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Convergent Synthesis of Potent Peptide Inhibitors of the Grb2-SH2 Domain by Palladium Catalyzed Coupling of a Terminal Alkyne

Joseph Schoepfer,* Brigitte Gay, Nicole End, Evelyne Muller, Gisela Scheffel, Giorgio Caravatti and Pascal Furet

Oncology Research, NOVARTIS Pharma AG, 4002 Basel, Switzerland

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Abstract—A new strategy was developed to prepare in a very efficient and convergent manner C-terminal modified tripeptides with high affinities for the Grb2-SH2 domain. Using $Pd(PPh_3)_2Cl_2$ as catalyst, selected naphthyl iodides and triflates were coupled to Ac-Pmp(*t*-Bu)$_2$-Ac$_6$c-Asn-NH(prop-2-ynyl). The resulting alkyne derivatives were hydrogenated and deprotected to afford potent Grb2-SH2 inhibitors. © 2001 Elsevier Science Ltd. All rights reserved.

The signal transduction pathways of tyrosine kinase growth factor receptors offer different possibilities of intervention in anticancer drug research. Besides targeting the kinase enzymatic activity of these receptors,[1,2] inhibiting events downstream in the signalling cascade constitutes another approach of potential interest in the search for new antitumor agents. A possible strategy in this direction is to block the interaction between the phosphotyrosine-containing activated receptors and the Src homology **2** (SH2) domain of the growth factor receptor-bound protein 2. Recent papers reported significant advances in the use of SH2 inhibitors in cancer therapy[3–5] and osteoporosis.[6] Phosphonopeptide **1**, the corresponding prodrug,[3,4] and the peptide **2** containing a malonic acid isostere of pTyr[5] showed encouraging cellular activities (Fig. 1). Furthermore, combining Src-SH2 inhibition and the bone targeting properties of phosphonates, compound **3** displayed in vivo anti-resorptive activity.[6]

The X-ray structure of the Grb2-SH2 domain in complex with a specific phosphopeptide ligand[7] has revealed an extended hydrophobic area adjacent to the primary binding site. Therefore, hydrophobic residues were added to the terminal Asn of the minimal peptide sequence Ac-pTyr-X_{+1}-Asn-NH_2 recognised by the Grb2-SH2 domain.[8,9] This modification resulted in phosphopeptides with affinity and selectivity.

Continuing along this line, we have designed, synthesized, and tested C-terminal modified tripeptides [general structure Ac-Pmp-Ac$_6$c-Asn-NH-(3-naphthalenyl-propyl)] **4**. These compounds contain 4-(phosphonomethyl)phenylalanine (Pmp) as a stable pTyr

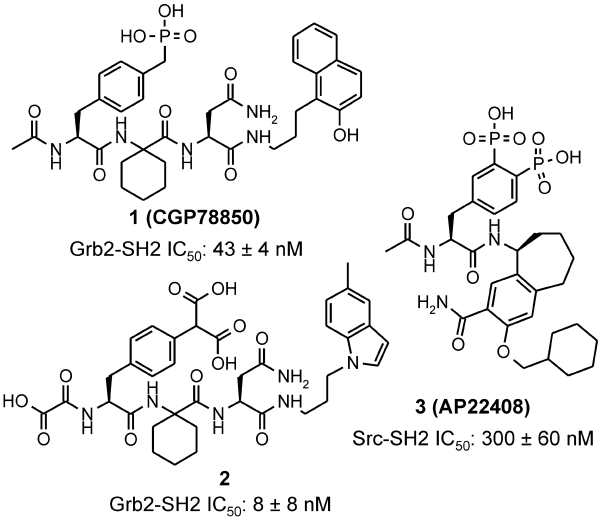

Figure 1. Examples of biologically profiled SH2 inhibitors.

*Corresponding author. Tel: +41-61-696-36-09; fax: +41-61-696-62-46; e-mail: joseph.schoepfer@pharma.novartis.com

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replacement and maintain high affinities for the Grb2-SH2 domain.

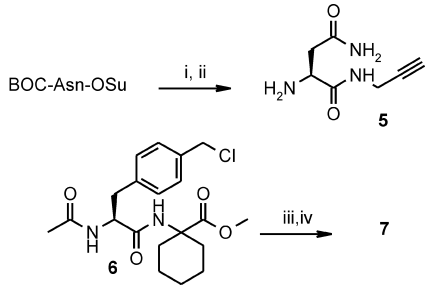
Chemistry

A new strategy was developed to synthesize naphthalenyl peptides in a convergent and efficient way.

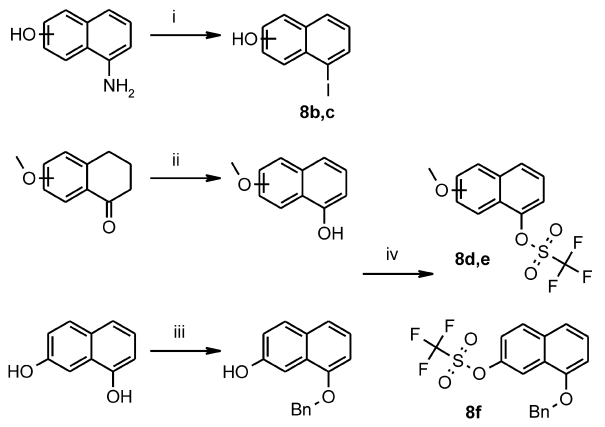
Starting from commercial BOC-Asn-OSu, the suitable alkyne amino acid **5** was obtained by reaction with propargylamine and subsequent TFA deprotection (Scheme 1). From the chloromethyl derivative **6**,[10] reaction with deprotonated di-*tert*-butylphosphite and coupling of the resulting free acid with **5** afforded the common intermediate **7**.

The iodonaphthols **8b**,**c** were obtained by diazotation[11] of the commercially available aminonaphthols. The triflates **8d**,**e** were obtained by aromatization of the corresponding methoxy-tetralone with Pd(C) in either triisopropylbenzenze[12] or β-methylstyrene,[13] followed by treatment with triflic anhydride in pyridine (Scheme 2).

Triflate **8f** was prepared the same way from 8-benzyloxy-naphthalen-2-ol, the major isomer obtained after alkylation of naphthalene-1,7-diol and chromatographic separation.



Scheme 1. (i) Propargylamine, DMF, rt; (ii) TFA, 0 °C; (iii) NaH, $HOP(Ot\text{-}Bu)_2$, DMF, rt; (iv) EDC, HOBT, NMM, **5**, DMF, rt.



Scheme 2. (i) $NaNO_2$, HCl, KI, H_2SO_4, THF/H_2O; (ii) for precursor of **8d**: Pd(C), triisopropylbenzene, 240 °C; for precursor of **8e**: Pd(C), β-methylstyrene, 250 °C; (iii) benzylbromide, Cs_2CO_3, dioxan reflux; (iv) Tf_2O, pyridine, 0 °C.

The terminal alkyne of **7** was then coupled with the different naphthyl iodides or triflate using Sonogashira[14] reaction conditions: $Pd(PPh_3)_2Cl_2$, copper iodide, and triethylamine in DMF; in the presence of tetrabutylammonium iodide[15] (Scheme 3). The alkyne derivative was then hydrogenated, deprotected and purified with reverse phase medium pressure liquid chromatography.

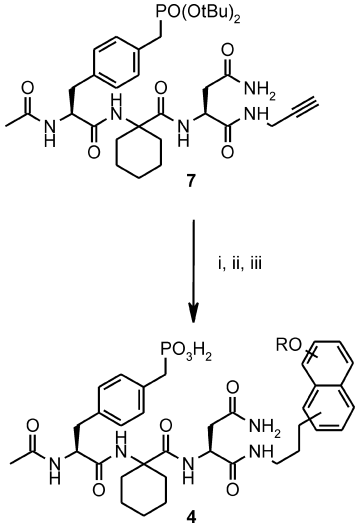
Results and Discussion

Earlier results have already shown that targeting the hydrophobic patch was a successful strategy.[8,10] Introduction at the C-terminus of 3-naphthalenyl-propyl substituents, whose size and shape allow to bury a significant portion of the surface of the hydrophobic patch, led to high affinity peptides (Table 1). The introduction of hydroxyl and methoxy groups, in order to have electron-rich naphthols[16] afforded in the case of **4f** a compound with a significantly lower IC_{50} value than **4a**.

The gain in activity obtained with **4f** can be ascribed to a favorable conformational restriction effect. Modeling suggests the possibility of an intramolecular hydrogen bond between the hydroxyl group of the naphthol and the C-terminal carbonyl of the compound. This internal hydrogen bond preorganizes the naphthol moiety in the conformation giving the most extended surface of contact with the hydrophobic patch, and thus burying the largest amount of surface (Fig. 2).

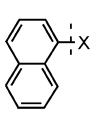
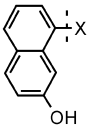
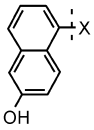
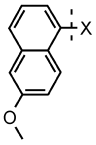
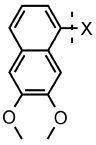
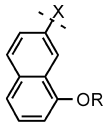
Conclusions

Structure base design in conjunction with a new synthetic strategy to incorporate C-terminal groups has allowed us to improve the Grb2-SH2 affinity of the Ac-Pmp-Ac_6c-Asn-NH-(3-naphthalenyl-propyl) compound class. Peptide **7** with a terminal alkyne group can be used for further exploration of the Grb2-SH2 hydrophobic patch.



Scheme 3. (i) **8**, $Pd(PPh_3)_2Cl_2$, CuI, TBAI, NEt_3, DMF, rt; (ii) Pd(C), H_2, MeOH; (iii) TFA, 0 °C.

Table 1. Starting naphthalenes and IC_{50}'s from Grb2-SH2 ELISA binding assay[9]

						
Compound	X = I **8a**	X = I **8b**	X = I **8c**	X = OTf **8d**	X = OTf **8e**	X = OTf, R = Bn **8f**
	X = Peptide					R = H
Compound	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
IC_{50} [nM]	42.9 ±2.0	24.6 ±2.9	42.7 ±2.9	129.9 ±5.9	84.4 ±5.0	11.1 ±0..4

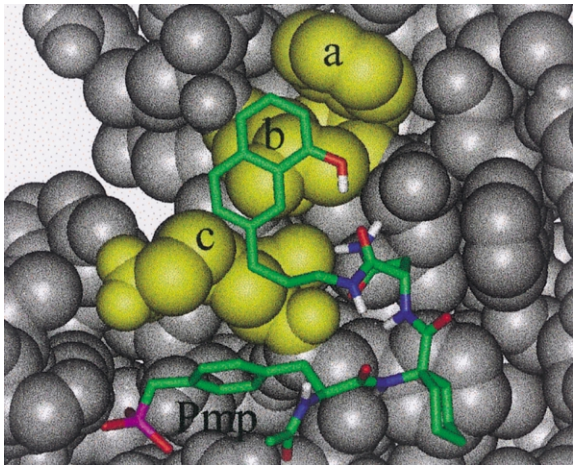


Figure 2. Compound **4f** minimized in the Grb2-SH2 binding site. Residues of the hydrophobic patch: a = Phe βE3, b = Leu βD′1, c = Lys βD6.

Acknowledgements

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