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SYNTHESIS, CHARECTERISATION AND ANTI CANCER AND ANTIHELMINTHETIC ACTIVITY OF 5-METHYL -2, 4-DIHYDRO-3H-PYRAZOL-3-ONE-4-(4-SUBTITUTED) BENZYLPIPERAZINE DERIVATIVES

Chidara Mahesh[1]*& Satla Shobha Rani[2]

[1]Department of Pharmaceutical Chemistry, Vaagdevi Pharmacy College, Bollikunta, Warangal.

[2]Department of Pharmaceutical Chemistry, Center for Pharmaceutical Sciences, IST, JNTUH, Hyderabad

***Corresponding Author Email: maheshchidara@gmail.com**

ABSTRACT

*Synthesis of 6-Methyl-2, 4-dihydro-3H-pyrazol-3-one- 4- (4-substituted) Benzylpiperazine derivatives IVP a-e was carried out by bromination of Ethyl aceto acetate (I) with KBr. The reaction was carried out in the presence of Hydrochloric acid and tolune to produce Bromo-ethyl aceto acetate (II), it is further condensed with substituted Benzylpiperazines in presence of ethanol to obtain condensed compound (III). This upon cyclization with excess of hydrazine hydrate will produce title compounds. All the title compounds IV a-j were screened for possible antihelminthic activity against and anti cancer activity against. Among the compounds synthesized **IV**a and **IV**h demonstrated good antihelminthic activity and **IV**a, **IV**d, and **IV**h showed good anticancer activity. The activities of the synthesized compounds are compared with the standard and other test compounds. The structures of synthesized compounds were established by elemental analysis, IR, H NMR and Mass spectral data.*

KEY WORDS

Benzylpiperazine, antihelminthic activity, anticancer activity.

INTRODUCTION

Benzylpiperazines and its derivatives are versatile type of ligands have attracted considerable pharmaceutical interest due to their antibacterial[1,2,3] antifungal [4,5,6] antitumor and anthelmintic[7] activities . Benzylpiperazines have drawn great interests for their high potential biological activity especially for their antitumor activity when linked with thiosemicarbazides increases their antimicrobial and antitumor activity[9].

MATERIALS AND METHODS

Chemistry: Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by capillary method and are uncorrected. Infrared (IR) spectra were taken on a Fourier Transform Infrared Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000 to 400 cm-1 using KBr disks. 1 H-NMR spectra were recorded at 400 MHz in DMSO-d6 using a Bruker Avance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at d units (ppm) relative to Tetra-methylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd. Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analysis was performed on a Vario EL III Elemental Analyser (Elementar, Germany) using sulfanilamide as standard. All chemicals were purchased from Merck, Spectrochem, or CDH, India. Solvents were of reagent grade and were purified and dried by standard procedure. Reactions were monitored by thin-layer chromatography on silica gel plates in either iodine or UV chambers. Intermediates were characterized by IR spectroscopic analysis and Elemental Analysis for CHNS. In the elemental analysis, the observed values were within ±0.4 % of

the calculated values. Final compounds were characterized by 1H-NMR and EI-MS.

Synthesis of α-Bromo ethyl aceto acetate (II)

Mix 1.5 mM of ethyl aceto acetate **(I)**, 7.5 mM of KBr, 7.5 ml of 1M HCl and 7.5 ml of tolune then stir them well at room temperature and add saturated solution of NaHCO3 sufficiently finally extracted with ethyl acetate

Synthesis of Ethyl 2-(4-(4-substituted) benzylpiperazin-1-yl)-3-oxobutanoate (III)

SCHEME

Mix 0.012M of Br-EAA **(II)** and 0.01M of substituted benzylpiperazine in ethanol and reflux for 1-2 hours finally compleation of reaction was confirmed by TLC and separate

Synthesis of 4-(4-(4-substituted) Benzylpiperazin-1-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one (IV)

Take 0.01M of Ethyl 2-(4-(4-substituted) benzylpiperazin-1-yl)-3-oxobutanoate **(III)** and excess of hydrazine hydrate in acetic acid and reflux to produce title compounds

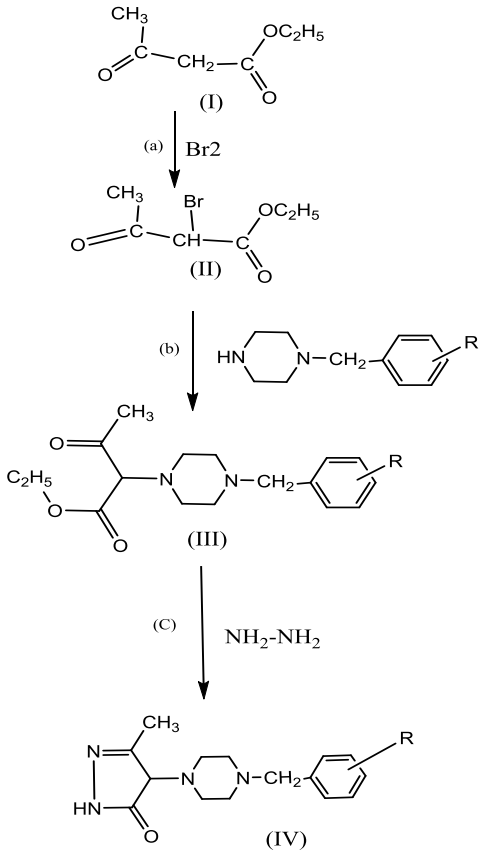

Table-I; Physical data of 4-(4-Substituted-4-benzylpiperazin-1-yl)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one (IVP a-e)

CODE	R	Solubility	MOL. Formula	MOL. Wt	Rf *	(%)YIELD	M.P
IVPa	H	DMSO	$C_{15}H_{20}ON_4$	272	0.64	73.3	219-221
IVPb	Cl	DMSO	$C_{15}H_{19}OClN_4$	306	0.81	73.8	215-217
IVPc	Br	DMSO	$C_{15}H_{19}OBrN_4$	351	0.77	71.1	225-227
IVPd	OH	DMSO	$C_{15}H_{20}O_2N_4$	288	0.71	69.5	200-203
IVPe	NO_2	DMSO	$C_{15}H_{19}O_3N_5$	317	0.78	68.6	197-199

Spectral data

IVPa - 4- (4-Benzylpiperazin-1-yl)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one:

1H-NMR (DMSO-d_6, δppm): 1.94(t, 3H, -CH3), 2.71 (t, 4H, pip-CH_2), 2.73 (m, 4H, pip- CH_2), 3.60(s, 1H, -CH), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 7.33 (s, 1H, 4–H), 12.34 (s, 1H, N–H);EI-MS (m/z): 273[M^{+1}]

IVPb- 4-(4-(4-Chlorobenzyl) piperazin-1-yl)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one:

1H-NMR (DMSO-d_6, δppm): 1.94(t, 3H, -CH3), 2.71 (t, 4H, pip-CH_2), 2.73 (m, 4H, pip- CH_2), 3.60(s, 1H, -CH), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 12.34 (s, 1H, N–H); EI-MS (m/z): 307[M^{+1}]

IVPc- 4-(4-(4-Bromobenzyl) piperazin-1-yl)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one:

1H-NMR (DMSO-d_6, δppm): 1.94(t, 3H, -CH3), 2.71 (t, 4H, pip-CH_2), 2.73 (m, 4H, pip- CH_2), 3.60(s, 1H, -CH), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 12.34 (s, 1H, N–H);EI-MS (m/z): 352[M^{+1}]

IVPd- 4-(4-(4-Hydroxybenzyl) Spiperazin-1-yl)-5-methyl-2, 4-dihydro-3H-pyrazol-3-one:

1H-NMR (DMSO-d_6, δppm): 1.94(t, 3H, -CH3), 2.71 (t, 4H, pip-CH_2), 2.73 (m, 4H, pip- CH_2), 3.60(s, 1H, -CH), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 12.34 (s, 1H, N–H);EI-MS (m/z): 289[M^{+1}]

IVPe- 4-(4-(4-Nitrobenzyl) piperazin-1-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one:

1H-NMR (DMSO-d_6, δppm): 1.94(t, 3H, -CH3), 2.71 (t, 4H, pip-CH_2), 2.73 (m, 4H, pip- CH_2), 3.60(s, 1H, -CH), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 12.34 (s, 1H, N–H);EI-MS (m/z): 318[M^{+1}]

ANTICANCER ACTIVITY

MTT Assay method

The synthesized products are subjected to anticancer activity and it is done by MTT assay by using HBL-100 (Breast cancer) cell lines. Absorbance directly correlates with the number. This is applicable for adherent cells cultured in MTP.

MTT (3(4, 5-dimethylthiazol-2yl) -2, 5-diphenyltetrazolium bromide) measures the metabolic activity of the viable cells. The assay is non radioactive and can be performed entirely in a microtiter plate (MTP). It is suitable for measuring cell proliferation, cell viability or cytotoxicity. The reaction between MTT and mitochondrial dehydrogenase produces water-insoluble frozamine salt. Procedure involves culturing the cells in a 96-well microtiterplate and then incubates them with MTT solution for two hours. During incubation period viable cells convert MTT to water insoluble frozamine dye. The frozamine dye in the MTP is solubilized and quantified with an ELISA plate reader.

Procedure

The adherent cells were trypsinized according to protocol and were re suspended in fresh medium after centrifugation. Cell suspension was mixed thoroughly by peppetting several times to get a uniform single cell suspension. Different dilutions of extracts were made in media with final DMSO concentrations in the well to be less than one percent. 100 µl of cell suspension was transferred aseptically to each well of a 96wellplate and to it 100µl of one percent media /extracts (in duplicate) in media was added. The plate was then incubated at 370^{o}C for 72 hours in CO_2 incubator. After 72 hours of incubation, 20µl of MTT was added to each well and plate was wrapped in aluminum foil to prevent the oxidation of the dye. The plate was again incubated for 2 hours. 80 µl of lysis buffer was added to each well and the plate was placed on a shaker for overnight. The absorbance was recorded on the ELISA reader at 562 nm wavelength. The absorbance of the test was compared with that of DMSO control to get the percentage inhibition. The **IC_{50}** represented in Table-2.

Table 2- IC50 values of compounds

Compound	R	$IC_{50}(\mu M)$
IVPa	H	0.142
IVPb	Cl	0.426
IVPc	Br	0.826
IVPd	OH	1.122
IVPe	NO_2	1.347
Methotrexate		0.043

Anthelmintic activity

Indian adult earthworms were collected from moist soil & washed with normal saline to remove all fecal matter were used for anthelmintic activity. The earthworm of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocols due to their anatomical and physiological resemblance with the intestinal round worm parasites of human beings.

Method: Anthelmintic studies were carried out against Eudrilus species of earthworms by Garg and Atal method at 4 mg/ml concentrations. Suspensions of samples (AT1-AT9) were prepared by triturating synthesized compounds (200 mg) with Tween 80 (0.5 %) and distilled water. The resulting mixtures were stirred using a mechanical stirrer for 30 min. The suspensions were diluted to contain 0.4 % w/v of the test samples. Suspension of reference drug albendazole was prepared with same concentration in a similar way. Five earthworms of almost similar sizes were placed in petriplates of 4 inch diameter containing 50 ml of suspension of test samples and reference drug at room temperature. Another set of five earthworms was kept as control in 50 ml suspension of distilled water and Tween 80 (0.5 %).

Observation was made for the time taken to paralysis and death of individual worms. Time for paralysis was note when no movement of any sort could be observed except when the worms were shaken vigorously. The death time was ascertained by placing the earthworms in warm water (50°C), which stimulate the movement if the worm was alive. The time in minutes to paralyze and death were shown in Table-3.

TABLE -3: Anthelmintic Studies Of Compound Against Eudrilus Species

Code of Compounds	Mean paralyzing time*(min)	Mean death time *(min)
IVPa	13.79	20.88
IVPb	14.88	33.38
IVPc	14.75	34.38
IVPd	15.22	25.06
IVPe	16.15	30.56
Albendazole (Std)	9.35	21.15

RESULTS AND DISCUSSION

Anticancer activity:

The cancer activity of test compounds shows that the newly synthesized Benzylpiperazine derivatives **(IVP a–e)** exhibited mild to moderate anticancer activity against the test organisms employed in the present investigation. All the test compounds i.e., **(IVP a–e)** showed a varied degree of anticancer activity against the test organisms employed. However, among this series of compounds **IVa and IVb** show high acivity

against all the organisms, whereas the test compounds **IVc, IVd** and **IVe** exhibited mild to moderate activity against the test organisms.

Antihelminthetic activity:

Antihelminthetic activity of the test compounds were showed that the newly synthesized Benzylpiperazine derivatives **(IVP a–e)** exhibited mild antihelminthetic activity against the test organism employed in the present investigation. Among the test compounds **IVa** and **IVd** shows good activity, whereas the test compounds **IVb, IVc** and **IVe** exhibited mild to moderate activity against the test organisms

Conclusion

In the present study new Benzylpiperazines were synthesized by conventional method as mentioned in the scheme and evaluated for their antihelminthic and anticancer activities. Among the compounds synthesized **IVP**a, **IVP**b and **IV**Pc demonstrated good anticancer activity and **IVP**a and **IV**Pd showed good antihelminthetic activity.

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***Corresponding Author:**
Chidara Mahesh
Email: maheshchidara@gmail.com