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Irreversible Enzyme Inhibitors. XCI. Candidate Active-site Directed Irreversible Inhibitors of Thymidylate Synthetase. II. 2-Amino-6-methyl-5-(*p*-tolylsulfonamidopropyl)-4-pyrimidinols with *N*-Substituents on the Sulfonamido Moiety (1,2).

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Three derivatives of 2-amino-6-methyl-5-(*p*-tolylsulfonamidopropyl)-4-pyrimidinol (I) with *N*-substituents on the sulfonamide group, namely bromoacetamidopropyl (XVI), *m*-bromoacetamidobenzyl (XXIVa), and *p*-bromoacetamidobenzyl (XXIVb), were synthesized as candidate active-site-directed irreversible inhibitors of thymidylate synthetase. The bromoacetamidopropyl derivative, (XVI), the *p*-bromoacetamidobenzyl derivative (XXIVb), and iodoacetamide showed irreversible inhibition of thymidylate synthetase, but XXIVa did not. Since iodoacetamide did inactivate the enzyme, but XXIVa did not, it cannot be ascertained whether XXIVb and XVI inactivate the enzyme by a random bimolecular mechanism or by the active-site-directed mechanism without evaluation of additional candidate inhibitors.

Two synthetic routes were employed. The key intermediates for the bromoacetamidobenzyl sulfonamides (XXIV) were the corresponding nitrobenzyl sulfonamides (XXI); the latter were best prepared by reductive alkylation of 2-amino-5-aminopropyl-6-methyl-4-pyrimidinol (XXV) with a nitrobenzaldehyde followed by tosylation. The key intermediate for XVI was a toluenesulfonamide with a carbobenzoxyaminopropyl substituent on the nitrogen (XIV); the latter was synthesized via *N*-carbobenzoxy-*N'*-tosyl-1,3-diaminopropane (XI).

Both 2-amino-6-methyl-5-(*p*-tolylsulfonamidopropyl)-4-pyrimidinol (I) (3) and its *N*-butyl derivative (4) were relatively good inhibitors of thymidylate synthetase, showing 50% inhibition at a ratio of 11 and 17, respectively, of inhibitor to 5,10-methylene-*l*-tetrahydrofolate. In order to convert I to an active-site-directed irreversible inhibitor (5,6) of thymidylate synthetase, a leaving group could be placed either on the benzene ring of I or on the R substituent of I; the former types were explored in the previous paper (2). The synthesis and enzymic evaluation of irreversible inhibitors with the leaving group on the R moiety are the subjects of this paper.

Two routes have been developed for compounds of type II. The tosyl group can be introduced at the first step and was used as a blocking group at that time (7); alternately, the R group of I could be introduced by reductive alkylation of 2-amino-4-hydroxy-6-methylpyrimidinyl-5-propionaldehyde with an amine (4,8-10) followed by selective tosylation (4) of the resultant amine. A simple route to the *N*-benzylsulfonamide (IX) would be selective benzylation of I. When I was treated with α-chloro-*m*-nitrotoluene in dimethyl sulfoxide in the presence of potassium carbonate, the sole product resulted from N_3-alkylation on the pyrimidine ring, as indicated by the characteristic ultraviolet spectra (11,12); with sodium hydride in dimethyl sulfoxide, the minor product was a result of N_3-alkylation and a major amount of N_3, sulfonamide bis-alkylation product was obtained.

The sequence starting with *N*-benzyl benzenesulfonamide (III) was investigated. The desired 4-pyrimidinol (IX) was obtained in 25-30% overall yield via IV and V. This sequence was also satisfactory for the *N*-aminopropyl sulfonamide (XV). The first key intermediate for synthesis of XV was a 1,3-propanediamine blocked by a carbobenzoxy group on one nitrogen and a tosyl group on the other (XI); XI was smoothly prepared from 3-bromopropylamine (VI) via VII and VIII. Alternate preparation of XI by monoacylation of 1,3-propanediamine with carbobenzoxy chloride gave XI in yields of 10% or less by the controlled *p*H method of Moore *et al.* (13). The remainder of the sequence to XIV proceeded by the standard method via XII and XIII. The carbo-

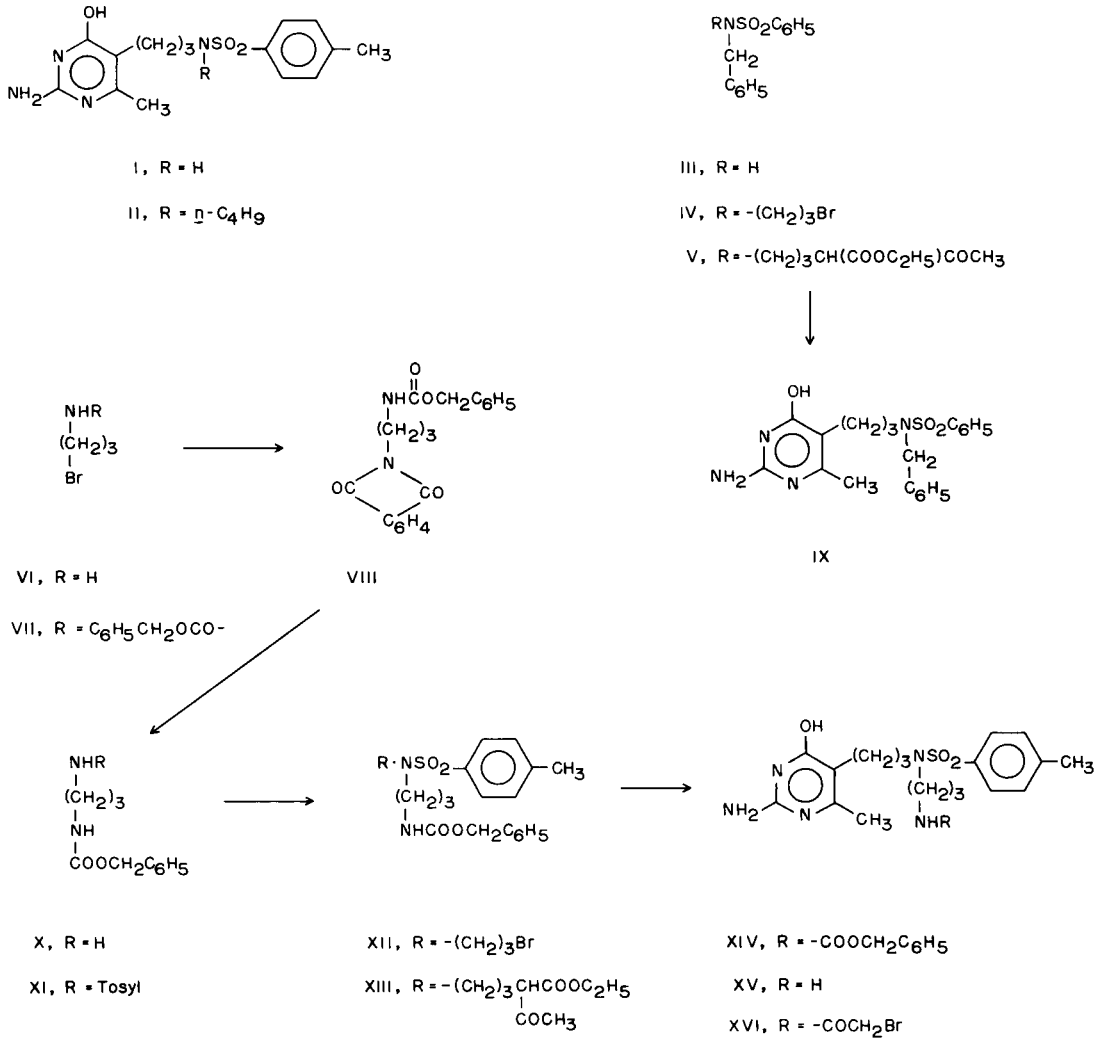
benzoxy group of XIV was removed with anhydrous hydrogen bromide in acetic acid (14). The resultant aminopropyl sulfonamide (XV) was isolated as its crystalline dipicrate in 15-20% overall yield from XI; the dipicrate could be converted to XV dihydrochloride in near quantitative yield. Selective bromoacetylation of the aliphatic amino group of XV to give XVI was achieved with *p*-nitrophenyl bromoacetate in aqueous acetone (12).

The second type of candidate irreversible inhibitor for thymidylate synthetase was an *N*-benzylsulfonamide, XXIV, bearing a bromoacetamido group on the *m*- or *p*-position. The key intermediate was the corresponding nitrobenzyl sulfonamide, XXI. In order to use the synthesis that introduces the tosyl group first (7), a suitable method for the *p*-nitrobenzylamines (XVIII) was developed which was considered more practical than the Sommelet reaction (15) or aqueous ammonia (16) to convert the proper nitrobenzyl chloride to XVIII. Reaction of the nitrobenzyl chlorides with potassium phthalimide in *N*,*N*-dimethylformamide at ambient temperature (17) afforded XVII; cleavage with hydrazine (18) gave excellent overall yields of XVIIIa and XVIIIb. Tosylation of XVIII with tosyl chloride in chloroform with triethylamine as an acid-acceptor afforded over 90% yields of XIX.

The standard sequence from the sulfonamide (XIXa) to XXIa via XXa proceeded in 10-15% overall yield for the three steps. The sequence was even less successful with the *p*-nitrobenzyl isomer (XIXb) and the desired pyrimidine (XXIb) could not be isolated; as shown in an alternate synthesis of XXIb, the product was amorphous and therefore difficult to purify.

An alternate method for XXI was then investigated which was superior to the route from the sulfonamide

CHART I



XIX. Condensation of the aminopropyl-4-pyrimidinol (XXV) with *m*-nitrobenzaldehyde gave an 81% yield of the anil (XXVIa) which was reduced with sodium borohydride to the substituted benzylamine (XXIIIa) in 62% yield. Reaction with tosyl chloride in chloroform containing triethylamine afforded a 68% yield of XXIa that was identical with XXIa prepared via XIX. Similarly the *p*-isomer (XXIb) was synthesized from XXV in considerably better overall yield.

Hydrogenation of the nitro group of XXI to the amines XXII proceeded smoothly in ethanol containing excess hydrochloric acid in the presence of platinum oxide catalyst. Selective acylation of the amines (XXII) with bromoacetic anhydride in acetone at 0° (12), afforded the desired bromoacetamides (XXIV), which were isolated as their sulfate salts.

ENZYMATIC EVALUATION

The various *N*-substituted sulfonamides were first investigated as reversible inhibitors of thymidylate synthetase; the results are recorded in Table I. Substitution on the sulfonamide nitrogen results in a 19-fold spread in the concentration required for 50% inhibition; *n*-butyl (II) is the poorest and *m*-bromoacetamidobenzyl (XXIVa) being the best. In fact, XXIVa is the best 2-amino-4-pyrimidinol-type of inhibitor for thymidylate synthetase yet observed,

CHART II

a series, m-isomer

b series, p-isomer

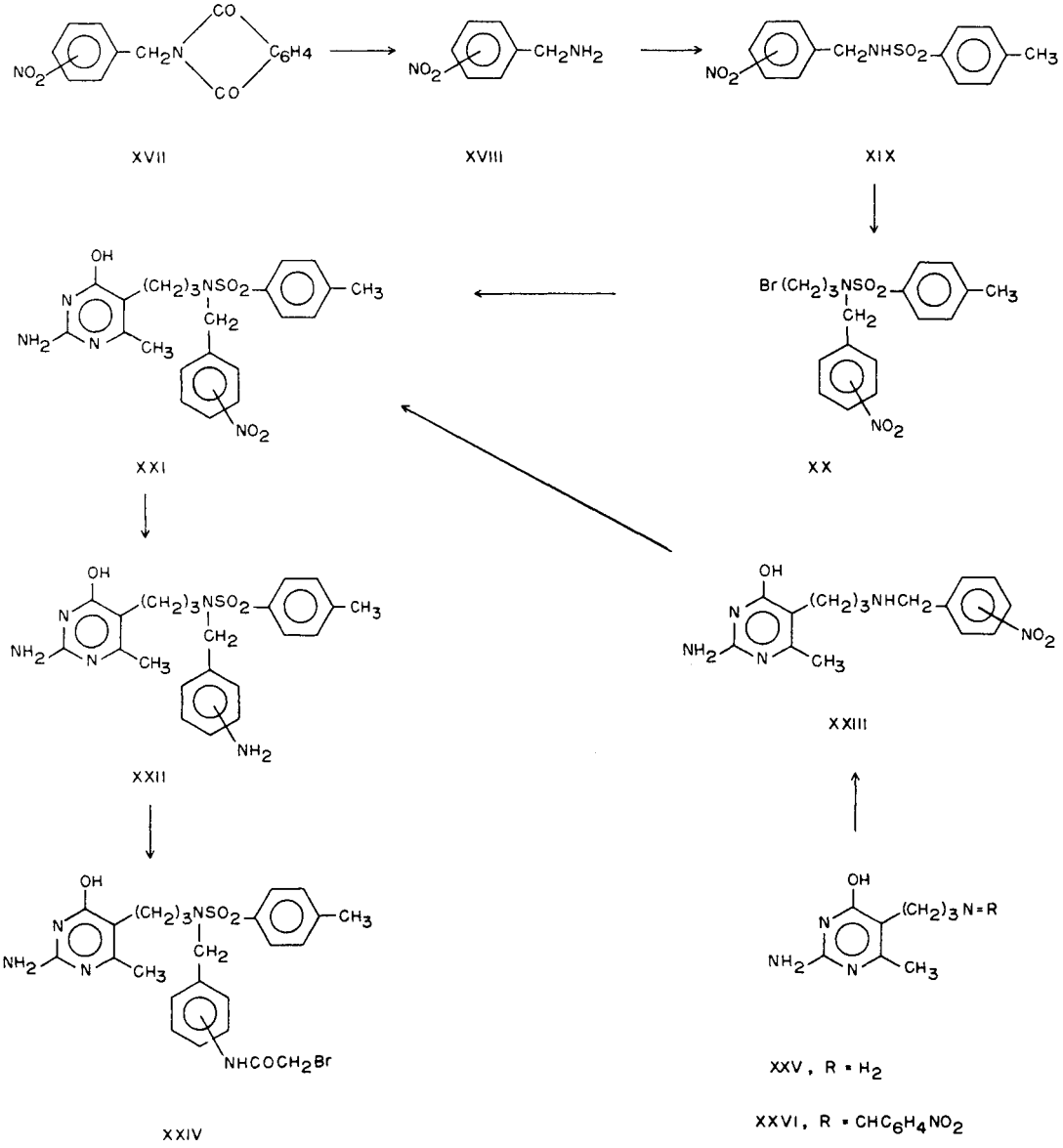
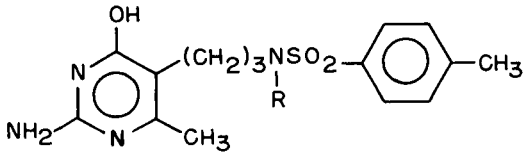


TABLE I

Reversible Inhibition of Thymidylate Synthetase By

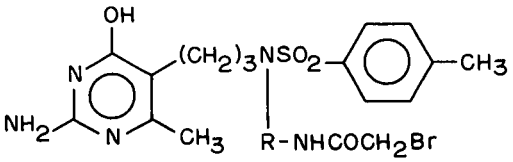


Compound	R	μM Conc. Inhibitor	% Inhibition (a)	Estimated μM Conc. for 50% Inhibition	Estimated $[I/S]_{0.5}$ (b)
I	H	150 (c)	50	150	5.8
II	n-C_4H_9	150 (d, e)	26	440	17
XV	$-(CH_2)_3NH_3^{\oplus}$	370	50	370	14
XVI	$-(CH_2)_3NHCOCH_2Br$	60 (d)	37	100	3.9
XXIa	$-CH_2C_6H_4NO_2$-m	100	50	100	3.9
XXIb	$-CH_2C_6H_4NO_2$-p	40 (d)	45	48	1.9
XXIIa	$-CH_2C_6H_4NH_2$-m	180	50	180	7.0
XXIIb	$-CH_2C_6H_4NH_2$-p	50 (d)	24	160	6.2
XXIVa	$-CH_2C_6H_4NHCOCH_2Br$-m	23	50	23	0.89
XXIVb	$-CH_2C_6H_4NHCOCH_2Br$-p	50 (d)	39	72	2.8

The technical assistance of Barbara Baine and Ann Jaqua with the assays in Tables I and II is acknowledged. (a) Thymidylate synthetase was a 45-90% ammonium sulfate fraction from *E. coli* B prepared (3) and assayed (19) as previously described with 80 μM 2'-deoxyuridylate and 51.4 μM 5,10-methylene-*dl*-tetrahydrofolate in 0.05 *M* Tris buffer (*p*H 7.4) containing 10 m*M* mercaptoethanol, 1 m*M* Versene, and 5% 2-methoxyethanol. (b) Ratio of the concentration of inhibitor to 25.7 μM 5,10-methylene-*l*-tetrahydrofolate giving 50% inhibition. (c) New data, previously $[I/S]_{0.5}$ = 11 was recorded. (d) Maximum solubility.

TABLE II

Irreversible Inhibition of Thymidylate Synthetase By



Compound	R	μM Conc. for 50% Reversible Inhibition	μM Conc.	Time (min.)	Percent Inactivation (a)
XVI	$-(CH_2)_3-$	100	100	60	10
XXIVa	m-$CH_2C_6H_4-$	23	23	60	0
XXIVb	p-$CH_2C_6H_4-$	72	50	60	27
XXIVb	p-$CH_2C_6H_4-$ (b)	72	50	60	30
Iodoacetamide	(b)	large ?	50	60	25

(a) For 37° incubation procedure see reference (2); the percent inactivation is corrected for thermal inactivation of a simultaneous enzyme control. (b) Simultaneous runs.

being complexed slightly better than the substrate. This 19-fold spread indicates that part of the R group on the sulfonamide can still be in contact with the enzyme surface. Since the *m*-bromoacetamidobenzyl group of XXIVa gives 8-fold better binding than the *m*-aminobenzyl group of XXIIa and 4-fold better binding than the *m*-nitrobenzyl group of XXIa, it is likely that the bromoacetamido function of XXIVa is complexing with the enzyme and unlikely that direct binding of the benzyl group is being influenced electronically. Additional studies on the mode of binding of these substituted benzyl groups might be rewarding, particularly to see if replacement of the *N*-benzyl group by 1-substituted-uracil-5-methylene groups would indicate that the benzyl group is in the area of the enzyme that complexes 2'-deoxyuridylate.

Irreversible inhibition studies are tabulated in Table II. When the *N*-bromoacetamidopropyl sulfonamide (XVI) was incubated at 100 μM ($[I/S]_{0.5}$ concentration) with thymidylate synthetase for 60 minutes at 37°, only 10% more inactivation than an enzyme control was observed. The *N*-(*m*-bromoacetamidobenzyl)sulfonamide (XXIVa) at 23 μM ($[I/S]_{0.5}$ concentration) showed no inactivation under the same conditions. In contrast, the *N*-(*p*-bromoacetamidobenzyl)sulfonamide (XXIVb) at 50 μM showed 27% inactivation. Unfortunately, iodoacetamide showed almost the same inactivation rate as XXIVb; this indicated that the irreversible inhibition was of the random bimolecular-type rather than the active-site-directed type proceeding through a neighboring group reaction within the enzyme-inhibitor complex. Even though the concentration of the *m*-bromoacetamido isomer (XXIVa) was less than XXIVb, XXIVa failed to show inactivation of the enzyme by the bimolecular mechanism; thus a bimolecular mechanism for inactivation of the enzyme by the *p*-bromoacetamido isomer is questionable, else XXIVa should have done likewise. It is therefore still possible that the *p*-isomer (XXIVb) inactivated the enzyme by the active-site-directed mechanism, but iodoacetamide inactivates by the bimolecular mechanism (6). A similar situation was observed earlier with lactic dehydrogenase; 4-(iodoacetamido)salicylic acid inactivated lactic dehydrogenase by the active-site-directed mechanism, iodoacetamide inactivated somewhat slower by the bimolecular mechanism, and 3-(iodoacetamido)oxanilic acid did not inactivate at all (5, 22-24). Therefore further studies on synthesis and evaluation of related compounds are in order to see if a better juxta-position of the inhibitor's leaving group and the supposed enzymic nucleophilic site will lead to a more accelerated inactivation by the active-site-directed mechanism.

EXPERIMENTAL

Methods.

Melting points were determined in capillary tubes on a Mel-temp block and are uncorrected. Infrared spectra were determined in potassium bromide pellet with a Perkin-Elmer 137B or 337 spectrophotometer. Ultraviolet spectra were determined in 10% alcohol with a Perkin-Elmer 202 spectrophotometer. Thin layer chromatograms (TLC) were run on Brinkmann silica gel GF and spots were detected by visual examination under ultraviolet light. All compounds designated as pure in the Experimental moved as single spots on TLC and had infrared and ultraviolet spectra compatible with their assigned structures.

N-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinylpropyl)-*N*-benzyl benzenesulfonamide (IX).

To a stirred solution of 1.89 g. (7.65 mmoles) of III (20) in 10 ml. of dimethyl sulfoxide protected from moisture was added 184 mg. (7.65 mmoles) of sodium hydride as a mineral oil dispersion. When solution was complete, 6.13 g. (31 mmoles) of 1,3-dibromopropane was added, then the mixture was stirred for 24 hours at ambient temperature. The reaction mixture was diluted with 50 ml. of water and extracted with 50 ml. of benzene. The separated organic layer was washed successively with 1 *N* aqueous sodium hydroxide (2 x 50 ml.) and water (2 x 100 ml.). Benzene was removed by spin-evaporation *in vacuo*, then excess 1,3-dibromopropane was removed at oil pump pressure at a bath temperature of 90°; yield, 2.35 g. (83%) of IV as an oil which could not be crystallized.

To a stirred solution of 1.04 g. (7.95 mmoles) of ethyl acetoacetate and 191 mg. (7.95 mmoles) of sodium hydride as a mineral oil dispersion in 25 ml. of *t*-butyl alcohol protected from moisture was added 2.35 g. (6.38 mmoles) of IV. After being refluxed for about 18 hours, the mixture was neutralized with glacial acetic acid and spin-evaporated *in vacuo*. The residue was partitioned between water and chloroform, then the latter was evaporated *in vacuo*; unchanged ethyl acetoacetate was removed at 1 mm. (bath 90°) leaving 2.45 g. (93%) of an oil containing V.

The crude V was dissolved in 25 ml. of *t*-butyl alcohol; after the addition of 0.53 g. (2.94 mmoles) of guanidine carbonate, the mixture was gently refluxed with stirring for 48 hours, then acidified with acetic acid and spin-evaporated *in vacuo*. The residue was partitioned between chloroform and water. The separated chloroform layer was washed several times with water, then dried with magnesium sulfate and spin-evaporated *in vacuo*. The residual oil was crystallized from 2-methoxyethanol; yield, 1.02 g. (42%), m.p. 177-184°. Recrystallization from ethanol afforded 0.91 g. (34%, 26% based on III) of white crystals, m.p. 196-199°.

Anal. Calcd. for $C_{21}H_{24}N_4O_3S \cdot {}^1/_2 H_2O$: C, 59.6; H, 5.95; N, 13.2. Found: C, 59.8; H, 5.98; N, 13.3.

1-Carbobenzyloxyamino-3-phthalimidopropane (VIII).

A mixture of 7.36 g. (27.1 mmoles) of VII (21), 75 ml. of *N*,*N*-dimethylformamide and 5.01 g. (27.1 mmoles) of potassium phthalamide was stirred for about 18 hours at ambient temperature, then poured into 150 ml. of water. The product (m.p. 90-96°) was collected on a filter and washed with water. Recrystallization from ethanol gave 6.66 g. (72%) of white crystals, m.p. 101-102°.

Anal. Calcd. for $C_{19}H_{18}N_2O_4$: C, 67.4; H, 5.36; N, 8.28. Found: C, 67.2; H, 5.22; N, 8.19.

N-Carbobenzyloxy-1,3-diaminopropane (X) Hydrochloride.

A mixture of 3.38 g. (10 mmoles) of VIII, 175 ml. of ethanol and 1.5 ml. of 95% hydrazine was refluxed for about 18 hours, then spin-evaporated *in vacuo*. The residue was dissolved in 100 ml. of water and acidified with acetic acid. After removal of the separated phthalhydrazide by filtration, the solution was spin-evaporated *in vacuo*. To the residual oil was added 1 *N* aqueous sodium hydroxide until *p*H 13 was reached, then the mixture was extracted with chloroform. The combined extracts were dried with magnesium sulfate, then spin-evaporated *in vacuo*. The residual X was dissolved in dry ether and the solution treated with hydrogen chloride. The hydrochloride was collected on a filter and washed with ether; yield, 2.13 g. (87%), m.p. 190-192°. Recrystallization from ethanol-ether gave 1.95 g. (79%) of white crystals, m.p. 193-194°.

Anal. Calcd. for $C_{11}H_{16}N_2O_2 \cdot HCl$: C, 53.9; H, 6.98; N, 11.4. Found: C, 53.7; H, 7.10; N, 11.1.

N-Carbobenzyloxy-*N'*-(*p*-tolylsulfonyl)-1,3-diaminopropane (XI).

A mixture of 445 mg. (4.2 mmoles) of anhydrous sodium carbonate, 5 ml. of water, 5 ml. of chloroform and 489 mg. (2 mmoles) of X hydrochloride was stirred for a few minutes to complete solution, then it was placed in an ice bath. To the stirred mixture was added 418 mg. (2 mmoles) of *p*-toluenesulfonyl chloride in portions over a period of about 15 minutes; the mixture was then stirred at ambient temperature for 18 hours. The aqueous layer was separated and

extracted with chloroform. The combined chloroform solutions were dried with magnesium sulfate, then spin-evaporated *in vacuo*. Crystallization of the residue from aqueous ethanol gave 484 mg. (67%) of white crystals, m.p. 68-69°.

Anal. Calcd. for $C_{18}H_{22}N_2O_4S$: C, 59.7; H, 6.12; N, 7.73. Found: C, 59.4; H, 6.03; N, 7.69.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(3-aminopropyl)-*p*-toluenesulfonamide (XV) Dipicrate.

Reaction of XI with 1,3-dibromopropane, ethyl acetoacetate and guanidine -- as described for the preparation of IX -- gave the *N*-carbobenzyloxy blocked 4-pyrimidinol (XIV). The crude product remaining after removal of the chloroform was dissolved in the minimum amount of ethanol, then poured into a large volume of water, and the turbid mixture was allowed to stand overnight. The amorphous XIV was collected on a filter and washed with water; yield, 2.18 g. (40% based on XI), which started to soften at 85° and decomposed at 220-245°. This amorphous product could not be purified further and was therefore converted to XV.

To 527 mg. (1 mmole) of crude XIV was added 5 ml. of 30% anhydrous hydrogen bromide in acetic acid (14); gas was evolved and solution was complete after 90 minutes of stirring at ambient temperature. The solution was poured into 25 ml. of ether, then the ether was decanted from the gummy hydrobromide salt. This salt was dissolved in 2 ml. of ethanol, then treated with a solution of 500 mg. (2.2 mmoles) of picric acid in 2 ml. of ethanol. After standing overnight at room temperature, the mixture was filtered and the crude product (395 mg., m.p. 220-231° dec.) was washed with ethanol. Recrystallization from 85% ethanol gave 327 mg. (38%) (15% based on XI) of bright yellow crystals, m.p. 239-241° dec.

Anal. Calcd. for $C_{30}H_{33}N_{11}O_{17}S$: C, 42.3; H, 3.91; N, 18.1. Found: C, 42.0; H, 4.09; N, 18.1.

The dipicrate could be converted to the dihydrochloride of XV in quantitative yield. The picrate was dissolved in 3 *N* aqueous hydrochloric by addition of a small amount of alcohol and heating. The solution was washed with chloroform until the washings and aqueous layer were colorless. The aqueous solution was then spin-evaporated *in vacuo* leaving XV dihydrochloride as a colorless, hygroscopic glass which could not be crystallized.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(3-bromoacetamidopropyl)-*p*-toluenesulfonamide (XVI).

To a solution of 466 mg. (1 mmole) of XV dihydrochloride in 0.67 ml. of 3 *N* aqueous sodium hydroxide and 0.5 ml. of water were added 3.5 ml. of acetone and 260 mg. of *p*-nitrophenyl bromoacetate (12); an intense yellow color immediately developed. After being stirred 1 hour at ambient temperature, the mixture was evaporated *in vacuo*. Trituration of the residue with water, then ether gave 134 mg. (26%) of a light yellow, amorphous solid, m.p. 99-101° dec. This material moved as a single spot on TLC in 2-methoxyethanol:ethanol (1:3) and gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen (12).

Anal. Calcd. for $C_{20}H_{28}BrN_5O_4S$: C, 46.7; H, 5.48; Br, 15.5. Found: C, 46.4; H, 5.95; Br, 15.7.

N-(*m*-Nitrobenzyl)-*p*-toluenesulfonamide (XIXa).

m-Nitrobenzylamine (XVIIIa) hydrochloride, m.p. 232 - 235°, was prepared from its *N*-phthalyl derivative (XVIIa) in 84% yield of ethanol recrystallized material as described for the preparation of X. The *N*-phthalyl derivative (XVIIa), m.p. 164-166°, was in turn prepared from α-chloro-*m*-nitrotoluene with a reaction time of 1 hour as described for the preparation of VIII.

Reaction of XVIIIa with *p*-toluenesulfonyl chloride -- as described for the preparation of XI -- gave XIXa. Recrystallization from ethanol afforded 4.69 g. (92%) of white crystals, m.p. 125-129°.

Anal. Calcd. for $C_{14}H_{14}N_2O_4S$: C, 54.9; H, 4.61; N, 9.15. Found: C, 55.0; H, 4.71; N, 9.06.

2-Amino-6-methyl-5-[3-(*m*-nitrobenzylideneamino)propyl]-4-pyrimidinol (XXVIa).

To a stirred solution of 255 mg. (1 mmole) of XXV dihydrochloride (3) in 0.67 ml. of 3 *N* aqueous sodium hydroxide, 0.50 ml. of water, and 3.5 ml. of methanol was added 151 mg. (1 mmole) of *m*-nitrobenzaldehyde. Within 2 minutes a thick precipitate had formed. The mixture was diluted with 10 ml. of methanol, then filtered after 30 minutes; yield, 257 mg. (81%), m.p. 180-184°, that was suitable for the next step. Recrystallization of a similar preparation from aqueous ethanol gave yellow crystals, m.p. 187-188°.

Anal. Calcd. for $C_{15}H_{17}N_5O_3 \cdot {}^1/_2 H_2O$: C, 55.5; H, 5.61; N, 21.6. Found: C, 55.6; H, 5.57; N, 21.4.

2-Amino-6-methyl-5-[3-(*p*-nitrobenzylideneamino)propyl]-4-pyrimidinol (XXVIb).

Reaction of 765 mg. (3 mmoles) of XXV dihydrochloride with 453 mg. (3 mmoles) *p*-nitrobenzaldehyde, as described for the preparation of XXVIa, gave 785 mg. (83%) of product, m.p. 215-219° dec. An analytical sample was obtained as yellow crystals, m.p. 224-225° dec., by recrystallization from methanol.

Anal. Calcd. for $C_{15}H_{17}N_5O_3$: C, 57.1; H, 5.43; N, 22.2. Found: C, 57.0; H, 5.29; N, 21.9.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(*p*-nitrobenzyl)-*p*-toluenesulfonamide (XXIb).

To a suspension of 785 mg. (2.5 mmoles) of XXVIb in 30 ml. of methanol was added 1.13 g. (30 mmoles) of sodium borohydride over a period of 40 minutes. After being stirred for 12 hours at ambient temperature, the mixture was adjusted to *p*H 8-9 with 1 *N* aqueous hydrochloric acid. The mixture deposited 670 mg. (84%) of low melting amorphous XXIIIb on standing.

A mixture of the crude XXIIIb, 10 ml. of chloroform, 0.8 ml. of triethylamine, and 437 mg. (2.3 mmoles) of *p*-toluenesulfonyl chloride was stirred at ambient temperature for 18 hours. After addition of 5 ml. of water, the mixture was filtered and the precipitate washed with chloroform. The chloroform layer was separated from the filtrate and the aqueous layer was extracted once more with chloroform. The combined chloroform extracts were dried with magnesium sulfate, then spin-evaporated *in vacuo* leaving 879 mg. (89%) of crude product, m.p. 130-140° with softening at 115° that was suitable for the next step. Reprecipitation from ethanol by addition of water gave a white amorphous solid of unchanged m.p.; yield, 426 mg. (42%).

Anal. Calcd. for $C_{27}H_{25}N_5O_5S$: C, 56.0; H, 5.34; N, 14.8. Found: C, 55.8; H, 5.49; N, 14.6.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(*m*-nitrobenzyl)-*p*-toluenesulfonamide (XXIa).

(A). Reaction of XIXa with 1,3-dibromopropane, ethyl acetoacetate, and guanidine -- as described for the preparation of IX -- gave an oily residue on evaporation of the chloroform. The amorphous product slowly separated from ethanol; yield, 20% based on XIXa, m.p. 102-112°.

Anal. Calcd. for $C_{22}H_{25}N_5O_5S$: C, 56.0; H, 5.34; N, 14.8. Found: C, 56.3; H, 5.18; N, 15.0.

(B). Reduction of XXVIa with sodium borohydride then reaction with *p*-toluenesulfonyl chloride -- as described for the preparation of XXIb -- gave a 42% overall yield of product identical with preparation (A) as shown by its infrared and ultraviolet spectra and by its co-movement on TLC.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(*p*-aminobenzyl)-*p*-toluenesulfonamide (XXIIb).

A mixture of 235 mg. (0.5 mmole) of XXIb, 100 ml. of ethanol, 1 ml. of 2 *N* aqueous hydrochloric acid, and 50 mg. of platinum oxide was shaken with hydrogen at 2-3 atm. for 3 hours when reduction was complete. The filtered solution was made strongly basic with aqueous ammonia, then concentrated *in vacuo* to about 10 ml. when the solution became turbid. After standing overnight at 5°, the mixture deposited 169 mg. (77%) of crude product, m.p. 184-192°. Recrystallization from ethanol afforded 122 mg. (55%) of nearly white crystals, m.p. 201-203°.

Anal. Calcd. for $C_{22}H_{27}N_5O_3S \cdot {}^1/_2 H_2O$: C, 58.7; H, 6.26; N, 15.5. Found: C, 58.5; H, 6.66; N, 15.6.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(*m*-aminobenzyl)-*p*-toluenesulfonamide (XXIIa).

Hydrogenation of XXIa -- as described for the preparation of XXIIb -- gave 75-80% of product suitable for the next step. Recrystallization from ethanol afforded white crystals, m.p. 213-214° dec.

Anal. Calcd. for $C_{22}H_{27}N_5O_3S$: C, 59.8; H, 6.16; N, 15.9. Found: C, 59.6; H, 6.00; N, 15.7.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(*m*-bromoacetamidobenzyl)-*p*-toluenesulfonamide (XXIVa) Sulfate.

To a stirred suspension of 110 mg. (0.25 mmole) of XXIIa in 2 ml. of acetone cooled in an ice-bath was added 71 mg. (0.28 mmole) of bromoacetic anhydride (12); the mixture was stirred at 0° for 1 hour when solution was complete. The solution was poured into 20 ml. of ether. The amorphous white solid (113 mg.) was collected and dissolved in a small volume of ethanol. The solution was clarified from a trace of insoluble material, then poured into 10 ml. of 1 *N* aqueous sulfuric acid. The product was collected on a filter and washed with

a small amount of the same solvent mixture; yield, 60 mg. (35%), of a white solid, m.p. 130-131°, that moved as a single spot on TLC in *t*-butyl alcohol-methylethylketone (4:3) and gave a positive 4-(*p*-nitrobenzyl)pyridine test for active halogen (12).

Anal. Calcd. for $C_{24}H_{28}BrN_5O_4S \cdot H_2SO_4 \cdot H_2O$: C, 42.5; H, 4.74; Br, 11.8. Found: C, 42.6; H, 4.68; Br, 11.7.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-(*p*-bromoacetamidobenzyl)-*p*-toluenesulfonamide (XXIVb) Sulfate.

Bromoacetylation of XXIIIb, as described for the preparation of XXIVa, gave 73 mg. (44%) of white crystals, m.p. 150-151°.

Anal. Calcd. for $C_{24}H_{28}BrN_5O_4S \cdot {}^1/_2 H_2SO_4$: C, 47.2; H, 4.79; Br, 13.0. Found: C, 47.5; H, 5.00; Br, 13.3.

2-Amino-5-(benzylideneaminopropyl)-6-methyl-4-pyrimidinol.

To a stirred solution of 510 mg. (2 mmoles) of XXV dihydrochloride (3) in 1.33 ml. of 3 *N* aqueous sodium hydroxide, 1 ml. of water and 7 ml. of methanol was added 212 mg. (2 mmoles) of benzaldehyde; after 5 hours during which time the product separated, the mixture was filtered; yield, 443 mg. (82%) of white crystals, m.p. 223-224°. Recrystallization of a similar preparation from methanol gave the analytical sample, m.p. 230-231°.

Anal. Calcd. for $C_{15}H_{16}N_4O$: C, 66.6; H, 6.71; N, 20.7. Found: C, 66.4; H, 6.77; N, 20.9.

N-[3-(2-Amino-4-hydroxy-6-methyl-5-pyrimidinyl)propyl]-*N*-benzyl-*p*-toluenesulfonamide.

Sodium borohydride reduction of 416 mg. of the preceding Schiff's base, followed by reaction with *p*-toluenesulfonyl chloride, as described for the preparation of XXIb, gave, after recrystallization from aqueous ethanol, 210 mg. (33%) of white crystals, m.p. 112-113°.

Anal. Calcd. for $C_{22}H_{26}N_4O_3S$: C, 62.0; H, 6.15; N, 13.1. Found: C, 61.8; H, 5.98; N, 13.0.

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