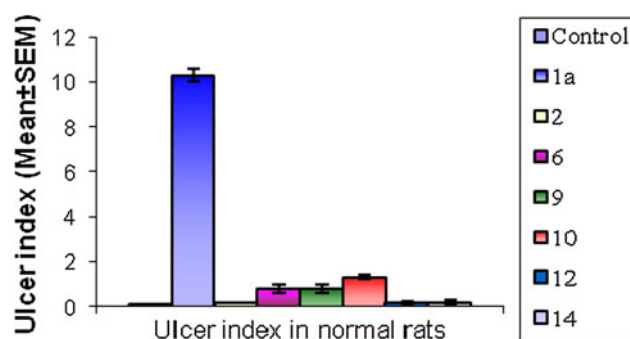
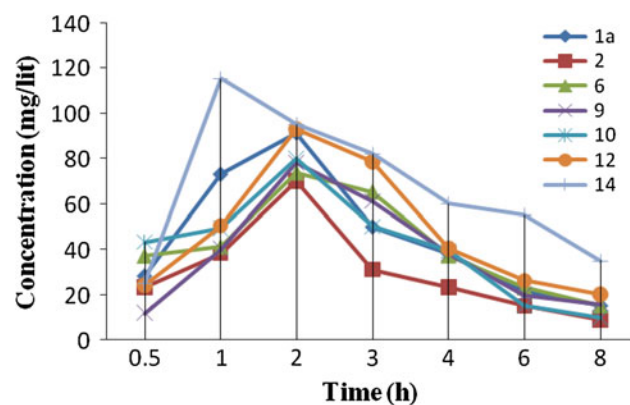
In vivo metabolic stability study (microsomal stability)

From experimental findings it is found that ester prodrugs of ibuprofen were highly metabolized however, standard ibuprofen was stable up to 60 min in liver microsomes (Table 6).

Table 4 Ulcer index of various prodrugs in normal rats

Treatment and dose (400 mg/kg p.o.)	Number of ulcer (Mean \pm SEM)	Ulcer index (Mean \pm SEM)
Normal control	0.00 \pm 0.0	0.10 \pm 0.01
Ibuprofen (1a)	7.2 \pm 0.20##	10.3 \pm 1.10##
2	0.2 \pm 0.03**	0.20 \pm 0.02**
6	0.6 \pm 0.01**	0.80 \pm 0.01**
9	0.5 \pm 0.04**	0.80 \pm 0.01**
10	0.7 \pm 0.01**	1.20 \pm 0.09**
12	0.2 \pm 0.01**	0.20 \pm 0.01**
14	0.02 \pm 0.01	0.20 \pm 0.01**

$p < 0.05$; ## $p < 0.01$ when compared with normal control group;
 ** $p < 0.01$, * $p < 0.05$ when compared with ibuprofen group

**Fig. 4** Ulcer index of various prodrugs in normal rats**Fig. 5** Plasma concentration of ibuprofen and prodrugs at various time intervals

Physicochemical properties

Aqueous solubility All the drugs administered by oral route are supposed to stable at various pH environments encountered in the GI to convert the intact prodrug to the systemic circulation and reach to site of action. Prodrugs of ibuprofen candidate (**2**, **6**, **9**, **10**, **12**, and **14**) were evaluated for solubility and stability in a series of buffer solutions

Table 5 Area under curve and plasma concentration of various test compounds

Compound	Area under curve (AUC)	Plasma concentration (mg/lit)
Ibuprofen (1a)	239	91.2
2	188	70.1
6	244	73.2
9	241	78.0
10	237	80.2
12	192	92.3
14	335	115.2

Table 6 Metabolic stability of various prodrugs of ibuprofen

Compound	Metabolic stability in rat liver microsomes at various time intervals (min)			
	0	30	60	90
Ibuprofen (1a)	100	92.1	79.2	58.3
2	100	8.1	0	0
6	100	9.2	0	0
9	100	1	0	0
10	100	1.2	0	0
12	100	2.1	0	0
14	100	0	0	0

The percentage of prodrugs remaining in rat microsomes was calculated by Prodrug remaining (%) = peak area at respective time (min)/peak area at 0 min \times 100

ranging from pH 1 to 9. All the ibuprofen prodrugs were found to be stable and sparingly soluble in buffer solutions (below detection limit). Aqueous solubility of **9**, **10**, **12**, and **14** was improved; however, it is lower than parent ibuprofen (Table 7). Furthermore, the degradation of prodrugs **9**, **10**, **12**, and **14** indicted a substantially higher chemical stability in an aqueous buffer solution.

Table 7 Aqueous solubility, stability of ibuprofen and various prodrugs (**2**, **6**, **9**, **10**, **12**, **14**) in buffer solutions at 40 °C for 4 h

Prodrug	Hydrolysis to parent drug ($\mu\text{g/mL}$) at various pH					Solubility ($\mu\text{g/mL}$) at various pH				
	1.0	3.0	5.0	7.4	9.0	1.0	3.0	5.0	7.4	9.0
Ibuprofen (1a)	—	—	—	—	—	BDL	BDL	BDL	BDL	BDL
2	0.0735	0.0122	0.1250	0.1256	0.0090	BDL	BDL	BDL	BDL	BDL
6	0.0936	0.0975	0.2965	0.1252	0.2521	BDL	BDL	BDL	BDL	BDL
9	0.2253	0.0255	0.2600	0.2510	0.2987	BDL	BDL	BDL	BDL	BDL
10	0.2361	0.1258	0.0556	0.0255	0.3565	BDL	BDL	BDL	BDL	BDL
12	0.2990	0.2415	0.0115	0.0241	0.3325	BDL	BDL	BDL	BDL	BDL
14	0.0323	0.0153	0.0026	0.2990	0.1988	BDL	BDL	BDL	BDL	BDL

Limit of detection is estimated by HPLC method; ibuprofen (**1a**) (0.0081 $\mu\text{g/mL}$), **2** (0.0162 $\mu\text{g/mL}$), **6** (0.0615 $\mu\text{g/mL}$), **9** (0.0071 $\mu\text{g/mL}$), **10** (0.0121 $\mu\text{g/mL}$), **12** (0.0072 $\mu\text{g/mL}$), **14** (0.0411 $\mu\text{g/mL}$)

BDL below detection limit

Table 8 Partition coefficients of ibuprofen and prodrugs (**2**, **6**, **9**, **10**, **12**, **14**) in buffer solutions at 20 °C and pH 4 and 7.4

Prodrug	Partition coefficients of prodrugs at various pH	
	3.0	7.4
Ibuprofen (1a)	3.75	2.45
2	3.68	4.11
6	4.12	3.49
9	4.09	4.21
10	4.76	4.57
12	3.91	4.02
14	4.05	4.25

Results reported are the average of three experiments ($n = 3$)

Partition coefficient (log P) The partition coefficients (log P) of ibuprofen **1a** and prodrugs **2**, **6**, **9**, **10**, **12**, **14** were determined by HPLC method in octanol-buffer system. Partition coefficient values for all prodrugs were found to be higher than ibuprofen at pH 7.4. Results of log P at pH 3.0 and pH 7.4 are summarized in Table 8. Higher log P values of all prodrugs at pH 7.4, indicated that prodrugs are more lipophilic than the parent drug.

Conclusions

A series of novel ester prodrugs of ibuprofen have been synthesized and evaluated in vivo for their anti-inflammatory potential using carrageenan-induced rat hind paw edema and cotton pellet-induced granuloma models inflammation along with non-ulcerogenic potential. These prodrugs emerged out as anti-inflammatory prodrugs with lesser degree of ulcerogenic potential than the parent drug ibuprofen. Ulcer index (UI) studies revealed that the ibuprofen prodrugs **2**, **12**, and **14** were substantially less ulcerogenic in both normal rats (UI = about 0.1) and inflammation-induced rats (cotton

pellets-induced granuloma; UI = about 0.2) than the parent ibuprofen **1a** (UI = 6 and 10.3 respectively). Metabolic stability study showed that prodrug **2**, **12** and **14** were rapidly transformed enzymatically into parent drug ibuprofen in both rat liver microsomes and rat plasma. Based on these experimental observations, prodrug **12** and **14** are potent and safe anti-inflammatory and analgesic agents with least GI irritation and ulceration therefore, can be preferred over parent drug ibuprofen for the treatment of inflammation

Acknowledgments The authors also gratefully acknowledge the support extended by Mr. Ashok Hiremath, Managing Director, Astec Lifesciences Ltd. Authors are also thankful to Dr. S. R. Naik and Dr. S.B. Bhise, Sinhgad Institute of Pharmaceutical Sciences, Lonavala for providing necessary facility for pharmacological experimentation.

References

- Abordo EA, Bowden K, Huntington AP, Powell SL (1998) Prodrugs. Part 3. 2-Formylphenyl esters of indomethacin, ketoprofen and ibuprofen and 6-substituted 2-formyl and 2-acylphenyl esters of aspirin. *Farmaco* 53:95–101
- Ashraf E, Ford L, Geetha R, Cooper S (1999) Safety profile of ibuprofen suspension in young children. *Inflammopharmacology* 7:219–225
- Bhosale D, Bharambe S, Gairola N, Dhaneshware SS (2006) Mutual prodrug concept: fundamentals and applications. *Ind J Pharm Sci* 3:286–294
- Borhade N, Pathan AR, Halder S, Karwa M et al (2012) NO-NSAIDs. Part 3: Nitric oxide-releasing prodrugs of non-steroidal anti-inflammatory drugs. *Chem Pharm Bull* 60:465–481
- Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanasa A, Konstam MA, Baron JA (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. *N Engl J Med* 352:1092–1102
- Bundgaard H (ed) (1986) Design of prodrugs. Elsevier, Amsterdam, pp 49–68
- Bundgaard H (1989) The double prodrug concept and its applications. *Adv Drug Rev* 3:39–65
- Chen Q, Gong T, Liu J, Wang X, Fu H, Zhang Z (2009) Synthesis, in vitro and in vivo characterization of glycosyl derivatives of ibuprofen as novel prodrugs for brain drug delivery. *J Drug Target* 17:318–328
- Cioli V, Putzolu S, Rossi V, Corradino CA (1980) Toxicological and pharmacological study of ibuprofen guaiacol ester (AF 2259) in the rat. *Toxicol Appl Pharmacol* 54:332–339
- Coruzzi G, Venturi N, Spaggiarim S (2007) Gastrointestinal safety of novel nonsteroidal anti-inflammatory drugs: selective COX-2 inhibitors and beyond. *Acta Biomed* 78:96–110
- Crunkhorn P, Meacock SER (1971) Mediators of inflammation induced in rat paw edema by carrageenan. *Br J Pharmacol* 42:392–402
- Gairola N, Nagpal D, Dhaneshwar SS, Dhaneshwar SR, Chaturvedi SC (2005) Synthesis, hydrolysis kinetics and pharmacodynamic profile of novel prodrugs of flurbiprofen. *Indian J Pharm Sci* 67:369–373
- Ganguly AK, Bhatnagar OP (1973) Effect of bilateral adrenalectomy on production of resistant ulcers in the stomach of albino rats. *Can J Physiol Pharmacol* 51:748–750
- Halen PK, Murumkar PR, Giridhar R, Yadav MR (2009) Prodrug designing of NSAIDs. *Mini-reviews in medicinal chemistry* 9:124–139
- Huang Z, Velazquez CA, Abdellatif KRA, Chowdhury MA, Reisz JA, DuMond JF, Bruce King S, Knaus EE (2011) Ethanedisulfonamide acid ester prodrugs of nonsteroidal anti-inflammatory drugs (NSAIDs): synthesis, nitric oxide and nitroxyl release, cyclooxygenase inhibition, anti-inflammatory, and ulcerogenicity index studies. *J Med Chem* 54:1356–1364
- Kawail S, Kojima F, Kusunoki N (2005) Recent advances in non-steroidal anti-inflammatory drugs. *Allergol Int* 54:209–215
- Kean WF, Buchanan WW (2005) The use of NSAIDs in rheumatic disorders 2005: a global perspective. *Inflammopharmacol* 13:343–370
- Kim H, Jeon H, Kong H, Yang Y, Choi B, Kim YM, Neckers L, Jung YA (2006) Molecular mechanism for the anti-inflammatory effect of taurine-conjugated 5-aminosalicylic acid in inflamed colon. *Mol Pharmacol* 69:1405–1412
- Klasser GD, Epstein J (2005) Nonsteroidal anti-inflammatory drugs: confusion, controversy and dental implications. *J Can Dent Assoc* 71:575–580
- Mahfouz NM, Omar FA, Aboul-Fadl T (1999) Cyclic amide derivatives as potential prodrugs II: *n*-hydroxymethylsuccinimide isatin esters of some NSAIDs as prodrugs with an improved therapeutic index. *Eur J Med Chem* 34:551–562
- Nielsen NM, Bundgaard H (1988) Glycolamide esters as biolabile prodrugs of carboxylic acid agents: synthesis, stability, bioconversion, and physicochemical properties. *J Pharm Sci* 77:285–298
- Nussmeier NA, Whelton AA, Brown MT, Langford RM, Hoeft A, Parlow JL, Boyce SW, Verburg KM (2005) Complications of the COX-2 inhibitors parecoxib and valdecoxib after cardiac surgery. *N Engl J Med* 352:1081–1091
- OECD (2006) Organization of Economic and Corporation Development guidelines for the testing of chemicals-acute oral toxicity up and down procedure. p 425
- Perioli L, Ambrogi V, Bernardini C, Grandolini G, Ricci M, Giovagnoli S, Rossi C (2004) Potential prodrugs of non-steroidal anti-inflammatory agents for targeted drug delivery to the CNS. *Eur J Med Chem* 39:715–727
- Rainsford KD (1988) Gastrointestinal damage from nonsteroidal anti-inflammatory drugs toxicol. *Pathology* 16:251–259
- Shaaya O, Magora A, Sheskin T, Kumar N, Domb A (2003) Anhydride prodrugs for nonsteroidal anti-inflammatory drugs. *J Pharm Res* 20:205–211
- Shanbhag VR, Rider AM, Gokhale R, Harpalanai A, Dick RM (1992) Ester and amide prodrugs of ibuprofen and naproxen: synthesis, anti-inflammatory activity, and gastrointestinal toxicity. *J Pharm Sci* 81:149–154
- Wallace JL (1994) The 1994 Merck Frost Award. Mechanisms of nonsteroidal anti-inflammatory drug (NSAID) induced gastrointestinal damage potential for development of gastrointestinal tract safe NSAIDs. *Can J Physiol Pharmacol* 72:1493–1498
- Winter CA, Porter CC (1957) Effect of alteration in side chain on anti-inflammatory and liver glycogen activities of hydrocortisone esters. *Am J Pharm Sci* 46:515–520
- Winter CA, Risley EA, Nuss GW (1962) Carrageenan induced edema in hind paw of rats as an assay for anti-inflammatory drugs. *Proc Soc Exp Biol Med* 111:544–547