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Original article

Bone-targeting glycol and NSAIDS ester prodrugs of rhein: Synthesis, hydroxyapatite affinity, stability, anti-inflammatory, ulcerogenicity index and pharmacokinetics studies

Jin Cai, Yanbing Duan, Jia Yu, Junqing Chen, Meng Chao, Min Ji*

School of Chemistry and Chemical Engineering, Institute of Pharmaceutical Engineering, Southeast University, 87 Dingjiaqiao, Nanjing, Jiangsu 210096, PR China

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ABSTRACT

Although rhein and NSAIDs are potent anti-inflammatory drugs, their use has been limited by the high incidence of gastrointestinal erosions and the necessity to deliver the drug to specific sites of target organ. Using the prodrug approach, a series of rhein–NSAIDs prodrugs containing anthraquinone bone-targeting moiety were synthesized by linking rhein with NSAIDs through glycol ester. The target compounds demonstrated significant capability of binding to HAP and were hydrolytically activated in physiological conditions. Hybrid rhein–NSAIDs prodrugs exhibited significant anti-inflammatory activity, moreover, the tested compounds were also found to possess less degree of ulcerogenic potential. Our pharmacokinetic studies of **7e** demonstrated this prodrug is a potential candidate for a slower and sustained release form of rhein.

1. Introduction

Osteoarthritis (OA) is a common intractable chronic degenerative disease characterized by the destruction of articular cartilage, chronic joint pain and inflammation [1,2]. Currently, this disease affects more than 20 million patients in the United States and this number is expected to double by 2020, making OA one of the most common health problems in this country [3]. However, in contrast to other tissues, the blood flow in bones is very low, thus drug hardly reaches with normal administration methods, osseous tissues are considered to be limited as therapeutic target sites due to their biological properties [4].

As it is known that tetracycline possesses bone affinity, which can be used as carrier of bone-targeting compounds [4]. A lot of bioactive compounds have been discovered from natural sources. Lead compounds can be further modified to enhance the biological profiles and developed as candidate drugs [5]. Rhein (**1**), a main constituent of Rhubarb isolated from *Rheum palmatum L.*, is a well-characterized anti-inflammatory agent with recognized effectiveness in a range of inflammatory diseases such as osteoarthritis and diabetic nephropathy [6]. In addition, the bone affinity of rhein has been confirmed by hydroxyapatite (HAP) affinity experiment *in vitro* due to the similar structure with tetracycline in our previous study. The acting mechanism at molecular level is still not clearly understood, however it is known that two molecules of rhein are able to complex bivalent ions such as calcium (Fig. 1) [7]. Although the pharmacological studies demonstrate rhein has anti-inflammatory activity and bone affinity, unfavorable physical characteristic and side effects such as gastrointestinal irritation have limited its extensive clinical use. Therefore diacerein (**3**), the prodrug of rhein, was developed as a new drug for the treatment of osteoarthritis. Diacerein can be completely metabolized by animals and humans to rhein *in vivo* and reduce the production of superoxide anions, chemotaxis, migration and phagocytic activity of neutrophils and macrophages [8–10]. Furthermore, diacerein inhibits the synthesis of proinflammatory cytokines, mainly of IL-1β [11–14].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used in the treatment of pain, fever and inflammatory diseases such as arthritis [15]. NSAIDs exert their therapeutic effects by inhibiting cyclooxygenase (COX) [16], the rate limiting enzyme of the prostanoid biosynthetic pathway catalyzes the conversion of arachidonic acid to important inflammatory mediators such as prostaglandins (PGs), prostacyclins and thromboxanes [17]. However, most currently used NSAIDs have limitations for therapeutic use since they cause gastrointestinal side effects, which are inseparable from

* Corresponding author.
E-mail address: jimin@seu.edu.cn (M. Ji).

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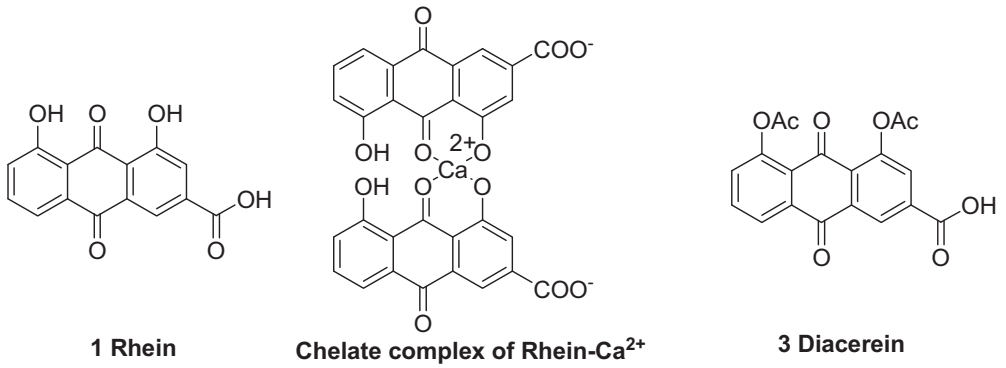


Fig. 1. Chemical structure of rhein, chelate complex and diacerein.

their pharmacological activities [18]. Previous reports on NSAIDs have demonstrated that gastric side effects are partly associated with the presence of free carboxylic acid group [19,20]. Rhein and diacerein have similar side effects because of the acidic moiety in their structure. To avert damage to the gastric mucosa while preserving satisfactory pharmacological activity, some scientists have created esteric and amidic derivatives bound to polymeric carriers or amino acids to mask the carboxylic acid group [20,21].

Consequently, on the basis of our past experience in the synthesis of prodrugs [22], in continuation of our work to get more potent and safe prodrugs, rhein was chosen in this paper as bone-targeting carrier and potentially anti-inflammatory lead compound. On the one hand, we made some simple modification with rhein or diacerein, the carboxyl group of which was esterified with ethylene glycol (diethylene glycol or triethylene glycol). On the other hand, rhein was chemically linked with some NSAIDs such as Aspirin, Diclofenac, Naproxen, Indomethacin and Ibuprofen (Fig. 2) through glycol ester as bone-targeting anti-inflammatory prodrugs, rhein and NSAIDs were expected to be released *in vivo* while exerting synergistic effects on inflammation (Fig. 3). Bone binding capabilities, hydrolytic activation of these prodrugs in simulated physiological conditions *in vitro* and mice auricle tumefaction experiment, ulcerogenic activity, pharmacokinetics *in vivo* were studied.

2. Chemistry

Compounds **2a**–**2c** were prepared as illustrated in Scheme 1. Using H_2SO_4 as catalyst, carboxyl group of rhein **1** was esterified with excessed ethylene glycol (diethylene glycol or triethylene glycol) to produce **2a**–**2c** in satisfactory yields (86–92%). In contrast, esterification using DCC or EDCI as condensing catalyst gave incomplete conversions, even after a long reaction time.

Our approach to the synthesis of **7a**–**7f** is depicted in Scheme 2. Rhein **1** as starting material was acetylated in presence of Ac_2O and H_2SO_4 to afford diacerein **3**, which was chloridated with oxalyl chloride and condensed with glycol to produce **5a**–**5c** in satisfactory yields. Chlorination of various NSAIDS (Ibuprofen, Aspirin, Naproxen, Indomethacin) with oxalyl chloride and esterification with **5a**–**5c** gave **6a**–**6g** (excluding **6f**) in high yields. There had an exception for diclofenac, the amino group of which was active and would be involved in the chlorination reaction. So condensation of diclofenac with **5a** using DCC and DMAP as catalyst straightforwardly got the ester **6f** in lower yield. Compounds **7a**–**7f** were achieved by hydrolysis of **6a**–**6g** using triethylamine as the most suitable catalyst with high reaction selectivity in satisfactory yields. All the target compounds were new chemical entities and showed in Tables 1 and 2.

3. Results and discussion

3.1. Determination of the levels of bone binding in vitro

Since the prodrugs were designed as bone-targeting drugs, we sought for a simple model system to simulate the bone tissue. Hydroxyapatite (HAP), a commercially available calcium mineral, is the major component of bone. A suspension of HAP in aqueous media was previously used as a bone model [23–25], so we adopted it as the simple model system in this study for evaluating the bone-targeting ability of the synthesized prodrugs. The affinity

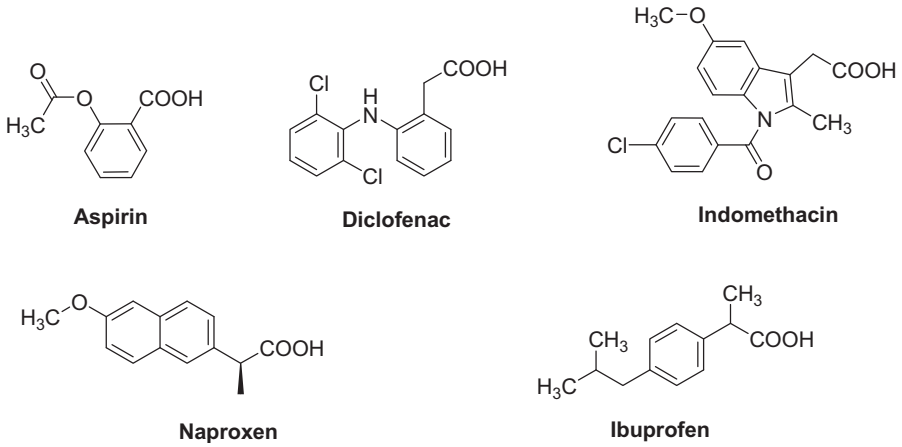


Fig. 2. Chemical structure of several NSAIDs.

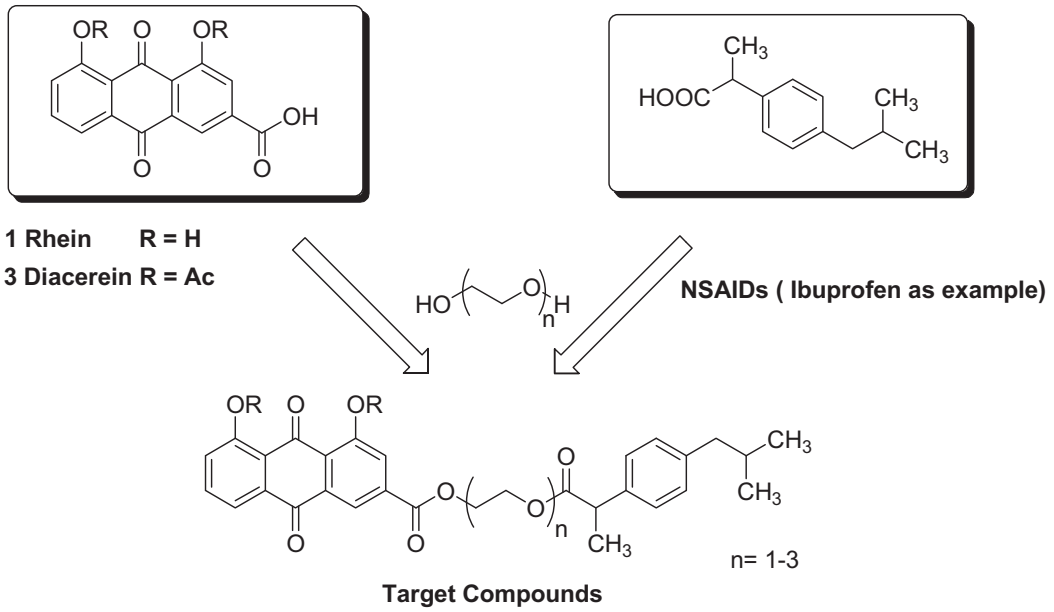


Fig. 3. A design for rhein–NSAIDs ester prodrugs.

of the prodrugs for HAP can be calculated by measuring the amount of unbound prodrugs in supernatant by HPLC relative to the total amount of input prodrugs.

As shown in Figs. 4 and 5, all prodrugs demonstrated significant binding capability to HAP, while the parent drug ibuprofen at negligibly bound. However, the prodrugs' binding capability to HAP was lower than rhein and diacerein. The binding efficiency was directly related with the quantity of HAP, the adsorption percents of compounds were increased sharply with the increasing amount of HAP.

Hybrid rhein–NSAIDs prodrugs **6a**–**6g** and **7a**–**7f** showed potent absorption percentage higher than the contrast ibuprofen, but a little lower than rhein–glycol esters **2a**–**2c** and **5a**–**5c**. By comparison the binding capability of **6a**–**6g** with **7a**–**7f**, we found that compounds **6a**–**6g** with acetyl substitution in hydroxyl group of rhein were lower than **7a**–**7f** (Fig. 5). We speculated the reason was substituents of the new compounds decomposed the chelation between HAP and rhein, which decreased the binding capability to HAP [26]. Overall, most new synthesized compounds showed obvious binding capability to HAP and could successfully carry the connected NSAIDs to absorption in HAP. From this we may certainly infer that the prodrugs had potent bone-targeting ability in accord with our previous design idea.

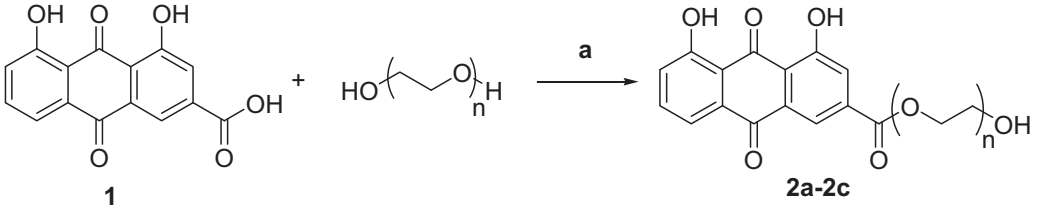
3.2. Hydrolysis of prodrugs at simulation physiological conditions

It was expected that the synthesized rhein ester prodrugs would be hydrolyzed *in vivo* at the targeting site to release the corresponding free rhein and NSAIDs which would then perform their anti-inflammatory activity. Therefore, it was desirable to determine the relative susceptibility of these compounds at 37 °C in acidic condition (simulating gastric juice, pH 1.2) and in neutral condition (simulating body fluid, pH 7.4).

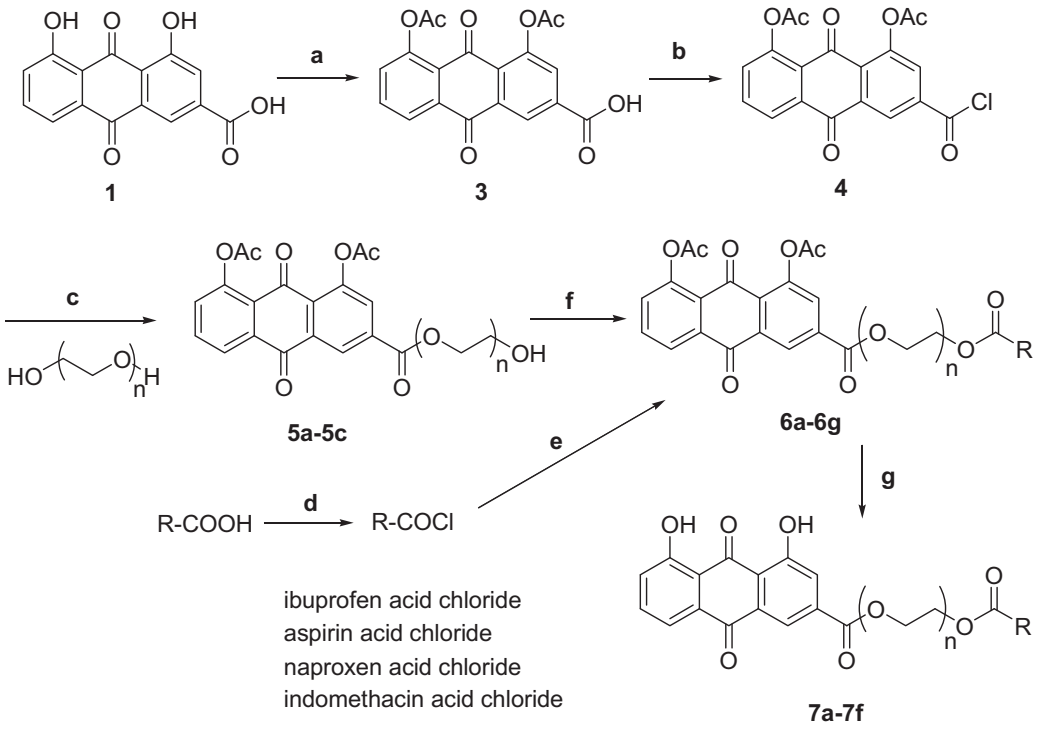
As shown in Figs. 6–8, all prodrugs were spontaneously hydrolyzed and released the active drugs at simulated physiological conditions by HPLC analysis of the reactions. The prodrugs, however, were more stable in acidic condition than in neutral condition, especially for compounds **5a**–**5c** and **6a**–**6g**, which had an acetyl in rhein skeletons. Rhein and NSAIDs were released slowly within 24 h.

3.3. Anti-inflammatory activity of the prodrugs

Table 3 revealed the *in vivo* acute anti-inflammatory activity of the target compounds at a dose of 0.2 mmol/kg in xylene-induced mice auricle tumefaction model. Compared with saline group, entire ester prodrugs of rhein exhibited moderate to good anti-inflammatory activity with the inhibition percentage of auricle tumefaction ranged from 13.92% to 43.98%. Rhein–glycol prodrugs **2a**–**2c** and **5a**–**5c** (13.92–19.51%) showed lower inhibition percentage compared with the contrast drug diacerein (29.22%), one plausible explanation for this could be due to the likely high hydrophilicity (log P = 058–0.94 range; see data in Table 3) of **2a**–**5c**, which maybe lead to the low bioavailability *in vivo* and lower the anti-inflammatory activity. Relatively, nearly all hybrid rhein–NSAIDs ester prodrugs **6a**–**6g** and **7a**–**7f** demonstrated good anti-inflammatory activity except for **6d** (19.92%) and **7d** (19.64%), it was worth pointing out that significant anti-inflammatory activity



Scheme 1. Synthesis of the compounds **2a**–**2c**. Reagents and conditions: (a) H_2SO_4, 1 h, 85 °C.



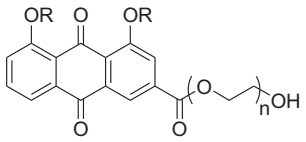
Scheme 2. Synthesis of the target compounds. Reagents and conditions: (a) Ac_2O, H_2SO_4, 3 h, reflux; (b) oxalyl chloride, DMF, CH_2Cl_2, 1 h, 30 °C; (c) Et_3N, 0.5 h, rt; (d) oxalyl chloride, DMF, CH_2Cl_2, 1 h, rt; (e) Et_3N, CH_2Cl_2, 0.5 h, rt; (f) diclofenac, DCC, DMAP, CH_2Cl_2, 1.5 h, rt; (g) Et_3N, acetone, H_2O, 6 h, 50 °C.

were achieved for **6f** (43.98%) and **7f** (43.89%) with potent inhibition percentage higher than the contrast drug diclofenac (41.92%) and diacerein (29.22%). So, the results of bone binding affinity *in vitro* and anti-inflammatory activity *in vivo* taken together, compounds **7a**–**7c** and **7e**–**7f** were selected for further evaluation of their acute ulcerogenic activity.

3.4. Ulcerogenic activity of the prodrugs

The most common side effects associated with the long-term administration of NSAIDs are gastrointestinal erosions, ulcer formation, and sometimes severe bleeding [16]. It was therefore essential to evaluate the potential *in vivo* ulcerogenicity of prodrugs **7a**–**7c** and **7e**–**7f** in comparison to the corresponding contrast drug ibuprofen. The severity of gastric damage, assessed using an ulcerogenicity assay, was expressed as an ulcer index (UI). Results were given in Fig. 9 that indicated these four compounds **7a**–**7c** and **7e** (ulcer index ranges from 5.8 to 6.0) cause less gastric ulceration and disruption of gastric epithelial cells as compared to ibuprofen (ulcer index 8.3). Hence gastric tolerance to these compounds was better than that of the contrast drug. This UI data suggested a much safer pharmacological profile for hybrid rhein–NSAIDs ester prodrugs relative to the parent drugs.

Table 1
Structure of compounds **2a**–**2c** and **5a**–**5c**.



Cpd.	R	*n*	Yield (%)[a]	Mp (°C)
2a	H	1	92	155–157
2b	H	2	88	139–141
2c	H	3	86	100–101
5a	Ac	1	85	200–203
5b	Ac	2	81	153–155
5c	Ac	3	83	96–97

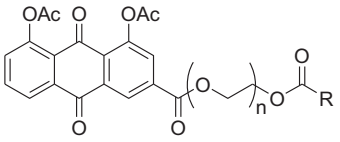
[a] Yield for last step.

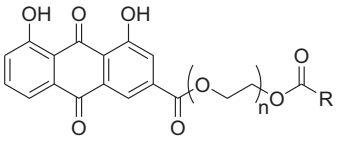
3.5. Pharmacokinetic studies

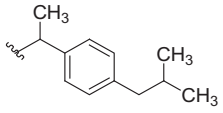
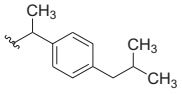
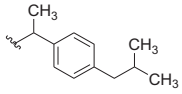
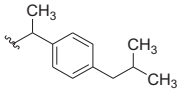
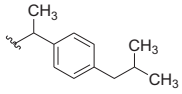
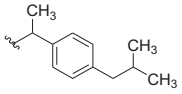
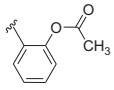
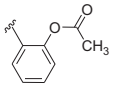
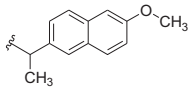
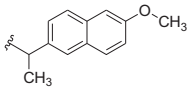
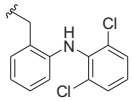
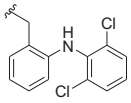
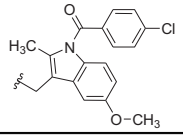
Therefore, all facts (include HAP affinity, hydrolysis stability, anti-inflammatory activity and ulcerogenic activity) taken together, the Rhein–Naproxen prodrug **7e** was selected for further pharmacokinetic studies to see whether this prodrug is a potential candidate for a slower and sustained release form of rhein. The pharmacokinetic profile of **7e** was compared to that of rhein according to plasma concentration after oral administration of **7e** (10 mg/kg) and rhein (5.26 mg/kg).

Rhein plasma concentration profile *versus* time is shown in Fig. 10, and pharmacokinetic parameters are reported in Table 4. A different pharmacokinetic profile was observed for the rhein, resulting from the prodrug **7e** hydrolysis and the rhein itself. A single dose of rhein, orally administrated, was rapidly absorbed and eliminated within 9 h. Instead, although plasma concentrations of the rhein released from **7e** were lower than those of rhein during the first 3.5 h, they nonetheless remained higher than that of the rhein itself throughout the remaining time of the experiments. Therefore, the prodrug **7e** demonstrated the typical profile of a controlled release drug system. The pharmacokinetic parameters related to these curves were calculated and are listed in Table 4. The areas under the curve from time 0 to 9 h (AUC_{0-9}), obtained from both curves, were very similar, thus indicating that the prodrug did not change the bioavailability of rhein. On the other hand, the pharmacokinetic parameters demonstrated that the pharmacokinetic behavior of the drug released from the prodrug and the rhein itself was different, thereby suggesting that the prodrug was

Table 2
Structure of compounds **6a**–**6g** and **7a**–**7f**.





Cpd.	*n*	R	Mp (°C)	Cpd.	*n*	R	Mp (°C)
6a	1		129–130	**7a**	1		85–87
6b	2		73–75	**7b**	2		89–91
6c	3		46–49	**7c**	3		62–65
6d	1		198–200	**7d**	1		132–135
6e	1		134–137	**7e**	1		122–124
6f	1		149–151	**7f**	1		164–166
6g	1		108–110				

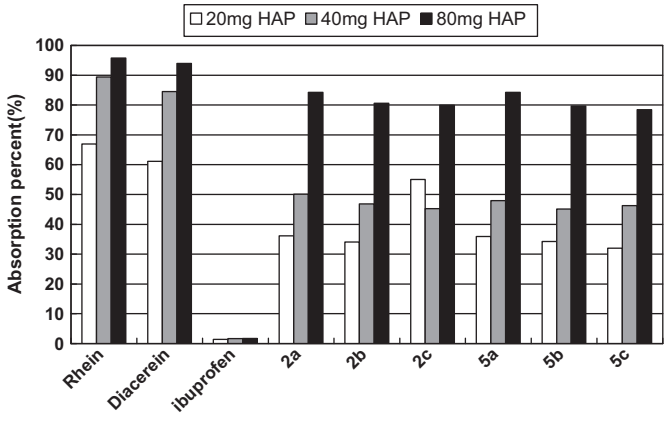


Fig. 4. *In vitro* HAP binding affinity ($n = 3$) of rhein, diacerein, ibuprofen and **2a**–**5c**.

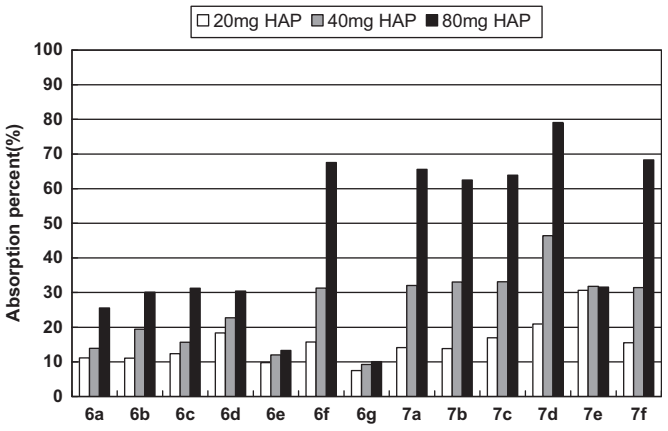


Fig. 5. *In vitro* HAP binding affinity ($n = 3$) of **6a**–**6g** and **7a**–**7f**.

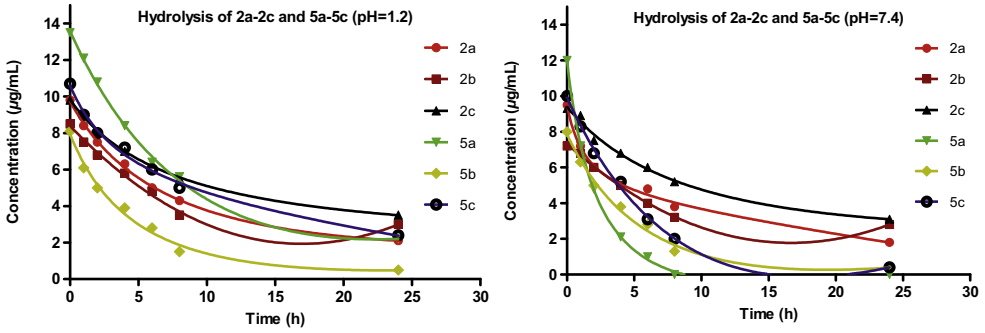


Fig. 6. Hydrolysis of compounds **2a**–**2c** and **5a**–**5c** in PBS (pH 1.2 and pH 7.4), 37 °C.

responsible for a sustained release of the drug and its longer half-life. Therefore, the introduction of the glycol and $NSAID_S$ promoiety on rhein elicited a slow and sustained release of rhein, thus prolonging its activity.

4. Conclusion

In the present paper, we reported the design, synthesis and pharmacological evaluation of a series of nineteen anti-inflammatory rhein ester prodrugs containing anthraquinone bone-targeting moiety. Rhein and NSAIDs were chemically linked through glycol ester. All prodrugs showed significant capability of binding to HAP and were hydrolytically activated in physiological conditions. The preliminary *in vivo* biological activities of these compounds evidenced our rational design of improved anti-inflammatory prodrugs with reduced gastric toxicity (ulcerogenicity). Among the prepared compounds, hybrid rhein–NSAIDs ester prodrugs (especially **6f** and **7f**) exhibited significant anti-inflammatory activity in model of acute inflammation such as xylene-induced mice auricle tumefaction. In addition, the tested compounds were also found to possess less degree of ulcerogenic potential as compared to ibuprofen. Our pharmacokinetic studies of **7e** have demonstrated that this prodrug is a potential candidate for a slower and sustained release form of rhein. Thus rhein–NSAIDs ester prodrugs may provide a promising alternative to the use of NSAIDs as an anti-osteoarthritis agent with more AI activity and less side effect.

5. Experimental protocols

5.1. Synthesis

All reagents were purchased from commercial sources and used without further purification. Melting points were measured in open capillaries and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker-ACF 300/500 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS), used as an internal standard. Mass spectra (MS) were obtained from Agilent 1100LC/MS Spectrometry Services. IR spectra were run on FI-IR Spectrometer (Perkin–Elmer). Elementary analyses were performed on Elementar Vario EL III instrument. All compounds were routinely checked by TLC with silica gel GF-254 glass plates and viewed under UV light at 254 nm.

5.1.1. 2-hydroxyethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**2a**)

Rhein **1** (0.5 g, 1.76 mmol) was dissolved in glycol (40 mL) and sulfuric acid (2 mL). The solution was heated to 85 °C for 1 h. After cooling to room temperature, the solution was poured into the ice water (150 mL). The water layer was extracted by dichloromethane, the organic layer was merged and evaporated under reduced pressure to get the crude product. Recrystallization from dichloromethane and methanol gave **2a** as orange-yellow solid (0.53 g, 92%). Mp: 155–157 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.01–4.03 (m, 2H, $CH_2\underline{CH_2}OH$), 4.53–4.54 (m, 2H, $\underline{CH_2}CH_2OH$), 7.34 (dd, 1H, $J_1 = 1.1$ Hz, $J_2 = 8.4$ Hz, Ar–H), 7.72–7.75 (m, 1H, Ar–H), 7.87 (dd, 1H, $J_1 = 1.1$ Hz, $J_2 = 7.5$ Hz, Ar–H), 7.96 (s, 1H, Ar–H), 8.42 (s, 1H, Ar–H), 11.95 (s, 1H, OH), 12.01 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 61.1, 67.6, 92.4, 120.2, 120.4, 125.0, 125.4, 137.8, 162.4, 162.9, 164.6, 180.9, 192.8; IR (cm^{-1}): 3490, 2924, 1703, 1674, 1627, 1475, 1392, 1275, 1248, 1209, 1156, 1062, 1021, 908, 840, 750, 733, 595; MS (ESI) m/z: 327.21 $[M - H]^+$; Anal. Calcd for $C_{17}H_{12}O_7$: C, 62.20; H, 3.68. Found: C, 61.97; H, 3.75%.

5.1.2. 2-(2-hydroxyethoxy)ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**2b**)

Preparation of **2b** is followed the procedure for **2a** described above. Yield: 88%. Mp: 139–141 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ:

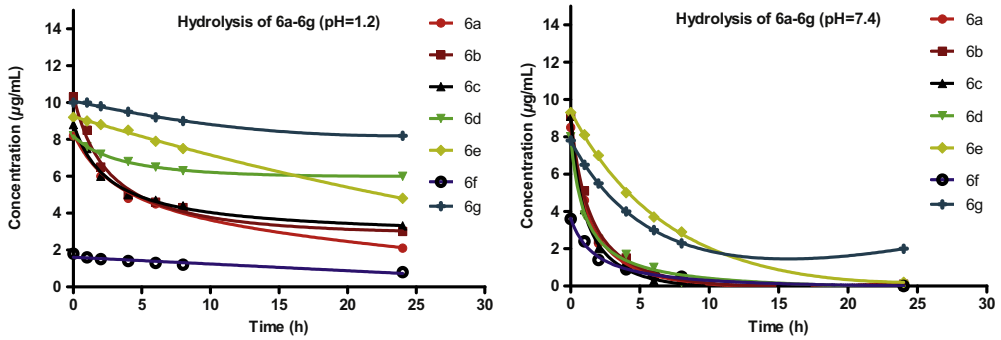


Fig. 7. Hydrolysis of compounds **6a**–**6g** in PBS (pH 1.2 and pH 7.4), 37 °C.

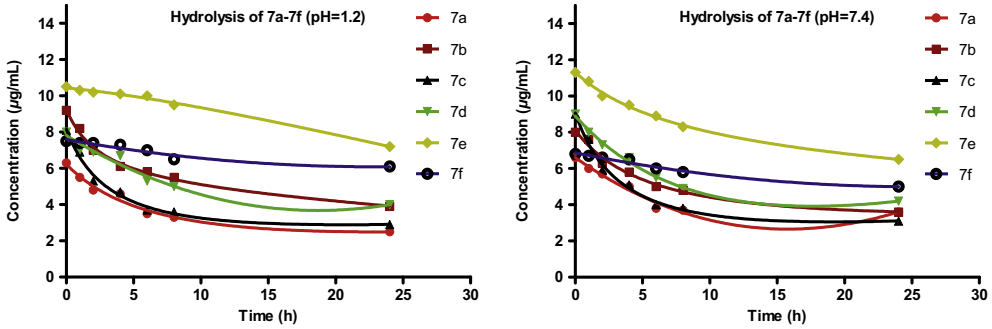


Fig. 8. Hydrolysis of compounds **7a**–**7f** in PBS (pH 1.2 and pH 7.4), 37 °C.

1.84 (s, 1H, $CH_2CH_2O\underline{H}$), 3.68–3.72 (m, 2H, $C\underline{H}_2CH_2OH$), 3.80–3.83 (m, 2H, $CH_2C\underline{H}_2OH$), 3.90–3.92 (m, 2H, $COOCH_2C\underline{H}_2O$), 4.57–4.60 (m, 2H, $COOC\underline{H}_2CH_2O$), 7.36 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, Ar–H), 7.75–7.78 (m, 1H, Ar–H), 7.89 (dd, 1H, J_1 = 1.1 Hz, J_2 = 7.5 Hz, Ar–H), 7.97 (s, 1H, Ar–H), 8.45 (s, 1H, Ar–H), 11.97 (s, 1H, OH), 12.04 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 61.8, 65.0, 68.9, 72.5, 76.6, 77.0, 77.2, 77.4, 118.3, 120.3, 120.4, 124.9, 125.4, 133.9, 137.8, 162.4, 162.8, 164.4, 192.8; IR (cm^{-1}): 3414, 3138, 1720, 1665, 1625, 1450, 1399, 1271, 1201, 1127, 1081, 1066, 901, 746, 600; MS (ESI) *m*/*z*: 371.3 $[M - H]^+$; Anal. Calcd for $C_{19}H_{16}O_8$: C, 61.29; H, 4.33. Found: C, 61.27; H, 4.29%.

5.1.3. 2-(2-(2-hydroxyethoxy)ethoxy)ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**2c**)

Preparation of **2c** is followed the procedure for **2a** described above. Yield: 86%. Mp: 100–101 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.99 (s, 1H, $CH_2CH_2O\underline{H}$), 3.62–3.64 (m, 2H, $CH_2C\underline{H}_2OH$), 3.65–3.87 (m, 6H, $C\underline{H}_2C\underline{H}_2OC\underline{H}_2CH_2OH$), 3.88–3.89 (m, 2H, $COOCH_2C\underline{H}_2O$), 4.54–4.56 (m, 2H, $COOC\underline{H}_2CH_2O$), 7.34 (dd, 1H, J_1 = 1.1 Hz, J_2 = 8.5 Hz, Ar–H), 7.71–7.75 (m, 1H, Ar–H), 7.88 (dd, 1H, J_1 = 1.1 Hz, J_2 = 7.5 Hz, Ar–H), 7.97 (s, 1H, Ar–H), 8.44 (s, 1H, Ar–H), 11.96 (s, 1H, OH), 12.02 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 65.0, 69.0, 70.5, 70.9, 72.6, 76.6, 77.0, 77.2, 77.5, 115.9, 120.3, 120.4, 124.9, 125.5, 133.6, 134.0, 137.8, 137.9, 162.5, 162.9, 164.4, 181.0, 192.9; IR (cm^{-1}): 3470, 3413, 3137, 2956, 2920, 2876, 1725, 1624, 1467, 1394, 1266, 1199, 1121, 1080, 1017, 928, 916, 869, 744, 706, 595; MS (ESI) *m*/*z*: 415.1 $[M - H]^+$; Anal. Calcd for $C_{21}H_{20}O_9$: C, 60.58; H, 4.84. Found: C, 60.69; H, 4.63%.

Table 3
In vivo inhibitory activity of target compounds in xylene-induced mice auricle tumefaction assay.

Cpd.	n[a]	Degree of tumefaction (mg)	Inhibition percentage (%)	log P[b]
Blank	10	10.64 ± 1.90	–	–
Diacerein	10	7.22 ± 1.59	29.22	1.15
Ibuprofen	10	7.23 ± 1.28	32.05	3.75
Diclofenac	10	6.18 ± 1.35	41.92	4.12
2a	10	8.78 ± 1.27	13.92	0.94
2b	10	8.45 ± 1.63	17.16	0.79
2c	10	8.43 ± 1.75	17.35	0.63
5a	10	8.57 ± 1.48	15.98	0.89
5b	10	8.67 ± 1.53	15.00	0.74
5c	10	8.21 ± 1.76	19.51	0.58
6a	10	6.89 ± 1.61	35.24	5.18
6b	10	7.13 ± 1.77	32.99	5.03
6c	10	7.52 ± 1.73	29.32	4.87
6d	10	8.52 ± 1.27	19.92	2.61
6e	10	8.04 ± 1.03	24.44	4.4
6f	10	5.96 ± 1.18	43.98	5.55
6g	10	8.15 ± 1.45	23.40	5.12
7a	10	6.26 ± 0.83	41.17	5.23
7b	10	7.16 ± 1.62	32.71	5.08
7c	10	7.03 ± 1.77	33.93	4.92
7d	10	8.55 ± 1.41	19.64	2.66
7e	10	8.10 ± 0.64	23.87	4.45
7f	10	5.97 ± 1.00	43.89	5.6

[a] Values are the means of the indicated number of experiments (n).
[b] The log P-value was calculated using the ChemDraw Ultra program, version 8.0, Cambridge Soft company.

5.1.4. 2-hydroxyethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**5a**)

To anhydrous dichloromethane (50 mL) were added oxalyl chloride (0.83 mL, 9.66 mmol) and DMF (0.5 mL). The solution was stirred for 0.5 h at room temperature and was added diacerein **3** (2 g, 5.43 mmol). The mixture was heated to 30 °C for 1 h. All solvents were removed under reduced pressure to get acyl chloride **4**, which was dissolved in THF (150 mL). This solution was added to glycol (50 mL) and triethylamine (1.3 mL, 9.25 mmol) by dripping at room temperature. The mixture was stirred for 0.5 h and water (3 mL) was added to terminate the reaction. The solvent was evaporated under reduced pressure and the residue was diluted with water and dichloromethane. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was recrystallized by petroleum ether and ethyl acetate to afford **5a** as a yellow solid (1.91 g, 85%). Mp: 200–203 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.45 (s, 6H, $OCOCH_3$), 4.01 (t, 2H, J = 4.6, $C\underline{H}_2CH_2OH$), 4.52–4.54 (m, 2H, $CH_2C\underline{H}_2OH$), 7.44 (dd, 1H, J_1 = 1.0 Hz, J_2 = 8.0 Hz, Ar–H), 7.80 (t, 2H, J = 7.9, Ar–H), 8.05 (s, 1H, Ar–H), 8.25 (dd, 1H, J_1 = 1.0 Hz,

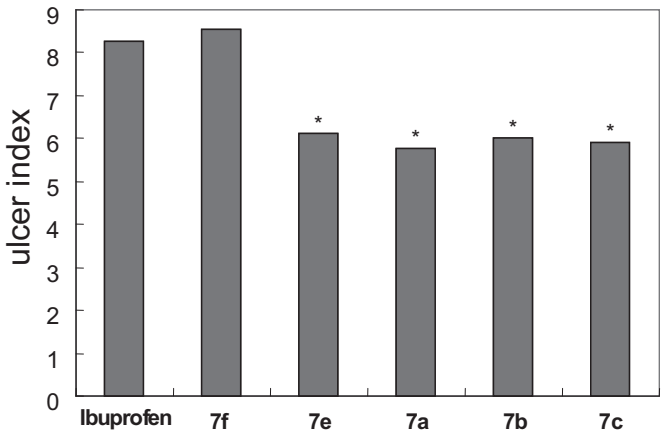


Fig. 9. Ulcer index of ibuprofen and rhein–NSAIDs prodrugs. Ulcer index: Calculated by adding the total length (L, in mm) of individual gastric lesions in each stomach and averaging over the number of animals in each group (n = 10).

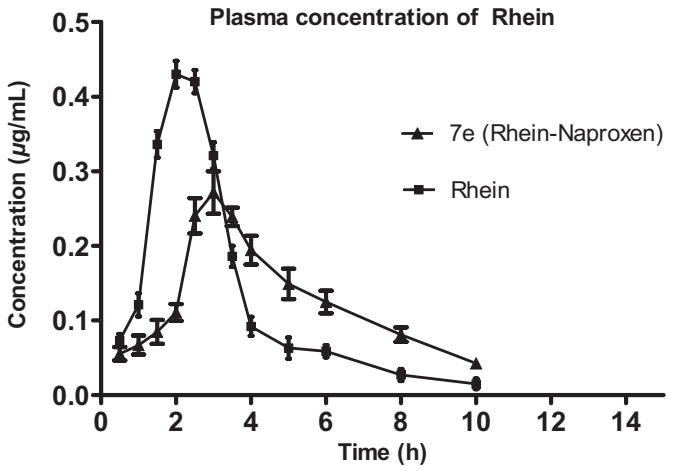


Fig. 10. Plasma concentration time profile of rhein upon oral administration of rhein itself (5.26 mg/kg) and of **7e** (Rhein–Naproxen) (10 mg/kg). Data are expressed as mean values ± SD ($n = 6$).

J_2 = 7.8 Hz, Ar–H), 8.83 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.9, 21.0, 61.1, 67.6, 125.7, 126.4, 128.6, 130.6, 130.9, 134.4, 134.8, 135.0, 135.6, 150.3, 150.4, 164.2, 169.1, 169.3, 181.2, 189.8; IR (cm^{-1}): 3434, 1759, 1724, 1680, 1593, 1372, 1327, 1280, 1254, 1204, 1074, 1025, 745, 701; MS (ESI) m/z: 435.0 $[M + Na]^+$, 451.0 $[M + K]^+$; Anal. Calcd for $C_{21}H_{16}O_9$: C, 61.17; H, 3.91. Found: C, 60.95; H, 4.08%.

5.1.5. 2-(2-hydroxyethoxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**5b**)

Preparation of **5b** is followed the procedure for **5a** described above. Yield: 81%. Mp: 153–155 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.89 (s, 1H, $CH_2CH_2O\underline{H}$), 2.45 (s, 6H, $OCOCH_3$), 3.65–3.68 (m, 2H, $C\underline{H}_2CH_2OH$), 3.76–3.79 (m, 2H, $CH_2C\underline{H}_2OH$), 3.86–3.89 (m, 2H, $COOCH_2C\underline{H}_2O$), 4.55–4.58 (m, 2H, $COOC\underline{H}_2CH_2O$), 7.44 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz, Ar–H), 7.80 (t, 2H, J = 7.9, Ar–H), 8.05 (s, 1H, Ar–H), 8.25 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, Ar–H), 8.84 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.0, 21.1, 61.9, 65.0, 69.0, 72.6, 76.6, 76.7, 77.0, 77.2, 77.5, 125.7, 126.4, 128.5, 130.6, 130.8, 134.8, 135.0, 135.7, 150.3, 150.4, 164.0, 169.1, 169.3, 180.4, 181.2; IR (cm^{-1}): 3545, 3471, 3414, 3136, 1767, 1729, 1674, 1621, 1605, 1405, 1394, 1323, 1270, 1192, 1123, 1068, 1021, 926, 890, 806, 740, 687, 621, 589; MS (ESI) m/z: 474.1 $[M + NH_4]^+$; Anal. Calcd for $C_{23}H_{20}O_{10}$: C, 60.53; H, 4.42. Found: C, 60.68; H, 4.28%.

5.1.6. 2-(2-(2-hydroxyethoxy)ethoxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**5c**)

Preparation of **5c** is followed the procedure for **5a** described above. Yield: 83%. Mp: 96–97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.17 (s, 1H, $CH_2CH_2O\underline{H}$), 2.45 (s, 6H, $OCOC\underline{H}_3$), 3.60–3.64 (m, 2H, $CH_2C\underline{H}_2OH$), 3.65–3.74 (m, 6H, $C\underline{H}_2C\underline{H}_2O\underline{C}H_2CH_2OH$), 3.85–3.89 (m, 2H, $COOCH_2C\underline{H}_2O$), 4.55–4.58 (m, 2H, $COOC\underline{H}_2CH_2O$), 7.44 (dd, 1H, J_1 = 1.1 Hz, J_2 = 8.0 Hz, Ar–H), 7.80 (t, 2H, J = 7.9, Ar–H), 8.06 (s, 1H, Ar–H), 8.25 (dd, 1H, J_1 = 1.1 Hz, J_2 = 7.8 Hz, Ar–H), 8.84 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.9, 21.0, 61.8, 65.0, 68.9, 70.4, 70.8, 72.5, 76.6, 77.0, 77.2, 77.4, 125.7, 126.4, 128.4, 130.5, 130.9, 134.3, 134.7, 135.0, 135.7, 150.2, 150.3, 163.9, 169.2, 169.3, 180.4, 181.2; IR (cm^{-1}): 3471, 3413, 3140, 2880, 1769, 1726, 1670, 1621, 1603, 1398, 1324, 1270, 1198, 1107, 948, 930, 883, 783, 749, 697, 627, 591; MS (ESI) m/z: 523.1 $[M + Na]^+$, 518.0 $[M + NH_4]^+$; Anal. Calcd for $C_{25}H_{24}O_{11}$: C, 60.00; H, 4.83. Found: C, 60.33; H, 4.92%.

5.1.7. 2-(2-(4-isobutylphenyl)propanoyloxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**6a**)

Ibuprofen (2-(4-isobutylphenyl)propanoic acid) (3 g, 14.56 mmol) and oxalyl chloride (1.84 mL, 21.4 mmol) were added into anhydrous dichloromethane (30 mL) and DMF (0.5 mL). This mixture was stirred at room temperature for 1 h, then the solvent and oxalyl chloride were removed under reduced pressure. The residue was dissolved in anhydrous dichloromethane (30 mL), this solution was added to **5a** (2 g, 4.85 mmol) in dichloromethane (100 mL) and triethylamine (7 mL, 49.81 mmol) by dripping at room temperature. The mixture was stirred for 0.5 h and poured into the ice water (150 mL). The water layer was extracted by dichloromethane, the organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by chromatography (AcOEt/pet = 2:5) on silica gel to afford **6a** as a pallid yellow solid (7.2 g, 83%). Mp: 129–130 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.82 (d, 6H, J = 6.6, $CH(C\underline{H}_3)_2$), 1.51 (d, 3H, J = 7.1, $CH_2C\underline{H}_3$), 1.76 (m, 1H, $C\underline{H}(CH_3)_2$), 2.35 (d, 2H, J = 7.2, $C\underline{H}_2CH(CH_3)_2$), 2.47 (s, 6H, $OCOCH_3$), 3.74–3.75 (m, 1H, $C\underline{H}CH_3$), 4.43–4.55 (m, 4H, CH_2CH_2), 7.02 (d, 2H, J = 8.1, Ar–H), 7.18 (d, 2H, J = 8.1, Ar–H), 7.43 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz, Ar–H), 7.78 (t, 1H, J = 7.9, Ar–H), 8.04 (s, 1H, Ar–H), 8.24 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, Ar–H), 8.83 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.3, 20.9, 21.0, 22.3, 30.1, 45.0, 61.9, 63.7, 125.6, 127.1, 128.5, 129.3, 130.5, 130.7, 134.4, 134.7, 135.4, 137.3, 140.6, 150.2, 150.3, 163.5, 163.6, 169.0, 169.2, 174.5, 180.4, 181.1; IR (cm^{-1}): 3454, 2958, 1776, 1729, 1676, 1594, 1370, 1332, 1284, 1257, 1197, 1020; MS (ESI) m/z: 618.2 $[M + NH_4]^+$, 623.1 $[M + Na]^+$, 639.0 $[M + K]^+$; Anal. Calcd for $C_{34}H_{32}O_{10}$: C, 67.99; H, 5.37. Found: C, 67.73; H, 5.67%.

5.1.8. 2-(2-(2-(4-isobutylphenyl)propanoyloxy)ethoxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**6b**)

Preparation of **6b** is followed the procedure for **6a** described above. Yield: 81%. Mp: 73–75 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.88 (d, 6H, J = 7.1, $CH(C\underline{H}_3)_2$), 1.48 (d, 3H, J = 7.2, $CHC\underline{H}_3$), 1.81–1.84 (m, 1H, $C\underline{H}(CH_3)_2$), 2.42 (d, 2H, J = 7.2, $C\underline{H}_2CH(CH_3)_2$), 2.45 (s, 6H,$OCOCH_3$), 3.68–4.46 (m, 9H, $C\underline{H}_2C\underline{H}_2$ and $C\underline{H}CH_3$), 7.07 (d, 2H, J = 8.1, Ar–H), 7.20 (d, 2H, J = 8.1, Ar–H), 7.44 (dd, 1H, J_1 = 1.1 Hz, J_2 = 8.0 Hz, Ar–H), 7.79 (t, 1H, J = 7.9, Ar–H), 8.03 (s, 1H, Ar–H), 8.25 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, Ar–H), 8.83 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.5, 21.0, 21.1, 22.4, 30.2, 42.7, 45.0, 45.1, 63.8, 64.9, 65.0, 68.8, 69.0, 69.1, 71.4, 76.6, 77.0, 77.2, 77.4, 125.7, 126.4, 127.2, 129.3, 130.5, 130.8, 134.4, 134.7, 135.0, 135.7, 137.6, 140.6, 150.2, 150.3, 163.9, 169.1, 169.3, 174.7, 180.4, 181.2; IR (cm^{-1}): 3546, 3469, 3413, 3135, 2962, 1771, 1729, 1670, 1621, 1605, 1449, 1309, 1325, 1273, 1196, 1149, 1085, 1021, 886, 742, 627, 593, 518; MS (ESI) m/z: 662.3 $[M + NH_4]^+$; Anal. Calcd for $C_{36}H_{36}O_{11}$: C, 67.07; H, 5.63. Found: C, 67.38; H, 5.72%.

Table 4
Pharmacokinetic parameters.[a]

Compound administered	Compound measured	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ug/mL)	AUC_{0-9} (μg h/mL)	$AUC_{0-\infty}$ (μg h/mL)	MRT_{0-9} (h)	$MRT_{0-\infty}$(h)
7e (Rhein–Naproxen)	**7e** (Rhein–Naproxen)	2.189 ± 0.460	1.750 ± 0.689	1.089 ± 0.161	4.423 ± 0.457	4.861 ± 0.639	3.465 ± 0.293	4.231 ± 0.711
	Rhein	2.771 ± 0.490	2.917 ± 0.376	0.291 ± 0.098	1.178 ± 0.371	1.394 ± 0.431	4.291 ± 0.368	5.699 ± 0.590
Rhein	Rhein	1.239 ± 0.127	2.035 ± 0.236	0.432 ± 0.125	1.166 ± 0.245	1.379 ± 0.436	2.918 ± 0.261	3.782 ± 0.786

[a] Each value represents the mean ± S.D. of 6 rats.

*5.1.9. 2-(2-(2-(2-(4-isobutylphenyl)propanoyloxy)ethoxy)ethoxy) ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**6c**)*

Preparation of **6c** is followed the procedure for **6a** described above. Yield: 80%. Mp: 46–49 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (d, 6H, J = 6.6, CH($C\underline{H}_3$)$_2$), 1.48 (d, 3H, J = 7.2, CH$C\underline{H}_3$), 1.78–1.85 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.43 (d, 2H, J = 7.2, C$\underline{H}_2$CH(CH$_3$)$_2$), 2.45 (s, 6H,OCO$C\underline{H}_3$), 3.57–4.54 (m, 13H, C$\underline{H}_2$C$\underline{H}_2$ and C$\underline{H}$CH$_3$), 7.07 (d, 2H, J = 8.1, Ar–H), 7.20 (d, 2H, J = 8.1, Ar–H), 7.43 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz, Ar–H), 7.79 (t, 1H, J = 7.9, Ar–H), 8.04 (s, 1H, Ar–H), 8.25 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, Ar–H), 8.83 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.5, 20.9, 21.0, 22.3, 30.1, 45.0, 63.8, 65.1, 68.9, 69.2, 70.6, 70.7, 76.7, 77.0, 77.3, 125.6, 125.7, 126.4, 127.2, 128.4, 129.3, 130.5, 130.8, 134.4, 134.7, 135.0, 135.8, 137.7, 140.5, 150.2, 150.3, 164.0, 169.1, 169.3, 174.7, 180.5, 181.2; IR (cm^{-1}): 3469, 3415, 3136, 3014, 2959, 2874, 1772, 1727, 1670, 1605, 1452, 1395, 1325, 1270, 1194, 1098, 1023, 928, 885, 872, 797, 779, 737, 684, 624, 584, 544; MS (ESI) m/z: 711.2 [M + Na]$^+$; Anal. Calcd for $C_{38}H_{40}O_{12}$: C, 66.27; H, 5.85. Found: C, 66.39; H, 5.52%.

*5.1.10. 2-(2-acetoxybenzoyloxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**6d**)*

Preparation of **6d** is followed the procedure for **6a** described above. Yield: 87%. Mp: 198–200 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.33 (s, 3H, OCO$C\underline{H}_3$), 2.45 (s, 6H, OCO$C\underline{H}_3$), 4.63–4.66 (m, 2H, –C$\underline{H}_2$), 4.69–4.72 (m, 2H, –C$\underline{H}_2$), 7.12 (d, 1H, J = 0.9, Ar–H), 7.33 (m, 1H, Ar–H), 7.44 (dd, 1H, J_1 = 1.3 Hz, J_2 = 8.0 Hz, Ar–H), 7.61 (m, 1H, Ar–H), 7.80 (t, 1H, J = 7.9, Ar–H), 8.04–8.05 (m, 2H, Ar–H), 8.25 (dd, 1H, J_1 = 1.3 Hz, J_2 = 7.8 Hz, Ar–H), 8.84 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.9, 21.0, 62.5, 63.7, 122.6, 123.9, 125.6, 126.1, 126.4, 130.5, 130.8, 131.9, 134.2, 134.3, 134.7, 135.0, 135.3, 150.2, 150.3, 150.9, 163.8, 164.0, 169.1, 169.2, 169.5, 181.0; IR (cm^{-1}): 3462, 1779, 1733, 1717, 1674, 1451, 1373, 1260, 1193, 1095, 1023, 920, 745, 701; MS (ESI) m/z: 592.3 [M + NH$_4$]$^+$, 597.3 [M + Na]$^+$, 613.2 [M + K]$^+$; Anal. Calcd for $C_{30}H_{22}O_{12}$: C, 62.72; H, 3.86. Found: C, 62.55; H, 3.73%.

*5.1.11. 2-(2-(6-methoxynaphthalen-2-yl)propanoyloxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**6e**)*

Preparation of **6e** is followed the procedure for **6a** described above. Yield: 81%. Mp: 134–137 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.56 (d, 3H, J = 7.1, CH$C\underline{H}_3$), 2.46 (s, 6H, OCO$C\underline{H}_3$), 3.70 (s, 3H, O$C\underline{H}_3$), 3.86–3.88 (m, 1H, C$\underline{H}$CH$_3$), 4.41–4.57 (m, 4H, C$\underline{H}_2$C$\underline{H}_2$), 6.84–6.86 (m, 2H, Ar–H), 7.46–7.55 (m, 5H, Ar–H), 7.73–7.80 (m, 2H, Ar–H), 8.22 (d, 1H, J = 1.2, Ar–H), 8.42 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.0, 21.0, 21.1, 45.3, 55.1, 61.7, 63.4, 100.7, 105.5, 118.8, 125.6, 125.8, 125.9, 126.1, 127.1, 128.9, 129.2, 130.3, 130.4, 133.5, 134.3, 134.4, 134.8, 134.9, 135.2, 150.0, 150.2, 157.4, 163.5, 169.1, 169.3, 174.4; IR (cm^{-1}): 3445, 3087, 2939, 2360, 2343, 1772, 1728, 1678, 1457, 1373, 1333, 1276, 1207, 1096, 1028, 921, 863, 809, 743, 693; MS (ESI) m/z: 647.2 [M + Na]$^+$; Anal. Calcd for $C_{35}H_{28}O_{11}$: C, 67.30; H, 4.52. Found: C, 67.65; H, 4.78%.

*5.1.12. 2-(2-(2-(2,6-dichlorophenylamino)phenyl)acetoxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**6f**)*

Diclofenac (4.3 g, 14.52 mmol), **5a** (2.0 g, 4.85 mmol), DCC (3.0 g, 14.54 mmol) and DMAP were added into anhydrous dichloromethane (150 mL). After stirring for 1.5 h at room temperature, water (20 mL) was added to stop the reaction. The precipitate was filtered, the filtrate was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by chromatography (AcOEt/pet = 1:2) on silica gel to afford **6f** as a pallid yellow solid (4.6 g, 46%). Mp: 149–151 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.46 (s, 6H, OCO$C\underline{H}_3$), 3.88 (s, 2H, OCO$C\underline{H}_2$), 4.52–4.55 (m, 2H, C$\underline{H}_2$C$\underline{H}_2$), 4.61–4.64 (m, 2H, C$\underline{H}_2$C$\underline{H}_2$), 6.48 (d, 1H, J = 8.4, Ar–H), 6.86–6.91 (m, 3H, Ar–H), 7.22–7.25 (m, 3H, Ar–H), 7.44 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.0 Hz, Ar–H), 7.80 (t, 1H, J = 7.9, Ar–H), 7.98 (s, 1H, Ar–H), 8.24 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.9 Hz, Ar–H), 8.78 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 20.7, 20.8, 36.9, 62.2, 63.8, 115.9, 120.6, 123.0, 124.8, 125.0, 125.3, 125.7, 127.7, 128.2, 129.0, 130.0, 130.5, 130.6, 130.8, 134.0, 134.5, 134.7, 135.5, 136.9, 142.7, 149.5, 149.7, 163.4, 168.8, 169.0, 171.4, 180.1, 180.5; IR (cm^{-1}): 3444, 3080, 2933, 2360, 1772, 1729, 1675, 1594, 1506, 1456, 1371, 1332, 1280, 1256, 1097, 1021, 921, 881, 770, 747; MS (ESI) m/z: 690.3 [M + H]$^+$, 728.3 [M + K]$^+$; Anal. Calcd for $C_{35}H_{25}Cl_2NO_{10}$: C, 60.88; H, 3.65; N, 2.03. Found: C, 60.76; H, 3.78; N, 2.12%.

*5.1.13. 2-(2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetoxy)ethyl 4,5-diacetoxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**6g**)*

Preparation of **6g** is followed the procedure for **6a** described above. Yield: 79%. Mp: 108–110 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.37 (s, 3H, CH_3), 2.46 (s, 6H, OCO$C\underline{H}_3$), 3.70 (s, 2H, CH_2), 3.74 (s, 3H, O$C\underline{H}_3$), 6.49 (s, 1H, Ar–H), 6.65–6.71 (m, 2H, Ar–H), 7.43–7.53 (m, 3H, Ar–H), 7.79–7.83 (m, 3H, Ar–H), 7.91 (s, 1H, Ar–H), 8.19 (dd, 1H, J_1 = 1.1 Hz, J_2 = 7.9 Hz, Ar–H), 8.65 (s, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.0, 21.1, 30.3, 55.6, 62.2, 63.6, 97.5, 101.2, 111.6, 112.1, 114.9, 125.7, 126.2, 128.4, 129.1, 130.4, 130.5, 130.6, 130.7, 131.2, 133.9, 134.4, 134.6, 135.0, 135.1, 136.1, 139.2, 150.1, 150.2, 156.0, 163.6, 168.2, 169.1, 169.3, 170.6, 180.4, 181.0; IR (cm^{-1}): 3442, 3081, 2934, 2359, 1775, 1733, 1679, 1594, 1476, 1456, 1368, 1326, 1257, 1195, 1166, 1094, 1022, 926, 746, 670; MS (ESI) m/z: 752.14 [M + H]$^+$; Anal. Calcd for $C_{40}H_{30}ClNO_{12}$: C, 63.88; H, 4.02; N, 1.86. Found: C, 63.67; H, 3.95; N, 1.75%.

*5.1.14. 2-(2-(4-isobutylphenyl)propanoyloxy)ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**7a**)*

Compound **6a** (0.55 g, 0.92 mmol) and triethylamine (3 mL, 21.35 mmol) were added to acetone (30 mL) and water (2 mL). The solution was heated to 50 °C for 6 h, the solvent was removed under reduced pressure and the residue was poured into water, the pH adjusted to 6 with 10% HCl. The water layer was extracted by dichloromethane and the organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure to give a crude product, which was purified by chromatography (AcOEt/pet = 1:3) on silica gel to afford **7a** as an orange-yellow solid (0.36 g, 76%). Mp: 85–87 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.83 (d, 6H, J = 6.6, CH(CH_3)$_2$), 1.51 (d, 3H, J = 7.2, $CHCH_3$), 1.72–1.81 (m, 1H, C$\underline{H}$(CH$_3$)$_2$), 2.36 (d, 2H, J = 7.1, C$\underline{H}_2$CH(CH$_3$)$_2$), 3.71–3.78 (m, 1H, C$\underline{H}$CH$_3$), 4.42–4.56 (m, 4H, CH_2CH_2), 7.03 (d, 2H, J = 8.1, Ar–H), 7.19 (d, 2H, J = 8.1, Ar–H), 7.34 (dd, 1H, J_1 = 1.2 Hz, J_2 = 8.4 Hz, Ar–H), 7.73–7.76 (m, 1H, Ar–H), 7.86 (s, 1H, Ar–H), 7.89 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.5 Hz, Ar–H), 8.37 (s, 1H, Ar–H), 11.96 (s, 1H, OH), 12.02 (s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 18.3, 22.3, 30.1, 45.0, 62.0, 63.6, 115.8, 118.3, 120.3, 120.4, 124.9, 125.4, 127.1, 129.3, 133.5, 133.9, 137.3, 137.5, 137.8, 140.5, 162.4, 164.0, 174.4, 180.7, 192.8; IR (cm^{-1}): 3447, 2953, 2931, 2360, 1735, 1672, 1625, 1507, 8451, 1411, 1375, 1269, 1238, 1200, 1158, 1088, 1034, 994, 903, 872, 842, 745, 597; MS (ESI) m/z: 517.2 [M + H]$^+$, 539.1 [M + Na]$^+$; Anal. Calcd for $C_{30}H_{28}O_8$: C, 69.76; H, 5.46. Found: C, 69.98; H, 5.22%.

*5.1.15. 2-(2-(2-(4-isobutylphenyl)propanoyloxy)ethoxy)ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**7b**)*

Preparation of **7b** is followed the procedure for **7a** described above. Yield: 67%. Mp: 89–91 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.87 (d, 6H, J = 6.7, CH($C\underline{H}_3$)$_2$), 1.48 (d, 3H, J = 7.1, CH$C\underline{H}_3$), 1.82 (m, 1H,

CH(CH$_3$)$_2$), 2.42 (d, 2H, J = 7.2, CH$_2$CH(CH$_3$)$_2$), 3.70–3.74 (m, 5H, CH$_2$CH$_2$ and CHCH$_3$), 4.25–4.26 (m, 2H, –CH$_2$–), 4.45–4.47 (m, 2H, –CH$_2$–), 7.06 (d, 2H, J = 8.0, Ar–H), 7.20 (d, 2H, J = 8.2, Ar–H), 7.34 (d, 1H, J = 7.4, Ar–H), 7.71–7.76 (m, 1H, Ar–H), 7.88 (d, 1H, J = 6.3, Ar–H), 7.96 (s, 1H, Ar–H), 8.44 (s, 1H, Ar–H), 11.97 (s, 1H, OH), 12.04 (s, 1H, OH); ^{13}C NMR (75 MHz, CDCl$_3$) δ: 18.5, 22.4, 30.2, 45.0, 63.8, 65.0, 68.8, 69.2, 76.6, 77.0, 77.2, 77.4, 118.3, 120.3, 120.4, 124.9, 125.4, 127.2, 129.3, 133.5, 134.0, 137.7, 137.8, 140.6, 162.4, 162.9, 164.4, 174.7, 192.9; IR (cm^{-1}): 3413, 3127, 2957, 2875, 1728, 1671, 1623, 1564, 1450, 1397, 1265, 1197, 1140, 1091, 1020, 838, 738, 715, 591, 510; MS (ESI) m/z: 559.3 [M – H]$^+$; Anal. Calcd for $C_{32}H_{32}O_9$: C, 68.56; H, 5.75. Found: C, 68.73; H, 5.52%.

5.1.16. 2-(2-(2-(2-(4-isobutylphenyl)propanoyloxy)ethoxy)ethoxy) ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**7c**)

Preparation of **7c** is followed the procedure for **7a** described above. Yield: 68%. Mp: 62–65 °C. ^{1}H NMR (500 MHz, CDCl$_3$) δ: 0.87–0.88 (d, 6H, J = 6.6, CH(CH$_3$)$_2$), 1.47 (d, 3H, J = 7.1, CH$_2$CH$_3$), 1.83 (m, 1H, CH(CH$_3$)$_2$), 2.42 (d, 2H, J = 7.2, CH$_2$CH(CH$_3$)$_2$), 3.58–4.53 (m, 13H, CH$_2$CH$_2$ and CHCH$_3$), 7.06 (d, 2H, J = 8.1, Ar–H), 7.18 (d, 2H, J = 8.1, Ar–H), 7.33 (d, 1H, J = 8.4, Ar–H), 7.73 (t, 1H, J = 8.0, Ar–H), 7.87 (d, 1H, J = 7.5, Ar–H), 7.95 (s, 1H, Ar–H), 8.43 (s, 1H, Ar–H), 11.96 (s, 1H, OH), 12.01 (s, 1H, OH); ^{13}C NMR (75 MHz, CDCl$_3$) δ: 18.6, 22.4, 30.2, 45.0, 63.9, 65.1, 69.0, 69.2, 70.6, 70.7, 76.8, 77.0, 77.3, 115.9, 118.3, 120.3, 120.4, 124.9, 125.4, 127.2, 129.3, 133.6, 134.0, 137.7, 137.8, 137.9, 140.5, 162.4, 162.9, 164.4, 174.7, 180.9, 192.9; IR (cm^{-1}): 3652, 3545, 3414, 3134, 2957, 2912, 2874, 1728, 1672, 1624, 1454, 1396, 1265, 1197, 1157, 1127, 1026, 867, 846, 744, 596; MS (ESI) m/z: 603.2 [M – H]$^+$, 627.1 [M + Na]$^+$; Anal. Calcd for $C_{34}H_{36}O_{10}$: C, 67.54; H, 6.00. Found: C, 67.32; H, 5.86%.

5.1.17. 2-(2-acetoxybenzoyloxy)ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**7d**)

Preparation of **7d** is followed the procedure for **7a** described above. Yield: 81%. Mp: 132–135 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ: 2.35 (s, 3H, OCOCH$_3$), 4.64–4.72 (m, 4H, CH$_2$CH$_2$), 7.10 (d, 1H, J = 0.9, Ar–H), 7.32–7.35 (m, 2H, Ar–H), 7.59–7.61 (m, 1H, Ar–H), 7.75–7.78 (m, 1H, Ar–H), 7.85 (s, 1H, Ar–H), 7.93 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.5 Hz, Ar–H), 8.03–8.06 (m, 1H, Ar–H), 8.45 (s, 1H, Ar–H), 11.97 (s, 1H, OH), 12.04 (s, 1H, OH); ^{13}C NMR (75 MHz, CDCl$_3$) δ: 21.0, 62.6, 63.7, 115.9, 118.4, 120.3, 120.4, 122.8, 123.9, 124.9, 125.5, 126.1, 131.9, 133.5, 134.0, 134.1, 137.5, 137.8, 150.9, 162.5, 162.9, 164.1, 169.5, 180.8, 192.8; IR (cm^{-1}): 3462, 2953, 2360, 2342, 1772, 1722, 1632, 1451, 1368, 1269, 1229, 1193, 1153, 1136, 1090, 1038, 747, 703, 671; MS (ESI) m/z: 489.1 [M – H]$^+$; Anal. Calcd for $C_{26}H_{18}O_{10}$: C, 63.68; H, 3.70. Found: C, 63.82; H, 3.86%.

5.1.18. 2-(2-(6-methoxynaphthalen-2-yl)propanoyloxy)ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**7e**)

Preparation of **7e** is followed the procedure for **7a** described above. Yield: 72%. Mp: 122–124 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ: 1.54–1.57 (m, 3H, CHCH$_3$), 3.67 (s, 3H, OCH$_3$), 3.85–3.88 (m, 1H, CHCH$_3$), 4.37–4.62 (m, 4H, CH$_2$CH$_2$), 6.74–6.78 (m, 2H, Ar–H), 7.33–7.54 (m, 6H, Ar–H), 7.75–7.78 (m, 1H, Ar–H), 7.87–7.90 (m, 1H, Ar–H), 7.98 (s, 1H, Ar–H), 11.90 (s, 1H, OH), 12.00 (s, 1H, OH); ^{13}C NMR (75 MHz, CDCl$_3$) δ: 18.0, 45.3, 55.0, 61.6, 63.0, 105.3, 115.9, 117.9, 118.5, 119.8, 120.1, 124.7, 124.8, 125.7, 125.9, 127.0, 128.6, 129.1, 133.3, 133.4, 133.5, 135.4, 136.7, 137.6, 157.2, 161.9, 162.8, 163.8, 174.2, 180.6, 192.7; IR (cm^{-1}): 3459, 3060, 2941, 2360, 2342, 1734, 1630, 1608, 1456, 1383, 1270, 1206, 1156, 1093, 1032, 748, 668; MS (ESI) m/z: 539.1 [M – H]$^+$; Anal. Calcd for $C_{31}H_{24}O_9$: C, 68.88; H, 4.48. Found: C, 68.97; H, 4.59%.

5.1.19. 2-(2-(2-(2,6-dichlorophenylamino)phenyl)acetoxy)ethyl 4,5-dihydroxy-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate (**7f**)

Preparation of **7f** is followed the procedure for **7a** described above. Yield: 77%. Mp: 164–166 °C. ^{1}H NMR (300 MHz, CDCl$_3$) δ: 3.88 (s, 2H, COCH$_2$), 4.52–4.63 (m, 4H, CH$_2$CH$_2$), 6.49 (d, 1H, J = 8.4, Ar–H), 6.75 (br, 1H, NH), 6.86–6.91 (m, 3H, Ar–H), 7.22–7.25 (m, 3H, Ar–H), 7.33 (dd, 1H, J_1 = 1.1 Hz, J_2 = 8.1 Hz, Ar–H), 7.72–7.75 (m, 1H, Ar–H), 7.85 (s, 1H, Ar–H), 7.87 (dd, 1H, J_1 = 1.2 Hz, J_2 = 7.8 Hz, Ar–H), 8.37 (s, 1H, Ar–H), 11.93 (s, 1H, OH), 11.96 (s, 1H, OH); ^{13}C NMR (75 MHz, CDCl$_3$) δ: 38.4, 62.5, 63.4, 115.8, 118.2, 118.3, 120.2, 120.4, 122.1, 123.9, 124.1, 124.8, 125.4, 128.1, 128.7, 129.5, 130.9, 133.4, 133.8, 137.3, 137.7, 142.5, 162.3, 162.8, 164.0, 172.1, 180.6, 192.7; IR (cm^{-1}): 3374, 3081, 2905, 2360, 2340, 1723, 1674, 1634, 1451, 1418, 1375, 1291, 1270, 1233, 1199, 1140, 1093, 1020, 777, 749, 666; MS (ESI) m/z: 606.0 [M + H]$^+$; Anal. Calcd for $C_{31}H_{21}Cl_2NO_8$: C, 61.40; H, 3.49; N, 2.31. Found: C, 61.72; H, 3.63; N, 2.26%.

5.2. Pharmacological procedure

5.2.1. In vitro studies

The HPLC system PE 200 series (Perkin Elmer, USA) equipped with the Totalchrom workstation software (Perkin Elmer) and comprised of a binary pump, an online vacuum degasser, an auto-sampler and a UV detector are used for the chromatographic analysis. All separations are carried out on an ODS-2 C_{18} column (250 mm * 4.6 mm, 5.0 μm particle size).

5.2.1.1. Bone affinity characteristics with HAP. Each of prodrugs in 10 mL (0.1 mmol/L) water was vigorously stirred with HAP (20 mg, 40 mg, 80 mg respectively) for 30 min in order to bind the compound to the HAP. The mixture was filtered and the residue was washed with water for removing any unbound prodrug. Each of the washing solutions containing unbound compound was diluted to 25 mL with water. The unbound compound in each water solution was then monitored by RP-HPLC using ibuprofen, rhein and diacerein as the authentic samples.

5.2.1.2. Hydrolysis of prodrugs in phosphate buffer. The hydrolytic stability of the test compounds was evaluated in different pH environments (pH = 1.2, pH = 7.4, pH value was adjusted with different phosphate buffer saline) at 37 °C up to 24 h. The samples were taken at specified time intervals during the period, and the active drugs released from the prodrugs in the samples were analyzed by using high performance liquid chromatography (HPLC). The mobile phase was V (CH$_3$OH):V (0.1% H_3PO_4 solution) = 90:10 (adjust pH to 2.3), the column temperature was maintained at 25 °C and wavelength was 254 nm. A constant mobile phase with flow rate of 1.0 mL/min was employed throughout the analyses. Each experiment was performed in triplicate.

5.2.2. In vivo studies

5.2.2.1. Anti-inflammatory assay. Male Kunming mice (18–22 g) were used through the studies. They were housed in room temperature of 25 ± 2 °C and humidity of 60% ± 5%. Animals were free access to feed and water *ad libitum* during the experimental period. The animal experiments were carried out according to (the Committee for the purpose of Control and Supervision of Experimentation on Animals guideline) the Regulation of Experimental Animal Administration issued by State Committee of Science and Technology of PR China on 14 November, 1988 and Institutional Animal Ethics Committee approved all the procedures for the investigating experimental pain in conscious animals.

Diacerein and ibuprofen (0.2 mmol/kg) was administered (0.1 mmol/L) as a positive comparator. The test compounds were administered (0.1 mmol/L) at the same dose (0.2 mmol/kg), respectively. 220 mice were randomly divided into 22 groups and were treated with 0.9% NaCl solution, diacerein and ibuprofen and test compounds once a day for 5 days, respectively. The buninoid filter with diameter of 7 mm is infiltrated by xylene. After 1 h of the last administration, the filter was clung to the right ear of the mice for 30 s. After 30 min the mice were executed by decollation and the ears were slotted wafers by 7 mm hole puncher. The inhibition percent of auricle tumefaction was calculated using the following formula. Percent of inhibition (%) = $(1 - a/b) * 100\%$, where a means tumefaction degree of control group and b means tumefaction degree of test group.

5.2.2.2. Acute ulcerogenesis assay. The ability of the test compounds **7a**–**7c** and **7e**–**7f** to produce gastric lesions was evaluated according to following procedure [27]. Kunming mice of either sex were divided into control and different test groups of ten animals each group (18–22 g). Ulcerogenic activity was evaluated after oral administration of test compounds at the dose of 1.0 mmol/kg. Ibuprofen and the test compounds **7a**–**7c** and **7e**–**7f** were suspended and administered in a 1% methylcellulose solution. Food and water were removed 24 h before administration of test compounds. All animals were sacrificed after 4 h of drug administration. Their stomachs were removed, immerged in 10% formaldehyde solution and 30 min later cut out along the greater curvature of the stomach, gently rinsed with water, and placed on ice. The number and the length of ulcers observed in each stomach were determined by using magnifier lenses. In this assay, the severity of each gastric lesion is measured along its greatest length (1 mm, rating of 1; 1–2 mm, rating of 2; and >2 mm, rating according to their length in millimeter). The UI for each test compound is calculated by adding the total length (L, in mm) of individual gastric lesions in each stomach and averaging over the number of animals in each group ($n = 10$): UI = (L1 + L2 + L3 + L4 + L5 + L6 + L7 + L8 + L9 + L10)/10.

5.2.2.3. Pharmacokinetic studies in rats

5.2.2.3.1. HPLC system. Chromatographic separations were performed using Waters Alliance liquid chromatograph (Waters, USA) equipped with a diode array detector 2996. All separations were accomplished on a phenomenex synergi 4u hydro-RP 80A (250 mm * 4.6 mm). The selected wavelength was 432 nm. The mobile phase consisted of 0.04% H_3PO_4/CH_3OH (5.5:94.5, v:v) for **7e**, 0.5% CH_3COOH/CH_3OH (14:86, v:v) for rhein. Column temperature, injection volume and flow rate were 35 °C, 25 μl and 1.0 mL/min, respectively. All reagents and solvents were analytical grade. Retention times were 5.8 min for **7c** and rhein.

5.2.2.3.2. Animals. Sprague–Dawley rats of either sex, obtained from QingLongChang experimental animal farms (Nanjing, China, qualified number: SCXK (Su) 2007–0008), were divided into different test groups of six animals each group (200 g ± 20 g). They were housed in room temperature of 25 ± 2 °C and humidity of 60% ± 5%. Animals were free access to feed and water *ad libitum* during the experimental period.

Under light anesthesia with ether, the femoral artery was cannulated with polyethylene tubing filled with 200 U/mL heparin in saline. After the rats recovered from anesthesia, dosing solutions were given by gastric intubation. Blood samples (approx. 0.5 mL) were collected in tubes at 0 (predose), 0.17 h, 0.33 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 4 h, 5 h, 7 h and 9 h after administration. Plasma samples were immediately separated by centrifugation from these arterial blood samples.

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References

[1] M.B. Goldring, S.R. Goldring, J. Cell. Physiol. 213 (2007) 626–634.
[2] J. Swierkot, J. Szechinski, Pharmacol. Rep. 58 (2006) 473–492.
[3] M. Fajardo, P.E. Di Cesare, Drugs Aging 22 (2005) 141–161.
[4] D.D. Perrin, Nature 208 (1965) 787–788.
[5] H. Itokawa, L. Susan, M. Natschke, T. Akiyama, K.H. Lee, J. Nat. Med. 62 (2008) 263–280.
[6] T. Tamura, T. Shirai, N. Kosaka, K. Ohmori, N. Takafumi, Eur. J. Pharmacol. 448 (2002) 81–87.
[7] E. Beccerica, G. Ferretti, G. Curatola, C. Cervini, Pharmacol. Res. 22 (1990) 277–285.
[8] P. Debord, K. Louchahi, M. Tod, A. Cournot, G. Perret, O. Petitjean, Eur. J. Drug Metab. Pharmacokinet. 19 (1994) 13–19.
[9] T. Tamura, N. Kosaka, J. Ishiwa, T. Sato, H. Nagase, A. Ito, Osteoarthr. Cartil. 9 (2001) 257–263.
[10] C.M. Spencer, M.I. Wilde, Drugs 53 (1997) 98–106.
[11] A. Moore, K. Greenslade, C. Alam, D. Willoughby, Osteoarthr. Cartil. 6 (1998) 19–23.
[12] J.P. Pelletier, D. Lajeunesse, P. Reboul, F. Mineau, J.C. Fernandes, P. Sabouret, J. Martel-Pelletier, J. Rheumatol. 28 (2001) 814–824.
[13] M. Mian, S. Brunelleschi, S. Tarli, A. Rubino, D. Benetti, R. Fantozzi, L.J. Zilletti, Pharm. Pharmacol. 39 (1987) 845–847.
[14] T. Tamura, K. Ohmori, Eur. J. Pharmacol. 419 (2001) 269–274.
[15] K.V. Nemmani, S.V. Mali, N. Borhade, A.R. Pathan, M. Karwa, V. Pamidiboina, S.P. Senthilkumar, M. Gund, A.K. Jain, N.K. Mangu, N.P. Dubash, D.C. Desai, S. Sharma, A. Satyam, Bioorg. Med. Chem. Lett. 19 (2009) 5297–5301.
[16] Z. Huang, C.A. Velazquez, K.R. Abdellatif, M.A. Chowdhury, J.A. Reisz, J.F. DuMond, S.B. King, E.E. Knaus, J. Med. Chem. 54 (2011) 1356–1364.
[17] J.R. Vane, R.M. Botting, Scand. J. Rheumatol. 102 (1996) 9–21.
[18] M.T. Chhabria, H.G. Bhatt, H.G. Raval, P.M. Oza, Bioorg. Med. Chem. Lett. 17 (2007) 1022–1024.
[19] M. Lazzaroni, P.G. Bianchi, Aliment. Pharmacol. Ther. 20 (2004) 48–58.
[20] A. Mishra, R. Veerasamy, P.K. Jain, V.K. Dixit, R.K. Agrawal, Eur. J. Med. Chem. 43 (2008) 2464–2472.
[21] S. Vyas, P. Trivedi, S.C. Chaturvedi, Acta Pharm. 57 (2007) 441–450.
[22] Y. Duan, J. Yu, S. Liu, M. Ji, Med. Chem. 5 (2009) 577–582.
[23] V. Gonnot, S. Tisserand, M. Nicolas, R. Baati, C. Mioskowski, Tetrahedron Lett. 48 (2007) 7117–7119.
[24] H. Fonge, L. Jin, H. Wang, Y. Ni, G. Bormans, A. Verbruggen, Bioorg. Med. Chem. Lett. 17 (2007) 4001–4005.
[25] R. Erez, S. Ebner, B. Attali, D. Shabat, Bioorg. Med. Chem. Lett. 18 (2008) 816–820.
[26] V. Kubicek, J. Rudovsky, J. Kotek, P. Hermann, E.L. Vander, R.N. Muller, Z.I. Kolar, H.T. Wolterbeek, J.A. Peters, I. Lukes, J. Am. Chem. Soc. 127 (2005) 16477–16485.
[27] C.A. Velazquez, P.N. Praveen Rao, M.L. Citro, L.K. Keefer, E.E. Knaus, Bioorg. Med. Chem. Lett. 15 (2007) 4767–4774.