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ANTICANCER AND ANTIMICROBIAL ACTIVITY OF 1-[(5-SUBSTITUTED-1,3,4 OXADIAZOL-2-YL) METHYL]-4-BENZYLPIPERAZINES

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ABSTRACT

The synthesis of 1-[(5-sustituted-1,3,4-oxadiazol-2-yl) methyl]-4-benzylpiperazines **VIa-j** was carried out by refluxing the 1-benzylpiperazine **II** with ethylchloroacetate **III** in dry acetone in the presence of potassium carbonate and subsequent hydrazinolysis with hydrazine hydrate. Finally 2-(4-benzylpiperazin-1-yl)aceto- hydrazide **V** was treated with appropriate carboxylic acids in the presence of phosphorous oxy chloride to produce title compounds. All the title compounds (VIa-j) were screened for anticancer activity using HBL-100 cell lines by MTT method and antibacterial activity against *B. subtiliis*, *S.aureus*, *E.coli* and *P.vulgaris*.The structures of newly synthesized compounds were established on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data.

Keywords:- Anticancer, antimicrobial, antifungal, piperazine and oxadiazole.

INTRODUCTION

Piperazine derivatives have been extensively investigated by the medicinal chemists due to their close association with various types of biological activities. Benzylpiperazine and Oxadiazole analogs are associated with a variety of pharmacological activities including anthelmintic, antibacterial, anti-HIV(El-Emam *et al*., 2004), antifungal, genotoxic (Maslat *et al*., 2002), antitubercular (Kucukguzel *et al*., 2002), virucidal (Chauhan *et al*., 2003), antimalarial (Kagthara *et al*., 1999), insecticidal (Mohan *et al*., 2004), anticonvulsant (Khan *et al*., 2001), sedative, hypnotic (Maillard *et al*., 1962), anticancer (Jessen *et al*., 2005) and lipid peroxidation inhibitor (Farghaly *et al*., 2000).

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The oxadiazole chemistry has been developed extensively and is still developing. Presently there are a number of drugs used clinically, which comprise oxadiazole moiety in association with various heterocyclic rings. In view of these, a project was undertaken to synthesize a new series of 1,3,4-oxadiazoles containing the 4-propyl- piperazine moiety by microwave irradiation and to evaluate the new compounds for their pharmacological activity. The title compounds were screened for anticancer activity by MTT method (Krief *et al*., 1989) and antimicrobial activity by cup plate method (Seely *et al*., 1975). Synthesis of title compounds was shown in Scheme 1**:** Synthesis of 1-[(5-sustituted-1,3,4-oxadiazol-2-yl) methyl]-4-benzylpiperazines.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes, using Toshniwal melting point apparatus and are uncorrected. IR spectra were recorded on Perkin – Elmer spectrum BX-I series, FT IR spectrophotometer using

KBr discs. PMR spectra were recorded on Brucker spectrospin 400 MHz spectrophotometer using TMS as an internal standard. Purity was checked by TLC using TLC aluminum sheets silica gel 60, supplied by E.Merk, Mumbai, India.

Anticancer Activity

1-[(5-sustituted-1,3,4-oxadiazol-2-yl) methyl]-4-benzylpiperazines were subjected to *in vitro* MTT [3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenyltetrazolium Bromide] assay to detect cytotoxic antitumor property and *in vivo* test using tumor mouse model to detect noncytotoxic antitumor property were used. MTT assay was used for *in vitro* cytotoxicity test. Cells were harvested from experimental-phase maintenance cultures. Four hundred cells were counted by trypan blue exclusion and dispensed within triplicate 96-well culture plates in 100 µl volumes for each venom concentration. The assay at each concentration was repeated twice. The cell proliferation activity was qualified on HBL-100 (ICLC NO. HTL 00004)- breast myoepithelial tumor cell line, by using Cisplatin as a standard. The results are represented in Table1.

Antimicrobial Activity

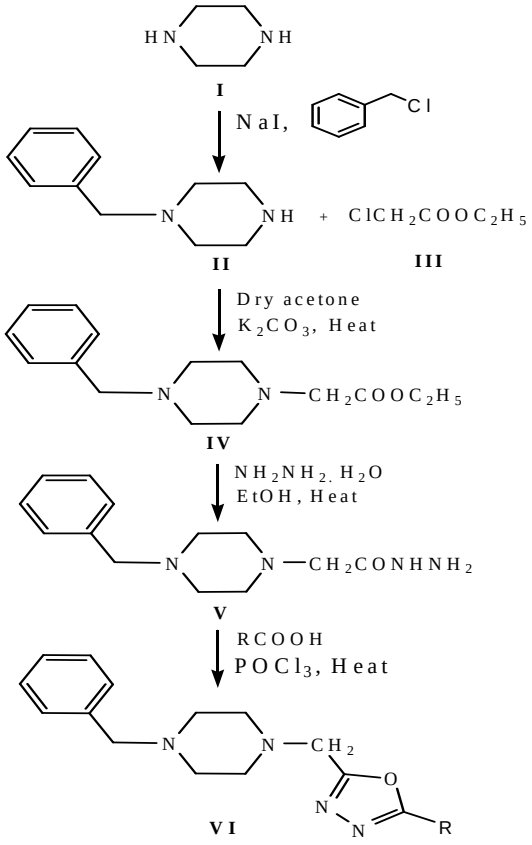
The antimicrobial activity of all the newly synthesized compounds were determined by well plate method in nutrient agar (Hi-Media) was used for antibacterial activity. The antibacterial activity of the test compounds was assayed against *Bacillus subtilis, Staphylococcus aureus* (gram – positive) and *Escherichia coli* and *Proteus vulgaris* (gram – negative) by CUP-plate method.

The compounds were tested at a concentration of a 100 µg/ml were prepared in dimethylformamide (DMF). The Petri dishes used for antibacterial screening were incubated at 37 ± 1º for 24 h; the diameters of zone of inhibition (mm) surrounding each of the wells were recorded. The results were compared with Ciprofloxacin of a 100 µg/ml concentration. The results are represented in Table1.

Preparation of 1-benzylpiperazine (II)

To a solution of piperazine **I** (10 g, 0.116 mol) in DMSO (30 ml), anhydrous cesium carbonate (60.0 g, 0.184 mol), sodium iodide (2.78 g, 0.0186 mol) and benzyl chloride **II** (13.32 ml, 0.116 mol) were added. The resulting mixture was stirred at 25-30 ^{0}C for 12 hours. The reaction mass was diluted with water (200 ml) and extracted with ethyl acetate (2 x 200 ml). The ethyl acetate layer was washed with water (2 x 100 ml), dried over anhydrous sodium sulfate (10.0 g) and concentrated under vacuum. The crude product thus obtained was purified by column chromatography (stationary phase silica gel 60-120 mesh; mobile phase 20% ethyl acetate in hexane). The compound **II** was confirmed by ^{1}H-NMR spectrum.

^{1}H-NMR (DMSO-d $_{6,}$ 400 MHz), δ (ppm): 2.0 (s, 1H, NH), 2.46 (m, 4H, $2CH_2$), 2.72 (m, 4H, $2CH_2$), 3.65 (m, 2H, CH_2), 7.11-7.30 (m, 5H, Ar-H).



Scheme 1

Preparation of Ethyl 2-(4-benzylpiperazin-1-yl) acetate (IV)

A mixture of 1-benzylpiperazine **II** (10g, 0.0567mol), ethyl chloroacetate **III** (6.06 g, 0.0567mol) and potassium carbonate (8g, 0.0817mol) in dry acetone (500 mL) was refluxed for 20 h. The reaction mixture was filtered hot and the solvent was distilled off from the filtrate. The crude ester thus obtained was purified by recrystalization from ethanol.

Preparation of 2-(4-benzylpiperazin-1-yl)acetohydrazide (V)

A mixture of Ethyl 2-(4-benzylpiperazin-1-yl) acetate **IV** (10 g, 0.0382mol) and hydrazine hydrate (99%, 1.2 ml, 0.0382mol) in ethanol (100mL) was refluxed for 8 h. The solution on cooling gave a solid mass of hydrazide **4**, which was collected by filtration, and recrystallized from ethanol. The analytical data:

IR (KBr) (cm $^{-1}$): 1016 (N-N), 1690 (C=O), 1611 (C=N), 3322 (-NH). ^{1} H-NMR (DMSO-d $_{6,}$ 400 MHz), δ (ppm): 2.44 (s, 8H, $4CH_2$), 3.32 (s, 2H, CH_2), 3.66 (s, 2H, CH_2), 4.4 (s, 2H, NH_2), 7.10-7.30 (m, 5H, Ar-H), 9.1 (s, 1H, NH)

Preparation of 1-[(5-sustituted-1,3,4-oxadiazol-2-yl) methyl]-4 benzylpiperazines (VIa-j)

To a mixture of substituted carboxylic acid (0·02 mol) and hydrazide **V** (0·02 mol), phosphorous oxychloride (5 mL) was added. The reaction mixture was refluxed for 4–6 h on an oil bath; the contents were cooled to room temperature and poured onto crushed ice. It was then neutralized by 5% sodium bicarbonate solution. The solid that separated was collected by filtration through a Büchner funnel and dried. Further purification was done by recrystalization from a mixture of ethanol-DMF to give title compounds (Priya *et al*., 2007). A typical compound **VIa** is described below.

2-phenyl-5-((4-benzylpiperazin-1-yl)methyl)-1,3,4-oxadiazole (VIa)

IR (KBr) (cm $^{-1}$): 1006(N-N), 1688 (C=O), 1611 (C=N), 1259 (C-O-C). ^{1} H-NMR (DMSO-d $_{6,}$ 400 MHz), δ (ppm): 2.48 (s, 8H, CH_2), 3.7 (s, 4H, $2CH_2$), 7.0-7.90 (m, 10H, Ar-H). LC-MS (m/z): 335.18 (M+1).

Table 1. Anticancer and antibacterial activity of 1-[(5-sustituted-1,3,4-oxadiazol-2-yl) methyl]-4-benzylpiperazines (VIa-j)

Compound	**R**	Cytotoxic activity IC_{50} (μM)	Antibacterial activity (Zone of inhibition in mm)			
			B. Subtilis	*S. aureus*	*E. coli*	*P. vulgaris*
VIa	Phenyl	78	16	10	--	06
VIb	*p*-Anisyl	98	08	12	10	11
VIc	*o*-Tolyl	38	20	18	16	15
VId	*p*-Tolyl	42	15	08	12	10
VIe	*p*-Chlorophenyl	34	10	10	11	09
VIf	3-Pyridyl	30	15	17	14	12
VIg	2-Furyl	66	17	18	15	14
VIh	*p*-Phenoxy methyl	96	11	12	11	--
VIi	*p*-Cresyloxy methyl	171	06	10	10	08
VIj	*o*-Cresyloxy methyl	135	--	08	02	06
Cisplatin	-	25	NA	NA	NA	NA
Ampicillin	-	NA	22	20	18	17

RESULTS AND DISCUSSION

The title compounds were characterized by their physical, analytical and spectral data and the title compounds were obtained in good yields and purity.

All the test compounds at the conc. of 20 μg/ml, 80 μg/ml, 100 μg/ml and 200 μg/ml were taken to evaluate the anticancer activity against HBL-100 cell lines and the results are presented as IC_{50} values. All the compounds showed anticancer activity in the range of 31 μM to 171 μM. The structure activity studies reveal that among the test compounds, the compound VIf with Pyridyl substitution at C-5 position on oxadiazole moiety showed relatively high degree of anticancer activity with IC_{50} of 30 μM. The compounds, VIe, VIc, VId, VIg was next in the order of anticancer activity with IC_{50} values of 34 μM and 38 μM, 42 μM respectively. The results are statistically significant and the activity of the compounds is compared with the standard Cisplatin.

The antibacterial data of 1-[(5-sustituted-1,3,4-oxadiazol-2-yl) methyl]-4-benzylpiperazines (VIa-l) given in Table 1 indicates that these compounds mild antibacterial activity at the concentration of 100 μg/disc against gram-positive organism (*B. subtilis*, *S. aureus*) and gram negative (*E. coli*, *P. vulgaris*) organisms. The compound VIc was more active among all the test compounds followed by compound VIc, VIg, VId.

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