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ARTICLE TYPE

Multi-substituted 8-Aminoimidazo[1,2-*a*]pyrazines by Groebke-Blackburn-Bienaymé Reaction and Their Hsp90 Inhibitory Activity

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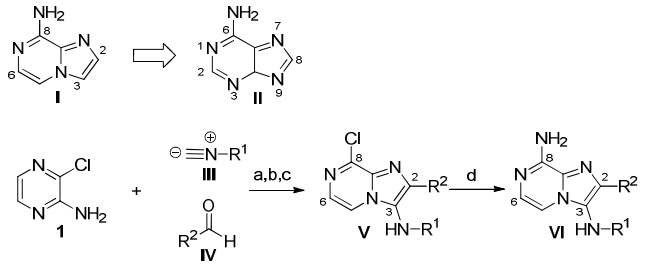
Using 2,3-diamino pyrazine substrate and yttrium triflate catalyst, various 2-alkyl and aryl substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines could be efficiently prepared through Groebke-Blackburn-Bienaymé MCR. In particular, a novel 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazine structure could be prepared exclusively with this new method and was found with moderate Hsp90 inhibitory activity. Crystal complex with N-terminus ATP domain of Hsp90 and one of the new Hsp90 inhibitors was also obtained to elucidate the activity origin of 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazines.

Introduction

8-Aminoimidazo[1,2-*a*]pyrazines **I** are highly useful for medicinal chemistry research, for it's obvious similarity with adenine **II**, which is ubiquitous in bioactive structures. Consequently, installation of different substituents on 8-aminoimidazo[1,2-*a*]pyrazine ring system become an intriguing topic, and this usually could be achieved via certain ring formation reactions from substituted fragments.[1] Preparation of multi-substituted 8-aminoimidazo[1,2-*a*]pyrazine ring system is more demanding and less reported. It is known that multicomponent reactions (MCRs) are advantageous in the syntheses of heavily substituted heterocycles. However, syntheses of multi-substituted 8-aminoimidazo[1,2-*a*]pyrazines through MCR pathway were rarely discussed and seemed to be less efficient: For example, David *et al.* [2] attempted the preparation of 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines through Groebke-Blackburn-Bienaymé MCR [3] and obtained poor yields. Interestingly, one recent article by Pirali *et al.* [4] described more efficient syntheses of 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines **VI** starting from chloropyrazine **1**, isocyanides **III** and aldehydes **IV** via two step sequences including Groebke-Blackburn-Bienaymé MCR and the following aminolysis of intermediate **V**. Nevertheless, it was found that only aryl carboxaldehydes were productive with the reported condition, and this meant that the R^2 on 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazine products **VI** could only be aryl groups (Scheme 1).



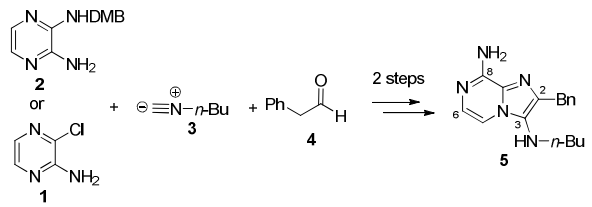
Scheme 1. A comparison of imidazo[1,2-*a*]pyrazines **I** and adenine **II** and one of the known synthetic method [4] to 8-aminoimidazo[1,2-*a*]pyrazines **VI**. Reaction conditions: a) compound **1** (1 equiv), R^2CHO (1.2 equiv), MeCN, reflux, 2 h; b) TMSCl (1.2 equiv), MeCN/DCM, 30 min; c) R^1NC (1.2 equiv), reflux, overnight; d) aq. NH_4OH/dioxane, 100 °C, overnight.

During our study of Groebke-Blackburn-Bienaymé MCR, [5] a serendipitous experiment indicated that 2,3-diaminopyrazine was a superior substrate for this MCR leading to 2-aryl, 2-benzyl, and 2-alkyl 3,8-diaminoimidazo[1,2-*a*]pyrazines. Using this efficient methodology, a panel of 2-piperonyl substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines could be prepared for the first time. Some of 2-piperonyl substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines were found with moderate Heat Shock Protein 90 (Hsp90) inhibitory activity, and could serve as leads for further optimization. These works will be described herein.

Results and discussion

Initially, using yttrium triflate catalyst, it was found that the *N*-2,4-dimethoxybenzyl (*N*-DMB) protected diamino pyrazine **2** reacted with isocyanide **3** and phenyl acetaldehyde **4** to give the desired product **5** with good yield, after removing the *N*-protection with acid (Table 1, entry 1). This was unique since in comparison experiments, TMSCl [4] failed to give the conversion and TfOH only gave low yield (Table 1, entry 2-3). Changing from diamino pyrazine **2** back to chloropyrazine **1** again failed to give the reaction with TMSCl, and this was consistent with the findings by Pirali *et al.* [4] (Table 1, entry 4). With TfOH or rare earth-metal triflate catalysts, some product **5** could be observed after aminolysis, however the yield was low (Table 1, entry 5-7). As phenyl acetaldehyde **4** is a special type of alkyl aldehyde, above discovery served as a successful example of Groebke-Blackburn-Bienaymé MCR leading to 2-alkyl 3,8-diaminoimidazo[1,2-*a*]pyrazines.

Table 1. MCR of pyrazines **1** or **2**, isocyanide **3**, and aldehyde **4**.[a]



No.	Pyrazine	Catalyst	Yield (for 2 steps) [b]
1	**2**	$Yb(OTf)_3$	75%
2	**2**	TMSCl	*n.d.*
3	**2**	TfOH	28%
4	**1**	TMSCl	*n.d.*
5	**1**	TfOH	4%
6	**1**	$Sc(OTf)_3$	8%
7	**1**	$Yb(OTf)_3$	11%

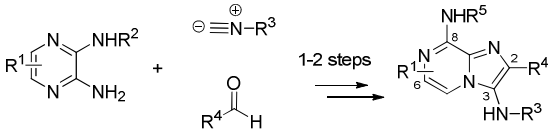
[a] Reaction conditions: For entry 1 and 3: a) compound **2** (1.0 equiv), compound **3** (1.2 equiv), compound **4** (1.2 equiv), catalyst (10 mol%), MeOH, 70 °C, 5 h; b) TFA, DCM, 50 °C, 1 h. For entry 2 and 4: a) compound **4** (1.2 equiv), MeCN, 100 °C, 2 h; b) TMSCl (1.2 equiv), MeCN/DCM, rt, 30 min; c) compound **3** (1.2 equiv), 100 °C, 12 h. For entry 5, 6, and 7: a) compound **1** (1.0 equiv), compound **3** (1.2 equiv), compound **4** (1.2 equiv), catalyst (10 mol%), MeOH, 100 °C, 12 h; b) aq. NH_4OH/dioxane, sealed tube, 120 °C, 24 h. [b] Yield was determined after flash chromatography.

To further explore the scope of the yttrium triflate catalyzed synthesis of 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines, different substrates, including pyrazines **6-8**, isocyanides **9-11**, as well as aldehydes **4** and **12-16**, were subjected to the new reaction condition described in Table 1, entry 1 (Scheme 2). For common electron rich substituted phenyl acetaldehydes, the yields were moderate to good with different isocyanides. Since the yields were still lower than that with phenyl acetaldehyde **4** (Table 1, entry 1), it might be speculated that the electron donating groups on the phenyl ring of the aldehyde component reduced the yield. Retaining phenyl acetaldehyde **4**, chloro-substituted pyrazine **6** gave product **20** again with good yield. Above examples (for products **17-21**) were all based on *N*-DMB protected pyrazines **6**, and several points were noteworthy: 1) Compared to chloropyrazine **1** starting material, *vic*-diamino substitution on diamino pyrazine **2** could significantly improve the MCR yield with various isocyanide and aldehyde components; 2) As illustrated in the general reaction schemes in Scheme 2 and Table 1, deprotection of N^8-DMB on 3,8-diaminoimidazo[1,2-*a*]pyrazine intermediate (formed from pyrazine **2**) gave better yield than aminolysis of 8-Cl on 3,8-diaminoimidazo[1,2-*a*]pyrazine intermediate (formed from chloropyrazine **1**), rendering the syntheses of 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines more efficient.

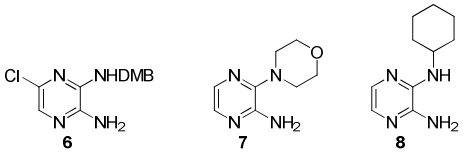
Next, morpholine substituted pyrazine **7** reacted with acetal aldehyde **15** and different isocyanides to give products **22** and **23** with good yields (Scheme 2). This was not trivial, since simple aldehydes such as **15** used to be poor partner for this type of reaction. Meanwhile, pyrazines **7** and **8** could react with aryl carboxaldehydes **14** and **16** as well to give the desired 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines **24-26** (Scheme 2). The reactions with morpholine substituted pyrazine **7** and *N*-cyclohexyl pyrazine **8** were highly practical for the following reasons: 1) The diamino substituted pyrazine substrates improved the MCR yield substantially, with both alkyl aldehyde and aryl carboxaldehyde; 2) It eliminated the necessity of *N*-deprotection, and yielded more interesting *N*-alkylated 2-substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines; 3) Morpholine or alkylated amino group was widely used in medicinal chemistry.

For Groebke-Blackburn-Bienaymé MCR, there might be several reasons accounting for the yield improvement using diamino substituted pyrazine substrates and yttrium triflate catalyst: 1) *vic*-Diamino substitution could enhance the nucleophilicity of pyrazine substrates and result in improved condensation reaction; 2) *vic*-Diamino substitution could provide a better chelation environment for yttrium metal, [6] which was an essential catalyst for Groebke-Blackburn-Bienaymé MCR. This might also explain why TMSCl failed to work with diamino substituted pyrazine substrates.

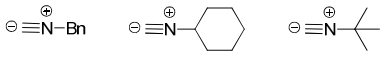
General scheme of the reaction: [a]



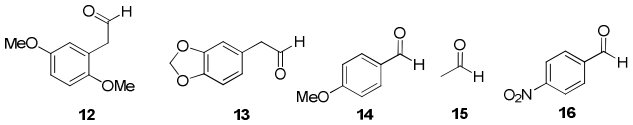
Pyrazines:



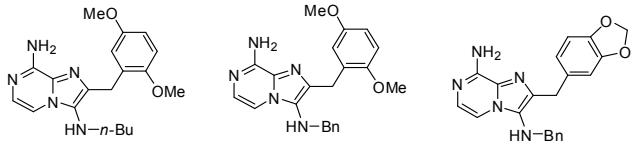
Isocyanides:

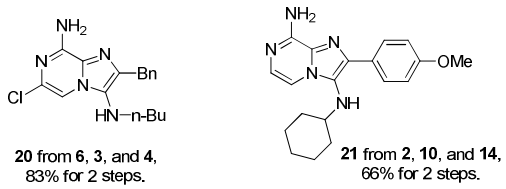


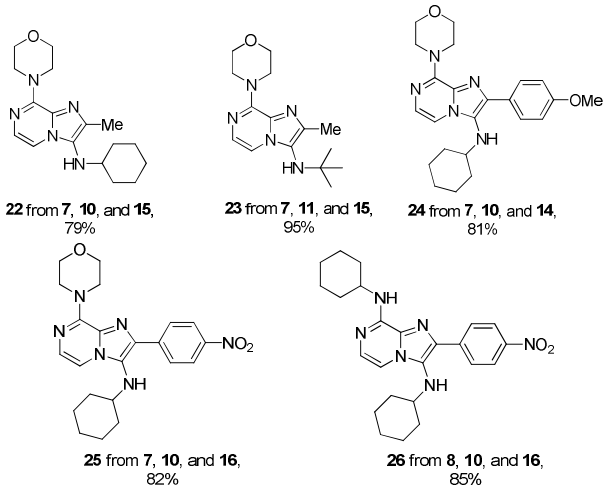
Aldehydes:



Products (Yields): [b]

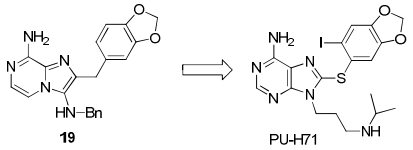






Scheme 2. New condition for Groebke-Blackburn-Bienaymé MCR led to various multisubstituted 3,8-diaminoimidazo[1,2-*a*]pyrazines. [a] Reaction conditions: For products **17-21**: a) pyrazines (1.0 equiv), isocyanides (1.2 equiv), aldehydes (1.2 equiv), $Yb(OTf)_3$ (10 mol%), MeOH, 70 °C, 5 h; b) TFA, DCM, 50 °C, 1 h. For products **22-26**: pyrazines (1.0 equiv), isocyanides (1.2 equiv), aldehydes (1.2 equiv), $Yb(OTf)_3$ (10 mol %), MeOH, 70 °C, 5 h. [b] Yield was determined after flash chromatography.

On the basis of above discovery, we looked for the applications in medicinal chemistry. It is known that Hsp90 belongs to a large family of heat shock proteins, which are evolutionarily conserved and required for essential housekeeping functions such as protein folding, assembly, and transportation.[7] Discovering new Hsp90 inhibitors is an important approach combating cancer. [8] We noticed that some ATP-competitive Hsp90 inhibitors contains 8,9-disubstituted adenine motif, [9] which might be replaced with 2,3-disubstituted 8-aminoimidazo[1,2-*a*]pyrazine scaffold, according to the structure similarity between adenine and 8-aminoimidazo[1,2-*a*]pyrazine. Further computational study using scaffold hopping concept also suggested that our new product **19** or similarly substituted 8-aminoimidazo[1,2-*a*]pyrazines might possess Hsp90 inhibitory activity due to the remarkable resemblance to PU-H71, [10] an Hsp90 inhibitor in Phase I clinical trial (Scheme 3).

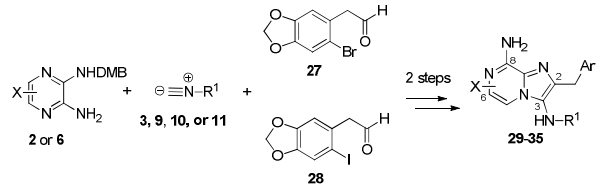


Scheme 3. The structure similarity between compound **19** and a known Hsp90 inhibitor PU-H71.

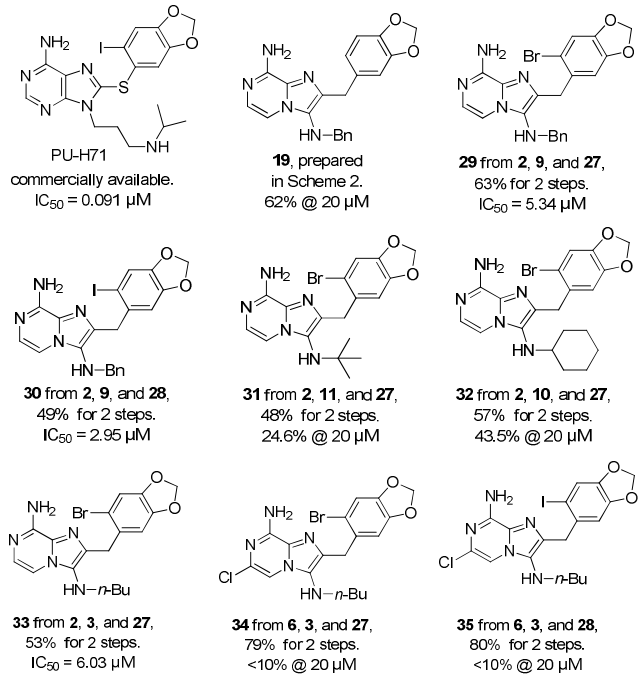
To test the hypothesis, a focused library of 8-aminoimidazo[1,2-*a*]pyrazines bearing 2-piperonyl and 3-alkyl amino substitutions was prepared using the new methodology (Scheme 4). With diamino pyrazine **2** and piperonals **27** and **28**, moderate to good yields were documented for products **29-32**. When chloro substituted diamino pyrazine **6** was used, the yields were again good for products **34** and **35**, and this was consistent with the observations in Scheme 2, conversions from **6**, **3**, and **4** to **22**.

The inhibitory activity of the library against Hsp90 was measured and analyzed, too (Scheme 4): 1) The halogenated 2-piperonyl substitution was found essential for Hsp90 binding, as suggested by the activity comparison of compounds **19**, **29**, and **30**: Compared to compound **19**, the bromine atom on compound **29** dramatically increased the activity to 5.34 μM, while the iodine on compound **30** further improves the value to 2.95 μM. 2) The substitution on C3 of 8-aminoimidazo[1,2-*a*]pyrazine scaffold affected the Hsp90 affinity, too, as demonstrated by the activities of compounds **29**, **31**, **32**, and **33**. The 2-*tert*-butyl amine group on compound **31** or the 2-cyclohexyl amino group on compound **32** may not be long enough to reach the van der Waals surface of Hsp90 protein, and resulted in decreased activtities. 3) The 6-chloro substitution on 8-aminoimidazo[1,2-*a*]pyrazine was found destructive to Hsp90 inhibitory activity, and this was demonstrated by the data of compounds **34** and **35**. Generally, the moderate inhibitory activity of 2-piperonyl and 3-alkyl amino 8-aminoimidazo[1,2-*a*]pyrazines suggested that this new scaffold could serve as a promising starting point for further structural optimization.

General scheme of the reaction: [a]



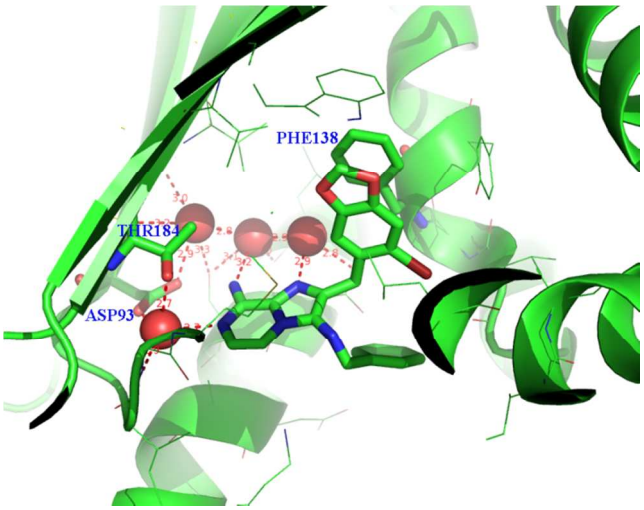
Products with yields [b] and Hsp90-*alpha* fluorescence polarization (FP) activities [c]:



Scheme 4. Synthesis and activity study of a Hsp90 targeted focused library. [a] Reaction conditions: a) pyrazines (1.0 equiv), isocyanides (1.2 equiv), aldehydes (1.2 equiv), $Yb(OTf)_3$ (10 mol%), MeOH, 70 °C, 5 h; b) TFA, DCM, 50 °C, 1 h. [b] Yield was determined after flash chromatography. [c] See supporting information for details.

For better understanding the interactions between 2,3-disubstituted 8-aminoimidazo[1,2-*a*]pyrazine and Hsp90 binding site, compound **29** was cocrystallized with N-terminus ATP domain of Hsp90 and the co-crystal structure was successfully solved (pdb entry: 4R3M).[11] As shown in Figure 1, in Hsp90 N-terminus ATP domain, the 8-aminoimidazo[1,2-*a*]pyrazine ring was situated at the same position as the adenine ring of PU-H71(Figure 1B). The 8-aminoimidazo[1,2-*a*]pyrazine ring system formed several indirect hydrogen bonds with the binding site of Hsp90 through four conserved water molecules (red spheres in Figure 1), and two direct hydrogen bonds with ASP93 and THR184 residues. The 2-piperonyl substitution showed typical π-π stacking interaction with PHE138 residue, and the bromine on 2-piperonyl substitution was close to GLY135 residue on the Hsp90 backbone. A halogen bond with the oxygen atom of GLY135 residue was thus speculated for the short Br-O distance of 3.4 Å. In addition, compared to the PU-H71 structure, discrepancy between the orientations of 3-benzyl amino group on compound **29** and 9-alkyl amino group on PU-H71 could be observed. This might account partially for the activity difference between these two compounds.

A:



B:

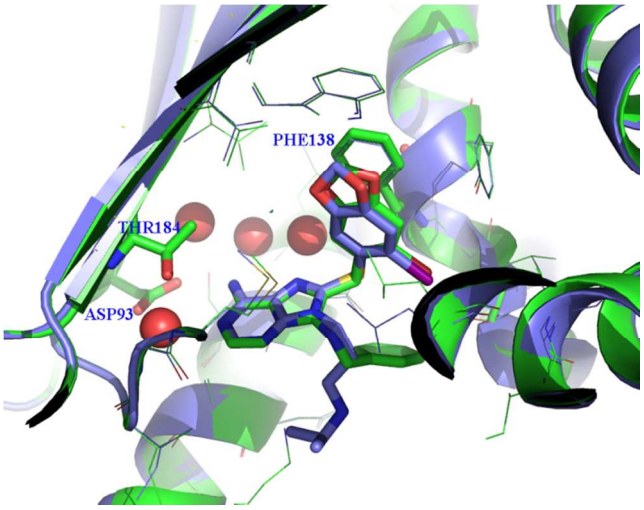


Figure 1. A: Co-crystal structure of Hsp90 with compound **29** (pdb entry: 4R3M). B: superimposition of crystal structures **29**-Hsp90[11] and PU-H71-Hsp90 (pdb entry: 2FWZ). Protein was shown in cartoon style, and the crystal water molecules were shown in red spheres. The carbon atoms of compound **29** were in green, and that of PU-H71 in blue.

Conclusions

In conclusion, with 2,3-diamino pyrazine substrate and yttrium triflate catalyst, various 2-alkyl and aryl substituted 3,8-diaminoimidazo[1,2-*a*]pyrazines could be efficiently prepared through Groebke-Blackburn-Bienaymé MCR. Elimination of the chloride aminolysis step made this method more favorable compared with previous synthetic approaches. We further demonstrate that 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazine structures, prepared exclusively with our new synthetic method, were potential Hsp90 inhibitors. Crystal complex with N-terminus ATP domain of Hsp90 and one of the new Hsp90 inhibitors was also obtained to elucidate the activity origin of 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazines. The new scaffold, moderate preliminary biological activity, and crystal structure details suggested together that 2-piperonyl 3,8-diaminoimidazo[1,2-*a*]pyrazines were promising leads for Hsp90 inhibitor discovery.

Acknowledgements

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental details, crystallographic data, and copies of NMR spectra. See DOI: 10.1039/b000000x/

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11. See supporting information for details.