Irreversible Enzyme Inhibitors. CLXVIII.[1,2] Irreversible Inhibition of Dihydrofolate Reductase and Cell Wall Transport by Pyrimidines and Dihydro-*s*-triazines with Pyridinium Side Chains

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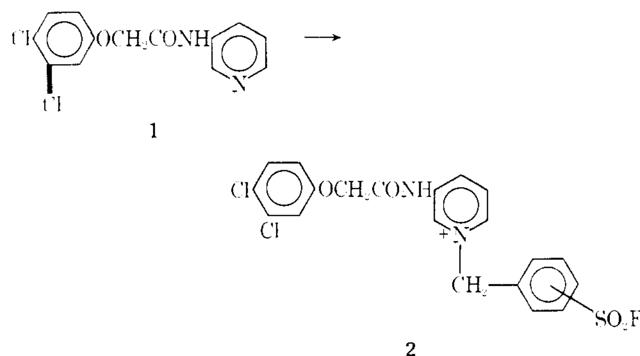
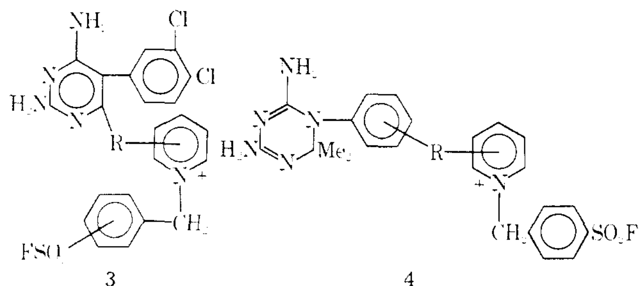
2,4-Diamino-5-(3,4-dichlorophenyl)pyrimidine with pyridylalkyl substituents on the 6-position were quaternized on the pyridine nitrogen with *p*- or *m*-fluorosulfonylbenzyl bromide. Four resultant compounds with a 3- or 4-pyridylethyl substituent (**6–9**) were not irreversible inhibitors of the dihydrofolate reductase from L1210 DF8 mouse leukemia. The fifth compound (**10**) derived from a 3-pyridylbutyl substituent was a good reversible and a fair irreversible inhibitor of the enzyme. All five compounds with their cationic side chains showed better cell wall penetration of L1210 cells than a sulfonyl fluoride attached to the 6-position of the pyrimidine ring through a nonpolar *p*-phenethyl–phenethyl bridge (**5**). Similarly, four pyridinium quaternaries bridged to the *meta* position of the phenyl moiety of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-*s*-triazine were synthesized and evaluated. Two (**16, 18**) were fair irreversible inhibitors of the enzyme, but all four compounds showed poor cell wall transport attributed to the dicationic character of these molecules.

One of the useful synthetic methods for introducing the terminal SO_2F group for candidate irreversible inhibitors is to quaternize a pyridine ring with a fluorosulfonylbenzyl bromide; for example, powerful irreversible inhibitors of chymotrypsin[5] and guinea pig complement[6,7] were synthesized by quaternization of **1** to **2**. Therefore quaternaries of type **3** and **4** that



might be useful irreversible inhibitors of dihydrofolate reductase were synthesized and evaluated for enzyme



inhibition and cell wall transport; the results are the subject of this paper.

Assay Results. Candidate irreversible inhibitors of type **3** (**6–10**) were measured as reversible and irreversible inhibitors of dihydrofolate reductase from L1210/DF8 mouse leukemia and mouse liver;[8] the results are presented in Table I. The closest structurally available compound for comparison that did not have a cationic charge in the side chain was **5**.[2b] About a ninefold loss in reversible binding occurred when **5** was compared with the quaternary **6**; furthermore, **6** showed no irreversible inhibition of the enzyme from L1210/DF8 and poor irreversible inhibition of the enzyme from mouse liver.

When the SO_2F group of **6** was moved from the *para* to the *meta* position, the resultant **7** was a threefold better reversible inhibitor than **6**; however, still no irreversible inhibition was observed with **7**. Similarly, when the ethane bridge was moved from the 4-position of the pyridinium ring to the 3-position (**8**, **9**), reversible inhibition was little changed and still no irreversible inhibition was observed.

The ethane bridge of **8** was then extended to butane; the resultant **10** was a fourfold better reversible inhibitor than **8**, that is, **10** was as effective as the inhibitor **5** with a hydrocarbon side chain. Furthermore, **10** showed appreciable—but not good—irreversible inhibition of the L1210/DF8 enzyme.

The compounds were then investigated for cell kill of L1210 cell culture;[9] as a first approximation these data can be related to the ability of the compounds to penetrate the L1210 cell wall.[10] As a second approximation differences in I_{50} between compounds can be normalized by comparing $ED_{50}:I_{50}$ ratios. Compounds **6–8** and **10** showed the same magnitude of ED_{50} as the reference compound **5** with a hydrocarbon side chain; however, these compounds were one magnitude better than **5** when the $ED_{50}:I_{50}$ ratios were compared. Since

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) (a) For the previous paper in this series see B. R. Baker and S. E. Hopkins, *J. Med. Chem.*, **13**, 87 (1970); (b) for the previous paper on dihydrofolate reductase see B. R. Baker and N. M. J. Vermeulen, *ibid.*, **13**, 82 (1970), paper CLXVI of this series.

(3) N. M. J. V. wishes to thank the Council of Scientific and Industrial Research, Republic of South Africa, for a tuition fellowship.

(4) On sabbatical leave from the University of Sydney, Australia.

(5) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 221 (1969), paper CL of this series.

(6) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 677 (1969), paper CLVI of this series.

(7) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 902 (1969), paper CLXI of this series.

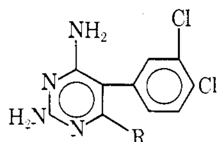
(8) See B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII of this series, for the assay methods used.

(9) We wish to thank Dr. Florence White of the CCNSC for these data obtained by Dr. Philip Himmelfarb, Arthur D. Little, Inc.

(10) For a more detailed discussion see (a) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 668 (1969), paper CLIV of this series; (b) B. R. Baker, E. E. Janson, and N. M. J. Vermeulen, *ibid.*, **12**, 898 (1969), paper CLX of this series.

TABLE I

INHIBITION OF DIHYDROFOLATE REDUCTASE[a] AND L1210 CELL CULTURE BY



No.	R[b]	Enzyme source	I_{50},[c] μM	Inhibitor, μM	Time, min	% inactivation[d]	ED_{50},[e] μM	$ED_{50}:I_{50}$
5[f]	p-$(CH_2)_2C_6H_4(CH_2)_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DF8	0.046	0.046	60	84	2	50
				0.046	60	5		
6	4-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DF8	0.43	1.1	60	5	3	7
			0.54	1.6	60	27		
7	4-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$m$	Mouse liver L1210/DF8	0.15	0.3	60	0	0.6	4
				0.45	60	0		
8	3-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DF8		0.22	60	0	1	5
			0.22	0.66	60	0		
9	3-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$m$	Mouse liver L1210/DF8	0.54	0.54	60	0	>2	>4
				0.54	60	0		
10	3-$(CH_2)_4C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DF8	0.050	0.10	60	42	0.4	8
				0.15	60	6		

[a] The technical assistance of Diane Shea and Sharon Lafler is acknowledged. [b] C_5H_4N = pyridyl. [c] $I_{50} \simeq 6K_i$ = concentration for 50% inhibition when measured with 6 μM dihydrofolate and 0.15 M KCl in pH 7.4 Tris buffer as previously described.[8] [d] Enzyme incubated with inhibitor at 37° in pH 7.4 Tris buffer containing 60 μM TPNH, then the remaining enzyme assayed as previously described.[8] [e] Concentration for 50% kill of L1210 cell culture.[9] [f] Data from ref 2b.

the $ED_{50}:I_{50}$ ratio reflects transport plus the irreversible effect on a target enzyme, and since an irreversible inhibitor enhances the $ED_{50}:I_{50}$ ratio,[2b,10b] it would appear that **6–8** and **10** are transported across the L1210 cell wall considerably more effectively than **5**. The failure to find good irreversible inhibitors in this pyrimidine series with quaternized side chains was unfortunate.

Attention was then directed to dihydro-*s*-triazine inhibitors of type **4**. Since the 1-phenyl-1,2-dihydro-*s*-triazine moiety of **4** is a strong base[11] with a pK_a about 11, a structure of type **4** would have two cationic groups at physiological pH; however, some organic structures with two cationic groups can still penetrate a cell wall.[12]

Four compounds of type **4** were synthesized and evaluated; the results are presented in Table II. When the pyridyl group of **11** was quaternized with *p*-fluorosulfonylbenzyl bromide, the resultant **12** was about fourfold less effective than **11** as a reversible inhibitor; furthermore **12** showed essentially no irreversible inhibition. Insertion of a methylene group in the bridge of **12** next to the pyrid'ne ring gave **14** which showed essentially the same inhibition as **12**. In contrast insertion of two methylene groups on the bridge of **11** next to the 1-phenyl moiety gave **16** which was a fourfold better reversible inhibitor than **12** and which showed fair irreversible inhibition of the L1210/DF8 enzyme; furthermore **16** showed specificity by not inactivating the mouse liver enzyme. The fourth compound (**18**) of the series showed enzyme inhibition similar to that of **16**.

All of the dihydro-*s*-triazines in Table II showed poor penetration of the L1210 cell wall, as approximated from $ED_{50}:I_{50}$ ratios; note that the quaternary **16** is about 15-fold less effective than its pyridine precursor (**15**). It was clearly apparent in this dihydro-*s*-triazine series that molecules of type **4** with two cationic groups would be unsuitable for *in vivo* use.

Chemistry.—The candidate irreversible inhibitors in Table I have the general structure **22**; these were synthesized as follows. Wittig condensation of **19**[13] with 3- or 4-pyridinecarboxaldehyde or 3-pyridylacrolein[14] gave **20**. Catalytic reduction of the bridge double bond(s) of **20** with a PtO_2 catalyst afforded **21**. Quaternization with *p*-fluorosulfonylbenzyl bromide[15] or its *meta* isomer[5] afforded the candidate irreversible inhibitors **22** (Scheme I); the diaminopyrimidine system was protected against reaction by protonation with HOAc.

The candidate irreversible inhibitors in Table II have the general structure **26** and were synthesized as follows. Acylation of 3-amino- or 3-aminomethylpyridine with *m*-nitrobenzoyl chloride or *m*-nitrocinnamoyl chloride afforded the nitro amides **23**. Catalytic reduction of the nitro group (and the C=C group in the base of **16**) gave the arylamines **24**. Condensation of **24** with cyanoguanidine in the presence of HBr[11] afforded the pyridyl-dihydro-*s*-triazines **25** as their monohydrobromides. Quaternization of **25** with *p*-fluorosulfonylbenzyl bromide[15] afforded the candidate irreversible inhibitors **26** (Scheme II).

(11) E. J. Modest, *J. Org. Chem.*, **21**, 1 (1956).

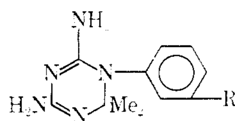
(12) L. S. Schanker, *Pharmacol. Rev.*, **14**, 501 (1962).

(13) B. R. Baker and N. M. J. Vermeulen, *J. Med. Chem.*, **12**, 82 (1969), paper CXXXVI of this series.

(14) Prepared from 3-pyridinecarboxaldehyde as described for *m*-nitrocinnamaldehyde by B. R. Baker and J. H. Jordaan, *ibid.*, **8**, 35 (1965); see M. Strell and E. Kopp, *Chem. Ber.*, **91**, 2854 (1958).

(15) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **11**, 666 (1968), paper CXXVII of this series.

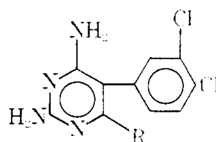
TABLE II
INHIBITION OF DIHYDROFOLATE REDUCTASE[a] AND L1210 CELL CULTURE BY



No.	R[b]	Enzyme source	I_{50},[c] μM	Inhibitor μM	Time, min	% inactivation[d]	ED_{50},[e] μM	ED_{50}/I_{50}
11	3-$CONHC_5H_4N$	L1210/DFS	0.18					
12	3-$CONHC_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DFS	0.85	1.7	60	0	>100	>100
				2.6	60	14		
13	3-$CONHCH_2C_5H_4N$	L1210/DFS	3.3				>100	>30
14	3-$CONHCH_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DFS	0.50	1.0	60	11	>100	>200
				1.5	60	0		
15	3-$(CH_2)_2CONHC_5H_4N$	L1210/DFS	0.089				2	30
16	3-$(CH_2)_2CONHC_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DFS	0.16	0.33	60	53	75	500
				0.33	60	0		
17	3-$CH{=}CHCONHCH_2C_5H_4N$	L1210/DFS	0.14					
18	3-$CH{=}CHCONHCH_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$	Mouse liver L1210/DFS	0.15	0.3	60	33	70	500
				0.45	60	0		

[a] For footnotes a-e see Table I.

TABLE III
PHYSICAL CONSTANTS OF



No.	R	Method	Yield, %	Mp, °C dec	Formula
6	4-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$ · $\overset{-}{Br}$ · HBr	C	23[a]	240-242	$C_{24}H_{21}BrCl_2FN_5O_2S \cdot HBr$[b]
7	4-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$m$ · $\overset{-}{Br}$ · HBr	C	30[c]	235-240	$C_{24}H_{21}BrCl_2FN_5O_2S \cdot HBr$[b]
8	3-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$ · $\overset{-}{Br}$ · HBr	C	29[c]	>250[d]	$C_{24}H_{23}BrCl_2FN_5O_2S \cdot HBr \cdot H_2O$[b]
9	3-$(CH_2)_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$m$ · $\overset{-}{Br}$ · HBr	C	15[a]	>238[d]	$C_{24}H_{21}BrCl_2FN_5O_2S \cdot HBr$[b]
10	3-$(CH_2)_4C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p$ · $\overset{-}{Br}$ · HBr	C	39[e]	225-228	$C_{26}H_{25}BrCl_2FN_5O_2S \cdot HBr \cdot 0.5EtOH$[b]
20a	4-$CH{=}CHC_5H_4N$	A	80[c]	261-263	$C_{17}H_{13}Cl_2N_5$[f]
20b	3-$CH{=}CHC_5H_4N$	A	75[c]	>240[d]	$C_{17}H_{13}Cl_2N_5$[f]
20c	3-$(CH{=}CH)_2C_5H_4N$	A	72[g]	>208[d]	$C_{19}H_{15}Cl_2N_5$[f]
21a	4-$(CH_2)_2C_5H_4N$	B	72[h]		
21b	3-$(CH_2)_2C_5H_4N$	B	66[h]		
21c	3-$(CH_2)_4C_5H_4N$	B	54[e]	210-214	$C_{19}H_{19}Cl_2N_5$[f]

[a] Recrystallized from EtOH-H_2O. [b] Analyzed for C, H, S. [c] Recrystallized from EtOH. [d] Gradually decomposes starting at this temperature. [e] Recrystallized from EtOH-THF. [f] Analyzed for C, H, N. [g] Recrystallized from Me_2CO-THF. [h] Crude product obtained by trituration with THF that was uniform on tlc.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had proper uv and ir spectra and moved as a single spot on tlc with Brinkman silica gel GF or polyamide MN. Each gave combustion values for C, H, and N or S within 0.4% of theoretical.

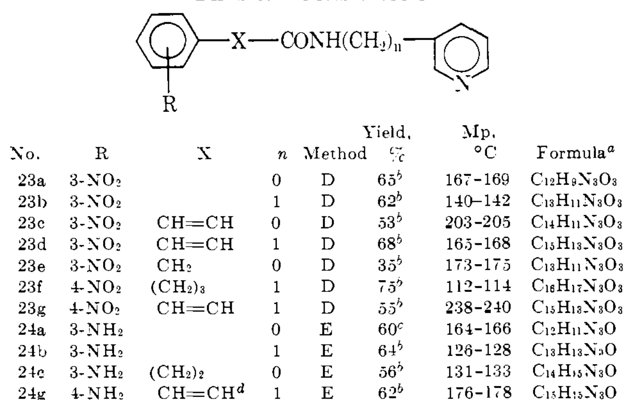
1-[2,4-Diamino-5-(3,4-dichlorophenyl)-6-pyrimidyl]-4-(3-pyridyl)butadiene (20c) (Method A).—To a mixture of 5.60 g (9.3 mmol) of **19**,[13] 1.33 g (10 mmol) of 3-pyridylacrolein,[14] and 40 ml of DMF was added 1.24 g (10 mmol) of 1,5-diazabicyclo[4.3.0]-nonene (DBN).[16] After being stirred for 20 hr at ambient temperature and protected from moisture the mixture was diluted with 80 ml of H_2O. The product was collected on a filter, washed with H_2O, then recrystallized from Me_2CO-THF; yield, 2.60 g (72%) which gradually decomposed >208°. For additional data and other compounds prepared by this method see Table III.

3-[2,4-Diamino-5-(3,4-dichlorophenyl)-6-pyrimidylbutyl]-pyridine (21c) (Method B).—A mixture of 0.96 g (2.5 mmol) of **20c**, 100 ml of MeOEtOH, and 60 mg of PtO_2 was shaken with H_2 at 2-3 atm for 2 hr when reduction was complete. The filtered solution was evaporated *in vacuo*. Recrystallization from EtOH-THF gave 0.58 g (54%) of product, mp 210-212°. See Table III for additional data and other compounds prepared by this method.

***N*-(*p*-Fluorosulfonylbenzyl)-3-[2,4-diamino-5-(3,4-dichlorophenyl)-6-pyrimidylbutyl]pyridinium Bromide Hydrobromide (10) (Method C).**—To a solution of 0.23 g (0.60 mmol) of **21c** and

(16) H. Oediger, H. Kabbe, F. Möller, and K. Eiter, *Chem. Ber.*, **99**, 2012 (1966).

TABLE IV
PHYSICAL CONSTANTS OF



No.	R	X	n	Method	Yield, %	Mp, °C	Formula[a]
23a	3-NO_2		0	D	65[b]	167–169	$C_{12}H_9N_3O_3$
23b	3-NO_2		1	D	62[b]	140–142	$C_{13}H_{11}N_3O_3$
23c	3-NO_2	CH=CH	0	D	53[b]	203–205	$C_{14}H_{11}N_3O_3$
23d	3-NO_2	CH=CH	1	D	68[b]	165–168	$C_{15}H_{13}N_3O_3$
23e	3-NO_2	CH_2	0	D	35[b]	173–175	$C_{13}H_{11}N_3O_3$
23f	4-NO_2	$(CH_2)_3$	1	D	75[b]	112–114	$C_{16}H_{17}N_3O_3$
23g	4-NO_2	CH=CH	1	D	55[b]	238–240	$C_{15}H_{13}N_3O_3$
24a	3-NH_2		0	E	60[c]	164–166	$C_{12}H_{11}N_3O$
24b	3-NH_2		1	E	64[b]	126–128	$C_{13}H_{13}N_3O$
24c	3-NH_2	$(CH_2)_2$	0	E	56[b]	131–133	$C_{14}H_{15}N_3O$
24g	4-NH_2	CH=CH[d]	1	E	62[b]	176–178	$C_{15}H_{15}N_3O$

[a] Analyzed for C, H, N. [b] Recrystallized from EtOH–H_2O; [c] Recrystallized from H_2O. [d] Presence of double bond indicated by long wavelength peak in uv.

0.036 g (0.60 mmol) HOAc in 3 ml of DMF was added 0.15 g (0.60 mmol) of *p*-fluorosulfonylbenzyl bromide[15] in 1 ml of DMF. After being stirred in a bath at 90° for 1 hr and at ambient temperature for 18 hr, the solution was evaporated *in vacuo*. The residue was dissolved in 10 ml of EtOH; the solution was treated with excess HBr gas, then evaporated *in vacuo*. The residue was stirred with THF until solidification occurred. The crude product was collected on a filter, washed with THF, then recrystallized from EtOH; yield, 0.18 g (39%), mp 225–228° dec.

SCHEME I

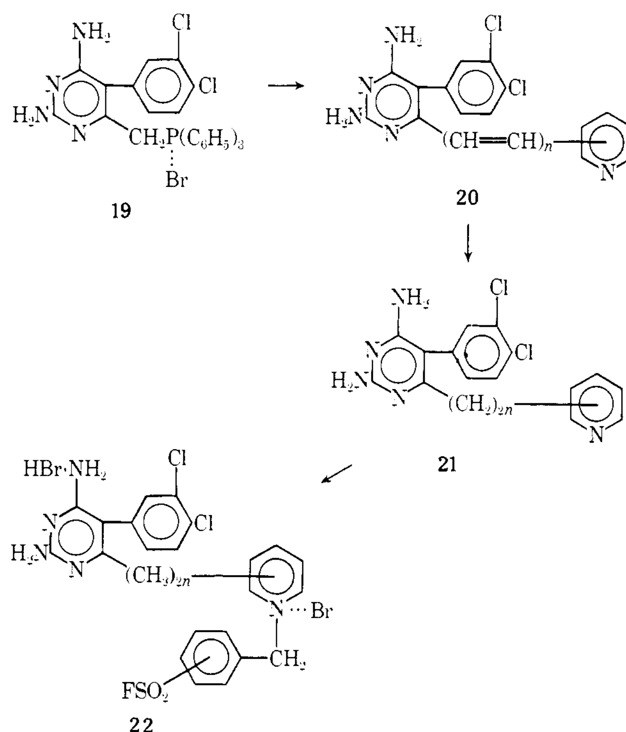
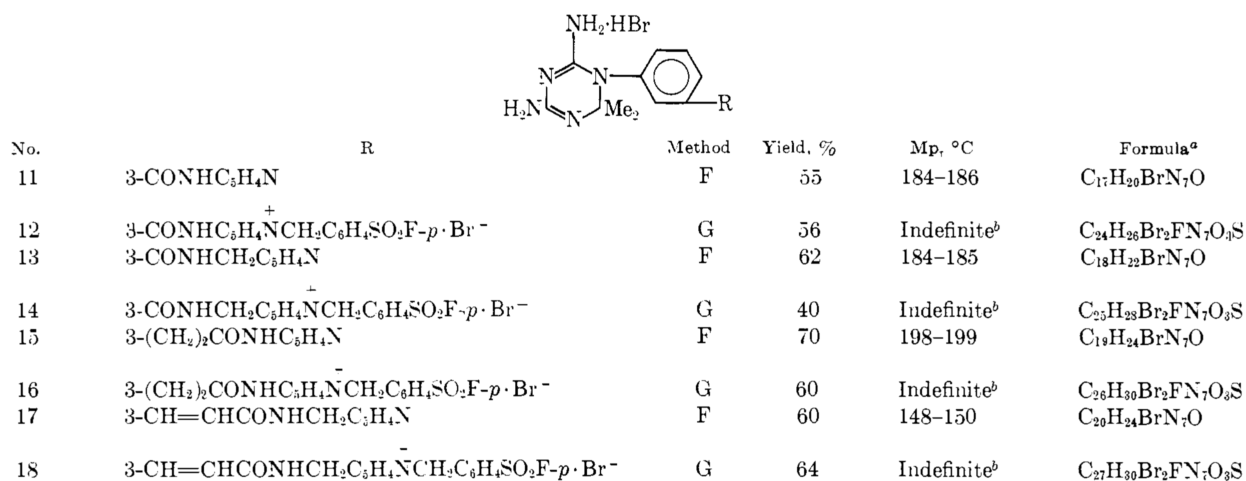


TABLE V
PHYSICAL CONSTANTS OF



No.	R	Method	Yield, %	Mp, °C	Formula[a]
11	3-$CONHC_5H_4N$	F	55	184–186	$C_{17}H_{20}BrN_7O$
12	3-$CONHC_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p\cdot Br^-$	G	56	Indefinite[b]	$C_{24}H_{26}Br_2FN_7O_3S$
13	3-$CONHCH_2C_5H_4N$	F	62	184–185	$C_{18}H_{22}BrN_7O$
14	3-$CONHCH_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p\cdot Br^-$	G	40	Indefinite[b]	$C_{25}H_{28}Br_2FN_7O_3S$
15	3-$(CH_2)_2CONHC_5H_4N$	F	70	198–199	$C_{19}H_{24}BrN_7O$
16	3-$(CH_2)_2CONHC_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p\cdot Br^-$	G	60	Indefinite[b]	$C_{26}H_{30}Br_2FN_7O_3S$
17	3-CH=$CHCONHCH_2C_5H_4N$	F	60	148–150	$C_{20}H_{24}BrN_7O$
18	3-CH=$CHCONHCH_2C_5H_4\overset{+}{N}CH_2C_6H_4SO_2F$-$p\cdot Br^-$	G	64	Indefinite[b]	$C_{27}H_{30}Br_2FN_7O_3S$

[a] Analyzed for C, H, N. [b] Single spot on tlc on polyamide with EtOH.

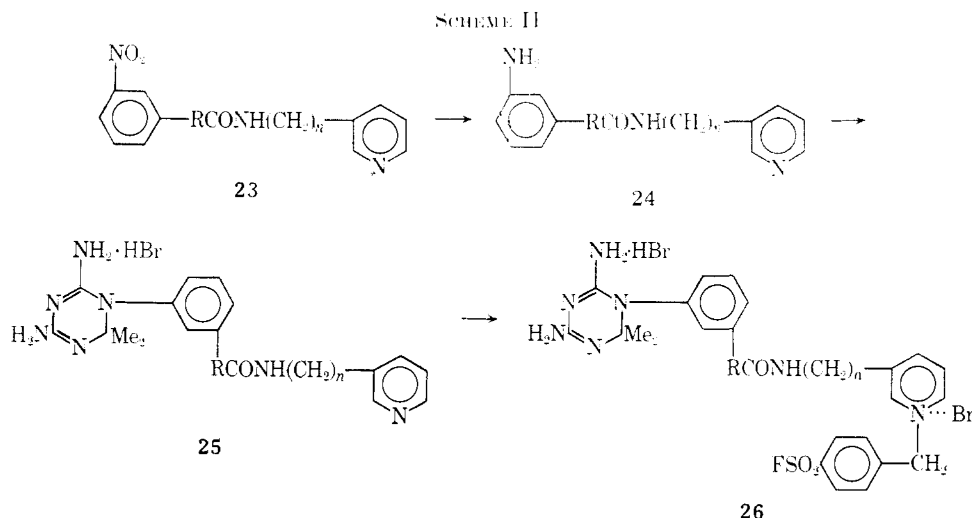
See Table III for additional data and other compounds prepared by this method.

***m*-Nitro-*N*-(3-pyridyl)cinnamamide (23c) (Method D).**—A mixture of 2.0 g (10 mmol) of *m*-nitrocinnamic acid and 15 ml of $SOCl_2$ was refluxed until gas evolution ceased (about 30 min). The solution was evaporated *in vacuo* and the residual acid chloride dissolved in 50 ml of $CHCl_3$. This solution was added dropwise with stirring to an ice-cooled solution of 0.94 g (10 mmol) of 3-aminopyridine and 2.5 g (25 mmol) of Et_3N in 50 ml of $CHCl_3$ at such a rate that the temperature did not exceed 15° (15 min). The mixture was then refluxed for about 10 min, then cooled and washed with two 200-ml portions of H_2O. Dried with Na_2SO_4, the $CHCl_3$ solution was evaporated *in vacuo*. Recrystallization from EtOH–H_2O gave 1.4 g (53%) of crystals, mp 203–205°. See Table IV for additional data and other compounds prepared by this method.

***m*-Amino-*N*-(3-pyridyl)hydrocinnamamide (24c) (Method E).**—A mixture of 2.69 g (10 mmol) of **23c**, 50 ml of MeOEtOH, and about 2 g of Raney Ni was shaken with H_2 at 2–3 atm until reduction was complete (2 hr). The filtered solution was evaporated to dryness *in vacuo* and the residue recrystallized from EtOH–H_2O; yield, 1.35 g (56%), mp 131–133°. See Table IV for additional data and other compounds prepared by this method.

***m*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)-*N*-(3-pyridyl)hydrocinnamamide (15) (Method F).**—A mixture of 2.4 g (10 mmol) of **24c**, 1.2 g (11 mol) of cyanoguanidine, 25 ml of Me_2CO, 10 ml of MeOH, and 1.7 ml (10 mmol) of 48% aqueous HBr was refluxed with stirring for 17 hr. The solution was evaporated *in vacuo*. The residue was crystallized by solution in the minimum amount of hot MeOH, then addition of Me_2CO to turbidity; yield, 3.0 g (70%), mp 198–199°. See Table V for additional data and other compounds prepared by this method.

***N*-(*p*-Fluorosulfonylbenzyl)-3-[*m*-(4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)phenylpropionamido]pyridinium Bromide Hydrobromide (16) (Method G).**—To a solution of 1.00 g (2.3 mmol) of **15** in 10 ml of dry DMF was added 0.57 g (2.2 mmol) of *p*-fluorosulfonylbenzyl bromide.[15] After 24 hr at ambient temperature, the solution was poured into 50 ml of dry Et_2O. The solvent was decanted from the gummy product. Solution



of the gum in hot *i*-PrOH, then cooling gave an amorphous solid that was collected by centrifugation and immediately dried over P_2O_5 *in vacuo;* yield, 1.00 g (64%), of white solid that gradually decomposes over 130° without melting. Two more precipitations from hot *i*-PrOH by cooling gave 0.7 g (42%) with no definite melting point; tlc on polyamide with EtOH showed one spot. See Table V for additional data and other compounds prepared in this way.

Mixed Bifunctionality. II. Relation of Antitumor Activity to Structure in Alkylating Agents Derived from Polynuclear Aromatic Hydrocarbons[1]

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Investigations of the antitumor properties of a variety of polynuclear aromatic hydrocarbon derivatives containing a single alkylating function have been continued. The existence of a parallelism between the carcinogenic and carcinostatic potentialities of several anthracene and benzanthracene moieties is indicated from studies with their S and N mustard derivatives. Various halomethyl aromatic hydrocarbons have also been found to be highly effective against ascites tumors in mice.

Antitumor activity due to mixed bifunctionality has been shown to occur when a "carrier" group embodying an acridine (or related) nucleus or an anthracene (or related) nucleus is convalently joined, preferably through a basic side chain, with a 2-chloroethylamine or a 2-chloroethyl sulfide alkylating function.[2-4]

Independent variation of each of these two structural features has already led to distinct new classes of antitumor agents. Further variations of the "carrier" portion have been made and, as shown in Table I, this feature when combined with a simple alkylating function as its complement has given agents with properties superior in many respects to any we have ever tested.

Two synthetic routes to the hydroxy precursors of the mustards in Table II were found to be superior to those previously employed.[4] In the first (method A in the Experimenal Section), the anil formed from the aromatic aldehyde and 2-(2-aminoethylthio)ethanol was reduced with $LiBH_4$. In the second (method B), the iodomethyl derivatives, available from the corresponding anthraquinones by the method of Badger and Pierce,[5] on reaction with the side chain gave the precursor in one step. In spite of generally poor yields and sometimes difficult isolations, this was, in some cases, the most convenient synthesis; often the choice of route was dictated by the accessibility of intermediates. Where two isomers are theoretically possible in the synthesis of the iodomethylanthracenes, the position of the side chain as determined by that of the iodomethyl group is tabulated as being at the *meso* position *not* adjacent to the substituent in the outer ring. This conclusion is not rigorously proved but is deduced from two lines of evidence both of which harmonize with the obvious rationale of steric hindrance: (1) Sandin and Fieser proved[6] that the iodomethyl group so introduced occupies the 7-position of benz[*a*]anthracene and (2) in contrast to the eight anthraquinones successfully subjected to the conditions of synthesis in this laboratory (not all of these are reported herein), only 1,4-dimethylanthraquinone, where there is a

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