Synthesis and Copper-Dependent Antimycoplasmal Activity of Amides and Amidines Derived from 2-Amino-1,10-phenanthroline

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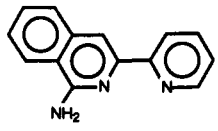
A series of both aliphatic and aromatic amides and aromatic amidines derived from 2-amino-1,10-phenanthroline (3) according to the Topliss scheme were synthesized and subsequently tested for antimycoplasmal potency. Although the compounds themselves showed no activity, in the presence of a nontoxic copper concentration of 40 μM all compounds appeared to be very active against *Mycoplasma gallisepticum* K154. The most active compounds were found in the amide series and show growth inhibition in the nanomolar range. These compounds are 4 times more active than tylosin, a macrolide antibiotic, which is used therapeutically in veterinary practice. In the presence of copper, amides derived from 3 are more active than corresponding amidines. Increased activity following derivatization of 3 may be due to the presence of a third coordination site for copper in the title compounds. Evaluation of biological data revealed that antimycoplasmal activity of amides derived from 3 is dependent on lipophilicity. For these amides a good linear correlation was found between antimycoplasmal activity and hydrophobic fragmental values for substituents considered. This quantitative structure-activity relationship study indicated that antimycoplasmal activity was increased upon a decrease of these hydrophobic fragmental values.

Introduction

It has been known for many years now that compounds containing a 2,2′-bipyridyl moiety possess antimycoplasmal activity in the presence of a small nontoxic amount of copper.[1] In a proposed mechanism of action[2-4] extracellular copper is bound by the compound containing the 2,2′-bipyridyl moiety and subsequently transported across the cell membrane into the cytosol. Once intracellular, copper itself inhibits enzymes involved in the energy providing metabolism.

In recent years we have investigated antimycoplasmal activity of amides[5] and amidines[6] derived from the easily accessible 1-amino-3-(2-pyridyl)isoquinoline (1). For these compounds, too, antimycoplasmal activity appeared to be dependent on the presence of copper. Furthermore it was found that all derivatives are more active against *Mycoplasma gallisepticum* K514 than the parent compound, whereas the amidines are 2–3 times more active than the corresponding amides. It was established that the most active compound within these series, viz. *N*-[3-(2-pyridyl)isoquinolin-1-yl]-2-pyridinecarboxamidine (2), is 3 times more active than the antimycoplasmal therapeutic tylosin.



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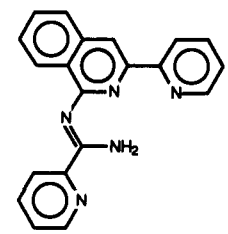
Structure–activity relationship (SAR) studies revealed that antimycoplasmal activity of both amides and amidines is dependent on the hydrophobic fragmental value ($\sum f$) of the amide or amidine residue. This dependency was parabolic in nature and for both types of compounds regression analysis revealed a good correlation between antimycoplasmal activity and $\sum f$ and $\sum f^2$.

Although we have already obtained a great increase of antimycoplasmal activity by structure optimization within a series of 1-amino-3-(2-pyridyl)isoquinoline derivatives, now we focus our attention to the corresponding 2-amino-1,10-phenanthroline (3) derivatives in an attempt to obtain even more active compounds.

Comparison of the antimycoplasmal activity of 2-substituted 1,10-phenanthrolines with the corresponding 1-substituted 3-(2-pyridyl)isoquinolines revealed that 1,10-phenanthroline derivatives are equal or somewhat less active than the corresponding 3-(2-pyridyl)isoquinolines.[1] However, when antimycoplasmal activity of 2-substituted 1,10-phenanthrolines is compared with antimycoplasmal activity of the corresponding 6-substituted 2,2′-bipyridyls it becomes clear that the 1,10-phenanthroline derivatives are 2–4 times more active than their 2,2′-bipyridyl analogues. This observation was explained by the fixed cisoid form of the two pyridine rings in the case of 1,10-phenanthroline derivatives. This cis coplanarity of the two pyridine rings is a prerequisite for complex formation with copper and accordingly for antimycoplasmal activity.



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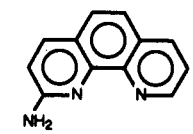

3

In a structure–activity relationship study by Pijper[7] it was shown that for several compounds containing a 2,2′-bipyridyl moiety, including 2-substituted 1,10-phenanthrolines and 1-substituted 3-(2-pyridyl)isoquinolines, an optimal lipophilicity exists with regard to antimycoplasmal activity. As in general the lipophilicity of 1-substituted 3-(2-pyridyl)isoquinolines, included in this study, is closer to this optimal value than the lipophilicity of the analogous 2-substituted 1,10-phenanthrolines, the antimycoplasmal activity of the latter is lower despite their

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(1) Pijper, P. J.; Van der Goot, H.; Timmerman, H.; Nauta, W. T. *Eur. J. Med. Chem.* **1984**, *19*, 389.
(2) Smit, H.; Van der Goot, H.; Nauta, W. T.; Timmerman, H.; De Bolster, M. W. G.; Jochemsen, A.-G.; Stouthamer, A. H.; Vis, R. D. *Antimicrob. Agents Chemother.* **1981**, *20*, 455.
(3) Smit, H.; Van der Goot, H.; Nauta, W. T.; Timmerman, H.; De Bolster, M. W. G.; Stouthamer, A. H.; Vis, R. D. *Antimicrob. Agents Chemother.* **1982**, *21*, 881.
(4) Gaisser, H.-D.; Van der Goot, H.; Timmerman, H. *Eur. J. Med. Chem.* **1985**, *20*, 513.
(5) De Zwart, M. A. H.; Van der Goot, H.; Timmerman, H. *J. Med. Chem.* **1988**, *31*, 716.
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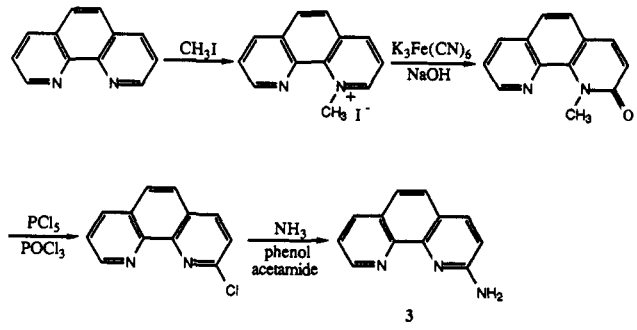
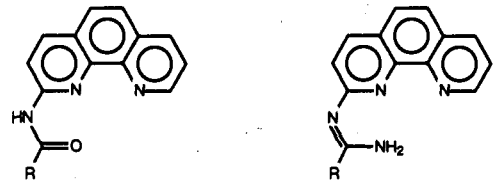
Scheme I



advantageous cis coplanarity.

Since we have demonstrated that for aromatic amides and amidines derived from 1-amino-3-(2-pyridyl)isoquinoline antimycoplasmal activity increases with decreasing lipophilicity,[5,6] the lower lipophilicity of 1,10-phenanthrolines as compared to the analogous 3-(2-pyridyl)isoquinolines may be advantageous in the case of amides and amidines derived from 2-amino-1,10-phenanthroline with regard to antimycoplasmal activity.

In the present paper we therefore report on the synthesis and antimycoplasmal activity of amides (**4**) and amidines (**5**) derived from 2-amino-1,10-phenanthroline. Structure optimization was performed according to the method proposed by Topliss.[8] Biological data are evaluated and eventually a quantitative structure–activity relationship is presented.



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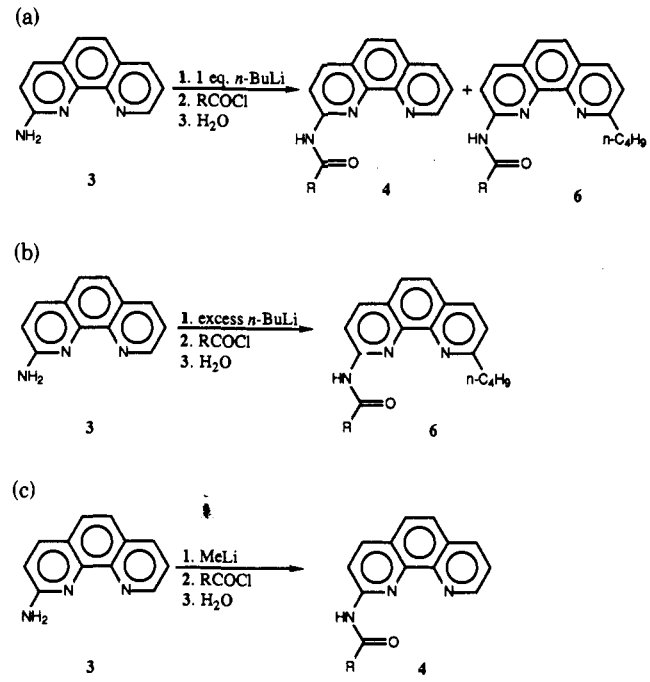
Chemistry

Although there are several possibilities for the synthesis of amides, we chose to prepare the desired amides **4** in the same way as we synthesized amides derived from 1-amino-3-(2-pyridyl)isoquinoline, i.e. by acylation of an amine with acyl chlorides.[5,9] The required acyl chlorides were obtained from the corresponding acids,[10,11] and 2-amino-1,10-phenanthroline (**3**) was prepared according to literature procedures[12,13] from 1,10-phenanthroline in an overall yield of 65% (Scheme I).

For the preparation of amides **4**, one of the amine protons is abstracted by first using *n*-butyllithium as a base to increase the nucleophilicity of the amine nitrogen. Subsequently, the anion obtained is treated with one of the acyl chlorides at −15 °C in anhydrous tetrahydrofuran (Scheme II part a). By this method, referred to as method A, amides **4a–e** were obtained in moderate yields.

It appeared that in some reactions considerable amounts of the 9-*n*-butyl derivative **6** were formed. Besides these 9-*n*-butyl-1,10-phenanthrolines minor amounts of diacylated 2-amino-1,10-phenanthrolines were also detected as undesired side products.

Scheme II



Although alkylation by nucleophilic substitution in pyridines by alkyllithiums is very well known, the available literature of the analogous reaction with 2,2′-bipyridyl or 1,10-phenanthroline is very sparse. Kauffmann et al.[14] described the alkylation of 2,2′-bipyridyl by nucleophilic substitution for the first time. Reaction of 1 equiv of alkyllithium with 2,2′-bipyridyl followed by hydrolysis and subsequent oxidation yielded very selectively the 6-monoalkylated compound. When an excess *n*-butyllithium was used, the 6,6′-dibutylated 2,2′-bipyridyl was obtained, whereas an excess methyllithium still resulted in the 6-monoalkylated compound only. Furthermore Kauffmann et al.[14,15] established that 2,2′-bipyridyl is more electrophilic than pyridine.

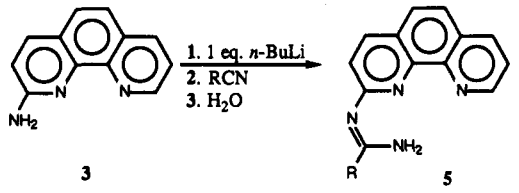
The synthesis of 6-methyl- and 6-ethyl-2,2′-bipyridyl via nucleophilic substitution followed by thermolytic aromatization has been described by Schmalzl et al.[16]

Dietrich-Buchecker et al.[17] found that the reaction of butyl- or phenyllithium with 1,10-phenanthroline followed by hydrolysis and rearomatization with manganese dioxide gave good yields of the 2,9-dibutyl-1,10-phenanthroline and 2,9-diphenyl-1,10-phenanthroline, respectively. It was found that yields were almost entirely independent of the solvent used, but that the nature of the dehydrogenating agent was of prime importance.[17] Nucleophilic alkylation appeared also to be applicable for the preparation of other alkyl-substituted 1,10-phenanthrolines.

Furthermore, the same authors found that alkylation of 1,10-phenanthroline gave higher yields than 2,2′-bipyridyl or 2,2′,6′,2″-terpyridine.[17] Because of the additional ethenylene bridge the negative charge introduced by the nucleophile can apparently be better stabilized by resonance than in the case of 1,10-phenanthrolines. This and the fixed cis coplanarity of the pyridyl rin s, which is an ad-

(8) Topliss, J. G. *J. Med. Chem.* **1977**, *20*, 463.
(9) Zabicky, J. *The Chemistry of Functional Groups*; Patai, S., Ed.; Interscience: New York, 1970.
(10) Patai, S. *The Chemistry of Acyl Halides*; Interscience: New York, 1972.
(11) Brown, H. C. *J. Am. Chem. Soc.* **1938**, *60*, 1325.
(12) Halcrow, B. E.; Kermack, W. O. *J. Chem. Soc.* **1946**, 155.
(13) Ogawa, S.; Yamaguchi, T.; Gotoh, N. *J. Chem. Soc. Perkin 1* **1974**, 976.
(14) Kauffmann, T.; König, J.; Woltermann, A. *Chem. Ber.* **1976**, *109*, 3864.
(15) Kauffmann, T.; König, J.; Körber, D.; Lexy, H.; Streitberger, H.-J.; Vahrenhorst, A.; Woltermann, A. *Tetrahedron Lett.* **1977**, 389.
(16) Schmalzl, K. J.; Summers, L. A. *Aust. J. Chem.* **1977**, *30*, 657.
(17) Dietrich-Buchecker, C. O.; Marnot, P. A.; Sauvage, J. P. *Tetrahedron Lett.* **1982**, *23*, 5291.

Scheme III



vantage for the formation of the transition state in which lithium is coordinated by the two nitrogen atoms, may be the reason why ortho alkylation is observed in case of the 2-amino-1,10-phenanthrolines and not in case of the 1-amino-3-(2-pyridyl)isoquinolines.

Initially we isolated the 9-*n*-butyl derivatives **6a–d** as a side product in the synthesis of amides **4a–e**. This was probably due to a slight excess of *n*-butyllithium which was used as a base and added as a 1.6 M solution in hexane. In an attempt to obtain these compounds as the main reaction product, we performed the same reaction using an excess of *n*-butyllithium (Scheme II part b). As expected, the 9-*n*-butylated amides **6a** and **6b** were the main reaction products and these compounds could be obtained by this method in satisfactory yields (30–35%).

Due to the formation of considerable amounts of side products in the synthesis of amides of 2-amino-1,10-phenanthroline by the method used, we tried to improve the synthesis of these compounds by using another base, viz. methyllithium (Scheme II part c). As mentioned before, use of an excess methyllithium in alkylation of 2,2′-bipyridyl still resulted only in the monoalkylated product.[14] Accordingly, use of methyllithium instead of *n*-butyllithium in the synthesis of amides of 2-amino-1,10-phenanthroline should give rise to less alkylation because of the decreased nucleophilic power of the base used.

Synthesis of amides **4a–c** from 2-amino-1,10-phenanthroline by this method, referred to as method B, resulted in yields 2–3 times higher as compared to method A. Hence, method B indeed proved to be a good method for the synthesis of amides of 2-amino-1,10-phenanthroline and should be preferred to method A.

Since not only amides of 1-amino-3-(2-pyridyl)isoquinoline but also the corresponding amidines showed antimycoplasmal activity in the presence of copper,[5,6] we also synthesized some amidines of 2-amino-1,10-phenanthroline. The easiest way to obtain these kind of compounds consists of the reaction of an amine, viz. 2-amino-1,10-phenanthroline with an electron-deficient nitrile[18-20] (Scheme III). By this method amidines **5a–c** were synthesized and isolated in rather low yields. Although no alkylated product could be isolated a high number of unidentified side products were formed during this reaction. As for the amides, yields might be expected to be improved by using methyllithium as a base.

As is generally known, 1,10-phenanthrolines form hydrates very easily because of the cis coplanarity of the two pyridine rings facilitating H-bond formation between H_2O and the N-atoms of the heterocyclic rings.[21] Also the title compounds were isolated as hydrates. Spectroscopic analysis revealed that formation of the iminol form as is described for amides of 1-amino-3-(2-pyridyl)isoquinoline does not take place in case of the amides **4a–e**. Due to complexation with a water molecule the nitrogen atom which is also involved in stabilizing the iminol form is already occupied by hydrogen bond formation. So, formation of hydrates might be the reason for the absence of the iminol form in case of the aforementioned amides.

Scheme IV

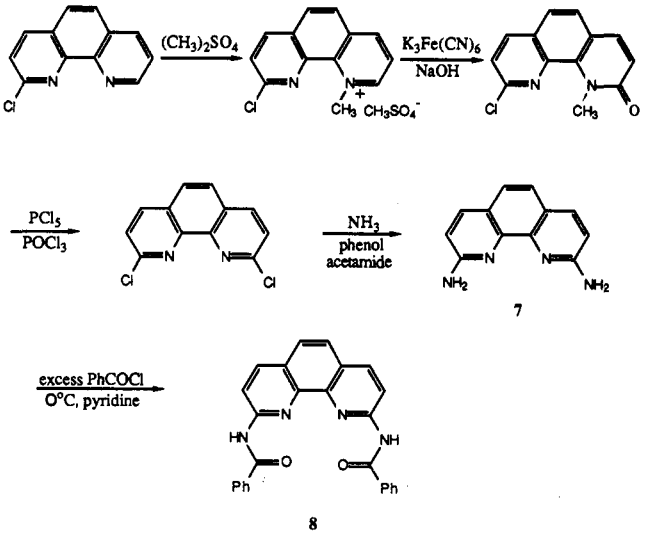


Table I. MIC Values[a] (μM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C

compd	without extra copper	extra copper added[b]
$CuSO_4 \cdot 5H_2O$	700	–
tylosin	0.1	0.1
3	>100	3.1
7	>100	0.8

[a] Number of determinations of MIC values is four. [b] 40 μM $CuSO_4$.

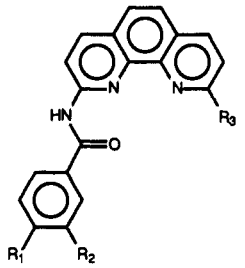
Yamada et al.[22] recently described the synthesis of 6,6′-bis(benzoylamino)-2,2′-bipyridyl and its Cu(II)-complex. It was found that a 1:1 complex was formed in which the copper atom is planar coordinated at the two ring-nitrogen atoms and at the two amide-oxygen atoms. This supports our hypothesis that the amide or amidine moiety of the respective derivatives of 2-amino-1,10-phenanthroline may be involved in the complex formation as a third coordination site. Because of the remarkable resemblance of 2,9-bis(benzoylamino)-1,10-phenanthroline **8** to both 6,6′-bis(benzoylamino)-2,2′-bipyridyl and to the compounds which are subject of this investigation, we synthesized compound **8** according to the procedure described by Yamada et al.[21] (Scheme IV) and subsequently investigated its antimycoplasmal activity. The required amine, viz. 2,9-diamino-1,10-phenanthroline **7**, was synthesized according to Ogawa et al.[13] starting from 2-chloro-1,10-phenanthroline (Scheme IV).

Biological Activity

As reported earlier, antimycoplasmal activity for a series of compounds containing a 2,2′-bipyridyl moiety is copper dependent. Amides and amidines derived from 2-amino-1,10-phenanthroline (**3**) and 2,9-diamino-1,10-phenanthroline (**7**) were tested with and without addition of extra copper to the test medium. Without addition of extra copper, the copper concentration of the modified

(18) Shriner, R. L.; Neumann, F. W. *Chem. Rev.* **1944**, *35*, 351.
(19) Gautier, J.-A.; Miocque, M.; Fauran, C.; Le Cloarec, A. Y. *Bull. Soc. Chim. Fr.* **1970**, 200.
(20) Cooper, F. C.; Patridge, M. W. *J. Chem. Soc.* **1953**, 255.
(21) Summers, L. A. *Advances in heterocyclic chemistry*; Katritzy, A. R., Boulton, A. J., Eds.; Academic Press: New York, 1978; Vol. 22, pp 1–69.
(22) Yamada, M.; Araki, K.; Shiraishi, S. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 3149.

Table II. MIC Values[a] (μM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C



compd	R_1	R_2	R_3	without extra copper	extra copper added[b]
4a	H	H	H	>100	0.023
4b	CH_3	H	H	>100	0.023
4c	OCH_3	H	H	>100	0.046
4d	Cl	H	H	>100	0.023
4e	Cl	Cl	H	>100	0.095
6a[c]	H	H	n-C_4H_9	>100	0.20
6b	CH_3	H	n-C_4H_9	>100	0.37
6c	Cl	H	n-C_4H_9	>100	0.75
6d	Cl	Cl	n-C_4H_9	>100	1.50
8	H	H	C_6H_5CONH	>100	0.050

[a] Number of determinations of MIC values is two. [b] 40 μM $CuSO_4$. [c] Compound tested as HCl salt.

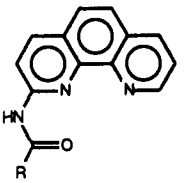
Adler medium was less than 3 μM.[23] For determination of the antimycoplasmal activity in the presence of copper, all compounds were tested in a test medium containing 40 μM $CuSO_4 \cdot 5H_2O$. This copper concentration is far below the toxic level, as the minimal inhibitory concentration was established to be 700 μM (Table I). All compounds were tested in a 3 nM to 100 μM concentration range. They were not tested for their antimycoplasmal activity in concentrations higher than 100 μM because at this concentration they tend to precipitate when added to the growth medium.

Besides the title compounds the butylated amides **6** seem very interesting with respect to antimycoplasmal activity, as it is known from investigations by Pijper et al. that diortho substitution of the 2,2′-bipyridyl moiety may be very advantageous for antimycoplasmal activity.[1] For this reason besides the desired amides we also isolated these 9-*n*-butyl analogues.

MIC values of the aromatic *N*-(1,10-phenanthrolin-2-yl)benzamides **4a–e**, their 9-*n*-butyl analogues **6a–d**, and 2,9-bis(benzoylamino)-1,10-phenanthroline (**8**) are presented in Table II. MIC values of the aliphatic *N*-(1,10-phenanthrolin-2-yl)amides **4f,g** and the *N*-(1,10-phenanthrolin-2-yl)benzamidines **5a–c** are presented in Tables III and IV, respectively.

Without addition of extra copper none of the compounds under investigation showed any antimycoplasmal activity in the concentration range tested, i.e. MIC's > 100 μM. However, as was shown for other 2,2′-bipyridyl containing compounds, these compounds are very active against *M. gallisepticum* K514 in the presence of a nontoxic amount of copper. Addition of 40 μM of copper to the test medium resulted in a tremendous increase of antimycoplasmal activity. Both amides and amidines are more active than the parent compound 2-amino-1,10-phenanthroline. In fact the most active compounds **4a,b,d** are 4 times more active on a molar basis than the reference compound tylosin, an antimycoplasmal drug used in veterinary practice for treatment of mycoplasmal infections.

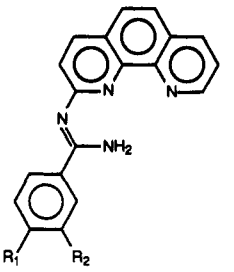
Table III. MIC Values[a] (μM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C



compd	R	without extra copper	extra copper added[b]
4f	$CH(C_2H_5)C_4H_9$	>100	0.23
4g	C_9H_{19}	>100	0.37

[a] Number of determinations of MIC values is two. [b] 40 μM $CuSO_4$.

Table IV. MIC Values[a] (μM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C



compd	R_1	R_2	without extra copper	extra copper added[b]
5a	H	H	>100	0.2
5b	H	Cl	>100	0.2
5c	Cl	Cl	>100	0.2

[a] Number of determinations of MIC values is two. [b] 40 μM $CuSO_4$.

Furthermore, amides derived from 2-amino-1,10-phenanthroline **4a,d,e** are 2–8 times more active than the corresponding amidines **5a–c**. This is in contrast with what had been found for a series of amides and amidines derived from 1-amino-3-(2-pyridyl)isoquinoline, the amidines showing a 2–3 times higher activity than the corresponding amides.[5,6]

Although amidines of 2-amino-1,10-phenanthroline appear to be fairly active against *M. gallisepticum* K514 in the presence of copper, we focused our attention on the corresponding amides because of their considerable higher antimycoplasmal activity.

Structure–Activity Relationships

As can be seen from the MIC values reported in Table II–IV, amides and amidines derived from 2-amino-1,10-phenanthroline themselves are not active against *M. gallisepticum* K514. However, addition of 40 μM copper increases their activity approximately 70–4000 times. Therefore it is clear that these compounds act via their copper complexes as do other 2,2′-bipyridyl analogues.[1,5,6]

The increase of activity of the amides and amidines compared to that of their parent compound 2-amino-1,10-phenanthroline (**3**) might be attributed to the presence of a third coordination site for the copper atom in the amide and amidines derivates of **3**. This feature was already discussed in the previous papers.[5,6] Further indication for the occurrence of this third coordination site for copper is found in the work of Yamada et al.[22] Their studies on the closely related 6,6′-bis(benzoylamino)-2,2′-bipyridine and its copper(II) complex revealed that 6,6′-bis(benzoylamino)-2,2′-bipyridine acts as a tetraden-

(23) Antić, B. M.; Van der Goot, H.; Nauta, W. T.; Balt, S.; De Bolster, M. W. G.; Stouthamer, A. H.; Verheul, H.; Vis, R. D. *Eur. J. Med. Chem.* **1977**, *12*, 573.

tate copper ligand, where both amide residues are deprotonated to form a neutral complex. It is very likely that the 2,9-bis(benzoylamino)-1,10-phenanthroline also acts as a tetradentate copper ligand, being able to neutralize the positive charge of the copper atom by the deprotonated amide residues. Formation of this neutral, lipophilic copper complex may be responsible for the remarkable increase of antimycoplasmal activity of 2,9-bis(benzoylamino)-1,10-phenanthroline as compared to the parent compound 2,9-diamino-1,10-phenanthroline.

Previous investigations on amides and amidines derived from 1-amino-3-(2-pyridyl)isoquinoline showed that the antimycoplasmal activity was dependent on the hydrophobic fragmental value of the amide or of the amidine residue, respectively.[5,6] Therefore antimycoplasmal activity of the closely related amides derived from 2-amino-1,10-phenanthroline (**3**) is likely to be controlled by the lipophilicity of the amide residue.

All amides synthesized, both aliphatic and aromatic, exist as the amide tautomer, since spectral data indicate the absence of the iminol tautomer. Consequently we may consider the contribution of the lipophilicity of the 1,10-phenanthroline part with the amide moiety attached to it, to be constant for all compounds when structure–activity relationships are considered. So, for both quantitative and qualitative considerations of a possible structure–activity relationship we only take into account the part of the molecule that is varied within the series, viz. the substituent of the amide moiety and the substituent at position 9 of the 1,10-phenanthroline system.

Since these compounds show antimycoplasmal activity in the presence of copper only, this copper-dependent activity was made the subject of both qualitative and quantitative structure–activity relationship considerations, which resulted in the following findings. The activity sequence within the original Topliss series of benzamides derived from 2-amino-1,10-phenanthroline did not clearly show a dependency on the lipophilicity, as only the 3,4-dichlorobenzamide (**4e**) was significantly less active than the four other benzamides. Therefore we decided to synthesize some more lipophilic amides. The two long-chain aliphatic amides **4f,g** indeed are less active than the more hydrophilic benzamides (Table II and III), with the more lipophilic decanamide **4g** being less active than the 2-ethylhexanamide **4f**.

Also in a series of 9-*n*-butylated amides **6a–d** the increase of activity is paralleled by a decrease of lipophilicity of the aromatic nucleus. This qualitative approach to a structure–activity relationship shows that the activity of the *N*-(1,10-phenanthrolin-2-yl)amides apparently is dependent on the contribution to lipophilicity of the amide residue and of the substituent at position 9 of 1,10-phenanthroline.

Combination of the three series, the benzamides **4a–e**, the 9-*n*-butylated benzamides **6a–d**, and the aliphatic amides **4f,g**, shows no clear evidence for the existence of an optimal lipophilicity as we observed for both amides and amidines derived from 1-amino-3-(2-pyridyl)isoquinoline. Yet the equal antimycoplasmal activity of the benzamide **4a**, the 4-methylbenzamide **4b**, and the 4-chlorobenzamide **4d** may be in accordance with the existence of such an optimal lipophilicity, if this optimum for lipophilicity is rather broad.

In a series of amides and amidines derived from 2-amino-1,10-phenanthroline (**3**) the amides are 2–8 times more active than their more hydrophilic amidine analogues (Table III and IV). From the limited series of amidines of 2-amino-1,10-phenanthroline a structure–activity relationship cannot be deduced, since all amidines are equally active.

Although amidines are somewhat more hydrophilic than corresponding amides, amidines **5a–c** possess comparable lipophilicity to some of the most active amides, due to the contribution of the substituents in the benzamide moiety. So, lipophilicity alone cannot account for the difference in antimycoplasmal activity of amidines as compared to amides. For an explanation of this difference, the influence of electronic and steric features should also be considered.

The series of amides and amidines of this investigation differs from the corresponding series derived from 1-amino-3-(2-pyridyl)isoquinoline in that the order of potency is reversed, the amides being more active than the corresponding amidines. This difference may be explained in terms of lipophilicity. In the isoquinoline series the amides appeared to be too lipophilic for high antimycoplasmal activity, whereas the lipophilicity of the corresponding amidines appeared to be optimal with respect to antimycoplasmal activity.[5,6]

As the contribution of the pyridylisoquinoline skeleton to lipophilicity is larger than the contribution of the phenanthroline skeleton,[7] amides derived from 2-amino-1,10-phenanthroline are less lipophilic than the corresponding derivatives of 1-amino-3-(2-pyridyl)isoquinoline. Consequently amides in the 1,10-phenanthroline series are not too lipophilic to show almost optimal antimycoplasmal activity. As the amidines derived from 2-amino-1,10-phenanthroline are also less lipophilic than the corresponding derivatives of 1-amino-3-(2-pyridyl)isoquinoline, these amidines have become too hydrophilic for displaying optimal antimycoplasmal activity. In this way lipophilicity may account for the reversed order of potency of amides and amidines derived from 2-amino-1,10-phenanthroline as compared to the corresponding 1-amino-3-(2-pyridyl)-isoquinoline derivatives.

Due to the limited number of amidines a reliable quantitative structure–activity relationship can only be achieved for the amides **4a–g** and **6a–d**. Since compound **8** is the sole representative of a series of compounds with two amide moieties among a series of compounds containing only one amide moiety, this compound is omitted from the quantitative structure–activity relationship study. To establish a quantitative structure–activity relationship we tried to find a correlation between the antimycoplasmal activity and lipophilicity. Parameters chosen were –log MIC values for biological activity and hydrophobic fragmental values ($\sum f$) for lipophilicity, which were calculated according to Rekker.[24] As only two parts of the molecule are varied, viz. the benzamide moiety and the substituent at position 9 of the 1,10-phenanthroline skeleton, being either a hydrogen atom or a *n*-butyl chain, only their contribution to lipophilicity is considered in this quantitative structure–activity relationship study. So the $\sum f$ used in this regression analysis is the sum of the fragmental values of these two substituents: $\sum f = \sum f_{R1} + \sum f_{R2}$ (Table V). According to Pijper et al.,[7] $1 \times c_m$ has to be substracted from the hydrophobic fragmental values of alkyl substituents when present in the ortho position of 2,2′-bipyridyl and related compounds. By multiple regression analysis, the following equation is obtained

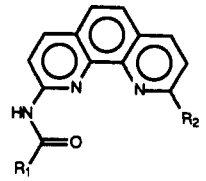
$$-\log \text{MIC} = 8.825\ (\pm 0.228) - 0.545\ (\pm 0.061)\sum f \quad (1)$$

$$n = 11 \qquad r = 0.948 \qquad s = 0.215 \qquad F = 80.612$$

So, within the lipophilicity range tested, we find a good linear correlation between antimycoplasmal activity and

(24) Rekker, R. F.; De Kort, H. M. *Eur. J. Med. Chem.* **1979**, *14*, 479.

Table V. Hydrophobic Fragmental Values[a]



compd	R_1	R_2	$\sum f_{R1,R2}$[c]	MIC_{calcd}[b]	MIC_{obsd}
4a	C_6H_5	H	2.022	0.019	0.023
4b	4-$CH_3(C_6H_4)$	H	2.541	0.036	0.023
4c	4-$OCH_3(C_6H_4)$	H	2.102	0.021	0.046
4d	4-$Cl(C_6H_4)$	H	2.764	0.049	0.023
4e	3,4-$Cl_2(C_6H_3)$	H	3.506	0.121	0.095
4f	$CH(C_2H_5)C_4H_9$	H	3.815	0.18	0.23
4g	C_9H_{19}	H	4.853	0.66	0.37
6a	C_6H_5	n-C_4H_9	3.809	0.18	0.20
6b	4-$CH_3(C_6H_4)$	n-C_4H_9	4.328	0.34	0.37
6c	4-$Cl(C_6H_4)$	n-C_4H_9	4.551	0.45	0.75
6d	3,4-$Cl_2(C_6H_3)$	n-C_4H_9	5.293	1.14	1.50

[a] See ref 24. [b] Calculated from eq 1. [c] $\sum f_{R1,R2} = f_{R1} + f_{R2}$ for compounds 4 and $\sum f_{R1,R2} = f_{R1} + f_{R2}-(1 \times c_m)$ for compounds 6.

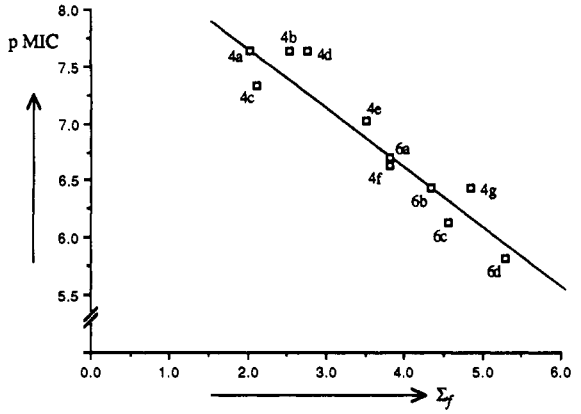


Figure 1. pMIC vs $\sum f$. For the identity of 4a–6d, see Tables II and III.

$\sum f$ of the substituents considered. Antimycoplasmal activity is increased when the contribution to lipophilicity of the amide residue and of the substituent on carbon-9 of the 1,10-phenanthroline is decreased (Figure 1). On the basis of the data available to us now, we cannot exclude the possibility that the above linear relationship obtained is in fact part of a relationship between antimycoplasmal activity and lipophilicity that is parabolic in nature, meaning that for these compounds, too, an optimal lipophilicity with regard to antimycoplasmal activity might exist. Obviously more hydrophilic amides need to be synthesized to establish the true nature of the activity–lipophilicity relationship in a larger lipophilicity range.

Conclusions

Although amides and amidines derived from 2-amino-1,10-phenanthroline are not active themselves, they are very potent antimycoplasmal agents in the presence of a small amount of copper. All derivatives of 2-amino-1,10-phenanthroline are more active against *M. gallisepticum* K514 than the parent compound. It was also shown that amides are more active than the corresponding amidines. In fact, the most active *N*-(1,10-phenanthrolin-2-yl)benzamides **4a,b,d** are 4 times more active than the antimycoplasmal therapeutic tylosin.

Antimycoplasmal activity of these compounds appeared to be dependent on their lipophilicity. For the amides, a good linear correlation was found between antimycoplasmal activity and the contribution to lipophilicity of the amide residue and of the substituent on carbon-9 of the 1,10-phenanthroline skeleton. From this relationship it was clear that antimycoplasmal activity increases with a decrease in the hydrophobic fragmental value of these substituents.

Experimental Section

Chemistry. Melting points were determined with use of a Mettler FP5/FP52 apparatus. NMR spectra were recorded on a Bruker WH-90 90 MHz spectrometer at 21 °C. Chemical shifts are expressed in ppm relative to tetramethylsilane. Infrared spectra were recorded on a Perkin-Elmer 580B spectrophotometer. Recording of mass spectra and peak matching were performed with a Varian CH 5 DI and a Finnigan MAT 90 mass spectrometer. All starting materials were commercially available and of the highest purity obtainable. Acyl chlorides were prepared from the corresponding carboxylic acids by standard methods[10,11] and distilled prior to use in the acylation reaction. 3,4-Dichlorobenzonitrile was synthesized from the corresponding aldehyde.[25] 2-Amino-1,10-phenanthroline and 2,9-diamino-1,10-phenanthroline were prepared according to literature procedures starting from 1,10-phenanthroline[12,13] (Schemes I and IV) and obtained in overall yields of 65% and 28%, respectively. Melting points and spectral data were in accordance with literature data. Water content of the compounds was not only determined by titration but also by a thermogravimetric method. Results obtained were in agreement with elemental analysis data. Analytical results for compounds indicated by the molecular formula were within ±0.4% of the theoretical values.

Synthesis. General Procedure for the Synthesis of Amides from 2-Amino-1,10-phenanthroline (4a–e, 6b–d). Method A. In a thoroughly dried three-necked flask, 0.02 mol of 2-amino-1,10-phenanthroline·HCl in 40 mL of anhydrous THF was stirred under nitrogen and cooled to −15 °C. Subsequently 25 mL of 1.6 M *n*-butyllithium in hexane was added dropwise, while maintaining the temperature at −15 °C. When the addition was complete, stirring was continued for 1 h. Then 0.02 mol of freshly distilled acyl chloride in 10 mL of anhydrous THF was added, and stirring was continued for another hour. The ice bath was removed, and when the mixture had reached room temperature it was hydrolyzed by the addition of a small amount of water. After evaporation of the organic solvent, the pH of the reaction mixture was adjusted to 8 with a dilute sodium bicarbonate solution and the water layer was extracted twice with chloroform. The combined chloroform layers were dried with anhydrous potassium carbonate and after filtration evaporated to dryness.

Improved General Procedure for the Synthesis of Amides from 2-Amino-1,10-phenanthroline (4a–c,f,g). Method B. In this procedure methyllithium was used as a base instead of *n*-butyllithium. A suspension of 0.01 mol of 2-amino-1,10-phenanthroline·HCl in 40 mL of anhydrous THF was stirred under a nitrogen atmosphere and cooled to 5 °C. Subsequently, 18.8 mL of 1.6 M methyllithium in diethyl ether was added dropwise, and stirring was continued for 1.5 h. Then the reaction mixture was cooled to −15 °C and 0.01 mol of freshly distilled acyl chloride in 10 mL of anhydrous THF was added dropwise. While the temperature was kept at −15 °C, stirring was continued for 1.5 h. The ice bath was removed, and when the mixture had reached room temperature, it was hydrolyzed with water. The organic solvents were removed by evaporation, and the remaining water layer was extracted with chloroform. The combined chloroform layers were washed with a diluted sodium bicarbonate solution, dried with anhydrous potassium carbonate, and after filtration, evaporated to dryness.

***N*-(1,10-Phenanthrolin-2-yl)benzamide (4a).** This compound was synthesized from 2-amino-1,10-phenanthroline and benzoyl chloride according to method A. The crude reaction mixture was crystallized twice from $CH_3OH/CH_3COOC_2H_5$. The crystals obtained first, appeared to be the diacylated, 9-*n*-butylated product. The filtrate was concentrated and the residue crystallized from $CH_3OH/CH_3COOC_2H_5$: yield 1.73 g (27%) of the monohydrate.

(25) Van Es, T. *J. Chem. Soc.* **1965**, 1564.

Prepared by Method B. The product obtained as the monohydrate was crystallized twice from CH_3OH: yield 1.71 g (54%) of white needles; mp 73.7–74.7 °C; NMR ($CDCl_3$) δ 7.44–7.60 (m, 3 H, φ H-3, φ H-4, φ H-5), 7.60 (dd, J = 8.1, 4.3 Hz, 1 H, H-8), 7.67 and 7.77 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 7.96–8.08 (m, 2 H, φ H-2, φ H-6), 8.22 (dd, J = 8.5, 1.8 Hz, 1 H, H-7), 8.30 (d, J = 9.0 Hz, 1 H, H-3), 8.82 (d, J = 9.0 Hz, 1 H, H-4), 9.14 (dd, J = 4.1, 1.8 Hz, 1 H, H-9), 9.45 (s br, 1 H, NH); IR[26] (KBr, cm^{-1}) 3380 (br) (H_2O), 3280 (sh) (NH), 3060, 3040 (CH(ar)), 1675 (s) (C=O), 1610, 1590, 1575, 1535, 1505 (s), 1480 (s) (C=C, C=N), 1440, 1420, 1390, 1340, 1325, 1310, 1270, 1255, 1190, 1140, 1130, 1085, 1030, 920, 900, 850, 840 (oop (out of plane) CH), 825, 795, 770, 740, 720, 700, 665, 630, 550, 510, 415, 290; MS m/e 299.11 ± 0.01 (M^+), 299.1059 ($C_{19}H_{13}N_3O$). Anal. ($C_{19}H_{13}N_3O \cdot H_2O$) C, H, N.

4-Methyl-*N*-(1,10-phenanthrolin-2-yl)benzamide (4b). This compound was synthesized from 2-amino-1,10-phenanthroline and 4-methylbenzoyl chloride according to method A. The crude reaction mixture, containing **4b** and its 9-*n*-butylated analogue **6b** was purified via column chromatography by use of silica gel 60 H with diethyl ether saturated with ammonia as eluent. Fractions with the same components were pooled, evaporated to dryness and crystallized from $CH_3OH/CH_3COOC_2H_5$; yield 0.64 g (10%) of 4-methyl-*N*-(1,10-phenanthrolin-2-yl)benzamide·$^1/_2H_2O$ and 0.64 g (10%) of 4-methyl-*N*-(1,10-phenanthrolin-2-yl)benzamide·H_2O; mp (hemihydrate) 109.4–110.0 °C, (monohydrate) 75.3–76.2 °C.

Prepared by Method B. The crude reaction mixture was crystallized from CH_3OH: yield 1.6 g (48%) of the monohydrate of **4b**; mp 75.0–76.1 °C; NMR ($CHCl_3$) δ 2.46 (s, 3 H, CH_3), 7.25 and 7.98 (AA'BB' system, J_{ab} = 8.6 Hz, 4 H), 7.64 (dd, J = 8.1, 4.3 Hz, 1 H, H-8), 7.69 and 7.82 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 8.26 (dd, J = 8.5, 1.8 Hz, 1 H, H-7), 8.32 (d, J = 9.0 Hz, 1 H, H-3), 8.86 (d, J = 9.0 Hz, 1 H, H-4), 9.18 (dd, J = 4.1, 1.8 Hz, 1 H, H-9), 9.42 (s br, 0.8 H, NH); IR (KBr, cm^{-1}) 3430 (br) (H_2O), 3300 (br), 3140 (NH), 3040, 3020 (CH(ar)), 2920 (CH_3), 1675 (C=O), 1610, 1590, 1575, 1540, 1505 (s), 1480 (s) (C=C, C=N), 1440, 1420, 1385 (s), 1325, 1310 (s), 1265, 1250, 1190, 1135, 1120, 1090, 1080, 1020, 920, 900, 845 (s) (oop CH), 825, 810, 790, 770, 745, 730, 720, 685, 645, 625, 610, 550, 510, 495, 480, 415, 260; MS m/e 313.12 ± 0.01 (M^+), 313.1215 ($C_{20}H_{15}N_3O$). Anal. (hemihydrate) ($C_{20}H_{15}N_3O \cdot {}^1/_2H_2O$) C, H, N.

4-Methoxy-*N*-(1,10-phenanthrolin-2-yl)benzamide (4c). This compound was synthesized from 2-amino-1,10-phenanthroline and 4-methoxybenzoyl chloride according to method A. The crude reaction mixture was crystallized from $CH_3OH/CH_3COOC_2H_5$. The precipitate consisted mainly of diacylated, 9-*n*-butylated amide. The filtrate was purified via column chromatography by use of silica gel 60 H with diethyl ether/chloroform/triethylamine (8:1:1) as eluent. The fractions with the same components were pooled and crystallized several times from CH_3OH or CH_3COCH_3; yield 1.05 g (15%) of the monohydrate of **4c**; mp 82.5–83.5 °C.

Prepared by Method B. The crude reaction mixture was crystallized from CH_3OH: yield 1.56 g (45%) of the monohydrate of **4c**; mp 82.3–83.4 °C; NMR ($CHCl_3$) δ 3.91 (s, 3H, CH_3), 7.02 and 8.02 (AA'BB' system, J_{ab} = 8.6 Hz, 4 H), 7.64 (dd, J = 8.1, 4.3 Hz, 1 H, H-8), 7.69 and 7.83 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 8.26 (dd, J = 8.5, 1.8 Hz, 1 H, H-7), 8.31 (d, J = 9.0 Hz, 1 H, H-3), 8.82 (d, J = 9.0 Hz, 1 H, H-4), 9.18 (dd, J = 4.1, 1.8 Hz, 1 H, H-9), 9.24 (s br, 0.9 H, NH); IR (KBr, cm^{-1}) 3580 (br) (H_2O), 3400 (br), 3330 (br) (NH), 3020 (CH(ar)), 2850 (w) (OCH_3), 1670 (C=O), 1605 (s), 1590, 1575, 1540, 1510, 1505 (s), 1485 (s) (C=C, C=N), 1440, 1420, 1385, 1325, 1310, 1270 (sh), 1250 (s), 1230 (sh), 1190, 1140, 1100, 1090, 1080, 1020, 920, 900, 850, 840 (oop CH), 800, 780, 760, 740, 720, 705, 680, 645, 615, 550, 445, 420, 305, 290; MS m/e 329.120 ± 0.01 (M^+), 329.1164 ($C_{20}H_{14}N_3O_2$). Anal. ($C_{20}H_{14}N_3O_2 \cdot H_2O$) C, H, N.

4-Chloro-*N*-(1,10-phenanthrolin-2-yl)benzamide (4d). This compound was synthesized from 2-amino-1,10-phenanthroline and 4-chlorobenzoyl chloride according to method A. The crude reaction mixture was crystallized from $CH_3OH/CH_3COOC_2H_5$. The compound which precipitated first appeared to be the 9-*n*-butylated amide **6c**. After filtration of these crystals the mother liquor was concentrated and the precipitate was recrystallized from $CH_3OH/CH_3COOC_2H_5$: yield 1.79 g (26%) of the monohydrate of **4d**; mp 96.1–97.1 °C; NMR ($CHCl_3$) δ 7.52 and 8.02 (AA'BB' system, J_{ab} = 8.6 Hz, 4 H), 7.66 (dd, J = 8.1, 4.3 Hz, 1 H, H-8), 7.74 and 7.85 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 8.30 (dd, J = 8.5, 1.8 Hz, 1 H, H-7), 8.36 (d, J = 9.0 Hz, 1 H, H-3), 8.81 (d, J = 9.0 Hz, 1 H, H-4), 9.18 (dd, J = 4.1, 1.8 Hz, 1 H, H-9); IR (KBr, cm^{-1}) 3370 (br) (H_2O), 3270 (sh) (NH), 3050, 3020 (CH(ar)), 1675 (C=O), 1610, 1595, 1575, 1540, 1505 (s), 1480 (s) (C=C, C=N), 1440, 1420, 1400, 1385, 1320, 1305, 1270, 1250, 1185, 1140, 1130, 1110, 1095, 1080, 1010, 920, 900, 850 (s) (oop CH), 825, 800, 775, 750, 740, 720, 630, 540, 405, 330; MS m/e 333.055 ± 0.01 (M^+), 333.0669 ($C_{19}H_{12}ClN_3O$, ^{35}Cl). Anal. ($C_{19}H_{12}ClN_3O \cdot H_2O$) C, H, N, Cl.

3,4-Dichloro-*N*-(1,10-phenanthrolin-2-yl)benzamide (4e). This compound was synthesized from 2-amino-1,10-phenanthroline and 3,4-dichlorobenzoyl chloride according to method A. The crude reaction mixture was crystallized from $CH_3OH/CH_3COOC_2H_5$. The precipitate consisted of two compounds, **4e** and **6d**, with **6d** as the major component. The filtrate was concentrated and the precipitate consisted for the most part of 4e and contained only a small amount of **6d**. Both crops were purified via column chromatography by use of silica gel 60 H with diethyl ether/chloroform/triethyl amine (8:1:1) as eluent: yield 0.88 g (11%) of monohydrate of 4e; mp 246.1–247.4 °C; NMR ($CHCl_3$) δ 7.62 (d, J = 8.1 Hz, 1 H, φ H-5), 7.65 (dd, J = 8.1, 4.3 Hz, 1 H, H-8), 7.74 and 7.84 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 7.91 (dd, J = 8.1, 1.8 Hz, 1 H, φ H-6), 8.18 (d, J = 1.8 Hz, 1 H, φ H-2), 8.30 (dd, J = 8.5, 1.8 Hz, 1 H, H-7), 8.36 (d, J = 9.0 Hz, 1 H, H-3), 8.75 (d, J = 9.0 Hz, 1 H, H-4), 9.18 (dd, J = 4.1, 1.8 Hz, 1 H, H-9); IR (KBr, cm^{-1}) 3370 (H_2O), 3290 (sh) (NH), 3060 (CH(ar)), 1680 (C=O), 1640, 1620, 1610, 1590, 1570, 1545, 1505 (s), 1485 (C=C, C=N), 1460, 1440, 1420, 1395, 1340, 1325, 1295, 1275, 1230, 1140, 1095, 1085, 1030, 910, 900, 850 (sh), 845 (oop CH), 830, 815, 775, 735, 705, 680, 650, 625, 575, 545, 415, 400, 330; MS m/e 367.030 ± 0.01 (M^+), 367.0279 ($C_{19}H_{11}Cl_2N_3O$, ^{35}Cl). Anal. ($C_{19}H_{11}Cl_2N_3O \cdot H_2O$) C, H, N, Cl.

2-Ethyl-*N*-(1,10-phenanthrolin-2-yl)hexanamide (4f). This compound was synthesized from 2-amino-1,10-phenanthroline and 2-ethylhexanoyl chloride according to method B. The crude product was purified via column chromatography by use of silica gel 60 H with diethyl ether saturated with ammonia as eluent. The fractions containing **4f** were pooled and after evaporation of the diethyl ether, the product was crystallized from petroleum ether (60–80 °C): yield 1.43 g (45%) of white needles; mp 193.5–194.3 °C; NMR ($CDCl_3$) δ 0.84–1.98 (m, 14 H, C_2H_5, C_4H_9), 1.98–2.38 (m, 1 H, CH), 7.63 (dd, J = 8.1, 4.3 Hz, 1 H, H-8), 7.66 and 7.78 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 8.24 (dd, J = 8.5, 1.8 Hz, 1 H, H-7), 8.25 (d, J = 9.0 Hz, 1 H, H-3), 8.65 (s br, 0.8 H, NH), 8.76 (d, J = 9.0 Hz, 1 H, H-4), 9.18 (dd, J = 4.1, 1.8 Hz, 1 H, H-9); IR (KBr, cm^{-1}) 3195, 3120, 3095 (NH), 3025, 3010 (CH(ar)), 2960, 2935, 2875, 2860 (CH(al)), 1685 (s) (C=O), 1605, 1590, 1570, 1530, 1505 (s), 1480 (s) (C=C, C=N), 1440, 1385, 1330, 1305 (s), 1175, 1140, 1100, 1080, 850 (oop CH), 830, 795, 770, 740, 720, 655, 625, 415; MS m/e 321.187 ± 0.008 (M^+), 321.1841 ($C_{20}H_{23}N_3O$). Anal. ($C_{20}H_{23}N_3O$) C, H, N.

***N*-(1,10-Phenanthrolin-2-yl)decanamide (4g).** This compound was synthesized from 2-amino-1,10-phenanthroline and decanoyl chloride according to method B. The product was obtained in the same way as compound **4f** and was isolated as the monohydrate: yield 1.32 g (38%); mp 72.2–73.3 °C; NMR ($CDCl_3$) δ 0.76–2.44 (m, 19 H, C_9H_{19}), 7.62 (dd, J = 8.1, 4.3 Hz, 1 H, H-8), 7.66 and 7.80 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 8.25 (dd, J = 8.5, 1.8 Hz, 1 H, H-7), 8.26 (d, J = 9.0 Hz, 1 H, H-3), 8.66 (d, J = 9.0 Hz, 1 H, H-4), 8.74 (s br, 0.7 H, NH), 9.16 (dd, J = 4.1, 1.8 Hz, 1 H, H-9); IR (KBr, cm^{-1}) 3380 (H_2O), 3215 (br) (NH), 3040 (CH(ar)), 2960, 2920, 2850 (CH(al)), 1700 (s) (C=O), 1645, 1610, 1570, 1540, 1505 (s), 1480 (s) (C=C, C=N), 1470, 1440, 1420, 1390, 1330, 1310, 1275, 1230, 1140, 1130, 1095, 1080, 850 (oop CH), 825, 815, 770, 740, 720, 655, 625; MS m/e 349.212 ± 0.04 (M^+), 349.2154 ($C_{22}H_{27}N_3O$). Anal. ($C_{22}H_{27}N_3O \cdot H_2O$) C, H, N; C: calcd, 7190, found, 71.33.

***N*-(9-*n*-Butyl-1,10-phenanthrolin-2-yl)benzamide (6a).** The following procedure can be used as a general procedure for the synthesis of 9-*n*-butylated amides from 2-amino-1,10-

(26) Grigg, M. C. E.; Hall, J. R. *Aust. J. Chem.* **1962**, *15*, 864.

phenanthroline. A suspension of 0.01 mol 2-amino-1,10-phenanthroline·HCl in 40 mL of anhydrous THF was stirred under a nitrogen atmosphere and cooled to 5 °C. Subsequently, 18.8 mL of 1.6 M *n*-butyllithium in hexane was added dropwise, and stirring was continued for 1.5 h. Then the reaction mixture was cooled to −15 °C and 0.01 mol of freshly distilled benzoyl chloride in 10 mL of anhydrous THF was added dropwise. While the temperature was kept at −15 °C, stirring was continued for 1.5 h. The ice bath was removed, and when the mixture had reached room temperature, it was hydrolyzed with water. The organic solvents were removed by evaporation, and the remaining water layer was extracted with chloroform. The combined chloroform layers were washed with a diluted sodium bicarbonate solution, dried with anhydrous potassium carbonate, and after filtration, evaporated to dryness. The crude reaction mixture was purified via column chromatography by use of silica gel 60 H with diethyl ether/chloroform (7:3) saturated with ammonia as eluent. Fractions containing **6a** were pooled and evaporated to dryness, the residue was dissolved in anhydrous diethyl ether and hydrogen chloride was bubbled through the solution. The precipitate, the hydrochloride of **6a**, was dried and crystallized from C_2H_5OH: yield 1.18 g (30%); mp 201.3–201.7 °C; NMR (salt) (DMSO-d_6) δ 0.99 (t, J = 7.2 Hz, 3 H, CH_3), 1.26–1.66 (m, 2 H, γ-CH_2), 1.78–2.11 (m, 2 H, β-CH_2), 3.26 (t, J = 7.2 Hz, 2 H, α-CH_2), 7.37–7.77 (m, 3 H, ϕ H-3, ϕ H-4, ϕ H-5), 7.98 (d, J = 8.1 Hz, 1 H, H-8), 8.16 (s, 2 H, H-5, H-6), 8.28–8.37 (m, 2 H, ϕ H-2, ϕ H-6), 8.59 (d, J = 9.0 Hz, 1 H, H-3), 8.76 (d, J = 8.5 Hz, 1 H, H-7), 8.96 (d, J = 9.0 Hz, 1 H, H-4); NMR (free base) ($CDCl_3$) δ 0.98 (t, J = 7.2 Hz, 3 H, CH_3), 1.28–2.06 (m, 4 H, β-CH_2, γ-CH_2), 3.16 (t, J = 7.2 Hz, 2 H, α-CH_2), 7.42–7.58 (m, 4 H, H-8, ϕ H-3, ϕ H-4, ϕ H-5), 7.62 and 7.73 (AB system, J_{ab} = 10.8 Hz, 2 H, H-5, H-6), 8.02–8.12 (m, 2 H, ϕ H-2, ϕ H-6), 8.14 (d, J = 8.5 Hz, 1 H, H-7), 8.38 (d, J = 9.0 Hz, 1 H, H-3), 8.76 (d, J = 9.0 Hz, 1 H, H-4), 9.68 (s br, 0.5 H, NH); IR (salt) (KBr, cm^{-1}) 3430 (br) (NH-amide), 3060, 3020 (CH(ar)), 2960, 2930, 2875, 2855 (CH(al)), 2730 ($^+$NH-ring), 1660 (C=O), 1640 (s), 1630 (s), 1605, 1580, 1540 (s), 1520, 1500, 1480 (C=C, C=N), 1470, 1450, 1430, 1400, 1365, 1320, 1275 (s), 1265 (s), 1245 (sh), 1230, 1220, 1200, 1185, 1160, 1095, 1075, 1030, 100, 970, 900, 875 (s), 850 (oop CH), 815, 800, 790, 780, 725, 715, 710 (s), 700 (sh), 685, 670, 655, 635, 570, 420, 400, 370, 320; MS m/e 355.167 ± 0.007 (M^+), 355.1685 ($C_{23}H_{21}N_3O$). Anal. ($C_{23}H_{21}N_3O$·HCl) C, H, N, Cl.

4-Methyl-*N*-(9-*n*-butyl-1,10-phenanthrolin-2-yl)benzamide (6b). This compound was obtained as a side product in the synthesis of **4b**. Following the chromatographic procedure described in the synthesis of **4b**, compound **6b** was crystallized from $CH_3OH/CH_3COOC_2H_5$: yield 0.19 g (3%). Compound **6b** was also synthesized and isolated in the same way as **6a** by using 4-methylbenzoyl chloride instead of benzoyl chloride: yield 1.31 g (34%) of the hydrochloride of **6b**; mp (salt) 207.0–208.0 °C; mp (free base) 165.7–166.6 °C; NMR (salt) ($CHCl_3$) δ 1.05 (t, J = 7.2 Hz, 3 H, CH_3), 1.35–1.75 (m, 2 H, γ-CH_2), 1.89–2.22 (m, 2 H, β-CH_2), 2.46 (s, 3 H, CH_3), 3.39 (t, J = 7.2 Hz, 2 H, α-CH_2), 7.41 and 8.51 (AA'BB' system, J_{ab} = 8.6 Hz, 4 H), 7.44 (d, J = 8.5 Hz, 1 H, H-8), 7.94 (s, 2 H, H-5, H-6), 8.41 (d, J = 8.5 Hz, 1 H, H-7), 8.62 (d, J = 9.0 Hz, 1 H, H-3), 9.43 (d, J = 9.0 Hz, 1 H, H-4), 13.78 (s br, 0.8 H, NH); NMR (free base) ($CHCl_3$) δ 1.00 (t, J = 7.2 Hz, 3 H, CH_3), 1.26–1.70 (m, 2 H, γ-CH_2), 1.74–2.08 (m, 2 H, β-CH_2), 2.46 (s, 3 H, CH_3), 3.18 (t, J = 7.2 Hz, 2 H, α-CH_2), 7.31 and 7.97 (AA'BB' system, J_{ab} = 8.6 Hz, 4 H), 7.52 (d, J = 8.1 Hz, 1 H, H-8), 7.66 and 7.74 (AB system, J_{ab} = 10.8 Hz, 2 H, H-5, H-6), 8.15 (d, J = 8.5 Hz, 1 H, H-7), 8.28 (d, J = 9.0 Hz, 1 H, H-3), 8.77 (d, J = 9.0 Hz, 1 H, H-4), 9.42 (s br, 0.8 H, NH); IR (free base) (KBr, cm^{-1}) 3300 (br) (H_2O), 3230 (sh) (NH), 3050 (CH(ar)), 2960, 2930, 2860 (CH(al)), 1675 (sh) (C=O), 1660 (s), 1610, 1590, 1570, 1565, 1525, 1510, 1490 (s) (C=C, C=N), 1465, 1425, 1365, 1325, 1305 (s), 1280, 1265, 1215, 1190, 1140, 1105, 1085, 1020, 905, 880, 850 (oop CH), 835 (sh), 720, 745, 640, 610, 590, 540, 425; MS m/e 369.181 ± 0.009 (M^+), 369.1841 ($C_{24}H_{23}N_3O$). Anal. (free base) ($C_{24}H_{23}N_3O·H_2O$) C, H, N. Anal. (salt) ($C_{24}H_{23}N_3O$·HCl) C, H, N.

4-Chloro-*N*-(9-*n*-butyl-1,10-phenanthrolin-2-yl)benzamide (6c). This compound was isolated as a side product in the synthesis of **4d**, by crystallization of the crude reaction mixture from $CH_3OH/CH_3COOC_2H_5$: yield 1.81 g (23%) of the monohydrate of **6c**; mp 186.1–186.2 °C; NMR ($CHCl_3$) δ 0.98 (t, J = 7.2 Hz, 3 H, CH_3), 1.23–1.62 (m, 2 H, γ-CH_2), 1.64–1.97 (m, 2 H, β-CH_2), 3.04 (t, J = 7.2 Hz, 2 H, α-CH_2), 7.30 and 7.79 (AA'BB' system, J_{ab} = 8.6 Hz, 4 H), 7.44 (d, J = 8.1 Hz, 1 H, H-8), 7.66 (d, J = 8.5 Hz, 1 H, H-7), 7.72 (s, 2 H, H-5, H-6), 8.08 (d, J = 8.6 Hz, 1 H, H-3), 8.19 (d, J = 8.6 Hz, 1 H, H-4); IR (KBr, cm^{-1}) 3370 (w), 3320 (w), 3070 (NH), 3040 (CH(ar)), 2950, 2930, 2860 (CH(al)), 1700 (s) (C=O), 1665 (s), 1620, 1610, 1585 (s), 1570 (sh), 1550, 1505, 1495 (s), 1485 (sh) (C=C, C=N), 1465, 1425, 1400, 1360, 1325 (sh), 1035 (s), 1285 (s), 1260 (s), 1245 (s), 1335 (s), 1230 (sh), 1210 (sh), 1170, 1145, 1125, 1105, 1090 (s), 1055, 1010, 970, 950, 905, 880, 865, 855 (s), 845 (s) (oop CH), 835 (sh), 795, 780, 755, 745, 725, 700, 690, 675, 635, 610, 590, 560, 540, 480, 450, 355, 275; no MS data are available due to the occurrence of a thermal reaction during heating the sample. Techniques used: CI, using isobutane as the reagent gas, and EI, with direct introduction under electron-impact conditions. Anal. ($C_{23}H_{20}ClN_3O·H_2O$) C, H, N, Cl.

3,4-Dichloro-*N*-[9-*n*-butyl-1,10-phenanthrolin-2-yl]benzamide (6d). This compound was obtained as a side product in the synthesis of **4e**. Compound **6d** was isolated by the same chromatographic procedure as described in the synthesis of **4e**: yield 0.88 g (11%) of the monohydrate of **6d**; mp 149.8–150.5 °C; NMR ($CHCl_3$) δ 0.096 (t, J = 7.2 Hz, 3 H, CH_3), 1.26–1.66 (m, 2 H, γ-CH_2), 1.72–2.05 (m, 2 H, β-CH_2), 3.13 (t, J = 7.2 Hz, 2 H, α-CH_2), 7.45 (d, J = 8.1 Hz, 1 H, H-8), 7.50 (d, J = 8.1 Hz, 1 H, ϕ H-5), 7.69 (s, 2 H, H-5, H-6), 7.83 (dd, J = 8.1, 1.8 Hz, 1 H, ϕ H-6), 8.11 (d, J = 8.5, 1.8 Hz, 1 H, H-7), 8.12 (d, J = 1.8 Hz, 1 H, ϕ H-2), 8.26 (d, J = 9.0 Hz, 1 H, H-3), 8.56 (d, J = 9.0 Hz, 1 H, H-4); IR (KBr, cm^{-1}) 3300 (br), 3280, 3070 (NH), 3050 (CH(ar)), 2960, 2930, 2860 (CH(al)), 1685 (C=O), 1635, 1610 (sh), 1595 (s), 1575, 1560, 1535, 1500 (sh), 1490 (s) (C=C, C=N), 1470, 1425, 1390, 1370, 1330, 1305, 1290 (sh), 1265, 1230, 1175, 1140, 110, 1030, 990, 915, 905, 885, 850 (oop CH), 820, 790, 770, 740, 710, 675, 640, 590, 460, 590, 460 (w), 420 (w); no MS data are available due to the occurrence of a thermal reaction during heating the sample. Techniques used: CI, using isobutane as the reagent gas, and EI, with direct introduction under electron-impact conditions. Anal. ($C_{23}H_{19}Cl_2N_3O·H_2O$) C, H, N, Cl.

General Procedure for the Synthesis of Amidines from 2-Amino-1,10-phenanthroline and Electron-Deficient Nitriles (5a–c). A solution of 0.02 mol 2-amino-1,10-phenanthroline·HCl in 50 mL of anhydrous THF was stirred under a nitrogen atmosphere and cooled to −10 °C. Subsequently 25 mL of 1.6 M *n*-butyllithium in hexane was added dropwise and stirring was continued for 10 min. Then 0.02 mol nitrile in a minimal amount of THF was added and, while keeping the reaction mixture at −10 °C, stirring was continued for 10 min. When the mixture had reached room temperature, it was refluxed for 8 h. After cooling, the mixture was hydrolyzed by the addition of a small amount of water. The organic phase was evaporated, and the remaining water layer was extracted with chloroform, after adjusting the pH to 8 with a dilute bicarbonate solution. The combined chloroform layers were dried with anhydrous potassium bicarbonate and after filtration, evaporated to dryness.

***N*-(1,10-Phenanthrolin-2-yl)benzamidine (5a).** This compound was synthesized from 2-amino-1,10-phenanthroline and benzonitrile. The crude reaction mixture was purified via column chromatography by use of silica gel 60 H with diethyl ether saturated with ammonia as eluent. The fractions containing **5a** were pooled, and after evaporation of the solvent, the product was crystallized from CH_3OH/H_2O: yield 0.23 g (4%) of the monohydrate; mp 168.3–170.1 °C; NMR ($CDCl_3$) δ 7.35–7.62 (m, 5 H, H-3, H-8, ϕ H-3, ϕ H-4, ϕ H-5), 7.60 and 7.71 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 7.88–8.05 (m, 2 H, ϕ H-2, ϕ H-6), 8.13 (d, J = 8.46 Hz, 1 H, H-4), 8.18 (dd, J = 8.46, 1.8 Hz, 1 H, H-7), 9.06 (dd, J = 5.4, 1.8 Hz, 1 H, H-9); IR (KBr, cm^{-1}) 3370 (broad) (NH), 3050 (broad) (CH), 1620, 1585, 1580, 1570, 1560, 1540, 1520, 1500 (C=C, C=N), 1490, 1450, 1420, 1340, 1292, 1270, 1223, 1190, 1140, 1105, 1080, 1035, 1005, 925, 850, 830, 783, 738, 703, 690, 658, 624; MS m/e 297.125 ± 0.01 (M − H^+), 297.1141 ($C_{19}H_{13}N_4$). Anal. ($C_{19}H_{16}N_4O$) C, H, N.

4-Chloro-*N*-(1,10-phenanthrolin-2-yl)benzamidine (5b). This compound was synthesized from 2-amino-1,10-phenanthroline and 4-chlorobenzonitrile. A little petroleum ether (60–80 °C) was poured on the crude reaction mixture to obtain yellow solid material from the brown oil. The precipitate was isolated by filtration and crystallized from $CHCl_3$/petroleum ether

(60–80 °C): yield 0.16 g (2.4%) of the monohydrate; mp 84.0–86.4 °C; NMR ($CDCl_3$) δ 7.40 and 7.90 (AA'BB' system, J_{ab} = 8.1 Hz, 4 H, φ H), 7.44–7.63 (m, 2 H, H-3, H-8), 7.61 and 7.73 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 8.12 (d, J = 8.1 Hz, 1 H, H-4), 8.20 (dd, J = 8.1, 1.8 Hz, 1 H, H-7), 9.02 (dd, J = 4.14, 1.8 Hz, 1 H, H-9); IR (KBr, cm^{-1}) 3400 (NH), 3060 (broad) (CH), 1620, 1585, 1530, 1505 (C=C, C=N), 1490, 1450, 1420, 1395, 1345, 1305, 1290, 1268, 1228, 1210, 1135, 1108, 1093, 1075, 1015, 927, 850, 840, 830, 787, 718, 690, 650, 630; MS m/e 331.080 ± 0.01 ($M - H^+$), 331.0751 ($C_{19}H_{12}N_4Cl$, ^{35}Cl). Anal. ($C_{19}H_{15}N_4ClO$) C, H, N, Cl.

3,4-Dichloro-*N*-(1,10-phenanthrolin-2-yl)benzamidine (5c). This compound was synthesized from 2-amino-1,10-phenanthroline and 3,4-dichlorobenzonitrile. A little petroleum ether (60–80 °C) was poured on the crude reaction mixture to obtain yellow solid material from the brown oil. After filtration, the precipitate was purified via column chromatography by use of silica gel 60 H with diethyl ether saturated with ammonia as eluent. The fractions containing **5c** were pooled and after evaporation of the solvent, the product was crystallized from $CHCl_3$/petroleum ether (60–80 °C): yield 1.68 g (23%); mp 185.8–187.5 °C; NMR ($CDCl_3$) δ 7.45–7.91 (m, 4 H, H-3, H-8, φ H-5, φ H-6), 7.68 and 7.78 (AB system, J_{ab} = 9.0 Hz, 2 H, H-5, H-6), 8.13 (d, J = 1.8 Hz, 1 H, φ H-2), 8.19 (d, J = 8.1 Hz, 1 H, H-4), 8.23 (dd, J = 8.1, 1.8 Hz, 1 H, H-7), 9.09 (dd, J = 3.96, 1.8 Hz, 1 H, H-9); IR (KBr, cm^{-1}) 3350 (broad) (NH), 3160, 3060 (broad) (CH), 1635, 1620, 1588, 1525, 1505 (C=C, C=N), 1490, 1455, 1420, 1385, 1345, 1290, 1270, 1230, 1215, 1145, 1135, 1105, 1080, 1030, 980, 930, 905, 850, 840, 825, 780, 740, 660, 630; MS m/e 336.051 ± 0.01 (M^+), 366.0439 ($C_{19}H_{12}N_4Cl_2$, ^{35}Cl). Anal. ($C_{19}H_{12}N_4Cl_2$) C, H, N, Cl.

2,9-Bis(benzoylamino)-1,10-phenanthroline (8). This compound was synthesized according to Yamada et al.[21] To obtain a better yield the reaction time was extended from 0.15 h to 2 h and the work-up procedure was slightly changed. To a suspension of 7.9 mmol 2,9-diamino-1,10-phenanthroline in 100 mL pyridine was added dropwise 90 mmol benzoyl chloride, while keeping the reaction mixture at 0 °C. Stirring was continued for 2 h. Subsequently, 600 mL diethyl ether was added to the reaction mixture and a white/yellow precipitate was obtained. This precipitate was filtered off and after washing with cold methanol dissolved in chloroform. The chloroform was washed three times with an aqueous solution of sodium bicarbonate, dried with anhydrous potassium carbonate, and after filtration, evaporated to dryness. The residue was crystallized twice from CH_3OH: yield 1.83 g (53%) of pale yellow needles; mp 242.8–244.1 °C; NMR (DMSO-d_6) δ 7.50–7.75 (m, 6 H, φ H-3, φ H-4, φ H-5), 7.96 (s, 2 H, H-5, H-6), 8.15–8.24 (m, 4 H, φ H-2, φ H-6), 8.51 and 8.53 (AA'BB' system, J_{ab} = 9.0 Hz, 4 H, H-3, H-4, H-7, H-8), 11.10 (s br, 2 H, NH).

Biological Activity. Nutrient Medium. All experiments with *Mycoplasma gallisepticum* were done in a growth medium which was a modification of the medium used by Adler[27] to cultivate this microorganism. This modified Adler medium contained 14.8 g of bacteriological peptone, 5.0 g of yeast extract powder, 8.16 g of D-glucose H_2O, 3.7 g of NaCl, 1.79 g of $Na_2HPO_4 \cdot 2H_2O$, 21 mg of phenol red (pH range 6.8–8.4), 150 mL of heat-inactivated (56 °C for 30 min) horse serum, and 10^6 IU benzylpenicillin G per liter of final medium. The medium components were dissolved in twice distilled water and the pH of the solution was adjusted to 8.0 with a concentrated sodium hydroxide solution. Before the horse serum and the benzylpenicillin were added, sterilization was performed by heating at 110 °C for 30 min.

Materials. Bacteriological peptone and yeast extract powder were purchased from OXOID Ltd, Basingstoke, Hampshire, England. Sterile donor horse serum was obtained from Flow Laboratories. Benzylpenicillin G was a generous gift from Gist-brocades N.V., Delft, The Netherlands. All chemicals used were of the highest quality obtainable.

Apparatus. Optical density of growing cultures were determined at 660 nm by using a Zeiss PMQ3 spectrophotometer. pH measurements were performed with a combined glass electrode. Test tubes were incubated in a waterbath at 37 °C.

Test Organism. *Mycoplasma gallisepticum* K514, kindly supplied by the research management of Gist-brocades N.V., Delft, The Netherlands, was used as the test organism. *Mycoplasma gallisepticum* strains can be stored at −20 °C for several months.[28] After thawing at room temperature the culture was transferred to a bottle with fresh Adler medium in such a way that the original culture was diluted 10 times. The culture was incubated overnight at 37 °C. When the pH of the culture had dropped to 6.8 and the density (determined as A_{660nm}) had reached a value of 0.22, the culture was used for inoculation purposes. The remaining part was stored at −20 °C.

Determination of Antimycoplasmal Activity. The antimycoplasmal activity of all compounds was determined in the presence or the absence of copper and expressed as the minimal inhibitory concentration (MIC). In the former case the final concentration of $CuSO_4$ in the test tube was 40 μM. Tylosin and compound 1 were included as controls in every test. All compounds were dissolved in dimethyl sulfoxide whereas tylosin was dissolved in water. It was established that DMSO in the final concentration in the Adler medium (1.25%) has no effect on mycoplasmal growth. Serial 2-fold dilutions (in duplicate) of test compounds were made in Adler medium. Each tube, containing 3 mL of medium, was inoculated with 1 mL of a fresh culture of *Mycoplasma gallisepticum* K514, and these mixtures were incubated at 37 °C for 24 h. Mycoplasmal growth was indicated by a change in color of the indicator present in the medium. The minimal inhibitory concentration was determined as the lowest concentration that did not cause a change in color.

Data Processing. Statistical correlations were performed by using a commercial multiple linear regression program (Statworks, Cricket Software, Inc., Philadelphia, PA). The figures in parentheses are the standard errors of regression coefficients. The parameters included in each equation are significant on a 1% level. For a given equation, n is the number of compounds, r is the multiple correlation coefficient, s is the standard error of estimate, and F represents the value of the F-test.

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