NEW PLEUROMUTILIN DERIVATIVES WITH ENHANCED ANTIMICROBIAL ACTIVITY

II. STRUCTURE-ACTIVITY CORRELATIONS

HELMUT EGGER and HELLMUTH REINSHAGEN

Sandoz Forschungsinstitut, A-1235 Vienna, Austria

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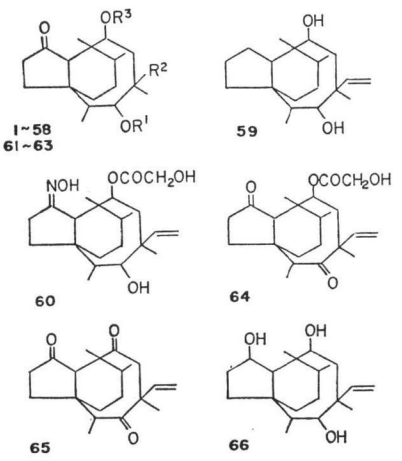
Structural modification of the antibiotic pleuromutilin has afforded several derivatives with considerably enhanced activity against bacteria and mycoplasmas, and has permitted conclusions to be reached about structure-activity relationships. The carbonyl group in the five-membered ring and the hydroxyl group at C_{11} seem to be essential for activity. The vinyl group can be hydrogenated without loss of activity. Chemical modification at C_{14} offers the most possibilities for achieving the best activity and solubility properties. Mutilin, and other compounds with a free OH at C_{14}, are inactive. It was shown that mutilin esters of substituted thioglycolic acids had distinctly superior MIC values, especially in combination with a tertiary amino group in the side chain, the latter group of derivatives having MIC values better than pleuromutilin by a factor of more than 10. Further variation within this group led to the development of 14-deoxy-14-[(2-diethylaminoethyl) thioacetoxy]-mutilin hydrogen fumarate (81.723 hfu, tiamulin) for extensive investigation of its chemotherapeutic potential.

In the previous paper[1] the chemical modification of pleuromutilin, an antibiotic produced by some species of Basidiomycetes has been reported. The present paper deals with the results of a systematic modification of the mutilin skeleton. In this investigation we have defined the basic structural requirements for antimicrobial activity and its enhancement.

Mutilin (**45**), the product of the alkaline hydrolysis of pleuromutilin, was found in earlier studies to be inactive. All other derivatives with a free C-14 hydroxy group subsequently prepared also proved inactive. Other chemical modifications that yield inactive products include pleuromutilin oxime (**60**), esterification of the C-11 hydroxy group (*e.g.* 11-O-acetates **62** and **63**) and oxidation to the C-11 ketone (**64**). Further derivatives with no antibiotic activity are listed in Table 1.

Chart 1. Derivatives of pleuromutilin



Conversely, hydrogenation of the vinyl group left the activity essentially unaltered in all compounds studied. Compounds obtained by variation of the ester group at C-14 were all antimicrobially active, although the activity varied widely and, as it seemed at first, inconsistently.

It was concluded from these results that modification of the side chain at C-14 would be the most promising approach to obtain optimum activity. It can be seen by comparing the various side chains linked through the ester group to C-14 that the antimicrobial activity of a derivative depends on

Table 1. Antimicrobial activities of pleuromutilin derivatives

No.	R^1	R^2	R^3	MIC (mcg/ml)			
				Staph. aureus	*Mycoplasma hominis*	*Mycoplasma gallisepticum*	*Mycoplasma hyorhinis*
1	H	vinyl	$COCH_2OH$	0.5	0.3	0.3	2
2	H	Et	$COCH_2OH$	0.3	0.8	0.3	3
3	H	vinyl	$COCH_2OSO_2C_6H_4$-4-Me	0.02	10	5	> 100
4	H	Et	$COCH_2OSO_2C_6H_4$-4-Me	0.3	2	0.6	25
5	H	vinyl	$COCH_2OSO_2CH_3$	0.2	0.2	0.2	12
6	H	vinyl	$COCH_2NEt_2$	1.4	5	5	3
7	H	vinyl	$COCH_2$(N,O)	0.2	0.2	0.2	10
8	H	vinyl	$COCH_2$(N,N)$(CH_2)_2OH$	0.8	0.08	0.3	10
9	H	vinyl	$COCH_2NH(CH_2)_2NEt_2$	2	15	3	—
10	H	vinyl	$COCH_2NH(CH_2)_3$(N,O)	10	60	1	25
11	H	vinyl	$COCH_2O(CH_2)_2NEt_2$	1	0.04	0.3	12
12	H	vinyl	$COCH_2S(CH_2)_2NMe_2$	0.05	0.03	0.02	0.2
13	H	vinyl	$COCH_2S(CH_2)_2NEt_2$	0.05	0.01	0.006	0.3
14	H	vinyl	$COCH_2S(CH_2)_2N(i\text{-}Pr)_2$	0.08	0.006	0.03	1
15	H	vinyl	$COCH_2S(CH_2)_2N(n\text{-}Bu)_2$	0.08	0.08	0.08	5
16	H	vinyl	$COCH_2S(CH_2)_2N(2\text{-}Et\text{-}hexyl)_2$	0.5	5	10	> 100
17	H	vinyl	$COCH_2S(CH_2)_3NMe_2$	0.1	0.002	0.006	0.04
18	H	vinyl	$COCH_2S(CH_2)_3N(n\text{-}Bu)_2$	0.1	0.02	0.08	5
19	H	vinyl	$COCH_2S(CH_2)_5NMe_2$	0.02	0.001	0.006	0.04
20	H	Et	$COCH_2S(CH_2)_2NMe_2$	0.1	0.06	0.06	3
21	H	Et	$COCH_2S(CH_2)_2NEt_2$	0.1	0.02	0.02	0.6
22	H	Et	$COCH_2S(CH_2)_2N(n\text{-}Bu)_2$	0.2	0.3	0.3	30
23	H	vinyl	$COCH_2S(CH_2)_2$(N)	0.3	0.002	0.006	0.2
24	H	vinyl	$COCH_2S(CH_2)_2$(N)	0.1	0.01	0.01	—
25	H	vinyl	$COCH_2S(CH_2)_2$(N)	0.07	0.006	0.01	0.6
26	H	vinyl	$COCH_2S(CH_2)_2$(N,O)	0.03	0.03	0.03	0.6
27	H	vinyl	$COCH_2S(CH_2)_2$(N,S)	0.03	0.02	0.04	1
28	H	vinyl	$COCH_2S(CH_2)_2$-(N,N)-Me	0.02	0.002	0.004	0.3
29	H	vinyl	$COCH_2S(CH_2)_2$-(N,N)$(CH_2)_2OH$	0.05	0.004	0.02	0.2
30	H	vinyl	$COCH_2S(CH_2)_2$-(N,N)$(CH_2)_2OAc$	0.02	0.004	0.008	0.2
31	H	vinyl	$COCH_2S(CH_2)_2$-(N,N)-n-Bu	0.02	0.003	0.003	0.1
32	H	vinyl	$COCH_2S(CH_2)_2$-(N,N)-R^4 R^4: $(CH_2)_2OCO(CH_2)_2COOH$	0.1	0.006	0.006	0.1
33	H	Et	$COCH_2S(CH_2)_2$-(N,N)-Me	0.02	0.002	0.004	0.3
34	H	Et	$COCH_2S(CH_2)_2$-(N,N)$(CH_2)_2OH$	0.07	0.002	0.008	0.5
35	H	Et	$COCH_2S(CH_2)_2$-(N,N)$(CH_2)_2OAc$	0.07	0.003	0.003	0.2
36	H	vinyl	$COCH_2S\ C_6H_4$-2-OH	0.01	0.03	0.03	12
37	H	vinyl	$COCH_2S\ C_6H_4$-4-OH	0.02	0.04	0.02	0.8
38	H	vinyl	$COCH_2S\ C_6H_4$-2-COOH	1	> 100	0.2	100
39	H	Et	$COCH_2S\ C_6H_4$-4-OH	0.02	0.08	0.6	25
40	H	vinyl	$COCH_2S\ C_6H_4$-2-$O(CH_2)_2NEt_2$	0.02	0.006	0.006	0.2
41	H	vinyl	$COCH_2S\ C_6H_4$-4-$O(CH_2)_2NMe_2$	0.02	0.004	0.008	0.3
42	H	vinyl	$COCH_2S\ C_6H_4$-4-$O(CH_2)_2NEt_2$	0.01	0.001	0.003	0.2
43	H	vinyl	$COCH_2S\ C_6H_4$-4-$O(CH_2)_3$(N)	0.02	0.002	0.002	0.2

(to be continued)

Table 1. (Continued)

No.	R^1	R^2	R^3	MIC (mcg/ml)			
				Staph. aureus	*Mycoplasma hominis*	*Mycoplasma gallisepticum*	*Mycoplasma hyorhinis*
44	H	Et	$COCH_2S\ C_6H_4\text{-}4\text{-}O(CH_2)_2NEt_2$	0.01	0.004	0.004	0.1
45	H	vinyl	H	>10	>10	>10	>100
46	H	Et	H	>10	>10	>10	>100
47	H	vinyl	$COCH_2OCO(CH_2)_5NMe_2$	0.3	0.4	0.4	12
48	H	vinyl	$COCH_2S(CH_2)_2CN$	0.1	0.08	0.04	1
49	H	vinyl	$CO(CH_2)_5NMe_2$	0.1	0.04	0.08	0.6
50	$CO(CH_2)_5NMe_2$	vinyl	H	>10	3	>10	100
51	$CO(CH_2)_5NMe_2$	vinyl	$CO(CH_2)_5NMe_2$	>10	6	>10	12
52	H	vinyl	$COCH_2OCO(CH_2)_2COOH$	1	>10	4	6
53	H	vinyl	$COCH_2OCONHR^4$ R^4: $CHCH_2CH(CH_3)_2$ COOH	>10	>10	>10	>100
54	H	vinyl	R^4: $CH(CH_2)_2COOH$ COOH	>10	>10	>10	>100
55	H	vinyl	R^4: $CHCH_3$ COOH	>10	>10	4	100
56	H	vinyl	$COCH_2S(CH_2)_2NH_2$	0.4	0.008	0.03	6
57	H	vinyl	$COCH_2S(CH_2)_2NHC(=NH)NH_2$	5	0.08	0.2	1
58	H	vinyl	$COCH_2S(CH_2)_2NHC$ 	0.2	0.08	0.08	1
59				>10	>10	>100	>10
60				>10	>10	>100	>100
61	H	vinyl	$COCH_2OAc$	0.5	0.5	8	0.5
62	Ac	vinyl	$COCH_2OAc$	>10	>10	10	—
63	Ac	vinyl	Ac	>10	>10	>10	>100
64				>10	>10	>10	>10
65				>10	>10	>10	>100
66				>10	>10	>10	>100

whether this side chain is neutral, acidic or basic. Neutral side chains, including those with highly polar groups like sulphonate or nitrile, have by and large little effect on the activity of the derivative with respect to the parent compound. An exception to this generalization are thioethers of type **36**, **37** and **39**, in which group the first significantly improved antibiotic activity was observed.

As possible reasons for the poor results of pleuromutilin *in vivo*[2], either rapid inactivation in the animal or inefficient transport to the site of infection, because of the strongly hydrophobic nature of the molecule might be postulated.

Derivatives **52**~**55** incorporating side chains with acid salt-forming groups (-COOH) have much poorer MIC values than pleuromutilin, although their salts are freely water-soluble. Derivatives with basic side chains can be subdivided into various groups, some of which also form highly soluble salts. Although the glycine derivatives **6**, **7** and **8** were not a notable improvement, and compounds such as **9** and **10** with two basic centres were even worse, combination of the above-mentioned thioether group (thioglycolic acid derivatives) with a basic side chain brought about a decisive improvement.

Basic alkylation of the thiophenol derivatives **36**, **37** and **39** makes accessible compounds of type

40～**44** with excellent inhibitory activities. (see Table). Comparison with the thiosalicylic acid derivative **38**, which is inferior even to the parent substance pleuromutilin, makes the decisive importance of the basic alkyl group even clearer.

More detailed studies soon showed that the aromatic ring in the side chain is unnecessary, derivatives such as **12**～**35** with purely aliphatic side chains being comparably active. The latter compounds are in addition more readily accessible, more soluble and more easily crystallized, so that within this series chemical variation was undertaken.

The distance between the tertiary basic centre and the ester group seems not to be critical unless radically shortened as in the above-mentioned weakly active compounds **6**, **7** and **8**. This assumption is supported by the observation that the benzene ring can be omitted, as stated above, and the remaining aliphatic chain lengthened without marked alteration of the antibiotic activity (*e. g.* **19**). Drastic reduction of activity results from replacement of the thioether sulphur by oxygen (**11**). Compounds with an uninterrupted carbon chain between the ester group and the basic nitrogen, *i. e.* those in which the sulphur atom is replaced by methylene, display only a minor loss of activity (**49**) with respect to the corresponding thioethers. An additional (secondary) basic nitrogen in the chain, as in the compounds **9** and **10**, has an unfavourable effect. Comparison of compounds **13**, **26** and **29** with the weakly active analogues **6**, **7** and **8** which lack both thioether group and methylene chain best shows the impressive dependence of activity on optimal structural elements. A certain favourable relationship between pK value and lipophilic properties in the side chain must clearly be adhered to.

Progressive lengthening of the alkyl substituents at the tertiary nitrogen atom caused a steep decline in activity. Within the group of cyclic amine derivatives **23**～**27**, structure-activity relationship is less clear. The piperazine derivatives **28**～**35** have an additional tertiary basic centre, and therefore form salts which are more soluble in water and crystallize more readily. Their activity spectra and inhibitory values, however, deviate little from those of the other members of the series.

Derivatives with other basic substituents in the side chain, such as the guanidine derivatives **57** and **58** and the primary amine **56**, are also highly active but do not quite reach the standard set by types **12**～**35**. Other derivatives studied include those in which the tertiary amino group is linked to a second ester group introduced by acylation of the primary hydroxy group of pleuromutilin, *e. g.* **47**. Such compounds are also distinctly inferior to those of types **12**～**35** and are in addition sensitive to hydrolysis, especially the lower homologues.

Extensive studies in animal models[3,4] led finally to the selection of the derivative **13** in the form of its crystalline hydrogen fumarate for further development under the designation 81.723 hfu, tiamulin.

Antimicrobial Activities

Staphylococcus aureus (tetracycline and penicillin resistant) was obtained from the collection of strains of the Veterinärmedizinische Universität Vienna (Strain No. 503), *Mycoplasma hominis* from the American Type Culture Collection (ATCC 15,488), *Mycoplasma gallisepticum* from the same source (ATCC 19,989), *Mycoplasma hyorhinis* from the Veterinär-Untersuchungsamt Koblenz.

MIC values for bacteria and mycoplasmas were determined by serial twofold dilution tests in the appropriate media. Bacteria were grown and tested on Trypticase soy broth (Baltimore Biological Laboratory). MIC values for mycoplasmas were determined on mycoplasma broth base or on agar base (Oxoid).

The antimicrobial activities of the pleuromutilin derivatives[4] are listed in Table 1.

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