European Journal of Medicinal Chemistry 46 (2011) 907–913

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

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

Original article

Investigations on cytotoxicity and anti-inflammatory potency of licofelone derivatives

Wukun Liu [a,b,c], Jinpei Zhou [a,**], Kerstin Bensdorf [b], Huibin Zhang [a], Haoran Liu [c], Yubin Wang [a], Hai Qian [a], Yanchun Zhang [a], Anja Wellner [b], Gerhard Rubner [b], Wenlong Huang [a], Cancheng Guo [c], Ronald Gust [b,d,*]

[a] Department of Medicinal Chemistry, China Pharmaceutical University, Tongjia Xiang 24, 210009 Nanjing, PR China
[b] Institute of Pharmacy, Freie Universität Berlin, Königin-Luise-Str, 2+4, 14195 Berlin, Germany
[c] College of Chemistry and Chemical Engineering, Hunan University, 410082 Changsha, PR China
[d] Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Innsbruck, Innrain 52, A-6020 Innsbruck, Austria

ARTICLE INFO

Article history:
Received 22 September 2010
Received in revised form
22 November 2010
Accepted 5 January 2011
Available online 11 January 2011

Keywords:
2,3-dihydro-1*H*-pyrrolizine derivatives
Anti-inflammatory activity
Cytotoxicity
COX inhibition

ABSTRACT

A series of C5-substituted licofelone ([2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1*H*-pyrrolizin-5-yl]acetic acid) derivatives were developed by a parallel synthesis approach and investigated for cytotoxicity against MCF-7 and MDA-MB-231 cells as well as for anti-inflammatory potency *in vitro* and *in vivo*. Dependent on the C5-substituent, the compounds showed high selectivity for MCF-7 cells. Especially 2-oxoethyl benzoate derivatives were inactive at the MDA-MB-231 cell line and as active as 5-FU at MCF-7 cells. C5-acetyl (**8a**), −2-oxoethyl formiate (**8e**), −2-oxoethyl acetate (**8f**) and −2-oxoethyl propionate (**8g**) derivatives showed growth inhibition at both cell lines, comparable with cisplatin. Modifications significantly reduced the inhibitory potency at COX-1 and COX-2 *in vitro* and in the xylene-induced ear swelling assay in mice. Only compound **8a** was equipotent to licofelone, ibuprofen and celecoxibe *in vivo*.

1. Introduction

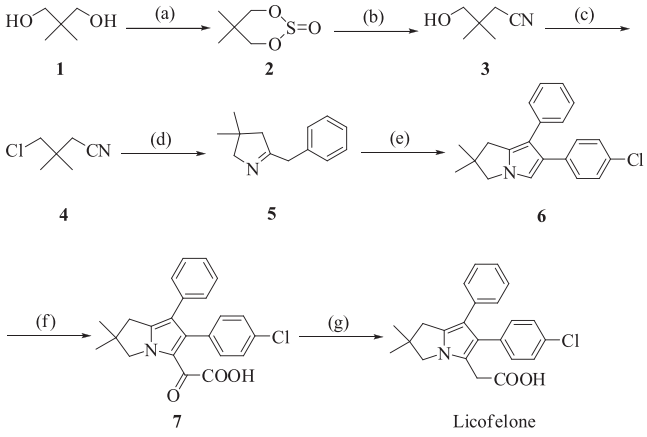
Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications worldwide. They are considered as effective anti-inflammatory, anti-pyretic and analgesic drugs. In recent years there is a growing body of evidence suggesting a correlation between NSAIDs use and lower incidence of cancer. Epidemiological studies also demonstrated that NSAIDs appear to be effective in chemoprevention and treatment of many tumoral diseases, e.g. breast cancer [1–3].

The prevention and treatment mechanism of NSAIDs against tumors is still not clear. However, some hypotheses have been proposed. Several groups postulated that both, anti-inflammatory activities and anticancer effects are due to a reduction of products formed by cyclooxygenase (COX) or lipoxygenase (LOX) catalyzed reactions [4–11]. Some experiments demonstrated that COX-2 expression correlates with a significant increased risk of metastasis and other parameters of aggressive disease such as large tumor size, axillary nodes involvement, ductal histology, receptor-negative disease and human epidermal growth factor 2 (HER-2) amplification [4]. HER-2-positive breast cancer and its association with aromatase production suggest that COX-2 inhibition may play a role in the management of breast cancer [5,6]. Moreover, our previous results also showed that COX inhibition especially COX-2 inhibition probably plays a major role in the anti-breast cancer effects of compounds [7–10]. A number of research also demonstrated that NSAIDs could inhibit LOX, thereby inhibiting proliferation and inducing apoptosis of many malignant tumors [11]. In addition to COX and LOX inhibition, these small molecules can also target other molecular pathways. For example, celecoxib can block phosphoinositide 3-kinase (PI3K)/phosphoinositide-dependent kinase (PDK)/Akt pathway to induce apoptosis in prostate cancer cells [12,13].

2,3-Dihydro-1*H*-pyrrolizines are widely investigated inhibitors of the arachidonic acid pathways [14–22]. A compound of these series, [2,2-dimethyl-6-(4-chlorophenyl)-7-phenyl-2,3-dihydro-1*H*-pyrrolizin-5-yl]acetic acid (licofelone, Scheme 1) showed in clinical trials anti-inflammatory and analgesic activity in osteoarthritis comparable to conventional NSAIDs with a safer gastrointestinal profile. It is a potent, competitive inhibitor of 5-LOX, COX-1 and COX-2 [14,15]. Researches also addressed that licofelone appears to suppress inflammatory PGE2 formation preferentially by inhibiting microsomal prostaglandin E2 synthase-1 (mPGES-1) at

* Corresponding author. Department of Pharmaceutical Chemistry, Institute of Pharmacy, University of Innsbruck, Innrain 52, A-6020 Innsbruck, Austria. Tel.: +43 512 507 5245; fax: +43 512 507 2940.
** Corresponding author. Tel.: +86 25 83471532; fax: +86 25 83271480.
E-mail addresses: jpzhou668@163.com (J. Zhou), ronald.gust@uibk.ac.at (R. Gust).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.01.002



Scheme 1. Synthetic routes of 6,7-diaryl-2,3-dihydro-1*H*-pyrrolizine compound **6** and licofelone.
Reagents and conditions: (a) $SOCl_2$, CH_2Cl_2, 5–10 °C, 77%; (b) NaCN, DMSO, 100 °C, 36 h, 45%; (c) $SOCl_2$, pyridine, 0 °C, 77%; (d) benzylmagnesium chloride (Grignard species provided in situ from benzylchloride and Mg 1:1), initially absolute Et_2O, 2 h, reflux, then toluene, 3 h, reflux, 70%; (e) 2-bromo-1-(4-chlorophenyl)ethanone, absolute ethanol, $NaHCO_3$, 36 h, rt, 25%; (f) oxalyl chloride, THF, 10–15 °C, then add H_2O; 25–30 °C, 20 min; (g) $N_2H_4 \cdot H_2O$, KOH, ethylene diglycol, 85 °C, 5 h, then to 140–145 °C, 2 h, 55%.

concentrations that do not affect COX-2, implying attractive and thus far unique molecular pharmacological dynamics as an inhibitor of COX-1, 5-LOX, and mPGES-1 [16,17]. Furthermore, it enhanced apoptosis in prostate cancer cells as well as HCA-7 colon cancer cells through the mitochondrial pathway. All these results show that licofelone has also a good perspective as anti-tumor drug [18,19].

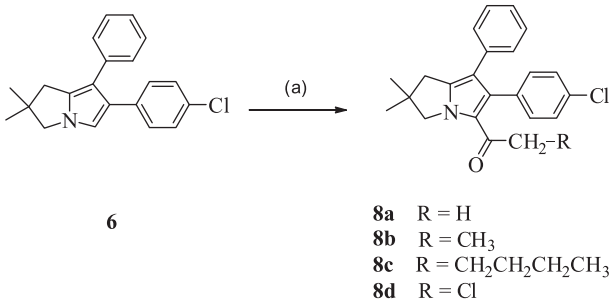
Since up to now no data are available concerning the efficacy of licofelone derivatives in breast cancer treatment (for anti-tumor activity see [18,19]) we focused our interest on this tumor identity. Here we first describe the variation of substituents at C5 of licofelone. The carboxymethyl group was exchanged by an acetyl residue which was further substituted with lipophilic groups (alkyl or chlorine), formiate, acetate, propionate, benzoate, and phenylacetate. A parallel synthesis approach which is recently widespread as tool for the discovery of new therapeutic agents was used [23]. All synthesized compounds were characterized by IR, ESI-MS, ^1H NMR, elementary analysis and tested for cytotoxicity as well as anti-inflammatory potency *in vitro* and *in vivo*.

2. Chemistry

6,7-Diaryl-2,3-dihydro-1*H*-pyrrolizine (**6**) and licofelone were synthesized according to previously published methods [14,24] and outlined in Scheme 1.

Thus, 4-chloro-3,3-dimethylbutyronitrile (**4**) was obtained in 3 steps starting from 2,2-dimethylpropane-l,3-diol (**1**). We condensed **4** with a commercially available benzyl-Grignard, and accomplished ring closure to the rather unstable 5-benzyl-3,3-dimethyl-3,4-dihydro-2*H*-pyrrole (**5**). Subsequently, **5** and 2-bromo-1-(4-chlorophenyl)ethanone was cycled with moderate yield to 6,7-diaryl-2,3-dihydro-1*H*-pyrrolizine (**6**) in ethanol/aqueous $NaHCO_3$ solution at room temperature (rt). Friedel-Craft acylation of **6** with oxalyl chloride and subsequent Wolff–Kishner reduction with hydrazine hydrate yielded licofelone (Scheme 1).

Analogously, **8a**–**d** were obtained by reaction of **6** with the respective substituted anhydride or chloroacetyl chloride by means of BF_3-Et_2O or $AlCl_3$ as catalysts (Scheme 2). The reaction yield depended on the catalyst. While in the case of **8d** both catalysts work as well (40% ($AlCl_3$) and 59% (BF_3-Et_2O)), only $AlCl_3$ led to



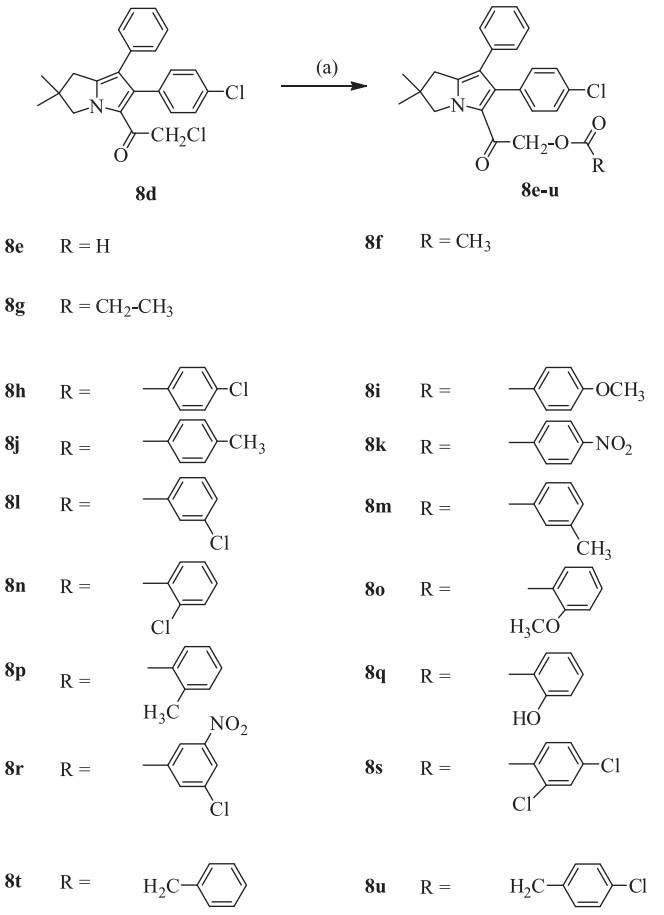
Scheme 2. Synthetic routes of compounds **8a**–**d**.
Reagents and conditions: (a) $(RCH_2CO)_2O$ (R = H, CH_3, $CH_2CH_2CH_2CH_3$) or $ClCH_2COCl$, Lewis acid, absolute Et_2O, rt, 3–12 h, 43–73%.

satisfying amounts of **8a** (43% compared to 10% (BF_3-Et_2O)) and **8b** (73% compared to 0% (BF_3-Et_2O)). Interestingly, **8c** is only available using BF_3-Et_2O as catalyst (56% compared to 0% ($AlCl_3$)).

Compound **8d** was used as synthon for the synthesis of **8e**–**u**. Substituted aromatic and alkyl acid were neutralized with triethylamine to give the corresponding triethylamine salt, which was then reacted with **8d** at rt to afford the compounds **8e**–**u** with high yields (Scheme 3).

3. Results and discussion

Hormone dependent MCF-7 and hormone independent MDA-MB-231 breast cancer cell lines were selected for the



Scheme 3. Synthetic routes of compounds **8e**–**u**.
Reagents and conditions: (a) Substituted aromatic or alkyl acid, Et_3N, absolute acetone, rt, over 24 h, 50–95%.

Table 1
Growth inhibitory effects against MCF-7 and MDA-MB-231 human breast cancer cells.

Compound	IC_{50} value [μM][a,b]		Compound	IC_{50} value [μM][a,b]	
	MDA-MB-231	MCF-7		MDA-MB-231	MCF-7
5-FU	9.6 ± 0.3	4.7 ± 0.4	**8j**	>50	>50
Cisplatin	3.8 ± 0.1	1.7 ± 0.1	**8k**	7.3 ± 0.7	4.8 ± 0.1
Licofelone	36.7 ± 3.2	5.5 ± 0.6	**8l**	>50	5.2 ± 0.3
6	19.7 ± 0.2	5.6 ± 0.2	**8m**	>50	3.8 ± 0.4
8a	3.3 ± 0.8	2.0 ± 0.3	**8n**	31.8 ± 0.4	5.2 ± 0.3
8b	19.2 ± 1.4	8.7 ± 0.8	**8o**	>50	4.5 ± 0.1
8c	31.3 ± 0.1	5.4 ± 0.5	**8p**	12.9 ± 2.9	6.2 ± 1.2
8d	35.4 ± 3.1	5.8 ± 0.5	**8q**	>50	8.2 ± 1.0
8e	1.8 ± 0.2	1.5 ± 0.2	**8r**	43.8 ± 4.7	31.7 ± 11.6
8f	4.4 ± 0.1	2.5 ± 0.1	**8s**	>50	6.7 ± 1.4
8g	3.1 ± 0.1	1.7 ± 0.1	**8t**	>50	4.7 ± 0.4
8h	>50	3.9 ± 0.4	**8u**	>50	7.1 ± 1.1
8i	>50	11.9 ± 1.5			

[a] The IC_{50} values represent the concentration which results in a 50% decrease in cell growth after 72 h incubation.
[b] Values are the means of at least 2–3 experiments.

evaluation of the growth inhibitory potency. MCF-7 cells have a basal level of COX-1 and a barely detectable and transient COX-2 inducible expression, whereas MDA-MB-231 cells show a low expression of COX-1 but a constitutive level of COX-2 [25]. Therefore, their growth is sensitive to NSAID treatment [2,3].

The experiments were performed according to established procedures [26]. In addition to compounds **6**, **8a**–**u** and licofelone the established anti-tumor drugs 5-fluorouracil (5-FU) and cisplatin were used as references. IC_{50} values for these compounds were calculated (OriginPro 8) and are presented in Table 1.

Licofelone showed at the MCF-7 cell line an IC_{50} = 5.5 μM very similar to 5-FU (IC_{50} = 4.7 μM). Against MDA-MB-231 cells it was only marginally active (IC_{50} = 36.7 μM) indicating eight-fold selectivity for MCF-7 cells. Exchange of the C5-carboxymethyl moiety by an acetyl residue (**8a**) enormously increased the growth inhibitory effects. Compound **8a** was more active than 5-FU and as active as cisplatin. The selectivity for MCF-7 (IC_{50} = 2.0 μM) cells was lost. **8a** influenced the growth of MDA-MB-231 cells to the same extent (IC_{50} = 3.3 μM). Additional alkyl groups at the acetyl moiety (**8b** and **8c**) or a chlorine substituent (**8d**) reduced the growth inhibitory effects, especially at the MDA-MB-231 cell line. In contrast, formiate (**8e**), acetate (**8f**), or propionate (**8g**) at the 2-oxoethyl residues did not reduce the inhibitory potency but even led in the case of **8e** to a more active compound.

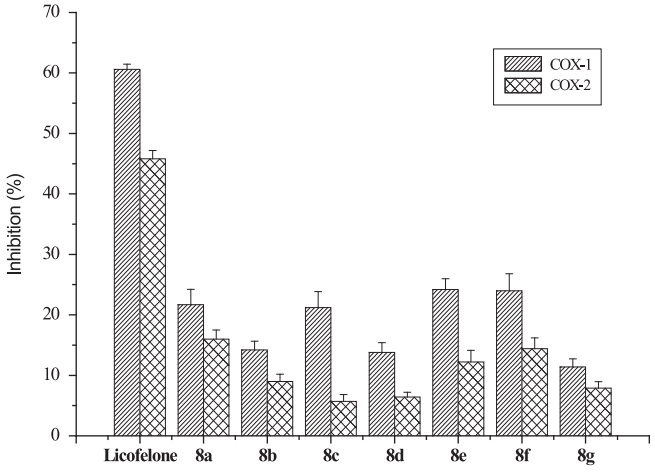
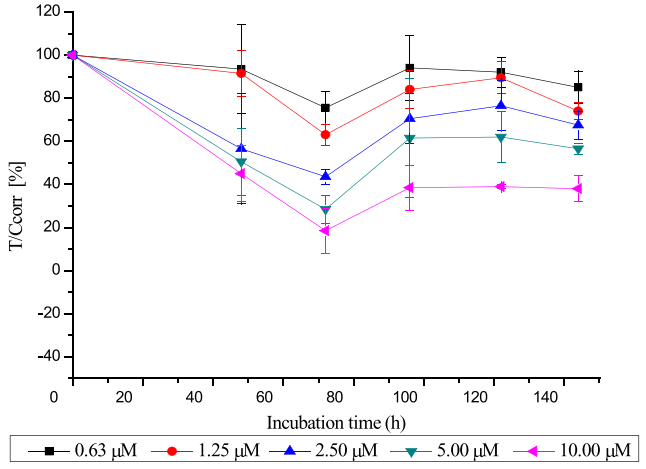
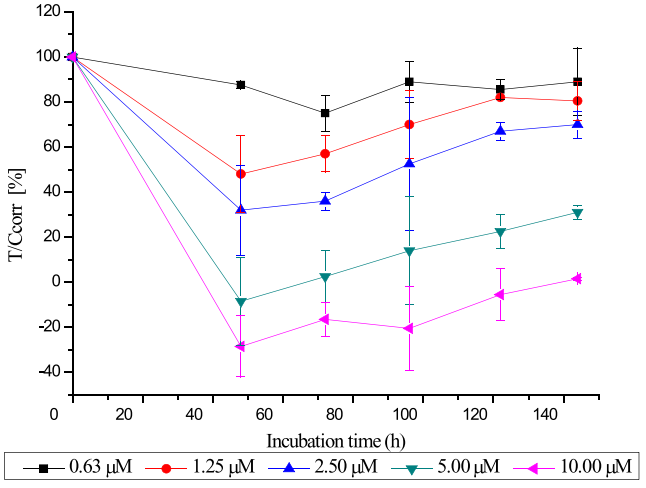


Fig. 1. Inhibition of COX-1 (ovine) and COX-2 (human recombinant) activity after treatment with the compounds at the concentration of 10 μM (negative control (DMSO) was set as 0%).

High tumor cell selectivity can be obtained using substituted benzoates as terminal groups. Unfortunately no clear structure–activity relation can be deduced from their results. The most selective compounds **8h** (4-Cl, IC_{50} = 3.9 μM), **8m** (3-CH_3, IC_{50} = 3.8 μM), **8o** (2-OCH_3, IC_{50} = 4.5 μM), and **8l** (3-Cl, IC_{50} = 5.2 μM) showed IC_{50} value ≤ 5 μM at the MCF-7 cell line and





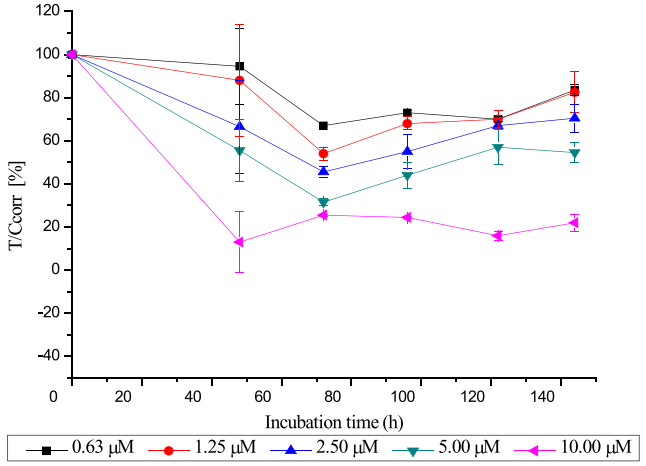


Fig. 2. Time dependent antiproliferative effects of compounds **8a** (up), **8e** (middle) and **8g** (below) on the human MCF-7 breast cancer cell line.

no growth inhibition of MDA-MB-231 cells ($IC_{50} > 50$ μM). Only a little less active ($IC_{50} = 5–10$ μM) were compounds **8i** (4-OCH_3), **8q** (2-OH), and **8s** (2-Cl) with retained selectivity ($IC_{50} > 50$ μM at MDA-MB-231 cells). Further modifications led to compounds with good activity and less selectivity (**8n** (2-Cl)), bad activity and low selectivity (**8r** (3-Cl, 5-NO_2)) and good activity with no selectivity (**8k** (4-NO_2) and **8p** (2-CH_3)). The exchange of the benzoate by a phenylacetate residue did not led to substantially changed results (compare **8h** and **8u**).

In order to study if growth inhibitory effects correlated with the inhibition of COX-1/2 enzymes licofelone and its derivatives **8a**–**g** were evaluated in an ELISA using the isolated isoenzymes (Fig. 1).

A drug concentration of 10 μM was used for the experiments since licofelone inhibited the COX at this concentration by about 50% (COX-1 (60.6%) and COX-2 (45.8%)). As depicted in Fig. 1, the selected compounds were distinctly less active (COX-1: 11–24%; COX-2: 9–16%) than licofelone. In each case, the COX-1 isoenzyme was more affected by the inhibitor than COX-2 indicating a comparable selectivity as demonstrated for licofelone. Furthermore, these results are also in accordance with those of Laufer et al. who found an occasionally decrease of COX inhibition after modification at position 5 [17]. However, a correlation of COX inhibition and growth inhibition of breast cancer cells were not visible.

Comparable to other cytostatics, the onset of activity in the growth inhibitory assay of the compounds **8a**–**g** was relatively slow. The time over activity (T/C_{corr}) correlation of **8a**, **8e** and **8g** shown as examples in Fig. 2 indicated a minimal T/C_{corr} (maximum of growth inhibition) after an incubation time 72 h which remained unchanged or increased only slowly. Based on our experience we therefore assume the selective interference with intracellular targets. The missing correlation with the inhibition of isolated COX enzymes, however, makes this target very unlikely.

Nevertheless, the anti-inflammatory effects of the licofelone derivatives were studied *in vivo* using the xylene-induced ear swelling in mice. As shown in Table 2, licofelone influenced the ear swelling in a dose-dependent manner, with an about 50% inhibition at a dose of 100 mg/kg. At the same dose, compound **8a** was as active as licofelone and the NSAIDs ibuprofen and celecoxibe. Compounds **8g**, **8i**, **8k** and **8l** showed the inhibition less than 16%. All further derivatives were half as active as licofelone with anti-inflammatory effects of about 20–30%.

Table 2
Effect of the compounds at 100 mg/kg on xylene-induced ear swelling in mice ($n = 6$, $\bar{x} \pm S$).

	Dose [mg/kg]	Swollen extent; weight [mg]	Inhibition (%)	Swollen extent; thickness [mm]	Inhibition (%)
Control		5.7 ± 1.4		0.138 ± 0.017	
Licofelone	25	3.8 ± 2.2*	33.3%	0.070 ± 0.032**	49.2%
	100	3.2 ± 0.8**	43.9%	0.066 ± 0.013**	52.2%
	200	1.3 ± 1.9**	77.2%	0.030 ± 0.029**	74.6%
8a	100	2.6 ± 1.2**	54.4%	0.068 ± 0.029**	50.7%
8b	100	4.3 ± 0.3*	24.6%	0.107 ± 0.033**	22.5%
8c	100	5.0 ± 0.1	14.0%	0.087 ± 0.036*	37.0%
8d	100	4.1 ± 2.3*	28.1%	0.104 ± 0.027*	24.6%
8e	100	4.6 ± 0.1	19.3%	0.110 ± 0.028*	20.3%
8f	100	4.6 ± 0.3	19.3%	0.087 ± 0.015**	37.0%
8g	100	5.5 ± 0.5	3.5%	0.121 ± 0.017	12.3%
8h	100	4.3 ± 0.6*	24.6%	0.097 ± 0.018**	29.7%
8i	100	4.8 ± 0.7	15.8%	0.124 ± 0.05	10.1%
8j	100	4.4 ± 2.7*	22.8%	0.109 ± 0.008**	21.0%
8k	100	5.6 ± 1.2	1.8%	0.129 ± 0.023	6.5%
8l	100	5.5 ± 1.8	3.5%	0.136 ± 0.010	1.4%
8m	100	4.7 ± 2.0*	17.5%	0.068 ± 0.046**	50.7%
8n	100	5.0 ± 1.4	12.3%	0.110 ± 0.025*	20.3%
8o	100	3.9 ± 0.7*	31.6%	0.113 ± 0.025*	18.1%
8p	100	5.0 ± 1.9*	12.3%	0.091 ± 0.10	34.1%
8q	100	3.9 ± 1.1*	31.6%	0.118 ± 0.010*	14.5%
8r	100	5.3 ± 1.3	7.0%	0.101 ± 0.029*	26.8%
8s	100	3.2 ± 0.6*	43.9%	0.127 ± 0.26**	8.0%
8t	100	4.2 ± 0.6*	26.3%	0.090 ± 0.015**	35.8%
8u	100	3.7 ± 1.7*	35.1%	0.087 ± 0.039*	37.0%
Ibuprofen	100	2.78 ± 0.37**	51.2%	0.063 ± 0.006**	54.3%
Celecoxibe	100	3.06 ± 0.49**	46.3%	0.072 ± 0.013*	47.9%

*$P < 0.05$, **$P < 0.01$. Data were subjected to one-way ANOVA.

4. Conclusion

In conclusion, an efficient method has been developed for the parallel synthesis of diversified novel 2,3-1*H*-dihydropyrrolizine derivatives. This parallel synthesis approach is highly efficient and suitable for the synthesis of large libraries of analogs. Through biological activity evaluation of the compound library, all novel licofelone derivatives compounds showed reduced anti-inflammatory effects. Only the acetyl derivative **8a** was as active as licofelone. The anti-inflammatory properties and the COX-1/2 inhibition did not correlate with the growth inhibition of MCF-7 and MDA-MB-231 cells. However, it was demonstrated that the substituent at C5 of licofelone determined the selectivity for MCF-7 cells (compared to MDA-MB-231 cells). This is a clear indication of selective interference with specific intracellular targets. The highest selectivity/growth inhibition showed the benzoate derivatives with 3-CH_3 (**8m**) and 4-Cl (**8h**) substituents which were inactive at MDA-MB-231 cells and caused $IC_{50} = 3.8$ and 3.9 μM, respectively at MCF-7 cells. The highest growth inhibition was determined with **8a** (C5-acetyl), and **8e**–**g** (C5-2-oxoethyl formiate/acetate/or propionate), however, independent on the cell line used. These results clearly documented that modification at position 5 at the 2,3-dihydro-1*H*-pyrrolizine core allows an optimization of licofelone for an effective and probably selective tumor therapy. Additional investigations to get insight into the mode of action as well as into structure–activity relationships are in progress and will be part of a forthcoming paper.

5. Experimental section

5.1. Chemistry

5.1.1. General

All reagents were purchased from Shanghai Chemical Reagent Company. 6-(4-Chlorophenyl)-2,2-dimethyl-7-phenyl-2,3-dihydro-1*H*-pyrrolizine (**6**) and licofelone were synthesized according to previous published method [14,24]. Column chromatography (CC): silica gel 60 (200–300 mesh). Thin-layer chromatography (TLC): silica gel 60 F254 plates (250 mm; Qingdao Ocean Chemical Company, China). Melting point: capillary tube; uncorrected. IR spectra: Shimadzu FTIR-8400S spectrophotometer.^{1}H NMR spectra: Bruker ACF-300 Q apparatus at 300 MHz (internal standard, TMS). Mass spectrometry (MS): Hewlett–Packard 1100 LC/MSD spectrometer; in *m*/*z*. Elemental analyses: CHN-O-Rapid instrument.

5.1.2. General procedures for the synthesis of the target compounds ***8a–d***

The reactions were performed in a 100 mL three neck round bottom flask fitted with a gas inlet port. The flask was charged with 50 mL of absolute diethyl ether, 0.4 g (1.27 mmol) of 6-(4-chlorophenyl)-2,2-dimethyl-7-phenyl-2,3-dihydro-1*H*-pyrrolizine (**6**), 0.25 mL of corresponding anhydride or chloroacetyl chloride and 3.72 mmol of Lewis acid. The resulting solution was magnetically stirred at room temperature and purged by nitrogen gas for 3–12 h. Subsequently, the reaction mixture was hydrolyzed with 60 mL of water and the product was extracted with diethyl ether. The organic layer was dried over Na_2SO_4 and the solvent was distilled off to give the crude product, which was then purified by flash

column chromatography with mixture eluent of petroleum ether and ethyl acetate to give the product.

*5.1.2.1. 1-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)ethanone (**8a**).* Yield 43.2%; mp 152–154 °C; MS (ESI, *m/z*): $[M + H]^+$ 364.1, $[M + Na]^+$ 386.1; IR (KBr, cm^{-1}): 2959, 2914, 2844, 1645, 1455, 843, 753, 694; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 1.87 (s, 3H, $-CH_3$), 2.81 (s, 2H, $-CH_2-$), 4.20 (s, 2H, $-CH_2-$), 6.95–7.3 3 (m, 9H, Ar–H). Anal. calcd for $C_{23}H_{22}ClNO$: C, 75.92; H, 6.09; N, 3.85%; found C, 75.71; H, 6.35; N, 4.03%.

*5.1.2.2. 1-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)propan-1- one (**8b**).* Yield 73.2%; mp 143–145 °C; MS (ESI, *m/z*): $[M + H]^+$ 378.2, $[M + Na]^+$ 400.2; IR (KBr, cm^{-1}): 3452, 2959, 2929, 2869, 1595, 1505, 948, 848, 694; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.935 (t, 3H, $J = 7.2$ Hz, $-CH_3$), 1.30 (s, 6H, 2-CH_3), 2.14 (q, 2H, $J = 7.2$ Hz, $-CH_2-$), 2.82 (s, 2H, $-CH_2-$), 4.22 (s, 2H, $-CH_2-$), 6.95–7.34 (m, 9H, Ar–H). Anal. calcd for $C_{24}H_{24}ClNO$: C, 76.28; H, 6.40; N, 3.71%; found C, 76.52; H, 6.39; N, 3.35%.

*5.1.2.3. 1-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)hexan-1- one (**8c**).* Yield 56.3%; mp 137–140 °C; MS (ESI, *m/z*): $[M + H]^+$ 420.1, $[M + Na]^+$ 442.3; IR (KBr, cm^{-1}): 2949, 2919, 2854, 1640, 1595, 967, 843, 694; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.82 (t, 3H, $J = 7.2$ Hz, $-CH_3$), 1.01 (m, 2H, $J = 7.2$ Hz, $-CH_2-$), 1.03 (m, 2H, $-CH_2-$), 1.15 (s, 6H, 2-CH_3), 1.44 (m, 2H, $-CH_2-$), 2.11 (t, 2H, $-CH_2-$), 2.81 (s, 2H, $-CH_2-$), 4.22 (s, 2H, $-CH_2-$), 6.95–7.34 (m, 9H, Ar–H). Anal. calcd. for $C_{27}H_{30}ClNO\cdot H_2O$: C, 74.04; H, 7.36; N, 3.20%; found C, 74.22; H, 7.18; N, 3.13%.

*5.1.2.4. 2-Chloro-1-(2-(4-chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)ethanone (**8d**).* Yield 59.3%; mp 157–159 °C; MS (ESI, *m/z*): $[M + H]^+$ 398.2, $[M + Na]^+$ 420.2; IR (KBr, cm^{-1}): 2919, 2839, 1645, 1595, 1450, 1012, 748, 704; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.31 (s, 6H, 2-CH_3), 2.84 (s, 2H, $-CH_2-$), 3.85 (s, 2H, $-CH_2-$), 4.24 (s, 2H, $-CH_2-$), 6.95–7.38 (m, 9H, Ar–H). Anal. calcd. for $C_{23}H_{21}Cl_2NO$: C, 69.35; H, 5.31; N, 3.52%; found C, 69.07; H, 5.41; N, 3.71%.

*5.1.3. General procedures for the synthesis of the target compounds **8e–u***

The flask was charged with 10 mL of absolute acetone, 1 mmol of triethylamine, 0.8 mmol of corresponding substituted aromatic and alkyl acid, after stirred for 15 min, 0.5 mmol of 2-chloro-1-(2-(4-chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5*H*-pyrrolizin-3-yl)ethanone (**8d**) was added. The resulting solution was stirred at room temperature for over 24 h. Then the solvent was distilled off to give the crude product, which was then purified by flash column chromatography with mixture eluent of petroleum ether and ethyl acetate to give the product, followed by recrystallization from acetone.

*5.1.3.1. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl formate (**8e**).* Yield 85.1%; mp 164–168 °C; MS (ESI, *m/z*): $[M + H]^+$ 406.2; IR (KBr, cm^{-1}): 3457, 2949, 1729, 1640, 958, 928, 694; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.33 (s, 6H, 2-CH_3), 2.84 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.45 (s, 2H, $-CH_2-$), 6.95–7.39 (m, 9H, Ar–H), 8.11 (s, 1H, $-CH$). Anal. Calcd. for $C_{24}H_{22}ClNO_3$: C, 70.64; H, 5.44; N, 3.43%; found C, 70.17; H, 5.31; N, 3.78%.

*5.1.3.2. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl acetate (**8f**).* Yield 86.7%; mp 186–188 °C; MS (ESI, *m/z*): $[M + H]^+$ 422; IR (KBr, cm^{-1}): 2954, 2924, 2869, 1744, 1645, 704; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.30 (s, 6H, 2-CH_3), 2.18 (s, 3H, $-CH_3$), 2.83 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.36 (s, 2H, $-CH_2-$), 6.95–7.38 (m, 9H, Ar–H). Anal. Calcd. for $C_{25}H_{24}ClNO_3$: C, 71.17; H, 5.73; N, 3.32%; found C, 69.92; H, 5.89; N, 3.54%.

*5.1.3.3. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl propionate (**8g**).* Yield 95.1%; mp 159–160 °C; MS (ESI, *m/z*): $[M + H]^+$ 436; IR (KBr, cm^{-1}): 2969, 2939, 2879, 1744, 1645, 1540, 972, 699; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.15 (t, 3H, $-CH_3$, $J = 7.8$ Hz)1.30 (s, 6H, 2-CH_3), 2.43 (q, 2H, $-CH_2-$, $J = 7.8$ Hz), 2.83 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.37 (s, 2H, $-CH_2-$), 6.95–7.38 (m, 9H, Ar–H). Anal. Calcd. for $C_{26}H_{26}ClNO_3$: C, 71.63; H, 6.01; N, 3.21%; found C, 71.52; H, 6.01; N, 3.45%.

*5.1.3.4. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 4-chlorobenzoate (**8h**).* Yield 70.3%; mp 224–226 °C; MS (ESI, *m/z*): $[M + H]^+$ 518; IR (KBr, cm^{-1}): 2964, 2919, 2854, 1729, 1650, 1600, 953, 743; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.84 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.60 (s, 2H, $-CH_2-$), 6.97–8.00 (m, 13H, Ar–H). Anal. Calcd. for $C_{30}H_{25}Cl_2NO_3$: C, 69.50; H, 4.86; N, 2.70%; found C, 69.07; H, 4.91; N, 3.10%.

*5.1.3.5. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 4-methoxybenzoate (**8i**).* Yield 56.0%; mp 230–240 °C; MS (ESI, *m/z*): $[M + H]^+$ 514, $[M + Na]^+$ 536; IR (KBr, cm^{-1}): 2955, 2914, 2854, 1719, 1729, 1600, 1510, 963, 768, 748; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.28 (s, 6H, 2-CH_3), 2.83 (s, 2H, $-CH_2-$), 3.84 (s, 3H, $-CH_3$), 4.21 (s, 2H, $-CH_2-$), 4.57 (s, 2H, $-CH_2-$), 6.87–8.01 (m, 13H, Ar–H). Anal. Calcd. for $C_{31}H_{28}ClNO_4$: C, 72.44; H, 5.49; N, 2.72%; found C, 72.07; H, 5.61; N, 3.11%.

*5.1.3.6. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 4-methylbenzoate (**8j**).* Yield 60.1%; mp 246–248 °C; MS (ESI, *m/z*): $[M + H]^+$ 498, $[M + Na]^+$ 520; IR (KBr, cm^{-1}): 2954, 1724, 1645, 1276, 958, 753, 694, 639; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.40 (s, 3H, $-CH_3$), 2.84 (s, 2H, $-CH_2-$), 4.22 (s, 2H, $-CH_2-$), 4.59 (s, 2H, $-CH_2-$), 6.97–7.95 (m, 13H, Ar–H). Anal. Calcd. for $C_{31}H_{28}ClNO_3$: C, 74.76; H, 5.67; N, 2.81%; found C, 74.29; H, 5.78; N, 3.22%.

*5.1.3.7. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 4-nitrobenzoate (**8k**).* Yield 75.8%; mp 104–107 °C; MS (ESI, *m/z*): $[M + H]^+$ 529; IR (KBr, cm^{-1}): 2929, 2849, 1734, 1660, 1276, 748; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.30 (s, 6H, 2-CH_3), 2.85 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.65 (s, 2H, $-CH_2-$), 6.98–8.30 (m, 13H, Ar–H). Anal. Calcd. for $C_{30}H_{25}ClN_2O_5$: C, 68.12; H, 4.76; N, 5.30%; found C, 67.97; H, 5.15; N, 5.42%.

*5.1.3.8. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 3-chlorobenzoate (**8l**).* Yield 75.4%; mp 199–200 °C; MS (ESI, *m/z*): $[M + Na]^+$ 540.1; IR (KBr, cm^{-1}): 2954, 2859, 1724, 1650, 958, 778, 748; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.83 (s, 2H, $-CH_2-$), 4.20 (s, 2H, $-CH_2-$), 4.60 (s, 2H, $-CH_2-$), 6.97–8.03 (m, 13H, Ar–H). Anal. Calcd. for $C_{30}H_{25}Cl_2NO_3$: C, 69.50; H, 4.86; N, 2.70%; found C, 69.21; H, 5.24; N, 2.48%.

*5.1.3.9. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 3-methylbenzoate (**8m**).* Yield 60.8%; mp 102–108 °C; MS (ESI, *m/z*): $[M + H]^+$ 498, $[M + Na]^+$ 520; IR (KBr, cm^{-1}): 2954, 2934, 2879, 1729, 1645, 967, 763, 704; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.18 (s, 6H, 2-CH_3), 2.51 (s, 3H, $-CH_3$), 2.76 (s, 2H, $-CH_2-$), 4.15 (s, 2H, $-CH_2-$), 4.51 (s, 2H, $-CH_2-$), 6.89–7.92 (m, 13H, Ar–H). Anal. Calcd. for $C_{31}H_{28}ClNO_3$: C, 74.76; H, 5.67; N, 2.81%; found C, 74.98; H, 5.34; N, 2.62%.

*5.1.3.10. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 2-chlorobenzoate (**8n**).* Yield 80.1%;

mp 180–181 °C; MS (ESI, *m/z*): $[M + H]^+$ 518.1, $[M + K]^+$ 556.1; IR (KBr, cm^{-1}): 2964, 2919, 2864, 1749, 1650, 1595, 958, 753; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.83 (s, 2H, $-CH_2-$), 4.22 (s, 2H, $-CH_2-$), 4.61 (s, 2H, $-CH_2-$), 6.96–8.00 (m, 13H, Ar–H). Anal. Calcd. for $C_{30}H_{25}Cl_2NO_3$: C, 69.50; H, 4.86; N, 2.70%; found C, 69.38; H, 5.13; N, 2.54%.

*5.1.3.11. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 2-methoxybenzoate (**8o**).* Yield 78.0%; mp 177–179 °C; MS (ESI, *m/z*): $[M + H]^+$ 514, $[M + Na]^+$ 536; IR (KBr, cm^{-1}): 2954, 2929, 2864, 1714, 1655, 958, 763, 694; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.83 (s, 2H, $-CH_2-$), 3.89 (s, 3H, $-CH_3$), 4.22 (s, 2H, $-CH_2-$), 4.58 (s, 2H, $-CH_2-$), 6.94–7.95 (m, 13H, Ar–H). Anal. Calcd. for $C_{31}H_{28}ClNO_4$: C, 72.44; H, 5.49; N, 2.72%; found C, 72.09; H, 5.54; N, 3.21%.

*5.1.3.12. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 2-methylbenzoate (**8p**).* Yield 73.2%; mp 178–180 °C; MS (ESI, *m/z*): $[M + H]^+$ 498, $[M + Na]^+$ 520; IR (KBr, cm^{-1}): 2959, 2929, 1724, 1650, 963, 793, 748, 699; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.18 (s, 6H, 2-CH_3), 2.51 (s, 3H, $-CH_3$), 2.76 (s, 2H, $-CH_2-$), 4.15 (s, 2H, $-CH_2-$), 4.51 (s, 2H, $-CH_2-$), 6.89–7.92 (m, 13H, Ar–H). Anal. Calcd. for $C_{31}H_{28}ClNO_3 \cdot CH_3COCH_3$: C, 73.43; H, 6.16; N, 2.52%; found C, 73.37; H, 6.42; N, 2.68%.

*5.1.3.13. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 2-hydroxybenzoate (**8q**).* Yield 78.7%; mp 180–182 °C; MS (ESI, *m/z*): $[M + H]^+$ 500, $[M + Na]^+$ 522; IR (KBr, cm^{-1}): 2964, 2939, 2874, 1689, 1645, 1605, 753, 694; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.84 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.62 (s, 2H, $-CH_2-$), 6.83–7.88 (m, 13H, Ar–H), 10.47 (s, 1H, –OH). Anal. Calcd. for $C_{30}H_{26}ClNO_4$: C, 72.07; H, 5.24; N, 2.80%; found C, 72.34; H, 5.03; N, 2.91%.

*5.1.3.14. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 3-methyl-5-nitrobenzoate (**8r**).* Yield 55.8%; mp 149–152 °C; MS (ESI, *m/z*): $[M + H]^+$ 543; IR (KBr, cm^{-1}): 2969, 2849, 1734, 1540, 963, 743; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.30 (s, 6H, 2-CH_3), 2.51 (s, 3H, $-CH_3$), 2.85 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.65 (s, 2H, $-CH_2-$), 6.89–8.86 (m, 12H, Ar–H). Anal. Calcd. for $C_{31}H_{27}ClN_2O_5 \cdot H_2O$: C, 66.37; H, 5.21; N, 4.99%; found C, 66.67; H, 5.13; N, 4.75%.

*5.1.3.15. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 2,4-dichlorobenzoate (**8s**).* Yield 56.0%; mp 140–141 °C; MS (ESI, *m/z*): $[M + H]^+$ 552; IR (KBr, cm^{-1}): 3068, 2944, 2864, 1739, 1655, 1585, 958, 833, 773; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 3.00 (s, 2H, $-CH_2-$), 4.22 (s, 2H, $-CH_2-$), 4.72 (s, 2H, $-CH_2-$), 6.98–7.95 (m, 12H, Ar–H). Anal. Calcd. for $C_{30}H_{24}Cl_3NO_3 \cdot CH_3COCH_3$: C, 64.87; H, 4.95; N, 2.29%; found C, 64.51; H, 4.62; N, 2.64%.

*5.1.3.16. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 2-phenylacetate (**8t**).* Yield 68.5%; mp 146–147 °C; MS (ESI, *m/z*): $[M + H]^+$ 498, $[M + Na]^+$ 520; IR (KBr, cm^{-1}): 3059, 2969, 2869, 1749, 1645, 1550, 958, 704; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.82 (s, 2H, $-CH_2-$), 3.72 (s, 2H, $-CH_2-$), 4.21 (s, 2H, $-CH_2-$), 4.38 (s, 2H, $-CH_2-$), 6.93–7.34 (m, 14H, Ar–H). Anal. Calcd. for $C_{31}H_{28}ClNO_3 \cdot H_2O$: C, 72.15; H, 5.86; N, 2.71%; found C, 72.01; H, 5.96; N, 2.82%.

*5.1.3.17. 2-(2-(4-Chlorophenyl)-6,6-dimethyl-1-phenyl-6,7-dihydro-5H-pyrrolizin-3-yl)-2-oxoethyl 2-(4-chlorophenyl)acetate (**8u**).* Yield 50.2%; mp 159–161 °C; MS (ESI, *m/z*): $[M-H]^-$ 530, $[M + Na]^+$ 554; IR (KBr, cm^{-1}): 2964, 2924, 2869, 1744, 1645, 748; ^1H NMR ($CDCl_3$, 300 MHz): δ 1.29 (s, 6H, 2-CH_3), 2.82 (s, 2H, $-CH_2-$), 3.68 (s, 2H, $-CH_2-$), 4.20 (s, 2H, $-CH_2-$), 4.37 (s, 2H, $-CH_2-$), 6.93–7.33 (m, 13H, Ar–H). Anal. Calcd. for $C_{31}H_{27}Cl_2NO_3$: C, 69.93; H, 5.11; N, 2.63%; found C, 69.57; H, 5.54; N, 2.71%.

5.2. Pharmacology

5.2.1. Cell culture

The human MCF-7 and MDA-MB-231 breast cancer cell lines were obtained from the American Type Culture Collection. Both cell lines were maintained as a monolayer culture in L-glutamine containing Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/L glucose (PAA Laboratories, Austria), supplemented with 5% fetal bovine serum (FBS; Biochrom, Germany) in a humidified atmosphere (5% CO_2) at 37 °C.

5.2.2. Cytotoxicity

The experiments were performed according to established procedures with some modifications [26]. In 96 well plates 100 μL of a cell suspension in culture medium at 7500 cells/mL (MCF-7 and MDA-MB-231) were plated into each well and were incubated for three days under culture conditions. After the addition of various concentrations of the test compounds, cells were incubated for up to 144 h. Then the medium was removed, the cells were fixed with glutardialdheyde solution 1% and stored under phosphate buffered saline (PBS) at 4 °C. Cell biomass was determined by a crystal violet staining, followed by extracting of the bound dye with ethanol and a photometric measurement at 590 nm. Mean values were calculated and the effects of the compounds were expressed as % Treated/$Control_{corr}$ values according to the following equations:

$$T/C_{corr}[\%] = \frac{T - C_0}{C - C_0} \cdot 100$$

(C_0 control cells at the time of compound addition; C control cells at the time of test end; T probes/samples at the time of test end).

The IC_{50} value was determined as the concentration causing 50% inhibition of cell proliferation and calculated as mean of at least two or three independent experiments (OriginPro 8).

5.2.3. Inhibition of COX enzymes

The inhibition of isolated ovine COX-1 and human recombinant COX-2 was determined with 10 μM of the respective compounds by ELISA ("COX inhibitor screening assay", Cayman Chemicals). Experiments were performed according to the manufacturer's instructions. (http://www.caymanchem.com/app/template/Product.vm/catalog/560131). Absorption was measured at 415 nm (Victor 2, Perkin Elmer). Results were calculated as the means of duplicate determinations.

5.2.4. Xylene-induced ear edema

5.2.4.1. Animals. The experiments applied with animals were approved by Research Ethic Committee of Jiang-Shu province, China. Kunming male mice of approximately 20 g were obtained from experimental animal center of ChinaPharmaceutical University, and fed with rat food and water ad libitum. All animals were fasted for 12 h before the experiments. The temperature (25 °C) and humidity (60%) in the animal room were well controlled.

5.2.4.2. Method. Mice were allotted to groups of 6 animals each. Thirty minutes after i.p. injection of the compounds **8a**–**u**, licofelone, ibuprofen and celecoxibe, 0.015 mL of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. Two hours after xylene application, mice were killed and both ears were removed. Circular sections were taken,

using a cork borer with a diameter of 7 mm, weighed and measured. The degree of ear swelling was calculated based on the weight and thickness of left ear without xylene.

5.2.4.3. Statistical analysis. The measurement data were expressed as the mean ± SD. Data were subjected to one-way analysis of variance (ANOVA), followed by multiple comparison with least significant differences (LSD) test or Dunnett's test as appropriate. Statistical significance was considered with $P < 0.05$. The analysis of data was performed by software SPSS 13.0.

Acknowledgments

The authors are grateful for the financial supports of National Natural Science Foundation of China (No. 30973638). The China scholarship council and the Deutsche Forschungsgemeinschaft (FOR 630) is gratefully acknowledged.

References

[1] A. Mantovani, P. Allavena, A. Sica, F. Balkwill, Nature 454 (2008) 436–444.
[2] D. Pereg, M. Lishner, J. Intern. Med. 258 (2005) 115–123.
[3] A. Agrawal, I.S. Fentiman, Int. J. Clin. Pract. 62 (2008) 444–449.
[4] A.P. Dicker, T.L. Williams, D.S. Grant, Am. J. Clin. Oncol. 24 (2001) 438–442.
[5] M.B. Terry, M.D. Gammon, F.F. Zhang, H. Tawfik, S.L. Teitelbaum, J.A. Britton, K. Subbaramaiah, A.J. Dannenberg, A.I. Neugut, JAMA 291 (2004) 2433–2440.
[6] A. Ristimaki, A. Sivula, J. Lundin, M. Lundin, T. Salminen, C. Haglund, H. Joensuu, J. Isola, Cancer Res. 62 (2002) 632–635.
[7] I. Ott, B. Kircher, P. Schumacher, K. Schmidt, R. Gust, J. Med. Chem. 48 (2005) 622–629.
[8] T. Wiglenda, I. Ott, B. Kircher, P. Schumacher, D. Schuster, T. Langer, R. Gust, J. Med. Chem. 48 (2005) 6516–6521.
[9] G. Rubner, K. Bensdorf, A. Wellner, S. Bergemann, I. Ott, R. Gust, Eur. J. Med. Chem. 45 (2010) 5157–5163.
[10] I. Ott, B. Kircher, C.P. Bagowski, D.H.W. Vlecken, E.B. Ott, J. Will, K. Bensdorf, W.S. Sheldrick, R. Gust, Angew. Chem. Int. Ed. 48 (2009) 1160–1163.
[11] L. Goossens, N. Pommery, J.P. Hénichart, Curr. Top. Med. Chem. 7 (2007) 283–296.
[12] J. Zhu, X. Song, H.P. Lin, D.C. Young, S. Yan, V.E. Marquez, C.S. Chen, J. Natl. Cancer Inst. 94 (2002) 1745–1757.
[13] X. Song, H.P. Lin, A.J. Johnson, P.H. Tseng, Y.T. Yang, S.K. Kulp, C.S. Chen, J. Natl. Cancer Inst. 94 (2002) 585–591.
[14] S.A. Laufer, J. Augustin, G. Dannhardt, W. Kiefer, J. Med. Chem. 37 (1994) 1894–1897.
[15] H. Ulbrich, B. Fiebich, G. Dannhardt, Eur. J. Med. Chem. 37 (2002) 953–959.
[16] A. Koeberle, U. Siemoneit, U. Buhring, H. Northoff, S. Laufer, W. Albrecht, O. Werz, J. Pharmacol. Exp. Ther. 326 (2008) 975–982.
[17] A.J. Liedtke, P.R. Keck, F. Lehmann, A. Koeberle, O. Werz, S.A. Laufer, J. Med. Chem. 52 (2009) 4968–4972.
[18] N.K. Narayanan, D. Nargi, M. Attur, S.B. Abramson, B.A. Narayanan, Anticancer Res. 27 (2007) 2393–2402.
[19] S. Tavolari, M. Bonafe, M. Marini, C. Ferreri, G. Bartolini, E. Brighenti, S. Manara, V. Tomasi, S. Laufer, T. Guarnieri, Carcinogenesis 29 (2008) 371–380.
[20] S. Laufer, H.G. Striegel, K. Neher, P. Zechmeister, C. Donat, K. Stolingwa, S. Baur, S. Tries, T. Kammermeier, G. Dannhardt, W. Kiefer, Arch. Pharm. (Weinheim) 330 (1997) 307–312.
[21] S. Laufer, H.G. Striegel, G. Dannhardt, Patent 94-4419247(4419247) 22 E. 1-6-1994 (1995) (Merckle GmbH, Germany).
[22] A. Kirschning, M. Ries, S. Domann, W. Martin, W. Albrecht, P. Arnold, S. Laufer, Bioorg. Med. Chem. Lett. 7 (1997) 903–906.
[23] K.S. Lam, Anti-Cancer Drug Des. 12 (1997) 145–167.
[24] G. Dannhardt, T. Kammermaier, P. Merckle, H.G. Striegel, S. Laufer, WO Patent 2003/018583 Chem. Abstr. 138 (2003) 204938.
[25] X.H. Liu, D.P. Rose, Cancer Res. 56 (1996) 5125–5127.
[26] A. Hille, R. Gust, Eur. J. Med. Chem. 45 (2010) 5486–5492.