Electronic Supplementary Material (ESI) for RSC Advances.

Screening of β-hairpin peptide-engrafted 1,2,3-triazoles to identify APEH enzyme inhibitors

A. Sandomenico, V. Celentano, L. D. D'Andrea, G. Palmieri, M. Ruvo,

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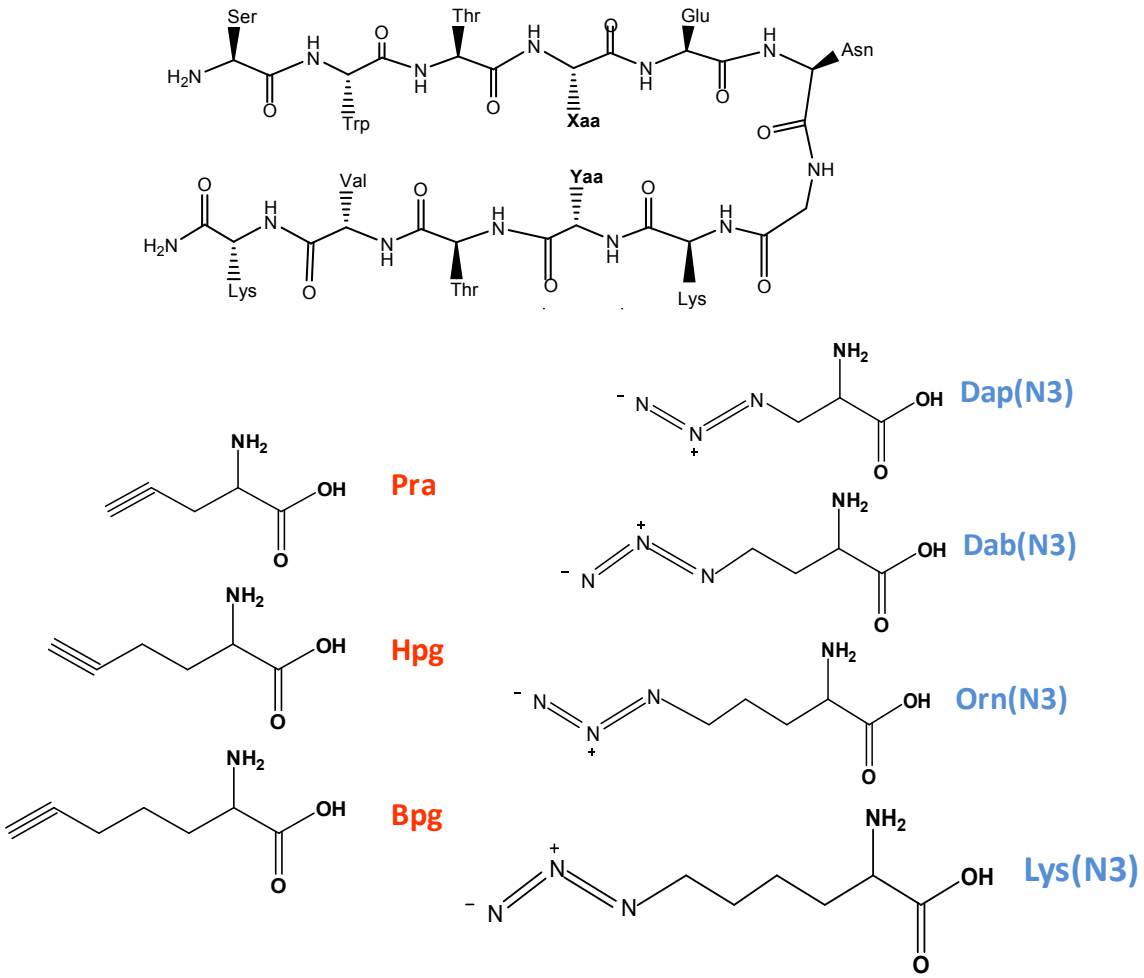
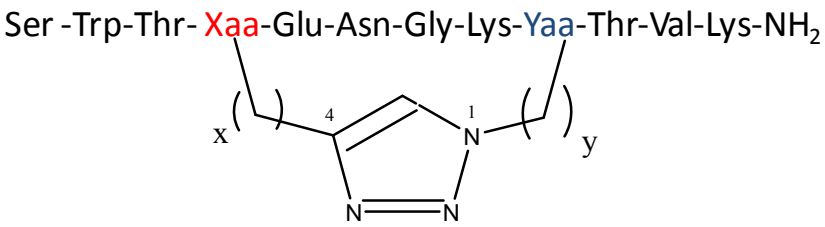


Figure S1: Molecular structure of the NHB scaffold and unnatural amino acids used. Top: Backbone of the NHB scaffold with the indication of the identity of the residue. Residues involved in a the click reaction (Xaa and Yaa) are in highlighted bold. Bottom: Molecular structure of the Xaa and Yaa amino acids.

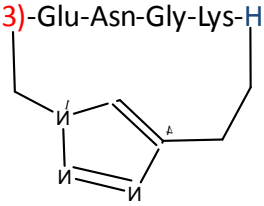


N°	Name	Xaa	Yaa	x	y
1	NHB 1.1	Pra	Dap(N3)	1	1
2	NHB 1.2	Pra	Dab(N3)	1	2
3	NHB 1.3	Pra	Orn(N3)	1	3
4	NHB 1.4	Pra	Lys(N3)	1	4
5	NHB 2.1	Hpg	Dap(N3)	2	1
6	NHB 2.2	Hpg	Dab(N3)	2	2
7	NHB 2.3	Hpg	Orn(N3)	2	3
8	NHB 2.4	Hpg	Lys(N3)	2	4
9	NHB 3.1	Bpg	Dap(N3)	3	1
10	NHB 3.2	Bpg	Dab(N3)	3	2
11	NHB 3.3	Bpg	Orn(N3)	3	3
12	NHB 3.4	Bpg	Lys(N3)	3	4

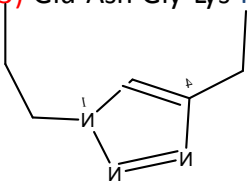
Figure S2: General formulas for NHB peptidomimetic #1 - #12. Top: General sequence bearing different substitutions on positions 4 and 9. Xaa and Yaa are unnatural amino acids possessing respectively alkyno and azide group. The identity of the Xaa and Yaa amino acids and the nomenclature for the peptidomimetics are reported in the table.

N°	Name	Xaa	Yaa	x	y
13	NHB 1.2 rev	Dap(N3)	Hpg	1	2
14	NHB 2.1.rev	Dab(N3)	Pra	2	1
15	NHB 2.1 W3	Hpg	Dap(N3)	2	1
16	NHB 3.3_C	Bpg	Orn(N3)	3	3
17	NHB 3.3_N	Bpg	Orn(N3)	3	3
18	NHB 3.3_CN	Bpg	Orn(N3)	3	3
19	NHB 3.3 Δ turn	Bpg	Orn(N3)	3	3
20	NHB 3.3 open	Bpg	Orn(N3)	3	3

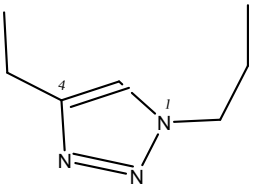
N°13 : NHB 1-2rev Ser -Trp-Thr- Dap(N3)-Glu-Asn-Gly-Lys-Hpg-Thr-Val-Lys-NH_2



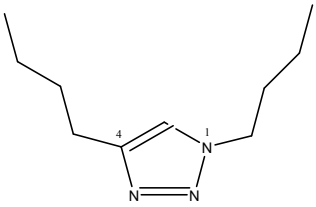
N°14 : NHB 2-1rev Ser -Trp-Thr- Dab(N3)-Glu-Asn-Gly-Lys-Pra-Thr-Val-Lys-NH_2



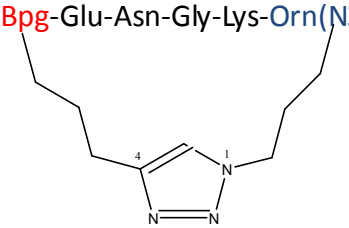
N°15 : NHB 2-1 W3 Ser -Thr-Trp- Pra-Glu-Asn-Gly-Lys-Dab(N3)-Thr-Val-Lys-NH_2



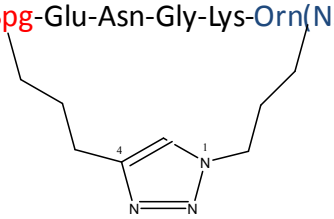
N°16 : NHB 3-3 _C Ser -Trp-Thr- Bpg-Glu-Asn-Gly-Lys-Orn(N3)-NH_2



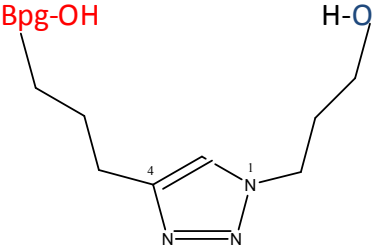
N°17 : NHB 3-3_N Trp-Bpg-Glu-Asn-Gly-Lys-Orn(N3)-Thr-Val-Lys-NH_2



N°18 : NHB 3-3_CN Trp- Bpg-Glu-Asn-Gly-Lys-Orn(N3)-NH_2



N°19 : NHB 3-3 Δ turn Ser -Trp-Thr- Bpg-OH H-Orn(N3)-Thr-Val-Lys-NH_2



N°20 : NHB 3-3 open Ser-Trp-Thr-Bpg-Glu-Asn-Gly-Lys-COOH H_2N-Orn(N3)-Thr-Val-Lys-NH_2

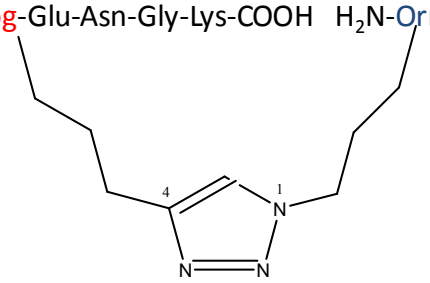


Figure S3: Molecular structures of NHB peptidomimetic #13 - #20. Top: The identity of the Xaa and Yaa amino acids and the nomenclature for the peptidomimetics are reported in the table. Bottom: Molecular structure of NHB peptidomimetics #13 - #20. Xaa and Yaa amino acids are highlighted in red and blue respectively.

Table S1: Analytical characterization of NHB linear and cyclized peptidomimetics.

N°	Name	Rt precursors (min.)	Rt clicked compound (min.)	HPLC method[a]	M.W. $_{Calc}$ (amu)[c]	$[M+H]^{+}{}_{Exp.}$ (amu)[c]
1	NHB 1.1	13.84	13.42	A	1354.4	1355.7
2	NHB 1.2	14.22	13.06	A	1368.5	1369.7
3	NHB 1.3	19.18	15.93	A	1382.5	1383.7
4	NHB 1.4	14.69	13.5	A	1396.5	1397.5
5	NHB 2.1	14.9	13.26	A	1368.4	1369.7
6	NHB 2.2	14.37	11.11	A	1382.5	1383.9
7	NHB 2.3	12.58	12.02	A	1396.5	1397.8
8	NHB 2.4	15.09	13.34	A	1410.5	1411.5
9	NHB 3.1	17.05	13.39	A	1382.5	1383.8
10	NHB 3.2	14.79	11.07	A	1396.6	1397.8
11	NHB 3.3	14.9	13.22	A	1410.6	1411.7
12	NHB 3.4	15.51	13.55	A	1424.4	1425.4
13	NHB 1.2 rev	12.69	5.5	B	1368.5	1368.7
14	NHB 2.1.rev	14.18	13.9	A	1368.4	1369.7
15	NHB 2.1 W3	14.12	12.04	A	1368.4	1369.7
16	NHB 3.3_C	17.69	15.01	C	1082.2	1083.3
17	NHB 3.3_N	16.87	14.45	C	1222.4	1223.6
18	NHB 3.3_CN	17.09	14.07	C	894.0	895.4
19	NHB 3.3 Δ turn	----	14.5	D	999.1	1000.6
20	NHB 3.3 open[b]	14.9	13.35	A	1429.6	1430.7

[a]All analytical RP-HPLC method consist of a linear gradients of CH3CN/TFA 0.1% (solvent A) in H2O/TFA 0.1% (solvent B). Method A: solvent A from 5% to 70% in 20 min; Method B: solvent A from 15% to 30% in 20 min; Method C: solvent A from 5% to 50% in 20 min; Method D: solvent A from 5% to 70% in 20 min.

[b]NHB 3.3 open was obtained by trypsin digestion of clicked NHB3.3. The Rt of the precursor is the same as NHB3.3.

[c]Calculated and experimental masses refer to the cyclic peptidomimetics.