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Synthesis and evaluation of diverse analogs of amygdalin as potential peptidomimetics of peptide T

Eyleen Araya,[a] Alex Rodriguez,[b] Jaime Rubio,[b] Alessandro Spada,[a] Jesus Joglar,[a] Amadeu Llebaria,[a] Carmen Lagunas,[d] Andres G. Fernandez,[d] Susanna Spisani[e] and Juan J. Perez[c,*]

[a]*RUBAM, Dept. de Química Organica Biologica, (IIQAB-CSIC), Jordi Girona Salgado, 18-26, E-08034 Barcelona, Spain*
[b]*Departament de Quimica fisica, Universitat de Barcelona, Mari i Franques, 1, E-08028 Barcelona, Spain*
[c]*Departament d'Enginyeria Quimica, Technical University of Catalonia, Av. Diagonal, 647, E-08028 Barcelona, Spain*
[d]*Departamento de Investigacion preclinica, Laboratorios Salvat SA, Gall, 30, E-08950 Esplugues de Llobregat, Spain*
[e]*Department of Biochemistry and Molecular Biology, Universita di Ferrara, I-44100 Ferrara, Italy*

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Abstract—Peptide T (ASTTTNYT) is a promising molecule to prevent the neuropsychometric symptoms of patients suffering AIDS and for the treatment of psoriasis. In order to fully prove its therapeutic benefits, efforts were put forward to design peptidomimetics of the peptide. In this direction, in a recent computational study the natural product amygdalin was identified as a prospective peptidomimetic of the peptide and later proved to exhibit a similar chemotactic profile to the peptide. However, the cyanide moiety of amygdalin provides to the molecule a toxic profile. The present study reports the synthesis of a set of amygdalin analogs lacking the cyanide group with improved chemotactic profiles.

1. Introduction

Peptide T (ASTTTNYT) is a fragment of the gp120 envelop protein of the human immunodeficiency virus (HIV), shown to inhibit binding of gp120 to the CD4 receptors expressed in T4 helper/inducer lymphocytes,[1] as well as to trigger human monocyte chemotaxis through the CD4/T4 antigen.[2] Different studies suggest that peptide T is a promising bioactive molecule for preventing the neuropsychometric symptoms of patients with AIDS,[3] and for the treatment of psoriasis.[4] In this direction, the beneficial effects of peptide T on keratinocytes have recently been demonstrated.[5,6] However, due to the poor pharmacological profile of peptides, it is sometimes difficult to prove fully their therapeutic benefits. To this end, efforts were recently put forward to find peptidomimetics of peptide T. From previous structure–activity studies based on the chemotactic properties of diverse peptide T analogs,[7] using a computational approach, a model of the bioactive conformation of the peptide was recently proposed.[8] This study led to define a pharmacophore incorporating all the chemical moieties, as well as their relative positions necessary to exhibit good monocyte chemotactic activity.[9] This pharmacophore was used for in silico screening of different databases of compounds, leading to the identification of amygdalin as a candidate peptidomimetic of peptide T. A subsequent monocyte chemotaxis study proved the compound to exhibit a chemotactic profile similar to that of peptide T.[10]

Amygdalin is a natural product that exhibits a toxic profile known to be due to the release of cyanide ions in vivo, produced in the hydrolysis of the glycoside bond by the action of glucosidases, releasing in a first step glucose and mandelonitrile, that is, later transformed into benzaldehyde and cyanhydric acid.[11] The goal of the present work was to design amygdalin analogs with the cyanide group replaced by other functionalities aimed at reducing its toxic profile. Accordingly, different structural modifications were studied, including the

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* Corresponding author. Tel.: +34 93 4016111; fax: +34 93 4016210; e-mail: juan.jesus.perez@upc.edu

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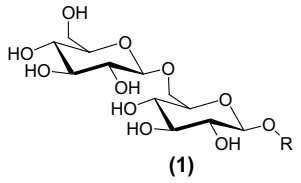
removal or substitution of this moiety. Compounds reported in the present work have the structural formula (**1**):



(1)

where R are benzyl or arylalkyl alcohol derivatives, substituted or not by diverse groups.

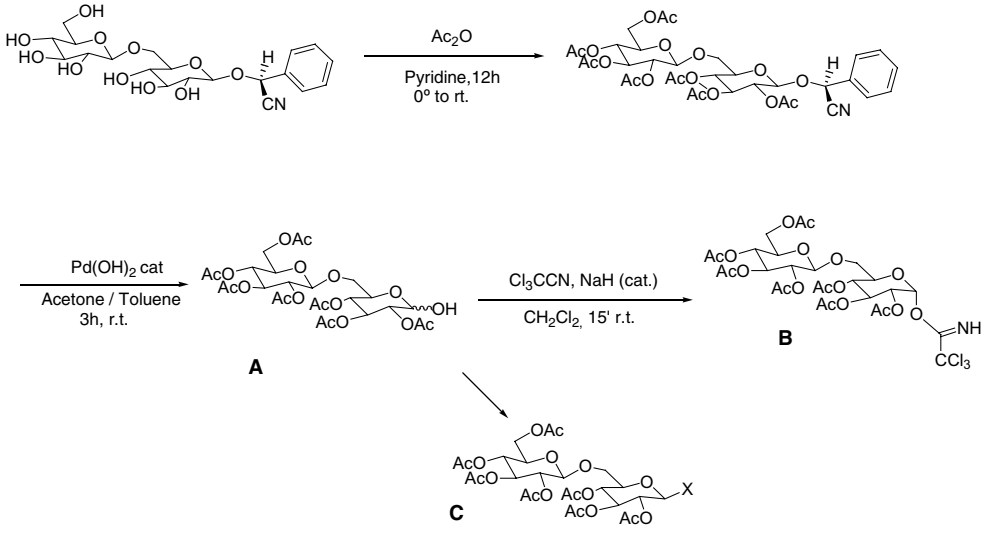
Compounds were synthesized as glycosylation products of diverse alcohols with hydroxyl protected gentiobiose (the corresponding disaccharide of amygdalin), using amygdalin itself as starting product. For this purpose, it was required to produce a glycosyl donor intermediate as starting product. Starting from the protected gentiobiose (**A**), two different intermediates were considered (**B** and **C**). The synthetic route used is shown in Scheme 1.

The protection of the hydroxyl groups of amygdalin was performed by acetylation,[12] followed by hydrolysis of the glycoside bond to produce heptaacetylgentiobiose (**A**). This compound was the precursor to produce an intermediate that was used to glycosylate the selected alcohols.

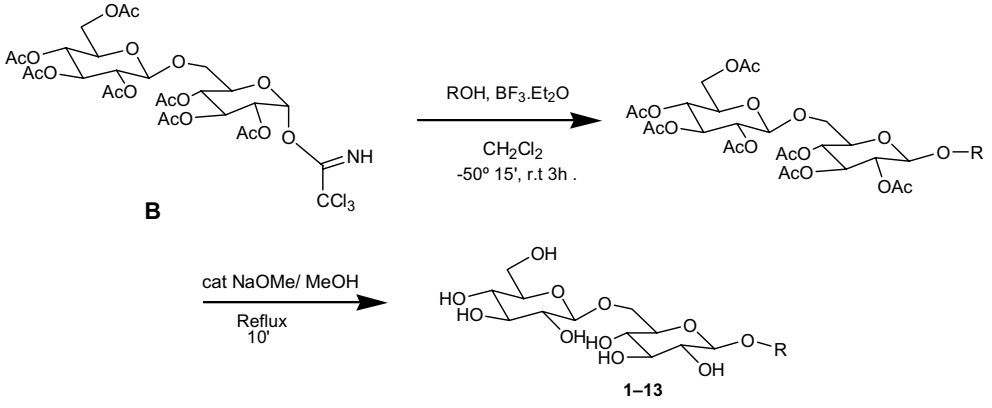
Of the derivatives of heptaacetylgentiobiose (**A**) tested in the glycosylation reaction (Scheme 1), the halides (**C**) (where X = Br, F) used in the Koenigs–Knorr method, showed poorer results than the trichloracetimidate (**B**) in yield, generality, and selectivity with the different alcohols tested. From the trichloroacetimido intermediate (**B**) the diverse derivatives can be synthesized by alcohol glycosylation according to Scheme 2.

2. Results and discussion

Analogs synthesized in the present work as well as results of the monocyte chemotaxis assays are listed in Table 1. Some of the analogs, like compounds **9**, **10**, and **11** (the only three compounds with a substituted aromatic ring) exhibit an improved monocyte chemotactic activity in regard to amygdalin at sub-nanomolar concentra-

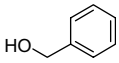
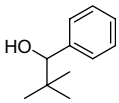
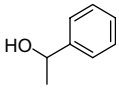
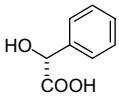
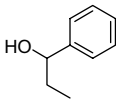
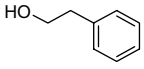
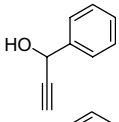
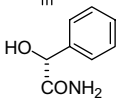
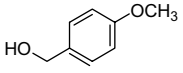
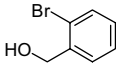
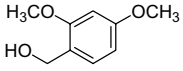
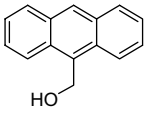


Scheme 1.



Scheme 2.

Table 1. Peak values of the chemotactic index together with the corresponding concentrations (−log[A]) for the different analogs synthesized

Molecule	Substituent	Chemotactic index	p[A]	Rmsd (Å)
1		0.76	8	0.72
2[a]		0.84	9	0.70
3[b]		0.77	8	0.72
4		0.71	9	0.27
5[b]		0.75	6	0.74
6		0.70	9	0.69
7[b]		0.79	6	0.74
8		0.84	7	0.32
9		0.87	10	0.74
10		0.94	10	0.72
11		0.84	10	0.81
12		0.94	8	0.64
13	Amygdalin	0.63	10	0.17
14	Peptide T	1.09	11	
15	[Dala[1]]pepTNH$_2$	0.89	11	

[a] Assayed as 3:2 mixture of epimers at the benzylic carbon.
[b] Assayed as 1:1 mixture of epimers at the benzylic carbon.

tions. This can be seen in more detail in Figure 1, where the chemotactic index of these compounds, measured as a function of the concentration is compared to that of peptide T, [DAla[1]]peptide T, and amygdalin. Compounds **2**, **4**, and **6** exhibit lower activity than amygdalin, being their chemotactic activity one order of magnitude lower. Similarly, compounds **1**, **3**, and **12** exhibit even poorer activities, two orders of magnitude lower than amygdalin. Compounds **5** and **7** both assayed as a 1:1 mixture of epimers show chemotactic

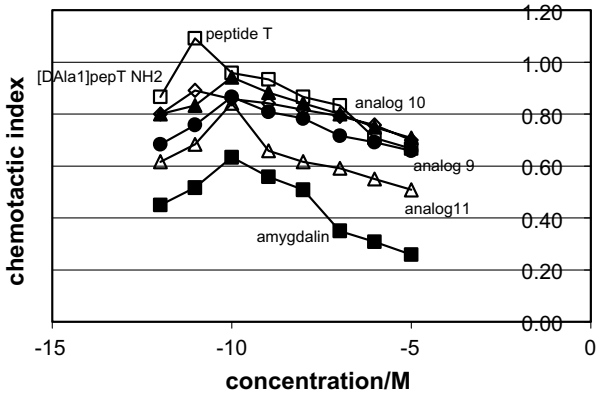


Figure 1. Plot of the chemotactic index of different compounds versus their concentration. Compounds displayed are: peptide T (□); [DAla[1]]peptideTNH$_2$ (◊); Amygdalin (■); analog 9 (●); analog 10 (▲); and analog 11 (△).

activity at micromolar concentrations, although it may the case that only one of the enantiomers is active. Although this may explain in part the difference of activity observed between compounds **2** and **5**, it should also be argued that the higher chemotactic activity exhibited by compound **2** is due to the constrained conformation adopted by the aromatic ring due to its interaction with the bulky neighboring *tert*-butyl group. Similarly, compound **8** shows a loss of chemotactic activity in regard to compound **4**, suggesting that the pharmacophore requires only a proton accepting moiety in this position.

In order to get some insights into the structure–activity relationships among this set of compounds, for each of the analogs, the conformation that best fitted the pharmacophore requirements was computed. This was carried out by finding the geometry with the lowest root mean square deviation between the corresponding chemical moieties of the analogs and the pharmacophore groups, by varying all rotatable angles without energy minimization. Last column of Table 1 lists the values of the root mean square deviation (rmsd) between the most fitted conformation of each analog and the four point pharmacophore. Analysis of the results suggests that compounds can be classified into two categories, those that exhibit rmsd values around 0.7 Å and those with values of 0.3 Å and lower. Analogs in the latter category fulfill one of the proton accepting centers with the carboxyl (**4**), carboxamide (**8**), and cyanide (**13**) moieties, respectively, whereas those compounds of the former category use the ether oxygen as proton accepting center. As can be seen, good fitting of the compounds to the pharmacophore is not enough to explain the chemotactic indexes observed. Accordingly, steric hindrance, the energy burden to access the most fitted conformation or electronic effects on the aromatic ring, are features that are not included in the pharmacophore in its present status and may account for the activity of the compounds.

In summary, there were identified several analogs of amygdalin that can be considered peptidomimetics of peptide T, based on the similar chemotactic profile

exhibited. Moreover, since these analogs lack the cyanide group they are expected not to be toxic. However, further studies need to be carried out in order to identify differences in the pharmacological profile in regard to peptide T. Work in this direction is presently being undertaken.

3. Experimental section

Monocyte chemotaxis measurements were carried out following the procedure described in Ref. 9.

4. Synthesis

4.1. 2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl (1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside, hepta-*O*-acetyl-D-gentiobiose (A)

Following an adaptation of a literature procedure describing the synthesis of the benzoyl derivative,[13] a suspension of heptaacetylgentiobiose[14] (9.0 g, 12 mmol) and 20% $Pd(OH)_2/C$ (3.6 g) in a toluene–acetone mixture (3:2 v:v, 500 mL) is hydrogenated at 25 °C and 1.0 bar until total consumption of the staring material (TLC). The reaction mixture is filtered and evaporated. The residue obtained is dissolved in EtOAc (200 mL) and washed with 1 N HCl and saturated NaCl. Drying and evaporation produce a yellow solid that is re-crystallized from ethanol to give 6.80 g (86%) of product as white crystals.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 5.50 (1H, dd, $J = 9.6$, $J = 10.2$), 5.19 (1H, t, $J = 9.6$), 5.07 (1H, t, $J = 9.6$), 4.94 (1H, dd, $J = 8.1$, $J = 9.6$), 4.88–4.81 (2H, m), 4.55 (1H, d, $J = 8.0$), 4.26–4.12 (2H, m), 3.85–3.80 (1H, m), 3.72–3.65 (1H, m), 3.63–3.56 (2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 170.27, 170.17, 170.09, 169.70, 169.39, 169.36, 169.21, 101.05, 89.95, 72.55, 71.96, 71.18, 71.00, 69.85, 69.13, 68.76, 68.07, 67.96, 62.51, 61.60, 20.5–20.3 (7C).

4.2. *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl (1→6)-2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)trichloroacetimidate (B)

A catalytic amount of 60% dispersion of NaH in mineral oil (approx 0.025 mmol) is added to a solution of hepta-*O*-acetylgentiobiose (500 mg, 0.8 mmol) and trichloroacetonitrile (0.5 mL, 5.0 mmol) in dichloromethane (20 mL) and the mixture is stirred at 25 °C under nitrogen until the total reaction (TLC) of the protected disaccharide (usually 15–30 min). The mixture is evaporated to dryness and the residue employed in the next step without further purification.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.12.071. Typical procedure for glycosylation of alcohols and characteristics of the analogs described in the present work is available as supplementary data.

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