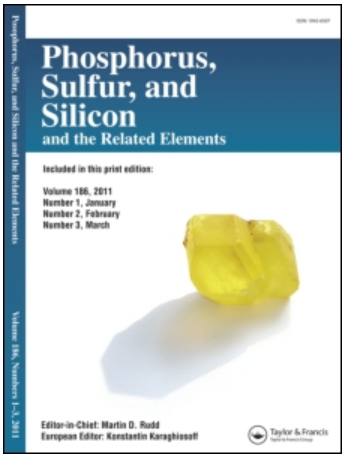

Phosphorus, Sulfur, and Silicon and the Related Elements

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SUBSTRATE RELATED *O,O*-DIALKYLDIPEPTIDYLAMINOPHOSPHONATES, A NEW TYPE OF THROMBIN INHIBITOR

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Abstract The facile reduction of *O,O*-dialkyl 1-hydroxyiminoalkanephosphonate precursors, using $LiBH_4/Me_3SiCl$ in THF at ambient temperature, conveniently affords *O,O*-dialkyl 1-aminoalkanephosphonates in good yield and high state of purity. *O,O*-Dialkyl 1-aminobenzylphosphonates may be prepared in high yield and purity from catalytic hydrogenolysis of their 1-benzylaminobenzylphosphonate precursors. These biologically active aminophosphonates, when coupled to substrate derived dipeptides, produced a range of novel phosphonotripeptides based upon the 'fibrinogen-like' sequence H-D-Phe-Pro-Arg; where the phosphorus structural units replace the 'P1'Arg. These tripeptides showed a marked inhibitory specificity towards the trypsin-like serine protease thrombin, a ubiquitous enzyme that plays a crucial role in the cardiovascular system. The compounds possess an initial K_i *in-vitro* in the micromolar range against thrombin. Further enzyme kinetic analysis of the compound Z-D-Dpa-Pro-$Pgl^P(OiPr)_2$ (IC_{50} 11.7 micromolar), showed that it displayed competitive inhibition characteristics toward thrombin, in contrast to the two stage slow-tight binding kinetics that had been shown by the analogous *O,O*-diphenyl derivative.

Key Words: Aminophosphonates, phosphonotripeptides, thrombin inhibition, anti-thrombotic agents.

INTRODUCTION

Cardiovascular disease is a prevalent cause of mortality across the world; recorded cases being higher than that of cancer. Advanced stages of the disease state such as myocardial infarcation, stroke, peripheral arterial occlusion and venous thromboembolic disease have been found to be as a result of formation of thromboembolic clots[1]. The anatomy of the disease state, and in particular clot formation is moderated by the coagulation serine proteases which are also responsible for the normal haemostatic equilibrium of the blood[2]. However, reversible inhibition of thrombin, a centrally acting multifunctional serine protease, and penultimate enzyme in the blood coagulation cascade, facilitates amelioration of thrombotic events that would

otherwise be extremely debilitating or potentially fatal[3]. Thrombin has the essential role of cleaving fibrinogen to liberate fibrin, which initiates blood clot formation; and stimulates platelet aggregation at the onset of vessel wall injury[4]. As a result of its pivotal role, thrombin is an ideal target for the development of an anticoagulant protease inhibitor.

Phosphorus containing inhibitors such as diisopropyl phosphorofluoridate[5], have often been used to characterise the physiological properties of hydrolytic enzymes. Furthermore Oleksyszyn and Powers showed that peptide derivatives of (α-aminoalkyl) phosphonic acids had the greatest potential for affording selective serine protease inhibitors, since they are closely related analogues of α-amino carboxylic acids. They prepared a number of peptidyl (α–aminoalkyl)phosphonate diphenyl esters with substrate related sequences, which in fact were potent and specific irreversible inactivators of a range of serine proteases; forming very stable derivatives with the enzymes[6,7]. This prompted our investigation into the synthesis and use of novel phosphorus-containing peptidomimetics as potentially useful antithrombotic agents.

RESULTS AND DISCUSSION

Earlier work had shown that mixed anhydride coupling of *O,O*-diphenyl α–aminoalkanephosphonates to Z-D-Dpa-Pro-OH efficiently generated phosphonotripeptides modelled on the 'fibrinogen-like' sequence H-D-Phe-Pro-Arg. Here the positively charged side chain of 'P1' Arg could be replaced with a neutral side chain protected by a phosphorus nucleus without a serious loss of inhibition[8]; and the 'P3' Phe could be replaced by the hydrophobic Dpa (ß,ß–diphenylalanine) to effect better interaction with the apolar binding site of thrombin. Although thrombin is known to cleave exclusively Arg and Lys bonds it was interesting that good inhibition could be obtained with a 'P1' structural unit having a neutral side chain, and that this contributed toward producing enhanced selectivity for thrombin. Initial K_i's were found to be in the micromolar range, and with 1h pre-incubation of the enzyme and inhibitor, these values improved, falling to the nanomolar range. The compounds showed two stage slow-tight binding behaviour, suggesting that the mechanism maybe ordered by hydrolysis of one of the phenyl ester groups to give a more stable enzyme-inhibitor complex[9].

An examination was later made to ascertain whether *O,O*-dialkoxy groups in $P(O)(OR)_2$ would interact with the catalytic triad of residues Asp, His, Ser in the active site of thrombin more favourably than the diphenyl groups of the earlier series (particularly as the free phosphonic acid derivatives were found to be less potent against thrombin). *O,O*-Dialkyl 1-aminoalkanephosphonates derived from the facile reduction of *O,O*-dialkyl 1-hydroxyiminoalkanephosphonate precursors, using $LiBH_4/Me_3SiCl$ in THF at ambient temperature[10]; and *O,O*-dialkyl 1-aminobenzylphosphonates derived from catalytic hydrogenolysis of their 1-benzylaminophosphonate precursors, were similarly coupled to Z-D-Dpa-Pro-OH, to form a new range of *O,O*-dialkyldipeptidylaminophosphonates (Scheme 1).

Z-D-Dpa-Pro-OH $\xrightarrow{1.\ iBuOCOCl,\ 2.\ H_2NCH(R)^P(OR')_2}$ Z-D-Dpa-Pro-$NHCH(R)^P(OR')_2$

Scheme 1

TABLE 1

O,O-DIALKYLDIPEPTIDYLAMINOPHOSPHONATE INHIBITORS OF THROMBIN

entry	R	R'	K_i (μM)	$\delta^{31}P(CDCl_3)$/ppm
1	CH_2CH_3	CH_2CH_3	27.2	25.23, 25.59
2	CH_2CH_3	$CH(CH_3)_2$	18.1	23.43, 23.58
3	CH_2CH_3	$(CH_2)_3CH_3$	4.7	25.23, 25.65
4	$(CH_2)_2CH_3$	CH_2CH_3	3.65	25.48, 25.98
5	$(CH_2)_2CH_3$	$CH(CH_3)_2$	1.18	23.68, 23.97
6	$(CH_2)_4CH_3$	CH_2CH_3	0.944	25.39, 25.93
7	$(CH_2)_4CH_3$	$CH(CH_3)_2$	2.8	23.65, 23.93
8	$(CH_2)_4CH_3$	CH_2CH_2Cl	18.6	26.09, 26.84
9	$(CH_2)_4CH_3$	C_6H_5	0.854	27.66, 28.54
10	C_6H_5	CH_2CH_3	1.5	21.91, 22.07
11	C_6H_5	$(CH_2)_2CH_3$	11.2	21.86, 22.04
12	$4\text{-}CH_3OC_6H_4$	CH_2CH_3	4.9	22.15, 22.31
13	$4\text{-}CH_3OC_6H_4$	$CH(CH_3)_2$	30.0	20.42, 20.55
14	$3,4,5\text{-}(CH_3O)_3C_6H_2$	CH_2CH_3	42.7	21.68, 22.13
15	$4\text{-}FC_6H_4$	CH_2CH_3	insoluble	21.60, 21.81
16	$4\text{-}CF_3C_6H_4$	CH_2CH_3	560.0	20.87, 21.20
17	$3\text{-}CF_3OC_6H_4$	CH_2CH_3	7.04	20.90, 21.13
18	$4\text{-}CF_3OC_6H_4$	$CH(CH_3)_2$	22.2	19.42, 19.67
19	C_6F_5	$CH(CH_3)_2$	19.2	16.22
20	C_6F_5	$(CH_2)_3CH_3$	10.6	18.41

The *O,O*-dialkyldipeptidylaminophosphonates prepared (see Table 1) were purified by flash chromatography through sephadex LH 20 (MeOH eluant). All the compounds were fully characterised by ^{1}H, ^{13}C, ^{31}P and where appropriate ^{19}F N.M.R. spectroscopy; FABMS, electrospray MS and C, H, N analysis. ^{31}P N.M.R. spectroscopy showed that the tripeptides were isolated for the most part, as a mixture of diastereoisomers. The compounds when assayed (apart from **9** and **15**) displayed competitive inhibition toward thrombin[11]. The use of different dialkoxy groups in the 'P1' position did not have a detrimental effect on the potency and innate specificity of the inhibitors. It is envisaged that resolution of chirally 'pure' products and further structural modification, may generate phosphonotripeptides that would be extremely useful therapeutic agents for use as antithrombotic drugs.

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