Enzyme Inhibitors XXIII: Syntheses of 9-(Substituted Aralkyl)-6-Substituted Purines as Inhibitors of Adenosine Deaminase

HOWARD J. SCHAEFFER*, EUGENE ODIN, and SHMUEL BITTNER

Abstract □ A series of 9-(substituted aralkyl)-6-substituted purines was prepared as potential inhibitors of adenosine deaminase. The adenine derivatives were reversible inhibitors of adenosine deaminase, with potencies not strikingly different than the corresponding benzyl derivatives. However, 9-(*p*-bromoacetamidophenethyl)-adenine (XV) and 9-(3-*o*- and *p*-bromoacetamidophenylpropyl)-adenines (XXIX*a* and XXIX*b*) were incapable of causing irreversible inhibition of adenosine deaminase, whereas the corresponding *ortho*- and *para*-benzyl derivatives (I and II) and 9-(*m*-bromoacetamidophenethyl)adenine (IV) caused rapid irreversible inactivation of this enzyme. These experiments demonstrated the selectivity of irreversible inhibition that can be obtained by site-directed irreversible enzyme inhibitors.

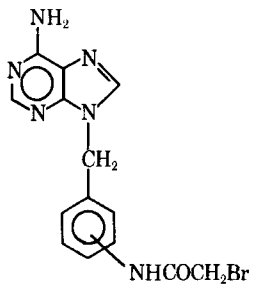
Keyphrases □ Adenosine deaminase inhibitors, irreversible—9-(substituted aralkyl)-6-substituted purines □ Purines, 9-(substituted aralkyl)-6-substituted—syntheses, adenosine deaminase irreversible inhibitors □ Enzymes, irreversible site-directed inhibitors—9-(substituted aralkyl)-6-substituted purines

Previous studies on the irreversible inactivation of adenosine deaminase established that the *para*- and *ortho*-isomers of 9-(bromoacetamidobenzyl)adenines (I and II) were excellent irreversible inhibitors, whereas the corresponding *meta*-isomer (III) was a poor irreversible inhibitor (1–3). In contrast to III, the *meta*-isomer of 9-(bromoacetamidophenethyl)adenine (IV) was capable of causing rapid, irreversible inactivation of adenosine deaminase (4). With a view toward further exploration of the hydrophobic region and the irreversible inhibition of adenosine deaminase, a series of 9-(*para*-substituted phenethyl)-6-substituted purines and 9-(*ortho*- and *para*-substituted phenylpropyl)-6-substituted purines was synthesized and evaluated.

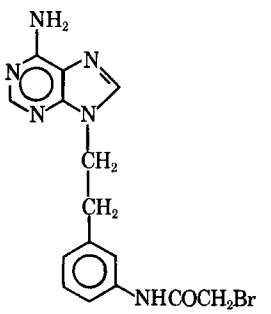
CHEMISTRY

The synthesis of these compounds was accomplished by a modification of procedures previously employed (1–6) (Schemes I and II).

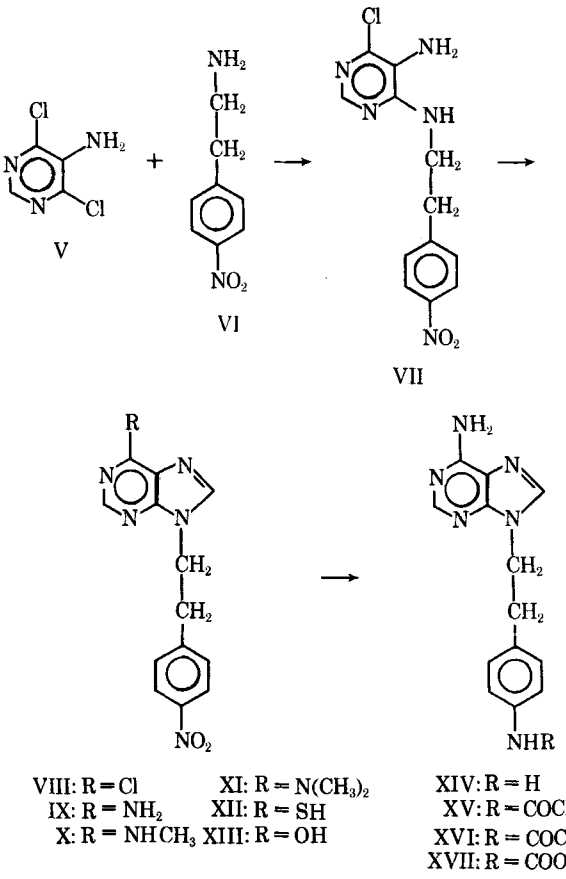
Condensation of 5-amino-4,6-dichloropyrimidine (V) with *p*-nitrophenethylamine (VI) gave the appropriately substituted pyrimidine (VII) which, upon treatment with triethyl orthoformate



I = *para*
II = *ortho*
III = *meta*



IV



VIII: R = Cl
IX: R = NH_2
X: R = $NHCH_3$
XI: R = $N(CH_3)_2$
XII: R = SH
XIII: R = OH
XIV: R = H
XV: R = $COCH_2Br$
XVI: R = $COCH_3$
XVII: R = $COOC_6H_5$

Scheme I

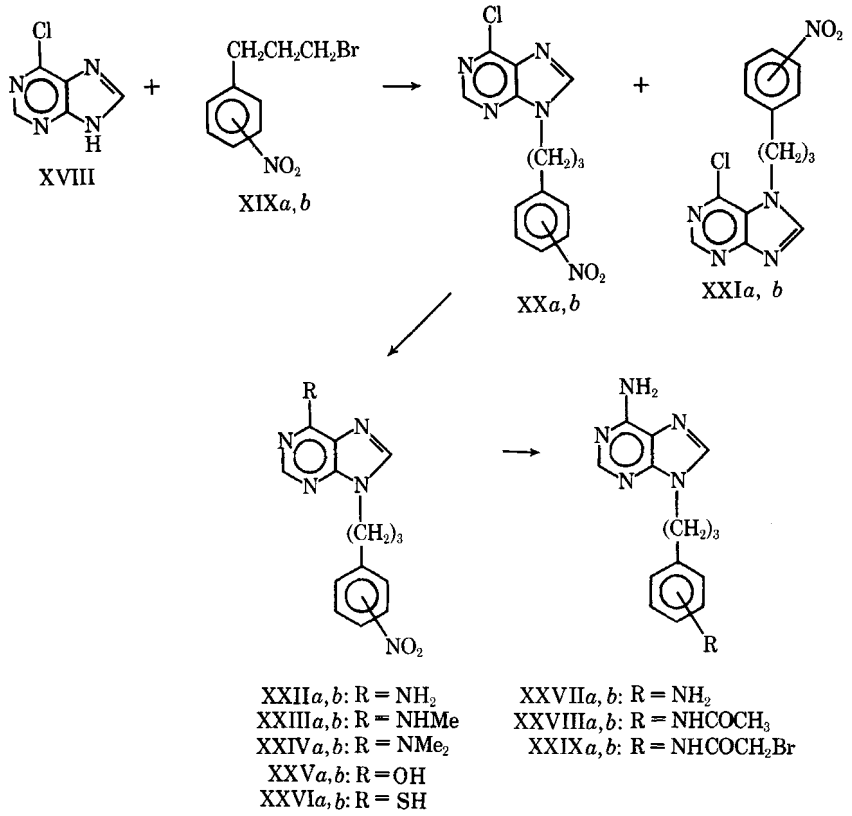
and ethanesulfonic acid, gave 9-(*p*-nitrophenethyl)-6-chloropurine (VIII). Treatment of the 6-chloro derivative (VIII) with ammonia, methylamine, dimethylamine, thiourea, and aqueous hydrochloric acid gave the corresponding 6-substituted derivatives (IX–XIII). Catalytic hydrogenation of IX gave 9-(*p*-aminophenethyl)adenine (XIV) which, upon reaction with bromoacetic anhydride, acetic anhydride, and phenyl chloroformate, gave the *para*-*N*-bromoacetamido, acetamido, and phenoxycarbonyl derivatives (XV, XVI, and XVII).

The 9-(*o*- and *p*-substituted-3-phenylpropyl)-6-substituted purines were prepared by condensation of the appropriately substituted 3-phenylpropyl bromide (XIX*a* and XIX*b*) with 6-chloropurine (XVIII). The resultant mixture of 9- and 7-isomers (XX and XXI) was separated, and XX was converted into XXII through XXVI by reaction with the appropriate nucleophile. Finally, the nitro group of XXII was reduced with hydrogen using a palladium catalyst, and the amino group was acetylated and bromoacetylated to give XXVIII and XXIX.

By a similar procedure, several 6-substituted-9-(3-phenylpropyl)-purines were prepared.

RESULTS AND DISCUSSION

When these compounds were tested as reversible inhibitors of adenosine deaminase, it was found that compounds with an amino group at the 6-position of the purine nucleus caused reversible



XVIII

XIX*a*, *b*

XX*a*, *b*

XXI*a*, *b*

XXII*a*, *b*: R = NH_2
XXIII*a*, *b*: R = NHMe
XXIV*a*, *b*: R = NMe_2
XXV*a*, *b*: R = OH
XXVI*a*, *b*: R = SH

XXVII*a*, *b*: R = NH_2
XXVIII*a*, *b*: R = $NHCOCH_3$
XXIX*a*, *b*: R = $NHCOCH_2Br$

a series = *ortho* substituted
b series = *para* substituted

Scheme II

inhibition of the enzyme (Table I), whereas compounds (VIII and X–XIII) with substituents at the 6-position other than an amino group were either noninhibitory or too weakly inhibitory to allow an accurate evaluation of $[I/S]_{0.5}$.

The lack of irreversible inhibition by the *p*-phenoxycarbonylamino derivative (XVII) is not surprising since irreversible inhibition with this type of compound had not been observed previously (1–4). However, even though 9-(*p*- and *o*-bromoacetamidobenzyl)adenines (I and II) and 9-(*m*-bromoacetamidophenethyl)adenine (IV) are capable of causing rapid irreversible inactivation of adenosine deaminase (1, 4), none of the structurally similar bromoacetamido derivatives (XV, XXIX*a*, and XXIX*b*) is capable of causing irreversible inhibition. Thus, the lack of irreversible enzyme inactivation by XV, XXIX*a*, and XXIX*b* represents another example of the selectivity in active-site-directed irreversible enzyme inhibitors (7).

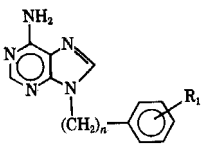
EXPERIMENTAL[1]

5-Amino-6-chloro-4-(*p*-nitrophenethylamino)pyrimidine (VII)—A solution of 405 mg. (2.00 mmoles) of *p*-nitrophenethylamine hydrochloride, 328 mg. (2.00 mmoles) of V, and 1.21 g. (12.0 mmoles) of triethylamine in 10 ml. of *n*-propyl alcohol was heated under reflux for 6 hr. The solvent was evaporated *in vacuo;* recrystallization of the residue from methanol and water gave the analytical sample; yield: 321 mg. (54.8%), m.p. 205–207°.

Anal.—Calc. for $C_{12}H_{12}ClN_5O_2$: C, 49.15; H, 4.12; Cl, 12.05; N, 23.80. Found: C, 49.22; H, 4.12; Cl, 12.21; N, 24.04.

6-Chloro-9-(*p*-nitrophenethyl)purine (VIII)—To a mixture of 150 mg. (0.510 mmole) of VII in 2 ml. of triethyl orthoformate w s added 13.3 mg. (0.121 mmole) of ethanesulfonic acid, and the mixture was stirred at 50° for 1.5 hr. The crystalline material which precipitated was collected by filtration; after recrystallization from

Table I—Inhibition of Adenosine Deaminase by:



Compound[a]	*n*	R_1	$[I/S]_{0.5}$[b]
IX	2	NO_2	6.9 ± 0.4[c]
XIV	2	NH_2	4.1 ± 0.1
XV	2	$NHCOCH_2Br$	1.9 ± 0.2
XVI	2	$NHCOCH_3$	2.5 ± 0.2
XVII	2	$NHCOOC_6H_5$	1.4 ± 0.1
XXX	2	H	5.0 ± 0.6
XXII*a*	3	NO_2	0.94 ± 0.06
XXVII*a*	3	NH_2	4.6 ± 0.2
XXIX*a*	3	$NHCOCH_2Br$	6.3 ± 1.3
XXII*b*	3	NO_2	5.3 ± 0.1
XXVII*b*	3	NH_2	1.4 ± 0.1
XXVIII*b*	3	$NHCOCH_3$	0.59 ± 0.1
XXIX*b*	3	$NHCOCH_2Br$	0.45 ± 0.02
XXXI	3	H	0.78 ± 0.02

[a] *a* series = *ortho*-substituted; *b* series = *para*-substituted. [b] The concentration of adenosine in all experiments was 0.066 m*M*. In no experiment of reversible inhibition did the concentration of inhibitor exceed 0.12 m*M*. The inhibition index $[I/S]_{0.5}$ is the ratio of the millimolar concentration of the inhibitor giving 50% inhibition to the millimolar concentration of the substrate. The inhibitor solutions were prepared in 0.05 *M* phosphate buffer (pH 7.6) containing 10% dimethyl sulfoxide. [c] Average of two determinations of the I_{50} obtained from a plot of V_0/V_i *versus* [I] with five different inhibitor concentrations. V_0 = initial velocity of the uninhibited enzymatic reaction, and V_i = initial velocity of the inhibited enzymatic reaction at various inhibitor concentrations.

[1] The melting points, unless noted otherwise, were taken in open capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had IR and UV spectra compatible with their assigned structures and moved as a single spot on TLC on Brinkmann silica gel. The analyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn.

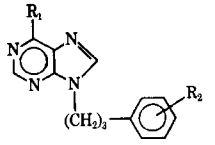


Table II—Physical Constants and Analytical Data of:

Compound	R_1	R_2	Method	Melting Point	Yield, %	Recrystallization Solvent	Formula	Analysis, % Calc.	Found
XXII*a*	NH_2	NO_2	A	216–217°	76	MeOH	$C_{14}H_{14}N_6O_2$	C, 56.37 H, 4.73 N, 28.17	C, 56.19 H, 4.90 N, 28.21
XXIII*a*	NHMe	NO_2	B	159–160°	49	2-PrOH	$C_{15}H_{16}N_6O_2$	C, 57.68 H, 5.16 N, 26.91	C, 57.78 H, 5.24 N, 27.31
XXIV*a*	NMe_2	NO_2	C	119–120°	85	H_2O	$C_{16}H_{18}N_6O_2$	C, 58.87 H, 5.55 N, 25.74	C, 59.02 H, 5.55 N, 25.87
XXV*a*	OH	NO_2	E	203–204°	57	H_2O–EtOH	$C_{14}H_{13}N_5O_3$	C, 56.18 H, 4.38 N, 23.40	C, 56.25 H, 4.54 N, 23.56
XXVI*a*	SH	NO_2	D	269–270°	88	Precipitate	$C_{14}H_{13}N_5O_2S$	C, 53.28 H, 4.15 N, 22.21 S, 10.02	C, 53.27 H, 4.13 N, 22.12 S, 10.26
XXVII*a*	NH_2	NH_2	F	165–166°	69	H_2O	$C_{14}H_{16}N_6$	C, 62.67 H, 6.01 N, 31.32	C, 62.87 H, 5.88 N, 31.14
XXVIII*a*	NH_2	$NHCOCH_3$	H	231–232°	48	Tetrahydrofuran	$C_{16}H_{18}N_6O$	C, 61.93 H, 5.85 N, 27.08	C, 62.05 H, 5.77 N, 27.24
XXIX*a*	NH_2	$NHCOCH_2Br$	G	230°[a]	58	MeOH–H_2O	$C_{16}H_{17}BrN_6O$	C, 49.36 H, 4.40 N, 21.56 Br, 20.52	C, 49.12 H, 4.50 N, 21.39 Br, 20.42
XXII*b*	NH_2	NO_2	A	223–224°	78	MeOH	$C_{14}H_{14}N_6O_2$	C, 56.37 H, 4.73 N, 28.17	C, 56.19 H, 4.73 N, 28.37
XXIII*b*	NHMe	NO_2	B	175–176°	82	2-PrOH	$C_{15}H_{16}N_6O_2$	C, 57.68 H, 5.16 N, 26.91	C, 57.54 H, 5.42 N, 26.70
XXIV*b*	NMe_2	NO_2	C	168–169°	76	H_2O–MeOH	$C_{16}H_{18}N_6O_2$	C, 58.87 H, 5.55 N, 25.74	C, 58.89 H, 5.73 N, 25.59
XXV*b*	OH	NO_2	E	249–250°	72	H_2O–MeOH	$C_{14}H_{13}N_5O_3$	C, 56.18 H, 4.38 N, 23.40	C, 56.03 H, 4.45 N, 23.19
XXVI*b*	SH	NO_2	D	281–288°	91	Precipitate	$C_{14}H_{13}N_5O_2S$	C, 52.38 H, 4.15 N, 22.21 S, 10.02	C, 53.40 H, 4.16 N, 22.23 S, 9.96
XXVII*b*	NH_2	NH_2	F	235–236°	75	H_2O	$C_{14}H_{16}N_6$	C, 62.67 H, 6.01 N, 31.32	C, 62.71 H, 6.24 N, 31.22
XXVIII*b*	NH_2	$NHCOCH_3$	H	220–221°	67	H_2O[b]	$C_{16}H_{18}N_6O$	C, 61.93 H, 5.85 N, 27.08	C, 61.84 H, 5.65 N, 27.38
XXIX*b*	NH_2	$NHCOCH_2Br$	G	240–275°[a]	61	MeOH–H_2O	$C_{16}H_{17}BrN_6O$	C, 49.36 H, 4.40 N, 21.56 Br, 20.52	C, 49.32 H, 4.60 N, 21.48 Br, 20.44
XXXI[c]	NH_2	H	A	185–186°	65	MeOH	$C_{14}H_{15}N_5$	C, 66.40 H, 5.93 N, 27.67	C, 66.47 H, 6.08 N, 27.74
XXXIV	NHMe[d]	H	B	207–208°	54	2-PrOH	$C_{15}H_{18}ClN_5$	C, 59.29 H, 5.97 N, 23.05 Cl, 11.67	C, 59.21 H, 6.09 N, 22.88 Cl, 11.75
XXXV	NMe_2[d]	H	C	220–221°	81	2-PrOH	$C_{16}H_{20}ClN_5$	C, 60.46 H, 6.34 N, 22.04 Cl, 11.15	C, 60.30 H, 6.34 N, 22.04 Cl, 11.09
XXXVI	OH	H	E	256–257°	56	2-PrOH	$C_{14}H_{14}N_4O$	C, 66.12 H, 5.51 N, 22.05	C, 65.97 H, 5.48 N, 21.90
XXXVII	SH	H	D	318–319°	88	Precipitate	$C_{14}H_{14}N_4S$	C, 62.18 H, 5.21 N, 20.72 S, 11.86	C, 62.14 H, 5.19 N, 20.95 S, 11.96

[a] No well-defined melting point, decomposition temperature given. [b] Recrystallized from H_2O which was adjusted to pH ≃8 with sodium bicarbonate. [c] B. R. Baker and E. H. Erickson prepared XXXI by a different procedure and reported m.p. 184–186°[*J. Pharm. Sci.*, **56**, 1075(1967)]. [d] Isolated as the hydrochloride salt.

methanol and then from 2-propanol, it gave the analytical sample; yield: 98 mg. (63%), m.p. 167–168°.

Anal.—Calc. for $C_{13}H_{10}ClN_5O_2$: C, 51.41; H, 3.32; Cl, 11.67; N, 23.06. Found: C, 51.65; H, 3.51; Cl, 11.46; N, 22.89.

Method A: 9-(*p*-Nitrophenethyl)adenine (IX)—A mixture of 1.00 g. (3.29 mmoles) of VIII in 40 ml. of methanolic ammonia (20%) was heated at 95° in a stainless steel bomb for 24 hr. After the reaction mixture was cooled, the insoluble material was collected by filtration. Two recrystallizations of the crude material from methanol gave the analytical sample; yield: 615 mg. (56.9%), m.p. 260–261°.

Anal.—Calc. for $C_{13}H_{12}N_6O_2$: C, 54.92; H, 4.26; N, 29.57. Found: C, 55.16; H, 4.33; N, 29.58.

Method B: 6-Methylamino-9-*p*-nitrophenethylpurine (X)—A mixture of 304 mg. (1.00 mmole) of VIII in 10 ml. of ethanol and 10 ml. of aqueous methylamine (40%) was heated in a stainless steel bomb at 90° for 22 hr. The volatile material was evaporated *in vacuo*, and the crystalline residue was recrystallized from 2-propanol. Another recrystallization from methanol gave the analytical sample; yield: 153 mg. (51.4%), m.p. 220–222°.

Anal.—Calc. for $C_{14}H_{14}N_6O_2$: C, 56.37; H, 4.73; N, 28.18. Found: C, 56.11; H, 4.80; N, 28.45.

Method C: 6-Dimethylamino-9-*p*-nitrophenethylpurine (XI)—A mixture of 304 mg. (1.00 mmole) of VIII in 10 ml. of ethanol and 10 ml. of aqueous dimethylamine (25%) was heated in a stainless steel bomb at 90° for 22 hr. The volatile material was evaporated *in vacuo*, leaving a yellow crystalline material which was recrystallized with 2-propanol. Another recrystallization from methanol gave the analytical sample; yield: 215 mg. (69.0%), m.p. 169–170°.

Anal.—Calc. for $C_{15}H_{16}N_6O_2$: C, 57.68; H, 5.16; N, 26.92. Found: C, 57.42; H, 5.19; N, 26.76.

Method D: 6-Mercapto-9-*p*-nitrophenethylpurine (XII)—A solution of 304 mg. (1.00 mmole) of VIII and 84 mg. (1.10 mmoles) of thiourea in 15 ml. of *n*-propyl alcohol was heated under reflux for 1 hr. and then cooled in an ice bath. The precipitate which formed was collected by filtration. Recrystallization of the solid material from methanol gave the analytical sample; yield: 179 mg. (59.7%), m.p. 300–301° dec.

Anal.—Calc. for $C_{13}H_{11}N_5O_2S$: C, 51.81; H, 3.68; N, 23.25; S, 10.64. Found: C, 51.69; H, 3.80; N, 23.06; S, 10.65.

Method E: 6-Hydroxy-9-*p*-nitrophenethylpurine (XIII)—A solution of 304 mg. (1.00 mmole) of VIII in 25 ml. of 3 *N* hydrochloric acid was heated under reflux for 1.5 hr. and then evaporated *in vacuo*. Recrystallization of the solid residue from methanol gave the analytical sample; yield: 200 mg. (70.3%), m.p. 294–295° dec.

Anal.—Calc. for $C_{13}H_{11}N_5O_3$: C, 54.73; H, 3.89; N, 24.55. Found: C, 54.62; H, 3.96; N, 24.51.

Method F: 9-(*p*-Aminophenethyl)adenine (XIV)—A solution of 988 mg. (3.48 mmoles) of IX in 150 ml. of glacial acetic acid was added to 400 mg. of 5% palladium on carbon, and the mixture was hydrogenated at room temperature in a Parr hydrogenator at an initial pressure of 4 kg./cm.[2]. After 1 hr., the catalyst was removed by filtration through a diatomaceous earth[2] pad, and the solvent was concentrated to 50 ml. *in vacuo*. The solution was cooled in an ice bath, and the pH was adjusted to 13 with 1 *N* sodium hydroxide. The insoluble material was collected by filtration; yield: 714 mg. (80.8%), m.p. 219–221°. The crude product recrystallized from water; yield: 548 mg. (62.1%), m.p. 221.5–223°.

Anal.—Calc. for $C_{13}H_{14}N_6$: C, 61.39; H, 5.55; N, 33.05. Found: C, 61.28; H, 5.56; N, 32.83.

Method G: 9-(*p*-Bromoacetamidophenethyl)adenine (XV)—To a cold solution of 153 mg. (0.60 mmole) of XIV in 5 ml. of tetrahydrofuran and 0.6 ml. of 10% aqueous acetic acid was added dropwise 271 mg. (1.05 mmoles) of bromoacetic anhydride in 1 ml. of tetrahydrofuran. After 1 hr. of stirring at 0°, the insoluble material was collected by filtration and dissolved in hot methanol to which sodium bicarbonate solution (2%) was added until the solution was basic. Upon cooling in an ice bath, a precipitate formed which, after recrystallization from methanol, gave the analytical sample as the hemisolvate; yield: 44 mg. (20%), m.p. 230–240° dec. with evolution of gas.

Anal.—Calc. for $C_{15}H_{15}BrN_6O \cdot 1/2CH_4O$: C, 47.58; H, 4.38; Br, 20.43; N, 21.48. Found: C, 47.72; H, 4.25; Br, 19.99; N, 21.14.

[2] Celite.

Method H: 9-(*p*-Acetamidophenethyl)adenine (XVI)—To a cold solution of 152 mg. (0.60 mmole) of XIV in 0.6 ml. of 10% aqueous acetic acid and 5 ml. of tetrahydrofuran was added dropwise 602 mg. (5.90 mmoles) of acetic anhydride in 4 ml. of tetrahydrofuran. The mixture was stirred in an ice bath for 1 hr. and the insoluble material which precipitated was collected by filtration and dried *in vacuo* at 100°; yield: 105 mg. (59.4%), m.p. 237–238°. Recrystallization of the crude product from chloroform gave the analytical sample; yield: 73.4 mg. (41.4%), m.p. 239–240°.

Anal.—Calc. for $C_{15}H_{16}N_6O$: C, 60.79; H, 5.44; N, 28.26. Found: C, 60.65; H, 5.39; N, 28.19.

9-(*p*-Phenoxycarbonylaminophenethyl)adenine (XVII)—To a cold solution of 152 mg. (0.60 mmole) of XIV and 60.0 mg. (0.60 mmole) of triethylamine in 150 ml. of *p*-dioxane was added slowly 366 mg. (2.35 mmoles) of phenyl chloroformate, and the mixture was stirred at room temperature for 26 hr. The insoluble material was collected by filtration and dried *in vacuo* at 100° for 48 hr. Recrystallization of the residual solid from methanol and water gave the analytical sample; yield: 99.5 mg. (44.4%), m.p. 163° softened with decomposition, solidified, melted 310–312° with decomposition and evolution of gas.

Anal.—Calc. for $C_{20}H_{18}N_6O_2$: C, 64.20; H, 4.85; N, 22.44. Found: C, 63.92; H, 4.96; N, 22.32.

Method I: 6-Chloro-9- and 7-*p*-nitrophenylpropylpurines[3] (XX*b* and XXI*b*)—6-Chloropurine (2.90 g., 0.019 mole) was dissolved in dimethylformamide (50 ml.), and 3-*p*-nitrophenylpropyl bromide (6.1 g., 0.025 mole) and triethylamine (2.52 g., 0.025 mole) were added to the solution. The yellow reaction mixture was left at room temperature for 24 hr. and then poured into a cooled mixture of 2-propanol–water (1:2) (300 ml.) and stirred vigorously. The insoluble white material was filtered off (4.7 g., 84%). This material was dissolved in chloroform (50 ml.) and introduced on a column (29.5 mm. i.d.) of silica gel (140 g. of 100–200 mesh) in chloroform. The column was eluted with chloroform, and 60–125-ml. fractions were collected. Then a 1.5% solution of methanol in $CHCl_3$ was used to elute the column, and 11–125-ml. fractions were collected. Eluent fractions 18–67 were combined and evaporated *in vacuo*, which gave 3.2 g. (57%) of XX*b*. Recrystallization from 2-propanol gave pure XX*b* (2.57 g., 50%), m.p. 138–139°.

Anal.—Calc. for $C_{14}H_{12}ClN_5O_2$: C, 52.92; H, 3.80; Cl, 11.16; N, 22.04. Found: C, 52.90; H, 3.82; Cl, 10.99; N, 21.91.

Eluent fractions 69–71 were combined and evaporated *in vacuo*, which gave the crude 7-isomer. This material was recrystallized from methanol and gave 70 mg. (1.5%) of pure XXI*b*, m.p. 152–153°.

Anal.—Calc. for $C_{14}H_{12}ClN_5O_2$: C, 52.92; H, 3.80; Cl, 11.16; N, 22.04. Found: C, 53.02; H, 3.90; Cl, 11.00; N, 21.97.

6-Chloro-9- and 7-(3-*o*-nitrophenylpropyl)purines (XX*a* and XXI*a*)—These were prepared from 6-chloropurine and 3-*o*-nitrophenylpropyl bromide by Method I; yield of XX*a*: 21%, m.p. 141–142°/2-propanol.

Anal.—Calc. for $C_{14}H_{12}ClN_5O_2$: C, 52.92; H, 3.80; Cl, 11.16; N, 22.04. Found: C, 53.05; H, 4.04; Cl, 11.04; N, 21.94.

Yield of XXI*a*: 11%, m.p. 142–143°/2-propanol.

Anal.—Found: C, 53.06; H, 3.69; Cl, 11.40; N, 22.11.

6-Chloro-9- and 7-(3-phenylpropyl)purines (XXXII and XXXIII)—These were prepared by Method I from 6-chloropurine and 3-phenylpropyl bromide; yield of XXXII: 49%, m.p. 62–63°/hexane.

Anal.—Calc. for $C_{14}H_{13}ClN_4$: C, 61.65; H, 4.80; Cl, 13.00; N, 20.55. Found: C, 61.70; H, 4.95; Cl, 12.91; N, 20.41.

Yield of XXXIII: 4%, m.p. 68–69°/hexane–methanol. Found: C, 61.80; H, 4.93; Cl, 13.17; N, 20.75.

ENZYME ASSAY

Adenosine deaminase (Type I, calf intestinal mucosa) was purchased[4]. The assay procedure for the study of reversible inhibitors was previously described (3) and is a modification of the procedure of Kaplan (8) based on the method of Kalckar (9).

[3] The structures of the isomers were assigned by analogy to similar alkylations. See, for example, *Reference 2*.

[4] Sigma Chemical Co.

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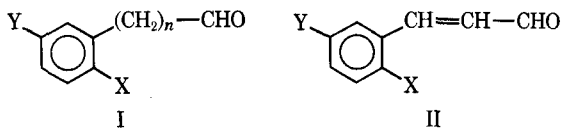
Substituted Aralkyl Aldehydes: Preparation and Antitumor Evaluation

JOHN H. BILLMAN and JOHN A. TONNIS*

Abstract □ A series of substituted aralkyl aldehydes—substituted phenylacetaldehydes, hydrocinnamaldehydes, and cinnamaldehydes—was prepared and tested for antitumor activity. The substituted phenylacetaldehydes were prepared from the corresponding benzaldehydes *via* the Darzen glycidic ester synthesis, followed by hydrolysis and decarboxylation. The dihydrocinnamaldehydes were prepared by the lead tetraacetate oxidation of the corresponding alcohols. The cinnamaldehydes were prepared from the substituted benzaldehydes by reaction with ethyl vinyl ether. All intermediates in the preparation of the aralkyl aldehydes were also screened for antitumor activity.

Keyphrases □ Antitumor activity evaluation—substituted aralkyl aldehydes □ Phenylacetaldehydes, substituted—synthesis, antitumor activity evaluation □ Hydrocinnamaldehydes, substituted—synthesis, antitumor activity evaluation □ Cinnamaldehydes, substituted—synthesis, antitumor activity evaluation

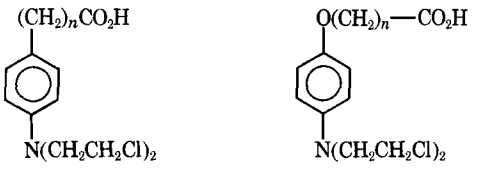
A considerable number of aliphatic and aromatic aldehydes and their derivatives were shown to possess appreciable antitumor activity (1–6). Several of these aldehydes and their derivatives were also used in the clinic as antitumor agents (7–9). All of the aldehydes that have shown activity have been alkyl or aromatic aldehydes. No substituted aralkyl aldehydes of general types I and II have been tested as antitumor agents and, indeed, few have even been synthesized.



I II

n = 1–2
X = OH, OCH_3, $-OCOCH_3$
Y = electron-donating or withdrawing groups

Various studies showed that the length of the alkyl chain in aralkyl compounds similar to I and II plays an important role in the antitumor activity. Some



III IV

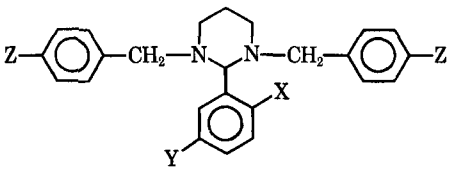
n = 0–3

investigators (10–12) prepared a series of *N*,*N*-bis(2-chloroethyl)phenylalkanoic acids (III) and *N*,*N*-bis-(2-chloroethyl)phenoxyalkanoic acids (IV).

In type III compounds, they found that when n is 0, the compound was only slightly active; as n increased the activity likewise increased rapidly and reached a maximum at n = 3. The compound of Structure III, when n is 3, is called chlorambucil and has been used clinically for treatment of chronic lymphocytic leukemia (13, 14).

Type IV compounds also show the same increase of activity as the alkyl chain length increases and reaches a maximum when n is 2.

This relationship of antitumor activity to alkyl chain length has been demonstrated on other compounds similar to type I and II compounds.



V

X = OH, OCH_3
Y = electron-donating or withdrawing groups
Z = OCH_3, $-N(CH_3)_2$, $-N(CH_2CH_2Cl)_2$