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Anticancer and Antimicrobial Studies on Mannich Bases of β-Diketones

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Anticancer and antimicrobial activities of some 2-(N-aryl/heteroarylaminomethyl)-1,3-diphenyl/1-phenyl-3-(3-nitrophenyl)/1-phenyl-3-(pyridin-3-yl) propan-1,3-diones were determined by adopting standard methods. Among the compounds tested the compounds **IX** and **VI** excel in their anticancer activity. In antibacterial screening, the same compounds showed more appreciable activity than the standard. In antifungal screening, all the compounds showed activity at 100 μ/mL.

Key Words: Mannich base, Diketones, Anticancer, antimicrobial.

β-diketones are considered to be the most important group of dicarbonyl compounds because of their usefulness as versatile intermediates for the synthesis of various heterocycles with different biological activities[1, 2]. Mannich bases of β-diketones are reported to possess various biological activities such as antimicrobial[3], anticancer[4] and analgesic[5]. Prompted by these findings, and as a continuation of our earlier reported substituted propan-1,3-diones[6] which has shown significant *in vitro* short term cytotoxicity, the anticancer and antimicrobial activities of some 2-(N-aryl/heteroarylaminomethyl)-1,3-diphenyl/1-phenyl-3-(3-nitrophenyl)/1-phenyl-3-pyridin-3-yl-) propan-1,3-diones is explored. The title compounds (Fig. 1) were prepared using the methods that were reported earlier[6].

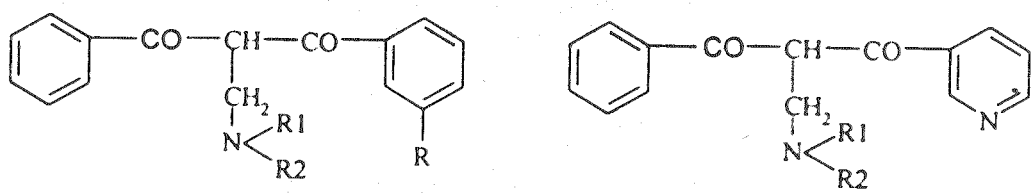

Fig. 1. Structures of Title Compounds

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Compounds	R	
I	H	Anilino
II	H	Morpholino
III	H	(1,3-thiazolin-2-yl-) amino
IV	NO_2	Anilino
V	NO_2	Morpholino
VI	NO_2	(1,3-thiazolin-2-yl-) amino
VII	—	Anilino
VIII	—	Morpholino
IX	—	(1,3-thiazolin-2-yl-) amino

EXPERIMENTAL

Melting points were determined in open capillary tubes on a Veego VMP-1 melting point apparatus and are uncorrected. UV data were recorded on UV-Shimadzu spectrophotometer 160 Å, IR spectra were recorded in KBr on a Perkin-Elmer infrared 1600 spectrophotometer (cm^{-1}), PMR spectra were recorded on a Perkin Elmer EM-390 (90 MHz) instrument.

The title compound 2-[N-(1,3-thiazolin-2-yl)-aminomethyl]-1-phenyl-3-(pyridin-3-yl) propan-1,3-dione (**IX**) was prepared by dissolving 1-phenyl-3-(pyridin-3-yl) propan-1,3-dione (2.37 g, 0.01 mol), 30% aqueous formaldehyde (0.3 g, 0.012 mol), concentrated HCl (2 mL) and 2-amino-2-thiazoline (1.1 g, 0.012 mol) in methanol (20 mL); the solution was stirred for 1 h at room temperature and refluxed on a water bath for 5 h. The reaction mixture was poured on to crushed ice, with stirring; the resultant solution was neutralized with sodiumbicarbonate solution (10%) and the product obtained was filtered, washed thoroughly with cold water, dried and recrystallized from ethyl acetate. Yield: 1.8 g (55%), m.p. 225–226°C; IR (KB, cm^{-1}): 3396 ν(NH, str.), 1652, 1670 ν(C=O, str.), 1590 ν(NH, bend.), 1631(C=N, str.). UV (λ_{max}) 267 nm. PMR (DMSO-d_6) spectrum exhibited characteristic bands (in δ ppm) at 1.75 (t, 1H, CO—CH—CO), 3.29 (d, 2H, —CH_2—), 5.95 (s, 1H, —NH) and 6.50–8.50 (m, 13H, Ar—H). Similarly, the compounds **I–VIII** were prepared. The starting material, 1-phenyl-3-(pyridin-3-yl) propan-1,3-dione was synthesized from 2,3-dibromo-1-phenyl-3-(pyridin-3-yl) propan-1-one, as reported earlier[6].

Encouraged by the results of *in vitro* studies that were reported earlier[6], all the title compounds were selected for *in vivo* studies. Acute toxicity studies were carried on Swiss albino mice using standard method[7] by oral administration of various doses of test compounds (250–1000 mg/kg body weight). Acute toxicity studies indicated that all the test compounds were found to be non-toxic up to a dose as high as 1000 mg/kg body weight which was chosen as a standard test dose in the *in vivo* anticancer studies.

In vivo anticancer studies[8, 9] were performed on Swiss albino mice using DLA cells as cancer cell lines. All the test compounds were prepared as a fine suspension in 0.3% w/v CMC and administered orally 100 mg/kg doses. The DLA cells were propagated in the intraperitoneal cavity of the mice by injecting 1×10^6 cells/mL. Treatment was started 24 h after tumour inoculation once daily for 10 d. The body weights of the individual mice were recorded at daily intervals and also increase in

life span of mice was calculated from the formula, % ILS = [(T – C/C] × 100, where T is the number of days the treated animals had survived and C is the number of days the controlled animals had survived.

RESULTS AND DISCUSSION

All the animal experiment protocols have met with the approval of the Institutional Animals Ethics Committee. Increase in body weight, mean survival time (MST) and percentage increase in life span (% ILS) of the experimental animals were taken as main parameters in the study. It was found that all the test compounds considerably opposed the average increase in the body weight of the carcinoma induced mice, which was comparable to the reference standard methotrexate, however, at hundred-fold higher concentrations.

Particularly, compounds **IX** and **VI** were found to be superior in their action. A drastic increase in the body weight was noted in DLA tumor bearing mice. On treatment with **IX**, a significant reduction in body weight was observed when compared with the positive control on the day 15.

Treatment revealed that the MST was a maximum of 24 d for compound **IX** in comparison with the untreated group. The % ILS of the treated groups (administered with a dose of 100 mg/kg body weight) was 60% for compond **IX** and showed significant anticancer property. The above result indicated that compound **IX** is excellent in its anticancer activity, while compound **VI** is next in the order of potency with 18.83 d MST and 26% ILS.

Even certain other parameters like the significance of difference between survival time of control and groups treated with reference to standard and the test compounds were also taken into consideration to evaluate the anticancer profile of the test compounds and these data were also in agreement with the data given in Table-1.

TABLE-1
EFFECT OF TEST COMPOUNDS ON MICE INOCULATED WITH DLA CELLS

Group	Drug and dose (mg/kg)	Increase in body weight	% Increase in body weight	MST (d)	% ILS
1	CMC (0.3% w/v)	18.06 ± 0.86	95.60	15.16 ± 0.35	—
2	Methotrexate (1.3)	9.60 ± 0.56*	50.50	19.13 ± 0.42*	28.80
3	I (100)	7.83 ± 0.60*	41.20	18.00 ± 0.68*	20.00
4	II (100)	8.20 ± 0.40*	43.20	17.30 ± 0.50†	15.33
5	III (100)	8.50 ± 0.42*	44.70	16.83 ± 0.40‡	12.20
6	IV (100)	8.70 ± 0.45*	45.80	16.00 ± 0.35†	06.60
7	V (100)	8.20 ± 0.41*	43.20	16.50 ± 0.36†	10.00
8	VI (100)	7.70 ± 0.49*	40.50	18.83 ± 0.48*	25.53
9	VII (100)	9.20 ± 0.47*	48.40	16.40 ± 0.38‡	09.30
10	VIII (100)	9.50 ± 0.56*	50.00	16.67 ± 0.33**	11.13
11	IX (100)	07.50 ± 0.42*	39.40	24.00 ± 0.48†	60.00

Values are Mean ± SEM (n = 6), *p < 0.001, †p < 0.01, ‡p < 0.02, **p < 0.05, *vs.* control group.
The *in vivo* anticancer activity of the synthesized compounds have been evaluated against Dalton's lymphoma ascites cells in Swiss albino mice by injecting 1×10^6 cells in the intraperitoneal cavity.

A regular increase in ascites tumour volume was noted in tumour bearing mice (increase in body weight). Ascites fluid is a direct nutritional source for tumour cells and the faster increase in ascites fluid with tumour growth would possibly be a mean to meet more nutritional requirement of tumour cells. The test compounds increased the MST and lowered the ascites fluid volume (decrease in body weight) to a considerable extent.

The title compounds **I–IX** were investigated for antibacterial activity by agar dilution technique[10] against 15 pathogenic bacteria, procured from National Chemical Laboratory, Pune, India. The medium was prepared as per the instructions of the manufacturer of dry Mueller hint on agar powder (Hi-media). The concentrations of the test samples used were from 5000 μg/mL to lower concentrations made by serial dilutions with DMSO. The minimum inhibitory concentration (MIC) was taken as the lowest concentration (higher dilution) without visible growth. The study was simultaneously performed for the pure standard drugs (pyrimethamine and sulphadoxine) also. The MICs are reported in Table-2.

TABLE-2
ANTIBACTERIAL ACTIVITY OF THE TEST COMPOUNDS

Microorganism	I	II	III	IV	V	VI	VII	VIII	IX	PYRI	SULD
Bacillus subtilis	39	19.5	2.44	312.5	39	2.44	19.5	39	2.44	1250	0.15
Staph. aureus	4.88	39	19.5	78	9.76	2.44	39	4.88	0.075	2500	156.25
Staph. albus	2.44	39	78	312.5	19.5	0.3	2.44	39	0.075	2500	0.3
E. coli	1250	78	625	2.44	78	2.44	625	39	2.44	2500	156.25
Shigella boydi	312.5	78	156.25	9.76	2.44	0.075	19.5	78	0.3	2500	19.5
Sh. dysentriae	156.25	625	78	0.3	39	0.075	19.25	39	0.075	2500	312.5
Enterobacter	1250	625	156.25	39	78	0.3	19.5	312.5	2.44	1250	19.5
P. vulgaris	1250	625	2.44	2.44	19.5	2.44	625	78	2.44	2500	19.5
P. aeruginosa	>5000	625	39	625	312.5	0.15	156.25	78	0.075	>5000	1.22
Sal. typhimurium	312.5	312.5	39	625	312.5	0.075	156.25	19.5	0.3	>5000	>5000
Sal. paratyphi A	2.44	625	78	19.5	78	2.44	39	19.5	0.075	2500	19.58
Sal. paratyphi B	156.25	625	78	39	156.3	0.3	625	488	0.3	2500	19.5
A. hydrophile	9.76	625	78	19.25	78	0.075	39	625	0.3	>5000	1.22
M. morgnaii	625	78	2.44	156.3	39	0.3	78	19.5	0.3	2500	>5000
S. murscesonse	1250	78	4.88	4.88	19.5	2.44	312.5	78	2.44	>5000	>5000

By adopting the same procedure, antifungal screening was also done for the title compounds against three pathogenic fungi at a concentration of 100 μg/mL. The compounds were solubilized in DMSO.

The results in Table-2 indicate that all the compounds exhibited appreciable activity against a variety of microorganisms as compared with the pure standard drugs (pyrimethamine and sulphadoxine). The compounds **VI** and **IX** were more potent than pyrimethamine and sulphadoxine. In antifungal screening, all the compounds showed activity at 100 μg/mL *Candida albicans, Cryptococcus neoformans* and *Aspergillus niger.*

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