1,2-Dihydro-4-quinazolinamines: Potent, Highly Selective Inhibitors of Inducible Nitric Oxide Synthase Which Show Antiinflammatory Activity in Vivo

Alan C. Tinker,*,† Haydn G. Beaton,†
Nigel Boughton-Smith,‡ Tony R. Cook,†
Sally L. Cooper,† Lynne Fraser-Rae,‡ Kay Hallam,‡
Peter Hamley,† Tom McInally,† David J. Nicholls,‡
Austen D. Pimm,† and Alan V. Wallace‡

Departments of Medicinal Chemistry and BioScience, AstraZeneca R & D Charnwood, Bakewell Road, Loughborough, Leicestershire LE11 5RH, UK

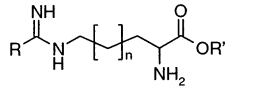
Received October 9, 2002

Abstract: The discovery of a novel class of nitric oxide synthase (NOS) inhibitors, 2-substituted 1,2-dihydro-4-quinazolinamines, and the related 4′-aminospiro[piperidine-4,2′(1′*H*)-quinazolin]-4′-amines is described. Members of both series exhibit nanomolar potency and high selectivity for the inducible isoform of the enzyme (i-NOS) relative to the constitutive isoforms in vitro. Efficacy in acute and chronic animal models of inflammatory disease following oral administration has also been demonstrated using these compounds.

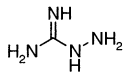
Introduction. Nitric oxide synthases (NOS) are a family of closely related heme-based oxygenases, which synthesize nitric oxide from the natural amino acid L-arginine.[1] Three isoforms of the enzyme have been characterized. Two of these, endothelial NOS (e-NOS) and neuronal NOS (n-NOS), are constitutive and calcium dependent. The third isoform, inducible NOS (i-NOS), is formed in response to pathological challenges. It is not dependent on calcium and produces much higher concentrations of nitric oxide than the others. Overexpression of i-NOS has been implicated in a number of inflammatory diseases,[2] for example, septic shock and rheumatoid arthritis. Inhibition of i-NOS should be a useful approach to treatment of these conditions. However, reported studies using i-NOS inhibitors in disease models have not demonstrated efficacy unequivocally.[3] This can largely be accounted for by the fact that the inhibitors studied lack sufficient selectivity over the other isoforms, particularly e-NOS, which is essential in maintaining vascular homeostasis, to produce uncomplicated pharmacology.

The best known inhibitors of NOS are amino acids related to the substrate arginine, for example L-NMMA (**1**),[4] L-NA (**2**)[5] (or its methyl ester L-NAME, **2′**), and L-NIL (**3**).[6] However, none of them is particularly potent or selective, and cardiovascular effects due to e-NOS inhibition[7] limit their usefulness in vivo. Aminoguanidine (**4**) selectively inhibits i-NOS relative to e-NOS but has many additional biological effects that are not related to NOS inhibition.[8] More recently, other inhibitors based on guanidines,[9] isothioureas,[10] or amidines[11] have been reported with various levels of selectivity and potency in vitro. However, their use in in vivo models has generally been limited by poor bioavailability or toxicity.

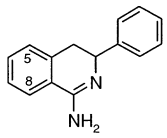
We have previously reported the discovery of two new inhibitor classes, the dihydroisoquinolines (e.g., **5**)[12] and thienopyridines (e.g., **6**),[13] as part of a program aimed at the development of potent, truly selective i-NOS inhibitors for use in the treatment of inflammatory arthritis, preferably by oral administration. Therefore, the reported[14] dihydroquinazoline (**7**), directly analogous to (**5**), was of obvious interest to us.



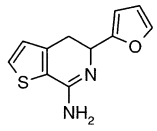
1. R = MeNH; n =1; R' = H
2. R = NO_2NH; n =1; R' = H (**2'** R'=Me)
3. R = Me; n =2; R' = H



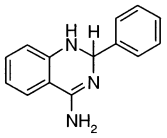
4



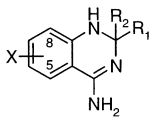
5



6



7



8

While (**7**) had only modest i-NOS inhibitory activity (IC_{50} = 2.5 μM against human i-NOS enzyme), this compared sufficiently favorably with the measured potency of (**5**) (IC_{50} = 8.5 μM)[12] to encourage further investigation. The elaboration of this lead to afford selective, potent inhibitors of i-NOS that display anti-inflammatory activity in vivo is described here.

Chemistry. Very few examples of the 1,2-dihydro-4-quinazolinamine ring-system have been reported in the literature, and only one practical synthetic approach is described, the reaction of 2-aminobenzamidine with benzaldehyde[14] or acetone[15] (Scheme 1). Fortunately, this method has proved to be very versatile in our hands: a variety of amidines (**9**) react with a wide range of aldehydes and ketones in refluxing ethanol to produce the required products (**8**) in generally excellent yield and purity.

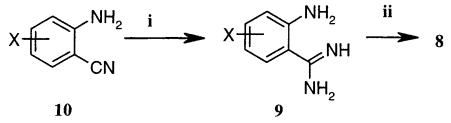
The reaction is quite insensitive to solvent, and the method is easily adapted to parallel synthesis in 96-well 2 mL microtiter plates by using DMSO solutions heated to 65 °C. Amidines (**9**) themselves can be prepared from 2-aminobenzonitriles (**10**) by reaction with hydroxylamine followed by hydrogenation of the resulting amidoxime over a Raney nickel catalyst.[15]

Biology. Inhibition of NOS enzymes was determined by measuring the formation of L-[^{3}H]citrulline from L-[^{3}H]arginine using an adaptation of the method of Förstermann et al.[16] Compound libraries in solution were first tested at a single concentration against an

* For correspondence: Tel: (44)1509-644882, Fax: (44)1509-645576. E-mail: Alan.C.Tinker@Astrazeneca.com.
† Department of Medicinal Chemistry.
‡ Department of BioScience.

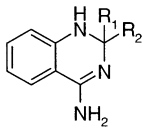
10.1021/jm0255926 CCC: $25.00
Published on Web 02/07/2003

Scheme 1[a]



[a] Reagents (i) (a) NH_2OH, NaOMe, MeOH. (b) H_2 Raney Ni, 60 °C (ii) $R_1R_2C{=}O$, ethanol, reflux.

Table 1. i-NOS Inhibitor Activity of Representative Quinazolinamines



compd no.	R_1	R_2	i-NOS IC_{50} (μM)[a]
7	H	Ph	2.5
8a	H	H	40
8b	H	CH_3	9.8
8c	H	C_2H_5	0.9
8d	H	c-propyl	1.1
8e	H	c-butyl	1.1
8f	H	c-pentyl	4.5
8g	H	2-furanyl	0.2
8h	H	2-thienyl	0.4
8i	CH_3	CH_3	52
8j	CH_3	C_2H_5	32

[a] Inhibition of isolated human enzyme (Mean of at least $n = 2$). Data for single, purified compounds.

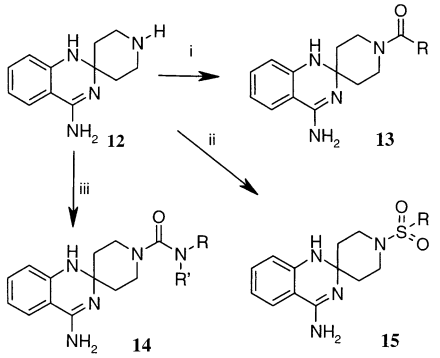
isolated i-NOS enzyme preparation. Compounds from wells showing inhibition similar to or greater than the product from an included control reactant (benzaldehyde or ethyl chloroformate) were prepared individually for accurate IC_{50} and selectivity measurements against human recombinant enzymes. Test compounds which showed $IC_{50} < 0.5$ μM vs i-NOS were also assayed for ability to inhibit NO production in human DLD-1 cells preincubated with a cytokine cocktail.[17] Those with $IC_{50} < 5$ μM in the intact cell assay were tested for inhibition of LPS induced NO production in conscious rats following iv and oral administration (see Supporting Information).

Discussion. Structure−activity relationships within the dihydroquinazoline series were very readily explored by a parallel synthesis approach. A library of dihydroquinazolines (**8,** X = H) was first prepared by reaction of amidine (**9**, X = H) with a collection of 400 commercial aldehydes and ketones.[18] Many of the aldehydes gave active products (**8**, R_1 = H), the SAR for these being very much that expected from the earlier isoquinoline work,[12] i.e., compounds with small (particularly five-membered) aromatics or small alkyl or cycloalkyl groups (2−4 C atoms) at R_2 were the most potent (Table 1).

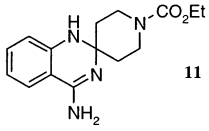
Since most of these compounds contain an asymmetric carbon atom, we undertook the resolution of several representative examples by chiral LC. Inhibitory activity against all three NOS isoforms was found to reside exclusively in one of the enantiomers.[19] Oxidation of all of these compounds (**8**; R = H) to the fully aromatic quinazoline derivatives was associated with total abolition of activity.

Ketone-derived compounds (e.g., **8i**, **8j**) generally had very low activity. However, 1-carbethoxy-4-piperidone uniquely gave a product with surprisingly high i-NOS inhibitor potency, the spirocycle (**11**). This had IC_{50} = 0.7 μM against human i-NOS and most excitingly proved to have no significant effects on either e-NOS or n-NOS at concentrations below 100 μM.

Scheme 2[a]



[a] Reagents. (i). RCOCl, base. (ii) RSO_2Cl, base. (iii) RNCO (for R′ = H) or RR′NCOCl, base.



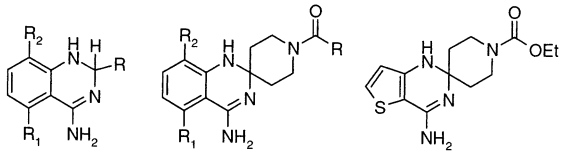
As very few simple, 1-substituted analogues of 4-piperidone are available commercially, we decided to explore the SAR of this intriguing lead by a second round of parallel synthesis, based on reaction of the parent spirocycle (**12**) (prepared from (**9**; X = H) and commercial 4-piperidone hydrate) with a range of electrophilic reagents (Scheme 2).

Screening the resulting libraries showed that members of only two compound classes possess i-NOS inhibitor potency comparable to that of the product from ethyl chloroformate (i.e., **11**): other alkyl carbamates (**13**, R = *O*-alkyl) and aryl amides (**13**, R = Aryl). Alkyl amides (**13**, R = Alkyl) and ureas (**14**) are significantly weaker, while aryl carbamates (**13**, R = OAr) and sulfonamides (**15**, R = Me, Ph) show little or no activity in the assay. (Compound **12** itself shows only very weak i-NOS inhibitor activity ($IC_{50} \sim 50$ μM). For the carbamates, alkyl groups derived from primary alcohols (except benzyl) are best. For the aryl amides, a wide range of meta- or para-substituted phenyl or heteroaryl rings appear to be acceptable. Finally the effect of quinazoline benzo ring substitution was investigated by reaction of selected aldehydes, piperidone aryl amides, and piperidone alkyl carbamates with a number of substituted analogues of (**9**). Once again, the results were qualitatively similar to those seen previously in the isoquinoline series;[12] substitution in position 5 of the aromatic ring (i.e., ortho to the cyclic amidine functionality) by halogens (particularly F) increases potency considerably, whereas other substitutions have either no effect or are detrimental to activity.[20]

Although the detailed SAR in these series is quite complex (and will be discussed in full elsewhere), these experiments readily identified compounds having optimal potency and selectivity in each of three compound classes (Table 2):

(a) 2-Monosubstituted Dihydroquinazolinamines (16−18). This series contains the most potent compounds in vitro some having IC_{50} as low as approxi-

Table 2. Biological Activity of Selected 2-Monsubstituted Quinazolinamines and Spirocyclic Compounds



16-18 **19-25** **26**

compd[a]	R	R_1	R_2	i-NOS[b] IC_{50} (μM)	e-NOS[b] IC_{50} (μM)	n-NOS[b] IC_{50} (μM)	i-NOS cell[c] IC_{50} (μM)	ID_{50} po in rat[d] (4 h postdose) (μmol/kg)
16	cyclobutyl	F	H	0.02	4.6	1	1.2	>30
17	2-furanyl	F	F	0.002	0.18	0.06	0.2	16
18	4-FPh	F	H	0.05	n.s. @ 100[e]	3.5	4.0	>30
19	OEt	F	H	0.035	100	40	9.6	n.d.
20	OEt	Cl	H	0.047	>100[e]	>100[e]	9.2	n.d.
21	$O(CH_2)_2SMe$	F	H	0.039	>100[e]	>100[e]	11	n.d.
22	3-thienyl	F	H	0.067	n.s. @ 100[e]	70	12	n.d.
23	4-CNPh	F	H	0.027	>100[e]	12	4.5	14
24	4-CNPh	F	F	0.048	>100[e]	3.3	1.6	3
25	6-CN-3-pyridyl	F	F	0.037	>100[e]	1	0.9	3
26				0.3	100	50	>500	n.d.
1 (L-NMMA)				0.34	0.33	0.09	21	700
2′ (L-NAME)				14	2.7	0.15	>300	>300
3 (L-NIL)				0.1	2.6	1.5	2.8	9
4 (AG)				3.9	100	5.9	>1000	n.d.

[a] All compounds gave satisfactory spectral and analytical data. [b] Inhibition of recombinant human enzyme at 37 °C. [c] Inhibition of NO production by intact human DLD-1 cells. [d] Inhibition of LPS induced nitrite production in rat following oral administration. [e] >100 indicates between 25% and 50% inhibition observed by 100 μM compound. n.s. = <25% inhibition at 100 μM. n.d. = not determined. All IC_{50}s are means of at least two separate measurements. For details, see Supporting Information.

mately 2 nM. Fair selectivity was also seen in some cases (e.g., **18**). However, as was seen previously in the thienopyridine series,[13] the most potent compounds are generally least selective and oral bioavailability of compounds of this type is generally very low. Additionally, the separated enantiomers of compounds with an aromatic 2-substituent, such as **17** and **18**, were found to be configurationally unstable, with partial racemization being detectable after a few hours in both aqueous and alcoholic solution (in contrast, 2-alkyl compounds (e.g., **16**) seem not to epimerize).

(b) Spirocyclic Carbamates (19−21). These are less potent but very selective. Some of these compounds (e.g., **20**, **21**) with inhibitory potency for i-NOS more than 2000-fold greater than for either of the other isoforms are among the most "pure" inhibitors of i-NOS in vitro that we have tested. Regrettably most show relatively low potency in intact cells.

(c) Spirocyclic Amides (22−25). These are intermediate between the first two types both in potency and selectivity. Excellent selectivity (commonly 2000−5000-fold) over e-NOS is seen for the best compounds, but potency of inhibition of n-NOS is usually higher than for the carbamates. The 5,8-difluoro substitution pattern usually increases relative potency in intact cells, possibly as a result of increased permeability resulting from the lower pK_a of the amidine group (e.g., **23** has pK_a 9.1, **24** has pK_a 8.4). This is usually at the cost of a further slight decrease in selectivity vs n-NOS, however.

In our earlier investigations[13] we had observed large increases in i-NOS inhibitor potency (typically from 10 to over 100-fold) when compounds from the thieno[2,3-*c*]pyridine series were compared with analogous derivatives of the isoquinoline type. In marked contrast, however, in this series i-NOS inhibitor potencies and selectivities against e-NOS and n-NOS of dihydrothienopyrimidines (e.g., **26**) did not differ significantly from those of their simple dihydroquinazoline analogues.

Activity in Vivo. Although a wide range of 1-aroyl groups appear acceptable for good in vitro potency in the spirocyclic amide series, cyano-substituted benzoyl and nicotinoyl analogues, were found consistently to confer exceptionally high potency in vivo following both iv and oral administration. Thus compounds **23**−**25** given orally to conscious rats produced a dose-dependent inhibition of NO production induced by lipopolysaccharide (LPS), their relative potency mirroring that seen in the whole cell assay (Table 2). This inhibition persisted for at least 6 h postdose in the case of **23** and **25**; however, the duration of action of the most lipophilic compound (**24**) was somewhat shorter, its effects decreasing after 4 h. No adverse effects on blood pressure or heart rate were observed during these experiments. In this model these compounds are up to 200 times more potent than the nonselective standard inhibitor L-NMMA and are both more selective and more potent than the standard selective i-NOS inhibitor L-NIL.

Compounds (**23**, AR-C85016) and (**25**, AR-C102222) were chosen for further investigation in acute and chronic models of inflammatory arthritis. Both compounds produce clear and reproducible protective effects in several of these models (full details will be published elsewhere). Typical effects are those seen in adjuvant-induced arthritis[21] in rat. In this model challenge with Freund's complete adjuvant produces, after an induction period of approximately 10 days, a severe polyarthritis leading to inflammation, edema, and joint destruction. Administration of **23** or **25** (30−300 μmol/kg or 10−100 μmol/kg po, respectively, twice daily) commencing on the day of challenge both delayed the onset of observable symptoms and reduced their severity in a dose-dependent manner (estimated ED_{50} of **25** at the 20 day

timepoint was ~10 μmol/kg). At the highest dose tested (100 μmol/kg) (**25**) completely abolished all indications of developing arthritis for the full 20 day duration of the experiment. At the end of this period joints from animals that had received the highest dose of **25** were histologically indistinguishable from those of sham-treated controls, suggesting that **25** may have disease-modifying effects in addition to its antiinflammatory actions in this model. It is noteworthy that this degree of protection was not achieved by a maximally effective dose of the standard antiinflammatory agent indomethacin. AR-C102222 was also effective when used in a more therapeutic context: significant suppression of arthritis was observed when dosing of **25** was started only when symptoms of the developing arthritis were first detected (10 days after adjuvant challenge).

Acknowledgment. We thank Phil Mallinder and his colleagues in the Molecular Biology section at AZ Charnwood for producing the recombinant human NOS enzymes used in this study; also David Blake and Paul Mapp (University of Bath, UK) for carrying out the evaluation of compounds **23** and **25** in their rat adjuvant-induced arthritis model.

Supporting Information Available: Synthetic methods and biological screen protocols. Microanalytical data and representative spectra for compounds **11** and **16**−**26**. Details of parallel synthesis experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) For reviews, see: (a) Kerwin, J. F., Jr.; Lancaster, J. R.; Feldman, P. L., Jr. Nitric Oxide: A new paradigm for second messengers. *J. Med. Chem.* **1995**, *38*, 4343−4362. (b) Southan, G. J.; Szabó, C. Selective Pharmacological Inhibition of distinct nitric oxide synthase isoforms. *Biochem. Pharmacol.* **1996**, *51*, 383−394. (c) Alderton, W. K.; Cooper, C. E.; Knowles, R. G. Nitric Oxide Synthases: Structure, Function and Inhibition. *Biochem. J.* **2001**, *357*, 593−615.

(2) Cochran, F. R.; Selph, J.; Sherman, P. Insights into the role of nitric oxide in inflammatory arthritis. *Med. Res. Rev.* **1996**, *16*, 547−563.

(3) Boughton-Smith, N. K.; Tinker, A. C. Inhibitors of Nitric Oxide Synthase in inflammatory arthritis. *IDrugs* **1998**, *1*, 321−334.

(4) Olken, N. M.; Rusche, K. M.; Richards, M. K.; Marletta, M. A. Inactivation of macrophage nitric oxide synthase activity by N^G-methyl-L-arginine. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 828−833.

(5) Furfine, E. S.; Harmon, M. F.; Paith, J. E.; Garvey, E. P. Selective inhibition of constitutive nitric oxide synthase by L-N^G-nitroarginine. *Biochemistry* **1993**, *32*, 8512−8517.

(6) Moore, W. M.; Webber, R. K.; Jerome, G. M.; Tjoeng, F. S.; Misko, T. P.; Currie, M. G. L−N^6-(1-iminoethyl)lysine: A selective inhibitor of inducible nitric oxide synthase. *J. Med. Chem.* **1994**, *37*, 3886−3888.

(7) Ruetten, H.; Thiemermann, C.; Nitric Oxide and septic shock. In *Nitric Oxide*; Ignarro, L. J., Ed.; Academic Press: San Diego, 2000; pp 747−757.

(8) (a) Corbett, A.; Tilton, R. G.; Chang, K.; Hasan, K. S.; Ido, Y.; Wang, J. L.; Sweetland, M. A.; Lancaster, J. R.; Williamson, J. R.; McDaniel, M. L.: Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. *Diabetes* **1992**, *41*, 552−556. (b) Wolff, D. J.; Lubeskie, A. Aminoguanidine is an isoform selective, mechanism based inactivator of nitric oxide synthase. *Arch. Biochem. Biophys.* **1995**, *316*, 290−301.

(9) Hasan, K.; Heesen, B. J.; Corbett, J. A.; McDaniel, M. L.; Chang, K.; Allison, W.; Wolfenbuttel, B. H. R.; Williamson, J. R.; Tilton, R. G. Inhibition of nitric oxide formation by guanidines. *Eur. J. Pharmacol.* **1993**, *249*, 101−106.

(10) (a) Garvey E. P.; Oplinger, J. A.; Tanoury, G. J.; Sherman, P. A.; Fowler, M.; Marshall, S.; Harmon, M. F.; Paith, J. E.; Furfine, E. S. Potent and selective inhibition of human nitric oxide synthases: inhibition by non-amino acid isothioureas. *J. Biol. Chem.* **1994**, *269*, 26669−26676. (b) Southan, G. J.; Szabó, C.; Thiemermann, C. Isothioureas: potent inhibitors of nitric oxide synthases with variable isoform selectivity. *Br. J. Pharmacol.* **1995**, *114*, 510−516.

(11) (a) Southan, G. J.; Szabó, C.; O'Connor, M. P.; Salzman, A. L.; Thiemermann, C. Amidines are potent inhibitors of nitric oxide synthases: Preferential inhibition of the inducible isoform. *Eur. J. Pharmacol.* **1995**, *291*, 311−318. (b) Moore, W. M.; Webber, R. K.; Fok, K. F.; Jerome, G. M.; Kornmeier, C. M.; Tjeong, F. S.; Currie M. G. Inhibitors of human nitric oxide synthase with the carbamidine moiety as a common structural element. *Bioorg. Med. Chem.* **1996**, *4*, 1559−1564. (c) Garvey, E. P.; Oplinger, J. A.; Furfine, E. S.; Kiff, R. J.; Laszlo, F.; Whittle, B. J. R.; Knowles, R. G. 1400W is a slow, tight binding inhibitor of inducible nitric oxide synthesis in vitro and in vivo. *J. Biol. Chem.* **1997**, *21*, 4959−4963.

(12) Beaton, H. G.; Hamley, P.; Nicholls, D. J.; Tinker, A. C.; Wallace, A. V. 3,4-Dihydro-1-isoquinolinamines: A novel class of Nitric Oxide Synthase Inhibitors with a range of isoform selectivity and potency. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1023−1026.

(13) Beaton, H. G.; Boughton-Smith, N.; Hamley, P.; Ghelani, A.; Nicholls, D. J.; Tinker, A. C.; Wallace, A. V. Thienopyridines: Nitric oxide synthase inhibitors with potent in vivo activity. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1027−1030.

(14) Finch, N.; Gschwend, H. W. Rearrangement of 3-amino-1-benzylindazole to 4-amino-2-phenylquinazoline. *J. Org. Chem.* **1971**, *36*, 1463−1465.

(15) Carrington, H. C. 1,2-Dihydro-2,2-dimethylquinazolines. Condensation of acetone with anthranilamide derivatives. *J. Chem. Soc.* **1955**, 2527−2528.

(16) Förstermann, U.; Schmidt, H. H. H. W.; Kohlhaas, K. L.; Murad, F. Induced RAW264.7 macrophages express soluble and particulate nitric oxide synthase: inhibition by transforming growth factor-β. *Eur. J. Pharm.* **1992**, *225*, 161−165.

(17) Sherman, P. A.; Laubach, V. E.; Reep, B. R.; Wood, E. R. Purification and cDNA sequence of an inducible nitric oxide synthase from a human tumor cell line. *Biochemistry* **1993**, *32*, 11600−11605.

(18) Neither **9** nor any member of the aldehyde and ketone collection used in this experiment gave a significant inhibition of isolated i-NOS enzyme at the concentrations used in this study.

(19) This enantiomer was identified as having the *R*-configuration from X-ray studies of enzyme inhibitor complexes (unpublished).

(20) Note that the ring systems are numbered differently, such that C-5 in the quinazoline ring is spatially equivalent to C-8 of the isoquinoline.

(21) Blake, D.; Mapp, P. Unpublished work.

JM0255926