At this point the color of the bromine persisted for 2–3 min after each addition. The unreacted bromine was blown off with a stream of nitrogen, and the reaction mixture was concentrated to a syrup *in vacuo*, bath temperature less than 50°. The residue was evaporated three times with 10-ml portions of ethanol, whereupon it crystallized. The product was triturated with cold ethanol and with ether to obtain 340 mg of a crystalline hydrobromide salt, mp 135–138°. Recrystallization from methanol–ether gave an analytical sample, mp 136–138°. *Anal.* Calcd for $C_9H_{11}BrN_2O_5$: Br, 26.0. Found: Br, 25.71. Concentration of the original mother liquors yielded 170 mg of the starting material, 2-thiouracilarabinoside. Treatment of a concentrated ethanolic solution of the hydrobromide with a slight excess of ethanolic NH_3 yielded 2,2′-anhydro-1-(β-D-arabinofuranosyl)-uracil (IIb), mp 238–241°, identical in all respects with an authentic sample.[31]

Bromination of 1-(β-D-Arabinofuranosyl)-2-thiocytosine. 2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine Hydrobromide (IXb).—Bromination of 80 mg of VII in the manner described in the previous example yielded, after recrystallization from ethanol, 17 mg of IXb, mp 240° dec, $\lambda_{max}^{H_2O}$ 264 mμ (ε 9900) and 231 mμ (ε 9100), $\lambda_{min}^{H_2O}$ 244 mμ (ε 6350), $\lambda_{max}^{pH\,13}$ 275 mμ (ε 9200), $\lambda_{min}^{pH\,13}$ 251 mμ (ε 4800).

Anal. Calcd for $C_9H_{12}BrN_3O_4$: C, 35.31; H, 3.95; N, 13.71; Br, 26.11. Found: C, 34.55; H, 3.76; N, 13.69; Br, 25.81.

After acidification of the alkaline uv solution the following constants were obtained: $\lambda_{max}^{pH\,2}$ 281 mμ (ε 12,100), $\lambda_{min}^{pH\,2}$ 242 mμ (ε 1500).

Iodination of IV.—A solution of 260 mg (1 mmole) of IV in a mixture of 28 ml of water and 7 ml of pH 6.84 buffer was treated dropwise with 0.8 ml of 1 *N* I_2 solution which was 2.4 *N* in KI. A solution of *N* K_2CO_3 was added simultaneously to maintain the pH near neutrality. The slightly turbid solution was deionized by treatment with Dowex 3 (OH^-) and Dowex 50 WX (H^+) resins. Concentration *in vacuo* yielded a mixture from which IIb (66 mg) was separated by virtue of its very limited solubility in ethanol. From the mother liquor, 123 mg of the starting material (IV) was obtained.

Substituted 2,3-Dihydro-4(1H)-quinazolinones. A New Class of Inhibitors of Cell Multiplication

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The synthesis of 23 2-aryl-2,3-dihydro-4(1H)-quinazolinones is reported. A number of these are highly active in inhibiting the multiplication of Earle's L cells growing in suspension; nine have $ED_{50} \leqq 6$ mg/ml and of these two have $ED_{50} = 0.1$ mg/ml in this screening procedure.

The literature contains a limited number of references to 2,3-dihydro-4(1H)-quinazolinones, and several of these reports are concerned with the evaluation of these compounds for possible pharmacodynamic, insecticidal, and antifungal activity.[1] Our objective in synthesizing a series of 2-aryl-substituted derivatives of that heterocycle was to study them as inhibitors of multiplication of the Earle's L cell line of mouse fibroblasts growing in suspension.[2] It will be seen in Table I that a significant number of these derivatives showed very high *in vitro* activity: compounds **1** and **11** with $ED_{50} = 0.1$ μg/ml and **2–5**, **10**, **13**, and **21** with $ED_{50} \leqq 6$ μg/ml[3] were the most potent. For comparison, 2,3-dihydro-2-phenyl-4H-1,3-benzoxazin-4-one,[4] the 1-oxa analog of **1**, had $ED_{50} > 50$ μg/ml; actinomycin IV, one of the highly cytotoxic antibiotics, had $ED_{50} = 0.006$ μg/ml.[5]

The compounds were screened as inhibitors of cell multiplication by the procedure of Perlman, *et al.*[6] To aliquots of sterile cells were added serial dilutions of aqueous dimethyl sulfoxide solutions of the quinazolone and the mixtures were incubated for 3 days at 37°. The amount of compound needed to give a 50% inhibition was determined graphically by means of dose–response curve.

Several methods were employed for the synthesis of the 2-aryl-2,3-dihydro-4(1H)-quinazolinones. Method A, anil formation in ethanol between the 2-aminobenzamide and the aromatic aldehyde followed by the base-catalyzed cyclization[1a] was the most generally applicable. This method failed with **13** and **22**; the successful procedure (method C) employed catalytic amounts of *p*-toluenesulfonic acid in boiling chlorobenzene and made use of a special device which allowed the condensed solvent to be dried by percolation through a bed of calcium hydride before returning to the reaction flask. In method B, saturated ethanolic hydrogen chloride was the reaction medium; while successful with **1** and **12**, the procedure failed with several other compounds. The two amino derivatives, **14** and **17**, were obtained by catalytic hydrogenation of the corresponding nitro compounds.

Structure–Activity Relationships.—The only structural modification of **1** which did not adversely affect activity was the replacement of a hydrogen at position 6 by a chlorine atom; somewhat decreased activity was found when the substituent in that position was NO_2 or Br and even less activity when the substituent was H_2N or CH_3CONH. A high order of activity was retained when the 2-phenyl group of **1** was replaced by

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(2) The inhibition studies were carried out by Dr. D. Perlman of these laboratories.

(3) J. Leitner, B. J. Abbott, and S. A. Schepartz, *Cancer Res.*, **25**, 1779 (1965), state that in the CCNSC cell culture screening procedure for the selection of compounds for further study, $ED_{50} \leqq 6$ μg/ml is the requirement to pass the first stage of evaluation.

(4) A. W. Titherley, *J. Chem. Soc.*, **91**, 1419 (1907).

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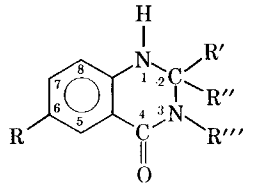
TABLE I

2,3-DIHYDRO-4(1H)-QUINAZOLINONES



No.	R	R′	R″	R‴	Method[a]	Recrystn solvent	Mp, °C	Yield, %	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N	ED$_{50}$, μg/ml (Earle's L cells)
1	H	H	Ph	H	A	*b*	224–226[c]	86	$C_{14}H_{12}N_2O$	74.98	5.65	12.49	75.10	5.78	12.19	0.1
2	H	H	*p*-$CH_3C_6H_4$	H	A	*d*	230–233[e]	66	$C_{15}H_{14}N_2O$	75.60	5.92	11.76	75.75	5.98	11.84	1
3	H	H	*o*-ClC_6H_4	H	A	*f*	209–211	78	$C_{14}H_{11}ClN_2O$	64.99	4.28	13.70	64.93	4.63	13.85	2.5
4	H	H	*p*-ClC_6H_4	H	A	*f*	205–208	70	$C_{14}H_{11}ClN_2O$	64.99	4.28	13.70	64.87	4.21	13.56	2.5
5	H	H	*trans*-PhCH=CH	H	A	*g*	168–172[h]	38	$C_{16}H_{14}N_2O$	76.78	5.64	11.19	76.68	5.87	11.05	0.5
6	H	H	2,3,5-$HO(Cl)_2C_6H_2$	H	A	*i*	261–262	65	$C_{14}H_{10}Cl_2N_2O_2$	54.38	3.26	9.07	54.07	2.96	8.93	30
7	H	H	3,4-$(CH_3)_3C(CH_3O)C_6H_3$	H	A	*j*	167–170	65	$C_{19}H_{22}N_2O_2$	73.52	7.14	9.03	73.42	7.22	8.89	30
8	H	H	2,4,5-$[(CH_3)_3C]_2(CH_3O)C_6H_2$	H	A	*k*	246–248	38	$C_{23}H_{30}N_2O_2$	75.37	8.25	7.64	75.31	8.17	7.72	10
9	H	H	2-Furyl	H	A	*l*	163–165	34	$C_{12}H_{10}N_2O_2$	67.28	4.71	13.08	67.22	4.85	13.38	30
10	H	H	2-Thenyl	H	A	*d*	213–216	75	$C_{12}H_{10}N_2OS$	62.60	4.38	12.17	62.83	4.53	12.08	6
11	Cl	H	Ph	H	A	*m*	255–257[n]	75	$C_{14}H_{11}ClN_2O$	64.99	4.28	...[o]	65.12	4.41	...	0.1
12	Br	H	Ph	H	B	*d*	255–256	66	$C_{14}H_{11}BrN_2O$	55.46	3.66	9.25	55.53	3.96	9.31	7.5
13	O_2N	H	Ph	H	C	*j*	267–268	74	$C_{14}H_{11}N_3O_3$	62.45	4.12	15.61	62.38	4.24	15.41	2
14	H_2N	H	Ph	H	D	*g*	192–195	42	$C_{14}H_{13}N_3O$	70.27	5.48	17.56	70.29	5.79	17.71	20
15	CH_3CONH	H	Ph	H	E	*p*	310–315	20	$C_{16}H_{15}N_3O_2$	...	...	14.94[q]	...	...	14.87	100
16	H	H	*o*-$O_2NC_6H_4$	H	A	*l*	191–194[r]	65	$C_{14}H_{11}N_3O_3$	62.45	4.12	15.61	62.75	4.34	15.40	10
17	H	H	*o*-$H_2NC_6H_4$	H	D	*s*	170–173	39	$C_{14}H_{13}N_3O$	70.27	5.48	17.56	70.42	5.70	17.34	20
18	H	H	Ph	CH_3	A	*l*	167–169[t]	50	$C_{15}H_{14}N_2O$	75.60	5.92	11.67	75.43	5.91	11.81	25
19	H	H	Ph	$PhCH_2$	A	*b*	163–165	76	$C_{21}H_{18}N_2O$	80.23	5.77	8.91	80.04	5.61	8.64	20
20	H	H	Ph	*o*-$CH_3C_6H_4$	A	*u*	173–175	80	$C_{21}H_{18}N_2O$	80.23	5.77	8.91	80.38	5.77	8.80	10
21	H	H	Ph	2,6-$(CH_3)_2C_6H_3$	A	*v*	219–222	63	$C_{22}H_{20}N_2O$	80.46	6.14	8.53	80.60	6.19	8.40	5
22	H	CH_3	Ph	H	C	*l*	225–229	47	$C_{15}H_{14}N_2O$	75.60	5.92	11.76	75.48	6.02	11.76	10
23	H	H	Ph	4-Pyridylmethyl	A	*g*	194–196	68	$C_{20}H_{17}N_3O$	76.17	5.44	13.33	76.40	5.61	13.20	10

[a] See Experimental Section for details. [b] 2-Propanol–H_2O (1:1). [c] Böhme and Böing[1b] report mp 223–224°; their synthetic] procedure was different. [d] 2-Propanol. [e] Lit.[1a] mp 230°. [f] Ethanol–H_2O (3:1). [g] Toluene. [h] Lit.[1a] mp 210° and lit.[1h] mp 224–226° for the product of the reaction between anthranilamide and cinnamaldehyde. The product now being described was prepared from *trans*-cinnamaldehyde and showed the expected absorption in the infrared of a *trans* >C=C< at 10.4 μ. [i] Propanol. [j] 95% Ethanol. [k] 2-Propanol–H_2O (2:1). [l] 2-Propanol–H_2O (1:2). [m] Ethanol–H_2O (1:1). [n] Lit.[1h] mp 246–248° for the product obtained by a different procedure. [o] *Anal.* Calcd: Cl, 11.70. Found: Cl, 11.90. [p] Acetonitrile. [q] *Anal.* Calcd: acetyl value, 15.30. Found: acetyl value, 15.61. [r] Lit.[1a] mp 192°. [s] Toluene–Skellysolve E (3:1). [t] Böhme and Böing[1b] employing a different synthetic procedure report mp 165°. [u] Skellysolve E. [v] Propanol–H_2O (1:1).

p-tolyl, *o*- or *p*-chlorophenyl, thenyl, or *trans*-styryl; replacement by *o*-nitrophenyl, *o*-aminophenyl, a polysubstituted phenyl, or 2-furyl gave compounds with decreased activity. When the hydrogen at position 2 in **1** was replaced by methyl, activity was decreased. Finally, replacement of the hydrogen at position 3 of **1** by 2,6-xylyl gave an active compound while substituents like methyl, benzyl, *o*-tolyl, and 4-pyridylmethyl in the same position gave less active derivatives. The 1-oxa analog of **1** was inactive at the highest level tested.

Experimental Section

All melting points were taken in capillary tubes heated by means of an oil bath and are uncorrected.

2,3-Dihydro-2-(*p*-tolyl)-4(1H)-quinazolinone (2). Method A.—To a solution of 3.40 g (0.025 mole) of anthranilamide and 3.00 g (0.025 mole) of *p*-tolualdehyde in 25 ml of 95% ethanol was added 1.6 ml of 20% aqueous NaOH, the mixture was heated under reflux for 1 hr and cooled, and the precipitated solid was filtered and air dried to give 5.10 g of crude product, mp 230–233°. Recrystallization from 600 ml of 2-propanol gave 3.90 g of **2**.

6-Bromo-2,3-dihydro-2-phenyl-4(1H)-quinazolinone (12). Method B.—A mixture of 2.16 g (0.01 mole) of 2-amino-5-bromobenzamide and 25 ml of 3.24 *N* ethanolic HCl was stirred 0.25 hr at 25°, 1.06 g (0.01 mole) of benzaldehyde was added, and the stirring at 25° was continued for 36 hr. The solid product was filtered; when air dried it weighed 3.00 g and melted 255–256°. Recrystallization from 900 ml of 2-propanol gave 2.07 g of **12**.

2,3-Dihydro-6-nitro-2-phenyl-4(1H)-quinazolinone (13). Method C.—A mixture of 0.20 g of *p*-toluenesulfonic acid monohydrate and 250 ml of chlorobenzene was distilled in a special apparatus constructed so that the condensed vapors percolated downward through a supported bed of 8–14 mesh CaH_2 and were dried by that reagent before returning to the reaction flask. When the evolution of hydrogen from the CaH_2 indicated the absence of water in the distillate, 3.62 g (0.02 mole) of 2-amino-5-nitrobenzamide and 2.12 g (0.02 mole) of benzaldehyde were added. The distillation of this mixture in the same apparatus was continued until there was no evidence of reaction with the CaH_2 (1 hr). The solid which separated on cooling was filtered; when air dried it weighed 4.80 g and melted 263–270° dec. Recrystallization from 500 ml of propanol–water (2:1) gave 4.00 g of **13**.

6-Amino-2,3-dihydro-2-phenyl-4(1H)-quinazolinone (14). Method D.—2,3-Dihydro-6-nitro-2-phenyl-4(1H)-quinazolinone (2.90 g, 0.011 mole), 200 ml of absolute ethanol, and 1.0 g of 5% Pd–C were shaken for 0.25 hr under 3.3 kg/cm² of hydrogen, the catalyst was filtered, and the filtrate was concentrated to dryness *in vacuo*. Successive recrystallizations from 700 ml and from 100 ml of toluene gave 1.10 g of **14**.

6-Acetamido-2,3-dihydro-2-phenyl-4(1H)-quinazolone (15). Method E.—Crude **14** (6.5 g), 200 ml of acetic anhydride, and 4 ml of pyridine were heated under reflux for 0.5 hr and concentrated to dryness *in vacuo*. Recrystallization from 1300 ml of acetonitrile gave 1.41 g of **15**.

The procedure described below, the reaction of an isatoic anhydride with ammonia or an amine, was employed to prepare all but two of the intermediate 2-aminobenzamides used to synthesize the 2,3-dihydro-4(1H)-quinazolones.

2-Amino-5-nitrobenzamide.—To a stirred suspension of 31.2 g (0.15 mole) of 6-nitroisatoic anhydride in 500 ml of 2-propanol was added dropwise 50 ml of aqueous ammonia (*d* 0.9). When the addition was complete, the mixture was concentrated from the steam bath first under atmospheric pressure and then *in vacuo*. The air-dried residue weighed 28.3 g, mp 225–230°, and was crystallized from 2830 ml of aqueous 2-propanol (2:3) to give 17.0 g (63% yield) of product, mp 232–235°.

Anal. Calcd for $C_7H_7N_3O_3$: C, 46.41; H, 3.90; N, 23.20. Found: C, 46.35; H, 3.90; N, 23.03.

In similar fashion were prepared the following 2-aminobenzamides.

2-Amino-5-chlorobenzamide, 75% yield, mp 166–169°, after recrystallization from toluene (50 ml/g). *Anal.* Calcd for $C_7H_7ClN_2O$: Cl, 20.66; N, 16.42. Found: Cl, 20.62; N, 16.56.

2-Amino-5-bromobenzamide, 82% yield, mp 184–186°, after recrystallization from toluene (70 ml/g). *Anal.* Calcd for $C_7H_7BrN_2O$: Br, 37.07; N, 12.98. Found: Br, 37.30; N, 12.85.

2-Amino-N-[(4-pyridyl)methyl]benzamide, 81% yield, mp 152–155°, after recrystallization from toluene (60 ml/g). *Anal.* Calcd for $C_{13}H_{13}N_3O$: C, 68.71; H, 5.77; N, 18.49. Found: C, 68.95; H, 5.90; N, 18.27.

2-Amino-N-benzylbenzamide, 86% yield, mp 121–122°, after recrystallization from Skellysolve E (40 ml/g). *Anal.* Calcd for $C_{14}H_{14}N_2O$: N, 12.38. Found: N, 12.58.

2-Amino-N-(2,6-xylyl)benzamide.—To 36.3 g (0.30 mole) of 2,6-dimethylaniline in 300 ml of dry chloroform was added dropwise a dry $CHCl_3$ solution of *o*-nitrobenzoyl chloride [prepared from 25 g (0.15 mole) of *o*-nitrobenzoic acid]. Subsequently, the mixture was stirred and refluxed for 1 hr and then worked up to give 35.5 g of crude product, mp 210–212°; recrystallization from 1600 ml of 2-propanol gave 25.5 g (63% yield) of 2-nitro-N-(2,6-xylyl)benzamide, mp 210–211°.

Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.49; H, 4.97; N, 10.42. Found: C, 66.38; H, 5.23; N, 10.37.

The nitro derivative (25.4 g), 3.0 g of 5% Pd–C, and 750 ml of absolute ethanol were shaken at 45° under 3.3 kg/cm² of hydrogen; reduction was rapid, and there was isolated in the usual manner 21.4 g (94% yield) of 2-amino-N-(2,6-xylyl)benzamide, mp 128–130° after recrystallization from 600 ml of Skellysolve E.

Anal. Calcd for $C_{15}H_{16}N_2O$: C, 74.97; H, 6.71; N, 11.66. Found: C, 74.87; H, 6.71; N, 11.60.

When the amino derivative was dissolved in 10% aqueous HCl, the solution filtered, and the filtrate cooled, there was obtained the **hydrochloride**, mp 238–240°.

Anal. Calcd for $C_{15}H_{16}N_2O \cdot HCl$: Cl, 12.81. Found: Cl, 12.68.

2-Amino-N-(*o*-tolyl)benzamide.—The above procedure, modified only in that 32.1 g (0.3 mole) of *o*-toluidine was used in place of 2,6-dimethylaniline, gave 33.0 g (85% yield) of the nitrobenzamide, mp 171–173° after recrystallization from 1400 ml of 2-propanol.

Anal. Calcd for $C_{14}H_{12}N_2O_3$: C, 65.62; H, 4.73; N, 10.93. Found: C, 65.76; H, 4.85; N, 11.06.

Reduction of 32.9 g of the nitro derivative gave 26.0 g (89% yield) of 2-amino-N-(*o*-tolyl)benzamide, mp 115–117° after recrystallization from 600 ml of Skellysolve E.

Anal. Calcd for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38. Found: C, 74.29; H, 6.24; N, 12.26.

2,3-Dihydro-2-(*o*-nitrophenyl)-4(1H)-quinazolinone (16). Method E. A.—A mixture of 6.80 g (0.05 mole) of anthranilamide, 7.55 g (0.05 mole) of *o*-nitrobenzaldehyde, and 45 ml of 95% ethanol was warmed until solution occurred and filtered, and the filtrate was allowed to cool. The yellow crystalline solid which separated was filtered to give 11.0 g (82% yield) of **2-[(*o*-nitrobenzylidene)amino]benzamide**, mp 171–175°.

Anal. Calcd for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12; N, 15.61. Found: C, 62.68; H, 4.41; N, 15.26.

B.—The product from A (2.0 g, 0.0075 mole), 45 ml of 95% ethanol, and 1.8 ml 20% aqueous NaOH were heated under reflux for 1 hr and cooled, and the solid was filtered to give 1.1 g of crude **13**. Recrystallization from 80 ml of aqueous ethanol (1:1) gave 0.8 g of pure **16**.