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Novel Antiallergic and Antiinflammatory Agents. Part I: Synthesis and Pharmacology of Glycolic Amide Derivatives

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Abstract—A series of mono-glycoloylamino derivatives was synthesized by treatment of the corresponding aromatic monoamine derivatives with glycoloyl chloride derivatives in pyridine or dichloromethane, in the presence of a base such as triethylamine or pyridine. Hydrolysis of acetoxy compounds in aqueous ammonia and methanol solution produced hydroxy derivatives with ease. These compounds were tested in the rat PCA (passive cutaneous anaphylaxis) assay by oral administration. Thiazole and thiadiazole derivatives showed moderate inhibition in this assay. In contrast, benzothiazole and benzonitrile derivatives exhibited marked inhibition. In particular, compound **5t** also showed marked inhibition of eosinophil adhesion to TNF (tumor necrosis factor) -α-treated HUVEC (human umbilical vein endothelial cells) in the range of 10^{-8}–10^{-5} M.

Introduction

Recently, eosinophils have come to be thought of as contributing to the pathogenesis of allergic disease such as asthma and atopic dermatitis. Eosinophils accumulate in the lungs and nasal airway after allergic challenges.[1–3] The initial phase in the process of eosinophil accumulation is the adhesion of eosinophils to endothelial cells.[4,5] Many antiallergic drugs have been developed but few drugs causing the adhesion of eosinophil to HUVEC (human umbilical vein endothelial cells) have been investigated.[6] To develop a new antiallergic and antiinflammatory drug, we have investigated a series of amide compounds. In a previous paper,[7] we reported that bis(glycoloylamino)pyrimidine derivatives showed marked inhibition in the rat PCA (passive cutaneous anaphylaxis) assay.[8] These compounds possess two active sites in their molecules as does DSCG (disodium cromoglycate)[9] (Figure 1). Though they have neither anti-histamine nor histamine release inhibitory effects at less than 10^{-4} M, they strongly inhibit the PCA reaction at 30 mg/kg p.o. In order to search for a more active compound in vivo, we synthesized new mono-glycoloylamino compounds[10,11] having other aromatic rings, and investigated these compounds in the PCA assay and for their effects on eosinophil adhesion to TNF (tumor necrosis factor)-α-treated HUVEC. In particular, 3-amino-4-chloro-5-hydroxyacetylaminobenzonitrile (**5t**) inhibited the PCA reaction as strongly as ketotifen fumarate and showed marked inhibition of eosinophil adhesion to TNF-α-treated HUVEC in the range of 10^{-8}–10^{-5} M. It is a metabolite of TYB-2285 (Figure 1), which is under investigation in Japan for asthma and atopic dermatitis in phase II clinical studies. In this paper, the synthesis, structure–activity relationships and some pharmacological evaluations of this series of compounds are described.

Chemistry

The compounds shown in Table 1 were prepared by treatment of five-membered heterocyclic amines with methoxyacetyl chloride or acetoxyacetyl chloride in pyridine or dichloromethane in the presence of base such as triethylamine or pyridine (General method A, Scheme 1). Starting amines were commercially available. Hydroxy compound **5h** was prepared by hydrolysis of acetoxyacetyl compound **4h** in aqueous ammonia and methanol solution (General method B, Scheme 1). The

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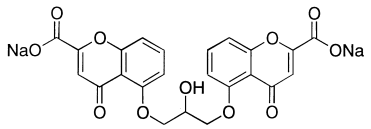
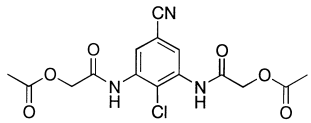


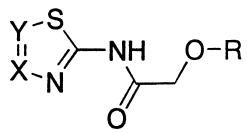
DSCG



TYB-2285

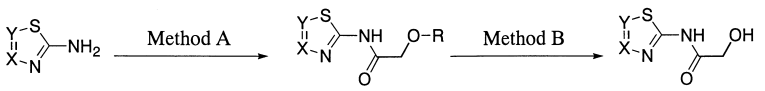
Figure 1. Structures of DSCG and TYB-2285.

Table 1. Physical and pharmacological data of thiazole, thiadiazole, and benzothiazole derivatives



Compd no.	X	Y	R	mp (°C)	Formula	Yield (%)	Recryst. solvent[a]	Rat PCA inhibition (%) 30 mg/kg p.o.
3a	CH	CH	Me	107	$C_6H_8N_2O_2S$	73	A	50
3b	CPh	CH	Me	115	$C_{12}H_{12}N_2O_2S$	79	B	72
3c	CPh	N	Me	116	$C_{11}H_{11}N_3O_2S$	58	B	52
3d	N	CCH_3	Me	220	$C_6H_9N_3O_2S$	24	A	55
3e	C-CH=CH-CH=CH-C		Me	100	$C_{10}H_{10}N_2O_2S$	78	B	96
3f	CCOCOOH	N	Me	261	$C_8H_2O_5S$	44	C	NA
3g	$CNHCOCH_2OMe$	CPh	Me	111	$C_{15}H_{17}N_3O_4S$	65	C	NA
4h	C-CH=CMe-CMe=CH-C		Ac	215	$C_{13}H_{14}N_3O_3S$	77	A	84
5h	C-CH=CMe-CMe=CH-C		H	208	$C_{11}H_{12}N_2O_2S$	90	A	80
	ketotifen fumarate							95

[a]A: EtOH; B: EtOH/H_2O; C: EtOAc/Hexane.
NA, Not active.



2 **3a-g, 4h** **5h**

2a: X = CH, Y = CH
2b: X = CPh, Y = CH
2c: X = CPh, Y = N
2d: X = N, Y = CCH_3
2e: X + Y = C-CH=CH-CH=CH-C
2f: X = C-COCOOEt, Y = N
2g: X = C-NH_2, Y = CPh
2h: X + Y = C-CH=CMe-CMe=CH-C

3a: X = CH, Y = CH, R = Me
3b: X = CPh, Y = CH, R = Me
3c: X = CPh, Y = N, R = Me
3d: X = N, Y = CCH_3, R = Me
3e: X + Y = C-CH=CH-CH=CH-C, R = Me
3f: X = C-COCOOEt, Y = N
3g: X = C-NH_2, Y = CPh, R = Me
4h: X + Y = C-CH=CMe-CMe=CH-C, R = Ac

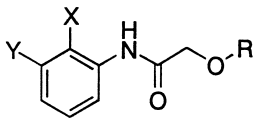
5h: X + Y = C-CH=CMe-CMe=CH-C

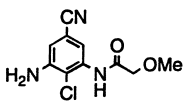
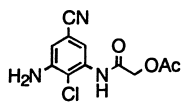
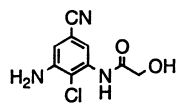
Scheme 1. Synthesis of thiazole, thiadiazole, and benzothiazole derivatives.

compounds shown in Table 2 were prepared in the same manner as the five-membered heterocyclic amides except for compounds **3t**, **4t** and **5t** (General method A, Scheme 2). Starting amines were prepared by the method of Klaubert et al.[12] Compound **4k** containing an amino group was prepared by the reduction of the corresponding nitro compound **4j**. Hydroxyacetylamino compounds **5m**, **5n**, **5o**, **5q** and **5r** were easily synthesized in the same manner as **5h** (General method B, Scheme 2).

Compounds **3t** and **4t** were synthesized by the Schotten–Baumann method (General method C, Scheme 3). The monoamide compound was selectively obtained without bisamide formation in a two phase system of ether and 1N NaOH(aq). In pyridine or dichloromethane in the presence of triethylamine, a mixture of monoamide, bisamide and starting amine was formed. Hydroxy compound **5t** was prepared in the same manner as **5h** (General method B, Scheme 3).

Table 2. Physical and pharmacological data of benzene glycolic amides

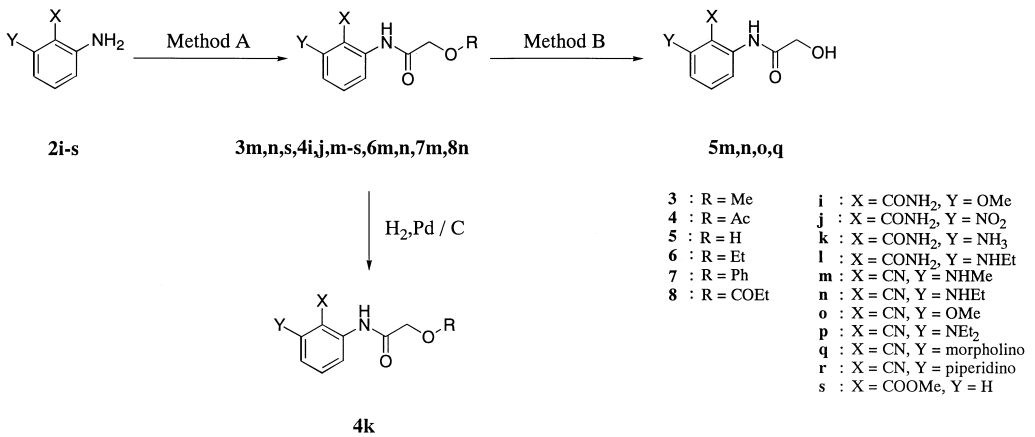


Compd no.	X	Y	R	mp (°C)	Formula	Yield (%)	Recryst. solvent[a]	Rat PCA inhibition (%) 30 mg/kg p.o.
4i	$CONH_2$	OMe	Ac	163–165	$C_{12}H_{14}N_2O_5$	77	A	79
4k	$CONH_2$	NH_2	Ac	181–184	$C_{11}H_{13}N_3O_4$	24	A	8
4l	$CONH_2$	NHEt	Ac	162–163	$C_{13}H_{14}N_3O_4$	60	A	NA
3m	CN	NHMe	Me	109–111	$C_{11}H_{13}N_3O_2$	39	B	91
6m	CN	NHMe	Et	133–134	$C_{12}H_{15}N_3O_2$	61	B	ND
7m	CN	NHMe	Ph	201–202	$C_{16}H_{15}N_3O_2$	22	A	NA
4m	CN	NHMe	Ac	144–145	$C_{12}H_{13}N_3O_3$	69	B	ND
5m	CN	NHMe	H	160–162	$C_{10}H_{11}N_2O_2$	84	A	ND
3n	CN	NHEt	Me	118–119	$C_{13}H_{15}N_3O_2$	59	B	ND
6n	CN	NHEt	Et	84–87	$C_{13}H_{17}N_3O_2$	69	B	ND
4n	CN	NHEt	Ac	110–113	$C_{13}H_{15}N_3O_3$	72	B	95
5n	CN	NHEt	H	111–112	$C_{11}H_{13}N_3O_2$	69	A	93
8n	CN	NHEt	COEt	111-112	$C_{14}H_{H_{17}}N_3O_3$	41	A	96
4o	CN	OMe	Ac	131–132	$C_{12}H_{12}N_2O_4$	87	A	30
5o	CN	OMe	H	142–145	$C_{10}H_{10}N_2O_3$	87	A	ND
4p	CN	NEt_2	Ac	50–51	$C_{15}H_{19}N_3O_3$	24	C	97
4q	CN	morpholino	Ac	131–132	$C_{15}H_{17}N_3O_4$	56	D	98
5q	CN	morpholino	H	126	$C_{13}H_{15}N_3O_3$	54	E	ND
4r	CN	piperidino	Ac	92	$C_{16}H_{19}N_3O_3$	24	B	ND
5r	CN	piperidino	H	124	$C_{14}H_{17}N_3O_2$	54	F	ND
3s	COOMe	H	Me	71–72	$C_{11}H_{13}NO_4$	39	E	NA
								
3t				163–165	$C_{10}H_{10}N_3O_2Cl$	48	G	90
								
4t				185–188	$C_{11}H_{10}N_3O_3Cl$	45	A	92
								
5t				203–205	$C_9H_8N_3O_2Cl$	83	A	93
	ketotifen fumarate							95

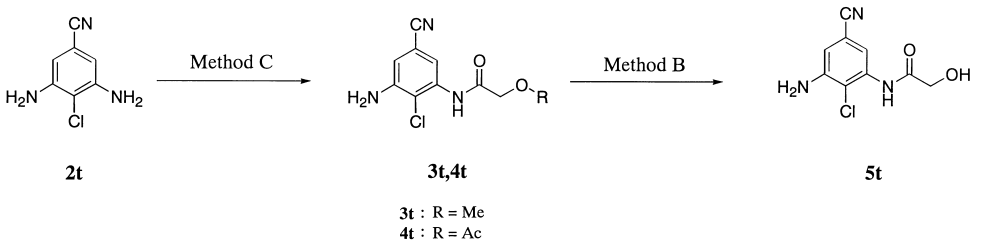
[a]A: EtOH; B: EtOA/Hexane; C: Hexane; D: EtOH/Toluene/Hexane; E: EtOH/H_2O; F: Toluene/Hexane; G: EtOH/EtOAc.
NA, Not active; ND, Not done.

Results and Discussion

Effects of five-membered heterocyclic methoxy-, acetoxy- and hydroxyacetylamino compounds are shown in Table 1. From the table it can be seen that thiazole derivatives **3a** and **3b** and thiadiazole derivatives **3c** and **3d** inhibit the PCA by oral administration, and that benzothiazoles (**3e**, **4h**, **5h**) inhibit the PCA more strongly than thiazoles and thiadiazoles. Among these compounds, **3e** inhibited as strongly as ketotifen fumarate. Compounds **3f** and **3g** were not active. Table 2 shows the activity of benzene derivatives having methoxy-ethoxy-, phenoxy-, acetoxy-, propionyloxy and hydroxy-acetylamino groups. In these cases, methoxy-, acetoxy-



Scheme 2. Synthesis of benzene glycolic amides.



Scheme 3. Synthesis of compounds **3t**, **4t** and **5t**.

and hydroxyacetylamino compounds inhibited the PCA reaction as strongly as ketotifen fumarate. But 2-carbamoyl derivative **4i** was less active in the rat PCA assay than 2-cyano derivative **4n**. Compound **3s**, having a methoxycarbonyl group in its benzene ring was not active. In the cyano series, it was found that a bigger R group (ethyl, phenyl) reduced activity in the rat PCA assay. Hydrogen, methyl and acetyl derivatives have marked or moderate effects. At the six-position, large amino groups, such as diethylamino and morpholino, are favored. Compound **4o** is less active than its amino compounds. Compounds **3t**, **4t** and **5t**, *m*-glycoloylaminobenzonitriles also showed marked inhibition in the rat PCA assay by oral administration.

We investigated the effect of these compounds on eosinophil adhesion to TNF-α-treated HUVEC. The results are shown in Table 3. Compounds **4h**, **4q** and **5o** showed a slight inhibition at 10^{-5} M and compound **5t** showed a marked inhibition of eosinophil adhesion to TNF-α-treated HUVEC in the range 10^{-8}–10^{-5} M in a concentration dependent manner. DSCG did not show an effect at any concentration tested.[13] These results indicated that meta-substituted benzonitrile derivatives (**3t**, **5t**) were more active than ortho-substituted ones (**4h**, **4q**, **5o**) in this assay. Recently, Tominaga et al.[13] showed that compound **5t** did not inhibit the adhesion of neutrophil at the same range (10^{-8}–10^{-5} M). Eosinophil adhesion to TNF-α-treated HUVEC was blocked by monoclonal antibody (mAb) against VLA-4 (anti-VLA-4), but not by that against Mac-1 (anti-Mac-1). However, neutrophil adhesion was blocked by anti-Mac-1,

Table 3. The effect of compounds in eosinophil adhesion to TNF-α-treated HUVEC

Compd no.	Percentage of cell adhesion Concentration of test compounds			
	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
4q	86	NA	NA	
5o	90	NA	NA	
4h	70	NA	NA	
3t	60	63	66*	
5t	20***	28***	42***	61**
DSCG	NA	NA	NA	NA
Ketotifen[a]	49**	NA		
Tranilast	38*	22**	NA	

NA, Not active.
[a]Ketotifen fumarate.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus control (%) adhesion of eosinophils to TNF-α treated HUVEC in the absence of test compounds.

but not by anti-VLA-4. These results suggest that compound **5t** might block the VLA-4/VCAM-1 pathway selectively. Compound **5t** is a metabolite of TYB-2285,[14] which is being investigated in Japan for asthma and atopic dermatitis in phase II clinical studies.

Recently, Yasuda et al.[15] reported that anti-VLA-4 antibodies, in combination with other anti-adhesion molecule antibodies, inhibited the PCA reaction. We speculate that **5t** and its derivatives might inhibit the PCA reaction by a mechanism related to the inhibition of the VLA-4/VCAM-1 pathway. Further studies are required to determine the precise mechanism of these compounds.

Conclusion

We have investigated a number of aromatic glycolic amide derivatives to find a unique type of antiallergic and antiinflammatory drug. Compound **5t** showed antiallergic activities in the PCA assay at 30 mg/kg p.o. and marked inhibitory effects on eosinophil adhesion to TNF-α-treated HUVEC at 10^{-8}–10^{-5} M. These compounds are anticipated to be a new type of antiallergic and antiinflammatory drug for the treatment of asthma and atopic dermatitis.

Experimental

Melting points were determined with a Mettler capillary melting-point apparatus (Model FP 61) and uncorrected. ^{1}H NMR spectra were recorded on a Varian FT 80A spectrometer or a Varian Gemini-200 spectrometer using TMS as an internal standard. Elemental analyses were performed at Kyoto University and TOYOBO Analytical Center. All starting materials were commercially available unless otherwise noted.

Passive cutaneous anaphylaxs (PCA) assay. Male Wistar rats (weighing about 200 g) were passively sensitized by intradermal injection of rat anti-egg albumin antiserum (100 μL/site) in the dorsal skin. After 48 h, each rat was challenged by injecting a mixture (1 mL) of egg albumin and Evans blue solution via the tail vein. Thirty minutes after the challenge, the rats were sacrificed to analyze the bluing region, and the amount of pigment from the bluing region was measured by the method of Katayama et al.[16] Test compounds were orally given at 30 mg/kg, 30 min before the antigen challenge.

Eosinophil adhesion to TNF-α-treated HUVEC. CSFE-labeled eosinophils (1×10^5 cells/well) were layered over HUVEC prestimulated with TNF-α. After 20 min of incubation, unbound cells were removed by plate inversion in RPMI-1640 for 30 min at room temperature. After adding 0.3% Triton X to the adherent cells, the intensity of fluorescence was measured using an automated microplate fluorometer with 485/22 nM excitation and a 530/25 nM emission filter. HUVEC were incubated with each compound for 6 h during treatment with 100 U/mL TNF-α, and were washed before the adhesion assay. Eosinophils were also preincubated for 20 min with each compound, and were then recoincubated with HUVEC in the presence of compounds. For the experiments evaluating the compounds, the percentage of control was calculated according to the following formula:

Percentage of control = (the percentage adhering to 100 U/mL of TNF-α-treated HUVEC in the presence of compounds − the percentage adhering to untreated HUVEC)/(the percentage adhering to 100 U/mL of TNF-α-treated HUVEC in the absence of compounds − the percentage adhering to untreated HUVEC).

General method A. 2-(Methoxyacetylamino)thiazole (3a). Methoxyacetyl chloride (5.0 mL) was added dropwise to a solution of 2-aminothiazole (5 g) in pyridine (100 mL) at room temperature. After the mixture was stirred at room temperature for 3 h, the solvent was distilled off under reduced pressure. Chloroform and water were added to the resulting oil. The organic layer was washed with water and saturated sodium chloride solution. Thereafter, the organic layer was dried over sodium sulfate and the solvent was removed. The resulting solids were washed with water. The solids were recrystallized from ethanol to give 6.3 g of **3a**; mp 105–107 °C; ^{1}H NMR (DMSO-d_6) δ: 11.91 (1H, s), 7.44 (1H, d, J = 6 Hz), 7.18 (1H, d, J = 6 Hz), 4.11 (2H, s), 3.33 (3H, s). Anal. calcd for $C_6H_8N_2O_2S$: C, 41.85; H, 4.68; N, 16.27; S, 18.62, found: C, 41.74; H, 4.62; N, 16.35; S, 18.68.

Compounds **3b–g**, **m**, **n**, **s**, **4h–r**, **6m**, **7m** and **8n** were prepared in the same manner as **3a** (General method A).

2-(Methoxyacetylamino)-4-phenylthiazole (3b). From 2-amino-4-phenylthiazole hydrobromide monohydrate (5.5 g) and methoxyacetyl chloride (2.0 mL): 4.2 g; mp 113–115 °C. ^{1}H NMR (DMSO-d_6) δ: 12.05 (1H, s), 7.95–7.20 (6H, m), 4.15 (2H, s), 3.35 (3H, s). Anal. calcd for $C_{12}H_{12}N_2O_2S$: C, 58.05; H 4.87; N, 11.28; S, 12.91, found: C, 57.99; H, 4.89; N, 11.32; S, 12.65.

5-(Methoxyacetylamino)-3-phenyl-1,2,4-thiadiazole (3c). From 5-amino-3-phenyl-1,2,4-thiadiazole (4.4 g) and methoxyacetyl chloride (3.4 mL): 3.6 g; mp 113–116 °C. ^{1}H NMR ($CDCl_3$) δ: 10.15 (1H, s), 8.30–7.35 (5H, m), 4.15 (2H, s), 3.45 (3H, s). Anal. calcd for $C_{11}H_{11}N_3O_2S$: C, 53.00; H, 4.45; N, 16.86; S, 12.86, found: C, 53.12; H, 4.72; N, 16.86; S, 12.81.

2-(Methoxyacetylamino)-5-methyl-1,3,4-thiadiazole (3d). From 2-amino-5-methyl-1,3,4-thiadiazole (9.2 g) and

methoxyacetyl chloride (5.0 mL): 3.6 g; mp 219–220 °C; ^{1}H NMR (DMSO-d_6) δ: 12.21 (1H, s), 4.16 (2H, s), 3.35 (3H, s), 2.10 (3H, s). Anal. calcd for $C_6H_9N_3O_2S$: C, 38.49; H, 4.89; N, 22.44; S, 17.12, found: C, 38.34; H, 5.00; N, 22.75; S, 17.22.

2-(Methoxyacetylamino)benzothiazole (3e). From 2-aminobenzothiazole(4.5 g) and methoxyacetyl chloride (3.0 mL): 5.2 g; mp 99–100 °C. ^{1}H NMR (DMSO-d_6) δ: 11.20 (1H, s), 8.10–7.12 (4H, m), 4.18 (2H, s), 3.36 (1H, s). Anal. calcd for $C_{10}H_{10}N_2O_2S$: C, 54.04; H, 4.53; N, 12.60; S, 14.42, found: C, 54.29; H, 4.48; N, 12.59; S, 14.27.

2-(Methoxyacetylamino)-4-thiazoleglyoxylic acid (3f). From ethyl 2-amino-4-thiazoleglyoxylate (6.0 g) and methoxyacetyl chloride (3.0 mL). The resulting oil was dissolved 1N aqueous sodium hydroxide (30 mL), and the solution was stirred at room temperature for 3 h. Thereafter, to the solution was added conc. HCl to be acidic. The resulting solids were filtered and washed with water. The solids were recrystallized from ethyl acetate/hexane to give 3.2 g of **3f**; mp 178 °C (Dec.). ^{1}H NMR (DMSO-d_6) δ: 12.46 (1H, s), 8.38 (1H, s), 4.13 (2H, s), 3.33 (3H, s). Anal. calcd for $C_8H_8N_2O_5S$: C, 39.34; H, 3.30; N, 11.47; S, 13.12, found: C, 39.31; H, 3.15; N, 11.61; S, 13.16.

2,4-Bis(methoxyacetylamino)-5-phenylthiazole (3g). From 2,4-diamino-5-phenylthiazole monohydrogen bromide (4.1 g) and methoxyacetyl chloride (3.0 mL): 3.3 g; mp 109–111 °C. ^{1}H NMR(DMSO-d_6) δ: 12.15 (1H, s), 9.19 (1H, s), 7.65–7.00 (5H, m), 4.14 (2H, s), 3.93 (1H, s), 3.34 (3H, s), 3.31 (3H, s). Anal. calcd for $C_{15}H_{17}N_3O_4S$: C, 53.72; H, 5.12; N, 12.53; S, 9.56, found: C, 53.98; H, 5.15;N, 12.45; S, 9.57.

2-(Acetoxyacetylamino)-5,6-dimethylbenzothiazole (4h). From 2-amino-5,6-dimethylbenzothiazole (4.5 g) and acetoxyacetyl chloride (3.0 mL): 5.4 g; mp 214–215 °C. ^{1}H NMR (DMSO-d_6) δ: 12.39 (1H, s), 7.67 (1H, s), 7.51 (1H, s), 4.78 (2H, s), 2.30 (6H, s), 2.13 (3H, s). Anal. calcd for $C_{13}H_{14}N_2O_3S$: C, 56.10; H, 5.07; N, 10.06; S, 11.52, found: C, 56.06; H, 5.07; N, 10.06; S, 11.43.

2-(Acetoxyacetylamino)-6-methoxybenzamide (4i). From 2-amino-6-methoxybenzamide (1.7 g) and acetoxyacetyl chloride (1.1 mL): 2.1 g; mp 163–165 °C. ^{1}H NMR (DMSO-d_6) δ: 11.45 (1H, s), 8.05–6.84 (3H, m), 7.91 (2H, s), 4.60 (2H, s), 3.82 (3H, s), 2.20 (3H, s). Anal. calcd for $C_{12}H_{14}N_2O_5$: C, 54.13; H, 5.30; N, 10.52, found: C, 54.19; H, 5.36; N, 10.57.

2-(Acetoxyacetylamino)-6-nitrobenzonitrile (4j). From 2-amino-6-nitrobenzonitrile (6.5 g) and acetoxyacetyl chloride (4.4 mL): 5.8 g; mp 157–158 °C. ^{1}H NMR (DMSO-d_6) δ: 10.52 (1H, s), 835–7.95 (3H, m), 4.72 (2H, s), 2.13 (3H, s). Anal. Calcd for $C_{11}H_9N_3O_5$: C, 54.05; H, 4.54; N, 12.61, found: C, 54.03; H, 4.56; N, 12.57.

2-(Acetoxyacetylamino)-6-aminobenzamide (4k). Cyclohexene (10.5 m) and 10% palladium-carbon (4.7 g) were added to a mixture of 2-(acetoxyacetylamino)-6-nitrobenzonitrile (5.7 g) and ethanol (50 mL). The mixture was refluxed for 20 min, and the reaction mixture was cooled to room temperature, and the solid materials were filtered off. The filtrate was distilled under reduced pressure. The residual crude solids were recrystallized from ethanol to give 1.2 g of **4k**, mp 181–184 °C. ^{1}H NMR (DMSO-d_6) δ: 10.00 (1H, s), 7.53 (2H, s), 7.03–6.44 (3H, m), 5.26 (2H, s), 2.14 (3H, s). Anal. calcd for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.21; N, 16.73, found: C, 52.57; H, 5.22; N, 16.64.

2-(Acetoxyacetylamino)-6-ethylaminobenzamide (4l). From 2-amino-6-ethylaminobenzamide (4.4 g) and acetoxyacetyl chloride (8.6 mL): 4.1 g; mp 124–125 °C. ^{1}H NMR (DMSO-d_6) δ: 9.70 (1H, s), 7.52–7.11 (3H, m), 4.72 (2H, s), 3.36–3.05 (3H, m), 2.10 (3H, s), 1.09 (3H, t, J = 8 Hz). Anal. calcd for $C_{13}H_{14}N_3O_4$: C, 55.33; H, 4.93; N, 12.10, found: C, 55.34; H, 4.94; N, 12.09.

2-(Methoxyacetylamino)-6-methylaminobenzonitrile (3m). From 2-amino-6-methylaminobenzonitrile (3.3 g) and methoxyacetyl chloride (4.2 mL): 1.9 g; mp 109–111 °C. ^{1}H NMR (DMSO-d_6) δ: 9.42 (1H, s), 7.40 (1H, dd, J = 8 Hz and J = 8 Hz), 6.92 (1H, d, J = 8 Hz), 6.48 (1H, d, J = 8 Hz), 6.20–6.01 (1H, m), 4.02 (2H, s), 3.45 (3H, s), 2.75 (3H, d, J = 5 Hz). Anal. calcd for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98; N, 19.17, found: C, 60.09; H, 5.93; N, 19.46.

2-(Ethoxyacetylamino)-6-methylaminobenzonitrile (6m). From 2-amino-6-methylaminobenzonitrile (3.3 g) and ethoxyacetyl chloride (5.0 mL): 3.2 g; mp 133–134 °C. ^{1}H NMR (DMSO-d_6) δ: 9.32 (1H, s), 7.40 (1H, dd, J = 8 Hz and J = 8 Hz), 7.14 (1H, d, J = 8 Hz), 6.49 (1H, d, J = 8 Hz), 6.36–6.03 (1H, m), 4.03 (2H, s), 3.48 (2H, q, J = 7 Hz), 2.25 (3H, d, J = 5 Hz), 1.22 (3H, t, J = 8 Hz). Anal. calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01, found: C, 61.71; H, 6.37; N, 18.04.

6-Methylamino-2-(phenoxyacetylamino)benzonitrile (7m). From 2-amino-6-methylaminobenzonitrile (3.3 g) and phenoxyacetyl chloride (6.2 mL): 1.4 g; mp 201–202 °C. ^{1}H NMR (DMSO-d_6) δ: 9.76 (1H, s), 7.52–6.48 (8H, m), 6.12 (1H, q, J = 5 Hz), 4.68 (2H, s), 2.74 (3H, d, J = 5 Hz). Anal. calcd, for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.37; N, 14.94, found: C, 68.20; H, 5.38; N, 14.86.

2-(Acetoxyacetylamino)-6-methylaminobenzonitrile (4m). From 2-amino-6-methylaminobenzonitrile (4.4 g) and

acetoxyacetyl chloride (6.5 mL): 5.1 g; mp 144–145 °C. ^{1}H NMR (DMSO-d_6) δ: 9.82 (1H, s), 7.34–6.46 (3H, m), 6.06 (1H, q, J = 5 Hz), 4.64 (2H, s), 2.74 (3H, d, J = 5 Hz), 2.09 (3H, s). Anal. calcd for $C_{12}H_{13}N_3O_3$: C, 58.28; H, 5.30; N, 16.99, found: C, 58.21; H, 5.28; N, 17.08.

2-Ethylamino-6-(methoxyacetylamino)benzonitrile (3n). From 2-amino-6-(ethylamino)benzonitrile (3.7 g) and methoxyacetyl chloride (2.8 mL): 3.2 g; mp 118–119 °C. ^{1}H NMR (DMSO-d_6) δ: 9.31 (1H, s), 7.35 (1H, dd, J = 8 Hz and J = 8 Hz), 6.82 (1H, d, J = 8 Hz), 6.54 (1H, d, J = 8 Hz), 5.86 (1H, t, J = 5 Hz), 3.94 (2H, s), 3.44 (3H, s), 3.34–3.02 (3H, m), 1.13 (3H, t, J = 8 Hz). Anal. calcd for $C_{12}H_{15}N_3O_2$: C, 61.79; H, 6.48; N, 18.01, found: C, 61.95; H, 6.49; N, 17.99.

6-(Ethoxyacetylamino)-2-(ethylamino)benzonitrile (6n). From 2-amino-6-(ethylamino)benzonitrile (4.0 g) and methoxyacetyl chloride (3.9 mL): 4.1 g; mp 84–87 °C. ^{1}H NMR (DMSO-d_6) δ: 9.32 (1H, s), 7.45 (1H, dd, J = 8 Hz and J = 8 Hz), 7.18 (1H, d, J = 8 Hz), 6.61 (1H, d, J = 8 Hz), 5.97 (1H, m), 4.03 (2H, s), 3.38–3.05 (3H, m), 3.17 (2H, q, J = 8 Hz), 1.22 (3H, t, J = 8 Hz), 1.15 (3H, t, J = 8 Hz). Anal. calcd for$C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99, found: C, 63.10; H, 6.98; N, 16.97.

2-(Acetoxyacetylamino)-6-(ethylamino)benzonitrile (4n). From 2-amino-6-(ethylamino)benzonitrile (6.7 g) and acetoxyacetyl chloride (4.8 mL): 7.8 g; mp 110–113 °C. ^{1}H NMR (DMSO-d_6) δ: 9.69 (1H, s), 7.19–6.42 (3H, m), 6.02–5.87 (1H, m), 4.52 (2H, s), 3.14–3.01 (2H, m), 2.02 (3H, s), 1.08 (3H, t, J = 8 Hz). Anal. calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08, found: C, 59.79; H, 5.79; N, 16.07.

2-Ethylamino-6-(propionyloxyacetylamino)benzonitrile (8n). From 2-amino-6-(etylamino)benzonitrile (2.3 g) and propionyloxyacetyl chloride (3.0 mL): 1.6 g; mp 103–105 °C. ^{1}H NMR (DMSO-d_6) δ: 9.69 (1H, s), 7.35 (1H, dd, J = 8 Hz and J = 8 Hz), 6.72 (1H, d, J = 8 Hz), 6.51 (1H, d, J = 8 Hz), 5.86 (1H, t, J = 5 Hz), 4.64 (2H, s), 3.35–2.98 (2H, m), 2.42 (2H, q, J = 8 Hz), 1.14 (3H, t, J = 8 Hz), 1.06 (3H, t J = 8 Hz). Anal. calcd for $C_{14}H_{17}N_3O_3$: C, 61.08; H, 622; N, 15.26, found: C, 61.06; H, 621; N, 15.28.

2-(Acetoxyacetylamino)-6-methoxybenzonitrile (4o). From 2-amino-6-methoxybenzonitrile (3.7 g) and acetoxyacetyl chloride (2.7 mL): 5.4 g; mp 131–132 °C. ^{1}H NMR (DMSO-d_6) δ: 9.94 (1H, s), 7.54–6.96 (3H, m), 4.65 (2H, s), 3.85 (3H, s), 2.10 (3H, s). Anal. calcd for $C_{12}H_{12}N_2O_4$: C, 58.06; H, 4.87; N, 11.28, found: C, 58.02; H, 4.92; N, 11.29.

2-(Acetoxyacetylamino)-6-diethyaminobenzonitrile (4p). From 2-amino-6-diethylaminobenzonitrile (3.0 g) and acetoxyacetyl chloride (1.8 mL): 1.1 g; mp 50–51 °C. ^{1}H NMR (DMSO-d_6) δ: 9.91 (1H, s), 7.45 (1H, dd, J = 8 Hz and J = 8 Hz), 7.06 (1H, d, J = 8 Hz) 6.87 (1H, d, J = 8 Hz), 4.66 (2H, s), 3.29 (4H, q, J = 8 Hz), 2.12 (3H, s), 1.08 (6H, t, J = 8 Hz). Anal. calcd for $C_{15}H_{19}N_3O_3$: C, 62.27; H, 6.62; N, 14.52, found: C, 62.18; H, 6.63; N, 14.51.

2-(Acetoxyacetylamino)-6-morpholinobenzonitrile (4q). From 2-amino-6-morpholinobenzonitrile (5.6 g) and acetoxyacetyl chloride (3.3 mL): 4.7 g; mp 131–132 °C. ^{1}H NMR (DMSO-d_6) δ: 10.07 (1H, s), 7.55 (1H, d, J = 8 Hz), 7.21 (1H, d, J = 8 Hz), 6.89 (1H, d, J = 8 Hz), 4.68 (2H, s), 3.82–3.58 (4H, m), 3.22–3.00 (4H, m), 2.13 (3H, s). Anal. calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85, found: C, 59.31; H, 5.60; N, 13.89.

2-(Acetoxyacetylamino)-6-piperidinobenzonitrile (4r). From 2-amino-6-piperidinobenzonitrile (8.8 g) and acetoxyacetyl chloride (3.3 mL): 3.2 g; mp 90–92 °C: ^{1}H NMR (DMSO-d_6) δ: 9.99 (1H, s), 7.50 (1H, dd, J = 8 Hz and J = 8 Hz), 7.15 (1H, d, J = 8 Hz), 6.85 (1H, d, J = 8 Hz), 4.68 (2H, s), 3.25–2.95 (4H, m), 1.91–1.48 (6H, m). Anal. calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.34; N, 13.94, found: C, 63.99; H, 6.04; N, 13.99.

Methyl 2-(methoxyacetylamino)benzoate (3s). From methyl anthranilate (4.5 g) and methoxyacetyl chloride (5.2 mL): 2.6 g; mp 71–72 °C. ^{1}H NMR (DMSO-d_6) δ: 11.65 (1H, s), 8.85–6.95 (4H, m), 4.05 (2H, s), 3.90 (3H, s), 3.45 (3H, s). Anal. calcd for $C_{11}H_{13}NO_4$: C, 59.19; H, 5.87; N, 6.28, found: C, 59.11; H, 5.92; N, 6.25.

General method B. 6-Methylamino-2-(hydroxyacetylamino)benzonitrile (5m). To a solution of **4m** (2 g) in methanol (250 mL) was added dropwise 28% aqueous ammonia (5.5 mL). The mixture was stirred at room temperature for 1 h. Thereafter, the solvent was distilled off under reduced pressure. The resulting solids were recrystallized from ethanol to give 1.4 g of **5m**; mp 160–162 °C. ^{1}H NMR (DMSO-d_6) δ: 9.25 (1H, s), 7.50–7.05 (2H, m), 6.50–6.28 (1H, m), 6.06 (1H, q, J = 4 Hz), 3.95 (2H, s), 2.72 (3H, d, J = 4 Hz). Anal. calcd for $C_{10}H_{11}N_2O_2$: C, 58.53; H, 5.40; N, 20.48, found: C, 58.28; H, 5.47; N, 20.43.

Compounds **5h** and **5n–r** were prepared in the same manner as **5m** (General method B).

2-(Hydroxyacetylamido)-5,6-dimethylbenzothiazole (5h). From the compound **4h** (4.2 g): 3.2 g; mp 214–215 °C. ^{1}H NMR (DMSO-d_6) δ: 11.28 (1H, s), 7.66 (1H, s), 7.49 (1H, s), 5.49 (1H, t, J = 7 Hz), 4.13 (2H, d, J = 7 Hz), 2.31 (6H, s). Anal. calcd for $C_{11}H_{12}N_2O_2S$: C; 55.92; H, 5.12; N, 11.86; S, 13.57, found: C, 55.82; H, 5.19; N, 11.79; S, 13.50.

2-Ethylamino-6-(hydroxyacetylamino)benzonitrile (5n). From the compound **4n** (4.5 g): 2.6 g; mp 111–112 °C. ^{1}H NMR (DMSO-d_6) δ: 9.38 (1H, s), 7.19–6.42 (3H, m), 5.91 (1H, m), 3.94 (2H, s), 3.35–3.13 (3H, m), 1.14 (3H, t, J = 7 Hz). Anal. calcd for $C_{11}H_{13}N_3O_2$: C, 60.30; H, 5.98; N, 19.18, found: C, 60.19; H, 6.01; N, 19.17.

2-(Hydroxyacetylamino)-6-methoxybenzonitrile (5o). From the compound **4o** (2.5 g): 1.8 g; mp 142–145 °C; ^{1}H NMR (DMSO-d_6) δ: 9.57 (1H, s), 7.72–7.62 (2H, m), 7.22–6.90 (1H, m), 6.06 (1H, s), 4.03 (2H, s), 3.90 (3H, s). Anal. calcd for $C_{10H_{10}}N_2O_3$: C, 58.25; H, 4.88; N, 13.58, found: C, 58.03; H, 4.91; N, 13.59.

2-(Hydroxyacetylamino)-6-morpholinobenzonitrile (5q). From the compound **4q** (3.0 g): 1.4 g; mp 126 °C. ^{1}H NMR (DMSO-d_6) δ: 9.60 (1H, s), 7.74–7.48 (2H, m), 7.00–6.78 (1H, m), 6.08 (1H, t, J = 6 Hz), 4.02 (2H, d, J = 6 Hz), 3.85–3.65 (4H, m), 3.27–3.00 (4H, m). Anal. calcd for $C_{13}H_{15}N_3O_3$: C, 59.76; H, 5.79; N, 16.08, found: C, 59.84; H, 5.78; N, 16.15.

2-(Hydroxyacetylamino)-6-piperidinobenzonitrile (5r). From the compound **4r** (3.0 g): 1.4 g; mp 121–124 °C. ^{1}H NMR (DMSO-d_6) δ: 9.60 (1H, s), 7.65–7.48 (2H, m), 6.95–6.78 (1H, m), 6.08 (1H, t, J = 6 Hz), 4.02 (2H, d, J = 6 Hz), 3.30–2.95 (4H, m), 1.98–1.48 (6H, m). Anal. calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.21, found. C, 64.89; H, 6.62; N, 16.24.

General method C. 3-Amino-4-chloro-5-(methoxyacetylamino)benzonitrile (3t). To a solution of 4-chloro-3,5-diaminobenzonitrile (5.5 g) in diethylether (200 mL) was added 1N sodium hydroxide solution (50 mL) and thereto was added dropwise methoxyacetyl chloride (3.0 mL) which was cooled in an ice bath. Thereafter, the mixture was stirred at room temperature for 15 min. The resulting solids were filtered and washed with water and diethylether. The solids were recrystallized from ethanol/ethyl acetate to give 3.8 g of **3t**; mp 163–165 °C. ^{1}H NMR (DMSO-d_6) δ: 9.18 (1H, s), 7.54 (1H, d, J = 3 Hz), 6.93 (1H, d, J = 3 Hz), 5.95 (2H, s), 4.05 (2H, s), 3.44 (3H, s). Anal. Calcd for $C_{10}H_{10}N_3O_2Cl$: C, 50.12; H, 4.21; N, 17.53; Cl, 14.79, found: C, 50. 12; H, 4.3 1; N, 17.47; Cl, 14.82.

Compound (**4t**) was prepared in the same manner as general method C, compound (**5t**) was prepared in the same manner as general method B.

3-Amino-4-chloro-5-(acetoxyacetylamino)benzonitrile (4t). From 4-chloro-3,5-diaminobenzonitrile (16.7 g) and acetoxyacetyl chloride (10.8 mL): 12.1 g; mp 185–188 °C. ^{1}H NMR (DMSO-d_6) δ: 9.10 (1H, s), 7.27 (1H, d, J = 3 Hz), 6.92 (1H, d, J = 3 Hz), 5.80 (2H, s), 4.18 (2H, s), 2.08 (3H, s). Anal. calcd for $C_{11}H_{10}N_3O_3Cl$: C, 49.36; H, 3.77; N, 15.70; Cl, 13.24, found: C, 49.20; H, 3.78; N, 15.52; Cl, 13.23.

3-Amino-4-chloro-5-(hydroxyacetylamino)benzonitrile (5t). From the compound **4t** (2.7 g): 1.9 g; mp 203–205 °C. ^{1}H NMR (DMSO-d_6) δ: 9.30 (1H, s), 7.72 (1H, d, J = 3 Hz), 6.89 (1H, d, J = 3 Hz), 6.23 (2H, s), 5.93 (2H, s), 4.02 (2H, s). Anal. calcd for $C_9H_8N_3O_2Cl$: C, 47.91; H, 3.57; N, 18.62; Cl, 15.71, found: C, 47.67; H, 3.68; N, 18.57; Cl, 15.78.

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