Synthesis and Pharmacological Activity of Triazolo[1,5-*a*]triazine Derivatives Inhibiting Eosinophilia

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In continuation of our previous work on eosinophilia inhibitors, we synthesized an additional series of inhibitors, which consisted of 5-amino-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole derivatives and a newly developed series of 1,2,4-triazolo[1,5-*a*]-1,3,5-triazine derivatives. We evaluated their inhibitory activity on the airway eosinophilia model, which was induced by the intravenous (iv) injection of Sephadex particles. In the 1,2,4-triazole series with various substituents at the 3 position of the triazole ring such as 2-furyl, pyridyl, and phenoxy, none of derivatives had comparable activity to the previously reported compound GCC-AP0341, 5-amino-3-(4-chlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole. In the triazolo-[1,5-*a*]triazine series, 2-(4-chlorophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (**3h**) was highly potent, and when given orally it had an ID_{50} value of 0.3 mg/kg, which is comparable to that of GCC-AP0341. The fact that the structure−activity relationship of these two series was quite similar suggests that a common substructure, such as the 1,2,4-triazole ring with a substituted phenyl ring at the 3 position and a thiocarbonyl moiety at the 1 position, could contribute to the activity. Our selected compound **3h** was less active than GCC-AP0341 in the antigen-induced hyper-responsiveness model in guinea pigs; however, we plan to carry out further studies on eosinophil functions, especially on their activation, using our two compounds, **3h** and GCC-AP0341.

Introduction

In a previous study, we described the synthesis and pharmacological activity of GCC-AP0341 [5-amino-3-(4-chlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole], which was very potent in the Sephadex-induced lung eosinophilia model in rats.[1,2] GCC-AP0341 blocked the allergen-induced airway hyper-responsiveness in guinea pigs.[1] To the best of our knowledge, this was the first evidence that an eosinophilia inhibitor alone could be a potent agent to treat the asthmatic response. We previously demonstrated that GCC-AP0341 was a well-defined agent for the treatment of asthma;[1] therefore we have attempted to identify other potent compounds based on the GCC-AP0341 molecule in order to confirm that eosinophilia is necessary for chronic asthma and airway hyper-responsiveness and that an inhibitor of eosinophilia could be a novel agent for the treatment of asthma.

Chemistry

Our synthetic designs based on the structure of GCC-AP0341 mainly involved 5-amino-1-[(methylamino)-thiocarbonyl]-1*H*-1,2,4-triazole derivatives **2**, which had various substituents at the 3 position of the triazole ring, such as 2-furyl, 2-thienyl, pyridyl, phenoxy, and benzyl, and triazolo[1,5-*a*]triazine derivatives **3**−**5**, as shown in Schemes 1 and 2. The compounds synthesized are listed in Tables 1−4. In the 3-substituted triazole derivatives **2**, most of the compounds, i.e., **2a**,**b**,**d**,**e**,**g**−**j**, were prepared according to the reported method shown in Scheme 1 (route a → f).[3] For synthesis of the phenoxy derivative **1f** (R^1 = OPh), the cyclization of diphenyl cyanocarbonimidate with hydrazine was used (Scheme 1, route e).[4] The triazoles obtained were treated with methyl isothiocyanate to obtain good yields of the target compounds.

The synthesis of triazolo[1,5-*a*]triazine derivatives is shown in Scheme 2. The triazolo[1,5-*a*]triazine-7(6*H*)-thione derivatives **3** were prepared from the corresponding triazole compounds **2** by the reaction with diethoxymethyl acetate and good yields obtained.[5] The compounds that had methyl or phenyl groups at the 5 position of the triazolo[1,5-*a*]triazine ring, **4a**,**b**, **5a**,**b**, were prepared by the reaction of 5-amino-1-[(methyl-amino)thiocarbonyl]-1*H*-1,2,4-triazole or 5-amino-3-(4-chlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole with triethyl orthoacetate or triethyl ortho-benzoate. As the synthesis of the triazolo[1,5-*a*]triazin-7(6*H*)-one **8** from 5-amino-1-[(methylamino)carbonyl]-1*H*-1,2,4-triazole gave a low yield, an alternative synthetic route reported by Hirata et al. was used.[5] Compound **8** was prepared by the reaction of diazomethane with triazolo[1,5-*a*]triazin-7(6*H*)-one (**7**), which was synthesized from the 5-amino-1-(aminocarbonyl)-1*H*-1,2,4-triazole (**6**). The synthesis of 7-ethoxy-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine (**9**) was produced using **6** with triethyl orthoformate. The isomers **10a**,**b** were synthesized from 3-amino-1*H*-1,2,4-triazole and di-

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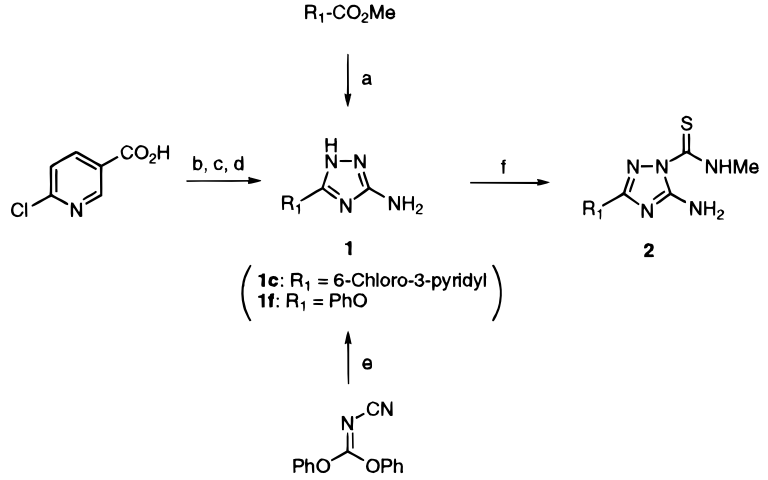
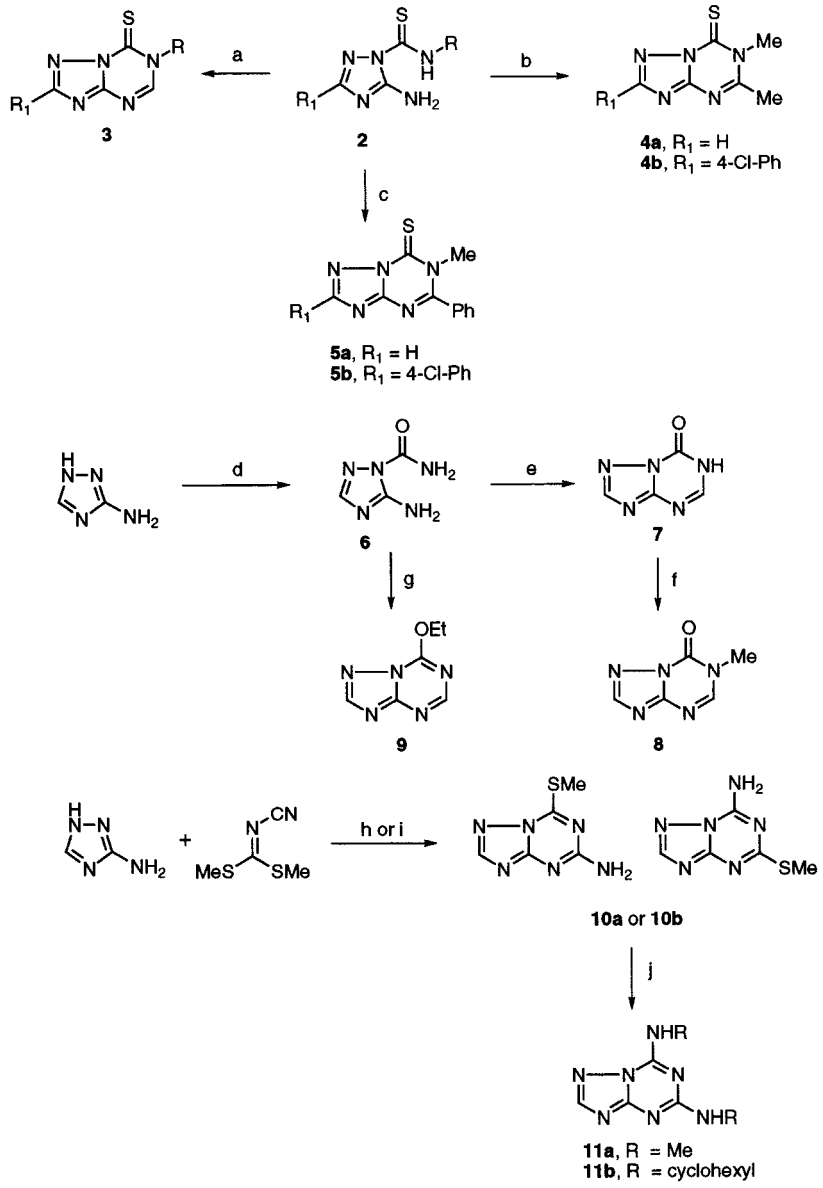
Scheme 1[a]



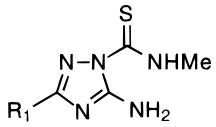
[a] Reagents: (a) aminoguanidine hydrochloride or nitrate, NaOMe, MeOH; (b) $SOCl_2$, DMF (cat.), CH_2Cl_2, reflux; (c) aminoguanidine hydrochloride, NaOH, H_2O, 0 °C; (d) DMSO, 180 °C; (e) hydrazine, MeOH, 0 °C to room temperature; (f) MeNCS, 1 N NaOH, THF.

Scheme 2[a]



[a] Reagents: (a) $(EtO)_2CHOAc$, room temperature to 90 °C; (b) $MeC(OEt)_3$, 145 °C; (c) $PhC(OMe)_3$, 150 °C; (d) KOCN, HCl (aq), room temperature; (e) $(EtO)_2CHOAc$, 100 °C; (f) CH_2N_2, ether, 0 °C to room temperature; (g) $(EtO)_3CH$, 140 °C; (h) pyridine, 50 °C; (i) pyridine, reflux; (j) RNH_2, 90−125 °C.

Table 1. Physical Data and Biological Activity for 3-Substituted 5-Amino-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole Derivatives



no.	R_1	mp[a] (°C)	formula[b]	inhibition[c] (%)
2a	2-furyl	173−175	$C_8H_9N_5OS$	18
2b	2-thienyl	170−172	$C_8H_9N_5S2$	−7
2c	6-chloro-3-pyridyl	208−211	$C_9H_9ClN_6S$	−9
2d	5-chloro-2-pyridyl	215−225	$C_9H_9ClN_6S{\cdot}0.1H_2O$	−2
2e	4-chloro-1-naphthyl	270−272	$C_{14}H_{12}ClN_5S$	14
2f	PhO	184−186	$C_{10}H_{11}N_5OS$	−5
2g	4-Cl-Ph-CH=CH	209−210	$C_{12}H_{12}ClN_5S$	25
2h	Ph-CH_2	173−175	$C_{11}H_{13}N_5S$	−24
2i	4-Ph-Ph	275−277	$C_{16}H_{15}N_5S$	58
2j	Ph-C≡C	212−214	$C_{12}H_{11}N_5S$	0
2k	Ph-CH=N	183−185	$C_{11}H_{12}N_6S$	37
12	SMe			(54)[d]
GCC-AP0341				88[e]
dexamethasone				(95)[f]

[a] Uncorrected. [b] Compounds were analyzed for C, H, N ±0.4% using the formula indicated. [c] Percent inhibition value on the Sephadex-induced lung eosinophilia model at an intraperitoneal (ip) dose of 3 mg/kg. [d] Value in parentheses is percent inhibition at an ip dose of 30 mg/kg. [e] SE value for GCC-AP0341 was determined to be ±4.2% from six experiments. [f] Value in parentheses is percent inhibition at an ip dose of 0.1 mg/kg.

methyl *N*-cyanodithioiminocarbonate in pyridine by controlling the reaction temperature at 50 °C and reflux, but a structural determination of these two isomers could not be obtained by spectroscopy. The amination of **10a** or **10b** with methylamine or cyclohexylamine, respectively, produced **11a** or **11b**.

Results and Discussion

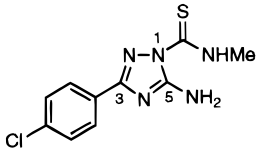
In continuation of our previous work on eosinophilia inhibitors,[1,2a−d] we modified various compounds based on GCC-AP0341 and investigated their effect on a Sephadex-induced lung eosinophilia model, in which the eosinophilia was induced in the airway by intravenous injection of Sephadex particles on days 0, 2, and 5.

In a previous study,[1] we reported that GCC-AP0341 [5-amino-3-(4-chlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole] (Chart 1) is a highly potent eosinophilia inhibitor and showed its structure−activity relationship (SAR). Typical characteristics of the SAR are as follows. (1) The amino function at the 5 position of the triazole ring is necessary for the high potency. (2) For the 3 position of the triazole ring, a phenyl ring that is substituted by a halogen at the 4 position facilitates the activity. (3) Concerning the (methylamino)thiocarbonyl moiety at the 1 position, the thiocarbonyl moiety and methylamino substitute facilitate the activity. Considering these results, we initiated a program to investigate other potent molecules based on the GCC-AP0341 structure.

We performed a screening test using the Sephadex model with the compounds injected intraperitoneally (ip) at doses of 30 or 3 mg/kg. The percent inhibition values obtained in this model are summarized in Tables 1−4.

Since the 4-chlorophenyl moiety in GCC-AP0341 contributes to the high potent activity, we determined whether another ring system, such as pyridine, thiophene, or naphthalene, in place of the phenyl ring had an effect, and the results are shown in Table 1 (**2a**−**e**). For this set of compounds, we prepared 2-furyl (**2a**), 2-thienyl (**2b**), 6-chloro-3-pyridinyl (**2c**), 5-chloro-2-pyridinyl (**2d**), and 4-chloro-1-naphthyl (**2e**) derivatives. Although compounds **2c**,**d** were expected to have similar activity to GCC-AP0341, they in fact showed less activity at a dose of 3 mg/kg. These results, especially the low activity of **2c**,**d**, indicate that the electronic and not the steric factor may determine the activity of GCC-AP0341. The result[1] that 5-amino-1-[(methylamino)-thiocarbonyl]-3-(methylthio)-1*H*-1,2,4-triazole (**12**) had an inhibition of 54% at a dose of 30 mg/kg ip prompted us to study the effect of introducing a junctional moiety between the triazole ring and the phenyl ring of GCC-AP0341 as shown in Table 1 (**2f**−**k**). This series of compounds has oxygen (**2f**), vinylene (**2g**), methylene (**2h**), phenylene (**2i**), acetylene (**2j**), and imine (**2k**) as junctional moieties. None of the derivatives was more active than GCC-AP0341, although the phenylene derivative (**2i**) showed comparable activity with an inhibition of 58%. This was the first evidence that a substituent with a moiety sterically larger than 4-(trifluoromethyl)phenyl[1] at the 3 position of the triazole ring had potent activity.

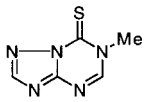
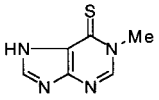
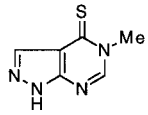
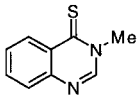
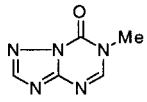
Chart 1. Structure of GCC-AP0341



GCC-AP0341

Aminophylline[2a] was reported to have an inhibitory effect in the Sephadex model, and this prompted us to study a series of fused bicyclic derivatives incorporating the 5-amino-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole function. Typical examples were triazolo[1,5-*a*]triazine molecules as shown in Tables 2 and 3. Table 2 lists 6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (**3a**) which had an inhibition of 69% at a dose of 30 mg/kg. Analogous compounds, which included purine **3b**, pyrazolo[3,4-*d*]pyrimidine **3c**, and quinazoline **3d**, were ineffective. For 1,2,4-triazolo[1,5-*a*]-1,3,5-

Table 2. Physical Data and Biological Activity of the 1,2,4-Triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione Derivatives

no.	structure	mp[a] (°C)	formula[b]	inhibition[c] (%)
3a		238-240	$C_5H_5N_5S$	69
3b		>285	$C_6H_6N_4S$	9
3c		>235	$C_6H_6N_4S$	-13
3d		142-143	$C_9H_8N_2S$	22
8		223-226	$C_5H_5N_5O$	17

[a] Uncorrected. [b] Compounds were analyzed for C, H, N ±0.4% using the formula indicated. [c] Percent inhibition value on the Sephadex-induced lung eosinophilia model at an ip dose of 30 mg/kg.

triazin-7(6*H*)-one (**8**), where its thiocarbonyl moiety was replaced with a carbonyl one in **3a**, the inhibitory activity was decreased. The introduction of methylthio, amino, or ethoxy substituents at the 5 and/or 7 position of the triazolo[1,5-*a*]triazine ring (**9**, **10a**,**b**, **11a**,**b**) reduced the activity, which indicated that the 1,2,4-triazole[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione moiety is essential for the activity.

Other structure−activity relationships for the 1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione are shown in Table 4, and their effects follow. (1) A shorter length substituent at the 6 position, such as methyl, contributes to the activity (**3a** > **3e** > **3f**). (2) At the 5 position, introduction of substituents, such as methyl **4a** and phenyl **5a**, reduces the activity. (3) Substituents at the 2 position, which are a 4-substituted phenyl moiety, such as 4-chlorophenyl **3h**, 4-trifluoromethyl **3l**, 2,4-dichlorophenyl **3m**, and 3,4-dichlorophenyl **3n**, contribute to the activity, and the activity is comparable to that of GCC-AP0341, whereas the nonsubstituted phenyl ring **3g** completely abolished the activity. The introduction of an ethoxalyl moiety at the 4 position of the phenyl ring reduced the activity, which was also observed in the derivatives of GCC-AP0341.[1] On the basis of these results, we selected compound **3h**, and a dose−response study on the Sephadex-induced lung eosinophilia model was conducted (Figure 1).

Like GCC-AP0341, the oral administration of **3h** potently suppressed the eosinophilia with an ID_{50} value of 0.3 mg/kg (Figure 1). The efficacy of **3h** against bronchial asthma in antigen (ascaris)-induced hyper-responsiveness in guinea pigs was performed at the ip dose of 1 mg/kg as shown in Figure 2.[6] The result showed that **3h** suppressed the hyper-responsiveness less than GCC-AP0341, and although the reason for this is not clearly understood, absorption and/or metabolism might be a cause. We have firmly believed that eosinophilia is a critical phenomenon for airway hyper-responsiveness and that an inhibitor of eosinophilia should be a potent inhibitor of airway hyper-responsiveness.[2e,7] However, compound **3h** showed lower activity compared to that of GCC-AP0341 in the ascaris-induced hyper-responsiveness model despite having highly potent activity for eosinophilia inhibition. Initially we could not interpret this result; however recent reports may offer some explanations. A report that multiple antigen challenge produces pulmonary eosinophilia without any pulmonary hyper-responsiveness in actively sensitized guinea pigs shows that eosinophilia is not always a necessary factor in the airway hyper-responsiveness model.[8] Also, we have noticed that it is not eosinophil recruitment (eosinophilia) but eosinophil activation that is linked to airway hyper-responsiveness.[9] On the basis of these results, we plan to conduct further examinations on the eosinophil functions, such as their activation, with compound **3h** and GCC-AP0341. We have examined the effect of **3h** on IL-5-mediated human eosinophil survival, and **3h** manifested less activity compared to GCC-AP0341 as shown in Figure 3.[10] Further research based on these results is being conducted.

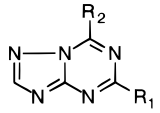
In conclusion, we screened several compounds modified from our previously reported compound GCC-AP0341, using the Sephadex-induced lung eosinophilia model, and found a highly potent eosinophilia inhibitor, 2-(4-chlorophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (**3h**). In the structure **3h**, we found that a thiocarbonyl moiety in the 7 position and a 4-chlorophenyl moiety in the 3 position of the triazolo-[1,5-*a*]-1,3,5-triazine molecule play an important role in the inhibitory activity on the eosinophilia model. Since the structure−activity relationships described in our present study and those obtained for GCC-AP0341 seem to be quite similar, we suggest that these two compounds should have a common substructure for exerting the highly potent eosinophilia inhibition. Compound **3h** showed highly potent activity in the Sephadex model but was less effective on antigen-induced airway hyper-responsiveness model compared to GCC-AP0341. In further examinations on the eosinophil functions, such as their activation, using both **3h** and GCC-AP0341 will be valuable.

Experimental Section

Chemistry. Melting points were determined with a Yanaco melting point apparatus and are uncorrected. ^{1}H NMR spectra were measured on Bruker AC-200 and AMX-500 NMR spectrometers with tetramethylsilane as the internal standard; chemical shifts are given on the δ (ppm) scale. Infrared (IR) spectra were obtained on a Shimazu IR-420 instrument.

5-Amino-3-(2-furyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole (2a). To a cooled (0 °C) solution of NaOMe prepared from sodium (10.9 g, 0.476 mol) and MeOH (150 mL), was added aminoguanidine hydrochloride (52.6 g, 0.476 mol). Then methyl 4-furoate (15.0 g, 0.119 mol) in MeOH (100 mL) was added dropwise to the resultant mixture. The reaction mixture was stirred at reflux for 24 h and poured into ice−water. The pH value of the water layer was adjusted to 3−4 with 3 N HCl. The solvent was distilled away under reduced pressure. MeOH was added to the residue for extraction. The

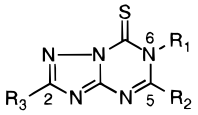
Table 3. Physical Data and Biological Activity of the 1,2,4-Triazolo[1,5-*a*]-1,3,5-triazine Derivatives



no.	R_1	R_2	mp[a] (°C)	formula[b]	inhibition[c] (%)
9	H	OEt	108−109	$C_6H_7N_5O$	6
10a[d]	NH_2 (SMe)	SMe (NH_2)	228−233	$C_5H_6N_6S\cdot0.5H_2O$	−29
10b[d]	SMe (NH_2)	NH_2 (SMe)	264−266	$C_5H_6N_6S$	−7
11a	NH-Me	NH-Me	248−250	$C_6H_9N_7\cdot0.2H_2O$	38
11b	NH-cyclohexyl	NH-cyclohexyl	194−195	$C_{16}H_{25}N_7$	1

[a] Uncorrected. [b] Compounds were analyzed for C, H, N ±0.4% using the formula indicated. [c] Percent inhibition value on the Sephadex-induced lung eosinophilia model at an ip dose of 30 mg/kg. [d] Structural determination of these isomers (**10a**,**b**) could not be obtained by spectroscopy.

Table 4. Physical Data and Biological Activity of the 1,2,4-Triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione Derivatives



no.	R_1	R_2	R_3	mp[a] (°C)	formula[b]	inhibition[c] (%)
3a	Me	H	H	238−240	$C_5H_5N_5S$	69
3e	*n*-Pro	H	H	168−169	$C_7H_9N_5S$	49
3f	*n*-Bu	H	H	132−134	$C_8H_{11}N_5S$	23
4a	Me	Me	H	147−148	$C_6H_7N_5S$	18
5a	Me	Ph	H	246−248	$C_{11}H_9N_5S$	29
3g	Me	H	Ph	>300	$C_{11}H_9N_5S$	−1
3h	Me	H	Ph(4-Cl)	>300	$C_{11}H_8ClN_5S$	92 (90)[d]
3i	Me	H	Ph(4-F)	>300	$C_{11}H_8FN_5S$	(38)[d]
3j	Me	H	Ph(4-Br)	>300	$C_{11}H_8BrN_5S$	(84)[d]
3k	Me	H	Ph(4-CN)	>300	$C_{12}H_8N_6S$	79
3l	Me	H	Ph(4-CF_3)	270−272	$C_{12}H_8F_3N_5S$	(87)[d]
3m	Me	H	Ph(2,4-Cl_2)	277−279	$C_{11}H_7Cl_2N_5S$	(90)[d]
3n	Me	H	Ph(3,4-Cl_2)	282−284	$C_{11}H_7Cl_2N_5S$	(89)[d]
3o	Me	H	$NHCOCO_2Et$	280−282	$C_9H_{10}N_6O_3S$	−2
4b	Me	Me	Ph(4-Cl)	>300	$C_{12}H_{10}ClN_5S\cdot0.2H_2O$	(60)[d]
5b	Me	Ph	Ph(4-Cl)	267−270	$C_{17}H_{12}ClN_5S$	(5)[d]
GCC-AP0341						(88)[d]

[a] Uncorrected. [b] Compounds were analyzed for C, H, N ±0.4% using the formula indicated. [c] Percent inhibition value on the Sephadex-induced lung eosinophilia model at an ip dose of 30 mg/kg. [d] Value in parentheses is percent inhibition at an ip dose of 3 mg/kg.

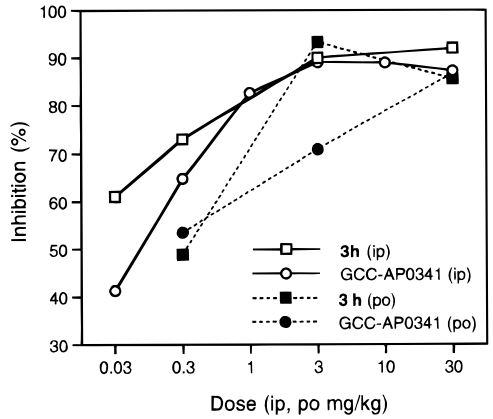


Figure 1. Dose−response curve of **3h** and GCC-AP0341 (ip or po) on the Sephadex-induced lung eosinophilia model. Each point was calculated using the same procedure as described in the Experimental Section.

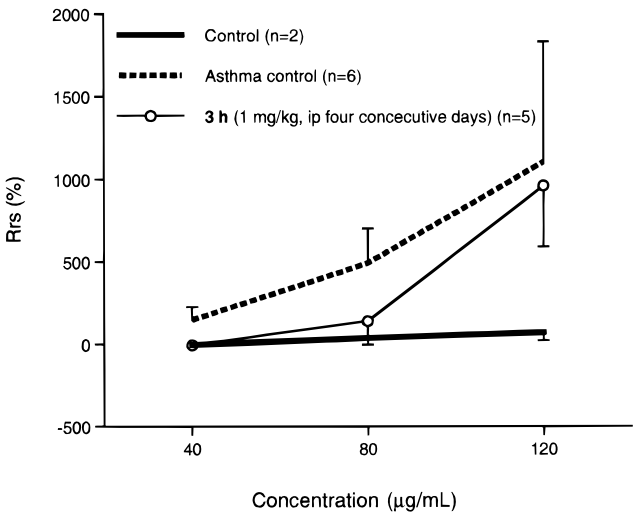


Figure 2. Effect of **3h** on the ascaris-induced hyperresponsiveness model in guinea pigs. Values are the mean ± SE. Statistical analysis was performed using Bonferroni's method. **P* < 0.05 vs asthma control. ***P* < 0.01 vs asthma control.

insoluble inorganic salt was removed by filtration. The filtrate was concentrated under reduced pressure to give 3-amino-5-(2-furyl)-1*H*-1,2,4-triazole which was used without further purification. To a mixture of the compound obtained above in 1 N NaOH (30.7 mL) and THF (60 mL) was added methyl isothiocyanate (11.0 g, 0.15 mol). The reaction mixture was stirred at room temperature for 1 h and neutralized with 3 N HCl, and the precipitated solid was collected by filtration. The solid obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (3.68 g, 16%): mp 173−175 °C; IR (KBr, cm^{-1}) 3300, 3050, 1650, 1620;

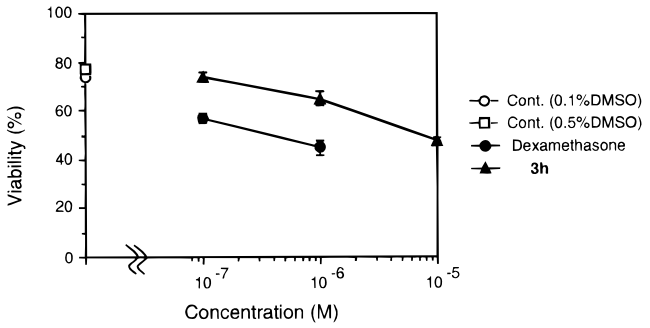


Figure 3. Effect of **3h** and dexamethasone on IL-5-mediated human eosinophil viability. Each point represents the mean ±SD (n = 4).

^{1}H NMR (DMSO-d_6) δ 3.07 (s, 3H, CH_3), 6.67 (d d, 1H, J = 3.4, 1.8 Hz, CH), 7.00 (d, 1H, J = 3.4 Hz, CH), 7.86 (d, 1H, J = 1.8 Hz, CH), 8.35 (brs, 2H, NH_2), 9.96 (brs, 1H, NH).

5-Amino-1-[(methylamino)thiocarbonyl]-3-(2-thienyl)-1*H*-1,2,4-triazole (2b). The procedure used for the preparation of **2a** was repeated with methyl 2-thiophenecarboxylate as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) to give white crystals (13%): mp 170−172 °C; IR (KBr, cm^{-1}) 3300, 1650, 1520; ^{1}H NMR (DMSO-d_6) δ 3.08 (s, 3H, CH_3), 7.2 (m, 1H, CH), 7.66 (d, 1H, J = 3.4 Hz, CH), 7.71 (d, 1H, J = 5.0 Hz, CH), 8.36 (brs, 2H, NH_2), 9.91 (brs, 1H, NH).

5-Amino-3-(6-chloro-3-pyridyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole (2c). To a solution of 6-chloronicotinic acid (10.0 g, 63.5 mmol) in CH_2Cl_2 (100 mL) were added dropwise DMF (10 mL) and thionyl chloride (5.1 mL, 70 mmol). The reaction mixture was stirred at reflux for 1 h and poured into a solution of aminoguanidine hydrochloride (24.6 g, 222 mmol) in 2.2 N NaOH (100 mL) under ice cooling. The precipitated solid was collected by filtration to give 6-chloronicotinic acid 2-amidinohydrazide as a pale-brown solid (6.87 g, 51%). A mixture of this material (5.40 g, 25.3 mmol) in DMSO (50 mL) was stirred at 180 °C for 1 h. After the reaction, to this reaction mixture were added 1 N NaOH (20 mL) and methyl isothiocyanate (1.5 g, 20 mmol). The reaction mixture was stirred at room temperature for 1 h and neutralized with 3 N HCl, and the precipitated solid was collected by filtration. The solid obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (2.9 g, 29%): mp 208−211 °C; IR (KBr, cm^{-1}) 3300, 3050, 1640, 1520; ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 3H, CH_3), 7.69 (d, 1H, J = 8.0 Hz, CH), 8.37 (d d, 1H, J = 8.0, 2.0 Hz, CH), 8.42 (brs, 2H, NH_2), 8.99 (d, 1H, J = 2.0 Hz, CH), 10.1 (brs, 1H, NH).

5-Amino-3-(5-chloro-2-pyridyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole (2d). The procedure used for the preparation of **2a** was repeated with ethyl 5-chloro-2-pyridinecarboxylate as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 250/1) and recrystallized from $CHCl_3$ to give white crystals (16%): mp 215−225 °C; IR (KBr, cm^{-1}) 3300, 3050, 1635, 1520; ^{1}H NMR (DMSO-d_6) δ 3.09 (s, 3H, CH_3), 8.09 (s, 2H, CH), 8.34 (brs, 2H, NH_2), 8.72 (s, 1H, CH), 10.1 (brs, 1H, NH).

5-Amino-3-(4-chloro-1-naphthyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole (2e). The procedure used for the preparation of **2a** was repeated with methyl 4-chloro-1-naphthoate as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (5%): mp 270−272 °C; IR (KBr, cm^{-1}) 3300, 3080, 1660, 1570, 1515; ^{1}H NMR (DMSO-d_6) δ 3.13 (s, 3H, CH_3), 7.62−7.90 (m, 3H, CH), 8.20 (d, 1H, J = 7.9 Hz, CH), 8.25−8.50 (m, 3H, CH, NH_2), 9.21 (m, 1H, CH), 10.1 (brs, 1H, NH).

5-Amino-1-[(methylamino)thiocarbonyl]-3-phenoxy-1*H*-1,2,4-triazole (2f). To a solution of diphenyl cyanocarbonimidate (9.53 g, 38.8 mmol) in MeOH (150 mL) was added hydrazine (1.5 mL, 48 mmol) at 0 °C. After stirring at 0 °C to room temperature for 2 h, MeOH was removed under reduced pressure and the residue was chromatographed on silica gel ($CHCl_3$/MeOH, 7/1) to give 3-amino-5-phenoxy-1*H*-1,2,4-triazole as white crystals (6.84 g, 100%). The procedure used for the preparation of **2a** was repeated with this compound as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 30/1) and recrystallized from hexane and $CHCl_3$ to give white crystals (41%): mp 184−186 °C; IR (KBr, cm^{-1}) 3280, 3120, 3060, 1660, 1570, 1515; ^{1}H NMR (DMSO-d_6) δ 3.00 (s, 3H, CH_3), 7.21 (t, 1H, J = 7.7 Hz, CH), 7.27 (d, 2H, J = 7.7 Hz, CH), 7.41 (t, 2H, J = 7.7 Hz, CH), 8.48 (brs, 2H, NH_2), 9.67 (brs, 1H, NH).

5-Amino-3-(4-chlorostyryl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole (2g). The procedure used for the preparation of **2a** was repeated with methyl 4-chlorocinnamate as a starting material. After the neutralization, the precipitated solid was collected by filtration to give white crystals (32%): mp 209−210 °C; IR (KBr, cm^{-1}) 3330, 3270, 3010, 1655, 1520; ^{1}H NMR (DMSO-d_6) δ 3.06 (s, 3H, CH_3), 6.93 (d, 1H, J = 16.1 Hz, CH), 7.46 (d, 2H, J = 8.5 Hz, CH), 7.49 (d, 1H, J = 16.1 Hz, CH), 7.68 (d, 2H, J = 8.5 Hz, CH), 8.25 (brs, 2H, NH_2), 9.93 (brs, 1H, NH).

5-Amino-3-benzyl-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole (2h). The procedure used for the preparation of **2a** was repeated with methyl phenylacetate as a starting material. After the neutralization, the precipitated solid was collected by filtration to give white powdery crystals (24%): mp 173−175 °C; IR (KBr, cm^{-1}) 3300, 3050, 1635, 1505; ^{1}H NMR (DMSO-d_6) δ 3.03 (s, 3H, CH_3), 3.81 (s, 2H, CH_2), 7.22 (m, 1H, CH), 7.27−7.32 (m, 4H, CH), 8.14 (brs, 2H, NH_2), 9.88 (brs, 1H, NH).

5-Amino-1-[(methylamino)thiocarbonyl]-3-(4-phenylphenyl)-1*H*-1,2,4-triazole (2i). The procedure used for the preparation of **2a** was repeated with methyl 4-phenylbenzoate as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (24%): mp 275−277 °C; IR (KBr, cm^{-1}) 3250, 3000, 1725, 1645, 1520, 1500; ^{1}H NMR (DMSO-d_6) δ 3.10 (s, 3H, CH_3), 7.30−7.58 (m, 3H, CH), 7.75 (d, 2H, J = 7.0 Hz, CH), 7.82 (d, 2H, J = 8.4 Hz, CH), 8.14 (d, 2H, J = 8.4 Hz, CH), 8.36 (brs, 2H, NH_2), 10.1 (brs, 1H, NH).

5-Amino-1-[(methylamino)thiocarbonyl]-3-(2-phenylethynyl)-1*H*-1,2,4-triazole (2j). The procedure used for the preparation of **2a** was repeated with methyl phenylpropiolate as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 50/1) and recrystallized from $CHCl_3$ to give white crystals (4%): mp 212−214 °C; IR (KBr, cm^{-1}) 3220, 2220, 1630, 1540, 1500; ^{1}H NMR (DMSO-d_6) δ 3.04 (s, 3H, CH_3), 7.44−7.54 (m, 3H, CH), 7.60 (d, 2H, J = 6.7 Hz, CH), 8.33 (brs, 2H, NH_2), 10.1 (brs, 1H, NH).

5-Amino-3-(*N*-benzylideneamino)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole (2k). A mixture of 3,5-diamino-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] (3.00 g, 17.4 mmol), benzaldehyde (1.99 g, 18.8 mmol), and DL-camphor-10-sulfonic acid (110 mg, 0.474 mmol) in EtOH (100 mL) was stirred at 60 °C for 5 h. After cooling to room temperature, the precipitated solid was collected by filtration to give pale-yellow crystals (2.13 g, 47%): mp 183−185 °C; IR (KBr, cm^{-1}) 3320, 3180, 1635, 1500; ^{1}H NMR (DMSO-d_6) δ 3.05 (s, 3H, CH_3), 7.56 (t, 2H, J = 7.2 Hz, CH), 7.62 (t, 1H, J = 7.2 Hz, CH), 7.99 (d, 2H, J = 7.2 Hz, CH), 8.36 (brs, 2H, NH_2), 9.16 (s, 1H, CH), 10.1 (brs, 1H, NH).

6-Methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3a). A mixture of 5-amino-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] (1.49 g, 9.48 mmol) and diethoxymethyl acetate (12 mL) was stirred at 80 °C for 2 h. After cooling to room temperature, the precipitated solid was collected by filtration to give white crystals (1.39 g, 88%): mp 238−240 °C; IR (KBr, cm^{-1}) 2300, 1585, 1560; ^{1}H NMR (DMSO-d_6) δ 3.35 (s, 3H, CH_3), 8.57 (s, 1H, CH), 8.86 (s, 1H, CH).

1-Methylpurine-6(1*H*)-thione (3b). A mixture of 4-amino-*N*-methylimidazole-5-carbothioamide[1] (1.2 g, 7.7 mmol) and

triethyl orthoformate (15 mL, 90 mmol) was stirred at 140 °C for 4 h. After cooling to room temperature, the precipitated solid was collected by filtration and recrystallized from $CHCl_3$ and MeOH to give gray crystals (340 mg, 27%): mp >285 °C; IR (KBr, cm^{-1}) 3400, 3000, 1600, 1565; ^1H NMR (DMSO-d_6) δ 3.90 (s, 3H, CH_3), 8.39 (s, 1H, CH), 8.73 (s, 1H, CH), 13.6 (s, 1H, NH).

5-Methylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione (3c). The procedure used for the preparation of **3a** was repeated with 3-amino-*N*-methylpyrazole-4-carbothioamide[1] as a starting material at 140 °C. After the reaction, the precipitated solid was collected by filtration to give white crystals (64%): mp >235 °C; IR (KBr, cm^{-1}) 3400, 3150, 3100, 1605, 1565, 1505; ^1H NMR (DMSO-d_6) δ 3.83 (s, 3H, CH_3), 8.26 (s, 1H, CH), 8.66 (s, 1H, CH), 14.0 (brs, 1H, NH).

3-Methylquinazoline-4(3*H*)-thione (3d). A mixture of 2-amino-*N*-methylbenzenecarbothioamide[1] (500 mg, 3.01 mmol) and diethoxymethyl acetate (5 mL) was stirred at room temperature for 2 h. The reaction mixture was chromatographed on silica gel (hexane/AcOEt, 3/1) and recrystallized from benzene to give yellow crystals (208 mg, 59%): mp 142−143 °C; IR (KBr, cm^{-1}) 1600, 1550; ^1H NMR (DMSO-d_6) δ 3.90 (s, 3H, CH_3), 7.64 (d t, 1H, J = 1.3, 7.5 Hz, CH), 7.75 (d, 1H, J = 7.5 Hz, CH), 7.90 (d t, 1H, J = 1.5, 7.5 Hz, CH), 8.70 (d d, 1H, J = 1.5, 7.5 Hz, CH), 8.71 (s, 1H, CH).

5-Amino-1-(aminocarbonyl)-1*H*-1,2,4-triazole (6). A solution of 3-amino-1*H*-1,2,4-triazole (8.41 g, 0.100 mol) and potassium cyanate (8.11 g, 0.100 mol) in 0.64 N HCl (159 mL) was stirred at room temperature for 2 h. After the reaction, the precipitated crystals were collected by filtration to give a mixture of **6** and 3-amino-1-(aminocarbonyl)-1*H*-1,2,4-triazole. Recrystallization from hot water gave **6** as white crystals (2.95 g, 23%): mp 160−163 °C; IR (KBr, cm^{-1}) 3440, 3360, 3170, 3090, 1740, 1645, 1615, 1515; ^1H NMR (DMSO-d_6) δ 7.15 (brs, 2H, NH_2), 7.51 (s, 1H, CH), 7.56 (brs, 1H, NH), 7.73 (brs, 1H, NH).

1,2,4-Triazolo[1,5-*a*]-1,3,5-triazin-7(6*H*)-one (7). The procedure used for the preparation of **3a** was repeated with **6** as a starting material at 100 °C. After the reaction, the precipitated solid was collected by filtration and recrystallized from MeOH to give white crystals (57%): mp 247 °C dec; IR (KBr, cm^{-1}) 3370, 3200, 2620, 1765, 1600, 1560; ^1H NMR (DMSO-d_6) δ 8.39 (s, 1H, CH), 8.41 (s, 1H, CH), 13.3 (brs, 1H, NH).

6-Methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazin-7(6*H*)-one (8). To a cooled (0 °C) solution of **7** (637 mg, 4.65 mmol) in Et_2O (10 mL) was added an ethereal solution of diazomethane (0.6 M, 26 mL). The mixture was stirred at 0 °C to room temperature for 17 h. The precipitated solid was collected by filtration to give yellow crystals (592 mg, 84%): mp 223−226 °C dec; IR (KBr, cm^{-1}) 3090, 3060, 1760, 1600, 1555; ^1H NMR (DMSO-d_6) δ 3.54 (s, 3H, CH_3), 8.42 (s, 1H, CH), 8.61 (s, 1H, CH).

7-Ethoxy-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine (9). A mixture of **6** (1.69 g, 13.3 mmol) and triethyl orthoformate (20 mL, 0.12 mol) was stirred at 140 °C for 20 h. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. The crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 30/1) and recrystallized from $CHCl_3$ to give white crystals (1.44 g, 60%): mp 108−109 °C; IR (KBr, cm^{-1}) 2990, 1740, 1600, 1550; ^1H NMR (DMSO-d_6) δ 1.33 (t, 3H, J = 7.2 Hz, CH_3), 4.04 (q, 2H, J = 7.2 Hz, CH_2), 8.42 (s, 1H, CH), 8.66 (s, 1H, CH).

5-Amino-7-(methylthio)-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine (10a) or 7-Amino-5-(methylthio)-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine (10b). A solution of 3-amino-1*H*-1,2,4-triazole (1.55 g, 18.5 mmol) and dimethyl *N*-cyanodithioiminocarbonate (3.61 g, 24.7 mmol) in pyridine (75 mL) was stirred at 50 °C for 36 h. After cooling to room temperature, the precipitated solid was collected by filtration to give white crystals (211 mg, 8.4%): mp 228−233 °C; IR (KBr, cm^{-1}) 3060, 1690, 1665, 1590, 1540; ^1H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH_3), 8.89 (s, 2H, NH_2), 9.01 (s, 1H, CH).

A solution of 3-amino-1*H*-1,2,4-triazole (1.61 g, 19.2 mmol) and dimethyl *N*-cyanodithioiminocarbonate (3.30 g, 22.6 mmol) in pyridine (65 mL) was stirred at reflux for 2.5 h. After removal of the solvent, the crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 10/1) and recrystallized from AcOEt to give yellow crystals (909 mg, 26%): mp 264−266 °C; IR (KBr, cm^{-1}) 3850, 3350, 3100, 2300, 1860, 1650, 1550; ^1H NMR (DMSO-d_6) δ 2.50 (s, 3H, CH_3), 8.34 (s, 1H, CH), 8.84 (s, 2H, NH_2).

5,7-Bis(methylamino)-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine (11a). A mixture of **10a** or **10b** (928 mg, 5.09 mmol) and methylamine (30 wt % solution in EtOH, 30 mL) was stirred in a sealed tube at 125 °C for 20 h. After cooling to room temperature, the solvent was removed under reduced pressure. Recrystallization from EtOH gave white crystals (560 mg, 61%): mp 248−250 °C; IR (KBr, cm^{-1}) 3300, 1640, 1605, 1515; ^1H NMR (DMSO-d_6, 60 °C) δ 2.83 (brs, 3H, CH_3), 2.95 (brs, 3H, CH_3), 7.18 (brs, 1H, NH), 8.00 (s, 1H, CH), 8.14 (s, 1H, NH).

5,7-Bis(cyclohexylamino)-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine (11b). A mixture of **10a** or **10b** (2.2 g, 12 mmol) and cyclohexylamine (12 mL, 105 mmol) was stirred at 90 °C for 19 h. The reaction mixture was chromatographed on silica gel (hexane/AcOEt, 1/3) and recrystallized from AcOEt to give white crystals (594 mg, 9.3%): mp 194−195 °C; IR (KBr, cm^{-1}) 3230, 2910, 2850, 1640, 1600, 1570; ^1H NMR (DMSO-d_6, 60 °C) δ 1.18−1.90 (m, 20H, CH_2), 3.95 (m, 2H, CH), 5.12 (brs, 1H, NH), 5.94 (brs, 1H, NH), 7.92 (s, 1H, CH).

6-*n*-Propyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3e). The procedure used for the preparation of **3a** was repeated with 5-amino-1-[(*n*-propylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole[1] as a starting material at room temperature. After the reaction, the precipitated solid was collected by filtration to give white crystals (90%): mp 168−169 °C; IR (KBr, cm^{-1}) 3050, 2970, 1585, 1560; ^1H NMR (DMSO-d_6) δ 0.94 (t, 3H, J = 7.5 Hz, CH_3), 1.85 (sext, 2H, J = 7.5 Hz, CH_2), 4.39 (d d, 2H, J = 6.3, 7.4 Hz, CH_2), 8.57 (s, 1H, CH), 8.88 (s, 1H, CH).

6-*n*-Butyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3f). The procedure used for the preparation of **3a** was repeated with 5-amino-1-[(*n*-butylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole[1] as a starting material at room temperature. After the reaction, the precipitated solid was collected by filtration to give white crystals (66%): mp 132−134 °C; IR (KBr, cm^{-1}) 3080, 3040, 2970, 1585, 1560; ^1H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.4 Hz, CH_3), 1.38 (sext, 2H, J = 7.4 Hz, CH_2), 1.80 (m, 2H, CH_2), 4.43 (t, 2H, J = 7.6 Hz, CH_2), 8.57 (s, 1H, CH), 8.88 (s, 1H, CH).

5,6-Dimethyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (4a). A mixture of 5-amino-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] (2.00 g, 12.7 mmol) and triethyl orthoacetate (13 mL, 71 mmol) was stirred at 140 °C for 3 h. After cooling to room temperature, the precipitated solid was collected by filtration to give pale-brown crystals (497 mg, 22%): mp 147−148 °C; IR (KBr, cm^{-1}) 3530, 3430, 1585; ^1H NMR (DMSO-d_6) δ 2.71 (s, 3H, CH_3), 3.96 (s, 3H, CH_3), 8.51 (s, 1H, CH).

6-Methyl-5-phenyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (5a). The procedure used for the preparation of **4a** was repeated with 5-amino-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] and trimethyl orthobenzoate as a starting material. After the reaction, the precipitated solid was collected by filtration and recrystallized from MeOH to give white crystals (40%): mp 246−248 °C; IR (KBr, cm^{-1}) 3410, 1590; ^1H NMR (DMSO-d_6) δ 3.73 (s, 3H, CH_3), 7.57−7.66 (m, 3H, CH), 7.72 (m, 2H, CH), 8.61 (s, 1H, CH).

6-Methyl-2-phenyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3g). The procedure used for the preparation of **3a** was repeated with 5-amino-1-[(methylamino)thiocarbonyl]-3-phenyl-1*H*-1,2,4-triazole[1] as a starting material at room temperature. After the reaction, the precipitated solid was collected by filtration to give white crystals (92%): mp >300 °C; IR (KBr, cm^{-1}) 1595, 1585, 1560; ^1H NMR (DMSO-d_6) δ

3.85 (s, 3H, CH_3), 7.55−7.59 (m, 3H, CH), 8.19 (m, 2H, CH), 8.88 (s, 1H, CH).

2-(4-Chlorophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3h). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(4-chlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] as a starting material at 90 °C. After the reaction, the precipitated solid was collected by filtration to give white crystals (96%): mp >300 °C; IR (KBr, cm^{-1}) 3040, 1585, 1555; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, CH_3), 7.64 (d, 2H, J = 8.5 Hz, CH), 8.19 (d, 2H, J = 8.5 Hz, CH), 8.89 (s, 1H, CH).

2-(4-Fluorophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3i). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(4-fluorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] as a starting material at 90 °C. After the reaction, the precipitated solid was collected by filtration to give white crystals (96%): mp >300 °C; IR (KBr, cm^{-1}) 1600, 1565; ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 3H, CH_3), 7.41 (t, 2H, J = 8.5 Hz, CH), 8.23 (d d, 2H, J = 6.0, 8.5 Hz, CH), 8.90 (s, 1H, CH).

2-(4-Bromophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3j). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(4-bromophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] as a starting material. After the reaction, the precipitated solid was collected by filtration to give white crystals (97%): mp >300 °C; IR (KBr, cm^{-1}) 3400, 3010, 1600, 1550, 1505; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H, CH_3), 7.78 (d, 2H, J = 8.5 Hz, CH), 8.12 (d, 2H, J = 8.5 Hz, CH), 8.90 (s, 1H, CH).

2-(4-Cyanophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3k). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(4-cyanophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] as a starting material. After the reaction, the precipitated solid was collected by filtration to give white crystals (90%): mp >300 °C; IR (KBr, cm^{-1}) 3400, 2210, 1600, 1550; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, CH_3), 8.05 (d, 2H, J = 8.5 Hz, CH), 8.35 (d, 2H, J = 8.5 Hz, CH), 8.93 (s, 1H, CH).

2-(4-(Trifluoromethyl)phenyl)-6-methyl-1,2,4-triazolo-[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3l). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(4-(trifluoromethyl)phenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole as a starting material at 90 °C. After the reaction, the precipitated solid was collected by filtration to give white crystals (93%): mp 270−272 °C; IR (KBr, cm^{-1}) 1600, 1580, 1530; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, CH_3), 7.95 (d, 2H, J = 8.0 Hz, CH), 8.39 (d, 2H, J = 8.0 Hz, CH), 8.92 (s, 1H, CH).

2-(2,4-Dichlorophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3m). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(2,4-dichlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] as a starting material. After the reaction, the precipitated solid was collected by filtration to give white crystals (84%): mp 277−279 °C; IR (KBr, cm^{-1}) 3350, 3005, 1730, 1600, 1540, 1500; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, CH_3), 7.64 (d d, 1H, J = 8.4, 2.0 Hz, CH), 7.86 (d, 1H, J = 2.0 Hz, CH), 8.02 (d, 1H, J = 8.4 Hz, CH), 8.92 (s, 1H, CH).

2-(3,4-Dichlorophenyl)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3n). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(3,4-dichlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] as a starting material. After the reaction, the precipitated solid was collected by filtration to give white crystals (80%): mp 282−284 °C; IR (KBr, cm^{-1}) 3400, 3050, 1600, 1565, 1550, 1500; ^{1}H NMR (DMSO-d_6) δ 3.85 (s, 3H, CH_3), 7.85 (d, 1H, J = 8.4 Hz, CH), 8.13 (d d, 1H, J = 8.4, 1.9 Hz, CH), 8.28 (d, 1H, J = 1.9 Hz, CH), 8.92 (s, 1H, CH).

2-(Ethoxalylamino)-6-methyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (3o). The procedure used for the preparation of **3a** was repeated with 5-amino-3-(ethoxalylamino)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole as a starting material at 90 °C. After the reaction, the precipitated solid was collected by filtration to give white crystals (78%): mp 280−282 °C; IR (KBr, cm^{-1}) 3400, 3300, 1725, 1600, 1580, 1545; ^{1}H NMR (DMSO-d_6) δ 1.31 (t, 3H, J = 7.1 Hz, CH_3), 3.82 (s, 3H, CH_3), 4.32 (q, 2H, J = 7.1 Hz, CH), 8.86 (s, 1H, CH), 11.9 (brs, 1H, NH).

2-(4-Chlorophenyl)-5,6-dimethyl-1,2,4-triazolo[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (4b). A mixture of 5-amino-3-(4-chlorophenyl)-1-[(methylamino)thiocarbonyl]-1*H*-1,2,4-triazole[1] (1.50 g, 5.60 mmol), triethyl orthoacetate (10 mL, 53 mmol), and acetic acid (0.5 mL) was stirred at 140 °C for 5 h. After cooling to room temperature, the precipitated solid was collected by filtration and chromatographed on silica gel ($CHCl_3$/MeOH, 100/1) to give white crystals (910 mg, 56%): mp >300 °C; IR (KBr, cm^{-1}) 1590; ^{1}H NMR (TFA-*d*) δ 2.96 (s, 3H, CH_3), 4.19 (s, 3H, CH_3), 7.66 (d, 2H, J = 8.4 Hz, CH), 8.10 (d, 2H, J = 8.4 Hz, CH).

2-(4-Chlorophenyl)-6-methyl-5-phenyl-1,2,4-triazolo-[1,5-*a*]-1,3,5-triazine-7(6*H*)-thione (5b). The procedure used for the preparation of **4b** was repeated with trimethyl orthobenzoate as a starting material. The precipitated solid was collected by filtration, chromatographed on silica gel ($CHCl_3$/MeOH, 100/1), and recrystallized from $CHCl_3$ to give white crystals (1.26 g, 59%): mp 267−270 °C; IR (KBr, cm^{-1}) 1580, 1575; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 3H, CH_3), 7.59−7.67 (m, 5H, CH), 7.74 (d, 2H, J = 8.4 Hz, CH), 8.22 (d, 2H, J = 8.4 Hz, CH).

Sephadex-Induced Lung Eosinophilia. Sephadex G-200 (fine) particles (Pharmacia) in sterilized saline (0.5 mg/mL) were fully swollen by boiling in water for 5 h, and 1 mL of the suspension was injected into each rat (Male, Wistar strain, five rats in each group) through a tail vein on days 0, 3, and 5. A drug or vehicle was ip administered 10 min before Sephadex injections. On day 7 each rat was sacrificed by CO_2 gas, and the trachea were cannulated. Three to four milliliters of phosphate-buffered saline (PBS) containing 6 units/mL heparin prewarmed to 37 °C was injected into the airways from a syringe connected to the cannula, and the solution was collected into a conical tube put on ice. The same procedure was repeated twice, and the solution was combined together followed by centrifugation at 150*g* for 10 min. The cell pellet thus obtained was suspended in 0.5 mL of RPMI-1640, and the suspension was diluted 10-fold with Hinkelman reagent. The number of eosinophils and the number of total cells were counted under a microscope. The number of rats used for a compound or vehicle group was five, and a mean value in each group was applied to the equation to calculate inhibitory activity. The inhibitory activity of a compound was calculated from the following equation: inhibition (%) = 100 − [(no. of eosinophils/no. of total cells)compound/(no. of eosinophils/no. of total cells)vehicle] × 100.

Airway Hypersensitivity in Actively Sensitized Guinea Pigs. Male guinea pigs (Hartley strain) were actively sensitized by the ip injection of ascaris antigen (20 μg) suspended in a silica gel on days 0 and 14. Three weeks after the first sensitization, ascaris antigen (0.25 μg/mL) was inhaled into the airway of guinea pigs by the ultrasonic neblizar, and 1 week later a similar procedure was carried out. **3h** or vehicle was ip injected for 4 consecutive days. Metpyron (50 mg/kg, ip) was injected 24 and 4 h before carbachol inhalation;24 h after the second inhalation, carbachol at a concentration of 40, 80, or 120 μg/mL was inhaled in a chamber to induce bronchoconstriction, which was monitored for the detection of airway resistance (Rrs). The concentration of the constrictor to be inhaled was increased step by step from 40 to 120 μg/mL after checking that Rrs had returned to baseline level. As a control animal, a carbachol-inhaled animal with no sensitization was used.

Eosinophil Survival Assay. Human eosinophils obtained from heparinized peripheral blood were purified by centrifugation through six discontinuous Metrizamide gradients as described before,[8] and the purity was more than 90%. The purified cells were then transferred to a 96-well microplate after washing them thoroughly with Tyrode solution containing 0.1% gelatin followed by suspension in RPMI-1640 supplemented with 10% fetal calf serum and 25 mM HEPES (1.25 ×

10^5 cells/mL). The inoculated cells were maintained in the presence or absence of recombinant human IL-5 (Genzyme) for 4 days in an atmosphere of 5% CO_2 in air. The ratio of viable cells was determined by the trypan blue exclusion test. **3h** or dexamethasone dissolved in DMSO was added to the microplate at the time of inoculation, and care was taken so that the concentration of DMSO might not exceed 0.5%.

References

(1) Naito, Y.; Akahoshi, F.; Takeda, S.; Okada, T.; Kajii, M.; Nishimura, H.; Sugiura, M.; Fukaya, C.; Kagitani, Y. Synthesis and pharmacological activity of triazole derivatives inhibiting eosinophilia. *J. Med. Chem.* **1996**, *39*, 3019−3029.

(2) (a) Spicer, B. A.; Baker, R. C.; Hatt, P. A.; Laycock, S. M.; Smith, H. The effects of drugs on Sephadex-induced eosinophilia and lung hyper-responsiveness in the rat. *Br. J. Pharmacol.* **1990**, *101*, 821−828. (b) Laycock, S. M.; Smith, H.; Spicer, B. A. Airway hyper-reactivity and blood, lung and airway eosinophilia in rats treated with Sephadex particles. *Int. Arch. Allergy Appl. Immunol.* **1986**, *81*, 363−367. (c) Spicer, B. A.; Baker, R.; Laycock, S. M.; Smith, H. Correlation between blood eosinophilia and airways hyper-responsiveness in rats. *Agents Actions* **1989**, *26*, 63−65. (d) Cook, R. M.; Musgrove, N. R.; Smith, H. Eosinophils and the granulomatous reaction in rats injected with Sephadex particles. *Pulm. Pharmacol.* **1989**, *2*, 185−190. (e) Desreumaux, P.; Capron, M. Eosinophils in allergic reactions. *Curr. Opin. Immunol.* **1996**, *8*, 790−795.

(3) Mullican, M. D.; Wilson, M. W.; Connor, D. T.; Kostlan, C. R.; Schrier, D. J.; Dyer, R. D. Design of 5-(3,5-di-*tert*-butyl-4-hydroxyphenyl)-1,3,4-thiadiazoles, -1,3,4-oxadiazoles, and -1,2,4-triazoles as orally active, nonulcerogenic antiinflammatory agents. *J. Med. Chem.* **1993**, *36*, 1090−1099.

(4) Webb, R. L.; Labaw, C. S. Diphenyl cyanocarbonimidate. A versatile synthon for the construction of heterocyclic ststems. *J. Heterocycl. Chem.* **1982**, *19*, 1205−1206.

(5) Hirata, T.; Twanmoh, L.; Wood, H. B.; Goldin, A.; Driscoll, J. S. 5-Azapurines and the structures of *sym*-triazole intermediates. *J. Heterocycl. Chem.* **1972**, *9*, 99−106.

(6) Inoue, H.; Aratani, H.; Takishima, T.; Kagoshima, M.; Iwahisa, Y.; Terasawa, M. Effect of a potent PAF antagonist, Y-24180, on an allergen-induced late asthmatic response in guinea-pigs. *J. Lipid Mediat.* **1992**, *5*, 173−176.

(7) Teixeira, M. M.; Williams, T. J.; Hellewell, P. G. Mechanisms and pharmacological manipulation of eosinophil accumulation in vivo. *Trends Pharmacol. Sci.* **1995**, *16*, 418−423.

(8) Lefort, J.; Bachelet, C.-M.; Leduc, D.; Vargaftig, B. B. Effects of antigen provocation of IL-5 transgenic mice on eosinophil mobilization and bronchial hyperresponsiveness *J. Allergy Clin. Immunol.* **1996**, *97*, 788−799.

(9) Watson, J. W.; Cohan, V. L.; Shay, A. K.; Freiert, K. W. Multiple antigen challenge produces pulmonary eosinophilia but not pulmonary hyperresponsiveness in actively sensitized guinea pigs. *Agents Actions* **1992**, *37*, 174−177.

(10) Wallen, N.; Kita, H.; Weiler, D.; Gleich, G. J. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J. Immunol.* **1991**, *147*, 3490−3495.

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