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Iterative Oxime Bond Chemistry Leads to Protease Inhibitors

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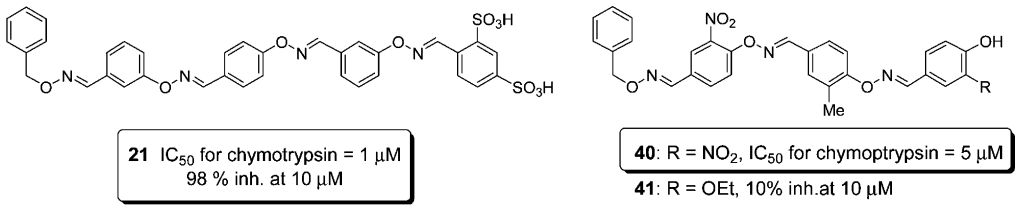
ABSTRACT



In the present paper, we have looked at iterative coupling as a strategy to form new druglike molecules. We have developed an iterative coupling chemistry based on oxime bond formation between hydroxyaromatic aldehyde building blocks to form linear oxime oligomers. The strategy is validated by the discovery of micromolar protease inhibitors.

The discovery of new bioactive organic molecules requires the exploration of molecular diversity from natural sources or by synthesis.[1] Nature very often uses peptides to bind selectively to various targets. Selectivity and potency results from the diversity created by the linear combinations of as few as 20 amino acid building blocks. This diversification strategy has been used in a number of peptide-like oligomers.[2] Such peptidomimetics include peptoids,[3] β-peptides,[4] vinylogous,[5] vinylogous sulfonyl,[6] permethylated[7] and β-sulfonyl[8] polypeptides, oligoureas,[9] oligocarbamates,[10] oligosulfones,[11] azapeptides,[12] azatides,[13] hydrazinoaza,[14] or α/β-aminooxy peptoids.[15] All of these synthetic oligomers are based on amide bond-type linkages between amino acid-like units. In principle, however, the iterative coupling strategy should also be applicable to building blocks unrelated to amino acids and linked by any type of bond amenable to iterative coupling. Herein, we report a new type of oligomer based on iterative oxime bond formation to build chains of hydroxyaromatic aldehydes. The strategy is validated by the discovery of micromolar protease inhibitors in a small oxime oligomer library.

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Oxime bond formation has been used for the assembly of synthetic proteins,[16] dendrimers,[17] glycopeptides,[18] lipopeptides,[18b] and oligonucleotide−peptide conjugates[19] and for the selective tagging of cell-surface glycoconjugates.[20] The oxime linkage is formed rapidly and quantitatively between an oxyamine and an aldehyde or ketone under mild conditions. Moreover, this linkage can be found in several drugs and is stable to chemical degradation and in vivo. We envisioned the preparation of oxime oligomers by sequential assembly of aldehyde building blocks bearing a masked or protected oxyamine functionality. Hydroxy-substituted aromatic aldehydes were selected after preliminary experiments showed that these formed only *E*-configured oxime linkages, which would allow us to obtain stereochemically homogeneous oligomers.[21] The aromatic hydroxyl group could be used as a masked oxyamine since it can be aminated by various reagents.[22]

The synthesis of oxime oligomers was realized by an iterative two-step procedure consisting in the amination of the phenolic hydroxyl group, followed by quantitative quenching of the resulting oxyamine by the next hydroxyaromatic aldehyde (Figure 1).[23] The elongated oxime product was then separated from the unreacted phenol by column chromatography and was immediately available for the next amination cycle. This procedure avoided handling of the free oxyamines, which react rapidly with any trace of carbonyl compounds such as acetone. Amination of the phenol hydroxyl group was best achieved by reacting the potassium phenolate with *O*-(mesitylenesulfonyl)-hydroxylamine (MtsONH$_2$) at 0 °C in DMF.[24] The modest amination yields (30−60%) were compensated by the quantitative oxime coupling (TLC), the overall simplicity of the elongation cycle, and the ease of product separation. Oligomers were assembled starting with *O*-benzylhydroxylamine using various 3- and 4-hydroxybenzaldehydes as building blocks (Figure 1).[25]

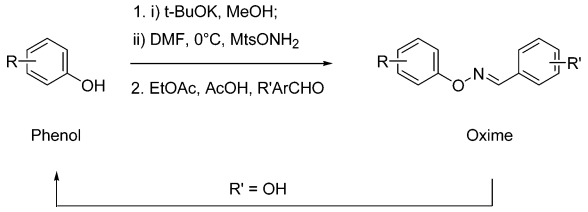

Figure 1. Synthesis of oxime oligomers by iterative O-amination/oxime bond formation. The following building blocks were used: (a) chain initiation: benzyloxyamine; (b) optional chain elongation: *m*(OH)C$_6$H$_4$CHO, *p*(OH)C$_6$H$_4$CHO, *m*(NO$_2$)*p*(OH) C$_6$H$_3$CHO, *m*(Me)*p*(OH)C$_6$H$_3$CHO, *m*(OMe)*p*(OH)C$_6$H$_3$CHO, *m* (OEt)-*p*(OH)C$_6$H$_3$CHO; (c) chain termination: *m*(Me)*m*(Me)*p*(OH) C$_6$H$_2$CHO, *m*(MeO)(MeO)*p*(OH)C$_6$H$_2$CHO, *o*(SO$_3$H)*p*(SO$_3$H) C$_6$H$_3$CHO; (d) termination with optional dimerization or addition of β-D-glucose-1-ONH$_2$: *m*(CHO)C$_6$H$_4$CHO, *p*(CHO)C$_6$H$_4$CHO.

Structural diversity was increased at the last building block by also using benzaldehyde-2,5-disulfonate and phthalic aldehydes. Phthalaldehyde-terminated oligomers were optionally functionalized with β-aminoxyglucose.[26] A series of 43 oxime oligomers spanning di-, tri-, tetra-, penta-, hexa-, and heptameric oximes was thus obtained. Structures were confirmed by NMR, MS, and in selected cases by X-ray crystallography (Figure 2).

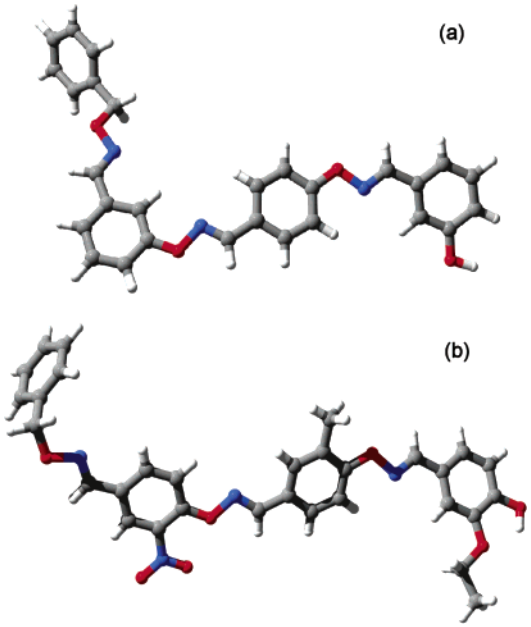

Figure 2. X-ray structure of compounds **19** (a) and **41** (b).

Due to their extended shape and abundance of aromatic functionality, we expected that our oxime oligomers might be suited to bind to proteins interacting with linear polymers,

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(23) The following procedure is typical. To a solution of **5** (0.423 g, 1.9 mmol) in methanol (5 mL) was added *t*BuOK (0.209 g, 1.9 mmol), and the solution was evaporated to dryness. The resulting solid was dissolved in DMF (4 mL), and MtsONH$_2$ (0.400 g, 1.9 mmol) was added at 0 °C. After being stirred for 30 min, the mixture was taken up with ethyl acetate (50 mL), washed several times with water, and dried over Na$_2$SO$_4$. A few drops of acetic acid and 3-hydroxybenzaldehyde (0.227 g, 1.86 mmol) were added to the organic layer, and then the solvent was evaporated under vacuum. Flash chromatography on silica gel (hexane/ethyl acetate 7: 3) gave **8** as a colorless oil (0.325 g, 51%).

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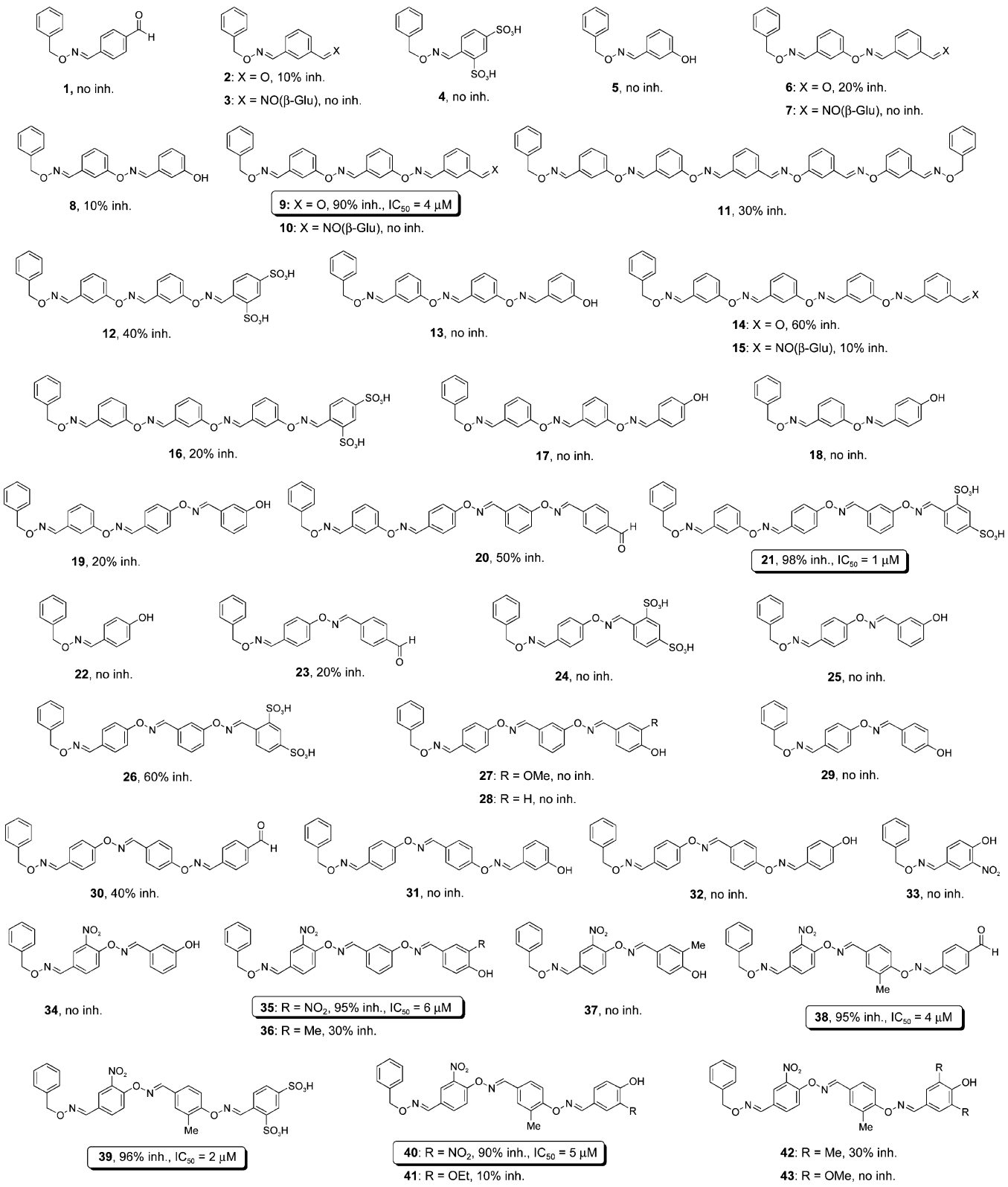

Figure 3. Oxime oligomers tested for inhibition. Inhibition data of proteolytic activity for α-chymotrypsin in the presence of 10 μM inhibitor are given for each oligomer as well as IC_{50} values of compounds with more than 90% inhibition.

in particular proteases, which possess channel-like binding pockets interacting with several residues on each side of the scissile peptide bond of their substrates.[27] Protease inhibition is relevant to the treatment of human diseases such as viral infections and cancers.[28] Six different proteases were assayed for inhibition of proteolysis using the copper−calcein

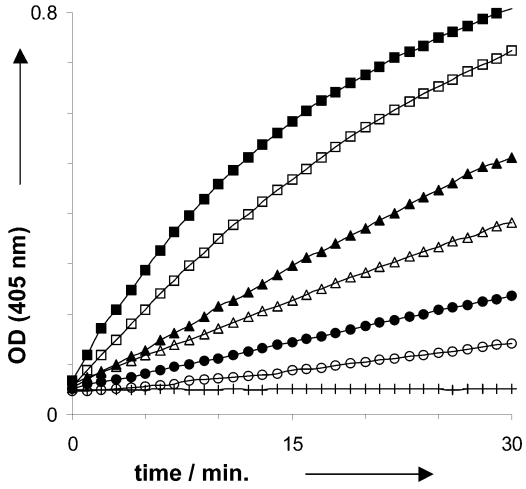

Figure 4. Inhibition of α-chymotrypsin by oxime oligomer **38**. Assay conditions: 5 μg·mL^{-1} α-chymotrypsin (from bovine pancreas, Sigma C-7762), 500 μM *N*-succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide (Sigma S-7388), 5 mM aq Bis Tris, pH 7.2, 26 °C, and inhibitor **38** at (■) 0 μM, (□) 2 μM, (▲) 4 μM, (△) 6 μM, (●) 8 μM, (○) 10 μM, (+) no enzyme.

fluorescent sensor system and bovine serum albumin as substrate.[29] Oxime oligomers and their building blocks were tested in triplicate at 10 μM concentration in the presence of 8% v/v DMSO as cosolvent, which was sufficient to ensure good solubility. While there was no effect on most enzymes (subtilisin, elatase, thermolysin, trypsin, papain), α-chymotrypsin was inhibited by several of the longer oxime oligomers. α-chymotrypsin inhibition was confirmed using purified enzyme and *N*-Succinyl-Ala-Ala-Pro-Phe *p*-nitroanilide[30] as a chromogenic substrate. Each inhibitor was also resynthesized. According to the nitroanilide assay, six inhibitors exhibited protease inhibition with IC_{50} values in the low micromolar range (Figures 3 and 4).

The inhibition observed is unlikely to be of a nonspecific type induced by aggregation.[31] Indeed inhibition was observed only with chymotrypsin and no other protease, and in the presence of excess BSA as substrate. In addition, no inhibition was observed with any of the compounds on β-glucosidase, an unrelated enzyme.

The observation of protease inhibition by tetramers (**9**, **35**, **38**, **39**, and **40**) or pentamer (**21**) suggests that activity is caused by multiple interactions. The terminal disulfonate substituent primarily enhances aqueous solubility, but does not seem to be directly responsible for activity. For example, the sulfonate-terminated pentameric oligomer **21** is a micromolar inhibitor, but its close analogue **16** is not, although it only differs by the meta- versus para-substitution at the third building block. The 3-nitro-4-hydroxybenzaldehyde and 3-methyl-4-hydroxybenzaldehyde building blocks in position two and three are found in three (**38**, **39**, **40**) of the six active compounds.

The iterative coupling procedure leading to oxime oligomers is remarkably simple since it does not require any protecting group operations. The suitability of the resulting oligomers for bioactivity is highlighted by the fact that active compounds were discovered using only a small subset of all compounds accessible by exhaustive combination of the building blocks (43 out of over 600 possible oligomers). The building blocks used here were those commercially available. Structural diversity can be increased by including 3-OH and 4-OH benzaldehydes bearing one additional substituent at position 2, 5, 6, and 3 or 4. This substituent may be a halogen, nitro, or an alkyl group of various size and substitution, securing a broad structural diversity. Nucleophilic groups such as unprotected amines and pyridines should however be avoided since these would react with the aminating reagent used for oxyamination.

In summary, the first oxime oligomers were assembled from readily available aromatic hydroxyaldehydes building blocks by an iterative two-step oxime bond formation protocol. A small oxime oligomer library provided micromolar inhibitors of the serine protease α-chymotrypsin, demonstrating the potential of these compounds as drug-like molecules. By contrast to amides, oximes are only hydrogen-bond acceptors but not hydrogen-bond donors. This might help to prevent self-aggregation and thus favor target binding. Synthesis optimization toward a solid-phase supported protocol and structural investigation of the enzyme−inhibitor complexes are currently underway.

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Supporting Information Available: Spectroscopic data of active compounds **9**, **21**, **35** and **38**−**40**, protease assay conditions, and basic crystallographic details of oligomers **19** and **41** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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