Articles

Synthesis and Evaluation of 2-(Arylamino)imidazoles as α_2-Adrenergic Agonists

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A series of 2-(arylamino)imidazoles was synthesized and evaluated for activity at α_1- and α_2-adrenoceptors. This class of agents has been shown to have potent and selective agonist activity at the α_2-adrenoceptors. The most potent member of this class, 2-[(5-methyl-1,4-benzodioxan-6-yl)amino]imidazole, proved efficacious for the reduction of intraocular pressure upon topical administration and for the reduction of blood pressure upon intravenous administration. During the course of our studies, we developed a new reagent that allowed rapid assembly of the target compounds. This reagent, *N*-(2,2-diethoxyethyl)carbodiimide, was convenient to prepare and was stable under low-temperature storage conditions.

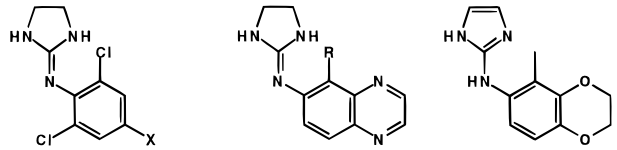
Introduction

Agents stimulating α_2-adrenoceptors have been shown to mediate a variety of physiological functions including reduction of blood pressure, sedation, and inhibition of intestinal fluid secretion.[1,2] We were interested in designing novel α_2-adrenoceptor agonists to reduce elevated intraocular pressure (IOP), a condition often associated with glaucoma.[3] Reduction in IOP is the only currently accepted outcome for a glaucoma medication. Studies conducted by Makabe[4] have demonstrated that agents acting at α_2-adrenoceptors such as clonidine, shown in Chart 1, are potent ocular antihypertensive agents. Many investigators have subsequently confirmed this observation.[5] The α_2-adrenoceptor therefore represents an attractive therapeutic target for the treatment of glaucoma. The ideal agent would reduce elevated intraocular pressure without other biological action. The α_2-adrenoceptors, however, can modulate many biological processes.[1] The most limiting side effects for the topical treatment of glaucoma with α_2-adrenoceptor agonists are those responses mediated within the central nervous system (CNS).

One approach toward glaucoma drugs possessing an enhanced therapeutic index relative to clonidine is to minimize access to the CNS. That approach led to the design of *p*-aminoclonidine and AGN 193080.[6,7] Both of these agents have limited access to the CNS and can reduce intraocular pressure upon topical administration while inducing minimal centrally mediated responses.

An alternative approach to agents possessing enhanced therapeutic indices relies on the recent recognition that the α_2-adrenoceptor has multiple subtypes.[8] Correlation of a given α_2-adrenoceptor subtype with biological function is a promising area of research; however, few subtype specific agonists have been described in the literature. Discovery of subtype selective ligands may allow development of α_2-adrenrgic drugs with enhanced therapeutic indices relative to currently available compounds.

Chart 1



X = H: **1**, Clonidine
X = NH_2: **2**, *p*-Aminoclonidine

R = Br: **3**, Brimonidine
R = CH_3: **4**, AGN 191103

5, AGN 192836

We initially prepared and evaluated 2-(arylamino)-imidazoles as potential metabolites of brimonidine and **4**.[9] Those agents proved to have modest affinity and selectivity for the α_{2A}-adrenoceptor. We therefore prepared and evaluated a set of compounds to define the features of the class responsible for activity. We developed *N*-(2,2-diethoxyethyl)carbodiimide as a convenient, new reagent for the assembly of these compounds. Agent **5** proved to be the most potent compound in the series. Herein, we describe the synthesis and evaluation of **5** and related agents as α_2-adrenergic receptor agonists. Binding assays demonstrated that **5** was 1200-fold selective for the α_{2A}-receptor relative to the α_1-receptor, 50-fold selective for the α_{2A}-receptor relative to the α_{2B}-receptor, and 10-fold selective for the α_{2A}-receptor relative to the α_{2C}-receptor. We provide details of the *in vitro* studies conducted with **5** and related analogs. Results of the preliminary *in vivo* evaluation of this agent demonstrated that **5** lowers both IOP and blood pressure.

Agent Synthesis

We used two approaches to assemble 2-(arylamino)-imidazoles. We utilized a multistep route to assemble the imidazole subunit of that class during the course of our synthetic studies toward brimonidine metabolites.[9] The 2-(arylamino)imidazoles showed promise as α_2-adrenergic receptor agonists. As a consequence of that activity, we developed a convergent route that would enhance the efficiency of the preparation.

The multistep approach to the synthesis is illustrated in Scheme 1 for the assembly of **16** and was also used

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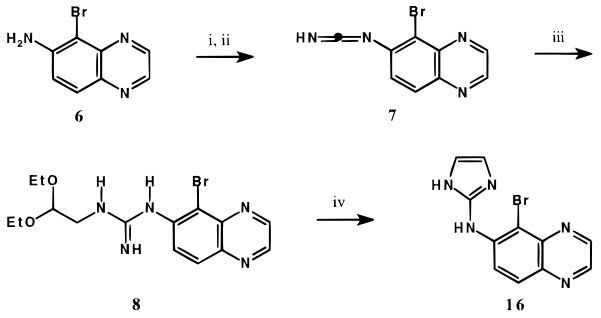
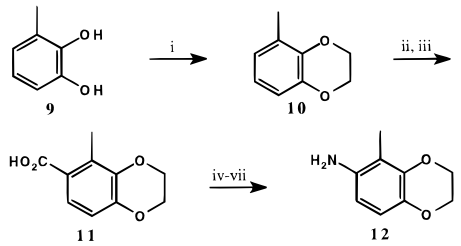
Scheme 1[a]



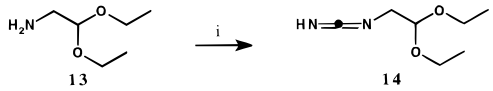
[a] Reagents and conditions: (i) NaH; (ii) phenyl cyanate; (iii) aminoacetaldehyde diethyl acetal, methanesulfonic acid; (iv) HCl−H_2O, then NaOH.

Scheme 2[a]



[a] Reagents and conditions: (i) 1,2-dibromoethane, NaI, K_2CO_3; (ii) Ac_2O, polyphosphoric acid; (iii) NaOCl; (iv) ethyl chloroformate; (v) NaN_3; (vi) benzyl alcohol, toluene, reflux; (vii) H_2, Pd−C.

Scheme 3[a]



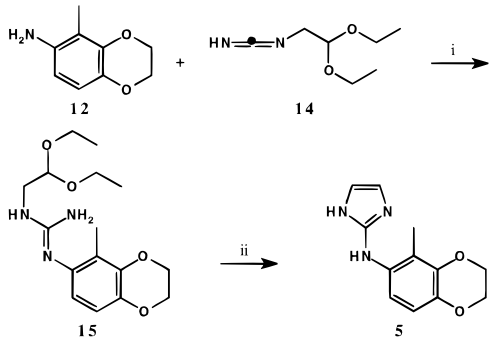
[a] Reagents and conditions: (i) CNBr.

to construct **18**−**20**. 6-Amino-5-bromoquinoxaline was treated with sodium hydride and then phenyl cyanate, which was derived from cyanogen bromide and phenol, to afford an electrophilic intermediate carbodiimide.[10,11] This intermediate is analogous to the intermediate isothiourea that Leonard employed during his assembly of 2-(arylamino)imidazoles.[12] The carbodiimide was treated with aminoacetaldehyde diethyl acetal to give guanidine **8**. Liberation of the aldehyde with aqueous hydrochloric acid and then treatment with base afforded the desired imidazole **16** in good overall yield.

Synthesis of the intermediate amine **12** for assembly of **5**, the most potent agent in the series, is presented in Scheme 2. 3-Methylcatechol, **9**, was treated with 1,2-dibromoethane to afford the benzodioxane core. The amine functionality was introduced by first acylating the aromatic ring and then oxidizing the newly introduced methyl ketone to the corresponding carboxylic acid.[13,14] The regiochemistry was confirmed at the methyl ketone stage by single-crystal X-ray analysis. Treatment of the carboxylic acid under standard Curtius conditions provided amine **12**.[15]

We prepared *N*-(2,2-diethoxyethyl)carbodiimide, **14**, as a reagent that could be used for the conversion of amines into the same *N*-(diethoxyethyl)guanidine intermediate previously discussed for the synthesis of 2-(arylamino)imidazoles. That reagent afforded a convergent approach to the agents. The reagent was prepared from aminoacetaldehyde diethyl acetal and cyanogen bromide, Scheme 3. Aminoacetaldehyde diethyl acetal was treated with 100 mol % of cyanogen

Scheme 4[a]



[a] Reagents and conditions: (i) methanesulfonic acid; (ii) HCl−H_2O, then NaOH.

bromide. The solid amine hydrobromide was removed by filtration followed by rapid chromatography to afford the desired reagent of sufficient purity to be used in subsequent reactions. The reagent was stored in an anhydrous ether solution at −78 °C as it was unstable at room temperature. The reagent solution was stable for 3 months at −78 °C.

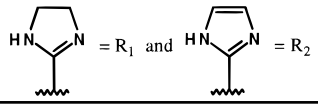
An example of the application of reagent **14** in the synthesis of 2-(arylamino)imidazoles is illustrated in Scheme 4. Treatment of amine **12** with carbodiimide **14** in the presence of methanesulfonic acid provided intermediate *N*-(diethoxyethyl)-*N*′-arylguanidine **15** in good yield. This intermediate was then treated as described previously to afford the 2-(arylamino)imidazole **5**. In addition to the preparation of **5**, the new reagent was used for the synthesis of **17** and also for **21**−**26**.

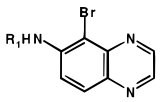
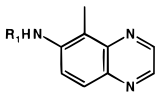
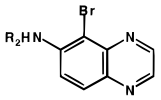
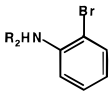
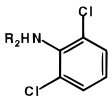
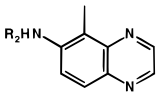
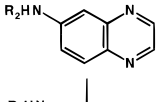
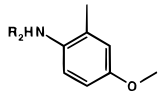
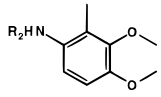
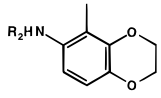
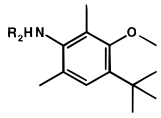
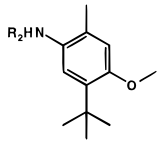
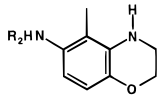
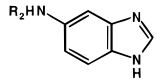
In Vitro Evaluation of Agents

Membrane suspensions for α_1-adrenoceptor binding assays were prepared from human cerebral cortex (HCC). This preparation provided a mixed population of α_1-adrenoceptor subtypes, and affinities were determined relative to the displacement of radiolabeled prazosin. Membranes from Chinese hamster ovary (CHO) cells expressing the human α_{2A}-adrenoceptor (CHO-C10) and α_{2C}-adrenoceptor (CHO-C4) as well as the rat α_{2B}-adrenoceptor (CHO-RNG) were used for α_2 binding assays.[8] The rat α_{2B}-adrenoceptor was employed in our studies as the human ortholog has been patented.[16] Affinity constants were measured relative to radiolabeled rauwolscine. The data were analyzed using the Beckman nonlinear least-squares curve-fitting program AccuFit Competition/Saturation. Table 1 summarizes the *in vitro* binding affinities of the agents resulting from at least three separate determinations.

Potent agents were evaluated for functional activity using the Cytosensor microphysiometer.[17] That technique has been applied in related G-protein-coupled receptor systems for the generation of functional responses of receptors to agonists.[18] The methodology allows the generation of functional response data for all three α_2-adrenergic receptor subtypes. We were not able to generate data for all of the agents as the assay is not amenable to a high-throughput format. Receptor expression vectors were identical with those utilized in the binding assays. Table 2 summarizes the EC_{50}s from

Table 1. Binding Affinities (K_is) for α-Adrenergic Receptors[a]

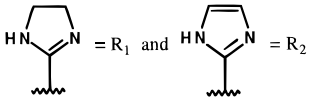


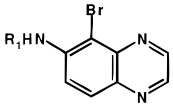
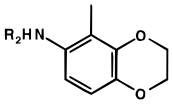
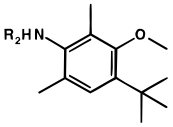
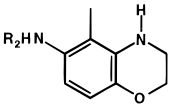
No.	Structure	K_i; nM α_1 Human Brain	α_{2A} CHO-C10	α_{2B} CHO-RNG	α_{2C} CHO-C4
3		1,800 ± 320	2.7 ± 0.32	52 ± 6.0	44 ± 3.3
4		1,400 ± 200	2.0 ± 0.58	17 ± 1.9	27 ± 7.0
16		>100,000	510 ± 49	>10,000	>10,000
17		11,000 ± 1,400	210 ± 45	1,500 ± 120	2,400 ± 241
18		6,000 ± 1,100	95 ± 23	590 ± 51	1,100 ± 48
19		>28,000	31 ± 4.0	1,200 ± 88	340 ± 61
20		>100,000	3,100 ± 620	9,300 ± 1,500	8,800 ±1,000
21		8,400 ± 860	10 ±1.4	434 ± 27	512 ± 81
22		6,600 ± 270	130 ± 16	2,500 ± 140	1,500 ± 67
5		2,100 ± 440	1.7 ± 0.15	82 ± 8.0	19 ± 3.3
23		4,200 ± 790	4.9 ± 0.12	220 ± 22	88 ± 5.6
24		16,000 ± 2,500	39 ± 4.1	380 ± 19	290 ± 22
25		16,000 ± 2,500	6 ± 0.83	421 ± 66	230 ± 43
26		24,000 ± 1,100	76 ± 6.1	640 ± 120	400 ± 29

[a] Affinities were determined relative to prazosin for the α_1-adrenoceptors and relative to rauwolscine for the α_2-adrenoceptors.

functional studies with some of the most potent 2-(arylamino)imidazolines. Intrinsic activities relative to norepinephrine are also included. The data shown are the results from at least three separate experiments.

Table 2. Functional Activity (EC_{50}s) at α_2-Adrenergic Receptors



No.	Structure	EC_{50}; nM (IA) α_{2A} CHO-C10	α_{2B} CHO-RNG	α_{2C} CHO-C4
3		4.1 ± 0.90 (0.91)	55 ± 13 (0.89)	3.4 ± 1.0 (0.82)
5		8.7 ± 4.0 (0.89)	41 ± 22 (1.0)	6.6 ± 3.0 (0.50)
23		>5,000	1,100 ± 110 (0.65)	>5,000
25		124 ± 16 (0.49)	470 ± 220 (0.88)	110 ± 14 (0.64)

In Vivo Evaluation of Agents

A single drop of either brimonidine or **5** was applied unilaterally to rabbit eyes, and the intraocular pressure was monitored for 6 h postadministration. Figure 1, panel A, details the effect that **5** had on intraocular pressure in the rabbit following topical administration of the agent. Comparative data for brimonidine are also shown. Brimonidine and **5** proved to be potent hypotensive agents following intravenous administration to cynomolgus monkeys. These results, Figure 1, panel B, demonstrated that **5** and brimonidine are both able to cross the blood−brain barrier.

Discussion

Table 1 summarizes the binding affinities of the agents for the α-adrenergic receptors. Comparison of the binding affinities for brimonidine (**3**) and methyl-substituted analog **4** suggested that the substitution of an electron-withdrawing subunit for an electron-releasing subunit of comparable size has little effect on the α_{2A}-adrenoceptor activity of the imidazoline-based agents. This is in contrast to the comparison of the imidazoles. Agents **16**−**18** all have electron-withdrawing substituents *ortho* to the aminoimidazole subunit. These agents are all less potent than **19** which has the same quinoxaline subunit found in **4**. In this case, the substitution of an electron-releasing methyl group for an electron-withdrawing bromine afforded an agent that had over a 10-fold greater affinity for the α_{2A}-adrenoceptor than the corresponding bromo analog **16**. A dramatic decrease in potency was observed upon removal of the *ortho* substituent, **20**. Substitution at the *ortho* position has been shown to facilitate a twist of the imidazoline relative to the quinoxaline nucleus in a series of brimonidine analogs.[12] Further enhancements in potency are obtained upon incorporation of additional electron-donating substituents onto the aromatic core. Incorporation of a *para* methoxy group, **21**, afforded a 3-fold enhancement in potency relative to **19**. Addition of a

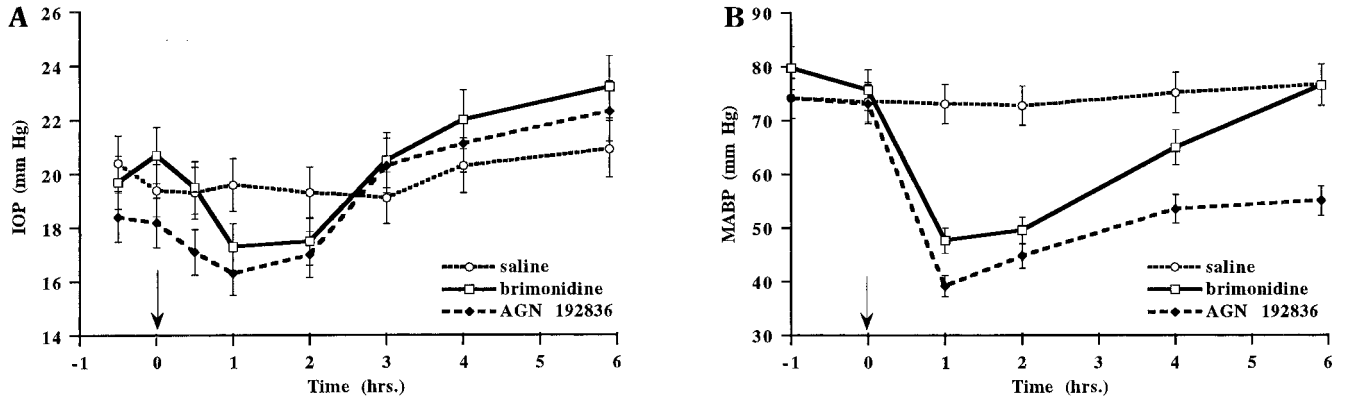


Figure 1. *In vivo* evaluation of brimonidine and **5** (AGN 192836). Panel A: Intraocular pressure (IOP) upon topical, unilateral administration of a single drop of a 0.001% solution of the specified agent to the rabbit eye. The mean values reported are the result from six animals in each group. Panel B: Mean arterial blood pressure (MABP) versus time upon intravenous administration of the specified agent to the cynomolgus monkey. Saline served as a control. Each agent was evaluated at a concentration of 50 μg/kg. The mean values reported are the result from six animals in each group.

second methoxy substituent, **22**, afforded an agent of decreased potency and selectivity relative to **21**. The most potent α_{2A}-adrenoceptor agent in this series was obtained upon preorganization of the methoxy subunits (a benzodioxane nucleus), agent **5**. This compound was over 40-fold selective for the α_{2A}-adrenoceptor relative to the α_{2B}-adrenoceptor and over 10-fold selective for the α_{2A}-adrenoceptor relative to the α_{2C}-adrenoceptor in binding studies. Agent **5** proved over 1000-fold selective for the α_{2A}-adrenoceptor relative to the α_1-adrenoceptor in binding studies. This agent was also selective for the the α_{2A}-adrenoceptor relative to the α_{2B}-adrenoceptor in the functional assay system, Table 2. The agent did not, however, prove selective for the the α_{2A}-adrenoceptor relative to α_{2C}-adrenoceptor in the functional system. Utilization of an aromatic core incorporating bulk and related oxymetazoline, **23**, Table 1, afforded a very selective α_{2A}-adrenoceptor probe in binding assays; however, that agent proved inactive in the functional assay systems, Table 2. Agent **24** is an analog of **21** with the addition of a bulky substituent in the 5-position of the phenyl nucleus. This agent had both reduced selectivity and potency relative to **21**. Incorporation of the benzoxazine subunit, **25**, afforded an agent that proved selective for the α_{2A}-adrenoceptor in binding studies but possessed reduced potency and efficacy in functional assay systems, Table 2. The difference between binding and functional results was similar to the observations with **23**. The benzimidazole nucleus, **26**, had enhanced binding activity relative to the corresponding quinoxaline nucleus, **20**, but reduced binding selectivity compared to the *ortho*-substituted benzodioxanyl nucleus, **5**.

Jeon *et al*. have evaluated a series of iminoimidazolines related to brimonidine (**3**) using subtype specific α-adrenergic assay systems.[19] The results that we obtained with brimonidine are similar to the reported values. Both studies serve to illustrate the requirements for subtype specific functional assay systems as binding affinities do not always correlate with the results obtained from functional studies within related series of agents.

Conclusions

We have synthesized and evaluated a series of 2-(arylamino)imidazoles as α_2-adrenoceptor agonists. These agents displayed modest to good selectivity for α_{2A}-adrenoceptors in binding studies. Binding affinity has not proved an accurate measure of functional activity in this study. Data presented herein highlight the requirement for both binding and functional assay systems for the selection of viable candidates for subsequent *in vivo* evaluation.

The most potent member of the class of compounds synthesised, 2-[(5-methyl-1,4-benzodioxan-6-yl)amino]-imidazole, **5**, proved very selective for the α_{2A}-adrenoceptor relative to the α_1-adrenoceptor preparation in binding assays. The compound was comparable in activity to brimonidine in α_2-adrenoceptor functional assay systems. Agent **5** was equally efficacious when compared to brimonidine for the reduction of intraocular pressure upon topical administration to the rabbit and more efficacious than brimonidine for the reduction of blood pressure upon intravenous administration to monkey. During the course of our studies, we developed a new reagent that allowed rapid assembly of the target 2-(arylamino)imidazoles from arylamines. This reagent, *N*-(2,2-diethoxyethyl)carbodiimide, will prove of value for the preparation of related compounds.

Experimental Section

General. Reagents used were the highest quality available commercially. Reaction solvents were anhydrous and were obtained from Aldrich in Sure-Seal bottles. Unless otherwise noted, all reactions were carried out under an argon atmosphere and temperatures refer to the temperature of the bath. Organic extracts were dried with magnesium sulfate ($MgSO_4$) or potassium carbonate (K_2CO_3). Flash chromatography was conducted using the procedure described by Still.[20] Proton and carbon NMR spectra were measured on either a Varian Gemini 300 or a Varian Unity Plus 500 spectrometer in the solvents specified. Chemical shifts are reported in ppm downfield from TMS as an internal standard, and coupling constants are reported in hertz. Mass spectra (LRMS and HRMS) were recorded on a VG 7070E Sector Magnetic 70 eV mass spectrometer. Combustion analyses were conducted by Robertson Microlit Laboratories, Madison, NJ.

***N*-(5-Bromoquinoxalin-6-yl)carbodiimide (7).** To 6-amino-5-bromoquinoxaline (1.55 g, 6.92 mmol) in dry THF (150 mL) was added NaH (0.61 g, 60% in oil, 15.2 mmol) in portions. The mixture turned from clear yellow/orange to red, but no H_2 liberation was observed. The reaction mixture was then warmed at 55 °C until H_2 was no longer liberated. A cherry red solution resulted. The reaction mixture was cooled to room temperature, and phenyl cyanate (1.81 g, 15.2 mmol)

was added. The solution gradually lightened to yellow brown, and a precipitate was observed. After 2 h at room temperature, the mixture was warmed at reflux for 2 h. The solid was isolated by filtration. The solid was then slurried in a minimum amount of water, and HCl (6 M) was added dropwise until the pH reached 6. Filtration and drying afforded 860 mg (50%) of the carbodiimide. ^{1}H NMR (DMSO): 7.77 (d, J = 9.1 Hz, 1H), 8.18 (d, J = 9.1 Hz, 1H), 8.91 (d, J = 1.8 Hz, 1H), 9.01 (d, J = 1.8 Hz, 1H), 10.35 (br s, 1H). ^{13}C NMR (DMSO): 107.5, 111.3, 120.1, 130.3, 138.5, 139.4, 140.4, 144.6, 146.8. IR (KBr): 2227 cm^{-1}.

2-[(5-Bromoquinoxalin-6-yl)amino]imidazole (16). Carbodiimide **7** (0.255 g, 0.94 mmol) and aminoacetaldehyde diethyl acetal (0.163 g, 1.22 mmol) were dissolved in ethanol (25 mL) and treated with methanesulfonic acid (0.109 g, 1.13 mmol). The reaction mixture was warmed at reflux overnight. Traces of the starting material remained. An additional portion of aminoacetaldehyde diethyl acetal (0.032 g, 0.24 mmol) and methanesulfonic acid (1 drop) were added. After an additional 2 h warming at reflux, no further starting material remained. The reaction mixture was concentrated to dryness, dissolved in ethyl acetate, and then washed with NaOH (0.5 M). The organic layer was dried, concentrated, and subjected to flash chromatography (gradient: EtOAc → 40% THF in EtOAc) to afford a foam that was dissolved in HCl (5 mL, 6 M) at 0 °C and then stirred for 2 h. After the starting material was consumed (2 h), NaOH (25%) was added until a precipitate formed (pH of 14). This mixture was stirred for 30 min. The reaction mixture was then acidified to a pH of 5 using concentrated HCl. The resulting yellow solid was collected and dried. Chromatography over basic alumina (gradient: EtOAc → THF) afforded pure **16** (0.109 g, 40% overall) as a yellow solid. ^{1}H NMR (DMSO): 6.65−7.00 (br s, 2H), 7.99 (d, J = 9.3 Hz, 1H), 8.62 (s, 1H), 8.73 (d, J = 9.3 Hz, 1H), 8.74 (d, J = 1.9 Hz, 1H), 8.89 (d, J = 1.9 Hz, 1H), 11.29 (br s, 1H). ^{13}C NMR (DMSO): 104.3, 121.3, 128.8, 138.3, 140.8, 142.4, 142.5, 142.8, 146.0. Anal. Calcd ($C_{11}H_8BrN_5$) C, H, N.

***N*-(2,2-Diethoxyethyl)carbodiimide (14).** A solution of the aminoacetaldehyde diethyl acetal (2.66 g, 20 mmol) was dissolved in a mixture of ether (10 mL) and pentane (10 mL). This was then treated with solid CNBr (2.11 g, 20 mmol). A solid precipitated from solution. The reaction mixture was magnetically mixed for 18 h at room temperature. The solid was removed by filtration, and the reaction mixture was concentrated. Flash chromatography of the concentrated residue (5% MeOH in CH_2Cl_2) afforded 1.2 g of the reagent (38%; note that one-half of the starting amine served as a sacrificial base in the reaction). After analysis by ^{1}H NMR and IR, the material was dissolved in anhydrous ether to afford a 1 M solution that was stable to storage at −78 °C for 3 months. ^{1}H NMR ($CDCl_3$): 1.24 (t, J = 7 Hz, 3H), 3.17 (app t, J = 5.4 Hz, 2H), 3.57 (m, 2H), 3.74 (m, 2H), 3.85 (br s, 1H), 4.59 (t, J = 5.4 Hz, 1H). IR (cm^{-1}): 1064 (s, s), 1130 (s, s), 2227 (s, s), 2978 (s, m), 3226 (br, m).

5-Methyl-1,4-benzodioxane (10). A mixture of 3-methylcatechol, **9** (12.4 g, 100 mmol), 1, 2-dibromoethane (9.5 mL, 110 mmol), potassium carbonate (34.6 g, 250 mmol), and sodium iodide (0.75 g, 5.0 mmol) in acetone (200 mL) was warmed at reflux with mechanical stirring in a Morton flask overnight. The black slurry was poured into water and extracted with ether three times. The ether layer was washed with 5% sodium thiosulfate, dried, and distilled via Kugelrohr (80 °C, 0.05 mmHg) to afford 10.5 g of a gold oil. Flash chromatography (SiO_2, 2 in. × 4 in., 5% EtOAc in hexane) afforded 9.0 g (60%) of pure 5-methyl-1,4-benzodioxane. ^{1}H NMR ($CDCl_3$): 2.20 (s, 3H), 4.23−4.29 (m, 4H), 6.72 (br s, 3H).

6-Acetyl-5-methyl-1,4-benzodioxane. A mixture of 5-methyl-1,4-benzodioxane, **10** (9.0 g, 60 mmol), and acetic anhydride (6.14 g, 60 mmol) was warmed at 60 °C with polyphosphoric acid (70 g) using mechanical mixing in a Morton flask for 2 h. The reaction mixture was cooled and then the reaction quenched by the slow addition of H_2O (100 mL). The mixture was poured into water (200 mL) and extracted with ether. The ether was washed with $NaHCO_3$ (saturated) and then NaCl (saturated). The ether was dried and concentrated to afford a black oil. Flash chromatography with 10% EtOAc in hexane afforded 4.0 g (35%) of the desired product as the most mobile product. Recrystallization of a sample from ethanol afforded crystalline material which was used for confirmation of the regiochemistry via single-crystal X-ray analysis. ^{1}H NMR ($CDCl_3$): 2.38 (s, 3H), 2.53 (s, 3H), 4.28 (s, 4H), 6.75 (d, J = 8.6 Hz, 1H), 7.25−7.27 (d, J = 8.6 Hz, 1H).

5-Methyl-1,4-benzodioxane-6-carboxylic Acid (11). Fresh sodium hypochlorite was prepared by slurrying $Ca(ClO)_2$ (11.4 g, 80 mmol) in H_2O (50 mL) and treating the mixture with Na_2CO_3 (8.05 g, 76 mmol) and NaOH (0.96 g, 24 mmol) in H_2O (25 mL). The slurry was warmed and then filtered through a plug of glass wool to afford a homogeneous solution. Solid 6-acetyl-5-methyl-1,4-benzodioxane (3.84 g, 20 mmol) was added, and the resulting slurry was warmed at 60 °C overnight, at which point a homgeneous solution was observed. The aqueous reaction mixture was washed with CH_2Cl_2 (2×). The aqueous solution was treated dropwise with concentrated HCl until the pH reached 3. The solid was collected by filtration and washed well with water. The solid, 3.95 g (quantitative yield), was dried and used for the subsequent Curtius reaction without further purification. ^{1}H NMR ($CDCl_3$): 2.50 (s, 3H), 4.29 (s, 4H), 6.75 (d, J = 8.7 Hz, 1H), 7.61 (d, J = 8.7 Hz, 1H).

6-Amino-5-methyl-1,4-benzodioxane (12). Acid **11** (3.95 g, 20 mmol) was dissolved in acetone (25 mL) and Hunig's base (4.35 mL, 25 mmol). The reaction mixture was chilled to 0 °C, and ethyl chloroformate (2.01 mL, 21 mmol) was added dropwise and then stirred for 1 h. Sodium azide (2.6 g, 40 mmol) in H_2O (6 mL) was added, and the reaction mixture was stirred for an additional 1 h. The reaction mixture was poured into ice−water, and the organic material was extracted with CH_2Cl_2. Drying and concentration afforded the azide (IR: 2139, 2038 cm^{-1}). The product was dissolved in toluene (40 mL), and benzyl alcohol (2.16 g, 20 mmol) was added. After warming at reflux overnight, the reaction mixture was poured into ether and the organic solution was washed once with H_3PO_4 and once with $NaHCO_3$ (saturated), dried, and concentrated to afford a solid. Recrystallization from ethanol afforded 3.7 g (47%) of the protected amine. The carbamate was an excellent point to store the intermediate as aniline **12** darkened on standing. In a typical procedure, amine **12** was liberated upon treatment of the carbamate (1.0 g) in THF (20 mL, degassed using a Firestone valve) with an atmosphere of H_2 and 10% Pd−C (200 mg) overnight. The catalyst was removed by filtration through Celite. Concentration of the solution led to the amine **12** (550 mg, 95%) that rapidly turned purple on standing in air. ^{1}H NMR ($CDCl_3$): 2.0 (s, 3H), 3.35 (br s, 2H), 4.19 (m, 2H), 4.25 (m, 2H), 6.21 (d, J = 8.7 Hz, 1H), 6.58 (d, J = 8.7 Hz, 1H).

2-[(5-Methyl-1,4-benzodioxan-6-yl)amino]imidazole (5). Amine **12** (350 mg, 2.11 mmol) was dissolved in ethanol (25 mL), and *N*-(2,2-diethoxyethyl)carbodiimide, **14** (2.4 mL of a 1 M solution in ether), was added dropwise. Methanesulfonic acid (208 mg, 2.11 mmol) was then added, and the mixture was warmed at reflux for 24 h. The reaction mixture was poured into NaOH (0.5 M) and extracted with CH_2Cl_2. Drying and concentration afforded a product that was subjected to flash chromatography (5% MeOH in CH_2Cl_2 to remove mobile impurities and then 5% NH_3-saturated MeOH in CH_2Cl_2) to give the intermediate guanidine. The guanidine was dissolved in HCl (5 mL, 6 M) at 0 °C and then stirred for 2 h. After the starting material was consumed, NaOH (25%) was added until a precipitate formed (pH = 14). This mixture was stirred for 30 min. The reaction was then poured into NaOH (0.5 M), extracted with CH_2Cl_2, dried, and concentrated. Flash chromatography (5% NH_3-saturated MeOH in CH_2Cl_2) afforded **5** (292 mg, 60%). ^{1}H NMR (DMSO): 2.00 (s, 3H), 4.22 (m, 4H), 6.55−6.59 (m, 3H), 7.13 (d, J = 8.8 Hz, 1H), 7.45 (s, 1H), 10.4 (s, 1H). ^{13}C NMR (DMSO): 9.7, 63.5, 64.37, 110.8, 113.7, 114.6, 134.8, 137.6, 141.3, 146.5. Anal. Calcd ($C_{12}H_{13}N_3O_2$) C, H, N.

2-[(2-Bromophenyl)amino]imidazole (17). ^{1}H NMR (DMSO): 6.74 (m, 1H), 6.79 (s, 2H), 7.17 (m, 1H), 7.47 (d, J = 6.9 Hz, 1H), 7.61 (d, J = 6.9 Hz, 1H) 10.0 (br s, 1H). ^{13}C NMR

(DMSO): 111.0, 116.4, 119.3 (br), 121.7, 128.5, 132.6, 139.6, 143.8. Anal. Calcd ($C_9H_8BrN_3$) C, H, N.

2-[(2,6-Dichlorophenyl)amino]imidazole (18). ^{1}H NMR ($CDCl_3$): 6.40−6.80 (br s, 2H), 6.69 (s, 2H), 6.99 (t, J = 8.1 Hz, 1H), 7.30 (d, J = 8.1 Hz, 2H). ^{13}C NMR ($CDCl_3$): 118.7, 125.4, 129.0, 129.6, 135.7, 144.9. Anal. Calcd ($C_9H_7Cl_2N_3$) C, H, N.

2-[(5-Methylquinoxalin-6-yl)amino]imidazole (19). ^{1}H NMR (DMSO): 2.62 (s, 3H), 6.70−6.90 (br s, 2H), 7.81 (d, J = 9.3 Hz, 1H), 8.30 (s, 1H), 8.44 (d, J = 9.3 Hz, 1H), 8.66 (d, J = 1.8 Hz, 1H), 8.80 (d, J = 1.8 Hz, 1H), 11.0 (br s, 1H). ^{13}C NMR (DMSO): 10.5, 118.1, 118.4, 122.0, 126.7, 138.2, 141.4, 141.8, 141.9, 144.1, 144.3. Anal. Calcd ($C_{12}H_{10}N_5$) C, H, N.

2-(Quinoxalin-6-ylamino)imidazole (20). ^{1}H NMR (DMSO): 6.74 (br s, 1H), 6.85 (br s, 1H), 7.70 (dd, J = 9.3, 2.5 Hz, 1H), 7.89 (d, J = 9.3 Hz, 1H), 8.22 (d, J = 2.5 Hz, 1H), 8.61 (d, J = 1.9 Hz, 1H), 8.73 (d, J = 1.9 Hz, 1H), 9.41 (br s, 1H), 11.05 (br s, 1H). ^{13}C NMR (DMSO): 108.4, 122.5, 129.3, 137.7, 141.5, 143.5, 143.9, 144.2, 145.4. Anal. Calcd ($C_{11}H_9N_5$) C, H, N.

2-[(2-Methyl-4-methoxyphenyl)amino]imidazole (21). ^{1}H NMR (DMSO): 2.18 (s, 3H), 3.68 (s, 3H), 6.62 (s, 1H), 6.65−6.74 (m, 2H), 7.45 (s, 1H), 7.68 (d, J = 8.7 Hz, 1H), 10.47 (s, 1H). ^{13}C NMR (DMSO): 19.4, 56.6, 112.6, 117.3, 119.8, 128.3, 135.7, 147.8, 154.6. Anal. Calcd ($C_{11}H_{13}N_3O$) C, H, N.

2-[(2-Methyl-3,4-dimethoxyphenyl)amino]imidazole (22). ^{1}H NMR (DMSO): 2.08 (s, 3H), 3.66 (s, 3H), 3.72 (s, 3H), 6.63 (s, 2H), 6.77 (d, J = 8.9 Hz, 1H), 7.42 (d, J = 8.9 Hz, 1H). ^{13}C NMR (DMSO): 10.4, 56.0, 59.9, 110.5, 112.8, 120.2, 135.1, 146.4, 146.7, 147.1. Anal. Calcd ($C_{12}H_{15}N_3O_2$) C, H, N.

2-[(2,6-Dimethyl-3-methoxy-4-*tert*-butylphenyl)amino]-imidazole (23). ^{1}H NMR: 1.39 (s, 9H), 2.19 (s, 3H), 2.21 (s, 3H), 3.73 (s, 3H), 6.05 (br s, 2H), 6.62 (s, 2H), 7.06 (s, 1H). ^{13}C NMR: 12.7, 18.1, 30.9, 34.8, 60.9, 126.7, 126.8, 129.1, 129.7, 135.9, 141.0, 148.7, 157.4. Anal. Calcd ($C_{16}H_{22}N_3O\cdot0.25H_2O$) C, H, N.

2-[(2-Methyl-4-methoxy-5-*tert*-butylphenyl)amino]imidazole (24). ^{1}H NMR (DMSO) 1.28 (s, 9H), 2.15 (s, 3H), 3.74 (s, 3H), 6.50−6.69 (m, 2H), 6.76 (s, 1H), 7.38 (s, 1H), 7.62 (s, 1H). ^{13}C NMR (DMSO) 18.9, 31.2, 35.6, 57.0, 116.0, 118.5, 126.0, 134.9, 136.4, 148.2, 153.7, 156.5. Anal. Calcd ($C_{15}H_{21}N_3O\cdot0.5H_2O$) C, H, N.

2-[(5-Methylbenzoxazin-6-yl)amino]imidazole (25). ^{1}H NMR (acetone): 2.00 (s, 3H), 3.41 (m, 2H), 4.08 (t, J = 4.4 Hz, 2H), 4.60 (br s, 1H), 6.48 (d, J = 8.6 Hz, 1H), 6.58 (s, 2H), 6.77 (d, J = 8.6 Hz, 1H), 6.98 (br s, 1H), 9.5 (br s, 1H). ^{13}C NMR (acetone): 11.3, 41.9, 65.2, 112.1, 114.6, 116.5, 118.3, 133.7, 134.5, 141.0, 149.5. MS (low res): obsd m/z at 230 (EI^+); (high res) mass calcd for $C_{12}H_{14}N_4O$ ($M^{\bullet+}$) = 230.1168; obsd m/z at 230.1196, deviation from theoretical = 1.8 mmu. Anal. Calcd for $C_{12}H_{15}ClN_4O$: C, 54.03; H, 5.67; N, 21.01. Found: C, 53.94; H, 5.52; N, 20.23.

2-(Benzimidazol-5-ylamino)imidazole (26). ^{1}H NMR (DMSO): 6.68 (s, 2H), 7.0 (d, 1H), 7.01 (dd, J = 8.7, 2.0 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.99 (s, 1H), 8.56 (s, 1H), 11.5 (br s, 2H). ^{13}C NMR (DMSO): 99.2 (br), 112.5, 117.1 (br), 118.0 (br), 138.1, 140.7, 145.6. Anal. Calcd ($C_{11}H_{10}N_4\cdot0.2H_2O$) C, H, N.

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