A Series of Diarylsubstituted Oximes As Potential Substrate For New Aldose Reductase Inhibitors

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Received September 10, 1999

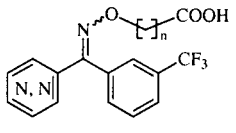
In the course of our research aimed at the discovery of new compounds acting as aldose reductase inhibitors, we tested a series of some (*E*)- and (*Z*)-ω-[[(aryldiazinylmethylene)amino]oxy]alkanoic acids, which were found to have moderate *in vitro* inhibitory activity. On this basis we have now prepared several new derivatives modified both at the length of the chain and at its terminal carboxylic group, together with compounds carrying various substituents at the phenyl ring. This paper describes their synthesis and biological properties.

J. Heterocyclic Chem., **37**, 1089 (2000).

Introduction.

Aldose reductase (ALR2, EC 1.1.1.21) is the first enzyme of the "polyol pathway"; in the presence of β-nicotinamide adenine dinucleotide phosphate (reduced form) it converts glucose to sorbitol, which is further processed by sorbitol dehydrogenase to fructose. ALR2 activity has been involved in the appearance of long-term diabetic complications, such as neuropathy, retinopathy, nephropathy and cataract. In fact, over two decades of intensive studies on several experimental diabetic animal models have demonstrated a link between glucose metabolism *via* the polyol pathway and diabetic complications [1].

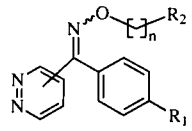
During studies aimed at finding both thromboxane synthetase inhibitors and thromboxane A_2 receptor antagonists [2], we developed a number of (*E*)- and (*Z*)-ω-[[(aryldiazinylmethylene)amino]oxy]alkanoic acids bearing a methylene chain consisting of 4 or 5 units. From the literature data it is well known that an acidic side chain is a characteristic of several aldose reductase inhibitors (ARIs). However, in the carboxylic acid series the length of the chain is important for the activity; in particular, for simple aliphatic carboxylic acids, maximum activity was associated with a chain length of 8 to 12 carbon atoms [3]. On this basis, we decided to test our compounds of general formula (**A**) as ARIs.



(A)

Compounds **1-7**

Given the significant properties associated with these derivatives, we decided to further exploit this substrate. In particular, modification both at the length of the chain and at its terminal carboxylic group were considered, together with the introduction of various substituents at the phenyl ring (**B**). Moreover, on the basis of the slightly better inhibitory properties associated with the pyridazine moiety versus other substituents, the latter was kept unchanged.



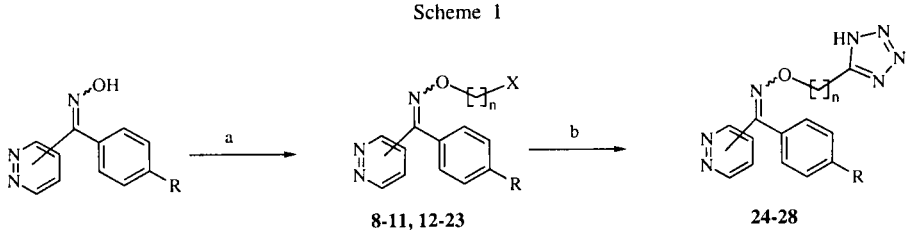
(B)

R_1 = H, Cl, OCH_3
R_2 = CH_3 (**8-11**), CN (**12-23**), tetrazolyl (**24-28**)

This paper describes the biological testing of compounds **1-7** [2] and the synthesis, structural characterisation and biological testing of compounds **8-28**.

Compounds **8-11** and **12-23** were synthesised starting from the appropriate phenylpyridazinylmethanone oxime [4,5] (Scheme 1) by reaction with the appropriate alkyl iodide in presence of potassium *tert*-butoxide or commercially available ω-haloalkylnitrile in presence of KOH, respectively. Nitriles **(*E*)-14, (*Z*)-14, (*Z*)-17, (*Z*)-19** and **(*Z*)-23** were then converted into the tetrazolyl derivatives **24-28** by treatment with trimethylsilyl azide in presence of dibutyltin oxide according to reference [6]. The (*E*)- and (*Z*)-isomers were separated by means of column chromatography (See Table 2 for eluents).

Scheme 1

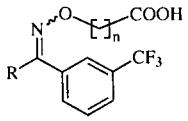


8-11, 12-23 24-28

a: I-$(CH_2)_n$-CH_3 for **8-11**; X-$(CH_2)_n$CN for **12-23**. b: trimethylsilylazide/dibutyltin

Table 1

Inhibitory activities of compounds **1-7** against ALR2



Compound	R	configuration	n	IC_{50} (μM) (95% C.L.)
1		*E*	4	51 (46-57)
2		*Z*	4	29 (20-44)
3		*E*	5	12 (11-14)
4		*Z*	5	10 (9-11)
5		*E*	4	110 (83-145)
6		*Z*	4	49 (33-72)
7		*E*	5	24(16-37)
Sorbinil				1.2 (1.0-1.4)
Tolrestat				0.096 (0.079-0.12)

Results.

The (*E*) and (*Z*)-ω-[[(aryldiazinylmethylene)amino]oxy]-alkanoic acids **1-7** possess some activity against bovine lens ALR2, even if lower than that of Sorbinil or Tolrestat (Table 1). Better properties were associated with the presence of both the pyridazine ring and a longer chain (n = 5). On the contrary, the geometry around the double bond did not affect the biological properties (for **3** and **4** IC_{50} values of 12 and 10 μM were found, respectively). Shortening the acidic side chain to 4 methylenes decreases the activity, though by different degrees, depending on the isomer considered (IC_{50} for **1** and **2**: 51 and 29 μM, respectively). Substitution of the pyridazine moiety by a pyrimidine ring always led to less potent compounds. However, also in this case better activity was found when n = 5. Finally, all the compounds lacking the terminal carboxylic group were found inactive at the concentration of 100 μM.

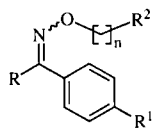
EXPERIMENTAL

Chemistry.

Melting points were determined with a Kofler hot-stage microscope (Reichert) and are uncorrected. Solvents were purified by distillation and stored over molecular sieves. All reactions were run under a dry nitrogen atmosphere. Infrared spectra (KBr pellets) were recorded on a Mattson Galaxy Series FTIR 3000 spectrophotometer. Mass spectra were obtained on a Finnigan MAT SSQ 7000 spectrometer (EI, 70 eV). All NMR spectra were

Table 2

Yields and Analytical Data of Compounds **8-28**



Compound	R	R^1	R	n	Isomer	Yield (%)	m.p. (°C)	Formula (MW)	Eluent	Elemental Analyses Calcd./Found		
										C	H	N
8	3-pyridazinyl	H	CH_3	3	*Z*	11	oil	$C_{15}H_{17}N_3O$ (255.32)	CH_2Cl_2/EA 1:1	70.56 70.83	6.71 6.65	16.46 16.26
					E	20	oil			70.56 70.86	6.71 6.59	16.46 16.35
9	3-pyridazinyl	H	CH_3	4	*Z*	33	oil	$C_{16}H_{19}N_3O$ (269.35)	CH_2Cl_2/EA 9:1	71.35 71.06	7.11 6.99	15.60 15.52
					E	29	83-84			71.35 71.51	7.11 7.16	15.60 15.68

Table 2 (continued)

Compound	R	R^1	R	n	Isomer	Yield (%)	m.p. (°C)	Formula (MW)	Eluent	Elemental Analyses Calcd./Found C	H	N
10	4-pyridazinyl	H	CH_3	3	*Z*	32	oil	$C_{15}H_{17}N_3O$ (255.32)	CH_2Cl_2/EA 5:1	70.56 70.59	6.71 6.77	16.46 16.33
11	4-pyridazinyl	H	CH_3	4	*Z*	11	oil	$C_{16}H_{19}N_3O$ (269.35)	CH_2Cl_2/EA 9:1	71.35 71.03	7.11 6.88	15.60 15.34
12	3-pyridazinyl	H	CN	4	*Z*	33	59-60	$C_{16}H_{16}N_4O$ (280.33)	EA	68.54 68.79	5.76 5.71	19.99 19.97
					E	19	88-92			68.54 68.61	5.76 5.83	19.99 19.84
13	3-pyridazinyl	H	CN	5	*Z*	39	43-45	$C_{17}H_{18}N_4O$ (294.36)	EA	69.35 69.61	6.17 5.96	19.04 18.96
					E	20	78-79			69.35 69.61	6.17 5.96	19.04 18.96
14	3-pyridazinyl	H	CN	6	*Z*	38	74-76	$C_{18}H_{20}N_4O$ (308.38)	CH_2Cl_2/EA 5:1	70.09 70.19	6.54 6.39	18.18 18.26
					E	34	73-76			70.09 70.38	6.54 6.40	18.18 18.17
15	4-pyridazinyl	H	CN	4	*Z*	43	76-78	$C_{16}H_{16}N4O$ (280.33)	EA	68.54 68.62	5.76 5.74	19.99 20.08
					E	12	oil	$C_{16}H_{16}N_4O$ x 0.2 EA (297.95)		67.72 67.33	5.95 5.56	18.80 19.06
16	4-pyridazinyl	H	CN	5	*Z*	28	22-25	$C_{17}H_{18}N_4O$ (294.36)	CH_2Cl_2/EA 1:1	69.35 69.41	6.17 5.99	19.04 18.86
					E	17	oil	$C_{17}H_{18}N_4O$ x 0.1 H_20 x 0. 1 CH_3COOH (304.97)		68.53 68.52	6.28 5.90	18.37 18.17
17	4-pyridazinyl	H	CN	6	*Z*	64	86-89	$C_{18}H_{20}N_4O$ (308.38)	CH_2Cl_2/EA 1:1	70.09 70.36	6.54 6.34	18.18 18.24
					E	16	oil			70.09 70.41	6.54 6.26	18.18 17.84
18	4-pyridazinyl	Cl	CN	4	*Z*	37	62-65	$C_{16}H_{15}ClN_4O$ (314.78)	EA	61.05 60.83	4.80 5.05	17.80 17.80
					E	26	84-88	$C_{16}H_{15}ClN_4O$ x 0.2 H_2O (318.38)		60.36 60.51	4.88 4.89	17.60 17.50
19	4-pyridazinyl	Cl	CN	5	*Z*	29	oil	$C_{17}H_{17}ClN_4O$ (328.8)	CH_2Cl_2/EA 1:1	62.10 61.84	5.21 5.17	17.04 16.80
					E	10	oil			62.10 62.09	5.21 5.45	17.04 16.79
20	4-pyridazinyl	Cl	CN	6	*Z*	34	oil	$C_{18}H_{19}ClN_4O$ (342.83)	CH_2Cl_2/EA 1:1	63.06 63.30	5.59 5.56	16.34 16.26
					E	10	oil	$C_{18}H_{19}ClN_4O$ x 0.3 EA (369.26)		62.45 62.36	5.84 5.65	15.17 15.30
21	4-pyridazinyl	OCH_3	CN	4	*Z*	80	oil	$C_{17}H_{18}N_4O_2$ x 0.3 H_2O (315.76)	EA	64.67 64.77	5.94 5.88	17.74 17.39
22	4-pyridazinyl	OCH_3	CN	5	*Z*	79	oil	$C_{18}H_2ON_4O_2$ x 0.3 EA (350.82)	EA	65.74 65.61	6.44 6.41	15.97 16.22
23	4-pyridazinyl	OCH_3	CN	6	*Z*	81	oil	$C_{19}H_{22}N_4O_2$ x 0.8 H_2O (352.82)	EA	64.68 64.85	6.74 6.90	15.88 15.57
24	3-pyridazinyl	H	5-tetrazolyl	6	*Z*	66	oil	$C_{18}H_{21}N_7O$ x 0.3 EA (377.85)	EA/MeOH 4:1	61.03 60.72	6.24 5.95	25.95 26.11
25	3-pyridazinyl	H	5-tetrazolyl	6	*E*	83	oil	$C_{18}H_{21}N_7O$ x 0.2 EA x 0.6 H_2O (379.84)	EA/MeOH 4:1	59.45 59.50	6.32 6.01	25.81 25.81
26	4-pyridazinyl	H	5-tetrazolyl	6	*Z*	44	oil	$C_{18}H_{21}N_7O$ x 0.3 EA x 0.3 H_2O (383.25)	EA/MeOH 4:1	60.17 60.14	6.31 6.02	25.58 25.67
27	4-pyridazinyl	Cl	5-tetrazolyl	5	*Z*	82	oil	$C_{17}H_{18}ClN_7O$ x 0.2 EA (389.45)	EA/MeOH 4:1	54.90 55.26	5.07 5.01	25.18 25.31
28	4-pyridazinyl	OCH_3	5-tetrazolyl	6	*Z*	43	oil	$C_{19}H_{23}N_7O_2$ x 0.3 EA x 0.3 MeOH (423.89)	EA/MeOH 4:1	58.65 58.55	6.52 6.34	23.13 23.17

EA = ethyl acetate, MeOH = methanol.

Table 3

Spectroscopic Data of Compounds **8-28**

Compound	IR (cm^{-1})	MS (m/z)	^{1}H-NMR	^{13}C-NMR
8 (*Z*)	no characteristical absorptions	255.2 (M^+)	9.21 (dd, 1H, J_{46} = 1.9 Hz, J_{56} = 4.8 Hz, pyridazine H-6), 7.66 (dd, 1H, J_{45} = 8.4 Hz, J_{46} = 1.9 Hz, pyridazine H-4), 7.55 (dd, 1H, J_{45} = 8.4 Hz, J_{56} = 4.8 Hz, pyridazine H-5), 7.53-7.30 (m, 5H, phenyl H), 4.20 (t, 2H, J = 6.6 Hz, OCH_2), 1.72-1.58 (m, 2H, $CH_2CH_2CH_3$), 1.42-1.23 (m, 2H, $CH_2CH_2CH_3$), 0.89 (t, 3H, J = 7.4 Hz, CH_3)	156.0 (pyridazine C-3), 152.9 (C=N), 150.8 (pyridazine C-6), 134.4 (phenyl C-1), 129.5 (phenyl C-4), 128.4, 125.7 (pyridazine C-4, pyridazine C-5), 128.3, 127.3 (phenyl C-2/6, phenyl C-3/5), 75.0 (OCH_2), 31.1 ($CH_2CH_2CH_3$), 19.0 ($CH_2CH_2CH_3$), 13.7 (CH_3)
8 (*E*)	no characteristical absorptions	255.2 (M^+)	9.10 (dd, 1H, J_{46} = 1.7 Hz, J_{56} = 5.0 Hz, pyridazine H-6), 8.04 (dd, 1H, J_{45} = 8.7 Hz, J_{46} = 1.7 Hz, pyridazine H-4), 7.48-7.37 (m, 6H, pyridazine H-5, phenyl H), 4.26 (t, 2H, J = 6.7 Hz, OCH_2), 1.78-1.64 (m, 2H, $CH_2CH_2CH_3$), 1.49-1.30 (m, 2H, $CH_2CH_2CH_3$), 0.93 (t, 3H, J = 7.3 Hz, CH_3)	157.8 (pyridazine C-3), 153.7 (C=N), 150.7 (pyridazine C-6), 131.1 (phenyl C-1), 129.5, 127.7 (phenyl C-2/6, phenyl C-3/5), 128.9 (phenyl C-4), 126.1, 125.3 (pyridazine C-4, pyridazine C-5), 75.2 (OCH_2), 31.1 ($CH_2CH_2CH_3$), 18.9 ($CH_2CH_2CH_3$), 13.7 (CH_3)
9 (*Z*)	no characteristical absorptions	269.2 (M^+)	9.21 (dd, 1H, J_{46} = 2.0 Hz, J_{56} = 5.0 Hz,pyridazine H-6), 7.66 (dd, 1H, J_{45} = 8.4 Hz, J_{46} = 2.0 Hz, pyridazine H-4), 7.55 (dd, 1H, J_{45} = 8.4 Hz, J_{56} = 5.0 Hz, pyridazine H-5), 7.53-7.32 (m, 5H, phenyl H), 4.19 (t, 2H, J = 6.7 Hz, OCH_2), 1.70-1.62 (m, 2H, CH_2), 1.33-1.24 (m, 4H, 2x CH_2), 0.87 (t, 3H, J = 6.6 Hz, CH_3)	156.0 (pyridazine C-3), 152.9 (C=N), 150.8 (pyridazine C-6), 134.4 (phenyl C-1), 129.5 (phenyl C-4), 128.4, 125.7 (pyridazine C-4, pyridazine C-5), 128.3, 127.3 (phenyl C-2/6, phenyl C-3/5), 75.2 (OCH_2), 28.6, 27.9, 22.2 (3x CH_2), 13.8 (CH_3)
9 (*E*)	no characteristical absorptions	269.2 (M^+)	9.11 (dd, 1H, J_{46} = 1.7 Hz, J_{56} = 4.9 Hz, pyridazine H-6), 8.04 (dd, 1H, J_{45} = 8.7 Hz, J_{46} = 1.7 Hz, pyridazine H-4), 7.50-7.38 (m, 6H, pyridazine H-5, phenyl H), 4.25 (t, 2H, J = 6.7 Hz, OCH_2), 1.77-1.66 (m, 2H, CH_2), 1.39-1.29 (m, 4H, 2x CH_2), 0.89 (t, 3H, J = 7.1 Hz, CH_3)	157.8 (pyridazine C-3), 153.7 (C=N), 150.7 (pyridazine C-6), 131.1 (phenyl C-1), 129.5, 127.8 (phenyl C-2/6, phenyl C-3/5), 128.9 (phenyl C-4), 126.1, 125.3 (pyridazine C-4, pyridazine C-5), 75.5 (OCH_2), 28.6, 27.9, 22.2 (3x CH_2), 13.8 (CH_3)
10 (*Z*)	no characteristical absorptions	255.2 (M^+)	9.30 (dd, 1H, J_{36} = 0.8 Hz, J_{56} = 5.2 Hz, pyridazine H-6), 9.20 ("d", 1H, pyridazine H-3), 7.44 (dd, 1H, J_{35} = 2.2 Hz, J_{56} = 5.2 Hz, pyridazine H-5), 7.44-7.33 (m, 5H, phenyl H), 4.23 (t, 2H, J = 6.6 Hz, OCH_2), 1.74-1.60 (m, 2H, $CH_2CH_2CH_3$), 1.45-1.26 (m, 2H, $CH_2CH_2CH_3$), 0.92 (t, 3H, J = 7.2 Hz, CH_3)	151.2, 151.0 (pyridazine C-6, pyridazine C-3), 150.8 (C=N), 134.1 (phenyl C-1), 131.9 (pyridazine C-4), 129.9 (phenyl C-4), 128.6, 127.3 (phenyl C-2/6, phenyl C-3/5), 126.1 (pyridazine C-5), 75.1 (OCH_2), 30.9 ($CH_2CH_2CH_3$), 19.0 ($CH_2CH_2CH_3$), 13.7 (CH_3)
11 (*Z*)	no characteristical absorptions	269.2 (M^+)	9.30 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.3 Hz, pyridazine H-6), 9.19 (dd, 1H, J_{35} = 2.2 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.45 (dd, 1H, J_{35} = 2.2 Hz, J_{56} = 5.3 Hz, pyridazine H-5), 7.42-7.29 (m, 5H, phenyl H), 4.22 (t, 2H, J = 6.7 Hz, OCH_2), 1.73-1.65 (m, 2H, CH_2), 1.34-1.27 (m, 4H, 2x CH_2), 0.89 (t, 3H, J = 6.6 Hz, CH_3)	151.2, 151.0 (pyridazine C-6, pyridazine C-3), 150.8 (C=N), 134.1 (phenyl C-1), 131.9 (pyridazine C-4), 129.9 (phenyl C-4), 128.6, 127.6 (phenyl C-2/6, phenyl C-3/5), 126.1 (pyridazine C-5), 75.4 (OCH_2), 28.5, 27.9, 22.2 (3x CH_2), 13.8 (CH_3)
12 (*Z*)	2240 (n C≡N)	279.1 (M^+ -1)	9.27-9.24 (m, 1H, pyridazine H-6), 7.64-7.29 (m, 7H, pyridazine H-4, pyridazine H-5, phenyl H), 4.24 (t,2H, J = 6.0 Hz, OCH_2), 2.32 (t, 2H, J = 6.9 Hz, CH_2CN), 1.91-1.63 (m, 4H, 2x CH_2)	156.0 (pyridazine C-3), 153.8 (C=N), 151.0 (pyridazine C-6), 134.0 (phenyl C-1), 129.9 (phenyl C-4), 128.5, 127.3 (phenyl C-2/6, phenyl C-3/5), 128.0, 126.0 (pyridazine C-4, pyridazine C-5), 119.4 (C≡N), 73.9 (OCH_2), 27.9, 22.2, 16.9 (3x CH_2)
12 (*E*)	2246 (n C≡N)	279.1 (M^+ -1)	9.16 ("d", 1H, pyridazine H-6), 8.04 ("d", 1H, pyridazine H-4), 7.53-7.38 (m, 6H, pyridazine H-5, phenyl H), 4.31 (t, 2H, J = 5.9 Hz, OCH_2), 2.34 (t, 2H, J = 6.8 Hz, CH_2CN), 1.90-1.70 (m, 4H, 2x CH_2)	157.7 (pyridazine C-3), 154.8 (C=N), 150.9 (pyridazine C-6), 131.0 (phenyl C-1), 129.4, 128.1 (phenyl C-2/6, phenyl C-3/5), 129.3 (phenyl C-4), 126.2, 125.5 (pyridazine C-4, pyridazine C-5), 119.4 (C≡N), 74.2 (OCH_2), 27.9, 22.3, 16.9 (3x CH_2)
13 (*Z*)	2239 (n C≡N)	293.1 (M^+ -1)	9.24 (dd, 1H, J_{46} = 2.2 Hz, J_{56} = 4.8 Hz, pyridazine H-6), 7.66 (dd, 1H, J_{45} = 8.5 Hz, J_{46} = 2.2 Hz, pyridazine H-4), 7.59 (dd, 1H, J_{45} = 8.5 Hz, J_{56} = 4.8 Hz, pyridazine H-5), 7.53-7.31 (m, 5H, phenyl H), 4.21 (t, 2H, J = 6.2 Hz, OCH_2), 2.31 (t, 2H, J = 6.8 Hz, CH_2CN), 1.78-1.42 (m, 6H, 3x CH_2)	156.1 (pyridazine C-3), 153.5 (C=N), 150.9 (pyridazine C-6), 134.2 (phenyl C-1), 129.8 (phenyl C-4), 128.5, 127.3 (phenyl C-2/6, phenyl C-3/5), 128.2, 126.0 (pyridazine C-4, pyridazine C-5), 119.6 (C≡N), 74.5 (OCH_2), 28.1, 25.1, 25.0, 17.1 (4x CH_2)
13 (*E*)	2241 (n C≡N)	293.1 (M^+ -1)	9.15 (dd, 1H, J_{46} = 1.6 Hz, J_{56} = 5.0 Hz, pyridazine H-6), 8.04 (dd, 1H, J_{45} = 8.6 Hz, J_{46} = 1.6 Hz, pyridazine H-4), 7.52-7.44 (m, 6H, pyridazine H-5, phenyl H), 4.28 (t, 2H, J = 6.2 Hz, OCH_2), 2.31 (t, 2H, J = 6.8 Hz, CH_2CN), 1.81-1.54 (m, 6H, 3x CH_2)	157.8 (pyridazine C-3), 154.4 (C=N), 150.9 (pyridazine C-6), 131.1 (phenyl C-1), 129.5, 128.0 (phenyl C-2/6, phenyl C-3/5), 129.2 (phenyl C-4), 126.2, 125.4 (pyridazine C-4, pyridazine C-5), 119.5 (C≡N), 74.9 (OCH_2), 28.2, 25.2, 25.1, 17.0 (4x CH_2)

Table 3 (continued)

Compound	IR (cm^{-1})	MS (m/z)	^{1}H-NMR	^{13}C-NMR
14 (*Z*)	2241 (n C≡N)	307.1 (M^+ -1)	9.24 (dd, 1H, J_{46} = 2.0 Hz, J_{56} = 4.7 Hz, pyridazine H-6), 7.65 (dd, 1H, J_{45} = 8.4 Hz, J_{46} = 2.0 Hz, pyridazine H-4), 7.58 (dd, 1H, J_{45} = 8.4 Hz, J_{56} = 4.7 Hz, pyridazine H-5), 7.53-7.31 (m, 5H, phenyl H), 4.20 (t, 2H, J = 6.5 Hz, OCH_2), 2.31 (t, 2H, J = 6.9 Hz, CH_2CN), 1.75-1.33 (m, 8H, 4x CH_2)	156.2 (pyridazine C-3), 153.3 (C=N), 151.0 (pyridazine C-6), 134.4 (phenyl C-1), 129.8 (phenyl C-4), 128.5, 127.4 (phenyl C-2/6, phenyl C-3/5), 128.3, 125.9 (pyridazine C-4, pyridazine C-5), 119.8 (C≡N), 74.9 (OCH_2), 28.7, 28.4, 25.3, 25.2, 17.1 (5x CH_2)
14 (*E*)	2240 (n C≡N)	307.1 (M^+ -1)	9.15 (dd, 1H, J_{46} = 1.6 Hz, J_{56} = 4.9 Hz, pyridazine H-6), 8.05 (dd, 1H, J_{45} = 8.7 Hz, J_{46} = 1.6 Hz, pyridazine H-4), 7.52-7.34 (m, 6H, pyridazine H-5, phenyl H), 4.27 (t, 2H, J = 6.5 Hz, OCH_2), 2.30 (t, 2H,J = 6.9 Hz, CH_2CN), 1.79-1.42 (m, 8H, 4x CH_2)	157.9 (pyridazine C-3), 154.2 (C=N), 150.9 (pyridazine C-6), 131.1 (phenyl C-1), 129.6, 128.0 (phenyl C-2/6, phenyl C-3/5), 129.2 (phenyl C-4), 126.2, 125.4 (pyridazine C-4, pyridazine C-5), 119.6 (C≡N), 75.2 (OCH_2), 28.7, 28.3, 25.2, 25.1, 17.0 (5x CH_2)
15 (*Z*)	2242 (n C≡N)	280.1 (M^+)	9.33 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.2 Hz, pyridazine H-6), 9.18 (dd, 1H, J_{35} = 2.3 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.46-7.39 (m, 6H, pyridazine H-5, phenyl H), 4.27 (t, 2H, J = 5.9 Hz, OCH_2), 2.36 (t, 2H, J = 6.8 Hz, CH_2CN), 1.91-1.66 (m, 4H, 2x CH_2)	151.7 (C=N), 151.4 (pyridazine C-6), 150.9 (pyridazine C-3), 133.8 (phenyl C-1), 131.9 (pyridazine C-4), 130.3 (phenyl C-4), 128.8, 127.4 (phenyl C-2/6, phenyl C-3/5), 126.0 (pyridazine C-5), 119.2 (C≡N), 74.0 (OCH_2), 28.0, 22.2, 16.9 (3x CH_2)
15 (*E*)	2244 (n C≡N)	280.1 (M^+)	9.40 ("d", 1H, pyridazine H-3), 9.15 ("d", 1H, pyridazine H-6), 7.51-7.29 (m, 6H, pyridazine H-5, phenyl H), 4.31 (t, 2H, J = 6.0 Hz, OCH_2), 2.33 (t, 2H, J = 6.9 Hz, CH_2CN), 1.93-1.69 (m, 4H, 2x CH_2)	152.6 (C=N), 151.2 (pyridazine C-6), 149.0 (pyridazine C-3), 134.3 (pyridazine C-4), 130.3 (phenyl C-1), 129.9 (phenyl C-4), 128.7, 128.6 (phenyl C-2/6, phenyl C-3/5), 119.3 (C≡N), 74.5 (OCH_2), 27.9, 22.3, 16.9 (3x CH_2)
16 (*Z*)	2243 (n C≡N)	294.1 (M^+)	9.32 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.4 Hz, pyridazine H-6), 9.18 (dd, 1H, J_{35} = 2.2 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.45 (dd, 1H, J_{35} = 2.2 Hz, J_{56} = 5.4 Hz, pyridazine H-5), 7.43-7.34 (m, 5H, phenyl H), 4.23 (t, 2H, J = 6.3 Hz, OCH_2), 2.34 (t, 2H, J = 6.8 Hz, CH_2CN), 1.81-1.45 (m, 6H, 3x CH_2) 151.4 (C=N),	151.4 (pyridazine C-6), 151.0 (pyridazine C-3), 134.0 (phenyl C-1), 132.0 (pyridazine C-4), 130.2 (phenyl C-4), 128.8, 127.4 (phenyl C-2/6, phenyl C-3/5), 126.1 (pyridazine C-5), 119.4 (C≡N), 74.9 (OCH_2), 28.1, 25.1, 25.0, 17.0 (4x CH_2)
16 (*E*)	2243 (n C≡N)	294.1 (M^+)	9.40 (dd, 1H, J_{35} = 2.1 Hz, J_{36} = 1.2 Hz, pyridazine H-3), 9.14 (dd, 1H, J_{36} = 1.2 Hz, J_{56} = 5.4 Hz, pyridazine H-6), 7.51-7.27 (m, 6H, pyridazine H-5, phenyl H), 4.28 (t, 2H, J = 6.3 Hz, OCH_2), 2.32 (t, 2H, J = 6.8 Hz, CH_2CN), 1.83-1.48 (m, 6H, 3x CH_2)	152.2 (C=N), 151.2 (pyridazine C-6), 149.1 (pyridazine C-3), 134.4 (pyridazine C-4), 130.4 (phenyl C-1), 129.8 (phenyl C-4), 128.8, 128.7 (phenyl C-2/6, phenyl C-3/5), 123.7 (pyridazine C-5), 119.4 (C≡N), 75.1 (OCH_2), 28.1, 25.1, 25.0, 17.0 (4x CH_2)
17 (*Z*)	2240 (n C≡N)	308.1 (M^+)	9.32 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.2 Hz, pyridazine H-6), 9.19 (dd, 1H, J_{35} = 2.2 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.46-7.34 (m, 6H, pyridazine H-5, phenyl H), 4.22 (t, 2H, J = 6.5 Hz, OCH_2), 2.33 (t, 2H, J = 6.8 Hz, CH_2CN), 1.75-1.36 (m, 8H, 4x CH_2)	151.4 (pyridazine C-6), 151.2 (C=N), 151.1 (pyridazine C-3), 134.0 (phenyl C-1), 132.0 (pyridazine C-4), 130.2 (phenyl C-4), 128.7, 127.4 (phenyl C-2/6, phenyl C-3/5), 126.1 (pyridazine C-5), 119.6 (C≡N), 75.1 (OCH_2), 28.7, 28.3, 25.2, 25.1, 17.0 (5x CH_2)
17 (*E*)	2244 (n C≡N)	308.1 (M^+)	9.40 ("d", 1H, pyridazine H-3), 9.14 ("d", 1H, pyridazine H-6), 7.52-7.28 (m, 6H, pyridazine H-5, phenyl H), 4.27 (t, 2H, J = 6.5 Hz, OCH_2), 2.31 (t, 2H, J = 6.9 Hz, CH_2CN), 1.81-1.39 (m, 8H, 4x CH_2)	151.9 (C=N), 151.2 (pyridazine C-6), 149.1 (pyridazine C-3), 134.5 (pyridazine C-4), 130.5 (phenyl C-1), 129.7 (phenyl C-4), 128.9, 128.6 (phenyl C-2/6, phenyl C-3/5), 123.7 (pyridazine C-5), 119.6 (C≡N), 75.4 (OCH_2), 28.6, 28.3, 25.2, 25.1, 17.0 (5x CH_2)
18 (*Z*)	2240 (n C≡N)	314.1 (M^+)	9.34 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.2 Hz, pyridazine H-6), 9.16 (dd, 1H, J_{35} = 2.3 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.44 (dd, 1H, J_{35} = 2.3 Hz, J_{56} = 5.2 Hz, pyridazine H-5), 7.38 (s, 4H, phenyl H), 4.27 (t, 2H, J = 6.2 Hz,OCH_2), 2.36 (t, 2H, J = 6.8 Hz, CH_2CN), 1.92-1.66 (m, 4H, 2x CH_2)	151.3 (pyridazine C-6), 150.6 (pyridazine C-3), 150.6 (C=N), 136.4, 132.2, 131.3 (phenyl C-1, phenyl C-4, pyridazine C-4), 129.0, 128.5 (phenyl C-2/6, phenyl C-3/5), 125.9 (pyridazine C-5), 119.2 (C≡N), 74.1 (OCH_2), 27.8, 22.0, 16.8 (3x CH_2)
18 (*E*)	2241 (n C≡N)	314.1 (M^+)	9.32 (dd, 1H, J_{35} = 2.4 Hz, J_{36} = 1.4 Hz, pyridazine H-3), 9.10 (dd, 1H, J_{36} = 1.4 Hz, J_{56} = 5.3 Hz, pyridazine H-6), 7.45-7.41 (m, 2H, phenyl H), 7.35 (dd, 1H, J_{35} = 2.4 Hz, J_{56} = 5.3 Hz, pyridazine H-5), 7.26-7.21 (m, 2H, phenyl H), 4.27 (t, 2H, J = 6.0 Hz, OCH_2), 2.32 (t, 2H, J = 6.8 Hz, CH_2CN), 1.92-1.61 (m, 4H, 2x CH_2)	151.3 (C=N), 151.1 (pyridazine C-6), 148.8 (pyridazine C-3), 135.8, 133.8, 128.4 (phenyl C-1, phenyl C-4, pyridazine C-4), 130.2, 128.9 (phenyl C-2/6, phenyl C-3/5), 123.5 (pyridazine C-5), 119.2 (C≡N), 74.5 (OCH_2), 27.8, 22.0, 16.8 (3x CH_2)

Table 3 (continued)

Compound	IR (cm^{-1})	MS (m/z)	^{1}H-NMR	^{13}C-NMR
19 (*Z*)	2240 (n C≡N)	328.1 (M^+)	9.34 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.3 Hz, pyridazine H-6), 9.16 (dd, 1H, J_{35} = 2.4 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.44 (dd, 1H, J_{35} = 2.4 Hz, J_{56} = 5.3 Hz, pyridazine H-5), 7.37 (s, 4H, phenyl H), 4.23 (t, 2H, J = 6.4 Hz, OCH_2), 2.34 (t, 2H, J = 6.7 Hz, CH_2CN), 1.80-1.45 (m, 6H, 3x CH_2)	151.4 (pyridazine C-6), 150.8 (pyridazine C-3), 150.4 (C=N), 136.4, 132.4, 131.5 (phenyl C-1, phenyl C-4, pyridazine C-4), 129.0, 128.5 (phenyl C-2/6, phenyl C-3/5), 126.0 (pyridazine C-5), 119.4 (C≡N), 74.9 (OCH_2), 28.1, 25.0, 25.0, 17.0 (4x CH_2)
19 (*E*)	2244 (n C≡N)	328.1 (M^+)	9.39 (dd, 1H, J_{35} = 2.2 Hz, J_{36} = 1.1 Hz, pyridazine H-3), 9.16 (dd, 1H, J_{36} = 1.1 Hz, J_{56} = 5.4 Hz, pyridazine H-6), 7.51-7.45 (m, 2H, phenyl H), 7.40 (dd, 1H, J_{35} = 2.2 Hz, J_{56} = 5.4 Hz, pyridazine H-5), 7.32-7.26 (m, 2H, phenyl H), 4.29 (t, 2H, J = 6.3 Hz, OCH_2), 2.35 (t, 2H, J = 6.8 Hz, CH_2CN), 1.85-1.48 (m, 6H, 3x CH_2)	151.2 (pyridazine C-6), 151.1 (C=N), 148.9 (pyridazine C-3), 135.8, 134.0, 128.6 (phenyl C-1, phenyl C-4, pyridazine C-4), 130.3, 129.0 (phenyl C-2/6, phenyl C-3/5), 123.6 (pyridazine C-5), 119.4 (C≡N), 75.1 (OCH_2), 28.1, 24.9, 24.9, 17.0 (4x CH_2)
20 (*Z*)	2240 (n C≡N)	342.1 (M^+)	9.33 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.3 Hz, pyridazine H-6), 9.17 (dd, 1H, J_{35} = 2.3 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.45 (dd, 1H, J_{35} = 2.3 Hz, J_{56} = 5.3 Hz, pyridazine H-5), 7.42-7.32 (m, 4H, phenyl H), 4.27 (t, 2H, J = 6.6 Hz, OCH_2), 2.33 (t, 2H, J = 7.0 Hz, CH_2CN), 1.75-1.33 (m, 8H, 4x CH_2)	151.4 (pyridazine C-6), 150.7 (pyridazine C-3), 150.0 (C=N), 136.0, 132.3, 131.3 (phenyl C-1, phenyl C-4, pyridazine C-4), 128.8, 128.4 (phenyl C-2/6, phenyl C-3/5), 125.9 (pyridazine C-5), 119.4 (C≡N), 75.1 (OCH_2), 28.4, 28.1, 25.0, 24.9, 16.8 (5x CH_2)
20 (*E*)	2240 (n C≡N)	342.1 (M^+)	9.39 (dd, 1H, J_{35} = 2.4 Hz, J_{36} = 1.2 Hz, pyridazine H-3), 9.15 (dd, 1H, J_{36} = 1.2 Hz, J_{56} = 5.4 Hz, pyridazine H-6), 7.50-7.45 (m, 2H, phenyl H), 7.39 (dd, 1H, J_{35} = 2.4 Hz, J_{56} = 5.4 Hz, pyridazine H-5), 7.30-7.25 (m, 2H, phenyl H), 4.27 (t, 2H, J = 6.4 Hz, OCH_2), 2.33 (t, 2H, J = 6.6 Hz, CH_2CN), 1.88-1.36 (m, 8H, 4x CH_2)	151.2 (pyridazine C-6), 150.9 (C=N), 149.0 (pyridazine C-3), 135.8, 134.1, 128.7 (phenyl C-1, phenyl C-4, pyridazine C-4), 130.4, 129.0 (phenyl C-2/6, phenyl C-3/5), 123.5 (pyridazine C-5), 119.5 (C≡N), 75.6 (OCH_2), 28.6, 28.3, 25.1, 25.1, 17.0 (5x CH_2)
21 (*Z*)	2241 (n C≡N)	310.1 (M^+)	9.32 (dd, 1H, J_{36} = 1.4 Hz, J_{56} = 5.3 Hz, pyridazine H-6), 9.16 (dd, 1H, J_{35} = 2.4 Hz, J_{36} = 1.4 Hz, pyridazine H-3), 7.44 (dd, 1H, J_{35} = 2.4 Hz, J_{56} = 5.3 Hz, pyridazine H-5), 7.39-7.33 (m, 2H, phenyl H-2/6), 6.92-6.86 (m, 2H, phenyl H-3/5), 4.23 (t, 2H, J = 6.1 Hz, OCH_2), 3.83 (s, 3H, OCH_3), 2.35 (t, 2H, J = 6.8 Hz, CH_2CN), 1.93-1.62 (m, 4H, 2x CH_2)	161.2 (phenyl C-4), 151.3 (pyridazine C-6), 150.8 (pyridazine C-3, C=N), 132.1 (phenyl C-1), 128.7 (phenyl C-2/6), 126.2 (pyridazine C-4), 126.0 (pyridazine C-5), 119.2 (C≡N), 114.1 (phenyl C-3/5), 73.7 (OCH_2), 55.3 (OCH_3), 27.8, 22.1, 16.8 (3x CH_2)
22 (*Z*)	2242 (n C≡N)	324.1 (M^+)	9.31 (dd, 1H, J_{36} = 1.3 Hz, J_{56} = 5.3 Hz, pyridazine H-6), 9.16 (dd, 1H, J_{35} = 2.3 Hz, J_{36} = 1.3 Hz, pyridazine H-3), 7.45 (dd, 1H, J_{35} = 2.3 Hz, J_{56} = 5.3 Hz, pyridazine H-5), 7.38-7.32 (m, 2H, phenyl H-2/6), 6.91-6.85 (m, 2H, phenyl H-3/5), 4.20 (t, 2H, J = 6.3 Hz, OCH_2), 3.83 (s, 3H, OCH_3), 2.34 (t, 2H, J = 6.9 Hz, CH_2CN), 1.79-1.44 (m, 6H, 3x CH_2)	161.1 (phenyl C-4), 151.2 (pyridazine C-6), 150.9 (C=N), 150.8 (pyridazine C-3), 132.1 (phenyl C-1), 128.6 (phenyl C-2/6), 126.3 (pyridazine C-4), 126.0 (pyridazine C-5), 119.4 (C≡N), 114.0 (phenyl C-3/5), 74.3 (OCH_2), 55.2 (OCH_3), 28.0, 24.9, 24.9, 16.8 (4x CH_2)
23 (*Z*)	2243 (n C≡N)	338.1 (M^+)	9.31 (dd, 1H, J_{36} = 1.4 Hz, J_{56} = 5.3 Hz, pyridazine H-6), 9.17 (dd, 1H, J_{35} = 2.4 Hz, J_{36} = 1.4 Hz, pyridazine H-3), 7.44 (dd, 1H, J_{35} = 2.4 Hz, J_{56} = 5.3 Hz, pyridazine H-5), 7.38-7.34 (m, 2H, phenyl H-2/6), 6.91-6.87 (m, 2H, phenyl H-3/5), 4.19 (t, 2H, J = 6.5 Hz, OCH_2), 3.83 (s, 3H, OCH_3), 2.33 (t, 2H, J = 6.9 Hz, CH_2CN), 1.76-1.26 (m, 8H, 4x CH_2)	161.1 (phenyl C-4), 151.3 (pyridazine C-6), 151.0 (pyridazine C-3), 150.8 (C=N), 132.2 (phenyl C-1), 128.7 (phenyl C-2/6), 126.4 (pyridazine C-4), 126.0 (pyridazine C-5), 119.5 (C≡N), 114.1 (phenyl C-3/5), 74.7 (OCH_2), 55.3 (OCH_3), 28.5, 28.2, 25.1, 25.1, 16.9 (5x CH_2)
24 (*Z*)	no characteristical absorptions	352.1 (M^+)	9.30 (dd, 1H, J_{46} = 2.0 Hz, J_{56} = 4.9 Hz, pyridazine H-6), 7.76 (dd, 1H, J_{45} = 8.6 Hz, J_{56} = 4.9 Hz, pyridazine H-5), 7.68 (dd, 1H, J_{45} = 8.6 Hz, J_{46} = 2.0 Hz, pyridazine H-4), 7.49-7.31 (m, 5H, phenyl H), 4.20-4.07 (m, 2H, OCH_2), 2.95 (t, 2H, J = 7.5 Hz, CH_2), 1.77-1.22 (m, 8H, 4x CH_2)	
25 (*E*)	no characteristical absorptions	352.1 (M^+)	9.17 (dd, 1H, J_{46} = 1.7 Hz, J_{56} = 5.0 Hz, pyridazine H-6), 7.95 (dd, 1H, J_{45} = 8.7 Hz, J_{46} = 1.7 Hz, pyridazine H-4), 7.57 (dd, 1H, J_{45} = 8.7 Hz, J_{56} = 5.0 Hz, pyridazine H-5), 7.41-7.37 (m, 5H, phenyl H), 4.21 (t, 2H, J = 6.5 Hz, OCH_2), 2.91 (t, 2H, J = 7.5 Hz, CH_2), 1.78-1.25 (m, 8H, 4x CH_2)	
26 (*Z*)	no characteristical absorptions	352.1 (M^+)	9.38-9.31 (m, 2H, pyridazine H-6, pyridazine H-3), 7.51-7.32 (m, 6H, pyridazine H-5, phenyl H), 4.24 (t, 2H, J = 6.0 Hz, OCH_2), 2.99 (t, 2H, J = 7.7 Hz, CH_2), 1.87-1.26 (m, 8H, 4x CH_2)	

Table 3 (continued)

Compound	IR (cm^{-1})	MS (m/z)	^{1}H-NMR	^{13}C-NMR
27 (*Z*)	no characteristical absorptions	372.1 (M^+)	9.35 ("s", 1H, pyridazine H-6), 9.20 ("s", 1H, pyridazine H-3), 7.50 ("d", 1H, pyridazine H-5), 7.38-7.28 (m, 4H, phenyl H), 4.20 (t, 2H, J = 6.0 Hz, OCH_2), 3.04 (t, 2H, J = 7.4 Hz, CH_2), 1.93-1.36 (m, 6H, 3x CH_2)	
28 (*Z*)	no characteristical absorptions	382.1 (M^+)	9.33 ("d", 1H, J = 5.2 Hz, pyridazine H-6), 9.24 ("s", 1H, pyridazine H-3), 7.49 (dd, 1H, J_{35} = 2.0 Hz, J_{56} = 5.2 Hz, pyridazine H-5), 7.38-7.32 (m, 2H, phenyl H-2/6), 6.92-6.86 (m, 2H, phenyl H-3/5), 4.18 (t, 2H, J = 6.1 Hz, OCH_2), 3.82 (s, 3H, OCH_3), 2.97 (t, 2H, J = 7.5 Hz, CH_2), 1.88-1.26 (m, 8H, 4x CH_2)	

recorded in deuteriochloroform solution in 5 mm tubes at 30°C on a Varian Gemini 200 MHz spectrometer (199.98 MHz for ^{1}H, 50.29 MHz for ^{13}C) with the deuterium signal of the solvent as the lock and TMS as internal standard. Chemical shifts are expressed in parts per million (ppm). The DEPT spectra were run in a standard manner using only the $\theta = 135°$ pulse to separate the CH/CH_3 and CH_2 lines phased "up" and "down", respectively. Reactions were monitored by thin layer chromatography using Polygram® SIL G/UV_{254} (Macherey-Nagel) plastic-backed plates (0.25 mm layer thickness) and visualised using an UV lamp. Column chromatography was conducted on Merck silica gel 60 (230-400 mesh) and medium pressure liquid chromatography (mplc) was carried out using Merck LiChroprep® Si 60 (40 – 63 µm) silica gel as stationary phase. Chromatographic separation of *E/Z*-isomers was at ratio resulting in R_f-values of about 0.3 for the faster eluted component (*Z*-isomer). The yields given and separations of *E/Z*-isomers are not optimised. Elemental analyses were performed by Mag. J. Theiner, Institute of Physical Chemistry, University of Vienna, Austria.

General procedure for the preparation of ketoxime ethers (**8-11**).

Potassium *tert*-butoxide (1.2 equivalents) was added to a suspension of the appropriate phenylpyridazinylmethanone oxime [1,2] in dry 1,4-dioxane (15-20 mL for each g of starting material) under an atmosphere of nitrogen and the mixture was stirred for 30 minutes at room temperature. After addition of 2.1 equivalents of the alkyl iodide, the solution was heated to 50°C until TLC (solvents see Table 2) indicated no further conversion. Then the mixture was poured into cold 1*N* HCl (50 mL) and was extracted with ethyl acetate (3x 25 mL). The organic layer was washed with water (2x 25 mL), brine (1x 25 mL), dried over anhydrous sodium sulphate and evaporated to dryness *in vacuo*. The products were separated and purified by column chromatography (see Table 2 for yields and analytical data and Table 3 for spectroscopic data).

General procedure for the preparation of ketoxime ethers (**12-23**).

Powdered potassium hydroxide (2 equivalents) was added to a solution of the appropriate phenylpyridazinylmethanone oxime [1,2] (1 equivalent) in dry dimethyl sulfoxide (2 mL for each 0.5 g of starting material) under an atmosphere of nitrogen. After stirring for 1 hour at room temperature, the suitable ω-haloalkylnitrile (1.1 equivalents) was added and stirring was continued for an additional 30 minutes. Then the mixture was poured into cold 1*N* HCl (50 mL) and was extracted with ethyl acetate (3x 25 mL). The organic layer was washed with water (2x 25 mL), brine (1x 25 mL), dried over anhydrous sodium sulphate and evaporated to dryness *in vacuo*. The products were separated and purified by column chromatography and/or recrystallization from diisopropylether/ethyl acetate (See Table 2 for yields and analytical data and Table 3 for spectroscopic data).

General procedure for the preparation of ketoxime ethers (**24-28**).

Trimethylsilyl azide (6 equivalents) and dibutyltin oxide (0.3 equivalents) were added to a solution of the appropriate nitrile [*i.e.* (*E*)-**14**, (*Z*)-**14**, (*Z*)-**17**, (*Z*)-**19**, and (*Z*)-**23** respectively] in dry 1,4-dioxane (2 mL for each 0.5 g of starting material) under an atmosphere of nitrogen. The reaction mixture was refluxed for 24 hours and was then extracted with dichloromethane. The organic layer was diluted two times with 2*N* NaOH (50 mL), the aqueous extract was neutralised with 5*N* HCl and extracted exhaustively with dichloromethane. The combined organic layers were washed with water (1x 25 mL), brine (1x 25 mL), dried over anhydrous sodium sulphate and evaporated to dryness *in vacuo*. The residues thus obtained were purified by column chromatography (see Table 2 for yields and analytical data and Table 3 for spectroscopic data).

Enzyme Section.

Sorbinil was a gift from Pfizer (Groton, Conn.). Calf lenses for the purification of ALR2 were obtained locally from freshly-slaughtered animals. The capsule was incised and the frozen lenses were suspended in sodium potassium phosphate buffer, *p*H 7 (standard buffer) containing 5 mM DTT (1 g tissue/3.5 mL) and stirred in an ice-cold bath for 1 hour. The suspension was then centrifuged at 22,000 x g at 4°C for 40 minutes and the supernatant was subjected to ion exchange chromatography on DE52. Enzyme activity for all tested enzymes was measured by monitoring the change in absorbance at 340 nm which accompanies the oxidation of β-nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) catalysed by ALR2. The assay was performed at 37°C as previously described [7], using 4.7 mM D,L-glyceraldehyde as substrate in 0.25 *M* sodium phosphate buffer *p*H 6.8, containing 0.38 *M* ammonium sulphate and 0.11mM NADPH. The sensitivity of ALR2 to inhibition by different ARIs was tested in the above assay conditions by including the inhibitors dissolved in dimethyl sulfoxide (DMSO) at the desired concentration in the reaction mixture. The DMSO in the assay mixture was kept at constant concentration of 1%. A reference blank containing all the above reagents except the substrate was used to correct for the non enzymatic oxidation of NADPH [7]. IC_{50} values (the concentration of the inhibitor required to

produce 50%inhibition of the enzyme catalysed reaction) were determined from least squares analyses of the linear portion of the log dose-inhibition curves. Each curve was generated using at least three concentrations of inhibitor causing an inhibition between 20%and 80%with two replicates at each concentration. The 95%confidence limits (95%CL) were calculated from T values for n-2, where n is the total number of determinations [8].

REFERENCES AND NOTES

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