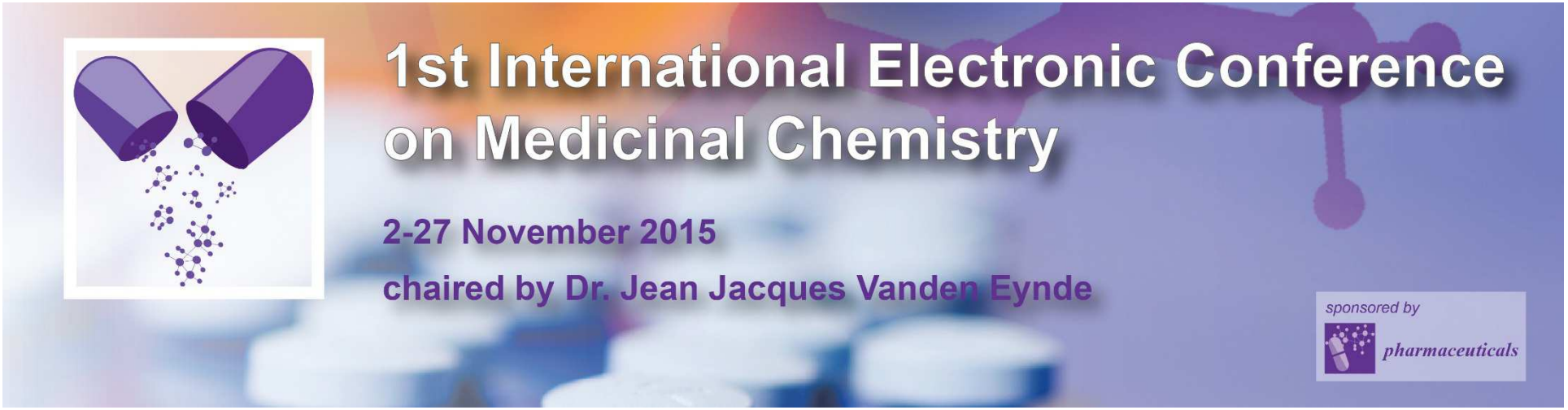

Design, Synthesis and Biological Activity of Selective PHEX Inhibitors

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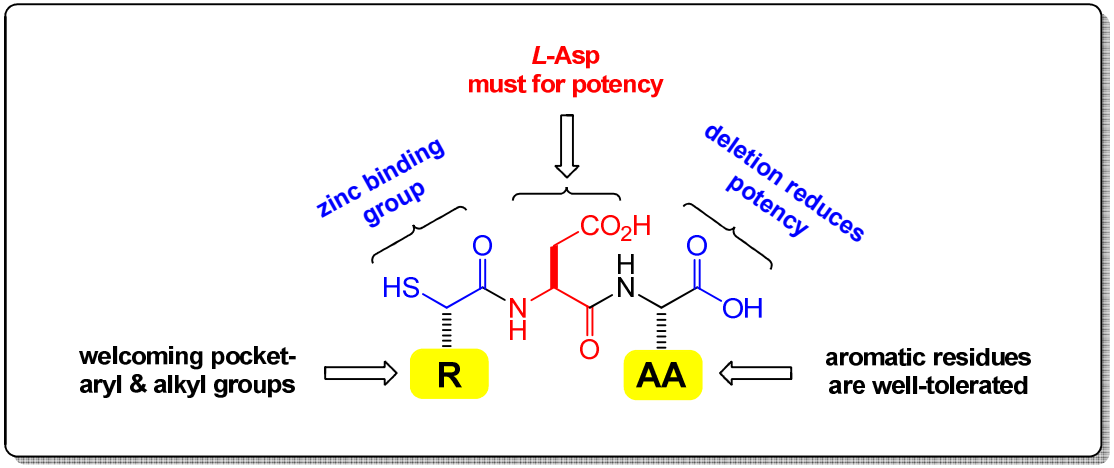
Graphical Abstract



Abstract: Here we report on the design, synthesis, and *in vitro* biological activity of mercaptoacyl dipeptide-based inhibitors of PHEX. A parallel solid phase peptide synthesis approach was used for producing focused compound libraries resulting in single digit nanomolar PHEX inhibitors. Structure activity relationships studies revealed that the P1' aspartic acid residue is critical and its deletion or modification lead to a large decrease in activity. The stereochemistry of the aspartic acid residue at P1' is also important. Replacing L-aspartic acid with its D enantiomer led to about a seven fold loss of potency. We explored multiple sites of diversity around the central aspartic acid and these results are also reported. In assessing selectivity for PHEX versus NEP, all the derivatives tested were highly selective for PHEX. Such compounds may have potential usage in regulating bone mineralization and/or as osteogenic agents.

Keywords: Osteogenesis; bone mineralization; peptide synthesis; PHEX inhibitors

Introduction

- **Osteogenesis is a complex biological process that includes:**
  - ✓ **proliferation and differentiation of bone-forming cells (osteoblasts),**
  - ✓ **synthesis of an organic matrix composed mainly of type I collagen, and**
  - ✓ **mineralization of the organic matrix by deposition of hydroxyapatite crystals.**
- **X-linked hypophosphatemic rickets (XLH), a human genetic disease, results from mutated PHEX gene.**
- **XLH is characterized by under mineralization of the bone extracellular matrix.**

Introduction

- **Several experimental observations support a role for PHEX in mineralization**:
  - ✓ **PHEX mRNA was detected in bones by Northern blot hybridization.**
  - ✓ **A soluble form of PHEX inhibits mineralization of rat calvaria osteoblast cultures.**
- **Thus, PHEX is involved**
- **Decreasing PHEX peptidase activity with specific inhibitors might therefore potentiate the mineralization process**

Results and discussion

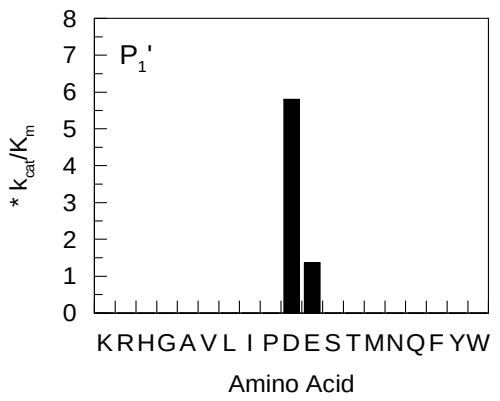
Subsite Specificity of PHEX:

- PHEX shows a very restricted specificity for its S'_1 and S'_2
- Several putative PHEX substrates (approximately 15 different peptides) were tested but only $PTHrp_{107-139}$ was cleaved.
- $PTHrp_{107-139}$ cleavage:

T R S A W L D S G V T G S G L E G D H L S D T S T T S L E L D S R

- Strong specificity of the S'_1 pocket for D (aspartic acid).

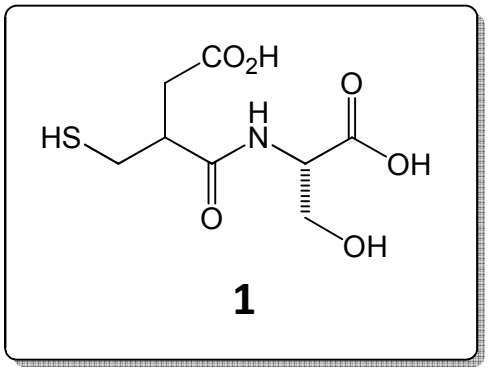
Specificity of the S'1 pocket of PHEX



Results and discussion

Design and Synthesis of PHEX Inhibitors: 1st generation

- Design and prepare a lead based on the above PHEX substrate results
- Prepare derivative 1 : it contains the P'1 aspartic acid side chain mimic, the thiol zinc-binding group, and serine in P'2
- 1 inhibited PHEX at 1 μM
- Prepare a compound library with a whole host of amino acids at P'2
- Activities ranged from low μM to sub μM

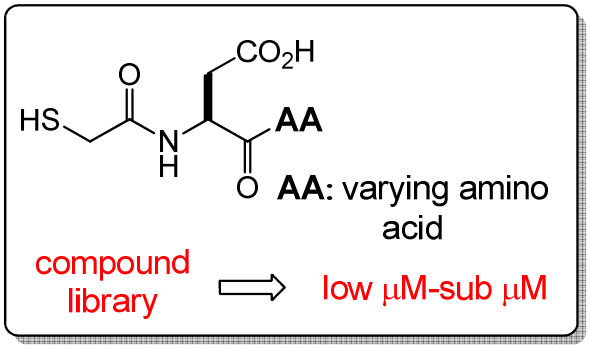


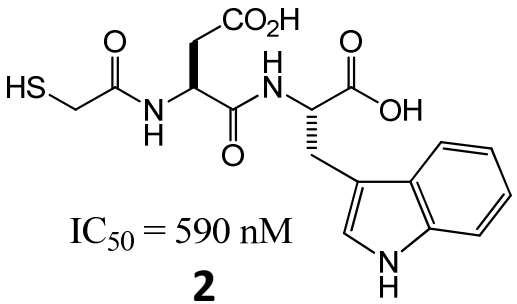
1

Results and discussion

Design and Synthesis of PHEX Inhibitors: 2nd generation

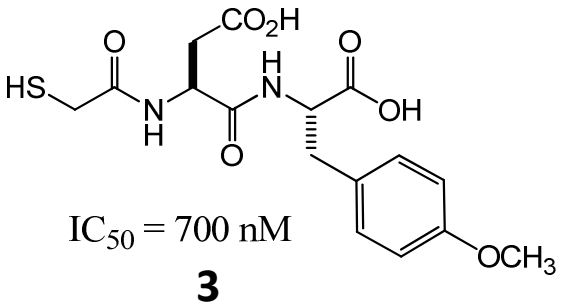
- **Explore the mercaptoacyl moiety as the zinc- binding group**





IC_{50} = 590 nM

2



IC_{50} = 700 nM

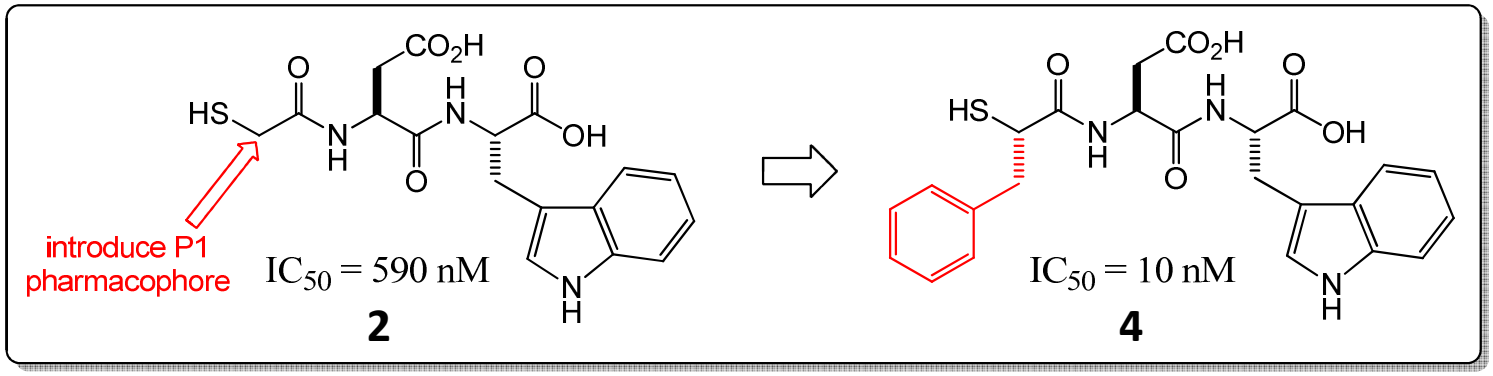
3

- **Prepare a compound library to explore P'1 (example: compounds 2 & 3)**
- **Activity still at the sub μM level**

Results and discussion

Design and Synthesis of PHEX Inhibitors: 2nd generation

- Can we gain additional binding by introducing a ligand in S1 pocket?
- Breakthrough: 60 fold increase in activity (compare compounds 2 and 4)



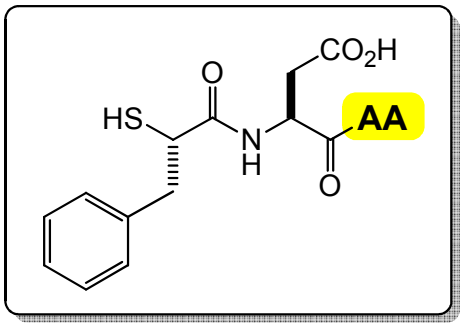
- Gained additional binding by the P1 pharmacophore

Results and discussion

Design and Synthesis of PHEX Inhibitors: 2nd generation

- **Optimize the P'1 site in presence of the P1 pharmacophore**

Compound	AA	IC_{50} (nM)
4	Trp	10
5	Phe	70
6	Bi-Phe	38
7	2-naphthyl-Ala	6
8	Tyr	72
9	Ser	170
10	Pro	>100000
11	Phenyl alaninol	90
12	Trptamine	140
13	Biphenyl methylamine	230



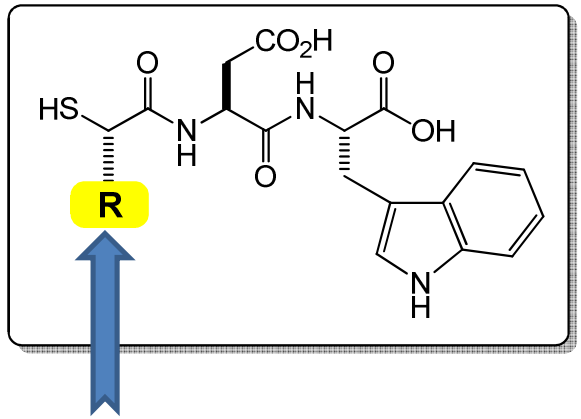
- Aromatic amino acids are well-tolerated (compounds 4-7)
- Removal of terminus CO_2H reduces potency (compounds 12 & 13)
- Proline eliminates activity (compound 10) Suggesting importance of H-bonding and/or conformational constraints

Results and discussion

Design and Synthesis of PHEX Inhibitors: 2nd generation

- **Optimize the P1 pharmacophore**

Compound	R	IC_{50} (nM)
4	Benzyl	10
14	4-Methoxybenzyl	20
15	4-F-Benzyl	6
16	Phenyl	2
17	2-Naphthylmethyl	15
18	2-Indolylmethyl	23
19	Biphenmethyl	8
20	4-Benzoloxybenzyl	8
21	Phenethyl	4
22	Methyl	68
23	Isopropyl	6
24	Isobutyl	94
25	n-Butyl	7
26	Cyclohexylmethyl	20



very welcoming pocket

Conclusions

- **Potent and selective inhibitors were rationally designed and synthesized for the first time.**
- **The P1 pharmacophore is a major contributor to the activity of the inhibitors of PHEX with clear preference to aromatic and alkyl substituents having the *S* stereochemistry.**
- **The P'1 sub-site is very restrictive with high specificity for *L*-aspartic acid.**
- **The P'2 sub-site shows clear preference for aromatic moieties and modification or deletion of the carboxyl terminus at this site leads to loss in potency.**

Acknowledgments

$$$$$

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