16 hr, cooled, and diluted with H_2O. The precipitated solid was dissolved in Et_2O, and this solution was washed (H_2O) and evaporated. Crystallization of the residue from Et_2O–hexane gave **5**-cyclohexyl-1-**indancarboxylic acid** (11c), the characterization of which is given in Table IV.

6-Chloro-5-cyclohexyl-1-indancarboxylic Acid (5). To a soln of 244 mg (1.0 mmole) of 5-cyclohexyl-1-indancarboxylic acid (11c) in 12 ml of MeCN was added a trace of sublimed $FeCl_3$ and then 0.74 ml of a soln of MeCN containing 71 mg of Cl_2. The soln was stirred at ambient temperature for 2.5 hr, and then diluted by dropwise addition of 11 ml of H_2O. The precipitated solid was collected as 150 mg (54%) of white crystals, mp 146–148°. Recrystallization from Et_2O–petr ether did not alter the melting point. Noguchi and his coworkers[8] record mp 151–152° and Juby and his collaborators[7] report mp 150.5–152.5°. The 4-chloro isomer has mp 120–123°.[8] *Anal.* ($C_{16}H_{19}ClO_2$) C, H, Cl.

Methyl 5-Cyclohexyl-1-indancarboxylate. To a stirred soln of 488 mg (2.0 mmoles) of 5-cyclohexyl-1-indancarboxylic acid in 10 ml of CH_2Cl_2 was added dropwise a soln of 360 mg of 3-methyl-1-*p*-tolyltriazine in 10 ml of CH_2Cl_2.[14] The soln was stirred at ambient temperature for 2 hr, diluted with 50 ml of Et_2O, and washed successively with 1 *N* HCl, H_2O, 1 *N* NaOH, and H_2O. The dried soln was evaporated, and the residual gum was crystallized from dilute MeOH affording 442 mg (85%) of white crystals, mp 45–47°. A sample recrystallized from dilute MeOH had mp 46–47°. *Anal.* ($C_{17}H_{22}O_2$) C, H.

Methyl 6-Acetyl-5-cyclohexyl-1-indancarboxylate (12a). To a soln of 258 mg (1.0 mmole) of methyl-5-cyclohexyl-1-indancarboxylate in 5 ml of CS_2 was added 0.09 ml of AcCl and 380 mg of $AlCl_3$. The mixture was stirred and heated under reflux for 2 hr and then evaporated. To the residue was added 30 ml of iced, dilute H_2SO_4, and the resulting gum was rubbed to a solid. The solid was crystallized from dilute MeOH to give 205 mg (69%) of white crystals, mp 72–73°. *Anal.* ($C_{19}H_{24}O_3$) C, H.

Methyl 6-Acetyl-5-cyclohexyl-1-indancarboxylate Oxime. To a soln of 100 mg (0.33 mmole) of methyl 6-acetyl-5-cyclohexyl-1-indancarboxylate in 5 ml of MeOH was added 62 mg of hydroxylamine hydrochloride and 0.066 ml of pyridine. The mixture was stirred and heated under reflux for 18 hr and then evaporated. The residue was partitioned between EtOAc and H_2O. The organic solution was washed with saline, dried, and evaporated. The residual gum was triturated with petr ether, and the resulting solid was collected to give 80 mg (76%) of white crystals, mp 136–140°. The 80 mg of crystals were recrystallized from dil MeOH to afford 56 mg of white needles, mp 142–143°. *Anal.* ($C_{19}H_{25}NO_3$) C, H, N.

Methyl 6-Acetamido-5-cyclohexyl-1-indancarboxylate (12b). To a stirred, ice-chilled soln of 1.15 g (3.65 mmoles) of methyl 6-acetyl-5-cyclohexyl-1-indancarboxylate oxime in 55 ml of Et_2O was added 1.15 g of PCl_5. The mixture was stirred at ambient temperature for 2 hr and then poured into 120 ml of ice H_2O. The Et_2O soln was separated and washed successively with 1 *N* HCl, H_2O, 1 *N* NaOH, and H_2O. The dried organic soln was evaporated under reduced pressure, and the residual gum was crystallized from Et_2O–petr ether affording, in two crops, 788 mg (68%) of white crystals, mp 167–169°. A sample of this material recrystallized from Et_2O–petr ether had mp 170–171°. *Anal.* ($C_{19}H_{25}NO_3$) C, H, N.

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Irreversible Enzyme Inhibitors. 194.†[1] Hydrophobic Bonding to Some Dehydrogenases by Substituted 5-Phenylethyl-4-hydroxyquinoline-3-carboxylic Acids

B. R. Baker‡ and Ray R. Bramhall*

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106.
Received March 10, 1972.

Nineteen derivatives of 4-hydroxyquinoline-3-carboxylic acid bearing substituted phenylethyl groups at the 5 position and Cl at the 8 position were prepared as inhibitors of glutamate, glyceraldehyde-3-phosphate, lactate, and malate dehydrogenases. The 2,6-$Cl_2C_6H_3CH_2CH_2$- and the 2,4-$Cl_2C_6H_3CH_2CH_2$-substituted compounds (**20** and **22**) were the best inhibitors of malate dehydrogenase. Both were complexed 5000-fold more effectively than the parent compound and 20,000-fold more effectively than the substrate L-malate. The best inhibitor of glutamate dehydrogenase was the *o*-$C_6H_5C_6H_4CH_2CH_2$-substituted compound (**8**), being complexed 250-fold more effectively than the parent and 1000-fold more than the substrate L-glutamate.

In a previous paper of this series, the possible utility of inhibitors of glutamate, glyceraldehyde-3-phosphate, lactate, and malate dehydrogenases as target enzymes for the treatment of cancer cells in the resting phase (G_0) was discussed.[2] The study of hydrophobic bonding of derivatives of **1** with substituents at the 5, 6, 7, and 8 positions has shown good hydrophobic bonding to malate dehydrogenase by **2** and good to excellent bonding to three of the four enzymes by **3**, **4**, and **5**, the exception being glyceraldehyde-3-phosphate dehydrogenase.[3,4]

A series of 5-phenylethyl derivatives with the Cl at the 8 position (**3**) was extended to include several compounds bearing substituents on the 5-phenylethyl ring. The phenyl-

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‡Deceased Oct 1971.

Table I. Inhibition[a] of Four Dehydrogenases by I

No.	R	I_{50},[b] μM			
		Glu-DH	GPDH	LDH	MDH
1[c]	H (no 8-Cl)	600	>1600[d]	440	520
3[e]	$C_6H_5CH_2CH_2$	11	310	69	22
4[e]	$C_6H_5(CH_2)_4$	5.2	140	6.7	1.0
5[e]	$C_6H_5(CH_2)_6$	2.4	>40[d]	6.8	0.46
6[e]	$C_6H_5CH=CH$	5.2	170	24	4.7
7	$4\text{-}CH_3C_6H_4CH_2CH_2$	8.9	90[f]	>120[d]	4.0
8	$o\text{-}C_6H_5C_6H_4CH_2CH_2$	1.8	260	18	1.7
9	$m\text{-}C_6H_5C_6H_4CH_2CH_2$	2.8	56	29	0.41
10	$p\text{-}C_6H_5C_6H_4CH_2CH_2$	12	230	17	0.25
11	$\alpha\text{-}C_{10}H_7CH=CH$	6.8	130	80	1.1
12	$\alpha\text{-}C_{10}H_7CH_2CH_2$	2.8	120	47	3.0
13	$\beta\text{-}C_{10}H_7CH_2CH_2$	3.4	100	18	1.2
14	$3,4\text{-}CH_2O_2C_6H_3CH=CH$	12	>600[d]	39[f]	3.1
15	$3,4\text{-}CH_2O_2C_6H_3CH_2CH_2$	10	>800[d]	>160[d]	0.79
16	$3\text{-}FC_6H_4CH_2CH_2$	28	>600[d]	>40[d]	4.3
17	$3\text{-}ClC_6H_4CH_2CH_2$	7.9	>80[d]	>20[d]	0.55
18	$4\text{-}ClC_6H_4CH_2CH_2$	13	>240[d]	>40[d]	2.2
19	$2,6\text{-}Cl_2C_6H_3CH=CH$	4.0	>80[d]	44	0.75
20	$2,6\text{-}Cl_2C_6H_3CH_2CH_2$	5.5	240[c]	>40[d]	0.1
21	$2,5\text{-}Cl_2C_6H_3CH_2CH_2$	2.0	>80[d]	~ 24[c]	0.19
22	$2,4\text{-}Cl_2C_6H_3CH_2CH_2$	8.8	>120[d]	>40[d]	0.11
23	$3,4\text{-}Cl_2C_6H_3CH_2CH_2$	6.4	58	38	0.48

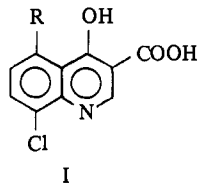
[a]The technical assistance of Nancy Middleton, Pauline Minton, and Diane Shea with these assays is acknowledged. [b]I_{50} = concentration for 50% inhibition of Glu-DH = glutamate dehydrogenase, GPDH = glyceraldehyde-3-phosphate dehydrogenase, LDH = lactate dehydrogenase, and MDH = malate dehydrogenase when assayed with 2 m*M* L-glutamate, 0.25 m*M* glyceraldehyde 3-phosphate, 2 m*M* pyruvate, and 2 m*M* L-malate, respectively. [c]Data from ref 2. [d]No inhibition at 0.25 this concentration, the maximum solubility. [e]Data from ref 4. [f]Estimated from V_0/V_I observed at maximum solubility which is less than I_{50}.

Table II. Physical Properties of $RC_6H_4CH_2P(C_6H_5)_3$ X

No.	R	X	Solvent[a]	Mp, °C	Yield, %	Formula[b]
24	$2\text{-}C_6H_5$	Cl	A	275-278	68	$C_{31}H_{26}ClP$
25	$3\text{-}C_6H_5$[c]	Br	A	294-297 dec	68	$C_{31}H_{26}BrP$
26	$2,5\text{-}Cl_2$[c]	Br	B	268-270	63	$C_{25}H_{20}BrCl_2P$

[a]Recrystallization solvents: A, from reaction mixture; B, EtOH-EtOAc. [b]Analyzed for C and H. [c]Prepared by previously described method.[4]

ethyl series was chosen over the phenylbutyl and phenylhexyl series because of the availability of the required



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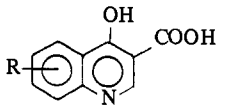


1, R = H
2, R = 6-$C_6H_5O(CH_2)_5O$
3, R = 8-Cl-5-$C_6H_5(CH_2)_2$
4, R = 8-Cl-5-$C_6H_5(CH_2)_4$
5, R = 8-Cl-5-$C_6H_5(CH_2)_6$

benzaldehydes or toluenes necessary for the syntheses (see the Experimental Section).

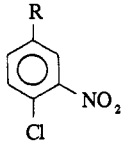
All of the new compounds listed in Table I were excellent inhibitors of malate dehydrogenase. The best inhibitors of this enzyme were the $2,6\text{-}Cl_2C_6H_3CH_2CH_2$ and $2,4\text{-}Cl_2C_6H_3CH_2CH_2$ derivatives (**20** and **22**) which were complexed about 5000-fold more effectively than the parent 4-hydroxyquinoline-3-carboxylic acid (**1**) and 20,000-fold more than the substrate L-malate. The $2,5\text{-}Cl_2C_6H_3CH_2CH_2$ (**21**) and the $p\text{-}C_6H_5C_6H_4CH_2CH_2$ (**10**) derivatives were about one-half as effective as **20** and **22**.

These compounds were good to excellent inhibitors of glutamate dehydrogenase. The best inhibitors were the $o\text{-}C_6H_5C_6H_4CH_2CH_2$ and $2,5\text{-}Cl_2C_6H_3CH_2CH_2$ derivatives (**8** and **21**) which were complexed 250-fold more effectively than **1** and 1000-fold more than the substrate L-glutamate. This is slightly better than the best previously reported compound, the $C_6H_5(CH_2)_6$ derivative (**5**).

The three best inhibitors of lactate dehydrogenase listed in Table I are the $o\text{-}C_6H_5C_6H_4CH_2CH_2$, $p\text{-}C_6H_5C_6H_4CH_2CH_2$, and $\beta\text{-}C_{10}H_7CH_2CH_2$ derivatives (**8**, **10**, and **13**, respectively) These compounds are not as strongly bound as the previousl reported $C_6H_5(CH_2)_4$ and $C_6H_5(CH_2)_6$ derivatives (**4** and **5**) which are bound 2- to 3-fold more effectively. Comparing **3** with **4** and **5** shows that limiting the methylene bridge to 2 carbons causes a 10-fold loss in binding.

The best inhibitors of glyceraldehyde phosphate were **9**

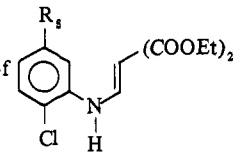
Table III. Physical Properties of



No.	R	Method[a]	Mp, °C[b]	Yield, %[c]	Formula[d]
27	$4\text{-}CH_3C_6H_4CH=CH$[e]	A	97-99	62	$C_{15}H_{12}ClNO_2$
28	$o\text{-}C_6H_5C_6H_4CH=CH$	A	*f*	97	
29	$m\text{-}C_6H_5C_6H_4CH=CH$	A	*f*	91	
30	$p\text{-}C_6H_5C_6H_4CH=CH$	B	183-185	70	$C_{20}H_{14}ClNO_2$
31	$\alpha\text{-}C_{10}H_7CH=CH$	B	124-125	81	$C_{18}H_{12}ClNO_2$
32	$\beta\text{-}C_{10}H_7CH=CH$	B	150-152	82	$C_{18}H_{12}ClNO_2$
33	$3,4\text{-}CH_2O_2C_6H_3CH=CH$	B	146-147	41[g]	$C_{15}H_{10}ClNO_4$
34	$3\text{-}FC_6H_4CH=CH$	A	119-121	110[h]	$C_{14}H_9ClFNO_2$
35	$3\text{-}ClC_6H_4CH=CH$	A	141-142	100[h]	$C_{14}H_9Cl_2NO_2$
36	$4\text{-}ClC_6H_4CH=CH$[e]	A	138-140	105[h]	$C_{14}H_9Cl_2NO_2$
37	$2,6\text{-}Cl_2C_6H_3CH=CH$	A[i]	172-173	80	$C_{14}H_8Cl_3NO_2$
38	$2,5\text{-}Cl_2C_6H_3CH=CH$	A	157-158	98	$C_{14}H_8Cl_3NO_2$
39	$2,4\text{-}Cl_2C_6H_3CH=CH$[j]	A	182-183	79	$C_{14}H_8Cl_3NO_2$
40	$3,4\text{-}Cl_2C_6H_3CH=CH$	B	228-229	70	$C_{14}H_8Cl_3NO_2$

[a]Method A, see Experimental Section; B, NaOMe in MeOH, see ref 4. [b]Recrystallized from EtOH unless noted otherwise. Determined only for trans isomer. [c]Yield based on total usable material, both cis and trans isomers. [d]Analyzed for C, H, N. [e]See ref 5 for starting Wittig reagent. [f]Oil. [g]Yield for trans isomer. [h]Cis and trans mixture of oil contains some solvent. [i]From reaction mixture, not recrystallized. [j]See ref 6 for the Wittig reagent.

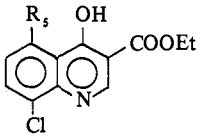
Table IV. Physical Properties[a] of



No.	R_5	Mp, °C[b]	Yield, %	Formula[c]
41	4-$CH_3C_6H_4CH_2CH_2$	93-94	45	$C_{23}H_{26}ClNO_4$
42	o-$C_6H_5C_6H_4CH_2CH_2$	76-78	29	$C_{28}H_{28}ClNO_4$
43	m-$C_6H_5C_6H_4CH_2CH_2$	*d*	100[e]	
44	p-$C_6H_5C_6H_4CH_2CH_2$	130-131	61	$C_{28}H_{28}ClNO_4$
45	α-$C_{10}H_7CH=CH$	110-114	30	$C_{26}H_{24}ClNO_4$[f]
46	β-$C_{10}H_7CH_2CH_2$	84-86	30	$C_{26}H_{26}ClNO_4$
47	3,4-$CH_2O_2C_6H_3CH=CH$	158-159	91	$C_{23}H_{22}ClNO_6$
48	3,4-$CH_2O_2C_6H_3CH_2CH_2$	*g*	55	
49	3-$FC_6H_4CH_2CH_2$	60-61	38	$C_{22}H_{23}ClFNO_4$
50	3-$ClC_6H_4CH_2CH_2$	*d*	108[e]	
51	4-$ClC_6H_4CH_2CH_2$	93-94	44	$C_{22}H_{23}Cl_2NO_4$
52	2,6-$Cl_2C_6H_3CH=CH$[h]	146-148	25	$C_{22}H_{20}Cl_3NO_4$
53	2,6-$Cl_2C_6H_3CH_2CH_2$[h]	96-98	36	$C_{22}H_{22}Cl_3NO_4$
54	2,5-$Cl_2C_6H_3CH_2CH_2$	108-109	40	$C_{22}H_{22}Cl_3NO_4$
55	2,4-$Cl_2C_6H_3CH_2CH_2$	97-98	56	$C_{22}H_{22}Cl_3NO_4$
56	3,4-$Cl_2C_6H_3CH_2CH_2$	73-75	30	$C_{22}H_{22}Cl_3NO_4$

[a] Prepared by previously described methods,[2] all had reaction times of 1 hr. [b] All recrystallized from EtOH. [c] Analyzed for C, H, N. [d] Oil. [e] Not homogeneous on tlc, but suitable for further transformations. [f] Analyzed for C, H only. [g] From EtOH at −78°, oil at room temperature. [h] Hydrogenation of 37 did not go to completion. The methylenemalonates are readily separable by recrystallization.

Table V. Physical Properties of



No.	R_5	Time, min[a]	Mp, °C	Solvent[b]	Yield, %	Formula[c]
57	4-$CH_3C_6H_4CH_2CH_2$	30	197-198	A	40	$C_{21}H_{20}ClNO_3$[d]
58	o-$C_6H_5C_6H_4CH_2CH_2$	60	178-180	B	25	$C_{26}H_{22}ClNO_3$
59	m-$C_6H_5C_6H_4CH_2CH_2$	60	165-167	A	23	$C_{26}H_{22}ClNO_3$
60	p-$C_6H_5C_6H_4CH_2CH_2$	45	204-205	A	56	$C_{26}H_{22}ClNO_3$
61	α-$C_{10}H_7CH=CH$	45	277-279	C	56	$C_{24}H_{18}ClNO_3$
62	α-$C_{10}H_7CH_2CH_2$	*e*	212-215	A	35	$C_{24}H_{20}ClNO_3$
63	β-$C_{10}H_7CH_2CH_2$	45	204-209	A	50	$C_{24}H_{20}ClNO_3$
64	3,4-$CH_2O_2C_6H_3CH=CH$	40	268-271	C	50	$C_{21}H_{16}ClNO_5$
65	3,4-$CH_2O_2C_6H_3CH_2CH_2$	30	197-199	A	76	$C_{21}H_{18}ClNO_5$
66	3-$FC_6H_4CH_2CH_2$	40	155-156	A	44	$C_{20}H_{17}ClFNO_3$
67	3-$ClC_6H_4CH_2CH_2$	45	162-163	A	9[f]	$C_{20}H_{17}Cl_2NO_3$
68	4-$ClC_6H_4CH_2CH_2$	45	196-197	A	38	$C_{20}H_{17}Cl_2NO_3$
69	2,6-$Cl_2C_6H_3CH=CH$	30	231-234	A	64	$C_{20}H_{14}Cl_3NO_3$
70	2,6-$Cl_2C_6H_3CH_2CH_2$	30	200-203	D	56	$C_{20}H_{16}Cl_3NO_3$
71	2,5-$Cl_2C_6H_3CH_2CH_2$	40	197-199	A	48	$C_{20}H_{16}Cl_3NO_3$
72	2,4-$Cl_2C_6H_3CH_2CH_2$	30	214-216	C	69	$C_{20}H_{16}Cl_3NO_3$
73	3,4-$Cl_2C_6H_3CH_2CH_2$	60	191-192	A	32	$C_{20}H_{16}Cl_3NO_3$

[a] Unless stated otherwise, time is for thermal ring closure; see ref 2. [b] Recrystallization solvents: A, EtOH; B, C_6H_5; C, 2-methoxyethanol; D, IPA. [c] Analyzed for C, H, N. [d] Calcd for N, 3.79%; found, 4.23%. [e] 62 prepared by hydrogenation of 61. [f] Yield over 3 steps.

and 23, the m-$C_6H_5C_6H_4CH_2CH_2$ and 3,4-$Cl_2C_6H_3CH_2CH_2$ derivatives, which were bound 5-fold better than the substrate. These values are strikingly poorer than the large increases in the inhibition of the other enzymes.

Retention of the double bond in the bridge between the phenyl and quinoline rings had variable results on binding. For example, the $C_6H_5CH=CH$ derivative (6) is bound 5-fold and 2-fold more effectively to MDH and GDH, respectively, than the saturated derivative, $C_6H_5CH_2CH_2$ (3). However, the α-$C_{10}H_7CH=CH$ derivative (11) is a 3-fold better inhibitor of MDH and a 2-fold poorer inhibitor of GDH than the α-$C_{10}H_7CH_2CH_2$ derivative (12). The 2,6-$Cl_2C_6H_3CH=CH$ derivative (19) is bound with essentially the same value as the 2,6-$Cl_2C_6H_3CH_2CH_2$ derivative (20) to GDH but is a 7-fold poorer inhibitor of MDH.

From these data it is apparent that MDH and GDH have large hydrophobic bonding areas. The binding to MDH is much greater than that to GDH and lends some specificity in that 22 is 80-fold more effectively bound to MDH than to GDH. Specificity for any enzyme over malate dehydrogenase has not been observed. In order to prepare better inhibitors of LDH it will be necessary to examine the phenylbutyl series.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had an ir spectrum compatible with its structure and was homogeneous on tlc on Brinkmann silica gel GF. All analytical samples gave combustion values for C, H, N within 0.4% of theoretical.

Synthesis of the nitro compounds listed in Table III by the Wittig reaction from 4-chloro-3-nitrobenzaldehyde and from 4-chloro-3-nitrotoluene has been previously described.[3,4] Conversion

Table VI. Physical Properties of I

No.	R[a]	Solvent[b]	Mp, °C	Yield, %	Formula[c]
7	4-$CH_3C_6H_4CH_2CH_2$	A	241-244	11	$C_{19}H_{16}ClNO_3$
8	o-$C_6H_5C_6H_4CH_2CH_2$	B	274-277	28	$C_{24}H_{18}ClNO_3$
9	m-$C_6H_5C_6H_4CH_2CH_2$	B	270-272	75	$C_{24}H_{18}ClNO_3$
10	p-$C_6H_5C_6H_4CH_2CH_2$	B	272 dec	73	$C_{24}H_{18}ClNO_3$
11	α-$C_{10}H_7CH=CH$	B	284 dec	36	$C_{22}H_{14}ClNO_3 \cdot 0.5C_3H_8O_2$
12	α-$C_{10}H_7CH_2CH_2$	C	255-256 dec	78	$C_{22}H_{16}ClNO_3 \cdot C_2H_5OH$
13	β-$C_{10}H_7CH_2CH_2$	B	274-276 dec	41	$C_{22}H_{16}ClNO_3$
14	3,4-$CH_2O_2C_6H_3CH=CH$	B	294 dec	62	$C_{19}H_{12}ClNO_5$
15	3,4-$CH_2O_2C_6H_3CH_2$-CH_2	B	260-261 dec	69	$C_{19}H_{14}ClNO_5$
16	3-$FC_6H_4CH_2CH_2$	B	264-265	74	$C_{18}H_{13}ClFNO_3$
17	3-$ClC_6H_4CH_2CH_2$	B	270-271 dec	30	$C_{18}H_{13}Cl_2NO_3$
18	4-$ClC_6H_4CH_2CH_2$	B	257-258 dec	47	$C_{18}H_{13}Cl_2NO_3$
19	2,6-$Cl_2C_6H_3CH=CH$	B	286-289 dec	54	$C_{18}H_{10}Cl_3NO_3$
20	2,6-$Cl_2C_6H_3CH_2CH_2$	C	259-262	41	$C_{18}H_{12}Cl_3NO_3$
21	2,5-$Cl_2C_6H_3CH_2CH_2$	B	265-266 dec	45	$C_{18}H_{12}Cl_3NO_3$
22	2,4-$Cl_2C_6H_3CH_2CH_2$	B	265-266 dec	51	$C_{18}H_{12}Cl_3NO_3$
23	3,4-$Cl_2C_6H_3CH_2CH_2$	B	274-275 dec	54	$C_{18}H_{12}Cl_3NO_3$

[a]Prepared by previously described method, ref 2. [b]Recrystallization solvents: A, C_6H_5N-hexane; B, 2-methoxyethanol; C, EtOH. [c]Analyzed for C, H, N.

of these nitro compounds to the anilinomethylenemalonates, the ethyl 4-hydroxyquinoline-3-carboxylates, and the 4-hydroxyquinoline-3-carboxylic acids (Tables IV, V, and VI, respectively) has also been previously reported.[2,4]

2-Phenylbenzyl Triphenylphosphonium Chloride (24). A mixt of 5.0 g (25.2 mmoles) of 2-phenylbenzoic acid and 1.5 g of LAH in 200 ml of THF was refluxed for 4 hr. The excess LAH was decompd by adding in order: 1.5 ml of H_2O, 1.5 ml of 15% NaOH, and 4.5 ml of H_2O. The crystalline salts were filtered off, and the THF was concentrated to an almost colorless oil of 4.9 g (105%) of 2-phenylbenzyl alcohol. To this alcohol was added 6 ml (10 g, 84 mmoles) of $SOCl_2$ and 20 ml of C_6H_6. The reaction mixt was placed on the steam bath for 15 min then spin evaporated. More benzene was added, and the mixt was spin evaporated again. Then 100 ml of toluene contg 6.9 g (25 mmoles) of $P(C_6H_5)_3$ was added, and the reaction refluxed for 18 hr to give a first crop of 5.53 g (47%), mp 269-275°. Refluxing the mother liquors for an addl 24 hr gave 1.56 g (13%), mp 275-278°. *Anal.* ($C_{31}H_{26}ClP$) C, H. In like manner a third crop of 0.93 g, mp 275-278°, was collected for a total of 8.02 g (68%) of useable material.

trans-3-Nitro-4,2',5'-trichlorostilbene (38) (Method A). To a mixt of 12.5 g (25 mmoles) of 26 and 4.6 g (25 mmoles) of 4-chloro-3-nitrobenzaldehyde in 50 ml of DMF was added 3.2 g (25 mmoles) of diazobicyclononane, and the mixt was stirred at room temp for 18 hr. The soln was poured into 50 ml of H_2O and stirred until all the oil had solidified. This yellow semisolid was collected and recrystallized from EtOH to give 1.83 g (22%) of yellow crystals, mp 157-158°. *Anal.* ($C_{14}H_8Cl_3NO_2$) C, H, N. The mother liquors (EtOH) were concentrated to an oil, dissolved in benzene, and placed on 100 g of silica. The silica was eluted with benzene until the tlc indicated no more product was present. The benzene was concentrated to 6.1 g of oily cis-trans mixt that was suitable for further transformations, total yield 7.9 g (96%).

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Irreversible Enzyme Inhibitors. 195.†,[1] Inhibitors of Thymidine Kinase from Walker 256 Carcinoma Derived from Thymidine 5'-Acetate

B. R. Baker‡ and John P. Neenan*

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106. *Received March 10, 1972*

Seventeen derivatives of thymidine 5'-carbamate and 27 5'-esters of thymidine were synthesized and investigated as inhibitors of thymidine kinase from Walker 256 rat tumor. Derivatives of thymidine 5'-acetate were good inhibitors of the enzyme. The inhibition displayed was attributed in part to an interaction of the inhibitor with an enzymic hydrophobic region adjacent to the active site. The binding exhibited by thymidine 5'-α-thionaphthyloxyacetate and thymidine 5'-*p*-benzyloxyphenoxyacetate was approximately equal to that of thymidine. These compounds could serve as prototypes in the design of more potent inhibitors.

There are two metabolic pathways to the intracellular thymidylate needed for DNA synthesis. The first is the thymidylate synthetase-dihydrofolate reductase pathway using deoxyuridylate and the second is the scavenge pathway from thymidine kinase. A blockade of dihydrofolate reductase, in effect, prevents formation of thymidylate from deoxyuridylate and has been extensively studied in this laboratory.[2-5]

Some studies on inhibition of thymidine kinase from *Escherichia coli* B were reported earlier from this laboratory;[6,7] apparently both the 3'- and 5'-hydroxyls of thymidine were binding points to the enzyme. No bulk tolerance for the large substituents needed for the design of active-site-directed irreversible inhibitors could be found that

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‡Deceased.