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Synthesis, characterisation and anti-microbial activity of 1-[2-Substituted hydrazine carbothioamido]- 4- benzyl piperazines

Chidara. Mahesh[1]*, Satla. Shobha Rani[2]
1. Department of PharmaceuticalChemistry, Vaagdevi Pharmacy College, Bollikunta, Warangal.
2. Department of Pharmaceutical Chemistry, Center for Pharmaceutical Sciences, IST, JNTUH, Hyderabad
***Corresponding author: E mail id: maheshchidara@gmail.com**

ABSTRACT

Synthesis of 1-[2-Substituted hydrazine carbothioamido]-4-benzyl piperazines IV a-j was carried out by reacting Methyl 2-substituted hydrazine carbodithioate II a-j with piperazine in ethanol, finally 1-[2-substituted hydrazine carbodithioamido] piperazines III a-j with required benzyl chloride in presence of propanol to produce title compounds.All the title compounds IV a-j were screened for possible anti-bacterial activity against P.vulgaris, S.aureas, E.coli, B.subtillus and anti-fungal activity against Altenaria, Culvalaria, C. albicans and A. niger. Among the compounds synthesized IVc, IVd and IVh demonstrated good antibacterial activity, IVb, IVf, and IVg showed good antifungal activity. The activities of the synthesized compounds are compared with the standardl and other test compounds. The structures of synthesized compounds were established by elemental analysis, IR, H NMR and Mass spectral data.

Key words: Benzylpiperazine, antimicrobial activity, anti-bacterial, anti-fungal

INTRODUCTION

Benzylpiperazines and its derivatives are versatile type of ligands have attracted considerable pharmaceutical interest due to their antibacterial (Rajeev Kharb, 2012; Thakran,2012; Bakhtmah, 2011), antifungal (Chetan B, 2010; Liu MC, 1995; Shyamkumar Immadi, 2010), antitumorand anthelmintic (Mostafa A. Hussein, 2005) activities.Benzylpiperazines have drawn great interests for their high potential biological activity especially for their antitumor activity when linked withm thiosemicarbazides increases their antimicrobial and antitumor activity by their ability to form chelates withspecific metallic ions (Rollas, 2007)

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.

General procedure for synthesis of methyl hydrazinecarbodithioate (I): To a cooled solution of potassium hydroxide (0.1 M, 6.6 g/7 mL) in 2-propanol (7 mL), hydrazine hydrate (85 %solution, 0.1 M, 6 mL) was added with stirring. Ice-cooled carbondisulfide (0.1 M, 10 mL) was added drop wise to the above stirred solution that was maintained below 10 ^{o} C over 1.5 h. The bright yellow mixture obtained was further stirred for 1 h, and then, ice-cooled iodomethane (0.1 M, 7 mL) was added drop wise over a period of 2 h. Stirring was continued for an additional 1.5 h to obtain a whiteprecipitate of 1. Filtered, washed with ice-cooled water,and recrystallized from dichloromethane. Yield: 43 %;m.p.: 90–92 ^{o}C (Klayman et al., 1979)

General procedure for synthesis of Schiff bases methylhydrazine carbodithioate (II a-j): Methyl hydrazinecarbodithioate I (0.01 M, 1.22 g) and (un)-substituted aromatic aldehydes/ketone (0.012 M) were dissolved in methanol (10 mL). To this mixture, catalytic amount of concentrated sulfuric acid was added and refluxed for 6–7 h. The reaction mixture turned yellow, as the methylhydrazine carbodithioate dissolved, and the yellow product began to precipitate. The solid obtained was filtered, dried, and recrystallized from suitable solvent. (Klayman et al., 1979)

General procedure for synthesis of N-arylmethylidene-piperazine-1-carbothio-hydrazide (III a-j): Piperazine (0.005 M, 0.685 g) was added to appropriate Schiff's base (II a-e, 0.005 M) in ethanol (25 mL) and refluxed until the evolution of methyl mercaptane almost completely ceased. Solvent present in the reaction mixture was evaporated under vacuum, and the solid was collected and washed with cold ethanol, further purified by recrystal-lization from suitable solvent
(Kulandaivelu et al., 2011).

General procedure for synthesis of 1-[2-Substituted hydrazine carbothioamido]-4-benzyl piperazines (IV a-j): Benzyl chloride (0.005 M, 0.844 g) was added to N-arylmethylidene-piperazine-1-carbothio-hydrazide (IIIa-e 0.005M)in propanol 20 mL and maintain the reaction condition 30 to 40 ºC for 5-6 hours and the completion of reaction is estimated by TLC and the solid was collected and washed with ethanol, further purified by recrystallization from suitable solvent. The synthesized compounds physical data shown in table 1.

Scheme: Reagents and conditions: a- KOH/i-PrOH, CS, stirring <10 ºC, 2.5 h; CH_3I, stirring,<10 º C, 3.5 h; b- R-C_6H_4–CO-R/MeOH, H_2SO_4[cat], reflux, 6–7 h; c- Piperazine/EtOH, reflux ; d-Benzyl chloride/ PrOH, reflux, 5-6 h.

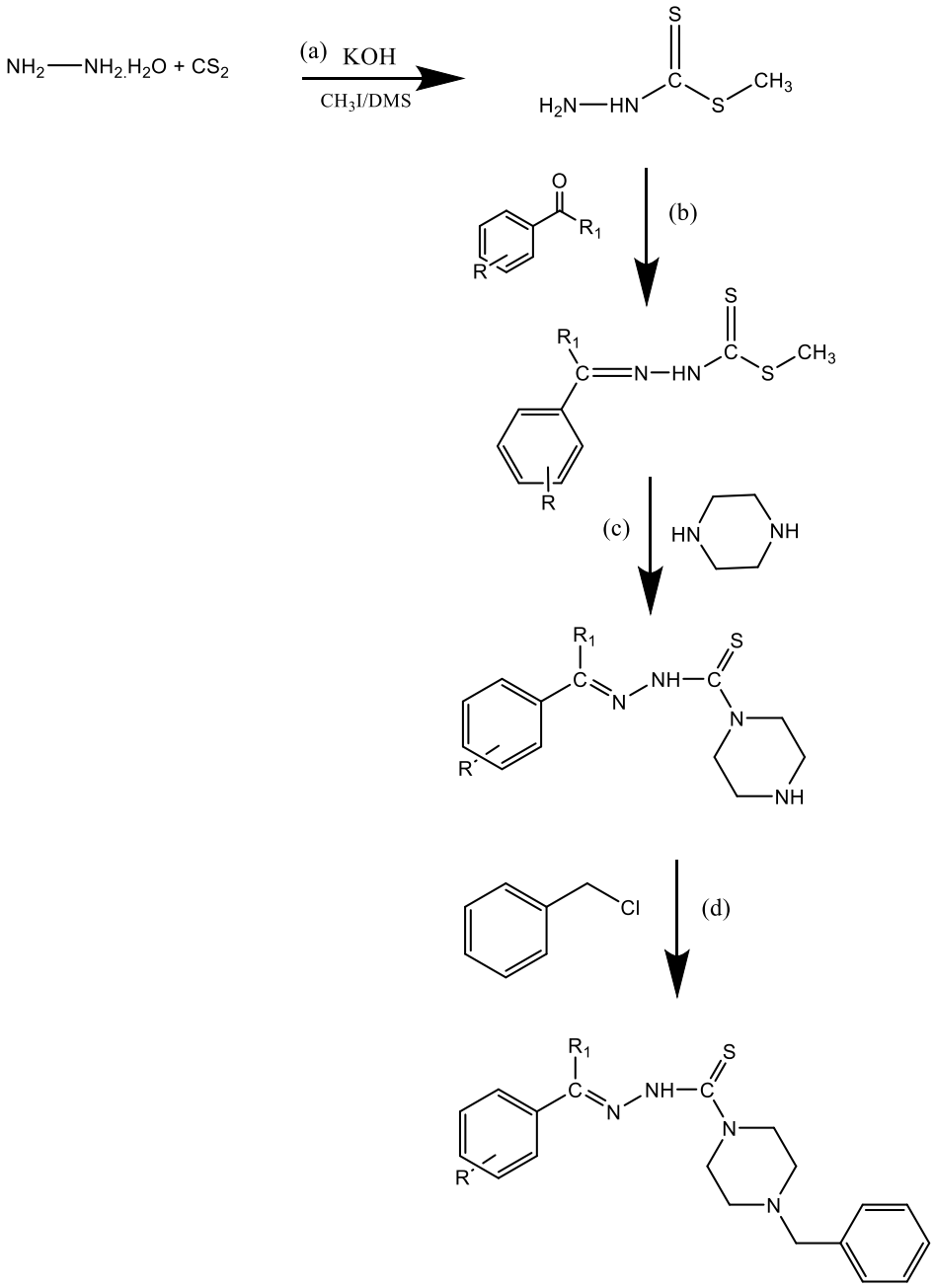

Table.1.Physical data of compounds VIa to VIj

CODE	R	R1	MOL. Formula	MOL. Wt	Rf *	M.P(°c)
IVa	OH	H	$C_{19}H_{22}OSN_4$	355	0.67	212-214
IVb	OH	CH_3	$C_{20}H_{24}OSN_4$	369	0.72	222-224
IVc	NO_2	CH_3	$C_{20}H_{23}O_2SN_5$	398	0.77	185-187
IVd	OCH_3	CH_3	$C_{21}H_{26}OSN_4$	382	0.68	216-218
IVe	Cl	H	$C_{19}H_{21}ClSN_4$	383	0.74	195-197
IVf	H	CH_3	$C_{20}H_{24}SN_4$	353	0.56	213-215
IVg	H	H	$C_{19}H_{22}SN_4$	339	0.65	198-200
IVh	Cl	CH_3	$C_{20}H_{23}ClSN_4$	387	0.66	223-225
IVi	H	CH_3	$C_{20}H_{24}SN_4$	353	0.68	200-202
IVj	H	C_6H_5	$C_{25}H_{26}SN_4$	414	0.74	198-200

Rf- Retention factor, M.P- Melting point

Spectral data:

VIa-4-Benzyl-N-(4-hydoxy benzylidine) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO-d_6, δppm): 2.26 (t, 4H, pip-CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H) 6.85-7.66 (m, 4H,benzylidine Ar–H), 8.41 (s, 1H, =C–H), 9.68 (s, 1H, O–H) 9.97 (s, 1H, CS–N–H);
EI-MS (m/z): 355[M^{+1}]

VIb-4-Benzyl-N(1-(4-hydoxy phenyl)ethylidine) piperazine-1-carbothiohydrazide:1H-NMR (DMSO--d_6, δppm): 2.32(t, 3H, -CH_3), 2.46 (t, 4H, pip- CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 6.85-7.66 (m, 4H,benzylidine Ar–H), 9.68 (s, 1H, O–H), 11.29 (s, 1H, CS–N–H);
EI-MS (m/z): 369[M^{+1}]

VIc-4-Benzyl-N(1-(4-nitro phenyl)ethylidine) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO--d_6, δppm): 2.32(t, 3H, -CH_3), 2.46 (t, 4H, pip- CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 6.85-7.66 (m, 4H,benzylidine Ar–H), 11.29 (s, 1H, CS–N–H);
EI-MS (m/z): 398[M^{+1}]

VId-4-Benzyl-N(1-(4-methoxy phenyl)ethylidine) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO--d_6, δppm): 2.32(t, 3H, -CH_3), 2.46 (t, 4H, pip- CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 3.81(t, 3H,-OCH3), 7.23 (m, 4H,benzyl benzene Ar–H), 6.85-7.66 (m, 4H,benzylidine Ar–H), 11.29 (s, 1H, CS–N–H);EI-MS (m/z): 382[M^{+1}]

VIe-4-Benzyl-N-(4-chloro benzylidine) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO-d_6, δppm): 2.26 (t, 4H, pip-CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23-7.29 (m, 5H,benzyl benzene Ar–H) 6.85-7.66 (m, 4H,benzylidine Ar–H), 8.41 (s, 1H, =C–H), 9.97 (s, 1H, CS–N–H);EI-MS (m/z): 383[M^{+1}]

VIf-4-Benzyl-N(1- phenylethylidine) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO--d_6, δppm): 2.32(t, 3H, -CH_3), 2.46 (t, 4H, pip- CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 6.85-7.66 (m, 4H,benzylidine Ar–H), 11.29 (s, 1H, CS–N–H);EI-MS (m/z): 353[M^{+1}]

VIg-4-Benzyl-N- benzylidinepiperazine-1-carbothiohydrazide: 1H-NMR (DMSO-d_6, δppm): 2.26 (t, 4H, pip-CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23-7.29 (m, 5H,benzyl benzene Ar–H) 6.85-7.66 (m, 4H,benzylidine Ar–H), 8.41 (s, 1H, =C–H), 9.97 (s, 1H, CS–N–H);EI-MS (m/z): 339[M^{+1}]

VIh-4-Benzyl-N(1- phenylethylidine) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO--d_6, δppm): 2.32(t, 3H, -CH_3), 2.46 (t, 4H, pip- CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23 (m, 4H,benzyl benzene Ar–H), 6.85-7.66 (m, 4H,benzylidine Ar–H), 11.29 (s, 1H, CS–N–H);EI-MS (m/z): 387[M^{+1}]

VIi-4-Benzyl-N-(4-methyl benzylidine) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO-d_6, δppm): 2.32(t, 3H, -CH_3), 2.46 (t, 4H, pip-CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.23-7.29 (m, 5H,benzyl benzene Ar–H) 6.85-7.66 (m, 4H,benzylidine Ar–H), 8.41 (s, 1H, =C–H), 9.97 (s, 1H, CS–N–H);EI-MS (m/z): 353[M^{+1}]

VIj-4-Benzyl-N-(4-diphenyl methelene) piperazine-1-carbothiohydrazide: 1H-NMR (DMSO-d_6, δppm): 2.46 (t, 4H, pip-CH_2), 3.68 (m, 4H, pip- CH_2), 3.66 (m, 2H,benzyl- CH_2), 7.21-7.29 (m, 5H,benzyl benzene Ar–H) 7.35-7.57 (m, 5H,phenyl, Ar–H),7.60-7.95(m, 5H,phenyl Ar–H), 9.97 (s, 1H, CS–N–H);EI-MS (m/z): 414[M^{+1}]

Antimicrobial study: The antibacterial activities of the newly synthesized compounds (4a–j) 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 40ºC 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 Norfloxacin (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 ºC 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 VIa to VIj

Code	R	R1	IC_{50} (µM)			
			P.vulgaris	***S.aureas***	***E.coli***	***B.subtillus***
IVa	OH	H	1.64	1.63	1.7	1.62
IVb	OH	CH_3	2.48	3.51	1.58	1.49
IVc	NO_2	CH_3	0.57	0.6	0.86	0.32
IVd	OCH_3	H	1.59	1.57	0.79	0.66
IVe	Cl	H	1.52	0.6	0.54	0.13
IVf	H	CH_3	1.76	2.11	2.07	1.78
IVg	H	H	2.91	1.97	1.36	0.93
IVh	Cl	CH_3	0.88	0.71	1.04	0.98
IVi	CH_3	H	1.74	1.7	1.66	1.7
IVj	H	C_6H_5	2.03	1.44	1.33	1.38
Norflaxacin			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 VIa to VIj

compound	R	R_1	IC_{50}(µM)			
			Altenaria	**Culvalaria**	**C.albicans**	**Asp.niger**
IVa	OH	H	3.69	3.62	1.94	1.97
IVb	OH	CH_3	3.49	1.54	2.56	3.55
IVc	NO_2	CH_3	3.12	3.05	2	1.56
IVd	OCH_3	H	3.39	3.38	2.15	2.09
IVe	Cl	H	0.345	3.33	1.85	2.14
IVf	H	CH_3	0.97	0.76	3.75	3.76
IVg	H	H	1.07	3.81	1.85	2.21
IVh	Cl	CH_3	1.1	0.63	3.37	3.32
IVi	CH_3	H	1.32	3.62	3.72.	3.79
IVj	H	C_6H_5	2.86	2.96	1.47	1.62
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 (IVa-j) 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., (IVa-j) showed a varied degree of antibacterial activity against the test organisms employed. However, among this series of compounds IVc, IVd and IVh show high acivity against all the organisms, whereas the test compounds IVa, IVe, IVf and IVi 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 IVe was relatively more potent.The compound IVc and IVd were relatively more active against S. aureus.

Antifungal activity: Antifungal activity among the test compounds were showed that the newly synthesized Benzylpiperazine derivatives (IVa-j) exhibited mild antifungal activity against the test organism employed in the present investigation.

Among the test compounds Iva, VIj shows moderate activity to *C. albicans* and *Asp. niger* and IVf , IVh 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 antimicrobial and antifungal activities. Among the compounds synthesized IVc, IVd and IVh demonstrated good antibacterial, IVb, IVf, and IVg showed good antifungal activity.

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