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Synthesis of 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones and their chemiluminescent properties

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Abstract—A series of 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones were prepared from 2-amino-3,5-dibromopyrazine. The concise synthesis of coelenterazine (**5j**), in three steps, 48% overall yield and >99% purity exemplifies the strategy. Further, the synthetic approach facilitated the regiospecific incorporation of carboxyalkyl linkers on the 3,7-dihydroimidazo[1,2*a*]pyrazine-3-one nucleus that are required for bioconjugation. Peroxymonocarbonate, an electrophilic oxidant, was used to initiate 'pseudo-flash' chemiluminescence from this class of molecules.
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1. Introduction

3,7-Dihydroimidazo[1,2*a*]pyrazine-3-ones (Fig. 1) are natural products occurring in a broad array of luminous marine organisms;[1] coelenterazine, 2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)-8-(phenylmethyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one, is found in jellyfish (*Aequorea victoria)*, sea pansy (*Renilla reniformis*), and squid (*Watasenia scintillans*) to name a few, while the Cypridina luciferin (*Cypridina hilgendorfii*) is more limited in its distribution. Each of these organisms utilizes the imidazopyrazinone substrate in an oxidative process that generates visible light. The phenomenon has intrigued scientists for nearly fifty years and is still an area of intense interest. While the bioluminescent reaction may have precipitated behavioral changes in these organisms relating to their feeding or reproductive habits, the underlying physiological anti-oxidant activity of the compounds may have been equally significant in the evolution of the species. The utility of antioxidants as therapeutic reagents that minimize the cellular damage caused by reactive oxygen species is well documented. It is only recently that imidazolpyrazinones

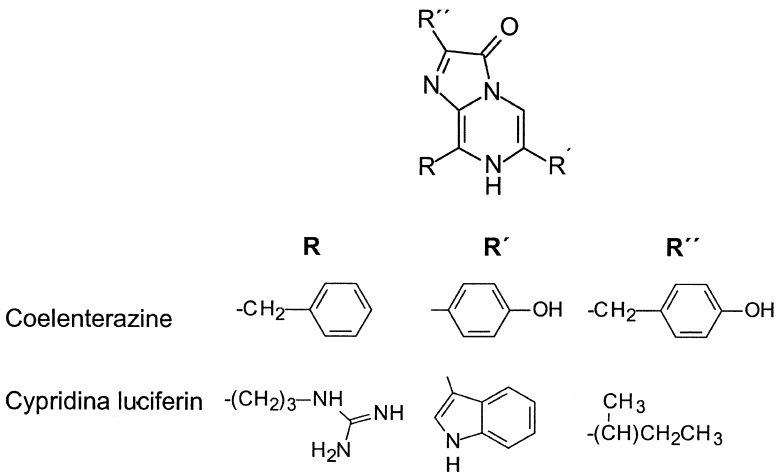

Figure 1. 3,7-Dihydroimidazo[1,2*a*]pyrazine-3-one natural products.

Keywords: chemiluminescence; bioluminescence; coelenterazine; peroxymonocarbonate; assays.
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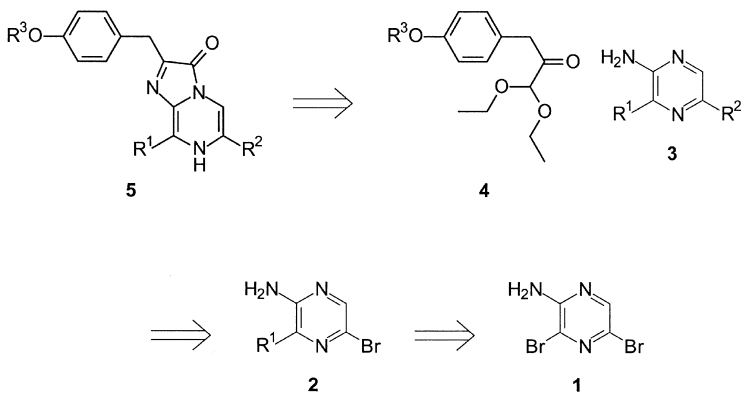


Figure 2. Retrosynthesis of 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones.

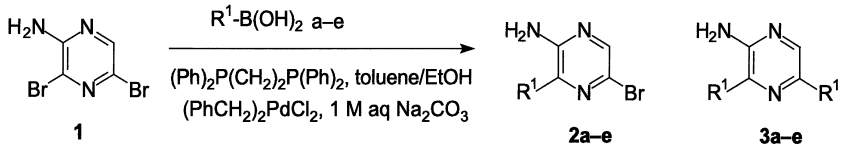
have been recognized as potential pharmacophores.[2,3] Beyond the potential therapeutic uses, the luminescent properties have shown utility as signaling reagents in biological research and clinical analysis.[4–6] Our continuing interest in this area prompted earlier studies pairing the imidazopyrazinone/coelenterazine-containing photoprotein aequorin and an acridinium-9-carboxamide chemiluminescent label in a multi-analyte prototype assay.[7] Both signal generating species delivered flash luminescence (<10 s) when they were triggered sequentially using Ca^{2+} (aequorin) followed by basic peroxide (acridinium). The use of 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones as standalone flash chemiluminescent labels has yet to be described. Unfortunately, coelenterazine emits a glowing chemiluminescence with oxygen in the absence of the photoprotein under a variety of conditions and is not directly suitable for bioconjugation. In this work we report our recent results in developing a concise and versatile synthesis of several 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones, including those with functionality for conjugation.

2. Results and discussion

The approach taken for the preparation of the 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones (**5**) of interest is presented in Figure 2. In the retroanalysis the penultimate step is comprised of a condensation between a α-ketoacetal (**4**) and a suitably substituted 2-aminopyrazine (**3**). The synthesis of a small library of α- ketoacetal based on the addition of substituted phenyl Grignard reagent to ethyl diethoxyacetate was recently described by us.[8] Ideally, introduction of the 3 and 5-substituents on the pyrazine nucleus should be sequential to allow for the greatest diversity of derivatives. The readily available 2-amino-3,5-dibromopyrazine (**1**) would serve as an attractive precursor.

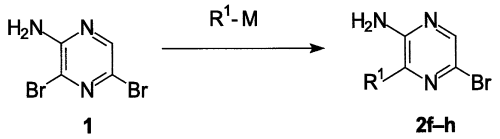
2.1. Preparation of 2-amino 3,5-disubstituted pyrazines

The classical synthesis 2-amino 3,5-disubstituted pyrazines involved the construction of the pyrazine nucleus via the condensation of an α-ketooxime with an α-aminonitrile. The resulting 2-amino-3,5-disubstituted pyrazine-*N*-oxide was then reduced.[9] This method was complicated by the difficulty in preparing the requisite starting materials and overall low yield. Other approaches started with a preformed pyrazine ring with subsequent introduction of substituents at the 3 and 5-positions. For instance, 2-halopyrazines were metallated at the 3-position and subsequently condensed with aldehydes.[10] The 2-halo group was then displaced by aminolysis under high temperature and pressure, or treated with azide and reduced[11] with accompanying tetrazole formation to yield the 2-amino-3-substituted pyrazine derivatives. Alkyl lithium reagents add to unsubstituted 2-amino-pyrazines at the 3-position to give similar derivatives.[12] The 5-position substitution proceeds via a two step process, (1) bromination followed by (2) Suzuki[10,12] or Stille coupling.[13] Alternatively, the sequence can be reversed, starting from 2-amino-5-bromopyrazines.[2,3] Recently, commercially available 2-amino-3,5-dibromopyrazine was reacted with an excess of aryl boronic acid under Suzuki coupling conditions to produce symmetrical 2-amino-3,5-substituted pyrazines.[2,3,14] The use of the Suzuki conditions to prepare unsymmetrical 2-amino-3,5-substituted pyrazines was not explored, however, sequential Stille coupling with 1 equiv. of different tin



	R^1	% Yield 2	3
a	4-(TBDMS-O)Ph-	42	32
b	4-F-Ph-	52	13
c	4-CF_3Ph-	41	12
d	4-CH_3Ph-	38	13
e	Ph-	57	13

Scheme 1.



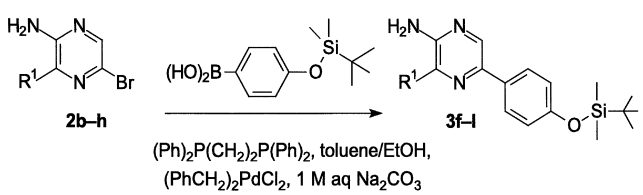
	Conditions	R1	% Yield
f	$PhCH_2MgCl$, $ZnCl_2$, $(Ph_3P)_2PdCl_2$, THF	$PhCH_2$-	78
g	1) 4-(TBDMS-O)$PhCH_2MgCl$, $ZnCl_2$ $(Ph_3P)_2PdCl_2$, THF; 2) TBAF; 3) NaH, $Br(CH_2)_3CO_2Et$	4-[$EtO_2C(CH_2)_3O$]$PhCH_2$-	43
h	$HC{\equiv}C(CH_2)_2CO_2Et$, CuI $Pd(PPh_3)_4$, Et_3N, DMF	$EtO_2C(CH_2)_2C{\equiv}C$-	74

Scheme 2.

reagents has led to a limited number of unsymmetrical 2-amino-3,5-substituted pyrazines.[15]

The ready availability of aryl boronic acids were attractive substrates for building the library of 3,7-dihydroimidazo-[1,2*a*]pyrazine-3-ones of our interest. We first chose to explore the regioselectivity of Suzuki coupling conditions with a limiting amount of aryl boronic acid. Addition of one equivalent aryl boronic acid [$R^1B(OH)_2$ **a**–**e**] in the presence of bis(benzonitrile)dichloropalladium catalyst afforded the desired 2-amino-3-aryl pyazines (**2a**–**e**) as the major product in 38–57% yield (Scheme 1). The regioselectivity favored substitution at the 3-position due to the electron deficiency at that position and coordination with the amino group at position-2; however, the symmetrically substituted pyrazines (**3a**–**e**) were also produced in 12–32% yield.

Better regioselectivity was seen using in situ generated organozinc reagents[16,17] (Scheme 2, entries f–g) for the introduction of aralkyl substituents. Thus, benzylmagnesium chloride was first treated with anhydrous zinc chloride to generate the benzylzinc chloride reagent, which was then reacted with 2-amino-3,5-dibromo-pyrazine (**1**) at room temperature in the presence of bis(triphenylphosphine)palladium (II) dichloride catalyst. The 2-amino-3-benzyl-5-bromo-pyrazine (**2f**) was purified by silica gel column chromatography in 78% yield and >99% purity. The unreacted starting material 3,5-dibromo-2-pyrazinamine (**1**) was recovered in 20% from this coupling reaction. Based on the recovered starting material, the yield of **2f** was >95%. Analysis of ^1H and ^{13}C NMR spectra indicated that it was a single isomer and consistent with the reported spectral data of a sample prepared by the previously reported strategies using benzyllithium[12,18] or via classical pyrazine ring formation.[9] The more highly functionalized benzylzinc reagent (entry **2g**) gave similar results, and upon further elaboration to incorporate a linker group, gave the derivative **2g** (43%, over three steps). Likewise, introduction of an alkyne linker at the 3-position was accomplished selectively under the Sonogashira conditions[19,20] (entry **2h**) in good yield.



3	R1	% Yield
f	4-FPh-	82
g	4-CF_3Ph-	92
h	4-CH_3Ph-	83
i	Ph-	68
j	$PhCh_2$-	98
k	4-[$EtO_2C(CH_2)_3O$]$PhCH_2$-	73
l	$EtO_2C(CH_2)_2C{\equiv}C$-	66

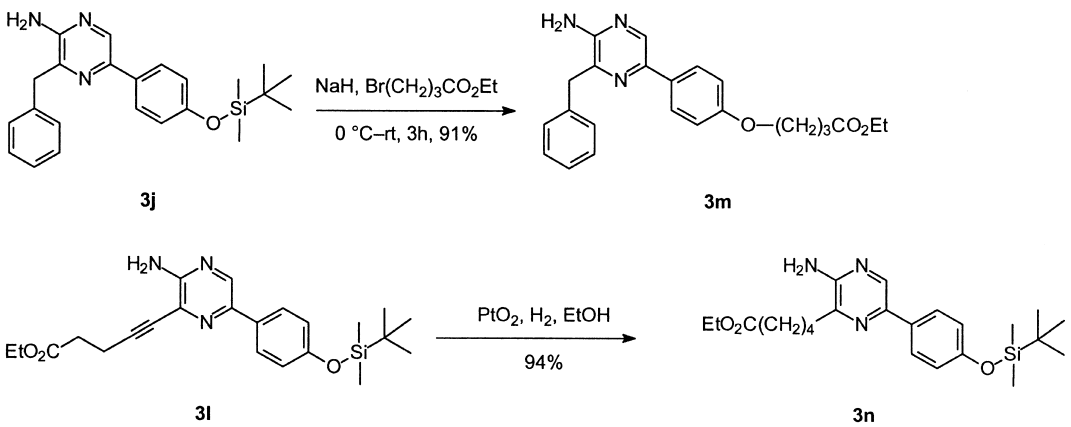
Scheme 3.

Having prepared a series of 3-substituted-5-bromopyrazines, introduction of the second substituent was efficiently (66–98%) accomplished under Suzuki coupling conditions (Scheme 3) as shown to afford the 2-amino-3,5-disubstituted pyrazines **3f**–**l**. Before proceeding to formation of the imidazolpyrazine nucleus, compound **3j** was deprotected and alkylated with ethyl 4-bromobutyrate to give the linker-bearing derivative **3m** (Scheme 4) and the alkynyl linker of compound **3l** was reduced to the saturated compound **3n**.

2.2. Condensation of 2-amino 3,5-disubstituted pyrazines with α-ketoacetals

The acid catalyzed addition of α-ketoacetal **4a**[12] to (Scheme 5, entries a–j) afforded the desired 3,7-dihydroimidazo-[1,2*a*]pyrazine-3-ones **5a**–**j** in 17–63% isolated yield. The α-ketoacetal **4a** was de-silylated and alkylated with ethyl 4-bromobutyrate to provide compound **4b** bearing a latent R^3 linking group. Condensation with 2-amino-pyrazine **3j** proceeded in 56% yield, while **4a** reacted with the 2-amino-pyrazine with R^2 (**3l**) and R^1 (**3m**, **3n**) linking groups to complete the examples of 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones with points for bioconjugation. In all cases the protecting groups were removed in the condensation reaction.

It is important to note that entry **5j** is the natural product coelenterazine. There are four previous reports on the total synthesis of coelenterazine[12,21–23] and as well as improvements to its synthesis.[10,13,24–26] The first total synthesis was reported by Inoue et al.,[21] which involved the construction of an appropriately substituted 2-pyrazinamine derivative



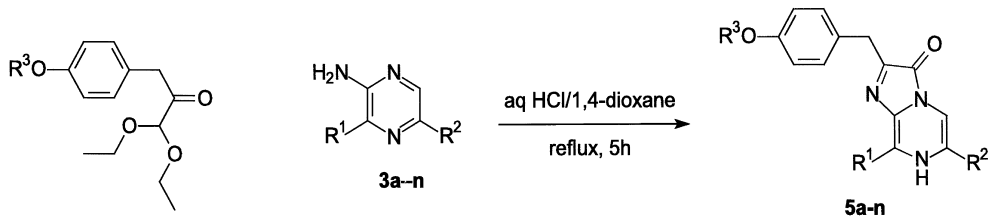
Scheme 4.

via a classical pyrazine-ring formation[9,27] and subsequent elaboration to the imidazo[1,2-*a*]pyrazin-3(*7H*)-one ring system by treatment with a 1,2-dicarbonyl compound in 4.6% overall yield. Later, Jones and co-workers[22] accomplished the synthesis of coelenterazine in a total of 14 steps and 11% overall yield starting from 2-chloropyrazine. Recently, we[12] and Kojima et al.,[23] simultaneously reported an improved syntheses of coelenterazine starting from 2-pyrazinamine.

In the work presented here, two critical modifications to our previous synthesis[12] of coelenterazine (**5j**) allowed the route to be shortened to 3 steps with an overall yield of 48%. First, introduction of a benzyl group at the 3-position of the pyrazine ring via palladium catalyzed coupling reaction of benzylzinc chloride 2-amino-3,5-dibromopyrazine (**1**), so that the need for generation of unstable and low yielding benzyllithium reagent could be avoided. Second, use of 4-*tert*-butyldimethylsilyloxyphenyl boronic acid reagent in the place of 4-methoxyphenylboronic acid, which was used in Suzuki coupling reaction to introduce 4-hydroxyphenyl group at the 5-position of the pyrazine ring. This modification eliminated the cleavage of methyl ether using pyridinium hydrochloride at high temperature (200°C) when 4-methoxyphenylboronic acid was used. The silyl protecting group was cleaved simultaneously under acidic conditions during the final condensation step.

2.3. Chemiluminescence evaluation

There are many conditions for eliciting chemiluminescence from 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones. Dissolved oxygen in DMSO,[28,29] acetate buffer (pH: 5.6) in diglyme[30] or aq. NaOH in DMSO,[31] acetate buffer (pH: 5.6) in DMSO,[32] *t*-BuOK in *t*-BuOH and DMSO,[33] and water



4a R^3= *t*-butyldimethylsilyl
4b R^3= $EtO_2C(CH_2)_3$-

5	R^1	R^2	R^3	% Yield
a	4-HOPh-	4-HOPh-	H	43
b	4-FPh-	4-FPh-	H	32
c	4-CF_3Ph-	4-CF_3Ph-	H	33
d	4-CH_3Ph-	4-CH_3Ph-	H	26
e	Ph-	Ph-	H	–
f	4-FPh	4-HOPh-	H	25
g	4-CF_3Ph-	4-HOPh-	H	21
h	4-CH_3Ph-	4-HOPh-	H	17
i	Ph-	4-HOPh-	H	43
j	$PhCH_2$-	4-HOPh-	H	63
k	$PhCH_2$-	4-HOPh-	$HO_2C(CH_2)_3$-	56
l	$PhCH_2$-	4-[$HO_2C(CH_2)_3O$]Ph-	H	41
m	$HO_2C(CH_2)_4$-	4-HOPh-	H	41
n	4-[$HO_2C(CH_2)_3O$]$PhCH_2$-	4-HOPh	H	32

Scheme 5.

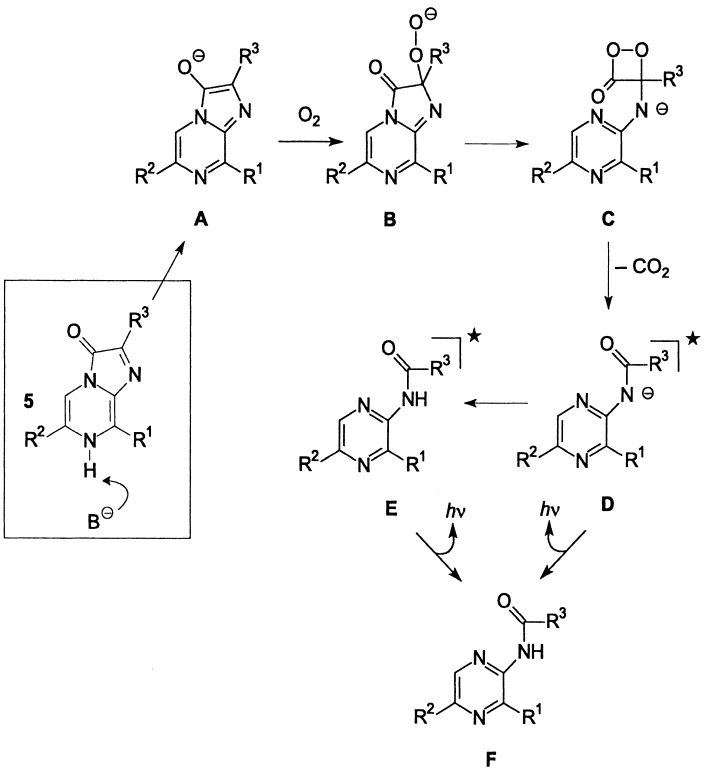


Figure 3. Mechanism of coelenterazine light emission.

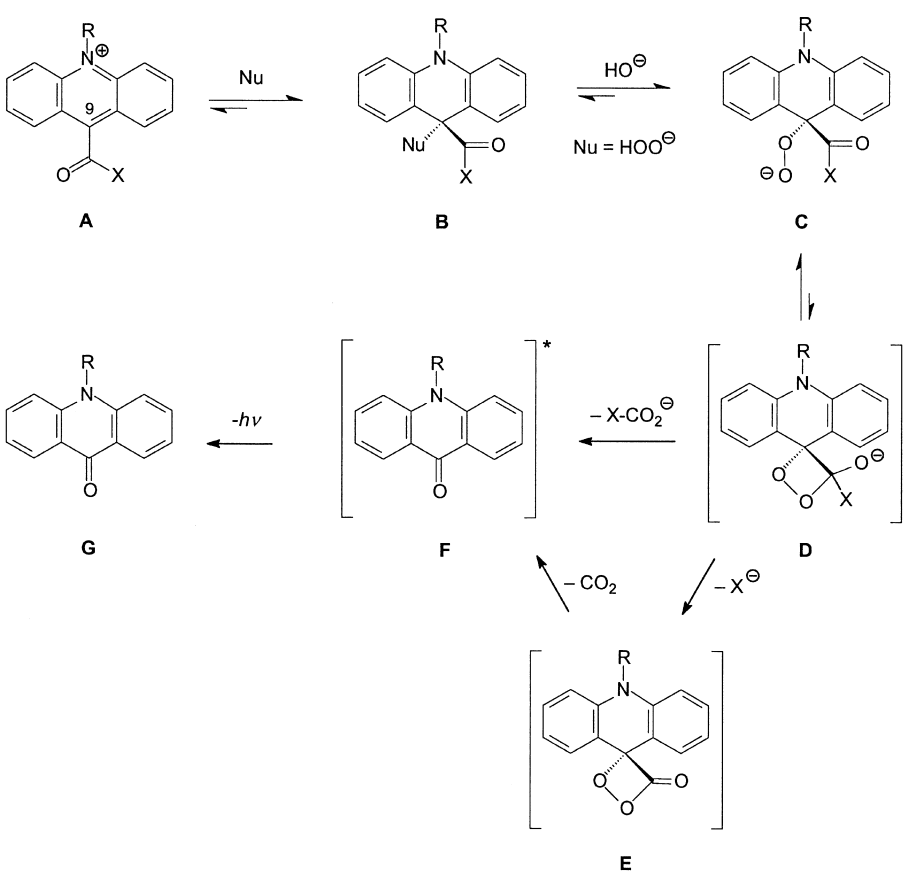


Figure 4. Acridinium chemiluminescence mechanism.

Table 1. Total and relative chemiluminescence signal

Compound	Total RLU/mol (10^{14})	Normalized RLU/mol
5a	49.4	26.0
5b	46.3	24.4
5c	1.32	0.69
5d	Not soluble	–
5e	Not soluble	–
5f	0.47	0.25
5g	1.17	0.62
5h	0.92	0.48
5i	61.8	32.5
5j	1.9	1.0
5k	0.834	0.44
5l	184	96
5m	1.34	0.68
5n	1.78	0.94

in DMF,[34] have all been used to induce a dim, glowing light. In contrast, the bioluminescent reaction of coelenterazine bound to its photoprotein, aequorin, in the presence of molecular oxygen, emits blue light (λ_{max} 465 nm) in a flash upon triggering with Ca^{2+}. The mechanism for light emission (Fig. 3) is similar for both the bioluminescent and chemiluminescent routes.[30,35,36] 3,7-Dihydroimidazo-[1,2*a*]pyrazine-3-one **5** is deprotonated to give the imidazo-[1,2*a*]pyrazine-3-ol anion **A** which does a formal nucleophilic addition to molecular oxygen. It has been suggested this may proceed via a one-electron transfer from the anion to oxygen and subsequent recombination of the radical ion pair. The 2-hydroperoxide anion **B** rearranges to the excited anion **D** via a putative dioxetanone **C**. The excited anion **D** can be protonated to give excited intermediate **E**. Both **D** and **E** emit light upon relaxing to ground state **F**. Formation of hydroperoxide **B** appears to be the slow step. In order for light to be observed as a flash, the rate of formation of hydroperoxide **B** must be increased or the slow step must be changed. This intermediate is stable enough for isolation and characterization at temperatures under −50°C.[37] Under bioluminescent conditions **B** is stabilized at ambient temperature by the photoprotein until a Ca^{2+} induced conformational change in the protein takes place and a flash of light is produced.

In contrast, the flash chemiluminescence from acridinium-9-carboxamides proceeds according to the mechanism in Figure 4.[38] The nucleophilic addition of the hydroperoxide anion to the electron-deficient 9-position of the acridinium nucleus leads to the tetrahedral intermediate **C** that ultimately proceeds through the dioxetanone **E** to the excited acridone **F** and light generation. Triggering of 3,7-dihydro-imidazo[1,2*a*]pyrazine-3-ones with basic hydrogen peroxide (nucleophilic conditions) failed to give flash chemiluminescence (data not shown). We thought that a stabilized electrophilic oxidant might mimic aequorin leading to flash chemiluminescence in the absence of the protein mediator.

Recently, bicarbonate-activated hydrogen peroxide was identified as an electrophilic oxidant useful in neutral aqueous medium for the conversion of alkenes to epoxides[39] and sulfides to sulfoxides.[40,41] The structure of the oxidizing species, $HOOCO_2^-$ was confirmed by ^{13}C NMR and numerous kinetic studies. To test this oxidant as a chemiluminescence triggering reagent, the 3,7-dihydro-imidazo[1,2*a*]pyrazine-3-ones **5a**–**n** were dissolved in methanol/0.2% aqueous dodecyltrimethylammonium bromide (DTAB) (1:1), then serially diluted in 0.2% aqueous DTAB. (After completion of this work, Richardson et al.,[42] reported the advantages of cetyltrimethylammonium bicarbonate in this oxidation system.) A triggering solution consisting of 60:40 acetonitrile/water containing 200 mM ammonium bicarbonate and 875 mM hydrogen peroxide was prepared 15 min prior to use. The chemiluminescence signal was measured over 10 min. Table 1 lists the total signal recorded for each compound. In line with previous observations, the chemiluminescence efficiency of the natural product coelenterazine (**5j**) is much lower than in its photoprotein aequorin or unrelated acridinium-9-carboxamide species. In this case **5j** produced only 10^{14} RLU per mole compared to the 10^{20} RLU per mole observed with acridinium-9-carboxamides. The chemiluminescence efficiency of compounds **5a**, **5b** and **5i** all exceeded coelenterazine by 24–32-fold. The inclusion of a conjugatable functional group had no significant effect on chemiluminescence with the exception of compound **5l**, which was nearly two orders of magnitude more efficient than coelenterazine. Replacing the free carboxylate in

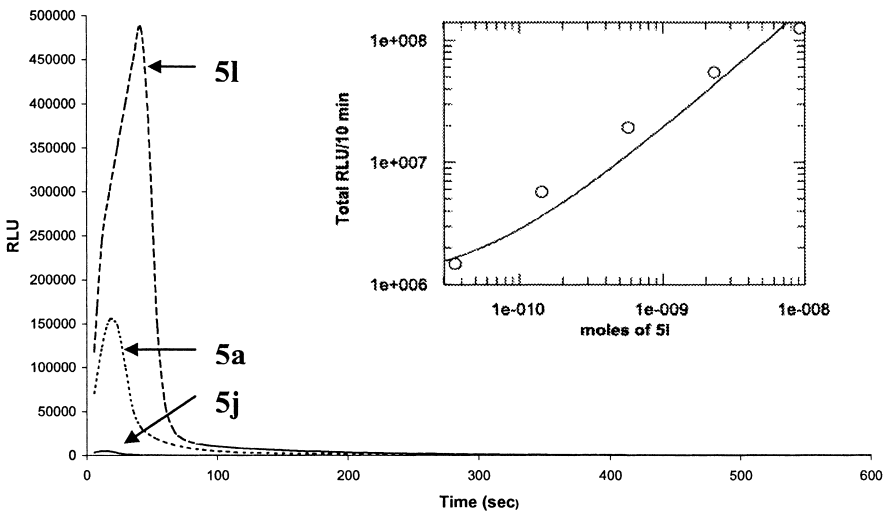


Figure 5. Chemiluminescence profiles.

structure **5l** with an ethyl ester had little detrimental effect on the signal (data not shown).

Under these triggering conditions all of the soluble 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones approached flash-type kinetics, emitting most of the observed signal in under 100 s, but were still far slower than the acridinium-9-carboxamide chemiluminophore (<10 s) or aequorin used in our previous report.[7] Typical chemiluminescence profiles are shown in Figure 5. Chemiluminescence response was linear over the concentration ranged tested (see inset).

3. Conclusion

The synthetic approach to 3,7-dihydroimidazo[1,2*a*]pyrazine-3-ones presented here led to a concise synthesis of coelenterazine (**5j**), an important luciferin found in a variety of marine organism in three steps, 48% overall yield and >99% purity. Further, the approach facilitated the regiospecific incorporation of carboxyalkyl linkers on the 3,7-dihydroimidazo[1,2*a*]pyrazine-3-one nucleus that are suitable for activation and subsequent conjugation. One of these, **5l**, generated a 100-fold more signal than coelenterazine using peroxymonocarbonate as an electrophilic oxidant. This oxidant was successful in converting the 'glow' luminescence normally reported for non-protein-mediated chemiluminescence of 3,7-dihydroimidazo[1,2*a*]-pyrazine-3-ones, to 'pseudo-flash' luminescence. However, even under these conditions, 3,7-dihydroimidazo[1,2*a*]-pyrazine-3-ones fell short of the efficiency (signal/mole) and speed (duration of signal) of acridinium-9-carboxamide chemiluminophores used in commercial analyzers.[43] For chemiluminescent assays of high concentration (>μM) analytes, e.g. theophylline, phenytoin, or those not requiring high throughput, these limitations may not be of consequence, but an advantage.[44,45]

4. Experimental

4.1. General methods

^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer (300 MHz) and the chemical shifts (δ) reported in ppm relative to TMS. Electrospray ionization mass spectrometry (ESI-MS) was carried out on a Perkin–Elmer (Norwalk, CT) Sciex API 100 Benchtop system, employing a Turbo IonSpray ion source and the HRMS were obtained on a Nermang 3010 MS-50, JEOL SX102-A. Thin layer chromatography was performed on a pre-coated Whatman MK6F silica gel 60 Å plates (layer thickness: 250 μm) and visualized with UV light and/or using 0.2% ninhydrin in ethanol. Column chromatography was performed on silica gel, Merck grade 60 (230–400 mesh). Anhydrous solvents were freshly distilled [(THF from a purple solution of sodium and benzophenone) and (CH_2Cl_2 from CaH_2)] under nitrogen. All reagents were obtained from Aldrich Chemical Co. (Milwaukee, WI) unless otherwise noted. Analytical reversed phase RP-HPLC was performed using a Waters (Milford, MA) Novapak C_{18}, 6.0 μm (3.9×150 mm) column or Waters Symmetry C_{18} 7.0 μm (8×100 mm) column (solvents ratio v/v reported) unless otherwise stated. Preparative RP-HPLC was performed using a Waters Novapak, C_{18}, 6.0 μm (40×100 mm) column or Waters Symmetry C_{18} 7.0 μm (40×100 mm) column (solvents ratio v/v reported) unless otherwise stated. Melting points were recorded in open capillary tubes on an Electrothermal (Barnstead International, Dubuque, IA) melting point apparatus and were uncorrected. Chemiluminescence analysis was carried out using a Wallac Inc. (Gaithersburg, MD) model LB96V2 microplate chemiluminometer. IUPAC names for all new compounds were obtained using ACD/Ilab Web service version 3.5 at http://www.acdlabs.com/ilab

4-(*tert*-Butyldimethylsiloxy)phenyl-2-oxopropanal diethyl acetal (**4a**) was prepared according to the published procedure.[12]

4.2. General procedure for Suzuki coupling using 2-amino-3,5-dibromopyrazine (1)

1,4-Bis(diphenylphosphino)butane (BDPB, 0.049 g, 0.114 mmol, 5.8 mol%) was added to a suspension of bis(benzonitrile)dichloro palladium (0.038 g, 0.099 mmol, 5 mol%) in toluene (4.0 mL) and the mixture was stirred for 30 min under nitrogen at room temperature. 2-Amino-3,5-dibromopyrazine (**1**, 0.5 g, 1.976 mmol), arylboronic acid (2.075 mmol, 105 mol%), ethanol (0.82 mL), 1.0 M aqueous Na_2CO_3 (1.976 mL) and toluene (6.0 mL) were added sequentially and the mixture was heated to reflux for 7 h. The mixture was cooled to room temperature, diluted with water (10 mL) and extracted with ethyl acetate (60 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated on rotary evaporator. The residue was purified by silica gel column chromatography (conditions given below).

4.2.1. 5-Bromo-3-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-2-pyrazinamine (2a) and 3,5-bis(4-{[*tert*-butyl(dimethyl)-silyl]oxy}phenyl)-2-pyrazinamine (3a). The title compounds were obtained from 4-(*tert*-Butyldimethylsilyloxy)-phenylboronic acid [250 mol%, 23 h reaction time, silica gel chromatography (20% ethyl acetate in hexanes)].

Compound **2a**. 42% yield; pale yellow solid; TLC R_f 0.53 (25% ethyl acetate in hexanes); mp 128–129°C; analytical RP-HPLC (70:30 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 14.89 min, 99%; ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.63–7.58 (m, 2H), 6.96–6.92 (m, 2H), 1.00 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 156.9, 151.1, 142.1, 140.8, 129.5, 128.7, 126.9, 120.7, 25.6, 18.2, −4.4; ESI-MS *m*/*z* 380, 382 $(M+H)^+$, 759, 761, 763 $(2M+H)^+$.

Compound **3a**. 32% yield; pale yellow solid; TLC R_f 0.38 (25% ethyl acetate in hexanes); mp 138–141°C; analytical RP-HPLC (65:35 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 14.72 min, 99%; ^{1}H NMR ($CDCl_3$) δ 8.34 (s, 1H), 7.83 (d, 2H, *J*=8.5 Hz), 7.71 (d, 2H, *J*=8.5 Hz), 6.94 (d, 2H, *J*=8.7 Hz), 6.91 (d, 2H, *J*=8.5 Hz), 4.78 (br s, 2H), 1.01 (s, 9H), 0.99 (s, 9H), 0.24 (s, 6H), 0.21 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 156.5, 155.9, 150.4, 142.9, 139.4, 136.6, 130.5, 130.4, 129.6, 126.9, 120.5, 120.4, 25.7, 25.6, 18.3, 18.2, −4.4, −4.3; ESI-MS *m*/*z* 508

$(M+H)^+$, 1015 $(2M+2)^+$; HRMS (FAB) *m/z* calcd for $C_{28}H_{41}N_3O_2Si_2$, 507.2737 $(M)^+$, observed 507.2734.

4.2.2. 5-Bromo-3-(4-fluorophenyl)-2-pyrazinamine (2b) and 3,5-bis(4-fluorophenyl)-2-pyrazinamine (3b). The title compounds were obtained from 4-fluorophenylboronic after silica gel chromatography (18–20% ethyl acetate in hexanes).

Compound **2b**. 52% yield; pale yellow solid; TLC R_f 0.4 (25% ethyl acetate in hexanes); mp 145–146°C; analytical RP-HPLC (50:50 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 5.93 min, 99%; ^1H NMR ($CDCl_3$) δ 8.05 (s, 1H), 7.74–7.69 (m, 2H), 7.21–7.15 (m, 2H), 4.85 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 164.9, 161.6, 151.1, 142.8, 139.6, 131.8, 131.7, 130.2, 130.1, 126.9, 116.3, 116.0; ESI-MS *m/z* 268, 270 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{10}H_8N_3F^{79}Br$, 267.9886 $(M+H)^+$, observed 267.9877; calcd for $C_{10}H_8N_3F^{81}Br$, 269.9865 $(M+H)^+$; observed, 269.9864.

Compound **3b**. 13% yield; pale yellow solid; TLC R_f 0.19 (25% ethyl acetate in hexanes); mp 160–162°C; analytical RP-HPLC (50:50 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 9.8 min, 96%; ^1H NMR ($CDCl_3$) δ 8.39 (s, 1H), 7.95–7.87 (m, 2H), 7.84–7.79 (m, 2H), 7.24–7.08 (m, 4H), 4.87 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 164.7, 164.6, 161.4, 161.3, 150.8, 144.1, 142.1, 138.6, 137.5, 133.3, 133.2, 133.1, 133.0, 131.7, 130.3, 130.2, 127.4, 127.2, 116.1, 115.9, 115.8, 115.5; ESI-MS *m/z* 284 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{16}H_{11}F_2N_3$, 283.0921 $(M)^+$; observed, 283.0911.

4.2.3. 5-Bromo-3-[4-(trifluoromethyl)phenyl]-2-pyrazinamine (2c) and 3,5-bis[4-(trifluoromethyl)phenyl]-2-pyrazinamine (3c). The title compounds were obtained from 4-trifluoromethylphenylboronic acid after silica gel chromatography (15% ethyl acetate in hexanes).

Compound **2c**. 41% yield; pale yellow solid; TLC R_f 0.25 (20% ethyl acetate in hexanes); mp 160–161°C; analytical RP-HPLC (50:50 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 12.33 min, 99%; ^1H NMR (CD_3OD) δ 8.09 (s, 1H), 7.88–7.86 (m, 2H), 7.78–7.75 (m, 2H), 5.09 (br s); ESI-MS *m/z* 316, 318 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{11H8}N_3F_3^{79}Br$, 317.9854 $(M+H)^+$, observed 317.9848; calcd for $C_{11H8}N_3F_3^{81}Br$, 319.9833 $(M+H)^+$, observed, 319.9823.

Compound **3c**. 12% yield; pale yellow solid; TLC R_f: 0.1 (20% ethyl acetate in hexanes); mp 200–201°C; analytical RP-HPLC (65:35 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 4.65 min, 99%; ^1H NMR (CD_3OD) δ 8.50 (s, 1H), 8.07 (d, 2H, *J*=7.9 Hz), 7.97 (d, 2H, *J*=7.9 Hz), 7.81 (d, 2H, *J*=8.5 Hz), 7.71 (d, 2H, *J*=8.5 Hz); ESI-MS *m/z* 384 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{18}H_{11}F_6N_3$, 383.0857 $(M)^+$ observed, 383.0861.

4.2.4. 5-Bromo-3-(4-methylphenyl)-2-pyrazinamine (2d) and 3,5-bis(4-methylphenyl)-2-pyrazinamine (3d). The title compounds were obtained from *p*-tolylboronic acid after silica gel chromatography (15% ethyl acetate in hexanes).

Compound **2d**. 38% yield; pale yellow solid; TLC R_f 0.44 (25% ethyl acetate in hexanes); mp 118–120°C; analytical RP-HPLC (acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 7.56 min, 99%; ^1H NMR ($CDCl_3$) δ 8.09 (s, 1H), 7.61–7.58 (m, 2H), 7.29–7.27 (m, 2H), 4.88 (br s, 2H), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 151.2, 142.3, 140.9, 139.6, 132.9, 129.7, 127.9, 126.8, 21.3; ESI-MS *m/z* 264, 266 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{11}H_{11}N_3^{79}Br$, 264.0136 $(M+H)^+$, observed 264.0137; calcd for $C_{11}H_{11}N_3^{81}Br$ 266.0116 $(M+H)^+$, observed 266.0118.

Compound **3d**. 13% yield; pale yellow solid TLC R_f 0.29 (25% ethyl acetate in hexanes); mp 160–161°C; analytical RP-HPLC (50:50 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL, $UV_{225\ nm}$) 13.02 min, 99%; ^1H NMR ($CDCl_3$) δ 8.38 (s, 1H), 7.83 (d, 2H, *J*=8.2 Hz), 7.70 (d, 2H, *J*=7.9 Hz), 7.29 (d, 2H, *J*=7.9 Hz), 7.23 (d, 2H, *J*=7.9 Hz), 4.87 (br s, 2H), 2.41 (s, 3H), 2.40 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 150.7, 142.9, 139.6, 138.9, 137.8, 137.1, 134.6, 134.3, 129.6, 129.4, 128.1, 125.5, 21.3, 21.2; ESI-MS *m/z* 276 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{18}H_{17}N_3$, 275.1422 $(M+H)^+$, observed 275.1432.

4.2.5. 5-Bromo-3-phenyl-2-pyrazinamine (2e)[15] and 3,5-diphenyl-2-pyrazinamine (3e).[11,15,46,47] The title compounds were obtained from phenylboronic acid after silica gel chromatography (20–30% ethyl acetate in hexanes).

Compound **2e**. 57% yield; TLC R_f 0.52 (20% ethyl acetate in hexanes); analytical RP-HPLC (50:50 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 5.16 min, 92%; ^1H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.70–7.66 (m, 2H), 7.50–7.42 (m, 3H), 4.99 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 151.2, 142.6, 140.6, 135.7, 129.4, 129.0, 128.7, 127.9; ESI-MS *m/z* 250, 252 $(M+H)^+$, 551 $(2M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{10}H_9N_3^{79}Br$, 249.9980 $(M+H)^+$ observed, 249.9986; calcd for $C_{10}H_9N_3^{81}Br$, 251.9959 $(M+H)^+$ observed, 251.9949.

Compound **3e**.[11,15,46,47] 13% yield; TLC R_f 0.36 (20% ethyl acetate in hexanes); analytical RP-HPLC (50:50 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 6.5 min, 99%; ^1H NMR ($CDCl_3$) δ 8.36 (s, 1H), 7.92–7.85 (m, 2H), 7.76–7.71 (m, 2H), 7.47–7.26 (m, 6H), 4.82 (br s, 2H); ^{13}C NMR ($CDCl_3$) δ 150.9, 142.9, 139.6, 137.7, 137.3, 137.0, 129.0, 128.9, 128.7, 128.3, 128.1, 125.7; ESI-MS *m/z* 248 $(M+H)^+$; HRMS (FAB, *m/z*) calcd for $C_{16}H_{13}N_3$, 247.1109 $(M)^+$; observed, 247.1111.

4.3. Organozinc coupling with 2-amino-3,5-dibromopyrazine (1)

4.3.1. 3-Benzyl-5-bromo-2-pyrazinamine (2f).[12] A solution of zinc chloride (1.0 M solution in ether, 12.1 mL, 12.1 mmol, 240 mol%) was added to a solution of benzylmagnesium chloride (2.0 M solution in THF, 5.5 mL, 11.0 mmol, 220 mol%) in THF (20 mL) at room temperature under nitrogen. The resulting turbid mixture was stirred at room temperature for 15 min. To this mixture, bis(triphenylphosphine)palladium (II) dichloride (0.175 g, 0.25 mmol, 5 mol%) and a solution of 2-amino-3,5-dibromopyrazine (**1**, 1.26 g, 5.0 mmol) in THF (5 mL) were added

sequentially at room temperature. The resulting orange-colored reaction mixture was stirred for 54 h at room temperature and then quenched with water (10 mL) at 0°C. The mixture was diluted with ethyl acetate (200 mL) and water (60 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2×100 mL). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated on a rotary evaporator. The residue was dissolved in dichloromethane (12 mL) and purified by silica gel column chromatography (25–40% ethyl acetate in hexanes) to afford compound **2f** (1.028 g, 78%) as viscous yellow oil. 2-Amino-3,5-dibromopyrazine (**1**, 0.262 g, 20%) was recovered. The yield of 3-benzyl-5-bromo-2-pyrazinamine (**2f**) based on recovered starting material, was 98%. TLC R_f 0.39 (35% ethyl acetate in hexanes); analytical RP-HPLC (Waters Symmetry C_{18}, 50:50 acetonitrile/0.1% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 5.26 min, 99.4%; ^{1}H NMR ($CDCl_3$) δ 8.04 (s, 1H), 7.34–7.21 (m, 5H), 4.37 (br s, 2H), 4.08 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 152.1, 142.6, 141.8, 135.8, 129.2, 128.5, 127.4, 126.4, 40.8; ESI-MS *m/z* 264, 266 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{11}H_{11}N_3^{79}Br$ 264.0136 $(M+H)^+$, observed 264.0143; calcd for $C_{11}H_{11}N_3^{81}Br$ 266.0116 $(M+H)^+$, observed 266.0125.

4.3.2. Ethyl 4-{4-[(3-amino-6-bromo-2-pyrazinyl)-methyl]phenoxy}butanoate (2g). Magnesium (turnings, 0.36 g, 15.0 mmol, 125 mol%) and 1,2-dibromomethane (0.010 mL) were added to a solution of *tert*-butyl[4-(chloromethyl)phenoxy]dimethylsilane[12] (3.02 g, 11.8 mmol) in THF (24 mL) at room temperature under nitrogen. The mixture was sonicated for 2 min to initiate the reaction and then stirred for another 10 min. The reaction flask was then placed in an oil bath and gently heated at 50–55°C (bath temperature) for 30 min. The oil bath was removed and the mixture was allowed to cool to room temperature. Anhydrous zinc chloride (1.77 g, 12.98 mmol, 110 mol%) was added at room temperature and the mixture was stirred for 15 min.

Bis(triphenylphosphine)palladium (II) chloride (0.207 g, 0.295 mmol, 2.5 mol%) and a solution of 2-amino-3,5-dibromopyrazine (**1**, 1.48 g, 5.9 mmol, 50 mol%) in THF 15 mL were added to the above prepared organozinc reagent at room temperature and the resulting orange-colored mixture was stirred for 22 h. Reaction was then quenched with water (10 mL) and diluted with ethyl acetate (200 mL) and water (40 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layers were washed with 20% aqueous sodium chloride (30 mL), dried over anhydrous magnesium sulfate and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatography (30% ethyl acetate in hexanes) to afford of 5-bromo-3-(4-{[*tert*-butyl(dimethyl)silyl]oxy}benzyl)-2-pyrazinamine (1.84 g).

This silyl-ether was dissolved in a minimum amount of THF then treated with tetra-*n*-butylammonium fluoride (1.0 M in THF, 4.67 mL, 100 mol%) at 0°C under nitrogen. The resulting orange-red colored reaction mixture was stirred for 35 min and then quenched with water (15 mL). The mixture was diluted with ethyl acetate (100 mL) and water (25 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (100 mL). The combined organic layers were washed with brine (30 mL), dried over anhydrous magnesium sulfate and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatography (30–40% ethyl acetate in hexanes) to afford 4-[(3-amino-6-bromo-2-pyrazinyl)methyl]phenol (0.953 g, 58% yield for two steps) as a gummy yellow material. TLC R_f 0.22 (40% ethyl acetate in hexanes); analytical RP-HPLC (Waters Symmetry C_{18}, 50:50 acetonitrile/0.1% aq. trifluoroacetic acid, 2.0 mL/min, $UV_{215\ nm}$) 2.53 min, 99%; ^{1}H NMR ($CDCl_3$) δ 8.03 (s, 1H), 7.11–7.06 (m, 2H), 6.81–6.76 (m, 2H), 5.12 (br s, 1H), 4.39 (br s, 2H), 4.00 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 154.9, 152.1, 142.4, 142.1, 129.7, 128.9, 127.7, 126.4, 116.0, 115.4, 40.0; ESI-MS *m/z* 280, 282 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{11}H_{10}^{81}BrN_3O$, 282.0065, observed, 282.0069.

NaH (95%, 0.145 g, 5.72 mmol, 200 mol%) was added to a solution of 4-[(3-amino-6-bromo-2-pyrazinyl)methyl]phenol (0.800 g, 2.86 mmol) in anhydrous DMF (20 mL) at 0°C under nitrogen. After stirring the mixture for 40 min, ethyl 4-bromobutyrate (1.0 mL, 7.0 mmol, 240 mol%) was added. The cooling bath was removed; the mixture was allowed to warm to room temperature and then stirred for 1 h. The reaction mixture was quenched with 20% aqueous sodium chloride (20 mL) and extracted with ethyl acetate (3×50 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatography (45% ethyl acetate in hexanes) to afford ethyl 4-{4-[(3-amino-6-bromo-2-pyrazinyl)methyl]phenoxy}butanoate (**2g**, 0.840 g, 74% yield). TLC R_f: 0.35 (60% ethyl acetate in hexanes); analytical RP-HPLC (Waters Novapak C_{18}, 50:50 acetonitrile/0.1% aq. trifluoroacetic acid, 1.0 mL/min, $UV_{215\ nm}$) 5.23 min, 96%; ^{1}H NMR ($CDCl_3$) δ 8.02 (s, 1H), 7.15–7.09 (m, 2H), 6.87–6.81 (m, 2H), 4.38 (br s, 2H), 4.18–4.11 (m, 2H), 4.01 (s, 2H), 3.98 (t, 2H, J=6.0 Hz), 2.50 (t, 2H, J=7.2 Hz), 2.14–2.05 (m, 2H), 1.28–1.22 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 173.1, 158.0, 152.1, 142.3, 142.1, 129.4, 128.6, 127.6, 126.2, 115.0, 114.5, 66.7, 60.3, 40.0, 30.7, 24.5, 14.2; ESI-MS *m/z* 394, 396 $(M)^+$; HRMS (FAB) *m/z* calcd for $C_{17}H_{20}^{80}BrN_3O_3$ 396.0746, observed 394.0752.

4.4. Sonogashira coupling with 2-amino-3,5-dibromopyrazine (1)

4.4.1. Ethyl 5-(3-amino-6-bromo-2-pyrazinyl)-4-pentynoate (2h). A solution of ethyl 4-pentynoate (2.61 g, 23.3 mmol, 120 mol%) in anhydrous DMF (10 mL), triethylamine (32 mL), copper (I) iodide (0.444 g, 2.33 mmol, 12 mol%) and tetrakis(triphenylphosphine)-palladium (0) catalyst (1.12 g, 0.97 mmol, 5 mol%) were added sequentially to a solution of 2-amino-3,5-dibromopyrazine (**1**, 4.90 g, 19.4 mmol) in anhydrous DMF (70 mL) at room temperature under nitrogen. The mixture was gently heated in an oil bath at 120°C (bath temperature) for 20 min. The solvent was removed on a rotary evaporator and the resulting residue was dissolved in dichloromethane (20 mL) and purified by silica gel column chromatography (40% ethyl acetate in hexanes) to afford compound **2h** (4.093 g, 74% yield) as a pale yellow solid. TLC R_f 0.23 (40% ethyl acetate in hexanes); analytical RP-HPLC (Waters

NovaPak C_{18}, 40:60 acetonitrile/0.1% aq. trifluoroacetic acid, 1.0 mL/min, $UV_{215\ nm}$) 3.46 min, 97.8%; ^1H NMR ($CDCl_3$) δ 8.00 (s, 1H), 5.22 (br s, 2H), 3.73 (s, 3H), 2.81 (t, 2H, J=6.3 Hz), 2.67 (t, 2H, J=6.6 Hz); ^{13}C NMR ($CDCl_3$) δ 172.3, 154.7, 143.2, 125.1, 124.4, 97.2, 76.1, 52.0, 32.5, 15.6; ESI-MS m/z 284, 286 $(M+H)^+$; HRMS (FAB) m/z calcd $C_{10}H_{10}^{79}BrN_3O_2$, 284.0035 $(M+H)^+$, observed 284.0030.

4.5. General procedure for Suzuki coupling of 5-bromo-2-pyrazinamines

1,4-Bis(diphenylphosphino)butane (BDPB, 0.023 g, 0.053 mmol, 5.8 mol%) was added to a suspension of bis(benzonitrile)dichloro palladium (0.018 g, 0.046 mmol, 5 mol%) in toluene (2.0 mL) and the mixture was stirred for 30 min under nitrogen at room temperature. 5-Bromo-2-pyrazinamine (0.92 mmol), 4-(*tert*-butyldimethylsilyloxy)-phenylboronic acid (0.3 g, 1.19 mmol, 130 mol%), ethanol (0.38 mL), aqueous sodium carbonate (1.0 M, 0.92 mL) and toluene (4.0 mL) were added sequentially and the mixture was heated to reflux for 7 h. The mixture was cooled to room temperature, diluted with water (10 mL) and extracted with ethyl acetate (50 mL). The organic layer was separated, dried over anhydrous sodium sulfate, and concentrated on a rotary evaporator.

4.5.1. 5-(4-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-(4-fluorophenyl)-2-pyrazinamine (3f). The title compound was obtained from 5-bromo-3-(4-fluorophenyl)-2-pyrazinamine (**2b**). The crude compound was purified by silica gel column chromatography (25% ethyl acetate in hexanes) to afford **3f** (82%) as a colorless solid. TLC R_f 0.26 (25% ethyl acetate in hexanes); mp 134–135°C; analytical RP-HPLC (75:25 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 13.9 min, 96%; ^1H NMR ($CDCl_3$) δ 8.37, (s, 1H), 7.84–7.78 (m, 4H), 7.21–7.15 (m, 2H), 6.93–6.89 (m, 2H), 4.83 (br s, 2H), 0.99 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 164.6, 161.3, 156.1, 150.3, 143.1, 138.3, 137.2, 133.5, 130.3, 130.2, 126.9, 120.4, 116.0, 115.7, 25.6, 18.2, −4.4; ESI-MS m/z 396 $(M+H)^+$; HRMS (FAB) m/z calcd for $C_{22}H_{26}FN_3OSi$, 395.1829 $(M)^+$, observed 395.1820.

4.5.2. 5-(4-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-(4-trifluoromethylphenyl)-2-pyrazinamine (3g). The title compound was obtained from 5-bromo-3-[4-(trifluoromethyl)phenyl]-2-pyrazinamine (**2c**). The compound was purified by silica gel column chromatography (20–30% ethyl acetate in hexanes) to afford **3g** (92%) as a colorless solid. TLC R_f 0.43 (30% ethyl acetate in hexanes); mp 189–91°C; analytical RP-HPLC (75:25 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 23.31 min, 99%; ^1H NMR ($CDCl_3/CD_3OD$) δ 8.4 (s, 1H), 8.00–7.97 (m, 2H), 7.86–7.74 (m, 4H), 6.97–6.92 (m, 2H), 1.00 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 156.1, 150.3, 143.0, 140.8, 137.8, 129.8, 126.8, 125.7, 125.6, 120.4, 25.5, 18.1, −4.6; ESI-MS m/z 446 $(M+H)^+$; HRMS (FAB) m/z calcd for $C_{23}H_{26}F_3N_3OSi$, 445.1797 $(M)^+$, observed 445.1780.

4.5.3. 5-(4-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-(4-methylphenyl)-2-pyrazinamine (3h). The title compound was obtained from 5-bromo-3-(4-methylphenyl)-2-pyrazinamine (**2d**). The compound was purified by silica gel column chromatography (20–30% ethyl acetate in hexanes) to afford **3h** (83%) as a colorless solid. TLC R_f 0.36 (35% ethyl acetate in hexanes); mp 122–124°C; analytical RP-HPLC (75:25 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 14.37 min, 99%; ^1H NMR ($CDCl_3$) δ 8.33 (s, 1H), 7.85–7.81 (m, 2H), 7.69 (d, 2H, J=7.9 Hz), 7.28 (d, 2H, J=7.9 Hz), 6.93–6.88 (m, 2H), 4.89 (br s, 2H), 2.39 (s, 3H), 0.99 (s, 9H), 0.21 9 s, 6H); ^{13}C NMR (CDCl3) δ 155.9, 150.4, 142.7, 139.4, 138.8, 136.8, 134.6, 130.4, 129.5, 128.1, 126.8, 120.3, 25.6, 21.3, 18.2, −4.4; ESI-MS m/z 392 $(M+H)^+$, 783 $(2M+H)^+$; HRMS (FAB) m/z calcd for $C_{23}H_{29}N_3OSi$, 391.2080 $(M)^+$, observed 391.2093.

4.5.4. 5-(4-{[*tert*-Butyl(dimethyl)silyl]oxy}phenyl)-3-phenyl-2-pyrazinamine (3i). The title compound was obtained from 5-bromo-3-phenyl-2-pyrazinamine **2e**. The compound was purified by silica gel column chromatography (20–25% ethyl acetate in hexanes) to afford **3i** (68% yield) as a colorless solid. TLC R_f: 0.31 (25% ethyl acetate in hexanes); mp 114–115°C; analytical RP-HPLC (60:40 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 11.28 min, 96%; ^1H NMR ($CDCl_3$) δ 8.35 (s, 1H), 7.83 (d, 2H, J=8.8 Hz), 7.80–7.77 (m, 2H), 7.50–7.37 (m, 3H), 6.89 (d, 2H, J=8.8 Hz), 4.91 (br s, 2H), 0.99 (s, 9H), 0.21 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 155.9, 150.4, 142.8, 139.3, 137.4, 137.1, 130.3, 128.8, 128.2, 126.8, 120.3, 25.6, 18.2, −4.5; ESI-MS m/z 378 $(M+H)^+$; HRMS (FAB) m/z calcd for $C_{22}H_{27}N_3OSi$, 377.1923 $(M)^+$, observed 377.1934.

4.5.5. 3-Benzyl-5-(4-*tert*-butyldimethylsilyloxyphenyl)-2-pyrazinamine (3j). The title compound was obtained from 3-benzyl-5-bromo-2-pyrazinamine (**2f**). The compound was dissolved in dichloromethane (10 mL) and purified by silica gel column chromatography (35% ethyl acetate in hexanes) to afford **3j** (98%) as a pale yellow solid. TLC R_f 0.36 (35% ethyl acetate in hexanes); mp 98–101°C; analytical RP-HPLC (Waters Symmetry C_{18}, 90:10 acetonitrile/water, 2.0 mL/min, $UV_{225\ nm}$) 4.91 min, 99.4%; ^1H NMR ($CDCl_3$) δ 8.33 (s, 1H), 7.84–7.80 (m, 2H), 7.36–7.25 (m, 5H), 6.95–6.91 (m, 2H), 4.33 (br s, 2H), 4.18 (s, 2H), 1.00 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 156.0, 151.2, 142.7, 140.4, 137.0, 136.8, 130.6, 129.0, 128.6, 127.0, 126.9, 120.5, 41.3, 25.7, 18.2, −4.4; ESI-MS m/z 392 $(M+H)^+$; HRMS (FAB) m/z calcd for $C_{23}H_{29}N_3OSi$ 391.2080 $(M)^+$, observed 391.2079.

4.5.6. Ethyl 4-(4-{[3-amino-6-(4-{[*tert*-butyl(dimethyl)-silyl]oxy}phenyl)-2-pyrazinyl] methyl}phenoxy)butanoate (3k). The title compound was obtained from ethyl 4-{4-[(3-amino-6-bromo-2-pyrazinyl)methyl] phenoxy}-butanoate (**2g**). The compound was dissolved in dichloromethane (5 mL) and purified by silica gel column chromatography (50% ethyl acetate in hexanes) to afford **3k** (73%). TLC R_f 0.39 (60% ethyl acetate in hexanes); analytical RP-HPLC (Waters Novapak C_{18}, 80:20 acetonitrile/0.1% aq. trifluoroacetic acid, 1.0 mL/min, $UV_{215\ nm}$) 3.48 min, 99%; ^1H NMR ($CDCl_3$) δ 8.32 (s, 1H), 7.82–7.79 (m, 2H), 7.18–7.14 (m, 2H), 6.94–6.90 (m, 2H), 6.86–6.82 (m, 2H), 4.33 (br s, 2H), 4.17–4.11 (m, 2H), 4.10 (s, 2H),

3.98 (t, 2H, J=6.3 Hz), 2.50 (t, 2H, J=7.5 Hz), 2.15–2.05 (m, 2H), 1.28–1.22 (m, 3H), 1.00 (s, 9H), 0.22 (s, 6H); ESI-MS m/z 522 $(M+H)^+$; HRMS (FAB) m/z calcd for $C_{29}H_{39}N_3O_4Si$, 521.2710; Observed: 521.2734.

4.5.7. Ethyl 5-[3-amino-6-(4-{[*tert*-butyl(dimethyl)silyl]-oxy}phenyl)-2-pyrazinyl]-4-pentynoate (3l). The title compound was obtained from ethyl 5-(3-amino-6-bromo-2-pyrazinyl)-4-pentynoate (**2h**). The compound was dissolved in dichloromethane (8 mL) and purified by silica gel column chromatography (40% ethyl acetate in hexanes) to afford **3l** (66%). TLC R_f 0.21 (40% ethyl acetate in hexanes); analytical RP-HPLC (Waters Novapak C_{18}, 70:30 acetonitrile/0.1% aq. trifluoroacetic acid, 1.0 mL/min, $UV_{215\ nm}$) 5.88 min, 96.4%; ^1H NMR ($CDCl_3$) δ 8.32 (s, 1H), 7.76–7.71 (m, 2H), 6.91–6.86 (m, 2H), 5.16 (br s, 2H), 3.74 (s, 3H), 2.84 (t, 2H, J=6.9 Hz), 2.70 (t, 2H, J=6.6 Hz), 0.99 (s, 9H), 0.21 (s, 6H); ^{13}C NMR ($CDCl_3$): δ 172.4, 156.1, 154.0, 142.6, 137.9, 130.0, 127.0, 123.9, 120.4, 95.4, 77.2, 51.9, 32.8, 25.6, 18.2, 15.6, −4.4; ESI-MS m/z 412 $(M+H)^+$, 434 $(M+Na)^+$; HRMS (FAB) m/z calcd for $C_{22}H_{29}N_3O_3Si$, 411.1978 $(M)^+$, observed, 411.1981.

4.6. General procedure for the condensation of 2-aminopyrazines with α-ketoacetals

A mixture of 2-aminopyrazine (0.6 mmol) and α-ketoacetal (200 mol%) in 1:10 6N aq. HCl/1,4-dioxane (10 mL) were heated to reflux for 5 h. The mixture was cooled to room temperature and purified by preparative RP-HPLC (v:v acetonitrile/water 45.0 mL/min, $UV_{225\ nm}$). The product fractions were combined and lyophilized.

4.6.1. 2-(4-Hydroxybenzyl)-6,8-bis(4-hydroxyphenyl)-imidazo[1,2-*a*]pyrazin-3(7*H*)-one (5a). The title compound was obtained from 2-aminopyrazine **3a** and α-ketoacetal **4a** after preparative RP-HPLC (18:82). Compound **5a** (43%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (20:80 acetonitrile/water, 2.0 mL/min, $UV_{225\ nm}$) 11.37 min, 96%; ^1H NMR (CD_3OD) δ 7.92–7.86 (m, 2H), 7.66–7.57 (m, 3H), 7.17–7.13 (m, 2H), 6.98–6.88 (m, 4H), 6.69–6.65 (m, 2H), 4.04 (s, 2H); ESI-MS m/z 426 $(M+H)^+$, 448 $(M+Na)^+$, 851 $(2M+H)^+$, 873 $(2M+Na)^+$; HRMS (FAB) m/z calcd for $C_{25}H_{19}N_3O_4$, 425.1376 $(M)^+$, observed 425.1382.

4.6.2. 6,8-Bis(4-fluorophenyl)-2-(4-hydroxybenzyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (5b). The title compound was obtained from 2-aminopyrazine **3b** and α-ketoacetal **4a** after preparative RP-HPLC (40:60). Compound **5b** (32%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (40:60 acetonitrile/water, 2.0 mL/min, $UV_{225\ nm}$) 10.93 min, 97%; ^1H NMR (CD_3OD/1,4-dioxane-d_8) δ 8.45–8.36 (m, 2H), 8.12–7.98 (m, 3H), 7.38–7.24 (m, 4H), 7.20–7.15 (m, 2H), 6.71–6.67 (m, 2H); ESI-MS m/z 430 $(M+H)^+$, 859 $(2M+H)^+$; HRMS (FAB) m/z calcd for $C_{25}H_{17}F_2N_3O_2$, 429.1289 $(M)^+$, observed 429.1292.

4.6.3. 2-(4-Hydroxybenzyl)-6,8-bis[4-(trifluoromethyl)-phenyl]imidazo[1,2-*a*]pyrazin-3(7*H*)-one (5c). The title compound was obtained from 2-aminopyrazine **3c** and α-ketoacetal **4a** after preparative RP-HPLC (65:35). Compound **5c** (33%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (65:35 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 7.86 min, 99%; ^1H NMR (CD_3OD) δ 8.56 (s, 1H), 8.40 (d, 2H, J=8.2 Hz), 8.24 (d, 2H, J=8.2 Hz), 7.85 (d, 2H, J=8.2 Hz), 7.79 (d, 2H, J=8.2 Hz), 7.15–7.10 (m, 2H), 6.73–6.68 (m, 2H), 4.11 (s, 2H); ESI-MS (m/z): 503 $(M+H)^+$, 1059 $(2M+H)^+$; HRMS (FAB, m/z): calcd for C27H17$F_6N_3O_2$, 529.1225 $(M)^+$, observed 529.1246.

4.6.4. 2-(4-Hydroxybenzyl)-6,8-bis(4-methylphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (5d). The title compound was obtained from 2-aminopyrazine **3d** and α-ketoacetal **4a** after preparative RP-HPLC (40:60). Compound **5d** (26%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (40:60 acetonitrile/0.05% aqueous trifluoroacetic acid, 2.0 mL/min, $UV_{225\ nm}$) 11.36 min, 95%; ^1H NMR (CD_3OD/1,4-dioxane-d_8) δ 8.02–8.98 (m, 2H), 7.83–7.68 (m, 3H), 7.42 (d, 2H, J=7.9 Hz), 7.34 (d, 2H, J=7.9 Hz), 7.20–7.16 (m, 2H), 6.70–6.66 (m, 2H), 4.06 (s, 2H), 2.46 (s, 3H), 2.41 (s, 3H); ESI-MS m/z 422 $(M+H)^+$, 843 853 $(2M+H)^+$; HRMS (FAB) m/z calcd for $C_{27}H_{23}N_3O_2$, 421.1790 $(M)^+$, observed 421.1799.

4.6.5. 2-(4-Hydroxybenzyl)-6,8-diphenylimidazo[1,2-*a*]-pyrazin-3(7*H*)-one (5e). The title compound was obtained from 2-aminopyrazine **3e** and α-ketoacetal **4a** after preparative RP HPLC (35:65 acetonitrile/0.05% aq. trifluoroacetic acid, 45.0 mL/min, $UV_{225\ nm}$). Compound **5e** was isolated as an orange red fluffy powder, which was found to mixture by analytical RP HPLC due to decomposition. ESI-MS m/z 397 $(M+H)^+$, 787 $(2M+H)^+$.

4.6.6. 8-(4-Fluorophenyl)-2-(4-hydroxybenzyl)-6-(4-hydroxyphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (5f). The title compound was obtained from 2-aminopyrazine **3 g** and α-ketoacetal **4a** after preparative RP-HPLC (27:73). Compound **5f** (25%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (30:70 acetonitrile/water, 2.0 mL/min, $UV_{225\ nm}$) 8.34 min, 99%; ^1H NMR (CD_3OD) δ 8.15–8.07 (m, 2H), 7.80 (br s, 1H), 7.70–7.64 (m, 2H), 7.35–7.28 (m, 2H), 7.16–7.11 (m, 2H), 6.93–6.89 (m, 2H), 6.69–6.65 (m, 2H), 4.04 (s, 2H); ESI-MS m/z 428 $(M+H)^+$, 856 $(2M+H)^+$; HRMS (FAB) m/z calcd for $C_{25}H_{18}FN_3O_3$, 427.1332 $(M)^+$, observed 427.1349.

4.6.7. 2-(4-Hydroxybenzyl)-6-(4-hydroxyphenyl)-8-[4-(trifluoromethyl)phenyl]imidazo [1,2-*a*]pyrazin-3(7*H*)-one (5g). The title compound was obtained from 2-aminopyrazine **3g** and α-ketoacetal **4a** after preparative RP-HPLC (38:62). Compound **5g** (21%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (40:60 acetonitrile/water, 2.0 mL/min, $UV_{225\ nm}$) 7.09 min, 99%; ^1H NMR (CD_3OD) δ 8.25 (d, 2H, J=8.2 Hz), 8.13 (s, 1H), 7.89 (d, 2H, J=8.2 Hz), 7.82–7.77 (m, 2H), 7.15–7.05 (m, 2H), 6.94–6.90 (m, 2H), 6.72–6.67 (m, 2H), 4.09 (s, 2H); ESI-MS m/z 478 $(M+H)^+$, 955 $(2M+H)^+$; HRMS (FAB) m/z calcd for $C_{26}H_{18}F_3N_3O_3Na$, 500.1198 $(M+Na)^+$, observed 500.1208.

4.6.8. 2-(4-Hydroxybenzyl)-6-(4-hydroxyphenyl)-8-(4-methylphenyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one (5h). The title compound was obtained from 2-aminopyrazine **3h** and α-ketoacetal **4a** after preparative RP-HPLC (25:75).

Compound **5h** (17%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (30:70 acetonitrile/water, 2.0 mL/min, $UV_{225\ nm}$) 9.95 min, 96%; ^1H NMR (CD_3OD) δ 7.89–7.85 (m, 2H), 7.75 (s, 1H), 7.65–7.60 (m, 2H), 7.38–7.34 (m, 2H), 7.16–7.12 (m, 2H), 6.91–6.86 (m, 2H), 6.69–6.65 (m, 2H), 4.04 (s, 2H), 2.41 (s, 3H); ESI-MS *m/z* 424 $(M+H)^+$, 446 $(M+Na)^+$, 847 $(2M+H)^+$, 869 $(2M+Na)^+$; HRMS (FAB) *m/z* calcd for $C_{26}H_{21}N_3O_3$, 423.1583 $(M)^+$, observed 423.1588.

4.6.9. 2-(4-Hydroxybenzyl)-6-(4-hydroxyphenyl)-8-phenylimidazo[1,2-*a*]pyrazin-3(7*H*)-one (5i). The title compound was obtained from 2-aminopyrazine **3i** and α-ketoacetal **4a** after preparative RP-HPLC (25:75). Compound **5i** (43%) was isolated as an orange red fluffy powder. Analytical RP-HPLC (25:75 acetonitrile/water, 2.0 mL/min, $UV_{225\ nm}$) 12.74 min, 99%; ^1H NMR (CD_3OD) δ 8.02–7.98 (m, 2H), 7.83 (s, 1H), 7.69–7.65 (m, 2H), 7.61–7.56 (m, 3H), 7.16–7.12 (m, 2H), 6.92–6.87 (m, 2H), 6.70–6.66 (m, 2H), 4.05 (s, 2H); ESI-MS *m/z* 410 $(M+H)^+$, 432 $(M+Na)^+$819, $(2M+H)^+$841, $(2M+Na)^+$; HRMS (FAB) *m/z* calcd for $C_{25}H_{19}N_3O_3$ 409.1426 $(M)^+$, observed 409.1413.

4.6.10. 2-(4-Hydroxybenzyl)-6-(4-hydroxyphenyl)-8-(phenylmethyl)imidazo[1,2-*a*]pyrazin-3(7*H*)-one, coelenterazine (5j). The title compound was obtained from 2-aminopyrazine **3j** and α-ketoacetal **4a** after preparative RP-HPLC (30:70). Compound **5j** (63%) was isolated as an orange red fluffy powder. TLC R_f 0.4 (10% methanol in dichloromethane); mp 176–181°C (dec), lit.[21] 175–178°C (dec); analytical RP-HPLC (Waters NovaPak C_{18}, 25:75 acetonitrile/water, 2.0 mL/min, $UV_{254\ nm}$) 7.55 min, >99%; IR (KBr): 3191, 3063, 3029, 1611, 1564, 1513, 1453, 1242, 1172, 838, 698 cm^{-1}; ^1H NMR (CD_3OD) δ 7.58 (br s, 1H), 7.47 (d, 2H, *J*=8.2 Hz), 7.38 (d, 2H, *J*=6.9 Hz), 7.28 (d, 2H, *J*=7.7 Hz), 7.24 (m, 1H), 7.15 (d, 2H, *J*=8.7 Hz), 6.87 (d, 2H, *J*=8.8 Hz), 6.69 (d, 2H, *J*=8.5 Hz), 4.39 (s, 2H), 4.06 (s, 2H); ^1H NMR (dimethylformamide-d_7) δ 8.01 (s, 2H), 7.69 (br s, 2H), 7.53 (d, 2H, *J*=7.1 Hz), 7.29 (t, 2H, *J*=7.3 Hz), 7.22 (d, 2H, *J*=8.5 Hz), 6.94 (d, 2H, *J*=8.5 Hz), 6.76 (d, 2H, *J*=8.5 Hz), 4.40 (s, 2H), 4.03 (s, 2H); ESI-MS *m/z* 424 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{26}H_{21}N_3O_3$ 423.1583, observed: 423.1598; analysis calcd for $C_{26}H_{21}N_3O_3H_2O$: C, 70.74, H, 5.25, N, 9.52, found: C, 71.15, H, 5.26, N, 9.67.

4.6.11. 4-(4-{[8-Benzyl-6-(4-hydroxyphenyl)-3-oxo-3,7-dihydroimidazo[1,2-*a*]pyrazin-2-yl]methyl}phenoxy)-butanoic acid (5k). The title compound was obtained from 2-aminopyrazine **3k** and α-ketoacetal **4b** after preparative RP-HPLC (35:65). Compound **5k** (56%) was isolated as a yellow fluffy powder. Analytical RP-HPLC (Waters Novapak C_{18}, 25:75 acetonitrile/water, 2.0 mL/min, $UV_{254\ nm}$) 11.0 min, >99%; ^1H NMR (CD_3OD) δ 7.53 (br s, 1H), 7.46 (br s, 2H), 7.38 (d, 2H, *J*=6.6 Hz), 7.28 - 7.21 (m, 5H), 6.88 (d, 2H, *J*=8.8 Hz), 6.83 (d, 2H, *J*=8.8 Hz), 4.39 (s, 2H), 4.09 (s, 2H), 3.96 (t, 2H, *J*=8.1 Hz), 2.45 (t, 2H, *J*=7.2 Hz), 2.04–2.01 (m, 2H); ESI-MS *m/z* 510 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{30}H_{27}N_3O_5$, 509.1951 $(M)^+$, observed 509.1974.

4.6.12. 4-{4-[8-Benzyl-2-(4-hydroxybenzyl)-3-oxo-3,7-dihydroimidazo[1,2-*a*]pyrazin-6-yl]phenoxy}butanoic acid (5l). The title compound was obtained from 2-aminopyrazine **3k** and α-ketoacetal **4a** after preparative RP-HPLC (35:65). Compound **5l** (41%) was isolated as a yellow fluffy powder. Analytical HPLC (Waters Novapak C_{18}, 25:75 acetonitrile/water, 2.0 mL/min, $UV_{254\ nm}$) 7.27 min, >99%; ^1H NMR (CD_3OD) δ 7.68 (s, 1H), 7.58 (d, 2H, *J*=7.6 Hz), 7.37 (d, 2H, *J*=6.6 Hz), 7.27–7.20 (m, 5H), 7.15 (d, 2H, *J*=8.8 Hz), 7.01 (d, 2H, *J*=8.8 Hz), 6.67 (d, 2H, *J*=8.5 Hz), 4.39 (s, 2H), 4.05 - 4.02 (m, 4H), 2.39 (t, 2H, *J*=7.2 Hz), 2.08–2.03 (m, 2H); ESI-MS *m/z* 510 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{30}H_{27}N_3O_5$, 519.1951 $(M)^+$, observed 519.1955.

4.6.13. 4-[2-(4-Hydroxybenzyl)-6-(4-hydroxyphenyl)-3-oxo-3,7-dihydroimidazo[1,2-*a*]pyrazin-8-yl]butanoic acid (5m). The title compound was obtained from 2-aminopyrazine **3m** and α-ketoacetal **4a** after preparative RP-HPLC (25:75). Compound **5m** (41%) was isolated as a yellow fluffy powder. Analytical RP-HPLC (Waters Nova-Pak C_{18}, 20:70 acetonitrile/0.1% aq. trifluoroacetic acid, 1.0 mL/min, $UV_{215\ nm}$) 4.78 min, 97%; ^1H NMR (CD_3OD) δ 7.89 (s, 1H), 7.62 (d, 2H, *J*=8.7 Hz), 7.10 (d, 2H, *J*=8.4 Hz), 6.90 (d, 2H, *J*=8.4 Hz), 6.68 (d, 2H, *J*=8.7 Hz), 4.08 (s, 2H), 3.14 (t, 2H, *J*=7.5 Hz), 2.38 (t, 2H, *J*=7.5 Hz), 1.90–1.84 (m, 2H), 1.80–1.68 (m, 2H); ESI-MS *m/z* 434 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{24}H_{23}N_3O_5$, 433.1638 $(M)^+$, observed 433.1646.

4.6.14. 4-(4-{[2-(4-Hydroxybenzyl)-6-(4-hydroxyphenyl)-3-oxo-3,7-dihydroimidazo[1,2-*a*]pyrazin-8-yl]methyl}-phenoxy)butanoic acid (5n). The title compound was obtained from 2-aminopyrazine **3m** and α-ketoacetal **4a** after preparative RP-HPLC (27:73 acetonitrile/0.1% aq. trifluorocaetic acid/, 40 mL/min, $UV_{215\ nm}$). Compound **5n** (32%) was isolated as a yellow fluffy powder. Analytical RP-HPLC (Waters Novapak C_{18}, 30:70 acetonitrile/0.1% aq. trifluoroacetic acid, 1.0 mL/min, $UV_{215\ nm}$) 3.37 min, 93%; ^1H NMR (CD_3OD) δ 7.94 (s, 1H), 7.60 (d, 2H, *J*=8.7 Hz), 7.32–7.28 (m, 2H), 7.15–7.10 (m, 2H), 6.92–6.82 (m, 4H), 6.74–6.69 (m, 2H), 4.39 (s, 2H), 4.12 (s, 2H), 3.97 (t, 2H, *J*=6.3 Hz), 2.46 (t, 2H, *J*=7.2 Hz), 2.0 −1.98 (m, 2H); ESI-MS *m/z* 526 $(M+H)^+$; HRMS (FAB) *m/z* calcd for $C_{30}H_{27}N_3O_6$, 525.1900, observed 525.1905.

4.6.15. Ethyl 4-[4-(5-amino-6-benzyl-2-pyrazinyl)phenoxy]butanoate (3m). NaH (95%, 0.104 g, 4.33 mmol, 150 mol%) was added to a solution of 4-(5-amino-6-benzyl-2-pyrazinyl)phenol (**3j**, 0.800 g, 2.89 mmol) in anhydrous DMF (20 mL) at 0–5°C (ice bath) under nitrogen. After stirring for 30 min, ethyl 4-bromobutylate (1.033 mL, 7.22 mmol, 250 mol%) was added at 0–5°C via syringe. After stirring an additional 5 min, the cooling bath was removed and the mixture was allowed warm to room temperature. The mixture was stirred for an additional for 3 h then quenched with saturated aqueous sodium chloride (30 mL). The mixture was extracted with dichloromethane (3×50 mL); the combined organic layers were dried over anhydrous sodium sulfate and concentrated on a rotary evaporator. The residue was purified by silica gel column chromatograph (50% ethyl acetate in hexanes) to afford compound **3m** (1.028 g, 91%). ^1H NMR ($CDCl_3$) δ 8.31 (s, 1H), 7.86 (d, 2H, *J*=4.7 Hz), 7.30–7.23 (m, 5H), 6.96 (d, 2H, *J*=4.7 Hz), 4.34 (s, 2H), 4.18–4.14 (m, 4H), 4.04 (t, 2H,

J=6.1 Hz), 2.50 (t, 2H, J=7.4 Hz), 2.11–2.15 (m, 2H), 1.25 (t, 3H, J=7.2 Hz); ESI-MS m/z 393 $(M+H)^+$; HRMS (FAB) m/z calcd for $C_{23}H_{26}N_3O_3$ 392.1974 $(M)^+$, observed 392.1988.

4.6.16. Ethyl 5-[3-amino-6-(4-{[*tert*-butyl(dimethyl)silyl]-oxy}phenyl)-2-pyrazinyl] pentanoate (3n). Platinum oxide (0.020 g) was added to an ethanolic solution of ester **3l** (0.200 g, 0.486 mmol, 10 mL) and the mixture was stirred at room temperature under hydrogen atmosphere (15–20 psi). After stirring for 4.5 h, the mixture was filtered through diatomaceous earth and washed with ethanol (3 mL). The combined filtrate was concentrated on a rotary evaporator and dried on a vacuum pump (0.1 mm/Hg) to afford pyrazine **3n** (0.189 g, 94%). TLC R_f 0.20 (50% ethyl acetate in hexanes); analytical RP-HPLC (Waters Novapak C_{18}, 70:30 acetonitrile/0.1% aq. trifluoroacetic acid, 1.0 mL/min, $UV_{215\ nm}$) 3.10 min, 95.2%; ^1H NMR ($CDCl_3$) δ 8.25 (s, 1H), 7.80- 7.76 (m, 2H), 6.92–6.88 (m, 2H), 4.55 (br s, 2H), 3.68 (s, 3H), 2.72 (t, 2H, J=7.2 Hz), 2.43 (t, 2H, J=6.9 Hz), 1.96–1.76 (m, 4H), 1.00 (s, 9H), 0.22 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 174.0, 155.8, 150.7, 142.2, 141.2, 135.7, 130.6, 126.7, 120.3, 51.5, 33.7, 32.7, 25.6, 25.4, 24.6, 18.1, −4.5; (17 carbons); ESI-MS m/z 416 $(M+H)^+$; HRMS (FAB) m/z calcd for $C_{22}H_{33}N_3O_3Si$, 415.2291 $(M)^+$, observed 415.2273.

4.6.17. Ethyl 4-[4-(3,3-diethoxy-2-oxopropyl)phenoxy]-butanoate (4b). A solution of tetra-*n*-butylammonium fluoride (1.0 M in THF, 1.5 mL, 1.5 mmol, 50 mol%) was added to a 0°C (ice bath) cooled solution of 3-(4-{[*tert*-butyl(dimethyl)silyl]oxy}phenyl)-1,1-diethoxyacetone (**4a**, 1.051 g, 2.98 mmol) in THF (25 mL) under nitrogen. After stirring the mixture for 30 min, the reaction was quenched with saturated aqueous sodium chloride (30 mL) and extracted with dichloromethane (3×30 mL). The combined organic layers were dried over anhydrous sodium sulfate and the solvent was removed on a rotary evaporator. The resulting residue was purified by silica gel column chromatograph (33% ethyl acetate in hexanes) to afford 3-(4-hydroxyphenyl)-1,1-diethoxyacetone (0.41 g, 58%) as a viscous oil. ^1H NMR ($CDCl_3$) δ 7.06 (d, 2H, J=8.2 Hz), 6.74 (d, 2H, J=8.5 Hz), 4.64 (s, 1H), 3.81 (s, 2H), 3.69–3.66 (m, 2H), 3.57–3.52 (m, 2H), 1.24 (t, 6H, J=7.0 Hz); ESI-MS m/z 256 $(M+NH_4)^+$.

Anhydrous K_2CO_3 (0.156 g, 1.13 mmol, 150 mol%) and ethyl 4-bromobutyrate (0.221 g, 1.13 mmol, 150 mol%) were added sequentially to a solution of 3-(4-hydroxyphenyl)-1,1-diethoxyacetone (0.180 g, 0.756 mmol) in anhydrous DMF (3.0 mL) under nitrogen. The reaction mixture was gently heated at 110°C (bath temperature) for 3 h and the mixture was allowed to cool to room temperature. The mixture was then filtered and washed with methanol (3.0 mL). The filtrate was purified by preparative RP-HPLC (Waters Novapak C_{18}, 6.0 μm, 40×100 mm column using 55:45 acetonitrile/water, 45 mL/min, $UV_{220\ nm}$). The product was collected and lyophilized to afford α-ketoacetal derivative **4b** (0.063 g, 23%) as a viscous oil. Analytical HPLC (Waters Novapak C_{18}, 6.0 μm, 3.9×150 mm column, 45:55 acetonitrile/water, 2.0 mL/min, UV $_{254\ nm}$) 9.3 min, >98%; ^1H NMR ($CDCl_3$) δ 7.11 (d, 2H, J=4.4 Hz), 6.83 (d, 2H, J=4.4 Hz), 4.62 (s, 1H), 4.12 (q, 2H, J=7.1 Hz), 3.98 (t, 2H, J=6.1 Hz), 3.81 (s, 2H), 3.69–3.64 (m, 2H), 3.56–3.50 (m, 2H), 2.50 (t, 2H, J=7.4 Hz), 2.11–2.07 (m, 2H), 1.27–1.21 (m, 9H); ESI-MS m/z 370 $(M+NH_4)^+$.

4.7. Chemiluminescence evaluation

4.7.1. Peroxymonocarbonate triggering solution. Ammonium bicarbonate (0.636 g, 8 mmol) was dissolved in water (12.0 mL) and combined with 30% aqueous hydrogen peroxide (4.0 mL, 35 mmol) and acetonitrile (24 mL) at room temperature. The solution was allowed to stand for 15 min prior to the use.

4.7.2. 3,7-Dihydroimidazo[1,2*a*]pyrazine-3-one stock solutions. Compounds **5a**–**n** were dissolved in methanol to give a 1 mg/mL solution then diluted 1:1 with 0.2% aqueous dodecyltrimethylammonium bromide (DTAB).

4.7.3. Chemiluminescence measurement. Each stock solution (50 μL, 0.5 mg/mL) was deposited on a 96-well white polystyrene microtiter plate (Wallac Inc., Gaithersburg, MD) and diluted with 0.2% aqueous DTAB (175 μL). Serial dilution (50 μL+175 μL diluent) of each sample was continued across the plate. Chemiluminescence was initiated by the addition of peroxymonocarbonate triggering solution (100 μL/well). Light emission (Relative Light Units, RLU) was recorded for 10 min automatically following the injection of trigger solution. Typical profiles of the emission (RLU/time) are seen in Figure 5. The integrated signal (total RLU over 10 min) was plotted versus moles of substrate as a measure of the chemiluminescence efficiency (see Figure 5 inset, and Table 1) and linearity of the response.

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