crystallized from acetone. The resulting ester (IXc) weighed 2.0 g. (75%) and melted at 138–140°.[24b] Five additional recrystallizations from acetone gave an analytical sample; colorless crystals, m.p. 141–142°,[25b] $\nu_{max}^{CHCl_3}$ 2900–2450 (broad) and 1710 cm.$^{-1}$

Anal. Calcd. for $C_{14}H_{21}Cl_2NO_5$: C, 47.43; H, 5.98; Cl, 20.03; N, 3.95. Found: C, 47.41; H, 6.17; Cl, 19.60; N, 3.98.

N-Bis(2-chloroethyl)-3,4,5-trimethoxybenzylamine (XI) Hydrochloride.—Lithium aluminum hydride (1.3 g.)–aluminum chloride (4.7 g.) reduction of N-bis(2-chloroethyl)-3,4,5-trimethoxybenzamide (IXb, 11 g.) was carried out as illustrated for reduction of N-bis(2-chloroethyl)-3,4-methylenedioxybenzamide (VIIIb). However, in this example, chloroform was substituted for benzene in the isolation procedure. The crude amine (XI) hydrochloride weighed 6.4 g. (54%) and melted at 172–175°.[25c] Five recrystallizations from methanol–acetone yielded a pure sample as colorless crystals; m.p. 181–182°,[24d] ν_{max}^{KBr} 2700, 2650 and 2450 (broad band over the 2900–2450 region) cm.$^{-1}$.

Anal. Calcd. for $C_{14}H_{22}Cl_3NO_3$: C, 46.88; H, 6.18; Cl, 29.65; N, 3.90. Found: C, 46.86; H, 6.16; Cl, 29.32; N, 3.77.

The Syntheses and Pharmacological Activities of Amide, Sulfamide, and Urea Derivatives of 4,6-Diaminopyrimidines

JOHN J. TRAVERSO, E. BROWN ROBBINS, AND CALVERT W. WHITEHEAD

The Lilly Research Laboratories, Indianapolis, Indiana

Received June 10, 1961; Revised Manuscript Received February 23, 1962

Primary and secondary amino groups of 4,6-diaminopyrimidines were converted to amide or amide-like derivatives. Their diuretic activities were determined in dogs. The amides were generally less active than the parent amines with the exception of N-(4-amino-6-pyrimidyl)acetamide. The latter compound was further studied for pathological and blood pressure effects because of its greatly increased potency.

A series of 6-alkylamino-4-amino-pyrimidines and 4-amino-6-arylaminopyrimidines was shown[1] previously to have diuretic activity. In the present work the diaminopyrimidines were converted to amide, sulfonamide and urea derivatives and their biological activities compared to those of the parent amines. The appropriate derivatives were prepared by the usual reactions with acid anhydrides, acid chlorides and isocyanates. When both primary and secondary amine groups were present the primary amine reacted with the first equiva-

(1) C. W. Whitehead and J. J. Traverso, *J. Am. Chem. Soc.*, **80**, 2185 (1958).

lent of reagent, and the secondary amine reacted only with a second equivalent to yield the respective mono- and diamides. The monoacylated derivatives were characterized by converting N-(6-chloro-4-pyrimidyl) acetamide to N-(6-alkylamino-4-pyrimidyl)acetamides, identical with the products obtained from 4-amino-6-*sec*-aminopyrimidines and acetic anhydride or acid chloride.

Reactions of diethylamine, di-*n*-butylamine, morpholine and piperidine with N-(4-amino-6-pyrimidyl)chloroacetamide yielded N-(4-amino-6-pyrimidyl)-α-aminoacetamides. Benzylmercaptan and 4-amino-6-chloropyrimidine in the presence of sodium yielded 4-amino-6-benzylmercaptopyrimidine. Acylation of this gave N-(6-benzylmercapto-4-pyrimidyl)acetamide and acylation of 4-amino-6-hydroxypyrimidine gave N-(6-hydroxy-4-pyrimidinyl)-acetamide. Bromination of 4,6-diaminopyrimidine, 4-amino-6-isopropylaminopyrimidine and 4-amino-6-benzylaminopyrimidine yielded the corresponding 5-bromo derivatives. 5-Bromo-4,6-diaminopyrimidine was converted to N-(4-amino-5-bromo-6-pyrimidinyl) acetamide.

Experimental

N-(4-Amino-6-pyrimidinyl)amides (Table I).—Monoamides were prepared by dissolving 0.1 mole of the diaminopyrimidine and 0.1 mole of the appropriate acid anhydride or acid chloride in 150 ml. of dioxane and heating the solution under reflux for 4–5 hr. Diamides were obtained when two or more molar equivalents of the acid anhydride or acid chloride were employed. The solutions were concentrated and neutralized with dilute NaOH solution. The solid product was collected and crystallized from dilute alcohol.

N-(4-Benzylamino-6-pyrimidinyl)acetamide.—Ten grams (0.06 mole) of N-(4-chloro-6-pyrimidinyl)acetamide (Table I) was refluxed for 8 hr. with 12.5 g. (0.12 mole) of benzylamine in 200 ml. of dioxane. After the solution was cooled, the benzylamine hydrochloride was filtered off and the dioxane was removed under reduced pressure. The residue was dissolved in 50% ethanol to which a few drops of ammonium hydroxide was added. The solution was treated with carbon and the clear filtrate was concentrated. The solid which crystallized from the cooled solution was recrystallized several times from ethyl acetate, yield 6 g. (41%), m.p. 181°. The melting point was not depressed when the compound was mixed with the product from 4-amino-6-benzylaminopyrimidine and acetic anhydride.

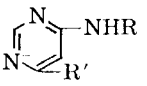
Anal. Calcd. for $C_{13}H_{14}N_4O_4$: C, 64.44; H, 5.82; N, 23.13. Found: C, 64.21; H, 5.69; N, 23.20.

N-(4-Amino-6-pyrimidinyl)ureas and N-(4-Amino-6-pyrimidinyl)thioureas (Table I).—4,6-Diaminopyrimidine (11 g., 0.1 mole) and 0.1 mole of the appropriate isocyanate or isothiocyanate were dissolved in 150 ml. of dioxane and refluxed for 4–5 hr. The dioxane was removed by reduced pressure distillation and the product crystallized from 50% alcohol.

N-(4-Amino-6-pyrimidinyl)formamide.—4,6-Diaminopyrimidine (11 g., 0.1 mole) was heated at 170° for 3 hr. with 25 ml. of formamide in an oil bath. After cooling, the solid was washed with ether and filtered. It was digested with hot

TABLE I

AMIDE DERIVATIVES OF 4,6-DIAMINOPYRIMIDINES



R	R'	Formula	Yield, %	M.p., °C.	Analyses % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
CH_3CO	Cl	$C_6H_6ClN_3O$	75	156	41.99	41.74	3.52	3.58	24.49	24.49
CH_3CO	OH	$C_6H_7N_3O_2$[f]	56	281 d.	47.05	47.07	4.61	4.57	27.44	27.45
CH_3CO	NH_2	$C_6H_8N_4O$[f]	87[a]	325 d.	47.36	47.45	5.30	5.33	36.83	37.52
C_2H_5CO	NH_2	$C_7H_{10}N_4O$[f]	60[a]	231	50.59	50.75	6.07	6.23	33.72	33.52
C_2H_5NHCO	NH_2	$C_7H_{11}N_5O$	15	[c]	46.40	46.39	6.12	6.17	38.65	38.53
CH_3CO	CH_3CONH	$C_8H_{10}N_4O_2$	78[a]	276	49.48	49.66	5.19	5.36	28.85	28.68
$HO_2C(CH_2)_2CO$	NH_2	$C_8H_{10}N_4O_3$	62[a]	260[c]	45.71	45.52	4.80	5.04	26.66	26.98
$CH_2{=}CHCH_2NHCS$	NH_2	$C_8H_{11}N_5S$	20	211	45.95	46.26	5.30	4.62	33.48	33.27
n-C_3H_7CO	NH_2	$C_8H_{12}N_4O$	33[a]	147	53.32	53.05	6.71	6.24	31.09	30.78
n-C_4H_9NHCS	NH_2	$C_9H_{15}N_5S$	20	231	49.98	48.66	6.71	6.35	31.08	30.65
C_6H_5CO	NH_2	$C_{11}H_{10}N_4O$	20[b]	217	61.67	61.31	4.71	4.52	26.16	25.93
C_6H_5NHCO	NH_2	$C_{11}H_{11}N_5O$	65	[c]	57.63	57.78	4.84	4.43	30.55	30.73
C_6H_5NHCS	NH_2	$C_{11}H_{11}N_5S$	33	216	53.87	53.71	4.52	4.74	28.56	28.51
CH_3CO	$C_5H_{10}N$[e]	$C_{11}H_{16}N_4O$	54[a]	203	59.98	60.06	7.32	6.93	25.44	25.88
CH_3CO	i-$C_5H_{11}NH$	$C_{11}H_{18}N_4O$	45[a]	169	59.43	59.42	8.16	8.13	25.21	24.94
o-$HO_2CC_6H_4CO$	NH_2	$C_{12}H_{10}N_4O_3$	41[a]	250 d.	55.81	56.18	3.90	3.91	21.70	22.10
$C_6H_5CH_2CO$	NH_2	$C_{12}H_{12}N_4O$	20[b]	194	63.14	63.60	5.30	4.96	24.55	25.01
CH_3CO	C_6H_5NH	$C_{12}H_{12}N_4O$	35[a]	231	63.14	63.39	5.30	5.44	24.55	24.81
$C_6H_5OCH_2CO$	NH_2	$C_{12}H_{12}N_4O_2$	64[a]	204	59.01	58.82	4.95	4.54	22.94	23.16
$C_6H_5SO_2$	CH_3CONH	$C_{12}H_{12}N_4O_3S$	95[a]	311 d.	49.30	49.01	4.14	4.19	19.17	18.92

TABLE I (*Continued*)

p-$CH_3OC_6H_4NHCO$	NH_2	$C_{12}H_{13}N_5O_2$	40	[c]	55.59	55.84	5.05	5.25	27.02	26.81
$C_6H_5CH{=}CHCO$	NH_2	$C_{13}H_{12}N_4O$	25[b]	255	64.98	64.86	5.03	5.33	23.32	23.39
CH_3CO	$C_6H_5CH_2S$	$C_{13}H_{13}N_3OS$	80	115	60.22	60.28	5.05	5.16	16.20	16.35
CH_3CO	$CH_3CONC_5H_{11}$	$C_{13}H_{20}N_4O_2$	68[a]	81	59.07	60.22	7.63	8.03	21.20	21.24
CH_3CO	$CH_3CONC_6H_4$-*p*-Cl	$C_{14}H_{13}ClN_4O_2$	55[a]	164	55.18	54.97	4.30	4.36	18.40	18.59
$C_6H_5OCH_2CO$	$C_6H_5OCH_2CONH$	$C_{20}H_{18}N_4O_4$	30	197	63.48	62.80	4.80	4.65	14.81	14.43
$C_6H_5CH_2(CH_3CO)$	$C_6H_5CH_2(CH_3CO)N$	$C_{22}H_{22}N_4O_2$	53	98	70.75	70.54	5.92	6.04	14.95	15.12
$C_6H_5SO_2$[j]	NH_2	$C_{10}H_{10}N_4O_2S$	68	[d]	47.99	47.72	4.03	3.83		
CH_3SO_2[j]	NH_2	$C_5H_8N_4O_2S$	11	305 d.	31.92	31.44	4.29	4.63	29.78	29.90
$C_6H_{10}N_2O$[g]	NH_2	$C_{10}H_{15}N_5O_2$	63	201					29.52	29.87
$C_7H_{12}NO$[h]	NH_2	$C_{11}H_{17}N_5O$	75	183					27.77	29.44
$(C_2H_5)_2NCH_2CO$	NH_2	$C_{10}H_{17}N_5O$	80	112					31.43	31.59
$(C_4H_7)_2NCH_2CO$	NH_2	$C_{14}H_{25}N_5O$	90	140					25.10	25.17
		These compounds have a bromine in the 5-position.								
H	NH_2	$C_4H_5BrH_4$	48	209	25.41	25.67	2.67	2.77	29.64	29.55
i-C_3H_7	NH_2	$C_7H_{11}BrN_4$	47	138	36.38	36.43	4.80	4.80	24.24	24.22
$C_6H_5CH_2$	NH_2	$C_{11}H_{11}BrN_4$	64	145	47.33	46.95	3.97	4.16	20.07	19.85
CH_3CO	NH_2	$C_6H_7BrN_4O$	50	181	31.19	31.33	3.05	3.21	24.25	24.41

[a] Prepared by the anhydride. [b] Prepared by the acid chloride. [c] Chars when heated. [d] Melts with decomposition. [e] Piperidino. [f] J. J. Traverso and C. W. Whitehead, U. S. Patent 2,874,157 (1959). [g] Morpholinoacetyl. [h] Piperidinoacetyl. [j] Prepared by the Schotten–Baumann method using the sulfonyl chloride with pyridine in dioxane solution.

dioxane and then was washed with ethanol and ethyl acetate. A tan solid (7 g., 50%), m.p. 276° dec.,[2] was obtained.

Anal. Calcd. for $C_5H_6N_4O$: C, 43.47; H, 4.38; N, 40.56. Found: C, 43.77; H, 4.41; N, 40.41.

Reactions of N-(4-Amino-6-pyrimidinyl)formamide and 1-(4-Amino-6-pyrimidinyl)-3-phenylurea with Acetic Anhydride.—Ten grams of 4-amino-6-formamidopyrimidine, or of 4-amino-6-phenylureidopyrimidine, respectively, was treated for 1 hr. with 30 ml. of boiling acetic anhydride. The resulting solutions were concentrated and cooled. In each case the product was 4,6-bis-acetamidopyrimidine.

N - (4 - Acetamido - 6 - pyrimidyl)succinimide.—N - (4 - Amino - 6 - pyrimidinyl)succinamic acid (14 g., 0.07 mole) (Table I) was refluxed for 8 hr. in 100 ml. of acetic anhydride. The excess acetic anhydride was removed under reduced pressure. The crystalline product was washed with ether, collected and recrystallized from ethanol, yield 10 g. (60%), m.p. 235°. The infrared band assignments were: 5.61 μ (weak) and 5.80 μ (strong) for the cyclic imide carbonyls; 2.99 μ and 3.07 μ for the amide NH; 5.89 μ and 6.60 μ for amide I and II bands.

Anal. Calcd. for $C_{10}H_{10}N_4O_3$: C, 51.28; H, 4.30; N, 23.92. Found: C, 51.46; H, 4.28; N, 23.72.

N-(4-Amino-6-pyrimidyl)chloroacetamide.—4,6-Diaminopyrimidine (30 g.) was added to 120 ml. of chloroacetyl chloride and the mixture allowed to stand for 4 days. The solid was filtered free of liquid. The dry solid was dissolved in cold water and the solution made slightly basic with dilute NH_4OH. The resulting precipitate was collected, washed with cold water and dried; yield 40 g. (90%). A sample was crystallized several times from alcohol, m.p. 175° dec.

Anal. Calcd. for $C_6H_7ClN_4O$: C, 38.63; H, 3.79; N, 30.05. Found: C, 38.58; H, 3.56; N, 30.33.

N-(4-Amino-6-pyrimidyl)-α-aminoacetamides (Table I).—To 100 ml. of alcohol was added 3.72 g. (0.02 mole) of N-(4-amino-6-pyrimidyl)chloroacetamide and *ca.* 0.05–0.10 mole of the appropriate amine. The alcohol solution was boiled under reflux for 4–6 hr. The alcohol was evaporated and the residue extracted with ethyl acetate. The ethyl acetate solution was filtered and concentrated.

4-Amino-6-benzylmercaptopyrimidine.—4-Amino-6-chloropyrimidine (13 g., 0.1 mole) was added to 500 ml. of dry xylene containing 2.3 g. (0.1 g.-atom) of sodium metal cut into small pieces. Benzylmercaptan (12.4 g.) was added and the mixture was refluxed overnight. The xylene was removed under reduced pressure. The residue was dissolved in 150 ml. of 50% ethanol, treated with carbon, and the ethanol solution was filtered several times. The filtrate was concentrated and, after cooling, 13 g. of product was obtained, yield 60%. A sample was recrystallized from dilute ethanol giving needles, m.p. 133–134°.

Anal. Calcd. for $C_{11}H_{11}N_3S$: C, 60.80; H, 5.10; N, 19.34. Found: C, 60.98; H, 4.91; N, 18.66.

5-Bromo-4,6-diaminopyrimidines (Table I).—The appropriate 4,6-diaminopyrimidine[1] (5–10 g.) was dissolved in 10–20 ml. of glacial acetic acid. Bromine was added dropwise to the clear solution until a faint yellow color persisted. The warm solution was cooled and then neutralized with 2 *N* sodium hydroxide. The solid was collected and recrystallized from water.

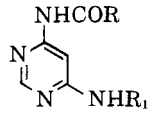
Diuretic Assay Method.—The method was essentially as reported by Robbins

(2) E. Richter and E. C. Taylor, *J. Am. Chem. Soc.*, **78**, 5851 (1956), reported m.p. 285° dec.

and Chen.[3] It differed in that all doses of the compounds under study as well as the barbiturate anesthetic were given by the oral route and on a slightly different time schedule.

Female mongrel dogs were deprived of food but not water for 24 hr. They were hydrated with 20 ml./kg. of 0.9% saline by stomach tube on the morning of a test day and given the dose of test compound in a capsule 30 min. later. Within 15 min. a dose of 35 mg./kg. of secobarbital sodium in 10% aqueous solution was given by stomach tube. When anesthesia was complete, a Foley bag retention catheter was placed in the urinary bladder which was emptied every 30 min. Beginning 90 min. after the dose of test compound, urine volumes were recorded each half hr. for 4.5 hr. and the output in ml./kg./4.5 hr. recorded. Each dog was standardized previous to, and during, the diuretic studies in order to establish a control urine output, when untreated, for each animal. Individual responses then were the increases in urine output during 4.5 hr. over mean control values in ml./kg. (Table II).

TABLE II



DIURETIC ACTIVITY

R	R_1	Dose, mg./kg.	No. of dogs	Mean response	Relative potency, %
CH_3	H	2.5	3	7.00	100
		5.0	13	5.82	
		10.0	10	9.89	
C_2H_5	H	5.0	3	0.84	38
		10.0	6	7.59	
CH_3	$C_6H_5CH_2$	5.0	3	0.00	11
		10.0	3	1.77	
$C_6H_5CH_2$	H	5.0	3	0.81	9
		10.0	7	0.81	
C_6H_5	H	5.0	3	0.00	0–9
		10.0	3	0.68	
$CH_3(CH_2)_2$	H	5.0	3	0.00	0
		10.0	4	0.00	
C_6H_5NH	H	5.0	3	0.00	13
		10.0	3	3.03	
C_2H_5NH	H	10.0	4	0.00	9
		20.0	4	5.03	
n-C_4H_9NH (Thiocarbamyl)	H	5.0	3	0.38	7
		10.0	3	0.55	
		20.0	3	1.24	
p-$CH_3OC_6H_4NH$	H	5.0	3	0.00	0
		10.0	4	0.71	

(3) E. B. Robbins and K. K. Chen, *J. Am. Pharmaceut. Assn., Sci. Ed.*, **40**, 249 (1951).

TABLE III

ACUTE ORAL TOXICITY OF N-(4-AMINO-6-PYRIMIDINYL)ACETAMIDE IN MICE, OBSERVED FOR SEVEN DAYS

Dose, mg./kg.	No. Died/ No. Used	Days of death	Pathology[a]
200	2/5	2,2	No crystalluria but granular precipitate in some glomerular capsules. In survivors, 5 of 6 kidneys showed groups of dilated but empty convoluted tubules with cellular proliferation or infiltration of some collecting tubules.
250	3/5	2,2,2	No crystals in tubules but some granular precipitate. Survivors had obstruction of some renal tubules without crystalluria.
300	2/5	2,4	Some convoluted tubules are slightly dilated but no crystals were seen. Survivors had obstruction of some renal tubules without crystalluria.
365	3/5	2,5,5	Fatty metamorphosis of liver and kidneys in one; necrosis and fatty metamorphosis of liver and early pyelonephritis in another; survivors had obstruction of renal tubules without crystalluria.

[a] The acute oral LD_{50} in mice was less than 275 mg./kg. with deaths occurring in 2 to 7 days.

TABLE IV

UROLOGICAL FINDINGS FOLLOWING ORAL ADMINISTRATION OF N-(4-AMINO-6-PYRIMIDINYL)ACETAMIDE

Dog no.	Daily dose, mg./kg.	Body wt.	Albumin	Sugar	Pus	Blood	Cast
1	10	loss	..	..	occasional	rare	1+
2	10	loss	..	..	1+	1+	2+
3	25	loss	3+	..	loaded	loaded	4+
4	25	loss	1+	..	loaded	4+	1+
5	50	loss	..	..	occasional	rare	..

NECROPSY FINDINGS: Crystalluria was noted in all cases. In the dogs which received 10 mg./kg., there were crystals in the collecting tubules with no damage to the tubular epithelium. Following 25 mg./kg., there were crystals in many of the convoluted and some of the collecting tubules without damage to the epithelium. In the one that received 50 mg./kg. daily, there was a reddish brown pigment in the convoluted tubules and a very few convoluted tubules contained one or two necrotic cells.

For estimating relative diuretic potency, at least three dogs per dose and at least two doses of each compound were used. Combining the information available regarding log dose-response slopes in the entire pyrimidine series, a combined slope figure of 7.3 was obtained.[4]

(4) C. I. Bliss, "The Statistics of Bioassay," Academic Press, Inc., New York, N. Y., 1952.

In a previously reported series,[1] 4-amino-6-anilinopyrimidine (L-25043) was assigned a potency of 100% and used as a reference compound. In this report N-(4-amino-6-pyrimidyl)acetamide was used as the reference compound; it was twice as potent as L-25043, so all relative potency figures in this report can be referred to the previous report if multiplied by 2.

Effect of N-(4-Amino-6-pyrimidinyl)acetamide on Blood Pressure, Respiration and the Electrocardiogram.—The blood pressure, respiration and electrocardiogram were studied in two dogs anesthetized with sodium phenobarbital. Following 5 mg./kg. given by tube into the duodenum, there was no significant change in mean arterial pressure in one dog during a 1 hr. observation period. In the second dog this dose was followed by a gradual fall in blood pressure (48%) reaching a maximum in 40 min. The respiration and electrocardiogram were not altered. In this dog a second dose of 10 mg./kg. was administered 4 hr. after the original dose. This was followed by an increase in mean arterial pressure of 14% in 40 min. Respiration and the electrocardiogram were not altered. In spite of a fall in pressure after 5 mg./kg. in one dog, it appears this compound has very little effect on blood pressure, respiration and the electrocardiogram. Since the blood pressure in this same dog showed a slight increase in mean artery pressure, it indicates that depressor action following 5 mg./kg. was due to anesthesia.

Acknowledgments.—The toxicity and pathology results were obtained by Drs. R. C. Anderson, P. N. Harris and F. G. Henderson of this laboratory. The microanalyses were done by William Brown, Howard Hunter, George Maciak and Miss Gloria Beckman.

Antidiabetic Agents. N^4-Arylsulfonylsemicarbazides

JOHN B. WRIGHT AND ROBERT E. WILLETTE

Research Laboratories of The Upjohn Company, Kalamazoo, Michigan

Received March 5, 1962

A series of N^4-arylsulfonylsemicarbazides (I, Table I) was prepared by reaction of arylsulfonylurethanes with suitably substituted hydrazines (II). The requisite hydrazines were prepared by reduction of nitrosamines (III) with lithium aluminum hydride. A number of the compounds prepared showed appreciable antidiabetic activity.

During the course of continuing work in these laboratories on antidiabetic compounds, we were interested in investigating arylsulfonylsemicarbazides of the general type I. Our initial experiments in this area produced compounds showing several times the blood sugar