STRUCTURE-ACTIVITY RELATIONSHIPS AMONG DERIVATIVES OF ARPHAMENINES, INHIBITORS OF AMINOPEPTIDASE B

Sir:

Arphamenines A (**1-a**) and B (**1-b**), which were isolated from the culture filtrate of *Chromobacterium violaceum* BMG361-CF4, inhibit aminopeptidase B but not leucine-aminopeptidase. Arphamenines A and B are competitive inhibitors with a *Ki* value of 2.5×10^{-9} M and 8.4×10^{-10} M for aminopeptidase B.[1)]

The structures of arphamenines A and B are 5-amino-8-guanidino-4-oxo-2-phenylmethyloctanoic acid and 5-amino-8-guanidino-2-(4-hydroxyphenylmethyl)-4-oxooctanoic acid,[1,2)] respectively. Arphamenines are a new class of aminopeptidase inhibitors, with a methylene ketone (–CO–CH_2–) in the place of the scissile bond (–CO–NH–) of the substrate. In this communication, we report on some chemical modifications of arphamenines, and their structure-activity relationships. We found that some of them have high inhibitory activities for carboxypeptidases A and B.

Three reactive functional groups, amino, carboxy and guanidino were modified (Scheme 1). The modification of the amino group of arphamenines was performed by acetylation (Ac), benzoylation (Bz) or benzyloxycarbonylation (Z). Acetylation of the amino group of arphamenine A with acetic anhydride in alkaline water (pH 8.0), gave *N*-acetylarphamenine A (**2-a**, $C_{18}H_{26}N_4O_4$) in 79% yield, FDMS: *m/z* 363 (MH^+). The amino group of arphamenine A was modified by benzoylation with benzoyl chloride in ice cold aq sodium carbonate to give *N*-benzoylarphamenine A (**3-a**, $C_{23}H_{28}N_4O_4$) in 93% yield, FDMS: *m/z* 425 (MH^+). The amino group of arphamenine A was protected with benzyloxycarbonyl group by reaction with *S*-benzyloxycarbonyl-4,6-dimethyl-2-mercaptopyrimidine (Kokusan Chemical Works) in dioxane-aq sodium bicarbonate, followed by column chromatography on CM-Sephadex C-25 (H^+) to give *N*-benzyloxycarbonylarphamenine A (**4-a**) as the monohydrate ($C_{24}H_{30}N_4O_5\cdot H_2O$) in 72% yield, FDMS: *m/z* 455 (MH^+).

Modification of the amino group of arphamenine B with acetyl, benzoyl or benzyloxycarbonyl group by the methods described above gave *N*-acetylarphamenine B [(**2-b**, $C_{18}H_{26}N_4O_5$),

Scheme 1. Derivatives of arphamenines A and B.

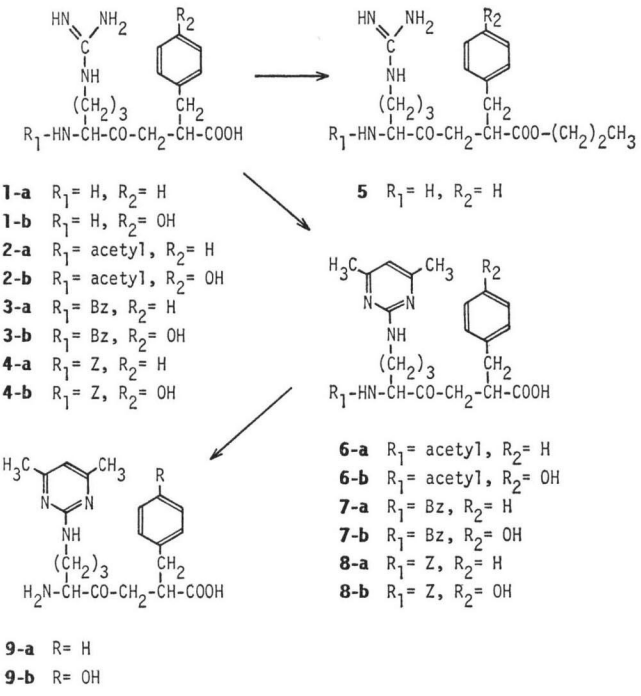
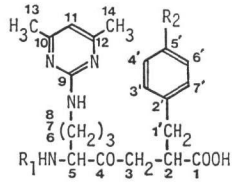


Table 1. Significant ^1H NMR chemical shifts of compounds **6-a**, **6-b**, **7-a**, **7-b**, **8-a** and **8-b**.



R_1=Ac or Bz or Z
R_2=H or OH

	6-a[a]	**6-b**[a]	**7-a**[a]	**7-a**[b]	**8-a**[a]	**8-b**[c]
5-CH	4.59 (m)	4.51 (m)	4.70 (m)	4.70 (m)	4.26 (m)	4.15 (m)
11-CH	6.34 (s)	6.36 (s)	6.27 (s)	6.31 (s)	6.17 (s)	6.36 (s)
13,14-CH_3	2.34 (s)	2.34 (s)	2.25 (s)	2.22 (s)	2.23 (s)	2.24 (s)
Phenyl (5H)	7.1~7.3 (m)	—	7.1~7.3 (m)	—	7.05~7.27 (m)	—
Phenyl (4H)	—	6.89 (m)	—	6.80 (m)	—	6.83 (m)
Ac	1.99 (s)	1.98 (s)	—	—	—	—
Bz	—	—	7.54 (m)	7.59 (m)	—	—
Z $\begin{cases}CH_2\\Phenyl\end{cases}$	—	—	—	—	5.03 (d)	5.06 (s)
	—	—	—	—	7.05~	7.26~
					7.27 (m)	7.38 (m)

[a] In $CDCl_3$, [b] in CD_3OD - $CDCl_3$, [c] in CD_3OD.

Table 2. Inhibitory activity of derivatives of arphamenines.

	IC_{50} (μg/ml)		
	Amino-peptidase B	Carboxy-peptidase A	Carboxy-peptidase B
1-a	0.006	90	>100
1-b	0.002	5.2	>100
2-a	>100	2.6	>100
2-b	>100	3.6	>100
3-a	28	0.10	80
3-b	40	0.32	26
4-a	51	1.1	>100
4-b	2.9	2.4	>100
5	0.72	>100	>100
6-a	>100	0.40	>100
6-b	>100	0.10	6.8
7-a	>100	0.020	2.6
7-b	>100	0.0088	0.70
8-a	>100	0.064	90
8-b	>100	0.35	9.0
9-a	1.5	40	>100
9-b	0.4	30	>100

FDMS: m/z 379 (MH^+), 80% yield], *N*-benzoylarphamenine B [(**3-b**, $C_{23}H_{28}N_4O_5$), FDMS: m/z 441 (MH^+), 80% yield] and *N*-benzyloxycarbonylarphamenine B [(**4-b**, $C_{24}H_{30}N_4O_6 \cdot 1/2H_2O$), FDMS: m/z 471 (MH^+), 92% yield], respectively.

Esterification of the carboxyl group of arphamenine A with boron trifluoride 1-propanol reagent 15 (Tokyo Kasei Co.) gave arphamenine A 1-propionyl ester (**5**, $C_{19}H_{30}N_4O_3 \cdot 2HCl \cdot H_2O$), SIMS: m/z 363 (MH^+).

The guanidino group of *N*-protected arphamenines was modified with acetylacetone in aq potassium carbonate to yield dimethylpyrimidyl derivatives. The properties of these *N*-substituted arphamenines which had been converted to dimethylpyrimidyl derivatives are as follows: *N*-Acetyldimethylpyrimidyl arphamenine A (**6-a**, $C_{23}H_{30}N_4O_4$), EIMS: m/z 426 (M^+), 25% yield; *N*-acetyldimethylpyrimidyl arphamenine B (**6-b**, $C_{23}H_{30}N_4O_5$), EIMS: m/z 442 (M^+), 24% yield; *N*-benzoyldimethylpyrimidyl arphamenine A (**7-a**, $C_{28}H_{32}N_4O_4$), SIMS: m/z 489 (MH^+), 54% yield; *N*-benzoyldimethylpyrimidyl arphamenine B (**7-b**, $C_{28}H_{32}N_4O_5$), SIMS: m/z 505 (MH^+), 23% yield; *N*-benzyloxycarbonyldimethylpyrimidyl arphamenine A (**8-a**, $C_{29}H_{34}N_4O_5 \cdot 1/2H_2O$), SIMS: m/z 519 (MH^+), 66% yield; *N*-benzyloxycarbonyldimethylpyrimidyl arphamenine B (**8-b**, $C_{29}H_{34}N_4O_6 \cdot 1/2H_2O$), SIMS: m/z 544 (MH^+), 67% yield. These structures were confirmed by ^1H NMR spectroscopy (Table 1).

Removal of the Z-group of *N*-Z-dimethylpyrimidyl arphamenines A and B with HBr-AcOH afforded dimethylpyrimidyl arphamenines A and B, respectively: Dimethylpyrimidyl arphamenine A (**9-a**, $C_{21}H_{28}N_4O_3 \cdot 2HBr$), SIMS: m/z 385 (MH^+), 55.1% yield; dimethylpyrimidyl arphamenine B (**9-b**, $C_{21}H_{28}N_4O_4 \cdot 2HBr \cdot H_2O$), SIMS: m/z 401 (MH^+), 67.7% yield.

The structure-activity relationships were studied by comparing the inhibiting activities of these derivatives for aminopeptidase B (Table 2). The *N*-Ac-arphamenine A (**2-a**) did not inhibit at 100 μg/ml, but *N*-Bz-arphamenine A (**3-a**) and *N*-Z-arphamenine A (**4-a**) had very weak activity (IC_{50} 28 and 51 μg/ml, respectively). These data and evidence that aminoacyl arginine shows anti-aminopeptidase B activity[3] indicate that this enzyme has a weakly interacted lipophilic pocket near its active site.

The dimethylpyrimidyl derivative (**9-a**) and the 1-propionyl ester (**5**) of arphamenine A had weak activity (IC_{50} 1.5 and 0.74 μg/ml, respectively). Arphamenine B and its derivatives are similar. These data indicate that the free amino group is essential for activity and the carboxyl and guanidino groups also are important; as in the case of bestatin the free amino and carboxyl groups are also needed for activity.[4]

Many carboxypeptidases A and/or B inhibitors, such as benzylmalic acid,[5] benzylsuccinic acid,[6] 2-benzyl-3-mercaptopropanoic acid[7] and 2-mercaptomethyl-5-guanopentanoic acid[7] have been reported. As arphamenine B inhibits carboxypeptidase A, we studied anti-carboxypeptidases A and B activity of related derivatives of arphamenines A and B (Table 2). We found that some derivatives were potent inhibitors of carboxypeptidases A and B. **7-b** showed especially strong anti-carboxypeptidases A and B activity (*Ki* 1.9×10^{-8} M vs. carboxypeptidase A, competitive and *Ki* 1.1×10^{-6} M vs. carboxypeptidase B, competitive), and **8-a** was a specific inhibitor of carboxypeptidase A (*Ki* 1.2×10^{-7} M, competitive). There are some trends in the inhibiting activities of these derivatives for carboxypeptidase A. Acetyl, carbobenzoxy, benzoyl groups gave, in that order, increasing activity against carboxypeptidase A. Both *N*-protection and modification of the guanidyl group with acetylacetone gave rise to stronger activity. These observations indicate that the *N*-benzoyl group plays a significant role in the inhibition of carboxypeptidase A. This is a new class of carboxypeptidases inhibitors; the high potency does not rely on two carboxyl groups or a sulfhydryl group.

Acknowledgment

This work was partly supported by a contact from the Division of Cancer Treatment, National Cancer Institute, NO1-CM-57009, U.S.A.

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(Received August 29, 1984)

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