Synthesis and Xanthine Oxidase Inhibitory Activity of 4,6-Disubstituted 1-*p*-Chlorophenylpyrazolo[3,4-*d*]pyrimidines

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Several 4,6-disubstituted 1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidines were prepared from 4,6-dichloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine and their xanthine oxidase inhibitory activity were tested *in vitro*.

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A hypoxanthine analog, 4-hydroxypyrazolo[3,4-*d*]pyrimidine (allopurinol) (1) has been found as a slow substrate and a good inhibitor of enzyme xanthine oxidase (2). Allopurinol has been clinically used for the treatment of gout (3). It has also been established that an isomer of xanthine, 4,6-dihydroxypyrazolo[3,4-*d*]pyrimidine, is a metabolite of allopurinol, as well as an excellent inhibitor of this enzyme (2). The xanthine oxidase inhibitory activity of the pyrazolo[3,4-*d*]pyrimidines has been studied (4a-c). Baker's group (5) investigated the effects of polar and hydrophobic substituents at positions 1 and 6, and it was observed that the mode of binding was dependent upon the position and the nature of these substituents. Furthermore, Kobayashi (6) reported the structural activity relationship of this ring system, and certain 3-phenyl derivatives were found to be as potent as allopurinol in inhibiting the xanthine oxidase. On the basis of these findings, we were interested in determining the effect of an alkylamino, alkoxy or alkylthio group in positions 4 or 6. In order to maintain the necessary binding factor, as suggested by Baker's work (5), the *p*-chlorophenyl group was retained in the position 1.

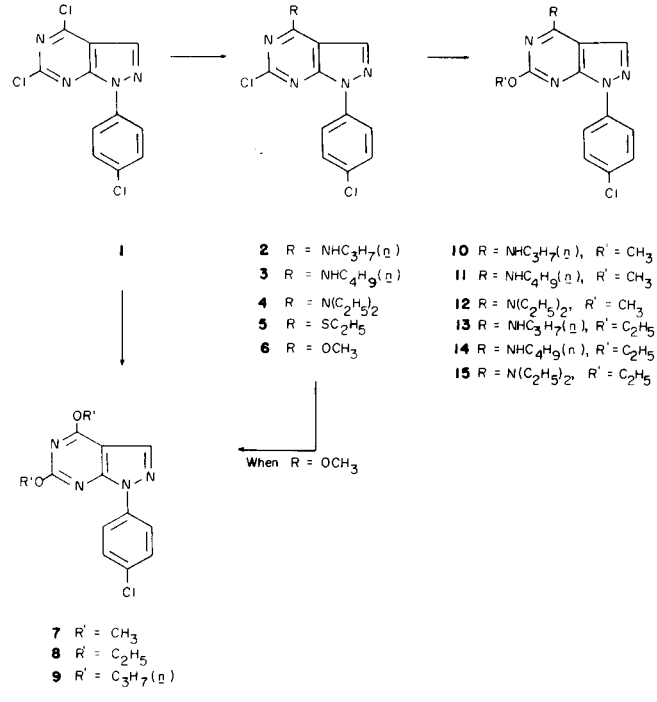
Chemistry.

It was reported that the selective nucleophilic displacement of 4,6-dichloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine (**1**) occurred at the 4 position under mild conditions. The displacement of the second chlorine atom at the 4 position requires a higher temperature (7). On the basis of these facts, 4-alkylamino-6-chloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidines (**2, 3** and **4**) and 4-ethylthio-6-chloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine (**5**) were prepared by the treatment of **1** with the corresponding nucleophile. When **1** was treated with sodium alkoxides, 4,6-dialkoxy-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidines (**8** and **9**) were obtained as described in the literature (7). It is interesting to note, however, the reaction of **1** with sodium methoxide displaced only one chlorine to afford 6-chloro-1-*p*-chlorophenyl-4-methoxypyrazolo[3,4-*d*]-pyrimidine (**6**). Compound **6** was easily converted to 1-*p*-chlorophenyl-4,6-dimethoxypyrazolo[3,4-*d*]pyrimidine (**7**) by further treatment with sodium methoxide. Furthermore, the 4-alkylamino-6-alkoxy-1-*p*-chlorophenylpyrazolo-[3,4-*d*]pyrimidines (**10, 11, 12, 13, 14** and **15**) were prepared by the reaction of the appropriate 4-alkylamino-6-chloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine with sodium alkoxide or alcoholic potassium hydroxide (Scheme).

Xanthine Oxidase Inhibitory Activity.

The compounds were tested *in vitro* for inhibititon of enzyme xanthine oxidase, which was isolated from bovine milk. The results shown in the Table inidcate that none of the compounds showed significant inhibition. Some degree of inhibitory activity was observed only in the case of the 4-alkylamino derivatives **2-4** and **10-14**.

Scheme



Table

1-*p*-Chlorophenylpyrazolo[3,4-*d*]pyrimidines and their Xanthine Oxidase Inhibitory Activity

Compound Number	Yield (%)	Mp (°C) (a)	Solvent of Crystallization	Calcd. (%) C	H	N	Formula	Found (%) C	H	N	Xanthine Oxidase Inhibition (%) (b)
2	87	194-195	Ethanol	52.17	4.07	21.73	$C_{14}H_{13}Cl_2N_5$	51.95	4.16	21.75	13
3	86	152-154	Benzene	53.57	4.51	20.83	$C_{15}H_{15}Cl_2N_5$	53.49	4.49	20.62	14
4	77	142-143	Ethanol	53.57	4.51	20.83	$C_{15}H_{15}Cl_2N_5$	53.45	4.64	20.65	18
5	85	124-126	*n*-Heptane	47.98	3.10	17.23	$C_{13}H_{10}Cl_2N_4S$	48.00	3.05	17.22	0
6	86	170-171	Benzene	48.82	2.73	18.98	$C_{12}H_8Cl_2N_4O$	48.80	2.72	19.02	0
7	69	194-195	Benzene	53.70	3.82	19.27	$C_{13}H_{11}ClN_4O_2$	53.42	3.67	19.28	0
8	77	127-128	Ethanol	56.51	4.75	17.57	$C_{15}H_{15}ClN_4O_2$	56.35	4.84	17.65	0
9	81	70-72	Methanol	58.84	5.53	16.16	$C_{17}H_{19}ClN_4O_2$	58.60	5.34	16.12	0
10	100	173-175	Ethanol	56.58	5.07	22.00	$C_{15}H_{16}ClN_5O$	56.51	5.14	21.98	18
11	87	166-168	Benzene	57.90	5.47	21.10	$C_{16}H_{18}ClN_5O$	57.89	5.52	21.10	23
12	75	134-136	Benzene-Petroleum ether	57.90	5.47	21.10	$C_{16}H_{18}ClN_5O$	58.05	5.52	21.21	18
13	57	184-185	Ethanol	57.90	5.47	21.10	$C_{16}H_{18}ClN_5O$	57.90	5.51	21.17	26
14	68	166-168	Benzene	59.03	5.84	20.25	$C_{17}H_{20}ClN_5O$	59.20	5.99	20.23	29
15	83	112-114	Ethanol-Water	59.03	5.84	20.25	$C_{17}H_{20}ClN_5O$	58.78	5.85	20.32	0
Allopurinol							$C_5H_4N_4O$				80

(a) All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. (b) At the concentration of 13 μM.

EXPERIMENTAL

Synthesis.

4-Alkylamino-6-chloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimdine (**2-4**).

Five mmoles of 4,6-dichloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine (**1**) was added to absolute ethanol (30 ml) containing a primary or secondary alkylamine (20 mmoles). The solution was stirred at room temperature for 3 hours. The filtered precipitate was then washed with ethanol, dried and recrystallized from the solvent indicated in the Table.

6-Chloro-1-*p*-chlorophenyl-4-ethylthiopyrazolo[3,4-*d*]pyrimidine (**5**).

To a solution of **1** (4.5 g, 15 mmoles) in absolute ethanol (30 ml) containing potassium hydroxide (2.76 g, 60 mmoles) was added ethylmercaptan (3.7 g, 60 mmoles). The solution was stirred at room temperature for 3 hours. The filtered precipitate was then washed with water (100 ml), dried and recrystallized from the indicated solvent in the Table to give pure product (4.1 g).

6-Chloro-1-*p*-chlorophenyl-4-methoxypyrazolo[3,4-*d*]pyrimidine (**6**).

To a solution of sodium (0.115 g, 5 × 10^{-3} g-atom) dissolved in absolute methanol (10 ml) was added **1** (1.5 g, 5 mmoles). The solution was stirred at room temperature for 1 hour and then diluted with water (50 ml). The filtered precipitate was washed with water, dried and recrystallized from the solvent indicated in the Table to give pure product (1.23 g).

4,6-Dialkoxy-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine (**7-9**).

Method A (**7**).

To a solution of sodium (0.35 g, 15 × 10^{-3} g-atom) dissolved in absolute methanol (10 ml) was added **6** (1.48 g, 5 mmoles). The solution was stirred at room temperature for 4 hours. The filtered precipitate was washed with water, dried and recrystallized from benzene to give 1 g of pure **7**.

Method B (**8** and **9**).

To a solution of sodium (2 × 10^{-3} g-atom) dissolved in the appropriate absolute alcohol (50 ml) was added **1** (10 mmoles). The solution was stirred at room temperature for 3 hours and then boiled for 5 minutes on the steam bath. The reaction mixture was evaporated to dryness *in vacuo*. The residue was triturated with water and filtered. The crude product was recrystallized from the solvent indicated in the Table.

4-Alkylamino-6-alkoxy-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine (**10-15**).

Method A (**10, 11** and **14**).

To a solution of sodium (15 × 10^{-3} g-atom) dissolved in appropriate absolute alcohol (30 ml) was added 4-alkylamino-6-chloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine (5 mmoles). The mixture was refluxed for 3 hours and then evaporated to dryness *in vacuo*. The residue was triturated with water, filtered and dried. The product was recrystallized from the indicated solvent.

Method B. (**12, 13** and **15**).

To a solution of potassium hydroxide (3 g) dissolved in the appropriate absolute alcohol (100 ml) was added 4-alkylamino-6-chloro-1-*p*-chlorophenylpyrazolo[3,4-*d*]pyrimidine (10 mmoles). The solution was heated on the steam bath for 1 hour, diluted with water (100 ml). The crude product from the cooled solution was recrystallized from the solvent indicated in the Table.

Assay of Xanthine Oxidase Inhibition.

The assay was a modification of the assay method of Kalcker (8). The 3 ml reaction contained (in μmoles): tris-hydrochloride, *p*H 7.5, 150; EDTA, 0.1; bovine milk xanthine oxidase (ICN Nutritional Biochemicals, 0.44 units/mg), 20-40 μg; xanthine, 0.013; and varying concentrations of the pyrazolo[3,4-*d*]pyrimidine being tested as an inhibitor. The assay was carried out aerobically at 25° at 290 nm with a Cary 15 recording spectrophotometer using the 0 to 0.1 OD slide wire in the synchronous drive mode. Reactions were started by adding enzyme.

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REFERENCES AND NOTES

(1) R. K. Robins, *J. Am. Chem. Soc.*, **78**, 784 (1956).

(2) G. B. Elion, A. Kovensky, G. H. Hitchings, E. Metz and R. W. Rundles, *Biochem. Pharmacol.*, **15**, 863 (1966).

(3) R. W. Rundles, J. B. Wyngaarden, G. H. Hitchings, G. B. Elion and H. R. Silberman, *Trans. Assoc. Am. Physicians*, **76**, 126 (1963).

(4a) R. McCollister, W. R. Gilbert, D. M. Ashton and J. B. Wyngaarden, *J. Biol. Chem.*, **239**, 1560 (1964); (b) P. Feigelson, J. D. Davidson and R. K. Robins, *ibid.*, **226**, 993 (1957); (c) G. B. Elion, F. M. Benezra, I. Carellas, L. O. Carrington and G. H. Hitchings, *Israel J. Chem.*, **6**, 787 (1968); *Chem. Abstr.*, **70**, 86069 (1969); (d) B. R. Baker, *J. Pharm. Sci.*, **56**, 959 (1967); (e) B. R. Baker and J. A. Kozma, *J. Med. Chem.*, **11**, 656 (1968).

(5) B. R. Baker, W. F. Wood and J. A. Kozma, *ibid.*, **11**, 661 (1968).

(6) S. Kobayashi, *Chem. Pharm. Bull.*, **21**, 941 (1973).

(7) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, **23**, 852 (1958).

(8) H. M. Kalckar, *J. Biol. Chem.*, **167**, 429 (1947).