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Synthesis of Mannich Bases of Antineoplaston A10 and their Antitumor Activity

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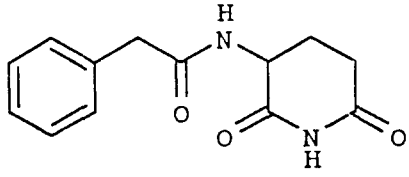
Some Mannich bases of Antineoplaston A10 which is antitumor agent under clinical investigation were synthesized and tested for cytotoxicity. The tested compounds **(2a, 2b, 2d)** showed good activity comparable to that of carboplatin.

Key words: Antineoplastic peptides, Antineoplaston A10, Piperidinedione derivatives, Mannich base, Antitumor agent

INTRODUCTION

Antineoplaston A10, 3-(N-phenylacetylamino)-2,6-piperi-dinedione is a natural compound isolated from human urine, identified, synthesized and shown to have various biologic activity such as antitumor, anti-AIDS, anti-Parkinsonism, and anti-neuropsychiatric disorders (Burzynski *et al.*, 1983; Burzynski, 1992a; Burzynski, 1992b; Hendry *et al.*, 1987). Accordingly to Burzynski, antineoplastons are small peptides and amino acid derivatives produced by the living organism and constitute a natural biochemical defense system against cancer (Burzynski, 1976; Burzyski, 1986a; Burzynski, 1986b).



A10

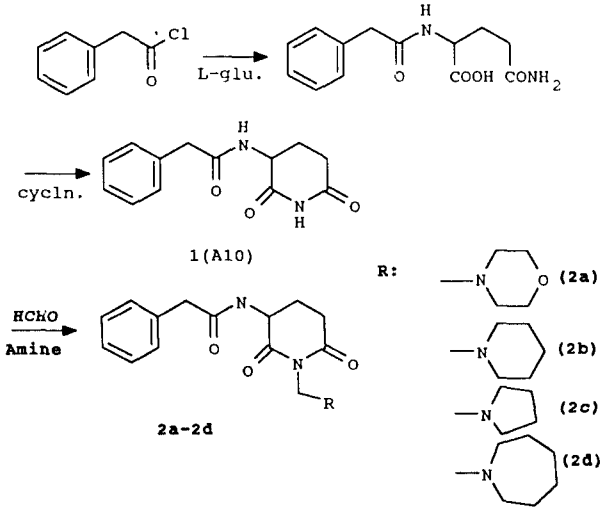
Recently, chemical synthesis and antineoplastic activity of A10 derivatives introducing some substituents to phenyl ring moiety of A10 have been studied by Hendry et al (Hendry *et al.*, 1990). According to the report, p-hydroxyphenyl and p-aminophenyl derivatives of A10 showed good antineoplastic activity.

On the other hand Mannich bases of NH-acidic compounds such as bis(2,6-dioxopiperazines) (Jun *et al.*, 1987) and thalidomide (Werner and Fritzshe, 1969) have been synthesized for finding improved antitumor activity.

In the present study, we synthesized the Mannich bases of A10 and tested their cytotoxicity against five solid tumor cell lines as potential antitumor agents.

MATERIALS AND METHODS

Phenylacetyl chloride and L-glutamine were purchased from Aldrich chemical Co. and other reagents and solvents were used of extra pure grade.



Scheme 1.

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Melting points were determined on a Gallenkamp MFB 030 G apparatus and are uncorrected. The IR spectra were recorded on a PERKIN-ELMER 783 spectrophotometer using KBr pellets. ^{1}H-NMR spectra were obtained on a BRUCKER AC80 fourier transform spectrometer for the 80-MHz ^{1}H-NMR spectra, using TMS as internal standard; chemical shifts are reported in parts per million(δ) and signals are quoted as a s(singlet), d(doublet), t(triplet), q(quartet) or m(multiplet). Mass spectra were recorded on a SHIMAZU GCMS QP2000A spectrometer. TLC was carried out using plates coated with silicagel 60F 254 purchased from MERCK Co.

3-Phenylacetylamino-2,6-piperidine-dione(A10,1)

A10 was synthesized from phenylacetyl chloride and L-glutamine via N-phenylacetyl-L-glutamine according toliterature (Burzynski, 1990).

mp.: 200-201℃ (200-202℃) (Xu *et al.*, 1988)

yield: 5.06 g (29.4%)

^{1}H NMR (DMSO-d_6): 1.94 (2H, m, $CH_2\underline{CH_2}CO$), 2.50 (2H, m, $CH_2\underline{CH_2}CO$), 3.44 (2H, s, $Ar\underline{CH_2}CON$), 4.38-4.70 (1H, m, $N\underline{CH}CO$), 7.28 (5H, S, Arom.H), 8.38 (1H, d, J=10 Hz, $CO\underline{NH}$-ring), 10.75 (1H, s, $CO\underline{NH}CO$)

MS: m/e 246(M^+)

The N-Hydroxymethylamines(a-d)

General procedure: To a solution of 37% formaline (2.26 ml, 30 mMol) cooled in an ice-salt bath, amines (2.60 ml, 30 mMol) was added dropwise with stirring. The mixture was stirred at 0℃ for 150 min. Anhydrous K_2CO_3 was then added in small portions until an oily layer formed. This was separated and dried over anhydrous K_2CO_3. During the entire preparation the temperature was kept below 5℃, and the product was kept in a refrigerator.

The Mannich bases of A10 (2a-2d)

Genaral procedure: A mixture of 0.49 g (2 mMol) of A10, 0.47 g (4 mMol) of N-hydroxymethylamines and 20 ml of ethyl acetate was refluxed for 20 min and evaporated to dryness according to the literature (Wemer and Fritzsche, 1969). To this oily residue benzene was added for crystallization. Analytical sample was recrystalized from benzene (see Table 1).

Antitumor test *in vitro*

The cell used for the experiment, ie A-549 (human non-small cell lung), SKOV-3 (ovarian carcinoma), HCT-15(colon), XF-498(CNS), SK-MEL-2(melanoma) were obtained from the National Cancer Institute(NCI) in USA. The cells were grown at 37℃ in RPMI 1640 medium supplemented with 10% FBS and separated using PBS containing 0.25% typsin and 3 mM EDTA. 5×10^3-2×10^4 cells were added to each well of 96 well plate and incubated at 37℃ for 24 h. For the bioassay, the desired quantity of each compound was dissolved in DMSO and diluted with the above medium at five different concentrations with the range of 10-1000 g /ml. The final DMSO concentration was below 0.5%.

Table I. Physical data for Mannich bases of A10

No.	mp. (℃)	Yield (%)	IR (KBr, cm $^{-1}$)	^{1}H-NMR ($CDCl_3$, δ)	MS (m/z, M^+)
2a	147-148	74.3	3280(amide NH) 1680, 1740 (imide C=O) 1640(amide C=O)	1.54-1.99(2H, m, $\underline{CH_2}CH_2CO$), 2.58 (4H, t, J=4.8 Hz, $\underline{CH_2}N\underline{CH_2}$), 2.68-2.90(2H, m, $CH_2\underline{CH_2}CO$), 3.64(2H, s, Ar-$\underline{CH_2}CO$), 3.64(4H, t, J=4.8 Hz, CH_2OCH_2), 4.46(1H, m, $N\underline{CH}CO$), 4.68(2H, d, J=3.6 Hz, $N\underline{CH_2}N$), 6.28(1H, b, $CO\underline{NH}CH$), 7.33 (5H, s, arom.H)	345
2b	113-115	99.4	3330(amide NH) 1680, 1730 (imide C=O) 1660(amide C=O)	1.44-1.55(6H, m, $\underline{CH_2CH_2}CH_2$), 1.55-1.90(2H, m, $\underline{CH_2}CH_2CO$), 2.42-2.53(4H, m, $\underline{CH_2}N\underline{CH_2}$), 2.66-2.92(2H, m, $CH_2\underline{CH_2}CO$), 3.64(2H, s, Ar-$\underline{CH_2}CO$), 4.50(1H, m, $N\underline{CH}CO$), 4.69(2H, d, J=3.2 Hz, $N\underline{CH_2}N$), 6.40(1H, b, $CO\underline{NH}CH$), 7.33(5H, s, arom.H)	343
2c		26.3	3280(amide NH) 1730(imide C=O) 1650(amide C=O)	1.60-1.86(5H, m, $\underline{CH_2}CH_2CO$, $CH_2\underline{CH_2CH_2CH_2}CH_2$), 2.30-2.50(2H, m, $CH_2\underline{CH_2}CO$), 2.51-2.86(4H, m, $\underline{CH_2}N\underline{CH_2}$), 3.62(2H, s, $Ar\underline{CH_2}CO$), 4.51(1H, m, $N\underline{CH}CO$), 4.75(2H, d, J=2.8 Hz, $N\underline{CH_2}N$), 6.48(1H, b, $CO\underline{NH}CH$), 7.31(5H, s, arom.H)	329
2d	125-126	94.2	3280(amide NH) 1680, 1730 (imide C=O) 1660(amide C=O)	1.33-1.65(8H, m, $\underline{CH_2CH_2CH_2}CH_2$), 1.73-1.90(2H, m, $\underline{CH_2}CH_2CO$), 2.40-2.64(2H, m, $CH_2\underline{CH_2}CO$), 2.63-2.94(4H, m, $\underline{CH_2}N\underline{CH_2}$), 3.64(2H, s, Ar-$\underline{CH_2}CO$), 4.50(1H, m, $N\underline{CH}CO$), 4.78(2H, d, J=2.4 Hz, $N\underline{CH_2}N$), 6.37(1H, b, $N\underline{CH}CO$), 7.33(5H, s, arom.H)	357

Table II. *In vitro* Antitumor Activity

No.	ED_{50} (μg/ml) A549	SK-OV-3	SK-MEL-2	XF498	HCT15
(A10)	>1000	>1000	>1000	>1000	>1000
2a	28.60	28.63	24.25	34.51	25.34
2b	26.35	52.11	24.99	48.78	26.59
2c	251.10	275.60	258.38	172.52	197.83
2d	25.59	25.57	27.09	28.43	27.26
carboplatin	34.59	11.56	12.55	33.75	37.31

A portion 200 L of the solution was added to above well plates. The plates were placed in 5% CO_2 incubator for 48 h. Thereafter, the protein stain assay was performed according to SRB method.

RESULT AND DISCUSSION

Chemistry

The synthesized product of A10 had an identical melting point, TLC pattern, IR spectrum and mass spectrum as the 3-phenylacetylamino-2,6-piperidinedione obtained from Aldrich Chemical Co. The aminomethylation of A10 by morpholine, piperidine, pyrrolidine and hexamethyleneimine affords the final products (**2 a-2d**). By means of IR spectroscopy it is shown that absorption bands at 3180 cm^{-1} corresponding to N-H vibrations of the cyclic imide was disappeared. The ^{1}H-nuclear magnetic resonance(NMR) spectra exhibited cyclic CH_2 proton of amines of Mannich bases as multiplet at 1.44-1.99 ppm, whereas NH peak of cyclic imide of A10 near 10.75 ppm was disappeared by bond formation. The mass spectra showed the correct molecular peak for the products respectively. These results confirmed the formation of Mannich bases.

Biological Activity

Several papers reported that A10 showed cytostatic activity against human carcinoma of breast cell line MDA-MB-231 (Burzynski *et al.*, 1983; Burzynski, 1985). In present reseach, A10(1) has been shown to devoid of cytotoxicity against five solid tumor cell lines tested in 10-1000 μg/ml range. However, the morpholinomethyl (**2a**), piperidinomethyl (**2b**) and homopipiridinomethyl derivatives (**2d**) of Mannich bases exhibited appreciable cytotoxic activity in tested tumor cell lines. But pyrrolidinomethyl derivative (**2c**) showed weak activity. Compared with carboplatin, two Mannich bases (**2a** and **2d**) exhibit similar cytotoxicities against A-549 (lung) and HCT-15(colon) tumor cell lines.

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