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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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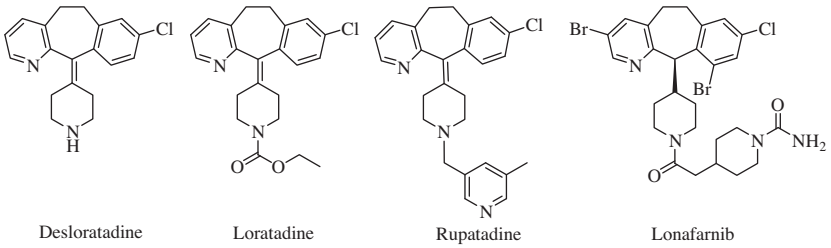
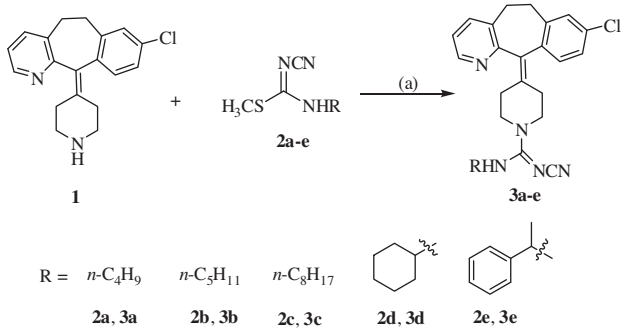


Figure 1. Representatives of benzo[5,6]cyclohepta[1,2-*b*]pyridine derivatives.



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

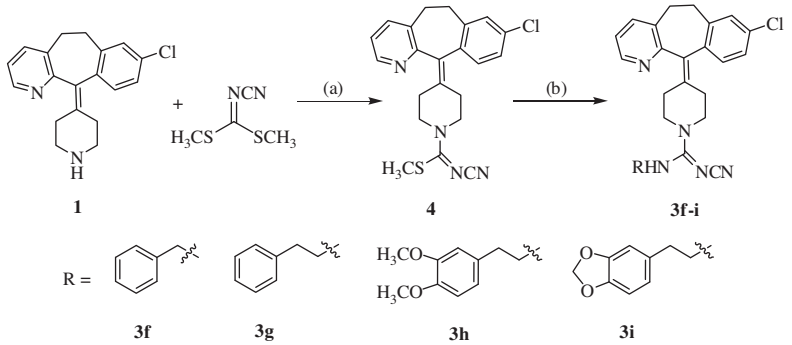
Compounds	Cytotoxicity IC_{50}[a], (μM)		
	MCF-7	MDA-MB 231	HT-29
3a	3.9 ± 0.3	3.1 ± 0.5	4.0 ± 0.2
3b	3.5 ± 0.1	1.7 ± 0.1	4.4 ± 0.2
3c	1.4 ± 0.2	4.1 ± 1.1	2.0 ± 0.2
3d	3.9 ± 1.0	1.9 ± 0.4	4.0 ± 0.9
3e	4.0 ± 0.3	6.9 ± 1.0	3.7 ± 1.0
3f	2.9 ± 0.4	2.7 ± 1.0	3.1 ± 0.5
3g	2.3 ± 0.7	2.6 ± 0.4	3.2 ± 0.7
3h	7.1 ± 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%.

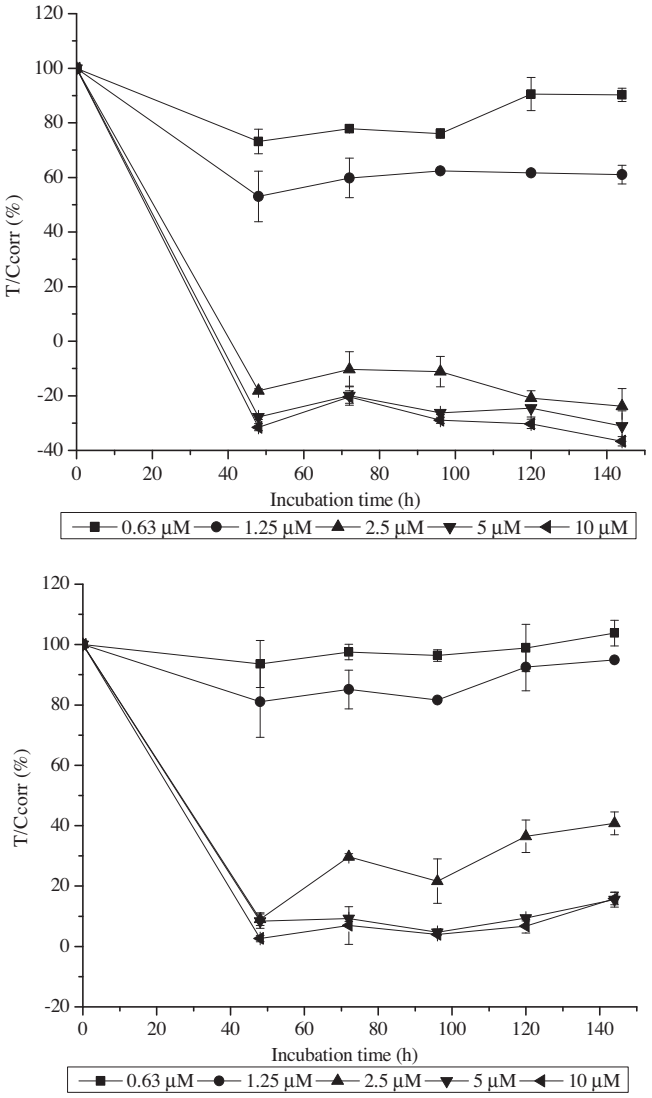


Figure 2. Time activity curves of **3c** on MCF-7 (up) and HT-29 (left) cell lines. Error bars are hidden behind the symbols in some cases.

Table 2
Effect of the compounds on xylene-induced ear swelling in mice ($n = 8$, $\bar{x} \pm S$)

	Dose (mg/kg)	Swollen extent; weight (g)	Inhibition (%)
Control		0.023 ± 0.003	
3a	4	0.019 ± 0.005*	17.39
3b	4	0.020 ± 0.006	13.04
3c	4	0.019 ± 0.004*	15.55
3d	4	0.020 ± 0.001	17.39
3e	4	0.017 ± 0.008*	26.09
3f	4	0.020 ± 0.003	13.04
3g	4	0.020 ± 0.003	13.04
3h	4	0.018 ± 0.006*	21.73
3i	4	0.020 ± 0.002	13.04
Ibuprofen	4	0.021 ± 0.002*	8.22
Ibuprofen	30	0.014 ± 0.004*	39.13

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

the thiourea derivatives.[12] Especially the high anti-inflammatory inhibition rate of **3e** (26.09%) is worthy to mention. In addition, all compounds (13.04–26.09% inhibition) were more active than ibuprofen (8.22% inhibition) used at the same dose (4 mg/kg b.w.). To achieve the same activity as **3e** ibuprofen has to be used at a dose of 30 mg/kg b.w. (39.13% inhibition).

Finally, in order to study if the inhibition of COX-1/2 enzymes (common targets for both antitumor and anti-inflammation) contributed to the anti-inflammatory and antitumor properties, we exemplarily examined **3e** by ELISA.[12,27,28,31] In these experiments, **3e** caused only a weak COX inhibition (COX-1 (4.9%) and COX-2 (18.9%)) at 10 μM (indomethacin as reference: COX-1 inhibition: 56%; COX-2 inhibition: 100%). Therefore, **3e** and its derivatives may not interfere in the arachidonic acid cascade via COX enzymes.

In conclusion, a series of cyanoguanidine derivatives of loratadine was designed and synthesized. All compounds have potential antitumor activity in the preliminary cytotoxic bioassay. **3c** was the most cytotoxic compound against tumor cells used in this study. Furthermore, the in vivo anti-inflammatory tests indicated that these compounds were more active than ibuprofen in the xylene-induced ear swelling assay. Additional investigations on the biological activity as well as an extended structure–activity relationship study are in progress and will be part of a forthcoming paper.

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References and notes

1. Smith, R. A.; Cokkinides, V.; Brooks, D.; Saslow, D.; Shah, M.; Brawley, O. W. *CA Cancer J. Clin.* **2011**, *61*, 8.
2. Gelfand, E. W.; Appajosyula, S.; Meeves, S. *Curr. Med. Res. Opin.* **2004**, *20*, 73.
3. Agrawal, D. K. *Clin. Exp. Allergy* **2004**, *34*, 1342.
4. Wang, Y.; Wang, J.; Lin, Y.; Si-Ma, L. F.; Wang, D. H.; Chen, L. G.; Liu, D. K. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4454.
5. Liu, G. Z.; Xu, H. W.; Chen, G. W.; Wang, P.; Wang, Y. N.; Liu, H. M.; Yu, D. Q. *Bioorg. Med. Chem.* **2010**, *18*, 1626.
6. Soule, B. P.; Simone, N. L.; DeGraff, W. G.; Choudhuri, R.; Cook, J. A.; Mitchell, J. B. *Radiat. Oncol.* **2010**, *5*, 8.
7. Chen, J. S.; Lin, S. Y.; Tso, W. L.; Yeh, G. C.; Lee, W. S.; Tseng, H.; Chen, L. C.; Ho, Y. S. *Mol. Carcinog.* **2006**, *45*, 461.
8. Robak, T.; Szmigielska-Kaplon, A.; Pluta, A.; Grzybowska-Izydorczyk, O.; Wolska, A.; Czemerska, M.; Wierzbowska, A. *Curr. Med. Chem.* **2011**, *18*, 638.
9. Castaneda, C.; Meadows, K. L.; Truax, R.; Morse, M. A.; Kaufmann, S. H.; Petros, W. P.; Zhu, Y.; Statkevich, P.; Cutler, D. L.; Hurwitz, H. I. *Cancer Chemother. Pharmacol.* **2011**, *67*, 455.
10. Zhu, H. Y.; Cooper, A. B.; Desai, J.; Njoroge, G.; Kirschmeier, P.; Bishop, W. R.; Strickland, C.; Hruza, A.; Doll, R. J.; Girijavallabhan, V. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1134.
11. Basso, A. D.; Mirza, A.; Liu, G.; Long, B. J.; Bishop, W. R.; Kirschmeier, P. *J. Biol. Chem.* **2005**, *280*, 31101.
12. Liu, W.; Zhou, J.; Zhang, T.; Zhu, H.; Qian, H.; Zhang, H.; Huang, W.; Gust, R. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2701.
13. Sasse, B. C.; Mach, U. R.; Leppaenen, J.; Calmels, T.; Stark, H. *Bioorg. Med. Chem.* **2007**, *15*, 7258.
14. Piwinski, J. J.; Wong, J. K.; Green, M. J.; Ganguly, A. K.; Billah, M. M.; West, R. E., Jr.; Kreutner, W. *J. Med. Chem.* **1991**, *34*, 457.
15. Geyer, R.; Buschauer, A. *Arch. Pharm.* **2011**, *344*, 775.
16. Igel, P.; Dove, S.; Buschauer, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7191.
17. Birnkammer, T.; Spickenreither, A.; Brunskole, I.; Lopuch, M.; Kagermeier, N.; Bernhardt, G.; Dove, S.; Seifert, R.; Elz, S.; Buschauer, A. *J. Med. Chem.* **2012**, *55*, 1147.
18. Binderup, E.; Bjorkling, F.; Hjarnaa, P. V.; Latini, S.; Baltzer, B.; Carlsen, M.; Binderup, L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2491.
19. Li, S. L.; Huang, C. H.; Lin, C. C.; Huang, Z. N.; Chern, J. H.; Lien, H. Y.; Wu, Y. Y.; Cheng, C. H.; Chang, C. Y.; Chuu, J. J. *Invest. New Drugs* **2011**, *29*, 195.
20. Wolin, R.; Connolly, M.; Kelly, J.; Weinstein, J.; Rosenblum, S.; Afonso, A.; James, L.; Kirschmeier, P.; Bishop, W. R. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2521.
21. Cooper, A. B.; Strickland, C. L.; Wang, J.; Desai, J.; Kirschmeier, P.; Patton, R.; Bishop, W. R.; Weber, P. C.; Girijavallabhan, V. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 601.
22. Florence, X.; Sebille, S.; Tullio, P.; Lebrun, P.; Pirotte, B. *Bioorg. Med. Chem.* **2009**, *17*, 7723.

23. Shi, Y.; Zhang, J.; Shi, M.; O'Connor, S. P.; Bisaha, S. N.; Li, C.; Sitkoff, D.; Pudzianowski, A. T.; Chong, S.; Klei, H. E.; Kish, K.; Yanchunas, J., Jr.; Liu, E. C.; Hartl, K. S.; Seiler, S. M.; Steinbacher, T. E.; Schumacher, W. A.; Atwal, K. S.; Stein, P. D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4034.

24. *General procedure for the synthesis of* ***3a–e***. 2.0 g (6.4 mmol) of **1** (desloratadine) and 3.8 mmol of substituted methyl *N*′-cyanocarbamimidothioate **2a–e** were combined and refluxed in 25 mL of toluene for 6.5 h. Then the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography with a mixture eluent of petroleum ether and ethyl acetate to give the corresponding product.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-(n-butyl)-1-piperidinecarboximidamide (**3a**)*. Yield: 42.1% of a white solid; mp: 151–152 °C. ESI-MS: 434.1 $[M+H]^+$, 456.1 $[M+Na]^+$. IR (cm^{-1}): 3443, 3292, 2954, 2926, 2868, 2163, 1574, 1556, 1436, 1324, 1298, 1246, 997, 828. ^1H NMR ($CDCl_3$) δ: 0.9–0.94 (t, 3H, CH_3); 1.25–1.42 (m, 2H, CH_2); 1.51–1.60 (m, 2H, CH_2); 2.33–2.43 (m, 2H, $=C(CH_2)_2$); 2.48–2.67 (m, 2H, $=C(CH_2)_2$); 2.76–2.89 (m, 2H, $N(CH_2)_2$); 3.26–3.40 (m, 6H, $PhCH_2$, NCH_2, $N(CH_2)_2$); 3.67–3.71 (m, 2H, CH_2); 4.81 (br, 1H, NH); 7.09–7.17 (m, 4H, Ar-H, pyridine-H); 7.49 (dd, 1H, J = 7.6 Hz, pyridine-H); 8.40 (dd, 1H, J = 1.4 Hz, pyridine-H). Anal. Calcd for $C_{25}H_{28}ClN_5$: C, 69.19; H, 6.50; N, 16.14. Found C, 69.14; H, 6.52; N, 16.20.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-(n-amyl)-1-piperidinecarboximidamide (**3b**)*. Yield: 37.2% of a yellow solid; mp: 155–156 °C. ESI-MS: 448.2 $[M+H]^+$, 470.1 $[M+Na]^+$. IR (cm^{-1}): 3290, 2953, 2928, 2868, 2158, 1573, 1553, 1503, 1435, 1322, 1295, 999, 993, 827, 810. ^1H NMR ($CDCl_3$) δ: 0.89 (t, 3H, CH_3); 1.25–1.33 (m, 4H, $2CH_2$); 1.52–1.61 (m, 2H, CH_2); 2.36–2.39 (m, 2H, $=C(CH_2)_2$); 2.41–2.81 (m, 2H, $=C(CH_2)_2$); 2.83–2.84 (m, 2H, $N(CH_2)_2$); 3.27–3.39 (m, 6H, $PhCH_2$, NCH_2, $N(CH_2)_2$); 3.67–3.73 (m, 2H, CH_2); 4.82 (br, 1H, NH); 7.09–7.17 (m, 4H, Ar-H, pyridine-H); 7.48 (dd, 1H, J = 7.5 Hz, pyridine-H); 8.40 (dd, 1H, J = 3.9 Hz, pyridine-H). Anal. calcd for $C_{26}H_{30}ClN_5$: C, 69.70; H, 6.75; N, 15.63. Found C, 69.69; H, 6.81; N, 15.71.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-(n-octyl)-1-piperidinecarboximidamide (**3c**)*. Yield: 41.4% of a white solid; mp: 136–137 °C. ESI-MS: 490.2 $[M+H]^+$, 512.3 $[M+Na]^+$. IR (cm^{-1}): 3285, 2952, 2920, 2852, 2161, 1571, 1538, 1436, 1329, 1297, 1254, 1174, 1085, 993, 829, 783. ^1H NMR ($CDCl_3$) δ: 0.86 (t, 3H, CH_3); 1.25–1.26 (m, 10H, $5CH_2$); 1.53–1.57 (m, 2H, CH_2); 2.35–2.40 (m, 2H, $=C(CH_2)_2$); 2.50–2.78 (m, 2H, $=C(CH_2)_2$); 2.80–2.84 (m, 2H, $N(CH_2)_2$); 3.24–3.39 (m, 6H, $PhCH_2$, NCH_2, $N(CH_2)_2$); 3.65–3.72 (m, 2H, CH_2); 4.88 (br, 1H, NH); 7.08–7.16 (m, 4H, Ar-H, pyridine-H); 7.46 (dd, 1H, J = 7.6 Hz, pyridine-H); 8.38 (dd, 1H, J = 3.5 Hz, pyridine-H). Anal. calcd for $C_{29}H_{36}ClN_5$: C, 71.07; H, 7.40; N, 14.29. Found C, 71.01; H, 7.38; N, 14.32.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-cyclohexyl-1-piperidinecarboximidamide (**3d**)*. Yield: 71.1% of a white solid; mp: 219–220 °C. ESI-MS: 460.2 $[M+H]^+$, 482.1 $[M+Na]^+$. IR (cm^{-1}): 3293, 3117, 2924, 2853, 2159, 1576, 1528, 1478, 1436, 1329, 1298, 1246, 1174, 1086, 995, 826. ^1H NMR ($CDCl_3$) δ: 1.12–2.00 (m, 10H, $5CH_2$); 2.34–2.43 (m, 2H, $=C(CH_2)_2$); 2.51–2.77 (m, 2H, $=C(CH_2)_2$); 2.80–2.92 (m, 2H, $N(CH_2)_2$); 3.30–3.40 (m, 4H, $N(CH_2)_2$, $PhCH_2$); 3.67–3.77 (m, 3H, CH, CH_2); 4.56 (br, 1H, NH); 7.12–7.20 (m, 4H, Ar-H, pyridine-H); 7.56 (dd, 1H, J = 7.4 Hz, pyridine-H); 8.43 (dd, 1H, J = 4.9 Hz, pyridine-H). Anal. calcd for $C_{27}H_{30}ClN_5{\cdot}H_2O$: C, 67.84; H, 6.75; N, 14.65. Found C, 67.79; H, 6.79; N, 14.52.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-(1-phenylethyl)-1-piperidinecarboximidamide (**3e**)*. Yield: 38.3% of a white solid; mp: 135–136 °C. ESI-MS: 482.1 $[M+H]^+$, 504.1 $[M+Na]^+$. IR (cm^{-1}): 3425, 3259, 2974, 2920, 2853, 2168, 1572, 1527, 1478, 1436, 1329, 1292, 1174, 1087, 993, 829, 762, 699. ^1H NMR ($CDCl_3$) δ: 1.56 (d, 3H, CH_3, J = 6.5 Hz); 2.30–2.38 (m, 2H, $=C(CH_2)_2$); 2.48–2.76 (m, 2H, $=C(CH_2)_2$); 2.78–2.88 (m, 2H, $N(CH_2)_2$); 3.30–3.38 (m, 4H, $N(CH_2)_2$, $PhCH_2$); 3.66–3.85 (m, 2H, CH_2); 4.98–5.08 (m, 2H, NHCH); 7.14–7.33 (m, 9H, Ar-H, pyridine-H); 7.55 (dd, 1H, J = 3.4 Hz, pyridine-H); 8.41 (dd, 1H, J = 4.4 Hz, pyridine-H). Anal. calcd for $C_{29}H_{28}ClN_5$: C, 72.26; H, 5.86; N, 14.53. Found C, 72.31; H, 5.89; N, 14.45.

25. *4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-1-piperidinecarboximidothioic acid methyl ester (**4**)*. The flask was charged with 60 mL of absolute ethanol, 6.0 g (19.3 mmol) of **1** (desloratadine) and 2.9 g (20 mmol) of dimethyl cyanocarbonimidodithioate. The resulting solution was stirred at rt for over 2 h. Then the white suspension was filtered under suction and the solid was washed with a small amount of cold ethanol to give the white product **4** (6.4 g). Yield: 81.2% of a white solid; mp: 162–164 °C. ESI-MS: 409.0 $[M+H]^+$. IR (cm^{-1}): 3080, 2929, 2894, 2852, 2180, 1548, 1540, 1456, 1434, 991, 834. ^1H NMR ($CDCl_3$) δ: 2.36–2.44 (m, 2H, $=C(CH_2)_2$); 2.47–2.72 (m, 2H, $=C(CH_2)_2$); 2.76 (s, 3H, SCH_3); 2.78–2.90 (m, 2H, $N(CH_2)_2$); 3.28–3.39 (m, 2H, $N(CH_2)_2$); 3.50–3.60 (m, 2H, $PhCH_2$); 4.03–4.15 (m, 2H, CH_2); 7.09–7.18 (m, 3H, Ar-H); 7.26 (m, 1H, pyridine-H); 7.47 (dd, 1H, J = 7.3 Hz, pyridine-H); 8.40 (dd, 1H, J = 4.7 Hz, pyridine-H).

26. *General procedure for the synthesis of* ***3f–i***. 1.0 g (2.4 mmol) of **4** and 3.3 mol of the respectively substituted amine were combined and refluxed in 25 mL of toluene for 9 h. Then the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography with a mixture eluent of petroleum ether and ethyl acetate to give the corresponding product.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-benzyl-1-piperidinecarboximidamide (**3f**)*. Yield: 53.2% of a white solid; mp: 177–178 °C. ESI-MS: 468.1 $[M+H]^+$. IR (cm^{-1}): 3238, 3116, 3055, 3028, 2924, 2856, 2156, 1556, 1536, 1495, 1435, 1356, 1301, 990, 828, 695. ^1H NMR ($CDCl_3$) δ: 2.33–2.39 (m, 2H, $=C(CH_2)_2$); 2.46–2.65 (m, 2H, $=C(CH_2)_2$); 2.75–2.87 (m, 2H, $N(CH_2)_2$); 3.27–3.37 (m, 4H, $N(CH_2)_2$, $PhCH_2$); 3.69–3.73 (m, 2H, CH_2); 4.52–4.54 (m, 2H, NCH_2); 5.41–5.50 (br, 1H, NH); 7.06–7.16 (m, 4H, Ar-H); 7.26–7.33 (m, 5H, Ar-H, pyridine-H); 7.44 (dd, 1H, J = 7.6 Hz, pyridine-H); 8.35 (dd, 1H, J = 4.6 Hz, pyridine-H). Anal. calcd for $C_{28}H_{26}ClN_5$: C, 71.86; H, 5.50; N, 14.96. Found C, 71.77; H, 5.62; N, 14.21.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-(2-phenylethyl)-1-piperidinecarboximidamide (**3g**)*. Yield: 79.2% of a white solid; mp: 178–180 °C. ESI-MS: 482.1 $[M+H]^+$. IR (cm^{-1}): 3226, 3085, 2925, 2882, 2856, 2163, 1556, 1456, 1436, 995, 829, 699. ^1H NMR ($CDCl_3$) δ: 2.27–2.34 (m, 2H, $=C(CH_2)_2$); 2.39–2.60 (m, 2H, $=C(CH_2)_2$); 2.75–2.84 (m, 2H, $N(CH_2)_2$); 2.89 (t, 2H, CH_2Ar); 3.15–3.22 (m, 2H, $N(CH_2)_2$); 3.27–3.37 (m, 2H, $PhCH_2$); 3.55–3.65 (m, 2H, CH_2); 3.67–3.71 (m, 2H, NCH_2); 4.82–4.95 (br, 1H, NH); 7.06–7.32 (m, 9H, Ar-H, pyridine-H); 7.46 (dd, 1H, J = 6.9 Hz, pyridine-H); 8.38 (dd, 1H, J = 2.2 Hz, pyridine-H). Anal. calcd for $C_{29}H_{28}ClN_5$: C, 72.26; H, 5.86; N, 14.53. Found C, 72.23; H, 5.74; N, 14.61.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-(2-(3,4-dimethoxyphenyl)ethyl)-1-piperidinecarboximidamide (**3h**)*. Yield: 65.3% of a yellow solid; mp: 112–113 °C. ESI-MS: 542.3 $[M+H]^+$, 564.2 $[M+Na]^+$. IR (cm^{-1}): 3372, 3268, 2931, 2858, 2832, 2165, 1571, 1545, 1510, 1437, 1261, 1236, 1156, 1141, 1028, 993, 829, 811, 746, 557. ^1H NMR ($CDCl_3$) δ: 2.27–2.36 (m, 2H, $=C(CH_2)_2$); 2.45–2.65 (m, 2H, $=C(CH_2)_2$); 2.77–2.89 (m, 4H, $N(CH_2)_2$); 3.21–3.28 (m, 2H, $ArCH_2$); 3.30–3.38 (m, 2H, $PhCH_2$); 3.57–3.70 (m, 4H, NCH_2, CH_2); 3.84 (s, 3H, OCH_3); 3.87 (s, 3H, OCH_3); 4.78 (br, 1H, NH); 6.71–6.83 (m, 3H, Ar-H); 7.11–7.24 (m, 4H, Ar-H, pyridine-H); 7.58 (dd, 1H, J = 7.1 Hz, pyridine-H); 8.42 (dd, 1H, J = 4.8 Hz, pyridine-H). Anal. calcd for $C_{31}H_{32}ClN_5O_2{\cdot}H_2O$: C, 66.48; H, 6.12; N, 12.50. Found C, 66.35; H, 6.23; N, 12.39.
*4-(8-Chloro-5,6-dihydro-11H-benzo[5,6]cyclohepta[1,2-b]pyridin-11-ylidene)-N-cyano-N′-(2-(3,4-methylenedioxyphenyl)ethyl)-1-piperidinecarboximidamide (**3i**)*. Yield: 65.5% of a white solid; mp: 214–216 °C. ESI-MS: 526.2 $[M+H]^+$, 548.1 $[M+Na]^+$. IR (cm^{-1}): 3428, 3272, 2922, 2889, 2164, 1548, 1500, 1488, 1438, 1261, 1247, 1158, 1042, 992, 814, 779, 549. ^1H NMR ($CDCl_3$) δ: 2.23–2.36 (m, 2H, $=C(CH_2)_2$); 2.42–2.66 (m, 2H, $=C(CH_2)_2$); 2.73–2.89 (m, 4H, $N(CH_2)_2$); 3.17–3.28 (m, 2H, $ArCH_2$); 3.30–3.39 (m, 2H, $PhCH_2$); 3.57–3.69 (m, 4H, NCH_2, CH_2); 4.95 (br, 1H, NH); 5.91 (s, 2H, OCH_2O); 6.61–6.74 (m, 3H, Ar-H); 7.06–7.17 (m, 4H, Ar-H); 7.46 (dd, 1H, J = 6.8 Hz, pyridine-H); 8.38 (dd, 1H, J = 1.5 Hz, pyridine-H). Anal. calcd for $C_{30}H_{28}ClN_5O_2$: C, 66.50; H, 5.37; N, 13.31. Found C, 66.62; H, 5.41; N, 13.27.

27. Liu, W.; Zhou, J.; Bensdorf, K.; Zhang, H.; Liu, H.; Wang, Y.; Qian, H.; Zhang, Y.; Wellner, A.; Rubner, G.; Huang, W.; Guo, C.; Gust, R. *Eur. J. Med. Chem.* **2011**, *46*, 907.

28. Liu, W.; Zhou, J.; Liu, Y.; Liu, H.; Bensdorf, K.; Guo, C.; Gust, R. *Arch. Pharm.* **2011**, *344*, 487.

29. *Cell culture*: The human MCF-7, MDA-MB 231 breast and HT-29 colon cancer cell lines were obtained from the American Type Culture Collection. All 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.
Cytotoxicity: In 96 well plates, 100 μL of a cell suspension in culture medium at 7500 cells/mL (MCF-7 and MDA-MB 231) or 3000 cells/mL (HT-29) were plated into each well and 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 glutardialdehyde 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 % *T*reated/*C*ontrol$_{corr}$ 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 three independent experiments (OriginPro 8).

30. Xylene-induced ear edema. 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 centre of China Pharmaceutical University, and fed with 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.
Method: Mice were allotted to groups of 8 animals each. One group of mice, which served as control was given vehicle (0.5% CMC in water in a volume of 20 mL/kg) only. Test compounds (4 mg/kg b.w.) and ibuprofen (4 mg/kg b.w. and 30 mg/kg b.w.) suspended in vehicle (20 mL/kg) were administered ip to respective groups for 5 days. Thirty minutes after the last administration, 0.1 mL of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. One hour after xylene application, mice were killed and both ears were removed. Circular sections were taken, using a cork borer with a diameter of 9 mm, weighed and measured. The degree of ear swelling was calculated based on the weight of left ear without xylene.
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.

31. The inhibition of isolated ovine COX-1 and human recombinant COX-2 was determined with 10 μM of the respective compound by ELISA ('COX inhibitor screening assay', Cayman Chemicals). Experiments were performed according to the manufacturer's instructions. Absorption was measured at 415 nm (Victor2, Perkin Elmer). Results were calculated as the means of duplicate determinations.