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

Marcel A. H. de Zwart, Henk van der Goot, and Henk Timmerman*

Department of Pharmacochemistry, Vrije Universiteit, 1083 De Boelelaan, 1081 HV Amsterdam, The Netherlands.
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In our search for new compounds with antimycoplasmal activity, a series of aromatic amidines derived from 1-amino-3-(2-pyridyl)isoquinoline (1) was synthesized. In the presence of 40 μM copper the most active compounds show growth inhibition of *Mycoplasma gallisepticum* in the nanomolar range. These compounds are 3 times as active as tylosin, an antimycoplasmal therapeutic agent that is used in veterinary practice. In the presence of copper, amidines derived from 1 are 2–3 times more active than the corresponding amides. Furthermore it was established that for these compounds too, the presence of a 2,2′-bipyridyl moiety is a necessary prerequisite for antimycoplasmal activity. As for the amides, antimycoplasmal activity of amidines derived from 1 is dependent on the hydrophobic fragmental value of the aromatic nucleus of the amidine moiety. A quantitative structure–activity relationship established the optimal hydrophobic fragmental value of this part of the molecule to be zero.

Previous studies from our laboratory revealed that copper complexes of compounds structurally related to 2,2′-bipyridyl are growth inhibitors of *Mycoplasma gallisepticum* in vitro.[1-4] In subsequent studies we established that copper is a very potent inhibitor in vitro of NADH-oxidase and lactate dehydrogenase,[5] two enzymes involved in the energy providing metabolism of fermentative mycoplasmas.[6] In a proposed mechanism of action of these copper complexes by Smit[7] and Gaisser,[8] copper is the ultimate toxic agent, whereas the ligand facilitates the penetration of copper into the cytosol.

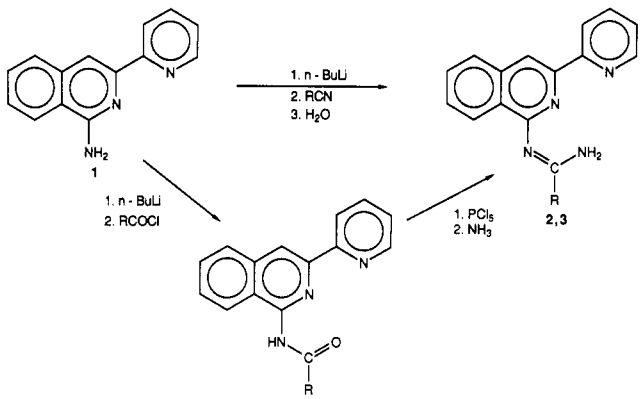
After the discovery of the high degree of antimycoplasmal activity of amides and amidines derived from 4-amino-2-(2-pyridyl)quinazoline by Linschoten,[4] we focused our attention on the structurally related derivatives of 1-amino-3-(2-pyridyl)isoquinoline. In part 1 of the present series of papers[9] we have reported on the synthesis and antimycoplasmal activity of both aliphatic and aromatic amides derived from 1-amino-3-(2-pyridyl)isoquinoline. A qualitative structure–activity relationship study initially revealed that antimycoplasmal activity is dependent on the hydrophobic fragmental value of the amide residue. This dependency was parabolic in nature and the optimal hydrophobic fragmental value was established by a subsequent regression analysis to be $\sum f = 0.30$. This value was approximated best by *N*-[3-(2-pyridyl)isoquinolin-1-yl]benzamide, which had a minimal inhibitory concentration (MIC) of 0.1 μM. This value is comparable with the MIC value of tylosin, a macrolide antibiotic that is used in veterinary practice for treatment of mycoplasmal infections in poultry.[10]

In the present paper we report on the synthesis and antimycoplasmal activity of amidines derived from 1-amino-3-(2-pyridyl)isoquinoline. Structure optimization was performed according to the Topliss scheme.[11] This was followed by an attempt to establish a quantitative structure–activity relationship.

Chemistry. Basically there are three ways to obtain amidines. They consist of the addition of ammonia or amines to nitriles, to (thio)amides, or to imido esters or imido halides.[12-15] Simple addition of ammonia and amines to nitriles are only observed in the case of nitriles activated by electron-attracting substituents in the position α to the CN bond. As an alternative, metal derivatives of ammonia or amines may be used as reactive nucleophiles.[16,17]

When it is not possible to obtain amidines by the addition of amine to nitrile, they can be synthesized from the corresponding amide. Treatment of mono-N-substituted amides with halogenating agents gives imido halides, which

Scheme I



* To whom correspondence should be sent.

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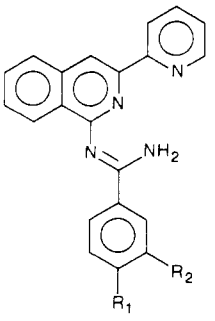
Table I. MIC Values[a] (μM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C

compd	without extra copper	extra copper added[b]
$CuSO_4 \cdot 5H_2O$	700	
tylosin	0.1	0.1
1	452	0.45
4	>500	250
5	>100	>100

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

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



2a-h

compd	R_1	R_2	without extra copper	extra copper added[b]
2a	H	H	195	63.4
2b	CH_3	H	780	68.3
2c	OCH_3	H	780	104.0
2d	Cl	H	6250	195.0
2e	Cl	Cl	>10000	780.0
2f	H	CH_3	780	48.8
2g	H	OCH_3	1560	78.0
2h	H	Cl	6250	195.0

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

react with ammonia to yield the corresponding amidines.[18,19] As halogenating agent phosphorus pentachloride is preferred. So, mono-N-substituted aromatic amidines **2**, **3** can be obtained either by direct addition of 1-amino-3-(2-pyridyl)isoquinoline (**1**) to electron-deficient nitriles or by treating amides derived from **1** with phosphorus pentachloride and subsequently with ammonia (Scheme I).

Previously we have described the synthesis of 1-amino-3-(2-pyridyl)isoquinoline (**1**) and amides derived thereof.[9] 1-Amino-3-(2-pyridyl)isoquinoline (**1**) was obtained from 2-methylbenzonitrile and pyridine-2-carbonitrile. Amides could be isolated from the reaction of the anion of 1-amino-3-(2-pyridyl)isoquinoline (**1**) and various acyl chlorides. Amidines **2b**, **2c**, **2f**, and **2h** were obtained from the corresponding amides. After conversion of these amides with phosphorus pentachloride in anhydrous chloroform to the corresponding imido chlorides, amidines were obtained by the subsequent addition of ammonia. All the other amidines **2a**, **2d**, **2e**, **2g**, and **3a–g** were obtained by abstracting a proton from 1-amino-3-(2-pyridyl)isoquinoline (**1**) with *n*-butyllithium followed by the addition of electron-deficient aromatic nitriles to this anion. An attempt to obtain compounds **2b** and **2c** in this way failed. This is in accordance with the electronic influence of the 4-CH_3 and 4-OCH_3 substituent on the carbonitrile moiety.

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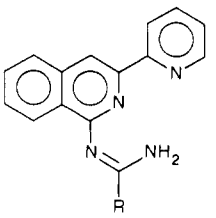
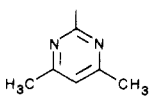
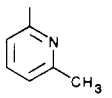
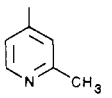
Table III. MIC Values[a] (nM) against *M. gallisepticum* K514 in a Modified Adler Medium at 37 °C



3a-g

compd	R	without extra copper	extra copper added[b]
3a		390	32.5
3b		390	39.0
3c		195	32.5
3d		195	39.0
3e		195	45.5
3f		390	65.0
3g		390	65.0

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

N-(2-Pyridyl)-2-pyridinecarboxamidine (**4**) and *N*-(5-methyl-3-phenylisoquinolin-1-yl)-2-pyridinecarboxamidine (**5**) could be obtained by direct addition of 2-pyridinecarbonitrile to the anions of the respective amines.

Biological Activity. Since antimycoplasmal activity is apparently copper dependent for compounds containing a 2,2′-bipyridyl moiety,[1,2] all of the compounds under investigation have been tested with and without the addition of copper to the growth medium. Without the addition of copper, the copper concentration was less than 3 μM.[1] For determination of antimycoplasmal activity of these compounds in the presence of copper, copper was added as $CuSO_4 \cdot 5H_2O$ to obtain a final concentration of 40 μM. The MIC value for copper was established to be 700 μM (Table I). This value is somewhat higher than values presented previously,[9] which is most probably due to minor variations in the growth medium constituents.

MIC values for *N*-[3-(2-pyridyl)isoquinolin-1-yl]amidines **2a–h**, **3a–g** are presented in Tables II and III, respectively. All compounds were tested up to a concentration of 100 μM. At higher concentrations some compounds tend to precipitate when added to growth medium.

In contrast to the structurally related amides,[9] amidines derived from **1** except for compound **2e** possess appreciable antimycoplasmal activity without the addition of extra copper. This activity is most probably due to the small amount of copper, which is present in the growth medium. Without the addition of extra copper, *N*-[3-(2-pyridyl)-

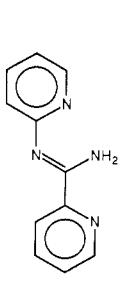
isoquinolin-1-yl]benzamidines are about 100 times more active than the corresponding amides. In the presence of 40 μM copper, all amidines derived from 1-amino-3-(2-pyridyl)isoquinoline show considerable antimycoplasmal activity. Also in this case antimycoplasmal activity is remarkably enhanced by the addition of a small amount of copper. Thus in the presence of copper, compounds **2a–h** and **3a–g** are about 5–30 times more active than in the absence of copper.

All compounds including **2e**, which shows least activity, are more active than the parent compound **1**. In fact, the most active compounds **3a** and **3c** are 3 times more active than the reference compound tylosin, which is used in veterinary practice for treatment of mycoplasmal infections. Benzamidines derived from **1** are 2–3 times more active than the corresponding amides at a copper concentration of 40 μM.

For *N*-[3-(2-pyridyl)quinazolin-4-yl]-2-pyridinecarboxamidine (**6**) we found a MIC value 23 times as high as the value reported by Linschoten.[4] This is probably due to the same features, which are also responsible for the increased MIC value for copper. Comparison of the antimycoplasmal activity of the structurally analogous **3a** and **6** shows that at least in this case amidines derived from 1-amino-3-(2-pyridyl)isoquinoline are more active than amidines derived from 4-amino-2-(2-pyridyl)quinazoline.

Structure–Activity Relationships. Just like for a variety of other compounds containing a 2,2′-bipyridyl moiety, antimycoplasmal activity of amidines derived from 1-amino-3-(2-pyridyl)isoquinoline is copper dependent.

Due to this remarkable copper effect, it is very likely that these compounds also act via their copper complexes. As proposed for the corresponding amides, the increase in activity of these amidines compared to that of the parent compound **1** may be due to the presence of a third coordination site for the copper atom. In fact, it is known that amidines are able to participate as a ligand in copper complexes.[20] To verify the correctness of this hypothesis, we investigated the antimycoplasmal activity of *N*-(2-pyridyl)-2-pyridinecarboxamidine (**4**) and *N*-(5-methyl-3-phenylisoquinolin-1-yl)-2-pyridinecarboxamidine (**5**), two amidines comparable with the ones under investigation but lacking a 2,2′-bipyridyl moiety. Surprisingly these compounds have negligible antimycoplasmal activity (Table I). So, although we cannot exclude participation of the amidine moiety in complex formation, contribution of this part of the molecule is small compared to that of the 2,2′-bipyridyl moiety.



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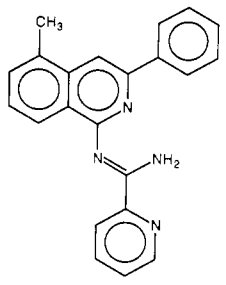

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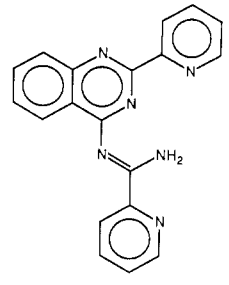

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Amidines exist as a mixture of the imino and the amino tautomer with *N*-aryl-substituted derivatives occurring predominantly in the amino form.[21–23] Since we are only dealing with *N*-aryl-substituted amidines, we may consider this part of the molecule, i.e. the 3-(2-pyridyl)isoquinoline part with the amidine 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 influence of the part of the molecule that is varied within these series, viz. the aromatic nucleus of the amidine moiety symbolized by R in the general form **2**, **3** (Scheme I). Since antimycoplasmal activity of these compounds is copper dependent for structure–activity relationship considerations, we focus our attention to antimycoplasmal activity in the presence of copper.

When we consider the activity sequence of the original Topliss series, that is compounds **2a–e**, an increase of activity is paralleled by a decrease in lipophilicity of the aromatic nucleus. When we compare antimycoplasmal activity of the 4-substituted benzamidines **2b–d** with the corresponding 3-substituted benzamidines **2f–h**, it is obvious that the position of the substituent in the aromatic nucleus has no influence on biological activity. As a consequence of this dependency on lipophilicity, a further increase of antimycoplasmal activity could be achieved by making the compounds less lipophilic. Therefore we decided to synthesize some N-heterocyclic aromatic amidines (**3a–g**). Indeed, by doing so we did establish an increase of antimycoplasmal activity. As for the benzamidines, a decrease of lipophilicity is paralleled by an increase of antimycoplasmal activity of these N-heterocyclic aromatic amidines. However when lipophilicity is decreased further, antimycoplasmal activity does not increase anymore. The very hydrophilic compounds **3c** and **3d** show antimycoplasmal activity comparable to that of the far more lipophilic analogues **3a** and **3b**. So, this qualitative approach to a structure–activity relationship suggests the existence of an optimal lipophilicity for antimycoplasmal activity of these amidines. An analogous dependency was found for antimycoplasmal activity of amides derived from 1-amino-3-(2-pyridyl)isoquinoline (**1**).[9]

In an attempt to establish a quantitative structure–activity relationship, we tried to find a correlation between the antimycoplasmal activity and lipophilicity. Parameters chosen were MIC values for biological activity and hydrophobic fragmental values ($\sum f$) for lipophilicity. Hydrophobic fragmental values were calculated for the substituted aromatic nucleus according to Rekker (Table IV).[24]

Table IV. Hydrophobic Fragmental Values[a]

compd	R	$\sum f$	MIC_{calcd},[b] nM	MIC_{obsd}, nM
2a	C_6H_5	1.840	88.5	63.4
2b	4-$CH_3(C_6H_4)$	2.359	156.8	68.3
2c	4-$OCH_3(C_6H_4)$	1.920	95.8	104.0
2d	4-$Cl(C_6H_4)$	2.582	209.4	195.0
2e	3,4-$(Cl)_2(C_6H_3)$	3.324	662.0	780.0
2f	3-$CH_3(C_6H_4)$	2.359	156.8	48.8
2g	3-$OCH_3(C_6H_4)$	1.920	95.8	78.0
2h	3-$Cl(C_6H_4)$	2.582	209.4	195.0
3a	C_5H_4N	0.520	39.1	32.5
3b	C_5H_4N	0.520	39.1	39.0
3c	$C_4H_3N_2$	−0.380[c]	37.8	32.5
3d	$C_4H_3N_2$	−0.380[c]	37.8	39.0
3e	3,5-$(CH_3)_2C_4HN_2$	0.658	40.8	45.5
3f	CH_3-C_5H_3N	1.040	48.3	65.0
3g	CH_3-C_5H_3N	1.040	48.3	65.0

[a] See ref 24. [b] Calculated from eq 2. [c] Personal communication with R. F. Rekker.

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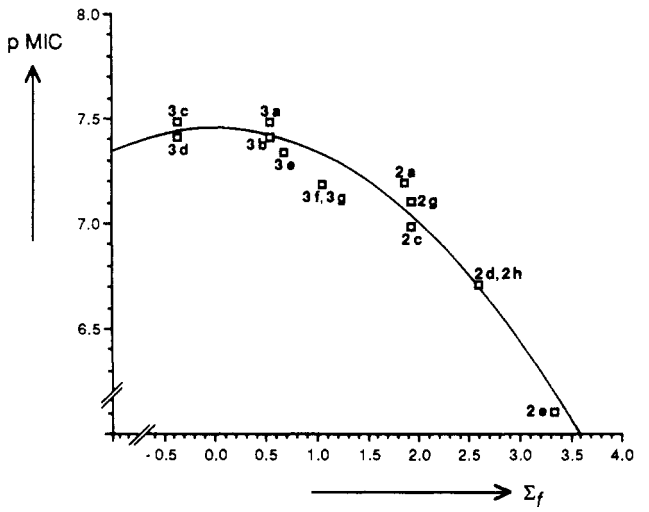

Figure 1. p MIC vs $\sum f$. For the identity of **2a–3g**, see Tables II and III.

By multiple regression analysis the following equation is obtained:

$$-\log \text{MIC} = 7.454\ (\pm 0.065) - 0.101\ (\pm 0.014)(\textstyle\sum f)^2 \quad (1)$$

$$F = 50.566,\ r = 0.892,\ s = 0.174,\ n = 15$$

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

$$-\log \text{MIC} = 7.439\ (\pm 0.033) - 0.114\ (\pm 0.007)(\textstyle\sum f)^2 \quad (2)$$

$$F = 236.369,\ r = 0.978,\ s = 0.087,\ n = 13$$

So for 13 of the original series of 15 compounds, a very good correlation is found between antimycoplasmal activity and hydrophobic fragmental values of the aromatic nucleus of the amidine moiety. As we did not observe anomalous physicochemical properties for both methyl-substituted compounds **2b** and **2f** compared to other benzamidines synthesized, we have no explanation for the fact that these compounds have to be omitted for statistical reasons from the equation obtained. It is not very likely that this anomalous behavior can be ascribed to electronic features, since we did not find any correlation between antimycoplasmal activity and an electronic parameter like σ. The dependency of antimycoplasmal activity on hydrophobic fragmental values is consistent with a parabolic nature, which means that an optimal lipophilicity for antimycoplasmal activity exists (Figure 1). However, as data on the left side of the parabola are not included in regression analysis, another relationship is also possible, e.g. of such a nature that in this range of $\sum f$ values (i.e. $\sum f < -0.38$) antimycoplasmal activity does not decrease with increasing hydrophilicity. According to the equation obtained the optimal contribution to lipophilicity of the substituted aromatic nucleus is $\sum f = 0$. Realizing that the optimum for lipophilicity is rather broad and taking into account the difference in lipophilicity between aromatic amides and corresponding amidines ($\sum f_{\text{amide}} - \sum f_{\text{amidine}} = 0.5$), this value corresponds very well with the optimal lipophilicity that was found for antimycoplasmal activity of amides derived from **1**. So apparently this optimal lipophilicity with regard to antimycoplasmal activity is more generally applicable to compounds derived from **1**.

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

Conclusions. For amidines derived from **1**, the presence of a 2,2′-bipyridyl moiety is a necessary prerequisite for antimycoplasmal activity. In the presence of a small nontoxic amount of copper amidines derived from **1** are very potent antimycoplasmal agents with MIC values in the nanomolar range. Even without the addition of extra copper some of these compounds show complete growth inhibition in the micromolar range. In the presence of copper amidines are 2–3 times as active as the corresponding amides.

Antimycoplasmal activity of amidines derived from **1** is dependent on lipophilicity as was found for the structurally related amides and this dependency is parabolic in nature. A quantitative structure–activity relationship was established, which revealed that the optimal hydrophobic fragmental value of the substituted aromatic nucleus of the amidine moiety is zero.

Experimental Section

Chemistry. Melting points were determined with 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. All starting materials were commercially available and of the highest purity obtainable. 1-Amino-3-(2-pyridyl)isoquinoline and amides derived thereof were synthesized as described earlier.[9] 1-Amino-5-methyl-3-phenylisoquinoline was taken from the laboratory stock.[25] 3,4-Dichlorobenzonitrile was synthesized from the corresponding aldehyde.[26] Pyrimidine-2-carbonitrile and 3,5-dimethylpyrimidine-2-carbonitrile were synthesized from the corresponding 2-chloro compounds by treatment of the quarternary ammonium salt, prepared from these compounds and trimethylamine, with potassium cyanide in acetamide.[27,28] 6-Methylpyridine-2-carbonitrile and 6-methylpyridine-4-carbonitrile were obtained by cyanation of 2-picoline 1-oxide.[29,30] Analytical results for compounds indicated by the molecular formula were within ±0.4% of the theoretical values.

Synthesis. General Procedure for the Synthesis of Amidines from 1-Amino-3-(2-pyridyl)isoquinoline and Electron-Deficient Nitriles (2a,d,e,g). A solution of 0.05 mol of 1-amino-3-(2-pyridyl)isoquinoline (**1**) in 100 mL of anhydrous THF was stirred under a nitrogen atmosphere and cooled to −10 °C. Subsequently 31.25 mL of 1.6 M *n*-butyllithium in hexane was added dropwise and stirring was continued for 10 min. Then 0.05 mol of nitrile in a minimal amount of THF was added, and while the reaction mixture was kept at −10 °C, stirring was continued for 10 min. When the mixture had reached room temperature, it was reluxed for several hours, varying from 2 to 10 h depending on the nitrile. 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 the pH was adjusted to 8 with a dilute bicarbonate solution. The combined chloroform layers were dried with anhydrous potassium bicarbonate and, after filtration, evaporated to dryness.

General Procedure for the Synthesis of Amidines from Corresponding Amides Derived from 1-Amino-3-(2-pyridyl)isoquinoline (2b,c,f,h). A solution of 0.02 mol of phosphorus pentachloride in 50 mL of freshly distilled chloroform was stirred under a nitrogen atmosphere at room temperature. A solution of 0.01 mol of amide in 50 mL of chloroform was added

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dropwise and the mixture was refluxed for half an hour. The mixture was cooled in an ice bath and anhydrous ammonia was bubbled through the mixture for 1 h. Subsequently an ice-cold saturated bicarbonate solution was added slowly. The chloroform layer was separated, washed three times, dried with anhydrous potassium carbonate, and, after filtration, evaporated to dryness.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]benzamidine (2a).** This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and benzonitrile. Time of reflux was 4 h. The crude reaction mixture was washed with a little methanol to remove traces of unreacted starting material. The remaining solid material was filtered off and crystallized from $CHCl_3$/petroleum ether (60–80 °C): yield 4.1 g (25%); mp 187.7–186.7 °C; NMR ($CDCl_3$) δ 7.36 (m, 1 H, 2-pyr H-5), 7.54–8.0 (m, 7.5 H), 8.13–8.30 (m, 3 H), 8.42 (s, 1 H, H-4), 8.79 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 9.06 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3400 (NH), 3040 (CH), 1605, 1570, 1560, 1540, 1520 (C=C, C=N), 1470, 1460, 1440, 1420, 1380, 1330, 1140, 1045, 1025, 925, 900, 880, 780, 750, 740, 700, 690, 620, 580; MS, m/e 324.1368 (M^+), 324.1375 ($C_{21}H_{16}N_4$). Anal. ($C_{21}H_{16}N_4$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-4-methylbenzamidine (2b).** This compound was synthesized from the corresponding amide. The crude product was crystallized from CH_3OH/$CH_3COOC_2H_5$: yield 1.9 g (60%); mp 188.1–188.3 °C; NMR ($CDCl_3$) δ 2.44 (s, 3 H, CH_3), 7.29 (m, 1 H, 2-pyr H-5), 7.33 and 8.06 (AA'BB' system, J_{ab} = 8.0 Hz, 4 H, Ph H), 7.54–7.95 (m, 5 H), 8.21 (d, J = 8.0 Hz, 1 H, H-8), 8.39 (s, 1 H, H-4), 8.76 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 9.03 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3450 (NH), 3060 (CH), 1600 (s), 1575, 1560, 1545, 1520, 1495 (C=C, C=N), 1475, 1420, 1380, 1330, 1245, 1180, 1145, 1045, 1015, 990, 965, 920, 900, 880, 860, 840, 825, 785, 755, 740, 690, 670, 640, 615, 580; MS, m/e 338.1522 (M^+), 338.1531 ($C_{22}H_{18}N_4$). Anal. ($C_{22}H_{18}N_4$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-4-methoxybenzamidine (2c).** This compound was synthesized from the corresponding amide. The crude reaction mixture was purified via column chromatography using silica gel 60 H with $CHCl_3$/$CH_3COOC_2H_5$/NH_3 (10:10:1) as eluent. The fractions containing **2c** were pooled, and after evaporation of the solvent, the product was crystallized from CH_3OH/$CH_3COOC_2H_5$: yield 0.85 g (24%); mp 199.3–199.6 °C; NMR ($CDCl_3$) δ 3.90 (s, 3 H, OCH_3), 7.05 and 8.12 (AA'BB' system, J_{ab} = 10.0 Hz, 4 H, Ph H), 7.30 (m, 1 H, 2-pyr H-5), 7.50–7.96 (m, 5 H), 8.21 (d, J = 8.0 Hz, 1 H, H-8), 8.38 (s, 1 H, H-4), 8.76 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 9.0 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3330 (NH), 3160, 3040 (CH), 3000, 1610, 1575, 1530, 1500 (C=C, C=N), 1480, 1470, 1415, 1380, 1365, 1330, 1305, 1245, 1180, 1165, 1140, 1025, 995, 955, 925, 885, 860, 835, 785, 755, 740, 690, 675, 640, 580, 560, 545, 525; MS, m/e 354.1480 (M^+), 354.1480 ($C_{22}H_{18}N_4O$). Anal. ($C_{22}H_{18}N_4O$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-4-chlorobenzamidine (2d).** This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 4-chlorobenzonitrile. Time of reflux was 6 h. The precipitate that appears after hydrolysis was filtered off and crystallized from $CHCl_3$/petroleum ether (60–80 °C): yield 9.2 g (51%); mp 162.0–163.0 °C; NMR ($CDCl_3$) δ 7.33 (m, 1 H, 2-pyr H-5), 7.53 and 8.11 (AA'BB' system, J_{ab} = 8.0 Hz, 4 H, Ph H), 7.50–8.08 (m, 5 H), 8.22 (d, J = 8.0 Hz, 1 H, H-8), 8.42 (s, 1 H, H-4), 8.78 (m, J = 4.8 Hz, 1 H, 2-pyr H-6), 9.0 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3480 (NH), 3050 (CH), 1610, 1580, 1560, 1550, 1540, 1510 (C=C, C=N), 1485, 1470, 1445, 1425, 1395, 1380, 1365, 1335, 1140, 1090, 1050, 1010, 990, 960, 940, 885, 860, 845, 790, 775, 750, 680, 620, 580; MS, m/e 358.0967 (M^+), 358.0985 ($C_{21}H_{15}ClN_4$, ^{35}Cl). Anal. ($C_{21}H_{15}ClN_4$) C, H, N, Cl.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-3,4-dichlorobenzamidine (2e).** This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 3,4-dichlorobenzonitrile and isolated in the same way as compound **2d**. Time of reflux was 2 h. **2e**: yield 6.5 g (33%); mp 180.0–181.0 °C; NMR ($CDCl_3$) δ 7.30 (m, 1 H, 2-pyr H-5), 7.50–7.95 (m, 5 H), 7.55 (d, J = 8.0 Hz, 1 H, Ph H-5), 7.88 (d, J = 8.0 Hz, 1 H, Ph H-6), 8.15 (d, J = 8.0 Hz, 1 H, H-8), 8.23 (d, J = 1.8 Hz, 1 H, Ph H-2), 8.40 (s, 1 H, H-4), 8.76 (d, J = 4.8 Hz, 1 H, 2-pyr H-6), 8.93 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3460 (NH), 3050 (CH), 1610, 1580, 1560, 1545, 1505 (C=C, C=N), 1480, 1465, 1420, 1385, 1365, 1345, 1240, 1200, 1170, 1145, 1020, 990, 960, 930, 905, 885, 865, 830, 790, 755, 740, 720, 665, 620, 580; MS, m/e 392.0582 (M^+), 392.0595 ($C_{21}H_{14}Cl_2N_4$, ^{35}Cl). Anal. ($C_{21}H_{14}Cl_2N_4$) C, H, N; Cl: calcd, 18.03; found, 18.48.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-3-methylbenzamidine (2f).** This compound was synthesized from the corresponding amide. The crude reaction mixture was purified via column chromatography using silica gel 60 H with $CHCl_3$/$CH_3COOC_2H_5$/NH_3 (10:10:1) as eluent. The fractions containing **2f** were pooled, and after evaporation of the solvent, the product was crystallized from CH_3OH/$CH_3COOC_2H_5$: yield 1.05 g (31%); mp 153.3–155.0 °C; NMR ($CDCl_3$) δ 2.50 (s, 3 H, CH_3), 7.31 (m, 1 H, 2-pyr H-5), 7.39–8.0 (m, 10 H), 8.24 (d, J = 8.0 Hz, 1 H, H-8), 8.42 (s, 1 H, H-4), 8.78 (d, J = 4.8 Hz, 1 H, 2-pyr H-6), 9.05 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3400 (NH), 3040 (CH), 1600, 1580, 1560, 1525 (C=C, C=N), 1485, 1470, 1425, 1380, 1330, 1145, 1080, 1045, 990, 960, 925, 885, 860, 790, 745, 700, 675, 620, 585, 525; MS, m/e 338.1539 (M^+), 338.1531 ($C_{22}H_{18}N_4$). Anal. ($C_{22}H_{18}N_4$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-3-methoxybenzamidine (2g).** This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 3-methoxybenzonitrile. Time of reflux was 10 h. A little diethyl ether was poured on the crude reaction mixture to obtain yellow solid material from the black oil. The precipitate was isolated by filtration and crystallized from CH_3OH/$CH_3COOC_2H_5$: yield 5.5 g (31%); mp 149.4–149.6 °C; NMR ($CDCl_3$) δ 3.94 (s, 3 H, OCH_3), 7.11 (ddd, J = 7.8, 2.5, 1.8 Hz, 1 H, Ph H-6), 7.26–7.40 (m, 1 H, 2-pyr H-5), 7.45–7.96 (m, 8 H), 8.22 (d, J = 8.0 Hz, 1 H, H-8), 8.42 (s, 1 H, H-4), 8.77 (d, J = 4.8 Hz, 1 H, 2-pyr H-6), 9.02 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3400 (NH), 3040 (CH), 1600, 1575, 1510 (C=C, C=N), 1480, 1470, 1420, 1380, 1365, 1342, 1310, 1285, 1235, 1190, 1160, 1140, 1080, 1045, 990, 960, 925, 880, 855, 810, 780, 747, 738, 700, 672, 618, 565, 500, 470; MS, m/e 354.1452 (M^+), 354.1480 ($C_{22}H_{18}N_4O$). Anal. ($C_{22}H_{18}N_4O$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-3-chlorobenzamidine (2h).** This compound was synthesized from the corresponding amide. The crude product was crystallized twice from CH_3OH/$CH_3COOC_2H_5$: yield 2.9 g (80%); mp 147.6–148.2 °C; NMR ($CDCl_3$) δ 7.31 (m, 1 H, 2-pyr H-5), 7.44–8.17 (m, 8 H), 8.17 (s, 1 H, Ph H-2), 8.21 (d, J = 8.0 Hz, 1 H, H-8), 8.42 (s, 1 H, H-4), 8.78 (d, J = 4.8 Hz, 1 H, 2-pyr H-6), 8.99 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3400 (NH), 3040 (CH), 1600, 1578, 1555, 1535, 1510 (C=C, C=N), 1480, 1470, 1420, 1405, 1380, 1330, 1140, 1090, 1075, 1045, 1025, 1010, 990, 925, 900, 885, 860, 785, 755, 748, 720, 670, 618, 582, 520, 445; MS, m/e 358.0980 (M^+), 358.0985 ($C_{22}H_{15}ClN_4$, ^{35}Cl). Anal. ($C_{22}H_{15}ClN_4$) C, H, N, Cl.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-2-pyridinecarboxamidine (3a).** This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 2-pyridinecarbonitrile. Time of reflux was 2 h. The crude reaction mixture was crystallized from $CHCl_3$/petroleum ether (60–80 °C): yield 8.1 g (50%); mp 185.0–186.0 °C; NMR ($CDCl_3$) δ 7.22–8.00 (m, 7 H), 8.20–8.44 (br, 1 H, NH), 8.23 (d, J = 8.0 Hz, 1 H, H-8), 8.40 (s, 1 H, H-4), 8.61–8.86 (m, 3 H), 9.01 (m, 1 H, 2-pyr H-3), 10.95 (br, 1 H, NH); IR (KBr, cm^{-1}) 3380 (NH), 3050 (CH), 1615, 1580, 1560, 1545 (C=C, C=N), 1480, 1465, 1435, 1385, 1365, 1330, 1245, 1200, 1140, 1040, 990, 960, 925, 895, 885, 860, 800, 785, 755, 740, 705, 675, 650, 620, 560, 550, 525; MS, m/e 325.1322 (M^+), 325.1327 ($C_{20}H_{15}N_5$). Anal. ($C_{20}H_{15}N_5$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-4-pyridinecarboxamidine (3b).** This compound was synthesized and isolated in the same way as compound **3a**: yield 13.8 g (85%); mp 221.5–222.3 °C; NMR ($CDCl_3$) δ 7.36 (m, 1 H, 2-pyr H-5), 7.57–8.05 (m, 5 H), 8.0 and 8.85 (AA'BB' system, J_{ab} = 6.1 Hz, 4 H, 4-pyr H), 8.22 (d, J = 8.0 Hz, 1 H, H-8), 8.44 (s, 1 H, H-4), 8.73–8.87 (m, 1 H, 2-pyr H-6), 8.98 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3300 (NH), 3050 (CH), 1630, 1580, 1550, 1530, 1520 (C=C, C=N), 1480, 1470, 1425, 1410, 1385, 1365, 1345, 1145, 1060, 995, 955, 935, 840, 785, 750, 745, 730, 705, 670, 620, 585, 560, 525; MS, m/e 325.1336 (M^+), 325.1327 ($C_{20}H_{15}N_5$). Anal. ($C_{20}H_{15}N_5$) C, H, N.

***N*-[3-(2-Pyridyl)isoquinolin-1-yl]-2-pyrimidinecarboxamidine (3c).** This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 2-pyrimidinecarbonitrile. Time of reflux was 8 h. The crude reaction mixture was acidified with concentrated hydrochloric acid and extracted with diethyl ether. Then the water layer was neutralized with sodium bicarbonate to pH 8.0 and extracted with diethyl ether. This organic layer

contained mainly the major side product formed during this reaction. Subsequently, the water layer was extracted with chloroform. This chloroform layer was dried with anhydrous sodium carbonate, filtered, and evaporated to dryness. The residue was crystallized from ethanol/diethyl ether. The precipitate formed was filtered off and the filtrate was evaporated. The residue was crystallized from $CHCl_3$/petroleum ether (60–80 °C). The precipitate consisted for the most part of the starting compound 1-amino-3-(2-pyridyl)isoquinoline. Again the mother liquor was evaporated and a small volume of ethanol was poured on the residue. The solid material formed was filtered off and crystallized from ethanol: yield 2.1 g (13%); mp 182.3–183.8 °C; NMR ($CDCl_3$) δ 7.31 (m, 1 H, 2-pyr H-5), 7.46 (t, J = 5.0 Hz, 1 H, pyrimidyl H-4), 7.61–7.97 (m, 6 H), 8.07–8.36 (br s, 1 H, NH), 8.26 (d, J = 8.0 Hz, 1 H, H-8), 8.47 (s, 1 H, H-4), 8.78 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 8.99 (d, J = 5.0 Hz, 2 H, pyrimidyl H-3 and H-5), 9.08 (m, 1 H, 2-pyr H-3), 11.08 (br s, 1 H, NH); IR (KBr, cm^{-1}) 3440 (NH), 3040 (CH), 1615, 1575, 1555, 1540 (C=C, C=N), 1480, 1420, 1395, 1380, 1360, 1330, 1265, 1220, 1180, 1140, 1040, 1020, 1010, 990, 965, 925, 885, 860, 820, 785, 760, 745, 705, 670, 630, 620, 590, 570, 560, 525, 470; MS, m/e 326.1284 (M^+), 326.1280 ($C_{19}H_{14}N_6$). Anal. ($C_{19}H_{14}N_6$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-2-pyrazinecarboxamidine (3d). This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 2-pyrazinecarbonitrile. Time of reflux was 4 h. The crude product was crystallized from $CH_3OH/CH_3COOC_2H_5$: yield 15.5 g (95%); mp 231.5–232.0 °C; NMR ($CDCl_3$) δ 7.32 (m, 1 H, 2-pyr H-5), 7.62–7.97 (m, 5 H), 8.20 (d, J = 8.0 Hz, 1 H, H-8), 8.44 (s, 1 H, H-4), 8.61 (dd, J = 2.7, 1.4 Hz, 1 H, 2-pyrazinyl H-5), 8.77 (m, 2 H, 2-pyr H-6 and 2-pyrazinyl H-6), 9.06 (m, 1 H, 2-pyr H-3), 10.05 (d, 1 H, J = 1.4 Hz, 2-pyrazinyl H-3), 11.08 (br s, 1 H, NH); IR (KBr, cm^{-1}) 3400 (NH), 3050 (CH), 1625, 1575, 1560, 1550, 1540 (C=C, C=N), 1485, 1465, 1425, 1380, 1360, 1345, 1170, 1145, 1015, 925, 890, 850, 785, 765, 750, 730, 715, 625, 615, 525, 495; MS, m/e 326.1284 (M^+), 326.1280 ($C_{19}H_{14}N_6$). Anal. ($C_{19}H_{14}N_6$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-3,5-dimethyl-2-pyrimidinecarboxamidine (3e). This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 4,6-dimethyl-2-pyrimidinecarbonitrile. Time of reflux was 4 h. After hydrolysis of the reaction mixture, the organic phase was evaporated and some diethyl ether was poured on the remaining water layer, resulting in precipitation of the crude product. The precipitate was filtered off and crystallized from ethanol. The pure compound was obtained after recrystallization from ethanol: yield 6.8 g (39%); mp 195.5–197.0 °C; NMR ($CDCl_3$) δ 2.66 (s, 6 H, CH_3), 7.16 (s, 1 H, pyrimidyl H-4), 7.30 (m, 1 H, 2-pyr H-5), 7.56–7.96 (m, 4 H), 8.26 (d, J = 8.0 Hz, 1 H, H-8), 8.46 (s, 1 H), 8.76 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 9.06 (m, 1 H, 2-pyr H-3), 11.19 (br s, 1 H, NH), 8.11–8.47 (br s, 1 H, NH); IR (KBr, cm^{-1}) 3420, 3040 (CH), 1620, 1580, 1560, 1540 (C=C, C=N), 1480, 1420, 1380, 1360, 1345, 1330, 1280, 1140, 1030, 990, 960, 925, 905, 865, 805, 785, 765, 740, 730, 720, 670, 620, 580, 570, 555, 535, 485; MS, m/e 354.1571 (M^+), 354.1593 ($C_{21}H_{18}N_6$). Anal. ($C_{21}H_{18}N_6$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-6-methyl-2-pyridinecarboxamidine (3f). This compound was synthesized from 1-amino-3-(2-pyridyl)isoquinoline and 6-methyl-2-pyridinecarbonitrile. Time of reflux was 1 h. The crude product was crystallized from methanol: yield 1.7 g (10%); mp 158.0–159.3 °C; NMR ($CDCl_3$) δ 2.65 (s, 3 H, CH_3), 7.25–7.28 (m, 2 H, 2-pyr H-5 and 2-pyr H-4′), 7.56–8.00 (m, 5 H), 8.22–8.47 (br, 1 H, NH), 8.27 (d, J = 8.0 Hz, 1 H, H-8), 8.42 (s, 1 H, H-4), 8.66 (d, J = 7.5 Hz, 1 H, 2-pyr H-5′), 8.79 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 9.06 (m, 1 H, 2-pyr H-3), 11.02 (br, 1 H, NH); IR (KBr, cm^{-1}) 3430 (NH), 2980 (CH), 1620, 1585, 1565, 1550 (C=C, C=N), 1480, 1450, 1425, 1400, 1380, 1365, 1345, 1145, 1080, 1015, 990, 930, 895, 885, 860, 810, 615, 590, 570, 530; MS, m/e 339.1455 (M^+), 339.1484 ($C_{21}H_{17}N_5$). Anal. ($C_{21}H_{17}N_5$) C, H, N.

N-[3-(2-Pyridyl)isoquinolin-1-yl]-6-methyl-4-pyridinecarboxamidine (3g). This compound was synthesized and isolated in the same way as compound **3f**: yield 15.3 g (90%); mp 206.6–209.2 °C; NMR ($CDCl_3$) δ 2.70 (s, 3 H, CH_3), 7.30 (m, 1 H, 2-pyr H-5), 7.61–7.96 (m, 6.5 H), 8.18 (d, J = 8.0 Hz, 1 H, H-8), 8.42 (s, 1 H, H-4), 8.68 (d, J = 6.3 Hz, 1 H, 4-pyr H-5), 8.75 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 8.94 (m, 1 H, 2-pyr H-3); IR (KBr, cm^{-1}) 3340 (NH), 3040 (CH), 1615, 1605, 1580, 1565, 1540, 1510 (C=C, C=N), 1480, 1465, 1420, 1380, 1350, 1330, 1145, 1090, 1040, 925, 895, 875, 845, 780, 750, 725, 700, 615, 585, 570, 545, 525. Anal. ($C_{21}H_{17}N_5$) C, H, N.

N-(2-Pyridyl)-2-pyridinecarboxamidine (4). A solution of 0.1 mol of 2-aminopyridine in 100 mL of anhydrous THF was stirred under a nitrogen atmosphere and cooled to −10 °C. Subsequently 62.5 mL of 1.6 M n-butyllithium in hexane was added dropwise and stirring was continued for 10 min. Subsequently 0.1 mol of 2-pyridinecarbonitrile in 50 mL of anhydrous THF was added and the mixture was refluxed for 3 h. After cooling to room temperature, the mixture was hydrolyzed by the addition of a small amount of water. The organic phase was evaporated and the pH of the remaining water layer was adjusted to 8 with a dilute bicarbonate solution. The mixture was extracted with chloroform. The combined chloroform layers were dried with anhydrous potassium carbonate and, after filtration, evaporated to dryness. The residue was crystallized from ethanol: yield 13.9 g (70%) of yellow needles; mp 105.3–106.4 °C; NMR ($CDCl_3$) δ 6.92 (ddd, J = 7.5, 5.0, 0.75 Hz, 1 H, H-5′), 7.34 (m, 2 H, H-3′, H-4′), 7.74 (m, 2 H, H-4, H-5), 7.62–8.12 (1 H, NH), 8.38 (dd, J = 5.0, 2.0 Hz, 1 H, H-6′), 8.56 (m, 2 H, H-3, H-6), 10.27 (1 H, C=NH); IR (KBr, cm^{-1}) 3350, 3180 (NH), 1620, 1580, 1560 (C=C, C=N), 1455, 1420, 1295, 1270, 1245, 1140, 1065, 1045, 985, 785, 745, 735, 655, 620, 555, 535, 470; MS, m/e 198.0888 (M^+), 198.0905 ($C_{11}H_{10}N_4$). Anal. ($C_{11}H_{10}N_4$) C, H, N.

N-(5-Methyl-3-phenylisoquinolin-1-yl)-2-pyridinecarboxamidine (5). This compound was synthesized from 0.01 mol of 1-amino-5-methyl-3-phenylisoquinoline and 0.01 mol of 2-pyridinecarbonitrile according to the procedure described for the synthesis of amidines from 1-amino-3-(2-pyridyl)isoquinoline and electron-deficient nitriles. Time of reflux was 3 h. The precipitate that appears after hydrolysis of the reaction mixture was filtered off and crystallized from $CH_3OH/CH_3COOC_2H_5$: yield 1.55 g (46%) of yellow needles; mp 201.3–203.0 °C; NMR ($CDCl_3$) δ 2.74 (s, 3 H, CH_3), 7.35–7.67 (m, 6 H), 7.81–8.14 (m, 3 H), 7.83 (s, 1 H), 8.14–8.38 (br s, 1 H, NH), 8.64 (d, J = 4.5 Hz, 1 H, 2-pyr H-6), 8.83 (d, J = 8.0 Hz, 1 H), 8.94 (m, 1 H, 2-pyr H-3), 10.98–11.32 (br s, 1 H, NH); IR (KBr, cm^{-1}) 3360 (NH), 3040, (CH), 1620, 1585, 1560, 1540, 1495 (C=C, C=N), 1465, 1440, 1375, 1350, 1320, 1245, 1205, 1140, 1060, 1040, 995, 930, 920, 850, 800, 765, 740, 690, 650, 630, 580, 570; MS, m/e 338.1539 (M^+), 338.1531 ($C_{22}H_{18}N_4$). Anal. ($C_{22}H_{18}N_4$) C, H, N.

Biological Activity. Nutrient Medium. All experiments with *Mycoplasma gallisepticum* were done in a growth medium that was a modification of the medium used by Adler[31] 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 hydrate, 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 of 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 addition of the horse serum and the benzylpenicillin, sterilization was performed by heating 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 quality obtainable.

Apparatus. Optical density of growing cultures were determined at 660 nm using 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. *Mycoplasma gallisepticum* K514, kindly supplied by the research management of Gist-brocades N.V., was used as the test organism. *Mycoplasma gallisepticum* strains can be stored at −20 °C for several months.[25] 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 (de-

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termined 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 if 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**, 118112-02-0; **2b**, 118112-03-1; **2b** (amide), 112575-49-2; **2c**, 118112-04-2; **2c** (amide), 112575-50-5; **2d**, 118112-05-3; **2e**, 118112-06-4; **2f**, 118112-07-5; **2f** (amide), 112575-53-8; **2g**, 118112-08-6; **2h**, 118112-09-7; **2h** (amide), 112575-55-0; **3a**, 118112-10-0; **3b**, 118112-11-1; **3c**, 118112-12-2; **3d**, 118112-13-3; **3e**, 118112-14-4; **3f**, 118112-15-5; **3g**, 118112-16-6; **4**, 79240-63-4; **5**, 118112-17-7; benzonitrile, 100-47-0; 4-chlorobenzonitrile, 623-03-0; 3,4-dichlorobenzonitrile, 6574-99-8; 3-methoxybenzonitrile, 1527-89-5; 2-pyridinecarbonitrile, 100-70-9; 4-pyridinecarbonitrile, 100-48-1; 2-pyrimidinecarbonitrile, 14080-23-0; 2-pyrazinecarbonitrile, 19847-12-2; 4,6-dimethyl-2-pyrimidinecarbonitrile, 22126-16-5; 6-methyl-2-pyridinecarbonitrile, 1620-75-3; 6-methyl-4-pyridinecarbonitrile, 2214-53-1; 2-aminopyridine, 504-29-0; 1-amino-5-methyl-3-phenylisoquinoline, 58814-44-1; *p*-toluoyl chloride, 874-60-2; *p*-methoxybenzoyl chloride, 100-07-2; *m*-toluoyl chloride, 1711-06-4; *m*-chlorobenzoyl chloride, 618-46-2.

Quaternary Salts of 2-[(Hydroxyimino)methyl]imidazole. 2. Preparation and in Vitro and in Vivo Evaluation of 1-(Alkoxymethyl)-2-[(hydroxyimino)methyl]-3-methylimidazolium Halides for Reactivation of Organophosphorus-Inhibited Acetylcholinesterases

Clifford D. Bedford,*,† Ralph N. Harris, III,*,‡ Robert A. Howd,‡ Dane A. Goff,‡ Gary A. Koolpe,‡ M. Petesch,‡ Alexi Miller,‡ Harold W. Nolen, III,‡ H. A. Musallam,° Robert O. Pick,△ Dennis E. Jones,§ Irwin Koplovitz,§ and Walter E. Sultan§

Organic Chemistry Department, SRI International, Menlo Park, California 94025, Drug Testing and Evaluation Branch, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, Maryland 21010, and Department of Medicinal Chemistry, Walter Reed Army Institute of Research, Washington, D.C. 20307. *Received February 29, 1988*

A series of structurally related mono- and bis-1,3-disubstituted 2-[(hydroxyimino)methyl]imidazolium halides were evaluated in vitro for their ability to reactivate electric eel, bovine, and human erythrocyte (RBC) acetylcholinesterases (AChE) inhibited by ethyl *p*-nitrophenyl methylphosphonate (EPMP) and 3,3-dimethyl-2-butyl methylphosphonofluoridate (soman, GD). All new compounds were characterized for (hydroxyimino)methyl acid dissociation constant, nucleophilicity, octanol–buffer partition coefficient, reversible AChE inhibition, and kinetics of reactivation of EPMP-inhibited AChEs. For GD-inhibited AChEs, maximal reactivation was used to compare compounds since rapid phosphonyl enzyme dealkylation "aging" complicated interpretation of kinetic constants. For comparison, we also evaluated three known pyridinium therapeutics, 2-PAM, HI-6, and toxogonin. In vivo evaluation in mice revealed that when selected imidazolium compounds were coadministered with atropine sulfate, they were effective in providing lifesaving protection against both GD and EPMP challenges. This was a major accomplishment in the search for effective anticholinesterase therapeutics—the synthesis and preliminary evaluation of the first new monoquaternary soman antidotes with potencies superior to 2-PAM. Significantly, there was an apparent inverse relationship between in vitro and in vivo results; the most potent in vivo compounds proved to be the poorest in vitro reactivators. These results suggested that an alternative and possibly novel antidotal mechanism of protective action may be applicable for the imidazolium aldoximes. Selected compounds were also evaluated for their inhibition of AChE phosphonylation by GD and antimuscarinic and antinicotinic receptor blocking effects.

Organophosphorus (OP) pesticides and chemical warfare (CW) nerve agents are powerful inhibitors of synaptic acetylcholinesterase (AChE).[1-7] Though pyridinium oximes effectively reverse intoxication symptoms in cases of accidental pesticide or nerve agent poisoning by many OP compounds, they are ineffective in preventing or treating

† Organic Chemistry Department, SRI International. Present Address: Head, Synthesis and Formulations Branch, Naval Surface Warfare Center, Code R11, White Oak Laboratory, Silver Spring, MD 20903-5000.

‡ Organic Chemistry Department and Biomedical Research Laboratory, SRI International.

° Walter Reed Army Institute of Research.

△ Present Address: U.S. Army Medical Research and Development Command, Fort Detrick, Frederick, MD.

§ U.S. Army Medical Research Institute of Chemical Defense.

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