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SYNTHESIS OF ECHIGUANINE ANALOGS AND THEIR RIBOFURANOSYL GLYCOSIDES THAT INHIBIT PHOSPHATIDYLINOSITOL 4-KINASE

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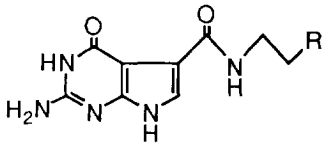
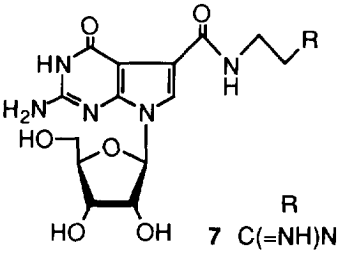
Abstract: *N*-carboxamide-substituted 7-deazaguanine-7-carboxamides and their ribofuranosyl compounds have been synthesized as echiguanine derivatives, and evaluated for inhibition of phosphatidylinositol (PI) 4-kinase. The ethylamide derivative and the corresponding ribofuranosyl compound inhibited PI 4-kinase with IC_{50} values of 0.02 and 2.4 μg/ml, respectively. The latter was suggested to also inhibit the enzyme in cultured human epidermoid carcinoma cells. © 1997 Elsevier Science Ltd.

The signaling pathways associated with activation of cell growth are considered to be new targets for cancer chemotherapy. Phosphatidylinositol (PI) 4-kinase is an enzyme involved in PI turnover, which turnover often regulates the growth of cells. PI 4-kinase activity was reported to be elevated in rat hepatomas[1] and human ovarian carcinoma cells.[2] We previously isolated novel PI 4-kinase inhibitors, echiguanine A**1** and B**2**, from *Streptomyces*[3], but they did not show any cellular effect, possibly because of poor permeation of the membrane. So, we synthesized their ribofuranosyl glycosides, **7** and **8**, to improve cell permeability to them.[4] However, they lost the inhibitory activity on the enzyme. To prepare effective and permeable inhibitors of PI 4-kinase, we became interested in modification of the terminal amidine moiety of echiguanine A. In this letter, we report on the chemical synthesis of echiguanine derivatives with or without a ribose moiety and their biological activities.



R

1 $C(=NH)NH_2$ (Echiguanine A)
2 CH_2NH_2 (Echiguanine B)
3 CN
4 COOMe
5 $CONH_2$
6 $CH_2NHC(=NH)NH_2$

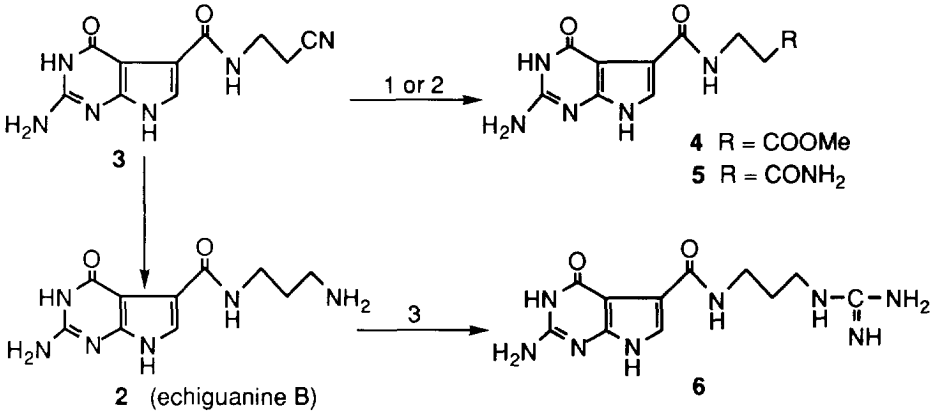


R

7 $C(=NH)NH_2$
8 CH_2NH_2
9 CN
10 COOMe
11 $CONH_2$
12 $CH_2NHC(=NH)NH_2$
13 $CH_2NHCONH_2$
14 $CSNH_2$

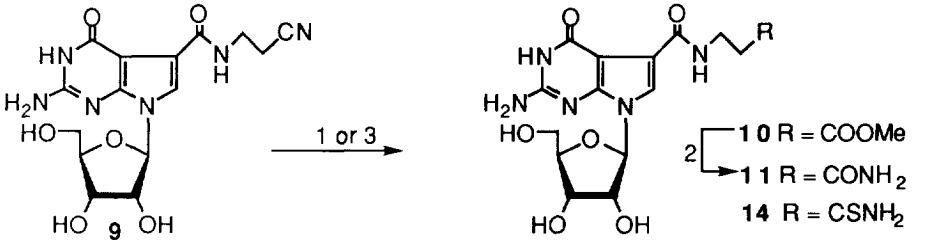
Chemistry

The scheme for the synthesis of target compounds **4**, **5**, and **6** are outlined in Scheme 1. Cyanoethylamide-deazaguanine **3**, which served as a starting material, was prepared from 7-iodo-7-deazaguanine and 3-aminopropionitrile under a carbon monoxide atmosphere in the presence of bis-(triphenylphosphine)palladium (II) chloride according to the protocol of Shih and Hu.[5] The cyano group of **3** was treated with saturated methanolic hydrogen chloride followed by hydrolysis of the imidate with water to give methyl ester **4** in 96 % yield. Initial attempts to obtain amide **5** from compound **4** by treatment with ammonia under various conditions were unsuccessful because of the low solubility of **4**. Alternatively, compound **3** was converted to **5** by use of 35 % H_2O_2 in aqueous base, resulting in an 82 % yield.[6] Compound **2** (echiguanine B) was obtained from **3** by hydrogenation on PtO_2 in acetic acid, and **2** was treated with aminoiminomethanesulfonic acid (AIMSA) in the presence of aqueous potassium carbonate to give guanidine **6** in 64 % yield.[7]



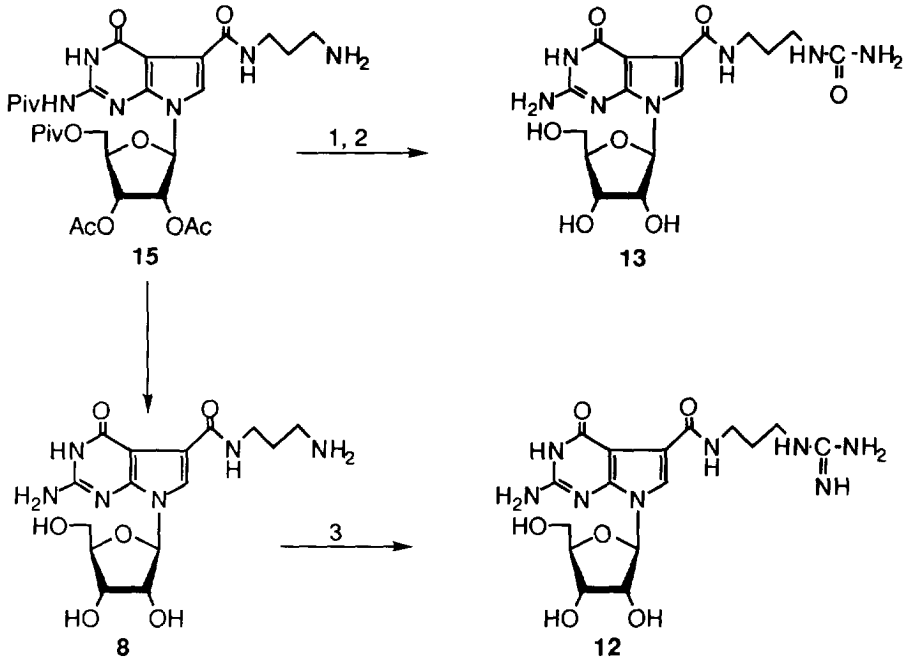
Scheme 1 *Reagents and Conditions*: 1) a: HCl gas, MeOH, 0 °C, 2 h, b: H_2O, 0 °C, 1.5 h; 2) 35 % H_2O_2, NH_4OH, H_2O, MeOH, rt, 2 h; 3) aminoiminomethanesulfonic acid, K_2CO_3, H_2O, 35 °C, 5 h.

Ribofuranosyl compounds **10**, **11**, **12**, **13**, and **14** were prepared as shown in Schemes 2 and 3. By employing a reaction similar to that described for compound **4** from **3**, we obtained ribo-methylester **10** in 92 % yield from **9** which was prepared as described before.[4] Treatment of **10** with methanolic ammonia provided ribo-amide **11** in 99 % yield. Conversion of the cyano group of **9** to thioamide **14** was accomplished by the reaction with H_2S in pyridine in 85 % yield.[8]



Scheme 2 *Reagents and Conditions* : 1) a) HCl gas, MeOH, 0 °C, 2 h, b) H_2O, 0 °C, 2h; 2) NH_3 gas, MeOH, rt, 14 h, 3) H_2S gas, Et_3N, pyridine, rt, 14h.

Guanidination of compound **8** prepared from **15**[4] with AIMSA in the presence of aqueous potassium carbonate provided ribo-guanidine **12** in 64 % yield. Ribo-urea **13** could be prepared from compound **15**[4] by treatment with trimethylsilyl isocyanate in CH_2Cl_2 followed by deprotection with methanolic ammonia in 52 % overall yield.

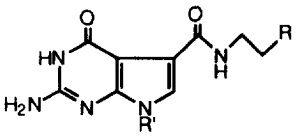


Scheme 3 *Reagents and Conditions* : 1) TMS-N=C=O, CH_2Cl_2, rt, 14 h; 2) NH_3 gas, MeOH, sealed tube, 70 °C, 72 h; 3) aminoiminomethanesulfonic acid, K_2CO_3, H_2O, rt, 5h.

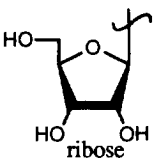
Biological activities

The newly synthesized compounds **3-6** and **9-14** were evaluated for their inhibition of PI 4-kinase. As shown in Table 1, compounds **5** and **6** were found to inhibit the enzyme approximately at the same level as echiguanines A and B, having IC_{50} values of 0.02 and 0.15 μg/ml, respectively. It is also noteworthy that their ribofuranosides **11**, **12**, and **13** effectively inhibited PI 4-kinase, although ribofuranosyl echiguanines **7** and **8** only weakly inhibited or did not inhibit the enzyme. Next, we examined the activity of compound **11** in cultured human epidermoid carcinoma A431 cells. Treatment of the cells with 100 μg/ml of **11** increased the amount of PI, the substrate of PI 4-kinase, when the cells were labeled with ^{32}P-labeled phosphate. The product, PI monophosphate, could not be detected in this assay, possibly because it would be readily metabolized. Thus, **11** was suggested to inhibit PI 4-kinase in cultured A431 cells.

Table 1 Inhibition of PI 4-kinase by echiguanine and ribofuranosyl analogs.



echiguanine analogs



ribose

R	echiguanine analogs (R'=H) IC_{50} (μg/ml)	ribosyl echiguanine analogs (R'=ribose) IC_{50} (μg/ml)
$C(=NH)NH_2$	0.03 (echiguanine A)	100
CH_2NH_2	0.18 (echiguanine B)	>100
CN	0.45	>100
COOMe	0.30	25
$CONH_2$	0.02	2.4
$CH_2NHC(=NH)NH_2$	0.15	7.5
$CH_2NHCONH_2$	N. P.*[9]	1.0
$CSNH_2$	N. P.[9]	>100

The membrane fraction of A431 cells was incubated with [γ-^{32}P] ATP and the chemical for 30 min at 20°C[3].

* N.P. , not prepared

In conclusion, a series of novel *N*-carboxamide-substituted 7-deazaguanine-7-carboxamides and their ribofuranosyl glycosides was prepared, and several compounds reported herein showed significant improvement in biological properties compared with echiguanines and their glycosides. Particularly, ribofuranosyl amide **11** effectively inhibited PI 4-kinase *in vitro* and was also suggested to inhibit the enzyme in cultured cells. Thus, it may possess increased ability to permeate the cell membrane. This encouraging result prompts us to discover further improved inhibitors and to study comprehensive structure-activity relationships.

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