1.79 (dd, 2 H, J = 4, 15 Hz), 2.02–2.21 (br m, 6 H), 5.41 (m, 1 H, $CHCO_2C_6H_5$), 7.45 (m, 3 H, Ar), 8.05 (dd, 2 H, J = 1.5, 8 Hz, Ar). Anal. ($C_{22}H_{30}O_5$) C, H.

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Synthesis and Copper-Dependent Antimycoplasmal Activity of 1-Amino-3-(2-pyridyl)isoquinoline Derivatives. 1. Amides

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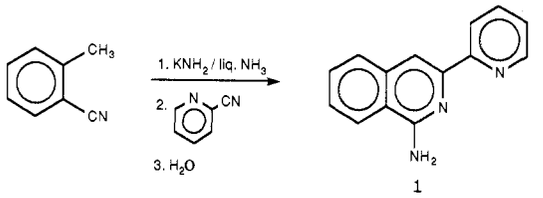
In order to investigate the antimycoplasmal activity of compounds structurally related to 2,2′-bipyridyl, a series of both aliphatic and aromatic amides derived from 1-amino-3-(2-pyridyl)isoquinoline were synthesized. The most active compounds appeared to be as active as Tylosin, an antimycoplasmal therapeutic that is used in veterinary practice, in the presence of a small nontoxic amount of copper. Furthermore, it was found that antimycoplasmal activity depends on the hydrophobic fragmental value of the amide residue. A quantitative structure–activity relationship established the optimal hydrophobic fragmental value of the amide residue to be 0.30.

Mycoplasmas are known to be causative agents of many infectious diseases not only in plants and animals but in humans as well.[1-3] Broad-spectrum antibiotics from the small polyene type (34–37 carbon atoms) and the tetracycline type are inhibitory to mycoplasmas in vitro as well as in vivo. Unfortunately, these broad-spectrum antibiotics induce resistance rapidly.[1] Tylosin, a macrolide antibiotic is often used in therapy of mycoplasmal infections in poultry.[1] Furthermore, Pijper et al.[4] have shown that in the presence of a small and nontoxic amount of copper certain 2,2′-bipyridyl derivatives are highly active against mycoplasmas. Due to the low activity of these compounds in the absence of copper, it was concluded that growth inhibition is caused by their copper complexes rather than by these compounds themselves. In a study on the mechanism of action of these copper complexes, Smit et al.[5] and Gaisser et al.[6,7] discovered that copper itself is the ultimate toxic agent, whereas ligands facilitate copper transport across the membrane through the formation of lipophilic complexes. The toxicity of copper is most probably based on the inhibition of enzymes involved in the energy providing metabolism like NADH-oxidase and lactate dehydrogenase.[7]

In a recent study from our laboratory, Linschoten et al.[8] reported on the antimycoplasmal activity of a series of amides and amidines derived from 4-amino-2-(2-pyridyl)quinazoline. It was found that the most active compound, *N*-[2-(2-pyridyl)quinazolin-4-yl]-2-pyridinecarboxamidine, was on a molar basis 40 times as active as Tylosin, which was used as a reference compound. On the basis of these results, we decided to continue our search for new antimycoplasmal therapeutics with the synthesis of amides and amidines derived from 1-amino-3-(2-pyridyl)isoquinoline (1), which has certain advantages over the structurally related 4-amino-2-(2-pyridyl)quinazoline from a synthetic point of view.

Scheme I



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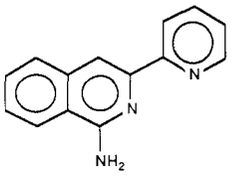

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In the present paper, we report on the synthesis and antimycoplasmal activity of both aliphatic and aromatic amides derived from 1-amino-3-(2-pyridyl)isoquinoline (1). In order to establish the structure with optimal activity, we used the efficient method proposed by Topliss.[9] This method is an application of the Hansch approach and is based on a proper selection of an initial small group of compounds. Analysis of the potency order provides a rational basis for the selection of more potent analogues.

Chemistry. A general method for the synthesis of amides consists of the acylation of amines by agents like acyl chlorides.[10] While these acyl chlorides can be obtained from the corresponding acids very easily,[11,12] our major

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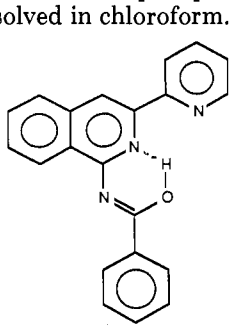
concern was the synthesis of the required amine. Although the synthesis of 1-amino-3-(2-pyridyl)isoquinoline (1) has been described, the yields are rather poor (25–28%).[13,14] Therefore, we decided to improve the synthesis of this compound by starting from the method used by Van der Goot in which 1-amino-3-(2-pyridyl)isoquinoline (1) was synthesized from 2-methylbenzonitrile and pyridine-2-carbonitrile (Scheme I).

Potassium amide is prepared in situ by adding potassium at −33 °C to liquid ammonia, to which some crystals of ferric nitrate had been added. When the formation of potassium amide is complete, the mixture is cooled to −78 °C, and an equimolar quantity of 2-methylbenzonitrile in anhydrous diethyl ether is added, while the temperature is kept at −78 °C. Subsequently, an equimolar quantity of pyridine-2-carbonitrile in anhydrous THF is added at that temperature. Then, stirring is continued at room temperature to evaporate the ammonia. According to this procedure the yield of 1-amino-3-(2-pyridyl)isoquinoline (1) was increased to 60%.

For the preparation of amides **2a–f** and **3a–m** from acyl chlorides and 1-amino-3-(2-pyridyl)isoquinoline (1) (Scheme II), we decided to abstract one of the amine protons first to increase the nucleophilic power of the amino nitrogen atom. The reaction of this anion with acyl chlorides is then carried out at −10 °C. Application of higher reaction temperatures resulted in increasing amounts of diacylated products. By use of this method, these amides could be obtained, however, in moderate yields (10–60%). This is probably due to delocalization of the negative charge of the anion of 1-amino-3-(2-pyridyl)isoquinoline (1) (Scheme II).

N-[3-(2-Pyridyl)isoquinolin-1-yl]acetamide (**2a**) was not only prepared as has been described above but also by refluxing 40 mmol of 1-amino-3-(2-pyridyl)isoquinoline (1) for 1 h in 100 mL of acetic acid/acetic anhydride (1:1 v/v). However, this procedure resulted in poor yields of **2a**, and high amounts of diacylated product were obtained. The structure of the synthesized compounds was established by IR, NMR, and mass spectrometry. It was remarkable that, in contrast with aliphatic amides, aromatic amides except for **3k** are for the most part present in the iminol form (**4**) when dissolved in chloroform. This can be con-



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cluded from the NMR spectra, which show an absorption at low field (~16.3 ppm), which can be assigned to a proton participating in an intramolecular hydrogen bond. This iminol form is favored by conjugation of the phenyl group with the isoquinoline moiety. This may also account for the relatively low predominance of the iminol form in the case of these aliphatic amides. IR spectrophotometry (KBr platelets) revealed that the aromatic amides except

Scheme II

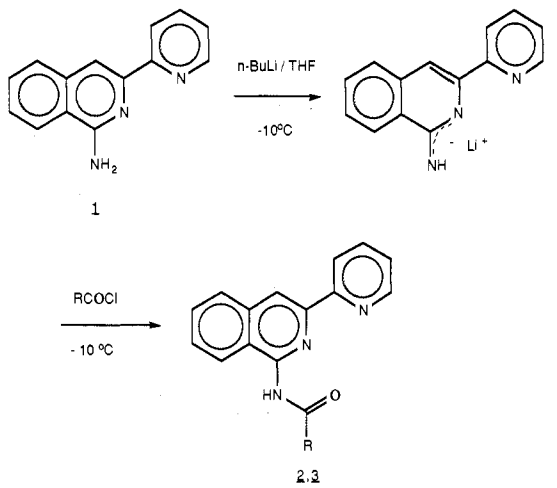

Table I. MIC Values[a] (μM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C

compd	without extra copper	extra copper added[b]
$CuSO_4 \cdot 5H_2O$	400	
Tylosin	0.1	0.1
1	452	0.45

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

for **3a**, **3b**, and **3i–k** are obtained after recrystallization in the iminol form. However, when IR spectrophotometry was performed with solutions of **3a**, **3b**, **3i**, and **3j** in chloroform, the carbonyl absorption disappeared (**3a**, **3b**) or was markedly reduced (**3i**, **3j**) (results not shown), indicating once again that these amides are present in the iminol form when dissolved in chloroform. The same features have been described for amides derived from 1-aminoisoquinoline.[15–18]

As was expected from NMR data, the carbonyl absorption did not disappear in the case of compound **3k**. This compound is present in the amide form in the solid state as well as in solution. This phenomenon is caused by the presence of two ortho substituents in the benzamide moiety. These substituents force the amide moiety and the phenyl group into perpendicular planes, disrupting the overlap of the π orbitals of the carbonyl moiety and the phenyl group. Consequently, conjugation of aromatic units in the iminol form is no longer apparent. Although π overlap of the iminol moiety with the isoquinoline nucleus and hydrogen bonding still contribute to the stabilization of the iminol tautomer, the loss of conjugation of these two aromatic units may be the reason that the iminol form is not favored over the amide form in the case of compound **3k**.

Biological Activity. All of these compounds have been tested with and without the addition of copper to the medium. Without the addition of copper, the copper concentration of Adler medium[19] was less than 3 μM.[20] To determine the antimycoplasmal activity of these com-

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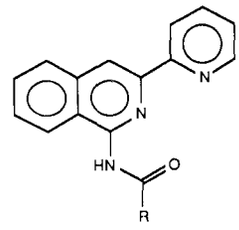
Table II. MIC Values[a] (μM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C



2a-f

compd	R	without extra copper	extra copper added[b]
2a	CH_3	>25	1.90
2b	C_2H_5	>25	0.45
2c	$CH(CH_3)_2$	>25	0.22
2d	$C(CH_3)_3$	>25	0.21
2e	$CH(C_2H_5)C_4H_9$	>170	0.36
2f	C_9H_{19}	>170	0.34

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

pounds in the presence of copper, 40 μM $CuSO_4$ was added to the Adler growth medium. This copper concentration, however, is far below the toxic level. The minimal inhibitory concentration (MIC) for copper was established to be 400 μM (Table I). MIC values for aliphatic and aromatic *N*-[3-(2-pyridyl)isoquinolin-1-yl]amides (**2a–f** and **3a–m**) are presented in Tables II and III, respectively. Without copper, none of these compounds were active in the concentration range tested. It was not possible to test these compounds in higher concentrations than indicated because they appeared to be poorly soluble in the growth medium. In the presence of copper, however, all of these amides had about the same antimycoplasmal activity as the parent compound 1. As a matter of fact, under these conditions all compounds except for **2a**, **2b**, **3e**, and **3k** appeared to be somewhat more active than compound **1**.

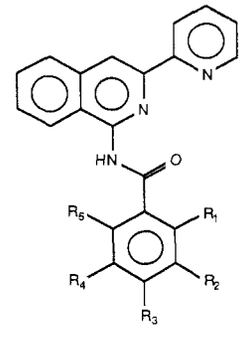
Comparison of compounds **2a** and **3a** with the corresponding *N*-[2-(2-pyridyl)quinazolin-4-yl]amides[8] revealed that they are 20 and 5 times less active respectively. The activity sequence for the aliphatic amides (**2a–f**) is as follows: $CH_3 < C_2H_5 < CH(CH_3)_2$, $C(CH_3)_3 > CH(C_2H_5)C_4H_9$, C_9H_{19}. Antimycoplasmal activity increases with chain size from the acetamide derivative (**2a**) up to the *tert*-butylamide (**2d**). A further elongation of the aliphatic chain results in a decrease of antimycoplasmal activity.

In the case of aromatic amides (**3a–m**) activity increases in the following order: 2,6-$(CH_3)_2$ < 3,4-Cl_2 < other $(CH_3)_2$, 3-Cl, 4-Cl < 4-CH_3, 3-OCH_3, 4-OCH_3 < 3-CH_3, H. In the presence of copper, compounds **3a** and **3f** are as active as the reference compound Tylosin.

Structure–Activity Relationships. Antimycoplasmal activity is copper dependent for a variety of compounds containing a 2,2′-bipyridyl moiety. Without the addition of a small amount of copper, all compounds of the present series except for **3a** are at least more than 200 times less active against *Mycoplasma gallisepticum* than the reference compound Tylosin. However, upon addition of a little copper, all compounds are fairly active against *M. gallisepticum*, the most active compounds being as active as Tylosin. Due to this remarkable copper effect, it is very likely that these compounds act via their copper complexes as do other 2,2′-bipyridyl analogues.[4]

Comparison of the antimycoplasmal activity of these compounds with the activity of the parent compound 1-amino-3-(2-pyridyl)isoquinoline (1) shows that most of the amides are more active. This is probably due to the presence of a third coordination site for the copper atom. It is known that 2,2′-bipyridyl compounds are able to chelate copper very well and that both nitrogen atoms play an important role in the formation of such copper complexes. However, when apart from the bipyridyl nitrogen atoms, the oxygen atom of the amide moiety is involved in the complex formation, the positive charge is more shielded, which might favor the transportation of these complexes across the lipophilic cell membrane.

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



3a-m

compd	R_1	R_2	R_3	R_4	R_5	without extra copper	extra copper added[b]
3a	H	H	H	H	H	12.31	0.10
3b	H	H	CH_3	H	H	>160	0.19
3c	H	H	OCH_3	H	H	>160	0.18
3d	H	H	Cl	H	H	>160	0.35
3e	H	Cl	Cl	H	H	>160	0.64
3f	H	CH_3	H	H	H	>23	0.09
3g	H	OCH_3	H	H	H	>23	0.18
3h	H	Cl	H	H	H	>23	0.35
3i	CH_3	H	CH_3	H	H	>100	0.39
3j	CH_3	H	H	CH_3	H	>100	0.39
3k	CH_3	H	H	H	CH_3	>25	0.78
3l	H	CH_3	CH_3	H	H	>100	0.39
3m	H	CH_3	H	CH_3	H	>100	0.39

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

In a qualitative consideration of a possible structure–activity relationship, we only took into account the influence of the part of the molecule that is varied within these series, viz. the amide residue, regarding the influence of the 3-(2-pyridyl)isoquinoline part to be constant. When we consider the activity sequence of these aliphatic amides **2a–d**, an increase of activity is paralleled by an increase in lipophilicity of the aliphatic chain. However, a further increase in lipophilicity (**2e,f**) results in a decrease in antimycoplasmal activity.

The influence of the lipophilicity of the amide residue is even more pronounced in the case of these aromatic amides. Here an increase in lipophilicity of the aromatic nucleus parallels a decrease in antimycoplasmal activity. Furthermore, it is obvious from these data that the position of the substituent in the aromatic nucleus has no influence on the biological activity. This also supports the hypothesis that the lipophilicity of the acyl residue is the predominant parameter that determines the antimycoplasmal activity and that other substituent contributions such as electronic features only play a minor role. Also, according to Topliss,[9] this activity sequence suggests a dependency on lipophilicity.

When we combine results of both aliphatic and aromatic amides, this qualitative approach to a structure–activity relationship suggests the existence of an optimal lipophilicity for antimycoplasmal activity of these amides. The existence of an optimal lipophilicity for antimycoplasmal activity is supported by results of a quantitative approach to a structure–activity relationship. As for the qualitative approach, we only took into account the influence of the alkyl or aryl amide moiety. Hydrophobic fragmental values ($\sum f$) were calculated for the NHCOR residue of these compounds according to Rekker.[21] Since lipophilicity is increased through conjugation in the iminol tautomer for aromatic amides only, $1 \times c_m$ (magic constant = 0.289) is added to the hydrophobic fragmental values of these aromatic amides (Table IV). By multiple regression analysis, the following equation is obtained:

$$-\log \text{MIC} = 6.720\ (\pm 0.075) + 0.159\ (\pm 0.047)\sum f - 0.178\ (\pm 0.036)(\sum f)^2 \quad (1)$$

$$F = 13.092,\ r = 0.797,\ s = 0.193,\ n = 18$$

When compounds **2f**, **3a**, and **3f** are omitted for legitimate statistical reasons (residual > 2 times the standard deviation), a much better equation is obtained:

$$-\log \text{MIC} = 6.754\ (\pm 0.039) + 0.135\ (\pm 0.020)\sum f - 0.225\ (\pm 0.020)(\sum f)^2 \quad (2)$$

$$F = 69.229,\ r = 0.959,\ s = 0.080,\ n = 15$$

So, for 15 of the original series of 18 compounds, a very good correlation is found between antimycoplasmal activity and lipophilicity.

It should be noted that compound **3k** is left out of this regression analysis deliberately. As explained above, this dimethyl-substituted benzamide, in contrast to all other dimethyl-substituted benzamides, is present in the amide form, and because of this, it is less lipophilic. So, according to the equation obtained from multiple regression analysis, one would expect this compound to be somewhat more active than the other dimethyl-substituted benzamides. However, compound **3k** is less active. Due to this anomalous behavior and to the different structure of compound **3k**, we apparently have to consider this compound as belonging to a different series of compounds. The dependency of the biological parameter on hydrophobic fragmental values is parabolic in nature, indicating that an optimal lipophilicity for antimycoplasmal activity exists (Figure 1). From this equation, the optimal lipophilicity for the amide residue is determined as $\sum f = 0.30$.

Table IV. Hydrophobic Fragmental Values of NHCOR Fragments[a]

compd	R	$\sum f$	$\text{MIC}_{\text{calcd}}$[b]	MIC_{obsd}
2a	CH_3	−1.745	1.47	1.90
2b	C_2H_5	−1.226	0.56	0.45
2c	i-C_3H_7	−0.707	0.28	0.22
2d	t-C_4H_9	−0.188	0.19	0.21
2e	C_7H_{15}	1.369	0.30	0.36
2f	C_9H_{19}	2.407	1.68	0.34
3a	C_6H_5	0.550	0.17	0.10
3g	3-$OCH_3(C_6H_4)$	0.630	0.18	0.18
3c	4-$OCH_3(C_6H_4)$	0.630	0.18	0.18
3f	3-$CH_3(C_6H_4)$	1.069	0.23	0.09
3b	4-$CH_3(C_6H_4)$	1.069	0.23	0.19
3h	3-$Cl(C_6H_4)$	1.292	0.28	0.35
3d	4-$Cl(C_6H_4)$	1.292	0.28	0.35
3e	3,4-$(Cl)_2(C_6H_3)$	2.034	0.80	0.64
3i	2,4-$(CH_3)_2(C_6H_3)$	1.588	0.40	0.39
3j	2,5-$(CH_3)_2(C_6H_3)$	1.588	0.40	0.39
3l	3,4-$(CH_3)_2(C_6H_3)$	1.588	0.40	0.39
3m	3,5-$(CH_3)_2(C_6H_3)$	1.588	0.40	0.39

[a] See ref 21. [b] Calculated from eq 2.

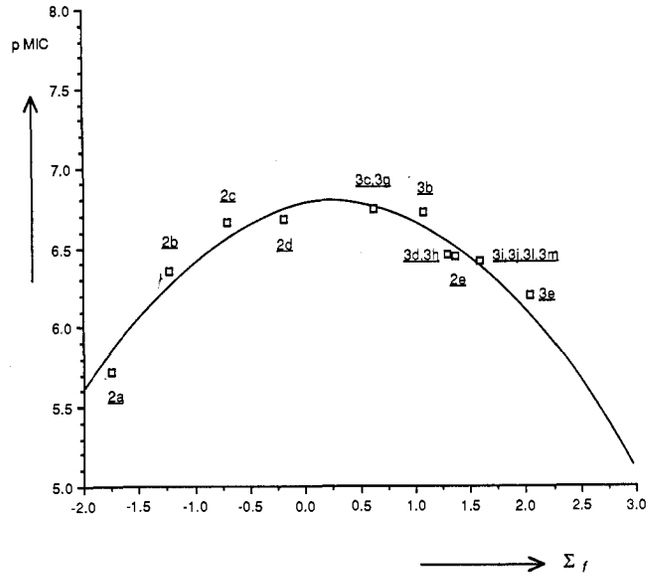


Figure 1. pMIC vs $\sum f$. For the meaning of **2a–3l**, see Tables II and III.

Conclusions

It can be concluded from this study that although amides **2a–f** and **3a–m** derived from 1-amino-3-(2-pyridyl)-isoquinoline are not active themselves, they are very potent antimycoplasmal agents in the presence of a small amount of copper. Therefore, it is very likely that copper complexes of these compounds are involved in the growth inhibiting process.

Acylation of 1-amino-3-(2-pyridyl)isoquinoline (**1**) resulted in the formation of the most potent derivatives having a fivefold increase in antimycoplasmal activity. The potency of the most active compounds, e.g. **3a**, **3f**, is comparable to the therapeutically useful antimycoplasmal drug Tylosin.

Furthermore, it is established that the antimycoplasmal activity is apparently dependent on the hydrophobic

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fragmental value of the amide residue. A quantitative approach to a structure–activity relationship revealed a good correlation between antimycoplasmal activity and lipophilicity parameters as $\sum f$ and $(\sum f)^2$. The optimal lipophilicity of the amide residue of *N*-[3-(2-pyridyl)isoquinolin-1-yl]amides regarding antimycoplasmal activity was calculated as $\sum f = 0.30$.

Experimental Section

Chemistry. All starting materials were commercially available and of the highest purity obtainable. Acyl chlorides were prepared from the corresponding carboxylic acids by standard methods[11,12] and distilled prior to use in the acylation reaction. Melting points were determined by using a Mettler FP5/FP52 apparatus. NMR spectra were recorded on a Bruker WH-90 90-MHz spectrophotometer at 21 °C. Chemical shifts are expressed in ppm relative to tetramethylsilane. Infrared spectra were recorded on a JASCO IRA II spectrophotometer. Recording and peak matching of mass spectra were performed with a Varian CH 5 DI mass spectrometer, electron impact 70 eV. Analytical results for compounds indicated by the molecular formula were within ±0.4% of the theoretical values.

Synthesis. 1-Amino-3-(2-pyridyl)isoquinoline (1). In a thoroughly dried three-necked flask equipped with a mechanical stirrer, 0.5 mol of potassium amide is freshly prepared in 500 mL of ammonia. The mixture is then cooled to −78 °C. Subsequently, 0.5 mol of 2-methylbenzonitrile in 200 mL of anhydrous diethyl ether is added slowly while the temperature is kept at −78 °C. When the addition is complete, a solution of 0.5 mol of 2-cyanopyridine in 250 mL of anhydrous THF is run into the flask within 20 min. The cooling device is then removed, and stirring is continued overnight to evaporate the ammonia. When necessary, a small amount of anhydrous THF is added, and the mixture is refluxed for 2 h. The mixture is cooled to room temperature and hydrolyzed by the addition of a small amount of water. After evaporation of the organic phase, diethyl ether is poured on the remaining mixture. Most of the 1-amino-3-(2-pyridyl)isoquinoline precipitated and was collected by filtration. A minor part could be isolated from the filtrate by extraction with diethyl ether. The product was crystallized from methanol: yield 66.3 g (60%) of white platelets; mp 152.0–153.0 °C; NMR ($CDCl_3$)[24] δ 5.32 (br s, 2 H, NH_2), 7.25 (ddd, $J_{3'-5'}$ = 1.2 Hz, $J_{5'-6'}$ = 4.8 Hz, $J_{4'-5'}$ = 7.4 Hz, 1 H, H-5′), 7.39–7.87 (m, 5 H, H-5, H-6, H-7, H-8, H-4′), 8.16 (d, J_{4-8} = 0.5 Hz, 1 H, H-4), 8.38 (ddd, $J_{3'-6'}$ = 0.9 Hz, $J_{3'-5'}$ = 1.4 Hz, $J_{3'-4'}$ = 7.8 Hz, 1 H, H-3′), 8.71 (ddd, $J_{3'-6'}$ = 0.9 Hz, $J_{4'-6'}$ = 1.75 Hz, $J_{5'-6'}$ = 4.8 Hz, 1 H, H-6′); IR (KBr, cm^{-1}) 3300 and 3190 (NH_2), 1635 (s), 1620 (s), 1580 (s), 1565 (s), 1495 (s) (C=C, C=N), 1475 (s), 1420, 990, 785 (s), 740 (s); MS, *m/e* 221.0964 (M^+), 221.0953 ($C_{14}H_{11}N_3$).

General Procedure for the Synthesis of Amides from 1-Amino-3-(2-pyridyl)isoquinoline (2a–f and 3a–m). A solution of 0.02 mol of 1-amino-3-(2-pyridyl)isoquinoline (1) in 40 mL of anhydrous THF was stirred under a nitrogen atmosphere and cooled to −10 °C. Subsequently, 12.5 mL of 1.6 M *n*-butyllithium in hexane was added dropwise, and stirring was continued for 10 min. Then, 0.02 mol of freshly distilled acyl chloride in 5 mL of anhydrous THF was added, and while the reaction mixture was kept at −10 °C, stirring was continued for 1 h. The ice bath was removed, and when the mixture had reached room temperature, it was hydrolyzed with water. The reaction mixture was extracted with chloroform. The combined chloroform layers were washed with a dilute sodium bicarbonate solution, dried with anhydrous potassium carbonate, and, after filtration, evaporated to dryness.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]acetamide (2a).** The crude product was crystallized from $CHCl_3$. The solid material was filtered and washed with a little diethyl ether to remove traces of 1-amino-3-(2-pyridyl)isoquinoline (1). After this purification step, the residue was crystallized from $CHCl_3$, yielding 2.76 g (53%) of very small, white needles, mp 186.0–187.4 °C: NMR ($CDCl_3$) δ 2.58 (s, 3 H, CH_3), 7.33 (ddd, J = 7.6, 4.8, 1.4 Hz, 1 H, H-5′), 7.51–8.07 (m, 5 H), 8.24 (br s, 0.4 H, NH), 8.38 (d, J = 7.2 Hz, 1 H, H-3′), 8.62 (s, 1 H, H-4), 8.73 (d, J = 4.5 Hz, 1 H, H-6′); IR (KBr, cm^{-1}) 3200 and 3100 (NH), 2960 (CH), 1680 (C=O), 1625, 1570 (C=C, C=N), 1500 (NH), 1470 (CH), 1425, 1375 (s), 1330 (s), 785, 750, 730, 670; MS, *m/e* 263.1057 (M^+), 263.1058 ($C_{16}H_{13}N_3O$). Anal. ($C_{16}N_{13}N_3O$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]propionamide (2b).** The crude product was crystallized from $CHCl_3$. The precipitate was recrystallized from $CHCl_3$ to remove trace amounts of 1-amino-3-(2-pyridyl)isoquinoline (1): yield 2.72 g (49%) of small white needles; mp 194.5–196.0 °C; NMR ($CDCl_3$) δ 1.35 (t, J = 7.2 Hz, 3 H, CH_3), 2.90 (q, J = 7.2 Hz, 2 H, CH_2), 7.32 (ddd, J = 7.4, 4.5, 1.0 Hz, 1 H, H-5′), 7.52–8.05 (m, 5 H); 8.25 (br s, 0.4 H, NH), 8.38 (d, J = 7.6 Hz, 1 H, H-3′), 8.65 (s, 1 H, H-4′), 8.74 (d, J = 4.5 Hz, 1 H, H-6′); IR (KBr, cm^{-1}) 3180 and 3080 (NH), 2960 (CH), 1680 (C=O), 1620 (s), 1580 (s), 1570 (s) (C=C, C=N), 1500 (s) (NH), 1470 (CH), 1420 (s), 1390, 1330 (s), 1290, 1270, 780, 750, 730; MS, *m/e* 277.1230 (M^+), 277.1215 ($C_{17}H_{15}N_3O$). Anal. ($C_{17}H_{15}N_3O$), C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-2-methylpropionamide (2c).** This product was obtained as small white needles in the same way as **2b**: yield 2.74 g (47%); mp 194.2–196.3 °C; NMR ($CDCl_3$) δ 1.38 (d, J = 7.2 Hz, 6 H, CH_3), 3.02 (m, 1 H, CH), 7.31 (ddd, J = 7.2, 4.5, 1.1 Hz, 1 H, H-5′), 7.49–8.0 (m, 5 H), 8.11 (br s, 0.5 H, NH), 8.36 (d, 7.6 Hz, 1 H, H-3′), 8.63 (s, 1 H, H-4), 8.72 (d, J = 4.5 Hz, 1 H, H-6′); IR (KBr, cm^{-1}) 3250 (NH), 2960 (CH), 1665 (C=O), 1625, 1580 (s), 1570 (s), 1510, 1490 (C=C, C=N), 1425, 1400 (s), 1340 (s), 1300, 1210, 1090, 790, 780, 740; MS, *m/e* 291.1380 (M^+), 291.1371 ($C_{18}H_{17}N_3O$). Anal. ($C_{18}H_{17}N_3O$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-2,2-dimethylpropionamide (2d).** The crude reaction mixture was dissolved in chloroform and subsequently extracted several times with a buffer solution, pH 5.0. The chloroform layer was dried with anhydrous potassium carbonate and, after filtration, evaporated to dryness. The residue was dissolved in dry diethyl ether. Diethyl ether saturated with hydrochloric acid was added dropwise to this solution. Initially, a small amount of the hydrochloride salt of 1-amino-3-(2-pyridyl)isoquinoline precipitated. The mixture was filtrated, and the filtrate was further acidified as described above. Eventually, the hydrochloride salt of **2d** was isolated by filtration of the acidified mixture and crystallized from ethanol: yield 2.12 g (31%); mp 156.6–158.3 °C, mp free base 56.0–58.3 °C;[25] NMR (DMSO-d_6) δ 1.40 (s, 9 H, CH_3), 7.65–8.39 (m, 6.5 H), 8.61 (d, J = 7.8 HZ, 1 H, H-3′), 8.85 (dd, J = 4.5 Hz, 1 H, H-6′), 8.93 (s, 1 H, H-8), 10.32 (br s, 0.5 H, NH); IR (KBr, cm^{-1}) (free base) 3280 (br), 3050 (NH), 2960 (CH), 1665 (C=O), 1620, 1580, 1535 (C=C, C=N), 1475, 1425, 1390, 1340, 1140, 790, 780, 740; MS, *m/e* 305.1520 (M^+), 305.1528 ($C_{19}H_{19}N_3O$). Anal. ($C_{19}H_{19}N_3O$) H, N; C: calcd, 74.73; found, 73.86.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-2-ethylhexanamide (2e).** The crude reaction product is crystallized several times from methanol, yielding 4.08 g (59%) of white needles: mp 177.3–178.8 °C; NMR ($CDCl_3$) δ 0.79–1.95 (m, 14 H, C_2H_5, C_4H_9), 2.43–2.83 (m, 1 H, CH), 7.32 (dd, J = 7.6, 4.7 Hz, 1 H, H-5′), 7.53–8.21 (m, 5.5 H), 8.42 (d, J = 7.6 Hz, 1 H, H-3′), 8.66 (s, 1 H, H-4′), 8.76 (d, J = 4.5 Hz, 1 H, H-6′); IR (KBr, cm^{-1}) 3240 (NH), 2960, 2920 (CH), 1665 (C=O), 1625, 1580, 1560, 1520 (C=C, C=N), 1425, 1380, 1340, 1215, 890, 790 (s), 780, 750, 740, 670, 620; MS, *m/e* 347.1988 (M^+), 347.1997 ($C_{22}H_{25}N_3O$). Anal. ($C_{22}H_{25}N_3O$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]decanamide (2f).** This product was obtained in the same way as **2e**: yield 3.75 g (50%); mp 122.4–124.5 °C; NMR ($CDCl_3$) δ 0.80–1.98 (m, 17 H, C_7H_{17}), 2.83 (t, J = 7.2 Hz, 2 H, CH_2), 7.33 (ddd, J = 7.6, 4.5, 1.1 Hz, 1 H, H-5′), 7.55–8.14 (m, 5.5 H), 8.34 (d, J = 7.2 Hz, 1 H, H-3′), 8.59 (s, 1 H, H-4), 8.77 (d, J = 4.5 Hz, 1 H, H-6′); IR (KBr, cm^{-1}) 3240 (NH), 2920, 2840 (CH), 1665 (C=O), 1625, 1580, 1520 (C=C, C=N), 1190, 890, 790, 740, 670; MS, *m/e* 375.2321 (M^+), 375.2310 ($C_{24}H_{29}N_3O$). Anal. ($C_{24}H_{29}N_3O$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]benzamide (3a).** This product was obtained by crystallization of the crude product from $CH_3OH/CH_3COOC_2H_5$: yield 2.80 g (43%); mp 189.9–191.0 °C; NMR ($CDCl_3$) δ 7.26–8.12 (m, 10 H), 8.55 (m, 2 H), 8.87 (d, J =

(24) Kook, A. M.; Smith, S. L.; Brown, E. V. *Org. Magn. Reson.* **1984**, *22*, 730.

(25) The melting point of the hydrochloride could not be determined accurately due to gradual formation of fine needles during the determination.

4.2 Hz, 1 H, H-6'), 9.01 (d, J = 7.5 Hz, 1 H, H-3'), 16.30 (br s, 0.6 H, OH); IR (KBr, cm^{-1}) 3240 (NH), 3040 (CH), 1660 (C=O), 1620, 1575, 1520 (C=C, C=N), 1470, 1420, 1390, 1335, 1300, 1145, 900, 885, 790, 780, 740, 720; MS, m/e 325.1212 (M^+), 325.1215 ($C_{21}H_{15}N_3O$). Anal. ($C_{21}H_{15}N_3O$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-4-methylbenzamide (3b). The crude product was crystallized from methanol, and the resulting precipitate was purified via column chromatography using silica gel 60 H with diethyl ether as eluent: yield 3.1 g (46%); mp 192.5–194.0 °C; NMR ($CDCl_3$) δ 2.43 (s, 3 H, CH_3), 7.28 and 8.42 (AA'BB' system, J_{ab} = 8.1 Hz, 4 H), 7.24–7.42 (m, 1 H, H-5'), 7.54–8.03 (m, 6.4 H), 8.86 (d, J = 4.5 Hz, 1 H, H-6'), 9.05 (d, J = 7.6 Hz, 1 H, H-3'), 16.33 (br s, 0.6 H, OH); IR (KBr, cm^{-1}) 3240 (NH), 3040 (CH), 1655 (C=O), 1625, 1610, 1575 (s), 1525 (C=C, C=N), 1490, 1470, 1400, 1340, 1300, 1145, 790, 745; MS, m/e 339.1375 (M^+), 339.1372 ($C_{22}H_{17}N_3O$). Anal. ($C_{22}H_{17}N_3O$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-4-methoxybenzamide (3c). The crude product was crystallized several times from $CH_3OH/CH_3COOC_2H_5$, yielding 3.69 g (52%) of the amide: mp 161.4–162.9 °C; NMR ($CDCl_3$) δ 3.91 (s, 3 H, OCH_3), 6.97 and 8.49 (AA'BB' system, J_{ab} = 8.4 Hz, 4 H), 7.49–8.07 (m, 7.2 H), 8.86 (d, J = 4.5 Hz, 1 H, H-6'), 9.01 (d, J = 7.6 Hz, 1 H, H-3'), 16.26 (br s, 0.6 H, OH); IR (KBr, cm^{-1}) 3400 (OH), 3040 (CH), 1620, 1570 (s), 1550 (s), 1520 (s) (C=C, C=N), 1465 (s), 1410, 1390, 1370, 1320 (s), 1245 (s), 1140 (s), 1100, 850, 775 (s), 755, 725, 690; MS, m/e 355.1320 (M^+), 355.1320 ($C_{22}H_{17}N_3O_2$). Anal. ($C_{22}H_{17}N_3O_2$), C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-4-chlorobenzamide (3d). The crude reaction mixture was crystallized several times from $CH_3OH/CH_3COOC_2H_5$, yielding 3.8 g (53%) of the amide: mp 166.2–167.4 °C; NMR ($CDCl_3$) δ 7.42 and 8.45 (AA'BB' system, J_{ab} = 8.3 Hz, 4 H), 7.26–8.08 (m, 8 H), 8.87 (d, J = 4.5 Hz, 1 H, H-6'), 9.01 (d, J = 7.6 Hz, 1 H, H-3'), 16.23 (br s, 0.6 H, OH); IR (KBr, cm^{-1}) 3400 (OH), 3040 (CH), 1610, 1575, 1525 (C=C, C=N), 1465, 1390, 1325, 1240, 1140, 1080, 1010, 880, 850, 780, 760, 740, 690; MS, m/e 359.0807 (M^+), 359.0825 ($C_{21}H_{14}ClN_3O$, ^{35}Cl). Anal. ($C_{21}H_{14}ClN_3O$) C, H, N; Cl: calcd, 9.85; found, 9.43.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-3,4-dichlorobenzamide (3e). The crude product was crystallized from acetone, yielding 2.36 g (30%) of very small, light-yellow needles: mp 207.4–208.7 °C; NMR ($CDCl_3$) δ 7.37 (ddd, J = 7.2, 4.7, 1.3 Hz, 1 H, H-5'), 7.54 (d, J = 8.1 Hz, 1 H, Phe H-5), 7.61–8.09 (m, 7 H), 8.33 (dd, J = 8.1, 1.8 Hz, 1 H, Phe H-6), 8.61 (d, J = 1.8 Hz, 1 H, Phe H-2), 8.88 (d, J = 4.7 Hz, 1 H, H-6'), 8.99 (d, J = 7.2 Hz, 1 H, H-3'), 16.23 (br s, 0.7 H, OH); IR (KBr, cm^{-1}) 3040 (CH), 1630, 1580, 1550, 1525 (C=C, C=N), 1470, 1440, 1375, 1325, 1140, 900, 840, 780 (s), 760, 740, 730, 670; MS, m/e 393.0456 (M^+), 393.0435 ($C_{21}H_{13}Cl_2N_3O$, ^{35}Cl). Anal. ($C_{21}H_{13}Cl_2N_3O$) C, H, N, Cl.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-3-methylbenzamide (3f). The crude product was first crystallized from $CH_3OH/CH_3COOC_2H_5$. The precipitate was purified via column chromatography using silica gel 60 H with diethyl ether as eluent. The fractions containing **3f** were pooled, and after evaporation of the solvent, the product was crystallized from $CH_3OH/CH_3COOC_2H_5$: yield 2.78 g (41%); mp 161.2–162.4 °C; NMR ($CDCl_3$) δ 2.49 (s, 3 H, CH_3), 7.27–8.13 (m, 9.6 H), 8.34 (m, 2 H), 8.87 (d, J = 4.5 Hz, 1 H, H-6'), 9.05 (d, J = 7.2 Hz, 1 H, H-3'), 16.32 (br s, 0.6 H, OH); IR (KBr, cm^{-1}) 3400 (OH), 3040 (CH), 1625, 1575 (s), 1550 (s), 1520 (C=C, C=N), 1470, 1440, 1390, 1315 (s), 1240, 1140, 780, 740, 730; MS, m/e 339.1345 (M^+), 339.1372 ($C_{22}H_{17}N_3O$). Anal. ($C_{22}H_{17}N_3O$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-3-methoxybenzamide (3g). The crude product is crystallized several times from $CH_3OH/CH_3COOC_2H_5$, yielding 2.56 g (36%) of the amide: mp 159.4–160.8 °C; NMR ($CDCl_3$) δ 3.93 (s, 3 H, OCH_3), 7.03–8.21 (m, 11.4 H), 8.85 (d, J = 4.5 Hz, H-6'), 9.01 (d, J = 7.5 Hz, 1 H, H-3'), 16.28 (br s, 0.6 H, OH); IR (KBr, cm^{-1}) 3400 (OH), 3040 (CH), 1625, 1575 (s), 1530 (s), (C=C, C=N), 1465, 1450, 1390, 1320 (s), 1270, 1240, 1140, 1040, 830, 775, 755, 745, 730, 670; MS, m/e 355.1290 (M^+), 355.1320 ($C_{22}H_{17}N_3O_2$). Anal. ($C_{22}H_{17}N_3O_2$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-3-chlorobenzamide (3h). The crude product was crystallized twice from $CH_3OH/CH_3COOC_2H_5$, yielding 2.75 g (38%) of the amide: mp 201.1–201.9 °C; NMR ($CDCl_3$) δ 7.26–8.01 (m, 9.3 H), 8.39 (dt, J = 7.2, 2.1 Hz, 1 H, Phe H-6), 8.53 (s, 1 H, Phe H-2), 8.86 (d, J = 4.5 Hz, 1 H, H-6'), 8.99 (d, J = 7.2 Hz, 1 H, H-3'), 16.23 (br s, 0.7 H, OH); IR (KBr, cm^{-1}) 3400 (OH), 3040 (CH), 1625, 1570 (s), 1545, 1520 (C=C, C=N), 1465, 1390, 1320, 1245, 1140, 1065, 1045, 890, 810, 780, 750, 730, 665; MS, m/e 359.0798 (M^+), 359.0825 ($C_{21}H_{14}N_3ClO$, ^{35}Cl). Anal. ($C_{21}H_{14}N_3ClO$) C, H, N, Cl.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-2,4-dimethylbenzamide (3i). After crystallization of the crude product from $CH_3OH/CH_3COOC_2H_5$, the compound was recrystallized from acetone: yield 0.78 g (11%); mp 172.6–173.5 °C; NMR ($CDCl_3$) δ 2.40 (s, 3 H, 4-CH_3), 2.56 (s, 1.7 H, 2-CH_3), 2.80 (s, 1.3 H, 2-CH_3), 7.10 (br s, 2 H, Phe H-3, Phe H-5), 7.20–7.45 (m, 1 H, H-5'), 7.53–8.50 (m, 7.7 H), 8.60–9.04 (m, 2 H, H-3', H-6'), 16.18 (br s, 0.3 H, OH); IR (KBr, cm^{-1}) 3400 (br) (OH), 3240 (s) (OH), 3000 (CH), 1660 (s) (C=O), 1635, 1610, 1580, 1510 (C=C, C=N), 1475, 1425, 1400, 1330, 1300, 1255, 1230, 1170, 1140, 885, 835, 790, 770, 740, 690, 670; MS, m/e 353.1531 (M^+), 353.1528 ($C_{23}H_{19}N_3O$). Anal. ($C_{23}H_{19}N_3O$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-2,5-dimethylbenzamide (3j). This product was obtained in the same way as compound **3i**: yield 0.92 g (13%); mp 186.1–187.4 °C; NMR ($CDCl_3$) δ 2.38 (s, 3 H, 5-CH_3), 2.52 (s, 1.8 H, 2-CH_3), 2.76 (s, 1.2 H, 2-CH_3), 7.20 (br s, 2 H, Phe H-3, Phe H-4), 7.26–7.45 (m, 1 H, H-5'), 7.59–8.35 (m, 7.7 H), 8.68–9.02 (m, 2 H, H-3', H-6'), 16.20 (br s, 0.3 H, OH); IR (KBr, cm^{-1}) 3400 (br) (OH), 3260 (s) (OH), 3000 (CH), 1660 (s) (C=O), 1625 (w), 1580, 1560, 1510, (C=C, C=N), 1485, 1425, 1400, 1340, 1300, 1280, 1200, 990, 940, 920, 900, 860, 840, 810, 790, 740, 675; MS, m/e 353.1531 (M^+), 353.1528 ($C_{23}H_{19}N_3O$). Anal. ($C_{23}H_{19}N_3O$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-2,6-dimethylbenzamide (3k). The crude product was crystallized from CH_3OH. The precipitate appeared to be the disubstituted product. This procedure was repeated, and after evaporation of the CH_3OH from the filtrate, compound **3k** was obtained after crystallization from $CH_3OH/CH_3COOC_2H_5$: yield 0.64 g (9%); mp 231.4–232.4 °C, NMR ($CDCl_3$) δ 2.43 (s, 6 H, CH_3), 7.06–7.35 (m, 4 H, H-5', Phe H-3, Phe H-4, Phe H-5), 7.60–8.25 (m, 7 H), 8.59–8.92 (m, 2 H); IR (KBr, cm^{-1}) 3170 (NH), 3040 (CH), 2920 (CH_3), 1640 (s) (C=O), 1565, 1500 (C=C, C=N), 1465, 1420, 1370, 1340, 1320, 1280, 1270, 1245, 1130, 1070, 990, 950, 880, 810, 755, 730, 710, 670; MS, m/e 353.1534 (M^+), 353.1528 ($C_{23}H_{19}N_3O$). Anal. ($C_{23}H_{19}N_3O$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-3,4-dimethylbenzamide (3l). This product was obtained in the same way as compound **3i**: yield 2.54 g (36%); mp 177.8–178.6 °C; NMR ($CDCl_3$) δ 2.35 (s, 3 H, 3-CH_3), 2.37 (s, 3 H, 4-CH_3), 7.16–7.40 (m, 2 H, H-5', Phe H-5), 7.50–8.06 (m, 6 H), 8.20–8.33 (d, 2 H, Phe H-6, Phe H-2), 8.61 (br s, 0.5 H, NH), 8.86 (d, J = 4.5 Hz, 1 H, H-6'), 9.03 (d, J = 7.0 Hz, 1 H, H-3'), 16.24 (br s, 0.5 H, OH); IR (KBr, cm^{-1}) 3400 (br) (OH), 3050 (CH), 1630 (w), 1575, 1525 (C=C, C=N), 1470, 1440, 1400, 1370, 1315, 1150, 1100, 900, 840, 785, 765, 735; MS, m/e 353.1534 (M^+), 353.1528 ($C_{23}H_{19}N_3O$). Anal. ($C_{23}H_{19}N_3O$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-3,5-dimethylbenzamide (3m). This product was obtained in the same way as compound **3i**: yield 2.90 g (41%); mp 188.6–189.4 °C; NMR ($CDCl_3$) δ 2.40 (s, 6 H, CH_3), 7.18 (br s, 1 H, Phe H-4), 7.24–7.40 (m, 1 H, H-5'), 7.53–8.36 (m, 5 H), 8.20 (br s, 2 H, Phe H-2, Phe H-6), 8.60 (br s, 0.3 H, NH), 8.82 (d, J = 4.2 Hz, 1 H, H-6'), 8.98 (d, J = 7.5 Hz, 1 H, H-3'), 16.22 (br s, 0.7 H, OH); IR (KBr, cm^{-1}) 3400 (br) (OH), 3040 (CH), 1630 (w), 1575, 1545, 1520 (C=C, C=N), 1465, 1450, 1440, 1390, 1370, 1320, 1270, 1240, 1140, 1115, 1080, 870, 850, 820, 785, 775, 760, 740, 730, 670; MS, m/e 353.1520 (M^+), 353.1528 ($C_{23}H_{19}N_3O$). Anal. ($C_{23}H_{19}N_3O$) C, H, N.

Biological Activity. Nutrient Medium. All experiments with *M. gallisepticum* were done in a growth medium that was a modification of the medium used by Adler[22] 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/liter 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 adding the horse serum and the benzylpenicillin, sterilization was per-

formed by heating the mixture at 110 °C for 30 min.

Materials. Bacteriological peptone and yeast extract powder were purchased from OXOID Limited, 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 obtainable quality.

Apparatus. Optical density of growing cultures were determined at 660 nm with a Zeiss PMQ3 spectrophotometer. pH measurements were performed with a saturated calomel electrode. Test tubes were incubated in a water bath at 37 °C.

Test Organism. *M. gallisepticum* K514, kindly supplied by the research management of Gist-brocades N.V., was used as the test organism. *M. gallisepticum* strains can be stored at −20 °C for several months.[23] After being thawed 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 in the test tube was 40 μM $CuSO_4$. 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 twofold 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 *M. 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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Registry No. 1, 37989-04-1; **2a**, 112575-42-5; **2b**, 112575-43-6; **2c**, 112575-44-7; **2d**, 112575-45-8; **2e**, 112575-46-9; **2f**, 112575-47-0; **3a**, 112575-48-1; **3b**, 112575-49-2; **3c**, 112575-50-5; **3d**, 112575-51-6; **3e**, 112575-52-7; **3f**, 112575-53-8; **3g**, 112575-54-9; **3h**, 112575-55-0; **3i**, 112575-56-1; **3j**, 112575-57-2; **3k**, 112575-58-3; **3l**, 112575-59-4; **3m**, 112575-60-7; H_3CCOCl, 75-36-5; H_5C_2COCl, 79-03-8; $(CH_3)_2CHCOCl$, 79-30-1; $(CH_3)_3CCOCl$, 3282-30-2; $C_4H_9CH(C_2H_5)COCl$, 760-67-8; $C_9H_{19}COCl$, 112-13-0; C_6H_5COCl, 98-88-4; 4-$H_3CC_6H_4COCl$, 874-60-2; 4-$H_3COC_6H_4COCl$, 100-07-2; 4-ClC_6H_4COCl, 122-01-0; 3,4-$Cl_2C_6H_3COCl$, 3024-72-4; 3-$H_3CC_6H_4COCl$, 1711-06-4; 3-$H_3COC_6H_4COCl$, 1711-05-3; 3-ClC_6H_4COCl, 618-46-2; 2,4-$(CH_3)_2C_6H_3COCl$, 21900-42-5; 2,5-$(CH_3)_2C_6H_3COCl$, 22328-43-4; 2,6-$(CH_3)_2C_6H_3COCl$, 21900-37-8; 3,4-$(CH_3)_2C_6H_3COCl$, 21900-23-2; 3,5-$(CH_3)_2C_6H_3COCl$, 6613-44-1; 2-$H_3CC_6H_4CN$, 529-19-1; 2-cyanopyridine, 100-70-9.

Using Shape Complementarity as an Initial Screen in Designing Ligands for a Receptor Binding Site of Known Three-Dimensional Structure

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Finding novel leads from which to design drug molecules has traditionally been a matter of screening and serendipity. We present a method for finding a wide assortment of chemical structures that are complementary to the shape of a macromoleculer receptor site whose X-ray crystallographic structure is known. Each of a set of small molecules from the Cambridge Crystallographic Database (Allen; et al. *J. Chem. Doc.* **1973**, *13*, 119) is individually docked to the receptor in a number of geometrically permissible orientations with use of the docking algorithm developed by Kuntz et al. (*J. Mol. Biol.* **1982**, *161*, 269). The orientations are evaluated for goodness-of-fit, and the best are kept for further examination using the molecular mechanics program AMBER (Weiner; Kollman *J. Comput. Chem.* **1981**, *106*, 765). The shape-search algorithm finds known ligands as well as novel molecules that fit the binding site being studied. The highest scoring orientations of known ligands resemble binding modes generated by interactive modeling or determined crystallographically. We describe the application of this procedure to the binding sites of papain and carbonic anhydrase. While the compounds recovered from the Cambridge Crystallographic Database are not, themselves, likely to be inhibitors or substrates of these enzymes, we expect that the structures from such searches will be useful in the design of active compounds.

The process of developing a new drug is long and complicated. The first step in this process is to find a "lead": a compound active in a particular therapeutic area. Analogues of the lead compound are made in order to study the way that its properties vary with structural changes. The resulting structure–activity data can be analyzed to optimize the overall profile of the potential drug. Lead compounds are usually found by screening many compounds or are discovered accidentally. There exists a good deal of expertise in making analogues and optimizing the lead compound's properties, but finding such compounds is still largely a matter of chance. In this paper, we attempt to assist drug design by finding structural precursors from which lead compounds can be designed when the leads are to be ligands for a receptor of

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