Synthesis and Pharmacological Activity of Triazole Derivatives Inhibiting Eosinophilia

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In order to develop novel antiasthmatic agents based on a new mechanism of action, a series of 3-substituted 5-amino-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole derivatives were synthesized and evaluated in a model in which eosinophilia was induced in the airway through intravenous (iv) injection of Sephadex particles on days 0, 2, and 5. After screening of several hundred derivatives, we finally identified the highly potent eosinophilia inhibitor 5-amino-3-(4-chlorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-triazole (**23c**, GCC-AP0341), which had ID_{50} values of 0.3 and 0.07 mg/kg when administered orally (os) and intraperitoneally (ip), respectively. This compound showed complete inhibition of the hypersensitivity induced by ascaris inhalation at an ip dose of 1 mg/kg as well as low toxicity, with an LD_{50} value of >2.0 g/kg in mice. Extensive study of its mechanism of action revealed that **23c** inhibited eosinophil survival induced by interleukin-5 (IL-5), but had little or no effect on leukotriene D_4 (LTD_4) or platelet-activating factor (PAF)-induced responses. Taken together, these results suggest **23c** as a novel candidate for the treatment of chronic asthma. Further studies are now underway.

Introduction

Recently, there has been increasing clinical and experimental evidence that human eosinophils play a central role in the pathogenesis of bronchial asthma and airway hyperresponsiveness.[1] An increased number of eosinophils in circulation and bronchoalveolar lavage fluids (BALFs) has been reported as a characteristic feature of chronic bronchial asthma.[2] Also, the mediators released by inflammatory cells such as sulfidopeptide leukotrienes (LTs), platelet activating factor (PAF), reactive oxygen species, and eosinophil cytotoxic cationic proteins can damage airway epithelial cells, which may cause airway hyperreactivity.[3]

The mechanism of eosinophilia in circulation and BALFs is unclear, but recent research findings offer several explanations. Firstly, activation of allergen-specific helper (CD_4^+) T-lymphocytes of the Th-2 subset and subsequent release of cytokines including interleukin-3 (IL-3), IL-5, and granulocyte macrophage colony-stimulating factor (GM-CSF),[4] with IL-5 as the most likely to be specific to eosinophil proliferation and activation,[5] links eosinophilia. This explanation is supported by the finding that the glucocorticoid dexamethasone, which has been shown to inhibit the activation of T-lymphocytes, is highly effective against pulmonary infiltration by eosinophils in actively sensitized rats.[6] Secondly, in a model of long-lasting eosinophil recruitment induced by intrathoracic injection of ovalbumins, the dual cyclooxygenase and lipoxygenase inhibitor BW755C, as well as the PAF acether antagonists BN52021 and WEB2086, have inhibitory activities,[7] which suggests that mediators, such as arachidonic acid metabolites produced by cyclooxygenase and lipoxygenase, or PAF acether, may be involved in eosinophilia. Thirdly, phosphodiesterase (PDE) inhibitors, especially inhibitors of PDE-IV, which is dominant in eosinophils, prevent allergen-induced lung eosinophilia in rats by directly or indirectly inhibiting the activity of eosinophils and/or of the cells responsible for attracting them into the lung.[8]

Several derivatives have been found effective in eosinophilia models, but the phenomenon of eosinophilia itself is not fully elucidated; the effect of glucocorticoids is not limited to T-lymphocytes or eosinophils,[6,7] and the action of mediators alone is not sufficient to explain several of the events characteristic of eosinophilia.

Since inhibitors of eosinophilia will hopefully provide novel agents for the treatment of chronic bronchial asthma, we initiated a program to modify various compounds using a rat lung model[9] in which eosinophilia was induced in the airway through intravenous (iv) injection of Sephadex particles on days 0, 2, and 5. In this model, desquamation of epithelial cells and mucus plugs was observed under histological examination, and hyperresponsiveness to serotonin was detected.[9]

Chemistry

Most of this series of compounds were prepared according to the reported method,[10] basically through reaction of substituted 3-amino-1*H*-1,2,4-triazole with alkyl isothiocyanates[11] as shown in Scheme 1 and Tables 1−4. Compound **1a** was prepared through reaction of commercially available 3-amino-1*H*-1,2,4-triazole with methyl isothiocyanate in 18% yield (Table 1, Scheme 1, method A). The low yield reflected the recovery of large amounts of the starting material together with the formation of the regioisomeric product 3-amino-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole. Compounds having various substituents at amide nitrogen of **1a**, such as **1b**−**i**, were similarly prepared using method A as shown in Table 1. The synthesis of corresponding alkyl isothiocyanates, such as *n*-hexyl isothiocyanate (**2**) is shown in Scheme 1.[12] In the preparation of the (dimethylamino)(thiocarbonyl) compound **3**, we adopted another method using reaction of

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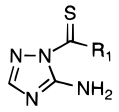
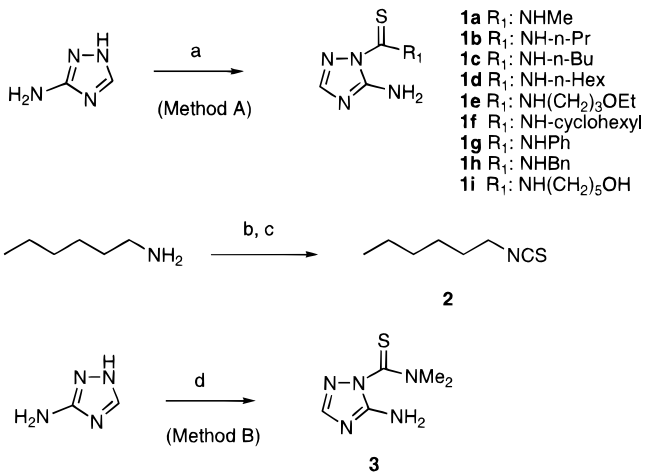
Table 1. Physical Data and Biological Activity for 5-Amino-1-[substituted amino(thiocarbonyl)]-1*H*-1,2,4-triazole



no.	R_1	method[a]	mp[b] (°C)	formula[c]	inhibn[d] (%)
1a	NH-Me	A	184−185	$C_4H_7N_5S$	93
1b	NH-*n*-Pr	A	117−118	$C_6H_{11}N_5S$	93
1c	NH-*n*-Bu	A	119−120	$C_7H_{13}N_5S$	21
1d	NH-*n*-Hex	A	122−123	$C_9H_{17}N_5S$	−1
1e	$NH(CH_2)_3OEt$	A	137−139	$C_8H_{15}N_5OS$	1
1f	NH-cyclohexyl	A	168−170	$C_9H_{15}N_5S$	31
1g	NH-Ph	A	146−148 dec	$C_9H_9N_5S$	−7
1h	NH-Bn	A	157−158	$C_{10}H_{11}N_5S$	40
1i	$NH(CH_2)_5OH$	A	108−109	$C_8H_{15}N_5OS$	−17
3	NMe_2	B	139.5−140.5	$C_5H_9N_5S$	34
dexamethasone					(95)[e]

[a] Methods A and B are general procedures described in the Experimental Section (see Scheme 1). [b] Uncorrected. [c] Compounds were analyzed for C, H, N ±0.4% for formula indicated. [d] Percent inhibition at ip dose of 30 mg/kg calculated from the equation described in Experiment. [e] Value in paretheses is percent inhibition at an ip dose of 0.1 mg/kg.

Scheme 1[a]

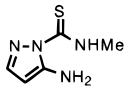
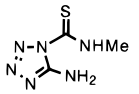
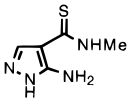
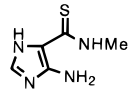
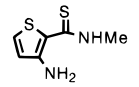
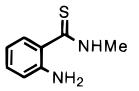
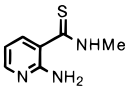
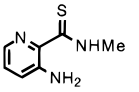
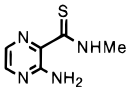


[a] Condition: (a) R_1NCS, DMF; (b) carbone disulfide, Et_3N, 0 °C; (c) $ClCO_2Et$, Et_3N, $CHCl_3$, 0 °C to room temperature; (d) dimethylthiocarbamoyl chloride, pyridine.

3-amino-1*H*-1,2,4-triazole with dimethylthiocarbamoyl chloride in pyridine (Table 1, Scheme 1, method B).

Analogous compounds having various heterocyclic ring systems instead of the triazole ring of **1a** were synthesized as shown in Table 2 and Scheme 2. Thioureas **4** and **5** were similarly prepared via method A using 3-aminopyrazole and 5-aminotetrazole, respectively, as starting material. Compound **7a** was prepared by reaction of phosphorus pentasulfide[13] with 3-amino-*N*-methylpyrazole-4-carbamide (**6a**). Thio amides **7b**−**e** were prepared from the corresponding amides **6b**−**e** using the same method as for **7a**. Compounds **7f**,**g** were prepared by thioamidation using a Lawesson's reagent[14] of the corresponding amides **6f**,**g**. The procedures used for the preparation of **6a**−**c**, **6d**,[15] **6e**, **6f**,[14,16] and **6g** are shown in Scheme 2. Modification of the (methylamino)(thiocarbonyl) moiety as well as the substituents at the triazole ring of **1a** was carried out as in Table 3 and Scheme 3. Compounds **8**, **14**, and **15** were similarly prepared using method A. Treatment of the triazole with carbon disulfide and potassium

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

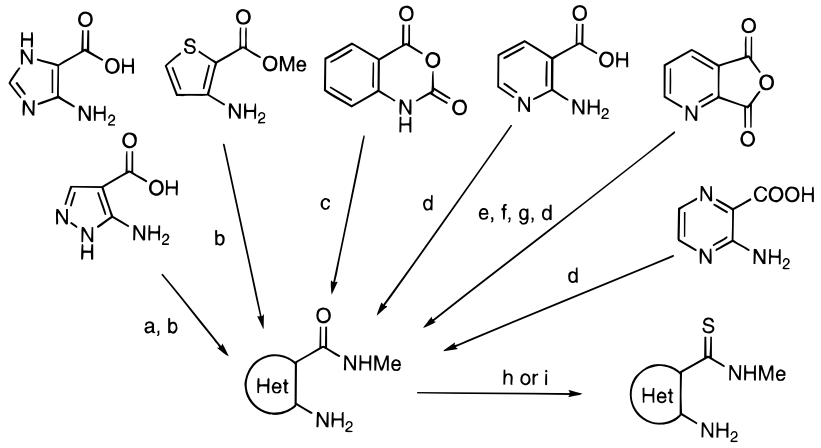
No.	Structure	mp[a] (°C)	Formula[b]	inhibition[c] (%)
4		Oil	$C_5H_8N_4S$	27
5		-[d]	$C_3H_6N_6S$	53
7a		235 - 237	$C_5H_8N_4S$	-22
7b		202 - 204	$C_5H_8N_4S$	5
7c		oil	$C_6H_8N_2S_2$	4
7d		97 - 99.5	$C_8H_{10}N_2S$	47
7e		134 - 135	$C_7H_9N_3S$	27
7f		oil	$C_7H_9N_3S$	9
7g		104 - 105	$C_6H_8N_4S$	27

[a] See Table 1, footnote *b*. [b] See Table 1, footnote *c*. [c] See Table 1, footnote *d*. [d] Mixture of **5** and 5-amino-2-[(methylamino)(thiocarbonyl)]-1*H*-tetrazole (10:1).

hydroxide followed by iodomethane gave **9** (Scheme 3, method C). Treatment of the triazole with methanesulfonyl chloride gave **10** (method D). The 5-methylamino derivative **12** was formed using method A from 3-(methylamino)-1*H*-1,2,4-triazole (**11**), which was obtained by reaction of 3-nitro-1*H*-1,2,4-triazole with concentrated HCl in a sealed tube at 100 °C for 15 h followed by treatment with aqueous methylamine at 180 °C for 24 h, as shown in Scheme 3. Compound **13** was formed as a byproduct of **1a**.

Method A was applied to 3-amino-5-methyl-1*H*-1,2,4-triazole, which was prepared from cyanamide and triethyl orthoacetate in two steps using the reported method[17] to give **18**, as shown in Scheme 4. The 3-methylthio derivative **19** was prepared through reaction of commercially available 3-amino-5-(methylthio)-1*H*-1,2,4-triazole with methyl isothiocyanate (Table 4, method A). Introduction of several substituents at the three position of **1a** was carried out as in Table 4 and Scheme 5. The synthesis of **23a**−**g**, **23h**,[18] and **23i**−**k** were carried out according to the reported method.[10,19] Since the preparation of 5-(substituted phenyl)-3-amino-1*H*-1,2,4-triazole **22a**−**f**,**j**,**k** involved two-step syntheses from benzhydrazides as shown in Scheme 5, an alternative route devised by Mullican *et al.* for one-step triazole synthesis from benzoates was utilized[20] for the synthesis of 3-amino-1*H*-1,2,4-triazole substituted at five position

Scheme 2[a]

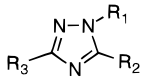


Het: pyrazole(3-amino-4-CONHMe) **6a**; imidazole(4-amino-5-CONHMe) **6b**; thiophene(3-amino-2-CONHMe) **6c**; phenyl(2-amino-1-CONHMe) **6d**; pyridine(2-amino-3-CONHMe) **6e**; pyridine(3-amino-2-CONHMe) **6f**; 1,4-pyrazine(3-amino-2-CONHMe) **6g**

Het: pyrazole(3-amino-4-CSNHMe) **7a**; imidazole(4-amino-5-CSNHMe) **7b**; thiophene(3-amino-2-CSNHMe) **7c**; phenyl(2-amino-1-CSNHMe) **7d**; pyridine(2-amino-3-CSNHMe) **7e**; pyridine(3-amino-2-CSNHMe) **7f**; 1,4-pyrazine(3-amino-2-CSNHMe) **7g**

[a] Condition: (a) HCl, MeOH; (b) aqueous 40% $MeNH_2$, 180 °C; (c) aqueous 40% $MeNH_2$; (d) 1,1′-carbonylimidazole, 30% $MeNH_2$ in EtOH, THF, 50 °C; (e) aqueous 28% NH_3, 50 °C; (f) acetic anhydride, 135 °C; (g) 10% NaOH, NaOBr, 80 °C; (h) P_2S_5, pyridine, 80 °C; (i) Lawesson's reagent, HMPA, 120 °C.

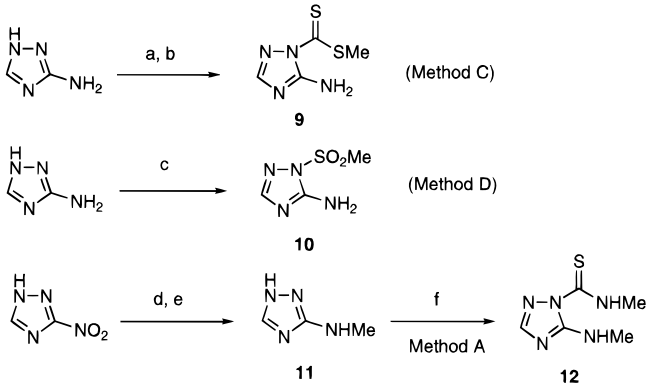
Table 3. Physical Data and Biological Activity for 5-Amino-1*H*-1,2,4-triazole Derivatives



no.	R_1	R_2	R_3	method[a]	mp[b] (°C)	formula[c]	inhibn[d] (%)
8	C(=O)NHMe	NH_2	H	A	192−193	$C_4H_7N_5O$	40
9	C(=S)SMe	NH_2	H	C	203−205	$C_4H_6N_4S_2$	36
10	$S(O_2)Me$	NH_2	H	D	132−133	$C_3H_6N_4O_2S$	−40
12	C(=S)NHMe	NHMe	H	A	98−99	$C_5H_9N_5S$	−1
13	C(=S)NHMe	H	NH_2	A	−[e]	$C_4H_7N_5S$	25
14	C(=S)NHMe	NH_2	NH_2	A	176−178	$C_4H_8N_6S$	53
15	C(=S)NH-cyclohexyl	H	H	A	74−76	$C_9H_{14}N_4S$	0

[a] See Table 1, footnote *a* and Scheme 3. [b−d] See Table 1. [e] Mixture of **13** and **1a** (85:15).

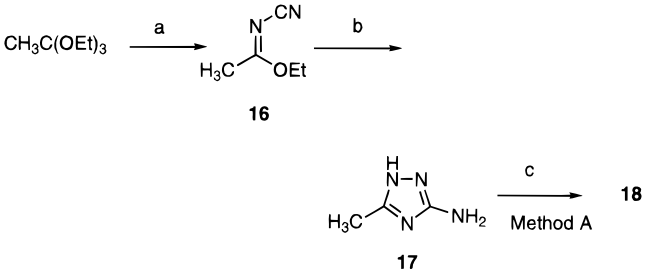
Scheme 3[a]



[a] Condition: (a) carbon disulfide, DMF, 0 °C; (b) aqueous KOH, MeI, DMF, 0 °C to room temperature; (c) $MeSO_2Cl$, pyridine, −78 °C; (d) concentrated HCl in sealed tube, 100 °C; (e) aqueous 40% $MeNH_2$, 180 °C; (f) MeNCS, pyridine.

with 4-fluorophenyl **24a**, 4-bromophenyl **24b**, 4-(trifluoromethyl)phenyl **24c**, 2,4-dichlorophenyl **24d**, 3,4-dichlorophenyl **24e**, and 4-chloro-2-methoxyphenyl **24f** as shown in Table 4 and Scheme 6. This method of synthesis improved efficiency. The obtained triazoles were treated with 1 N sodium hydroxide in THF and

Scheme 4[a]



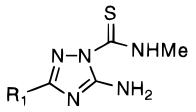
[a] Condition: (a) H_2NCN, Ac_2O, 140 °C; (b) H_2NNH_2, CH_3CN; (c) MeNCS, DMF.

methyl isothiocyanate to give **25a**−**f** in higher yields with shorter reaction time (method E).

Results and Discussion

In order to characterize the role of eosinophils in the pathophysiology of bronchial asthma and airway hyperresponsiveness, a variety of methods have been employed to induce pulmonary and peripheral blood eosinophilia in appropriate experimental animal models. So far, there have been reports on several models including actively sensitized guinea pigs,[21] a PAF-

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

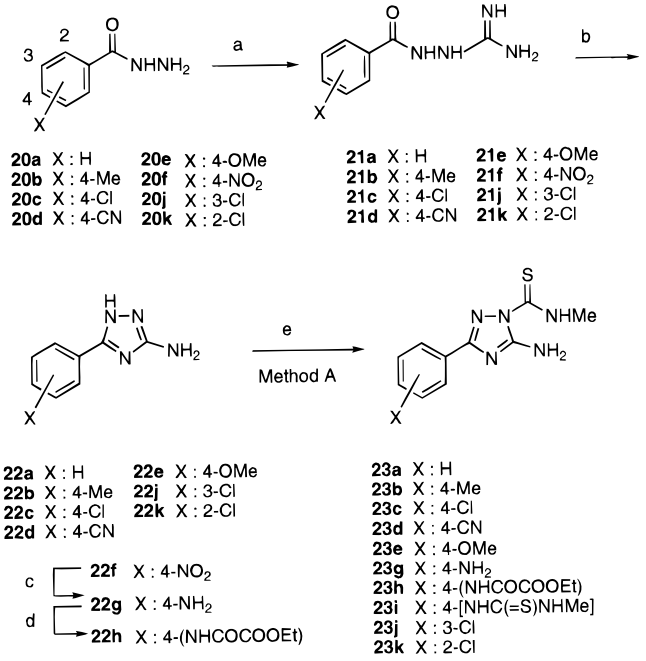


no.	R_1	method[a]	mp[b] (°C)	formula[c]	inhibn[d] (%)
18	Me	A	180−181	$C_5H_9N_5S$	13
19	SMe	A	173−175	$C_5H_9N_5S_2$	54
23a	Ph	A	174−175	$C_{10}H_{11}N_5S$	54
23b	Ph(4-Me)[e]	A	180−182	$C_{11}H_{13}N_5S$	65
23c	Ph(4-Cl)	A	203−206	$C_{10}H_{10}ClN_5S$	88 (88)[f,g]
23d	Ph(4-CN)	A	213−216 dec	$C_{11}H_{10}N_6S\cdot 0.3H_2O$	87
23e	Ph(4-OMe)	A	188−189	$C_{11}H_{13}N_5OS$	70
23g	Ph(4-NH_2)	A	172−174	$C_{10}H_{12}N_6S\cdot 0.3H_2O$	−[h]
23h	Ph(4-R_2[i])	A	261−262	$C_{14}H_{16}N_6O_3S$	49
23i	Ph(4-R_3[j])	A	190−192	$C_{12}H_{15}N_7S_2\cdot 0.2H_2O$	31
23j	Ph(3-Cl)	A	191−192	$C_{10}H_{10}ClN_5S$	(54)[f]
23k	Ph(2-Cl)	A	168−170	$C_{10}H_{10}ClN_5S$	(18)[f]
25a	Ph(4-F)	E	192−194	$C_{10}H_{10}FN_5S$	(73)[f]
25b	Ph(4-Br)	E	210−212	$C_{10}H_{10}BrN_5S$	(88)[f]
25c	Ph(4-CF_3)	E	193−195	$C_{11}H_{10}F_3N_5S$	(91)[f]
25d	Ph(2,4-Cl_2)	E	192−193	$C_{10}H_9Cl_2N_5S$	(91)[f]
25e	Ph(3,4-Cl_2)	E	216−218	$C_{10}H_9Cl_2N_5S$	(85)[f]
25f	Ph(4-Cl-2-OMe)	E	165−167 dec	$C_{11}H_{12}ClN_5OS$	(68)[f]

polyclonal rabbit anti-human IL-5

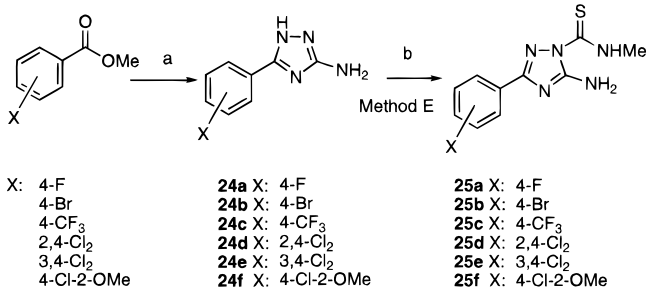
[a] See Table 1, footnote *a* and Scheme 4−6. [b−d] See Table 1. [e] Ph(4-Me) denotes 4-methylphenyl moiety. [f] Value in parentheses is percent inhibition at an ip dose of 3 mg/kg. [g] SE value for **23c** was determined to be ±4.2 from six experiments ($n = 30$). [h] All animals died. [i] R_2 denotes ethoxalylamino moiety. [j] R_3 denotes 3-methylthioureido moiety. [k] Percent inhibition at ip dose of 100 μg/kg.

Scheme 5[a]



[a] Condition: (a) S-methylisothiourea sulfate, aqueous NaOH; (b) 220 °C; (c) 10% Pd/C, MeOH; (d) ethyl oxalyl chloride, Et_3N, DMF, −78 °C; (e) MeNCS, DMF.

Scheme 6[a]



[a] Condition: (a) aminoguanidine nitrate or hydrochloride, NaOMe, MeOH; (b) MeNCS, 1 N NaOH, THF.

induced model,[22] and a Sephadex-induced model.[9] In the actively sensitized model, symptoms agreed well with those of allergic asthma, but the procedures involved were too complicated to allow evaluation of a wide variety of compounds. In the PAF-induced model, PAF alone seems to have been found insufficient as a basic mediator for the complex mechanism of eosinophilia, as it was reported that cytokines released from T-lymphocytes appeared to play a part in eosinophilia.[4] In addition, oral (os) administration of the potent PAF antagonist UK-74,505 to allergen-challenged asthmatics showed no effect on either the early (EAR) or late (LAR) asthmatic response.[23]

On this basis, we selected the rat model of Sephadex-induced eosinophilia reported on by Spicer *et al.* in which eosinophilia is induced in the airway through iv injection of Sephadex G200 particles.[9] In the present model, this procedure induced increased eosinophil counts in BAL fluids and airway hyperresponsiveness to 5-hydroxytryptamine as well as desquamation of epithelial cells and mucus plugs, which were observed under histological examination.[9]

The Sephadex model was originally developed from the recognition that parasitic infestations of animal and humans are associated with blood eosinophilia.[24] In the parasitic infection model as well as the Sephadex-induced model, eosinophilia is induced by particle- or larva-based embolism of the pulmonary vasculature.[25] The key factor is particle size, since eosinophilia is induced by live and dead larvae but not by homogenates.[25] The relationship between Sephadex particle size and eosinophilia is clear, with Sephadex G200 of diameter 66.8 ± 22.9 μm the most effective in inducing eosinophilia.[26] However, the fact that no eosinophilia

is observed when polystyrene beads of similar size are injected suggests that antigen interaction may be involved in the formation of eosinophilia.[26] Recent studies suggest that the relatively rapid onset of periarterial and peribronchial eosinophilia following Sephadex administration is most likely due to an eosinophilic chemotactic substance released by the lodging of the Sephadex beads in the pulmonary arteries.[27] In addition, anti-IL-5 monoclonal antibody (mAb) has been found to totally inhibit eosinophilia in the airway of a guinea pig model of Sephadex G50-induced eosinophilia.[28] In our preliminary experiment, the effect of 100 μg of polyclonal rabbit antihuman IL-5 was examined in this model. As shown in Table 4, moderate inhibition was observed in spite of the use of human antibody, suggesting that IL-5 plays some role.

Accordingly, this model was not only highly reproducible but presented an easy way of evaluating a large number of compounds in one operation. It also seemed recommended as a screening system by the fact that steroids, which have been demonstrated to be the most effective drugs for the treatment of asthma, showed potent inhibitory activity in it.

In the initial step of our screening, we used intraperitoneal (ip) administration of compounds at a dose of 30 mg/kg. During the course of the randomized screening test using this model, 5-amino-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole **1a** was identified as a potent inhibitor, with a 93% inhibition of eosinophilia at 30 mg/kg (ip). Applying further structural modification of the lead **1a**, we first examined the effect of alkyl substitution of the (methylamino)(thiocarbonyl) moiety of **1a** on activity. The results are shown in Table 1. The activity of compounds (**1c** and **1d**) having a linear alkyl chain longer than *n*-propane was reduced drastically. In addition, the weakened activity of compounds having hydrophilic substituents **1e** (3-ethoxypropyl) and **1i** (5-hydroxypentyl) in side chains suggested that not a hydrophilic but a steric effect plays an important role in this activity. When the effect of the steric shape of the amino substituent was examined, cyclohexyl **1f**, phenyl **1g**, and benzyl **1h** showed reduced activity, indicating that larger substituents might be unfavorable. Contrary to our supposition, the low activity of the dimethylamino derivative **3**, which is considered to have a steric substituent comparable to that of **1a**, indicated that amide protons play some role in the activity.

Secondly, we examined the effect of the ring systems of **1a** by introducing other rings in place of the triazole moiety without modifying the substituent pattern. The results are shown in Table 2. While **5**, having a tetrazole ring in place of the triazole ring of **1a**, showed a 53% inhibition, **4** and **7a**−**c**, having pyrazole, imidazole, or thiophene rings, showed drastically reduced activity. Furthermore, benzene **7d**, pyridine **7e**,**f**, and pyrazine **7g** all had weakened activity compared to that of **1a**, indicating that the triazole ring of **1a** is the most favorable of the ring systems examined. The reason for this is unknown at present, but detailed examinations are underway.

Thirdly, we examined the effect of the amino(thiocarbonyl) moiety of **1a** on activity. The results are shown in Table 3. Compounds **8** and **9**, having (methylamino)-carbonyl and methyl dithiocarbonate, respectively, in place of the (methylamino)(thiocarbonyl) moiety of **1a**, showed half the inhibitory activity of **1a**. In addition, the observation that the methyl sulfone derivative **10** showed complete loss of activity suggests that the thiocarbonyl moiety provides the most favorable function and that the sulfur atom in this moiety may have some role in producing a potent inhibitory activity.

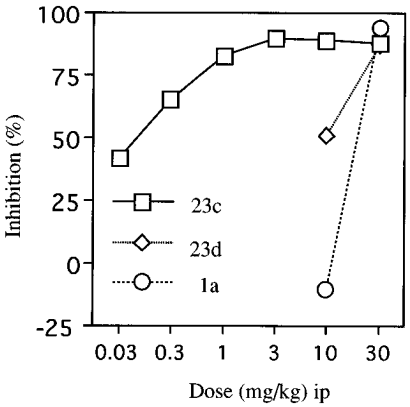


Figure 1. Dose−response examination of **1a**, **23c**, and **23d** on eosinophilia model. Each point was calculated by the same procedure described in Experimental Section.

To investigate the effect of amino substituent at the five position of **1a**, we prepared the methylamino derivative **12**, which showed complete loss of activity. This result, together with the low activity of the (dimethylamino)(thiocarbonyl) derivative **3**, suggests that the steric shape derived from the interaction between adjacent functional groups, amine at the 5-position and (methylamino)(thiocarbonyl), may play some part in inhibitory activity. A nuclear Overhauser effect was observed between the amino at the five position and the methyl at the methylaminothiocarbonyl moiety of **1a**[29] in NMR analysis, which also supports the explanation offered above.

Fourthly, we examined the effect of a substituent at the 3-position of the triazole ring of **1a** as shown in Table 4. Introduction of methyl (**18**) weakened activity, but methylthio (**19**) and phenyl (**23a**) showed higher activity than **18**, indicating that methylthio and phenyl moiety play some part in maintaining activity. These observations, together with synthetic accessibility, encouraged us to examine a series of phenyl derivatives with various substituents on the phenyl ring. While introduction of *p*-tolyl (**23b**) and 4-methoxyphenyl (**23e**) showed equipotent activity with the nonsubstituted **23a**, compounds with 4-chlorophenyl (**23c**) and 4-cyanophenyl (**23d**) showed more potent activity. The reason for this potentiation is unknown at present, but the presence of electron-withdrawing substituents at the 4-position of the phenyl ring would seem fairly favorable to activity. In order to use these findings to explore for further potent inhibitors, (ethoxalylamino)phenyl **23h** and 4-(methylthioureido)phenyl **23i** were synthesized and their activity evaluated. In contrast to **23c**, these derivatives showed lower activity at a dose of 30 mg/kg. Administration of 4-aminophenyl **23g** caused death. In view of the almost complete inhibition of **1a**, **23c**, and **23d** at a dose of 30 mg/kg, we conducted a dose−response examination as shown in Figure 1. The activity of **1a** was completely lost at a dose of 10 mg/kg, but **23c** showed 90% inhibition at a dose of 1 mg/kg, indicating that **23c** is a much more potent compound.

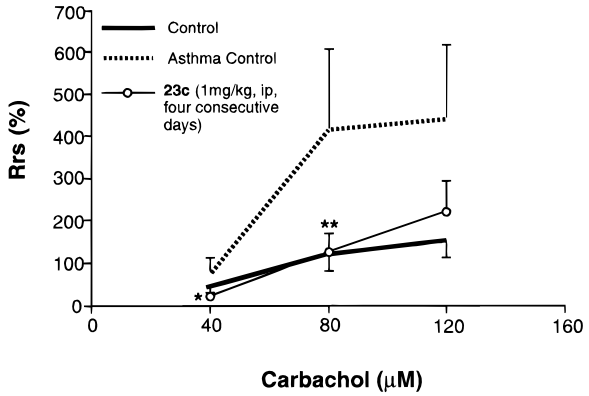


Figure 2. Effect of **23c** on ascaris induced hyperresponsiveness model in guinea pigs. Values are means ± SE (n = 8). Statistical analysis was carried out by Bonferroni's method. *P < 0.05 vs asthma control. **P < 0.01 vs asthma control.

Using the method proposed by Topliss,[30] substituted phenyl derivatives were synthesized as shown in Table 4. A series of compounds having 4-fluorophenyl (**25a**), 4-bromophenyl (**25b**), 4-(trifluoromethyl)phenyl (**25c**), 2,4-dichlorophenyl (**25d**), and 3,4-dichlorophenyl (**25e**) also showed high inhibition rates of 73, 88, 91, 91, and 85%, respectively, at a dose of 3 mg/kg. The effect of the position of the chlorine atom on the phenyl moiety was also examined: maximum activity was attained in **23c** with progressively weaker activity at the 3- (**23j**) and 2-positions (**23k**). These results suggest that electron-withdrawing substituents, preferably at the 4-position, are fairly favorable to activity.

On the strength of these biological results and its synthetic accessibility, **23c** was selected as a candidate drug for further development. When os administered, **23c** also suppressed eosinophilia very potently with an ID_{50} value of 0.3 mg/kg. In an examination of the efficacy of **23c** against bronchial asthma in antigen (ascaris)-induced hyperresponsiveness in guinea pigs using the modified method of sensitization reported by H. Inoue *et al.*,[31] it was found to inhibit airway hyperresponsiveness at a dose of 1 mg/kg (ip) without exhibiting any significant side effects, as shown in Figure 2. The compound showed low toxicity, with an LD_{50} value of >2.0 g/kg in mice. The mechanisms of action of **23c** have been extensively studied in our laboratory. No or only very weak activity has so far been observed on the binding of PAF[32] and LTD_4,[33] as well as the inhibition of 5-lipoxygenase.[34] To investigate the activity of IL-5 in the eosinophilia model, we examined the effect of **23c** on IL-5-mediated biological events. Among these, we detected activity of **23c** on IL-5-stimulated human eosinophil survival[35] with an IC_{50} value of 2 μM, as shown in Figure 3. Further extensive research based on this result is underway.

In conclusion, we used a Sephadex-induced lung eosinophilia model to screen for an antiasthmatic agent based on a novel mechanism of action. After examination of a series of triazole derivatives, we finally identified the highly potent eosinophilia inhibitor 5-amino-3-(4-chlorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-triazole **23c**. This compound, which showed complete inhibition of hypersensitivity induced by ascaris inhalation as well as low toxicity in mice, is now undergoing further developmental examination.

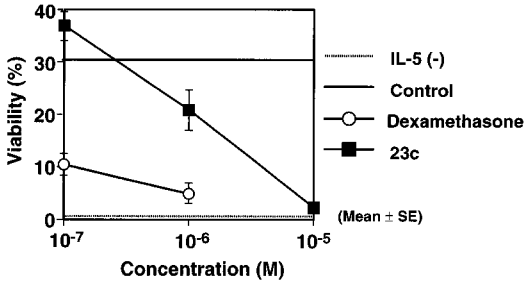


Figure 3. Effect of **23c** and dexamethasone on IL-5 (10 units/mL)-stimulated human eosinophil survival. Values are mean ± SE (n = 4).

Experimental Section

Chemistry. Melting points were determined with a Yanaco menting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured on a Bruker AC-200 and AMX-500 NMR spectrometer 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 spectrometer.

Method A. 5-Amino-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1a). To a mixture of 3-amino-1*H*-1,2,4-triazole (3.91 g, 46.5 mmol) in DMF (20 mL) was added methyl isothiocyanate (3.41 g, 46.6 mmol). The resultant mixture was stirred at room temperature for 60 h. After filtration of the precipitated solid, the filtrate was poured into water and extracted with ethyl acetate (AcOEt). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give a white powder. Upon recrystallization from EtOH, a mixture of **1a** and **13** (73:27, 3.46 g, 47%) was obtained. After further recrystallization from AcOEt, **1a** (1.34 g, 18%) was obtained as yellow crystals: mp 184−185 °C; IR (KBr, cm^{-1}) 3420, 3310, 1634, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.04 (s, 3H, CH_3), 7.63 (s, 1H, CH), 8.18 (brs, 2H, NH_2), 10.02 (brs, 1H, NH).

5-Amino-1-[(*n*-propylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1b). The procedure used for the preparation of **1a** (method A) was repeated with *n*-propyl isothiocyanate as a starting material. After the reaction, the mixture was poured into water and extracted with AcOEt. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to give a crude product which was purified by column chromatography on silica gel (hexane/AcOEt, 5/1) followed by reversed phase HPLC (CH_3CN/H_2O, 1/2) to give a mixture of **1b** and 3-amino-1-[(*n*-propylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1:1, 3.07 g, 35%). Upon recrystallization from AcOEt and hexane, **1b** was obtained as colorless crystals: mp 117−118 °C; IR (KBr, cm^{-1}) 3300, 1638, 1503; ^{1}H-NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7.4 Hz, CH_3), 1.63 (sext, 2H, J = 7.4 Hz, CH_2), 3.53 (t, 2H, J = 7.4 Hz, CH_2), 7.62 (s, 1H, CH), 8.18 (brs, 2H, NH_2), 10.03 (brs, 1H, NH).

5-Amino-1-[(*n*-butylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1c). The procedure used for the preparation of **1a** (method A) was repeated with *n*-butyl isothiocyanate as a starting material. After purification with column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) and HPLC (CH_3CN/H_2O, 1/1), a mixture of **1c** and 3-amino-1-[(*n*-butylamino)-(thiocarbonyl)]-1*H*-1,2,4-triazole (64:36, 6.5 g, 91%) was obtained. Upon recrystallization from $CHCl_3$ and hexane, **1c** was obtained as colorless crystals: mp 119−120 °C; IR (KBr, cm^{-1}) 3270, 3060, 1636; ^{1}H-NMR (DMSO-d_6) δ 0.90 (t, 3H, J = 7.4 Hz, CH_3), 1.31 (sext, 2H, J = 7.4 Hz, CH_2), 1.60 (quint, 2H, J = 7.4 Hz, CH_2), 3.52 (t, 2H, J = 7.4 Hz, CH_2), 7.62 (s, 1H, CH), 8.18 (brs, 2H, NH_2), 10.02 (brs, 1H, NH).

***n*-Hexyl Isothiocyanate (2).** To a cooled (0 °C) solution of carbon disulfide (40 mL) was added dropwise a solution of *n*-hexylamine (6.05 g, 59.8 mmol) and triethylamine (8.3 mL, 60 mmol) for 10 min. After stirring for 2 h at 0 °C, solvents were removed under reduced pressure. The residue obtained was treated with Et_2O to give a white solid which was dissolved in $CHCl_3$ (20 mL) and triethylamine (8.3 mL, 60 mmol). After being cooled to 0 °C, ethyl chloroformate (5.4

mL, 56 mmol) was added to the solution. Stirring further at 0 °C for 10 min and at room temperature for 30 min, the mixture was poured into 1 N HCl and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure to give a yellow oil (8.54 g, quantitative): IR (KBr, cm^{-1}) 2910, 2840, 2070; ^{1}H-NMR ($CDCl_3$) δ 0.90 (t, 3H, J = 7.1 Hz, CH_3), 1.28−1.36 (m, 4H, CH_2), 1.42 (quint, 2H, J = 7.0 Hz, CH_2), 1.70 (quint, 2H, J = 7.0 Hz, CH_2), 3.51 (t, 2H, J = 7.0 Hz, CH_2).

5-Amino-1-[(*n*-hexylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1d). The procedure used for the preparation of **1a** (method A) was repeated with **2** as a starting material. After purification with column chromatography on silica gel (hexane/AcOEt, 5/1), a mixture of **1d** and 3-amino-1-[(*n*-hexylamino)-(thiocarbonyl)]-1*H*-1,2,4-triazole (1:1, 3.13 g, 29%) was obtained. Further purification with HPLC (CH_3CN/H_2O, 1/1) and recrystallization from hexane and AcOEt, **1d** was obtained as colorless crystals: mp 122−123 °C; IR (KBr, cm^{-1}) 3400, 1628, 1515; ^{1}H-NMR (DMSO-d_6) δ 0.86 (t, 3H, J = 6.9 Hz CH_3), 1.22−1.36 (m, 6H, CH_2), 1.61 (quint, 2H, J = 7.2 Hz, CH_2), 3.56 (t, 2H, J = 7.2 Hz, CH_2), 7.61 (s, 1H, CH), 8.18 (brs, 2H, NH_2), 10.02 (brs, 1H, NH).

5-Amino-1-[[(3-ethoxypropyl)amino](thiocarbonyl)]-1*H*-1,2,4-triazole (1e). The procedure used for the preparation of **1a** (method A) was repeated with 3-ethoxypropyl isothiocyanate as a starting material. After purification with column chromatography on silica gel ($CHCl_3$/MeOH, 20/1), a mixture of **1e** and 3-amino-1-[[(3-ethoxypropyl)amino](thiocarbonyl)]-1*H*-1,2,4-triazole (69:31, 5.41 g, 50%) was obtained. Purification with HPLC (CH_3CN/H_2O, 2/3) and recrystallization from hexane and $CHCl_3$ gave colorless crystals: mp 137−139 °C; IR (KBr, cm^{-1}) 3320, 3060, 2920, 1638, 1507; ^{1}H-NMR (DMSO-d_6) δ 1.12 (t, 3H, J = 7.0 Hz, CH_3), 1.86 (quint, 2H, J = 6.5 Hz, CH_2), 3.42 (q, 2H, J = 7.0 Hz, CH_2), 3.44 (t, 2H, J = 6.5 Hz, CH_2), 3.63 (t, 2H, J = 6.5 Hz, CH_2), 7.61 (s, 1H, CH), 8.18 (brs, 2H, NH_2), 10.02 (brs, 1H, NH).

5-Amino-1-[(cyclohexylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1f). The procedure used for the preparation of **1a** (method A) was repeated with cyclohexyl isothiocyanate as a starting material. After purification with column chromatography on silica gel (hexane/AcOEt, 1/1) and recrystallization from hexane and AcOEt, **1f** was obtained as colorless crystals (2.26 g, 14%): mp 168−170 °C; IR (KBr, cm^{-1}) 3380, 3270, 1633, 1562, 1505; ^{1}H-NMR (DMSO-d_6) δ 1.13 (t q, 1H, J = 3.4, 12.6 Hz, CH), 1.30 (t q, 2H, J = 3.4, 12.6 Hz, CH), 1.51 (d q, 2H, J = 3.4, 11.7 Hz, CH), 1.60 (d t, 1H, J = 12.8, 3.3 Hz, CH), 1.73 (d t, 2H, J = 13.5, 3.1 Hz, CH), 1.85 (d d, 2H, J = 2.9, 12.2 Hz, CH), 4.16 (brs, 1H, CH), 7.62 (s, 1H, CH), 8.18 (brs, 2H, NH_2), 9.62 (brs, 1H, NH).

5-Amino-1-[(phenylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1g). The procedure used for the preparation of **1a** (method A) was repeated with phenyl isothiocyanate as a starting material. After the reaction, the precipitated white solid was collected and recrystallization from MeOH gave **1g** as colorless crystals (0.21 g, 2.7%): mp 146−148 °C dec; IR (KBr, cm^{-1}) 3290, 3240, 3060, 1638, 1503; ^{1}H-NMR (DMSO-d_6) δ 7.31 (t, 1H, J = 7.2 Hz, CH), 7.41−7.50 (m, 4H, CH), 7.71 (s, 1H, CH), 8.26 (brs, 2H, NH_2), 11.50 (brs, 1H, NH).

5-Amino-1-[(benzylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (1h). The procedure used for the preparation of **1a** (method A) was repeated with benzyl isothiocyanate as a starting material. After recrystallization from EtOH, **1h** was obtained as white crystals (3.99 g, 36%): mp 157−158 °C; IR (KBr, cm^{-1}) 3380, 3270, 3070, 1633, 1618, 1512; ^{1}H-NMR (DMSO-d_6) δ 4.81 (s, 2H, CH_2), 7.25 (t t, 1H, J = 1.8, 6.8 Hz, CH), 7.30−7.36 (m, 4H, CH), 7.66 (s, 1H, CH), 8.20 (brs, 2H, NH_2), 10.54 (brs, 1H, NH).

5-Amino-1-[[(5-hydroxypentyl)amino](thiocarbonyl)]-1*H*-1,2,4-triazole (1i). The procedure used for the preparation of **1a** (method A) was repeated with 5-hydroxypentyl isothiocyanate as a starting material. Purification with column chromatography on silica gel ($CHCl_3$/MeOH, 10/1) and recrystallization from $CHCl_3$ gave white crystals (19%): mp 108−109 °C; IR (KBr, cm^{-1}) 3290, 3130, 2940, 2880, 1634, 1521; ^{1}H-NMR (DMSO-d_6) δ 1.27−1.36 (m, 2H, CH_2), 1.45 (quint, 2H, J = 6.5 Hz, CH_2), 1.62 (quint, 2H, J = 7.4 Hz, CH_2), 3.39 (d t, 2H, J = 5.2, 6.5 Hz, CH_2), 3.57 (q, 2H, J = 7.4 Hz, CH_2), 4.34 (t, 1H, J = 5.2 Hz, OH), 7.63 (s, 1H, CH), 8.19 (brs, 2H, NH_2), 10.02 (brs, 1H, NH).

Method B. 5-Amino-1-[(dimethylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (3). To a solution of 3-amino-1*H*-1,2,4-triazole (4.00 g, 47.6 mmol) in pyridine (30 mL) was added dimethylthiocarbamoyl chloride (6.46 g, 52.3 mmol). After stirring for 24 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was extracted with AcOEt, and the extract was washed with brine and dried over $MgSO_4$. After removal of the solvent, purification with column chromatography on silica gel ($CHCl_3$/MeOH, 10/1) gave a mixture of **3** and 3-amino-1-[(dimethylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (2:1, 1.63 g, 20%). Recrystallization from AcOEt gave **3** as white crystals: mp 139.5−140.5 °C; IR (KBr, cm^{-1}) 3360, 1645, 1516; ^{1}H-NMR (DMSO-d_6) δ 3.17 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 6.80 (brs, 2H, NH_2), 7.53 (s, 1H, CH).

5-Amino-1-[(methylamino)(thiocarbonyl)]pyrazole (4). The procedure used for the preparation of **1a** (method A) was repeated with 3-aminopyrazole as a starting material. The crude product obtained was purified by column chromatography on silica gel (hexane/AcOEt, 10/1 to 1/2) to give a colorless oil (2.03 g, 27%): IR (KBr, cm^{-1}) 3270, 1600, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.04 (d, 3H, J = 4.7 Hz, CH_3), 5.40 (d, 1H, J = 1.7 Hz, CH), 7.33 (brs, 2H, NH_2), 7.36 (d, 1H, J = 1.7 Hz, CH), 9.99 (brs, 1H, NH).

5-Amino-1-[(methylamino)(thiocarbonyl)]-1*H*-tetrazole (5). The procedure used for the preparation of **1a** (method A) was repeated with 5-amino-1*H*-tetrazole as a starting material. The crude product obtained was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 20/1) to give a mixture of **5** and 5-amino-2-[(methylamino)(thiocarbonyl)]tetrazole (10:1, 234 mg, 3.9%) as a white powder: IR (KBr, cm^{-1}) 3320, 3170, 1640, 1553; ^{1}H-NMR (DMSO-d_6) δ 3.08 (d, 3H, J = 4.2 Hz, CH_3), 6.03 (brs, 2H, NH_2), 10.70 (brs, 1H, NH).

3-Amino-*N*-methylpyrazole-4-carbothioamide (7a). A mixture of **6a** (3.6 g, 25.7 mmol) and phosphorus pentasulfide (8.5 g, 38.3 mmol) in pyridine (50 mL) was stirred at 80 °C overnight. After careful addition of 28% aqueous ammonia solution (50 mL), the mixture was stirred at 80 °C for 4 h. After the reaction, a solution of $CHCl_3$−MeOH (1:1, 300 mL) was added. The organic layer was collected by decantation and concentrated under reduced pressure. Purification with column chromatography on silica gel ($CHCl_3$/MeOH, 20/1 to 10/1) and recrystallization from $CHCl_3$ and MeOH gave white crystals (600 mg, 15%): mp 235−237 °C; IR (KBr, cm^{-1}) 3450, 3300, 3100, 1585, 1560, 1525; ^{1}H-NMR (DMSO-d_6) δ 3.01 (d, 3H, J = 4.5 Hz, CH_3), 6.85 (brs, 2H, NH_2), 7.76 (s, 1H, CH), 9.24 (s, 1H, NH), 11.84 (brs, 1H, NH).

4-Amino-*N*-methylimidazole-5-carbothioamide (7b). The procedure used for preparation of **7a** was repeated with **6b** as a starting material. The residue obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 20/1 to 10/1) and recrystallized from $CHCl_3$ and MeOH to give dark brown crystals (1.1 g, 33%): mp 202−204 °C; IR (KBr, cm^{-1}) 3300, 3200, 1610, 1540; ^{1}H-NMR (DMSO-d_6) δ 3.01 (d, 3H, J = 4.8 Hz, CH_3), 6.72 (brs, 2H, NH_2), 7.11 (s, 1H, CH), 9.05 (brs, 1H, NH), 11.47 (brs, 1H, NH).

3-Amino-*N*-methylthiophene-2-carbothioamide (7c). The procedure used for preparation of **7a** was repeated with **6c** as a starting material. The residue obtained was chromatographed on silica gel (AcOEt/hexane, 1/4 to 1/2) to give a brown oil (0.35 g, 51%): IR (KBr, cm^{-1}) 3300, 1590, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.00 (d, 3H, J = 4.4 Hz, CH_3), 6.63 (d, 1H, J = 5.5 Hz, CH), 7.43 (d, 1H, J = 5.5 Hz, CH), 7.57 (brs, 2H, NH_2), 8.75 (m, 1H, NH).

2-Amino-*N*-methylbenzenecarbothioamide (7d). The procedure used for the preparation of **7a** was repeated using **6d** as a starting material. A mixture of **6d** (2.0 g, 13.6 mmol) and phosphorus pentasulfide (3.03 g, 13.6 mmol) in pyridine (8 mL) was stirred at reflux for 1.5 h. After addition of H_2O, a precipitated yellow solid was collected by filtration. A suspension of the solid obtained in benzene (150 mL) and H_2O (15 mL) was stirred at reflux for 15.5 h. The benzene layer

was washed with H_2O and brine and dried over $MgSO_4$. The benzene layer obtained was concentrated to give a yellow solid. Recrystallization from benzene and hexane gave yellow crystals (1.18 g, 52%): mp 97−99.5 °C; IR (KBr, cm^{-1}) 3400, 3200, 3000, 1610, 1580; ^{1}H-NMR (DMSO-d_6) δ 3.06 (s, 3H, CH_3), 5.73 (brs, 2H, NH_2), 6.55 (t, 1H, J = 3 Hz, CH), 6.70 (d, 1H, J = 3 Hz, CH), 7.03 (dd, 1H, J = 3, 0.5 Hz, CH), 7.07 (dt, 1H, J = 0.5, 3 Hz, CH).

2-Amino-*N*-methylpyridine-3-carbothioamide (7e). The procedure used for the preparation of **7a** was repeated using **6a** as a starting material. A mixture of **6a** (5.0 g, 33 mmol) and phosphorus pentasulfide (11.0 g, 49 mmol) in pyridine (25 mL) was stirred at reflux for 4 h. After addition of H_2O, the precipitated yellow solid was collected by filtration. A suspension of the solid obtained in benzene (150 mL) and H_2O (15 mL) was stirred at reflux overnight. The solid obtained by filtration was treated with 28% aqueous ammonia solution (30 mL), and the mixture was stirred at reflux for 0.5 h. The mixture was extracted with benzene, and the organic layer was washed with brine (100 mL) and concentrated under reduced pressure. The residue was crystallized from hexane and AcOEt to give yellow crystals: mp 134−135 °C; IR (KBr, cm^{-1}) 3200, 3100, 1610, 1570, 1540; ^{1}H-NMR (DMSO-d_6) δ 3.09 (s, 3H, CH_3), 6.44 (brs, 2H, NH_2), 6.60 (dd, 1H, J = 7.5, 4.9 Hz, CH), 7.40 (dd, 1H, J = 7.5, 1.9 Hz, CH), 7.99 (dd, 1H, J = 4.9, 1.9 Hz, CH), 10.35 (brs, 1H, NH).

3-Amino-*N*-methylpyridine-2-carbothioamide (7f). A mixture of **6f** (1.0 g, 6.6 mmol) and Lawesson's reagent (2.6 g, 6.4 mmol) in HMPA (20 mL) was stirred at 120 °C overnight. The resultant mixture was treated with 28% aqueous ammonia solution (5 mL) for 10 min at 120 °C and was extracted with $CHCl_3$ by the usual manner. After removal of the solvents, the crude product was chromatographed on silica gel (AcOEt/hexane, 1/10 to 1/6) to give an oil (230 mg, 21%): IR (neat, cm^{-1}) 3350, 3200, 2900, 1600, 1570, 1500; ^{1}H-NMR (DMSO-d_6) δ 3.10 (d, 3H, J = 4.9 Hz, CH_3), 7.24 (m, 2H, CH), 7.51 (brs, 2H, NH_2), 7.81 (t, 1H, J = 2.8 Hz, CH), 10.62 (m, 1H, NH).

3-Amino-*N*-methylpyrazine-2-carbothioamide (7g). The procedure used for preparation of **7f** was repeated with **6g**. The Et_2O extract was washed with H_2O and brine and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on silica gel (AcOEt/hexane, 1/3) and recrystallized from $CHCl_3$ and hexane to give yellow crystals (220 mg, 15%): mp 104−105 °C; IR (KBr, cm^{-1}) 3300, 1610, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.12 (s, 3H, CH_3), 7.86 (d, 1H, J = 2.3 Hz, CH), 8.04 (brs, 2H, NH_2), 8.21 (d, 1H, J = 2.3 Hz, CH), 10.72 (s, 1H, NH).

5-Amino-1-[(methylamino)carbonyl]-1*H*-1,2,4-triazole (8). The procedure used for the preparation of **1a** (method A) was repeated with methyl isocyanate. After the reaction, the precipitated solid was collected by filtration and recrystallized from hexane and AcOEt to give colorless crystals (6.83 g, 81%): mp 192−193 °C; IR (KBr, cm^{-1}) 3370, 3100, 1707, 1643, 1555, 1504; ^{1}H-NMR (DMSO-d_6) δ 2.75 (d, 3H, J = 4.7 Hz, CH_3), 7.15 (brs, 2H, NH_2), 7.53 (s, 1H, CH), 8.11 (brs, 1H, NH).

Method C. Methyl (5-Amino-1,2,4-triazol-1-yl)dithiocarbonate (9). After a mixture of 3-amino-1*H*-1,2,4-triazole (8.41 g, 0.1 mol) and carbon disulfide (6.6 mL, 0.11 mol) in DMF (30 mL) was stirred at 0 °C for 0.5 h, aqueous KOH solution [KOH (5.70 g, 0.102 mol) in H_2O (10 mL)] was added. The resultant solution was stirred at 0 °C for 45 min, and then MeI (6.6 mL, 0.11 mol) was added. The mixture was stirred at 0 °C for 0.5 h and at room temperature for 1.5 h. After addition of water (30 mL), the precipitated solid was collected by fitration. The crude solid was recrystallized from EtOH to give yellow crystals (6.12 g, 35%): mp 203−205 °C; IR (KBr, cm^{-1}) 3350, 1655, 1510; ^{1}H-NMR (DMSO-d_6) δ 2.60 (s, 3H, CH_3), 7.71 (s, 1H, CH), 8.46 (brs, 2H, NH_2).

Method D. 5-Amino-1-(methylsulfonyl)-1*H*-1,2,4-triazole (10). To a cooled (−78 °C) mixture of 3-amino-1*H*-1,2,4-triazole (1.54 g, 8.3 mmol) in pyridine (10 mL) was added dropwise methanesulfonyl chloride (1.6 mL, 21 mmol) for 1 h. After stirring addtional 1 h, the reaction mixture was added H_2O and extracted with AcOEt. The organic layer was washed with water and brine and dried over $MgSO_4$. After evaporation of the solvent, the crude product was chromatographed on silica gel (AcOEt/hexane, 1/1 to 1/0) and recrystallized from AcOEt to give white crystals (484 mg, 18%): mp 132−133 °C; IR (KBr, cm^{-1}) 3420, 3300, 3150, 3040, 1650, 1570, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.49 (s, 3H, CH_3), 7.09 (brs, 2H, NH_2), 7.68 (s, 1H, CH).

5-(Methylamino)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (12). A solution of 3-nitro-1*H*-1,2,4-triazole (15.0 g, 132 mmol) in concentrated HCl (50 mL) was stirred in sealed tube for 15 h at 100 °C. After cooling to room temperature, the solution was extracted with AcOEt. The organic layer was washed with H_2O, dried over $MgSO_4$, and evaporated to give 3-chloro-1*H*-1,2,4-triazole as a white solid (5.24 g, 38%). The solid obtained (3.0 g, 29 mmol) in 40% aqueous methylamine (20 mL) was stirred in a sealed tube for 24 h at 180 °C. After cooling to room temperature, the mixture was concentrated under reduced pressure to give a crude product which was used without further purification. The procedure used for the preparation of **1a** (method A) was repeated with 3-(methylamino)-1*H*-1,2,4-triazole (5.17 g, 52.8 mmol), methyl isothiocyanate (4.27 g, 57.8 mmol), and pyridine (20 mL). The crude product obtained was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 50/1) followed by reversed phase HPLC (CH_3CN/H_2O, 1/4) and recrystallization from $CHCl_3$ and hexane to give white crystals (228 mg, 4.7%): mp 98−99 °C; IR (KBr, cm^{-1}) 3400, 3240, 3200, 1621, 1521; ^{1}H-NMR (DMSO-d_6) δ 3.01 (d, 3H, J = 4.5 Hz, CH_3), 3.03 (s, 3H, CH_3), 7.68 (s, 1H, CH), 8.62 (brs, 1H, NH), 9.96 (brs, 1H, NH).

3-Amino-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (13). Recrystallization from EtOH of the mother liguor residue used for the preparation of **1a** gave a mixture of **13** and **1a** (1.06 g, 85:15, 14.4%): IR (KBr, cm^{-1}) 3430, 3320, 1634, 1540; ^{1}H-NMR (DMSO-d_6) δ 3.07 (s, 3H, CH_3), 6.02 (brs, 2H, NH_2), 8.89 (s, 1H, CH), 9.76 (brs, 1H, NH).

3,5-Diamino-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (14). The procedure used for the preparation of **1a** (method A) was repeated with 3,5-diamino-1*H*-1,2,4-triazole. After the reaction, the reaction mixture was poured into water and exracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. Recrystallization from AcOEt gave white crystals (13.0 g, 68%): mp 176−178 °C; IR (KBr, cm^{-1}) 3430, 3280, 1620, 1520; ^{1}H-NMR (DMSO-d_6) δ 2.98 (d, 3H, J = 4.4 Hz, CH_3), 5.63 (brs, 2H, NH_2), 8.14 (brs, 2H, NH_2), 9.19 (m, 1H, NH).

1-[(Cyclohexylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (15). The procedure used for the preparation of **1a** (method A) was repeated with 1*H*-1,2,4-triazole and cyclohexyl isothiocyanate. The reaction was carried out at 100 °C for 30 h. After the reaction, the reaction mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$. The crude product obtained was purified by column chromatography on silica gel (hexane/AcOEt, 3/1 to 2/1) and recrystallization from Et_2O and hexane to give white crystals (455 mg, 3.7%): mp 74−76 °C; IR (KBr, cm^{-1}) 3400, 3150, 1538; ^{1}H-NMR ($CDCl_3$) δ 1.28 (t q, 1H, J = 3.6, 13.0 Hz, CH), 1.36−1.52 (m, 4H, CH), 1.69 (d t, 1H, J = 13.2, 4.1 Hz, CH), 1.80 (d t, 2H, J = 13.5, 4.1 Hz, CH), 2.12−2.18 (m, 2H, CH), 4.27−4.37 (m, 1H, CH), 8.00 (s, 1H, CH), 8.61 (brs, 1H, NH), 9.22 (s, 1H, CH).

5-Amino-3-methyl-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (18). The procedure used for the preparation of **1a** (method A) was repeated with **17** 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 (785 mg, 68%): mp 180−181 °C; IR (KBr, cm^{-1}) 3330, 3050, 1645, 1530; ^{1}H-NMR (DMSO-d_6) δ 2.13 (s, 3H, CH_3), 3.01 (s, 3H, CH_3), 8.11 (brs, 2H, NH_2), 9.86 (brs, 1H, NH).

5-Amino-1-[(methylamino)(thiocarbonyl)]-3-(methylthio)-1*H*-1,2,4-triazole (19). The procedure used for the preparation of **1a** (method A) was repeated with 3-methyl-5-(methylthio)-1*H*-1,2,4-triazole and methyl isothiocyanate as a starting material. The crude product which was obtained by extraction with AcOEt was chromatographed on silica gel

($CHCl_3$/MeOH, 50/1) and recrystallized from EtOH to give white crystals (310 mg, 4%): mp 173−175 °C; IR (KBr, cm^{-1}) 3330, 3300, 3090, 1657, 1630; ^{1}H-NMR (DMSO-d_6) δ 2.52 (s, 3H, CH_3), 3.03 (s, 3H, CH_3), 8.30 (brs, 2H, NH_2), 9.77 (brs, 1H, NH).

5-Amino-1-[(methylamino)(thiocarbonyl)]-3-phenyl-1*H*-1,2,4-triazole (23a). The procedure used for the preparation of **1a** (method A) was repeated with **22a** and methyl isothiocyanate as a starting material. The crude product which was obtained by extraction with AcOEt was chromatographed on silica gel ($CHCl_3$/MeOH, 100/1) and recrystallized from $CHCl_3$ to give white crystals (980 mg, 25.6%): mp 174−175 °C; IR (KBr, cm^{-1}) 3300, 3070, 1638, 1521; ^{1}H-NMR (DMSO-d_6) δ 3.10 (d, J = 4.7 Hz, 3H, CH_3), 7.4−7.5 (m, 3H, CH), 8.0−8.1 (m, 2H, CH), 8.31 (brs, 2H, NH_2), 10.03 (q, 1H, J = 4.7 Hz, NH).

5-Amino-1-[(methylamino)(thiocarbonyl)]-3-*p*-tolyl-1*H*-1,2,4-triazole (23b). The procedure used for the preparation of **1a** (method A) was repeated with **22b** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 50/1) and recrystallized from $CHCl_3$ and hexane to give white crystals (12%): mp 180−182 °C; IR (KBr, cm^{-1}) 3320, 3070, 1641, 1528, 1500; ^{1}H-NMR (DMSO-d_6) δ 2.36 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 7.30 (d, 2H, J = 8.1 Hz, CH), 7.94 (d, 2H, J = 8.1 Hz, CH), 8.28 (brs, 2H, NH_2), 9.99 (brs, 1H, NH).

5-Amino-3-(4-chlorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (23c). The procedure used for the preparation of **1a** (method A) was repeated with **22c** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 50/1) and recrystallized from AcOEt to give white crystals (43%): mp 203−206 °C; IR (KBr, cm^{-1}) 3270, 3070, 1635, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.09 (s, 3H, CH_3), 7.57 (d, 2H, J = 8.4 Hz, CH), 8.04 (d, 2H, J = 8.4 Hz, CH), 8.32 (brs, 2H, NH_2), 10.05 (brs, 1H, NH).

5-Amino-3-(4-cyanophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (23d). The procedure used for the preparation of **1a** (method A) was repeated with **22d** as a starting material. The crude product which was obtained by extraction with AcOEt was chromatographed on silica gel (AcOEt/hexane, 3/1 to 2/1) and recrystallized from $CHCl_3$ and hexane to give yellow crystals (4%): mp 213−216 °C dec; IR (KBr, cm^{-1}) 3250, 2220, 1650, 1530; ^{1}H-NMR (DMSO-d_6) δ 3.09 (d, 3H, J = 3.9 Hz,CH_3), 7.99 (d, 2H, J = 8.4 Hz, CH), 8.19 (d, 2H, J = 8.4 Hz, CH), 8.40 (brs, 2H, NH_2), 10.1−10.2 (brs, 1H, NH).

5-Amino-3-(4-methoxyphenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (23e). The procedure used for the preparation of **1a** (method A) was repeated with **22e** as a starting material. The crude product obtained was chromatographed on silica gel (AcOEt/hexane, 1/3 to 1/2) and recrystallized from AcOEt to give white crystals (4% from **21e**): mp 188−189 °C; IR (KBr, cm^{-1}) 3340, 3300, 3100, 1635, 1610, 1585, 1525, 1500; ^{1}H-NMR (DMSO-d_6) δ 3.08 (s, 3H, CH_3), 3.82 (s, 3H, CH_3), 7.05 (d, 2H, J = 7.0 Hz, CH), 7.98 (d, 2H, J = 7.0 Hz, CH), 8.31 (brs, 2H, NH_2), 9.99 (brs, 1H, NH).

5-Amino-3-(4-aminophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (23g) and 5-Amino-1-[(methylamino)(thiocarbonyl)]-3-[4-(3-methylthioureido)phenyl]-1*H*-1,2,4-triazole (23i). The procedure used for the preparation of **1a** (method A) was repeated with **22g** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$/MeOH, 50/1) and recrystallized from $CHCl_3$ and hexane to give **23g** as white crystals (5%): mp 172−174 °C; IR (KBr, cm^{-1}) 3400, 3300, 3190, 1604, 1518; ^{1}H-NMR (DMSO-d_6) δ 3.07 (s, 3H, CH_3), 5.51 (brs, 2H, NH_2), 6.60 (d, 2H, J = 8.6 Hz, CH), 7.71 (d, 2H, J = 8.6 Hz, CH), 8.19 (brs, 2H, NH_2), 9.38 (brs, 1H, NH).

Elution of the column with a solvent of ($CHCl_3$/MeOH, 30/1) and recrystallization from $CHCl_3$ gave **23i** as white crystals (8.6%): mp 190−192 °C; IR (KBr, cm^{-1}) 3260, 1635, 1550; ^{1}H-NMR (DMSO-d_6) δ 2.94 (s, 3H, CH_3), 3.09 (s, 3H, CH_3), 7.55 (d, 2H, J = 8.5 Hz, CH), 7.86 (brs, 1H, NH), 7.97 (d, 2H, J = 8.5 Hz, CH), 8.29 (brs, 2H, NH_2), 9.70 (brs, 1H, NH), 9.98 (brs, 1H, NH).

3-Amino-5-[4-(ethoxalylamino)phenyl]-1*H*-1,2,4-triazole (22h). To a cooled (−78 °C) solution of **22g** (36.9 g, 0.21 mol) and triethylamine (31 mL, 0.22 mol) in DMF (380 mL) was added dropwise ethyloxalyl chloride (26 mL, 0.23 mol) for 1 h. After gradually warming up to −10 °C, the mixture was extracted with AcOEt. The organic layer was washed with H_2O and dried over $MgSO_4$. The crude product obtained was chromatographed on silica gel (AcOEt/MeOH, 40/1 to 20/1) to give a white solid (4.0 g, 7%): IR (KBr, cm^{-1}) 3590, 3310, 3140, 1735, 1680, 1645, 1595, 1555; ^{1}H-NMR (DMSO-d_6) δ 1.32 (t, 3H, J = 7.1 Hz, CH_3), 4.31 (q, 2H, J = 7.1 Hz, CH_2), 6.07 (brs, 2H, NH_2), 7.77 (d, 2H, J = 8.7 Hz, CH), 7.85 (d, 2H, J = 8.7 Hz, CH), 10.87 (brs, 1H, NH), 12.05 (brs, 1H, NH).

5-Amino-3-[4-(ethoxalylamino)phenyl]-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (23h). The procedure used for the preparation of **1a** (method A) was repeated with **22h**, methyl isothiocyanate, and DMSO. The crude product obtained was chromatographed on silica gel (AcOEt/hexane, 1/2 to 1/1) and recrystallized from AcOEt to give white crystals (26%): mp 261−262 °C; IR (KBr, cm^{-1}) 3300, 3050, 1730, 1700, 1640, 1615, 1595, 1530, 1500; ^{1}H-NMR (DMSO-d_6) δ 1.33 (t, 3H, J = 7.1 Hz, CH_3), 3.09 (brs, 3H, CH_3), 4.32 (q, 2H, J = 7.1 Hz, CH_2), 7.88 (d, 2H, J = 8.8 Hz, CH), 8.02 (d, 2H, J = 8.8 Hz, CH), 8.34 (brs, 2H, NH_2), 10.02 (brs, 1H, NH), 10.97 (brs, 1H, NH).

5-Amino-3-(3-chlorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (23j). The procedure used for the preparation of **1a** (method A) was repeated with **22j** as a starting material with use of DMSO as a solvent. The crude product obtained was chromatographed on silica gel (AcOEt/hexane, 1/3) and recrystallized from AcOEt to give white crystals (24%): mp 191−192 °C; IR (KBr, cm^{-1}) 3450, 3300, 1640, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.09 (m, 3H, CH_3), 7.47−7.60 (m, 2H, CH), 7.90−8.03 (m, 1H, CH), 8.03−8.13 (m, 1H, CH), 8.37 (brs, 2H, NH_2), 10.1−12.2 (1H, NH).

5-Amino-3-(2-chlorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (23k). The procedure used for the preparation of **1a** (method A) was repeated with **22k** as a starting material. The crude product obtained was chromatographed on silica gel (AcOEt/hexane, 1/3) and recrystallized from AcOEt to give light yellow crystals (31%): mp 168−170 °C; IR (KBr, cm^{-1}) 3300, 3090, 1645, 1600, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.07 (d, 3H, J = 4.7 Hz, CH_3), 7.38−7.64 (m, 3H, CH), 7.80−7.90 (m, 1H, CH), 8.35 (brs, 2H, NH_2), 9.8−10.1 (m, 1H, NH).

3-Amino-5-(4-fluorophenyl)-1*H*-1,2,4-triazole (24a). To a cooled (0 °C) solution of NaOMe prepared from sodium (6.0 g, 0.26 mol) and MeOH (250 mL), was added aminoguanidine nitrate (35.6 g, 0.26 mol). Then methyl 4-fluorobenzoate (10.0 g, 0.065 mol) in MeOH (100 mL) was added dropwise to the resultant mixture. The reaction mixture was stirred at reflux for 18 h and poured into ice−water. The pH value of the water layer adjusted to 3−4 with 3 N HCl. The precipitated solid was collected by filtration and the solid obtained was washed with Et_2O to give a yellow solid (9.6 g, 83%): IR (KBr, cm^{-1}) 3600, 1720, 1600, 1500; ^{1}H-NMR (DMSO-d_6) δ 7.43 (t, 2H, J = 9.0 Hz, CH), 7.99 (d d, 2H, J = 9.0, 6.0 Hz, CH).

5-Amino-3-(4-fluorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (25a). To a mixture of **24a** (200 mg, 1.12 mmol) in 1 N NaOH (1.12 mL) was added methyl isothiocyanate (90 mg, 1.23 mmol) in THF (3 mL). The reaction mixture was stirred at room temperature for 1 h, neutralized with 1 N HCl, and extracted with AcOEt. After evaporation of the solvent, the residue was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (130 mg, 46%): mp 192−194 °C; IR (KBr, cm^{-1}) 3350, 3250, 3050, 1640, 1600, 1520, 1500; ^{1}H-NMR (DMSO-d_6) δ 3.09 (s, 3H, CH_3), 7.3−7.4 (m, 2H, CH), 8.08 (d d, 2H, J = 8.0, 6.0 Hz, CH), 8.35 (brs, 2H, NH_2), 10.1 (m, 1H, NH).

5-Amino-3-(4-bromophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (25b). The procedure used for the preparation of **25a** was repeated with **24b** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (17%): mp 210−212 °C; IR (KBr, cm^{-1}) 1630, 1595, 1515, 1500; ^{1}H-NMR (DMSO-d_6) δ 3.08 (d, 3H, J = 2.1

Hz, CH_3), 7.72 (d, 2H, J = 8.5 Hz, CH), 7.97 (d, 2H, J = 8.5 Hz, CH), 8.35 (brs, 2H, NH_2), 9.8−10.2 (m, 1H, NH).

5-Amino-1-[(methylamino)(thiocarbonyl)]-3-[4-(trifluoromethyl)phenyl]-1*H*-1,2,4-triazole (25c). The procedure used for the preparation of **25a** was repeated with **24c** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (47%): mp 193−195 °C; IR (KBr, cm^{-1}) 3300, 3000, 1645, 1520, 1500; ^{1}H-NMR (DMSO-d_6) δ 3.10 (s, 3H, CH_3), 7.89 (d, 2H, J = 8.2 Hz, CH), 8.24 (d, 2H, J = 8.2 Hz, CH), 8.40 (brs, 2H, NH_2), 10.2 (brs, 1H, NH).

5-Amino-3-(2,4-dichlorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (25d). The procedure used for the preparation of **25a** was repeated with **24d** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (35%): mp 192−193 °C; IR (KBr, cm^{-1}) 3400, 3250, 1650, 1590, 1550, 1515, 1500; ^{1}H-NMR (DMSO-d_6) δ 3.07 (s, 3H, CH_3), 7.56 (d d, 1H, J = 8.4, 2.1 Hz, CH), 7.70 (d, 1H, J = 2.1 Hz, CH), 7.90 (d, 1H, J = 8.4 Hz, CH), 8.36 (brs, 2H, NH_2), 9.97 (brs, 1H, NH).

5-Amino-3-(3,4-dichlorophenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (25e). The procedure used for the preparation of **25a** was repeated with **24e** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from $CHCl_3$ to give white crystals (44%): mp 216−218 °C; IR (KBr, cm^{-1}) 3250, 3000, 1660, 1520; ^{1}H-NMR (DMSO-d_6) δ 3.09 (s, 3H, CH_3), 7.79 (d, 1H, J = 8.4 Hz, CH), 7.96 (dd, 1H, J = 8.4, 1.9 Hz, CH), 8.22 (d, 1H, J = 1.9 Hz, CH), 8.38 (brs, 2H, NH_2), 10.16 (brs, 1H, NH).

5-Amino-3-(4-chloro-2-methoxyphenyl)-1-[(methylamino)(thiocarbonyl)]-1*H*-1,2,4-triazole (25f). The procedure used for the preparation of **25a** was repeated with **24f** as a starting material. The crude product obtained was chromatographed on silica gel ($CHCl_3$) and recrystallized from AcOEt to give white crystals (40%): mp 165−167 °C dec; IR (KBr, cm^{-1}) 3300, 3080, 1640, 1595, 1580, 1530, 1510; ^{1}H-NMR (DMSO-d_6) δ 3.07 (s, 3H, CH_3), 3.85 (s, 3H, CH_3), 7.10 (d d, 1H, J = 8.2, 1.9 Hz, CH), 7.22 (d, 1H, J = 1.9 Hz, CH), 7.74 (d, 1H, J = 8.2 Hz, CH), 8.25 (brs, 2H, NH_2), 9.87 (brs, 1H, NH).

Sephadex-Induced Lung Eosinophilia. Sephdex 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 canulated. Three to four milliliters of phosphate-buffered saline (PBS) containing 6 units/mL of 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 total cells were counted under microscopy. 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 an inhibitory activity. The inhibitory activity of a compound was calculated from the following equation:

$$\text{inhibition}(\%) = 100 - [(\text{no. of eosinophils/no. of total cells})\text{compound}/(\text{no. of eosinophils/no. of total cells})\text{vehicle}] \times 100$$

Airway Hypersensitivity in Actively Sensitized Guinea Pigs. Male guinea pigs (Hartley strain) were actively sensitized by the ip injection of ascaris antgen (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 the similar procedure was carried out. **23c** or vehicle was ip injected for four consecutive days. Metpyron (50 mg/kg, ip) was injected 24 and 4 h before carbacol inhalation. Twenty-four hours 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 being checked that Rrs was 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 discontinous Metrizamide gradients as described before,[35] 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. **23c** 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%.

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