Graphical Abstract

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Naamidine H, $IC_{50} = 4.94 \mu M$

Total Synthesis and Cytotoxicity of *Leucetta* Alkaloids

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ABSTRACT

The total synthesis of a number of representative natural products isolated from *Leucetta* and *Clathrina* sponges containing a polysubstituted 2-aminoimidazole are described. These syntheses take advantage of the site specific metallation reactions of 4,5-diiodoimidazoles resulting in the syntheses of three different classes of *Leucetta* derived natural products. The cytotoxicities of these natural products, along with several precursors in MCF7 cells were determined through an MTT growth assay. For comparative purposes a series of naphthimidazole-containing family members are included.

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1. Introduction

The *Leucetta* and *Clathrina* families of marine sponges have been investigated as sources of novel bioactive secondary metabolites for several years.¹ Broadly speaking, these sponges produce either polyunsaturated fatty acid derivatives or a growing family of 2-aminoimidazole containing alkaloids (e.g., **1–7**, Figure 1).² These alkaloids vary in complexity from the simple monobenzyl substituted derivatives such as clathridine A (**1**) to the highly condensed kealiquinone (**6**)³ or the highly oxidized spiroleucettadine (**7**) (Figure 1).⁴ In between these extremes are the two isomeric dibenzyl substituted systems exemplified by isonaamidine E (**2**)⁵ or naamidine H (**5**).⁶ The vast majority of these natural products are described as possessing “biological activity”, but rarely are either the assays or the cell types the same from one isolation group to the next.⁷ Furthermore, it is often times difficult to obtain extremely pure materials from natural isolates which influences both the magnitude and in some cases the reliability of these data. Lastly, the ability to conduct SAR studies is limited to derivatization of the natural material, which often can only be obtained in limited supply. To date, only the biological activity of naamidine A (**3**) has been extensively investigated by the Ireland lab which has demonstrated that it promotes caspase-dependent apoptosis in cultured tumor cells.⁸ An Australian team has reported a modest exploration of the SAR of the naamine/naamidine A framework.⁹ More recently, the Looper lab¹⁰ and our group¹¹ have begun to evaluate the biological activity of synthetic versions of various other family members, specifically the naphthimidazole group. Very recently Jiang and coworkers have applied our synthetic methods to access the naamine framework to prepare derivatives at the 2-amino group with activity against P-glycoprotein mediated drug resistance in various cancer cell lines.¹² In order to pursue a medicinal chemistry campaign of these molecules, we

needed reliable synthetic strategies that ideally were short and readily amenable to variation thus providing both the opportunity to access natural products but also permitting the construction of analogues around the imidazole core.

Several years ago, we began a synthetic program directed towards the total synthesis of several of the more complex members of the *Leucetta* alkaloids, specifically calcaridine A (**10**)¹³ and spirocalcaridines A (**8**) and (**9**),¹⁴ using a broadly bioinspired-synthetic strategy.^{1a, 15} As a result we needed to access the basic framework found in the naamine group of alkaloids to perform a variety of biomimetic rearrangements. As we considered our options, it became clear that any strategy along these lines would provide an opportunity for the preparation of a selection of different family members. In this manuscript, we detail this strategy with the total synthesis of an example from each major class of *Leucetta* alkaloids. In addition, we describe the cytotoxicity of the synthesized natural products and selected precursors using an MTT based cell viability assay in MCF7 breast cancer cells, thereby obtaining direct comparative biological data for several members of the *Leucetta* alkaloids and their precursors for the first time.

Figure 1: Characteristic members of the *Leucetta* alkaloids

2. Results and Discussion

Strategy: As noted above, we wished to develop an approach to this group of molecules which was flexible, would readily deliver analogs and above all would be scalable. Several general strategies can be envisioned for the preparation of the polysubstituted imidazoles, either through the intermediacy of α -substituted ketones (**12**→**11**), hydroamination of alkynes^{10b, 16} or through the functionalization of pre-formed imidazoles (**13**→**11**).¹⁷ In each case, the strategies are executed to avoid introducing the polar 2-amino substituent until the end of the synthetic sequence. In this context we pursued the latter approach, relying on halogen-Grignard exchange reactions of 4,5-diiodoimidazole derivatives and subsequent electrophilic quench.¹⁸ Based on literature reports, it was known that C5 would undergo exchange first, followed by C4, the remaining unsubstituted position at C2 could then be deprotonated with *n*-BuLi allowing the sequential and selective polyfunctionalization of the heterocycle. The majority of examples described in the literature prior to our work involved imidazoles with stabilizing *N*-substituents, whereas our substrates by necessity would have either methyl or benzyl groups which would not stabilize any adjacent negative charge character to any appreciable extent. As a consequence, when we initiated this effort it was uncertain whether this technique could be extrapolated to such systems.

Figure 2: Strategic approaches to the *Leucetta* alkaloids

Our initial target centered on the construction of the simple trisubstituted system clathridine A (**1**) and the related preclathridine A (**19**) (Scheme 1).¹⁹ Our synthesis began with the preparation of 4,5-diiodo-1-methylimidazole (**14**);²⁰ while this material is commercially available through various sources, it can be prepared easily and in large quantities through the iodination of imidazole in basic solution and then *N*-methylation. No chromatography is required for either step; simply washing with solvent provides material of sufficient purity for further use. In order to access clathridine A (**1**), the 4-iodoimidazole **15** was required, this can be obtained by treatment of **14** with ethylmagnesium bromide and quenching with water.²¹ At this stage we were in position to install the benzylic substituent. Previous experience in our lab had demonstrated that this would be difficult to achieve directly by using a benzyl halide with electron rich imidazoles due to competitive *N*-alkylation^{13a} and therefore we employed an aldehyde and reduced the corresponding alcohol. In practice this involved metallation of C4 of **15** by treatment with ethylmagnesium bromide followed by treatment with piperanal, which delivered the doubly benzylic alcohol **16** in excellent yield. Ionic reduction occurred smoothly upon treatment of **16** with Et₃SiH in a mixture of TFA and dichloromethane. At this stage the introduction of the 2-amino group was necessary and this was accomplished through a two-step sequence involving C2-metallation of **17** with *n*-BuLi and trapping with TsN₃.²² The resulting 2-azido derivative **18** was subjected to catalytic hydrogenation (Pd-C/H₂) affording preclathridine A (**19**). Clathridine A (**1**) was obtained by introduction of the methyl parabanic acid fragment using a literature procedure involving exposure of **19** to the silylated parabanic acid derivative **20**.²³ The resulting synthetic material had spectroscopic properties that nicely matched those reported for the natural material. In addition, the synthetic natural product was crystalline and so an X-ray crystal structure was obtained which nicely illustrated the location of all of the substituents and confirmed the structure of the natural product (Figure 3). Interestingly, the crystalline material obtained was tautomeric to the reported structure of clathridine A (**1**) (Figure 1) but this may simply reflect crystal packing forces.

Figure 3: X-ray crystal structure of clathridine

With the synthesis of the two clathridine derivatives complete, we turned our attention to the isonaamidine series.²⁴ In this subfamily, the imidazole nitrogen is substituted with a benzyl moiety and thus the synthesis commenced with the 4,5-diiodoimidazole by alkylation with *p*-methoxybenzyl chloride resulting in the formation of **22** (Scheme 2). Treatment of **22** with ethylmagnesium bromide followed by quenching with water provided the 4-iodoimidazole **23**.²⁵ Exposure of **23** to ethylmagnesium bromide and treatment of the metallated imidazole with benzaldehyde **24** gave a mixture of the desired alcohol **25** and the corresponding ketone. Treatment of this unpurified mixture with NaBH₄ reduced the ketone to the alcohol, which upon purification delivered 73% of the required product **25**. Reduction of the doubly benzylic alcohol with Et₃SiH and TFA provided **26**. At this stage we were in a position to introduce the C2-amine via metallation with *n*-BuLi and trapping with TrisN₃, however there was some concern *vis à vis* chemoselectivity as it is known that *N*-benzylimidazoles are prone to deprotonation at the benzylic position.²⁶ Fortunately, when **26** was exposed to *n*-BuLi followed by reaction with TrisN₃ the 2-azido derivative **27** was obtained in good yield. Reduction with Pd-C/H₂ delivered isonaamine C (**28**) which upon treatment with the silylated parabanic acid derivative **20** afforded isonaamidine E (**2**) in good yield (Scheme 2).

Scheme 2: Total synthesis of isonaamine (**28**) and isonaamidine E (**2**)

With approaches to examples of 4-substituted imidazoles secured, we turned our attention to the 4,5-disubstituted derivatives. Strategically, we anticipated that both benzyl moieties could be installed sequentially through metallation and trapping with the appropriate aldehydes. As initial targets, our attention was directed to naamidine G (**4**),²⁷ one of several molecules isolated by Pietra and coworkers.²⁸ The crude extract containing these molecules was described as being cytotoxic, but no details were described for the individual purified molecules and thus access to synthetic versions would help address the question of which of these several derivatives were responsible for the cytotoxicity. The synthesis commenced from the diiodoimidazole **14** which was subjected to transmetalation with EtMgBr. Exposure of the resulting organometallic to *p*-anisaldehyde delivered the corresponding alcohol **16**; ionic reduction of the doubly benzylic alcohol using Et₃SiH and trifluoroacetic acid provides **18** (Scheme 3). In the context of our calcaridine A (**10**) synthesis we had attempted to introduce the second benzylic substituent by reaction of the Grignard derived from **30** and a protected benzaldehyde derivative, but this was not very efficient.¹³ We were able to circumvent this problem by formylating first and then using an aryl Grignard reagent. Accordingly, formation of the Grignard reagent was accomplished by treatment of **30** with EtMgBr and then reaction with *N*-methyl formanilide (**31**) which delivered the aldehyde **32**

Scheme 1: Total synthesis of preclathridine A (**19**) and clathridine A (**1'**)

in excellent yield. Reaction of the aldehyde with *p*-anisylmagnesium bromide provided the corresponding alcohol **33** in good yield. Ionic reduction of the benzylic alcohol **33** was readily accomplished on exposure to Et₃SiH and TFA. The synthesis naamidine **5** (**4**) was completed in short order by metallation of imidazole **34** at C2 with *n*-BuLi and reaction of the thus formed organolithium with TsN₃. The resulting azido derivative **35** was hydrogenated with Pd-C to provide the 2-amino derivative **36** which upon treatment with the activated parabanic acid derivative **20** afforded naamidine **G** (**4**) (Scheme 3). Compound **36** has not been reported as a natural product, but the desmethyl derivative (naamidine **D**) and the dimethyl derivative (N,N-dimethylnaamidine **D**)² have been described.

Scheme 3: Total synthesis of naamidine **G** (**4**)

We noted that alcohol **33** might serve as a precursor to the C14-oxygenated derivatives exemplified by 14-methoxynaamidine **G** (**40**) (Scheme 4). Accordingly, alcohol **33** was converted to the methyl ether by treatment with TFA and methanol followed by C2-azidation and reduction to give the corresponding amine. Unfortunately, we were unable to obtain the naamidine derivative in useful amounts as the introduction of the parabanic acid fragment was not efficient using standard literature methods for its introduction. Presumably, residual BSA

from the activation of the parabanic acid leads to competitive ionization of the methyl ether and non-productive side reactions.

Scheme 4: Total synthesis of 14'-Methoxynaamidine

The last targets that we pursued were naamine **G** and naamidine **H** as these derivatives were reported to possess cytotoxicity against several cancer cell lines in the low micromolar range.²⁹ Our synthesis began from **14** and proceeded through halogen-metal exchange and subsequent reaction with the Bn-protected aldehyde **41** resulting in the formation of alcohol **42**. Attempts to reduce this alcohol under ionic conditions were not especially efficient, delivering less than 30% yield of the benzylic derivative **43**. In addition to the required product, the reductive debenzoylation product **43** was obtained in a similar yield. However, rather than searching for conditions to optimize this reduction, we chose to introduce the second benzylic substituent and then remove both hydroxyl groups simultaneously. We were able to introduce this second substituent through similar chemistry to that employed above wherein the imidazolyl aldehyde **45** was prepared first and then reaction with *p*-anisylmagnesium bromide to deliver diol **46** as a mixture of diastereomers. With the crude diol in hand it was subjected to the standard conditions that had been successful previous for the ionic reduction of related derivatives, however, rather than reduction, the diol underwent an intramolecular Friedel-Crafts reaction followed by elimination to provide the naphthimidazole **47**. Presumably, a carbocation is formed and then this is trapped by the more electron rich aromatic system, dehydration then affords **47**. The initial formation and rearrangement of an *ipso* addition adduct cannot be ruled out. Indeed, in recently published work we have evidence of such

rearrangements resulting in the formation of naphthimidazoles.¹⁴ While clearly in the context of our targets this outcome was not useful, the framework produced in this reaction maps very nicely onto other members of the *Leucetta* alkaloids, most notably the kealiinines and kealiquinones. Indeed, we have used related chemistry to access both families of natural products.^{11, 15}

Scheme 5: First generation approach to naamidine H (5)

Given the propensity of this electron-rich system to undergo cyclization rather than reduction chemistry, we needed to develop an approach to circumvent this problem. An obvious way to accomplish this task was to avoid the generation of these benzylic carbocations. One way that this can be achieved is to use benzyl halides as electrophiles rather than aldehydes. We attempted to execute such a strategy with *N*-methylimidazole derivatives, e.g., **14**+**48**→**43**. Unfortunately this chemistry was not successful and led to the formation of monoiodinated imidazolium salts. On the other hand, Lindell has demonstrated that C-alkylation can be feasible using dimethylsulfamoyl (DMAS)-protecting group via the corresponding organocuprate. While this was encouraging, this meant that an exchange of imidazole *N*-substituents was necessary and that this had to be accomplished in a position selective manner. Fortunately, Robiette,³⁰ Sames³¹ and more recently Knochel^{17b} have demonstrated that the DMAS-protecting group can be removed with a methyl group via the intermediacy of an imidazolium salt formed by reaction with methyl triflate or Meerwein's salt. Given this encouraging precedent, the corresponding DMAS-protected

4,5-diiodoimidazole was used as a starting material. Sequential treatment of **49** with EtMgBr then CuCN.2LiCl provided the corresponding cuprate which was then reacted with *p*-methoxybenzyl bromide to provide **50** in 65% yield. Essentially the same sequence of reactions was used to obtain the dibenzyl substituted imidazole this time using benzyl bromide **48** as the electrophile. At this stage, we chose to perform the protecting group exchange sequence by first converting **51** to the methyl imidazolium salt **52** by treatment with methyl triflate. Subsequent reaction with benzylamine in acetonitrile at reflux provided the *N*-methyl substituted derivative **53** in 90% yield for the two step sequence. At this point all that remained to be done was to introduce the 2-amino group and the parabanic acid moiety. Interestingly, it was found that metalation at C2 followed by treatment with TsN₃ resulted in sulfonylation, but substitution with TrisN₃ delivered the 2-azidoimidazole **55**. Catalytic hydrogenation resulted in reduction of the azido moiety to the corresponding amine and hydrogenolysis of the benzyl protecting group resulting in the synthesis of naamine G (**56**) in excellent yield. Treatment with activated *N*-methyl parabanic acid then delivered naamidine H (**5**) in good yield. The spectroscopic data for both synthetic naamine G and synthetic naamidine H were in good agreement with that obtained for the isolated materials.

Scheme 6: Second generation total syntheses of naamine G (**56**) and naamidine H (**5**)

With this group of natural products in hand they were subjected to cytotoxicity testing using an MTT assay with MCF7 cells; the outcomes of these experiments are reported in Table 1 (entries 1-10). In addition to the naamine/naamidine group of compounds, we also include the naphthimidazole based family that we have reported previously for comparative purposes (entries 12-23, Table 1).¹¹ Generally speaking for the synthetic versions of the natural products, the systems substituted with *N*-methyl parabanic acid are substantially more active than the corresponding 2-amino derivatives or desamino derivatives. Interestingly, there appears to be broad tolerance with respect to the identity and location of the other substituents around the imidazole moiety. In cases where cytotoxicity has been determined on material isolated from natural sources, our synthetic materials display similar values – typically $IC_{50} < 10 \mu M$. One notable exception was with isonaamine C (**28**) (entry 4), for which the naturally occurring material was reported to have an IC_{50} value of 2-5 μM , although this was obtained in different cell lines, whereas our synthetic material had an $IC_{50} > 100 \mu M$. Our data are consistent with respect to the naamine vs naamidine activities, with the latter being more active. In the cases of the naphthimidazole derivatives, the cytotoxicities were not determined on natural materials, only on the synthetic materials.^{11, 32} In general terms the presence of a C2-amino substituent increases their cytotoxicity as evidenced by the kealiinines, but the presence of quinone in the C-ring as found in kealiiquinone (**6**) and 2-deoxy-2-aminokealiiquinone (**68**) appears to reduce activity. Interestingly, kealiinine C (**63**) is almost devoid of activity, whereas the isomeric congener **65** (entry 19) is about equipotent with the other two family members **59** and **61** (entries 13 and 15). At one point in our synthetic studies we were concerned that the structure of kealiinine C had been misassigned, however, HPLC studies suggested that the original assignment does in fact appear to be correct.^{11, 32a}

Table 1. Cytotoxicity data for several Leucetta-derived alkaloids and analogs in MCF7 cells using an MTT growth assay.

Entry	Compound	IC ₅₀ /μMol	Entry	Compound	IC ₅₀ /μMol
1	17	>100	13	Kealiinine A (59)	23.4±1.9
2	Preclathridine A (19)	>100	14	60	28.0±1.3
3	Clathridine A (1)	5.78±0.04	15	Kealiinine B (61)	28.8±1.2
4	Isonaamine C (28)	>100	16	62	99.8±0.9
5	Isonaamidine E (2)	4.4±0.2	17	Kealiinine C (63)	>100
6	36	19.5±1.5	18	64	68.2±1.6

7	<p>Naamidine G (4)</p>	5.08±0.05	19	<p>Isokealiinine C (65)</p>	22.7±1.0
8	<p>39</p>	16.3±0.4	20	<p>2-Deamino-2-oxokealiinine C (66)</p>	29.9±0.3
9	<p>Naamine G (56)</p>	76.9±0.97	21	<p>2-Deoxykealiiquinone (67)</p>	41.1±0.1
10	<p>Naamidine H (5)</p>	4.94±0.12	22	<p>Kealiiquinone (6)</p>	91.9±0.5
11	<p>57</p>	92.0±2.2	23	<p>2-Deoxy-2-aminokealiiquinone (68)</p>	43.8±0.4
12	<p>58</p>	>100			

Conclusion

Our initial approach to these molecules relied on the formation of Grignards by *in situ* metal-halogen exchange reactions followed by trapping with carbonyl derivatives. In most cases

this was followed by ionic reduction to deliver the net C-alkylated product. This strategy worked well for the production a number of representative members of the *Leucetta* alkaloids, however, in at least one case the ionic reduction chemistry was

compromised by the presence of an electron rich aromatic ring that led to an unanticipated Friedel-Craft's pathway and the formation of a naphthimidazole. To circumvent this pathway, an electron poor imidazole derivative was alkylated at carbon thereby avoiding the non-productive cyclization pathway and ultimately delivering naamidine H. This latter strategy, while requiring the use of an *N*-protecting group, results in an overall shorter synthetic sequence and would appear to represent the best approach to this family of natural products. In general terms, synthetic variants of the natural products containing the methyl parabanic acid moiety exhibited higher cytotoxicities.

Experimental

Chemicals were used as received unless indicated otherwise. NMR spectra were obtained at either 500 MHz (for ^1H NMR spectra) and 125 MHz (for ^{13}C NMR spectra) in CDCl_3 unless indicated otherwise. Infrared (IR) spectra were obtained on neat samples (ATR spectroscopy) or using either KBr pellets for solids or neat films on NaCl plates for liquids (transmission). Analytical thin-layer chromatography (TLC) was performed on silica gel plates, 200 mesh with F254 indicator. Visualization was accomplished by UV light (254 nm), and/or a 10% ethanol solution of KMnO_4 . Flash column chromatography was performed with 230–400 silica gel. High resolution mass spectra (HRMS) were recorded by electrospray ionization (ESI-TOF) or atmospheric-pressure chemical ionization (APCI-TOF) unless otherwise indicated.

4-Iodo-1-methyl-1*H*-imidazole (15): EtMgBr (3.0 M solution in ether, 11.00 mL, 32.9 mmol) was added to a solution of **14** (10.00 g, 29.9 mmol) in dry CH_2Cl_2 (150 mL) at rt. over ~15 min. The resulting mixture was stirred for 20 min and water (20 mL) was added to the reaction mixture after running a TLC experiment to confirm all the starting material is consumed. Then, the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3x50 mL) and the combined organic layers were dried (Na_2SO_4) and concentrated to isolate **15** as pale yellow oil (5.86 g, 94%).

4-(Benzo[1,3]dioxol-5-yl)hydroxymethyl-1-methyl-1*H*-imidazole (16): EtMgBr (3.0 M solution in ether, 9.76 mL, 29.3 mmol) was added to a solution of **15** (5.80 g, 27.9 mmol) in dry THF (100 mL) at 0 °C over ~10 min. The resulting mixture was stirred at rt. for 30 min and piperonal (4.65 mL, 30.7 mmol) was added to the reaction slowly at rt. After stirring overnight at rt., satd. aq. NH_4Cl (20 mL) was added to the reaction and the layers were separated. The aqueous layer was extracted with EtOAc (3 x 50 mL) and the combined organic extracts were dried (Na_2SO_4) and concentrated to provide the crude product, which was purified by flash column chromatography on silica gel (100% EtOAc $\rightarrow$ 100% Acetone) to isolate **16** (5.82 g, 90%) as an orange semi-solid: ^1H NMR: δ = 7.35 (s, 1H), 6.90 (s, 1H), 6.87 (d, J = 7.8 Hz, 1H), 6.72 (d, J = 7.8 Hz, 1H), 6.45 (s, 1H), 5.88 (s, 2H), 5.66 (s, 1H), 5.27 (br, 1H), 3.54 (s, 3H); ^{13}C NMR: δ = 147.5, 146.8, 146.0, 137.8, 137.4, 120.1, 117.2, 107.9, 107.4, 101.0, 70.0, 33.5; IR (neat, cm^{-1}): = 3114, 2891, 1493, 1442, 1243, 1037, 927, 794, 756; HR-ESIMS (m/z): Calcd. for $\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_3$ [$\text{M}+\text{H}$] $^+$ 233.0921, found 233.0925.

4-(Benzo[1,3]dioxol-5-yl)methyl-1-methyl-1*H*-imidazole (17): TFA (7.00 mL, 91.4 mmol) and Et_3SiH (17.00 mL, 106.4 mmol) were added to a solution of **16** (5.31 g, 22.9 mmol) in dry CH_2Cl_2 (150 mL) at rt. The resulting mixture was stirred for 48 h; the reaction mixture was then quenched by the addition of sat.

NaHCO_3 solution until effervescence ceased. The resulting mixture was extracted with CH_2Cl_2 several times and the combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (acetone/EtOAc, 3/7) to isolate a pale yellow semi-solid: **17** (3.55 g, 72%); ^1H NMR: δ = 7.27 (s, 1H), 6.71 (s, 1H), 6.67 (s, 2H), 6.46 (s, 1H), 5.82 (s, 2H), 3.76 (s, 2H), 3.52 (s, 3H); ^{13}C NMR: δ = 147.6, 145.8, 142.8, 137.4, 134.4, 121.6, 117.0, 109.4, 108.2, 101.0, 34.7, 33.2; IR (neat, cm^{-1}): = 3367, 2900, 2778, 1492, 1441, 1246, 1185, 1038, 928, 815, 773; HR-ESIMS (m/z): Calcd. for $\text{C}_{12}\text{H}_{13}\text{N}_2\text{O}_2$ [$\text{M}+\text{H}$] $^+$ 217.0972, found 217.0991.

2-Azido-4-(benzo[1,3]dioxol-5-yl)methyl-1-methyl-1*H*-imidazole (18): *n*-Butyl lithium (1.4 M solution in hexanes, 11.34 mL, 15.9 mmol) was added dropwise to a solution of **17** (3.12 g, 14.4 mmol) in THF at -78 °C and TsN_3 (3.41 g, 17.3 mmol) were used to afford the crude product, which was purified by chromatography (15% $\rightarrow$ 20% EtOAc in hexanes) to isolate **18** (2.93 g, 78%) as a reddish brown oil: ^1H NMR: δ = 6.76 (s, 1H), 6.72 (s, 2H), 6.24 (s, 1H), 5.90 (s, 2H), 3.74 (s, 2H), 3.31 (s, 3H); ^{13}C NMR: δ = 147.7, 146.0, 140.2, 140.0, 133.6, 121.7, 116.1, 109.5, 108.2, 100.9, 34.8, 31.5; IR (neat, cm^{-1}): = 2896, 2154, 2125, 1494, 1441, 1245, 1039, 929, 812; HR-ESIMS (m/z): Calcd. for $\text{C}_{12}\text{H}_{12}\text{N}_5\text{O}_2$ [$\text{M}+\text{H}$] $^+$ 258.0986, found 258.0987.

2-Amino-4-(benzo[1,3]dioxol-5-yl)methyl-1-methyl-1*H*-imidazole (Preclathridine A) (19): Azide **18** (2.80 g, 10.9 mmol) was dissolved in EtOH (30 mL) and stirred under a hydrogen atmosphere (1 atm) in the presence of 10% Pd-C on charcoal (0.30 g) at rt overnight to afford **19** (2.59 g, quant) as a yellow solid: m.p. = 116–118 °C; ^1H NMR (CDCl_3): δ = 6.73 (s, 1H), 6.69 (s, 2H), 6.08 (s, 1H), 5.86 (s, 2H), 4.14 (br, 2H), 3.63 (s, 2H), 3.26 (s, 3H); ^{13}C NMR δ = 148.0, 147.5, 145.8, 137.1, 134.5, 121.7, 112.8, 109.5, 108.1, 100.8, 34.7, 31.3; IR (KBr, cm^{-1}): = 3432, 3299, 3109, 1643, 1547, 1505, 1442, 1244, 1034, 921, 814; HR-ESIMS (m/z): Calcd. for $\text{C}_{12}\text{H}_{14}\text{N}_3\text{O}_2$ [$\text{M}+\text{H}$] $^+$ 232.1081, found 232.1094.

2-(3-Methylimidazolidine-2,4-dione)imino-4-(4-benzo[1,3]dioxol-5-yl)methyl-1-methyl-1*H*-imidazole (Clathridine A) (1'): *N,O*-Bis(trimethylsilyl)acetamide (5.18 mL, 21.2 mmol) was added to a solution of 1-methylparabanic acid (2.26 g, 17.7 mmol) in dry CH_3CN (30 mL) under an N_2 atmosphere and the resulting mixture was heated at reflux temperature for 1.5 h. The solvent was removed by vacuum distillation, and to the resulting yellow residue **20 was dissolved in dry toluene (5 mL) and then added amine **19** (817 mg, 3.5 mmol) under N_2 atmosphere. After, this mixture was heated at 85 °C overnight (product starts to form after 10 min as a yellow solid at the bottom of the flask), the product was filtered and solid was washed with methanol (10 mL) to deliver **1'** as a yellow solid (730 mg, 61%): m.p. = 253–254 °C (lit.⁷ m.p. = 260–262 °C); ^1H NMR: δ = 6.75 (d, J = 8.3 Hz, 1H), 6.71 (s, 1H), 6.70 (d, J = 8.3 Hz, 1H), 6.52 (s, 1H), 5.93 (s, 2H), 3.80 (s, 2H), 3.71 (s, 3H), 3.18 (s, 3H); ^{13}C NMR: δ = 162.0, 155.0, 147.8, 147.1, 146.3, 144.3, 139.7, 132.7, 121.7, 117.7, 109.3, 108.4, 101.0, 34.5, 32.2, 24.8; IR (KBr, cm^{-1}): = 3204, 3122, 2914, 1789, 1738, 1665, 1444, 1391, 1323, 1249, 1209, 1114, 1034, 925, 732; HR-ESIMS (m/z): Calcd. for $\text{C}_{16}\text{H}_{16}\text{N}_5\text{O}_4$ [$\text{M}+\text{H}$] $^+$ 342.1197, found 342.1201.**

4,5-Diiodo-1-(4-methoxybenzyl)-1*H*-imidazole (22): 4,5-Diiodo-1*H*-imidazole **21** (0.50 g, 1.6 mmol) in THF (15 mL) was cooled to 0 °C and NaH (60% suspension in mineral oil) (0.10 g, 2.5 mmol) was added slowly. The reaction was allowed to come to rt and stir under N_2 for 30 min. 4-Methoxybenzyl chloride (0.28 mL, 2.0 mmol) was added and stirred at 50 °C for 18 h. The reaction was allowed to cool to rt, quenched with water

(10 mL) and extracted with EtOAc (2 x 10 mL). The combined organic solutions were washed with water (10 mL), brine (10 mL), dried (anhyd. Na₂SO₄) and concentrated. The resulting crude residue was purified by column chromatography (4:6 EtOAc/Hexane) to give **22** as a yellow solid (0.63 g, 91%). m.p. = 158-160 °C; ¹H NMR: δ = 7.55 (s, 1H), 7.10 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.07 (s, 2H), 3.80 (s, 3H); ¹³C NMR: δ = 159.9, 141.2, 129.2, 126.7, 114.5, 96.1, 82.4, 55.4, 53.1; IR (cm⁻¹): 3099, 2929, 2832, 1611, 1510, 1433, 1298, 1241, 1174, 1024, 816, 760, 629; HR-ESIMS (*m/z*): Calcd. for [M+H]⁺ C₁₁H₁₁N₂O₂ is 440.8955 found 440.8956.

4-Iodo-1-(4-methoxybenzyl)-1H-imidazole (23):^{25b}

Diiodoimidazole **22** (16.2 g, 36.8 mmol) in CH₂Cl₂ (250 mL) was cooled to 0 °C under N₂ and EtMgBr (3.0 M in Et₂O) (12.3 mL, 36.8 mmol) was added slowly. The reaction mixture was allowed to come to rt and stirred for 1.5 h. Water (5 mL) was slowly added and then the mixture was stirred at rt for an additional 1 h. The reaction was partitioned with water and the organic layer separated. The aqueous layer was extracted with CH₂Cl₂ (2 x 100 mL). The combined organic extracts were washed with brine, dried (anhyd. Na₂SO₄), and concentrated. The resulting residue was purified by column chromatography (1:1 EtOAc/Hexane) to give **23** as a pale yellow solid (9.6 g, 83%). m.p. = 49-51 °C; ¹H NMR (CDCl₃+DMSO-*d*₆): δ = 7.37 (d, *J* = 1.7 Hz, 1H), 7.08, (d, *J* = 8.6 Hz, 2H), 6.91 (d, *J* = 1.7 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 4.97 (s, 2H), 3.76 (s, 3H); ¹³C NMR (CDCl₃+DMSO-*d*₆): δ = 159.9, 138.7, 129.2, 127.2, 124.7, 114.5, 81.9, 55.4, 50.8; IR (cm⁻¹): 3127, 3005, 2959, 2836, 1612, 1512, 1441, 1244, 1223, 1179, 1095, 1033, 934, 837, 813, 760, 619.

4-[Hydroxy-(3,4-dimethoxyphenyl)]methyl-1-(4-methoxybenzyl)-1H-imidazole (25):

Iodoimidazole **23** (1.0 g, 3.2 mmol) in THF (15 mL) was added EtMgBr (3.0 M in Et₂O) (1.28 mL, 3.83 mmol) under N₂ and stirred at rt for 30 min. The resulting Grignard solution was then added to aldehyde **24** (0.53 g, 3.2 mmol) in THF (30 mL) and the mixture was stirred at rt for 18 h. The reaction was quenched with water (30 mL) and the aqueous phase was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with brine, dried (anhyd. Na₂SO₄), and concentrated. The crude residue was dissolved in MeOH (10 mL) and NaBH₄ (0.060 g, 1.6 mmol) was added and stirred at rt for 3 h. The reaction was quenched with water (10 mL) and extracted with CH₂Cl₂ (2 x 15 mL). The combined organics were dried (anhyd. Na₂SO₄) and concentrated. The resulting residue was purified by column chromatography (5% MeOH in EtOAc) to give **25** as an off-white solid (0.82 g, 73%). m.p. = 115-117 °C; ¹H NMR: δ = 7.45 (d, *J* = 1.2 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 2H), 7.02 (d, *J* = 2.3 Hz, 1H), 6.96 (dd, *J* = 2.3, 8.0 Hz, 1H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.82 (d, *J* = 8.0 Hz, 1H), 6.51 (s, 1H), 5.72 (s, 1H), 4.93 (s, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 3.79 (s, 3H); ¹³C NMR: δ = 159.7, 148.9, 148.5, 146.3, 137.0, 135.6, 129.0, 127.8, 119.0, 116.0, 114.4, 110.9, 109.9, 70.6, 56.0, 55.9, 55.4, 50.6; IR (cm⁻¹): 3125 (br), 2838, 1612, 1505, 1417, 1302, 1248, 1134, 792, 746, 630; HR-ESIMS (*m/z*): Calcd. for [M+H]⁺ C₂₀H₂₃N₂O₄ is 355.1652 found 355.1642.

4-(3,4-Dimethoxybenzyl)-1-(4-methoxybenzyl)-1H-imidazole (26):

Alcohol **25** (0.82 g, 2.3 mmol) in CHCl₃ (25 mL) was added TFA (1.10 mL, 13.9 mmol) followed by Et₃SiH (1.85 mL, 11.6 mmol) and heated to 55 °C under N₂ for 30 h. The reaction was quenched with 2M Na₂CO₃ until neutral and then extracted with CH₂Cl₂ (2 x 20 mL), dried (anhyd. Na₂SO₄), and concentrated. The resulting residue was purified by column chromatography (100% EtOAc to 5% MeOH in EtOAc) to give **26** as a colorless oil (0.67 g, 86%). ¹H NMR: δ = 7.42 (d, *J* = 1.2 Hz, 1H), 7.07 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.79

(d, *J* = 1.7 Hz, 1H), 6.78 (d, *J* = 1.7 Hz, 1H), 6.77 (s, 1H), 6.49 (d, *J* = 1.2 Hz, 1H), 4.93 (s, 2H), 3.83 (s, 5H), 3.81 (s, 3H), 3.78 (s, 3H); ¹³C NMR: δ = 159.6, 148.8, 147.4, 143.2, 136.8, 133.1, 128.9, 128.3, 120.8, 116.0, 114.3, 112.2, 111.2, 56.0, 55.8, 55.4, 50.4, 34.8; IR (cm⁻¹): 2998, 2933, 2834, 1611, 1510, 1463, 1246, 1175, 1137, 1025, 818, 764, 686; HR-ESIMS (*m/z*): Calcd. for [M+H]⁺ C₂₀H₂₃N₂O₃ is 339.1703 found 339.1718.

2-Azido-4-(3,4-dimethoxybenzyl)-1-(4-methoxybenzyl)-1H-imidazole (27):

Imidazole **26** (0.10 g, 0.30 mmol) in THF (15 mL) was cooled to -78 °C under N₂. *n*-BuLi (1.6 M in hexane) (0.22 mL, 0.36 mmol) was added and stirred at -78 °C for 7 min. TrisN₃ (0.11 g, 0.36 mmol) was added in one portion and then the mixture was stirred at -78 °C for 30 min. The reaction was quenched by the addition of water (15 mL) and extracted with EtOAc (2 x 10 mL). The combined organic extracts were washed with brine, dried (anhyd. Na₂SO₄), and concentrated. The resulting residue was purified by column chromatography (35% EtOAc in hexane) to give **27** as a yellow oil (0.072 g, 65%). ¹H NMR: δ = 7.08 (d, *J* = 8.6 Hz, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 6.80 (s, 1H), 6.78 (m, 2H), 6.24 (s, 1H), 4.72 (s, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 3.77 (s, 2H); ¹³C NMR: δ = 159.5, 148.8, 147.5, 140.7, 139.6, 132.2, 128.9, 128.2, 120.8, 114.9, 114.3, 112.3, 111.2, 56.0, 55.9, 55.4, 48.2, 34.8; IR (cm⁻¹): 2934, 2907, 2128, 1734, 1611, 1512, 1244, 1175, 1137, 1027; HR-ESIMS (*m/z*): Calcd. for [M+H]⁺ C₂₀H₂₂N₅O₃ is 380.1717 found 380.1727.

2-Amino-4-(3,4-dimethoxybenzyl)-1-(4-methoxybenzyl)-1H-imidazole (28):

Isonaamine **C** 10% Pd/C (0.020 g, 0.018 mmol) was added to azide **26** (0.069 g, 0.18 mmol) in MeOH (5 mL) and stirred under H₂ (1 atm) at rt for 20 h. The catalyst was filtered through Celite and washed with CH₂Cl₂. The filtrate was reduced and the resulting residue purified by column chromatography using Biotage KP-NH Silica (100% EtOAc to 1% MeOH in EtOAc) to give **28** as a yellow solid (0.055 g, 86%). m.p. = 103-106 °C; ¹H NMR: δ = 7.06 (d, *J* = 8.6 Hz, 2H), 6.85 (d, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 2.0 Hz, 1H), 6.79 (d, *J* = 2.0 Hz, 1H), 6.78 (s, 1H), 6.16 (s, 1H), 4.74 (s, 2H), 3.83 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 3.70 (s, 2H); ¹³C NMR: δ = 159.4, 148.8, 147.5, 147.4, 137.4, 132.9, 128.3, 128.1, 120.8, 114.5, 112.5, 112.3, 111.2, 56.0, 55.9, 55.4, 48.2, 34.6; IR (cm⁻¹): 3367, 3075, 2997, 2939, 2838, 1612, 1587, 1512, 1442, 1232, 1151, 1024, 803; HR-ESIMS (*m/z*): Calcd. for [M+H]⁺ C₂₀H₂₄N₃O₃ is 354.1812, found 354.1824.

4-(3,4-dimethoxybenzyl)-1-(4-methoxybenzyl)-2-(3-methylimidazolidine-2,4-dione)imino-1H-imidazole (Isonaamidine E) (2):

1-Methylparabanic acid (0.11 g, 0.85 mmol) in dry CH₃CN (8 mL) was added *N,O*-bis(trimethylsilyl)acetamide (0.25 mL, 1.0 mmol) and heated to reflux for 1.5 h. The solvent was removed by vacuum distillation and the resulting residue was dissolved in dry toluene (5 mL). Amine **28** (0.060 g, 0.17 mmol) was added and stirred at 85 °C under N₂ for 18 h. The reaction was quenched with water (10 mL) and extracted with EtOAc (2 x 10 mL). The combined organic extracts were dried (anhyd. Na₂SO₄) and concentrated. The resulting residue was purified by column chromatography (1:1 EtOAc/Hexane) to give **2** as an orange solid (0.078 g, 51%). m.p. = 121-124 °C; ¹H NMR: δ = 7.16 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 6.80-6.74 (m, 3H), 6.47 (s, 1H), 5.19 (s, 2H), 3.85 (s, 3H), 3.83 (s, 3H), 3.80 (s, 2H), 3.77 (s, 3H), 3.18 (s, 3H); ¹³C NMR: δ = 162.0, 159.5, 155.1, 149.0, 147.5, 147.7, 146.8, 144.6, 140.0, 131.3, 129.5, 128.4, 120.8, 116.1, 114.3, 112.1, 111.3, 56.0, 55.9, 55.4, 48.4, 34.4, 24.8; IR (cm⁻¹): 3001, 2936, 2839, 1789, 1728, 1661, 1609, 1512, 1440, 1389, 1343, 1245,

1226, 1137, 1105, 1025, 822, 603; HR-ESIMS (m/z): Calcd. for $[M+H]^+ C_{24}H_{26}N_5O_5$ is 464.1928 found 464.1915.

4-Iodo-5-(4-methoxybenzyl)-1-methyl-1H-imidazole (30): Et_3SiH (13.9 mL, 87.2 mmol) and TFA (5.60 mL, 72.6 mmol) were added to a solution of benzyl alcohol **29** (5.00 g, 14.5 mmol) in anhydrous CH_2Cl_2 (100 mL) at rt, then the resulting mixture was heated at reflux for 60 h under N_2 atmosphere. After cooling to rt, the reaction was quenched by the addition of sat. aq. solution of $NaHCO_3$. The resulting mixture was extracted with CH_2Cl_2 several times and the combined extracts were dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (hexane/EtOAc, 1/3) to isolate **30** (4.65 g, 97%) as a pale yellow solid: m.p. = 97–99 °C; 1H NMR (Acetone- D_6): δ = 7.50 (s, 1H), 7.09 (d, J = 8.7 Hz, 2H), 6.85 (d, J = 8.7 Hz, 2H), 3.92 (s, 2H), 3.74 (s, 3H), 3.51 (s, 3H); ^{13}C NMR: δ = 158.6, 139.4, 133.4, 129.3, 129.1, 114.3, 84.9, 55.4, 32.6, 30.0; IR (KBr, cm^{-1}): = 3005, 3001, 2957, 2834, 1608, 1508, 1444, 1280, 1247, 1176, 1030, 981, 821, 770, 694; HR-ESIMS (m/z): Calcd. for $C_{12}H_{14}IN_2O$ $[M+H]^+$ 329.0145, found 329.0142.

5-(4-Methoxybenzyl)-1-methyl-1H-imidazole-4-carbaldehyde (32): $EtMgBr$ (3.0 M in ether, 5.2 mL, 15.7 mmol) was added into a solution of **30** (4.90 g, 14.9 mmol) in dry THF (50 mL) at 0 °C, and the resulting mixture was stirred at rt. for 2 h. Then, *N*-methylformanilide (**31**) (2.23 mL, 17.9 mmol) was added at 0 °C and the resulting mixture was stirred at rt overnight. Saturated aq. NH_4Cl (20 mL) was added to quench the reaction and the organic layer was extracted with EtOAc, dried (Na_2SO_4) and concentrated to provide the crude product, which was purified through a short plug of silica gel (EtOAc) to isolate **32** (3.10 g, 90%) as a white solid: m.p. = 120–121 °C; 1H NMR: δ = 9.99 (s, 1H), 7.40 (s, 1H), 7.04 (d, J = 8.3 Hz, 2H), 6.79 (d, J = 8.3 Hz, 2H), 4.32 (s, 2H), 3.375 (s, 3H), 3.45 (s, 3H); ^{13}C NMR: δ = 187.7, 158.6, 138.8, 138.4, 137.7, 129.3, 128.5, 114.4, 55.3, 31.6, 28.5; IR (KBr, cm^{-1}): = 3105, 3022, 2957, 2818, 2770, 1669, 1552, 1511, 1344, 1244, 1022, 824, 789; HR-ESIMS (m/z): Calcd. for $C_{13}H_{15}N_2O_2$ $[M+H]^+$ 231.1128, found 231.1139.

4-[Hydroxy-(4-methoxyphenyl)]methyl-5-(4-methoxybenzyl)-1-methyl-1H-imidazole (33): *p*-Anisylmagnesium bromide was prepared from *p*-bromoanisole (6.67 mL, 52.1 mmol) and magnesium turnings (1.25 g, 52.1 mmol) in THF. A solution of **32** (3.00 g, 13.0 mmol) in THF (20 mL) was added dropwise. to afford a crude product, which was purified through a short plug of silica gel (EtOAc) to isolate **33** (4.34 g, 98%) as an off-white solid: m.p. = 134–135 °C; 1H NMR: δ = 7.33 (d, J = 8.7 Hz, 2H), 7.33 (s, 1H), 6.88 (d, J = 8.7 Hz, 2H), 6.81 (d, J = 8.7 Hz, 2H), 6.75 (d, J = 8.7 Hz, 2H), 5.74 (s, 1H), 3.81 (s, 2H), 3.75 (s, 6H), 3.30 (s, 3H); ^{13}C NMR: δ = 158.5, 158.1, 141.4, 136.8, 136.7, 130.2, 129.2, 127.8, 126.2, 113.9, 113.4, 69.4, 55.1, 55.1, 31.4, 28.0; IR (KBr, cm^{-1}): = 3112 (br), 2835, 2710, 1608, 1582, 1511, 1461, 1298, 1250, 1173, 1042, 846, 820, 787; HR-ESIMS (m/z): Calcd. for $C_{20}H_{23}N_2O_3$ $[M+H]^+$ 339.1703, found 339.1701.

4,5-Bis(4-methoxybenzyl)-1-methyl-1H-imidazole (34): Et_3SiH (1.88 mL, 11.8 mmol) and TFA (0.73 mL, 9.5 mmol) were added to a solution of **33** (800 mg, 2.4 mmol) in anhydrous CH_2Cl_2 (20 mL) at rt, then the resulting mixture was stirred overnight. The reaction was quenched by the addition of satd. aq. solution of $NaHCO_3$ and the resulting mixture was extracted with CH_2Cl_2 several times. The combined extracts were dried (Na_2SO_4), concentrated and the residue was purified by chromatography (acetone) to provide **34** (615 mg, 81%) as a pale yellow oil: 1H NMR: δ = 7.32 (s, 1H), 7.16 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 6.78 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 3.86 (s, 2H), 3.85 (s, 2H), 3.75 (s, 3H), 3.74 (s, 3H),

3.30 (s, 3H); ^{13}C NMR: δ = 158.3, 157.9, 139.0, 136.7, 133.2, 130.4, 129.6, 129.0, 125.7, 114.1, 113.9, 55.3, 33.1, 31.7, 28.3; IR (neat, cm^{-1}): = 3000, 2908, 2835, 1610, 1509, 1461, 1245, 1178, 1034, 819; HR-ESIMS (m/z): Calcd. for $C_{20}H_{23}N_2O_2$ $[M+H]^+$ 323.1754, found 323.1774.

2-Azido-4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazole (35): *n*-Butyl lithium (1.4 M solution in hexanes, 1.50 mL, 2.1 mmol) was added dropwise to a stirred solution of **34** (613 mg, 1.9 mmol) in dry THF (15 mL) at -78 °C. The reaction was stirred for 1 h at the same temperature. The cooling bath was removed for 10 min, then the reaction mixture was re-cooled to -78 °C, and TsN_3 (0.80 g, 4.1 mmol) in THF (3 mL) was added dropwise. After stirring for 40 min at -78 °C, the reaction mixture was allowed to come to rt. and was quenched by the addition of satd. aq. NH_4Cl (3 mL). The aqueous layer was extracted with EtOAc (3x20 mL), and the combined organic extracts were dried (Na_2SO_4) and concentrated to provide a pale brown oil, which was purified through a short column of silica gel (hexane/EtOAc, 4:1) to isolate **35** (615 mg, 89%) as a pale yellow oil: 1H NMR: δ = 7.17 (d, J = 8.3 Hz, 2H), 6.90 (d, J = 8.3 Hz, 2H), 6.79 (d, J = 8.3 Hz, 2H), 6.77 (d, J = 8.3 Hz, 2H), 3.84 (s, 2H), 3.79 (s, 2H), 3.76 (s, 6H), 3.06 (s, 3H); ^{13}C NMR: δ = 158.3, 158.0, 139.0, 136.3, 132.8, 130.2, 129.5, 129.0, 125.0, 114.1, 113.9, 55.3, 32.8, 29.5, 28.7; IR (neat, cm^{-1}): = 3000, 2944, 2835, 2132, 1609, 1508, 1247, 1176, 1034, 821; HR-ESIMS (m/z): Calcd. for $C_{20}H_{22}N_5O_2$ $[M+H]^+$ 364.1773, found 364.1768.

2-Amino-4,5-bis(4-methoxybenzyl)-1-methyl-1H-imidazole (36): Azide **35** (475 mg, 1.3 mmol) was dissolved in EtOH (10 mL) and stirred overnight under a hydrogen atmosphere (1 atm) in the presence of 10% Pd-C on charcoal (47 mg) at rt. The catalyst was removed by filtration through a pad of Celite and the filtrate was concentrated to isolate amine, **36** (440 mg, quant) as a pale yellow solid: m.p. = 175–176 °C; 1H NMR: δ = 7.09 (d, J = 8.3 Hz, 2H), 6.93 (d, J = 8.3 Hz, 2H), 6.77 (d, J = 8.3 Hz, 2H), 6.74 (d, J = 8.3 Hz, 2H), 5.79 (br, 2H), 3.75 (s, 2H), 3.74 (s, 3H), 3.70 (s, 3H), 3.68 (s, 2H), 3.03 (s, 3H); ^{13}C NMR: δ = 158.4, 158.0, 147.4, 132.4, 130.4, 130.4, 129.6, 128.9, 121.0, 114.1, 113.9, 55.3, 55.3, 31.8, 29.5, 28.5; IR (KBr, cm^{-1}): = 3374, 3296, 3123, 2954, 2838, 1764, 1610, 1550, 1510, 1462, 1249, 1178, 1033, 819, 758; HR-ESIMS (m/z): Calcd. for $C_{20}H_{24}N_3O_2$ $[M+H]^+$ 338.1863, found 338.1871.

4,5-Bis(4-methoxybenzyl)-1-methyl-2-(3-methylimidazolidine-2,4-dione)imino-1H-imidazole (Naamidine G) (4): *N,O*-Bis(trimethylsilyl)acetamide (1.00 mL, 4.1 mmol) was added to a solution of 1-methylparabanic acid (526 mg, 4.1 mmol) in dry CH_3CN under an N_2 atmosphere and the resulting mixture was heated at reflux for 1 h. Then, the solvent was removed by distillation and to the resulting yellow residue in toluene (3 mL) was added amine **36** (277 mg, 0.8 mmol) under N_2 . After, this mixture was heated at 85 °C overnight, water (5 mL) was added and the organic layer was extracted in to EtOAc. The dried organic layer (Na_2SO_4) was concentrated to afford a yellow residue, which was purified over silica gel (EtOAc/hexanes, 3/7) to provide **4** (287 mg, 78%) as a yellow powder: m.p. = 195–196 °C (lit.²⁸ m.p. = 94 °C); 1H NMR: δ = 10.04 (br, 1H), 7.10 (d, J = 8.7 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 6.80 (d, J = 8.7 Hz, 2H), 6.77 (d, J = 8.7 Hz, 2H), 3.89 (s, 2H), 3.88 (s, 2H), 3.76 (s, 6H), 3.47 (s, 3H), 3.15 (s, 3H); ^{13}C NMR: δ = 162.3, 158.6, 158.3, 155.7, 146.5, 145.0, 135.7, 131.5, 129.4, 129.1, 129.0, 127.0, 114.3, 114.1, 55.4, 32.3, 30.0, 28.7, 24.7; IR (KBr, cm^{-1}): = 3241, 2931, 2835, 1788, 1738, 1656, 1511, 1451, 1302, 1248, 1177, 1036, 744, 697; HR-ESIMS (m/z): Calcd. for $C_{24}H_{26}N_5O_4$ $[M+H]^+$ 448.1979, found 448.1984.

2-Azido-5-[4-methoxybenzyl]-4-[methoxy-(4-methoxy-phenyl)]methyl-1-methyl-1H-imidazole (38): TFA (0.60 mL, 7.8 mmol) was added to a solution of **33** (1.31 g, 3.9 mmol) in anhyd MeOH (20 mL) at rt. and the mixture was then heated at 55 °C overnight. Satd. aq. solution of NaHCO₃ was used to neutralize the above reaction mixture, and the aqueous layer was extracted with EtOAc (3x 30 mL). Combined organic layers were washed with water and brine, then dried (Na₂SO₄) and concentrated to provide **37** (1.37 g, >95%) as a pale yellow oil which was used directly in the next step: ¹H NMR: δ = 7.34 (d, *J* = 8.7 Hz, 2H), 7.23 (s, 1H), 6.86 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.71 (d, *J* = 8.7 Hz, 2H), 5.26 (s, 1H), 3.93 (s, 2H), 3.75 (s, 6H), 3.34 (s, 3H), 3.29 (s, 3H). Following the general procedure for azidation, *n*-Butyl lithium (1.4 M solution in hexanes, 2.66 mL, 3.7 mmol), **37** (1.45 g, 3.4 mmol) in dry THF (20 mL) and TsN₃ (0.80 g, 4.1 mmol) were used to obtain the crude product, which was purified through a short column of silica gel (hexane/EtOAc, 4:1) to isolate **38** (891 mg, 67%) as a reddish brown oil: ¹H NMR: δ = 7.41 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.76 (d, *J* = 8.7 Hz, 2H), 5.23 (s, 1H), 3.85 (s, 2H), 3.78 (s, 3H), 3.76 (s, 3H), 3.35 (s, 3H), 3.03 (s, 3H); ¹³C NMR: δ = 159.0, 158.4, 139.8, 136.9, 133.4, 129.9, 129.1, 128.4, 126.1, 114.1, 113.7, 79.1, 56.8, 55.3, 55.3, 29.4, 28.6; IR (neat, cm⁻¹) = 2937, 2834, 2138, 1508, 1246, 1174, 1087, 1033, 829; HR-ESIMS (*m/z*): Calcd. for C₂₁H₂₃N₅NaO₃ [M+Na]⁺ 416.1693, found 416.1677.

2-Amino-5-(4-methoxybenzyl)-4-[methoxy-(4-methoxy-phenyl)]methyl-1-methyl-1H-imidazole (39): Following the general procedure for catalytic reduction, azide **38** (800 mg, 2.0 mmol) in EtOH (10 mL) and 10% Pd-C on charcoal (80 mg) at 1 atm hydrogen were used to afford amine **39** (745 mg, quant) as a pale yellow solid: m.p. = 139-140 °C; ¹H NMR (CD₃OD): δ = 7.29 (d, *J* = 8.7 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 2H), 6.82 (d, *J* = 8.7 Hz, 2H), 6.78 (d, *J* = 8.7 Hz, 2H), 5.21 (s, 1H), 3.86 (s, 2H), 3.74 (s, 3H), 3.73 (s, 3H), 3.30 (s, 3H), 3.08 (s, 3H); ¹³C NMR: δ = 159.0, 158.4, 148.9, 133.8, 131.8, 130.7, 128.7, 127.9, 123.0, 113.6, 113.1, 78.5, 55.4, 54.4, 54.4, 28.0, 27.7; IR (KBr, cm⁻¹) = 3129, 1671, 1510, 1459, 1248, 1176, 1084, 1031, 830, 746; HR-ESIMS (*m/z*): Calcd. for C₂₁H₂₆N₃O₃ [M+H]⁺ 368.1969, found 368.1970.

5-(4-Methoxybenzyl)-4-[methoxy-(4-methoxy-phenyl)]methyl-1-methyl-2-(3-methylimidazolidine-2,4-dione)imino-1H-imidazole (4-Methoxynaamidine G) (40): Following the general procedure, *N,O*-Bis(trimethylsilyl)acetamide (0.33 mL, 1.4 mmol) and 1-methylparabanic acid (174 mg, 1.4 mmol) in dry CH₃CN (15 mL) were used to produce a yellow residue, which was treated with amine **39** (100 mg, 0.3 mmol) to obtain the product **40** (13 mg, 11%) as a yellow solid after the purification over silica gel (EtOAc/hexanes, 2/3): ¹H NMR: δ = 7.34 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.8 Hz, 2H), 6.87 (d, *J* = 8.8 Hz, 2H), 6.78 (d, *J* = 8.8 Hz, 2H), 5.29 (s, 1H), 3.98 (s, 2H), 3.79 (s, 3H), 3.77 (s, 3H), 3.45 (s, 3H), 3.35 (s, 3H), 3.17 (s, 3H);

5-(4-Benzyloxy-3,5-dimethoxyphenyl)hydroxymethyl-4-iodo-1-methyl-1H-imidazole (42): EtMgBr (3.0 M solution in ether, 7.54 mL, 22.6 mmol) was added to a solution of **14** (7.19 g, 21.5 mmol) in dry CH₂Cl₂ (100 mL) at rt. over ~10 min. After stirring at rt. for 20 min, aldehyde **41** (6.46 g, 23.7 mmol) was added to the reaction followed by 48 h stirring. Then, satd. aq. NH₄Cl (10 mL) was added to the reaction and the resulting pale yellow solid was filtered and the filtrate was partitioned with CH₂Cl₂. The organic layer was dried (Na₂SO₄) and concentrated to provide a pale yellow solid. The resulting solid was triturated with hexanes, recrystallized with CH₂Cl₂ to isolate **42** (9.68 g,

95%) as a white solid: m.p. = 173-175 °C; ¹H NMR: δ = 7.44 (d, *J* = 7.8 Hz, 2H), 7.31-7.24 (m, 4H), 6.57 (s, 2H), 5.98 (s, 1H), 5.18 (s, 1H), 4.98 (s, 2H), 3.75 (s, 6H), 3.43 (s, 3H); ¹³C NMR: δ = 153.6, 141.1, 137.8, 136.6, 135.9, 135.2, 128.6, 128.2, 127.9, 102.7, 84.6, 75.0, 67.1, 56.3, 33.5; IR (neat, cm⁻¹): = 3253 (br), 3104, 1585, 1501, 1411, 1338, 1226, 1140, 1033, 908; HR-DARTMS (*m/z*): Calc. for C₂₀H₂₁IN₂O₄ [M]⁺ 480.0546; found: 480.0546; Calc. for C₂₀H₂₂IN₂O₄ [M+H]⁺ 481.0624, found 481.0624.

5-(4-Benzyloxy-3,5-dimethoxybenzyl)-4-iodo-1-methyl-1H-imidazole (43): Et₃SiH (1.00 mL, 6.2 mmol) and TFA (0.40 mL, 5.2 mmol) were added to a solution of **42** (0.50 g, 1.0 mmol) in anhydrous CHCl₃ (20 mL) at rt. and the resulting mixture was heated at reflux temperature for 24 h under nitrogen atmosphere. After cooling to rt., reaction was quenched by the addition of satd. aq. NaHCO₃. The aqueous layer was extracted several times with CHCl₃ and the combined organic extracts were dried (Na₂SO₄) and concentrated. The residue was purified by chromatography (EtOAc) to isolate **43** (0.14 g, 28%) as a pale brown semi solid: ¹H NMR: δ = 7.44 (d, *J* = 6.9 Hz, 2H), 7.36 (s, 1H), 7.30 (t, *J* = 6.9 Hz, 2H), 7.25 (d, *J* = 6.9 Hz, 1H), 6.31 (s, 2H), 4.95 (s, 2H), 3.87 (s, 2H), 3.73 (s, 6H), 3.40 (s, 3H); ¹³C NMR: δ = 153.8, 139.6, 137.9, 135.9, 133.1, 128.5, 128.2, 127.9, 105.2, 85.0, 75.1, 56.3, 32.7, 31.0; IR (neat, cm⁻¹): = 3107, 2939, 1588, 1494, 1460, 1420, 1237, 1215, 1187, 1100, 978, 758; HR-ESIMS (*m/z*): Calc. for C₂₀H₂₂IN₂O₃ [M+H]⁺ 465.0670, found 465.0669.

5-[(3,5-Dimethoxy-4-hydroxy)benzyl]-4-iodo-1-methyl-1H-imidazole (44): A second product was obtained from above reaction in which the *O*-benzyl protecting group has been removed **44** (0.11 g, 27%) as a pale yellow solid: m.p. = 210-212 °C; ¹H NMR (DMSO-*d*₆): δ = 7.40 (s, 1H), 6.34 (s, 2H), 5.47 (br, 1H), 3.90 (s, 2H), 3.82 (s, 6H), 3.45 (s, 3H); ¹³C NMR: δ = 148.6, 140.6, 134.7, 133.7, 128.4, 106.1, 85.1, 56.5, 32.6, 30.0; IR (neat, cm⁻¹): = 3107, 2936 (br), 1595, 1514, 1499, 1413, 1242, 1214, 1113, 1037, 838, 811, 765, 737; HR-ESIMS (*m/z*): Calc. for C₁₃H₁₅IN₂O₃ [M+H]⁺

5-(4-Benzyloxy-3,5-dimethoxyphenyl)hydroxymethyl-1-methyl-1H-imidazole-4-carbaldehyde (45): EtMgBr (3.0 M in ether, 13.8 mL, 41.4 mmol) was added into a solution of **42** (9.03 g, 18.8 mmol) in dry THF (200 mL) at rt., and the resulting mixture was stirred for 20 min. Then, *N*-methylformanilide (**30**) (2.78 mL, 22.6 mmol) was added to the reaction mixture followed by stirring for 33 h. Half saturated NH₄Cl (30 mL) was added to quench the reaction and the organic layer was extracted with EtOAc, dried (Na₂SO₄) and concentrated to provide the crude product, which was purified through a short plug of silica gel (EtOAc→Acetone) to isolate **45** as a pale yellow solid (5.52 g, 77%): m.p. = 132-134 °C; ¹H NMR: δ = 9.82 (s, 1H), 7.42 (d, *J* = 7.3 Hz, 2H), 7.38 (s, 1H), 7.29 (t, *J* = 7.3 Hz, 2H), 7.25 (m, 1H), 6.50 (s, 2H), 6.23 (s, 1H), 4.94 (s, 2H), 3.71 (s, 6H), 3.47 (s, 3H); ¹³C NMR: δ = 188.4, 153.8, 141.7, 139.9, 138.0, 137.7, 136.6, 136.2, 128.5, 128.2, 127.9, 103.4, 75.1, 66.7, 56.3, 33.1; IR (neat, cm⁻¹): = 3253 (br), 3106, 2938, 1680, 1587, 1502, 1450, 1415, 1230, 1100, 1056, 824; HR-DARTMS (*m/z*): Calc. for C₂₁H₂₃N₂O₅ [M+H]⁺ 383.1601, found 383.1597.

6-Benzyloxy-5,7-dimethoxy-4-(4-methoxyphenyl)-1-methyl-1H-naphtho[2,3-*d*]imidazole (47): A Grignard reagent was prepared from *p*-bromoanisole, (7.23 mL, 56.5 mmol), magnesium turnings (1.35 g, 56.5 mmol) and a small crystal of iodine in THF (100 mL) and a solution of **45** (5.40 g, 14.1 mmol) in THF (50 mL) were used to obtain a crude product, which was purified through a short plug of silica gel (EtOAc) to isolate **46** (4.38 g, 63%) as a pale yellow oil, which was used in the next

step directly without further characterization. Et₃SiH (11.41 mL, 71.4 mmol) and TFA (4.81 mL, 62.5 mmol) were added to a solution of **45** (4.38 g, 8.9 mmol) in anhydrous CH₂Cl₂ (100 mL) at rt. and the resulting mixture was stirred for 24 h under nitrogen atmosphere. Then, the reaction was quenched by the addition of satd. aq. solution of NaHCO₃. The aqueous layer was extracted with CH₂Cl₂ several times and the combined extracts were dried (Na₂SO₄), and concentrated. The residue was purified by chromatography (EtOAc → acetone) to provide **47** (3.30 g, 81%) as a pale brown solid; m.p. = 194-197 °C; ¹H NMR: δ = 7.87 (s, 1H), 7.61 (s, 1H), 7.53 (d, *J* = 8.3 Hz, 2H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.36 (t, *J* = 7.3 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 1H), 7.08 (s, 1H), 7.05 (d, *J* = 8.7 Hz, 2H), 5.10 (s, 2H), 3.96 (s, 3H), 3.89 (s, 3H), 3.74 (s, 3H), 3.37 (s, 3H); ¹³C NMR: δ = 158.2, 152.1, 150.6, 146.6, 142.9, 139.8, 138.0, 134.4, 132.4, 131.6, 130.9, 129.7, 128.4, 128.3, 127.9, 119.6, 112.7, 104.0, 102.3, 75.2, 60.9, 55.8, 55.4, 31.0; IR (neat, cm⁻¹): = 2929, 2831, 1607, 1514, 1451, 1330, 1274, 1236, 1145, 1075, 1027, 826, 740; HR-DARTMS (*m/z*): Calc. for C₂₈H₂₇N₂O₄ [M+H]⁺ 455.1971, found 455.1983.

1-(*N,N*-dimethylaminosulfonyl)-4-iodo-5-(4-methoxybenzyl)-1H-imidazole (50): EtMgBr (3.0 M solution in ether, 8.60 mL, 25.8 mmol) was added to a solution of **49** (7.19 g, 21.5 mmol) in dry CH₂Cl₂ (150 mL) at rt. The resulting mixture was stirred at rt. for 20 min and 1.0 M solution of CuCN.2LiCl in dry THF (26.0 mL, 26.0 mmol) was added followed by *p*-methoxybenzyl bromide (3.80 mL, 25.8 mmol). The orange reaction solution was stirred at rt. for 48 h and poured into half sat. NH₄Cl containing 2% concentrated NH₃ (50 mL). After stirring for 20 min, the resulting solid was filtered off and the filtrate was partitioned with CH₂Cl₂ (3x50 mL). The organic layer was dried (Na₂SO₄), concentrated and purified by chromatography (EtOAc/hexane, 3:7) to afford **50** (6.41 g, 65%) as a pale yellow solid; m.p. = 76-78 °C; ¹H NMR: δ = 7.87 (s, 1H), 6.99 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.37 (s, 2H), 4.09 (s, 2H), 3.71 (s, 3H), 2.49 (s, 6H); ¹³C NMR: δ = 158.5, 139.7, 137.9, 132.5, 129.1, 114.0, 90.6, 55.4, 37.6, 29.9; IR (neat, cm⁻¹): = 3111, 2919, 1514, 1459, 1415, 1240, 1173, 1174, 1095, 960, 802; HR-DARTMS (*m/z*): Calc. for C₁₃H₁₇IN₃O₃S [M+H]⁺ 422.0030, found 422.0047.

4-(4-Benzyloxy-3,5-dimethoxybenzyl)-1-(*N,N*-dimethylsulfonyl)-5-(4-methoxybenzyl)-1H-imidazole (51): EtMgBr (3.0 M solution in ether, 4.98 mL, 14.9 mmol), **50** (5.72 g, 13.6 mmol) in dry CH₂Cl₂ (150 mL), 1.0 M solution of CuCN.2LiCl in dry THF (16.3 mL, 16.3 mmol) and 206 (6.87 g, 20.4 mmol) were used to synthesize **51** (5.18 g, 70%) as a pale yellow oil after the purification by chromatography (EtOAc:hexane, 1:1): ¹H NMR: δ = 7.94 (s, 1H), 7.48 (d, *J* = 7.3 Hz, 2H), 7.33 (t, *J* = 7.3 Hz, 2H), 7.27 (t, *J* = 7.33 Hz, 1H), 6.94 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.37 (s, 2H), 4.94 (s, 2H), 4.14 (s, 2H), 3.78 (s, 2H), 3.75 (s, 3H), 3.71 (s, 6H), 2.57 (s, 6H); ¹³C NMR: δ = 158.4, 153.4, 141.3, 138.1, 138.0, 135.6, 134.8, 130.3, 129.9, 128.9, 128.5, 128.2, 127.8, 114.0, 106.0, 75.1, 56.1, 55.4, 37.5, 34.0, 28.1; IR (neat, cm⁻¹): = 2929, 2857, 1691, 1507, 1393, 1252, 1124, 909, 836, 779; HR-DARTMS (*m/z*): Calc. for C₂₉H₃₄N₃O₆S [M+H]⁺ 552.2163, found 552.2180.

5-(4-Benzyloxy-3,5-dimethoxybenzyl)-4-(4-methoxybenzyl)-1-methyl-1H-imidazole (53): Methyl trifluoromethanesulfonate (0.95 mL, 8.7 mmol) was added dropwise to a solution of **51** (3.96 g, 7.2 mmol) in CH₂Cl₂ (50 mL), at 0 °C under N₂ and stirred for 4 h at the same temperature. Then, the solvent was evaporated under reduced pressure and the crude pale yellow oil was dissolved in dry acetonitrile (30 mL), and benzylamine (0.95 mL, 8.7 mmol) was added to it. After heating at 80 °C for 10 h, the solvent was evaporated to provide a

crude oil, which was purified with a gradient column (EtOAc:hexanes, 3:1→EtOAc:acetone; 1:1) to isolate **53** (2.96 g, 90%) as a light brown oil: ¹H NMR: δ = 7.45 (d, *J* = 7.3 Hz, 2H), 7.40 (s, 1H), 7.31 (t, *J* = 7.3 Hz, 2H), 7.27 (t, *J* = 7.3 Hz, 1H), 7.19 (d, *J* = 8.7 Hz, 2H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.15 (s, 2H), 4.96 (s, 2H), 3.88 (s, 2H), 3.87 (s, 2H), 3.75 (s, 3H), 3.64 (s, 6H), 3.34 (s, 3H); ¹³C NMR: δ = 157.9, 153.8, 138.8, 137.9, 136.8, 135.5, 134.1, 133.1, 129.5, 128.5, 128.2, 127.9, 125.5, 113.9, 105.1, 75.1, 56.1, 55.3, 32.8, 31.9, 29.5; IR (neat, cm⁻¹): = 2929, 2857, 1691, 1507, 1391, 1251, 1176, 1150, 910; HR-ESIMS (*m/z*): Calc. for C₂₈H₃₁N₂O₄ [M+H]⁺ 459.2278, found 459.2278.

2-Azido-5-(4-benzyloxy-3,5-dimethoxybenzyl)-4-(4-methoxybenzyl)-1-methyl-1H-imidazole (55): *n*-Butyl lithium (1.6 M solution in hexanes, 1.31 mL, 2.1 mmol) was added dropwise to a stirred solution of **52** (870 mg, 1.9 mmol) in dry THF (20 mL) at -78 °C, and the reaction was stirred for 1 h. The cooling bath was removed for 10 min, then the reaction mixture was re-cooled to -78 °C, and then TrisN₃ (706 mg, 2.3 mmol) was added. After stirring for an additional 45 min at -78 °C, the reaction mixture was quenched by the addition of satd. aq. NH₄Cl (5 mL). The aqueous layer was extracted with EtOAc (3x15 mL), and the combined organic extracts were dried (Na₂SO₄) and concentrated to provide a pale brown oil, which was purified through a short column of silica gel (hexane/EtOAc, 7:3) to isolate azide **55** (600 mg, 63%) as a pale brown oil: ¹H NMR: δ = 7.46 (d, *J* = 7.4 Hz, 2H), 7.38-7.26 (m, 3H), 7.21 (d, *J* = 8.5 Hz, 2H), 6.79 (d, *J* = 8.5 Hz, 2H), 6.16 (s, 2H), 4.95 (s, 2H), 3.85 (s, 2H), 3.80 (s, 2H), 3.76 (s, 3H), 3.64 (s, 6H), 3.08 (s, 3H); ¹³C NMR: δ = 158.0, 153.7, 139.2, 137.9, 136.5, 135.5, 134.0, 132.9, 129.5, 128.6, 128.2, 127.9, 124.6, 113.9, 105.0, 75.0, 56.1, 55.3, 32.7, 30.0, 29.6; IR (neat, cm⁻¹): = 2929, 2857, 2129, 1691, 1507, 1391, 1252, 1150, 1124, 909, 836, 779; HR-ESIMS (*m/z*): Calc. for C₂₈H₃₀N₃O₄ [M+H]⁺ 500.2292, found 500.2290.

2-Amino-5-(3,5-dimethoxy-4-hydroxybenzyl)-4-(4-methoxybenzyl)-1-methyl-1H-imidazole (Naamine G) (56): Azide **55** (600 mg, 1.2 mmol) was dissolved in EtOH (15 mL) and stirred overnight under a hydrogen atmosphere (55 psi) in the presence of 20% Pd(OH)₂ on charcoal (100 mg) at rt. The catalyst was filtered through a pad of Celite and the filtrate was concentrated to isolate naamine G, (**56**) (430 mg, 95%) as a greenish-yellow solid; m.p. = 218-220 °C; ¹H NMR (CD₃OD): δ = 7.17 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.34 (s, 2H), 3.92 (s, 2H), 3.84 (s, 2H), 3.74 (s, 3H), 3.69 (s, 6H), 3.23 (s, 3H); ¹³C NMR: δ = 158.8, 148.3, 146.5, 134.3, 129.8, 129.2, 127.3, 122.8, 122.4, 114.0, 105.2, 55.5, 54.5, 28.8, 28.3, 27.9; IR (neat, cm⁻¹): = 3244 (br), 3004, 2836, 1667, 1654, 1609, 1500, 1461, 1429, 1245, 1216, 1110, 1022; HR-DARTMS (*m/z*): Calc. for C₂₁H₂₆N₃O₄ [M+H]⁺ 384.1918, found 384.11907.

5-(3,5-Dimethoxy-4-hydroxybenzyl)-4-(4-methoxybenzyl)-1-methyl-2-(3-methylimidazolidine-2,4-dione)imino-1H-imidazole (Naamidine H) (5): Following the general procedure for this reaction, *N,O*-Bis(trimethylsilyl)acetamide (0.63 mL, 2.6 mmol) and 1-methylparabanic acid (331 mg, 4.1 mmol) in dry CH₃CN (10 mL) were used to produce 3-trimethylsilyl-1-methylparabanic acid. After removing the solvent, naamine G, 1 h (198 mg, 0.5 mmol) was added and the mixture was heated at 80 °C overnight in dry toluene (5 mL). Usual workup and purification over silica gel (EtOAc/hexanes, 4/6) provided naamidine H (**5**) as a yellow amorphous solid (205 mg, 80%): m.p. = 204-205 °C; ¹H NMR: δ = 7.14 (d, *J* = 8.7 Hz, 2H), 6.98 (br, 1H), 6.78 (d, *J* = 8.7 Hz, 2H), 6.14 (s, 2H), 3.89 (s, 2H), 3.88 (s, 2H), 3.75 (s, 3H), 3.69 (s, 6H), 3.49 (s, 3H), 3.47 (s, 3H), 3.16 (s, 3H); ¹³C NMR: δ = 162.3, 158.3, 155.5, 147.4, 146.6, 144.7, 136.1, 133.7, 131.7, 129.4, 128.1, 126.7, 114.1, 104.7, 56.3, 55.4,

32.3, 30.0, 29.7, 24.8; IR (neat, cm^{-1}): = 3501, 3212, 2929, 2837, 1784, 1718, 1652, 1511, 1392, 1113, 1039, 1020, 918 ; HR-DARTMS (m/z): Calc. for $\text{C}_{25}\text{H}_{28}\text{N}_5\text{O}_6$ $[\text{M}+\text{H}]^+$ 494.2034, found 494.2049.

Cytotoxicity assay (IC_{50} measurement): Human breast adenocarcinoma cell line (MCF7, ATCC) was maintained in growth medium containing Dulbecco's modified Eagles media (DMEM, Sigma) with 10% heat inactivated fetal bovine serum (FBS, Sigma), 2 mM L-glutamine, 100 units of penicillin, and 0.1 mg/mL streptomycin. Cells were incubated in a water-jacketed humidified CO_2 incubator with 5% CO_2 and at 37 °C. To measure the cytotoxicity of the test compounds, approximately 10,000 MCF7 cells were cultivated in each well of a 96-well microtiter plate and maintained overnight in 150 μL of the growth medium and the conditions described above. Each compound was initially dissolved in DMSO and then was serially diluted also made (in DMSO) for (10 μL for each concentration). 10 μL of each diluted stock was further then diluted within 990 μL of above growth medium to have desired concentration. To treat the cells, 50 μL of growth medium containing the appropriate amount of each compound was added to each well to obtain required final concentration (200 μL final volume and 0.1 μL of DMSO in each well). An equivalent volume of the vehicle (0.1 % DMSO) was added to each control well. After 96 h of incubation, the cell viability was analyzed by using the MTT assay. In brief, 20 μL of MTT solution (stock 5 mg/ml in PBS) was added into each well and incubated for 2 h under normal growth conditions to allow the viable cell to

convert MTT to formazan. Then the media was discarded and formazan crystals were dissolved by adding 100 μL of DMSO and incubating 2 h with continuous shaking. The absorbance of the lysates was measured directly at 560 nm using a micro plate reader (Fluostar-omega, BMG Labtech). The percent viable cells (calculated based on absorbance of the untreated control sample) were plotted as a function of concentration to obtain the IC_{50} values. Each experiment was repeated at least twice (with 5 replicates each time).

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