Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 2865–2870

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Structure-Based Design of Peptidomimetic Ligands of the Grb2-SH2 Domain

Joseph Schoepfer, Brigitte Gay, Giorgio Caravatti, Carlos Garcia-Echeverria, Heinz Fretz, Joseph Rahuel and Pascal Furet*

Novartis Pharma Inc., Oncology Research Department, 4002 Basle, Switzerland

Received 8 July 1998; accepted 3 September 1998

Abstracts: We have designed and synthesized a (3-aminomethyl-phenyl)-urea scaffold to mimic the X_{+1}-Asn part of the minimal phosphopeptide sequence, Ac-pTyr-X_{+1}-Asn-NH2, recognized by the Grb2-SH2 domain. The resulting compounds show the same degree of affinity as their peptide counterparts for the Grb2-SH2 domain. This is the first example reported to date of ligands of the Grb2-SH2 domain with substantially reduced peptidic character.

Introduction: 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 signaling cascade constitutes another approach of potential interest in the search for new antitumor agents.[3,4] Our current strategy in this direction is to block the interaction between the phosphotyrosine (pTyr) containing-activated receptors and the Src homology 2 (SH2) domain of the growth factor receptor-bound protein 2 (Grb2). This strategy which targets a key component of the Ras activation pathway is conceptually attractive.[5,6] Thus, we have initiated a medicinal chemistry project along this line starting from the minimal peptide sequence Ac-pTyr-X_{+1}-Asn-NH2[7] recognized by the Grb2-SH2 domain.[8,9] The main objective for chemistry in the project is the identification of low molecular weight compounds with reduced peptidic character that can efficiently disrupt these protein-protein interactions. As part of this effort, we report here the design, synthesis and binding affinities of peptidomimetics containing a (3-aminomethyl-phenyl)-urea moiety as a substitute for the X_{+1}-Asn part of the minimal sequence.

E-mail: pascal.furet@pharma.novartis.com Fax: +41 61 696 62 46

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00513-7

Design/Modeling: The recent determination in our group of the X-ray crystal structure of the Grb2-SH2 domain in complex with the phosphopeptide Lys-Pro-Phe-pTyr-Val-Asn-Val-NH2 has revealed the structural basis of ligand recognition by this SH2 domain, offering opportunities for structure-based design.[10] Key elements for recognition are the phosphotyrosine and asparagine residues of the ligand whose side chains are seen to make multiple electrostatic and hydrogen bond interactions with the protein in the X-ray structure. In particular, the side chain carboxamide group of the asparagine expresses its full hydrogen bonding potential by making 3 hydrogen bonds with residues of the protein. Additional ligand-protein hydrogen bonds involve the backbone carbonyl of the X_{-1} residue and the backbone NH of the X_{+1} residue. In contrast, the peptide bond between the X_{+1} and asparagine amino acids does not interact with the SH2 domain. Therefore, the idea of replacing these two contiguous residues by a peptidomimetic moiety in the minimal sequence Ac-pTyr-X_{+1}-Asn-NH2 appeared to be attractive.

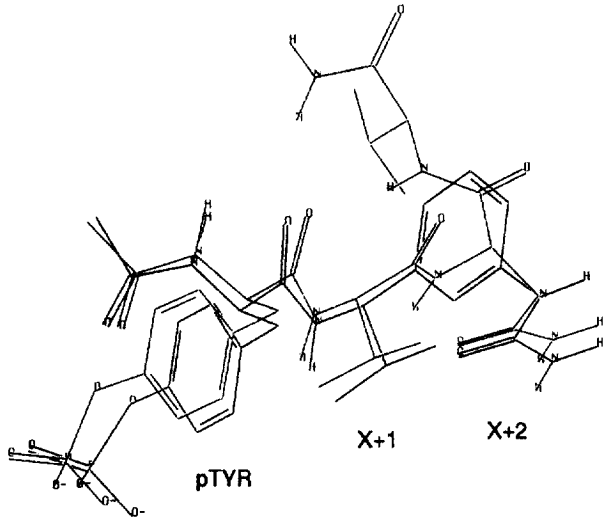
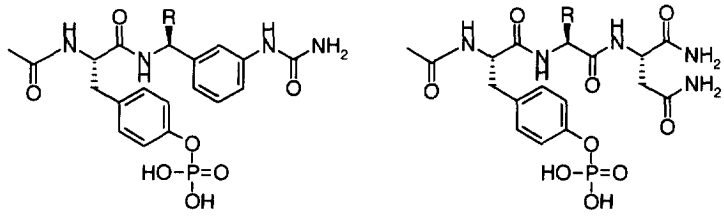


Figure 1. Overlaying of designed peptidomimetic **1b** (blue) with the X-ray structure phosphopeptide ligand (red). For clarity, the residues N-terminal to the phosphotyrosine are not represented. The model for **1b** comes from a fully minimized ligand-protein complex.

Interactive design in MacroModel[11] in conjunction with energy minimization using the AMBER force field,[12] led to the identification of (3-aminomethyl-phenyl)-urea as an adequate, geometric as well as conformational, mimetic of the X_{+1}-Asn sequence bound to the Grb2-SH2 domain (Figure 1). In this moiety, the urea function mimics the side chain carboxamide of Asn while the benzyl group acts as a spacer connecting this crucial pharmacophore element to the amino group corresponding to the backbone NH of residue X_{+1} in the phosphopeptide sequence. In addition, the benzylic position offered possibilities to

introduce substituents mimicking the side chain of residue X_{+1}. The X-ray structure of the phosphopeptide-SH2 domain complex shows that phosphotyrosine, residue X_{+1} and Asn occupy the i, i_{+1} and i_{+2} positions of a type I β-turn respectively.[13] Modeling clearly demonstrated that molecules resulting from the coupling of phosphotyrosine to a (3-aminometyl-phenyl)-urea moiety can adopt a low-energy conformation mimicking the minimal motif pTyr-X_{+1}-Asn in the type I β-turn conformation, without making steric clashes with the Grb2-SH2 domain. The ability of the designed molecules to mimic pTyr-X_{+1}-Asn in the type I β-turn conformation was supported by the occurrence of the required conformation in the X-ray crystal structures of small molecules presenting a N-benzylacetamide substructure (Cambridge Crystallographic Database codes BEXYOS and CUJVIM[14]) taken as a model for the conformationally flexible central part of the mimetics. In the X-ray structures of these molecules the torsion angles corresponding to the Φ and Ψ angles of residue X_{+1} of the peptide ligand show similar values as those observed in the latter.

The urea mimetic, unlike the reference peptide, cannot form the β-turn intramolecular hydrogen bond between the backbone carbonyl of the phosphotyrosine and the backbone NH of residue X_{+3}. We reasoned that the rigidity of the phenylurea moiety replacing the conformationally more flexible asparagine amino acid would compensate in terms of conformational restriction the loss of this intramolecular hydrogen bond.

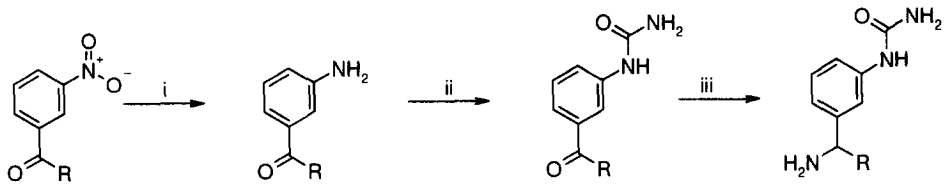


R=			
	H	**1a**	**2a**
	$CH(CH_3)_2$	**1b**	**2b**
	$CH_2CH_2CONH_2$	**1c**	**2c**

Figure 2. Peptidomimetics **1a-c** and reference peptides **2a-c**

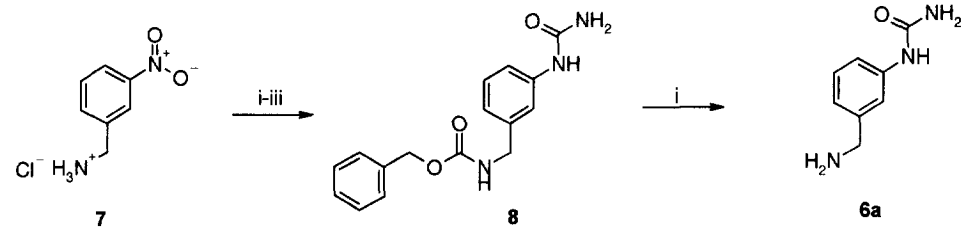
Synthesis. The α-substituted (3-aminomethyl-phenyl)-urea derivatives **6b** and **6c** were prepared as racemic mixtures starting from readily available 3-nitroacetophenones derivatives **3b** and **3c**.

The compounds **3b** and **3c** were prepared according to the literature[15,16] and hydrogenated over Raney-Nickel catalyst to afford the 3-aminoacetophenone derivatives **4b** and **4c** (Scheme 1). Compounds **4b** and **4c** were treated with 1 equivalent of hydrochloric acid and potassium cyanate to provide the ureas **5b** and **5c**.[17] The conversion of **5b** and **5c** to the corresponding amines **6b** and **6c** was achieved by formation of the oxime and its subsequent reduction with aluminum-nickel alloy in a one pot procedure.[18]



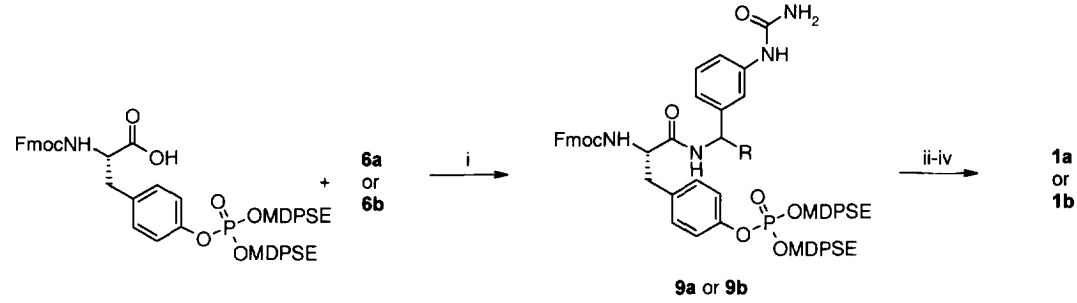
Scheme 1. Reagents and conditions: i) Raney-Ni, H_2, EtOH (**4b**: 88%, **4c**: 74%); ii) HCl 1 eq., KOCN, H_2O (**5b**: 61%, **5c**: 77%); iii) $NH_2OH{\cdot}HCl$, NaOH 2N, EtOH then Ni-Al alloy (**6b**: 60%, **6c**: 74%).

For the synthesis of the (3-aminomethyl-phenyl)-urea **6a**: commercial 3-nitrobenzylamine **7** was protected with Z-Cl. The nitro group was then hydrogenated in presence of Raney-Ni catalyst, and the resulting aniline was treated with 1 equivalent of hydrochloric acid and potassium cyanate to afford the urea **8**. Catalytic hydrogenation of **8** with Pd(C) furnished the urea **6a** (Scheme 2).



Scheme 2. Reagents and conditions: i) Z-Cl, K_2CO_3 10%, dioxane; ii) Raney-Ni, H_2, MeOH; iii) KOCN, HCl 1 eq., H_2O (74%); iv) Pd(C), H_2, MeOH (quant.).

The amine **6a** and **6b** were coupled with commercially available Fmoc-Tyr[PO_3(MDPSE)$_2$] using PyBOP, in presence of DIPEA in DMF, to afford the protected intermediates **9a**, and **9b** (Scheme 3).



Scheme 3. Reagents and conditions: i) PyBOP, DIPEA, DMF; ii) MeCN, $NHEt_2$; iii) Ac_2O, NEt_3, DMF; iv) TFA, DCM.

The FMOC group was cleaved with diethylamine in acetonitrile, the free base was reacted with acetic anhydride in presence of triethylamine in DMF, and the acetylated derivative treated with TFA in dichloromethane to afford the phosphate monoesters **1a** and **1b**. The two diastereoisomers of **1b** were

separated using reverse phase medium pressure liquid chromatography. The stereochemistry of **1b** was assigned based on the structure-activity relationships reported in Table 1 (the IC_{50} value of one of the diastereoisomers was above 50 μM).

The free amino acid **6c** was protected as a Fmoc derivative, and coupled on solid phase using standard solid phase procedure to afford **1c** as a diastereoisomeric mixture.[8] The reference peptides **2a-c** were synthesized in the same manner.

Results and discussion. The binding affinity of ligands for the Grb2-SH2 domain was assessed by an ELISA-type assay that measures their ability to inhibit the binding of the phosphorylated C-terminal intracellular domain of the epidermal growth factor receptor (EGFR) to this SH2 domain.[19] Three mimetics were synthesized: a reference X_{+1}= Gly mimetic (**1a**) and two other ones mimicking the side chains of two of the preferred natural amino acids (Val and Gln) (**1b** and **1c**) at X_{+1}.[9] The corresponding tripeptides were also synthesized for comparison. The biological data reported in Table 1, shows that our peptidomimetic strategy was successful. The urea derivatives **1a-c** exhibit micromolar IC_{50} values remarkably similar to those of the corresponding reference tripeptides **2a-c.** Introduction at the benzylic position of substituents mimicking the side chains of valine and glutamine (**1b** and **1c** compared to **1a**) leads to an increase of the binding affinity of the same magnitude (approximately 10-fold) as in the peptide series when glycine at X_{+1} is replaced by the former amino acids, thus confirming the validity of the design. The improvement in activity may be explained in the case of valine by additional side chain hydrophobic contacts with the Grb2-SH2 domain seen in the X-ray structure. For glutamine, modeling based on the X-ray crystal structure of the homologous SH2 domain of Lck in complex with a phosphopeptide having a glutamic acid at the X_{+1} position,[20] suggests the existence of a beneficial water mediated hydrogen bond between the side chain carboxamide group of the ligand X_{+1} residue and an amino acid of the SH2 domain belonging to the central beta sheet (His βD4).

Table 1. Grb2-SH2 Domain Inhibitory Activity of the Peptidomimetics and the Related Peptides

Compounds	R	IC_{50} (μM)
1a	H	48.66 ± 0.64
2a (Gly)		67 ± 8.42
1b	$CH(CH_3)_2$	6.19 ± 1.02
2b (Val)		4.32 ± 0.86
1c (dias. mix)	$CH_2CH_2CONH_2$	6.5 ± 0.29
2c (Gln)		5.44 ± 0.26

In conclusion, the present work demonstrates that it is possible to replace two out of the three amino acids forming the minimal phosphopeptide sequence recognized by the Grb2-SH2 domain by a (3-aminomethyl-phenyl)-urea moiety without loss of affinity. This finding represents an important step forward in the search for non peptidic ligands of the Grb2-SH2 domain that can efficiently block the signal transduction pathways of tyrosine kinase growth factor receptors.

References and Notes

1. Traxler, P.; Lydon, N. *Drugs Future* **1996**, *20*, 1261.
2. Groundwater, P. W.; Solomons, K. R. H.; Drewe, J. A.; Munawar, M. A. *Prog. Med. Chem.* **1996**, *33*, 233.
3. Smithgall, T. E. *J. Pharmacol. Toxicol. Methods* **1995**, *34*, 125.
4. Gishizky, M. L. *Annu. Rep. Med. Chem.* **1995**, *30*, 247.
5. Rojas, M.; Yao, S.; Lin, Y.-Z. *J. Biol. Chem.* **1996**, *271*, 27456.
6. Lowenstein, E. J.; Daly, R. J.; Batzer, A. G.; Li, W.; Margolis, B.; Lammers, R.; Ullrich, A.; Skolnik, E. Y.; Bar-Sagi, D.; Schlessinger, J. *Cell (Cambridge, Mass.)* **1992**, *70*, 431.
7. Phosphopeptide residues are numbered relative to the position of phosphotyrosine which is denoted 0. Positive numbers are used for amino acids C-terminal to phosphotyrosine.
8. Furet, P.; Gay, B.; Garcia-Echeverria, C.; Rahuel, J.; Fretz, H.; Schoepfer, J.; Caravatti, G. *J. Med. Chem.* **1997**, *40*, 3551.
9. Peptide studies in our group have established that the minimal sequence retaining micromolar affinity for the Grb2-SH2 domain is the tripeptide Ac-pTyr-X_{+1}-Asn-NH2. Asparagine at X_{+2} is absolutely required while position X_{+1} is more versatile: valine, isoleucine, glutamine and glutamic acid being the preferred natural amino acids at this position (Garcia-Echeverria, C.; et al. Novartis Pharma Inc., Oncology Research Department, unpublished results).
10. Rahuel, J.; Gay, B.; Erdmann, D.; Strauss, A.; Garcia-Echeverria; Furet, P.; Caravatti, G.; Fretz, H.; Schoepfer, J.; Gruetter, M. G. *Nat. Struct. Biol.* **1996**, *3*, 586. PDB code 1TZE.
11. MacroModel v.4.0: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.
12. Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S. Jr.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765. A dielectric constant of 4r was used in the calculations.
13. For the definition of β-turn types see: Wilmot, C. M.; Thornton, J. M. *Protein Eng.* **1990**, *3*, 479.
14. Allen, F. H.; Bellard, S.; Brice, M. D.; Cartwright, B. A.; Doubleday, A.; Higgs, H.; Hummelink, T.; Hummelink-Peters, B. G.; Kennard, O.; et al. *Acta Crystallogr., Sect. B* **1979**, *B35*, 2331.
15. Martin, E. L. *J. Am. Chem. Soc.* **1936**, *58*, 1438.
16. Curran, W. V.; Ross, A. *J. Med. Chem.* **1974**, *17*, 273.
17. Florence, G. *J. Bull. Sci. Pharm.* **1933**, *40*, 325.
18. Staskun, B.; Van Es, T. *J. Chem. Soc. (C)* **1966**, *40*, 531.
19. Gay, B.; Furet, P.; Garcia-Echeverria, C.; Rahuel, J.; Chene, P.; Fretz, H.; Schoepfer, J.; Caravatti, G. *Biochemistry* **1997**, *36*, 5712.
20. Eck, M. J.; Shoelson, S. E.; Harrison, S. C. *Nature (London)* **1993**, *362*, 87.