(Pyrimidinyloxy)acetic Acids and Pyrimidineacetic Acids as a Novel Class of Aldose Reductase Inhibitors

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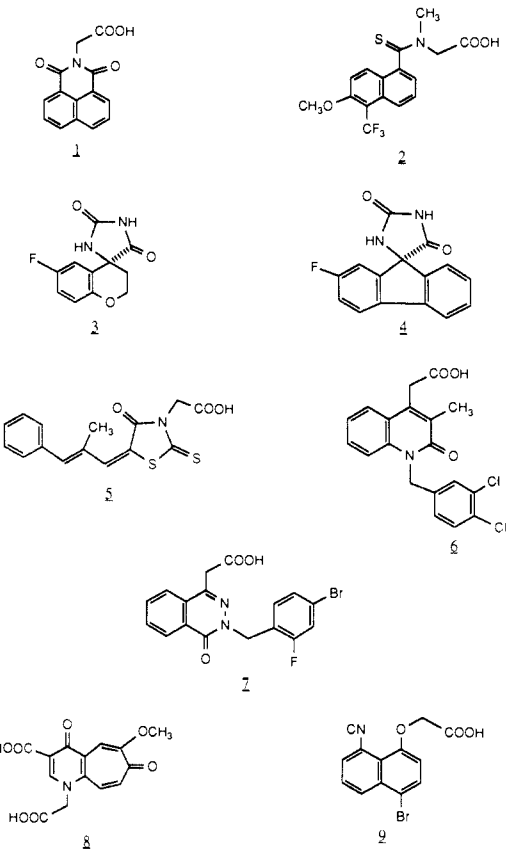
Pyrimidineacetic acids and (pyrimidinyloxy)acetic acids were synthesized by alkylation, with methyl bromoacetate or *tert*-butyl bromoacetate as alkylating agents. Alkylation reaction at the nitrogen or oxygen atom for different substrates was found to be solvent dependent. N-Alkylation was favored in ethereal solvent, e.g., tetrahydrofuran and dimethoxyethane, whereas O-alkylation was predominant in dimethylformamide. These compounds were tested in vitro to determine their ability to inhibit bovine lens aldose reductase. Selected compounds were assayed in vivo, in a 4-day galactose-fed rat model. The decrease in galactitol from the control was determined in lens, nerve, and diaphragm. Several of the 6-oxopyrimidine-1-acetic acids and (pyrimidinyl-4-oxy)acetic acids were found to be potent inhibitors of bovine lens aldose reductase. A study was also undertaken to determine in vitro the transport behavior of selected compounds in the isolated rat sciatic nerve. A discussion of the structure–activity relationship of this class of compounds with reference to their intrinsic biochemical activity is reported. It is concluded, in general, that ability of a compound to penetrate the tissue membrane plays an important role in the genesis of in vivo lens aldose reductase (LAR) inhibitory activity.

Despite the beneficial effects of the insulin therapy, the long-term diabetic complications, such as neuropathy, nephropathy, retinopathy, and cataracts, continue to develop. This mainly results from the fact that the tissues involved in these complications do not require insulin for glucose uptake. In patients treated with insulin, normalization of glucose level can only be effected partially. High intracellular sugar concentrations in the diabetics or in experimentally induced galactosemia increases the activity of the enzyme aldose reductase and the flux of the sugar through the polyol pathway.[1] There is a great deal of scientific evidence to suggest that the accumulation of polyol in tissue contributes to the development of diabetic complications, and inhibition of aldose reductase is a useful strategy for the prevention of the long-term diabetic disorders.

The first aldose reductase inhibitor found to be orally active was alrestatin[2] (1). Structurally, it is an *N*-acetic acid derivative of a cyclic imide. Following that lead, a highly potent *N*-acetic acid derivative tolrestat (Alredase) 2 was developed[3] in our laboratories.

The presence of an acidic proton appears to be mandatory for the expression of the inhibition of aldose reductase. This is substantiated by the structures of several inhibitors, presently undergoing clinical studies. Sorbinil[4] (3) and alconil[5] (4) possess the acidic hydrogen attached to the imidic nitrogen. In contrast, all the other agents, 1, 2, epalrestat (5),[6] ICI-105552 (6),[7] and statil (7),[8] carry an acetic acid moiety attached either to a carbon or nitrogen atom. Also, we have observed in vitro aldose reductase inhibitory (ARI) activity associated with molecules such as 8 and 9. Whereas 8 was devoid of in vivo activity,[9] 9 displayed weak in vivo activity.[10] These observations prompted us to synthesize pyrimidine derivatives having *N*- and *O*-acetic acid substituents. In this article we report the synthesis and biological results of some pyrimidine-*N*-acetic acids and (pyrimidinyloxy)acetic acids.

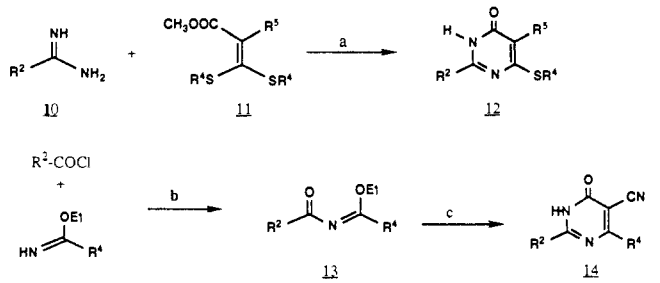
Chart I



Chemistry

In the past, 3,4-dihydro-2-methyl-4-(methylthio)-6-oxo-5-pyrimidinecarbonitrile (12) (R^5 = CN) has served us well, as a key synthon, in other unrelated synthetic programs.[11] It was also utilized as a starting material in the present work.

Scheme I[a]



[a] (a) NaH, DMF. (b) $(Et)_3N$, toluene. (c) $CNCH_2CONH_2$, NaOEt/ethanol.

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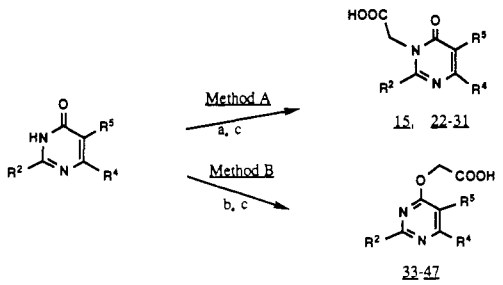
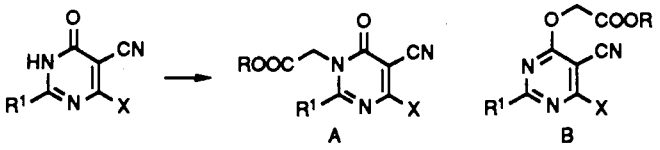
Scheme II[a]



[a] (a) NaH, dimethoxyethane or tetrahydrofuran, $BrCH_2COOX$. (b) NaH, dimethylformamide, $BrCH_2COOX$. (c) 1 N NaOH, methanol or dioxane (X = CH_3) or CF_3COOH (X = *tert*-butyl).

Table I. Effect of Solvent on Alkylation



compound no.	R^1	X	solvent[a]	A	B
46	1-naphthyl	CH_3	THF	5	1
			DMF	1	4
47	1-naphthyl	phenyl	THF	13	1
			DMF	1	5
28	5-Br-1-naphthyl	phenyl	THF	15	1
			DMF	1	11
	5-Br-1-naphthyl	isopropyl	THF	2	1
			DMF	1	2
25	cyclohexyl	SCH_3	DME	20	1
			DMF	1	1.4
33	phenyl	SCH_3	THF		100
			DMF		100
34	*tert*-butyl	SCH_3	THF		100
			DMF		100

[a] THF, tetrahydrofuran; DMF, dimethylformamide; DME, dimethoxyethane.

The compounds described in this article were synthesized as follows. Two general strategies were used for the preparation of oxopyrimidines (Scheme I). The synthesis of 6-oxopyrimidines **12** bearing a 4-alkylthio substituent and having different substituents at C-5 was achieved in a manner described earlier.[11] Amidine **10** was condensed with an appropriately substituted 3,3-bis(alkylthio)-2-acrylic acid methyl ester **11**. The compounds **11** were synthesized from the corresponding dipotassium salts as described before.[12]

The preparation of oxopyrimidines **14**, where alkyl or aryl groups are directly substituted on the pyrimidines, was carried out by an alternative route (Scheme I). The corresponding imino ethers were acylated with an appropriate acid chloride to yield the *N*-acyl derivative **13**. Condensation of **13** with cyanoacetamide under basic conditions yielded[13] 4-alkyl- or -aryl-6-oxopyrimidines **14**.

Alkylation of oxopyrimidines **12** and **14** led to *N*- and *O*-acetic acid esters, which were hydrolyzed to the corresponding acids (Scheme II). Alkylation was carried out with methyl or *tert*-butyl bromoacetate in the presence of sodium hydride. It was noted that the proportion of N- and O-alkylated products varied depending on the solvent employed. Thus, using ethereal solvents such as dimethoxyethane or tetrahydrofuran we observed a preferential formation of N-alkylated product. In contrast, when the

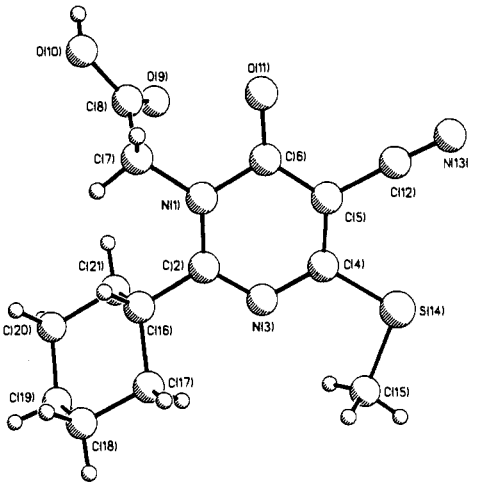
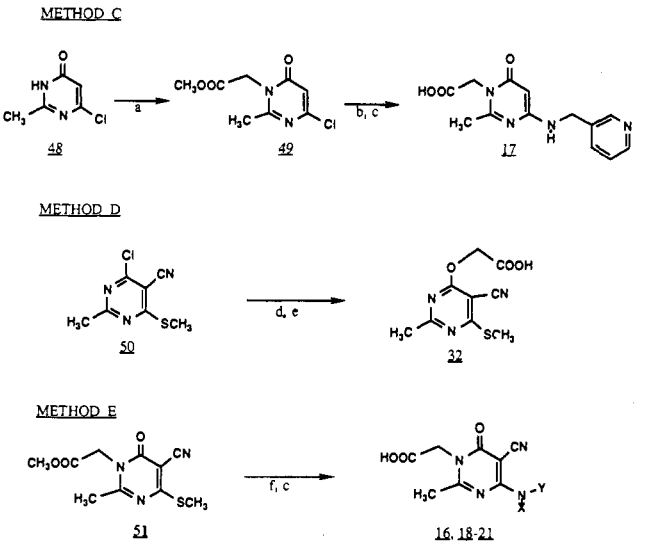


Figure 1. Single-crystal X-ray structure of pyrimidineacetic acid **25**.

Scheme III[a]



[a] (a) NaH, $BrCH_2COOCH_3$/DMF. (b) 3-(Aminomethyl)-pyridine/THF. (c) NaOH, water/methanol. (d) NaH, $HOCH_2COOC_2H_5$/THF. (e) NaOH, water/dioxane. (f) HNXY, dimethoxyethane.

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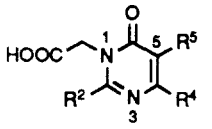
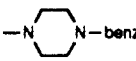
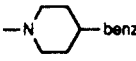
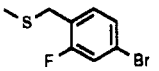
(4) Sarges, R. U.S. Patent 4 130 714, December 19, 1978.
(5) Griffin, B. W.; Chandler, M. L.; Desantis, L. *Invest., Ophthalmol., Vis. Sci.* **1984**, *25* (Suppl 2), 136.
(6) Terashima, H.; Hama, K.; Yamamoto, R.; Tsuboshima, M.; Hikkawa, R.; Hatanaka, I.; Shigeta, Y. *J. Pharmacol. Exp. Ther.* **1984**, *229*, 226.
(7) Poulsom, R.; Heath, H. *Biochem. Pharmacol.* **1983**, *32*, 1495.
(8) Stribling, D.; Mirrlus, D. J.; Earl, D. C. N. *Diabetologia* **1983**, *25*, 196.
(9) Bagli, J. Unpublished results.
(10) Sestanj, K. Personal communication.
(11) Bagli, J.; Bogri, T.; Palameta, B.; Rakhit, S.; Peseckis, S.; McQuillan, J.; Lee, D. K. H. *J. Med. Chem.* **1988**, *31*, 814.
(12) Jensen, K. A.; Henrikson, L. *Acta Chem. Scand.* **1968**, *22*, 1107.
(13) Soto, J. L. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2447.

Table II. In Vitro Results and Physical Properties of Substituted 6-Oxopyrimidine-N^1-acetic Acids



no.	R^2	R^4	R^5	% yield	method	formula (anal.)	mp, °C	cryst solv[a]	in vitro[b] 10^{-5} M	10^{-6} M	10^{-7} M
15	CH_3	SCH_3	CN	45	B	$C_9H_9N_3O_3S$	236–8	M	86	84	74
16	CH_3	$HNCH_2$-3-pyridinyl	CN	68	E	$C_{14}H_{13}N_5O_3$	>300	D/M	88	90	30
17	CH_3	$HNCH_2$-3-pyridinyl	H	51	C	$C_{13}H_{14}N_4O_2$	300 dec	W/M	53	18	
18	CH_3	$HNCH_2$-cyclohexyl	CN	62	E	$C_{15}H_{20}N_4O_2$	>290	W/M	93	91	72
19	CH_3	$HNCH_2$ Ph	CN	83	E	$C_{15}H_{14}N_4O_3$	222–4	M/E	96	90	78
20	CH_3		CN	67	E	$C_{19}H_{21}N_5O_3$	276–8	M/E	92	89	64
21	CH_3		CN	19	E	$C_{20}H_{22}N_4O_3$	183–4	C/H	90	88	81
22	isopropyl	SCH_3	CN	30	A	$C_{11}H_{13}N_3O_3S$	226–8	M	96	96	94
23	isopropyl	S-*i*-Pr	CN	26	A	$C_{13}H_{17}N_3O_3S$	192.5–3	AN	96	97	91
24	isopropyl	benzylthio	CN	38	A	$C_{17}H_{17}N_3O_3S$	194–5	AN	96	98	94
25	cyclohexyl	SCH_3	CN	47	A	$C_{14}H_{17}N_3O_3S$	198–9	AN	98	94	89
26	cyclohexyl		CN	73	A	$C_{20}H_{19}BrFN_3O_3S$	183–4	T	96	94	92
27	α-naphthyl	Ph	CN	49	A	$C_{23}H_{15}N_3O_3$	235–6	EA/H	84	68	29
28	5-bromo-α-naphthyl	Ph	CN	48	A	$C_{23}H_{14}BrN_3O_3$	234–6	EA/H	82	71	22
29	5-bromo-α-naphthyl	α-naphthalenylmethyl	CN	55	A	$C_{28}H_{18}BrN_3O_3$	192–3	EA/H	93	85	46
30	cyclohexyl	SCH_3	SO_2-phenyl	35	A	$C_{19}H_{22}N_2O_5S_2$	239–241	AN	3	0	
31	cyclohexyl	SCH_3	$COOCH_3$	52	A	$C_{15}H_{20}N_2O_5S$	208–10	AN	5	1	
tolrestat (2)[c]									98	94	65

[a] M = methanol, D = dimethylformamide, E = ether, AN = acetonitrile, T = toluene, EA = ethyl acetate; W = water, H = hexanes, C = chloroform. [b] The number represents percent inhibition of aldose reductase at the concentrations used. [c] In vitro IC_{50} for tolrestat 3.5×10^{-8} M (see ref 18).

reaction was conducted in dimethylformamide, a preponderance of O-alkylation was observed. Some examples of this solvent effect are shown in Table I. The ratio of N-alkylation to O-alkylation varied from 2:1 to greater than 20:1 in ethereal solvents. Alkylation of 2-phenyl- and 2-*tert*-butyl-4-(methylthio)-6-oxo-5-pyrimidinecarbonitriles exclusively yielded O-alkylated products **33** and **34**, respectively, regardless of the solvents used. 2-Cyclohexyl-4-(methylthio)-6-oxo-5-pyrimidinecarbonitrile was found to demonstrate the least selectivity (1:1.4 N-alkyl:O-alkyl) for O-alkylation.

We have reported earlier[11] that oxopyrimidines of the type **12** and **14** are protonated at nitrogen in the position α to the carbonyl. This was earlier confirmed by the single-crystal X-ray structure of pelrinone. On the basis of our earlier observation we have assumed N-alkylation to occur at the nitrogen α to the carbonyl. This was further confirmed by the single-crystal X-ray structure of 2-cyclohexyl-5-cyano-1,6-dihydro-4-(methylthio)-6-oxo-1-pyrimidineacetic acid (**25**, Figure 1).

Compound **17** was synthesized by alkylation of oxopyrimidine **48**[11] to yield *N*-alkyl ester **49** (Scheme III). Treatment of **49** with 3-(aminomethyl)pyridine, followed by hydrolysis, led to the formation of **17**. Treatment of chloropyrimidine **50** with glycolic acid ethyl ester, in the presence of sodium hydride, in tetrahydrofuran, led to an ester which upon hydrolysis generated acid **32**. Treatment of *N*-acetic acid ester **51** with appropriate amines led to the corresponding 5-aminated pyrimidines. These, upon hydrolysis, yielded compounds **16** and **18–21**.

Biological Results and Discussion

The goal of the present study was to identify orally active inhibitors of aldose reductase. The compounds described in this article were first tested for their in vitro activity, as inhibitors of partially purified bovine lens aldose reductase,[14] at three different concentrations. These results are shown in Tables II and III.

In general, at a concentration of 10^{-5} M, all the *N*- and *O*-acetic acids having a nitrile group at the C-5 position showed >80% inhibition of enzyme activity. In order to establish the importance of this group of the biological response, a descyano compound **17** was synthesized. Compared to the nitrile analogue **16**, the descyano compound **17** showed only marginal inhibitory activity (53% inhibition) at 10^{-5} M. Replacement of the nitrile of compounds **25** and **34** by other electron-withdrawing moieties such as phenylsulfonyl (**30**), carbomethoxy (**31**, **45**), and carboxamide (**44**) in both *N*- and *O*-acetic acid series led to complete loss or significant drop in the inhibitory activity. The amide **44** did retain activity at the higher concentration (10^{-5} M), but a marked drop was observed at the lower (10^{-6}, 10^{-7} M) concentration compared to nitrile analogue **34**.

Extensive conjugation of pyrimidineacetic acids with naphthalene at C-2 and phenyl at C-4 (**27**, **28**) or with naphthalene alone as in **29** leads to a marked drop in the in vitro activity at 10^{-7} M. A similar drop in the in vitro potency is also observed for aryl conjugation in (pyrimidinyloxy)acetic acids **46** and **47**. This loss of activity cannot be attributed to the steric bulk of the aromatic nucleus. Compounds with bulky substituents at C-2 and/or C-4 such as **26** and **21** in the *N*-acetic acid series and **39** and **42** in the oxyacetic acids demonstrate good in vitro potency at 10^{-7} M. A possible explanation for the reduced potency of these compounds may be the purturbation of the electronic character of the pyrimidine ring due to conjugation with aromatic ring. This electronic interaction may be crucial for the generation of optimal biological response. Similar loss of biological activity due to aryl conjugation of pyrimidines was also observed in other biological studies.[11]

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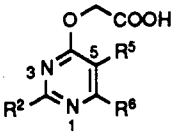
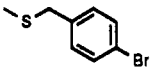
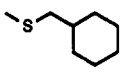
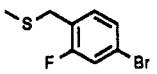
Table III. In Vitro Results and Physical Properties of (Pyrimidinyl-4-oxy)acetic Acids



no.	R^2	R^5	R^6	% yield	method	formula (anal.)	mp, °C	cryst[a] solv	in vitro[c] 10^{-5} M	10^{-6} M	10^{-7} M
32	CH_3	CN	SCH_3	15	D	$C_9H_9N_3O_3S$	205–6	M/W	90	84	55
33	phenyl	CN	SCH_3	74	A	$C_{14}H_{11}N_3O_3S$	223–4	M/E	92	87	75
34	*tert*-butyl	CN	SCH_3	35	B	$C_{12}H_{15}N_3O_3S$	212–5	M/E	89	88	82
35	neopentyl	CN	SCH_3	44	B	$C_{13}H_{17}N_3O_3S$	155–7	M/E	95	96	85
36	isopropyl	CN	SCH_3	88	B	$C_{11}H_{13}N_3O_3S$	158–60	M/E	98	97	88
37	phenyl	CN	CH_3	46	B	$C_{14}H_{11}N_3O_3$	228–9	M	92	88	79
38	*tert*-butyl	CN	SCH_2Ph	70	B	$C_{18}H_{19}N_3O_3S$	126–9	M/E	86	85	82
39	*tert*-butyl	CN		50	B	$C_{18}H_{18}BrN_3O_3S$	160–2	T/H	95	95	95
40	*tert*-butyl	CN	-S-*n*-C_6H_{13}	20	B	$C_{17}H_{25}N_3O_3S$	91–2	E/H	94	94	91
41	*tert*-butyl	CN	-S-*i*-Pr	36	B	$C_{16}H_{21}N_3O_3S$	120–2	T/H	92	91	89
42	*tert*-butyl	CN		40	B	$C_{18}H_{25}N_3O_3S$	126–8	AN	97	96	88
43	*tert*-butyl	CN		52	B	$C_{18}H_{17}BrFN_3O_3S$	154–5	T/H	98	93	93
44	*tert*-butyl	$CONH_2$	SCH_3	24[b]		$C_{12}H_{17}N_3O_4S$	210–2	AN	81	42	10
45	*tert*-butyl	$COOCH_3$	SCH_3	15	A	$C_{13}H_{18}N_2O_5S$	103	T/H	13	2	
46	α-naphthyl	CN	CH_3	32	B	$C_{18}H_{13}N_3O_3$	198–9.5	M	93	85	46
47	α-naphthyl	CN	phenyl	23	B	$C_{23}H_{15}N_3O_3$	187–8	EA/H	79	76	53
tolrestat (**2**)									98	94	65

[a] See footnote *a* of Table II. [b] See Experimental Section for method of preparation. [c] See footnote *b* of Table II.

It is apparent that a heteroatom at C-4 in the *N*-acetic acid series and at C-6 in the *O*-acetic acid series has a beneficial effect on the biological activity. This is confirmed in compounds having a sulfur atom at these positions. Thus, compounds in both series with an alkylthio or aralkylthio substituent retain excellent activity. Furthermore, the biological activity is insensitive to change from methylthio (**22** and **25**) to aralkylthio (as in **24** and **26**) in N-substituted compounds. A similar effect is observed in *O*-acetic acids. Changing the methylthio moiety (**34**) to aralkylthio in **38**, **39**, and **43** caused little change in the in vitro potency of the compounds at 10^{-7} M. Also altering the acyclic grouping of **40** to a cyclic moiety in **42** did not affect the potency of the compound in vitro at low concentration. These observations suggest that this portion of the pyrimidine molecule may interact with a secondary lipophilic site rather than with the active site of the enzyme. An analogous pattern of structure–activity was observed for pyrimidine series in other biological areas.[11]

Increasing the steric bulk at the C-2 position in both *N*- and *O*-acetic series appears to improve the in vitro activity at low concentration. Thus changing the methyl group of **15** to isopropyl (**22**) or to cyclohexyl (**25**) resulted in improved potency in vitro. Similarly, replacement of methyl in oxyacetic acid **32** by isopropyl (**36**), *tert*-butyl (**34**), and neopentyl (**35**) also resulted in improvement of biological activity at 10^{-7} M.

The in vivo activity of these compounds was assayed in a 4-day galactose-fed rat model.[15,18] The lowering of the galactitol levels was measured in lens, nerve, and diaphragm. The high in vitro activity observed for these compounds was not accompanied by equally high activity in the in vivo model. At high doses modest in vivo activity was observed. In general, oxygen-substituted analogues had better in vivo activity than the *N*-acetic acids. Marginal activity was observed for compound **26** in the rat lens.

The results of the in vivo assay are shown in Table IV. Of the five *N*-acetic acids tested, three showed activity in the nerve and two of these (**24** and **25**) were also found to

Table IV. In Vivo Inhibition of Polyol Accumulation by Pyrimidine-*N*-acetic Acids and (Pyrimidinyloxy)acetic Acid in Galactosemic Rats

no.	dose, mg/kg po	% reduction in galactitol levels: lens[a]	nerve	diaphragm
	Pyrimidine-*N*-acetic Acids			
15	71			
24	102			38
25	90		47	42
26	93	15	50	
29	151		30	
	(Pyrimidinyloxy)acetic Acids			
32	71			
33	96			55
34	83		35	32
36	78		37	
38	97		46	67
39	135			58
40	112		43	60
41	82		37	19
42	105		70	60
43	99		28	47
tolrestat[b]	6		53	90

[a] Except for **26**, all other compounds were inactive for reducing galactitol levels in lens. [b] ID_{50} for tolrestat in sciatic nerve was 8 mg/kg per day, in the lens was 178 mg/kg per day (see ref 18) and in diaphragm was 1.5 mg/kg per day.

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be active in the diaphragm. In the case of *O*-acetic acid series most of the compounds were active in the diaphragm and nerve. It is apparent that the oxygen-substituted derivatives can be transported to the active site in the nerve and diaphragm better than the *N*-acetic acids.

Although neither of the C-2 methyl analogues **15** (*N*-acetic acid) nor **32** (*O*-acetic acid) had in vitro activity at 71 mg/kg, an increase of the lipophilicity of the C-2 substituent in a systematic manner conferred in vivo activity on the cyclohexyl analogue **25**. Further change of the lipophilicity at C-6 from SCH_3 to S-aralkyl (**26**) made little change in the in vivo activity in the nerve.

In the case of *O*-acetic acid series, a systematic change from a C-2 methyl substituent to a C-2 *tert*-butyl (**34**) led to the appearance of in vivo activity. Retaining *tert*-butyl at C-2, efforts were made to vary the liphophilicity of the group at C-6 (**38–43**). As seen from Table IV, the best in vivo activity was observed for compound **42**, having a *tert*-butyl group at C-2 and (cyclohexylmethyl)thio group at C-6, at 105 mg/kg po.

The effectiveness of a compound in vivo depends on a number of pharmacokinetic factors including its ability to cross a biological membrane. The in vitro nerve assay was therefore designed to examine this parameter.

In an effort to study the penetration of selected compounds into the nerve tissue they were tested for their ability to inhibit polyol accumulation in an in vitro assay using isolated rat sciatic nerve. In general, it was observed that compounds which demonstrated no vivo activity in the nerve also exhibited low inhibition in the in vitro nerve assay. For example, compound **24** showed 39 ± 8% inhibition of polyol accumulation at 10^{-5} M and **33** showed 66 ± 3% inhibition at 5×10^{-5} M. In contrast, compounds **25**, **34**, and **42**, which were active in the nerve in vivo, showed 80 ± 3, 82 ± 5, and 80 ± 1% inhibition, respectively, at 10^{-5} M concentration. It is important to note that tolrestat (see Table IV) demonstrated 82% inhibition at 10^{-5} M in the in vitro sciatic nerve assay. The descrepency between excellent intrinsic activity in the LAR assay but low oral activity most probably results from an inefficient transport to the site of activity in the nerve.

In conclusion, we have reported in this article a novel class of pyrimidine-*N*-acetic acids and (pyrimidinyloxy)-acetic acids having a high order of intrinsic biological activity to inhibit lens aldose reductase. On the basis of the results of our in vitro sciatic nerve assay, it is concluded that tissue penetration at the active site has an important influence on the in vivo activity.

Experimental Section

The infrared spectra were recorded on a Perkin-Elmer diffraction grating or a Perkin-Elmer 784 spectrophotometer. The ultraviolet spectra were recorded on a Zeiss-DMR-21 spectrometer. The melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The NMR spectra were recorded on either a Varian VXR200 or a Brucker AM-400 instrument. The mass spectra were recorded on a LKB-9000S or a Finigan 8230 high-resolution mass spectrometer. Organic extracts were dried over magnesium sulfate, and the solvents were always removed under vacuum. Merck silica gel 60 (70–230 mesh) was used for column chromatography.

The preparation of oxopyrimidines of type **12** where R^5 = CN has been described.[11] The corresponding derivative with R^5 = $COOCH_3$ was synthesized in an analogous manner using methyl 2-carbomethoxy-3,3 bis(methylthio)acrylate.[12] In a similar manner the 5-phenylsulfonyl analogues were also synthesized by reaction of appropriate amidine with methyl 2-(phenylsulfonyl)-3,3-bis-(methylthio)acrylate.

The preparation of oxopyrimidines via the imino ether pathway (Scheme I) is exemplified by the synthesis of **1,6-dihydro-4-methyl-2-(1-naphthalenyl)-6-oxo-5-pyrimidinecarbonitrile** (14, R^2 = 1-naphthalenyl, R^4 = methyl).

A suspension of ethyl acetimidate hydrochloride (4.54 g, 36.7 mmol) in dry toluene (100 mL) was treated with triethylamine (8.16 g, 11.2 mL, 80.8 mmol) at room temperature. After the mixture was stirred for 5 min, a solution of 1-naphthoyl chloride (7.0 g, 36.7 mmol) in toluene (30 mL) was added dropwise and the mixture was allowed to stir at room temperature for 40 h. The precipitate was removed by filtration and washed with toluene. The filtrate was evaporated to give an orange oil (8.39 g, 34.8 mmol, 95%) which was used without purification: ^{1}H NMR (Me_2SO-d_6) δ 8.95 (1 H, d, J = 8 Hz, arom), 8.17 (1 H, d, J = 8 Hz, arom), 8.13 (1 H, d, J = 7 Hz, arom), 8.02 (1 H, d, J = 8 Hz, arom), 7.6 (3 H, dd, J = 8 Hz, J_2 = 7 Hz, arom), 4.25 (2 H, q, OCH_2), 2.03 (3 H, s, N=CCH_3), 1.32 (3 H, t, J = 7 Hz, CCH_3).

Cyanoacetamide (2.93 g, 34.8 mmol) was added in one portion to a solution of NaOEt freshly prepared from sodium (0.80 g, 34.8 mmol) and absolute EtOH (120 mL). The mixture was stirred 5 min at room temperature, and then a solution of the imidate ester (8.39 g, 34.8 mmol) obtained above in EtOH (20 mL) was added dropwise. After the addition was complete, the mixture was allowed to stir for 60 h at room temperature and then neutralized with concentrated sulfuric acid (1 mL, 36 mmol). After 10 min, a precipitate formed. The solvent was evaporated and the product collected by filtration and washed with water (300 mL). The yellow powder was recrystallized from EtOAc/hexane to give the desired product (6.39 g, 24.5 mmol, 70%). Analytically pure material was obtained upon recrystallization three times from ethanol: mp 272–273 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.19 (2 H, m, arom), 8.03 (1 H, m, arom), 7.79 (1 H, dd, J_1 = 7 Hz, J_2 = 1.3 Hz, arom), 7.62 (3 H, m, arom), 2.52 (3 H, s, C=CCH_3); IR (KBr) 2220, 1660 cm^{-1}; UV (MeOH) λ max 321.5 (11 984), 218.5 (53 746) nm; MS m/z 262 $(M + H)^+$. Anal. ($C_6H_{11}N_3O$) C, H, N.

In a similar manner the following pyrimidine derivatives were synthesized. **1,6-Dihydro-4-methyl-6-oxo-2-phenyl-5-pyrimidinecarbonitrile**: mp 287–289 °C; ^{1}H NMR (Me_2SO-d_6) δ 13.5 (1 H, br s, NH), 8.14 (2 H, d, J = 8.2 Hz, arom), 7.6 (3 H, m, arom), 2.52 (3 H, s, CCH_3); IR (KBr) 2220, 1650 cm^{-1}; MS m/z 211 $(M)^+$, 183 $(M - CO)^+$. **1,6-Dihydro-2-(1-naphthalenyl)-6-oxo-4-phenyl-5-pyrimidinecarbonitrile**: mp 270–271 °C; ^{1}H NMR (Me_2SO-d_6) δ 13.9 (1 H, br s, NH), 8.3 (1 H, m, arom), 8.18 (1 H, d, J = 8.2 Hz, arom), 8.07 (1 H, m, arom), 7.98 (2 H, dd, J_1 = 7.9 Hz, J_2 = 1.7 Hz, arom), 7.87 (1 H, d, J = 7 Hz, arom), 7.62 (5 H, m, arom); MS m/z 324 $(M + H)^+$. Anal. ($C_{21}H_{13}N_3O$) C, H, N.

5-Cyano-2-methyl-6-oxo-4-(methylthio)-1(6*H*)-pyrimidineacetic Acid (15). Method A. Sodium hydride (5.04 g of 50% suspension in oil, 105 mmol) was washed twice with hexanes and then suspended in DMF (50 mL). Pyrimidone **12** (R^2 = R^4 = CH_3, R_5 = CN, 18.1 g, 100 mmol) was added in portions and DMF (20 mL) was used to wash the last of the solid into the reaction. The reaction was then stirred at 23 °C for 30 min. At the end of this time, methyl bromoacetate (14.2 mL or 22.95 g, 150 mmol) was added dropwise over 40 min. TLC showed the reaction to be complete after 4.5 h.

The reaction mixture was then quenched with a few milliliters of methanol and water, and most of the DMF was removed under vacuum. The residue was partitioned between water (150 mL) and chloroform (100 mL). The chloroform layer was separated, dried, and filtered, and the solvent was evaporated. The residue was triturated with ether (100 mL) and filtered to give the crude product (18.5 g).

A portion of the crude product (2 g) was recrystallized twice from a chloroform/hexane mixture to give a pure sample (1.58 g): mp 150–152 °C; ^{1}H NMR ($CDCl_3$) δ 4.83 (2 H, s, CH_2CO), 3.85 (3 H, s, OCH_3), 2.65 and 2.58 (3 H, s, each CCH_3, SCH_3); IR ($CHCl_3$) 2240, 1760, 1690 cm^{-1}; UV (MeOH) λ max 240.0 (18 371), 285.0 (10 989), 317.5 (7571) nm; MS m/z 253 $(M)^+$, 222 $(M - OCH_3)^+$.

The above ester (2.28 g, 9 mmol) was suspended in methanol (6 mL), and sodium hydroxide solution (3 N, 3.3 mL, 9.9 mmol) was added. The reaction was warmed to 30 °C for 1.5 h and then rotavaped to remove methanol. The residue was dissolved in water (20 mL) and neutralized to pH 7 (3.3 mL of 3 N HCl). A precipitate formed and was filtered to give the product (1.9 g). Recrystallization of crude product from methanol–ether–hexane (two times) yielded pure product (1.0 g): mp 236–238 °C; ^{1}H NMR

(Me_2SO-d_6) δ 2.60 (3 H, s, CH_3), 2.68 (3 H, s, SCH_3), 4.05 (1 H, s, OH), 4.84 (2 H, s, CH_2); IR (Nujol) 3050, 2230, 1740, 1660 cm^{-1}; UV λ max (MeOH) 286.5 (10 260), 239.8 (14 216), 212.8 (13 845) nm; MS m/z 239 $(M)^+$. Anal. ($C_9H_9N_3O_3S$) C, H, N.

5-Cyano-2-methyl-6-oxo-4-[(3-pyridinylmethyl)amino]-1-(6*H*)-pyrimidineacetic Acid (16). Method E. The methylthio ester **51** (0.25 g, 1 mmol) was suspended in dimethoxyethane (DME) (1 mL), and 3-(aminomethyl)pyridine (0.11 mL, 0.119 g, 1.1 mmol) was added. The reaction was then heated to reflux. After 19 h more DME (0.5 mL) was added and reflux continued for 4 h. The reaction was then cooled to room temperature, diluted with ether (5 mL), and filtered, and the filtercake was washed to give the product (0.20 g): ^1H NMR (Me_2SO-d_6) δ 8.6 (2 H, m, arom), 7.85 (1 H, m, arom), 7.4 (1 H, m, arom), 4.75 (3 H, m, NH, NCH_2CO), 3.85 (3 H, s, OCH_3), 3.5 (2 H, m, CH_2N), 2.45 (3 H, s, CCH_3); IR (KBr) 2100, 1735, 1660 cm^{-1}.

The above ester (1.5 g, 5 mmol) was suspended in methanol (5 mL). Aqueous sodium hydroxide (1.8 mL, 3 N, 5.5 mmol) was added and the reaction warmed to 50 °C for 1.5 h. The reaction was then cooled to room temperature and acidified (pH 4) with aqueous HCl (1.8 mL, 3 N), yielding the crude product as a precipitate (1.53 g). The crude product was crystallized twice from DMF/methanol to yield pure product (0.97 g, 68%): ^1H NMR (TFA) δ 8.9 (3 H, m, arom), 8.2 (1 H, m, arom), 5.25 (2 H, s, CH_2), 5.05 (2 H, s, CH_2) 2.7 (3 H, s, CH_3); IR (KBr) 3450, 3000, 3275, 2210, 1660, 1615 cm^{-1}; UV (MeOH) λ max 293.5 (4883), 269.5 (6028), 227.5 (23 713). Anal. ($C_{14}H_{13}N_5O_3$) C, H, N.

2-Methyl-6-oxo-4-[(3-pyridinylmethyl)amino]-1(6*H*)-pyrimidineacetic Acid (17). Method C. Sodium hydride (5.04 g, 50% in oil, 105 mmol) was washed with hexanes and suspended in DMF (60 mL). Chloropyrimidone **48** (14.45 g, 100 mmol) was suspended in DMF (50 mL) and added dropwise to the sodium hydride suspension. After the addition was complete, methyl bromoacetate (14.2 mL, 150 mmol) was added, and the reaction mixture was stirred for 18 h at room temperature. A few milliliters of water was added and the DMF was removed under vacuum. The residue was partitioned between chloroform and water, and the organic layer was dried and evaporated to give an oil (39.4 g). The oil was chromatographed over silica gel (500 g) in 20% ethyl acetate/hexane and the product eluted with 40% ethyl acetate/hexane to yield the desired compound (**49**) (12.1 g, 38%): ^1H NMR ($CDCl_3$) δ 6.5 (1 H, s, C=CH), 4.85 (2 H, s, CH_2CO), 3.85 (3 H, s, $OCH)_3$, 2.55 (3 H, s, CCH_3); IR ($CHCl_3$) 1730, 1680, 1560 cm^{-1}.

To a solution of pyrimidone **49** (3.9 g, 19 mmol) in dry tetrahydrofuran (18 mL) was added 3-(aminomethyl)pyridine (4.6 mL, 45 mmol). The reaction mixture was heated to reflux for 20 h. The mixture was cooled, diluted with saturated saline, and extracted with ethyl acetate. The organic layer was dried and the solvent removed under vacuum. The residue (4 g) was filtered through silica gel (120 g) in 10% methanol/EtOAc and eluted with the same solvent to yield pure product (3.7 g): ^1H NMR ($CDCl_3$) δ 8.55 (2 H, m, arom), 7.18–7.83 (2 H, m, arom), 5.85 (1 H, m, NH), 5.22 (1 H, s, vinylic H), 4.7 (2 H, m, CH_2CO), 4.4 (2 H, d, br, CH_2N), 3.78 (3 H, s, OCH_3) 2.35 (3 H, s, CCH_3); IR ($CHCl_3$) 1745, 1650, 1595, 1500 cm^{-1}.

The above ester (3.46 g, 12 mmol) was dissolved in methanol (12 mL). Sodium hydroxide solution (4.4 mL, 3 N, 13.2 mmol) was added and the reaction stirred for 40 min. The mixture was then neutralized with hydrochloric acid (4.4 mL, 3 N, 13.2 mmol) and evaporated under reduced pressure. The residue was triturated with ethanol, filtered, washed with ether, triturated with water, filtered, and dried to give crude product (2.3 g). Crystallization from water/methanol yielded pure product (2.1 g, 51%): mp 300 °C dec; ^1H NMR (Me_2SO-d_6 + TFA) δ 8.6 (3 H, m, arom), 7.9 (1 H, m, arom), 4.58 (4 H, m, NCH_2CO, CH_2N), 2.25 (3 H, s, C=CCH_3); IR (KBr) 3240, 3040, 2860, 2950, 1650–1550 cm^{-1}; MS m/z 274 $(M)^+$, 92 $(C_6H_6N)^+$. Anal. ($C_{13}H_{14}N_4O_2$) C, H, N.

4-[(Cyclohexylmethyl)amino]-5-cyano-1,4-dihydro-2-methyl-6-oxo-1-pyrimidineacetic Acid (18). Method E. The (methylthio)pyrimidone **51**, (2.53 g, 10 mmol) was suspended in dimethoxyethane (10 mL). Cyclohexylmethylamine (1.95 mL, 15 mmol) was added, and the reaction was heated to reflux for 2.5 h. The mixture was then cooled to room temperature, diluted with ether, and filtered. The product (2.33 g) was used without further purification: ^1H NMR ($CDCl_3$) δ 5.7 (1 H, br, NH), 4.8 (2 H, s, $COCH_2$), 3.8 (3 H, s, OCH_3), 3.4 (2 H, dd, CH_2C), 2.45 (3 H, s, CCH_3), 0.8–1.8 (11 H, m, cyclohexyl); IR ($CHCl_3$) 3400, 2200, 1740, 1660, 1590 cm^{-1}.

The above ester (2.45 g, 7.7 mmol) was suspended in methanol (20 mL). Sodium hydroxide solution (2.8 mL, 3 N, 8.5 mmol) was added. The reaction was stirred at room temperature for 1.5 h and then diluted with water (20 mL), neutralized with hydrochloric acid (2.8 mL, 3 N, 8.5 mmol), and filtered to give crude product (2.06 g). Recrystallization twice from water/methanol gave pure product (1.56 g, 63%): mp >290 °C; ^1H NMR (Me_2SO-d_6) δ 7.8 (1 H, s, NH), 4.5 (2 H, s, CH_2CO), 3.2 (2 H, dd, CH_2C), 2.3 (3 H, s, CH_3), 0.8–1.8 (11 H, m, cyclohexyl); IR (KBr) 3340, 2930, 2200, 1670, 1600 cm^{-1}; UV (MeOH) λ max 2.75 (7400), 290.5 (6310) nm; MS m/z 304 $(M)^+$, 221 $(M - C_6H_{11})^+$. Anal. ($C_{15}H_{20}N_4O_2 \cdot H_2O$) C, H, N.

5-Cyano-1,4-dihydro-2-(1-methylethyl)-4-[(1-methylethyl)thio]-6-oxopyrimidineacetic Acid (23). Method A. To a cooled (0 °C) suspension of prewashed sodium hydride (0.703 g, 50% suspension, 15 mmol) in dimethoxyethane (75 mL) was added pyrimidone (**14**, R^2 = isopropyl, R^4 = isopropylthio, 2.9 g, 12 mmol) portionwise. Methyl bromoacetate (1.62 mL, 17 mmol) was added and the solution heated at 85–90 °C for 4 h. Water was added and the mixture was extracted with ethyl acetate. The combined organic layers were washed with saturated saline solution, dried, and concentrated to produce a viscous semisolid (2.5 g, 68%). The product was used without further purification in the next step: ^1H NMR ($CDCl_3$) δ 4.84 (2 H, s, CH_2CO), 4.13 (1 H, m, SCH), 3.74 (3 H, s, OCH_3), 2.83 (1 H, m, C=CCH), 1.47 (6 H, d, J = 6.2 Hz, $C(CH_3)_2$), 1.33 (6 H, d, J = 6.11 Hz, $C(CH_3)_2$).

To a stirred solution of the above ester (2.5 g, 8.1 mmol) in dioxane (200 mL) was added 1 N NaOH (8.9 mL, 8.9 mmol). Stirring was continued at room temperature for 4 h. The mixture was concentrated, water was added, and the mixture was extracted with ethyl acetate. The combined organic layers were dried and concentrated to yield a yellow powder. Recrystallization from acetonitrile produced white crystals (610 mg): mp 192.5–193.5 °C; ^1H NMR (Me_2SO-d_6) δ 4.8 (2 H, m, NCH_2CO), 4.1 (1 H, m, SCH), 3.2 (1 H, m, C=CCH), 1.41 (6 H, d, J = 6.34 Hz, $C(CH_3)_2$), 1.21 (6 H, d, J = 6.28 Hz, $C(CH_3)_2$; IR (KBr) 2220, 1690, and 1740 cm^{-1}; UV (CH_3OH) λ max 317.5 (6361), 287.5 (12 008), 241.5 (19 236) nm; MS m/z 295 $(M)^+$, 280 $(M - CH_3)^+$. Anal. ($C_{13}H_{17}N_3O_3S$) C, H, N.

5-Cyano-1,4-dihydro-2-(1-naphthalenyl)-6-oxo-4-phenyl-1-pyrimidineacetic Acid (27). Method A. The pyrimidone **14** (R^2 = 1-naphthalenyl, R^4 = phenyl, 6.8 g, 21 mmol) was added portionwise to a suspension of sodium hydride (1.24 g, 32 mmol, 60% suspension, washed 3 × 5 mL hexane) in dry tetrahydrofuran (225 mL) at room temperature. The mixture was allowed to stir for 1 h, and then neat *tert*-butyl bromoacetate (6.2 g, 32 mmol) was added in one portion and stirring continued for 72 h. Additional *tert*-butyl bromoacetate (0.97 g, 5.0 mmol) was added and the mixture heated to 60 °C for 18 h. After cooling to room temperature the reaction was quenched by the addition of water (300 mL) and extracted with chloroform (3 × 125 mL). The combined organic layers were washed with saturated ammonium chloride, water, brine, dried, and chromatographed on silica gel. Elution with EtOAc/hexanes (20:80) gave the ester (4.95 g, 54%): ^1H NMR ($CDCl_3$) δ 8.12 (2 H, d, J = 6.9 Hz, arom), 8.05 (1 H, m, arom), 7.99 (1 H, m, arom), 7.58 (8 H, m, arom), 4.78 (1 H, d, J = 16.2 Hz, NCHCO), 4.10 (1 H, d, J = 16.2 Hz, NCHCO) 1.34 (9 H, s, *t*-Bu); IR (KBr) 2235, 1735, 1670 cm^{-1}.

The above ester (4.9 g, 11.2 mmol) was treated with trifluoroacetatic acid (30 mL) at room temperature for 3 h. The solvent was removed in vacuo and the yellow oil triturated with ether. The product crystallized twice from EtOAc/hexane to give the pure acid (2.1 g, 49%): mp 234.5–236 °C; ^1H NMR (Me_2SO-d_6) δ 8.19 (1 H, m, arom), 8.08 (1 H, d, J = 7 Hz, arom), 7.97 (2 H, d, J = 7 Hz, arom), 7.85 (1 H, d, J = 8.3 Hz, arom), 7.67 (2 H, d, J = 5.4 Hz, arom), 7.57 (5 H, m, arom), 4.63 (1 H, d, J = 17.1 Hz, NCH_2CO), 4.23 (1 H, d, J = 17.1 Hz, NCHCO); IR (KBr) 3430, 2230, 1725, 1690 cm^{-1}. Anal. ($C_{23}H_{15}N_3O_5$) C, H, N.

[[5-Cyano-2-(1-naphthalenyl)-6-phenyl-4-pyrimidinyl]oxy]acetic Acid (47). Method B. A solution of the pyrimidone (**14**, R^2 = 1-naphthalenyl, R^4 = phenyl, 6.8 g, 21 mmol) in dry DMF (125 mL) was added dropwise to a suspension of sodium hydride

(1.84 g, 60% suspension, 46 mmol) and washed with hexanes in DMF (100 mL) at room temperature. The mixture was stirred 45 min, then neat *tert*-butyl bromoacetate (6.15 g, 31.6 mmol) was added, and the mixture was stirred for 18 h. Additional *tert*-butyl bromoacetate (0.97 g, 4.96 mmol) was added and the mixture heated to 60 °C for 3 h. After cooling to room temperature the reaction mixture was quenched with water (300 mL) and extracted with chloroform. The combined organic layers were washed with saturated ammonium chloride, water, and brine, dried, and filtered through a short plug of Florisil. The filtrate was concentrated in vacuo to give an orange oil (16.3 g). Flash chromatography on silica gel and elution with EtOAc/hexanes (15:85) gave the ester as a white solid (4.51 g, 49%): ^{1}H NMR ($CDCl_3$) δ 8.95 (1 H, d, J = 8 Hz, arom), 8.25 (2 H, m, arom), 8.07 (1 H, d, J = 7 Hz, arom), 7.97 (2 H, d, J = 8 Hz), 7.6 (6 H, m, arom), 5.1 (2 H, s, OCH_2CO), 1.42 (9 H, s, *tert*-Bu); IR (KBr) 2235, 1740 cm^{-1}.

The above *tert*-butyl ester (4.5 g, 10.3 mmol) was dissolved in trifluoroacetic acid (25 mL) and the mixture was stirred at room temperature for 16 h. The solvent was removed in vacuo to give a yellow oil which was triturated with ether to induce crystallization. The product was recrystallized three times from EtOAc/hexanes to yield the pure acid as an off-white crystalline solid (0.92 g, 23%): mp 187–188 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.78 (1 H, d, J = 7.5 Hz, arom), 8.25 (1 H, d, J = 7.3 Hz, arom), 8.17 (1 H, d, J = 8.2 Hz, arom), 8.1 (2 H, dd, J = 7.9 Hz, J_2 = 1.9 Hz, arom), 8.05 (1 H, dd, J_1 = 7.2 Hz, J_2 = 2.2 Hz, arom), 7.65 (6 H, m, arom), 5.2 (2 H, s, OCH_2CO); IR (KBr) 3430, 2225, 1725 cm^{-1}. Anal. ($C_{23}H_{15}N_3O_3$) C, H, N.

[[5-Cyano-2-(1,1-dimethylethyl)-6-(methylthio)-4-pyrimidinyl]oxy]acetic Acid (34). Method B. To a cooled (10 °C), stirred suspension of NaH (50% dispersion, 0.71 g, 15 mmol) in DMF (8 mL) was added pyrimidone 14 (R^2 = *tert*-butyl, R^4 = methylthio, 3.0 g, 13.4 mmol) in several portions. After 15 min, a solution of methyl bromoacetate (3.1 g, 20 mmol) in DMF (3 mL) was added and the resulting mixture was stirred at room temperature for 4 h. Water was added and the mixture was extracted with ethyl acetate. The combined extracts were dried and concentrated. Purification of the crude product by flash chromatography (eluent 5% EtOAc/hexane) gave 3.2 g of desired product: ^{1}H NMR ($CDCl_3$) δ 4.97 (2 H, s, OCH_2CO), 3.77 (3 H, s, OCH_3), 2.65 (3 H, s, SCH_3), 1.31 (9 H, s, *t*-Bu); IR (Nujol) 2220, 1760 cm^{-1}.

To a stirred solution of the above methyl ester (2.5 g, 8.5 mmol) in dioxane (10 mL) was added sodium hydroxide (1 N, 9.3 mL). After 3 h, 2 N HCl (4.6 mL) was added and the resulting precipitate was collected by filtration. Recrystallization from methanol/ether (three times) gave 0.83 g: mp 212–213 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.0 (2 H, s, OCH_2CO), 2.66 (3 H, s, SCH_3), 1.38 (9 H, s, *t*-Bu); IR (KBr), 2210, 1725 cm^{-1}; UV (MeOH) λ max 236.5 (26 986), 269 (8679) nm; MS m/z 281 (M)$^+$, 208 (M - CH_2COOCH_3)$^+$. Anal. ($C_{12}H_{15}N_3O_3S$) C, H, N.

2-[(5-Cyano-2-methyl-6-(methylthio)-4-pyrimidinyl)oxy]acetic Acid (32). Method D. To a cooled (0 °C) suspension of hexane-washed sodium hydride (3.17 gm, 50% suspension, 66 mmol) in THF (30 mL) was added a solution of ethyl glyoxalate (6.86 g, 66 mmol) in THF (30 mL) dropwise over 20 min. After 10 min, chloropyrimidine **50** (11.97 g, 60 mmol) was added portionwise over 30 min followed by THF (90 mL). The reaction mixture was stirred at room temperature for 10 min. The mixture was diluted with saturated saline (100 mL) followed by water and extracted with ethyl acetate. Organic liquor dried and solvent was evaporated to yield a yellow solid (16.1 g). A sample was purified by chromatography for analysis: mp 100–103 °C; ^{1}H NMR ($CDCl_3$) δ 5.02 (2 H, s, OCH_2CO), 4.3 (2 H, q, J = 7 Hz, OCH_2), 2.6 and 2.68 (2 × 3 H, s, C=CCH_3, SCH_3), 1.3 (3 H, t, J = 7 Hz, CCH_3).

The above ester (2.67 g, 10 mmol) was suspended in dioxane (5 mL). Sodium hydroxide solution (3 N, 3.67 mL, 11 mmol) was added, and the mixture was stirred for 15 min and warmed for the last 5 min. The mixture was then diluted with water and filtered. The filtrate was charcoaled and filtered through Celite. The filtrate was neutralized with 3 N HCl (3.7 mL), and the resulting precipitate was filtered and crystallized from methanol/water to yield pure product: 0.81 g (15.5%); mp 205–206 °C; ^{1}H NMR (Me_2SO-d_6) δ 5.1 (2 H, s, OCH_2), 2.6 and 2.68 (2 × 3 H, s, C=CCH_3, SCH_3); IR (Nujol) 3700, 2300, 2220, 1740, 1085 cm^{-1}; UV (CH_3OH) λ max 270 (8968), 236.5 (26 195) nm. Anal. ($C_9H_9O_3N_3S$) C, H, N.

2-[[5-Carbamoyl-2-methyl-6-(methylthio)-4-pyrimidinyl]oxy]acetic Acid (44). A stirred solution of acid **34** (3.7 g, 12.8 mmol) in concentrated H_2SO_4 (13 mL) was heated at 70 °C for 16 h, cooled, and poured onto ice. The resulting precipitate was collected by filtration and dissolved in 1 N sodium hydroxide (13 mL). After extraction with ether, the aqueous phase was neutralized with 1 N hydrochloric acid and the precipitate was filtered. Recrystallization from acetonitrile gave 0.9 g: mp 210–212 °C ^{1}H NMR (Me_2SO-d_6) δ 7.7 (2 H, d, J = 15 Hz, NH_2), 4.86 (2 H, s, OCH_2CO), 2.46 (3 H, s, SCH_3), 1.28 (9 H, s, *t*-Bu); IR (KBr) 3420, 1720, 1640 cm^{-1}; UV (MeOH) λ max 298 (11 000) nm; MS m/z 299 (M)$^+$, 282 (M - OH)$^+$. Anal. ($C_{12}H_{17}N_3O_4S$) C, H, N.

2-[[5-Carbomethoxy-2-(1,1-dimethylethyl)-6-(methylthio)-4-pyrimidinyl]oxy]acetic Acid (45). To a cooled (0 °C) stirred suspension of prewashed sodium hydride (1.59 g, 33 mmol, 50% suspension) in dimethoxyethane (150 mL) was added pyrimidone **12** (R^2 = *t*-Bu, R^4 = CH_3, R^5 = $COOCH_3$, 7.09 g, 27 mmol) portionwise. The mixture was stirred at room temperature for 1 h. *tert*-Butyl bromoacetate (7.3 mL, 39 mmol) was added and the solution was heated at reflux overnight. The solution was cooled, water was added, and the mixture was extracted with ethyl acetate. The combined organic layers were washed, dried, and concentrated to produce an orange oil. This was purified by flash chromatography (20% EtOAc/hexanes). Recrystallization of the resulting yellow solid from CH_3CN produced: white crystals (10 g, 93%); ^{1}H NMR ($CDCl_3$) δ 4.84 (2 H, s, OCH_2CO), 3.93 (3 H, s, OCH_3), 2.54 (3 H, s, SCH_3), 1.46 and 1.33 (9 H each, 2 × s, *t*-Bu).

The above ester (5.55 g, 15 mmol) was combined with trifluoroacetic acid (35 mL, 45 mmol) and stirred at room temperature for 1 h. The solution was concentrated and azeotroped with toluene. Recrystallization from toluene/hexane gave a white powder (0.69 g, 15%): mp 103 °C ^{1}H NMR ($CDCl_3$) δ 4.97 (2 H, s, OCH_2CO), 3.93 (3 H, s, OCH_3), 2.54 (3 H, s, SCH_3), 1.32 (9 H, s, *t*-Bu); IR (KBr) 1710, 1730 cm^{-1}; UV (MeOH) λ max 235 (21 800), 267 (10 000) nm; MS m/z 314 (M)$^+$. Anal. ($C_{13}H_{18}N_2O_5S$) C, H, N.

Biological Evaluation. The test compounds were evaluated as inhibitors of partially purified bovine lens aldose reductase by the spectrophotometric method of Hayman and Kinoshita.[14] The in vitro LAR assay was routinely done in duplicates.

Using an in vitro sciatic nerve incubation, compounds were tested for their ability to inhibit sorbitol accumulation in isolated tissue. Sciatic nerves from rats (Sprague–Dawley) were quickly removed and incubated for 3 h in 4 mL of Hanks salt buffer (GIBCO) at 37 °C under an atmosphere of 95% air and 5% CO_2. The buffer contained 5 mM or 50 mM glucose and 0–10^{-5} M of test compound. At the end of the incubation period sorbitol levels were determined by a spectrofluorometric enzymatic assay.[15]

The inhibition of aldose reductase in vivo was assessed in rats that were fed 20% galactose for 4 days. The test compounds were administered in the diet. At the termination of the experiment, the galactitol levels were determined in trichloroacetic acid extracts of the lenses and sciatic nerves and diaphragm by a modification of the method of Kraml and Cosyns.[16] Galactitol is not detected in rats fed normal rodent chow. Following 4 days of feeding of galactose-rich diet, however, the levels of galactitol (μg/gm of wet weight of tissues) are 12.5 for lens, 1.79 for sciatic nerve, and 3.0 for diaphragm. The galactitol levels in the drug-treated galactosemic animals were compared to that in the galactosemic control animals by Dunnett's multiple comparison.

X-ray Crystallographic Analyses. Compound **25** was crystallized from ethyl acetate/hexane at room temperature. A single crystal of $C_{14}H_{17}N_3O_3S \cdot {}^1/_2C_4H_8O_2$ measuring 0.14 mm × 0.48 mm × 0.54 mm was mounted on a glass fiber and centered on a Nicolet R3m diffractometer. Cell constants and their esd's were determined by a least-squares fit of 15 diffractometer-measured reflections with 45° ≤ 2θ ≤ 50°. The material belongs to the monoclinic crystal class, space group $C2/c$, with a = 25.369 (10) Å, b = 8.283 (3) Å, c = 23.387 (10) Å, and β = 134.34 (2)°. A density of 1.33 g/cm^3 was calculated for Z = 8, FW = 351.5 g, and a unit cell volume of 3515 (2) Å.

All intensity measurements were made at room temperature

using graphite-monochromated Cu Kα radiation (λ = 1.541 78 Å) and an ω scan technique with a variable scan rate of 3.91–29.30°/min. Background counts were taken for half the scan time at each extreme of the scan range. All data (2630) having $h, k \geq 0$ with $3° \leq 2\theta \leq 114°$ were measured in this manner. Crystal decomposition was monitored throughout data collection by remeasuring two standard reflections after every 50 data measurements; no significant variations in intensity were observed. The intensities were reduced by applying Lorentz and polarization corrections. Empirical absorption corrections were applied on the basis of azimuthal scans of 15 reflections representing the range of 2θ values. The maximum and minimum transmission coefficients were 0.840 and 0.428, respectively. Systematically absent reflections were eliminated, and equivalent reflections were averaged to give 2364 unique data of which 2183 were considered to be observed [$F_o > 3\sigma(F_o)$].

The structure was solved by direct methods using the SHELXTL software. Following refinement of the carbon, nitrogen, and oxygen atoms with isotropic temperature factors, a different Fourier map displayed a number of large peaks near the inversion center. The residual peaks were assigned to a molecule of the solvent ethyl acetate which is disordered about the inversion center. In the final stages of refinement, the solvent atoms were refined with isotropic temperature factors and all other nonhydrogen atoms were refined with anisotropic temperature factors. The carboxylic acid proton was located on a difference map and fixed at the observed position. All other hydrogens were included at idealized positions (C–H 0.96 Å, C–CH 109° or 120°) and allowed to ride on the carbon to which they were attached. Solvent hydrogens were not included. Refinement converged (shift/error ≤ 0.05) at $R = 0.065$, where R is $\sum||F_o| - |F_c||/\sum|F_o|$, and $R_w = 0.084$, where R_w is $\sum[w(|F_o| - |F_c|)^2]^{1/2}/\sum(wF_o^2)^{1/2}$. The four largest peaks in a final difference map (e/Å^3 = 0.29–0.60) were in the vicinity of the disordered solvent molecule. The quantity minimized by the least-square program was $w(|F_o| - |F_c|)^2$ where w is the weight of a given observation ($w^{-1} = \sigma^2(|F_o|) + 0.0015|F_o|^2$). The analytical forms for the scattering factors of the neutral atoms were used.[17]

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Supplementary Material Available: The atomic coordinates, anisotropic and isotropic thermal parameters, bond lengths, and bond angles for **25** (7 pages); calculated structure factors (13 pages). Ordering information is given on any current masthead page.

2-Phenyl-2-(1-hydroxycycloalkyl)ethylamine Derivatives: Synthesis and Antidepressant Activity

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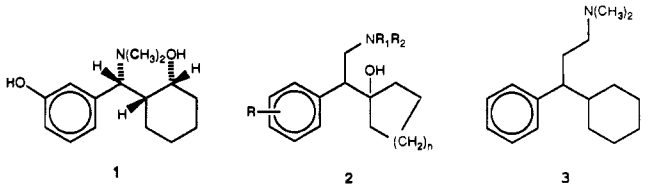
A series of 2-phenyl-2-(1-hydroxycycloalkyl)ethylamine derivatives was examined for the ability to inhibit both rat brain imipramine receptor binding and the synaptosomal uptake of norepinephrine (NE) and serotonin (5-HT). Neurotransmitter uptake inhibition was highest for a subset of 2-phenyl-2-(1-hydroxycyclohexyl)dimethylethylamines in which the aryl ring has a halogen or methoxy substituent at the 3- and/or 4-positions. Potential antidepressant activity in this subset was assayed in three rodent models—the antagonism of reserpine-induced hypothermia, the antagonism of histamine-induced ACTH release, and the ability to reduce noradrenergic responsiveness in the rat pineal gland. An acute effect seen in the rat pineal gland with several analogues, including 1-[1-(3,4-dichlorophenyl)-2-(dimethylamino)ethyl]cyclohexanol (**23**) and 1-[2-(dimethylamino)-1-(4-methoxyphenyl)ethyl]cyclohexanol (**4**), was taken as a possible correlate of a rapid onset of antidepressant activity. Compound **4** (venlafaxine) is presently undergoing clinical evaluation.

Introduction

Recent years have seen the development of a number of nontricyclic antidepressants with diminished cardiovascular and anticholinergic liability.[1] However, the goal of a rapid-onset antidepressant remains elusive. Indeed, the discrepancy in time course between the onset of clinical effectiveness and the pharmacologically observable increase in monoamine levels characteristic of most antidepressants has led to an extensive reappraisal of the simple "monoamine hypothesis" of depression. Current theories of antidepressant action focus on the gradual adaptational changes in neurotransmitter receptors, particularly the down-regulation of β-adrenoceptors coupled to adenylate cyclase, caused by chronic antidepressant therapy.[2]

The present study evolved from investigations on the mixed opiate agonist–antagonist ciramadol (**1**)[3] and was primarily aimed at structural simplification. Thus, the variants (**2**) identified in Table I contain a single stereogenic carbon, while a three-carbon interval between the amine and hydroxyl functions is maintained. The target structures proved inactive as analgesics; however, since



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minor structural alterations can frequently induce profound changes in the profile of central nervous system drugs,[4] we conducted further studies. In particular, structural similarities to gamfexine (**3**)[5] suggested the evaluation of **2** for antidepressant activity. Demonstration

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