http://dx.doi.org/10.5012/bkcs.2013.34.1.271

2-Amino-1,3-thiazoles Suppressed Lipopolysaccharide-Induced IL-β and TNF-α

Kee Dal Nam,[†] Minsoo Han,[†,§] Jun Yoon,[†,§] Eun-A Kim,[‡] Sung-Woo Cho,[‡] and Hoh-Gyu Hahn[†,*]

[†]*Organic Chemistry Laboratory, Korea Institute of Science and Technology, Seoul 136-791, Korea*
[*]*E-mail: hghahn@kist.re.kr*
[‡]*Department of Biochemistry and Molecular Biology, University of Ulsan college of Medicine, Seoul 138-736, Korea*
[§]*Department of Chemistry, Korea University, Seoul 136-701, Korea*
Received September 19, 2012, Accepted October 8, 2012

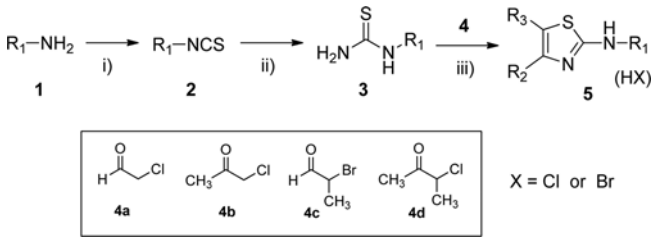
Key Words : Cytokines, Neuroinflammation, IL-1β, TNF-α, 2-Alkylaminothiazole

Heterocycles are significant chemical entities for research on small molecule drugs and their development in the pharmaceutical industry. Among the broad range of templates available, heterocyclic scaffolds represent the most promising molecules as leading structures for the discovery of novel synthetic drugs. Thiazole derivatives are highly interesting molecules for drug development, as their usefulness has already been demonstrated for treating various diseases including neurodegenerative disorders.[1] The benzothiazole derivative AS601245 has shown beneficial effects in models of global and focal brain ischemia.[2] Benzothiazoles were identified as huntingtin aggregation inhibitors in high-throughput in vitro screening and subsequently PGL-135 (2-amino-4,7-dimethylbenzothiazole) and riluzole (2-amino-6-trifluoromethoxy benzothiazole) were shown to inhibit huntingtin aggregation in cell culture.[1(a)] Riluzole has been tested for therapy of Huntington's disease patients, where treatment with benzothiazole has positive effects on choreatic hyperkinesia.[3] Various thiazole derivatives have also been reported as drugs to suppress the immune system and as inhibitors of p38 MAP kinase.[4] However, their action mechanisms have not been completely elucidated yet. In addition, structurally distinct classes will be necessary for strengthening our therapeutic concept. We have screened new drugs with a view to developing effective drugs against neuroinflammation-mediated degeneration of neuronal cells. We previously reported that 2-cyclopropylimino-3-methyl-1,3-thiazoline hydrochloride suppressed glutamate-induced excitotoxicity in rat glial cultures[5] and inhibited glutamate dehydrogenase activity in cultured islets.[6] As an extension of our efforts to investigate the biological activities of the analogues, we report here that 2-aminothiazole derivatives effectively suppress the lipopolysaccharide (LPS)-induced interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) level.

Cytokines, as major effectors of the inflammatory cascade, have key roles in the nerve cell response to brain injury.[7] Elevated levels of cytokines have been associated with the pathological effects of a variety of infectious, neurological, neurodegenerative, and neurotoxic conditions. LPS injected into the rat hippocampus CA1 region activated microglial cells, leading to an increased production of IL-1β and TNF-α in the hippocampus.[8] IL-1 ligands (IL-1α and IL-1β, collectively referred to as IL-1) are pluripotent, proinflammatory cytokines that orchestrate inflammatory and host defense responses in the body.[9] IL-1 augments T-cell responses to mitogens (and indirectly activates B cells), increases expression of vascular adhesion molecules, and induces a number of other proinflammatory cytokines, chemokines, and inflammation-associated molecules that form an amplifying cascade to stimulate an immune response.[9] IL-1β has been reported to show toxicity to oligodendrocytes, and can exacerbate inflammation of the brain.[10] The cytokine possesses both growth-stimulating and -inhibitory properties, and it appears to have self regulatory properties as well. For instance, TNF-α induces neutrophil proliferation during inflammation, but it also induces neutrophil apoptosis upon binding to the TNF-R55 receptor.[11] The pathological activities of TNF-α have attracted much attention. For instance, although TNF-α causes necrosis of some types of tumors, it promotes the growth of other types of tumor cells. High levels of TNF-α are correlated with increased risk of mortality.[12]

Chemistry

2-Alkylamino-1,3-thiazole derivatives **5** were synthesized by the method reported in the literature[13] and an overall synthetic route is shown in Scheme 1. Treatment of alkylamine **1** with an excess amount of thiophosgene in the presence of aqueous calcium carbonate in methylene chloride at room temperature gave the corresponding isothiocyanate **2** in good yield (55-94%). The reaction of **2** with ethanolic ammonia solution under reflux afforded the corresponding thiourea **3**. The reaction of **3** with either the commercially available or prepared α-haloketone **4** (**4c** was prepared by bromination of propionaldehyde (see Experimental section)) gave the corresponding 2-alkylaminothiazole **5** in quantitative yield. The desired products **5** could be isolated either by crystallization from ethyl acetate/diethyl ether or by flash chromatography (*n*-hexane:ethyl acetate = 4:1) from the reaction mixture, with isolated yields ranging from 17 to 93%. The obtained products were found to be 2-amino-1,3-thiazole hydrogen halide salts (HCl or HBr) when they were isolated by crystallization. The structures were confirmed by ^{1}H NMR spectroscopy. We synthesized 31 analogues of 2-alkylaminothiazole **5** in this manner and Table 1 lists the



Scheme 1. Reagents and conditions: i) thiophosgene, aq. $CaCO_3$, CH_2Cl_2, rt; ii) NH_3, EtOH, 80 °C; iii) **4**, EtOH, 80 °C.

prepared compounds and yields.

Biological Screening. LPS-induced microglial activation is shown by increased production of proinflammatory cytokines such as IL-1β and TNF-α. We examined the effects of 2-amino-1,3-thiazoles **5** on the proinflammatory cytokine production induced by LPS in cultured BV-2 microglial cells. The IL-1β level of BV-2 cells was increased by LPS treatment. 2-Amino-1,3-thiazoles treatment led to a reduction in the IL-1β level induced by LPS (see Table 1). Similarly, TNF-α was upregulated by LPS, but this increase was significantly attenuated by 2-amino-1,3-thiazoles (see Table 1). 2-Amino-1,3-thiazoles **5** alone did not display significant changes in the proinflammatory cytokine production and cytotoxicity in BV-2 cell cultures at the concentration examined (data not shown). These results indicate that the mechanisms underlying the effects of 2-amino-1,3-thiazoles **5** are closely related to the inhibition of proinflammatory cytokine production in LPS-induced inflammation.

TNF-α is the major neurotoxic agent causing neuronal cell death, both directly and indirectly *via* induction of NO and free radicals in neuronal cells.[14] IL-1β is a key proinflammatory cytokine produced by activated resident glia, and increased expression levels are observed in activated micro-

Table 1. A list of the prepared 2-amino-1,3-thiazole derivatives 5, the yields and the *in vitro* effects on LPS-induced upregulation of proinflammatory cytokines

Entry	Compounds	R_1	R_2	R_3	HX	Yields[a] (%)	*In vitro* effects[b] (%)	
							IL-1β	TNF-α
1	KHG26679	CH_3	H	H	HCl	38	70	73
2	KHG26680	CH_2CH_3	H	H	-	53	94	91
3	KHG26681	cyclopropyl	H	H	-	33	89	94
4	KHG26682	cyclopentyl	H	H	-	77	88	105
5	KHG26683	cyclohexyl	H	H	-	93	53	87
6	KHG26684	cycloheptyl	H	H	-	17	40	78
7	KHG26685	cyclooctyl	H	H	-	89	43	86
8	KHG26686	CH_3	CH_3	H	HCl	54	86	94
9	KHG26687	CH_2CH_3	CH_3	H	-	53	71	93
10	KHG26688	cyclopropyl	CH_3	H	-	28	68	89
11	KHG26689	cyclopentyl	CH_3	H	-	89	77	115
12	KHG26690	cyclohexyl	CH_3	H	-	28	82	138
13	KHG26691	cycloheptyl	CH_3	H	-	72	55	79
14	KHG26692	cyclooctyl	CH_3	H	-	87	38	69
15	KHG26693	1-adamantyl	CH_3	H	-	92	44	58
16	KHG26694	CH_3	H	CH_3	HBr	26	110	96
17	KHG26695	CH_2CH_3	H	CH_3	-	73	92	86
18	KHG26696	cyclopropyl	H	CH_3	HBr	41	65	73
19	KHG26697	cyclopentyl	H	CH_3	-	38	64	85
20	KHG26698	cyclohexyl	H	CH_3	HBr	39	72	80
21	KHG26699	cycloheptyl	H	CH_3	HBr	75	62	68
22	KHG26700	cyclooctyl	H	CH_3	HBr	68	46	66
23	KHG26701	1-adamantyl	H	CH_3	HBr	22	49	83
24	KHG26702	CH_3	CH_3	CH_3	HCl	97	87	94
25	KHG26703	CH_2CH_3	CH_3	CH_3	HCl	82	87	76
26	KHG26704	cyclopropyl	CH_3	CH_3	HCl	92	88	83
27	KHG26705	cyclopentyl	CH_3	CH_3	HCl	62	59	80
28	KHG26706	cyclohexyl	CH_3	CH_3	HCl	89	59	83
29	KHG26707	cycloheptyl	CH_3	CH_3	-	92	42	64
30	KHG26708	cyclooctyl	CH_3	CH_3	HCl	90	32	61
31	KHG26709	1-adamantyl	CH_3	CH_3	-	21	30	58
		Control					12	6
		LPS 1 ug/mL					100	100

[a]Isolated yields. We did not try to enhance the yields. [b]Results are expressed as a percentage of LPS response and from three independent experiments.

glia associated with brain injury.[7(a)] Recent experiments have established increased IL-1β activity as an important factor in acute neuroinflammation.[15] Therefore, targeting the upregulation of proinflammatory cytokines by activated glia should alter disease progression by attenuating the subsequent neuronal synaptic dysfunction. However, no such therapies or consensus molecular targets are currently available. Our results suggest a possibility for the potential therapeutic efficacy of 2-amino-1,3-thiazoles **5** for the treatment of neuroinflammation *via* the targeting of key proinflammatory cytokine production in microglial activation pathways. Further studies on the anti-inflammatory effects of these drugs *in vivo* are in progress in our laboratory.

Structure-activity Relationship. The dependence of the structure-activity relationship on the substituents (R_1, R_2 and R_3) of the aminothiazole scaffolds could not be precisely determined due to lack of prepared compounds, but the bulkiness of the substituents seemed to exert an important role in the activity. For example, when the large, bulky cycloheptyl, cyclooctyl or adamantyl was substituted in R_1, the *in vitro* effects on LPS-induced upregulation of proinflammatory cytokines were higher than those of the corresponding compounds when the relatively small substituent were present in the same position (compare entries 6, 7 with entries 1, 2; compare entries 8, 9 with entries 14, 15; compare entries 22, 23 with entries 16, 17; and compare entries 24, 25 with entries 30, 31, respectively). Further studies on expanding the library of the 2-amino-1,3-thiazole derivatives and on elucidating the structure-activity relationship of these series are in progress.

Experimental Section

Preparation of 2-Alkylaminothiazole Derivatives 5.

Synthesis of Isothiocyanate 2 (General Procedure): To a mixture of a solution of amine **1** (23 mmol) in methylene chloride (20 mL) and calcium carbonate ($CaCO_3$) (138 mmol) in water (80 mL) was added thiophosgene (45 mmol). The mixture was stirred for 2 h at room temperature, and then extracted with methylene chloride. The organic extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was removed by evaporation to afford **2** (55-94% yields).

Synthesis of *N*-Alkylthiourea 3 (General Procedure): To a solution of isothiocyanate **2** (8 mmol) in ethanol (10 mL) was added ammonia (2.0 M solution in ethanol, 16 mL, 32 mmol), and the mixture was heated to reflux for 12 h. The reaction mixture was cooled to room temperature and the precipitates were filtered, washed with cold ethyl ether, and then dried in air to afford the corresponding alkylthiourea **3** (52-78% yields).

Synthesis of 2-Bromopropanal (4c): To a solution of propionaldehyde (9 mmol) in methylene chloride (10 mL) at 0 °C under N_2 atmosphere was added a solution of Br_2 (9 mmol) dissolved in methylene chloride (3 mL). The reaction mixture was stirred for 30 min, washed sequentially with saturated aqueous $NaHCO_3$ solution and brine, and then dried over anhydrous $MgSO_4$. The solvent was removed by evaporation to afford **4c**.

Yield 99%, oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.78 (d, 3H, J = 6.9 MHz, CH_3), 4.40 (q, 1H, J = 6.9 MHz, CH), 9.22 (s, 1H, CHO).

Synthesis of 2-Alkylaminothiazole Derivatives 5 (General Procedure): To a solution of alkylthiourea **3** (10 mmol) in ethanol (5 mL) was added α-haloketone **4** (10 mmol). The reaction mixture was heated to reflux for 12 h, and cooled to room temperature. The solvent was removed by evaporation, and the residue was purified either by crystallization from ethyl acetate/diethyl ether or by flash chromatography (*n*-hexane:ethyl acetate = 4:1) on silica gel to obtain the corresponding 2-alkylaminothiazole **5** (17-93% isolated yields).

Biological Screening Method.

Cell Cultures: Murine BV-2 microglial cells were maintained in DMEM supplemented with 5% fetal bovine serum, and antibiotics at 37 °C in a humidified incubator under 5% CO_2. The medium was changed day by day, and the cells were plated at an appropriate density according to each experimental scale.

Cytokine Assays: The BV-2 cells were subcultured in 6-well plates (5×10^5 cells/ml) and incubated with 2-amino-1,3-thiazoles **5** in the presence or absence of 1 μg/mL LPS for 24 h. The cell-free supernatant was collected after 24 h stimulation with LPS, IL-1β and TNF-α were measured by ELISA kits (R&D, Minneapolis, MN, USA) according to the manufacturer's instructions. The absorbance at 450 nm was determined using a microplate reader.

Acknowledgments. This research was supported by both Korea Institute of Science and Technology and the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and the Technology (2012R1A1A2041484).

References

1. (a) Heiser, V.; Engemann, S.; Brocker, W.; Dunkel, I.; Boeddrich, A.; Waelter, S.; Nordhoff, E.; Lurz, R.; Schugard, N.; Rautenberg, S.; Herhaus, C.; Barnickel, G.; Bottcher, H.; Lehrach, H.; Wanker, E. E. *Proc. Natl. Acad. Sci.* **2002**, *99*, 16400, (b) Mortimer, C. G.; Wells, G.; Crochard, J.-P.; Stone, E. L.; Bradshaw, T. D.; Stevens, M. F. G.; Westwell, A. D. *J. Med. Chem.* **2006**, *49*, 179. (c) Pietrancosta, N.; Moumen, A.; Dono, R.; Lingor, P.; Planchamp, V.; Lamballe, F.; Bahr, M.; Kraus, J.-L.; Maina, F. *J. Med. Chem.* **2006**, *49*, 3645.
2. Carboni, S.; Hiver, A.; Szyndralewiez, C.; Gaillard, P.; Gotteland, J.-P.; Vitte, P.-A. *J. Pharmacol. Exp. Ther.* **2004**, *310*, 25.
3. (a) Keita, H.; Boczkowski, J.; Samb, A.; Lanone, S.; Lang-Lazdunski, L.; Rouelle, D.; Desmonts, J.-M.; Mantz, J. *Brain Res.* **2000**, *881*, 237. (b) Rosas, H. D.; Koroshetz, W. J.; Jenkins, B. G.; Chen, Y. I.; Hayden, D. L.; Beal, M. F.; Cudkowicz, M. E. *Movement Disord.* **1999**, *14*, 326.
4. (a) Chihiro, M.; Nagamoto, H.; Takemura, I.; Kitano, K.; Komatsu, H.; Sekiguchi, K.; Tabusa, F.; Mori, T.; Tominaga, M.; Yabuuchi, Y. *J. Med. Chem.* **1995**, *38*, 353. (b) Millan, D. S.; Prage, R. H.; Brand, C.; Hart, P. H. *Tetrahedron* **2000**, *56*, 811. (c) Miwatashi, S.; Arikawa, Y.; Naruo, K.; Igaki, K.; Watanabe, Y.; Kimura, H.; Kawamoto, T.; Ohkawa, S. *Chem. Pharm. Bull.* **2005**, *53*, 410.
5. Kim, E.-A.; Hahn, H.-G.; Kim, K.-S.; Kim, T. U.; Choi, S. Y.;

Cho, S.-W. *Cell. Mol. Neurobiol.* **2010**, *30*, 807.
6. Yang, S.-J.; Hahn, H.-G.; Choi, S. Y.; Cho, S.-W. *BMB Rep.* **2010**, *43*, 245.
7. (a) Wang, C.; Shuaib, A. *Prog. Neurobiol.* **2002**, *67*, 161. (b) Yuan, L.; Neufeld, A. H. *Glia* **2000**, *32*, 42. (c) Muñoz-Fernández, M. A.; Fresno, M. *Prog. Neurobiol.* **1998**, *56*, 307. (d) Venters, H. D.; Dantzer, R.; Kelley, K. W. *Trends Neuroscience* **2000**, *23*, 175.
8. Tanaka, S.; Ide, M.; Shibutani, T.; Ohtaki, H.; Numazawa, S.; Shioda, S.; Yoshida, T. *J. Neurosci. Res.* **2006**, *83*, 557.
9. Basu, A.; Krady, J. K.; Levison, S. W. *J. Neurosci. Res.* **2004**, *78*, 151.
10. Morganti-Kossmann, M. C.; Rancan, M.; Stahel, P. F.; Kossmann, T. *Immune and Inflammatory Responses in the Nervous System*; Rotherwell, N., Lodick, S., Eds.; Oxford University Press: Oxford, 2002; p106.
11. Murray, J.; Barbara, J. A. J.; Dunkley, S. A.; Lopez, A. F.; Van Ostade, X.; Condliffe, A. M.; Dransfield, I.; Haslett, C.; Chilvers, E. *Blood* **1997**, *90*, 2772.
12. Rink, L.; Kirchner, H. *Int. Arch. Allergy Immunol.* **1996**, *111*, 199.
13. Wiley, R. H.; England, D. C.; Behr, L. C. *Organic Reactions*; John Wiley and Sons, Inc.: New York, U.S.A.; 1951, Vol. 6, p 367.
14. Bhat, N. R.; Zhang, P.; Lee, J. C.; Hogan, E. L. *J. Neurosci.* **1998**, *18*, 1633.
15. Allan, S. M.; Tyrrell, P. J.; Rothwell, N. J. *Nat. Rev. Immunol.* **2005**, *5*, 629.