Eur. J. Med. Chem. **24** (1989) 455–457

Effect on DNA synthesis and cell proliferation of some Mannich bases derived from 1-phenyl 2-propanones

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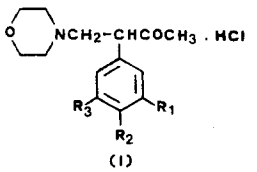
(Received September 22, 1988, accepted January 30, 1989)

cytotoxic activity (Mannich bases) / NMR spectroscopy / 1-phenyl-2-propanones

Introduction

Mannich bases exhibit various biological responses [1] and are active against certain carcinomas, including the Ehrlich ascites carcinoma [2], the sarcoma 180 screen in mice [3], and more recently P388 lymphocytic leukemia [4].



(I)

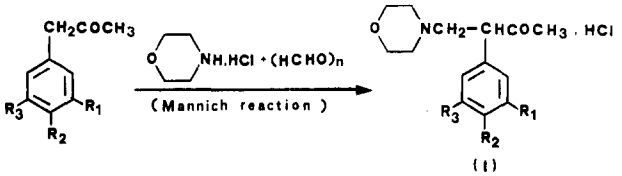
In addition, the same category of compounds has shown interesting anti-viral activity [5]. These observations led us to prepare a series of Mannich bases derived from 1-phenyl-2-propanones. The compounds prepared correspond to the general formula **I**. These compounds were studied for their effect on the DNA synthesis and cell division using cycling Chinese hamster ovary (CHO) cells [6]. The experiments performed [7] showed that the action of these products is as follows: 1) they induced DNA damage, and 2) cell proliferation was greatly reduced.

Chemistry

We previously reported [8] that, when the Mannich reaction is applied to phenylpropanones, the direction of the reaction depends upon the site of the substituents on the phenyl moiety. When the 2′-position of the phenyl propanone is not substituted, the $N-CH_2-$ group is directed to the methylene of the ketone with the formation of compounds of formula **A**. On the other hand, when there is substitution on the 2′,3′-position of the phenylpropanone, the reaction is directed to the methyl of the ketone which eventually leads to the formation of compounds of formula **B**.

(A) (B)

In the present study, the morpholine Mannich bases prepared corresponded to formula **A**, as confirmed by the NMR data. They were prepared by refluxing the appropriate 1-phenyl-2-propanone with morpholine hydrochloride and paraformaldehyde in absolute ethanol [8] (Scheme 1).



(I)

Scheme 1. The phenyl-2-propanones were prepared by reductive hydrolysis of the corresponding nitroalkenes [8, 9].

Conclusion

The products tested (T_{5310}, T_{5311}, T_{5313}, T_{5314}) were dissolved in McCoy's 5A medium without calf serum. CHO cells were cultured for 24 h as described under Experimental protocols. Increasing amounts of T_{5310}, T_{5311}, T_{5313} and T_{5314} were added after incubations for 2 h and were then incubated for an additional 22 h.

The data obtained for 15 and 30 $\mu g/ml$ of culture medium for T_{5310} have shown that the number of labeled

cells was decreased by almost a factor of 3 compared the untreated cells. On the other hand, the number of labeled cells when T_{5311} was used exhibited a reduction by a factor greater than 4 for the 15 μg / ml concentration and it was drastically reduced when the 30 μg / ml concentration was used. The mitotic indices were lowered as well.

In the case of T_{5313} the differences were not as pronounced compared to the untreated cells for both concentrations used. However, when T_{5314} was added, the number of labeled cells and the mitotic indices exhibited considerable decreases and, in the 15 μg / ml concentration, the culture cells were almost destroyed.

Table I.

Product	Dose (μg / ml)	Counts per min (cpm)	MI
		Control 67370	
T_{5310a}	15	23304	100
T_{5310b}	30	22122	98
T_{5311a}	15	15132	26
T_{5311b}	30	842	6.5
T_{5313a}	3	60942	85
T_{5313b}	5	46524	80
T_{5314a}	3	8644	46
T_{5314b}	5	430	0

Experimental protocols

Chemistry

All melting points and boiling points are uncorrected. Elemental analysis were within ±0.4 of the theoretical values and were carried out by the Service Central de Microanalyses du CNRS (Paris). IR spectra were recorded using a Perkin–Elmer 177 infrared spectrophotometer. NMR spectra were determined on a Varian 60 Hz spectrometer.

1-Phenyl-2-propanones
The method of Shepard *et al.* [9] for the reductive hydrolysis of nitroalkenes was used. The only ketone not mentioned earlier in the literature was the 1-(3′-methoxy-4′-*n*-propoxy phenyl)-2-propanone, bp: 130–135°C (0.4 mm Hg) Analysis. for $C_{13}H_{18}O_3$: C, H.

3-(3′,4′-Dialkoxyphenyl)-4-(4-morpholinyl)-2-butanones (Table II)
The synthesis of the derivatives listed in Table II was accomplished by the following general method: a mixture of the appropriate 2-propanone (0.025 mol), morpholine hydrochloride (0.041 mol), paraformaldehyde (0.041 mol) and conc. hydrochloric acid (0.5 ml) in absolute ethanol (50 ml) was heated under reflux for 5–6 h. After the evaporation of the solvent, the residue was extracted with ether to remove the unreacted ketone. The residue was alkalinized with potassium carbonate and extracted with ether. The ethereal extracts were washed with water until neutral to litmus and were dried with anhydrous sodium sulfate. Removal of the solvent gave the Mannich bases which were converted into the corresponding hydrochlorides. The salts were crystallized from EtOH–Et_2O.

NMR spectroscopy

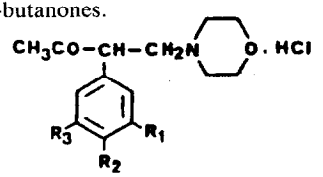
T_{5310}
^{1}H NMR ($CDCl_3$, 60 Mz) δ ppm: 1.20–1.90 (m, 3H, CH_3CH_2O); 2.33 (s, 3H, CH_3–C=O); 2.46–3.10 (m, 6H, 3,5-morpholine-H, CH_2N); 3.50–4.48 (m, 7H, CH_3CH_2O, *CH*-Ar, 2,6-morpholine-H); 4.00 (s, 3H, CH_3O); 6.73–7.15 (m, 3H, aromatic).

T_{5311}
^{1}H NMR ($CDCl_3$, 60 Mz) δ ppm: 2.03 (s, 3H, CH_3–C=O); 2.14–2.75 (m, 6H, 3,5-morpholine-H, CH_2N); 3.39–3.95 (m, 14H, 2,6-morpholine-H, *CH*-Ar, 3×CH_3O); 6.30 (s, 2H, aromatic).

T_{5312}
^{1}H NMR ($CDCl_3$, 60 Mz) δ ppm: 2.10 (s, 3H, CH_3–C=O); 2.25–2.66 (m, 6H, 3.5-morpholine-H, CH_2N); 3.33–3.98 (m, 5H, 2,6-morpholine-H, *CH*–Ar); 5.90 (s, 2H, OCH_2O); 6.33–7.06 (m, 3H, aromatic).

T_{5313}
^{1}H NMR ($CDCl_3$, 60 Mz) δ ppm: 0.90–1.50 (m, 3H, $CH_3(CH_2)_2O$); 1.60–2.35 (m, 2H, $CH_3CH_2CH_2O$); 2.24 (s, 3H, CH_3–C=O); 2.40–2.90 (m, 6H, 3,5-morpholine-H, CH_2N); 3.55–4.25 (m, 7H, $CH_3CH_2CH_2O$; *CH*–Ar, 2.6-morpholine-H); 3.94 (s, 3H, CH_3O); 6.50–7.25 (m, 3H, aromatic).

Table II. 3-(3′,4′-Dialkoxyphenyl)-4-(4-morpholinyl)-2-butanones.



Code no.	R_1	R_2	R_3	Formula	Yield (%)	mp (°C)	Analyses			
							% Calc.		% Found	
							C	H	C	H
T_{5310}	OCH_3	OC_2H_5	H	$C_{17}H_{26}ClNO_4$	45	135	59.38	7.62	59.30	7.82
T_{5311}	OCH_3	OCH_3	OCH_3	$C_{17}H_{26}ClNO_5$	20	167–8	56.74	7.28	56.70	7.19
T_{5312}	>O–CH_2–O<		H	$C_{15}H_{20}ClNO_4$	47	154	57.42	6.42	57.21	6.41
T_{5313}	OCH_3	OC_3H_7	H	$C_{18}H_{28}ClNO_4$	38	143	60.41	7.89	60.35	7.82
T_{5314}	OCH_3	OC_4H_9	H	$C_{19}H_{30}ClNO_4$	37	151–2	61.36	8.13	61.27	8.08

T_{5314}
^{1}H NMR ($CDCl_3$, 60 MHz) δ ppm: 0,70–1.18 (m, 3H, $CH_3(CH_2)_3O$); 1.21–2.26 (m, 4H, $CH_3(CH_2)_2CH_2O$); 2.13 (s, 3H, $CH_3-C=O$); 2.30–2.80 (m, 6H, 3,5-morpholine-H, CH_2N); 3.51–4.19 (m, 7H, $CH_3(CH_2)_2CH_2O$, CH–Ar, 2,6-morpholine-H); 3.83 (s, 3H, CH_3O); 6.75 (s, 3H, aromatic).

Pharmacology

Methods used to estimate biological activities
CHO culture. Chinese hamster ovary (CHO) cells were grown in McCoy's 5A culture medium supplemented with 10% FCS (fetal calf serum) and antibiotics. Approximately 1×10^6 CHO cells were transferred to a flask with 5 ml of medium and gassed with 5% CO_2. The flasks were incubated at 37°C for 24 h.

After 20 h, [^{3}H]thymidine (1 μCi / ml; sp. act. 5 Ci / mM) was added to the culture medium and, at 22 h, 0.1 ml of colcemid (Gibco) was added.

The cells were subsequently incubated for 2 h. At the end of this labeling period, a fraction (0.5 ml) of the culture was removed, the cells were washed twice with Hanks' salt solution, then taken up in a solution of sodium dodecyl sulfate (1.5%) in 0.01 M Tris buffer, pH 7.4, with 0.01 M EDTA, sodium hydroxide was added at a final concentration of 0.3 M, the mixture was precipitated with trichloroacetic acid at a final concentration of 5% and collected on Millipore filters. These were washed 3 times with 5% trichloroacetic acid, dried and counted in a scintillation counter.

From the remaining culture (4.5 ml) about 1×10^6 mitotic cells were harvested by selective detachment and transferred to a culture tube. The cells were harvested by hypotonic solution (0.075 M KCl) treatment and fixed with acetic acid-methanol. Slides were prepared by the air dried method and stained with Giemsa.

Mitotic indices (MI). Indices were calculated after analysis of 500 cells for each sample. Early prophases were not included in the determination of *MI* because of uncertainty in recognition.

The experiments were performed in triplicate for each concentration of the products.

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