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MICROWAVE ASSISTED SYNTHESIS AND MICROBIAL EVALUATION OF -2H-PYRROLO [2, 3-C: 5, 4 C'] DIPYRAZOLE-2, 5 (7H)-BIS-CARBOTHIOAMIDE DERIVATIVES

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ABSTRACT

In this study the synthesis of novel carbothiomides derivatives containing pyrazole moiety have been carried out by use of microwave assisted green and efficient process. The starting compound N-phenyl substituted succinamides 5a-b were condensed with substituted benzaldehydes furnished in to bis-heterocyclic chalcones which on treatment with thiosemicarbazide hydrochloride leads to formation of pyrralo dipyrazole carbothiomide derivatives. The structures of all synthesized compounds were determined by FT IR, ^{1}H NMR, ^{13}CNMR spectral analysis techniques.

The pyrazole carbothiomide derivatives were selected for evaluation of antimicrobial activities. The *in vitro* antibacterial screening was carried out against Gram positive bacteria *Staphylococcus auras*, *Bacillus subtilis* and Gram negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*. The antibacterial activity was measured in term of zone of inhibition in mm unit and compared with standard antibiotic drug Chloramphenicol.

Similarly antifungal evaluation was also evaluated *in vitro* against fungi *Aspergillus niger* and *Candida albicans*. The zone of inhibition was measured in mm and compared with standard antifungal drug Amphotericin-B.

The all carbothiomide derivatives showed good anti-bacterial activities against gram positive bacteria and good anti-fungal activities. The compounds 9ai and 9aiv showed potent antifungal activity against *Candida albicans* than standard drug amphotericin-B at 100 µgm/mL concentration.

Keywords: Cyclic imide, Chalcones, Pyrazoles, Carbothiomides.

1. INTRODUCTION

The heterocyclic compounds have been significantly incorporated in large number of drugs and pharmaceutical compounds. The substituted pyrazole is an important class of heterocyclic compounds and the pyrazole carbothioamide has remarkable applications in the field of medicine and drug designing. It shows anti-mycobacterial activity used for treatment of pulmonary tuberculosis [1-2], Cytostatic [3], Anti-inflammatory [4-5], Antifungal [6-7], Anti-malarial [8-9] and antiamoebic activities [10]. The pyrazole carbothiamide ring is present as the core in variety of leading drug. They found to have Anti-nociceptive [11] Anti-convulsant [12] Anti-oxidant [13], Antiviral [14], Antimicrobial [15], Analgesic [16] and Antidepressant activity [17]. The pyrazole analogues have been used as building block in organic synthesis for development of important pharmaceuticals compounds and drugs. The molecule incorporated pyrazole carbothiamide moiety serves as MAO-B inhibitor [18-19], Lipoprotein oxidation inhibitor [20], Adenosine receptor Antagonist [21], Antihypertensive [22], Cytotoxic [23] and Anti-cancer [24-25]. On account of such enormous applications of pyrazole so here in this research work we attempt to synthesize pyrazole incorporated carbothiomide derivatives. For this synthesis we have developed an efficient and green method of synthesis and carried out preparation of di-carbothiamides containing pyrazole moiety from N-phenyl substituted succinamide chalcone with rationale to get promising anti-microbial activities.

The Succinamides belong to drug family so we selected as starting compounds for synthesis of pyrazole which was achieved by formation of succinamide chalcones; an important synthone for preparation pyrazole carbo-thiomides. MAOS (microwave assisted organic synthesis) is very useful tool in organic synthesis that enhance rate of reaction and many organic reactions driven with the help of microwave radiation. So here, we developed

combine techniques which incorporated grinding with irradiation which afford easy workup and solvent free synthetic protocol.

2. MATERIAL AND METHODS

The melting points were taken in to open capillaries and are uncorrected. The IR spectra were recorded on FTIR shimazdu spectrophotometer using KBr disc method. The ^{1}HNMR spectrum were recorded on Bruker 500 MHz instrument and TMS is used as an internal standard the chemical shift were recorded in terms of δ value relative to TMS in solvent DMSO-d_6. Similarly ^{13}C NMR Spectrum was recorded on Bruker 400 MHz instrument and TMS was used as an internal standard. The chemical shift was recorded in terms of δ value relative to TMS in solvent DMSO-d_6. The reactions were carried out in domestic microwave oven of Model Samsung-MW73AD-B/XTL-800W instrument. The starting compound cyclic imide was used for synthesis of the succinamide chalcones **7ai-v**and **7bi-v** which was synthesized by using microwave irradiated solid phase synthetic method. The reactions were monitored by thin layer chromatography by using pre-coated silica gel aluminium plates and mixture of n-hexane: ethyl acetate 7:3 proportion was used as mobile phase. The identification of spots was done by visualizing plate in U.V chamber. All the chemicals used in this experiment were of AR grade, purchased from Loba Chem Pvt Ltd. The chemicals used in this research work were of analytical grade and of high purity

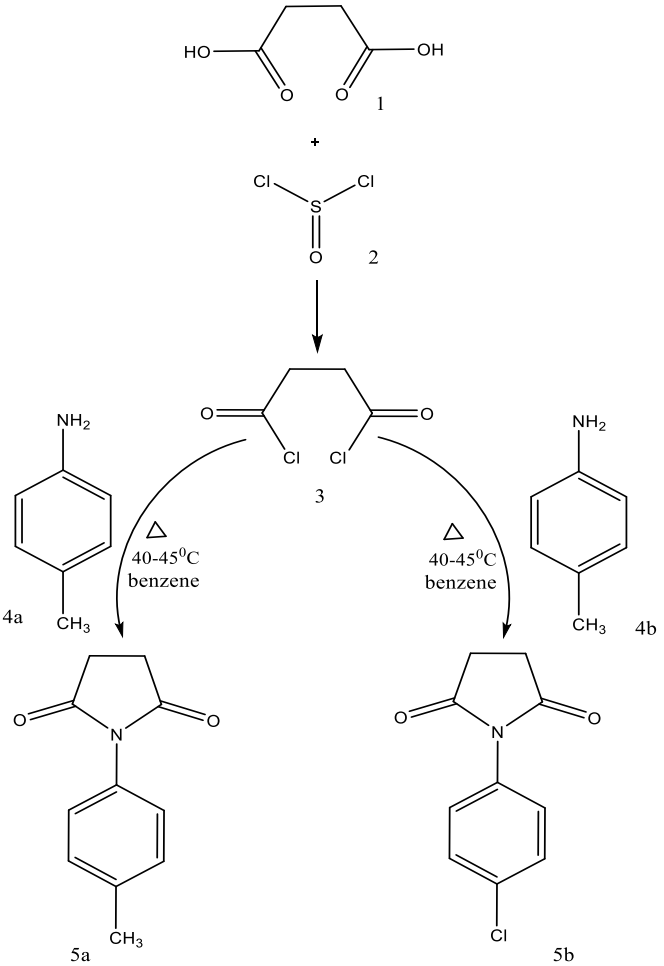
2.1. Experimental

2.1.1. General procedure for synthesis of N-substituted phenyl succinamides 1-p-tolylpyrrolidine-2, 5-dione (5a)

A mixture of 0.1 M Succinic acid **1** and 0.2 M Thionyl chloride **2** was taken in two neck round bottom flask attached with reflux condenser and refluxed for 30 min at 40-45$^{°C}$ temperature then conversion of succinyl chloride **3** in situ. The 0.1 M p-Toluidine**4a** was dissolved in 5-10 ml benzene this solution was added slowly in above reaction mixture. The reaction mixture was then refluxed till complete evolution of HCl gas thus the product N-phenyl substituted succinamide **5a** was obtained cooled and recrystallized from ethyl alcohol.

2.1.2. Synthesis of 1-(4-chlorophenyl) pyrrolidine-2,5-dione (5b)

A mixture of 0.1 M Succinic acid **1** and 0.2 M Thionyl chloride **2** was taken in two neck round bottom flask attached with reflux condenser and refluxed for 30 min at 40-45°C temperature then there was formation of succinyl chloride **3** in situ. The 0.1 M p- Chloro aniline **4b** was dissolved in 5-10 ml benzene. The solution was added slowly in above reaction mixture. The reaction mixture was then refluxed till complete evolution of HCl gas, thus the product N-phenyl substituted succinamide **5b** was obtained, cooled and recrystallized from ethyl alcohol **(Scheme 1).**



SCHEME-1

Table 1: Physical properties of cyclic imides

Entry	R_1	Colour	Recrys. solvent	M.P$^{°C}$	% Yield	M.W
5a	4-CH_3	Yellow	Ethanol	117	87	189.21
5b	4-Cl	Yellow	Ethanol	154	84	209.63

2.1.3. **1-p-tolylpyrrolidine-2, 5-dione (5a)**

Anal Cal. for $C_{11}H_{11}NO_2$: C, 69.83; H, 5.86; N, 7.40; O, 16.91 F**ound:** C, 69.13; H, 5.27; N, 7.12; O, 16.36, **FTIR (KBr, cm^{-1}):** 1700.31 (C=O), 2931.90 (Ar-CH3**)** 1611 (Ar, C=C).

2.1.4. **1-(4-chlorophenyl) pyrrolidine-2,5-dione (5b)**

Anal Cal. For $C_{10}H_8ClNO_2$**:** C, 69.83; H, 5.86; N, 7.40; **Found:** C, 69.73; H, 5.46; N, 7.60; **FTIR (KBr,cm^{-1}):** 1700.31 (C=O), 720.44 (Ar-Cl), 1611.58 (Ar, C=C), Cyclic CH2-CH2: 2972.40 Cyclic imide.

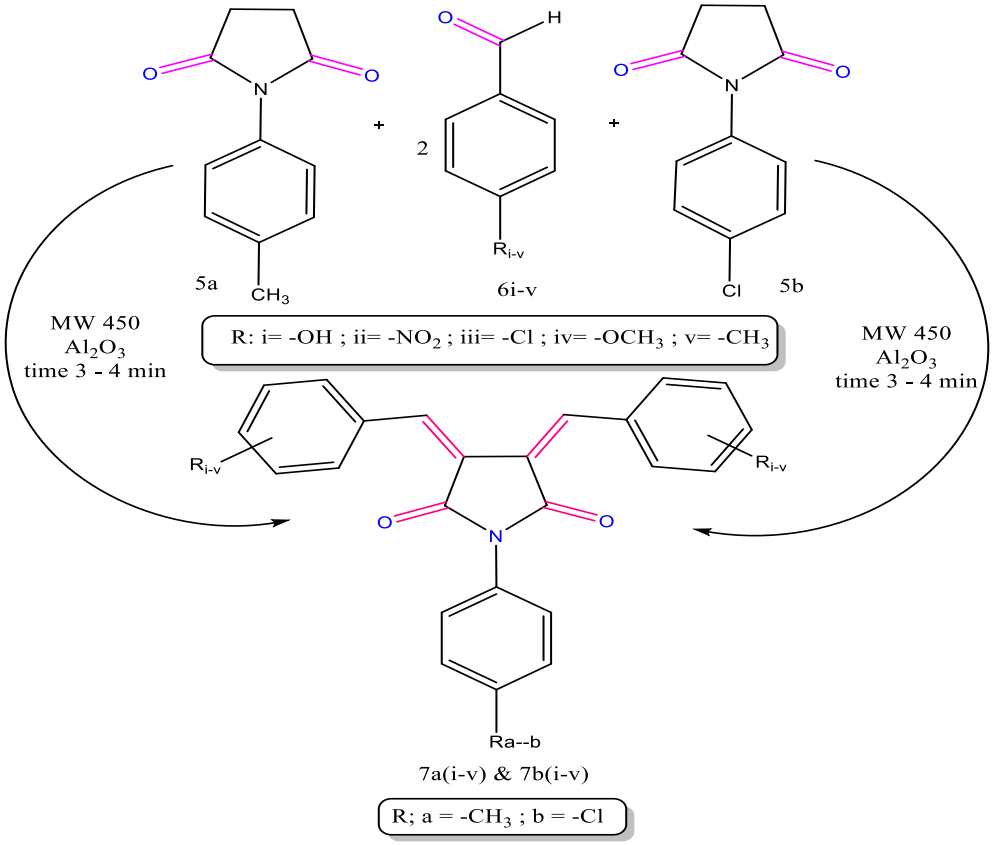
2.2. General procedure for Synthesis of (3Z, 4Z)-3,4-bis (substituted benzylidene)-1-p-tolyl-pyrrolidine-2, 5-dione(7a-e)

2.2.1. Synthesis of (3Z, 4Z)-3,4-bis (substituted benzylidene)-1-p-tolylpyrrolidine-2, 5-dione (7a-e)

For preparation of compounds **7ai-v,** 0.01 M of cyclic imide; **5a** (1.89 gm) and 0.02 M substituted aldehydes; **6ai-v** (2-hydroxy benzaldehyde 2.08 mL; **6ai**, 3-nitro benzaldehyde 3.03 gm; **6aii**, 2-chloro benzadehyde 2.26 mL; **6aiii**, 4-methoxy benzaldehyde 2.44 mL; **6aiv**, 4-methyl benzaldehyde 2.36 mL; **6av**) were taken and then 1.5-2 gm of neutral alumina was added and homogenized in a mortar with pestle. Then reaction mixture was added in borosilicate glass 100mL conical flask and covered with borosilicate glass Petri dish. The beaker was placed in microwave oven and irradiated at 450 watt power for 3-4 min thus yellow colour fused solid mixture of bis-heterocyclic chalcone **7ai-v** obtained which was recrystalised from ethyl alcohol.

2.2.2. Synthesis of (3Z, 4Z)-3, 4-bis (substituted benzylidene)-1-(4-chlorophenyl) pyrrolidine-2,5-dione (8a-e)

For preparation of compounds **7bi-v,** 0.01 M of (2.09 gm) succinamide; **5b** and 0.02 M substituted aldehydes; **6ai-v** were taken and then 1.5-2 gm of neutral alumina was added and homogenized in a mortar. Then reaction mixture was added in borosilicate glass 100mL conical flask and covered with borosilicate glass petri dish. The beaker was placed in microwave oven and irradiated at 450 watt power for 3-4 min thus yellow colour fused solid mixture of bis-heterocyclic chalcone; **7bi-v** obtained which was recrystallized from ethyl alcohol.



SCHEME-2

Table 2: Physical properties of bis-chalcones

Entry	R_{a-b}	R_{i-v}	Colour	Recrys. solvent	M.P (°C)	% Yield	M.W
7a(i)	4-CH_3	2-OH	Yellow solid	Ethanol	168	86	397.42
7a(ii)	4-CH_3	3-NO_2	Yellow solid	Ethanol	200	89	455.42
7a(iii)	4-CH_3	2-Cl	Yellow solid	Ethanol	173	84	434.31
7a(iv)	4-CH_3	4-OCH_3	Yellow solid	Ethanol	162	87	425.48
7a(v)	4-CH_3	4-CH_3	Yellow solid	Ethanol	144	83	393.48
7b(i)	4-Cl	2-OH	Yellow solid	Ethanol	118	85	417.84
7b(ii)	4-Cl	3-NO_2	Yellow solid	Ethanol	68	82	475.84
7b(iii)	4-Cl	2-Cl	Yellow solid	Ethanol	191	84	454.73
7b(iv)	4-Cl	4-OCH_3	Yellow solid	Ethanol	156	81	445.89
7b(v)	4-Cl	4-OCH_3	Yellow solid	Ethanol	134	83	413.9

2.2.3. (3Z,4Z)-3,4-bis(2-hydroxybenzylidene)-1-p-tolylpyrrolidine-2,5-dione(7a(i))

Anal Cal. for $C_{25}H_{19}NO_4$: C, 75.55; H, 4.82; N, 3.52. Found: C, 75.15; H, 4.22; N, 3.72; FTIR (KBr, cm^{-1}) :1705 (C=O), 3368 (-OH), 2937(-CH_3), 1611 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3), 5.1 (s, 1H, -OH), 7.31-7.17 (m, 6H, Ar-H and 1H of =CH).; ^{13}C NMR (400 MH_Z; DMSO-d_6 ; δ ppm): 20.26 (-CH_3), 28.36, 39.09 (OCH_3), 127.15, 129.85, 133.65, 139.61 (Ar,C), 178.35 (C=O).

2.2.4. (3Z, 4Z)-3,4-bis(3-nitro benzylidene)-1-p-tolylpyrrolidine-2,5-dione(7a(ii))

Anal Cal. for $C_{25}H_{17}N_3O_6$: C, 65.93; H, 3.76; N, 9.23 Found: C, 65.33; H, 3.21; N, 9.53; FTIR (KBr, cm^{-1}): 2937 (-CH_3), 1705 (C=O), 1611 (C=C) 1345 (Ar-NO_2); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3), 8.47-6.67 (m, 6H, Ar-H and 1H of =CH); ^{13}C NMR (400 MH_Z; DMSO-d_6 ; δ ppm): 24.26 (-CH_3), 22.36, 39.99 (-OCH_3),129.15, 139.15, 140.27, 143.16 (Ar, C), 169.12(C=O).

2.2.5. (3Z, 4Z)-3,4-bis(2-chlorobenzylidene)-1-p-tolylpyrrolidine-2,5-dione(7a(iii))

Anal Cal. For $C_{25}H_{17}Cl_2NO_2$: C, 69.14; H, 3.95; N, 3.23 Found: C, 69.34; H, 3.86; N, 3.73; FTIR (KBr, cm^{-1}): 1705 (C=O), 2937 (-CH_3), 713 (C-Cl), 1611 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3), 7.40-7.12 (m, 6H,Ar-H and1H of =CH); ^{13}C NMR (400 MH_Z; DMSO-d_6 ; δ ppm): 24. 62 (-CH_3), 30.31, 38.19 (-OCH_3), 127. 58, 129.11, 133.65, 136.81 (Ar, C), 179.53 (C=O).

2.2.6. (3Z, 4Z)-3,4 –bis(4-methoxybenzylidene)-1-p-tolylpyrrolidine-2,5-dione(7a(iv))

Cal. for $C_{27}H_{23}NO_4$: C, 76.22; H, 5.45; N, 3.29 Found: C, 76.42; H, 5.75; N, 3.59; FTIR (KBr, cm^{-1}): 1705 (C=O), 2937(Ar-CH_3), 1154 (C-O ether) 1611 (C=C); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.40 (s, 3H, CH_3), 3.7 (s, 3H, -OCH_3), 8.22-6.43 (m, 6H, Ar-H and 1H of =CH); ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 24.22 (-CH_3), 28.36, 40.19 (-OCH_3), 123. 85, 127.52, 131.65, 138.71 (Ar,C), 178.45 (C=O).

2.2.7. (3Z,4Z)-3,4-bis(4-methylbenzylidene)-1-p-tolylpyrrolidine-2,5-dione(7a(v))

Anal Cal. for $C_{27}H_{23}NO_2$: C, 82.42; H, 5.89; N, 3.56 Found: C, 82.62; H, 5.69; N, 3.76; FTIR (KBr, cm^{-1}): 1705 (C=O), 2937 (-CH_3), 1611(C=C); ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.40 (s, 1H, CH_3), 8.22-6.43 (m, 6H, Ar-H and 1H of =CH); ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 21.26 (-CH_3), 27.16, 39.19 (-OCH_3), 123.63, 128.16, 132.85, 138.41 (Ar, C), 174.26 (C=O).

2.2.8. (3Z, 4Z)-3, 4-bis (2-hydroxybenzylidene)-1-(4-chlorophenyl) pyrrolidine-2,5-dione (7b(i))

Anal Cal. for $C_{24}H_{16}ClNO_4$: C, 68.99.55; H, 3.86; N, 3.35; Found: C, 68.79; H, 3.66; N, 3.55; FTIR (KBr, cm^{-1}): 1705.79 (C=O), 3431.26 (-OH), 725.33 (Ar-Cl), ^{1}HNMR (500 MH_Z; DMSO-d_6; δ ppm): 9.1 (s, 1H, -OH), 7.31-7.17 (m, 6H,Ar-H and 1H of =CH); ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 126.85, 129.25, 130.05, 137.61 (Ar, C), 177.05 (C=O) 28.36, 39.99.

2.2.9. (3Z, 4Z)-3, 4-bis (3-nitrobenzylidene)-1-(4-chlorophenyl) pyrrolidine-2, 5-dione (7b(ii))

Anal Cal. for $C_{24}H_{14}ClN_3O_6$: C, 60.58; H, 2.97; N, 8.83, Found: C, 60.38; H, 2.67; N, 8.73; FTIR (KBr, cm^{-1}): 1700.07 (C=O), 1381.03 (Ar-NO_2), 1612.49 (Ar, C=C), 729.09 (Ar-Cl). ^{1}HNMR (500 MH_Z; DMSO-d_6; δ ppm): 8.57-7.3 (m, 6H, Ar-H and 1H

=CH). ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 126. 85, 129.25, 131.05, 137.61 (Ar, C), 166.05 (C=O).

2.2.10. (3Z, 4Z)-3, 4-bis (2-chlorobenzylidene)-1-(4-chlorophenyl) pyrrolidine-2, 5-dione (7biii))

Anal Cal. for $C_{24}H_{14}Cl_3NO_2$: C, 63.39; H, 3.10; N, 3.08, Found: C, 63.59; H, 3.21; N, 3.28; FTIR (KBr, cm^{-1}): 1710.86 (C=O), 717.52 (Ar-Cl), 1649.14 (Ar, C=C), ^{1}HNMR (500 MH_Z; DMSO-d_6 ; δ ppm): 7.40-7.12 (m, 6H, Ar-H and 1H =CH). ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 126.85, 129.25, 130.05, 137.61 (Ar; -C), 169.05 (C=O).

2.2.11. (3Z, 4Z)-3, 4-bis (4-methoxybenzylidene)-1-(4-chlorophenyl) pyrrolidine-2, 5-dione (7b(iv))

Anal Cal. For $C_{26}H_{20}ClNO_4$: C, 70.03; H, 4.52; N, 3.14; Found: C, 70.23; H, 4.62; N, 3.34; FTIR (KBr, cm^{-1}): 1703.14 (C=O), 1184.29 (-OCH_3), 825.53 (Ar-Cl). ^{1}HNMR (500 MH_Z; DMSO-d_6; δ ppm): 7.20-6.9 (m, 6H , Ar-H and 1H =CH), 3.83 (s, 3H, OCH_3), ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 39.99 (-OCH_3), 126.85, 129.25, 130.05, 137.61 (Ar, C), 167.05 (C=O).

2.2.12. (3Z, 4Z)-3, 4-bis(4-methyl benzylidene)-1-(4-chlorophenyl) pyrrolidine-2,5-dione (7b(v))

Anal Cal. for $C_{26}H_{20}C$ lNO_2: C, 75.45; H, 4.87; N, 3.38; Found: C, 75.65; H, 4.67; N, 3.58; FTIR (KBr, cm^{-1}): 1710.86 (C=O), 713.66 (Ar-Cl), 3290.56 (Ar-CH_3). ^{1}HNMR (500 MH_Z; DMSO-d_6; δ ppm): 2.3 (s, 3H, CH_3), 7.6-7.2(m, 6H, Ar-H and 1H =CH). ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 28.36 (-CH_3), 126.85, 129.25, 130.05, 137.61 (Ar; C), 187.05 (C=O).

2.3. Preparation of pyrrolo pyrazole carbothioamide

2.3.1. General procedure for Synthesis of 7-(p-tolyl) -2H-pyrrolo [2, 3-c: 5, 4-c'] dipyrazole-2, 5(7H)-bis (carbothioamide) 9ai-v

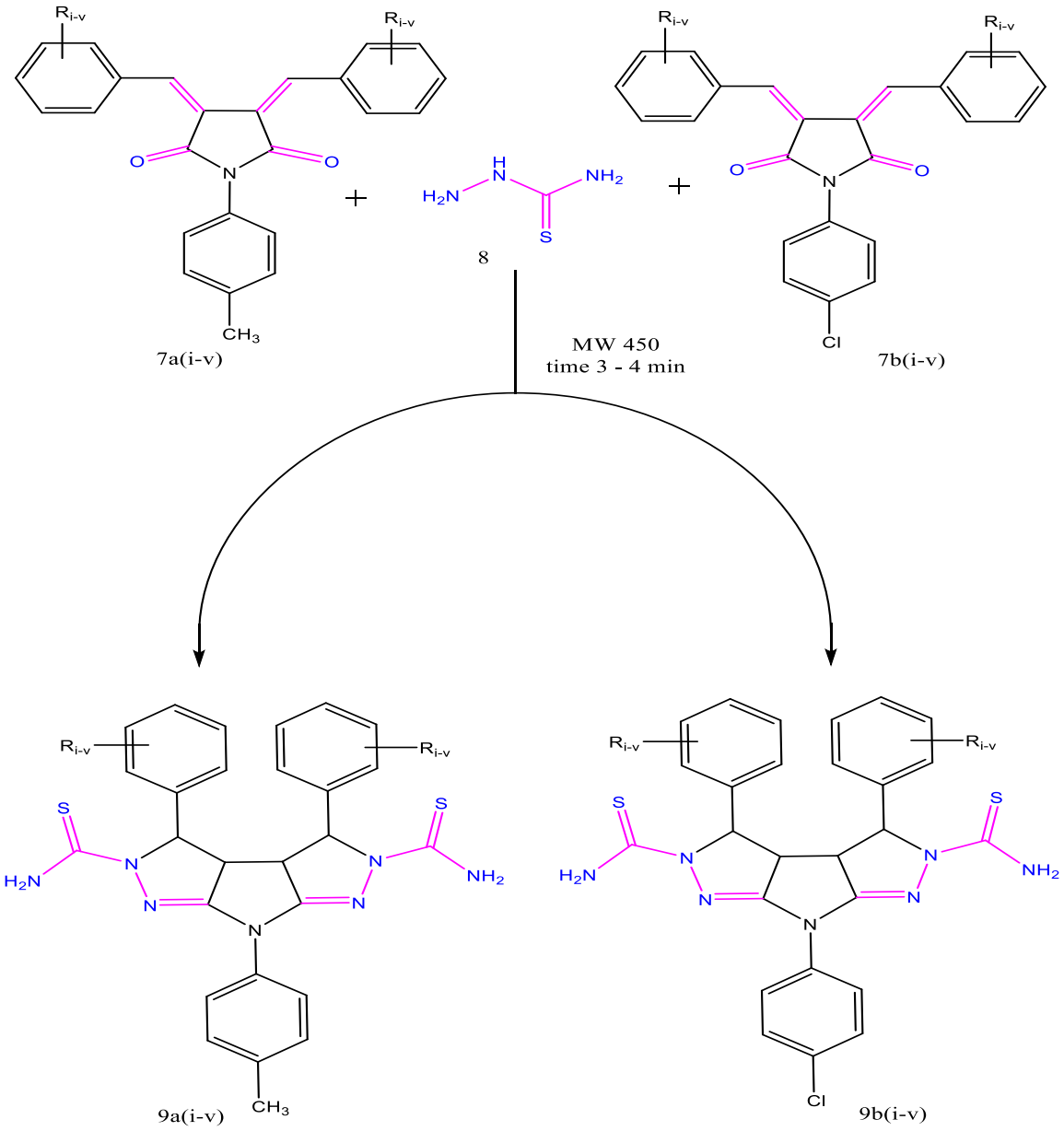
The dipyrazole dicarbothiomide derivatives; **9ai-v** were synthesized by reaction of 0.02M of Thiosemicarbazide hydrochloride with 0.01M of Bis-chalcones; **7ai-v** (0.39 gm of 7ai, 0.45 gm of 7aii, 0.43 gm of 7aiii, 0.42 gm of 7aiv, and 0.39 gm of 7av) in the presence of 2 grams of neutral alumina and homogenized with mortar, then irradiated in microwave oven as solid phase solvent free condition at 450 watt power for 4 to 5 min, thus fused mixture of dipyrazole-2, 5(7H)-bis (carbothioamide) was obtained and recrystallized from ethanol.

2.3.2. General procedure for Synthesis of 7-(4-chlorophenyl) -2H-pyrrolo [2, 3-c: 5, 4 c'] dipyrazole-2, 5 (7H)-bis (carbothioamide) 9bi-v

The dipyrazole dicarbothiomide derivatives; **9bi-v** were synthesized by reaction of 0.02M of Thiosemicarbazide hydrochloride with 0.01M of Bis-chalcones; **7bi-v** (0.42 gm of 7bi, 0.47 gm of 7bii, 0.45 gm of 7biii, 0.44 gm of 7biv and 0.41gm of 7bv) in the presence of 2 grams of neutral alumina and homogenized in mortar and irradiated in microwave oven as solid phase solvent free condition at 450 watt power for 4 to 5 min, thus fused mixture of dipyrazole-2, 5(7H)-bis (carbothioamide) was obtained and recrystallized from ethanol.

Table 3: Physical Data of Pyrazole Carbothiamides

Entry	R_{a-b}	R_{i-v}	Colour	Recrys. solvent	M.P°C	% Yield	M.W
9a(i)	4-CH_3	2-OH	brown solid	Ethanol	75	70.18	543.66
9a(ii)	4-CH_3	3-NO_2	brown solid	Ethanol	111	72.62	601.66
9a(iii)	4-CH_3	2-Cl	brown solid	Ethanol	136	71.42	580.55
9a(iv)	4-CH_3	4-OCH_3	Yellow solid	Ethanol	150	73.11	571.72
9a(v)	4-CH_3	4-CH_3	brown solid	Ethanol	173	74.21	564.08
9b(i)	4-Cl	2-OH	yellow solid	Ethanol	164	73.64	564.08
9b(ii)	4-Cl	3-NO_2	yellow solid	Ethanol	101	73.12	622.08
9b(iii)	4-Cl	2-Cl	yellow solid	Ethanol	127	70.11	600.97
9b(iv)	4-Cl	4-OCH_3	brown solid	Ethanol	107	70.18	592.13
9b(v)	4-Cl	4-OCH_3	brown solid	Ethanol	89	73.32	560.14



SCHEME-3

2.3.3. *3,4-bis(2-hydroxyphenyl)-7-(p-tolyl)-3,3a, 3b,4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c'] dipyrazole-2,5(7H)-bis(carbothioamide) (9a(i))*

Anal Cal. for: $C_{27}H_{25}N_7O_2S_2$: C, 59.65; H, 4.64; N, 18.03, Found: C, 59.25; H, 4.64; N, 18.13, FTIR (KBr, cm^{-1}): 1519.91 (C=N), 3392.79 (N-H), 2924.09 (Ar-CH_3), 3608.81 (-OH), 1394.53 (C=S)., ^1H NMR (500 MHz ; DMSO d6 ; δ ppm): 2.3 (s, 3H, -CH_3), 2.76 (dd, 2H, *J*= 8.1, 7.7 Hz -CH Pyrazole), 3.36 (d, 2H, *J* = 4.1 Hz Pyrazole), 6.91 (ddd, 4H, *J*= 8.2, 1.4, 0.5 Hz) , 7.18 (ddd, 4H, *J*= 8.3, 1.3, 0.5 Hz), 8.59 (ddd, 4H, *J*= 8.4, 1.6, 0.5 Hz), 9.01 (s, 4H, of two -NH_2), 11.34 (s, 2H, of two -OH).^{13}C NMR (400 MH_Z; DMSO-d6; δ ppm): 176.19, 162.75, 158.61, 137.55, 133.21, 130.79, 130.08, 129.23, 126.89, 119.58, 118.57, 116.50, 114.46, 111.56, 104.32, 88.61, 40.27, 38.18

2.3.4. *3,4-bis(3-nitrophenyl)-7-(p-tolyl)-3,3a,3b, 4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c'] dipyra-zole-2,5(7H)-bis (carbothioamide) (9a(ii))*

Anal Cal. for: $C_{27}H_{23}N_9O_4S_2$: C, 53.90; H, 3.85; N, 20.95, Found: C, 23.90; H, 3.75; N, 20.83, FTIR (KBr, cm^{-1}): 1517.93 (C=N), 1288.45(Ar-NO_2),

1388.75 (C=S), 2931.80 (Ar-CH_3), 3469.94 (-NH_2), ^{1}H NMR (500 MHz; DMSO d6; δ ppm): 2.34 (s, 3H, -CH_3), 2.76 (dd, 2H, *J*= 8.2, 7.4 Hz -CH Pyrazole), 3.36 (d, 2H, *J*= 4.2 Pyrazole), 7.29-7.11 (m, 12H, Ar-H) 7.12 (ddd, 4H, *J*= 8.3, 1.3, 0.5 Hz), 7.18 (ddd, 4H, *J*= 8.4, 1.5, 0.5 Hz), 7.28 (ddd, 4H, *J*= 8.6, 1.8, 0.5 Hz), 9.39 (s, 2H, of two -NH_2), ^{13}C NMR (400 MH_Z; DMSO-d6; δ ppm): 178.11, 163.56, 156.11, 135.45, 133.21, 131.79, 130.08, 129.23, 126.19, 119.68, 118.57, 117.50, 115.46, 112.56, 104.62, 88.61, 40.17, 38.28.

2.3.5. 3,4-bis(2-chlorophenyl)-7-(p-tolyl)-3,3a, 3b,4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c'] dipyrazole-2,5(7H)-bis(carbothioamide) (9a(iii))

Anal Cal. for $C_{27}H_{23}Cl_2N_7S_2$: C, 55.86; H, 3.99; N, 16.89. Found: C, 55.32; H, 3.49; N, 16.19 FTIR (KBr, cm^{-1}): (Spectrum No: 54): 1512.19 (C=N), 819.75 (Ar-Cl), 1342.43 (C=S), 2933.73 (Ar-CH_3), 3325.28 (- NH_2), ^{1}H NMR (500 MH_Z; DMSO d6; δ ppm): 2.34 (s, 3H, -CH_3), 2.76 (d, 2H, *J*= 8.3, 7.2 Hz -CH Pyrazole), 3.35 (d, 2H, *J*= 4.4 Hz -CH Pyrazole), 7.29-6.93 (m , 12H, Ar-H), 6.91 (ddd, 4H, *J*= 8.2, 1.4, 0.5 Hz), 7.18 (ddd, 4H, *J*= 8.3, 1.3, 0.5 Hz), 8.59 (ddd, 4H, *J*= 8.4, 1.6, 0.5 Hz) 8.4 (s, 4H, of two -NH_2), ^{13}C NMR (400 MH_Z; DMSO-d6; δ ppm): 174.19, 163.65, 157.63, 138.45, 134.21, 134.79, 130.08, 129.23, 126.19, 121.28, 118.17, 116.59, 114.46, 111.56, 104.32, 88.31, 40.17, 39.18.

2.3.6. 3,4-bis(4-methoxyphenyl)-7-(p-tolyl)-3,3a, 3b,4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c'] dipyrazole-2,5(7H)-bis(carbothioamide) (9a(iv))

Anal Cal. for $C_{29}H_{29}N_7O_2S_2$: C, 60.92; H, 5.11; N, 17.15. Found: C, 60.22; H, 5.51; N, 17.45. FTIR (KBr, cm^{-1}): 1512.19 (C=N), 1372.43 (C=S), 2931.53 (Ar-CH_3), 3312.19 (-NH_2), 1178.21 (-OCH_3), ^{1}H NMR (500 MHZ ; DMSO d6; δ ppm): 2.3 (s, 3H, -CH_3), 3.7 (s, 6H, of two -OCH_3), 2.6 (dd, 2H, *J*= 8.2, 7.3 Hz -CH Pyrazole), 4.1 (d, 2H., *J*= 4.2 Hz -CH Pyrazole) 7.11 (ddd, 4H, *J*= 8.2, 1.4, 0.5 Hz), 7.28 (ddd, 4H, *J*= 8.4, 1.5, 0.5 Hz), 7.49 (ddd, 4H, *J*= 8.5, 1.8, 0.5 Hz) 9.1 (s, 4H, of two -NH2), ^{13}C NMR (400 MH_Z; DMSO-d6; δ ppm): 177.39, 164.15, 157.63, 137.55, 134.23, 132.79, 131.18, 129.33, 127.19, 120.58, 118.47, 116.20, 113.66, 111.26, 104.42, 88.71, 40.67, 38.78.

2.3.7. 7-(4-chlorophenyl)-3,4-bis(2-hydroxyphenyl)-3,3a,3b,4-tetrahydro-2H-pyrrolo [2,3-c:5,4-c']dipyrazole-2,5(7H)-bis (carbothioamide) (9a(v))

Anal Cal. for $C_{29}H_{29}N_7S_2$: C, 55.36; H, 3.93; N, 17.38. Found: C, 55.16; H, 3.37; N, 17.58. FTIR (KBr, cm^{-1}): 1517.29 (C=N), 1329.83 (C=S), 2946.13 (Ar-CH_3), 3544.87 (-OH), 3352.69 (-NH_2), 746.56 (Ar-Cl), ^{1}H NMR (500 MHZ ; DMSO d6 ; δ ppm): 2.3 (s, 9H, of three -CH_3), 2.76 (dd, 2H, *J*= 8.2, 7.3 Hz -CH, pyrazole), 3.35 (d, 2H, *J*= 4.3, -CH, pyrazole), 6.93 (ddd, 4H, *J*= 8.3, 1.5, 0.5 Hz), 7.48 (dd, 4H, *J*= 8.4, 1.6, 0.5 Hz), 7.69 (ddd, 4H, *J*= 8.6, 1.8, 0.5 Hz), 9.11 (s, 4H, -of two -NH_2), ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 178.29, 168.15, 154.21, 134.65, 131.21, 130.89, 130.08, 129.16, 125.19, 119.58, 117.57, 116.10, 113.26, 110.66, 104.22, 88.71, 40.37, 38.28.

2.3.8. 7-(4-chlorophenyl)-3,4-bis(2-hydroxyphenyl)-3,3a,3b,4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole-2,5(7H)-bis(carbothioamide) (9b(i))

Anal Cal. for $C_{26}H_{22}ClN_7O_2S_2$: C, 55.36; H, 3.93; N, 17.38. Found: C, 55.16; H, 3.37; N, 17.58. FTIR (KBr, cm^{-1}): 1517.29 (C=N), 1329.83 (C=S), 2946.13 (Ar-CH_3), 3544.87 (-OH), 3352.69 (-NH2), 746.56 (Ar-Cl). ^{1}H NMR (500 MHZ; DMSO d6; δ ppm): 2.76 (dd, 2H, *J*= 8.1, 7.7 Hz -CH Pyrazole), 3.36 (d, 2H, *J* = 4.1 Hz Pyrazole), 6.91 (ddd, 4H, *J*= 8.2, 1.4, 0.5 Hz), 7.18 (ddd, 4H, *J*= 8.3, 1.3, 0.5 Hz), 8.59 (ddd, 4H, *J*= 8.4, 1.6, 0.5 Hz), 9.01 (s, 4H, of two -NH_2), 11.34 (s, 2H, of two -OH), ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 176.19, 162.75, 158.61, 137.55, 133.21, 130.79, 130.08, 129.23, 126.89, 119.58, 118.57, 116.50, 114.46, 111.56, 104.32, 88.61, 40.17, 38.18.

2.3.9. 7-(4-chlorophenyl)-3,4-bis(3-nitrophenyl)-3,3a,3b,4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole-2,5(7H)-bis(carbothioamide) (9b(ii))

Anal Cal. for $C_{26}H_{20}ClN_9O_4S_2$:C, 50.20; H, 3.24; N, 20.26. Found: C, 50.43; H, 3.14; N, 20.37. FTIR (KBr, cm^{-1}): 1512.37 (C=N), 1380.46 (C=S), 725.14(Ar-Cl) , 3325.28 (-NH_2), 1288.45(Ar-NO_2). ^{1}H NMR (500 MHz, DMSO d_6, δ ppm): 2.67 (dd, 2H, *J*= 8.3, 7.2 Hz -CH Pyrazole), 3.45 (d, 2H, *J*= 4.2 Hz-CH Pyrazole), 8.18 (ddd, 4H, *J*= 8.4, 1.5, 0.5 Hz), 8.54

(ddd, 4H, *J*= 8.6, 1.8, 0.5 Hz), 9.2 (s, 4H, of two -NH_2). ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 179.33, 164.75, 158.71, 137.55, 133.21, 130.79, 130.08, 129.23, 126.89, 119.58, 118.57, 116.50, 114.46, 111.56, 104.32, 88.61, 40.17, 38.18.

2.3.10. 3,4-bis(2-chlorophenyl)-7-(4-chlorophenyl)-3,3a,3b,4-tetrahydro-2H-pyrrolo [2,3-c:5,4-c']dipyrazole-2,5(7H)-bis(carbothioamide) (9b(iii)

Anal Cal. for $C_{26}H_{20}Cl_3N_7S_2$, FTIR (KBr, cm^{-1}): 1492.27 (C=N), 1390.46 (C=S), 723.44(Ar-Cl) , 3315.28 (-NH_2); C H N Analysis: Cal.: C, 51.96; H, 3.35; N, 16.31 Obs.: C, 51.43; H, 3.13; N, 16.34, ^{1}H NMR (500 MHz, DMSO d_6, δ ppm): 2.77 (dd, 2H, *J*= 8.2, 7.1 Hz -CH Pyrazole), 3.35 (d, 2H, *J*= 4.3 Hz -CH Pyrazole), 7.23 (ddd, 4H, *J*= 8.3, 1.3, 0.5 Hz) , 7.38 (ddd, 4H, *J*= 8.5, 1.6, 0.5 Hz), 8.56 (ddd, 4H, *J*= 8.6, 1.8, 0.5 Hz), 9.3 (s, 4H, of two -NH_2). ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 177.29, 163.55, 157.68, 136.55, 133.31, 130.19, 130.28, 129.43, 126.19, 119.58, 117.57, 115.50, 114.26, 111.16, 104.42, 87.11, 41.17, 39.18.

2.3.11. 7 (4-chlorophenyl)-3,4-bis(4-methoxyphenyl)-3,3a,3b,4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c']dipyrazole-2,5(7H)-bis(carbothioamide) (9b(iv))

Anal Cal. for $C_{28}H_{26}ClN_7O_2S_2$**:** C, 60.04; H, 4.68; N, 17.50. Found: C, 60.24; H, 4.23; N, 17.25. FTIR (KBr, cm^{-1}): 1490 (C=N), 1174.66 (O-CH_3), 1396.46 (C=S), 713.66 (Ar-Cl). 3345.28 (-NH_2), 749.15 (Ar-Cl); C H N Analysis: Cal.: C, 56.79; H, 4.43; N, 16.56 Obs.: C, 56.37; H, 4.16; N, 16.86, ^{1}H NMR (500 MHz, DMSO d_6, δ ppm): 2.57 (dd, 2H, *J*= 8.3, 7.4 Hz -CH Pyrazole), 3.5 (d, 2H, *J*= 4.2 Hz CH Pyrazole), 3.81 (s, 6H, of two -OCH_3), 7.21 (ddd, 4H, *J* = 8.4, 1.3, 0.5 Hz) , 7.38 (ddd, 4H, *J*= 8.5, 1.4, 0.5 Hz), 7.59 (ddd, 4H, *J*= 8.6, 1.8, 0.5 Hz), 9.01 (s, 4H, of two -NH_2). NMR (400 MH_Z; DMSO-d_6; δ ppm): 174.29, 160.15, 158.41, 134.65, 133.11, 131.79, 130.08, 129.53, 126.19, 119.88, 118.17, 115.51, 114.6, 111.56, 104.32, 88.61, 40.17, 38.18.

2.3.12. 7-(4-chlorophenyl)-3,4-di-p-tolyl-3,3a,3b,4-tetrahydro-2H-pyrrolo[2,3-c:5,4-c'] dipyrazole-2,5(7H)-bis(carbothioamide) (9b(v))

Anal Cal. for $C_{28}H_{26}ClN_7S_2$: C, 60.04; H, 4.68; N, 17.50; Found: C, 60.44; H, 4.28; N, 17.10. FTIR (KBr, cm^{-1}):1480 (C=N), 1376.46 (C=S), 2938. 78 (-CH_3 , Ar- CH_3) 733.26(Ar-Cl). ^{1}H NMR (500 MHz, DMSO d_6, δ ppm): 2.31 (s, 6H, of two -CH_3), 2.57 (dd, 2H, *J*= 8.2, 7.3 Hz -CH Pyrazole), 3.5 (d, 2H, *J*= 4.2 Hz CH Pyrazole), 7.21 (ddd, 4H, *J* = 8.4, 1.3, 0.5 Hz), 7.31 (ddd, 4H, *J*= 8.2, 1.4, 0.5 Hz), 7.39 (ddd, 4H, *J*= 8.5, 1.8, 0.5 Hz), 9.01 (s, 4H, of two -NH_2). ^{13}C NMR (400 MH_Z; DMSO-d_6; δ ppm): 170.19, 161.75, 158.61, 138.55, 133.21, 130.79, 131.08, 129.23, 126.89, 119.58, 118.57, 116.50, 114.46, 111.56, 104.32, 88.61, 40.17, 38.18.

3. RESULTS AND DISCUSSION

3.1. Chemistry

This work of synthesis is divided in to three steps; in first step, synthesis of cyclic imides 5a and 5b was carried out by conventional method. The succinic acid refluxed with thionyl chloride which afforded the succinyl chloride, then p-toluidine and p-chloro anilines were dissolved in 5mL of benzene and slowly added in to the reaction mixture till complete evolution of HCl gas and formation of compound 5a and 5b, which is recrystallized from ethanol. The reactions shown in (Scheme -I) and physical data is shown in table 1. The band of IR stretching frequency is obtained at 1700.31 cm^{-1} indicating the cyclisation and corresponds to carbonyl group of cyclic imides. Similarly, the IR stretching band was obtained at 2972.40 cm^{-1}due to presence of CH_2-CH_2 group of cyclic imide. This cyclic imides have two carbonyl and two active methylene groups which provided better circumstances for conducting claisen- schmidt type of condensation reaction.

In second step, the succinamide derivatives were reacted with substituted benzaldehydes in the presence of neutral alumina. Solvent free microwave assisted claisen- Schmidt type of condensation reaction took place leading to formation of bis-succinamide chalcone which is shown in (Scheme-II) and physical data is shown in table 2. The IR starching band obtained at 1705.03 cm^{-1} is due to presence of C=C in conjugation with C=O groups. ^{1}HNMR spectrum of chalcone derivatives shown peak at 2.3 δ singlet due to presence of -CH_3 group at aromatic ring and peak obtained at 7.6-7.2 δ multiplate indicates the presence of 6H aromatic proton Ar-H and 1H of =CH methyne proton. Similarly ^{13}C NMR spectrum showed that peak at 180 δ corresponds to presence of carbonyl carbon. Spectral analysis confirmed the structures of chalcone

derivatives; an important synthon which is required for synthesis of carbothioamide derivatives in further step.

In third step of this research study, the synthesis of important analogue of substituted pyrazole derivative of dipyrazole-2, 5 (7H)-bis (carbothioamide) was done from chalcones; **7ai-v**and **7bi-v**. The two moles of Thiosemicarbazide hydrochloride reacted with one mole chalcones; **7ai-v** in presence of neutral alumina.

Grinding in mortar with pestle and irradiation in microwave oven converted into 7-(p-toly) -2H-pyrrolo [2, 3-c: 5, 4-c'] dipyrazole-2, 5(7H)-bis (carbothioamide); **9ai-v**. In the same way, the one mole of Chalcones; **7bi-v** treated with two moles of Semicarbazide hydrochloride was converted in to 7-(4-chlorophenyl) -2H-pyrrolo [2, 3-c: 5, 4 c'] dipyrazole-2, 5 (7H)-bis (carbothioamide); **9bi-v** which is shown in **(Scheme-III)** and physical data is shown in table 3.

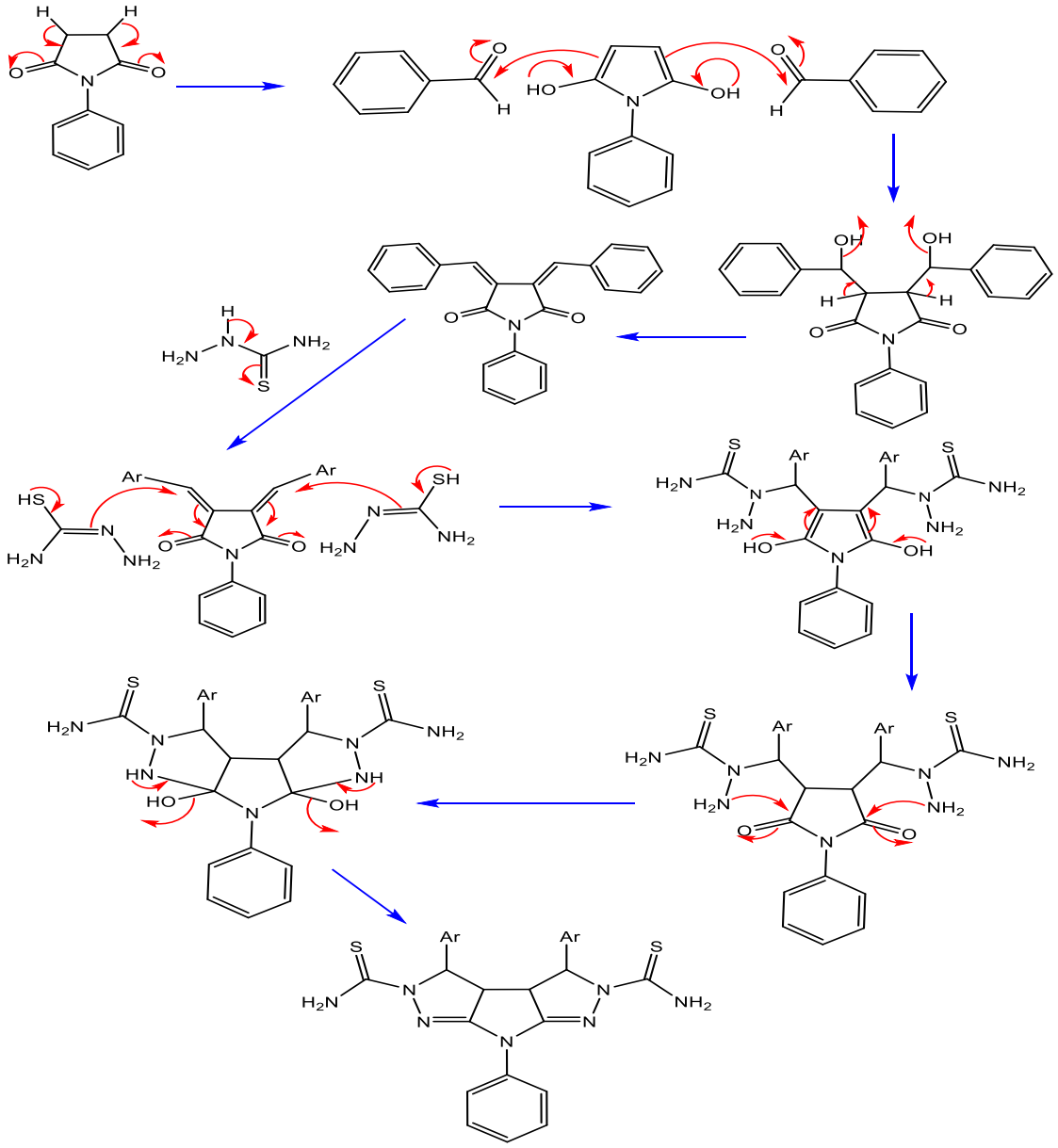


Fig.1: Possible mechanism of pyrazole carbothiomide from succinamide

The condensation with ring closure reaction takes place and formation of carbothiomide derivatives have been confirmed by spectral analysis. The FTIR starching frequency obtained at 1490 cm^{-1} indicated the presence of C=N group which gives an evidence of cyclisation and formation of pyrazole ring. Similarly the band appeared at 1396.46 cm^{-1} is due to presence of thoi carbonyl group (C=S), and absorption peak obtained at

3345.28 cm^{-1} is due to presence of (-NH_2) group. The ^{1}H NMR spectral analysis of compound 9bii showed peak at 2.67 δ doublet 2H, for-CH Pyrazole and peak obtained at 3.45 δ doublet, 2H, indicate the presence of -CH Pyrazole which clearly indicated the formation of five membered pyrazole ring. The peak obtained at 9.2 δ singlet shown the presence of 4H atoms of two -NH_2 group and ^{13}C NMR spectral analysis shown the peak at 165.57 δ indicated the presence of thiocarbonyl carbon of carbothiomide. All spectral analysis data gave an evidence that synthesis of pyrazole carbothiomide derivatives was successfully carried out by using this greener synthetic approach. The possible mechanism of conversion of succinamide to carbothiomide derivative via chalcone formation is that firstly succinamide is converted into enol from which reacted with two moles of benzaldehyde leading to formation of beta- hydroxyl ketone of cyclic imide which goes to dehydration and converted in to chalcone this chalcone reacted with enol form of thiosemicarbazide (Z)-carbamohydrazonothioic acid. The nucleophilic attack of nitrogen C=N- of (Z)-carbamohydrazonothioic acid on C=C bond of chalcone leads into formation an enol form intermediate which converted in to keto form then nucleophilic attack of nitrogen of -NH_2 group to carbonyl of chalcone leading to ring closure and further loss of water molecule formation of pyrazole carbothiomide takes place. All the reactions were carried out in presence of neutral alumina. It is powerful dehydrating agent and hence all transformations are possible due to dehydration and it is evident that the reaction mechanism given in fig. 1 is possible mechanism.

3.2. Antibacterial evaluation of dipyrazole bis-carbothioamide (9ai-v) and (9bi-v)

The series of 2H-pyrrolo [2, 3-c: 5, 4 c'] dipyrazole-2, 5 (7H)-bis (carbothioamide) derivatives were selected for screening of their antibacterial activities *in vitro* against pathogenic bacteria species. The Gram positive bacteria *Staphylococcus auras* (NCIM 2079), *Bacillus subtilis* (NCIM 2250) and Gram negative bacteria *Pseudomonas aeruginosa* (NCIM 2036), *Escherichia coli* (NCIM 2109) were selected for antibacterial activities. The solution of all the compounds; **(9ai-v)** and **(9bi-v)** were prepared in DMSO solvent. The assay was carried by taking 100μgm/mL per disc by using disc diffusion method. For this purpose, nutrient agar media was employed. The results were obtained in the form of zone of inhibition and noted after period of incubation (at 37°C for 24-28 hours). The zone of inhibition was measured in mm with help of venire calliper and compared with standard antibiotic Chloramphenicol (Chmpl).

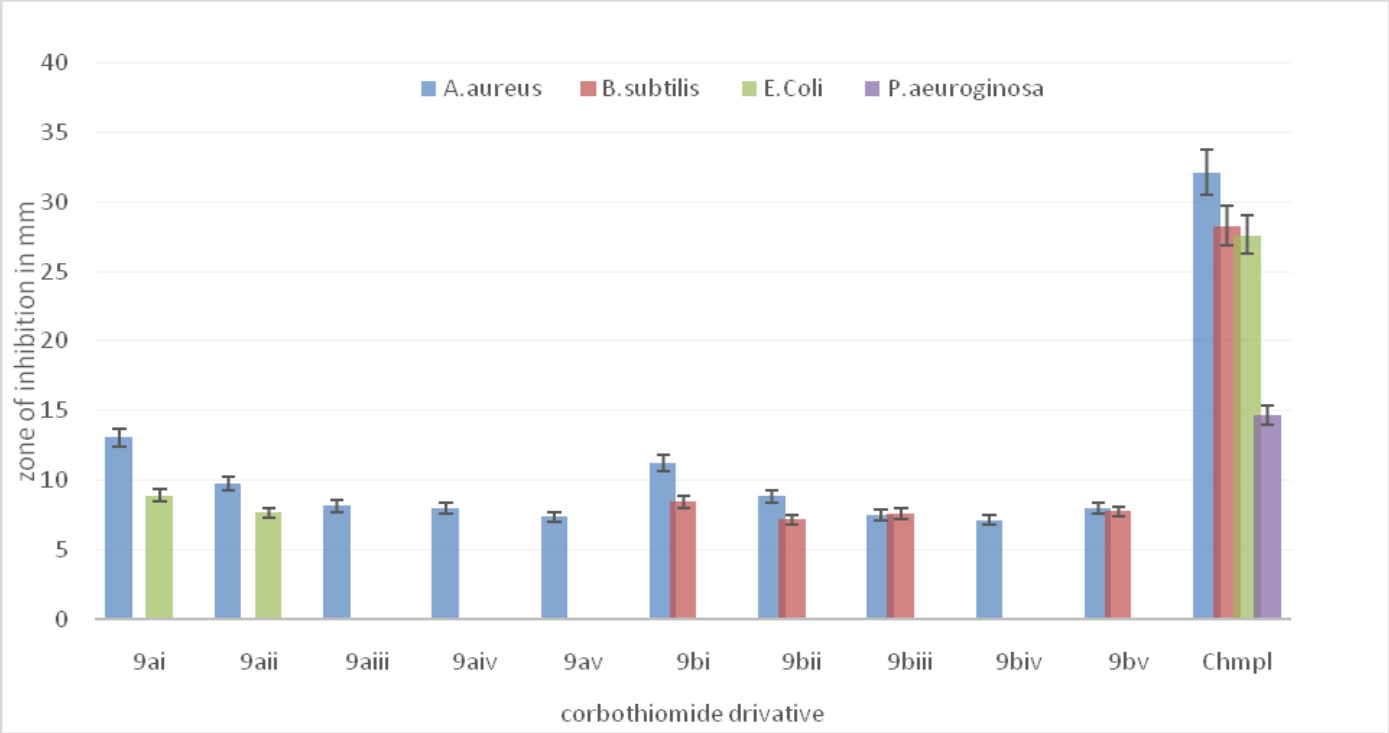
3.3. Antifungal evaluation

Antifungal evaluation was also carried out *in vitro* against fungal species *Aspergillus niger* (NCIM 545) and *Candida albicans* (NCIM 3471) in Hi-Media at conc. of 100μg/mL per disc. The zone of inhibition was measured in mm and compared with standard drug Amphotericin-B. The anti-bacterial and anti-fungal results obtained are mentioned in table 4.

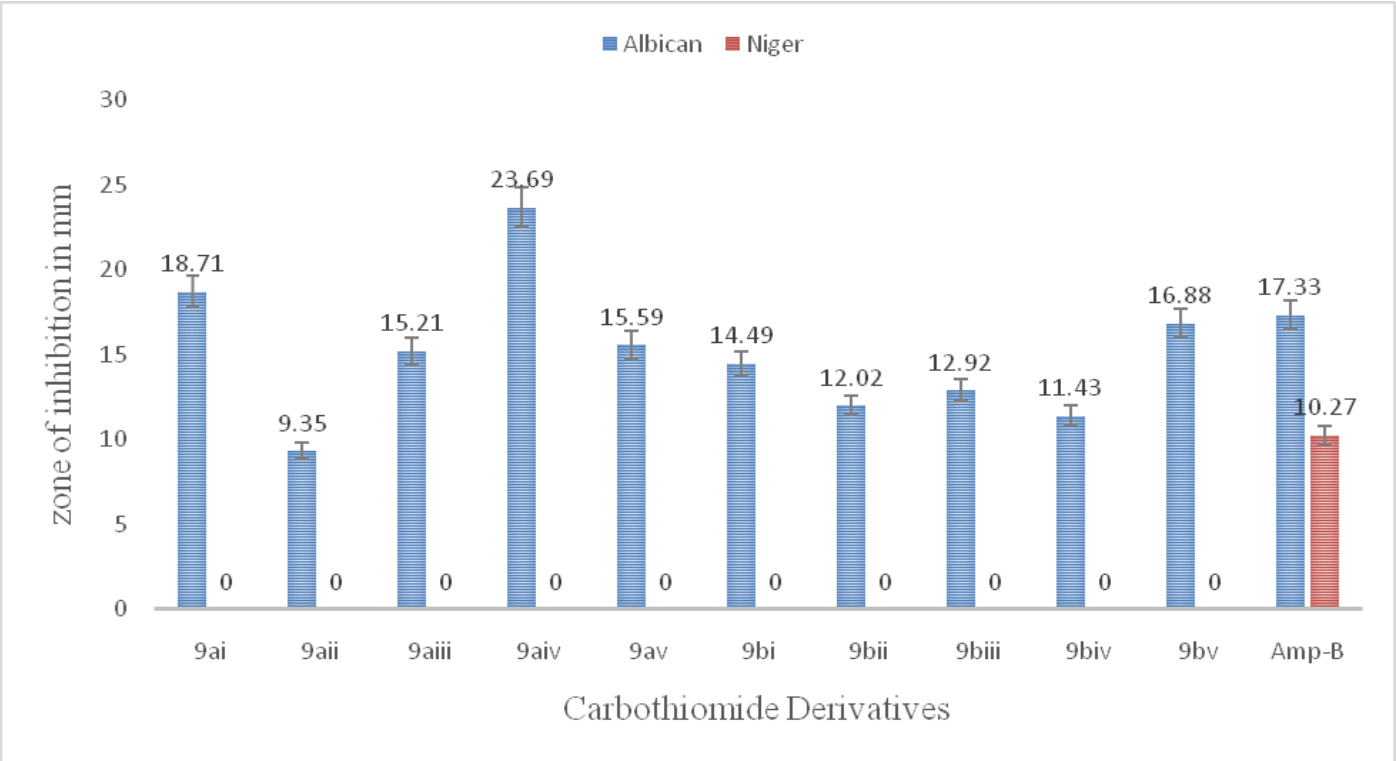
Compound	Bacterial Strains				Fungal Strains	
	Gram +ve		Gram -ve			
	A aureus	*B subtilis*	*E Coli*	*P aeuroginosa*	*C Albican*	*A Niger*
9ai	13.09	--	8.89	--	18.71	--
9aii	9.76	--	7.65	--	9.35	--
9aiii	8.15	--	--	--	15.21	--
9aiv	8.02	--	--	--	23.69	--
9av	7.37	--	--	--	15.59	--
9bi	11.22	8.45	--	--	14.49	--
9bii	8.85	7.18	--	--	12.02	--
9biii	7.53	7.62	--	--	12.92	--
9biv	7.14	--	--	--	11.43	--
9bv	8.01	7.75	--	--	16.88	--
Chmpl	32.13	28.30	27.62	14.68	NA	NA
Amp-B	NA	NA	NA	NA	17.33	10.27

Zone of inhibition in mm, 'NA' means not applicable, '-' no activity, 'Chmpl' means Chloramphenicol, 'Amp-B' means Amphotericin-B

The results obtained from microbial evaluation reveals that all carbothioamide derivatives show antibacterial activities against *A. aureus* and did not show antibacterial activities against *P. aeuroginosa* at 100 µgm/mL concentration.



Graph 1: Antibacterial activities of pyrazole carbothiamide derivatives



Graph 2: Antifungal activities of pyrazole carbothiamide derivatives

4. CONCLUSION

The present study concluded that carbothiomides incorporated with pyrazole moiety have been successfully synthesised by using green and solvent free microwave assisted method of synthesis. Microbial evaluation study reveals that all the carbothiomide derivatives exhibited antibacterial activities against and potent antifungal activities. The compounds **9bv, 9av, 9aii** showed good antifungal activity and the compound 3,4-bis(4-methoxyphenyl)-7-(p-tolyl)-3,3a,3b,4-tetra-hydro-2H-pyrrolo[2,3-c:5,4-c'] dipyrazole-2,5(7H)-bis (carbothioa-mide); **9aiv** and 3,4-bis(2-hydroxyphenyl)-7-(p-tolyl)-3,3a,3b,4-tetrahydro-2H-pyrrolo [2,3-c:5, 4-c'] dipyrazole-2,5(7H)-bis(carbothioamide); **9ai** displayed potent antifungal activities than standard antifungal drug amphotericin-B against fungal strain *C. albicans*. The objective of research was to enhance potency of pyrazole moiety which was achieved by converting them in to carbothiamide derivatives. The formulated derivatives have future scope in designing new pharmaceutical compounds this extends great possibilities in future.

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Conflict of interest

Declared none

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