Bioorganic & Medicinal Chemistry Letters 22 (2012) 7582–7587

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and pharmacokinetic profile of rhein- boswellic acid conjugate

Dhaneshwar Suneela *, Patil Dipmala

Department of Pharmaceutical Chemistry, Bharati Vidyapeeth Deemed University, Poona College of Pharmacy, Pune 411038, Maharashtra, India

ARTICLE INFO

Article history:
Received 18 August 2012
Revised 21 September 2012
Accepted 2 October 2012
Available online 11 October 2012

Keywords:
Diacerein
Inflammation
In vitro/vivo kinetics
Mutual prodrug
Osteoarthritis

ABSTRACT

Rhein, an active metabolite of diacerein, down-regulates the gene-expression and production of pro-matrix metalloproteinases and up-regulates the tissue inhibitors of metalloproteinase-1 production. The therapeutic effects of diacerein on osteoarthritis are, at least in part, due to the chondroprotective effect of rhein. Boswellic acid is a specific, non-redox inhibitor of leukotriene synthesis. It is claimed to possess good anti-inflammatory, anti-arthritic, analgesic, and anti-ulcer activities. It prevents the destruction of articular cartilage by decreasing degradation of glycosaminoglycans. Therefore, rhein and boswellic acid were linked chemically through a bioreversible ester linkage to synthesize their mutual prodrug by reported procedure. In vitro release profile of this prodrug was extensively studied in aqueous buffers of varied pH, upper GIT homogenates and 80% human plasma. In vivo release studies were undertaken in blood, urine and feces of rats. The prodrug was stable in HCl buffer (pH 1.2) and stomach homogenates of rats. However; in phosphate buffer (pH 7.4) and in intestinal homogenates the prodrug exhibited 91% and 96% release of rhein and 27.5% and 38% release of boswellic acid respectively over a period of 6 h following first order kinetics. In 80% human plasma (in vitro) and rat blood (in vivo) also 96.35% and 91% release of rhein and 78% and 86.41% release of boswellic acid respectively was observed. The 24 h pooled samples of rat urine revealed presence of 6.2% intact prodrug, 7.1% of rhein and 8.9% of boswellic acid indicating their renal excretion. Samples of rat feces pooled over a period of 24 h showed absence of rhein and presence of 3.1% of intact boswellic acid and 4.6% of boswellic acid emphasizing their intestinal excretion. The in vivo release kinetics of prodrug in rat clearly indicated activation of prodrug to be occurring in blood, being catalyzed by the weak alkaline pH of blood (7.4) in combination with esterases present therein.

Osteoarthritis (OA) is a multifactorial, complex disease of synovial joints.[1] It is characterized by loss of articular cartilage due to degradation resulting in reduced joint space, remodeling of the subchondral bone, formation of osteophytes, cyst formation, joint misalignment and inflammation of the synovial membrane; the later being the main cause of chronic pain which is the major symptom of OA.[2–4] Ligament laxity, meniscal degradation and bone marrow edema are other important characteristics which lead to joint impairment and disability. All of these are responsible more or less for the generation of local, chronic pain with insidious onset characterized by aching with episodic stabbing.[5–8] OA generally occurs when the normal cartilage matrix is overloaded or when cartilage matrix is vulnerable. Interleukin- 1 (IL-1) is principally involved in degradation while transforming growth factor (TGF-β) is implicated in the excessive repair of synovium and chondrocytes. Degradation and repair are simultaneously occurring phenomenon.[9]

A large number of palliative treatment modalities are available for OA which mainly use non-steroidal anti-inflammatory drugs (NSAIDs) but steroidal anti-inflammatory agents and immunosuppressants are also equally important components of OA therapy.[10] Long-term administration of NSAIDs has deleterious effects on the vital organs like gastrointestinal tract (GIT), kidney, liver, central nervous system and immune system.[11–13] Intra-articular, long acting corticosteroid injections although used in the management of knee OA, can be given only three to four times in year as repeated injections lead to progressive cartilage damage in weight-bearing joints.[14–17] Chondro-protective or disease-modifying drugs preserve normal joint function by slowing down the rate of anatomical progression of OA.[18] Chondroitin sulfate, glucosamine, diacerein, doxycycline and minocycline are the frontline disease modifying anti-osteoarthritic drugs (DMOADs).[19]

Diacerein [4,5-bis(acetyloxy)-9, 10-dioxo-2-anthracene carboxylic acid], a low molecular weight anthraquinone derivative has been introduced as an effective chondroprotective for the management of OA. Since then it has emerged as a better and safer alternative for the treatment of the OA, which provides symptomatic treatment along with modification of underlying pathological process.[20,21] It has demonstrated efficacy on the functional and structural manifestations of OA by inhibition of IL-1 which is mainly involved in cartilage destruction and acting on the synthesis of

* Corresponding author. Tel.: +91 20 25437237; fax: +91 20 25439383.
E-mail address: suneeladhaneshwar@rediffmail.com (D. Suneela).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.10.017

principal components of cartilage like proteoglycans and hyaluronic acid.[22] Diacerein inhibits interleukin-1β (IL-1β) and down-regulates the IL-1β-induced inflammatory pathways and cartilage breakdown in OA as opposed to inhibiting the cyclooxygenase (COX) pathway as NSAIDs do. In addition, it down-regulates the gene-expression and production of pro-matrix metalloproteinases (proMMPs) that are involved in cartilage degradation and up-regulates the tissue inhibitor of metalloproteinase-1 (TIMP-1) production.[10,23–25] Orally administered diacerein is completely converted into its active metabolite rhein by deacetylation before reaching systemic circulation.[26] Rhein being an IL-1 inhibitor, reduces collagenase production in articular cartilage, dose-dependently inhibits superoxide anion production, chemotaxix and phagocytic activity of neutrophils, macrophage migration and phagocytosis.[27] The therapeutic effects of diacerein on OA may be due, at least in part, to the chondroprotective effect of rhein.[28]

Therefore in the present work, rhein was chosen as potential candidate for development of a mutual prodrug which would have quick onset of action with improved absorption and bioavailability than diacerein. Boswellic acid was selected as the promoiety in this mutual prodrug design owing to its diverse pharmacological activities that would potentiate the chondroprotetive, disease modifying effect of rhein.

Boswellic acid and its acetates are ursane-type pentacyclic triterpene acids isolated from the gummy exudates of *Boswellia serrata* Roxb and *Boswellia carterri* Birdw.[29] The resin of *Boswellia serrata* is used in India for the treatment of chronic inflammatory arthritis. Ethanolic extract of the resin contains boswellic acid as the main active constituent.[30,31]Gum resin extracted from *Boswellia serrata* Roxb possesses good anti-inflammatory, anti-arthritic and analgesic activities.[32] Boswellic acid is reported to possess anti-ulcer property as well.[33] It also has protective effect on articular cartilage by the virtue of its inhibitory effect on degradation of glycosaminoglycans.[34]

We have reported the synthesis and the chondroproctective effect of mutual prodrugs of diacerein with glucosamine[35,36] and essential amino acids in monosodium iodoacetate-induced osteoarthritis in Wistar rats.[37] Two hydrolytically activated antraquinone-diclofenac prodrugs have been designed and synthesized by Daan et al. (2009) for bone targeting.[38] Synthesis and pharmacological activities of anthraquinone-ibuprofen prodrugs targeting osseous tissues were also reported by Duan et al. (2009).[39] Singh et al. (2007) have revealed the synergistic effect of mixture of boswellic acid and glucosamine for anti-inflammatory and anti-arthritic activities in rats.[40]

There are no reports of any prodrugs of rhein in the literature so far. The present work reports synthesis, in vitro and in vivo release kinetics of mutual prodrug of rhein with boswellic acid with aims of decreasing local irritant effect, potentiating anti-inflammatory activity, anti-arthritic effect and increasing lipophilicity of rhein in order to increase its bioavailability.

Diacerein was generously gifted by Glenmark Pharmaceuticals Ltd., Mumbai, India. Rhein was synthesized in our lab from diacerein by reported procedure. Boswellic acid was purchased from Jai Rhade Sales, Ahmedabad, Gujrat, India. All chemicals and solvents used in the study were purchased from Merck Chemical Corporation, USA and were of analytical reagent grade (AR) or higher purity and were used as it is. Thin layer chromatography was performed on precoated silica gel plates-60 F264 (Merck) for purity check and monitoring of reactions. The IR spectrum of the synthesized compound was recorded on Jasco V-530 FTIR in anhydrous IR grade potassium bromide. Proton and ^{13}C NMR spectra of the synthesized prodrug were recorded in DMSO-d_6 using Bruker Avance II 400 NMR spectrometer at Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh, India. Chemical shifts are reported in ppm downfield on δ scale. The mass spectrum was recorded employing Waters Q-Tof Macromass spectrometer, LC–MS system at SAIF, Panjab University, Chandigarh, India. The elemental analysis of synthesized prodrug was performed on Elemental Analyzer (Vario MICRO CUBE, Germany) at Department of Pharmaceutical Chemistry, Poona College of Pharmacy, Pune (India). The absorbance maxima (λ_{max}) of synthesized compound in various solvents were determined on Jasco V-530 UV–Visible double-beam spectrophotometer. Partition coefficient was determined in *n*-octanol/phosphate buffer (pH 7.4) whereas the aqueous solubility was determined in distilled water at room temperature 25 ± 1 °C using Jasco V-530 UV–Visible double-beam spectrophotometer. For release kinetic studies, a new HPLC method was developed for simultaneous estimation of prodrug and its active metabolites (rhein and boswellic acid). In vitro release was studied in aqueous buffers of varied pH, rat's upper GIT homogenates and 80% human plasma. In vivo release was studied in rat blood, feces and urine. Human plasma was procured from Bharati Vidyapeeth Medical College, Dhankavadi, Pune. The HPLC system used for this purpose consisted of a pump (Jasco PU model 2080), with autosampler programmed at 20 μL capacity per injection and a UV/VIS detector (Jasco UV 2075). Data was integrated using Jasco Borwin version 1.5, LC-net-II/ADC system. XTerra RP18 column (4.6 × 150 mm; 3.5 μM) in the reversed phase partition chromatographic condition was used for HPLC analysis. The system was used in an air-conditioned HPLC laboratory atmosphere (20 ± 1 °C). Before analysis, the mobile phase was degassed using sonicator and filtered through a 0.45 μM Millipore filter. Sample solutions were also filtered through the same. The system was equilibrated before making an injection. The column was monitored for UV absorbance at a detection wavelength selected after taking the overlay spectra of all the components.

Rhein **1** was synthesized from diacerein by standard procedure.[41] The mutual ester prodrug **3** of rhein **1** with boswellic acid **2** was synthesized by using *N*,*N*′-dicyclohexylcarbodiimide (DCC) coupling (Fig. 1) and the structure was confirmed by spectral analysis.[42–44]

In vitro hydrolysis kinetics of prodrug was studied in aqueous buffers pH 1.2 and 7.4. A reversed-phase HPLC method was developed for the simultaneous estimation of prodrug and its hydrolytic metabolites rhein and boswellic acid. The mobile phase composed of a phosphate buffer: acetonitrile (55:45 v/v, pH 3.0 ± 0.1) at a flow rate of 1.0 mL/min and the column effluent was monitored at 211 nm. The retention times for rhein, boswellic acid and prodrug were found to be 7.1, 5.4 and 8.2 min respectively. Aqueous buffers of pH 1.2 (simulating the pH of stomach) and pH 7.4 (corresponding to physiological pH of small intestine and blood) were prepared to study the release profile of prodrug. The prodrug was incubated in these media at 37 ± 0.5 °C with continuous stirring at 100 rpm. Prodrug (10 mg) was introduced in the medium. Aliquots (5 mL) were withdrawn and replaced with an equal volume of fresh incubation medium by a sampler at different time intervals up to 7 h. The release studies were performed in triplicate.

In vitro release of rhein from the prodrug was further studied in stomach and small intestinal homogenates of rats. A solution of prodrug was prepared in respective buffers (250 μg/mL) and 0.8 mL of it was added to 0.2 g of stomach or small intestinal homogenate placed in 1 mL centrifuge tubes that were pre-equilibrated at 37 ± 0.5 °C. Samples were withdrawn at appropriate time intervals, centrifuged at 10,000 rpm for 10 min. The supernatants were filtered through membrane filter (0.45 μM) and estimated by HPLC for the amount of prodrug remaining and also rhein and/or boswellic acid that might have released after hydrolysis of prodrug. Rate constants for the hydrolysis of prodrug were determined from the slopes of linear plots of the logarithm of residual prodrug concentration versus time. These studies were performed in triplicate and the mean of the rate constant was calculated.

In vitro release kinetic studies were performed in human plasma also. For this purpose 80% human plasma was prepared in

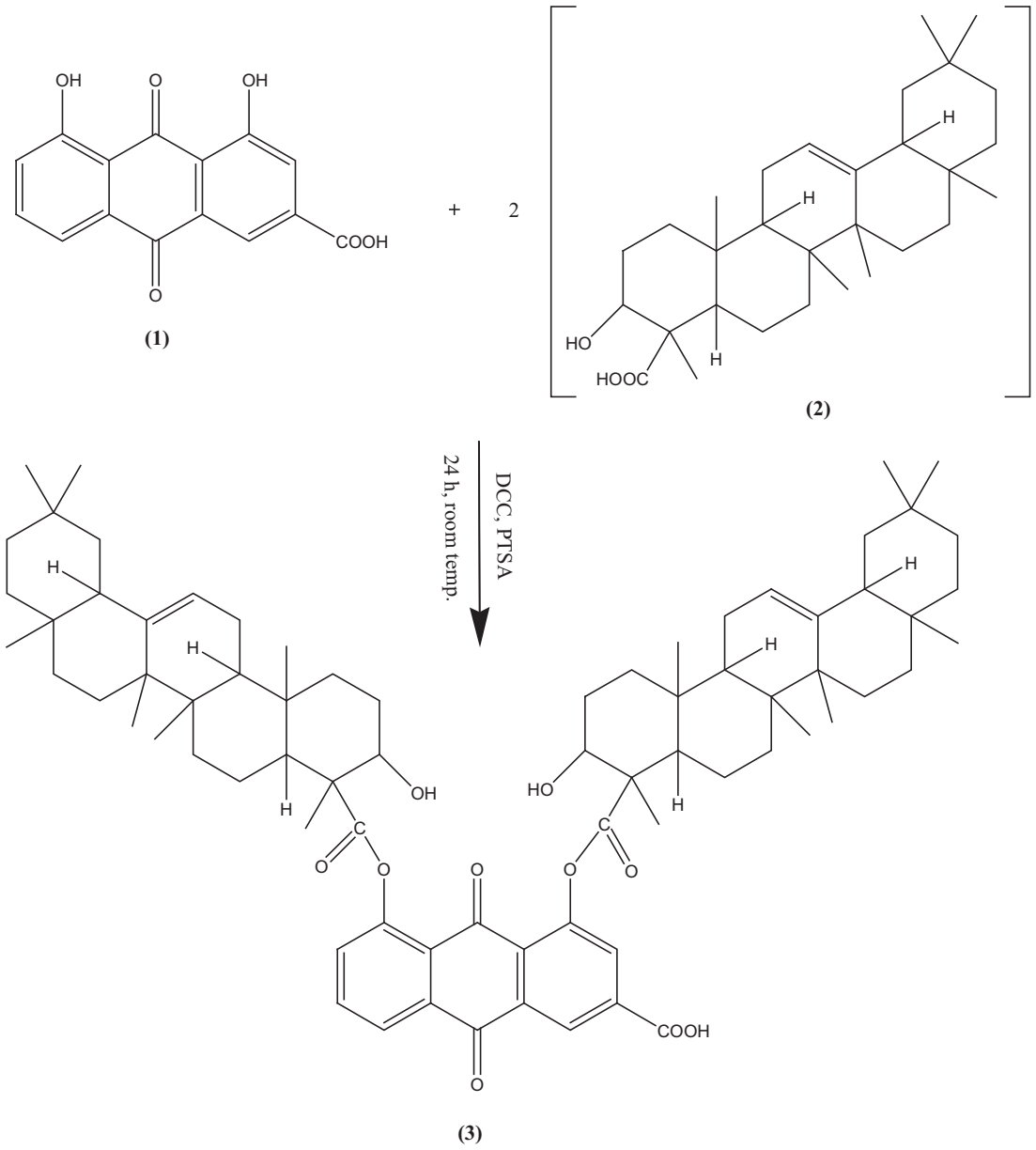

Figure 1. Scheme of synthesis.

0.05 M phosphate buffer (pH 7.4). Stock solution of prodrug was prepared in methanol (250 μg/mL) and 100 μL of it was added to 100 μL of 80% plasma placed in 1 mL eppendorf tubes. The tubes were kept in incubator at 37 ± 0.5 °C and removed at appropriate time intervals and then centrifuged at 5000 rpm at 0–5 °C for 10 min. The supernatant (0.1 mL) was added to another eppendorf tube (1 mL capacity) and 0.9 mL methanol was added to it for immediate plasma protein precipitation. All the solutions were then vortexed for 2 min and again centrifuged at 10,000 rpm for 5 min at 0–5 °C in order to precipitate other impurities or solid matter present in the biological samples. The clear supernatant (20 μL) was filtered through a membrane filter (0.45 μM) and analyzed by HPLC for presence of residual prodrug and released rhein and/or boswellic acid. These studies were performed in triplicate and the mean of the rate constant was calculated.

In vivo behavior of orally administered prodrug was investigated in Wistar rats at animal facilities of Poona College of Pharmacy, Pune, India which were approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The protocols for the same were approved by Institutional Animal Ethics Committee of Poona College of Pharmacy, Pune. Wistar rats (200–250 g; $n = 3$) were housed in metabolic cages individually under normal conditions (at 27 ± 0.5 °C and a relative humidity of 70 ± 0.5% under natural light/dark conditions). The rats were fasted overnight before the experiment but had free access to water. Prodrug (2.5 mg) was dissolved in saline (0.5 mL) and was administered to rats by oral route. Blood was drawn by retro-orbital puncture from both the eyes of the rats and collected in eppendorf tubes over a period of 8 h. Same procedure as described for 80% human plasma was followed to study the fate of the prodrug in terms of its absorption/distribution in the body. Urine and feces of the rats treated with the prodrug were collected at different time intervals from the metabolic cages over a period of 24 h and pooled together and diluted with phosphate buffer (pH 7.4) by 10 and 100-fold respectively. Same procedure as described for 80% human plasma was followed to study the excretion profile of the prodrug.

In this study, a novel covalently bound conjugate of rhein and boswellic acid was synthesized and structurally characterized by spectral analysis wherein satisfactory FTIR, ^{1}H and ^{13}C NMR spectra were obtained. The IR spectrum showed characteristic peaks at 1721 cm^{-1} for C=O stretching ester, broad peak at 3300 cm^{-1} for carboxylic OH stretching (rhein backbone) and 3538 cm^{-1} for alcoholic OH stretching (boswellic acid backbone). The chemical shifts observed in ^{1}H NMR of prodrug were in accordance with the anticipated structure. ^{13}C NMR exhibited all the relevant chemical shifts as per the anticipated number of carbon atoms present in prodrug. Elemental analysis, ^{13}C NMR and mass spectroscopy results confirmed the formation of prodrug by reaction of one molecule of rhein with two molecules of boswellic acid. The molecular weight of prodrug was calculated from the results of elemental analysis and mass spectroscopy and it was in accordance with the anticipated molecular weight. The lipophilicity of rhein (Log*P* 0.2402) was markedly enhanced in prodrug (Log*P* 2.568) due to its conjugation with boswellic acid. This would improve absorption and bioavailability of rhein.

The results of in vitro kinetics studies are compiled in Table 1. The prodrug resisted acid-catalyzed hydrolysis on incubation with HCl buffer and stomach homogenates. However, the prodrug was susceptible to weak alkaline hydrolysis in phosphate buffer (pH 7.4) accomplishing 91% release of rhein and 27.5% release of boswellic acid over a period of 6 h following first order kinetics with a half life of 192 min. The result of release kinetics of prodrug in small intestinal homogenates was slightly better (96% of rhein and 38% of boswellic acid) than in phosphate buffer (pH 7.4). The release kinetics was further studied in 80% human plasma over a period of 7 h offering 96.35% of rhein and 77.31% of boswellic acid (Fig. 2).

In vivo behavior of the orally administered prodrug was studied in Wistar rats over a period of 7 h in blood. The concentrations of prodrug remaining after hydrolysis and released rhein and boswellic acid were determined in blood which was withdrawn at various time intervals by the retro-orbital puncture and also in 24 h pooled samples of urine and feces collected from the metabolic cages. First appearance of prodrug in blood was noted at 2 h (93.6%) indicating its absorption in intact form from the stomach, which decreased consistently at 3 h (70.43%), 4 h (34.5%), 5 h (22%) and 6 h (12%) eventually reaching a negligible level at the end of 7 h (3.4%) owing to its hydrolysis (activation) into rhein and boswellic acid. Presence of rhein and boswellic acid (approximately 29%) was first observed in blood at 3 h. A constant increase in the concentration of rhein and boswellic acid was observed from 4 h onwards reaching the highest concentration at the end of 7 h. Approximately 91% of the administered dose of prodrug was recovered in blood as rhein and 86.41% as boswellic acid in 7 h (Fig. 3). The in vivo release profile of prodrug in rat clearly indicates that its activation is mediated by hydrolysis which in turn is catalyzed by the weak alkaline pH of blood (7.4) in combination with esterases present therein (Fig. 4). The results of in vitro release kinetics of prodrug in small intestinal homogenates and 80% human plasma correlate well with its in vivo

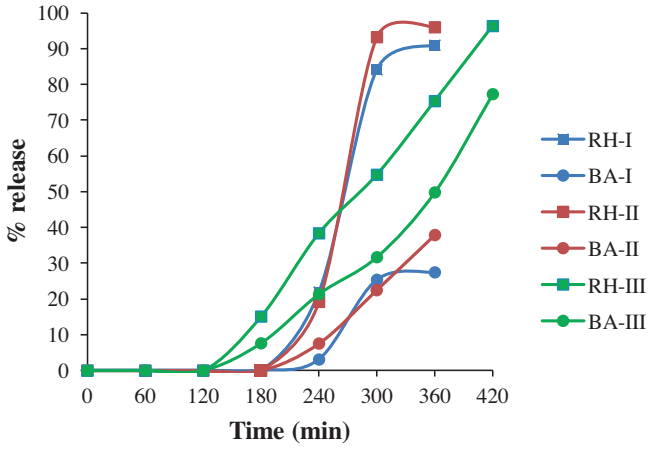

Figure 2. In vitro percent release of rhein and boswellic acid from prodrug in different incubation media. RH: Rhein; BA: Boswellic acid; I-Phosphate buffer pH-7.4; II-Intestinal homogenates; III-80% human plasma.

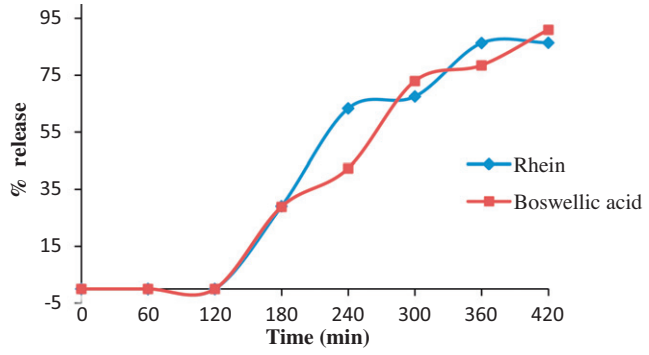

Figure 3. In vivo percent release of rhein and boswellic acid from orally administered prodrug in rat blood.

behavior because of the similarity in their enzymatic set up (esterases) and pH of the environment (7.4). The 24 h pooled samples of rat urine revealed presence of 6.2% of intact prodrug, 7.1% of rhein and 8.9% of boswellic acid indicating their renal excretion. The urine also indicated presence of negligible concentration of unknown metabolites. Samples of rat feces collected and pooled over a period of 24 h showed absence of rhein and presence of 3.1% of intact prodrug and 4.59% of boswellic acid emphasizing their intestinal excretion.

In conclusion, the release kinetics has supported the bioreversible nature of the ester linkage which upon alkaline and enzymatic (esterases in small intestine and blood) hydrolysis is almost completely releasing rhein and boswellic acid at the end of 7 h. Markedly enhanced lipophilicity of prodrug as compared to rhein and its stability in stomach might be responsible for its quick absorption from

Table 1
In vitro release kinetics data of prodrug at 37 °C

S.N.	Incubation medium	Order of reaction	K ± S.D. (min^{-1})*	$t_{1/2}$ (min)	Av % release	
					Rhein	Boswellic acid
1	Hydrochloric acid buffer (pH 1.2)	Stable	–	–	–	–
2	Phosphate buffer (pH 7.4)	First	$0.003 \pm 3\times10^{-4}$	192.3	91	27.5
3	Stomach homogenates	Stable	–	–	–	–
4	Small intestinal homogenates	First	$0.005 \pm 2\times10^{-3}$	160.3	96	38
5	80% Human plasma	First	$0.004 \pm 3\times10^{-4}$	186.1	96.35	77.31

* Average of three readings.

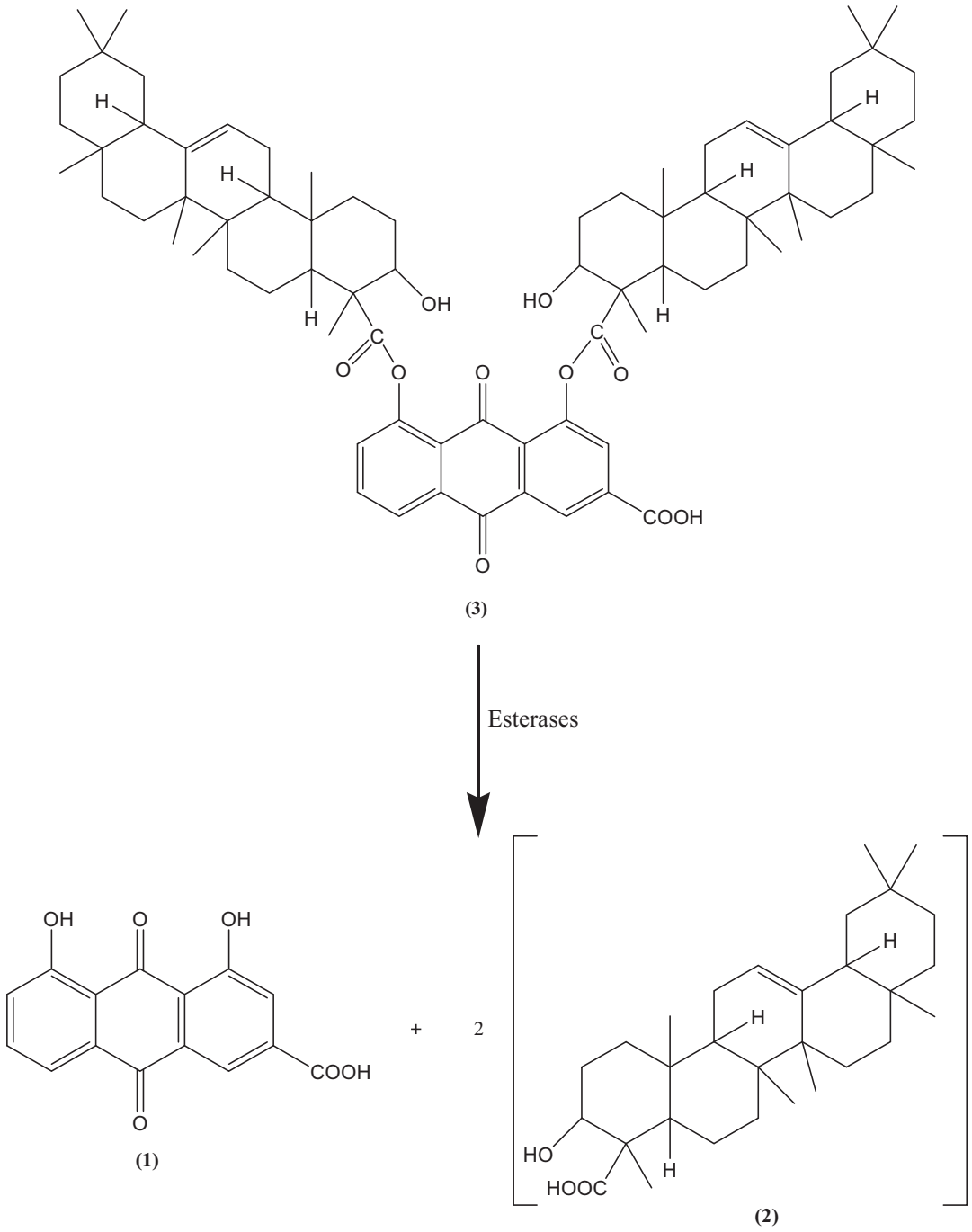

Figure 4. Proposed scheme of activation of prodrug.

the gastric mucosa which is characterized by appearance of 93.6% of intact prodrug in blood during first 2 h of its oral administration. The in vivo release kinetics of prodrug in rat clearly indicates that the activation of prodrug is occurring in blood and is being catalyzed by the weak alkaline pH of blood (7.4) in combination with esterases present therein. The results of evaluation of disease-modifying effect of prodrug in collagenase (type-II)-induced OA in Wistar rats are very promising and will be published separately.

Acknowledgment

Authors thank the UGC for providing financial assistance and Glenmark Pharmaceutical Pvt. Ltd, Mumbai, India, for providing the gift sample of diacerein.

References and notes

1. Boileau, C.; Tat, S. K.; Pelletier, J. P.; Cheng, S.; Martel-Pelletier, J. *Arthritis Res. Ther.* **2008**, *10*, R71.
2. Pelletier, J. P.; Martel-Pelletier, J. *Ann. Rheum. Dis.* **2003**, *62*, 79.
3. Pelletier, J. P.; Martel-Pelletier, J.; Abramson, S. B. *Arthritis Rheum.* **2001**, *44*, 1237.
4. Verbruggen, G. *Am. Coll. Rheumatol.* **2006**, *45*, 129.
5. Guermazi, A.; Zaim, S.; Taouli, B.; Miaux, Y.; Peterfy, C. G.; Genant, H. G. *Eur. Radiol.* **2003**, *13*, 1370.
6. Dieppe, P. A.; Lim, K. Osteoarthritis and Related Disorders; Clinical Features and Diagnostic Problems. In *Rheumatology*; Dequeker, J., Dieppe, P. A., Eds., 2nd ed.; Mosby: London, 2000; pp 831–836.
7. Felson, D. T.; Lawrence, R. C.; Dieppe, P. A.; Hirsch, R.; Helmick, C. G.; Jordan, J. M.; Kington, R. S.; Lane, N. E.; Nevitt, M. C.; Zhang, Y.; Sowers, M.; McAlindon, T.; Spector, T. D.; Poole, A. R.; Yanovski, S. Z.; Ateshian, G.; Sharma, L.; Buckwalter, J. A.; Brandt, K. D.; Fries, J. F. *Ann. Intern. Med.* **2000**, *133*, 635.

8. Felson, D. T.; Lawrence, R. C.; Hochberg, M. C.; McAlindon, T.; Dieppe, P. A.; Minor, M. A.; Blair, S. N.; Berman, B. M.; Fries, J. F.; Weinberger, M.; Lorig, K. R.; Jacobs, J. J.; Goldberg, V. *Ann. Intern. Med.* **2000**, *133*, 726.
9. Creamer, P. *Curr. Opin. Rheumatol.* **2000**, *12*, 450.
10. Solignac, M. *Presse Med.* **2004**, *33*. 9 pt 2, S10.
11. Jieyun, J.; Qiang, X. J. *Ethnopharmacology* **2003**, *85*, 53.
12. Lichtenstein, D. R.; Syngle, S.; Wolfe, M. *Arthritis Rheum.* **1995**, *38*, 5.
13. Lester, R. S.; Konwles, S. R.; Shear, N. H. *Dermatol. Clin.* **1998**, *16*, 277.
14. Patino, F. G.; Olivieri, J.; Allison, J. J. *J. Rheumatol.* **2003**, *30*, 2680.
15. http://www.rheumatology.org/publications/guidelines/oa-mgmt/oa-mgmt.asp?aud=mem.
16. Jordan, K. M.; Arden, N. K.; Doherty, M.; Bannwarth, B. *Ann. Rheum. Discov.* **2003**, *62*, 1145.
17. Schnitzer, T. J. *Am. Coll. Rheumatol.* **2002**, *24*, 24.
18. http://www.bly.org/journal/2002_4404_oct/gps_670.htm.
19. Altman, R. D.; Hochberg, M. C.; Moskowitz, R. W.; Schnitzer, T. J. *Arthritis Rheum* **1905**, *2000*, 43.
20. http://www.realage.com/joint-pain/disease-modifying-drugs-for-osteoarthritis-dmoads.
21. Spencer, C. M.; Wilde, M. I. *Drugs* **1997**, *53*, 98.
22. Nicolas, P.; Tod, M.; Padoin, C.; Petitjean, O. *Clin. Pharmacokinet.* **1998**, *35*, 347.
23. http://www.cipladoc.com/therapeutic/admin.php?mode=prod&action=disp&id=551.
24. Pelletier, J. P.; Yaron, M.; Haraoui, B.; Cohen, P.; Nahir, M. A.; Choquette, D.; Wigler, I.; Rosner, I. A.; Beaulieu, A. D. *Arthritis Rheum.* **2000**, *43*(10), 2339.
25. Tamura, T.; Kosaka, N.; Ishiwa, J.; Sato, T.; Nagase, H.; Ito, A. *Osteoarthritis Cartilage* **2001**, *9*, 257.
26. Nicholas, P.; Tod, M.; Padoin, C.; Petitijean, O. *Clin. Pharmacokinet.* **1998**, *35*(5), 347.
27. Spencer, C. M.; Wilde, M. I. *Drugs* **1997**, *53*, 98. Discussion 107.
28. Tamura, T.; Ohmori, K. *Jpn. J. Pharmacol.* **2001**, *85*, 101.
29. Krohn, K.; Rao, M. S.; Raman, N. V.; Khalilullah, M. *Phytochem. Anal.* **2001**, *12*, 374.
30. Majeed, M.; Badmaev, V.; Gopinathan, S.; Rajendran, R.; Norton, T.; Braly, J. *Anti-inflammatory Phytonutrient*; Nutriscience Publishers Inc.: Piscataway, NJ, 1996.
31. Henderson, W. R. *Ann. Intern. Med.* **1994**, *121*, 684.
32. Selected medicinal plants of India- A Monograph of identity, safety and clinical usage. Compiled by Swami Prakashanand Ayurved Reserch Centre (SPARC) for Chemixil. Tata Press: India, 1992, 65–66.
33. Singha, S.; Khajuriaa, A.; Tanejab, S. C.; Khajuriab, R. K.; Singha, J.; Johria, R. K.; Qazi, G. N. *Phytomedicine* **2008**, *15*, 408.
34. Reddy, G. K.; Chandrakashan, G.; Dhar, S. C. *Biochem. Pharmacol.* **1989**, *38*, 3527.
35. Dhaneshwar, S. S.; Patil, D.; Mengi, S.; Mulay, G.; Lahane, J. *J. Drug Del. Sci. Tech.* **2009**, *19*, 25.
36. Dhaneshwar, S. S.; Patil, D.; Mengi, S.; Mulay, G.; Lahane, J. *J. Drug Del. Sci. Tech.* **2009**, *19*, 425.
37. Dhaneshwar, S.; Patil, D. *Med Chem.* **2012**, *8*, doi:1573-4064/12 $58.00+.00, (In press).
38. Duan, Y.; Yu, J.; Liu, H.; Ji, M. *Lett. Drug Des. Discov.* **2009**, *6*, 393.
39. Duan, Y.; Yu, J.; Liu, S.; Ji, M. *Med. Chem.* **2007**, *5*, 577.
40. Singh, S.; Khajuria, A.; Taneja, S. C.; Khajuria, R. K.; Singh, J.; Qazi, G. N. *Bioorg. Med. Chem. Lett.* **2007**, *17*(13), 3706.
41. Synthesis of rhein from diacerein: Diacerein (0.012 M, 4.5 g) was dispersed in 80 ml of 10% aqueous sodium hydroxide solution. The solution was heated on a boiling water bath for 30 min and then poured into 120 mL of 10% HCl. Rhein was obtained in the form of yellow flakes which were recrystallized twice with pyridine. Rhein. mp 318–319 °C, R_f 0.58 (chloroform: methanol; 3:0.5 v/v),% yield 60, Log*P* 0.2402. IR (ν, cm^{-1}, KBr) 3566 (Phenolic OH stretch.), 3100, 2850 (Broad OH stretch. of COOH), 1682 (C=O stretch. of –COOH), 1193 (C–O stretch. of phenol), ^{1}H NMR (δ, ppm, DMSO-d_6) 8.57 carboxylic OH [s; 1H], 8.26 benzene CH_2 [s; 2H], 8.25 benzene CH_2 [s; 2H], 7.32–7.83 benzene CH_2 [t; 3H], 3.79 phenolic OH [d; 2H].
42. Holmberg, K.; Hansen, B. *Acta Chem. Scand.* **1979**, *B33*, 410.
43. Fieser, L. F.; Fieser, M. In *Reagents for organic synthesis*; Wiley: New York, 1967; vol. 1,
44. Synthesis of prodrug DSRB (3): Rhein (**1**) (0.002 M, 0.57 g) and boswellic acid (**2**) (0.008 M, 3.65 g) were dissolved in 30 mL of DMF. *p*-Toluene sulfonic acid (PTSA) 0.004 g was added to above mixture and stirred for 15 min. DCC (0.004 M, 1.15 g) was added to above solution and stirred for 30 h at room temperature. Then 20 mL glacial acetic acid was added and reaction mixture was kept at 0–5 °C overnight. The mixture was filtered and the crystals were washed with pyridine (2–4 mL). Mixture of ethyl acetate: ether: ice (30 mL: 30 mL: 30 g) was added to the filtrate followed by slow addition of 5 M HCl with stirring to adjust the pH to 2.5. Then the organic phase was washed first with water, then 0.5 M aqueous potassium carbonate solution and again water. The solvent was evaporated under vacuum and the product was recrystallized with ethyl acetate. DSRB. mp 380 °C (d, uncorrected), R_f 0.76, chloroform: methanol: acetic acid (4:1:3 drops v/v/v),% yield 46.15, aqueous solubility 92 μg/ml, Log*P* 2.568, IR (ν, cm^{-1}, KBr) 3538 (OH stretch.), 3300 (broad OH-stretch. of –COOH), 1721 (C=O stretch. ester), 1628, 1451(aromatic C=C stretch.), 1267 (C–O stretch. ester), 1249 (C–O stretch. COOH), 1081 (C–O stretch alcoholic OH), ^{1}H NMR (δ, ppm, DMSO-d_6) rhein backbone: 11.8 carboxylic OH [s; 1H], 8.59 benzene CH_2 [s; 2H], 8.26 benzene CH_2 [s; 2H], 7.37–7.88 benzene CH_2 [t; 3H]; boswellic acid backbone: 5.22 cyclohexene 2 × CH [t; 2H], 3.77–3.79 cyclohexanol 2 × CH [t; 2H], 2.01–2.05 cyclohexanol 2 × OH [d; 2H], 1.44–2.11 cyclohexane 2 × CH [m; 6H], 1.24–1.93 cyclohexane 2 × CH_2 [m; 40H], cyclohexane 2 × CH_3 [m; 46H]. ^{13}C NMR (δ, ppm, DMSO-d_6) rhein backbone: 124.9, 125.1, 125.2, 127.6, 133.8, 134.2, 135.2, 149.5, 149.7, 164.9, 180.0; boswellic acid backbone: 20.6, 38.9, 39.1, 39.3, 39.5, 39.7, 39.9, 40.1, 78.3, 78.6, 78.8, 124.9, 149.5, 187.0, 187.6. MS, m/z 1161.5901 ($C_{75}H_{100}O_{10}$, calc. 1161.5905). Elemental Anal. calcd: C, 77.55; H, 8.68. Found: C, 77.72; H, 8.65.