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# Synthesis of a 200-member library of squaric acid *N*-hydroxylamide amides

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ABSTRACT

We report here the parallel synthesis of 200 compounds based on squaric acid template. These compounds are obtained via a one-step solution-phase procedure starting from three squaric acid N-hydroxylamide esters precursors. The set of diverse reagents qualified (amines, anilines, amino-alcohols and amino-esters) makes this strategy suitable for the search of biologically active compounds. The library was screened on the zinc metalloenzyme ADAMTS-5 and hits with $IC_{50}$ in the range of 1–50 μM were identified.



The squaric template has been introduced in chemistry by the pioneering work of Cohen in 1959.[1] Since then, several examples of the use of squaric template have been reported in the fields of bioorganic and medicinal chemistry.[2] The conjugate base of squaric acid can serve as a mimic of negatively charged groups that are common in biology, including carboxylates and phosphate mono- and di-esters and of growing importance in medicinal chemistry such as hydroxamic acids. As a result, derivatives of squaric acid have been used as a replacement for these groups in medicinal applications. Squaric acid itself is an inhibitor of glyoxylase I, and semisquaric acid (3-hydroxy-3-cyclobutenedione) is an inhibitor of pyruvate dehydrogenase and transketolase.[3,4] Recently, Sekine and co-workers have used a diamide of squaric acid as a replacement for one of the phosphate diester linkages in an oligodeoxynucleotide.[5]

Our group focuses on the synthesis of acidic functions and zinc chelating groups like hydroxamates.[6,7] In that context, we investigated the synthesis of squaric acid *N*-hydroxylamide amides as acidic groups. We describe here the preparation of a library of such derivatives, based on a convergent solution-phase synthesis.

Many metalloenzyme inhibitors contain a hydroxamic acid to chelate the zinc ion present in the catalytic site of hydrolases. However with the exception of vorinostat (SAHA, suberoylanilide hydroxamic acid), most of them did not live up to expectations in the development phases. There has been considerable interest in discovering alternative groups to the hydroxamic acid.[8] Bruckner and co-workers have observed that vinylogous hydroxamic acids that are derived from squaric acids (the squaric acid *N*-hydroxylamide amide) bind iron (III) in aqueous system, without demonstrating the binding mode (Fig. 1).[9]

Inhibitors of matrix metalloproteases and analogues of SAHA bearing squaric acid- based moiety have been synthesized but they revealed to be poorly active.[10,11] In all, only few squaric acid *N*-hydroxylamines amides, mainly peptidic, have been prepared and described until now.[12]

Target compounds can be obtained from squaric acid N-substituted hydroxylamides esters. These non symmetrical building blocks are synthesized using a squaric acid diester precursor. The diester used is usually symmetrical. Indeed, the use of disymmetrical ester is not required because squarate bis-*N*-hydroxylamides could not be prepared by nucleophilic displacement of diester as reported by Bruckner[9] and Grünefeld.[12] For this study, dibutyl squarate was used as the starting point because of its good solubility in most classical organic solvents and its lower cost compared with other diesters.

Reaction of the dibutyl squarate with a series of N-substituted hydroxylamines was conducted as described in Scheme 1. Vinylo-

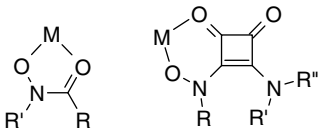


**Figure 1.** Known binding mode of hydroxamic acid and putative binding mode of squaric acid (six membered zinc chelation model) to metal ion.

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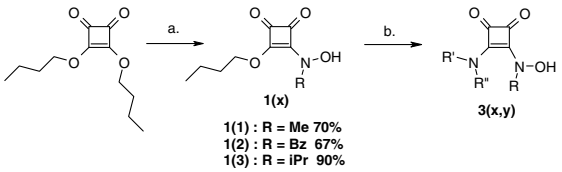



**Scheme 1.** Reagents and conditions: (a) R-NH-OH·HCl, 1.5 equiv, KOH, 1.5 equiv, MeOH, rt, 5 h; (b) amine R′R″NH 2(y) (1.1 equiv).

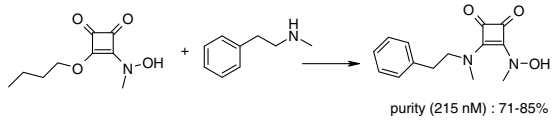
gous hydroxamic acids **1(1–3)** were obtained with good to excellent yields. On the contrary, squaric acid *N*-hydroxylamide ester (R = H) was not obtained although the substitution of the butyl ester by the hydroxylamine was complete.[13] Onaran et al. did describe the synthesis of methyl analogue from dimethylester in reasonable yield (47%) but failed to obtain final products in yields compatible with library synthesis.[14]

Compounds **1(1–3)** could then be engaged in reaction with various amines using a general solution-phase method as described in Scheme 1.

We looked for the best conditions for the synthesis of target compounds **3(x,y)** from **1(x)** and amines **2(y)** in microplates at a 15 μmol scale. Using precursor **1(1)** and *N*-methyl-phenethylamine we rationalized reported reaction conditions by testing several parameters such as solvent, final concentration, temperature and reaction time (Scheme 2).

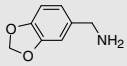
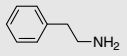
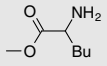
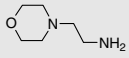
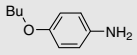
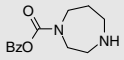
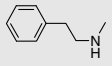
Concentration did not prove critical for synthesis and we decided to work at the lowest tested concentration to recruit the largest number of amines. Heating the reaction mixture did not improve results or even reduced purity in some cases. For ease of setting we chose to work at 20 °C. Shorter time of reaction (5 h vs overnight) slightly increased the yield. At last, effect of the solvent was not critical and we decided to select MeOH which is a better solvent than EtOH for most drug relevant amines.

Using these optimized conditions, we evaluated the scope of the reaction by reacting compounds **1(1–3)** with 8 various amines representative from different classes. Classification was based on both expected reactivity and chemical origin (Table 1). Among primary amines (classes A–F) were selected non conjugated amines (classes A–E) or anilines (class F) were selected. *N*-Alkylamines were either benzylamine derivatives (class A) that display high nucleophilicity but have a high sensitivity to oxidation and other *N*-alkylamines like phenethylamines that display medium to good nucleophilicity and a good stability (class B). Derivatives of amino-acids are represented by classes C and D (α-amino-esters and β-amino-alcohols, respectively). Other *N*-alkylamines that bear a second function (like a tertiary amine) are gathered in class E, called 'bifunctional amines'. Secondary amines are divided into two classes: cyclic amines that are very good nucleophiles (class G) and *N*-methylalkylamines (class H) that display lower nucleophilicity. As can be seen in Table 1, with squaric precursors **1(1)** and **1(2)** the reaction tolerates a broad diversity of amines, the desired product being formed quantitatively in all cases. In the case of precursor **1(3)**, only primary amines gave the desired products with good purity. Consequently for the library synthesis, only primary amines have been selected to react with this precursor. Figures 2 and 3 show the primary amines **2(1–54)** and the secondary amines **2(55–74)** selected for library synthesis.




**Scheme 2.** Reagents and conditions: solvent: MeOH or EtOH, concentration: 0.1 M or 0.25 M, temperature: 20 or 40 °C, reaction time: 5 or 18 h.

**Table 1**
Purity[a] of target compound in reaction mixture

| Class | Representative amine | Squaric precursor | | |
|---|---|---|---|---|
| | | **1(1)** | **1(2)** | **1(3)** |
| A |  | 98 | 96 | 94 |
| B |  | 87 | 94 | 92 |
| C |  | 90 | 93 | 95 |
| D |  | 98 | 98 | 99 |
| E |  | 100 | 93 | 82 |
| F |  | 98 | 97 | 93 |
| G |  | 95 | 97 | 72 |
| H |  | 85 | 93 | 72 |

[a] HPLC monitoring at 215 nM.

The library members were synthesized in polypropylene deepwell plates at a 15 μmol scale as described in Scheme 3. The squaric acid *N*-hydroxylamides esters **1(1–2)** and **1(3)** were reacted, respectively, with amines **2(1–74)** and **2(1–54)** to yield 200 squaric acid *N*-hydroxylamide amides (**3(1, 1–74), 3(2, 1–74), 3(3, 1–54)**, Scheme 3).

All compounds were characterized by LC/MS analysis (detection at 215 nm). Positive and negative electrospray (ESI+ and ESI−) MS showed the presence of a single parent ion, which confirmed the identity of the library members. Out of the 200 library members, 86% displayed purity (215 nM) above 80%. Figure 4 represents mean and standard deviation of purity in each series of compounds sorted by both the squaric precursor and the class of amines incorporated in the library. As can be seen, our conditions are very robust. Purity for all classes is excellent and only low variability is observed. When using isopropyl **1(3)** precursor, nevertheless, a greater variability in purity must be expected depending on the substituents of on the amine. To further characterize the library, 11 structurally diverse compounds were analyzed by $^{1}H$ and $^{13}C$ NMR and displayed very good spectra.

Many groups work on the systematic assessment of bioavailability for molecules early in the drug discovery cycle.[15] We calculated the physical properties that are traditionally been considered to be related to membrane permeability or bioavailability: molecular weight, log*P*, polar surface area, number of rotatable bonds and number of hydrogen bond donors and acceptors, using PipelinePilot™ from Scitegic for all members of the library.[16] All compounds pass the Rule of Five[17] and 97.5% of the library complies with Veber's criteria.[18,19]

It is often interesting to determine experimentally the key physicochemical parameters for a prototypal compound of the library.[20] The partition coefficient (log *D*) between 1-octanol and PBS buffer (pH 7.4), p$K_a$ and solubility in PBS buffer were measured for compound **3(1,22)** (Table 2). Results are compatible with drug-like properties and p$K_a$ is in the range expected for analogues of hydroxamates.

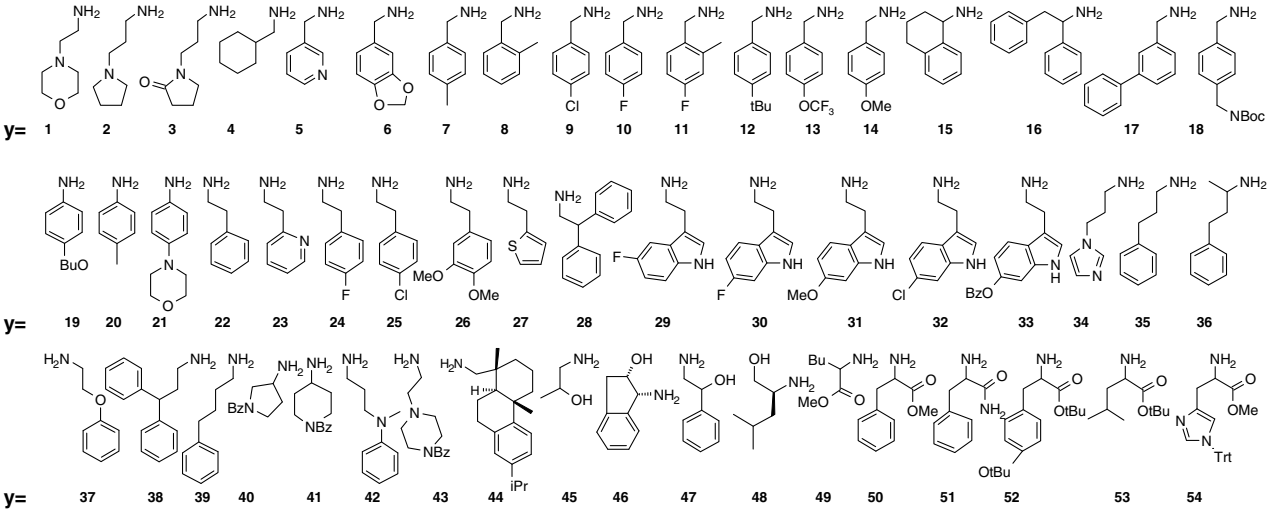



**Figure 2.** Set of primary amines **2(1–54)** for the library. Class A **2**(**5–18**); Class B (**4**,**22–39**,**44**); Class C (**49–54**); Class D (**45–48**); Class E (**1–3**,**40–43**); Class F (**19–21**).

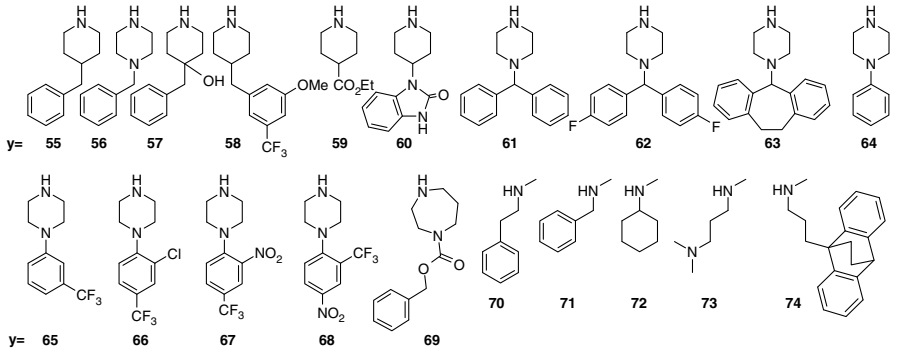
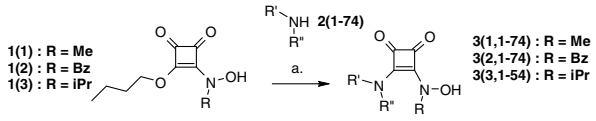



**Figure 3.** Set of secondary amines **2(55–74)** for the library. Class G **2**(**55–69**); Class H **2**(**70–74**).




**Scheme 3.** Reagents and conditions: (a) amines (1.1 equiv, 16.5 μmol), MeOH, rt, 5 h, evaporation (Genevac TM, $T$ = 30 °C).

This library was screened on ADAMTS-5,[21] a Zinc metalloprotease that degrades, like MMPs, the extracellular matrix.[22,23] Inhibitors of this enzyme could result in efficient treatments for osteoarthritis. Only a few inhibitors, mainly micromolar, with the noteworthy exception of hydroxamates and some thioxothiazolidinone, have been published so far.[24,25] The screening of our library led to the identification of two original, structurally related, ADAMTS-5 inhibitors with $IC_{50}$ in low micromolar range (respectively, 2.6 and 37 μM). $IC_{50}$ were confirmed on resynthesized and purified compounds (Table 3). Interestingly, these compounds are derived from *p*-butoxyaniline. Preferably, the substituent of the hydroxylamine should not be sterically hindered like iPr and should bear an aromatic ring. In particular, compound **3(2,19)** seems to be a good starting point for hit-to-lead optimization.

We developed a practical and efficient strategy to synthesize a library of squaric acid *N*-hydroxylamide amides. The scope of the amine set that can be used has been considerably extended relative to earlier data. Each compound was obtained in a 15 μmol scale suitable for biological screening and with very good purity and yield. Our library was screened on ADAMTS-5 and allowed the identification of a totally new micromolar inhibitor ($IC_{50}$ = 2.6 μM). Our goal is yet the optimization of this primary hit to obtain a lead with a submicromolar activity and good pharmacokinetic properties.

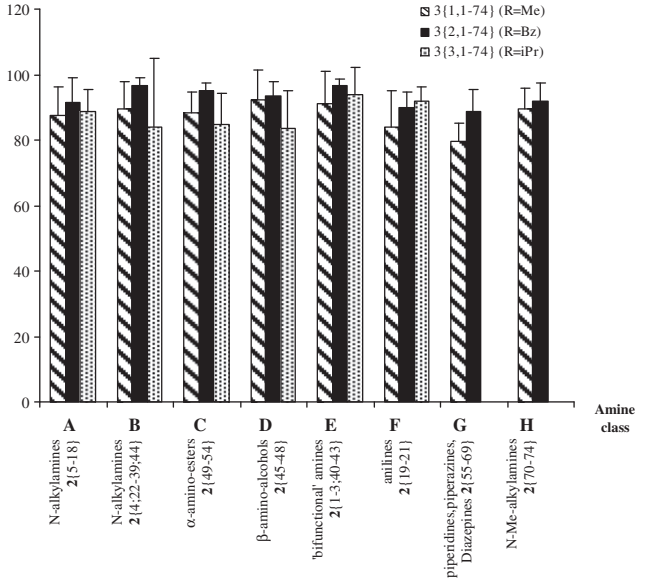
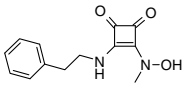


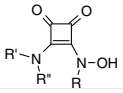

**Figure 4.** Purity of library members according to the amine input and the squaric precursor (% at 215 nm).

**Table 2**
p$K_a$, log$D$ and solubility measured for compound **3**(**1**,**22**)



| p$K_a$ | Log$D$ (pH 7.4) | Solubility (μg/mL) |
|---|---|---|
| 8.5 | 0.5 | 24 |

**Table 3**
Inhibition on ADAMTS-5



| Compound | R′R″*N*- | R= | ADAMTS-5 $IC_{50}$[a] (μM) |
|---|---|---|---|
| **3**(**1**,**19**) | *p*(Bu-O)-$C_6H_4$-NH– | $CH_3$– | 37.0 |
| **3**(**2**,**19**) | *p*(Bu-O)-$C_6H_4$-NH– | Ph-$CH_2$– | 2.6 |
| **3**(**3**,**19**) | *p*(Bu-O)-$C_6H_4$-NH– | *i*-Pr– | >100.0 |

[a] $IC_{50}$ were measured on resynthesized and purified compounds.[21]

## Acknowledgements

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.08.025.

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13. The product was completely degraded during evaporation of the solvent under reduced pressure after the purification step on silica gel. A one pot, sequential reaction was attempted. The dibutyl squarate was reacted with hydroxylamine during 1 h 30 min, the amine was then added and the reaction mixture was stirred at room temperature overnight. The very low conversion observed (<20%) was consistent with the yields (<15%) obtained by Onaran.[10,14]
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