# Synthesis and Antitumor Activities of Heterocycle-substituted alaninine amides

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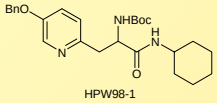
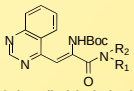
## Purpose:

Azatyrosine, isolated from Streptomyces chibanensis, was reported to selectively inhibit the growth of ras-transformed NIH3T3 cells. However antitumor activity was limited (IC50 at 16.5 mM) due to its low intracellular bioavailability. Structural modification of azatyrosine in this laboratory lead to a series of amide analogues, which demonstrated selective inhibition against ras-transformed NIH3T3 cells (HP Wang, et al,. 2005). In order to evaluate the antitumor activity, the compounds were subjected for in vitro screening on ras-mutated human cancer cell lines and the lead compound was evaluated for antitumor activity in mice bearing human tumor.

L-azatyrosine



HPW98-1



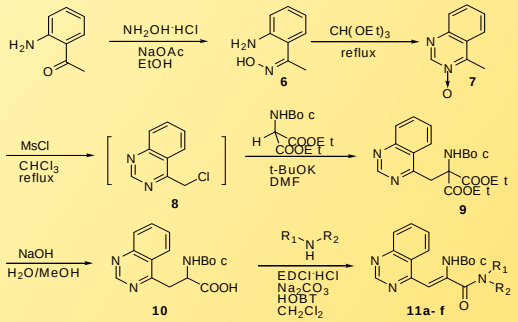
4-Quinazolinylalaninylamides

## Method:

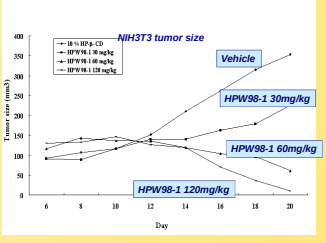
Thirteen pyridylalanine amides and six 4-quinazolinylalanine amides were synthesized in this laboratory. The compounds were screened on SW480 human colon cancer, T24 human bladder cancer and PC-3 human prostate cancer cell lines and tumor growth was evaluated with MTT assay. One of the pyridylalanine amides HPW98-1, was tested for in vivo activity in SCID mice bearing SW620 human colon cancer by i. p. injection at a dose level of 25mg/kg/day 3 days after dorsal subcutaneous inoculation of cancer cells (1.5X107 cells) to the animals.

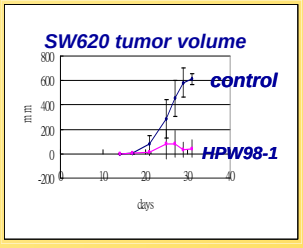
## Results:

### Chemical Synthesis:

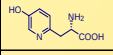
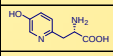
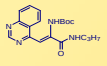
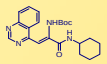
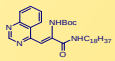
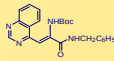
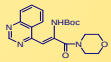



### Tumor Inhibition:




### Cytotoxicity:

| Compound | Structure | $IC_{50}$ (μM) NIH3T3-ras | $IC_{50}$ (μM) NIH3T3-wild | T24 IC50(μM) | % growth at $10^{-4}$ M |
|---|---|---|---|---|---|
| azatyrosine |  | 7554±417 | 10793±471 | 3455.0 | -- |
| 11a |  | 10 | >100 | >100 | -- |
| 11b |  | 30 | 100 | >100 | 28% NCI-H460 lung 31% MCF7 breast63% SF-268 CNS |
| 11c |  | 2 | 60 | 32 | 1% NCI-H460 lung 14% MCF7 breast17% SF-268 CNS |
| 11d |  | 13 | 23 | 17 | -- |
| 11e |  | 14 | 16 | 100 | -- |
| 11f |  | >100 | 15 | >100 | -- |

## Discussion:

The pyridylalanine amides and 4-quinazolinylalanine amides exhibited in vitro activity against SW480 human colon cancer, T24 human bladder cancer and PC-3 human prostate cancer cell lines, with $IC_{50}$ ranged between $10^{-5}$-$10^{-6}$M (mean ±SD of 3-6 experiments). HPW98-1 significantly inhibited the growth SW620 colon cancer cells ($p<0.05$). Tumor mass was detected in 5 out of 10 mice in the test group after 21 days of drug treatment, while it was observed in all ten mice of the control group. The tumor volumes of the control and the test groups after 58 days of drug treatment were 2634.7±549.9 mm3 (n=5) and 518.9±722.3 $mm^3$ (n=10), respectively. This compound also proved to have wide safety margin with maximum tolerated dose over 400 mg/kg.

## Conclusion:

HPW 98-1, an azatyrosinamide, greatly inhibited tumor size in animal models. Tumor size were 100% inhibited in NIH3T3 animal model with 60 mg/kg of oral administrated HPW98-1, and in SW620 animal model, tumor size were reduced to 7% to that of control. However, 4-quinazolinyl alaninylamides, modified from HPW98-1, showed marginal antitumor activities. Chemical modifications for optimization of biological activities need to be further invistigated.

## Acknowledgement:

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