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Microwave Assisted Facile One Pot Synthesis and Antimicrobial Activity of Some New Pyrazolo [3, 4-d] Pyrimidine Derivatives

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ABSTRACT

*3-methyl-1-phenyl-pyrazol-5-one **1** was used for the preparation of some new pyrazolo (3,4-d) pyrimidines derivatives **2a-2i**. Microwave assisted three component condensation of 3-methyl-1-phenyl-pyrazol-5-one **1**, thiourea and aromatic aldehydes gave 4-substituted pyrazolo (3,4-d) pyrimidines resulting from cyclization. The structures of the products obtained were confirmed by spectral data. All compounds of the series have been screened for their antibacterial (Gram positive and Gram negative) and antifungal studies. The most active compounds are **2b** against the bacterial strain P. aeruginosa and **2d** against the bacterial strains; B. subtilis and P.glabrum at M.I.C. 8μg mL^{-1} Rest of the compounds showed moderate activity against tested microbial strains at M.I.C. of 32-16 μg mL^{-1}.*

Keywords: Pyrazolo (3,4-d) pyrimidines, microwave, Synthesis, antimicrobial activity.

INTRODUCTION

Several pyrazole derivatives received great attention by organic chemists due to their biological and pharmacological activities like antimicrobial [1, 2], anti-inflammatory [3], antiviral [4], antihypertensive [5] and anticancer agents [6, 7]. Moreover, in recent years these compounds find much importance as starting material for the synthesis of other fused heterocyclic systems, among these pyrazolo(3,4-d) pyrimidines derivatives is of interest as potential bioactive molecules. These fused heterocyclic derivatives have been found to possess various pharmacological activities such as antitumor [8,9], antimicrobial [10,11], CNS (Central Nervous System) depressants [12,13], xanthine oxidase inhibitors [14]. The methods reported in literature for the synthesis of these compounds are associated with many drawbacks like multistep synthetic route, longer reaction time with drastic conditions, difficult work-up, low yield. With the increasing community concern over the possible influences of chemicals and chemical practices on the environment and present economic conditions, we have modified the synthetic route of the title compounds to one-pot multicomponent reaction under microwave irradiation. From this method new targets were synthesized which are structurally/ chemically related to allopurinol, a potent inhibitors of xanthine oxidase and the growth of several human tumour cell lines [15].

MATERIALS AND METHODS

Melting and boiling points were taken in open capillary and are uncorrected. Infra red spectra were recorded in KBr disc on Perkin Elmer FTIR-spectrometer and ν_{max} was recorded in cm^{-1} Proton Magnetic Resonance spectra were recorded on BRUKER AVANCE II 400 NMR Spectrometer with auto sampler in $CDCl_3$ using tetramethylsilane (TMS) as internal standard and the chemical shifts values were recorded in ppm on δ scale. Mass spectra were recorded by GC-MS system with model GC-MS-QP2010 Plus (Shimadzu Corporation, Kyoto, Japan). For all the reactions, chemicals of SD fine standards were used. All solvents were distilled before use.

Procedure for the synthesis of 3-methyl-1-phenyl-pyrazol-5-one pyrazolo 1: 0.0384mol of ethyl acetoacetate and 0.037mol of phenyl hydrazine was heated on boiling water bath in a conical flask for 3 h [16, 17]. The reaction mixture was stirred with glass rod. To the cooled reaction mixture 20mL solvent ether was added and reaction mixture was stirred again. The separated product was filtered, washed with ether and recrystalized from ethanol (1:1).

General procedure for the synthesis of tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2a-2i: The desired compounds **2a-2i** were synthesized by the condensation of 0.01mol of thiourea, 0.01mol of appropriate aromatic aldehyde and 0.01mol of 3-methyl-1-phenyl pyrazol-5-one **1**, catalyzed by conc. HCl in the presence of small amount of ethanol. The reaction mixture was irradiated under microwave pulse at 320W for 5-7 min. It was followed by Thin Layer Chromatography and conditions were standardized. The reaction mixture was kept overnight and product was extracted from alcohol. The crude product was filtered, washed with alcohol and recrystalized from appropriate solvents. All the compounds were obtained in good yield (57 % - 74 %).

3-Methyl-1, 4-diphenyl-1, 4, 5, 7-tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2a: White solid, Yield - 2.0 g, 64 %(methanol);m.p. 155-158^0 C; IR ν_{max} (KBr,cm^{-1})**:** 3351 (N-H), 3063 (Aromatic C-H), 2962, 2885 (Aliphatic C-H), 1597 (C=C Aromatic skeleton vib.), 1579 (C=N), 1451 (C-H def.), 1369 (C-N), ^{1}H NMR: (400 MHz, DMSO- d_6), δ (ppm): 13.84 (1H, s, NH, exchangeable with D_2O), 12.35 (1H, s, NH, exchangeable with D_2O), 7.72 (4H, d, H- a ,b, a', b', J_o=7.8 Hz), 7.41 (4H, t, H- c, d, c', d', $J_{o,m}$=7.72,1.56 Hz), 7.25-7.14 (2H, m, H- e, e'), 4.92 (1H, s, C-4), 2.32 (3H, s, CH_3), GC-MS (m/z): 321, 262 (100%), 185, 165, 128, 91, 77 and 51; Anal. Calc. for $C_{18}H_{16}N_4S_1$: C, 67.5; H, 5.0; N, 17.5; found: C, 67.44; H, 5.04; N, 17.47.

4-(2, 4-dichlorophenyl)-3-methyl-1-phenyl-1, 4, 5, 7-tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2b: Shining white crystals, Yield – 2.2 g, 57%(methanol);m.p. 208-209^0 C ; IR ν_{max} (KBr,cm^{-1})**:** 3423 (N-H), 3058 (Aromatic C-H), 2920, 2803 (Aliphatic C-H), 1598(C=C Aromatic skeleton vib.), 1573 (C=N), 1500 (N-H def.), 1470 (C-H def.), 1380 (C-N); ^{1}H NMR: (400 MHz, DMSO- d_6), δ (ppm): 13.81 (1H, s, NH, exchangeable with D_2O), 12.57 (1H, s, NH, exchangeable with D_2O), 7.82 (1H, s, H- d), 7.72 (3H, d, H- c', d', e', J_o=8.00 Hz), 7.42 (2H, dd, H-a', b', $J_{o,m}$=8.4,2.1 Hz), 7.31 (1H, dd, H- c, $J_{o,m}$=8.5, 2.2 Hz), 7.24 (1H, d, H- a, J_o=6.52 Hz), 5.10 (1H, s, C-4), 2.31 (3H, s, CH_3); GC-MS (m/z): 389, 310 (100%), 282, 254,156, 91, and 77, 51; Anal. Calc. for $C_{18}H_{14}N_4Cl_2S$: C, 55.52; H, 3.59; N, 14.39; found: C, 55.47; H, 3.63; N, 14.35.

4-(4-methoxyphenyl)-3-methyl-1-phenyl-1, 4, 5, 7-tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2c: Yellow solid, Yield - 2.5 g, 73 %(methanol);m.p. 208-209^0 C ; IR ν_{max} (KBr,cm^{-1})**:** 3350 (N-H), 3063 (Aromatic C-H), 2960, 2880 (Aliphatic C-H), 1590 (C=C Aromatic skeleton vib.), 1580 (C=N), 1517 (N-H def.), 1450 (C-H def.), 1368 (C-N), ^{1}H NMR: (400 MHz, DMSO- d_6), δ (ppm): 13.80(1H, s, NH, exchangeable with D_2O), 12.30 (1H, s, NH, exchangeable with D_2O), 7.60 (2H, d, H- a', b'), 7.58 (3H, dd, H- c', d', e'), 7.55 (2H, d, H- a, b), 7.20 (2H, d, H- c, d), 4.71 (1H, s, C-4), 3.73 (3H, s, OCH_3), 2.31 (3H, s, CH_3); Anal. Calc. for $C_{19}H_{18}N_4OS$: C, 65.14; H, 5.14; N, 16.0; found: C, 65.08; H, 5.1; N, 15.96.

Synthesis of 3-methyl-4-(3-nitrophenyl)-1-phenyl-1, 4, 5, 7-tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2d: Yellow solid, Yield - 2.1 g, 60 % ($CHCl_3$: MeOH 1:1) ;m.p. 230-232^0 C ; IR ν_{max} (KBr,cm^{-1})**:** 3361 (N-H), 3073 (Aromatic C-H), 2968, 2885 (Aliphatic C-H), 1545 (NO_2 antisymmetric.), 1598 (C=C Aromatic skeleton vib.), 1595 (C=N), 1545 (N-H def.), 1450 (C-H def.), 1369 (C-N), 1342 (NO_2 symmetric). ^{1}H NMR: (400 MHz, DMSO- d_6) δ (ppm): 13.81 (1H, s, NH, exchangeable with D_2O), 12.45 (1H, s, NH, exchangeable with D_2O), 7.78 (1H, d, H- b), 7.72 (1H, d, H- e), 7.60 (2H, dd, H- a,c), 7.51 (2H, d, H- a', b'), 7.49 (3H, dd, H- c', d', e'), 5.0 (1H, s, C-4), 2.33 (3H, s , CH_3); Anal. Calc. for $C_{18}H_{15}N_5O_2S$: C, 59.17; H, 4.10; N, 19.17; found: C, 59.13; H, 4.13; N, 19.12.

3-Methyl-4-(2-nitrophenyl)-1-phenyl-1, 4, 5, 7-tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2e: Orange solid, Yield - 2.6 g, 74 % ($CHCl_3$: MeOH 1:1) ;m.p. 155-157^0 C ; IR ν_{max} (KBr,cm^{-1})**:** 3361 (N-H), 3070 (Aromatic C-H), 2969, 2885 (Aliphatic C-H), 1598 (C=C Aromatic skeleton vib.), 1595 (C=N), 1548 (N-H def.), 1547 (NO_2 antisymmetric), 1450 (C-H def), 1372 (C-N), 1343 (NO_2 Symmetric), ^{1}H NMR: (400 MHz, DMSO-d_6), δ(PPM): 13.80 (s, 1H, NH, exchangeable with D_2O), 12.47 (s, 1H, NH, exchangeable with D_2O), 7.77 (dd, 1H, H- d), 7.70 (dd, 1H, H- e), 7.56 (dd, 2H, H- a, c), 7.50 (d, 3H, H- a', b'), 7.48 (dd, 3H, H- c', d', e'), 5.02 (s, 1H, C-4), 2.32 (s, 3H, CH_3); Anal. Calc. for $C_{18}H_{15}N_5O_2S$: C, 59.17; H, 4.10; N, 19.17; found: C, 59.12; H, 4.07; N, 19.11.

Synthesis of 4-(4-hydroxyphenyl)-3-methyl-1-phenyl-1, 4, 5, 7-tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2f: Orange solid, Yield - 2.6 g, 74 % (methanol) ;m.p. 155-157^0 C ; IR ν_{max} (KBr,cm^{-1})**:** 3435 (O-H), 3350 (N-H), 3055 (Aromatic C-H), 2962, 2880.18 (Aliphatic C-H), 1595 (C=C Aromatic skeleton vib.), 1591 (C=N)), 1518 (N-H def.), 1457 (C-H def.), 1369 (C-N), ^{1}H NMR: (400 MHz, DMSO- d_6), δ (ppm): 13.80 (1H, s, NH, exchangeable with D_2O), 12.32 (1H, s, NH, exchangeable with D_2O), 7.62 (2H, d, H- a', b'), 7.60 (3H, dd, H- c', d', e'), 7.58 (2H, d, H- a, b), 7.25 (2H, d, H- c, d), 5.0 (1H, s, OH), 4.75 (1H, s, C-4), 2.31 (3H, s, CH_3); Anal. Calc. for $C_{18}H_{16}N_4OS$: C, 64.28; H, 4.76; N, 16.66; found: C, 64.23; H, 4.79; N, 16.62.

Synthesis of 4-(4-hydroxy-3-methoxyphenyl)-3-methyl-1-phenyl-1, 4, 5, 7-tetrahydro -6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2g: yellowish orange solid, Yield - 2.2 g, 63 % (methanol); m.p. 238-240^0 C; IR ν_{max} (KBr,cm^{-1})**:** 3432 (O-H), 3345 (N-H), 3052 (Aromatic C-H), 2959, 2879 (Aliphatic C-H), 1588 (C=C Aromatic skeleton vibration), 1572 (C=N), 1510 (N-H def.), 1455 (C-H def.), 1368 (C-N). ^{1}H NMR: (400 MHz, DMSO- d_6), δ(ppm): 13.75 (1H, s, NH, exchangeable with D_2O), 12.25 (1H, s, NH, exchangeable with D_2O), 7.27-7.12 (5H, m, Aromatic H- a', b', c', d', e'), 7.10 (2H, d, Aromatic H- a, c), 6.98 (1H, s, Aromatic H- b), 5.0 (1H, s, OH), 4.5 (1H, s, C-4), 3.73 (3H, s, OCH_3), 2.30 (3H, s, CH_3); Anal. Calc. for $C_{19}H_{18}N_4O_2S$: C, 62.29; H, 4.91; N, 15.30; found: C, 62.25; H, 4.94; N, 15.26.

Synthesis of 4-(2, 3-methylene-dioxy-phenyl)-3-methyl-1-phenyl-1, 4, 5, 7-tetrahydro -6H-pyrazolo [3, 4-d] pyrimidine-6-thione 2h: Yellow solid, Yield - 2.1 g, 60 % ($CHCl_3$: MeOH 1:1); m.p. 142-145^0 C; IR ν_{max} (KBr,cm^{-1})**:** 3351 (N-H), 3058 (Aromatic C-H), 2966, 2882 (Aliphatic C-H), 1596 (C=C Aromatic skeleton vib.), 1579 (C=N), I510 (N-H def.), 1369 (C-N).^{1}H NMR: (400 MHz, DMSO- d_6), δ(ppm): 13.78 (1H, s, NH, exchangeable with D_2O), 12.30 (1H, s, NH, exchangeable with D_2O), 7.27 (2H, d, H- a', b'), 7.25 (2H, d, H- c', d'), 7.18-7.20 (1H, m, H- e'), 6.98 (2H, d, H-a, c), 6.88 (1H, dd, H- e), 5.1 (2H, s, CH_2), 4.75 (1H, s, C-4), 2.32 (3H, s, CH_3); Anal. Calc. for $C_{19}H_{16}N_4O_2S$: C, 62.63; H, 4.39; N, 15.38; found: C, 62.58; H, 4.42; N, 15.35.

Synthesis of 4-(2-hydroxyphenyl)-3-methyl-1-phenyl-1, 4, 5, 7-tetrahydro-6H-pyrazolo [3, 4-d] spyrimidine-6-thione 2i: Orange solid, Yield - 2.3 g, 72 % (methanol) ;m.p. 208-209^0 C ; IR ν_{max} (KBr,cm^{-1})**:** 3432 (O-H), 3350 (N-H), 3055 (Aromatic C-H), 2965, 2880 (Aliphatic C-H), 1595 (C=C Aromatic skeleton vib.), 1589 (C=N), 1517 (N-H def.), 1455 (C-H def.), 1369 (C-N).^{1}H NMR: (400 MHz, DMSO- d_6), δ(ppm): 13.80 (1H, s, NH,. exchangeable with D_2O), 12.32 (1H, s, NH, exchangeable with D_2O), 7.63 (2H, d, H-a', b'), 7.61 (3H , dd, H-c', d', e'), 7.60 (2H, d, H- a, c), 7.35 (1H, dd, H-e), 7.32

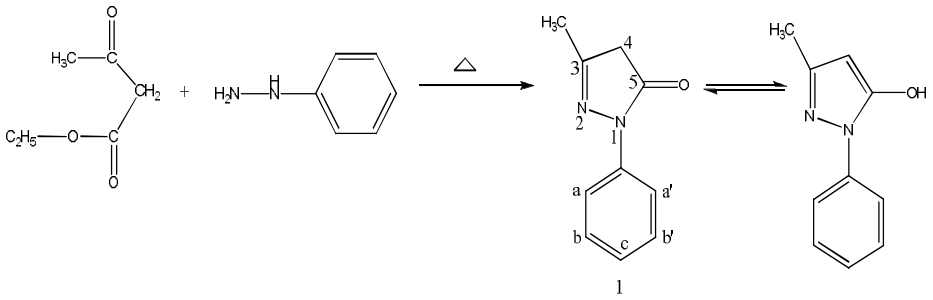
(1H, dd, H-d), 5.0 (1H, s, OH), 4.75 (1H, s, C-4), 2.31 (3H, s, CH_3); Anal. Calc. for $C_{18}H_{16}N_4OS$: C, 64.28; H, 4.76; N, 16.66; found: C, 64.24; H, 4.72; N, 16.61.

Antimicrobial Studies: The synthesized compounds **2a-2i** was screened for antimicrobial activity by "Serial Tube Dilution Technique" [14, 18, 19] The bacterial strains used in the present study were *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 3384), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 424) and *Penicillium glabrum* (MTCC 4951). The fungal strains used are *Aspergillus Janus* (MTCC 2751) and *Fusarium oxysporum* (MTCC 248). In this technique, the tubes of broth medium, containing graded doses of compounds are inoculated with the test organisms. After suitable incubation, growth will occur in those tubes where concentration of compounds is below the inhibitory level and the culture will become turbid (cloudy). The tube, in which growth will not occur above the inhibitory level, will remain clear. Therefore, M.I.C. was determined by choosing lowest concentration in which no growth occurs (Table 2).

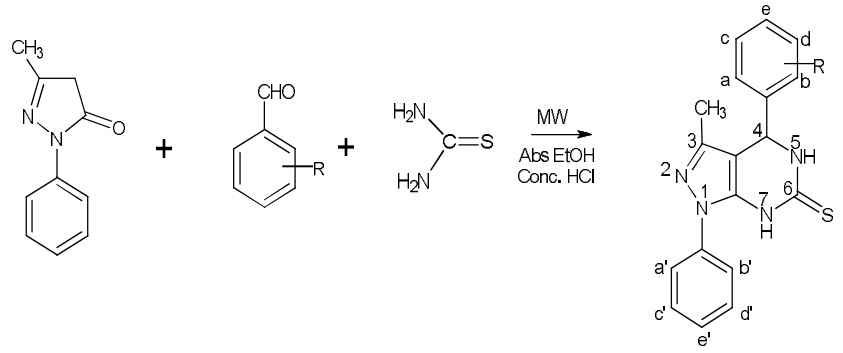
The test bacteria grown at 37^0C in nutrient agar medium were diluted in sterile nutrient broth medium in such a manner that the suspension contains about 10^7cells mL^{-1}. This suspension was used as the inoculums. 5 test tubes were taken for anti-bacterial activities.1mL of nutrient broth medium was poured to each of the tube. These test tubes were cotton plugged and sterilized in an autoclave for 15lbs sq^{-1} Inch pressure. After cool, 1mL of sample solution (made from tested compound and DMSO) was added to 1st tube and mixed well and then 1mL of this content was transferred to 2nd tube. The content of the 2nd test tube was mixed well and again 1mL of this mixture was transferred to 3rd test tube. This process of serial dilution was continued up to 5th tube.1mL of the contents was removed from tube 5th and discarded. The tubes were inoculated by 0.1mL of the bacterial suspension and then mixed well. All the test tubes were inoculated at 37^0C for 24 h. The highest dilution without growth is the M.I.C. All the samples were tested at the concentration of 128, 64, 32, 16, 8, 4, 2, and 1. The whole process was repeated for anti-fungal activities. The results of antimicrobial studies are summarised in table-2.

RESULTS AND DISCUSSION

Chemistry: The starting material 3-methyl-1-phenyl-pyrazol-5-one **1** was synthesized in good yield as per details given in experimental section by the reaction of ethyl acetoacetate and phenyl hydrazine (scheme-1) The compound **2a-2i** were synthesized by reacting compound **1** with thiourea and substituted aromatic aldehyde in the presence of few drops of conc. HCl and 10 ml of ethanol under microwave irradiation for few minutes (scheme-2). The optimum reaction conditions determined for the synthesis are summarized in table-1 and the proposed mechanism for the above reaction is given in (scheme-3) Formation of pyrazolo [3, 4-d] pyrimidine compounds **2a-2i** were characterized from the rigorous analysis of their spectral data (I.R, ^{1}H NMR and Mass spectra). I.R spectrum of compound **2a** exhibited absorption band at 3351.30 for N-H, the disappearance of C=O band of compound **1** confirmed that this group had been used in the ring formation. Moreover aromatic C-H stretching was observed at 3063 cm^{-1}, aliphatic C-H stretching at 2962, 2885 cm^{-1} and C=C and C=N stretching at 1597 and 1579 cm^{-1}. The ^{1}H NMR of compound **2a** exhibited two broad singlets at δ 13.84 and δ12.35 ppm due to NH proton which are exchangeable with D_2O. In the aromatic region most downfield resonance appeared as doublet for four protons at δ 7.70 ppm with $J_{o=}$7.8 Hz for protons a, b, a', b'. Aromatic protons c, d, c', d' appeared as triplet at δ 7.41 ppm with $J_{o,m}$ = 7.72, 1.56 Hz and a multiplet appeared at δ 7.25-7.14 ppm for the protons e and e'. Moreover it's ^{1}H NMR spectrum revealed characteristic singlet signal at δ 4.92 ppm due to proton at C-4 of pyrimidine moiety which confirmed the formation of pyrazolo pyrimidine compound. A singlet signal appeared at δ 2.31 which confirmed the presence of CH_3 protons. Mass spectrum showed m/z values at 321 (n+1), 262 (100%), 185, 165, 128, 91, 77, 51.

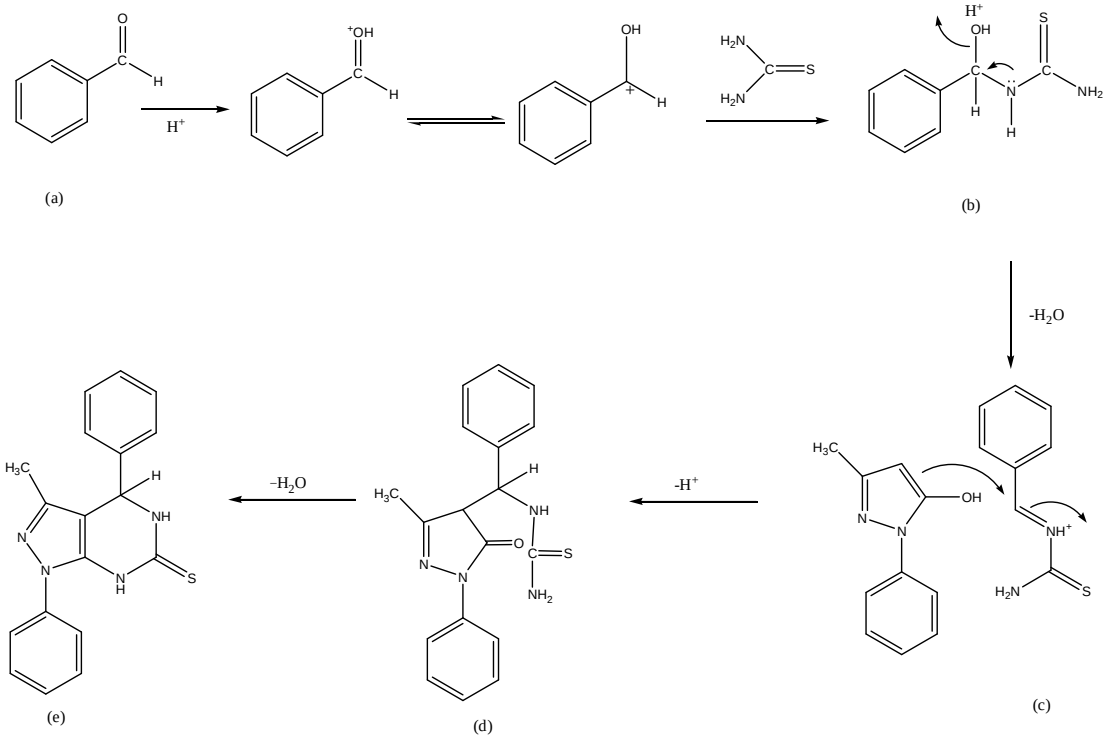


Scheme 1: Synthesis of 3-methyl-1-phenyl-pyrazol-5-one pyrazolo **1**



Scheme 2: Synthesis of tetrahydro-6H-pyrazolo [3, 4-d] pyrimidine-6-thione **2a-2i**

R: (a= H; b= 2, 4-Cl; c= 4-OCH_3; d= 3-NO_2; e= 2-NO_2; f= 4-OH; g= 4-OH, 3-OCH_3; h= 2, 3-OCH_2O; i= 2-OH)



Scheme 3: Possible mechanism for the synthesis of compounds **2a-2i**

Table-1: Physical and Analytical Data of products **2a-2i**

Product	R	Reaction Time (MW)	Product m.p (^{0}C)	Yield (%)	Molecular formula
2a	H	5	155-157	64	$C_{18}H_{16}N_4S_1$
2b	2, 4-Cl	5	208-209	58	$C_{18}H_{14}N_4Cl_2S_1$
2c	4-OCH_3	6	198-200	74	$C_{19}H_{18}N_4S_1O_1$
2d	3-NO_2	5	230-232	60	$C_{18}H_{15}N_5O_2S_1$
2e	2-NO_2	5	155-157	74	$C_{18}H_{15}N_5O_2S_1$
2f	4-OH	5	228-230	72	$C_{18}H_{16}N_4S_1O_1$
2g	4-OH, 3-OCH_3	6	238-240	63	$C_{19}H_{18}N_4O_2S_1$
2h	2, 3-OCH_2O	7	142-145	60	$C_{19}H_{16}N_4O_1S_1$
2i	2-OH	6	208-209	-	$C_{18}H_{16}N_4O_1S_1$

MW = microwave irradiation (320 W)

APPLICATIONS

Antimicrobial Activity: All the prepared compounds were tested for their anti-microbial activities by the "Serial Tube Dilution Technique" and the M.I.C. (Minimum Inhibitory Concentration) was determined in μg mL^{-1}. Standard antibacterial Amoxicillin and fungicide Fluconazole were also screened under the similar conditions for comparison. it has been observed that the significant activity of compound **2b** against the bacterial strain *P. aeruginosa* is may be due to the presence of chloro group alone in **2b** and in **2d** activity against the bacterial strains; *B. subtilis* and *P.glabrum* may be due to the presence and orientation of nitro group at meta position since **2c** in which the nitro group is at ortho position did not show any activity. The results with other test compounds show some degrees of antimicrobial activity as given in table-2(figure-1).

Table-2: In vitro antimicrobial MIC (μg/mL) of prepared compounds **2a-2h**

	Gram - negative bacteria			Gram- positive bacteria		Fungi	
Compound	***P. aeruginosa***	***E. coli***	***K. pneumoniae***	***B. subtilis***	***P. glabrum***	***A. janus***	***F. oxysporum***
2a	32	32	16	16	32	32	32
2b	**8**	32	32	16	32	32	32
2c	32	32	16	32	32	32	32
2d	16	16	32	**8**	**8**	16	32
2e	16	32	16	32	32	32	32

2f	16	32	32	16	32	32	32
2g	16	32	32	32	32	32	32
2h	16	32	32	16	32	32	32
Amoxicillin	4	4	4	4	4	-	-
Fluconazol	-	-	-			2	2

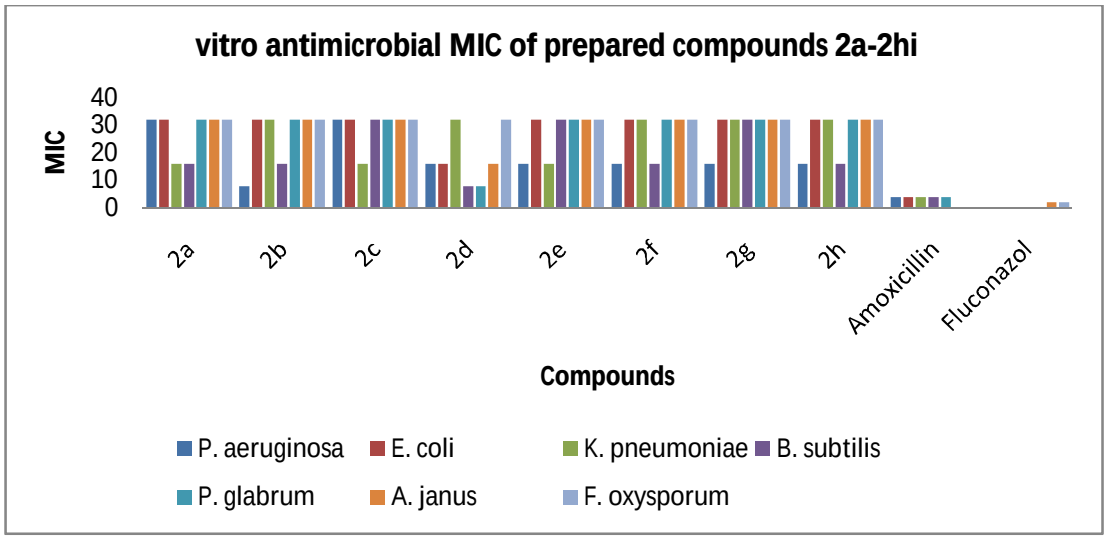


Figure-1: In Vitro Antimicrobial MIC (µg/mL) Of Prepared Compounds **2a-2h**

CONCLUSIONS

A novel series of Pyrazolo (3, 4-d) pyrimidine derivatives were synthesized through one pot multi component cyclo condensation reaction under microwave irradiation. These were characterized by IR, NMR, mass and elemental analysis. All the compounds **2a-2i** were screened for antimicrobial activity by "Serial Tube Dilution Technique" and the M.I.C. (Minimum Inhibitory Concentration) was determined in µg/mL and it was found that the nitro-substituted compound **2d** exhibited significant activity against the bacterial strains; *B. subtilis* and *P.glabrum* (gram positive) at M.I.C. of 8µg mL^{-1} at M.I.C. The chloro substituted compound **2b** was found to be the most active against the bacterial strain *P. aeruginosa* (gram negative) at M.I.C. 8µg mL^{-1}. None o the prepared compound showed antifungal activity.

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