Design and Synthesis of 2-(Arylamino)-4(3*H*)-quinazolinones as Novel Inhibitors of Rat Lens Aldose Reductase

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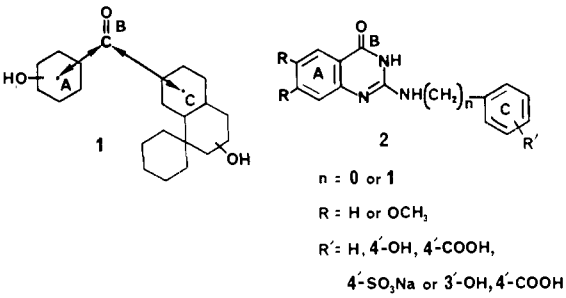
A number of 2-(arylamino)-4(3*H*)-quinazolinones (**2a–i**) that possess several of the pharmacophore moieties necessary for binding to the inhibitor site of the enzyme aldose reductase were synthesized and tested for their ability to inhibit crude aldose reductase obtained from rat lens. Only those quinazolinones that possess an acidic moiety on the 2-(arylamino) substituent were found to display significant inhibitory activity. Of these, the most potent compound is the 4′-CO_2H derivative (**2i**) with an IC_{50} of 34 μM, while the least potent is the 4′-OH derivative (**2c**) with an IC_{50} of 75 μM. All of the 2-(arylamino)-4(3*H*)-quinazolinones tested are less potent than other known inhibitors of aldose reductase, such as alrestatin and sorbinil, indicating that the pharmacophore moieties present in these compounds may not be positioned optimally relative to one another for maximal interaction with the enzyme.

There is a growing body of evidence establishing a link between the formation and accumulation of sorbitol in ocular tissue (lens, retina) and nerve tissue and the development of pathologies associated with chronic diabetes (cataract formation, retinopathy, and neuropathy).[1] Sorbitol is formed in these tissues from glucose in a reaction mediated by the NADPH-dependent enzyme aldose reductase. Therefore, one approach that has been explored to prevent, or at least delay, the onset of diabetic complications has involved the development of specific inhibitors of aldose reductase.[1] A variety of compounds of diverse structure, including alrestatin, sorbinil, tolrestat, statil, and several flavonoids and chromones, have been found to possess significant aldose reductase inhibitory activity.[2] These compounds display either noncompetitive or uncompetitive inhibition, indicating that they do not interact with either the substrate or cofactor binding site on the enzyme.[3] Furthermore, competition studies suggest that all of these compounds interact at a common site on the enzyme—a site designated as the "inhibitor site".[3] Kador and Sharpless determined the pharmacophoric requirements of this inhibitory site by identifying key steric and electronic similarities between structurally different aldose reductase inhibitors.[2,3] They proposed that the minimum pharmacophore requirements include a primary aromatic region and a carbonyl group separated by 2.8–3.8 Å from the center of the primary aromatic region (**1**). They also noted that the presence of a secondary lipophilic moiety located 2.8–6.1 Å from the carbonyl group and hydroxy groups positioned 2.8–3.8 and 8.9–9.3 Å from the primary aromatic region enhanced binding to the inhibitory site.[2,3] In an attempt to obtain novel, more potent and specific inhibitors of aldose reductase, we have prepared a number of 2-(arylamino)-4(3*H*)-quinazolinones **2** that possess several of these basic pharmacophoric moieties; they contain a "primary aromatic region" (A), carbonyl moiety (B), and "secondary aromatic ring" (C), all positioned at the appropriate distances for interaction with the complimentary binding sites on the inhibitory site of aldose reductase.

Chemistry

Synthesis of the 2-(arylamino)-4(3*H*)-quinazolinones **2a–i** is outlined in Scheme I. The target compounds were prepared from the corresponding 2-chloro-4(3*H*)-quinazolinones **7a–b** by a displacement reaction employing commercially available anilines or benzylamine (Tables I and II). The 2-chloro-4(3*H*)-quinazolinones were synthesized from the quinazolinediones **5a–b** by using the general procedures described by Hess and co-workers.[4] Treatment of quinazolinediones **5a–b** with $POCl_3$ in the presence of *N*,*N*-dimethylaniline provided the dichloroquinazolines **6a** and **6b** in greater than 80% yield. Selective hydrolysis of **6a** and **6b** with sodium hydroxide in aqueous THF gave the 2-chloro-4(3*H*)-quinazolinones **7a,b** in near-quantitative yield.



The intermediate 6,7-dimethoxy-2,4(1*H*,3*H*)-quinazolinedione (**5b**) was prepared from methyl 3,4-dimethoxybenzoate (**3**) as described by Curd et al.[5] Nitration of **3** with 70% nitric acid, followed by reduction of the nitro group with stannous chloride, gave methyl 2-amino-4,5-dimethoxybenzoate (**4**). Hydrolysis of the ester moiety of **4**, followed by treatment with potassium cyanate, yielded quinazolinedione **5b**.

Results and Discussion

The ability of the 2-(arylamino)-4(3*H*)-quinazolinones **2a–i** to inhibit crude aldose reductase obtained from rat lens was determined as previously reported by Kador and Sharpless.[6] For those compounds displaying greater than 50% inhibition at a concentration of 100 μM, IC_{50} values were determined from simple linear regressions of the log dose–response curves, using the LINEFIT least-squares program of Barlow.[16] The results of these evaluations are presented in Table II. The quinazolinones displaying the highest level of inhibitory activity are those that possess a carboxyl and/or hydroxy group on the aryl moieties attached to the 2-position of the quinazolinone ring (**2c,d,f,i**). These results are consistent with the observation that virtually all known inhibitors of aldose reductase are

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(1) Lipinski, C. A.; Hutson, N. J. *Annu. Rep. Med. Chem.* **1984**, *19*, 169.
(2) Kador, P. F.; Kinoshita, J. H.; Sharpless, N. E. *J. Med. Chem.* **1985**, *28*, 841.
(3) Kador, P. F.; Sharpless, N. E. *Mol. Pharmacol.* **1983**, *24*, 521.
(4) Hess, H.-J.; Cronin, T. H.; Scriabine, A. *J. Med. Chem.* **1968**, *11*, 130.
(5) Curd, F. H. S.; Landquist, J. K.; Ross, F. L. *J. Chem. Soc.* **1948**, 1759.
(6) Kador, P. F.; Sharpless, N. E. *Biophys. Chem.* **1978**, *8*, 81.

0022-2623/86/1829-0627$01.50/0

Table I. 2-(Arylamino)-4(3*H*)-quinazolinones

compd	R	R[1]	*n*	mp,[a] °C (lit. mp, °C)	recrystn solvent	yield,[b] %	formula[c]	anal.
2a	H	H	1	212–215 (213–214.5)[d]	EtOH	83	$C_{15}H_{13}N_3O$	C, H, N
2b	H	H	0	234–238 (260–263)[e]	EtOH–H_2O	65	$C_{14}H_{11}N_3O\cdot0.5H_2O$	C, H, N
2c	H	4′-OH	0	>300 (334–336)[e]	DMF–H_2O	83	$C_{14}H_{11}N_3O_2\cdot0.17DMF\cdot H_2O$	C, H, N
2d	H	4′-CO_2H	0	>300	EtOH–H_2O	89	$C_{15}H_{11}N_3O_3$	C, H, N
2e	H	4′-SO_3Na	0	>300	EtOH–H_2O	57	$C_{14}H_{10}N_3O_4SNa\cdot0.5H_2O$	C, H, N, S
2f	H	3′-OH,4′-CO_2H	0	>300	EtOH	72	$C_{15}H_{11}N_3O_4$	C, H, N
2g	OCH_3	H	1	245–247 (245–247)[f]	EtOH	87	$C_{17}H_{17}N_3O_3\cdot0.5EtOH$	C, H, N
2h	OCH_3	H	0	260–265 (267–270)[f]	EtOH	86	$C_{16}H_{15}N_{303}\cdot0.5H_2O$	C, H, N
2i	OCH_3	4′-CO_2H	0	>300	EtOH–H_2O	56	$C_{17}H_{15}N_3O_5\cdot1.75H_2O$	C, H, N

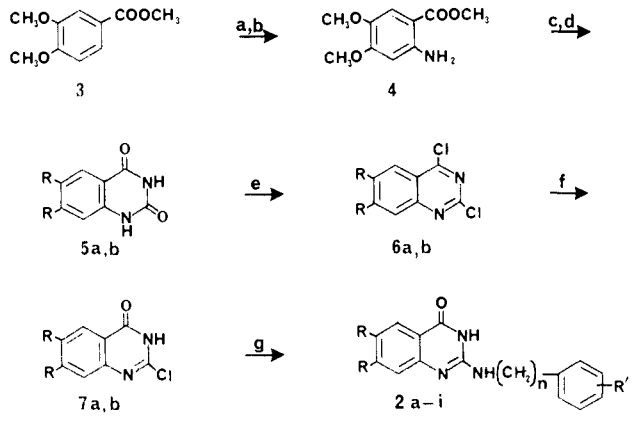
[a] All melting points were determined in open capillary tubes and are uncorrected. [b] These values represent the yield of analytically pure product obtained from the final displacement reactions (**7a** or **7b** to **2a–i**). No attempt was made to optimize yields. [c] All products exhibited IR and ^1H NMR spectra consistent with assigned structures. The homogeneity of these products was determined by reverse-phase HPLC with two different mobile phase solvent systems (see Experimental Section). All products gave satisfactory C, H, N analyses. The presence of DMF in crystalline **2c**, EtOH in **2g**, and H_2O in **2b, e, h,** i was also demonstrated in the ^1H NMR spectra of these compounds. [d] Reference 14. [e] Reference 15. [f] Reference 4.

Table II. Aldose Reductase Inhibitory Activity of the 2-(Arylamino)-4(3*H*)-quinazolinones[a]

compd	% inhibn at 100 μM (SEM)[b]	IC_{50},[c] μM	compd	% inhibn at 100 μM (SEM)[b]	IC_{50},[c] μM
2a	3.9 (2.3)		**2g**	22.4 (1.1)	
2b	5.9 (1.3)		**2h**	17.4 (6.1)	
2c		75	**2i**		34
2d		50	**10**		214
2e	42.6 (8.1)		sorbinil		0.02[d]
2f		57			

[a] The ability of **2a–i** to inhibit crude aldose reductase obtained from rat lens was determined by using the procedures described by Kador and Sharpless.[6] [b] Standard error of the mean (SEM). [c] IC_{50} values were determined by linear regression of the log dose–response curves by using the LINEFIT least-square curve-fitting program of Barlow.[16] In each case, the significance level for the least square fit was <0.01 (determined from the *T* value). The log dose–response curves were generated by using at least three concentrations of each inhibitor, with three replicates at each inhibitor concentration. [d] The IC_{50} value reported in the literature for sorbinil is 0.07 μM.[3]

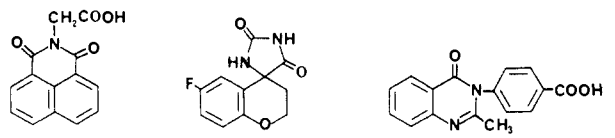
Scheme I



[a] 70% HNO_3, HOAc. [b] $SnCl_2$, HCl. [c] NaOH, MeOH, H_2O. [d] KOCN, H_2O, HOAc. [e] $PhNMe_2$, $POCl_3$. [f] NaOH, H_2O, THF. [g] Anilines or benzylamine, DMF.

weak organic acids. Quinazolinones **2c,d,f,i** display similar potencies with IC_{50} values in the 10–100-μM range. The most potent compound of this series is the 4′-CO_2H derivative **2i** with an IC_{50} value of 34 μM, while the least potent compound is the 4′-OH derivative **2c** with an IC_{50} of 75 μM.

Many known inhibitors of aldose reductase have significantly greater potencies in the rat lens assay than quinazolinone derivatives **2a–i**. For example, alrestatin (**8**) and sorbinil (**9**) have IC_{50} values of 1.5[7] and 0.02 μM, respectively, in this assay. Therefore, it is clear that, even though quinazolinones **2a–i** possess three of the basic pharmacophoric moieties for optimal interaction with the enzyme's inhibitory site, they are considerably less potent than aldose reductase inhibitors such as alrestatin and sorbinil, which have only the minimal pharmacophore requirements (a "primary aromatic ring" linked to a carbonyl moiety).



One possible explanation for the low inhibitory activity observed for compounds **2a–i** may be that the pharmacophoric moieities present in these compounds are not optimally positioned relative to one another for simultaneous interaction with complimentary binding sites present on the enzyme. For example, the spatial relationship between the aromatic ring of the quinazolinone nucleus and the aromatic ring of the 2-(arylamino) substituent may be such that it is not possible for both rings to interact simultaneously with the enzyme. To explore this possibility, we synthesized and evaluated several 3-aryl-substituted quinazolinones that represent derivatives of **2a–i** in which the relative positions of the two aromatic rings are varied. These derivatives, however, were found to be even less potent as inhibitors of aldose reductase than the corresponding 2-(arylamino)-4(3*H*)-quinazolines; compound **10** (see Experimental Section) has an IC_{50} value of only 214 μM in the rat lens assay. Nevertheless, this finding supports the conclusion that the relative positions of key pharmacophoric moieties present in the quinazolinones are important in determining the ability of these compounds to interact with the enzyme. Therefore, we are continuing our studies using these, and related heterocycles, in an attempt to determine how varying the spatial relationship of key pharmacophores influences aldose reductase inhibitory activity.

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded with a Beckman 4230 spectrophotometer, and ^1H NMR spectra were recorded on a Varian T-60A spec-

(7) Kador, P. F.; Goosey, J. D.; Sharpless, M. E.; Kolish, J.; Miller, O. D. *Eur. J. Med. Chem.* **1981**, *16*, 293.

trometer with tetramethylsilane as an internal standard. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and are within ±0.4 of the theoretical percentages. HPLC analyses of all products were carried out with a Waters Model 6000A liquid chromatograph equipped with a Zorbax ODS C-18 column (4.6 mm × 15 cm) and a Waters Model 440 absorbance detector set at 254 nm. Two different eluting solvent systems (60% MeOH–H_2O and 80% MeOH–H_2O) were used for these analyses. Common reagent grade chemicals were purchased from Aldrich Chemical Co. and were used as received.

Methyl 2-Amino-4,5-dimethoxybenzoate (4). This compound was prepared from methyl 3,4-dimethoxybenzoate (58.5 g, 300 mmol) as described by Curd et al.[5] Recrystallization from benzene afforded 26.3 g (68%) of 4 as fine yellow needles: mp 125–128 °C (lit.[9] mp 128–133 °C).

6,7-Dimethoxy-2,4(1*H*,3*H*)-quinazolinedione (5b). This compound was prepared from 4 (13.0 g, 62 mmol) as described by Curd et al.[5] Recrystallization from absolute ethanol gave 9.0 g (98%) of **5b** as a fine white powder: mp 275–280 °C (lit.[5] mp 270–280 °C).

2,4-Dichloro-6,7-dimethoxyquinazoline (6b). This compound was prepared from **5b** (6.5 g, 29 mmol) as described by Hess et al.[4] Recrystallization from methanol gave 5.8 g (85%) of **6b** as long yellow needles: mp 173–176 °C (lit.[4] mp 175–177 °C).

2-Chloro-6,7-dimethoxy-4(3*H*)-quinazolinone (7b). This compound was prepared from **5b** (5.3 g, 20 mmol) as described by Hess et al.:[4] mp 269–272 °C (lit.[4] mp 270–272 °C).

2,4-Dichloroquinazoline (6a). This compound was synthesized from **5a** (9.3 g, 57 mmol) by using the same procedure used for the preparation of **6b**. Recrystallization from methanol gave 9.8 g (87%) of **6a** as feathery yellow needles: mp 115–117 °C (lit.[11] mp 117–118 °C).

2-Chloro-4(3*H*)-quinazolinone (7a). This compound was prepared from **6a** (7.96 g, 40 mmol) by employing the same procedure used for the preparation of **7b**. Recrystallization from methanol yielded 6.96 g (96%) of **7a** as long white needles: mp 214–218 °C (lit.[12] mp 216–218 °C).

2-Substituted 4(3*H*)-Quinazolinones 2a–i. All of the target quinazolinones were prepared by stirring a heated (80–100 °C) mixture of the 2-chloroquinazolinone derivative (**7a** or **7b**, 2 mmol) with the appropriate aniline (6.0 mmol) or benzylamine (6.0 mmol) either neat (with liquid amines) on in DMF (10 mL) for 1–5 h. The reaction mixtures were then cooled to room temperature, and the solvent was removed in vacuo. The residual solid was then suspended in warm H_2O (10 mL), filtered, and washed with ethanol. The solvents employed for recrystallization, as well as the physical properties of the quinazolinone products, are provided in Table I.

2-Methyl-3-(4-carboxyphenyl)-4(3*H*)-quinazolinone (10). This compound was prepared by the general method reported by Grimmel et al.[13] A solution of PCl_3 (1.15 g, 25 mmol) in toluene (10 mL) was added dropwise, over a 15-min period, to a stirred suspension of *N*-acetylanthranilic acid (4.48 g, 25 mmol) and 4-aminobenzoic acid methyl ester (3.78 g, 25 mmol) in toluene (50 mL). The mixture was stirred at reflux for 1.5 h and then concentrated in vacuo to yield a yellow solid. The solid was suspended in 10% Na_2CO_3 (200 mL) and extracted with $CHCl_3$ (2 × 200 mL). The combined $CHCl_3$ extracts were washed with H_2O (200 mL) and evaporated to dryness to give a yellow powder. Column chromatography using Florisil (50 g) and benzene as the eluting solvent (2 L) yielded 2-methyl-3-(4-carbomethoxyphenyl)-4(3*H*)-quinazolinone (4.6 g, 63%): mp 199–200 °C; IR (Fluorolube) 1715, 1690 cm^{-1} (C=O); ^1H NMR ($CDCl_3$ + $(CD_3)_2SO$) δ 2.13 (s, 3 H), 3.82 (s, 3 H), 7.1–8.1 (complex m, 8 H). The intermediate methyl ester (1.47 g, 5 mmol) in 6 N HCl (75 mL) was stirred at 65–70 °C for 20 min. Cooling of this solution (ice bath), followed by the addition of crushed ice (100 mL), yielded a white precipitate, which was isolated by filtration. The solid was suspended in H_2O (50 mL), NH_4OH (2 mL) was added, and the resulting solution was washed with CH_2Cl_2 (2 × 50 mL). The aqueous solution was then acidified (concentrated HCl), yielding a white, granular solid. This solid was isolated by filtration, washed with a small quantity of cold H_2O, and recrystallized from ethanol to give 1.1 g (79%) of **10** as fine white needles: mp 265–270 °C dec; IR (Fluorolube) 1695 cm^{-1} (broad absorbance C=O); ^1H NMR ($CDCl_3$ and $(CD_3)_2SO$) δ 2.12 (s, 3 H), 7.2–8.1 (complex m, 9 H). Anal. ($C_{16}H_{12}N_2O_3$) C, H, N.

Enzyme Assay. Crude aldose reductase was obtained from rat lens as described by Kador and Sharpless.[6] Enzyme activity was assayed spectrophotometrically, determining the decrease in NADPH concentration at 340 nm in a Guilford 2400-2 automated compensating double-beam spectrophotometer. The assay mixture contained 0.1 M phosphate buffer, pH 6.2, 0.104 mM NADPH (sigma type I), 1.5 mM DL-glyceraldehyde, and 0.1 mL of supernatant containing the enzyme, in a total volume of 1.0 mL. The reference blank contained all of the above compounds except for the substrate glyceraldehyde. The percent inhibition of each compound was calculated by comparing the reaction rate of the solution containing the substrate as well as the inhibitor with that of control solutions containing only the substrate. Inhibitor IC_{50} values were obtained for quinazolinones **2c,d,f,i**, **10**, and sorbinil from simple linear regressions of the log dose–response curves by using the LINEFIT least-square curve-fitting program of Barlow.[16]

Acknowledgment. We thank Dr. Nancy Hutson of Pfizer Central Research, Groton, CT, for providing us with a sample of sorbinil.

Registry No. **2a**, 1791-50-0; **2b**, 4248-15-1; **2c**, 6499-59-8; **2d**, 100448-60-0; **2e**, 100466-13-5; **2f**, 100448-61-1; **2g**, 20198-38-3; **2h**, 20198-40-7; **2i**, 100448-62-2; **3**, 2150-38-1; **4**, 26759-46-6; **5a**, 86-96-4; **5b**, 28888-44-0; **6a**, 607-68-1; **6b**, 27631-29-4; **7a**, 607-69-2; **7b**, 20197-86-8; **10**, 4005-05-4; 4-sulfoaniline sodium salt, 515-74-2; 2-hydroxy-4-aminobenzoic acid, 65-49-6; *N*-acetylanthranilic acid, 89-52-1; 2-methyl-3-(4-carbomethoxyphenyl)-4(3H)-quinazolinone, 35218-84-9; benzylamine, 100-46-9; aniline, 62-53-3; 4-hydroxyaniline, 123-30-8; 4-carboxyaniline, 150-13-0; methyl 4-aminobenzoate, 619-45-4; aldose reductase, 9028-31-3.

(8) Pollock, J. R. A.; Stevens, R. "Dictionary of Organic Compounds", 4th ed.; Oxford University Press: New York, 1965; p 1132.
(9) Pollock, J. R. A.; Stevens, R. "Dictionary of Organic Compounds", 4th ed.; Oxford University Press: New York, 1965; p 120.
(10) Fetscher, C. A.; Bogert, M. T. *J. Org. Chem.* **1939**, *4*, 71.
(11) Scarborough, H. C.; Lawes, B. C.; Minielli, J. L.; Compton, J. L. *J. Org. Chem.* **1962**, *27*, 957.
(12) Curd, F. H. S.; Landquist, J. H.; Rose, F. L. *J. Chem. Soc.* **1947**, 775.
(13) Grimmel, H. W.; Guenther, A.; Morgan, J. F. *J. Am. Chem. Soc.* **1946**, *68*, 542.
(14) Grout, R. J.; Partridge, M. W. *J. Chem. Soc.* **1960**, 3540.
(15) Manolov, E. *Khim. Ind.* **1965**, *37*, 372.
(16) Barlow, R. B. "Biodata Handling with Microcomputers"; Elsevier: Amsterdam, 1983; p 76.