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Highly Potent Inhibitors of the Grb2-SH2 Domain

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Abstract: Highly potent inhibitors of the Grb2-SH2 domain have been synthesized. They share the common sequence: Ac-Pmp-Ac_6c-Asn-NH-(3-indolyl-propyl). Different substituents at the 3-indolyl-propylamine C-terminal group were explored to further improve the activity. This is the first example of inhibitors of SH2 domains with sub-nanomolar affinity reported to date.

Introduction: Possibilities to inhibit transduction pathways of tyrosine kinase growth factor receptors are investigated intensively in cancer therapy research.[1-3] Blocking the interactions between the phosphotyrosine (pTyr) activated receptors and the Src homology 2 (SH2) domain of the growth factor receptor-bound protein 2 (Grb2) constitutes an attractive strategy. In fact, this approach targets a key component of mitogenesis, the Ras pathway.[4,5] We initiated a medicinal chemistry project along these lines and this has led to the identification of small and potent inhibitors of the Grb2-SH2 domain.

The present work is based on the X-ray crystal structure of Grb2-SH2 with a phosphopeptide ligand[6] and on structural information from previous structure-activity relationship (SAR) data.[7-10] In one of these recent papers[10] we have shown how to exploit an extended hydrophobic area adjacent to the Grb2-SH2 primary binding site. Hydrophobic C-terminal groups allowed us to increase substantially the affinity of these peptide mimics in comparison with the non-substituted minimal peptide Ac-pTyr-Ile-Asn-NH2. This work was extended by incorporation of a variety of different amines as the C-terminal group and using the sequence containing 4-phosphonomethyl-phenylalanine (Pmp), 1-aminocyclohexane carboxylic acid (Ac_6c), and asparagine substituted at the C-terminal carboxyl group. The compounds can be represented by the general

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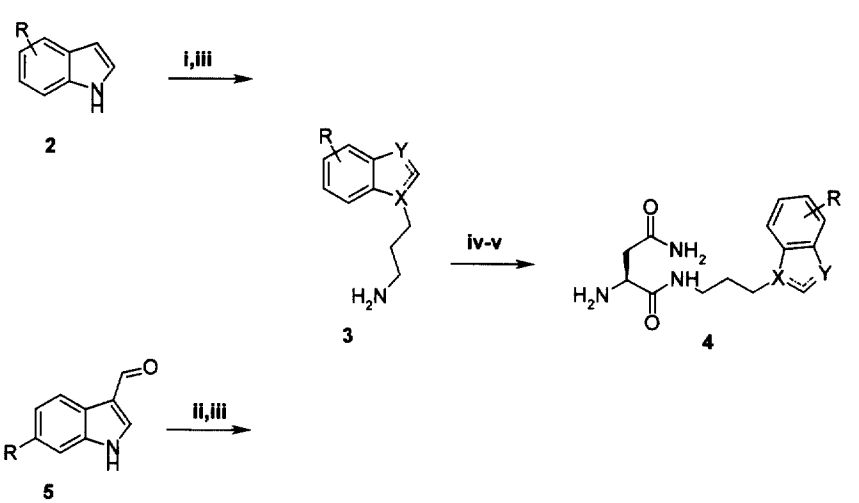
structure Ac-Pmp-Ac_6c-Asn-NHR. Among these compounds, the analog **1a** (R = 3-indol-1-yl-propyl) showed high potency. We decided to study more precisely the SAR of this indole series and we also describe a synthesis of Ac-Pmp-Ac_6c-OH based on the chloromethylation of Ac-Phe-Ac_6c-OMe.

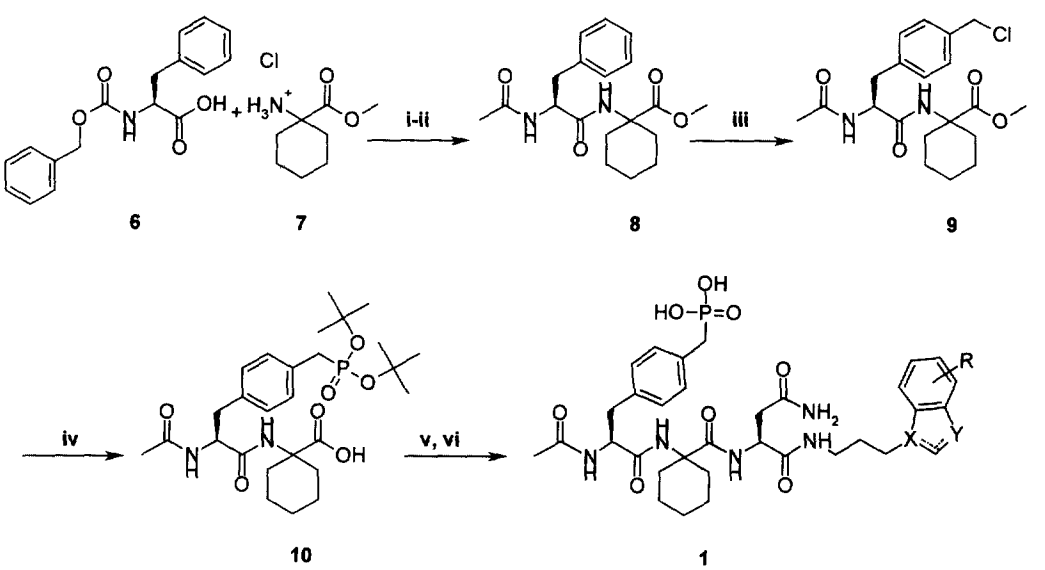
Chemistry: The 3-indol-1-yl-propylamine derivatives **3** (X= N, Y= C) were synthesized following the literature procedure (Scheme 1, **3** R= H, 5-Me, 5-Cl, 5-OMe, X= N, Y= C are described in [11]). A mixture of the indole **2** and acrylonitrile in dioxane is treated with a solution of benzyltrimethylammonium hydroxide in methanol (Triton® B). The resulting nitrile derivative was hydrogenated over Raney-Ni to afford the free amine **3** (X= N, Y= C) which was crystallized as the hydrochloride salt or distilled. For the 3-indol-3-yl-propylamine derivatives **3** (X= C, Y= N), aqueous sodium carbonate was added to a mixture of the indol-3-carbaldehyde **5** (R= 6-OMe is described in [12]) and diethyl cyanomethylphosphonate[13] to afford an isomeric mixture of nitriles that was hydrogenated to obtain the indole derivative **3** (X= C, Y= N). In general, the amine **3** was coupled with 4-nitrophenol activated benzyloxycarbonyl-asparagine and hydrogenated to afford the asparagine derivative **4**. The only exception is the chlorine derivative **4** (R= 5-Cl, X= N, Y= C) which was obtained using the 4-nitrophenyl ester of fluorenylmethyloxycarbonyl-asparagine and deprotection with diethylamine in acetonitrile.



Scheme 1. Reagents and conditions: i) acrylonitrile, Triton® B, dioxane. 0°C -> RT; ii) $NCCH_2PO(OEt)_2$, K_2CO_3 6M, RT; iii) H_2, Raney-Ni, MeOH; iv) Z-Asn-ONp, DMF, (NEt_3 if HCl salt), RT; v) H_2, Pd(C) 10%, DMF/MeOH/THF 12:4:1, RT; except for R= 5-Cl, X= N, Y= C then iv) Fmoc-Asn-ONp, DMF, RT; v) Et_2NH, MeCN.

Considering the enzymatic instability of the pTyr, we intended to replace it with its well-known biological analog Pmp.[14,15] Since Pmp is not commercially available, we were looking for an easy synthesis of this non-natural amino acid. A possibility is the chloromethylation of phenylalanine,[16] and subsequent Arbuzov reaction with the appropriate phosphite. In order to get a more convergent synthesis, we have extended this approach and examined a direct chloromethylation of Ac-Phe-Ac_6c-OMe **8**, which has the appropriate final acetyl protection (Scheme 1). In this case the presence of an α-di-substituted amino acid at the C-terminus allows us to do a proper fragment coupling of Ac-Pmp-Ac_6c-OH **10** with the substituted asparagine-amide derivatives without racemization. In addition this synthetic strategy avoids tedious protecting group chemistry.

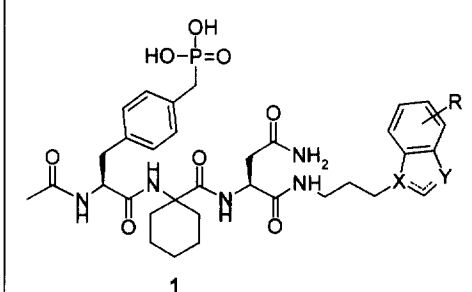
For the synthesis of Ac-Phe-Ac_6c-OH **8** commercially available benzyloxycarbonyl-phenylalanine **6** was coupled to H-Ac_6c-OMe hydrochloride **7**[17], using TPTU[18], and Hünig base in DMF. The dipeptide was then hydrogenated and protected in one step to afford crystalline **8** in 85% yield over the two steps. Chloromethylation of **8** was attempted using zinc chloride as described in the literature,[16] but with little success. However, addition of solid **8** to a preactivated solution of titanium tetrachloride and chloromethyl methyl ether afforded crystalline **9** in 50% yield. Treatment of the chloromethyl derivative **9** with di-*tert*-butyl phosphite[19] in the presence of sodium hydride in DMF unexpectedly afforded directly the free acid **10** in 54% yield.



Scheme 2. Reagents and conditions: i) TPTU, DIPEA, DMF; ii) H_2, Pd(C) 10%, Ac_2O, EtOH, (85% over 2 steps); iii) MOMCl, $TiCl_4$, neat 50°C (50%); iv) di-*tert*-butyl phosphite, NaH, DMF RT (54%); v) EDC, HOBT, NMM, **4**, DMF; vi) MeCN, HCl 4M.

The 3-indolyl-propylamine **4** was coupled to **10** using EDC and HOBT in the presence of N-methyl morpholine in DMF. Deprotection of the di-*tert*-butyl phosphonate has to be done carefully, using aqueous HCl in acetonitrile. Due to the instability of the indolyl residue under strong acidic conditions, the use of TFA or HCl in organic solvent has to be avoided. In addition, the phosphonate **1** was isolated as the ammonium salt. The acidic acetonitrile solution was neutralized with aqueous ammonium hydroxide, lyophilized and submitted to medium-pressure liquid chromatography, using a C_{18} reverse phase column.

Results and discussion. The structures of peptide mimics **1a-k** described in the above section are reported in table 1 together with their inhibitory activity (IC_{50} values, [nM]) towards the Grb2-SH2 domain. These values were assessed by an ELISA-type assay that measures their ability to inhibit the binding of the phosphorylated carboxy-terminal intracellular domain of the epidermal growth factor receptor (EGFR) to the Grb2-SH2 domain.[20]



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	R=	X=	Y=	IC_{50} [nM]
1a	H	N	C	1.2 ± 0.1
1b	2-Me	N	C	14.7 ± 0.6
1c	3-Me	N	C	81.0 ± 9
1d	5-Me	N	C	0.4 ± 0.1
1e	5-Cl	N	C	59.0 ± 9
1f	5-OH	N	C	0.3 ± 0.1
1g	5-OMe	N	C	0.9 ± 0.1
1h	5-NMe_2	N	C	3.4 ± 0.4
1i	H	C	N	155.0 ± 7
1j	6-OMe	C	N	14.0 ± 0.7
1k	1-Me	C	N	9.2 ± 0.3

Table 1. Grb2-SH2 Domain IC_{50} of the Peptides **1a-k**

As it can be seen in the table, we were able to reach unprecedented affinities between a short peptide mimic and a SH2 domain.[21-23] In the indol-1-yl-propylamine format **1a-h** a methyl group at position 5 (**1d**) shows a slight increase in the affinity compared to the parent compound **1a**, but the methyl group is detrimental at position 2 (**1b**) and even more so at position 3 (**1c**). According to our binding mode model,[10] the indolyl

moiety is involved in hydrophobic interactions with the side chains of residues Lys βD6 and Leu βD'1 of the SH2 domain which brings it in proximity to the phosphotyrosine residue due to the β-turn conformation. The model suggests the possibility of steric clashes of the substituents in positions 2 and 3 of the indolyl group with the edge of the phosphotyrosine ring exposed to solvent. This provides an explanation for the observed detrimental effects. The affinity is enhanced with increasing electron donating character of the substituents at position 5: OH, Me > OMe > H >> Cl (IC_{50} of **1f**, **1d** > **1g** > **1a** >> **1e**), except for 5-NMe_2 (**1h**). This trend in affinity may be interpreted in the context of specific CH/π interactions[24] between the aromatic system of the indolyl moiety and alkyl groups of the side chains of Lys βD6 and Leu βD'1 in conformity to our binding mode hypothesis. Electron enrichment of the acceptor aromatic system is expected to increase the strength of such interactions.

For the indol-3-yl-propylamine **1i**, the affinity is lower. We can assume that for the indole with the free NH, the decreased lypophilicity of the C-terminal moiety of the inhibitor is responsible for the reduced activity (desolvation of the free NH upon binding of the ligand is not compensated by formation of a hydrogen bond with the SH2 domain). In agreement with this, the activity can be recovered by the methylation of the nitrogen atom (compound **1k**). As for the geometrical isomer **1c**, the 1,3-substitution pattern of **1k** is not optimal. However, the IC_{50} of **1i** can also be improved by a factor of ten by the introduction of an electron donating methoxy group at position 6 (**1j**). This parallels the increase in activity observed when **1a** is substituted with electron donating groups at position 5 (**1d, 1f-h**).

The compounds presented here are a major step towards inhibitors of the Grb-SH2 domain and these might effectively block the signaling pathways of tyrosine kinase growth factor receptors.

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