(30 mL), washed with 1 N HCl solution, saturated aqueous $NaHCO_3$ solution, and brine, and dried; the solvent was removed in vacuo. Flash chromatography (33% EtOAc/toluene) gave 210 mg (11%) of the desired bicyclic nucleus **6c** as a yellow oil. NMR ($CDCl_3$): δ 5.98 (m, 1, OCH_2CHCH_2), 5.5–5.2 (m, 2, OCH_2CHCH_2), 5.25 (br, 1, NH), 4.85 (d, 2, J = 6, OCH_2CHCH_2), 4.56 (m, 1, $NCH(CH_2)CO$), 4.38 (d, 1, J = 13, NCH_2C), 3.95 (d, 1, J = 13, NCH_2C), 3.15 (m, 2, CH_2CH_2N), 2.80 (m, 1, CH_2CH_2N), 1.65 (m, 1, CH_2CH_2N), 1.45 (s, 9). IR ($CHCl_3$): 2230, 1747, 1704 cm^{-1}. UV (EtOH): λ_{max} 321 nm (ε 5490). FABMS: calcd for $C_{17}H_{23}N_4O_5$ 363.1668, found 363.1638, M + 1. Anal. ($C_{17}H_{22}N_4O_5$): C, H, N.

Preparation of Bicyclic Pyridazinone 11c. Acylation of Pyridazinone 6c. Pyridazinone **6c** (181 mg, 0.5 mmol) was dissolved in 5 mL of 3 N HCl(g) in glacial acetic acid. The mixture was allowed to stand for 10 min then concentrated in vacuo to remove the acetic acid. Toluene (25 mL) was added to the residue and after brief sonication was removed in vacuo. In the meantime, 2-[[(allyloxy)carbonyl]amino]-α-methoximino-4-thiazoleacetic acid[1c] (171 mg, 0.6 mmol) was slurried in CH_2Cl_2 (5 mL) and cooled to 0 °C. *N*-Methylmorpholine (66 μL, 0.6 mmol) was added followed by $POCl_3$ (57 μL, 0.6 mmol), and the resulting solution was stirred at 0 °C for 20 min. Additional *N*-methylmorpholine (200 μL, 1.8 mmol) was added followed by a solution of the above prepared deblocked nucleus in CH_2Cl_2 (3 mL). The mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. The mixture was diluted with EtOAc (50 mL) and washed with water, 1 N HCl solution, saturated aqueous $NaHCO_3$ solution, and brine. Drying followed by concentration in vacuo gave a yellow powder. Flash chromatography (3% MeOH/CH_2Cl_2) gave 150 mg (57%) of the desired acylation product **11c** as a yellow powder. NMR ($CDCl_3$): δ 9.20 (s, 1, NH), 7.86 (br d, 1, J = 9, NH), 7.18 (s, 1, SCHC), 5.95 (m, 2, OCH_2CHCH_2), 5.35 (m, 4, OCH_2CHCH_2), 5.13 (q, 1, J = 9, $NCH(CH_2)CO$), 4.83 (d, 2, J = 6, OCH_2CHCH_2), 4.74 (d, 2, J = 6, OCH_2CHCH_2), 4.44 (d, 1, J = 13, NCH_2C), 4.00 (d, 1, J = 13, NCH_2C), 3.97 (s, 3, $NOCH_3$), 3.20 (m, 2, CH_2CH_2N), 2.85 (m, 1, CH_2CH_2N), 1.88 (m, 1, CH_2CH_2N). IR ($CHCl_3$): 2225, 1747, 1733, 1694, 1673 cm^{-1}. UV (EtOH): λ_{max} 313 nm (ε 8010), 266 (ε 14 100), 225 (ε 21 100). FDMS: m/e 529, M+.

Preparation of 13c. Deprotection of Pyridazinone 11c. To a solution of acylated pyridazinone **11c** (140 mg, 0.26 mmol) in CH_2Cl_2 (3 mL) was added $(Ph_3P)_2PdCl_2$ (14 mg, 0.02 mmol), glacial AcOH (58 μL, 1 mmol), and *n*-Bu_3SnH (157 μL, 0.58 mmol). This mixture was stirred at room temperature overnight at which time a precipitate had formed. This material was collected by filtration and then purified by reverse-phase medium-pressure liquid chromatography (C18 Lobar column, material was dissolved in water containing 1 mmol of $NaHCO_3$ and eluted with 20% MeOH/water) to give after freeze-drying: 100 mg (90%) of **13c** as a light tan powder. Analytical HPLC on a Waters C18 μ-Bondpak column (30% MeOH/1% AcOH/water) showed a single peak with t_R = 2.48 min. NMR (D_2O) partial: δ 7.16 (s, 1, SCHC), 4.27 (d, 1, J = 13, NCH_2C), 4.09 (d, 1, J = 13, NCH_2C), 3.94 (s, 3, $NOCH_3$), 3.25 (m, 2, CH_2CH_2N), 2.59 (m, 1, CH_2CH_2N), 1.95 (m, 1, CH_2CH_2N). IR (KBr): 2220, 1646, 1642 cm^{-1}. UV (EtOH): λ_{max} 299 nm (ε 10 600), 232 (ε 13 000). FABMS: calcd for $C_{15}H_{15}N_7O_5SNa$ 428.0753, found 428.0752, M + 1.

Computational Chemistry. The SYBYL molecular modeling software (versions 3.5 and 5.3) was run on a VAX 8800 minisupercomputer. Macintosh II, Modgraph GX1000, and Evans and Sutherland PS330 terminals were used for molecular graphics. MOPAC (Version 4.0) was run on the Cray X-MP/48 supercomputer at the National Center for Supercomputing Applications (University of Illinois, Urbana—Champaign).

Acknowledgment. We are grateful to Dr. F. T. Counter and C.-Y. E. Wu for the antibacterial activity evaluations, to the scientists in our Physical Chemistry Department for chemical and spectroscopic analyses, and to Mike Krogh (National Center for Supercomputing Applications) for developing software on the Cray supercomputer to facilitate use of MOPAC. This was the first project completed on the Cray supercomputer after Lilly's establishment of an industrial partnership at NCSA.

Supplementary Material Available: Four tables listing the MNDO-optimized Cartesian atomic coordinates (4 pages). Ordering information is given on any current masthead page.

Synthesis and Quantitative Structure–Activity Relationship Analysis of 2-(Aryl or Heteroaryl)quinolin-4-amines, a New Class of Anti-HIV-1 Agents

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Thirty-eight 2-(aryl or heteroaryl)quinolin-4-amines, N,N-disubstituted, N-monosubstituted, and without a substituent at the amino group have been synthesized with use of novel chemistries developed by us recently. Some of these derivatives show anti-HIV-1 activity at a concentration level of 1 μM and low cell toxicity in vitro. The most active and least toxic compounds are derivatives of 2-(3-pyridyl)quinoline. The results of the quantitative structure–activity relationship analyses, including several classical, linear regression correlations and a Free–Wilson approach of de novo model, provide guidelines for the design of new active compounds of this class.

Recently we analyzed a short series of heteropolyaromatic compounds as potential anti-HIV-1 agents.[1] Several moderately active derivatives were identified which contained alkylamino, dialkylamino, or alkoxy substituents located ortho or para to the ring nitrogen atoms. By contrast, the alkylthio-substituted analogues and non-substituted parent heteropolyaromatic systems [e.g., 4,6-di-2-thienylpyrimidine] generally were inactive. These results were interpreted in terms of different electronic effects in the two sets of molecules. It is known that the amino and alkoxy groups are strongly conjugated with an aromatic ring system while this conjugation is relatively

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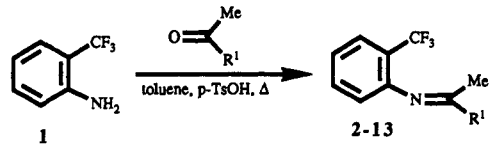
[∥] Deceased.

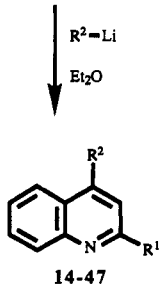
(1) Strekowski, L.; Mokrosz, M. J.; Harden, D. B.; Mokrosz, J. L.; Wilson, W. D. In *Advances in Chemotherapy of AIDS*; Diasio, R. B., Sommadossi, J.-P., Eds.; Pergamon: New York, 1990; Chapter 5, p 43.

0022-2623/91/1834-1739$02.50/0

Scheme I[a]

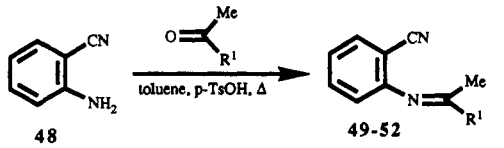


2-13	R^1
2	phenyl
3	4-F-Ph
4	4-Cl-Ph
5	4-Br-Ph
6	4-MeO-Ph
7	4-Me-Ph
8	4-Ph-Ph
9	2-pyridyl
10	3-pyridyl
11	4-pyridyl
12	2-furanyl
13	2-thienyl

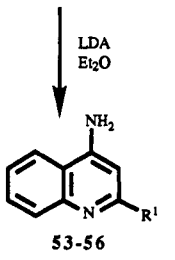


[a] See Table I for R^2 in 14-47.

Scheme II



49-56	R^1
49, 53	phenyl
50, 54	2-pyridyl
51, 55	3-pyridyl
52, 56	2-thienyl



weak for the thio derivatives.[2] A working hypothesis was formulated that the increased electron density at the ring nitrogen atoms, due to the conjugation, has a favorable effect on the anti-HIV-1 activity. This increased electron density may stabilize a hydrogen-bond complex of the active molecule with a biological receptor, possibly the viral RNA.[1]

In continuation of our studies, we now report the synthesis and anti-HIV-1 activity in vitro of quinolin-4-amines **14-47** (Scheme I) and **53-56** (Scheme II), a set of molecules with the structural characteristics suggested previously to have a favorable effect on the activity. The biological activity is also analyzed using quantitative structure-activity relationships (QSAR). The rationale for this study is to delineate factors, in addition to the already suggested stereoelectronic effect, that influence the anti-HIV-1 potency of this and related classes of compounds.

Chemistry

Recently we described a new, highly efficient synthetic route to *N*-alkyl- or *N,N*-dialkyl-substituted 2-phenylquinolin-4-amines.[3] For example, 2-(trifluoromethyl)aniline (1, Scheme I) was condensed with acetophenone to give ketimine **2**. Lithium isopropylamide mediated cyclization of **2** furnished *N*-isopropyl-2-phenylquinolin-4-amine **14** (Scheme I, R_2 = Me_2CHNH, and Table I) in a high yield. As an extension of this study we now report the scope and limitations of this novel quinoline synthesis for the preparation of other derivatives. Complete mechanistic studies will be reported elsewhere.[4]

As can be seen from Scheme I a large number of aryl methyl ketones and heteroaryl methyl ketones was condensed successfully with **1** to give the corresponding ketimines **2-13**, all in high yields. As shown by NOE experiments, compounds **2-13** are single *E* diasteromers. The treatment of these ketimines with a large variety of lithium alkylamide or lithium dialkylamide reagents produced the expected quinoline products **14-47** in all cases studied. With few exceptions the yields of isolated quinolines were good to excellent. Although a chromatographic separation was often required, the use of simple flash chromatography usually gave satisfactory results.

The lithium amide reagents were conveniently prepared in situ from *n*-butyllithium and the corresponding amines. The cyclization reactions of ketimines **2-13** with lithium dialkylamides thus prepared were efficient regardless of the presence or absence of a free dialkylamine in the reagent mixture. By contrast, the use of an alkylamine-free lithium alkylamide reagent was necessary to obtain a corresponding 4-alkylamino-substituted quinoline in a high yield.[3,4]

On the other hand, this method is not suitable for the preparation of quinolin-4-amines with a primary amino group, such as **53-56** (Scheme II). We could not cyclize the ketimine **2** in attempted reactions with lithium, sodium, or potassium amide reagents and under various solvent and temperature conditions. The desired compounds **53-56** were obtained, however, in a lithium diisopropylamide mediated cyclization of ketimines **49-52** derived from 2-aminobenzonitrile (**48**). A preliminary account of this cyclization reaction has been published.[5]

Biological Evaluations

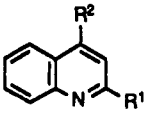
The quinolinamines were evaluated for their potential toxic effects on uninfected phytohemagglutinin (PHA) stimulated human PBM cells and for the antiviral activity in the PHA-stimulated PBM cells infected with HIV-1 (strain LAV-1) as described previously.[1,6-8] The preliminary cell toxicity results expressed as minimum toxic concentrations (MTC) and the antiviral median effective concentrations (EC_{50}) are given in Table I. The uncertainty in the reported EC_{50} values is estimated to gradually increase from ±3 μM for the more active quinolinamines (EC_{50} < 10 μM) to ±10 μM for derivatives with a marginal activity (EC_{50} > 50 μM).

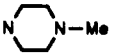
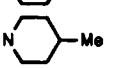
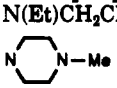
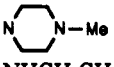
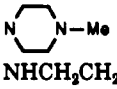
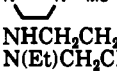
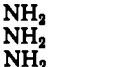
QSAR

Classical, linear regression analyses were conducted with inductive substituent constants[9] σ^* and calculated[10] va-

(2) Strekowski, L.; Mokrosz, J. L.; Tanious, F. A.; Watson, R. A.; Harden, D.; Mokrosz, M. Edwards, W. D.; Wilson, W. D. *J. Med. Chem.* **1988**, *31*, 1231.
(3) Strekowski, L.; Wydra, R. L.; Cegla, M. T.; Czarny, A.; Harden, D. B.; Patterson, S. E.; Battiste, M. A.; Coxon, J. M. *J. Org. Chem.* **1990**, *55*, 4777.
(4) A paper in preparation of *J. Org. Chem.*
(5) Strekowski, L.; Kong, S.-B.; Cegla, M. T.; Harden, D. B. *Heterocycles* **1989**, *29*, 539.
(6) Schinazi, R. F.; Cannon, D. L.; Arnold, B. H.; Martino-Saltzman, D. *Antimicrob. Agents Chemother.* **1988**, *32*, 1784.
(7) Sommadossi, J.-P.; Carlisle, R.; Schinazi, R. F.; Zhou, Z. *Antimicrob. Agents Chemother.* **1988**, *32*, 997.
(8) Chou, J.; Chou, T.-C. Dose-effect analysis with microcomputers: Quantitation of ED_{50}, LD_{50} synergism, antagonism, low-dose risk, receptor binding and enzyme kinetics. *A Computer Software for Apple II Series and IBM PC and Instruction Manual*; Elsevier-Biosoft, Elsevier Science: Cambridge, U.K., 1985.
(9) Perrin, D. D.; Dempsey, B.; Serjeant, E. P. *pKa Prediction for Organic Acids and Bases*; Chapman and Hall: London, 1981.

Table I. In Vitro Anti-HIV-1 Activity (EC_{50}) and Toxicity (MTC) and Structural Parameters Used in Correlation Analyses of Quinolin-4-amines **14–47, 53–56**



no.	substituents		biological data		structural parameters		
	R^1	R^2	EC_{50}, μM	MTC, μM	σ^{*a}	$^1\chi^v$	$^3\chi_p{}^v$
14	Ph	$NHCHMe_2$	100	>100	0.75		0.577[b]
15	Ph	$NHCH_2CHMe_2$	50.6	100	0.75		0.612[b]
16	Ph	$NHCMe_3$	100		0.75		0.750[b]
17	Ph		26.4	100	0.75		2.135[b]
18	Ph		16.7	100	0.75		1.860[b]
19	Ph	$NHCH_2CH_2NMe_2$	10.0	100	0.75		3.660
20	Ph	$N(Et)CH_2CH_2NMe_2$	34.3	100			
21	Ph		34.8				
22	4-F-C_6H_4	$NHCH_2CH_2NMe_2$	13.2	100	0.81		3.731
23	4-Cl-C_6H_4	$NHCH_2CH_2NMe_2$	100		0.92		3.983
24	4-Br-C_6H_4	$NHCH_2CH_2NMe_2$	44.2	100	0.86		4.259
25	4-MeO-C_6H_4	$NHCH_2CH_2NMe_2$	10.4	10	0.60		3.976
26	4-Me-C_6H_4	$NHCH_2CH_2NMe_2$	4.47	10	0.59		3.938
27	2-pyridyl		4.24	100	0.56	8.406	2.135[b]
28	2-pyridyl		4.86	100	0.56	8.372	1.860[b]
29	2-pyridyl		4.08	100	0.56	8.372	2.007[b]
30	2-pyridyl		18.5			7.556	
31	2-pyridyl		2.81			8.710	
32	2-pyridyl	$NHCH_2CH_2NMe_2$	1.35	100	0.56		3.518
33	2-pyridyl	$N(Et)CH_2CH_2NMe_2$	13.2			8.058	
34	2-pyridyl		5.51			8.396	
35	3-pyridyl		33.7	100	0.73		1.330[b]
36	3-pyridyl		20.0	100	0.73		1.580[b]
37	3-pyridyl	$NHCH_2CH_2NMe_2$	1.51	>100	0.73		3.548
38	3-pyridyl	$N(Et)CH_2CH_2NMe_2$	24.2	100			
39	3-pyridyl		1.00	>100			
40	4-pyridyl	$NHCH_2CH_2NMe_2$	36.9	100			
41	4-pyridyl	$N(Et)CH_2CH_2NMe_2$	100	100			
42	4-pyridyl		68.2				
43	2-furanyl	$NHCH_2CH_2NMe_2$	0.91	>1	0.25		3.364
44	2-furanyl		15.8				
45	2-thienyl	$NHCH_2CH_2NMe_2$	0.57	10	0.31		3.364
46	2-thienyl	$N(Et)CH_2CH_2NMe_2$	32.4	100			
47	2-thienyl		9.07				
53	phenyl	NH_2	25.7				
54	2-pyridyl	NH_2	8.56	100			
55	3-pyridyl	NH_2	1.00	85.7			
56	2-thienyl	NH_2	34.3	10			

[a] Inductive constants for substituents R^1 at position 2 of the quinoline, taken from ref 9. [b] Calculated for substituents R^2 at position 4 of the quinoline including the C4 atom.

lence molecular connectivity indices[11,12] of the first order $^1\chi^v$ and third order path type $^3\chi_p{}^v$ given in Table I. The $^1\chi^v$ indices encode efficiently the additive and constitutive nature of complex molecules or substituents including their basic stereochemical[12] and electronic[13] properties. The $^3\chi_p{}^v$ indices characterize well a saturated hydrocarbon fragment, and are sensitive to the position and type of branching.[11,14] The molecular connectivity indices are also helpful in estimation of the lipophilic character of substituents and molecules.[12,15]

In this work the quality of all single and multiparameter correlations are characterized by a regression coefficient r, a standard deviation s, and by an F_x ratio at the given probability level x.

Since the quinolines under study contain diverse substituents, a Free–Wilson approach of de novo model[16,17] was also applied to the analysis of the anti-HIV-1 activity (eq 1). In this nonparameter model, for every compound

$$\log BA_i = \mu + \sum_j \alpha_{jk} X_{jk} \quad (1)$$

of the series the biological activity values BA_i used in the logarithmic scale are expressed as the sum of the biological activity contributions α_{jk} of the substituents R_k in each position j, referring to the overall average μ. In eq 1, X_{jk} has a value of 1 when the substituent R_k is present in the position j; otherwise its value is zero.[17] A computer program[18] has been developed recently for a convenient determination of the constant μ and individual substituent contributions α_{jk}.

Results and Discussion

As can be seen from Table I the anti-HIV-1 activity in vitro of quinolinamines **14–47** and **53–56** is strongly dependent on the structure of both the substituent at position 2 and the amino group at position 4 of the quinoline. For the available pairs of compounds with the same aromatic group at position 2 and with 2-(dimethylamino)-ethylamino or *N*-ethyl-2-(dimethylamino)ethylamino group at position 4, the former amino derivatives are much more active: **19** > **20**, **32** > **33**, **37** > **38**, and **45** > **46**. Interestingly, these relative activities parallel the relative electron densities in the quinoline ring system for all four pairs of compounds as obtained from analysis of the ^{1}H NMR spectra. It is known that chemical shifts of the aromatic protons are sensitive to π-electron density in the aromatic system, and in the absence of other factors, they shift upfield in the electron-rich environment.[19] The electron resonance effect in quinolin-4-amines increases electron densities at N1 and C3 atoms of the quinoline. Thus, in all cases the signal for H3 for the more active alkylamino derivative is shifted upfield 0.50 ± 0.05 ppm in comparison to the chemical shift of the corresponding, less active dialkylamino analogue. Even the chemical shift for the H8 atom adjacent to N1 is affected by this structural change and is located 0.06 ± 0.02 ppm upfield in the spectrum of the alkylamino derivative in comparison to the absorption position of the corresponding dialkylamino analogue. These results suggest that the electron-density distribution in the aromatic system of a quinolinamine greatly affects the anti-HIV-1 activity. The more active molecules are also more electron-rich in the quinoline portion of the molecule due to a stronger resonance effect. This conjugation is efficient for the quinoline ring system substituted at C4 with an alkylamino group. By contrast, the more sterically demanding dialkylamino group cannot attain a conformation favorable for an efficient overlap of the lone electron pair of the amino nitrogen with the aromatic π-system of the quinoline. As a result, such sterically hindered derivatives are less electron-rich in the quinoline part of the molecule and show lower anti-HIV-1 activity.

The generally low activities of the dialkylamino derivatives discussed above may also be caused, in part, by unfavorable steric interactions of these sterically hindered molecules with a biological receptor. Although this possibility cannot be ruled out, the additional information obtained with the help of QSAR analyses is consistent with the suggested importance of the electron-density distribution on the anti-HIV-1 activity of this class of compounds. The QSAR analyses also show that the polarization effect, although important, is not the only factor responsible for the biological activity of a quinolinamine.

Significant correlations were obtained for compounds **19**, **22–26**, **32**, **37**, **43**, **45**, all containing 2-(dimethylamino)ethylamino group at position 4 of the quinoline and differing in the inductive substituent constants σ^* for their 2-aryl or 2-heteroaryl substituents (eqs 2 and 3). As can

$$\log (1/EC_{50}) = 1.06\ (\pm 0.42) - 2.83\ (\pm 0.62)\sigma^* \quad (2)$$

compounds **19**, **22–26**, **32**, **37**, **43**, **45**

$n = 10$, $r = 0.849$, $s = 0.416$, $F_{0.002} = 20.8$

$$\log (1/EC_{50}) = 5.06\ (\pm 1.64) - 1.62\ (\pm 0.69)\sigma^* - 1.28\ (\pm 0.52)^3\chi_p{}^v \quad (3)$$

compounds **19**, **22–26**, **32**, **37**, **43**, **45**

$n = 10$, $r = 0.923$, $s = 0.324$, $F_{0.001} = 20.1$

be seen from the one-parameter equation 2, the anti-HIV-1 activity of this set of quinolinamines increases with decreasing electron-withdrawing character of the substituent at position 2 of the quinoline. The same conclusion remains valid for the two-parameter equation 3. Additionally, this correlation shows that a decreased lipophilicity of the 2-substituent increases the anti-HIV-1 activity. Since both the lipophilicity and the electronic effect determine the activity, it is not surprising that the two-parameter correlation 3 is much better than the one-parameter correlation 2 for the same set of compounds.

2-Pyridyl derivatives **27–31**, **33**, and **34** all contain a disubstituted amino group with similar steric hindrance at position 4 of the quinoline. This structural feature results in a similar electron density distribution in the quinoline ring system in all compounds of the series. These compounds were chosen, thus, for the initial analysis of the structural effect of the amino group on the biological

(10) Hall, L. H. *Molconn 2, Version 2.0., a program for Molecular Topology Analysis*; Hall Associates Consulting: Quincy, MA 02170, 1989.
(11) Kier, L. B.; Hall, L. H. *Molecular Connectivity in Chemistry and Drug Research*; Academic: New York, 1976.
(12) Kier, L. B.; Hall, L. H. *Molecular Connectivity in Structure–Activity Analysis*; Wiley: New York, 1986.
(13) For a correlation of $^1\chi^v$ indices of various groups with their electronegativities taken in Mulliken scale, see: Kier, L. B.; Hall, L. H. *J. Pharm. Sci.* **1981**, *70*, 583. The polarity indice $-^1\chi^v$ permits quantitative evaluation of the polarity of compound directly from its structural formula: Kier, L. B. *J. Pharm. Sci.* **1981**, *70*, 930.
(14) Mokrosz, J. L. *Quant. Struct.-Act. Relat.* **1989**, *8*, 305.
(15) Kaliszan, R. *Quantitative Structure–Chromatographic Retention Relationships*; Wiley: New York, 1987.
(16) Free, S. M.; Wilson, J. W. *J. Med. Chem.* **1964**, *7*, 395.
(17) Kubinyi, H. *Quant. Struct.-Act. Relat.* **1988**, *7*, 121.
(18) Coburn, R. A. *QSAR-PC: PAR. Quantitative Structure–Activity Relationship Studies. Physicochemical-Activity Relationship Methods for IBM PC*; Biosoft: Cambridge, U.K., 1987.
(19) Kowalewski, A.; Strekowski, L.; Szajda, M.; Walenciak, K.; Brown, D. J. *Aust. J. Chem.* **1981**, *34*, 2629.

activity (eq 4). A statistically valid correlation was ob-

$$\log (1/EC_{50}) = -7.14\ (\pm 0.92) + 0.77\ (\pm 0.11)^1\chi^v \quad (4)$$

compounds **27–31, 33, 34**

$$n = 7,\ r = 0.952,\ s = 0.100,\ F_{0.001} = 47.9$$

tained with use of the molecular connectivity indices $^1\chi^v$ as the only structural descriptor. This result is consistent with a qualitative observation that the size and shape of the amino substituents is important for the activity. This conclusion is even better formulated in the analysis of closely related compounds **14–18, 27–29, 35, 36** using both the structural descriptors $^3\chi_p{}^v$ for the 4-substituents and the inductive constants σ^* for the 2-substituents of the quinoline (eq 5). This statistically highly significant

$$\log (1/EC_{50}) = 0.53\ (\pm 0.53) - 3.56\ (\pm 0.64)\sigma^* + 0.41\ (\pm 0.09)^3\chi_p{}^v \quad (5)$$

compounds **14–18, 27–29, 35, 36**

$$n = 10,\ r = 0.972,\ s = 0.140,\ F_{0.001} = 60.82$$

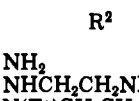
correlation was obtained with quinolines substituted at position 2 with isosteric phenyl, 2-pyridyl, and 3-pyridyl groups. The C-4 amino function is substituted with a bulky alkyl group or is disubstituted with an α,ω-alkanediyl moiety to form a cyclic structure. Similar steric conditions around the amino group minimize differences in the electron densities at the quinoline, due to the conjugation effect. Also, the purely hydrocarbon substituents of the amino function allow analysis of their hydrophobic effect without complication by the presence of a heteroatom. This hydrophobic effect[12,15] is characterized well by the structural descriptors $^3\chi_p{}^v$ used in eq 5. As can be seen from this correlation, the activity generally increases with increasing steric bulk of the hydrocarbon moiety, which is known to parallel hydrophobicity. This is an interesting result because, as shown previously in eq 3, increasing lipophilicity of the 2-substituent decreases the anti-HIV-1 activity.

The delineation of the electronic effect–activity relationship is another important result of these studies. In general, the activity increases with decreasing steric hindrance around the C4-amino substituent, thus increasing electron density in the quinoline part of the molecule, due to resonance.

The Free–Wilson analysis was conducted with a series of compounds **19–21, 32–34, 37–47, 53–56** for which 2-(phenyl or heteroaryl)quinoline systems were substituted with common amino groups found in most of the molecules. The results (eq 6) are not only consistent with the conclusions given above but all provide an additional insight into the structure–activity relationship of this class of compounds.

$$\log (1/EC_{50}) = 0.97 + \alpha_{R^1} + \alpha_{R^2} \quad (6)$$

compounds **19–21, 32–34, 37–47, 53–56**

R^1	α_{R^1}	R^2	α_{R^2}
phenyl	−0.37	NH_2	−0.14
2-pyridyl	0.27	$NHCH_2CH_2NMe_2$	0.56
3-pyridyl	0.61	$N(Et)CH_2CH_2NMe_2$	−0.49
4-pyridyl	−0.84		
2-furyl	0.14		−0.06
2-thienyl	0.06		

$$n = 21,\ r = 0.889,\ s = 0.41,\ F_{0.01} = 5.67$$

The most striking observation is a high contribution of the 3-pyridyl, 2-pyridyl, and 2-furyl substituents into the activity, which sets these substituents apart from other aromatic groups. While the 2-thienyl substituent appears to be neutral, the activity is decreased for phenyl-substituted quinolines and strongly decreased for 4-pyridyl derivatives. These findings suggest that the distance between the quinoline N1 atom and the heteroatom of the aromatic substituent is a pivotal structure feature in the compounds investigated for their anti-HIV-1 activity. It is tempting to speculate that the quinoline N1 atom and the heteroatom (N or O) of the heteroaryl group in the most active 2-heteroarylquinolines are favorably positioned to form a specific hydrogen-bonded complex with a biological receptor. A strong conjugation effect of the 4-amino group with the quinoline would increase the stability of such a complex by increasing electron density at the quinoline nitrogen atom. A specific interaction can also be suggested for the terminal dimethylamino moiety of the 2-(dimethylamino)ethylamino substituent. As can be seen from eq 6, this substituent is the only one with a strong positive contribution to the antiviral activity.

Conclusions

In summary, the quinolinamines described in this paper represent a new class of anti-HIV-1 agents. Some of these compounds have good selectivity against this virus in culture. The finding of a new class of nonnucleoside antiviral agents is important, as these compounds act by a mechanism that is different from that of nucleosides. Drug resistance to zidovudine, a nucleoside currently in use for the treatment of HIV-1 infections, has been reported.[20,21] This is not surprising since there is significant genetic variation in HIV-1 over time in patients with AIDS or at risk for AIDS, and the rate of evolution of HIV-1 was estimated to be a million-fold greater than for most DNA viruses and about 10-fold greater than for some other RNA viruses.[22]

Although the mechanism of the anti-HIV-1 activity for quinolinamines is not known, the QSAR analysis results clearly provide guidelines for the design of new derivatives with the expected activity. On the basis of the activity and cell toxicity results, 2-(3-pyridyl)quinolin-4-amines appear to be the most promising candidates for these drug-development studies. For example, 2-(3-pyridyl)quinolines with an additional electron-donating substituent at position 4 or 6 of the pyridine as expected to be more active than the model compounds analyzed in this work. Since 4-alkoxyquinolines are highly polarized molecules, due to conjugation of the oxygen electrons with the quinoline system, they also are expected to be active. Such quinolines are available in the cyclization reaction of the ketimines described in this work in the presence of alkoxide bases.[3,4]

Experimental Section

All reagents were obtained from Aldrich. Amines were stored over pellets of sodium hydroxide. Reactions with *n*-butyllithium (2.6 M in hexanes) were conducted in ether distilled from sodium benzophenone ketyl immediately before use and under static pressure of nitrogen. The glassware was dried at 140 °C, assembled hot, and cooled in a stream of nitrogen. The liquids were transferred with syringes.

Melting points (Pyrex capillary) are uncorrected. Unless stated otherwise, ^{1}H NMR spectra were obtained on a Varian VXR-400 (400 MHz) at 25 °C in $CDCl_3$ solutions with Me_4Si as an internal reference. Coupling constants smaller than 1.5 Hz are not re-

(20) Larder, B. A.; Darby, G.; Richman, D. D. *Science* **1989**, *243*, 1731.
(21) Larder, B. A.; Kemp, S. D. *Science* **1989**, *246*, 1155.
(22) Hahn, B. H.; Shaw, G. M.; Taylor, M. E.; Redfield, R. R. *Science* **1986**, *232*, 1548.

ported. Mass spectra (70 eV) were recorded on a Varian MAT spectrometer. Elemental analyses (Atlantic Microlab, Inc., Atlanta, GA) were within 0.3%, 0.1%, and 0.2% for C, H, and N, respectively.

General Procedure A. Preparation of Ketimines 2–13. A solution of 2-(trifluoromethyl)aniline (1; 1.61 g, 10 mmol), and methyl aryl (or heteroaryl) ketone (13 mmol), and *p*-toluenesulfonic acid (50 mg) in toluene (50 mL) was heated under reflux for 10 h with azeotropic removal of water. The mixture was then concentrated on a rotary evaporator, and the oily residue was fractionated on a Kugelrohr (100–150 °C/0.1–0.5 mmHg). Solid products **2**, **4–8**, **10–13** were additionally crystallized from toluene/hexanes.

***N*-(1-Phenylethylidene)-2-(trifluoromethyl)aniline (2)**: yield 90%; mp 22–25 °C (reported[23] as an oil).

***N*-[1-(4-Fluorophenyl)ethylidene]-2-(trifluoromethyl)-aniline (3)**: yield 95%; an oil; ^{1}H NMR δ 2.18 (s, 3 H), 6.75 (d, J = 8 Hz, 1 H), 7.10–7.17 (m, 3 H), 7.48 (t, J = 8 Hz, 1 H), 7.64 (d, J = 8 Hz, 1 H), 7.97 (m, 2 H). Anal. ($C_{15}H_{11}F_4N$) C, H, N.

***N*-[1-(4-Chlorophenyl)ethylidene]-2-(trifluoromethyl)-aniline (4)**: yield 71%; mp 48–49 °C; ^{1}H NMR δ 2.18 (s, 3 H), 6.76 (d, J = 8 Hz, 1 H), 7.17 (t, J = 8 Hz, 1 H), 7.43 (d, J = 9 Hz, 2 H), 7.50 (t, J = 8 Hz, 1 H), 7.66 (d, J = 8 Hz, 1 H), 7.91 (d, J = 9 Hz, 2 H). Anal. ($C_{15}H_{11}ClF_3N$) C, H, N.

***N*-[1-(4-Bromophenyl)ethylidene]-2-(trifluoromethyl)-aniline (5)**: yield 50%; mp 63–65 °C; ^{1}H NMR δ 2.17 (s, 3 H), 6.75 (d, J = 8 Hz, 1 H), 7.16 (t, J = 8 Hz, 1 H), 7.50 (t, J = 8 Hz, 1 H), 7.59 (d, J = 8 Hz, 2 H), 7.65 (d, J = 8 Hz, 1 H), 7.84 (d, J = 8 Hz, 2 H). Anal. ($C_{15}H_{11}BrF_3N$) C, H, N.

***N*-[1-(4-Methoxyphenyl)ethylidene]-2-(trifluoromethyl)aniline (6)**: yield 53%, mp 69–71 °C; ^{1}H NMR δ 2.16 (s, 3 H), 3.87 (s, 3 H), 6.96 (d, J = 9 Hz, 2 H), 7.13 (t, J = 8 Hz, 1 H), 7.48 (t, J = 8 Hz, 1 H), 7.64 (d, J = 8 Hz, 1 H), 7.94 (d, J = 9 Hz, 1 H). Anal. ($C_{16}H_{14}F_3NO$) C, H, N.

***N*-[1-(4-Methylphenyl)ethylidene]-2-(trifluoromethyl)-aniline (7)**: yield 78%; mp 45–47 °C; ^{1}H NMR δ 2.17 (s, 3 H), 2.41 (s, 3 H), 6.76 (d, J = 8 Hz, 1 H), 7.14 (t, J = 8 Hz, 1 H), 7.26 (d, J = 8 Hz, 2 H), 7.48 (t, J = 8 Hz, 2 H), 7.64 (d, J = 8 Hz, 1 H), 7.87 (d, J = 8 Hz, 2 H). Anal. ($C_{16}H_{14}F_3N$) C, H, N.

***N*-[1-(4-Biphenylyl)ethylidene]-2-(trifluoromethyl)aniline (8)**: yield 83%; mp 137–138 °C; ^{1}H NMR δ 2.23 (s, 3 H), 6.79 (d, J = 8 Hz, 1 H), 7.16 (t, J = 8 Hz, 1 H), 7.39 (t, J = 8 Hz, 1 H), 7.45–7.53 (m, 3 H), 7.64–7.72 (m, 5 H), 8.05 (d, J = 9 Hz, 2 H). Anal. ($C_{21}H_{16}F_3N$) C, H, N.

***N*-[1-(2-Pyridyl)ethylidene]-2-(trifluoromethyl)aniline (9)**: yield 80%; an oil; ^{1}H NMR (60 MHz) δ 2.37 (s, 3 H), 6.70–6.93 (m, 1 H), 7.03–7.97 (m, 5 H), 8.20–8.47 (m, 1 H), 8.60–8.80 (m, 1 H). Anal. ($C_{14}H_{11}F_3N_2$) C, H, N.

***N*-[1-(3-Pyridyl)ethylidene]-2-(trifluoromethyl)aniline (10)**: yield 85%; mp 45–47 °C; ^{1}H NMR δ 2.22 (s, 3 H), 6.77 (d, J = 8 Hz, 1 H), 7.17 (t, J = 8 Hz, 1 H), 7.38 (m, 1 H), 7.50 (t, J = 8 Hz, 1 H), 7.66 (d, J = 8 Hz, 1 H), 8.29 (d, J = 8 Hz, 1 H), 8.71 (d, J = 5 Hz, 1 H), 9.15 (s, 1 H). Anal. ($C_{14}H_{11}F_3N$) C, H, N.

***N*-[1-(4-Pyridyl)ethylidene]-2-(trifluoromethyl)aniline (11)**: yield 84%; mp 72–74 °C; ^{1}H NMR δ 2.21 (s, 3 H), 6.76 (d, J = 8 Hz, 1 H), 7.21 (t, J = 8 Hz, 1 H), 7.53 (t, J = 8 Hz, 1 H), 7.68 (t, J = 8 Hz, 1 H), 7.79 (d, J = 6 Hz, 2 H), 8.76 (d, J = 6 Hz, 1 H). Anal. ($C_{14}H_{11}F_3N_2$) C, H, N.

***N*-[1-(2-Furanyl)ethylidene]-2-(trifluoromethyl)aniline (12)**: yield 80%; mp 25–27 °C; ^{1}H NMR (60 MHz) δ 2.08 (s, 3 H), 6.43–6.55 (m, 1 H), 6.70–6.90 (m, 1 H), 6.97–7.78 (m, 5 H). Anal. ($C_{13}H_{10}F_3NO$) C, H, N.

***N*-[1-(2-Thienyl)ethylidene]-2-(trifluoromethyl)aniline (13)**: yield 82%; mp 67–69 °C; ^{1}H NMR δ 2.20 (s, 3 H), 6.79 (d, J = 8 Hz, 1 H), 7.10 (m, 1 H), 7.16 (t, J = 8 Hz, 1 H), 7.46–7.51 (m, 3 H), 7.64 (d, J = 8 Hz, 1 H). Anal. ($C_{13}H_{10}F_3NS$) C, H, N.

General Procedure B. Preparation of Quinolin-4-amines 14–47. A solution of an amine (6 mmol) in ether (15 mL) was treated with the commercial solution of *n*-butyllithium (2.3 mL, 6 mmol) at −10 °C, and the resultant mixture was stirred at −10 °C for 20 min before treatment with a solution of a ketimine (**2–13**, 1.5 mmol) in ether (5 mL). The mixture was stirred at −5 °C (with a primary amine) or at −10 °C (with a secondary amine) for an additional 45 min and then quenched with water (0.5 mL). The organic layer was concentrated on a rotary evaporator, and the residue was purified by silica gel chromatography (hexanes/Et_3N/EtOH, 7:2:1). A simple removal of colored polymeric materials on a short silica gel column was sufficient for a crystalline product which was further purified by crystallization from hexanes. The purification of an oily quinolin-4-amine required the use of a conventional chromatographic procedure. The noncrystalline product was additionally purified by crystallization of its hydrobromide salt. Thus, a solution of a quinolin-4-amine in EtOH was treated with a solution of hydrobromic acid (4 molar equiv) in EtOH/H_2O (9:1), and the resultant mixture was concentrated to precipitate the hydrobromic salt. The salt was crystallized twice from EtOH or EtOH/hexanes. The composition was determined by elemental analysis.

Yields, melting points, and ^{1}H NMR spectra of *N*-isopropyl-2-phenylquinolin-4-amine (**14**), *N*-isobutylquinolin-4-amine (**15**), and *N*-*tert*-butyl-2-phenylquinolin-4-amine (**16**) are given in a preliminary communication.[3]

4-(2-Methylpiperidino)-2-phenylquinoline (17): obtained from 2-methylpiperidine and **2**; yield 73%; an oil; ^{1}H NMR δ 1.02 (d, J = 6 Hz, 3 H), 1.78 (m, 6 H), 2.68–3.95 (m, 3 H), 7.30–7.82 (m + s at δ 7.37, 6 H); 8.03–8.25 (m, 4 H); MS m/e 302 (39, M^+), 287 (100), 245 (12), 204 (19). **17**·HBr: mp 255–257 °C. Anal. ($C_{21}H_{22}N_2$·HBr) C, H, N.

4-(3-Methylpiperidino)-2-phenylquinoline (18): obtained from 3-methylpiperidine and **2**; yield 62%; an oil; ^{1}H NMR (60 MHz) δ 0.95 (d, J = 6 Hz, 3 H), 1.83 (m, 5 H), 2.17–2.97 (m, 2 H), 3.55 (m, 2 H), 7.23 (s, 1 H), 7.33–7.77 (m, 5 H), 7.88–8.22 (m, 4 H); MS m/e 302 (100, M^+), 247 (23), 232 (13), 205 (36). **18**·HBr: mp 240–243 °C. Anal. ($C_{21}H_{22}N_2$·HBr) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-phenylquinolin-4-amine (19)**: obtained from *N*,*N*-dimethylethylenediamine and **2**; yield 93%; an oil; ^{1}H NMR δ 2.32 (s, 6 H), 2.72 (t, J = 6 Hz, 2 H), 3.39 (m, 2 H), 5.90 (br s, 1 H), 6.85 (s, 1 H), 7.42 (m, 2 H), 7.49 (m, 2 H), 7.64 (t, J = 8 Hz, 1 H), 7.80 (d, J = 8 Hz, 1 H), 8.08 (m, 3 H); MS m/e 291 (6, M^+), 58 (100). **19**·2HBr·$2H_2O$: mp 244–246 °C. Anal. ($C_{10}H_{21}N_3$·2HBr·$2H_2O$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-*N*-ethyl-2-phenylquinolin-4-amine (20)**: obtained from *N*,*N*-dimethyl-*N*′-ethylethylenediamine and **2**; yield 85%; an oil; ^{1}H NMR δ 1.20 (t, J = 6.5 Hz, 3 H), 2.26 (s, 6 H), 2.56 (t, J = 7 Hz, 2 H), 3.49 (m, 4 H), 7.36 (s, 1 H), 7.45 (m, 2 H), 7.52 (m, 2 H), 7.65 (d, J = 8 Hz, 1 H), 8.09 (m, 4 H); MS m/e 319 (4, M^+), 261 (48), 58 (100). **20**·2HBr·$2H_2O$: mp 233–235 °C. Anal. ($C_{21}H_{25}N_3$·2HBr·$2H_2O$) C, H, N.

4-(4-Methylpiperazino)-2-phenylquinoline (21): obtained from *N*-methylpiperazine and **2**; yield 96%; an oil; ^{1}H NMR δ 2.44 (s, 3 H), 2.75 (br s, 4 H), 3.34 (br s, 4 H), 7.31 (s, 1 H), 7.45–7.54 (m, 4 H), 7.67 (t, J = 8 Hz, 1 H), 8.02 (d, J = 8 Hz, 1 H), 8.11 (m, 3 H); MS m/e 347 (2, M^+), 303 (58), 288 (12), 204 (16), 70 (100). **21**·2HBr: mp 320–325 °C. Anal. ($C_{20}H_{21}N_3$·2HBr) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-(4-fluorophenyl)quinolin-4-amine (22)**: obtained from *N*,*N*-dimethylethylenediamine and **3**; yield 81%; mp 134–136 °C; ^{1}H NMR δ 2.33 (s, 6 H), 2.73 (t, J = 6.5 Hz, 2 H), 3.39 (m, 2 H), 5.94 (br s, 1 H), 6.79 (s, 1 H), 7.17 (m, 2 H), 7.43 (t, J = 8 Hz, 1 H), 7.65 (t, J = 8 Hz, 1 H), 7.80, (d, J = 8 Hz, 1 H), 8.04 (d, J = 8 Hz, 1 H), 8.08 (m, 2 H); MS m/e 309 (5, M^+), 58 (100). Anal. ($C_{19}H_{20}FN_3$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-(4-chlorophenyl)-quinolin-4-amine (23)**: obtained from *N*,*N*-dimethylethylenediamine and **4**; yield 98%; mp 135–137 °C; ^{1}H NMR δ 2.33 (s, 6 H), 2.73 (t, J = 6.5 Hz, 2 H), 3.40 (m, 2 H), 5.96 (br s, 1 H), 6.80 (s, 1 H), 7.45 (m, 3 H), 7.65 (t, J = 8 Hz, 1 H), 7.80 (d, J = 8 Hz, 1 H), 8.05 (m, 3 H); MS m/e 325 (12, M^+), 58 (100). Anal. ($C_{19}H_{20}ClN_3$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-2-(4-bromophenyl)quinolin-4-amine (24)**: obtained from *N*,*N*-dimethylethylenediamine and **5**; yield 98%; mp 119–121 °C; ^{1}H NMR δ 2.33 (s, 6 H), 2.73 (t, J = 6 Hz, 2 H), 3.39 (m, 2 H), 5.95 (br s, 1 H), 6.79 (s, 1 H), 7.43 (t, J = 8 Hz, 1 H), 7.62 (d, J = 8 Hz, 2 H), 7.65 (t, J = 8 Hz, 1 H), 7.80 (d, J = 8 Hz, 1 H), 7.98 (d, J = 8 Hz, 2 H), 8.04 (d, J = 8 Hz, 1 H); MS m/e 369 (7, M^+), 371 (6, M^+), 203 (10), 58 (100). Anal. ($C_{19}H_{20}BrN_3$) C, H, N.

(23) Satterthwait, A. C.; Westheimer, F. H. J. *J. Am. Chem. Soc.* **1980**, *102*, 4464.

N-[2-(Dimethylamino)ethyl]-2-(4-methoxyphenyl)quinolin-4-amine (25): obtained from *N,N*-dimethylethylenediamine and **6**; yield 88%; mp 121–122 °C; ^{1}H NMR δ 2.32 (s, 6 H), 2.72 (t, J = 6.5 Hz, 2 H), 3.38 (m, 2 H), 3.88 (s, 3 H), 5.87 (br s, 1 H), 6.80 (s, 1 H), 7.02 (d, J = 9 Hz, 2 H), 7.40 (t, J = 8 Hz, 1 H), 7.63 (t, J = 8 Hz, 1 H), 7.79 (d, J = 8 Hz, 1 H), 8.04 (d, J = 8 Hz, 1 H), 8.06 (d, J = 9 Hz, 2 H); MS m/e 321 (25, M$^+$), 263 (10), 58 (100). Anal. ($C_{20}H_{23}N_3O$) C, H, N.

N-[2-(Dimethylamino)ethyl]-2-(4-methylphenyl)quinolin-4-amine (26): obtained from *N,N*-dimethylethylenediamine and **7**; yield 93%; mp 108–110 °C; ^{1}H NMR δ 2.33 (s, 6 H), 2.42 (s, 3 H), 2.72 (t, J = 6 Hz, 2 H), 3.40 (m, 2 H), 5.89 (br s, 1 H), 6.83 (s, 1 H), 7.30 (d, J = 8 Hz, 2 H), 7.41 (t, J = 7 Hz, 1 H), 7.64 (t, J = 8 Hz, 1 H), 7.79 (d, J = 8 Hz, 1 H), 8.00 (d, J = 8 Hz, 2 H), 8.05 (d, J = 8 Hz, 1 H); MS m/e 305 (22, M$^+$), 247 (12), 58 (100). Anal. ($C_{20}H_{23}N_3$) C, H, N.

4-(2-Methylpiperidino)-2-(2-pyridyl)quinoline (27): obtained from 2-methylpiperidine and **9**; yield 57%; an oil; ^{1}H NMR (60 MHz) δ 1.03 (d, J = 6 Hz, 3 H), 1.78 (m, 6 H), 2.78–4.08 (m, 3 H), 7.22–8.27 (m + s at δ 8.18, 7 H), 8.60–8.83 (m, 2 H); MS m/e 303 (29, M$^+$), 288 (100), 246 (10), 205 (11). **28**·HBr: mp 225–226 °C. Anal. ($C_{20}H_{21}N_3$·HBr) C, H, N.

4-(3-Methylpiperidino)-2-(2-pyridyl)quinoline (28): obtained from 3-methylpiperidine and **9**; yield 48%; an oil; ^{1}H NMR (60 MHz) δ 0.97 (d, J = 6 Hz, 3 H), 1.87 (m, 5 H), 2.30–3.08 (m, 2 H), 3.60 (m, 2 H), 7.13–8.23 (m + s at δ 8.05, 7 H), 8.53–8.95 (m, 2 H); MS m/e 303 (100, M$^+$), 260 (16), 248 (20), 234 (16), 206 (52). **29**·HBr: mp 238–239 °C. Anal. ($C_{20}H_{21}N_3$·HBr) C, H, N.

4-(4-Methylpiperidino)-2-(2-pyridyl)quinoline (29): obtained from 4-methylpiperidine and **9**; yield 47%; an oil; ^{1}H NMR (60 MHz) δ 1.03 (d, J = 6 Hz, 3 H), 1.73 (m, 5 H), 2.97 (m, 2 H), 3.70 (m, 2 H), 7.20–8.27 (m + s at δ 8.12, 7 H), 8.62–8.85 (m, 2 H); MS m/e 303 (100, M$^+$). **30**·HBr·$^1/_2$H$_2$O: mp 243–246 °C. Anal. ($C_{20}H_{21}N_3$·HBr·$^1/_2$H$_2$O) C, H, N.

4-Morpholino-2-(2-pyridyl)quinoline (30): obtained from morpholine and **9**; yield 30%; mp 116–117 °C; ^{1}H NMR (60 MHz) δ 3.33 (m, 4 H), 4.03 (m, 4 H), 7.07–8.27 (m + s at δ 8.13, 7 H), 8.55–8.82 (m, 2 H); MS m/e 291 (100, M$^+$), 260 (18), 233 (37), 206 (88), 116 (12), 102 (13), 78 (14). Anal. ($C_{18}H_{17}N_3O$) C, H, N.

4-Thiomorpholino-2-(2-pyridyl)quinoline (31): obtained from thiomorpholine and **9**; yield 15%; mp 142–143 °C; ^{1}H NMR (60 MHz) δ 2.97 (t, J = 5 Hz, 4 H), 3.63 (t, J = 5 Hz, 4 H), 7.27–8.33 (m + s at δ 8.17, 7 H), 8.60–8.88 (m, 2 H); MS m/e 307 (100, M$^+$), 260 (19), 234 (65), 206 (83), 102 (12), 78 (15). Anal. ($C_{18}H_{17}N_3S$) C, H, N.

N-[2-(Dimethylamino)ethyl]-2-(2-pyridyl)quinolin-4-amine (32): obtained from *N,N*-dimethylethylenediamine and **9**; yield 82%; an oil; ^{1}H NMR δ 2.33 (s, 6 H), 2.74 (t, J = 6.5 Hz, 2 H), 3.50 (m, 2 H), 5.96 (br s, 1 H), 7.33 (m, 1 H), 7.45 (t, J = 8 Hz, 1 H), 7.61 (s, 1 H), 7.66 (t, J = 8 Hz, 1 H), 7.85 (m, 2 H), 8.07 (d, J = 8 Hz, 1 H), 8.62 (d, J = 8 Hz, 1 H), 8.72 (m, 1 H); MS m/e 234 (18, M$^+$), 58 (100). **33**·2HBr·$^3/_2$H$_2$O: mp 279–282 °C. Anal. ($C_{18}H_{20}N_4$·2HBr·$^3/_2$H$_2$O) C, H, N.

N-[2-(Dimethylamino)ethyl]-*N*-ethyl-2-(2-pyridyl)quinolin-4-amine (33): obtained from *N,N*-dimethyl-*N'*-ethylethylenediamine and **9**; yield 72%; an oil; ^{1}H NMR δ 1.21 (t, J = 7 Hz, 3 H), 2.25 (s, 6 H), 2.57 (t, J = 7 Hz, 2 H), 3.53 (m, 4 H), 7.34 (m, 1 H), 7.47 (t, J = 8 Hz, 1 H), 7.66 (t, J = 8 Hz, 1 H), 7.86 (m, 1 H), 8.09–8.13 (m + s at δ 8.12), 8.63 (d, J = 8 Hz, 1 H), 8.72 (m, 1 H); MS m/e 320 (14, M$^+$), 262 (65), 58 (100). **34**·2HBr·2H$_2$O: mp 228–230 °C. Anal. ($C_{20}H_{24}N_4$·2HBr·2H$_2$O) C, H, N.

4-(4-Methylpiperazino)-2-(2-pyridyl)quinoline (34): obtained from *N*-methylpiperazine and **9**; yield 74%; an oil; ^{1}H NMR δ 2.44 (s, 3 H), 2.75 (m, 2 H), 3.40 (m, 2 H), 7.35 (m, 1 H), 7.48 (t, J = 8 Hz, 1 H), 7.67 (t, J = 8 Hz, 1 H), 7.86 (m, 1 H), 8.04 (d, J = 8 Hz, 1 H), 8.06 (s, 1 H), 8.13 (d, J = 8 Hz, 1 H), 8.63 (d, J = 8 Hz, 1 H), 8.73 (m, 1 H); MS m/e 304 (100, M$^+$), 260 (11), 248 (19), 234 (38), 98 (11), 70 (59), 56 (13), 43 (98). **35**·2HBr·H$_2$O: mp 316–320 °C. Anal. ($C_{19}H_{20}H_4$·2HBr·H$_2$O) C, H, N.

4-Pyrrolidino-2-(3-pyridyl)quinoline (35): obtained from pyrrolidine and **10**; yield 60%; mp 160–162 °C; ^{1}H NMR (60 MHz) δ 2.05 (m, 4 H), 3.75 (m, 4 H), 6.88 (s, 1 H), 7.22–7.83 (m, 3 H), 8.02–8.82 (m, 4 H), 9.33 (d, 1 H); MS m/e 275 (100, M$^+$), 246 (57), 220 (13), 205 (18), 101 (10). Anal. ($C_{18}H_{17}N_3$) C, H, N.

4-Piperidino-2-(3-pyridyl)quinoline (36): obtained from piperidine and **10**; yield 46%; mp 98–100 °C; ^{1}H NMR (60 MHz) δ 1.83 (m, 6 H), 3.27 (m, 4 H), 7.22–8.78 (m + s at δ 7.30, 8 H), 9.37 (d, 1 H); MS m/e 289 (100, M$^+$), 260 (15), 246 (11), 206 (36), 101 (11), 75 (11). Anal. ($C_{19}H_{19}N_3$) C, H, N.

N-[2-(Dimethylamino)ethyl]-2-(3-pyridyl)quinolin-4-amine (37): obtained from *N,N*-dimethylethylenediamine and **10**; yield 85%; an oil; ^{1}H NMR (60 MHz) δ 2.32 (s, 6 H), 2.65 (t, J = 6 Hz, 2 H), 3.33 (m, 2 H), 6.1 (m, 1 H), 6.78 (s, 1 H), 7.27–8.70 (m, 7 H), 9.32 (d, 1 H); MS m/e 234 (15, M$^+$), 58 (100). **38**·3HBr: mp 281–283 °C. Anal. ($C_{18}H_{20}N_4$·3HBr) C, H, N.

N-[2-(Dimethylamino)ethyl]-*N*-ethyl-2-(3-pyridyl)quinolin-4-amine (38): obtained from *N,N*-dimethyl-*N'*-ethylethylenediamine and **10**; yield 74%; an oil; ^{1}H NMR δ 1.23 (t, J = 7 Hz, 3 H), 2.59 (s, 6 H), 2.57 (t, J = 7 Hz, 2 H), 3.51 (m, 4 H), 7.34 (s, 1 H), 7.46 (m, 2 H), 7.68 (t, J = 8 Hz, 1 H), 8.10 (m, 2 H), 8.46 (d, J = 8 Hz, 1 H), 8.69 (d, J = 5 Hz, 1 H), 9.28 (d, J = 2 Hz, 1 H); MS m/e 320 (2, M$^+$), 262 (13), 58 (100). **39**·3HBr·3H$_2$O: mp 131–133 °C. Anal. ($C_{20}H_{24}N_4$·3HBr·3H$_2$O) C, H, N.

4-(4-Methylpiperazino)-2-(3-pyridyl)quinoline (39): obtained from *N*-methylpiperazine and **10**; yield 74%; mp 127–128 °C; ^{1}H NMR δ 2.45 (s, 3 H), 2.76 (m, 4 H), 3.36 (m, 4 H), 7.29 (s, 1 H), 7.48 (m, 2 H), 7.70 (t, J = 8 Hz, 1 H), 8.04 (d, J = 8 Hz, 1 H), 8.13 (d, J = 8 Hz, 1 H), 8.69 (d, J = 5 Hz, 1 H), 9.29 (d, J = 2 Hz, 1 H); MS m/e 304 (53, M$^+$), 289 (10), 206 (13), 70 (100). Anal. ($C_{19}H_{20}N_4$) C, H, N.

N-[2-(Dimethylamino)ethyl]-2-(4-pyridyl)quinolin-4-amine (40): obtained from *N,N*-dimethylethylenediamine and **11**; yield 82%; mp 110–112 °C; ^{1}H NMR δ 2.34 (s, 6 H), 2.75 (t, J = 6 Hz, 2 H), 3.41 (m, 2 H), 6.06 (br s, 1 H), 6.86 (s, 1 H), 7.48 (t, J = 8 Hz, 1 H), 7.69 (t, J = 8 Hz, 1 H), 7.83 (d, J = 8 Hz, 1 H), 8.00 (d, J = 5 Hz, 2 H), 8.08 (d, J = 8 Hz, 1 H), 8.74 (d, J = 5 Hz, 2 H); MS m/e 292 (2, M$^+$), 262 (13), 58 (100). Anal. ($C_{18}H_{20}N_4$) C, H, N.

N-[2-(Dimethylamino)ethyl]-*N*-ethyl-2-(4-pyridyl)quinolin-4-amine (41): obtained from *N,N*-dimethyl-*N'*-ethylethylenediamine and **11**; yield 88%; an oil; ^{1}H NMR δ 1.22 (t, J = 7 Hz, 3 H), 2.26 (s, 6 H), 2.56 (t, J = 7 Hz, 2 H), 3.52 (m, 4 H), 7.37 (s, 1 H), 7.49 (t, J = 8 Hz, 1 H), 7.69 (t, J = 8 Hz, 1 H), 8.00 (d, J = 5 Hz, 2 H), 8.08 (d, J = 8 Hz, 1 H), 8.13 (d, J = 8 Hz, 1 H), 8.76 (d, J = 5 Hz, 2 H); MS m/e 320 (5), 262 (27), 58 (100). **42**·3HBr·H$_2$O: mp 248–250 °C. Anal. ($C_{20}H_{24}N_4$·3HBr·H$_2$O) C, H, N.

4-(4-Methylpiperazino)-2-(4-pyridyl)quinoline (42): obtained from *N*-methylpiperazine and **11**; yield 94%; an oil; ^{1}H NMR δ 2.45 (s, 3 H), 2.77 (m, 4 H), 3.37 (m, 4 H), 7.31 (s, 1 H), 7.52 (t, J = 8 Hz, 1 H), 7.70 (t, J = 8 Hz, 1 H), 8.01 (d, J = 5 Hz, 2 H), 8.04 (d, J = 8 Hz, 1 H), 8.14 (d, J = 8 Hz, 1 H), 8.76 (d, J = 5 Hz, 2 H); MS m/e 304 (80, M$^+$), 233 (12), 206 (20), 70 (100). **43**·2HBr·$^3/_2$H$_2$O: mp 226–229 °C. Anal. ($C_{19}H_{20}N_4$·2HBr·$^3/_2$H$_2$O) C, H, N.

N-[2-(Dimethylamino)ethyl]-2-(2-furanyl)quinoline (43): obtained from *N,N*-dimethylethylenediamine and **12**; yield 66%; mp 93–95 °C; ^{1}H NMR δ 2.32 (s, 6 H), 2.72 (t, J = 6 Hz, 2 H), 3.39 (m, 2 H), 5.91 (br s, 1 H), 6.56 (m, 1 H), 6.87 (s, 1 H), 7.16 (d, J = 3 Hz, 1 H), 7.39 (t, J = 8 Hz, 1 H), 7.58 (m, 1 H), 7.62 (t, J = 8 Hz, 1 H), 7.76 (d, J = 8 Hz, 1 H), 8.01 (d, J = 8 Hz, 1 H); MS m/e 281 (10, M$^+$), 58 (100). Anal. ($C_{17}H_{19}N_3O$) C, H, N.

4-(4-Methylpiperazino)-2-(2-furanyl)quinoline (44): obtained from *N*-methylpiperazine and **12**; yield 21%; an oil; ^{1}H NMR δ 2.44 (s, 3 H), 2.74 (m, 4 H), 3.31 (m, 4 H), 6.58 (m, 1 H), 7.20 (d, J = 3 Hz, 1 H), 7.31 (s, 1 H), 7.43 (t, J = 8 Hz, 1 H), 7.60 (m, 1 H), 7.64 (t, J = 8 Hz, 1 H), 7.98 (d, J = 8 Hz, 1 H), 8.07 (d, J = 8 Hz, 1 H); MS m/e 293 (53, M$^+$), 278 (17), 222 (22), 149 (11), 125 (12), 70 (100). **45**·2HBr·H$_2$O: mp 335–340 °C. Anal. ($C_{18}H_{19}N_3O$·2HBr·H$_2$O) C, H, N.

N-[2-(Dimethylamino)ethyl]-2-(2-thienyl)quinolin-4-amine (45): obtained from *N,N*-dimethylethylenediamine and **13**; yield 95%; mp 109–110 °C; ^{1}H NMR δ 2.33 (s, 6 H), 2.73 (t, J = 6 Hz, 2 H), 3.38 (m, 2 H), 5.89 (br s, 1 H), 6.81 (s, 1 H), 7.13 (m, 1 H), 7.39 (t, J = 8 Hz, 1 H), 7.42 (d, J = 5 Hz, 1 H), 7.61 (t, J = 8 Hz, 1 H), 7.69 (t, J = 4 Hz, 1 H), 7.75 (d, J = 8 Hz, 1 H), 7.98 (d, J = 8 Hz, 1 H); MS m/e 297 (3, M$^+$), 58 (100). Anal. ($C_{17}H_{19}N_3S$) C, H, N.

N-[2-(Dimethylamino)ethyl]-N-ethyl-2-(2-thienyl)-quinolin-4-amine (46): obtained from N,N-dimethyl-N'-ethylethylenediamine and 13; yield 95%; an oil; ^{1}H NMR δ 1.20 (t, J = 7 Hz, 3 H), 2.26 (s, 6 H), 2.54 (t, J = 7 Hz, 2 H), 3.46 (m, 4 H), 7.15 (m, 1 H), 7.31 (s, 1 H), 7.40 (t, J = 8 Hz, 1 H), 7.44 (d, J = 5 Hz, 1 H), 7.61 (t, J = 8 Hz, 1 H), 7.69 (d, J = 4 Hz, 1 H), 8.02 (m, 2 H); MS m/e 325 (7, M$^+$), 267 (52), 58 (100). **47**·2HBr·2H$_2$O: mp 263-264 °C. Anal. ($C_{19}H_{23}N_3S$·2HBr·2H$_2$O) C, H, N.

4-(4-Methylpiperazino)-2-(2-thienyl)quinoline (47): obtained from N-methylpiperazine and 13; yield 91%; an oil; ^{1}H NMR δ 2.44 (s, 3 H), 2.74 (m, 4 H), 3.32 (m, 4 H), 7.15 (m, 1 H), 7.26 (s, 1 H), 7.42 (t, J = 8 Hz, 1 H), 7.45 (d, J = 5 Hz, 1 H), 7.63 (t, J = 8 Hz, 1 H), 7.70 (d, J = 4 Hz, 1 H), 7.97 (d, J = 8 Hz, 1 H), 8.04 (d, J = 8 Hz, 1 H); MS m/e 309 (51, M$^+$), 294 (12), 238 (15), 70 (100). **48**·2HBr·H$_2$O: mp 330-334 °C. Anal. ($C_{18}H_{19}N_3S$·2HBr·H$_2$O) C, H, N.

General Procedure C. Preparation of Ketimines 49-52. The procedure for condensation of 2-aminobenzonitrile (**48**) with methyl ketones has been published.[5] Yields, melting points, and ^{1}H NMR spectra of 2-[(1-phenylethylidene)amino]benzonitrile (**49**), 2-[[1-(2-pyridyl)ethylidene]amino]benzonitrile (**50**), and 2-[[1-(2-thienyl)ethylidene]amino]benzonitrile (**52**) have also been presented previously.[5]

2-[[1-(3-Pyridyl)ethylidene]amino]benzonitrile (51): obtained from 3-acetylpyridine and **48**; yield 87%; mp 45-46 °C; ^{1}H NMR (60 MHz) δ 2.30 (s, 3 H), 6.75-7.73 (m, 4 H), 8.30 (m, 1 H), 8.68 (m, 1 H), 9.17 (m, 1 H). Anal. ($C_{14}H_{11}N_2$) C, H, N.

General Procedure D. Preparation of Quinolin-4-amines 53-56. The procedure for LDA-mediated cyclization of ketimines **49-52** has been published.[5] Yields, melting points, and ^{1}H NMR spectra for 2-phenylquinolin-4-amine (**53**), 2-(2-pyridyl)-quinolin-4-amine (**54**), and 2-(2-thienyl)quinolin-4-amine (**56**) have also been presented previously.[5]

2-(3-Pyridyl)quinolin-4-amine (55): obtained from **51**; yield 52%; mp 185-188 °C; ^{1}H NMR (60 MHz, DMSO-d_6) δ 7.03 (br s, 2 H), 7.27 (s, 1 H), 7.42-8.80 (m, 7 H), 9.38 (d, J = 2 Hz, 1 H); MS m/e 221 (100, M$^+$), 195 (16). Anal. ($C_{14}H_{11}N_3$) C, H, N.

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Communications to the Editor

Synthesis and Bioactivity of N^{ω}-Hydroxyarginine: A Possible Intermediate in the Biosynthesis of Nitric Oxide from Arginine

Nitric oxide (NO) has recently been found to be an endogenous molecule of extreme biological importance in mammalian cells. It has been demonstrated to play a vital role in a variety of physiological responses[1] including smooth muscle relaxation.[2] Though a significant amount of research has been conducted in determining the physiological role of NO, little work has been performed in elucidating the mechanistic pathway of NO formation. Early studies have demonstrated that NO generation is the result of the enzymatic oxidation of a terminal guanidinium nitrogen on arginine with citrulline being the other product. To date, two mechanisms for the generation of NO have been put forth (Figure 1) with little supporting evidence. One pathway (A-B-C-D, Figure 1), proposed by Marletta et al.,[3] involves initial N^{ω}-hydroxylation of arginine to give N^{ω}-hydroxyl-L-arginine (NOHA) followed by a series of reactions to generate NO. The other pathway (A-E-F, Figure 1), proposed by DeMaster et al.,[4] also

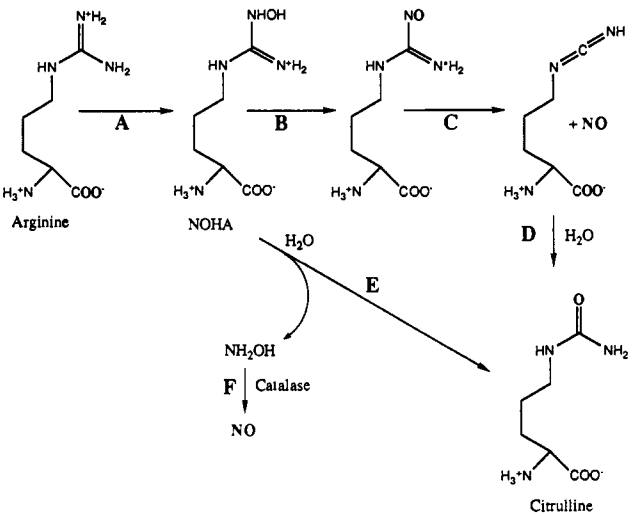

Figure 1. Proposed pathways for the biosynthesis of NO from arginine.

requires an initial N-hydroxylation of arginine to generate NOHA. This pathway differs from the first in that free hydroxylamine is generated and subsequently oxidized by another enzyme (possibly catalase) to give NO. Both pathways have as their first step the formation of NOHA. In the macrophage, it is unlikely that either mechanism is entirely correct since oxygen isotope studies have indicated that the oxygen in citrulline originates from molecular oxygen and not water.[5] Although NOHA is proposed to be a critical biosynthetic intermediate, no reports on its synthesis or bioactivity have appeared in the literature. Herein, we describe the synthesis of NOHA and report our preliminary findings regarding its biological activity.

NOHA was synthesized according to the scheme outlined in Figure 2 and is described below. NMR spectra

(1) For the most recent examples, see: James, S. L.; Hibbs, J. B., Jr. *Parasitol. Today* **1990**, *6* (9), 303. Collier, J.; Vallance, P. *Trends Pharmacol. Sci.* **1989**, *10*, 427. Radomski, M. W.; Palmer, R. M. J.; Moncada, S. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5193. Humphries, R. G.; Tomlinson, W.; O'Connor, S.; Leff, P. *J. Cardiovasc. Pharmacol.* **1990**, *16*, 292. Mollace, V.; Salvemini, D.; Anggard, E.; Vane, J. R. *Biochem. Biophys. Res. Commun.* **1990**, *172* (2), 564. Green, S. J.; Mellouk, S.; Hoffman, S. L.; Meltzer, M. S.; Nacy, C. *Immunol. Lett.* **1990**, *25*, 15. Beckman, J. S.; Beckman, T. W.; Chen, J.; Marshall, P. A.; Freeman, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1620.

(2) Gardiner, S. M.; Compton, A. M.; Bennett, T.; Palmer, R. M. J.; Moncada, S. *Hypertension* **1990**, *15* (5), 486. Kelm, M.; Schrader, J. *Circ. Res.* **1990**, *66* (6), 1561.

(3) Marletta, M. A.; Yoon, P. S.; Iyengar, R.; Leaf, C. D.; Wishnok, J. S. *Biochemistry* **1988**, *27*, 8706.

(4) DeMaster, E. G.; Raij, L.; Archer, S. L.; Weir, E. K. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 527.

(5) Kwon, N. S.; Nathan, C. F.; Gilker, C.; Griffith, O. W.; Matthews, D. E.; Stuehr, D. J. *J. Biol. Chem.* **1990**, *265* (23), 13442.

0022-2623/91/1834-1746$02.50/0