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NONPEPTIDE SMALL-MOLECULAR INHIBITORS OF DIPEPTIDYL PEPTIDASE IV: N-PHENYLPHTHALIMIDE ANALOGS

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Abstract: A novel series of nonpeptide small-molecular dipeptidyl peptidase IV (DPP-IV) inhibitors with an *N*-phenylphthalimide skeleton has been developed. Some of the compounds, including 4-amino-(2,6-dimethylphenyl)phthalimides (**7**), 4- and 5-hydroxy-(2,6-diethylphenyl)phthalimide (**11** and **14**), 4-hydroxy-(2,6-diisopropylphenyl)phthalimide (**12**), and thiocarbonyl analogs of (2,6-diisopropylphenyl)phthalimide and their 4,5,6,7-tetrafluorinated derivative (**18**, **19** and **20**), were more potent than the well-known DPP-IV-specific inhibitor, Pro-boroPro (PBP). Among them, **18** was revealed to be a DPP-IV-specific inhibitor, while the others also showed inhibitory activity toward another peptidase, aminopeptidase N (APN).

Dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5) is a membrane-associated serine protease which is widely distributed in mammalian tissues and body fluids.[1] DPP-IV preferentially liberates Xaa-proline or Xaa-alanine dipeptides from the N-termini of some polypeptides, and is identical to the T cell activation marker (or the leukocyte differentiation antigen) CD26 in the human immune system.[2] Recent investigation of DPP-IV/CD26 has indicated its involvement in various pathophysiological effects, including tumor cell adhesion and the entry of human immunodeficiency virus into $CD4^+$ T cells.[3] Thus, DPP-IV inhibitors are expected to be immunomodulators and to have potential in pharmacological/clinical applications.

The most potent reversible specific inhibitors of DPP-IV are boronic acid derivatives of peptides, including Pro-boroPro (PBP), which are considered to be transition state analogs.[4] Though some other DPP-IV inhibitors, including cyanopyrrolidide and diphenyl phosphonate derivatives, have been developed, they are all peptide-derived analogs in both their structure and chemical nature.[5] Generally, peptides have drawbacks for clinical application, including low bioavailability, proteolytical lability, rapid biliary excretion, and short duration of action. Therefore, it is important from a medicinal-chemical point of view to discover nonpeptide derivatives.

Recently, we have reported 2-(2,6-diethylphenyl)-1,2,3,4-tetrahydroisoquinoline-1,3-dione

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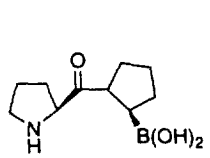
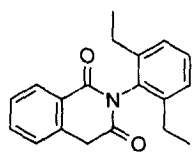
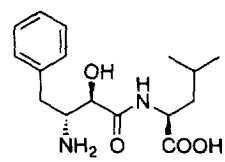
(PIQ-22) as a potent specific inhibitor of aminopeptidase N (APN); this small-molecular nonpeptide homophthalimide analog is more potent than the natural APN inhibitor bestatin.[6] Similar to DPP-IV, APN (EC 3.4.11.2, also known as the myeloid differentiation antigen CD13) is a membrane-associated peptidase, but it can be distinguished from DPP-IV on the basis of its substrate specificity, *i.e.*, APN releases a N-terminal single amino acid residue (preferentially alanine), not dipeptide as DPP-IV does. During our previous studies on the structure-activity relationship of protease inhibitors with a homophthalimide and phthalimide skeletons,[8] we found that some *N*-phenylphthalimide analogs show DPP-IV-inhibitory activity, though PIQ-22, its analogs with a homophthalimide skeleton, and bestatin are all inactive toward DPP-IV.[8] In this report, we describe the structural development of phthalimide analogs as small-molecular nonpeptide DPP-IV inhibitors.



PBP



PIQ-22



bestatin

N-Phenylphthalimide analogs were prepared by condensation of appropriate amines with phthalic anhydride or substituted phthalic anhydride as described previously.[7] Mono- and dithiocarbonyl analogs were prepared from the corresponding phthalimides by treatment with diphosphorus pentasulfide in xylene under reflux for 5 h. All the compounds prepared gave appropriate analytical values (details will be published elsewhere).

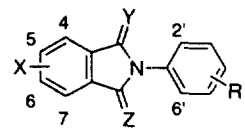
The DPP-IV-inhibitory activity of the prepared compounds was evaluated in intact-cell assays using the human acute lymphoblastic leukemia cell line MOLT-4 (5 x 10^4 cells/well), by measuring 7-amino-4-methylcoumarin (AMC: quantified on the basis of its fluorescence intensity) liberated from glycyl-L-proline 4-methylcoumaryl-7-amide (Gly-Pro-AMC: 200 μM, incubated with cells in the presence or absence of a test compound for 1 h at 37°C, pH 7.6).[7,8] APN-inhibitory activity was also determined by using MOLT-4 cells with L-alanine 4-methylcoumaryl-7-amide (Ala-AMC). Cytotoxicity of the compounds were assessed by means of WST-1 viability assays using human embryonic lung fibroblast WI-38 cells as described.[7,9] Though the results (IC_{50} values) showed some variation from experiment to experiment, they were basically reproducible, and a typical set of data is shown in Table 1.

N-(Dialkylphenyl)phthalimides (**1** and **2**) were inactive in both DPP-IV and APN inhibitory activity assays. Introduction of a nitro group at the phthalimide moiety (**3**–**6**) had no effect. However, introduction of an electron-donating group (an amino group for **7**–**9**, a hydroxyl group for **10**–**15**) caused the appearance of inhibitory activity. The 4-hydroxyl derivatives (**11** and **12**), 4-amino derivative (**7**), and 5-hydroxyl derivative (**14**) all showed potent DPP-IV-inhibitory activity (IC_{50} of 12.8 – 16.0 μg/ml under the experimental conditions), being more potent than

PBP (IC_{50} of 18.7 μg/ml under the same conditions), though they are also active toward APN. The enhancing effect of a hydroxyl group introduced at the 4-position on DPP-IV/APN-inhibitory activity is also observed for 2'-thiomethyl analogs (**16** and **17**).

The inhibitory activity towards DPP-IV of all of the compounds mentioned above (**1**–**17**) seems to be correlated with the inhibitory activity towards APN.

Table I. DPP-IV-inhibitory Activity of *N*-Phenylphthalimide Derivatives



Compound	X	Y/Z	R	DPP-IV inhibition IC_{50} [μg/ml (μM)]	APN inhibition IC_{50} [μg/ml (μM)]	Cytotoxicity IC_{50} [μg/ml (μM)]
1	H	O/O	2',6'-diMe	>100 (>398.0)	>100	>100
2	H	O/O	2',6'-(iPr)$_2$	>100 (>325.3)	>100	>100
3	4-NO_2	O/O	2',6'-diMe	>100 (>337.5)	>100	>100
4	4-NO_2	O/O	2',6'-(iPr)$_2$	>100 (>283.8)	>100	93.1 (264.2)
5	5-NO_2	O/O	2',6'-diMe	>100 (>337.5)	>100	>100
6	5-NO_2	O/O	2',6'-(iPr)$_2$	>100 (>283.8)	>100	42.7 (121.2)
7	4-NH_2	O/O	2',6'-diMe	*16.0* (*60.1*)	29.0 (108.9)	>100 (>375.5)
8	5-NH_2	O/O	2',6'-diMe	23.4 (87.9)	15.0 (56.3)	>100 (>375.5)
9	5-NH_2	O/O	2',6'-(iPr)$_2$	81.0 (251.2)	5.4 (16.8)	>100 (>310.2)
10	4-OH	O/O	2',6'-diMe	>100 (>374.1)	70.7 (264.5)	>100
11	4-OH	O/O	2',6'-diEt	*12.8* (*43.3*)	10.2 (34.5)	>100 (>338.6)
12	4-OH	O/O	2',6'-(iPr)$_2$	*14.1* (*43.6*)	4.3 (13.3)	>100 (>309.2)
13	5-OH	O/O	2',6'-diMe	19.8 (74.1)	10.3 (38.5)	>100 (>374.1)
14	5-OH	O/O	2',6'-diEt	*12.8* (*43.3*)	9.6 (32.5)	52.0 (176.1)
15	5-OH	O/O	2',6'-(iPr)$_2$	21.3 (65.9)	15.0 (46.4)	12.7 (39.3)
16	4-NO_2	O/O	2'-SMe	>100 (>318.2)	>100	>100
17	4-OH	O/O	2'-SMe	64.4 (225.7)	38.2 (133.9)	>100 (>350.5)
18	H	S/O	2',6'-(iPr)$_2$	**18.7** (**57.8**)	**>100** (>309.2)	60.5 (187.1)
19	4,5,6,7-F_4	S/O	2',6'-(iPr)$_2$	*12.0* (*30.4*)	11.4 (28.8)	>100 (>252.9)
20	4,5,6,7-F_4	S/S	2',6'-(iPr)$_2$	*15.1* (36.7)	40.5 (98.4)	1.4 (3.4)
PBP				18.7 (88.2)	>100 (>471.6)	>100
bestatin				>100 (>324.3)	0.8 (2.6)	>100

Interestingly, conversion of one carbonyl group of **2** to a thiocarbonyl group (compound **18**), resulted in a DPP-IV-specific potent inhibitor, *i.e.*, **18** showed potent DPP-IV-inhibitory activity comparable to that of PBP, but showed no activity toward APN. We cannnot interprete this phenomena at this stage. We have already shown that compound **18** possesses potent TNF-α production-regulating activity and antiangiogenic activity.[7,10] The specific DPP-IV-inhibitory activity of **18** might contribute to the latter activity, at least in part, because peptidases, including DPP-IV, have been reported to play a role in angiogenesis. Tetrafluorination of **18** (compound **19**) did not have any marked effect on DPP-IV-inhibitory activity, but resulted in dramatic appearance of APN-inhibitory activity. Conversion of the remaining carbonyl group of **19** to a thiocarbonyl group (compound **20**) did not increase the activities, but resulted in the appearance of potent cytotoxicity. The protease-inhibiting activity of the corresponding analogs of **2**, [*i.e.*, 4,5,6,7-tetrafluoro-(2,6-diisopropylphenyl)phthalimide], was very weak, though the activity could not be assessed accurately because of its high toxicity.

In conclusion, we have prepared potent DPP-IV/APN dual protease inhibitors, **7, 11, 12, 14, 19**, and **20**, all of which possess stronger activity than PBP toward DPP-IV and moderate activity toward APN, as well as a potent DPP-IV-specific inhibitor, **18**, which possesses comparable activity to that of PBP. Further studies on the structure-activity relationship, the mechanism of the enzyme inhibition, and potential clinical/pharmaceutical applications are in progress.

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