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Synthesis and biological evaluation of cyanoguanidine derivatives of loratadine

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ABSTRACT

Cyanoguanidine derivatives of loratadine (**3a–i**) were synthesized and screened for antitumor and anti-inflammatory activity. The most promising compound **3c** (R = *n*-C_8H_{17}) possessed at least twofold higher in vitro cytotoxicity than 5-fluorouracil against mammary (MCF-7 and MDA-MB 231) as well as colon (HT-29) carcinoma cells. The mode of action, however, is so far unclear. The participation of the COX-1/2 enzymes on the cytotoxicity, however, is very unlikely. Nevertheless all compounds showed stronger in vivo anti-inflammatory activity than ibuprofen in the xylene-induced ear swelling assay in mice.

Cancer, a group of malignant diseases is responsible for tremendous health costs associated with high levels of mortality and morbidity and remains the second leading cause of death in the world.[1] Although chemotherapy is the mainstay of cancer therapy, the use of available chemotherapeutics is often limited due to undesirable side effects and the development of resistance during the therapy. Therefore, the successful treatment of cancer still remains a challenge in the 21st century, and clearly underscores the need of novel chemotherapeutic agents.

Benzo[5,6]cyclohepta[1,2-*b*]pyridine derivatives (for examples see Fig. 1) exhibited various biological effects and had therefore attracted considerable pharmaceutical interest.[2–14] Recently, it was demonstrated that the second-generation H_1 histamine antagonist loratadine (Fig. 1) induces cell cycle arrest of tumor cells in G2/M phase.[6] Then further investigations documented its potential as a chemotherapeutic agent as well as a modifier of radiation responsiveness in the treatment of cancer and might warrant further clinical evaluation.[7] In preclinical studies lonafarnib (Fig. 1), a farnesyl protein transferase inhibitor showed selective anticancer activity in a broad range of solid and hematologic tumor cell lines, including those with wild-type Ras. This cellular Ras plays an important role in cellular proliferation mediated by growth factor receptor. Moreover, lonafarnib showed activity against human lung, prostate, pancreas, colon, bladder and glioblastom also in in vivo studies.[8–11]

In our group, we started a project to optimize loratadine as chemotherapeutic agent for the treatment of inflammatory cancerous diseases (e.g. the mammary carcinoma). In the first part of this study we designed a series of thiourea derivatives containing the benzo[5,6]cyclohepta[1,2-*b*]pyridine moiety of loratadine. These compounds were as active as 5-fluorouracil (5-FU) in vitro against tumor cells and caused stronger anti-inflammatory effects than ibuprofen in vivo.[12]

In the next step we combined the benzo[5,6]cyclohepta[1,2-*b*]pyridine core with a cyanoguanidine moiety which is a highly efficient pharmacophore.[15–23] The influence of alkyl chains and substituted aromatic rings at the cyanoguanidine on the in vitro cytotoxicity and the anti-inflammatory potency were studied.

The synthetic routes to get the target compounds **3a–i** are outlined in Schemes 1 and 2. The synthon desloratadine (**1**), synthesized according to previously published methods[12–14] was reacted with substituted methyl *N*′-cyanocarbamimidothioates **2a–e** in toluene under reflux for 6.5 h yielding **3a–e** (Scheme 1).[24] Compounds **2a–e** were previously obtained from substituted amines and dimethyl cyanocarbonimidodithioate.

Unfortunately, this synthetic route failed in the synthesis of **3f–i**. So we used the *N*-cyano-1-piperidinecarboximidothioic acid methyl ester **4**[25] as intermediate. Reaction with substituted amines in toluene under reflux for 9 h gave the corresponding compounds **3f–i** (Scheme 2).[26]

All new compounds (**3a–i**) as well as loratadine, desloratadine, and the established antitumor drug 5-FU were screened for

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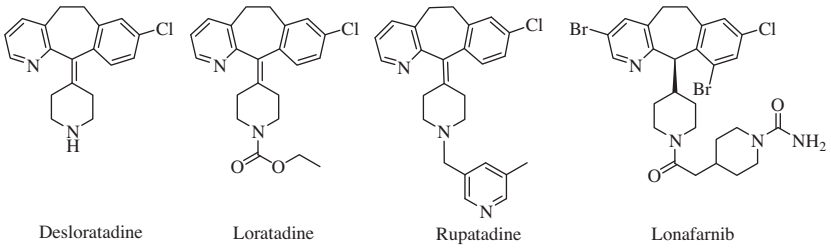
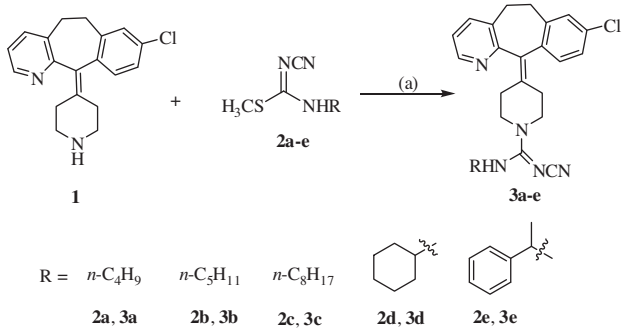


Figure 1. Representatives of benzo[5,6]cyclohepta[1,2-*b*]pyridine derivatives.



Scheme 1. Synthetic routes of **3a–e**. Reagents and conditions: (a) toluene, 6.5 h, reflux, 37.2–71.1%.

cytotoxicity against breast (MCF-7 and MDA-MB 231) and colon (HT-29) cancer cell lines. The experiments were performed according to established procedures.[12,27–29]

Loratadine caused comparable effects as 5-FU against MDA-MB 231 (loratadine: IC_{50} = 8.4 μM; 5-FU: IC_{50} = 9.6 μM) and HT-29 (loratadine: IC_{50} = 6.2 μM; 5-FU: IC_{50} = 7.3 μM) cells. At MCF-7 cells, loratadine (IC_{50} = 7.5 μM) was less active than 5-FU (IC_{50} = 4.7 μM). For desloratadine IC_{50} values of about 10–12 μM were calculated at all cell lines.

As outlined in Table 1, most of the target compounds (**3a** to **3g**) displayed IC_{50} values (1.4–6.9 μM) lower than 5-FU and loratadine. Especially the most promising compound **3c** (IC_{50} (MCF-7) = 1.4 μM; IC_{50} (MDA-MB 231) = 4.1 μM; IC_{50} (HT-29) = 2.0 μM) possessed at least 2-fold higher cytotoxic potential than 5-FU and loratadine. In addition, the IC_{50} values of **3a–g** were lower than those of the thiourea derivatives (IC_{50} = 4.7–10.4 μM) which indicate that the introduction of the substituted cyanoguanidine moiety in loratadine was more effective than the substituted thiourea moiety.[12]

Table 1
Cytotoxicity against MCF-7, MDA-MB 231 and HT-29 cells

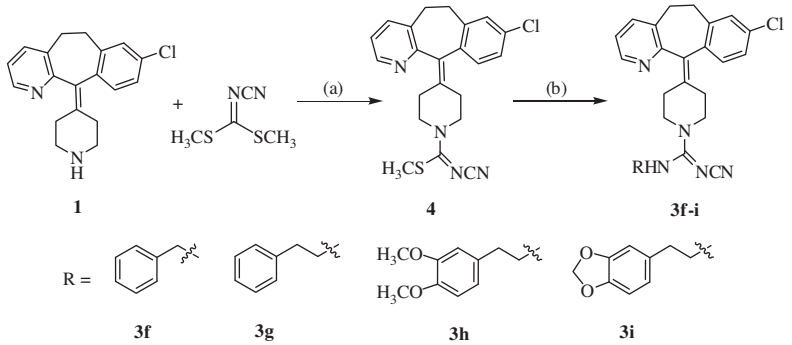
Compounds	Cytotoxicity IC_{50}[a], (μM)		
	MCF-7	MDA-MB 231	HT-29
3a	3.9 ± 0.3	3.1 ± 0.5	4.0 ± 0.2
3b	3.5 ± 0.1	1.7 ± 0.1	4.4 ± 0.2
3c	1.4 ± 0.2	4.1 ± 1.1	2.0 ± 0.2
3d	3.9 ± 1.0	1.9 ± 0.4	4.0 ± 0.9
3e	4.0 ± 0.3	6.9 ± 1.0	3.7 ± 1.0
3f	2.9 ± 0.4	2.7 ± 1.0	3.1 ± 0.5
3g	2.3 ± 0.7	2.6 ± 0.4	3.2 ± 0.7
3h	7.1 ± 0.4	18.2 ± 0.2	5.7 ± 1.6
3i	8.0 ± 0.3	12.9 ± 1.2	6.5 ± 1.1
Loratadine	7.5 ± 0.7	8.4 ± 1.3	6.2 ± 2.4
Desloratadine (**1**)	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 of incubation.

The experimental cytotoxic activity data suggested that substituents at the aromatic ring of the *N*′-phenylethyl residue might reduce the cytotoxic potency. The 3,4-dimethoxy (**3h**) and the 3,4-methylenedioxy (**3i**) derivatives were less effective than the unsubstituted **3g**.

Compound **3c** was further investigated in a time-dependent test for antiproliferative activity. The time over activity (T/C_{corr}) correlation is shown in Figure 2 and indicates only a marginal recuperation of cells after a prolonged time of exposition. The minimal T/C_{corr} (maximum of growth inhibition) after an incubation time of 48 h remained nearly unchanged or increased only slowly. Therefore, the development of drug resistance is very unlikely.

Next, as the thiourea derivatives containing a benzo[5,6]cyclohepta[1,2-*b*]pyridine moiety showed anti-inflammatory activity in the well-known xylene-induced ear swelling assay in mice[12,27,30] we investigated the cyanoguanidine derivatives in the same in vivo assay, too. As shown in Table 2, **3a–i** exhibited anti-inflammatory activity at a dose of 4 mg/kg b.w. comparable to



Scheme 2. Synthetic routes of **3f–i**. Reagents and conditions: (a) absolute ethanol, rt, over 2 h, 81,2%; (b) substituted amine, toluene, 9 h, reflux, 53.2–79.2%.