TABLE 2: ANALYSIS OF CARVEDILOL TABLETS

Tablet Formulation	Label Claim (mg/tab)	Amount found (mg/tab)	% label claim ±SD	SEM	% Recovery ±SD
Brand A					
Carloc 25 (Cipla)	25	24.72	99.03±0.14	0.063	99.2±0.15
Brand B					
Cardivas 25 (Sun)	25	24.78	99.21±0.07	0.033	99.4±0.12

*Average of five determination

let powder solution containing the equivalent of 25 mg/ml of drug. From the amount of drug found, percentage recovery was calculated.

Carvedilol exhibited maximum absorption at 285 nm and obeyed Beer's law in the concentration range of 4-36 μg/ml. The percentage recovery value between 99.2% and 99.4% (Table 2) indicates that there is no interference of the excipients present in the formulations. The study was made to test ruggedness of the method through an interday and intraday analysis of samples. Results obtained confirmed ruggedness of the method. The developed method was found to be accurate, precise, repeatable, reproducible and stability indicated and can be used for the routine analysis of carvedilol in bulk drug and formulations.

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In Vitro Cytotoxic Studies of Mannich Bases of β-Diketones

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***In vitro* cytotoxic activity of some 2-(N-aryl/heteroaryl aminomethyl)-1,3-diphenyl/1-phenyl-3-(3-nitrophenyl)/1-phenyl-3-(pyridin-3-yl)propan-1,3-diones were determined by adopting three methods (Trypan blue dye exclusion, Lowry, MTT, 3-(4,5-dimethyl thiazol-2-y1)-2,5-diphenyl tetrazo-**

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lium bromide assay) using HEp-2 cell cultures. Among the compounds tested the compound VIII was found to be superior in its cytotoxicity in all the three methods.

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], 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, we have aimed to study the *in vitro* cytotoxic activity of a some 2-(N-aryl/heteroarylaminomethyl)-1,3-diphenyl/1-phenyl-3-(3-nitrophenyl)/1-phenyl-3-pyridin-3-yl-) propan-1,3-diones. The title compounds (fig. 1) were prepared using the methods that were reported earlier from our laboratory[6].

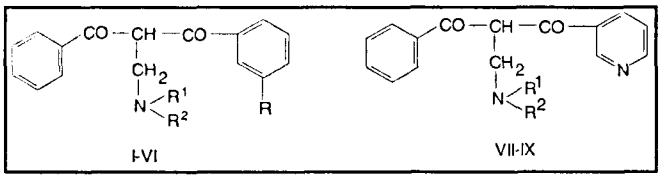

Fig. 1: Structures of title compounds

For compound I, R=H, R^1 and R^2=anilino; for compound II R=H, R^1 and R^2=morpholino; for compound III, R=H, R^1 and R^2=(1,3-thiazolin-2-y1-)amino; for compound IV, R=NO_2, R^1 and R^2=anilino; for compound V, R=NO_2, R^1 and R^2=morpholino; for compound VI, R=NO_2, R^1 and R^2=(1,3-thiazolin-2-yl-)amino; for compound VII, $R^1$and R^2=anilino; for compound VIII, R^1 and R^2=morpholino and for compound IX, R^1 and R^2=(1,3-thiazolin-2-yl-)amino.

Melting points were determined in open capillary tubes on a Veego VMP-1 melting point apparatus and are uncorrected. UV data were recorded on a Shimadzu UV-spectrophotometer 160-A°, IR spectra were recorded in KBr on a Perkin Elmer infra red 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-(pydridin-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 onto crushed ice, with stirring, the resultant solution was neutralized with sodium bicarbonate solution (10%) and the product obtained was filtered, washed thoroughly with cold water, dried and recrystallized from ethyl acetate. Yield: 1.8 g (55%), mp: 225-226°; IR (KBr): 3396 (NH-Stretching), 1652, 1670 (C=0 stretching), 1590 (NH-bending), 1631 (C=N stretching). 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 to 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 we have reported earlier[6].

The compounds (I-IX) were investigated for their cytotoxic activity to determine the cytotoxic tolerance of the HEp-2 cell cultures (procured from Pasteur Institute, Coonoor, Tamilnadu) and grown in Eagle's minimum essential medium (MEM) and CTC_{50} (concentration producing 50% cytotoxicity) was determined by the following methods.

In the trypan blue dye exclusion method[7], cell suspension (0.1 ml/well) containing $6x10^4$ cells in MEM was incubated in microtiter plate for 24 h at 37° in a humidified 5% carbon dioxide incubator (ISW, Mumbai). Different concentrations of test compounds (dilutions made with MEM) were added into 96 well plate, then incubated further for 24 h. At the end of 24 h, trypan blue (0.4%) was added and percentage inhibition was calculated and shown in Table 1.

In the determination of cell metabolite functions by protein estimation (Lowry method)[8], cell suspension (0.1ml/well) containing $6x10^4$ cells/ml in MEM was incubated for 24 h at 37° in a humidified carbon dioxide incubator in presence of various concentrations of compounds (dilutions made with MEM) in a 96 well microtiter plates. Total protein content was estimated by precipitating trypsinated cells with ice cold 11% trichloro acetic acid to remove amino acid pool and serum proteins by treating with alkaline cupric sulphate and folin catechaeu phenol reagent after centrifugation. The results are presented in Table 1.

In the MTT assay[9], the mitochondrial synthesis of the cell was estimated by MTT (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) dye. MTT reacts with succi-

TABLE 1: *IN VITRO* CYTOTOXIC ACTIVITY OF TEST COMPOUNDS

Compoundcode	Trypan Blue [a]CTC_{50}	Protein estimation CTC_{50}	MTT assay CTC_{50}	[b]Avg CTC_{50}
I	1000	803	860	887
II	909	802	833	848
III	829	883	803	838
IV	819	829	791	813
V	893	703	767	787
VI	925	815	847	862
VII	944	714	713	790
VIII	956	606	662	741
IX	833	842	804	826

[a]CTC_{50} is the concentration of the compound in micrograms required to inhibit 50 % of cell growth [b]Avg CTC_{50} is the average CTC_{50} value of all the three methods. Test compounds were dissolved in DMSO. Control indicates the activity of DMSO.

nate dehydrogenase present in the mitochondria and forms a blue colour formazan compound. This was later dissolved in DMSO and estimated colorimetrically at 540 nm. The results are presented in Table 1.

The results of cytotoxic studies revealed that the test compounds exhibited varying degree of activity (CTC_{50} 741 to 887 µg/ml). The compounds 2-(N-aryl/heteroarylamino methyl)-1-phenyl-3-(pyridin-3-y1) propan-1,3-diones (VII–IX) shown better activity (CTC_{50} 741 to 826 µg/ml) than 2-(N-aryl/heteroarylamino methyl)-1-phenyl-3-(3-nitrophenyl) propan-1,3-diones (I–VI, CTC_{50} 787 to 887 µg/ml). The compound VIII was found to be the most active molecule (CTC_{50} 741 µg/ml) of this series, hence it could serves as lead molecule for the future studies.

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