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SYNTHESIS, CHARECTERIZATION AND ANTIMICROBIAL ACTIVITY OF 5-METHYL -2, 4-DIHYDRO-3H-PYRAZOL-3-ONE-4-(4-SUBTITUTED) BENZYLPIPERAZINE DERIVATIVES

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Keywords:

Benzylpiperazine, Antimicrobial Activity, Anti Bacterial, Anti Fungal

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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 IVP a-e were screened for possible antibacterial activity against *P. Vulgaris, S. Aureas, E. Coli, B. Subtillus* and antifungal activity against *Altenaria, Culvalaria, C. Albicans and A. Niger*. Among the compounds synthesized IVPb and IVPc demonstrated good antibacterial activity, IVb, IVc, and IVe showed good antifungal 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.

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].

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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).

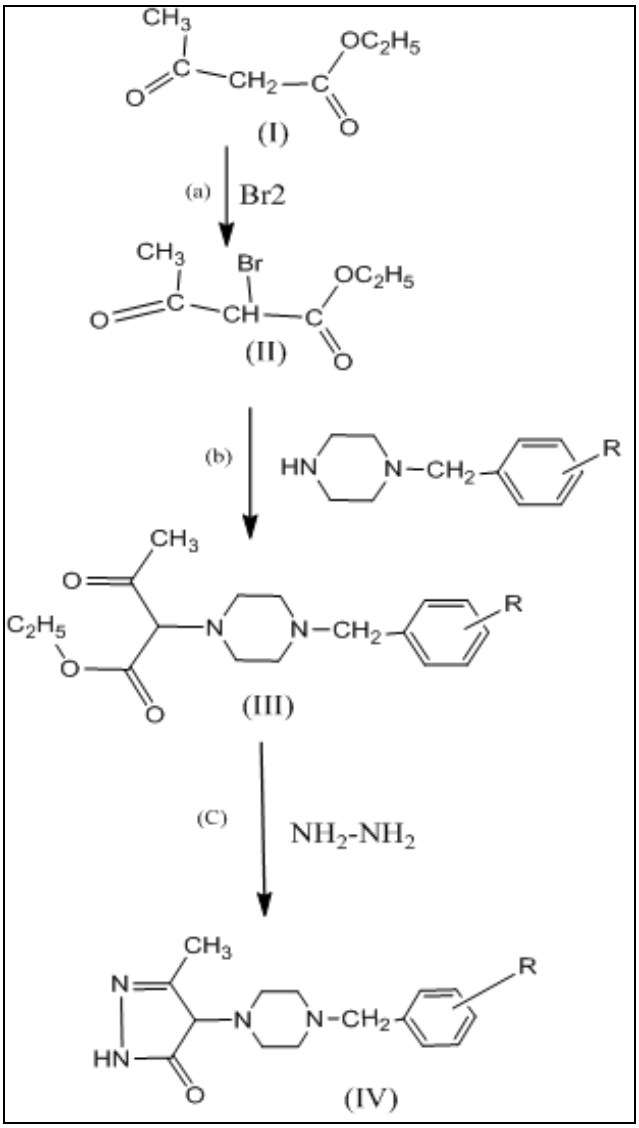
Chemical shifts were measured at d units (ppm) relative to Tetra-methylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd. Akishima, Tokyo, Japan) using argon/xenon (6 kV, 10 mA) as FAB gas, m-nitrobenzyl alcohol as

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 pur-chased 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) benzyl piperazin-1-yl)-3-oxobutanoate (III): 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) Benzyl piperazin-1-yl)-5-methyl - 2, 4 - dihydro-3H-pyrazol-3-one (IV): Take 0.01M of Ethyl 2-(4-(4-substituted) benzyl piperazin-1-yl)-3-oxobutanoate **(III)** and excess of hydrazine hydrate in acetic acid and reflux to produce title compounds.



SCHEME: 1

TABLE 1: PHYSICAL DATA OF 4-(4-SUBSTITUTED-4-BENZYLPIPERAZIN-1-YL)-5-METHYL-2,4-DIHYDRO-3H-PYRAZOL-3-ONE (IVP a-e)

CODE	R	Soubility	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, -CH_3), 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, -CH_3), 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) piperazin-1-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one:

1H-NMR (DMSO-d_6, δppm): 1.94(t, 3H, -CH_3), 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, -CH_3), 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}]

Antimicrobial study:

Antibacterial studies:

The antibacterial activities of the newly synthesized compounds (IVP a–e) were tested using serial double dilution method against strains of *P.vulgaris*, *S.aureas*, *E.coli*, *B.subtillus* in nutrient agar medium by Cup-plate method. Sterilized media was cooled to 40^{o}C and 0.5 mL of inoculum for 100 mL of media was added.

The flasks were shaken gently to avoid formation of air bubbles. This medium was transferred to Petri dishes of 9-cm diameter in 25 mL portions, so as to obtain 4-5 mm thickness of the media layer. The plates were left at room temperature to allow solidification of the media. In each Petri plate, four cups of suitable diameter were made with a sterile borer. All these procedures were conducted aseptically under laminar air flow workstation. The test com-pounds and Ciprofloxacin (Symed Lab India Pvt Ltd.,

Hyderabad, India) were dissolved in DMSO (0.5 %) and the entire test compounds equivalent to concentration of 1500, 1000, 500 and 250µg/ml were prepared by dissolving in dimethylsulphoxide. Weight equivalent to concentration of 100µg/ml was prepared by dissolving in DMSO. DMSO control was also maintained. Test compounds (40 µL) and standard (40 µL) were added into each cup with the help of a micropipette. Plates were kept undisturbed for at least 2 h at room temperature to allow for proper diffusion. Petri plates were then incubated at 37 ± 1 ^{o}C for 24 h. Zone inhibitions (in mm) were measured after incubation [8], and IC_{50} values are calculated by plotting a graph between log concentrations and percentage inhibition values. All the studies were performed in triplicate and results were presented in **Table 2**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF COMPOUNDS VIP (a-e)

Code	R	IC_{50} (µM)			
		P.vulgaris	***S.aureas***	***E.coli***	***B.subtillus***
IVPa	H	1.64	1.63	1.7	1.62
IVPb	Cl	0.78	0.51	1.58	1.49
IVPc	Br	0.57	0.6	0.86	0.82
IVPd	OH	1.59	1.57	0.79	1.66
IVPe	NO_2	1.52	1.6	1.54	1.13
Ciproflaxacin		0.04	0.05	0.20	0.27

Antifungal studies:

The antifungal activities of the test compounds were assayed using serial double dilution method against *Altenaria, Culvalaria C. albicans* and *A. niger* in Sabouraud dextrose agar medium by Cup–plate method. The sterile medium was inoculated using 24 h slant cultures of test organisms and transferred into sterile petri dishes and allowed to solidify. Four cups of suitable diam-eter were made on the solidified media. The Fluconazole (Symed Lab India Pvt. Ltd., Hyderabad, India) was dissolved in DMSO (0.5 %) and the entire test compounds equivalent to concentration of 1500, 1000, 500 and 250µg/ml were prepared by dissolving in dimethylsulphoxide. Weight equivalent to concentration of 100µg/ml was prepared by dissolving in DMSO solution ranging. DMSO control was also maintained. Test compounds (40 µL) and standard (40 µL) were added into each cup with the help of a micropipette. Zones of inhibition (in mm) were measured after 24 h of incubation [8] and IC_{50} values are calculated by plotting a graph between log concentrations and percentage inhibition value. All the studies were performed in triplicate and results were presented in **Table 3**.

TABLE 3: ANTIFUNGAL ACTIVITY OF COMPOUNDS VIP (a-e)

Compound	R	IC_{50}(µM)			
		Altenaria	***Culvalaria***	***C.albicans***	***Asp.niger***
IVPa	H	3.69	3.62	1.94	1.97
IVPb	Cl	1.49	1.54	1.96	1.05
IVPc	Br	1.12	1.05	1.44	1.56
IVPd	OH	3.39	3.38	2.15	2.09
IVPe	NO_2	1.45	1.33	1.85	2.14
Fluconazole		0.20	0.32	0.96	0.91

RESULTS AND DISCUSSION:

Antibacterial activity: The antibacterial activity of test compounds shows that the newly synthesized Benzylpiperazine derivatives (IVP a–e) exhibited mild to moderate antibacterial activity against the test organisms employed in the present investigation. However, the degree of inhibition varied with the test compound and the test bacterium.

All the test compounds i.e., **(**IVP a–e) showed a varied degree of antibacterial activity against the test organisms employed. However, among this series of compounds IVb and IVc show high acivity against all the organisms, whereas the test compounds IVa, IVd and IVe exhibited mild to moderate activity against the test organisms. Among the test compounds employed IVc was relatively more active against all the test organisms. All the test compounds were equipotent against *B. subtillis,* but IVd was relatively more potent.

Antifungal activity:

Antifungal activity among the test compounds were showed that the newly synthesized Benzylpiperazine derivatives (IVP a–e) exhibited mild antifungal activity against the test organism employed in the present investigation.

Among the test compounds IVb, IVc shows moderate activity to *C. albicans* and *Asp. niger* and IVc , IVe was more potent against *Culvalaria* and potent against *altenaria*.

CONCLUSION: In the present study new Benzylpiperazines were synthesized by conventional method as mentioned in the scheme

and evaluated for their antibacterial and antifungal activities. Among the compounds synthesized IVb and IVc demonstrated good antibacterial, IVb, IVc, and IVe showed good antifungal activity.

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