Eur. J. Med. Chem. **23** (1988) 45—52

Original paper

Gastric anti-secretory, anti-ulcer and cytoprotective properties of substituted *(E)*-4-phenyl- and heteroaryl-4-oxo-2-butenoic acids[a]

Mario BIANCHI[1†], Alina BUTTI[1*], Yani CHRISTIDIS[1**], Jacques PERRONNET[1***], Fernando BARZAGHI[2], Raffaele CESANA[2] and Alberto NENCIONI[2]

[1] *Department of Chemistry, and*
[2] *Department of Pharmacology, Roussel—Maestretti S.p.A., Research Center, Viale Gran Sasso 18, Milano, Italy*

(Received December 5, 1986, accepted September 1, 1987)

Summary — A class of anti-secretory, anti-ulcer and cytoprotective agents, the substituted *(E)*-4-phenyl and heteroaryl-4-oxo-2-butenoic acids, is described. This chemical structure is not related to those of any known anti-cholinergic drugs, histamine H_2-receptor antagonists or prostaglandins. Five compounds, 4-(2-methoxyphenyl)- **15**, 4-(4-methoxyphenyl)- **22**, 4-(3,4-dimethoxyphenyl)- **32**, 4-(3,4,5-trimethoxyphenyl)- **40**, and 4-(2-furanyl)-4-oxo-2-butenoic acid **44**, have cytoprotective activity at a very low dose (0.6 mg/kg, *p.o.*) and anti-secretory and anti-ulcer activities at higher doses.

One of these compounds, RU 38086 **40**, has been selected for clinical evaluation.

Résumé — **Propriétés anti-sécrétoire gastrique, anti-ulcéreuse et cytoprotectrice d'acides *(E)*phenyl-4 et hétéroaryl-4 oxo-4 butène-2 oïques.** *Une classe d'agents anti-secréteurs, anti-ulcéreux et cytoprotecteurs, les acides* E-*phényl-4 et hétéroaryl-4 oxo-4 butène-2 oïques est décrite. Cette structure chimique n'est liée à celle d'aucune substance anti-cholinergique connue, ni d'antagonistes du récepteur H_2 de l'histamine, pas plus que de prostaglandines. Cinq composés, les acides (méthoxy-2 phényl)-4* ***15****, (méthoxy-4 phényl)-4* ***22****, (diméthoxy-3,4 phényl)-4* ***32****, (triméthoxy-3,4,5 phényl)-4* ***40*** *et (furanyl-2)-4* ***44****, oxo-4 butène-2 oïques, manifestent une activité cytoprotectrice à des doses très faibles (0,6 mg/kg* per os*) et des activités anti-secrétrice et anti-ulcéreuse à plus fortes doses.*

L'un de ces composés, RU 38086 ***40****, a été sélectionné en vue d'essais cliniques.*

anti-ulcer agents / cytoprotective agents / *(E)*-4-phenyl- and heteroaryl-4-oxo-2-butenoic acids

Introduction

During our investigation of gastric secretion inhibitors, we found that some 4-substituted-phenyl-4-oxo-2-butenoic acids have strong gastric anti-secretory, anti-ulcer and cytoprotective activities in animals. This chemical structure is not related to those of any known anti-cholinergic drugs, histamine H_2-receptor antagonists or prostaglandins. For this reason, we have synthesized a series of compounds to determine the extent of structural variation consistent with retention of pharmacological activity. Some of these compounds are already reported in the literature, but were not studied for this kind of activity. Fumaric acid is the only related unsaturated carboxylic acid exhibiting an anti-ulcer activity [1]. We decided to examine the effects of substituents at different positions on the aryl ring and of replacement of the phenyl by a heterocyclic ring. We also modified the vinyl chain and replaced the carboxyl with ester, amide or hydroxamic acid groups. From this series of compounds, we have selected *(E)*-4-oxo-4-(3,4,5-trimethoxyphenyl)-2 butenoic acid, RU 38086 **40** for further pharmacological investigation [2] and for clinical evaluation, as a potential gastric cytoprotective, anti-secretory and anti-ulcer agent.

---

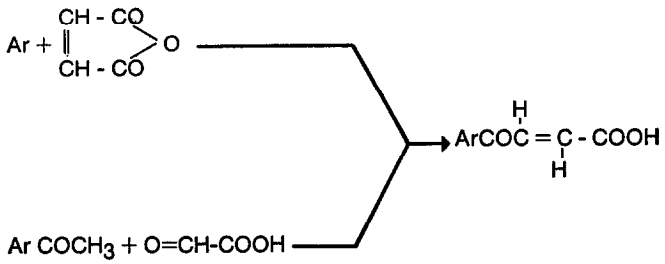
[a] This paper was presented in part. See: *Proceedings of the XIX*[e] *Rencontres Int. de Chim. Ther. Pamplona, Sept. 1983*, Posters 131, 132 and *Proceedings of the VIIth Int. Symp. Med. Chem. Uppsala, Aug. 1984*, poster 188.

† *Deceased.*

* *Author to whom correspondence should be addressed.*
** *Société Française Hoechst, Strains, France.*
*** *Present address*: Centre de Recherches Roussel—Uclaf, Romainville, France.

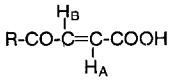
Chemistry

The *(E)*-4-aryl and heteroaryl-4-oxo-2-butenoic acids shown in Table I were synthesized by known methods following Scheme 1. Two pathways were essentially followed, depending upon the aryl substituent or the starting material: A) A Friedel—Crafts reaction between a substituted aromatic or heteroaromatic hydrocarbon, maleic anhydride and $AlCl_3$ in dichloroethane. B) Crotonic condensation of an aryl-(heteroaryl)-methylketone with glyoxylic acid monohydrate. This reaction was generally carried out by heating the reactants in refluxing acetic acid (method B_1) and it



Scheme 1. General synthesis of *(E)*-4-aryl-4-oxo-2-butenoic acids.

Table I. *(E)*-4-Aryl- and heteroaryl-4-oxo-2-butenoic acids.



compd	R	method	yield %	mp, °C (lit. mp.)	recrystn solvent	formula (ref.)	anal.	^{1}H NMR δH_A (ppm)	δH_B (ppm)	J_{AB} (Hz)
13	C_6H_5	A[a]	84	94-5 (94-6)	C_6H_6	$C_{10}H_8O_3$ (19)		6.67	7.80	15
14	2-HOC_6H_4	(b)	32	172-5 (172-5)	C_6H_6	$C_{10}H_8O_4$ (20)		6.55	7.78	16
15	2-$CH_3OC_6H_4$	B_2	36	146-8	Et_2O	$C_{11}H_{10}O_4$	C,H.	6.43	7.53	15
16	2-$O_2NC_6H_4$	B_2	43	169-71 (170-3)	EtOAc	$C_{10}H_7NO_5$ (21)		6.50	7.40	16
17	4-$CH_3C_6H_4$	A	53	133-4 (137-8)	C_6H_6	$C_{11}H_{10}O_3$ (22)		6.53	7.73	15
18	4-ClC_6H_4	B_2	45	158-60 (154-5)	EtOAc	$C_{10}H_7ClO_3$ (22)		6.55	7.68	15
19	4-$CF_3C_6H_4$	B_4	67	154-6 (149-51)	EtOAc	$C_{11}H_7F_3O_3$ (23)		6.57	7.71	16
20	4-FC_6H_4	B_4	45	133-5 (132-8)	$CHCl_2$-$CHCl_2$	$C_{10}H_7FO_3$ (24)		6.55	7.72	15
21	4-HOC_6H_4	B_1	35	193-5 (197-8)	H_2O	$C_{10}H_8O_4$ (22)		6.53	7.75	15
22	4-$CH_3OC_6H_4$	B_4	52	134-6 (138-9)	$CHCl_2CHCl_2$	$C_{11}H_{10}O_4$ (22)		6.50	7.73	15
23	4-$O_2NC_6H_4$	B_1	30	174-6 (173-4)	95% EtOH	$C_{10}H_7NO_5$ (25)		6.53	7.67	15
24	4-$CH_3COOC_6H_4$	C	62	154-6	70% EtOH	$C_{12}H_{10}O_5$	C,H.	6.72	7.80	15
25	4-$C_2H_5COOC_6H_4$	C	58	140-2	H_2O	$C_{13}H_{12}O_5$	C,H.	6.55	7.75	15
26	4-$(CH_3)_2NC_6H_4$	B_6	20	182-4	iso-PrOH	$C_{12}H_{13}NO_3$	C,H,N.	6.47	7.73	15
27	4-$NH(COCH_3)C_6H_4$	A	45	243-6 (242-4)	70% EtOH	$C_{12}H_{11}NO_4$ (22)		6.55	7.77	15
28	4-$C_6H_5C_6H_4$	A[c]	28	172-3 (167-8)	HOAc	$C_{16}H_{12}O_3$ (26)		6.67	7.77	15
29	3,4-$(HO)_2C_6H_3$	A	20	220-2 dec (218-20 dec)	H_2O	$C_{10}H_8O_5$ (27)		6.60	7.75	16
30	(3-CH_3O,4-HO)C_6H_3	B_1	24	164-5	EtOAc+light petr	$C_{11}H_{10}O_5$	C,H.	6.53	7.80	15
31	(2-HO,4-CH_3O)C_6H_3	B_4	22	177-9	EtOAc	$C_{11}H_{10}O_5$	C,H.	6.60	7.83	15
32	3,4-$(CH_3O)_2C_6H_3$	B_1	47	181-2 (181)	HOAc	$C_{12}H_{12}O_5$ (10)		6.58	7.83	16
33	2,5-$(CH_3O)_2C_6H_3$	B_4	58	150-2 (151-2)	EtOAc	$C_{12}H_{12}O_5$ (22)		6.60	7.75	16
34	2,6-$(CH_3O)_2C_6H_3$	B_3	22	145-50	iso-Pr_2O	$C_{12}H_{12}O_5$	C,H.	6.17	6.87	15
35	3,4-$(C_2H_5O)_2C_6H_3$	B_2	50	144-6	toluene	$C_{14}H_{16}O_5$	C,H.	6.70	7.83	16
36	3,4-$(CH_3COO)_2C_6H_3$	C	36	164-6	EtOAc-Et_2O	$C_{14}H_{12}O_7$	C,H.	6.75	7.70	15
37	3,4-$(Cl)_2C_6H_3$	B_2	54	141-4 (142-3)	$CHCl_2CHCl_2$	$C_{10}H_6Cl_2O_3$ (22)		6.57	7.73	15
38	3,4-$(OCH_2O)C_6H_3$	B_1	28	206-8	HOAc	$C_{11}H_8O_5$	C,H.	6.51	7.70	15
39	3,4-$(OCH_2CH_2O)C_6H_3$	A	47	186-7	95% EtOH	$C_{12}H_{10}O_5$	C,H.	6.50	7.68	15
40	3,4,5-$(CH_3O)_3C_6H_2$	B_4	70	143-5	aq. EtOH	$C_{13}H_{14}O_6$	C,H.	6.55	7.82	16
41	2,3,4-$(CH_3O)_3C_6H_2$	B_4	62	99-101	aq. HOAc	$C_{13}H_{14}O_6$ (28)		6.50	7.76	16
42	2,4,5-$(CH_3O)_3C_6H_2$	B_4	62	209-11	HOAc	$C_{13}H_{14}O_6$	C,H.	6.30	7.60	16

Table I. Continued.

compd	R	method	yield %	mp, °C (lit. mp.)	recrystn solvent	formula (ref.)	anal.	^{1}H NMR δH_A (ppm)	δH_B (ppm)	J_{AB} (Hz)
43	2,4,6-$(CH_3O)_3C_6H_2$	B_5	53	151-3	MEK	$C_{13}H_{14}O_6$	C,H.	6.20	6.95	16
44	2-furanyl	B_4	50	158-60 (153-5)	iso-PrOH	$C_8H_6O_4$ (29)	C,H.	6.63	7.60	15
45	3-furanyl	B_4	43	188-91	EtOAc	$C_8H_6O_4$ (29b)	C,H.	6.63	7.43	16
46	5-CH_3-2-furanyl	B_3	15	159-62	EtOAc	$C_9H_8O_4$	C,H.	6.60	7.57	15
47	2,5-$(CH_3)_2$-3-furanyl	B_3	15	144-7	EtOAc	$C_{10}H_{10}O_4$	C,H.	6.55	7.30	16
48	2-thienyl	B_4	65	151 (139-42)	EtOAc	$C_8H_6O_3S$ (29b)	C,H.	6.95	7.90	16
49	(N-$SO_2C_6H_5$)-3-pyrrolyl	A	27	178-9	C_6H_6:EtOAc:HOAc 5 : 5 : 1	$C_{14}H_{11}NO_5S$	C,H,N.	6.42	7.47	15
50	2,5-$(CH_3)_2$-4-oxazolyl	B_1	26	183-5	EtOAc	$C_9H_9NO_4$	C,H,N.	6.60	7.67	15
51	3,5-$(CH_3)_2$-4-isoxazolyl	B_2[d]	42	138-40	C_6H_6:EtOAc 3:1	$C_9H_9NO_4$	C,H,N.	6.43	7.37	15
52	1,3,5-$(CH_3)_3$-4-pyrazolyl	B_1	41	200-1	H_2O	$C_{10}H_{12}N_2O_3$	C,H,N.	6.37	7.40	15
53	3-pyridyl-N-oxide	B_2	10	192-4	60% EtOAc	$C_9H_7NO_4$	C,H,N.	6.63	7.67	15
54	4-HO-6-CH_3-2-oxo-3-pyranyl	A[e]	27	228 dec	EtOAc	$C_{10}H_8O_6$	C,H.	6.62	8.05	15
55	2-benzofuranyl	B_1	37	182-4 (184)	95% EtOH	$C_{12}H_8O_4$ (30)		6.67	7.93	15
56	3-indolyl	Ex[f]	63	237-8 dec	95% EtOH	$C_{12}H_9NO_3$	C,H,N.	6.65	7.72	15
57	2-benzothiazolyl	B_4	31	186 dec	C_6H_6	$C_{11}H_7NO_3S$	C,H,N.	6.85	8.02	15

[a]Benzene in excess was used as solvent.
[b]Synthesized as described in the literature [20].
[c]Benzene was used as solvent, 4 h at 70°C.
[d]15 h at reflux.
[e]6 h at reflux.
[f]Experimental procedure described.

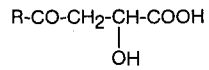
could be accelerated by adding concentrated hydrochloric acid to the reaction mixture (method B_2). The same condensation also took place when a methylketone and glyoxylic acid monohydrate were melted at 120—5°C under reduced pressure (method B_3), while at lower temperature (95°C) aldol condensation occurred. The aldol was easily dehydrated in refluxing acetic and hydrochloric acids (method B_4). Aldols not yet described in the literature are listed in Table II. Condensation of an aryl-(heteroaryl)-methylketone with glyoxylic acid monohydrate was also carried out in alkaline medium (method B_5), also utilizing glyoxylic acid formed *in situ* by oxidation of tartaric acid with periodic acid (method B_6). In this case, either a crotonic or an aldol condensation could take place, according to the starting material. With these synthetic procedures, we also prepared compounds already described in the literature by other methods. All the new compounds in Table III were prepared with methods described later under experimental protocols. Compounds **58**, **64** and **66** were synthesized as described in the literature, whereas **65** was commercially available (see Table III for details).

The *trans*-configuration of the butenoic acids was established by ^{1}H NMR spectroscopy. Olefinic protons of the side chain showed an AB system spectrum, in which the first doublet at δ 6.17—6.85 ppm was ascribable to a proton (H_A) near the carboxyl function, while the other doublet at δ 6.87—8.05 ppm was ascribable to the proton in the 3 position (H_B). The spin—spin coupling constant $J_{A,B}$ was 15—16 Hz, which is in agreement with a *trans*-configuration. This constant was 11 Hz for *cis*-isomers. These values are in full agreement with those reported for similar structures [3].

Pharmacology

The anti-secretory activity of the compounds was evaluated in two animal models: pylorus-ligated rats [4] and the perfused stomach of the anesthetized rat [5]. In the first model, the compounds were administered orally at 10 mg/kg, 1 h before ligation of the pylorus and the changes in volume and in the titratable acidity were measured after 3 h. In the second model, the compounds were tested as inhibitors of histamine-stimulated gastric acid secretion. The compounds were inserted into the stomach at a dose of 1.2 mg/kg and left *in situ* for 1 h or were administered by bolus intravenous injection of 5 mg/kg. The anti-ulcer activity was tested in stressed rats [6, 7]. The compounds were administered orally at 10 mg/kg and 1 h later the rats were immobilized in a mesh corset and placed for 2 h in a refrigerator at 8°C. The cytoprotective activity was evaluated against ethanol-induced gastric necrosis in rats [8]. The compounds were administered orally at 0.6 mg/kg 1 h before the oral administration of 1 ml of absolute ethanol and the effect of the compound was determined 1 h later (see Experimental protocols).

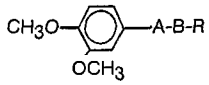
Table II. 2-Hydroxy-4-oxo-4-aryl- and heteroaryl-butanoic acids.



compd	R	yield %	mp, °C	recrystn solvent	formula	anal.
1	2-$O_2NC_6H_4$	60	107-9	$CHCl_2$-$CHCl_2$	$C_{10}H_9NO_6$	C,H,N.
2	4-ClC_6H_4	60	136-8	$CHCl_2$-$CHCl_2$	$C_{10}H_9ClO_4$	C,H.
3	4-FC_6H_4	45	126-8	$CHCl_2$-$CHCl_2$	$C_{10}H_9FO_4$	C,H.
4[a]	(2-HO,4-CH_3O)-C_6H_3	100 (crude)	119-20	C_6H_6	$C_{11}H_{12}O_6$	C,H.
5	3,4-$(CH_3O)_2C_6H_3$	52	106-8	$CHCl_2CHCl_2$	$C_{12}H_{14}O_6$	C,H.
6	2,5-$(CH_3O)_2C_6H_3$	51	88-90	$CHCl_2CHCl_2$	$C_{12}H_{14}O_6$	C,H.
7	3,4-$(Cl)_2C_6H_3$	54	145-7	EtOAc	$C_{10}H_8Cl_2O_4$	C,H.
8	2,3,4-$(CH_3O)_3C_6H_2$	38	93-5	$CHCl_2CHCl_2$	$C_{13}H_{16}O_7$	C,H.
9	2,4,5-$(CH_3O)_3C_6H_2$	67	145-7	$CHCl_2CHCl_2$	$C_{13}H_{16}O_7$	C,H.
10	3,4,5-$(CH_3O)_3C_6H_2$	56	119-20	$CHCl_2CHCl_2$	$C_{13}H_{16}O_7$	C,H.
11	3-furanyl	22	104-6	EtOAc-light petr.	$C_8H_8O_5$	C,H.
12	2-benzothiazolyl	41	145 dec.	H_2O	$C_{11}H_9NO_4S$	C,H,N.

[a]Prepared by method B_6.

Table III.



compd	A	B	R	yield %	mp, °C (lit. mp.)	recrystn solvent	formula (ref.)	anal
58	CO	CH_2-CH_2	COOH	42	164-6 (159-161)	80% EtOH	$C_{12}H_{14}O_5$ (31)	
59	C=$NOCH_3$	CH=CH	COOH	57	160-2	50% EtOH	$C_{13}H_{15}NO_5$	C,H,N.
60	S	CH=CH	COOH	68	154-6	$CHCl_3$-hexane	$C_{11}H_{12}O_4S$	C,H.
61	SO	CH=CH	COOH	56	130-2	C_6H_6	$C_{11}H_{12}O_5S$	C,H.
62	SO_2	CH=CH	COOH	80	147-9	C_6H_6	$C_{11}H_{12}O_6S$	C,H.
63	CHOH	CH=CH	COOH	67	105-7	$CHCl_3$-hexane	$C_{12}H_{14}O_5$	C,H.
64	CH=CH	CO	COOH	43	154-6 (155)	H_2O	$C_{12}H_8O_5$ (32)	
65	-	CH=CH	COOH		181-3		$C_{11}H_{12}O_4$ (a)	
66	CO	CH=CH	$COOCH_3$	61	92-4 (91)	95% EtOH	$C_{13}H_{14}O_5$ (10)	
67	CO	CH=CH	CONHOH	24	189 dec	95% EtOH	$C_{12}H_{13}NO_5$	C,H,N.
68	CO	CH=CH	$CONH_2$	20	189-91	acetone	$C_{12}H_{13}NO_4$	C,H,N.

[a]Purchased from Aldrich-Chemie GmbH & Co. KG, D-7924 Steinheim/Albuch (F.R.G.).

Results and Discussion

The gastric anti-secretory, anti-ulcer and cytoprotective effects of *(E)*-4-aryl and 4-heteroaryl-4-oxo-2-butenoic acids and other correlated structures are shown in Table IV. Most of the compounds administered orally to pylorus-ligated rats increased the volume of gastric juice and decreased the acid concentration. Some compounds also showed an anti-secretory activity against histamine-stimulated acid secretion in the perfused stomach of the anesthetized rats. In the latter model, compounds with a gastric anti-secretory activity when left *in situ* in the stomach at a low dose of 1.2 mg/kg were inactive when administered intravenously at a dose of 5 mg/kg.

The simplest structure, the 4-phenyl-4-oxo-2-butenoic acid **13** had remarkable cytoprotective activity against

Table IV. Gastric anti-secretory, anti-ulcer and cytoprotective activities of 4-aryl- and heteroaryl-4-oxo-2-butenoic acids. Results are expressed as var. % *versus* control.

Compound	Antisecretory Act. Pylorus-ligated rat 10 mg/kg p.o. Volume	Antisecretory Act. Pylorus-ligated rat 10 mg/kg p.o. Ac. Conc.	Antisecretory Act. Perfused stomach of rat (Histam.-stim.) 1.2 mg/kg «in situ»	Antiulcer Act. Cold-restraint ulcers 10 mg/kg p.o.	Cytoprotective Act. Ethanol-induced lesions 0.6 mg/kg p.o.
13	+ 130**	- 66**	- 32*	- 54	- 86**
14	+ 40*	- 65**	- 62**	- 35	- 82**
15	+ 110**	- 78**	- 87**	- 80**	- 77**
16	+ 47*	- 63**	- 48**	- 5	- 44**
17	+ 27	- 61**	- 13	- 39	- 53*
18	- 1	- 46**	+ 9	- 19	- 98**
19	+ 36*	- 29**	+ 26	+ 15	- 46
20	+ 12	- 65**	- 9	- 56*	- 99**
21	- 47	- 69**	- 41*	+ 77	- 37
22	+ 51*	- 66**	- 63**	- 73*	- 92**
23	+ 11	- 63**	- 16	+ 29	- 44*
24	+ 105**	- 77**	- 71**	- 13	- 50*
25	+ 64*	- 73**	- 60**	- 76*	- 56**
26	+ 18	- 58**	- 54*	- 61*	- 58**
27	- 33	- 28**	- 2	- 16	- 23
28	+ 15	- 6	- 5	- 7	- 13
29	- 44*	- 17	- 11	- 57*	- 23
30	+ 64	- 89**	- 32	- 89**	- 62**
31	+ 92**	- 66**	- 37	0	- 81**
32	+ 79**	- 72**	- 74**	- 86**	- 90**
33	- 16	- 60**	- 72**	- 5	- 54**
34	+ 82**	- 83**	- 64**	- 19	- 18
35	+ 15	- 52**	- 68**	- 48	- 78**
36	+ 91**	- 55**	- 28*		- 16
37	+ 17	- 44**	- 17	- 60	- 98**
38	+ 65**	- 30**	- 61*	- 18	- 58*
39	+ 64**	- 41**	- 45*	- 33	- 75**
40	+ 75**	- 83**	- 66**	- 70**	- 64**
41	+ 105**	- 63**	- 90**	- 52	- 51*
42	+ 42	- 23*	- 2	- 33	- 13
43	+ 97**	- 67**	- 65**	- 12	- 57**
44	+ 83**	- 94**	- 70**	- 83**	- 81**
45	+ 115**	- 82**	- 80**	- 57**	- 77**
46	+ 86**	- 87**	- 73**	- 52*	- 84**
47	+ 88**	- 70**	- 68**	- 2	- 74**
48	+ 70**	- 78*	- 43**	- 69	- 71**
49	+ 100*	- 54**	- 63**	- 13	- 41*
50	+ 36	- 77**	- 84**	- 54*	- 80**
51	+ 83*	- 77**	- 84**	- 51	- 66**
52	+ 67*	- 87**	- 47**	- 3	- 30
53	0	- 6	- 3	- 65	- 9
54	+ 77*	- 70**	- 61**	- 67	- 38**
55	+ 56*	- 53**	- 41	+ 16	- 46**
56	+ 16	- 11	- 3	+ 5	- 16
57	+ 7	- 12	- 32**	+ 16	- 9
58	+ 9	+ 12	+ 34	- 40	- 58**
59	- 11	+ 16	- 7	+ 36	- 54*
60	- 5	- 1	+ 42	- 62*	- 32*
61	- 30*	- 9	+ 4	+ 54	- 19
62	- 17	- 20		- 77*	- 18
63	- 19	- 3	+ 49	- 38	- 63**
64	- 1	- 27**	+ 18	+ 77	+ 9
65	- 6	- 4	- 13	- 64*	- 22
66	+ 56**	- 36**	- 25	+ 9	- 59**
67	+ 20	+ 4	+ 28	- 46	- 41
68	+ 88**	- 31*	- 14	- 41	- 10

Significant difference from controls: $*P < 0.05$, $**P < 0.01$.

ethanol-induced gastric lesions and less pronounced effects against stress ulcers and gastric secretion. Cytoprotective activity was retained and both anti-secretory and anti-ulcer activities improved when a methoxy group was introduced at the 2-position **15** or the 4-position **22** of the phenyl ring. Introduction onto the phenyl ring of **15** of one other methoxy substituent, in position 5 or 6 (2,5- and 2,6-dimethoxy compounds, **33** and **34**) and of two other methoxy groups in positions 3,4, 4,5 or 5,6 (2,3,4-, 2,4,5- and 2,5,6-trimethoxy compounds, **41**, **42** and **43**) led to compounds less active than **15** in one or more tests. The activities were enhanced by changing the positions of the methoxy substituents: in fact, 4-(3,4-dimethoxyphenyl)- and (3,4,5-trimethoxyphenyl)-4-oxo-2-butenoic acids (**32** and **40**) were equipotent with **15**. Replacement of the dimethoxy groups of **32** by the more lipophilic diethoxy ones (**35**) decreased all activities. The same results were obtained with the more hydrophilic OH in positions 2,4 and 3,4 (**14**, **21** and **29**). Interestingly, 4-Cl **18**, 4-F **20** and 3,4-Cl_2 **37** derivatives completely inhibited ethanol-induced gastric lesions.

Introduction of other electron-withdrawing (**16**, **19** and **23**) and electron-donating substituents (**17**, **26**, **27**, **28**, **38** and **39**) onto the phenyl ring of **13** led to less active compounds. In conclusion, the importance of the aryl-methoxy substituent(s) and its positions was made apparent by the very high activities of 2-methoxy **15**, 4-methoxy **22**, 3,4-dimethoxy **32** and 3,4,5-trimethoxy **40** analogues, in comparison with other substituents or positions, which resulted in a loss of activity in one or more tests.

Isosteric replacement of the phenyl ring in **13** with the 2-thienyl nucleus resulted in analogue **48**. These two compounds exhibited comparable activities. Among the other heteroaryl derivatives, the most active compound was 4-(2-furanyl)-4-oxo-2-butenoic acid **44**, which had very high activities in all tests, equivalent to those of **15**, **22**, **32** and **40**. Introduction of a methyl substituent at the 5-position of the furanyl ring **46** decreased the anti-ulcer potency. The benzofuran analogue **55** of **44** was inactive. Replacement of the 2-furanyl group in **44** with the isomeric 3-furanyl substituent resulted in analogue **45**, which had less anti-ulcer activity than **44**. Introduction of two methyl groups at positions 2 and 5 of **45** led to the less active analogue **47**. The 2,5-dimethyl-4-oxazolyl **50** and the 3,5-dimethyl-4-isoxazolyl **51** derivatives had activities comparable to that of **44** except for a reduction of the anti-ulcer potency. The other heteroaryl compounds in Table IV had less activity than **44**.

All the modifications of the side chain on the 3,4-dimethoxyphenyl derivative **32**, reported in Table III, led to marked decreases of all the activities (Table IV). Therefore, the simultaneous presence in the chain of a 4-carbonyl group, a CH=CH double bond and of a carboxyl appeared to be necessary for the best activity.

The above results led to the identification of five compounds, **15**, **22**, **32**, **40** and **44** with cytoprotective activity at a very low dose (0.6 mg/kg *p.o.*) and anti-secretory and anti-ulcer activities at higher doses. One of these compounds, *(E)*-4-(3,4,5-trimethoxyphenyl)-4-oxo-2-butenoic acid, RU 38086 **40**, has been selected for clinical evaluation.

Experimental protocols

Chemistry

Melting points were determined with a Tottoli apparatus and are uncorrected. IR spectra were recorded in nujol on a Perkin—Elmer 247 spectrophotometer and NMR spectra on a Varian T-60 A spectrometer, using tetramethylsilane (TMS) as the internal standard and DMSO-d_6 as the solvent. Analyses, indicated by elemental symbols, were within $\pm$ 0.4% of the theoretical values and were made by the Analytical Service of the Università degli Studi di Milano, Dipartimento di Chimica Organica e Industriale, Via Venezian 21, Milan. Unless otherwise noted, all the compounds gave rise to a single spot on thin—layer chromatography TLC (precoated silica gel plates, Merck 60 F_{254}, 0.25 mm).

Preparation of 4-aryl-2-hydroxy-4-oxo-butanoic acids (Table II)
Glyoxylic acid monohydrate (0.1 mol) and an acetophenone (0.2 mol) were heated at 95°C under reduced pressure (50 mm Hg) for 1.5—3 h. During this time, water was continually removed. After cooling, the reaction mixture was taken up in aq Na_2CO_3 and extracted with Et_2O. The aqueous layer was acidified with 15% HCl and extracted with EtOAc. The organic layer was evaporated to dryness and the residue directly dehydrated with HOAc—HCl. The analytical samples were recrystallized from dichloroethane or EtOAc.

Preparation of 4-heteroaryl-2-hydroxy-4-oxo-butanoic acids (Table II)
A solution of $NaHCO_3$ (0.2 mol) in 150 ml of H_2O was added to a mixture of glyoxylic acid monohydrate (0.1 mol) and a heteroarylmethylketone (0.1 mol) in 250 ml of 95% EtOH and the mixture was heated for 2.5 h at 50°C (solution was complete). After cooling, the reaction mixture was acidified with 2 N HCl, the alcohol was removed under reduced pressure and the remaining solution was extracted with EtOAc. The organic layer was evaporated to dryness and the residue recrystallized.

Preparation of (E)*-4-aryl- and heteroaryl-4-oxo-2-butenoic acids (Table I)*
Method A: (E)-4-(2,3-dihydro-1,4-dioxanaphthalen-6-yl)-4-oxo-2-butenoic acid ***39***. 2,3-Dihydro-1,4-dioxanaphthalene [9] (10.2 g, 0.075 mol) and maleic anhydride (8 g, 0.08 mol) were added to a suspension of $AlCl_3$ (37.5 g, 0.275 mol) in 1,2-dichloroethane (100 ml). The temperature spontaneously rises to about 60°C. Stirring at room temperature was continued for 8 h. After standing overnight, the reaction mixture was treated with ice and conc. HCl, the precipitated solid filtered, washed with water and recrystallized. Yield 8.3 g (47%); mp: 186—7°C (from 95% EtOH).

Method B_1*: (E)-4-(3,4-dimethoxyphenyl)-4-oxo-2-butenoic acid* ***32*** [*10*]. From 3,4-dimethoxyacetophenone (3.6 g, 0.02 mol) and glyoxylic acid monohydrate (1.84 g, 0.02 mol) in glacial HOAc (4 ml), heated under reflux for 20 h. The solid that separated after cooling was filtered, washed with acetic acid and recrystallized. Yield 2.2 g (47%); mp: 181—2°C (from HOAc).

Method B_2*: (E)-4-(2-methoxyphenyl)-4-oxo-2-butenoic acid* ***15***. A mixture of 5 g (0.03 mol) of 2-methoxyacetophenone [11] and 3.06 g (0.03 mol) of glyoxylic acid monohydrate in 50 ml of glacial HOAc and 5 ml of conc. HCl was heated under reflux for 8 h. The solvents were removed under reduced pressure, the residue was taken up in Et_2O, the solid that formed was filtered and recrystallized. Yield 2.5 g (36%); mp: 146—8°C (from Et_2O).

Method B_3*: (E)-4-(2,6-dimethoxyphenyl)-4-oxo-2-butenoic acid* ***34***. 2,6-Dimethoxyacetophenone [12] (3.6 g, 0.02 mol) and glyoxylic acid monohydrate (1.84 g, 0.02 mol) were heated at 120°C and 15 mm Hg for 7 h. After cooling, the reaction mixture was taken up with aq $NaHCO_3$, the solution was washed with EtOAc, the aqueous layer was acidified with 2 N HCl and extracted with EtOAc. The organic layer was evaporated to dryness and the residue chromatographed on silica gel ($MeOH/CHCl_3$, 3:7) to give 1.04 g (22%) of **34**, mp: 145—50°C (from *i*-Pr_2O).

Method B_4*: (E)-4-(2-hydroxy-4-methoxyphenyl)-4-oxo-2-butenoic acid* ***31***. Crude **4** (10 g) in a mixture of glacial HOAc (20 ml) and conc. HCl (2 ml) was heated under reflux for 1.5 h. After cooling, the reaction mixture was diluted with H_2O, the solid that precipitated was filtered,

washed with HOAc and recrystallized to give 2 g (22%) of pure **31**, mp: 177—9°C (from EtOAc).

Method B_5: (E)-*4-(2,4,6-trimethoxyphenyl)-4-oxo-2-butenoic acid* ***43***. To 3.3 g (0.05 mol) of KOH pellets dissolved in 100 ml of absolute EtOH, 31.5 g (0.15 mol) of 2,4,6-trimethoxyacetophenone [13] were added. To the resulting solution, 4.6 g (0.05 mol) of glyoxylic acid monohydrate dissolved in absolute EtOH (50 ml) were added dropwise in 30 min at 20°C. The solution (pH 6.8) was heated under reflux for 6 h, the solvent was removed under reduced pressure and the residue was taken up in H_2O (300 ml). Following filtration, the filtrate was acidified (pH = 1) with conc. HCl. The solid that formed was isolated by filtration to give 0.7 g (53%) of **43**, mp: 151—3°C (from methylethylketone).

Method B_6: (E)-*4-(4-dimethylaminophenyl)-4-oxo-2-butenoic acid* ***26***. Sodium metaperiodate (17.12 g, 0.08 mol) was dissolved in 96 ml of H_2O and 1.6 ml of conc. H_2SO_4. The solution was cooled to — 5—0°C and 12 g (0.08 mol) of tartaric acid dissolved in 24 ml of H_2O were added in 5 min. After stirring for 30 min at room temperature, 13.04 g (0.08 mol) of 4-dimethylaminoacetophenone [14] and a solution of 12 g (0.3 mol) of NaOH pellets in 400 ml of 50% EtOH were added. After stirring for 5 h at room temperature, the reaction mixture was heated at 60°C for 15—20 min, then cooled and diluted with H_2O. The aqueous solution was washed with Et_2O, acidified with 2 N HCl, extracted with $CHCl_3$ and the solvent removed. The residue was a solid that was recrystallized from *i*-PrOH to give 3.5 g (20%) of **26**, mp: 182—4°C.

Method C: (E)-*4-(4-acetoxyphenyl)-4-oxo-2-butenoic acid* ***24***. A mixture of 6 g (0.03 mol) of **21** and 50 ml of Ac_2O was heated at 50°C for 1.5 h. After evaporation to dryness, the residue was taken up in a 5% aqueous solution of $NaHCO_3$, the solution was washed with Et_2O, acidified with 2 N HCl and the precipitate was filtered and recrystallized from 70% EtOH. Yield 4.5 g (62%), mp: 154—6°C.

(E)-*4-(3-Indolyl)-4-oxo-2-butenoic acid* ***56***
A solution of 23 g (0.2 mol) of indole in 50 ml of anisole was added with stirring to a cooled (5—10°C) solution of 38 g (0.21 mol) of C_2H_5MgI in 50 ml of anisole. Stirring was continued for 20 min at room temperature and 20 min at 60—70°C. A hot solution of maleic anhydride in 100 ml of anisole was added and the reaction mixture was heated at 100°C for 1.5 h. After cooling, 30 ml of HOAc and 150 ml of H_2O were added, the precipitate was filtered and recrystallized from 95% EtOH. TLC on silica gel (C_6H_6:EtOAc:HOAc, 5:5:1) revealed two components that were separated by column chromatography, yielding 2.8 g (63%) of pure **56**, mp: 237—8°C dec. (from 95% EtOH). The other product was the *cis*-isomer [15].

(E)-*4-(3,4-Dimethoxyphenyl)-4-methoxyimino-2-butenoic acid* ***59***
From 7.1 g (0.03 mol) of **32** and 9 g (0.108 mol) of *O*-methylhydroxylamine hydrochloride in 600 ml of 95% EtOH at room temperature for 40 h. After removal of the solvent, the residue was taken up in H_2O to give a solid that was filtered and recrystallized from 50% EtOH. Yield 4.5 g (57%), mp: 160—2°C. ^1H NMR: olefinic protons at δ 6.07 and 7.87 ppm (J = 15 Hz).

(E)-*3-(3,4-Dimethoxyphenylthio)-propenoic acid* ***60***
3,4-Dimethoxythiophenol (4 g, 0.023 mol) [16] and 2.6 g (0.024 mol) of *(E)*-3-chloroacrylic acid [17] in 80 ml of absolute EtOH and 6 g (0.071 mol) of $NaHCO_3$ were refluxed for 2 h. After removal of solvent, the residue was dissolved in H_2O and acidified with dil. H_2SO_4 to give a solid that was filtered and recrystallized from $CHCl_3$:hexane. Yield 3.8 g (68%), mp: 154—6°C. ^1H NMR: olefinic protons at δ 5.47 and 7.80 ppm (J = 15 Hz).

(E)-*3-(3,4-Dimethoxyphenylsulfinyl)-propenoic acid* ***61***
To a solution of 5 g (0.02 mol) of **60** in 100 ml of MeOH and 25 ml of H_2O, 6.8 g (0.03 mol) of sodium metaperiodate dissolved in 75 ml of H_2O were added. The reaction mixture was stirred at 50°C for 5 h, left to stand overnight at room temperature, filtered from the precipitated salts and concentrated to about half volume. The residue was diluted with aq $NaHCO_3$, the solution filtered, the filtrate acidified with 2 N HCl and extracted with $CHCl_3$. Hexane was added to the organic extract. The solid that formed was isolated by filtration. Yield 3 g (56%), mp: 130—2°C (from benzene). ^1H NMR: olefinic protons at δ 6.55 and 7.35 ppm (J = 15 Hz).

(E)-*3-(3,4-Dimethoxyphenylsulfonyl)-propenoic acid* ***62***
Compound **60** (2 g, 0.008 mol) in 40 ml of AcOH and 4 ml (0.04 mol) of 36% H_2O_2 was heated at 90°C for 30 min. The solvent was removed under reduced pressure and the residue was recrystallized from benzene to give 1.8 g (80%) of **62**, mp: 147—9°C. ^1H NMR: olefinic protons at δ 6.60 and 7.28 ppm (J = 15 Hz).

(E)-*4-(3,4-Dimethoxyphenyl)-4-hydroxy-2-butenoic acid* ***63***
A suspension of 11.8 g (0.05 mol) of **32** in 50 ml of H_2O and 6 g (0.15 mol) of $NaBH_4$ was kept at room temperature for 48 h. Following filtration, the filtrate was acidified with dil H_2SO_4, the solid that formed was filtered and recrystallized from $CHCl_3$:hexane, yielding 8 g (67%) of **63**, mp: 105—7°C. ^1H NMR δ 6.83 (m, 4H); 5.90 (dd, J = 16 Hz, 1H); 5.20 (dd, J = 5 Hz, 1H); 3.70 (s, 6H). A by-product of this reaction was 4-(3,4-dimethoxyphenyl)-γ-butyrolactone [18], mp: 120—1°C (from *i*-PrOH).

(E)-*4-(3,4-Dimethoxyphenyl)-4-oxo-2-butenohydroxamic acid* ***67***
Compound **32** (5.9 g, 0.025 mol) was dissolved in 20 ml of dimethylformamide (DMF). The solution was cooled to —5°C and 3.5 ml (0.025 mol) of Et_3N followed by 2.46 ml (0.025 mol) of ethyl chloroformate were added. After stirring for 10 min, the salt that precipitated was filtered out and to the filtrate a solution of hydroxylamine (obtained from 1.65 g (0.025 mol) of hydrochloride and 3.5 ml (0.025 mol) of Et_3N) in 20 ml of DMF was added. After stirring at room temperature for 12 h, the reaction mixture was diluted with Et_2O (300 ml), the oil that formed was triturated with H_2O to give a solid that was filtered and recrystallized from 95% EtOH. Yield 1.5 g (24%); mp: 189°C dec. ^1H NMR: olefinic protons at δ 6.77 and 7.75 ppm (J = 15 Hz).

(E)-*4-(3,4-Dimethoxyphenyl)-4-oxo-2-butenamide* ***68***
A suspension of 11.8 g (0.05 mol) of **32** and 12.5 g (0.06 mol) of PCl_5 in 150 ml of CS_2 was refluxed until solution was complete (15 min). After removal of the solvent, the residue was dissolved in 150 ml of $CHCl_3$ and the solution was cooled to —10°C. The chloroform solution was treated with gaseous ammonia for 0.5 h, the solvent was removed under reduced pressure, the residue was washed with water and recrystallized twice from 95% EtOH and then from acetone. Yield 2.35 g (20%); mp: 189—91°C. ^1H NMR: olefinic protons at δ 6.82 and 7.70 ppm (J = 15 Hz).

Pharmacological methods

Pylorus-ligated rats
Sprague—Dawley CD male rats (Charles River) weighing 220—240 g, were housed in individual cages and fasted for 48 h (water *ad libitum*). After the first 24 h of fasting, the water was replaced by an 8% glucose solution. Groups of 5 animals were used.

The pylorus was ligated under ether anesthesia, according to the method of Shay *et al.* [4], care being taken not to cause trauma to the surrounding tissue. After 3 h, the animals were killed with ether, the esophagus ligated, and the stomach dissected out. The gastric juice from each stomach was collected in a centrifuge tube, by opening the stomach along the greater curvature, and the volume after centrifugation was recorded. Acid concentration was measured by titrating 100 μl of centrifuged gastric juice to pH 7 with N/100 NaOH, using an automatic titration instrument (Mettler Instrumente AG).

The compounds suspended in an aqueous solution of 0.5% carboxymethyl cellulose (or 5 ml/kg of vehicle for the controls) were administered orally 1 h before ligation of the pylorus. Using this procedure, the absolute values for the controls were: volume = 4.4 $\pm$ 0.2 ml/3 h; acid concentration = 119 $\pm$ 5 μeq H^+/ml. Cimetidine at 25 mg/kg intraduodenally produced the following reduction: —33% volume; —29% acid concentration.

Histamine-stimulated gastric secretion by the perfused stomach of the anesthetized rat
Male rats (8/group), weighing 200—240 g, fasted for 24 h (water *ad libitum*) and anesthetized with 1.5 g/kg subcutaneous ethylurethane were used. After intubation of the trachea, the esophagus and the duodenum (next to the pylorus) were catheterized according to the method of Ghosh and Schild [5]. The stomach was perfused with physiological saline (pH 7.4, 37°C) at a constant rate of 1 ml/min, by a peristaltic pump. The liquid perfusing the stomach was collected

every 10 min and titrated to pH 7. The acidity was measured in μeq H^+/10 min. The effects of the compounds were studied by the two following procedures:

a. After 3 determinations of baseline values, 0.6 mg/kg/h of histamine were infused intravenously for 4 h. 1 h after starting the stimulation, gastric perfusion was discontinued. The duodenal cannula was then closed and the compounds (or 10 ml/kg of vehicle for the controls) were inserted into the stomach and left *in situ* for 1 h, while still infusing the agonist. The duodenal cannula was then opened to resume gastric perfusion, and after 10 min of washing, titration was started again.

b. After 3 determinations of baseline values, 0.6 mg/kg/h of histamine were infused intravenously for 3 h. 1 h after starting the stimulation, the compounds, dissolved in NaOH, pH 5—6 (or saline 2 ml/kg for the controls) were administered by bolus intravenous injection.

Results are reported as percent variations (treated *versus* control) of acid output evaluated on the 6th sampling period after reperfusion. In the controls, the acid output was 7.45 $\pm$ 0.74 μeq H^+/10 min. Cimetidine, at 0.5 mg/kg intravenously, reduced the acid output by 49%.

Cold-restraint stress ulcers in rats

Groups of 5 female rats, weighing 100—110 g, were housed and fasted as described above. The compounds (or 5 ml/kg of vehicle for the controls) were administered orally. 1 h after treatment, the rats were immobilized in a wire mesh corset and placed for 2 h in a refrigerator at 8°C [6, 7]. The animals were then killed with ether, their stomachs dissected out and opened along the greater curvature. The gastric mucosa was examined under a stereoscopic microscope (American Optical, Buffalo, N.Y., U.S.A.) and the ulcers counted and measured. Each ulcer was scored on the following scale: 1 = ulcer less than 0.5 mm; 2 = ulcer between 0.5 and 1.5 mm; 3 = ulcer longer than 1.5 mm. The total ulceration index for each stomach was the sum of these scores. In this procedure, the total ulceration index for the controls was 9.25 $\pm$ 1.7. Cimetidine, given at 25 mg/kg orally, inhibited stress-induced ulcers by 67%.

Ethanol-induced gastric lesions in rats

Female rats weighing 200—220 g were used. After 6 h of fasting (water *ad libitum*), the animals were placed in individual cages and also deprived of water for an additional 18 h. The rats, in groups of 10, were treated orally. Controls were treated with 5 ml/kg of vehicle. 1 h after treatment, 1 ml of absolute ethanol was administered orally [8]. After 1 h the animals were killed, their stomachs dissected out and the severity of the gastric lesions scored on the following scale: 1 = ulcer less than 0.5 mm; 2 = ulcer between 0.5 and 1.5 mm; 3 = ulcer between 1.5 and 3 mm; 4 = ulcer longer than 3 mm. The total lesion index for each stomach was the sum of these scores. Using this procedure, the total lesion index for the controls was 67 $\pm$ 5. Cimetidine was inactive at 100 mg/kg, given orally; PGE_2, at 0.1 mg/kg given orally, reduced the total lesion index by 63% and at 0.5 mg/kg by 92%.

Statistical evaluation

Dunnet's *t*-test was used for the analysis of data from pylorus-ligated rats. Student's *t*-test was used for analysis of data from stomach perfused rats. Non-parametric data were evaluated using the Kruskal—Wallis test.

Acknowledgments

The authors would like to thank Mr. C. Parini, F. Selva, F. Cella, R. Maggi and A. Crippa for technical assistance and Miss M. T. Ranucci for typing the manuscripts.

References

1 Kuroda K. & Akao M. (1977) *Arch. Int. Pharmacodyn.* 226, 324
2 Barzaghi F., Cesana R., Delevallée F., Fournex R., Nencioni A., Vincent J. C. & Deraedt R. (1985) *Arzneim. Forsch. Drug Res.* 35, 1412
3 Sugiyama N., Gasha T., Kataoka H. & Kashima C. (1968) *Bull. Chem. Soc. Jpn.* 41, 971
4 Shay H., Komarov S. A., Fels S., Meranze D., Gruenstein M. & Siplet H. (1945) *Gastroenterology* 5, 43
5 Ghosh M. N. & Schild H. O. (1958) *Br. J. Pharmacol.* 13, 54
6 Rossi G., Bonfils S., Lieffooch F. & Lambling A. (1956) *C. R. Soc. Biol.* 150, 2124
7 Senay E. C. & Levine R. J. (1967) *Proc. Soc. Exp. Biol. Med.* 124, 1221
8 Robert A., Nezamis J., Lancaster C. & Hanchar A. J. (1979) *Gastroenterology* 77, 433
9 Dallacher F. & Bloemen J. (1961) *Monatsh. Chem.* 92, 640
10 Dave K. P. & Nargund K. S. (1938) *J. Univ. Bombay* 7, Pt 3, 191; (1939) *Chem. Abstr.* 33, 3779
11 Prey V. & Pieh G. (1949) *Monatsh. Chem.* 80, 790
12 Shamshurin A. A. (1946) *J. Gen. Chem. (U.S.S.R.)* 16, 99; (1947) *Chem. Abstr.* 41, 104
13 v. Kostanecki St. & Tambor J. (1899) *Chem. Ber.* 32, 2260
14 Lienhard U., Fahrni H. P. & Neuenschwander M. (1978) *Helv. Chim. Acta* 61, 1609
15 Kost A. N., Mitropol'Skaya V. N., Portnova S. L. & Krasnova V. A. (1964) *Zh. Obshch. Khim.* 34, 2989
16 Lilly E. & Co. (1967) Fr. Patent 1,481,052; (1968) *Chem. Abstr.* 69, 18840
17 Baudrowski E. (1882) *Chem. Ber.* 15, 2698
18 Trivedi J. J. & Nargund K. S. (1941) *J. Univ. Bombay* 10, Pt3, 99; (1942) *Chem. Abstr.* 36, 3801
19 Grummitt O., Becker E. I. & Miesse C. (1955) *in: Organic Syntheses* Vol. III, Wiley & Sons, New York, p. 109
20 Ziegler E., Henning G. & Müller A. K. (1973) *Liebigs Ann. Chem.* 1552
21 Sakan T. (1942) *J. Chem. Soc. Jpn.* 63, 1545; (1947) *Chem. Abstr.* 41, 3072
22 Papa D., Schwenk E., Villani F. & Klingsberg E. (1948) *J. Am. Chem. Soc.* 70, 3356
23 Markovac A., La Montagne M. P., Blumbergs P., Ash A. B. & Stevens C. L. (1972) *J. Med. Chem.* 15, 918
24 Kirchner F. C. & Alexander E. J. (1959) *J. Am. Chem. Soc.* 81, 1721
25 Ginsberg H. F., Lederman I. & Papa D. (1953) *J. Am. Chem. Soc.* 75, 4587
26 Oddy H. G. (1923) *J. Am. Chem. Soc.* 45, 2156
27 Baddeley G., Makar S. M. & Ivinson M. G. (1953) *J. Chem. Soc.* 3969
28 Raabe T., Stachel A., Scholtholt J. & Nitz R. E. (Cassella Farbwerke Mainkur A.G.) (1973) Ger. Offen. 2,116,293; (1973) *Chem. Abstr.* 78, 16219
29 a) Ichihara A., Hasegawa H., Sato H., Koyama M. & Sakamura S. (1973) *Tetrahedron Lett.* 37
b) Rovnyak G. C. (E. R. Squibb & Sons, Inc., Princeton, N.J.) (1981) U.S. Patent 4,254,267
30 Aurozo A., Faure R. & Mattioda G. (1975) *Eur. J. Med. Chem.* 10, 182
31 Ghosh R. & Robinson R. (1944) *J. Chem. Soc.* 506
32 Reimer M., Tobin E. & Schaffner M. (1935) *J. Am. Chem. Soc.* 57, 211