gave (-)-4e as its hydrochloride (67%): mp 234 °C; $[\alpha]^{25}_{546}$ -45.6° (c 1, MeOH); ^{1}H NMR identical with (+)-4e and (±)-4e. Anal. ($C_{24}H_{36}N_2O_2 \cdot 2HCl$) H, N; C: calcd, 63.01; found, 63.66.

(±)-N,N'-Dibutyl-4,5-bis(4-methoxyphenyl)-imidazolidine-2-thione (8). (±)-4e (3.0 g, 7.7 mmol) was dissolved in CS_2 and stirred for 1 day. After evaporation of the solvent, the residue was dissolved in dilute EtOH (50%, 60 mL), and HCl (0.3 mL) was added. The solution was boiled under reflux for 60 h. After the addition of aqueous ammonia, the solution was extracted with ether. After drying and evaporation of the organic phase, etheral HCl was added and the precipitate was filtered. The solvent was removed from the filtrate, and the white solid obtained was dried in vacuo: yield 2.2 g (67%); mp 114-116 °C. The product was used for the ether cleavage without further purification.

Biological Methods. Thymidine Incorporation Study. Chemicals: [*Me*-^{3}H]thymidine (40-60 Ci/mol) was obtained from New England Nuclear Corp. Cell Culture: MCF-7 cells, a gift of Dr. Marc Lippman (NIH, Bethesda, MD), were grown in Falcon plastic flasks in 5% CO_2 in humidified air at 37 °C. The growth medium consisted of Richter's improved minimal essential medium with zinc and without insulin (Associated Biomedic Systems, Buffalo, NY), 10% fetal calf serum (FCS; Gibco), 2.2 g/L $NaHCO_3$, and 40 μg/mL gentamycin (Sigma Chemical Co.). Thymidine incorporation: Cells, growing in log phase, were harvested in 0.02% EDTA in Ca^{2+}/Mg^{2+}-free PBS salt solution and plated in 6-well Linbro dishes (Bellco) in Richter's medium with 10% FCS. The next day the medium was changed to Richter's medium with 5% calf serum stripped of endogenous hormones by 30-min incubation at 45 °C with a dextran-coated charcoal (DCC) pellet (0.25% Norit A, 0.0025% dextran in 0.01 M Tris/HCl, pH 7.4). The medium was changed every 72 h. When the cells were grown preconfluent (60% confluent), the test compounds, dissolved in absolute ethanol, were added; the final ethanol concentration was 0.1%. The incubation time was 60 h; 2 h before harvesting, 1 μCi of [^{3}H]-thymidine was added to each Linbro well. The cells were harvested with EDTA, washed 3 times with PBS, and sonicated (Branson, Cell disruptor B 15). [^{3}H]Thymidine incorporation into the acid-precipitable fraction of the cell sonicate was determined by the addition of 10% Cl_3AcOH and collection of the precipitate by filtering over a 0.45-μm filter (Millipore). The filters were transfered to scintillation vials and counted in a Liquid Scintillation Counter (Beckman LS 8000).

All other applied procedures have already been described in detail and were used without alterations. These methods are: E2-receptor binding assay,[23] Allen-Doisy test,[24] Dorfman uterine weight test,[23], inhibition of the DMBA-induced mammary tumor growth,[23] determination of enzyme activities of tumors,[16] assay of serum 17β-estradiol,[25] assay of prolactin,[26] inhibition of DNA, RNA, and protein syntheses of Walker 256 carcinosarcoma cells in vitro,[24] and growth inhibition of the Ehrlich ascites carcinoma in vivo.[27]

Acknowledgment. The authors thank Dr. M. Lippman, Medicine Branch, NCI, NIH, for the supply of MCF-7 cells, J. Curran, NIH, for providing the prolactin kit, Professor W. Wuttke, MPI for Biophysical Chemistry, Göttingen, BRD, for his advise concerning the prolactin assay, Mrs. M. Horn for technical assistance, and the Deutsche Forschungsgemeinschaft and the Verband der Chemischen Industrie, Fonds der Chemischen Industrie, for financial support.

(23) Hartmann, R. W.; Kranzfelder, G.; von Angerer, E.; Schönenberger, H. *J. Med. Chem.* **1980**, *23*, 841.
(24) Kranzfelder, G.; Schneider, M.; von Angerer, E.; Schönenberger, H. *J. Cancer Res. Clin. Oncol.* **1980**, *97*, 167.
(25) Cameron, E. H. D.; Jones, D. A. *Steroids* **1972**, *20*, 737.
(26) Cassedy, J. M.; Li, G. S.; Spitzer, E. B.; Floss, H. G.; Clemens, J. A. *J. Med. Chem.* **1974**, *17*, 300.
(27) Lettré, H. *Ergeb. Physiol., Biol. Chem. Exp. Pharmakol.* **1950**, *46*, 379.

Novel Orally Active Inhibitors of Passive Cutaneous Anaphylaxis in Rats: *N*-[2-(4-Pyridinyl)-4-pyrimidinyl]ureas and Dialkyl [[[2-(4-Pyridinyl)-4-pyrimidinyl]amino]methylene]malonates

George Y. Lesher,† Baldev Singh,*† and Zigurd E. Mielens‡

Medicinal Chemistry Department and Department of Pharmacology, Sterling-Winthrop Research Institute, Rensselaer, New York 12144. *Received October 8, 1981*

4-Chloro-2-(4-pyridinyl)pyrimidines were treated with alkylamines to afford the corresponding N-substituted amino derivatives. 4-Amino-2-(4-pyridinyl)pyrimidines and their N-substituted analogues were converted to amides, carbamates, amimiomethylenemalonates, and ureas. Many of these compounds were found to have potential antiallergic activity as indicated by the rat passive cutaneous anaphylaxis screen. The most potent compounds were found in the last two series.

The introduction of disodium cromoglycate[1] in 1968 marked a major advancement for the treatment of allergic disease, such as extrinsic bronchial asthma; however, this compound does not have any significant oral activity. Since then, extensive efforts have been made to find more potent and orally effective compounds.[2]

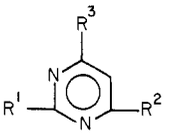
Following the initial discovery of the antiallergic activity of 4-amino-2-(4-pyridinyl)pyrimidine (**4a**), an intermediate required for another project, we embarked on a program to explore this lead. We will describe here some of our work with this novel pyridinyl-substituted pyrimidine system which covers mainly the various substituents on the amino group of **4a** represented by general structure **1**. There is, we believe, only a superficial resemblance between **1** and both **2**[3] and **3**[4] (M + B 22948)[5] because the

† Medicinal Chemistry Department.
‡ Department of Pharmacology.

(1) J. B. L. Howell and R. E. C. Altounyan, *Lancet*, **2**, 539 (1967); J. S. G. Cox, *Nature (London)*, **216**, 1328 (1967); J. S. G. Cox in "Proceedings of Symposium of Disodium Cromoglycate in Allergic Airways Disease", J. Pepys and A. W. Frankland, Eds., Butterworths, London, 1970, pp 13-27; H. Cairns, C. Fitzmaurice, D. Hunter, P. B. Johnson, J. King, T. B. Lee, G. H. Lord, R. Minshull, and J. S. G. Cox, *J. Med. Chem.*, **15**, 583 (1972).
(2) J. P. Devlin, *Annu. Rep. Med. Chem.*, **16**, 61 (1981); J. P. Devlin, *ibid.*, **15**, 59 (1980); see also chapters on pulmonary and antiallergy drugs in previous volumes of *Annu. Rep. Med. Chem.*

0022-2623/82/1825-0837$01.25/0

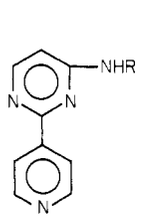
Table I. 4-Amino-2-pyridinylpyrimidines 4a–o and Intermediates



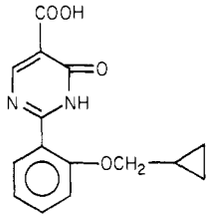
no.	R^1	R^2	R^3	mp, °C	yield, %	formula	anal.	recrystn solvent	PCA[a] shift[b]
4a	4-py[c]	NH_2	H						8.6 (1)
4b	3-py[c]	NH_2	H						4.2 (2)
4c	2-py[c]	NH_2	H						2.5 (2)
4e	4-py	NH_2	CH_3	192–194	81	$C_{10}H_{10}N_4$	C, H, N	*i*-PrOH	10.2 (5)
4f	4-py	$NHCH_3$	CH_3	145–146	97	$C_{11}H_{12}N_4$	C, H, N	Et_2O–hexane	4.9 (2)
4g	4-py	$N(CH_3)_2$	CH_3	274–276	67	$C_{12}H_{14}N_4 \cdot 2HCl \cdot H_2O$	C, H, N	EtOH	5.0 (2)
4h	4-py	$N(CH_3)_2$	H	109–110	77	$C_{11}H_{12}N_4$	C, H, N	cyclohexane	1.5 (1)
4i	4-py	$NHC(CH_3)_3$	H	202–204	65	$C_{13}H_{16}N_4$	C, H, N	cyclohexane	1.5 (1)
4j	4-py	$NHCH_3$	H	148–150	57	$C_{10}H_{10}N_4$	C, H, N	*i*-PrOH–hexane	7.4 (1)
4k	4-py	NHC_2H_5	H	123–125	89	$C_{11}H_{12}N_4$	C, H, N	*i*-PrOH–hexane	8.4 (1)
4l	4-py	$NHCH(CH_3)_2$	H	117–120	72	$C_{12}H_{14}N_4$	C, H, N	Et_2O–hexane	9.0 (2)
4m	4-py	$NH(CH_2)_3CH_3$	H	102–104	76	$C_{13}H_{16}N_4$	C, H, N	Et_2O	7.6 (2)
4n	4-py	$N(C_2H_5)_2$	CH_3	205–208	59	$C_{14}H_{18}N_4 \cdot 2HCl$	C, H, N	*i*-PrOH–Et_2O	2.2 (1)
4o	4-py	$N(CH_2CH_2OH)_2$	CH_3	135–137	69	$C_{14}H_{18}N_4O_2$	C, H, N	*i*-PrOH–Et_2O	6.3 (2)
4p	4-py	$NHNH_2$	CH_3	228–230	89	$C_{10}H_{11}N_5 \cdot 2CH_3SO_3H$	C, H, N	EtOH	2.0 (2)
6a	4-py	OH	CH_3	236–238[d]	75	$C_{10}H_9N_3O$	C, H, N	EtOH	2.8 (2)
7a	4-py	Cl	CH_3	128–130	100	$C_{10}H_8ClN_3$	C, H, N	Et_2O–hexane	1.4 (2)
10	4-py(N–>o)	NH_2	H	>310	96	$C_9H_8N_4O$	C, H, N	*e*	3.8 (1)

[a] PCA = passive cutaneous anaphylaxis in rats. [b] Shift of antibody dose–response curve to the right in multiples of antibody concentration (number of replicate experiments). [c] See ref 6. [d] Literature[12] mp 234–235 °C. [e] Described under Experimental Section.

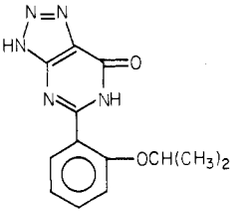
H bonding described as structurally important for the antiallergic activity in these compounds is not possible in 1.



1

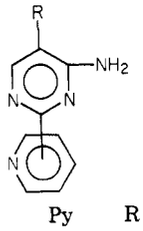


2



3

Chemistry. Scheme I outlines the preparation of some of the 4-aminopyrimidines **4e–o** and related intermediates screened in this work. We have previously reported the synthesis of 4-amino-2-pyridinylpyrimidines (**4**).[6] 4-



	Py	R
4a	4	H
b	3	H
c	2	H
d	4	CH_3

Amino-6-methyl-2-(4-pyridinyl)pyrimidine (**4e**) was prepared by a multistep sequence outlined in Scheme IA. Diazotization of 4-amino-2-(4-pyridinyl)pyrimidine (**4a**) gave a mixture of the corresponding hydroxy **6b** and chloro **7b** compounds. This mixture was treated with phenylphosphonic dichloride to give the pure chloro compound **7b** (Scheme IB).

The intermediate chloropyrimidines (**7a,b**) were allowed to react with several amines to afford the corresponding N-substituted amino derivatives **4f–o** (Scheme IC).

The [[[2-pyridinyl-4-pyrimidinyl]amino]methylene]-malonates and their analogues **8** were prepared by heating the amines **4** with ethoxymethylenemalonates or related compounds (Table II). Amides and carbamates **9** were prepared by reacting amine **4a** with acid chlorides or acid anhydrides in pyridine (Table III).

Ureas **11** were prepared by reaction of the sodium salts of the amines **4** with isocyanates in dimethylformamide or dimethyl sulfoxide (Scheme IIA). Alternatively, when the isocyanates were not readily available, the sodium salts of the amines **4** were first treated with 1,1′-carbonyldiimidazole[7] and then with the desired amines (Scheme IIB).

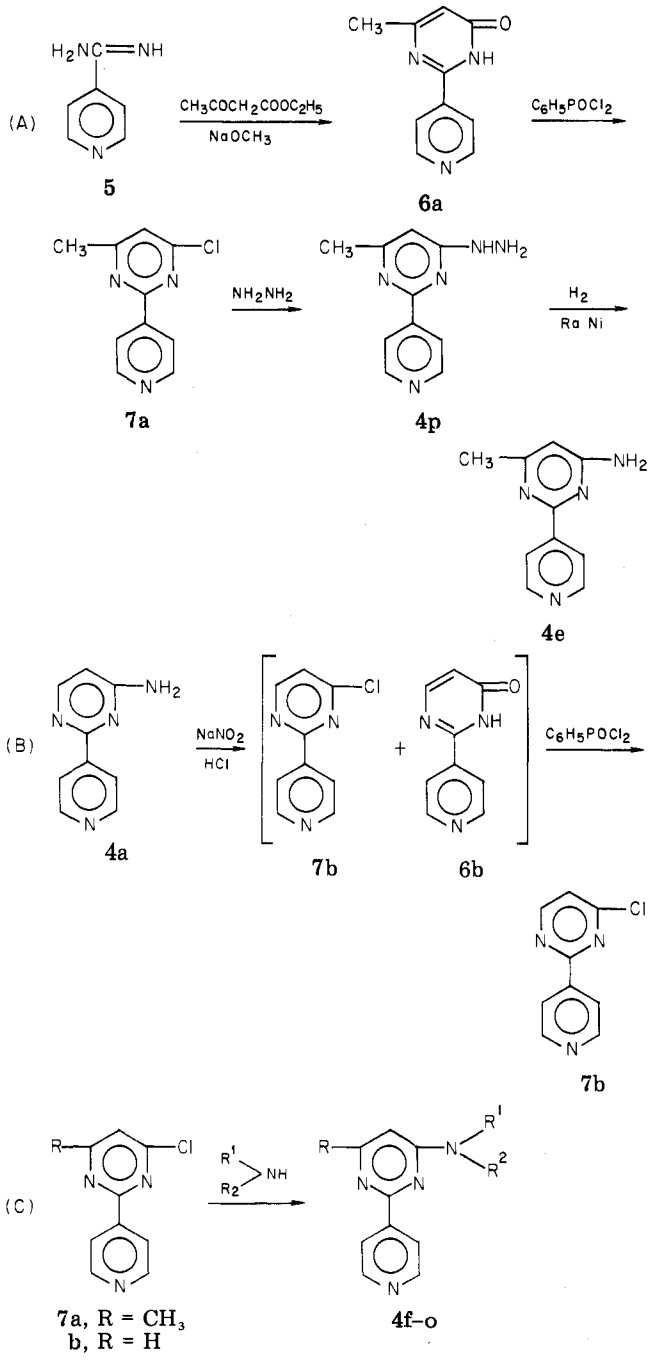
Results and Discussion

The activity of 4-amino-2-pyridinylpyrimidines (**4**) and their derivatives in the PCA test decreased progressively from 4-pyridinyl to 3-pyridinyl to 2-pyridinyl compounds. Therefore, most of the structural modifications were made on 4-amino-2-(4-pyridinyl)pyrimidine (**4a**). Replacement of the amino group by an hydroxy (**6a**), chloro (**7b**), or hydrazino (**4p**) group caused a marked reduction in activity (Table I). Acylation of the amino group lowered the activity in most cases (Table III).

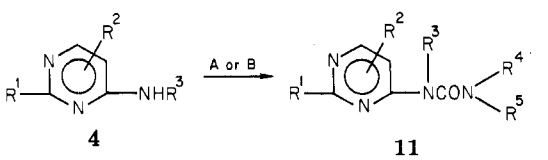
The most potent inhibitors of IgE-mediated PCA in rats were found among [[(2-pyridinyl-4-pyrimidinyl)amino]-methylene]malonates (**8**) (Table II) and *N*-(2-pyridinyl-4-pyrimidinyl)ureas (**11**) (Table IV). The antiallergic activity of these compounds (**11a**, **11b**, **11f**, **11l**, **11m**, **8a**,and **8b**) was substantiated by inhibition of IgE-mediated histamine release from human basophiles by these

(3) P. F. Juby, F. W. Hudyma, M. Brown, J. M. Essery, and R. A. Partyka, *J. Med. Chem.*, **22**, 263 (1979).
(4) B. J. Broughton, P. Chaplen, P. Knowles, E. Lunt, S. M. Marshall, D. L. Pain, and K. R. H. Wooldridge, *J. Med. Chem.*, **18**, 1117 (1975).
(5) H. Bergstrand, B. Lundquist, and A. Schurmann, *Mol. Pharmacol.*, **14**, 848 (1978).
(6) B. Singh and G. Y. Lesher, *J. Heterocycl. Chem.*, **14**, 1413 (1977).
(7) H. A. Staab, *Angew. Chem., Int. Ed. Engl.*, 1, 351 (1962).

Scheme I



Scheme II

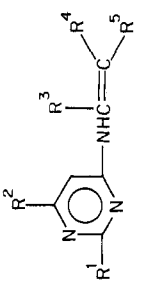


compounds. The AEC_{50}'s (approximate effective dose for 50% inhibition of histamine release) ranged from 1.4 to 6.5 × 10^{-5} M. These data suggest potential antiallergic utility of some of these [[[2-(4-pyridinyl)-4-pyrimidinyl]-amino]methylene]malonates and *N*-[2-(4-pyridinyl)-4-pyrimidinyl]ureas. The optimum compound was found to be **11m**.

Experimental Section

Melting points are uncorrected and were determined in open capillary tubes in an oil bath. All the compounds were analyzed

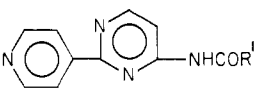
Table II. [[(2-Pyridinyl-4-pyrimidinyl)amino]methylene]malonates 8a–k



no.	R'	R^2	R^3	R^4	R^5	mp, °C	yield, %	formula	anal.	recrystn solvent	PCA[a] shift[b]	PCA[a] potency[c]
8a	4-py	H	H	$COOC_2H_5$	$COOC_2H_5$	138–140	61	$C_{17}H_{18}N_4O_4$	C, H, N	EtOH–Et_2O	>10 (4)	2.4 ± 0.7 (6)
8b	4-py	H	H	$COOCH_3$	$COOCH_3$	209–211	78	$C_{15}H_{14}N_4O_4$	C, H, N	MeOH	>10 (3)	5.8 ± 0.9 (4)
8c	3-py	H	H	$COOC_2H_5$	$COOC_2H_5$	115–117	82	$C_{17}H_{18}N_4O_4$	C, H, N	*i*-PrOH–Et_2O	>10 (2)	0.8 (1)
8d	3-py	H	H	$COOCH_3$	$COOCH_3$	183–184	75	$C_{15}H_{14}N_4O_4$	C, H, N	MeOH–$CHCl_3$	>10 (4)	2.9 ± 1.0 (4)
8e	2-py	H	H	$COOC_2H_5$	$COOC_2H_5$	162–164	47	$C_{17}H_{18}N_4O_4 \cdot CH_3SO_3H$	C, H, N	EtOH–Et_2O	4.1 (1)	
8f	4-py	H	H	CN	$COOC_2H_5$	194–196	72	$C_{15}H_{13}N_5O_2$	C, H, N	EtOH	9.4 (4)	
8g	4-py	H	CH_3	CN	$COOC_2H_5$	203–205	19	$C_{16}H_{15}N_5O_2$	C, H, N	EtOH	5.0 (2)	
8h	4-py	H	H	$COCH_3$	$COCH_3$	182–183	49	$C_{15}H_{14}N_4O_2$	C, H, N	EtOH	5.6 (2)	
8i	4-py	H	H	$COCH_3$	$COOC_2H_5$	198–200	42	$C_{16}H_{16}N_4O_3$	C, H, N	EtOH	2.9 (1)	
8j	4-py	CH_3	H	$COOC_2H_5$	$COOC_2H_5$	169–171	76	$C_{18}H_{20}N_4O_4$	C, H, N	EtOH	7.8 (3)	
8k	4-py	H	H	$COOC[(CH_3)_2]OOC$		>240 dec	70	$C_{16}H_{14}N_4O_4$	C, H, N	DMF	2.2 (1)	

[a, b] See corresponding footnotes in Table I. [c] Potency in terms of aminophylline plus or minus standard error (number of replicate experiments).

Table III. *N*-[2-(4-Pyridinyl)-4-pyrimidinyl]amides 9a-h



no.	R^1	mp, °C	yield, %	formula	anal.	recrystn solvent	PCA[a] shift[b]
9a	CH_3	218–220	44	$C_{11}H_{10}N_4O$	C, H, N	EtOH	1.7 (1)
9b	$CH(CH_3)_2$	215–217	65	$C_{13}H_{14}N_4O$	C, H, N	EtOH	2.3 (1)
9c	$CH_2CH(CH_3)_2$	180–182	78	$C_{14}H_{16}N_4O$	C, H, N	*i*-PrOH	5.0 (1)
9d	$C(CH_3)_3$	248–250	67	$C_{14}H_{16}N_4O$	C, H, N	EtOH	4.2 (1)
9e	$CH_2C(CH_3)_3$	190–192	83	$C_{15}H_{18}N_4O$	C, H, N	EtOH	2.2 (1)
9f	$(CH_2)_4CH_3$	119–120	100	$C_{15}H_{18}N_4O$	C, H, N	*i*-PrOH	7.0 (2)
9g	$O(CH_2)_3CH_3$	180–182	79	$C_{14}H_{16}N_4O_2$	C, H, N	CH_3CN	3.0 (1)
9h	$OC(CH_3)_3$	193–195 dec	84	$C_{14}H_{16}N_4O_2$	C, H, N	EtOH	1.6 (1)

[a,b] See corresponding footnotes in Table I.

for C, H, and N, and the analytical results obtained for these elements were within ±0.4% of the theoretical values. Yields given are those of the purified products and are not optimized. Compounds that failed to analyze properly as free bases or failed to crystallize were converted to methanesulfonic acid or hydrochloric acid salts. All the compounds gave the expected IR and NMR spectra.

Biological Test Methods. IgE-Mediated Passive Cutaneous Anaphylaxis in Rats (PCA). The PCA procedure as described by Mielens et al.[8] was used. Female Sprague–Dawley rats, weighing 90–125 g each, were passively sensitized with id injections of graded concentrations of IgE directed to egg albumin. Forty-eight hours later, the rats were injected iv with 10 mg/kg of egg albumin and Evans Blue. The rats were killed 30 min later, and the diameters of the blueing in the skin sites were recorded as a dose–response vs. antibody concentrations.

For primary screening, the rats were fasted overnight, and compounds were administered orally at 100 mg (base)/kg as a suspension in 0.5% gum tragacanth. Each compound was administered to four rats 1 h before the iv challenge. Activity was measured as the shift of the antibody dose–response curve to the right, toward the region of higher antibody concentrations. The shift was expressed in multiples of antibody concentrations. Most of the compounds resulting in a shift of >10 were examined for their potency in multiple dose tests side by side with aminophylline. The most potent oral inhibitors of IgE-mediated PCA in rats were found among aminomethylenemalonates (**8**) and ureas (**11**).[9]

IgE-Mediated Histamine Release from Human Basophiles.[10] The procedure has been described by Magro,[11] except for minor modifications. Basophiles from allergic donors, 1–2 × 10^4/tube, were incubated at 37 °C for 4 min with purified ragweed protein, antigen E, anti-IgE, or extracts from grasses. Released histamine was assayed by an automated spectrofluorometric technique. Seven of the most potent compounds against PCA (**11a**, **11b**, **11f**, **11l**, **11m**, **8a**, or **8b**) were added to the basophile suspensions 5 min before the antigenic challenge at 3 × 10^{-6}, 1 × 10^{-5}, or 1 × 10^{-4} M, and percent inhibition was calculated with respect to negative controls. AEC_{50}'s were calculated for each compound from the dose–response lines. All of the seven compounds inhibited IgE-mediated histamine release from human basophiles, and their AEC_{50}'s ranged from 1.4 to 6.5 × 10^{-5} M. These data substantiate the antiallergic properties of these compounds as seen in the PCA test.

4-Hydroxy-6-methyl-2-(4-pyridinyl)pyrimidine (6a). A mixture of 31.6 g (0.2 mol) of isonicotinamidine hydrochloride (**5**), 22 g (0.4 mol) of CH_3ONa, 27 g (0.2 mol) of ethyl acetoacetate, and 200 mL of MeOH was refluxed with stirring for 24 h and then concentrated. The residue was dissolved in H_2O and acidified with AcOH. The white solid that precipitated was collected and recrystallized from EtOH to give 28.2 g of **6a**, mp 236–238 °C.

4-Chloro-6-methyl-2-(4-pyridinyl)pyrimidine (7a). A mixture of 48 g (0.25 mol) of **6a** and 80 g (0.4 mol) of phenylphosphonic dichloride was heated at 150–170 °C for 6 h, cooled to room temperature, quenched with ice–H_2O, and made basic by adding aqueous NH_3. The precipitate was collected, washed with H_2O, and dried to give **7a** in quantitative yield of 51.6 g, mp 126–129 °C. The analytical sample (Et_2O–hexane) melted at 128–130 °C.

4-Hydrazino-6-methyl-2-(4-pyridinyl)pyrimidine (4p). A solution of 36 g (0.17 mol) of **7a**, 20 mL of $NH_2NH_2{\cdot}H_2O$, and 100 mL of EtOH was refluxed for 2 h and then stripped to dryness. The residue was partitioned between H_2O and $CHCl_3$. Removal of $CHCl_3$ gave 31.2 g of **4p** as a yellow solid, mp 150–152 °C. It was analyzed as a dimethanesulfonate salt (EtOH), mp 228–230 °C.

4-Amino-6-methyl-2-(4-pyridinyl)pyrimidine (4e). A mixture of 31 g (0.15 mol) of **4p**, 2 g of Raney nickel, and 150 mL of EtOH was hydrogenolized in a Parr apparatus at 60–65 °C until the uptake of H_2 stopped. This was cooled to room temperature, and the catalyst was filtered. The filtrate was concentrated, and the solid residue was recrystallized from *i*-PrOH to yield 22.6 g of **4e**, mp 192–194 °C.

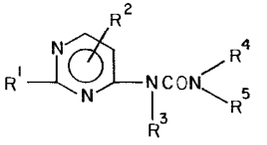
4-Chloro-2-(4-pyridinyl)pyrimidine (7b). To a stirred mixture of 100 g (0.58 mol) of 4a and 400 mL of concentrated aqueous HCl cooled in an ice bath was added a solution of 70 g (1 mol) of $NaNO_2$ in 100 mL of H_2O over 4 h. The temperature was kept below 5 °C during the addition. This was allowed to stand at room temperature overnight and then concentrated. The residue was quenched with aqueous K_2CO_3 and acidified with AcOH. The precipitate was collected, washed with H_2O, and dried: yield 102 g (a mixture of 4-hydroxy derivative **6b** and 4-chloro derivative **7b**, confirmed by TLC and MS). The above mixture (24.7 g) and 40 g of phenylphosphonic dichloride was heated at 155–170 °C for 5 h, cooled to room temperature, and treated with aqueous NH_3. This was extracted with CH_2Cl_2, treated with charcoal, and concentrated to give 19.2 g (overall 68%) of **7b**, mp 130–132 °C. Anal. ($C_9H_6ClN_3$) C, H, N.

Preparation of N-Substituted Amines 4f–o Is Illustrated by the Following Representative Example: 4-(Methylamino)-2-(4-pyridinyl)pyrimidine (4j). A mixture of 15 g (0.08 mol) of **7b**, 25 mL of 40% aqueous CH_3NH_2, and 100 mL of EtOH was refluxed for 5 h and then concentrated. The residue was partitioned between H_2O and $CHCl_3$. The concentrate left after the evaporation of $CHCl_3$ was crystallized from *i*-PrOH–hexane to give 8.4 g of **4j**, mp 148–150 °C.

The Following Three Examples Illustrate the Preparation of [[(2-Pyridinyl-4-pyrimidinyl)amino]methylene]malonates and Their Analogues (8): Dimethyl [[[2-(3-Pyridinyl)-4-pyrimidinyl]amino]methylene]malonate (8d). A mixture of 8.6 g (0.05 mol) of **4b** and 15 mL of dimethyl ethoxymethylenemalonate was heated with stirring at 140–150 °C for 4 h, cooled, and dissolved in MeOH. The dark solution was treated with charcoal and then chilled, whereupon a pale yellow solid crys-

(8) Z. E. Mielens, E. W. Ferguson, and F. J. Rosenberg, *Int. Arch. Allergy*, **47**, 633 (1974).
(9) J. F. Baker, A. F. Jensen, B. W. Chalecki, S. Clemans, D. P. Benziger, P. O'Melia, and J. Edelson, *Arch. Int. Pharmacodyn. Ther.*, **236**, 4 (1978).
(10) Performed under a Sterling-Winthrop Research Institute grant by Dr. A. M. Magro, New York State Kidney Disease Institute, Empire State Plaza, Albany, NY 12237.
(11) A. M. Magro, *Clin. Exp. Immunol.*, **29**, 436 (1977).
(12) K. Sirakawa, T. Tujikawa, and T. Tsuda, *Takedo Kenkyusho Nempo*, **22**, 27 (1963); *Chem. Abstr.*, **60**, 12011*b* (1964).

Table IV. *N*-(2-Pyridinyl-4-pyrimidinyl)ureas **11a–w**



no.	R^1	R^2	R^3	R^4	R^5	mp, °C	yield, %	method	formula	anal.	recrystn solvent	PCA[a] shift[b]	PCA[a] potency[c]
11a	3-py	H	H	H	C_2H_5	220–222	56	A	$C_{12}H_{13}N_5O$	C, H, N	EtOH	>10 (3)	4.8 (2)
11b	3-py	H	H	H	$CH(CH_3)_2$	224–225	70	A	$C_{13}H_{15}N_5O$	C, H, N	EtOH	>10 (3)	1.6 (2)
11c	3-py	H	H	H	$(CH_2)_3CH_3$	180–182	25	A	$C_{14}H_{17}N_5O$	C, H, N	*i*-PrOH	8.5 (3)	
11d	3-py	H	H	H	$C(CH_3)_3$	260–261	82	A	$C_{14}H_{17}N_5O$	C, H, N	EtOH	5.8 (2)	
11e	4-py	H	H	H	H	>340	26	B	$C_{10}H_9N_5O$	C, H, N	DMF	4.8 (2)	
11f	4-py	H	H	H	CH_3	273 dec	55	A	$C_{11}H_{11}N_5O$	C, H, N	EtOH	>10 (4)	3.8 (2)
11g	4-py	H	H	H	C_2H_5	233 dec	70	A	$C_{12}H_{13}N_5O$	C, H, N	EtOH	>10 (5)	7.0 (3)
11h	4-py	H	H	H	$CH(CH_3)_2$	220 dec	66	A	$C_{13}H_{15}N_5O$	C, H, N	EtOH	>10 (4)	2.4 (2)
11i	4-py(N->0)	H	H	H	$CH(CH_3)_2$	125–150	36		$C_{13}H_{15}N_5O_2 \cdot 2CH_3SO_3H$	C, H, N	*i*-PrOH	5.4 (3)	1.8 (2)
11j	4-py	H	H	H	$(CH_2)_2CH_3$	233 dec	72	A	$C_{13}H_{15}N_5O$	C, H, N	EtOH	>10 (4)	
11k	4-py	H	H	C_2H_5	C_2H_5	165–166	17	A	$C_{14}H_{17}N_5O$	C, H, N	*i*-PrOH	8.9 (2)	
11l	4-py	H	H	H	$(CH_2)_3CH_3$	210–212	63	A	$C_{14}H_{17}N_5O$	C, H, N	EtOH	>10 (4)	2.4 (2)
11m	4-py	H	H	H	$C(CH_3)_3$	>350	79	A	$C_{14}H_{17}N_5O$	C, H, N	MeOH	>10 (8)	3.9 ± 1.6 (4)
11n	4-py(N->0)	H	H	H	$C(CH_3)_3$	>300 dec	82		$C_{14}H_{17}N_5O_2$	C, H, N	*d*	1.5 (1)	
11o	4-py	H	H	H	$C(CH_3)_2CH_2OH$	233 dec	43	B	$C_{14}H_{17}N_5O_2$	C, H, N	DMF	>10 (3)	1.0 (1)
11p	4-py	H	CH_3	H	$CH(CH_3)_2$	143–145	47	A	$C_{14}H_{17}N_5O$	C, H, N	*i*-PrOH	>10 (1)	
11q	4-py	H	H	H	$CH_2C(CH_3)_3$	>250 dec	52	B	$C_{15}H_{19}N_5O$	C, H, N	*i*-PrOH–$CHCl_3$	2.2 (1)	
11r	4-py	5-CH_3	H	H	$C(CH_3)_3$	227–228	73	A	$C_{15}H_{19}N_5O$	C, H, N	EtOH	3.5 (1)	
11s	4-py	6-CH_3	H	H	$C(CH_3)_3$	>310	70		$C_{15}H_{19}N_5O$	C, H, N	*i*-PrOH	3.2 (1)	
11t	4-py	H	CH_3	H	$C(CH_3)_3$	152–154	57	A	$C_{15}H_{19}N_5O$	C, H, N	*i*-PrOH	4.0 (1)	
11u	4-py	H	H	H	$CH(C_2H_5)_2$	147–149	36	B	$C_{15}H_{19}N_5O \cdot 2CH_3SO_3H$	C, H, N	EtOH–Et_2O	6.4 (2)	
11v	4-py	H	$(CH_2)_3CH_3$	-H	$CH(CH_3)_2$	122–124	44	A	$C_{17}H_{23}N_5O \cdot 2CH_3SO_3H$	C, H, N	EtOH–Et_2O	4.2 (1)	
11w	2-py	H	H	H	$C(CH_3)_3$	223–225 dec	69	A	$C_{14}H_{17}N_5O$	C, H, N	*i*-PrOH	4.5 (1)	

[a,b] See corresponding footnotes in Table I. [c] Potency in terms of aminophylline plus or minus standard error (number of replicate experiments). [d] Described under Experimental Section.

tallized and was collected and dried to give 11.8 g of **8d**, mp 183–184 °C.

Ethyl 2-Cyano-3-[[2-(4-pyridinyl)-4-pyrimidinyl]amino]-2-propenoate (8f). A mixture of 10 g (0.058 mol) of **4a**, 13 g of ethyl 2-cyano-3-ethoxy-2-propenoate, and 700 mL of xylene was refluxed with stirring for 72 h, treated with charcoal, and concentrated to a yellow solid residue, which was recrystallized from EtOH to give 12.4 g of **8f**, mp 194–196 °C.

Ethyl 3-Oxo-2-[[[2-(4-pyridinyl)-4-pyrimidinyl]amino]methylene]butanoate (8i). A mixture of 8.6 g (0.05 mol) of **4a**, 13 g of ethyl acetoacetate, 15 g of triethyl orthoformate, 100 mg of *p*-toluenesulfonic acid, and 700 mL of xylene was refluxed with stirring for 92 h, treated with charcoal, and evaporated to dryness. The yellow residue was recrystallized from EtOH to yield 7.2 g of **8i**, mp 198–200 °C.

Preparation of Amides and Carbamates (9). 2-Methyl-*N*-[2-(4-pyridinyl)-4-pyrimidinyl]propionamide (9b). After a mixture of 8.6 g (0.05 mol) of **4a**, 10 mL of isobutyryl chloride, and 50 mL of pyridine was left at room temperature overnight, it was poured into ice-cold H_2O. The solid was collected and recrystallized from EtOH to yield 7.9 g of white crystals of **9b**, mp 215–217 °C.

Butyl *N*-[2-(4-Pyridinyl)-4-pyrimidinyl]carbamate (9g). A mixture of 9 g (0.052 mol) of **4a**, 10 mL of butyl chloroformate, and 50 mL of pyridine was stirred in an ice bath for 1 h and then left at room temperature overnight; this was poured into ice-cold H_2O, and the precipitate was collected and recrystallized from CH_3CN to give 11.2 g of **9g**, mp 180–182 °C.

Preparation of Ureas 11. Scheme IIA. *N*-(1,1-Dimethylethyl)-*N'*-methyl-*N'*-[2-(4-pyridinyl)-4-pyrimidinyl]urea (11t). After a mixture of 8.5 g (0.045 mol) of **4f**, 5 mL (0.044 mol) of *tert*-butyl isocyanate, 2.4 g of 50% NaH/oil, and 75 mL of DMF had been stirred for 2 h, it was treated with 5 mL of glacial AcOH and then the solvent was removed under reduced pressure. The residue was diluted with H_2O, and a solid was precipitated, which after washing with hexane was recrystalized from *i*-PrOH to give 7.4 g of **11t** as white needles, mp 152–154 °C.

***N*-(1-Methylethyl)-*N'*-[2-(3-pyridinyl)-4-pyrimidinyl]urea (11b).** A mixture of 17.2 g (0.1 mol) of **4b**, 4.8 g of 50% NaH/oil, and 100 mL of Me_2SO was stirred for 25 min and then 10 mL (0.1 mol) of isopropyl isocyanate was added slowly. The resulting mixture was further stirred for 5 h, acidified with AcOH, and poured into ice-cold H_2O. The precipitate was collected, washed with hexane, and recrystallized from EtOH to afford 17.9 g of **11b** as a white solid, mp 224–225 °C.

Scheme IIB. *N*-(2,2-Dimethylpropyl)-*N'*-[2-(4-pyridinyl)-4-pyrimidinyl]urea (11q). A slurry of 8.6 g (0.05 mol) of **4a**, 2.4 g (0.05 mol) of 50% NaH/oil, and 50 mL of DMF was stirred until the evolution of H_2 had ceased (20 min), and then the resulting solution was slowly stirred into a solution of 8.2 g (0.05 mol) of 1,1'-carbonyldiimidazole and 50 mL of DMF over a 15-min period. This was followed by the addition of 4.4 g (0.05 mol) of neopentylamine. The resulting mixture was allowed to stand at room temperature overnight and then treated with 6 mL of glacial AcOH. The solvent was removed under reduced pressure, and the residual semisolid material was treated with H_2O. The solid was filtered, washed with H_2O and then hexane (to remove oil), and recrystallized from *i*-PrOH–$CHCl_3$ to give 7.4 g of **11q** as white needles, mp > 250 °C dec.

Preparation of Pyridine *N*-Oxide Derivatives is Illustrated by the Following Examples: *N*-(1,1-Dimethylethyl)-*N'*-[2-(4-pyridinyl)-4-pyrimidinyl]urea *N*(py)-Oxide (11n). A mixture of 15 g (0.055 mol) of **1m**, 15 g of 85% *m*-chloroperbenzoic acid, and 500 mL of $CHCl_3$ was stirred at room temperature for 2 h and then concentrated. The residue was stirred in 200 mL of 10% aqueous K_2CO_3 solution. The resulting yellow solid was collected, washed with EtOH, and dried to give 13.1 g of **11n**, mp >300 °C dec.

4-Amino-2-(4-pyridinyl)pyrimidine *N*(py)-Oxide (10). A mixture of 8 g (0.03 mol) of butyl *N*-[2-(4-pyridinyl)-4-pyrimidinyl]carbamate (**9g**), 6.5 g of 85% *m*-chloroperbenzoic acid, and 100 mL $CHCl_3$ was stirred overnight and then extracted with aqueous K_2CO_3 solution. The $CHCl_3$ extract was concentrated. The residue (5.8 g) thus obtained was dissolved in 50 mL of EtOH and 10 mL of 35% aqueous NaOH solution and then heated under reflux of 4 h; the solid that separated was collected, washed with H_2O and then EtOH, and dried to give 4.8 g of **10**, mp >310 °C.

Acknowledgment. We express our thanks to Chester J. Opalka, Jr., for his help in the preparation of some of these compounds and Louise J. Klein for technical assistance in the PCA experiments.

Arylhydroxamic Acid Bioactivation via Acyl Group Transfer. Structural Requirements for Transacylating and Electrophile-Generating Activity of *N*-(2-Fluorenyl)hydroxamic Acids and Related Compounds

Heui-mei Yeh and Patrick E. Hanna*

Departments of Medicinal Chemistry and Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455.
Received October 5, 1981

The synthesis of a series of 12 *N*-(2-fluorenyl)hydroxamic acids, *N*-(2-fluorenyl)-*N*-hydroxyureas, and *N*-(2-fluorenyl)-*N*-hydroxycarbamates is reported. The compounds were evaluated for their ability to serve as substrates for a partially purified hamster hepatic arylhydroxamic acid *N,O*-acyltransferase preparation. Transacylating activity was measured spectrophotometrically with 4-aminoazobenzene as the acyl group acceptor, and electrophile-generating activity was quantified by the *N*-acetylmethionine trapping assay. Only the *N*-acetyl, *N*-propionyl, and *N*-methoxyacetyl derivatives exhibited relatively high levels of activity as measured by either of the assay methods. These results are generally consistent with previously reported conclusions regarding the steric and electronic characteristics of acyl groups that are required for activation by this enzyme system. *N,O*-Acyltransferase inactivation by *N*-hydroxy-2-acetamidofluorene depressed the bioactivation of the *N*-acetyl compound to a greater extent than either the *N*-propionyl or *N*-methyloxyacetyl derivative.

N-(2-Fluorenyl)acetohydroxamic acid (1; Table I) is an *N*-arylhydroxamic acid which, upon metabolic activation, is converted to electrophilic reactants capable of covalent binding to nucleophilic sites on biological macromolecules. The latter process is believed to be responsible for the toxic and carcinogenic activity of 1 and related *N*-arylhydroxamic acids.[1] *N*-Arylhydroxamic acid *N,O*-acyltransferase (AHAT) is a widely distributed mammalian enzyme system that is capable of converting certain *N*-arylhydroxamic acids into electrophilic intermediates that react with biological nucleophiles, including those present on AHAT itself.[2-5]

(1) Miller, E. C. *Cancer Res.* **1978**, *38*, 2479–96.

(2) Bartsh, H.; Dworkin, M., Miller, J. A.; Miller, E. C. *Biochim. Biophys. Acta* **1972**, *286*, 272–98.

(3) King, C. M. *Cancer Res.* **1974**, *34*, 1503–15.

0022-2623/82/1825-0842$01.25/0