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Design and synthesis of thiourea derivatives containing a benzo[5,6]cyclohepta[1,2-*b*]pyridine moiety as potential antitumor and anti-inflammatory agents

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ABSTRACT

Thiourea derivatives (**6a–e**) were developed and screened for antitumor and anti-inflammatory activity. Most of the compounds exhibited growth inhibitory effects comparable to 5-fluorouracil in vitro against mammary (MCF-7 and MDA-MB 231) as well as colon (HT-29) carcinoma cells. They also showed stronger anti-inflammatory activity than ibuprofen in vivo in the xylene-induced ear swelling assay in mice.

Cancer is a group of malignant diseases responsible for tremendous health costs associated with high level of mortality and morbidity.[1] Apart from the use of surgical treatment and irradiation, chemotherapy still remains an important option for its treatment. However, the use of available chemotherapeutics is often restricted mainly due to undesirable side effects and a limited choice of available anticancer drugs. Still, the successful treatment of cancer remains a challenge in the 21st century, and this clearly underlies the urgent need of developing novel and safe chemotherapeutic agents with more potent antitumor activities.

The thiourea derivatives represent one of the most promising classes of anticancer agents with a wide range of activities against various leukemia and solid tumors.[2–10] They play an important role as anticancer agents because of their good inhibitory activity against protein tyrosine kinases (PTKs),[3–6] human sirtuin type proteins 1 and 2 (SIRT1 and SIRT2),[7] topoisomerase II[8] and DNA repair synthesis.[9] For example, Liu and Jiang's group[3,4] reported a series of N-substituted-thiourea derivatives as epidermal growth factor receptor (EGFR) (one of important PTKs) inhibitors. The antitumor activity studies focused on optimizing activity against EGFR as this kinase plays an important role in tumor angiogenesis. In addition, thiourea derivatives also exhibit other various biological properties such as antiviral,[11] antimalaria,[12] antibacterial[13,14] and anti-inflammatory[14] activities and have therefore attracted considerable pharmaceutical interest.[11–16] For these reasons, the synthesis of thiourea and their functionalized derivatives is a primary objective.

The benzo[5,6]cyclohepta[1,2-*b*]pyridine is a highly efficient pharmacophore and widely used in drug molecular design. Derivatives containing this group such as loratadine (**4**), desloratadine (**5**), rupatadine and lonafarnib (Sch-66336) could exhibit antihistamine as well as antitumor and anti-inflammatory activities.[17–26] For instance, it has been demonstrated that loratadine, a second-generation H_1 histamine antagonist used to treat allergies, induced a cell cycle arrest in G2/M by interfering with the activity of these regulatory proteins.[19] Further investigations indicated that this drug had potential as a chemotherapeutic agent and as a modifier of radiation responsiveness in the treatment of cancer and may warrant further clinical evaluation.[20] In addition, desloratadine, a third-generation H_1 antihistamine, has been shown to have direct effects on inflammatory mediators in vitro.[21]

The above mentioned results induced us to investigate whether there would be some new beneficial properties if the benzo[5,6]cyclohepta[1,2-*b*]pyridine moiety was introduced in thiourea derivatives. Here we described the synthesis and the preliminary in vitro cytotoxic activity as well as the in vivo anti-inflammatory effects.

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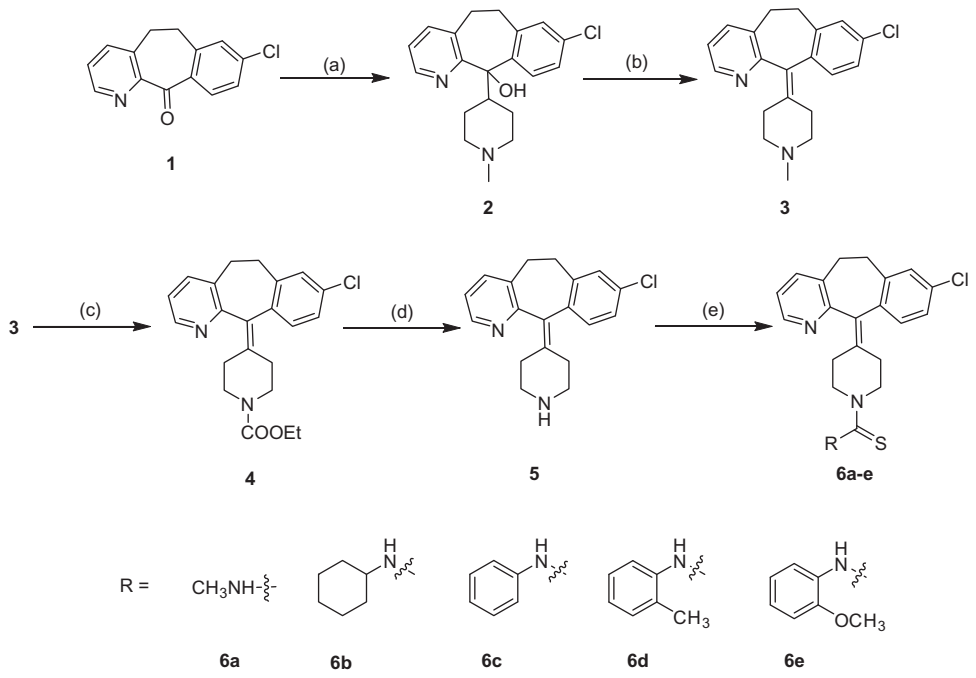

Scheme 1. Synthetic routes of thiourea derivatives **6a–e**. Reagents and conditions: (a) (1-methylpiperidin-4-yl)magnesium chloride (Grignard species provided in situ from 4-chloro-1-methylpiperidine and Mg 1:1), absolute THF, 3 h, reflux, 95%; (b) H_2SO_4 (85%), 4.5 h, rt, 65%; (c) $ClCO_2CH_2CH_3$, absolute toluene, Et_3N, 3 h, reflux, 72%; (d) KOH, EtOH (80%), 6 h, reflux, 93%; (e) RNCS, absolute EtOH, over 1 h, rt, 69–76%.

The synthetic routes to the thiourea derivatives **6a–e** are outlined in Scheme 1. Compounds **4** (loratadine) and **5** (desloratadine) were synthesized according to previously published methods.[24–26] The commercially available ketone **1** was treated with a Grignard reagent to give the corresponding tertiary carbinol **2** which was dehydrated with 85% H_2SO_4 affording the 8-chloro-11-piperidinylidene derivative **3**. Then **3** was converted to the corresponding carbamate **4** by employing ethyl chloroformate in toluene. The carbamate **4** was further cleaved under alkaline or acidic conditions to release the amine **5**. The yield of this reaction depended on the hydrolytic agent and solvent. In this study, several experimental conditions were investigated (Table 1). Among them using KOH as hydrolytic agent and EtOH (80%) as solvent led to the best yield (93%). Finally, **5** was treated with isothiocyanate derivatives in ethanol at room temperature (rt) to give the target compounds **6a–e** in high yield (69–76%).[27]

In vitro cytotoxicity assays were performed with **6a–e** according to established procedures[28–31] to get an insight into the antitumor activity. In addition, loratadine (**4**), desloratadine (**5**), the isothiocyanate derivatives and the antitumor drug 5-FU were screened against hormone-dependent MCF-7, hormone-independent MDA-MB 231 breast cancer and HT-29 colon cancer cell lines. In this assay, a known number of cells were exposed to increasing concentrations of compounds on a 96-well tissue culture plate and incubated for a given period of time. IC_{50} values for these compounds were calculated (OriginPro 8) and are presented in Table 2.

Interestingly, as mentioned in Table 2, our target compounds **6a–e** displayed IC_{50} values in the range of 4.7–10.4 μM in the tested cell lines. Therefore, these thiourea derivatives have a comparable activity as 5-FU which is widely employed in the treatment of cancer. Besides the compounds without benzo[5,6]cyclohepta[1,2-*b*]pyridine moiety (isothiocyanate derivatives, IC_{50} >40 μM, data not shown) were inactive against the tumor cells.

In addition, loratadine (**4**) caused comparable effects to compounds **6a–e** with IC_{50} values between 6.2 and 8.4 μM, while desloratadine (**5**) was less active with IC_{50} values of about 10 μM. These results indicated that the high growth inhibitory effects of **6a–e** might be due to the combination of benzo[5,6]cyclohepta[1,2-*b*]pyridine group with the thiourea structure.

Moreover, **6a–e** showed promising antiproliferative activities at MCF-7 cells (IC_{50} values of 5.1–9.4 μM; 5-FU: IC_{50} 4.7 μM). While **6a** (IC_{50} = 9.4 μM) and **6e** (IC_{50} = 7.6 μM) were less active than

Table 1
Experimental conditions to prepare **5** and its yields

Entry	Solvent	Hydrolytic agent	Reagent (**4**) (g)	Solvent volume (mL)	Reaction time (h)	Yield (%)
1	H_2O	KOH	2.0	10	24	85.4
2	EtOH (80%)	KOH	2.0	17	6	93.0
3	EtOH	KOH	2.0	17	10	92.3
4	EtOH	NaOH	2.0	84	56	75.2
5	EtOH (80%)	NaOH	2.0	17	24	73.8
6	H_2O	HCl	2.0	8	24	61.6
7	EtOH	HCl	2.0	17	8	55.4

Table 2
Antiproliferative effects against MCF-7, MDA-MB 231 and HT-29 cells

Compound	Cytotoxicity IC_{50}, (μM)[a]		
	MCF-7	MDA-MB 231	HT-29
6a	9.4 ± 0.7	7.1 ± 1.9	9.6 ± 2.6
6b	5.1 ± 0.2	8.1 ± 0.5	6.2 ± 0.4
6c	5.6 ± 0.5	6.4 ± 1.1	6.7 ± 0.5
6d	5.4 ± 1.9	4.7 ± 0.6	6.6 ± 0.6
6e	7.6 ± 0.1	10.4 ± 0.1	9.2 ± 1.4
Loratadine (**4**)	7.5 ± 0.7	8.4 ± 1.3	6.2 ± 2.4
Desloratadine (**5**)	10.5 ± 0.6	12.1 ± 1.1	11.2 ± 0.7
5-FU	4.7 ± 0.4	9.6 ± 0.3	7.3 ± 1.0

[a] The IC_{50} values represent the concentration which results in a 50% decrease in cell growth after 72 h incubation.