Synthesis and Biological Activity of a Series of Diaryl-Substituted α-Cyano-β-hydroxypropenamides, a New Class of Anthelmintic Agents†

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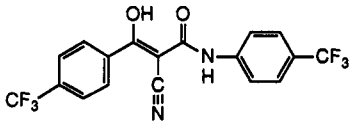
A series of α-cyano-β-hydroxypropenamides was prepared and tested for anthelmintic activity. α-Cyano-β-hydroxy-*N*-[4-(trifluoromethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]propenamide (1) showed good activity against the nematode *Nematospirodes dubius* in a mixed parasite infection in mice; several of the analogues were also effective against the cestode *Hymenolepis nana*. In sheep trials, 1 caused 100% reduction of the hematophagous nematode *Haemonchus contortus* after a single dose of 20 mg/kg but did not show satisfactory control of *Trichostrongylus colubriformis* or *Ostertagia circumcincta*. Against the liver fluke *Fasciola hepatica*, 1 suppressed egg production but only temporarily, suggesting that the adult flukes were not eliminated. Mechanism of action studies on 1 using *Ascaris* mitochondria showed it to be an uncoupler of oxidative phosphorylation.

There are currently a number of potent and broad-spectrum agents available for the treatment of parasitic nematodes in domestic animals. Unfortunately, the effectiveness of many of these agents has diminished due to the emergence of resistant strains. This problem has become especially serious on Australian sheep farms with the development of parasites resistant to the benzimidazole and levamisole classes of anthelmintics.[1-3] Our recent research efforts have been directed at identifying structurally novel classes of anthelmintics for treatment of both resistant and nonresistant parasites. One series of compounds that emerged from this work centered around the lead compound 1, an α-cyano-β-hydroxypropenamide which was discovered through routine anthelmintic screening. This report describes the biological activity and structure–activity relationships of analogues related to 1.



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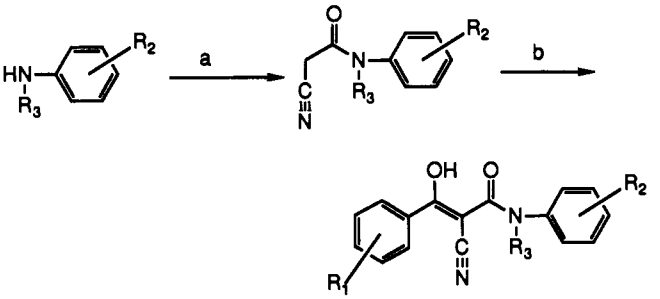
Chemistry

Two synthetic routes were utilized for the synthesis of 1 and its analogues. The principal route (Scheme I) proceeded via condensation of the appropriate aniline with cyanoacetic acid in the presence of diisopropylcarbodiimide to give the corresponding cyanoacetanilide. Treatment of the cyanoacetanilides with sodium hydride in THF or DMF followed by addition of the appropriate acid chloride afforded the α-cyano-β-hydroxypropenamide upon acidic workup. An alternative synthesis, used for the preparation of **24**, **26**, **28–30**, and **53** is shown in Scheme II. Reaction of the lithium salt of acetonitrile with 4-(trifluoromethyl)benzoyl chloride gave [4-(trifluoromethyl)benzoyl]acetonitrile, which was condensed with the appropriate isocyanate or isothiocyanate in the presence of sodium hydride to give the product. The isothiocyanate analogue **36** was prepared by reduction of **33** with stannous chloride followed by treatment of the resulting amine with thiophosgene.

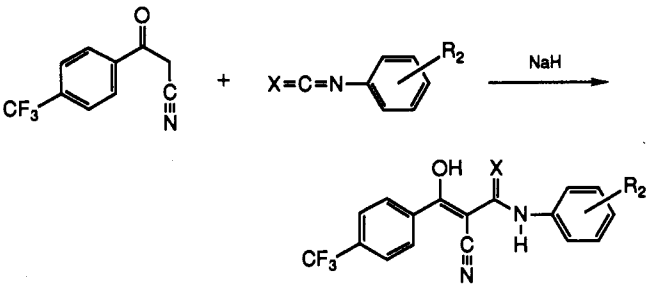
Compound 1 and its analogues exist primarily in the enol tautomer as determined by ^1H and ^{13}C NMR in DMSO-d_6.[4] These compounds are weakly acidic, forming salts with

Scheme I[a]



[a] (a) Cyanoacetic acid, diisopropylcarbodiimide; (b) NaH, THF; then ArCOCl.

Scheme II



triethylamine and ammonia. The parent compound **2** has been reported as a chelating agent, generating colored solutions in the presence of ferric ion.[5] For this reason

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(1) Waller, P. J.; Prichard, R. K. Drug Resistance in Nematodes. In *Chemotherapy of Parasitic Diseases*; Campbell, W. C., Rew, R. S., Eds.; Plenum Press: New York, 1986; pp 339–362.
(2) Edwards, J. R.; Wroth, R.; de Chaneet, G. C.; Bresier, R. B.; Karlsson, J.; Morcombe, P. W.; Dalton-Morgan, G.; Roberts, D. Survey of Anthelmintic Resistance in Western Australian Sheep Flocks 1. Prevalence. *Aust. Vet. J.* **1986**, *63*, 135–138.
(3) Prichard, R. K.; Hall, C. A.; Kelly, J. D.; Martin, I. C. A.; Donald, A. D. The Problem of Anthelmintic Resistance in Nematodes. *Aust. Vet. J.* **1980**, *56*, 239–251.
(4) The chemical shift of the hydroxyl-bearing carbon of 1 at considerably higher field (183.9 ppm) than the chemical shift of the carbonyl carbon of 4-(trifluoromethyl)acetophenone (197.5 ppm). α-Formylcyanoacetanilides are also reported to exist in the enol tautomer: Mansour, T. S. Selective Carbon Formylation of Cyanoacetanilides. *Syn. Commun.* **1987**, *17*, 1315–1322.
(5) Shoukry, M. M.; Elnagdi, M. H.; El Said Mahgoub, A. Iron-(III)-α-Cyanobenzoylacetanilide Complex and its Utility for Spectrophotometric Determination of Iron (III). *Ann. Chim. (Rome)* **1979**, *69*, 211–217.

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silica gel chromatography was usually avoided in the purification of these compounds as colored product fractions were often obtained, apparently due to leaching of small amounts of iron from the silica.

Biological Data and Structure–Activity Relationships

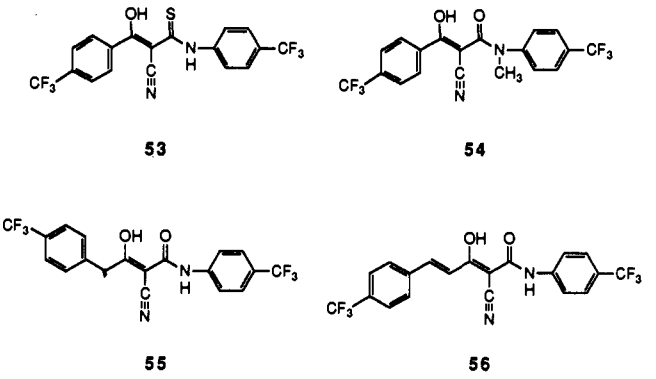
The compounds in Table I were assayed for anthelmintic activity using a mixed parasite infection in mice consisting of the nematode *Nematospirodes dubius* and the cestode *Hymenolepis nana*.[6] Animals were placed on medicated feed at the indicated dose (in parts per million in the diet) for 18 days postinfection followed by necropsy to determine the remaining parasite burden. Oxfendazole was used as a positive control in all assays. The lead compound 1 showed good activity at 62 and 31 ppm against *N. dubius*. Removing one or both of the trifluoromethyl groups (**2–4**) resulted in loss of activity, though **4** showed activity against the tapeworm *H. nana* at high concentration. For further evaluation of the series one of the ring substituents was held as 4(or 4')-CF_3 and the other ring substituent varied. With R_2 = 4'-CF_3, moving the 4-CF_3 group to the 3-position (**5**) diminished activity while moving it to the 2-position resulted in loss of activity (**6**). Replacement of the 4-CF_3 group with a halogen (**7–10**) gave active compounds, with chlorine and bromine being the most effective substituents. The 3-chloro compound (**11**) was inactive; however, the 3,5-dichloro and other dichloro analogues (**12–14**) showed good activity. The OCF_3 group proved equipotent to the CF_3 group (**15**). More hydrophilic electron-withdrawing groups such as nitro (**16**) and cyano (**17**) were less effective substituents, while the electron-donating substituents methyl (**18**) and methoxy (**19**) were inactive. A brief investigation of larger substituents (**20, 21**) failed to provide active compounds.

Qualitatively similar results were observed upon varying the substituent on the aniline ring with a fixed 4-CF_3 group, though a stricter requirement for an electron-withdrawing group in the 4'-position was evident (compare **5** with **22**). Again, halogen and dihalogen analogues were active (**24–29**), whereas the methoxy (**30**) derivative was not. Nitro and cyano substituents were more effective in the 4'-position than in the 4-position (compare **17** with **31**), and in the case of the nitro group it was found that an ortho methyl group (**34**) enhanced potency. Replacement of the 4-CF_3 group with other hydrophilic electron-withdrawing groups (**37–40**) did not give active compounds. Active compounds were also obtained if both CF_3 groups of 1 were replaced by a halogen or an OCF_3 substituent, though the 4,4'-difluoro analogue (**44**) was inactive at 125 ppm. A surprising result was obtained with **52**, which was expected to be active on the basis of the results with **13** and **29**. Testing of this compound in vitro against the nematode *C. elegans* showed it to be highly active, and it also proved to be active in sheep (vide infra). It is not clear why this compound was inactive in the mouse model.

Chart I shows a series of miscellaneous analogues of 1 that were also prepared and tested. The thioamide analogue (**53**) was approximately one-fourth as active as 1, and the *N*-methyl analogue **54** was nearly equipotent. Incorporation of a methylene (**55**) or vinyl (**56**) spacer into 1 gave less active analogues.

The results of testing 1 and several analogues against a mixed parasite infection in sheep are shown in Table II.

Chart I



Although 1 had good activity against *Haemonchus contortus*, it was inactive against the other parasitic nematodes *Ostertagia circumcincta* and *Trichostrongylus colubriformis*. This narrow spectrum was also found with the other analogues examined, including **43** and **55**, which are structurally somewhat more divergent from 1. We were, however, encouraged by the finding that 1 showed activity against a benzimidazole resistant strain of *H. contortus* as well as against the susceptible strain. Thus, though these compounds lacked broad-spectrum activity, our goal of circumventing the acquired resistance to the benzimidazoles was achieved with this new class of agents.

Compounds 1, **9**, **43**, and **45** were also tested against the trematode *Fasciola hepatica* using a model infection in mice.[7] None of the compounds tested showed a statistically significant reduction of liver pathology in the mice at dose levels where efficacy was seen against *N. dubius* or *H. nana*. A somewhat more encouraging result was obtained against a 15-week-old artificial infection of *F. hepatica* in sheep. Flukes achieve maximum egg production from 13 to 17 week postinfection, with a plateauing effect occurring to approximately 27 weeks.[8] Treatment with 30 mg/kg of 1 on day 105 postinfection gave a 52% reduction of egg count on day 119 and an 80% reduction on day 125 after treatment. By day 136, however, egg counts had returned to that of the nontreated controls, suggesting that no permanent reduction of adult flukes had taken place.

Mechanism of Action Studies

The enol form of 1 bears some structural similarity to the salicylanilide class of anthelmintic agents, which are known to be uncouplers of oxidative phosphorylation.[9-13]

(6) Brody, G.; Elward, T. E. Comparative Activity of 29 Known Anthelmintics Under Standardized Drug-Diet and Gavage Medication Regimens Against Four Helminth Species in Mice. *J. Parasit.* **1971**, *57*, 1068–1077.

(7) Hayes, T. J.; Bailer, J.; Mitrovic, M. Murine Fascioliasis: Laboratory Model for the Detection of Fasciolicidal Activity of Chemical Compounds. *J. Parasit.* **1973**, *59*, 314–318.

(8) Happich, F. A.; Boray, J. C. Quantitative Diagnosis of Chronic Fasciolosis. 2. The Estimation of Daily Total Egg Production of *Fasciola hepatica* and the Number of Adult Flukes in Sheep by Faecal Egg Counts. *Aust. Vet. J.* **1969**, *45*, 329–331.

(9) Agrawal, V. K.; Sharma, S. Salicylanilides in the Treatment of Helminth Diseases. *Pharmazie* **1984**, *39*, 373–378.

(10) Saz, H. J. Anaerobic Phosphorylation in *Ascaris* Mitochondria and the Effects of Anthelmintics. *Comp. Biochem. Physiol. B* **1971**, *39B*, 627–637.

(11) Williamson, R. L.; Metcalf, R. L. Salicylanilides: A New Group of Active Uncouplers of Oxidative Phosphorylation. *Science* **1967**, *158*, 1694–1695.

(12) Kane, H. J.; Behm, C. A.; Bryant, C. Metabolic Studies of the New Fasciolicidal Drug, Closantel. *Mol. Biochem. Parasitol.* **1980**, *1*, 347–355.

(13) Van den Bossche, H.; Verhoeven, H.; Vanparijs, O.; Lauwers, H.; Thienpont, D. Closantel, a New Antiparasitic Hydrogen Ionophore. *Arch. Int. Physiol. Biochim.* **1979**, *87*, 851–853.

As a result, a series of experiments were carried out on mitochondria isolated from the body wall muscle of the nematode *Ascaris suum*. Energy generation in these mitochondria results from a site 1, electron transport associated phosphorylation coupled to the NADH-dependent reduction of fumarate, giving succinate as the end product.[14] Site 1 inhibitors, such as rotenone, inhibit ATP synthesis and succinate formation, while uncouplers of oxidative phosphorylation, such as 2,4-dinitrophenol and the salicylanilides, inhibit ATP synthesis but stimulate succinate formation. As shown in Table III, 1 inhibited ATP synthesis and stimulated succinate formation in a concentration-dependent manner. This suggests that the anthelmintic action of 1 is may be due to its ability to act as an uncoupler of oxidative phosphorylation.

Conclusions

The narrow spectrum exhibited by 1 and analogues in the sheep studies is paralleled by the salicylanilide class of anthelmintics. Like 1, the salicylanilides closantel and rafoxanide show activity only against *H. contortus*.[15,16] Since these salicylanilides are highly serum bound,[17] it has been proposed that only the blood-engorging *H. contortus* is exposed to enough of the drug to be affected.[18] A similar explanation for the narrow spectrum of 1 probably applies. Van den Bossche has shown that albumin prevents the uncoupling effect of closantel in vitro, and suggests that the formation of a closantel–albumin complex protects the host from the drug.[13,18] If the selective toxicity shown by these drugs is indeed due to the protective effects of serum binding, it may be difficult to devise a broad-spectrum agent that acts by uncoupling oxidative phosphorylation. Any structural modification made to reduce serum binding in an attempt to attain better distribution to the other nematodes will likely also result in higher toxicity to the host.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. ^{1}H NMR spectra were measured on a Varian EM 390 or Bruker WM 300 spectrometer and are expressed in ppm downfield from internal TMS. Microanalyses were performed by the Syntex Analytical Department. Column chromatography was performed on silica gel 60 (230–400 mesh) using a forced flow (flash chromatography) of the indicated solvent. THF was distilled from sodium metal/benzophenone ketyl. Reagents and other solvents were used as obtained without further purification. All reactions were run under a nitrogen atmosphere.

The following intermediates were prepared according to published procedures: 4-(4-chlorophenoxy)benzoic acid[19] (for **20**); 4-thiocyanatoaniline[20] (for **35**); 4-aminodiphenyl sulfide[21] (for 41); 4-chloro-4′-aminodiphenyl sulfide[21] (for **42**).

***N*-[4-(Trifluoromethyl)phenyl]cyanoacetamide: General Procedure.** To a stirred, 0 °C solution of 4-aminobenzotrifluoride (21.2 g, 0.135 mol) and cyanoacetic acid (18.2 g, 0.214 mol) in DMF (140 mL) was added diisopropylcarbodiimide (18.7 g, 0.148 mol) portionwise over 10 min. The reaction was allowed to warm to room temperature over 1 h and then diluted with 150 mL of hexane–EtOAc (1:1). The reaction was filtered to remove the urea byproduct, and the filtrate was partitioned between dilute HCl and EtOAc. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated to give a solid which was recrystallized from EtOH to give 21.2 g (60%) of *N*-[4-(trifluoromethyl)-phenyl]cyanoacetamide: mp 191–3 °C; ^{1}H NMR (DMSO-d_6) 10.75 (br s, 1 H, NH), 7.75 (s, 4 H, ArH), 3.95 (s, 2 H, CH_2). Anal. ($C_{10}H_7F_3N_2O$) C, H, N.

α-Cyano-β-hydroxy-*N*-[4′-(trifluoromethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]propenamide (1). General Procedure, Scheme I. To a mechanically stirred, 0 °C solution of *N*-[4-(trifluoromethyl)phenyl]cyanoacetamide (21.75 g, 0.095 mol) in 450 mL of THF was added portionwise 8.8 g (0.22 mol) of a 60% oil dispersion of NaH. The mixture was stirred for 15 min and then 4-(trifluoromethyl)benzoyl chloride (20.9 g, 0.10 mol) in CH_2Cl_2 (50 mL) was added dropwise over 30 min. After the addition was complete, the reaction was stirred at 0 °C for an additional 30 min and then cautiously treated with 1 L of 0.5 N HCl. The solid that formed was filtered off, washed with water and ethanol, and dried under vacuum. Recrystallization of this material from toluene gave 34 g (89%) of 1: ^{1}H NMR (DMSO-d_6) δ 11.8 (br s, 1 H, NH), 8.5 (br s, 1 H, OH), 7.7–8.0 (m, 8 H, ArH).

[4-(Trifluoromethyl)benzoyl]acetonitrile. To a stirred, −78 °C solution of acetonitrile (17.0 mL, 320 mmol) in THF (800 mL) was added over 15 min a 1.6 M solution of *n*-BuLi in hexane (150 mL, 240 mmol). The resultant slurry was stirred at −78 °C for 15 min and then treated over 15 min with 4-(trifluoromethyl)-benzoyl chloride (12.0 mL, 80.5 mmol). After 40 min, the reaction mixture was treated with NH_4Cl (20 g) in water (100 mL). The reaction mixture was partitioned between 1 N HCl and hexane–EtOAc (50/50). The organic phase was separated, washed with brine, dried (Na_2SO_4), and concentrated to an oil. This oil was redissolved in ether and treated with concentrated NH_4OH to give a white precipitate. This precipitate was filtered off and partitioned between dilute HCl and EtOAc. The organic phase was separated, dried (Na_2SO_4), and concentrated to an oil which upon trituration with warm hexane afforded 11.4 g (66%) of [4-(trifluoromethyl)benzoyl]acetonitrile as an off-white solid: mp 46–47 °C (lit.[22] mp 44–45 °C).

α-Cyano-β-hydroxy-*N*-[4′-(trifluoromethyl)phenyl]-3-[4-(trifluoromethyl)phenyl]propenethioamide (53). General Procedure, Scheme II. To a stirred, 0 °C solution of [4-(trifluoromethyl)benzoyl]acetonitrile (0.50 g, 2.3 mmol) in THF (20 mL) was added NaH (0.10 g of a 60% oil dispersion, 2.5 mmol). After 20 min 4-(trifluoromethyl)phenyl isothiocyanate (0.48 g, 2.4 mmol) was added and the mixture stirred for another 2 h. The solution was partitioned between dilute HCl and EtOAc. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated to a solid. Recrystallization from toluene yielded 0.40 g (41%) of **53** as a yellow solid.

4-(Phenylsulfonyl)benzoic Acid (for 21). A solution of benzenesulfinic acid sodium salt (34.4 g, 0.21 mmol) and 4-fluoroacetophenone (20.0 g, 0.145 mol) in DMF (200 mL) was heated at 125 °C for 2 days. The solution was cooled, poured into water, and extracted with toluene. The organic phase was washed with dilute aqueous NaOH, dried (Na_2SO_4), and concentrated to a solid. This solid was digested with ether to give 19.0 g (50%)

(14) Kohler, P.; Bachman, R. Mechanism of Respiration and Phosphorylation in *Ascaris* Muscle Mitochondria. *Mol. Biochem. Parasitol.* **1980**, *1*, 75–90.

(15) Hall, C. A.; McDonell, P. A.; Graham, J. M. Anthelmintic Activity of Closantel against Benzimidazole Resistant Strains of *Haemonchus contortus* and *Trichostrongylus colubriformis* in Sheep. *Aust. Vet. J.* **1980**, *56*, 461–462.

(16) Guerrero, J. Closantel: A Review of its Antiparasitic Activity. *J. Preventive Vet. Med.* **1984**, *2*, 317–327.

(17) Mohammed-Ali, N. A. K.; Bogan, J. A. The Pharmacodynamics of the Flukicidal Salicylanilides, Rafoxanide, Closantel and Oxyclosanide. *J. Vet. Pharmacol. Ther.* **1987**, *10*, 127–133.

(18) Van den Bossche, H. Mode of Action of Anticestodal Agents. In *Chemotherapy of Parasitic Diseases*; Campbell, W. C., Rew, R. S., Eds.; Plenum Press: New York, 1986; pp 495–503.

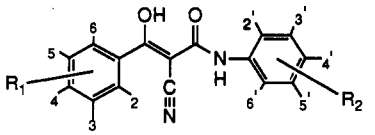
(19) Haydock, D. B.; Mulholland, T. P. C.; Telford, B.; Thorp, J. M.; Wain, J. S. Analogues of Clofibrate and Clobuzarit Containing Fluorine in the Side Chains. *Eur. J. Med. Chem.-Chim. Ther.* **1984**, *19*, 205–214.

(20) Kaufmann, H. P. Weitere Mitteilung uber meine Method der Rhodanierung Organischer Verbindung. *Chem. Ber.* **1929**, *62*, 390–401. Ammonium thiocyanate was used instead of sodium thiocyanate for this preparation.

(21) Gilman, H.; Broadbent, H. S. Some Basically Substituted Diaryl Sulfides and Sulfones. *J. Am. Chem. Soc.* **1947**, *69*, 2053–2057.

(22) Ridge, D. N.; Hanifin, J. W.; Harten, L. A.; Johnson, B. D.; Menschik, J.; Nicolau, G.; Sloboda, A. E.; Watts, D. E. Potential Antiarthritic Agents. 2. Benzoylacetonitriles and β-Aminocinnamonitriles. *J. Med. Chem.* **1979**, *22*, 1385–1389.

Table I. Anthelmintic Activity of α-Cyano-β-hydroxypropenamides



no.	R_1	R_2	formula[a]	mp, °C (solv)[b]	% yield	dose (ppm in feed)	in vivo mouse screen: % reduction[c] *N. dubius*	*H. nana*
1	4-CF_3	4′-CF_3	$C_{18}H_{10}F_6N_2O_2$	221–223 (g)	89	62	100	0
						31	76	0
2	H	H	$C_{16}H_{12}N_2O_2$	205–207 (g)	57	1000	0	0
3	H	4′-CF_3	$C_{17}H_{11}F_3N_2O_2$	245–248 (g)	24	875	0	0
4	4-CF_3	H	$C_{17}H_{11}F_3N_2O_2$	236–238 (o)	39	500	32	100
5	3-CF_3	4′-CF_3	$C_{18}H_{10}F_6N_2O_2$	165–166 (g)	57	125	89	100
						62	0	0
6	2-CF_3	4′-CF_3	$C_{18}H_{10}F_6N_2O_2$	151–153 (g)	70	125	0	0
7	4-F	4′-CF_3	$C_{17}H_{10}F_4N_2O_2$	211–213 (g)	58	125	100	100
						62	0	0
8	4-Cl	4′-CF_3	$C_{17}H_{10}ClF_3N_2O_2$	218–220 (g)	68	62	53	0
9	4-Br	4′-CF_3	$C_{17}H_{10}BrF_3N_2O_2$	213–214 (g)	77	125	99	100
						62	45	92
10	4-I	4′-CF_3	$C_{17}H_{10}F_3IN_2O_2$	239–240 (g)	50	125	97	89
						62	0	0
11	3-Cl	4′-CF_3	$C_{17}H_{10}ClF_3N_2O_2$	201–202 (o)	65	125	0	0
12	2,4-Cl_2	4′-CF_3	$C_{17}H_9Cl_2F_3N_2O_2$	187–188 (i)	37	125	*d*	*d*
						62	32	52
13	3,4-Cl_2	4′-CF_3	$C_{17}H_9Cl_2F_3N_2O_2$	187–188 (g)	43	125	95	0
						62	0	0
14	3,5-Cl_2	4′-CF_3	$C_{17}H_9Cl_2F_3N_2O_2$	208–210 (p)	78	125	100	100
						62	0	0
15	4-OCF_3	4′-CF_3	$C_{18}H_{10}F_6N_2O_3$	188–190 (g)	63	125	100	100
						62	86	0
16	4-NO_2	4′-CF_3	$C_{17}H_{10}F_3N_3O_4$	211–214 (g)	72	125	*d*	*d*
						62	0	69
17	4-CN	4′-CF_3	$C_{18}H_{10}F_3N_3O_2$	252–254 (o)	72	125	28	78
18	4-CH_3	4′-CF_3	$C_{18}H_{13}F_3N_2O_2$	259–261 (m)	36	125	0	0
19	4-OCH_3	4′-CF_3	$C_{18}H_{13}F_3N_2O_3$	248–249 (m)	31	125	0	0
20	4-(4-ClC_6H_4O)	4′-CF_3	$C_{23}H_{14}ClF_3N_2O_3$	233–235 (o)	80	125	30	0
21	4-$C_6H_5SO_2$	4′-CF_3	$C_{23}H_{15}F_3N_2O_4S$	230–232 (o)	72	125	0	0
22	4-CF_3	3′-CF_3	$C_{18}H_{10}F_6N_2O_2$	138–140 (g)	54	125	0	0
23	4-CF_3	2′-CF_3	$C_{18}H_{10}F_6N_2O_2$	122–124 (q)	62	125	0	0
24	4-CF_3	4′-F	$C_{17}H_{10}F_4N_2O_2$	196–198 (i)	71	125	100	0
						62	0	0
25	4-CF_3	4′-Cl	$C_{17}H_{10}ClF_3N_2O_2$	221–222 (g)	45	125	100	100
						62	65	78
26	4-CF_3	4′-Br	$C_{17}H_{10}BrF_3N_2O_2$	237–238 (j)	62	125	100	95
						62	56	0
27	4-CF_3	4′-I	$C_{17}H_{10}F_3IN_2O_2$	256–258 (o)	78	125	100	0
						62	0	0
28	4-CF_3	2′,4′-Cl	$C_{17}H_9Cl_2F_3N_2O_2$	148–150 (i)	57	125	89	100
						62	0	63
29	4-CF_3	3′,4′-Cl_2	$C_{17}H_9Cl_2F_3N_2O_2$	187–188 (h)	79	125	100	93
						62	0	0
30	4-CF_3	4′-OCH_3	$C_{18}H_{13}F_3N_2O_3$	248–250 (n)		125	0	0
31	4-CF_3	4′-CN	$C_{18}H_{10}F_3N_3O_2$	206–208 (o)	67	125	100	100
						62	43	0
32	4-CF_3	2′-CH_3, 4′-CN	$C_{19}H_{12}F_3N_3O_2$	216–218 (o)	66	125	90	100
						62	0	0
33	4-CF_3	4′-NO_2	$C_{17}H_{10}F_3N_3O_4$	245–247 (p)	80	250	100	100
						125	50	0
34	4-CF_3	2′-CH_3, 4′-NO_2	$C_{18}H_{12}F_3N_3O_4$	190–191 (p)	82	125	97	99
						62	35	0
35	4-CF_3	4′-SCN	$C_{18}H_{10}F_3N_3O_2S$	199–200 (g)	45	125	0	0
36	4-CF_3	4′-NCS	$C_{18}H_{10}F_3N_3O_2S$	230–231 (j)	63	125	80	0
37	4-CF_3	4′-SO_2CH_3	$C_{18}H_{13}F_3N_2O_4S$	248–250 (o)	74	125	0	0
38	4-CF_3	4′-$COCH_3$	$C_{19}H_{13}F_3N_2O_3$	232–233 (o)	68	125	0	0
39	4-CF_3	4′-CO_2CH_3	$C_{19}H_{13}F_3N_2O_4$	252–253 (o)	70	125	0	0
40	4-CF_3	4′-$CON(C_2H_5)_2$	$C_{22}H_{20}F_3N_3O_3$	176–178 (i)	34	125	0	0
41	4-CF_3	4′-C_6H_5S	$C_{23}H_{15}F_3N_2O_2S$	190–191 (k)	64	250	0	0
42	4-CF_3	4′-(4-ClC_6H_4S)	$C_{23}H_{14}ClF_3N_2O_2S$	198–199 (j)	65	250	94	33
						125	0	0
43	4-CF_3	3′-Cl, 4′-(4-ClC_6H_4O)	$C_{23}H_{13}Cl_2F_3N_2O_3$	210–211 (k)	75	500	100	83
						125	0	0
44	4-F	4′-F	$C_{16}H_{10}F_2N_2O_2$	261–263 (o)	61	125	0	0
45	4-Cl	4′-Cl	$C_{16}H_{10}Cl_2N_2O_2$	225–226 (m)	61	125	100	100
						62	59	30

Table I (Continued)

no.	R_1	R_2	formula[a]	mp, °C (solv)[b]	% yield	dose (ppm in feed)	in vivo mouse screen: % reduction[c] *N. dubius*	*H. nana*
46	4-Cl	4′-Br	$C_{16}H_{10}BrClN_2O_2$	243–244 (g)	46	125	100	0
						62	47	0
47	4-Br	4′-Cl	$C_{16}H_{10}BrClN_2O_2$	234–235 (o)	72	125	100	0
						62	76	0
48	4-Br	4′-Br	$C_{16}H_{10}Br_2N_2O_2$	251–253 (o)	51	125	100	0
						62	29	0
49	4-OCF_3	4-OCF_3	$C_{18}H_{10}F_6N_2O_4$	198–200 (o)	41	62	100	56
						31	39	0
50	3,4-Cl_2	4′-Cl	$C_{16}H_9Cl_3N_2O_2$	235–237 (o)	84	125	100	100
						62	32	0
51	4-Cl	3′, 4′-Cl_2	$C_{16}H_9Cl_3N_2O_2$	234–236 (l)	74	125	57	47
52	3, 4-Cl_2	3′, 4′-Cl_2	$C_{16}H_8Cl_4N_2O_2$	229–230 (l)	72	125	0	0
53			$C_{18}H_{10}F_6N_2OS$	208–210 (g)	41	250	100	90
						125	0	0
54			$C_{19}H_{12}F_6N_2O_2$	118–119 (k)	31	125	100	0
						62	76	0
55			$C_{19}H_{12}F_6N_2O_2$	141–143 (q)	29	250	90	0
						125	35	0
56			$C_{20}H_{12}F_6N_2O_2$	264–267 (m)	31	250	48	0
oxfendazole						125	100	100
						31	100	0

[a] The analysis for C, H, and N for all compounds was within ±0.4% of the calculated values. [b] Recrystallized from (g) toluene, (h) toluene–EtOAc, (i) EtOH, (j) EtOAc, (k) hexane–EtOAc, (l) EtOAc–DMF, (m) DMF, (n) acetic acid, or crude solid digested with (o) MeOH, (p) EtOH, (q) 2-propanol. [c] Reduction of worms ≤25% recorded as 0. [d] Toxic.

Table II. Anthelmintic Activity in Sheep

no.	dose, mg/kg	% reduction vs control[a] *H. contortus*	*O. circumcincta*	*T. colubriformis*
1	30	100	0	0
1	20	100[c]	66[d]	0[d]
14	30	100	0	0
31	30	87	7	26
34	30	0	0	0
43	30	0	0	0
52	30	100	0	0
55	30	78	0	0

[a] Mean values (four to seven sheep per group). A reduction >95% is considered significant. [b] Dosed as ammonium salt. [c] Benzimidazole-resistant strain. [d] Benzimidazole-, levamisole-, and morantel-resistant strain.

Table III. Effect of 1 on Incorporation of $^{32}P_i$ and Succinate Formation by Isolated *A. summ* Mitochondria

concn of 1, μM	$^{32}P_i$ incorporated[a]	succinate[b]
0	0.39 ± 0.003	0.45–0.48
3	0.35 ± 0.007	0.63–0.63
10	0.28 ± 0.007	0.72–0.75
25	0.15 ± 0.003	0.77–0.86
50	0.05 ± 0.004	1.13–1.17
2,4-dinitrophenol (200 μM)	0.14 ± 0.002	0.91–0.97

[a] μmol/15 min/mg of protein; values reported are mean ± standard error (n = 3). [b] μmol/15 min/mg of protein; assay run in duplicate.

of 4-(phenylsulfonyl)acetophenone, mp 130–132 °C (lit.[23] mp 137–138 °C). To a suspension of this ketone (10.0 g, 38.4 mmol) in EtOH (80 mL) at 60 °C was added aqueous sodium hypochlorite (5.25%, 300 mL) dropwise over 2 h. The solution was heated for another 2 h and then quenched by dropwise addition of aqueous Na_2SO_3. After acidification with concentrated HCl, the mixture was extracted with EtOAc and the organic phased dried (Na_2SO_4) and concentrated to a solid. The solid was digested with MeOH to give 7.4 g (73%) of 4-(phenylsulfonyl)benzoic acid, mp 270–272 °C (lit.[24] mp 273 °C).

4-Cyano-2-methylaniline (for 32). To a solution of 3-methyl-4-nitrobenzonitrile[25] (24 g, 0.148 mol) in acetic acid (250 mL) was added dropwise a solution of $SnCl_2 \cdot 2H_2O$ (133 g, 0.59 mol) in concentrated HCl (250 mL). After stirring for 3 h, the reaction mixture was added carefully to excess cold NH_4OH. The reaction mixture was extracted several times with ether. The organic extracts were dried (Na_2SO_4) and evaporated under reduced pressure to afford 12 g (61%) of 4-cyano-2-methylaniline, mp 92–93 °C (hexane) (lit.[26] mp 94–5 °C).

***N*-[3-Chloro-4-(4′-chlorophenoxy)phenyl]cyanoacetamide (for 43).** To a solution of 4-chlorophenol (11.4 g, 0.089 mol) in 80 mL of DMSO was added portionwise NaH (3.5 g of a 60% oil suspension, 0.088 mol). When gas evolution was complete, the solution was treated with 3,4-dichloronitrobenzene (15.3 g, 0.080 mol) and heated at 85 °C for 24 h. The reaction mixture was cooled to room temperature and partitioned between ether and water. The organic phase was washed with aqueous NaOH (0.5 N) and aqueous HCl (1 N), dried ($MgSO_4$), and concentrated to a solid. The solid was washed with EtOH to give 18.5 g (74%) of 4-(4′-chlorophenoxy)-3-chloronitrobenzene. To a suspension of 4-(4′-chlorophenoxy)-3-chloronitrobenzene (7.2 g, 0.025 mol) in 125 mL of acetic acid was added a solution of $SnCl_2 \cdot 2H_2O$ (16 g, 0.071 mol) in 125 mL of concentrated HCl. The reaction mixture was heated at reflux for 3 h, cooled to room temperature, and filtered, and the solid was washed with a small amount of cold water. The solid was then partitioned between aqueous NaOH (0.5 N) and ether. The organic phase was dried ($MgSO_4$) and concentrated to give 4.0 g (62%) of 4-(4′-chlorophenoxy)-3-chloroaniline. Condensation of this material with cyanoacetic acid as described above and recrystallization from EtOH gave 4.0 g (80%) of *N*-[3-chloro-4-(4′-chlorophenoxy)phenyl]cyanoacetamide, mp 188–90 °C.

***N*-Methyl-*N*-[4-(trifluoromethyl)phenyl]cyanoacetamide (for 54).** A mixture of 4-(trifluoromethyl)aniline (6.7 g, 41.6

(23) Ulman, A.; Urankar, E. Novel Synthesis of 4-[Alkyl(aryl)-sulfonyl]benzaldehydes: Alkyl(aryl)sulfinate Anion as a Nucleophile in Aromatic Substitutions. *J. Org. Chem.* **1989**, *54*, 4691–4692.

(24) Bradsher, C. K.; Turner, J. D. Acridizinium Ion Chemistry. V. Sulfonation. *J. Org. Chem.* **1966**, *31*, 565–567.

(25) Beilstein, F.; Kreusler, U. Untersuchungen uber Isomerie in der Benzoereihe. Siebente Abhandlung. Ueber Para-Nitrotoluylsaure and deren Derivate. *Ann.* **1867**, *144*, 163–184.

(26) Bartoli, G.; Bosco, M.; Dal Pozzo, R.; Petrini, M. One-Pot Chemoselective Reductive Alkylation of Nitroarenes: A New General Method of Synthesis of Alkylanilines. *Tetrahedron* **1987**, *43*, 4221–4226.

mmol), trimethyl orthoformate (6.6 g), and sulfuric acid (0.22 g) was heated at 90 °C and the MeOH formed in the the reaction was allowed to distill off.[27] Additional trimethyl orthoformate (4 mL) was added twice more. After each addition the reaction mixture was distilled. A final aliquot trimethyl orthoformate (5 mL) was added and the reaction mixture was heated at 150 °C for 4 h, cooled, and partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic layer was dried (Na_2SO_4) and concentrated to a yellow oil. This oil was then suspended in 2 N aqueous HCl (100 mL) and then heated at 80 °C for 8 h. The reaction mixture was then cooled, made basic to pH 11 with aqueous NaOH, and extracted with ether. The organic layer was dried ($MgSO_4$) and concentrated to an oil which was purified by chromatography on silica gel (350 g, hexane–ether 3:2) to give 5.8 g (79%) of *N*-methyl-4-(trifluoromethyl)aniline as a low-melting solid. Condensation of this material with cyanoacetic acid as described above yielded 6.0 g (75%) of *N*-methyl-*N*-[4-(trifluoromethyl)phenyl]cyanoacetamide, mp 68–70 °C.

α-Cyano-β-hydroxy-*N*-(4′-isothiocyanatophenyl)-3-[4-(trifluoromethyl)phenyl]propenamide (36). A suspension of **33** (7.33 g, 19.4 mmol) in glacial acetic acid (40 mL) was treated with a solution of $SnCl_2 \cdot 2H_2O$ (15 g, 66.5 mmol) in concentrated HCl (45 mL). The reaction mixture was heated at 80 °C for 8 h and then allowed to cool to room temperature. The reaction mixture was poured into a mixture of water (1 L) and EtOAc (100 mL) and shaken in a separatory funnel. The solid was filtered off, washed with water (2 × 100 mL) and ether (2 × 100 mL), and dried under vacuum to give 6.94 g (92%) of α-cyano-β-hydroxy-*N*-(4-aminophenyl)-3-[4-(trifluoromethyl)phenyl]propenamide hydrochloride as a tan solid. A suspension of this material (1.33 g, 3.46 mmol) in acetone (40 mL) was treated with $NaHCO_3$ (0.87 g, 10.4 mmol) and thiophosgene (0.5 g, 4.3 mmol). The mixture was heated at reflux for 5 h, allowed to cool, and then partitioned between 0.5 N aqueous HCl (200 mL) and EtOAc (300 mL). The organic layer was washed with brine and dried (Na_2SO_4), and the solvent was evaporated to afford a yellow solid. Recrystallization from EtOAc afforded 0.85 g (63%) of **36**.

Mixed-Parasite Infection in Mice. Third stage larvae of the nematode *Nematospirodes dubius* were collected from the feces of infected mice and suspended in sterile water to a concentration of 160–180 larvae/mL of water. Eggs of the tapeworm *Hymenolepis nana* were collected from ground adult tapeworms that were kept at 4 °C after removal from infected mice. The eggs were suspended in sterile water at a concentration of 10 000 eggs/mL of water. Equal volumes of the *N. dubius* and *H. nana* suspensions were mixed together to form the combined helminth inoculum. Male Swiss-Webster mice, 18–20 g, were challenged orally with 0.5 mL of the inoculum. Starting at 24 h postinfection, the mice were treated for 18 days ad libitum with the test compound mixed in the food at the indicated concentration. The treatment group for each compound contained four mice. The mice were monitored daily for any visible signs of toxicity and to ensure that all the food had been consumed. On day 19 postinfection the mice were sacrificed, and the entire intestine was removed and placed between two glass plates. Using a dissecting microscope, the number of *N. dubius* and *H. nana* were counted in the duodenum and ileum, respectively. The percent reduction in the worm burden was calculated as follows:

$$[\text{mean no. worms (control group)} - \text{mean no. worms (treated group)}]/\text{mean no. worms (control group)} \times 100$$

Mixed-Parasite (Nematode) Infection in Sheep. Merino ewes and wethers, approximately 12–15 months of age and weighing 25–35 kg, were maintained at the Australian Research Unit field facility, Syntex Research, New South Wales, Australia. The animals were fed an ad libitum ration of lucern and oaten chaff and allowed free access to water. For each compound challenge, benzimidazole susceptible third stage larvae (L3) of *Haemonchus contortus* (6000), *Ostertagia circumcincta* (8000), and *Trichostrongylus colubriformis* (8000) were administered via interruminal injection (day 0). For the studies with resistant strains, benzimidazole resistant larvae of *H. contortus* (8000), *O. circumcincta* (9000), and *T. colubriformis* (9000) were administered. On the basis of fecal nematode egg counts at day 27, the animals were ranked, weighed, and allocated to treatment groups (n = 4–7). Compounds for testing were suspended in 0.5% carboxymethylcellulose saline (0.9% NaCl) solution. The indicated dose was delivered via intraruminal injection. In every assay, a control group received no treatment. On day 35 after infection, the sheep were sacrificed, the abomasum and small intestine were removed, and the total number of adult *H. contortus*, *O. circumcinta*, and *T. colubriformis* was determined. The percent reduction in worm burden was calculated as above.

Trematode Infection in Sheep. Merino ewes and wethers, approximately 6–8 months of age and weighing 23–29 kg, were maintained as described above. Triclabendazole- and closantel-susceptible *Fasciola hepatica* metacercaria (125) were administered to each sheep via intraruminal injection (day 0). On the basis of faecal *F. hepatica* egg counts (eggs per gram of feces, epg) at day 98, the animals were ranked, weighed, and allocated to experimental (n = 5) and control (n = 7) groups. Compound 1 was suspended in a 0.5% sodium carboxymethylcellulose saline (0.9% NaCl) solution, and the required dose (30 mg/kg) was delivered via intraruminal injection (day 105). The control group received no treatment. On days 105, 112, 119, 125, 129, and 136, faecal *F. hepatica* egg counts were determined. The percent reduction in egg burden was calculated as

$$\frac{\text{mean epg (control)} - \text{mean epg (treated)}}{\text{mean epg (control)}} \times 100$$

***Ascaris suum* Mitochondria Assays.** Body wall muscle was obtained from adult *A. suum* by dissection and mitochondria were isolated as described previously.[28] Isolated mitochondria (4–5 mg of protein) were preincubated at 30 °C for 5 min in 170 mM mannitol, 50 mM sucrose, 10 mM $^{32}P_i$-potassium phosphate, 1 mM $MgCl_2$, 0.4 mM ADP, 0.1 unit of hexokinase and inhibitors, as indicated, at pH 7.4 in a final volume of 2 mL. The reaction was initiated with the addition of 10 mM malate and terminated after 15 min by the addition of 0.2 mL of 14% perchloric acid. ^{32}P-Organic phosphates were extracted and counted for radioactivity according to Kohler[14] and Saz.[10] Succinate was determined in neutralized perchloric extracts according to Kmetec.[29]

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Registry No. 1, 136185-95-0; **2**, 136185-96-1; **3**, 136185-97-2; 4, 136185-98-3; **5**, 136185-99-4; **6**, 136186-00-0; **7**, 136186-01-1; **8**, 136186-02-2; **9**, 136186-03-3; **10**, 136213-69-9; 11, 136186-04-4; **12**, 136186-05-5; **13**, 136186-06-6; 14, 136186-07-7; **15**, 136186-08-8; **16**, 136186-09-9; 17, 136186-10-2; **18**, 136186-11-3; **19**, 136186-12-4; **20**, 136186-13-5; **21**, 136186-14-6; **22**, 136186-15-7; **23**, 136186-16-8; **24**, 136186-17-9; **25**, 136186-18-0; **26**, 136186-19-1; **27**, 136186-20-4; **28**, 136186-21-5; **29**, 136186-22-6; **30**, 136186-23-7; **31**, 136186-24-8; **32**, 136186-25-9; **33**, 136186-26-0; **34**, 136186-27-1; **35**, 136186-28-2; **36**, 136186-29-3; **37**, 136186-30-6; **38**, 136186-31-7; **39**, 136186-32-8; **40**, 136186-33-9; **41**, 136186-34-0; **42**, 136186-35-1; **43**, 136186-36-2; 44, 136186-37-3; **45**, 136186-38-4; **46**, 136186-39-5; **47**, 136186-40-8; **48**, 136186-41-9; **49**, 136186-42-0; **50**, 136186-43-1; **51**, 136186-44-2; **52**, 136186-45-3; **53**, 136186-46-4; **54**, 136186-47-5; **55**, 136186-48-6; **56**, 136186-49-7; 4-aminobenzotrifluoride, 455-14-1; cyanoacetic acid, 372-09-8; *N*-[4-(trifluoromethyl)phenyl]cyanoacetamide, 24522-30-3; 4-(trifluoromethyl)benzoyl chloride, 329-15-7; acetonitrile, 75-05-8; [4-(trifluoromethyl)benzoyl]acetonitrile, 71682-

(27) Marcincal-Lefebvre, A.; Gesquiere, J. C.; Lemer, C.; Dupuis, B. (Phenylthio)phenylamine Derivatives as Potential Antiinflammatory Compounds. *J. Med. Chem.* **1981**, *24*, 889–893.

(28) Campbell, T.; Rubin, N.; Komuniecki, R. Succinate-dependent Energy Generation in *Ascaris suum* Mitochondria. *Mol. Biochem. Parasitol.* **1989**, *33*, 1–12.

(29) Kmetec, E. Spectrophotometric Method for the Enzymic Microdetermination of Succinic Acid. *Anal. Biochem.* **1966**, *16*, 474–480.

94-5; 4-(trifluoromethyl)phenyl isothiocyanate, 1645-65-4; 4-(phenylsulfonyl)benzoic acid, 5361-54-6; 4-cyano-2-methylaniline, 78881-21-7; N-[3-chloro-4-(4'-chlorophenoxy)phenyl]cyanoacetamide, 136186-50-0; N-methyl-N-[4-(trifluoromethyl)phenyl]-cyanoacetamide, 136186-51-1; benzenesulfinic acid sodium salt, 873-55-2; 4-fluoroacetophenone, 403-42-9; 4-(phenylsulfonyl)-acetophenone, 65085-83-8; 3-methyl-4-nitrobenzonitrile, 96784-54-2; 4-chlorophenol, 106-48-9; 3,4-dichloronitrobenzene, 99-54-7; 4-(4'-chlorophenoxy)-3-chloronitrobenzene, 22544-07-6; N-methyl-4-(trifluoromethyl)aniline, 22864-65-9.

Gossypol and Derivatives: A New Class of Aldose Reductase Inhibitors†

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Gossypol and 17 derivatives were tested as inhibitors of aldose reductase from human placenta. Gossypol and a number of the derivatives were potent inhibitors. The order of inhibitory activity was interpreted in relation to the Kador-Sharpless pharmacophor model for the aldose reductase inhibitor site. The structural but not the electronic aspects of the model were found to apply to this series of compounds.

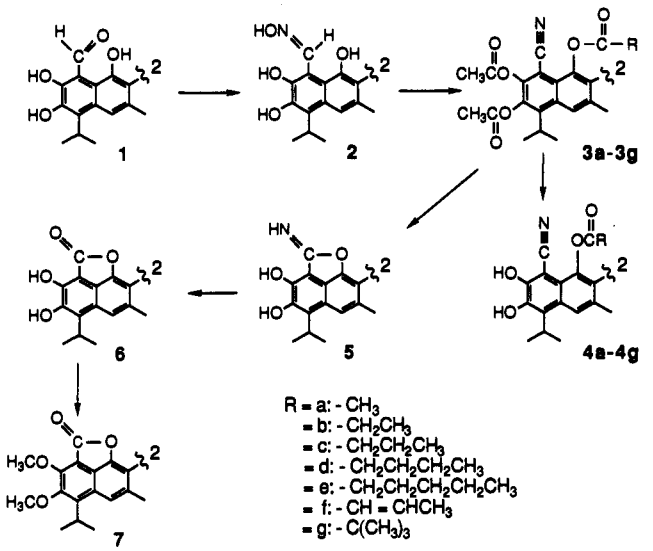
The complications of diabetes mellitus can affect many organs, but especially eye, nerve, and kidney.[1] The mechanisms whereby prolonged hyperglycemia induces these complications are not known, although a number of pathogenic mechanisms have been proposed.[1,2] One contributing mechanism might involve the polyol pathway in which glucose is reduced by NADPH to sorbitol. This reduction is catalyzed by aldose reductase (alditol:NADP$^+$ oxidoreductase, EC 1.1.1.21). Numerous studies with experimental animals support the idea that inhibition of aldose reductase can prevent the development of diabetic complications.[3-6] Therefore, aldose reductase has become a prime target for the development of therapeutic approaches to the treatment of the complications of diabetes.

Aldose reductase inhibitors are structurally diverse compounds. Kador and Sharpless[7] proposed a pharmacophor model for aldose reductase inhibitors based upon examination of the structural and electronic features of some of the known inhibitors of rat lens aldose reductase. Most kinetic studies have used aldose reductase from animal sources.[8,9] It has been shown that the sensitivity of aldose reductase to inhibitors varies with different tissues and species.[10,11] Studies carried out with an enzyme from human tissues might have greater relevance to human disease. The instability of the drug binding site of human aldose reductase has been a problem in the past.[12]

A rapid procedure for the purification of human placental aldose reductase with stabilization of the drug binding site has been described recently.[13,14] The presence of NADP at all stages of purification and during storage of the enzyme stabilizes the inhibitor binding site, making it possible to use human enzyme for kinetic studies of inhibition.

In this paper we examine a set of structurally related compounds as inhibitors in relation to the Kador-Sharpless pharmacophor model. The objective of the work was to test the model with inhibitors of human aldose reductase. The set of compounds chosen was the natural product gossypol (1) and a series of derivatives. Gossypol fits the pharmacophor model reasonably well. The structural features of the model[7,15] and the molecular dimensions of gossypol are indicated in Figure 1. Key structural features of the Kador-Sharpless model include the following: (1) a primary lipophilic (aromatic) region separated (center to center) by approximately 2.8-3.8 Å (d_1) from an electrophilic group; (2) a secondary lipophilic region located about 2.8-6.1 Å (d_2) from the electrophilic group and coplanar with the primary lipophilic region; and

Scheme I



† This paper is dedicated to Professor Herbert C. Brown on the occasion of his 80th birthday.

(1) Crabbe, M. J. C. The complications of diabetes. In *Diabetic Complications*; Crabbe, M. J. C., Ed.; Churchill Livingstone: London, 1985; pp 1-24.
(2) Baynes, J. W.; Thorpe, S. R.; Murtiashaw, M. H. Nonenzymatic Glucosylation of Lysine Residues in Albumin. In *Methods in Enzymology: Posttranslational Modifications*; Wold, F., Moldave, K., Eds.; Academic Press: New York, 1984; pp 88-98.
(3) Culebras, A.; Alio, J.; Herrera, J. L.; Lopez-Fraile, M. I. Effect of an Aldose Reductase Inhibitor on Diabetic Peripheral Neuropathy. *Arch. Neurol.* **1981**, *38*, 133-134.
(4) Chylack, L. T.; Henriques, H. F.; Cheng, H. M.; Kinoshita, J. H. Efficacy of Alrestatin, an Aldose Reductase Inhibitor, in Human Diabetic and Nondiabetic Lenses. *Ophthalmology* **1979**, *86*, 1579-1585.
(5) Beyer-Mears, A.; Cruz, E. Reversal of Diabetic Cataract by Sorbinil, an Aldose Reductase Inhibitor. *Diabetes* **1985**, *34*, 15-21.
(6) Christensen, J. E. J.; Varnek, L.; Gregersen, G. The Effect of an Aldose Reductase Inhibitor (Sorbinil) on Diabetic Neuropathy and Neural Function of the Retina: A Double-blind Study. *Acta Neurol. Scand.* **1985**, *71*, 164-167.
(7) Kador, P. F.; Sharpless, N. E. Pharmacophor Requirements of the Aldose Reductase Inhibitor Site. *Mol. Pharmacol.* **1983**, *24*, 521-531.
(8) Doughty, C. C.; Conrad, S. M. A Reaction Mechanism for Aldose Reductase from Lens. *Biochim. Biophys. Acta* **1982**, *708*, 358-364.

0022-2623/91/1834-3301$02.50/0