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Synthesis and evaluation of aminophosphinic acid derivatives as inhibitors of renal dipeptidase

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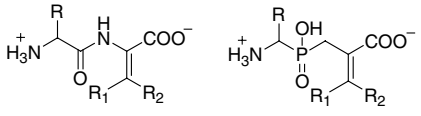
Abstract—Renal dipeptidase (RDP) is an enzyme overexpressed in benign and malignant colorectal tumors. In an effort to identify potent inhibitors of this enzyme, a series of aminophosphinic acid derivatives were synthesized. Compounds **3a** and **3c** in which the phenyl ring was *para* substituted with F and Br and olefin with *Z* geometry, showed better inhibitory activity against RDP enzyme ($IC_{50} = 5$–6 nM).

Renal dipeptidase (dehydropeptidase I, EC 3.4.13.11) is a zinc-containing hydrolytic enzyme that exhibit preference for dipeptide substrates with dehydro amino acids at the carboxy position. However, it can accommodate substrate with both D or L amino acids at that position as well.[1] This enzyme is responsible for the hydrolytic scission of the lactam bond in carbapenems, potent broad-spectrum antibiotics that are resistant to the action of microbial β-lactamases.[2] It has been shown that enzymatic turnover of carbapenems in vivo poses a serious obstacle to clinical efficacy of these bactericidal agents. Therefore, specific inhibitors for this enzyme are widely sought.[3] Serial analysis of gene expression (SAGE) studies have indicated RDP to be over expressed in both benign and malignant tumor in comparison to normal colonic epithelium,[4] providing an excellent opportunity for the development of specific probes for its detection in vivo to detect colon cancer at early stage.

The synthesis of phosphinic acids and phosphonic acids are currently an area of great interest due to the ability of these acids to function as effective bioesters of the carboxylic acid moiety in certain biological system. A successful approach for the inhibition of a number of peptidases has been to utilize phosphorus analogues to mimic unstable tetrahedral intermediates[5,6] or naturally occurring peptide inhibitors.[7] For example, potent inhibitors of the zinc peptidases thermolysin and carboxypeptidase A are obtained when the scissile carbonyl group of a substrate is replaced with a phosphonic acid moiety. In addition to their potency, these compounds appear to be transition-state analogues.[6]

We sought to extend this strategy to inhibitors of RDP by synthesizing dehydropeptide analogues in which scissile carboxamide has been replaced with a $PO(OH)CH_2$ group (Fig. 1). In continuation of our studies toward RDP inhibitors,[8] this communication describes design, synthesis and the in vitro evaluation of novel aminophosphinic acid derivatives.

The aminophosphinic acid analogues **3a**–**t** was synthesized following the synthetic strategy (Scheme 1). (1-*tert*-Butoxycarbonylamino-2-cyclohexyl-ethy)phosphinic acid methyl ester (**1**) was prepared according to reported procedure.[9] Treatment of **1** with NaOMe followed by trimethylphosphonoacrylate gave an intermediate,



Dehydropeptide Aminophosphinic acid analog

Figure 1. General structures of dehydropeptide and aminophosphinic acid analogue.

Keywords: Aminophosphinic acid derivatives; RDP inhibitors.
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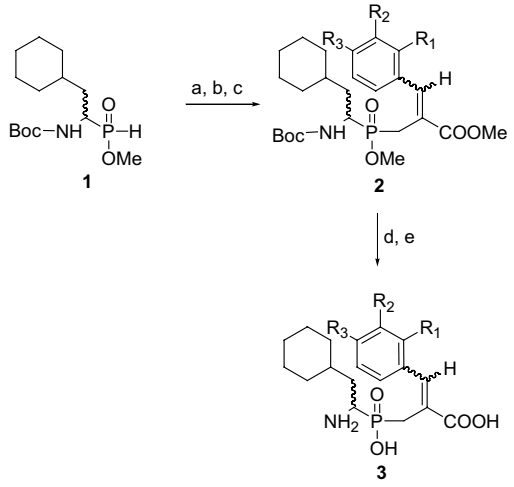

Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, 0 °C, 10 min; (b) trimethyl-2-phosphonoacrylate, 0 °C, 30 min; (c) $(R_1R_2R_3)$PhCHO, rt, 1 h; (d) CF_3COOH, CH_2Cl_2, rt, 30 min; (e) concd HCl, 50 °C, 8 h.

which subsequently underwent a Wittig–Horner type olefination[10] with substituted benzaldehydes to give mixture of *E* and *Z* isomers. These isomers were separated by column chromatography over silica gel. The yields were 40% for *Z* isomers and 30% for the *E* isomers. The protecting groups were removed by treatment with CF_3COOH followed by concd HCl[9] in 95% yield. The known compounds **3e** and **3f** have been prepared[8] and used for activity comparison. All the compounds were purified by column chromatography over silica gel and their structure were unambiguously confirmed by spectroscopic methods.[11]

The RDP inhibition activity of these compounds was determined using crude lysates prepared from human colon cancers according to reported procedure.[8] Human colon cancer extracts were prepared by homogenizing 1 cm^3 of frozen colon tissue in 10 mL of 20 mM Tris, pH 8.0, 10 μM $ZnCl_2$, 0.1% Triton X 100. The extract was clarified by centrifugation at 13,000*g* for 5 min at 4 °C and, for each measurement, 20 μL was diluted into 158 μL of 20 mM Tris, pH 8.0, 10 μM $ZnCl_2$. The synthesized compounds (20 μL) were added to each reaction to obtain final concentrations ranging from 0 to 10 μM. The mixtures were incubated at room temperature for 30 min to allow enzyme–inhibitor complex formation, and the reactions were initiated by the addition of 2 μL of 1 mM substrate (∈DNP-L-Lys-D-Amp).[12] While incubating at 37 °C, fluorescence ($\lambda_{ex} = 320$ nm, $\lambda_{em} = 405$ nm) measurements were determined at 30 s intervals and the relative reaction rate was taken as the rate of increase of fluorescence over time. The results are expressed as the concentration of inhibitor needed to inhibit enzyme activity by 50% (IC_{50}). The data for the synthesized compounds are shown in Table 1.

From the data in Table 1, it follows that the substitution pattern on the phenyl ring has influence on the RDP inhibition activity. Compounds **3a** and **3c** showed potent inhibitory activity against RDP. Iodo substitution on *para* position of the phenyl ring had better effect compare to *ortho* and *meta* position. In general compounds with the *Z* configuration were more active than their *E* counterparts.

Table 1. RDP inhibition (IC_{50})[a] values of compounds

Compound	R_1	R_2	R_3	Olefin geometry	IC_{50} (nM)
3a	H	H	F	*Z*	5
3b	H	H	F	*E*	15
3c	H	H	Br	*Z*	6
3d	H	H	Br	*E*	30
3e	H	H	I	*Z*	8
3f	H	H	I	*E*	25
3g	H	I	H	*Z*	45
3h	H	I	H	*E*	250
3i	I	H	H	*Z*	100
3j	I	H	H	*E*	1000
3k	H	H	CF_3	*Z*	10
3l	H	H	CF_3	*E*	200
3m	H	Cl	Cl	*Z*	20
3n	H	Cl	Cl	*E*	200
3o	H	Cl	F	*Z*	30
3p	H	Cl	F	*E*	350
3q	H	Br	F	*Z*	40
3r	H	Br	F	*E*	3000
3s	H	H	$N(Et)_2$	*Z*	70
3t	H	H	$N(Et)_2$	*E*	150

[a] IC_{50} values are determined using colon cancer lysate.

In conclusion, novel aminophosphinic acid analogues were synthesized and evaluated for in vitro RDP inhibition activity. Compounds **3a** and **3c** were found to be best inhibitors among the tested compounds with IC_{50} values 5 and 6 nM, respectively. Modification of active compounds with radiolabel and fluorescence tag to use them as biomarkers to detect early stage colon tumors are underway. Structure–activity relationship studies of this new class of compounds are in progress and will be reported in future communication.

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11. Representative examples (a) Compound **2a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, $J = 4.4$ Hz, 1H), 7.68 (m, 1H), 7.12 (t, $J = 8.4$ Hz, 1H), 5.06 (d, $J = 10$ Hz, 1H), 4.18 (m, 1H), 3.86 (s, 3H), 3.70 (d, $J = 10$ Hz, 3H), 3.14 (m, 2H), 0.71–2.04 (m, 22H); ^{13}C NMR (125 MHz, $CDCl_3$): 167.5, 160.2, 158.6, 134.5, 132.2, 130.3, 128.6, 116.4, 72.3, 58.4, 52.3, 44.3, 32.3, 30.3, 29.4, 28.7, 27.5, 25.3, 24.1; ^{31}P NMR (162 MHz, $CDCl_3$): δ 51.35; LC–MS m/z 497 $[M]^+$. (b) Compound **2b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.64 (m, 1H), 7.24 (t, $J = 8.4$ Hz, 1H), 6.94 (d, $J = 4.4$ Hz, 1H), 4.87 (d, $J = 10$ Hz, 1H), 4.15 (m, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.08 (m, 2H), 0.70–2.00 (m, 22H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.8, 161.4, 158.3, 134.7, 132.6, 129.7, 128.3, 116.8, 71.5, 59.6, 52.8, 43.4, 32.7, 30.8, 29.6, 28.5, 27.3, 25.6, 23.8; ^{31}P NMR (162 MHz, $CDCl_3$): δ 50.79; LC–MS m/z 497 $[M]^+$. (c) Compound **3a**: ^{1}H NMR (400 MHz, CD_3OD): δ 7.98 (d, $J = 4.4$ Hz, 1H), 7.68 (m, 1H), 7.18 (t, $J = 8.4$ Hz, 1H), 3.67 (m, 1H), 3.27 (m, 2H), 0.87–1.83 (m, 13H); ^{13}C NMR (125 MHz, CD_3OD): δ 170.6, 163.3, 138.7, 132.3, 129.3, 128.5, 117.2, 46.7, 32.3, 31.8, 29.3, 27.8, 26.4, 24.8; ^{31}P NMR (162 MHz, CD_3OD): δ 34.46; LC–MS m/z 369 $[M]^+$; HRMS calcd for $C_{18}H_{25}FNO_4PNa$ $[M+Na]^+$: 392.1397, found: 392.1382. (d) Compound **3b**: ^{1}H NMR (400 MHz, CD_3OD): δ 7.65 (m, 1H), 7.25 (t, $J = 8.4$ Hz, 1H), 6.95 (d, $J = 4.4$ Hz, 1H), 3.65 (m, 1H), 3.25 (m, 2H), 0.88–1.84 (m, 13H); ^{13}C NMR (125 MHz, CD_3OD): δ 170.2, 162.8, 138.2, 131.8, 128.7, 127.4, 116.7, 45.8, 31.6, 31.2, 28.6, 27.5, 25.8, 24.3; ^{31}P NMR (162 MHz, CD_3OD): δ 33.58; LC–MS m/z 369 $[M]^+$; HRMS calcd for $C_{18}H_{25}FNO_4PNa$ $[M+Na]^+$: 392.1397, found: 392.1385.
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