Synthesis and biological evaluation of Boswellic acid-NSAID hybrid molecules as anti-inflammatory and anti-arthritic agents

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · MAY 2015

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2015.05.001

5 AUTHORS, INCLUDING:

Suvarna Shenvi
Vittal Mallya Scientific Research Foundation
6 PUBLICATIONS **31** CITATIONS

SEE PROFILE

European Journal of Medicinal Chemistry 98 (2015) 170–178

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

Synthesis and biological evaluation of boswellic acid-NSAID hybrid molecules as anti-inflammatory and anti-arthritic agents

Suvarna Shenvi [a], K.R. Kiran [b], Krishna Kumar [b], Latha Diwakar [b], G.Chandrasekara Reddy [a, *]

[a] Chemical Sciences Division, Vittal Mallya Scientific Research Foundation, BTM II Stage, Bangalore 560076, India
[b] Department of Biological Sciences, Vittal Mallya Scientific Research Foundation, BTM II Stage, Bangalore 560076, India

ARTICLE INFO

Article history:
Received 30 December 2014
Received in revised form
16 March 2015
Accepted 2 May 2015
Available online 5 May 2015

Keywords:
Boswellia serrata
Boswellic acids
NSAIDs
Anti-arthritic
Anti-inflammatory

ABSTRACT

Methyl esters of the β-boswellic acid (BA) and 11-keto-β-boswellic acid (KBA) obtained from *Boswellia serrata* resin were subjected to Steglich esterification with the different non-steroidal anti-inflammatory drugs (NSAID) viz., ibuprofen, naproxen, diclophenac and indomethacin. The novel hybrids of methyl boswellate (**5**–**8**) and that of methyl 11-keto boswellate (**9**–**12**) were evaluated for anti-inflammatory activity by carrageenan-induced rat hind paw edema model and anti-arthritic activity by Complete Freund's Adjuvant (CFA) induced arthritis in Wister albino rat. Significant inhibition on carrageenan-induced paw edema has been observed with **5**, **6** and **10** where as in CFA induced rats, hybrids **5**, **8**, **9** and **12** exhibited pronounced antiarthritic activity. Hybrid molecules **5** and **9** have been found to be more effective in inhibiting in-vivo COX-2 than ibuprofen by itself, thus showing the synergistic effect. Hybrid **5** and **9** tested for *in-vitro* lipoxygenase and cyclooxygenase-2 (LOX/COX-2) inhibitory activity. The studies revealed that both **5** and **9** inhibited COX-2 relatively better than LOX enzyme.

1. Introduction

Salai guggal, an oleo-gum-resin obtained from *Boswellia serrata* (family Burseraceae) has been used in Ayurvedic preparations as an anti-inflammatory agent since antiquity [1–6]. Extract of *B. serrata* showed various pharmacological properties against asthma [7], colitis [8], crohn's disease [9], cancer [10], hyperlipidemia [11] etc. Boswellic acids which are the main constituents of *B. serrata* are specific, non-redox, noncompetitive selective inhibitors of 5-lipoxygenase (5-LOX) either interacting directly with the 5-LOX or blocking its translocation [12–15]. To enhance the biological profile of boswellic acids, glucosamine salts were made. These salts exhibited significant synergistic anti-arthritic effect in chronic model of inflammation in rats but showed no significant synergistic effect in acute inflammation [16]. Clinical studies using herbal formulations with *Boswellia* have yielded good results in osteoarthritis as well as in rheumatoid arthritis [17,18].

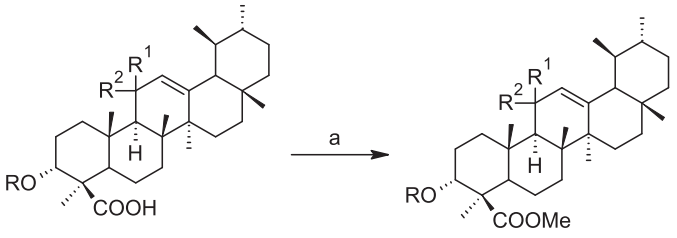
Since boswellic acids salts of glucosamine exhibited no significant synergistic effect in acute inflammation, we made covalent hybrid molecules of boswellic acids with non-steroidal anti-inflammatory drugs (NSAID). The hybridization approach is a technique where two distinct drug entities are connected covalently in one molecule [19,20]. Design and development of hybrid molecules with the intention of exerting dual drug action or enhancement in activity is a current trend [21–26]. When a drug candidate has a weak bioavailability, the hybrid strategy is also useful to correct the pharmacokinetic and pharmacodynamic profiles leading to a valuable lead [27].

Inflammation is the initial trigger of several different diseases. Although inflammation is not the direct cause of these disorders, but inflammatory processes often increase related pain and suffering [28]. The first line of clinical treatment for the inflammatory disorders is NSAIDs via COX pathway. Multiple-ligand drugs using hybridization techniques can act on a single or multiple targets with synergistic action and minimize toxicity or adverse reactions [29,30].

In continuation of our research work on boswellia gum resin [31,32], we have synthesized a series of hybrid molecules containing important pharmacophores such as non steroidal anti-inflammatory drugs (NSAIDS) and boswellic acids and described their potentiated anti-inflammatory and anti-arthritic activity.

* Corresponding author.
E-mail address: gcreddy@vmsrf.org (G.Chandrasekara Reddy).

http://dx.doi.org/10.1016/j.ejmech.2015.05.001
0223-5234/



1. R^1, R^2=H, R=H (β-BA)
2. R^1, R^2=O, R=H (β-KBA)
3. R^1, R^2=H, R=OAc (β-ABA)
4. R^1, R^2=O, R=OAc (β-AKBA)

1a. R^1, R^2=H, R =H
2a. R^1, R^2=O, R=H
3a. R^1, R^2=H, R=OAc
4a. R^1, R^2=O, R=OAc

Scheme 1. Methylation of boswellic acids. Reagents and conditions a) DMS/K_2CO_3/DMF at ~80 °C for 6 h.

2. Results and discussion

2.1. Chemistry

Boswellic acids from *B. serrata* resin were isolated and converted into their methyl esters using the reported procedure [31]. The total acid content of the crude resin estimated by acid base titration was found to be 94 ± 2% consisting a mixture of β-boswellic acid (BA, **1**), 11-keto-β-boswellic acid (KBA, **2**), 3-acetyl-β-boswellic acid (ABA, **3**) and 3-acetyl-11-keto-β-boswellic acid (AKBA, **4**) [33–35]. The entire mixture was subjected to methylation using DMS/K_2CO_3 in DMF at 80 °C for 6 h. After work up, the reaction mass was subjected to silica column chromatography using hexane/EtOAc mixture as eluent to obtain pure major methyl esters of BA (**1a**), KBA (**2a**) and minor methyl esters of ABA (**3a**) and AKBA (**4a**) as in Scheme 1.

Compounds **1a** (Methyl ester 3α-hydoxy-urs-12ene-24β-oic acid) and **2a** (Methyl ester 3α-hydroxy-urs-12ene-11-oxo-24β-oic acid) were reacted with different NSAIDs (Fig. 1) viz., (±) ibuprofen, (±) naproxen, indomethacin and diclofenac in presence of DCC and DMAP in dichloromethane at 0 °C for an hr (Steglich estrification, Scheme 2) to form the hybrids (Fig. 2). Hybrids were purified by column chromatography and characterized them by recording NMR, GC–MS, IR and HR-MS. However, the hybrid molecules **5**, **6**, **9** and **10** which are diastereomers could not be separated, hence these were subjected as such for evaluating their biological activity.

Molecular mass of all the hybrids were determined by GC–MS and confirmed by HR-MS. Hybrids (**5**–**8**) showed a fragment peak at *m*/*z* 218 indicating double bond in ring C at 12–13 position, while hybrids (**9**–**12**) showed fragment peaks at *m*/*z* at 273 and 232 indicating vinylic keto group at position 11. Further, NMR spectra showed down field shift of H-3 proton of methyl ester of boswellic acids from around δ 4.10 to around δ 5.50 indicating the formation of hybrid esters.

2.2. Biology

Hybrid molecules (**5**–**12**) were subjected to acute anti-inflammatory study and chronic anti-arthritic study in carrageenan induced paw edema model and adjuvant induced arthritis in rats.

a) Anti-inflammatory

The carrageenan test was selected because of its sensitivity in identifying orally active anti-inflammatory agents, particularly in the acute phase of inflammation [36]. Carrageenan-induced rat hind paw edema was used as the model of acute inflammation according to the method of Winter [37].

The intraplantar injection of carrageenan in rats leads to paw edema. The first phase in biphasic event of inflammation (0–2.5 h after injection of carrageenan) is due to the concomitant release of mediators like histamine, serotonin and kinins, which have the effect on vascular permeability. The second phase is correlated with leukotrienes and prostaglandins [38]. The oral administration suppresses inflammation during the second phase presumably by inhibiting the COX-2 activity.

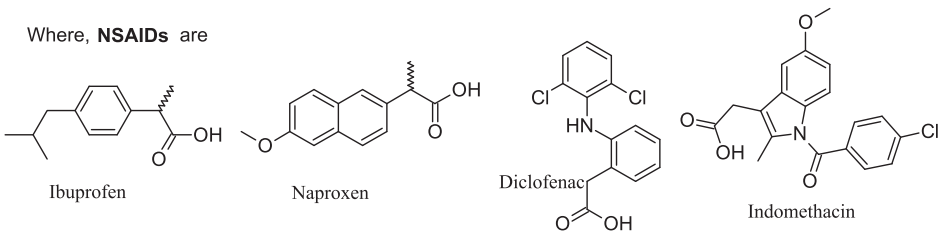
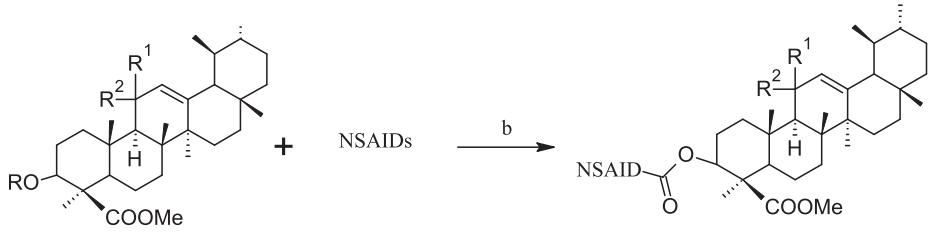


Fig. 1. NSAID molecules.



1a. R^1, R^2=H, R=H
2a. R^1, R^2=O, R=H

Hydrid molecule

Scheme 2. Strategy for synthesis of hybrids. Reagents and condition: (b) DCC/DMAP/DCM/0 °C for 1 h.

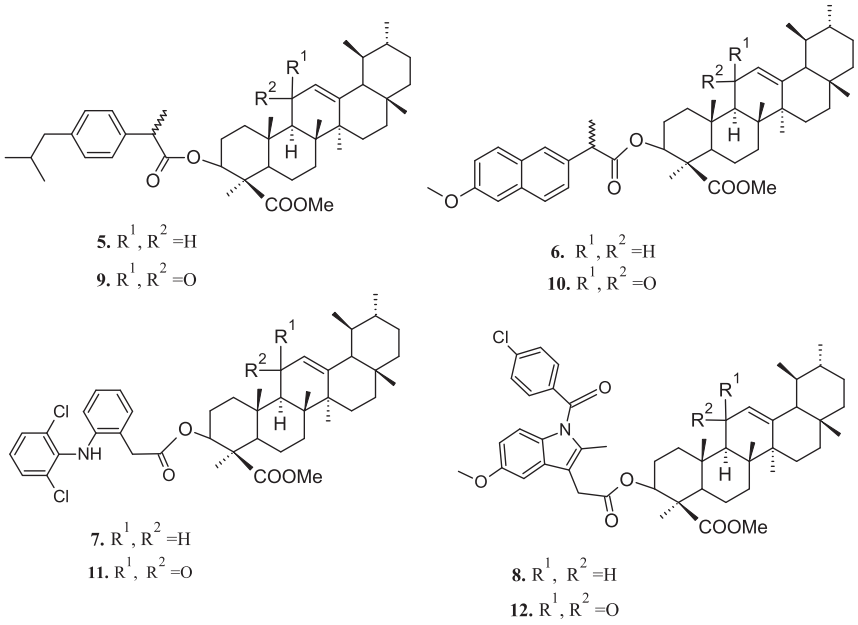


Fig. 2. Methyl boswellate (**5**–**8**) and methyl 11-keto boswellate (**9**–**12**) hybrid molecules.

Table 1
Paw volume of carrageenan induced inflammatory rats at different time points.

Hybrids	Dose in mg/kg body weight	Paw edema volume			
		At different time point after injecting carrageenan			
		0th	2 h	4 h	24 h
5	10	1.08 ± 0.09	1.31 ± 0.09	1.45 ± 0.16	1.26 ± 0.06***
6	10	1.10 ± 0.04	1.47 ± 0.11	1.54 ± 0.27	1.21 ± 0.07***
7	10	1.10 ± 0.02	1.53 ± 0.22	1.52 ± 0.19	1.30 ± 0.14***
8	10	1.10 ± 0.02	1.71 ± 0.08	1.62 ± 0.40	1.41 ± 0.04**
9	10	1.11 ± 0.04	1.88 ± 0.13	1.97 ± 0.17	1.83 ± 0.12*
10	10	1.19 ± 0.02	1.40 ± 0.06	1.61 ± 0.12	1.26 ± 0.16***
11	10	1.10 ± 0.02	1.71 ± 0.27	1.81 ± 0.18	1.55 ± 0.20**
12	10	1.10 ± 0.02	1.75 ± 0.08	1.60 ± 0.10	1.47 ± 0.11**
[a]NC1	–	1.10 ± 0.04	1.10 ± 0.04	1.10 ± 0.04	1.10 ± 0.04
[b]NC2	–	1.07 ± 0.01	2.17 ± 0.07	2.21 ± 0.12	1.69 ± 0.09
[c]PC1	10	1.10 ± 0.04	1.65 ± 0.07	1.65 ± 0.10	1.52 ± 0.05
[d]PC2	10	1.18 ± 0.03	1.58 ± 0.12	1.69 ± 0.06	1.62 ± 0.08

[a] NC1: healthy rats.
[b] NC2: carrageenan injected rats.
[c] PC1: Ibuprofen treated rats.
[d] PC2: KBA treated rats. Each value represents the mean ± S.D and level of significance was determined by *Bonferroni* test $P < 0.001$. * No effect; ** moderate effect; ***significant effect.

All the hybrid molecules except compound **9** reduced the induced paw volume (Table 1) at the end of 24 h. The highest decrease in paw volume was observed with **5**, **6** (hybrids of ibuprofen and naproxen with BA) and **10** (hybrid of naproxen with KBA) indicating synergistic effect. However, compound **9** (hybrid of ibuprofen with KBA) has no anti-inflammatory activity after 24 h as compared to KBA and ibuprofen individually indicating no synergistic effect. Overall it has been observed that compounds **5** and **6** appear to be the best hybrid molecules in this series.

b) Anti-arthritic activity

As human arthritis and adjuvant induced arthritis in rat model resembles each other, inhibition of disease parameters in rat model is one of the most suitable procedure to screen anti-arthritic compounds [39]. Complete Freund's Adjuvant (CFA) induced arthritis is one of the most widely used model as it has been shown to share a number of immunological and clinical features with human arthritis [40].

Due to the structural similarity between mycobacteria and cartilage proteoglycan, Freund's Adjuvant induced arthritis is thought to occur through cell-mediated autoimmunity. It activates macrophages and lymphocytes by adjuvant inoculation. Cyclooxygenase (COX) is the key enzyme required for the conversion of arachidonic acid to pro inflammatory prostaglandins (PGs) [41]. Prostaglandins mediate various manifestations of the inflammatory response, including fever, hyperalgesia, increase in vascular permeability and edema [42] PGs are involved in a number of inflammatory conditions and diseases, such as arthritis and skin inflammation. COX-2 inhibitors are powerful anti-inflammatory drugs.

As seen from the Table 2, the paw volume was reduced

Table 2
Paw volume of CFA induced arthritic rats at different days interval.

Hybrids	Dose in mg/kg body weight (mg/kg)	Paw edema volume			
		Days			
		0	**7**	**14**	**21**
5	10	1.84 ± 0.11	2.53 ± 0.08	2.43 ± 0.06	2.36 ± 0.12***
6	10	1.77 ± 0.07	2.56 ± 0.07	3.01 ± 0.13	3.05 ± 0.40*
7	10	1.79 ± 0.08	3.04 ± 0.24	2.92 ± 0.23	2.72 ± 0.15**
8	10	1.79 ± 0.08	2.70 ± 0.16	2.85 ± 0.15	2.58 ± 0.13***
9	10	1.97 ± 0.16	2.64 ± 0.11	3.12 ± 0.35	2.66 ± 0.26**
10	10	1.77 ± 0.07	2.59 ± 0.09	2.79 ± 0.31	2.95 ± 0.10*
11	10	1.80 ± 0.08	2.61 ± 0.11	2.84 ± 0.05	2.73 ± 0.19**
12	10	1.89 ± 0.05	2.86 ± 0.14	2.89 ± 0.18	2.58 ± 0.18***
[a]NC1	–	1.80 ± 0.08	1.81 ± 0.09	1.81 ± 0.08	1.82 ± 0.07
[b]NC2	–	1.86 ± 0.13	2.99 ± 0.24	3.23 ± 0.17	3.17 ± 0.21
[c]PC1	10	1.80 ± 0.08	2.70 ± 0.14	2.73 ± 0.08	2.22 ± 0.04
[d]PC2	10	1.78 ± 0.06	2.73 ± 0.14	2.85 ± 0.08	2.75 ± 0.08

[a] NC1: healthy rats.
[b] NC2: CFA induced rats.
[c] PC1: Ibuprofen treated rats.
[d] PC2: KBA treated rats. Each value represents the mean ± S.D and level of significance was determined by *Bonferroni* test, $P < 0.001$. * less effect; ** moderate effect; ***significant effect.

significantly at 21 days after induction of paw edema, with compounds **5**, **7**, **8**, **9**, **11** and **12**. The highest reduction in paw volume was seen with compound **5** (2.36 ± 0.12) followed by **8** and **12**. Though the reduction in paw edema was not too high, rest of the hybrids also showed moderate anti-arthritic activity. But all the hybrids showed less effect compared with ibuprofen by itself.

c) COX-2 activity

The serum from 21 day old treated animals stored at −20 °C was used for the analysis of COX-2 activity (Fig. 3). The COX-2 activity was then measured using a commercial COX activity assay kit measuring the peroxidase activity.

It was evident from the graph that the highest COX-2 inhibitory activity was recorded for **5** (132.73 ± 7.58) and **9** (131.97 ± 3.96) among others, which was almost equivalent to ibuprofen itself (126.69 ± 8.04). Remaining hybrid molecules though showed moderate COX-2 inhibitory activity but were found to be better than KBA showing the synergistic effect.

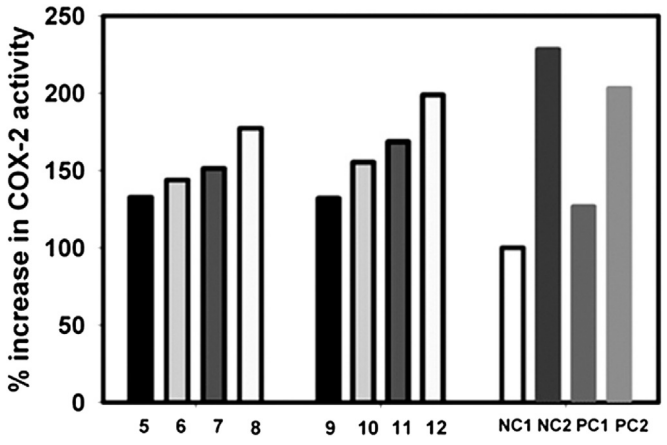


Fig. 3. COX-2 inhibitory activity of methyl boswellate (**5**–**8**) and methyl 11-keto boswellate (**9**–**12**) hybrids.

d) Histopathology

Neutrophil infiltration is one of the main histological parameter in assessing the inflammatory condition since these leukocytes migrate to the site of inflammation. In the hybrid molecules treated groups there was a significant decrease in the density of infiltrated neutrophils, which was a sign of reduction in inflammation. The reduction in neutrophil infiltration results in reduced secretion of leukotrienes, which were in turn, strong chemo attractant for neutrophils.

The ankle joints were used for histopathological studies (Fig. 4). The joints were embedded in paraffin blocks. 7 μm sections were taken and routine Haematoxylin eosin staining (H&E) was performed. The stained sections were observed under microscope. Based on COX-2 inhibitory activity, hybrid molecules **5** and **9** were chosen for histopathological study. Decrease in neutrophil infiltration with hybrids **5** and **9** were found to be better than KBA but less compared to PC1 again indicating synergistic effect.

e) *In-vitro* COX-2 and LOX enzyme activity

The LD_{50} values for BA, KBA, Ibuprofen, hybrids **5** and **9** on Synovial sarcoma cell line (SW-982) were found to be 70.19 ± 3.66, 81.97 ± 2.23, 92.97 ± 4.59, 62.65 ± 8.87, 40.64 ± 0.12 respectively. To evaluate the enzyme activity, cells were pre-treated with the test compound at their respective LD_{50} dose followed by IL-1β treatment.

As shown in Table 3, hybrid **5** and **9** inhibited LOX as well as COX-2 activity. Interleukin-1β treated cells which exhibited high levels of COX-2 and LOX enzyme has been taken as 100%. Untreated cells served as control with less enzyme activity. **5** and **9** pre-treated cells inhibited enzyme activity post interleukin treatment. However, **5** and **9** showed better inhibition against COX-2 than LOX activity.

In summary, all hybrid molecules have been found to exhibit synergistic effect in acute inflammatory and chronic arthritic conditions. However, hybrid molecules of ibuprofen with BA **(5)** and KBA **(9)** exhibited higher synergistic effect in COX-2 studies. Though COX-2 inhibitory activities of **5** and **9** were on par with ibuprofen at dose levels of 10 mg/kg body weight but in terms of molar concentrations, these hybrids were found superior.

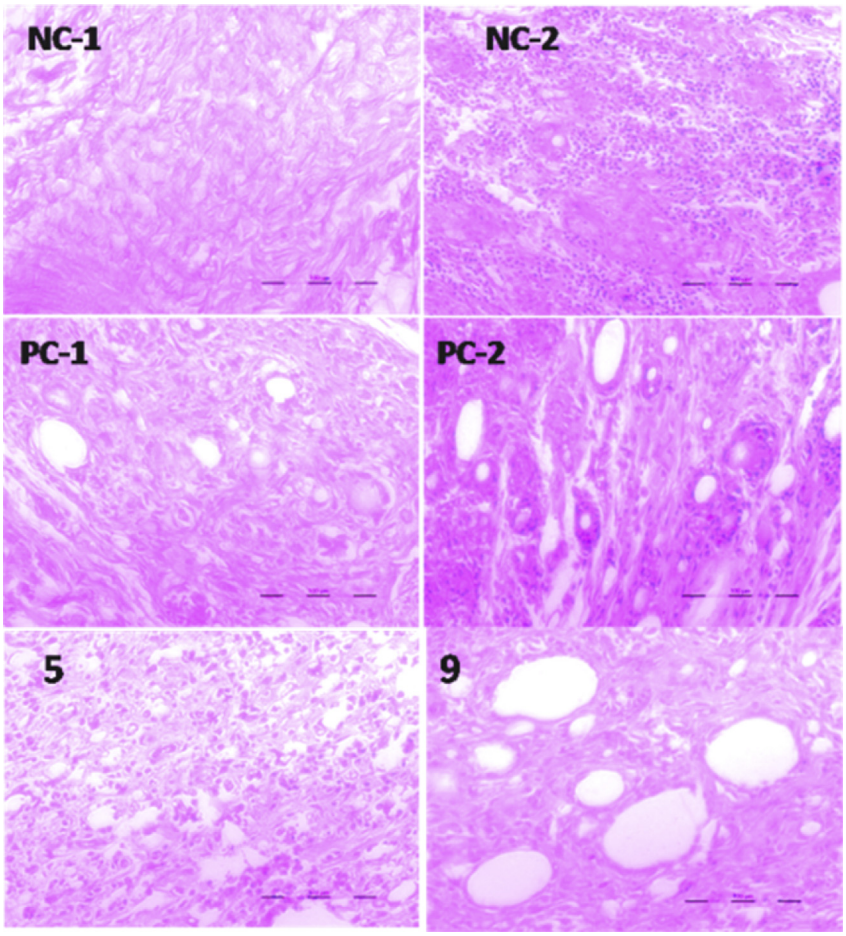


Fig. 4. Histopathology of ankle joint of control and hybrids treated rats.

Compounds **5** (15.2 μmol/kg) and **9** (14.8 μmol/kg) showed the same effect as that of ibuprofen at concentration level of 48 μmol/kg. In other words compounds **5** and **9** exhibited the same effect on COX-2 at one-third concentration levels of ibuprofen. *In-vitro* lipoxygenase and cyclooxygenase-2 (LOX/COX-2) inhibitory activity studies revealed that both **5** and **9** inhibited COX-2 relatively better than LOX enzyme. This was an interesting finding that while reducing the adverse effect of ibuprofen, increase in anti-arthritic activity occurred when compared to either boswellic acids or ibuprofen individually. This study will open up avenues to go for hybrid molecules for reducing the side effects of NSAIDs in long term usage in chronic inflammatory diseases.

Table 3
In-Vitro COX-2 and LOX enzyme activity of hybrid **5** and **9**.

Sl.No	Sample	% COX-2 activity	% LOX activity
1	[a]IL-1β	100.00	100.00
2	[b]BA + IL-1β	50.83 ± 2.07	44.02 ± 0.05
3	KBA + IL-1β	27.43 ± 0.10	72.65 ± 3.42
4	Hybrid 5 + IL-1β	30.23 ± 0.09	65.85 ± 1.08
5	Hybrid 9 + IL-1β	27.66 ± 0.18	60.22 ± 2.16
6	Control	18.20 ± 0.99	26.05 ± 0.27

[a] Cell lysate from Interleukin-1β treated cells.
[b] Cell lysate obtained from cells pre-treated with test compound followed by Interleukin-1β treatment. Each value represents the mean ± S.D from three different test results in triplicate.

3. Experimental

Melting points were recorded on an Acro melting point apparatus using a calibrated thermometer. Thin layer chromatography (TLC) was performed on [TLC silica gel 60 F_{254} Merck]. Chromatograms were developed using hexane-EtOAc (8:2, v/v) and compounds were detected by H_2SO_4 solution (10%) with subsequent heating at 100–120 °C for 4–5 min. IR spectra were recorded on Thermo-Nicolet instrument in KBr discs. Mass spectra were recorded using GCMS-QP2010S (direct probe). PMR spectra were recorded in $CDCl_3$ with TMS (tetra methyl silane) as internal standard on a Bruker AG spectrometer at 200 MHz and chemical shifts are recorded in δ units.

3.1. Isolation of β-boswellic acids

Extracted the *B. serrata gum* resin (1 Kg) with methanol (2 × 2 L) in a percolator and combined extracts were evaporated under reduced pressure at 45 °C to obtain thick brown residue (450 g). Residue was stirred with 3% NaOH (5 L) until it is uniform emulsion. Aqueous layer was extracted with CH_2Cl_2 to remove non-acidic part. Aqueous layer was acidified with 1N HCl to precipitate the total organic acids. Filtered acids were washed with water several times until neutral pH. Crude total acids redissolved in 3% NaOH solution and repeated the process to give off white powder. Then the product was dried in vacuum oven below 50 °C to get crude powder of boswellic acids (260 g).

3.2. Methylation mixture of acids

Mixture of acids were dissolved (100 g) in DMF (300 ml), added K_2CO_3 (45 g, 32.6 mol) heated up to 80 °C and then added DMS (27.5 g, 21.8 mol) and maintained at this temperature for 6 h. The reaction mass was cooled to room temperature and quenched into ice, acidified till pH is 3–4 and extracted with ethyl acetate (3 × 500 ml). Combined extract was dried over Na_2SO_4, vacuum evaporated to get methyl ester of Boswellic acids (105 g).

Separation: Mixture of methyl ester of boswellic acids (100 g) was loaded on silica gel column (60–120 mesh, 1 Kg) and the elution of column was carried out by hexane/ethyl acetate (95/5 to 85/15) and obtained pure methyl ester of BA (22.3 g) and KBA (3.9 g) was islotated.

3.2.1. Methyl ester 3α-hydoxy-urs-12ene-24β-oic acid (1a)

White solid; $C_{31}H_{50}O_3$; mp 189–190 °C (lit., 189 °C) [34].

IR (KBr): ν_{max} 3565, 2926, 1712, 1453, 1358, 1245, 1057, 954, 755 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz); δ 0.70 (3H, s, CH_3), 0.83 (3H, s, CH_3), 0.87–0.91 (4H, m), 0.99 (3H, s, CH_3), 1.03 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.15 (3H, s, CH_3), 1.18 (3H, s, CH_3), 1.25–1.33 (4H, m), 1.38–1.53 (4H, m), 1.59–1.67 (3H, m), 1.61 (3H, s, CH_3), 1.74–1.94 (3H, m), 2.09 (3H, s, CH_3), 3.67 (3H, s, $COOCH_3$), 4.13 (1H, bs, H-3, C<u>H</u>OH), 5.12 (1H, bs, vinylic H at C-12).

GC–MS (*m/z*): 470 [M^+] (12), 453 (5), 437 (5), 393 (5), 346 (4), 297 (14), 252(32), 234 (20), 218 (100), 203 (28), 189 (31), 175 (31), 161 (5), 147(14), 133 (12), 119 (18).

3.2.2. Methyl ester 3α-hydroxy-urs-12ene-11-oxo-24β-oic acid (2a)

White solid; $C_{31}H_{48}O_4$; mp 218–219 °C (lit., 217 °C) [34].

IR (KBr): ν_{max} 3469, 2921, 1724, 1655, 1610, 1453, 1387, 1230, 1062, 838 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz); δ 0.77 (3H, s, CH_3), 0.81 (3H, s, CH_3), 0.88–0.88 (3H, m), 0.94 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.17 (3H, s, CH_3), 1.31–1.26 (8H, m), 1.40–1.51 (4H, m), 1.42–1.51 (3H, m), 1.60 (3H, s, CH_3), 1.68–1.86 (3H, m), 2.42 (1H, s), 3.66 (3H, s, $COOCH_3$), 4.10 (1H, bs, H-3, C<u>H</u>OH), 5.54 (1H, s, H-12, C=C<u>H</u>–CO).

GC–MS (*m/z*): 484 [M^+] (10), 469 (6), 451 (4), 424 (5), 407 (5), 391(3), 299 (5), 273 (100), 232 (60), 189 (5), 135 (50), 161 (35).

3.3. Synthesis and characterization of hybrids

3.3.1. General procedures for the synthesis of hybrids

To a solution of methyl ester of boswellic acid (1.0 g, 2.12 mmol) and methyl ester of 11-keto Boswellic acid (1.0 g, 2.06 mmol) in DCM (15 ml), corresponding NASIDs (1.5 equivalent) and added DMAP (120 mg, 0.1 mmol) stirred at 0 °C till clear solution then, a solution of DCC (660 mg, 3.2 mmol) in DCM (10 ml) added slowly. The reaction mixture was maintained at 0 °C under stirring for 1 h and at 25–30 °C for 30–40 min. Completion of the reaction was confirmed by TLC. The reaction mixture was filtered and filtrate (diluted with 30 ml of DCM) was poured into ice water, then the mixture was acidified with 0.1 N HCl and separated the organic layer, washed with water and dried over Na_2SO_4. The organic layer was evaporated under vacuum the crude was purified on silica column using hexane/ethyl acetate ratio with increasing order of polarity and isolated the pure hybrids. Boswellic acid hybrids (5–10) and 11-keto boswellic acid hybrids (11–16).

3.3.2. (±) Methyl, 3-[2′-(4′-isobutylphenyl) propanoyl]-12-ursen-24-oate (5)

White solid; $C_{44}H_{66}O_4$; 72% yield; mp 80–82 °C.

IR (KBr): ν_{max} 3456, 2956, 1738,1458, 1377, 1240, 1174, 1118, 1052 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.75 (3H, s, CH_3), 0.82 (3H, s, CH_3), 0.84–0.87 (8H, m), 0.95 (3H, s, CH_3), 0.97–1.05 (6H, m), 1.13 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.37–1.42 (8H, m), 1.46–1.50 (5H, m), 1.53 (3H, s, CH_3), 1.61 (3H, s, CH_3), 1.74–1.96 (3H, m), 2.18–2.25 (3H, m), 2.34–2.44 (2H, m, benzylic), 3.69 (3H, s, $COOCH_3$), 3.69–3.75 (1H, m), 5.19 (1H, bs, vinylic H at C-12), 5.51 (1H, bs, H-3, –C<u>H</u>-O-C=O), 7.02–7.08 (2H, m, Ar), 7.16–7.22 (2H, m, Ar).

GC–MS (*m/z*): 658 [M^+] (55), 534 (5), 452 (7), 440 (8), 234 (82), 218 (100), 203 (22), 189 (15), 175 (12), 161 (44).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{44}H_{66}O_4Na$; calculated 681.4859; observed 681.4858.

3.3.3. (±) Methyl, 3-[6′-methoxy-α-methyl-2-naphthyleneacetyl]-12-ursen-24-oate (6)

Pale yellow solid; $C_{45}H_{62}O_5$; 75% yield; mp 169–172 °C.

IR (KBr): ν_{max} 3437, 2943, 1733, 1607, 1461, 1380, 1224, 1194, 1037, 856 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.72 (3H, s, CH_3), 0.82 (3H, s, CH_3), 0.83–0.87 (4H, m), 0.96 (3H, s, CH_3), 0.98–1.03 (5H, m), 1.12 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.38–1.46 (2H, m), 1.42–1.50 (3H, m), 1.53 (3H, s, CH_3), 1.56 (3H, s, CH_3), 1.60 (3H, s, CH_3), 1.62–1.69 (5H, m), 1.89–2.08 (2H, m), 3.63 (3H, s, $COOCH_3$), 3.85 (1H, s, Ar-C<u>H</u>–CO–), 3.89 (3H, s, OCH_3), 5.08 (1H, bs, vinylic H at C-12), 5.22 (1H, bs, H-3, –C<u>H</u>–O–C=O), 7.01–7.08 (1H, m, Ar), 7.12–7.14 (1H, m, Ar), 7.37–7.42 (1H, m, Ar), 7.52–7.70 (3H, m, Ar).

GC–MS (*m/z*): 682 [M^+] (15), 464 (35), 453 (4), 438 (5), 234 (44), 218 (26), 203 (18), 185 (100), 175 (24).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{45}H_{62}O_5Na$; calculated 705.4495; observed 705. 4494.

3.3.4. Methyl, 3-[{2-[(2′, 6′-dichlorophenyl)amino] phenyl} acetyl]-12-ursen-24-oate (7)

White solid; $C_{45}H_{59}Cl_2NO_4$; 83% yield; mp 135–138 °C.

IR (KBr): ν_{max} 3342, 2923, 1733, 1456, 1244, 1148, 987, 987, 745 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.78 (3H, s, CH_3), 0.81 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.93 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.12 (3H, s), 1.15 (3H, s, CH_3), 1.26 (3H, s), 1.55 (3H, s, CH_3), 1.35–1.42 (8H, m), 2.17–1.60 (10H, m), 3.66 (3H, s, $COOCH_3$), 3.83 (2H, s, CH_2), 5.14 (1H, bs, vinylic H at C-12), 5.36 (1H, bs, H-3, –C<u>H</u>-O-C=O), 6.93 (1H, d, *J* = 8.0 Hz, Ar), 6.55 (1H, d, *J* = 8.0 Hz, Ar), 6.73 (1H, s, Ar), 6.97–7.01 (1H, m, Ar), 7.07–7.15 (1H, m, Ar), 7.31 (1H, s, Ar), 7.35 (1H, s, Ar).

GC–MS (*m/z*): 747 [M^+] (45), 13 (5), 529 (25), 453 (15), 437 (5), 295 (100), 234 (24), 218 (32), 214 (72), 203 (28), 189 (15), 175 (38).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{45}H_{59}Cl_2NO_4Na$; calculated 770.3719; observed 770.3717.

3.3.5. Methyl, 3-[1-(4′-chlorobenzoyl)-5′-methoxy-2′-methyl-1H-indole-3′acetyl]-12-ursen-24-oate (8)

White solid; $C_{50}H_{64}ClNO_6$; 84% yield; mp 195–198 °C.

IR (KBr): ν_{max} 3458, 2918, 1738, 1481, 1330, 1264, 1219, 1143, 755 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.62–0.73 (3H, m), 0.78 (3H, s, CH_3), 0.80 (3H, s, CH_3), 0.86 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.11–1.48 (12H, m), 1.56 (3H, s, CH_3), 1.62–2.15 (8H, m), 2.48 (3H, s, CH_3), 3.64 (3H, s, $COOCH_3$), 3.69 (2H, s), 3.81 (3H, s, OCH_3), 5.13 (1H, bs, vinylic H at C-12), 5.31 (1H, bs, H-3, –C<u>H</u>–O–C=O), 6.65 (1H, d, *J* = 2.0 Hz, Ar), 6.77 (1H, s, Ar), 6.94 (1H, d, *J* = 2.0 Hz, Ar), 7.42–7.47 (2H, m, Ar), 7.40–7.64 (2H, m, Ar).

GC–MS (*m/z*): 809 [M^+] (12), 591 (23), 452 (12), 357 (10), 312 (8), 234 (12), 218 (8), 203 (12), 187 (11), 175 (21)139 (100).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{50}H_{64}ClNO_6Na$; calculated 832.4320; observed 832.4318.

3.3.6. (±) Methyl, 3-[2′-(4′-isobutylphenyl)propanoyl]-11-oxo-12-ursen-24-oate (9)

White solid; $C_{44}H_{64}O_5$; 83% yield; mp 108–111 °C.

IR (KBr): ν_{max} 3454, 2951, 1732, 1672, 1463, 1209, 1052, 884 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.70–0.74 (5H, m), 0.77 (3H, s, CH_3), 0.80–0.85 (4H, m), 0.87 (3H, s, CH_3), 0.89–0.94 (3H, m), 0.99–1.05 (3H, m), 1.16 (3H, s, CH_3), 1.20–1.27 (4H, m), 1.35–1.40 (6H, m), 1.42 (3H, s, CH_3), 1.50 (3H, s, CH_3), 1.51–1.54 (5H, m), 1.56 (3H, s, CH_3), 1.66–2.10 (4H, m), 2.41 (2H, m, benzylic), 3.66 (3H, s, $COOCH_3$), 3.68–3.74 (1H, m), 5.17 (1H, bs, H-12, C=C<u>H</u>–CO), 5.22 (1H, bs, H-3, –C<u>H</u>-O-C=O), 7.05–7.09 (2H, m, Ar), 7.18–7.23 (2H, m, Ar).

GC–MS (*m/z*): 672 [M^+] (52), 657 (10), 483 (10), 467 (35), 435 (15), 407 (32), 287 (22), 273 (78), 232 (42), 189 (12), 175 (15), 161 (100), 135 (38).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{44}H_{64}O_5Na$; calculated 695.4651; observed 695.4650.

3.3.7. (±) Methyl, 3-[6′-methoxy-α-methyl-2-naphthyleneacetyl]-11-oxo-12-ursen-24-oate (10)

Pale yellow solid; $C_{45}H_{60}O_6$; 82% yield; mp 146–148 °C.

IR (KBr): ν_{max} 3463, 2928, 1728, 1642, 1466, 1224, 1190, 1037, 745 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.78 (3H, m), 0.82–0.88 (3H, m), 0.93 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.05 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.15–1.37 (6H, m), 1.39–1.50 (6H, m), 1.52 (3H, s, CH_3), 1.56 (3H, s, CH_3), 1.60–1.69 (7H, m), 2.08–2.27 (2H, m), 3.64 (3H, s, $COOCH_3$), 3.83 (1H,s, Ar–C<u>H</u>–CO–), 3.89 (3H, s, OCH_3), 5.24 (1H, bs, H-12, C=C<u>H</u>–CO), 5.44 (1H, bs, H-3, –C<u>H</u>–O–C=O), 7.03–7.08 (1H, m, Ar), 7.11–7.12 (1H, d, J = 2.0 Hz, Ar), 7.33–7.38 (1H, d, J = 2.0 Hz, Ar), 7.62–7.67- (3H, m, Ar).

GC–MS (*m/z*): 696 [M^+] (52), 467 (13), 407 (10), 273 (44), 232 (30), 185 (100), 135 (48).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{45}H_{60}O_6Na$; calculated 719.4288; observed 719.4280.

3.3.8. Methyl, 3- [{2-[(2′, 6′-dichlorophenyl) amino] phenyl} acetyl]-11-oxo-12-ursen-24-oate (11)

White solid; $C_{45}H_{57}Cl_2NO_5$; 79% yield; mp 106–108 °C.

IR (KBr): ν_{max} 2926, 1737, 1717, 1667,1453, 1240, 1062 cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.82 (3H, s, CH_3), 0.84–0.92 (2H, m), 0.96 (3H, s, CH_3), 0.99 (3H, s, CH_3), 1.02 (3H, s, CH_3), 1.11 (3H, s), 1.16 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.57 (3H, s, CH_3), 1.20–1.29 (2H, m), 1.42–1.52 (5H, m), 1.65–1.96 (6H, m), 2.04 (3H, s), 2.08–2.52 (1H, m), 3.66 (3H, s, $COOCH_3$), 3.83 (2H, s, CH_2), 5.37 (1H, bs, H-12, C=C<u>H</u>–CO), 5.55 (1H, bs, H-3, –C<u>H</u>-O-C=O), 6.55 (1H, d, J = 8.0 Hz, Ar), 6.88–6.92 (1H, d, J = 2.0 Hz, Ar), 6.93–6.96 (1H, d, J = 8.0 Hz, Ar), 7.10 (1H, d, J = 2.0 Hz, Ar), 7.19–7.20 (1H, m, Ar), 7.30 (2H, m, Ar).

GC–MS (*m/z*): 761 [M^+] (22), 725 (3), 467 (30), 435 (10), 407 (35), 295 (100), 242 (21), 218 (30), 214 (64), 203 (8), 189 (31), 175 (11).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{45}H_{57}Cl_2NO_5Na$; calculated 784.3511; observed 784.3515.

3.3.9. Methyl, 3-[1-(4′-chlorobenzoyl)-5′-methoxy-2′-methyl-1H-indole-3′acetyl]-11-oxo-12-ursen-24-oate (12)

White solid; $C_{50}H_{62}ClNO_7$; 77% yield; mp 133–135 °C.

IR (KBr): ν_{max} 3447, 2933, 1733, 1646, 1456, 1315, 1234, 1068, 832, cm^{-1}.

^1H NMR ($CDCl_3$, 200 MHz): δ 0.70–0.73 (3H, m), 0.79 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.97 (3H, s, CH_3), 1.04 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.22–1.54 (12H, m), 1.57 (3H, s, CH_3), 2.20–2.24 (5H, m), 2.44 (3H, s, CH_3), 2.47–2.54 (4H, m), 3.65 (3H, s, $COOCH_3$), 3.70 (2H, s), 3.79 (3H, s, OCH_3), 5.33 (1H, bs, H-12, C=C<u>H</u>–CO), 5.49 (1H, bs, H-3, –C<u>H</u>-O-C=O), 6.64 (1H, d, J = 2.0 Hz, Ar), 6.73 (1H, s, Ar), 6.93 (1H, d, J = 2.0 Hz, Ar), 7.43–7.48 (2H, m, Ar), 7.56–7.62 (2H, m, Ar).

GC–MS (*m/z*): 823 [M^+] (26), 685 (3), 467 (5), 407 (12), 357 (10), 312 (16), 273 (10), 232 (6), 174 (21), 139 (100).

HRMS-ESI: *m/z* [M+Na]$^+$ for $C_{50}H_{62}ClNO_7Na$; calculated 846.4113; observed 846.4117.

3.4. Biological activity

As a part of our biological evaluation for hybrid molecules acute anti-inflammatory study by carrageenan induced paw edema model and chronic anti-arthritic efficacy by Complete Freund's adjuvant (CFA) induced developing arthritis model were used.

3.5. Materials and methods

All the chemicals and reagents used in the experiment were of analytical grade and purchased from Sigma–Aldrich Corporation, MO, USA and Merck KGaA. The Carrageenan and Complete Freund's adjuvant (CFA) were purchased from Sigma–Aldrich Corporation, MO, USA. DMEM GlutaMAX media and Fetal Bovine Serum were procured from Life Technologies Inc., For testing COX-2 activity and Lipoxygenase Inhibitor Screening Assay kits were purchased from Cayman Chemical Company, MI, USA.

3.5.1. Experimental animals

A total of 96 male albino Wistar rats, weighing between 230 g and 250 g, were used in this study. The animals were housed in polypropylene cages with sterile paddy husk as bedding, in a climate-controlled room at temperature 24±1 °C and a 12 h light/dark cycle. Rats were fed on standard pellets of rat diet and water *ad libitum*. The animals were allowed to acclimatize to the environmental conditions for one week before experiments. The animals were randomized into experimental and control groups. Animal experiments were performed in accordance with the national guidelines for the use and care of laboratory animals and approved by Institutional Ethical Committee.

3.5.2. Anti-inflammatory activity

In this study, carrageenan-induced rat hind paw edema was used as the model of acute inflammation according to the method of Winter [37]. Edema induced by carrageenan is highly sensitive to NSAIDs and has been accepted as a useful indicator for identifying the new anti-inflammatory molecules. The animals were weighed and randomly divided into 12 groups of 4 rats each. NC1: healthy rats (negative control); NC2: carrageenan injected rats (negative control); PC1: ibuprofen treated rats (positive control); PC2: KBA treated rats (positive control); groups 5, 6, 7, 8, 9, 10, 11 and 12 were treated with compound 5, 6, 7, 8, 9, 10, 11 and 12 respectively at the dose of 3 mg/kg and 10 mg/kg bodyweight (result of 10 mg/kg dose were significant, so the same were tabulated as in Table 1). After 45 min of intraperitoneal administration of respective test compounds to the respective groups, 0.1 ml of 10 mg/ml of carrageenan solution prepared in normal saline was injected intradermally into the sub-plantar region of the right hind paw of rats. The level of inflammation was quantified at 0 h, 2nd, 4th and 24th h after injection of carrageenan, using plethysmometer (IIHC life sciences, USA). Mean paw volume was noted at the respective time.

3.5.3. Anti-arthritic activity

Complete Freund's adjuvant (CFA) induced arthritis is one of the most widely used model as it has been shown to share a number of immunological and clinical features with human arthritis [40]. The rats were randomly grouped into 12 groups as in the anti-inflammatory study, mentioned earlier. Arthritis process was

induced in rats with the intradermal injection of 0.2 ml of Freund's adjuvant containing heat killed *Mycobacterium tuberculosis* in paraffin oil into the right hind paw. The day on which adjuvant was injected was taken as day 0. From day 7, the respective compounds dissolved in dimethyl sulfoxide were gavaged daily till day 21, at the dose of 3 mg/kg and 10 mg/kg bodyweight (result of 10 mg/kg dose were significant, so the same were tabulated as in Table 2). The inflammation was quantified by measuring paw volume at day 0, 7, 14 and 21, using plethysmometer and on the same day, serum was collected and stored at −20 °C till analysis for COX-2 activity. At the end of the study, the animals were sacrificed and tissues were collected and stored in 10% formalin solution. The ankle joints were used for histopathological studies.

3.5.4. COX-2 activity assay

The serum stored at −20 °C were used for the analysis of COX-2 activity. Cell pellets were homogenized in 50 μl of cold buffer (0.1 M Tris–HCl, pH 7.8 containing 1 mM ethylene diamine tetra acetic acid) and centrifuged at 10 000 g for 15 min at 4 °C. Supernatants were collected and frozen at −80 °C before assay. The COX activity was then measured using a commercial test COX activity assay (Cayman Chemical Company USA) that measures the peroxidase activity of COX. The assay was performed according to the manufacturer's instructions. Briefly, the peroxidase activity was assayed colorimetrically by the monitoring appearance of oxidized N, N, N, N′-tetramethyl-p-phenylenediamine (TMPD) at 590 nm. COX activity is expressed as the rate of oxidation of TMPD in nmol/min/mg protein.

3.5.5. Histopathological study

The ankle joints were used for histopathological studies. The joints were embedded in paraffin blocks. 7 μm sections were taken and routine Haematoxylin eosin staining (H&E) was performed. The stained sections were observed under microscope at the magnification of 100X.

3.5.6. Statistical analysis

All values are reported as means ± SD in groups of four rats. Kinetic changes of each parameter were analyzed by one-way analysis of variance (ANOVA) to compare the values recorded at the individual time-points with those of control (NC1). Differences between control and compound treated rats were analyzed by *Bonferroni* test. Differences were considered statistically significant at $\mathbf{P} \leq 0.001$.

3.5.7. In-vitro COX-2 and LOX enzyme activity

LD_{50} of the test molecules was evaluated in-vitro by MTT assay on Synovial sarcoma (SW-982) cell line. Briefly, SW-982 cells were seeded into 96-well plate at density of 10^4 cells/well. After 24 h, media was replaced with media containing test compounds at different concentrations (10–150 μM) and incubated for 24 h. Cytotoxicity was measured by adding MTT at 5 mg/ml and incubated for 3 h. The formazan crystals were dissolved in DMSO and absorbance was read at 570 nm. DMSO was maintained below 0.5%.

In order to study the effect of IL-1β in activating COX-2 and LOX enzymes, SW-982 cells were treated with IL-1β for different time points ranging from 30 m, 1 h, 3 h, 6 h, upto 24 h and studied the enzyme activity wherein, we found maximum activity between 6 h and 24 h.

To determine the enzyme activity, SW-982 cells were pretreated with these molecules at their respective inhibitory concentration, incubated for 24 h and treated with Interleukin-1β at 2 ng/ml concentration for 6 h. Cells lysate was prepared and protein concentration was estimated. Enzyme activity was performed according to the manufacturer's instructions.

Acknowledgments

We express our sincere gratitude to Dr. Anil Kush, CEO, Vittal Mallya Scientific Research Foundation, for his keen interest and encouragement. Mr. A.C. Karunakara and Ms.Aparna Bhat for analytical help. Dept. of Biotechnology, India (Grant No. BT/PR11910/BRB/10/697/2009) is gratefully acknowledged for financial assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2015.05.001.

References

[1] G.B. Singh, C.K. Atal, Pharmacology of an extract of salai guggul ex- Boswellia serrata, Ind. J. Pharmacol. 16 (1984) 51–54.
[2] G.K. Reddy, S.C. Dhar, G.B. Singh, Urinary excretion of connective tissue metabolites under the influence of a new non-steroidal anti-inflammatory agent in adjuvant induced arthritis, Agents Actions 22 (1987) 99–105.
[3] H.P.T. Ammon, H. Safayhi, T. Mack, J. Sabieraj, Mechanism of antiinflammatory actions of curcumine and boswellic acids, J. Ethnopharmacol. 38 (1993) 113–119.
[4] H.P.T. Ammon, Boswellic acids in chronic inflammatory diseases, Planta Med. 72 (2006) 1100–1116.
[5] M.L. Sharma, S. Bani, G.B. Singh, Anti-arthritic activity of boswellic acids in bovine serum albumin (BSA)-induced arthritis, Int. J. Immunopharmacol. 11 (1989) 647–652.
[6] B.A. Shah, G.N. Qazi, S.C. Taneja, Boswellic acids: a group of medicinally important compounds, Nat. Prod. Rep. 26 (2009) 72–89.
[7] I. Gupta, V. Gupta, A. Parihar, S. Gupta, R. Lüdtke, H. Safayhi, H.P. Ammom, Effects of Boswellia serrata gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study, Eur. J. Med. Res. 3 (1998) 511–514.
[8] I. Gupta, A. Parihar, P. Malhotra, H. Safayhi, Effects of gum resin of Boswellia serrata in patients with chronic colitis, Planta Med. 67 (2001) 391–395.
[9] H. Gerhardt, F. Seifert, P. Buvari, R. Repges, Therapy of active crohn disease with Boswellia serrata extract H 15, Z. Gastroenterol. 39 (2001) 11–17.
[10] Y. Takada, H. Ichikawa, V. Badmaev, B.B. Aggarwal, Acetyl-11-keto-β-Boswellic acid potentiates apoptosis, inhibits invasion, and abolishes Osteoclastogenesis by suppressing NF-κB and NF-κB-regulated gene expression, J. Immunol. 176 (2006) 3127–3140.
[11] R.S. Pandey, B.K. Singh, Y.B. Tripathi, Extract of gum resins of Boswellia serrata L. inhibits lipopolysaccharide induced nitric oxide production in rat macrophages along with hypolipidemic property, Indian J. Exp. Biol. 43 (2005) 509–516.
[12] H. Safayhi, T. Mack, J. Sabieraj, M.I. Anazodo, L.R. Subramanian, H.P. Ammon, Boswellic acids: novel, specific, nonredox inhibitors of 5-lipoxygenase, J. Pharmacol. Exp. Ther. 261 (1992) 1143–1146.
[13] H.P. Ammon, T. Mack, G.B. Singh, H. Safayhi, Inhibition of leukotriene B4 formation in rat peritoneal neutrophils by an ethanolic extract of the gum resin exudate of Boswellia serrata, Planta Med. 57 (1991) 203–207.
[14] H. Safayhi, E.R. Sailer, H.P. Ammon, Mechanism of 5-lipoxygenase inhibition by acetyl-11-keto-beta-boswellic acid, Mol. Pharmacol. 47 (1995) 1212–1216.
[15] E.R. Sailer, L.R. Subramanian, B. Rall, R.F. Hoernlein, H.P. Ammon, H. Safayhi, Acetyl-11-keto-beta-boswellic acid (AKBA): structure requirements for binding and 5-lipoxygenase inhibitory activity, Br. J. Pharmacol. 117 (1996) 615–618.
[16] S. Singh, A. Khajuria, S.C. Taneja, R.K. Khajuria, J. Singh, G.N. Qazi, Boswellic acids and glucosamine show synergistic effect in preclinical anti-inflammatory study in rats, Bioorg. Med. Chem. Lett. 17 (2007) 3706–3711.
[17] Graus I.M.F., Smit H.F., patent No US6492429 B1, 2002.
[18] J.E. Chrubasik, B.D. Roufogalis, S. Chrubasik, Evidence of effectiveness of herbal antiinflammatory drugs in the treatment of painful osteoarthritis and chronic low back pain, Phytother. Res. 21 (2007) 675–683.
[19] B. Meunier, Hybrid molecules with a dual Mode of action: dream or reality? Acc. Chem. Res. 41 (2008) 69–77.
[20] A. Bisi, A. Rampa, R. Budriesi, S. Gobbi, F. Belluti, P. Ioan, et al., Cardiovascular hybrid drugs: new benzazepinone derivatives as bradycardic agents endowed with selective beta1-non-competitive antagonism, Bioorg. Med. Chem. 11 (2003) 1353–1361.
[21] D. Schellenberg, S. Abdulla, C. Roper, Current issues for anti-malarial drugs to control P. falciparum malaria, Curr. Mol. Med. 6 (2006) 253–260.
[22] M. Decker, B. Kraus, J. Heilmann, Design, synthesis and pharmacological evaluation of hybrid molecules out of quinazolinimines and lipoic acid lead to highly potent and selective butyrylcholinesterase inhibitors with antioxidant properties, Bioorg. Med. Chem. 16 (2008) 4252–4261.
[23] F.W. Muregi, A. Ishih, Next-generation antimalarial drugs: hybrid molecules as a new strategy in drug Design, Drug Dev. Res. 71 (2010) 20–32, http://

dx.doi.org/10.1002/ddr.20345.
[24] E.M.H. Yee, E. Pasquier, G. Iskander, K. Wood, D.S. Black, N. Kumar, Synthesis of novel isoflavene-propranolol hybrids as anti-tumor agents, Bioorg. Med. Chem. 21 (2013) 1652–1660.
[25] S. Rizzo, M. Bartolini, L. Ceccarini, L. Piazzi, S. Gobbi, A. Cavalli, et al., Targeting Alzheimer's disease: novel indanone hybrids bearing a pharmacophoric fragment of AP2238, Bioorg. Med. Chem. 18 (2010) 1749–1760.
[26] J.G. Ghogare, S.V. Bhandari, K.G. Bothara, A.R. Madgulkar, G.A. Parashar, B.G. Sonawane, et al., Design, synthesis and pharmacological screening of potential anticonvulsant agents using hybrid approach, Eur. J. Med. Chem. 45 (2010) 857–863.
[27] P. Ettmayer, G.L. Amidon, B. Clement, B. Testa, Lessons learned from marketed and investigational prodrugs, J. Med. Chem. 47 (2004) 2393–2404, http://dx.doi.org/10.1021/jm0303812.
[28] P.L. Bosquesi, T.R.F. Melo, E.O. Vizioli, J.L. dos Santos, M.C. Chung, Anti-inflammatory drug design using a molecular hybridization approach, Pharmaceuticals 4 (2011) 1450–1474.
[29] G. Melagraki, A. Afantitis, O. Igglessi-Markopoulou, A. Detsi, M. Koufaki, C. Kontogiorgis, et al., Synthesis and evaluation of the antioxidant and anti-inflammatory activity of novel coumarin-3-aminoamides and their alpha-lipoic acid adducts, Eur. J. Med. Chem. 44 (2009) 3020–3026.
[30] K.R.A. Abdellatif, M.A. Chowdhury, Y. Dong, D. Das, G. Yu, C.A. Velázquez, et al., Dinitroglyceryl and diazen-1-ium-1,2-diolated nitric oxide donor ester prodrugs of aspirin, indomethacin and ibuprofen: synthesis, biological evaluation and nitric oxide release studies, Bioorg. Med. Chem. Lett. 19 (2009) 3014–3018.
[31] S. Shenvi, G.C. Reddy, Reagent optimization for allylic oxidation of 3-O-acetyl-β-boswellic acid into 3-O-acetyl-11-oxo-β-boswellic acid, Chem. Nat. Compd. 48 (2013) 1008–1012.
[32] S. Shenvi, K. Rijesh, L. Diwakar, G.C. Reddy, Beckmann rearrangement products of methyl 3-oxo-tirucall-8, 24-dien-21-oate from Boswellia serrata gum and their anti-tumor activity, Phytochem. Lett. 7 (2014) 114–119.
[33] R.S. Pardhy, S.C. Bhattacharyya, Tetracyclic triterpene acids from the resin of Boswellia serrata Roxb, Indian J. Chem. 16B (1978) 174–175.
[34] R.S. Pardhy, S.C. Bhattacharyya, β-Boswellic acid, acetyl- β-boswellic acid, acetyl-11-keto-β-boswellic acid and 11-keto-β-boswellic acid, four pentacyclic triterpenic acids from the resin of Boswellia serrata Roxb, Indian J. Chem. 16B (1978) 176–178.
[35] J. Jauch, J. Bergmann, An Efficient method for the large-scale preparation of 3-O-Acetyl-11-oxo-β-boswellic acid and other boswellic acids, Eur. J. Org. Chem. 2003 (2003) 4752–4756.
[36] M. Di Rosa, Biological properties of carrageenan, J. Pharm. Pharmacol. 24 (1972) 89–102.
[37] C.A. Winter, E.A. Risley, G.W. Nuss, Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[38] A. Sharma, S. Bhatia, M.D. Kharya, V. Gajbhiye, N. Ganesh, A.G. Namdeo, et al., Anti-inflammatory and analgesic activity of different fractions of Boswellia serrata, Int. J. Phytomedicine 2 (2011).
[39] N.K. Mishra, S. Bstia, G. Mishra, K.A. Chowdary, S. Patra, Anti-arthritic activity of Glycyrrhiza glabra, Boswellia serrata and their synergistic activity in combined formulation studied in freund's adjuvant induced arthritic rats, J. Pharm. Educ. Res. 2 (2011) 92–98.
[40] V. Nair, Antiarthritic activity of majoon suranjan (a polyherbal unani formulation) in rat, Indian J. Med. Res. 134 (2011) 384–388.
[41] M.E. Turini, R.N. DuBois, Cyclooxygenase-2: a therapeutic target, Annu. Rev. Med. 53 (2002) 35–57.
[42] O. Morteau, Prostaglandins and inflammation: the cyclooxygenase controversy, Arch. Immunol. Ther. Exp. (Warsz.) 48 (2000) 473–480.